UNIVERSIDADE FEDERAL DO PARANÁ

AMANI ABRAHEEM ALSADIQ ALYASEER

THE ROLE OF NLRP3 INFLAMMASOME ACTIVATION IN THE EPITHELIAL TO MESENCHYMAL TRANSITION PROCESS DURING THE FIBROTIC PROCESS

Dissertação apresentada ao curso de PósGraduação em Microbiologia, Parasitologia ePatologia, Setor de Ciências Biológicas,Universidade Federal do Paraná, como requisitoparcial à obtenção do título de Mestre em Microbiologia, Parasitologia e Patologia.

Orientador: Prof. Dr. Tárcio Teodoro Braga

CURITIBA 2020

AMANI ABRAHEEM ALSADIQ ALYASEER

THE ROLE OF NLRP3 INFLAMMASOME ACTIVATION IN THE EPITHELIAL TO MESENCHYMAL TRANSITION PROCESS DURING THE FIBROTIC PROCESS

Dissertação apresentada ao curso de Pós-Graduação em Microbiologia, Parasitologia e Patologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Microbiologia, Parasitologia e Patologia.

Orientador: Prof. Dr. Tárcio Teodoro Braga

CURITIBA

2020

Universidade Federal do Paraná. Sistema de Bibliotecas.
Biblioteca de Ciências Biológicas.
(Rosilei Vilas Boas – CRB/9-939).

Alyaseer, Amani Abraheem Alsadiq.
The role of NLRP3 inflammasome activation in the epithelial to mesenchymal transition process during the fibrotic process. / Amani Abraheem Alsadiq Alyaseer. – Curitiba, 2020.
72 f. : il.

Orientador: Tárcio Teodoro Braga.

Dissertação (Mestrado) – Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Microbiologia, Parasitologia e Patologia.

1. Fibrose. 2. Inflamassomos. 3. Inflamação. I. Título. II. Braga, Tárcio Teodoro. III. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Microbiologia, Parasitologia e Patologia.

CDD (20.ed.) 616.0473

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS BIOLÓGICAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO MICROBIOLOGIA, PARASITOLOGIA E PATOLOGIA - 40001016044P0

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em MICROBIOLOGIA, PARASITOLOGIA E PATOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **AMANI ABRAHEEM ALSADIQ ALYASEER** intitulada: **The role of NLRP3 Inflammasome Activation in the Epithelial to Mesenchymal Transition Process during the Fibrotic Process**, sob orientação do Prof. Dr. TÁRCIO TEODORO BRAGA, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 30 de Novembro de 2020.

Assinatura Eletrônica
01/12/2020 13:26:54.0
TÁRCIO TEODORO BRAGA
Presidente da Banca Examinadora (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
01/12/2020 14:18:17.0
MARCELO BELTRÃO MOLENTO
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
01/12/2020 11:54:47.0
ADRIANA FROHLICH MERCADANTE
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

I would like to dedicate this work to every person that was here for me ; my husband, family, Orientador and colleagues.

ACKNOWLEDGMENT

First I want to thank my Husband who made this possible, for his great support and love in every step, and for being there when it was stressful.

I want to thank my kids for keeping me up and pushing me to be a better me even though they don't know that.

I want to thank my very faraway family , my mom, my dad my sisters brothers and my In-laws for being a support although far but through the internet .

I want to thank my beautiful Orientador Prof Tarcio for opening his hands wide for me supporting me , pushing me to improve, having great patience with me and making me feel I have a friend I can rely on .

I want to thank Profa.Giseli and Profa. Edneia for accepting me and giving me the opportunity to be a part of this program, and everyone in the lab including Lucas, Andressa , Felipe and Sledz.

I want to thank my small family in the University ,Lais and Claudia who were more than sisters to me , thank you for all the love and laughs and the great times, Lucas , Jason and Adelino who helped me out a lot throughout this program, Murilo the best partner anyone can have, and a great friend .

I want to thank everyone in LIFE lab, Ollavo, Rebecca, Mariana, Thais, Laura, Maria-Clara and Caio such a beautiful great team and keeping me energetic.

I want to thank the beautiful girls in Prof. Silvio Zanata's Lab for all the help and putting up with my mistakes, Silvia, Soraia and Jordana. And the girls in Profa. Andreas lab especially Regi.

I want to thank Prof Wandersson and his team mainly Jose and Israel for being such great help without complaining.

I want to thank the Postgraduate Microbiology, parasitology and pathology program for this great opportunity and thank every professor that gave us lectures in this program and helped me out to pass and made my path easier, And I love to thank everyone for putting up with my weak Portuguese.

I want to thank UFPR, Capes and Cnpq for the opportunity to be in their program and do my research and Masters.

I want to thank my Portuguese professor Andre who gave his time and energy to teach me Portuguese. And to everyone that made me feel more than welcome In Brazil.

What your mind can conceive and believe, it can ACHIEVE ☺

Abdulsalam Eshmaila

RESUMO

A fibrose é considerada uma forma complexa de dano tecidual comumente presente em o estágio final de muitas doenças. Também está relacionado a uma alta porcentagem de morte, cujas características predominantes são uma deposição excessiva e anormal de fibroblastos e componentes da matriz extracelular (MEC) derivados de miofibroblastos. A transição epitelial para mesenquimal (EMT), um processo no qual as células epiteliais mudam gradualmente para as mesenquimais, é um dos principais contribuintes na patogênese da fibrose. O mediador principal da EMT é uma citocina multifuncional chamada fator de crescimento transformador-β (TGF-β) que atua como o principal indutor da montagem e remodelação da ECM por meio da fosforilação de Smad2 / 3, que, em última análise, forma um complexo com Smad4 e se transloca em O núcleo. Por outro lado, a proteína-7 morfogênica óssea (BMP-7), um membro da família TGF, reverte a EMT ao neutralizar diretamente a sinalização celular dependente de Smad induzida por TGF-β. O NLRP3 (proteína 3 contendo domínios NACHT, LRR e PYD), por sua vez, atua como sensores citosólicos de moléculas microbianas e auto-derivadas e forma um complexo imunológico denominado inflamassoma no contexto de comprometimentos inflamatórios. A montagem do inflamassoma NLRP3 é desencadeada por ATP extracelular, espécies reativas de oxigênio (ROS), efluxo de potássio, desequilíbrio de cálcio e ruptura de lisossoma. Devido ao seu envolvimento em várias doenças, o NLRP3 tornou-se um dos receptores de reconhecimento de padrões (PRRs) mais estudados. No entanto, o papel do NLRP3 no desenvolvimento da fibrose não foi completamente elucidado. Nosso objetivo nesta pesquisa foi estudar a formação do complexo inflamassoma após a indução de EMT por TGF-β em células epiteliais tubulares renais humanas e murinas. Para tanto, tentamos transfectar as células epiteliais tubulares humanas HK-2 e as murinas mm55.k com proteínas inflamatórias NLRP3 e ASC marcadas com GFP e Cerulean, respectivamente. A fim de investigar o processo EMT, tanto E-caderina quanto α-SMA foram tentados para serem detectados por western blot, e isso foi feito pela exclusão dos genes smad 3 e BMP-7 e detecção da formação da oligomerização do complexo inflamassoma por o uso da microscopia confocal.

Palavras chave: Fibrose, Inflammasome, NLRP3, EMT, TGF-β, SMAD

ABSTRACT

Fibrosis is considered a complex form of tissue damage commonly present in the end stage of many diseases. It is also related to a high percentage of death, whose predominant characteristics are an excessive and abnormal deposition of fibroblasts and myofibroblasts -derived extracellular matrix (ECM) components. Epithelial-to-mesenchymal transition (EMT), a process in which epithelial cells gradually change to mesenchymal ones, is a major contributor in the pathogenesis of fibrosis. The key mediator of EMT is a multifunctional cytokine called transforming growth factor-β (TGF-β) that acts as the main inducer of the ECM assembly and remodeling through the phosphorylation of Smad2/3, which ultimately forms a complex with Smad4 and translocates into the nucleus. On the other hand, the bone morphogenic protein-7 (BMP-7), a member of the TGF family, reverses EMT by directly counteracting TGF-β induced Smad-dependent cell signaling. NLRP3 (NACHT, LRR, and PYD domains-containing protein 3), in turn, acts as cytosolic sensors of microbial and self-derived molecules and forms an immune complex called inflammasome in the context of inflammatory commitments. NLRP3 inflammasome assembly is triggered by extracellular ATP, reactive oxygen species (ROS), potassium efflux, calcium misbalance, and lysosome disruption. Due to its involvement in multiple diseases, NLRP3 has become one of the most studied pattern-recognition receptors (PRRs). Nevertheless, the role of NLRP3 in fibrosis development has not been completely elucidated. Our aim in this research was to study the formation of the inflammasome complex following the induction of EMT by TGF-β in both human and murine renal tubular epithelial cells. For that we tried to transfect the human tubular epithelial cells HK-2 and the murine ones mm55.k with GFP and Cerulean labeled NLRP3 and ASC inflammatory proteins, respectively. In order to investigate the EMT process, both *E-cadherin* and *α-SMA* were tried to be detected by western blot, and this was done by deleting bothe the smad 3 and BMP-7 genes and detecting the formation of the inflammasome complex oligomerization by the use of the confocal microscopy.

Key Words: Fibrosis, Inflammasome, NLRP3, EMT, TGF-β, SMAD

LIST OF FIGURES

LIST OF SYMBOLS

α :Alpha

β :Beta

ABBREVIATIONS

ECM: Extracellular matrix

IPF: Idiopathic pulmonary fibrosis

IBD: Inflammatory bowel disease

CKD: Chronic kidney disease

α SMA: Alpha smooth muscle actin

FSP 1: Fibroblast specific protein 1

E-cadherin: Cell surface epithelial cadherin

N-cadherin: Mesenchymal neural cadherin

EMT: Epithelial mesenchymal transition

MET: Mesenchymal epithelial transition

TGF-β: Transforming growth factor beta

BMP: Bone morphogenic protein

CCA: Coloangiocarcinoma

NLRP3: NOD-, LRR- and pyrin domain-containing protein 3

ASC: Apoptosis-speck like protein containing a CARD.

Th2:T-hlper 2 cells

M1 macrophages: the classical macrophages

M2 macrophages: the anti-inflammatory macrophages

MMP: Matrix metaloproeinase

TF: Transcription factors

HDAC: Histone deactylase

CDH1: e-cadherin encoding gene

PAI-1: Plasminogen activator inhibitor-1

AMH: anti-mullerin hormone

CCL4: Carbon tetrachloride

TIMP:Tissue inhinitor of metalloproteinase

MET: Mesenchymal epithelial transition

ERK : Extracellular-signal-regulated kinase

MAPK : Mtogen activated protein kinase

DAMP: Danger associated molecular pattern

PAMP:Pathogen associated molecular pattern.

PRR: Pathogen recognition receptor

NOD: Nucleotide-binding oligomerization

CAPS : Cryopyrin-associated periodic syndromes

GSMD: Gasdermin-D

BMDM : Bone marrow derived macrophages

ICE: Interleukin converting enzyme

UUO: Unilateral Uretric obstruction

oxPAPC: 1-palmitoyl-2- arachidonoyl-sn-glycero-3-phosphorylcholine

LPS: Lipopolysaccharide

LPG: Lipophosphoglycan

TRIF: TIR domain containing adapter inducing interferon β

Nek7: NIMA-related kinase 7

Table of Contents

frontiers
in Immunology

REVIEW
published: 20 May 2020
doi: 10.3389/fimmu.2020.00883

The Role of NLRP3 Inflammasome Activation in the Epithelial to Mesenchymal Transition Process During the Fibrosis

Amani Abraheem Alsadiq Alyaseer[1], *Murilo Henrique Saturnino de Lima*[1,2] and *Tarcio Teodoro Braga*[1,2]*

[1] *Department of Pathology, Federal University of Parana, Curitiba, Brazil,* [2] *Instituto Carlos Chagas, Fiocruz-Parana, Curitiba, Brazil*

Fibrosis is considered a complex form of tissue damage commonly present in the end stage of many diseases. It is also related to a high percentage of death, whose predominant characteristics are an excessive and abnormal deposition of fibroblasts and myofibroblasts -derived extracellular matrix (ECM) components. Epithelial-to-mesenchymal transition (EMT), a process in which epithelial cells gradually change to mesenchymal ones, is a major contributor in the pathogenesis of fibrosis. The key mediator of EMT is a multifunctional cytokine called transforming growth factor-β (TGF-β) that acts as the main inducer of the ECM assembly and remodeling through the phosphorylation of Smad2/3, which ultimately forms a complex with Smad4 and translocates into the nucleus. On the other hand, the bone morphogenic protein-7 (BMP-7), a member of the TGF family, reverses EMT by directly counteracting TGF-β induced Smad-dependent cell signaling. NLRP3 (NACHT, LRR, and PYD domains-containing protein 3), in turn, acts as cytosolic sensors of microbial and self-derived molecules and forms an immune complex called inflammasome in the context of inflammatory commitments. NLRP3 inflammasome assembly is triggered by extracellular ATP, reactive oxygen species (ROS), potassium efflux, calcium misbalance, and lysosome disruption. Due to its involvement in multiple diseases, NLRP3 has become one of the most studied pattern-recognition receptors (PRRs). Nevertheless, the role of NLRP3 in fibrosis development has not been completely elucidated. In this review, we described the relation of the previously mentioned fibrosis pathway with the NLRP3 inflammasome complex formation, especially EMT-related pathways. For now, it is suggested that the EMT happens independently from the oligomerization of the whole inflammasome complex, requiring just the presence of the NLRP3 receptor and the ASC protein to trigger the EMT events, and we will present different pieces of research that give controversial point of views.

Keywords: NLRP3, EMT—epithelial to mesenchymal transition, fibrosis, inflammasome, TGF-β

OPEN ACCESS

Edited by:
Robson Coutinho-Silva,
Federal University of Rio de Janeiro, Brazil

Reviewed by:
Alberto Baroja-Mazo,
Biomedical Research Institute of Murcia (IMIB), Spain
Junya Masumoto,
Ehime University Graduate School of Medicine, Japan

*Correspondence:
Tarcio Teodoro Braga
tarcio_tt@yahoo.com.br

Specialty section:
This article was submitted to Inflammation,
a section of the journal Frontiers in Immunology

Received: 04 February 2020
Accepted: 14 April 2020
Published: 20 May 2020

Citation:
Alyaseer AAA, de Lima MHS and Braga TT (2020) The Role of NLRP3 Inflammasome Activation in the Epithelial to Mesenchymal Transition Process During the Fibrosis. Front. Immunol. 11:883. doi: 10.3389/fimmu.2020.00883

1. INTRODUCTION

Fibrosis is a major global health issue, and a worldwide health burden. It is an end result of a variety of disease resulting in a decrease of quality of life of the patient and sometimes even ending up in death. It has been reported that around 45% of deaths in developed countries were found to be due to fibrotic diseases Distler et al. (2019),where chronic kidney disease alone was found to affect almost 47 million patients in the United states alone Murphy et al. (2016), and until today we can say that we still suffer the lack of the therapeutic approaches that target the pathogenesis of fibrosis. A mechanism that was found to be triggered during fibrosis is the epithelial mesenchymal transition (EMT), a phenomenon that was found to play a role in embryogenesis, cancer and fibrosis, as mentioned Kalluri; Neilson (2003)Zeisberg et al. (2008). During the EMT, cells switch form from non-migratory epithelial cells to motile mesenchymal cells and gain their characters Cruz-Solbes; Youker (2017). When an injury happens triggered by different stimuli, the immune cells will release a number of cytokines and growth factors, and one of these growth factors is the TGF-β1, which was found to be the key element of the activation of this EMT process Wang et al. (2017). This pro-fibrotic factor, is a potent inducer of EMT and a part of the TGF-β-induced EMT program, which changes the cell basement membrane adhesion relation Li et al. (2003)Strutz et al. (2002). TGF-β1 is considered a master regulator of fibrosis through the accumulation of extracellular matrix components (ECM) and a potential key driver of fibrosis Bartram; Speer (2004). Another member of this large family is the BMP7, which shares with TGF-β1 the capability that they both bind to serine/threonine kinase trans membrane receptors and can activate a canonical SMAD dependent or a non-canonical non SMAD dependent pathway Boon et al. (2011). This member is considered anti-fibrotic and was found that it plays a role in contra versing the effect of TGF-β1 in reversing the EMT and eventually the fibrosis from happening Zeisberg; Hanai; et al. (2003). Although a number of researches have been focused on this opposing relation between TGF-β1 and BMP7, it still remains a

controversy. Both of them activate a canonical and non-canonical pathway, being the canonical one activated via proteins known as SMAD's, where TGF-β1 phosphorylated receptors bind to the regulatory SMAD -2 and -3, whilst the BMP7 phosphorylated receptors bind to the SMAD-1, -5, and -8. Moreover, in both pathways the SMAD's will bind to the co-SMAD, the SMAD4 to translocate into the nucleus and activate the related genes Guo; Wang (2009). In case of TGF-β1, as mentioned, it will play a role in activating the EMT, that is why it was found that SMAD-3 may be considered a key regulator of fibrosis Latella et al. (2009). These two pathways in a normal physiological condition create a balance by opposing each other. Another important structure that plays a major role in the bodies' defense once triggered by a stimuli whether a PAMP or DAMP, is the NLRP3 inflammasome, which is considered a recent discovered structure Schroder; Tschopp (2010). Once the inflammasome is assembled, it results in the proteolytic cleavage of the pro-inflammatory cytokines IL1-β, IL18 and the activation of the programmed cell death pyroptosis Schroder; Tschopp (2010). The linkage between the NLRP3 inflammasome and the activation of EMT is still under study, but how it works exactly still remains unclear. Up to now the relation has either been described as inflammasome independent explaining the fact that it only requires NLRP3 protein without the requirement of the inflammasome assembly Wang et al. (2013). While other researchers have proven with their results that the inflammasome assembly is mandatoryTian et al. (2017), in addition, some have shown a role of IL1-β in induction of TGF-β1 to activate EMT Doerner; Zuraw (2009).And based on the gathered information, we have tried in our research, although not considered perfectly clarified , but we might have given a small clue of this relation.

1.1 JUSTIFICATION

Based on the knowledge we have collected from previous researches on the role of the NLRP3 inflammasome in fibrosis through the activation of epithelial mesenchymal transition (EMT), and due to the controversy on how this relation happens whether it is inflammasome dependent or independent, this research was based on finding a more clear answer to this question, and this was based on detecting the role of Smad3 through it's deletion to detect the influence on the TGF-β 1 pathways, and it's opposing BMP7 pathway through the deletion of the BMP-7 gene. And this was based on studying the

formation of the NLRP3 in tubular renal cells after trying to induce EMT using TGF-β.and this is shown in the figure below. (FIGURE 1)

FIGURE 1

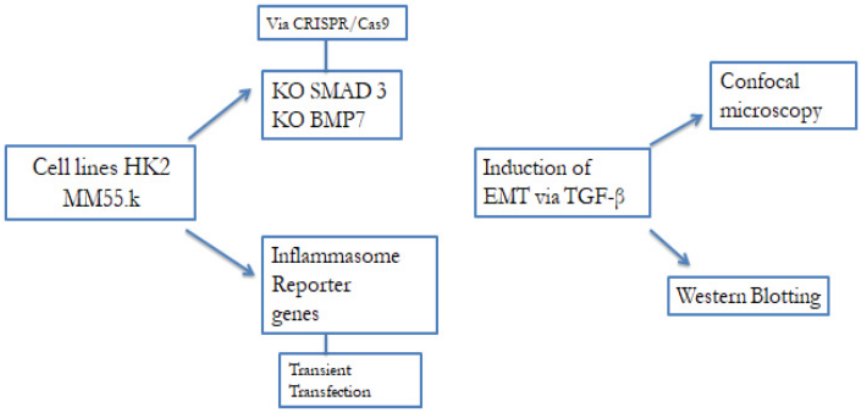

Figure 1 : Chronogram of the planned experiments during a research , showing the aim of study to detect the influence of deleting the SMAD3 gene that is of key importance in the TGF-β pathway, and deleting the BMP-7 gene , that is opposing the TGF-β pathway and detect the income on the formation of the NLRP 3 inflammasome and vice versa.BMP-7 : Bone morphogenic protein 7, TGF-β: Transforming growth factor Beta.

OBJECTIVES

1.1.1 General objectives

To observe the formation of the Inflammasome complex after EMT induction via TGF-β1 in tubular renal cells.

1.1.2 Specific Objectives:

- To construct renal epithelial cells carrying reporter genes (NLRP3-GFP and ASC-Cerulean) and SMAD3 and BMP-7 silenced genes;
- To study the EMT process in human (HK-2) and murine (mm55.k) renal epithelial cells in the presence or absence of SMAD3 and BMP-7 via TGF-β;
- To investigate the formation of inflammasome complex in HK-2 cells and mm55.K after induction of the EMT.

2. LITERATURE REVISION

2.1 Fibrosis

Fibrosis is the final pathological outcome of many chronic inflammatory diseases. It can be defined as an excessive accumulation of fibrous connective tissue, such as collagen and fibronectin, in the areas of inflamed and injured tissues, which results in the formation of scar, and so a destruction of the normal tissue architecture, organ damage and even death Wynn (2011). Fibrosis can also be defined in other words, as a chronic inflammation followed by a persistent immune response, where inflammation, tissue remodeling, and the repair processes occurs concomitantly Wynn (2007). Wynn and colleagues also found that fibrotic disorders all share a common feature, and that is the presence of a persistent irritant resulting in the continued production of growth factors, cytokines, proteolytic enzymes and angiogenic factors ,which work simultaneously and result in the accumulation of the connective tissue elements Wynn (2007)Tomasek et al. (2002). Fibrosis is considered the end stage of multiple diseases, naming; most chronic kidney disease, ends up with renal fibrosis, leading patients to dialysis or even requiring kidney transplantation Liu (2006). Any chronic liver disease also ends up with liver fibrosis Liu (2006), that might develop to liver failure or cancer Aydin; Akcali (2018). And lung disease too mentioning of them idiopathic pulmonary fibrosis Martinez et al. (2017). In addition to the chronic organ diseases, there is also a number of autoimmune diseases such as rheumatoid arthritis Scott et al. (2010), inflammatory bowel disease Torres et al. (2017), and any other chronic inflammatory reaction due to allergic responses, chemical insults, and radiation was found to develop fibrosis Wynn (2008). Although until these days fibrotic diseases have a large impact on human pathology, being considered a health care burden with a high morbidity

and a mortality that reaches up to 45% of all deaths in the developed world Distler et al. (2019), there is no approved effective treatment strategies that target fibrosis Wynn; Ramalingam (2012). The strategies to deal with fibrotic diseases rely nowadays mostly on an attempt to neutralize the aggressor, but not all of them are easily diffusible or with a known origin, which forces clinicians to try to find ways to control and reverse the fibrosis Wynn; Ramalingam (2012), or target common mechanisms and core pathways that are of central pathophysiological relevance across different fibrotic diseases Eyden (2001)Distler et al. (2019). For example, two drugs that have been approved for the use in idiopathic pulmonary fibrosis are *Pirefenidone* and *Nintedanib*, but the clinical experience with these drugs is indecisive, and the long-term safety and efficacy of these medications is not determined yet, not to mention the long list of their side effects Martinez et al. (2017).

Wound healing and fibrosis are in the beginning of their pathways somehow similar. After injury, the wound healing mechanism will be activated, that initiates a reparative coagulation anti-fibrinolytic cascade, which triggers blood-clot formation and formation of a provisional extracellular matrix (ECM) Wynn; Ramalingam (2012), when endothelial or epithelial damage occurs, platelets will be exposed to the ECM components, such as collagen and vonwilbrands factor, and this will initiate the platelet degranulation and hence promote vasodilation and increased vascular permeability Wynn (2008). Epithelial and endothelial cells together with the myofibroblasts produce a number of chemotactic factors, such as MMPs, which disrupt the basement membrane that recruit inflammatory monocytes and neutrophils to the site of tissue damage Wynn (2008). But what distinguishes the fibrosis is the presence of myofibroblasts which are the positive αSMA activated fibroblasts, that instead of vanishing after a successful repair they persist Hinz (2015).

Although different fibrotic conditions might have different inflammatory responses, it was found that they all share polarization towards a T helper 2 (Th2) cell and M2 macrophage-mediated response with the abundant release of pro-fibrotic mediators as a common feature McAnulty (2007). Such mediators are responsible for controlling the response triggered against aggressor agents, and for starting the tissue repair process McAnulty (2007). In chronic inflammatory diseases, macrophages are the main characters in expressing these mediators Fenyo; Gafencu (2013). These cells are differentiated, mainly

from monocytes when they leave the circulatory system and invade the tissue to become phagocytes and are able to produce inflammatory cytokines Yang et al. (2014). Besides bone marrow hematopoietic cells, different sources were found to be the sources of macrophages, such as local proliferation of embryonic progenitor cells, which is responsible for the generation of tissue-resident macrophages Braga et al. (2015) .They are plastic cells and functionally subdivided into profiles that vary from an inflammatory subpopulation the classical (usually called M1 macrophages) to the alternative known as an anti-inflammatory one (usually referred as M2 macrophages Mosser; Edwards (2008). Noting that anti-inflammatory macrophages can be derived from inflammatory ones or resident cells Martinez; Gordon (2014)Atri et al. (2018). Inflammatory macrophages importance also lies in the fact in that they express IL-1β, IL-23, TNF-α, and lipid-derived mediators. Adding to it , the production of reactive oxygen species (ROS), which is necessary to kill the invader and therefore responsible for the injury during inflammation Martinez; Gordon (2014)Atri et al. (2018). Mediators prom the activation and accumulation of the Myofibroblasts (activated fibroblasts) which can be determined as the main effector in fibrosis and source of physiological accumulation of extracellular matrix in the granulation tissue and for the excess deposition of interstitial extracellular matrix under pathologic conditions Hinz (2015)Roberts et al. (1997). Myofibroblasts can be defined as specialized fibroblast like contractile cells with the characters of smooth muscle cells. Myofibroblasts are spindle-shaped cells, which are specified by the presence of microfilament bundles (stress fibers) and α-smooth muscle actin (α-SMA). Their surface is specified by their fibronexus junctions and prominent fibronectin, and present abundant rough endoplasmic reticulum Eyden (2001)Desmoulière et al. (2003). Compared to other cells they have shown to have a higher proliferation rate, the ability of migration, and cytokine expression, Guarino et al. (2009). They synthesize a variety of inflammatory and anti-inflammatory cytokines, chemokines, growth factors, inflammatory mediators, as well as extracellular matrix components such as collagens , and proteases involved in matrix degradation Desmoulière et al. (2003). The source of the myofibroblasts are either from the bone marrow derived progenitors (fibrocytes), resident fibroblast responding to stress Chapman (2004), or from different organs such as the lung, liver or kidney due to the transitional process that occurs in the epithelial/endothelial cells known as the EMT Hinz

(2015). Ghatak and colleagues described myofibroblasts, that it acts like a double-edged sword Ghatak et al. (2018). Where a fine balance has to be maintained. On one side, it is beneficial because it helps normal wound healing, but on the other side excessive action can result in undesirable fibrosis resulting in contracture, scarring and so the loss of the tissue architecture Ghatak et al. (2018). Fibrosis occurs when the synthesis of new collagen by myofibroblasts exceeds that rate in which it will normally be degraded Ghatak et al. (2015). Noting that normally myofibroblasts undergo apoptosis once the repair is accomplished in a normal wound healing process but in fibrotic lesions where the aggressor is persistent, apoptosis was found to be resistant Ghatak et al. (2015). Until the day we can say that it's controversial whether fibrosis can be completely reversed to the extent where normal tissue structure is restored completely. Indeed, that's because researches have given evidence that advanced fibrosis is hypo-cellular , it has been suggested that incomplete ECM degradation (irreversible fibrosis) develops when the appropriate cellular mediators (the source of MMPs) are no longer present, and that means that inflammation should be ongoing Issa et al. (2004).

2.2 Epithelial to mesenchymal transition

In the late 1960's , a researcher under the name Elizabeth Hay made an observation of changes in the primitive streak of chick embryos , where she described the epithelial to mesenchymal phenotype changes, giving the idea that epithelial cells can down-regulate their epithelial characteristics and acquire mesenchymal one's "hay1995" (). Epithelial mesenchymal transition (EMT) is a biological process, that is characterized by the changing in the phenotype of polarized epithelial cells into mobile migratory mesenchymal cells, and this occurs through a switch of the expressed proteins, ending up with motile, collagen producing cells from an epithelial originCruz-Solbes; Youker (2017).This phenomenon is a response to pleiotropic signaling factors that induce the expression of specific transcription factors (TFs) named the EMT-TFs (e.g., Snail, Zeb..etc) and miRNAs together with epigenetic and post-translational regulators Cano et al. (2000).What happens during EMT is that the epithelial cells start losing their junctions, and apical basal polarity, they also start to reorganize their cytoskeleton, reprogram their gene expression and shape,

resulting in the loss of their epithelial characteristics and gaining mesenchymal ones such a motility and collagen productionThiery; Sleeman (2006) Thiery et al. (2009). Basically the epithelial cells have a structural function, and are impermeable; therefore, their most prominent characteristic is apical basal polarity, and are held together laterally by tight junctions, and adherent junctions, which are formed by cell surface epithelial cadherin (e-cadherin) molecules Dongre; Weinberg (2019). They are also known by their adherence to the basement membrane and to each other, and hence providing and anchor in the tissues and lack the motility ability Xu et al. (2009).Upon the activation of EMT, the expression of e-cadherin, which is considered a hallmark of EMT, together with certain cytokeratins, will be repressed and gradually lost, while the expression of the genes associated with the mesenchymal state will start expressing and gradually up-regulated, such as α smooth muscle actin a N-cadherin integrin, fibronectin, and matrix metalloproteinase Yilmaz; Christofori (2009) Xu et al. (2009). The cells morphological appearance changes too, where they will start to reorganize their cortical actin cytoskeleton , and formation of invadopodia or podosomes, that enables dynamic cell elongation and directional motility Yilmaz; Christofori (2009).These changes in gene expression and repression of e-cadherin, will prevent the repeated formation of epithelial cell–cell junctions and result in the loss of the epithelial barrier function Peinado et al. (2007). This decrease in the e-cadherin is usually balanced by the increase of the mesenchymal neural cadherin (n-cadherin), which results in what's known as a 'cadherin switch' that will alter the cell-cell adhesion Wheelock et al. (2008).

The transcriptional factors that play a role in the changes that result in the repression of the epithelial phenotype and activation of the mesenchymal phenotype involve master regulators, including SNAIL, SLUG,ERK, TWIST and zinc-finger E-box- binding (ZEB) transcription factors Lamouille et al. (2014)**.** SNAIL represses the e-cadherin-encoding gene, CDH1, through the binding to the E boxes in the CDH1 promoter and recruiting the histone deacetylase (HDAC) activity Peinado et al. (2004). The EMT process does not represent bipolarity between epithelial and mesenchymal cells, but instead is considered a spectrum of different protein expression throughout the time that culminates in a complete transition. Both EMT and the opposite process, known as mesenchymal to epithelial transition (MET), are considered integral stages during development of many physiological

processes that are closely and firmly coordinated by a number of molecular regulators Pei et al. (2019)Hogan; Kolodziej (2002). EMT plays a main role in a diversity of biological processes, in addition to the fibrotic process Kalluri; Neilson (2003)that we mainly pay attention to here, it has also great and important roles in embryogenesis Hogan; Kolodziej (2002),and cancer metastasis Saitoh (2018). It is separated into three types, each of them presenting a different function in the mesenchymal cells at the end of the process. The type I EMT is present in embryogenesis implantation, during the initiation of the placenta development and organ development; the type II EMT, on the other hand, occurs during fibrotic diseases in organs such as the liver, kidney, lung, and intestine, and it is also associated with tissue regeneration and wound healing; the type III EMT is conceptually present in the progression of cancer and in the invasion- metastasis cascade Kalluri; Neilson (2003) Zeisberg et al. (2008). In the early embryonic life mesoderm formation and neural crest development represent the key EMT programs, where their mesenchymal changes allows them to further develop and differentiate into various cell types Yang; Weinberg (2008). In cancer, the cells activate the EMT program to allow them to gain the migratory ability, for either local invasion to nearby tissues, or distant organs via vessels through blood or lymph Yang; Weinberg (2008). This ability is attributed to the type III EMT that promotes a loss of cell adhesion and helps the invasion Yang; Weinberg (2008). Both type I and type III EMT processes are reversible by the MET process after a tissue invasion, and regain the epithelial characteristics, a reversion that does not happen in type II EMT Campbell (2018). During fibrotic diseases, the main triggered mechanism is the type II EMT. Here, the mesenchymal type of cells formed after complete transitions are myofibroblasts and fibroblasts, responsible for expressing ECM components in tissue repair after an injury Kalluri; Neilson (2003).Figure two shows the types and shows the difference in the cells structure between bothe epithelial and mesenchymal cells.

FIGURE 2

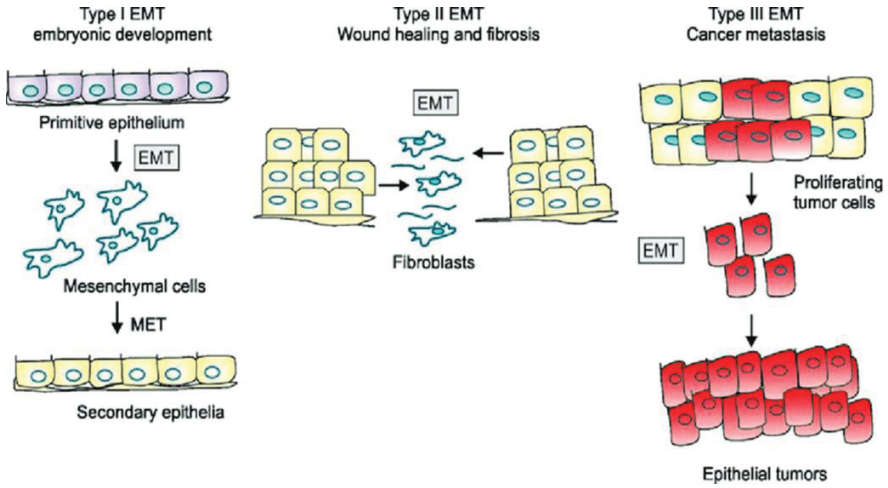

Figure 2 : Showing the three types of EMT that have been recorded, with TYPE 1 to occur in the embryonic development , TYPE 2 is the type that occurs during the process of fibrosis, and TYPE 3 has shown to occur in cancer development and responsible for metastasis. The image also shows the diference of stability between the epithelial and mesenchymal cells. EMT: epithelial mesenchymal transition. *Nishad et al.,2017*

2.2.1 Type 2 Epithelial to mesenchymal transition

One of the driving forces behind fibrosis is the epithelial mesenchymal transition, which as mentioned is considered the type two EMT. Once the tissue is triggered by different pathogens whether viruses or bacteria, a series of signaling cascades happens, which will activate the immune system .The immune system once activated results in the activation of an inflammatory response that will result in EMTSun et al. (2017) Zeisberg; Hanai; et al. (2003). Immune cells such as neutrophils, and macrophages recruits to the site of injury, release an array of growth factors and cytokines, one of these growth factors is the transforming growth factor beta (TGFβ1), which is considered a key element in fibrotic EMT Atala (2017)Wang et al. (2017). TGF-β1 binds to target molecules on the cellular membrane known as TGF-β type I and type II receptors (TBR-I and II) creating a cascade

of signals, and performs these various actions by directly activating a range of signaling pathways including either Smad proteins, or the ERK/MAP kinases and micro RNAsHeldin et al. (1997). During the past decade, a lot of evidence both in vitro and in vivo have supported the important roles for TGF-β1 as an inducer of EMT. Subsequently , in various lung diseases the underlying cause of fibroblast, goblet cell and pneumocyte hyperplasia, which eventually ends up with fibrosis, has been linked to be under the influence of EMT Kim et al. (2006)Liu et al. (2017).

For instance, a study using bronchial epithelial cells, TGF-β1 was found to stimulate bronchial epithelial cells morphological change to mesenchymal type, and that was proved by the down-regulation of *e-cadherin* and an up-regulation of *αSMA* expression quantified by quantitative real time PCR Doerner; Zuraw (2009). And in another study targeting asthmatic patients, primary airway epithelial cells from normal and asthmatic patients were studied under the influence of TGF-β1 Hackett et al. (2009). Where the results showed that after the exposure to TGF-β1 the epithelial markers such as *e-cadherin* was decreased, while mesenchymal markers including *fibronectin* and *α-sma* showed to be increased. indicating the occurrence of EMT Hackett et al. (2009). Another lung fibrotic disease, is the idiopathic pulmonary fibrosis, the most common type of idiopathic interstitial pneumonia Richeldi et al. (2017), where research in both animal models and human ,have shown that activated alveolar epithelial cells secrete a variety of cytokines and growth factors, including from these the TGF-β1, and that this leads to the recruitment and activation of myofibroblasts Thannickal et al. (2004) Richeldi et al. (2017), with the fact one of the main sources of the myofibroblasts here is the EMT/EndMT. And it was found that the myofibroblasts derived from epithelial cells by epithelial–mesenchymal transition (EMT), exhibit abnormal proliferation and ECM overproduction, resulting in the development of IPF Lamouille et al. (2014) Thannickal et al. (2004). In kidneys, fibrosis estimated that during the repair process, around 35% of fibroblast originate from epithelial/endothelial mesenchymal transition Lamouille et al. (2014).Grande and colleagues also highlighted the importance of EMT in the pathogenesis of renal fibrosis, and reported that Snail1 reactivation mediates the induction of cytokines and TGF-β1 Grande et al. (2015). And that this fibrosis *in vivo* animal models can be reversed through the inhibition of SNAIL1 Grande et al. (2015).

Studies in the liver too has shown that epithelial to mesenchymal transition occurs in human liver fibrosis, such as biliary atresia and primary biliary cirrhosis with prominent bile ductular proliferationDíaz et al. (2008) .Where Zeisberg et al. demonstrated that liver cells that express albumin ,under the effect of the profibrotic growth factor TGF-β1 undergo phenotypical changes in vitroZeisberg et al. (2008), and that these hepatocytes in vivo in response CCL4 to acquire the expression of fibroblast specific protein 1(FSP1)Kaimori et al. (2007).Coming up to the conclusion that EMT is a major step in development of fibrosis and that TGF-β1 can be considered a key regulator.

2.2 TGF-β1 - transforming growth factor beta1

A lot of triggering factors have been implicated to the activation of EMT, one of the most important is the TGF-β1 dependent pathway. TGF-β1 is a multifunctional growth factor ,it's a member of a large family that regulates a variety of key events in normal development and physiology, mentioning from them; proliferation, differentiation, apoptosis, adhesion and migration in various cells, including macrophages, activated T and B cells, immature hematopoietic cells, neutrophils and dendritic cells Morikawa et al. (2016).It has also been implicated to embryogenesis, connective tissue diseases ,fibrosis and cancer Clark; Coker () Morikawa et al. (2016). Early researches since the 1980s , guided by Todaro, Holley, Moses, Roberts/Sporn, Derynck, and Massague´ laboratories, to study the TGF-β1 led to the discovery of its characterization, pathways, and family members Moses et al. (2016), and changed the incorrect belief that this cytokine was only expressed in transformed cells during the establishment of cancer when it was studied in cancer biology Moses et al. (2016).Until the day it was found that almost thirty three different human genes have been identified that encode structurally related homomeric (and also heteromeric) TGF-β family members Hinck et al. (2016). Other members that have been recorded to belong to this family are the bone morphogenic proteins (BMPs), Activin, nodal and anti-mullerian hormone (AMH), they share the similarity in their molecular structure and are all reported to be involved in a number of cellular functions Chang et al. (2002)Kingsley (1994).

TGF-β exists in three main isoforms in humans (TGF-β1, TGF-β2, and TGF-β3), and they share about 70-82% amino acid homology Lichtman et al. (2016). All secreted as inactive precursors, high molecular weight complexes, that require activation before binding to the TGF-β receptors Miyazono; Heldin (1991) ,and although they are encoded by distinct genes, they have found to all share a similar homology and activate the same intracellular signaling pathway (Barton et al 1998). TGF-β1 is the most common studied and has been defined as a master regulator of ECM accumulation and consequently a potential key driver of fibrosis Bartram; Speer (2004). TGF-β1 has also been shown to play a dramatic role in regulation of inflammation Gorelik; Flavell (2000)Slater, A. F. G., & Cerami (1992), where the deletion of TGF-β1 gene in mice led to a rapid demise due to a sever systemic inflammation followed by their death.

TGF-β1 comprises an important role in wound healing, where it plays a role in macrophages chemo taxis to site of wound, increase of ECM synthesis, angiogenesis, and the expression of proteinase inhibitors, including plasminogen activator inhibitor-1 (PAI-1), and by decreasing the ECM-degrading proteins, such as collagenaseLichtman et al. (2016). According to both Li et al. and Strutz et al. this pro-fibrotic activity is a potent inducer of EMT and part of the TGF-β-induced EMT program, which changes the cell basement membrane adhesion relation Li et al. (2003)Strutz et al. (2002). TGF-β1 have reported to increase the expression of the extracellular matrix proteins fibronectin and both epithelial and mesenchymal cells in vitro through transcriptional activation of relevant genes Ignotzs; Massague8 (1986). In both experimental and human studies of fibrosis in in different organs, TGF-β1 has shown to be up-regulated, Sanderson et al. (1995)Roberts et al. (1986)(Kopp et al 1996). In one research subcutaneous injection of TGF-β in new born mice, led to angiogenesis, collagen accumulation through the activation of fibroblasts, and a fibrotic tissue response (Roberts et al. 1986). And it was also found that TGF-β1 is responsible for the activation of both epithelial mesenchymal transition (EMT) and Endothelial mesenchymal transition (EndMT) Zeisberg et al. (2008)Zavadil; Böttinger (2005), and hence responsible for around 30-50% of myofibroblasts through the activation of resident fibroblasts in renal fibrosis Zeisberg et al. (2008).

Transgenic mice overexpressing TGF-β1 in the kidneys developed an in increase in extracellular matrix material, including collagen fibrils, in subendothelial and mesangial locations (Kopp et al 1996). Giving the evidence that TGF-β1 induced progressive glomerular sclerosis, and is a cause of progressive renal fibrotic disease (Kopp et al 1996). Similarly in the transgenic mouse model in which expression of mature TGF-β1 was targeted to the liver, the mice developed elevated levels of type 1 collagen and sever hepatic fibrosis Sanderson et al. (1995).

In other experimental models, where they used knockout mice, it led to a lethal multifocal inflammatory disease, which later led to death ,and this gives an idea of how it also plays different roles in maintaining homeostasis and in embryo genic development Kulkarni et al. (1993) “kaartinen1995” (). TGF-β 1 signaling has also been blocked in T cells and the bone marrow and the results were similar, where they ended up with sever multifocal inflammatory responses Gorelik; Flavell (2000) Slater, A. F. G., & Cerami (1992). And according to these evidence we can say that TGF-β1 is a master regulator of ECM accumulation and consequently a potential key driver of fibrosis Bartram; Speer (2004). The three isoforms to work binds to the transmembrane receptors the type I receptor (TGF-βRI) and the type II receptor (TGF-βRII), both being threonine/serine kinase receptors Maity et al. (2020). Where TGF-βRII is usually kinetically active while TGF-βRI requires activation via TGF-β Maity et al. (2020). When TGF-β link to TGF-βRII this promotes a phosphorylation of TGF-βRI, which will phosphorylate the SMAD2 and SMAD3 to form a hetero-dimeric complex with the common smad known as SMAD4, together this complex translocate into the nucleus and result in the activation and transcription of the related genes to perform different biological functions which include; ECM production, epithelial mesenchymal transition induction, apoptosis and fibroblast proliferation Maity et al. (2020). But in addition to its SMAD signaling pathway it also has a known non-SMAD pathway through other transducers, and although they play a different biological role they may also play a role in regulating the SMAD pathway Lan (2011) .

2.3.1 SMAD

SMAD, named after the members Mad (the Drosophila gene Mothers Against Decapentaplegic) and Sma (the Mad homologs observed in the nematode Caenorhabditis elegans) Newfeld et al. (1996), have shown to be activated by a variety of cytokine upstream pathways through pleiotropic molecules and are thought to constitute the core of regulation for multiple genes with diverse activities Massagué et al. (2005).

SMAD's are proteins, in the vertebrates they are eight known SMAD 1 -8,characterized by a transcription factor function that responds to TGF-β signaling, which appears after the phosphorylation of their receptors Massagué et al. (2005). The SMAD are classified according to their function into two subgroups; the R-SMAD are what is known as receptor activated sMADs, and they include SMAD's 1,2,3,5 ,and 8, and the I-SMAD known as the inhibitory SMAD's and this group includes SMAD 6 and SMAD 7.And then there is a CO-SMAD or a common smad and that is SMAD4 Guo; Wang (2009). The R-SMAD's are activated via the phosphorylation of their Carboxy terminal by either TGF-βRII (including SMAD2 and SMAD-3) or by BMP receptor (BMPR) (and include SMAD-1, SMAD-5, and SMAD-8).And once activated they will bind to the Co-SMAD forming a heterodimer, this heterodimeric complex translocates into the nucleus and activates the targeted transcription genes Guo; Wang (2009). R-SMAD and Co-SMAD contain two highly conserved domains, the Mad-homology domain 1 (MH1) and the MH2 domain, which are connected together by linker region. Where their MH1 domains interact with the DNA, the MH2 domains are endowed with transcriptional activation properties Piek et al. (2001).

I-SMAD(which includes SMAD-6 and SMAD-7 as mentioned), on the other hand, inhibit the signaling activity of the R-Smads and the formation of the Co-SMAD/R-SMAD complex, which is considered important for creating a balance during non-fibrotic functionsMacias et al. (2015). These two SMAD's are activated by BMP and TGF-β through the binding of their MH2 domain the type 1 receptor, thus preventing recruitment and phosphorylation of effector SMAD's. And hence can be described as an auto-inhibitory feedback mechanism for ligand induced signaling Massagué et al. (2005), Howell et al. (1999) Macias et al. (2015).

Going back to the R-SMAD's, specifically SMAD-2 and SMAD-3, both SMAD's have shown to play a role in EMT in the context of fibrosis, and that is through the TGF-β1 signaling pathways. The different development roles of these two SMAD have been reported in a number of studies, such as the absence of SMAD-2 or SMAD-3 expression resulting from targeted deletion of the respective SMAD genes in mice models. For instance, targeted deletion of either MH1 or the MH2 domain of the smad2 gene, was found to be lethal to the embryos , due to failure to set up an anterior-posterior axis, gastrulation and mesoderm formation "li1998" (), Piek et al. (2001)Weinstein et al. (1998).While the mice targeted disruption of the SMAD-3 gene on the other hand where found to be viable and survived for several months, which indicates that SMAD-3 is dispensable for embryonic development Datto et al. (1999) Yang et al. (1999). With the fact that SMAD-3 knockout mice were found to be a lot smaller than the wild type ones, with the presence of some type of limb malformation Datto et al. (1999)Yang et al. (1999).

Although the mice lacking of SMAD-3 weren't embryonically lethal, several organs were affected by a sever chronic inflammation and this led to their death after a while, as a consequence of impaired immune function including defects in mucosal immunity Yang et al. (1999). Therefore, SMAD-3 is not important to embryological development but it is crucial in the pathobiology of fibrosis development Xu et al. (2016). This was explained that there was a lack of responsiveness of SMAD3-deficient T cells to the growth inhibitory effects of TGF-β1, and also due to the lack of the chemotactic response of smad3 deficient neutrophils Datto et al. (1999) Yang et al. (1999).

In an investigation done using mouse embryo-derived fibroblasts deficient in expression of Smad2 and Smad3, in order to detect the effect of loss of each of these key signaling intermediates on induction of target gene expression by TGF-β1 Piek et al. (2001). The experiment revealed that both SMAD-2 and SMAD-3 have distinct roles in TGF-β1 signaling, dependent on target gene and cellular context Piek et al. (2001). TGF-β1-induced fibronectin synthesis occurs in the absence of SMAD-2 or SMAD-3 expression, but they both showed a role in induction of PAI-1 protein Piek et al. (2001). The study also showed induction of matrix metalloproteinase MMP-2 is selectively dependent on SMAD-2 ,and on

the other hand that auto induction of TGF-β1 in fibroblasts was strongly suppressed in the absence of SMAD-3 Piek et al. (2001) .

And although both SMAD-2 and SMAD-3 are found to be activated during fibrosis, SMAD-3 is considered the key element in the signal transduction pathways that are responsible for fibrosis .Fore, SMAD-3 target deletion in mice models has shown to prevent the TGF-β1 induced epithelial to mesenchymal transition and caused attenuation of the development of their fibrotic squeal Roberts et al. (2006). SMAD-3 activation leads to an increased expression of fibrogenic genes, including α-sma and collagen I and III Latella et al. (2009). Besides, SMAD-3 activation through TGF-β1 signaling triggers the induction of tissue inhibitor of metalloproteinase (TIMP) Leivonen et al. (2013) and thus prevents the degradation of ECM Xu et al. (2016).

SMAD-4 as mentioned is considered a common smad in both the TGFβ/BMP 7 pathway and the key molecule for shuttling the SMAD-2/SMAD-3 or SMAD-1/SMAD-5/SMAD-8 into the nucleus, “massague2000” (). Although to the day the role of SMAD-4 remains unclear but the KO of SMAD-4 has also shown lethality in in early embryonic development Sirard et al. (1998),but studies done on kidney disease has given a glimpse of its role, where the distribution of the smad4 gene in kidney tubules decreased ECM synthesis *in vivo* in obstructed kidneys, and *in vitro* in TGF-β1-treated kidney interstitial fibroblasts Meng et al. (2012). Whereas the deletion of the SMAD-4 gene in in mesengial cells result in inhibition of the ECM deposition that was induced by the TGF-β1 Tsuchida et al. (2003).

These differences have also been apparent in carcinogenesis, where SMAD-2 has been implied as a tumor suppressor based on its mutation frequency that was reported in a number of tumors Hata et al. (1998) Massagué (1998). Inactivating mutation of both SMAD-2 and SMAD-4 were found to be associated early in colorectal carcinoma Eppert et al. (1996), Hata et al. (1998). SMAD-3 absence whether of acquired mutations, insertions, or micro-deletions have been reported in both skin and parathyroid tumors (Tannehil-gregg et al. 2004)(Shattuck etal.2002). It is also reported that its somatic mutation in association with both SMAD-2 and SMAD-4 mutations can cause sporadic colorectal carcinoma Fleming et al. (2013). In addition to this it was also found that SMAD-3 through its inhibition E4BP4-mediated NK cell development, can promote cancer

progression. Accordingly, SMAD-3 functions as both a negative and positive regulator of carcinogenesis depending on the cell type and on the clinical stage of the tumor (Millet and Zhang 2007).

2.3.2 Bone morphogenic protein7 BMP7

Another important member of the TGF-β family is the Bone morphogenic protein (BMP) which was named so in the 1970's by Marshall Urist, when he made a huge discovery of its role in osteogenesis and bone formation (Urist 1965). In addition they have found that members of this family also have roles in cell growth and differentiation in tissue repair and embryogenesis Harland (1994). There are around 20 BMP's that have been identified, and they are subdivided into four groups, 1) BMP-2/4, 2) BMP-5/6/7/8a/8b, 3) BMP-9/10, and 4) BMP-12/13/14 based on their function and amino acid sequence similarity Bragdon et al. (2011).

Our main concern of this large family is BMP 7, or what's formerly known as Osteogenic protein1. The Bmp-7 compromises an anti-inflammatory, anti-fibrotic, anti-apoptotic, and a proliferating stimulating function Sugimoto et al. (2007)Zeisberg; Bottiglio; et al. (2003). BMP 7 is expressed in a variety of organs, including sensory, reproductive , lymphoid and organ protectors Dudley et al. ()Nakase; Yoshikawa (2006) Luo; J J (), where it is considered a pleiotropic growth factor and plays a crucial role in the development of various tissues and organs, plus it plays a role where it maintains a number of physiological processes such as bone development through calcium regulation and the healing of fractures Nakase; Yoshikawa (2006). In addition it has shown to have an important role in kidney and eye formation, where Bmp-7 KO mice die directly after birth due to renal failure Dudley et al. ()Luo; J J ().While other studies has shown that the reduction of BMP7 expression leads to Mesenchymal epithelial transition (MET) a number of diseases, mentioning osteoporosis "winkler2003" (), diabetes Chattopadhyay et al. (2017), and even cardiovascular disease Aluganti Narasimhulu; Singla (2020). The pathway by which BMP 7 works is similar to that of the TGF-β, where it binds to bone morphogenetic protein receptor II (BMPRII) on the surface of cells and activates two major signaling pathways the Canonical/SMAD dependent and the Non-canonical/Smad independent pathway Boon et al.

(2011). In the canonical /SMAD dependent pathway the phosphorylation of the BMP R I via BMPRII results in the activation of the regulatory Smads that are specific for BMP (SMAD-1, 5, and 8). Thereafter, phosphorylated regulatory SMAD proteins form a complex together with the co-stimulatory molecule Smad-4. This complex is then transported to the nuclei to activate the related genes including genes responsible for and genes that regulate osteogenic gene expression and consequently influences osteoblast differentiation Bragdon et al. (2011)Boon et al. (2011).

In addition to its canonical pathway like TGF-β1, it can also activate a non-canonical cascade that results in the activation of ERK (extracellular-signal-regulated kinase) and MAPK (mitogen activated protein kinase), P38 and JNK kinase pathways, which plays either a role in the regulation of the smad pathway or result in an induction of an unrelated biological activity Derynck; Zhang (2003). Going back to the roles of BMP-7 as an anti-fibrotic, it has been reported in a number of researches that BMP-7 is effective in reversing both EMT and the fibrotic effect that is induced by TGF-β, in other words it counteracts the pro-fibrotic effect of TGF-β1.According to this , it is assumed that in a normal physiological state there is balance between TGF-β1 and BMP-7, and that this balance will shift during inflammation and fibrosis towards TGF-β1 Weiskirchen; Meurer (2013). This has made it a therapeutic target in the treatment of both acute and chronic injuries. Zeisberg et al. using mice with acute glomerular nephritis, showed that BMP7 has counteracts TGF-β ability to induce EMT by increasing the expression of e-cadherin and leading to ECM degradation Zeisberg; Hanai; et al. (2003). The same team used rhBMP-7 on two mice models that were genetically modified to mimic long term renal disease, and also found that it inhibited the progression of renal fibrosis Zeisberg; Bottiglio; et al. (2003). Where in previous study it was mentioned that it inhibits the release of pro-inflammatory cytokines in renal tubular cells Gould et al. (2002), preserves the kidney function and increases the patient's survival rate Vukicevic et al. (1998), but the precise mechanism remained unclear. Concerning the liver, studying the anti-fibrotic effect of recombinant BMP7 in CCL4-treated mouse (carbon tetrachloride) models of liver fibrosis, the models showed an increase in the levels of MMp2 and a decreased level of αSMA, and collagen type1 with an improvement of the liver function. Yang et al. (2012).

Lou and colleagues studied the effect of BMP-7 on response to TGF-β1 in the proximal tubular cell line (HK-2) Luo et al. (2010) and came up with the conclusion that BMP7 specifically results in limitation of SMAD-3, with no effect on SMAD-2, and that this limitation didn't alter SMAD-3 phosphorylation, accumulation in the nucleus nor did it alter the phosphorylated SMAD-3 Luo et al. (2010).But what BMP7 did here, was it reduced SMAD-3 DNA binding to a consensus SMAD binding element probe, related to a failure to degrade the transcriptional repressor SnoN in BMP-7-treated cells Luo et al. (2010).Noting that SnoN is a related protein,, that binds to SMAD proteins and to SMAD binding elements in DNA and repress Smad-dependent transcriptional activation. SnoN seems to be restricted to TGF-dependent Smad inhibition. Recent work demonstrated that SnoN degradation is required for Smad3- but not Smad2-dependent transcription Luo et al. (2010). Whereas a study of mesengial cells, showed that the induction of the inhibitory Smad6 by BMP-7 led to loss of TGF- signaling and failure of nuclear Smad3 accumulation Wang; Hirschberg (2004). Higgings on the other hand reported that this inhibition is done through the decreased activation of PI3K signaling via Akt and also the inhibition of SMAD-3 phosphorylation without any effect reported on SMAD-2 or the SMAD2/3/4 complex Higgins et al. (2017). These results might give an idea of the opposing effect between BMP-7 and TGF-β and is shown in figure1.

Reversing metastasis through blocking EMT has also been widely studied mentioning in that field; Na et al. showed that BMP-7 inhibits migration and invasion ability of WM-266-4 melanoma cell lines by decreasing the expression of Snail, Slug, and Twist transcription factors Na et al. (2009) and in a study of choloangicarcinoma (CCA) where the TGF-β1 enhanced the migration behavior of CCA cells associated with an up-regulation of Twist , the BMP 7 showed to decrease it in comparison to the control, with a decreased Twist nuclear expression. Suggesting that BMP-7 might be used as an EMT inhibitor to prevent CCA metastasis Duangkumpha et al. (2014). Miettinen et al. showed that TGF-β can induce EMT-related cell migration and invasion in normal mammary epithelial cell lines by the changing of cell morphology and decreasing the expression of the epithelial marker, E-cadherin, whereas the expression of mesenchymal markers were increased Miettinen et al. (),and since the TGF-β1 receptor signaling pathway appears to play a critical role in the carcinogenesis of breast cancer, in a research done using two

different human breast cancer cell lines. They found that BMP7 did not alter the TGFβ1-stimulated phosphorylation of TGF-β receptor, but what it did is that it inhibited the TGFβ1-induced activation of epithelial-mesenchymal transition (EMT) associated genes in these breast cancer cells, resulting in a significant reduction in TGFβ1-triggered cell growth and cell metastasis Ying et al. (2015).

FIGURE 3

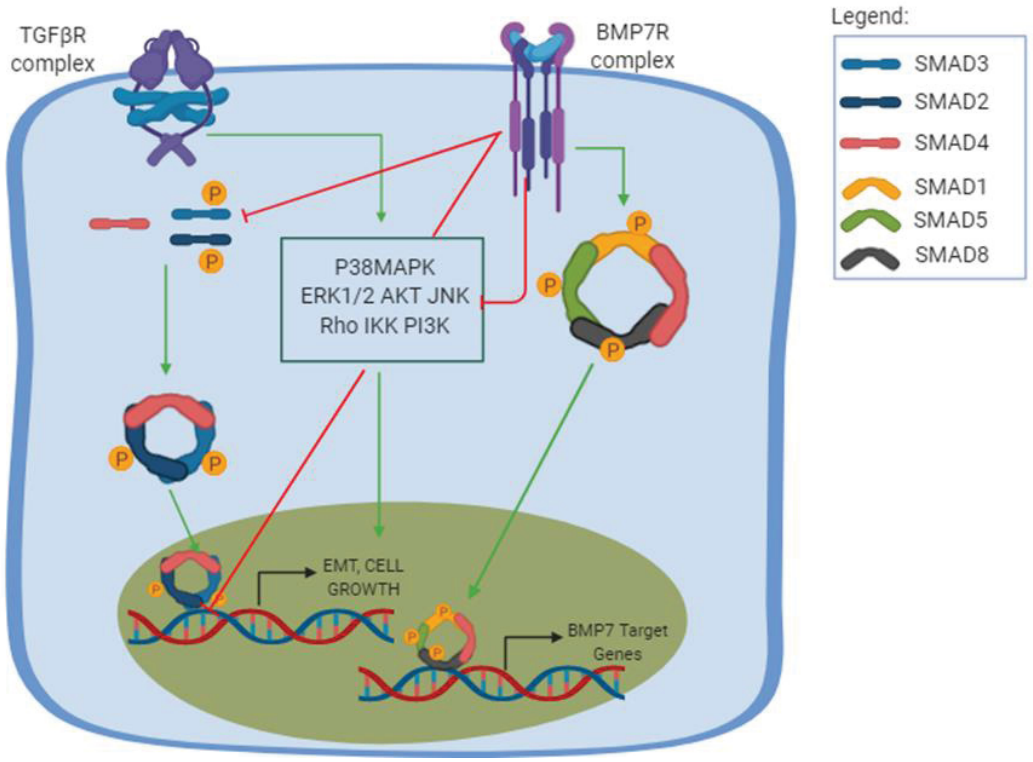

Figure 3: TGF-β and BMP-7 pathways. The TGF-β pathway is shown in its canonical and non-canonical forms. The canonical pathway is Smad-dependent, in which the TGF-β binds to the TGF-βRI and promotes a phosphorylation of TGF-βRII, which will posteriorly recruit downstream signals that results in the phosphorylation of both Smad2 and Smad3 to form a trimer complex with the Smad 4, a common Smad. This results in the trimer translocation into the nucleus and activation of a number of genes related to cellular functions and profibrotic effect including EMT, cell growth and apoptosis. The non-canonical pathway, also known as the non-Smad signaling pathways, is mediated by other

transducers, such as the p38 mitogen-activated protein kinase (P38MAPK) pathways, that includes each of the extracellular signal regulated kinases (ERKs), AKT, c-Jun amino terminal kinase (JNK), Rho family GTPases, as well as the IkB kinase (IKK) and phosphatidylinositol-3 kinase (PI3K). The non-canonical pathway results in different cellular functions and can amplify the Smad pathway. The BMP-7 pathway is activated after the binding of the BMP-7 to the BMP-7R complex and results in the phosphorylation of the Smad1/5/8 to form a complex with the common Smad4 and translocate in the nucleus to activate the opposing function to TGF-β, creating a normal physiological balance between the TGF-β pathway, ultimately leading to an anti-fibrotic effect, increasing ECM degradation and inducing MET.

TGF-β:Transforming growth facro beta,BMP-7:Bone morphogrnic protein7,P38 MAPK: P38 mitogen activated protein kinase, IKK:Ikb kinase,JNK: c-Jun amino terminal kinase,PI3K:phosphatidyl inositol-3 kinase,ERK: extracellular signal regulated kinase.

2.4 NLRP3 Inflammasome

The innate immune system being the first line of defense against any harmful stimuli, becomes activates once exposed to one via the pattern recognition receptors (PRR). Takeuchi; Akira (2010). The PRR is characterized by the ability of sensing different pathogens and their components, which are either pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs) that are generated by endogenous stressTakeuchi; Akira (2010). This recognition is then followed by an activation of a downstream inflammatory pathway, which will end up in removal and elimination of harmful microbes , and the repair of the damaged tissues Franchi et al. (2009). One of these inflammatory pathways is what is known as the inflammasome, that causes the cleavage of pro-caspase 1 ending up with the maturation of the inflammatory cytokines IL1-β and IL18 Franchi et al. (2009).

A decade ago, Jurg Schopp and his team introduced to us what today is known as the inflammasome ‘a sensor of danger’ which was described as a protein complex composed of nucleotide-binding oligomerization (Nod) receptor, capable of activating an inflammatory caspase that will eventually lead to IL-1 β production Martinon et al. (2002).Members of

this Nod receptor family, which are shortly known as the NLRs are composed of three domains, a carboxy-terminal leucine-rich repeat (LRR), which was described in its early discovery as the one responsible for the sensing of the pathogen Ng; Xavier (2011). A central NACHT domain (also known as NOD domain) which has an ATPase activity required for NLRP3 oligomerization following activation Duncan et al. (2007), and an effector amino-terminal (N-terminal) domain (PYRIN or caspase recruitment domain CARD) which is required for protein– protein interactions Proell et al. (2008). Hafner-Bratkovicˇ has reported that the LRR domain function is still to be considered unclear, since it was found that the inflammasome was fully activated in the absence of LRR, suggesting that its sensor function is probably executed by another domain Hafner-Bratkovič et al. (2018).

In the humans, the NLR family is a large family of 22 members, but it's classified into 4 subfamilies according to their effector N-terminal domain configuration; the NLRA (with the acidic domain), NLRB (BIR domain), NLRC (CARD domain) and NLRP (Pyrin domain) subfamilies Ting et al. (2008).

The subfamilies which constitutes of Pyrin as an amino terminal are considered to be the major forms of the inflammasome: NLRP1, NLRP3, Aim-2, and Pyrin S.A.; K.A. (2011)Zoete et al. (2014). In general we can describe it that once these inflammasomes are exposed to either a PAMP or DAMP , their proteins bind and the pyrin domain of the NLRP interact with ASC adaptor protein resulting in the inflammasome assembly. Which then causes the activation of the caspase-1 through the binding and cleavage of its inactive precursor the procaspase-1 Zoete et al. (2014) Manji et al. (2002). Procaspase-1clustering results in the auto-cleavage to lead to the formation of the active form caspase-1 p10/p20 tetramer, to cause the processing of the pro-forms of IL1-β and IL-18 cytokines, making them active Schroder; Tschopp (2010). In addition to the release of the inflammatory cytokines, it was found that it also causes the cleavage of gasdermin-D, which causes the N-terminal domain of GSDMD to form pores in the plasma membrane, and triggering pyroptosis a type of pro-inflammatory programmed cell death Fink; Cookson (2006).

The most studied inflammasome is the NLRP3 inflammasome. This is linked to the fact that NLRP3 inflammasome has been found to be involved in a variety of inflammatory

disorders when deregulated or activated, including cryopyrin-associated periodic syndromes (CAPS) Booshehri; Hoffman (2019), Alzheimer's disease Heneka et al. (2013), diabetes Ding et al. (2019), gout Dalbeth et al. (2016) and atherosclerosis Grebe et al. (2018). Figure 5 shows the structure of the mostly studied NLRP3.

Activation of the NLRP3 inflammasome is divided into a canonical and non-canonical. NLRP3 activators alone are found to be insufficient for the inflammasome assembly ,and for the complete activation it was found that a priming signal is required .This is activated by a priming stimuli, such as ligands for toll-like receptors (TLRs), NLRs (e.g. NOD1 and NOD2), or cytokine receptors, which activate the nuclear factor (NF- κB) that controls the transcription Bauernfeind et al. (2009). NF-κB is considered a key activator of the inflammasome , and plays a critical role through the transcription of NLRP3, and pro-IL-1β in addition to an anti-inflammatory role that was reported where it was found to prevent immature and excessive NLRP-3 inflammasome activation Zhong et al. (2016). The second step in the canonical pathways, following the NLRP3 activation is the oligomerization phase of the complex, this step results in PYD domain clustering, which leads to a homotypic interaction with the PYD- and CARD- containing adaptor ASC, and this results that the CARD- domain in the ASC will recruit with the CARD- of the procaspase1, hence it's cleavage and activation Schroder; Tschopp (2010). After the inflammasome assembly it will be activated and start performing its functions, which are converting the inactive pro-IL-1β and pro-IL18 to their active forms Pellegrini et al. (2017). In addition and as mentioned it will also result in the cleavage of the pore forming protein known as Gasdermin-D (GSMD) to result in a type of programmed cell death called Pyroptosis Shi et al. (2015). This type of cell death is and inflammatory form characterized by cell swelling followed by lysis, and ending up with the release of the cells intracellular contents. What happens it the once the GSMD protein is cleaved, it's N-terminal domain is oligomerized and translocates to the inner cell membrane, where it binds to the phospholipids (phosphatidyl-serine and phosphatidylinositol), this will promote the pore formation and thus the release of the pro-inflammatory cytokines IL-1β and IL18 Liu et al. (2016). This pore formation and release will gradually result in the cell swelling and the overflow of the cytosol content resulting in a disruption of its osmotic potential leading to the cells death Liu et al. (2016). In a study done by Shi and colleagues they demonstrated

that this release of IL-1β was increased during pyroptosis. Where they used *gsmd-/-* bone marrow derived macrophages (BMDM) and observed a conserved caspase-1 action downstream the NLRP3 activation. And the results however showed that the secretion of IL-1β in these knockout cells was reduced when compared to the wild type ones Shi et al. (2015).

Another pathway mentioned is the non-canonical pathway , which is different from the canonical pathway in both the activating ligand and the underlying activation mechanism , Which is considered a caspase -1 independent ,but a caspase 11(in mice) and caspase (4/5 in humans)dependent. It was found that caspase-11 non-canonical inflammasome is the only molecular platform capable of inducing an inflammatory response when triggered by the Gram negative bacteria Lipopolysaccharide (LPS), and that is through the interaction between Lipid A of the cell wall and the CARD domain of the caspase 11 Yi (2020).r Other activators of the non-canonical inflammasome include the lipophosphoglycan (LPG) which is found in the protozoa leishmania cell wall, and the oxidized form of endogenous phospholipids, 1-palmitoyl-2- arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC) but until the day they are unclear and further studies about them seem to be required (Carvalho et al.2019).

This pathway is also first initiated through an intracellular TLR adaptor, TIR-domain-containing adapter-inducing interferonβ(TRIF),which is capable of recognizing gram-negative bacteria, and results in the induction and activation of the non-canonical pathway Yi (2020).In this pathway, in addition to the classical translocation of the NF-κB transcription factor into the nucleus, promoting the transcription of NLRP3 and pro IL-1β that occurs in the priming signaling ,here it will also result in the transcription of regulatory factors of interferon's 3 and 7 (IRF3 and IRF7) Pellegrini et al. (2017). Once this extracellular LPS derived from the gram-negative bacteria reaches the host cells it enters by receptor-mediated endocytosis, and then this internalized LPS directly interacts with caspase11Yi (2020)forming the LPS-non-canonical inflammasome Yi (2020). And although it was reported that caspase 11 and caspase 4/5 don't play a direct role in the activation of IL-1β or IL18 Viganò; Mortellaro (2013). But Viganò et al has confirmed that a functional caspase 11 is required in LPS primed macrophages for the processing of caspase 1 and the release of IL-1β and IL18, as much as it requires NLRP3 and ASC,

whereas IL-1α production is fully dependent on caspase-11Viganò; Mortellaro (2013).And that Casp11-/- mice, exhibited defects in IL-1β production as reported by Kayagaki1 et al Kayagaki et al. (2011). Yi et al also supported this recently reporting that the non-canonical pathway activation plays a role in the proteolytic activation of GSDMD generating N-GSDMD-mediated membrane pores, allowing the secretion of the pro-inflammatory cytokines IL-1β and IL18, followed by increase in the osmotic pressure, cell swelling and eventually cell lysis known as the programmed cell death the pyroptosis Yi (2020).

A more recent structure that has been implicated to the inflammasome, is the NIMA-related kinase 7 (Nek7) and specifically the NLRP3 inflammasome, where it was reported that it doesn't interact with either NLRC4 or AIM2 inflammasome He et al. (2016). Nek7 was reported by Schmid-Burgk et al. in an experiment based on a CRISPR/Cas9 technology that it is essential for the activation of NLRP3Schmid-Burgk et al. (2016),and Nek7 was found to bound to the NLRP3 inflammasome through the interaction with the LRR region Shi et al. (2016). It was also reported that the deficiency of Nek7 in macrophages led to the failure of activation of caspase1 and hence the absence of the release of IL-1β and IL 18 He et al. (2016). But until the day the structure and the pathways of the inflammasome remains controversial proving the requirements of more further studies.

FIGURE 4

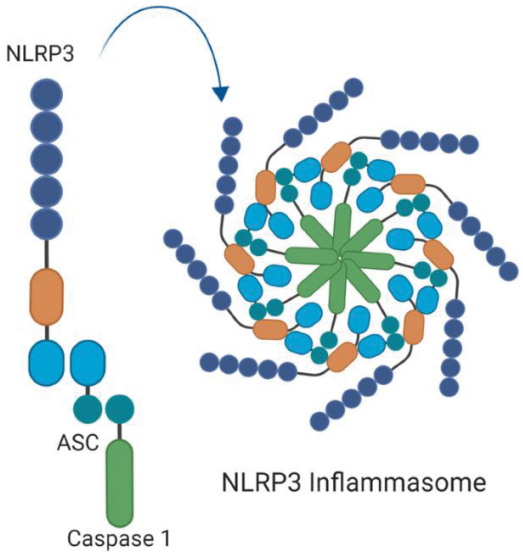

Figure 4 : Showing the Inflammasome NLRP3 main confirmed structures after assembly which are three domains, a carboxy-terminal leucine-rich repeat (LRR), which was described in its early discovery as the one responsible for the sensing of the pathogen A central NACHT domain (also known as NOD domain) which has an ATPase activity required for NLRP3 oligomerization following activation and an effector amino-terminal (N-terminal) domain (PYRIN or caspase recruitment domain CARD) which is required for protein– protein interactions.Silvis et al 2020.

2.4.1Interleukin-1β (IL-1β)

Menkin and Beeson studied in the 1940s the pathogenesis of fever. In their studies they reported that once the rabbit peritoneal exudate cells were stimulated, they were able to release factors, which caused the induction of fever (Dinarello 2002). Several years later another researcher Waksman, studying myeloid cells described a soluble factor which was released from them, this factor was found to activate lymphocytes , and they named them accordingly lymphocyte activation factor, which was renamed interleukin in 1979 (Dinarello 2002).

The interleukin family consists of 11 members (IL-1α, IL-1β, IL-1RA, IL-18, IL-36Ra, IL-36α, IL-37, IL-36β, IL-36γ, IL-38, and IL-33) Dinarello (2011), the importance of these interleukin family members was noticed when they discovered that that the cytoplasmic domain of the IL-1 receptor type I (IL-1R1) is also found in the Toll protein of the fruit fly. Both the TLR and IL-1 families nonspecifically were found to play a role in both the augmentation of the antigen recognition and the activation of lymphocyte function “gay1991” ().These members are divided into three subfamilies based on their consensus sequence and the primary ligand receptor to which they bind. With the exception of IL-1Ra, all members of the IL-1 family lack a signal peptide and are not secreted active Dinarello (2018).They are usually found diffusely in the cytoplasm as precursors Towne et al. (2011).

There are 10 members of the IL-1 family of receptors. IL-1R1 binds IL-1α, IL-1β, and IL-1Ra. IL-1R3 (formerly IL-1R accessory protein) is the co-receptor for forming a trimeric signaling complex with IL-1α or IL-1β. IL-1R1 and IL-1R3 are present on the cell membraneBoraschi et al. (2018). Over the last years many studies have been focused on it,

and came with the conclusion that it doesn't only mediate fever, but it has a role in mediating acute and chronic inflammatory disease Dinarello (2011), including osteoarthritis Kapoor et al. (2011) (Vincent TL), rheumatoid arthritis, and diabetes (Ruscitti et al 2018). Where this pro-inflammatory cytokine was found to be synthesized as a precursor protein, and for activation it's cleaved together with IL-1α and expelled from the cell very early in the course of inflammation, playing a role in assisting host defense against infection and inducing first localized inflammation, and eventually leading to a systemic inflammation and fever. Both IL-1α and IL-1β share IL-1R1 as their common receptor (Dinarello 1996), which were proven when a low dose of recombinant IL1β protected mice from lethal bacterial infection Van Der Meer et al. (1988). In 1992 the fact that IL-1β was secreted inactive and became activated by caspase-1 was still unknown, where Thornberry identified the requirement of an enzyme for the maturation of IL-1β, and at that time caspase was still being identified as Interleukin 1-converting enzyme (ICE) "thornberry1992" (). It wasn't until ten years later that Fabio Martinon and Jurg Tschopp introduced to us to what is known as the inflammasome, as a cytosolic protein complex responsible for the processing of the pro-inflammatory cytokines through the activation of caspases Martinon et al. (2002).After the activation via caspase-1 , the IL-1 β bind to IL-1 receptor 1 (IL-1R1). IL-1R1 which belongs to the large family of Toll-like/IL-1 receptors(TIR)superfamily receptors. And similar to the Toll like receptors (TLRs), IL-1R1 signals through the adaptor protein MyD88, which will then lead to the activation of the transcription factor NF_kB to initiate the inflammationRe et al. ().

The inflammasome and The IL-1β are involved in the pathogenesis of many inflammatory disorders, although yet the mechanism by which IL-1βis involved in fibrosis can be considered still unclear and complex. One of the main hypothesis, explains that this relationship is between the inflammatory cytokine and TGF- β Luo et al. (2009). According to Luo et al. the short exposure of IL-1β inhibits the action of TGF-β via the NF-κB pathway, and consequently, the Smad pathway, responsible for the transcription of the TGF- β -encoding genes. However, this situation is transient due to a change in the NF-κB heterodimer from p65/p50 at 24h exposure to IL-1βLuo et al. (2009).The newly formed heterodimer is not capable of inactivating Smad and thus the prolonged exposure to IL-1β will ultimately promote the expression of TGF-β via SMAD Lee et al. (2006). And so such

data can be considered to represent the first finding regarding the potential of IL-1β in activating TGF-β in vascular muscle cells Lee et al. (2006).

Similar and considered the most identical to IL-1β is the IL18, which also requires to be cleaved for activation. This cleavage was also found to be mediated via the inflammatory caspase, such as caspase1,which is activated via the inflammasome, and which converts the inactive full length Il-18 to its 17 kDa mature form to be secreted Novick et al. (2013).Once its activated, it is released mainly by the myeloid cells, binding to its own receptor, the IL18 receptor, but yet considered similar to the IL-1R1.IL18 was found to induce an increase in the (IFN)-γ production. Accordingly, in autoimmune diseases such as lupus erythematous, psoriasis, or inflammatory bowel disease, that are provoked by interferons, the levels of IL18 has been found to be raised in inflamed tissue Dinarello (2009).This pro-inflammatory cytokine has proved its relation to EMT in a study performed by Bani-Hani and colleagues, using transgenic mice overexpressing human IL-18-binding protein in HK2 cells, where fibrosis was enhanced by ureteral obstruction, and led to a significant increase in IL-18 production, collagen deposition, α-sma, TGF-β1, and TNF-α production, whereas E-cadherin expression was simultaneously decreasedBani-Hani et al. (2009).Further analysis revealed that neutralization of IL18 led to reductions in these indicators of obstruction-induced renal fibrosis and EMT, without any alterations in TGF-β1 or TNF-α activityBani-Hani et al. (2009). Another study done by Zhang and colleagues showed that the IL-18 is highly expressed in bleomycin-induced pulmonary fibrosis, here they used an IL-18 binding protein to block IL-18, and, as result, it prevented the EMT induction through the reduction of α-sma and the reestablishing of e-cadherin Zhang et al. (2019). The downstream signaling pathway of IL18in the induction of EMT, is believed to be through the transcriptor SNAIL1, where this is supported by the finding that this factor was found to be up-regulated after IL18 treatment. In addition the SNAIL1 knockout approach reduced the α-sma and increased the e-cadherin using the same IL18 treatmentZhang et al. (2019).

2.4.2The role of EMT on inflammasome assembling

The NLRP3 protein is best known as the functional component of the inflammasome, which recruits together with ASC and cleaves pro-caspase1, to result in the maturation of the pro-inflammatory cytokines IL1-β and IL18.And until the day the role of the inflammasome in fibrotic disease still remains a question and whether this relation is inflammasome dependent or independent requiring only the presence of NLRP3 protein is still controversial .In a study of chronic kidney injury where fibrosis is the main cause of the end stage of the disease, a group of researchers came up with the result that the genetic ablation of NLRP3 protected the kidney from chronic injury and reduced fibrosis following unilateral ureteric obstruction(UUO) in mice Vilaysane et al. (2010).Where the results showed a significant decrease in all of the inflammasome components, including caspase1, IL1-β and IL18 Vilaysane et al. (2010).But the exact role was unknown ,and that the tubular epithelial cells showed a significant expression of NLRP3 that contributed to the experimental disease pathogenesis Vilaysane et al. (2010). While another research led by Wang showed that NLRP3 promotes renal tubular EMT by enhancing TGF-β1 signaling and R-Smad activation. And that Nlrp3-/- mice showed a decrease in the EMT via TGF-β1 to induction and so did the expression of MMP-9, MMP-2, and aSMA decrease Wang et al. (2013),but in this case , the effect of Nlrp3 on MMP-9 and aSMA expression was inflammasome independent, whereas MyD88, IL-1b, IL-18, and caspase-1 were all dispensable. Wang et al. (2013).They also noted a significant reduction in SMAD2/3 phosphorylation in NLRP3 -/- mice, following stimulation with TGF- β1, confirming that Nlrp3 affects early TGF-β signal activation in epithelial cellsWang et al. (2013).These results are also supported by another study, but in colorectal cancer cells, demonstrating that the NLRP3 expression alone , but not the NLRP3 inflammasome complex activation as a whole was required for EMT, in a model in which cancer cells were transfected with inflammasome-related genes, such as NLRP3Wang et al. (2016) .And it was found that cleaved caspase-1 after the induction of EMT via TGF-β1 was not detected supporting the fact that it was inflammasome independent Wang et al. (2016).

Hackett et al. demonstrated that TGFβ-1 induces EMT and that was found in both normal and asthmatic primary bronchial epithelial cells in vitro and he reported that it was smad3 dependent Hackett et al. (2009). In a further investigation based on this finding, Doerner and colleagues tried to link this relation between both TGF-β1 and EMT, and investigated whether the TGF-β1 stimulates bronchial epithelial cells to undergo transition to mesenchymal phenotype, and they also wanted to know if this transition is abrogated by the effect of corticosteroids and if it was enhanced by the pro-inflammatory cytokine IL-1βDoerner; Zuraw (2009).depending on the fact that TGF-β1 levels were found to be high in bronchial alveolar lavage(BAL) obtained from asthmatic patients and that the levels increased simultaneously with the increased allergensRedington et al. (1997) They came out with the result that the induction via TGF-β1 results in an increase in the mesenchymal markers such as collagen I, tenascin C,fibronectin and α-smooth muscle actin, and a decrease in the expression of the epithelial marker e-cadherin, with a significant change in the cell shape Doerner; Zuraw (2009). And that IL1-β enhanced these changesDoerner; Zuraw (2009).Another common fibrotic disease, Idiopathic pulmonary fibrosis , where epithelial mesenchymal transition has shown to play an important role in its development, and EMT being one of the three main sources of fibroblast Salton et al. (2019),a study revealed that the levels of NLRP3 and caspase-1 were significantly increased together with IL-1β in the untreated broncho-alveolar lavage fluid macrophages of IPF patients Lasithiotaki et al. (2016).Based on these information a study that took place in 2017 where they studied the relation between inflammasome NLRP3 regulation of EMT and bleomycin induced fibrosis Tian et al. (2017).Inflammasome related proteins such as NLRP3, ASC and caspase 1 were found to be up-regulated after treatment with bleomycin in the alveolar epithelial cells type2. The treatment with bleomycin also showed a change in the epithelial mesenchymal markers, where e-cadherin decreased and α-SMA increased. And when measuring the levels of IL-1β and IL18 the results showed a significant increase in the levels of IL-1β compared to IL18 which might indicate its role in the progression of pulmonary fibrosis. Later to link the relation of the NLRP3 inflammasome to the EMT , they silenced the NLRP3 and the measurements came back with results, that there was a significant decrease in the NLRP3 proteins and IL-1β together with the mesenchymal markers, while the epithelial markers such as e-cadherin started increasingTian et al.

(2017) .Following these results, measurement of the key regulator TGF-β1 which also showed a reduction after silencing NLRP3.These results suggested that the NLRP3 inflammasome was activated in type II alveolar epithelial cells and that NLRP3 may modulate EMT through TGF-β1 signaling pathwayTian et al. (2017).

And since NLRP3 inflammasome is the best characterized and most widely implicated inflammasome in acute or chronic lung diseases, especially in inflammatory or fibrotic lung diseases Pinkerton et al. (2017). Concerning fibrotic lung disease too, mainly silicosis, a research took place in order to link a direct relation between NLRP3 inflammasome and EMT. Silica proved to be capable of inducing the transition of epithelial cells to the mesenchymal phenotype and it also up-regulated the expression of the NLRP3 inflammasome where both IL-1β and Il18 levels were high Li et al. (2018). And although previous studies may have linked the relation via IL-1β could also activate Wnt/GSK3β signaling ,TGF-/Smad signaling ,and augment TGF-β-induced EMTKaler et al. (2009).In this study the inhibition of the NLRP3 ended up inhibiting the EMT process via IL-1β-TAK1-MAPK-Snail/NF-κB pathwayLi et al. (2018).Romero et al suggests that there is a physical interaction between the NLRP3 and the smad 2/3 transcription factor in the cytosol in order to regulate the expression of genes responsible for EMTRomero et al. (2017).And came out with the conclusion that NLRP3/ASC expression was increased in kidney fibrosis and that it was both inflammasome related caspase-1 and non-inflammasome related P-Smad 2/3 pathways Romero et al. (2017). According to these studies and until the day , we can say that the relation between the inflammasome and EMT remains controversial , and for better clarification more studies are required, especially the point of the dependence of the NLRP3 complex formation for the EMT process to occur.

3. MATERIALS AND METHODS

3.1 Cell lines

The cell lines used in the project were the human proximal tubular cell line HK2 cells that were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and at 37°C in a humidified atmosphere of 21% O^2 and 5% CO^2, These cells were chosen for their ability to be easy moved to in *vivo*. The murine proximal tubular epithelial cells MM55.7 that were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 %fetal bovine serum and at 37^o C in a humidified atmosphere of 21% O^2 and 5% CO^2. And the human embryonic kidney cell line HEK-293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and the at 37°C in a humidified atmosphere of 21% O^2 and 5% CO^2.

3.2 Preparation of the inflammasome reporter genes

In the present work the genes of interest are those encoding the inflammasome-forming proteins NLRP3 and ASC. The plasmids were obtained by donation from researcher Eicke Latz, from the University of Bonn's innate immunity laboratory, on circularly designed filter paper in the center where the plasmids were located. The circle was cut using scissors and inserted into a 1 mL tube along with 40 µL of buffer and centrifuged for 30 seconds to recover the plasmid on the filter paper. The next step was to insert the recovered plasmid into E.coli. To this end, 3 µL of the plasmid was added to 1 mL tubes containing E.coli in solution located in an ice box. For the plasmid to be transformed it was necessary to provide a thermal shock to the bacteria containing the plasmid. The thermal shock was performed at 42ºC for 42 seconds. After this shock the contents of the tube were transferred to a new tube all containing SOC medium and centrifuged at 37 ° C for 45 minutes at 1200 rpm. The last step was to transfer the contents of the SOC medium tube to an ampicillin-containing petri dish, as it is the antibiotic in which the plasmids contain the resistance gene.

3.3 Knockout of Smad3 and BMP-7 via CRISPR

The plasmid obtained was the PX601 and the restriction enzyme used was the BsaI, The gRNA of both smad3 and BMP-7 was designed using the CRISPR design website CHOPCHOP.com ,the sequence of the primers for smad3 is F*; 5'CACCGAATCCGATGTCCCCAGCAC3'* , R; *5'AAACGTGCTGGGGACATCGGATTC3'* and for BMP-7; *F; 5'-CACCCATGAGCTTCGTCAACCTCG-3'* R; 5'*AAACCGAGGTTGACGAAGCTCATG3'*. To open the plasmid we used 1µl of the restriction enzyme BsaI, 5µl of the plasmid, 2µl of buffer and completed to 20 µl with water, the mix was left for 2 hours in 37^{o} c before running the gel and purified.

3.4 Oligo DNA phosphorylation and annealing

For the primers annealing, we used 50µl of the designed primer forward and reverse together kept at 95^{o}c for 10 minutes followed by 25^{o} c for 45 minutes. The binding was done where the designed primers were diluted , and then 10 µl of this diluted primer was prepared with the DNA , T4 ligase buffer and ligase and completed with water left in 4^{o}c for three hours followed by 16^{o}c overnight and then at 22^{o}c for four hours before plating and 72^{o}c for 15 minutes to inactivate the enzyme.

3.5 Miniprep

For the next steps of the project it was necessary to recover these transformed plasmids into E.coli. For this, the miniprep protocol was performed for the formation of a plasmid DNA concentrate. Before starting the protocol, inoculum were prepared to be used in miniprep. These consist of removing a colony from the petri dish and placing them in inoculum glassware with 5mL of LB medium (Luria-Bertani), 2.5 µl of ampicillin. The inoculum glass remains in a shaker at 37ºC overnight (16h to 20h after inoculum preparation).The miniprep protocol started by removing 1.5 ml from the inoculum and adding it to a volume of 1.5 ml. Then centrifuge the tube and discard the supernatant, keeping the pellet in the tube. After discarding, we added 200 µL of the first solution of protocol P1 to suspend the pellet, then we added solution P2 and homogenized the tube. Then the last P3 solution was added. The next step was to centrifuge the tube at full speed (15000 rpm) for 12 minutes. In a new 1.5 mL tube, 600 µL of 70% chloroform was prepared to add 600 µL of the supernatant formed in the last centrifugation. Under the same conditions a new spin in the

centrifugation was done of maximum rotation for 12 minutes. The next step we added 1 mL of ethanol into a new 1.5 mL all which will receive 400 µL of the previous centrifugation supernatant. Then it was incubated at -20 ° C for at least 15 minutes. Finally, it underwent a maximum rotation spin for 20 minutes, the supernatant was discarded and the ethanol was dried and then suspended with 30 µl of MiliQ water. The end result was then kept frozen at -20ºC.

3.6 Transduction

The DNAs of interest were transfected into the HK-2 cells, the transduction was done using Lentivirus delivery, the Lentivirus was constructed using the DNA sequence that encode the viral proteins, that are the capsid and effector moiety. This construction is performed by transforming the DNA of the virus component into the E.coli bacteria to synthesize the proteins that make up Lentivirus. After transformation into the E.coli the DNA was recovered via miniprep for lentivirus assembly, which was transfected into HEK-293 cells next to the DNA of interest. Following the transfection into the HEK 293 cells, the supernatant from the lentivirus containing medium was collected with the DNA to be overexpressed in HK-2 cells. This supernatant was added to the HK-2 cells medium, where Lentivirus delivers the DNA of interest for expression in HK-2.In the present project, the DNA of interest are the inflammasome components, being NLRP3-GFP and ASC-Cerulean. The production of the Lentivirus follows the same steps of obtaining the inflammasome genes. Plasmid DNAs containing the sequence coding for P-CMV and VSV-G viral proteins were obtained by donation from the Department of immunology located at the Institute of Biomedical Sciences of the University of Sao Paulo (USP). The contents were transported on filter paper to Curitiba. The filter paper containing the plasmid was cut with scissors and added into 1mL tube along with 40µl of buffer and centrifuged for 30 seconds to recover the plasmid on the filter paper. The next step was to insert the recovered plasmid into E.coli. To this end the , 3µl of the plasmid was added to 1 mL tubes containing E.coli in solution and put in an ice box .For the plasmid to be transformed it was necessary to provide a thermal shock to the bacteria containing the plasmid , the thermal shock performed was at 42^{o}C for 42 seconds. After the shock the contents of the tube were transferred to a new all containing SOC medium and centrifuged at 37^{o}Cfor 45 minutes at 1200 rpm. The last step was to transfer the SOC medium tube to an ampicillin

containing petri dish as it is the antibiotic in which the plasmid contains the resistance gene. The plate was kept in a temperature controlled colony growing green house that contains only the genes of interest, in this case the genes that encode the proteins to be used to construct the temperature controlled lentivirus, P-CMV, VSV-G .and overnight period .The next day the plates were removed from the green house and pre inoculations were prepared for a miniprep and only the recovering DNA was present in the colonies.

3.7 Sorting

Following Transfection , only cells that have successfully delivered DNAs of interest should have been recovered , for this the cells were sorted using the FACS\Melody equipment at the CTAF(Center for advanced Fluorescence Technlogies) located in the biological Science sector of UFPR. To undergo the process of sorting the cells were removed from their culture media and added to PBS for the passage in the equipment, and should be returned to the culture media after passing through the equipment.

3.8 Induction of EMT using TGF-β

The MM55.7 cells were divided into three groups a control group and a EMT induced group were 5 ng/ml of TGF-β was used to induce the epithelial mesenchymal transition, and a third group that was supposed to be transfected, and following the transfection , EMT was going to be induced via TGF-β . The pictures were taken using microscope Image J at 24 and 48 hours for both the control and induced. The same step was supposed to be repeated on the HK-2 cells using either 5 ng/ml or 10 ng/ml of TGF-β.

3.9 Confocal microscopy

The cells after being transfected with genes encoding the fluorescently labeled inflammasome proteins, NLRP3 GFP + and ASC Cerulean +. After induction of EMT via TGF-β, it was expected to observe the placement of inflammasome proteins in the confocal microscope

3.10 Western blotting

Western blotting was performed on cell lysates collected from murine MM55.7 cells after the induction of EMT using TGF-β for detection of α-SMA, and E-cadherin. Proteins were separated on a Sodium dodecyl sulfate/Polyacrylamide gel electrophoresis gel

(SDS/PAGE), transferred onto a polyvinylidene fluoride (PVDF) membrane and subjected to immunoblot analysis. Blotting was performed using antibodies against α-SMA (1:1000 Dako), E-cadherin (1:2000, Dako). After rinsing with Tris-buffered saline supplemented with 0.1% BSA in (TBST), the membranes were incubated with a fluorescent anti-mouse antibody for 60 min at room temperature. The detection of specific signals was performed using enhanced fluorescent detector this was normalized to the band intensity of GADPH.

4. RESULTS

4.1 The sorting process

The Lentivirus production and the cell sorting plasmids which contain the inflammasome reporter genes were recovered. The plasmids were inserted into E.coli for a further expansion of the plasmid DNA and then retrieved by Miniprep. HEK-293 cells were transfected with the plasmid DNA for Lentivirus production which already contained the reporter genes. After the viruses with the NLRP3-GFP and ASC-Cerulean inflammasome genes were harvested from the HEK-293 cells supernatant and seeded into the HK-2 cells and maintained for 7 days. The next step was preparing the cells for sorting to only recover the cells with GFP and or cerulean, in the end of this step using the CTAF we lost the cells during the procedure were only PBS came out in the tubes, which was a result of a technical problem in the plugs , they were placed in the wrong place.

FIGURE 5

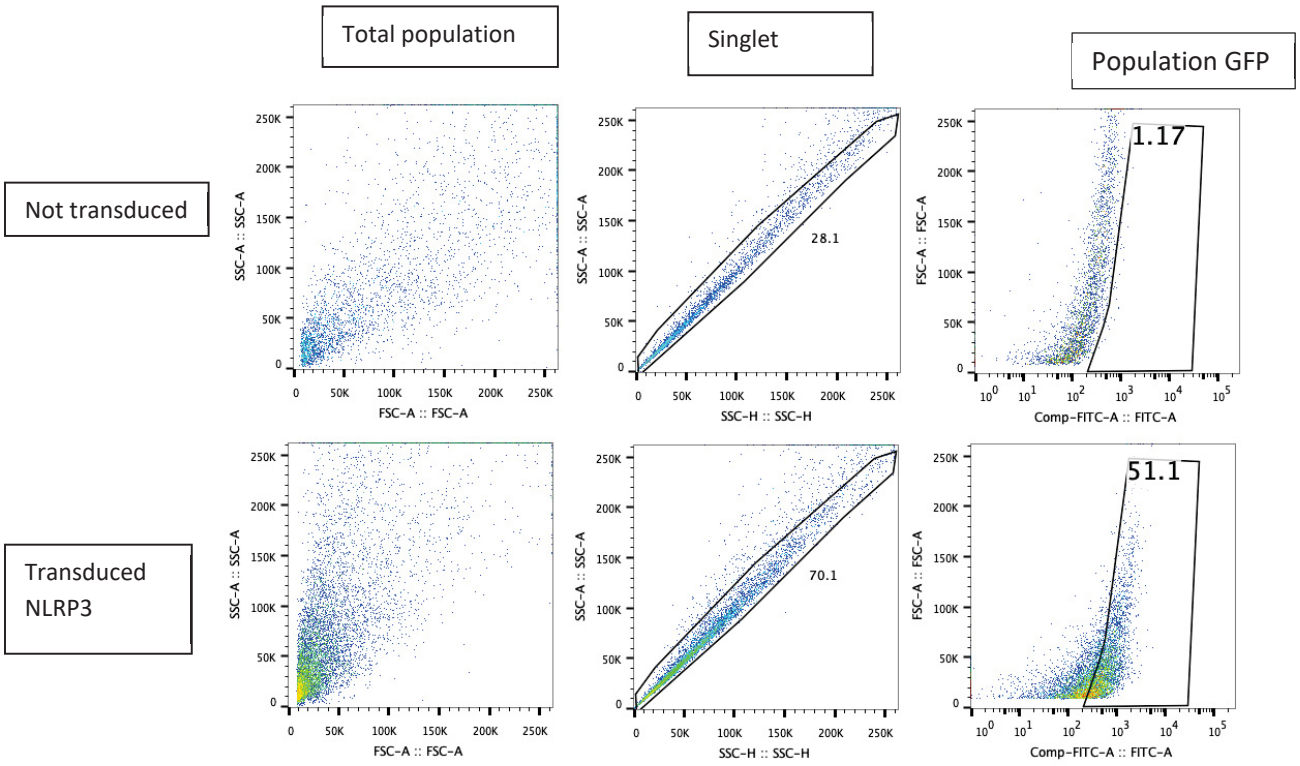

Figure 5: Dot plot. The figure shows the gate strategy and the percentage of GFP positive cells after the transduction protocol.

4.2 Deleting the SMAD3 gene

After cutting the plasmid Px-601 with the restriction enzyme Bsa1, the Smad3 gene deletion guide sequence was designed, and the annealing reaction that makes up the guided sequence was prepared to be inserted in the Bsa1 cleaved site, the next step after that the sequence was ligated into the vector and transformed into E.coli for transfer to an ampicillin containing agar plate in which colonies with the cas9 vector and the smad3 guide sequence grew. After the colony growth a colony PCR was performed to confirm the successful insertion. The PCR product was observed, enabling the mini prep to recover the DNA contained within the bacteria and which will be used to perform the CRISPR-cas9 technique in the chosen cell model. Prior the transfection a new PCR was performed to confirm that the DNA recovered through the Miniprep is our DNA with the inserted guided sequence. The pictures below although showing according to us a successful cut, but due

the results prior the cut , we can expect the fact that some cut-plasmids could have been not successfully cut and open.

FIGURE 6

Figure 6: Cutting of the plasmid Px601. 1: represents the marker used . 2: represents the plasmid obtained from the Lab before the opening and being cut. 3: represents the plasmid after the cut using the restriction enzyme Bsa1.

FIGURE 7

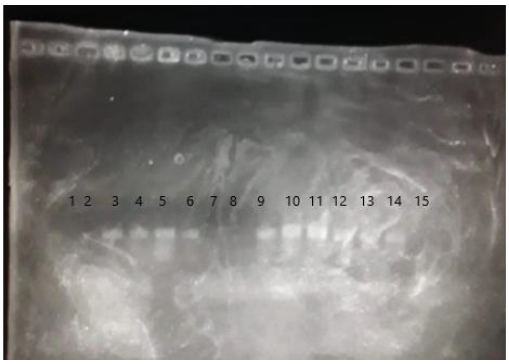

Figure 7: PCR verification. The PCR colony for inserting the SMAD3 was performed showing the success of the insertion in the numbers as shown 3,4,5,6,9,10,11,12,13,and 14.

4.3 Induction of EMT in MM55.k cells

As mentioned the induction of EMT was performed on the murine MM55.K cells using 5 ng/ml of TGF-β, pictures were taken using microscope Image J at 24 and 48 hours for both the control and induced.

FIGURE 8

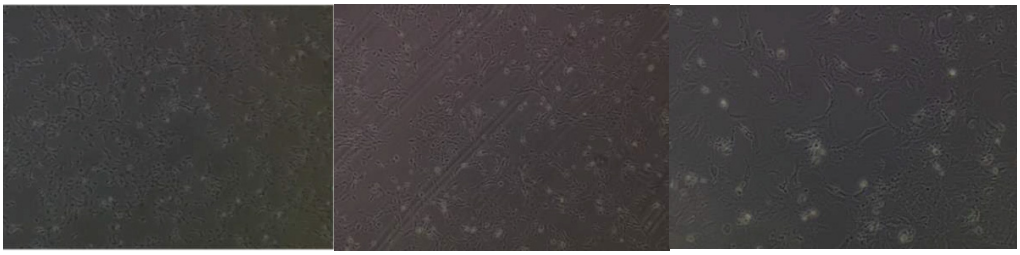

Figure 8: Cells under EMT induction. Showing the MM55.K cells after the EMT induction via TGFβ and in order from left to right they are; The control, after 24 hours, and after 48 hours. * With the note that we wanted to repeat this step but we suffered from contamination of the cells a couple of times .EMT: epithelial mesenchymal transition

4.4 Opening another plasmid for the BMP 7 primer insertion

Another plasmid Px-601 was cut with the restriction enzyme Bsa1, the BMP7 gene deletion guide sequence was designed, and the annealing reaction that makes up the guided sequence was prepared to be inserted in the Bsa1 cleaved site, the cutting showed a success According to the appeared results , but we can't exclude the fact that the plasmid was not open, since there was a failure with the insertion. Unfortunately theses experimets were supposed to be repeated but we never got the chance. The figure below shows only the cutting of the plasmid.

FIGURE 9

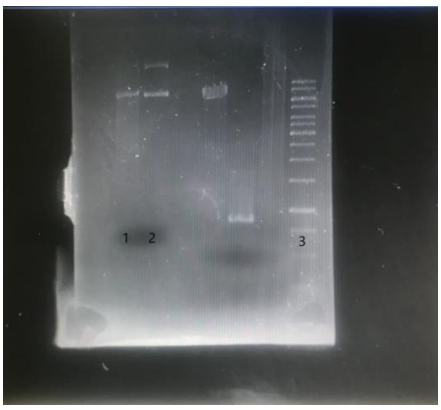

Figure 9: PCR verification. The figure shows the cutting of another plasmid, representing: 1- the plasmid Px601 as obtained from the lab (before the cut). 2- represents the plasmid after the cut using the restriction enzyme as mentioned . 3- represents the marker.

4.5 EMT verification through western blotting analysis

After the induction of EMT in the control Murine MM55.K cells we performed a western blot to detect αSMA and e-cadherin and using NADPH for the normalizing intensity.

When the bands were quantified using the software only the control sheet came out with detection of e-cadherin. Here the e-cadherin is and indicator of the epithelial cells and not the EMT process, our goal was to the detect if EMT was induced , were the α-SMA was supposed to appear in case of the success of the EMT and E-cadherin was to appear in the control since it’s the key indicator here for epithelial cells.

FIGURE 10

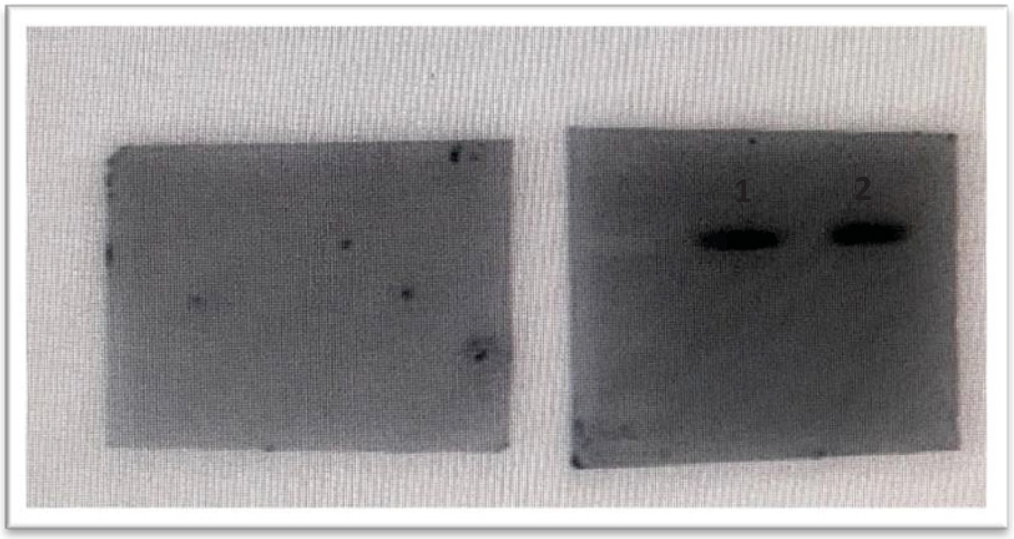

Figure 10: Western blotting analysis. The sheet on the left shows loss of the results. The loss might be due to technical process during our western blot since both the NADPH nor the expected α-SMA are not seen.

In the right sheet: 1- showed the normalizing band NADPH, 2- shows e-cadherin band

αSMA: alpha smooth muscle actin.

5. DISCUSSION

The aiming for therapeutic approaches in controlling fibrotic disease to improve their outcome has been under study for several years, and looking up different pathways that might play a significant role had been a goal of multiple studies. In studying the relation of the inflammasome with fibrosis we can still say that it is not clear yet and it might require a couple more years to reach a definite conclusion. In literature until nowadays the relation between the inflammasome complex and the onset and progression of fibrosis is considered confusing, where researchers such as Wang clarifies that this relation does not require the activation of the inflammasome as whole, but only requires the NLRP3 (W. Wang et al. 2013). And although the results are considered somewhat significant, other studies such as the one performed by Tian links EMT type 2, which is the source of fibroblasts and

myofibroblasts in fibrosis, to the activation of the NLRP3 inflammasome complex and not just to NLRP3 protein and that its induced via TGF-β1 (Tian et al. 2017). Moreover, the relation between TGF-β1 and the inflammasome complex is also considered under the study for the understanding that the inflammasome assembly and activation leads to IL-1β activation and this leads to increased TGF-β1 expression. We can link the fact that the inflammasome complex triggers EMT for fibrosis via TGF-β1 that result from the IL-1β production (Zhang etal.2016). TGF-β1 has also been reported to induce EMT in peritoneal fibrosis via modulation of the NLRP3 inflammasome and oxidative stress in a study of the effect of paricalcitol on EMT (Ko et al.2019). Giving the results as seen, we have faced during our work a couple of difficulties, including the loss of the cells in the sorting which came out only as PBS during the FACS Melody processing. Another difficulty we faced a lot and led to a postponing of a lot of the methods is the contamination of the cells. We also repeated the opening of the plasmid more than once before we got a successful result. After the success of the cut, the ligation reaction of the oligonucleotide sequence was performed and then transformed into *E. coli* so that it could be recovered via the miniprep and later used to silence the Smad3 gene. But as seen, we never got the chance to finish our experiments, and most of them we only performed once. With the information we gained during our work we know that the relation we are trying to study exists, and we have tried to summarized it in figure 9, and that if this relation could be understood properly will have a great influence on the therapeutic approaches for fibrotic disease.

FIGURE 11

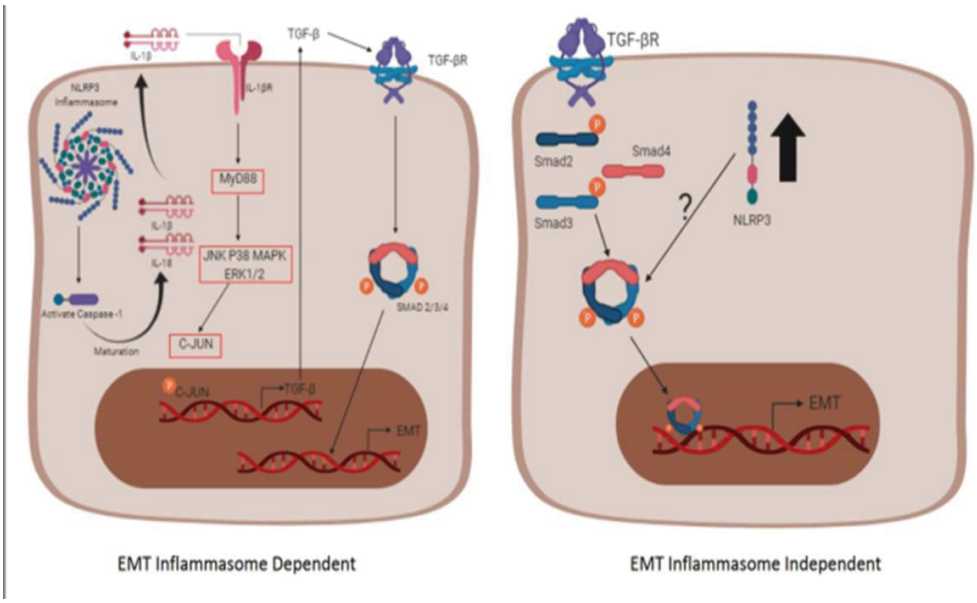

Figure 11: The inflammasome -dependent and -independent pathways involved in EMT. The inflammasome complex are activated after the two signal, priming that promote the transcription of the inflammasome related proteins (NLRP3, Pró-IL1β and Pró-IL18) through NF-κB, and, the activating that promote the oligomerization of the complex, after the inflammasome formation, the maturation of the cytokines IL-1β and IL-18 trough caspase-1. After it, the IL-1β reach its receptor and promote the transcription of the TGF-β gene. The TGF-β, after secretion, binds to its receptor triggering the pathway dependent of the Smads, promoting the Epithelial-Mesenchymal Transition (left cell). There is a pathway that not depend of the inflammasome oligomerization (right cell), this pathway happens when TGF-β reach its receptor and together with the Smads signaling, the expression of NLRP3 are enhanced (black arrow). Another relationship is between the NLRP3 and the Smad3 (Arrow with interrogation) but to fully understand this step more studies are required.

6. FUTURE PROSPECTIVES

In the end, the conclusion we came out with is that EMT process might happen in two ways: one is dependent on the inflammasome activation when the IL-1β executes its pro-inflammatory action by up-regulating the TGF-β pathway; another is independent of the inflammasome activation, being necessary only in the presence of the NLRP3 receptor which enhances R-Smads in the TGF-β/Smad pathway. And on a future aspect I might add that a proper organized plan based on our objectives and experiments in addition to our literature conclusions, with the opportunity to correct our mistakes , we might have better results.

And the correction of our mistakes whether based on having a proper non-contaminated cell environment , or correcting the technical problems we faced by having a proper knowledge on the protocols and equipment, and repeat the experiments we didn't have the ability to repeat. We will hopefully reach a future proper understanding of this pathway and we will have greater understanding of the pathway that can be targeted to have a better outcome from fibrosis, and increasing the patient's quality of life.

REFRENCES

ALUGANTI NARASIMHULU, C.; SINGLA, D. K. The Role of Bone Morphogenetic Protein 7 (BMP-7) in Inflammation in Heart Diseases. **Cells**, v. 9, n. 2, p. 280, 2020. MDPI AG.

ATALA, A. Re: Infiltrating Macrophages Increase RCC Epithelial Mesenchymal Transition (EMT) and Stem Cell-Like Populations via AKT and mTOR Signaling. **The Journal of urology**, v. 197, n. 1, p. 51–52, 2017.

ATRI, C.; GUERFALI, F. Z.; LAOUINI, D. Role of human macrophage polarization in inflammation during infectious diseases. **International Journal of Molecular Sciences**, 19. jun. 2018. MDPI AG.

AYDIN, M. M.; AKCALI, K. C. Liver fibrosis. **Turkish Journal of Gastroenterology**, 1. jan. 2018. AVES.

BANI-HANI, A. H.; LESLIE, J. A.; ASANUMA, H.; et al. IL-18 neutralization ameliorates obstruction-induced epithelial-mesenchymal transition and renal fibrosis. **Kidney International**, v. 76, n. 5, p. 500–511, 2009.

BARTRAM, U.; SPEER, C. P. The Role of Transforming Growth Factor β in Lung Development and Disease. **Chest**, v. 125, n. 2, p. 754–765, 2004. American College of Chest Physicians.

BAUERNFEIND, F. G.; HORVATH, G.; STUTZ, A.; et al. Cutting Edge: NF-κB Activating Pattern Recognition and Cytokine Receptors License NLRP3 Inflammasome Activation by Regulating NLRP3 Expression. **The Journal of Immunology**, v. 183, n. 2, p. 787–791, 2009.

BOON, M. R.; VAN DER HORST, G.; VAN DER PLUIJM, G.; et al. Bone morphogenetic protein 7: A broad-spectrum growth factor with multiple target therapeutic potency. **Cytokine and Growth Factor Reviews**, ago. 2011.

BOOSHEHRI, L. M.; HOFFMAN, H. M. CME REVIEW CAPS and NLRP3. , p. 277–286,

2019. Journal of Clinical Immunology.

BORASCHI, D.; ITALIANI, P.; WEIL, S.; MARTIN, M. U. The family of the interleukin-1 receptors. **Immunological Reviews**, 1. jan. 2018. Blackwell Publishing Ltd.

BRAGA, T. T.; AGUDELO, J. S. H.; CAMARA, N. O. S. Macrophages during the fibrotic process: M2 as friend and foe. **Frontiers in Immunology**, 2015. Frontiers Research Foundation.

BRAGDON, B.; MOSEYCHUK, O.; SALDANHA, S.; et al. Bone Morphogenetic Proteins: A critical review. **Cellular Signalling**, abr. 2011.

CAMPBELL, K. Contribution of epithelial-mesenchymal transitions to organogenesis and cancer metastasis. **Current Opinion in Cell Biology**, 1. dez. 2018. Elsevier Ltd.

CANO, A.; PÉREZ-MORENO, M. A.; RODRIGO, I.; et al. **The transcription factor Snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression**. 2000.

CHANG, H.; BROWN, C. W.; MATZUK, M. M. Genetic analysis of the mammalian transforming growth factor-β superfamily. **Endocrine Reviews**, v. 23, n. 6, p. 787–823, 2002.

CHAPMAN, H. A. Disorders of lung matrix remodelling. **Journal of Clinical Investigation**, 2004. The American Society for Clinical Investigation.

CHATTOPADHYAY, T.; SINGH, R. R.; GUPTA, S.; SUROLIA, A. Bone morphogenetic protein-7 (BMP-7) augments insulin sensitivity in mice with type II diabetes mellitus by potentiating PI3K/AKT pathway. **BioFactors**, v. 43, n. 2, p. 195–209, 2017. Blackwell Publishing Inc.

CLARK, D. A.; COKER, R. **Transforming growth factor-beta (TGF-b)**. .

CRUZ-SOLBES, A. S.; YOUKER, K. Epithelial to mesenchymal transition (EMT) and endothelial to mesenchymal transition (EndMT): Role and implications in kidney fibrosis. **Results and Problems in Cell Differentiation**. v. 60, p.345–372, 2017. Springer Verlag.

DALBETH, N.; MERRIMAN, T. R.; STAMP, L. K. Gout. **The Lancet**, v. 388, n. 10055, p. 2039–2052, 2016.

DATTO, M. B.; FREDERICK, J. P.; PAN, L.; et al. **Targeted Disruption of Smad3 Reveals an Essential Role in Transforming Growth Factor-Mediated Signal Transduction Downloaded from**. 1999.

DERYNCK, R.; ZHANG, Y. E. **Smad-dependent and Smad-independent pathways in TGF-b family signalling**. 2003.

DESMOULIÈRE, A.; DARBY, I. A.; GABBIANI, G. Normal and Pathologic Soft Tissue Remodeling: Role of the Myofibroblast, with Special Emphasis on Liver and Kidney Fibrosis. **Laboratory Investigation**, dez. 2003.

DÍAZ, R.; KIM, J. W.; HUI, J. J.; et al. Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis. **Human Pathology**, v. 39, n. 1, p. 102–115, 2008. W.B. Saunders.

DINARELLO, C. A. Immunological and inflammatory functions of the interleukin-1 family. **Annual Review of Immunology**, 2009.

DINARELLO, C. A. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. **Blood**, v. 117, n. 14, p. 3720–3732, 2011.

DINARELLO, C. A. Overview of the IL-1 family in innate inflammation and acquired immunity. **Immunological Reviews**, 1. jan. 2018. Blackwell Publishing Ltd.

DING, S.; XU, S.; MA, Y.; et al. Modulatory mechanisms of the NLRP3 inflammasomes in diabetes. **Biomolecules**, v. 9, n. 12, p. 1–15, 2019.

DISTLER, J. H. W.; GYÖRFI, A. H.; RAMANUJAM, M.; et al. Shared and distinct mechanisms of fibrosis. **Nature Reviews Rheumatology**, 1. dez. 2019. Nature Research.

DOERNER, A. M.; ZURAW, B. L. TGF-β1induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells is enhanced by IL-1β but not abrogated by corticosteroids. **Respiratory Research**, v. 10, 2009.

DONGRE, A.; WEINBERG, R. A. New insights into the mechanisms of epithelial–mesenchymal transition and implications for cancer. **Nature Reviews Molecular Cell Biology**, 1. fev. 2019. Nature Publishing Group.

DUANGKUMPHA, K.; TECHASEN, A.; LOILOME, W.; et al. BMP-7 blocks the effects of TGF-β-induced EMT in cholangiocarcinoma. **Tumor Biology**, v. 35, n. 10, p. 9667–9676, 2014. Kluwer Academic Publishers.

DUDLEY, A. T.; ~ Y O N S , ', K. M. ~; ~ O B E R T S O N ~, E. J. **A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye**. .

DUNCAN, J. A.; BERGSTRALH, D. T.; WANG, Y.; et al. and requires ATP binding to mediate inflammatory signaling. **Pnas**, v. 104, p. 8041–8046, 2007.

EPPERT, K.; SCHERER, S. W.; OZCELIK, H.; et al. **MADR2 Maps to 18q21 and Encodes a TGF-Regulated MAD-Related Protein That Is Functionally Mutated in Colorectal Carcinoma carcinoma and melanoma cell lines have demonstrated a progressive loss of responsiveness to TGF growth-inhibitory effects as tumor aggressiveness increases (re-viewed by Filmus and Kerbel, 1993; Roberts and Sporn, Lap**. 1996.

EYDEN, B. **The Myobroblast: An Assessment of Controversial Issues and a Denition Useful in Diagnosis and Research**. 2001.

FENYO, I. M.; GAFENCU, A. V. The involvement of the monocytes/macrophages in chronic inflammation associated with atherosclerosis. **Immunobiology**, v. 218, n. 11, p. 1376–1384, 2013.

FINK, S. L.; COOKSON, B. T. Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. **Journal of Immunology**, v. 202, n. 7, p. 1913–1926, 2006.

FLEMING, N. I.; JORISSEN, R. N.; MOURADOV, D.; et al. SMAD2, SMAD3 and SMAD4 mutations in colorectal cancer. **Cancer Research**, v. 73, n. 2, p. 725–735, 2013.

FRANCHI, L.; EIGENBROD, T.; MUÑOZ-PLANILLO, R.; NUÑEZ, G. The inflammasome: A caspase-1-activation platform that regulates immune responses and disease pathogenesis. **Nature Immunology**, v. 10, n. 3, p. 241–247, 2009.

gay1991. .

GHATAK, S.; HASCALL, V. C.; MORENO RODRIGUEZ, R.; MARKWALD, R. R.; MISRA, S. **Inflammation, Wound Healing, and Fibrosis**. 2018.

GHATAK, S.; MAYTIN, E. V.; MACK, J. A.; et al. Roles of Proteoglycans and Glycosaminoglycans in Wound Healing and Fibrosis. **International Journal of Cell Biology**, 2015. Hindawi Publishing Corporation.

GORELIK, L.; FLAVELL, R. A. **Abrogation of TGF Signaling in T Cells Leads to Spontaneous T Cell Differentiation and Autoimmune Disease**. 2000.

GOULD, S. E.; DAY, M.; JONES, S. S.; DORAI, H. **BMP-7 regulates chemokine, cytokine, and hemodynamic gene expression in proximal tubule cells 1**. 2002.

GRANDE, M. T.; SÁNCHEZ-LAORDEN, B.; LÓPEZ-BLAU, C.; et al. Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. **Nature Medicine**, v. 21, n. 9, p. 989–997, 2015. Nature Publishing Group.

GREBE, A.; HOSS, F.; LATZ, E. NLRP3 inflammasome and the IL-1 pathway in atherosclerosis. **Circulation Research**, v. 122, n. 12, p. 1722–1740, 2018.

GUARINO, M.; TOSONI, A.; NEBULONI, M. Direct contribution of epithelium to organ fibrosis: epithelial-mesenchymal transition. **Human Pathology**, v. 40, n. 10, p. 1365–1376, 2009.

GUO, X.; WANG, X. Signaling cross-talk between TGF- β / BMP and other path- ways. , p. 71–88, 2009.

HACKETT, T. L.; WARNER, S. M.; STEFANOWICZ, D.; et al. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by

transforming growth factor-β1. **American Journal of Respiratory and Critical Care Medicine**, v. 180, n. 2, p. 122–133, 2009.

HAFNER-BRATKOVIČ, I.; SUŠJAN, P.; LAINŠČEK, D.; et al. NLRP3 lacking the leucine-rich repeat domain can be fully activated via the canonical inflammasome pathway. **Nature Communications**, v. 9, n. 1, 2018.

HARLAND, R. M. **Commentary The transforming growth factor , family and induction of the vertebrate mesoderm: Bone morphogenetic proteins are ventral inducers**. 1994.

HATA, A.; LAGNA, G.; MASSAGUÉ, J.; HEMMATI-BRIVANLOU, A. **Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor**. 1998.

hay1995. .

HE, Y.; HARA, H.; NÚÑEZ, G. Mechanism and Regulation of NLRP3 Inflammasome Activation. **Trends in Biochemical Sciences**, v. 41, n. 12, p. 1012–1021, 2016.

HELDIN, C.-H.; MIYAZONO, K.; PETER, &. **TGF-signalling from cell membrane to nucleus through SMAD proteins**. 1997.

HENEKA, M. T.; KUMMER, M. P.; STUTZ, A.; et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. **Nature**, v. 493, n. 7434, p. 674–678, 2013. Nature Publishing Group. Disponível em: <http://dx.doi.org/10.1038/nature11729>. .

HIGGINS, D. F.; EWART, L. M.; MASTERSON, E.; et al. BMP7-induced-Pten inhibits Akt and prevents renal fibrosis. **Biochimica et Biophysica Acta - Molecular Basis of Disease**, v. 1863, n. 12, p. 3095–3104, 2017. Elsevier B.V.

HINCK, A. P.; MUELLER, T. D.; SPRINGER, T. A. Structural biology and evolution of the TGF-β family. **Cold Spring Harbor Perspectives in Biology**, v. 8, n. 12, 2016. Cold Spring Harbor Laboratory Press.

HINZ, B. Myofibroblasts. **Experimental Eye Research**, 14. maio 2015. Academic Press.

HOGAN, B. L. M.; KOLODZIEJ, P. A. Molecular mechanisms of tubulogenesis. **Nature Reviews Genetics**, 2002.

HOWELL, M.; ITOH, F.; PIERREUX, C. E.; et al. **Xenopus Smad4 Is the Co-Smad Component of Developmentally Regulated Transcription Factor Complexes Responsible for Induction of Early Mesodermal Genes**. 1999.

IGNOTZS, R. A.; MASSAGUE8, J. **THE JOURNAL Transforming Growth Factor-@ Stimulates the Expression of Fibronectin and Collagen and Their Incorporation into the Extracellular Matrix***. 1986.

ISSA, R.; ZHOU, X.; CONSTANDINOU, C. M.; et al. Spontaneous recovery from micronodular cirrhosis: Evidence for incomplete resolution associated with matrix cross-linking. **Gastroenterology**, v. 126, n. 7, p. 1795–1808, 2004. W.B. Saunders.

kaartinen1995. .

KAIMORI, A.; POTTER, J.; KAIMORI, J. Y.; et al. Transforming growth factor-β1 induces an epithelial-to-mesenchymal transition state in mouse hepatocytes in vitro. **Journal of Biological Chemistry**, v. 282, n. 30, p. 22089–22101, 2007.

KALER, P.; AUGENLICHT, L.; KLAMPFER, L. Macrophage-derived IL-1B stimulates Wnt signaling and growth of colon cancer cells: A crosstalk interrupted by vitamin D"3. **Oncogene**, v. 28, n. 44, p. 3892–3902, 2009.

KALLURI, R.; NEILSON, E. G. Epithelial-mesenchymal transition and its implications for fibrosis. **Journal of Clinical Investigation**, dez. 2003.

KAPOOR, M.; MARTEL-PELLETIER, J.; LAJEUNESSE, D.; PELLETIER, J. P.; FAHMI, H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. **Nature Reviews Rheumatology**, jan. 2011.

KAYAGAKI, N.; WARMING, S.; LAMKANFI, M.; et al. Non-canonical inflammasome activation targets caspase-11. **Nature**, v. 479, n. 7371, p. 117–121, 2011. Nature Publishing

Group. Disponível em: <http://dx.doi.org/10.1038/nature10558>. .

KIM, K. K.; KUGLER, M. C.; WOLTERS, P. J.; et al. **Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix**. 2006.

KINGSLEY, D. M. The TGF-β superfamily: New members, new receptors, and new genetic tests of function in different organisms. **Genes and Development**, v. 8, n. 2, p. 133–146, 1994.

KULKARNI, A. B.; HUH, C.-G.; BECKER, D.; et al. **Transforming growth factor 181 null mutation in mice causes excessive inflammatory response and early death**. 1993.

LAMOUILLE, S.; XU, J.; DERYNCK, R. Molecular mechanisms of epithelial-mesenchymal transition. **Nature Reviews Molecular Cell Biology**, mar. 2014.

LAN, H. Y. Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. **International Journal of Biological Sciences**, v. 7, n. 7, p. 1056–1067, 2011.

LASITHIOTAKI, I.; GIANNARAKIS, I.; TSITOURA, E.; et al. NLRP3 inflammasome expression in idiopathic pulmonary fibrosis and rheumatoid lung. **European Respiratory Journal**, v. 47, n. 3, p. 910–918, 2016. European Respiratory Society.

LATELLA, G.; VETUSCHI, A.; SFERRA, R.; et al. Smad3 loss confers resistance to the development of trinitrobenzene sulfonic acid-induced colorectal fibrosis. **European Journal of Clinical Investigation**, v. 39, n. 2, p. 145–156, 2009.

LEE, K.-Y.; ITO, K.; HAYASHI, R.; et al. NF-κB and Activator Protein 1 Response Elements and the Role of Histone Modifications in IL-1β-Induced TGF-β1 Gene Transcription. **The Journal of Immunology**, v. 176, n. 1, p. 603–615, 2006. The American Association of Immunologists.

LEIVONEN, S. K.; LAZARIDIS, K.; DECOCK, J.; et al. TGF-β-Elicited Induction of Tissue Inhibitor of Metalloproteinases (TIMP)-3 Expression in Fibroblasts Involves Complex Interplay between Smad3, p38α, and ERK1/2. **PLoS ONE**, v. 8, n. 2, 2013.

LI, X.; YAN, X.; WANG, Y.; et al. NLRP3 inflammasome inhibition attenuates silica-induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells. **Experimental Cell Research**, v. 362, n. 2, p. 489–497, 2018. Elsevier Inc.

LI, Y.; YANG, J.; DAI, C.; WU, C.; LIU, Y. Role for integrin-linked kinase in mediating tubular epithelial to mesenchymal transition and renal interstitial fibrogenesis. **Journal of Clinical Investigation**, v. 112, n. 4, p. 503–516, 2003. The American Society for Clinical Investigation.

li1998. .

LICHTMAN, M. K.; OTERO-VINAS, M.; FALANGA, V. Transforming growth factor beta (TGF-β) isoforms in wound healing and fibrosis. **Wound Repair and Regeneration**, 1. mar. 2016. Blackwell Publishing Inc.

LIU, T.; LIU, Y.; MILLER, M.; et al. Autophagy plays a role in FSTL1-induced epithelial mesenchymal transition and airway remodeling in asthma. **American Journal of Physiology - Lung Cellular and Molecular Physiology**, v. 313, n. 1, p. L27–L40, 2017. American Physiological Society.

LIU, X.; ZHANG, Z.; RUAN, J.; et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. **Nature**, v. 535, n. 7610, p. 153–158, 2016. Nature Publishing Group. Disponível em: <http://dx.doi.org/10.1038/nature18629>. .

LIU, Y. Renal fibrosis: New insights into the pathogenesis and therapeutics. **Kidney International**, jan. 2006.

LUO, D. D.; FIELDING, C.; PHILLIPS, A.; FRASER, D. Interleukin-1 beta regulates proximal tubular cell transforming growth factor beta-1 signalling. **Nephrology Dialysis Transplantation**, v. 24, n. 9, p. 2655–2665, 2009.

LUO, D. D.; PHILLIPS, A.; FRASER, D. Bone morphogenetic protein-7 inhibits proximal tubular epithelial cell Smad3 signaling via increased SnoN expression. **American Journal of Pathology**, v. 176, n. 3, p. 1139–1147, 2010. Elsevier Inc.

LUO, G.; J J, A. L. **BMP-7 is an inducer of ne~hro~enesis, and is also reauired for eve**

d e d o ~ m e n t and skeletal &tterning. .

MACIAS, M. J.; MARTIN-MALPARTIDA, P.; MASSAGUÉ, J. Structural determinants of Smad function in TGF-β signaling. **Trends in Biochemical Sciences**, 1. jun. 2015. Elsevier Ltd.

MAITY, S.; DAS, F.; KASINATH, B. S.; GHOSH-CHOUDHURY, N.; GHOSH CHOUDHURY, G. TGFb acts through PDGFRb to activate mTORC1 via the Akt/PRAS40 axis and causes glomerular mesangial cell hypertrophy and matrix protein expression. **Journal of Biological Chemistry**, p. jbc.RA120.014994, 2020.

MANJI, G. A.; WANG, L.; GEDDES, B. J.; et al. PYPAF1, a PYRIN-containing Apaf1-like protein that assembles with ASC and regulates activation of NF-κB. **Journal of Biological Chemistry**, v. 277, n. 13, p. 11570–11575, 2002.

MARTINEZ, F. J.; COLLARD, H. R.; PARDO, A.; et al. Idiopathic pulmonary fibrosis. **Nature Reviews Disease Primers**, 20. out. 2017. Nature Publishing Group.

MARTINEZ, F. O.; GORDON, S. The M1 and M2 paradigm of macrophage activation: Time for reassessment. **F1000Prime Reports**, v. 6, 2014. Faculty of 1000 Ltd.

MARTINON, F.; BURNS, K.; RG TSCHOPP, J. **The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-that they possess several distinct protein/protein inter-action domains which are used to assemble large multi-component complexes. Apaf-1, for example, contains an N-terminal CARD followed by a NBS/self-oligomer-ization domain and a C-terminal WD-40 repeat (Jaro**. 2002.

MASSAGUÉ, J. **TGF-β SIGNAL TRANSDUCTION**. 1998.

MASSAGUÉ, J.; SEOANE, J.; WOTTON, D. Smad transcription factors. **Genes and Development**, 1. dez. 2005.

massague2000. .

MCANULTY, R. J. Fibroblasts and myofibroblasts: Their source, function and role in

disease. **International Journal of Biochemistry and Cell Biology**, 2007.

VAN DER MEER, J. W. M.; BARZA, M.; WOLFF, S. M.; DINARELLO, C. A. **A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal Gram-negative infection (immunomodulation/natural resistance/macrophages)**. 1988.

MENG, X. M.; HUANG, X. R.; XIAO, J.; et al. Disruption of Smad4 impairs TGF-B/Smad3 and Smad7 transcriptional regulation during renal inflammation and fibrosis in vivo and in vitro. **Kidney International**, v. 81, n. 3, p. 266–279, 2012. Nature Publishing Group.

MIETTINEN, L. J.; EBNER, R.; LOPEZ, A. R.; DERYNCK, R. **TGF-/3 Induced Transdifferentiation of Mammary Epithelial Cells to Mesenchymal Cells: Involvement of Type I Receptors**. .

MIYAZONO, K.; HELDIN, C.-H. **Latent forms of TGF-P: molecular structure and mec han isms of activation**. 1991.

MORIKAWA, M.; DERYNCK, R.; MIYAZONO, K. TGF- β and the TGF-β family: Context-dependent roles in cell and tissue physiology. **Cold Spring Harbor Perspectives in Biology**, 1. maio 2016. Cold Spring Harbor Laboratory Press.

MOSES, H. L.; ROBERTS, A. B.; DERYNCK, R. The discovery and early days of TGF-b: A historical perspective. **Cold Spring Harbor Perspectives in Biology**, v. 8, n. 7, 2016. Cold Spring Harbor Laboratory Press.

MOSSER, D. M.; EDWARDS, J. P. Exploring the full spectrum of macrophage activation. **Nature Reviews Immunology**, dez. 2008.

MURPHY, D.; MCCULLOCH, C. E.; LIN, F.; et al. Trends in prevalence of chronic kidney disease in the United States. **Annals of Internal Medicine**, v. 165, n. 7, p. 473–481, 2016.

NA, Y. R.; SEOK, S. H.; KIM, D. J.; et al. Bone morphogenetic protein 7 induces mesenchymal-to-epithelial transition in melanoma cells, leading to inhibition of metastasis. **Cancer Science**, v. 100, n. 11, p. 2218–2225, 2009.

NAKASE, T.; YOSHIKAWA, H. Potential roles of bone morphogenetic proteins (BMPs) in skeletal repair and regeneration. **Journal of Bone and Mineral Metabolism**, nov. 2006.

NEWFELD, S. J.; RAFTERY, L. A.; CHARTOFF, E. H.; GELBART, W. M. **Caenorhabditis elegans genes sma-2, sma-3, and sma-4 define a conserved family of transforming growth factor 3 pathway components (signal transduction/pattern formation/bone morphogenetic protein/multigene family)**. 1996.

NG, A.; XAVIER, R. J. Leucine-rich repeat (LRR) proteins: Integrators of pattern recognition and signaling in immunity. **Autophagy**, v. 7, n. 9, p. 1082–1084, 2011.

NOVICK, D.; KIM, S.; KAPLANSKI, G.; DINARELLO, C. A. Interleukin-18, more than a Th1 cytokine. **Seminars in Immunology**, 15. dez. 2013.

PEI, D.; SHU, X.; GASSAMA-DIAGNE, A.; THIERY, J. P. Mesenchymal–epithelial transition in development and reprogramming. **Nature Cell Biology**, v. 21, n. 1, p. 44–53, 2019. Springer US. Disponível em: <http://dx.doi.org/10.1038/s41556-018-0195-z>. .

PEINADO, H.; BALLESTAR, E.; ESTELLER, M.; CANO, A. Snail Mediates E-Cadherin Repression by the Recruitment of the Sin3A/Histone Deacetylase 1 (HDAC1)/HDAC2 Complex. **Molecular and Cellular Biology**, v. 24, n. 1, p. 306–319, 2004. American Society for Microbiology.

PEINADO, H.; OLMEDA, D.; CANO, A. Snail, ZEB and bHLH factors in tumour progression: An alliance against the epithelial phenotype? **Nature Reviews Cancer**, jun. 2007.

PELLEGRINI, C.; ANTONIOLI, L.; LOPEZ-CASTEJON, G.; BLANDIZZI, C.; FORNAI, M. Canonical and non-canonical activation of NLRP3 inflammasome at the crossroad between immune tolerance and intestinal inflammation. **Frontiers in Immunology**, v. 8, n. JAN, 2017.

PIEK, E.; JU, W. J.; HEYER, J.; et al. Functional Characterization of Transforming Growth Factor β Signaling in Smad2- and Smad3-deficient Fibroblasts. **Journal of Biological Chemistry**, v. 276, n. 23, p. 19945–19953, 2001.

PINKERTON, J. W.; KIM, R. Y.; ROBERTSON, A. A. B.; et al. Inflammasomes in the lung. **Molecular Immunology**, v. 86, p. 44–55, 2017. Elsevier Ltd.

PROELL, M.; RIEDL, S. J.; FRITZ, J. H.; ROJAS, A. M.; SCHWARZENBACHER, R. The Nod-Like Receptor (NLR) family: A tale of similarities and differences. **PLoS ONE**, v. 3, n. 4, p. 1–11, 2008.

RE, F.; MUZIO, M.; DE L%SSI, M.; et al. **The Type H "Receptor" As a Decoy Target for Interleukin 1 in Polymorphonuclear Leukocytes: Characterization of Induction by Dexamethasone and Ligand Binding Properties of the Released Decoy Receptor**. .

REDINGTON, A. E.; MADDEN, J.; FREW, A. J.; et al. **Brief Communications Transforming Growth Factor-1 in Asthma Measurement in Bronchoalveolar Lavage Fluid**. 1997.

RICHELDI, L.; COLLARD, H. R.; JONES, M. G. Idiopathic pulmonary fibrosis. **The Lancet**, 13. maio 2017. Lancet Publishing Group.

ROBERTS, A. B.; SPORN, M. B.; ASSOIAN, R. K.; et al. **Transforming growth factor type ,8: Rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro**. 1986.

ROBERTS, A. B.; TIAN, F.; BYFIELD, S. D. C.; et al. Smad3 is key to TGF-β-mediated epithelial-to-mesenchymal transition, fibrosis, tumor suppression and metastasis. **Cytokine and Growth Factor Reviews**, v. 17, n. 1–2, p. 19–27, 2006. Elsevier BV.

ROBERTS, I. S. D.; BURROWS, C.; SHANKS, J. H.; VENNING, M.; MCWILLIAM, L. J. **Interstitial myofibroblasts: predictors of progression in membranous nephropathy**. 1997.

ROMERO, C. A.; REMOR, A.; LATINI, A.; et al. Uric acid activates NRLP3 inflammasome in an in-vivo model of epithelial to mesenchymal transition in the kidney. **Journal of Molecular Histology**, v. 48, n. 3, p. 209–218, 2017. Springer Netherlands.

S.A., S.; K.A., F. The PYHIN protein family as mediators of host defenses. **Immunological Reviews**, v. 243, n. 1, p. 109–118, 2011. Disponível em:

<http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L362440545%0Ahttp://dx.doi.org/10.1111/j.1600-065X.2011.01053.x>. .

SAITOH, M. Saitoh M. , , n. May, 2018.

SALTON, F.; VOLPE, M. C.; CONFALONIERI, M. Epithelial-mesenchymal transition in the pathogenesis of idiopathic pulmonary fibrosis. **Medicina (Lithuania)**, v. 55, n. 4, 2019. MDPI AG.

SANDERSON, N.; FACTOR, V.; NAGY, P.; et al. **Hepatic expression of mature transforming growth factor (81 in transgenic mice results in multiple tissue lesions**. 1995.

SCHMID-BURGK, J. L.; CHAUHAN, D.; SCHMIDT, T.; et al. A genome-wide CRISPR (clustered regularly interspaced short palindromic repeats) screen identifies NEK7 as an essential component of NLRP3 inflammasome activation. **Journal of Biological Chemistry**, v. 291, n. 1, p. 103–109, 2016.

SCHRODER, K.; TSCHOPP, J. The Inflammasomes. **Cell**, v. 140, n. 6, p. 821–832, 2010.

SCOTT, D. L.; WOLFE, F.; HUIZINGA, T. W. J. Rheumatoid arthritis. The Lancet. **Anais...** . v. 376, p.1094–1108, 2010. Lancet Publishing Group.

SHI, H.; WANG, Y.; LI, X.; et al. NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. **Nature Immunology**, v. 17, n. 3, p. 250–258, 2016. Nature Publishing Group. Disponível em: <http://dx.doi.org/10.1038/ni.3333>. .

SHI, J.; ZHAO, Y.; WANG, K.; et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. **Nature**, v. 526, n. 7575, p. 660–665, 2015.

SIRARD, C.; LUIS DE LA POMPA, J.; ELIA, A.; et al. **The tumor suppressor gene Smad4/Dpc4 is required for gastrulation and later for anterior development of the mouse embryo**. 1998.

SLATER, A. F. G., & CERAMI, A. [2]»ÉøÍ¸ĐÔµÄ © 19 9 2 Nature Publishing Group.

Nature, v. 359, p. 167–169, 1992.

STRUTZ, F.; ZEISBERG, M.; ZIYADEH, F. N.; et al. **Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation with the technical assistance of**. 2002.

SUGIMOTO, H.; YANG, C.; LEBLEU, V. S.; et al. BMP-7 functions as a novel hormone to facilitate liver regeneration. **The FASEB Journal**, v. 21, n. 1, p. 256–264, 2007. Wiley.

SUN, Q.; FAN, J.; BILLIAR, T. R.; SCOTT, M. J. Inflammasome and autophagy regulation: A two-way street. **Molecular Medicine**, v. 23, p. 188–195, 2017.

TAKEUCHI, O.; AKIRA, S. Pattern Recognition Receptors and Inflammation. **Cell**, v. 140, n. 6, p. 805–820, 2010. Elsevier Inc. Disponível em: <http://dx.doi.org/10.1016/j.cell.2010.01.022>. .

THANNICKAL, V. J.; TOEWS, G. B.; WHITE, E. S.; LYNCH, J. P.; MARTINEZ, F. J. Mechanisms of pulmonary fibrosis. **Annual Review of Medicine**, 2004.

THIERY, J. P.; ACLOQUE, H.; HUANG, R. Y. J.; NIETO, M. A. Epithelial-Mesenchymal Transitions in Development and Disease. **Cell**, 25. nov. 2009.

THIERY, J. P.; SLEEMAN, J. P. Complex networks orchestrate epithelial-mesenchymal transitions. **Nature Reviews Molecular Cell Biology**, fev. 2006.

thornberry1992. .

TIAN, R.; ZHU, Y.; YAO, J.; et al. NLRP3 participates in the regulation of EMT in bleomycin-induced pulmonary fibrosis. **Experimental Cell Research**, v. 357, n. 2, p. 328–334, 2017. Academic Press Inc.

TING, J. P. Y.; LOVERING, R. C.; ALNEMRI, E. S.; et al. The NLR Gene Family: A Standard Nomenclature. **Immunity**, v. 28, n. 3, p. 285–287, 2008.

TOMASEK, J. J.; GABBIANI, G.; HINZ, B.; CHAPONNIER, C.; BROWN, R. A. Myofibroblasts and mechano: Regulation of connective tissue remodelling. **Nature Reviews Molecular Cell Biology**, 2002.

TORRES, J.; MEHANDRU, S.; COLOMBEL, J. F.; PEYRIN-BIROULET, L. Crohn's disease. **The Lancet**, 29. abr. 2017. Lancet Publishing Group.

TOWNE, J. E.; RENSHAW, B. R.; DOUANGPANYA, J.; et al. Interleukin-36 (IL-36) ligands require processing for full agonist (IL-36α, IL-36β, and IL-36γ) or antagonist (IL-36Ra) activity. **Journal of Biological Chemistry**, v. 286, n. 49, p. 42594–42602, 2011.

TSUCHIDA, K.-I.; ZHU, Y.; SIVA, S.; DUNN, S. R.; SHARMA, K. **HORMONES-CYTOKINES-SIGNALING Role of Smad4 on TGF-induced extracellular matrix stimulation in mesangial cells**. 2003.

VIGANÒ, E.; MORTELLARO, A. Caspase-11: The driving factor for noncanonical inflammasomes. **European Journal of Immunology**, v. 43, n. 9, p. 2240–2245, 2013.

VILAYSANE, A.; CHUN, J.; SEAMONE, M. E.; et al. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. **Journal of the American Society of Nephrology**, v. 21, n. 10, p. 1732–1744, 2010. American Society of Nephrology.

VUKICEVIC, S.; STAVLJENIC, A.; SAMPATH, T. K. protein-7) reduces severity of injury after ischemic acute renal failure in rat . Find the latest version : , v. 1, n. 1, p. 202–214, 1998.

WANG, H.; WANG, Y.; DU, Q.; et al. Inflammasome-independent NLRP3 is required for epithelial-mesenchymal transition in colon cancer cells. **Experimental Cell Research**, v. 342, n. 2, p. 184–192, 2016. Academic Press Inc.

WANG, S.; HIRSCHBERG, R. Bone morphogenetic protein-7 signals opposing transforming growth factor β in mesangial cells. **Journal of Biological Chemistry**, v. 279, n. 22, p. 23200–23206, 2004.

WANG, W.; WANG, X.; CHUN, J.; et al. Inflammasome-Independent NLRP3 Augments TGF-β Signaling in Kidney Epithelium. **The Journal of Immunology**, v. 190, n. 3, p. 1239–1249, 2013. The American Association of Immunologists.

WANG, Y.; JIA, M.; YAN, X.; et al. Increased neutrophil gelatinase-associated lipocalin (NGAL) promotes airway remodelling in chronic obstructive pulmonary disease. **Clinical**

Science, v. 131, n. 11, p. 1147–1159, 2017. Portland Press Ltd.

WEINSTEIN, M.; YANG, X.; LI, C.; et al. **Developmental Biology Communicated by Igor B. Dawid, National Institute of Child Health and Human Development**. 1998.

WEISKIRCHEN, R.; MEURER, S. K. **] 1407 BMP-7 counteracting TGF-beta 1 activities in organ fibrosis**. 2013.

WHEELOCK, M. J.; SHINTANI, Y.; MAEDA, M.; FUKUMOTO, Y.; JOHNSON, K. R. Cadherin switching. **Journal of Cell Science**, 15. mar. 2008.

winkler2003. .

WYNN, T. A. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. **Journal of Clinical Investigation**, 1. mar. 2007.

WYNN, T. A. Cellular and molecular mechanisms of fibrosis. **Journal of Pathology**, jan. 2008.

WYNN, T. A. Integrating mechanisms of pulmonary fibrosis. **Journal of Experimental Medicine**, v. 208, n. 7, p. 1339–1350, 2011.

WYNN, T. A.; RAMALINGAM, T. R. Mechanisms of fibrosis: Therapeutic translation for fibrotic disease. **Nature Medicine**, jul. 2012.

XU, F.; LIU, C.; ZHOU, D.; ZHANG, L. TGF-β/SMAD Pathway and Its Regulation in Hepatic Fibrosis. **Journal of Histochemistry and Cytochemistry**, 1. mar. 2016. Histochemical Society Inc.

XU, J.; LAMOUILLE, S.; DERYNCK, R. TGF-B-induced epithelial to mesenchymal transition. **Cell Research**, fev. 2009.

YANG, J.; WEINBERG, R. A. Epithelial-Mesenchymal Transition: At the Crossroads of Development and Tumor Metastasis. **Developmental Cell**, 10. jun. 2008.

YANG, J.; ZHANG, L.; YU, C.; YANG, X.-F.; WANG, H. **Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory**

diseases. 2014.

YANG, T.; CHEN, S. L.; LU, X. J.; et al. Bone morphogenetic protein 7 suppresses the progression of hepatic fibrosis and regulates the expression of gremlin and transforming growth factor β1. **Molecular Medicine Reports**, v. 6, n. 1, p. 246–252, 2012.

YANG, XIAO; LETTERIO, JOHN J; LECHLEIDER, R. J.; et al. **Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-β**. 1999.

YI, Y. S. Caspase-11 non-canonical inflammasome: Emerging activator and regulator of infection-mediated inflammatory responses. **International Journal of Molecular Sciences**, v. 21, n. 8, p. 1–11, 2020.

YILMAZ, M.; CHRISTOFORI, G. EMT, the cytoskeleton, and cancer cell invasion. **Cancer and Metastasis Reviews**, 2009.

YING, X.; SUN, Y.; HE, P. Bone morphogenetic protein-7 inhibits EMT-associated genes in breast cancer. **Cellular Physiology and Biochemistry**, v. 37, n. 4, p. 1271–1278, 2015. S. Karger AG.

ZAVADIL, J.; BÖTTINGER, E. P. TGF-β and epithelial-to-mesenchymal transitions. **Oncogene**, 29. ago. 2005.

ZEISBERG, E. M.; POTENTA, S. E.; SUGIMOTO, H.; ZEISBERG, M.; KALLURI, R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. **Journal of the American Society of Nephrology**, v. 19, n. 12, p. 2282–2287, 2008.

ZEISBERG, M.; BOTTIGLIO, C.; KUMAR, N.; et al. Bone morphogenic protein-7 inhibits progression of chronic renal fibrosis associated with two genetic mouse models. **Am J Physiol Renal Physiol**, v. 285, p. 1060–1067, 2003. Disponível em: <http://www.ajprenal.org>. .

ZEISBERG, M.; HANAI, J.-I.; SUGIMOTO, H.; et al. **BMP-7 counteracts TGF-β1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury**. 2003.

ZHANG, L. M.; ZHANG, Y.; FEI, C.; et al. Neutralization of IL-18 by IL-18 binding protein ameliorates bleomycin-induced pulmonary fibrosis via inhibition of epithelial-mesenchymal transition. **Biochemical and Biophysical Research Communications**, v. 508, n. 2, p. 660–666, 2019. Elsevier B.V.

ZHONG, Z.; UMEMURA, A.; SANCHEZ-LOPEZ, E.; et al. NF-κB Restricts Inflammasome Activation via Elimination of Damaged Mitochondria. **Cell**, v. 164, n. 5, p. 896–910, 2016. Elsevier Inc. Disponível em: <http://dx.doi.org/10.1016/j.cell.2015.12.057>. .

ZOETE, M. R. DE; PALM, N. W.; ZHU, S.; et al. Marcel R. de Zoete, Noah W. Palm, Shu Zhu and Richard A. Flavell Cold Spring Harb Perspect Biol published online October 16, 2014. , 2014.