Epigenetic Pharmacology

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Contents

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Abstract

Decades of research focused on the genetic basis for development of pancreatic ductal adenocarcinoma have yielded tremendous discoveries. Clues to increase our understanding of the underlying biology of disease, the time along which the disease develops, and the potential vulnerabilities of disease are being elucidated daily. Alongside this genetically driven paradigm, researchers have uncovered the phenomenon of dramatically altered protein expression in the absence of an associated gene mutation. Through a mechanism termed epigenetics, the transcription and translation of genes can be dramatically altered by a variety of mechanisms including DNA methylation and histone modification. The fundamental concepts of epigenetics and major molecular agents that participate in setting the epigenome are reviewed herein. For each mechanism, the pharmacologic agents available for current use and the research underlying their approval are discussed. The potential impact of epigenetic pharmacology in pancreatic cancer is discussed in turn, and future directions of current research efforts are outlined.

Keywords

Pancreatic ductal adenocarcinoma · Epigenetics · Epigenetic pharmacology · DNA methylation · Histone modification · DNA methyltransferase · DNA methyltransferase inhibitor · Histone deacetylase inhibitors

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the United States [1]. With a mortality rate that approaches the incidence, the outcomes following diagnosis are dismal. There are many reasons that account for this statistic: advanced stage at presentation, aggressive underlying tumor biology, and relative inefficacy of standard therapies. It is the latter that often drives mortality. Whereas progress with systemic therapies has led to prolonged survival in many malignancies (including breast, colon, and gastrointestinal stromal tumors), cytotoxic chemotherapeutics have negligible benefit in survival after a diagnosis with PDAC. Research to associate genetic profiles with treatment response has also yielded disappointing findings. Alternative mechanisms of disease biology and treatment response are in active development.

Introduction to Epigenetics

The central dogma of molecular biology posits that genetic information coded in DNA is transcribed into RNA and translated into protein. Protein then functions in

such a way to ensure that the phenotype expressed by a cell accurately reflects the cell's underlying genotype. The recognition of this oversimplification occurred in parallel with the discovery of the genome itself, as it was clear that cells containing the same genome expressed widely disparate phenotypes (e.g., note the differences between a hepatocyte and a melanocyte). Even today the forces driving the development of a particular phenotype remain incompletely understood; however, the mechanisms used by cells to establish these differences are increasingly being unraveled. Examples of these mechanisms include variable transcription from the DNA, regulation of RNA translation, and regulation of protein expression.

With transcription alone, it is important to remember that the DNA is not always freely available for copy into RNA. At baseline, portions of the genetic code are twisted and wrapped around alkaline proteins, termed histones [2]. These histones, together with the DNA and other nuclear proteins, form tightly spiraled nuclear structures, called nucleosomes, which can promote or restrict access to DNA by the translational machinery of a cell. Further, even when not tightly bound to histones, specific residues of the DNA can be shrouded behind methyl groups (CH_3) prohibiting their transcription (as discussed later in this chapter). In cases such as these, when DNA is wrapped into tight complexes or covered by methylation, the expression of genes can be significantly altered.

Epigenetics is the term used to characterize the mechanisms of variable gene expression leading to disparate cellular phenotypes due to changes in a chromosome, without changes in the underlying sequence of DNA [2]. Though chromatin structure and nucleotide methylation are commonly cited examples of epigenetic variability, there are many other potential cellular processes with the capacity to exert epigenetic influence on a cell. These include changes in RNA or microRNA profiles that bind and augment the structure or function of histones, changes in nuclear protein composition that may fundamentally alter the microarchitecture between histones, or metabolic changes that can modify epigenetic protein binding or affinity. Commonly, these global changes within a cell can result in histone modifications by way of acetylation, ubiquitylation, sumoylation, and methylation.

Epigenetic changes are believed to be heritable with a potential impact just as great as germ line mutations in the DNA sequence [2]. Even after gestation and throughout the duration of life, epigenetic events are durable and persist from one cell division to the next. Importantly, however, the epigenetic profile of a cell (i.e., the epigenome) can be dynamic, reacting to environmental signals and allowing for changes to accumulate. At times this is likely a protective mechanism, helping to guide cellular fate during embryogenesis and adult cell renewal [2]. In stark contrast, alongside genetic mutations that drive malignancy, there are changes to the epigenome that appear to be early events in cancer tumorigenesis. In this chapter, the rationale for broadening research into novel therapeutics based on recent epigenetic studies is highlighted. The current mechanisms of epigenetic control are detailed as a framework from which to discuss potential pharmacologic therapies. Finally, ongoing studies and anticipated future work are highlighted.

Epigenetics: Definitions and Basic Mechanisms

Despite an increasing understanding of the DNA mutational landscape driving cancer, the progress made in developing therapeutics has been disappointing. While there are many reasons for this, one prominent hypothesis rests on the vast machinery that regulates the expression of the cell's genotype. In a simplified model, each gene encoded by DNA would be transcribed into RNA, be translated into protein, and then contribute to a cell's fate through the protein-protein interactions detailed in biochemical and molecular biologic texts. In reality however, there are dramatic differences in the ultimate production of protein encoded from one gene to the next on the chromosome. Some of this variability is due to regulation of RNA translation or protein-level degradation. However, much of this variability is due to differences in the amount of DNA transcription that occurs at each gene location on the chromosome and is controlled by local factors. These local factors, that change the gene expression patterns in a cell, can be due to two nuclear phenomena in the epigenome. First, changes in gene expression can result from the nuclear protein interactions with DNA that form chromatin (the local arrangement or "micro-architecture" of the chromosomes). The resulting microarchitecture is sometimes referred to as the "histone code" [3]. Second, gene expression can be augmented by the direct methylation of DNA residues. Finally, microRNA and other noncoding RNA molecules can have profound effects on gene expression.

Chromatin Modification: Histone Modification, The "Histone Code"

The microarchitecture of chromosomes within the nucleus of a cell is dependent upon the relationship between the DNA and nuclear proteins (Fig. 1). In some cases, the DNA may be loosely splayed open in a bath of transcription factors and electrolyte solution, termed euchromatin. In other areas, the DNA is tightly bound to spherical nuclear proteins with the nucleotides shielded from view, termed heterochromatin. It is this relationship, between the DNA and alkaline-rich proteins called histones, which is the major determinant of chromosome shape and function. Around each histone core, approximately 160 base pairs of DNA are wrapped. Together this complex is called the nucleosome. Each nucleosome may also bind tightly to a neighbor or be distanced from each other and stand apart at length. The positioning of nucleosomes in relation to their neighbors helps to form macrostructures termed chromatin. Chemical modifications to the core of histone proteins are the major determinants of chromatin arrangement (Fig. 1) [4].

Over the past two decades, major strides have been made to increase understanding of the mechanisms controlling the epigenome. Expression of genes along any length of DNA is dependent upon the arrangement of the chromatin and nucleosomes. As transcription start sites are wrapped tightly, the transcription machinery cannot intercalate with the DNA to facilitate gene expression. In contrast, as the start sites in the DNA move away from the nucleosome, they become more available for transcription. Nuclear proteins that function within intricate complexes control these

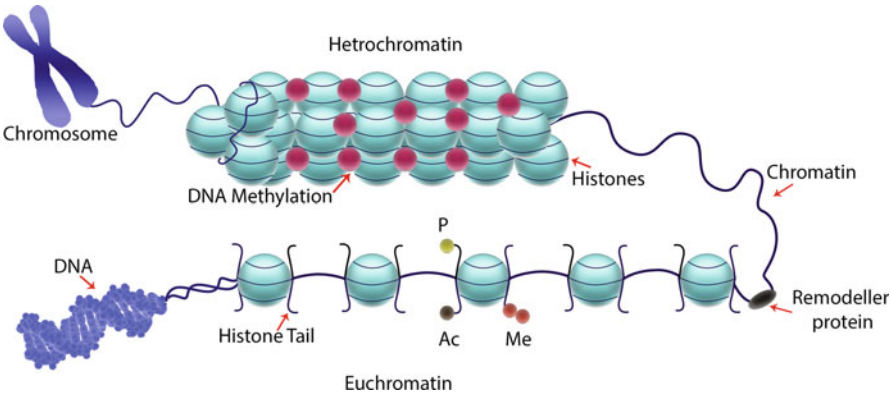

Fig. 1 The architecture of epigenomic landscape. The chromatin platform is an agile hub of activities switching genes "on" and "off" by regulating positioning of nucleosomes (*blue circles*). The unwinding of the chromatin leaves the transcription start site nucleosome free for transcriptional activities. Modifications of nucleosome histone tails (*blue lines* extending from *circle*) regulate the process, including DNA methylation (*red circles*), serine phosphorylation (P; *yellow circle*), lysine acetylation (Ac; *brown circle*) and lysine methylation (Me; *orange circle*), and nucleosome remodeler complexes protein required for moving nucleosomes (*black oval*)

epigenetic factors. These proteins are known as the writers, erasers, readers, and remodeler proteins and are discussed further below (Figs. 1 and 2) [4]. In general, these proteins are vital to cell maturation as their function in manipulating the epigenome can have profound effects on the proteome and phenotype of the cell. Through functions to add, remove, and interpret the "histone code," the proteins in these four classes are at the core of epigenetic determinants of cellular fate (such as maturation) [3].

Beyond maturation however, alteration of the epigenome by these proteins can also have profound effects during the dedifferentiation that leads to carcinogenesis. Two potential examples of this would include epigenome-based inactivation of tumor suppressor genes or activation of oncogenes [4]. The great promise in targeting therapy toward these epigenetic events is based on their potentially reversible nature. As discussed later in this chapter, the reversibility of these epigenetic events mirrors the flexibility seen in cellular differentiation during development [5]. For example, as mammalian cells mature from pluripotent progenitor cells to a differentiated phenotype, epigenetic control of gene expression through mechanisms such as histone modification, DNA methylation, and changes to noncoding RNA is key to appropriate differentiation. These epigenetic mechanisms are flexible, being modified as cells reach their differentiated states before settling into a more permanent epigenome [5]. Just as the epigenome is modified during development, data is mounting to support the role of epigenome modification in the dedifferentiating process that is the hallmark of the cancer phenotype. Further, once a gene is silenced, it remains heritable in somatic cells.

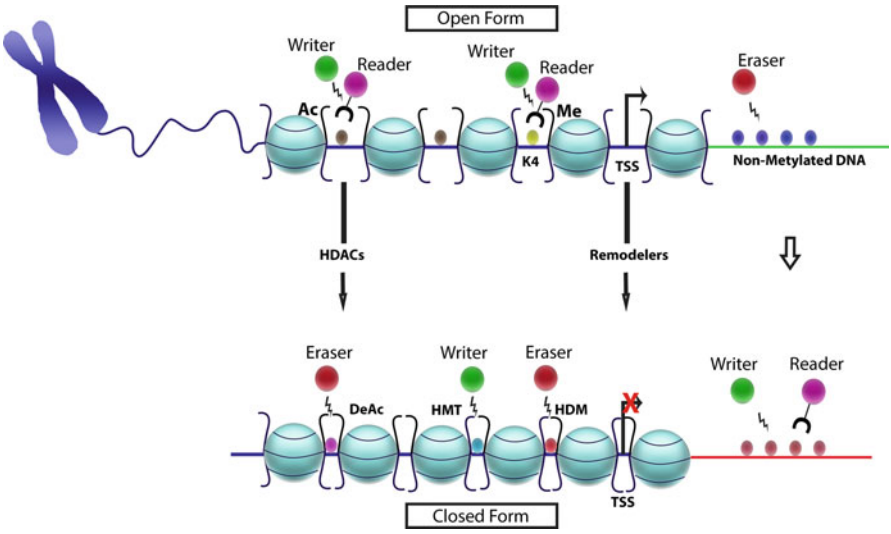

Fig. 2 The epigenomic 4Rs. For open chromatin form (*top*), which exposes the promoter region for transcriptional epigenetic switch in the form of writers (*green circles*), readers (*pink circles*), and erasers (*red circles*), and generally no DNA methylation in associated CpG islands (*yellow circle*). Nucleosomes (*blue circles*) are in an open conformation around the transcription start site (TSS). Writer enzymes in the form of histone methyltransferases (HMTs) add acetyl (Ac), methyl (me), and phosphorylation (P) marks to histone proteins (acetylated lysine, *brown circles*; methylated lysine, *yellow circles*). These regulated chromatin architectural (open and closed form) changes and gene expression regulation. Readers containing specialized domains bind to these distant marks, which are critical for binding to specific modification states. Erasers such as histone deacetylases (HDACs), lysine demethylases (KDMs), and phosphatases are involved in the removal of epigenetic marks. As the chromatin is modulated to the inactive state (bottom), with promoter DNA hypermethylation, it is associated with a more closed form of chromatin near transcription start site (TSS). HDACs, which erase histone acetylation (pink circle), writers (HMTs), which change active histone methylation marks to repressive ones such as H3K9me3 (blue circle) and HDMs, acting as antagonist to HMTs can all impact the epigenome. Another set of writers (DNMT) establish methylation of CpGs at promoter regions (*small red circle*), and readers for this methylation are methylcytosine-binding proteins (MBDs). *Abbreviations*: *HDACs* histone deacetylase, *HMT* histone methyltransferase, *HDMs* histone demethylases

DNA Methylation

DNA methylation refers to the state in which a methyl group (CH3) is bound to a nucleotide on the chromosome. This occurs almost exclusively on cytosine residues that precede guanine in the sequence CpG in the mammalian genome (Fig. 3). Both the distribution of CpG sequences across the genome and the degree to which these sequences are methylated are highly variable [6, 7]. The vast majority of the DNA is relatively poor in CpG density. There are, however, small regions of DNA with highly concentrated repeats of CpG that are known as CpG islands. These islands are frequently found adjacent to gene promoter regulatory sites. The CpG islands adjacent to gene promoter sites remain relatively free of methylation. In stark contrast, CpG dinucleotides in the vast majority of the remaining genome (i.e., not

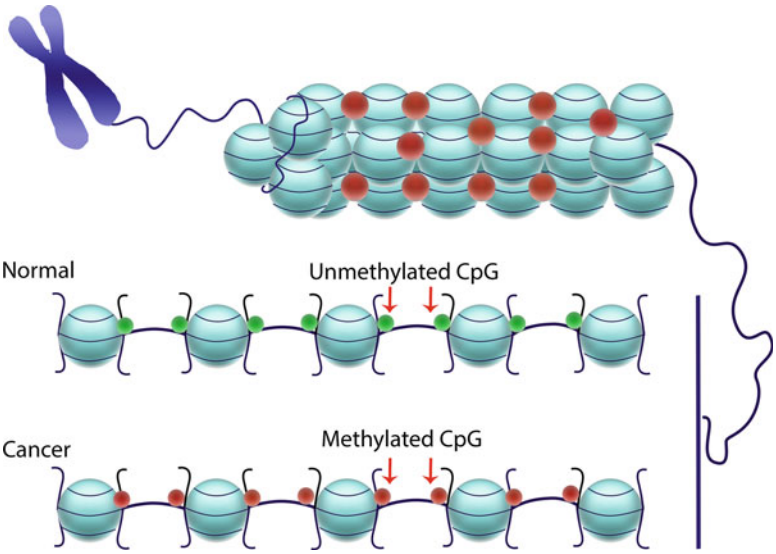

Fig. 3 DNA methylation patterns in normal and disease condition. In a normal cell, the promoter CpG islands (*top*) generally lack CpG site DNA methylation (*green circle*), whereas gene body is heterogeneous for DNA methylation in CpG dinucleotides. In cancer (*bottom*), many genes are heavily methylated in the promoter region of CpG islands, which represses chromatin landscape and leads to abnormal gene silencing. Whereas surrounding region is hypermethylated in the promoter regions with a gain in function

near gene promoter sites) tend to be heavily methylated. This includes heavily methylated areas present at repetitive DNA elements such as Alu (*Arthrobacter luteus* restriction endonuclease-characterized short DNA stretches), long interspersed nuclear elements (i.e., LINEs), and pericentromeric repeats [4, 8].

A growing body of literature is characterizing the effects of CpG island methylation in the cell during embryogenesis, mature cell division, and cellular dedifferentiation found in cancer. The key mechanistic association links increasing methylation of the dinucleotide sequences in CpG islands and decreased gene expression. Methylation-directed gene silencing is critical during embryogenesis, not only directing proper differentiation and maintaining cell lineage but also in ensuring genome stability [5]. Additionally, the phenomenon of gene imprinting, when heritable gene expression is controlled through epigenetic mechanisms (i.e., parental strand-specific expression), is reestablished during this period of embryogenesis [4].

Disorders in methylation can have profound effects on the fate of the cell and host. For example, certain inherited diseases are a result of gene imprinting rather than gene mutation. The neurodevelopmental disorders, Prader-Willi and Angelman syndromes, are two often cited examples of diseases of imprinting [9]. In Prader-Willi, for example, one predominant mechanism is driven by aberrant DNA methylation that silences genes along the maternal allele of 15q11-13 and loss of paternal

genes. This leads to a disorder characterized by mild to moderate cognitive defects (affecting speech, attention, executive function, and mood) that occurs in approximately 1 in 20,000 live births.

As fully differentiated cells divide and renew, opportunities for alterations in DNA methylation profiles exist. As cancer develops, DNA methylation is commonly altered. Fundamental changes in the epigenome include a relative global hypomethylation paired commonly with focal hypermethylation of CpG islands typically in gene promoters [2, 8]. These changes alter the nucleosome structure and global gene expression profiles. Additionally, specific hypermethylation in the promoter region of tumor suppressor genes, such as *Breast Cancer 1 (BRCA1)* or *Von Hippel-Lindau Tumor Suppressor (VHL)*, is commonly encountered and results in silencing of genes critical to the integrity of a cell. It is important to note that once DNA methylation is acquired, it is heritable in somatic cells and can contribute to malignancy [5]. Contemporary research efforts aimed at understanding the hypermethylome of cancer have shown that methylation-associated gene silencing is commonly seen in many tumor types, including colorectal, breast, pancreas, and gastric, amongst others [4, 8]. Generally, hundreds of genes show methylation in many cancer subtypes as demonstrated by the efforts by The Cancer Genome Atlas (TCGA) consortium [10]. Work is now progressing in understanding which of these gene-silencing events are epigenetic drivers rather than simply passenger events.

Beyond the focal hypermethylation, there are associated changes in histone marks including trimethylated histone 3 lysine 27 (H3K27me3), trimethylated histone 3 lysine 9 (H3K9me3), and many others [4]. Finally methylation in selected promoter regions, such as that adjacent to *MutL homolog 1 (MLH1)*, can drive changes to the underlying genome itself. Work by Herman and colleagues demonstrated that *MLH1* promoter hypermethylation drives microsatellite instability in selected carcinomas [11].

Epigenetic Mechanisms in Pancreatic Cancer Carcinogenesis

Original investigations into the role of the tumor suppressor genes, such as *p16*, in PDAC suggested that this family of proteins played a pivotal role in tumorigenesis [12]. Mechanistically, p16 is involved in a cell cycle regulatory complex that functions to arrest the cell at the G1 phase of division. The p16 protein, in particular, is responsible for control of cyclin-dependent kinase 4 (Cdk4) binding to cyclin D1 and subsequent progression through G1. Initial work by Caldas and colleagues found that genetic inactivation was present in 82% of tumors studied [12]. Nevertheless, one-fifth of tumors possessed wild-type (WT) *p16*, which led subsequent investigators to study other potential mechanisms of inactivation of this pathway [12].

The role of gene silencing through epigenetic mechanisms, such as DNA methylation patterns (Fig. 3), was of particular interest in follow-up studies [11, 13]. After confirmation of *p16* WT status in seven PDAC samples, a PCR-based methylation screen targeting the 5′-CpG islands of *p16* was used to investigate the

epigenome. In all but one, homogenous methylation patterns were detected for all *p16* transcripts, which resulted in a loss of downstream p16 protein and subsequent loss of growth suppressor function [14]. DNA methylation patterns were subsequently evaluated in depth for pancreatic cancer. Global methylation profiling assays identified nearly 60 candidate genes, which had altered expression due potentially to changes in methylation [14]. In the same work, candidate methylation markers of gemcitabine responsiveness were also proposed. Subsequent data have similarly shown extensive epigenetic changes in pancreatic cancer with methylation-associated transcriptional activation of many genes that are silenced early during cancer development [15]. These hypermethylated genes are often preferentially poised toward bivalency with both active and silencing histone marks, and environmental pressures may push toward inactivation of many of these genes by DNA methylation [16].

Similarly, the role of the epigenome in oncogene activation has been demonstrated in cell culture and xenograft models of PDAC [17]. Affecting a similar point in the cell cycle, G1-phase progression (as well as G1-S transition), the oncogene *c-myc* is a transcription factor responsible for upregulation of a variety of gene products with function in cell cycle progression, apoptosis, and cellular transformation [18]. In a study by Koenig et al., the regulation of *c-myc* gene expression demonstrated epigenetic changes driven by intracellular calcium concentration that controls the response of the calcineurin/cellular nuclear factor of activated T-cell (NFAT) pathway [17]. Specifically, NFAT binds to an element of the DNA adjacent to a *c-myc* proximal promoter and induces chromatin structural modification to allow for protein-promoter interactions driving c-myc protein translation. Importantly, and in a manner that provides insight into the pharmacologic rationale of targeting the epigenome, the depletion of NFAT abrogated c-myc protein expression leading to G1 arrest and decreased tumor growth in both in vitro and xenograft models of PDAC [17].

While a full review of the epigenetic mechanisms of disease is outside the scope of this chapter, and can be found in detail in chapter ▶ "Epigenetics and Its Applications to the Progression Model of Pancreatic Cancer", it is worth noting that the pancreas methylome clearly plays a role in PDAC [15]. In both in vitro models and patient tumor specimens, Yi et al. showed that cancer-specific promoter DNA methylation for two particular genes, *Basonuclin 1 (BNC1)* and *A Disintegrin-Like and Metalloprotease with Thrombospondin Type 1 (ADAMTS1)*, corresponds with early-stage PDAC [15]. The presence of PDAC-specific methylome changes may in fact hold promise in new early detection (disease-specific biomarker) and treatment paradigms. As such, it is this work in particular that makes a chapter such as this, focusing on epigenetic pharmacology, particularly relevant [15]. Lastly, there are important germ line mutations of critical regulatory elements of the epigenome that occur with some frequency in pancreatic cancer [19]. For example, the *AT-Rich Interaction Domain 1A* (*ARID1A*) gene is frequently mutated in many cancers of gastrointestinal cell origin, including from 2% to 8% of pancreatic tumors, and suggests that aberrant chromatin remodeling in this disease may be driven in part by acquisition of somatic mutations [19].

Pharmacological Strategies

Though there is clearly interplay and cross talk between the various effectors of epigenetics, for the purposes of a pharmacologic discussion, these will be addressed independently. It is important to remember, however, that the mechanisms of action for many of the agents discussed in the ensuing section are multifaceted. To facilitate discussion and understanding, a list of commonly researched agents and their current research point/approval status is noted in Table 1.

Table 1 Commonly researched agents, the current status of research and approval status if applicable

	Drug	Preclinical / Early phase	Approved / Disease
Single Agents			
DNMTi	Azacitidine		Approved/MDS
	DAC		Approved/MDS
	SGI110	Phase I/II	*MDS, AML, Ovarian, hepatocellular, Colon*
HDACi	Vorinostat		Approved/CTCL
	Romidepsin		Approved/CTCL
	Valproic acid		Approved/CTCL
	Pivanex (AN-9)	Phase I/II	*CLL, NSCLC*
	Entinostat	Phase I/II	*Approved/AML, MDS*
	Panobinostat	Phase III	*Hodgkin's Lymphoma, Kidney Cancer*
	Belinostat	Phase I/II	*Relapsed or refactory acute myeloid leukemia*
	Givinostat	Phase I/II	*Chronic myeloproliferative neoplasms*
	Pracinostat	Phase I/II	*AML, MDS, Metastatic sarcoma*
	Panobinostat	Phase III	*Hodgkins Lymphoma, multiple myeloma*
	Rocilinostat	Phase III	*Multiple Myeloma, CRC, Melanoma*
HATi	Curcumin	Phase III	*Breast Cancer, CRC, multiple myeloma*
HMTi	Tazemetostat	Phase I	*ALL, MLL*
	EPZ-5676	Phase I	*NHL, Breast cancer*
	GSK126	Preclinical	*Hematological Malignancies, NHL*
BETi	GSK525762	Phase I	*NUT midline carcinoma*
	JQ1	Preclinical	*AML, Multiple myeloma, NUT midline carcinom*
Combination			
Epi-Chemo	Vorinostat/5FU/Leucovorin		*CRC*
	SGI-110/Irinotecan		*CRC*
Epi-Immune	Aza/Romidepsin/PD-1		*CRC*
	SGI-110/GVAX/CY		*MDS*
Epigenetic priming with other drugs	AZA/Entinostat		*NSCLC, CRC*
	Romidepsin/Aza		*NSCLC*
	Radiotherapy/Vorinostat		*GI cancer*
	Vorinostat/Gemcitabine/paclitaxel/Sorafenib		*Pancreatic Cancer*
	Valprolic acid/hydralazine/Cisplatin		*Cervical Cancer*
	Vorinostat/Capecitabine/Cisplatin		*Gastric Cancer*

Targeting the Effectors of DNA Methylation

In general, there are several unique effectors of DNA methylation that play prominent roles in different biologic systems or at different times during cell maturation. While small noncoding RNA can play a role in directing DNA methylation (and is discussed later in this chapter), the family of catalysts that does the majority of work is known as DNA methyltransferases (DNMTs) [20]. These enzymes facilitate transfer of a methyl group from a donor (commonly *S*-adenosyl-L-methionine or SAM) to the 5′ position of the cytosine in CpG elements. Of note for the discussion to follow regarding pharmacotherapy, SAM exists in a balance with *S*-adenosyl-L-homocysteine (SAH). There are three primary DNMTs identified in mammalian studies: DNMT1, DNMT3A, and DNMT3B. Isoforms of DNMT3A and DNMT3B contribute to DNA imprinting and de novo methylation, while DNMT1 appears to be most important in maintenance of methylation [21].

The conserved elements of DNMT across family members appear to include a conserved sequence motif that binds to SAM [21]. Similarly, all family members have motifs toward the N-terminus, which serves to localize the protein to its nuclear target. For DNMT1, function includes interaction with the DNA replication complex at the replication fork whereby methylation maintenance is carried out as DNA is newly synthesized [22]. As each methylated CpG dipeptide is replicated, DNMT1 rests at the methylation site, flips the cytosine into its catalytic pocket, and facilitates methyl group transfer from SAM before moving along with the DNA replication complex [22].

Preclinical rationale for manipulation of DNMT family members in oncologic therapy is derived from several early studies to elucidate function of the protein. Following discovery of the gene, studies investigating function in cell lines demonstrated that mutation of DNMT1 caused no noticeable changes in embryonic stem cells [23]. Drastically, however, when a similar mutation was bred into the germ line of mice, a uniformly lethal phenotype was obtained. This initial work demonstrated that DNA methylation via DNMT1 function was both necessary and sufficient for preserved in vivo cellular maturation.

Interestingly, further work on methylation has demonstrated the agility of these enzymatic complexes. For example, when studying methylation after replication of X chromosome in cells passaged in tissue culture models, Riggs et al. demonstrated that omissions and errors occurred in as many as 5% of sites for each cell division [24]. These data raised the rational interest in targeting methylation as an oncologic therapy for several reasons. First, the tumorigenesis model whereby spontaneous epigenetic changes may impact phenotype alongside genetic mutations was recognized. Second, the flexibility of cellular processes controlling methylation and subsequent gene expression was proposed to be more "accessible" (or targetable) than corresponding changes in the underlying genome.

Given that initial studies associated oncogenesis with tumor suppressor gene hypermethylation, initial attempts to target DNMT function have focused on inhibition of the protein. Compounds found to inhibit DNMT can be broadly divided into

two categories: nucleoside analogs and non-nucleoside inhibitors [25]. The first generation to be discovered was nucleoside analog compounds initially believed to function as antimetabolites in cytotoxic regimens for leukemia [26]. The hypomethylation that results from therapy with two analogs of cytidine, 5-azacitidine and 2′-deoxy-5-azacitidine (DAC), was discovered after cellular differentiation was noted as a by-product of treatment in embryonic cell line studies [26]. Work to clarify the mechanism of action of these two agents has subsequently been elucidated. After entry into the cell, azacitidine and DAC are incorporated into the RNA and DNA of proliferating cells and recognized by DNMT during replication. Rather than catalyzing methylation, DNMT is irreversibly bound to the nucleotide analog due to substitution of nitrogen for the standard carbon on position 5 of the ring [25]. The differences between azacitidine and DAC are due to their molecular makeup. Azacitidine is a ribonucleoside that is incorporated preferentially into RNA rather than DNA. DAC, in contrast, is a deoxyribonucleoside and can only incorporate into DNA. These compounds both tend to have different mechanisms with different doses. Traditional use with high-dose administration causes direct cytotoxicity due to antimetabolite and DNA intercalation effects. In contrast, low-dose administration has been shown to effect demethylation with little cytotoxicity [27].

The US Food and Drug Administration has approved both azacitidine and DAC for the treatment of myelodysplastic syndrome and certain classes of lymphoma. Additionally, in the European Union, DAC is approved for acute myelogenous leukemia. Work by Silverman and colleagues in hematologic malignancies has shown us that the efficacy of these drugs is slow and responses are seen after several months [28]. As such, testing the efficacy of these epigenetic drugs in solid tumors has to be done carefully with the caveat that current clinical trials are performed in advanced cancers in patients who are rapidly progressing.

Utility of these compounds in solid tumors is under active investigation, but results have been hampered by early use of high doses of these drugs in the paradigm of using maximally tolerated doses similar to cytotoxic drugs and the resultant frequent side effects on bone marrow suppression from high doses [27]. However, in recent years low doses of these compounds have been tested in some solid cancers. Recently the Stand Up To Cancer/AACR consortium funded several trials with combination epigenetic therapy with a DNMT inhibitor, 5-azacitidine, along with an HDAC inhibitor entinostat in lung, colorectal, and breast cancers (discussed in detail below). In pancreatic cancer, for example, there is a wealth of preclinical data that suggests promise for DNMT inhibition either as a single agent or in multi-agent combination therapies. In cell culture models, administration of DNMT inhibitors has been repeatedly demonstrated to have profound effects on cellular growth and tumorigenicity of pancreatic cancer stem cells [29–31]. Additionally, preclinical models suggest a profound sensitization to other cytotoxic chemotherapeutics can be conveyed by low-dose DNMT inhibition. Telomerase activity, critical for cellular immortalization, has also been shown to be impacted by DNMT inhibition [32]. Finally, in vivo testing of DNMT inhibition has validated much of the data from cell culture

experiments: slowing progression of PDAC, extending survival, and sensitizing tumors to combination therapy [33].

A recent search of clinicaltrials.gov notes two trials evaluating the efficacy of DNMT inhibition in human subjects with pancreatic cancer. The first, NCT01845805, evaluates azacitidine in a phase II setting as monotherapy (versus an observation control) after completion of adjuvant therapy in resected pancreatic adenocarcinoma. First opening in April 2013 through the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, this trial is due to accrue 80 patients. The intended patient population for this trial includes those with node-positive disease, margin-positive disease, and/or elevation in CA 19-9. The second trial, NCT02847000, evaluates DAC in combination with tetrahydrouridine (to improve biodelivery) in a phase 0/I setting. Accrual for this second trial has not yet begun. Studies in pancreatic cancer so far have been limited with single-agent therapy given the rapidly aggressive nature of the disease and the slow onset of action seen with these compounds.

The toxicities that are encountered when using cytidine analogs are well documented from use in other settings. In general, there are two distinct profiles that arise from azacitidine and DAC therapy and depend on dose. At high dose, myelosuppressive effects are most common and reflect the cytotoxic antimetabolite profile that characterized their early discovery and use [27]. Importantly, however, the goal of epigenetically directed therapy is to avoid overt cytotoxicity by using low-dose therapy [27]. In these settings, the frequency of side effects are few and morbidity is low [34]. Ongoing work with second-generation nucleoside analogs (such as the DAC prodrug, guadecitabine or SGI-110) aims to increase bioavailability, limit cytotoxicity at higher doses, and improve efficacy [25, 35]. An initial trial testing guadecitabine in hematologic malignancies has shown promising bioavailability of this drug [35].

Non-nucleoside analogs are also of interest in epigenetic drug discovery. While sharing the core mechanism of action, inhibition of DNMT, non-nucleoside analogs do not require DNA intercalation to exert pharmacologic effect. In general, the majority of compounds in this class were discovered to have effects on the methylation profile of cells as a secondary finding [25]. Examples of compounds include certain flavonoids, hydralazine, procainamide, and curcumin. Each compound, or compound family, is purported to have their own distinct mechanism of action. For flavonoids, an indirect effect due to catechol-*O*-methyltransferase (COMT)-mediated accumulation of *S*-adenosyl-L-homocysteine (SAH) is thought to cause DNMT inhibition from SAM/SAH disequilibrium [25]. Hydralazine is thought to be a direct enzyme inhibitor through binding of the active site of DNMT, though this remains highly debated in the field [36]. In general, the use of flavonoids, hydralazine, and curcumin has all demonstrated the capacity to impact pancreatic cancer cell growth and induce apoptosis in vitro [37].

The efficacy of non-nucleoside analogs in the clinic is also promising, though data lags behind that of their nucleoside analog counterparts. Perhaps the best data are from trials involving hydralazine administration in combination with other

antitumor agents. Combination with valproate, for example, has demonstrated a limited capacity to resensitize patients to chemotherapeutics (a topic which will be discussed further later in the chapter), and hydralazine monotherapy was associated with reestablishment of tumor suppressor gene expression in otherwise untreated cervical cancer [36]. To date, there are no ongoing clinical trials evaluating the efficacy of non-nucleoside analogs for the prevention or treatment of pancreatic cancer.

Targeting the Effectors of Chromatin Structure and Function

The structure of chromatin can vary based on the markers which are affixed to the individual histone protein. These conformal rearrangements can dramatically alter the function of chromatin, including its capacity to bind nearby structures such as adjacent chromatin or nearby DNA strands. Based on this structure and function, the expression of genes can be regulated. In a simplistic view, the effectors that mark histones and change chromatin function can be divided into four classes. These are sometimes referred to as the "four Rs of epigenetics" and include the *r*emodelers, w*r*iters, e*r*asers, and *r*eaders (Figs. 1 and 2) [4].

These broad categories reflect differences in the function of the various proteins involved. For example, remodelers can be protein or noncoding RNA that often work in complexes to initiate the process of chromatin remodeling [38]. Epigenetic writers and erasers also often function in complexes of larger proteins as the enzymatic catalysts of histone modification [38]. As implied by the name, writers are responsible for labeling the histones with epigenetic marks. This family of catalysts has many members and can mark by facilitating transfer of acetyl, phosphoryl, hydroxyl, methyl, and many other moieties to the histone. In general, the focus of histone modification occurs at the amino-terminal peptide regions that are exposed at the periphery of the chromatin complex. Erasers are a family of enzymatic proteins that remove the marking of histones. Finally, epigenetic readers are responsible for identifying the epigenetic information laid down and facilitating changes in gene expression profiles (Figs. 1 and 2) [4, 8]. Remodelers help to arrange the histone and chromatin structure.

A historical view of epigenetics posited that increased marking of histones resulted in chromatin unfolding and directly correlated with increased gene expression. We now know that the relationship is complex and that both down- and upregulation of gene expression can be seen with histone modification [39]. Nevertheless, research has begun to wade into the nuanced world of these four protein families in attempts to discover new therapies for pancreatic cancer. While all four (remodelers, writers, erasers, and readers) may represent druggable targets, there are certain classes that lend themselves to therapeutic manipulation easier than others. For example, the enzymatic function of writers and erasers has enabled researchers to screen for and identify inhibitors of these enzymes (many of which are clinically approved for use and discussed below) [38, 40]. An additional class of epigenetic pharmacologic agents being studied focuses on disruption of the protein-protein

interactions central to the function of the reader proteins. The bromodomain inhibitors (or bromodomain and extraterminal, BET, inhibitors of reader protein function) are the classic example of this latter class of agents and will also be discussed later in this chapter [41].

Histone Deacetylase (HDAC) Inhibition: The Prototypical Agent for Histone Modification

In the eraser family of proteins, histone deacetylase (HDAC) and histone lysine demethylases are the two major members [40]. While work to target lysine demethylases is limited [42, 43], the HDAC inhibitors are a particularly well-described and well-studied class of medications that act on this epigenetic eraser family of proteins. There are several HDAC inhibitors that are approved for clinical use for various hematologic malignancies including vorinostat and panobinostat (Table 1). The original discovery of this class of agents was made following empiric compound screens for antitumor agents; only subsequently were the mechanisms of action elucidated [44]. Follow-up work has demonstrated that most of these agents have little-to-no sensitivity for targeting individual HDACs (as opposed to the whole class of proteins) and have potent effects on "off-target" enzymes in related classes [45]. Nevertheless, enthusiasm for this pharmacologic class has not waned, and there are currently more HDAC inhibitors in clinical trials than any other class of epigenetic agent.

The effects of HDAC inhibition on tumorigenesis is an area that has grown exponentially over the past decade. Proposed mechanisms of action include a direct effect on cell death via apoptosis and DNA damage accumulation, cell cycle arrest, reversal of dedifferentiation, and enhanced tumor immunogenicity [40]. Induction of apoptosis can occur via both the intrinsic and extrinsic pathway through gene modification of proteins such as the death receptors (DR4, DR5, FAS) and their ligands [46]. DNA damage repair mechanisms can also be fundamentally altered, and the resulting accumulation of errors can lead to apoptosis or autophagy [47]. The same line of investigation also discovered a toxic accumulation of reactive oxygen species was associated with increased DNA damage and proposed a role of HDAC in native metabolic homeostasis. Work on the mechanistic drivers of cell cycle arrest implicated direct transcriptional changes in genes such as *p21*, *p15*, *p19*, and *p57* [40]. Finally, an immunomodulatory component contributing to HDAC inhibitor efficacy was recently suggested after studies of murine models of carcinogenesis found an intact immune system was necessary for antitumor effect [48].

There are several classes of medications with a proposed mechanism of HDAC inhibition. The two broad categories include pan inhibitors (not HDAC isotype specific and with significant "off-target" effects) and inhibitors that purport to target a specific class of HDAC enzyme. The latter are far less common. Historically, hydroxamates and their derivatives were the most common HDAC inhibitors. These agents are composed of three domains: a cap region with surface recognition motifs, an active zinc-binding group that acts to perform its catalytic function, and a nonspecific linker region. Compounds belonging to this class include vorinostat and panobinostat. These agents generally target several classes of HDAC in addition

to having effects on other cellular lysine deacetylases that act on both nuclear and cytoplasmic protein targets [40, 45]. The nonspecific nature of these agents is principally due to the relative availability of the catalytic domain when these compounds are in their native forms.

The second class of HDAC inhibitors belong to a family known as the benzamides. These agents are characterized by more complex cap and linker regions which increase specificity of binding and limit the activity of the zinc-binding group for a particular HDAC class (generally class I HDAC). The most commonly studied agents in this family of medications are entinostat and mocetinostat [40, 49]. Novel compounds in this family are being frequently described and tested, such as the HDAC class 3 inhibitors RG2833 and RGFP966 [40]. Finally, other attempts to develop HDAC-specific therapies involve agents that architecturally abandon the traditional cap-linker-zinc catalyst mold of prior generations of agents. Thiol derivatives, which shroud the zinc-binding region within a complex ring structure, are one example of this class. The most well-described agent in the thiol class is romidepsin [40, 50].

The clinical utility of HDAC inhibition is limited thus far to patients with hematologic malignancies. Vorinostat, for example, has demonstrated modest efficacy in the treatment of refractory cutaneous T-cell lymphoma [51]. In this supporting work, 8 of 33 patients achieved a partial response with a median time to disease progression beyond 6 months in heavily pretreated patients. These findings, along with work done by many other groups, warranted granting of approval for use in this disease by the United States Food and Drug Administration [40]. The study of other HDAC inhibitors, such as romidepsin and belinostat, has also led approval of these agents for clinical use in selected hematologic malignancies [52]. A recent comprehensive review of HDAC inhibitor trials notes that over 350 clinical trials are currently ongoing to evaluate the efficacy of these agents, with most focused on hematologic tumors [40].

Belinostat is an interesting case study that represents a novel process of clearance for clinical use: accelerated approval. In July 2014, the FDA granted accelerated approval to belinostat (a relatively nonspecific HDAC inhibitor) for relapsed or refractory peripheral T-cell lymphoma [53]. The dose was chosen through a standard phase I dose escalation study that characterized the common side effects of nausea, vomiting, fatigue, fever, and anemia. As a monotherapy in second line or beyond disease, belinostat was found to convey an overall response in approximately one-quarter of patients. Given the accelerated approval paradigm, the end points of overall or progression-free survival were not reported. Importantly, this agent was never tested against control in any of the pre-approval trials, and as such a comparison end point of overall or progression-free survival would be inappropriate (and was not used to determine FDA status). Finally, subsequent studies of combination therapy of belinostat (and other HDAC inhibitors) with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) in early-phase clinical trials have been encouraging [54]. This experience clearly reflects the dire outcomes in relapsed and refractory peripheral T-cell lymphoma. The parallels (regarding the devastating

prognosis) with advanced pancreatic adenocarcinoma are glaring, and interest in accelerated approval for novel therapeutics in that disease is growing.

The use of these agents in solid tumors is still investigatory, though early reports are promising in selected diseases and when HDAC inhibition is combined with other agents. In breast cancer, for example, there is mounting evidence that targeted epigenetic therapy with HDAC inhibitors can reestablish sensitivity of tumors to antiestrogen therapy. This work was spearheaded in part by Merino and colleagues after successful results from early-phase clinical trials [55]. There are currently trials actively recruiting subjects in phase III for entinostat in combination with an aromatase inhibitor for patients who develop endocrine therapy resistance [56]. This trial is not alone as a recent search of clinicaltrials.gov reveals over a dozen trials registered testing entinostat in breast cancer, with correlative translational research providing clues to the underlying mechanistic rationale for treatment response or failure. Recent work from another of these trials suggests that combination therapy with immunomodulatory agents may be a rational strategy [57].

Combination therapy may be efficacious in other solid tumor models. Data from a phase I/II trial at Johns Hopkins University found that combination epigenetic therapy with azacitidine and entinostat produced responses in some patients with refractory advanced non-small cell lung cancer [58]. Data showed a median survival of 6.4 months in heavily pretreated patients, more than 2 months longer than historical controls. Of the 45 patients enrolled in the study, all of whom received the epigenetic treatment, 19 were able to undergo subsequent chemotherapy, and several had positive responses to treatment. In all, seven patients remain alive, including two who began the therapy nearly 4 years ago. Two other notable results combining azacitidine and entinostat include a phase II trial in advanced breast cancer (NCT01349959) and a phase II trial in metastatic colorectal cancer (NCT01105377) which have recently been completed as part of the Stand Up To Cancer consortium. The breast cancer trials included randomization by hormone receptor status and an optional continuation arm to investigate if epigenetic therapy can resensitize hormone-resistant patients to therapy [59].

In pancreatic cancer models, there has been little published to date suggesting that HDAC inhibition is a viable single-agent strategy for in vivo tumor response [38, 40]. This is despite growing in vitro data suggesting that HDAC plays an important role in pancreatic cancer cell growth, apoptosis, and downregulation of selected tumor suppressor genes [60]. Recapitulating the models developed in other tumor systems, there is in vitro evidence to suggest that combination strategies with HDAC inhibition and nucleoside analogues are promising in pancreatic cancer [61]. In this work by Arnold et al., vorinostat treatment of three pancreatic cancer cell lines resulted in cell cycle arrest and gemcitabine sensitization that appeared to be *p21* dependent.

There are other compounds that demonstrate histone acetyltransferase inhibition that are also worth noting. Many of these are derivatives from natural compounds such as curcumin, anacardic acid, and garcinol [4]. Other compounds, such as BIX-01294, chaetocin, and 3-deazaneplanocin A (i.e., DZNep), can be included in the

category of histone methyltransferase (HMT) and histone demethylase (HDM) inhibitors and are at various preclinical stages of development [8].

Targeting the Reader Proteins, a Relatively New Approach

The importance of the reader proteins in the structure and function of chromatin was highlighted by the discovery of mutations in the PHD domain (plant homeodomain – Cys4-His-Cys3 motif). PHD fingers are involved in chromatin-mediated gene regulation. Co-effectors of this function include the transcriptional coactivators p300 and CBP, polycomb-like protein (Pcl), trithorax group, the Mi-2 complex, the corepressor TIF1, the JARID1 family of demethylases, and many more [62]. Specific mutations in the PHD finger have been found to abrogate the protein's ability to bind protein effector partners and result in various disease conditions including carcinogenesis and immunodeficiency syndromes [62]. Thus, chromatin readers give us a unique opportunity for targeted therapies.

The best example of targets in the reader family of proteins are the bromodomains and extraterminal (BET) family of proteins. In brief, BET protein studies demonstrate a range of activity with the capacity to impact molecular function across a wide array of cellular processes [63]. They not only interact with the chromatin but also seem to function alongside other core nuclear protein complexes to affect DNA damage repair and transcriptional regulation. These findings have paved the way for the identification of potential BET bromodomain inhibitors as novel anticancer agents. Currently three BET inhibitors (I-BET762, JQ1, and I-BET151) are currently in preclinical models [4, 64]. These agents have been shown to bind to BRD2, BRD3, and BRD4 with a capacity to inhibit their engagement with acetyl-lysine residues. To date, effective antitumor properties have been demonstrated in several murine models of carcinogenesis and nearly two-dozen clinical trials are underway in a variety of advanced malignancies as tracked by clinicaltrials.gov.

Targeting the Associated Complexes in Epigenetics: Noncoding RNA and Protein-Protein Interaction

The role of ancillary pathways of epigenetic control to complement DNA methylation and histone modification is a relatively recent discovery. For example, it is becoming more evident that noncoding RNA plays an important role in the regulation of epigenetic processes [65]. In contrast to the central dogma of molecular biology, wherein RNA is supposed to code for amino acids, this family of nucleotides contains members that impart direct effects on cellular function or phenotype without translation into protein. These RNA transcripts are variable in length and can function both within the nucleus and in the cytoplasm. Effector functions of noncoding RNA can vary from epigenetic control (including chromatin remodeling or direction of methylation) to direct gene expression through transcriptional control and binding of DNA or posttranscriptional processing [66]. Examples include tRNAs, snRNAs, miRNAs, siRNAs, piRNAs, tiRNAs, spliRNAs, and sdRNAs among others. In general, the letters preceding RNA in each family provide clues

as to function. For example, siRNA tends to have a gene-silencing function. There are several key transcripts with known function via epigenetic mechanisms of control: Kcnq1ot1, Airn, Xist, and HOTAIR, for example [66]. Importantly, however, the role of microRNAs can be broad as nonspecific binding and "off-target" effects are as likely with this mechanism (as they are with other mechanisms of epigenetic control).

Perhaps one of the first studies to establish a potential role for noncoding RNA in oncogenesis was performed by Yu et al. and published in 2008 [67]. In this work a leukemia model of tumorigenesis was used to demonstrate the power of antisense RNA to silence tumor suppressor gene function. Specifically, with exogenous overexpression of an antisense noncoding RNA targeting *p15*, investigators demonstrated decreased gene expression and increased tumor growth associated with heterochromatin formation and DNA methylation [67]. A translational link was provided in that natural expression of this antisense construct appeared to be associated with decreased *p15* expression from patient samples.

There is strong preclinical rationale to support the role of noncoding RNA transcripts in solid tumors such as pancreatic cancer. First, global transcriptome analyses suggest that as many as 70% of all genes are susceptible to silencing through the effects of naturally occurring siRNA products present in nearby genetic code [68]. Second, members of another noncoding RNA family have already been shown to have effects on the development of pancreatic cancer [69]. MicroRNAs (miRNAs) are generally short RNA transcripts with the capacity to alter gene expression through any of the mechanisms described above. In pancreatic cancer, miRNA-17-92 has been suggested to be a key molecule in the restriction of tumorigenesis of cancer stem cells [31]. Interestingly, the discovery of this link was made after analysis of cancer stem cells' response to therapy aimed at targeting another epigenetic mechanism of gene expression, methylation through DNMT1. Another suggestion of the role that microRNA plays in pancreatic cancer derives from classic high-throughput discovery, necessity, and sufficiency experiments performed in cell line studies of pancreatic cancer [69]. These authors used a methylated DNA immunoprecipitation chip assay to discover that miRNA-615-5p was hypermethylated and silenced. Overexpression of this particular microRNA led to growth inhibition and decreased migration and invasion. Mechanistic studies suggested that miRNA-615-5p acts through effects on insulin-like growth factor 2 (IGF2), itself a heavily imprinted gene that is subject to epigenetic control. The direct influence, whether epigenetic, transcriptional, or posttranscriptional, between miRNA-615-5p and IGF2 is not clear, though the driver of expression (or silencing) of the actual microRNA is clearly through epigenetic mechanisms.

Drug Resistance in Pancreatic Cancer: An Epigenetic Problem?

There are four core mechanisms that have been proposed for acquired drug resistance in cancer therapy: reactivation of an oncogenic pathway, activation of parallel signaling pathways (i.e., bypass mechanisms), pathway-independent tumor cell

growth, and secondary alterations in the targets of selected drug therapy [70]. Classically, these have been described as mechanisms driven by genetic drift in tumorigenesis. It is increasingly being recognized, however, that epigenetic mechanisms of acquired resistance to therapy are important [71]. It is plausible that the relatively quick changes in cancer phenotype that occur during development of therapeutic resistance are driven more by the quick and directed epigenetic mechanisms of gene expression rather than the relatively slow and undirected process of acquired novel gene mutations [71]. Preventing or reversing these epigenetic mechanisms of acquired resistance could lead to more effective systemic therapy and extend survival [6, 71].

In pancreatic cancer there are two core bodies of work that support the hypothesis of epigenome-controlled therapeutic resistance. The first, led by Qin and colleagues, investigated the patterns of resistance that develop in pancreatic cancer cell line models to treatment with gemcitabine (until recently, the gold standard monotherapy in pancreatic cancer) [72]. Results demonstrated a cellular phenotype with dramatically upregulated expression of the 14-3-3σ protein. This protein is one member of a family that is known to bind a number of signaling proteins including key oncogenic effectors. Crucially, the σ isoform has been associated with particularly poor prognosis in pancreatic adenocarcinoma [73]. Mechanistic work to uncover the driver of 14-3-3σ overexpression implicated epigenetic regulation as the root cause. Under gemcitabine therapy, *14-3-3σ* is demethylated by DNA methyltransferase 1 and ubiquitin like with PHD and ring finger domains 1 (Uhrf1) [72]. When gemcitabine therapy was suspended, the epigenome partially reverted to its previous state of heavy methylation of *14-3-3σ*. These findings implicate epigenetic control of gene expression in the acquisition of therapeutic resistance and highlight the promise of targeted epigenetic therapy in combination treatments for this disease.

The use of combination chemotherapeutics using epigenetic agents with standard chemotherapeutics is beginning to show promise in selected tumor systems. As mentioned previously for breast cancer, the combined use of entinostat with all-trans-retinoic acid (ATRA) and doxorubicin resulted in significant tumor regression in xenograft modeling [55, 59]. This work has consequently led to clinical trials that are ongoing, including one successful phase II and an ongoing phase III trial [59]. Additionally, in ovarian cancer patients with platinum-resistant tumors, administration of low-dose 5-aza-2′-deoxycytidine was associated with resensitization to platinum agents (improved objective response rates and progression-free survival) which has led to an ongoing phase III trial (NCT00477386) [74]. Finally, work at Johns Hopkins in heavily pretreated metastatic colon cancer is now trialing guadecitabine (SGI-110) with irinotecan versus standard of care in a randomized phase II setting (NCT01896856). These trials reinforce the notion that future work in PDAC will focus on combination therapy utilizing epigenetic pharmacotherapy with standard cytotoxic, immunotherapy, or future targeted approaches [65].

Future Directions

While current epigenetic therapeutic approaches in solid tumors have showed minimal responses, the future for this therapy remains full of potential. Previous research, focused mainly on the effect of changes in DNA sequence on drug efficacy, failed to account for the changes in the proteome that were not driven by mutational burden. An increasing recognition of the importance that epigenetic factors play on disease biology and treatment response is driving current research. There are several barriers that remain, however, including a deeper understanding of the biology of the epigenome, a recognition of which epigenetic players are targetable and which are bystanders, and the pharmacodevelopment of novel compounds.

Additionally, the integration of targeted epigenetic therapies into clinical patient care will require multidisciplinary cooperation. Similar to data supporting multimodality treatment (surgery, cytotoxic chemotherapeutics, and radiation therapy) to maximize outcomes in pancreatic cancer, the goal of future epigenetic therapeutics will be to integrate novel drugs into a clinically relevant treatment model to allow for continued multidisciplinary care. In this respect, one would expect that epigenetic therapy should be well tolerated with few side effects. This is in keeping with work described earlier in this chapter in which maximal epigenetic benefits could be achieved at relatively low, noncytotoxic doses. Other than the aforementioned approaches, hormone therapy, immunotherapy, and other molecularly targeted therapies may change the landscape of treatment for pancreatic cancer in the future, and it is imperative that epigenetic therapies "play nice" with these other novel treatments as well.

Finally, it is well recognized that pancreatic ductal adenocarcinoma is a disease in need of better biomarkers. This would aid in both the early detection of disease and determining an optimal treatment paradigm. The traditional model of characterizing patient disease largely ignores the underlying biology of a patient's tumor and relies instead on needle biopsy for histopathologic diagnosis, blood measurement of a cell-surface carbohydrate (CA19-9), and imaging. One could certainly envision a future where a more robust analysis of disease biology is performed at key points in a patient's course of disease (from diagnosis to key points in treatment algorithms and therapeutic switches). It is becoming increasingly evident that an analysis of the epigenome would provide valuable data in this future paradigm.

Conclusion

Epigenetic influence on oncogenesis is becoming accepted as an increasingly important aspect of disease onset and progression. The biology responsible for epigenetic control is now becoming clear with key underlying mechanisms that include DNA methylation, histone modification, and noncoding RNA interactions. With clarification of the mechanisms, proteins involved are being characterized with increasing

detail. Targeting of key players is already in use in the clinic for certain tumors, and work is ongoing to broaden the utility of these FDA-approved agents. Importantly, epigenetic targeting appears to have a key role in both direct cellular cytotoxicity and in maintaining tumor response to current chemotherapeutics. As such, the future role of targeted epigenetic therapy in pancreatic cancer will likely include a multimodality approach and take advantage of improving surgical, cytotoxic chemotherapeutic, and radiotherapeutic advancements.

Cross-References

- ▶ Emerging Therapeutic Targets in Pancreatic Adenocarcinoma
- ▶ Epigenetics and Its Applications to the Progression Model of Pancreatic Cancer
- ▶ The Molecular Pathology of Precursor Lesions of Pancreatic Cancer

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