Tumour epithelial vimentin expression and outcome of pancreatic ductal adenocarcinomas

A Handra-Luca*,1,2, S-M Hong1, K Walter1, C Wolfgang3, R Hruban1,4 and M Goggins*,1,4,5

1Department of Pathology, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins Medical Institutions, Baltimore, MD, USA; 2The UFR SMBH Universite Paris 13/Nord Medecine APHP, Paris, France; 3Department of Surgery, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins Medical Institutions, Baltimore, MD, USA; 4Department of Medicine, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins Medical Institutions, Baltimore, MD, USA; 5Department of Oncology, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

PURPOSE: Tumour epithelial vimentin expression is a marker of mesenchymal differentiation and may be a useful marker of carcinomas with more aggressive behaviour. The aim of this study was to determine the extent and prognostic significance of vimentin expression in pancreatic ductal adenocarcinomas.

METHODS: Vimentin expression was detected by immunohistochemistry on tissue microarrays of surgically resected pancreatic ductal adenocarcinomas from 387 patients. The percentage of vimentin-immunolabelled neoplastic cells was correlated with outcome and with clinico-pathological factors using the Kaplan–Meier method and Cox multivariate survival models.

RESULTS: In all, 45% of primary pancreatic adenocarcinomas contained neoplastic cells that expressed vimentin, and in 27.5% of the cancers > 10% of cells expressed vimentin. Vimentin expression was correlated with poor histological differentiation. By both univariate and multivariate survival analysis, neoplastic vimentin expression (P < 0.01, HR 1.52, 95% confidence interval 1.14–2.04) was an indicator of a shorter postsurgical survival independent of other clinico-pathological variables.

CONCLUSION: The presence of vimentin-expressing tumour epithelial cells in surgically resected pancreatic adenocarcinomas independently predicted a shorter postsurgical survival.

Keywords: pancreas; adenocarcinoma; prognosis; vimentin

Pancreatic adenocarcinoma is the fourth leading cause of cancer death in the United States. Although there have been numerous advances in our understanding of pancreatic cancer development and progression in recent years, therapies for pancreatic cancer generally still provide only modest benefit. Surgical resection is currently the most effective treatment but is undertaken in only 15–20% of patients highlighting the need for early detection strategies. Even among patients with resectable pancreatic cancer survival is poor, with < 20% of patients alive at 5 years. Among patients undergoing pancreatic resection with curative intent, the main prognostic factors are histological grade, resection margin status, tumour size, location and lymph node metastasis (Winter et al, 2006; Corsini et al, 2008; Herman et al, 2008; Goggins, 2011 (in press); Vincent et al, 2011 (in press)).

Although very useful, these pathological risk factors do not sufficiently predict outcome and most of these prognostic factors reflect tumour stage rather than tumour epithelial biology. Multiple studies have attempted to identify markers that may assist in predicting outcome after pancreatic cancer resection and that may also provide clues as to biological mechanisms that contribute to pancreatic cancer aggressiveness. For example, mutations or loss of expression of tumour-suppressor proteins expressed by tumour epithelial cells such as Smad4 (Tascilar et al, 2001; Biankin et al, 2002; Blackford et al, 2009; Iacobuzio-Donahue et al, 2009), or patterns of stromal fibroblast-expressed proteins such as Sparc have been shown to predict outcome (Sato et al, 2003; Infante et al, 2007).

Vimentin is expressed by normal mesenchymal tissue and is considered a marker of mesenchymal differentiation (Leader et al, 1987). Vimentin is an intermediate-sized filament polypeptide, which along with desmin, keratin, glial acidic protein and neurofilament intermediate filaments are distinguished by their chemical characteristics, immunological specificities and cell-type distribution (Dellagi et al, 1983; Lazarides et al, 1982). Epithelial neoplasms can occasionally express both cytokeratin and vimentin, features often associated with the acquisition of mesenchymal histologic features. There is evidence that carcinomas with markers of mesenchymal differentiation have different biological and clinical behaviour (Domagala et al, 1990a,b; Medeiros et al, 1988; Liu et al, 2010). Pancreatic cancer vimentin expression patterns have been investigated in small series. Schussler et al (1992) reported a lack of expression in pancreatic cancers, while Nakajima et al (2004) observed sparse cell labelling in 30 primary pancreatic adenocarcinomas with prominent expression in 15 liver metastases. Similarly, vimentin labelling was more pronounced in
widely metastatic pancreatic cancers as compared with locally destructive ones (Naito and Iacobuzio-Donahue, 2010). Khoury et al found that vimentin expression in 34 pancreatic cancers correlated with poor survival (Javle et al, 2007). Interestingly, although mesenchymal differentiation is associated with reduced E-cadherin expression in vitro, in liver metastasis of pancreatic ductal adenocarcinomas Nakajima et al (2004) found a correlation between vimentin expression and N-cadherin but not E-cadherin expression. More recent reports suggest that, in vitro, most pancreatic cancers with well-defined glandular differentiation do not show the downregulation of E-cadherin, although focal loss of E-cadherin expression is observed in some primary pancreatic cancers (Li et al, 2010; Hong et al, 2011 (in press)) and undifferentiated pancreatic cancers often display complete loss of E-cadherin expression (Winter et al, 2008).

In this study, we investigated vimentin expression in a large series of pancreatic ductal adenocarcinomas treated by surgery and assessed its relationship to survival.

**PATIENTS AND METHODS**

**Patients and samples**

Patients having conventional pancreatic ductal adenocarcinoma (Kloppel et al, 2000), treated by surgical resection were retrieved from the database of the Pathology Department, of the Johns Hopkins Medical Institutions, Baltimore, MD, USA. Between 1998 and 2006, 387 patients had available neoplastic tissue blocks to construct tissue microarrays.

This study was designed and performed according to current recommendations for tumour markers (McShane et al, 2005). The patients were analysed for clinical and pathological data according to standard criteria (Kloppel et al, 2000; AJCC, 2010). The clinical data analysed included: age, gender, date of surgery, type of surgery, stage, date of death or last consultation for the postsurgical survival. As information on adjuvant therapy was not complete for some patients who received their postoperative therapy at other centres, postoperative therapy was not included in the analysis of the prognostic significance of vimentin. The analysed pathological data were: tumour size, histological differentiation, vascular invasion, perineural invasion, pathological T stage, lymph node metastases and N stage as well as for distant metastases (M stage). As their outcome is significantly different patients were excluded if they had a peri-operative death (occurring within the first 30 days postoperatively, 4 patients) or with follow-up of <30 days (25 patients), a distal splenopancreatectomy (1 patients) or if they received neoadjuvant radiotherapy (2 patients).

**Immunohistochemistry**

Vimentin protein expression was assessed in the tumour epithelial tissues by immunohistochemistry. Tissue microarrays were constructed from representative areas of neoplastic epithelial cells and normal pancreas as previously described (Infante et al, 2007; Matsuyoshi et al, 2007; Waller et al, 2010). Each patient's tissue was represented on the tissue microarrays by two cores of pancreatic cancer and two cores of non-neoplastic pancreas.

Immunohistochemistry was performed using an antibody to vimentin protein (Dakocytomation, Carpenteria, CA, USA, clone V9, dilution 1:200). Protein expression in the cytoplasm or perinuclear membrane and membrane in epithelial malignant cells was assessed by an experienced pancreatic pathologist (AHL) at an Olympus BX51 microscope (Olympus, Center Valley, PA, USA). The percentage of labelled tumour epithelial cells was determined. Spindle-shaped isolated cells with bland, regular nuclei were considered as stromal fibroblasts. Representative neoplastic zones were photographed and were included in the figures (Photo Olympus DP20). For 14 patients, there were no data on vimentin neoplastic cell expression because of tissue loss during the immunolabelling protocol. For each neoplasm, the core with the highest expression of vimentin was taken into consideration for subsequent statistical analysis. The percentage of labelled neoplastic epithelial cells ranged from 0 to 95%.

**Statistical analysis**

For the statistical analysis, the variables were considered as categorical. For continuous variables such as tumour size or lymph node metastases, the cutoff for classifying the tumours was the median. We also determined the median percentage of vimentin-expressing neoplastic cells, which was 1% and used this as a cutoff for classifying cancers. We also evaluated other cutoffs (0, 10, 20, 30, 40, 50, 60, 70, 80 and 90%). According to the distribution of the percentage of vimentin-expressing neoplastic cells in this series of tumours, the cutoff of 10% was accepted as most predictive. The relationships between clinico–morphological variables and the expression of vimentin by neoplastic cells were analysed by using the Fisher’s or χ²-tests (Medcalc v11.1.1 software, Medcalc, Mariakerke, Belgium). For univariate survival analysis we used the Kaplan–Meier method, the survival curves being compared by the log-rank test. For multivariate survival analysis we used the Cox method. Variables found on univariate analysis to be related to postsurgical survival with a P-value of <0.05 were included in Cox models. Colinearity (redundancy) of variables being significantly correlated on Fisher’s or χ²-tests was tested (neoplastic differentiation and vimentin expression, microscopic vascular expression and lymph node metastases, tumour size and type of surgery). Patients with unavailable data were included in the analysis as ‘unknown’. For all the statistical tests and methods, a P-value of <0.05 was used for defining statistical significance (Christensen, 1987; Chen and Wang, 1991; Hosmer and Lemeshow, 2000).

**RESULTS**

**Patients’ and tumour characteristics**

The patients’ demographics are listed in Table 1. The median follow-up was 14.4 months (range, 1.1–101.95 months). During the study period, 246 patients died (excluding patients with perioperative death) and the median survival was 14.4 months and the time to death was 12.90 months (range, 1.21–59.24 months). The 3-year postsurgical survival was 19.32% and the 5-year survival 8.0%.

Median tumour size was 30 mm (range, 10–120 mm). Tumour size was significantly higher in those patients having total pancreatectomy surgery as compared with those treated by pancreaticoduodenectomy (P = 0.04). Lymph node metastases were observed in 85.7% of the patients with the median number of metastatic lymph nodes being 3 (range, 1–25). The presence of lymph node metastases was correlated to vascular and perineural invasion (P <0.01 and P = 0.05, respectively), whereas presence of >3 lymph node metastases was correlated to vascular invasion and increased neoplastic size (P <0.01 and P = 0.02, respectively). Three patients showed distant metastasis (pericaval, mesocolon and subcostal skin metastases, respectively).

**Vimentin expression by pancreatic cancer cells**

Vimentin expression by the neoplastic cells was observed in 154 (45%) pancreatic ductal adenocarcinomas (Figure 1), and included a wide variation in the extent of cancer cell expression varying from 1 to 95%, with the median percentage of vimentin-labelled cancer cells being 1%. In 94 pancreatic cancers (27.5%), vimentin was expressed in >10% of neoplastic cells. There was not a specific labelling pattern, vimentin being expressed by cells with varying degrees of cytonuclear atypia, with or without intracellular...
Table 1  Clinico-pathological characteristics of the patients with pancreatic ductal adenocarcinomas treated by surgical resection

|                | Number of patients n = 356* | Median postsurgical survival (months) | Log-rank P |
|----------------|-----------------------------|--------------------------------------|------------|
| Gender         |                             |                                      | 0.83       |
| Women          | 160                         | 16.76                                |            |
| Men            | 196                         | 18.08                                |            |
| Age (years)    |                             |                                      |            |
| Range          | 32–90                       |                                      |            |
| Median         | 66.66                       |                                      |            |
| Outcome        |                             |                                      |            |
| Follow-up      | 14.4                        |                                      | 0.12       |
| Range, months  | 1.08–101.95                 |                                      |            |
| Median, months | 14.4                       |                                      |            |
| Dead           | 246                         |                                      |            |
| Alive          | 110                         |                                      |            |
| T Stage        |                             |                                      |            |
| pT1            | 4                           |                                      |            |
| pT2            | 7                           | 17.44                                |            |
| pT3            | 336                         | 17.98                                |            |
| pT4            | 9                           | 10.29                                |            |
| Differentiation|                             |                                      | <0.01      |
| Well, moderate | 199                         | 21.89                                |            |
| Poor           | 157                         | 13.01                                |            |
| Vascular invasion|                            |                                      | 0.02       |
| Present        | 170                         | 20.38                                |            |
| Indeterminate  | 176                         | 15.32                                |            |
| Surgery type   |                             |                                      | 0.04       |
| Pancreatoduodenectomy | 335                  | 17.98                                |            |
| Total pancreatectomy    | 21                       | 10.75                                |            |
| Tumour size     |                             |                                      | 0.02       |
| Range, median   | 30 mm                      | 19.59                                |            |
| > 30 mm         | 139                         | 14.66                                |            |
| Surgical margins|                            |                                      | <0.01      |
| Non-tumoral     | 238                         | 20.31                                |            |
| Tumoral         | 118                         | 14                                   |            |
| Lymph node metastasis |                   |                                      | 0.01       |
| N0              | 51                          | 26.4                                 |            |
| N1              | 305                         | 16.63                                |            |

Abbreviations: n = number of patients; N = node; T = tumour; p = pathology; TNM = tumour node metastasis classification. *Patients with perioperative death (within the 30 days after surgery), were not included for the statistical analysis.

DISCUSSION

Our analysis of a large series of pancreatic adenocarcinomas treated by surgical resection indicated that tumour epithelial vimentin expression is a powerful predictor of outcome than the N TNM stage, the type of surgery, and tumour size, the risk of death being 1.53. High tumour epithelial vimentin expression correlated with 3- and 5-year survival (P < 0.01 for both comparisons) as well as tumour size (P = 0.03 and P = 0.05), N TNM stage (P = 0.01 and P = 0.03), differentiation (P < 0.01 for both comparisons), surgery type (P = 0.03 and P = 0.05), and margin status (P < 0.01 and P = 0.02).

The association of positive vimentin expression with a shorter survival remained among the main subgroups of patients studied including those treated by pancreatoduodenectomy, with T3 stage cancers, those patients with lymph node metastasis (N1 TNM stage) and those patients having margin negative, stage 2 or stage 2B disease or in those patients having cancers with moderate or poor differentiation (Table 3) (Figure 3).

Similarly, in the group of patients having cancers with high vimentin expression (> 10% of cancer cells expressing), margin-positive surgical resection (P = 0.04) and tumour size (P = 0.04) were also predictors of a shorter postsurgical survival.
Vimentin expression is an indicator of adverse outcome both on univariate and multivariate survival analysis, independently of classical tumour characteristics such as differentiation, tumour size, resection margin status and type of surgical treatment.

The most powerful predictors of a shorter postsurgical survival in our series were positive margin status and poor histological differentiation, in agreement with the results of Herman et al (2008), and Winter et al (2006), in previous studies from our institution. We also found that lymph node metastasis predicted a shorter postsurgical survival, consistent with already reported results (Winter et al, 2006; Infante et al, 2007; Chang et al, 2009).

Vimentin expression by neoplastic cells was observed in 45% of the pancreatic adenocarcinomas and an expression level of >10% was noted in 27.5% of the pancreatic cancers. We found this 10% cutoff to be more predictive of outcome than other cutoffs of percentage expression or the absolute cutoff (of presence vs absence of neoplastic cell expression). Although the expression concerned a limited percentage of neoplastic cells in some tumours, vimentin was significantly related to postsurgical survival probability (%). The continuous line indicates the patients with high vimentin (>1% or >10%) expression whereas the discontinuous lines those patients with low vimentin expression.

**Figure 2**  Kaplan–Meier curves for postsurgical survival according to tumour epithelial vimentin expression when considering the cutoff of 1% (A) and that of 10% (B). The continuous line indicates the patients with high vimentin (>1 or >10%) expression whereas the discontinuous lines those patients with low vimentin expression.

**Table 2** Cox model including as variables tumour and surgical resection parameters

| Parameter                          | P-value | Hazard ratio | 95% confidence interval |
|------------------------------------|---------|--------------|-------------------------|
| Differentiation                    | <0.01   | 1.54         | 1.20 to 1.96            |
| Surgical margin status             | <0.01   | 1.62         | 1.24 to 2.12            |
| Tumour vimentin expression         | <0.01   | 1.53         | 1.14 to 2.05            |
| Type of surgery                    | 0.03    | 1.81         | 1.07 to 3.08            |
| N TNM stage                        | 0.05    | 1.48         | 1.00 to 2.18            |
| Tumour size, > 3 mm                | 0.14    | 1.22         | 0.94 to 1.59            |

Abbreviations: N = node; TNM = tumour node metastasis classification.

**Table 3** Univariate survival analysis for vimentin expression in pancreatic ductal adenocarcinomas

| Parameter                          | Median postsurgical survival (months) | Log-rank P-value |
|------------------------------------|--------------------------------------|-----------------|
| Patients treated by pancreaticoduodenectomy (N = 322) | Low tumour vimentin 19.72 | <0.01 |
|                                    | High tumour vimentin 12.82            |                 |
| T3 TNM stage patients (N = 322)    | Low tumour vimentin 20.35             | <0.01 |
|                                    | High tumour vimentin 12.49            |                 |
| N1 TNM stage patients (N = 293)    | Low tumour vimentin 19.29             | <0.01 |
|                                    | High tumour vimentin 12.09            |                 |
| Stage 2 patients (AJCC 7) (N = 328) | Low tumour vimentin 20.38             | <0.01 |
|                                    | High tumour vimentin 12.49            |                 |
| Stage 2B patients (AJCC 7) (N = 279)| Low tumour vimentin 20.35             | <0.01 |
|                                    | High tumour vimentin 12.29            |                 |
| Patients with margin negative surgical resections (N = 230) | Low tumour vimentin 14.95 | 0.02 |
|                                    | High tumour vimentin 10.25            |                 |
| Patients having tumours with moderate or poor histological differentiation (N = 322) | Low tumour vimentin 19.46 | <0.01 |
|                                    | High tumour vimentin 12.49            |                 |

Abbreviations: AJCC = American Joint Committee on Cancer; n = number of patients.
Molecular Diagnostics

neoplastic tissues showed a three-fold higher expression of vimentin (Lahat et al., 2010). Proapoptotic effects in sarcoma and carcinoma cell lines expressed vimentin degradation and slowed the growth of sarcomas and had a therapeutic target. A natural compound, withaferin-A-induced protein have been recently reported (Hong et al., 2005). More promising as a marker of neoplasia is vimentin promoter methylation, which is being evaluated as a candidate marker of colorectal carcinomas (Chen et al., 2005; Zou et al., 2007; Shirahata et al., 2009).

As vimentin is highly expressed in stromal fibroblasts it is not likely to be useful as a marker for differentiating pancreatic cancers from pancreatitis, but interestingly autoantibodies to this protein have been recently reported (Hong et al., 2006). Pancreatic neoplastic tissues showed a three-fold higher expression of vimentin than neoplasms of other origins (lung, colon and ovary), and had higher levels of an isoform with demonstrable immunogenicity (Hong et al., 2006). More promising as a marker of neoplasia is vimentin promoter methylation, which is being evaluated as a candidate marker of colorectal carcinomas (Chen et al., 2005; Zou et al., 2007; Shirahata et al., 2009).

In conclusion, the results of our study suggest that de novo tumour epithelial expression of vimentin in pancreatic ductal adenocarcinoma is an independent predictor of adverse postsurgical outcome.

ACKNOWLEDGEMENTS

We thank Conrad Lubek for assistance in immunohistochemistry procedures and Claude Lesly, Cristina Goia and Amanda Blackford for advice for statistical aspects. This work was supported by the National Cancer Institute grants (CA62924, CA120432), and the Michael Rolfe Foundation.

REFERENCES

AJCC (2010) American Joint Committee on Cancer: AJCC Cancer Staging Manual. 7th edn. In: Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A (eds) Springer: New York

Biankin AV, Morey AL, Lee CS, Kench JG, Biankin SA, Hook HC, Head DR, Hugh TB, Sutherland RL, Henshall SM (2002) DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. J Clin Oncol 20: 4531 – 4542

Figure 3 Kaplan–Meier curves for postsurgical survival according to tumour epithelial vimentin expression when considering varied groups of patients: groups of patients treated by pancreaticoduodenectomy (A), patients with pathological T3 TNM stage (B), patients with tumour-free surgical resection (C) as well as patients with stage 2 disease (D). The continuous line indicates the patients with high vimentin (> 10%) expression whereas the discontinuous lines those patients with low vimentin expression.
Blackford A, Serrano OK, Wolfgang CL, Parmigiani G, Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Eshleman JR, Goggins M, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Cameron JL, Olini K, Schultk R, Winter J, Herman JM, Laheru D, Klein AP, Vogelstein B, Kinzler KW, Velculescu VE, Hruban RH (2009) SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res* 15: 4674 – 4679

Braucksiepe B, Muija A, Herrmann H, Schmidt ER (2008) The serine/threonine kinase Stk33 exhibits autophosphorylation and phosphorylates the intermediate filament protein vimentin. *BMC Biochem* 9: 25

Chen CH, Wang PC (1991) Diagnostic plots in Cox’s regression model. *Biometrics* 47: 841 – 850

Chen WD, Han ZJ, Sokotezky J, Olson J, Sah J, Myeroff L, Platter P, Lu S, Dawson D, Willis J, Pretlow TP, Lutterbaugh J, Kasturi L, Willson JK, Rao JS, Shuber A, Markowitz SD (2005) Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. *J Natl Cancer Inst* 97: 1124 – 1132

Christensen E (1987) Multivariate survival analysis using Cox’s regression model. *Hepatology* 7: 1346 – 1358

Colucci-Guyon E, Portier MM, Duna i, Paulin D, Pourpin S, Babinet C (1983) Alteration of SPARC expression and patient outcome with resectable pancreatic adenocarcinomas. In: Johns Hopkins Hospital.

Crosini MM, Miller RC, Haddock MG, Donohue JH, Farnell MB, Nagorney DM, Jatoi A, McWilliams RR, Kim GP, Bhatia S, Iott MJ, Gunderson LL (2008) Adjunctive radiotherapy and chemotherapy for pancreatic carcinoma: the Mayo Clinic experience (1975 – 2005). *J Clin Oncol* 26: 3511 – 3516

Delagi K, Vainchenker W, Vinci G, Weber K, Osborn M (1990a) Vimentin expression appears to be associated with elevated circulating miR-200a and miR-200b levels. *Clin Cancer Res* 6: 1588 – 1593

Dellagi K, Vainchenker W, Paulin D, Brouet JC (1983) Intermediate filament protein vimentin. *A Handra-Luca Tumoral vimentin and pancreatic cancer prognosis*.

Domagala W, Yuml;niak L, Lasota J, Weber K, Osborn M (1990b) Vimentin is preferentially expressed in high-grade ductal and medullary, but not in lobular breast carcinomas. *Am J Pathol* 137: 1059 – 1064

Goggins M (2011) Markers of pancreatic cancer: working toward early detection. *Clin Cancer Res* (in press)

Heatley M, Maxwell P, Whiteside C, Toner P (1993) Vimentin expression in benign and malignant breast epithelium. *J Clin Pathol* 46: 441 – 445

Hereman JM, Swartz MJ, Hsu CC, Winter J, Pawlik TM, Sugar E, Robinson R, Laheru DA, Jaffee E, Hruban RH, Campbell KA, Wolfgang CL, Asrari F, Donehower R, Hidalgo M, Diaz JR LA, Yeo C, Cameron JL, Schulick RD, Abrams R (2008) Analysis of fluorouracil-based adjunct chemotherapy and radiation after pancreaticoduodenectomy for ductal adenocarcinoma of the pancreas: results of a large, prospectively collected database at the Johns Hopkins Hospital. *J Clin Oncol* 26: 3530 – 3532

Hong SH, Misek DE, Wang H, Purav E, Hinderer R, Giordano TJ, Heatley M, Maxwell P, Whiteside C, Toner P (1993) Vimentin expression in pancreatic carcinoma. *Clin Cancer Res* 1: 175 – 183

Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K (2009) Vimentin expression in pancreatic carcinoma: the Mayo Clinic experience (1975 – 2005). *J Natl Cancer Inst* 101: 1588 – 1593

Javle MM, Gibbs JB, Ivuta KK, Pak Y, Rutledge P, Yu J, Black JD, Tan D, Kennedy TC (2007) Epithelial-mesenchymal transition (EMT) and activated extracellular signal-regulated kinase (p-Erk) in surgically resected pancreatic cancer. *Ann Surg Oncol* 14: 3527 – 3533

Kloppel G, Hruban RH, Longnecker DS, Adler G, Kern SE, Partanen TJ (2000) Ductal adenocarcinoma of the pancreas. In: Hamilton S, Aaltonen L (eds). *WHO Classification of Diagnostic Neoplasms* pp 220 – 230. IARC Press Lyon.

Klymowsky MW, Savagner P (2009) Epithelial-mesenchymal transition: a cancer researcher’s conceptual friend and foe. *Am J Pathol* 174: 1588 – 1593

Lahat G, Zhu QS, Huang KL, Wang S, Bolsahov S, Liu J, Torres K, Langley RR, Lazar AJ, Hung MC, Lev D (2010) Vimentin is a novel anti-cancer therapeutic target: insights from in vitro and in vivo mice xenograft studies. *PLoS One* 16: e10105

Lazarides E, Grander BL, Gar D, O’Connor CM, Breekler J, Price M, Danto SI (1982) Desmin- and vimentin-containing filaments and their role in the assembly of the Z disk in muscle cells. *Cold Spring Harb Symp Quant Biol* 1: 351 – 378

Leader M, Collins M, Patel J, Henry K (1987) Vimentin: an evaluation of its role as a tumour marker. *Histopathology* 11: 63 – 72

Li A, Omura N, Hong SM, Vincent A, Walter K, Griffith M, Borges M, Goggins M (2010) Pancreatic cancers epigenetically silence SIP1 and hypomethylate and overexpress miR-200a/200b in association with elevated circulating miR-200a and miR-200b levels. *Cancer Res* 70: 5226 – 5237

Liu JK, Jiang XY, Zhou XX, Wang DM, Song XL, Jiang HB (2010) Upregulation of vimentin and aberrant expression of E-cadherin/beta-catenin complex in oral squamous cell carcinomas: correlation with the clinicopathological features and patient outcome. *Mod Pathol* 23: 213 – 224

Matsubayashi H, Inafune JR, Winter J, Klein AP, Schultk R, Hruban R, Vriavanathan K, Goggins M (2007) Neoplastic COX-2 expression and prognosis of patients with resectable pancreatic cancer. *Cancer Biol Ther* 6: 1569 – 1575

McNroy L, Määttä A (2007) Down-regulation of vimentin expression inhibits carcinoma cell migration and adhesion. *Biochem Biophys Res Commun* 345: 109 – 114

McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics (2005) Reporting recommendations for neoplastic marker prognostic studies. *J Clin Oncol* 23: 9067 – 9072

Medeiros LJ, Michie SA, Johnson DE, Warnke RA, Weiss LM (1987) Intermediate filament protein vimentin. *Am J Pathol* 129: 259 – 281

Mesnil P, Hadju J, Lebrun A, Albiges L, Henshall SM, Eveno J, Eriksson JE, Marttila-Ichihara F, Jalkanen S (2006) Vimentin function in lymphocyte adhesion and transmigration. *Nat Cell Biol* 8: 156 – 162

Nakajima S, Mori Y, Toyoda E, Tsuji S, Wada M, Koizumi M, Tulachan SS, Ito T, Moriyuki K, Mori T, Kawaguchi Y, Fujimoto K, Hosotani R, Imamura M (2004) N-cadherin expression and epithelial-mesenchymal transition in pancreatic cancer. *Clin Cancer Res* 10: 4125 – 4133

Neureiter D, Zopf S, Dimmler A, Stintzing S, Hahn EG, Kirchner T, Herold C, Ocker M (2005) Different capabilities of morphological pattern formation and its association with the expression of differentiation markers in a xenograft model of human pancreatic cancer cell lines. *Pancreatology* 5: 387 – 397

Nieminen M, Henttinen T, Merinien M, Marttila-Ichihara F, Eriksson JE, Jalkanen S (2006) Vimentin function in lymphocyte adhesion and transmigrational cell. *Nat Cell Biol* 8: 156 – 162

Norton P, Fujishima N, Maehara N, Matsubayashi H, Koopmann J, Su GH, Hruban RH, Goggins M (2003) SPARC/osteonectin is a frequent target for aberrant methylation in pancreatic adenocarcinoma and a mediator of neoplastic-stromal interactions. *Oncogene* 22: 5021 – 5030

Schüßler MH, Skoudy A, Ramaerkers F, Real FX (1992) Intermediate filaments as differentiation markers of normal pancreas and pancreatic cancer. *Am J Pathol* 140: 559 – 568

Shibuya H, Sakata M, Sakuraba K, Goto T, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K (2009) Vimentin methylation as a marker for advanced colorectal carcinoma. *Anticancer Res* 29: 279 – 281

Tascilar M, Skinner HG, Roesty C, Soltan, T, Willetz RE, Offerhaus GJ, Adsay V, Abrams RA, Cameron JL, Kern SE, Yeo CJ, Hruban RH, Goggins M (2001) The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res* 7: 4115 – 4121

Tumor vimentin and pancreatic cancer prognosis A. Handra-Luca et al.
Traub PG, Perides G, Scherbarth A, Traub U (1985) Tenacious binding of lipids to vimentin during its isolation and purification from Ehrlich ascites neoplastic cells. FEBS Lett 193: 217–221

Vincent A, Herman JM, Schulick R, Hruban R, Goggins M (2011) Pancreatic cancer. Lancet (in press)

Walter K, Omura N, Hong SM, Griffith M, Vincent A, Borges M, Goggins M (2010) Overexpression of smoothened activates the sonic hedgehog signaling pathway in pancreatic cancer-associated fibroblasts. Clin Cancer Res 16: 1781–1789

Winter JM, Cameron JL, Campbell KA, Arnold MA, Chang DC, Coleman J, Hodgkin MB, Sauter PK, Hruban RH, Riall TS, Schulick RD, Choti MA, Lillemoe KD, Yeo CJ (2006) 1423 Pancreaticoduodenectomies for pancreatic cancer: a single-institution experience. J Gastrointest Surg 10: 1199–1210

Winter JM, Ting AH, Vilardebell F, Gallmeier E, Baylin SB, Hruban RH, Kern SE, Jcobuzio-Donahue CA (2008) Absence of E-cadherin expression distinguishes noncohesive from cohesive pancreatic cancer. Clin Cancer Res 14: 412–418

Zhao Y, Yan Q, Long X, Chen X, Wang Y (2008) Vimentin affects the mobility and invasiveness of prostate cancer cells. Cell Biochem Funct 26: 571–577

Zhu QS, Rosenblatt K, Huang KL, Lahat G, Brobey R, Bolshakov S, Nguyen T, Ding Z, Belousov R, Bill K, Luo X, Lazar A, Dicker A, Mills GB, Hung MC, Lev D (2011) Vimentin is a novel AKT1 target mediating motility and invasion. Oncogene 30: 457–470

Zou H, Harrington JJ, Shirc AM, Rego RL, Wang L, Campbell ME, Ober A, Ahlquist DA (2007) Highly methylated genes in colorectal neoplasia: implications for screening. Cancer Epidemiol Biomarkers Prev 16: 2686–2696