Genetic alterations in pancreatic cancer

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Abstract

The diagnosis of pancreatic cancer is devastating for patients and their relatives as the incidence rate is approximately the same as mortality rate. Only a small percentage, which ranges from 0.4% to 4% of patients who have been given this diagnosis, will be alive at five years. At the time of diagnosis, 80% of pancreatic cancer patients have unresectable or metastatic disease. Moreover, the therapeutic alternatives offered by chemotherapy or radiotherapy are few, if not zero. For all these reasons, there is an imperative need of analyzing and understanding the primitive lesions that lead to invasive pancreatic adenocarcinoma. Molecular pathology of these lesions 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. This review focuses on medical research on pancreatic cancer models and the underlying genetic alterations.

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INTRODUCTION

The diagnosis of pancreatic cancer is devastating for patients and their relatives as the incidence rate is approximately the same as mortality rate. Only a small percentage, which ranges from 0.4% to 4% of patients who have been given this diagnosis, will be alive at five years[1,2]. At the time of diagnosis, 80% of pancreatic cancer patients have unresectable or metastatic disease[3]. Moreover, the therapeutic alternatives offered by chemotherapy or radiotherapy are few, if not zero. For all these reasons, there is an imperative need of analyzing and understanding the primitive lesions that lead to invasive pancreatic adenocarcinoma. Molecular pathology of these lesions 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. This review focuses on medical research on pancreatic cancer models and the underlying genetic alterations.

CARCINOGENESIS IN PANCREAS

Histologically the development of adenocarcinoma of the pancreas has its roots in cuboidal ductal epithelium alterations. These alterations are named PanIN (pancreatic intraepithelial neoplasia) and are classified into different progressive types (Figure 1). The PanIN-1A lesions present only minimal alterations from the normal epithelium, such as tall columnar cells with some crowding while the PanIN-1B lesions present increased crowding of columnar cells with papillary projections. The PanIN-2 lesions apart from previous alterations develop nuclear atypia. Finally, the PanIN-3 lesions present atypical ductal hyperplasia with severe atypia and are more likely to progress to invasive carcinoma[4].

With the example of proposed progression model for colorectal neoplasia in mind, scientists tried to propose a model of progression for pancreatic neoplasia using the multi-hit hypothesis. The concept is the following: the first hit seems to be the point when mutations in the K-ras oncogene and overexpression of the HER-2/neu gene product occur. If some of these altered cells survive, they are susceptible to the second hit which is the inactivation of the p16 tumor suppressor gene. The third hit is represented by the loss of the tumor suppressor genes p53, DPC4, and BRCA2[5-10]. This theory is supported by the experimental work of Rozenblum *et al*[11] who analyzed the DNA from 42 pancreatic adenocarcinomas for alterations in the K-ras, p53, p16, and DPC4. They found that all 42 (100%) carcinomas presented point mutations in the K-ras oncogene, 82% genetic alterations in p16, 76% in p53, and 53% in DPC4. Concomitant activation of K-ras gene with inactivation of all three suppressor genes was presented in 38% of the tumors studied. Moreover, all these mutations had their origin in somatic cells[11].

These genetic alterations are correlated with histological findings of metaplasia, hyperplasia, dysplasia, and neoplasia. In addition, they most likely represent the precursor lesions for pancreatic adenocarcinoma. We shall try to present the genetic alterations of pancreatic cancer in more detail with the aim of better understanding and thus, earlier intervention.

CELL-CYCLE REGULATORS

The cell division cycle in pancreatic carcinoma, as other tumors, is an extremely complicated process. It is regulated by three major protein players, which act at particular checkpoints and permit, or not, the progression of cell division: (1) The cyclin dependent kinases (CDKs); (2) The cyclins; (3) The cyclin-dependent kinase inhibitors (CKIs).

In general, CDKs form complexes with their regulatory subunits named cyclins in order to help the cell to enter the S-phase. CDKs phosphorylation and CKIs are inhibitory signals for the complex activation process and consequently, for cell division progression. When the cell is found at G_1 checkpoint, before starting DNA replication, it has two possibilities: the first two possibilities: the first to progress to cell division and the other to remain in a quiescence state. The activation of CDK4 by cyclinD with the formation of CDK4/cyclinD complex leads the cell beyond the restriction point. The next step is hyperphosphorylation of the retinoblastoma protein, Rb, catalyzed by CDK4/cyclinD or CDK2/cyclinE complex. The phosphorylation results in the dissociation of Rb from its complex with transcription factors such as E2F with immediate consequences on activation of target genes that are required for G_1/S transition[12,13].

The oncogene products (p21, p16, p27) act as CKIs by blocking the hyperphosphorylation of the Rb oncogene *via* inactivation of CDK4/cyclinD and CDK2/cyclinE complexes. The cell thus cannot traverse the G_1/S checkpoint. Moreover, the p53 tumor suppressor gene can activate CKIs. When DNA alterations or negative external signals are present, p53 gene product is increased and stimulates transcription of the p21 gene, as a CKIs[14-17].

TELOMERASE ACTIVITY

Enzymes like telomerase play pivotal roles in cell-cycle regulation and have important implications in cell immortality. Telomeres have the property of not being reattached once they have been cut off from their fellow chromosome. Chromosomes lose 50-100 nucleotides from their telomeric sequence with every division. In this way, chromosomal length is reduced and programmed cell death may ensue. The stabilization of telomeric sequences is attributed to telomerase activation.

All normal somatic cells, with the exception of proliferating cells of self-regenerating tissues, do not present telomerase activity compared to malignant tissues. Reactivation or upregulation of telomerase has been detected in many types of cancer such as breast, lung, and bladder, gastric and colorectal cancer. Hiyama *et al*[18] using the TRAP assay (telomeric repeat amplification protocol), a highly sensitive PCR-based telomerase assay, tried to detect
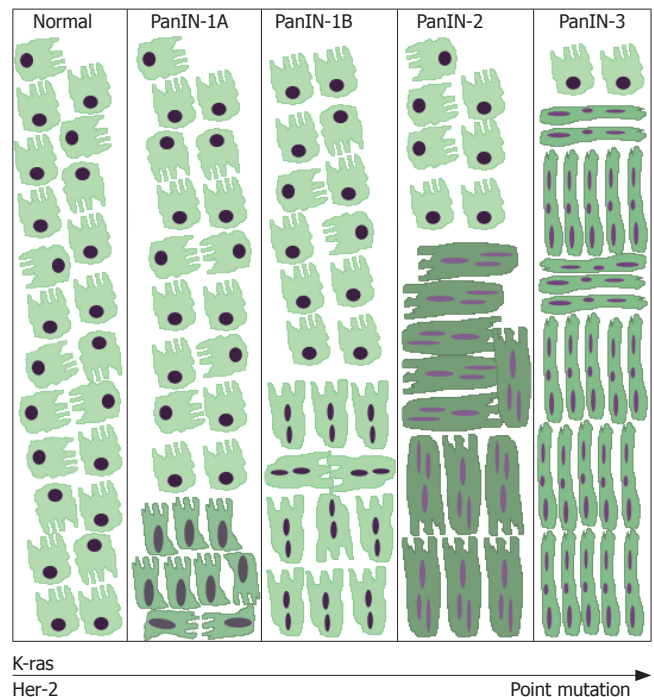
Figure 1 The most important genetic abnormalities associated with pancreatic cancer.

if there was telomerase activity in pancreatic tissue cancer and if possible, to correlate these results with telomere length. The authors studied 43 pancreatic cancer tissues, 11 benign tumors tissues, 3 chronic pancreatitis tissues, and 6 metastatic lesions from patients with pancreatic cancer. Telomerase activity was detected in 41 out of 43 (95%) pancreatic cancer samples analyzed, in all metastatic lesions (100%), but in none of the benign lesions. Unfortunately, the range of telomere length was the same in the malign and the benign lesions. This study showed the future utility of this enzyme in pancreatic cancer diagnosis.

ONCOGENES

Oncogenes are genomic sequences that are activated under special conditions. Activation results in initiation of carcinogenesis either through encoding protein up-regulation or through encoding proteins with altered function.

The family of the ras protein is synthesized in the cytoplasm and arrives at the inner surface of cytoplasmic membrane with the role of transforming an inactive guanosine 5'-diphosphate (GDP)-bound form into an active guanosine 5'-triphosphate (GTP)-bound form. Ras oncogene, when it is found in its active form or under pressure of external signals, activates several downstream effectors such as Raf-1, Rac, Rho, or phosphatidynil-3 kinase (P13K) with important implications for cell differentiation and proliferation[19,20].

Mutations in K-ras oncogene are point mutations, a single amino acid change. They are located mainly on codon 12 and, rarely, on codon 13 and 61. K-ras gene mutations are found not only in 70%-95% of pancreatic carcinoma tissues but also in pancreatic juice, fine-needle aspirations of the pancreas, endoscopic retrograde

cholangiopancreatography brushings, duodenal fluid, and blood and stool of pancreatic cancer patients[21,22]. Wilentz *et al*[23] examined the duodenal fluid of patients with periampullary cancer for K-ras mutations. The results of this study showed a high specificity (100%) but a low sensitivity of K-ras mutations[23]. Of crucial clinical importance is the observation by Berthelemy *et al*[24] that pancreatic secretions may present cells with these genetic alterations even one year before the diagnosis of pancreatic cancer. Contrary to that, K-ras mutations may be present as benign condition in chronic pancreatitis without evidence of progression to pancreatic cancer even after 78 mo of follow-up[25].

K-ras mutations seem to be prerequisite for pancreatic carcinogenesis. The K-ras mutation rate increases with advancing cellular atypia. This mutation, in association with other genetic alterations, may help to identify precursor lesions in future.

p21

There is evidence that p21 acts in cyclinD1 synthesis, where overexpression is a marker of poor outcome in many human cancers including pancreatic cancer. Expression of p21 is regulated by other suppressor genes which are implicated in pancreas carcinogenesis. Biankin *et al*[26], using immunohistochemical methods, examined the expression of p21 in 451 PanIN lesions from 60 pancreatic cancer tissues and tried to correlate this expression with the histopathological grade of the lesions. Overexpression of p21 was present at 9% of the normal ducts, 16% of PanIN-1A, 32% of PanIN-1B, 56% of PanIN-2, 80% of PanIN-3 lesions and, finally, in 85% of invasive carcinoma. These observations suggest that p21 overexpression is an early event in this type of cancer and that there is a relationship between overexpression and progressive lesions. In addition, this study showed that overexpression of p21 is controlled by mutant K-ras and HER-2/neu genes rather than by p53 overexpression[26].

TUMOR SUPPRESSOR GENES

Tumor suppressor genes encode proteins with a protective role against malignant phenotypes. Their inactivation may lead to initiation and progression of carcinogenesis. When the balance between oncogenes and tumor suppressor genes is disrupted, the result is the initiation of carcinogenesis.

DPC4/SMAD4

SMAD4, known as DPC4 (deleted in pancreatic carcinoma locus4) and as a tumor suppressor gene, is located at 18q21.1. SMAD4 encodes a protein with major implications in signal transduction, through activating members of the TGF-β superfamily[27].

The SMAD family consists of nine members with a central role in the transduction of the TGF-β signaling from the cell surface to the nucleus. SMAD2 and SMAD3 are also named "receptor-regulated SMADs" because of their property of being phosphorylated by receptor kinases forming heteromeric complexes with SMAD4.

These complexes enter the nucleus and bind to DNA - a prerequisite step for transcriptional activation of TGF-β responsive genes. Moreover, SMAD2/SMAD4 and SMAD3/SMAD4 complexes can downregulate c-myc proto-oncogene and upregulate p21 and p15 expression. p21 does not permit the formation of CDK4/cyclinD and CDK6/cyclinD complexes and their subsequent transcription[28-30].

TGF-β (transforming growth factor-β) is a member of the dimeric polypeptide growth factor family that regulates cell proliferation and differentiation, embryonic development, wound healing, and angiogenesis. In normal cells, TGF-β promotes differentiation and apoptosis and does not permit the cell to go beyond the G_1 phase. Contrary to that, tumor cells that encode for proteins participating in this signaling pathway are altered and the protective role of TGF-β against tumor phenotypes is abolished. The tumor cells begin to proliferate without restriction and with an increased production of TGF-β. A vicious cycle begins: an increased amount of TGF-β leads to increased invasiveness of tumor cells by destruction of extracellular matrix and promotion of molecular adhesive proceedings. The results of two studies show that 100% of pancreatic adenocarcinomas and 83% of colon cancers have a mutation which affects at least one gene involved in the TGF-β pathway[32].

Due to this process, SMAD4 expression is well-examined in human cancers. It is found that 50% of pancreatic cancers and 30% of colorectal and biliary cancers present mutant genes. It has been shown that in pancreatic adenocarcinomas, 30% present homozygous deletions while 20% present intragenic mutations in one allele coupled with loss of heterozygosity[33,34].

The protective character of SMAD4 expression against carcinogenesis was studied by Tascilar *et al*[35]. They examined the SMAD4 expression in patients with pancreatic carcinoma who had undergone surgical resection. Patients with positive SMAD4 expression survived 4.5 mo longer than patients with negative SMAD4 expression. For a patient with a very poor prognosis, this gain is significant.

Wilentz *et al*[36] studied the expression of SMAD4 gene in 188 PanIN lesions from 40 adenocarcinomas using immunohistochemical methods. All three "early" PanIN-1A, PanIN-1B, PanIN-2 lesions expressed DPC4 but it was only seen in one third of the PanIN-3 lesions. The conclusions from this study suggest that the loss of DPC4 gene expression occurs late in pancreatic carcinogenesis and, unfortunately, cannot be used for the differential diagnosis of the benign lesions from the malignant ones[36].

Finally, the last property of SMAD4 restoration is its influence on angiogenesis. It seems to decrease VEGF and to increase TSP-1 (trombospondin) expression, an angiogenesis inhibitor[37].

p16

On chromosome 9q21, there is a locus called p16[INK4A]/p14[ARF], which encodes for two tumor suppressor genes. Genetic alterations of this locus through gene mutation, deletion, or promoter hypermethylation are found in 80%

to 95% of sporadic pancreatic cancers[38]. Additionally, expression of p16 has been studied in many types of cancer such as melanomas, gliomas, and leukemias.

p16 suppressor gene is also named cdkn2 (cyclin-dependent kinase-2) because it is a cyclin-dependent kinase 4 inhibitor. Loss of its expression results in an increasing activity of cyclin dependent kinase 4 with the direct consequence of Rb protein hyperphosphorylation and subsequent uncontrolled cell proliferation.

There are three different mechanisms for p16 inactivation: small mutations as seen in 40% of the cases, deletion of both alleles in the following 40%, and gene silencing through hypermethylation in the remaining 20% of the cases[38-40].

Genetic analyses have shown that p16 alterations are very common in pancreatic adenocarcinomas but these alterations are not necessarily seen in cultured cell lines. The question is whether p16 mutations and deletions are prerequisite for the establishment of such a cell line. Several studies on p16 expression in pancreatic adenocarcinomas have opposing results. Huang et al[41] report that only 26.7% of examined pancreatic cancers present deletions or mutations on this tumor suppressor gene. In a study by Bartch et al[42], this percentage increased to 34.4%. Later, Hu et al[43] studied 62 pancreatic cancer tissues using immunohistochemical methods and reported that 42% of the examined tissues did not express the gene at all. Moreover, loss of p16 expression could be correlated with less differentiated tumors, shorter overall survival, and the presence of metastatic disease[43].

It appears that there are at least two genetic alterations that must be present: K-ras mutations and p16 mutations. Human cancers hardly present simultaneous alterations in these two genes. This information may be useful in the future in differential diagnosis of adenocarcinomas of unknown origin.

p53

In human cancers, the most frequent mutant gene is the p53. It is located on the short arm of chromosome 17 and its mutations are either due to loss of heterozygosity in 95% of pancreatic adenocarcinomas or to sequence alterations in 75% of cases with small changes most likely in amino acid sequence such as G: C→A: T (transition)[44-46].

p53 is a nuclear phosphoprotein with the ability to bind to specific DNA elements and to activate gene transcription. It has a central position in cell cycle regulation through its role in inactivating a variety of genes and interrupting cell proliferation at G_1/S checkpoint.

Mutant status of p53 has been examined in pancreatic adenocarcinomas indirectly through p53 immunostaining and directly through molecular analyses using sequence analyses or polymerase chain reaction. The results of these studies show that mutant p53 correlates with shorter postoperative survival of patients and metastatic disease. However, all these studies have two main drawbacks. One is that the number of examined tissues was not adequate and the other is that the results obtained by the two methods - immunohistochemistry and molecular analyses

- are not consistent. Using both techniques, Ruggeri et al[46] studied 126 cases of sporadic adenocarcinomas, 10 cases of familial adenocarcinomas, 77 cases of non-neoplastic but histologically abnormal pancreatic lesions, and 23 cases of metastatic lesions. The results of this published study show that p53 mutations were present at 56% of sporadic pancreatic adenocarcinomas, 33% of familial pancreatic adenocarcinomas. However, p53 alterations did not correlate with tumor grade, stage, or metastatic disease[46].

Generally speaking, genetic alterations of p53 tumor suppressor gene are an early event in pancreas carcinogenesis but not an initiating event.

Mdm-2

The mdm-2 gene encodes a protein with possible implications in appearance of malignant character of a cell. Its overexpression in absence of gene amplification has been studied in sarcomas and gliomas as well as in the presence of DNA-damaging agents. It was suggested that expression of mdm-2 gene is regulated by p53 tumor suppressor gene but Ruggeri et al[47] proved that there is no association between these two genes and moreover amplification and overexpression of mdm-2 is an infrequent event in the development of pancreatic adenocarcinomas.

MATRIX METALLOPROTEINASES (MMPS)

MMPs comprise a family of at least twenty members that act as zinc-dependent enzymes. The well-known collagenases, stromelysins, and gelatinases are members of this family. Their principal role is the degradation of extracellular matrix components. MMPs play a role only under special conditions such as tissue remodeling, embryonic development, and wound healing. Cytokines, growth factors and mechanical stress could be the triggers for MMPs production[48]. Abnormal expression of MMPs has been described in periodontitis, rheumatoid arthritis, tumor cell invasion, and metastasis[49].

At a structural level, MMPs consist of a signal peptide and a catalytic domain. At the functional level, proteolytic processes must be present in order to activate the enzymes.

MMPs have a pivotal position in carcinogenesis as well as in angiogenesis. Firstly, they degrade the basement membrane and the extracellular matrix components, offering tumor cells the best nutritive conditions for their establishment at the primary site and permitting the circulation of tumor cells and their extravasation at distant, metastatic sites[48]. In addition, MMPs are capable of removing sites of adhesion, exposing new binding sites, and releasing chemoattractants[50].

It seems MMPs play a role in 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[48]. Due to these properties, the inhibition of MMPs represents the scientific rationale for the development of chemotherapeutic agents against pancreatic cancer.

Table 1 The most important pancreatic-prone syndromes

Syndrome	Mutation	Inheritance	Manifestations
Familial atypical mole-malignant melanoma syndrome (FAMMM)	CDKN2A	AD	Multiple atypical nevi Malignant melanoma Extracutaneous cancers
Hereditary pancreatitis	PRSS1 Kazal type 1 (SPINK1)	AD	Relapsing pancreatitis Young age of onset Associated pancreatic insufficiency, diabetes and pseudocysts
Hereditary non-polyposis colon cancer (Lynch Ⅱ)	HMSH2, HMLH1, HPMS2, p16 BRCA2	AD	Adenocarcinoma of the colon and extracolonic adenocarcinomas (endometrium, ovary)
Familial adenomatous polyposis	APS	AD	Innumerable colonic polyps with highly possible malignant transformation
Ataxia-telangiectasia	ATM	AR	Progressive cerebral ataxia, telangiectasias, sinopulmonary infections, oculomotor apraxia, immune deficiencies, 3-fold operative risk for PC
Li-Fraumeni	p53	AD	Predisposition to several neoplasms
Peutz-Jeghers	LKB1/STK11	AD	Multiple oromucosal and intestinal hamartomas

Epidermal growth factor receptors

The family of epidermal growth factor receptors (EGFR) consists of four types of receptors: HER-1, HER-2, HER-3, and HER-4, which have been studied in detail due to their implications in carcinogenesis. These four structurally similar receptor tyrosine kinase proteins are present on various domains: extracellular, transmembrane, and intracytoplasmic. Ligands of these proteins are EGF (betacellulin), TGFα (epiregulin), HB-EGF (amphiregulin) and three neuregulins (1, 2 and 3)[51,52].

Upon binding to ligands, these receptors undergo homo- or hetero-dimerization at the cell surface with subsequent phosphorylation of serine residues in the intracytoplasmic domain. This phosphorylation is translated into a downstream signal with resultant gene activation that leads to cell proliferation, decreased apoptosis, angiogenesis, and metastasis[53].

Overexpression of EGFR is a common characteristic in epithelial tumors such as breast, lung, and colorectal cancer. This expression has been associated with aggressive tumor growth and poor clinical outcome. Safran *et al*[54] studied 154 patients with metastatic pancreatic cancer for HER-2 overexpression by immunohistochemical means. They reported positive results for 21% of the cases studied.

All these important implications of HER-2 gene in carcinogenesis constitute the scientific rationale for new approaches in targeted therapy of pancreatic cancer.

FAMILIAL PANCREATIC CARCINOMA

It has been statistically observed that 5%-10% patients with pancreatic cancer have a close relative with the same cancer while this rate among controls is only about 0.6%[55]. Lynch *et al*[56] have shown that the risk for a person to develop pancreatic cancer is increased by 30% when there is a family history of any cancer among first-degree relatives. The European Registry of Hereditary Pancreatic Diseases (EUROPAC) identifies an individual at high risk for developing pancreatic cancer (PC) when he/she has two or more first-degree relatives with PC, or has three or more relatives of any degree with PC, or has any two

relatives who have been given this diagnosis and the sum of their ages is under 110 years.

Studies of family histories might lead us to a better understanding of genetic alterations in human pancreatic adenocarcinoma. Patients with cancer in their families present an inherited germ-line genetic mutation in a cancer-causing gene. Among genes which have shown to be involved with familial pancreatic carcinogenesis are BRCA-2 and a large genetic area on locus 4q32-q34. Germ-line BRCA-2 mutations (mainly 6147delT) are present in approximately 17%-19% of familial pancreatic families in accordance with the results of recent studies[57-59].

Table 1 summarizes the most important pancreatic-prone syndromes. Much additional work needs to be done before the genetic basis of pancreatic cancer is completely understood in sporadic cases as well as in familial cases. This information will help us to identify the primary genetic factor and if possible to organize a counseling program for individuals at high risk.

NOVEL THERAPEUTIC AGENTS

Since 1997, the standard first-line chemotherapeutic agent for pancreatic adenocarcinoma has been gemcitabine (2' 2'-difluorodeoxycytidine), a difluorinated analogue of deoxycytidine, which is a member of the antimetabolites. The patient's benefit using this chemotherapeutic agent is an improvement in quality of life; however, the survival benefit is marginal. Antimetabolites cannot prolong the median survival time of patients with metastatic disease for more than six months.

The rationale for further understanding of genetic alterations of pancreatic cancer is based on the need for earlier diagnosis and development of more effective therapies. MMPs present very interesting links with extracellular matrix participating in its degradation and in the process of neovascularization. Marimastat, a MMPs inhibitor, was administrated in 414 patients with advanced pancreatic cancer as first-line chemotherapy in different doses (5, 10, and 25 mg orally twice a day) compared to the standard chemotherapy, gemcitabine, in a clinical study. Unfortunately, the study results are not encouraging. There

is no difference in the median survival interval between the two agents or with regard to marimastat dose escalation. The most important clinical information from this study is the longer overall survival time of patients with no metastatic disease *versus* patients with metastatic disease (200 *versus* 89 d). Thus, it is concluded that marimastat should be used in an adjuvant and not in a first-line setting[60,61].

Inhibition of EGFR by monoclonal antibodies (MoABs) that inhibit ligand binding or by tyrosine kinase inhibitors (TKIs) that bind to the adenosine triphosphate binding site of the growth factor receptor represents another therapeutic approach for pancreatic adenocarcinoma. Cetuximab (Erbitux) is the first human-mouse chimeric IgG1 antibody which has been approved for EGFR-positive expression in colorectal cancer. Currently, it is used in large clinical trials for EGFR-positive expression in pancreatic cancer. This novel agent presents more than one mechanism of action such as arrest of cell-cycle, activation of apoptosis, inhibition of angiogenesis, and inhibition of distant metastasis. It is interesting that EGFR inhibition contributes to angiogenic inhibition[62]. The next step is a clinical study comparing gemcitabine alone and in combination of an EGFR-inhibitor. Another novel agent which could be used as targeted therapy in pancreatic carcinoma is ABX-EGF, a fully humanized IgG2 monoclonal antibody that has a higher binding affinity to EGFR than the previous one. There is evidence that ABX-EGF, in combination with chemotherapy, could eradicate some tumors and prolong overall survival. Unfortunately, the number of patients with pancreatic cancer and EGFR overexpression is limited[63].

Several TKIs (gefitinib, erlotinib, PKI-166) have been tried as targeted therapies in pancreatic adenocarcinoma. Oral administration of PKI-166 in combination with intraperitoneal injections of gemcitabine in nude mice with implanted human pancreatic carcinoma cells into their pancreas showed significant regression of tumor growth and inhibition of metastasis. This inhibition was mediated directly by antitumor effect and indirectly by anti-angiogenic effects. Some clinical phase III studies are in process, which compare a combination of TKIs and gemcitabine versus gemcitabine alone as a first-line treatment for pancreatic cancer[64,65].

Due to rapid cancer cell division, the tumor growth increases rapidly. The young cells need oxygen and nutrients supplied by newly made vessels, otherwise, they will die. This information represents the scientific rationale for the development of new drugs that will target several points along the angiogenic pathway. The targeted therapy advantage is that it applies only to new vessels, and will not present widespread toxicity. It acts by blocking vascular epithelial growth factor (VEGF) through monoclonal antibodies or through agents responsible for VEGF receptor tyrosine kinase inhibition. A multicentre phase II trial, which studies the efficacy of bevacizumab plus gemcitabine for advanced pancreatic cancer, is currently taking place with satisfactory results: the median time to progression is 5.5 mo and the estimated 1-year survival rate is 54%[66].

Another therapeutic approach to pancreatic cancer is the antisense therapy. The mechanism of action is the inhibition of protein expression through trapping mRNA by specific RNA sequences. There are ongoing trials on murine xenografts on the human pancreatic cancer cell line, AsPC-1, where liposome-mediated gene transfer of antisense K-ras is used[67].

CONCLUSION

During the past decade, important steps have been made towards understanding the primary lesions that may lead to pancreatic adenocarcinoma. Molecular biology is the major key in this effort. Furthermore, a biologic and molecular staging of this disease may lead us to earlier diagnoses, efficient familial counseling, better management, and new therapeutic approaches.

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