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Confirmation of DNA Microarray-Derived Differentially Expressed Genes in Pancreatic Cancer Using Quantitative RT-PCR

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Abstract
The fact that pancreatic ductal adenocarcinoma (PDAC) is still an exceptionally lethal disease with an annual mortality almost equivalent to its annual incidence has stimulated intense research efforts directed at understanding the underlying molecular mechanisms. By enabling simultaneous expression analysis of thousands of genes, microarray technology has significantly contributed to illuminating the pathophysiology of PDAC. Gene expression profiling studies have been performed for molecular classification of clinically relevant tumor subtypes and have shed light on various signaling pathways associated with tumor progression. Altered expression levels of several genes have been identified as correlating with functional in vitro data as well as patient survival, indicating the potential clinical value of transcriptional profiling. However, broad clinical use of array techniques for patient characterization has been hampered by their cost intensity and by limited inter-study comparability. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR), as the most sensitive technique for mRNA detection and quantification, will complement arrays for the confirmation of individual transcripts in larger sample cohorts. This review highlights recent studies that addressed gene expression analysis with both methodologies and that identified components of the TGF-β signaling pathway, BNIP3, or periostin to be differentially expressed in PDAC. These studies demonstrated that the combination of microarray and RT-PCR technologies is a highly efficient and reliable approach for the identification of clinically important diagnostic and prognostic biomarkers, as well as for the discovery of novel therapeutic target candidates.

Introduction

Among the most common carcinomas, pancreatic ductal adenocarcinoma (PDAC) remains the one with the worst prognosis. PDACs represent about 95% of all pancreatic malignancies; the remaining 5% are tumors of the exocrine pancreas such as mucinous/serous cystic neoplasm, acinar cell cancers, and pancreatic neuroendocrine tumors. One major reason for the dismal prognosis of PDAC is that the development of the disease is associated with non-specific and variable symptoms. As a consequence, PDACs are usually diagnosed at a late stage, when surrounding tissues have already been infiltrated. Frequent metastasis, difficult resectability, broad resistance to conventional therapies, and a high incidence of local recurrence following resection are other reasons.

In almost all cases of pancreatic cancer, activating mutations of the K-ras oncogene have been identified as early events in tumor development [1]. Mutations in tumor

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suppressor genes, including p16 [2], p53 [3], and DPC4/SMAD4 [4], appear to occur as secondary genetic lesions in advanced stages of tumorigenesis.

In addition to the occurrence and accumulation of key genetic mutations in oncogenes and tumor suppressor genes, deregulated gene expression has been attributed to the pathogenesis of pancreatic cancer. In particular, the activity of cell surface receptor proteins and related signaling pathways were demonstrated to be perturbed through altered gene expression levels. Among others, the epidermal growth factor receptor itself (EGFR [5, 6]) as well as HER2/ErbB2 [7] and HER3/ErbB3 [8], along with other EGFR family members, has been proven to be frequently up-regulated in pancreatic tumors, which correlates with reduced survival and a worsened prognosis in PDAC patients. In addition to increased expression of the receptors themselves, up-regulation of the corresponding ligands, such as EGF [5, 6], TGF-α [5, 6], HB-EGF [9], epiregulin [10], betacellulin [11], amphiregulin [12], and heregulins [13], has also been observed in PDAC samples. Through autocrine or paracrine action, these factors stimulate tumor cell growth and contribute to enhanced aggressiveness and disease progression.

The development of DNA microarray technology made it possible to simultaneously analyze the expression status of thousands of genes. Reflecting entire transcriptional profiles, it is an extremely powerful tool for comparative studies and systematic screens of different tissue samples for molecular differences at the mRNA level. All presently available DNA chip or microarray technologies share the same general concept, regardless of whether oligonucleotides or full-length coding sequences are employed as immobilized substrates: complementary DNA (cDNA) pools prepared from samples of interest and labeled with fluorescent dyes are allowed to hybridize to solid-state DNA elements on the microarray chip. These elements derived from the coding sequence of individual genes are spotted to defined positions on the array surface. After hybridization, the fluorescence intensity at each spot is quantified. This value enumerated either as ratio to a reference sample or as an absolute intensity value specifically represents the abundance of the respective gene transcript.

The fact that several studies reported contradictory results caused the US Food and Drug Administration (FDA) to initiate a project to address the technical reproducibility of microarray measurements within and between laboratories, as well as across different microarray platforms [14]. These results show that measurements are highly reproducible and therefore sufficient reliable to be used for clinical and regulatory purposes. Nevertheless, further doubts have been raised regarding its application in disease diagnostics, staging or treatment prediction after discrepant results were obtained. These discrepancies were caused by the use of different microarray platforms with different probe sets and data normalization methods, as well as differences in study populations. Nonetheless, the microarray technology represents an ideal opportunity to start performing comprehensive or even genome-wide analysis of cellular transcription activities.

Quantitative real-time RT-PCR is the most sensitive technique currently available for mRNA detection and quantification and is therefore frequently used for the extended analysis of individual transcripts in large sample populations. In real-time RT-PCR, signals (generally fluorescent) are monitored as they are generated and are tracked after they rise above background levels, but before the reaction reaches a plateau. The cycle fraction at which fluorescence first rises above a defined background is known as the threshold cycle (C_t) or crossing-point. Consequently, the lower the C_t, the more abundant the initial target. Compared to other techniques for quantifying mRNA like Northern blot or RNase protection assay, RT-PCR can be used to quantify mRNA levels from much smaller samples. Although improvements are necessary to create internal standards, references for data normalization as well as common models for data analysis, the method is attractive for a clinical use, since it can be automated and performed on fresh or archived formalin-fixed, paraffin-embedded tissue samples. The outcome might accelerate the application of basic research findings into daily clinical practice through translational research and may have an impact on foreseeing the clinical outcome, predicting response to therapy, and discovering targets for the development of novel therapies.

A combination of the techniques therefore provides an efficient and reliable approach for gene expression profile-based tumor classification, the identification of clinically important diagnostic/prognostic biomarkers and target candidates.

Microarray-Based Identification of Differentially Expressed Genes in Pancreatic Cancer

One of the systems frequently used for comprehensive transcription profiling analysis is the GeneChip HuGeneFL array, which was developed by Affymetrix and contains full-length sequences from 5,600 human genes. This system was also utilized by our group to apply mi-

croarray technology to the field of pancreatic oncology [15, 16]: Friess and colleagues investigated the mRNA expression patterns of these 5,600 genes in 8 human PDAC samples and compared them with those obtained from 8 chronic pancreatitis (CP) and 8 normal pancreas control samples. Whereas CP samples showed partial similarities to both normal and cancer transcriptional profiles, expression patterns from cancer samples and normal tissues were clearly distinguishable in almost all cases. The detailed analysis of PDAC expression profiles and comparison with those of CP and normal tissues thus revealed gene expression alterations that most likely were directly related to the malignant phenotype, but which are distinct from those observed in CP.

In another approach to identify genes involved in pancreatic cancer progression, Buchholz et al. [17] utilized oligonucleotide microarray for the analysis of different grades of pancreatic intraepithelial neoplasias (PanIN-1 through -3), PDAC, and normal pancreatic ducts. Comparing the respective expression profiles, the authors suggested that PanIN-2 represents the first truly preneoplastic stage in the process of pancreatic carcinogenesis.

As of today, more than a dozen studies evaluating pancreatic cancer with DNA microarray technology as well as a meta-analysis have been published [15, 16, 18–27]. The results are somewhat difficult to compare, as different hybridization platforms were used and patient cohorts or cell lines sometimes were less well defined. Kuo et al. [28] demonstrated a poor correlation of data obtained with two different technologies, for instance cDNA versus oligonucleotide-based microarrays, even though the same samples were analyzed. Nevertheless, a comparison of these studies revealed some similarities in expression profiles, and a catalogue of 148 genes was extracted containing genes that were most up-regulated in at least two studies [29]. Among them are genes encoding the S100 calcium-binding protein P (S100P) and annexin-A1, laminin-γ2 or lipocalin-2, whose expression levels were found to be increased in seven and five studies, raising the possibility that these genes play an important role in PDAC development.

In order to improve the inter-study comparability of microarray-derived data and to establish microarray technology as a general clinical tool for the prediction of PDAC patient outcome and therapeutic response, more efforts should be made to standardize patient selection and technical parameters, such as the array platform or detection chemistry. Moreover, different techniques should be applied to validate expression differences, ideally in an extended sample cohort. As mentioned above, quantitative RT-PCR currently appears to be most suitable to affirm expression data reliability. Recent results from the combined application of both methods to three PDAC-related signaling modules – the TGF-β pathway, periostin and BNIP3 – will be discussed in the following sections.

The Transforming Growth Factor-β Signaling Pathway

The transforming growth factor-β (TGF-β) signaling pathway has been identified as one of the major contributors to the pathogenesis of pancreatic cancer. Paradoxically, increased expression of TGF-β was observed to accompany the progression of several cancer types, and disruption of normal TGF-β signaling has been attributed to many malignancies, such as breast, colon, and gastric cancer and melanoma [30]. Thus, TGF-β appears to exert opposing effects, both acting as a tumor suppressor in normal cells by inhibiting tumor initiation, and acting as a tumor promoter in cancerous cells, furthering the advancement of established tumors [31]. Comprehensive microarray analysis has confirmed the previously described overexpression of TGF-β_1 in pancreatic tumor samples as well as the up-regulation of Smad2 and Smad7, components of the TGF-β signaling pathway [32–34].

Additionally, these array experiments have revealed enhanced expression of TGF-β-activated kinase 1 (TAK1) and TAK1 binding protein-1 (TAB-1). TAK1 is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family, which has emerged as a key regulator of signaling cascades triggered in response to various stimuli [35, 36]. Activated through association with TAK1 binding proteins (TAB1–3) [37], TAK1 was identified as a mediator of TGF-β-induced, rapid activation of the MAPKs p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK). As a mediator of the I-κ B (IκB) kinase (IKK)/nuclear factor-κB (NF-κB) signaling pathway [38], TAK1 was also suggested to be critically involved in TGF-β_1-controlled regulation of matrix metalloproteinase-9 (MMP-9) expression. This is in line with experiments that demonstrate reduced MMP-9 expression levels upon suppression of TAK1 signaling [39]. As MMPs are established as functionally important components of the angiogenic switch during multistage pancreatic carcinogenesis [40], this metalloproteinase may provide the molecular link explaining the role of TAK1 in tumor angiogenesis and metastasis [41]. Thus, TGF-β_1 and TAK1 may represent potential targets for therapeutic antibodies and small molecules, respectively. Together with members

of the Smad and TAB families, they may define subsets of molecular signatures for progressive pancreatic cancer or other invasive malignancies that can be identified through microarray technology.

Periostin as a Regulator of Pancreatic Stellate Cell Activity

Since the first reports on the identification, isolation and characterization of pancreatic stellate cells (PSCs) [42], numerous studies have provided strong evidence that PSCs play a central role in fibrogenesis [43]. Stimulated by pancreatic cancer cells, PSCs excessively synthesize extracellular matrix proteins that create a tumor-supportive microenvironment and promote tumorigenesis [44, 45]. One of the PSC-specific matrix proteins, periostin (POSTN), is secreted as a disulfide-linked 90-kDa cell adhesion molecule. Consistent with its strong induction by TGF-β, POSTN context-dependently exerts pro-metastatic effects by increasing cell motility, cell survival and angiogenesis [46–48] as well as increasing anti-metastatic and tumor-suppressive activities such as anchorage-independent growth inhibition [49, 50]. Previously, POSTN was identified as a differentially expressed gene in PDAC by microarray profiling [16, 51], and in the following, RT-PCR analysis of POSTN mRNA levels was performed in a larger cohort of cancer patients treated with or without neoadjuvant chemoradiation (20 normal pancreas, 45 PDAC) [52]. POSTN expression was confirmed to be significantly increased in PDAC, more precisely two thirds showed a higher copy number than the donors. Patients with elevated expression levels showed a tendency towards shorter survival. Moreover, in an autocrine manner, POSTN has been shown to stimulate its own synthesis in PSCs as well as the expression of α-smooth muscle actin, collagen-1, fibronectin and TGF-β_1. In cancer cells, POSTN turned out to induce growth and to mediate resistance to hypoxia and to serum deprivation. In addition, collagen-1, as a downstream target of POSTN, significantly increased in vitro chemoresistance to 5-fluorouracil and gemcitabine. Increased POSTN expression may thus indicate a more aggressive tumor phenotype.

Loss of BNIP3 and Chemoresistance

BNIP3 is a member of the apoptosis-regulating Bcl-2 family, and is induced by hypoxia. Altered expression of this protein has been linked to several malignancies. The decreased BNIP3 expression in pancreatic cancer as compared to normal tissue was shown to be a consequence of *BNIP3* promoter hypermethylation [53], and restoration of BNIP3 expression through demethylation leads to hypoxia-inducible cell death [54]. In a study by Erkan et al. [55], quantitative RT-PCR was employed to address BNIP3 expression levels in whole tissue extracts from 26 healthy pancreas, 20 CP, and 23 PDAC samples. In comparison to normal tissue, there was a statistically significant reduction of BNIP3 mRNA levels in CP and an even more pronounced decrease in PDAC. Comparable relative expression levels were observed by microarray analysis of microdissected ductal epithelium from CP and PDAC patients versus microdissected normal ducts or normal pancreatic tissue: cancer cells revealed significantly lower BNIP3 expression compared to normal ducts or normal pancreatic tissue. In agreement with these findings, the comparison of the immunohistochemically assessed tumor BNIP3 expression status with corresponding clinical patient follow-up revealed that loss of BNIP3 expression significantly correlated with poorer survival of PDAC patients. These data suggest that BNIP3 – similar to periostin – plays an important role in the poor response of pancreatic cancer patients to gemcitabine treatment.

Conclusion

There is no doubt that gene expression profiling will have a great impact on the improvement of pancreatic cancer management and our understanding of the related disease biology. However, other important technologies are being developed that allow the analysis of, for instance, genetic and epigenetic alterations, microRNAs, and protein patterns in PDACs. All these platforms should help to provide a much more comprehensive understanding of the pathobiology of individual tumors. The challenge, however, will be to evaluate these molecular data, and to combine them with clinical parameters for more accurate prediction of disease outcomes and responses to anticancer drugs. Only close collaborations and the concerted actions of clinicians, clinical researchers and basic scientists will help to translate molecular findings into clinical applications. In order to facilitate interdisciplinary research, endeavors leading to improved survival and quality of life of pancreatic cancer patients, MolDiag-PaCa (www.moldiagpaca.eu), a multinational and EU-funded consortium, was launched in 2006.

References

1 Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M: Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 1988;53:549–554.

2 Caldas C, Hahn SA, da Costa LT, Redston MS, Schutte M, Seymour AB, Weinstein CL, Hruban RH, Yeo CJ, Kern SE: Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. Nat Genet 1994;8:27–32.

3 Scarpa A, Capelli P, Mukai K, Zamboni G, Oda T, Iacono C, Hirohashi S: Pancreatic adenocarcinomas frequently show p53 gene mutations. Am J Pathol 1993;142:1534–1543.

4 Wilentz RE, Iacobuzio-Donahue CA, Argani P, McCarthy DM, Parsons JL, Yeo CJ, Kern SE, Hruban RH: Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. Cancer Res 2000;60:2002–2006.

5 Korc M, Chandrasekar B, Yamanaka Y, Friess H, Buchier M, Beger HG: Overexpression of the epidermal growth factor receptor in human pancreatic cancer is associated with concomitant increases in the levels of epidermal growth factor and transforming growth factor-α. J Clin Invest 1992;90:1352–1360.

6 Yamanaka Y, Friess H, Kobrin MS, Büchler M, Beger HG, Korc M: Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. Anticancer Res 1993;13:565–569.

7 Friess H, Berberat P, Schilling M, Kunz J, Korc M, Büchler MW: Pancreatic cancer: the potential clinical relevance of alterations in growth factors and their receptors. J Mol Med 1996;74:35–42.

8 Friess H, Yamanaka Y, Kobrin MS, Do DA, Büchler MW, Korc M: Enhanced erbB-3 expression in human pancreatic cancer correlates with tumor progression. Clin Cancer Res 1995;1:1413–1420.

9 Ito Y, Higashiyama S, Takeda T, Yamamoto Y, Wakasa KI, Matsuura N: Expression of heparin-binding epidermal growth factor-like growth factor in pancreatic adenocarcinoma. Int J Pancreatol 2001;29:47–52.

10 Zhu Z, Kleeff J, Friess H, Wang L, Zimmermann A, Yarden Y, Büchler MW, Korc M: Epiregulin is up-regulated in pancreatic cancer and stimulates pancreatic cancer cell growth. Biochem Biophys Res Commun 2000;273:1019–1024.

11 Miyagawa J, Hanafusa O, Sasada R, Yamamoto K, Igarashi K, Yamamori K, Seno M, Tada H, Nammo T, Li M, Yamagata K, Nakajima H, Namba M, Kuwajima M, Matsuzawa Y: Immunohistochemical localization of betacellulin, a new member of the EGF family, in normal human pancreas and islet tumor cells. Endocr J 1999;46:755–764.

12 Ebert M, Yokoyama M, Kobrin MS, Friess H, Lopez ME, Büchler MW, Johnson GR, Korc M: Induction and expression of amphiregulin in human pancreatic cancer. Cancer Res 1994;54:3959–3962.

13 Kolb A, Kleeff J, Arnold N, Giese NA, Giese T, Korc M, Friess H: Expression and differential signaling of heregulins in pancreatic cancer cells. Int J Cancer 2007;120:514–523.

14 MAQC Consortium: The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 2006;24:1151–1161.

15 Friess H, Ding J, Kleeff J, Liao Q, Berberat PO, Hammer J, Büchler MW: Identification of disease-specific genes in chronic pancreatitis using DNA array technology. Ann Surg 2001;234:769–779.

16 Friess H, Ding J, Kleeff J, Fenkell L, Rosinski JA, Guweidhi A, Reidhaar-Olson JF, Korc M, Hammer J, Büchler MW: Microarray-based identification of differentially expressed growth- and metastasis-associated genes in pancreatic cancer. Cell Mol Life Sci 2003;60:1180–1199.

17 Buchholz M, Braun M, Heidenblut A, Kestler HA, Kloppel G, Schmiegel W, Hahn SA, Luttges J, Gress TM: Transcriptome analysis of microdissected pancreatic intraepithelial neoplastic lesions. Oncogene 2005;24:6626–6636.

18 Han H, Bearss DJ, Browne LW, Calaluce R, Nagle RB, Von Hoff DD: Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. Cancer Res 2002;62:2890–2896.

19 Iacobuzio-Donahue CA, Ashfaq R, Maitra A, Adsay NV, Shen-Ong GL, Berg K, Hollingsworth MA, Cameron JL, Yeo CJ, Kern SE, Goggins M, Hruban RH: Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies. Cancer Res 2003;63:8614–8622.

20 Iacobuzio-Donahue CA, Maitra A, Shen-Ong GL, van Heek T, Ashfaq R, Meyer R, Walter K, Berg K, Hollingsworth MA, Cameron JL, Yeo CJ, Kern SE, Goggins M, Hruban RH: Discovery of novel tumor markers of pancreatic cancer using global gene expression technology. Am J Pathol 2002;160:1239–1249.

21 Tan ZJ, Hu XG, Cao GS, Tang Y: Analysis of gene expression profile of pancreatic carcinoma using cDNA microarray. World J Gastroenterol 2003;9:818–823.

22 Logsdon CD, Simeone DM, Binkley C, Arumugam T, Greenson JK, Giordano TJ, Misek DE, Kuick R, Hanash S: Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. Cancer Res 2003;63:2649–2657.

23 Grutzmann R, Foerder M, Alldinger I, Staub E, Brummendorf T, Ropcke S, Li X, Kristiansen G, Jesnowski R, Sipos B, Lohr M, Luttges J, Ockert D, Kloppel G, Saeger HD, Pilarsky C: Gene expression profiles of microdissected pancreatic ductal adenocarcinoma. Virchows Arch 2003;443:508–517.

24 Crnogorac-Jurcevic T, Missiaglia E, Blaveri E, Gangeswaran R, Jones M, Terris B, Costello E, Neoptolemos JP, Lemoine NR: Molecular alterations in pancreatic carcinoma: expression profiling shows that dysregulated expression of S100 genes is highly prevalent. J Pathol 2003;201:63–74.

25 Terris B, Blaveri E, Crnogorac-Jurcevic T, Jones M, Missiaglia E, Ruszniewski P, Sauvanet A, Lemoine NR: Characterization of gene expression profiles in intraductal papillary-mucinous tumors of the pancreas. Am J Pathol 2002;160:1745–1754.

26 Iacobuzio-Donahue CA, Maitra A, Olsen M, Lowe AW, van Heek NT, Rosty C, Walter K, Sato N, Parker A, Ashfaq R, Jaffee E, Ryu B, Jones J, Eshleman JR, Yeo CJ, Cameron JL, Kern SE, Hruban RH, Brown PO, Goggins M: Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. Am J Pathol 2003;162:1151–1162.

27 Grutzmann R, Boriss H, Ammerpohl O, Luttges J, Kalthoff H, Schackert HK, Kloppel G, Saeger HD, Pilarsky C: Meta-analysis of microarray data on pancreatic cancer defines a set of commonly dysregulated genes. Oncogene 2005;24:5079–5088.

28 Kuo WP, Jenssen TK, Butte AJ, Ohno-Machado L, Kohane IS: Analysis of matched mRNA measurements from two different microarray technologies. Bioinformatics 2002;18:405–412.

29 Brandt R, Grutzmann R, Bauer A, Jesnowski R, Ringel J, Lohr M, Pilarsky C, Hoheisel JD: DNA microarray analysis of pancreatic malignancies. Pancreatology 2004;4:587–597.

30 Massague J, Blain SW, Lo RS: TGF-β signaling in growth control, cancer, and heritable disorders. Cell 2000;103:295–309.

31 Truty MJ, Urrutia R: Basics of TGF-β and pancreatic cancer. Pancreatology 2007;7:423–435.

32 Friess H, Yamanaka Y, Büchler MW, Ebert M, Beger HG, Gold LI, Korc M: Enhanced expression of transforming growth factor-β isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology 1993;105:1846–1856.

33 Kleeff J, Friess H, Simon P, Susmallian S, Büchler P, Zimmermann A, Büchler MW, Korc M: Overexpression of Smad2 and colocalization with TGF-β_1 in human pancreatic cancer. Dig Dis Sci 1999;44:1793–1802.

34 Kleeff J, Ishiwata T, Maruyama H, Friess H, Truong P, Büchler MW, Falb D, Korc M: The TGF-β signaling inhibitor Smad7 enhances tumorigenicity in pancreatic cancer. Oncogene 1999;18:5363–5372.

35 Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, Ueno N, Taniguchi T, Nishida E, Matsumoto K: Identification of a member of the MAPKKK family as a potential mediator of TGF-β signal transduction. Science 1995; 270:2008–2011.

36 Sakurai H, Suzuki S, Kawasaki N, Nakano H, Okazaki T, Chino A, Doi T, Saiki I: Tumor necrosis factor-α-induced IKK phosphorylation of NF-κB p65 on serine 536 is mediated through the TRAF2, TRAF5, and TAK1 signaling pathway. J Biol Chem 2003;278: 36916–36923.

37 Singhirunnusorn P, Suzuki S, Kawasaki N, Saiki I, Sakurai H: Critical roles of threonine-187 phosphorylation in cellular stress-induced rapid and transient activation of transforming growth factor-β-activated kinase 1 (TAK1) in a signaling complex containing TAK1-binding protein TAB1 and TAB2. J Biol Chem 2005;280:7359–7368.

38 Karin M, Greten FR: NF-κB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005;5:749–759.

39 Safina A, Ren MQ, Vandette E, Bakin AV: TAK1 is required for TGF-β_1-mediated regulation of matrix metalloproteinase-9 and metastasis. Oncogene 2008;27:1198–1207.

40 Abdollahi A, Schwager C, Kleeff J, Esposito I, Domhan S, Peschke P, Hauser K, Hahnfeldt P, Hlatky L, Debus J, Peters JM, Friess H, Folkman J, Huber PE: Transcriptional network governing the angiogenic switch in human pancreatic cancer. Proc Natl Acad Sci USA 2007;104:12890–12895.

41 Choo MK, Sakurai H, Koizumi K, Saiki I: TAK1-mediated stress signaling pathways are essential for TNF-α-promoted pulmonary metastasis of murine colon cancer cells. Int J Cancer 2006;118:2758–2764.

42 Bachem MG, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, Siech M, Beger H, Grunert A, Adler G: Identification, culture, and characterization of pancreatic stellate cells in rats and humans. Gastroenterology 1998;115:421–432.

43 Apte MV, Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Wilson JS: Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. Gut 1999; 44:534–541.

44 Lohr M, Trautmann B, Gottler M, Peters S, Zauner I, Maillet B, Kloppel G: Human ductal adenocarcinomas of the pancreas express extracellular matrix proteins. Br J Cancer 1994;69:144–151.

45 Bissell MJ, Radisky D: Putting tumours in context. Nat Rev Cancer 2001;1:46–54.

46 Bao S, Ouyang G, Bai X, Huang Z, Ma C, Liu M, Shao R, Anderson RM, Rich JN, Wang XF: Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. Cancer Cell 2004;5:329–339.

47 Gillan L, Matei D, Fishman DA, Gerbin CS, Karlan BY, Chang DD: Periostin secreted by epithelial ovarian carcinoma is a ligand for $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins and promotes cell motility. Cancer Res 2002;62:5358–5364.

48 Tai IT, Dai M, Chen LB: Periostin induction in tumor cell line explants and inhibition of in vitro cell growth by anti-periostin antibodies. Carcinogenesis 2005;26:908–915.

49 Kim CJ, Yoshioka N, Tambe Y, Kushima R, Okada Y, Inoue H: Periostin is down-regulated in high grade human bladder cancers and suppresses in vitro cell invasiveness and in vivo metastasis of cancer cells. Int J Cancer 2005;117:51–58.

50 Yoshioka N, Fuji S, Shimakage M, Kodama K, Hakura A, Yutsudo M, Inoue H, Nojima H: Suppression of anchorage-independent growth of human cancer cell lines by the TRIF52/periostin/OSF-2 gene. Exp Cell Res 2002;279:91–99.

51 Amatschek S, Koenig U, Auer H, Steinlein P, Pacher M, Gruenfelder A, Dekan G, Vogl S, Kubista E, Heider KH, Stratowa C, Schreiber M, Sommergruber W: Tissue-wide expression profiling using cDNA subtraction and microarrays to identify tumor-specific genes. Cancer Res 2004;64:844–856.

52 Erkan M, Kleeff J, Gorbachevski A, Reiser C, Mitkus T, Esposito I, Giese T, Büchler MW, Giese NA, Friess H: Periostin creates a tumor-supportive microenvironment in the pancreas by sustaining fibrogenic stellate cell activity. Gastroenterology 2007;132: 1447–1464.

53 Okami J, Simeone DM, Logsdon CD: Silencing of the hypoxia-inducible cell death protein BNIP3 in pancreatic cancer. Cancer Res 2004;64:5338–5346.

54 Abe T, Toyota M, Suzuki H, Murai M, Akino K, Ueno M, Nojima M, Yawata A, Miyakawa H, Suga T, Ito H, Endo T, Tokino T, Hinoda Y, Imai K: Upregulation of BNIP3 by 5-aza-2′-deoxycytidine sensitizes pancreatic cancer cells to hypoxia-mediated cell death. J Gastroenterol 2005;40:504–510.

55 Erkan M, Kleeff J, Esposito I, Giese T, Ketterer K, Büchler MW, Giese NA, Friess H: Loss of BNIP3 expression is a late event in pancreatic cancer contributing to chemoresistance and worsened prognosis. Oncogene 2005;24:4421–4432.