Introduction

With the rapid establishment of immunohistology as an indispensable adjunct to histological diagnosis, an ever-increasing range of antibodies has been developed to complement highly sensitive methods for demonstrating cellular antigens in fixed tissues. Space limitation restricts this coverage to those reagents that are more important for cancer diagnosis and prognostication - the latter often an integral component of diagnosis. For convenience, the antibodies will be discussed in the context of specific neoplasms and markers for diagnosis of metastatic tumours will not be specifically addressed. Details of antibodies discussed are available elsewhere.

B-cell lymphoma

A major development in lymphoma diagnosis relates to the separation of the small cell lymphomas where newer antibodies that are immunoreactive in fixed tissue sections are now available. This group of lymphomas include small lymphocytic lymphoma/well differentiated lymphocytic lymphoma (SLL/WDL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), small cell follicle centre lymphoma (FCCL) and lymphocytic/plasmacytoid lymphoma (LPL). The application of a panel of antibodies shown in table one allows the distinction of the small cell lymphomas, which have different prognoses.

Table 1: Antibody panel for small cell lymphomas

<table>
<thead>
<tr>
<th></th>
<th>CD43</th>
<th>CD5</th>
<th>CD23</th>
<th>Cyclin D1</th>
<th>CD10</th>
<th>CD38</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLL/WDL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCL</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MZL</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LPL</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>FL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

SLL/BCLL = small lymphocytic lymphoma/B-cell lymphocytic leukaemia
MCL = mantle cell lymphoma
MZL = marginal zone lymphoma
LPL = lymphoplasmacytic lymphoma
FL = follicle centre cell lymphoma

The CD5 molecule is a transmembrane glycoprotein that is expressed on T and some B cells. CD5 is a marker of pre-germinal B cells. SLL/WDL (and chronic lymphocytic leukaemia) is the most common CD5+ B cell malignancy and it is thought that the small population of CD5+ B cells found in normal healthy adults and prominent in cord blood is the normal counterpart of this neoplasm. CD5 expression may be lost when large cell lymphoma of Richter’s syndrome supervenes. CD5 expression is also seen in MCL but not in nodal or extranodal MZL, the latter largely identified by the absence of most of the markers in the panel shown in table one. De novo expression of CD5 in diffuse large B cell lymphoma was shown to be an indicator of poor prognosis associated with centroblastic phenotype, interfollicular growth pattern, and intravascular or sinusoidal infiltration.

CD23 is invariably expressed in SLL/WDL and is an important marker for the separation of the small cell lymphomas. Activated B cells within germinal centres strongly express CD23 but mantle cells and MCL are negative. CD23 expression may be seen in a small number of other non-SLL B cell lymphomas and in Reed-Sternberg cells but staining is weak and only in a small proportion of cells.

Cyclin D1 is the most specific marker for MCL whose identification is important for prognostic and therapeutic reasons. The G1 cyclin gene (cyclin D1, PRAD-1, CCND-1), located on chromosome 11q13, exhibits characteristics of cellular oncogenes and plays an integral role in normal cell growth control. Many neoplasms including MCL, parathyroid adenomas, and a spectrum of carcinomas such as breast, supradiaphragmatic squamous cell, ovarian, endometrial and bladder transitional carcinomas, demonstrate over-expression of cyclin D1. The nuclear expression of this protein is found in over 75% of MCL and may be seen in rare cases of plasma cell myelomas, Reed-Sternberg cells and anaplastic lymphoma. It is found in hairy cell leukaemia but this over-expression is not associated with t(11;14) or bcl-1 rearrangement.

The bcl-6 gene product is highly expressed in germinal centre cells and their neoplastic counterparts. Hence, bcl-6 immunoreexpression is found in follicular lymphoma, Burkitt’s lymphoma, some diffuse B cell lymphoma, nodular lymphocyte-predominant Hodgkin’s lymphoma. MZL and MCL are negative.

DBA.44 recognises an unknown fixation-resistant B cell antigen expressed by mantle cells, immunoblasts, monocytoid B cells and a small proportion of low and high-grade lymphomas. It is principally employed for the identification of hairy cell leukaemia and among the node-based lymphomas, the strongest membrane staining is seen in centroblastic, immunoblastic and monocytoid B cell lymphomas.

Immunoglobulin light chain restriction was one of the earliest and most specific methods of identifying neoplastic B cell populations. Although the staining of immunoglobulin in plasma cells and immunoblasts was readily achieved, the demonstration of immunoglobulin light chain restriction in other B cell types in fixed tissue sections was beset with inconsistencies, and the technique was all but abandoned by most diagnostic laboratories. The recent description of a method that employs trypsinisation followed by antigen retrieval in 4 M urea allows consistent demonstration of light chain restriction in B cell lymphomas of small and large cell types.

AS-Y Leong
Hunter Area Pathology Service and University of Newcastle
New Lambton Heights, NSW

FJW-M Leong
NIffield Department of Clinical and Laboratory Sciences
University of Oxford
Oxford, UK
T-cell lymphoma

The anaplastic lymphoma kinase (ALK) protein is the most important prognostic indicator in anaplastic large cell lymphoma and ALK expression is also pathognomonic for anaplastic large cell lymphoma. This chimeric protein is due to genetic alteration of the ALK locus on chromosome 2 with the most frequent alteration being a translocation involving ALK and NPM (nucleophosmin) gene on chromosome 5. Those tumours that carry the t(2;5) show localisation of the ALK protein to the nucleus and cytoplasm but variant translocations and inversions involving ALK and other partner genes on chromosomes 1, 2, 3, and 17 occur less frequently and result in different localisation of the chimeric protein to only the cytoplasm and/or cell membrane. CD1 molecules are expressed in 70% of thymocytes, largely cortical thymocytes and this is reflected in the neoplastic population where precursor T-ALL/BLs expressing cortical or immature phenotypes are CD1+, in contrast to those with prothymocyte or medullary phenotypes. Post-thymic or TDT-negative T-cell neoplasms such as T-CLL, T-PLL, cutaneous T-cell lymphoma and node-based T-cell lymphoma are CD1−. CD1a immunoreactivity is thus useful for the classification of thymomas and T-cell precursor neoplasms. S100 positivity has been used as the conventional marker to distinguish Langerhans’ histiocytosis and non-Langerhans’ histiocytosis but it is now clearly recognised that abnormal histiocytes may also stain for S100. CD1a is an important discriminator in this context.

Malignant melanoma

Conventional markers for melanoma include S100 protein and HMB45. S100 is not specific and is expressed in a variety of tumours whereas, HMB45, which is highly tissue selective is fixation-dependent and may not be detectable following prolonged fixation. Furthermore, HMB45 is not expressed in desmoplastic melanomas. Melan-A/Mart-1 was cloned from a human melanoma cell line and appears to show more homogenous staining of melanoma and nevus cells compared to HMB45, which stains mainly intradermal and superficial dermal cells of compound nevi. While Melan-A/Mart-1 is a useful adjunctive marker for melanomas, it too fails to label desmoplastic melanoma and is also expressed in adenocortical and Leydig/Sertoli cell tumours. Both HMB45 and Melan-A stain an expanding group of tumours of vascular epithelioid cells, the so-called PEComas, including angiomyolipoma, lymphangiomyomatosis and ‘sugar’ tumours. Microphthalmia Transcription Factor (MiTF) shows high sensitivity and specificity for melanoma and appears to be equally sensitive for cutaneous nevi and metastatic melanoma. The results compare favourably with HMB-45, Melan-A and tyrosinase. However, the situation is less defined in the case of desmoplastic melanoma with positivity in the range of 3-55%, allegedly inversely related to the size of the tumour. MiTF is also expressed in the group of PEComas and other tumours that show melanocytic differentiation such as melanotic schwannomas and clear cell sarcomas. Tyrosinase is an enzyme involved in the initial stages of melanin biosynthesis. Immunopositivity for tyrosinase is high in melanoma but appears to show inverse correlation with the clinical stage of the disease with homogenous staining in the early stages to a more heterogenous pattern in later stages. Tyrosinase is highly expressed in epithelioid melanoma but is rarely expressed in spindle melanoma and is not useful in desmoplastic melanoma and PEComas.

Soft tissue tumours

Soft tissue tumours can be difficult to diagnose and there has been a continued search for newer markers particularly as the repertoire of markers for the separation of this group of tumours is limited. Traditional markers for myogenic (skeletal and smooth muscle) differentiation include desmin, smooth muscle actin, muscle specific actin and myoglobin. These markers have varying degrees of sensitivity and a number of new antibodies have been developed to detect myogenic differentiation. Activation of MyoD1 is an early event that commits the cell to skeletal muscle differentiation and MyoD1 nuclear immunoreactivity has been shown to be inversely related to the degree of differentiation of rhabdomyosarcoma and is a useful marker, particularly in the context of the small round blue cell tumours of childhood. Staining is more consistent in alveolar compared to embryonal rhabdomyosarcomas. An earlier claim that the marker is highly fixation-resistant has been retracted and only as many as 35% of such tumours will stain in fixed tissue sections. Myogenin is another regulatory protein essential for skeletal muscle differentiation. It is a better marker for skeletal muscle differentiation than MyoD. Although all rhabdomyosarcomas show nuclear immunostaining, similar to MyoD1, this protein shows strongest immunostaining in alveolar rhabdomyosarcoma compared to the embryonal variant of this tumour. No reactivity has been reported in other small round blue cell tumours in children. Calponin and caldesmon are two new markers to identify smooth muscle differentiation and are largely used in the context of distinguishing spindle cell tumours and for labelling myoepithelial cells and myofibroblasts.

Small round cell tumours of childhood

Besides MyoD1 and myogenin discussed above, two other newer markers have been added to the panel for the separation of this category of tumours. Ewing’s sarcoma/primitive neuroectodermal tumour (ES/PNET) is characterised by a reciprocal translocation t(11;22)(q24;q12), which results in the fusion of EWS to FLI-1. The FLI-1 protein has been demonstrated in 71% of ES/PNET and not in the other small round cell tumours with the exception of lymphoblastic lymphoma that showed 88% positivity. A variety of vascular tumours also showed nuclear immunoreactivity of this protein. Wilms’ tumour gene protein (WT1) has largely been used to identify mesothelial cells discussed below. The desmoplastic small round cell tumour is characterised by t(11;22) involving WT1 and EWS genes and has been shown to immunostain for the WT1 protein. This contrasts with no staining in ES/PNET. However, there was staining of 71% of nephroblastomas.

Mesothelioma

While immunohistology has largely usurped the role of electron microscopy in the diagnosis of mesothelioma, the panel of antibodies for mesothelioma continues to expand, largely because no single marker has proven to be specific for this tumour and a panel approach is essential for accurate diagnosis. Epithelial membrane antigen (EMA) is not a new marker but deserves revisiting because it is often not employed in the manner that we had originally described. Cytoplasmic immunoreactivity for EMA is not a discriminator between mesothelioma and metastatic carcinoma. Both tumours are positive for EMA. However in mesothelioma, EMA localisation...
Cytokeratin 20 (CK20) and cytokeratin 7 (CK7) are useful markers to distinguish ovarian sex cord tumours, which stain positive compared to fibrothecomas and granulosa cell tumours. Initial studies in mesothelioma suggested that cytoplasmic and nuclear localisation of the antigen was only found in about 42% of cases and also in 6% of adenocarcinomas but it appears that these figures vary with the antibody clone employed. In our experience the positivity in mesothelioma is closer to 85%.

WT1 mentioned previously, has also been employed in the panel for mesothelioma and is reported to be immunoexpressed in the nuclei of 75% of cases. While nuclear immunolocalisation is not seen in pulmonary adenocarcinoma, cytoplasmic staining may occur in up to 86% of cases and in the context of peritoneal tumours, 93% of ovarian serous carcinomas may show varying degrees of immunoreactivity.

**Epithelial tumours**

Breast carcinomas immunoexpress GCDFP-15 in up to 74% of cases and this marker, unlike the oestrogen receptor, serves as a relatively specific marker. Other tumours that express GCDFP-15 include those of the salivary, eccrine, apocrine and bronchial glands, seminal vesicles and prostate.

Amplification of HER-2/neu is a poor prognostic factor found in 20-30% of human breast cancers. The availability of trastuzumab, a recombinant monoclonal antibody against the HER-2 oncprotein offers a novel therapeutic approach. While fluorescence in situ hybridisation (FISH) is the gold standard to assess amplification of the gene, immunostaining for HER-2 is an expedient screening method. Tumours with a HER-2 score of 3+ correspond to FISH+ and score 0 and 1+ corresponding to FISH+ tumours. A proportion of tumours with score 2+ by immunostaining are FISH+ so that such cases require examination by FISH. There is controversy surrounding the method of scoring, with some laboratories taking into account the normal immunopexpression of benign mammary epithelium. Field discusses the application of HER-2 FISH in detail in this edition of Cancer Forum.

E-cadherin and the cadherin-catenin complex have been employed as markers to predict behaviour of breast cancers. E-cadherin is not only useful for the understanding breast cancer pathobiology but has been shown to be a diagnostic discriminator between lobular and ductal carcinoma, the former showing an absence of E-cadherin immunostaining. Conversely, lobular carcinomas express high molecular weight cytokeratins, whereas ductal carcinomas do not.

High molecular weight cytokeratin (CK5/6, 34BE12, caluks keratin) immunostaining has also been used effectively to identify the basal cells in prostatic acini. Malignant prostatic acini are lined by a single layer of cells with loss of the basal cell layer a diagnostic feature that sometimes difficult to discern with immunostaining. High molecular weight cytokeratins are also expressed in mesothelial and mesothelioma cells compared to carcinoma cells, which express these proteins much less frequently.

Cytokeratin 20 (CK20) is a low molecular weight cytokeratin that is expressed in a restricted number of carcinomas, including carcinomas of the gastrointestinal tract and urinary bladder. The combined application of CK20 and CK7 allows the separation of a number of epithelial tumours as shown in table two.

**Table 2: Cytokeratin 20 (CK20) and cytokeratin 7 (CK7) immunoexpression in epithelial tumours**

<table>
<thead>
<tr>
<th>CK7+</th>
<th>CK20+</th>
<th>CK20-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>Cervical</td>
<td>Merkel cell</td>
</tr>
<tr>
<td>Breast</td>
<td>Endometrium</td>
<td>Stomach</td>
</tr>
<tr>
<td>Colon</td>
<td>Esophagus</td>
<td>Colon</td>
</tr>
<tr>
<td>Bile duct</td>
<td>Breast</td>
<td>Liver</td>
</tr>
<tr>
<td>Ovary mucinous</td>
<td>GIT carcinoid</td>
<td>Lung carcinoma</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Bile duct</td>
<td>Neuroendocrine carcinoma</td>
</tr>
<tr>
<td>Stomach</td>
<td>Pancreas</td>
<td>Lung squamous</td>
</tr>
<tr>
<td>Kidney</td>
<td>Adrenal cortex</td>
<td>Lung small</td>
</tr>
<tr>
<td>Liver</td>
<td>Esophagus</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>Lung carcinoid</td>
<td>GIT carcinoid</td>
<td>Ovary</td>
</tr>
<tr>
<td>Neuroendocrine carcinoma</td>
<td>Germ cell</td>
<td>Salivary gland</td>
</tr>
<tr>
<td>Lung squamous</td>
<td>Liver</td>
<td>Thyroid</td>
</tr>
<tr>
<td>Lung small</td>
<td>Mesothelioma</td>
<td>Prostatic</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>Soft tissue epithelioid sarcoma</td>
<td>Thymus</td>
</tr>
</tbody>
</table>

Poorly differentiated tumours in the liver include hepatocellular, cholangio- and metastatic carcinomas. A number of markers including alpha-fetoprotein, carcinoembryonic antigen and CK19 have been purported to separate these entities but the panel is not foolproof. Hep Par 1 has been shown to be specific for hepatocytes and their neoplastic counterparts. The marker is also immunoexpressed in a small number of gastric carcinomas.

Thyroid transcription factor-1 (TTF-1) was originally demonstrated in follicular thyroid cells and subsequently in respiratory epithelial cells, and has since been shown to be a useful marker of pulmonary adenocarcinoma and thyroid neoplasms, including papillary, follicular, medullary and insular carcinomas. Anaplastic carcinomas are negative and TTF-1 has been observed in occasional gastric and endometrial carcinomas.
Tumour immunohistochemistry and predictive pathology

The recent introduction of a new targeted therapy, STI-571, a receptor tyrosine kinase inhibitor that inhibits the activated Kit protein, provides effective treatment for recurrent or metastatic gastrointestinal stromal tumours (GISTS). It is now appreciated that Kit (CD117) immunoreactivity in the specific context of mesenchymal lesions of the gastrointestinal tract defines a group of tumours that are collectively called GISTS so that this marker is necessary for both diagnosis and therapy. In a recent consensus meeting, it was concluded that “indeed the term ‘GIST’ should apply only to neoplasms displaying Kit immunoreactivity with very rare exceptions”76. CD34, which was previously a component of the definition, is only found in 60-70% of cases. The role of c-kit in the diagnosis of GISTS is discussed further by Waring in this edition of Cancer Forum.

Tumour immunohistochemistry and the diagnosis of familial cancer syndromes

Germline mutations of the genes responsible for mismatch repair proteins MLH1, MSH2 and MSH6 account for over 90% of mutations in hereditary nonpolyposis colorectal carcinoma (HNPPC), which makes up 1-5% of all carcinomas in the colon. Deficiency of these mismatch repair proteins leads to an increased rate of mutations in microsatellite regions and may affect crucial genes that regulate growth, differentiation and apoptosis. Patients with colorectal carcinomas showing abnormalities of one of these proteins have an increased incidence of synchronous and metachronous tumours in the colon, as well as other sites such as ovary, endometrium and urinary bladder80.

Conclusions

By necessity this has been a brief listing of newer antibodies that are immunoreactive and applicable for routine tumour diagnosis. The continual development of increasingly sensitive antibodies contributes significantly to more accurate tissue diagnosis, better prognostication and greater individualisation of treatment in cancer.

References

9. M Oshawa, M Kanno, T Machii, K Aozasa. “Immunoreactivity on continual development of increasingly sensitive antibodies immunoreactive and applicable for routine tumour diagnosis. The recent introduction of a new targeted therapy, STI-571, a receptor tyrosine kinase inhibitor that inhibits the activated Kit protein, provides effective treatment for recurrent or metastatic gastrointestinal stromal tumours (GISTS). It is now appreciated that Kit (CD117) immunoreactivity in the specific context of mesenchymal lesions of the gastrointestinal tract defines a group of tumours that are collectively called GISTS so that this marker is necessary for both diagnosis and therapy. In a recent consensus meeting, it was concluded that “indeed the term ‘GIST’ should apply only to neoplasms displaying Kit immunoreactivity with very rare exceptions”76. CD34, which was previously a component of the definition, is only found in 60-70% of cases. The role of c-kit in the diagnosis of GISTS is discussed further by Waring in this edition of Cancer Forum.


