Gastrointestinal stromal tumour and other mesenchymal tumours of the gastrointestinal tract: the role of immunohistochemistry in an evolving era of molecular diagnostics

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Abstract

Significant progress has been made in the molecular characterization of soft tissue tumours arising in the gastrointestinal tract, primarily with the identification of recurrent translocations, gene amplifications and mutations in different tumour types. Translational studies have resulted in the development of many diagnostically useful immunohistochemical markers that reflect these underlying genetic changes. In addition, expression of some such markers is associated with distinctive clinical and histologic features, which may impart prognostic or predictive information, particularly for gastrointestinal stromal tumour. The advent of 'next-generation' immunohistochemistry has reduced the need for additional, more costly, molecular studies; however, it is important to understand the complementary role of ancillary molecular studies, which may be needed for diagnostic or therapeutic information. This review outlines the different roles of immunohistochemistry in the evaluation of select soft tissue neoplasms of the gastrointestinal tract, emphasizing their utility and limitations in clinical practice, molecular correlates, and the role of immunohistochemistry in guiding the appropriate application of ancillary molecular studies.

Keywords fluorescence in situ hybridization; gastrointestinal tract; immunohistochemistry; molecular genetics; sarcoma; soft tissue; tumour

Introduction

Over the last ten to twenty years, the genetic basis of a variety of different soft tissue tumours of various anatomic sites has been elucidated, advancing the field of soft tissue tumour pathology significantly and resulting in improved tumour classification and prognostication. Many of these findings have been translated into the development of diagnostically useful immunohistochemical markers that reflect underlying genetic changes, primarily recurrent translocations, gene amplifications, and mutations. In addition, expression of some markers is associated with distinctive clinical and histologic features, particularly in gastrointestinal stromal tumour, and may impart prognostic or predictive information. Within the GI tract, examples include the protein products KIT, and more recently DOG1, for the diagnosis of gastrointestinal stromal tumour. Fusion protein products resulting from recurrent translocations may also be useful diagnostic markers, such as ALK and ROS1 for inflammatory myofibroblastic tumour, which not infrequently arises within the GI tract. Inflammatory fibroid polyp shows consistent expression of platelet-derived growth factor receptor alpha (PDGFRA) due to the presence of mutations of this gene in this tumour. In addition, metabolic enzyme disturbances in the clinicopathologically distinct group of 'SDH deficient GIST' can be identified by immunohistochemistry (IHC) for SDHB; identification of this distinct subgroup provides not only diagnostic information, but also important prognostic and predictive information. Finally, clear cell sarcoma-like tumour of the GI tract (also known as gastrointestinal neuroectodermal tumour) has been genetically characterized, and in addition to IHC, fluorescence in situ hybridization (FISH) to identify the *EWSR1* rearrangement characteristic of this tumour type is often needed to confirm the diagnosis.

The advent of 'next-generation' IHC has reduced the need for additional, more costly, molecular studies; however, it is important to understand the complementary role of ancillary molecular studies, which may be needed for diagnostic or therapeutic information. This article reviews the different and complementary roles of IHC in the evaluation of the above listed tumour types, in the context of available molecular studies such as FISH, RT-PCR and other sequencing assays, along with discussion of the appropriate utility of these markers in clinical practice, limitations, and corresponding molecular alterations.

Gastrointestinal stromal tumour

Gastrointestinal stromal tumour (GIST) is the most common mesenchymal tumour of the gastrointestinal tract. The clinical behaviour of GIST is variable, and many factors help predict behaviour, including location, size and mitotic activity.[1] Genotype is predictive of treatment response, and in some cases is also predictive of clinical behaviour. Approximately 80% of GISTs harbour oncogenic mutations in *KIT*, and 5–10% in *PDGFRA*, resulting in constitutive kinase activation in the absence of natural ligands (stem-cell factor for KIT, and PDGFA for PDGFRA). The discovery of *KIT* mutations in GIST has served as an excellent model for the development of effective molecularly targeted chemotherapy in solid tumours, as exemplified by the tyrosine kinase inhibitor imatinib mesylate. Before the use of tyrosine kinase inhibitor therapy, the median survival for advanced GIST was only 18 months; the median survival for patients with advanced disease who are treated with imatinib is now around 5 years, with one-third of such patients surviving more than 9 years. However, approximately 10% of GISTs lack *KIT* and *PDGFRA* mutations, so called "wild-type" GIST; such tumours are generally resistant to treatment with imatinib but may respond better to second or third generation tyrosine kinase inhibitors. Wild-type (WT) GIST includes SDH-deficient GIST, neurofibromatosis

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type 1 (NF1)-associated GIST, and *BRAF*-mutant GIST, as well as other tumours in which the underlying genetic basis is unknown.

The most commonly used immunohistochemical markers in the evaluation of GIST are KIT and DOG1, which are used to confirm the diagnosis of this tumour type, both being highly sensitive and specific markers. More recently, IHC has proved to be a useful tool to help identify the clinicopathologically and biologically distinct group of '*SDH-deficient GIST*', a diagnosis that carries significant prognostic and predictive implications and identifies a group of patients in whom genetic testing for inherited germline mutations is indicated. The role of molecular studies in GIST is primarily to identify the presence and specific type of *KIT* or *PDGFRA* mutations and similarly identify those tumours that are wild-type for both. This imparts significant predictive information with regards to the use of targeted drug therapies but has a limited diagnostic role. Other molecular studies that may be useful in certain clinical settings, as discussed below, are *BRAF* and *SDH* mutational analysis.

Immunohistochemistry in the evaluation of GIST

GIST arises from the interstitial cells of Cajal (ICC), or their precursors, which reside in the myenteric plexus of the muscularis propria, where they function as pacemaker cells of peristalsis. The relationship between GIST and ICC was recognized with the identification of expression of the cell-surface transmembrane receptor KIT on tumour cells, a feature shared by ICC. In addition to KIT, both GIST and ICC usually express CD34, DOG1, the intermediate filament nestin, and ETV1, a member of the ETS family of transcription factors. KIT is an extremely useful marker for confirming a diagnosis of GIST. Expression of KIT is seen in approximately 95% of GISTs, usually with a diffuse cytoplasmic pattern (Figure 1A–B). Less common patterns include membranous staining and Golgi accentuation resulting in a dot-like pattern. Of the 5% of GISTs that lack KIT expression, the majority (approximately 70%) are *PDGFRA* mutant tumours that arise in the stomach and show epithelioid cytomorphology (Figure 2A–C). The remaining KIT-negative GISTs are usually wild-type for *KIT* and *PDGFRA*. *KIT*-mutant GIST lacking KIT expression is extremely rare, but dedifferentiated GIST loses expression of KIT, DOG1 and CD34.

DOG1 (ANO1; anoctamin 1) is a recently discovered chloride channel protein that was found to be overexpressed in GIST by gene expression studies and shows cytoplasmic and membranous expression in >95% of GISTs (Figure 1C).[2] DOG1 is expressed in the majority of KIT-negative GISTs and is slightly more sensitive for gastric epithelioid GISTs (including *PDGFRA*-mutant GISTs) than KIT.[3,4] In contrast, KIT is slightly more sensitive than DOG1 for intestinal GISTs. The specificity of DOG1 for GIST is also relatively high, particularly among other mesenchymal neoplasms that may mimic GIST. Other mesenchymal tumours that rarely show expression of DOG1 include leiomyosarcoma, synovial sarcoma, uterine-type retroperitoneal leiomyomas, and some PEComas, and is usually only focal when present, unlike the diffuse staining seen in GIST. Only 2–3% of GISTs are negative for both DOG1 and KIT. In cases where the diagnostic suspicion remains high for GIST, molecular testing for *KIT* or *PDGFRA* mutations is advisable to confirm the diagnosis.

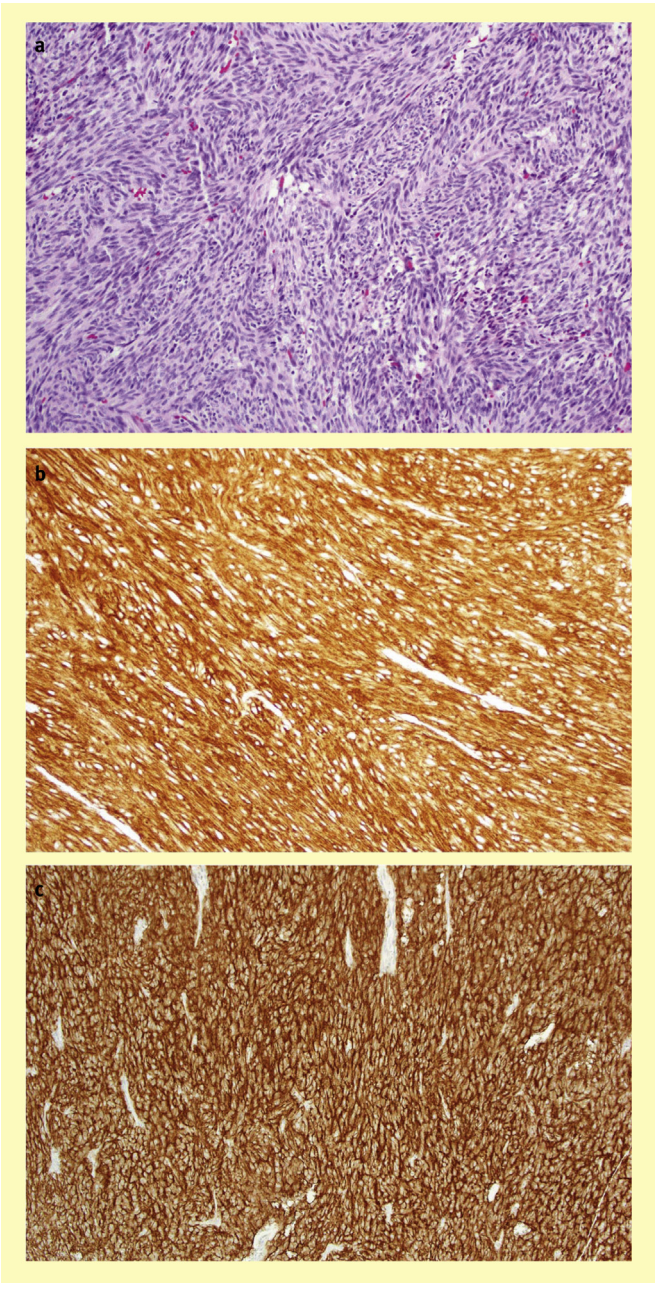

Figure 1 GIST with spindle cell morphology (**a**) showing diffuse cytoplasmic and membranous expression of KIT (**b**) and DOG1 (**c**).

KIT or *PDGFRA* mutations occur in approximately 50% of DOG1-negative GISTs.

In addition to the diagnostic utility of KIT and DOG1, IHC for SDHB and SDHA has proven to be an extremely useful tool to identify SDH-deficient GIST and is being increasingly used in routine practice as an effective and efficient substitute for tumour sequencing to identify GISTs with *SDH* mutations, as discussed in detail below.

SDH-deficient GIST: defects in the succinate dehydrogenase (SDH) metabolic pathway have been found to occur in most paediatric GISTs and a subset of adult WT gastric GISTs,