Clinical implication of immunohistochemical markers

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Abstract

The role of the pathologist is to establish accurate diagnosis more and more with the help of complementary techniques. At present immunohistochemical expression of some diagnostic and prognostic factors may possibly predict the response to specific therapies. We focus here on the expression of three of these markers : epidermal growth factor receptor which overexpression is correlated with aggressive tumoral behaviour and with the possibility of a targeted therapy, cytokeratins 7 and 20 with their diagnostic implication in carcinomatous differentiation, and we close this review with the identification of markers related to hereditary non-polyposis colorectal cancer involving microsatellite instability. (Acta gastroenterol. belg., **2006**, 69, **52-54**).

Implication of the epidermal growth factor receptor expression in digestive carcinomas

Epidermal growth factor receptor (EGFR) is highly expressed in a variety of solid malignant tumours and its expression has been correlated with disease progression and poor survival.

Activation of the proto-oncogene encoding the EGFR may contribute to the transformation of cellular phenotypes providing growth and survival advantages (1).

EGFR is a transmembrane protein consisting in an extracellular ligand binding domain, a transmembrane region and an intracellular domain with intrinsic tyrosine kinase activity. Ligand binding activates an intracellular signalling cascade that leads to the activation of the tyrosine kinase activity of EGFR. The EGFR family consists in four members, so called : HER1/erbB1, HER2/erbB2, HER3/erbB3 and HER4/erbB4. EGFR is expressed in many epithelial cell types and carcinoma but also in non epithelial tissue, for instance smooth muscle cells, fibroblasts and perineurium (2).

Many cancers are associated with EGFR activation, which can result from mutation of the receptor, for instance mutation that alters the transmembrane region causes dimerisation of two receptors in the absence of the normal ligand, making the protein constitutively active as a kinase. This activation can also be due to increased EGFR expression as a result of gene amplification or transcriptional events.

In oesophageal cancer, the association between elevated EGFR levels and poor patient outlook is particularly strong, showing that increased EGFR expression is correlated with reduction in recurrence-free survival or overall survival rates. In gastric and colorectal cancer, EGFR seems to be a modest prognostic factor particularly in term of overall survival (1).

In gastric carcinoma, EGFR and c-erbB-2 expression correlates with different clinicopathological characteristics of tumours including depth of penetration, metastatic behaviour and stage of tumour.

Taken together, these molecules are considered as poor prognostic factors in gastric cancer (4).

In pancreatic ductal adenocarcinoma, detection of EGFR expression is helpful to judge malignancy, progression and metastasis. Expression increases significantly in the ductal carcinoma compared to normal pancreas or chronic pancreatitis (5).

HER-1 and HER-2 over-expression contributes to a more aggressive phenotype, in contrast the lack of HER-4 expression might increase the metastatic capacity of pancreatic cancer cells (6).

The role of EGFR in hepatocarcinoma remains controversial ; indeed, EGFR could be involved in the development or progression of human hepatocarcinoma as well as on hepatic regeneration (8).

Various strategies have been used to inhibit EGFR activity, targeting both the extra-cellular domain of the receptor or the kinase activity (2).

In colorectal cancer, for instance, a humanised mouse monoclonal antibody : IMC-C225 (cetuximab) is used again the ectodomain of the EGF receptor (3). Of the range of more than ten different methods to evaluate the tumour EGFR status, detection of the protein expression by immunohistochemistry on paraffin sections seems to be the more appropriate method, but the lack of standardised detection remains problematic (1).

In order to achieve the reproducibility and reliability required for diagnostic tests with potential impact on therapy, the Dako EGFRpharmDx™ has been developed and contains quality control steps based on the evaluation of control cell lines (2).

A same quality control test can be made with appropriate tissue section in routine laboratories.

The mouse monoclonal anti-EGFR clone 2-18C9 used in the Dako EGFRpharmDx™ is highly specific and recognises both the wild type and the EGFRvIII mutant form of the receptor.

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EGFR stains cell membrane, demonstrating both complete and incomplete circumferential staining. The immunostaining pattern is frequently heterogeneous, exhibiting various staining intensities within a single specimen. Staining has also been observed in the cytoplasm and the extra-cellular space.

Positivity for EGFR expression is defined as any membrane staining above background level.

A scoring system in three grades is then applied evaluating the intensity of the tumour cell membrane and whether this staining is complete or incomplete.

An absence of specific membrane staining within the tumour or cytoplasmic staining without associated membrane staining has to be considered as EGFR negative specimen (1).

It is interesting to note that the immunohistochemical evaluation of EGF-R on biopsies may provide reliable information with the surgical resection despite a heterogeneous expression in most of the specimen (7).

Actually, oncologists consider that an expression of 1% is enough to treat the patient.

Implication of cytokeratins 7 and 20 in carcinomatous differentiation

The task of the pathologist is to diagnose correctly neoplasms. This task is complicated by the fact that neoplasms demonstrate a wide variety of tissue patterns. In surgical pathology and cytopathology, the starting point for diagnostic interpretation is to categorise a neoplasm as a carcinoma, germ cell tumour, sarcoma, lymphoma or melanoma (6,8).

Once the tumour is identified as one of these major categories, further information about the cellular differentiation may be obtained from specific cellular defining maturation markers. Of the four main categories of malignant neoplasms, carcinomas compose the largest single group of metastatic tumours of unknown primary site. The simple and broad spectrum of cytokeratins are the initial antibodies of choice for detecting carcinomatous differentiation. More specific subcategorisation of the tumour origin is then possible using a variety of site-specific cytokeratins like CK7 and CK20 (4,5,6).

The soft epithelial keratin intermediate filaments comprise approximately 20 different keratin polypeptides of the approximately 30 keratin polypeptides.

The polypeptides numbered 1 to 20 comprise the type II basic keratins and the type I acidic keratins.

Keratin filaments are formed by tetrameric heteropolymers of two different keratins, two from type I and two from type II.

CK7 is a type II keratin with restricted distribution, present in simple, pseudostratified, ductal epithelia and mesothelia. The restricted topography of CK7 makes it especially useful in evaluating the origin of adenocarcinomas as this keratin is present in most breast, lung, ovarian, pancreatobiliary and transitional cell carcinomas, but it is either absent or only decorates rare cells in colorectal and prostatic carcinomas. The specific diagnostic utility of CK7 lies in the fact that there are three dominant patterns of immunostaining : strongly and diffusely positive in lung, breast, ovary, endometrium, bladder, thymus carcinomas as well as in mesotheliomas, neuroendocrine tumours, pancreatobiliary and parotid adenocarcinomas and the fibrolamellar variant of hepatocarcinoma (4,8,9).

Carcinomas that may immunostain a minority of cells include biliary and gastric tumours.

Carcinomas that are almost invariably negative but may occasionally show rare CK7 positive cells include hepatomas, duodenal ampulla carcinomas, colorectal carcinomas and adrenal tumours.

The distribution of CK20 is limited predominantly to gastrointestinal epithelium and its tumours, mucinous tumours of the ovary and Merkel cell neoplasms. This limited distribution when combined with the specific tissue distribution of CK7 is useful to identifies metastatic tumours (7).

In colorectal carcinomas, the frequency of CK 20+ is reported in about 70% to nearly 100%. This range could be due to differences in tumour characteristics (grade), small number of studied cases, technical factors (fixation time, method of antigen retrieval, different clones and dilution of primary antibodies) and criteria for positive staining. In colorectal carcinomas, CK7/CK20 expression is correlated with histological grade and location, CK7+/CK20- appeared higher in high-grade and right-sided tumours. Half of the high-grade large intestinal adenocarcinomas exhibited aberrant expression. In hereditary non polyposis colorectal cancer syndrome (HNPCC), reduced or absent CK20 expression appears to be additional phenotypic characteristics of MSI-H colorectal carcinomas (7). In small intestine, the CK7, CK20 expression has not been often studied. While normal small intestinal mucosa is diffusely positive for CK20 and totally negative for CK7, all adenocarcinomas reveal a variable degree of CK7 expression (5). Gastric adenocarcinomas show various combinations of CK7/CK20 expression with none difference in the intestinal versus diffuse type (5). It has also been suggested that CK7/CK20 expression could distinguish intestinal metaplasia in Barrett's oeosophagus or in gastric cardia. However, this was not confirmed in subsequent studies (6). Nevertheless, it should be emphasised that clinical information is essential : the knowledge of a previously diagnosed primary tumour and the location of the lesions are the basis for proper use of immunohistochemistry and may lead to inclusion of additional markers.

Moreover, literature makes clear that additional cases with identified location, histological type and subtype are necessary to confirm the results of previous studies. Intratumoral heterogeneity also has to be investigated, as well as the stability of antigen expression in the primary tumour and their metastases.

Identification of colorectal adenocarcinoma of HNPCC type

Colorectal cancer has been shown to arise through at least two distinct genetic pathways : one involving chromosomal instability and the other involving microsatellite instability (MSI) (10). Hereditary non-polyposis colorectal cancer (HNPCC) due to MSI is an autosomal dominant disease with high risk for colorectal, endometrial and ovary cancer caused by germline mutations in DNA mismatch-repair genes (MMR). Increased risk for tumours of the ureter, renal pelvic and small bowel is also observed. HNPCC account for approximately 2 to 5% of all colorectal cancers (11-12). Numerous studies reveal that MSI is associated with better prognosis of patients with stage III colon cancer and some suggest a greater chemosensitivity of MSI colon cancers (10,13).

The increased risk for malignancy in HNPCC is caused by mutation in one of the following DNA mismatch repair genes : MLH1, MSH2, MSH6, PMS1 and PMS2. Germ-line mutations of MLH1 and MSH2 account for > 90% of all known MMR mutations in HNPCC (14). The most sensitive technique to detect such mutations is DNA sequencing. However, this procedure is expensive and is not recommended for the examination of individuals with a very low probability of carrying a mutation (15). In the routine diagnosis of HNPCC, three methods have been proposed : evaluation of clinical and pedigree data, microsatellite instability analysis and examination of protein expression by immunohistochemistry (IHC) (16). A few studies have shown that immunohistochemical staining of tumours using antibodies against the MMR proteins is a sensitive method to identify families eligible for mutation analysis, even in paraffin blocks (14,16). Most studies reported so far used antibodies against MLH1, MSH2 and MSH6, some included also PMS2. Because the PMS2 protein forms a heterodimer with the MLH1 protein, absence of the MLH1 protein due to a mutation also leads to loss of the PMS2 protein caused by abrogation of the total protein complex. Absence of PMS2 staining might therefor suggest the presence of a hMLH1 or PMS2 germline mutation or somatic abrogation of hMLH1 (14).

In recent literature, overall, immunohistochemical staining using four, three (MLH1, MSH2, MSH6) or even two (MLH1, MSH2) antibodies confirmed the results of MSI analysis in about 93% of the cases. IHC cannot achieve 100% sensitivity in the detection of the mutation because of the occurrence of missense mutations, approximately in 30% of all hMLH1 and 10% of all hMSH2. Missense mutations also do not always cause abnormalities in protein expression which is measured by HIC. Other limitations of IHC are the technical problems related to weak staining of currently available antibodies against hMLH1 (16).

IHC has the main advantage to be much less expensive and more rapid than MSI testing. Another advantage is that IHC may guide the clinicians to the correct gene for genetic testing. Additionally, immunohistochemistry can be performed on tiny fragments such as those typically obtained from a needle or a colonoscopic biopsy. This type of fragment would frequently yield insufficient DNA to conduct MSI testing (17).

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