Pseudomyxoma Peritonei Is a Disease of MUC2-Expressing Goblet Cells

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Pseudomyxoma peritonei, a syndrome first described by Karl F. Rokitsky in 1842, is an enigmatic, often fatal intra-abdominal disease characterized by dissecting gelatinous ascites and multifocal peritoneal epithelial implants secreting copious globules of extracellular mucin. Although past interest in the syndrome has focused on the questions of the site of origin (appendix *versus* ovary), mechanisms of peritoneal spread (multicentricity, redistribution phenomenon, or metastasis), and the degree of malignant transformation present (adenoma, borderline tumor, or carcinoma), another important question is the mechanism behind the accumulation of extracellular mucin, the real cause of the disease's morbidity and mortality irrespective of the site of origin, mechanism of peritoneal spread, or transformed status of its epithelium. Taking advantage of the recently cloned human mucin genes, we decided to investigate this question. Our studies revealed that pseudomyxoma peritonei is a disease of MUC2-expressing goblet cells. These cells also express MUC5AC but the latter mucin is not specific for pseudomyxoma peritonei. MUC2 expression accounts for the voluminous deposits of extracellular mucin (mucin:cell ratios exceeding 10:1) and distinguishes pseudomyxoma peritonei secondarily involving the ovary from primary ovarian mucinous tumors with peritoneal implants. Because mucinous tumors of the appendix similarly express MUC2, the MUC2 expression profile also supports an appendiceal rather than ovarian origin for pseudomyxoma peritonei. Increased steady-state mRNA is observed in pooled cases of pseudomyxoma peritonei but does not occur on the basis of gene rearrangement or gene amplification. Primary epithelial cell cultures obtained from pseudomyxoma peritonei express MUC2 whose levels can be epigenetically regulated. These lines up-regulate MUC2 expression in response to both methylation inhibition by 5-azacytidine and exposure to *Pseudomonas aeruginosa* lipopolysaccharide, both of whose effects can be suppressed by genistein pretreatment. Both

immunocytochemical as well as *in situ* hybridization studies with ancillary digital image analysis reveal that MUC2 expression in cases of pseudomyxoma peritonei is independent of the degrees of malignant transformation that are present and, in fact, reflects the constitutive levels of expression observed in normal goblet cells of the appendix. Extracellular mucin accumulates dramatically in pseudomyxoma peritonei because the number of MUC2-secreting cells dramatically increase and because this MUC2 has no place to drain. These studies suggest that pseudomyxoma peritonei should be regarded as a disease of MUC2-expressing goblet cells whose MUC2 expression might be susceptible to pharmacological targeting. (*Am J Pathol* 2002, 161:551-564)

Pseudomyxoma peritonei, a syndrome first described by Karl F. Rokitsky in 1842,¹ is an enigmatic, often fatal intra-abdominal disease characterized by dissecting mucinous ascites and multifocal peritoneal epithelial implants secreting copious globules of extracellular mucin.²⁻⁵ In classic cases >90% of the globular masses are mucin by volume. Although past interest in the syndrome has focused on the questions of the site of origin (appendix *versus* ovary)⁶⁻¹⁰ and the mechanisms of peritoneal spread (multicentricity, redistribution phenomenon, or metastasis),¹⁰⁻¹³ another important question that has not been addressed is the mechanism behind the accumulation of extracellular mucin, the real cause of the disease's morbidity and mortality irrespective of the site of origin or mechanism of peritoneal spread. Recent clinical studies have argued for more narrowly limiting the definition of this syndrome to histologically benign peritoneal tumors arising from appendiceal adenomas (disseminated peritoneal adenomucinosis) to distinguish these from the overtly malignant peritoneal carcinomatoses arising from true carcinomas on the basis of the former group's more favorable response to cytoreductive surgery and intraperitoneal chemotherapy.¹⁴⁻¹⁶ Whatever definition one accepts, the accumulation of extracellular mucin with its resulting obstruction of abdominal viscera and adhesion formation is really the disease's main feature, re-

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sponsible for its morbidity and mortality irrespective of the transformed status of the epithelium secreting it. Because of this and because of the recent discovery and cloning of a series of specific human mucin genes responsible for mucin secretion and extracellular deposition,^{17,18} we decided to analyze cases of pseudomyxoma peritonei and companion mucinous tumors of the appendix, primary ovarian mucinous tumors, and normal tissue controls with specific mucin cDNAs and corresponding antibodies to investigate the mechanism and specificity of this mucin accumulation. We extended our studies to primary epithelial cell cultures derived from pseudomyxoma peritonei as well. Our study specifically investigated MUC2 and MUC5AC because these two mucins possessed the physicochemical property of being gel forming,^{19,20} a property exhibited by pseudomyxoma peritonei grossly.

Materials and Methods

Selection of Cases

Cases were obtained both retrospectively and prospectively. We retrospectively retrieved 25 cases of pseudomyxoma peritonei, 15 of which had a past or synchronous mucinous tumor of the appendix and 10 of which had no such documentation because either the appendix was not sampled or represented pseudomyxomatous recurrences without clinical information of the initial surgery. Of the 10 cases with no documented appendiceal primary, 3 exhibited ovarian involvement. Of the 15 cases with a mucinous tumor of the appendix, 6 had synchronous ovarian involvement. We also retrospectively retrieved 5 cases of solitary mucinous tumors of the appendix without pseudomyxoma peritonei and 25 cases of primary ovarian mucinous tumors, 5 of which had peritoneal implants and 1 of which had pseudomyxoma ovarii (extracellular mucin pools within ovarian stroma) but not generalized pseudomyxoma peritonei. The primary ovarian mucinous tumors, the mucinous tumors of the appendix and the cases of pseudomyxoma peritonei retrieved for study exhibited a range of histologies that included benign, borderline, and malignant histologies. The histological distribution of the selected cases are summarized in Table 1. Sections of normal appendix, normal peritoneum, and normal ovary served as controls.

We prospectively collected 10 cases of pseudomyxoma peritonei. The tissue was obtained fresh from the operating room at the UCLA Center for the Health Sciences, Los Angeles, CA, and triaged for diagnostic purposes and epithelial cell isolation as enumerated in a subsequent section.

Routine Microscopic and Histochemical Studies

All cases were initially evaluated with routine hematoxylin and eosin staining. Alcian blue staining (pH = 2.5), which was hyaluronidase-resistant, was used to better visualize the extracellular mucin pools. This histochemical stain helped distinguish extracellular mucin accumulations from other nonmucinous extracellular proteinaceous accumulations. All of the retrospectively retrieved and pro-

Table 1. Histologic Distribution and Numbers of Positive Cases

Category	No.	MUC2		MUC5AC	
		AB [†]	IS [§]	AB [†]	IS [§]
Pseudomyxoma peritonei	25	24	23	23	22
Benign	17	16	16	17	16
Borderline	5	5	4	4	4
Malignant	3	3	3	2	2
Ovarian involvement	9	9	9	8	8
Associated with MTA*	15	15	15	15	15
Solitary MTA*	5	5	5	5	5
Benign	2	2	2	2	2
Borderline	2	2	2	2	2
Malignant	1	1	1	1	1
POMT ^{†¶}	25	2	2	24	24
Benign	11	0	0	11	11
Borderline*	9	1	1	8	8
Malignant*	5	1	1	5	5
Peritoneal implants [¶]	5	1	1	5	5
Pseudomyxoma ovarii	1	0	0	1	1

*Mucinous tumor of the appendix; †primary ovarian mucinous tumor; ‡antibody; §*in situ* hybridization. ¶Positivity of MUC2 in this category was limited to weak or focal positivity (positive in <5% of the cells). No case exhibited strong or diffuse positivity.

spectively obtained cases of pseudomyxoma peritonei exhibited varying degrees of extracellular mucin accumulation by Alcian blue staining.

Immunocytochemical Studies

The identity of the specific mucin(s) involved in each of the cases was investigated with antibodies to MUC2 and MUC5AC. Anti-MUC2 (Research Diagnostics, Inc., Flanders, NJ), a murine monoclonal antibody (clone Ccp58) made to a synthetic peptide corresponding to a site on the MUC2 human core protein, and which was nonreactive to both MUC1 and MUC5AC, was used at a dilution of 1/100 to 1/200. Anti-MUC5AC (Research Diagnostics, Inc.), a murine monoclonal antibody (clone 45M1) made to a peptide core corresponding to a site on the MUC5AC human core protein, and which was nonreactive to both MUC1 and MUC2, was used at a dilution of 1/100 to 1/200. Paraffin-embedded sections were deparaffinized, rehydrated, and processed for antigen retrieval by citrate pretreatment (0.1 mol/L sodium citrate buffer, pH 6.0) followed by microwave heating²¹ for 10 minutes. Sheep anti-mouse IgG was used as a secondary antibody (1/200 dilution). Antigen-binding sites were then revealed by incubating with peroxidase-polymerizing diaminobenzidine-producing insoluble brownish-black staining at sites of antigen presence. Immunoreactivity staining patterns were categorized as negative (absent staining), weak or focal (positive in <5% of the cells), or strong (positive in >75% of the cells). All of the staining patterns fit into one of these three categories.

In Situ Hybridization Studies

In situ hybridization studies²² were conducted with riboprobes of MUC2 and MUC5AC. The pBluescript SK plas-

mid (Stratagene, La Jolla, CA) containing an *EcoRI* 90-bp repeat region of the human MUC2 gene, termed "HAM1,"²³ was linearized with *XbaI* for anti-sense strand preparation from the T7 promotor and with *HindIII* for sense strand preparation from the T3 promotor. A pCRII plasmid (Invitrogen, Carlsbad, CA) containing an *EcoRI* 298-bp tandem repeat fragment of the 3' end of the MUC5AC gene, was processed for riboprobe preparation identically except that the SP6 promotor was used for sense probe preparation. [³⁵S]UTP-labeled RNA transcripts were synthesized at concentrations of 4 to 6 × 10⁵ cpm/μl. Paraffin-embedded sections were deparaffinized, rehydrated, washed in 0.5× standard saline citrate (SSC), and digested with proteinase K at room temperature for 10 minutes. The sections were acetylated using 0.25% acetic anhydride and 0.1 mol/L triethanolamine HCl, rinsed with 0.5× SSC, and dehydrated with ethanol and finally air-dried. A MUC riboprobe was applied in a hybridization mixture containing deionized formamide (50%), dextran sulfate (10%), tRNA (0.5 mg/ml), salmon sperm DNA (1 mg/ml), 10 mmol/L dithiothreitol, 0.3 mol/L NaCl, 5 mmol/L ethylenediaminetetraacetic acid (EDTA), 20 mmol/L Tris-HCl, and 10 mmol/L NaPO₄ (pH 6.8). The mixture was heated to 65°C for 15 minutes and chilled on ice. Fresh dithiothreitol was added to achieve a concentration of 20 mmol/L. The 120 μl of the mixture was applied to each section and parafilm coverslips were applied. Hybridizations were performed in humidified chambers overnight at 55°C. Coverslips were removed in 5× SSC, 10 mmol/L dithiothreitol at 55°C. Sections were washed three times in 2× SSC containing 2-mercaptoethanol and EDTA, treated with RNase A, and washed in 0.1× SSC at 62°C for 3 hours (high stringency). Slides were then washed for 5 minutes at room temperature in 0.5× SSC without 2-mercaptoethanol or EDTA. The sections were dehydrated with ethanol and air-dried. The slides were exposed to Ilford K5D emulsion (Ilford Imaging USA, Paramus, NJ) and stored in the dark at 4°C until developed, which took at least 10 to 14 days.

Digital Image Analysis Studies

Digital image analysis was used to quantitate the amount of extracellular mucin present and the ratio of extracellular mucin to cells. In the *in situ* hybridization studies, digital image analysis was used to compare the signal intensities of the anti-sense probe with the signal intensities of the sense probe (background) over the corresponding areas of neoplastic and control epithelium. Signal intensities were expressed as anti-sense fold increase over sense. Signal intensity per cell was calculated. Digital image analysis used a digital imaging system, composed of a Leitz Dialux microscope linked to a Vidicon camera, an IBM PC with PCVision digitizer, and Microscience software.

Isolating Epithelial Cells from Pseudomyxoma Peritonei

The globules of mucin obtained fresh from cytoreductive surgeries of pseudomyxoma peritonei were mixed with

5% dextran sulfate (Sigma Chemical Co., St Louis, MO). This approach has shown some mucolytic activity *in vitro*.²⁴ After this the loosened mucin globules were minced into small 1-cm³ cubes and further digested with 200 U/ml of collagenase type IV from *Clostridium histolyticum* (EC 3.4.24.3, Sigma Chemical Co.) and 200 U/ml of hyaluronidase type V from sheep testis (EC 3.2.1.35, Sigma Chemical Co.). Digested tissue was pushed through a Collector tissue sieve (Bellco Glass, Inc., Vineland, NJ) and further minced. The cells that were liberated by these approaches were centrifuged at 100 × g, pelleted, and resuspended. This method yielded the epithelial cells that were originally embedded within the mucinous matrix of pseudomyxoma peritonei. Depending on the cellularity of the original material, ~10⁵ to 10⁷ epithelial cells could be obtained from 1 kg of starting material.

Northern and Southern Analysis

Epithelial cells obtained from the 10 cases of pseudomyxoma peritonei were pooled and processed for DNA and RNA extraction. Genomic DNA was extracted using standard methods^{25,26} and digested overnight with *EcoRI*, *BamHI*, or *PstI* (Life Technologies, Inc., Gaithersburg, MD) and run on a 1.0% agarose gel. Total RNA was extracted using Trizol reagent (Life Technologies, Inc.), 10 μg of total RNA was loaded onto a denaturing formaldehyde 1.2% agarose gel, and Northern blot analysis was performed. In the Northern blot experiments, we attempted initially to add equal amounts of total RNA per lane, ~10 μg. Despite our efforts, sometimes slightly unequal amounts of RNA were loaded and to correct for this we attempted to normalize with a common housekeeping probe, 36B4 (a gift of Dr. Judy Berliner, UCLA, Los Angeles, CA). In our hands we have found the 36B4 gene to be very reliable as a housekeeping gene, that is, its steady-state mRNA levels do not vary among most cells in culture. The pBluescript SK plasmid (Stratagene) containing a 90-bp repeat region of the human MUC2 gene, termed "HAM1,"²³ was digested with *EcoRI*, which liberated the insert that was gel-purified using an electroeluter (International Biotechnologies, Inc., New Haven, CT). Labeling was accomplished by random-prime labeling (Multiprime DNA Labeling System; Amersham, Arlington Heights, IL) of 25 ng of probe with [α -³²P]dCTP (ICN Pharmaceuticals, Irvine, CA) at a specific activity of ≥5.0 × 10⁹ cpm/μg. Sephadex TE Select-D G-50 columns (5 Prime-3 Prime, Inc., Boulder, CO) were used to remove unincorporated nucleotides.

Primary Cell Cultures and Cell Lines

The epithelial cells that were isolated fresh from the cases of pseudomyxoma peritonei were grown in monolayer culture in minimal essential medium with 10% fetal calf serum (Life Technologies, Inc., Gaithersburg, MD). In 3 of 10 attempts we were successful in getting the epithelial cells to grow. After several hours the cells in suspension attached to the tissue culture flask and formed monolayer-

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ers. For subculture, cell monolayers were washed in $\text{Ca}^{++}/\text{Mg}^{++}$ -free Hanks' balanced salt solution and detached with 0.05% trypsin-EDTA. Trypsinized cells were resuspended in medium containing 0.5% fetal calf serum, allowed to attach overnight, and maintained thereafter in minimal essential medium with 10% fetal calf serum. Cells were generally seeded to tissue culture flasks at 1/6 to 1/12 the confluent density or 0.5 to 1×10^4 cells/cm². The cultured cells were examined by phase contrast microscopy and electron microscopy using standard techniques.²⁷ After 2 to 3 weeks in culture, portions of the cultured cells were pelleted and studied for MUC2 expression by both *in situ* hybridization as well as Northern blot. We used other established cell lines as controls. These included the human colonic carcinoma cell lines, Colo205 and HT29 [American Type Culture Collection (ATCC), Rockville, MD] and human mammary epithelial cells (HMEC) (Clonetics, San Diego, CA). The Colo205 and HT29 cells were grown in minimal essential medium with 10% fetal calf serum. Human mammary epithelial cells were grown in keratinocyte serum-free medium (Life Technologies, Inc.) with the supplements, recombinant human epidermal growth factor (5 ng/ml) and bovine pituitary extract (50 $\mu\text{g}/\text{ml}$). All lines were grown at 37°C in an air-5% CO₂ atmosphere at constant humidity.

Pharmacological Manipulations

To demonstrate the possible epigenetic regulation of MUC2 expression, the primary epithelial cell cultures of pseudomyxoma peritonei were grown in regular media with and without added 5-azacytidine (10 $\mu\text{mol}/\text{L}$) (Sigma Chemical Co.) for 10 days. Fresh drugs with media changes were added every fourth day. The primary cultures were also grown in media containing lipopolysaccharide (LPS), a bacterial lipopolysaccharide²² purified from *P. aeruginosa* (serotype 10) (Sigma). LPS (5 $\mu\text{g}/\text{ml}$) was added to the cultures for varying times ranging from 0 to 10 hours. In parallel experiments the primary epithelial cell cultures were pretreated with genistein (100 $\mu\text{g}/\text{ml}$) (Sigma), a tyrosine kinase inhibitor, for 4 hours before treatment with either 5-azacytidine (10 $\mu\text{mol}/\text{L}$) or LPS (5 $\mu\text{g}/\text{ml}$). During these treatments cells were harvested at regular time intervals and subjected to RNA extraction and MUC2 Northern blot analysis.

Statistical Analysis

Experiments were performed by counting 10 microscopic fields per case with the appropriate magnification (mucin:

cell ratio, $\times 250$; *in situ* hybridization, $\times 250$ and $\times 450$) and results were expressed as mean $\pm$ SD. Other experiments combined the results of the individual cases into groups and group means $\pm$ standard deviations were calculated. All results were analyzed with standard tests of significance, including the two-tailed Student's *t*-test and a one-way analysis of variance.

Institutional Certifications

For the retrospective aspects of the study, informed patient consent was waived. For the prospective aspects of the study, informed patient consent was obtained and the study was approved by the UCLA Human Subject Protection Committee and the UCLA Institutional Biosafety Committee.

Results

A striking expression of both MUC2 and MUC5AC by both immunocytochemistry as well as *in situ* hybridization was observed in nearly all cases of pseudomyxoma peritonei of unknown and appendiceal origin (Figure 1; A to F, and Table 1) ($P < 0.01$). In these cases, however, MUC2 gene expression, by *in situ* hybridization, was uniformly more prominent (Figure 1D) than MUC5AC expression (Figure 1F) ($P < 0.05$). In analyzing the associated mucinous tumors of the appendix, a similar expression of both MUC2 and MUC5AC was observed ($P < 0.01$). The solitary mucinous tumors of the appendix without pseudomyxoma peritonei similarly exhibited a striking expression of both MUC2 and MUC5AC in all cases (Table 1) ($P < 0.01$). In both pseudomyxoma peritonei and solitary mucinous tumors of the appendix, the extracellular mucin deposits adjacent to the MUC2-immunopositive cells interestingly did not exhibit MUC2 immunoreactivity (Figure 1C).

Primary ovarian mucinous tumors including benign (Figure 2A), borderline, and malignant ovarian tumors essentially did not express MUC2 (Figure 2C) but expressed MUC5AC (Figure 2E and Table 1) ($P < 0.01$). The extracellular mucin deposits adjacent to the MUC5AC-immunopositive cells did express focal MUC5AC immunoreactivity.

All cases of pseudomyxoma peritonei (Table 1), however, on the other hand that metastasized to the ovary (Figure 2B) gave a pattern of mucin gene expression (Figure 2, D and F) identical to that exhibited by mucinous tumors of the appendix and pseudomyxoma peritonei involving the peritoneum, namely expression of both

Figure 1. The typical gross appearance of pseudomyxoma peritonei illustrates the so-called "jelly-belly" (A); the hallmark of the disease is copious accumulations of mucin deposits in which the intestinal lining cells are sometimes difficult to see (B); the cells do give intense signals and can be readily seen at all magnifications when the appropriate antibody or probe is used: for example, anti-MUC2 clearly delineates the intestinal lining cells responsible for this massive mucin secretion and accumulation. The extracellular mucin deposits paradoxically are not immunoreactive because their extensive glycosylation blocks antibody access to the core protein (C); anti-sense MUC2 *in situ* hybridization demonstrates intense MUC2 signals within this lining epithelium (D); similarly anti-MUC5AC also delineates the intestinal lining cells responsible for mucin secretion; the extracellular mucin deposits close to the cells are, however, focally immunoreactive presumably because their glycosylation status is less restrictive to antibody access than is MUC2 (E); anti-sense MUC5AC *in situ* hybridization demonstrates less intense but still significant MUC5AC signals within this lining epithelium (F). The extracellular mucin specifically was not recognized by these probes and antibodies and hence was difficult to visualize. To quantitate the mucin volumes and the mucin/cell ratios depicted in the subsequent Figure 5, we used Alcian blue staining, which stained the extracellular mucin more intensely permitting accurate digital measurements. A, Gross photograph; Original magnifications, $\times 250$, B, H&E; C, anti-MUC2, immunoperoxidase; D, MUC2 anti-sense riboprobe; E, anti-MUC5AC, immunoperoxidase; F, MUC5AC anti-sense riboprobe.

Figure 2. The mucin immunocytochemical profile of a primary ovarian mucinous tumor, in this case a mucinous cystadenoma (**A**) contrasts with the mucin immunocytochemical profile of pseudomyxoma peritonei with secondary ovarian involvement (**B**); the primary ovarian mucinous cystadenoma is MUC2 nonimmunoreactive (**C**) whereas the pseudomyxoma peritonei ovarian metastasis is strongly MUC2 immunoreactive (**D**); both the primary ovarian mucinous cystadenoma (**E**) and the pseudomyxoma peritonei metastasis (**F**) exhibit MUC5AC immunoreactivity. On close inspection some of the MUC5AC immunoreactivity appears extracellularly. Original magnifications: $\times 250$ (**A–E**); $\times 450$ (**F**). **A** and **B**, H&E; **C** and **D**, anti-MUC2, immunoperoxidase; **E** and **F**, anti-MUC5AC, immunoperoxidase.

Figure 3. **A:** Southern blot of 10 pooled cases of pseudomyxoma peritonei (PP) compared to Southern blots of the Colo205 and HT29 colonic carcinoma cell lines. High molecular weight DNA (20 μ g) was separately digested with the 6-cutters, *EcoRI*, *BamHI*, and *PstI* and probed with MUC2. No gene rearrangements or amplifications were observed in pseudomyxoma peritonei. **B:** Northern blot of 10 pooled cases of pseudomyxoma peritonei (PP) compared to Northern blots of the Colo205, HT29, and human mammary epithelial cells (HMEC). Equal amounts (10 μ g) of total RNA were added to each lane and probed with MUC2. Only pseudomyxoma peritonei showed high steady-state mRNA levels.

MUC2 and MUC5AC. Primary malignant ovarian mucinous tumors, on the other hand, even when they were associated with peritoneal implants essentially did not express MUC2 but only MUC5AC ($P < 0.01$). When morphological analysis was equivocal, both MUC2 immunostaining and MUC2 *in situ* hybridization were useful in distinguishing an intestinal *versus* ovarian primary in problematic cases involving the ovary.

The mechanism behind this expression of MUC2 in pseudomyxoma peritonei was not gene amplification or rearrangement (Figure 3A). Pooled cases of freshly obtained pseudomyxoma peritonei exhibited high steady-state MUC2 mRNA levels (Figure 3B). It seemed to us that there was either increased transcription of MUC2 per cell or that the number of cells capable of MUC2 transcription had increased in this disease or both. To investigate these two alternate possibilities we performed both immunocytochemistry studies and *in situ* hybridization studies in cases of pseudomyxoma peritonei where we could visualize the evolution of the primary appendiceal tumor.

In cases of primary mucinous tumors of the appendix that contained an *in situ* mucinous cystadenoma or mucinous borderline tumor, the immunocytochemical and *in situ* hybridization signals for MUC2 were overall in-

creased in the mucinous tumor compared to the adjacent appendix but were not increased on a per cell basis by digital image analysis when compared to the signals observed over normal goblet cells in the appendix (Figure 4; A to F). This suggested that pseudomyxoma peritonei is a neoplastic disease of MUC2-expressing goblet cells that collectively overexpress MUC2 because of their increase in number.

The mucin:cell ratio averaged 10 to 1 in representative cases of pseudomyxoma peritonei that was similar to the ratio observed in the mucinous tumors of the appendix. Primary ovarian mucinous tumors, in contrast, gave rise to implants in which the mucin:cell ratio was only 1 to 1 (Figure 5A) ($P < 0.05$).

In nearly all of the cases of pseudomyxoma peritonei, whether there was an associated mucinous tumor of the appendix or ovarian involvement (Figure 5B), *in situ* hybridization studies with anti-sense MUC2 and MUC5AC revealed strong MUC2 and MUC5AC signals (10-fold and fourfold to fivefold increases, respectively) ($P < 0.01$; $P < 0.05$). Solitary mucinous tumors of the appendix exhibited a similar mucin signal pattern by *in situ* hybridization. Primary ovarian mucinous neoplasms, in contrast, gave rise to implants in which only the anti-sense MUC5AC revealed strong MUC5AC signals. In these cases MUC5AC signals were slightly greater than in the cases of pseudomyxoma peritonei but this difference was not statistically significant (Figure 5B) ($P > 0.1$).

In situ MUC2 and MUC5AC expressions were independent of the degrees of malignant transformation present within the different pseudomyxoma peritonei subcategories of benign (adenoma), borderline, and malignant (carcinoma) (Figure 5C) ($P > 0.1$).

When cases of pseudomyxoma peritonei were compared to the control cases of normal appendix, normal ovary, and normal peritoneum, *in situ* hybridization studies revealed strong MUC2 and MUC5AC signals (10-fold and fourfold to fivefold increases, respectively; $P < 0.01$; $P < 0.05$) (Figure 5D) in pseudomyxoma peritonei (Figure 5E) but similarly fold increases (Figure 5D) were observed within the normal goblet cells of the appendix (Figure 5F). Nongoblet cell of the appendix showed no MUC2 or MUC5AC signals. Surface ovarian cells and peritoneal mesothelial cells showed no signals for MUC2 but weak signals for MUC5AC (data not shown).

Primary short-term cultures of epithelial cells could be obtained from cases of pseudomyxoma peritonei in 3 of 10 attempts. These cells exhibited mucin vacuoles in culture visible by both phase contrast (Figure 6A) and ultrastructural studies (Figure 6B). We have been able to successfully pass these lines 5 to 10 times. We have not yet been successful however in producing an immortalized cell line of pseudomyxoma peritonei. Still we have been able to use these primary cell cultures of pseudomyxoma peritonei to demonstrate that they express strong MUC2 signals by *in situ* hybridization (Figure 6C). We have also been able to study the regulation of MUC2 gene expression by pharmacological manipulation of these lines. The primary cell cultures constitutively express high levels of steady-state MUC2 mRNA. In addition this MUC2 expression can be epigenetically regu-

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lated. 5-Azacytidine, an inhibitor of DNA methylation, induced a twofold increase in MUC2 mRNA levels (Figure 7A) throughout 10 days suggesting that methylation of the MUC2 promoter repressed MUC2 expression in pseudomyxoma peritonei. Exposure of the pseudomyxoma peritonei cells to LPS, the major outer membrane component of gram-negative bacteria²² throughout a 10-hour time period up-regulated MUC2 mRNA levels in a more dramatic manner causing threefold to fivefold increases in a much shorter period of time (Figure 7B). Genistein, a potent tyrosine kinase inhibitor, when used to pretreat the cells for 4 hours, blocked the MUC2-enhancing effects of both 5-azacytidine (Figure 7C) as well as LPS (Figure 7D).

In the 3 of the 10 attempts in which we were successful in getting the epithelial cells from cases of pseudomyxoma peritonei to grow, the cells were subcultured at least three times throughout 2 to 3 weeks and grown to >80% confluent density before pharmacological treatment was initiated. The different pharmacological treatments, eg, 5-azacytidine, LPS, and genistein had no obvious morphological effects on the cells during the course of treatment. We did not notice any cellular changes either in the form of flattening or growth arrest. We did not examine apoptosis specifically by terminal dUTP nick-end labeling but did not notice any morphological changes that would suggest apoptosis. MUC2 expression, in the absence of treatment, was independent of both time in culture and cell density. Steady-state MUC2 mRNA levels were similar in the cultured pseudomyxoma peritonei cells as in the freshly isolated cells from pseudomyxoma peritonei cases.

Discussion

MUC2 gene expression in pseudomyxoma peritonei confirms an intestinal origin, identifies a pathogenesis pathway responsible for the disease's morbidity/mortality, and offers up a therapeutic target.

The vast majority of studies of pseudomyxoma peritonei throughout the past two decades have been primarily descriptive in nature.²⁸⁻³² Past interest in the syndrome has focused on the questions of the site of origin (appendix *versus* ovary),⁶⁻¹⁰ the mechanisms of peritoneal spread (multicentricity, redistribution phenomenon, or metastasis),¹⁰⁻¹³ and the transformed status of the epithelium responsible for the disease.¹⁴⁻¹⁶

Studies addressing the first question, the site of origin, first observed that pseudomyxoma peritonei occurred more commonly in women and frequently involved the ovary either unilaterally or bilaterally. Because of the ovarian involvement, for years the disease was assumed

to take origin from the ovary.⁶⁻¹⁰ Because of the frequency of ovarian involvement and the high female/male ratio of pseudomyxoma peritonei the physician most often caring for the patient was the gynecologist. Recent studies of cytokeratin immunostaining patterns,^{33,34} clonality studies with loss of heterozygosity,³⁵ and molecular studies of Ki-ras mutations^{12,35} have all supported the conclusion that pseudomyxoma peritonei usually takes origin from the appendix and not the ovary. Our findings with respect to MUC2 expression would also support this same conclusion. Our studies have demonstrated that MUC2 is a reliable molecular marker for pseudomyxoma peritonei that can be used to help make a diagnosis of this disease and distinguish metastatic involvement of the ovary by pseudomyxoma peritonei from a primary ovarian mucinous tumor with peritoneal implants. We are currently conducting a much larger clinical study comparing MUC2 immunocytochemistry and MUC2 *in situ* hybridization in the evaluation of ovarian, appendiceal, and pseudomyxoma peritonei mucinous neoplasms. So far our studies have indicated that mucinous tumors of the appendix, primary ovarian mucinous tumors, and pseudomyxoma peritonei all express MUC5AC but only mucinous tumors of the appendix and their associated pseudomyxoma peritonei express MUC2. This pattern of mucin expression reflects the putative cells of origin. Goblet cells of the appendix express both MUC2 and MUC5AC. This suggests that they are the cells of origin of mucinous tumors of the appendix and pseudomyxoma peritonei. Mesothelial cells and cells that line the surface of the ovary express only MUC5AC. Specifically this pattern of mucin gene expression suggests that pseudomyxoma peritonei does not originate from either the epithelium of the ovary or the mesothelial cells of the peritoneum as has been suggested in some studies.^{36,37}

Not only does MUC2 serve as a molecular marker of pseudomyxoma peritonei but likely accounts for the differences in the gross appearances of the mucinous deposits of pseudomyxoma peritonei compared to the mucinous implants of ovarian carcinoma. Our findings revealed that the mucin:cell ratio averaged or exceeded 10 to 1 in pseudomyxoma peritonei. We have observed some cases of pseudomyxoma peritonei in which the mucin:cell ratio was as much as 100:1 or even 1000:1. Sometimes the mucin accumulations were so abundant that intestinal cells could not even be identified. We did not include those cases in this study for obvious reasons. Ovarian neoplasms, in contrast, gave rise to implants in which the mucin:cell ratio was only 1 to 1. Because both pseudomyxoma peritonei and ovarian neoplasms express MUC5AC, it likely is the MUC2 and not the

Figure 4. An origin of pseudomyxoma peritonei within the appendix is demonstrated. Here, a mucinous borderline tumor of the appendix is observed arising *in situ* from a normal appendix (**A**). The mucinous tumor is present at the **top right** and the normal appendix is present in the **bottom left**. **B:** Anti-sense MUC2 *in situ* hybridization reveals strong MUC2 signals in the corresponding areas of the mucinous borderline tumor (**top right**) compared to weak to absent signals in the normal appendix (**bottom left**). **C:** MUC2 immunocytochemistry reflects intense but focal MUC2 immunoreactivity limited to the goblet cells of the normal appendix compared to intense and homogeneous immunoreactivity in all of the proliferating epithelial cells of the mucinous borderline tumor of the appendix (**D**); this observation was further reflected by the anti-sense MUC2 *in situ* hybridization findings of intense but focal signals in the goblet cells of the normal appendix (**E**) compared to intense and homogeneous signals in all of the proliferating epithelial cells of the mucinous borderline tumor of the appendix (**F**). Original magnifications: $\times 150$ (**A**, **B**); $\times 250$ (**C-F**). **A**, H&E; **B**, MUC2 anti-sense riboprobe; **C** and **D**, anti-MUC2, immunoperoxidase; **E** and **F**, MUC2 anti-sense riboprobe.

Figure 5. **A:** The extracellular mucin:cell ratio is quantitated in five individual cases of mucinous tumors of the appendix, primary ovarian mucinous tumors, and pseudomyxoma peritonei (PP). In each, results depict mean $\pm$ SD of 10 medium-power microscopic fields ($\times 250$). Higher mucin:cell ratios averaging 10:1 are in evidence in pseudomyxoma peritonei and in mucinous tumors of the appendix, suggesting that these two entities are related. In primary ovarian mucinous tumors, which included ovarian carcinomas with peritoneal implants, the mucin:cell ratio was much less and hovered around unity. **B:** The MUC2 and MUC5AC anti-sense *in situ* hybridization signals [fold increase over sense (background)] are quantitated in various mucinous lesions. Here mean signals from all of the individual cases were pooled and an overall mean $\pm$ SD was calculated. Mucinous tumors of the appendix and pseudomyxoma peritonei (PP) both exhibit strong signals with MUC2 more than MUC5AC. Primary ovarian mucinous tumors, in contrast, exhibit no MUC2 but slightly higher MUC5AC. **C:** Anti-sense MUC2 and MUC5AC *in situ* hybridization signals are quantitated in pseudomyxoma peritonei lesions showing varying degrees of transformation (benign, borderline, and malignant). Here mean signals from all of the individual cases of a given histology were pooled and an overall mean $\pm$ SD was calculated. The signals for MUC2 and MUC5AC do not vary with the degrees of transformation. **D:** Anti-sense MUC2 and MUC5AC *in situ* hybridization signals in pseudomyxoma peritonei compared to signals in goblet and nongoblet cells in the normal appendix are quantitated. Signals are increased in both pseudomyxoma peritonei as well as in normal goblet cells; digital image analysis of the *in situ* hybridization data from pseudomyxoma peritonei (**E**) and goblet cells in the normal appendix (**F**) confirm that on a per cell basis, there is no difference in the constitutive levels of expression (**D**). **A–D**, Comparative histograms; Original magnifications, $\times 250$ (**E**), $\times 450$ (**F**). **E** and **F**, MUC2 anti-sense riboprobe.

A

B

C

Figure 6. **A:** Short-term cultures of pseudomyxoma peritonei grow as monolayers in which prominent cytoplasmic vacuoles are observed; **B,** these vacuoles are mucin vacuoles on ultrastructural analysis; **C,** anti-sense MUC2 *in situ* hybridization of a cell pellet of these cultured cells confirms intense MUC2 signals. Original magnifications: $\times 200$ (**A**); $\times 3500$ (**B**); $\times 150$ (**C**). **A,** Phase contrast; **B,** uranyl acetate, lead citrate; **C,** MUC2 anti-sense riboprobe.

MUC5AC that is responsible for the high degree of gelation within the pseudomyxoma peritonei deposits. The complete lack of anti-MUC2 to detect the core protein within the extracellular mucin deposits of pseudomyxoma peritonei compared to the limited ability of anti-MUC5AC to reach its core protein is indirect evidence that extracellular MUC2 is more extensively

glycosylated than MUC5AC and therefore sterically occupies a greater volume than MUC5AC on an equimolar basis. It would then be anticipated that extracellular mucin deposits of MUC2 would occupy a greater volume than the extracellular mucin deposits of MUC5AC and cause pseudomyxoma peritonei to have its gross jelly-belly appearance.

Figure 7. Northern blots (10 μ g of total RNA per lane) of pseudomyxoma peritonei cell cultures pretreated and treated with various agents. **A:** Treatment with an inhibitor of DNA methylation, 5-azacytidine (10 μ mol/L), for 0 to 10 days increased steady-state MUC2 mRNA levels. **B:** Treatment with *P. aeruginosa* LPS (5 μ g/ml), for 0 to 10 hours increased steady-state MUC2 mRNA levels approximately threefold to fivefold. Genistein (100 μ g/ml), a tyrosine kinase inhibitor, when used to pretreat the cells, blocked the up-regulation of MUC2 $\times$ 5-azacytidine (**C**) as well as LPS (**D**).

Studies addressing the second question, the mechanisms of peritoneal spread, have focused on hypotheses of field defects giving rise to multicentric neoplasms,³⁸ rupture of the appendix with seeding of epithelial cells and a redistribution phenomenon related to the ovarian cycle of ovulation and intestinal peristalsis,^{39,40} and metastases related to properties of transformed cells to invade and colonize distant sites.^{41,42} Our observations concerning the high constitutive level of MUC2 expression in both the normal goblet cells of the appendix, in primary mucinous tumors of the appendix, and in pseudomyxoma peritonei but not in mesothelial cells nor in the surface cells of the ovary would weaken support for the field defect hypothesis and instead support either a rupture and redistribution phenomenon or an invasion and metastasis mechanism. Because our cases of pseudomyxoma peritonei included cases with both benign as well as malignant histologies, probably both mechanisms of dissemination are at play. Because MUC2 was observed in nearly all of our cases of

pseudomyxoma peritonei, it follows that MUC2 expression has nothing to do with the specific mechanisms of peritoneal spread.

Studies addressing the third question, the transformed status of the epithelium responsible for the disease, have observed that the prognosis of patients and the responses to cytoreduction and intraperitoneal chemotherapy depend on the histology and the degree of malignant transformation that is present. These studies have argued for more narrowly limiting the definition of this syndrome to histologically benign peritoneal tumors arising from appendiceal adenomas (disseminated peritoneal adenomucinosis) to distinguish these from the overtly malignant peritoneal carcinomatoses arising from true carcinomas.^{14–16} It is interesting that in our studies, MUC2 expression was independent of the degree of malignant transformation that was present suggesting that MUC2 expression has nothing to do with the mechanism or pathways involved in the transformation of the epithelial cells of pseudomyxoma peritonei. Very little is known concerning the genetic alterations contributing to the development and progression of mucinous tumors of the appendix and the ensuing development of pseudomyxoma peritonei. Whatever they are, they do not seem to be related to mucin expression. Mucin expression requires no genetic alterations because the goblet cells that give rise to mucinous tumors of the appendix and pseudomyxoma peritonei already constitutively express MUC2 at high levels.

Although the first three questions are undoubtedly important, the most important question of all which has not been addressed in previous studies is the mechanisms behind the accumulation of extracellular mucin, the real cause of the disease's morbidity and mortality irrespective of the site of origin, mechanism of peritoneal spread, or transformed status of the secreting epithelium. We have attempted to address this question in the present study. In the past pathologists were limited in their study of mucin by having to rely on conventional histochemical stains including periodic acid-Schiff (diastase resistance) and Alcian blue positivity that would not distinguish among the different mucin gene products. With the availability of specific mucin probes and antibodies this question could finally be addressed. Our studies indicated that pseudomyxoma peritonei is a neoplastic disease of MUC2-expressing goblet cells that collectively overexpress MUC2 because of their increase in number but on a per cell basis maintain the constitutive level of MUC2 expression observed in normal goblet cells. Whereas goblet cells comprise only a minority of epithelial lining cells in colonic epithelium and can secrete their MUC2 gene product into the colonic lumen where the mucin washes away in the fecal stream, in pseudomyxoma peritonei goblet cells comprise the totality of the epithelial lining cells and secrete their MUC2 gene product within the confines of the peritoneum (Figure 8).

The mechanism of increased MUC2 expression in pseudomyxoma peritonei is not genetic but rather epigenetic. There was no apparent evidence of gene rearrangement or amplification in the pooled cases of pseudomyxoma peritonei. We pooled the cases for DNA

Figure 8. Schematic depicts our hypothesis that pseudomyxoma peritonei is a disease of MUC2-expressing goblet cells. In the normal colon (**A**), goblet cells comprise a minority of intestinal lining cells and constitutively express high levels of MUC2. This extracellular mucin is washed out in the colonic lumen and does not accumulate. Pseudomyxoma peritonei is a disease in which these MUC2-expressing goblet cells transform and proliferate, so instead of representing only a minority of the intestinal lining cells now represent all of the lining cells (**B**). The overall MUC2 expression and secretion rise dramatically in pseudomyxoma peritonei but, on a per cell basis, there is no difference in the constitutive levels of expression of MUC2, reflecting only the levels expressed by normal goblet cells. These MUC2-expressing goblet cells spread to the omentum and the mesentery either through appendiceal rupture or invasion, subsequent redistribution, or metastasis. In the omentum and mesentery the extracellular mucin cannot wash out the colonic lumen and instead accumulates as the gelatinous deposits of pseudomyxoma peritonei.

and RNA determinations because we could not get enough DNA or RNA from individual cases to perform any meaningful studies. It should be remembered that pseudomyxoma peritonei consists mainly of extracellular mucin and one must process at least 1 kg of starting material to get 10^5 to 10^7 cells. In the vast majority of our cases, our starting material was considerably less than 1 kg. We certainly would have preferred to analyze each of the individual cases but could not. Minor rearrangements present in some individual cases certainly could have been missed by our pooled Southern blot but even if present would not likely be the cause of the global MUC2 expression observed. We would anticipate finding a common rearrangement in our pooled cases if the rearrangement were the cause of the MUC2 expression.

Furthermore the levels of MUC2 expression could be epigenetically regulated in primary cultures of pseudomyxoma peritonei cells. Although these studies are preliminary, the effects of 5-azacytidine suggested that promoter methylation may repress MUC2 expression in pseudomyxoma peritonei. The similar but more potent effects of LPS in increasing MUC2 expression remind us that in the respiratory tract *P. aeruginosa* and other similar bacterial pathogens containing lipopolysaccharides account for increased MUC2 expression and mucous production during infection.²² Similarly in the lower gastrointestinal tract, which is colonized by gram-negative organisms, LPS or a similar bacterial lipopolysaccharide may enhance MUC2 expression by goblet cells. LPS or a similar lipopolysaccharide may also stimulate MUC2 expression in pseudomyxoma peritonei as undoubtedly bacterial breakdown products exist in the mesenteric circulations.

The molecular mechanisms responsible for the control of mucin transcription and expression are just beginning to be understood as mucin gene promoters and regulatory regions are characterized.^{43,44} Four mucin genes, including MUC2 and MUC5AC, are clustered on the p15 arm of chromosome 11. These mucin genes are regulated at the transcriptional level by proinflammatory cytokines (interleukin-1 β , IL-6, tumor necrosis factor- α), pleiotropic cytokines (IL-4, IL-13, IL-9), bacterial exoproducts (LPS), growth factors (epidermal growth factor, transforming growth factor- α), lipid mediator platelet-activating factors (PAF), retinoids, and hormones. To date, the only downstream cascade known to activate mucin gene transcription is the Src/Ras/MAPK/pp90rsk cascade, which leads to the activation of the transcription factor nuclear factor- κ B. Mucin gene transcription is also regulated by ATF-1, CREB, and RAR- α transcription factors. The identification of the specific signaling cascades and the active *cis* and *trans* elements within the mucin promoters offers potential therapeutic opportunities to affect mucin gene expression. When one notes that the mucin:cell ratio of pseudomyxoma peritonei exceeded 10 to 1, and that no mucolytic agent exists presently that can be applied *in vivo* to remove the mucin deposits of this disease, a reasonable adjuvant therapeutic approach is to inhibit MUC2 transcription.

Relapse in pseudomyxoma peritonei has been correlated with a number of clinicopathological parameters^{45,46} including extent of initial disease, histology, adequacy of the cytoreduction, the use of intraperitoneal chemotherapy, and other factors. Still the ability to inhibit mucin reaccumulation would transcend all of these prognostic factors. We can think of no setting more appropriate for new translational approaches than that offered by pseudomyxoma peritonei where MUC2 is the clear target and the cause of the disease's morbidity and mortality.

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References

- Weaver CH: Mucocele of the appendix with pseudomucinous degeneration. *Am J Surg* 1937, 36:523-526
- Young RH, Gilks CB, Scully RE: Mucinous tumors of the appendix associated with mucinous tumors of the ovary and pseudomyxoma peritonei. A clinicopathological analysis of 22 cases supporting an origin in the appendix. *Am J Surg Pathol* 1991, 15:415-429
- Carleton CC: Mucoceles of the appendix and peritoneal pseudomyxoma. *Arch Pathol* 1955, 60:39-48
- Parsons J, Gray GF, Thorbjarnarson B: Pseudomyxoma peritonei. *Arch Surg* 1970, 101:545-549
- Carr NJ, Sobin LH: Unusual tumors of the appendix and pseudomyxoma peritonei. *Semin Diagn Pathol* 1996, 13:314-325
- Klemi PJ: Pathology of mucinous ovarian cystadenomas. I. Argrophil and argentaffin cells and epithelial mucosubstances. *Acta Pathol Microbiol Scand A* 1978, 86A:465-470
- Gough DB, Donohue JH, Schutt AJ, Gonchoroff N, Goellner JR, Wilson TO, Naessens JM, O'Brien PC, van Heerden JA: Pseudomyxoma peritonei. Long-term patient survival with an aggressive regional approach. *Ann Surg* 1994, 219:112-119
- Costa MJ: Pseudomyxoma peritonei. Histologic predictors of patient survival. *Arch Pathol Lab Med* 1994, 118:1215-1219
- Seidman JD, Elsayed AM, Sobin LH, Tavassoli FA: Association of mucinous tumors of the ovary and appendix. A clinicopathologic study of 25 cases. *Am J Surg Pathol* 1993, 17:22-34
- Sumithran E, Susil BJ: Concomitant mucinous tumors of appendix and ovary. Result of a neoplastic field change? *Cancer* 1992, 70:2980-2983
- Esquivel J, Sugarbaker PH: Clinical presentation of the pseudomyxoma peritonei syndrome. *Br J Surg* 2000, 87:1414-1418
- Cuatrecasas M, Matias-Guiu X, Prat J: Synchronous mucinous tumors of the appendix and the ovary associated with pseudomyxoma peritonei. A clinicopathologic study of six cases with comparative analysis of c-Ki-ras mutations. *Am J Surg Pathol* 1996, 20:739-746
- Sugarbaker PH, Yan H, Shmookler B: Pedunculated peritoneal surface polyps in pseudomyxoma peritonei syndrome. *Histopathology* 2001, 39:525-528
- Ronnett BM, Zahn CM, Kurman RJ, Kass ME, Sugarbaker PH, Shmookler BM: Disseminated peritoneal adenomucinosis and peritoneal mucinous carcinomatosis. A clinicopathologic analysis of 109 cases with emphasis on distinguishing pathologic features, site of origin, prognosis, and relationship to "pseudomyxoma peritonei." *Am J Surg Pathol* 1995, 19:1390-1408
- Sugarbaker PH: Cyto-reductive surgery and peri-operative intraperitoneal chemotherapy as a curative approach to pseudomyxoma peritonei syndrome. *Eur J Surg Oncol* 2001, 27:239-243
- Ronnett BM, Yan H, Kurman RJ, Shmookler BM, Wu L, Sugarbaker PH: Patients with pseudomyxoma peritonei associated with disseminated peritoneal adenomucinosis have a significantly more favorable prognosis than patients with peritoneal mucinous carcinomatosis. *Cancer* 2001, 92:85-91
- Verma M, Davidson EA: Mucin genes: structure, expression and regulation. *Glycoconj J* 1994, 11:172-179
- Gendler SJ, Spicer AP: Epithelial mucin genes. *Annu Rev Physiol* 1995, 57:607-634
- Kim YS, Gum Jr JR: Diversity of mucin genes, structure, function, and expression. *Gastroenterology* 1995, 109:999-1001
- O'Connell JT, Shao ZM, Drori E, Basbaum CB, Barsky SH: Altered mucin expression is a field change that accompanies mucinous (colloid) breast carcinoma histogenesis. *Hum Pathol* 1998, 29:1517-1523
- Taylor CR, Shi SR, Chaiwun B, Young L, Imam SA, Cote RJ: Strategies for improving the immunohistochemical staining of various intranuclear prognostic markers in formalin-paraffin sections: androgen receptor, estrogen receptor, progesterone receptor, p53 protein, proliferating cell nuclear antigen, and Ki-67 antigen revealed by antigen retrieval techniques. *Hum Pathol* 1994, 25:263-270
- Li JD, Dohrman AF, Gallup M, Miyata S, Gum JR, Kim YS, Nadel JA, Prince A, Basbaum CB: Transcriptional activation of mucin by *Pseudomonas aeruginosa* lipopolysaccharide in the pathogenesis of cystic fibrosis lung disease. *Proc Natl Acad Sci USA* 1997, 94:967-972
- Chang SK, Dohrman AF, Basbaum CB, Ho SB, Tsuda T, Toribara NW, Gum JR, Kim YS: Localization of mucin (MUC2 and MUC3) messenger RNA and peptide expression in human normal intestine and colon cancer. *Gastroenterology* 1994, 107:28-36
- Beller FK, Zimmerman RE, Nienhaus H: Biochemical identification of the mucus of pseudomyxoma peritonei as the basis for mucolytic treatment. *Am J Obstet Gynecol* 1986, 155:970-973
- Sharma RC, Murphy AJM, DeWald MG, Schimke RT: A rapid procedure for isolation of RNA-free genomic DNA from mammalian cells. *BioTechniques* 1993, 14:176-178
- Reed KC, Mann DA: Rapid transfer of DNA from agarose gels to nylon membranes. *Nucleic Acids Res* 1985, 13:7207-7221
- Hayat MA: Principles and Techniques of Electron Microscopy: Biological Applications, ed 3. Boca Raton, CRC Press, 1989, p 137
- Bechtold RE, Chen MY, Loggie BW, Jackson SL, Geisinger K: CT appearance of disseminated peritoneal adenomucinosis. *Abdom Imaging* 2001, 26:406-410
- Clark R: Pseudomyxoma peritonei. *Cancer Control* 1995, 2:227
- Hemet J, Lemoine F, Irimescu D, Michot F, Dunet F: Pseudomyxoma extraperitonei. Report of 3 cases. *Ann Pathol* 1998, 18:137-140
- Jackson SL, Fleming RA, Loggie BW, Geisinger KR: Gelatinous ascites: a cytohistologic study of pseudomyxoma peritonei in 67 patients. *Mod Pathol* 2001, 14:664-671
- Lee KR, Scully RE: Mucinous tumors of the ovary: a clinicopathologic study of 196 borderline tumors (of intestinal type) and carcinomas, including an evaluation of 11 cases with 'pseudomyxoma peritonei.' *Am J Surg Pathol* 2000, 24:1447-1464
- Guerrieri C, Fralund B, Fristedt S, Gillooley JF, Boeryd B: Mucinous tumors of the vermiform appendix and ovary, and pseudomyxoma peritonei: histogenetic implications of cytokeratin 7 expression. *Hum Pathol* 1997, 28:1039-1045
- Guerrieri C, Fralund B, Boeryd B: Expression of cytokeratin7 in simultaneous mucinous tumors of the ovary and appendix. *Mod Pathol* 1995, 8:573-576
- Szych C, Staebler A, Connolly DC, Wu R, Cho KR, Ronnett BM: Molecular genetic evidence supporting the clonality and appendiceal origin of pseudomyxoma peritonei in women. *Am J Pathol* 1999, 154:1849-1855
- Urbanski SJ: In support of findings on pseudomyxoma peritonei. *Am J Pathol* 1999, 155:2182
- Urbanski SJ, Robertson DI: Pseudomyxoma peritonei: a primary tumor of peritoneal mesothelium? *Lab Invest* 1999, 79:126A
- Chuaqui RF, Zhuang Z, Emmert-Buck MR, Bryant BR, Nogales F, Tavassoli FA, Merino MJ: Genetic analysis of synchronous mucinous tumors of the ovary and appendix. *Hum Pathol* 1996, 27:165-171
- Sugarbaker PH: Pseudomyxoma peritonei. A cancer whose biology is characterized by a redistribution phenomenon. *Ann Surg* 1994, 219:109-111
- Sugarbaker PH: Observations concerning cancer spread within the peritoneal cavity and concepts supporting an ordered pathophysiology. *Cancer Treat Res* 1996, 82:79-100
- Sugarbaker PH, Ronnett BM, Archer A, Averbach AM, Bland R, Chang D, Dalton RR, Ettinghausen SE, Jacquet P, Jelinek J, Koslowe P, Kurman RJ, Shmookler B, Stephens AD, Steves MA, Stuart OA, White S, Zahn CM, Zoetmulder FA: Pseudomyxoma peritonei syndrome. *Adv Surg* 1996, 30:233-280
- Sugarbaker PH, Shmookler B, Ronnett BM, Chang D: Pseudomyxoma peritonei. *Br J Surg* 1999, 86:842
- Seunigen IV, Pigny P, Perrais M, Porchet N, Aubert JP: Transcriptional regulation of the 11p15 mucin genes. Towards new biological tools in human therapy, in inflammatory diseases and cancer? *Front Biosci* 2001, 6:D1216-D1234
- Sylvester PA, Myerscough N, Warren BF, Carlstedt I, Corfield AP, Durdey P, Thomas MG: Differential expression of the chromosome 11 mucin genes in colorectal cancer. *J Pathol* 2001, 195:327-335
- Yan H, Pestieau SR, Shmookler BM, Sugarbaker PH: Histopathologic analysis in 46 patients with pseudomyxoma peritonei syndrome: failure versus success with a second-look operation. *Mod Pathol* 2001, 14:164-171
- Sugarbaker PH, Fernandez-Trigo V, Shamsa F: Clinical determinants of treatment failure in patients with pseudomyxoma peritonei. *Cancer Treat Res* 1996, 81:121-132