Published in final edited form as:

Curr Opin Gastroenterol. 2008 September ; 24(5): 597–602. doi:10.1097/MOG.0b013e32830b111d.

The Sunset of Somatic Genetics and the Dawn of Epigenetics: A New Frontier in Pancreatic Cancer Research

Gwen Lomberk, Angela J. Mathison, Adrienne Grzenda, and Raul Urrutia

Laboratory of Chromatin Dynamics and Epigenetics, Gastroenterology Research Unit, Departments of Biochemistry and Molecular Biology, Biophysics and Medicine, Mayo Clinic, Rochester, MN 55901, phone: (507) 358-5636, fax: (507) 255-6318

Abstract

Purpose of Review—The excitement of finding a cancer modulator which is either mutated or deleted *in vivo* (Genetics), unfortunately, is shadowed by the fact that we scientists have failed to live to the promise of gene therapy, and therefore, these genes cannot be replaced to cure the patients. On the other hand, both DNA methylation and chromatin-mediated inactivation of tumor suppressor genes (Epigenetics), for example, is reversible as demonstrated by the relative success of emerging therapies. Therefore, epigenetics, and its molecular basis (DNA methylation and chromatin modification) is among the most promising areas of cancer research and is a nascent field in pancreatic cancer research.

Recent Findings—Here, we review and update novel findings on epigenetics as it applies to pancreatic cancer.

Summary—Special focus has been given to novel potential therapeutic targets and currently available drugs which are emerging from this exciting new field of pancreatic cancer research.

Keywords

Epigenetics; Pancreatic Cancer; Chromatin Modifying Proteins; HP1; Polycomb

Introduction

During the last three decades, primarily as a result of Dr. Volgestein's significant leadership in somatic genetics of cancers, we have significantly advanced in identifying the major tumor suppressors and oncogenes for many neoplasias, including pancreatic cancer [1]. Fortunately, a new generation of investigators interested in modeling the effects of these mutations *in vivo* using genetically engineered mice is confirming the validity of these observations [2]. Unfortunately, somatic genetics has currently become significantly limited for further exploring the key changes that associate not only to transformation but also with other cancer-associated functions. A valid concern is that, through genetics, we have: 1. already isolated the critical oncogenes and tumor suppressors leaving only minor contributors of unknown importance, and 2. limited ourselves to techniques of genetics which do not allow us to get the best information on epigenetics. In other words, using genetic techniques has given us approximately a dozen of well-trusted oncogenes and tumor suppressors. However, due to the nature of epigenetic pathways (defined as heritable changes in gene expression that are not due to any alteration in the DNA sequence), alterations in these pathways are by far more prevalent in cancer than genetic alterations and in addition, can be reversible [3]. Therefore, the focus of this review is to summarize general concepts of epigenetics and highlight emerging and important contribution of this field as it relates to pancreatic cancer. When appropriate, developing therapeutic approaches based upon targeting epigenetic phenomena in pancreatic cancer, are discussed.

DNA Methylation

DNA methylation was the first epigenetic change to be studied in more detail as a mechanism for the inactivation of tumor suppressors [4]. DNA methylation occurs on cytosines that precede guanines; these are called dinucleotide CpGs. This process involves the addition of a methyl group to DNA, such as to the number 5 carbon of the cytosine pyrimidine ring, ultimately silencing gene expression. Noteworthy, however, this type of DNA modification has significant physiological significance. Genomic imprinting, for instance, also requires DNA hypermethylation at one of the two parental alleles of a gene to ensure monoallelic expression, and a similar gene-dosage reduction is involved in X-chromosome inactivation in females. The hypermethylation of repetitive genomic sequences prevents chromosomal instability, translocations, and gene disruption caused by the reactivation of transposable DNA sequences, a process that has been observed in certain organisms when exposed to stress.

Molecular Pathway

DNA methylation has been classified into two types, namely *de novo* and maintenance methylation [4]. In humans, there are three DNA methylases, DNA methyltransferase 1, 3a, and 3b (DNMT1, DNMT3a, DNMT3b) [5]. The *de novo* pathway is mediated by DNMT3a and DNMT3b. The major role of these enzymes is to establish embryonic methylation patterns and this function has been exploited during carcinogenesis. For *de novo* methylation, DNMT1 works at replication forks during DNA replication and maintains methylation. However, DNMT1 appears to be inefficient at maintaining the methylation of many CpG dense regions. Therefore, the *de novo* activities of DNMT3a and DNMT3b are also necessary in somatic cells in order to reestablish the methylation patterns so that they are not lost due to the inefficient activity of DNMT1.

Potential Therapeutic Agents that Target Methylation

There are two types of DNMTs inhibitors, namely, nucleoside and non-nucleoside (small molecule) inhibitors [6,7]. Pharmacologic DNMTs inhibitors are being tested in phase I–III clinical trials. More importantly, the prototypical DNMT inhibitor 5-azacytidine (i.e. Vidaza) has recently been approved by the US Food and Drug Administration (FDA) for the treatment of myelodysplastic syndrome.

Novel contributions to Pancreatic Cancer

DNA methylation in pancreatic cancer has been known for a long time as a mechanism to inactivate tumor suppressor genes, such as p16 [1]. Initial studies focused on experiments at the single cell level, but fortunately, recent methodological developments are helping to perform genome-wide scale gene methylation analysis. For instance, KLF11, a tumor suppressor gene for pancreatic cancer and leukemia, was found to be methylated in these neoplastic diseases using large scale methylation analysis [8,9]. While both methodologies are valid, the single gene methylation analysis is a candidate gene approach while the power of the genome-wide analysis is its unbiased approach. Recent relevant examples of the candidate approach for analyzing gene methylation have been provided. Techniques used for this purpose include methylation-specific PCR, sequencing after bisulfite treatment, and mass spectrometry. However, we would like to highlight advances in using methylation analysis for pancreatic cancer due to the significant power underlying these techniques.

Two studies are worth discussion because they indicate that, besides individual genes being methylated in advanced pancreatic cancer, aberrant methylation occurs very early during the histopathological progression of this neoplasia. In the first study, using a candidate approach, Rosty *et al* show that PanINs arising in patients with chronic pancreatitis show loss of p16 expression [10]. This alteration, common to pancreatic cancer-associated PanINs, may

contribute to the predisposition of patients with chronic pancreatitis to develop pancreatic ductal adenocarcinoma.

Similarly, using methylation-specific PCR to confirm a study that initially involved large scale methylation analysis, Sato, *et al* [11] studied DNA samples from 65 PanIN lesions for methylation status of eight genes recently identified by a larger scale microarray approaches as aberrantly hypermethylated in invasive pancreatic cancer. Methylation at any of these genes was identified in 68% of all the PanIN lesions examined. More importantly, aberrant methylation was present in approximately 70% of earliest lesions (PanIN-1A). Among the genes analyzed, NPTX2 demonstrated an increase in methylation prevalence from PanIN-1 to PanIN-2, and from PanIN-2 to PanIN-3 for SARP2, Reprimo, and LHX1. Thus, the results of these studies suggest that aberrant CpG island hypermethylation begins in early stages of PanINs, and its prevalence progressively increases during neoplastic progression.

Thus, the evidence supports a clear role for methylation in the silencing of tumor suppressor genes that are key in the progression of pancreatic cancer. In the past, some of the genes like p16 were found to be methylated in transformed pancreatic tissue. However, current evidence indicate that methylation can occur earlier at the preneoplastic stage suggesting that pharmacological agents that target methylation may be useful not only for treatment but perhaps also for chemoprevention.

Chromatin Modifying Protein Complexes

Chromatin must undergo various forms of change in its structure to facilitate transcription, DNA repair and other nuclear processes. Histone proteins, which are the foundation of chromatin, are modified by various posttranslational modifications to alter the way DNA is compacted. The following paragraphs will reflect on three chromatin modifying protein complexes, which are key machinery in influencing these changes: histone acetylation/deacetylation, polycomb group and HP1.

A. Histone Acetylation and Deacetylation

Acetylation and deacetylation of lysine residues within histone tails is an important mechanism underlying the epigenetic regulation of gene expression [3]. Acetylation mediated by enzymes known as histone acetylases (HATs), such as CBP, P300, and pCAF results in gene expression activation, whereas deacetylation mediated by two different families of histone deacetylases (HDACs) is responsible for gene silencing. Thus, together, these enzymes offer a fine-tuned mechanism for the activation of oncogenic pathways and the silencing of tumor suppression. However, differently than other epigenetic regulators, such as HP1 and polycomb complexes discussed below, HATs and HDACs appear to mediate short-term responses, a fact that should be taken into consideration when thinking about these molecules as potential therapeutic targets in cancer [3,12].

Molecular Pathway

In mammals, HDACs are classified into three subfamilies [13–17]; the Rpd3-like or Class I HDACs, Hda1-like or Class II HDACs and the sirtuin family. Class I members, which are related to the first HDAC to be ever identified, the yeast rpd3, include HDAC1, -2, -3 and -8. Class II members, which on the other hand, are related to yeast Hda1, include HDAC4, -5, -6, -7, -9 and -10. Class II is further divided into two subclasses: IIa (HDAC4, -5, -7 and -9) and IIb (HDAC6 and -10). Sirtuin family members are related to the Sir proteins from *Saccharomyces cerevisiae*. These proteins differ from the better understood Class I and II family of proteins in the sense that their deacetylase activity is NAD⁺-dependent. Noteworthy, these proteins are primarily recruited to promoters through corepressor molecules, which

include Sin3, NUR-D, N-COR, Co-REST, SMART, CtBP, Groucho and other less understood silencing complexes [3,12]. Therefore, the potential permutations that can be achieved by complexing different HDACs with distinct co-repressors suggest that the biochemistry, biology, and pathobiology of these enzymes combined with how they are coordinated within the cell to maintain homeostasis and act in diseased states will turn out to be more complex than previously suspected.

Potential Therapeutic Agents that Target Histone Deacetylation

HDAC inhibitors (HDACIs), currently, are among the most promising epigenetic treatments for cancer [13–17]. The first proposed mechanisms of action for HDACs highlight their ability to reactivate transcription of multiple genes found to be silenced in human tumors. However, the fact that these proteins show pleiotropic anti-tumor effects selectively in cancer cells along with its potential to be beneficial for other diseases has raised the possibility that they also act in other biological processes, though currently these other mechanisms remain poorly understood. HDACIs are well-tolerated and several show promising anti-tumor activity. Currently, more than 50 naturally occurring or synthetic HDACIs have been developed. The best known among these agents are hydroxamic acid compounds, trichostatin A (TSA), and suberoylanilide hydroxamic acid (SAHA). However, other less-known, yet equally promising, drugs can be classified into wider groups, such as short-chain fatty acids (e.g: valproic acid), epoxides (e.g: trapoxin), cyclic peptides (e.g: Apicidin), benzamides (e.g: CI-994, N-acetyldinaline), and hybrid compounds (e.g: SK-7068).

While the cellular, biochemical, and molecular work dealing with the characterization of histone deacetylases in pancreatic cancer remains almost unexplored, more attention has been given to the potential of HDAC inhibitors as potential agents to fight this disease. For instance, recent work indicates that certain HDACIs can induce death of cultured pancreatic cells [18]. In addition, combination of some of these agents with conventional chemotherapeutic drugs, such as Gemcitabine, can achieve a synergistic inhibition of pancreatic adenocarcinoma cell growth [19]. This promising work has therefore fuelled the discovery of new agents that belong to this family of drugs, such as FR235222, which potentially could be used as therapeutic tools for this cancer [20]. Lastly, HDACIs are in advanced phases of clinical trials and may soon become part of the therapeutic arsenal for the medical oncologists treating this disease. Thus, in summary, this area of investigation is exciting, novel, and highly promising with the added value for researchers that investigations in this field still remain an underrepresented area of research.

B. Polycomb

Components of megadalton complexes, collectively called Polycomb [3,21–23], are responsible of long-term gene silencing, similar to HP1 proteins described below. Long-term gene silencing is important for fixing the blueprint of development, and its effectors are emerging as key players in stem cell biology and cancer. For instance, some genes which are silenced early by any of these complexes during embryonic development remain non-inducible for the entire life of the organism. This phenomenon, which is called transcriptional memory, implicates these proteins as attractive candidates to silenced pathways in early stages of cancer development along with maintenance in this manner through promotion, progression and possibly, participation in the response to chemotherapy.

Molecular Pathway

Polycomb proteins silence gene expression by methylating, in particular, histone 3, on lysine (K) 27 (methyl-K27-H3) [3,21–23]. This epigenetic mark can be mono-, di-, or trimethylated, with the last two forms of modifications as the ones believed to mediate the gene silencing function of Polycomb. Noteworthy, the Polycomb pathway is conserved between flies (where

most of the work on these complexes has been done to date) and human [3]. In flies, for instance, there are DNA sequences known as Polycomb Response Elements or PREs. However, bona fide PREs remain to be identified in human. Polycomb complexes primarily comprise two functionally and biochemically distinct multimeric Polycomb repressive complexes (PRCs), called PRC1 and PRC2. A simple pathway for PcG protein involves the stepwise recruitment to chromatin of PRC2, which contains histone H3 K27 methylase activity. Subsequently, the gene silencing complex formation is completed by the recruitment of PRC1 to the trimethyl-K27-H3 mark deposited by PRC2. The enzymatic activity of the PRC2 complex involves the function of the K27-H3 histone methylase, EZH2, together with Suz12, EED, and the RbAp46 and RbAp48 proteins. The PRC1 complex contains the oncogene BMI1, as well as HPC1-3, HPH1-3, SCMH1 and the methyl-K27-H3-binding proteins Cbx 2, 4, 6, 7, and 8. However, it is not clear which of the Cbx proteins is active at different loci under different circumstances.

Potential Therapeutic Agents that Target Polycomb Complexes

Significant efforts are being made to identify small molecules that target and modulate the function of Polycomb members. However, these efforts are at very early stages. Recently, the S-adenosylhomocysteine hydrolase inhibitor, 3-Deazaneplanocin A (DZNep), induces efficient apoptotic cell death in cancer cells, but not in normal cells, by effectively depleting PRC2 levels (decreased EZH2, SUZ12, and EED) and thereby inhibiting associated histone H3 K27 methylation [24]. The discovery of this new molecule could serve as a robust template upon which modification can help to develop new direct therapies based upon the inhibition of these proteins. Currently, however, in spite of how promising drugs that directly target Polycomb proteins may be for the treatment of cancer, the most common way to manipulate this pathway is indirectly. For instance, Bmi1 and EZH2 are downstream targets of Sonic hedgehog (Shh) [25] as well as estrogen and Wnt [26] signaling, respectively, providing a connection between epigenetic change regulators (PcG) and developmental-signaling pathways (Shh, Wnt). Finally, potential therapies may include using inhibitors acting on cancer stem cell populations, such as cyclopamine, an inhibitor of hedgehog signaling, 6-bromoindirubin-3'-oxime (BIO), which acts on GSK3, inhibitors of beta-catenin signaling, such as exisulind, and the tyrosine-kinase inhibitor, STI571/Gleevec/Imatinib. Thus, taking into consideration the pivotal role of Polycomb complexes in modulating the phenotype of both stem and cancer cells, we predict that intense efforts will be given to the development of compounds that target this pathway and impact cancer-associated processes.

Novel contributions to Pancreatic Cancer

The role of Polycomb proteins in pancreatic cancer has just begun exploration. For instance, new polycomb proteins are being discovered in pancreatic cancer cells [27]. More importantly, recently it has been shown that, loss of trimethylation at lysine 27 of histone H3, which is achieved by EZH2, is a predictor of poor outcome in pancreatic cancers [28]. In fact, the level of trimethyl-K27-H3 was shown to be a strong and independent prognostic influence ($P = 0.001$) in pancreatic cancer, together with tumor size and lymph node status. Thus, although this initial work indicates that much more is yet to be learned about the composition and function of polycomb complexes in pancreatic cancer, the association of this pathway to poor survival of patients affected by this disease makes this area of research one of paramount importance.

C. HP1

Heterochromatin Protein 1, or HP1, was originally discovered in *Drosophila* through studies of mosaic gene silencing resulting from a euchromatic gene placed near or within heterochromatin [3,29]. This phenomenon is known as position effect variegation (PEV), and HP1 is a dominant suppressor of this effect. In human and other mammals, there are three HP1

isoforms, namely HP1 α , HP1 β and HP1 γ . These proteins have been well-studied for their localization, as well as their roles, within heterochromatic regions which associate with gene silencing. More recent studies have made it increasingly unmistakable, however, that HP1 proteins not only localize to heterochromatic regions, but also euchromatic regions. This appears to be in an isoform specific manner. In mammalian cells, HP1 α and HP1 β display mainly heterochromatic localization, while HP1 γ is observed in both heterochromatin and euchromatin. Thus, these proteins provide an appropriate repertoire to mediate gene silencing in both euchromatin and heterochromatin. In addition, HP1 have been reported to participate in DNA stability, DNA repair, maintenance of telomeres, cancer cell migration.

Molecular Pathway

Functionally, the N-terminal chromodomain binds methylated K9 of histone H3, causing transcriptional repression [3,29]. The highly-related C-terminal chromoshadow domain allows for dimerization of these HP1 molecules and serves as a docking site for factors involved in a wide variety of nuclear functions, from transcription to nuclear architecture. One mechanism of HP1 chromoshadow domain binding is through a PXVXL motif present in interacting proteins. This pentapeptide motif is sufficient for interaction with dimerized chromoshadow domains. Targeting of HP1 to heterochromatin has been demonstrated to require this interaction with PXVXL-containing proteins in addition to the necessity of methyl K9 histone H3 recognition. Interestingly, interactions of HP1 with various partners can occur in either an HP1 isoform-specific manner or universally with all three isoforms. The diversity of binding partners combined with isoform specificity implicates HP1 in a myriad of nuclear processes.

To form heterochromatin, HP1 isoforms must interact with two different K9 histone methylases [3,29]. In euchromatin, the methylase partnering with HP1 is G9a (EuHMTase-2) and in heterochromatin, the dominant methylase is Suv39h1. HP1 and either of these methylases work together in a circular manner to form silenced chromatin. For instance, either of the methylases adds methyl groups to K9 of histone H3, which in turn forms an HP1 docking site on chromatin. Subsequently, HP1 can recruit more methylase and thereby by repeating this cycle, the HP1-methylase pair can spread the formation of silenced chromatin to adjacent nucleosomes, coating and silencing entire genes whether in heterochromatin or euchromatin.

Potential Therapeutic Agents

Although not yet tested in the pancreas, exciting developments have been made with the first generation of inhibitors of this pathway, through the G9a methyltransferase. Recently, in recombinant non-pancreatic cells, using G9a as the target enzyme, investigators have isolated 7 compounds from a chemical library comprising 125,000 preselected molecules [30]. One inhibitor, BIX-01294 (diazepin-quinazolin-amine derivative), selectively impairs the G9a HMTase and the generation of dimethyl-H3-K9 *in vitro*. In cellular assays, incubation with BIX-01294 lowers overall dimethyl-H3-K9 levels. Importantly, chromatin immunoprecipitation at several G9a target genes demonstrates reversible reduction of promoter-proximal dimethyl-H3-K9 in inhibitor-treated mouse ES cells and fibroblasts. Thus, this data identifies a promising HMTase inhibitor that can modulate dimethyl-H3-K9 marks in mammalian chromatin. The testing of this agent in pancreatic cancer remains to be done.

HP1 and Pancreatic Cancer

Evidence for the existence and function of HP1 proteins in both normal and tumor pancreatic cells is emerging. For instance, with the three human isoforms having over 80% similarity between them, the factors that influence these differences remain unknown. Unfortunately, in spite of the identification of all these HP1 binding partners, defined signaling cascades that mediate the interaction with these proteins in order to ultimately “switch on” or “switch off” gene silencing remains poorly understood. Thus, it is essential to define these pathways to map

useful networks of membrane-to-chromatin signaling cascades for better understanding the regulation of activation, repression, as well as other cellular processes. Our laboratory has recently described that HP1 proteins are amenable to the same posttranslational modifications that create the histone code (i.e. phosphorylation, acetylation, ubiquitination, sumoylation, and methylation), which likely regulate these distinct functions, thereby creating a subcode within the context of the histone code [31]. This HP1-mediated subcode in conjunction with the histone code constitutes a significant step in this field of research. This article describes, for the first time, molecular mechanisms that operate as subcodes within the histone code triggering nuclear instructions imparted by K9-H3 methylation, which are then translated as silencing. This mechanism appears to participate in the silencing of tumor suppressor genes.

Other Players

Substantial work on the role of Sin3a-HDAC system in pancreatic cancer suppression has been performed by our laboratory [32]. We have learned that pancreatic cells express three different Sin3 proteins that are recruited by tumor suppressors, such as the Myc antagonistic, Mad1, and KLF11. We have also provided evidence that these tumor suppressor proteins have an absolute requirement for binding to the Sin3a-HDAC complex to perform their function. Thus, all this evidence demonstrates that this system is both active and important for antagonizing pancreatic carcinogenesis. Numerous other gene silencing complexes exist in mammalian cells [12]. These proteins have been shown to participate in several cancer-associated functions in other organs. However, studies on these proteins in the pancreas remain underrepresented and in most cases, absent. Some of these proteins include corepressors such as the Mi-2/NuRD complex, N-CoR, SMART, Groucho, CtBP, TGIF, c-Ski, SnoN, and many others. Thus, we anticipate that studies of these complexes in pancreas may reveal a significant contribution of these proteins to the initiation, maintenance, or spreading of pancreatic cancer or to cancer-associated function such as stem cell maintenance, DNA repair, metastasis, and therapeutic response.

Concluding Remarks

Studies on chromatin dynamics are revealing the existence of robust machinery that can mediate epigenetic changes in pancreatic cells. Unfortunately, however, a large part of our field continues to focus only on studying aspects of somatic genetics (e.g: loss of heterozygosity), which appears to only represent a minor mechanism for gene inactivation in pancreatic cancer. The era of Epigenetics has taken this year a well-justified and energetic kick-off. Through these initial studies, we are entering into a frontier area for pancreatic cancer research. We anticipate that the recruitment of new investigators to this area of research will uncover important and potentially reversible mechanisms for fighting this disease. Mechanisms that only a few years ago, we could not even imagine existed.

References

- 1••. Singh M, Maitra A. Precursor lesions of pancreatic cancer: molecular pathology and clinical implications. *Pancreatology* 2007;7:9–19. Outstanding review covering key aspects of the biology and pathobiology of early pancreatic preneoplastic lesions. [PubMed: 17449961]
2. Tuveson DA, Hingorani SR. Ductal pancreatic cancer in humans and mice. *Cold Spring Harb Symp Quant Biol* 2005;70:65–72. [PubMed: 16869739]
- 3•••. Allis, CD.; Jenuwein, T.; Reinberg, D.; Caparros, ML., editors. *Epigenetics*. 1. Cold Spring Harbor Laboratory Press; 2007. p. 507. Recently released outstanding book, which covers from basic to advanced molecular concepts of Epigenetics.
- 4••. Esteller M. Epigenetics in Cancer. *N Engl J Med* 2008;358:1148–59. An Excellent review offering a robust description of DNA methylation. [PubMed: 18337604]

5. Cheng X, Blumenthal RM. Mammalian DNA Methyltransferases: A Structural Perspective. *Structure* 2008;16:341–350. [PubMed: 18334209]
6. Mund C, Brueckner B, Lyko F. Reactivation of epigenetically silenced genes by DNA methyltransferase inhibitors: basic concepts and clinical applications. *Epigenetics* 2006;1:7–13. [PubMed: 17998812]
7. Griffiths EA, Gore SD. DNA Methyltransferase and Histone Deacetylase Inhibitors in the Treatment of Myelodysplastic Syndromes. *Seminars in Hematology* 2008;45:23–30. [PubMed: 18179966]
8. Fernandez-Zapico M, Molina E JR, Ahlquist D, Urrutia R. Functional Characterization of KFL11, a Novel TGF β -Regulated Tumor Suppressor for Pancreatic Cancer. *Pancreatol* 2003;3:429–441.
9. Gebhard C, Schwarzfischer L, Pham TH, Schilling E, Klug M, Andreesen R, Rehli M. Genome-wide profiling of CpG methylation identifies novel targets of aberrant hypermethylation in myeloid leukemia. *Cancer Res* 2006;66(12):6118–28. [PubMed: 16778185]
10. Rosty C, Geradts J, Sato N, Wilentz RE, Roberts H, Sohn T, Cameron JL, Yeo CJ, Hruban RH, Goggins M. p16 Inactivation in pancreatic intraepithelial neoplasias (PanINs) arising in patients with chronic pancreatitis. *Am J Surg Pathol* 2003;27:1495–501. [PubMed: 14657708]
11. Sato N, Fukushima N, Maitra A, Matsubayashi H, Yeo CJ, Cameron JL, Hruban RH, Goggins M. Discovery of Novel Targets for Aberrant Methylation in Pancreatic Carcinoma Using High-Throughput Microarrays. *Cancer Res* 2003;63:3735–3742. [PubMed: 12839967]
12. Privalsky, ML., editor. *Transcriptional Corepressors*. 1. Springer; 2001. p. 190
13. Berger SL. Histone modifications in transcriptional regulation. *Curr Opin Genet Dev* 2002;12:142–148. [PubMed: 11893486]
14. Hildmann C, Riester D, Schwienhorst A. Histone deacetylases—an important class of cellular regulators with a variety of functions. *Applied Microbiology and Biotechnology* 2007;75:487–497. [PubMed: 17377789]
15. Bruserud O, Stapnes C, Ersvær E, Gjertsen BT, Rynningen A. Histone Deacetylase Inhibitors in Cancer Treatment: A Review of the Clinical Toxicity and the Modulation of Gene Expression in Cancer Cells. *Current Pharmaceutical Biotechnology* 2007;8:388–400. [PubMed: 18289048]
16. Glaser KB, Staver MJ, Waring JF, Stender J, Ulrich RG, Davidsen SK. Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. *Mol Cancer Ther* 2003;2:151–163. [PubMed: 12589032]
17. Pan L, Lu J, Huang B. HDAC Inhibitors: A Potential New Category of Anti-Tumor Agents. *Cell & Mol Imm* 2007;4:337–343.
18. Ouaiissi M, Cabral S, Tavares J, Cordeiro da Silva A, Mathieu-Daude F, Mas E, Bernard J, Sastre B, Lombardo D, Ouaiissi A. Histone deacetylase (HDAC) encoding gene expression in pancreatic cancer cell lines and cell sensitivity to HDAC inhibitors. *Cancer Biol Ther* 2007;7. [PubMed: 17612005]
- 19••. Donadelli M, Costanzo C, Beghelli S, Scupoli MT, Dandrea M, Bonora A, Piacentini P, Budillon A, Caraglia M, Scarpa A, Palmieri M. Synergistic inhibition of pancreatic adenocarcinoma cell growth by trichostatin A and gemcitabine. *Biochim Biophys Acta* 2007 Jul;1773(7):1095–106. This manuscript offers a solid proof-of-principle on the utility of combining HDAC inhibitors with Gemcitabine (the first line of treatment for patients affected by pancreatic cancer) to reach a synergistic effect. [PubMed: 17555830]
20. Singh EK, Ravula S, Pan CM, Pan PS, Vasko RC, Lapera SA, Weerasinghe SV, Pflum MK, McAlpine SR. Synthesis and biological evaluation of histone deacetylase inhibitors that are based on FR235222: a cyclic tetrapeptide scaffold. *Bioorg Med Chem Lett* 2008 Apr 15;18(8):2549–54. [PubMed: 18381239]
21. Breiling A, Sessa L, Orlando V, Kwang WJ. Biology of Polycomb and Trithorax Group Proteins. *International Review of Cytology* 2007;258:83–136. [PubMed: 17338920]
22. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* 2002;298:1039–1043. [PubMed: 12351676]
23. Ringrose L. Polycomb comes of age: genome-wide profiling of target sites. *Current Opinion in Cell Biology* 2007;19:290–297. [PubMed: 17481880]

- 24•••. Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL, Karuturi RK, Tan PB, Liu ET, Yu Q. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes Dev* 2007;21:1050–1063. This manuscript constitutes the first description of a pharmacological agent that can directly affect polycomb complexes. While exciting, additional experiments must be done to define the specificity of the drug described. The data serves as a reminder of the fact that, similar to DNA methylation, long term chromatin-mediated epigenetic changes can be reversible, a fact of great benefit when trying to reactivate tumor suppressor genes. [PubMed: 17437993]
25. Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, Suri P, Wicha MS. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res* 2006 Jun 15;66(12):6063–71. [PubMed: 16778178]
26. Shi B, Liang J, Yang X, Wang Y, Zhao Y, Wu H, Sun L, Zhang Y, Chen Y, Li R, Zhang Y, Hong M, Shang Y. Integration of estrogen and Wnt signaling circuits by the polycomb group protein EZH2 in breast cancer cells. *Mol Cell Biol* 2007;27:5105–19. [PubMed: 17502350]
27. Grzenda AL, Lomberk G, Urrutia R. Different EZH2 Isoforms Are Expressed in Pancreatic Cells: Evidence for a Polycomb-Mediated Subcode within the Context of the Histone Code. *Pancreas* 2007;35:404.
- 28••. Wei Y, Xia W, Zhang Z, Liu J, Wang H, Adsay NV, Albarracin C, Yu D, Abbruzzese JL, Mills GB, Bast RC Jr, Hortobagyi GN, Hung MC. Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. *Mol Carcinog*. 2008 Jan 4; This manuscript looks at tri-methyl-K27 Histone H3 as a predictor of poor outcome in pancreatic cancer. This work also supports the idea that Polycomb complexes, which bind to this epigenetic mark, may play a key role in pancreatic cancer development and progression.
29. Lomberk G, Wallrath L, Urrutia R. The Heterochromatin Protein 1 Family. *Genome Biol* 2006;7:228. [PubMed: 17224041]
- 30•••. Kubicek S, O'Sullivan RJ, August EM, Hickey ER, Zhang Q, Teodoro ML, Rea S, Mechtler K, Kowalski JA, Homon CA, Kelly TA, Jenuwein T. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Mol Cell* 2007 Feb 9;25(3):473–81. This is another example of the use of small drugs to target epigenetic complexes that are involved in gene silencing, potentially of tumor suppressor genes. The inhibitor described in this manuscript targets the HP1-mediating silencing system. Since Polycomb and HP1 may together be the most important system for silencing tumor suppressor genes, it would be interesting to define whether drugs against both systems synergize. [PubMed: 17289593]
31. Lomberk G, Bensi D, Fernandez-Zapico ME, Urrutia R. Evidence for the existence of an HP1-mediated subcode within the histone code. *Nat Cell Biol* 2006;8:407–415. [PubMed: 16531993]
32. Lomberk G, Urrutia R. The family feud: turning off Sp1 by Sp1-like proteins. *Biochem J* 2005;392:1–11. [PubMed: 16266294]