

## *Editorial*

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### Molecular Profiling of Tumours by Immunohistochemistry

In recent decades, molecular techniques have defined the genetic profiles of many tumours and contributed significantly to the advancement of clinical management and therapeutic approaches. But, due to lack of facilities, only a few molecular techniques have reached from the bench to the bedside. Immunohistochemistry (IHC) has emerged as a major 'doable' tool for pathologists. The application of IHC to detect various antigenic components using specific antibodies has revolutionized diagnostic pathology.<sup>1</sup> IHC can be used to define molecular alterations in cancers, which not only confirms the diagnosis of certain tumours, but also helps in their prognostication and therapeutic management, especially in this era of targeted molecular therapy. The greatest benefit of this technique seems to be in countries such as India where molecular laboratories are not viable everywhere. Understanding the concept of 'molecular histochemistry' can thus modernize the practice of oncology. Another advantage of IHC is that it can be easily applied to formalin-fixed, paraffin-embedded tissues, which form the backbone of tissue pathology. On the other hand, most molecular techniques require fresh cryopreserved tissue for extraction of good quality DNA, RNA or protein, which is often difficult to obtain. However, the results of IHC need to be interpreted with caution because the level of protein expression may not exactly correlate with the corresponding genetic alterations.

In the traditional practice of diagnostic pathology, the role of IHC is mainly to localize specific epitopes in tissue sections by either monoclonal or polyclonal antibodies. However, it is indispensable in the diagnosis of poorly differentiated carcinomas (cytokeratin-positive), sarcomas (vimentin-positive) and lymphomas (CD45-positive).<sup>1</sup> In addition, IHC has a specific role in detecting tumour differentiation by identifying specific intermediate filaments. It also helps to identify the source of a tumour in case of metastasis from an unknown primary, e.g. if the origin is from the breast (oestrogen/progesterone receptor-positive), thyroid/lung (thyroid transcription factor-1-positive) or from the gastrointestinal tract (by analysing the panel of CK 7/CK 20 antibodies).<sup>1</sup>

Genomic alterations, which are molecular signatures of specific malignancies, can be identified using 'molecular histochemistry'. Some common genetic alterations in cancer are deletion, mutation and silencing of tumour suppressor genes, tumour-specific translocations and proto-oncogene amplifications. These alterations manifest as loss, gain or abnormal subcellular localization of proteins, which can be studied using IHC and available antibodies.<sup>1</sup> This information may contribute immensely to the diagnosis, prognosis and treatment of cancers.

#### *Markers for diagnosis*

E-cadherin is a transmembrane glycoprotein that mediates calcium-dependent intercellular adhesion and is specifically involved in epithelial cell-to-cell adhesion in the breast. Loss of E-cadherin expression is the molecular signature of lobular carcinomas of the breast and helps to differentiate them from infiltrating duct carcinomas. E-cadherin alterations can occur either by somatic mutation, allelic loss or methylation of the gene. Molecular techniques to detect these alterations can be

costly and cumbersome. Whatever the mechanism of the genetic alteration, the end result is the loss of expression of E-cadherin, which can be easily detected by IHC.<sup>2,3</sup>

Rhabdoid tumours are highly malignant neoplasms which occur in children in the kidney, brain and extrarenal soft tissues. Histologically, rhabdoid tumours need to be differentiated from primitive neuroectodermal/embryonal tumours (PNET) because they have a very poor prognosis and are non-responsive to chemotherapeutic regimens used for the management of other malignant small round cell tumours. The genetic hallmark of atypical teratoid/rhabdoid tumours (AT/RTs) is mutation or loss of the *INI 1* (*hSNF5/SMARCB1*) gene locus at chromosome 22q11.2.<sup>4</sup> This results in the loss of INI 1 protein expression in almost all AT/RTs. In normal tissue and in most tumours, INI 1 is expressed as a nuclear protein. However, in AT/RTs, there is loss of nuclear expression of INI 1 in tumour cells. Immunohistochemical staining for expression of INI 1 protein is a sensitive and specific marker for the diagnosis of AT/RTs. Hence, it has become mandatory to identify the INI 1 protein as a molecular signature of *hSNF5/INI 1* gene mutation in AT/RTs of the brain or in renal/extrarenal rhabdoid tumours.<sup>4</sup>

IHC can distinguish a particular tumour type from a subset of tumours with similar histomorphology by identifying the unique tumour-specific fusion protein formed out of a specific chromosomal translocation. A characteristic recurrent chromosomal translocation—t(11;22)—between *EWSR1/FLI-1* genes in Ewing sarcoma (ES) leads to the formation of a fusion protein, where the usual RNA-binding function is replaced by DNA-binding activity of FLI-1, resulting in prolonged transcription factor activity in this tumour.<sup>1</sup> Immunohistochemical detection of FLI-1 protein in tumours (normally expressed in endothelial and haematopoietic cells) confirms the diagnosis of ES/PNET.<sup>1</sup>

Similarly, IHC for ALK-1 protein in anaplastic large cell lymphoma is an indicator of the translocation t(2;5) (*NPH/ALK-1*) and helps in diagnosis.<sup>5</sup> Also, the characteristic IHC expression of bcl-2 due to t(14;18) seen in synovial sarcoma helps to differentiate it from other soft tissue sarcomas.<sup>1</sup>

Molecular histochemistry is essential in classifying lymphomas according to the current WHO lymphoma classification.<sup>5</sup> For example, t(11;14) is seen in mantle cell, hairy cell and in some splenic marginal zone lymphomas. This translocation is reflected by IHC expression of bcl-1 in these tumours. Similarly, bcl-2 expression represents t(14;18) in follicular lymphomas and helps to differentiate these from reactive follicles.<sup>5</sup>

The tumour suppressor gene *p53* is mutated in a large number of cancers. The normal *p53* gene codes for the wild-type protein, which has a short half-life, and hence cannot be detected by IHC. However, mutation of the *p53* gene leads to expression of a mutant protein which, by virtue of its stability and longer half-life, can be easily detected by IHC in tissue sections. Hence, IHC for *p53* protein can be used as a surrogate marker for detection of *p53* mutation in tumours.<sup>6</sup> Astrocytic tumours of grades II to IV show an immunopositivity for *p53*, while grade I pilocytic astrocytomas are immunonegative. This can be used to differentiate grade I from grade II tumours, which differ in biological behaviour and management; radiotherapy is a must for grade II tumours following surgical resection.<sup>7-9</sup> The 2 types of glioblastomas differ in their molecular pathways of genesis—primary glioblastomas are associated with epidermal growth factor receptor (*EGFR*) amplification and secondary glioblastomas with *p53* mutations. Clinically, the differentiation is important because secondary glioblastomas occur more commonly in young patients and have a better prognosis while primary glioblastomas arise in the elderly and have a poor outcome. IHC can detect *p53* and *EGFR* protein expression and aid in this differentiation.<sup>7-9</sup>

#### Markers of prognosis

IHC can identify mutations or alterations in methylation of the promoter sequence of mismatch repair genes in colorectal adenocarcinomas in Lynch syndrome and in 10%–15% of sporadic adenocarcinomas. These alterations result in loss of expression of the specific mismatch repair genes (*MMRs*)—*hMSH1*, *hMSH2*, *hMLH1*, *hPSM1* or *hPSM2*. The absence of a properly functioning mismatch repair mechanism may

result in microsatellite instability (MSI). Loss of MMR activity corresponds to the loss of protein expression (as detected by IHC). Hence, this phenotype can be detected (in 93% of cases) without resorting to complicated molecular techniques.<sup>10</sup> Even though molecular MSI testing is the gold standard for assessing tumour DNA mismatch repair competency, studies show that IHC for detecting hMLH1 and hMSH2 is a sensitive, rapid and cost-effective method. Adjuvant chemotherapy improves overall 5-year survival in patients with colorectal microsatellite-stable tumours or tumours exhibiting low-frequency microsatellite instability. However, there is no benefit of adjuvant chemotherapy in those with high-frequency microsatellite instability.<sup>11</sup> The detection of loss of MMRs is being increasingly used in routine clinical practice.

Overexpression of p53 has been detected in 91% of colorectal carcinoma patients in northern India. Routine assessment of this marker is helpful as it is indicative of a poor postoperative course in colorectal carcinomas.<sup>6</sup>

Adenoma polyposis coli (*APC*) gene mutation has been shown to be one of the earliest events in the progression of familial adenomatous polyposis and Gardner syndrome. The *APC* gene acts through interaction with  $\beta$ -catenin.<sup>9</sup> Mutation of the *APC* gene can be identified early in colorectal carcinogenesis by using monoclonal antibodies against  $\beta$ -catenin in colon biopsies. Increased and ectopic expression of  $\beta$ -catenin in colorectal carcinoma has been associated with a reduction of E-cadherin

TABLE I. Immunohistochemistry as a window to molecular alterations

Marker	Molecular alteration	Disease	Advantage
E-cadherin (epithelial transmembrane glycoprotein); N-cadherin (neural)	Germline mutation/deletion	Breast cancer, gastric cancer	Diagnostic
INI 1	<i>hSNF5/INI 1</i> gene mutation	Atypical teratoid/rhabdoid tumours of brain; renal and extrarenal rhabdoid tumours	Diagnostic
FLI-1	t(11;22) (EWS; FLI-1)	Ewing sarcoma/PNET	Diagnostic
ALK-1	t(2;5) (ALK; NPM)	Anaplastic large cell lymphoma, inflammatory myofibroblastic tumour	Diagnostic
bcl-1	t(11;14)	Mantle cell lymphoma and CLL/SLL 10%	Diagnostic
bcl-2	t(14;18)	Follicular lymphoma	Diagnostic and prognostic
p53	Deletion/LOH 17p	CRC, astrocytomas, cervical carcinomas	Diagnostic (GBM and cervical cancer); prognostic (CRC)
hMSH1, hMSH2, hMLH1, hPSM1, hPSM2	Methylation of promoter sequence of mismatch repair genes	Lynch syndrome and some sporadic CRC	Diagnostic and prognostic
b-catenin	<i>APC</i> mutation	Adenoma, CRC	Prognostic
Her-2/neu	<i>EGFR</i> receptor mutation, c-erb-2 overexpression	Breast, lung, CRC and gastric carcinoma	Prognostic and therapeutic (trastuzumab)
MYCC and MYCN	Amplification	Neuroblastoma, rhabdomyosarcoma, medulloblastoma	Prognostic
mdm2	Chromosome 12q mutation	CRC, soft tissue sarcoma	Prognostic and therapeutic (anti-mdm2 oligonucleotide)
EGFR, EGFRvIII receptor	Amplification	Lung, GBM	Therapeutic (erlotinib, gefitinib)

PNET primitive neuroectodermal/embryonal tumours    CLL chronic lymphocytic leukaemia  
 SLL small lymphocytic lymphoma    CRC colorectal carcinoma    GBM glioblastoma multiforme  
 APC adenoma polyposis coli    EGFR epidermal growth factor receptor

level and loss of cell-to-cell adhesion, resulting in deep tumour invasion and distant metastasis.<sup>12</sup>

The *c-erbB-2* proto-oncogene, a member of the RTK-I family, encodes the human EGFR 2 (Her 2), structurally homologous to the *EGFR*. *Her-2/neu* is amplified in about 40% cases of breast cancer. There is a significant correlation between levels of the Her-2/neu protein and amplification of the gene itself—*Her-2/neu* IHC was positive in 56% of *Her-2/neu*-amplified breast cancers and in only one of the non-amplified tumours.<sup>13</sup> Node-positive patients and those with higher Her-2/neu protein had a shorter disease-free and overall survival than patients with lower levels of the protein. However, in node-negative patients, the Her-2/neu protein failed to predict disease outcome. The availability of trastuzumab (herceptin) as a therapeutic agent that binds the Her-2/neu protein and results in a 33% lower risk of mortality in patients with breast cancer has further increased the importance of this diagnostic technique.<sup>13</sup>

Various oncogene amplification products can be identified by IHC. For example, *MYCN* amplification can be detected in neuroblastomas and rhabdomyosarcomas by overexpression of the protein by IHC. Expression of this marker indicates a poor prognosis in these tumours. Chromosomal gains of *MYCC* and *MYCN* locus have been identified in medulloblastoma in 4%–17% of patients. Amplification of *MYCC* or *MYCN* is associated with the aggressive large cell tumour variant and a poor clinical outcome. Overexpression of *erbB-2* receptors has been proposed as an independent indicator of aggressive behaviour. All these can be detected by IHC in formalin-fixed tissues.<sup>14</sup>

Mutation of *p53* gene characterizes serous adenocarcinomas of the uterus and ovary, which often are histologically indistinguishable from endometrioid carcinomas. However, this distinction is important as serous adenocarcinomas have a poor prognosis, with increased myometrial invasion and a poor response to chemotherapy. Mutation of *p53* gene in serous carcinomas occurs relatively early and is central to the development of this tumour. However, mutation of *p53* gene is only a late event in higher grade endometrioid carcinomas. Hence, p53 IHC is used to differentiate these tumours.<sup>15</sup>

Mutation of *p53* gene is also important in the prognostication of astrocytic tumours of the brain. Chattopadhyay *et al.*<sup>16</sup> and Sarkar *et al.*<sup>17</sup> have shown that *p53* mutations are not only an early event in astrocytic tumorigenesis, but are also involved in the progression of low grade tumours to a higher grade. Sarkar *et al.*<sup>17</sup> have also reported a correlation between immunopositivity of p53 and the interval to recurrence in astrocytic tumours. It has also been shown that p53-immunopositive tumours and tumours with loss of heterozygosity of a locus in the chromosomal region 17p13.3 have higher proliferation indices.<sup>8,9,18</sup> Thus, p53 IHC can be used as a surrogate marker of *p53* mutations to predict the biological behaviour of astrocytic tumours.

#### *Markers for targeted molecular therapy*

Molecular histochemistry can contribute to targeted therapy. Non-small cell lung cancers, colorectal carcinomas and squamous cell carcinomas of the head and neck respond well to small molecule tyrosine kinase inhibitors (TKI) specific for *EGFR* such as gefitinib or erlotinib. Similarly, anti-MDM2 (anti-double minute 2 gene) antisense oligonucleotides as cancer therapeutic agents alone or in combination with conventional chemotherapeutics have a therapeutic potential.<sup>19</sup>

*EGFR* is amplified in 21% of lung cancers and in 50% of glioblastoma multiforme (GBM). This is reflected by overexpression of EGFR protein, which can be detected by IHC. Similarly, a mutant form of *EFGR* is described in GBMs which can also be detected by IHC using a corresponding antibody. This has led to the use of EGFR-targeted therapies in these tumours. Results of a phase III study, comparing gefitinib and erlotinib with a placebo in lung carcinoma showed a 1-year survival benefit of 31.2% with erlotinib v. 21.5% with placebo.<sup>20</sup> In GBMs, a phase III trial revealed that only a small percentage of patients responded to gefitinib and in these the response could be predicted by EGFRvIII and phosphatase and tensin homologue (*PTEN*) co-expression.<sup>21</sup>

### Conclusion

IHC can be used to identify genetic alterations which are molecular signatures of various tumours. This technique can differentiate genetic changes that manifest as similar phenotypic variations. IHC is cheaper, faster and easy to use in comparison with molecular studies. However, fallacies of this technique, for example, the level of protein expression may not exactly correlate with the corresponding genetic alterations, need to be kept in mind while interpreting results. By rapidly evolving as a tool for 'standard of care' in the management of various tumours, molecular histochemistry is contributing to the paradigm shift to a molecular classification of tumours.

### REFERENCES

- 1 Weiss SW, Goldblum RJ (eds). *Enzinger and Weiss soft tissue tumours*. 4th edn. Toronto: Mosby; 2001:109.
- 2 De Leeuw WJ, Berx G, Vos CB, Peterse JL, Van de Vijver MJ, Litvinov S, et al. Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma *in situ*. *J Pathol* 1997;**183**:404–11.
- 3 Kowalski PJ, Rubin MA, Kleer CG. E-cadherin expression in primary carcinomas of the breast and its distant metastases. *Breast Cancer Res* 2003;**5**:R217–22. Epub 2003 Sep 26.
- 4 Biegel JA, Tan L, Zhang F, Wainwright L, Russo P, Rorke LB. Alterations of the *hSNF5/IN11* gene in central nervous system atypical teratoid/rhabdoid tumors and renal and extrarenal rhabdoid tumors. *Clin Cancer Res* 2002;**8**:3461–7.
- 5 Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). *World Health Organization classification of tumours. Pathology and genetics of tumours of hematopoietic and lymphoid tissues*. Lyon: IARC Press; 2001.
- 6 Das P, Vaiphei K, Jain D, Wig JD. *p53* and *mdm2* expression in colorectal carcinoma: A correlative analysis with clinical staging and histological parameters. *Int J Surg Pathol* 2007;**15**:335–45.
- 7 Nayak A, Ralte AM, Sharma MC, Singh VP, Mahapatra AK, Mehta VS, Sarkar C. p53 protein alterations in adult astrocytic tumors and oligodendrogliomas. *Neurol India* 2004;**52**:228–32.
- 8 Sarkar C, Karak AK, Nath N, Sharma MC, Mahapatra AK, Chattopadhyay P, et al. Apoptosis and proliferation: Correlation with p53 in astrocytic tumours. *J Neurooncol* 2005;**73**:93–100.
- 9 Sarkar C, Chattopadhyay P, Ralte AM, Mahapatra AK, Sinha S. Loss of heterozygosity of a locus in the chromosomal region 17p13.3 is associated with increased cell proliferation in astrocytic tumors. *Cancer Genet Cytogenet* 2003;**144**:156–64.
- 10 de Jong AE, van Puijtenbroek M, Hendriks Y, Tops C, Wijnen J, Ausems MG, et al. Microsatellite instability, immunohistochemistry, and additional PMS2 staining in suspected hereditary nonpolyposis colorectal cancer. *Clin Cancer Res* 2004;**10**:972–80.
- 11 Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003;**349**:247–57.
- 12 Utsunomiya T, Doki Y, Takemoto H, Shiozaki H, Yano M, Inoue M, et al. Clinical significance of disordered beta-catenin expression pattern in human gastric cancers. *Gastric Cancer* 2000;**3**:193–201.
- 13 Kulka J, Tökés AM, Kaposi-Novák P, Udvarhelyi N, Keller A, Schaff Z. Detection of *HER-2/neu* gene amplification in breast carcinomas using quantitative real-time PCR—a comparison with immunohistochemical and FISH results. *Pathol Oncol Res* 2006;**12**:197–204. Epub 2006 Dec 25.
- 14 Bruggers CS, Tai KF, Murdock T, Sivak L, Le K, Perkins SL, et al. Expression of the c-Myc protein in childhood medulloblastoma. *J Pediatr Hematol Oncol* 1998;**20**:18–25.
- 15 Soong R, Knowles S, Williams KE, Hammond IG, Wysocki SJ, Iacopetta BJ. Overexpression of p53 protein is an independent prognostic indicator in human endometrial carcinoma. *Br J Cancer* 1996;**74**:562–7.
- 16 Chattopadhyay P, Rathore A, Mathur M, Sarkar C, Mahapatra AK, Sinha S. Loss of heterozygosity of a locus on 17p13.3, independent of p53, is associated with higher grades of astrocytic tumours. *Oncogene* 1997;**15**:871–4.
- 17 Sarkar C, Ralte AM, Sharma MC, Mehta VS. Recurrent astrocytic tumours—a study of p53 immunoreactivity and malignant progression. *Br J Neurosurg* 2002;**16**:335–42.
- 18 Sarkar C, Rathore A, Chattopadhyaya P, Mahapatra AK, Sinha S. Role of 17p13.3 chromosomal region in determining p53 protein immunopositivity in human astrocytic tumors. *Pathology* 2000;**32**:84–8.
- 19 Wang H, Nan L, Yu D, Lindsey JR, Agrawal S, Zhang R. Anti-tumor efficacy of a novel antisense anti-MDM2 mixed-backbone oligonucleotide in human colon cancer models: p53-dependent and p53-independent mechanisms. *Mol Med* 2002;**8**:185–99.
- 20 Garfield DH. Modern treatment of lung cancer: Case 2. Response to erlotinib after failure of gefitinib in a patient with advanced non-small-cell lung carcinoma. *J Clin Oncol* 2005;**23**:7738–40.
- 21 Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 2005;**353**:2012–24. Erratum in: *N Engl J Med* 2006;**354**:884.

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