HIGHLIGHT ARTICLE

Translational Research in Pancreatic Cancer

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Summary

Pancreatic cancer is an aggressive type of cancer ranking as the 10th most common cancer and the 4th cause of cancer related deaths. Due to disappointing treatment results and outcome of pancreatic cancer patients there is urgent need for better understanding of pathogenesis, mechanisms of tumor progression and resistance to treatment in order to achieve etiological approach. The development of the field of translational research and pharmacogenomics during the last several years has revealed many molecular pathways being aberrant in pancreatic cancer. This knowledge has led to the identification of biomarkers with prognostic or predictive value and the development of novel drugs against specific abnormal targets of pancreatic tumors. In this year's ASCO Gastrointestinal Cancers Symposium, researchers presented data showing evidence of biomarkers with prognostic value (Abstracts #166, #140, and #126) and genetic polymorphisms predicting possibly efficacy of gemcitabine treatment (Abstract #166). The development of the new small molecule CRT0066101 targeting the protein kinase D (PKD), which is upregulated significantly in pancreatic cancer cells was also presented (Abstract #159). This small molecule blocked specifically PKD and inhibited potently *in vitro* and *in vivo* the growth of pancreatic cancer cells. Furthermore, a retrospective analysis of *KRAS* status on resected pancreatic cancer specimens showed that frequency of *KRAS* mutations was 67% (57% of those were on codon 12), lower than previous reported in more advanced stages (Abstract #169). In this paper we present details and comment on these works.

Introduction

Pancreatic cancer is a devastating and aggressive malignancy with high mortality almost equal to its incidence; estimation for 37,000 new cases and 34,000 deaths in 2008 only in United States [1]. Though remarkable advances have been achieved based on clinical and preclinical research, these have not translated to equal noteworthy improvements at the bedside. Many initially promising agents have failed to prove some benefit despite being tested in numerous studies. There are many novel drugs that are evaluated in preclinical and clinical studies and hopefully some of them will eventually show some activity against pancreatic cancer. At the moment, strong evidence of efficacy exists only for some chemotherapy drugs such as gemcitabine, platinum agents and capecitabine, one targeted agent (erlotinib) and possibly for radiotherapy in the locally advanced disease [2]. As long as the prognosis remains poor, increasing understanding of carcinogenesis and molecular abnormalities of this disease is needed along with improvement in diagnostic means and treatment options. There are quite a few translational and pharmacogenomic advances that have been recently developed. For example, polymorphisms of reductase ribonuclease M1 (*RRM1*) and cytidine deaminase (*CDA*) genes are known to predict toxicity of gemcitabine. Similarly, genetic changes of *HuR* and deoxycytidine kinase (*dCK*) are predictors of response to this cytotoxic drug. In this paper we will discuss the incidence of *KRAS* mutations in early pancreatic cancer, the predictive value of *RRM1*, the prognostic value of human equilibrative nucleoside transporter 1 (*hENT-1*) expression, caveolin-1 expression and sonic hedgehog (*SHH*) polymorphisms and the potential of inhibiting protein kinase D (PKD) in pancreatic cancer patients.

Key words Biological Markers; Biological Therapy; gemcitabine; Pancreatic Neoplasms; Pharmacogenetics

Abbreviations *Cav-1*: Caveolin-1; CDA: cytidine deaminase; CI: confidence interval; dCK: deoxycytidine kinase; dFdCDP: difluorodeoxycytidine diphosphate; dFdCTP: difluorodeoxy-cytidine 5'-triphosphate; GWAS: genome-wide association study; *hENT-1*: human equilibrative nucleoside transporter 1; HR: hazard ratio; OS: overall survival; PFS: progression free survival; PKD: protein kinase D; *RRM1*: reductase ribonuclease M1; *SHH*: sonic hedgehog; SNP: single nucleotide polymorphism

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Translational Studies

Gemcitabine is a fluorine-substituted deoxycytidine analog which is activated intracellularly by the enzyme deoxycytidine kinase (dCK) and metabolised then to

the cytotoxic nucleotide difluorodeoxycytidine 5'-triphosphate (dFdCTP). Quite a few DNA polymerases are inhibited by the dFdCTP metabolite interfering to DNA chain synthesis, elongation or repair. The diphosphate form (dFdCDP) inhibits the enzyme ribonucleotide reductase, resulting in decreased levels of essential deoxyribonucleotides for DNA synthesis and function. Gemcitabine is inactivated by cytidine deaminase (CDA) to its inactive product 2',2'-difluorodeoxyuridine (dFdU). Polymorphisms of the CDA enzyme have been previously reported and have been associated with decreased clearance and increased toxicity of gemcitabine [3].

Similar to other antineoplastic drugs, cancer cells may develop resistance to gemcitabine effect via various ways, commonly decreased transport into the cells by the human equilibrative nucleoside transporters (hENT), increased catabolism by CDA or decreased activation by dCK, etc. [4].

The studies with translational interest presented in this year's ASCO Gastrointestinal Cancers Symposium are summarized in Table 1.

Abstract #169: KRAS mutations in a population of patients operated for pancreatic adenocarcinoma [5]

Researchers from Denmark presented a translational study on *KRAS* mutations in early pancreatic cancer. Previous reports had showed that the frequency of KRAS gene mutations in pancreatic cancer was as high as 75-90% [2]. This high percentage of *KRAS* mutations was representative of all pancreatic cancer stages, which is in fact consisted mainly of stage IV, due to the usual late presentation and diagnosis of these patients. In this study, tumor samples from 277 operated patients for pancreatic adenocarcinoma (n=161) and ampullary cancer (n=116) were tested for *KRAS* mutations by polymerase chain reaction (PCR) and LightScanner®, Idaho Technology Inc., Salt Lake City, UT, USA. For the pancreatic cancer patients group, *KRAS* was found mutated in 67%, mainly involving codon 12 (57%) and less so codons 13 and 61. Univariate analysis showed that *KRAS* mutation was associated with short progression free survival (PFS) (hazard ratio (HR): 1.36, 95% confidence interval (CI): 1.01-1.38; P=0.043) and no statistically significant short overall survival (OS) (P=0.10). The authors suggested that the early stage pancreatic cancer group may represent a subpopulation with fewer genetic changes, thus why the lower frequency of *KRAS* mutations, than the advanced disease group. The prognostic significance of *KRAS* mutations is rather unclear as the data from literature are conflicting.

Abstract #166: Significance of hENT-1 and RRM1 expression in resected pancreatic cancer [6]

Apart from their known role in gemcitabine resistance and toxicity, *hENT-1* and *RRM1* were studied for their potential prognostic value in early resected pancreatic cancer. From 179 patients having undergone pancreaticoduodenectomy for pancreatic adenocarcinoma at the Cleveland Clinic, Ohio, in the United States between 1999 and 2006, RNA was isolated in 84 of them and the expression levels of *hENT-1* and *RRM1* were evaluated by reverse transcriptase-polymerase chain reaction (RT-PCR). From this cohort of patient, only 18 received adjuvant gemcitabine chemotherapy. Using Cox proportional hazard analysis high *hENT-1* levels were associated with better OS (P=0.007) and PFS (P=0.016) compared to low *hENT-1* expression. The independent prognostic significance of *hENT-1* was confirmed on multivariate analysis. As far as *RRM1* is concerned, no prognostic value was demonstrated, but patients with low *RRM1* levels were likely to benefit from adjuvant gemcitabine. This is consistent with previous studies which had showed that overexpression of *RRM1* was associated with poor response to gemcitabine likely due to the increased concentration of nucleotide deoxycytidine triphosphate which is competing the action of gemcitabine.

Table 1. Summary of abstracts with translational and pharmacogenomic interest.

Abstract	Researcher, Institute	Title	Main findings *Comments*
#169 Schultz NA, *et al.* [5]	Hervel Hospital, Copenhagen, Denmark	KRAS mutations in a population of patients operated for pancreatic adenocarcinoma	**Frequency of *KRAS* mutations in early pancreatic ductal adenocarcinoma is 67%; mutations may be related to shorter PFS** *Prognostic value based on univariate analysis*
#166 Tan A, *et al.* [6]	Cleveland Clinic Found, Cleveland, OH, USA	Significance of hENT-1 and RRM1 expression in resected pancreatic cancer	**High *hENT-1* expression is associated with better OS and PFS. Patients with low *RRM1* expression possibly derive benefit from adjuvant gemcitabine** *Retrospective study, small sample (n=84)* *Only 18 patients treated with gemcitabine*
#126 McWilliams RR, *et al.* [7]	Mayo Clinic, Rochester, MN, USA	Association of sonic hedgehog variant with survival in pancreatic cancer	**Germline SNP of *SHH, rs1233556*, is associated with increased OS** *Results were based on GWAS*
#140 Williams TM, *et al.* [8]	University of Michigan, Ann Arbor, MI, USA	Caveolin-1: A potential biomarker of poor prognosis in pancreatic cancer	**Caveolin-1 expression is upregulated in poorly differentiated pancreatic tumors and related to short TTP** *Potential biomarker of poor prognosis*
#159 Guha S, *et al.* [10]	M. D. Anderson Cancer Center, Houston, TX, USA	Protein kinase D: A novel therapeutic target in pancreatic cancer	**PKD is upregulated in pancreatic cancer (PaCa) cell lines. PKD inhibitor CRT0066101 blocked PaCa growth** *Preclinical study. No previous similar data*

GWAS: genome-wide association study; *hENT-1*: human equilibrative nucleoside transporter 1; OS: overall survival; PFS: progression free survival; PKD: protein kinase D; *RRM1*: reductase ribonuclease M1; *SHH*: sonic hedgehog; SNP: single nucleotide polymorphism; TTP: time to progression

Abstract #126: Association of sonic hedgehog variant with survival in pancreatic cancer [7]

Using data from a genome-wide association study (GWAS), McWilliams *et al.*, tried to identify possible polymorphisms affecting the *SHH* genes as the hedgehog pathway is thought to be activated and related to the extensive stromal reaction in pancreatic cancer, and thus facilitate resistance to chemotherapy. Pancreatic tumors from 605 patients treated at Mayo Clinic were studied by GWAS and multivariate analysis including factors such as tumor stage, age, sex and performance status was performed. It was found that presence of the single nucleotide polymorphism (SNP) *rs1233556*, which is carrying minor alleles, in the *SHH* gene was associated with decreased desmoplasia (P=0.029) and increased overall survival (HR: 0.835, 95% CI: 0.71-0.98; P=0.033). Therefore, this germline mutation of the *SHH* gene may serve as a good prognostic factor but may also justify targeting of the activated SHH pathway with novel drugs in future.

Abstract #140: Caveolin-1: A potential biomarker of poor prognosis in pancreatic cancer [8]

Cancer growth and progression is often sustained due to uncontrolled activation of important signalling proteins, enzymes, kinases and other molecules which participate in complex and cross-talking signalling pathways. Caveolin-1 (*Cav-1*) belongs to the family of caveolins which are membrane proteins and play role in signal transduction and tumorigenesis [9]. In pancreatic tumor samples from xenografts and from patients operated radically, *Cav-1* expression was tested using immunohistochemistry study. *Cav-1* was significantly up-regulated in both the preclinical model and the resected specimen from patients. The overexpression was even more profound in poorly differentiated tumors as compared to well differentiated ones (P<0.05) and directly correlated to preoperative CA 19-9 tumor marker levels. The statistical analysis revealed also a trend of *Cav-1* overexpression for shorter time to progression (P=0.088). In conclusion, *Cav-1* is another poor prognostic biomarker which may be used in future when a more personalized management is adopted.

Abstract #159: Protein kinase D: A novel therapeutic target in pancreatic cancer [10]

The role of the serine/threonine PKD family in carcinogenesis, angiogenesis, tumor growth and survival is increasingly recognized. The PKD family includes three members, PKD1, 2 and 3. In this year's ASCO Gastrointestinal Cancers Symposium, the potential of the small-molecule CRT0066101 as a PKD inhibitor was presented. Protein PKD1 was found significantly upregulated in pancreatic cancer cells (PaCa) as compared to normal cells (91% versus 22%, P<0.001). CRT0066101 blocked proliferation of PaCa cells *in vitro* and abrogated the activity of nuclear factor-kappaB (NF-kappaB). Similarly, when CRT0066101 was given orally to orthotopic and xenograft models carrying subcutaneous pancreatic cancer tumors, at a dose of 80 mg/kg/day for 3-4 weeks, the expression of activated PKD (pS916PKD1) was inhibited (P<0.05) and tumor growth was significantly attenuated (P<0.01). The authors demonstrated that the small molecule CRT0066101 is a PKD inhibitor and concluded that these *in vitro* and *in vivo* results prove PKD as a new therapeutic target in pancreatic cancer which needs further evaluation.

Discussion

The dismal outcome of patients with pancreatic cancer may be a result of poorly understood biological and genetic events that determine its aggressive nature and the poor response to treatments. There are labor efforts from clinicians and researchers to improve understanding and therapeutic efficacy. Of the above selected abstracts presented in this paper we can draw some useful conclusions.

We learnt that early pancreatic cancers may carry fewer genetic changes than advanced stages which actually influence prognosis. Though it sounds quite logical, its impact in our current clinical practice is minimal. The prognostic significance of *KRAS* mutations (mainly in codon 12) is still unknown as the data presented in Abstract #169 [5] were based on univariate analysis and therefore cannot be reliably accepted, as other prognostic factors may have influenced the reported results. Furthermore, the retrospective nature of this study along with the rather small patients' sample weakens the level of evidence. The importance of SHH pathway and the significance of *SHH* gene polymorphisms were studied in Abstract #126 [7]. The hypothesis of SHH pathway involvement in resistance to treatment is very interesting. A negative point of this study is the fact that the results were based on data from the novel GWAS. There are quite a few challenges concerning the genome wide association studies such as the high rate of false-positive results due to massive number of statistical tests required, the genotyping errors, bias related to population selection, the lack of replication of the results, lack of facilities and the high cost [11]. All these make the findings difficult to be reproduced and necessary to validate them in prospective translational studies.

The prognostic value of *hENT-1* was demonstrated in Abstract #166 [6] and that was statistically significant. Again, validation in more prospective and large studies is needed as the sample size used was rather small. In the same study, low levels of RRM1 suggested some benefit from adjuvant chemotherapy, but only 18 patients out of 84 had received gemcitabine and therefore this finding should be viewed with skepticism. Nevertheless, it is consistent with previous reports suggesting high levels of RRM1 may confer resistance to gemcitabine.

The role of caveolin-1 as a biomarker of poor prognosis is a new finding in pancreatic cancer [8] and therefore need further replication and validation in large prospective studies in order to be definitely

accepted. Its role in cancer growth and resistance to treatment may offer some ground for developing agents able to block its action and overcome the treatment failure. Lastly, the development of a new targeted agent [10] is quite a significant fact as it offers hope for a potentially active weapon in the battle of cancer. It will greatly anticipate to see the tolerance, safety and efficacy of the PKD inhibitor CRT0066101 in human trials. The experience so far has showed that only 1 in every 10 drugs, tested in drug development units, will actually justify the initial optimism, and eventually benefit the suffering patients. This fact should not disappoint and restrain researchers from continuing their fight.

In the middle of financial crisis, joined forces globally can assure reasoned use of resources and conduction of large studies able to produce faster, reliable, and cost-effective results.

Conflict of interest The authors declare no conflicts of interest

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