

HIGHLIGHT ARTICLE

Translational Research in Pancreatic Adenocarcinoma

Highlights from the "2014 ASCO Gastrointestinal Cancers Symposium".

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Summary

Pancreatic cancer is ranked as the fourth leading cause of cancer-related deaths in the United States despite intensive basic and clinical research over the last decade. In this paper we summarize the Abstracts #176, #234, and #206 presented at the 2014 ASCO Gastrointestinal Cancers Symposium focussing on *BRCA* 1 and 2 mutations, *PABL* gene mutations and expression of microRNAs to matrix metalloproteinase. There is an intense need for new findings in the translational research field with prognostic, predictive and therapeutic value.

Introduction

Pancreatic cancer is the 4th amongst cancer deaths in America, with annual mortality rate being equivalent to annual morbidity [1]. Cigarette smoking, increased body mass index, alcohol use, and diabetes are some of the risk factors of pancreatic cancer [2]. Underlying theme is that genetics play a major role in cancer development and also influence the response to treatment. Because of its lethality, high mortality, and late presentation, it is vital that novel drug targets are developed to combat pancreatic cancer.

What We Knew Before the 2014 ASCO Gastrointestinal Cancers Symposium

BRCA2

Pancreatic cancer is a genetic disease, caused by inherited germline mutations and acquired somatic mutations. Ninety-five percent cases of pancreatic cancer are sporadic while 5% are familial [3]. Mutations in genome maintenance genes (i.e., genes

responsible for identifying and repair damaged DNA) like *BRCA2*, which aids in DNA strand and interstrand repair lead to genomic instability and ultimately to tumorigenesis. Seven to ten percent of pancreatic ductal adenocarcinomas carry a mutated copy of *BRCA2* gene which, when accompanied by loss of heterozygosity, leads to tumorigenesis [4]. Recent studies have shown that *BRCA1/BRCA2* deficient cells have increased sensitivity to poly(ADP-ribose)phosphate (PARP) inhibitors through the concept of synthetic lethality. Synthetic lethality refers to a genetic interaction when the combination of mutations in two genes leads to cell death, yet the mutation in either gene is viable with life [1].

PARP inhibitors have shown promise in *BRCA1* and *BRCA2* deficient ovarian and breast cancers. In xenograft and *in vitro* studies, PARP inhibitors killed *BRCA2* deficient cells at doses that were nontoxic to normal cells. Furthermore, sensitivities of the *BRCA2* deficient cells to PARP inhibitors were 90 times more than wild type cells. Olaparib and MK 4827 are two PARP inhibitors that have activity in *BRCA1* and *BRCA2* tumors when used as single agents [5]. In cancer cells with *BRCA* mutations, PARP inhibitors are specific to tumor cells because most *BRCA* mutations are heterozygous and after a second hit, may become homozygous, which is nonviable [5]. However, *BRCA1* and *BRCA2* deficient cells can also develop resistance to PARP inhibitors, and escape synthetic lethality if the mutation reverses and restores wild type *BRCA* function [6].

Key words Genes, *BRCA1*; Genes, *BRCA2*; Matrix Metalloproteinases; Pancreas; Pancreatic Neoplasms

Abbreviations miR-21: microRNA-21; PARP: poly(ADP-ribose)phosphate

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MicroRNA

MicroRNAs are 18-23 nucleotide non coding RNAs which can function as tumor suppressor genes (*TSGmiRs*) or oncogenes (*OncomiRs*). They bind to target RNAs and induce post-transcriptional gene silencing by either cleaving the target microRNA or by inhibiting the translation process [4]. Sicard *et al.* were the first to demonstrate that microRNA-21 (*miR-21*) is up-regulated during tumorigenesis of pancreatic cancer and participates in cancer cell proliferation, migration, invasion, metastasis and resistance to chemotherapy. Pancreatic tumor growth was reduced in pancreatic cancer cell lines in which *miR-21* was targeted [7]. MicroRNAs have been investigated for their role as potential diagnostic and prognostic markers as well. For example, *miR-452*, *105*, *127*, *518 a-2*, and *187*, are up-regulated and can aid in distinguishing long-term survivors in patients who have nodal involvement of their pancreatic cancer. MicroRNA-196a-2 is also up-regulated and predicts poor survival. *MiR-200c* is associated with better survival rates [8].

Matrix Metalloproteinases

Pancreatic cancer has a dense desmoplastic stroma and hypoxic microenvironment due to lack of vascularization. This dense stroma plays a major role in cancer progression and resistance to chemotherapy agents as it makes up of eighty percent of pancreatic tumor [1]. Increased matrix metalloproteinase (MMP) activity along with the increased desmoplastic reaction in the extracellular matrix lead to an abnormal composition and quality of the extracellular matrix in many cancers. MMPs, vital to normal tissue homeostasis, have very diverse and conflicting roles depending on type of local source and stage of cancer [9]. Multiple trials using novel MMP agents as new drug therapies have been conducted [1]. However, the toxicity profile of these agents is unknown. Unlike PARP inhibitors, MMPs are nonspecific and can target normal cells as

well as the abnormal cells. A phase III clinical trial of marimastat (a broad MMP inhibitor) did not demonstrate any improvement in overall survival compared to treatment with gemcitabine plus marimastat. Another phase III trial of Bay 129566, specific inhibitor of MMP-2, 3, 9 and 13 showed no improvement as well. MMPs as novel drug target therapies have not performed well in clinical trials. However, they can be utilized to service as markers of disease response [10].

What We Learned at the 2014 ASCO Gastrointestinal Cancers Symposium

PARP Inhibitors in BRCA1/BRCA2 CancerFU (Abstract #206 [11])

Studies have shown that pancreatic adenocarcinoma associated with *BRCA1/BRCA2* and *PALB2* mutations is sensitive to DNA damaging agents like PARP inhibitors. Lowery *et al.* [11] tried to identify *BRCA1/BRCA2* germline or somatic mutations in unselected pancreatic cancers. All patients who underwent surgical resection at Memorial Sloan Kettering Cancer Center (New York, NY, USA) for pancreatic cancer after the year 2000 were analyzed to find out if they have *BRCA1/BRCA2*, *PALB2* and *KRAS* mutations. The germ line mutations are found in 7% of patients with pancreatic cancer, with the most common being the Ashkenazi founder mutation 6174delT. It was discovered that bi-allelic loss of *BRCA2/PALB2* is not required for pancreatic cancer development. *KRAS* mutations were found in all *BRCA2*, *PALB2*, and *RAD51c* mutated cancers, emphasizing that *KRAS* mutations may be the ultimate driver of malignancy. Benefit of PARP inhibitors in *BRCA1/BRCA2* cancer is limited to the cancers that show loss of heterozygosity.

Effect of MicroRNA-21 (miR-21) Gene on Clinical OutcomesFU (Abstract #234 [12])

Expression of microRNA-21 (*miR-21*) gene and its effects on clinical outcomes was evaluated in 41 patients who underwent curative surgery and then adjuvant therapy with gemcitabine. Expression levels of *miR-21* were based on staining intensity, which was scored from 1 to 3 ranging from weak to strongly positive. Percentage of positive tumor cells were also semi-quantified, with less than 50% equal to 1, 50-80% equal to 2, and more than 80% equal to 3. The sum of scores from the staining intensity for *miR-21* and the scores from the percentage of positive tumor cells made up the composite score. Less than 4 was described as low expression of microRNA expression group and more than 4 was the high *miR* group. It was concluded that high *miR* expression in pancreatic cancer is associated with shorter disease free survival and overall survival (Figure 1).

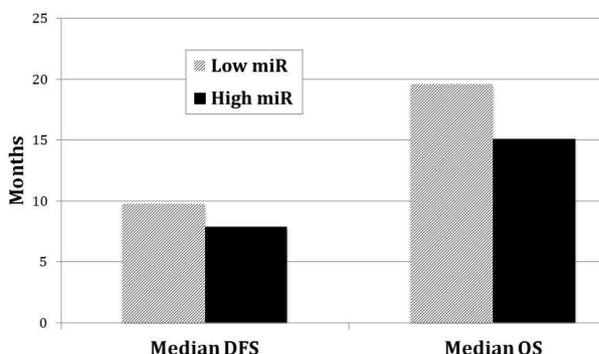


Figure 1. Survival vs. intensity of microRNA-21 staining plus severity of cancer. High microRNA-21 expression is associated with shorter disease free survival (DFS) and overall survival (OS) as graded by expression of microRNA (*miR*).

Impact of Low Levels of Matrix Metalloproteinase-7 on Adjuvant Therapy with Gemcitabine or 5-FU (Abstract #176 [13])

Heestand *et al.* conducted a trial in which they determined pancreatic cancer with low levels of MMP-7 might benefit from adjuvant therapy with gemcitabine. In this study, stage I-III resected cancer patients were randomized to either F-5U or gemcitabine to study 42 key proteins that corresponded with survival or predicted response. Decreased CEA and CA 19-9 determined markers of overall survival benefit. They also concluded that proximity ligation assay is a powerful tool that can help identify potential biomarkers.

Discussion

Pancreatic cancer is a deadly disease with a 5-year survival of less than 5% [10]. Research in the past two decades has shown that pancreatic cancer is essentially a genetic disease and understanding molecular genetics has allowed to the development of new drug targets [4]. The abstracts discussed above highlight the importance of using molecular genetics to find novel drug targets or markers of disease [8]. An ideal biomarker is something can help with an early diagnosis but also one that has good prognostic indication [3]. Currently, CA 19-9 is the most commonly used marker for pancreatic cancer. It is 90% specific and 80% sensitive. The use of CA 19-9 as a diagnostic tool is limited because it can be elevated in benign obstructive jaundice, chronic pancreatitis, liver cirrhosis, and cholangitis and in gastrointestinal cancers. It is also not sensitive to cancer sizes less than 3 cm [8].

The future of pre-clinical research in pancreatic cancer should continue to focus on developing markers that not only aid in diagnosing pancreatic cancer at earlier stages, like MMP-7, but can also assess treatment response [8]. A plasma microRNA profile can be developed as some microRNAs are up-regulated and others are down-regulated in pancreatic cancer. Research is also underway to analyze pancreatic juice for markers of disease. However, obtaining pancreatic juice will require an invasive procedure. Cytokeratin-20 is epithelium specific marker that is elevated in precursor lesions

to pancreatic cancer. It is expressed in 30-50% in pancreatic cancer but not expressed in normal adult pancreas and can also predict prognosis because cytokeratin-20 cancers are associated with a poor prognosis [3].

Conflicts of interest The authors have no potential conflicts of interest

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