Immunohistochemistry — a new era in diagnostic surgical pathology

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Pathologists have used many special techniques over the years to confirm, complement and refine the information they were able to obtain with their old faithful armamentarium i.e. formalin fixation, paraffin embedding and haematoxylin eosin staining. These techniques include special staining, tissue culture, electron microscopy, immunohistochemistry and molecular biology methods. It could be fair to say that today no special technique has influenced the way pathology is practiced as profoundly as immunohistochemistry or has even come close to it. It would not be an exaggeration to speak of a revolution, particularly in the field of tumour pathology.

The combination of the precision of immunohistochemistry with the visualization provided by a sensitive detection system, is a science that has its origins in the work of Albert Coons, 60 years ago.[1] Building upon the work of Coons, who initially sought to prove the presence of immunoglobulin on plasma cells, the past half century has seen tremendous refinements in the development of the antibody tools and detection systems. Immunohistochemistry started with a brilliant yet disarmingly simple idea; to have antibodies bind the specific antigens being sought and to make those antibodies visible by hooking them to a fluorescent compound. All subsequent modification's such as use of non-fluorescent chromogen, the amplification of reaction and unmasking of antigens are technical improvements.

The advancement of monoclonal antibody technology has been of great significance in assuring the place of immunohistochemistry in modern accurate microscopic diagnosis of human neoplasms as a method of choice in histopathology.[2]

The production of antibodies enabling the detection of genetic abnormalities including mutations, gene amplifications or specific chromosomal translocations associated with novel chimeric proteins promises to yield further insights into the genesis and behaviour of tumours.[3]

In gene mutations, it is used to show the loss of protein expression, reflecting the final common pathway that results from mutation, promoter methylation or other genetic mechanisms. The loss of mismatch repair gene proteins in colorectal carcinoma is one such example. Hereditary non-polyposis colon cancer forms a small subset of colorectal cancer that do not arise from the multistep process of carcinogenesis in familial adenomatous polyps. Instead they arise by a process of alterations in DNA mismatch repair genes, commonly hMLH1 and hMSH2, giving rise to the status of micro-satellite instability (MSI). There is absence of MLH1 nuclear immunohistochemical staining in tumours of patients with MS1. More recently, the detection of MS1 has been used a prognostic marker. Tumours with MS1 have a better prognosis than those with intact mismatch repair and show a better resonse to Fluoropyrimidine - based chemotherapy.[3,4]

Chromosomal translocation resulting in gene fusions encoding novel chimeric proteins of diagnostic and prognostic significance can be determined by immunohistochemistry e.g. Alk t(2;5) translocation in Anaplastic large cell lymphoma, indicates favourable prognosis. ZAP-70, is a tyrosine kinase that participates in early B-Cell differentiation. In Chronic Lymphocytic Leukaemia, its expression has been shown to be a poor prognostic factor, associated with non-mutated configuration of the IgVH genes. Other translocations that can be determined by immunohistochemistry include WT-1 (t11;22) in desmoplastic small round cell tumour and FL-1 over expression in t(11;22) q (24;12) translocation in Ewing's sarcoma /PNET.

The molecular signature of lobular carcinoma of breast is a genetic alteration which results in loss of E Cadherin protein, which can be determined by immunohistochemistry.[5]

Therapeutic targets for treatment of Cancer: e.g. Her2/neu in breast cancer. Her2neu (C-erb-B-2) oncogene protein is a transmembrane glycoprotein in the epidermal growth factor receptor family.[5] It is expressed at low levels in a variety of normal epithelia, including that of mammary duct cells. Tumours with its over-expression respond to Transtuzumab. Moreover, its overexpression is an independent prognostic marker specially for node positive patients.[5] Some studies also suggest that Her2neu status predicts response to Adriamycin based adjuvant — chemotherapy and poor response to Tamoxifen even in the setting of positive Estrogen receptor expression.[5] Gleevac targets BCR-ABL gene product, characteristic of Chronic Myeloid Leukaemia and is also effective in blocking the

"Kinase Pocket" of the platelet derived growth factor receptor and C-Kit. The latter is expressed in (& highly characteristic of) Gastrointestinal Stromal Tumours. C-Kit can be demonstrated by immunohistochemistry.[5]

The above examples explain Genogenic immunohistochemistry which describes application of immunohistochemistry, where this protein based technique is employed to answer questions about alterations at the molecular level.[5]

The mouse — human chimeric antibody Rituximab has been used in aggressive B-cell lymphomas which express CD20.[3] Gemtuzumab Ozogamicin is the humanized anti CD33 antibody. CD33 is expressed in about 90% of blasts in Acute myeloid leukaemia.[3] There are extensive, constantly developing applications of immunohistochemistry to diagnostic haematopathology. These applications are useful in diagnosis and in determining prognosis of haematologic malignancies as well as evaluating residual/relapsing disease. Some of the examples include differentiation of various forms of B-Cell hyperplasia from B-cell Lymphoma and defining Hodgkin's Lymphoma etc.[6]

Other important uses of immunohistochemistry include determination of unknown primary tumours[6] diagnosis of central nervous system tumours, diagnosis of Paediatric small round blue cell tumours, carcinomas, Melanoma and sarcomas.[7-10]

Small round blue cell tumours and solid paediatric malignancies require Immunohistochemistry for the diagnosis, evaluation of recurrent or metastatic disease and in some cases prognostic classification. It is only one of the very important tools available to the pathologists to categorize solid tumours of childhood and adolescence. Immunohistochemistry has been shown to have impact on patient diagnosis and has been reported to be a cost effective ancillary test in patient diagnosis.[11] The discovery of neoplasm associated antigens has not only made the more accurate diagnosis of human cancer feasible but has also shed the light on extensive immunophenotypic heterogenicity of even the most closely linked human malignancies.[11] Future antineoplastic therapeutical appearances should see the inclusion of a variety of immunotherapies, in the form of an individualized "Cocktail" specific for the particular immunophenotypical pattern associated with each individual patient's neoplastic disease.

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