MUC1 and MUC2 in pancreatic neoplasia

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MUCs are glycoproteins with various roles in homeostasis and carcinogenesis. Among other actions, MUC1 may inhibit cell–cell and cell–stroma interactions and function as a signal transducer, participating in cancer progression. In contrast, MUC2 is normally found only in goblet cells, where it contributes to the protective barrier function of these cells. Recently, a tumour suppressor role has been demonstrated for MUC2, and both MUC1 and MUC2 appear to have important roles in pancreatic neoplasia. MUC1 appears to be a marker of aggressive phenotype and may facilitate the vascular spread of carcinoma cells. In contrast, MUC2 is rarely detectable in aggressive pancreatic tumours, but is commonly expressed in intraductal papillary mucinous neoplasms (IPMNs), which are rare, indolent tumours, in intestinal IPMNs, and in indolent colloid carcinomas. MUC2 appears to be not only a marker of this indolent pathway, but also partly responsible for its less aggressive nature. Thus, in pancreatic neoplasia, MUC1 and MUC2 have potential diagnostic and prognostic value as markers of aggressive and indolent phenotypes, respectively, and have potential as therapeutic targets.

“Both MUC1 and MUC2 seem to have important roles in pancreatic neoplasia”

In contrast, MUC2 is referred to as intestinal-type mucin because its expression is normally limited to goblet cells, where it contributes to the protective barrier function of these cells. Recently, MUC2 has been shown to have tumour suppressor activity, as demonstrated by the neoplastic transformation seen in the gastrointestinal system of MUC2 knockout mice. Both MUC1 and MUC2 seem to have important roles in pancreatic neoplasia. MUC1 appears to be a marker of aggressive phenotype. It is commonly expressed in high grade but not low grade pancreatic intraepithelial neoplasia (PanIN; microscopic/ incidental precursors of invasive carcinoma). Furthermore, it is abundant in most all examples of conventional ductal adenocarcinoma. In fact, dense cytoplasmic immunostaining for MUC1 can be used as a diagnostic tool, because such staining is not usually seen in normal or preinvasive neoplasia or other carcinoma types in this region. This may also be helpful in the subclassification of ampullary neoplasia. It is also possible that MUC1 facilitates the vascular spread of carcinoma cells by helping them to remain detached from the endothelial cells.

Thus, in pancreatic neoplasia, MUC1 and MUC2 may have diagnostic and prognostic value as markers of aggressive and indolent phenotypes, respectively, and may have potential as therapeutic targets.

MUC1 AND MUC2: BIOCHEMICAL AND PHYSIOLOGICAL PROPERTIES

Mucins are the major components of mucous, the visco-elastic substance that protects and lubricates epithelial mucosa, including that of the gastrointestinal tract. They are highly

Abbreviations: EGFR, epidermal growth factor receptor; IPMN, intraductal papillary mucinous neoplasia; MAPK, mitogen activated protein kinase; PanIN, pancreatic intraepithelial neoplasia; TCF, T cell factor
glycosylated molecules; up to 80% of their mass consists of O-linked glycosyl residues. The mucins constitute an expanding family of proteins that can be grouped into “transmembrane mucins” and “secretory mucins.” So far, 19 different mucin apoproteins have been identified. The transmembrane mucins are emerging as signal transducer proteins that regulate the growth, adhesion, and motility of cells.

The best studied transmembrane mucin is MUC1, also known as epithelial membrane antigen, which was first identified in human milk. MUC1 is synthesised as a single polypeptide that undergoes proteolytic cleavage, creating a heterodimer that remains associated during its post-translational processing in the Golgi. The larger component contains most of the extracellular domain—the tandem repeat domain—which comprises 30–90 repeat sequences of 20 amino acids. Approximately 50–90% of the mass of MUC1 results from the O-glycosylation that occurs on the amino acid repeat units. The smaller component of the heterodimer consists of an extracellular stem that joins the larger component to the transmembrane domain and a 72 amino acid cytoplasmic tail, which has signal transduction properties as a result of the presence of docking sites for SH2 domain containing proteins, in addition to the presence of tyrosine phosphorylation sites.

MUC1 is expressed by almost all human glandular epithelial tissues and throughout all regions of the gastrointestinal tract. MUC1 is also expressed in normal pancreatic intralobular ducts, together with MUC5AC, MUC5B, and MUC6. One major role of the MUC1 protein is to act as an anti-adhesive protein to maintain the luminal integrity of the lining epithelium. Therefore, in normal epithelia its expression is largely limited to the apical borders of secretory cells. In contrast, MUC2 is a secreted form of mucin, which is exclusively expressed in the goblet cells lining the gastrointestinal mucosa. MUC2 is characterised by tandem and irregular repeat sequences rich in threonine and serine, which are sites of attachment of the oligosaccharide chains. The MUC2 gene product is more than 5100 amino acids long in its most common allelic form, which accounts for one fifth of the mucin glycoprotein molecule by weight. The MUC2 protein is polymerised end to end through disulphide bridges to form large secreted polymeric gel forming mucins. It acts as a protective barrier at mucosal surfaces. It is also expressed in areas of intestinal metaplasia, such as those seen in Barrett’s oesophagus and long standing *Helicobacter pylori* gastritis. MUC2 is not expressed in the normal pancreas.

**MUC1 AND MUC2: THEIR POSSIBLE ROLES IN MOLECULAR CARCINOGENESIS**

MUC1 is a signal transducer molecule, which can sense environmental changes and transduce messages into the cell. It is normally expressed on the apical borders of secretory epithelial cells. However, in tumour cells, MUC1 expression loses its polarity, being expressed throughout the entire cell surface. The presence of extracellular, transmembrane, and cytoplasmic domains makes it an ideal candidate as a signal transduction unit.

MUC1 has been shown to be associated with the epidermal growth factor receptor (EGFR) members EGFR, ErbB2 (HER2/neu), ErbB3, and ErbB4 receptor tyrosine kinases through its cytoplasmic tail. In addition, binding of Grb2/sos and c-src to the cytoplasmic tail of MUC1 has been demonstrated. These findings suggest that the Ras–Raf–mitogen activated protein kinase (MAPK) signalling system can be triggered by MUC1. The importance and the context of these interactions are not completely clear. It is tempting to speculate that the extracellular domain of the MUC1 protein can sense some distinct changes in the extracellular environment, such as adhesion status or chemical composition of the milieu (for example, the presence of oxygen radicals).

In addition to binding to EGFR family members, MUC1 can physically interact with β catenin, a key molecule of the Wnt signalling pathway, and also a key molecule in colorectal tumorigenesis. β Catenin is normally bound to cadherins, which regulate cell to cell adhesion. Upon activation of the Wnt pathway, β catenin translocates to the nucleus, binding the lymphoid enhancer factor (TF) complex, thereby initiating the transcription of growth promoting genes. MUC1 is associated with increased nuclear localisation of β catenin. In fact, Wen et al recently demonstrated the translocation of the cytoplasmic tail of MUC1 to the nucleus in conjunction with β catenin. These findings suggest that, overall, MUC1 is associated with the detachment of β catenin from the cadherins and its translocation to the nucleus. This translates into loss of adhesion, and perhaps contact inhibition, and increased proliferation.

The association of MUC1 with the cadherin–catenin–TF complex, the EGFR–Raf–Ras–MAPK, signalling pathways, two important transduction modules that regulate cell proliferation and diverse functions, strongly suggests that MUC1 has an active and functional role in carcinogenesis in a variety of tissues. MUC1 is overexpressed and differentially glycosylated by many tumours. There are several studies demonstrating the adverse prognostic effect of MUC1 expression on survival in various tumours, including those of the stomach, breast, kidney, colon, endometrium, lung, bile duct, and even lymphomas. In a recent study, MUC1 tandem repeats of the extracellular portion and the cytoplasmic tail were shown to be responsible for the invasive properties of a pancreatic carcinoma cell line.

In contrast to MUC1, MUC2 has no transmembrane domain or signal transduction properties. Recently, there has been increased interest in the MUC2 protein as the result of the demonstration that MUC2 knockout mice develop colorectal tumours, including tubular and villous adenomas and invasive carcinomas. This finding indicates that MUC2 has tumour suppressor properties.

The expression of MUC2 is regulated by homeobox genes CDX1 and CDX2. These transcription factors are key in understanding normal intestinal development and the intestinal metaplasia that is often seen in *H pylori* gastritis, Barrett’s oesophagus, and cholecystitis. In fact, it has been shown that intestinal metaplasia of the stomach is always preceded by activation of the transcription factor CDX2. CDX2 expression also precedes the expression of MUC2 in Barrett’s epithelium. In a recent study by Seno et al, CDX2 expression in gastric cancer was associated with intestinal-type gastric cancer, a lower proliferation rate, and better survival compared with non-expressors. By binding to enhancer sequences in the promoter regions of target genes, CDX2 triggers the transcription of genes that encode intestine specific proteins, such as MUC2, sucrase/isomaltase, lactase, villin, KLF4, and carbonic anhydrase I. CDX2 also upregulates its own expression. Another way that CDX2 promotes intestinal differentiation is by upregulating the expression of WAF1/p21, causing cell cycle arrest. Overall, CDX2 seems to
promote the differentiation and maintenance of intestinal epithelial cells.\(^5^4\)

MUC2 expression is also regulated by p53.\(^5^5\) The MUC2 gene has been shown to have p53 binding sites.\(^5^5\) In cellular stress conditions, p53 is activated to control cell proliferation and allow DNA repair.\(^5^6\) It also triggers apoptosis when the repair efforts have been futile.\(^5^6\) The fact that p53 triggers MUC2 expression suggests that MUC2 is part of a stress response programme.\(^5^5\) Because MUC2 is part of the intestinal metaplasia programme, which itself is a response to stress conditions in gastric, oesophageal, and biliary mucosa, MUC2 expression may not be an oncogenic event per se, but a protective mechanism, which is activated to prevent further exposure to carcinogens.\(^5^4\) However, overall, the development of intestinal metaplasia in the stomach and oesophagus is associated with an increased risk of cancer.\(^5^4\)\(^5^7\)\(^5^8\)

Whereas mucin secretion and MUC2 expression are part of the many stress responses in airway epithelia, stomach, and biliary tract, conversely, loss of MUC2 gene activation has been attributed to receptor methylation.\(^2^4\)\(^5^9\)\(^6^0\) Siedow \textit{et al} have studied the mechanism of MUC2 expression in pancreatic carcinoma cell lines. They have shown that in non-expressor cell lines the MUC2 gene is methylated, whereas in the expressors it is demethylated. The investigators also treated non-expressor cell lines with demethylating agents, such as 5-aza-2-deoxycytidine, which caused expression of MUC2 in non-expressors.\(^5^9\)\(^6^1\) Demethylating agents may be useful as potential therapeutic agents in treating aggressive adenocarcinomas.

**MUC1 AND MUC2 EXPRESSION IN NORMAL ADULT PANCREAS**

In the normal adult pancreas, MUC1 expression is confined to the apical membrane of intralobular ductules, possibly a manifestation of the “lumen maintenance” role of MUC1. MUC1 expression is not detected in larger ducts or other pancreatic elements (islets or acini).\(^6^2\)\(^6^3\) It should be noted here that, in our opinion, it is these intralobular ductules that pancreatic ductal adenocarcinoma resembles most closely, both in its histochemical properties and its immunophenotype.

MUC2 is not expressed in normal adult pancreas.\(^6^6\)\(^6^7\)

**MUC1 AND MUC2 IN PREINVASIVE NEOPLASIA OF THE PANCREAS**

**Pancreatic intraepithelial neoplasia**

- MUC1: expressed in some PanIN-3 tumours only
- MUC2: virtually absent

PanIN is the term adopted recently for the microscopic/incidental form of intraductal proliferations that have been long suspected to be the precursors of invasive ductal adenocarcinoma.\(^6^8\) These proliferations have been previously classified under various names ranging from metaplasia (for the early forms) to hyperplasia (with or without atypia) to dysplasia.\(^6^9\)\(^7^0\) According to the scheme proposed recently, these lesions are unified under the term PanIN (to conform with the terminology used in other organs, such as the prostate), and classified into three grades: PanIN-1 includes but is not limited to changes previously regarded as mucinous metaplasia (but which were recently found to harbour several molecular alterations associated with neoplasia), and at the other end of the spectrum, PanIN-3, which includes carcinoma in situ.\(^6^8\)

MUC1 is expressed in a substantial number of PanIN-3 tumours but is almost absent in PanIN-1 or PanIN-2 (fig 1C). In contrast, MUC2 is not detected in PanINs.\(^6^8\)

**Intraductal papillary mucinous neoplasia**

- MUC1: rarely expressed
- MUC2: very frequently expressed

IPMN is now a well-recognised category in the pancreas, unifying tumours that are characterised by intraductal proliferation of neoplastic mucinous cells, which often form papillae, and lead to cystic dilatation of the pancreatic ducts,
forming clinically and macroscopically detectable masses.\textsuperscript{72–79} These tumours have previously been reported under a variety of names, such as villous adenoma, intraductal papillary tumour, mucin producing tumour, mucinous duct ectasia, etc.\textsuperscript{80–83} IPMN should be distinguished from the more widely recognised mucinous cystic neoplasms (see below).\textsuperscript{84–86}

Similar to PanINs, IPMNs are regarded as a form of preinvasive neoplasia, acting as a precursor and maybe as even a marker of invasive carcinoma.\textsuperscript{87–89} Both PanINs and IPMNs are intraductal lesions with papilla forming mucinous cells; however, in the case of IPMNs, the proliferation progresses to form large (clinically and grossly detectable) cystic or papillary masses, whereas PanINs are noted only as incidental findings.\textsuperscript{90} We think that this dichotomy represents two relatively distinct pathways of carcinogenesis, although there are some overlaps, as would be expected. This dichotomy is similar to that seen in other organs, such as: (1) in the urothelium, as flat versus papillary transitional cell carcinomas; or (2) in the breast, as intracystic papillary carcinoma versus ordinary intraductal proliferations; these are distinct pathways not only clinically and morphologically, but also biologically and at the molecular level.\textsuperscript{91–94}

‘MUC expression patterns not only support the dichotomy between pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasia (IPMN) but also confirm the existence of morphological subtypes in IPMN’

MUC1 and MUC2 expression patterns in IPMNs versus PanINs support this dichotomy. Whereas MUC2 expression is almost absent in PanINs, it is present in a substantial number of IPMNs.\textsuperscript{95} In particular, MUC2 expression is uniformly diffuse and strong in the most common subtype of IPMN (fig 1C), which is described as villous/intestinal type or compact cell type, and is characterised by finger-like papillary projections that are lined by columnar cells with cigar shaped nuclei, and is morphologically very similar to colonic villous adenomas. In contrast, MUC1, which is often present in PanIN-3, is much less frequently expressed in IPMNs.\textsuperscript{96–98} More interestingly, those IPMNs that express MUC1 are invariably of the rare subtype that we have independently referred to as pancreatobiliary, and which is characterised by more complex (arborising) papillae and cuboidal cells, similar to those seen in biliary papillomatosis (fig 1A, B). This differential expression of MUC1 and MUC2 in the subtypes of IPMN has been noted by various investigators, including Luttges et al.,\textsuperscript{99} Kloppe et al.,\textsuperscript{100} and Yonezawa et al.,\textsuperscript{101} who refer to these subtypes as dark cell, clear cell, and compact cell types. Because MUC2 is also referred to as intestinal type mucin and regarded as a marker of goblet cells, its consistent expression in the intestinal subtype of IPMN can be taken as further evidence of intestinal differentiation. In other words, MUC expression patterns not only support the dichotomy between PanINs and IPMNs but also confirm the existence of morphological subtypes in IPMNs.

Intraductal oncocytic papillary neoplasia (IOPN)

- MUC1: expressed (data limited)
- MUC2: expressed (data limited)

Oncocytic papillary tumours are characterised by mucin filled cysts containing nodular papillary projections. Dilated ducts communicating with the main tumour can also be seen. Microscopically, the cystic structures correspond to dilated ducts containing intraductal tumour. The tumours are characterised by variably complex, arborising papillary structures. The papillae have thin, delicate fibrovascular cores with focal myxoid changes, and are lined by stratified oncocytic cells. Goblet cells and intraepithelial mucin containing lumina are also present, with these last structures resulting in a characteristic cribriform pattern. The extent of epithelial proliferation varies from case to case and between different regions within individual tumours; solid sheets of cells can often be identified.\textsuperscript{102} Intraductal oncocytic papillary neoplasms have been studied for their mucin expression patterns and they were found to express both MUC1 and MUC2 by Luttges et al.\textsuperscript{99} However, Terris et al reported that oncocytic papillary neoplasms lacked MUC2 expression.\textsuperscript{103} Because they are rarely encountered, there is still a need for further work on these tumours.

Mucinous cystic neoplasms

- MUC1: rarely expressed
- MUC2: present only in goblet cells; no diffuse labelling

Mucinous cystic neoplasms are seen typically in perimenopausal women (> 95% women; mean age, 50 years) and present as multilocular, thick walled cysts in the tail of the pancreas. Ovarian-like stroma is invariably present.\textsuperscript{104–107} Luttges et al have studied the mucin expression profiles of mucinous cystic neoplasms comprising adenomas, borderline lesions, and carcinomas. They found that MUC1 is not expressed in non-invasive lesions of mucinous cystic neoplasms, but is a marker of invasion.\textsuperscript{108} MUC2 expression was seen in the interspersed goblet cells. There was no diffuse staining of the lining epithelium. They found that mucinous cystic neoplasms invariably expressed MUC5AC, which is also commonly expressed in ordinary ductal carcinoma.

MUC1 AND MUC2 IN INVASIVE CARCINOMAS OF THE PANCREAS

Conventional ductal adenocarcinomas

- MUC1: uniform and strong expression; apical membranous in tubule forming areas; intracytoplasmic in the poorly differentiated areas
- MUC2: virtually absent

MUC1 is expressed in almost all examples of ordinary ductal (pancreatobiliary) adenocarcinomas of the pancreas, which are characterised by infiltrating small tubular units embedded in desmoplastic stroma. Expression is predominantly confined to the luminal membrane in the duct forming areas, whereas it is also intracytoplasmic in the poorly differentiated areas (fig 1E).\textsuperscript{112, 115–118} MUC2 expression has been very rarely reported in ordinary ductal carcinomas of the pancreas.\textsuperscript{119}

Colloid carcinomas

- MUC1: rarely expressed
- MUC2: very frequently expressed

Colloid carcinoma of the pancreas was recently described as a distinct entity and was distinguished from signet ring cell carcinomas, ordinary ductal carcinomas, and mucinous cystadenocarcinomas.\textsuperscript{120–122} Like their counterparts in the breast, colloid carcinomas of the pancreas have a much better prognosis than regular adenocarcinomas, with a five year survival of 55% compared with 10% for ordinary ductal carcinomas.\textsuperscript{123} MUC2 expression is a hallmark of colloid carcinomas of the pancreas (fig 1F). We recently examined the ultrastructural properties of colloid carcinomas and showed that mucin secretion was towards the stroma.
Take home messages

- MUC1 versus MUC2 staining correlates with the morphological subtypes of intraductal papillary mucinous neoplasia (IPMN) and can predict prognosis because MUC1 expression is more often associated with pancreatobiliary-type IPMN and ductal adenocarcinoma
- If a papillary lesion has diffuse MUC2 positivity (not limited to goblet cells) it is probably an intestinal subtype of IPMN, whereas if an invasive carcinoma is positive for MUC2 staining, it is very likely to have at least a colloid carcinoma component
- MUC1 is a marker of an aggressive phenotype in pancreatic intraepithelial neoplasia (PanIN); it is expressed only in PanIN grade 3 lesions and is a harbinger of invasive carcinoma
- In ampullary carcinomas, MUC1 versus MUC2 staining can help to determine the origin of the lesion: diffuse intracytoplasmic staining for MUC1 is indicative of pancreatobiliary-type carcinoma, whereas MUC2 staining favours the intestinal type

MUC1 AND MUC2 IN AMPULLARY NEOPLASIA

- MUC1: frequently expressed in pancreatobiliary type, rarely expressed in intestinal type
- MUC2: commonly expressed in intestinal type (low sensitivity), rarely expressed in pancreatobiliary type

Ampullary carcinomas can arise from the intestinal or the pancreatobiliary epithelium. Determining the origin of adenocarcinomas arising in the papillary region is important because intestinal-type carcinomas behave like colonic adenocarcinomas, whereas pancreatobiliary types behave more like pancreatic adenocarcinomas. MUC1 and MUC2 immunostaining can be helpful in determining the origin of the carcinoma, particularly when tumour histomorphology is not discriminatory, because cases with mixed patterns are frequently encountered. In an unpublished study, we have shown that MUC1 is frequently expressed in the pancreatobiliary type, whereas MUC2 is absent. In contrast, the intestinal type lacks MUC1 expression, whereas almost half of these tumours express MUC2. In other words, our preliminary unpublished data suggest that MUC1 and MUC2 are very specific in differentiating intestinal from pancreatobiliary types, whereas the sensitivity of MUC2 expression is low. In our experience, patients with ampullary carcinomas that are MUC1+/MUC2− have a shorter survival than patients whose tumours are MUC1−/MUC2−.

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REFERENCES

23 Wason C, Van De Bovenkamp JH, Korteland-Van Male AM, et al. Barrett’s esophagus is characterized by expression of gastric-type mucins (MUC5AC, MUC6) and TFF peptides (TFF1 and TFF2), but the risk of carcinoma development may be indicated by the intestinal-type mucin, MUC2. Hum Pathol 2002;33:660–8.
MUC1/2 in pancreatic neoplasia


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Cancer stem cell theory: pathologists’ considerations and ruminations about wasting time and wrong evaluations

The genomic revolution has changed the role of the pathologist. In daily practice, our work is no longer limited to reaching a correct diagnosis and we are asked to answer questions about the patient’s prognosis and treatment options through the evaluation of selected molecular targets (such as erbB2 for breast cancer) in tumour specimens. Thus, we have acquired a major role in the translation of novel gene findings from experimental model systems to their clinical application.

There is overwhelming evidence that only a subset of cells within a tumour clone, referred to as cancer stem cells, are tumorigenic and possess the metastatic phenotype.¹ The recent identification of human breast cancer initiating cells by Al-Hajj and colleagues² provided a major step forward in this field. With this knowledge, the stem cell compartment should represent the selected target for tumour eradication.

As pathologists we would like to share some considerations and ruminations about this scenario.

Currently, tissue microarray analysis generates gene profiles capable of differentiating tumours with different biological behaviours.³ However, this screening method is conducted on heterogeneous tumour tissue samples containing a mixture of non-neoplastic cells, non-tumorigenic cancer cells, and cancer stem cells. Similarly, until now, we have evaluated the immunohistochemical expression of a molecular marker in the bulk of the tumour, considering it as relatively homogeneous.

What is the clinical relevance of these results? Although new therapeutic approaches based on these studies have modified the prognosis of some neoplasms,³ conflicting results are still seen with many other tumours.⁴ We should start to feel worried about the value of the information retrieved from this type of tumour analysis.

The few cancer stem cells and the large number of cells constituting the tumour are morphologically similar but functionally heterogeneous. It is likely that we are still evaluating the main population of tumour cells, which are not cancer stem cells, and are thus probably wasting time and loosing essential treatment information. It is unlikely that gene expression profiles obtained using the currently available methods reflect those of the tumour stem cell population, which forms only 0.1–2% of the whole tissue sample.³ ⁴

The cancer stem cell hypothesis has started a new era in cancer research. Tumours contain functionally different subpopulations of cells. However, unique gene expression profiles are generated by current methods of evaluation. Probably, when the isolation and molecular characterisation of cancer stem cells from primary tissue becomes possible, the role of pathologists will change again. Collaboration between researchers and pathologists will be more widely practised and we will be able to rise to the next challenge: namely, assessing the prognosis of a patient from only one of 5000 tumour cells in a tissue sample.

Public opinion on the use of tissue samples

I read with interest and increasing concern the paper by Goodson and Vernon,⁵ “A study of public opinion on the use of tissue samples from living subjects for clinical research”. The paper demonstrates that the use of a vaguely worded and ambiguous questionnaire leads to misleading results. A few of the problems with the questions may be taken individually:

(1) “Would you be happy for pieces of any of the following body tissues or organs to be used in clinical research? (Eyes, lung, heart, tissue from head and neck, embryo, brain, ovary, testes, bone, and breast).”

The question could refer to postmortem tissue and the choice of organs is (as the authors confess) deliberately “emotional”, without any insight into every day pathological services. Heart, brain, and eyes are not exactly common surgical specimens, whereas embryos are subject to special guidelines. Surely, this question is almost designed to make the patient believe it refers to postmortem organs? The use of subjective words such as “happy” is extremely unhelpful. “Are you happy to vote Labour?” would not, for example, be acceptable in a comparable political survey?

(2) “What kind of research would you be happy for your tissues to be used for? (Cancer research, testing medicines, genetic cloning, general knowledge of body tissues, genetic research for diagnosis or treatment of, for example, Down’s syndrome.)”

Again scientific imprecision exists, because the writers of the questionnaire appear not to understand that these fields are interdependent. In particular, the lack of public understanding of cloning has caused them to reject this field, with no idea that this may include tissue culture or polymerase chain reaction.

(3) “Would you want to be informed if your tissues were to be stored beyond the time required for diagnosis?”

This question seems to show no knowledge of the necessity for longterm storage of samples after diagnosis. Tissue retention for medicolegal, audit, clinical governance, and comparison with later samples has been ignored. No explanation has been given to the patients of why this is in their best interests.

(4) “Would you be happy to give consent for a child’s tissues to be used for scientific research?”

Apart from the obvious flaw that it has not been stated whose child is being talked about, again the question appears almost deliberately ambiguous and could be taken to refer to postmortem tissue. Apparently, the designers of the questionnaire are interested in “scientific research” on children’s tissues, whereas in adults in question 1 it is only “clinical research”.

(5) “Would you be happy to give consent for your tissues to be used to teach medical students?”

The word happy is used again, in addition to a lack of explanation of how the tissues are “used”, and the vital role of histology in teaching medical students and pathology trainees. I suggest to the authors that their survey, in contrast to all other studies, shows that patients were unwilling to donate their tissues because they were presented with a poorly designed, misleading survey.

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References


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Limitations of the Wilcoxon matched pairs signed ranks test for comparison studies

We read the short report by Ellis et al with interest. However, we are unsure whether they have adequately proved that no significant difference was detected between the two outlined storage methods.

The hypothesis evaluated with the Wilcoxon matched pairs signed ranks test is whether or not the median of the difference scores equals zero. Let us consider the situation of x measurements tending to exceed y measurements in the low range, and vice versa in the high range, with similar values in the mid range. Such results may have a median of the difference scores of approximately zero; that is, there might be no significant differences by the Wilcoxon matched pairs signed ranks test, although there would be differences by linear regression (Deming or Passing-Bablok) and/or difference plots.

However, this short report lacks both a regression equation (proportional and constant error) and difference plots. Therefore, we believe that although the IgG anti-rubella activity in frozen serum stored in primary gel separation tubes may not be significantly different from that stored frozen in secondary tubes, this study did not sufficiently prove this. We recommend, in line with others, that differential evaluation be conducted for such comparative studies and that such studies are put into a clinical context. 1,4

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Basic Pathology: An Introduction to the Mechanisms of Disease


With the new “integrated” undergraduate medical curriculum being adopted by medical schools in many countries, there is an ever increasing need for an appropriate basic pathology textbook. The authors have produced a book which is based on the principles and objectives of the integrated curriculum. Consequently, it is an ideal basic pathology textbook for students in the integrated medical curriculum.

The book has a novel approach to basic pathology, which is different from the standard basic pathology textbooks. There are four parts: “Introduction—what is a disease?”, “Defence against disease”, “Circulatory disorders”, and “Disorders of cell growth”. Each part consists of a variable number of chapters containing several unique learning aids.

The material is presented in a format that is easy to read and can be read at leisure. In accordance with the integrated curriculum, some material is presented by using clinical cases—for example, myocardial infarction, breast lump, and prostatic hyperplasia, among others. Innovative additions are the excellent cartoons, selected “key facts,” “dictionary box,” and “small print”. The cartoons are well illustrated, extremely apt, and informative. There is also a selection of relevant tables that complement the text. The inclusion of appropriate colour diagrams, photomicrographs, and macroscopic pathology images aids the text. Clinopathological case studies are used as a tool to facilitate the integration of pathology with clinical medicine. At the end of each part, there is a selection of questions covering core material with answers and cross references.

There are six colour coded theme maps that cover the four main pathology disciplines—histology, histopathology, haematology, and microbiology—and two additional overview themes—science and disease and patient and disease.

The authors have produced a remarkable book, which deals with a difficult but important subject in a user friendly manner. The book ought to be prescribed reading for undergraduate students in the new integrated medical curriculum.

The Cytology of Soft Tissue Tumours


Akerman’s and Domanski’s text The Cytology of Soft Tissue Tumours from the Monographs in Clinical Cytology series is a beautifully illustrated, well referenced and written treatise on the interpretation of fine needle aspirations (FNAs) of these lesions. The text starts with a brief overview of the FNA of soft tissue tumours including accuracy, pitfalls, complications, and a discussion of the aspiration technique itself, with application of ancillary studies. This is followed by a concise review of the specific entities following standard histogenetic organisation. With each major entity, the salient cytological features and differential diagnostic considerations are clearly listed, with comments on the potential pitfalls admixed with helpful hints, providing a practical approach to the diagnosis of the lesions. The final chapter summarises in tabular form the salient diagnostic features and results of ancillary studies of the various entities in groupings based on a pattern recognition approach. Illustrations abound and include air dried May-Grünwald-Giemsa, in addition to alcohol fixed haematoxylin and eosin or occasionally cytological preparations. Little criticism of this text can be found and there is no question that this book should be in the library of those interpreting FNAs of soft tissue lesions.
CALENDAR OF EVENTS

Full details of events to be included should be sent to Maggie Butler, Technical Editor JCP, The Cedars, 36 Queen Street, Castle Hedingham, Essex CO9 3HA, UK. email: maggie.butler2@btopenworld.com

Practical Pulmonary Pathology
27–30 July, 2004, Brompton Hospital, London, UK
Further details: Professor B Corrin, Brompton Hospital, London SW3 6NP, UK. (Tel: +44 (0)20 7351 8420; Fax: +44 (0)20 7351 8293; Email: b.corrin@ic.ac.uk)

ACP Management Course for Pathologists, 2004
8–10 September 2004, Hardwick Hall Hotel, Sedgefield, County Durham, UK
Further details: V Wood, ACP Central Office, 189 Dyke Road, Hove, East Sussex BN3 1TL, UK. (Tel: +44 (0) 1273 775700; Fax: +44 (0) 1273 773303; Email: Jacqui@pathologists.org.uk)

Combined Adult and Congenital Cardiovascular Pathology Course
8–10 November 2004, Imperial School of Medicine, National Heart and Lung Institute, London, UK
Further details: Short Course Office, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK. (Tel: +44 (0)20 7351 8172; Fax: +44 (0)20 7351 8246; Email: shourtcourse.NHLI@IC.AC.UK)

Asian Pacific Association for Study of the Liver Biennial Conference
11–15 December 2004, New Delhi, India
Further details: Dr V Malhotra (General Secretary) or Dr P Sahuja (Treasurer and Pathology Coordinator), Room 325, Academic Block, Department of Pathology, GB Pant Hospital, New Delhi 110002, India. (Tel: +91 11 23237455; Email: welcome@apaslindia2004.com; Website: www.apaslindia2004.com)

CORRECTIONS

