We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Epigenetics: Dissecting Gene Expression Alteration in PDAC

Alia Abukiwan and Martin R. Berger

Abstract

Pancreatic cancer is the fourth leading cause of cancer deaths, with a low 5-year survival rate of about 7% due to its highly invasive nature. Pancreatic ductal adenocarcinoma (PDAC) comprises more than 90% of all pancreatic cancer cases. At the time of detection, around 80% of cases harbor metastases due to the lack of early diagnosis. For decades, scientists have primarily focused on dissecting the origin of pancreatic cancer through genetic alterations and their contribution to diagnosis. Recently, PDAC research has turned into epigenetics to revolutionize our understanding about the silencing of critical regulatory genes. Epigenetic events can be divided mechanistically into various components, including DNA methylation, histone posttranslational modification, nucleosome remodeling, and regulation of transcription or translation by microRNA. The identified epigenetic processes in PDAC contribute to its specific epigenotype and are correlated phenotypic features. Strikingly, some of them have been suggested to have potential as cancer biomarkers, for disease monitoring, prognosis, and risk validation. As epigenetic aberrations are reversible, their correction will become as a promising therapeutic target.

Keywords: PDAC, epigenetics, DNA methylation, histone modification, microRNA, 3' UTR

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) comprises more than 90% of all pancreatic cancer cases. It is highly aggressive, extremely lethal and shows resistance to chemotherapy [1–3]. At diagnosis, around 80% of PDAC cases have already metastasized, thus rendering the current therapeutic options practically ineffective. In line with this, potentially curative surgical resection is limited to a very small portion of patients [4].

On the other hand, cancer metastasis is associated not only with simple gene/protein expression models but also with the existence of epigenetic mechanisms [5], which complicates this process through DNA methylation, histone modifications, and microRNA regulation (see **Figure 1**). Recent studies uncovered the regulatory mechanisms of each process and their key role in EMT and cancer metastasis [6].

2. PDAC from genetics to epigenetics

Historically, the development of PDAC was attributed to DNA mutations, which are classified into three main types: oncogenes (KRAS, BRAF, AKT2, MYB, and AIBI),

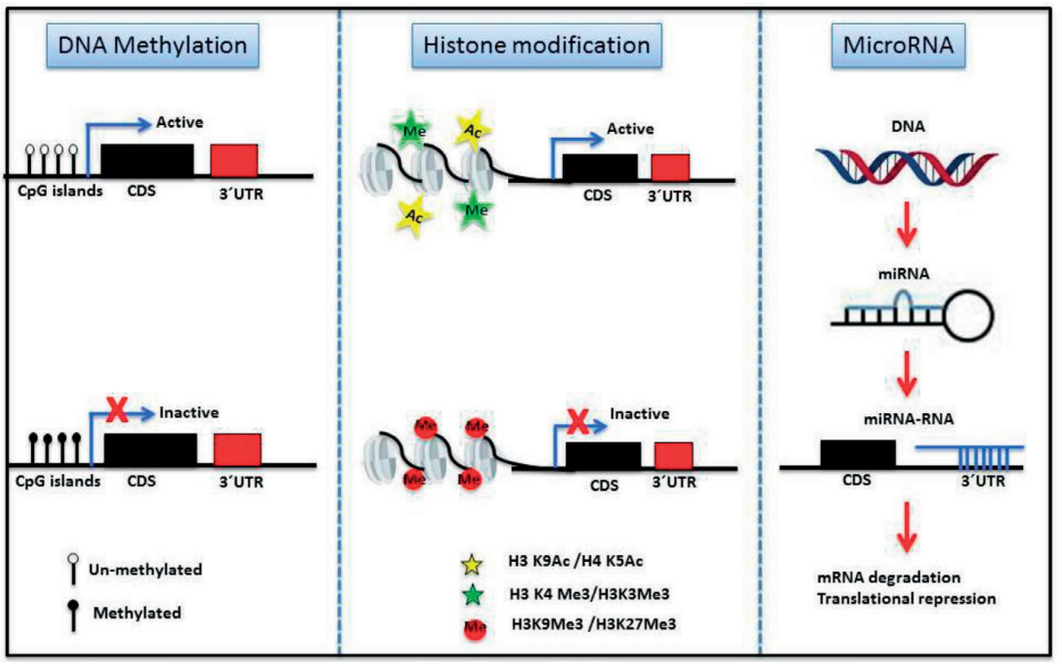

Figure 1.
Schematic diagram of epigenetic mechanisms influencing gene expression. DNA methylation is an epigenetic mechanism through which cytosine residues within CpG regions are covalently modified (left). In addition, the modification of histones has two consequences on genes, which pending on the type of modification and the target residues can either activate or repress the target gene (middle). The epigenetic mechanism is also influenced by microRNAs (miRNAs). These are small noncoding RNAs, which have a proximal length of 22 nucleotides. Functionally, the miRNAs influence gene expression through base pairing with 3′ UTRs of messenger RNAs (right).

tumor suppressor genes (p16, CDKN2A, p53, p21, BRCA2, and SMAD4), and genome maintenance and repair genes (MLH, MSH2, and BRCA2) [1, 2]. Several studies explained the complexity of genetic aberrations and their regulatory signaling pathways [3]. Although a large variety of signal transduction pathways have already been studied in PDAC, much less is known about the cross talk between epigenetic mechanism and signaling pathways typical for PDAC [1]. Strikingly, there are also particular cases where signaling pathways are altered, which directly affect important components of the epigenetic machinery. Therefore, a clear understanding of the epigenetic mechanisms and their implication in PDAC development will open new avenues of therapy. This approach will exploit the intricate process through which cells induce changes at transcription level [4–6].

Epigenetic mechanisms are defined in a way that they can both silence or activate genes without alteration to the DNA sequence itself. Mechanistically, epigenetic changes represent DNA hypermethylation or hypomethylation, histone-based mechanisms that include posttranslational modifications and nucleosome remodeling, as well as aberrant expression of microRNAs [5, 7]. These modifications affect chromatin structure and promoter accessibility, which contribute to genetic alterations [8].

In PDAC, the famous mutant gene KRAS and its downstream signaling cascade are an example for the low therapeutic effect, which is accomplished by current therapies against this gene and its downstream effectors. Interestingly, recent studies demonstrate that dysregulation of epigenetic regulators is essential for PDAC progression as well as for that of many other tumors [9]. Genomic deletions, mutations, and rearrangements frequently target genes encoding components of the chromatin remodeling complex (SWI/SNF), which have been identified in 10–15% of PDAC patients [10].

In general, the discovery of the involvement of the epigenetic effect in cancer added a new concept of gene therapy and informative markers for the diagnosis and prognosis for many malignancies [11]. Whole genomic sequencing studies have revealed driver mutations in epigenetic regulators in various cancer types such as IDH1/2, DNMT3A, KDM6A, DNMT3B, SMARCB1, and CREBBP/EP300 [12]. In PDAC, the sequencing experiments showed more pathogenic mutations in genes encoding multiple components of the SWI/SNF complexes, including ARID1A, ARID1B, PBRM1, SMARCA2, and SMARCA4 [13]. Additionally, recent studies found mutations in important epigenetic regulators like histone methyltransferase enzymes MLL1, MLL2, and MLL3 and histone demethylase KDM6A [13, 14]. Specifically, KDM6A has been found mutated in 5–10% of PDAC patients [15].

Several studies on familial PDAC have shown an association between DNA repair genes' dysfunction and those genes that are responsible for this inheritance (BRCA2, BRCA1, CDKN2A/p16, STK11/LKB1) [13, 16]. Strikingly, the altered DNA repair system is a hallmark of cancer, which causes genome instability and DNA damage [17]. Each cell contains a specific enzymatic system termed the DNA mismatch repair (MMR), which is responsible for detecting and correcting DNA replication errors [18, 19]. Loss or silencing of any protein in this system leads to the accumulation of gene mutations. In this regard, studies reported that MMR inactivation was caused by the epigenetic silencing of the hMLH1 and hMLH2 genes [20, 21]. The hMLH1 protein is one component of a family of seven members of MMR proteins that work coordinately to regulate DNA replication error in humans [20]. In this context, hypermethylation of the hMLH1 promoter has been shown to be an early detection marker of esophageal cancer and also a prognostic marker in colorectal and pancreatic cancers. On the other hand, this mechanism cannot be generalized, as methylation of the hMLH2 gene results less clear into gene inactivation, because the respective promoter is a weak target for CpG island methylation [16, 17, 20–22].

Another example of an affected DNA repair gene is the O^6-methylguanine-DNA transferase (MGMT), which is most intensively regulated by CpG promoter methylation [23]. MGMT is responsible for removing alkyl groups from O^6 in guanine and thus prevents mismatch errors during DNA replication. The silencing of the MGMT activity in human colorectal adenomas has been linked to K-ras GC → AT transition mutations [24, 25]. Interestingly, the epigenetic silencing of MGMT has two main effects in human cancer. First, it reveals a new mutator pathway that causes the accumulation of G-to-A transition mutations. Second, there is a strong and significant positive association between MGMT promoter hypermethylation and enhanced tumor sensitivity to alkylating drugs. These findings highlight the significance of MGMT promoter hypermethylation in translational cancer research [17, 26, 27].

3. DNA methylation

The first epigenetic modification to be identified was DNA methylation [28], which is based on stable and heritable changes in gene structure without a change in DNA sequence [12]. Methylation refers to the addition of a methyl group to the fifth carbon in cytosine forming 5-methylcytosine (5-mC), which is mediated by DNMTs [29]. Generally, methylation occurs in intergenic regions and repetitive sequences such as satellite repeats, and long and short interspersed nuclear elements, while CpG islands of gene promoters often are unmethylated [5, 7, 30]. Interestingly, the global effects of epigenetic alterations in gene regulatory sequences from over 100 cancer cell lines have been identified by the ENCODE project [31]. Normally, DNA

methylation is critical for maintaining pluripotency, X chromosome inactivation, and genome imprinting [12]. Aberrant DNA methylation is one of the hallmarks of cancer [32].

Methylation of DNA is catalyzed by the enzymes DNMT3A and DNMT3B and is then maintained by the major DNA methyltransferase DNMT1, which is also assisted by DNMT3A and DNMT3B [9, 12]. A recent study found that DNMT1, DNMT3A, and DNMT3B are themselves differentially methylated in PDAC [33]. Besides, a very recent finding suggests that the interactions between TP53 and H3K4, MLL3 and MOZ genes play a major role in chromatin regulation [34]. The methylation of tumor suppressor genes is the best-characterized epigenetic event in several malignancies, including PDAC [11]. In fact, several genes such as APC, BRCA1, P16INK4a, P15INK4b, RARβ, and p73 are frequently methylated [10]. Recent studies have revealed that apparent DNA methylation occurred in critical signaling pathways in PDAC such as TGFβ, WNT, integrin, cell adhesion, and axon guidance signaling pathways [35]. Likewise, TGFβ induces epithelial-mesenchymal transition (EMT) by enhancing hypermethylation of CpG islands in the VAV1 gene promoter [36] (see **Figure 2**). Furthermore, the WNT signaling pathway is a target of hypermethylation in PDAC. This has been found for WNT ligands WNT5A, WNT7A, and WNT9A, or the cell surface receptor FZD9, or the cytoplasmic transducer APC2, the nuclear factors SOX1, SOX7, SOX14, and SOX17, and the pathway inhibitors FRZB, SFRP1, SFRP2, KREMEN2, NKD2, and WIF1. Strikingly, the tumor suppressor candidate HIC1 is hypermethylated, which is acting as a transcriptional repressor for abnormal survival circuits of the transcription factors involved in the WNT signaling pathway [33, 37].

Furthermore, several studies demonstrated that promoter DNA hypermethylation is associated with the transcriptional repression of multiple microRNAs (miRNAs). This results into upregulation of oncogenic target genes of the microRNAs, such as observed for the downregulation of miR-181b, which promotes the

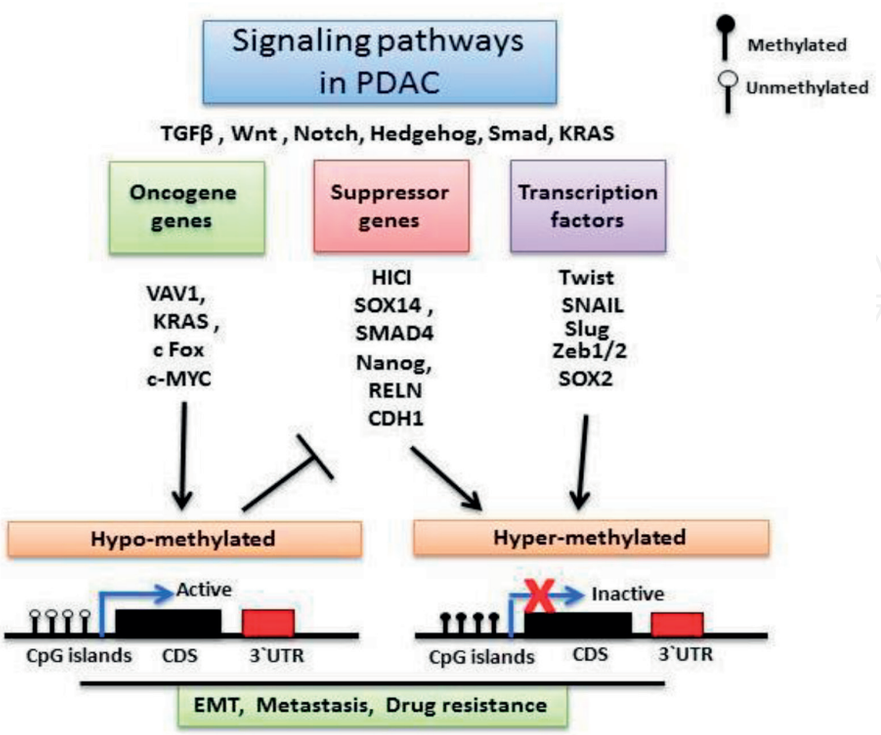

Figure 2.
Schematic diagram of signaling pathways in PDAC, which are deregulated by DNA methylation. Critical tumor suppressors and transcription factors are silenced, whereas oncogenes are activated. This deregulation promotes EMT and metastasis.

Gene	Gene name	Epigenetic alteration	Function	References
CADM1	Cell adhesion molecule 1	Hypermethylation	Cell-cell interaction	[41]
CDH1	Epithelial cadherin	Hypermethylation	Cell adhesion and invasion	[42]
DKK3	Dickkopf-related protein 3	Hypermethylation	Tumor suppressor	[43]
S100A4	S100 calcium-binding protein A4	Hypermethylation	Invasion, motility, and tubulin polymerization	[44]
P16	Cyclin-dependent kinase inhibitor 2A	Hypermethylation	Multiple tumor suppressor	[45]
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	Hypermethylation	Enzyme	[33]
BMP3	Bone morphogenetic protein 3	Hypermethylation	Growth factor	[33]
ST6GAL2	ST6 beta-galactosamide alpha-2,6-sialyltranferase 2	Hypermethylation	Generation of the cell surface carbohydrate determinants and differentiation antigens	[42]
ST8SIA5	ST8 alpha-N-acetyl-neuraminide α-2,8-sialyltransferase 5	Hypermethylation	A member of glycosyltransferase family	[42]
ST8SIA2	ST8 α-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2	Hypermethylation	A member of glycosyltransferase family	[42]
ST8SIA3	ST8 α-N-acetyl-neuraminideα-2,8-sialyltransferase 3	Hypermethylation	A member of glycosyltransferase family	[42]
AKT1	v-Akt murine thymoma viral oncogene homolog 1	Hypermethylation	Kinases	[30]
LCN2	Lipocalin 2	Hypomethylation	Epithelial differentiation	[33]
CCND2	Cyclin D2	Hypermethylation	Cell cycle control	[33]
CLDN4	Claudin-4	Hypomethylation	Cell adhesion	[44]
miR-9-1	MicroRNA-9	Hypomethylation	miRNA translation control	[40]
P59	Cyclin-dependent kinase inhibitor 1C	Hypermethylation	Cyclin-dependent kinase inhibitor	[34]
P16	Cyclin-dependent kinase inhibitor 2A	Hypermethylation	Cyclin-dependent kinase inhibitor	[33]
RARB	Retinoic acid receptor	Hypermethylation	Cell growth control	[33]
SFN	Stratifin (14-3-3sigma)	Hypomethylation	P53-induced G2/M cell cycle arrest	[9]
LCN2	Tissue factor pathway inhibitor	Hypomethylation	Epithelial differentiation	[9]

Gene	Gene name	Epigenetic alteration	Function	References
CDKN1C/ P57	Cyclin-dependent kinase inhibitor 1C	Hypomethylation	Cyclin-dependent kinase inhibitor	[42]
FOXE1	Forkhead box E1	Hypomethylation	Thyroid transcription factor	[42]

Table 1.
A series of methylated genes in PDAC.

expression of BCL 2 [38]. Moreover, downregulation of the miR-29 family was associated with the overexpression of the DNA methyltransferases DNMT3A and DNM3B [39]. The noncoding RNAs and antisense RNA sequences are strongly involved in the respective DNA hypermethylation process, which silences important genes such as polycomb group (PcGs), which in turn may expose these regions to DNA methylation changes [40]. More examples are listed in **Table 1**, all of which are related to PDAC.

4. Histone modification

Nucleosomes are considered to be the basic constituents of chromatin. Each nucleosome is an octamer of histones, which consist of two copies each of histone proteins H2A, H2B, H3, and H4 [46]. The most interesting epigenetic events in PDAC are histone modifications, since several studies revealed that the most frequently mutated epigenetic genes occurred in the histone family [13]. The posttranslational modifications include methylation, acetylation, citrullination, phosphorylation, SUMOylation, and ADP ribosylation. However, the most studied histone modifications in cancer are lysine alterations, including lysine methylation, acetylation, and phosphorylation [47–49]. In normal cell development, histone modifications regulate critical cell processes such as DNA replication and transcription or repair [46], while in cancer, histone modifications contribute to the maintenance of malignant phenotypes. In PDAC, the most common modification includes methylation and acetylation of lysine residues within the N terminal tails of histone proteins [11].

In the context of epithelial-mesenchymal transition (EMT) in PDAC, SNAIL is a critical transcription repressor of E-cadherin in EMT process. It plays a significant role in embryonic development and tumorigenesis [50]. Moreover, SNAIL has an essential function in histone modifications. This includes the activation of a set of chromatin modifiers such as lysine-specific demethylase, euchromatic histone lysine methyltransferase 2 (G 9a), suppressor of variegation 3–9 homolog 1 histone methyltransferases (Suv39H1), SIN3 transcription regulator family member A (SIN3A), and histone deacetylases (HDAC1 and HDAC2) [51, 52].

4.1 Histone methylation

Methylation of histones is coordinated by histone methyltransferases (HMTs) and histone demethylases (HDMs). There are at least 17 different HMTs, all of which share the conserved (Su (var) 3–9, enhancer-of-zeste, trithorax) motif. The lysine methylation residue is most common and is mediated by histone lysine methyltransferases (HKMTs) [53]. Particularly, methylation at H3K9, H3K27, and H3K20 is associated with transcriptional repression, while methylation of H3K4, H3K36, and H3K79 causes transcriptional activation [47]. The silencing of tumor suppressor genes in cancer is caused by the corresponding activities of the HMT and HDMT

enzymes. On the other hand, the H3K27me3-specific HMT EZH2 (enhancer of zeste homolog 2), the catalytic subunit of PRC2, is overexpressed in a broad range of solid tumors, including prostate, lung, breast, colon, skin, and pancreatic cancers [54, 55].

The most frequently altered histone methylated genes in PDAC are KDM6A and MLL2 [33]. KDM6A is an H3K27me3 demethylase, which has a role in endoderm differentiation by regulating the expression of WNT signaling and HOX genes [56]. Other studies found that the loss of trimethylation at K27 of histone H3, which causes nuclear accumulation of EZH2, is strongly correlated with a poor PDAC outcome [57]. Various interactions have been shown to occur between DNA methylation and histone methylation. For example, the interaction between EZH2 and DNMTs renders the EZH2 gene a potential therapeutic target. Mucins (MUCs) are also known to play essential roles in tumor growth and invasion in pancreatic neoplasms. MUC1 and MUC4 are high-molecular-weight transmembrane mucins. Overexpression of mucins in cancer is associated with poor prognosis. It has been shown that mucin expression changes in PDAC are due to DNA methylation of H3 at the lysine9 residue [58, 59].

4.2 Histone acetylation

Histone acetylation is the first discovered histone modification. The acetylation of lysine residues neutralizes their positive charge, which induces chromatin relaxation and activates a set of genes associated with transcription. On the other hand, removal of the acetyl groups is associated with gene silencing. Histone acetylases (HATs) and deacetylases (HDAC) are the required enzymes for this process [60, 61] (see **Figure 3a**).

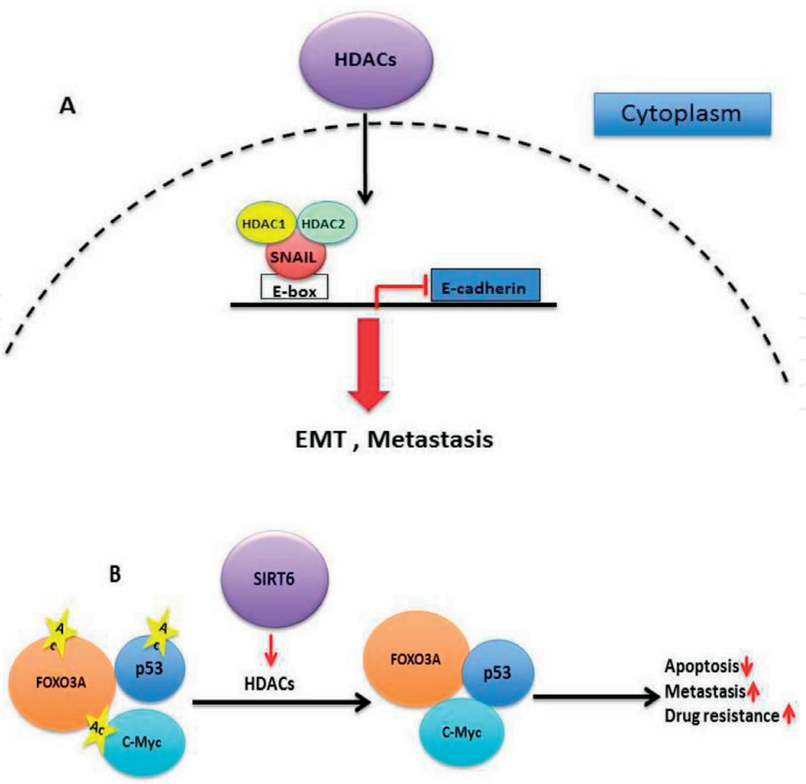

Figure 3.
Schematic diagram on the role of HDACs in PDAC. (A) HDACs mediate E-cadherin translational repression by activating the binding of EMT transcription factors to the E-boxes present in the E-cadherin promoter. (B) SIRT6 mediates the deacetylation of p53, FOXO3A, and C-Myc, which leads to increased metastasis and drug resistance.

Recent studies show a series of significant alterations of the acetylation process in PDAC, as well as mutations in the histone acetylase EP300 [33]. Furthermore, the SIRT6 gene is associated with the deacetylation of histone H3 at lysine residues 9 and 56, thus increasing the expression of the SIRT6 gene associated with PDAC metastasis by deacetylation of p53 and FOXOA3 [62] (see **Figure 3b**). For instance, the activation of KRAS and increased expression of the c-Myc transcription factor promote PDAC metastasis [13]. Also, expression of HDAC7 and HDAC2 has been found increased in PDAC [63]. In addition, HDACs/HATs play important roles in the activation of several tumor suppressor genes in PDAC, such as p53 and EP300 [11, 14, 53, 64].

A recent study identified the acetylation of glutamate oxaloacetate transaminases 2 (GOT2) at three lysine residues (K159, K185, and K404) in PDAC. This promotes the transfer of NADH from the cytoplasm into mitochondria, enhancing PDAC cell proliferation and tumor growth in vivo. On the other hand, the acetylation of GOT2 at only K159 is correlated with downregulation of SIRT3 expression [65].

4.3 Histone phosphorylation

Histone phosphorylation has been associated with different cell processes, including apoptosis, cell cycle, DNA transcription, DNA repair, chromosome condensation, gene regulation, cell signaling pathways, energy, and metabolic pathways [66]. Phosphorylation of histones occurs on serine, threonine, and tyrosine residues, a process mediated by different kinases and phosphatases [46]. In cell development, the most important site for histone phosphorylation is the serine 10 of histone H3 (H3S10P), which is mediated by the Aurora-B kinase. This modification is a critical event in cell mitosis and meiosis [67].

Several studies identified histone phosphorylation changes during DNA damage, such as the phosphorylation of serine 139 on the histone H2A(X). On the other hand, phosphorylation of serines, e.g., 10 and 28 on H3, and serine 32 on H2B have been contributed by the activation of the epidermal growth factor (EGF). Moreover, H3ser28p mediated the expression of c-fos and α-globin [68–70].

It has been shown that H2A T120 is phosphorylated in PDAC by VRK1 on the promoter region of CCND1, which consequently activates the transcription of cyclin D1 [71]. Besides, KRAS is most well-studied and known activated oncogene in PDAC [72]. Other studies have implicated the activation of the Ras-MAPK pathway with the upregulation of phospho-ERK1/2 and their downstream levels of H3 S10ph [73].

5. MicroRNA

MicroRNAs (miRNAs) are small (20–23 nucleotides), endogenous, noncoding, single-stranded RNA molecules, which control the expression of around 60% of the protein-coding genes [74]. Moreover, they can control both physiological and pathological processes, such as development and cancer [75]. In addition, the miRNA machinery is of great importance for drug development, since a functional miRNA machinery is a compulsory prerequisite for any RNA interference (RNAi)-based therapy approach. A total of 700 miRNAs have been discovered in human diseases, and more than 1000 predicted miRNA genes are yet to be experimentally validated [76].

Mature microRNAs require several steps of preprocessing before they can become functional. After they are transcribed by RNA polymerase II/III from intragenic regions or from regions that code for introns, the primary transcript (pri-miRNA) is processed by the ribonuclease Drosha and DGCR8 in the nucleus. The process

produces pre-microRNAs, hairpin-shaped intermediates of 70–100 nucleotides. Exportin-5, a Ran-GTP-dependent dsRNA-binding protein, transports pre-microRNAs into the cytoplasm where they are further processed by the ribonuclease Dicer and TRBP (Tar RNA-binding protein) into a double-stranded miRNA. The strands separate and a mature single-stranded molecule join an RNA-induced silencing complex (RISC). The double miRNA strands are required to interact with RISC complex or to be degraded. Ordinarily, one miRNA strand can give rise to two individual mature miRNA sequences with different targets due to complementary seed sequence [74, 77, 78]. The single-stranded mature microRNA remains stable on the miRISC and induces posttranscriptional silencing of one or more target genes, usually through imperfect pairing with a target sequence in the 3' UTR [74]. However, this is not the only binding region for miRNAs, as there are also binding sites located in 5' UTR or even within the coding DNA sequence of mRNAs [77]. The seed sequence or seed region is a conserved heptametrical sequence, which is mostly situated at positions 2–7 from the miRNA 5' end [79, 80]. Furthermore, degradation of mature miRNAs appears to depend on their activity; in the absence of complementary targets, the miRNA could be released from miRNA-RISC complex, and then its 5′ end becomes accessible to the 5′ → 3′ exonuclease XRN2, which degrades the miRNA [81].

Cancer represents a heterogeneous group of diseases characterized by uncontrolled growth of cells, high proliferation rates, and apoptosis resistance. All of these features result from a complex of structural and expression abnormalities of genes, including those encoding microRNAs [75, 82]. The classification of cancer is more accurately defined with microRNA profiling than with mRNA profiling because of the strong correlation between microRNA expression signatures and tumor origin [75]. In general, microRNAs have two main functions in cancer; they can act as tumor suppressors (TSmiRs) or oncogenes (OncomiRs) [75, 76, 82, 83].

One of the first indications that miRNAs serve as tumor suppressors (TSmiRs) came from Calin and colleagues when they discovered that miR-15a and miR-16-1 were deleted or downregulated in about 68% of chronic lymphocytic leukemia (CLL) samples. MiR-15a and miR-16-1 have been shown to control the expression of VEGF, a key proangiogenic factor involved in tumor angiogenesis. Furthermore, both of them induce the apoptosis of leukemic cells by affecting the antiapoptotic protein BCL2 [75, 84]. Another prominent TSmiR is the let-7 family, located at a chromosomal region, which is usually deleted in human cancers. It has been reported as a TSmiR in lung, breast [84], urothelial, and cervical cancers [85]. Recent studies found that let-7 was able to regulate the RAS oncogene in lung cancer. In addition, let-7 regulates late embryonic development by suppressing a number of genes such as c-Myc, RAS, and HAMGA2 [76, 82]. Taken together, reduced expression of TSmiRs in cancer releases oncogenic genes and promotes tumor initiation and progression.

In contrast, oncogenic miRNAs (OncomiRs) promote tumorigenesis by inhibiting tumor suppressor genes that play roles within other functions, such as cell differentiation and apoptosis. The first OncomiR that was discovered is the miR-17-92 cluster, which encodes miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, miR19b-1, and miR-92-1. This cluster is located on chromosome 13 and is commonly found to be amplified in human B-cell lymphomas, lung cancer, and anaplastic thyroid cancer cells [86]. Another oncogenic miRNA, miR-21, has been validated in nine solid tumor types (lung, breast, head and neck, prostate, colon, pancreas, esophagus, stomach, and brain). Experimental data confirmed that miR-21 plays a significant role in cancer cell proliferation, apoptosis, and invasion. Accordingly, inhibition of miR-21 induces cell cycle arrest, increased apoptosis, and increased chemosensitivity to anticancer agents [87].

The important emerging role of miRNAs in many cancer types, together with the fact that they can function as TSmiRs or OncomiRs, supports the potential of

miRNAs as a new class of targets in the development of cancer therapies. Several studies have focused on targeting miRNAs as an experimental therapy in vitro or in vivo [85]. Notably, to modulate cancer-associated miRNAs in vivo, two main approaches were established: first, miRNA replacement therapy, which is based on adding the miRNAs missing in cancer cells for restoring their normal functions; second, inhibition of oncogenic miRNAs by using single-stranded chemically modified anti-miR oligonucleotides [85, 88]. The first successful in vivo experiment using anti-miRs in conjunction with locked nucleic acids was successfully applied in African green monkeys with hypercholesterolemia. The experiments resulted in the successful control of triglyceride and cholesterol levels, together with the management of disease manifestations with minimal side effects to herald a new research approach that is equally applicable in cancer [89].

PDAC shares many features with other solid tumors. Numerous studies have reported the significant roles, which miRNAs play in PDAC progression. Furthermore, these studies have also provided important information about cellular features, such as growth, invasive, and metastatic behavior that have been modified or altered in PDAC as a result of miRNAs, thus highlighting, to a large extent, the significance of miRNAs in PDAC progression [90]. High-throughput microarray technologies have been used to extensively profile miRNA signatures in cell lines, normal frozen tissues, formalin-fixed paraffin-embedded tissues (FFPE), blood, and fine needle aspiration biopsy (FNAB) samples, in order to establish a common expression pattern in PDAC [91]. Recently, a meta-analysis reviewed 11 miRNA profiling studies in PDAC and reported 439 miRNAs as deregulated in the 538 PDAC samples that were evaluated [92]. This analysis defines a common pool of

miRNAs	Expression status	Target genes	References
Let-7 family	Downregulated	KRAS, MAPK, c-Myc, STAT3	[94, 104]
miR-181s	Upregulated	TIMP3, TCL1, TGFBI, TRIM2, SIRT1, Bcl2	[91, 105]
miR-26s	Downregulated	MMP2, MMP14, cyclin D1, Mcl-1, Bcl2	[91, 106, 107]
miR-125a	Upregulated	Bcl-w, Bcl2	[108]
miR-192	Downregulated	SERPINE1	[109]
miR-148a,b	Downregulated	DNMT3B, Mtif, CCKBR, BCL2	[90, 91, 110]
miR-200 family	Downregulated	VEGF-A, KRAS, KDR, VEGFR2, ZEB1/2	[100, 111–113]
miR-34a	Downregulated	Notch1/2, Bcl2, SIRIT, CDK4, VEGF	[99, 114–117]
miR-375	Downregulated	PDK1	[90]
miR-124	Downregulated	ITGB1, Rac1, RocK2, EZH1, Bcl2, CDK6	[91, 101, 118]
miR-217	Downregulated	KRAS, SIRIT, c-MYC	[119, 120]
miR-21	Upregulated	PTEN	[121]
miR-132	Upregulated	Rb1, SMAD2	[122, 123]
miR-208	Upregulated	E-cadherin	[106]
miR-196-a	Upregulated	NFKBIA	[124]
miR-100	Upregulated	IGFR1	[90]
miR-155	Upregulated	TP53INP1	[125]
miR-10b	Upregulated	TIP30	[91]

Table 2.
Top frequently deregulated miRNAs in PDAC.

miRNAs that are atypically expressed in PDAC, and the potential renormalization of these miRNAs and/or expression patterns could help create a therapeutic approach in managing this aggressive disease [93].

The commonly deregulated miRNAs are associated with major regulatory genes in several signaling pathways (**Table 2**), which are involved in most aspects of cellular physiology including regulation of cell cycle, differentiation, proliferation, and apoptosis. Notably, altered miRNA expression in PDAC contributes to metastasis and drug resistance [92, 94, 95]. The more frequently deregulated miRNAs in PDAC include miR-21, the expression of which is regulated by KRAS, and correlates with the degree of tumor progression [90]. KRAS is an important molecule in PDAC and is a direct target of miR-96, miR-217, miR-126, and miR-200c. The overexpression of these miRNAs reduces the level of KRAS expression, resulting in decreased cell invasion, migration, and tumor growth [96, 97]. Strikingly, two of these miRNAs, miR-145 and miR-200c, function as a regulatory network in the AKT-PI3K signaling pathway [98]. Conversely, it has been reported that KRAS activation suppresses the expression of the miR-134/145 cluster via the Ras responsive element-binding protein (RREB1) [99]. The miR-200 family is also frequently deregulated in PDAC and plays a significant role in EMT inhibition. One study demonstrated that miR-200 negatively regulates ZEB1 and ZEB2, which are both direct repressors of E-cadherin [100]. In the context of epigenetic modifications, several studies have found TSmiRs in PDAC, including miR-9-1, miR-124s, miR-192, miR-615-5p, and miR-1247, which were hypermethylated [44, 101–103].

6. Conclusions

For the high mortality, poor prognosis, and undefined therapeutic targets in PDAC, the unraveling of the complex molecular layers driving this lethal cancer is a prerequisite for more effective therapeutic strategies and consistent diagnostic markers. The recent research on epigenetic mechanisms has significantly enriched our knowledge about the regulatory characteristics involved in the initiation, progression, and metastasis of PDAC. This book chapter has focused on the most critical epigenetics mechanisms, including DNA methylation, histone modifications, and modulated expression of miRNAs that play a significant role in PDAC tumorigenesis, and could serve as future therapeutic targets. Currently, significant emphasis is still given on detecting somatic genetic alterations in PDAC. However, it seems also promising to investigate the underlying epigenetic mechanisms for completing the full puzzle of altered gene expression in PDAC. The epigenetics field has developed strongly and will continue to advance into a frontier field for PDAC research. Additionally, it is essential to highlight the features of epigenetic mechanisms of gene regulation—their reversibility. This feature provides a ground for specifically targeting the epigenetic changes contributing to PDAC.

Acknowledgements

Alia Abukiwan was funded by the Heidrun-Seibert-Stiftung.

Conflict of Interest

The authors declare no conflict of interest.

Author details

Alia Abukiwan and Martin R. Berger*
Toxicology and Chemotherapy Unit, German Cancer Research Center (DKFZ), Heidelberg, Germany

*Address all correspondence to: m.berger@dkfz.de

IntechOpen

References

[1] Ying H et al. Genetics and biology of pancreatic ductal adenocarcinoma. Genes & Development. 2016;**30**(4):355-385

[2] Cowan RW, Maitra A. Genetic progression of pancreatic cancer. Cancer Journal. 2014;**20**(1):80-84

[3] Yuan QY et al. Identification of dysregulated pathways associated with pancreatic cancer by survival analysis. Molecular Medicine Reports. 2015;**11**(1):277-282

[4] Lomberk GA, Urrutia R. The triple-code model for pancreatic cancer: Cross talk among genetics, epigenetics, and nuclear structure. The Surgical Clinics of North America. 2015;**95**(5):935-952

[5] Paradise BD, Barham W, Fernandez-Zapico ME. Targeting epigenetic aberrations in pancreatic cancer, a new path to improve patient outcomes? Cancers (Basel). 2018;**10**(5)

[6] Trager MM, Dhayat SA. Epigenetics of epithelial-to-mesenchymal transition in pancreatic carcinoma. International Journal of Cancer. 2017;**141**(1):24-32

[7] Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010;**31**(1):27-36

[8] Shen H, Laird PW. Interplay between the cancer genome and epigenome. Cell. 2013;**153**(1):38-55

[9] Feinberg AP, Koldobskiy MA, Gondor A. Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. Nature Reviews. Genetics. 2016;**17**(5):284-299

[10] Silverman BR, Shi J. Alterations of epigenetic regulators in pancreatic cancer and their clinical implications. International Journal of Molecular Sciences. 2016;**17**(12)

[11] McCleary-Wheeler AL et al. Insights into the epigenetic mechanisms controlling pancreatic carcinogenesis. Cancer Letters. 2013;**328**(2):212-221

[12] Bird A. DNA methylation patterns and epigenetic memory. Genes & Development. 2002;**16**(1):6-21

[13] Biankin AV et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature. 2012;**491**(7424):399-405

[14] Sausen M et al. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. Nature Communications. 2015;**6**:7686

[15] Kugel S et al. SIRT6 suppresses pancreatic cancer through control of Lin28b. Cell. 2016;**165**(6):1401-1415

[16] Borazanci E et al. Pancreatic cancer: "a riddle wrapped in a mystery inside an enigma". Clinical Cancer Research. 2017;**23**(7):1629-1637

[17] Lahtz C, Pfeifer GP. Epigenetic changes of DNA repair genes in cancer. Journal of Molecular Cell Biology. 2011;**3**(1):51-58

[18] Geisler JP et al. Mismatch repair gene expression defects contribute to microsatellite instability in ovarian carcinoma. Cancer. 2003;**98**(10):2199-2206

[19] Saez GT. DNA injury and repair systems. International Journal of Molecular Sciences. 2018;(7):**19**

[20] Hitchins MP et al. Epigenetic inactivation of a cluster of genes flanking MLH1 in

microsatellite-unstable colorectal cancer. Cancer Research. 2007;**67**(19):9107-9116

[21] Kondo E et al. Not hMSH2 but hMLH1 is frequently silenced by hypermethylation in endometrial cancer but rarely silenced in pancreatic cancer with microsatellite instability. International Journal of Oncology. 2000;**17**(3):535-541

[22] Ye P, Shi Y, Li A. Association between hMLH1 promoter methylation and risk of gastric cancer: A meta-analysis. Frontiers in Physiology. 2018;**9**:368

[23] Shalaby SM et al. Promoter methylation and expression of DNA repair genes MGMT and ERCC1 in tissue and blood of rectal cancer patients. Gene. 2018;**644**:66-73

[24] De Maglio G et al. MGMT promoter methylation status in brain metastases from colorectal cancer and corresponding primary tumors. Future Oncology. 2015;**11**(8):1201-1209

[25] Schmitt AM et al. Prognostic and predictive roles of MGMT protein expression and promoter methylation in sporadic pancreatic neuroendocrine neoplasms. Neuroendocrinology. 2014;**100**(1):35-44

[26] Jin J et al. Aberrant DNA methylation of MGMT and hMLH1 genes in prediction of gastric cancer. Genetics and Molecular Research. 2014;**13**(2):4140-4145

[27] Zuo C et al. O6-methylguanine-DNA methyltransferase gene: Epigenetic silencing and prognostic value in head and neck squamous cell carcinoma. Cancer Epidemiology, Biomarkers & Prevention. 2004;**13**(6):967-975

[28] Rivenbark AG et al. Epigenetic reprogramming of cancer cells via targeted DNA methylation. Epigenetics. 2012;**7**(4):350-360

[29] Lujambio A et al. A microRNA DNA methylation signature for human cancer metastasis. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(36):13556-13561

[30] Tan AC et al. Characterizing DNA methylation patterns in pancreatic cancer genome. Molecular Oncology. 2009;**3**(5-6):425-438

[31] Consortium EP. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;**489**(7414):57-74

[32] Zhang MW et al. DNA methylation in the tumor microenvironment. Journal of Zhejiang University. Science. B. 2017;**18**(5):365-372

[33] Vincent A et al. Genome-wide analysis of promoter methylation associated with gene expression profile in pancreatic adenocarcinoma. Clinical Cancer Research. 2011;**17**(13):4341-4354

[34] Zhu J et al. Gain-of-function p53 mutants co-opt chromatin pathways to drive cancer growth. Nature. 2015;**525**(7568):206-211

[35] Rajamani D, Bhasin MK. Identification of key regulators of pancreatic cancer progression through multidimensional systems-level analysis. Genome Medicine. 2016;**8**(1):38

[36] Huang PH et al. TGFbeta promotes mesenchymal phenotype of pancreatic cancer cells, in part, through epigenetic activation of VAV1. Oncogene. 2017;**36**(16):2202-2214

[37] Galamb O et al. Aberrant DNA methylation of WNT pathway genes in the development and progression

of CIMP-negative colorectal cancer. Epigenetics. 2016;**11**(8):588-602

[38] Zhu W et al. miR-181b modulates multidrug resistance by targeting BCL2 in human cancer cell lines. International Journal of Cancer. 2010;**127**(11):2520-2529

[39] Morita S et al. miR-29 represses the activities of DNA methyltransferases and DNA demethylases. International Journal of Molecular Sciences. 2013;**14**(7):14647-14658

[40] Ramassone A et al. Epigenetics and MicroRNAs in cancer. International Journal of Molecular Sciences. 2018;**19**(2)

[41] Thompson MJ et al. Pancreatic cancer patient survival correlates with DNA methylation of pancreas development genes. PLoS One. 2015;**10**(6):e0128814

[42] Iguchi E et al. Pancreatic cancer, a Mis-interpreter of the epigenetic language. The Yale Journal of Biology and Medicine. 2016;**89**(4):575-590

[43] Gu YM et al. Dickkopf3 overexpression inhibits pancreatic cancer cell growth in vitro. World Journal of Gastroenterology. 2011;**17**(33):3810-3817

[44] Omura N, Goggins M. Epigenetics and epigenetic alterations in pancreatic cancer. International Journal of Clinical and Experimental Pathology. 2009;**2**(4):310-326

[45] Schutte M et al. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. Cancer Research. 1997;**57**(15):3126-3130

[46] Audia JE, Campbell RM. Histone modifications and cancer. Cold Spring Harbor Perspectives in Biology. 2016;**8**(4):a019521

[47] Shanmugam MK et al. Role of novel histone modifications in cancer. Oncotarget. 2018;**9**(13):11414-11426

[48] Kurdistani SK. Histone modifications in cancer biology and prognosis. Progress in Drug Research. 2011;**67**:91-106

[49] Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000;**403**(6765):41-45

[50] Zheng M et al. Snail and slug collaborate on EMT and tumor metastasis through miR-101-mediated EZH2 axis in oral tongue squamous cell carcinoma. Oncotarget. 2015;**6**(9):6797-6810

[51] Lin Y, Dong C, Zhou BP. Epigenetic regulation of EMT: The snail story. Current Pharmaceutical Design. 2014;**20**(11):1698-1705

[52] Peinado H et al. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. Molecular and Cellular Biology. 2004;**24**(1):306-319

[53] Suganuma T, Workman JL. Crosstalk among histone modifications. Cell. 2008;**135**(4):604-607

[54] Grzenda A, Ordog T, Urrutia R. Polycomb and the emerging epigenetics of pancreatic cancer. Journal of Gastrointestinal Cancer. 2011;**42**(2):100-111

[55] Wang W et al. Polycomb group (PcG) proteins and human cancers: Multifaceted functions and therapeutic implications. Medicinal Research Reviews. 2015;**35**(6):1220-1267

[56] Van der Meulen J, Speleman F, Van Vlierberghe P. The H3K27me3 demethylase UTX in normal development and disease. Epigenetics. 2014;**9**(5):658-668

[57] Wei Y et al. Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. Molecular Carcinogenesis. 2008;**47**(9):701-706

[58] Yamada N et al. MUC1 expression is regulated by DNA methylation and histone H3 lysine 9 modification in cancer cells. Cancer Research. 2008;**68**(8):2708-2716

[59] Yokoyama S et al. Aberrant methylation of MUC1 and MUC4 promoters are potential prognostic biomarkers for pancreatic ductal adenocarcinomas. Oncotarget. 2016;**7**(27):42553-42565

[60] Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997;**389**(6649):349-352

[61] Marmorstein R, Zhou MM. Writers and readers of histone acetylation: Structure, mechanism, and inhibition. Cold Spring Harbor Perspectives in Biology. 2014;**6**(7):a018762

[62] Demir IE, Ceyhan GO, Friess H. Epigenomic therapies: The potential of targeting SIRT6 for the treatment of pancreatic cancer. Expert Opinion on Therapeutic Targets. 2017;**21**(1):1-3

[63] Campbell MJ, Turner BM. Altered histone modifications in cancer. Advances in Experimental Medicine and Biology. 2013;**754**:81-107

[64] Rivera CM, Ren B. Mapping human epigenomes. Cell. 2013;**155**(1):39-55

[65] Yang H et al. SIRT3-dependent GOT2 acetylation status affects the malate-aspartate NADH shuttle activity and pancreatic tumor growth. The EMBO Journal. 2015;**34**(8):1110-1125

[66] Banerjee T, Chakravarti D. A peek into the complex realm of histone phosphorylation. Molecular and Cellular Biology. 2011;**31**(24):4858-4873

[67] Sawicka A, Seiser C. Histone H3 phosphorylation—a versatile chromatin modification for different occasions. Biochimie. 2012;**94**(11):2193-2201

[68] Prigent C, Dimitrov S. Phosphorylation of serine 10 in histone H3, what for? Journal of Cell Science. 2003;**116**(Pt 18):3677-3685

[69] Choi HS et al. Phosphorylation of histone H3 at serine 10 is indispensable for neoplastic cell transformation. Cancer Research. 2005;**65**(13):5818-5827

[70] Chadee DN et al. Increased Ser-10 phosphorylation of histone H3 in mitogen-stimulated and oncogene-transformed mouse fibroblasts. The Journal of Biological Chemistry. 1999;**274**(35):24914-24920

[71] Aihara H et al. Histone H2A T120 phosphorylation promotes oncogenic transformation via upregulation of Cyclin D1. Molecular Cell. 2016;**64**(1):176-188

[72] Bryant KL et al. KRAS: Feeding pancreatic cancer proliferation. Trends in Biochemical Sciences. 2014;**39**(2):91-100

[73] Espino PS et al. Genomic instability and histone H3 phosphorylation induction by the Ras-mitogen activated protein kinase pathway in pancreatic cancer cells. International Journal of Cancer. 2009;**124**(3):562-567

[74] Winter J et al. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nature Cell Biology. 2009;**11**(3):228-234

[75] Calin GA, Croce CM. MicroRNA signatures in human cancers. Nature Reviews. Cancer. 2006;**6**(11):857-866

[76] Lujambio A, Lowe SW. The microcosmos of cancer. Nature. 2012;**482**(7385):347-355

[77] Wilczynska A, Bushell M. The complexity of miRNA-mediated repression. Cell Death and Differentiation. 2015;**22**(1):22-33

[78] Ha M, Kim VN. Regulation of microRNA biogenesis. Nature Reviews. Molecular Cell Biology. 2014;**15**(8):509-524

[79] Witkos TM, Koscianska E, Krzyzosiak WJ. Practical aspects of microRNA target prediction. Current Molecular Medicine. 2011;**11**(2):93-109

[80] Shin C et al. Expanding the microRNA targeting code: Functional sites with centered pairing. Molecular Cell. 2010;**38**(6):789-802

[81] Chatterjee S, Grosshans H. Active turnover modulates mature microRNA activity in *Caenorhabditis elegans*. Nature. 2009;**461**(7263):546-549

[82] Kunej T et al. Cross talk between microRNA and coding cancer genes. Cancer Journal. 2012;**18**(3):223-231

[83] Esquela-Kerscher A, Slack FJ. Oncomirs—microRNAs with a role in cancer. Nature Reviews. Cancer. 2006;**6**(4):259-269

[84] Zhang B et al. microRNAs as oncogenes and tumor suppressors. Developmental Biology. 2007;**302**(1):1-12

[85] Wu WL et al. Suppressive effects of microRNA-16 on the proliferation, invasion and metastasis of hepatocellular carcinoma cells. International Journal of Molecular Medicine. 2015;**36**(6):1713-1719

[86] Wu W et al. MicroRNA and cancer: Current status and prospective. International Journal of Cancer. 2007;**120**(5):953-960

[87] Pan X, Wang ZX, Wang R. MicroRNA-21: A novel therapeutic target in human cancer. Cancer Biology & Therapy. 2010;**10**(12):1224-1232

[88] Wang Z et al. Targeting miRNAs involved in cancer stem cell and EMT regulation: An emerging concept in overcoming drug resistance. Drug Resistance Updates. 2010;**13**(4-5):109-118

[89] Akbari Moqadam F, Pieters R, den Boer ML. The hunting of targets: Challenge in miRNA research. Leukemia. 2013;**27**(1):16-23

[90] Bloomston M et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA. 2007;**297**(17): 1901-1908

[91] Yonemori K et al. MicroRNA in pancreatic cancer. Journal of Human Genetics. 2016

[92] Hawa Z et al. The miRacle in pancreatic cancer by miRNAs: Tiny angels or devils in disease progression. International Journal of Molecular Sciences. 2016;**17**(6)

[93] Taucher V, Mangge H, Haybaeck J. Non-coding RNAs in pancreatic cancer: Challenges and opportunities for clinical application. Cellular Oncology (Dordrecht). 2016;**39**(4):295-318

[94] Szafranska AE et al. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene. 2007;**26**(30):4442-4452

[95] Li Z, Rana TM. Therapeutic targeting of microRNAs: Current status and future challenges. Nature Reviews. Drug Discovery. 2014;**13**(8):622-638

[96] Zhu M et al. microRNA and gene networks in human pancreatic cancer. Oncology Letters. 2013;**6**(4):1133-1139

[97] Kopp F, Wagner E, Roidl A. The proto-oncogene KRAS is targeted by miR-200c. Oncotarget. 2014;**5**(1):185-195

[98] Drakaki A, Iliopoulos D. MicroRNA-gene signaling pathways in pancreatic cancer. Biomedical Journal. 2013;**36**(5):200-208

[99] Jamieson NB et al. MicroRNA molecular profiles associated with diagnosis, clinicopathologic criteria, and overall survival in patients with resectable pancreatic ductal adenocarcinoma. Clinical Cancer Research. 2012;**18**(2):534-545

[100] Park SM et al. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes & Development. 2008;**22**(7):894-907

[101] Wang P et al. Methylation-mediated silencing of the miR-124 genes facilitates pancreatic cancer progression and metastasis by targeting Rac1. Oncogene. 2014;**33**(4):514-524

[102] Gao W et al. miR-615-5p is epigenetically inactivated and functions as a tumor suppressor in pancreatic ductal adenocarcinoma. Oncogene. 2015;**34**(13):1629-1640

[103] Yi JM et al. Epigenetically altered miR-1247 functions as a tumor suppressor in pancreatic cancer. Oncotarget. 2017;**8**(16):26600-26612

[104] Patel K et al. MicroRNA let-7 downregulates STAT3 phosphorylation in pancreatic cancer cells by increasing SOCS3 expression. Cancer Letters. 2014;**347**(1):54-64

[105] Wald P et al. Prognostic value of microRNA expression levels in pancreatic adenocarcinoma: A review of the literature. Oncotarget. 2017;**8**(42):73345-73361

[106] Kent OA et al. A resource for analysis of microRNA expression and function in pancreatic ductal adenocarcinoma cells. Cancer Biology & Therapy. 2009;**8**(21):2013-2024

[107] Deng M et al. miR-26a suppresses tumor growth and metastasis by targeting FGF9 in gastric cancer. PLoS One. 2013;**8**(8):e72662

[108] Tang L et al. MiR-125a-5p decreases after long non-coding RNA HOTAIR knockdown to promote cancer cell apoptosis by releasing caspase 2. Cell Death & Disease. 2016;**7**:e2137

[109] Botla SK et al. Early epigenetic downregulation of microRNA-192 expression promotes pancreatic cancer progression. Cancer Research. 2016;**76**(14):4149-4159

[110] Zhang R et al. MiR-148a regulates the growth and apoptosis in pancreatic cancer by targeting CCKBR and Bcl-2. Tumour Biology. 2014;**35**(1):837-844

[111] Feng X et al. MiR-200, a new star miRNA in human cancer. Cancer Letters. 2014;**344**(2):166-173

[112] Uhlmann S et al. miR-200bc/429 cluster targets PLCgamma1 and differentially regulates proliferation and EGF-driven invasion than miR-200a/141 in breast cancer. Oncogene. 2010;**29**(30):4297-4306

[113] Chen Y, Zhang L. WITHDRAWN: MiR-200 family and cancer: Function, regulation and signaling. Surgical Oncology. 2014

[114] Luo G et al. Highly lymphatic metastatic pancreatic cancer cells possess stem cell-like properties. International Journal of Oncology. 2013;**42**(3):979-984

[115] Ji Q et al. MicroRNA miR-34 inhibits human pancreatic cancer

tumor-initiating cells. PLoS One. 2009;**4**(8):e6816

[116] Lodygin D et al. Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. Cell Cycle. 2008;**7**(16):2591-2600

[117] Nalls D et al. Targeting epigenetic regulation of miR-34a for treatment of pancreatic cancer by inhibition of pancreatic cancer stem cells. PLoS One. 2011;**6**(8):e24099

[118] Hunt S et al. MicroRNA-124 suppresses oral squamous cell carcinoma motility by targeting ITGB1. FEBS Letters. 2011;**585**(1):187-192

[119] Deng S et al. Chronic pancreatitis and pancreatic cancer demonstrate active epithelial-mesenchymal transition profile, regulated by miR-217-SIRT1 pathway. Cancer Letters. 2014;**355**(2):184-191

[120] Zhao WG et al. The miR-217 microRNA functions as a potential tumor suppressor in pancreatic ductal adenocarcinoma by targeting KRAS. Carcinogenesis. 2010;**31**(10):1726-1733

[121] Wei X et al. MicroRNA-21 induces 5-fluorouracil resistance in human pancreatic cancer cells by regulating PTEN and PDCD4. Cancer Medicine. 2016;**5**(4):693-702

[122] Park JK et al. miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. Biochemical and Biophysical Research Communications. 2011;**406**(4):518-523

[123] Zhao JL et al. miR-212/132 downregulates SMAD2 expression to suppress the G1/S phase transition of the cell cycle and the epithelial to mesenchymal transition in cervical cancer cells. IUBMB Life. 2015;**67**(5):380-394

[124] Huang F et al. MiR-196a promotes pancreatic cancer progression by targeting nuclear factor kappa-B-inhibitor alpha. PLoS One. 2014;**9**(2):e87897

[125] Gironella M et al. Tumor protein 53-induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(41):16170-16175