Genes and surgery in pancreatic cancer
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Gene expression profiling identifies markers of ampullary adenocarcinoma

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Background
Ampullary adenocarcinoma is an aggressive cancer with a poor prognosis. Without surgical resection, ampullary adenocarcinomas can be difficult to distinguish from ampullary adenomas. The aim of this study was to identify differentially expressed genes in ampullary adenocarcinoma in order to identify candidate markers of the disease.

Methods
The Affymetrix Human Genome U133 GeneChip set (HG-U133A and HG-U133B) was used to obtain gene expression profiles of 5 ampullary adenocarcinomas and 10 normal duodenal samples. Genes expressed at levels at least fivefold greater in the adenocarcinomas as compared to normal duodenum were identified and some of them confirmed.

Results
Using fold change analysis we identified 235 fragments expressed at least fivefold higher in ampullary cancers than in normal duodenum. The expression profiles of eight candidate overexpressed genes (osteopontin, mesothelin, tissue inhibitor of metalloproteinases 1, mucin-1, mucin-5, fascin, heat shock protein 47, fibronectin 1) were confirmed by immunohistochemistry or in situ hybridization on tissue microarrays (TMA) containing 54 ampullary adenocarcinomas. One of these genes, osteopontin, was expressed at 27-fold higher levels in ampullary adenocarcinomas compared to normal duodenum by genechip analysis. We therefore determined serum osteopontin levels in patients with ampullary neoplasms, patients with other periampullary diseases and in normal controls. Mean pre-operative serum osteopontin levels as measured by competitive ELISA were 906 ± 268 ng/ml in patients with ampullary cancer, 867 ± 160 ng/ml in patients with an ampullary adenoma, 327.1±195.6 ng/ml in patients with non-malignant periampullary diseases and 204 ± 65 ng/ml in age-matched healthy controls (p<0.001).

Conclusion
Measurement of markers of ampullary cancer such as osteopontin may aid in the early detection and differential diagnosis of patients with periampullary lesions.
Introduction

Ampullary adenocarcinomas comprise approximately 6% of the cancers arising in the region of the head of the pancreas. The overall 5-year survival of patients with ampullary adenocarcinomas after radical resection is better than that of other periampullary neoplasms and ranges from 30% to 50%. A possible explanation may be that even small lesions growing in the ampulla can cause obstruction of the bile duct, leading to early jaundice, and therefore early presentation. In addition, differences in tumor biology may also be an explanation for the relatively good survival of patients with this disease.

The biological characteristics of ampullary adenocarcinoma have not been extensively studied. Genetic alterations of the K-ras oncogenes, and the p53, p16, and Smad4 (DPC4) tumor suppressor genes, all commonly altered in pancreatic cancer, have also been described in ampullary cancer, although at lower frequencies. Perhaps reflecting its intestinal origin, ampullary cancers are thought to arise from adenomas and are known to inactivate the APC gene.

Pancreaticoduodenectomy is the treatment of choice for patients with an ampullary adenocarcinoma whereas ampullary adenomas should preferably be treated with local resection. Unfortunately, differentiating ampullary adenomas from adenocarcinomas pre-operatively is difficult histologically, but it is possible that with a better understanding of the molecular profiles of ampullary cancer immunohistochemical or serum markers could be developed that help distinguish ampullary adenocarcinomas from ampullary adenomas.

Several patient groups are at risk developing ampullary neoplasms including patients with polyposis syndromes as well as patients with a history of ampullary neoplasms. Such patients would likely benefit from a serum marker that could predict the presence of primary or recurrent ampullary cancer.

To better understand the molecular profiles of ampullary adenocarcinoma, and to search for potential markers of the disease, we utilized high-throughput gene expression analysis to identify the genes differentially expressed in ampullary carcinomas compared to normal duodenum.

Materials and Methods

Patients and Tissues

Tissue samples (0.5 g) of ampullary adenocarcinoma (n=5) and normal duodenal mucosa (n=10) surgically resected specimens from patients undergoing treatment at The Johns Hopkins Hospital were harvested within 10 minutes after resection, and snap-frozen in liquid nitrogen, and stored at -80°C. Bulk ampullary cancer tissue was used so that we could not only identify genes expressed by neoplastic epithelial cells but also identify genes expressed in the stromal compartment as a result of tumor-stromal interactions. All snap-frozen neoplasms were examined prior to gene expression profiling to confirm the presence of cancer in the specimen.

To confirm the differential expression of genes identified by oligonucleotide microarray analysis,
Table 1 Genechip data for the eight candidate genes selected for validation

<table>
<thead>
<tr>
<th>Fragment name</th>
<th>Known gene name</th>
<th>Fold change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>211738_x_at</td>
<td>fibronectin 1</td>
<td>10.15</td>
<td>0.00273</td>
</tr>
<tr>
<td>209386_at</td>
<td>hypothetical protein MGC4655, singed-like (fascin homolog, sea urchin) (Drosophila)</td>
<td>12.02</td>
<td>0.00002</td>
</tr>
<tr>
<td>205753_at</td>
<td>mesothelin</td>
<td>9.78</td>
<td>0.10139</td>
</tr>
<tr>
<td>205509_at</td>
<td>mucin 1, transmembrane</td>
<td>20.27</td>
<td>0.0052</td>
</tr>
<tr>
<td>205421_at</td>
<td>mucin 5, subtype B, tracheobronchial</td>
<td>11.56</td>
<td>0.1123</td>
</tr>
<tr>
<td>202628_s_at</td>
<td>secreted phosphoprotein 1 (osteopontin, bone sialoprotein 1, early T-lymphocyte activation 1)</td>
<td>26.95</td>
<td>0.00197</td>
</tr>
<tr>
<td>202363_at</td>
<td>serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47)</td>
<td>21.23</td>
<td>0.00613</td>
</tr>
<tr>
<td>201564_s_at</td>
<td>tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)</td>
<td>7.34</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 2 Immunohistochemical and in situ hybridization* profiles of genes identified as overexpressed in ampullary cancers by oligonucleotide microarray

<table>
<thead>
<tr>
<th>Gene</th>
<th>% Positive (focal / diffuse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fascin</td>
<td>77 (28/49)</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>86</td>
</tr>
<tr>
<td>HSP 47</td>
<td>85</td>
</tr>
<tr>
<td>Mesothelin</td>
<td>66 (42/24)</td>
</tr>
<tr>
<td>Mucin1</td>
<td>66 (30/36)</td>
</tr>
<tr>
<td>Mucin5</td>
<td>68 (39/29)</td>
</tr>
<tr>
<td>Timp 1*</td>
<td>22</td>
</tr>
<tr>
<td>Osteopontin*</td>
<td>100</td>
</tr>
</tbody>
</table>

tissue microarrays (TMAs) of ampullary adenocarcinoma and normal duodenal ampulla were created from formalin-fixed paraffin-embedded tumor blocks of 54 patients who previously underwent pylorus preserving pancreaticoduodenectomy (PPPD) for an infiltrating ampullary adenocarcinoma at the Johns Hopkins Hospital. The paraffin blocks were obtained from the surgical pathology archives of the Department of Pathology at the Johns Hopkins Hospital. Areas of ampullary cancer, and adjacent normal ampullary and stromal tissue were arrayed onto 3 TMA blocks. TMAs were constructed using a manual Tissue Puncher/Arrayer (Beecher Instruments, Silver Spring, MD) as previously described.21 For each selected lesion, a 1.4mm core was punched from the donor block to ensure that an adequate section of ampullary cancer and surrounding tissue could be incorporated into each spot. From each case four cores of ampullary cancer with matching normal ampulla, as well as control tissue cores from various organs were arrayed on one of the three ampullary adenocarcinoma recipient blocks. A total of 99 cores (72 cores of ampullary cancer with matching normal ampulla as well as 27 control tissue cores from various organs) were arrayed on each ampullary adenocarcinoma recipient block. Serial sections were cut from all three TMAs for immunostaining or in situ hybridization, and one was stained with hematoxylin and eosin (H&E) as a reference.
Figure 1 Expression patterns of candidate overexpressed genes in ampullary adenocarcinoma. (A) Fascin is overexpressed in the neoplastic epithelium, but not in adjacent normal epithelium (B) Mucin 5 is overexpressed in the neoplastic epithelium, but no expression is seen in retained strips of normal epithelium (arrows). (C) Fibronectin is overexpressed in the neoplastic stromal desmoplastic component; in contrast, the neoplastic epithelium is non-reactive (D) In situ hybridization demonstrates intense expression of tissue inhibitor of metalloproteinase-1 transcripts in neoplastic epithelium and adjacent desmoplastic stroma (27, page 261)

For the analysis of osteopontin, preoperative sera from patients with ampullary adenocarcinoma (n=28) and ampullary adenoma (n=6) undergoing pancreaticoduodenectomy (Whipple procedure) between 1998 and 2002 in the Johns Hopkins Hospital were collected after informed consent as part of an ongoing IRB-approved observational study. The diagnosis of ampullary neoplasm was confirmed for each patient by histopathological examination of the resection specimen. Sera from patients with pancreatic and periampullary diseases who had undergone surgical resection usually to rule out malignancy were also included in the serum osteopontin analysis including 6 patients with intraductal papillary mucinous neoplasms, 5 with mucinous cystic neoplasms, 3 with non-neoplastic pancreatic cysts, 11 with neuroendocrine neoplasms of the pancreas, and 6 with miscellaneous diseases (one each of those with cholangitis, omental fat necrosis, choledochal cyst, and pancreatic intraepithelial neoplasias (PanINs, n=3). Sera from healthy individuals (n=22) were obtained from the Johns Hopkins Bayview Medical Center General Clinical Research Center. All of these analyses were performed with approval of the Institutional Review Board.
mRNA Extractions and Affymetrix GeneChip hybridization
Sample preparation and processing were performed using the Affymetrix GeneChip Expression Analysis Manual (Santa Clara, CA), and was described previously by Iacobuzio-Donahue et al.\textsuperscript{22}

Immunohistochemistry
Immunohistochemical labeling was performed on the 54 ampullary cancers arrayed onto three ampullary TMA to validate the translation and differential expression of selected genes in archival tissue sections of ampullary adenocarcinomas. For each antibody immunolabeling was performed as previously described.\textsuperscript{14,22,23} The epitopes analyzed, and the antibodies used with their dilutions were as follows: fascin, DAKO Carpinteria, CA, dilution 1:3200;\textsuperscript{23} fibronectin, DAKO Carpinteria, CA, dilution 1:1200;\textsuperscript{24} heat shock protein 47 (hsp 47), Stressgen Biotechnologies, San Diego, CA, dilution 1:800;\textsuperscript{23} mesothelin, Novocastra Laboratories, Newcastle upon Tyne, UK, clone 5B2, dilution 1:800
Figur ee  3  OPN  seru m  level s  i n  patient s  wit h  ampullar y  carcino ma s  (AMP C A) ,  ampullar y  adenoma s  (AMP A D) ,  non-malignan t periampullar y  disease s  (controls )  an d  in  health y  control s  (normals) ,  plotte d  in  a  scattergra m.

(14); mucin 1 and mucin 5, Vector Laboratories, Burlingame, CA, clone nc1695, dilution 1:100, and clone CLH2, dilution 1:50, respectively. Staining was evaluated by two of the authors (NTvH and AM). The extent of immunolabeling was categorized into three groups: negative, focal positive, and diffuse positive. Negative labeling meant a virtual absence of labeling (<5% of neoplastic cells) on the tissue section. Diffusely positive labeling was present when at least 25% of the neoplastic cells were clearly positive and focally positive, if between 5-25% of cells labeled. If the antibody labeled only stromal cells, labeling was invariably diffuse.

**In situ hybridization**

The mRNA transcripts analyzed by in situ hybridization were tissue inhibitor of metalloproteinases 1 (timp-1) and osteopontin (OPN). Preparation of digoxigenin-labeled sense and antisense riboprobes were performed based on previously performed protocol. In brief: riboprobes were prepared by in vitro transcription from appropriate I.M.A.G.E. clones (Resgen, Birmingham, Alabama). For the in situ hybridization of formalin fixed tissue, sections were deparaffinized in xylene for 5 minutes, followed by hydration in graded ethanols for 5 minutes each. Next, sections were digested with Proteinase K (15-μg/ml) at 37°C for 20 minutes (timp-1) and 40 minutes (OPN), respectively. This was followed by hybridization overnight with a 300 ng/ml concentration of antisense or sense riboprobes in mRNA hybridization buffer, at 49°C for timp-1 and at 45°C for OPN. The following day, sections were washed in 2x standard saline citrate (SSC) at 58°C for timp-1 and at 45°C for OPN, followed by incubation with a 1/35 dilution of RNase A cocktail (Ambion, Austin, TX) in 10 mmol/L Tris, 500 mmol/L NaCl, 1 mmol/L ethylenediaminetetraacetic acid, pH 7.5, for 1 hour at 37°C. Next, slides were washed twice in 2x SSC/50% formamide at 55°C, followed by one wash at 0.08x SSC also at 55°C. Signal amplification was achieved by
incubation of sections with biotinyl-tyramide, followed by secondary streptavidin complex (GenPoint kit, DAKO). The final signal was developed with diaminobenzidine chromagen (GenPoint kit, DAKO). A tissue section was considered positive if any portion of the section labeled with the probe.

Osteopontin serum ELISA
A modified competitive serum ELISA procedure for OPN has been developed by one of the authors (NF) and performed as described previously. In brief: serum samples (100 ml) were diluted 1:10 in a 50% formamide-40 mM phosphate buffer (pH 7.4) and were reduced with 2 mM DTT at 100°C for 5 min to disrupt the binding of OPN by complement factor H in serum. Residual reducing agent and formamide were removed by strong anion exchange column chromatography (ToyoPearl QAE resin; Tosohas, Montgomeryville, PA). After loading the 1.0-ml sample, the column was washed with 6x column volumes of TBS-T solution. OPN was eluted with TBS-T containing 1.0 M NaCl. For the competitive ELISA Greiner high-binding plates (USA Scientific, Inc., Ocala, FL) were coated with 20 ng/well OPN overnight in 50 mM carbonate buffer (pH 8.0). The standard curves for OPN were constructed using recombinant protein. Samples and standards (100-μl volume) were incubated for 2 h with shaking at room temperature with 100 μl of a 1:100,000 of LF-124 antibody in TBS-T in polypropylene 96-well plates (USA Scientific, Inc.), and the antigen-coated plate blocked with TBS + 5% powdered milk. Antibody-coated plate was then rinsed three times with TBS-T, and the antibody-sample solution was added to the wells. After a second incubation for 1 h at room temperature with shaking, the plate was washed three times with TBS-T. A secondary antibody of goat antirabbit peroxidase-labeled antibody conjugate, human serum adsorbed (Kirkegaard & Perry, Gaithersburg, MD) at 1:2000 was then added, and the plates were incubated for 1 h. After three washes with TBS-T, substrate (TMB microwell peroxidase substrate; BioFX Laboratories) was added and after a final 20-min incubation, the color reaction was stopped by the addition of 25 μl of 1 N H2SO4. Absorbance was read at 450 nm. Serum CA 19-9 CA 19-9 levels in serum (25 ml) were measured by ELISA (Alpha Diagnostics Inc., San Antonio, TX) according to the manufacturer’s recommendations.

Statistical data analysis
The GeneExpress Software System Fold Change Analysis tool from Genelogic was used to identify genes expressed at least fivefold greater in the ampullary cancers compared to normal tissues. For each gene fragment, the ratio of the geometric means of the expression intensities in the normal control tissues and the ampullary cancer samples was calculated, and the fold change then calculated on a per fragment basis. We used a cutoff of at least fivefold overexpression in the cancer compared to normal tissues as the bulk tissues analyzed contain different proportions of epithelial and other cells types. Thus, many modest changes in gene expression identified by comparison of bulk tissues
may not represent true differences in tissue expression. Confidence limits were calculated using a
two-sided Welch modified t-test on the difference of the means of the logs of the intensities.
Means of the OPN sera levels were compared using the independent samples T-test with SPSS
10.0 for Windows; a p value of <0.05 was considered statistically significant.

Results

Data filtering

RNA samples were hybridized to the complete Affymetrix Human Genome U133 GeneChip set
(HG-U133A and HG-U133B) for simultaneous analysis of over 39,000 transcript variants, which
in turn represent greater than 33,000 well-substantiated human genes. The Genelogic GeneExpress
Software System Fold Change Analysis tool identified 235 fragments expressed at levels at least
fivefold greater in ampullary cancer samples than in normal duodenal tissues.

Identification of highly expressed genes in ampullary cancer

The 235 overexpressed fragments included fragments corresponding to ESTs and fragments
 corresponding to known genes. Among these fragments were genes represented by two or
more fragments, leaving the number of known genes identified as expressed at least fivefold or
greater in ampullary cancer as compared to normal duodenum at 189 (the top 50 of which are
listed at http://pathology2.jhu.edu/pancreas/ampullarytable.htm). These 189 genes represented
a variety of functional groups, including genes involved in cell membrane junctions (claudin 1,
claudin 18), signal transduction (hepatocyte nuclear factor 3, beta), calcium homeostasis (S100
calcium-binding protein P), cytoskeletal assembly (keratin 7, and pleckstrin), cell surface adhesion
and recognition (integrin B-like 1), DNA transcription (topoisomerase (DNA) I), and extracellular
matrix remodeling and function (collagens 1α1, 1α2, and X1α1, heat shock protein 47, MMP14,
and MMP7). Many of the genes identified as overexpressed have been similarly identified in
other cancers and in addition to the above include genes such as mesothelin, osteopontin, TIMP-
1, inhibin Beta A, interleukin 8, several kallikreins and sparcc.

Verification of selected candidate tumor markers by
immunohistochemistry and in situ hybridization

From the list of 189 genes we selected eight candidate genes (osteopontin, mesothelin, timp-1,
mucin-1, mucin-5, fascin, heat shock protein 47, fibronectin 1) chosen based on the availability
of antibodies suitable for immunohistochemistry (6 genes) as well as biological interest and
verified their expression patterns in formalin fixed paraffin embedded primary ampullary tissues
(table 1). The expression patterns of two additional genes (Timp-1 and OPN) were analyzed by in
situ hybridization (table 2).
An example of immunohistochemical labeling patterns for each marker is shown in figure 1. Staining for Fascin showed intensely positive cytoplasmic labeling of the neoplastic epithelium in 76% cases: 28% focal, and 48% diffuse. In all cases, normal duct epithelium did not express fascin protein (figure 1A). An example of in situ hybridization labeling patterns is shown in figure 2A and 2B, respectively. A strong OPN mRNA signal was identified in tumor-infiltrating cells with morphologic characteristics of monocytes/macrophages. This pattern was found in all examined tissue microarray cores. Immunohistochemistry for CD68 on the same tissue microarrays confirmed the macrophage origin of these cells expressing OPN. Interestingly, only the macrophages that directly infiltrated the tumor tissue expressed osteopontin. Macrophages in adjacent normal tissue were not positive for osteopontin mRNA by in situ hybridization (figure 2B).

Serum osteopontin and Ca19-9
Mean serum osteopontin levels were significantly higher in patients with ampullary adenocarcinoma (n=28)(905 ± 268 ng/ml), and in patients with ampullary adenomas (n=6) (866 ± 160 ng/ml) than in healthy individuals (n=22) (204 ± 65 ng/ml) (p<0.001)(figure 3). Using a cutoff level for osteopontin of 334 ng/ml (2 standard deviations above the mean of the healthy controls), sera from patients with an ampullary neoplasm could be distinguished from the healthy controls with a sensitivity of 100% and specificity of 95%. Osteopontin levels were also higher in patients with ampullary neoplasms than in a disease control group consisting of those with other benign pancreatic neoplasms such as intraductal papillary mucinous neoplasms (mean 348± 185 ng/ml), mucinous cystadenomas (mean 275± 222 ng/ml), and neuroendocrine tumors (mean 426 ± 278 ng/ml) and non-neoplastic pancreatic diseases such as pancreatic cysts (mean 370 ± 33 ng/ml) and other miscellaneous periampullary diseases (mean 217 ng/ml). The mean osteopontin level in this disease control group was 327.1 ± 195.6 ng/ml (figure 3). Surprisingly, there were no significant differences in the mean osteopontin level between patients with an ampullary adenocarcinoma and those with an ampullary adenoma. Primary ampullary adenocarcinoma tissues were available for osteopontin expression analysis on the tissue microarray for 7 of the 28 patients with ampullary carcinoma whose osteopontin serum levels were determined. All seven patients with an elevated serum osteopontin level by ELISA displayed overexpression of osteopontin in their ampullary carcinoma by in situ hybridization. There was no correlation between pre-operative serum bilirubin and OPN levels in the 15 patients from whom preoperative serum bilirubin was available. There was also no correlation between TNM status and the serum osteopontin level.

Since Ca19-9 levels are often used to as a tumor marker in patients with periampullary neoplasms, we compared Ca19-9 levels with those of osteopontin. Ca19-9 levels had been measured prior to surgery in 17 patients with ampullary adenocarcinoma and from all 6 patients with ampullary
adenoma. Using a cutoff level of 70 U/ml, four of the 17 patients with ampullary carcinomas and two of the six with ampullary adenomas had elevated Ca19-9, corresponding to a sensitivity of serum Ca19-9 for the identification of patients with an ampullary neoplasm of 26%.

Discussion

In this study we have identified multiple genes that are overexpressed in primary ampullary adenocarcinomas. We were able to confirm the overexpression patterns of all eight genes selected for confirmation using either immunohistochemistry or in situ hybridization. One of the most useful applications of global gene expression profiling is in the identification of overexpressed genes that could serve as diagnostic markers. In this regard, we were able to identify a potential serum marker of ampullary neoplasms, namely osteopontin. Serum osteopontin levels were elevated approximately fourfold in the serum of patients with an ampullary adenoma or carcinoma compared to healthy controls, corresponding to a diagnostic sensitivity of 100% and a specificity of 95% in this small sample set. In comparison, serum Ca19-9 levels were elevated in only 26% of the patients with an ampullary neoplasm. These results suggest that patients at risk of developing ampullary cancer such as patients who have had a previous resection of an ampullary neoplasm as well as patients with familial cancer syndromes such as familial adenomatous polyposis and the Peutz-Jeghers syndrome could potentially benefit from surveillance measurements of serum osteopontin in addition to standard cancer surveillance tests.

Osteopontin is a member of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of proteins and is a secreted glycoprophosphoprotein of 55-80kD that is bound to complement factor H in serum. Osteopontin is expressed in mineralized tissue, in apical surfaces of epithelia and in macrophages, and binds to collagen and integrins. Furthermore, osteopontin is known to be expressed in several malignant tissues including pancreatic, breast, prostate, thyroid, and skin cancers, often within the tumor-infiltrating macrophages as we found for ampullary neoplasms. And as we found for patients with ampullary cancers, patients with other cancers such as pancreatic, ovarian, colon, breast, and prostate cancer have all been shown to have elevations of serum osteopontin. However, because osteopontin is expressed in macrophages and in other non-neoplastic cells, elevations of osteopontin have been described in other inflammatory conditions such as atherosclerosis. The degree of osteopontin elevation in patients with atherosclerosis was mild compared to what we observed in patients with ampullary neoplasms and similar in magnitude to the mild elevations we observed in patients with non-neoplastic periampullary diseases. Thus, osteopontin is not a specific marker of ampullary neoplasms but is elevated in a variety of cancers. Nor is osteopontin a specific cancer marker, future studies will likely demonstrate that certain severe inflammatory conditions will cause serum elevations of osteopontin. However, our results also show that several periampullary non-malignant conditions only cause modest
elevations of osteopontin compared to that seen in patients with ampullary neoplasms. Many other genes were identified as overexpressed in ampullary cancer including several genes identified previously as overexpressed in pancreatic cancer. One such gene is mesothelin, which is consistently and highly overexpressed in virtually all pancreatic adenocarcinomas at both the mRNA and protein levels. Mesothelin is one of several genes identified as overexpressed in ampullary adenocarcinomas that have been shown to function as a tumor antigen (others include several cancer-testis antigens, see online table 1 (http://pathology2.jhmi.edu/ampullary). Mesothelin is a cell surface glycoprotein known to be overexpressed in several malignancies, but its biological function is not known. However, low concentrations of an immunotoxin targeting mesothelin appear to be cytotoxic to mesothelin-expressing human tumors by inducing apoptosis. Clinical trials with a recombinant antimesothelin immunotoxin have already been initiated for treatment of patients with mesothelin expressing malignancies.

Histology and preoperative investigation cannot always differentiate adenomas from those destined to recur as invasive ampullary adenocarcinomas. Surgical resection is the treatment of choice for managing ampullary adenocarcinomas, while many ampullary adenomas can be treated by local excision endoscopically. Ampullary carcinomas are managed surgically with a (pylorus preserving) pancreaticoduodenectomy and lymph node dissection according to the principles of cancer surgery. Although adenomas can be treated with local endoscopic or surgical resection, local resection may not be adequate for “suspicious” adenomas. Ampullary adenomas often relapse after local resection, with a frequency of 0% to 42%. When relapse occurs after surgery, patients tend to present with advanced disease. Thus, a marker of ampullary cancer could be useful in identifying patients with relapse of their ampullary cancer. While serum osteopontin levels do not appear to be effective in distinguishing these lesions, we anticipate that several of the markers identified as overexpressed in ampullary cancers could help predict the biological properties of ampullary neoplasms when histology is inconclusive for cancer. Such a marker could be readily applied to histological endoscopic biopsies of ampullary masses. For example, mesothelin which we found to be overexpressed in ampullary cancers is also overexpressed in pancreatic cancers, and has been shown to be useful in predicting which pancreatic non-diagnostic cytological specimens are from patients with pancreatic cancer and not from those without cancer. The detection of mesothelin by immunohistochemistry may also prove to be useful in differentiating high grade ampullary neoplasms from low grade adenomas, as in precancerous lesions of the pancreas, mesothelin is only expressed in high grade PanINs.

In summary, using global gene expression profiling, we have identified a number of genes that are overexpressed in ampullary adenocarcinomas. One such gene, osteopontin, is overexpressed in tumor-infiltrating macrophages of ampullary neoplasms and its protein product is elevated in the serum of both patients with ampullary neoplasms.


