NAFLD

Guide to a collection of the most important pathways leading to the disease

INTRODUCTION
Purpose and scope

Welcome to the NAFLD Booklet, a document that helps scientists and researchers appreciate and navigate the diversity of the molecular pathways associated with the development of non-alcoholic steatohepatitis (NASH) and its progression to fibrosis. NAFLD is a range of progressive liver diseases that includes steatosis, NASH, fibrosis, and ultimately, liver cirrhosis. Over the last few years, indications as to the complex nature of the disease and its many pathways have emerged. We hope the visuals provided in this document will help shed light on and clarify an otherwise complex disease.

This document is organized around the progression of NAFLD. A short introduction on the structure and function of the liver is immediately followed by a section dedicated to NAFL, NASH, with a final section on Fibrosis. Details are given concerning the key cell types and proteins involved in liver disease.

The collection of molecular pathways presented in the document was prepared based on authentic and highly regarded articles and journals. Footnotes at the bottom of each page correlate with the references used. All pathways have been curated for scientific knowledge and accuracy by Revvity's scientific team. Due to the expertise of its creators, you will find this document to be more than just a compiled and curated list of molecular pathways.

Although some of the molecular pathways vital for our full understanding of NASH and Fibrosis still remain to be elucidated, we believe this review will provide its reader with the most up to date snapshot of current NAFLD molecular pathophysiology.

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INTRODUCTION

Liver structure and function of hepatic cells

The liver is the most vital organ in the body. It governs whole-body energy homeostasis by performing many essential functions related to nutrient digestion, metabolism and storage, as well as detoxification and immunity. All nutrients along with drugs and other potentially harmful substances from the small intestine, stomach, pancreas, and spleen are absorbed by the liver through the portal vein.

The structural unit of the liver is the hepatic lobule, surrounded by portal tracts from which blood perfuses through capillaries called sinusoids. Liver sinusoids provide large surface areas for the exchange of metabolites between blood and hepatic cells. Bile secreted is collected in bile ducts and transported to the gut or stored in the gall bladder [1].

The organ is comprised of parenchymal cells and many different types of non-parenchymal cells, all of which play significant roles:

- Hepatocytes are the predominant and chief functional cells of the liver. These parenchymal cells are linked together to form a crucially important cell layer that separates sinusoidal blood from the bile duct. They are responsible for most of the liver functions such as the metabolism of lipids, carbohydrates and proteins, as well as detoxification by eliminating toxic compounds via the secretion of bile [2].
- Hepatic stellate cells (HSCs) are non-parenchymal cells that are located within the perisinusoidal space of Disse (or perisinusoidal space). In a normal liver, quiescent HSCs regulate metabolism and storage of vitamin A, extracellular matrix (ECM) synthesis under cytokine control, and sinusoidal blood flow. Upon liver injury, they become activated and transdifferentiate into proliferative and fibrogenic myofibroblasts [3].

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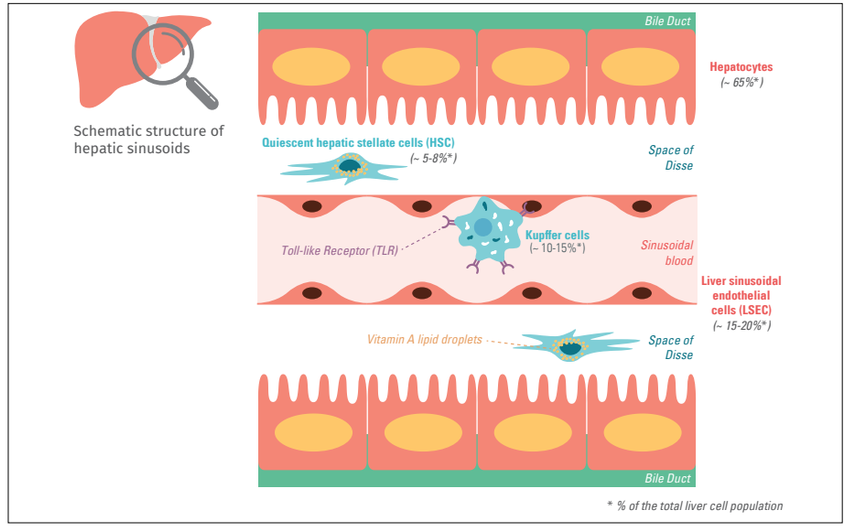
Liver structure and function of hepatic cells

- Kupffer cells (KCs) are resident and non-migratory liver macrophages serving as sentinels for liver homeostasis. These non-parenchymal intra-sinusoidal cells are in constant contact with gut-derived products, and thus are the body's primary line of defense against pathogens. They play an important role in innate immune response by secreting potent inflammatory mediators and they are essential for the phagocytosis of cellular debris, foreign material or pathogens. Upon liver injury, KCs rapidly produce cytokines and chemokines, which induce the recruitment of other immune cells, including monocytes, into the liver [4].
- Liver sinusoidal endothelial cells (LSECs) are non-parenchymal sinusoidal cells. These highly permeable cells with high endocytic capacity act as a filter to transfer molecules from the sinusoidal blood to the space of Disse. They regulate hepatic vascular tone, maintain hepatic stellate cell quiescence, and assist the metabolic function of hepatocytes [5].



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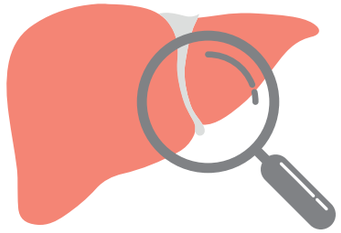
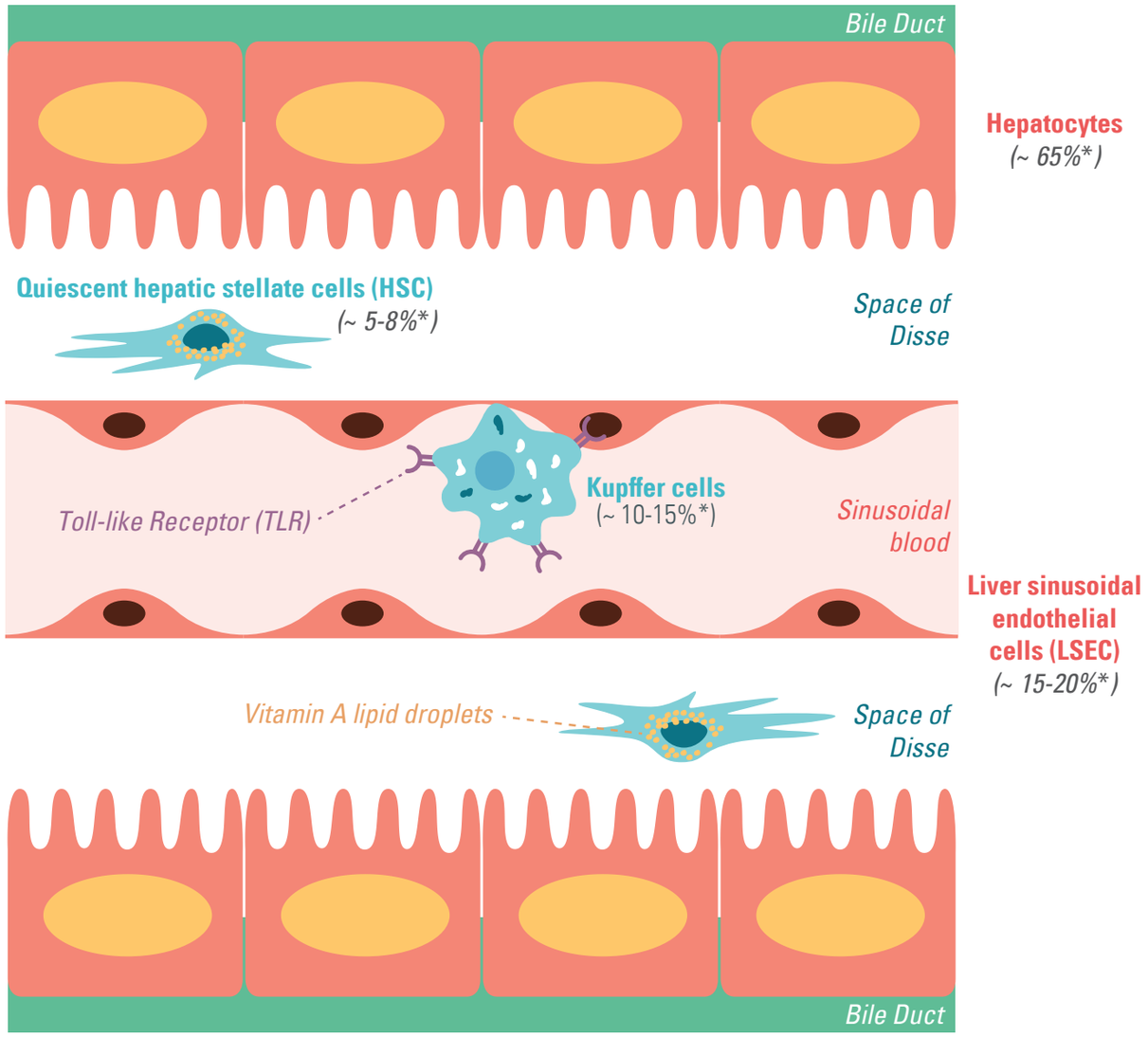


Schematic structure of hepatic sinusoids



* % of the total liver cell population

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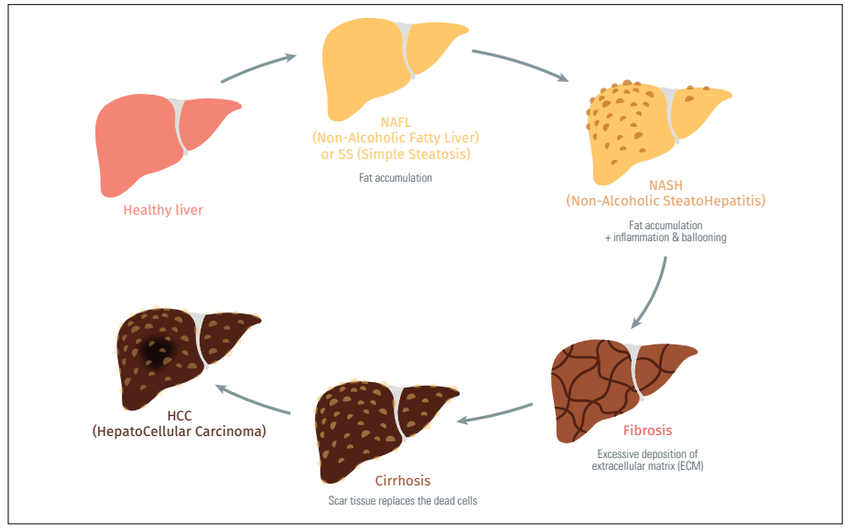
The spectrum of NAFLDs

- Non-alcoholic fatty liver disease is a term used to cover a spectrum that ranges from fatty liver to non-alcoholic steatohepatitis to fibrosis, and finally cirrhosis. Lipid accumulation, or steatosis, marks one end of the spectrum and is generally considered benign. The word steatosis comes from the Greek word “steato” meaning fat, or suet. Simple accumulation of fat in the liver is sometimes referred to as “Simple Steatosis” and does not include inflammation. Lipid accumulation is considered a hepatic manifestation of obesity and its related metabolic disorders such as diabetes and insulin resistance.
- The other end of the spectrum is characterized by the development of hepatocellular carcinoma (HCC), the most prevalent type of primary liver cancer. Surgery to remove the cancer plus a marginal section of the surrounding healthy tissue can in some cases constitute a therapeutic approach. More often than not, however, the diagnosis of HCC constitutes an indication for liver transplantation.
- Non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis are intermediary steps between simple steatosis and HCC. In NASH, the build-up of fat in the liver is accompanied by inflammation. NASH is considered a pivotal stage in NAFLD progression. If left untreated, NASH can progress to fibrosis, i.e. the deposition of scar tissues in the liver and in the blood vessels around the liver. If, over time, the scar tissue starts to replace a significant portion of the heathy tissues, the function of the liver may become affected. This can lead to cirrhosis and partial loss of liver function.



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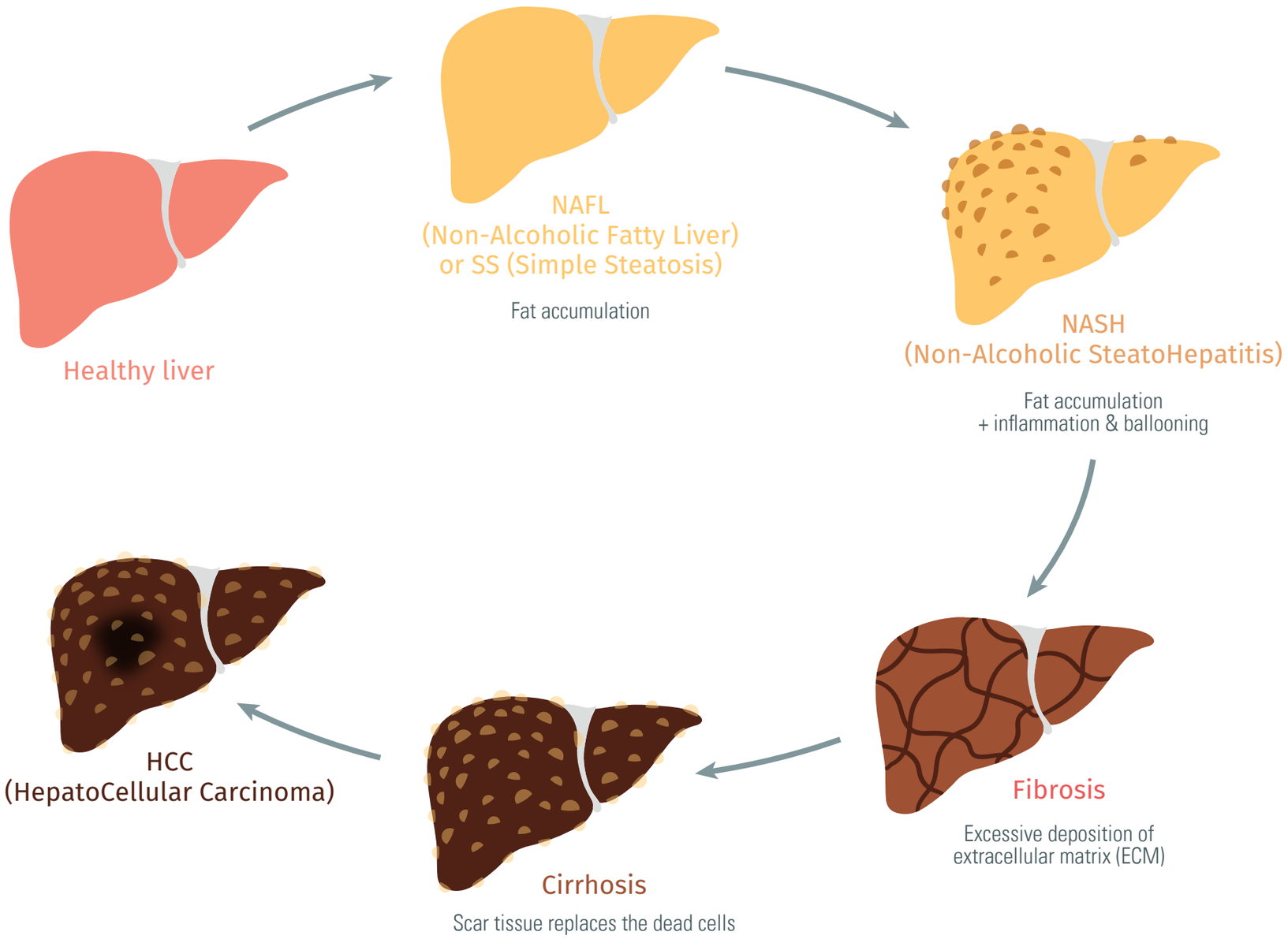

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MOLECULAR PATHOGENESIS OF NAFL

Overview

Accumulation of fat in the liver is the first and reversible event that occurs within the physio-pathological progression from a healthy liver to NASH. This abnormal and significant lipid accumulation in the liver, referred to by the terms hepatic steatosis, Simple Steatosis (SS) or (Non-Alcoholic) Fatty Liver (NAFL), is the result of a dramatic increase of free fatty acid fluxes into the organ. Due to the association of the prevalence of non-alcoholic fatty liver disease (NAFLD) with the increase in obesity worldwide, NAFLD is now the most common liver disease in Western societies and may predispose patient progress to non-alcoholic steatohepatitis.

Healthy liver

Under normal conditions, hepatocytes take up free fatty acids from circulating blood that are derived from the diet through intestinal adsorption and extra-hepatic metabolism, implicating chylomicrons and lipoprotein lipase, or from lipolysis of the adipose tissue in the fasting state. Dietary glucose and the subsequent insulin secretion stimulate the uptake and conversion of glucose into fatty acids through glycolysis and *de novo* lipogenesis (DNL) in hepatocytes. Fatty acids taken up from the circulation (diet, lipolysis) and those generated by DNL are esterified in triglyceride and cholesterol ester molecules to prevent cytotoxicity, and to enable storage as lipid droplets as well as export as very low density lipoproteins (VLDL). During the fasting state, fatty acids are not processed to triglycerides (TG) but metabolized through beta-oxidation to serve as a source of energy.

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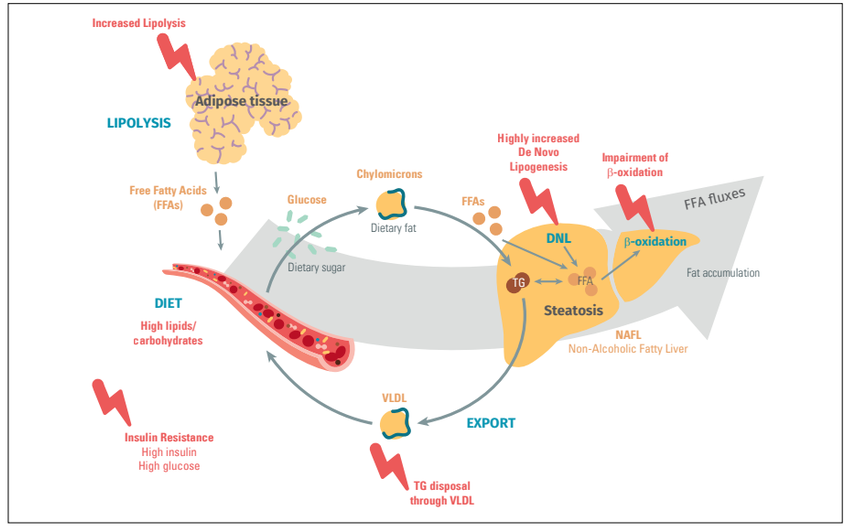
MOLECULAR PATHOGENESIS OF NAFL

Overview

Hepatic Steatosis and the metabolic syndrome

As the liver is instrumental in the uptake, synthesis, packaging, redistribution, storage, and even degradation of fatty acids, any dysregulation of these functions can contribute to hepatic steatosis, which is associated with obesity and insulin resistance. Additionally, as lipolysis in adipocytes is normally repressed by insulin, under pathological conditions such as metabolic syndrome, type 2 diabetes, or obesity, the insulino-resistance leads to an increase of adipocyte lipolysis. This results in higher and sustained concentrations of circulating free fatty acids that are taken up by hepatocytes, independently from the nutritional status (fed or fasting). In insulin-resistant states, the hepatic insulin resistance is accompanied by hepatic glucose production, an increased *de novo* lipogenesis and an impaired fatty acid beta-oxidation.

As a result, dysregulations of lipid metabolism encountered during over-alimentation with diets rich in lipids and carbohydrates, or in cases of obesity and insulin resistance, can lead to adverse consequences that alter and increase lipid and fatty acid fluxes into the hepatocyte, causing excessive TG accumulation and hepatic steatosis.



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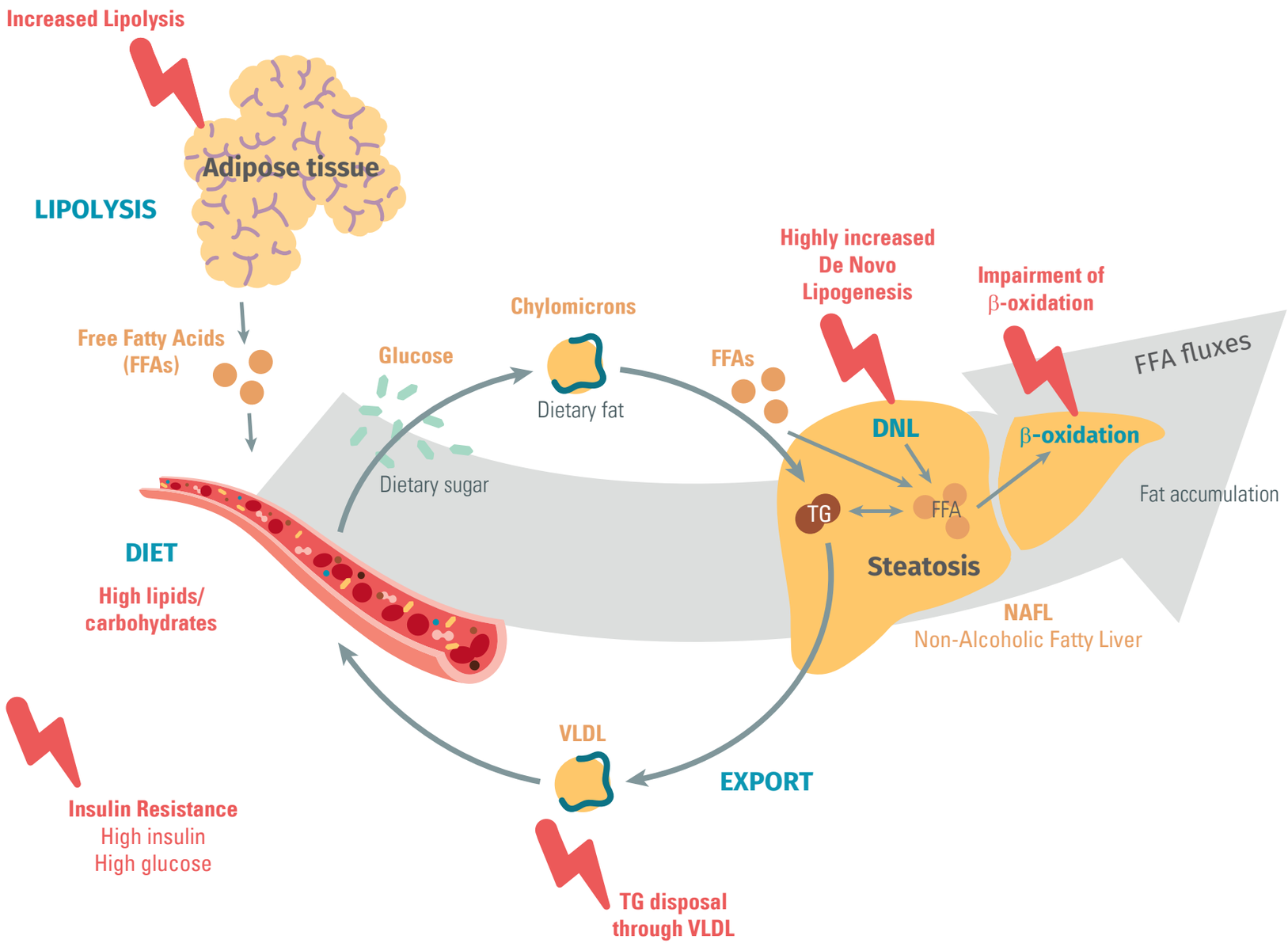

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MOLECULAR PATHOGENESIS OF NAFL

Hepatic steatosis

De Novo Lipogenesis (DNL)

DNL is a highly regulated pathway that leads to the generation of fatty acids from acetyl-coA coming from different pathways, mainly carbohydrate catabolism (glycolysis). Regulation occurs through hormones (insulin), nutrients (glucose, fructose), and energy status, and is controlled at the transcriptional level by enzymatic control and rapid post-translational modifications. The key Transcription Factors (TF) involved in DNL gene regulation are ChREBP, USF1, SREBP-1c, and LXRs. Their activation will activate the expression of ACC, FAS, ELOVL6 and SCD1, leading to the production of palmitate and stearate, and their subsequent unsaturated long chain FAs. Incorporation of acyl-coA through acylation of the glycerol chain by GPAT and other enzymes creates diacylglycerols (DAG) and leads to their processing by DGAT into triglycerides (TG). These moderate cytotoxicity and allow storage/export steps. During fasting, low insulin and AMPK activation results in the inhibition of DNL. In the fed state, glucose activates glycolysis and DNL through activation of ChREBP while insulin activates kinases including PI3K/AKT, triggering the transcription of DNL genes through SREBP1c as well as the inhibition of FoxO1 and GSK3b, repressing gluconeogenesis and glycogen synthesis respectively.

In pathological situations, insulin resistant states result in SREBP1c activation being maintained through mTOR(C1) leading to DNL, whereas the high glucose state with impairment of the IRS1/2/PI3K/AKT insulin signaling fails to suppress FoxO1 activation and on the contrary increases gluconeogenesis, leading to excessive lipogenesis. Subsequent FFA overloads can also activate JNK-1, which in turn blocks phosphorylation of IRS-1/2 and proper activation of the insulin signaling pathway.

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MOLECULAR PATHOGENESIS OF NAFL

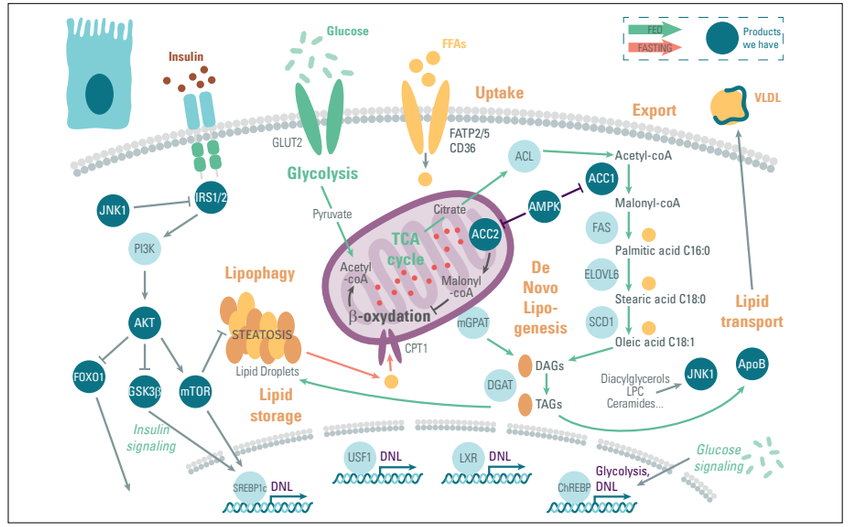
Hepatic steatosis

β-oxidation

FA oxidation corresponds to the degradation into acetyl-coA to generate energy (ATP), and takes place during the fasting state and to prevent FFA accumulation. The major β-oxidation of FAs imported through CPT1 takes place within the mitochondria, while β/α-oxidation of VLCFA occurs in the peroxisomes and minor ω-oxidation within the ER. As Malonyl-CoA generated by ACC during DNL is an allosteric inhibitor of CPT1, β-oxidation is inhibited during lipogenesis. In NAFLD, increased rates of DNL and mitochondrial dysfunction result in an impaired β-oxidation, exacerbating FA accumulation. Alternative peroxisomal and ω-oxidation pathways are then activated and contribute to ROS generation.

Export, storage and uptake

TG are incorporated into the ApoB100 protein through the MTP enzyme in the ER to generate VLDL particles that are secreted (under insulin control) into the space of Disse. TG can also be stored within the cytoplasm, in lipid droplets (LDs) that can be hydrolyzed via lipolysis or autophagy (lipophagy) to release FFAs. Prolonged availability of fat and carbohydrates and hyper insulinemia will result in autophagy/lipophagy inhibition mediated by mTOR activation. High concentrations of circulating FFAs also result in an increase in their uptake by hepatocytes via transport proteins FATP2/5 as well as the fatty acid translocase scavenger receptor CD36, which greatly contributes to FFA overload and a detrimental accumulation of toxic lipid intermediates.



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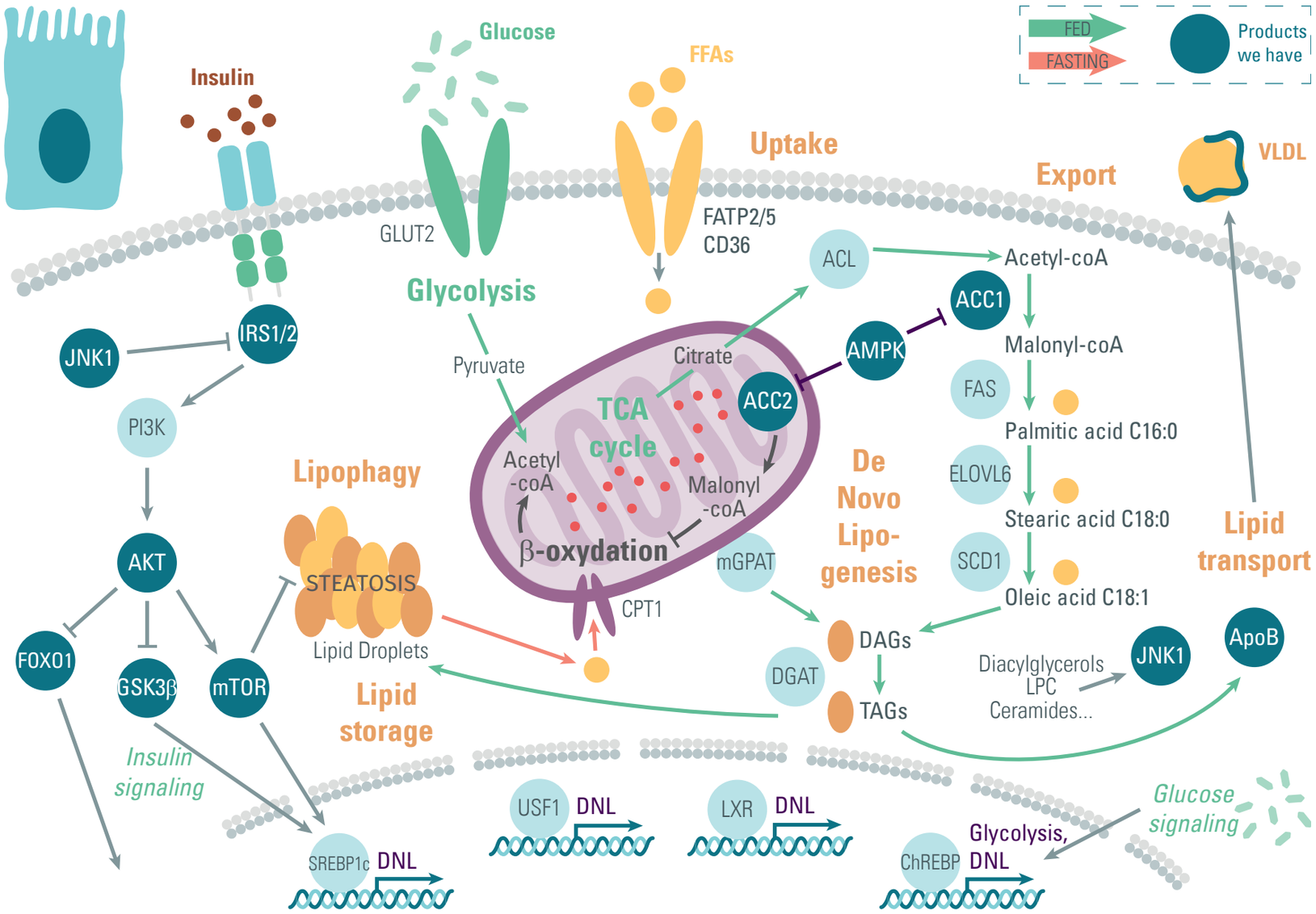

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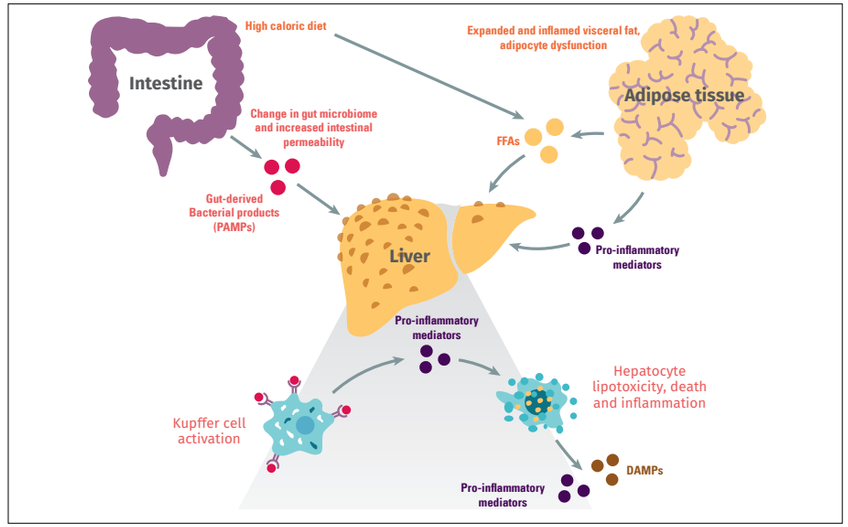
MOLECULAR PATHOGENESIS OF NASH

Overview

NASH is characterized by steatosis, hepatocyte injury (ballooning), inflammation and varying degrees of fibrosis. Its progression results from numerous events originating within the liver, as well as from signals derived from the adipose tissue and the intestine [1].

The pathogenic components of NASH are complex and multifactorial, and different theories have been debated in the literature. In the traditional 'two-hit' hypothesis, hepatic lipid accumulation is the first 'hit' and this leads to a consecutive second 'hit', which is responsible for hepatocellular damage and inflammation with associated fibrogenesis. Recent findings have challenged this view, now considered as too simplistic, and the 'multiple parallel hits' hypothesis involving organ-organ interactions is now favored. According to this model, a multitude of events occur in parallel, including genetic predisposition, insulin resistance, adipose tissue dysfunction, abnormal lipid metabolism, lipotoxicity, altered production of inflammatory mediators, and dysregulation of the gut-liver axis and innate immunity [2].

- Adipose tissue dysfunction caused by insulin resistance leads to an excessive production and secretion of pro-inflammatory mediators and FFAs (due to increased lipolysis). Circulating FFAs, which are also derived from high-fat diets, accumulate within hepatocytes and are responsible for lipotoxicity [3].
- Increased intestinal permeability as well as changes in gut microbiome contribute to the entry of gut-derived bacterial products into the liver. These pathogen-associated molecular patterns (PAMPs) activate Toll-like receptors (TLRs) on the surface of KCs and stimulate the production of pro-inflammatory mediators [4].
- Pro-inflammatory mediators secreted by both adipocytes and KCs cause hepatocyte damage and inflammation. Injured hepatocytes in turn release pro-inflammatory mediators and damage-associated molecular patterns (DAMPs), which are intracellular signals released from dying cells [5].



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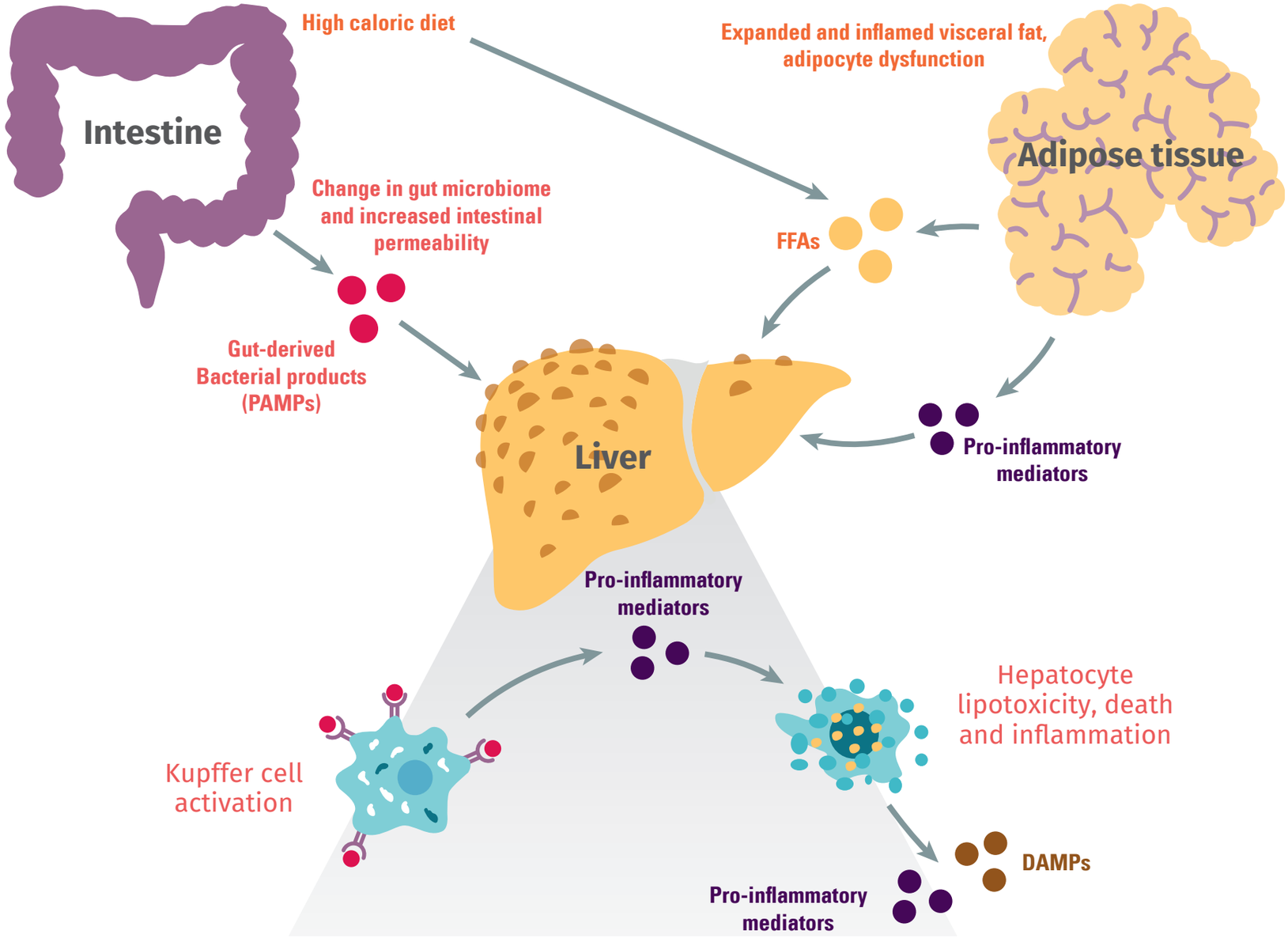

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MOLECULAR PATHOGENESIS OF NASH

Kupffer cell activation

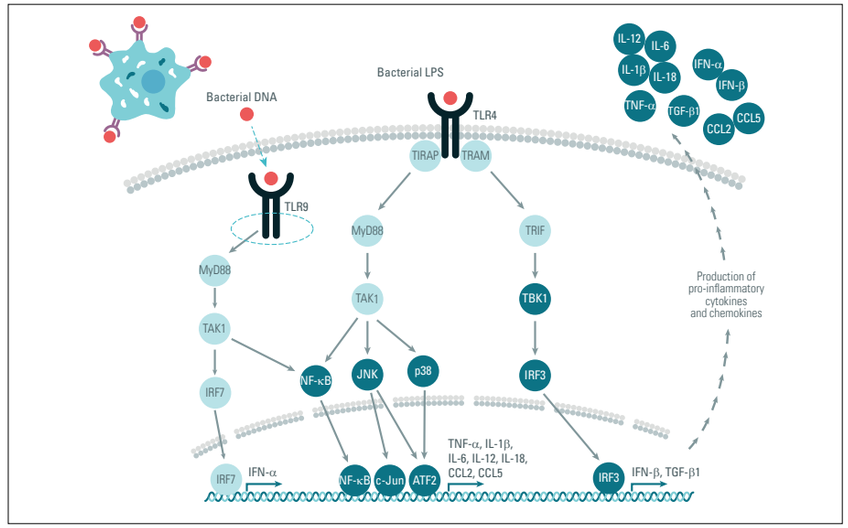
TLRs are pattern recognition receptors (PRRs) that detect PAMPs derived from various pathogens. The activation of these transmembrane proteins plays a crucial role in the innate immune system and inflammation [1].

KC activation mediated by TLR signaling is of critical importance in the development of NASH. In a healthy liver, KCs are alternatively activated by TLR ligands originating from the gut microbiota. In this case, the cells are mainly of the M2 phenotype and exhibit increased production of anti-inflammatory mediators. In NASH patients, the liver is continuously exposed to an overload of gut-derived bacterial products that constantly activate TLRs. This phenomenon leads to the switch of KCs from the M2 to the M1 phenotype, characterized by increased secretion of pro-inflammatory cytokines and chemokines, as well as fibrogenic mediators [2].

The pathogenesis of NASH is associated with several TLRs (2, 4, 5 and 9), but the TLR4 signaling has been identified as the principal actor in the progression of the disease [3].

TLR4 is a cell surface receptor for lipopolysaccharides (LPS) produced by Gram-negative bacteria. LPS binding triggers the recruitment of the adaptor molecules TIRAP and TRAM, which initiate the MyD88-dependent and TRIF-dependent pathways respectively. The first signaling cascade leads to the phosphorylation of TAK1, which in turn activates NF-κB as well as the MAP kinases JNK and p38. This results in the transcription of genes coding for pro-inflammatory mediators such as the cytokines TNF-α, IL-1β, IL-6, IL-12, IL-18, and the chemokines CCL2 and CCL5. The second pathway (TRIF-dependent) triggers the activation of TBK1, which phosphorylates the transcription factor IRF3, finally leading to the production of IFN-β and the fibrogenic cytokine TGF-β1 [4].

TLR9, which is an endosomal receptor recognizing bacterial DNA, also plays a role in the development of NASH. Activation of TLR9 directly leads to the recruitment of MyD88 and the phosphorylation of TAK1. In addition to activating NF-κB and the associated pro-inflammatory genes, TAK1 also phosphorylates IRF-7, resulting in IFN-α expression [5].



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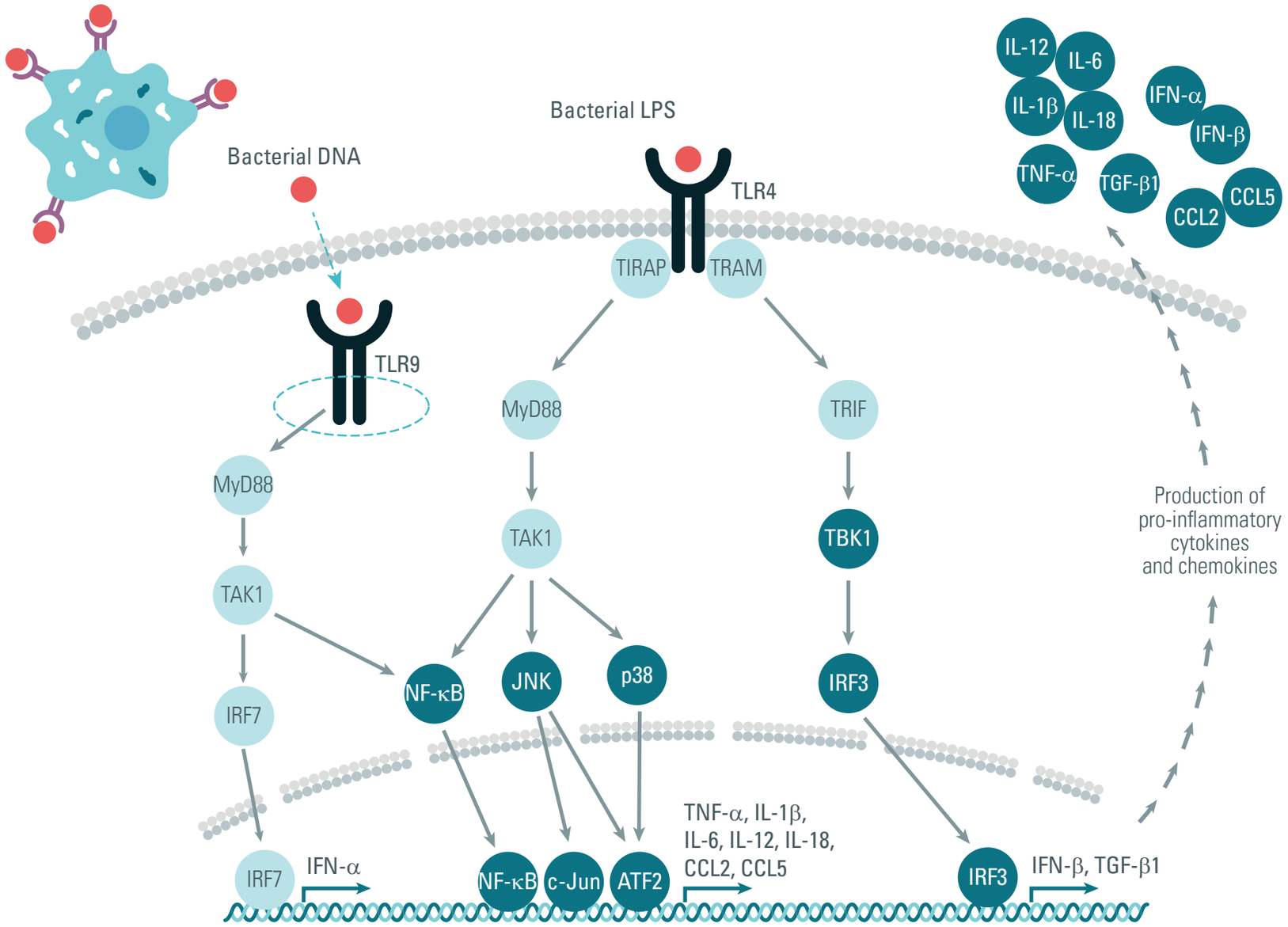

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MOLECULAR PATHOGENESIS OF NASH
Hepatocyte damage and inflammation

Excessive fat accumulation within hepatocytes leads to the generation of lipotoxic metabolites (e.g. ceramides, diacylglycerols, and oxidized cholesterol metabolites) which contribute to cell lipotoxicity. Lipotoxicity appears via mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum (ER) stress. Prolonged intracellular stress, together with pro-inflammatory mediators secreted by KCs and adipocytes, in turn drive hepatocyte inflammation and death [1].

Lipotoxicity

The overload of FFAs increases mitochondrial fatty acid β-oxidation, leading to an overproduction of reactive oxygen species (ROS). ROS contribute to oxidative damage and mitochondrial dysfunction (more particularly respiratory chain deficiency). The damaged respiratory chain generates more ROS and a vicious circle occurs. Oxidative stress mediated by ROS activates the ASK-1/JNK/FoxO1 pathway, leading to the upregulation of pro-apoptotic factors such as Bim, PUMA, TRAIL and FasL. Moreover, high concentrations of lipid-derived products overwhelm the ER, triggering prolonged ER stress and activation of the ER stress sensor PERK. The kinase PERK then phosphorylates EIF2α, resulting in the activation of the transcription factor ATF4 and the expression of specific proteins such as the stress-induced apoptotic factor CHOP. It should be noted that ER stress is exacerbated by ROS and also activates the ASK-1/JNK/FoxO1 signaling cascade [2].

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MOLECULAR PATHOGENESIS OF NASH

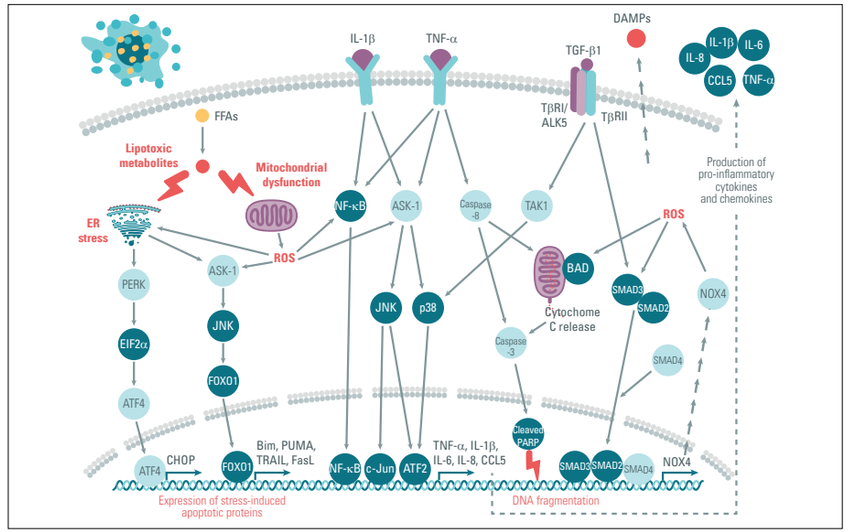
Hepatocyte damage and inflammation

Inflammation and death

IL-1β and TNF-α secreted by KCs and adipocytes, as well as intracellular ROS, trigger the activation of NF-κB, JNK and p38, leading to the expression and release of pro-inflammatory cytokines and chemokines, including TNF-α, IL-1β, IL-6, IL-8 and CCL5 [3].

TGF-β1 secreted by KCs binds on its receptors that activate the SMAD3 signaling pathway and the expression of NOX4 involved in the production of ROS, again increasing oxidative stress. TGF-β receptors also activate the TAK1-p38 pathway, increasing the production of pro-inflammatory mediators [4].

Hepatocyte apoptosis occurs via intrinsic and extrinsic pathways that all converge on caspase-3 activation, PARP cleavage, and DNA fragmentation. The intrinsic mechanism is triggered by oxidative/ER stress and mediated by mitochondrial permeabilization. The extrinsic pathway is initiated by the binding of death ligands to their respective receptors, with TNF-α being one of the principal mediators of hepatocyte death in NASH. Binding on its cell surface receptor leads to caspase-8 activation, cytosolic release of cytochrome c from the mitochondria, and finally leads to caspase-3 activation. Stressed or dying hepatocytes release DAMPs such as nuclear DNA, mitochondrial DNA, and high mobility group box 1 (HMGB1) [5].



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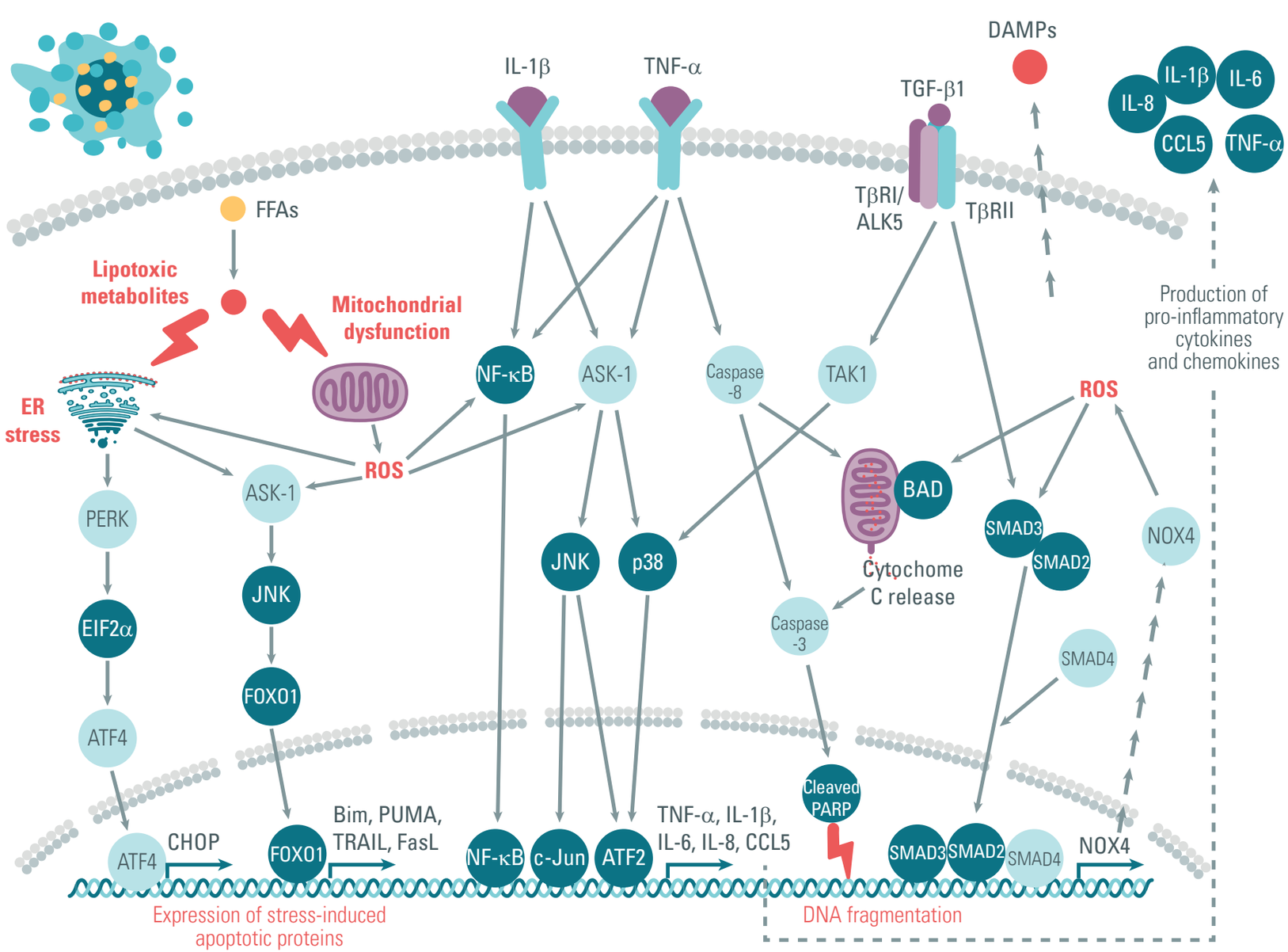

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MOLECULAR PATHOGENESIS OF LIVER FIBROSIS

Overview

Hepatic fibrosis that happens with NASH is a healing response to chronic liver injury and inflammation. It is characterized by the excessive deposition of ECM as the result of an imbalance between ECM synthesis and degeneration.

In its initial stages, hepatic fibrosis is a beneficial and reversible process that occurs to remove the causative wound. However, chronic liver aggression ultimately progresses to advanced and irreversible fibrosis, resulting in impaired hepatic function.

Different populations of cells, signaling pathways, and molecular processes are involved in liver fibrogenesis. However, the activation of HSCs, which are the primary source of ECM, plays a central role [1].

Recruitment and activation of inflammatory cells

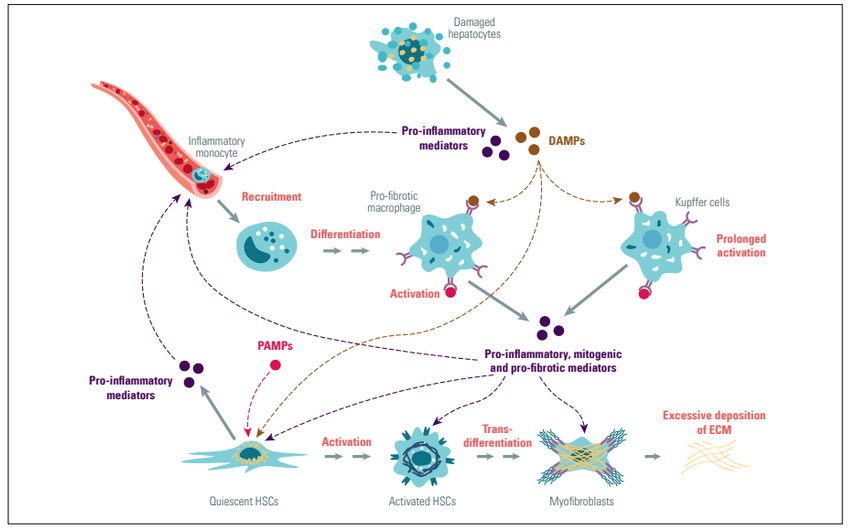
Pro-inflammatory chemokines released by injured hepatocytes trigger the recruitment of circulating inflammatory monocytes to the liver and their differentiation into pro-fibrotic macrophages.

In addition to the overload of gut-derived PAMPs, DAMPs secreted by dying hepatocytes activate the pro-fibrotic macrophages and amplify the activation of Kupffer cells. These inflammatory cells in turn produce and release pro-inflammatory, mitogenic, and pro-fibrotic mediators [2].

Activation of HSCs and transdifferentiation into fibrogenic myofibroblasts

Together, gut-derived PAMPs, DAMPs, pro-inflammatory, mitogenic and pro-fibrotic factors activate quiescent HSCs and trigger their transdifferentiation into proliferative myofibroblasts. In response to all these signals, HSCs also secrete pro-inflammatory chemokines that amplify the recruitment of inflammatory cells, creating a vicious circle.

Myofibroblasts are the major drivers of liver fibrosis. These contractile cells migrate to the sites of injury and secrete ECM molecules that progressively accumulate and form scar tissue. The excessive deposition of matrix proteins increases the density and the stiffness of ECM, which in turn serves as a mechanical stimulus to amplify the activation of HSCs, forming positive feedback loops. Excess scar tissue gradually disrupts normal cellular functional units and eventually causes liver failure [3].



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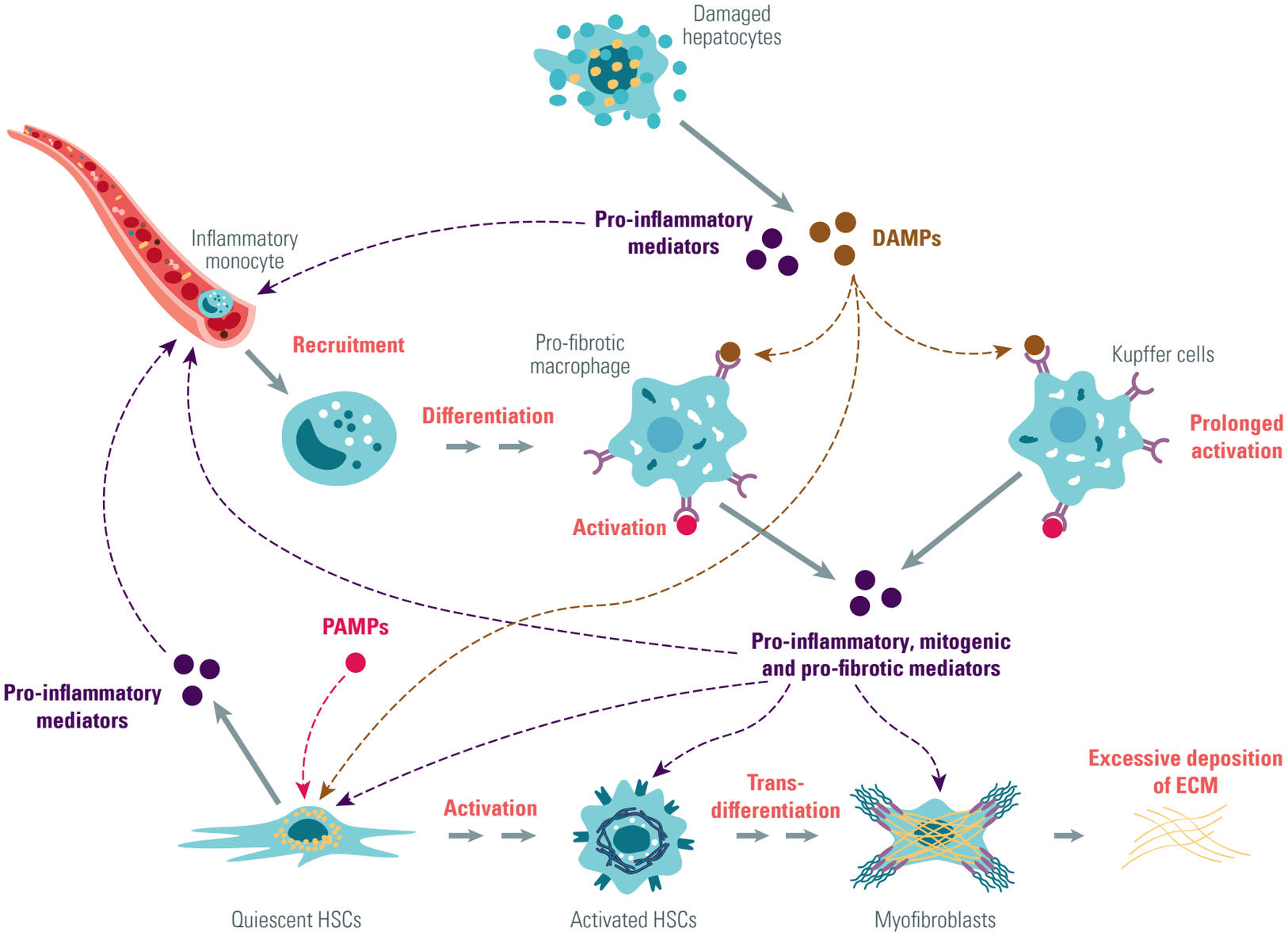

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MOLECULAR PATHOGENESIS OF LIVER FIBROSIS

Recruitment of inflammatory monocytes

The recruitment of specific immune cells from the bloodstream to the injured liver is one of the main events occurring in the early phases of hepatic fibrogenesis. It is orchestrated by chemokines, a family of small pro-inflammatory chemoattractant cytokines that are released from hepatic resident cells. The chemokines CCL2 (MCP-1) and CCL5 (RANTES) appear to be the central mediators in the initiation of this process by driving the migration and infiltration of circulating inflammatory monocytes into the liver. They are expressed and secreted by activated Kupffer cells, damaged hepatocytes, and most importantly by activated HSCs [1].

CCL2 and CCL5 induce their effects by interacting with their respective receptors CCR2 and CCR1/5 at the surface of monocytes. These receptors are members of the G-Protein Coupled Receptor (GPCR) superfamily and can interact with multiple G-protein subtypes. Conformational change upon chemokine binding induces the dissociation of G protein subunits, resulting in the activation of diverse signaling cascades [2].

- Gq-mediated PLC pathway: Activated GPCRs trigger the activation of phospholipase C (PLC) which produces the second messengers Inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 binds to receptors in the ER, releasing calcium (Ca2+). The increase in cytosolic Ca2+ and DAG leads to the activation of protein kinase C (PKC) which in turn phosphorylates the kinase Raf as well as the transcription factor NF-κB [3].
- Gi-mediated cAMP pathway: Activated chemokine receptors inhibit the enzyme adenylate cyclase (AC), leading to a decrease in the production of intracellular cyclic AMP (cAMP) and subsequently to an inhibition of cAMP-dependent cellular responses [4].

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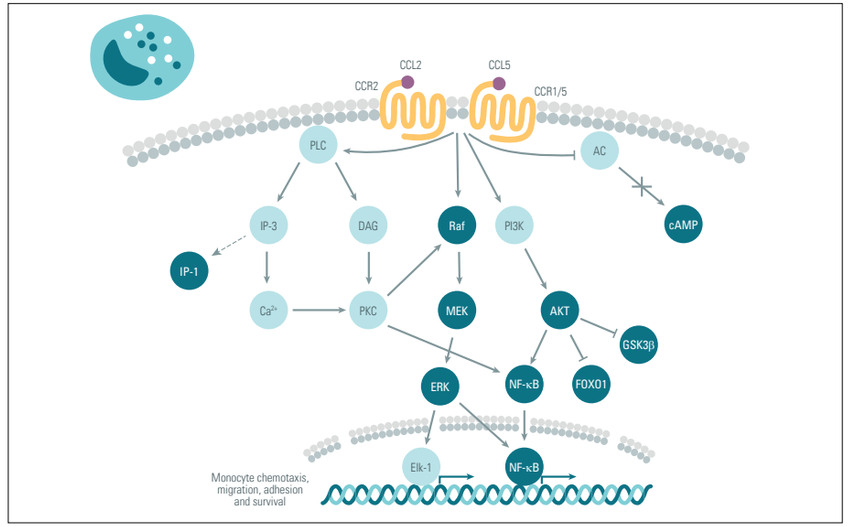
MOLECULAR PATHOGENESIS OF LIVER FIBROSIS

Recruitment of inflammatory monocytes

- PI3K/AKT pathway: CCL2 and CCL5 stimulate the PI3K-dependent signaling cascade, resulting in the activation of AKT which is the best-characterized downstream effector of PI3K. AKT in turn phosphorylates its own substrates, thus leading to the inhibition of FoxO1 and GSK3β, as well as the activation of NF-κB which translocates to the nucleus and promotes the transcription of target genes [5].
- MAPK/ERK pathway: Upon chemokine binding, CCR2 and CCR1/5 also trigger the activation of the Raf/MEK/ERK signaling axis, resulting in the activation of the transcription factors Elk-1 and NF-κB [6].

All these pathways lead to monocyte chemotaxis, migration to the liver, adhesion, and survival.



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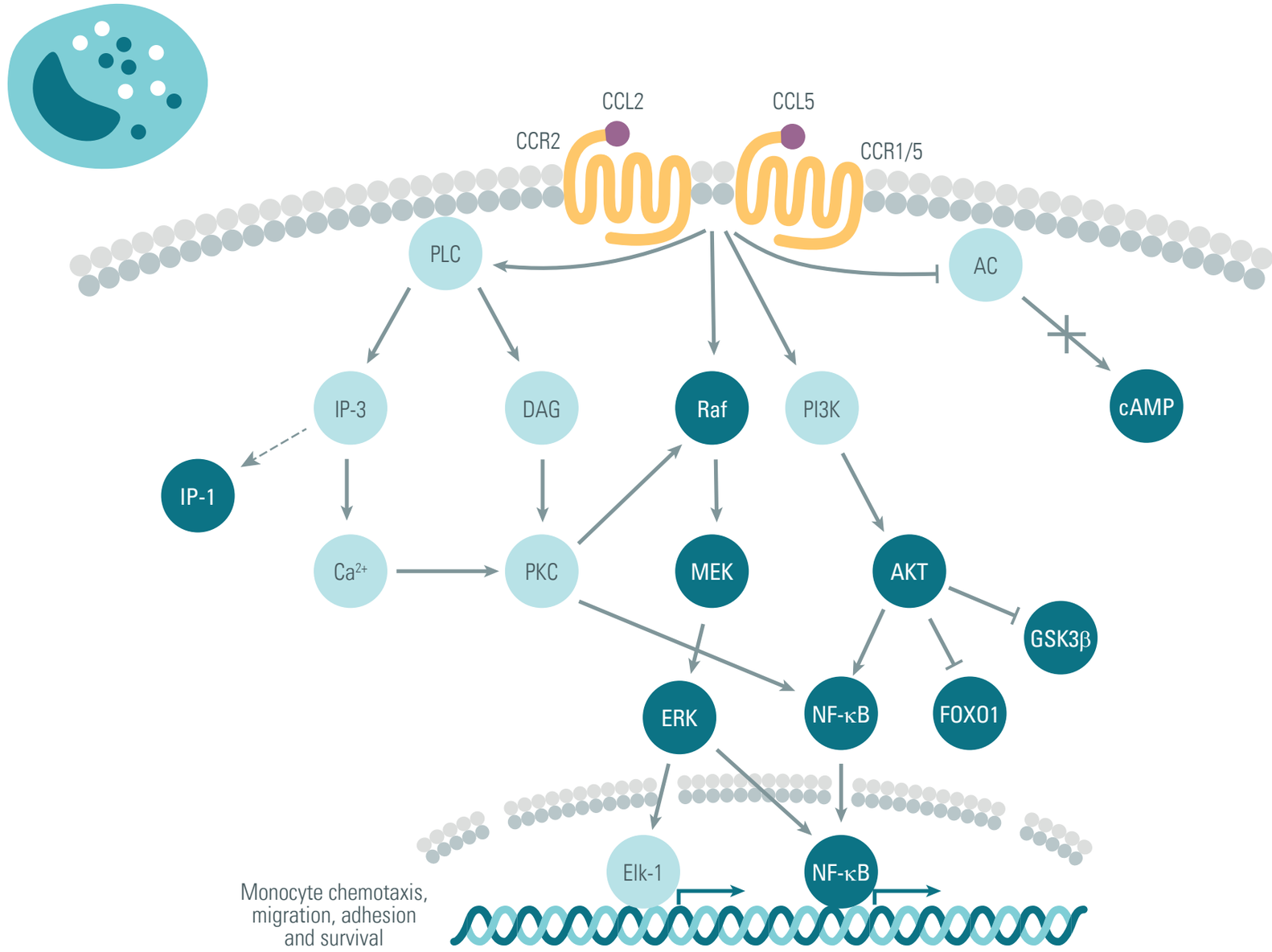

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MOLECULAR PATHOGENESIS OF LIVER FIBROSIS

Activation of hepatic macrophages

The macrophage pool of the injured liver is rapidly expanded by infiltrating monocytes that develop into inflammatory and fibrogenic macrophages.

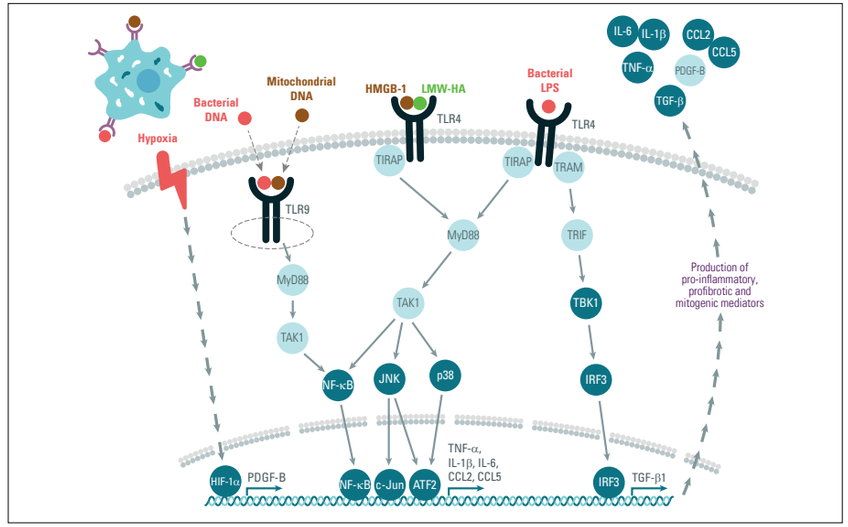
Both subtypes of hepatic macrophages, i.e. Kupffer cells and monocyte-derived macrophages, then become overactivated by gut-derived PAMPs such as LPS and bacterial DNA, as well as by DAMPs like mitochondrial DNA or HMGB-1 [1]. Moreover in the damaged liver, the ubiquitous ECM component hyaluronic acid (HA) is broken down into low molecular weight HA (LMW-HA) fragments that also activate liver macrophages [2].

All these molecules bind to their respective TLR, either the cell surface TLR4 or the endosomal TLR9, and induce the activation of the MyD88-dependent and/or TRIF-dependent signaling cascades.

The first pathway (MyD88-dependent) leads to the phosphorylation of TAK1, which then activates NF-κB as well as the MAP kinases JNK and p38. This finally results in the expression and secretion of pro-inflammatory mediators such as the cytokines TNF-α, IL-1β, IL-6, and the chemokines CCL2 and CCL5.

The second signaling axis (TRIF-dependent) induces the activation of TBK1, which in turn phosphorylates IRF3 which is responsible for the transcription of the gene coding for the profibrotic cytokine TGF-β1 [3].

In addition, regions of hypoxia develop in the injured liver. In hypoxic macrophages, the transcription factor Hypoxia-Inducible Factor 1α (HIF-1α) is upregulated, resulting in the increased expression of profibrotic mediators like PDGF-B [4].



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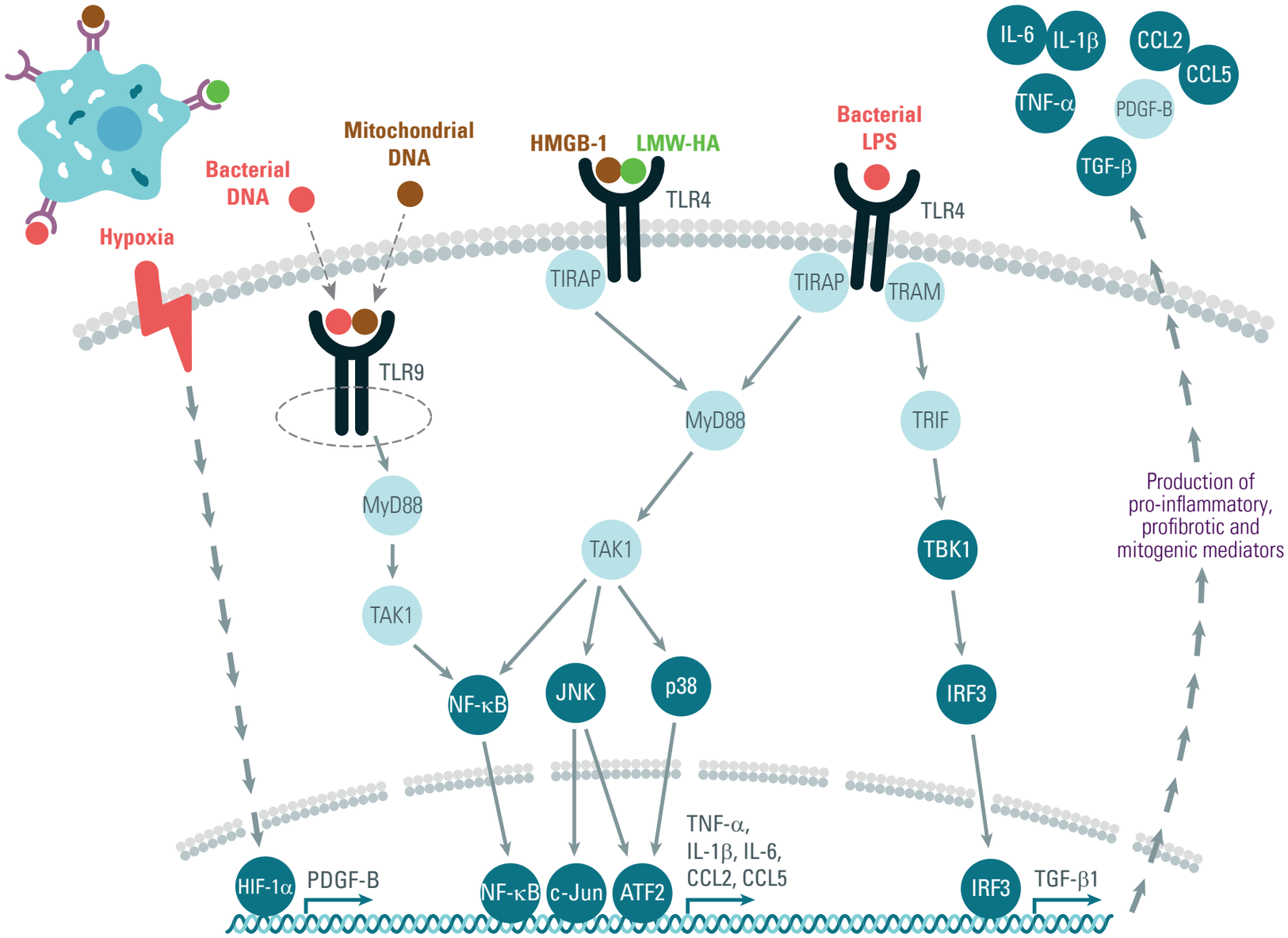

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MOLECULAR PATHOGENESIS OF LIVER FIBROSIS

HSC transdifferentiation into myofibroblasts

Activation of HSCs is a key event in liver fibrosis leading to trans-differentiation from quiescent vitamin-A-storing cells to myofibroblasts, which are proliferative, contractile, inflammatory and chemotactic, and are characterized by enhanced ECM production. [1] This activation is caused by a wide range of signals from several extra-cellular factors, and involves many interconnected signaling pathways. The main (non-exhaustive) mechanisms are briefly described in this section.

- TGF-β/SMAD signaling: TGF-b released in a latent form by several cell populations is cleaved and allows the binding of mature TGF-b to the receptor heterocomplex, leading to phosphorylation of the type I receptor (ALK5) which further recruits, phosphorylates and activates SMAD2/3 proteins. The subsequent translocation with SMAD4 into the nucleus promotes the transcription of fibrogenic genes: αSMA, which confers contractility, collagens I and III, HA, TIMP1 as secreted ECM components, and ROS generating enzymes (NOX4). H_2O_2 activates signaling proteins such as SMAD2/3, ERK1/2, JNK and Src, which subsequently induce the transcription of genes involved in ECM deposition, cell proliferation, migration, differentiation, and apoptosis [4]. TGF-β also activates MAPK pathways including ERK, participating in the differentiation and proliferation of HSCs [2].
- Signaling by Inflammatory cytokines: HSCs can maintain their activated state by autocrine and paracrine stimulation through cytokines. TNFα and IL-1b binding to the corresponding receptor leads to activation of the NF-κB pathway, promoting HSC survival [3].
- Gut-microbiota LPS/TLR4 signaling: increases in PAMPs such as LPS due to higher proportions of Gram negative endotoxin-producing bacteria (from a high fat diet or via higher intestinal permeability) activates TLR4 receptors that are highly expressed in HSCs. This results in activation of the MyD88 dependent pathway, turning on pro-inflammatory pathways NF-κB, and JNK, and the expression of chemokines CCL2 and CCL5. Recruitment is promoted, as well as interaction with inflammatory cells.

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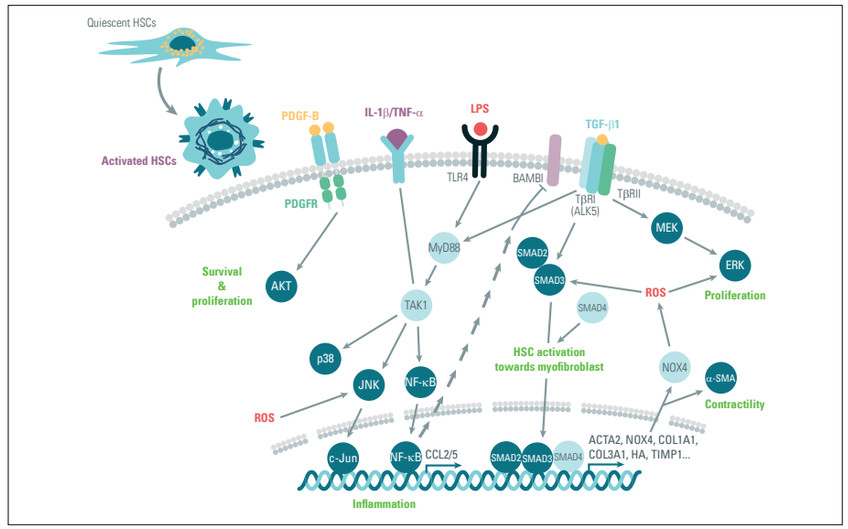
MOLECULAR PATHOGENESIS OF LIVER FIBROSIS

HSC transdifferentiation into myofibroblasts

- PDGF signaling: PDGF secreted from monocyte-derived macrophages and Kupffer cells cause AKT activation and subsequent proliferation and migration through PDGF receptor beta signaling. The receptor also increases during HSC activation, setting up a positive loop and contributing to sustained proliferation.
- Links between inflammation and fibrosis: in HSCs, JNK MAPKs are activated by several stimuli including TLRs, IL-1β, TNFα, and ROS. JNK promotes PDGF, TGF-β, αSMA, and collagen production, and plays important roles in SMAD2/3 phosphorylation [3]. NF-κB transcription factor is a key regulator of inflammation and cell death, and is also activated by TLRs, IL-1β and TNFα. NFKB activation results in down-regulation of the TGF-b decoy pseudo-receptor BAMBI that lacks the intracellular serine/threonine protein kinase domain, thereby acting as a negative regulator. This downregulation results in sensitization of the TGF-b activation. Both JNK and NF-κB play a crucial role by linking inflammation and fibrogenesis in HSCs.



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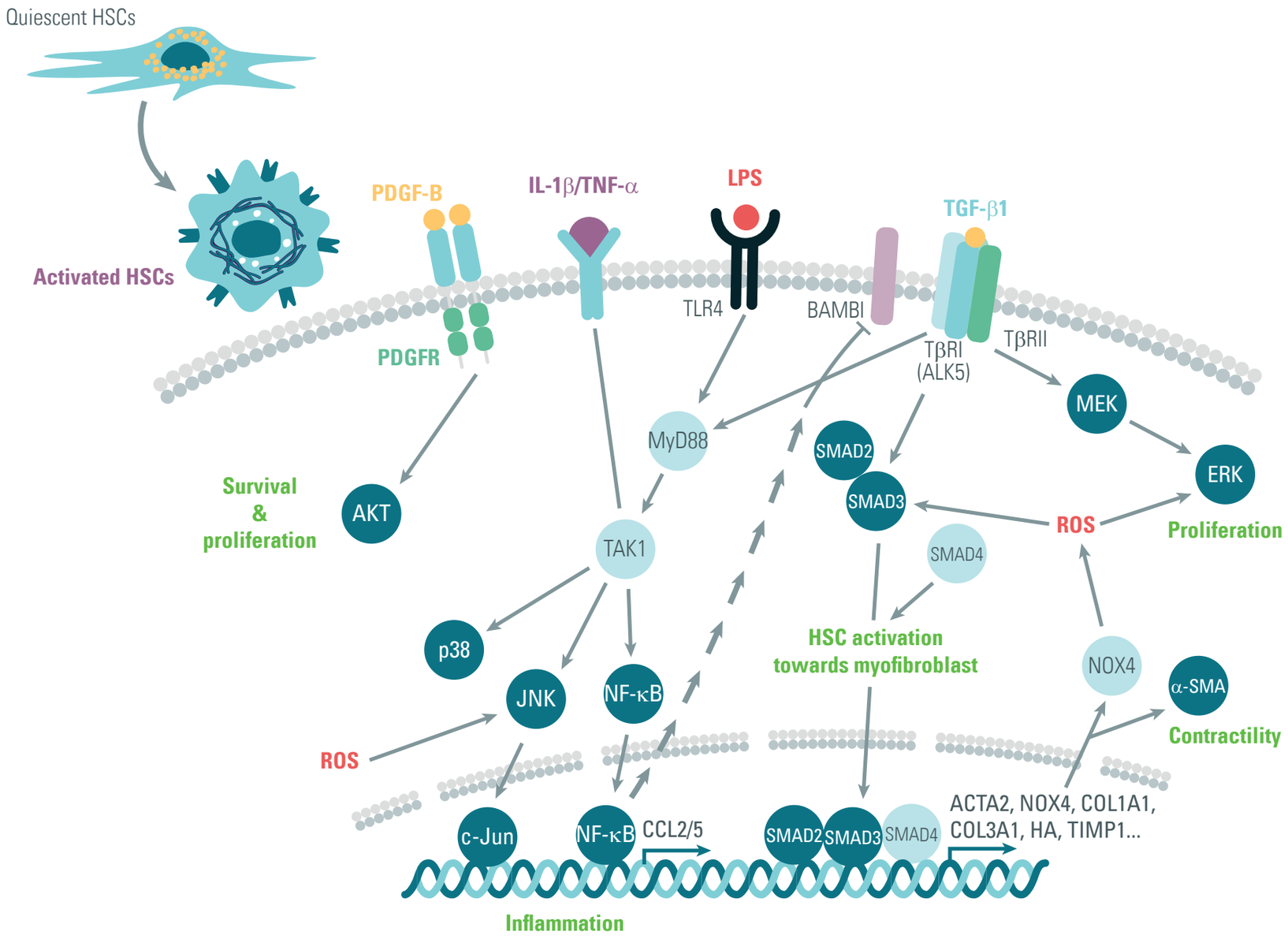

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MOLECULAR PATHOGENESIS OF LIVER FIBROSIS

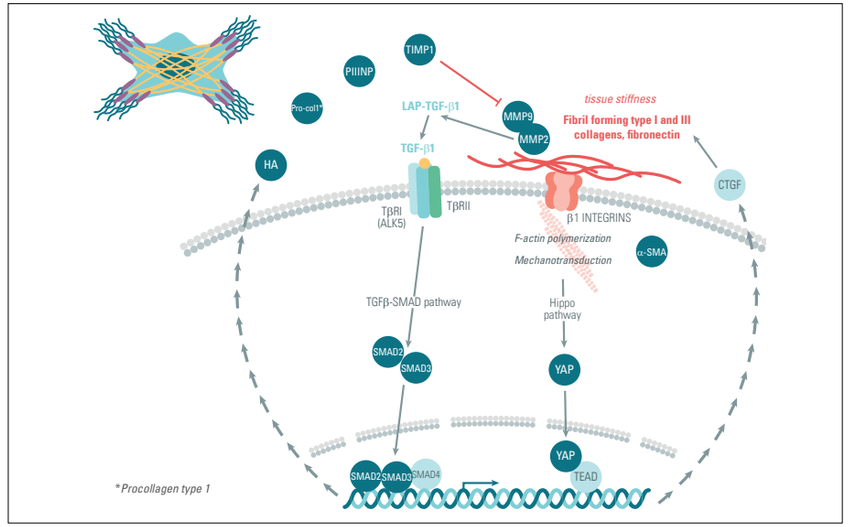
Myofibroblast activation and ECM secretion

Upon liver damage, HSC-derived myofibroblasts proliferate and migrate to promote wound healing by depositing ECM components such as fibrillar collagens I and III, and their remodeling by metalloproteinases (MMPs). The aberrant wound healing mechanism taking place due to chronic injury in NASH causes hyper-proliferation and reduced apoptosis of myofibroblasts, leading to increased ECM deposition, tissue stiffness, and mechano-transduction via contact with ECM molecules through αSMA fibers and focal adhesions. [1]

Growth factors such as TGF-β1 are trapped in the ECM until they are released during injuries by mechanical tensions and/or activation from their latent form (LAP-TGFb1) through proteolytic cleavages [2] by MMPs and alpha integrins. This results in an increased activation of the TGF-β/SMAD pathway that leads to even higher expression of αSMA stress fibers and the secretion of ECM molecules that can be measured though hyaluronic acid (HA), procollagen I or N-terminal peptide of collagen III (P3NP). Expression of TIMP-1 and -2 also increases with fibrosis, inhibiting MMPs and further favoring ECM deposition and tissue stiffness.

The Hippo pathway and its effector YAP is an early key pathway that is also involved in HSC activation [3]. Increases in matrix stiffness are sensed through cell surface receptors such as integrins, and there is a subsequent cytoskeletal reorganization through F-actin that alters the cytoplasmic core components of the Hippo pathway. Depolymerization of F-actin triggers translocation of YAP into the nucleus that serves as a co-activator with the TEAD transcription factors to promote expression of fibrogenic genes such as CTGF, leading to subsequent stimulation of HSC, ECM secretion, and even greater stiffness [1]. This results in a feedback-loop promoting self-sustained activation and ultimately the persistence of populations of myofibroblasts in the liver.

Chronic injury in NASH causes increased signaling through TGFβ1-SMAD3, YAP/TEAD, and also WNT/β-catenin, which are intricate pathways driving the activation and maintenance of myofibroblasts. The result is perpetuated HSC activation, a positive feedback loop causing over-accumulation of ECM, formation of fibrous scar tissue, and liver fibrosis.



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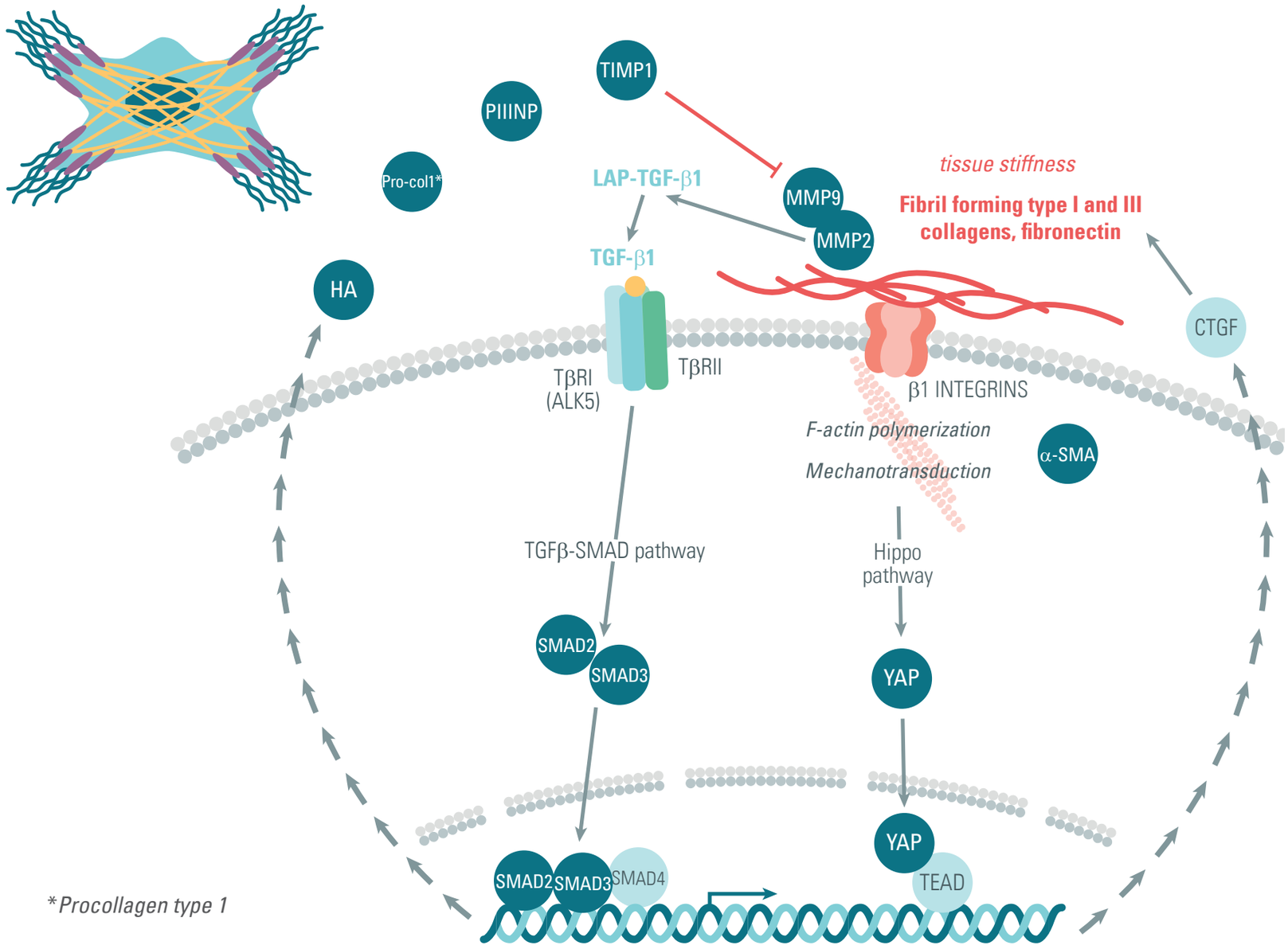

*Procollagen type 1

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APPLICATIONS

Kits, reagents, and services

Everyday, Revvity invests in NAFLD research and is offering you a lot of solutions for you to investigate each step of its development.

Phosphorylation assays

NFkB phospho-S536 kit
SMAD3 phospho-total kit
YAP phospho-S127 kit
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Fibrosis biomarker assays

Pro-collagen type 1 kit
Alpha-SMA kit
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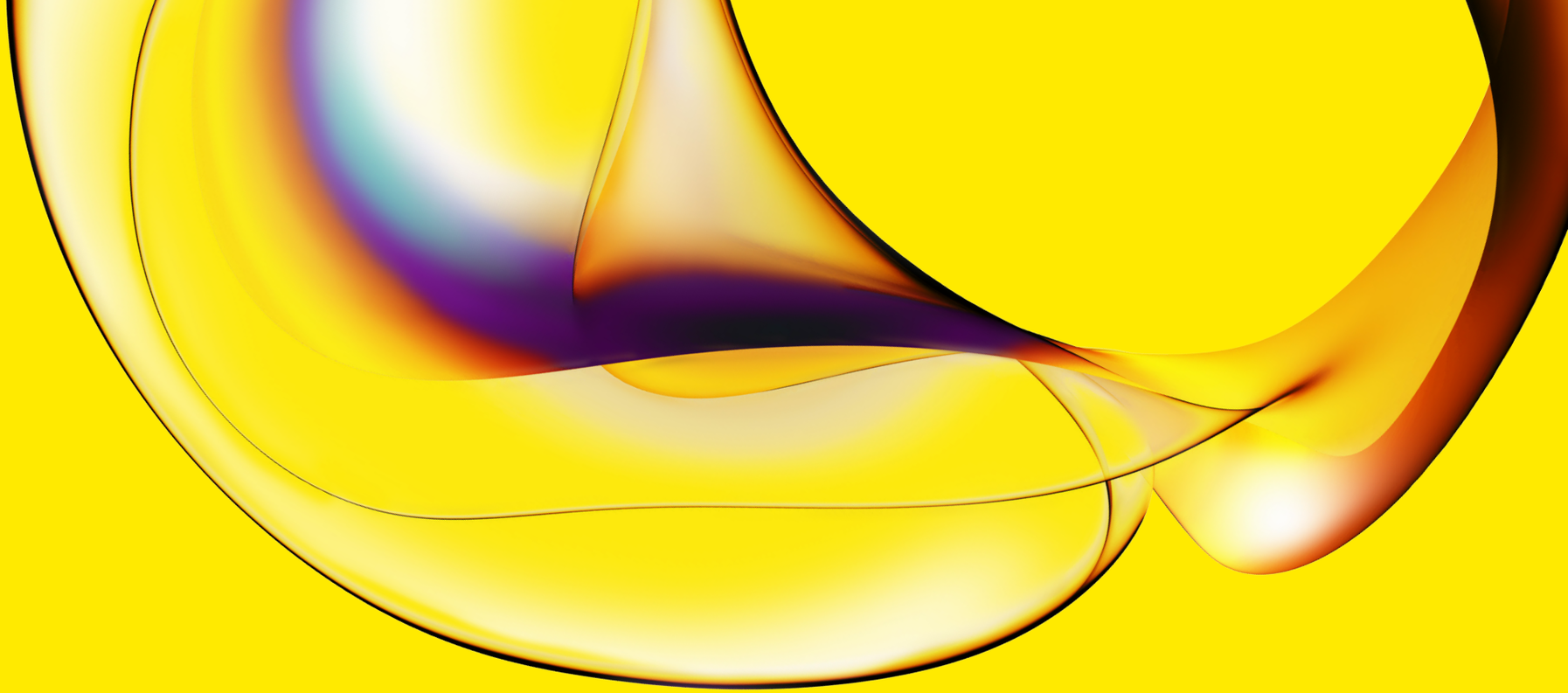

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