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Epigenetic Mechanisms of Pancreatobiliary Fibrosis

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Abstract

Purpose of review The goal of this manuscript is to review the current literature related to fibrogenesis in the pancreatobiliary system and how this process contributes to pancreatic and biliary diseases. In particular, we seek to define the current state of knowledge regarding the epigenetic mechanisms that govern and regulate tissue fibrosis in these organs. A better understanding of these underlying molecular events will set the stage for future epigenetic therapeutics.

Recent findings We highlight the significant advances that have been made in defining the pathogenesis of pancreatobiliary fibrosis as it relates to chronic pancreatitis, pancreatic cancer, and the fibro-obliterative cholangiopathies. We also review the cell types involved as well as concepts related to epithelial-mesenchymal crosstalk. Furthermore, we outline important signaling pathways (e.g., TGFβ) and diverse epigenetic processes (i.e., DNA methylation, non-coding RNAs, histone modifications, and 3D chromatin remodeling) that regulate fibrogenic gene networks in these conditions.

Summary We review a growing body of scientific evidence linking epigenetic regulatory events to fibrotic disease states in the pancreas and biliary system. Advances in this understudied area will be critical toward developing epigenetic pharmacological approaches that may lead to more effective treatments for these devastating and difficult to treat disorders.

Introduction

Tissue fibrosis occurs in the context of an exaggerated wound healing response to various chronic injuries. During this process, inflammatory mediators drive extracellular matrix (ECM) deposition in an effort to curtail the injury and allow repair processes to take place. Fibrosis is mediated by fibroblasts or

myofibroblasts, specialized mesenchymal cells that produce and lay down the ECM components including fibronectin (FN) and collagen (COL), as well as intracellular markers of activation such as alpha smooth actin (αSMA). In pathological conditions, fibrosis is excessive, resulting in structural tissue distortion and organ dysfunction. This pathological process is an integral part of many pancreatobiliary diseases. In the pancreas, fibrosis plays a significant role in the pathogenesis of chronic pancreatitis and pancreatic adenocarcinoma. Similarly, in the biliary system, the fibro-obliterative cholangiopathies result in peri-portal fibrosis which can progress to biliary cirrhosis. Currently, there are no effective treatments to prevent the progression of pancreatobiliary fibrosis. In this review, we will consider the key cells involved in pancreatobiliary fibrosis. We will then discuss the signaling pathways and epigenetic regulators that initiate and propagate pancreatobiliary fibrosis. We will also point out vast gaps in the current understanding of these epigenetic mechanisms. Ultimately, a more complete understanding of these pathobiologic mechanisms may be exploited in the future to develop novel therapeutic approaches to prevent the progression of fibrosis.

Part I: Pancreatic fibrosis

Chronic pancreatitis

Pancreatic fibrosis is an established driving force behind the pathogenesis of chronic pancreatitis. Chronic pancreatitis develops as a result of various chronic injuries to the pancreas including alcohol abuse, tobacco smoking, metabolic insults such as hypercalcemia and hypertriglyceridemia, autoimmune disorders, and injuries as a result of genetic predisposition [1•]. In Western countries, the majority of cases of chronic pancreatitis are due to alcohol abuse with tobacco smoking contributing as an independent risk factor [2, 3]. Multiple models of the pathophysiology of chronic pancreatitis have been hypothesized. These include (1) direct and chronic metabolic-toxic effects that accrue over time, (2) repeated episodes of acute pancreatitis that damage the pancreatic parenchyma, (3) a single sentinel acute pancreatitis in predisposed patients that causes ongoing injury, (4) oxidative stress perpetuating injury to the parenchyma, and (5) ductal dysfunction and plugging that cause persistent injury [1•]. The pathophysiology of chronic pancreatitis might involve components of several pathways. Regardless of the etiology or manner of pancreatic injury, the common result is pancreatic fibrosis. Fibrosis in chronic pancreatitis eventually replaces significant parts of the organized parenchyma including acinar cells, islet of Langerhans, and pancreatic ducts resulting in ductopenia and abnormal ducts [1•, 4].

Pancreatic cancer

Pancreatic cancer exists within a unique microenvironment, exhibiting excessive fibrogenesis referred to as the "desmoplastic reaction." In fact, the extent of ECM deposition in the primary pancreatic cancer has been associated with patient survival [5]. The desmoplastic reaction contains pancreatic cancer, immune, myofibroblast, and stromal cells. Up to 80–90% of the tumor volume can be a combination of stromal cells and ECM [6•, 7]. The extent of ECM in these tumors confers increased tumor stiffness which is thought to cause tissue hypoperfusion and hypoxia [7, 8]. This phenomenon may confer a survival advantage to tumor cells and

may also contribute to chemotherapy and radiotherapy resistance [7]. The desmoplastic reaction also appears to be required for metastasis. Targeting the signaling pathways regulating the fibrogenic microenvironment can curtail cancer migration and metastasis [9, 10].

Pancreatic stellate cells

Pancreatic stellate cells (PSCs) are resident, mesenchymal pancreatic cells and well-established mediators of pancreatic fibrosis. PSCs are found in the peri-acinar space of the exocrine pancreas forming 4–7% of the pancreas [11, 12]. PSCs are similar to their hepatic counterparts, hepatic stellate cells (HSCs); both of which are distinctly different from fibroblasts [13]. Both PSCs and HSCs exist in two states: quiescent or activated. Quiescent PSCs contain retinoid droplets [11, 12]. Their functions include maintenance of tissue architecture, vitamin A storage, phagocytosis of bacteria, and stimulation of amylase from the exocrine pancreas [14]. When activated, PSCs trans-differentiate into a myofibroblast phenotype and loose retinoid droplets; express αSMA, FN, and COL; and are capable of active migration and proliferation [14, 15]. Activated PSCs are the predominant cells responsible for ECM deposition and resultant fibrosis in the pancreas [1]. An increasing number of injurious stimuli have been shown to activate PSCs including alcohol and its metabolites, endotoxins, oxidative stress, hypoxia, hyperglycemia, proteases, and other toxic agents [15, 16]. Similarly, various cytokines and chemokines are also capable of activating PSCs. Notable among these are platelet-derived growth factor (PDGF), transforming growth factor beta (TGFβ), and tumor necrosis factor alpha (TNFα) [15, 16]. Multiple signaling pathways have been implicated in mediating PSC activation. These include the Wnt/β-catenin pathway, the mitogen-activated protein kinase (MAPK) pathway, p38 and c-jun N-terminal kinase (JNK/p38) signaling, the nuclear transcription factor AP-1, Hedgehog pathways, extracellular signal-regulated kinase (ERK) and Janus activated kinases/Signal induced activation of transcription (JAK/STAT) signaling, and the phosphoinositide 3-kinases/protein kinase B (PI3K/AKT) pathway [15]. PSCs also express toll-like receptors (TLRs) which, upon binding to endotoxins, activate the transcription factor NFκB and result in PSC activation [17, 18]. Importantly, the profibrotic growth factor TGFβ can activate PSCs in both autocrine and paracrine manner [15, 19–22]. This has been shown to take place through both canonical/SMAD-dependent pathways as well as non-canonical pathways [22].

PSCs are key mediators of the pathogenesis and ultimate fibrosis of chronic pancreatitis [1]. Human samples of chronic pancreatitis show strong staining for TGFβ in acinar cells that may then activate PSCs in a paracrine fashion [23]. Animal models of chronic pancreatitis show improvement with the prevention or reversal of PSC activation [24]. All-trans-retinoic acid (ATRA) induces quiescence in PSCs. ATRA treatment of a mouse model of chronic pancreatitis suppressed fibrosis by inhibition of PSC proliferation, induction of PSC apoptosis, and reduced ECM synthesis through the Wnt/β-catenin pathway [25]. Others have shown that inhibiting the canonical/SMAD-dependent TGFβ pathway results in

reduced PSC activation and pancreatic fibrosis in a rat model of chronic pancreatitis [26]. Peroxisome proliferator-activated receptor gamma (PPAR) activation, which is known to block PSC activation [27], has also been shown to prevent the development of chronic pancreatitis by modulating NFκB-mediated inflammatory cytokines and PSC activation [28].

Likewise, PSCs play an integral role in the development, invasion, and metastasis of pancreatic cancer [14]. This is thought to occur through a significant contribution of PSCs to the desmoplastic reaction that accompanies pancreatic cancer development. During this process, PSCs undergo activation and optimize the tumor microenvironment for progression and invasion by increased ECM synthesis and modulating the secretion of matrix metalloproteinases [14, 29]. Subpopulations of PSCs, for example, CD10+ or cadherin 11–expressing PSCs, have been linked with pancreatic cancer invasion and metastasis [9, 10]. Indeed, treatment of PSCs with quiescence-inducing ATRA prevented cancer invasion in in vitro models [30•].

Part II: Biliary fibrosis

Fibrotic cholangiopathies

Cholangiocytes are a heterogeneous group of epithelial cells that line the lumen of the intrahepatic and extrahepatic biliary system. Chronic disorders of cholangiocytes are collectively referred to as cholangiopathies [31]. The etiologic causes of the cholangiopathies are diverse and include genetic disorders (e.g., Alagille syndrome and cystic fibrosis), infections (e.g., Cryptosporidium-associated cholangiopathy), immune-mediated disorders (e.g., primary biliary cholangitis), idiopathic disorders (e.g., primary sclerosing cholangitis and biliary atresia), and vascular disorders (e.g., hepatic artery thrombosis after liver transplantation) [31, 32]. Regardless of etiology, the cholangiopathies share similar pathologic features that result from the initial injury to cholangiocytes, followed by inflammation, cholestasis, cholangiocyte proliferation and apoptosis, and ultimately ductopenia and fibrosis [32••, 33]. With persistent injury, the fibrosis can progress to biliary cirrhosis and its complications including malignancy (i.e., hepatocellular carcinoma or cholangiocarcinoma) and end-stage liver disease. Currently, therapies are targeted at preventing the initial injury to cholangiocytes; for example, ursodeoxycholic acid and obeticholic acid are approved treatments for primary biliary cholangitis. Other conditions, such as primary sclerosing cholangitis, have no effective therapy and are often progressive, requiring liver transplantation. A better understanding of the mechanistic underpinnings of biliary fibrosis should ultimately offer new therapeutic options to curtail the progression of the cholangiopathies.

Cholangiocyte injury and the resulting aberrant signaling cascades transform cholangiocytes into an activated form. Various inflammatory signals and angiogenic factors have been implicated in cholangiocyte activation [32••, 33]. Among these, TGFβ is abundantly expressed in the cholangiopathies and is known to activate cholangiocytes [33]. Activated cholangiocytes are characterized by active proliferation and a highly secretory state [34]. Cytokines and chemokines secreted by activated cholangiocytes may additionally recruit inflammatory cells and engage in crosstalk with mesenchymal cells [32••, 34]. The complex of activated cholangiocytes, inflammatory cells, and mesenchymal cells constitutes the histological lesion known as the ductular reaction [32••].

The ductular reaction further propagates the activation of cholangiocytes and myofibroblasts that ultimately lay down the ECM of fibrosis.

Hepatic stellate cells

The origin of the myofibroblasts that lay down the ECM of liver fibrosis has been a matter of some debate. Varying degrees of contribution have been proposed from activated HSCs, portal fibroblasts, bone marrow–derived fibrocytes, and epithelial-mesenchymal transition [35•, 36–38]. More recently, lineage tracing studies have supported HSCs as the dominant contributor to hepatic fibrosis, regardless of the etiology [39]. Therefore, while there is likely some contribution of portal fibroblasts, particularly in the earlier stages of biliary fibrosis, HSCs are thought to mediate significant parenchymal fibrosis in the latter stages of the disease [32••, 33].

HSCs are lipid-containing cells comprising about 10% of resident liver cells, located in the space of Disse, between liver sinusoidal endothelial cells and hepatocytes [40, 41]. Quiescent HSCs are thought to serve as vitamin A storage cells. When activated, they transform to a proliferative, contractile, and chemotactic myofibroblast-like cell type, capable of profound ECM synthesis. Their role in mediating fibrosis in various liver diseases, including cholangiopathies, chronic viral infections, alcoholic liver disease, and non-alcoholic liver disease (NAFLD), has been established [41•].

HSC activation is mediated by a wide variety of stimuli and modulated by a variety of signaling pathways and cell-cell interactions [41•]. Chief among these are fibrogenic, proliferative, and angiogenic cytokines such as TGFβ, PDGF, vascular endothelial growth factor, and connective tissue growth factor [41•]. Indeed, the selective deletion of PDGF receptor β in HSCs prevents hepatic fibrosis in the bile duct ligation model of biliary fibrosis [42]. Similarly, the inhibition of TGFβ signaling in HSCs attenuates hepatic fibrosis in hepatocellular models of fibrosis [43]. Hedgehog signaling is another pathway that regulates HSC activation and its inhibition in mice attenuates fibrosis during liver injury [44]. Other mechanisms of HSC activation include autophagy, endoplasmic reticulum and oxidative stress, free cholesterol formation, and various inflammatory cytokines and endotoxins [41•]. HSC interactions with injured hepatocytes, immune cells, LSECs, and platelets have been shown to result in HSC activation [41•], while the hypothesized interactions of cholangiocytes and HSCs in biliary fibrosis is less well studied.

Maintenance of or regression to HSC quiescence is mediated by adiponectin and nuclear receptors, liver X receptor, farnesoid X receptor, PPAR, vitamin D receptor, and nuclear receptor subfamily 4 group A member 1. Activation of these nuclear receptors in HSCs has been shown to attenuate fibrosis and inhibition has been shown to worsen fibrosis in hepatocyte injury models [41•], although these observations are yet to be replicated in models of biliary fibrosis.

TGFβ signaling

TGFβ signaling plays a dominant role in the activation of PSCs and HSCs. In fact, TGFβ signaling is a well-accepted regulator of organ fibrosis [45]. TGFβ is secreted by various cells in pancreatic and biliary diseases [33, 46]. The latent

form is activated by αvβ6 integrin, which is expressed by activated cholangiocytes [33]. Canonical TGFβ signaling occurs through the activation of SMADs, predominantly SMAD3, which promotes HSC and PSC activation [41•, 46]. Non-canonical TGFβ signaling involves activation of MAPK, including ERK, JNK/p38, and Rho-like GTPase signaling pathways, which also activate PSCs and HSCs [33, 46]. [47] Blocking TGFβ signaling through various strategies ameliorates pancreatobiliary fibrosis in preclinical studies [33, 46]. There are also ongoing clinical trials targeting TGFβ signaling in the treatment of pancreatobiliary fibrosis [33, 46]. The primary concern with this strategy is that TGFβ signaling is quite broad, affecting not only multiple cells and organs in the body, but also various, sometimes opposing, cellular processes. For example, while TGFβ signaling is integral for pancreatobiliary fibrosis, it also has an important role in pancreatic and biliary repair [33, 46]. Similarly, TGFβ may have pro- or anti-tumorigenic effects, depending upon the stage of pancreatic cancer [46]. Furthermore, TGFβ has an important role in regulating the immune system and therefore, non-specific blocking of TGFβ may have untoward immunological effects [45]. Thus, further understanding of the downstream regulators of TGFβ signaling is required in order to isolate the pathological mechanisms that are involved in pancreatobiliary fibrosis. In this context, targeting druggable epigenetic regulatory mechanisms holds significant promise in developing more specific therapeutics.

Part III: Epigenetic mechanisms of pancreatobiliary fibrosis

Epigenetics

All cells within a mammalian organism have an identical genomic DNA sequence. Yet, cells in different organs have vastly variable functions, structures, and gene expression patterns. Furthermore, cells respond differently to internal and external stimuli by activating or silencing various gene networks, without changes to the genomic DNA sequence. These cell type–specific responses are orchestrated primarily by epigenetic mechanisms. Epigenetics is defined as reversible changes that do not alter the underlying DNA sequence, modulate gene expression, and are heritable through cell division. Epigenetic mechanisms can be broadly categorized into DNA methylation, non-coding RNA–mediated gene modulation, histone modifications, and 3-dimensional/higher order chromatin remodeling. Since cells need to adapt and respond to various stimuli, these disparate mechanisms also appear to be both dynamic and overlapping [48]. The stimuli can be internal, such as autoimmunity, and external, such as environmental exposures. Therefore, it is likely that epigenetic mechanisms play a key role in the pathogenesis of many pancreatobiliary diseases including fibrosis.

DNA methylation

During DNA methylation, a methyl group is added to the cytosine ring of DNA to form 5-methylcytosine. This process is carried out by DNA methyltransferases and mainly takes place at CpG dinucleotides. DNA methylation of promoter regions is typically associated with suppression of downstream gene transcription, probably mediated by the recruitment

of suppressive proteins by the methylated promoter [49]. In contrast, DNA methylation within the gene body can be associated with activated genes [50]. DNA methylation is a dynamic process, and demethylation is chiefly carried out by Ten Eleven Translation (TET) demethylase [51]. DNA methylation has been well studied in many diseases and is well-known in the pathogenesis of cancers [52, 53].

Although genome-wide DNA methylation has been studied in pancreatic cancer, genome-wide or gene-specific analysis of DNA methylation has not been well studied in PSCs [54, 55]. In pancreatic cancer and chronic pancreatitis, the promoter of WNK2, a tumor suppressor gene, is highly methylated and correlates with low mRNA and protein expression [56]. This is one mechanism for cancer pathogenesis in at-risk chronic pancreatitis patients and for cell growth in patients with established pancreatic cancer. Further studies are required in models of chronic pancreatitis and pancreatic cancer to identify the role of DNA methylation in the pathogenesis and progression of these diseases.

Activated HSCs show global changes in DNA methylation patterns, which correlate with gene expression [57]. In HSC activation, methyl-CpG binding protein 2 (MeCP2) methylates the promoter of PPARγ, the master regulator of HSC quiescence. This process suppresses PPARγ expression by recruiting other epigenetic regulatory machinery, allowing for HSC activation and subsequent fibrogenesis (Fig. 1a) [58]. Furthermore, MeCP2 promotes pro-fibrogenic gene expression within HSCs by recruiting the histone methyltransferase, ASH1, which then methylates lysine 4 on histone 3 (H3K4), promoting gene expression (Fig. 1a) [59]. Notably, HSC activation was studied in models of hepatocyte injury and has not been replicated in biliary models yet. In biliary fibrosis animal models, DNA methyltransferases, DNMT1 and 3a, increase HSC activation and fibrosis, whereas TET demethylase is downregulated [60].

Non-coding RNAs

Non-coding RNAs (ncRNAs) are RNA molecules that are not translated into proteins. They are traditionally divided into small ncRNAs and long ncRNAs based on size cut off of 200 nucleotides. Small ncRNAs occur in several varieties, including small interfering RNAs (siRNAs), microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), and transfer RNAs (tRNAs). siRNAs can be experimentally introduced into a cell or formed endogenously to form a complex of RNA-induced silencing complexes (RISCs). siRNA guides the RISC complex to a target mRNA through base pair complementarity and results in mRNA degradation [61, 62]. Similarly, miRNAs form a miRISC complex and bind predominantly to the 3′ untranslated region (UTR) but also perhaps the coding region and the 5′UTR of target mRNA (Fig. 1b, c). Translation of target mRNA is silenced by suppressing protein translation, enhancing mRNA degradation, mRNA compartmentalization, or a combination of these processes (Fig. 1c) [61, 62]. miRNAs have been studied in many diseases including pancreatobiliary fibrosis.

The role of miRNAs in HSC quiescence, activation, proliferation, and ECM deposition is increasingly recognized [63, 64]. miRNAs can interact with the TGFβ and PPARγ signaling pathways to affect HSC quiescence or activation [63]. Some of these miRNAs have been directly implicated in the

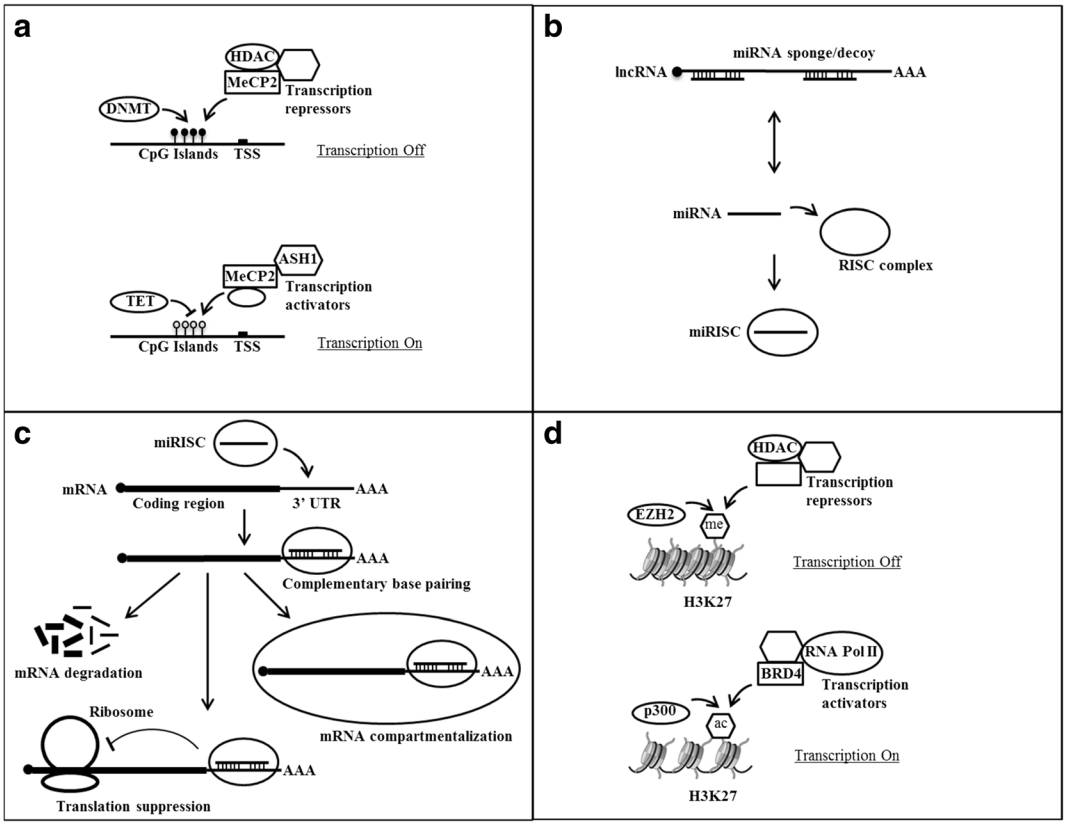

Fig. 1. Epigenetic mechanisms involved in pancreatobiliary fibrosis. **a** DNMTs mediate DNA methylation at the promoter CpG islands, which is recognized by MeCP2, recruiting further transcriptional repressive machinery, and silencing antifibrogenic genes. Unmethylated promoter regions are recognized by transcriptional activating machinery, in which case, MeCP2 serves to activate fibrogenic genes by recruiting ASH1. **b** LncRNAs have both profibrotic and anti-fibrotic effects by acting as sponge/decoy for miRNA. **c** miRNAs modulate both fibrogenic and antifibrogenic gene expression by predominantly binding to the 3'UTR of mRNAs and modulating their degradation, translational suppression, or compartmentalization. **d** Histone modifications are laid down by epigenetic writers (e.g., EZH2 or p300). H3K27me3 is mediated by EZH2 and can be associated with gene silencing. H3K27ac is mediated by p300 and associated with enhancers. BRD4 is the epigenetic reader of H3K27ac and activates gene expression.

pathogenesis of biliary fibrosis [65, 66]. Similarly, miRNAs have been implicated in pancreatic fibrosis and PSC activation through the TGFβ pathway [67, 68]. In one study, PSC activation was shown to cause upregulation of 42 miRNAs as well as downregulation of 42 miRNAs [69]. Of these miRNAs, 22 were affected in both pancreatic cancer and PSC activation. Predictably, the most affected signaling pathways were the canonical and non-canonical TGFβ signaling pathways.

Long ncRNAs (lncRNAs) are processed very similarly to mRNAs and are enriched in the nucleus. They are classified by their site of origin into sense, antisense, intronic, intergenic, enhancer, and circular RNAs [70]. Functionally, lncRNAs with signal and decoy capabilities serve in gene activation or suppression respectively. LncRNAs can act as guides to recruit epigenetic enzymes to regulate gene expression. Scaffold lncRNAs serve as platforms to assemble ribonucleoprotein complexes that act on chromatin or histones. These

capabilities are not mutually exclusive in any single lncRNA [70].

LncRNAs have both profibrotic and anti-fibrotic effects in modulating HSCs [63]. The mechanism for these effects typically occur through modulating miRNAs (Fig. 1b), but further research will likely uncover other mechanisms as well. LncRNA myocardial infarction–associated transcript (MIAT), which is overexpressed in chronic pancreatitis, is upregulated with TGFβ-mediated activation of PSCs and acts as a sponge for inhibitory miRNAs [71]. Syntaxin-12 (STX12) lncRNA is implicated in PSC activation in chronic pancreatitis by decreasing miR-148a levels and increasing SMAD5 [72]. These studies highlight the need for further exploration including RNA-sequencing studies to identify other lncRNAs involved in pancreatobiliary fibrosis.

Histone modifications

Post-transcriptional modifications of histones take place at amino acids both within the folded domains and on the histone tails that extend beyond the nucleosome. To date, there are more than 200 modifications known. These modifications are thought to regulate chromatin structure and thereby allow access to transcriptional regulatory machinery [73, 74]. They are also required at multiple steps of transcription where they directly recruit proteins involved in regulating transcription (Table 1) [75].

The most well-studied histone modifications are acetylation and methylation of lysine residues in the histone tails. Histone acetylation is generally associated with active promoters and downstream gene expression, while the effect of histone methylation depends on the site of methylation and the number of methyl components. Histone 3 lysine 9 acetylation (H3K9ac), histone 3 lysine 27 acetylation (H3K27ac), and histone 3 lysine 4 tri-methylation (H3K4me3) are examples of modifications associated with active genes. H3K9me2/3, H3K27me3, and H4K20me3 are associated with repressed genes. Histone modifications are laid down by "writers" such as histone acetyltransferases and methyltransferases. "Erasers" such as histone deacetylase and demethylases remove these modifications. "Readers" are proteins recruited by the modifications for downstream effects and modulation of gene expression. The bromodomain and extra-terminal domain (BET) family of bromodomain proteins are the most well-known group of readers implicated in many diseases [76].

Table 1. Selected well-known histone marks and associated transcriptional activity

Histone mark	Locus and associated transcriptional activity
H3K4me	Poised enhancers
H3K4me3	Transcriptionally active promoters
H3K9ac	Actively transcribed promoters
H3K9me3	Constitutively repressed genes
H3K27ac	Active enhancers
H3K27me3	Facultatively repressed genes
H3K36me3	Actively transcribed genes

The effect of histone modifications on PSC activation is beginning to be explored. HDAC inhibition abrogates fibrogenesis in chronic pancreatitis through TGFβ suppression and reduced PSC activation [77•]. In pancreatic cancer, the histone methyltransferase, G9a, mediates cancer resistance to gemcitabine [78]. G9a-dependent expression of IL-8 and paracrine PSC activation contributes to gemcitabine resistance. Indeed, G9a overexpression correlates with poor survival and early recurrence of pancreatic cancer [78]. Interestingly, G9a is responsible for repressive histone marks, notably H3K9me1/2. Therefore, G9a-mediated IL-8 production may occur through indirect effects. Further studies are required to evaluate the full spectrum of histone modifications and their role in pancreatic fibrosis.

In activated HSCs, promoter H3K27ac levels are globally decreased [57]. Paradoxically, H3K9ac was increased in a non-biliary model of HSC activation [79]. HDAC inhibitors are generally associated with reduced HSC activation, fibrogenic gene expression, and fibrosis, both in vitro and in vivo [63, 80, 81]. The exact mechanisms of these observations are under investigation and likely involve the interplay of TGFβ and PPARγ signaling. At the promoter level, HSC activation is regulated by the histone methyltransferases, enhancer of zeste homolog 2 (EZH2) and ASH1, which are both recruited by MeCP2 [81]. EZH2 is primarily responsible for H3K27me3, a repressive histone mark, which is recruited to the PPARγ promoter resulting in gene suppression, HSC activation, and liver fibrosis (Fig. 1d) [59, 82]. Similar to ASH1, a histone methyltransferase, MLL1, is also recruited to the promoter of fibrogenic genes in HSC activation resulting in H3K4me3 and gene expression [83]. TGFβ signaling is central to many of these mechanisms and also known to recruit P300, a transcriptional co-activator and acetyltransferase responsible for H3K27ac, which is required for HSC activation (Fig. 1d) [84]. Finally, at the reader level, targeting the BRD4 with a small molecule inhibitor, JQ1, prevents HSC activation, fibrogenic gene expression, and fibrosis [85].

Most of these observations have been made in hepatocyte injury models of HSC activation and not biliary injury induced HSC activation. This is an important caveat as there may be cell-specific implications and opposing functions of these epigenetic modifications. For example, unlike in HSCs, EZH2 genetic ablation in cholangiocytes results in worsened fibrosis [86•]. This appears to occur through TGFβ-mediated targeting of the EZH2 protein for proteasomal degradation in activated cholangiocytes. EZH2 degradation results in reduced promoter H3K27me3 and allows expression and release of pro-fibrogenic signals. These cholangiocyte signals then activate HSCs in a paracrine manner and ultimately mediate biliary fibrosis. Therefore, epigenetic pathways have to be studied simultaneously in the different cells within the hepatobiliary system in order to fully understand their contributions to hepatobiliary fibrosis.

3-dimensional and higher order chromatin remodeling

The DNA in the nucleus can be found in a heterochromatin form, which is tightly packaged comprising of suppressed genes, or in euchromatin, which is loosely packed, actively expressed genes. An example of this

type of regulation is the lamina-associated domains (LADs) in which heterochromatin and suppressed genes are compartmentalized to the nuclear periphery [87]. It is increasingly recognized that the chromatin structure is dynamic. On a smaller scale, partitioning of large segments of DNA resolve into topologically associated domains (TADs) wherein enhancer-promoter interactions take place [88]. Enhancers and super enhancers are regions of DNA marked by H3K27ac that are distant to the gene promoter but can form chromatin loops to bring transcriptional machinery to the promoter for transcription initiation [89, 90]. At the nucleosome level, ATP-dependent chromatin remodeling enzymes are part of the transcriptional machinery that modify nucleosome structure, composition, and positioning to regulate gene expression [91]. Switch/sucrose non-fermentable (SWI/SNF) proteins are among the most studied group of nucleosome remodelers that regulate the capacity to slide histone along the DNA and expose DNA for transcription [91, 92].

Whether pancreatobiliary fibrosis coincides with changes in heterochromatin and euchromatin has not yet been formally studied. Given the significant changes in gene expression that occur during pancreatobiliary fibrosis, there are likely to be many changes in LAD and TAD structure that allow enhancer-promoter interactions to occur and drive expression of fibrogenic genes. At the nucleosome level, the SWI/SNF nucleosome remodeling enzymes have been implicated in regulating HSC activation by modulating TGFβ/SMAD signaling [93]. Similar studies are required in PSCs and other cells involved in the pathophysiology of pancreatobiliary fibrosis.

Summary

In this article, we have reviewed the pathophysiology of the fibrotic disorders affecting the pancreatobiliary system. These disorders are the source of considerable morbidity, mortality, and cost, and yet they remain poorly understood and understudied. Despite this, major advances have been made in our understanding of the cell types that drive fibrosis and the aberrant signaling pathways that promote it. In particular, we note significant progress in unravelling the various epigenetic mechanisms that modulate signaling and regulate fibrogenic gene networks in these cells. As outlined in the above sections, several lines of evidence in preclinical models support epigenetic approaches for the treatment of pancreatobiliary fibrosis. There are several epigenetic inhibitors currently in clinical trials for solid tumors and lymphomas, including DNMT, EZH2, and HDAC inhibitors [63]. While arguably more specific than traditional small molecule inhibitors, the major concern with epigenetic inhibitors relates to off-target effects. These enzymes have critical regulatory functions in distinct cell types and across signaling pathways. Therefore, indiscriminate inhibition may produce unacceptable side effects. Ideally, epigenetic mechanisms can be modified in a cell-type or gene-specific manner. With the development of newer technologies, such as epigenetic editing techniques, such specificity may one day be achievable [94].

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Compliance with ethical standards

Conflict of interest
Sayed Obaidullah Aseem and Robert C. Huebert declare that they have no conflict of interest.

Human and animal rights and informed consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.• Kleeff J, Whitcomb DC, Shimosegawa T, Esposito I, Lerch MM, Gress T, et al. Chronic pancreatitis. Nat Rev Dis Primers. 2017;3:17060
This article provides a comprehensive review of chronic pancreatitis, its epidemiology, pathophysiology, diagnosis, and management.
2. Cote GA, Yadav D, Slivka A, Hawes RH, Anderson MA, Burton FR, et al. Alcohol and smoking as risk factors in an epidemiology study of patients with chronic pancreatitis. Clin Gastroenterol Hepatol. 2011;9(3):266–73 quiz e27.
3. Conwell DL, Banks PA, Sandhu BS, Sherman S, Al-Kaade S, Gardner TB, et al. Validation of demographics, etiology, and risk factors for chronic pancreatitis in the USA: a report of the North American Pancreas Study (NAPS) Group. Dig Dis Sci. 2017;62(8):2133–40.
4. Lee E, Ryu GR, Ko SH, Ahn YB, Song KH. A role of pancreatic stellate cells in islet fibrosis and beta-cell dysfunction in type 2 diabetes mellitus. Biochem Biophys Res Commun. 2017;485(2):328–34.
5. Whatcott CJ, Diep CH, Jiang P, Watanabe A, LoBello J, Sima C, et al. Desmoplasia in primary tumors and metastatic lesions of pancreatic cancer. Clin Cancer Res. 2015;21(15):3561–8.
6.• Neesse A, Michl P, Frese KK, Feig C, Cook N, Jacobetz MA, et al. Stromal biology and therapy in pancreatic cancer. Gut. 2011;60(6):861–8
This review provides a thorough discussion of the role of dysmoplasia, stroma microenvironment, and contribution of PSCs to these processes in pancreatic cancer.
7. Kanat O, Ertas H. Shattering the castle walls: anti-stromal therapy for pancreatic cancer. World J Gastrointest Oncol. 2018;10(8):202–10.
8. Kota J, Hancock J, Kwon J, Korc M. Pancreatic cancer: stroma and its current and emerging targeted therapies. Cancer Lett. 2017;391:38–49.
9. Ikenaga N, Ohuchida K, Mizumoto K, Cui L, Kayashima T, Morimatsu K, et al. CD10+ pancreatic stellate cells enhance the progression of pancreatic cancer. Gastroenterology. 2010;139(3):1041–51, 51 e1–8.
10. Birtolo C, Pham H, Morvaridi S, Chheda C, Go VL, Ptasznik A, et al. Cadherin-11 is a cell surface marker up-regulated in activated pancreatic stellate cells and is involved in pancreatic cancer cell migration. Am J Pathol. 2017;187(1):146–55.
11. Apte MV, Haber PS, Applegate TL, Norton ID, McCaughan GW, Korsten MA, et al. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. Gut. 1998;43(1):128–33.
12. Bachem MG, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, et al. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. Gastroenterology. 1998;115(2):421–32.
13. Buchholz M, Kestler HA, Holzmann K, Ellenrieder V, Schneiderhan W, Siech M, et al. Transcriptome analysis

of human hepatic and pancreatic stellate cells: organ-specific variations of a common transcriptional phenotype. J Mol Med (Berl). 2005;83(10):795–805.
14. Allam A, Thomsen AR, Gothwal M, Saha D, Maurer J, Brunner TB. Pancreatic stellate cells in pancreatic cancer: in focus. Pancreatology. 2017;17(4):514–22.
15. Apte MV, Pirola RC, Wilson JS. Pancreatic stellate cells: a starring role in normal and diseased pancreas. Front Physiol. 2012;3:344.
16. Apte M, Pirola RC, Wilson JS. Pancreatic stellate cell: physiologic role, role in fibrosis and cancer. Curr Opin Gastroenterol. 2015;31(5):416–23.
17. Vonlaufen A, Xu Z, Daniel B, Kumar RK, Pirola R, Wilson J, et al. Bacterial endotoxin: a trigger factor for alcoholic pancreatitis? Evidence from a novel, physiologically relevant animal model. Gastroenterology. 2007;133(4):1293–303.
18. Masamune A, Kikuta K, Watanabe T, Satoh K, Satoh A, Shimosegawa T. Pancreatic stellate cells express Toll-like receptors. J Gastroenterol. 2008;43(5):352–62.
19. Schneider E, Schmid-Kotsas A, Zhao J, Weidenbach H, Schmid RM, Menke A, et al. Identification of mediators stimulating proliferation and matrix synthesis of rat pancreatic stellate cells. Am J Physiol Cell Physiol. 2001;281(2):C532–43.
20. Mews P, Phillips P, Fahmy R, Korsten M, Pirola R, Wilson J, et al. Pancreatic stellate cells respond to inflammatory cytokines: potential role in chronic pancreatitis. Gut. 2002;50(4):535–41.
21. Apte MV, Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, et al. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. Gut. 1999;44(4):534–41.
22. Ohnishi H, Miyata T, Yasuda H, Satoh Y, Hanatsuka K, Kita H, et al. Distinct roles of Smad2-, Smad3-, and ERK-dependent pathways in transforming growth factor-beta1 regulation of pancreatic stellate cellular functions. J Biol Chem. 2004;279(10):8873–8.
23. Haber PS, Keogh GW, Apte MV, Moran CS, Stewart NL, Crawford DH, et al. Activation of pancreatic stellate cells in human and experimental pancreatic fibrosis. Am J Pathol. 1999;155(4):1087–95.
24. Pang TCY, Wilson JS, Apte MV. Pancreatic stellate cells: what's new? Curr Opin Gastroenterol. 2017;33(5):366–73.
25. Xiao W, Jiang W, Shen J, Yin G, Fan Y, Wu D, et al. Retinoic acid ameliorates pancreatic fibrosis and inhibits the activation of pancreatic stellate cells in mice with experimental chronic pancreatitis via suppressing the Wnt/beta-catenin signaling pathway. PLoS One. 2015;10(11):e0141462.
26. Xu M, Cai J, Wei H, Zhou M, Xu P, Huang H, et al. Scoparone protects against pancreatic fibrosis via TGF-beta/Smad signaling in rats. Cell Physiol Biochem. 2016;40(1–2):277–86.
27. Jaster R, Lichte P, Fitzner B, Brock P, Glass A, Karopka T, et al. Peroxisome proliferator-activated receptor gamma overexpression inhibits pro-fibrogenic activities of immortalised rat pancreatic stellate cells. J Cell Mol Med. 2005;9(3):670–82.
28. Hisada S, Shimizu K, Shiratori K, Kobayashi M. Peroxisome proliferator-activated receptor gamma ligand prevents the development of chronic pancreatitis through modulating NF-kappaB-dependent proinflammatory cytokine production and pancreatic stellate cell activation. Rocz Akad Med Bialymst. 2005;50:142–7.
29. Phillips PA, McCarroll JA, Park S, Wu MJ, Pirola R, Korsten M, et al. Rat pancreatic stellate cells secrete matrix metalloproteinases: implications for extracellular matrix turnover. Gut. 2003;52(2):275–82.
30.• Chronopoulos A, Robinson B, Sarper M, Cortes E, Auernheimer V, Lachowski D, et al. ATRA mechanically reprograms pancreatic stellate cells to suppress matrix remodelling and inhibit cancer cell invasion. Nat Commun. 2016;7:12630
Provides mechanistic understanding of the role of PSCs in pancreatic cancer metastasis.
31. Lazaridis KN, Strazzabosco M, Larusso NF. The cholangiopathies: disorders of biliary epithelia. Gastroenterology. 2004 Nov;127(5):1565–77.
32.•• Banales JM, Huebert RC, Karlsen T, Strazzabosco M, LaRusso NF, Gores GJ. Cholangiocyte pathobiology. Nat Rev Gastroenterol Hepatol. 2019 May;16(5):269–281
This review provides a comprehensive overview of cholangiocyte biology in health and disease. Mechanisms and signaling pathways involved in biliary disease are discussed.
33. Santos-Laso A, Munoz-Garrido P, Felipe-Agirre M, Bujanda L, Banales JM, Perugorria MJ. New advances in the molecular mechanisms driving biliary fibrosis and emerging molecular targets. Curr Drug Targets. 2017;18(8):908–20.
34. Pinto C, Giordano DM, Maroni L, Marzioni M. Role of inflammation and proinflammatory cytokines in cholangiocyte pathophysiology. Biochim Biophys Acta Mol Basis Dis. 2018;1864(4 Pt B):1270–8.
35.• Iwaisako K, Jiang C, Zhang M, Cong M, Moore-Morris TJ, Park TJ, et al. Origin of myofibroblasts in the fibrotic liver in mice. Proc Natl Acad Sci U S A. 2014;111(32):E3297–305
This study shows the contribution of portal fibroblasts and HSCs to the myofibroblast pool and consequently hepatobiliary fibrosis.
36. Lemoinne S, Cadoret A, El Mourabit H, Thabut D, Housset C. Origins and functions of liver myofibroblasts. Biochim Biophys Acta. 2013 Jul;1832(7):948–54.
37. Dranoff JA, Wells RG. Portal fibroblasts: underappreciated mediators of biliary fibrosis. Hepatology. 2010 Apr;51(4):1438–44.
38. Omenetti A, Porrello A, Jung Y, Yang L, Popov Y, Choi SS, et al. Hedgehog signaling regulates epithelial-mesenchymal transition during biliary fibrosis in rodents and humans. J Clin Invest. 2008;118(10):3331–42.
39. Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, et al. Fate tracing reveals hepatic stellate

cells as dominant contributors to liver fibrosis independent of its aetiology. Nat Commun. 2013;4:2823.
40. Wake K. "Sternzellen" in the liver: perisinusoidal cells with special reference to storage of vitamin A. Am J Anat. 1971;132(4):429–62.
41.• Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol. 2017;14(7):397–411
A comprehensive overview of the mechanisms of HSC activation.
42. Kocabayoglu P, Lade A, Lee YA, Dragomir AC, Sun X, Fiel MI, et al. Beta-PDGF receptor expressed by hepatic stellate cells regulates fibrosis in murine liver injury, but not carcinogenesis. J Hepatol. 2015;63(1):141–7.
43. Henderson NC, Arnold TD, Katamura Y, Giacomini MM, Rodriguez JD, McCarty JH, et al. Targeting of alphav integrin identifies a core molecular pathway that regulates fibrosis in several organs. Nat Med. 2013;19(12):1617–24.
44. Michelotti GA, Xie G, Swiderska M, Choi SS, Karaca G, Kruger L, et al. Smoothened is a master regulator of adult liver repair. J Clin Invest. 2013;123(6):2380–94.
45. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-beta: the master regulator of fibrosis. Nat Rev Nephrol. 2016;12(6):325–38.
46. Zhou Q, Xia S, Guo F, Hu F, Wang Z, Ni Y, et al. Transforming growth factor-beta in pancreatic diseases: mechanisms and therapeutic potential. Pharmacol Res. 2019;142:58–69.
47. Zhang YE. Non-Smad pathways in TGF-beta signaling. Cell Res. 2009;19(1):128–39.
48. Pujadas E, Feinberg AP. Regulated noise in the epigenetic landscape of development and disease. Cell. 2012;148(6):1123–31.
49. Lin JC, Jeong S, Liang G, Takai D, Fatemi M, Tsai YC, et al. Role of nucleosomal occupancy in the epigenetic silencing of the MLH1 CpG island. Cancer Cell. 2007;12(5):432–44.
50. Holmgren C, Kanduri C, Dell G, Ward A, Mukhopadhya R, Kanduri M, et al. CpG methylation regulates the Igf2/H19 insulator. Curr Biol. 2001;11(14):1128–30.
51. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science. 2011;333(6047):1300–3.
52. Koch A, Joosten SC, Feng Z, de Ruijter TC, Draht MX, Melotte V, et al. Analysis of DNA methylation in cancer: location revisited. Nat Rev Clin Oncol. 2018;15(7):459–66.
53. Lomberk GA, Iovanna J, Urrutia R. The promise of epigenomic therapeutics in pancreatic cancer. Epigenomics. 2016;8(6):831–42.
54. Mishra NK, Guda C. Genome-wide DNA methylation analysis reveals molecular subtypes of pancreatic cancer. Oncotarget. 2017;8(17):28990–9012.
55. McCleary-Wheeler AL, Lomberk GA, Weiss FU, Schneider G, Fabbri M, Poshusta TL, et al. Insights into the epigenetic mechanisms controlling pancreatic carcinogenesis. Cancer Lett. 2013;328(2):212–21.
56. Dutruel C, Bergmann F, Rooman I, Zucknick M, Weichenhan D, Geiselhart L, et al. Early epigenetic downregulation of WNK2 kinase during pancreatic ductal adenocarcinoma development. Oncogene. 2014;33(26):3401–10.
57. El Taghdouini A, Sorensen AL, Reiner AH, Coll M, Verhulst S, Mannaerts I, et al. Genome-wide analysis of DNA methylation and gene expression patterns in purified, uncultured human liver cells and activated hepatic stellate cells. Oncotarget. 2015;6(29):26729–45.
58. Mann J, Chu DC, Maxwell A, Oakley F, Zhu NL, Tsukamoto H, et al. MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. Gastroenterology. 2010;138(2):705–14, 14 e1–4.
59. Perugorria MJ, Wilson CL, Zeybel M, Walsh M, Amin S, Robinson S, et al. Histone methyltransferase ASH1 orchestrates fibrogenic gene transcription during myofibroblast transdifferentiation. Hepatology. 2012;56(3):1129–39.
60. Page A, Paoli P, Moran Salvador E, White S, French J, Mann J. Hepatic stellate cell transdifferentiation involves genome-wide remodeling of the DNA methylation landscape. J Hepatol. 2016;64(3):661–73.
61. Patil VS, Zhou R, Rana TM. Gene regulation by noncoding RNAs. Crit Rev Biochem Mol Biol. 2014;49(1):16–32.
62. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–97.
63. Massey V, Cabezas J, Bataller R. Epigenetics in liver fibrosis. Semin Liver Dis. 2017;37(3):219–30.
64. El Taghdouini A, van Grunsven LA. Epigenetic regulation of hepatic stellate cell activation and liver fibrosis. Expert Rev Gastroenterol Hepatol. 2016;10(12):1397–408.
65. Lin YC, Wang FS, Yang YL, Chuang YT, Huang YH. MicroRNA-29a mitigation of toll-like receptor 2 and 4 signaling and alleviation of obstructive jaundice-induced fibrosis in mice. Biochem Biophys Res Commun. 2018;496(3):880–6.
66. Zhao R, Dong R, Yang Y, Wang Y, Ma J, Wang J, et al. MicroRNA-155 modulates bile duct inflammation by targeting the suppressor of cytokine signaling 1 in biliary atresia. Pediatr Res. 2017;82(6):1007–16.
67. Yu P, Liu K, Gao X, Karmouty-Quintana H, Bailey JM, Cao Y, et al. Transforming growth factor-beta and bone morphogenetic protein 2 regulation of microRNA-200 family in chronic pancreatitis. Pancreas. 2018;47(2):252–6.
68. Xu M, Wang G, Zhou H, Cai J, Li P, Zhou M, et al. TGF-beta1-miR-200a-PTEN induces epithelial-mesenchymal transition and fibrosis of pancreatic stellate cells. Mol Cell Biochem. 2017;431(1–2):161–8.
69. Masamune A, Nakano E, Hamada S, Takikawa T, Yoshida N, Shimosegawa T. Alteration of the microRNA expression profile during the activation of

pancreatic stellate cells. Scand J Gastroenterol. 2014;49(3):323–31.
70. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell. 2011;43(6):904–14.
71. Liu H, Yu K, Ma P, Xiong L, Wang M, Wang W. Long noncoding RNA myocardial infarction-associated transcript regulated the pancreatic stellate cell activation to promote the fibrosis process of chronic pancreatitis. J Cell Biochem. 2019 Jun;120(6):9547–9555.
72. Wang H, Jiang Y, Lu M, Sun B, Qiao X, Xue D, et al. STX12 lncRNA/miR-148a/SMAD5 participate in the regulation of pancreatic stellate cell activation through a mechanism involving competing endogenous RNA. Pancreatology. 2017;17(2):237–46.
73. Thankam FG, Boosani CS, Dilisio MF, Agrawal DK. Epigenetic mechanisms and implications in tendon inflammation (review). Int J Mol Med. 2019;43(1):3–14.
74. Feinberg AP. The key role of epigenetics in human disease prevention and mitigation. N Engl J Med. 2018;378(14):1323–34.
75. Zhang G, Pradhan S. Mammalian epigenetic mechanisms. IUBMB Life. 2014;66(4):240–56.
76. Taniguchi Y. The Bromodomain and extra-terminal domain (BET) family: functional anatomy of BET paralogous proteins. Int J Mol Sci. 2016;7:17(11).
77.• Bombardo M, Chen R, Malagola E, Saponara E, Hills AP, Graf R, et al. Inhibition of class I histone deacetylases abrogates tumor growth factor beta expression and development of fibrosis during chronic pancreatitis. Mol Pharmacol. 2018;94(2):793–801
This study describes an epigenetic mechanism for fibrosis in chronic pancreatitis.
78. Pan MR, Hsu MC, Luo CW, Chen LT, Shan YS, Hung WC. The histone methyltransferase G9a as a therapeutic target to override gemcitabine resistance in pancreatic cancer. Oncotarget. 2016;7(38):61136–51.
79. Kim JS, Shukla SD. Histone h3 modifications in rat hepatic stellate cells by ethanol. Alcohol Alcohol. 2005;40(5):367–72.
80. Mannaerts I, Nuytten NR, Rogiers V, Vanderkerken K, van Grunsven LA, Geerts A. Chronic administration of valproic acid inhibits activation of mouse hepatic stellate cells in vitro and in vivo. Hepatology. 2010;51(2):603–14.
81. Moran-Salvador E, Mann J. Epigenetics and liver fibrosis. Cell Mol Gastroenterol Hepatol. 2017;4(1):125–34.
82. Martin-Mateos R, De Assuncao TM, Arab JP, Jalan-Sakrikar N, Yaqoob U, Greuter T, et al. Enhancer of zeste homologue 2 inhibition attenuates TGF-beta dependent hepatic stellate cell activation and liver fibrosis. Cell Mol Gastroenterol Hepatol. 2019;7(1):197–209.
83. Page A, Paoli PP, Hill SJ, Howarth R, Wu R, Kweon SM, et al. Alcohol directly stimulates epigenetic modifications in hepatic stellate cells. J Hepatol. 2015 Feb;62(2):388–97.
84. Dou C, Liu Z, Tu K, Zhang H, Chen C, Yaqoob U, et al. P300 acetyltransferase mediates stiffness-induced activation of hepatic stellate cells into tumor-promoting myofibroblasts. Gastroenterology. 2018;154(8):2209–21 e14.
85. Ding N, Hah N, Yu RT, Sherman MH, Benner C, Leblanc M, et al. BRD4 is a novel therapeutic target for liver fibrosis. Proc Natl Acad Sci U S A. 2015;112(51):15713–8.
86.• Jalan-Sakrikar N, De Assuncao TM, Shi G, Aseem SO, Chi C, Shah VH, Huebert RC. Proteasomal Degradation of Enhancer of Zeste Homologue 2 in Cholangiocytes Promotes Biliary Fibrosis. Hepatology. 2019 May 9. https://doi.org/10.1002/hep.30706
Describes a role for H3K27me3 and the methyltransferase EZH2 in biliary fibrosis.
87. Harr JC, Luperchio TR, Wong X, Cohen E, Wheelan SJ, Reddy KL. Directed targeting of chromatin to the nuclear lamina is mediated by chromatin state and A-type lamins. J Cell Biol. 2015;208(1):33–52.
88. Lupianez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, et al. Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. Cell. 2015;161(5):1012–25.
89. Hamdan FH, Johnsen SA. Super enhancers - new analyses and perspectives on the low hanging fruit. Transcription. 2018;9(2):123–30.
90. Hamdan FH, Johnsen SA. DeltaNp63-dependent super enhancers define molecular identity in pancreatic cancer by an interconnected transcription factor network. Proc Natl Acad Sci U S A. 2018;115(52):E12343–E52.
91. Struhl K, Segal E. Determinants of nucleosome positioning. Nat Struct Mol Biol. 2013;20(3):267–73.
92. Ribeiro-Silva C, Vermeulen W, Lans H. SWI/SNF: complex complexes in genome stability and cancer. DNA Repair (Amst). 2019;77:87–95.
93. Li H, Lan J, Han C, Guo K, Wang G, Hu J, et al. Brg1 promotes liver fibrosis via activation of hepatic stellate cells. Exp Cell Res. 2018;364(2):191–7.
94. Esvelt KM, Wang HH. Genome-scale engineering for systems and synthetic biology. Mol Syst Biol. 2013;9:641.

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