Epigenomics

The promise of epigenomic therapeutics in pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is often viewed to arise primarily by genetic alterations. However, today we know that many aspects of the cancer phenotype require a crosstalk among these genetic alterations with epigenetic changes. Indeed, aberrant gene expression patterns, driven by epigenetics are fixed by altered signaling from mutated oncogenes and tumor suppressors to define the PDAC phenotype. This conceptual framework may have significant mechanistic value and could offer novel possibilities for treating patients affected with PDAC. In fact, extensive investigations are leading to the development of small molecule drugs that reversibly modify the epigenome. These new 'epigenetic therapeutics' discussed herein are promising to fuel a new era of studies, by providing the medical community with new tools to treat this dismal disease.

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In mammals, nucleated somatic cells share identical genomes but different epigenomes, resulting in an incredible variation in morphology and functional plasticity. This diversity is defined initially by cell-specific patterns of gene expression, which are controlled by transcription factors binding to regulatory sites in the genome. Access to these sites is orchestrated via the remodeling of chromatin, which comprises the complex of DNA, RNA and proteins that constitutes the functional platform of the genome. In contrast to the DNA sequence, chromatin is highly dynamic, particularly brought about through modifications of both, the histones within nucleosomes and DNA cytosines, which together define the epigenome [1]. Although little remains known about how epigenetic features vary between different cell types in healthy and disease states, next-generation sequencing (NGS) tools have allowed the simultaneous profiling of the epigenome at high resolution. We now know that genes encoding components or regulators of the epigenetic machinery are frequently mutated in cancers and that these mutations, through their capacity to influence expression of many hundreds of genes, likely lead to heritable reconfigurations of gene expression (epimutations) many times priming healthy cells toward malignancy. Recent publications in pancreatic cancer demonstrate that many of these tumors have one or more mutations or copy number aberrations in genes that regulate such chromatin marks [2]. In this review, we focus on the possible epigenetic-based therapeutic strategies to treat patients with a pancreatic adenocarcinoma (PDAC).

A rationale for targeting the epigenome & its regulators for the treatment of PDAC

An emerging set of critical studies indicates that epigenetic mechanisms, which can both

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silence or activate genes in a heritable manner independently of the coding capacity of DNA, could play an important role in PDAC [3]. The discovery of epigenetic events that determine the outcome of this disease is important since, contrary to genetic changes, such molecular mechanisms are easily reversible using small molecule drugs and thus represent promising new therapeutic targets in the treatment of PDAC. Moreover, epigenetic markers that are informative for the diagnosis and prognosis of many malignancies continue to be discovered and will likely soon become applicable to the management of patients with PDAC. The concept that the cell obtains and carries out functional instructions, either through environmental signs or cell autonomous processes, by synthesizing regulatory noncoding RNAs, as well as reversibly marking DNA and proteins with distinct post-translational modifications is at the mechanistic core of epigenetics [4]. These modifications, referred to as 'marks', are deposited by 'writers', hydrolyzed or degraded by 'erasers' and recognized and bound by 'readers'. Remarkably, the combination of these histone marks was found to instruct the cell as a 'code' to establish and inherit distinct and stable patterns of gene expression in order to define a particular phenotype, either normal or diseased. These conditions, in which gene expression patterns correlated with their resulting phenotypic characteristics, became known as 'epigenetic landscapes' [5,6]. One prediction based on this concept is therefore that efficient manipulation of chromatin regulators may induce cells to transverse epigenetic landscapes. Aimed at achieving this goal, extraordinarily rapid discoveries of new concepts, methodologies and drugs have recently characterized a new and innovative avenue in biomedical research.

DNA methylation as promising therapeutic target

DNA methylation, which usually occurs on cytosines that precede guanines, called dinucleotide CpGs, was the first documented and studied epigenetic change [7]. The genome contains stretches of sequence enriched in CpG islands that are contained within the promoter regions of 76% of all mammalian genes [8]. Patterns of DNA methylation are established and maintained by various DNA methyltransferases (DNMTs), which work as writers of this mark under different circumstances. For example, to preserve patterns of DNA methylation, the ubiquitin-like protein UHRF1 recognizes hemi-methylated DNA and directs DNMT1 to methylate the correct cytosine in the newly synthesized DNA strand [9], whereas during embryogenesis, *de novo* DNA methylation is mediated by DNMT3A and DNMT3B (Figure 1) [10].

Noteworthy, DNA methylation usually has significant physiological impact, such as genomic imprinting to guarantee monoallelic expression and hypermethylation of repetitive genomic sequences to prevent chromosomal instability, translocations, and gene disruption through the reactivation of transposable DNA sequences. However, during tumorigenesis, abnormal DNA methylation can assist the development of the cancer phenotype. In PDAC, DNA methylation has long been recognized as a mechanism through which tumor suppressor genes such as *p16* are inactivated [11]. Recent methodological developments in gene methylation analysis have allowed our view to expand from the single gene level, which remains a valid specific candidate gene approach, to the genome-wide scale, which possesses power in its unbiased approach. Several techniques utilized for methylation analysis include methylation-specific PCR, array methodology and NGS after bisulfite treatment [12]. Although individual genes have been discovered as being methylated in advanced PDAC, as shown in the examples below, current evidence supports the notion that aberrant methylation takes place very early during the histopathological development of this neoplasia. Using a specific candidate gene approach, Rosty and colleagues reported a loss of *p16* expression in PanIN lesions of patients with chronic pancreatitis [13], suggesting that this modification may contribute to the predisposition of patients affected by this disease who transition to develop PDAC. In their study, involving large-scale methylation analysis with subsequent confirmation via methylation-specific PCR, Sato and colleagues examined DNA samples from 65 PanIN lesions for methylation status of eight genes previously recognized by a larger scale microarray study as being abnormally hypermethylated in invasive PDAC [14]. Strikingly, of the PanIN lesions inspected in this study, methylation at any of these genes was identified in 68%. The most remarkable finding from both of these studies was that aberrant CpG island hypermethylation begins at early stages of PanINs and its incidence progressively increases during neoplastic development. Congruent with the concept that aberrant methylation occurs early during pancreatic carcinogenesis, Gazin *et al.* pioneered the notion that epigenetic changes are necessary for the transformation of NIH3T3 cells by mutant *KRAS*, including the regulation of DNMT1 expression in a 5-aza-dC-sensitive manner [15]. Since then, other investigators have reported that such epigenetic changes are not restricted to Kras-induced neoplastic transformation, and have shown that DNA methylation is also required by other oncogenes to achieve their function (reviewed in [4,16,17]. Last, due to the fact that methylation occurs earlier at the preneoplastic stage,
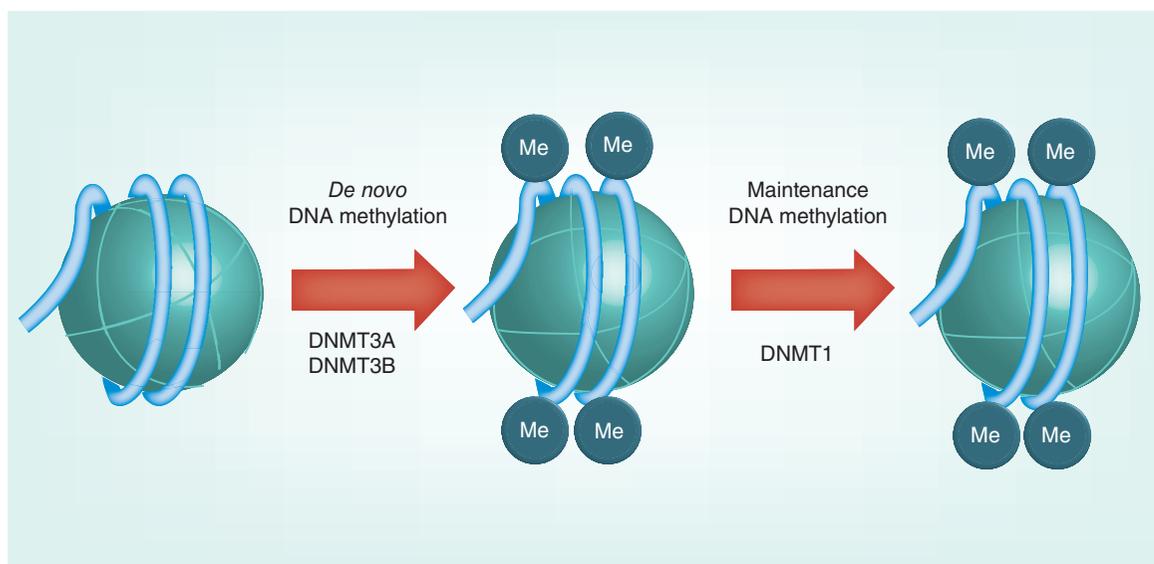
Figure 1. DNA methyltransferases function to preserve patterns of DNA methylation. DNMT1 methylates the correct cytosine in the newly synthesized DNA strand, whereas during embryogenesis, *de novo* DNA methylation is mediated by DNMT3A and DNMT3B.
Me: Methylation.

pharmacological agents that modulate this epigenetic process, as discussed below, may be useful not only in a treatment setting but perhaps also in chemoprevention.

Two types of DNMT inhibitors currently exist to modulate the function of these pathways, namely, nucleoside and non-nucleoside (small molecule drugs) [18]. Today, nucleoside analogs based on the first synthesized epigenetic inhibitors 5-azacytidine and 5-aza-2′-dC are being tested in Phase I–III clinical trials for many diseases [19]. More importantly, the US FDA have accepted the prototypical DNMT inhibitor, 5-azacytidine (i.e., Vidaza) for the treatment of myelodysplastic syndrome [20]. Thus, taking into consideration the importance of aberrant DNA methylation in PDAC, current or future members of this family of drugs are likely to find a place in the therapeutic arsenal against this disease.

The therapeutic value of inhibiting the acetylation & deacetylation of histones

Among the key epigenetic signals that regulate gene expression are the acetylation and deacetylation of lysine residues within histone tails and other nonhistone proteins [4]. Generally, acetylation functions to activate gene expression, whereas deacetylation occurs for gene silencing. The enzymes responsible for these reactions comprise HATs, which transfer an acetyl group from acetyl-CoA (donor) to the lysine residue (Box 1), and HDACs, serving to reverse this reaction [21]. A conserved central fold characterizes the core domains of all HATs, which contains the acetyl-CoA cofactor binding site and the catalysis pocket [22]. Struc-

tural differences among the HAT families are found N- and C-terminal to these core domains, which likely contribute to substrate specificity. Much effort has been devoted to targeting these enzymes, resulting in the identification of useful HAT inhibitors, from some less specific natural substances to covalently modifying isothiazolones [23]. To date, most of the possible therapies based on HAT inhibition focus on targeting CBP/p300, and currently all have remained in a preclinical phase, with the exception of curcumin, which has advanced into clinical trials as a potential anticancer therapy [24]. Thus, HAT inhibitors, though at their nascent state of testing, may also be a promising strategy for either chemoprevention or new combinatorial therapies for PDAC.

HDACs, which counteract the action of the HATs, are classified into four classes based on homology to yeast HDACs (Box 2). Interestingly, HDACs are present in various multiprotein complexes, which frequently include other HDAC family members, therefore numerous distinct complexes may exist at any given time, suggesting that the regulation of these proteins, by cell signaling events or pharmacological manipulation, is multifaceted. For example, the highly similar HDACs, HDAC1 and HDAC2 are present in complexes that can target either oncogenes or tumor suppressors [25]. Unfortunately, the currently available drugs inhibit their enzymatic activity independently of the complex, indicating that the effects of these drugs are somewhat unpredictable and highlighting the need to perform careful and extensive empirical trials. Nevertheless, HDAC inhibitors (HDACIs) are among the

Box 1. Histone acetyltransferases.

GNAT family
- Gcn5
- PCAF
- Hat1
- Elp3
- Hpa2
- Hpa3
- ATF-2
- Nut1

MYST family
- MOZ
- Ybf2 (Sas3)
- Sas2
- Tip60
- Esa1
- MOF
- MORF
- HBO1

p300/CBP
- p300
- CBP

HAT proteins are grouped into three main classes. The most important families among the nuclear HATs, are the GNAT family, which includes GCN5 and human PCAF, the MYST family, which includes Tat interacting protein Tip60 and monocytic leukemia zinc finger protein/MOZ-related factor protein MOZ/MORF and the p300/CBP family.
GNAT: Gcn5-related N-acetyltransferase.

best-characterized epigenetic drugs evaluated in cancers. HDACIs have been shown to induce hyperacetylation of histones and thus, reactivate tumor suppressor gene expression, leading to inhibition of cell proliferation, cell differentiation and apoptosis [26]. Furthermore, many proteins with different biological roles, in addition to histones, are HDAC substrates, which include p53, c-Myc, NF-κB and E2F, signaling mediators such as Stat3 and Smad7, HIF-1α, estrogen receptor α, androgen receptor, MyoD, HSP90, α-tubulin, β-catenin and Rb protein [25]. An increase in HDAC activity has been shown in several tumors compared with normal tissue. Blasco and colleagues examined the differential expression of genes in a PDAC-derived cell line upon induction of apoptosis, one of which they found to be HDAC1 [27]. By inhibiting HDAC activity, the authors demonstrated an increase in the level of apoptosis and thus proposed that HDAC1 could be a possible target to develop modulators in cancer chemotherapy that would increase or restore apoptosis. In another study, Ouaissi *et al.* reported a significant increase of HDAC7 mRNA and protein levels in approximately 80% of PDAC samples analyzed [28]. In addition, HDAC1 has been shown to mediate transcriptional repression of the *TGFβRII* promoter in PDAC cells via KLF14, one of the most important metabolic regulator proteins discovered to

date [29]. Altogether, these data demonstrate the important role played by HDACs in maintaining the proper balance of chromatin marks on a given promoter, and are an indicator of the extent to which a change in this balance, through altered HDAC expression in PDAC for instance, would be expected to affect promoters.

HDAC inhibitors are well tolerated, and several among the tested natural or synthetic agents have shown promising antitumor action. Some of these, recently entered into preclinical or clinical trials, are giving encouraging results as anticancer drugs, including abexinostat, pracinostat, resminostat, givinostat, panobinostat and CUDC-101 [25]. Whether isotype- and class-specific HDAC inhibitors would be more useful than, or preferred over, broad-spectrum HDAC inhibitors remains an important consideration.

Inhibiting the methylation & demethylation of histones to treat cancers

The methyl marks on histones have the potential to instigate long-term effects on cells through their strong signals for inheriting certain gene expression patterns. The function of some histone methylation pathways in PDAC has been recently described by our group [30,31]. Methylation occurs in several distinct histones at different residues in the context of not only gene promoters, but enhancers, silencers or gene bodies, and thus, the functional diversity provided by histone methylation is vast. Methylation in histones can occur on lysines, as mono-, di-, or tri-methylation or arginine residues, with up to two methyl groups in a symmetric or asymmetric position. As a general rule, transcriptional repression is associated with methylation marks at H3-K9, H3-K27 and H4-K20, while active gene transcription is linked to methylation of H3-K4, H3-K36 and H3-K79 [32]. Remarkably, as with lysine methylation, arginine methylation has different consequences depending on the residue modified. Both arginine and lysine methyl marks are written by S-adenosylmethionine (SAM)-dependent methyltransferases. Protein arginine methyltransferases (PRMTs) are classified as type I, II and III PRMTs (Box 3) [33]. Although potent PRMT1 and PRMT4 inhibitors have been documented [23], drug discovery efforts for PRMT inhibitors are still in their early stages.

Over 50 lysine methyltransferases (KMTs) have been reported [34], with all of them but one (Dot1), belonging to the SET domain-containing protein group. The Set domain is an approximately 130 amino acid evolutionarily preserved protein module (Box 4). Small sequence variances in its primary sequence and in other accompanying motifs outside of this domain convey specificity to particular KMTs [35]. One of the best-known examples of a SET domain-containing

KMT is the EZH2 protein, a writer enzyme within the Polycomb PRC2 complex, which is responsible for the deposition of the trimethyl K27 mark [36]. So far, human EZH2 proteins and their related homolog, EZH1, are the only two types of enzymes found to catalyze H3-K27 methylation, resulting in the formation of heterochromatin for gene silencing. Remarkably, the *EZH2* gene is mutated and deregulated in a large range of cancers [34] where it has been found to regulate stem cell biology, tumor cell growth and invasion making it a promising drug target.

The function of Polycomb proteins, of which many new ones have been revealed in PDAC cells, is an incipient area of investigation, including as a potential therapeutic target for the treatment of PDAC. Loss of the mark deposited by EZH2 has been shown to predict poor prognosis in PDAC [37], and the level of trimethyl-K27-H3 is a robust and independent predictor of this outcome. Poorly differentiated PDAC frequently displays nuclear accumulation of EZH2, which contributes toward PDAC cell growth [38]. These studies, in addition to the knowledge that distinct EZH2 isoforms exist and are functional [30], underscore the need to increase our understanding on the conformation and function of Polycomb complexes in PDAC. Mechanistically, one of the consequences of abnormal Polycomb regulation is the silencing of the *p16* gene, which could take place prior to DNA methylation [39]. The presence of Polycomb proteins on the *p16* promoter may result in the recruitment of DNA methylases, which would then further inactivate the expression of *p16* via DNA methylation. However, since EZH2 regulates entire gene networks, the silencing of a single tumor suppressor gene is only one of the phenomena that contributes to the development and progression of the cancer phenotype.

Early work showed that the global methyltransferase inhibitors adenosine dialdehyde (AdOx) and 3-deazaneplanocin (DZNep) inhibit EZH2 [40]. Both induce cell death upon decreasing the deposition of the trimethyl H3-K27 mark, which promotes the derepression of Polycomb-regulated genes. In fact, DZNep has been shown to synergistically enhance the antiproliferative effect of gemcitabine in primary cultures of cells derived from human PDAC tumors and PDAC cell lines [41]. However, EZH2 activity is also inhibited as a result of downregulation of several proteins from the PRC2 complex after DZNep treatment [36]. Studies on these inhibitors therefore offer limited information about the direct role of EZH2 activity in the general drug effects. Thus, more potent and selective S-adenosyl-methionine-competitive compound inhibitors of EZH2, such as GSK126, EPZ-6438, UNC-1999 and CPI-169, continue to be developed [36]. These inhibi-

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Box 2. Histone deacetylases.

Class I
- HDAC1
- HDAC2
- HDAC3
- HDAC8

Class II
- HDAC4
- HDAC5
- HDAC6
- HDAC7
- HDAC9
- HDAC10

Class III
- SIRT1
- SIRT2
- SIRT3
- SIRT4
- SIRT5
- SIRT6
- SIRT7

Class IV
- HDAC11

Class I members, comprising HDAC1, -2, -3 and -8, are mainly found in the nucleus and mediate gene repression as the catalytic component of multiprotein complexes, which include Sin3, Mi-2/NurD, N-CoR/SMRT and Co-REST among others. Class II HDACs are principally cytoplasmic proteins, with some cytoplasm to nucleus shuttling in response to their phosphorylation state. Class IV only comprises nuclear-based HDAC11, which shares some sequence homology with class I and II enzymes, but has specific structural distinctions. Class III, which differs from Classes I, II and IV since their enzymatic activity requires NAD+ as a cofactor, includes the Sirtuins, evolutionarily conserved and structurally related proteins to Sir2 from *Saccharomyces cerevisiae*. There are seven members of this family, SIRT1 through to SIRT7, which function in the nucleus, cytoplasm and mitochondria.

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tors have further confirmed the importance of EZH2 in tumor growth. Considering their high selectivity for EZH2 (more than 1000-fold over other methyltransferases) and effectiveness to inhibit EZH2 activity in the low nanomolar range, they represent auspicious candidate therapeutic tools for PDAC treatment.

Lysine demethylases (KDMs) control chromatin dynamics, as well as epigenetic and gene expression patterns by opposing the KMTs through removal of the methyl marks from histone lysine residues. Generally, the more than 20 known KDMs are classified based on sequence homology and catalytic mechanism into two functional groups (Box 5) [42]. KDM1A/LSD1 and KDM1B/LSD2, which are members of the amine oxidase KDM1 subgroup and related to the well-characterized monoamine oxidases (MAOs), erase mono- and dimethyl lysine marks, but not the trimethyl lysine mark [36]. Often the KDMs act upon several lysines, for example, KDM1A/LSD1 removes mono- and dimethylated H3-K4 and H3-K9, however, specificity is obtained by binding other factors. KDM1A/LSD1

Box 3. Protein arginine methyltransferases.

Type I
- PRMT1
- PRMT3
- PRMT4/CARM1
- PRMT6
- PRMT8

Type II
- PRMT5
- PRMT7
- PRMT9/FBXO11

Type III
- PRMT7

Protein arginine methyltransferases (PRMTs) are classified as type I and type II PRMTs, which catalyze the formation of a mono-methyl-arginine intermediate to ultimately produce a dimethylated product, as well as type III PRMTs, for which enzymatic activity is limited to monomethylation.

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has significantly higher expression in several tumor types, including PDAC, and correlates with poor prognosis [36]. The Jumonji C (JmjC) domain-containing proteins comprise the second group of KDMs, which demethylate distinct mono-, di- and tri-methyl lysine residues through an oxygenase activity required for this function [42]. Out of the 32 distinct JmjC domain-containing proteins encoded for in the human genome, 24 have documented demethylase activity to create this larger of the two KDM classes [43]. Several members of the JmjC domain-containing KDM class are implicated to have a role in carcinogenesis. KDM2B, KDM3A, the KDM4 family and KDM5B are overexpressed in cancer with some of their genes, such as *KDM4C*, being amplified in certain cancers [36]. Other members of this KDM class are frequently deleted or mutated in cancer. For example, *KDM6A* has a significant frequency of somatic mutations in multiple myeloma as well as several other cancers, and the *KDM6B* gene is regularly lost along with the often deleted *TP53* locus [36].

In regards to drug development, the KDM family is an optimal pharmacological target for two central reasons. First, since their mechanism of action involves oxidation, these enzymes have the potential to be inhibited by derivatives of existing drugs used to target other oxidases. Indeed, an early developed KDM1 inhibitor was tranylcypromine, which is an FDA-approved MAO inhibitor used to treat mood and anxiety disorders [42]. Second, these demethylases, in particular the JmjC domain-containing proteins, have multiple domains to provide various strategies for targeting, which can individually or collectively inactivate different members of the family. Structure-based computer-simulated screenings have facilitated identification of more selective KDM inhibitors, such as a collection

of novel N'-(1-phenylethylidene)-benzohydrazides that have reversible, nanomolar potency and selectivity against KDM1A compared with monoamine oxidases [42]. This screening produced one improved compounds in particular that increases dimethyl H3-K4 levels and inhibits cell proliferation of several cancer cell lines [42]. Development of drugs to inhibit KDMs continues to evolve with a particular focus on improving their selectivity, potency and pharmacokinetic properties in order to eventually obtain suitable compounds for investigations on individual KDMs.

Epigenetic regulation by noncoding RNAs

One of the most significant findings from the human genome mapping and sequencing has been the discovery that it gives rise to countless noncoding RNAs (ncRNAs), mainly miRNAs, which may regulate as much as 30% of all protein-coding genes in mammals [44,45]. In PDAC and desmoplasia, numerous miRNAs are abnormally expressed, as determined by global profiling [46]. Remarkably, some of these including miR-155, miR-21, miR-221 and miR-222 had been previously reported as being differentially expressed in other human cancers, while others such as miR-376a and miR-301 were novel. In another study, some miRNAs, including miR-205, -18a, -31, -93, -221 and -224, were revealed to be overexpressed in primary neoplastic ductal cells and PDAC-derived cell lines, and thus found to represent encouraging biomarkers for PDAC [47]. Additionally, the analysis of two among the 26 miRNAs significantly misregulated in PDAC, miR-217 and -196a, provided discrimination between normal and PDAC tissues, further supporting the possible use of miRNAs for the diagnosis of PDAC. In their global investigation comparing miRNA profiles of normal pancreas, chronic pancreatitis and PDAC, Bloomston and colleagues found that a set of 25 miRNAs was capable of differentiating PDAC from benign pancreatic tissues for 90% of the tested samples [48].

Current approaches to target small ncRNAs involve: small-molecule inhibitors, expression vectors (miRNA sponges) and antisense oligonucleotides (ASOs) [49]. Therapies exploiting miRNA sponges, which are based on the use of vectors that express mRNAs with multiple artificial miRNA-binding sequences to sequester endogenous miRNAs, have only been successful for utilization *in vitro* thus far [49]. The most promising approach and thus receiving the most attention currently, is the use of ASO technology that directly targets miRNAs to specifically inhibit their function (anti-miRs). Due to their high complementarity, anti-miRs efficiently interfere with binding of endogenous mRNA targets to the miRNA-RISC ribonucleopro-

tein silencing complex. In order to increase the stability of the anti-miRs against nucleases and improve their binding affinity for the target miRNA, chemical modification of oligonucleotides is necessary. One of these chemical modifications, locked nucleic acid (LNA), considerably increases thermal stability upon hybridization with complementary single-stranded RNA target molecules as a result of its locked ribose conformation [50]. The functional inhibition of miR-NAs has been achieved by additional oligonucleotide analogs, including 2′-O-methyl, 2′-O-methyoxyethyl and 2′-fluoro [50]. Although several challenges exist for the application of ncRNA-targeting therapies, their significant role in disease development and progression advocates the need to continue advancing this line of therapeutics.

New avenues for developing drugs targeting chromatin readers

For many years, enzymes have been the preferred target of both pharmaceutical industry and academic chemists, as they possess dynamically regulated cavities and pockets that work well as pharmacophores. As enzymes therefore, writers and erasers of the histone code are the most frequently considered druggable epigenetic targets. However, the targeting of histone mark readers remains a new field of drug discovery. The original belief that the marks themselves directly modulate the transition between transcriptionally active euchromatin and transcriptionally silent heterochromatin by altering the charge of the DNA–histone interaction surface [23] has since been replaced with experimental evidence establishing that these histone marks symbolize docking sites for other chromatin proteins. These proteins function to 'read' these post-translational alterations and thus, control the genome by coupling to specific molecular machineries to impact nuclear mechanisms such as nucleosome positioning and assembly, transcriptional initiation, elongation and splicing as well as DNA repair and replication [51]. The recognition of specific histone marks by histone mark readers is through specialized modules. Sophisticated structural studies have shown the existence of not only a huge variety of reader-binding pocket architectures, but also common principles underlying the readout of marks carrying methyl-lysine, methyl-arginine, acetyl-lysine and phospho-serine [51].

The desire for pharmacological targeting of reader complexes has already resulted in the development of several drugs for mechanistic bench-based studies and potential therapeutic interventions. Acetylated lysines can be recognized by bromodomains (BRD) and the tandem plant homeodomain (PHD) [52]. Contained within 46 proteins encoded by the human

Box 4. Lysine methyltransferases.
• SUV39H1
• SUV39H2
• EHMT2
• EHMT1
• SETDB1
• SETDB2
• KMT2A
• KMT2B
• KMT2C
• KMT2D
• KMT2E
• SETD1A
• SETD1B
• ASH1L
• SETD2
• NSD1
• SMYD2
• SMYD1
• SMYD3
• DOT1L
• SETD8
• SUV420H1
• SUV420H2
• EZH2
• EZH1
• SETD7
• PRDM2

Several lysine methyltransferases (KMTs) have been reported, with all of them but one (Dot1), belonging to the SET domain-containing protein group.

genome [53], BRDs are frequently found in proteins that also have enzymatic domains, such as HATs, or additional reader domains, such as PHDs, or up to six BRDs, in an organization that eases specific combinatorial recognition of multiple histone marks [23]. Robust biophysical, structural and molecular modeling studies have now rendered accessible numerous useful drugs that inhibit the function of BRD-containing proteins. High-resolution crystal structures of 29 of the 61 human BRDs, which spans all eight BRD families, have revealed a conserved hydrophobic pocket with a left-handed bundle of four α-helices that are associated by diverse loop regions of variable charge and length surrounding the acetylated lysine-binding site [54]. BRD inhibitors can be divided into two key types, based on whether or not the small molecules act as acetylated lysine mimetics. The nonacetylated lysine mimetic class includes small molecule drugs that interact with the acetylated lysine-binding pocket of the BRD without forming a hydrogen bond with the conserved asparagine that usually anchors acetylated lysines [53]. This type of inhibitor, which includes compounds such as NP1, ischemin, MS7072, MS436 and BID1, inhibits

Box 5. Lysine demethylases.

KDM1
- KDM1A
- LSD1
- BHC110
- AOF2
- KDM1B
- LSD2
- AOF1

KDM2/7
- KDM2A
- JHDM1A
- FBXL11
- KDM2B
- JHDM1B
- FBXL10
- KDM7A
- JHDM1D

KDM3
- KDM3A
- JMJD1A
- JHDM2A
- TSGA
- KDM3B
- JMJD1B
- JHDM2B
- 5qNCA
- KDM3C
- JMJD1C
- JHDM2C
- TRIP8

KDM4
- KDM4A
- JMJD2A

KDMs are classified based on sequence homology and catalytic mechanism into two functional groups known as KDM1A/LSD1 and KDM1B/LSD2. The JmjC domain-containing KDMs (KDM2–7 subfamilies) represent the larger KDM class, which are grouped into five subfamilies (KDM2/7, KDM3, KDM4, KDM5 and KDM6).

Box 5. Lysine demethylases (cont.).

KDM4 (cont.)
- JHDM3A
- KDM4B
- JMJD2B
- JHDM3B
- KDM4C
- JMJD2C
- JHDM3C
- GASC1
- KDM4D
- JMJD2D
- KDM4E
- KDM4DL
- JMJD2E

KDM5
- KDM5A
- JARID1A
- RBBP2
- KDM5B
- JARID1B
- PLU-1
- KDM5C
- JARID1C
- SMCX
- KDM5D
- JARID1D
- SMCY

KDM6
- KDM6A
- UTX
- KDM6B
- JMJD3

KDMs are classified based on sequence homology and catalytic mechanism into two functional groups known as KDM1A/LSD1 and KDM1B/LSD2. The JmjC domain-containing KDMs (KDM2–7 subfamilies) represent the larger KDM class, which are grouped into five subfamilies (KDM2/7, KDM3, KDM4, KDM5 and KDM6).

the reader function of the BRD by steric exclusion of the acetyl lysine peptide. The acetylated lysine mimetic class of small molecule drugs are competitive inhibitors, forming hydrogen bonds with the conserved asparagine residue [53]. The early development of BRD inhibitors focused almost entirely on targeting bromodomain and extra-terminal motif (BET) proteins; however, more recent studies have expanded to examine the druggability of the entire BRD family. Due to these extended efforts, all subfamilies have been determined to have relatively suitable druggability scores based on unique amino-acid BRD signatures, thereby supporting the feasibility of developing powerful inhibitors [53]. Even BAZ2B, a BRD predicted to be among the most problematic to target, has recently been successfully targeted for inhibition by the acetylated lysine mimetic

GSK2801 [53]. Notably, the study of BET BRD inhibitors in different diseases, including various cancers, has revealed novel insight into their function and the therapeutic potential of their targeting. These studies have produced the basis for the development of clinical trials with several BRD inhibitors. Remarkably, in this way it was recently shown that treatment with JQ1, an inhibitor of the BRD and extraterminal family of proteins, suppresses PDAC development by inhibiting both MYC activity and inflammatory signals [55]. Most importantly, combination with another epigenetic regulator such as suberoylanilide hydroxamic acid (SAHA; an HDAC inhibitor) synergizes with JQ1 to augment cell death and more potently suppress advanced PDAC. These data support a strong proof-of-concept that epigenetic modulation can be used as an efficient tool for treating PDAC development.

Why introduce epigenetics marks to understand PDAC development?

The revolution of somatic genetics in the field of cancer was brought about by the model developed by Fearon and Vogelstein in colon [56] and later adapted to the pancreas by Hruban *et al.* [57], which led to a productive period of PDAC research for approximately two decades. The basic principle of somatic genetics in cancer is that genes with a role associated to cancer via overexpression (through gene amplification, such as with *MYC* in brain), work as oncogenes, while those that are downregulated, such as *p16* in PDAC, work as tumor suppressors. According to this principle and to the Hruban model, the variations in expression of both oncogenes and tumor suppressors in PDAC were initially believed to occur via mutation or deletion and only later was promoter methylation integrated into the model [58–60]. The legitimacy of this model has been elegantly validated using genetically engineered models.

While we recognize the outstanding impact that this progression model of somatic genetics has had in progressing cancer research, we now recognize a model that also considers the theoretical context of epigenetics, and in particular, changes that occur at the protein level in the absence of DNA changes including deletion, mutation or even promoter methylation. For example, if we use the Hruban model to understand PDAC, in which the fundamental conceptual framework is genetic in nature, one could conclude that PDAC advances through multistep mechanisms with different lesions progressing via mutations in diverse genes. This model does not take into account what epigenetic changes, which can take place between the occurrences of landmark mutations, are responsible for cancer progression, nor can it prove that a later mutation is caused by an earlier one. Thus, a model of PDAC progression, which not only incorporates the elegant and extremely important data generated under the premise of the genetic model but also, in addition, includes epigenetic changes and overall nuclear structure, organization and dynamics [3], is essential to consider as we seek to treat this dismal disease. We believe that in the next few years, continued experiments aimed at addressing the contribution of these phenomena to PDAC progression, as well as devoting efforts toward their potential translation to clinical applications, including epigenetic-based therapeutics, will be among the most fruitful in the field.

Conclusion & future perspective

Genetic alterations crosstalk with epigenetic changes to not only give rise to neoplastic transformation but also are likely to determine many features of the cancer phenotype and its symptoms. This conceptualization has significant mechanistic value in our efforts to comprehend how this disease originates and evolves. A model for the progression of PDAC associates patterns of gene expression networks that define the PDAC phenotype being dictated by the combination of genetic and corresponding epigenetic instructions, and considers that both of these codes contribute to the development and progression of this disease. It is important to appreciate that new types of therapeutic approaches, which target the epigenome, could significantly ameliorate many epigenetic alterations. In fact, promising epigenetics-based therapies are currently being evaluated through different types of trials.

Executive summary

- Critical studies, emanating from the work of many laboratories including ours, indicate that epigenetic mechanisms can both silence or activate genes in a heritable manner independently of the coding capacity of DNA and thus influence the development and malignant behavior of pancreatic ductal adenocarcinoma.
- Taking into consideration the importance the pathogenic role that aberrant DNA methylation plays in pancreatic ductal adenocarcinoma, current or future small molecule drugs targeting DNMTs hold promise as part of the therapeutic arsenal against this dismal disease.
- The histone acetylase/deacetylase system serves as an important example of writers, readers, and erasers of histone marks that have a significant impact on the expression of the human genome, and constitutes the first widespread, histone-based therapies developed.
- The writing and erasing of methyl marks by histone methyltransferases and histone demethylases, respectively, confer strong signals for inheriting certain gene expression patterns in an inherited manner and thus are excellent targets for cancer therapy.
- Although several challenges exist for the application of ncRNA-targeting therapies, their significant role in disease development and progression advocates the need to continue advancing this line of therapeutics.
- The ability of chromatin readers to recognize and interpret the signals from specific histone marks to control the expression of the genome with long lasting consequences has increased the momentum to discover small molecules targeting these proteins.
- Epigenomic-based pharmacology and its translation to therapies have the potential to serve as a robust tool to improve the future treatment of pancreatic cancer.

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