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TOPIC HIGHLIGHT

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Epigenetics and pancreatic cancer: Pathophysiology and novel treatment aspects

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Abstract

An improvement in pancreatic cancer treatment represents an urgent medical goal. Late diagnosis and high intrinsic resistance to conventional chemotherapy has led to a dismal overall prognosis that has remained unchanged during the past decades. Increasing knowledge about the molecular pathogenesis of the disease has shown that genetic alterations, such as mutations of K-ras, and especially epigenetic dysregulation of tumor-associated genes, such as silencing of the tumor suppressor $p16^{ink4a}$, are hallmarks of pancreatic cancer. Here, we describe genes that are commonly affected by epigenetic dysregulation in pancreatic cancer *via* DNA methylation, histone acetylation or miRNA (microRNA) expression, and review the implications on pancreatic cancer biology such as epithelial-mesenchymal transition, morphological pattern formation, or cancer stem cell regulation during carcinogenesis from PanIN (pancreatic intraepithelial lesions) to invasive cancer and resistance development. Epigenetic drugs, such as DNA methyltransferases or histone deactylase inhibitors, have shown promising preclinical results in pancreatic cancer and are currently in early phases of clinical development. Combinations of epigenetic drugs with established cytotoxic drugs or targeted therapies are promising approaches to improve the poor response and survival rate of pancreatic cancer patients.

Key words: Pancreatic cancer; Epigenetics; DNA methylation; Histone modification; microRNA; Targeted therapy; Epithelial-mesenchymal transition

Core tip: Pancreatic cancer represents a devastating disease with poor overall survival at advanced stages, and new and effective treatment options are required. Besides genetic mutations, epigenetic dysregulation of oncogenes and tumor suppressor genes is recognized as a novel therapeutic target. Mechanisms underlying DNA methylation, histone acetylation and microRNA regulation and their contribution to pancreatic cancer development and resistance to treatment are highlighted in this review. Potential therapeutic interventions are discussed.

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BRIEF INTRODUCTION TO PANCREATIC CANCER AND ITS CURRENT MODEL FOR PATHOGENESIS

Overall, the incidence of pancreatic cancer is minimally increasing or stable[1]. Experimental and clinical investigations have been intensified in the last few years (1) to obtain more pathogenetic insights in this highly life-destructive cancer entity; (2) to improve the early detection rate of this often concealed disease; and (3) to identify new therapeutic strategies to improve quality of life and survival time[2,3]. Nevertheless, the fate of patients with a diagnosis of pancreatic cancer is miserable even with maximal application of possible combined therapeutic interventions such as surgery, radiation and/or chemotherapy[4,5]. The overall survival time of patients with pancreatic cancer is a mean of 1 year after diagnosis[6]. This leads to the unsettling question of whether patients with a diagnosis of pancreatic cancer can survive at all[7].

In the last few years, one therapeutic point of attack has been concerned with the role of cancer stem-cells and the epithelial-mesenchymal transition under the influence of epigenetic regulator mechanisms[8]. These approaches are interesting and promising as they could explain the chemotherapy refractiveness of most pancreatic cancers. We have shown previously that pancreatic cancer employs classical pathways of organ development and embryogenesis such as Hedgehog or WNT (wingless) signaling[9,10] which, amongst others, could be targets for novel therapeutic approaches.

In this review, we focus on epigenetic regulation mechanisms in pancreatic cancer providing possible novel treatment aspects by highlighting the pathophysiology of this special tumor entity for pathologists, clinicians and future therapeutic approaches.

Epidemiologic (re-)view of pancreatic cancer

Pancreatic cancer is associated with a high mortality rate and represents the 7th most frequent cause of cancer death, with approximately 265000 deaths and an incidence of 280000 per year worldwide in 2008[11,12]. Europe and Northern America have the highest incidence of pancreatic cancer, with slightly more males being affected.

Pancreatic cancer is usually diagnosed at an advanced stage due to a lack of symptoms in the early stages so that resection of the advanced tumor is often not possible. The overall 1-year survival rate for pancreatic cancer is 26%, and the 5-year survival rate is approximately 6% for advanced cancer and 22% for early stages when surgical removal of the tumor is still possible[13].

Therefore, new therapeutic approaches combining neoadjuvant chemotherapy and radiotherapy to significantly reduce the tumor size are promising to allow the option of surgical removal in selected patients[14].

Morphological aspects under respect of the precursor lesions

Classical malignant pancreatic tumors show heterogeneous glandular and duct-like, grading-dependent structures, mostly infiltrating the pancreatic parenchyma and exhibiting a partially prominent desmoplastic stroma. The widely accepted and routinely used grading system of pancreatic cancer is based on (1) glandular differentiation; (2) mucin production; (3) mitosis (per 10 microscopic high power fields), and (4) nuclear features (such as nuclear polymorphism, size or arrangement of the nucleus)[15,16]. So far, no definitive and routinely used immunohistochemical markers exist, although many biological markers in pancreatic ductal adenocarcinoma were tested as possible diagnostic and prognostic tools. However, the main limitations arise from the small number of patients studied and in the heterogeneity of the applied methods[17]. Further approaches for prognostic grading focused on different morphological patterns similar to Gleason's scoring system[18], included epithelial-mesenchymal characteristics, such as vimentin expression and tumor budding[19,20], or evaluated several gene expression signatures including downregulation of ASPM (abnormal spindle-like microcephaly associated) which could be detected by immunohistochemistry[21].

Detailed morphological analysis revealed prognostic subtypes of pancreatic cancer, with a group associated with better survival (colloid and medullary) and a group with a worse outcome (adenosquamous or undifferentiated)[22] (as described in Table 1).

It is important not only to give the diagnosis "pancreatic cancer", but to discriminate between tumor entities for patient communication, and to evaluate a possible family history of cancer in genetic counselling (as in cases of medullary carcinoma[23]) as well as to establish tumor-specific therapy modalities, since it is possible to link tumor sub-entities to specific genetic lesions (Table 1).

Pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN) are considered precursor lesions[24] (Figure 1). As IPMN and MCN are seen pretherapeutically in radiological investigations, therapeutic strategies/algorithms were established to weigh up the extent of surgical resection and patients' quality of life.

Further molecular analyses of different precursor lesions and their morphological variants revealed a stepwise model of carcinogenesis and possible epigenetic associations (Figure 1). Interestingly, a huge number of epigenetically regulated genes have been detected by global gene expression profiles in comparison with classical genetic alterations showing an association with cytological and architectural atypia of these precursor lesions. The challenge in the future is to analyze the complex mechanistic crosstalk of these genetic and epigenetic regulatory mechanisms[25] during pancreatic carcinogenesis for new drug development and administration, as shown by Breitkreutz *et al*[26] using network statistics.

Molecular aspects of pancreatic cancer: Oncogenes and suppressor genes

Intensive DNA analysis using genome- and epigenome-wide screening methods during the last few years have

Table 1 Histomorphologic subtypes of pancreatic cancer related to their genetic/epigenetics and possible prognostic characteristics

Variant (source)	Morphology	Genetics/epigenetics	Prognosis
MC	Well defined pushing border, syncytial growth pattern, and poorly differentiated cancer cells	Germline or somatic mutations as well as epigenetic silencing by promoter methylation of mismatch repair genes *MLH1* and *MSH2*	Better MC: Overall 2- and 5-yr survival rate of 29% and 13%[23]
CC	Suspension of well-differentiated cancer cells in extracellular mucin pools (at least 80%)	Unknown	CC: 2-yr and 5-yr survival rate of 70% and 57%[153]
AC	Combination of glandular and squamous (at least 30%) components	*K-RAS2* mutations, inactivation of *CDKN2A/p16*, *SMAD4/DPC4* and *TP53*	Worse AC/UC: Median survival of 5 mo after resection[154,155]
UC	Noncohesive cancer, lacking histological features of differentiation	*K-RAS2* gene mutations, loss of E-cadherin protein expression (promoter hypermethylation) Expression of *L1CAM*, *COX2*, and *EGFR* Subtype with osteoclast-like giant cells shows mutations like noninvasive precursor lesions	

MC: Medullary carcinoma; CC: Colloid carcinoma; AC: Adenosquamous carcinoma; UC: Undifferentiated carcinoma; COX: Cyclooxygenase; L1CAM: L1 cell adhesion molecule; EGFR: Epidermal growth factor receptor; MSH2: MutS homolog 2.

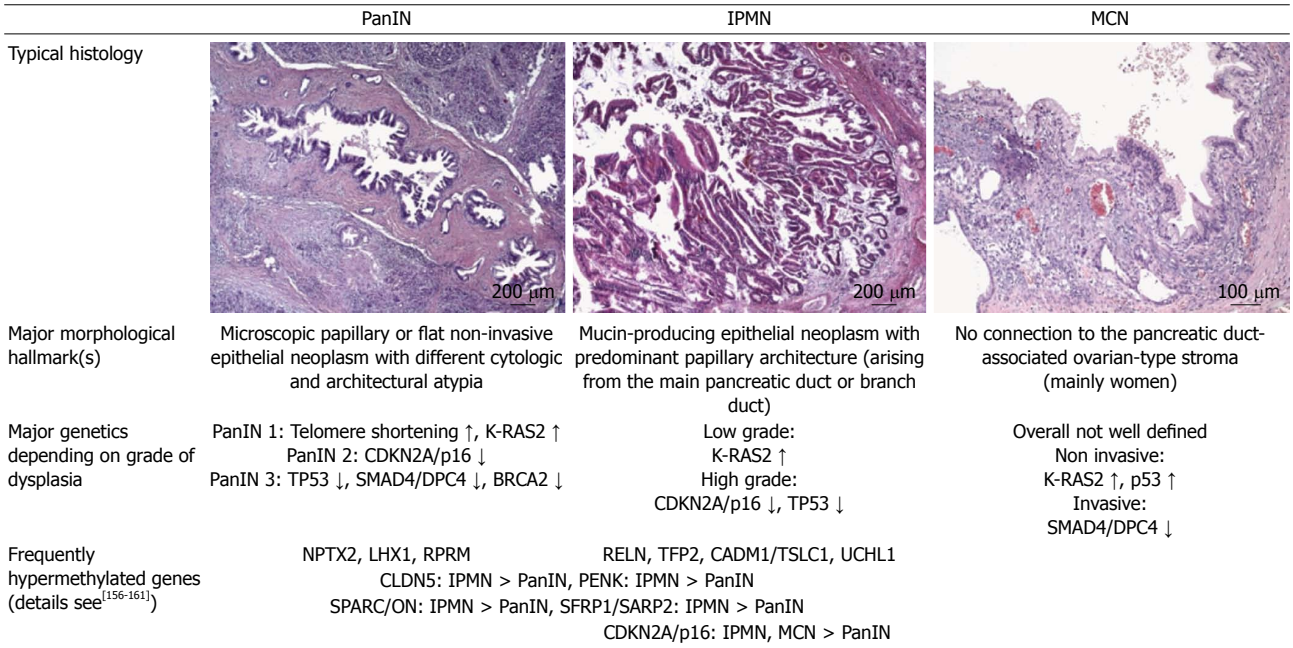

Figure 1 Precursor lesions of pancreatic cancer with their typical morphologic and genetic as well epigenetic characteristics. PanIN: Pancreatic intraepithelial lesions; IPMN: Intraductal papillary mucinous neoplasms; MCN: Mucinous cystic neoplasms; RELN: Reelin.

elucidated some major deregulated gate "drivers", "passengers" and "keepers" in pancreatic cancer (Table 2)[27-30]. Overall, more suppressor genes than oncogenes are involved in pancreatic cancer.

As recently described in depth by Hong *et al*[31], the most frequently mutated oncogene (> 95%) in pancreatic cancer is K-RAS (Kirsten rat sarcoma viral oncogene homolog), leading to constitutive downstream signaling of proliferation, cellular survival, motility and remodeling. On the other hand, the major deregulated suppressor genes in pancreatic cancer are *CDKN2A/p16* (cyclin-dependent kinase inhibitor 2A), *TP53* (tumor protein p53) and *SMAD4/DPC4* (SMAD family member 4) which are inactivated by 2 different, often independent, mechanisms. Whereas *CDKN2A/p16* and *TP53* are mainly involved in cell cycle checkpoint control and arrest, *SMAD4/DPC4* plays an important role in signal transduction of the transforming growth factor (TGF)-β pathway, and furthermore in cellular proliferation. Finally, when looking at epigenetically affected genes, it is obvious that the classical and most frequent pancreatic cancer genes are only partially epigenetically regulated (see Table 2). Interestingly, such cases with epigenetically silenced *MLH1* (mutL homolog 1) genes are associated with the distinctive medullary phenotype of pancreatic cancer[32-34].

Linking morphology and genetics to epigenetics in pancreatic cancer

As later described in detail (see Figure 1, Tables 3 and 4), epigenetics *via* DNA methylation, histone acetylation or

Table 2 Activated and deactivated key genes in pancreatic cancer according to hallmark of cancer, frequency as well as to kind of possible genetic and epigenetic alteration

Gene symbol (source)	Associated function according the hallmarks of cancer[1][35,36]	Frequency	Type of genetic alteration	Evidence for epigenetic regulation (reference)
Activation				
K-RAS2[131]	a, f, g	> 90	Point mutation	
AKT2[162,163]	a, j, g	10-20	Amplification	
BRAF[164]	a, b, g	5	Point mutation	
Deactivation				
CDKN2A/p16[165]	b, d, i	95	Homozygous deletion, intragenic mutation	Yes[166]
TP53[167-169]	b, d, i, h	50-70	Intragenic mutation an one allele and loss in the other allele	
SMAD4/DPC4[170,171]	b, c, f	55	Homozygous deletion, intragenic mutation	
MLH1[23,172]	h	3-15	Heterozygote mutations	Yes[34]
BRCA2[173]	a	7	Heterozygote mutations	
STK11/LKB1[174]	i	5	Homozygous deletion, intragenic mutation	
TGFBR2[175]	a, f	4	Homozygous deletion, homozygous frameshift mutation	
MAP2K4[176,177]	a	2	Homozygous deletion, missense mutation	

[1]Hallmarks of cancer: Sustaining proliferative signaling (a), evading growth suppressors (b), avoiding immune destruction (c), enabling replicative immortality (d), tumour promoting inflammation (e), activating invasion and metastasis (f), inducing angiogenesis (g), genome instability and mutations (h), resisting cell death (i), deregulating cellular energetics (j)[35,36].

Table 3 Overview of DNA hyper-/hypomethylation involved in pancreatic cancer

DNA modification	Material			Gene affected	Ref.
	Cancer samples	Cell lines	Other		
DNA hyper-methylation	√			*p16*	[166]
	√			*RASSF1A*	[178]
	√			*MDFI, hsa-miR-9-1, ZNF415, CNTNAP2, ELOVL4*	[179]
	√			*SOX15*	[180]
	√			HOP hoemobox (*HOPX*)	[181]
	√			*KLF10*	[182]
	√			*hMLH1*	[183]
	√			*miR-34a/b/c*	[184]
	√			*SPARC*	[185]
	√			*FoxE1, NPTX2, CLDN5, P16, TFPI-2, SPARC, ppENK*	[186]
	√			*SFRP*	[187]
	√	AsPC1, Hs766T, MiaPaCa2, Panc1		*UCHL1, NPTX2, SARP2, CLDN5, reprimo, LHX1, WNT7A, FOXE1, TJP2, CDH3, ST14*	[30,159]
	√	AsPC1, BxPC3, CFPAC1, Panc1		*NPTX2*	[188]
	√	Panc1, SW1990		*miR-132*	[121]
	√	BxPC3, Capan2, CFPAC1, HPACI, HPAFⅡ, MiaPaCa2, Panc1, PL45		*FOXA1/2*	[81]
		MiaPaCa2		*ARID1B*	[189][1]
		Panc1		*NPTX2*	[190][1]
		AsPC1, BxPC3, Panc1, MIA PaCa-2		*Dkk3*	[191]
		BXPC3, HPAFⅡ, HPAC, hTERT-HPDE, Panc1		*Cldn18*	[192]
		BxPC3, CFPAC1, Panc1, SW1990		*TNFRSF10C*	[193]
			Pancreatic juice	Neuronal pentraxin Ⅱ (*NPTX2*)	[194]
			Pancreatobiliary fluid	*UCHL1, RUNX3*	[195]
			PanIN	*p16*	[196]
			IPMNs	*BNIP3, PTCHD2, SOX17, NXPH1, EBF3, SPARC, SARP2, TSLC1, RELN, TFPI2, CLDN5, UCHL1*	[157,197]
			Blood, brush cytology	*NPTX2*	[198,199]
DNA hypo-methylation	√			*VAV1*	[79]
	√			Claudin4, lipocalin2, 14-3-3 sigma, trefoil factor 2, S100A4, mesothelin, prostate stem cell antigen	[78]
	√			*MUC4*	[77]
		SW1990		*ABCB1/MDR1, ABCC1/MRP1, ABCG2/BCRP*	[200]

[1]Only abstract available.

Table 4 Overview of miRNAs associated with specific targets/functions in pancreatic cancer

miRNA/function		Cell lines	Target gene(s)	Cellular effects	Ref.
Function as oncogene	-10a	AsPC1, Capan1, Capan2, MiaPaCa2, Panc1, Patu8988T, Patu8988S, Patu8902	*HOXB1, 3*	Metastasis ↑	[201]
	-21	AsPC1, BxPC3, Capan1, Capan2, CFPAC1, Hs776T, H48N, KP-1N, KP-2, KP-3, MiaPaCa2, NOR-P1, Panc1, SUIT-2, SW1990	*HOXA1*	Invasion ↑	[202]
		AsPC1, Capan1, Capan2, CFPAC1, H48N, HS766T, KP-1N, KP-2, KP-3, MiaPaCa2, NOR-P1, Panc1, SUIT-2, SW1990		Proliferation ↑, invasion ↑, chemoresistance ↑	[203]
		BxPC3		Proliferation ↑	[204]
		Capan1, HS766T, MiaPaCa2, MPanc96, Panc1, PL45, SW1990	*PTEN, RECK*	After miRNA inhibition: cell cycle arrest ↑, apoptosis ↑	[205]
	-132, -212	Panc1	*Rb1*	Proliferation ↑	[206]
	-155	Capan2, MiaPaCa2, MCF7, MEFs, 293T	*TP53INP1*	Apoptosis ↓	[207]
	-194, -200b, -200c, -429	AsPC1, A818, BxPC3, Capan1, Capan2, HPAFII, MiaPaCa2, MPanc96, Panc1, Patu8902, Patu8988T, Patu8988S, PT45, Suit 007, Su.86.86, Sut0028[1]	*EP300*	Metastasis ↑	[208]
	-197	AsPC1, Panc1	*p120 catenin*	EMT ↑	[209]
	-210	Panc1, MiaPaCa2, SUIT-2		Migration ↓, vimentin ↓, snai-1 ↓, membraneous β-catenin ↑	[210]
	-221	Capan1, HS766T, MiaPaCa2, MPanc96, Panc1, PL45, SW1990	*p27*	Chemosensitivity ↑	[205]
	-224, -486	AsPC1, A818, BxPC3, Capan1, Capan2, HPAFII, Su 86.86, MPanc96, MiaPaCa2, Panc1, Patu8902, Patu8988T, PT45, Patu8988S, Suit 007, Suit 0028[1]	*CD40*	Invasion ↑, metastasis ↑	[211]
Function as tumor suppressor	-301a	BxPC3, Hs766T	*Bim*	Proliferation ↑,	[212]
	-320c	AsPC1, Panc1[2]	*SMARCC1*	Chemoresistance ↑	[213]
	-421	SW1990, Panc1	*DPC4/Smad4*	Proliferation ↑, colony formation ↑	[214]
	-491-5p	AsPC1, Capan1, MiaPaCa2, SW1990	*Bcl-XL, TP53*	Proliferation ↓, apoptosis ↑, STAT3 ↓, PI-3K/Akt ↓	[215]
	let-7 let-7a	BxPC3, Capan1, Capan2, human HPNE (human pancreatic nestin-positive) cells MiaPaCa2, Panc1		Proliferation ↓, K-RAS ↓, MAPK ↓	[216]
		AsPC1	*RAS*	K-RAS ↓, radiosensitivity ↑	[217]
	-22	BxPC3	*SP1, ESR1*	Tumourigenesis ↓	[218]
	-26a	SW1990, Panc1	*HMGA1*	Proliferation ↓, invasion ↓, migration ↓, apoptosis ↑	[219]
	-34	BxPC3, MiaPaCa2	*Bcl-2, Notch-1/2*	Clonogenicity ↓, invasion ↓, apoptosis ↑, cell cycle arrest ↑, chemosensitivity ↑, radiosensitivity ↑, CSC ↓	[220]
	-34a	Panc1		Cell cycle arrest ↑, apoptosis ↑, migration ↓, E2F3 ↓, Bcl-2 ↓, c-myc ↓, cyclin D1 ↓	[221]
	-34b	AsPC1, MiaPaCa2	*Notch-1*	Proliferation, apoptosis	[222]
			Smad3	Progression *in vivo* ↑	[223][3]
	-107	MiaPaCa2, Panc1	*CDK6*	Proliferation ↓	[224]
	-126	AsPC1, BxPC3, KLM-1, MiaPaCa2, Panc1	*ADAM9*	Migration ↓, invasion ↓, E-cadherin ↑	[225]
	-132	BxPC3, HPAFII, HPAC, Panc1		Proliferation ↓, colony formation ↓, Akt ↓	[121]
	-143	AsPC1, BxPC3, Capan2, HPAFII, MiaPaCa2, Panc1	*COX-2*	Proliferation ↓, MEK/MAPK ↓	[226]
		Panc1	*ARHGEF1* (*GEF1*), *ARHGEF2* (*GEF2*), *K-RAS*	Migration ↓, invasion ↓, metastasis ↓, E-cadherin ↑	[227]
	-148a	IMIM-PC2	*CDC25B*	Proliferation ↓, colony formation ↓	[228]
	-148b	AsPC1, BxPC3, MiaPaCa2, Panc1, SW1990	*AMPKα1*	Proliferation ↓, apoptosis ↑, cell cycle arrest ↑, invasion ↓, chemosensitivity ↑, tumourigenicity ↓	[229]
	-150	Colo357, HPAF, Panc10.05	*MUC4*	Proliferation ↓, clonogenicity ↓, migration ↓, invasion ↓, cellular adhesion ↑	[230]

-200	AsPC1, BxPC3, Colo357, HPAC, MiaPaCa2, L3.6pl, Panc1[2]	EMT ↓ (ZEB1 ↓, slug ↓, vimentin ↓)	[231]
-375		Proliferation ↓, cell cycle arrest ↑, apoptosis ↑	[232][3]
-548d	Panc1	Proliferation ↓, apoptosis ↑, cell cycle arrest ↑	[233]

[1]Including an orthotopic murine model; [2]Gemcitabine-resistant cell line; [3]Only abstract available. Based on and updated from Park *et al*[120] 2011.

Table 5 Overview of Epigenetic mechanisms - see text for details and references

Mechanism	Enzyme	Subclasses/components	Effect on target gene expression
DNA (de-) methylation	DNMT	DNMT1 (methylation maintenance) DNMT3A and -3B (*de novo* methylation)	↓
	DNA de-methylase	Not known	↑
Histone (de-) acetylation	HAT	*e.g.*, CBP, p300	↑
Histone methylation	HDAC	Class Ⅰ (HDACs-1-3, -8), class Ⅱa (HDACs-4, -5, -7, -9), class Ⅱb (HDACs-6, -10), class Ⅲ (SIRT1-7), class Ⅳ (HDAC-11)	↑
	PcG→ H3-K27-me3	PRC1: CBX-2, 4, or 9, PHC-1, 2, or 3, BMI1, RING1A/B or RNF2 → H3-K27-me3 maintenance PRC2: EZH2, SUZ12, EED → *de novo* H3-K27-me3 maintenance	↓
	TrxG → H3-K4-me3	Several members	↑
Post-transcriptional	miRNAs	2578 mature miRNA (miRBase v20)	↓

HAT: Histone acetylase; HDAC: Histone deactylase; DNMT: DNA methyltransferase.

interacting regulative microRNAs (miRNAs) could essentially be linked to different morphological and genetic changes during pancreatic carcinogenesis. Extensive investigations are been carried out on epigenetic changes in pancreatic cancer precursor lesions, indicating that heterogeneous, non-linked pathways of carcinogenesis are regulated by epigenetics as summarized in Figure 1 and Table 3. Detailed analysis of the function of these epigenetically deregulated genes revealed that all hallmarks of cancer[35,36] such as self-sufficiency in growth signals (*e.g.*, SFRP1/SARP2; secreted frizzled-related protein 1), insensitivity to anti-growth signals [*e.g.*, CDKN2A/p16 or RPRM (reprimo)], tissue invasion and metastasis [*e.g.*, SPARC/ON (secreted protein, acidic, cysteine-rich (osteonectin)], limitless replicative potential [*e.g.*, LHX1 (LIM homeobox 1)], sustained angiogenesis [*e.g.*, CLDN5 (Claudin-5)] or evading apoptosis [*e.g.*, RPRM or CADM1/TSLC1 (cell adhesion molecule 1/tumor suppressor in lung cancer 1)] are involved in pancreatic cancer and affected by epigenetic (de)regulation. This supports our knowledge of the pleiotropic effects of systemic epigenetic mechanisms. The degree of cytological and architectural atypia correlated with the amount of methylated genes supporting the hypothesized multiple step model of pancreatic cancer even in the early disease stages[31,37].

Additionally, hypomethylation is also recognized in pancreatic cancer, leading to genomic instability by overexpression of genes and proteins in contrast to hypermethylation by silencing genes and subsequent protein expression. Serial genes like *SERPINB5* (serpin peptidase inhibitor, clade B, member 5), *CLDN4*, stratifin, lipocalin-2, trefoil factor2, *S100P* (S100 calcium-binding protein P), mesothelin or prostate stem cell antigen are hypomethylated which causes uncontrolled or "dys"-regulated cell cycle progression, proliferation, differentiation or adhesion[31].

EPIGENETICS OF PANCREATIC CANCER

Overview of epigenetic mechanisms

The identification of DNA methylation, histone modification and the action of miRNAs has profoundly increased the knowledge about the regulation of gene activity. Epigenetics studies the stable and inheritable patterns of altered gene expression independent of the primary DNA sequence[38], and has shown that dynamic traits of chromatin, reversible covalent modification of DNA, and post-transcriptional regulation centrally impact on gene expression and phenotypic characteristics[8,39]. With increasing evidence that tumorigenesis-associated cellular changes are caused by epigenetic alterations, the field of cancer research has evolved to incorporate oncogenic mechanisms beyond DNA mutations. Epigenetic mechanisms (see Table 5 for an overview about the major epigenetic mechanisms) are generally reversible. Together with the fact that epigenetic alterations may be even more prevalent than genetic aberrations, this is highly attractive in the conceptual approach of selecting and exploiting potential molecular targets for novel cancer therapeutics[8,40].

The methylation of DNA and subsequent silencing of a gene is catalyzed by DNA methyltransferases (DNMTs) which add a methyl group to the 5' carbon of the cytosine pyrimidine ring. This occurs preferably in regions containing cytosine-guanine dinucleotides (CpGs); these CpG islands are preferentially located in regions corresponding to regulatory regions of many genes[41].

While DNMT1 is responsible for maintenance of parental DNA methylation patterns following replication, *de novo* DNA methylation is catalyzed by DNMT3A and DNMT3B enzymes[42]. The identification of DNA demethylases which remove the methyl group and reverse the action of DNMTs still warrants further research. DNA methylation was the first type of epigenetic alteration identified as responsible for inactivation of a tumor suppressor gene[43], and it is suggested that 100-400 hypermethylated CpG islands may exist in a given tumor[44].

Compared with DNA-based epigenetics, alterations in DNA-associated histones offer a greater variety of covalent epigenetic modifications, including phosphorylation, methylation, acetylation, ubiquitination and sumoylation, all with different degrees of modification (*e.g.*, mono-, di-, and trimethylation)[45,46]. The nucleosome as the core building block of eukaryotic chromatin comprises histone octamers (dimers of H2A, H2B, H3 and H4, *i.e.*, the nucleosome core particle, NCP) and about 146 base pairs of DNA[47]. Modifications to histones determine the packing of DNA into either tight and transcriptionally silent heterochromatin or transcriptionally active and open-structured euchromatin. These modifications affecting chromatin mobility and stability have been termed "marks" whose type, position, and combinations determine whether a given gene is expressed or silenced, *i.e.*, the histone code[46,48]. This hypothesis postulates that local histone modifications determine the configuration of chromatin, alter the binding affinities of non-histone proteins, and subsequently influence the chromatin structure and accessibility of DNA for transcription[39,49]. Histone acetylation is thought to activate gene transcription and is catalyzed by histone acetyltransferases (HATs) which transfer an acetyl group from acetyl coenzyme A to the ε-amino group of lysine such as CREBBP (cAMP response element-binding protein binding protein), p300 and p300-CBP-associated factor (P/CAF). The reverse reaction is mediated by histone deacetylases (HDACs) which comprise a group of 18 isoenzymes identified to date. HDACs are classified into four groups (I -IV) based on their homology to yeast HDACs as summarized in Table 5[50,51].

The polycomb group proteins (PcG) repress gene activity by trimethylation of H3-K27 (histone 3 lysine 27) while TrxG proteins (Trithorax group) activate gene expression *via* H3-K4 histone trimethylation. The PcGs have 2 functions: polycomb repressive complex 1 (PRC1) maintains the silenced (H3-K27-me3, trimethylated) chromatin state and consists of CBX-2, 4, or 8 (chromobox homologue 2/4/8), PHC-1, 2, or 3 (polyhomeotic homologue 1/2/3), BMI1 (B-cell-specific Moloney murine leukemia virus integration site 1), and RING1A/B or RNF2 (RING finger domain protein). PRC2 initiates the repressive state by *de novo* trimethylation of H3-K27 and consists of EZH2 (enhancer of zeste homologue 2), SUZ12 (suppressor of zeste 12) and EED (embryonic ectoderm development)[39]. Together with other chromatin-modifying enzymes including DNMTs and HDACs, the initially constituted suppression *via* H3K27-3me by PRC2 is maintained by PRC1 and allows fine-tuned, context-dependent regulation of gene silencing.

miRNAs are short (18-25 nucleotides), phylogenetically conserved single-stranded RNA molecules without protein-coding functions involved in the silencing of messenger RNAs (mRNAs)[52,53]. This post-transcriptional repression is accomplished by (partial) base-pairing with the respective mRNA causing either (1) inhibition of translation initiation; (2) inhibition of translation elongation; or (3) mRNA decay initiated by deadenylation of the mRNA following recruitment of a deadenylase complex[54,55]. The functions added a new layer of regulatory mechanisms affecting virtually all cellular functions[56,57]. The interaction between miRNAs and their target mRNAs ultimately leads to reduced levels of the regulated mRNA/protein. The current release of miRBase (v20[58]) lists 2578 mature miRNA sequences and it is estimated that over 60% of human mRNAs are direct miRNA targets[59]. miRNAs can function as either suppressors or oncogenes and their regulatory importance in human tumorigenesis has been demonstrated for various cancer types[60,61]. While miRNAs are *per se* categorized as an epigenetic mechanism, their cancer-related expression itself may be subject to epigenetic regulation by the above-mentioned mechanisms of chromatin modulation[62,63].

In the following paragraphs, we focus on their role in tumorigenesis in pancreatic cancer including their potential therapeutic exploitation by "epidrugs". For a general overview on cancer-related epigenetic mechanisms, we kindly refer the reader to recent comprehensive reviews: DNA methylation[64-66], histone (de)acetylation[67-70], histone methylation[71-73], and miRNAs[74-76].

Epigenetic mechanisms

DNA methylation in pancreatic cancer: As discussed in McCleary-Wheeler *et al*[8] DNA methylation may occur early during tumorigenesis of pancreatic cancer as some epigenetic alterations are already observed in the earliest lesion, *i.e.*, PanIN-1A, and their importance may increase during further tumor progression[30]. In line with these findings, mucin 4 (MUC4) gene expression is increased from normal to precancerous lesions to pancreatic cancer associated with an increasing frequency of MUC4 promoter hypomethylation[77]. Furthermore, not only DNA methylation but also hypomethylation and thus aberrantly high expression of a gene may represent an epigenetic feature in pancreatic carcinoma[78,79]. Table 3 summarizes the currently available literature on (deregulated) DNA methylation in pancreatic cancer.

Aberrant activation of developmental signalling pathways such as Hedgehog represents a frequently observed trait in human cancers[9]. He *et al*[80] have shown that the Hedgehog transcription factor Gli1 (GLI family zinc finger 1) targets epigenetic modifiers in pancreatic cancer, namely DNMT1 and DNMT3a. After showing a concomitantly higher expression of Gli1 and the DN-

MTs in pancreatic tumor samples, the authors proved by pharmacological inhibition using cyclopamine, Gli1 overexpression and siRNA (small interfering RNA)-based Gli1 knockdown, that the DNMT proteins are positive targets of this oncogenic pathway, suggesting a cross-talk between aberrantly activated embryogenesis pathways and activation of oncogenic epigenetic mechanisms in pancreatic cancer.

Related to the important role of epithelial-mesenchymal transition (EMT) in pancreatic tumor progression, FOXA1 and FOXA2 (forkhead box A1/2) transcriptions factor were identified as effective antagonists of EMT in pancreatic cancer due to their ability to positively regulate E-cadherin expression: in a study by Song *et al*[81], FOXA1/2 expression was lost during malignant progression and their promoter was extensively hypermethylated in a pancreatic cancer cell line. As the demethylation-mediated reactivation of E-cadherin required concomitant FOXA2 expression, the authors concluded that suppression of FOXA1/2 is necessary and sufficient for EMT in the progression of pancreatic cancer[81].

From the data summarized in Table 3 and the mentioned examples, it is clear that numerous genes and signaling targets are epigenetically regulated by means of DNA methylation in pancreatic cancer. Importantly, not only does late-stage, malignant pancreatic cancer display aberrant DNA methylation, but also earlier, pre-malignant lesions. It is important to note that DNA methylation may act in cooperation with other epigenetic mechanisms (histone modification) to achieve stable silencing of, for example, individual tumor suppressor genes. In a series of studies, Yamada *et al*[82-84] have shown that different mucin variants are regulated by different and complementary epigenetic mechanisms: MUC1, MUC2 and MUC5AC by DNA methylation and H3-K9 histone methylation. This fact has to be considered in therapeutic approaches which aim at reversing deregulated DNA methylation patterns, for example.

Histone-based epigenetics in pancreatic cancer: Acetylation and methylation of histones represent the 2 epigenetic mechanisms based on histone modifications for which currently data exist in the context of pancreatic tumorigenesis. Several studies have demonstrated the general relevance of HDAC enzymes in pancreatic cancer by showing that (1) HDAC2 is increased in pancreatic ductal adenocarcinoma, especially in poorly differentiated tumors[85]; and (2) the expression of HDAC7 is significantly increased in pancreatic adenocarcinoma samples; HDAC7 expression furthermore allows for discrimination between pancreatic adenocarcinoma from other pancreatic tumors (serous cystadenoma, IMPN)[86]. The potential of therapeutic targeting of HDACs in pancreatic cancers has been reviewed by Schneider *et al*[87]. As discussed in this section, several studies have unequivocally demonstrated the relevance of histone-based epigenetic mechanisms including the (oncogenic) functions of PRC1/2 protein complexes to contribute to pancreatic tumorigenesis. These studies provided evidence of either altered/aberrant expression of these epigenetic regulators in pancreatic cancer samples or demonstrated the potential therapeutic exploitation of these mechanisms using cell-based *in vitro* studies.

An interesting cooperation between the ZEB1 (zinc finger E-box binding homeobox) transcription factor which is responsible for silencing of the E-cadherin gene (*CDH1*) and HDAC enzymes was investigated by Aghdassi *et al*[88]. In 25 surgical pancreatic cancer specimens, the authors found neither hypermethylation nor somatic mutations in the *CDH1* gene, but complexes of ZEB1/HDAC attached to the *CDH1* promoter. Knockdown of ZEB1 prevented this interaction resulting in histone acetylation and re-expression of E-cadherin. This study has provided additional insights into how EMT transcription factors cooperate with HDACs to silence E-cadherin, and thus promote EMT and tumor progression[89]. These data confirmed earlier results which demonstrated that downregulation of E-cadherin in metastatic pancreatic cancer cells is mediated by a repressor complex containing the EMT transcription factor Snail and the HDAC1 and HDAC2 enzymes[90].

The particular role of PRC proteins as epigenetic mechanisms has only recently become a focus in pancreatic cancer research. In several tumor types, overexpression of the *EZH2* gene is associated with poor prognosis, advanced stage, invasion and metastasis - for an overview see Crea *et al*[91]. As reviewed in Grzenda *et al*[39], *EZH2* overexpression promoted survival, angiogenesis, migration, proliferation and repression of E-cadherin. For pancreatic cancer, several studies demonstrated an involvement of components of PRC1/2 in malignant progression: Martínez-Romero *et al*[92] found the expression of the PRC1 proteins Bmi1 and Ring1b to be upregulated in PanIN lesions (Bmi1) and pancreatic adenocarcinoma (both components). These finding were confirmed by Song *et al*[93] showing that Bmi1 is correlated with lymph node metastasis and poor survival rates; furthermore, stable knockdown of Bmi1 reduced the levels of cyclin D1, CDK-2/4 (cyclin-dependent kinase), Bcl-2 (B-cell CLL/lymphoma 2) and phosphor-Akt while the expression of p21 and Bax was increased and associated with higher susceptibility towards apoptosis induction. Moreover, Wellner *et al*[94] showed that Bmi1 and other "stemness" factors were negative targets of the epithelial differentiation-linked miR-200c, -203 and -183 suggesting a possible mechanism for Bmi1 deregulation in pancreatic cancer. Another PRC1 member was investigated by Karamitopoulou *et al*[95]: CBX7 was analyzed in 210 ductal pancreatic adenocarcinomas and its expression was found to progressively decrease from normal pancreatic tissue, PanINs and invasive ductal adenocarcinoma-associated with increasing malignancy and a trend to shorter overall survival.

Using immunohistochemistry, Ougolkov *et al*[96] could show nuclear overexpression of EZH2 in pancreatic cancer cell lines and in 68% (71/104) of pancreatic ad-

enocarcinomas and that its nuclear accumulation was more prevalent in poorly differentiated pancreatic adenocarcinomas (91%, 31/34). Using RNA interference, genetic depletion of EZH2 sensitized pancreatic cancer cells to chemotherapy and caused re-expression of p27 [cyclin-dependent kinase inhibitor 1B (p27, Kip1)] and decreased proliferation. Similar results were obtained by Toll *et al*[97] who demonstrated an inverse relationship between expression of EZH2 and E-cadherin in 54 pancreatic adenocarcinomas, and significantly longer survival in gemcitabine-treated patients with low expression of *EZH2*. Interestingly, inactivation of RUNX3 (runt-related transcription factor 3), a component of TGF-β signalling, is mediated by at least 2 epigenetic mechanisms, both EZH2[98] and DNA hypermethylation[99], highlighting their cooperation to convey a malignant phenotype in pancreatic cancer. Recently, miR-218 was demonstrated to be negatively regulated by EZH2 in pancreatic ductal adenocarcinoma[100]. MiR-218 was significantly reduced in primary tumor samples compared with normal adjacent tissue, and its silencing was mediated by EZH2 which binds to the miR-218 promoter, promotes formation of heterochromatin, and recruits DNMT-1, -3A and -3B. MiR-218 expression reduces proliferation *in vitro* and tumor formation as well as metastasis in nude mice[100]. The inverse regulatory relationship has also been recently observed between EZH2 and pre-miR101. Overexpression of pre-miR-101 reduced the binding of EZH2 to the promoter of the E-cadherin gene (*CDH1*) and increased the levels of E-cadherin[101].

Epigenetics in pancreatic cancer stem cells: An interesting and possibly therapeutically relevant aspect is the role of EZH2 in maintenance of stemness characteristics in cancer, particular its role in maintaining the self-renewal capabilities of cancer stem cells (CSC)[102-108]. This subpopulation of cancer cells has been characterized in pancreatic cancer by surface markers CD44, CD24, CD133, ESA (EpCAM, epithelial cell adhesion molecule)[109,110] and is thought to represent the population of cancer cells responsible for tumor maintenance, tumorigenicity, metastasis, and resistance to conventional chemotherapeutic drugs, as well as recurrence[109,111]. Similar to studies with CSC derived from hepatocellular carcinoma and acute myeloid leukemia[104,112], recent studies have demonstrated the therapeutic potential of epidrugs to directly target this tumorigenic subpopulation of pancreatic cancer cells: Avan *et al*[102] used an inhibitor of the EZH2 methyltransferase (DZNep, deazaneplanocin-A) and showed that treatment with DZNep reduced spheroid formation of pancreatic cancer cells and decreased the $CD133^+$ subpopulation. Furthermore, the combination of DZNep and gemcitabine was shown to be highly synergistic and was accompanied by a reduced percentage of G2/M cells, reduced migration, increased E-cadherin expression and increased apoptosis[102]. The level of EZH2 has furthermore been suggested as an assay to effectively measure changes in the CSC subpopulation: using pancreatic and breast cancer cell lines, knockdown of EZH2 by RNA interference decreased the CSC subpopulation, confirming its role in CSC maintenance, and genes affected by EZH2 knockdown were inversely correlated with their expression in enriched CSC subpopulations[108]. The Hedgehog pathway has also been implicated in the maintenance of CSCs in various models[9,113]; interestingly a combination of Hedgehog inhibition (SANT-1) and SAHA (a pan-HDAC inhibitor; suberanilohydroxamic acid, Vorinostat) synergistically suppressed proliferation and colony formation in gemcitabine-resistant pancreatic adenocarcinoma cell lines by increased Bax expression, activation of caspase-3/7, increased p21 and p27 and reduced cyclin D1 expression. This study suggests that combined inhibition of stem cell-associated pathways (Hedgehog) and epigenetic drugs could be efficient in targeting the CSC subpopulation in pancreatic cancer[114]. A study by Nalls *et al*[115] could demonstrate that demethylating agents (5-aza-dC, 5-aza-2'-deoxycytidine) and the HDAC inhibitor SAHA restored the expression levels of miR-34a, which is reduced in pancreatic CSCs. These inhibitors caused a reduction in the EMT-related ZEB1, Snail, and Slug transcription factors, increased epithelial marker expression (E-cadherin) and, most importantly, reduced the number of viable pancreatic CSC, accompanied by reduced migration, colony formation and invasion of these cells.

Based on the above-mentioned functions and properties of CSC which is critical for tumor initiation, metastasis, progression and therapeutic resistance, these findings are of central importance and warrant further investigation to hopefully develop (epigenetics-based) therapeutic regimens specifically targeting this tumorigenic subpopulation in pancreatic cancer.

miRNA-based epigenetics in pancreatic cancer: Some reporters[116-119] and Park *et al*[120] have reviewed the available publications on differential miRNA expression in pancreatic cancer *vs* normal tissue culminating in a list of 64, partly overlapping individual miRNAs which were found to be deregulated in pancreatic cancer. Of these miRNAs, overexpression of miR-21, -155, -196a-2, -203, -210 and -222 was furthermore associated with poor outcome[120].

Table 4 provides an update (based on Park *et al*[120] 2011) of the currently available literature on the specific role of individual miRNAs in pancreatic cancer. All of these studies investigated the cellular/molecular mechanisms of the oncogenic or tumor-suppressive action of miRNAs, mainly by forced overexpression or knockdown of the respective miRNAs.

An example of how epigenetic mechanisms are employed to regulate the expression of tumor-suppressive miRNAs is shown in the study of Zhang *et al*[121]. From 12 miRNAs differentially expressed in pancreatic cancers *vs* adjacent normal tissue, miR-132 was downregulated in 16/20 pancreatic carcinomas accompanied by methylation of its promoter, as shown both in cell lines and tumor tissue. Sp-1 expression was correlated with miR-132

expression, and its binding affinity to the miR-132 promoter was significantly lower in pancreatic tumors relative to non-tumor samples.

As recently discussed[120,122], epigenetic features and especially miRNAs could also serve as biomarkers to allow specific and sensitive diagnosis of pancreatic cancer - an important approach as most patients with this disease remain without symptoms until the lesion has progressed to an advanced or metastatic stage. In this context, the use of miR-155 which showed upregulation in most IPMNs (83% of cases) has been analyzed in pancreatic juice by Habbe *et al*[123]. The authors confirmed upregulation of the miR-155 transcript in 60% (6/10) of IPMN-associated pancreatic juice samples but in none of the 5 control cases. Wang *et al*[124] profiled 4 miRNAs (miR-21, -210, -155, and -196a) in heparin-treated blood samples and found a sensitivity of 64% and a specificity of 89% to distinguish pancreatic cancer patients from healthy controls using this panel of miRNAs, thus proving the feasibility of plasma-based miRNA profiling as a potential biomarker for pancreatic cancer. Furthermore, Kawaguchi *et al*[125] investigated the utility of plasma miR-221 as a biomarker for cancer detection and monitoring tumor dynamics in 47 consecutive pancreatic cancer patients: similar to cancer tissue, plasma miR-221 levels were significantly higher in pancreatic cancer patients and correlated with distant metastasis and non-resectable status. Also, miR-21 serum levels were shown to be associated with overall survival of pancreatic cancer patients, and, in combination with 6 other miRNAs, allowed for correct classification of clinically suspected pancreatic cancer with a rate of 84%[126]. Similar results were obtained for miR-18a in plasma samples of patients with pancreatic cancer: miR-18a levels were significantly higher in 36 cancer patients compared with 30 healthy volunteers[127]. Kong *et al*[128] investigated the utility of several miRNAs as serum markers: while miR-21 distinguished pancreatic ductal adenocarcinoma patients from chronic pancreatitis and controls, miR-196a could distinguish resectable (stages Ⅰ and Ⅱ) and unresectable pancreatic ductal adenocarcinoma (Ⅲ and Ⅳ) as well as predict median survival time of pancreatic ductal adenocarcinoma patients (6.1 mo *vs* 12.0 mo for high *vs* low level miR-196a). Recently, miR-21 from pancreatic cyst fluid was investigated as a potential biomarker and could differentiate between benign, premalignant and malignant pancreatic cyst neoplasms[129].

POTENTIAL TARGETS FOR EPIGENETIC THERAPY IN PANCREATIC CANCER

The classic cancer progression model from PanIN to invasive carcinoma highlights genetic alterations in several oncogenes and tumor suppressor genes[130]. Hanahan *et al*[35,36] characterized additional distinct features of malignant tumor cells in their outstanding reviews on hallmarks of cancer that have also been identified in pancreatic cancer: sustaining proliferative signaling (*e.g.*, activating mutations of K-ras[131]), evading growth suppressors (*e.g.*, deletions or mutations of *CDKN2A/p16*^{Ink4A}[132]), activating invasion and metastasis (*e.g.*, expression of CXCL12/CXCR4 [chemokine (C-X-C motif) ligand 12/(CXC receptor 4)[133]], enabling replicative immortality (*e.g.*, telomerase activation *via* loss of ATRX in pancreatic neuroendocrine tumors[134]), inducing angiogenesis [*e.g.*, increase in serum vascular endothelial growth factor (VEGF)[135]] and resisting cell death (*e.g.*, overexpression of anti-apoptotic Bcl-2[136]. Many of these alterations have been explored as targets for novel therapies (*e.g.*, anti-angiogenesis using the anti-VEGF antibody bevacizumab or anti-epidermal growth factor receptor directed therapies using erlotinib or cetuximab) achieved only marginal survival benefits in pancreatic cancer patients compared with standard therapy[137-139]. As outlined above, recent data also suggest strong roles for non-genetic events in pancreatic carcinogenesis and resistance to current therapies[8], *e.g.*, by modulating ABC drug transporters or interfering with cell death pathways (see Tables 2-4 for details).

Consequently, these regulatory mechanisms could represent interesting and potent novel targets for therapy to overcome resistance and to improve treatment outcome further[140,141]. Inhibitors of DNMT are nucleoside analogues of cytidine and currently azacytidine and decitabine are available for clinical use (Table 6), although the number of current trials is very limited. Zebularine is in preclinical development[142] with promising experimental data in pancreatic cancer[143].

Inhibitors of protein and histone deacetylases have been established as a novel approach to target hematologic and solid tumors[144]. Several phase I studies using the first-in-class molecule vorinostat (SAHA) are currently ongoing, especially in combination with cytotoxic agents or radiotherapy. Other agents like belinostat (PXD-101), entinostat (MS-275) or panobinostat (LBH-589) are at various stages of early clinical development, too, with progression-free survival or maximum tolerated dose as study endpoints. As described above, in addition to deacetylases, HATs can also regulate gene transcription. Here, curcumin (derived from the South Asian plant turmeric) has been demonstrated to effectively inhibit the activity of the HAT p300/CBP in cancer cells[145,146]. Although its pharmacokinetic properties are unsatisfying so far, it demonstrated early signs of clinical efficacy in pancreatic cancer patients in a phase Ⅱ setting[147].

Other epigenetic modifiers besides DNMT, HAT or HDAC have been identified and the first lead compounds are currently being extensively studied preclinically or are in early clinical phases. However, clinical data for pancreatic cancer is not available[148].

While miRNAs are considered useful tools for diagnosis, prognosis and possibly patient stratification[149], miRNA-based therapeutics are currently not available. Although preclinical data suggests that antagomiRs or miRNA replacement therapy is promising for pancreatic cancer models, clinical use is hampered by unresolved

Table 6 Trials using epigenetic agents in pancreatic cancer

Compound	Combination	Phase	Endpoint	ClincialTrials.gov	Treatment
DNMT inhibitors					
Azacitidine	+ Gemcitabine	I	MTD	NCT01167816	
Azacitidine		II	PFS	NCT01845805	2
Decitabine			Various stages of development for solid tumors		3
Zebularine			Preclinical		
HDAC inhibitors					
Vorinostat	+ Marizomib	I	MTD	NCT00667082[234]	
Vorinostat	+ Radiation + 5-FU	I / II	MTD, PFS	NCT00948688	
Vorinostat	+ Radiation + Capecitabine	I	MTD	NCT00983268	
Vorinostat	+ Radiation	I / II	MTD	NCT00831493	
Belinostat			Various stages of development for solid tumors		
Entinostat		I	MTD	NCT00020579[235]	
Entinostat	13-*cis* retinoic acid	I	MTD		
Panobinostat	+ Bortezomib	II	PFS	NCT01056601[236]	1
HAT inhibitors					
Curcumin		II	Survival	NCT00094445[147]	1
Curcumin	+ Gemcitabine	II	TTP	NCT00192842	1
Curcumin	+ Gemcitabine + Celecoxib	III		NCT00486460	1
Curcumin		I	MTD	-[237]	
Curcumin	+ Gemcitabine	I	MTD	-[238]	
Curcumin	+ Gemcitabine	I / II	MTD	-[239]	

1: Palliative; 2: Postoperative adjuvant; 3: Both. MTD: Maximum tolerated dose; PFS: Progression-free survival; HDAC: Histone deacetylase; HAT: Histone acetyltransferase.

drug delivery and the fact that one miRNA also has several target mRNAs, thus possibly being too unspecific[150,151].

Overall, as most of the agents highlighted above are currently in early phases of clinical development, no clear data on efficacy of epigenetic agents in pancreatic cancer are available, but promising preclinical[152] and early clinical data warrant further development.

CONCLUSION

Due to the poor prognosis of pancreatic cancer patients, understanding the molecular events driving this devastating tumor disease is central for development of alternative and more effective treatment strategies and for the determination of reliable diagnostic markers. Recent research on epigenetic mechanisms has greatly enriched our knowledge about the regulatory traits involved in initiation, progression and metastasis of pancreatic cancer. As reviewed in this article, DNA-, histone- and miRNA-based epigenetic events have been demonstrated to play a role in pancreatic cancer and could serve as future therapeutic targets aiming at reversing the epigenetic deregulation of the cellular machinery. Initial clinical trials at stages I-III using inhibitors of DNMTs, HDACs and HATs are currently under way and open the door to development of novel and hopefully more effective 'epi-drugs' for patients with pancreatic cancer.

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