JOP. J Pancreas (Online) 2006; 7(3):324-326.

PANCREAS NEWS

Cells Lines *vs*. Animals Studies for Developing New Therapeutic Strategies in Human Pancreatic Cancer?

Raffaele Pezzilli, Lorenzo Fantini

Department of Internal Medicine, Sant'Orsola-Malpighi Hospital. Bologna, Italy

In the past, most of the knowledge gained regarding the physiology and the pathology of the pancreas has been evaluated in experimental studies on animals especially on rats/mice. This approach has been criticized in recent years because most of the data obtained from animals cannot be fully applied to humans [1]. A new approach to the physiology and pathology of the pancreas comes from studying its molecular biology, and the results obtained seem to be more reliable than those obtained in animals. An example of this assumption comes from studies on pancreatic ductal adenocarcinoma: this cancer seems to result from a progressive accumulation of mutations in genes such as K-ras, CDKN2A, p53, BRCA2, p164ink, and SMAD4 [2]; in particular, the SMAD4 mutations which result in the constitutive activation of transforming growth factor b1 signalling, are generally considered to be responsible for the desmoplastic response, which includes upregulated expression of the extracellular matrix, and type I collagen [3, 4, 5, 6]. Grzesiak *et al*. [7, 8] have also shown that a2b1 integrin-mediated adhesion on type I collagen promotes a malignant phenotype in FG pancreatic cells, as defined by increased proliferation and haptokinetic cell migration, downregulated expression and localization of E-cadherin and b-catenin in cell-cell contacts, increased phosphorylation of GSK3b and PKB/Akt, and downregulated expression of PTHrP, IL-6, and IL-8 as compared to fibronectin, type IV collagen, laminin, or vitronectin. These results are in agreement with previous studies demonstrating that type I collagen downregulates E-cadherin expression in Panc-1, BxPC-3, and PaTu8988s pancreatic cancer cells, resulting in increased proliferation and migration compared to fibronectin. Recently, the same group of researchers demonstrated similar phenotypic differences in BxPC-3, Colo-357, and CFPAC cells on type I collagen as compared to fibronectin; that is, increased haptokinetic cell migration, downregulated expression and localisation of E-cadherin and b-catenin in disrupted cell-cell contacts, increased phosphorylation of GSK3 and PKB/Akt, and decreased expression of PTHrP, IL-6, and IL-8. Furthermore, functional studies with pharmacological inhibitors for GSK3 and PKB/Akt suggest that these signalling effectors are involved in the mechanism of a2b1 integrin-mediated regulation of the malignant phenotype in FG cells. Type I collagen, in particular, seems to play an active role in vitro and in vivo in the pathophysiology of pancreatic cancer [9, 10] as demonstrated by in vitro studies which have shown how pancreatic cancer cell lines stimulate the production of type I collagen from adjacent stellate cells, which resulted in increased cancer cell proliferation and resistance to chemically induced apoptosis. However, the integrin specificity of this interaction between tumor cells and type I collagen was not identified. Thus, Grzesiak and Bouvet [11] examined eight pancreatic

cancer cell lines for adhesion, proliferation, and migration on types I and IV collagen, fibronectin, laminin, and vitronectin, as well as integrin expression. They have shown that type I collagen promotes the strongest adhesion, proliferation, and migration relative to the other substrates tested. Utilizing function-blocking monoclonal antibodies directed against particular integrin subunits in cell adhesion and migration inhibition assays, they further demonstrated that the malignant phenotype on type I collagen is mediated specifically by the a2b1 integrin. These results identify a2b1 integrin-mediated adhesion to type I collagen as a potential therapeutic target in the treatment of pancreatic cancer.

The main utility of molecular studies for clinicians is the introduction of new agents for controlling pancreatic cancer progression into clinical practice. One example is the finding that altered expression or constitutive activation of the epidermal growth factor receptor (EGFR/HER1/erbB1) commonly occurs in both primary and metastatic pancreatic cancers and is often a critical component in progressive growth and resistance to normal mechanisms of cell death [12, 13, 14]. Epidermal growth factor receptor expression in pancreatic cancer has been correlated with tumor aggressiveness [15]. On the basis of this information, it has been found that matuzumab, a humanised immuno-globulin G1 (IgG1) monoclonal antibody to the human EGFR binds the EGFR with high affinity, competitively blocking natural ligand binding and blocking receptor-mediated downstream signalling. In preclinical studies, matuzumab demonstrated activity in the PAXF546 xenograft model of human pancreatic cancer which expressed high levels of EGFR and demonstrated almost complete resistance to clinically available chemo-therapeutic drugs. Also, combining matuzumab with gemcitabine enhanced the effects of gemcitabine in a model of gemcitabine-sensitive pancreatic cancer and antitumor effects were mediated by both direct inhibition of tumor growth and inhibition of tumor-induced angiogenesis. In addition, because matuzumab is an IgG1 antibody to the EGFR capable of mediating antibody-dependent cellular cytotoxicity, additional cytotoxic mechanisms may be involved in its effects on pancreatic cancer. A phase I clinical trial with matuzumab demonstrated that it is well-tolerated as a single agent [16]; it blocks growth factor signalling through EGFR with a weekly dose of 800 mg and the dose-limiting toxicities occurred at 2,000 mg on a weekly schedule consisting of headache and fever. Skin reactions, a common effect of anti-EGFR, also occur with matuzumab, but they are less severe than those of the other anti-EGFR agents. The results of a phase I study for assessing the safety and potential benefit of combined treatment with matuzumab and standard-dose gemcitabine have recently been communicated [17]. Three groups of chemotherapy-naive advanced pancreatic adenocarcinoma patients received increasing doses of matuzumab (400 mg weekly, 800 mg biweekly, or 800 mg weekly) and gemcitabine (1,000 mg/m^2 weekly in weeks 1-3 of each 4-week cycle). The results of this study have shown that severe treatment-related toxicities were limited to grade 3 neutropenia, leucopenia, and a decreased white blood cell count. Common drug-related adverse events were skin toxicities and fever. Matuzumab inhibited phosphorylated EGFR and affected receptor-dependent signalling and transduct-ion, the effects being seen even in the lowest-dose group. Pharmacokinetic data were consistent with results of matuzumab monotherapy. Partial response or stable disease occurred in 66.7% of the treated patients, with three partial responses among six patients evaluated in the group receiving 800 mg weekly. In conclusion, matuzumab seems to be well tolerated in biologically effective doses with standard gemcitabine therapy; the combination is feasible and may have enhanced activity.

In summary, we hope that the search for new antitumoral strategies will be carried out on cellular lines rather than on animals; this approach seems to be better and easier for applying the positive results to humans.

Keywords Antibodies, Monoclonal; Gemcitabine; Pancreatic Neoplasms; Receptor, Epidermal Growth Factor

Correspondence
Raffaele Pezzilli
Dipartimento di Medicina Interna
Ospedale Sant'Orsola-Malpighi
Via Massarenti, 9
40138 Bologna
Italy
Phone: +39-051.636.4148
Fax:+39-051.636.4148
E-mail: pezzilli@aosp.bo.it

References

1. Case RM. Is the Rat Pancreas an Appropriate Model of the Human Pancreas? Pancreatology 2006; 6:180-90. [PMID 16534243]

2. Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. Nat Rev Cancer 2002; 2:897-909. [PMID 12459728]

3. Mollenhauer J, Roether I, Kern HF. Distribution of extracellular matrix proteins in pancreatic ductal adenocarcinoma and its influence on tumor cell proliferation in vitro. Pancreas 1987; 2:14-24. [PMID 3554225]

4. Menke A, Adler G. TGFbeta-induced fibrogenesis of the pancreas. Int J Gastrointest Cancer 2002; 31:41-6. [PMID 12622414]

5. Iacobuzio-Donahue CA, Ashfaq R, Maitra A, Adsay NV, Shen-Ong GL, Berg K, et al. Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies. Cancer Res 2003; 63:8614-22. [PMID 14695172]

6. Iacobuzio-Donahue CA, Maitra A, Olsen M, Lowe AW, van Heek NT, Rosty C, et al. Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. Am J Pathol 2003; 162:1151-62. [PMID 12651607]

7. Grzesiak JJ, Clopton P, Chalberg C, Smith K, Burton DW, Silletti S, et al. The extracellular matrix differentially regulates the expression of PTHrP and the PTH/PTHrP receptor in FG pancreatic cancer cells. Pancreas 2004; 29:85-92. [PMID 15257098]

8. Grzesiak JJ, Smith KC, Burton DW, Deftos LJ, Bouvet M. GSK3 and PKB/Akt are associated with integrin-mediated regulation of PTHrP, IL-6 and IL-8 expression in FG pancreatic cancer cells. Int J Cancer 2005; 114(4):522-30. [PMID 15609321]

9. Armstrong T, Packham G, Murphy LB, Bateman AC, Conti JA, Fine DR, et al. Type I collagen promotes the malignant phenotype of pancreatic ductal adenocarcinoma. Clin Cancer Res 2004; 10:7427-37. [PMID 15534120]

10. Bachem MG, Schunemann M, Ramadani M, Siech M, Beger H, Buck A, et al. Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. Gastroenterology 2005; 128:907-21. [PMID 15825074]

11. Grzesiak JJ, Bouvet M. The alpha(2)beta(1) integrin mediates the malignant phenotype on type I collagen in pancreatic cancer cell lines. Br J Cancer 2006; (Epub ahead of print). [PMID 16622460]

12. Lemoine NR, Hughes CM, Barton CM, Poulsom R, Jeffery RE, Kloppel G, et al. The epidermal growth factor receptor in human pancreatic cancer. J Pathol 1992; 166:7-12. [PMID 1538276]

13. Schmiegel W, Schmielau J, Henne-Bruns D, Juhl H, Roeder C, Buggisch P, et al. Cytokine-mediated enhancement of epidermal growth factor receptor expression provides an immunological approach to the therapy of pancreatic cancer. Proc Natl Acad Sci USA 1997; 94:12622-6. [PMID 9356499]

14. Xiong HQ, Abbruzzese JL. Epidermal growth factor receptor-targeted therapy for pancreatic cancer. Semin Oncol 2002; 29(5 Suppl 14):31-7. [PMID 12422311]

15. Tobita K, Kijima H, Dowaki S, Kashiwagi H, Ohtani Y, Oida Y, et al. Epidermal growth factor receptor expression in human pancreatic cancer: significance for liver metastasis. Int J Mol Med 2003; 11:305-9. [PMID 12579331]

16. Vanhoefer U, Tewes M, Rojo F, Dirsch O, Schleucher N, Rosen O, et al. Phase I study of the humanized antiepidermal growth factor receptor monoclonal antibody EMD72000 in patients with advanced solid tumors that express the epidermal growth factor receptor. J Clin Oncol 2004; 22:175-84. [PMID 14701780]

17. Graeven U, Kremer B, Sudhoff T, Killing B, Rojo F, Weber D, et al. Phase I study of the humanized anti-EGFR monoclonal antibody matuzumab (EMD 72000) combined with gemcitabine in advanced pancreatic cancer. Br J Cancer 2006; March 28th (Advance Online Publication) [PMID 16622465]