

REVIEW

Molecular Biology of Pancreatic Cancer: How Useful Is It in Clinical Practice?

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ABSTRACT

Context During the recent two decades dramatic advances of molecular biology allowed an in-depth understanding of pancreatic carcinogenesis. It is currently accepted that pancreatic cancer has a genetic component. The real challenge is now how these impressive advances could be used in clinical practice. **Objective** To critically present currently available data regarding clinical application of molecular biology in pancreatic cancer. **Methods** Reports about clinical implications of molecular biology in patients with pancreatic cancer were retrieved from PubMed. These reports were selected on the basis of their clinical relevance, and the data of their publication (preferentially within the last 5 years). Emphasis was placed on reports investigating diagnostic, prognostic, and therapeutic implications. **Results** Molecular biology can be used to identify individuals at high-risk for pancreatic cancer development. Intensive surveillance is indicated in these patients to detect pancreatic neoplasia ideally at a preinvasive stage, when curative resection is still possible. Molecular biology can also be used in the diagnosis of pancreatic cancer, with molecular analysis on samples of biologic material, such as serum or plasma, duodenal fluid or preferentially pure pancreatic juice, pancreatic cells or tissue, and stools. Molecular indices have also prognostic significance. Finally, molecular biology may have therapeutic implications by using various therapeutic approaches, such as antiangiogenic factors, purine synthesis inhibitors, matrix metalloproteinase inhibitors, factors modulating tumor-stroma interaction, inactivation of the hedgehog pathway, gene therapy, oncolytic viral therapy, immunotherapy (both passive as well as active) etc. **Conclusion** Molecular biology may have important clinical implications in patients with pancreatic cancer and represents one of the most active areas on cancer research. Hopefully clinical applications of molecular biology in pancreatic cancer will expand in the future, improving the effectiveness of treatment and prognosis of patients with pancreatic cancer.

Introduction

Pancreatic cancer is an aggressive and highly lethal malignant disease. Pancreatic cancer represents about 3% of new cancer cases, but it is responsible for 6% of deaths from malignant disease [1]. Nowadays, pancreatic cancer is the 4th (for females) or the 5th (for males) leading cause of death from cancer in the western world. Recent reports state that 5 year survival for all stages of pancreatic cancer is only 5% (15-20% for localized disease, 8% for pancreatic cancer with regional dissemination, and 2% for patients with distant metastases) [1]. Unfortunately, most pancreatic cancers are diagnosed late in their natural course;

indeed, about 80% of pancreatic cancers have metastases at the time of diagnosis, either locoregional (25%) or distant (55%) [1, 2].

There has been little improvement in prognosis over the past 20 years. Radical surgery is the only hope for cure and increases medial overall survival to 13-15 months [2]. However, due to disease dissemination, surgery with therapeutic intent is possible in a minority of patients with pancreatic cancer (10-20%) [3]. Chemotherapy still relies on few drugs (including gemcitabine) and may further prolong survival, but only for a few months [4].

Given the dismal prognosis of pancreatic cancer and the relative inefficacy of currently available therapeutic methods, the need to develop other more effective diagnostic and therapeutic approaches is clear. The rapid development of modern molecular biology during the last two decades uncovered the genetic mechanisms controlling pancreatic carcinogenesis; new diagnostic and therapeutic tools have been developed or are under intense investigation, which hopefully will improve in the future the outcome of patients with pancreatic cancer. The aim of this paper is to summarize and critically evaluate currently available data regarding

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Abbreviations FAMMM: familial atypical multiple mole melanoma; Hh: Hedgehog; HNPCC: hereditary non-polyposis colorectal cancer; SHh: sonic Hedgehog

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clinical implications of molecular biology of pancreatic cancer. Problems arising in everyday practice will also be discussed from a practical point of view.

Molecular Biology of Pancreatic Cancer: A Synopsis

An in depth discussion about molecular biology of pancreatic cancer is beyond the scope of this paper and has been presented in detail elsewhere [5, 6, 7]; therefore, only a brief description of our current knowledge regarding the molecular/genetic basis of pancreatic cancer will be presented.

Pancreatic cancer is a disease controlled by inherited and acquired mutations in cancer related genes, which could be classified into three categories:

- Oncogenes (*K-ras*, *BRAF*, *AKT 2*, *MYB*, and *AIB1*)
- Tumor-suppressor genes (*p16/CDKN2A*, *p53*, *p21*, and *SMAD4*)
- Genome-maintenance genes (*MLH*, *MSH2*, *BRCA2* and other Fanconi anemia genes).

Oncogenes

K-ras mutations are very common (>90%) in pancreatic cancer and are usually restricted to codon 12. *K-ras* mutations impair intrinsic GTPase activity resulting in a protein that is constitutively active in signal transduction, resulting in alterations in cell proliferation, survival, and migration. *K-ras* mutations are an early genetic event in pancreatic carcinogenesis and are considered as a 'signature' for pancreatic cancer. *BRAF* gene mutations are observed in about 30% of the pancreatic cancers with wild-type (normal) *K-ras* gene. *BRAF* and *K-ras* gene mutations are mutually exclusive, i.e. one of them is not observed in the presence of the other. Other oncogenes amplified in pancreatic cancer include the *AKT1* gene, *AKT2* gene, and *MYB* gene; amplification of these genes is observed in 60%, 10-15%, and 10% of pancreatic cancers, respectively. In addition to these genes, a number of amplicons (amplified fragments of DNA) have been identified in pancreatic cancers, including a localized region on long arm of chromosome 8, corresponding to the oncogenic transcription factor *CMYC*. Probably there are too many other oncogenes which still remain unknown.

Tumor Suppressor Genes

Inactivation of the p16 gene is observed in 80-95% of sporadic pancreatic cancers. The combination of p16 and *K-ras* mutations is uncommon in other human tumors and is considered as a molecular 'signature' for pancreatic cancer. p16 gene inactivation is observed at a later stage in pancreatic carcinogenesis compared to *K-ras* mutations (Figure 1). p53 gene inactivation is observed in 55-75% of pancreatic cancers and is a late event in pancreatic tumorigenesis. p21 gene inactivation is an early event in the development of pancreatic carcinoma, appearing at a progressively higher frequency during the progression from normal ducts (9%) to Pan IN to invasive pancreatic cancer

(>85%). *MTAP* gene function is completely lost in about one third of infiltrating pancreatic cancers, but concurrent (by chance) deletion during inactivation (again by deletion) of the p16 gene. *SMAD4* gene plays a critical role in signaling through the TGF-beta pathway. *SMAD4* gene inactivation occurs in about 55% of pancreatic cancers. *BRCA1* and *BRCA2* genes are mutated in most advanced PanIN lesions, whereas a germline mutation in either gene represents the earliest risk factor in many familial pancreatic cancer cases. *BRCA2* (*her-2/neu*) gene mutation carriers have an increased (10-fold) risk of developing pancreatic cancer than the general population. There are too many others tumor-suppressor genes involved in pancreatic carcinogenesis, including *STK11/LKBI*, *MKK4*, *TGF-beta-R1* (*ALK5*), *TGF-beta-R2*, *ACVRI-beta* (*ALK 4*), *ACVR2*, *FBXW7* (*CDC4*) and *EP300*.

Genome Maintenance Genes

These genes are responsible for the identification and repair of damage to DNA. As a result of inactivation of such a gene, the damaged DNA is not repaired efficiently and DNA damages remain accumulating, thereby contributing in carcinogenesis. This group of genes includes *MLH1* and *MSH2* and the Fanconi anemia genes (*FANCC*, *FANCG*, etc.), which are targeted in a small percentage of patients with pancreatic cancer (<10%).

Other Genetic Mechanisms Involved in Pancreatic Carcinogenesis

Telomere shortening, expression of specific proteins, upregulation and overexpression of growth factors and/or their receptors (such as EGFR, TGF-beta, etc.), altered expression of angiogenic factors and their receptors (VEGF-A, VEGFR1 and VEGF2, PEDF, etc.) are also mechanisms involved in the process of pancreatic carcinogenesis. Derangements of developmental signaling pathways (mainly the Hedgehog (Hh) signaling pathway) are also observed in pancreatic cancer, as observed by the expression of the sonic Hh (SHh) protein in 70% of human pancreatic cancer.

Currently, pancreatic carcinogenesis is considered to be a multistep phenomenon, characterized by specific genetic mutations that take place during the evolution of a pre-invasive pancreatic lesion to pancreatic cancer;

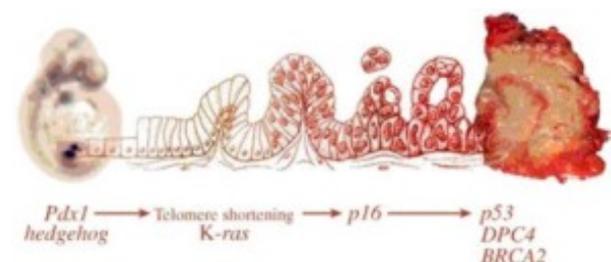


Figure 1. Multistep pancreatic carcinogenesis. Note the progression of pancreatic neoplasia from pre-malignant pancreatic lesions (PanIN, left) to carcinoma (right) (with permission from Maitra et al., 2006 [6]).

of note, these genetic alterations occur in an ordered sequence rather than a random fashion (Figure 1).

Applying Molecular Biology in Clinical Practice: Where Are We Today Regarding Pancreatic Cancer?

Evolution of molecular biology has been impressive during the last two decades. Sequencing of the human genome is now possible and a huge number of genetic markers can be identified in a single patient. From a clinical point of view, however, the real challenge remains to determine how these impressive progresses could improve clinical management of cancer patients. Nowadays, potential clinical implications include genetic counseling for individuals at high-risk for developing pancreatic cancer, early detection of pancreatic cancer, determination of prognosis, and mechanism-based therapies for pancreatic cancer.

Genetic Counseling

The identification of individuals with an increased risk for the development of pancreatic cancer is obviously of particular clinical importance. Hereditary pancreatic cancer represents about 5-15% of pancreatic cancers and is directly attributable to genetic alterations [8, 9]. There are three groups of patients characterized by an inherited predisposition for pancreatic cancer development [7, 8, 9, 10, 11]: i) patients with familial cancer syndromes associated with an increased risk of pancreatic cancer (i.e., familial atypical multiple mole melanoma (FAMMM) syndrome, Peutz-Jeghers syndrome, and hereditary non-polyposis colorectal cancer (HNPCC) syndrome); ii) individuals belonging to families with a history of familial pancreatic cancer. The risk of developing pancreatic cancer among first-degree relatives of an individual belonging to such families increases with the number of affected family members (18-fold with two; 57-fold with three affected members); iii) patients with benign diseases associated with inherited pancreatic cancer, such as chronic hereditary pancreatitis, cystic fibrosis, ataxia telangiectasia, etc.

As above noted, germline *p16* gene mutations are associated with the FAMMM syndrome (which is characterized by an increased incidence of melanoma and pancreatic cancer (from 20 to 34-fold)). Based on this, it has been suggested that patients with a family history of melanoma, multiple atypical nevi, and a family history of pancreatic cancer can obtain genetic testing for germline *p16/CDKN2A* gene mutations. Those found to carry a germline mutation would benefit from increased surveillance for skin cancer and, as tests for pancreatic cancer become available, for screening for early pancreatic neoplasia [6].

BRCA2 gene mutation carriers have an increased (10-fold) risk of developing pancreatic cancer compared to general population (see above), as well as an increased risk of developing breast, prostate, and ovarian cancer. Moreover, germline (inherited) mutations in *BRCA2* can cause the familial aggregation of pancreatic cancer [12]. Individuals with a family history of cancer can be

screened for a germline *BRCA2* mutation to establish cancer risk profiles; management options should then be discussed with the patient (for example, intensive surveillance, chemoprevention, or prophylactic mastectomy to prevent breast cancer or intensive surveillance for early detection of pancreatic cancer or preferentially preinvasive disease) [6, 13].

In addition to *p16* and *BRCA2*, genetic analysis for individuals with a family history of pancreatic cancer with or without other cancers could include genetic testing for inherited (germline) mutation in other genes known to predispose to familial pancreatic cancer, such as *STK11*, *PRSS1*, *BRCA1* and *MLH1*.

Practically, individuals found to carry specific gene mutations responsible for the aggregation of cancer in their family should follow a program of intense surveillance, aiming to detect pancreatic and extrapancreatic neoplasms at an early stage, ideally before the development of invasive cancer and before the development of clinical manifestations; at this early stage of pancreatic neoplasia radical surgical resection is still possible and cure can be achieved [14]. This surveillance program should include periodic examination of the patients using modern imaging methods, such as endoscopic ultrasonography and multidetector computed tomography. Molecular tests to detect genetic alterations found specifically in early pancreatic neoplasia could also be used.

From a clinical point of view, it should be noted that the concept of “prophylactic pancreatectomy” is currently considered by many as a too aggressive and basically unjustified approach, in contrast to prophylactic thyroidectomy or prophylactic mastectomy in individuals found to carry specific genetic alterations. This could be explained by the significantly higher surgical morbidity and mortality of a major pancreatectomy compared to total thyroidectomy or mastectomy. However, this strategy has been applied in clinical practice; Canto *et al.* at Johns Hopkins screened 38 asymptomatic patients with a strong history of family pancreatic cancer, while one had Peutz-Jeghers syndrome, using endoscopic ultrasonography (EUS); six pancreatic masses were found in this group of patients [15]. The same group found that noninvasive precursor lesions are more common in patients with a strong familial history of pancreatic cancer than in patients with sporadic disease; precursor lesions were of a higher grade in patients with a strong family history of pancreatic cancer [16]. Once detected, by using a combination of multi-detector CT scan and EUS, resection of these preinvasive lesions could achieve cure from a highly lethal disease (i.e., invasive pancreatic cancer). However, until now the target group of individuals which should be included in intensive surveillance programs remains undefined; moreover, the diagnostic strategy which is indicated during the follow-up of these individuals remains controversial and somehow ambiguous; therefore clear and robust recommendations are not possible at this time [17, 18].

To further emphasize the clinical applicability of the concept of prophylactic pancreatectomy, it should be noted that pancreatectomy in patients with noninvasive cystic pancreatic neoplasms (such as noninvasive mucinous cystic neoplasms or noninvasive intraductal papillary mucinous neoplasms) is essentially prophylactic, i.e. is performed when the disease is at the stage of preinvasive neoplasia to prevent the highly possible development of invasive disease. In this case, the risk associated with a major pancreatic resection is accepted and pancreatectomy is indicated [19, 20]. For the same reasons, prophylactic pancreatectomy aiming to resect preinvasive pancreatic lesions (such as PanIN) in individuals with specific mutation carriers is also justified; it appears that the concept of prophylactic pancreatectomy will be more appealing in the future. Needless to say that such a surgical procedure should be performed in hospitals and by surgeons with significant experience and interest in pancreatic surgery to minimize surgical morbidity and mortality rates.

Diagnosis

Given the dismal prognosis of pancreatic cancer, even in patients with resectable disease, early diagnosis at an early (preclinical) and potentially curable stage is required to improve therapeutic results. Molecular changes occurring early during tumor progression are preferred to diagnose pancreatic neoplasia at a stage where there is committal to invasive pancreatic cancer, but where the lesion is still treatable. The clinical impact of diagnosing preinvasive or even early pancreatic cancer could be tremendous; indeed, there is some evidence suggesting that resection of small (<1 cm) pancreatic cancers can achieve a 100% 5-year survival [21]. Molecular analysis could be performed on samples of biologic material, such as serum or plasma, duodenal fluid or (preferentially) pure pancreatic juice, pancreatic cells obtained by fine-needle aspiration (FNA) or cytological brushings, bile and stools, or at tissue level (on surgical specimens). ERCP allows collection of pure pancreatic juice for genetic analysis, brush cytology and biopsy [22]. Detection of *K-ras* mutations in this material has been proposed for the early diagnosis of pancreatic cancer [23, 24, 25]. Localization of *K-ras* mutations to a single codon (codon 12) in the vast majority of pancreatic cancers greatly facilitates genetic testing to detect specifically these mutations rather than to examine the entire gene [6]. However, *K-ras* mutations in the pure pancreatic juice are also observed in patients with benign diseases (albeit at a much lower frequency), such as chronic pancreatitis (up to 40% of patients) or even biliary tract stones, thereby substantially diminishing the specificity of this genetic test [23, 24, 26].

Expression of mesothelin, as detected by immunolabeling, can be used in the interpretation of difficult biopsies of the pancreas [27]. Mesothelin can also be detected in the serum [6]. Quantitative

determination of p16^{INK4a} promoter CpG island methylation has been examined as a screening modality for pancreatic cancer, with a reported specificity and sensitivity of about 90% and greater than 60%, respectively [22, 28]. Immunocytology can detect mutant p53 indirectly as a result of accumulation of mutant p53 protein within cells [29]. Mutations of p53 genes have also been detected in pancreatic juice; these mutations could be used to differentiate pancreatic cancer from chronic pancreatitis [29]. Increased telomerase activity in pure pancreatic juice has been observed in a high percentage of patients with pancreatic cancer (about 80%), but in none of patients with chronic pancreatitis or normal pancreas [30]. Immunohistochemical labeling for the *SMAD4* protein in biopsies and resected tissues could be used in the interpretation of histological findings in difficult cases [6, 31]. Loss of *SMAD4* expression strongly supports the diagnosis of pancreatic cancer, while intact *SMAD4* expression supports the diagnosis of a non-pancreatic malignancy. This could be useful in the evaluation of metastatic lesions (from the pancreas to other organs and conversely). Microsatellite instability in patients with pancreatic cancers has been associated with a risk of developing other cancers (as a part of multi-organ cancer syndromes, such as HNPCC); this association should be taken into consideration when treating patients with pancreatic cancer and microsatellite instability in order to plan a management strategy to achieve early detection of other synchronous or metachronous extrapancreatic cancers [32, 33].

From a surgical and practical point of view, it should be noted that frequently surgical resection is performed for a pancreatic mass which could be inflammatory (i.e., chronic pancreatitis) or neoplastic [26, 34, 35]. Practically, differential diagnosis of these two entities is not always possible, especially when the clinical presentation and laboratory/imaging findings are not typical. In this case the highly sophisticated modern diagnostic approach (which could include determination of molecular/genetic indices) may be unreliable and accurate diagnosis in the individual patient is often possible only following radical resection of the affected pancreatic parenchyma, which is appropriate therapy for both chronic pancreatitis as well as for pancreatic cancer [35, 36].

Prognosis

Several molecular alterations observed in pancreatic cancer have prognostic significance. For example, *K-ras* gene mutations have been reported as a negative prognostic factor after surgery and adjuvant chemoradiation, or surgery alone. Increased expression of EGFR or its ligands has been associated with an enhanced malignant phenotype and a worse prognosis [37, 38]; EGFR overexpression is also associated with a high probability to respond to anti-EGFR agents [39]. In contrast, pancreatic cancers with genome-maintenance genes mutations and microsatellite instability may have a better prognosis, but they may

show a different response to some chemotherapeutic regimens [6, 32]. Loss of *SMAD4* expression correlates with a better survival following resection of pancreatic cancer [39, 40]. Therefore, information based on genetic/molecular profile of pancreatic cancer could be used to determine cancer sub-types, classify tumor phenotype, and determine prognosis (in association with known prognostic factors) with a higher accuracy [41].

Therapy

Biochemical differences caused by the specific genetic alterations observed in pancreatic cancer could be used to target malignant cells, avoiding at the same time damage of normal cells. For example, the CAPANI pancreatic cancer cell line harboring *BRCA2* gene mutations is very sensitive to the DNA cross-linking agent mitomycin C [6, 42, 43]. In experimental models, purine synthesis inhibitors (such as L-alanosine) have been used to target the selective loss of Mtap function observed in cancers [6]. Since angiogenesis is essential for tumor growth, antiangiogenic therapy represents an interesting type of treatment of pancreatic cancer. Bevacizumab (a humanized monoclonal antibody against VEGF) has been used in combination with gemcitabine in pancreatic cancer, but without significantly prolonging survival [44]. Other angiogenic inhibitors that target other non-VEGF pathways (such as sorafenib which inhibits the VEGF receptor, platelet-derived growth factor receptor (PDGFR), stem-cell factor receptor (SCFR), Raf1, and FMS-like tyrosine kinase 3 (FLT3) have been used in the treatment of advanced pancreatic cancer with poor results [45]. Other antiangiogenic agents, such as axitinib, aflibercept, integrin, and cilengitide, have been used in the management of metastatic pancreatic cancer, without improving patient's outcome [44]. Erlotinib (an orally active anti-EGFR inhibitor) has demonstrated a small but statistically significant increase in the survival of patients with advanced pancreatic cancer [46]; in 2005, erlotinib was the first targeted therapy approved by the FDA for pancreatic cancer. Another anti-EGFR agent is cetuximab, which however was ineffective in phase III trial in patients with locally advanced and metastatic pancreatic cancers [47], but is still under investigation in the treatment of pancreatic cancer. Matrix metalloproteinase inhibitors (MMPIs) are other examples of angiogenesis inhibitors, which have been used in the treatment of pancreatic cancer, without prolonging survival [48]. Cyclooxygenase inhibitors reduce angiogenesis and invasiveness of cancer and enhance apoptosis; these antiangiogenic agents have also been used in the treatment of pancreatic cancer, with conflicting results [38].

Tumor-stroma interaction (which is important for the progress of pancreatic cancer) can mediate specific gene expression in the tumor, which could be used as a therapeutic target [49]. Cyclopamine specifically inactivates the hedgehog pathway (see above); it has

been shown that cyclopamine produces dramatic anti-tumor effects in xenograft models of human pancreatic cancers without demonstrable side effects in treated mice [50]. Intense research is focused in developing more potent inhibitors of the hedgehog pathway.

Other therapeutic approaches in pancreatic cancer based on molecular biology include gene therapy and signal transduction inhibition and oncolytic viral therapy [51]. Intensive research efforts are focused on the role of immunotherapy in cancer treatment; immunotherapy currently represents the most important and rapidly grown area of cancer research. Passive immunotherapy includes the use of antitumor agents that have been generated *in vitro* (antibodies, effector cells, etc.), whereas active immunotherapy aims to stimulate an antitumor response *in vivo* by means of vaccination [52, 53].

Conflict of interest The authors have no potential conflict of interest

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