J Hepatobiliary Pancreat Surg (2006) 13:286–295
DOI 10.1007/s00534-005-1057-1

Journal of HBP Surgery

The role of epigenetic alterations in pancreatic cancer

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Abstract
The past several years have witnessed an explosive increase in our knowledge about epigenetic features in human cancers. It has become apparent that pancreatic cancer is an epigenetic disease, as it is a genetic disease, characterized by widespread and profound alterations in DNA methylation. The introduction of genome-wide screening techniques has accelerated the discovery of a growing list of genes with abnormal methylation patterns in pancreatic cancer, and some of these epigenetic events play a role in the neoplastic process. The detection and quantification of DNA methylation alterations in pancreatic juice is likely a promising tool for the diagnosis of pancreatic cancer. The potential reversibility of epigenetic changes in genes involved in tumor progression makes them attractive therapeutic targets, but the efficacy of epigenetic therapies in pancreatic cancer, such as the use of DNA methylation inhibitors, remains undetermined. In this review, we briefly summarize recent research findings in the field of pancreatic cancer epigenetics and discuss their biological and clinical implications.

Key words Epigenetics · Hypermethylation · Hypomethylation · Pancreatic cancer

Introduction

In the United States, more than 30000 people develop pancreatic cancer each year and almost an equivalent number of patients die of this disease, making pancreatic cancer the fourth leading cause of cancer death.[1] Pancreatic ductal adenocarcinoma is an extremely aggressive and devastating neoplasm, which often invades to and destroys surrounding stromal components, including lymphatic, vascular, and perineural systems, ultimately metastasizing to distant organs. In contrast to the improvements in survival that have been realized for other gastrointestinal cancers, the survival rate for pancreatic cancer remains dismal, emphasizing the need for a better understanding of pancreatic cancer biology, which can provide the basis for the development of newer biomarkers and targets for therapeutic intervention.

Over the past two decades, tremendous effort has been devoted to identifying genetic alterations (at both the chromosomal and nucleotide levels) in pancreatic cancer, and these efforts have led to the discovery of gross chromosomal losses and gains at selected loci and mutations/deletions of oncogenes and tumor-suppressor genes, including *KRAS2*, *CDKN1A/p16*, *TP53*, *SMAD4/DPC4/MADH4*, and *BRCA2*.[2–5] In addition to these genetic changes, many alterations in gene expression and specific signaling pathways (such as the aberrant activation of the Hedgehog and Notch pathways) have been described in pancreatic cancer and its precursors.[6–19]

In recent years, the field of cancer epigenetics has attracted considerable interest among researchers and clinicians, especially after the introduction of tools for studying DNA methylation, such as the polymerase chain reaction (PCR) amplification of bisulfite-modified DNA. It is now apparent that epigenetic alterations, including DNA hyper- and hypomethylation, and the associated transcriptional changes of the affected genes are central to the evolution and progression of various human cancers.[20] With the use of genome-wide screening technologies, as well as conventional candidate gene approaches, we and other groups have identified a number of genes that are affected by aberrant DNA methylation in pancreatic cancer. Importantly, the detection of DNA methylation alterations has been proposed for cancer risk assessment, and for the early detection of cancer, as well as for tumor classification and prognostication; these alterations have also been suggested as therapeutic targets.[20–25] In this article, we will review recent advances in our understanding of the epigenetic

Offprint requests to: N. Sato
Received: August 1, 2005 / Accepted: September 1, 2005

features associated with pancreatic neoplastic progression, focusing on their biological and clinical relevance.

Aberrant hypermethylation in pancreatic cancer

Aberrant hypermethylation of promoter cytosine-phospho-guanine CpG islands is closely linked to gene silencing and loss of tumor suppressor function in cancer.[20] The first detailed analysis of aberrant DNA hypermethylation in pancreatic cancer was reported in 1997 by Schutte et al.,[26] who demonstrated aberrant hypermethylation of the *p16/CDKN1A* gene in a subset of pancreatic cancers. The *p16* methylation was found exclusively in the wild-type alleles and was associated with gene silencing,[26] suggesting DNA methylation as an alternative pathway to inactivate this important tumor-suppressor gene in pancreatic cancer. Ueki et al.[27] analyed a large panel of 45 pancreatic cancers for the methylation status of multiple genes (including *p16* and *hMLH1*) and CpG islands previously identified as aberrantly methylated in other cancers. This study was the first to show that specific genes are selectively hypermethylated in pancreatic cancer.[27] The results also revealed that a small subset (14%) of pancreatic cancers have a higher prevalence of DNA methylation, suggestive of the presence of a CpG island methylator (CIMP) phenotype.[23,27] Subsequent studies have demonstrated that pancreatic cancers with methylation of the highest proportion of CpG islands in a gene panel are larger in size and are found in older patients,[28] though distinct biological, clinical, or pathological differences have not yet been identified to support the use of a CIMP classification for pancreatic cancer. Nonetheless, numerous studies in recent years have demonstrated that the methylation-induced silencing of biologically relevant genes in pancreatic cancer is common and influences tumor behavior. Indeed, many investigators have used candidate gene approaches to identify various tumor-suppressor or cancer-related genes that undergo aberrant methylation in pancreatic cancer, including *APC*,[29] *TSLC1/IGSF4*,[30] *SOCS-1*,[31] *cyclin D2*,[32] *RASSF1A*,[33] *WWOX*,[34] *RUNX3*,[35] *CDH13*,[36] *DUSP6*,[37] and *HHIP* (Hedgehog interacting protein).[38] The introduction of genome-wide screening techniques has enabled us to search for novel sites for epigenetic alterations in pancreatic cancer. First, Ueki et al.[28] used methylated CpG island amplification coupled with representational difference analysis (MCA/RDA) to isolate a number of CpG islands differentially methylated in pancreatic cancer. One of the CpG islands identified was located in the 5′ region of the gene preproenkephalin (*ppENK*), encoding for a native opioid peptide with growth-suppressor properties,[39,40] which was found to be aberrantly methylated in the vast majority (>90%) of pancreatic cancers.[28,41] Aberrant methylation at *p16*, *ppENK*, and others was also detectable at various frequencies in pancreatic intraepithelial neoplasias (PanINs) and in intraductal papillary mucinous neoplasms of the pancreas (IPMNs), known precursors to invasive adenocarcinoma;[42,43] also, the aberrant methylation increased progressively with advancing stage of the neoplasms.[41,44] These findings suggest that aberrant methylation at some loci occurs at relatively early stages and accumulates during pancreatic neoplastic progression. Sato et al.[45] used oligonucleotide microarrays to search for novel methylation sites in pancreatic cancer. This high-throughput approach identified a total of 475 candidate genes that were induced by a DNA methyltransferase inhibitor (5-aza-2′-deoxycytidine 5-Aza-dC) in four pancreatic cancer cell lines, but not in HPDE, a non-neoplastic pancreatic ductal epithelial cell line, and subsequent analyses confirmed aberrant hypermethylation of 11 genes in a large number of established and primary pancreatic cancer.[45] One of the genes identified was *UCHL1/PGP9.5*, a member of the carboxyl-terminal ubiquitin hydrolase family, and this gene was recently shown to be methylated in other cancers, including head and neck[46] and esophageal squamous cell carcinoma.[47] Importantly, the methylation status of *UCHL1* was an independent prognostic factor for patients with esophageal cancer, emphasizing an important role of this gene in tumor progression.[47] Another gene of interest identified in this study was *Reprimo*, a p53-induced mediator of cell-cycle arrest at the G2 phase.[48] *Reprimo* was aberrantly hypermethylated in over 80% of pancreatic cancers, which is in striking contrast to the frequent hypomethylation and overexpression of *14-3-3sigma*, another p53-induced mediator of G2/M cell cycle arrest, in pancreatic cancer.[49] Recently, Takahashi et al.[50] extensively studied the methylation status of *Reprimo* in a wide spectrum of malignant tumors (total of 645 tumors representing 16 tumor types) and found frequent methylation in certain tumor types. In separate studies, we demonstrated the aberrant methylation and silencing of two additional genes (*SPARC* and *TFPI-2*), selected from the list of candidate methylation genes, in a vast majority of pancreatic cancers.[51,52] Using methylation-sensitive-representational difference analysis (MS-RDA), Hagihara et al.[53] successfully discovered 27 CpG islands that were aberrantly methylated and 13 genes that were silenced in pancreatic cancer. Finally, a global gene expression comparison of IPMNs and normal pancreatic ductal epithelium led to the identification of *CDKN1C/p57KIP2* as a gene commonly downregulated in pancreatic ductal neoplasms through one or more of the following mechanisms: *CDKN1C* promoter hypermethylation and histone deacetylation, and/or loss of the *CDKN1C*-expressing allele, as evidenced by

loss of heterozygosity (LOH) of the *CDKN1C* locus and hypomethylation of *LIT1*, an imprinting control region that silences *CDKN1C* when *LIT1* is hypomethylated and expressed.[54]

Mechanisms of aberrant hypermethylation in cancer

Although several lines of evidence suggest that aberrant DNA hypermethylation in cancer is maintained by DNA methyltransferase activity,[55,56] the mechanism by which such methylation occurs at specific loci during carcinogenesis remains unclear. The most likely scenario is that DNA methylation initially arises at discrete CpG sites independent of gene expression, but then spreads into promoter CpG islands, presumably through a loss of balance between factors that promote and those that protect against methylation spreading.[57] This model was supported by a study showing that *GSTP1* methylation in prostate cancer cells was initiated by a combination of transcriptional gene silencing (by removal of the Sp1 sites) and seeds of methylation that subsequently spread across the promoter CpG island.[58] It has been also suggested that establishing the transcriptional silencing of a gene involves a close interplay between DNA methylation and histone modifications, and that methylation change in cancer can be a secondary event that may occur as a consequence of genetic or other events, such as the loss of transcription factor(s), that alter the transcriptional activities of affected promoters. For example, the leukemia-promoting PML-RAR fusing protein, which functions as a transcriptional regulator of retinoic acid (RA) target genes, has been shown to induce *RARβ2* gene hypermethylation and silencing by recruiting DNA methyltransferases to target promoters.[59] Bachman et al.[60] have demonstrated, in a model system where DNA methyltransferase genes are disrupted in a colorectal cancer cell line, that histone modifications (methylation of histone H3 lysine-9) are the primary events associated with the re-silencing of the *p16* gene that occurs prior to DNA methylation. In addition, a recent study has shown that the loss of estrogen receptor (*ER*) α expression by RNA interference results in the silencing of downstream target genes (including progesterone receptor [*PR*]) through the recruitment of polycomb repressors and histone deacetylases to their promoters, followed by the progressive accumulation of DNA methylation in their promoter CpG islands.[61] These findings have suggested that, at least at some genetic loci, initial silencing events lead to chromatin modifications that may predispose promoter CpG islands to hypermethylation. However, it is not known whether this is true for all the CpG islands that are aberrantly methylated in cancer. Finally, recent evidence suggests that RNA interference, a highly conserved system mediating sequence-specific RNA degradation, can also drive the transcriptional silencing of target genes by inducing DNA methylation in human cells,[62,63] raising the possibility that microRNA alterations which occur during cancer development could also contribute to aberrant DNA methylation in cancer.[64]

Functional consequences of aberrant hypermethylation in pancreatic neoplastic progression

Among the substantial number of hypermethylated genes identified in pancreatic cancer, several may be functionally involved in tumor growth, invasion, metastasis, and chemoresistance (Table 1). One typical example is the classic tumor-suppressor gene *p16*, which undergoes methylation-induced silencing in almost all those pancreatic cancers (around 15%–20% of cases) that do not have bi-allelic genetic inactivation of *p16*. For many genes, there is ample evidence that their anti-cancer functions are silenced by methylation and not by genetic inactivation. For example, there is growing evidence that *SPARC*, a gene identified as silenced in most pancreatic cancers in association with aberrant methylation, has inhibitory effects on the growth of pancreatic and other cancers in vitro and in vivo.[51,65] Moreover, a recent study identified a novel function of SPARC as a sensitizer to chemotherapy and radiation therapy, suggesting the potential usefulness of SPARC-based gene or protein therapy for refractory pancreatic cancers.[66] Interestingly, SPARC is overexpressed in stromal fibroblasts adjacent to cancer cells and its expression in these fibroblasts may be regulated through tumor-stromal interactions,[51] though the biological significance of SPARC expression in the cancer stroma remains unknown. *WWOX* (WW domain containing oxidoreductase), a candidate tumor-suppressor gene that maps to the common fragile site *FRA16D*, was recently shown to be inactivated in pancreatic cancer by genetic (deletion) and/or epigenetic (promoter hypermethylation) mechanisms, and transfection of *WWOX* induced apoptosis and inhibited the colony formation of pancreatic cancer cell lines lacking *WWOX* expression.[34] Another gene of interest in *TFPI-2* (tissue factor pathway inhibitor 2), encoding for a broad-spectrum serine proteinase inhibitor, which was found to be commonly inactivated by aberrant methylation in pancreatic cancer.[52] Restored expression of *TFPI-2* in nonexpressing pancreatic cancer cells resulted in marked suppression of their proliferation, migration, and invasive potential in vitro.[52] Finally, recent studies have shown that *BNIP3*, a hypoxia-inducible proapoptotic gene, is silenced in pancreatic cancer,[67] and loss of BNIP3 function may increase cellular resistance to

Table 1. A selected list of genes that are aberrantly hypermethylated in pancreatic cancer

Gene	Chromosome	Known or predicted function	Methylation in pancreatic cancer cell lines	Methylation in primary or xenografted pancreatic cancer	Reference number
TFPI-2	7q22	Serine proteinase inhibitor	14/17 (82%)	102/140 (73%)	52
SPARC	5q31.3–q32	Cell-matrix interaction, cell-growth inhibition	16/17 (94%)	21/24 (88%)	51
BNIP3	10q26.3	Hypoxia-induced cell death	9/10 (90%)	8/10 (80%)	67
TSLC1/IGSF4	11q23.2	Cell-cell, cell-matrix interaction	4/17 (24%)	25/91 (27%)	30
CDKN1A/p16	9p21	Cyclin-dependent kinase inhibitor	3/9 (33%)	5/36 (14%)	26, 27
CDKN1C/p57KIP2	11p15.5	Cyclin-dependent kinase inhibitor	7/9 (%)	Not determined	54
ppENK	8q23–q24	Neuropeptide precursor	11/11 (100%)	43/47 (91%)	28, 41
SOCS-1	16p13.13	Inhibitor of JAK/STAT pathway	6/19 (32%)	13/60 (22%)	31
WWOX	16q23.3–q24.1	Steroid metabolism, apoptosis	2/9 (22%)	2/15 (13%)	34
DUSP6	12q21–q22	Negative regulator of MAPK	2/16 (13%)	5/12 (42%)	37
Reprimo	2q23.3	p53-Induced G2/M cell-cycle arrest	20/22 (91%)	16/20 (80%)	45
HHIP	4q28–q32	Negative regulator of hedgehog pathway	10/20 (50%)	33/70 (47%)	38
MLH1	3p21.3	DNA mismatch repair	0/9 (0%)	2/36 (6%)	27
RARβ	3p24	Cell-growth control	5/9 (56%)	4/36 (11%)	27
Cyclin D2	12p13	Cell-cycle control	19/22 (86%)	71/109 (65%)	32
FOXE1	9q22	Thyroid transcription factor	14/22 (64%)	15/20 (75%)	45
NPTX2	7q21.3–q22.1	Neuronal transport	21/22 (95%)	20/20 (100%)	45

hypoxia-induced cell death[67] and to certain chemotherapeutic agents, including gemcitabine.[68,69] These studies together suggest that the epigenetic inactivation of selected genes is an important mechanism that contributes to the aggressive phenotypes of pancreatic cancer.

On the other hand, it is also notable that genes whose expression should favor neoplastic progression, such as *COX2* and *CXCR4*, have been shown to be downregulated in a subset of pancreatic cancers in association with promoter hypermethylation.[70,71] The biological significance of aberrant methylation in these potential cancer-promoting genes is unknown, but this phenomenon could be part of a genome-wide process of CpG island hypermethylation that occurs during pancreatic carcinogenesis.[23,27]

Gene hypomethylation in pancreatic cancer

DNA hypomethylation is another major form of epigenetic alteration in human cancer.[72] This epigenetic alteration is detected both at the genomic level (global hypomethylation) and at specific sequences (regional or site-specific hypomethylation), such as normally methylated repeat sequences and 5′ regions of certain genes.[73] Global DNA hypomethylation has been considered to occur, at least in part, as a result of altered folate metabolism, and has been linked to genetic instability [74] and tumorigenesis.[75] Despite the lack of evidence supporting a causal relationship between folate status and DNA methylation abnormalities in cancer,[76] the deficiency of nutrients essential for methylation (such as vitamin B12 and folate) is associated with an increased risk of several cancers, including pancreatic cancer.[77] In addition, we have found that pancreatic cancers with the most deficient methylenetetrahydrofolate reductase (*MTHFR*) genotypes have more DNA hypomethylation and more chromosomal losses, supporting the hypothesis that global hypomethylation can promote genomic instability.[78]

Little is known about the role of site-specific hypomethylation in cancer, but increasing evidence linking decreased methylation at specific CpG sites and the overexpression of affected genes has led to an attractive hypothesis: that promoter hypomethylation can cause gene activation.[72,73] Table 2 provides a list of genes identified as aberrantly hypomethylated in pancreatic cancer. Rosty et al.[79] demonstrated that overexpression of the *S100A4* gene in pancreatic cancer was associated with hypomethylation at specific CpG sites within the first intron. An extensive methylation analysis of a large panel of genes with differing expression status in pancreatic cancer demonstrated frequent hypomethylation in seven genes (*claudin4, lipocalin2, 14-3-3sigma/*

Table 2. A selected list of genes that are aberrantly hypomethylated in pancreatic cancer

Gene	Chromosome	Known or predicted function	Methylation in pancreatic cancer cell lines	Methylation in xenografted pancreatic cancer	Reference number
14-3-3sigma/stratifin	1p36.11	p53-Induced G2/M cell-cycle arrest	17/20 (85%)	36/37 (97%)	11, 49
Maspin/SERPINB5	18q21.3	Regulation of cell motility and cell death	20/23 (87%)	32/34 (94%)	49, 82, 83
S100P	4p16	Cell-cycle progression and differentiation	13/23 (57%)	30/34 (88%)	49
Trefoil factor 2	21q22.3	Secretory polypeptide/epithelial repair	13/20 (65%)	31/37 (84%)	49
Claudin 4	7q11.23	Cell adhesion/invasion	17/20 (85%)	33/37 (89%)	49
Mesothelin	16p13.3	Cell-surface antigen/cell adhesion	8/20 (40%)	34/37 (92%)	49
PSCA	8q24.2	Cell-surface antigen/cell differentiation	6/20 (30%)	20/37 (54%)	49
S100A4	1q21	Motility, invasion, and tubulin polymerization	10/20 (50%)	28/37 (76%)	49, 79
Lipocalin2	9q34	Epithelial differentiation	17/20 (85%)	34/37 (92%)	49

stratifin, trefoil factor2, S100A4, mesothelin, and *prostate stem cell antigen* [*PSCA*]) that were overexpressed in the neoplastic epithelium of pancreatic cancers and not expressed in normal pancreatic ducts.[49] In an attempt to identify additional hypomethylation targets in pancreatic cancer, we used oligonucleotide microarrays to screen for genes that displayed expression patterns associated with hypomethylation.[80] This analysis identified two genes, *S100P* and *maspin*, as aberrantly hypomethylated in pancreatic cancer.[80] Interestingly, cell-type-restricted maspin expression appears to be regulated by DNA methylation,[81] and other investigators also reported an association between hypomethylation and the overexpression of *maspin* in pancreatic cancer,[82,83] supporting the role of hypomethylation in the transcriptional activation of this gene.

As is the case for aberrant DNA hypermethylation in cancer, however, it is not certain at this time whether gene-related hypomethylation is a cause or a consequence of altered transcriptional activity in cancer cells.[84] Recently, De Smet et al.[85] analyzed the mechanism of selective hypomethylation at the *MAGE-A1* promoter in tumor cells and provided evidence that site-specific hypomethylation in this gene may result from a transient process of demethylation (presumably as part of genomic hypomethylation) followed by a persistent local inhibition of remethylation, due to presence of transcriptional factors. Further studies will be required to determine the mechanism and the role of aberrant gene hypomethylation in pancreatic cancer.

Diagnostic potential of epigenetic markers in pancreatic cancer

The development of early detection strategies, using molecular markers, should lead to an improved clinical outcome for pancreatic cancer.[86,87] In this regard, epigenetic changes (aberrant DNA hypermethylation) hold promise as novel screening/diagnostic markers of pancreatic cancer, especially for high-risk individuals such as those with a strong family history of pancreatic cancer.[88,89] The diagnostic potential of epigenetic markers has been evaluated in clinical samples (i.e., pancreatic juice) from patients with different pancreatic diseases.[32, 45, 52, 90,91] Fukushima et al.[90] first demonstrated that, using a methylation-specific PCR (MSP) assay,[92] aberrant methylation of *ppENK* and *p16* was detected in 30 (67%) and 5 (11%) of 45 pancreatic juice samples, respectively, collected during surgery form patients with pancreatic cancer, while such methylation was not detected in 20 pancreatic juice samples from patients with benign pancreatic disorders, including chronic pancreatitis.[90] Using a panel of three genes (*NPTX2*,

SARP2, and *CLDN5*) identified by a microarray approach as very frequently methylated in pancreatic cancer, we were able to detect aberrantly methylated DNA in 18 (75%) of 24 pancreatic juice samples from patients with pancreatic cancer, but not in samples from benign counterparts.[45] These findings have highlighted the feasibility of detecting aberrantly methylated DNA (especially using multiple markers), in pancreatic juice for the diagnosis of pancreatic cancer. Yan et al.[93] recently used real-time quantitative MSP (QMSP) to demonstrate that 26 of 42 (62%) patients with pancreatic cancer had higher levels of *p16* promoter methylation in their pancreatic juice samples, compared with 3 of 24 (13%) controls (benign biliary disease) and 2 of 26 (8%) patients with pancreatitis. Our recent study also demonstrated that quantifying pancreatic juice methylation, using QMSP, could better predict pancreatic cancer than detecting methylation using conventional MSP.[94] It should be noted, however, that many genes (including *ppENK* and *p16*) that undergo methylation in pancreatic cancer are normally methylated in the non-neoplastic duodenum, albeit at low levels in most cases, and, therefore, such methylation is frequently detected in pancreatic secretions aspirated from the duodenum of patients with and without pancreatic cancer.[90,95] Thus, strategies to detect pancreatic cancer using aberrantly methylated genes should rely on the analysis of pure pancreatic juice collected through selective pancreatic duct cannulation rather than that of pancreatic secretion collected within the duodenal lumen.

From the standpoint of risk assessment, our observation that patients with pancreatic cancer have a greater propensity to methylate non-neoplastic duodenum, specifically at certain CpG islands, than patients without neoplasia is important.[95] This finding raises a possibility that determining mehtylation at selected genes in non-neoplastic tissues such as the duodenum could be a useful biomarker to assess future risk of developing pancreatic cancer. Additional studies are needed to identify the best set of methylation markers for early detection and/or risk assessment, to determine the detection technologies best suited for each application (as well as their cost performance) in the clinical setting, and to establish the sensitivity and specificity of these selected markers in larger studies.

Epigenetic alteration as a therapeutic target in pancreatic cancer

DNA methylation changes in cancer may have important therapeutic implications, because such epigenetic alterations, unlike genetic changes, are considered to be a reversible biological phenomenon.[24,25] For example, some potential cancer-accelerating genes activated through the hypomethylation of their corresponding promoters could be therapeutic targets for inactivation by strategies to induce de-novo methylation at specific CpG sites. A recent study demonstrated that treatment of hepatocellular carcinoma cells with a methylated oligonucleotide targeting the hypomethylated *IGF2* promoter inhibited its expression and markedly prolonged the survival of nude mice with an implanted tumor.[96]

On the other hand, inhibitors of DNA methylation and histone deacetylation (HDAC) have been considered promising chemotherapeutic agents, based on the rationale that these drugs could potentially restore some of the epigenetically silenced tumor-suppressor genes in cancer.[24,97] Indeed, a number of such inhibitors have been shown to suppress tumor growth in vitro and in vivo, and some of the inhibitors are being tested in clinical trials for patients with different types of solid and hematological cancers.[98,99] One of the most commonly used DNA methyltransferase inhibitors, 5-Aza-dC (Decitabine; Dacogen, MGI Pharma, Bloomington, MN, USA), has been extensively investigated for its effects on gene expression and for its antineoplastic potential.[100–102] This drug, however, is also known to have toxic side effects, as well as mutagenic potential.[103,104] Recently, a more chemically stable and orally administrable demethylating drug, zeburaline, has been demonstrated to inhibit the growth of bladder cancer in mice.[105] Only a few studies, however, have addressed the effects of epigenetic modifying drugs on pancreatic cancer. Missiaglia et al.[106] have recently shown that 5-Aza-dC inhibits the growth of pancreatic cancer cell lines and that this effect is associated with the activation of interferon-related genes. These authors also showed that pretreatment with 5-Aza-dC increased the sensitivity of pancreatic cancer cells to other chemotherapy agents, including tumor necrosis factor (TNF)-α, cisplatin, and gemcitabine.[106] It is also notable that many cancer testis antigens, such as G antigens (GAGE) and so forth, are robustly induced in pancreatic cancer cells by 5-Aza-dC treatment,[45,107] suggesting the possible use of this drug as an aid in immunotherapy directed against these antigens. Additionally, several HDAC inhibitors (such as trichostatin A [TSA] and FR901228) have been shown to inhibit growth and to induce apoptosis in pancreatic cancer cells.[108–110] However, the use of these epigenetic modifying drugs for the treatment of pancreatic cancer should be carefully evaluated in preclinical studies, because previous reports have suggested that these drugs could also reactivate potential cancer-promoting genes when silenced by methylation and, in some cases, accelerate tumor progression. In fact, we and other investigators have shown that treatment with a DNA methyltransferase inhibitor resulted in the upregulation of invasion-promoting genes (including *MMPs* and *uPA*), thereby leading to increased invasiveness in

certain cancer cell lines.[111–113] We have also demonstrated that genes favorable for tumor progression, such as *COX-2* and *CXCR4*, are silenced by aberrant hypermethylation in a subset of pancreatic cancers and are re-expressed in these cancers after treatment with 5-Aza-dC and/or TSA.[70,71] The efficacy of these epigenetic modifying drugs may vary among individual cancers, and could be determined by the balance between the activation of tumor-suppressor genes and that of cancer-promoting genes. Furthermore, a recent study, showing that global DNA hypomethylation can lead to tumor formation in mice, raises a question about the rationale for the use of demethylating agents for cancer.[75] Thus, these questions need to be further investigated before DNA methylation and HDAC inhibitors are moved into clinical use for patients with pancreatic cancer.

Summary

A growing body of evidence indicates that pancreatic cancer is characterized by widespread and profound epigenetic changes, including CpG island hypermethylation and gene hypomethylation. These aberrant methylation events could represent novel sdiagnostic and therapeutic targets for this devastating disease. Many fundamental questions about the biological and clinical significance of DNA methylation have yet to be answered, such as how and when such epigenetic defects occur during pancreatic ductal carcinogenesis, and how our knowledge of epigenetic features in pancreatic cancer should be translated into the clinical setting.

Acknowledgments. This work was supported by a National Cancer Institute grant (CA90709, CA62924), and by the Michael Rolfe Foundation.

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