Histograph

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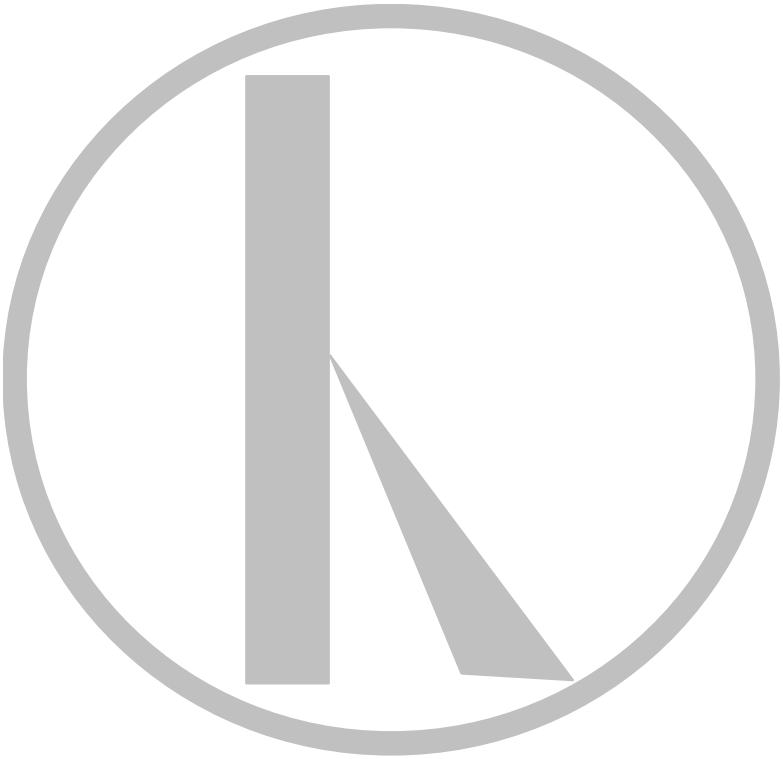

Contents

Editorial

What a great meeting in Mudgee: an extensive trade display and scrumptious food. Copies of the abstracts are included in this issue. You will also see several candid shots taken throughout the weekend.

The final part of Immunohistochemistry of Mesothelioma by Chow Heok P'Ng is also included.

The latest information on the National Histotechnology Meeting to be held on the Gold Coast in October and the New Zealand Meeting can also be found.

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A Review of the Immunohistochemistry of Mesothelioma- Part 2

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Registrar in Histopathology
Institute of Clinical Pathology and Medical Research, Westmead and
The Children's Hospital at Westmead.

Editor's note: This is part 2 of the review on the use of immunohistochemistry in the diagnosis of mesothelioma. Part 1 dealt with the positive markers for mesothelioma and the concluding part deals with the negative markers, those usually used to exclude the diagnosis of mesothelioma.

Negative markers for mesothelioma (markers usually expressed in adenocarcinomas)

Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA) is a complex glycoprotein commonly expressed in a wide variety of adenocarcinomas, especially those originating in the lung, gastrointestinal tract, pancreas, and breast, but not in mesotheliomas. According to most recent publications, monoclonal antibodies to CEA were expressed in 80% to 90% of adenocarcinomas of the lung, whereas mesotheliomas were almost invariably negative for this marker (5,6,7,13,28). In one of the largest series, Riera et al (13) reported immunoreactivity for CEA was observed in 82.9% (175 of 211) of the adenocarcinomas and in none (0 of 57) of the mesotheliomas. Among the positive cases, the majority of tumour cells showed intense cytoplasmic staining with apical enhancement. Its high sensitivity and specificity renders it one of the best "negative'' markers for discriminating epithelioid mesotheliomas from lung adenocarcinomas.

MOC-31

MOC 31 is a monoclonal antibody that recognizes a 38-kd epithelial associated glycoprotein of unknown function, frequently expressed in a wide variety of adenocarcinomas, including those originating in the lung (5). In 2000, Oates and Edwards (14) found MOC-31 to be positive in 5% of mesotheliomas (1/42) and in 90% of adenocarcinomas. Ordonez (5) also recorded similar results with strong MOC-31 reactivity in 85 of 95 (89%) adenocarcinomas, and weak reactivity (<10% of the neoplastic cells) in only 2 of 38 (5%) epithelioid mesotheliomas. In most adenocarcinomas, the staining was strong and diffuse (>50%) and occurred in the cytoplasm and along the cell membrane, while in mesothelioma cases, the staining was focal (<10%) or limited to a few scattered cells (5). Because of its high sensitivity and specificity for carcinomas, MOC-31 is one of the best " negative'' markers currently available for distinguishing between epithelioid mesotheliomas and carcinomas metastatic to the serosal membranes (29).

Leu-Mi (CD15)

Leu-M1 monoclonal antibody recognizes a specific sugar sequence (also known as X-hapten or Le), that occurs in the glycolipid lacto-N-fucopentaose 111 ceramide. LeuM1 antibody is expressed by Reed-Sternberg cells, by approximately one half of all carcinomas, and by cells in various myeloproliferative disorders (28). Some disagreement exists among investigators regarding the sensitivity and specificity of this marker in discriminating these malignancies. The reported percentages of Leu-M1 positivity have ranged from 40% to 100% in adenocarcinomas (5,6,11,13), while most studies have found mesotheliomas to be either consistently negative (5,6,11,20) or rarely positive (13). In the largest and one of the most recent studies, Riera et al (13) reported focal Leu-M1 positivity in 2 of 57 (4%) epithelioid mesotheliomas, and in 159 of 211 (75.3%) adenocarcinomas. Most positive cases exhibited a granular cytoplasmic staining pattern, with occasional membrane accentuation. Although Leu-M1 is highly specific for distinguishing between epithelial mesotheliomas and adenocarcinomas, its sensitivity is rather low when compared to other "negative'' mesothelioma markers, such as MOC-31, BG-8, or CEA (5,28).

B72.3

The B72.3 monoclonal antibody recognizes a tumour-associated glycoprotein (TAG-72) present in a wide variety of adenocarcinomas, including those originating in the lung, gastrointestinal tract, pancreas, breast and ovary (28). Irrespective of their sites of origin, the majority of adenocarcinomas show reactivity with B72.3 ranging from 80%-87% (6,13,28). The pattern of staining is coarse and granular and occurred throughout the cytoplasm. Only very rare examples of mesotheliomas (approximately 1% to 5%) show focal labelling with this reagent (9,13,28). One of the largest comparative studies with this antibody was by Riera et al (13), who reported B72.3 reactivity in 2 of 57 (3.5%) epithelioid malignant mesotheliomas and in 170 of 211 (80.5%) adenocarcinomas of various types. Because of its high specificity and sensitivity, B72.3 remains one of the best "negative'' markers for mesotheliomas in distinguishing between epithelioid malignant mesotheliomas and adenocarcinomas (5).

Ber-EP4

The Ber-EP4 monoclonal antibody recognizes an epitope on 2 non-covalently bound glycoproteins present in most epithelial cells, but absent in mesothelial cells (5). In adenocarcinomas, the reaction is usually strong and diffuse, and occurs in both the cytoplasm and cell membrane (6). In contrast the reaction seen in mesotheliomas show focal membranous staining usually restricted to a limited number of cells (28). A wide range of Ber-EP4 positivity has been reported for adenocarcinomas, ranging from as low as 32% in some series up to 100% in others (5,6,7,11,13,28). A wide range of Ber-EP4 positivity has also been reported for mesotheliomas, ranging from 0 to 26% (5,6,7,9,11,13,28). The discrepancy may be related to the pattern of staining. For example, Riera et al (13) considered only lateral membrane staining to be positive, whereas other investigators regarded any membrane staining as

positive. When only staining involving the lateral membranes of the tumour cells was regarded as truly positive, none of the mesotheliomas in Riera et al (13) series stained with this antibody. Because of the current availability of other "negative'' markers with a higher specificity for mesotheliomas, Ber-EP4 is usually not included in the routine panel of immunohistochemical markers commonly used.

BG-8

BG-8 is a monoclonal antibody that recognizes the blood group antigen Lewis. In a recent study (6), 48 of 50 (96%) lung adenocarcinomas stained with the BG-8 antibody. In most cases, the reactivity was strong and diffuse, and occurred in the cytoplasm and along the cell membrane. Only four (7%) of the mesotheliomas exhibited BG-8 positivity. In all four cases, the staining was focal or limited to a few cells. Riera et al (13) demonstrated similar results with 187 of 211 (88.6%) adenocarcinomas being BG-8 positive, whereas only 5 of 57 (8.7%) mesotheliomas stained with BG-8. Hence, it is a selective antibody for adenocarcinomas with high sensitivity and specificity.

Cadherins

Cadherins constitute a group of adhesion proteins that play an important role in the sorting of cells into specialized tissues during morphogenesis. The cadherin family includes several distinctive members including E (epithelial)-cadherin, N (nerve)-cadherin, P (placental)-cadherin, R (retina)-cadherin and OB (osteoblast)-cadherin (29). In a recent study (6), 44 of 60 (73%) mesotheliomas exhibited N-cadherin positivity along the cell membrane. Fifteen of 50 (30%) lung adenocarcinomas were also N-cadherin positive. Using anti-E-cadherin antibody, 88% of the adenocarcinomas and 40% of the mesotheliomas expressed this marker. These findings indicate that neither N-Cadherin, nor E-Cadherin is absolutely specific for separating epithelioid mesothelioma from pulmonary adenocarcinoma (6).

Other markers

CK7/CK20

Adding CK20 and CK7 to the panel of antibodies in the differential diagnosis of pleural mesotheliomas versus metastatic adenocarcinomas is useful, because diffuse CK20 positivity seems to be an indicator of metastasis. Furthermore, CK7 negativity most often is associated with metastasis, and the CK20+/7- pattern, typical of colorectal adenocarcinomas, is absent in pleural mesotheliomas. A study by Tot (26) showed 12 of 14 (86%) mesotheliomas to be CK7+. None of the mesotheliomas expressed the CK20/7- pattern. Mesotheliomas may show focal CK20 positivity, but strong diffuse staining favours the diagnosis of metastatic adenocarcinoma.

Epithelial membrane antigen (EMA)

EMA is derived as an antibody to a human milk fat globule membrane immunogen. As observed in most studies, mesothelioma cells displayed circumferential "thick" cell membrane staining for EMA, whereas benign mesothelium exhibited none or focal reactivity limited to one surface of the cells (10, 11, 24). Adenocarcinomas have been reported to show diffuse cytoplasmic immunopositivity (6).

However, overlapping staining patterns may limit the value of EMA immunostaining in distinguishing malignant mesotheliomas from reactive mesothelial proliferations and adenocarcinomas (24).

Thyroid transcription factor-1 (TTF-1)

TTF-1 is a tissue-specific transcription factor that is expressed in the normal lung and thyroid as well as in the tumours derived from these organs. In the lung, TTF-1 is present in type 11 cells and Clara cells, where it acts as a promoter factor for the transcription of surfactant proteins (A, B, and C), and Clara cell secretory protein (5). Investigation by Ordonez (6) reported 37 of 50 (74%) adenocarcinomas exhibited nuclear staining for TTF-1. None of the mesotheliomas were positive for this marker. Because of its high specificity for lung carcinomas and not in mesotheliomas, this marker could assist in the evaluation of pleural-based epithelial malignancies.

Vimentin

Vimentin represents the intermediate filament that is usually present in cells of mesenchymal derivation, and as such, could be a useful marker for distinguishing mesothelial cells from carcinomas. The largest and one of the recent studies by Riera et al (13), reported vimentin expression in 46 of 57 (81%) epithelioid mesotheliomas and in 66 of 211 (31%) adenocarcinomas. The series by Ordonez (6), reported that 33 of 60 (55%) mesotheliomas and 19 of 50 (38%) lung adenocarcinomas expressed vimentin. Therefore, vimentin does not seem to be substantially more specific and sensitive for mesothelioma than for adenocarcinoma, and thus has no utility in discriminating between these tumours.

CA19-9

CA 19-9 is a sialynated lacto-N-fucopentaose 11 related to the Lewis blood group that is commonly expressed in adenocarcinomas of the ovary, pancreas, and gastrointestinal tract (5). A series by Ordonez (6) reported that 24 of 50 (48%) lung adenocarcinomas were positive for CA19-9. No reactivity was seen in any of the mesotheliomas. Even though CA19-9 is highly specific for the diagnosis of epithelioid mesotheliomas, its sensitivity for distinguishing these tumours from lung adenocarcinoma is relatively low, thus limiting its diagnostic utility between the two malignancies. Another series (31) by the same author found that CA19-9 reactivity was observed in 31 of 45 (69%) serous carcinomas. None of the mesotheliomas showed CA19-9 expression. It should be emphasized however, that because CA19-9 is often expressed in serous carcinomas of the ovary and peritoneum, positivity for this marker can be useful in distinguishing these tumours from epithelioid peritoneal mesotheliomas, which similar to pleural mesotheliomas, are CA19-9 negative.

CD44S (CD44H)

CD44S (also known as CD44H) belongs to a family of glycoproteins associated with cell-cell adhesion, cell-matrix interactions, lymphocyte homing and activation, and metastasis formation (15). A current study (6) reported CD44S staining in 44 of 60 (73%) mesotheliomas, and 24 of 50 (48%) lung adenocarcinomas exhibited

positivity. Attanoos et al (15) reported an overall sensitivity for CD44S to be 47%. In the majority, the staining was strong and diffuse and occurred along the cell membrane. The results showed that CD44S has a low sensitivity and specificity for mesothelioma, and has no practical use in the diagnosis of this malignancy.

Human milk fat globule protein-2 (HMFG-2)

This marker is derived from milk fat globule membranes, and recognizes antigens in normal and neoplastic breast cells, as well as a variety of other adenocarcinomas. Riera et al (13) noted diffuse moderate intense cytoplasmic staining in 180 of 211 (85.3%) adenocarcinomas, and 16 of 57 (28%) mesotheliomas stained with HMFG-2. Most adenocarcinomas showed, in addition to the cytoplasmic staining, a membrane pattern of reactivity. Mesotheliomas on the other hand, showed membrane staining in 57.8% of the cases, often without or with minimal cytoplasmic staining.

Muscle markers

Afify et al (16) evaluated the diagnostic use of muscle markers in separating reactive mesothelial cells from malignant mesotheliomas and adenocarcinomas. Strong cytoplasmic reactivity for desmin was noted in 22 of 24 (92%) cases of reactive mesothelial cells. The reactive mesothelial cells did not express actin, myoglobin or myogenin. All cases of malignant mesothelioma and metastatic adenocarcinoma were negative for the four muscle markers. The mesothelial lining and scattered subserosal cells in the omental sections were positive for desmin. Because desmin is expressed only in benign mesothelial cells, it may serve as a reliable marker in distinguishing reactive mesothelial cells from mesotheliomas and adenocarcinomas.

Conclusion

Immunohistochemistry is generally considered the most useful ancillary techniques for the diagnosis of malignant mesothelioma if optimal fresh tissue required in electron microscopy is not available. To date, there is no single immunohistochemical marker that is entirely specific and sensitive for distinguishing mesothelioma, adenocarcinoma, sarcoma and reactive mesothelial hyperplasia. Because of their specificity and sensitivity for mesotheliomas, the best "positive'' markers for mesothelioma appear to be cytokeratin 5/6 and calretinin (or WT1). Among the antibodies which are considered to be "negative'' markers for mesothelioma, CEA, B72.3, MOC-31 seem to be best diagnostic discriminators. Although BG-8 has been shown to be useful in the diagnosis of mesothelioma, it is not usually available in most laboratories. Other markers, such as Leu-M1, Ber-EP4, and thrombomodulin can be used as secondary markers if the results obtained with previously mentioned markers are equivocal. Unfortunately, given the current range of available markers and their undoubted limitations, it is likely that the search will continue for the ultimate antibody specific for malignant pleural mesothelioma.

References can be obtained from the editor (anthonyh@chw.edu.au)

Advanced Notice – Kiwi Gold

New Zealand's Histology Group (HSIG) will be holding their annual meeting in Taupo, New Zealand. It will cover two days with a workshop(s) on the Friday followed by the actual meeting on the Saturday, 3rd and 4th of November, 2006. I was able to attend last years meeting and it was a hoot!

The Convenor would like to let everyone know that there will be a poster session and would also like to put out a call for presenters with an emphasis on the technical although anything Histology related would be welcomed. Please contact Joe direct with your interest of presenting or producing a poster on (09) 307 4949 ext 6142 or email joem@adhb.govt.nz.

Details as they become available will be posted on the NZIMLS website: http://www.nzimls.org.nz/

Some info on Taupo can be found here http://www.laketauponz.com/

For further details contact Steven Cooke [SCooke@adhb.govt.nz].

Abstracts from our Mudgee State Meeting

FISH, CISH and HER-2

LEAHY, B, MOREY, A.L., Anatomical Pathology, St. Vincent's Hospital, Sydney

Belinda Leahy completed her Bachelor of Science at Macquarie University in 1999, while training as an Andrologist at Sydney IVF. She then trained as a Cytogeneticist in London and in April 2002 began work in the departments of Anatomical Pathology and Cytogenetics at St Vincents Hospital, Sydney. She is Scientist-in-charge the HER-2 FISH Referral Service and also performs paraffin FISH on a range of other malignancies.

The HER-2 oncogene is a transmembrane tyrosine kinase receptor, coded on chr 17q and over-expressed (secondary to gene amplification) in around 15% of breast carcinomas. An accurate means of identifying HER-2 over-expressing tumours has become vitally important with the advent of Herceptin® (Roche), a humanized monoclonal antibody therapy specifically targeted at HER-2. Government funding for Herceptin treatment is currently available to Australian metastatic breast cancer patients with positive (3+) HER-2 immunohistochemistry (IHC), or equivocal IHC with HER-2 gene amplification confirmed by fluorescent in situ hybridization (FISH). IHC is still advocated as the appropriate "screening" test for HER-2 overexpression, however there are considerable issues with uniformity and reliability of testing. Acting as an Australian reference laboratory, we have performed dual probe FISH (PathVysion, Vysis) on paraffin embedded sections from over 3000 IHC-equivocal cases. In an analysis of 2000 recent cases, our repeat rate was 0.35% and our non diagnostic rate 0.6% (primarily decalcified samples). Only 21.4% of assessable IHC 2+ cases were amplified, in line with international data. Between the 74 referring labs, the percentage of 2+ cases found to be amplified by FISH varied significantly from 0-100% of cases. Almost all amplified cases are high grade ductal carcinomas. FISH is an expensive and labour-intensive diagnostic test, which is not suitable for application in all routine laboratories, however there is increasing demand for the accurate demonstration of molecular targets in a morphological context, and the ongoing development of in situ hybridization (ISH) assays represents an exciting interface between molecular biology and histopathology. Chromogenic ISH assays are under investigation as a possible alternative to FISH, but are also unlikely to be widely adopted unless automation is possible.

Flow Cytometry in the Clinical Laboratory

Mary Sartor

Flow cytometry is a technology that was first developed 30 years ago to provide a quantitative evaluation of cellular properties based on their light scatter properties and characteristics that could be determined using fluorescent probes. The first flow cytometers were developed for use in research. They were very expensive, quite large and required a dedicated room and an operator. Immunophenotyping is the identification of cells using fluorchrome-conjugated antibodies as probes to proteins expressed by cells. The flow cytometer was introduced into the clinical laboratory in the early 1980's when monoclonals became available and instruments were developed that were smaller, less expensive and user friendlier. In the last 20 years, there has been a virtual explosion in immunophenotying applications using flow cytometry in the clinical laboratory. This talk will highlight some of the uses of flow cytometry in the clinical laboratory

HAEMATOXYLIN – a dye to die for

Dr Phil Baird

Haematoxylin from the Greek haemato = blood; xylon = wood, is a dye, $C_{16}H_{14}O_6$, that is extracted from a tree in Central America called Haematoxylon campechianum,(Legumino sae), named after a town on the Yucatan peninsula of Mexico.
The Spanish carried Logwood, as it was known, back to Spain in the 16th century, as a dye for cloth, especially the purples and reds for royalty and the clergy.

England and Spain fought many naval battles over Logwood in the 16th and 17th centuries, sinking many ships in the waters near Belize and Dominican Republic
After Logwood was imported into England in vast quantities, eg 69, 290 tons in 1880, the guilds had the parliament pass a law prohibiting its use, (1581 to 1662).

In 1863 the dye was used for staining cells. The use of mordants (metal salts) by the dyers had shown how versatile this dye was. Unfortunately the molecule is very susceptible to oxidation by air and water, and so looses its potency.

It has been used for the detection of heavy metals such as lead. Various types of Haematoxylin stains are based on the use of various metals as mordants.

In 1912, Haiti exported 83,000,000 pounds of wood to the USA.
A synthetic haematoxylin is used in Cytyc's image analysis system, 2005.
Phil had plant material and raw extract from the heartwood of the tree available at the meeting.

Forensic Biology – CSI NSW

Sienna MARGAN & Lisa WEDERVANG

In recent years, DNA analysis has become an important instrument in criminal investigations and in the judicial process. Advances in technology mean today that stains do not have to be visible to recover a genetic profile. Invisible DNA, known as "trace" DNA, may be a valuable source of investigative information. Although a powerful tool, there are some limitations inherent in the DNA analysis. Despite the depiction of forensic biology on the current trend of TV crime fighting shows, cases cannot be solved and criminals arrested in a one-hour episode! An overview of the DNA process will be described and a case study presented in order to put this process into context.

Body Identification in Kosovo: the Investigation of Mass Disasters

Dianne Little

In the recent past, Forensic Pathologists from Australia have been called upon to help in the investigation of a number of mass disasters, both within Australia and overseas.

Disaster Victim Identification (DVI) procedures are performed under guidelines set out in the Interpol Protocol. There are 5 phases to the investigation:

1. Scene
2. Post mortem
3. Ante mortem
4. Reconciliation
5. Debrief

In a large disaster, all of these may proceed simultaneously.

The Forensic Pathologist may be involved in all or only a few of these phases depending on the scope of the disaster.

Typically, the Forensic Pathologist's work includes:
Visiting the site of the disaster to

- Help retrieve the bodies
- Look for evidence
- Obtain a general overview of the situation

Performing autopsies looking for

- Cause of death
- Features to aid in identification of the body
- Evidence relating to the cause of the disaster (eg bomb fragments)

Involvement in the reconciliation process
Involvement in the debrief process

In a large-scale disaster where the cause is clear (eg a natural phenomenon like the Asian tsunami of December 2004), the focus of the investigations is largely on identification of the deceased bodies. This primarily involves identification by fingerprint and dental comparisons and DNA analysis ("primary identifiers"), with contributions from physical description, medical evidence and personal effects.

This presentation outlined the DVI process using examples of large and small scale disasters both in Australia and overseas.

The Role of Electronmicroscopy in the Diagnosis of Renal Disease

Paul D Kirwan, EM Unit, Dept Anatomical Pathology, CRGH Concord NSW 2139

EM is a powerful diagnostic tool in the examination of percutaneous renal biopsies, especially when used in conjunction with immunofluorescence (or IPX) and various routine and special stains for light microscopy.
The usefulness of electronmicroscopy (EM), in the study of pathological specimens, lies in the fact that the electron microscope has an absolute resolution of 0.15 nm, which is approximately one thousand times greater than the light microscope. Hence the exact positions of immune complexes, as well as fine structural changes at the cellular level, within the renal biopsy can be more accurately determined by EM. However it is not possible to determine the type of immunoglobulin present by routine EM methods.

Firstly let me just say a word about fixation for EM. As we are viewing specimens at very high magnification the autolytic changes resulting from delayed fixation are apparent much sooner by EM than by light microscopy so it is imperative that the EM specimen is selected and placed in EM fixative as soon as possible. In liver, for example, the autolytic changes can be seen within minutes of cessation of blood oxygen to the specimen. The most commonly used fixative is 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (ph7.4). This must be cold and kept cold for at least the first hour of fixation but NEVER FROZEN. After that you can relax. Nevertheless if all else fails reprocessed paraffin embedded of frozen IF material can be used in most cases with good results.

As many of you will be involved in processing, cutting and staining renal biopsies for IF, IPX and various histological stains for light microscopy my aim in this talk will be to correlate the EM findings with the light microscopic and IF appearances to help you understand what is happening at the ultrastructural level. I will not attempt to cover the whole field or renal EM but simply present a few appropriate examples. EM is a powerful diagnostic tool in the examination of percutaneous renal biopsies, especially when used in conjunction with immunofluorescence (or IPX) and various routine and special stains for light microscopy.
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Cut-Up for Non-Pathologists

AE Woods PhD, Associate Professor, School of Pharmacy and Medical Sciences
University of South Australia, Adelaide, South Australia, Australia.

Since the National Pathology Accreditation Advisory Council (NPAAC) released guidelines for the Performance of Pathology Surgical Cut-up ('grossing' or 'macroscopic description') in 2001, we have seen a steady increase in non-pathologists taking up this role. More recently NPAAC released an update to the Guidelines for discussion – the final version of this document is not yet available. The original Guidelines recognised three classes of specimens, simple, non-complex and complex allowing non-pathologists access to the first two categories only. The update has relaxed this requirement. In 2003, The University of South Australia developed a Graduate Certificate in Surgical Pathology Preparation that provides a formal qualification for those undertaking cut-up training. The program can be delivered on-line, synchronously and has graduates from NSW, Qld and WA, as well as in SA. The move of scientific and technical staff into this area has lead to changes not only in the performance of cut up also in the perception of non-pathology staff engaging in the diagnostic process. Is this just the beginning of role extension in pathology?

Viruses, diets, hormones and breast cancer

Prof. Jim Lawson

There are 3 striking features of breast cancer. These are

1. Breast cancer is over 100 times more common in women than men,
2. THERE are over 6 fold differences in incidence and mortality between high and low risk populations such as the US and Japan and

These differences rapidly lessen to equalise within 2 generations of migration from low to high-risk countries. Changes in diet and patterns of reproduction after migration partly but not wholly explain these phenomena. Hence the emergence of hormone responsive viruses as prime suspects in breast carcinogenesis.

Viral suspects

Human papilloma viruses (HPVs), mouse mammary tumour virus (MMTV), and Epstein-Barr virus (EBV) and are prime candidate viruses for human breast cancer. HPV and MMTV have hormone responsive DNA elements which appear to be associated with enhanced replication of these viruses in the presence of corticosteroid and other hormones. This biological phenomenon is particularly relevant because of the hormone dependence of breast cancer.

Evidence of viral carcinogenesis in human breast cancer

Viral genetic material for each of these candidate viruses has been identified by polymerase chain reaction (PCR) in breast tumours but rarely in normal breast tissue controls. Pooled data from controlled studies show substantial odds ratios (ORs) for the presence of viral genetic material in breast tumours as compared to normal controls.

The adjusted (for differences in study sizes) OR for the presence of HPV gene sequences in breast cancer as compared with normal controls is 7.18 (CI 1.82-28.47).The adjusted OR for the presence of MMTV gene sequences in breast cancer as compared to normal controls is 27.55 (CI 12.26-61.91).

Histological characteristics of HPV positive human breast tumours are similar to HPV positive human cervical cancer. Histological characteristics of some (47%) MMTV positive human breast tumours are similar to MMTV positive mouse mammary tumours. MMTV sequences have been located in breast cancer epithelial cells.

Normal breast cell cultures are transformed by exposure to HPV. Normal human breast cell cultures may be transformed by exposure to MMTV sequences. In mice models, most MMTV related evidence points to such transformation by insertional mutagenesis with activation of oncogenes. Normal breast epithelial cells are immortalised by EBV.

Conclusion

These and additional data provide substantial, but not conclusive, evidence that HPV, MMTV, and EBV may have a role in the etiology of human breast cancer. If conclusive evidence for a role of these viruses in breast carcinogenesis can be developed, there is a practical possibility of primary prevention.

The Many Faces of Basal Cell Carcinoma

Ruma Dutta

Basal cell carcinomas (BCCs) are the commonest type of skin cancers and their incidence is increasing worldwide. Sun exposure remains the commonest aetiological factor, hence their high incidence in Australia. Correct specimen handling is essential for their accurate diagnosis and for the accurate identification of the different microscopic patterns. There are numerous different histological patterns/subtypes, several of which behave more aggressively, such as the micronodular, infiltrating and morphoeic subtypes. Identification of the different subtypes is therefore clinically important, and not only an academic exercise. Different subtypes are often present in the same lesion, hence the importance of adequate sampling. Treatment of BCCs depends to some extent on the microscopic subtype and includes surgery, cryotherapy, curettage and diathermy/cautery, radiotherapy, cytotoxic agents, and newer drugs such as Aldara. The majority of cases are easily cured, and the mortality rate is low.

Stain those Bugs

Its seems the demonstration of bacteria can be a major BUG-bear in the Histo Lab. Some interesting discussion recently took place on Pedpath, a listserver similar to our Histonet.

What started it:

Just a thought, I have found giemsa to be an excellent stain for viewing the morphology of bacteria. In typical sections, at "our lab" one can also make out about 100 x more bacteria that in "our" tissue gram stained sections.

One Pathologist comments:

In "our" lab (beyond my control) I am stuck with the Brown and Brenn tissue Gram stain (B&B). This stain is OK for Gram positive organisms but is not satisfactory for Gram negative organisms Like Fusobacterium.

Mixed infections are often MISSED because of this. In a effort to convince our lab to switch from the B&B to the Brown and Hopps tissue Gram (B&H) I did an in-house study with the following results.

B&B stained section	*1 X organisms in the slide.*
B&H stained section	*10 X organisms.*
Giemsa stained section	*100 X organisms.*

Also the Brown and Hopps is MUCH better than the Brown and Brenn with Gram negative organisms Like Fusobacterium. Giemsa is excellent for organisms Like Fusobacterium (really striking, almost as good as a Warthin-Starry)

When I want to look for bugs with special stains I have to order 3 B&B stained sections on one block with varying degrees of decolourization combined with a Giemsa stained section (the type used for helicobacter) to get a feel for how many organisms are present and to make such I don't miss some Gram negative organisms.

Bottom-line, I agree 100% the Brown and Hopps Gram stain smokes the Brown and Brenn Gram stain. The Giemsa stain also has important clinical utility under certain circumstances. Every lab is different and once the techs get used to preforming a stain like the B&H they can become very good at it with excellent results.

Another Pathologist comments:

In my experience, gram positives and gram negatives stain the same with giemsa-- dark. But one does see size, shape and structural arrangements; eg, streptococci vs. staphylococci, small rods aggregated suggesting diphtheroids vs. those more consistent with enterobacteriaceae, etc.

AGM Notice

The upcoming AGM (including voting for Office Bearers) will be held on 24 July, 2006 at 7.30 pm at Anatomical Pathology at ICPMR, Westmead.

3rd National Histotechnology Meeting Conference Programme

Holiday Inn, Gold Coast
Queensland

Workshops Friday 11th August

Morning 1000 - 1300

Workshop 1	Dr Eva Wojcik	Molecular Pathology
Workshop 2	Michael Adamson	Digital Imaging

Lunch 1300 - 1400

Afternoon 1400 - 1700

Workshop 3	Liz Baker	An outline of fluorescence in situ hybridisation (FISH) techniques and their applications in the diagnosis and treatment of human diseases.
Workshop 4	Michael Adamson	Digital Imaging(Repeat of W2)

Friday Evening

Welcome Drinks – Trade area 1830 - 2000

Conference Draft Programme

Saturday 12th August		
Session 1	**0900 – 1030**	
	Dr Eva Wojcik	A Review of Adjuvant Tests in Urologic Pathology
	Dominique Davidson	Ploidy Studies
Morning Tea	1030 - 1100	
Session 2	**1100-1230**	
	Suzanne Parry	Tissue Microarrays
	Prof Sunil Lukhani	Breast Research
	Dr Alex Olumbe	Comparison between Forensic Experiences Overseas and in Australia
Lunch	1230 - 1330	
Session 3	**1330-1500**	
	Proffered Papers and Cameo Presentations	
Session 4	**1530- 1700**	
	Liz Baker	How to make your own DNA Probe
	Dr Sue Edwards	Research on Fish- Histology, IHC and ISH
	Dr David Williams	Forensics in the Tropics
Sunday 13th August		
Session 1	**0900 – 1030**	
	Laurie Reilly	Haematoxylin
	Maxine Crook	"Fishing" Paraffin fixed tissues-Obtaining Genetic Information from Pathology Archival Material
	Paul Addison	Histology on Bats including Archival Slides from the 19th Century
	Bharathi Cheerala	Alpha-Methylacyl-Coa Racemase, a Novel Tumour Marker for the Diagnosis of Prostate Carcinoma.
Morning Tea		
Session 2	**1100-1230**	
	Dr Belinda Clarke	Mesothelioma
	Neil O'Callaghan	Histology: An Integral Part of New Molecular Pathology Tests
	Leigh Winsor	Taxanomic Histology
	Judging of Posters.	
Lunch		
Session 3		
	Leanne Giles	Digital Imaging
	Mary Graham and Lynn Tolley	SEM of Hair: Its use in Pathology, Biology and Forensics.
	Georgia Stamaratis	CATCH THE FISH WITH CISH: Using Chromogenic In Situ Hybridization as an alternative to FISH
CLOSE	**1500**	

Registration

The Histotechnology Group of Queensland supported by its major sponsors Dakocytomation and Vision Biosystems invites everybody with an interest in the field of Histotechnology to experience the sun, surf and sand of the Gold Coast; the science of Histotechnology; and the company of like minded people at the Conference Dinner. The dinner will be held at Q1 the Gold Coast's latest attraction. At 80 stories Q1 is the highest residential building in Australia offering views from its observation deck as far as Brisbane to the north and Byron Bay to the south. Experience this and more at the:

3rd National Histotechnology Meeting
Holiday Inn
Gold Coast, Queensland

On the accompanying form please complete your personal details, tick the relevant boxes then return with payment to:

Tony Reilly
The Secretary
Histotechnology Group of Queensland,
Level 2 Clinical Sciences Building
Prince Charles Hospital,
Rode Road
Chermside, QLD4032
Ph: 07 3350 8543
Email: tony_reilly@health.qld.gov.au

Proffered Papers and Posters can be submitted via our website at www.hgq.org.au or by mail to the above address. Closing date is 7th July

Registration for the both Workshops and Conference will close on the 7th July 2006 after which all registrations will incur a $75 late fee.

Note that there will be a limit on numbers for workshops.

Registrant details:

Full Name :_____

Preferred name for ID badge :_____

Organisation :_____

Postal address :_____

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Contact Telephone Number :_____

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Workshop Registration Friday 11th August

All Workshops $75

Morning 1000 - 1300

Workshop 1	Dr Eva Wojcik	*Molecular Pathology*	$_____
Workshop 2	Michael Adamson	*Digital Imaging*	$_____

Afternoon 1400 - 1700

Workshop 3	Liz Baker	*An outline on fluorescence in situ hybridisation (FISH) techniques and their applications in the diagnosis and treatment of human diseases.*	$_____
Workshop 4	Michael Adamson	*Digital Imaging (Repeat of W2)*	$_____

Workshop Sub Total A$_____

Trade Launch in the trade exhibition area commences at 1830.

Conference Registration 12th-13th August

☐ FULL REGISTRATION (Twin Share) $590 $_____

(Includes registration for Saturday and Sunday, accommodation for Friday and Saturday nights, all meals(including breakfast) and 1 entry to the trade launch on Friday night and 1 entry to the Conference Dinner on Saturday night.

I would like to share with:_____

☐ FULL REGISTRATION (Single Room) $640 $_____

(Includes registration for Saturday and Sunday, accommodation for Friday and Saturday nights, all meals(including breakfast) and 1 entry to the trade launch on Friday night and 1 entry to the Conference Dinner on Saturday night.

☐ +Accompanying Person $100 $_____
(Includes accommodation and breakfast only)

☐ FULL REGISTRATION ONLY $250

(Includes registration for Saturday and Sunday, lunches, Morning and afternoon teas and 1 entry to the trade launch on Friday night)

DAY REGISTRATION
(Includes lunches and morning teas and afternoon tea on Saturday)

☐ Saturday $150 $_____
☐ Sunday $125 $_____

☐ Extra Conference Dinner Tickets at Q1 (Saturday Night) $ 95 $_____

Quantity:_____Name(s) on Ticket_____

☐ Extra Trade Launch Ticket (Friday Night) $ 45 $_____

Quantity:_____Name(s) on Ticket_____

EXTRA NIGHTS ACCOMMODATION
(Cost per Room including Breakfast)

☐ Thursday night Single $171 $_____
☐ Sunday Night Twin Share $197 $_____

Conference Subtotal A$_____

Workshop Subtotal A$_____

Total **A$_____**

2005 - 2006

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PLEASE TICK

- ☐ $38.50 for annual subscription of $35.00 and $3.50 GST.
- ☐ $16.50 for student subscription of $15.00 and $1.50 GST
  (Full-time or working toward first qualification)
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