Review

Mutated Genes in Pancreatic Cancer

Song-bing He, De-chun Li*

Department of General Surgery, the First Affiliated Hospital, Soochow University, Suzhou 215006, China

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ABSTRACT

Pancreatic cancer continues to be a deadly malignancy with still high mortality and poor survival. Little progress has been made on the treatment of advanced pancreatic cancer despite the significant advances in understanding, diagnosis, and access to conventional and novel treatments. Molecular pathology of the lesion is the key of our understanding of the mechanisms underlying the development of this cancer and will probably help us in earlier diagnosis and better therapeutic results. New treatment strategies and a more careful evaluation of innovative therapies are clearly needed for this disease. In view of many findings pancreatic cancer should be regarded as a systemic disease, and over the last several years, investigators have gained a better understanding of the molecular biology and events that lead to the development of malignancies. We here review the novel developments in the systemic exploring for commonly mutated genes in pancreatic cancer.

Key words: Pancreatic cancer; Epithelial growth factor; Matrix metalloproteinases; Oncogenes

INTRODUCTION

Pancreatic cancer (PC) is one of the most lethal cancers worldwide, as indicated by its mortality incidence ratio of 98%[1]. The prognosis for pancreatic cancer is extremely poor, and the high mortality can be attributed to the ambiguous symptoms, a distinct lack of early diagnostic methods and a lack of effective therapy[2]. Surgery remains the only curative treatment option for this disease but 80% of pancreatic cancer patients have unresectable or metastatic disease[3,4]. Moreover, adjuvant chemotherapy or radiotherapy was assessed in several trials in order to improve patients' prognosis[5,6]. Thus, most patients receive palliative treatment with the aim of an improved quality of life.

In view of many findings pancreatic cancer should be regarded as a systemic disease, and over the past few decades, knowledge of the genetic defects involved in tumor formation and growth has increased rapidly. There is an imperative need of analyzing and understanding the primitive lesions that lead to invasive pancreatic cancer. Identification of cancer escape mechanisms and critical cell survival pathways has launched the development of novel antitumor agents. This review will focus on the recent developments in the systemic exploring for commonly mutated genes in pancreatic cancer.

K-ras

K-ras activation is known to contribute to cancer cell survival in a number of different tumor types and experimental systems. In mouse models of cancer, somatic activation of oncogenic K-ras is necessary for the early onset of tumors, and its continuous production is necessary for the maintenance of tumor viability[7]. Molecular genetic analysis has identified that activating K-ras mutations, as the earliest and most common genetic mutations in pancreas cell transformation and tumor progression, are found not only in 70% to 95% of pancreatic carcinoma tissues but also in pancreatic juice, fine-needle aspirations of

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*Corresponding author.
E-mail: hesongbing2@yahoo.com.cn

the pancreas, duodenal fluid, and blood and stool of pancreatic cancer patients[8,9]. Recent study demonstrated that the introduction of a promoterless full-length K-ras cDNA could efficiently suppress endogenous K-ras gene expression in human pancreatic cancer cells. This inhibition was achieved when the adequate levels of the responsible effectors were reached. Knockdown of K-ras expression in pancreatic cancer cells resulted in slower cell proliferation and lower tumorigencity in mice[10]. Moreover, K-ras mutations have been reported as a negative prognostic factor after surgery and adjuvant chemoradiation in pancreatic cancer[11,12].

p53

In human cancers, the most frequent mutant gene is p53, which has a central position in cell cycle regulation through its role in inactivating a variety of genes and interrupting cell proliferation at G1/S checkpoint. p53 mutations cause loss of the above functions and are central in carcinogenesis leading to uncontrolled cell growth and proliferation, increased cellular survival and chromosomal instability. It has been reported that p53 mutations are found in up to 76% in pancreatic cancer[13,14]. Usually, mutations of p53 are not specific but rather sporadic and are resulting in the production of a mutant p53 protein which lives longer than the wild type(normal) p53 protein. Furthermore, p53 mutations may lead to resistance to chemotherapy treatments due to impaired p53-induced apoptosis[15]. There is emerging evidence that the oncogenic potential of human and/or murine double minute-2 protein(hdm2)stems not only from its ability to counteract tumor suppressor p53 but also from its less understood p53-independent functions. Sui X et al concluded from their results that hdm2 is expressed in pancreatic cancer cells as a result of activated Ras signaling, and that it regulates cellular proliferation and the expression of three novel target genes by p53-independent mechanisms[16]. However, whether these mutations have also a prognostic significance remains unclear. Few researchers have suggested a shorter overall survival in pancreatic cancer patients carrying p53 mutation compared to p53(-) patients[13,17].

p16

Mutation and loss of p16 activity results in absence of the inhibitory effects at the level of cyclin D-CDK4 interaction, thereby promoting cell cycle progression. Genetic alterations of this locus through gene mutation, deletion, or promoter hypermethylation are found in 80% to 95% of sporadic pancreatic cancers[18]. Genetic analyses have shown that p16 alterations are very common in pancreatic adenocarcinomas but these alterations are not necessarily seen in cultured cell lines. Moreover, loss of p16 expression could be correlated with less differentiated tumors, shorter overall survival, and the presence of metastatic disease[19,20]. The polymorphic genotypes of the p16 gene were associated with significantly shorter time to tumor progression and poorer response to therapy in pancreatic cancer[21]. Functional inactivation of the tumour suppressor p16 marks a key event in pancreatic carcinogenesis. Conversely, reconstitution of the biological action of p16 provides an attractive therapeutic approach. Schulz P et al addressed the consequences of p16 re-expression in MiaPaca-2 pancreatic cancer cells in vivo in the orthotopic tissue context. They found p16 capable of reducing primary tumor growth. In addition, p16 restitution resulted in a marked reduction of tumor angiogenesis, largely accounted for by a p16-dependent inhibition of lymphangio-genesis[21].

DPC4/SMAD4

Another tumor suppressor gene which is involved in pancreatic cancer pathogenesis is the DPC4 gene also known as SMAD4. In normal cells the product of this gene plays a role in TGF-β mediated signal transduction, gene transcription and growth arrest[22]. Inactivation of DPC4 facilitates uncontrolled cellular growth and proliferation. It is found that about 50% of pancreatic cancers present mutant genes[24]. Loss of the DPC4 expression is a rather late event in the pathogenesis of pancreatic cancer, as this gene was expressed normally in PanIN1 and 2 and only in 30% of cases with PanIN3[23,24]. Cao D et al utilized the Gene Logic Inc. BioExpress™ platform and Affymetrix U133 GeneChip® set to determine the changes in gene expression associated with SMAD4 gene inactivation in a series of well-characterized pancreatic cancer cell lines to define the downstream effects associated with SMAD4 gene inactivation, which offers insight into the role played by SMAD4 in pancreatic cancer, as well as providing potential novel targets for the development of molecular therapeutics in pancreatic cancer[25]. Pancreatic cancers are represented by distinct genetic subtypes with significantly different patterns of failure. Determinations of DPC4 status at initial diagnosis may be of value in stratifying patients into treatment regimens related to local control versus systemic therapy[26]. Whether the DPC4 status has a prognostic value remains controversial, as in a few studies positive DPC4 status was associated with better outcome and survival post resection[25,27],

but in other studies DPC4 expression was associated with worse outcome after surgery or adjuvant chemotherapy[23,28].

HER-1/EGFR

Changes in the expression of a range of proteins involved in the control of the cell cycle, proliferation, apoptosis, and invasiveness, such as human epidermal growth factor receptor (HER-1/EGFR) play a critical role in the development of pancreatic cancer[29]. In pancreatic cancer, amplification of the EGFR gene has been demonstrated by many researchers but the incidence rate ranges from 16% to 65%[30]. The coexpression of HER-1/EGFR has been shown to stimulate tumor cell proliferation, and elevated HER-1/EGFR levels are linked to poor disease outcomes and lower sensitivity to chemotherapy[31]. The signal activated by EGFR, penetrating into cell nucleus, accelerates protooncogene phosphorylation, and it is therefore assumed that a serious prognosis of pancreatic cancer is also due to high expression of EGFR and its gene polymorphism[32]. Increased EGFR gene copy number and elevated EGF levels are present in a significant proportion of pancreatic cancer patients, and this may reflect increased EGFR pathway dependence with improved sensitivity to EGFR-targeted therapy[33,34]. Agents that target the HER-1/EGFR pathway are at the forefront of development, and a significant number of ongoing trials continue to explore the potential of these therapies for this disease[35].

COX-2

Cyclooxygenase(COX)-2 is inducible by cytokines and intracellular signals produced at sites of inflammation, it can also be induced in various normal tissues by the hormones of ovulation and pregnancy, growth factors, oncogenes and tumor promoters. Experimental data regarding COX-2 biology have also accumulated in pancreatic cancer[36]. Preclinical studies testing the COX-2 expression in pancreatic cancer cells in comparison to PanINs, chronic pancreatitis specimens and normal pancreatic cells, demonstrated overexpression of COX-2 in adenocarcinomas in contrast to non-malignant tissues; additionally, in adeno-carcinomas overexpressing COX-2 versus non-expressing ones there was a positive association with severity and poor outcome[37,38]. A recent study identified that the COX-2 expression is significantly correlated with the tumor size in pancreatic cancer, which offers a new insight into the regulation of growth and development of metastases in pancreatic cancer[39]. A correlation between COX-2 and p53 expression levels in carcinomas was revealed, and an accumulation of p53 was associated with COX-2 overexpression in premalignant and malignant ductal lesions, which offers opportunities for targeted therapy and chemoprevention of pancreatic cancer using COX-2 inhibitors，especially in lesions with functional p53[40].

NF-κB

Nuclear factor-kappa B (NF-κB) is a family of cytoplasmic molecules, in normal conditions, which is in inactive status by binding to proteins IkBa and p100. As in many other cancers, NF-κB is activated in pancreatic cancer cells, playing central role in its pathogenesis, progression, invasion and metastasis [41,42]. It has been reported that NF-κB overexpression is common in pancreatic cancer. During the last few years a new molecular pathway, the hedgehog (hh) signalling, has been found to play a role in pancreatic carcinogenesis. Inhibition of Shh by cyclopamine (a naturally occurring steroidal alkaloid) resulted in apoptosis induction and blockage of pancreatic cancer cell lines proliferation, both *in vivo* and *in vitro*[43]. It is postulated that Shh overexpression is regulated by activation of NF-κB and consequently inhibition of NF-κB results in down-regulation of Shh[44]. Another recent study demonstrated that pancreatic cancer cells resistant to gemcitabine exhibit a high basal NF-κB activity[45].

MMPs

Matrix metalloproteinases (MMPs) are a collection of proteases normally used for tissue remodeling that have been found to be upregulated in the setting of many tumors, including pancreatic cancer. MMP-2 and MMP-9 are likely the most implicated in cancer invasion and metastases among many MMPs[46]. As showed in a study by Schneiderhan W et al, pancreatic stellate cells (PSC) represent a significant source of MMP-2 in pancreatic cancer, which is known to promote tumor progression. Moreover, cancer cells release basigin (BSG) to stimulate MMP-2 in PSC, indicating that cross-talk of cancer cells and mesenchymal cells might further increase MMP-2 production in the desmoplasia[47]. Moreover, MMP-2 expression correlates with advanced stages of disease and poor prognosis[48]. TNF-related apoptosis-inducing ligand (TRAIL) can stimulate the expression of MMP-9 in pancreatic cancer cell lines resulting in the enhancement of invasion involving the signaling pathways of caspases, MEK1/2, PKC and NF-κB[49]. Along with

overexpression of specific MMPs in advanced cancer, there is production of the tissue inhibitor of the metalloproteinases (TIMP) which aims to counteract the untoward effects of MMPs in pathological situations[48]. The described pathways outline the perplexity of the molecular pathways and their cross talk especially in highly uncontrolled conditions as in malignancies. It seems MMPs play a role as an "angiogenic switch", to facilitate the expression of proangiogenic factors such as VEGF and bFGF in order to overcome the negative signals of angiogenic inhibitors such as trombospondins, angiostatins, and INFs[50]. Due to these properties, the inhibition of MMPs represents the scientific rationale for the development of chemotherapeutic agents against pancreatic cancer[51].

In conclusion, with the comprehensive understanding of its biology, a number of molecularly targeted agents have evolved, which specifically interfere with major pathways involved in pancreatic cancer pathogenesis. Targeted therapy is a promising strategy for pancreatic cancer, where current strategies have failed to significantly improve survival. Furthermore, a biologic and molecular staging of pancreatic cancer may lead us to earlier diagnoses, efficient counseling, and better therapeutic approaches. The ultimate goal would be a tailored-made treatment for maximal therapeutic results.

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