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REVIEW ARTICLE

Molecular Pathology and Methylation Assessment in Pancreatic Cancer; New Approach in Diagnosis and Treatment

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ABSTRACT: Pancreatic cancer is one of the deadliest cancers in world. Patient survival is less than 5%. However, early diagnosis of this cancer is very essential. In this article, we studied molecular pathology, epigenetic change in pancreatic cancer, and discussed the effect of methylation in inception and development of pancreatic cancer. By studying and identifying the genes methylated in this cancer, we can utilize them as biomarkers to be used to diagnose this cancer in a timely manner. Pancreatic cancer is realized as a multistage process characterized by the accumulation of genetic alterations companioned by typical histological conversion in pancreatic ductal cells. DNA methylation is one of the key changes in epigenetics in DNA structure. DNA methylation pattern as biomarker has explicit applications in diagnosis of cancers. Extensive disturbances of DNA methylation have been observed in cancer, causing changes in regulation of gene expression, developing oncogenesis. Understanding both epigenetic changes and DNA mutations promises for improving the characterization of malignancy to predict prognosis and treatment response. By recognition and understanding of molecular pathways and gene changes in this cancer, numerous drugs have been tested for targeted treatment that will allow identifying whole methylation patterns to recognize biomarkers for prognosis and early diagnosis of this cancer in future. By identifying pathways and aberrant methylation, screening and diagnosis are more and more necessary at early stages.

INTRODUCTION

Cancer is a considerable health problem and the second cause of death in the all of the world[1]. According to GLOBOCAN 2018, pancreatic cancer is the 11th cancer and causing 865000 new cases and deaths in developed and developing countries [2, 3]. Most patients are asymptomatic in the in this disease, and patients are identified when they are in the metastatic and at advanced stages of their disease. Thus, this cancer is one of the deadliest malady in world [4]. The 5-year permanence rate for this cancer is less than 5%. The outbreak and mortality of pancreatic cancer worldwide are associated with increasing age and more common in men than in women. Most cases of this disease happen at people over 60 years old [5, 6], and there is not much difference Between rich and poor countries [7]. Although the cause of pancreatic cancer are still not fully

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understood[8]. Despite improvement in the treatment of most solid malignancies, therapeutic targeting, and early detection of cancer in recent years, the mortality rate of pancreatic cancer is actually rising. Pancreatic cancer is expected to become the second prominent cause of death, due to poor prognosis by year 2030[9, 10]. A screening program is needed to progress the prognosis and diagnosis of the disease in the early [11]. Most patients are in advance stage and metastatic when their disease is diagnosed, with only 10% to 15% of patients being candidates for surgical resection. Unfortunately, despite surgery and adjuvant systemic therapies, most patients still relapse, often invading surrounding stromal components, eventually invading distant organs[12, 13]. Most of pancreatic tumors have onset from the epithelium of pancreas fruitful termed as pancreatic ductal adenocarcinoma [14]. The disease is characterized by nausea or vomiting, abdominal pain, and steatorrhea. Diagnosis was relying on a composition of symptoms, containing abdominal pain, high serum amylase or lipase and which be diagnosed as pancreatitis in patients with less serum amylase contents but imaging of acute pancreatitis and would over diagnose pancreatitis due to hyperamylasemia without pancreatitis [15, 16]. CA19-9 is the valid biomarker used in the handling of this cancer. CA19-9 is prosperously used for predicting clinical of this cancer, while this biomarker had sensitivity and specificity for pancreatic cancer diagnosis of 80% patients, progress in specificity and sensitivity for trusty early PDAC detection as favorable clinical benefits have been so far derived [17]. DNA methylation is a main component of the epigenetic mechanism regulating embryonic development, X-chromosome inactivation, chromatin structure, genomic imprinting, and improper DNA methylation associated with cancer development and progression[18, 19]. Identifying the genes and pathways involved in this cancer leads to finding pancreatic cancer therapeutic opportunities [20-22].

In this article, we studied molecular pathology and the genes in which the mutation causes this cancer, also the epigenetic change in pancreatic cancer, and discussed the effect of methylation in inception and development of this cancer. Since, unlike other cancers, no effective therapeutic target for pancreatic cancer has yet been identified, by studying and identifying the genes methylated in this cancer, we can use them as biomarkers to be used to diagnose this cancer in a timely manner.

Molecular pathology of pancreatic cancer

Pancreatic precursor lesions

pancreatic cancer phenotype divided to three distinctive regions with gene variations, mucinous cystic neoplasms, intraductal papillary mucinous neoplasms, and pancreatic intraepithelial neoplasia [23]. Perceived in 82% of pancreatic cancers, PanINs are the high frequent preneoplastic harms of the pancreas. Unlike microscopic lesions PanIN23, IPMNs and MCNs can be detected using CT and MRI to improve prognostic[24] .

Alterations in oncogenic molecular pathways

The pancreatic adenocarcinoma expands approximately particularly from the ductal epithelium cells it is responsible for causing cancer in 85% of patients. Other pancreatic cancers contain the acinar cell carcinoma originating from the exocrine acini of the pancreas, tumors of neuroendocrine arising from neuroendocrine cells, and other cells of pancreas [25, 26].

pancreatic cancer is currently realized as a multistage process determined by the accumulation of genetic variations companioned by typical histological and morphological conversion in pancreatic ductal cells[27]. Almost 10% of pancreatic cancers be develop to have an inherited component. Sporadic and familial pancreatic cancers contribute to the same driver mutations. The abundant variations of pancreatic ductal adenocarcinoma are SMAD4, TP53, CDKN2A, and KRAS. KRAS, is modified in the pancreatic cancers, most of patient about 95%, activated by point mutations at codon 12 and TP53 mutation at codon 273, mutation in KRAS have been observed in patients with chronic pancreatitis (CP) [7, 28-30]. KRAS variations are significant abnormalities in the development this cancer, while the change of p16/CDKN2A is formerly Recognizable at the early PanIN stages. The mutation of SMAD4/DPC4 and TP53 is related with cancer development. Common genetic mutations recognize in pancreatic cancer include activation of Her-2/neu, mutation in tumor suppressor genes such as p16, p53, and SMAD4 [31, 32].

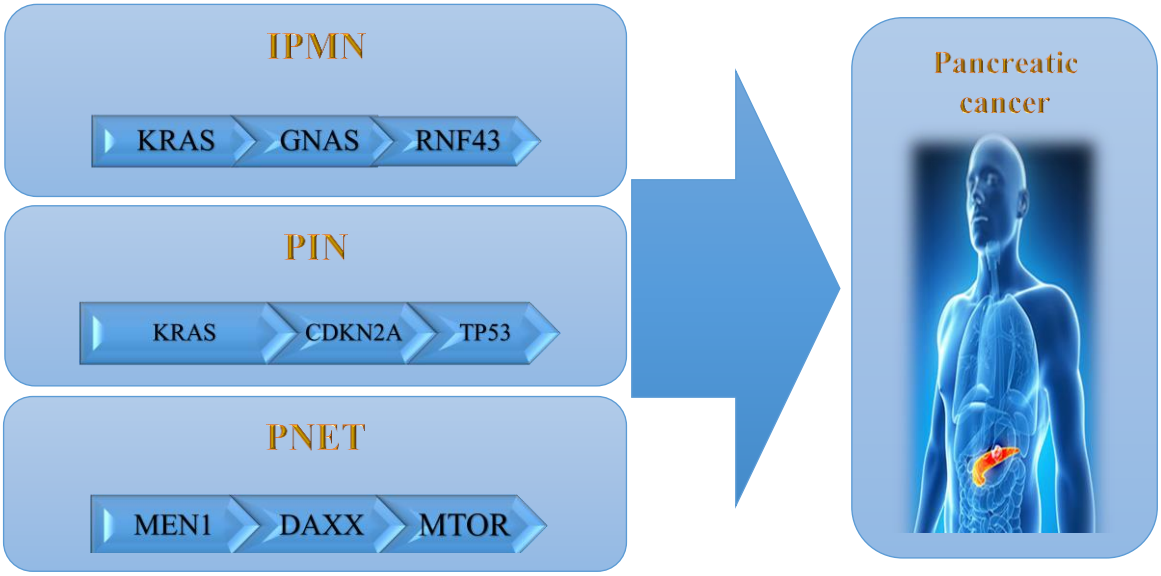

Figure 1. Molecular variation in three regions of pancreas.

Genes that mutate in PNETs are commonly diverse from mutated in type exocrine pancreatic cancers. For instance, KRAS mutation is invisible in type PNETs, as long as mutations in PNET tumors occur in DAXX, MTOR, and MEN1 genes. Genetic variation has been observed in PINs including CDKN2A, TP53, KRAS, and SMAD4. In IPMN mutations observed in KRAS, GNAS, and RNF43 genes (Figure 1) [27, 33]. Hereditary pancreatitis is caused by a variation in the trypsinogen gene Known by (PRSS1) whereas changes in the serine protease inhibitor gene Known by (SPINK1) reason an autosomal recessive form of hereditary form pancreatitis. hereditary form pancreatitis in patients have a considerable 58-fold increased risk of advancing pancreatic cancer and a risk pancreatic cancer of 30–40% at the age over of 60 [34, 35]. Sequencing technique and bioinformatics have converted our comprehension of genetic conversions associated with the genesis and development of cancer and pancreatic cancer. Besides, this is considerable that this abundant number of multiple gene mutations converges pathways and processes, including Hedgehog pathway, Wnt/β-catenin, axon conduction, DNA repair, NOTCH and chromatin remodeling pathways, it shows these conversions may function through certain processes, may suggest important note for therapeutic intervention[36, 37].

Pancreatic cancers an average of nearly 60 genetic alterations. Sequencing analyses of manifold pancreatic cancers have disclosed many pathways changed by mutations in PDAC, including many cases that have yet to be totally defined in the cancer. Moreover mutations and copy number alterations were found in genes affecting axon direction pathways. Hence, identifing the significant pathways controlling PDAC improvment is necessary for an improved conception of the malignancy [36, 38, 39]. A bout of 12 signaling pathways has been described by genomic analysis and the susceptibility loci have been identified by a genome association study pathways in cancer involves several pathways, including the Janus kinase-signal transducer, ErbB pathway, phosphoinositide 3-kinase-Akt pathway, activator of transcription (Jak-STAT) signaling pathway and p53 signaling pathway [21, 22, 40, 41]. It is known that similar to EGFR, TGF-β is a growth factor for pancreatic cancer. Enhanced levels of TGF-β are found in tumor tissue, and studies have revealed that it may serve as a marker for tumor progression and few survivals in patients with pancreatic cancer Hedgehog pathway has been involved as playing a significant impress in the progression of pancreatic cancer. Hh signaling is required for tissue patterning, morphogenesis, and stem cell maintenance in pluricellular embryos. The coincidence of uncontrolled activity of the RAS and Hh pathways in primary stages of this cancer indicate that cross-talk between these two pathways may be a very substantial mechanism for the initiation and extension of pancreatic cancer [42-44].

Transcription of the modified KRAS gene generates an unusual RAS protein, consequencing in the improper activation of proliferative signaling pathways. In addition, in 95% of cancers, CDKN2A gene has inactivation with the outcome loss of the p16 protein, leading to a gain cell proliferation. In 30-50 % of cancers, mutations have occurred in TP53permitting cells to bypass DNA damage control checkpoints and apoptotic signals and assistanceing to genomic inconstancy [24, 43, 45].

Mutated Genes in which the risk of cancer increase are BRCA2, BRCA1, FANCG, the Fanconi anaemia genes FANCC and ataxia telangiectasia mutated (ATM), are ingredients of the repair DNA. Variation in these genes (especially BRCA1 and BRCA2) increase instability of genome during damaged homologous recombination [46]. Hh pathway has a synergistic result with activated K-ras signaling by reducing tumor cell association on preserve activation of MAPK and phosphoinositide 3-kinase PI3-K/Akt/mTOR signals, thus enhancing the K-ras-induced pancreatic cancer increase. In pancreatic cancer, the downstream nuclear transcription factor GLI1 of the Hh signaling pathway is not exactly dependent on upstream Shh-Ptch-Smo signals and is arranged by TGF-β and KRAS signals[47].

Methylation and its role in Incidence of cancer

Epigenetic is described as inheritable conversions in expression of gene without alterations in sequence of DNA. The primary epigenetic mechanisms that may result gene expression include DNA methylation, microRNA expression and histone modification. Convenient methylation of DNA is substantial for progress and appropriate cell functioning; thus, any disorder in this process may lead to diverse diseases, containing cancer. Furthermore, tumor cells are defined by a different from that of normal cells [48-50].

In mammals' cells, DNA methylation is one of the key changes in epigenetics in DNA structure. After being built in DNA compound, cytosines within the CpGs islands are methylated at carbon 5 through enzymes DNA methyltransferase (Figure 2). Dinucleotide CpGs, has been methylated, could be either in two parts of the genome containing single or CpG islands. In recent studies, detract 5hmC has been reported in many cancers, containing kidney tumors, acute myeloid leukemia and other cancers [51, 52].

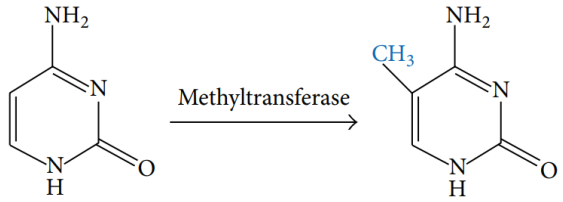

Figure 2. Addition of methyl group to cytosine by enzyme DNMT1

Cytosine in DNA structure can be methylated to 5-methylcytosine (5mC), initially observed, leading to the suggestion that variations in methylation may contribute to development of tumor. DNA change of methylation patterns in cancer is unusual hypermethylation into regions of genes, hypermethylation can be completely related with transcriptional silencing, an alternative mechanism to conversion for the inactivation of genes with tumor-suppressor function. Epigenetic mechanisms that arrange processes in eukaryotic cells change in apoptosis, cell cycles, proliferation, differentiation, and invasiveness; it is therefore substantial in the initiate and development of cancer [51, 53]. Aberrant DNA methylation was the first epigenetic marker to be associated with cancer as the result of the alteration leading in normal gene regulation. These changes are divided into three sections: loss of imprinting, hypermethylation, and hypomethylation[54]. Three DNMTs enzymes have been recognized in eukaryote calls. methyltransferase identifying hemimethylated DNA generated during replication of DNA and then methylating lately synthesized CpG [55-57]. Recently,

reduction of methylated cytosines has also been reported in human cancers, including kidney tumors, breast cancer, acute myeloid leukemia and liver cancer [30]. Several CpG island promoters become methylated during extension, outcoming in transcriptional silencing imprinted genes and X-chromosome silencing are common examples of such naturally occurring CpG methylation during DNA extension. Methylation may direction to gene silencing by either promoting or preventing the employment of regulatory proteins to DNA[58]. Loss of methylation of DNA in cancer commonly happens at centromeric repeats and repetitive sequences and it has been offered that it is associated with loss of imprinting, chromosomal instability and reactivation of DNA transposons[59].

Methylation in pancreatic cancer

Variations in methylation of genome have been observed in abundant cancers, including pancreatic cancer. These variations can be discovered in pancreatic cancer, in tissue, and in pancreatic conversions in methylation that have been also observed in chronic pancreatitis[60]. Multiple classic tumor suppressor genes, also increasing the number significant genes, show improper CpG Island of promotor hyper methylation in cancers of pancreati. DNA methylation of the p16 and APC genes is exclusively essential during multistage carcinogenesis of the pancreatic cancer, even from the early stages of pancreatic cancer. CDKN2A/p16 was the first gene demonstrated hypermethylation and silencing of promotor in pancreatic cancer patients. In patients of pancreatic cancer other genes of tumor suppressor like TP53, STK11/LKB1, and SMAD4 are inactivated and have not been displayed to silencing by methylation [61-63].

In actuality, various genes such as P16INK4a, APC, BRCA, P15INK4b, RARβ, and p73 are mostly methylated. novel studies have disclosed that DNA methylation occurred in significant signaling pathways in PDAC as TGFβ, WNT, cell adhesion, integrin, and axon conduction signaling pathways[64]. Using the candidate gene, seven overexpression genes LCN2, MSLN, CLDN4, PSCA, S100A4, SFN, and TFF2 were identified in pancreatic cancer when contrasted to normal pancreatic duct because of hypomethylation[65]. Improper promoter methylation was found in various genes in pancreatic carcinoma (p16, RARb, CACNA1G, TIMP-3, THBS1, and hMLH1), and occurred for each gene at a bout of 5–20%. These studies indicate that improper hypermethylation can be a regular mechanism of gene of tumor suppressor inactivation in pancreatic cancer. Methylation of these genes has been displayed to be correlated with damage of their expression. Aberrant methylation of the studied four CpG, for example (MINT1, -2, -31, and -32) occurred at 11–73% [66, 67].

Accordingly, 140 genes may be improperly inactivated by methylation in a pancreatic cancer, 60 of which would be anticipated to be CpG islands. Conversions in methylation templates presentation a definitive role in expansion and development of cancer[24]. The genes recognized as improperly methylated in pancreatic cancer have recognized to have substantial attributes involved in cell cycle such as apoptosis, cell cycle regulation and cell adhesion. Inactivating of genes and improper methylation may be significant for this carcinogenesis[68].

From 1200 nomination hypermethylated genes, almost genes were formely recognized using MCAM on the 44K microarray and evaluated by MSP in pancreatic cells of cancer, for example: SOX14, CNTNAP2, PKP1, BAI1, KCNV1, EYA4, BNC1, TLX3, ACTA1, MDFI, EVC2, NRN1, PENK, and ZNF415. Methylation profiles were confirmed by genes whose epigenetic silencing was disclosed by researchers in several studies. Identified genes include FOXE1, PAX6, CACNA1G, CCND2, NPTX2, CLDN5, LHX1, BNIP3, GADD45B, HS3ST2, TWIST1, CCNA1, ALPP, CEBPA, and TFPI-2. Moreover, like the included transcriptional repressor like CBFA2T3, genes of tumor suppressor like STK11, transcription factor, also RUNX3, secreted glycoprotein and aryl-hydrocarbon receptor repressor recognize genes generally and differentially methylated in this cancer as the selection criterion of these genes. Therefore, multiple important genes infrequently methylated in pancreatic cancer contain E-cadherin/CDH1, p16/CDKN2A, and hMLH1 since these genes are infrequent targets in this cancer. In examined patients and normal pancreas, hypermethylation in CDKN2A was perceived in 30% of patients. Finally, all genes that were methylated in

diverse kinds of pancreatic cancer (PIN, IPMN, CP, and invasive pancreatic cancer) are illustrated in Figure 3. Several genes were hypermethylated in pancreatic cancer were not detected because their promoters were not investigating by MCAM method (SPARC, MMP3, MMP2, MMP9, and MMP) [65, 69-71]

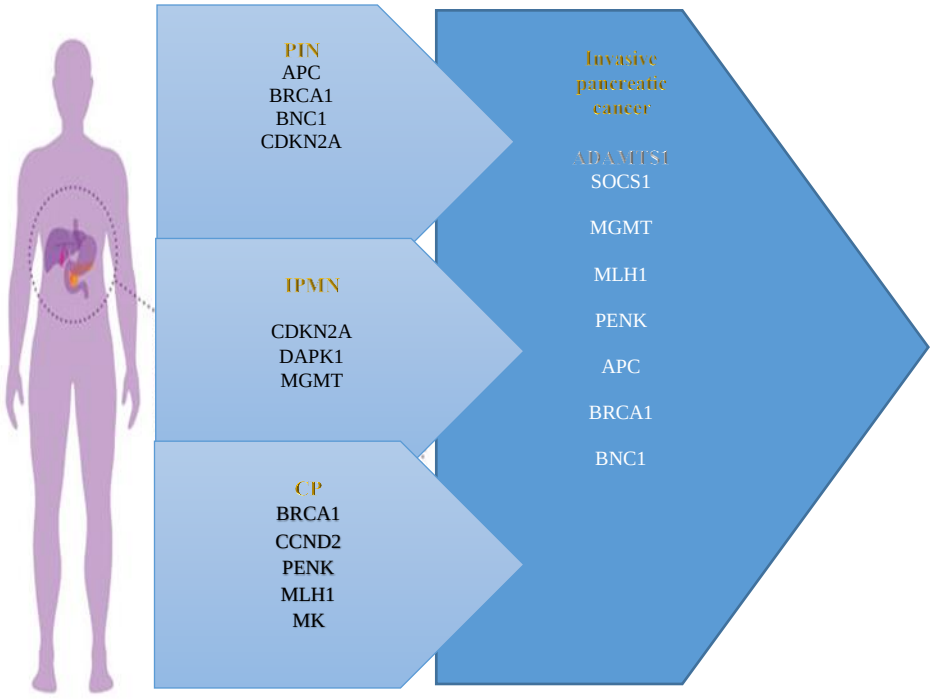

Figure3. Methylation pattern genes in stages of pancreatic cancer

DNA methylation as marker for diagnosis of pancreatic cancer

Describing the DNA methylation has been effective to recognize not only recent DNA methylation biomarker candidates but also tumor suppressor gene candidates. DNA methylation pattern as biomarker has explicit applications in diagnosis of cancers, but it can also indirectly contribute to therapeutics as predictors of response to therapy [72]. Methylation of genome is a regulator of gene expression usually resulting in gene silencing. Extensive disturbances of DNA methylation have been observed in cancer, causing changes in regulation of gene expression developing oncogenesis. Understanding both epigenetic changes and DNA mutations promises for improving the characterization of malignancy to predict prognosis and treatment response [73]. Compared to patients with benign pancreatic diseases, a highly considerable number of hypermethylated genes are in patients with pancreatic cancer. Hypermethylated genes such as MESTv2, BMP3, RASSF1A, BNC1, TFPI2, SFRP1, APC, and SFRP2 is capable to differentiate between patients with pancreatic adenocarcinoma and control group [67].

In patient for genes ADCY1, AK055957, KCNK12, ELMO1, and PRKCB, methylated candidates were more than 100-fold greater compared to the healthy people. In these patient, more than 10 hypermethylated genes have been observed in these patients with essentially shorter survival time , compared to patients with fewer hypermethylated genes Hypermethylation of TFPI2, SFRP1, and BNC1 were associated with poor survival for final stage (stage IV) disease; these genes can be significant biomarkers of pancreatic cancer[74, 75].

CONCLUSIONS

According to recent advances in targeted treatment of all types of cancers, pancreatic cancer remains an approximately lethal disease. Although there has been significant development in understanding the molecular biology of pancreatic cancer, these advances have not led to effective therapy of the cancer. Number of patients with cancer is permanently rising and pancreatic cancer will be second most reason of cancer-relevanted death by 2030. Therefore, recent therapeutic strategies for patients with pancreatic cancer are strongly needed. Diagnosis of cancer is based on liver biopsy or endoscopic ultrasound-guided fine-needle aspiration method is known (EUS-FNA). Thus, precise studies depend on well quality of tumor models.

This cancer is diagnosed when it is too late for surgery. Due to poor prognosis in pancreatic cancer, five classes of cytotoxic drug in treatment of these patients (gemcitabine, fluoropyrimidines, oxaliplatin, irinotecan-nanoliposomal or not-nab-paclitaxel) have now demonstrated their effect in PC.

Due to the recognition and understanding of molecular pathways and gene changes in this cancer, numerous drugs have been tested for targeted treatment, such as pembrolizumab in patients with the mutation ERBB2 gene, mfolfox-6 in patients with the mutation of BRCA2, or niraparib in those with mutation of PARP, but they have not yet approved by FDA. Therefore, there is currently no therapeutic target for treating this cancer; beside, methylation of tumor suppressors has a significant role in the development of this cancer. Detecting variation in DNA methylation can be used as a promising way to discover biomarkers for the recognition of pancreatic cancer. in recent years, advancement of sequencing methods in the diagnosis of diseases and development of personalized medicine have allowed identifying whole methylation patterns to recognize biomarkers for prognosis and early diagnosis of this cancer in future. By identify pathways and aberrant methylation, screening and diagnosis are more and more necessary at the primary stages. We hope to treat these patients at their early stages. (Strength points of article).

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