UNIVERSIDADE FEDERAL DO PARANÁ

ÉRIKA PEREIRA ZAMBALDE

ANÁLISE DE REGIÕES ULTRACONSERVADAS TRANSCRITAS (T-UCRs) NO CÂNCER DE MAMA

CURITIBA

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Genética do Departamento de Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial para obtenção do título de Doutor em Genética.

Orientadora: Profª. Drª. Jaqueline Carvalho de Oliveira.
Co-orientadora: Profª. Drª. Enilze M. S. F. Ribeiro.

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CURITIBA, 29 de Janeiro de 2020.

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“Every adventure requires a first step”

Cheshire Cat

RESUMO

No mundo todo, o câncer de mama é a neoplasia mais frequente entre as mulheres e compreende um grupo heterogêneo de doenças, resultando em prognósticos e respostas clínicas distintas. Atualmente existem vários sistemas de classificação, sendo um dos mais usados o baseado em imunoistoquímica. Nessa classificação os tumores de mama são divididos em quatro grupos principais: luminal A, luminal B, HER2+ e triplo negativo. Apesar da classificação, ainda há uma heterogeneidade dentre os pacientes de cada grupo, fazendo com que o curso da doença e a resposta ao tratamento se diversifique. No genoma humano, foram identificadas 481 regiões ultraconservadas (UCRs) que apresentam 100% de identidade entre humanos, camundongos e ratos, e também com alta taxa de conservação em outras espécies. Tais regiões possuem de 200-781 pares de bases. Essas regiões podem ser transcritas (T-UCRs: *transcribed ultraconserved regions*), e alterações em seus perfis de expressão têm sido associados a doenças, incluindo alguns tipos de tumores, porém, pouco se sabe da influência dessas regiões no câncer de mama. Além disso, apenas 4% dos transcritos foram totalmente descritos. Na busca por entender melhor o papel desses transcritos no desenvolvimento do câncer de mama, o nível de expressão das 481 T-UCRs foi analisado através de dados depositados no banco TCGA (The Cancer Genome Atlas). Através dessa análise foi verificado que a expressão diferencial de 302 (~63%) das T-UCRs estão associadas com algum parâmetro envolvido no desenvolvimento do câncer de mama como: subtipo molecular (43,45%), receptor de estrógeno (35,76%), receptor de progesterona (32,85%), amplificação de HER2 (16,63%), estadiamento (12,47%), terapia (36,59%) e sobrevida (9,56%). Dessas regiões analisadas fizemos um filtro em busca de regiões que estavam expressas em mais de 80% da amostra, localizadas em regiões intronicas e intergênicas, e qua apresentavam diferença de expressão enre subtipos para a confirmação da diferença de expressão em pacientes brasileiras (n=102) por RT-qPCR, e obtivemos a confirmação da diferença de expressão entre subtipos das regiões uc.147 e uc.193. O transcrito uc.147 está em uma região intrônica do gene *LRBA*, sendo transcrita no sentido contrário desse gene, e com expressão restrita ao núcleo. Já o transcrito uc.193 está na região 3´UTR do gene *SYNCRIP*, transcrita no sentido oposto desse gene, e com localização predominante citoplasmática. Adicionalmente, o aumento de expressão da uc.147 foi associada com uma pior sobrevida entre os pacientes luminal A. Com as técnicas de *northern blotting* e *rapid amplification of cDNA ends* (RACE), identificamos e descrevemos um transcrito de ~2800nt para a uc.147. O silenciamento da uc.147 nas linhagens celulares do subtipo luminal CAMA-1 e BT474, diminuiu a viabilidade celular. Além disso, na linhagem luminal A CAMA-1, esse silenciamento também provocou uma diminuição na formação de colônias, aumentou a apoptose e provocou uma parada na fase G1 do ciclo celular. Essas alterações não foram vistas quando o gene *LRBA* foi silenciado, sugerindo um importante papel do uc.147 em câncer de mama. Adicionalmente, através do ensaio de pull-down nós observamos que existem muitas proteínas que interagem fisicamente com o transcrito e estão envolvidas no processo de tumorigênese. Esse trabalho é o primeiro a fazer uma análise global das T-UCRs em câncer de mama, e o primeiro a caracterizar e sugerir uma importante função

ao uc.147, além de apresentar um potencial como biomarcador de prognóstico no câncer de mama.
Palavras-chave: Câncer de mama. T-UCRs. Biomarcadores. Tumorigênese.

ABSTRACT

Breast Cancer (BC) is the most commonly diagnosed cancer and the leading cause of cancer death among women. BC is a heterogeneous disease with a rage of clinical and molecular characteristics. There are many classification systems for BC. Based on immuno-histochemical analysis, BC is subdivided in four main groups: luminal A, luminal B, HER2 and triple negative. Although, the number of BC classification system and the stratification of patients improved the therapy, inside of each subtype, patients can still have different outcomes. So, the identification of new biomarkers is important to a better prognosis, and development of more specific therapy. There are 481 ultra-conserved regions (UCRs) described in the genome, being longer than 200bp and 100% conserved among human, rat, and mouse. Most UCRs are transcribed (T-UCR) and some are differentially expressed in tumors. In addition, T-UCRs have already been described participating of the tumorigenesis process. However, the influence that T-UCRs impose on BC remains unclear. Furthermore, only 4% of T-UCRs present information on molecular details. Through analysis of all T-UCRs in the TCGA (The Cancer Genome Atlas) BC data, we found 302 regions associated with important clinical features in BC: 43% associated with molecular subtype, 36% of estrogen-receptor-positive, 17% with ERBB2 expression, 12% with clinical staging and 10% with overall survival. Looking for regions that were expressed in more than 80% of the samples, located in noncoding region and with a greater difference among subtypes we chose 12 T-UCRs to confirm differential expression in Brazilian patients (n=102) by RT-qPCR. We confirmed the differential expression among subtypes in uc.147 and uc.193. Furthermore, the strand specific RT-qPCR indicated that uc.147 and uc.193 are transcribed in the opposite direction from their host genes, *LRBA* and *SYNCRIP*, respectively. RT-qPCR. Through subcellular fractionation we demonstrated that uc.147 is located in the nucleus and uc.193 is most cytosolic. Additionally, high expression of uc.147 is associated with poor survival in luminal A patients. Northern blotting results indicated that the uc.147 transcript is around 2800nt and its sequence was confirmed by rapid amplification of cDNA ends (RACE). In addition, the silencing of uc.147 in luminal cell lines CAMA-1 and BT474 reduces cell viability, and in luminal A CAMA-1 cells the silencing reduces colony formation, increases apoptosis, and arrests cells in G1 phase of cell cycle. The same effects are not observe knocking down the *LRBA*. Besides, using pull-down assay we discover that many proteins associated with tumorigenesis process are binding to uc.147. This study is the first screening of all T-UCRs in BC, the first to characterize uc.147, and demonstrate its potential as prognostic marker in BC.

Keywords: Breast Cancer. T-UCRs. Tumorigenesis. Biomarker.

LISTA DE ILUSTRAÇÕES

REVISÃO DE LITERATURA

CAPÍTULO I

CAPÍTULO II

LISTA DE TABELAS

CAPÍTULO I

CAPÍTULO II

LISTA DE SIGLAS

°C – grau Celsius

µg – micrograma

µL – microlitro

µm – micrômetro

BC – Breast cancer

BSA – Bovine Serum Albuin

CAAE - Certificado de Apresentação para Apreciação Ética

Cat# - Catalog number

DEPC - Diethyl pyrocarbonate

DNA - Deoxyribonucleic Acid

dNTPs - Deoxynucleotide Triphosphate

dTTP - Deoxythymidine Triphosphate

EGF – Epidermal Growth Factor

FBS – Fetal Bovine Serum

g – g force

h – hours

HER2 – Human Epidermal Growth Factor Receptor 2

KI-67 - Proliferation-related ki-67 antigen

LumA – Luminal A

LumB – Luminal B

miRNA - microRNAs

mL – mililitro

nM – nanomolar

pmol – picomol

RE – Receptor de estrógeno

RNA - Ribonucleic Acid

RP – Receptor de progesterona

RQ – Relative Quantification

RT-qPCR - Reverse Transcriptase quantitative Polymerase Chain Reaction

SDS-PAGE - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

TBST - Tris Buffered Saline with Tween® 20

TBST-leite - Tris Buffered Saline with Tween® 20 com leite desnatado

TEM – Transmission Electron Microscopy

TN – Triple negative

T-UCRs – Transcribed Ultraconserved Regions

U – units

UCRs – Ultraconserved Regions

UK – United Kingdom

USA – United States of America

V – Volts

WB – Western blotting

SUMÁRIO

1 INTRODUÇÃO

O câncer de mama (CM) é o segundo tipo de neoplasia mais frequente entre as mulheres em todo o mundo e estimativas para os próximos anos indicam um aumento no número de novos casos (Bray *et al.*, 2018). O CM compreende um grupo heterogêneo de doenças, que apresentam características histopatológicas, moleculares e genéticas distintas, resultando em prognósticos e respostas clínicas diferentes (Harbeck *et al.*, 2019). Atualmente existem vários sistemas de classificação, em um dos sistemas mais usados os tumores de mama são classificados em quatro grupos, de acordo com o padrão de expressão imunoistoquimica, sendo eles: luminal A, luminal B, HER2+ e triplo negativo (Goldhirsch *et al.*, 2011; Goldhirsch, 2013).

Essa classificação é essencial para um melhor prognóstico e a adequada terapia dos pacientes. Luminal A e luminal B são grupos sensitivos a terapia hormonal. Pacientes HER2+ se beneficiam de terapia com anticorpo monoclonal (Network, 2012). O grupo triplo negativo é o mais heterogêneo, mas recentemente novas terapias alvo estão se tornando disponíveis como, por exemplo, o uso de inibidores de PARP (Beniey *et al.*, 2019).

Apesar da disponibilidade de sistemas de classificação, o progresso da doença ainda pode variar entre pacientes de um mesmo grupo. Por exemplo, alguns pacientes luminal A evoluem bem, alguns podendo até ser poupados da quimioterapia, e outros podem ser mais agressivos e resistentes a esquemas poliquimioterápicos (Curigliano *et al.*, 2017; Andre *et al.*, 2019). Dessa forma, a identificação e validação de novos marcadores moleculares é importante para melhorar o diagnóstico e prognóstico, assim como para o desenvolvimento de tratamentos mais específicos para os diversos tipos tumorais.

Em 2004, Bejerano e colaboradores, descreveram 481 regiões do genoma humano que apresentam 100% de identidade entre humanos, camundongos e ratos e também com alta taxa de conservação em outras espécies, chamadas regiões ultraconservadas (UCRs). Tais regiões possuem de 200-781 pares de bases, e estão distribuídas em todos os cromossomos, com exceção dos cromossomos 21 e Y. A maioria das UCRs são transcritas (regiões transcritas

ultraconservadas – T-UCRs) em tecidos normais (Calin *et al.*, 2007), porém poucos desses transcritos são caracterizados (Pereira Zambalde *et al.*, 2019).

As T-UCRs participam de diversos processos biológicos como diferenciação celular (Wang *et al.*, 2018; Xiao *et al.*, 2018), metabolismo (Cui *et al.*, 2016; Guo *et al.*, 2018), resistência a drogas (Sekino *et al.*, 2019) e, no desenvolvimento e progressão ao câncer (Calin *et al.*, 2007; Olivieri *et al.*, 2016; Zhou *et al.*, 2018; Pereira Zambalde *et al.*, 2019). Além disso, a diferença de expressão das T-UCRs foi demonstrada em diversos tipos tumorais como, câncer cervical (Li, Q. *et al.*, 2017), câncer colorretal (Calin *et al.*, 2007), carcinoma hepatocelular (Bo *et al.*, 2016), neuroblastoma (Mestdagh *et al.*, 2010) e outros (Calin *et al.*, 2007; Honma *et al.*, 2017; Sekino *et al.*, 2017; Zhou *et al.*, 2018). No CM, a superexpressão da uc.63 foi associada com um pior prognóstico em pacientes luminal A (Marini *et al.*, 2016), já a uc.38 apresenta baixa expressão nos pacientes (Zhang *et al.*, 2017).

Sendo assim, a análise da expressão das T-UCRs tem se demonstrado interessante e com potencial utilização como biomarcadores. No entanto, a influência que as T-UCRs exercem sobre a progressão do câncer, principalmente no CM ainda precisa ser melhor investigada, adicionalmente, somente 4% das T-UCRs apresenta caracterização detalhada dos seus transcritos (Pereira Zambalde *et al.*, 2019). Sendo assim, a potencial importância desses RNAs no contexto tumoral e a pouca informação sobre a relação dos T-UCRs com CM atestam a relevância do presente trabalho.

2 REVISÃO DE LITERATURA

2.1. CÂNCER DE MAMA

O câncer pode ser definido como um conjunto de doenças caracterizado pelo crescimento desordenado de células com potencial de invadirem tecidos e órgãos (Inca, 2019). A etiologia destas doenças envolve fatores genéticos, ambientais e o estilo de vida da população. Segundo a Organização Mundial de Saúde (WHO), o câncer é a segunda causa de morte no mundo, sendo responsável por cerca de 9.6 milhões de óbitos no ano de 2018 (Bray *et al.*, 2018).

O CM é o tipo de câncer mais frequente entre mulheres, e uma das principais causas de morte por doenças malignas no mundo (Bray *et al.*, 2018; Ferlay *et al.*, 2018). No Brasil, segundo a última estatística do Instituto Nacional do Câncer (INCA), o número de novos casos de carcinomas mamários estimados para o ano de 2018 foi de 59.700 mil novos casos, o que corresponde à 29,5% dos cânceres entre as mulheres. Para a região Sul, a estimativa foi de 11.030 novos casos, um crescimento acentuado se comparado a dados de anos anteriores (Inca, 2019).

O carcinoma mamário compreende um grupo heterogêneo de doenças, que apresentam características histopatológicas, moleculares e genéticas distintas, resultando em prognósticos e respostas clínicas diferentes (Harbeck *et al.*, 2019). Sendo assim, diferentes tipos de classificação são utilizados na identificação e diferenciação do CM.

Quando consideramos suas características histopatológicas, o CM pode ser classificado como carcinoma *in situ* (não invasivo) ou invasivo, podendo ser subdividido, de forma geral, em ductal ou lobular (Rakha *et al.*, 2010). A Organização Mundial da Saúde classifica, em relação aos tumores invasivos, o carcinoma mamário invasivo de tipo não especial e mais de 20 tipos histológicos especiais definidos predominantemente por critérios morfológicos (Lakhani and M.J., 2016). Usualmente identificado na prática médica como carcinoma ductal invasivo, o carcinoma mamário invasivo de tipo não especial é o mais frequente,

correspondendo de 40 a 75% dos casos e incluem aqueles que não possuem nenhuma característica específica que os enquadrem entre os outros tipos especiais, sendo, portanto ainda bem heterogêneo (Geyer Fc, 2013).

Entre os tipos histológicos especiais invasivos, existem mais de 20 subtipos, incluindo o lobular invasivo (segundo mais prevalente, ~15%), tubular, mucinoso, metaplásico e carcinomas com características medulares, neuroendócrinas ou apócrinas (Lakhani and M.J., 2016).

Os carcinomas invasivos podem também ser classificados baseado no grau, de acordo com o sistema de classificação de Bloom e Richardson (1957) modificado por Elston e Ellis (1991). Nessa classificação, o Grau I (baixo) caracteriza-se por ser bem diferenciado e com melhor prognóstico; Grau II (intermediário), moderadamente diferenciado; e Grau III (alto), pouco diferenciado e com pior prognóstico. Os indivíduos que possuem CM de alto grau tendem a ter recaída e metástase mais precocemente do que aqueles que possuem baixo grau (Rakha *et al.*, 2010).

Outro sistema utilizado para a classificação dos tumores malignos é o sistema TNM, que leva em consideração características clínicas e patológicas como tamanho do tumor (T), comprometimento do linfonodo regional (N) e metástase para locais distantes (M). A cada uma destas categorias é adicionado um número para indicar a extensão da doença maligna, variando entre 0 a 4. A consoante X também é utilizada quando não foi possível fazer a análise em questão. Esta classificação é disponibilizada pelo INCA (2014) e está de acordo com os padrões da União Internacional de Combate ao Câncer (UICC) (Giuliano *et al.*, 2018).

Além desses protocolos, no ano 2000, surgiu uma nova classificação do CM a classificação molecular. Esse modelo iniciou-se em 2000, por um estudo realizado por Perou e colaboradores, e foi consolidado por Sorlie e colaboradores (2001), baseado no perfil de expressão gênica desses tumores. Nessa classificação há a divisão dos subtipos moleculares em 5 categorias: luminal A, luminal B, HER2 positivo, basal e normal *like*. Posteriormente outros dois subtipos foram adicionados: a claudina "*low"* (Prat *et al.*, 2010) e o molecular apócrino (Farmer *et al.*, 2005; Sanga *et al.*, 2009).

Os tumores do tipo luminal (A e B) apresentam expressão de receptor estrógeno e progesterona positivos e de outros marcadores como queratina 8/18, o luminal A geralmente apresenta níveis de Ki-67 baixos, e baixos graus histológicos, já o subtipo luminal B, apresentam maior proliferação e são, muitas vezes, de alto grau (Reis-Filho and Pusztai, 2011; Alizart *et al.*, 2012; Eroles *et al.*, 2012; Goldhirsch, 2013).

O subgrupo HER2 positivo possui uma superexpressão deste receptor e de outros genes como o *GRB7*. Pacientes classificados com o tipo basal são negativos para o receptor de estrógeno, progesterona e HER2, mas apresentam reatividade para citoqueratina 5, citoqueratina 17, integrina ß4 e laminina. O subtipo normal *like* expressam marcadores comuns aos tecidos adiposos, como por exemplo proteína 4 de ligação ao ácido graxo e PPARγ (Perou *et al.*, 2000; Sørlie *et al.*, 2001).

Adicionalmente, o subtipo molecular claudina "*low"* é caracterizado pela diminuição da expressão de claudina 3, 4 e 7, da E-caderina e de CD24 (Prat *et al.*, 2010), e o subtipo molecular apócrino não expressa receptor de estrógeno, mas sim de andrógeno em altos níveis (Farmer *et al.*, 2005; Sanga *et al.*, 2009).

Embora a classificação molecular seja de extrema importância para o prognóstico das pacientes, a implementação desta nos sistemas de saúde não é algo viável a curto e médio prazo. Dessa forma, na Conferência de St Gallen em 2011 foi sugerida uma classificação simplificada que leva em consideração características clinico-patológicas, que são estratificadas de acordo com a análise imunohistoquímica dos tumores, uma vez que este método é mais viável para a implementação nos sistemas de saúde. Através desta técnica é possível realizar uma aproximação dos subtipos moleculares de acordo com a expressão proteica dos marcadores: receptor de estrógeno (ER) e progesterona (PR), HER2 e Ki-67 (QUADRO 1).

QUADRO 1 CLASSIFICAÇÃO DO CANCÊR DE MAMA DE ACORDO COM CARACTERÍSTICAS CLINICOPATOLÓGICAS

Subtipo	**ER/PR/HER2**	**Ki-67**	**Grau Histológico**	**Prognóstico**
Luminal A	ER+ PR+ HER2-	<14%	I ou II	Bom
Luminal B	ER+ PR+ HER2+ ER+ PR+ HER2-	<14% >14%	II ou III	Intermediário/ Desfavorável
HER2 +	ER- PR- HER2+	>14%	II ou III	Desfavorável
Triplo Negativo	ER- PR- HER2-	>14%	III	Desfavorável

FONTE: Adaptado de Goldhirsch *et al.*, 2011; Reis-Filho e Pusztal, 2011; Alizart *et al.*, 2012; Eroles *et al.*, 2012.

O prognóstico e tratamento diferem para cada um dos subtipos, por isso a classificação do tipo tumoral na clínica é de extrema importância. O subtipo luminal A tem um prognóstico favorável e normalmente se utiliza a terapia hormonal, sendo a quimioterapia restrita a alguns casos específicos. O prognóstico do subtipo luminal B é pior quando comparado ao luminal A, e o tratamento comumente utilizado é a terapia hormonal, quimioterapia e trastuzumab (um anticorpo monoclonal direcionado ao receptor HER2) quando há amplificação de HER2. O subtipo HER2+ não possui um prognóstico favorável e normalmente se utiliza a terapia específica com trastuzumab e quimioterapia como forma de tratamento (Network, 2012). Os tumores do subtipo triplo negativo são mais heterogêneos e de pior caracterização, mas recentemente novas terapias estão disponíveis, como por exemplo, os inibidores de PARP (Beniey *et al.*, 2019).

Nos últimos 10 anos, foram disponibilizadas diversas drogas para o tratamento do câncer da mama. Adicionalmente, os esquemas de tratamento visam uma medicina mais personalizada, onde os planos de tratamento são desenvolvidos com base no subtipo de câncer do indivíduo, biomarcadores ou outras características. Isso tem implementado o tratamento, muitas vezes diminuindo o uso de quimioterapias citotóxicas tradicionais para agentes

biológicos direcionados que atuam sobre marcadores moleculares dentro da célula cancerosa (FDA, 2016). Hoje, por exemplo, alguns pacientes luminal A já podem se beneficiar de um tratamento sem quimioterapia, e menos agressivo o que melhora a qualidade de vida (Curigliano *et al.*, 2017; Andre *et al.*, 2019).

Por isso existe a importância de se identificar e estratificar os subtipos tumorais com base na identificação de novos biomarcadores. Contudo, a identificação de biomarcadores com relevância para o diagnóstico, prognóstico e tratamento das doenças humanas, como por exemplo o câncer, é um processo longo, trabalhoso, sendo dependente de uma variedade de análises laboratoriais e de bioinformática.

2.2. REGIÕES TRANSCRITAS ULTRACONSERVADAS (T-UCRs).

As regiões ultaconservadas (UCRs) foram descritas primeiramente em 2004 através de estudos de bioinformática da comparação entre genomas de camundongo, rato e humanos (Bejerano *et al.*, 2004). Nessa primeira descrição foram identificadas 481 regiões entre 200 e 781 pares de base, distribuídas em todos os cromossomos, com exceção dos cromossomos 21 e Y, e perfeitamente conservadas nas três espécies analisadas. Posteriormente, observou-se alta similaridade também em outros mamíferos e até em grupos mais distantes evolutivamente, como peixes e aves, o que sugere uma forte pressão seletiva na região e importante papel biológico (Bejerano *et al.*, 2006).

Além disso, foi visto que polimorfismos de nucleotídeo único (SNPs) estão sub-representados dentro desses *loci* (Bejerano *et al.*, 2004; Yang *et al.*, 2008). E, mesmo em condições de instabilidade genômica, elementos ultraconservados não acumulam mutações (De Grassi *et al.*, 2010).

Dispersas tanto em regiões intra como intergência, foi demonstrado que 53% das UCRs são não-exônicas e apenas 47% são exônicas ou possivelmente exônicas. Dessa maneira, 256 sequências são não codificantes, e desses ainda a maioria está mepeada em regiões intergênicas. Em uma re-anotação dessas regiões, baseada na versão do genoma hg19, as UCRs foram subdivididas em 5 grupos: intergênicas (38,7%), intrônicas (42,6%), exônicas (4,2%),

parcialmente exônicas (5%) e contendo éxon (5,6%). Para as 3,9% UCRs restantes, a anotação foi variável devido a variantes de *splicing* do gene hospedeiro, sendo classificadas como “múltiplas” (Mestdagh *et al.*, 2010).

Em 2007, Calin e colaboradores (2007), através da técnica de microarranjo, descreveram que uma grande quantidade destas regiões é transcrita, denominando-as como T-UCRs, do termo em inglês *transcribed ultraconserved regions*. De acordo com sua orientação genômica, eles podem ser classificados como senso (da mesma orientação do gene de referência) ou antisenso (de orientação oposta). Este estudo ainda demonstrou a alta especificidade da expressão dessas T-UCRs em determinados tecidos, o que sugere uma importante função para tais regiões nos tecidos em que são diferencialmente expressas, além do fato de serem conservadas.

A desregulação dessas T-UCRs é associada a diferentes processos fisiológicos e patológicos que envolvem termogênese (Cui *et al.*, 2016), apoptose (Nan *et al.*, 2016; Zhang *et al.*, 2017; Qin *et al.*, 2018), renovação da barreira intestinal (Wang *et al.*, 2018; Xiao *et al.*, 2018), isquemia (Ding *et al.*, 2019), dor neuropática (Jiang, B. C. *et al.*, 2016), doença de Crohn (Qian *et al.*, 2016), além do câncer (Pereira Zambalde *et al.*, 2019).

O primeiro trabalho a demonstrar a diferença de expressão entre tecido não tumoral e tecido tumoral foi de Calin e colaboradores em 2007. Nesse trabalho, 173 amostras foram testadas, sendo 133 derivadas de câncer humano (leucemia linfocítica crônica, câncer colorretal e hepatocarcinoma celular e 40 derivadas de tecido normal (Calin *et al.*, 2007). Assim, foram avaliados padrões diferenciais de expressão nestes tipos tumorais. Este foi o trabalho inicial que associou as T-UCRs com o câncer, e a partir disto, muitos outros trabalhos foram desenvolvidos a fim de avaliar suas funções nos diferentes tipos tumorais (Terracciano *et al.*, 2017).

A diferença de expressão de 286 de 481 (59,46%) T-UCRs já foi associada com algum tipo de tumor. A maioria das T-UCRs estão diferencialmente expressas em diferentes tumores, mas algumas ainda tem sua expressão diferencial em um único tipo tumoral (Revisado por Pereira Zambalde *et al.*, 2019). Além disso, diferentes T-UCRs apresentam influencia em

mecanismos celulares que são importantes para a tumorigeneses, como proliferação, apoptose, alteração do ciclo celular, migração e invasão (Pereira Zambalde *et al.*, 2019).

Até o momento, apenas duas T-UCRs foram associadas ao CM, a uc.63 e a uc.38. A uc.63 é uma T-UCR que também está envolvida em outros tipos de canceres, sendo hipoexpressa em câncer de bexiga e superexpressa em CM, próstata e de colón e reto. Em CM o aumento da expressão de uc.63 está associada a um pior prognóstico em pacientes luminal A. E através de ensaios *in vitro* pode se observar que o silenciamento de uc.63 está associado ao aumento de apoptose e diminuição de eventos G2/M no ciclo celular (Marini *et al.*, 2017).

A baixa expressão da uc.38 é associada ao CM, essa é uma T-UCR com diferença de expressão demonstrada apenas para esse tipo de tumor. A indução de expressão da uc.38 inibe a proliferação celular e aumenta a apoptose em experimentos *in vitro*. A indução da apoptose pode ser explicada pela regulação negativa da proteína PBX1 através de uc.38, o que afeta a expressão de membros da família BCL-2 (Zhang *et al.*, 2017).

Diante do exposto torna-se evidente a importância biológica das T-UCRs no contexto tumoral e o seu potencial como biomarcadores, porém, essas moléculas ainda precisam ser melhor caracterizadas e os mecanismos celulares melhor estudados, principalmente no CM.

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3 OBJETIVOS

3.1. OBJETIVO GERAL

Avaliar a expressão das regiões ultraconservadas transcritas (T-UCRs) nos subtipos de carcinomas mamários, assim como caracterizar e avaliar o efeito da modulação de uma dessas moléculas em células mamárias.

3.2. OBJETIVOS ESPECÍFICOS

- Analisar a expressão das 481 T-UCRs em amostras de CM a partir de resultados de sequenciamento global de RNA armazenados no banco de dados TCGA;

- Confirmar as diferenças de expressão de T-UCRs que apresentam expressão em mais de 80% das amostras sequenciadas pelo TCGA, localizadas em regiões intrônicas ou intergênicas e que apresentaram diferença de expressão entre os subtipos pós análise "*in sílico"* em amostras de tecido tumoral de pacientes brasileiras diagnosticados com CM e sua associação com subtipo tumoral;

- Caracterizar o transcrito derivado da UCR evidenciado nas análises de expressão dos pacientes com CM;

- Avaliar o efeito do silenciamento de uma T-UCR na viabilidade, taxas de apoptose e ciclo celular de linhagens derivadas de pacientes com CM;

- Identificar proteínas que interagem com a T-UCR analisada funcionalmente.

4 MATERIAL E MÉTODOS

4.1 ANALISES BIOINFORMÁTICA

As 481 T-UCRs descritas por Bejerano e colaboradores (2004) foram identificadas através da sua posição em relação ao genoma. As análises foram feitas de acordo com a posição no genoma de referencia h19, de acordo com Mestdagh e colaboradores (2010) e convertidas para a posição no genoma hg38 através da ferramenta LiftOver disponível no USCS genoma browser. A partir da anotação de posição da sequência, os níveis de expressão das T-UCRs foram analisadas em relação aos parâmetros clínicos: positividade para receptores de estrogênio e progesterona, amplificação de HER2, estadiamento, sobrevida e subtipos baseados na assinatura PAM50. A busca foi feita através do website TANRIC que facilita o acesso aos dados do TCGA (The Atlas of non-coding RNA in Cancer) (Li *et al.*, 2015), e contêm os dados de expressão de 837 tumores de mama e 135 amostras não tumorais.

4.2 CARACTERIZAÇÃO DA AMOSTRA

Foram utilizadas 102 amostras de tumores de pacientes com CM, sendo 16 luminal A, 36 luminal B, 10 HER2 positivo e 40 triplo negativo provenientes do hospital Nossa Senhora da Graças, Curitiba, Paraná, Brasil. As amostras foram obtidas de tecido cirúrgico congelados com RNA later e armazenados no -80ºC. Todas as amostras foram obtidas com consentimento das pacientes através da assinatura do termo de consentimento livre e esclarecido. A classificação tumoral de acordo com os subtipos, definidos por imunoistoquímica, foi realizada posteriormente, a partir dos dados obtidos dos prontuários e laudos histopatológicos (Receptor de Estrógeno, Receptor de Progesterona, HER2 e Ki-67) das pacientes na análise das amostras tumorais obtidas na cirurgia. Os critérios de inclusão foram: possuir idade acima de 18 anos, não apresentar história familiar de CM ou outra síndrome de câncer hereditário e apresentar diagnóstico clínico e histológico positivo para o tumor de

mama maligno. Os critérios de exclusão foram: infecção grave ou qualquer outra doença ativa, doença psiquiátrica que possa interferir na participação no estudo; gestantes ou pacientes em período de amamentação; presença de histórico pessoal ou familiar compatível com síndrome de câncer hereditário.

Este projeto foi aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos do Setor de Ciências da Saúde, UFPR, sob o número de CAAE: 19870319.3.0000.0102.

4.3 CULTIVO CELULAR

Para os experimentos com cultura celular, foram realizados testes em 9 linhagens celulares de mama: MCF-10A (não tumoral), MDA-MB-231 (TN), MDA-MB-436 (TN), SK-BR-3(HER2+), BT-474 (LB), MCF-7 (LA), T-47D (LA), ZR-75-1 (LA) e CAMA-1 (LA). Todas as linhagens tumorais exceto ZR-75-1 foram cultivadas em meio DMEM/F12 (Dulbecco's Modified Eagle's Medium) (LONZA, USA) suplementado com 10% de soro fetal bovino (SFB) (Gibco, USA) e 1% Penicillin-Streptomycin (Gibco, USA), respectivamente 100 U/mL e 100 µg/mL. A linhagem ZR-75-1 foi cultivada em meio RPMI suplementado com 10% de SFB, 1mM de piruvato de sódio, 10mM hepes, e mM de glutamina e 1% Penicillin-Streptomycin. As células MCF-10A foram cultivadas em meio DMEM/F12 (Gibco, USA) suplementadas com 2,5mM de L-glutamina, 20ng/mL de fator de crescimento epidérmico (EGF), 0,01mg/mL de insulina, 500ng/ml de hidrocortisona, 0.5% de penicilina/estreptomicina e 5% de soro de cavalo. Todas as células foram cultivadas em uma estufa úmida com 5% de CO_2 a 37°C. Após atingir confluência necessária de cerca de 80%, as células foram tripsinizadas para utilização em experimentos posteriores.

4.4 ISOLAMENTO DO RNA E SÍNTESE DE cDNA

O RNA foi extraído utilizando o kit Quick RNA Miniprep (ZymoResearch, USA). A qualidade e concentração do RNA foram mensuradas através de duas técnicas: o nanodrop ND-1000 instrument (NanoDrop Tachnologies, Termo

Scientific, USA) e o Bioanalyser (Agilent, USA). Todos os RNAs passaram por um tratamento com DNAse 1U (Ambion, USA), afim de garantir que a amostra está livre de qualquer contaminante de DNA.

Para a realização da reação de cDNA foi utilizado o kit da *Invitrogen Super Script III Reverse Transcriptase*. Para cada amostra, primeiramente foi adicionado 1µL de dNTP (10mM), 1µL de *Random Primers*, 12µL de RNA, numa concentração de cerca de 1µg. Essa reação foi então levada ao termociclador por 5 minutos a 65ºC e por 2 minutos a 4ºC. O passo posterior envolveu a transcrição reversa propriamente dita, em que foi adicionado 4µL do Buffer 5x, 1µL de DTT (0,1M), 0,5µL da enzima Super Script III (200U/µL) e 0,5 de RNase out. (5000U) Essa solução também foi levada ao termociclador por 5 minutos a 25ºC, 60 minutos a 50ºC e 15 minutos a 70ºC. Ao final, espera-se que o cDNA correspondente ao RNA de interesse esteja presente na amostra, o qual pode ser utilizado nos próximos experimentos.

A síntese de fita específica foi feita numa reação total para 20µl utilizando 100ng de RNA. Duas reações foram feitas para síntese do cDNA fita especifica: uma para detectar o uc.147 na direção sense (utilizando o primer reverso tanto para uc.147 e beta actina), e outra para uc.147 na direção antisense (usando os primers *forward* para uc.147 e reverso para beta actina). Nós utilizamos somente o primer reverso da beta actina porque em ambas as reações visavam detectar apenas o mRNA da beta actina. Antes de utilizar o cDNA para a qPCR, esse foi diluído 5 vezes.

4.5 QUANTIFICAÇÃO POR PCR EM TEMPO REAL (qPCR)

As reações de PCR foram realizadas utilizando o master *mix* iQ SYBR Green (Bio-Rad) SYBR *green* (*Applied Biosystems*), e os primers apropriados para cada reação (listados na página 162 desse trabalho). Os experimentos foram realizados em triplicata no equipamento CFX96™ (*Bio-Rad*®). A quantificação relativa foi avaliada a partir da comparação do ciclo em que o nível de fluorescência alcançou um limiar pré-estabelecido (ct) do gene alvo em relação a dois controles endógenos (GAPDH, beta-actina, TBP ou U6). Para

análise dos pacientes os controles endógenos utilizados foram TBP e U6, após uma análise através do programa NormFinder. Para os cálculos de expressão nas amostras tumorais, a linhagem celular MCF-7 foi utilizada como amostra calibradora. Já para as análises de silenciamento das linhagens celulares utilizamos a beta actina e o GAPDH como controles endógenos, e a amostra calibradora foi a linhagem transfectada com controle negativo. Portanto, a quantificação do gene alvo, normalizado em relação ao gene endógeno e em relação ao calibrador foi obtida pela fórmula $2^{-\Delta\Delta CT}$.

4.6 CLONAGEM POR *RACE*

No intuito de identificar e amplificar os finais 5´e 3´ do transcrito uc.147, foi primeiramente extraído RNA da linhagem BT474, e posteriormente tratado com DNAse I (Invitrogen). Foi o utilizado o kit SMARTer RACE Amplification cDNA (Clontech) seguindo as instruções do fabricante. Os cDNAs 5´e 3´ foram então amplificados com a enzima Platinum Taq DNA Polymerase High Fidelity (Invitrogen) e gene-primers (listados na página 163 desse trabalho) específicos foram utilizados na reação. Após a PCR, a amplificação foi visualizada em gel de agarose 1,5%, e o DNA foi extraído através do kit QIAquick Gel Extraction (Qiagen), de acordo com as instruções do fabricante. O produto da reação de RACE foi então clonado no vetor a TOPO® TA pCR®2.1 (Invitrogen), e sequenciado usando primers M13 (descrito na página 162).

4.7 NORTHERN BLOTTING

Para identificação do transcrito da uc.147, os RNAs foram separados por eletroforese em gel de agarose 15% utilizando um tampão de corrida contendo 1X MOPS (40 mM de ácido morfolinopropanosulfônico, pH 7,0; 10 mM de acetato de sódio e 1 mM de EDTA, pH 8,0). A agarose (15%) foi solubilizada em solução de 1X MOPS fervente (micro-ondas por 5 min), aguardando-se o esfriamento da solução até aproximadamente 60ºC, para adição do formaldeído (37%) e brometo de etídeo (0,5 µg/ml). O RNA total de cada amostra foi, então,

submetido a desnaturação por solução contendo formaldeído (17,5% vol/vol), formamida deionizada (50% vol/vol), 10X MOPS (5% vol/vol) e água Milli-Q tratada com dietilpirocarbonato (DEPC), sob aquecimento (65ºC). Após a eletroforese, os RNAs ribossômicos foram visualizados sobre a luz ultravioleta. Este procedimento permitiu verificar se as faixas do gel estavam carregadas com as mesmas quantidades de RNA, sendo possível então a comparação entre as amostras. Além disso, foi avaliada a integridade do RNA, por meio da visualização de bandas bem definidas dos RNA ribossomais 28S e 18S (Chin *et al.*, 1998).

Para realização do Northern blotting, o RNA fracionado pela eletroforese foi transferido para uma membrana de nylon, por capilaridade, empregando a técnica descrita por Thomas, modificada por Maniatis et al. (1989). Após a transferência (12-14 h), a membrana foi cuidadosamente lavada em solução de 10X SSC e deixada por 30 minutos em temperatura ambiente; em seguida, foi feito o *crosslink* da membrana a 12000μJ/cm^2 por 10 segundos para a fixação do RNA. Após o *crosslink*, a membrana foi submetida à pré-hibridação sendo incubada em tampão de pré-hibridação (Express Hyb^{TM}), por 30 minutos a 68ºC em forno de hibridação.

Para a hibridação a membrana foi deixada com a sonda por aproximadamente 16 horas. A membrana foi, então, lavada por 15 minutos em SSC (de 2 a 0,1X) e SDS (de 0,1 a 1%) três vezes, e 30 minutos em 1X SSC e 1% SDS a 50º C. Após a lavagem, a membrana foi colocada em chassis contendo um filme, que foi sensibilizado por aproximadamente 20 dias e depois revelado e fixado dentro de uma sala escura. A sonda da uc.147 foi marcada com dCTP α^{-32}P, por random priming, utilizando-se o kit comercial Genomic Labeling Kits (Aglient, USA).

4.8 FRACIONAMENTO CELULAR

A separação das frações nuclear e citosólica foi feita nas linhagens CAMA-1, BT474 e MCF-7 utilizando o kit PARIS (Life Technologies) de acordo com as instruções do fabricante.

4.9 TRANSFECÇÃO

O protocolo utilizado para o silenciamento da região uc.147 e do seu gene hospedeiro *LRBA*, envolveu a transfecção de RNAs pequenos de interferência (siRNAs), com o reagente lipofectamina.

Dessa forma, em placa de 6 poços, cerca de 200.000 células da linhagem CAMA-1 e BT474 foram plaqueadas em cada poço, um dia antes do tratamento com o siRNA. Após as células aderirem foi iniciado o protocolo de transfecção, sendo que como controle, as células foram tratadas com a lipofectamina 2000 (1,6%) (Invitrogen) contendo um siRNA inespecífico, ou *scramble (*100nM*),* o siRNA específico para a região uc.147 (100nM) e siRNA para o gene *LRBA* (20nM). As sequências dos siRNAs utilizados estão descritas na página 164 desse trabalho.

As células foram coletadas após 24, 48 e 96 horas de transfecção, para a realização da extração do RNA, reação de transcrição reversa e posterior confirmação do silenciamento por PCR em tempo real.

4.10 VIABILIDADE CELULAR

As linhagens celulares CAMA-1 e BT474 tratadas com si-SCR, si-uc.147.1, si-uc.147.2 ou si-*LRBA* foram colocadas em placas de 96 poços. Um total de 5000 células por poço. A leitura da viabilidade celular foi feita nos tempos de 24, 48, 72 e 96 horas após a transfecção. Para fazer a leitura, primeiro o meio é substituído por 80µl de meio com 20µl de MTS (5 mg/ml) (*CellTiter 96 AQueous One Solution Cell Proliferation Assay*, Promega) e incubado por 4 horas. Após 4 horas a leitura foi feita a 580nm.

4.11 APOPTOSE

A taxa de apoptose foi analisada usando o kit de detecção *the Annexin V-FITC Apoptosis*. Após 24 horas do tratamento das linhagens celulares CAMA-1 e BT474 com si-SCR, si-uc.147.1 ou si-*LRBA*, as células foram coletadas e ressuspendidas em tampão de ligação contendo Annexin V-FITC e iodeto de propídeo (PI) de acordo com as instruções do fabricante. As amostras foram analisadas na citometria de fluxo (BD Biosciences, USA). As células foram classificadas como: viáveis, necróticas, e apoptóticas usando BD FACSVantage™ cytofluorimeter (BD Biosciences, USA), e então as porcentagens apoptóticas de cada grupo foram comparadas.

4.12 CICLO CELULAR

As linhagens celulares CAMA-1 e BT474 foram primeiramente tratadas com si-SCR (100nM), si-uc.147 (100nM) ou si-*LRBA* (20nM), e após 48 horas fixadas em etanol 70%, e marcadas com uma solução contendo 10µg/ml de iodeto de propídeo, 10,000 U/ml de RNase (Sigma-Aldrich) e 0.01% de NP40 (Sigma-Aldrich). Depois de 30-60 minutos as amostras foram analisadas por citometria de fluxo usadno o citometro BD FACSVantage™ cytofluorimeter (BD Biosciences). As analises foram feitas observando 10.000/15.000 eventos/amostras no FACS Calibur (BD Biosciences). ModFit software foi utilizado para analisar as fases do ciclo celular.

4.13 FORMAÇÃO DE COLÔNIA

Foram colocadas 500 células por poço em uma placa de 6 poços, após 24 horas ao tratamento com si-SCR (100nM), si-uc.147 (100nM) ou si-*LRBA* (20nM), e a placa foi deixada na estufa por 20 dias a 37ºC em 5% de CO_2. Após 20 dias, as células foram fixadas com 100% de metanol a temperatura ambiente por 20 minutos, coradas com cristal violeta a 1% na temperatura ambiente por 5

minutos, e posteriormente lavada com PBS até retirar o excesso de corante. O número de colônias em cada poço foi contado.

4.14 ENSAIO *PULL-DOWN*

A região uc.147 foi clonada inicialmente em vector TOPO TA (TAKARA, USA) seguindo as instruções do fabricante.

A técnica para o *pull-down* consiste no uso de vetores pMS2 (Yoon and Gorospe, 2016). A região uc.147 inserida no vetor TOPO TA foi clonada no vetor pMS2, utilizando as enzimas de digestão Xba1 (1µl- 20000U/µl) e Spe1 (2µl – 10000U/µl) Para isso, foi usado o vetor TOPO TA + uc.147 e o vetor pMS2. O vetor pMS2 foi digerido pela enzima Xba1 (1µl- 20000U/µl). O vetor pMS2 contendo a região uc.147 e um vetor controle vazio foram co-transfectados na linhagem celular HEK293 com o vetor pMS2 ligado a GST (glutationa S transferase). A GST é utilizada para purificar as proteínas de interesse presentes na solução, devido sua alta afinidade com esferas de agarose GSH.

Após 48 horas o sobrenadante foi coletado e a concentração proteica foi determinada pelo método de Bradford. Uma quantidade de 2µg/µl foi utilizada para o ensaio de o *pull-down.*

As proteínas fusionadas à proteína solúvel GST foram imobilizadas na matriz de esferas de agarose GSH (GE healthcare; cat 17-0756-01). Em seguida essa matriz foi lavada (3x) com tampão isotônico e o complexo foi eluido pela adição de solução contendo glutationa reduzida. As frações foram analisadas em gel SDS-PAGE corado com prata (Pierce™ Silver Stain for Mass Spectrometry). Os complexos proteicos que
obtidos por ensaios de *pull-down* foram analisados por espectrometria de massas no departamento Proteomics and Metabolomics Facility do MD Anderson Cancer Center.

4.15 WESTERN BLOTTING

Primeiramente a linhagem celular CAMA-1 foi tratada com si-SCR (100nM), si-LRBA (20nM) e si-uc.147.1 (100nM). Após 24 horas as células foram coletadas, e a extração de proteína foi feita. As proteínas foram quantificadas pelo ensaio de Bradford (Bio-Rad, USA), lidas a uma absorbância de 595nm em um leitor de ELISA (BioTek, USA), localizado no Departamento de Fisiologia da UFPR. As concentrações foram determinadas baseadas em uma curva padrão (0 - 5mg/mL), diluída em tampão de lise.

As proteínas foram desnaturadas a 100ºC por 10 minutos, com tampão redutor com beta-mercaptoetanol. As amostras foram então aplicadas em gel *sodium dodecyl sulfate polyacrylamide gel electrophoresis* (SDS-PAGE), utilizando-se um sistema vertical de eletroforese (Bio-Rad, USA) durante 1hora e 30minutos, a 110V. As proteínas foram transferidas para uma membrana Amersham Protran Premium 0,2 de nitrocelulose (GE Healthcare Life Science, UK) por um sistema de transferência semi dry (Bio-Rad Trans-Blot SD Cell Semi-Dry Transfer Cell). As membranas foram coradas com solução de Ponceau (Sigma-Aldrich, USA) para confirmar que as proteínas foram devidamente transferidas. Em seguida, a membrana foi lavada 3x com *Tris Buffered Saline with Tween® 20* (TBST), para retirar o Ponceau, bloqueadas com tampão TBST misturado com 10% de leite (utilizamos leite desnatado molico) por 1h e incubada por cerca de 14 horas com anticorpo de camundongo primário anti-humano específico para LRBA (Cell Signaling, Cat.#16295), todos diluídos 1:1000 em TBST-leite e beta-actina (BioVision Cat.#3917-30T) na diluição 1:3000 . Após 16 horas, a membrana foi lavada 3x com TBST e incubada com anticorpo secundário de cabra anti-coelho, marcado com HRP (*Horseradish Peroxidase*) (Cell Signaling Cat.#: 7074S), diluído 1:3000 em TBST-leite. As proteínas foram detectadas utilizando-se o kit *SuperSignal™ West Pico PLUS Chemiluminescent Substrate* (Thermo Fisher Scientific, USA), e capturadas utilizando-se o fotodocumentador Amersham Imager 680 blot (GE healthcare).

4.16 ANÁLISES ESTATÍSTICAS

Os dados estão representados como média ± desvio padrão. As análises estatísticas foram feitas pelos testes t de student e ANOVA. Valores de $p \leq 0,05$ foram considerados significantes. Todos os testes foram feitos em triplicata biológica.

5 DESCRIÇÃO DOS CAPÍTULOS

A parte de Resultados e Discussão dessa tese será dividida em dois capítulos.

O capítulo I apresenta o artigo de revisão ***Highlighting transcribed ultraconserved regions in human diseases*** revisão que foi publicado no ano de 2019 na revista *WIREs RNA*. O artigo está formatado de acordo com as normas da revista. Este artigo apresenta uma revisão bibliográfica sobre as regiões ultraconservadas em diversas doenças humanas, incluindo o câncer. Através dessa revisão, podemos observar que as T-UCRs tem um envolvimento importante em diferentes patologias, e que alterando sua expressão, processos celulares também se alteram. Além disso, também optamos por listar àquelas regiões os quais os transcritos foram descritos e caracterizados de alguma forma, tornando um acesso fácil e claro para pesquisadores que optam por trabalhar com essas regiões.

O capítulo II, considerado o mais importante dessa tese, apresenta o artigo intitulado: ***Association and characterization of transcribed ultraconserved regions (T-UCRs) in Breast Cancer*.** Esse será submetido a revista *Genome Research*, e já se encontra no formato da revista. O capítulo representa todo o trajeto dessa tese, cada experimento desenhado a fim de responder a nossa pergunta: Qual a influência das T-UCRs no CM?

O artigo se inicia com uma análise global de todas as 481 T-UCRs em CM, através das análises de bioinformática de dados armazenados em bancos de dados. Em seguida, confirmamos esses dados em amostras de tumores de pacientes brasileiras e selecionamos duas T-UCRs localizadas em regiões não codificantes, e que apresentaram diferencialmente expressas em relação com a sobrevida de pacientes e entre os subtipos de CM para continuar o estudo. Caracterizamos o transcrito da uc.193 em relação a sua localização celular e direção da transcrição, já o transcrito da uc.147, foi caracterização quanto a sua localização celular, direção da transcrição, seu tamanho e a sequência. As duas T-UCRs que ainda não haviam sido descritas no CM.

Finalmente, através da manipulação da expressão da uc.147, foi evidenciada um papel importante na viabilidade de células derivadas de CM. Sendo assim, esse trabalho foi o primeiro a identificar a influência da uc.147 no CM a partir de uma análise global. E o primeiro a caracterizar esse transcrito.

6 CAPÍTULO I

Article Title: **Highlighting Transcribed Ultraconserved Regions (T-UCRs) in Human Diseases**

Article Type:

- ○ OPINION
- ◉ ADVANCED REVIEW
- ○ PRIMER
- ○ FOCUS ARTICLE
- ○ OVERVIEW
- ○ SOFTWARE FOCUS

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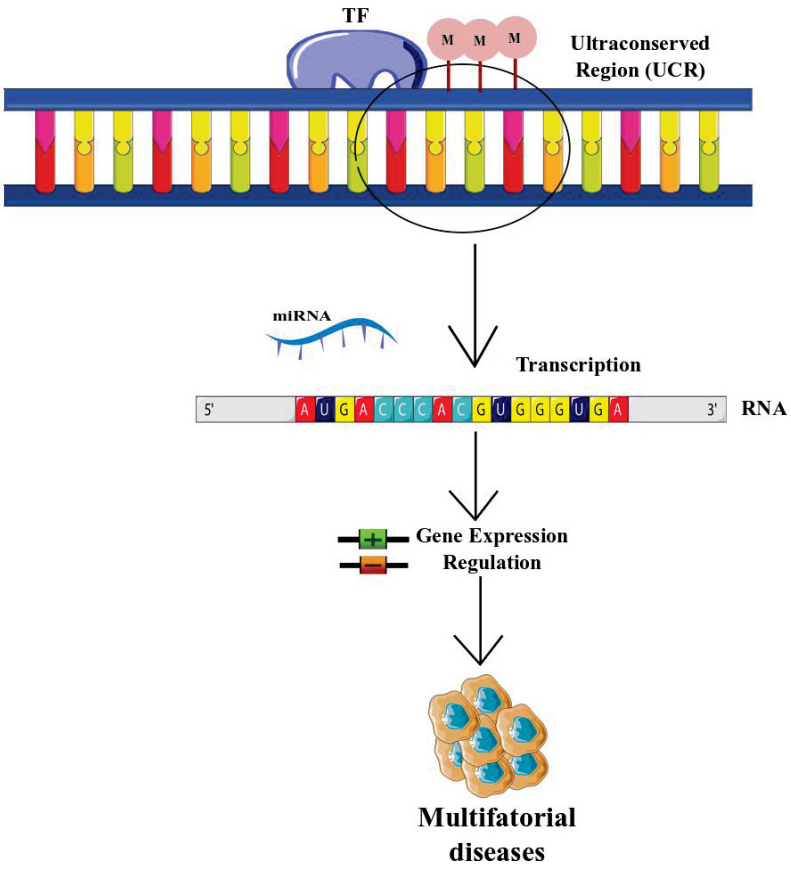
Abstract

Ultraconserved regions (UCRs) are 481 DNA segments longer than 200 bp in length that are completely conserved among human, mouse, and rat and, extremely conserved across disparate taxa. More than 90% of UCRs are transcribed (T-UCRs) in normal tissues, but most of them remain uncharacterized. In addition, it was demonstrated that T-UCRs have a tissue-specific expression, and a differential expression profile between tumors and other diseases, which suggests that most of T-UCRs may have an important role in cell processes. However, there is little information about T-UCR characterization or about their molecular mechanisms of action. Taking this into account, in this paper, we aim to summarize deregulated T-UCRs in human diseases, emphasizing the ones with stronger functional evidences that are associated with important cell pathways and have a detailed molecular characterization.

Graphical/Visual Abstract and Caption



Introduction

Ultraconserved regions (UCRs) are extremely conserved DNA segments which are 100% identical in human, mouse, and rat genomes.These regions also exhibit extremely high levels of conservation in orthologous regions of other species, such as fish, chicken, and fugu (Bejerano *et al.*, 2004). Single-nucleotide polymorphisms (SNPs) are under-represented within these loci (Bejerano *et al.*, 2004; Yang *et al.*, 2008). Moreover, ultraconserved elements do not accumulate mutations in somatic cells, even in genomic instability conditions (De Grassi *et al.*, 2010). Excluding ribosomal DNA (rDNA) regions, there are 481 UCRs, ranging in size from 200 bp to 781 bp. They are widely distributed in the human genome and, have been mapped on all chromosomes, except 21 and Y.

In the first UCR annotation (Bejerano *et al.*, 2004), which focused on overlap with protein-coding genomic regions, 23% of the UCRs overlapped mRNA known sequences (including the untranslated regions – UTRs). Fifty-three percent (53%) did not present mRNAs sequences from any species, and for 23% the overlap with a protein-coding sequence was inconclusive.

Mestdagh et al. (2010) re-organized all UCR sequences in six categories, providing more detailed genomic annotation for each region, whereas 38.7% of UCRs were intergenic, 42.6% were intronic, 4.2% were exonic, 5% partly exonic and 5.6% exon containing. For few UCRs (3.9%), the genomic annotation varied because of host gene splice variants. These UCRs were categorized as “multiple” (Mestdagh *et al.*, 2010).

In addition to this high degree of conservation, genome-wide profiling reveals extensive transcription of UCRs in normal human tissues . Therefore, these regions are also named transcribed UCRs (T-UCRs). In a pioneer study about UCR transcription, Calin et al. (2007) analyzed whether UCRs may be transcribed in normal human tissues, and whether the expression levels of UCRs are associated with tumorigenesis. To that end, the authors conducted a microarray analysis of 481 UCRs in 19 normal human tissues, in sense and antisense orientation.The majority of T-UCRs were expressed in normal human tissues, but there were a tissue-specific expression level, whereas 93% of the probes showed expression in at least one of the tissue samples analysed, and 34% were expressed in all tissues (Calin *et al.*, 2007). In this study, T-UCRs genome-wide profiling also revealed distinct signatures for chronic lymphoblastic leukemia (CLL), colorectal (CRC) and hepatocellular carcinomas (HCC) (Bullrich *et al.*, 2001; Calin *et al.*, 2007). Since then, T-UCR deregulated expression has been associated with several pathological conditions (Nan *et al.*, 2016; Fabris and Calin, 2017; Terracciano *et al.*, 2017; Ding *et al.*, 2019).

The extreme conservation of UCRs and the great number of deregulated T-UCRs associated with human diseases (summarized in Figure 1 and supplementary table 1) may be a sign of their importance in physiologic and pathophysiologic processes. As it is generally assumed that coding regions are more conserved than non-coding regions, it is interesting to highlight that more than 80 % of UCRs were intergenic or intronic. Additionally, among the intronic UCRs, almost 58% were detected in the antisense orientation when compared with the host gene, suggesting that most of these molecules did not represent only intronic transcription of the known host genes (Calin *et al.*, 2007). These numbers indicate that most T-UCRs may be long noncoding noncoding RNAs (Ling *et al.*, 2015), and they may have an important role in cell processes. However,there is little information about which T-UCR is characterized (in terms of deitaled information on RNA size and sequence), or has molecular mechanisms of action described. Here we are going to summarize deregulated T-UCRs in human diseases, highlighting T-UCRswith greater functional evidence, and detailed molecular characterization.

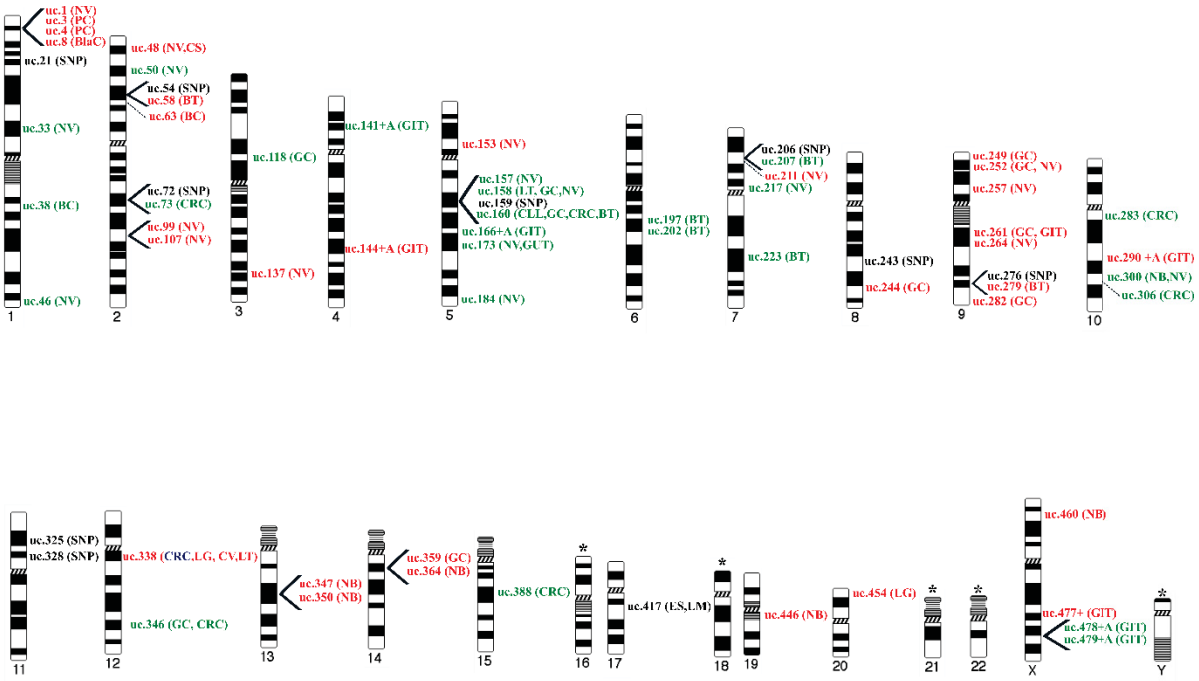


Figure 1 Ideogram representing the position of T-UCRs most studied in human diseases. T-UCRs are named based on their position according to the genome. ***IN RED:** upregulated. **IN GREEN**: down-regulated. **IN BLUE**: controversial. **IN BLACK**: associated with expression level in another diseases. **NV**: Nervous System; **PC**: Prostate Cancer; **BlaC**: Bladder Cancer; **SNP:** Single Nucleotide Polymorphism; **BC:** Breast Cancer; **CS**: Circulatory System; **BT**: Barret´s Adenocarcinoma; **CRC**: Colorectal Cancer; **GC**: Gastric Cancer; **GIT**:Gastro-Intestinal Tract; **LT**: Liver Cancer; **CLL**: Chronic Lymphocytic Leukemia; **GUT**: Gastro Urinary Tract; **NB**: Neuroblastoma; **LG**: Lung Cancer; **CV**: Cervical Cancer; **ES**: Esophagus Cancer; **LM**: Lipid Metabolism.

T-UCRs Deregulated in Cancer

In Calin et al.'s (2007) study, distinct signatures for types of tumor based on T-UCR expression were found .. In addition, this association showed that ubiquitously expressed T-UCRs are more frequently found in Cancer Associated Genomic Regions (CAGRs), when compared to UCRs. (Calin *et al.*, 2007).Since Calin et al.'s (2007) study, the differential expression of T-UCRs has been described in several types of cancer, and studies have been conducted in order to characterize the role of T-UCRs in carcinogenesis(Terracciano *et al.*, 2017). Several studies have identified distinct signatures in human carcinomas, such as: hepatocellular (HCC) (Braconi *et al.*, 2011; Carotenuto *et al.*, 2017; Luo *et al.*, 2017), neuroblastoma (NB) (Mestdagh *et al.*, 2010), chronic lymphocytic leukemia (CLL) (Calin *et al.*, 2007), prostate cancer (PC) (Hudson *et al.*, 2013) and bladder cancer (BlaC) (Olivieri *et al.*, 2016). Herein, we organized all T-UCRs described with differential expression in different types of cancer (Supplementary table 2) and figure 2 summarizes these studies, highlighting the T-UCRs that have stronger association with cancer types.

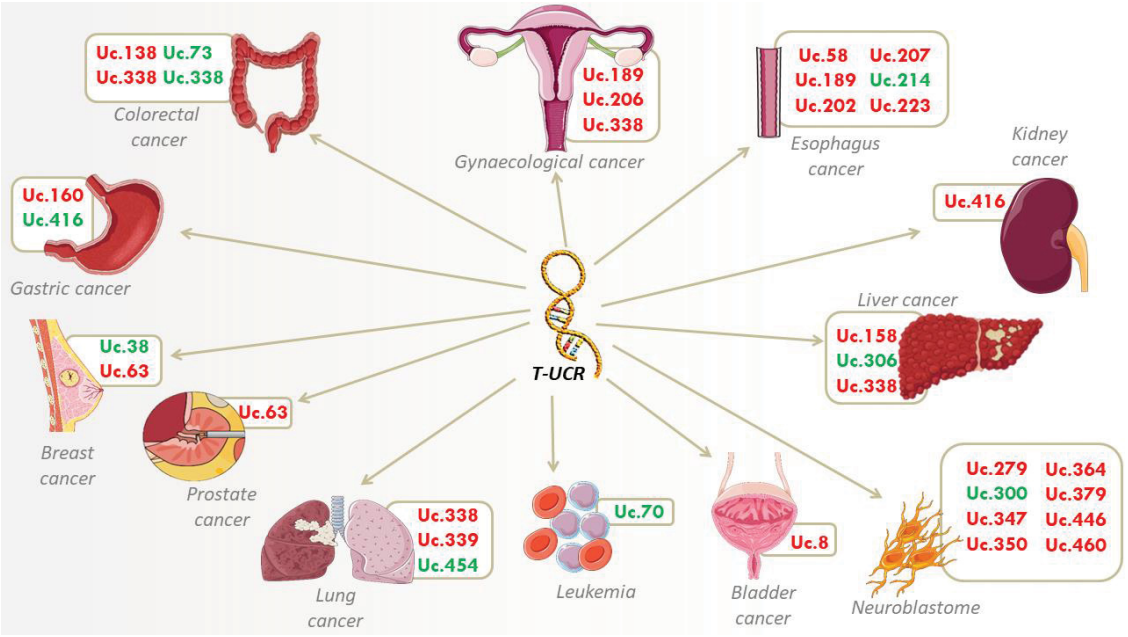


Figure 2 Repesentative T-UCRs associated with cancer. T-UCRs have differential expression in distinct types of tumor, some of them are associated with more than one type. Moreover, T-UCRs are proved to be involved with some cellular process, such as apoptosis, DNA methylation, cell cycle, proliferation. T-UCRs associated with more than one tumor type and/or tumor process are highlighted here. IN RED: upregulated. IN GREEN: down-regulated.

Not only were T-UCRs with deregulated expression found in several types of tumor, but many other T-UCRS also showed influence cell mechanisms that are important in the development of cancer, such as proliferation, apoptosis, disruption of the cell cycle, migration and invasion (Figure 3).

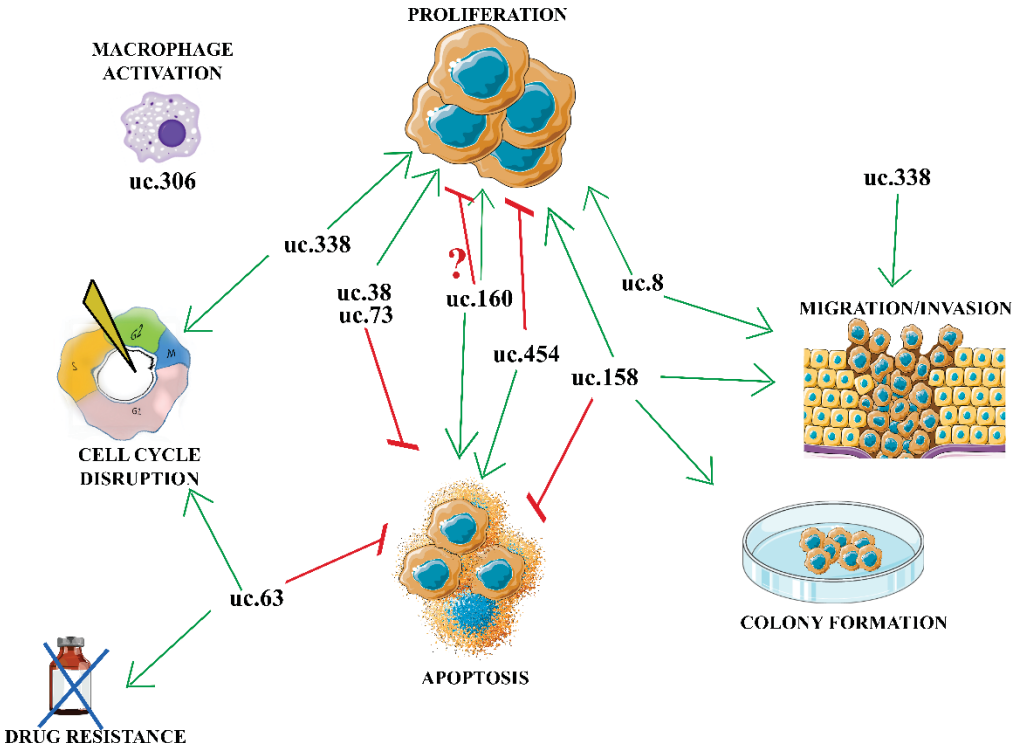


Figure 3 T-UCRs associated with the cellular processes. Tumor development depends on the combination of various cellular processes, especially the ones involving proliferation and apoptosis. T-UCRs have been proving to regulate some of these cellular processes. * green arrows are meaning promotion and red meaning suppression of physiological process.

Differential expression of 286 (59.46%) T-UCRs (out of 481) have been associated with some types of tumor. Many of them appear differentially expressed in two or more types, but few T-UCRs are differentially expressed in a specific type of tumor, for example, uc.8 in BlaC and uc.38 in breast cancer (BC).

Thus far, uc.8 is upregulated and,highlighted exclusively inBlaC.. Through functional cell assays, the inhibition of uc.8 expression decreased cell migration, proliferation, and invasion. Using bioinformatic tools, Olivieri et al. found that uc.8 acts as a decoy for miR-596, which is already known to regulate MMP9 expression (Olivieri *et al.*, 2016). Additionally, (Terreri *et al.*, 2016) showed that the transcription factor YY1, commonly overexpressed in cancer, is a mediator of binding between miR-596 and T-UCR uc.8

Downregulation of uc.38has only been demonstrated in BCThe induced expression of this T-UCR inhibitscell proliferation and induces cell apoptosis. Furthermore, uc.38 negatively regulates PBX1 protein expression and subsequently affects the expression of Bcl-2 family members, which induces BC cell apoptosis (Zhang *et al.*, 2017).

One hundred and twenty-six (126) T-UCRs are expressed in more than one tumor type, such as uc.63, uc.73, and uc.454, whichrepresents 44.05% of all differentially expressed T-UCRs. The pattern of expression can vary in each type of tumor, which means that the same T-UCR can be overexpressed in one type and down expressed in another.

For example, uc.63 is downregulated in BlaC, but overexpressed in PC, CRC, and BC. In both hormonal dependent tumors, BC and PC, uc.63 expression was associated with poor prognosis.. In BC, the overexpression of uc.63 is also associated with bad outcome in luminal A subtype patients and the silencing of uc.63 induces cell death and reduces G2/M events *in vitro* (Marini *et al.*, 2017). In serum of patients with PC, the expression of uc.63 is higher in the docetaxel-resistant patients. In addition, the induced overexpression of uc.63+ promotes resistance to docetaxel through androgen receptor regulation. Furthermore, overexpression in PC cells increases proliferation and migration, possibly by modulating MMP2 via miR-130b regulation (Sekino *et al.*, 2017).

Deregulated expression of uc.73 is associated with BlaC, CLL, and CRC (Calin *et al.*, 2007; Olivieri *et al.*, 2016). This T-UCR is overexpressed in BlaC and down expressed in CLL. In CRC, its expression is controversial, as Calin et al. (2007) demonstrated that uc.73 is upregulated, while Sana et al. (2012) indicated that uc.73 is down-regulated. upregulatedThe silencing of uc.73 reduces proliferation and increases apoptosis on COLO-320 cells (Calin *et al.*, 2007). Regarding clinical features,suc.73 was positively associated with overall survival rate (Sana *et al.*, 2012).

The deregulated expression of uc.388 is also controversial in CRC. Calin et al. (2007) analyzed 78 primary colorectal carcinomas and 21 normal colonic mucosas and proposed a CRC signature of 61 UCRs (59 up- and 2 down-regulated), in which uc.388 appears to be one of the most up regulated T-UCRs. upregulatedOn the other hand, Sana et al. (2012) evaluated T-UCRs in 54 CRC samples and 15 adjacent tissues, and found that uc.388 was downregulated and associated with the distal location of CRC. Moreover, the down-expression of uc.388 has also ben demonstrated in blaC (Olivieri *et al.*, 2016).

Uc.454 was found downregulated in all tumors studied, including BlaC, PC, and LC. This T-UCR was predicted to directly interact with Ras signaling pathway-related transcripts, such as RIN2 and RAB37 in PC (Hudson *et al.*, 2013). In addition, induced uc.454 expression in LC cell lines increased apoptosis rates and inhibited cell proliferation trough HSPA12B protein negative regulation (Zhou *et al.*, 2018). Furthermore, uc.454 was associated with tumor size and more advanced stages of LC (Zhou *et al.*, 2018).

Uc.338 expression also presents a deregulation in several types of tumor and is overexpressed in CRC, HCC, LC, GC, and CC .(Calin *et al.*, 2007; Braconi *et al.*, 2011; Li, Q. *et al.*, 2017; Wang *et al.*, 2017; Tian and Feng, 2018). The over-expression of uc.338 increases proliferation, migration, and invasion, and provoking alterations in the cell cycle.
Different pathways in the regulation of uc.338 that lead to inhibition of p21 are proposed: a) binding of p21 to polycomb complex 1, more specifically to BMI1, which suppresses p21 (Bo *et al.*, 2016); (b) promotion of p21 downregulation and cyclin D1 upregulation via the PI3K/AKT pathway in CRC cells (Zhang *et al.*, 2018); and c) negative regulation of TIMP-1 expression, which is a natural inhibitor of the matrix metalloproteinase, and also contributes to cell proliferation and migration in CRC (Wang *et al.*, 2017). Furthermore, uc.338 is associated with larger tumors, deeper tumor invasion, increased lymph node metastasis in CRC, and poorer prognosis in CRC and NSCLC (Tian and Feng, 2018).

In relation to drug resistance, , after the screening of samples from patients with CLL treated with toll-like receptor 9 agonist (CpG-ODN), it was observed that uc.216 was significantly highly expressed in CpG-ODN-stimulated versus control samples (Bomben *et al.*, 2018).

Retinoic acid is already known to induce cell differentiation (Thiele *et al.*, 1985). Watters et al. (2013) evaluated T-UCRs expression after treating NB cell lines with all-trans-retinoic acid (ATRA). As a result, they identified 32 T-UCRs that were differentially expressed (16 upregulated, 16 down-regulated). Among these T-UCRs, uc.300A expression was downregulated after ATRA treatment and, after siRNA knockdown, uc.300A was associated with proliferation and invasion. Moreover, it inhibits the differentiation of neuroblastoma cell lines prior to ATRA treatment (Watters *et al.*, 2013).

The role of the Wnt/β-catenin pathway in tumorigenesis is already known, and there are some T-UCRs involved in that pathway. Carotenuto et al (2017), through a microarray experiment in mousemodels with Wnt/β-catenin dependent HCC Carotenuto et al (2017) identified 22T-UCRs aberrantly expressed.Of these, uc.158 showed potential to differentiate Wnt/β-catenin dependent HCC. In human samples, uc.158 expression is increased in patients with CTNNB1-mutated HCC and, with nuclear localization of β-catenin. Overexpression of uc.158 was also associated with CRC. However, uc.158 is downregulated in GC, PC, and BlaC. The study also showed that theinhibition of uc.158 in HCC cells reduces anchorage cell growth, 3D-spheroid formation, cell migration, and increases apoptosis *in vitro*. In addition, the effect of uc.158 seems to be partially dependent on miR-193b. So, this study suggests that uc.158 is activated by the Wnt pathway in liver cancers and drives their growth (Carotenuto *et al.*, 2017).

Several T-UCRs were found to be associated with microRNA expression. This relationship may be essential for T-UCR rolewhen these RNAs acts as competitive endogenous and decrease the microRNA binding to other mRNA targets. This association may also suggest a possible mechanism for the regulation of T-UCR's expression. Additionally, specific transcription factors and epigenetics mechanism have been described to be regulating T-UCRs expression (Table 1).

Table 1 Regulation mechanisms of UCRs transcripts related to diseases

miR/Methylation/ Transcript Factor associated with T-UCRs	Disease	References
miR-24-1/uc.160	Leukemia	(Calin *et al.*, 2007)
miR-130b /uc.63	Prostate CA	(Sekino *et al.*, 2017)
miR-153 /uc.416	Colorectal and Renal CA	(Goto *et al.*, 2016; Sekino *et al.*, 2017)
miR-155/uc.160	Gastric CA	(Calin *et al.*, 2007; Pang *et al.*, 2018)

miR-155/uc346A	Leukemia	(Calin *et al.*, 2007)
mir-195 /uc.283	Bladder CA	(Liz *et al.*, 2014)
miR-195, miR-4668/uc.372	Lipid Metabolism	(Guo *et al.*, 2018)
Mir-195/uc.173	Gastro-Intestinal Tract	(Xiao *et al.*, 2018)
miR-214/uc.276	Colorectal CA	(Wojcik *et al.*, 2010)
miR-291a-3p/uc.173	Nervous System	(Nan *et al.*, 2016)
miR-29b/uc.173	Gastro-Intestinal Tract	(Wang *et al.*, 2018)
miR-339-3p, miR-663b-3p, and miR-95-5p/uc.339	Lung CA	(Vannini *et al.*, 2017)
miR-596/uc.8	Bladder CA	(Olivieri *et al.*, 2016)
DNA Methylation/uc.160, uc.283, and uc.346	Colorectal CA	(Kottorou *et al.*, 2018)
DNA Methylation/Uc.158+A, Uc.160+, Uc.241+A, Uc.283+A and Uc.346+A	Gastric CA	(Lujambio *et al.*, 2010; Goto *et al.*, 2016)
Transcription factor SP1/Uc.138 (TRA2β4)	Colorectal CA	(Kajita *et al.*, 2016)
Transcription factor YY1/Uc.8	Bladder CA	(Terreri *et al.*, 2016)

The most studied T-UCR associated with epigenetic regulation is the uc.160, which is a known UCR regulated by DNA methylation. Kottorou et al. (2018), for example, studied the expression and methylation of uc. 160, uc. 283 and uc. 346 in CRC. Using plasma samples of 64 CRC patients, the study showed that the methylation levels of uc.160, combined with uc.283 and uc.346, resulted in 45% sensitivity and 74.3% specificity to discriminate between cancer, adenoma and healthy control samples. Uc.160 is upregulated in CLL, and is the one of 19 T-UCRs used as a profile to differentiate CLL from normal tissue, and one of the 5 T-UCRs able to distinguish between two main subgroups of CLL patients with different prognosis (Calin *et al.*, 2007). The uc.160 is also upregulated in BlaC and downregulated in CRC and GC. Honma et al. (2007) demonstrated that the silencing of uc.160, significantly reduces cell growth. Incontrast, induced uc.160 expression in GC cells, decreases viability and proliferation, inducing even higher rates of apoptosis (Pang *et al.*, 2018). Additionally, both studies suggest that uc.160 regulates the phosphorylation of AKT by regulating tumor-suppressing *PTEN* expression in

GC(Honma *et al.*, 2017; Pang *et al.*, 2018). In addition to DNA methylation, uc.160 can also interact with miR-155 and miR-24-1 (Calin *et al.*, 2007; Pang *et al.*, 2018).

When investigating epigenetic mechanisms, (Hudson *et al.*, 2013) treated PC cells with two epigenetic drugs (the hypomethylating agent, 5-Aza 2'deoxycytidine, and the histone deacetylase inhibitor, trichostatin A) or a synthetic androgen, R1881. They showed that R1881 induces uc.287+ whereas uc.283 + A was upregulated following treatment with combined 5-Aza 2'deoxycytidine and trichostatin A.

Concerning regulation through transcription factors, (Kajita *et al.*, 2016) found that the overexpression of the alternative transcript of TRA2B gene, which contains a 419-bp UCR (uc.138), sequesters the transcription factor SP1, thus decreasing CDKN1A mRNA levels and accelerating cell growth. In order to investigate the mechanisms underlying the overexpression of uc.138 in colon cancer cells, Satake and colleagues (2018) verified that the nucleolin protein interacts with uc.338. This protein participates in the metabolism and function of RNA and cell destination (Ginisty *et al.*, 1999).

Important events, such as hypoxia, have been studied in order to evaluate the T-UCR role in cancer cells. Ferdin et al. (2013) identified a set of T-UCRs (uc.63, uc.73, uc.106, uc.134, and uc.475) that was induced after 48 hours of hypoxic conditions. Uc.475 was chosen to determine biological effects of hypoxia-induced noncoding ultraconserved transcripts. The authors demonstrated that the silencing of uc.475 decreases cell proliferation and enriches the G2/M fraction of cell cycle. Interestingly, the effects of uc.475 only occur under hypoxia and not under normal conditions (Ferdin *et al.*, 2013).

Related to tumor immunology , an important event in cancer is the recruitment of M2 macrophages to the tumor site, increasing tumor angiogenesis (Lewis and Pollard, 2006). This event is associated with poor prognosis (Hu *et al.*, 2016). In order to evaluate the relationship between T-UCRs and macrophages during polarization from the M2 (alternatively activated macrophages) to the M1 (classically activated macrophages) phenotype, Luo et al. (2017) identified 257 differently expressed T-UCRs. Among these, uc.306 was upregulated in M1 cells and appears to be involved in the Wnt pathway. Moreover, low uc.306 expression was found in BlaC and HCC, and in the latter, it was associated with shorter survival rate (Luo *et al.*, 2017).

T-UCRs Deregulated in Physiologic/Pathologic processes

Since most studies involving T-UCRs focus mainly on their functions in cancer, their role in other diseases and physiological conditions still needs to be studied in greater depth. However, there are studies describing T-UCR deregulation in diseases such as ischemia, neuropathic pain, Crohn's disease, and in the physiological processes including thermogenesis, apoptosis, and intestinal barrier renewal (Figure 4). All differentially expressed T-UCRs described in those situations are reviewed in supplementary table 3.

The T uc.173 is the most studied T-UCR in several conditions and it is highly correlated with apoptosis. Uc.173 was described as being downregulated in populations exposed to lead It was

also associated with lead-induced apoptosis in nerve cells (Nan *et al.*, 2016) and renal cells (Qin *et al.*, 2018). Induced uc.173 expression in non-treated nerve cells does not affect viability, cell cycle, and apoptosis. However, uc.173 induced expression has an inhibitory effect on lead-induced apoptosis, potentially through miR-291a-3p regulation (Nan *et al.*, 2016).

Uc.173 is also associated with the renewal of intestinal mucosal cells (Xiao *et al.*, 2018) and with intestinal barrier homeostasis (Wang *et al.*, 2018). In intestinal mucosa, induced uc.173 expression increases cell growth, whilesilencing uc.173 inhibited epithelial intestinal cell proliferation. In addition, uc.173 interacts with miR-195 and induces its degradation (Xiao *et al.*, 2018). The silencing of uc.173 causes dysfunction in the intestinal epithelial barrier by inhibiting the claudin-1 protein, which is important to determine the permeability of this tissue. The regulatory action of uc.173 is also caused by the decoy RNA effect for miR-29b, which represses claudin-1 translation (Wang *et al.*, 2018).

Regarding intestinal pathologies, Qian et al. (2016) evaluated the T-UCRs expression in mucosa from patients with Crohn's disease, where uc.290+A, uc.144+A, uc.261+A and, uc.477+ were upregulated while uc.166+A, uc.141+A, uc.478+ and, uc.479+ were down-regulated.In addition, overexpression of uc.261 participates in intestinal mucosa barrier damage, whileits silencing reverses the damage to a tight junction in inflammation, an important feature in Crohn's disease (Qian *et al.*, 2016).

Cui et al.´s (2016) study focused on the characterization of uc.417 and described its role in the thermogenesis process. The expression of uc.417 increases with age, and it has been observed that uc.417 can decrease the expression of adipogenic genes. Additionally, uc.417 was found to produce its inhibitory effect on brown adipose tissue (BAT) by suppressing phosphorylation of the p38MAPK protein, which is involved in the thermogenesis process. Therefore, it has been observed that uc.417 may be associated with loss of BAT function and thermogenesis (Cui *et al.*, 2016).

In terms of lipid metabolism, (Guo *et al.*, 2018) found that the uc.372 is upregulated in the livers of diabetic dyslipidemic mice, of mice with high-fat diet, and in patients with non-alcoholic fatty liver disease Functional assays after modulating the expression of uc.372 have shown that this T-UCR leads to hepatic lipid accumulation and promotes lipogenesis. Uc.372 binds and inhibits miR-195/miR-4668, and regulates genes related to lipid synthesis and absorption. Thus, uc.372 is an interesting target in the control of lipid accumulation in cases of steatosis.

Myocardial ischemia/reperfusion (MI/R) injury is another pathological condition that has already been associated with T-UCRs. In rats treated with a high-fat diet and MI/R, a higher expression of uc.48 was observed, as well as an upregulation of the P2X7R cation channel and consequent activation of NF-Kβ, accelerating apoptosis rates of cardiac cells. Therefore, uc.48 plays an important role in promoting apoptosis of cardiac cells and MI/R vulnerability to a high-fat diet(Ding *et al.*, 2019).

The relation between T-UCRs and systemic lupus erythematosus (SLE), which is an autoimmune disease with complex pathways, was studied by H. Lin et al. (2016).. The study aimed to investigate the influence of three factors - major histocompatibility complex (MHC), methylation of CpG islands and T-UCRs expression - on SLE regulation. Eight upregulated and 29 downregulated T-UCRs were found, but complete data from this analysis were not disclosed. Although this study integrating T-UCRs with MHC and the immune-correlated process, it did not correlate T-UCRs with common clinicopathological features of SLE patients. But differentially expressed T-UCRs were found in patients and must be better investigated (Lin *et al.*, 2016).

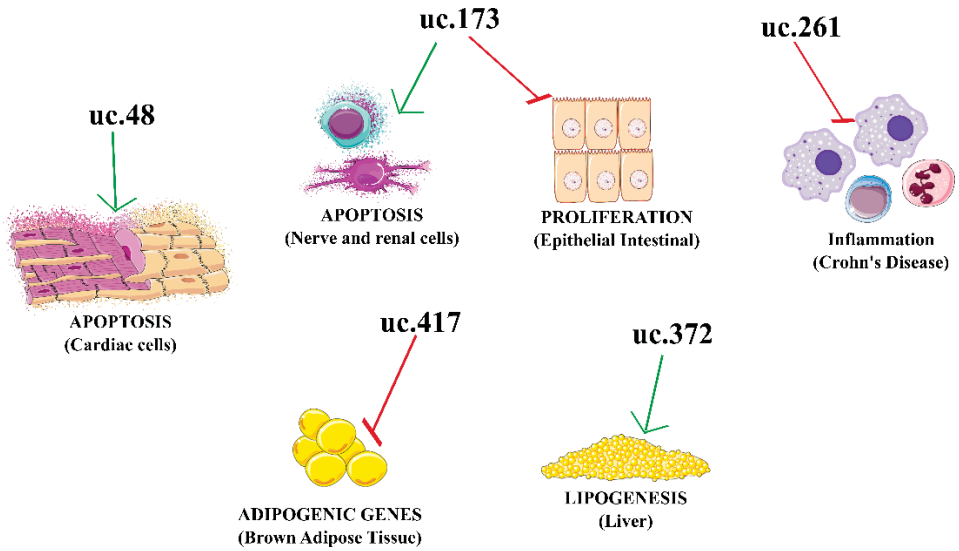


Figure 4 T-UCRs associated with physiologic/pathologic processes. The expression of T-UCRs has been described in the different physiologic and pathologic processes rather than cancer. They mostly affect cell proliferation and apoptosis in distinct tissues, but also the recruitment of inflammation cells. * green arrows meaning promotion and red meaning suppression of physiological process.

Single Nucleotide Variation in T-UCRs

As we know T-UCRs are extremely conserved, and have fewer mutations than any other regions in the genome and, in fact, ultraconserved regions tend to accumulate fewer mutations than flanking segments do, in both neoplastic and non-neoplastic samples from individuals with hereditary non-polyposis colorectal cancer (De Grassi *et al.*, 2010). Moreover, single nucleotide variations in UCR regions are associated with other diseases (Bhatia *et al.*, 2013)

Regarding familial breast cancer, Yang and colleagues (2008) evaluated the impact of six single nucleotide polymorphism (SNPs) on UCRs. In this study, they selected 1214 german women without mutations in *BRCA1* and *BRCA2* genes. The SNP rs2056116 (G>A) on uc.140 showed a significant association with familial BC risk. A stratified analysis considering rs2056116 and clinical features of BC patients revealed that BC was more predominant in premenopausal

women who were rs2056116 (G>A) carriers. This result indicate an age-related effect (Yang *et al.*, 2008). Catucci and colleagues, on the contrary, did not find a correlation between the SNPs rs2056116 and BC risk in 737 Italian women, all of whom had a history of familial BC without mutations in *BRCA1* and *BRCA2*. They observed a marginally significant protective effect for the heterozygous genotype of rs2056116 (A>G) analyzing 109 cases affected with bilateral BC compared with 1245 Italian women blood donors controls (Catucci *et al.*, 2009).

Nucleotide variations in T-UCRs were investigated in CLL patients by Wojcik et al. (2010). Of 39 genotyped CLL patients and 175 controls, two variants were identified in T-UCRs exclusively for CLL patients. These variations comprise uc.206 (G>A) and uc.243 (G>A) (Wojcik *et al.*, 2010).

Bao et al (2016) demonstrated that rs8004379 (A>C) in uc.368 exhibits a significant association in recurrence localized PC. The protective C allele was associated with a decreased risk of mortality in advanced prostate cancer patients. By bioinformatic approaches, rs8004379 probably affects NPAS3, a prostate cancer prognostic marker (Bao *et al.*, 2016).

Wojcik et al. (2010) genotyped 35 patients diagnosed with CRC to 28 SNPs located in T-UCRs. Among them, 4 ultraconserved regions exhibited variants with significant associations with CRC: uc.21 (T>C), uc.276 (A>G), uc.328 (G>T) and uc.483 (A>T). The alteration found in uc.276 disturbs the putative interaction with miR-214; however, more studies are needed to better understand the real influence of this variation on CRC (Wojcik *et al.*, 2010). Also, regarding CRC, a study with 787 CRC patients and 551 healthy controls found that rs7849 (A>G) in uc.298 is significantly associated with reducing CRC risk. In this study, the authors correlated the association between SNPs and tumor-site, and rs9784100, rs7849 and rs7033100 exhibited association with left-sided CRC susceptibility in the pooled analysis (Lin, Eng, Hawk, Huang, Greisinger*, et al.*, 2012). Additionally, rs10211390 in uc.54 was associated with more advanced CRC stages degrees for patients who had an increased recurrence risk after fluoropyrimidine-based chemotherapy (5FU) This variation was highlighted as a promising prognostic marker (Lin, Eng, Hawk, Huang, Lin*, et al.*, 2012).

A SNP (G>T) in the uc.325 site was found in a patient with aniridia, a disorder characterized by a complete or partial absence of the coloured part of the eye. Uc.325 is mapped 150 kb downstream from PAX6 and mutation in this region changes the binding site for PAX6. This mutation results in the deregulation of the gene, and may be associated with development defect (Bhatia *et al.*, 2013).

Why so conserved?

Although most UCRs are mapped in noncoding areas, it is possible that they are functional elements and, due their high conservation, an essential function for UCRs may be expected. However, (Ahituv *et al.*, 2007) did not find a decrease in viability and fertility in uc.248, uc.329, uc.467, and uc.482 null mice. They also did not find any alteration in phenotypes in terms of growth, metabolism, or apparent pathology. (Chen *et al.*, 2007) genotyped SNPs in UCRs in unrelated human DNA samples, but individuals homozygous for alleles that differ from the rodent reference genomes were viable and healthy. On the other hand, the deregulated

expression of UCRs associated with human diseases and UCR contributing to important cellular processes suggest the relevant role of these regions.

The explanations for the conservation of UCRs are intriguing. Usually, evolutionary conservation is a hallmark of biological function (Dermitzakis *et al.*, 2005) caused by a strong negative selection, since sequences that contribute to the fitness of the organism evolve slowly when compared to changes in selectively neutral sequences. Natural selection, which maintains almost the total conservation of nucleotides, is associated with multiple functions that are overlaid on the same DNA, but the mechanism for UCRs are still unknown. Extreme repairs or low mutation rates are possible explanation for UCR conservation. For conserved areas, the purifying selection is more acceptable (Drake *et al.*, 2006), so the mechanisms that maintain the conservation in UCRs remain a mystery.

Molecular Characterization of T-UCRs

Since the identification of UCRs, in 2004, several researchers have become interested in uncovering the biological function of these ultraconserved regions and in understanding how important they are in development, physiologic, and pathologic processes. An important role for most T-UCRs has been suggested in embryonic development and normal physiologic processes. The innumerous descriptions of T-UCR deregulation and DNA variations are associated with diseases and cell processes, which are reviewed herein. Such descriptions have provided evidence that T-UCRs are important molecules in normal and pathologic conditions.

Additionally, T-UCRs have been associate with tissue-specific expression, differential expression among tumors and other diseases, as well as with tumorigenesis and cellular processes. However, the molecular characterization of most T-UCRs remains unclear in terms of identification of the transcript size, cellular localization and complete sequence of these T-UCRs. Table 2 shows a summary of the T-UCRs that already present molecular characterization. There is information on molecular details for only 19 of them (3.95%), which shows the necessity of better understanding their molecular features.

Table 2 Characterization of UCRs transcripts

T-UCR	Orientation	Location	Size	Complete sequence	References
uc.8	sense	cytoplasmatic	2.435kb	available	(Olivieri *et al.*, 2016)
uc.63	antisense	nuclear	2.214kb	available	(Marini *et al.*, 2017)
uc.73	-	nuclear	-	-	(Ferdin *et al.*, 2013)

uc.106	-	nuclear	-	-	(Ferdin *et al.*, 2013)
uc.134	-	nuclear	-	-	(Ferdin *et al.*, 2013)
uc.138	-	nuclear	~0.7kb	available	(Kajita *et al.*, 2016)
uc.158	antisense	-	0.54kb	-	(Carotenuto *et al.*, 2017)
uc.190	sense	-	-	-	(Jiang, J. *et al.*, 2016)
uc.233	sense	-	-	-	(Jiang, J. *et al.*, 2016)
uc.246	sense	-	0.8kb	-	Calin et al. 2007
uc.260	sense	cytoplasmatic	-	-	(Lujambio *et al.*, 2010)
uc.269	antisense	-	1.8-2.8kb	-	(Calin *et al.*, 2007)
uc.270	antisense	-	-	-	(Jiang, J. *et al.*, 2016)
uc.283	antisene	cytoplasmatic	-	-	(Lujambio *et al.*, 2010)
uc.338	sense	nuclear	0.59kb	available	(Braconi *et al.*, 2011)
uc.339	antisense	cytoplasmatic	0.85kb	-	(Vannini *et al.*, 2017)
uc.346	sense	cytoplasmatic	-	-	(Lujambio *et al.*, 2010)
uc.417	-	nuclear	0.222kb	available	(Cui *et al.*, 2016)
uc.475	sense	nuclear	~9.5kb	-	(Ferdin *et al.*, 2013)

Conclusion

Most of the studies reviewed in this paper describe T-UCRs with deregulated expression in tumor cells. These T-UCRs have an important role in cell process that are essential for tumorigeneses, such as proliferation, apoptosis, and migration/invasion. Of 481 T-UCRs, 11 are associated with tumorigenesis processes, and 286 others present differential expression in

samples of patients with cancer. Moreover, 89 deregulated T-UCRs were also associated with other conditions, such as Crohn's disease, systemic lupus erythematosus, and myocardial ischemia/reperfusion injury. However, the role of T-UCRs in most complex diseases is still under investigated, and only 4% of T-UCRs present information on molecular details. This shows the necessity for better understanding of their molecular features and, also, their mechanisms of action. Furthermore, even with the increase of studies focusing on T-UCRs and their potential in molecular marker utility, real application in clinical contexts or strategies to target T-UCRs in trials have not beenexplore yet.

Based on this review, T-UCRs are deregulated in several human diseases, and are highlighted as important molecules in cellular environment T-UCRs studies are important for evolutionary approaches butshould be better investigated as potential diagnostic/prognostic markers in cancer and other diseases.Theirt potential impact as new targets for therapy should also be studied.

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Notes

Non

Supplementary table 1

T-UCRs	Annotation (genome H38)	Disease	References
uc.1	chr1:10537640-10537846	CRC; PC; BlaC; NP	Calin et al., 2007; Hudson et al., 2013; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.2	chr1:10672486-10672692	x	x
uc.3	chr1:10691108-10691332	CRC;PC;BlaC	Calin et al., 2007; Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.4	chr1:10698192-10698550	PC;BlaC	Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.5	chr1:10721294-10721507	BlaC	Olivieri et al., 2016
uc.6	chr1:10735064-10735364	BlaC	Olivieri et al., 2016
uc.7	chr1:10776076-10776331	x	x
uc.8	chr1:10791761-10791976	BlaC	Olivieri et al., 2016
uc.9	chr1:10865353-10865554	CRC;BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.10	chr1:10905517-10905791	CRC;BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.12	chr1:35184626-35184826	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.13	chr1:35893589-35893825	BlaC	Olivieri et al., 2016
uc.14	chr1:38029332-38029544	BlaC	Olivieri et al., 2016
uc.15	chr1:38095404-38095636	x	x
uc.16	chr1:38162527-38162737	BlaC	Olivieri et al., 2016
uc.17	chr1:38336479-38336715	BlaC	Olivieri et al., 2016
uc.18	chr1:44249989-44250226	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.19	chr1:44524640-44524895	BlaC	Olivieri et al., 2016
uc.20	chr1:44536700-44536968	LC;PC; BlaC	Calin et al., 2007; Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.21	chr1:48647314-48647548	BlaC	Olivieri et al., 2016
uc.22	chr1:50540559-50540866	PC; NP	Hudson et al., 2013; Jiang, Yang, He, Tao, & Gao, 2016
uc.23	chr1:50570105-50570333	LC	Calin et al., 2007
uc.24	chr1:50633473-50633808	BlaC	Olivieri et al., 2016

uc.25	chr1:50700362-50700596	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.26	chr1:62903975-62904186	PC	Hudson et al., 2013
uc.27	chr1:62904209-62904498	LC	Calin et al., 2007
uc.28	chr1:70231030-70231384	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.29	chr1:87356700-87356918	CRC	Calin et al., 2007
uc.30	chr1:87563317-87563559	BlaC	Olivieri et al., 2016
uc.31	chr1:88462335-88462587	BlaC	Olivieri et al., 2016
uc.33	chr1:96806181-96806492	PC;BlaC; NP	Hudson et al., 2013; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.34	chr1:96814616-96814823	PC;BlaC	Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.35	chr1:97536726-97536930	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.36	chr1:108697806-108698069	BlaC; NP	Olivieri et al., 2016
uc.37	chr1:114737432-114737633	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.38	chr1:163970718-163970941	BC	L. X. Zhang et al., 2017
uc.39	chr1:164054298-164054653	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.40	chr1:164668725-164668971	x	x
uc.41	chr1:213425424-213425639	x	x
uc.42	chr1:215715690-215715955	x	x
uc.43	chr1:243732733-243732989	CRC;BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.44	chr1:244054405-244054634	BlaC	Olivieri et al., 2016
uc.45	chr1:244853384-244853586	FT	Xiao et al., 2018
uc.46	chr1:244854301-244854517	NP; FT	Jiang, Yang, He, Tao, & Gao, 2016; Xiao et al., 2018
uc.47	chr2:7634520-7634746	PC;BlaC	Hudson et al., 2013; Olivieri et al., 2016
uc.48	chr2:20278572-20278869	BlaC; MI	Olivieri et al., 2016
uc.49	chr2:33588342-33588548	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.50	chr2:38749159-38749380	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.51	chr2:57745424-57745630	x	x
uc.52	chr2:58881051-58881324	x	x
uc.53	chr2:58906176-58906407	x	x

uc.54	chr2:58972268-58972476	BlaC	Olivieri et al., 2016
uc.55	chr2:59519443-59519682	x	x
uc.56	chr2:59720696-59720897	BlaC	Olivieri et al., 2016
uc.57	chr2:59912105-59912350	BlaC	Olivieri et al., 2016
uc.58	chr2:60070828-60071030	ES	Fassan et al., 2014
uc.59	chr2:60071248-60071467	BlaC	Olivieri et al., 2016
uc.60	chr2:60214425-60214641	x	x
uc.61	chr2:60460438-60460763	BlaC	Olivieri et al., 2016
uc.62	chr2:60553547-60553780	x	x
uc.63	chr2:61525366-61525643	CRC;NB; BC; PC;BlaC	Calin et al., 2007; Mestdagh et al., 2010; Marini et al., 2017; Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.64	chr2:62966956-62967200	x	x
uc.65	chr2:66071457-66071668	x	x
uc.66	chr2:72947874-72948120	x	x
uc.67	chr2:104120268-104120484	BlaC	Olivieri et al., 2016
uc.68	chr2:143157153-143157407	BlaC	Olivieri et al., 2016
uc.69	chr2:143354541-143354841	x	x
uc.70	chr2:143679770-143680006	CLL;BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.71	chr2:143955289-143955536	x	x
uc.72	chr2:143957405-143957811	CRC	Calin et al., 2007
uc.73	chr2:144004746-144004946	CLL;PC;BlaC	Calin et al., 2007; Hudson et al., 2013; Goto et al., 2016; Olivieri et al., 2016
uc.74	chr2:144068396-144068933	x	x
uc.75	chr2:144388283-144388518	x	x
uc.76	chr2:144403566-144403900	x	x
uc.77	chr2:144428198-144428493	BlaC	Olivieri et al., 2016
uc.78	chr2:144430787-144431034	BlaC	Olivieri et al., 2016
uc.79	chr2:144439610-144439904	x	x
uc.80	chr2:144443285-144443578	x	x

uc.81	chr2:146376497-146376707	x	x
uc.82	chr2:155870587-155870796	x	x
uc.83	chr2:156135115-156135410	x	x
uc.84	chr2:156338194-156338402	BlaC	Olivieri et al., 2016
uc.85	chr2:156694902-156695149	BlaC	Olivieri et al., 2016
uc.86	chr2:156803598-156803937	x	x
uc.87	chr2:157043757-157044046	x	x
uc.88	chr2:161238531-161238842	BlaC	Olivieri et al., 2016
uc.89	chr2:161382162-161382468	PC;BlaC	Hudson et al., 2013; Olivieri et al., 2016
uc.90	chr2:161416516-161416721	x	x
uc.91	chr2:162188512-162188718	CRC	Calin et al., 2007
uc.92	chr2:163594169-163594477	CRC;BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.93	chr2:163805397-163805659	x	x
uc.94	chr2:163987660-163987859	x	x
uc.95	chr2:170715020-170715270	x	x
uc.96	chr2:171964152-171964412	BlaC	Olivieri et al., 2016
uc.97	chr2:171966110-171966551	BlaC;NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.98	chr2:172091790-172092027	x	x
uc.99	chr2:172093653-172094050	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.100	chr2:173250052-173250258	x	x
uc.101	chr2:173909753-173910006	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.102	chr2:174081681-174082018	BlaC	Olivieri et al., 2016
uc.103	chr2:174104944-174105176	x	x
uc.104	chr2:174122206-174122421	x	x
uc.105	chr2:174125059-174125281	x	x
uc.106	chr2:174160695-174160900	CRC;PC;BlaC	Calin et al., 2007; Hudson et al., 2013; Olivieri et al., 2016
uc.107	chr2:174342880-174343116	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.108	chr2:176075629-176076002	x	x

uc.109	chr2:176638610-176638833	x	x
uc.110	chr2:236162738-236162980	x	x
uc.111	chr3:9429777-9430072	CRC;BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.112	chr3:18128072-18128417	CRC;BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.113	chr3:18634912-18635158	x	x
uc.114	chr3:18802958-18803251	x	x
uc.115	chr3:18992666-18992884	BlaC	Olivieri et al., 2016
uc.116	chr3:70517273-70517478	x	x
uc.117	chr3:70822689-70822939	x	x
uc.118	chr3:70822941-70823159	GC;PC	Goto et al., 2016; Hudson et al., 2013
uc.119	chr3:114714620-114714920	x	x
uc.120	chr3:114716192-114716461	x	x
uc.121	chr3:114856627-114856919	BlaC	Olivieri et al., 2016
uc.122	chr3:114893044-114893258	CLL; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.123	chr3:137264702-137265193	x	x
uc.124	chr3:137329602-137329888	x	x
uc.125	chr3:137349475-137349739	BlaC	Olivieri et al., 2016
uc.126	chr3:137406806-137407076	BlaC	Olivieri et al., 2016
uc.127	chr3:147312921-147313192	x	x
uc.128	chr3:147331851-147332149	BlaC	Olivieri et al., 2016
uc.129	chr3:152446598-152446809	BlaC	Olivieri et al., 2016
uc.130	chr3:158058785-158059008	x	x
uc.131	chr3:158272251-158272457	x	x
uc.132	chr3:158308370-158308577	x	x
uc.133	chr3:158308689-158308965	BlaC	Olivieri et al., 2016
uc.134	chr3:158528115-158528325	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.135	chr3:169116495-169116695	CLL	Calin et al., 2007
uc.136	chr3:169476164-169476510	BlaC	Olivieri et al., 2016

uc.137	chr3:180719069-180719453	BlaC	Olivieri et al., 2016
uc.138	chr3:185931508-185931926	BlaC; FT	Olivieri et al., 2016; Xiao et al., 2018
uc.139	chr4:4521713-4522050	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.140	chr4:13008246-13008468	BlaC	Olivieri et al., 2016
uc.141	chr4:24527539-24527833	BlaC; CD; FT	Olivieri et al., 2016; Qian et al., 2016; Xiao et al., 2018
uc.142	chr4:41748052-41748310	BlaC	Olivieri et al., 2016
uc.143	chr4:75653940-75654157	BlaC	Olivieri et al., 2016
uc.144	chr4:82425512-82425716	CLL;CRC;BlaC; CD; FT	Calin et al., 2007; Olivieri et al., 2016; Qian et al., 2016; Xiao et al., 2018
uc.145	chr4:104425156-104425403	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.146	chr4:110995320-110995533	BlaC	Olivieri et al., 2016
uc.147	chr4:150315231-150315538	x	x
uc.148	chr4:150572800-150573039	x	x
uc.149	chr4:150573041-150573244	BlaC	Olivieri et al., 2016
uc.150	chr5:3512507-3512768	BlaC	Olivieri et al., 2016
uc.151	chr5:32380031-32380244	BlaC	Olivieri et al., 2016
uc.152	chr5:51039804-51040004	x	x
uc.153	chr5:72899859-72900098	PC;BlaC; ES; NP	Hudson et al., 2013; Olivieri et al., 2016; Fassan et al., 2014; Jiang, Yang, He, Tao, & Gao, 2016
uc.154	chr5:72914188-72914390	x	x
uc.155	chr5:77638539-77638745	x	x
uc.156	chr5:77639594-77639806	BlaC	Olivieri et al., 2016
uc.157	chr5:77645336-77645542	BlaC	Olivieri et al., 2016
uc.158	chr5:77844429-77844652	LC;CRC;GC;PC; BlaC; ES; NP	Carotenuto et al., 2017; Goto et al., 2016; Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016; Fassan et al., 2014; Jiang, Yang, He, Tao, & Gao, 2016
uc.159	chr5:77852108-77852579	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.160	chr5:77973020-77973341	CLL;GC; BlaC; ES	Calin et al., 2007; Kottorou et al., 2018; Honma et al., 2017; Pang et al., 2018; Olivieri et al., 2016; Fassan et al., 2014

uc.161	chr5:78062705-78062982	x	x
uc.162	chr5:81851542-81851759	BlaC	Olivieri et al., 2016
uc.163	chr5:87872806-87873181	BlaC	Olivieri et al., 2016
uc.164	chr5:87944588-87944790	x	x
uc.165	chr5:88397116-88397339	ES	Fassan et al., 2014
uc.166	chr5:88665987-88666296	BlaC; CD	Olivieri et al., 2016; Qian et al., 2016
uc.167	chr5:88883807-88884007	x	x
uc.168	chr5:91632976-91633195	BlaC	Olivieri et al., 2016
uc.169	chr5:93585311-93585514	x	x
uc.170	chr5:93891964-93892273	BlaC	Olivieri et al., 2016
uc.171	chr5:94240142-94240349	x	x
uc.172	chr5:94315014-94315231	BlaC	Olivieri et al., 2016
uc.173	chr5:134390469-134390744	BlaC; ND; LRI; FT; IBH	Olivieri et al., 2016; Nan et al., 2016; Qin et al., 2018; J. Y. Wang et al., 2018; Xiao et al., 2018; Ding et al., 2019
uc.174	chr5:139307965-139308224	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.175	chr5:158914830-158915079	x	x
uc.176	chr5:167905690-167905935	x	x
uc.177	chr5:170990625-170990881	BlaC	Olivieri et al., 2016
uc.178	chr5:170990994-170991242	x	x
uc.179	chr5:171201208-171201426	BlaC	Olivieri et al., 2016
uc.180	chr5:171201485-171201709	BlaC	Olivieri et al., 2016
uc.181	chr5:171202475-171202752	CRC;PC; BlaC	Calin et al., 2007; Hudson et al., 2013; Olivieri et al., 2016
uc.182	chr5:171276075-171276313	BlaC	Olivieri et al., 2016
uc.183	chr5:171957516-171957751	CLL; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.184	chr5:173958289-173958518	PC; NP	Hudson et al., 2013; Jiang, Yang, He, Tao, & Gao, 2016
uc.185	chr5:178617306-178617716	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.186	chr5:179619196-179619500	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.187	chr6:10394444-10394655	x	x

uc.188	chr6:16299153-16299367	BlaC	Olivieri et al., 2016
uc.189	chr6:36599740-36600312	EA;OC;CC; BlaC; ES; NP	L. Wang et al., 2018; Olivieri et al., 2016; Guo et al., 2018; Jiang, Yang, He, Tao, & Gao, 2016
uc.190	chr6:41555702-41555901	BlaC; PCC	Olivieri et al., 2016; Jiang et al., 2016
uc.191	chr6:51109081-51109288	BlaC	Olivieri et al., 2016
uc.192	chr6:51284199-51284442	CLL; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.193	chr6:85611968-85612286	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.194	chr6:93259346-93259546	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.195	chr6:97215480-97215752	BlaC	Olivieri et al., 2016
uc.196	chr6:97668664-97668884	BlaC	Olivieri et al., 2016
uc.197	chr6:97914923-97915146	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.198	chr6:98272013-98272319	LC;CRC	Calin et al., 2007
uc.199	chr6:98365979-98366234	x	x
uc.200	chr6:98547657-98547910	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.201	chr6:99604108-99604347	BlaC	Olivieri et al., 2016
uc.202	chr6:100526107-100526336	CRC	Calin et al., 2007
uc.203	chr6:163570672-163570874	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.204	chr7:1226268-1226469	CRC	Calin et al., 2007
uc.205	chr7:20790170-20790421	x	x
uc.206	chr7:20964146-20964644	CRC;CC; BlaC; ES	Calin et al., 2007; Li, Li, & Wang, 2016; Olivieri et al., 2016; Fassan et al., 2014
uc.207	chr7:21771875-21772104	ES	Fassan et al., 2014
uc.208	chr7:23522051-23522268	BlaC	Olivieri et al., 2016
uc.209	chr7:23522269-23522518	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.210	chr7:26657459-26657715	x	x
uc.211	chr7:26689853-26690143	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.212	chr7:27102319-27102523	x	x
uc.213	chr7:27143513-27143713	BlaC; NP	Olivieri et al., 2016
uc.214	chr7:31362784-31363026	ES; NP	Fassan et al., 2014; Jiang, Yang, He, Tao, & Gao, 2016

uc.215	chr7:42152986-42153247	PC; BlaC	Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.216	chr7:50318559-50318870	x	x
uc.217	chr7:54568963-54569183	PC; BlaC; NP	Hudson et al., 2013; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.218	chr7:70337989-70338274	BlaC	Olivieri et al., 2016
uc.219	chr7:70515794-70516003	x	x
uc.220	chr7:97004604-97004860	BlaC	Olivieri et al., 2016
uc.221	chr7:97011989-97012337	CRC;BlaC; NP	Calin et al., 2007; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.222	chr7:114417116-114417316	x	x
uc.223	chr7:114418130-114418397	BlaC; ES	Olivieri et al., 2016; Fassan et al., 2014
uc.224	chr7:114422964-114423258	x	x
uc.225	chr7:114432800-114433000	BlaC	Olivieri et al., 2016
uc.226	chr7:114569263-114569467	x	x
uc.227	chr7:114655261-114655491	BlaC; ES	Olivieri et al., 2016; Fassan et al., 2014
uc.228	chr7:115476643-115476907	x	x
uc.229	chr7:115494591-115494886	BlaC	Olivieri et al., 2016
uc.230	chr7:115679407-115679644	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.231	chr7:115942063-115942286	CLL	Calin et al., 2007
uc.232	chr7:122304552-122304798	x	x
uc.233	chr7:151131227-151131492	CLL; PCC	Calin et al., 2007; Jiang et al., 2016
uc.234	chr7:157019691-157019962	CRC;PC; BlaC	Calin et al., 2007; Hudson et al., 2013; Olivieri et al., 2016
uc.235	chr8:25918405-25918631	x	x
uc.236	chr8:37392951-37393217	x	x
uc.237	chr8:52225342-52225809	x	x
uc.238	chr8:52254557-52254914	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.239	chr8:59029814-59030113	BlaC	Olivieri et al., 2016
uc.240	chr8:64579982-64580187	x	x
uc.241	chr8:64584573-64584774	PC;BlaC	Hudson et al., 2013; Goto et al., 2016; Olivieri et al., 2016
uc.242	chr8:65237062-65237326	x	x

uc.243	chr8:76778726-76778941	x	x
uc.244	chr8:104950121-104950441	GC;PC; BlaC	Goto et al., 2016; Hudson et al., 2013; Olivieri et al., 2016
uc.245	chr8:105321604-105321942	x	x
uc.246	chr8:118110979-118111262	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.247	chr9:969154-969514	BlaC	Olivieri et al., 2016
uc.248	chr9:974189-974410	PC; BlaC	Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.249	chr9:8095728-8095987	CRC;GC;PC; BlaC	Calin et al., 2007; Goto et al., 2016; Hudson et al., 2013; Olivieri et al., 2016
uc.250	chr9:13939911-13940119	x	x
uc.251	chr9:15874311-15874523	x	x
uc.252	chr9:16710755-16710985	LC;GC;PC; BlaC	Calin et al., 2007; Goto et al., 2016; Hudson et al., 2013; Olivieri et al., 2016
uc.253	chr9:17332214-17332435	BlaC	Olivieri et al., 2016
uc.254	chr9:23496727-23497005	x	x
uc.255	chr9:23691770-23692001	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.256	chr9:23692236-23692441	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.257	chr9:37215207-37215470	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.258	chr9:37324427-37324627	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.259	chr9:77012963-77013270	x	x
uc.260	chr9:78857508-78857738	x	x
uc.261	chr9:79256659-79256969	GC;PC; BlaC; CD	Goto et al., 2016; Hudson et al., 2013; Olivieri et al., 2016; Qian et al., 2016
uc.262	chr9:81112807-81113061	CLL	Calin et al., 2007
uc.263	chr9:83975369-83975575	BlaC	Olivieri et al., 2016
uc.264	chr9:83975577-83975843	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.265	chr9:105356190-105356406	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.266	chr9:106616011-106616253	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.267	chr9:122291611-122291813	x	x
uc.268	chr9:122844549-122844799	BlaC	Olivieri et al., 2016
uc.269	chr9:123775658-123775874	BlaC	Olivieri et al., 2016
uc.270	chr9:125541790-125542067	PCC	Jiang et al., 2016

uc.271	chr9:125542073-125542283	x	x
uc.272	chr9:125670309-125670521	x	x
uc.273	chr9:125755319-125755639	x	x
uc.274	chr9:125759592-125759918	LC; BlaC; ES; NP	Calin et al., 2007; Olivieri et al., 2016; Fassan et al., 2014; Jiang, Yang, He, Tao, & Gao, 2016
uc.275	chr9:125821837-125822091	BlaC	Olivieri et al., 2016
uc.276	chr9:125843533-125843964	BlaC	Olivieri et al., 2016
uc.277	chr9:125845431-125845706	x	x
uc.278	chr9:125883886-125884122	BlaC	Olivieri et al., 2016
uc.279	chr9:125910280-125910615	NB	Mestdagh et al., 2010
uc.280	chr9:125915727-125915946	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.281	chr9:132620138-132620375	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.282	chr9:137148038-137148244	GC;PC; BlaC	Goto et al., 2016; Hudson et al., 2013; Olivieri et al., 2016
uc.283	chr10:49396711-49396987	CRC; BlaC	Kottorou et al., 2018; Olivieri et al., 2016
uc.284	chr10:49398810-49399018	x	x
uc.285	chr10:68756234-68756465	CLL; BlaC; NP	Calin et al., 2007; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.286	chr10:75379266-75379517	x	x
uc.287	chr10:75736185-75736441	BlaC	Olivieri et al., 2016
uc.288	chr10:75967425-75967647	BlaC	Olivieri et al., 2016
uc.289	chr10:76230781-76231034	CLL; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.290	chr10:76282524-76282729	CD	Qian et al., 2016
uc.291	chr10:76523866-76524096	CLL;PC; BlaC	Calin et al., 2007; Hudson et al., 2013; Olivieri et al., 2016
uc.292	chr10:96955698-96955914	CRC; BlaC; NP	Calin et al., 2007; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.293	chr10:100612912-100613154	x	x
uc.294	chr10:100613860-100614303	x	x

uc.295	chr10:100615343-100615551	BlaC	Olivieri et al., 2016
uc.296	chr10:100655349-100655809	BlaC	Olivieri et al., 2016
uc.297	chr10:100659463-100659826	x	x
uc.298	chr10:100687901-100688259	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.299	chr10:100749678-100749887	BlaC	Olivieri et al., 2016
uc.300	chr10:100787361-100787568	CRC	Calin et al., 2007
uc.301	chr10:100808034-100808317	x	x
uc.302	chr10:101219423-101219763	BlaC	Olivieri et al., 2016
uc.303	chr10:101292670-101292941	x	x
uc.304	chr10:101322747-101323018	BlaC	Olivieri et al., 2016
uc.305	chr10:101451678-101451982	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.306	chr10:101452282-101452505	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.307	chr10:101484226-101484457	x	x
uc.308	chr10:101486055-101486331	PC	Hudson et al., 2013

uc.309	chr10:101507274-101507541	x	x
uc.310	chr10:112644464-112644692	BlaC	Olivieri et al., 2016
uc.311	chr10:118314890-118315108	BlaC	Olivieri et al., 2016
uc.312	chr10:118317025-118317346	x	x
uc.313	chr10:119580662-119580892	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.314	chr10:123092687-123092888	x	x
uc.315	chr10:123093099-123093333	x	x
uc.316	chr10:125216996-125217235	CLL	Calin et al., 2007
uc.317	chr10:129648074-129648291	PC; BlaC	Hudson et al., 2013; Olivieri et al., 2016
uc.318	chr10:129893322-129893642	x	x
uc.319	chr11:8283148-8283463	BlaC	Olivieri et al., 2016
uc.320	chr11:8296287-8296621	x	x
uc.321	chr11:15602878-15603081	BlaC	Olivieri et al., 2016
uc.322	chr11:16294805-16295027	BlaC	Olivieri et al., 2016
uc.323	chr11:16453789-16453988	BlaC	Olivieri et al., 2016
uc.324	chr11:30535974-30536198	BlaC	Olivieri et al., 2016
uc.325	chr11:31664096-31664330	BlaC	Olivieri et al., 2016
uc.326	chr11:31764132-31764446	BlaC	Olivieri et al., 2016

uc.327	chr11:31764736-31765003	PC; BlaC; ES	Hudson et al., 2013; Olivieri et al., 2016; Fassan et al., 2014
uc.328	chr11:31804115-31804345	x	x
uc.329	chr11:32176446-32176752	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.330	chr11:66626425-66626631	x	x
uc.331	chr11:83484116-83484333	x	x
uc.332	chr11:116361973-116362309	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.333	chr11:124774751-124775020	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.334	chr11:131998051-131998272	BlaC	Olivieri et al., 2016
uc.335	chr12:16562480-16562693	BlaC	Olivieri et al., 2016
uc.336	chr12:24139072-24139322	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.337	chr12:41355377-41355594	x	x
uc.338	chr12:53464705-53464927	LC;CRC;CC; LG	Braconi et al., 2011; Calin et al., 2007;C. Wang et al., 2017;Y. Zhang et al., 2018; Li, Shen, & Wang, 2017; Tian & Feng, 2018
uc.339	chr12:53677312-53677563	CRC; BlaC; LG; NP	Calin et al., 2007; Olivieri et al., 2016; Vannini et al., 2017; Jiang, Yang, He, Tao, & Gao, 2016
uc.340	chr12:53697048-53697306	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.341	chr12:53989134-53989447	CRC	Calin et al., 2007
uc.342	chr12:54016710-54016936	BlaC	Olivieri et al., 2016
uc.343	chr12:54028657-54029044	BlaC	Olivieri et al., 2016
uc.344	chr12:54033102-54033355	BlaC	Olivieri et al., 2016
uc.345	chr12:54053816-54054116	PC; BlaC	Hudson et al., 2013; Olivieri et al., 2016
uc.346	chr12:106582732-106582933	CRC;PC; BlaC; NP	Kottorou et al., 2018; Hudson et al., 2013; Goto et al., 2016; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.347	chr13:71219856-71220064	NB	Mestdagh et al., 2010
uc.348	chr13:71489225-71489464	x	x

uc.349	chr13:71547170-71547372	CLL;PC; BlaC	Calin et al., 2007; Hudson et al., 2013; Olivieri et al., 2016
uc.350	chr13:71681968-71682207	NB; BlaC	Mestdagh et al., 2010; Olivieri et al., 2016
uc.351	chr13:72094762-72095016	x	x
uc.352	chr13:72120027-72120226	CLL; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.353	chr13:72197415-72197737	x	x
uc.354	chr13:78402694-78402928	PC; BlaC; NP	Hudson et al., 2013; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.355	chr13:94966628-94966855	x	x
uc.356	chr13:97356566-97356816	PC; BlaC; FT	Hudson et al., 2013; Olivieri et al., 2016; Xiao et al., 2018
uc.357	chr13:112062023-112062264	x	x
uc.358	chr14:25908828-25909053	BlaC	Olivieri et al., 2016
uc.359	chr14:26445762-26446085	GC;PC; BlaC; NP	Goto et al., 2016; Hudson et al., 2013; Goto et al., 2016; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.360	chr14:26446176-26446462	BlaC	Olivieri et al., 2016
uc.361	chr14:28763929-28764195	x	x
uc.362	chr14:28879546-28879784	CRC;PC; BlaC	Calin et al., 2007; Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.363	chr14:29392113-29392377	PC; BlaC	Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.364	chr14:30243553-30243759	NB	Mestdagh et al., 2010
uc.365	chr14:30273156-30273433	x	x
uc.366	chr14:30913501-30913702	PC; BlaC; NP	Hudson et al., 2013; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.367	chr14:33375257-33375554	x	x
uc.368	chr14:33599370-33599597	x	x
uc.369	chr14:33653411-33653623	BlaC	Olivieri et al., 2016
uc.370	chr14:33733365-33733757	x	x
uc.371	chr14:35550983-35551278	BlaC	Olivieri et al., 2016
uc.372	chr14:35573829-35574105	BlaC; HA	Olivieri et al., 2016; Guo et al., 2018
uc.373	chr14:36112601-36112994	x	x
uc.374	chr14:37246708-37246931	PC; BlaC	Hudson et al., 2013; Olivieri et al., 2016

uc.375	chr14:37308015-37308314	x	x
uc.376	chr14:45096547-45096836	x	x
uc.377	chr14:45109820-45110036	CLL; BlaC; NP	Calin et al., 2007; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.378	chr14:79861134-79861384	BlaC	Olivieri et al., 2016
uc.379	chr14:96965031-96965282	NB	Mestdagh et al., 2010
uc.380	chr14:97296257-97296488	x	x
uc.381	chr14:97412953-97413190	BlaC	Olivieri et al., 2016
uc.382	chr15:35626711-35626910	PC; BlaC	Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.383	chr15:36527726-36527994	CRC	Calin et al., 2007
uc.384	chr15:36673192-36673457	x	x
uc.385	chr15:36893469-36893677	PC; BlaC	Hudson et al., 2013; Olivieri et al., 2016
uc.386	chr15:37229808-37230010	x	x
uc.387	chr15:41739856-41740093	x	x
uc.388	chr15:57133327-57133624	CRC; BlaC	Calin et al., 2007; Sana et al., 2012; Olivieri et al., 2016
uc.389	chr15:67373047-67373317	PC; BlaC	Hudson et al., 2013; Goto et al., 2016; Olivieri et al., 2016
uc.390	chr15:67585832-67586036	PC; BlaC	Goto et al., 2016; Olivieri et al., 2016
uc.391	chr15:67747966-67748276	x	x
uc.392	chr15:70099795-70100050	BlaC	Olivieri et al., 2016
uc.393	chr15:74621901-74622175	BlaC	Olivieri et al., 2016
uc.394	chr15:96692870-96693071	x	x
uc.395	chr16:24567682-24567930	x	x
uc.396	chr16:49060820-49061027	LC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.397	chr16:49701937-49702247	BlaC	Olivieri et al., 2016
uc.398	chr16:49856980-49857301	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.399	chr16:50815080-50815293	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.400	chr16:51637576-51637781	x	x
uc.401	chr16:52460803-52461052	x	x
uc.402	chr16:53620473-53620717	LC; BlaC	Calin et al., 2007; Olivieri et al., 2016

uc.403	chr16:54289943-54290148	BlaC	Olivieri et al., 2016
uc.404	chr16:55189445-55189679	PC; BlaC	Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.405	chr16:59541491-59541700	BlaC	Olivieri et al., 2016
uc.406	chr16:69646461-69646671	x	x
uc.407	chr16:69647252-69647577	x	x
uc.408	chr16:72787146-72787397	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.409	chr16:73058972-73059215	x	x
uc.410	chr17:36655773-36655991	BlaC	Olivieri et al., 2016
uc.411	chr17:36972320-36972548	x	x
uc.412	chr17:36979187-36979454	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.413	chr17:39410266-39410537	CRC	Calin et al., 2007
uc.414	chr17:40092921-40093166	BlaC	Olivieri et al., 2016
uc.415	chr17:48586544-48586750	x	x
uc.416	chr17:48593525-48593810	GC;PC;RC; BlaC	Goto et al., 2016; Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016
uc.417	chr17:48604950-48605171	BlaC; BAT	Olivieri et al., 2016; Cui et al., 2016
uc.418	chr17:58004867-58005083	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.419	chr17:58005354-58005642	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.420	chr17:64500653-64500885	CRC;PC; BlaC; NP	Calin et al., 2007; Hudson et al., 2013; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.421	chr18:25113191-25113535	BlaC	Olivieri et al., 2016
uc.422	chr18:25168224-25168449	CLL;CRC;BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.423	chr18:25176391-25176613	x	x
uc.424	chr18:25187815-25188029	x	x
uc.425	chr18:25285243-25285567	BlaC	Olivieri et al., 2016
uc.426	chr18:26335628-26335889	CRC	Calin et al., 2007
uc.427	chr18:26657235-26657449	CRC; PC; BlaC	Calin et al., 2007; Hudson et al., 2013; Olivieri et al., 2016
uc.428	chr18:32773246-32773484	x	x
uc.429	chr18:36900578-36900815	x	x

uc.430	chr18:37483687-37483899	x	x
uc.431	chr18:37598637-37598866	x	x
uc.432	chr18:37984968-37985178	BlaC	Olivieri et al., 2016
uc.433	chr18:38483668-38483873	x	x
uc.434	chr18:47244417-47244665	x	x
uc.435	chr18:55422700-55422926	CLL;CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.436	chr18:55587010-55587219	BlaC	Olivieri et al., 2016
uc.437	chr18:74645702-74645916	x	x
uc.438	chr18:74645918-74646158	x	x
uc.439	chr18:74651075-74651337	x	x
uc.440	chr18:74651409-74651737	BlaC	Olivieri et al., 2016
uc.441	chr18:74856636-74856883	x	x
uc.442	chr18:74880756-74881004	x	x
uc.443	chr19:8462385-8462623	BlaC	Olivieri et al., 2016
uc.444	chr19:30003872-30004259	x	x
uc.445	chr19:30075528-30075837	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.446	chr19:30256947-30257218	NB; BlaC	Mestdagh et al., 2010; Olivieri et al., 2016
uc.447	chr19:30276874-30277146	x	x
uc.448	chr19:30350623-30350854	BlaC	Olivieri et al., 2016
uc.449	chr19:30512634-30512923	BlaC	Olivieri et al., 2016
uc.450	chr19:31096720-31096930	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.451	chr19:31315714-31315938	BlaC	Olivieri et al., 2016
uc.452	chr19:31337041-31337244	BlaC	Olivieri et al., 2016
uc.453	chr19:41933165-41933489	BlaC	Olivieri et al., 2016
uc.454	chr20:4885794-4886001	PC; BlaC; LG	Hudson et al., 2013; Olivieri et al., 2016; Zhou et al., 2018
uc.455	chr20:35740457-35740701	FT	Xiao et al., 2018
uc.456	chr20:43459116-43459435	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.457	chr22:19408386-19408596	CRC; BlaC; FT	Calin et al., 2007; Olivieri et al., 2016; Xiao et al., 2018

uc.458	chr22:35752445-35752648	BlaC	Olivieri et al., 2016
uc.459	chrX:21516442-21516696	x	x
uc.460	chrX:24805394-24805668	NB	Mestdagh et al., 2010
uc.461	chrX:24846680-24847076	NP	Jiang, Yang, He, Tao, & Gao, 2016
uc.462	chrX:24876709-24877487	BlaC	Olivieri et al., 2016
uc.463	chrX:24897765-24898039	x	x
uc.464	chrX:24898041-24898810	x	x
uc.465	chrX:24899364-24899673	BlaC	Olivieri et al., 2016
uc.466	chrX:24928341-24928689	BlaC	Olivieri et al., 2016
uc.467	chrX:24990237-24990967	x	x
uc.468	chrX:24999446-24999934	CRC; BlaC	Calin et al., 2007; Olivieri et al., 2016
uc.469	chrX:24999936-25000157	BlaC	Olivieri et al., 2016
uc.470	chrX:25383099-25383439	CRC; NP	Calin et al., 2007; Jiang, Yang, He, Tao, & Gao, 2016
uc.471	chrX:41349118-41349356	BlaC; NP	Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016
uc.472	chrX:41520053-41520254	BlaC; ES	Olivieri et al., 2016;Fassan et al., 2014
uc.473	chrX:71153374-71153595	PC; BlaC; ES	Hudson et al., 2013; Olivieri et al., 2016; Fassan et al., 2014
uc.474	chrX:71248993-71249202	x	x
uc.475	chrX:71546205-71546601	CRC	Calin et al., 2007
uc.476	chrX:82534150-82534387	BlaC	Olivieri et al., 2016
uc.477	chrX:103786562-103786770	PC; BlaC;NP; CD; FT	Hudson et al., 2013; Sekino et al., 2017; Olivieri et al., 2016; Jiang, Yang, He, Tao, & Gao, 2016; Qian et al., 2016; Xiao et al., 2018
uc.478	chrX:123465606-123465857	CD	Qian et al., 2016
uc.479	chrX:123479900-123480201	BlaC; CD	Olivieri et al., 2016; Qian et al., 2016
uc.480	chrX:124101422-124101623	x	x
uc.481	chrX:124101625-124101828	BlaC; FT	Olivieri et al., 2016

Supplementary Table 2

Cancer	T-UCR	Up/Down	Metodology	References
Liver	uc.20+A	down	Microarray	Calin et al., 2007
	uc.23+	up	Microarray	Calin et al., 2007
	uc.27+	down	Microarray	Calin et al., 2007
	uc.158	up	microarray/RT-qPCR/In situ hybridisation	Carotenuto et al., 2017
	Uc.198+	down	Microarray	Calin et al., 2007
	uc.252+A	up	Microarray	Calin et al., 2007
	uc.274+	down	Microarray	Calin et al., 2007
	uc.338	up	Microarray/ In situ Hybridisation	Braconi et al., 2011
	uc.396+	down	Microarray	Calin et al., 2007
	uc.402+A	up	Microarray	Calin et al., 2007
Leukemia	uc.73+A	down	Microarray	Calin et al., 2007
	uc.122+	up	Microarray	Calin et al., 2007
	uc.135+	down	Microarray	Calin et al., 2007
	uc.144+	up	Microarray	Calin et al., 2007
	uc.160+	up	Microarray	Calin et al., 2007
	uc.183+	down	Microarray	Calin et al., 2007
	uc.192+	up	Microarray	Calin et al., 2007
	uc.231+	down	Microarray	Calin et al., 2007

	uc.233+	down	Microarray	Calin et al., 2007
	uc.262+	up	Microarray	Calin et al., 2007
	uc.285+	down	Microarray	Calin et al., 2007
	uc.289+	down	Microarray	Calin et al., 2007
	uc.291+	down	Microarray	Calin et al., 2007
	uc.316+	down	Microarray	Calin et al., 2007
	uc.349+A	down	Microarray	Calin et al., 2007
	uc.352+	up	Microarray	Calin et al., 2007
	uc.377+A	up	Microarray	Calin et al., 2007
	uc.422+A	up	Microarray	Calin et al., 2007
	uc.435+	down	Microarray	Calin et al., 2007
Colorectal	uc.1+	up	Microarray	Calin et al., 2007
	uc.3+	down	Microarray	Calin et al., 2007
	uc.9+	up	Microarray	Calin et al., 2007
	uc.10+	up	Microarray	Calin et al., 2007
	uc.18+	up	Microarray	Calin et al., 2007
	uc.29+A	up	Microarray	Calin et al., 2007
	uc.43+	down	Microarray	Calin et al., 2007
	uc.63+	up	Microarray	Calin et al., 2007
	uc.63+A	up	Microarray	Calin et al., 2007
	uc.73+A	up	Microarray	Calin et al., 2007
	uc.73+A	down	RT-qPCR	Calin et al., 2007
	uc.91+	up	Microarray	Calin et al., 2007
	uc.92+	up	Microarray	Calin et al., 2007
	uc.106+	up	Microarray	Calin et al., 2007
	uc.111+	up	Microarray	Calin et al., 2007

	uc.112+	up	Microarray	Calin et al., 2007
	uc.134+A	up	Microarray	Calin et al., 2007
	uc.144+	up	Microarray	Calin et al., 2007
	uc.145+A	up	Microarray	Calin et al., 2007
	uc.158+	up	Microarray	Calin et al., 2007
	uc.160	down	RT-qPCR	Kottorou et al., 2018
	uc.182+	up	Microarray	Calin et al., 2007
	uc.198+A	up	Microarray	Calin et al., 2007
	uc.200+	up	Microarray	Calin et al., 2007
	uc.202+A	up	Microarray	Calin et al., 2007
	uc.204+A	up	Microarray	Calin et al., 2007
	uc.206+	up	Microarray	Calin et al., 2007
	uc.221+A	up	Microarray	Calin et al., 2007
	uc.230+	up	Microarray	Calin et al., 2007
	uc.234+A	up	Microarray	Calin et al., 2007
	uc.246+	up	Microarray	Calin et al., 2007
	uc.249+	up	Microarray	Calin et al., 2007
	uc.249+A	up	Microarray	Calin et al., 2007
	uc.266+A	up	Microarray	Calin et al., 2007
	uc.281+	up	Microarray	Calin et al., 2007
	uc.283	down	RT-qPCR	Kottorou et al., 2018
	uc.292+	up	Microarray	Calin et al., 2007
	uc.298+A	up	Microarray	Calin et al., 2007
	uc.300+A	up	Microarray	Calin et al., 2007
	uc.305+A	up	Microarray	Calin et al., 2007
	uc.310+	up	Microarray	Calin et al., 2007

	uc.326+	up	Microarray	Calin et al., 2007
	uc.330+	up	Microarray	Calin et al., 2007
	uc.338+A	up	Microarray/ RT-qPCR	Calin et al., 2007;C. Wang et al., 2017;Y. Zhang et al., 2018.
	uc.339+	up	Microarray	Calin et al., 2007
	uc.340+A	up	Microarray	Calin et al., 2007
	uc.341+	up	Microarray	Calin et al., 2007
	uc.346	down	RT-qPCR	Kottorou et al., 2018
	uc.362+	up	Microarray	Calin et al., 2007
	uc.383+	up	Microarray	Calin et al., 2007
	uc.388+	up	Microarray	Calin et al., 2007
	uc.388+	down	RT-qPCR	Sana et al., 2012
	uc.398+	up	Microarray	Calin et al., 2007
	uc.399+A	up	Microarray	Calin et al., 2007
	uc.412+	up	Microarray	Calin et al., 2007
	uc.413+A	up	Microarray	Calin et al., 2007
	uc.420+A	up	Microarray	Calin et al., 2007
	uc.422+A	up	Microarray	Calin et al., 2007
	uc.426+	up	Microarray	Calin et al., 2007
	uc.427+A	up	Microarray	Calin et al., 2007
	uc.435+A	up	Microarray	Calin et al., 2007
	uc.445+	up	Microarray	Calin et al., 2007
	uc.457+A	up	Microarray	Calin et al., 2007
	uc.468+A	up	Microarray	Calin et al., 2007
	uc.470+A	up	Microarray	Calin et al., 2007

	uc.475+	up	Microarray	Calin et al., 2007
Gastric	uc.118	down	RT-qPCR	Goto et al., 2016
	uc.158	down	RT-qPCR	Goto et al., 2016
	uc.160	down	RT-qPCR	Honma et al., 2017; Pang et al., 2018
	uc.244	down	RT-qPCR	Goto et al., 2016
	uc.249	down	RT-qPCR	Goto et al., 2016
	uc.252	down	RT-qPCR	Goto et al., 2016
	uc.261	down	RT-qPCR	Goto et al., 2016
	uc.282	down	RT-qPCR	Goto et al., 2016
	uc.359	down	RT-qPCR	Goto et al., 2016
	uc.416	up	RT-qPCR	Goto et al., 2016
Neuroblastoma	uc.279	up	RT-qPCR	Mestdagh et al., 2010
	uc.347	up	RT-qPCR	Mestdagh et al., 2010
	uc.350	up	RT-qPCR	Mestdagh et al., 2010
	uc.364	up	RT-qPCR	Mestdagh et al., 2010
	uc.379	up	RT-qPCR	Mestdagh et al., 2010
	uc.446	up	RT-qPCR	Mestdagh et al., 2010
	uc.460	up	RT-qPCR	Mestdagh et al., 2010
Breast	uc.38	down	RT-qPCR	L. X. Zhang et al., 2017
	uc.63	up	RT-qPCR	Marini et al., 2017
Prostate	uc.1+	down	Microarray	Hudson et al., 2013
	uc.3+	up	Microarray	Hudson et al., 2013
	uc.3	up	RT-qPCR	Sekino et al., 2017
	uc.4	up	RT-qPCR	Sekino et al., 2017
	uc.4+	up	Microarray	Hudson et al., 2013

	uc.20+	up	Microarray	Hudson et al., 2013
	uc.20+A	up	Microarray	Hudson et al., 2013
	uc.20	down	RT-qPCR	Sekino et al., 2017
	uc.22+	up	Microarray	Hudson et al., 2013
	uc.26+	up	Microarray	Hudson et al., 2013
	uc.33+	up	Microarray	Hudson et al., 2013
	uc.34+	up	Microarray	Hudson et al., 2013
	uc.34	down	RT-qPCR	Sekino et al., 2017
	uc.47+	up	Microarray	Hudson et al., 2013
	uc.63+	up	Microarray	Hudson et al., 2013
	uc.63	up	RT-qPCR	Sekino et al., 2017
	uc.73+	down	Microarray	Hudson et al., 2013
	uc.73	down	RT-qPCR	Goto et al., 2016
	uc.89+	up	Microarray	Hudson et al., 2013
	uc.106+	up	Microarray	Hudson et al., 2013
	uc.106+A	up	Microarray	Hudson et al., 2013
	uc.118+A	down	Microarray	Hudson et al., 2013
	uc.118	down	RT-qPCR	Goto et al., 2016
	uc.153+A	up	Microarray	Hudson et al., 2013
	uc.158+	up	Microarray	Hudson et al., 2013
	uc.158+A	down	Microarray	Hudson et al., 2013
	uc.158	down	RT-qPCR	Goto et al., 2016
	uc.158	down	RT-qPCR	Sekino et al., 2017
	uc.181+A	down	Microarray	Hudson et al., 2013
	uc.184+	down	Microarray	Hudson et al., 2013
	uc.215+	up	Microarray	Hudson et al., 2013

	uc.215	down	RT-qPCR	Sekino et al., 2017
	uc.217+A	up	Microarray	Hudson et al., 2013
	uc.234+A	down	Microarray	Hudson et al., 2013
	uc.241+A	down	Microarray	Hudson et al., 2013
	uc.241	down	RT-qPCR	Goto et al., 2016
	uc.244+	down	Microarray	Hudson et al., 2013
	uc.244	down	RT-qPCR	Goto et al., 2016
	uc.248+	up	Microarray	Hudson et al., 2013
	uc.248	down	RT-qPCR	Sekino et al., 2017
	uc.249+	down	Microarray	Hudson et al., 2013
	uc.249	down	RT-qPCR	Goto et al., 2016
	uc.252+A	down	Microarray	Hudson et al., 2013
	uc.252	down	RT-qPCR	Goto et al., 2016
	uc.261+	down	Microarray	Hudson et al., 2013
	uc.261	down	RT-qPCR	Goto et al., 2016
	uc.282+	down	Microarray	Hudson et al., 2013
	uc.282	down	RT-qPCR	Goto et al., 2016
	uc.291+	down	Microarray	Hudson et al., 2013
	uc.308+A	up	Microarray	Hudson et al., 2013
	uc.317+	down	Microarray	Hudson et al., 2013
	uc.327+	down	Microarray	Hudson et al., 2013
	uc.345+A	down	Microarray	Hudson et al., 2013
	uc.346+A	down	Microarray	Hudson et al., 2013
	uc.346	down	RT-qPCR	Goto et al., 2016
	uc.349+	down	Microarray	Hudson et al., 2013
	uc.354+A	up	Microarray	Hudson et al., 2013

	uc.356+A	up	Microarray	Hudson et al., 2013
	uc.359+A	down	Microarray	Hudson et al., 2013
	uc.359	down	RT-qPCR	Goto et al., 2016
	uc.362+A	up	Microarray	Hudson et al., 2013
	uc.362	down	RT-qPCR	Sekino et al., 2017
	uc.363+A	up	Microarray	Hudson et al., 2013
	uc.363	down	RT-qPCR	Sekino et al., 2017
	uc.366+	down	Microarray	Hudson et al., 2013
	uc.374+A	up	Microarray	Hudson et al., 2013
	uc.382+A	up	Microarray	Hudson et al., 2013
	uc.382	down	RT-qPCR	Sekino et al., 2017
	uc.385+	down	Microarray	Hudson et al., 2013
	uc.389+	down	Microarray	Hudson et al., 2013
	uc.389	down	RT-qPCR	Goto et al., 2016
	uc.390+	down	RT-qPCR	Goto et al., 2016
	uc.404+	up	Microarray	Hudson et al., 2013
	uc.404	down	RT-qPCR	Sekino et al., 2017
	uc.416+A	down	Microarray	Hudson et al., 2013
	uc.416	down	RT-qPCR	Goto et al., 2016
	uc.420+A	down	Microarray	Hudson et al., 2013
	uc.427+A	down	Microarray	Hudson et al., 2013
	uc.454+A	down	Microarray	Hudson et al., 2013
	uc.473+A	up	Microarray	Hudson et al., 2013
	uc.473	down	RT-qPCR	Sekino et al., 2017
	uc.477+	up	Microarray	Hudson et al., 2013
	uc.477	down	RT-qPCR	Sekino et al., 2017

Renal	uc.416	up	RT-qPCR	Sekino et al., 2017
Endometrial Adenocarcinomas	uc.189	up	RT-qPCR	L. Wang et al., 2018
Ovarian Cystadenocarcinomas	uc.189	up	RT-qPCR	L. Wang et al., 2018
Cervical Cancer	uc.189	up	RT-qPCR	L. Wang et al., 2018
	uc.206	up	RT-qPCR	Li, Li, & Wang, 2016
	uc.338	up	RT-qPCR	Li, Shen, & Wang, 2017
Bladder	uc.1+	up	Microarray	Olivieri et al., 2016
	uc.3+	down	Microarray	Olivieri et al., 2016
	uc.4+	down	Microarray	Olivieri et al., 2016
	uc.5+A	down	Microarray	Olivieri et al., 2016
	uc.6+	down	Microarray	Olivieri et al., 2016
	uc.8+	up	Microarray/RT-qPCR/In situ hybridisation	Olivieri et al., 2016
	uc.9+A	up	Microarray	Olivieri et al., 2016
	uc.10+	down	Microarray	Olivieri et al., 2016
	uc.10+A	down	Microarray	Olivieri et al., 2016
	uc.12+	down	Microarray	Olivieri et al., 2016
	uc.12+A	up	Microarray	Olivieri et al., 2016
	uc.13+A	down	Microarray	Olivieri et al., 2016
	uc.14+	up	Microarray	Olivieri et al., 2016
	uc.16+	down	Microarray	Olivieri et al., 2016
	uc.17+A	down	Microarray	Olivieri et al., 2016
	uc.18+	up	Microarray	Olivieri et al., 2016
	uc.19+	down	Microarray	Olivieri et al., 2016

	uc.20+	down	Microarray	Olivieri et al., 2016
	uc.21+A	down	Microarray	Olivieri et al., 2016
	uc.24+	down	Microarray	Olivieri et al., 2016
	uc.24+A	up	Microarray	Olivieri et al., 2016
	uc.28+	down	Microarray	Olivieri et al., 2016
	uc.30+	down	Microarray	Olivieri et al., 2016
	uc.31+	up	Microarray	Olivieri et al., 2016
	uc.33+	down	Microarray	Olivieri et al., 2016
	uc.34+A	down	Microarray	Olivieri et al., 2016
	uc.36+A	down	Microarray	Olivieri et al., 2016
	uc.43+	down	Microarray	Olivieri et al., 2016
	uc.44+	up	Microarray	Olivieri et al., 2016
	uc.44+A	down	Microarray	Olivieri et al., 2016
	uc.47+	down	Microarray	Olivieri et al., 2016
	uc.48+A	down	Microarray	Olivieri et al., 2016
	uc.54+	up	Microarray	Olivieri et al., 2016
	uc.56+	up	Microarray	Olivieri et al., 2016
	uc.57+	down	Microarray	Olivieri et al., 2016
	uc.59+	down	Microarray	Olivieri et al., 2016
	uc.61+A	down	Microarray	Olivieri et al., 2016
	uc.63+	down	Microarray	Olivieri et al., 2016
	uc.67+	down	Microarray	Olivieri et al., 2016
	uc.67+A	down	Microarray	Olivieri et al., 2016
	uc.68+	up	Microarray	Olivieri et al., 2016
	uc.70+	down	Microarray	Olivieri et al., 2016
	uc.73+	up	Microarray	Olivieri et al., 2016

	uc.77+	down	Microarray	Olivieri et al., 2016
	uc.77+A	down	Microarray	Olivieri et al., 2016
	uc.78+	down	Microarray	Olivieri et al., 2016
	uc.84+A	up	Microarray	Olivieri et al., 2016
	uc.85+A	down	Microarray	Olivieri et al., 2016
	uc.88+	down	Microarray	Olivieri et al., 2016
	uc.88+A	down	Microarray	Olivieri et al., 2016
	uc.89+	down	Microarray	Olivieri et al., 2016
	uc.92+A	down	Microarray	Olivieri et al., 2016
	uc.96+	down	Microarray	Olivieri et al., 2016
	uc.97+A	down	Microarray	Olivieri et al., 2016
	uc.102+	down	Microarray	Olivieri et al., 2016
	uc.102+A	down	Microarray	Olivieri et al., 2016
	uc.106+A	down	Microarray	Olivieri et al., 2016
	uc.111+	down	Microarray	Olivieri et al., 2016
	uc.112+A	down	Microarray	Olivieri et al., 2016
	uc.115+A	down	Microarray	Olivieri et al., 2016
	uc.121+A	up	Microarray	Olivieri et al., 2016
	uc.122+	down	Microarray	Olivieri et al., 2016
	uc.122+A	down	Microarray	Olivieri et al., 2016
	uc.125+A	down	Microarray	Olivieri et al., 2016
	uc.126+	down	Microarray	Olivieri et al., 2016
	uc.126+A	down	Microarray	Olivieri et al., 2016
	uc.128+	down	Microarray	Olivieri et al., 2016
	uc.129+	up	Microarray	Olivieri et al., 2016
	uc.133+A	down	Microarray	Olivieri et al., 2016

	uc.134+	down	Microarray	Olivieri et al., 2016
	uc.136+	up	Microarray	Olivieri et al., 2016
	uc.137+A	down	Microarray	Olivieri et al., 2016
	uc.138+	down	Microarray	Olivieri et al., 2016
	uc.139+	up	Microarray	Olivieri et al., 2016
	uc.140+A	down	Microarray	Olivieri et al., 2016
	uc.141+	down	Microarray	Olivieri et al., 2016
	uc.142+A	down	Microarray	Olivieri et al., 2016
	uc.143+A	down	Microarray	Olivieri et al., 2016
	uc.144+	down	Microarray	Olivieri et al., 2016
	uc.145+A	up	Microarray	Olivieri et al., 2016
	uc.146+	down	Microarray	Olivieri et al., 2016
	uc.149+	down	Microarray	Olivieri et al., 2016
	uc.149+A	down	Microarray	Olivieri et al., 2016
	uc.150+	down	Microarray	Olivieri et al., 2016
	uc.151+A	down	Microarray	Olivieri et al., 2016
	uc.153+A	down	Microarray	Olivieri et al., 2016
	uc.156+A	down	Microarray	Olivieri et al., 2016
	uc.157+A	down	Microarray	Olivieri et al., 2016
	uc.158+A	down	Microarray	Olivieri et al., 2016
	uc.159+A	down	Microarray	Olivieri et al., 2016
	uc.160+	up	Microarray	Olivieri et al., 2016
	uc.162+	down	Microarray	Olivieri et al., 2016
	uc.162+A	down	Microarray	Olivieri et al., 2016
	uc.163+	down	Microarray	Olivieri et al., 2016
	uc.166+	down	Microarray	Olivieri et al., 2016

	uc.168+A	down	Microarray	Olivieri et al., 2016
	uc.170+	down	Microarray	Olivieri et al., 2016
	uc.170+A	down	Microarray	Olivieri et al., 2016
	uc.172+A	up	Microarray	Olivieri et al., 2016
	uc.173+A	down	Microarray	Olivieri et al., 2016
	uc.177+A	down	Microarray	Olivieri et al., 2016
	uc.179+	up	Microarray	Olivieri et al., 2016
	uc.180+	down	Microarray	Olivieri et al., 2016
	uc.180+A	down	Microarray	Olivieri et al., 2016
	uc.181+	down	Microarray	Olivieri et al., 2016
	uc.182+	down	Microarray	Olivieri et al., 2016
	uc.183+	down	Microarray	Olivieri et al., 2016
	uc.185+	down	Microarray	Olivieri et al., 2016
	uc.185+A	down	Microarray	Olivieri et al., 2016
	uc.188+	down	Microarray	Olivieri et al., 2016
	uc.189+	down	Microarray	Olivieri et al., 2016
	uc.190+A	down	Microarray	Olivieri et al., 2016
	uc.191+A	down	Microarray	Olivieri et al., 2016
	uc.192+A	up	Microarray	Olivieri et al., 2016
	uc.195+	down	Microarray	Olivieri et al., 2016
	uc.196+	down	Microarray	Olivieri et al., 2016
	uc.200+	down	Microarray	Olivieri et al., 2016
	uc.201+	up	Microarray	Olivieri et al., 2016
	uc.206+A	down	Microarray	Olivieri et al., 2016
	uc.208+	down	Microarray	Olivieri et al., 2016
	uc.208+A	down	Microarray	Olivieri et al., 2016

	uc.213+A	down	Microarray	Olivieri et al., 2016
	uc.215+A	down	Microarray	Olivieri et al., 2016
	uc.217+A	down	Microarray	Olivieri et al., 2016
	uc.218+	down	Microarray	Olivieri et al., 2016
	uc.220+	down	Microarray	Olivieri et al., 2016
	uc.221+	up	Microarray	Olivieri et al., 2016
	uc.223+A	down	Microarray	Olivieri et al., 2016
	uc.225+A	down	Microarray	Olivieri et al., 2016
	uc.227+A	down	Microarray	Olivieri et al., 2016
	uc.229+A	down	Microarray	Olivieri et al., 2016
	uc.230+	up	Microarray	Olivieri et al., 2016
	uc.234+	down	Microarray	Olivieri et al., 2016
	uc.238+	down	Microarray	Olivieri et al., 2016
	uc.238+A	up	Microarray	Olivieri et al., 2016
	uc.239+A	down	Microarray	Olivieri et al., 2016
	uc.241+A	up	Microarray	Olivieri et al., 2016
	uc.244+	up	Microarray	Olivieri et al., 2016
	uc.246+	down	Microarray	Olivieri et al., 2016
	uc.247+	up	Microarray	Olivieri et al., 2016
	uc.247+A	down	Microarray	Olivieri et al., 2016
	uc.248+A	down	Microarray	Olivieri et al., 2016
	uc.249+	down	Microarray	Olivieri et al., 2016
	uc.252+A	up	Microarray	Olivieri et al., 2016
	uc.253+	down	Microarray	Olivieri et al., 2016
	uc.257+	up	Microarray	Olivieri et al., 2016
	uc.261+	up	Microarray	Olivieri et al., 2016

	uc.263+A	down	Microarray	Olivieri et al., 2016
	uc.265+A	down	Microarray	Olivieri et al., 2016
	uc.266+A	up	Microarray	Olivieri et al., 2016
	uc.268+	down	Microarray	Olivieri et al., 2016
	uc.269+	down	Microarray	Olivieri et al., 2016
	uc.269+A	up	Microarray	Olivieri et al., 2016
	uc.274+	down	Microarray	Olivieri et al., 2016
	uc.274+A	down	Microarray	Olivieri et al., 2016
	uc.275+A	down	Microarray	Olivieri et al., 2016
	uc.276+	down	Microarray	Olivieri et al., 2016
	uc.276+A	down	Microarray	Olivieri et al., 2016
	uc.278+	up	Microarray	Olivieri et al., 2016
	uc.281+	down	Microarray	Olivieri et al., 2016
	uc.282+A	up	Microarray	Olivieri et al., 2016
	uc.283+	up	Microarray	Olivieri et al., 2016
	uc.283+A	up	Microarray	Olivieri et al., 2016
	uc.285+	down	Microarray	Olivieri et al., 2016
	uc.287+A	down	Microarray	Olivieri et al., 2016
	uc.288+A	down	Microarray	Olivieri et al., 2016
	uc.289+	down	Microarray	Olivieri et al., 2016
	uc.289+A	down	Microarray	Olivieri et al., 2016
	uc.291+	up	Microarray	Olivieri et al., 2016
	uc.292+	down	Microarray	Olivieri et al., 2016
	uc.295+A	down	Microarray	Olivieri et al., 2016
	uc.296+	down	Microarray	Olivieri et al., 2016
	uc.298+	down	Microarray	Olivieri et al., 2016

	uc.299+A	down	Microarray	Olivieri et al., 2016
	uc.302+	down	Microarray	Olivieri et al., 2016
	uc.302+A	up	Microarray	Olivieri et al., 2016
	uc.304+A	up	Microarray	Olivieri et al., 2016
	uc.305+	down	Microarray	Olivieri et al., 2016
	uc.305+A	down	Microarray	Olivieri et al., 2016
	uc.306+	down	Microarray	Olivieri et al., 2016
	uc.310+	down	Microarray	Olivieri et al., 2016
	uc.311+	down	Microarray	Olivieri et al., 2016
	uc.317+	up	Microarray	Olivieri et al., 2016
	uc.319+A	up	Microarray	Olivieri et al., 2016
	uc.321+A	down	Microarray	Olivieri et al., 2016
	uc.322+	down	Microarray	Olivieri et al., 2016
	uc.322+A	down	Microarray	Olivieri et al., 2016
	uc.323+	down	Microarray	Olivieri et al., 2016
	uc.324+	down	Microarray	Olivieri et al., 2016
	uc.324+A	down	Microarray	Olivieri et al., 2016
	uc.325+	up	Microarray	Olivieri et al., 2016
	uc.325+A	down	Microarray	Olivieri et al., 2016
	uc.326+	up	Microarray	Olivieri et al., 2016
	uc.327+A	up	Microarray	Olivieri et al., 2016
	uc.329+	down	Microarray	Olivieri et al., 2016
	uc.333+A	down	Microarray	Olivieri et al., 2016
	uc.334+	up	Microarray	Olivieri et al., 2016
	uc.334+A	down	Microarray	Olivieri et al., 2016
	uc.335+	up	Microarray	Olivieri et al., 2016

	uc.339+	up	Microarray	Olivieri et al., 2016
	uc.340+	down	Microarray	Olivieri et al., 2016
	uc.342+	down	Microarray	Olivieri et al., 2016
	uc.343+	up	Microarray	Olivieri et al., 2016
	uc.343+A	up	Microarray	Olivieri et al., 2016
	uc.344+A	up	Microarray	Olivieri et al., 2016
	uc.345+A	down	Microarray	Olivieri et al., 2016
	uc.346+	up	Microarray	Olivieri et al., 2016
	uc.346+A	up	Microarray	Olivieri et al., 2016
	uc.349+	up	Microarray	Olivieri et al., 2016
	uc.350+	down	Microarray	Olivieri et al., 2016
	uc.352+	up	Microarray	Olivieri et al., 2016
	uc.354+A	down	Microarray	Olivieri et al., 2016
	uc.356+	down	Microarray	Olivieri et al., 2016
	uc.358+	down	Microarray	Olivieri et al., 2016
	uc.359+	down	Microarray	Olivieri et al., 2016
	uc.359+A	up	Microarray	Olivieri et al., 2016
	uc.360+	down	Microarray	Olivieri et al., 2016
	uc.362+A	down	Microarray	Olivieri et al., 2016
	uc.363+A	down	Microarray	Olivieri et al., 2016
	uc.366+	up	Microarray	Olivieri et al., 2016
	uc.369+	up	Microarray	Olivieri et al., 2016
	uc.371+A	down	Microarray	Olivieri et al., 2016
	uc.372+	down	Microarray	Olivieri et al., 2016
	uc.372+A	down	Microarray	Olivieri et al., 2016
	uc.374+A	down	Microarray	Olivieri et al., 2016

	uc.377+	down	Microarray	Olivieri et al., 2016
	uc.377+A	down	Microarray	Olivieri et al., 2016
	uc.378+A	down	Microarray	Olivieri et al., 2016
	uc.381+	down	Microarray	Olivieri et al., 2016
	uc.382+A	down	Microarray	Olivieri et al., 2016
	uc.385+A	down	Microarray	Olivieri et al., 2016
	uc.388+A	down	Microarray	Olivieri et al., 2016
	uc.389+	down	Microarray	Olivieri et al., 2016
	uc.390+	up	Microarray	Olivieri et al., 2016
	uc.392+A	down	Microarray	Olivieri et al., 2016
	uc.393+	down	Microarray	Olivieri et al., 2016
	uc.396+	up	Microarray	Olivieri et al., 2016
	uc.397+A	down	Microarray	Olivieri et al., 2016
	uc.398+	up	Microarray	Olivieri et al., 2016
	uc.398+A	down	Microarray	Olivieri et al., 2016
	uc.399+	up	Microarray	Olivieri et al., 2016
	uc.399+A	up	Microarray	Olivieri et al., 2016
	uc.402+	down	Microarray	Olivieri et al., 2016
	uc.402+A	down	Microarray	Olivieri et al., 2016
	uc.403+	down	Microarray	Olivieri et al., 2016
	uc.403+A	down	Microarray	Olivieri et al., 2016
	uc.404+	down	Microarray	Olivieri et al., 2016
	uc.405+A	down	Microarray	Olivieri et al., 2016
	uc.408+A	down	Microarray	Olivieri et al., 2016
	uc.410+	down	Microarray	Olivieri et al., 2016
	uc.412+A	down	Microarray	Olivieri et al., 2016

	uc.414+	up	Microarray	Olivieri et al., 2016
	uc.416+	up	Microarray	Olivieri et al., 2016
	uc.417+	down	Microarray	Olivieri et al., 2016
	uc.419+	up	Microarray	Olivieri et al., 2016
	uc.420+	down	Microarray	Olivieri et al., 2016
	uc.421+	up	Microarray	Olivieri et al., 2016
	uc.422+	up	Microarray	Olivieri et al., 2016
	uc.425+A	down	Microarray	Olivieri et al., 2016
	uc.427+	down	Microarray	Olivieri et al., 2016
	uc.432+	down	Microarray	Olivieri et al., 2016
	uc.432+A	down	Microarray	Olivieri et al., 2016
	uc.435+	down	Microarray	Olivieri et al., 2016
	uc.436+	down	Microarray	Olivieri et al., 2016
	uc.440+A	down	Microarray	Olivieri et al., 2016
	uc.443+A	down	Microarray	Olivieri et al., 2016
	uc.445+A	down	Microarray	Olivieri et al., 2016
	uc.446+	down	Microarray	Olivieri et al., 2016
	uc.448+A	down	Microarray	Olivieri et al., 2016
	uc.449+A	down	Microarray	Olivieri et al., 2016
	uc.450+	down	Microarray	Olivieri et al., 2016
	uc.450+A	down	Microarray	Olivieri et al., 2016
	uc.451+	down	Microarray	Olivieri et al., 2016
	uc.452+	up	Microarray	Olivieri et al., 2016
	uc.453+A	down	Microarray	Olivieri et al., 2016
	uc.454+	down	Microarray	Olivieri et al., 2016
	uc.456+A	down	Microarray	Olivieri et al., 2016

	uc.457+	down	Microarray	Olivieri et al., 2016
	uc.458+	down	Microarray	Olivieri et al., 2016
	uc.458+A	up	Microarray	Olivieri et al., 2016
	uc.462+A	down	Microarray	Olivieri et al., 2016
	uc.465+	down	Microarray	Olivieri et al., 2016
	uc.466+A	up	Microarray	Olivieri et al., 2016
	uc.468+	down	Microarray	Olivieri et al., 2016
	uc.469+A	down	Microarray	Olivieri et al., 2016
	uc.471+A	down	Microarray	Olivieri et al., 2016
	uc.472+A	down	Microarray	Olivieri et al., 2016
	uc.473+A	down	Microarray	Olivieri et al., 2016
	uc.476+	down	Microarray	Olivieri et al., 2016
	uc.476+A	down	Microarray	Olivieri et al., 2016
	uc.477+A	down	Microarray	Olivieri et al., 2016
	uc.479+	up	Microarray	Olivieri et al., 2016
	uc.481+A	up	Microarray	Olivieri et al., 2016
Lung	uc.338	up	RT-qPCR	Tian & Feng, 2018
	uc.339	up	RT-qPCR	Vannini et al., 2017
	uc.454	up	RT-qPCR	Zhou et al., 2018
Esophagus	uc.58+A	up	Microarray	Fassan et al., 2014
	uc.153+A	up	Microarray	Fassan et al., 2014
	uc.158+A	up	Microarray	Fassan et al., 2014
	uc.161+A	down	Microarray	Fassan et al., 2014
	uc.165+A	down	Microarray	Fassan et al., 2014
	uc.189	up	RT-qPCR	Guo et al., 2018
	uc.202+A	up	Microarray	Fassan et al., 2014

	uc.206+A	up	Microarray	Fassan et al., 2014
	uc.207+A	up	Microarray	Fassan et al., 2014
	uc.214+	down	Microarray	Fassan et al., 2014
	uc.223+A	up	Microarray	Fassan et al., 2014
	uc.227+A	up	Microarray	Fassan et al., 2014
	uc.274+A	up	Microarray	Fassan et al., 2014
	uc.327+A	down	Microarray	Fassan et al., 2014
	uc.472+A	up	Microarray	Fassan et al., 2014
	uc.473+A	up	Microarray	Fassan et al., 2014
Pancreatic	uc.190	up	Microarray/RT-qPCR	Jiang et al., 2016
	uc.233	up	Microarray/RT-qPCR	Jiang et al., 2016
	uc.270	up	Microarray/RT-qPCR	Jiang et al., 2016

Supplementary table 3

Patologic Alterations	T-UCR	Down/Up	Metodology	References
Nerve Damage	uc.173	down	RT-qPCR	Nan et al., 2016
Neuropathic Pain	uc.1	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.12	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.22	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.25	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.28	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.33	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.35	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.36	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.37	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.39	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.46	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.49	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.50	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.97	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.99	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.101	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.107	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.139	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.153	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.158	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.174	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016

	uc.184	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.185	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.186	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.189	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.193	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.194	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.197	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.203	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.208	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.209	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.211	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.213	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.214	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.217	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.221	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.238	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.255	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.256	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.257	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.258	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.264	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.265	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.274	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.280	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.285	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.292	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016

	uc.305	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.306	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.313	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.329	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.332	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.333	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.336	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.339	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.346	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.354	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.359	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.366	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.377	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.408	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.418	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.419	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.420	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.450	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.456	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.461	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.470	down	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.471	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
	uc.477	up	Microarray	Jiang, Yang, He, Tao, & Gao, 2016
Lead-induced Renal Injury	uc.173	down	RT-qPCR	Qin et al., 2018
Chron´s Disease	uc.141+A	down	Microarray	Qian et al., 2016
	uc.144+A	up	Microarray	Qian et al., 2016

	uc.166+A	down	Microarray	Qian et al., 2016
	uc.261+A	up	Microarray	Qian et al., 2016
	uc.290+A	up	Microarray	Qian et al., 2016
	uc.477+	up	Microarray	Qian et al., 2016
	uc.478+	down	Microarray	Qian et al., 2016
	uc.479+	down	Microarray	Qian et al., 2016
Fasting	uc.45+A	down	Microarray	Xiao et al., 2018
	uc.46+A	down	Microarray	Xiao et al., 2018
	uc.138+A	down	Microarray	Xiao et al., 2018
	uc.141+A	down	Microarray	Xiao et al., 2018
	uc.144+A	down	Microarray	Xiao et al., 2018
	uc.173+	down	Microarray	Xiao et al., 2018
	uc.356+	down	Microarray	Xiao et al., 2018
	uc.455+A	down	Microarray	Xiao et al., 2018
	uc.457+A	up	Microarray	Xiao et al., 2018
	uc.475+	down	Microarray	Xiao et al., 2018
	uc.477+	up	Microarray	Xiao et al., 2018
	uc.481+A	down	Microarray	Xiao et al., 2018
Intestinal Barrier Homeostasis	uc.173	up	RT-qPCR	J. Y. Wang et al., 2018
Myocardial Ischemia/Reperfusion Injury	uc.48	up	In situ hybridization/ RT-qPCR	Ding et al., 2019
Brown Adipose Tissue Thermogenesis	uc.417	up	RT-qPCR	Cui et al., 2016
Hepatic Lipid Accumulation	uc.372	up	Microarray/RT-qPCR	Guo et al., 2018

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Further Reading

None

7 CAPÍTULO II

Association and characterization of transcribed ultraconserved regions (T-UCRs) in Breast Cancer

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Running title: Analysis of T-UCRs in Breast Cancer

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ABSTRACT

There are 481 ultra-conserved regions (UCRs) longer than 200 bp in the genome, and 100% conserved among human, rat, and mouse. Most UCRs are transcribed (T-UCR), and some are differentially expressed in tumors. However, the influence that T-UCRs impose on breast cancer (BC) remains unclear. Furthermore, only 4% of T-UCRs present information on molecular details. Through TCGA (The Cancer Genome Atlas) analysis, we found 302 regions associated with clinical features in BC: 43% molecular subtype, 36% estrogen-receptor positivity, 17% ERBB2 expression, 12% stage and 10% overall survival. Looking for regions that were expressed in 80% of the samples, located in noncoding regions and, with greater difference among subtypes, we chose 12 T-UCRs to confirm differential expression in Brazilian patients (n=102) by RT-qPCR. The differential expression of uc.147 and uc.193 were confirmed, and both were associated with overall survival. Strand-specific RT-qPCR showed that uc.147 and uc.193 are transcribed in the opposite direction from their host genes. Through subcellular fractionation, we demonstrated that uc.147 is located in the nucleus and uc.193 is most cytosolic. Northern blotting results indicated that uc.147 is around 2800nt and we described its sequence by rapid amplification of cDNA ends. Silencing of uc.147 increases apoptosis, arrests cell cycle and reduces cell viability and colony formation in BC cell lines. The pull-down assay indicated that 19 proteins associated with tumorigenesis are binding to uc.147. This is the first study to screening all T-UCRs in BC, to characterize uc.147, and to demonstrate its potential as a prognostic marker in BC.

INTRODUCTION

Breast Cancer (BC) is the most commonly diagnosed cancer and the leading cause of cancer death among women (Bray *et al.*, 2018). BC is a heterogeneous disease with a range of clinical and molecular characteristics (Harbeck *et al.*, 2019). Based on immuno-histochemical analysis, BC is subdivided into four main groups: luminal A, luminal B, HER2 (Human Epidermal Growth Factor Receptor 2) and triple-negative (Goldhirsch *et al.*, 2011; Goldhirsch, 2013). This classification is essential for prognosis and therapy decisions. Luminal A and luminal B are sensitive to hormone therapy. HER2 positive patients can benefit from monoclonal antibody therapy (Network, 2012). Triple-negative remains the most unclear group, but new target therapies are become available, for example, PARP inhibitors (Beniey *et al.*, 2019). Besides that, BC can also be classified according gene expression profile, called molecular classification. The molecular characteristics stratify BC in five groups: luminal A, luminal B, HER2 positive, basal, and normal-like (Perou *et al.*, 2000; Sørlie *et al.*, 2001). Later, two more groups were added: claudin-low (Prat *et al.*, 2010) and molecular apocrine (Farmer *et al.*, 2005; Sanga *et al.*, 2009). So, the number of BC classification system and stratification of patients inside each group can bring a more personalized therapy and improved BC survival rates, but new molecular markers are still needed. (Curigliano *et al.*, 2017; Andre *et al.*, 2019).

In 2004, (Bejerano *et al.*, 2004) described genome regions with 200-781 nucleotides that are completely conserved among human, mouse, and rat and, extremely conserved across disparate taxa, named ultra-conserved regions (UCRs). Most UCRs are known to be transcribed (transcribed ultra-conserved regions – T-UCRs) in normal tissue (Calin *et al.*, 2007), but most their transcripts remain uncharacterized (Pereira Zambalde *et al.*, 2019). Recently, these regions have been demonstrated to participate into several biological and cell process, such as differentiation (Wang *et al.*, 2018; Xiao *et al.*, 2018), metabolism (Cui *et al.*, 2016; Guo *et al.*, 2018), drug resistance (Sekino *et al.*, 2019) and, cancer development and progression (Calin *et al.*, 2007; Olivieri *et al.*, 2016; Zhou *et al.*, 2018; Pereira Zambalde *et al.*, 2019). Also, the differential expression of T-UCRs were demonstrated to be associate with different types of cancer such as cervical cancer (Li, Q. *et al.*, 2017), colorectal cancer(Calin *et al.*, 2007), hepatocellular carcinoma (Bo *et al.*, 2016), neuroblastoma (Mestdagh *et al.*, 2010) and others (Calin *et al.*, 2007; Honma *et al.*, 2017; Sekino *et al.*, 2017; Zhou *et al.*, 2018). In BC, the overexpression of uc.63 was associated with poor prognosis in luminal A patients (Marini *et al.*, 2016). Besides, uc.38 is downregulated in BC patients (Zhang *et al.*, 2017). Taken together, all these highlights the T-UCRs as essential molecules in cancer biology and cellular environment. Moreover, suggest the relevance of T-UCRs-expression profiles as useful tools to differentiate human cancer types and correlate with diagnosis and prognosis.

Nevertheless, the influence that T-UCRs impose in cancer progression is still under investigation, and only 4% of T-UCRs present information on transcript molecular details (Pereira Zambalde *et al.*, 2019). Additionally, in BC, just two specific T-UCR were investigated. In this study, we screened all 481 T-UCRs in BC patients and found around 63% of regions correlated with some clinical and/or molecular parameters of BC. In addition, we focused on characterizing and understanding the role of the uc.147 in BC development.

RESULTS

T-UCRs in Breast Cancer Patients

In order to investigate the expression level of T-UCRs and its association with clinical parameters, we evaluated the expression from the 481 regions in TCGA (The Cancer Genome Atlas) data.

In total, 302 (62.78%) are associate with some clinical parameter. With more details, 209 (43.45%) associated with PAM50, 172 (35.76%) with ER+, 158 (32.85%) with PR+, 80 (16.63%) with HER2+, 60 (12.47%) with stage, 176 (36.59%) with therapy and 46 (9,56%) with overall survival (Supplemental Table 1).

Trying to identify poorly characterized lncRNA and focusing on the most interesting regions for further analysis, we excluded regions mapped in known exons regions and remain with 125 intronic and 84 intergenic T-UCRs. After, T-UCRs that were effectively expressed in the majority of the samples (> 80%) were selected to avoid rare transcripts, generating a list of 33 T-UCRs. Analyzing the subgroups of PAM50 gene signature of these 33 T-UCRs we observed that 12 T-UCRs had a greater difference in the levels of expression among the subtypes. Finally, the expression level of these 12 T-UCRs (uc.84, uc.138, uc.147, uc.193, uc.268, uc.271, uc.311, uc.376, uc.378, uc.427, uc.456, uc.475) (Supplemental Figure 1) were evaluated in a Brazilian cohort (n= 102 tumor samples) and confirmed the differential expression of uc.147 and uc.193 (Figure 1A and 1B/ supplemental figure 2A and B).

Despite the differential expression among subtypes, the increased expression of uc.147 is associated with reduced disease-free survival in luminal A subtype (Figure 1C), and uc.193 is associated with reduced disease-free survival in TCGA cohort (Figure 1D). Additionally, univariate and multivariate analysis suggested that uc.147 is an independent prognostic factor in luminal A patients (Table 1).

Uc.147 is located in an intronic region, overlapping the *LRBA* gene. As well, uc.193 is located in the 3´UTR of the gene *SYNCRIP*. Interestingly, uc.147 and uc.193 expression are correlated with their host genes (Supplemental figure 2C and 2D), but *LRBA* and *SYNCRIP* expression are not associated with the free survival (Supplemental figure 2A and 2B), suggesting the specific association with uc.147/uc.193 transcript and not with the host genes.

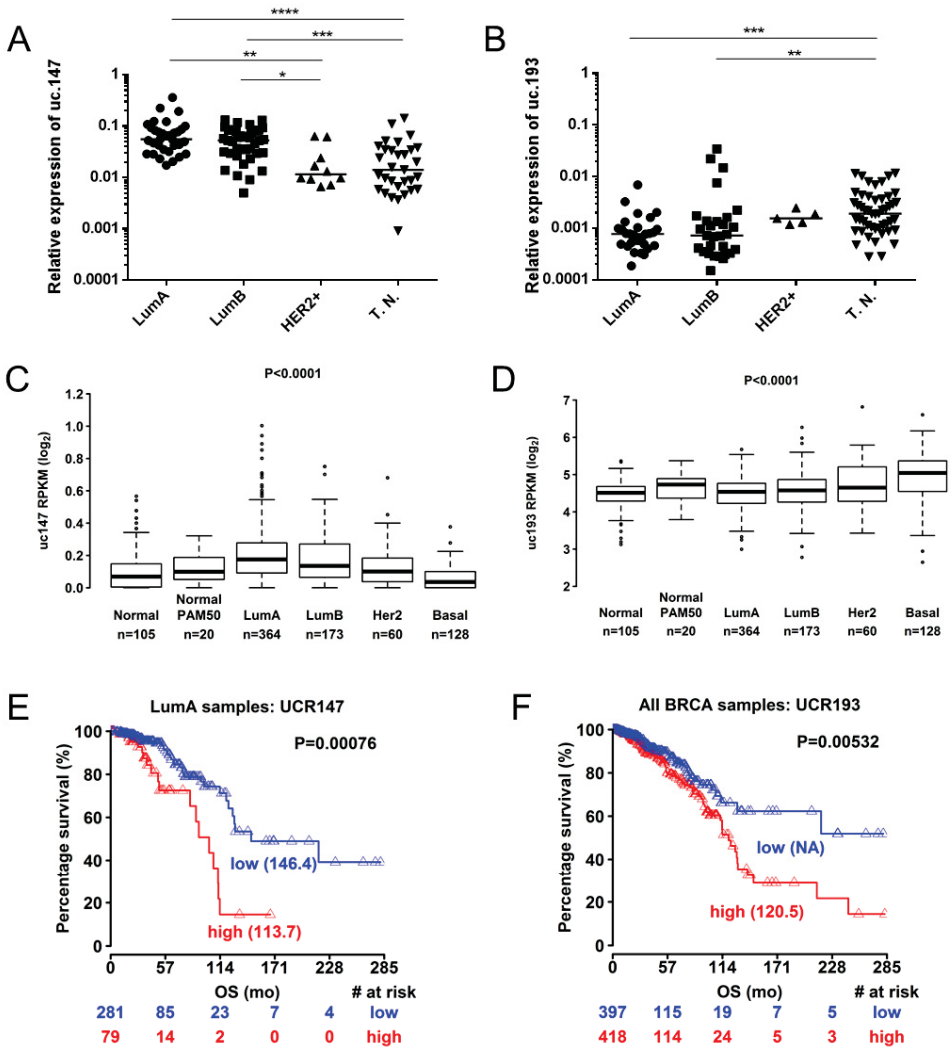

Figure 1 Expression analysis of uc.147 and uc.193. A and B. Relative expression of T-UCRs in Luminal A (LumA), Luminal B (LumB), HER2 overexpression (HER2+), and Triple Negative (T.N.) BC subtypes in a Brazilian cohort. A. uc.147. B. uc.193. C and D. Relative expression of T-UCRs in Normal, Normal PAM50, Luminal A (LumA), Luminal B (LumB), HER2 overexpression (HER2), Basal, breast cancer subtypes from TCGA patients. C. uc.147. D. uc.193. E. Overall survival information of LumA BC patients according to uc.147 expression. F. Overall survival information BC patients according to uc.193 expression. Kaplan-Meier method was used to generate a percentage of disease-free curves. *p<0.05.

Table 1. Summary of univariate and multivariate Cox regression analysis of overall survival and Luminal A survival.

		Univariate analysis				Multivariate analysis			
	Variable	**HR**	**lower .95**	**upper .95**	**p-value**	**HR**	**lower .95**	**upper .95**	**p-value**
BRCA	Age (continuous)	1.03	1.02	1.05	**1.92E-05**	2.34	1.55	3.52	**5.30E-05**
	Stage (III/IV vs I/II)	2.34	1.55	3.52	**5.30E-05**	1.04	1.02	1.05	**5.9E-07**
	uc.147	2.64	0.75	9.25	0.12971				
	LRBA	1.01	0.99	1.04	0.25445				
	uc.193	1.57	1.12	2.21	**0.00962**	1.75	1.21	2.52	**0.0026**
	SYNCRIP	1.01	0.99	1.03	0.22748				
Luminal A	Age (continuous)	1.03	1.01	1.05	**0.01059**	1.03	1.005	1.05	**0.01752**
	Stage (III/IV vs I/II)	1.75	0.89	3.45	0.10494				
	uc.147	8.25	1.57	43.47	**0.01276**	7.19	1.23	42.09	**0.028**
	LRBA	1.04	1.00	1.07	**0.04808**	1.03	0.99	1.07	0.123
	uc.193	2.42	1.17	4.99	**0.01692**	2.31	1.1	4.85	**0.02612**
	SYNCRIP	1.04	0.99	1.10	0.10606				

Characterization of uc.193 and uc.147

Uc.147 is an ultraconserved region of 308 bp located inside the 51st intron of the *LRBA* gene (Figure 2A). In addition, the ultraconserved region uc.193 is located in the 3´UTR of *SYNCRIP* gene, and it has 319 bp (Figure 2B). To characterize the uc.147 and uc.193, we asked whether these UCRs are transcribed in the same orientation of host genes in BC cell lines. To address this point, a strand-specific RT-qPCR was carried out. Beta-actin was used as endogenous control. We found that, in both cases, antisense transcripts according the host gene are the predominant form of uc.147 and uc.193 expressed in CAMA-1 and BT474, being sense transcript poorly detectable (Figure 2C and 2D). We also asked in which cell compartment these transcripts are most abundant. To find this out, the cytosolic and nuclear cell fractions were separated, and it was performed an RT-qPCR from each fraction. The expression of uc.147 was only detectable at nucleus (Figure 2E). The expression of uc.193 was detectable in both nucleus and cytosolic but with higher expression in the cytosolic fraction (Figure 2F).

As uc.193 is overlapping a 3´UTR of the *SYNCRIP* gene, the silencing of this transcript independently from the host gene would be more difficult, and we focused our further analysis in uc.147.

Uc.147 is an unknown transcript, and we interrogated the size and sequence of this molecule. To answer our question, we performed northern blotting and we detected a transcript around 3000nt and, with cloning by rapid amplification of cDNA ends (RACE) we uncovered 2745nt from the uc.147 (supplemental figure 3). Besides, we confirmed the conservation of this region

(supplemental figure 4). We also look for an enhancer site in the transcript through VISTA enhancer browser (https://enhancer.lbl.gov/), but possible enhancer activity was not found.

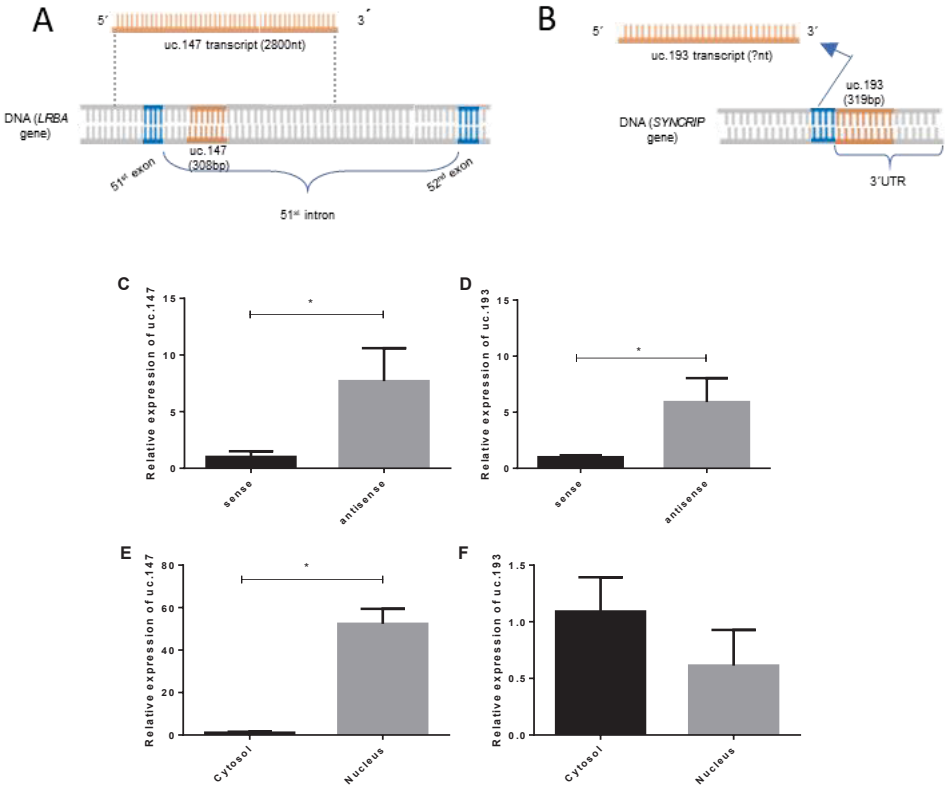

Figure 2 Characterization of uc.147 and uc.193. A. Scheme of uc.147 transcript and locus in chromosome 4 (grey: LRBA gene / red: uc.147 ultraconserved sequence and uc.147 transcript/ blue: LRBA exons). B. Scheme of uc.193 and locus in chromosome 6 (grey: SYNCRIP gene / red: uc.193 ultraconserved sequence/ blue: SYNCRIP exon). C and D. Strand-specific RT-qPCR. F and G. RT-qPCR assays for detection of uc.147 (F) and uc.193 (G) performed on RNA extracted from nuclear and cytoplasmic fractions obtained from two different cell lines CAMA-1 and BT474. The detection level of U6 and GAPDH was used as nuclear and cytosolic marker, respectively. *p<0.05

Biological effects of uc.147

RT-qPCR was performed in nine BC cell lines to determine the uc.147 high-expressing cell lines (Figure 3A). To verify the real activity of uc.147 transcript, the expression level of the *LRBA* exon and another intron from *LRBA*, with very low expression evidence, were analyzed (Figure 3B).

In order to investigate the biological role of uc.147 in BC, two luminal cell lines with high expressing levels of uc.147 were chosen (CAMA-1 and BT474). A siRNA-based approach, using two specific siRNAs targeting uc.147, was applied to investigate the phenotypic effects of uc.147 knockdown (Figure 3C-P). Both cell lines choose for functional essays are classified as a luminal subtype, corresponding with the subtype with high uc.147 expression in BC patients.

In CAMA-1, the siuc.147.1 does not have any effect, but the siuc.147.2 has the potential to inhibit 50% of uc.147 expression (figure 3C). On the other hand, in BT474, both siRNAs have inhibitory effect (Figure 3F). The siuc.147.1 inhibits 40% of uc.147 expression, and siuc.147.2 inhibits 60% (Figure 3F). Also, in CAMA-1 cells the siRNA against uc.147 has weak effect for the *LRBA* expression (Figure 3D). But, in BT474 we can see that *LRBA* expression drop, but not at the same intensity of the uc.147 (Figure 3G). Specific siRNA against *LRBA* gene was evaluated (Figure 3E and 3H). Furthermore, the silencing of *LRBA* gene also decreased the LRBA protein levels, but when we use the siRNA against the uc.147, the protein expression remains the same (supplemental figure 5).

After uc.147 downregulation, CAMA-1 and BT474 cell viability decreased, but this effect was not found in *LRBA* downregulation (Figure 3H-J). On BT474, both siRNAs demonstrated the same results on viability (Figure 3H). Additionally, in CAMA-1 cells, this inhibitory effect is also seen in colony formation assay (Figure 3K). Colony formation was not assessed on BT474 due its low ability to form colonies.

The silencing of uc.147 also leads to cell death, associated with an increase in G0/G1 cells and a reduction in G2/M events in CAMA-1, but not the same was observed in BT474 (Figure 3L-O). Of note, the events are only seen when uc.147, but not the *LRBA* host gene, is knocked down (Figure 3H-O). This suggests that uc.147 and *LRBA* are two independent transcripts with different roles. In addition, we performed a migration and invasion assay, but we couldn´t analyze due the fact that both cell lines, CAMA-1 and BT474, have lower migration and invasion activity (supplemental figure 6). Taken together, these findings show that uc.147 plays an important role in BC cells

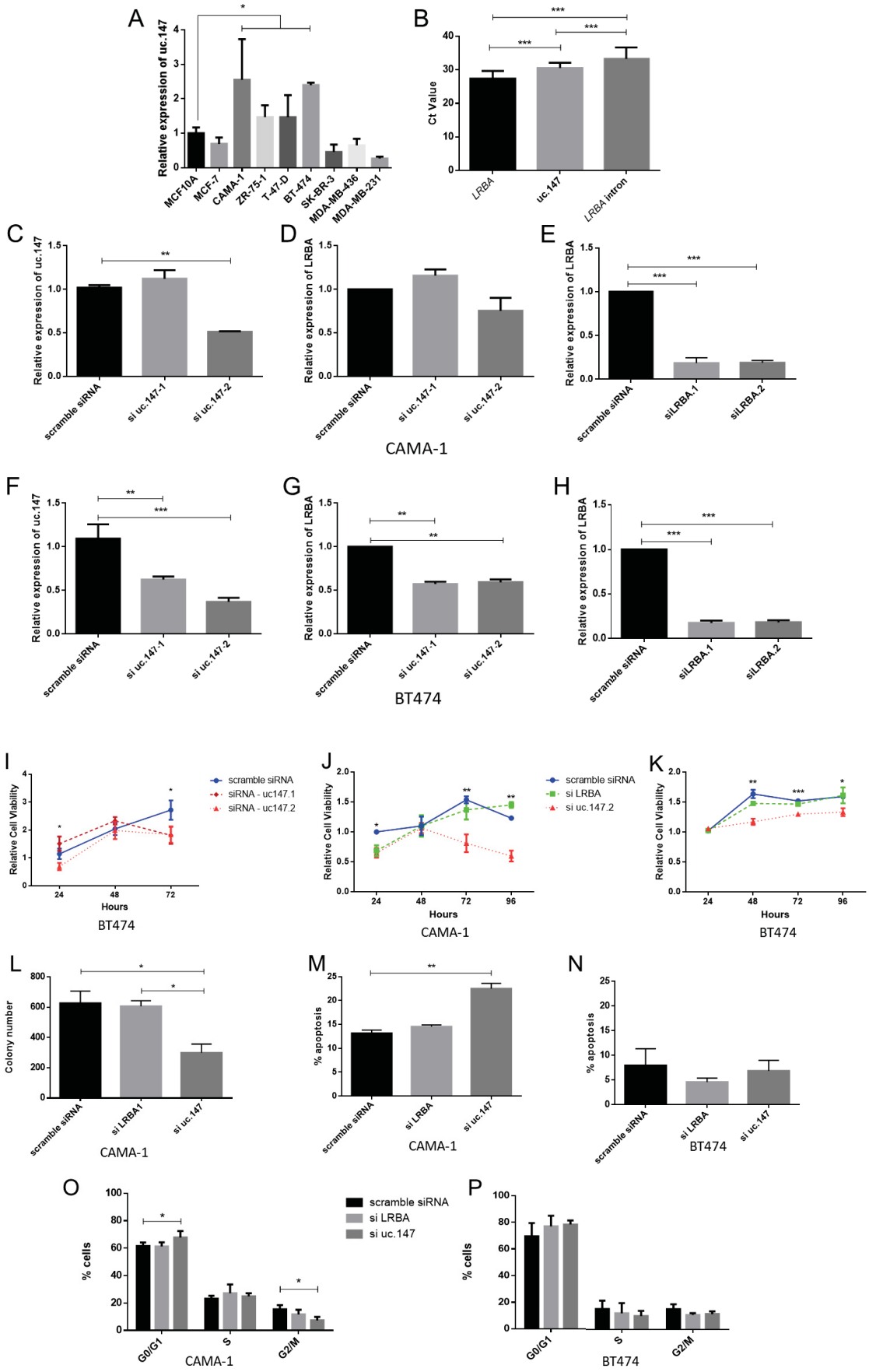
A
Relative expression of uc.147
MCF10A
MCF-7
CAMA-1
ZR-75-1
T-47-D
BT-474
SK-BR-3
MDA-MB-436
MDA-MB-231
B
Ct Value
LRBA
uc.147
LRBA Intron
C
Relative expression of uc.147
scramble siRNA
si uc.147-1
si uc.147-2
D
Relative expression of LRBA
CAMA-1
E
Relative expression of LRBA
siLRBA.1
siLRBA.2
F
BT474
G
H
I
Relative Cell Viability
Hours
scramble siRNA
siRNA - uc147.1
siRNA - uc147.2
J
si LRBA
si uc.147.2
K
L
Colony number
si LRBA1
si uc.147
M
% apoptosis
si LRBA
si uc.147
N
O
% cells
G0/G1
S
G2/M
P

Figure 3. uc.147 silence reduces the tumorigenesis process. A. Expression levels of uc.147 in BC cell lines. B. Ct values demonstrating expression levels of uc.147/LRBA exon/LRBA intron in BC cell lines. C and F. Relative expression of uc.147 after siRNA uc.147.1 and siRNA uc.147.2 (100nM) treatment in CAMA-1 (C) and BT474 (F). D and G. Relative expression of LRBA gene after siRNA uc.147.1 and siuc.147.2 (100nM) treatment in CAMA-1 (D) and BT474 (G). E and H. Relative expression of LRBA after siRNA LRBA.1 and siRNA LRBA.2 (100nM) treatment in CAMA-1 (E) and BT474 (H). I. Cell viability in BT474 after siRNA uc.147 1 and 2 (100nM) treatment 24, 48 and 72 hours. J and K. Cell viability in CAMA-1 (J) and BT474 (K) after siRNA uc.147.2 (100nM) and siRNA LRBA (20nM) treatment 24, 48, 72 and 96 hours. L. Number of CAMA-1 colonies after siRNA uc.147.2 (100nM) and siRNA LRBA treatment (20nM). M and N. Apoptosis ratio after CAMA-1 (M) and BT474 (N) treatment with siRNA uc.147.2 (100nM) and siLRBA (20nM). O and P. Cell cycle analysis in CAMA-1 (O) and BT474 (P) after siRNA uc.147.2 (100nM) and siLRBA (20nM). *p<0.05.

Protein binding sites in uc.147 transcript

We asked which proteins could be binding to this transcript and be involved in BC development. A pulldown assay was performed and generated a list of 19 bind proteins (Table 2). Of note, most of these proteins are recognized for their role in cancer and may help to explain uc.147 mechanism of action in BC cells.

Table 2. Results of pull-down assay indicating the coverage of proteins binding to the uc.147.

Protein	Initials	Coverage	Function
Nascent Polypeptide Associated Complex Subunit Alpha	NACA	40.85%	Epithelial-Mesenchymal Transition
Keratin 17	KRT17	26.16%	Cytoskeletal System
Dyskerin Pseudouridine Synthase 1	DKC1	21.4%	rRNA processing and TERC stabilization
ATP synthase subunit alpha	ATP1	19.48%	ATP production
Zinc finger antiviral 1	ZC3HAV1	17.6%	mRNAs Stability
Calmodulin Like 5	CALML5	16.44%	Epithelial-Mesenchymal Transition
Pescadillo Ribosomal Biogenesis factor 1	PES1	14.25%	Contain a BRCA1 Domain
Scaffolding protein	TDRD3	13.85%	Transcription Activation
Spermatid Perinuclear RNA Binding Protein	STRBP	11.25%	RNA binding
POM121 Transmembrane Nucleoporin Pseudogene	LOC102725072	8.66%	Transmembrane Nucleoporin
WD Repeat Domain 18	WDR18	7.84%	DNA replication
Ras Homolog Family Member A	RHOA	7.77%	Cytoskeletal System
Glioma Tumor Suppressor Candidate Region Gene 2 Protein	NOP53	4.60%	Chromosome Stability
Leucine Rich Repeat Containing 59	LRRC59	3.91%	ER binding
Centrosomal Protein 68kDa	CEP68	3.39%	Chromosome Stability
Chaperonin Containing TCP1 Subunit 8	CCT8	2.11%	Cytoskeletal System
Centrosomal protein	CEP131	2.1%	Chromosome Stability

Testis Expressed 10	TEX10	1.97%	Epithelial-Mesenchymal Transition
Citron Rho-Interacting Serine/Threonine Kinase	CIT	0.45%	Cytoskeletal System

DISCUSSION

Previously, only two T-UCRS were investigated in BC, the overexpression of uc.63 associated with poor prognosis in Luminal A patients (Marini *et al.*, 2016) and, downregulate of uc.38 associated with worst outcome of BC patients (Zhang *et al.*, 2017).

We analyzed all 481 T-UCRs in TCGA data and found that most of them (~63%) are associate with some clinical parameters. In addition, uc.193 and uc.147 differential expression were highlighted in a Brazilian cohort and survival analysis. Overexpression of uc.147 is associate with poor prognosis in luminal A patients and overexpression of uc.193 with BC poor survival.

Both uc.147 and uc.193 are non-characterized T-UCRs and our study help to better understand these transcripts. The uc.193 is found in a 3´UTR of the *SYNCRIP* gene but is transcribed in the opposite direction, indicating that they are two independent transcripts, although they have great expression correlation and both are found in the nucleus and cytoplasm. Uc.147 transcript is also antisense and independent from its host gene *LRBA*. This transcript has approximately 2800 nucleotides and, the sequence includes an intron and one exon of the *LRBA* gene.

As uc.147 was highly expressed in ER-positive subtypes and associated with poor survival in luminal A patients, we investigated its role in luminal cell lines. Uc.147 silencing decreased cell viability in both cell lines and CAMA-1, a luminal A cell line, uc.147 silencing also reduced colony formation, number of cells in G2/M during cell cycle and increased apoptosis, suggesting an oncogenic effect, mainly in luminal A. Moreover, the silencing of the host gene *LRBA* did not lead to the same cell phenotyping of the uc.147 silencing. Indeed, indicating that they are independent transcripts and have individuality.

Trying to understand the proper mechanism by which uc.147 controls viability in tumor cells, we performed a pull-down assay and identify that proteins linked to cancer development are binding in this RNA. Most proteins identified in pull-down assay are involved in cytoskeletal system, centrosomal organization, and epithelial-mesenchymal transition (EMT).

In the cytoskeletal system, we found KRT17, RhoA, CCT8 and CIT is a type I intermediate filament mainly expressed in the basal cells of epithelia. As a multifaceted cytoskeletal protein, KRT17 regulates many biological, such as cell proliferation and growth (Depianto *et al.*, 2010; Mikami *et al.*, 2015; Yang *et al.*, 2019). KRT17 is an oncogenic protein that reduces nuclear p27, associated with poor survival and aggressiveness of several cancers including BC (Escobar-Hoyos *et al.*, 2015; Merkin *et al.*, 2017).

The Rho family of GTPases are master regulators of the cytoskeleton and play critical roles in assembly and maintenance of cell-cell contacts and cell migration (Collins and Nelson, 2015). (Liu *et al.*, 2019). Aberrant regulation of the Rho-GTPases has been identified as an important contributing factor in the acquisition of the metastatic phenotype (Canel *et al.*, 2013; Little *et al.*, 2019) and drug resistance (Liu *et al.*, 2019). In addition, the RhoA can interact with CIT. CIT-

K regulates cytokinesis at a step after Rho in the contractile process (Madaule *et al.*, 1998) by maintaining RhoA localization at the cleavage site, which is necessary for proper RhoA activity and contractile ring dynamics (Bassi *et al.*, 2011) CIT was also associated with tumor progression and aggressiveness, one of the mechanisms involved in the regulation of tumor suppression p53 and it was also found precipitated with uc.147 (Mckenzie and D'avino, 2016; Wu *et al.*, 2017).

Another protein associate with the cytoskeletal system identified is the CCT8. CCT8 is a subunit of CCT complex (Vallin and Grantham, 2019), and its overexpression is already associated with cancer progression in BC, glioma, and hepatocellular carcinoma (Shaw *et al.*, 2013; Huang *et al.*, 2014; Qiu *et al.*, 2015).

The correct cell division depends on the organization of centrosome, and maintenance of chromosome stability. The proteins found involved in this process are NOP53, CEP131, and CEP38. NOP53 ribosome biogenesis factor is a nucleolus-localized protein (Kalt *et al.*, 2012) that translocate to the nucleoplasm in response to ribosomal stress and regulates the stability of stress-responsive proteins (Kim *et al.*, 2011). NOP53 participates in the maintenance of nuclear morphology, chromosomal stability and mitotic integrity during nuclear division. Besides, its expression is correlated with carcinogenesis. NOP53 physically interacts with p53 and increases p53 stability by inhibiting the mouse double minute 2 homolog (*MDM2*)-mediated polyubiquitination pathway, which contributes to cancer development (Lee *et al.*, 2018).

CEP131 is a centriolar satellite protein and is required for centrosome duplication, playing critical roles in the maintenance of genome stability. Due this characteristics CEP131 hint a potential role for this protein in cancer development and progression (Staples *et al.*, 2012; Li, X. *et al.*, 2017). Finally, CEP68 plays an essential role in centrosome formation in the cell cycle; thus it can be implicated in tumorigenesis process (Graser *et al.*, 2007).

The EMT is an important process in cancer metastasis, and the precipitate proteins CALML5, TEX10, and NACA are involved in this process. CALML5 is a protein involved in epidermal differentiation (Méhul *et al.*, 2001), and has been associate with BC (Debald *et al.*, 2011). Also, TEX10 is a protein from the group of tumor-related antigens (Ags), which are overexpressed in the testis and different tumor sites (Asgharzadeh *et al.*, 2019) and its expression is associate with BC (Dianatpour *et al.*, 2012; Asgharzadeh *et al.*, 2019), and EMT in bladder cancer (Wu *et al.*, 2019). Also, NACA participate in EMT in hematopoietic cells (Murayama *et al.*, 2015).

In addition, we found other proteins that participate in another regulatory process associate with cancer progressions like POM121, WDR18, and Diskerin. POM121 is a transmembrane nucleoporin (NUP) that anchors the nuclear pores complexes (NPC) to the mammalian nuclear membrane (Knockenhauer and Schwartz, 2016). POM121 influences adherens junction, axon guidance, cell cycle, hedgehog signaling, mismatch repair and lysine degradation. This indicates that POM121 may affect cancer progression and metastasis (Ma *et al.*, 2019).

Little is known about the WDR18, a protein that is required for DNA replication and cell viability (Huang *et al.*, 2016). A recent study associates WDR18 overexpression in hepatocellular carcinoma (HCC) by activating Wnt/β-catenin signaling pathway (Zhang and Chen, 2019). Another protein, dyskerin, is a component of small nucleolar ribonucleoprotein

particles (RNPs) crucial for rRNA processing. It binding to H/ACA sequence that is present in small nucleolar RNAs (snoRNAs) and telomerase RNA component (TERC). The snoRNA control diskerin-mediated pseudouridine in ribosomal RNA. Dyskerin allows TERC stabilization and proper functioning of the telomerase complex. As the functions of diskerin are essential to maintenance cell proliferation, its expression level have already been associate with BC (Montanaro *et al.*, 2008; Alawi and Lin, 2011).

Two of 19 found proteins have a relation with estrogen. PES1 contain a BC–associated gene 1 (BRCA1) C-terminal (BRCT) domain- protein domain and is estrogen inducible. The sensibility to estrogen is appealing because uc.147 was associated with ER-presence and overexpressed associated with survival only in Luminal A patients. Also, PES1 expression gradually increases during BC development and progression (Li *et al.*, 2009; Cheng *et al.*, 2012). The other, LRRC9 binds to estrogen receptor and is associated with poor prognosis in BC (Zhen *et al.*, 2012; Toda *et al.*, 2018).

Finally, we found proteins precipitated with uc.147 that regulate cancer progression by regulating transcriptional factors or epigenetics mechanisms. The TDRD3 is one of the major methyl-arginine effector molecules that recognize methylated arginine residues on histones and the C-terminal domain of RNA polymerase II and activates transcription. TDRD3 was shown to regulate cell proliferation and invasion in BC cells (Yang *et al.*, 2010; Morettin *et al.*, 2017; Narayanan *et al.*, 2017). Besides, ZAP is an RNA-binding protein that regulates the stability and translation of specific mRNAs. Its increase level is associate with HCC progression and poor survival (Liu *et al.*, 2018).

All of the proteins found has essential features for cancer development and progression, and give some clues for uc.147 mechanisms and reinforce the critical uc.147 role in BC cells. More studies are needed to better understand how the complexes are formed, and how these proteins interact with uc.147 regulating tumorigenesis process.

We show herein several proofs that neoplastic BC cells show unique expression profile of T-UCRs, suggesting a significant role of T-UCRs in the malignant process. In this study, we provide more evidence for the role of T-UCRs in BC. We also characterized the uc.147, a T-UCR that was never been described before, which elucidates one more fraction about these complex regions. Indeed, uc.147 has an oncogenic effect in luminal BC cell line. Furthermore, uc.147 expression may have potential as BC prognosis marker in luminal patients.

METHODS

Bioinformatic analysis

The 481 T-UCRs were described by Bejerano, 2004, and were identified by their position according to the genome. We first converted the position of T-UCRs of hg18 into the hg38 using the LiftOVer tool from USCS genome browser. We use the position of T-UCRs to search for their expression level and association with clinical parameters, and subgroups of PAM50 gene signature. We performed the search by using TANRIC website (The Atlas of non-coding RNA in Cancer) (Li *et al.*, 2015) that facilitate the access of the data from TCGA (The Cancer

Genome Atlas) portal, which contains expression profiles data of 837 BC specimens and 135 normal tissue.

Patients samples

For expression analysis, 16 luminal A, 36 luminal B, 10 HER2-enriched, and 40 triple-negative Brazilian BC samples were obtained from Hospital Nossa Senhora da Graças, Curitiba, Paraná, Brazil. Tissue samples were obtained from fresh surgical specimens frozen in RNA later and stored at -80°C. All the samples were collected with the patients' informed consent and the subtypes were immunohistochemically confirmed. The protocol for studying biological markers associated with disease outcome was approved by the medical ethics committee of the Universidade Federal do Paraná (19870319.3.0000.0102-CONEP).

Cell cultures and growth conditions

MDA-MB-231, MDA-MB-436, SK-BR-3, BT-474, MCF-7, T-47D, ZR-75-1 and CAMA-1 cells were cultured in Dulbecco's Modified Eagle's Medium DMEM (LONZA) supplemented with 10% FBS (Gibco) and Penicillin-Streptomycin (Gibco), respectively 100 U/mL and 100 µg/mL. ZR-75-1 were cultured in RPMI medium supplemented with 10% FBS and 1% Penicillin-Streptomycin. The MCF10A were cultured in DMEM/F12 (LONZA, USA) supplemented with 2.5mM of L-glutamine, 20ng/ml of epidermal factor growth (EFG), 0.01mg/ml of insulin, 500ng/ml of hydrocortisone, 0.5% of Penicillin-Streptomycin and 5% of horse serum.

RNA isolation, cDNA synthesis, and RT-qPCR

RNA from cells and tissues were extracted using the Quick RNA Miniprep Kit (ZymoResearch, USA). All RNAs passed by DNAse-digestion (Ambion, USA). The quality and concentration of the RNA were assessed using nanodrop ND-1000 instrument (NanoDrop Technologies, Thermo Scientific, USA) and Bioanalyser (Agilent, USA). The cDNA was synthesized using the SuperScript III cDNA kit (Invitrogen), and diluted cDNA was used for RT-PCR analysis using iQ SYBR Green Supermix (Bio-Rad) with the appropriate primers (Supplementary Table 2). The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative abundance of RNA genes compared with two of the following genes GAPDH, actin-beta, TBP and U6 expression.

Strand-specific RT was made in 20µL, where 100ng of RNA was transcribed to strand-specific cDNA. Two reverse transcription reaction mix was made for strand-specific cDNA synthesis: one to detect uc.147 sense-oriented transcript (using reverse primers both for uc.147 and actin-beta) and one for uc.147 antisense oriented transcript (using forward primer for uc.147 and reverse primer for actin beta). Before use in qPCR analysis, strand-specific cDNAs were diluted 5-times with nuclease-free water (Supplementary Table 2).

RACE Cloning and Northern blotting

To identify the 5'- and 3'-end of the uc.147 transcript, BT474 cell total RNAs were treated with DNase I (RNase-free) (Invitrogen) and the SMARTer RACE cDNA Amplification Kit (Clontech) was used, according to the manufacturer's instructions. The cDNA ends were amplified with the Platinum Taq DNA Polymerase High Fidelity (Invitrogen), and gene-specific primers (listed in Supplementary) were used. We performed a nested PCR with the nested universal primer provided with the kit and the nested gene-specific primers listed in Supplementary Table. The

PCR fragments were then run on a 1.5% agarose gel, and DNA was extracted with the QIAquick Gel Extraction Kit (Qiagen), according to the manufacturer's instructions. The RACE products were then cloned into a TOPO® TA pCR®2.1 cloning vector (Invitrogen) according to the manufacturer's instructions, and the inserts were sequenced by using the M13 primers. For northern blotting a total RNA was electrophoresed on 15% PAA-urea gels (Calin et al., 2002). RNA source was BT474 cell line with no treatment, and cells treated with uc.147 siRNA.

siRNA treatment

CAMA-1 and BT474 cells were seeded one day before onto six-well plates, reaching a total of 200000 for 50-70% confluence. The cells were transfected with 100nM siRNA scramble, siRNA uc.147.1, and siRNA uc.147.2, and 20nM siRNA *LRBA*.1, and siRNA *LRBA*.2. (Supplemental Table 2). By lipofectamine 2000 reagent (Invitrogen) for 24, 48 and 72 h. All siRNAs were provided by Sigma Aldrich.

Subcellular fractionation

The separation of nuclear and cytosolic fractions was performed using the PARIS Kit (Life Technologies) according to the manufacturer's instructions in three different cell lines CAMA-1, BT474, and MCF-7.

Cell viability

For cell viability assay, CAMA-1, and BT474 cells treated with siSCR, siuc.147 or si*LRBA* were plated in 96 well plates. A total of 5000 cells were seemed in each well. The cell viability was read after 24, 48, 72 and 96 hours after transfection. Each day the media were changed to 100 ul of media + MTS (5 mg/ml) (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega) incubated for 4 hours, and read at 580 nm.

Apoptosis

The apoptosis ratio was analyzed using the Annexin V-FITC Apoptosis Detection Kit. At 24 h after transfection, cells were harvested and resuspended in binding buffer containing Annexin V-FITC and PI according to the manufacturer's instructions. The samples were analyzed by flow cytometry (BD Biosciences, USA). Cells were discriminated into viable cells, necrotic cells, and apoptotic cells by using BD FACSVantage™ cytofluorimeter (BD Biosciences, USA), and then the percentages of apoptotic cells from each group were compared.

Cell Cycle

For cell-cycle analysis, the cells were harvested 48 h after treatment with siSCR, siuc.147 or si*LRBA*, fixed in 70% ethanol, and stained in a solution containing 10 μg ml−1 of propidium iodide (Sigma-Aldrich), 10,000 U ml−1 of RNase (Sigma-Aldrich) and 0.01% of NP40 (Sigma-Aldrich). After 30–60 min, the samples were analyzed by flow cytometry using a BD FACSVantage™ cytofluorimeter (BD Biosciences). Analysis of samples was carried out acquiring 10,000-15,000 events/samples on FACS Calibur (BD). ModFit software was used to analyze cell cycle phases.

Colony-forming assay

The CAMA-1 cells were seeded in a 6-well plate at 500 cells per well and allowed to grow for 20 days at 37 °C in a 5% CO_2 humidified incubator. The cells were fixed with 100% methanol at room temperature for 20 min, stained with 1% crystal violet at room temperature for 5 min, and washed with water until excess dye is removed. The number of colonies in each well was counted.

Pull-Down Assay

Uc.147 was first cloned into TOPO TA vector following manufactory´s instructions (TAKARA, USA). The pull-down protocol was adapted from (Yoon and Gorospe, 2016). The TOPO TA with uc.147 was cloned inside the pMS2 vector. The digestion of TOPO TA was done by enzymes Xba1 (1 µl - 20000U/µl) and Spe1 (2 µl – 10000U/ µl). Also, Xba1 (1 µl - 20000U/ µl) was used to digest pMS2 vector and generate complementary extremities. The pMS2 vector with uc.147 and an empty control vector were co-transfected with pMS2-GST vector in HEK293 cells. After 48 hours, the supernatant was collected and the protein concentration was determinate by Bradford method.

The GST fusion proteins were immobilized in the GSH beads (GE healthcare; cat 17-0756-01). The beads were washed (3X) with NT2 buffer. Reduced glutathione was used to elute the complexes. The fractions were analyzed in silver staining SDS-PAGE gel (Pierce™ Silver Stain for Mass Spectrometry). The obtained complexes were sent to Proteomics and Metabolomics Facility core of MD Anderson Cancer Center to mass spectrometry analysis.

Western blotting

For immunoblotting analysis, proteins were quantified using Bradford assay (Bio-Rad, USA), with absorbance read at 595 nm on an ELISA reader (BioTek, USA), and the concentrations calculated using a standard curve. Overall, 40 µg of proteins were loaded on 8% gel and transferred on a nitrocellulose membrane by using a semi-dry Transfer System, according to the manufacturer's instructions. The membrane was stained with Ponceau S to make sure that equal amounts of proteins were loaded in each lane. The membranes were then incubated for 2 h at room temperature with TBST containing 5% non-fat dry milk. The membrane was probed overnight at 4 °C with the primary antibody, then horseradish peroxidase-conjugated secondary antibody (Cell Signaling) was added at a dilution of 1:3000. The following primary antibodies were used: anti-*LRBA* rabbit monoclonal antibody (Bethyl Cat.# A304-478A) diluted 1:1000 and, anti-beta-actin rabbit monoclonal antibody (BioVision Cat.#3917-30T) diluted 1:3000, The used secondary antibody was: anti-rabbit HRP linked (Cell Signaling Cat.#: 7074S) diluted 1:5000. The bound antibodies were detected by enhanced chemiluminescence and by using the SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific, USA) according to the manufacturer's instructions.

Statistical Analysis

Data are presented as means ± SD. Statistical analysis of the data was performed by Student's t-test or ANOVA. p-values of ≤ 0.05 were considered statistically significant. All experiments were performed in triplicate.

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Author contributions: J.C.O, G.A.C., and E.P.Z. designed this study. E.P.Z, J.C.O and G.A.C wrote the manuscript. E.P.Z, R.B, T.S.J, A.C.R performed most of the wet-lab experiments. C.I. and C.M. reviewed and performed most bioinformatical analysis. D.F.G and E.M.S.R.F assisted with interpretation. E.P.Z., J.C.O. and G.A.C analyzed the data. All authors read and approved the manuscript's content.

DISCLOSURE DECLARATION

No conflicts of interest

Supplemental Materials for

Association and characterization of transcribed ultraconserved regions (T-UCRs) in Breast Cancer

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CONTENTS

Fig S1. **Relative expression of T-UCRs in BC subtypes Luminal A (LumA), Luminal B (LumB), HER2 overexpression (HER2+) and triple negative (T.N.) in Brazilian cohort**. A. uc.84. B. uc.138. C. uc.268. D. uc.271. E. uc.311. F. uc.376. G. uc.378. H. uc.427. I. uc.456. J. uc.475.

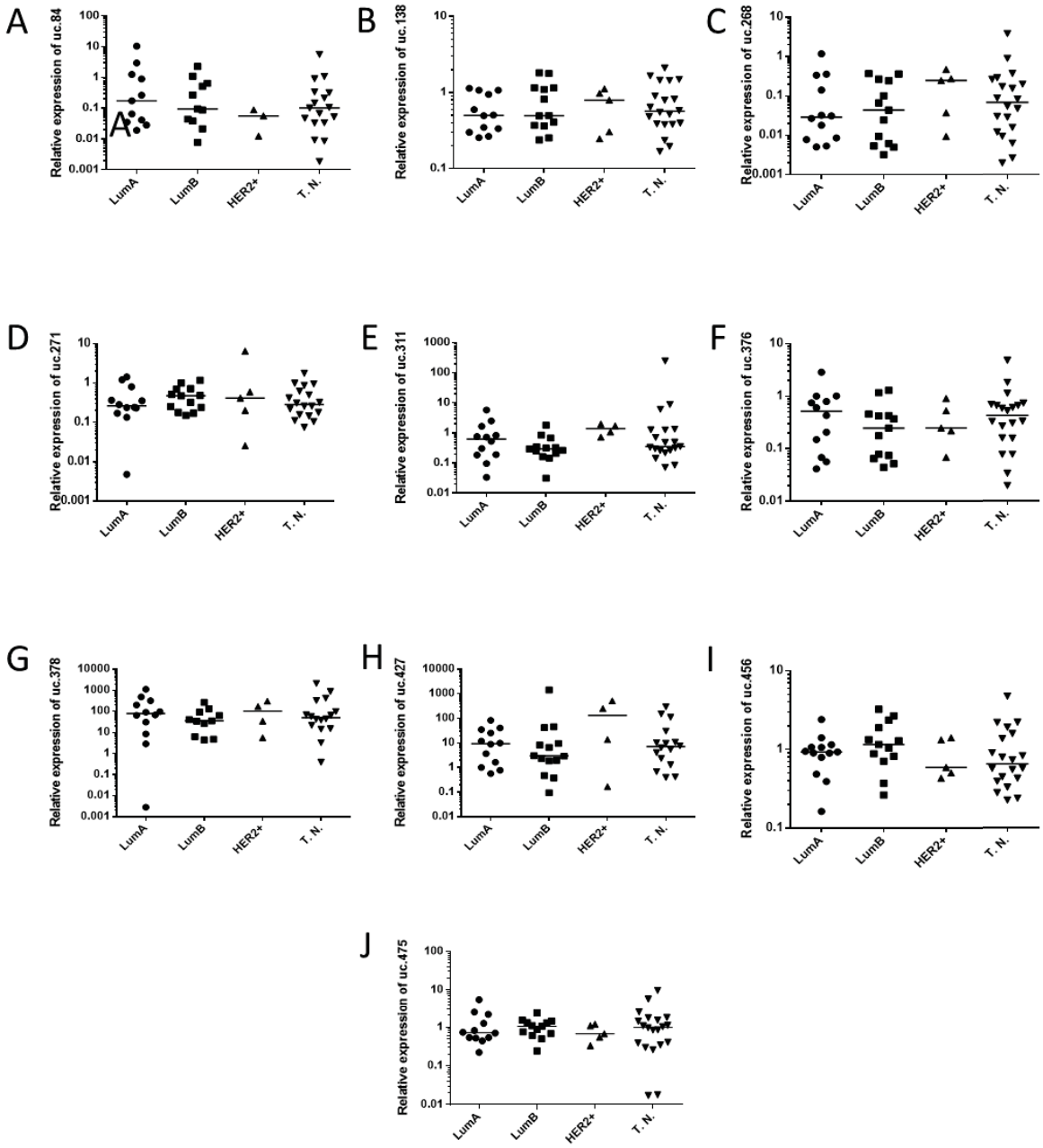


Fig S2. **Overall survival in breast cancer patients and correlation expression between T-UCRs and host genes**. A and B. Association between *LRBA* (A) and *SYNCRIP* (B) on overall breast cancer survival. Kaplan-Meier method was used to generate percentage disease-free curves. Data available on OncoLnc database (http://www.oncolnc.org/). C and D. Correlation between expression levels of T-UCR and host gene. *LRBA* and uc.147 (C). *SYNCRIP* and uc.193 (D).

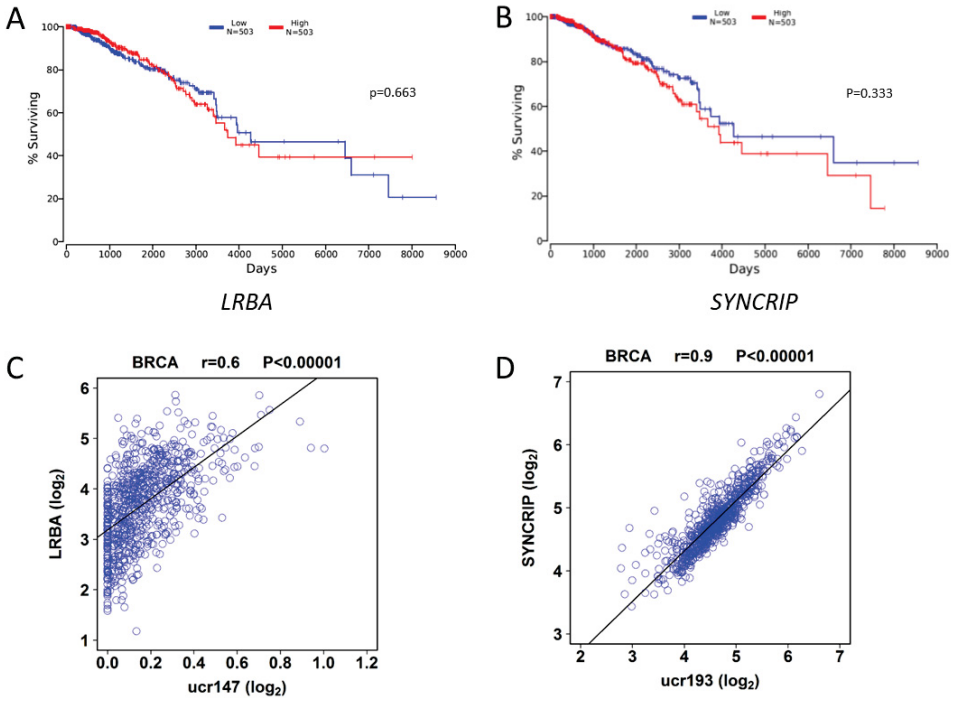


Fig S3. **Characterization of uc.147.** A. Northern blotting analysis of uc.147 expression in BC cell lines. RNA was isolated with Trizol (Invitrogen) and run on a MOPS/formaldehyde denaturing agarose gel. RNA was then blotted on a nitrocellulose blot and hybridized with a P32-labelled DNA oligo probe. The band around 3kb is the uc.147 signal. B. RACE PCR results of 3´ region from uc.147. C. PCR results of 5´region from uc.147. After a cDNA construct, following PCRs were done until the uncover of the 5´region that was not able to be amplified by RACE cloning. D. Sequencing results confirming the amplification of the specific uc.147 region. E. A sequence of 2475nt of uc.147(IN CAPS LOCK: exonic region/ in yellow: sequence of the uc.147 on DNA cited by Bejerano, 2004)

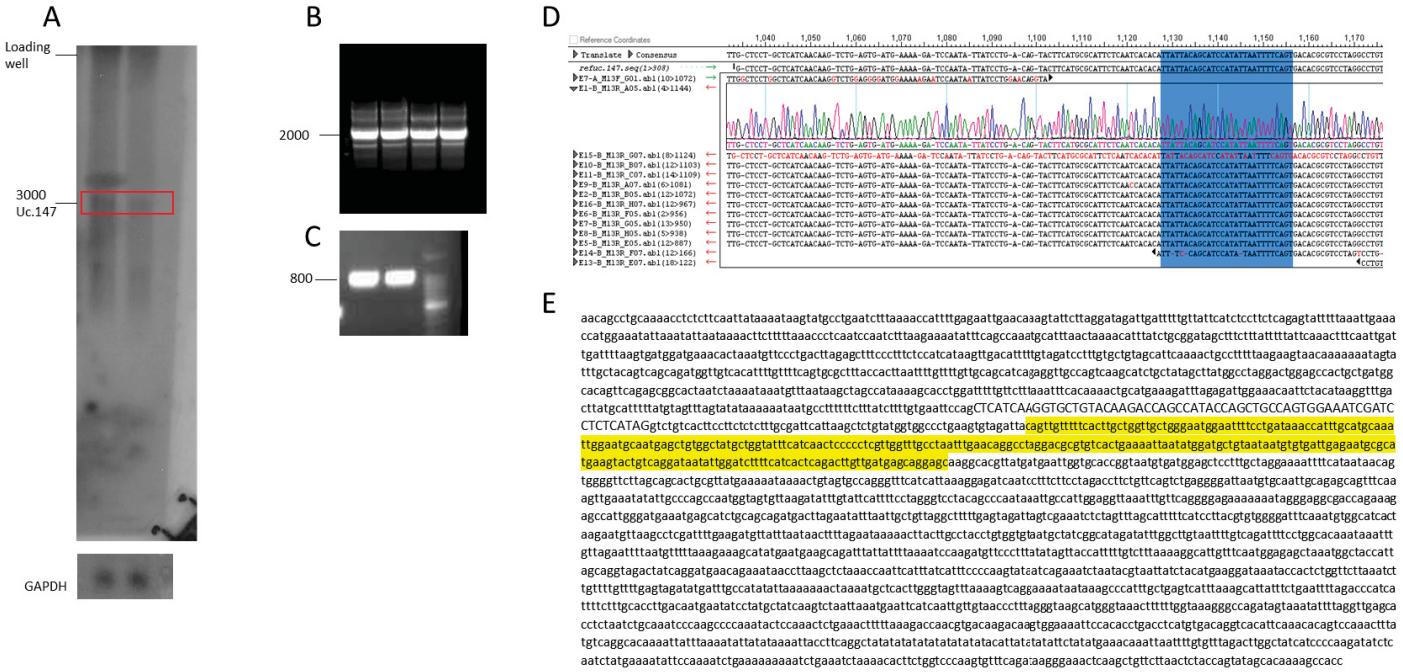


Fig S4. **Conservation among species of uc.147**.

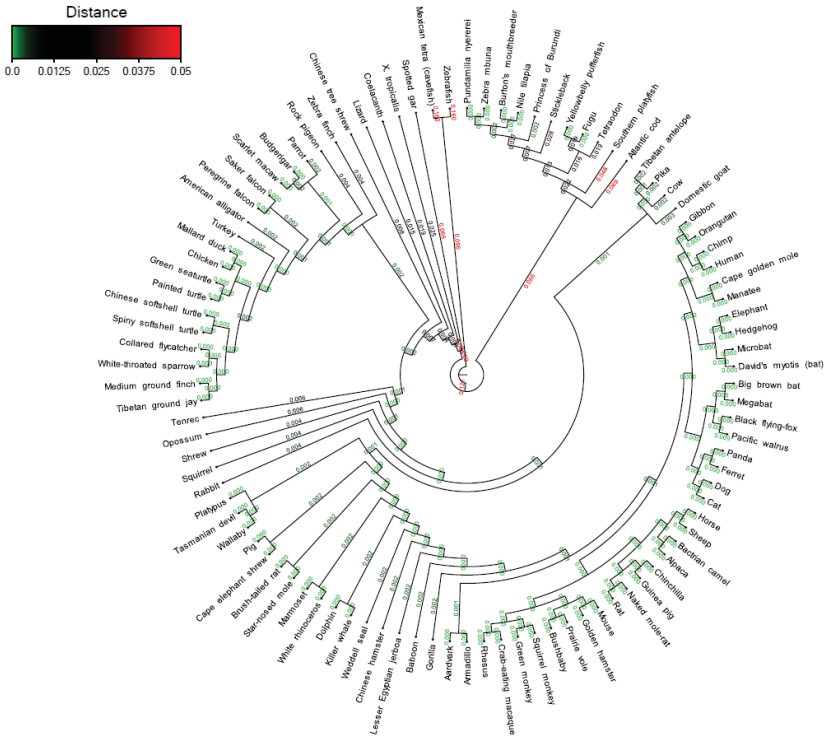


Fig S5. **Western blotting of cell line CAMA-1 after silencing of LRBA and uc.147.** The LRBA protein expression is knocked down when its gene is silencing. The expression of LRBA protein maintain after uc.147 silencing.

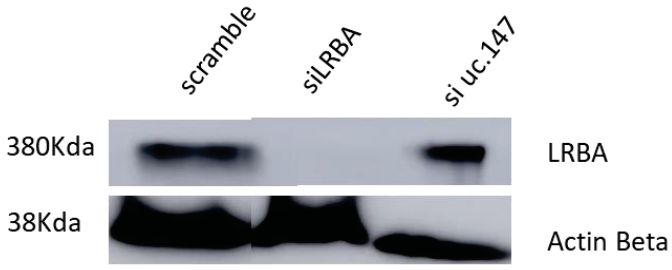
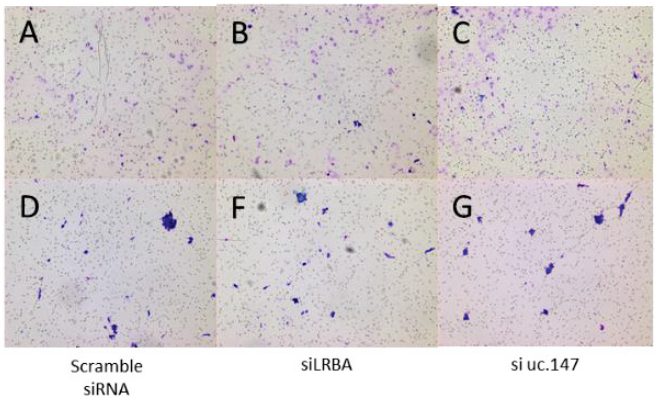
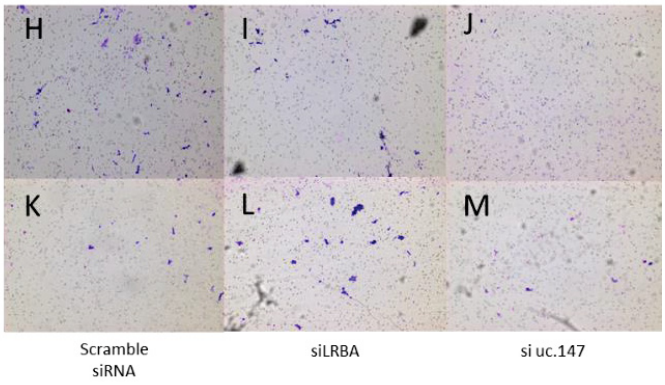


Fig S6. **Effect of knockdown of uc.147 on cells migration and invasion.** A, B and C. Migration of BT474 cells after 24hours of treatment with siRNA scramble (A), siLRBA (B) and siuc.147 (C). D, E and F. Invasion of BT474 cells after 24hours of treatment with siRNA scramble (D), siLRBA (E) and siuc.147 (F). G, H and I. Migration of CAMA-1 cells after 24hours of treatment with siRNA scramble (G), siLRBA (H) and siuc.147 (I). J, K and L. Invasion of CAMA-1 cells after 24hours of treatment with siRNA scramble (J), siLRBA (K) and siuc.147 (L).



BT474



CAMA-

Supplemental Table 1

Name	Type (MESTDAGH, 2010)	length	Position (hg16) (BEJERANO, 2004)	Postion (hg19)(MESTDAGH, 2010)	Position (hg 38) (AUTHOR, 2020)	distance of upstream gebe	Upstream gene	Host gene	Downstream gene	distance of downstream gene	Differential Expression	(p<0,05)	Association between expression and Overall Survival (p<0,05)	Association among PAM + ER;PR;HER2 and Stage or Survival
uc.2	intronic	207	chr1:10442089-10442295	chr1:10732543-10732749	chr1:10672486-10672692	34690	AK130996	FLJ20321	FLJ37118	273783	PAM50; HER;PR;Therapy	p<0.05	No	No
uc.3	intronic	225	chr1:10460711-10460935	chr1:10751165-10751389	chr1:10691108-10691332	53312	AK130996	FLJ20321	FLJ37118	255143	Stage;PAM50; HER	p<0.05	No	yes
uc.4	intronic	359	chr1:10467795-10468153	chr1:10758249-10758607	chr1:10698192-10698550	4286	FLJ20321	\N	FLJ37118	247925	PAM50;PR;ER; Therapy	p<0.05	No	No
uc.5	intronic	214	chr1:10490897-10491110	chr1:10781351-10781564	chr1:10721294-10721507	27388	FLJ20321	\N	FLJ37118	224968	PAM50; PR; ER; Therapy	p<0.05	No	No
uc.6	intronic	301	chr1:10504667-10504967	chr1:10795121-10795421	chr1:10735064-10735364	41158	FLJ20321	\N	FLJ37118	211111	PAM50;PR;ER; Therapy	p<0.05	No	No

uc.7	intronic	256	chr1:10545679-10545934	chr1:10836133-10836388	chr1:10776076-10776331	82170	FLJ20321	\N	FLJ37118	170144	PAM50;PR;ER; HER; Therapy	p<0.05	No	No
uc.8	intronic	216	chr1:10561364-10561579	chr1:10851818-10852033	chr1:10791761-10791976	97855	FLJ20321	\N	FLJ37118	154499	PAM50; PR; ER; HER; Therapy	p<0.05	No	No
uc.10	intergenic	275	chr1:10675120-10675394	chr1:10965574-10965848	chr1:10905517-10905791	211611	FLJ20321	\N	FLJ37118	40684	Therapy	p<0.05	No	No
uc.12	intronic	201	chr1:35077889-35078089	chr1:35650227-35650427	chr1:35184626-35184826	68766	FLJ23151	SFPQ	ZNF262	140539	PAM50; PR;ER;HER; Therapy	p<0.05	No	No
uc.13	exon_containing	237	chr1:35786852-35787088	chr1:36359190-36359426	chr1:35893589-35893825	36769	EIF2C4	EIF2C1	EIF2C3	36892	Stage, PAM50; HER; Therapy	p<0.05	No	No
uc.15	intergenic	233	chr1:37974370-37974602	chr1:38561076-38561308	chr1:38095404-38095636	48625	POU3F1	\N	RRAGC	742565		No	Yes	No
uc.17	intergenic	237	chr1:38215445-38215681	chr1:38802151-38802387	chr1:38336479-38336715	289700	POU3F1	\N	RRAGC	501486	Stage;	p<0.05	No	No

uc.19	intronic	256	chr1:44403606-44403861	chr1:44990312-44990567	chr1:44524640-44524895	169431	PRNPIP	\N	FLJ10597	107445	PAM50;	p<0.05	No	No
uc.21	intronic	235	chr1:48482903-48483137	chr1:49112986-49113220	chr1:48647314-48647548	175140	SPATA6	FLJ14442	FLJ11588	80321		No	Yes	No
uc.23	intergenic	229	chr1:50405695-50405923	chr1:51035777-51036005	chr1:50570105-50570333	148734	DMRTA2	FAF1	LOC51336	384224	Stage;	p<0.05	No	No
uc.24	intronic	336	chr1:50469063-50469398	chr1:51099145-51099480	chr1:50633473-50633808	212102	DMRTA2	FAF1	LOC51336	320749	Stage; HER; Therapy	p<0.05	No	No
uc.25	exon_containing	235	chr1:50535952-50536186	chr1:51166034-51166268	chr1:50700362-50700596	278991	DMRTA2	FAF1	LOC51336	253961	Stage;	p<0.05	No	No
uc.26	intergenic	212	chr1:62739563-62739774	chr1:63369646-63369857	chr1:62903975-62904186	38822	AUTL1	\N	FOXD3	419268	ER;	p<0.05	No	No
uc.27	intergenic	290	chr1:62739797-62740086	chr1:63369880-63370169	chr1:62904209-62904498	39056	AUTL1	\N	FOXD3	418956	PR;	p<0.05	No	No

uc.28	intronic	355	chr1:70066629-70066983	chr1:70696713-70697067	chr1:70231030-70231384	25453	FLJ20331	SFRS11	DKFZP566D1346	29852	HER2; Therapy	p<0.05	No	No
uc.29	intergenic	219	chr1:87244976-87245194	chr1:87822383-87822601	chr1:87356700-87356918	11120	LMO4	\N	AF118089	1371952	PAM50; ER; Therapy	p<0.05	No	No
uc.31	intergenic	253	chr1:88395168-88395420	chr1:88928018-88928270	chr1:88462335-88462587	116312	LMO4	\N	AF118089	221726	PAM50	p<0.05	No	No
uc.33	exon_containing	312	chr1:96743538-96743849	chr1:97271737-97272048	chr1:96806181-96806492	9890	BC030757	PTBP2	DPYD	271253	PAM50;HER; Therapy	p<0.05	No	No
uc.34	part_exonic	208	chr1:96751973-96752180	chr1:97280172-97280379	chr1:96814616-96814823	18325	BC030757	PTBP2	DPYD	262922	PAM50;ER; PR; Therapy	p<0.05	No	No
uc.36	intronic	264	chr1:108587040-108587303	chr1:109240428-109240691	chr1:108697806-108698069	36279	FLJ30525	FLJ10330	FLJ35838	24296	PAM50;HER; Therapy	p<0.05	No	No
uc.37	intronic/exon_containing	202	chr1:114578780-114578981	chr1:115280053-115280254	chr1:114737432-114737633	41876	AMPD1	D1S155E	FLJ21168	35142	PAM50; PR;ER;Therapy	p<0.05	No	No

uc.38	intergenic	224	chr1:161127332-161127555	chr1:163939955-163940178	chr1:163970718-163970941	614407	CDCA1	\N	PBX1	588881	Stage	p<0.05	No	No
uc.40	intronic	247	chr1:161825339-161825585	chr1:164637962-164638208	chr1:164668725-164668971	1312414	CDCA1	PBX1	LMX1A	534716	PAM50; ER; PR; Therapy	p<0.05	Yes	yes
uc.41	intergenic	216	chr1:210655265-210655480	chr1:213598767-213598982	chr1:213425424-213425639	151959	RPS6KC1	\N	PROX1	562303	ER; Therapy	p<0.05	No	No
uc.43	intronic	257	chr1:240842759-240843015	chr1:243896035-243896291	chr1:243732733-243732989	232641	SDCCAG8	AKT3	ZNF238	320290	Therapy	p<0.05	No	No
uc.44	exonic	230	chr1:241164431-241164660	chr1:244217707-244217936	chr1:244054405-244054634	211234	AKT3	ZNF238	AK124614	9695	PR;HER2; Therapy	p<0.05	No	No
uc.45	exonic	203	chr1:241963410-241963612	chr1:245016686-245016888	chr1:244853384-244853586	9728	LOC116228	HNRPU	AK095945	115772	PAM50, ER,PR,HER2,Therapy	p<0.05	No	No
uc.46	part_exonic/exon_containing	217	chr1:241964327-241964543	chr1:245017603-245017819	chr1:244854301-244854517	10645	LOC116228	HNRPU	AK095945	114841	Stage;PAM50; ER; HER2; Therapy	p<0.05	No	No

uc.47	intergenic	227	chr2:77963 90-7796616	chr2:7774651-7774877	chr2:76345 20-7634746	59042 6	RNF144	\N	AK125905	212606	ER;PR; Therapy	p<0.05	No	No
uc.49	part_exonic	207	chr2:33787 944-33788150	chr2:33813409-33813615	chr2:33588 342-33588548	23748	RASGRP3	AK074867	AK057470	137707	Stage;PAM50;ER;PR;HER2;Therapy	p<0.05	Yes	No
uc.50	intronic	222	chr2:38950 836-38951057	chr2:38976301-38976522	chr2:38749 159-38749380	14775	GALM	SFRS7	GEMIN6	28836	PAM50;HER2;Therapy	p<0.05	No	No
uc.52	intergenic	274	chr2:59082 720-59082993	chr2:59108186-59108459	chr2:58881 051-58881324	63969 5	FANCL	\N	BCL11A	156984 2	PAM50; PR	p<0.05	No	No
uc.55	intergenic	240	chr2:59721 112-59721351	chr2:59746578-59746817	chr2:59519 443-59519682	12780 87	FANCL	\N	BCL11A	931484	Stage	p<0.05	Yes	No
uc.56	intergenic	202	chr2:59922 365-59922566	chr2:59947831-59948032	chr2:59720 696-59720897	14793 40	FANCL	\N	BCL11A	730269	PR;	p<0.05	No	No
uc.58	intergenic	203	chr2:60272 497-60272699	chr2:60297963-60298165	chr2:60070 828-60071030	18294 72	FANCL	\N	BCL11A	380136	Stage	p<0.05	No	No

uc.60	intergenic	217	chr2:60416094-60416310	chr2:60441560-60441776	chr2:60214425-60214641	19730 69	FANCL	\N	BCL11A	236525	PAM50;ER;PR; Therapy	p<0.05	No	No
uc.61	part_exonic/exonic	326	chr2:60662107-60662432	chr2:60687573-60687898	chr2:60460438-60460763	22190 82	FANCL	BCL11A	PAPOLG	295706	PAM50;ER;PR; Therapy	p<0.05	No	No
uc.62	intergenic	234	chr2:60755216-60755449	chr2:60780682-60780915	chr2:60553547-60553780	23121 91	FANCL	BCL11A	PAPOLG	202689	PAM50;ER;PR; Therapy	p<0.05	No	No
uc.63	intronic	278	chr2:61727035-61727312	chr2:61752501-61752778	chr2:61525366-61525643	54511	USP34	XPO1	AK023367	310413	PAM50;ER;PR; Therapy	p<0.05	No	No
uc.66	intronic	247	chr2:73149541-73149787	chr2:73175003-73175249	chr2:72947874-72948120	13526	EMX1	SFXN5	GAF1	125259	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.70	intronic	237	chr2:144648108-144648344	chr2:144437339-144437575	chr2:143679770-143680006	63746 4	KYNU	ARHGAP15	AK126774	259532	PAM50;HER2	p<0.05	No	No
uc.71	intronic	248	chr2:144923625-144923872	chr2:144712856-144713103	chr2:143955289-143955536	12536	AK12677 4	BC01774 1	ZFHX1B	432481	PR;	p<0.05	No	No

uc.72	intronic	407	chr2:144925741-144926147	chr2:144714972-144715378	chr2:143957405-143957811	14652	AK126774	BC017741	ZFHX1B	430206	PAM50;HER2;Therapy	p<0.05	No	No
uc.74	intronic	538	chr2:145036732-145037269	chr2:144825963-144826500	chr2:144068396-144068933	125643	AK126774	BC017741	ZFHX1B	319084	PAM50;ER;Therapy	p<0.05	No	No
uc.75	exonic	236	chr2:145356619-145356854	chr2:145145850-145146085	chr2:144388283-144388518	55811	BC017741	ZFHX1B	AK098835	72593	PAM50;	p<0.05	No	No
uc.77	intronic	296	chr2:145396534-145396829	chr2:145185765-145186060	chr2:144428198-144428493	95726	BC017741	ZFHX1B	AK098835	32618	ER	p<0.05	No	No
uc.79	intronic	295	chr2:145407946-145408240	chr2:145197177-145197471	chr2:144439610-144439904	107138	BC017741	ZFHX1B	AK098835	21207	PAM50;ER	p<0.05	No	No
uc.80	intronic	294	chr2:145411621-145411914	chr2:145200852-145201145	chr2:144443285-144443578	110813	BC017741	ZFHX1B	AK098835	17533	PAM50	p<0.05	No	No
uc.81	intergenic	211	chr2:147344834-147345044	chr2:147134065-147134275	chr2:146376497-146376707	185684	ZFHX1B	\N	ACVR2	1467909	Stage	p<0.05	No	No

uc.84	intergenic	209	chr2:157397251-157397459	chr2:157194706-157194914	chr2:156338194-156338402	5522	NR4A2	\N	GPD2	97307	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.86	intergenic	340	chr2:157862655-157862994	chr2:157660110-157660449	chr2:156803598-156803937	220193	GPD2	\N	GALNT5	453890	ER	p<0.05	No	No
uc.87	intergenic	290	chr2:158102814-158103103	chr2:157900269-157900558	chr2:157043757-157044046	460352	GPD2	\N	GALNT5	213781	ER	p<0.05	No	No
uc.88	intergenic	312	chr2:162297586-162297897	chr2:162095042-162095353	chr2:161238531-161238842	2359	TANK	\N	PSMD14	69700	PAM50;PR;ER;Therapy	p<0.05	No	No
uc.89	intronic	307	chr2:162441217-162441523	chr2:162238673-162238979	chr2:161382162-161382468	145990	TANK	PSMD14	TBR1	33918	Stage	p<0.05	No	No
uc.90	exonic	206	chr2:162475571-162475776	chr2:162273027-162273232	chr2:161416516-161416721	4800	PSMD14	TBR1	AK095511	17152	PAM50	p<0.05	No	No
uc.92	intergenic	309	chr2:164653223-164653531	chr2:164450679-164450987	chr2:163594169-163594477	755438	KCNH7	\N	FIGN	13137	PAM50;ER;PR;Therapy	p<0.05	No	No

uc.93	intergenic	263	chr2:164864451-164864713	chr2:164661907-164662169	chr2:163805397-163805659	193762	FIGN	\N	GRB14	687163	Therapy	p<0.05	No	No
uc.94	intergenic	200	chr2:165046714-165046913	chr2:164844170-164844369	chr2:163987660-163987859	376025	FIGN	\N	GRB14	504963	Therapy	p<0.05	No	No
uc.95	intergenic	251	chr2:171774074-171774324	chr2:171571530-171571780	chr2:170715020-170715270	452	AK123485	\N	GAD1	101291	PAM50;ER;PR;Therapy	p<0.05	Yes	yes
uc.97	exon_containing	442	chr2:173025175-173025616	chr2:172822631-172823072	chr2:171966110-171966551	71885	SLC25A12	HAT1	DLX1	127168	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.100	intergenic	207	chr2:174317324-174317530	chr2:174114780-174114986	chr2:173250052-173250258	197164	RAPGEF4	ZAK	CDCA7	104593		No	Yes	No
uc.101	part_exonic	254	chr2:174977025-174977278	chr2:174774481-174774734	chr2:173909753-173910006	204375	M19503	SP3	AK095037	77599	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.102	part_exonic	338	chr2:175148953-175149290	chr2:174946409-174946746	chr2:174081681-174082018	91488	AK095037	PTD004	AK128215	252419	PAM50;ER	p<0.05	No	No

uc.105	intronic	223	chr2:175192331-175192553	chr2:174989787-174990009	chr2:174125059-174125281	134866	AK095037	PTD004	AK128215	209156	Stage;PAM50;ER;PR;Therapy	p<0.05	No	yes
uc.108	intergenic	374	chr2:177142901-177143274	chr2:176940357-176940730	chr2:176075629-176076002	73365	KIAA1715	\N	HOXD13	16801	Therapy	p<0.05	No	No
uc.109	intronic	224	chr2:177705882-177706105	chr2:177503338-177503561	chr2:176638610-176638833	678	BC013438	\N	hnRNPA3	573966	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.110	intergenic	243	chr2:237358132-237358374	chr2:237071382-237071624	chr2:236162738-236162980	37490	CENTG2	\N	GBX2	2843	PAM50;ER;PR;	p<0.05	No	No
uc.111	intronic	296	chr3:9446461-9446756	chr3:9471461-9471756	chr3:9429777-9430072	32708	AK128398	\N	AB051544	3920	PAM50	p<0.05	No	No
uc.113	intergenic	247	chr3:18651408-18651654	chr3:18676404-18676650	chr3:18634912-18635158	196199	SATB1	\N	KCNH8	513366	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.117	intergenic	251	chr3:70792683-70792933	chr3:70871840-70872090	chr3:70822689-70822939	192800	AK125942	\N	FOXP1	136271	Stage	p<0.05	Yes	No

uc.119	intronic	301	chr3:115754368-115754668	chr3:114433467-114433767	chr3:114714620-114714920	40423	AF116655	ZNF288	AF119886	405251	PAM50;PR;HER2	p<0.05	No	No
uc.121	intronic	293	chr3:115896375-115896667	chr3:114575474-114575766	chr3:114856627-114856919	97686	ZNF288	\N	AF119886	263252	PAM50	p<0.05	No	No
uc.122	intronic	215	chr3:115932792-115933006	chr3:114611891-114612105	chr3:114893044-114893258	134103	ZNF288	\N	AF119886	226913	Stage;HER2	p<0.05	No	No
uc.123	intergenic	492	chr3:138304453-138304944	chr3:136983544-136984035	chr3:137264702-137265193	253619	MGC34923	\N	SOX14	499543	ESTADIAMRNTO	p<0.05	No	No
uc.125	intergenic	265	chr3:138389226-138389490	chr3:137068317-137068581	chr3:137349475-137349739	338392	MGC34923	\N	SOX14	414997		No	Yes	No
uc.129	intronic/exon_containing	212	chr3:153485296-153485507	chr3:152164387-152164598	chr3:152446598-152446809	105607	AY358260	MBNL1	P2RY1	388137		No	Yes	No
uc.131	intronic	207	chr3:159310953-159311159	chr3:157990040-157990246	chr3:158272251-158272457	156935	AF257098	MGC12197	MLF1	298736	Stage; PAM50;ER;PR;Therapy	p<0.05	No	yes

uc.132	intronic	208	chr3:159347072-159347279	chr3:158026159-158026366	chr3:158308370-158308577	193054	AF257098	MGC12197	MLF1	262616	PAM50;PR; Therapy	p<0.05	No	No
uc.133	intronic	277	chr3:159347391-159347667	chr3:158026478-158026754	chr3:158308689-158308965	193373	AF257098	MGC12197	MLF1	262228	PAM50;PR	p<0.05	No	No
uc.134	intronic	211	chr3:159566817-159567027	chr3:158245904-158246114	chr3:158528115-158528325	412799	AF257098	MGC12197	MLF1	42868	ER	p<0.05	No	No
uc.135	exonic	201	chr3:170155196-170155396	chr3:168834283-168834483	chr3:169116495-169116695	1021196	GOLPH4	EVI1	ARPM1	650229	Stage;PAM50;ER;PR;Therapy	p<0.05	No	No
uc.136	intronic	347	chr3:170514865-170515211	chr3:169193952-169194298	chr3:169476164-169476510	94639	EVI1	AK096400	ARPM1	290414	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.138	intronic	419	chr3:186970209-186970627	chr3:185649296-185649714	chr3:185931508-185931926	106483	IMP-2	SFRS10	ETV5	115887	Stage;PAM50;ER;PR;HER2;Therapy	p<0.05	Yes	Yes
uc.141	exonic	295	chr4:24280045-24280339	chr4:24529162-24529456	chr4:24527539-24527833	637461	PPARGC1A	DHX15	SOD3	267763	PAM50	p<0.05	No	No

uc.142	intronic	259	chr4:41665611-41665869	chr4:41750069-41750327	chr4:41748052-41748310	48009	AL831962	PHOX2B	FLJ10525	186841	PAM50;ER;PR; Therapy	p<0.05	No	No
uc.143	intronic/exon_containing	218	chr4:77037519-77037736	chr4:76579124-76579341	chr4:75653940-75654157	23552	CDKL2	G3BP2	VDP	70440	PAM50;ER;PR;HER2;Therapy	p<0.05	Yes	No
uc.144	intronic/exon_containing	205	chr4:83805060-83805264	chr4:83346665-83346869	chr4:82425512-82425716	51837	HNRPD	HNRPDL	MASA	4893	Stage;PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.145	intergenic	248	chr4:105805133-105805380	chr4:105346313-105346560	chr4:104425156-104425403	705339	TACR3	\N	CXXC4	46784		No	Yes	No
uc.146	intergenic	214	chr4:112375296-112375509	chr4:111916476-111916689	chr4:110995320-110995533	372221	PITX2	\N	AK096400	106277	HER2; Therapy	p<0.05	No	No
uc.147	intronic	308	chr4:151814010-151814317	chr4:151236383-151236690	chr4:150315231-150315538	57799	MGC45428	LRBA	MAB21L2	266745	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.148	intronic	240	chr4:152071579-152071818	chr4:151493952-151494191	chr4:150572800-150573039	315368	MGC45428	LRBA	MAB21L2	9244	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No

uc.149	intronic	204	chr4:152071820-152072023	chr4:151494193-151494396	chr4:150573041-150573244	315609	MGC45428	LRBA	MAB21L2	9039	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.151	exon_containing	214	chr5:32425638-32425851	chr5:32380137-32380350	chr5:32380031-32380244	67061	PIP3AP	ZFR	PC4	205321	PAM50;ER;PR;Therapy	p<0.05	Yes	No
uc.153	exon_containing	240	chr5:72279759-72279998	chr5:72195686-72195925	chr5:72899859-72900098	392436	ZNF366	TNPO1	BC014311	55972	Stage;PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.154	part_exonic	203	chr5:72294088-72294290	chr5:72210015-72210217	chr5:72914188-72914390	5241	TNPO1	\N	BC014311	41680	PAM50;ER;PRHER2;Therapy	p<0.05	No	No
uc.158	intergenic	224	chr5:77224326-77224549	chr5:77140253-77140476	chr5:77844429-77844652	68082	AK128395	\N	AP3B1	157673		No	Yes	No
uc.161	intronic	278	chr5:77442602-77442879	chr5:77358529-77358806	chr5:78062705-78062982	286358	AK128395	AP3B1	SCAMP1	297666	ER	p<0.05	No	No
uc.163	intergenic	376	chr5:87252696-87253071	chr5:87168623-87168998	chr5:87872806-87873181	459925	CCNH	\N	MGC33214	322026	PR	p<0.05	No	No

uc.166	intronic	310	chr5:88045878-88046187	chr5:87961805-87962114	chr5:88665987-88666296	397139	MGC33214	\N	MEF2C	54051	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.167	intergenic	201	chr5:88263697-88263897	chr5:88179624-88179824	chr5:88883807-88884007	573	MEF2C	\N	CETN3	1509714	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.169	part_exonic	204	chr5:92995090-92995293	chr5:92921017-92921220	chr5:93585311-93585514	99030	AK096400	NR2F1	AK126015	14842	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.170	intronic	310	chr5:93301743-93302052	chr5:93227670-93227979	chr5:93891964-93892273	28138	AK022140	DKFZP564D172	AK130941	260691	HER2;	p<0.05	No	No
uc.171	intergenic	208	chr5:93649920-93650127	chr5:93575847-93576054	chr5:94240142-94240349	128607	DKFZP564D172	AK130941	AB020632	172636	PR;	p<0.05	No	No
uc.173	exonic	276	chr5:133802376-133802651	chr5:133726160-133726435	chr5:134390469-134390744	23394	CDKL3	UBE2B	MGC13017	11369	ER;PR;Therapy	p<0.05	No	No
uc.174	exonic	260	chr5:138719870-138720129	chr5:138643654-138643913	chr5:139307965-139308224	11460	AF116688	MATR3	PAIP2	33637	Stage;PAM50;ER;PR;Therapy	p<0.05	No	No

uc.176	intronic	246	chr5:167313590-167313835	chr5:167332695-167332940	chr5:167905690-167905935	303273	BC011998	\N	AB032953	212433	Therapy	p<0.05	No	No
uc.179	intronic	219	chr5:170609134-170609352	chr5:170628212-170628430	chr5:171201208-171201426	387190	GABRP	RANBP17	TLX3	107857	Stage;	p<0.05	No	No
uc.183	intronic/exon_containing	236	chr5:171365442-171365677	chr5:171384520-171384755	chr5:171957516-171957751	500343	FGF18	FBXW1B	STK10	85866	PAM50;ER;PR;Therapy	p<0.05	Yes	No
uc.184	exonic	230	chr5:173366215-173366444	chr5:173385292-173385521	chr5:173958289-173958518	341635	LOC91272	CPEB4	BC034971	30679	Stage;PAM50;ER;PR;Therapy	p<0.05	Yes	No
uc.185	exon_containing	411	chr5:178157908-178158318	chr5:178044307-178044717	chr5:178617306-178617716	26750	COL23A1	CLK4	ZNF354A	93878	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.186	exon_containing	305	chr5:179155889-179156193	chr5:179046197-179046501	chr5:179619196-179619500	9184	RUFY1	HNRPH1	AK093042	19772	Stage;PAM50;ER;	p<0.05	Yes	No
uc.187	intergenic	212	chr6:10502663-10502874	chr6:10394677-10394888	chr6:10394444-10394655	18283	OFCC1	PAK1IP1	TFAP2A	3274	PAM50;ER;Therapy	p<0.05	No	No

uc.188	exonic	215	chr6:16407363-16407577	chr6:16299384-16299598	chr6:16299153-16299367	3605	GMPR	SCA1	RNPC6	982175	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.189	intronic	573	chr6:36614372-36614944	chr6:36567517-36568089	chr6:36599740-36600312	52269	STK38	SFRS3	CDKN1A	78413	PAM50;HER2	p<0.05	No	No
uc.190	intronic	200	chr6:41570295-41570494	chr6:41523440-41523639	chr6:41555702-41555901	204814	AJ225109	FOXP4	BC009628	45050	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.193	intergenic	319	chr6:86317282-86317600	chr6:86321686-86322004	chr6:85611968-85612286	18082	SNX14	SYNCRIP	HTR1E	1325405	PAM50;ER;PR;Therapy	p<0.05	Yes	Yes
uc.194	exon_containing	201	chr6:93964662-93964862	chr6:93969064-93969264	chr6:93259346-93259546	2672287	MAP3K7	EPHA7	MANEA	2106148	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.196	intergenic	221	chr6:98162138-98162358	chr6:98116540-98116760	chr6:97668664-97668884	385487	C6orf167	\N	POU3F2	1165837	Therapy	p<0.05	Yes	No
uc.198	intergenic	307	chr6:98765487-98765793	chr6:98719889-98720195	chr6:98272013-98272319	988836	C6orf167	\N	POU3F2	562402	Therapy	p<0.05	SEM DADOS	No

uc.199	intergenic	256	chr6:98859453-98859708	chr6:98813855-98814110	chr6:98365979-98366234	108280 2	C6orf167	\N	POU3F2	468487	PAM50	p<0.05	SEM DADOS	No
uc.200	intergenic	254	chr6:99041131-99041384	chr6:98995533-98995786	chr6:98547657-98547910	126448 0	C6orf167	\N	POU3F2	286811	Stage	p<0.05	No	No
uc.202	intronic	230	chr6:101019581-101019810	chr6:100973983-100974212	chr6:100526107-100526336	62431	SIM1	HELIC1	GRIK2	872456	PAM50	p<0.05	No	No
uc.203	exon_containing/part_exonic	203	chr6:163900992-163901194	chr6:163991704-163991906	chr6:163570672-163570874	255180	PACRG	QKI	C6orf118	1701258	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.205	intergenic	252	chr7:20574105-20574356	chr7:20829789-20830040	chr7:20790170-20790421	3283	SP8	\N	SP4	637661	Stage	p<0.05	Yes	No
uc.208	intronic	218	chr7:23303942-23304159	chr7:23561670-23561887	chr7:23522051-23522268	30642	BC026177	TRA2A	AB052759	7598	Stage;PAM50;ER;PR;HER2;Therapy	p<0.05	No	yes
uc.209	intronic	250	chr7:23304160-23304409	chr7:23561888-23562137	chr7:23522269-23522518	30860	BC026177	TRA2A	AB052759	7348	Stage;PAM50;ER;PR;HER2;Therapy	p<0.05	No	yes

uc.211	intronic	291	chr7:26471744-26472034	chr7:26729472-26729762	chr7:26689853-26690143	151064	D42038	SCAP2	HOXA1	403171	PAM50	p<0.05	No	No
uc.212	part_exonic	205	chr7:26884210-26884414	chr7:27141938-27142142	chr7:27102319-27102523	6412	HOXA1	\N	HOXA3	3676	PAM50	p<0.05	No	No
uc.213	intronic/part_exonic/exon_containing	201	chr7:26925404-26925604	chr7:27183132-27183332	chr7:27143513-27143713	12779	HOXA4	HOXA5	HOXA6	1783	PAM50	p<0.05	No	No
uc.215	intronic	262	chr7:41933365-41933626	chr7:42192585-42192846	chr7:42152986-42153247	449909	INHBA	GLI3	C7orf25	756027	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.216	intronic	312	chr7:50102955-50103266	chr7:50358155-50358466	chr7:50318559-50318870	225327	ZPBP	\N	ZNFN1A1	8175	PAM50	p<0.05	No	No
uc.217	exon_containing/part_exonic	221	chr7:54378405-54378625	chr7:54636656-54636876	chr7:54568963-54569183	16432	MGC33530	\N	SEC61G	183072	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.220	intergenic	257	chr7:96245647-96245903	chr7:96633916-96634172	chr7:97004604-97004860	294712	SHFM1	\N	DLX5	15539	PAM50;ER;PR;Therapy	p<0.05	No	No

uc.221	intergenic	349	chr7:96253032-96253380	chr7:96641301-96641649	chr7:97011989-97012337	302097	SHFM1	\N	DLX5	8062	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.222	intronic	201	chr7:113611674-113611874	chr7:114057171-114057371	chr7:114417116-114417316	498119	PPP1R3A	FOXP2	HIC	504837	PAM50;PR	p<0.05	Yes	yes
uc.223	intronic	268	chr7:113612688-113612955	chr7:114058185-114058452	chr7:114418130-114418397	499133	PPP1R3A	FOXP2	HIC	503756	PAM50	p<0.05	No	No
uc.234	intergenic	272	chr7:156229240-156229511	chr7:156812385-156812656	chr7:157019691-157019962	3267	BC033117	\N	AK130204	85708	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.237	intronic	468	chr8:53187862-53188329	chr8:53137902-53138369	chr8:52225342-52225809	364104	LOC115294	ST18	AY358213	308330	ESTADIAMENNTO	p<0.05	Yes	No
uc.239	intronic	300	chr8:59992334-59992633	chr8:59942373-59942672	chr8:59029814-59030113	370177	NSMAF	AB018351	CA8	1159430	ER; Therapy	p<0.05	No	No
uc.240	intronic	206	chr8:65542500-65542705	chr8:65492539-65492744	chr8:64579982-64580187	1367194	FLJ31657	\N	BHLHB5	360	PAM50	p<0.05	No	No

uc.241	intergenic	202	chr8:65547091-65547292	chr8:65497130-65497331	chr8:64584573-64584774	2636	BHLHB5	\N	CYP7B1	11360	PAM50;ER;Therapy	p<0.05	No	No
uc.242	intergenic	265	chr8:66199258-66199522	chr8:66149297-66149561	chr8:65237062-65237326	437993	CYP7B1	\N	FLJ10511	365149	Stage	p<0.05	No	No
uc.244	intergenic	321	chr8:105918932-105919252	chr8:105962349-105962669	chr8:104950121-104950441	361220	ST7	\N	ZFPM2	368477	PAM50	p<0.05	No	No
uc.246	part_exonic	284	chr8:119079806-119080089	chr8:119123218-119123501	chr8:118110979-118111262	570718	THRAP6	EXT1	AK096777	266725	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.247	intergenic	361	chr9:959154-959514	chr9:969154-969514	chr9:969154-969514	65	DMRT1	\N	DMRT3	7872	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.248	intergenic	222	chr9:964189-964410	chr9:974189-974410	chr9:974189-974410	5100	DMRT1	\N	DMRT3	2976	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.249	intergenic	260	chr9:80857288085987	chr9:80957288085987	chr9:80957288095987	295960	AL137489	\N	PTPRD	221280	Stage;ER;Therapy	p<0.05	Yes	yes
uc.250	intronic	209	chr9:13929910-13930118	chr9:13939910-13940118	chr9:13939911-13940119	689552	MPDZ	\N	NFIB	147254	PAM50;ER;PR;Therapy	p<0.05	No	No

uc.251	intronic	213	chr9:15864309-15864521	chr9:15874309-15874521	chr9:15874311-15874523	363319	PSIP1	C9orf93	AK092247	544044	PR;	p<0.05	Yes	No
uc.254	intergenic	279	chr9:23486725-23487003	chr9:23496725-23497003	chr9:23496727-23497005	1044252	AK096011	\N	ELAVL2	193101	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.256	exonic	206	chr9:23682234-23682439	chr9:23692234-23692439	chr9:23692236-23692441	1239761	AK096011	ELAVL2	BC046919	1984528	PAM50	p<0.05	No	No
uc.259	intergenic	308	chr9:75084998-75085305	chr9:79627879-79628186	chr9:77012963-77013270	106875	C9orf65	\N	VPS13A	164174	Therapy	p<0.05	No	No
uc.260	intergenic	231	chr9:76929543-76929773	chr9:81472424-81472654	chr9:78857508-78857738	527416	PSAT1	\N	TLE4	714875	Therapy	p<0.05	SEM DADOS	No
uc.261	intergenic	311	chr9:77328693-77329003	chr9:81871574-81871884	chr9:79256659-79256969	926566	PSAT1	\N	TLE4	315645	Stage	p<0.05	Yes	No
uc.262	intergenic	255	chr9:79184841-79185095	chr9:83727722-83727976	chr9:81112807-81113061	1386067	TLE4	\N	TLE1	470622		No	Yes	No

uc.263	exon_containing	207	chr9:82047403-82047609	chr9:86590284-86590490	chr9:83975369-83975575	18382	C9orf64	HNRPK	C9orf76	5206	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.264	intronic	267	chr9:82047611-82047877	chr9:86590492-86590758	chr9:83975577-83975843	18590	C9orf64	HNRPK	C9orf76	4938	Stage;PAM50;ER;PR;HER2;TERPIA	p<0.05	No	yes
uc.265	exon_containing	217	chr9:103498309-103498525	chr9:108118471-108118687	chr9:105356190-105356406	427952	ABCA1	CDW92	CSDUFD1	91764	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.266	intronic	243	chr9:104758130-104758372	chr9:109378292-109378534	chr9:106616011-106616253	555366	AK130032	\N	AK023447	242502	ER;PR;Therapy	p<0.05	No	No
uc.267	part_exonic	203	chr9:120429935-120430137	chr9:125053890-125054092	chr9:122291611-122291813	26799	RBM18	MRRF	PTGS1	79219	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.268	intergenic	251	chr9:120982873-120983123	chr9:125606828-125607078	chr9:122844549-122844799	16017	PDCL	\N	MNAB	4653	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.270	intronic	278	chr9:123680114-123680391	chr9:128304069-128304346	chr9:125541790-125542067	176786	AB040954	MAPKAP1	LOC51145	202309	PAM50;ER;PR;Therapy	p<0.05	No	No

uc.271	intronic	211	chr9:123680397-123680607	chr9:128304352-128304562	chr9:125542073-125542283	177069	AB040954	MAPKAP1	LOC51145	202093	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.272	intronic	213	chr9:123808633-123808845	chr9:128432588-128432800	chr9:125670309-125670521	305305	AB040954	MAPKAP1	LOC51145	73855	Stage	p<0.05	No	No
uc.273	intronic	321	chr9:123893643-123893963	chr9:128517598-128517918	chr9:125755319-125755639	8766	LOC51145	PBX3	C9orf28	571209	PAM50	p<0.05	No	No
uc.274	intronic	327	chr9:123897916-123898242	chr9:128521871-128522197	chr9:125759592-125759918	13039	LOC51145	PBX3	C9orf28	566930	PAM50;PR;	p<0.05	No	No
uc.276	intronic	432	chr9:123981857-123982288	chr9:128605812-128606243	chr9:125843533-125843964	96980	LOC51145	PBX3	C9orf28	482884	PAM50;HER2	p<0.05	No	No
uc.277	intronic	276	chr9:123983755-123984030	chr9:128607710-128607985	chr9:125845431-125845706	98878	LOC51145	PBX3	C9orf28	481142	Stage	p<0.05	Yes*	yes
uc.278	intronic	237	chr9:124022210-124022446	chr9:128646165-128646401	chr9:125883886-125884122	137333	LOC51145	PBX3	C9orf28	442726	HER2;Therapy	p<0.05	No	No

uc.279	intronic	336	chr9:124048604-124048939	chr9:128672559-128672894	chr9:125910280-125910615	163727	LOC511455	PBX3	C9orf28	416233	HER2;Therapy	p<0.05	No	No
uc.280	part_exonic	220	chr9:124054051-124054270	chr9:128678006-128678225	chr9:125915727-125915946	169174	LOC511455	PBX3	C9orf28	410902	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.281	intronic	238	chr9:130771570-130771807	chr9:135495525-135495762	chr9:132620138-132620375	29884	BARHL1	DDX31	GTF3C4	49965	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.282	intronic	207	chr9:135399783-135399989	chr9:140042490-140042696	chr9:137148038-137148244	30899	AK128156	GRIN1	ANAPC2	26539	ER;PR;	p<0.05	Yes	yes
uc.283	intergenic	277	chr10:49949360-49949636	chr10:50604757-50605033	chr10:49396711-49396987	69224	AJ237663	\N	ERCC6	61675		No	Yes	No
uc.285	part_exonic	232	chr10:69860594-69860825	chr10:70515991-70516222	chr10:68756234-68756465	61752	CXXC6	CCAR1	C10orf24	71376	PAM50;HER2	p<0.05	No	No
uc.292	exonic	217	chr10:98380042-98380258	chr10:98715455-98715671	chr10:96955698-96955914	14188	AF338191	MLR2	C10orf12	25369	PAM50;ER;PR;Therapy	p<0.05	No	No

uc.296	intergenic	461	chr10:102079693-102080153	chr10:102415106-102415566	chr10:100655349-100655809	105343	HIF1AN	\N	PAX2	89901	ER;	p<0.05	No	No
uc.297	intergenic	364	chr10:102083807-102084170	chr10:102419220-102419583	chr10:100659463-100659826	109457	HIF1AN	\N	PAX2	85884	Stage	p<0.05	No	No
uc.298	intergenic	359	chr10:102112245-102112603	chr10:102447658-102448016	chr10:100687901-100688259	137895	HIF1AN	\N	PAX2	57451	Stage	p<0.05	No	No
uc.299	part_exonic	210	chr10:102174022-102174231	chr10:102509435-102509644	chr10:100749678-100749887	199672	HIF1AN	PAX2	C10orf6	162681	PAM50;ER;	p<0.05	No	No
uc.300	intronic	208	chr10:102211705-102211912	chr10:102547118-102547325	chr10:100787361-100787568	237355	HIF1AN	PAX2	C10orf6	125000	Stage	p<0.05	No	No
uc.304	intergenic	272	chr10:102747091-102747362	chr10:103082504-103082775	chr10:101322747-101323018	93786	LBX1	\N	BTRC	31125	Stage; PAM50;ER;PR;HER2;Therapy	p<0.05	No	yes
uc.305	intronic	305	chr10:102876022-102876326	chr10:103211435-103211739	chr10:101451678-101451982	222717	LBX1	BTRC	AF090931	62482	ER;PR	p<0.05	No	No

uc.306	intronic	224	chr10:102876626-102876849	chr10:103212039-103212262	chr10:101452282-101452505	223321	LBX1	BTRC	AF090931	61959	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.307	intronic	232	chr10:102908570-102908801	chr10:103243983-103244214	chr10:101484226-101484457	255265	LBX1	BTRC	AF090931	30007	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.308	intronic	277	chr10:102910399-102910675	chr10:103245812-103246088	chr10:101486055-101486331	257094	LBX1	BTRC	AF090931	28133	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.309	intronic	268	chr10:102931618-102931885	chr10:103267031-103267298	chr10:101507274-101507541	278313	LBX1	BTRC	AF090931	6923	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.310	intronic	229	chr10:114068810-114069038	chr10:114404223-114404451	chr10:112644464-112644692	197550	ZDHHC6	VTI1A	TCF7L2	305759	Stage	p<0.05	Yes	No
uc.311	intronic	219	chr10:119738989-119739207	chr10:120074402-120074620	chr10:118314890-118315108	215033	CASC2	C10orf84	GPR10	279023	Stage;PAM50;ER;PR;HER2;Therapy	p<0.05	No	yes
uc.312	intronic	322	chr10:119741124-119741445	chr10:120076537-120076858	chr10:118317025-118317346	217168	CASC2	C10orf84	GPR10	276785	Stage;PAM50;ER;PR;Therapy	p<0.05	No	yes

uc.313	intronic	231	chr10:121004761-121004991	chr10:121340174-121340404	chr10:119580662-119580892	38006	RGS10	TIAL1	BAG3	70499	HER2	p<0.05	No	No
uc.322	intronic	223	chr11:16280660-16280882	chr11:16316351-16316573	chr11:16294805-16295027	12124 62	CALCB	SOX6	AL136602	129166	PAM50;PR	p<0.05	No	No
uc.323	intronic	200	chr11:16439645-16439844	chr11:16475336-16475535	chr11:16453789-16453988	27533	AL136602	SOX6	SMAP	284658	PAM50;ER;PR	p<0.05	No	No
uc.324	exon_containing	225	chr11:30521830-30522054	chr11:30557521-30557745	chr11:30535974-30536198	19835 5	FLJ38968	C11orf8	FLJ46154	294179	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.325	intronic	235	chr11:31649953-31650187	chr11:31685644-31685878	chr11:31664096-31664330	15447 4	FLJ25059	ELP4	AK130563	19757	PAM50	p<0.05	No	No
uc.328	intronic	231	chr11:31789972-31790202	chr11:31825663-31825893	chr11:31804115-31804345	20335	ELP4	PAX6	RCN1	286797	Stage;PAM50;ER;PR;Therapy	p<0.05	No	yes
uc.330	part_exonic	207	chr11:66169256-66169462	chr11:66393896-66394102	chr11:66626425-66626631	20406	CCS	RBM14	MGC15912	1298	PAM50	p<0.05	No	No

uc.331	exon_containing	218	chr11:82921467-82921684	chr11:83195159-83195376	chr11:83484116-83484333	25952	FLJ37266	DLG2	HT007	2144264	PAM50;ER;Therapy	p<0.05	No	No
uc.333	part_exonic	270	chr11:124182299-124182568	chr11:124644647-124644916	chr11:124774751-124775020	12460	ESAM	FLJ23342	AY358681	14223	Stage;PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.335	intronic	214	chr12:16606681-16606894	chr12:16715414-16715627	chr12:16562480-16562693	198071	MGST1	DAT1	FLJ22655	151817 6	PAM50;PR	p<0.05	No	No
uc.336	intronic	251	chr12:24183273-24183523	chr12:24292006-24292256	chr12:24139072-24139322	118534 2	AK122798	SOX5	FLJ32894	427641	PAM50;ER;Therapy	p<0.05	No	No
uc.338	intronic/exon_containing	223	chr12:52144756-52144978	chr12:53858489-53858711	chr12:53464705-53464927	18067	DKFZP564J157	PCBP2	MAP3K12	15567	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.339	intergenic	252	chr12:52357363-52357614	chr12:54071096-54071347	chr12:53677312-53677563	4664	ATP5G2	\N	KIAA1536	33555	PAM50;ER;PR	p<0.05	No	No
uc.341	exon_containing	314	chr12:52669185-52669498	chr12:54382918-54383231	chr12:53989134-53989447	12716	HOXC11	HOXC10	HOXC9	10671	HER2; Therapy	p<0.05	No	No

uc.342	part_exonic	227	chr12:52696761-52696987	chr12:54410494-54410720	chr12:54016710-54016936	4370	HOXC8	HOXC4	SMUG1	163062	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.343	intronic/part_exonic/exon_containing	388	chr12:52708708-52709095	chr12:54422441-54422828	chr12:54028657-54029044	16317	HOXC8	HOXC4	SMUG1	150954	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.344	intronic/part_exonic	254	chr12:52713153-52713406	chr12:54426886-54427139	chr12:54033102-54033355	20762	HOXC8	HOXC4	SMUG1	146643	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.345	exon_containing/part_exonic	301	chr12:52733867-52734167	chr12:54447600-54447900	chr12:54053816-54054116	41476	HOXC8	HOXC4	SMUG1	125882	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.346	intergenic	202	chr12:105478977-105479178	chr12:106976510-106976711	chr12:106582732-106582933	72637	RPC2	\N	RFX4	320	PAM50; ER; PR; Therapy	p<0.05	Yes	yes
uc.348	intronic	240	chr13:69861358-69861597	chr13:72063357-72063596	chr13:71489225-71489464	1380765	KLHL1	DACH	FLJ22624	1238479	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.349	intronic	203	chr13:69919303-69919505	chr13:72121302-72121504	chr13:71547170-71547372	1438710	KLHL1	DACH	FLJ22624	1180571	PAM50; ER; PR; Therapy	p<0.05	No	No

uc.350	intronic	240	chr13:70054101-70054340	chr13:72256100-72256339	chr13:71681968-71682207	1573508	KLHL1	DACH	FLJ22624	104573 6	PAM5020; ER; PR; Therapy	p<0.05	No	No
uc.353	exon_containing/part_exonic	323	chr13:70569554-70569876	chr13:72771553-72771875	chr13:72197415-72197737	33022	DACH	\N	FLJ22624	530200		No	Yes	No
uc.354	intergenic	235	chr13:76774830-76775064	chr13:78976829-78977063	chr13:78402694-78402928	427164	EDNRB	\N	POU4F1	196168	PAM50	p<0.05	No	No
uc.356	intronic	251	chr13:95706821-95707071	chr13:98008820-98009070	chr13:97356566-97356816	368789	GPR80	MBNL2	MBNL2	665	PAM50	p<0.05	No	No
uc.357	intergenic	242	chr13:110664338-110664579	chr13:112716337-112716578	chr13:112062023-112062264	96974 2	MGC35169	\N	SOX1	5334	ER; PR; Therapy	p<0.05	No	No
uc.358	intergenic	226	chr14:24368162-24368387	chr14:26378034-26378259	chr14:25908828-25909053	858929	STXBP6	\N	NOVA1	536830	PAM50; ER; PR; HER2; Therapy	p<0.05	No	No
uc.359	part_exonic	324	chr14:24905096-24905419	chr14:26914968-26915291	chr14:26445762-26446085	1395863	STXBP6	NOVA1	FOXG1B	2319669	Stage; PAM50; ER; PR; HER2; Therapy	p<0.05	No	No

uc.360	exonic	287	chr14:24905510-24905796	chr14:26915382-26915668	chr14:26446176-26446462	139627	STXBP6	NOVA1	FOXG1B	231929	PAM50; ER; PR; HER2; Therapy	p<0.05	No	No
uc.361	intergenic	267	chr14:27223174-27223440	chr14:29233135-29233401	chr14:28763929-28764195	216652	NOVA1	\N	FOXG1B	1648	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.362	intergenic	239	chr14:27338791-27339029	chr14:29348752-29348990	chr14:28879546-28879784	86376	BX248251	\N	PRKCM	696696	Stage	p<0.05	No	No
uc.363	intergenic	265	chr14:27851358-27851622	chr14:29861319-29861583	chr14:29392113-29392377	598943	BX248251	\N	PRKCM	184103	Therapy	p<0.05	No	No
uc.365	intergenic	278	chr14:28732401-28732678	chr14:30742362-30742639	chr14:30273156-30273433	345462	PRKCM	\N	KIAA1333	285724	Stage	p<0.05	No	No
uc.366	exon_containing	202	chr14:29372746-29372947	chr14:31382707-31382908	chr14:30913501-30913702	18447	COCH	STRN3	AP4S1	111948	Stage; PAM50; ER; PR; Therapy	p<0.05	Yes	No
uc.368	intronic	228	chr14:32058615-32058842	chr14:34068576-34068803	chr14:33599370-33599597	766308	AKAP6	NPAS3	EGLN3	324618	Stage	p<0.05	No	No

uc.370	intronic	393	chr14:32192610-32193002	chr14:34202571-34202963	chr14:33733365-33733757	900303	AKAP6	NPAS3	EGLN3	190458		No	Yes	No
uc.371	intronic	296	chr14:34010228-34010523	chr14:36020189-36020484	chr14:35550983-35551278	13928	INSM2	BC042045	AB020691	124950	PAM50; ER; Therapy	p<0.05	No	No
uc.372	intronic	277	chr14:34033074-34033350	chr14:36043035-36043311	chr14:35573829-35574105	36774	INSM2	BC042045	AB020691	102123	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.374	intronic	224	chr14:35705952-35706175	chr14:37715913-37716136	chr14:37246708-37246931	74047	SLC25A21	MIPOL1	FOXA1	343056	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.375	exon_containing	300	chr14:35767259-35767558	chr14:37777220-37777519	chr14:37308015-37308314	135354	SLC25A21	MIPOL1	FOXA1	281673	PAM50; ER; PR; HER2; Therapy	p<0.05	No	No
uc.376	intronic	290	chr14:43555788-43556077	chr14:45565750-45566039	chr14:45096547-45096836	22116	KIAA0423	PRPF39	FKBP3	19069	PAM50; ER; PR; HER2; Therapy	p<0.05	No	No
uc.377	intronic	217	chr14:43569061-43569277	chr14:45579023-45579239	chr14:45109820-45110036	35389	KIAA0423	PRPF39	FKBP3	5869	PAM50; ER; PR; Therapy	p<0.05	No	No

uc.378	intronic	251	chr14:78317518-78317768	chr14:80327477-80327727	chr14:79861134-79861384	107249	BX248745	NRXN3	DIO2	336148	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.379	intergenic	252	chr14:95421409-95421660	chr14:97431368-97431619	chr14:96965031-96965282	83427	VRK1	\N	BX161483	667402	PAM50	p<0.05	No	No
uc.381	intergenic	238	chr14:95869331-95869568	chr14:97879290-97879527	chr14:97412953-97413190	531349	VRK1	\N	BX161483	219494	Stage	p<0.05	No	No
uc.382	intergenic	200	chr15:33634968-33635167	chr15:35918912-35919111	chr15:35626711-35626910	80547	MGC14798	\N	HH114	967983	HER2	p<0.05	No	No
uc.385	intronic	209	chr15:34901726-34901934	chr15:37185670-37185878	chr15:36893469-36893677	75009	BC028192	MEIS2	MGC35118	1040948	PAM50	p<0.05	No	No
uc.386	intergenic	203	chr15:35238065-35238267	chr15:37522009-37522211	chr15:37229808-37230010	128504	MEIS2	\N	MGC35118	704615	Stage	p<0.05	Yes	No
uc.387	part_exonic	238	chr15:39748110-39748347	chr15:42032054-42032291	chr15:41739856-41740093	10600	BC038449	AL713737	MGA	8728	Stage	p<0.05	Yes	No

uc.390	exon_containing	205	chr15:65593988-65594192	chr15:67878170-67878374	chr15:67585832-67586036	84028	FLJ12476	MAP2K5	AF130086	20215	PAM50; ER; PR; HER2; Therapy	p<0.05	No	No
uc.391	exon_containing	311	chr15:65756122-65756432	chr15:68040304-68040614	chr15:67747966-67748276	139957	AF130086	MAP2K5	PIAS1	305957	PAM50; ER; PR; HER2; Therapy	p<0.05	No	No
uc.393	part_exonic	275	chr15:72630059-72630333	chr15:74914242-74914516	chr15:74621901-74622175	23771	ARID3B	CLK3	FLJ21128	8383	PAM50	p<0.05	No	No
uc.395	exon_containing	249	chr16:24545554-24545802	chr16:24579003-24579251	chr16:24567682-24567930	205275	CACNG3	RBBP6	AK127191	93491	PAM50; ER; PR; Therapy	p<0.05	Yes	No
uc.397	intronic	311	chr16:49513876-49514186	chr16:49735848-49736158	chr16:49701937-49702247	302538	MGC33367	OAZ	FLJ38101	322989	HER2	p<0.05	No	No
uc.398	intergenic	322	chr16:49668919-49669240	chr16:49890891-49891212	chr16:49856980-49857301	126112	OAZ	\N	FLJ38101	167935	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.401	intronic	250	chr16:52273300-52273549	chr16:52494715-52494964	chr16:52460803-52461052	14626	TNRC9	\N	AK092208	638124	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No

uc.403	intergenic	206	chr16:54102457-54102662	chr16:54323855-54324060	chr16:54289943-54290148	3509	IRX3	\N	IRX5	641050	PAM50	p<0.05	No	No
uc.404	intergenic	235	chr16:55001959-55002193	chr16:55223357-55223591	chr16:55189445-55189679	254963	IRX5	\N	IRX6	134080	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.406	intronic/exon_containing	211	chr16:69456552-69456762	chr16:69680364-69680574	chr16:69646461-69646671	183762	CYB5-M	NFAT5	NQO1	62734	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.407	exonic	326	chr16:69457343-69457668	chr16:69681155-69681480	chr16:69647252-69647577	184553	CYB5-M	NFAT5	NQO1	61828	PAM50	p<0.05	No	No
uc.408	part_exonic	252	chr16:72597321-72597572	chr16:72821045-72821296	chr16:72787146-72787397	614984	PMFBP1	ATBF1	AK129695	339240	Stage;PAM50;ER;Therapy	p<0.05	Yes	No
uc.409	intergenic	244	chr16:72869147-72869390	chr16:73093871-73093114	chr16:73058972-73059215	886810	PMFBP1	ATBF1	AK129695	67422	PAM50;ER;PR	p<0.05	No	No
uc.411	intronic	229	chr17:35525169-35525397	chr17:35329619-35329847	chr17:36972320-36972548	29124	LHX1	AATF	ACACA	112084	PAM50;ER;	p<0.05	No	No

uc.412	intronic	268	chr17:35532036-35532303	chr17:35336486-35336753	chr17:36979187-36979454	35991	LHX1	AATF	ACACA	105178	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.413	exonic	272	chr17:37941482-37941753	chr17:37566519-37566790	chr17:39410266-39410537	8642	MGC15482	PPARBP	CRK7	51223	Stage;PAM50;ER;HER2;Therapy	p<0.05	Yes	No
uc.414	part_exonic	246	chr17:38624137-38624382	chr17:38249174-38249419	chr17:40092921-40093166	39047	THRAP4	THRA	CASC3	47207	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.415	intergenic	207	chr17:47138544-47138750	chr17:46663906-46664112	chr17:48586544-48586750	8162	HOXB4	\N	HOXB5	4507	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.416	part_exonic/exon_containing	286	chr17:47145525-47145810	chr17:46670887-46671172	chr17:48593525-48593810	15143	HOXB4	HOXB5	HOXB6	1994	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.417	part_exonic	222	chr17:47156950-47157171	chr17:46682312-46682533	chr17:48604950-48605171	6230	HOXB6	BC010732	HOXB7	2063	PAM50;ER;HER2;Therapy	p<0.05	No	No
uc.418	exonic	217	chr17:56556866-56557082	chr17:56082228-56082444	chr17:58004867-58005083	10016	AK126318	SFRS1	Dlc2	78354	PAM50;HER2;Therapy	p<0.05	No	No

uc.419	part_exonic/exon_containing/exonic	289	chr17:56557353-56557641	chr17:56082715-56083003	chr17:58005354-58005642	10503	AK126318	SFRS1	Dlc2	77795	PAM50;ER;HER2;Therapy	p<0.05	Yes	No
uc.420	part_exonic	233	chr17:63046872-63047104	chr17:62496771-62497003	chr17:64500653-64500885	3628	POLG2	DDX5	LOC90799	5876	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.421	intronic	345	chr18:20945142-20945486	chr18:22693155-22693499	chr18:25113191-25113535	633234	HRH4	EHZF	SS18	903078	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.422	intronic	226	chr18:21000175-21000400	chr18:22748188-22748413	chr18:25168224-25168449	68826 7	HRH4	EHZF	SS18	848164	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.423	intronic	223	chr18:21008342-21008564	chr18:22756355-22756577	chr18:25176391-25176613	696434	HRH4	EHZF	SS18	840000	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.424	intronic	215	chr18:21019766-21019980	chr18:22767779-22767993	chr18:25187815-25188029	707858	HRH4	EHZF	SS18	828584	PAM50;ER;PR	p<0.05	No	No
uc.425	intronic	325	chr18:21117194-21117518	chr18:22865207-22865531	chr18:25285243-25285567	805286	HRH4	EHZF	SS18	731046	PAM50;ER;Therapy	p<0.05	No	No

uc.426	intronic	262	chr18:22167579-22167840	chr18:23915592-23915853	chr18:26335628-26335889	142271	MGC26605	TAF4B	KCTD1	119023	Therapy	p<0.05	No	No
uc.427	intergenic	215	chr18:22489186-22489400	chr18:24237199-24237413	chr18:26657235-26657449	16890	KCTD1	\N	AQP4	198009	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.428	intergenic	239	chr18:28605196-28605434	chr18:30353209-30353447	chr18:32773246-32773484	234	AB037805	\N	LOC374864	163922	PAM50	p<0.05	No	No
uc.435	intronic	227	chr18:51238918-51239144	chr18:53089931-53090157	chr18:55422700-55422926	463293	SE57-1	TCF4	AK127787	353800	PAM50;ER;PR	p<0.05	No	No
uc.436	exon_containing	210	chr18:51403228-51403437	chr18:53254241-53254450	chr18:55587010-55587219	627603	SE57-1	TCF4	AK127787	189507	PAM50;ER;PR;Therapy	p<0.05	No	No
uc.439	intronic	263	chr18:70490008-70490270	chr18:72363031-72363293	chr18:74651075-74651337	89168	CN1	ZNF407	AK096231	452249	PAM50	p<0.05	No	No
uc.443	exon_containing	239	chr19:8433269-8433507	chr19:8527269-8527507	chr19:8462385-8462623	23371	LOC51257	HNRPM	PRAM-1	27433	PAM50	p<0.05	No	No

uc.446	intergenic	272	chr19:35439694-35439965	chr19:30747854-30748125	chr19:30256947-30257218	227030	AF116658	\N	AB002388	115202	Stage	p<0.05		No
uc.447	intergenic	273	chr19:35459621-35459893	chr19:30767781-30768053	chr19:30276874-30277146	246957	AF116658	\N	AB002388	95274	Stage	p<0.05	Yes	No
uc.450	intergenic	211	chr19:36279466-36279676	chr19:31587626-31587836	chr19:31096720-31096930	538660	AB002388	\N	AL137518	52524	HER2;Therapy	p<0.05	No	No
uc.451	intronic	225	chr19:36498460-36498684	chr19:31806620-31806844	chr19:31315714-31315938	161862	AL137518	ZNF537	BX640881	1029708	ER	p<0.05	No	No
uc.452	intronic	204	chr19:36519787-36519990	chr19:31827947-31828150	chr19:31337041-31337244	183189	AL137518	ZNF537	BX640881	1008402	Stage; PAM50	p<0.05	No	No
uc.453	intergenic	325	chr19:47129157-47129481	chr19:42437317-42437641	chr19:41933165-41933489	25713	ARHGEF1	\N	RABAC1	23197	ER	p<0.05	No	No
uc.455	intronic	245	chr20:35043808-35044052	chr20:34328379-34328623	chr20:35740457-35740701	39477	C20orf52	RNPC2	C20orf53	12079	PAM50; ER; PR; HER2; Therapy	p<0.05	Yes	yes

uc.456	intronic	320	chr20:42773185-42773504	chr20:42087756-42088075	chr20:43459116-43459435	269198	PTPRT	SFRS6	L3MBTL	55085	PAM50; ER; PR; Therapy	p<0.05	No	No
uc.457	exon_containing	211	chr22:17770463-17770673	chr22:19395909-19396119	chr22:19408386-19408596	116711	CLTCL1	HIRA	MRPL40	23916	PAM50; ER; PR	p<0.05	Yes	No
uc.458	intronic	204	chr22:34420305-34420508	chr22:36148492-36148695	chr22:35752445-35752648	22961	APOL5	RBM9	AK130887	191277	PAM50	p<0.05	No	No
uc.459	exon_containing	255	chrX:20895986-20896240	chrX:21534560-21534814	chrX:21516442-21516696	124980 9	RPS6KA3	CNK2	FLJ34960	138794	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.460	intronic	275	chrX:24184937-24185211	chrX:24823511-24823785	chrX:24805394-24805668	132719	PCYT1B	POLA	ARX	199004	HER2	p<0.05	No	No
uc.461	intronic	397	chrX:24226223-24226619	chrX:24864797-24865193	chrX:24846680-24847076	174005	PCYT1B	POLA	ARX	157596	PAM50;ER;PR	p<0.05	No	No
uc.471	exonic	239	chrX:40239309-40239547	chrX:41208371-41208609	chrX:41349118-41349356	12379	AK127902	DDX3X	NYX	98077	PR	p<0.05	Yes	No

uc.472	part_exonic	202	chrX:40410244-40410445	chrX:41379306-41379507	chrX:41520053-41520254	44342	NYX	CASK	S79449	155985	PAM50;ER;PR	p<0.05	No	No
uc.473	exon_containing	222	chrX:69240015-69240236	chrX:70373224-70373445	chrX:71153374-71153595	11076	TNRC11	NLGN3	GJB1	69630	PAM50;ER	p<0.05	No	No
uc.474	exon_containing	210	chrX:69335634-69335843	chrX:70468843-70469052	chrX:71248993-71249202	23798	GJB1	ZNF261	NONO	34500	Stage;PAM50;ER;PR;Therapy	p<0.05	Yes	No
uc.475	intronic	397	chrX:69632846-69633242	chrX:70766055-70766451	chrX:71546205-71546601	13830	TAF1	OGT	ACRC	31809	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.477	part_exonic	209	chrX:101813348-101813556	chrX:103041491-103041699	chrX:103786562-103786770	72535	MGC39655	PLP1	MGC39900	175505	PAM50;ER;PR;HER2;Therapy	p<0.05	No	No
uc.478	intronic/exon_containing	252	chrX:121297212-121297463	chrX:122599457-122599708	chrX:123465606-123465857	2415524	GLUD2	GRIA3	THOC2	145063		No	Yes	No
uc.480	exonic	202	chrX:121933027-121933228	chrX:123235272-123235473	chrX:124101422-124101623	193364	BIRC4	STAG2	SH2D1A	244720	PAM50;HER2;Therapy	p<0.05	No	No

uc.481	exonic	204	chrX:121933230-121933433	chrX:123235475-123235678	chrX:124101625-124101828	193567	BIRC4	STAG2	SH2D1A	244515	PAM50;HER2;Therapy	p<0.05	No	No

Supplemental Table 2

Name	Forward	Reverse	for what?	BRAND
uc.84	TAATTGCCAATTGAACAGCAA	TGATTAGCTCCCTCCCCAGT	qPCR	IDT
uc.138	CAAGTGGGACTTCTGGTCTGA	GGAAAATGCGGAAGTCGT	qPCR	IDT
uc.147	CGTCCTAGGCCTGTTCAAAT	TGGGAATGGAATTTTCCTGA	qPCR	IDT
uc.193	CTGAAGAAGCAAGGCTGCT	TTGCCTTTAGTTTCAGAGGCT	qPCR	IDT
uc.193 .2	GCCTTTAGTTTCAGAGGCTTG	AAGCAAGGCTGCTTTTTCAG	qPCR	IDT
uc.268	GTGCATGCAAATACATGTCAA	GCTTTGGCTGTTCACAAAATA	qPCR	IDT
uc.271	CAATTGCTCTCCTCTGCCAT	AGGACACGGATGAGCTGTCT	qPCR	IDT
uc.311	TTCAGGAACCAAGGGTGTAAA	TCCAACCAGCTAAAGCCCT	qPCR	IDT
uc.376	CTCTAATGGGTCTGTGCCCT	CCACGTGCAGAGTCTTGATC	qPCR	IDT
uc.378	ACCTTGCTTGTCGGACCA	ACCTTGCTTGTCGGACCA	qPCR	IDT
uc.427	CACATGCGTAAAAGCGAGTT	AAGATCTGACGGTTTGGGTT	qPCR	IDT
uc.456	TGGCTTCCGGTAAGGTCA	GCTGTGTCCACTGAGGCTAA	qPCR	IDT
uc.475	TGGAAGAGGAGGAGGAGGTT	CAGGTGGGTGAAACCTCCTA	qPCR	IDT
LRBA	CCAACTTCAGAGAT TTGTCCAAGC	ATGCTGCTCTTTTTGGGTTCAG	qPCR	IDT
SYNCRIP	TGCCTTTTTATGTGGAGTCATGA	AATCTGCTACTTTGGTCCCTTGTT	qPCR	IDT
B-actin	CACCAACTGGGACGACAT	ACAGCCTGGATAGCAACG	qPCR	IDT
GAPDH	GGATTTGGTCGTATTGGG	GGAAGATGGTGATGGGATT	qPCR	IDT
LRBA intron	TCTTGGGGTACACTGGAGGT	TATCACGGCTCTCCCCTCTC	qPCR	IDT
u6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT	qPCR	IDT
TBP	TCAAACCCAGAATTGTTCTCCTTAT	CCTGAATCCCTTTAGAATAGGGTAGA	qPCR	IDT
M13	GTAAAACGACGGCCAG	CAGGAAACAGCTATGAC	qPCR	IDT
Northern Blottting				
uc.147 Probe	GCTCCTGCTCATCAACAAGTC	GCGATTCATTAAGCTCTGTATGG	Northern Blotting	IDT

uc.193 Probe	AAAGGGTTGGTTAACTACCTCAG	CATCTTGCTAAACTATTATACATCCAA	Northern Blotting	IDT
GAPDH Probe	CTGCACCACCAACTGCTTAG	TTCTAGACGGCAGGTCAGGT	Northern Blotting	IDT
LRBA Probe	TAATCTGCAGAATGGGATGC	TAATCTGCAGAATGGGATGC	Northern Blotting	IDT
RACE primers				
5' UC147-GSP	TTCAGTGACACGCGTCCTAGGCCTGTT		RACE PCR	IDT
3' UC147-GSP	GCTCTGTATGGTGGCCCTGAAGTGTAGA		RACE PCR	IDT
5' UC147-NGSP	GATTACGCCAAGCTTTTAGGCAAACCAACGAGGGGGAGTTG		RACE PCR	IDT
3' UC147-NGSP	GATTACGCCAAGCTTCACTTGCTGGTTGCTGGGAATGGAAT		RACE PCR	IDT
5' UC.193-GSP	AGCAGAGGATTGAACTATACCCTGTCTG		RACE PCR	IDT
3' UC.193-GSP	GAAGCAAGGCTGCTTTTTCAGCCACA		RACE PCR	IDT
5' UC.193-NGSP	GATTACGCCAAGCTTAGTTGCCTTTAGTTTCAGAGGCTTGT		RACE PCR	IDT
3' UC.193-NGSP	GATTACGCCAAGCTTCAAGGCCTGTTATGATGGTCATGAGGAA		RACE PCR	IDT
RACE 5' UC147-GSP2	GATTACGCCAAGCTTTCCCAATGGCTCTTTCTGGTCGCCT		RACE PCR	IDT
RACE 3' UC147-GSP2	GATTACGCCAAGCTTGGGTCCTACAGCCCAATAAATTGCCA		RACE PCR	IDT
RACE 3 uc.147.3	GATTACGCCAAGCTTTCAGGAAAATTCCATTCCCA		RACE PCR	IDT
RACE 3.1 uc.147.3	GATTACGCCAAGCTTGCGCATTCTCAATCACACAT		RACE PCR	IDT
RACE 5 UC.147.3	GATTACGCCAAGCTTAAATCCCAAGCCCCAAATACT		RACE PCR	IDT
RACE 5.1 UC.147.3	GATTACGCCAAGCTTTGCTGCTAAGAACCCCACTG		RACE PCR	IDT
uc.147 5prime 1	ACAGCCTGCAAAACCTCTCTT		PCR	IDT
uc.147 5prime 2	CAGTCAAGCATCTGCTATAGCT		PCR	IDT
uc.147 5prime 3	CTTTATCTTTTGTGAATTCCAG		PCR	IDT
uc.147 5prime 4	CTGGCAAAACATGGCTGGAG		PCR	IDT
uc.147 5prime 5	AAGAGCCGTTGTGGTTTCTT		PCR	IDT
uc.147 5prime 6	TTGGGTAGACCAGAGGCTGTA		PCR	IDT
uc.147 5prime 7	TGACACTGGTATTGCTGGAAGT		PCR	IDT

			PCR	IDT
uc.147 pulldown	AACTCCCCCTCGTTGGTTTG	TGGCTTTTGTGCTATACTGGTAG	PCR	IDT
siRNA	sense	Antisense		
siRNA uc.147 1	CAAUAUUAUCCUGACAGUAdTdT	UACUGUCAGGAUAAUAUUGdTdT	iRNA	Sigma
siRNA uc.147 2	CCAGCAACCAGCAAGUGAAdTdT	UUCACUUGCUGGUUGCUGGdTdT	iRNA	Sigma
siRNA LRBA 1	GUCAACUUCAAUAAUUAUAdTdT	UAUAAUUAUUGAAGUUGACdTdT	iRNA	Sigma
siRNA LRBA 2	GUCAUCUUUGUACACAACAdTdT	UGUUGUGUACAAAGAUGACdTdT	iRNA	Sigma

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8 CONCLUSÃO

- A expressão de aproximadamente 63% das T-UCRs está associada a algum parâmetro clínico no CM, mais especificamente, associado ao subtipo molecular (43,45%), receptor de estrógeno (35,76%), receptor de

progesterona (32,85%), amplificação de HER2 (16,63%), estadiamento (12,47%), terapia (36,59%) e sobrevida (9,56%);

- A diferença de expressão entre os subtipos tumorais das T-UCRs uc.147 e uc.193 foram confirmadas em amostra brasileira;

- O transcrito da região uc.147 foi pela primeira vez caracterizado, enquanto o transcrito da região uc.193 foi parcialmente caracterizado. Ambos são transcritos na direção oposta em relação aos seus genes hospedeiro. A uc.147 está localizada no núcleo, enquanto a uc.193 é predominantemente citoplasmática. O RNA da região uc.147 tem aproximadamente 2800nt, e sua sequência foi descrita;

- A região uc.147 demonstrou um importante papel em linhagens luminal de mama, pois o silenciamento *in vitro* dessa região em linhagens celulares derivadas do CM diminuiu a viabilidade celular, aumentou a apoptose, provocou um aumento de células na fase G1 do ciclo celular e diminuiu a capacidade das células de formarem colônias;

- Proteínas conhecidamente envolvidas no processo de tumorigenese estão ligadas ao RNA uc.147.

9 PERSPECTIVAS

A análise de expressão das T-UCRs apresenta potencial para utilização como marcadores de prognóstico no CM, como apresentado neste estudo. O potencial de discriminação dos subtipos tumorais pelas T-UCRs, assim como identificação de pacientes com pior sobrevida dentro de um mesmo subtipo

tumoral. Além disto, as T-UCRs possuem um papel importante durante o processo neoplásico do CM e estudos funcionais que visem entender os mecanismos pelos quais essas T-UCRs atuam são importantes.

Considerando que uma série de outras T-UCRs, foram evidenciadas na fase de rastreamento desse trabalho, estas podem ser futuramente avaliados em relação aos seus potenciais prognósticos e funcionais. Adicionalmente, a caracterização das T-UCRs auxilia no entendimento dessas regiões, sobretudo na importância de sua conservação. Finalmente, este trabalho abre também uma ampla frente de investigação das T-UCRs específicos descritos neste trabalho em outros tipos de amostras tumorais, como já está sendo feito em projetos em desenvolvimento no grupo de pesquisa.

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