C-KIT MUTATIONS IN GASTROINTESTINAL STROMAL TUMORS - MORPHOLOGICAL, BIOLOGICAL AND GENETICAL ASPECTS

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Abstract. Gastrointestinal Stromal Tumors(GISTs) are defined as mesenchymal tumors and 95% of them showed mutation in KIT gene. Our aim is to investigate the significance of C-KIT mutation for GISTs, bio- genetical mehanizm and detected by immunohistochemistry. Three cases were observed in the stomach, per one of the small bowel, rectum, colon and the rest were extragastrointestinal. Of these tumors five were classified as benign , three as malignant and one was diagnosed as borderline tumor.Immunohistochemical analysis showed C-KIT (CD117) positivity in all cases. Indentification and expression of C-KIT mutation with immunohistochemical method is the gold standard for diagnosing GISTs.

Key words: Gastrointestinal Stromal Tumors, C-KIT, Immunohistochemical analysis

INTRODUCTION

Gastrointestinal Stromal Tumors (GISTs) are the most common mesenchymal tumors previously uniformly smooth muscle of the human gastrointestinal tract (GIT) [7]. From all tumors in GIT the GISTs are 0,1-3% [4]. A big contribution are the investigations about C-KIT protooncogen to the differentiation between these tumors, to understanding their biology, as well as to the following treatment.

AIM of the our study was investigate to the significance of C-KIT mutation for GISTs, bio-genetical mehanizm and Immunohistochemical expression of this mutation.

MATERIALS AND METHODS

Retrospectively for the period 2003-2006 in the Department of General and Clinical Pathology - MU- Pleven were examined 9 GISTs. Basically the materials were obtained by Department of Surgical Oncology and Second Surgical Clinic-UMBAL-Pleven, with clinical diagnoses leomyoma, leomyosarcoma and tumor abdominis.

For this purpose were used routine histological methods (hematoxylin&eosin-HE, Van Gieson) and Immunohistochemical with polyclonal antibodies from DAKO corp. for C-KIT (CD117), CD34, LSMA, Desmin, S100 protein. Histologic evaluation - 1 to 4 HE slides where reviewed for each case. Per every case was made a morphological mark including: type of the majority cells (spindle vs. epithelioid), skenoid fibres, mucosal ulceration, mitoses (counted from 50/HPF), coagulation necrosis, infiltrative grow pattern, limph node involvement, distant metastasis and size of tumor.

RESULTS

Three of the nine cases are women and the rest six are men. The age range is from 40-71 years. The sizes of the tumors varies between 2-15cm. Three cases were observed in the stomach, one in the small bowel, one in rectum-sigma, one colon, one omentum, and two mesentery. Of these tumors five were classified as benign (fig. 1), three as malignant based on size more than 10cm and metastases in liver,

coalagutive necrosis ulceration, infiltrative grow pattern, high mitotic activity (more than 5 mitoses per 50HPF) (fig. 2). One was diagnosed as borderline tumor (fig. 3). This tumors displayed different histological patterns and cells types. From the examined tumors, spindle cells pattern was the most common(fig. 4); however two tumors were epithelioid pattern (fig. 5).

Immunohistochemical findings-C-KIT(CDl 17) positivity was detected in all cases, typically in most of tumor cells in a membrane stain (fig. 6). CD34 positivity was seen in 7 cases, a small number were positive for LSMA (1/9) and Desmin (1/9).

DISCUSSION

According to Finland Annual Incidence, GISTs is around 10-20 per million and that of malignant GISTs is about 4 cases per million of population [6]. Both determining the C-KIT mutation and detecting the immunohistochemical analysis lead to diagnosing of new cases which used to be in the group of Smooth Muscle Tumors and Sarcoma [3,4,6]. This tumor affects middle-aged and elderly patients with a median age of 50-60 years. GIST is rare before the age of 40 and very rare in children which was confirmed in our study too [2,3]. 60-80% from GISTs are localized in the stomach, 20-30% in the small bowel, and less than 10% in oesophagus, colon, rectum, omentum and mesentery. 20-25% from the gastric GISTs are malignant [3,4].

Extragastrointestinal stromal tumors (EGIST) is rare [6]. But in our research EGIST is about 33% (omentum and mesentery). Larger lesions often show cystic degeneration or central necrosis. Ulceration of the overlying mucosa is common, observed in three of the our cases. Histologically 70-80% of the GISTs are from spindle cell type with a fascicular or storyform grow pattern [2,4,6,]. In our cases 80% were diagnosed as spindle storyform pattern. About 20-30% of tumors are predominantly composed of large round or polygonal epithelioid cells with often localization in the stomach, as two from our cases. Mixed-spindle and epithelioid tumors are common which contradicts with our research. Skenoid fibres, metaplastic bone formation and stromal calcification were not found in our cases.

Researchers in Japan and in Sweden independently discovered in 1998 that GISTs express the KIT receptor protein [3,6,8]. Mutations in the KIT gene that are relevant for GISTs are found in exons 9, 11, 13 and 17. These regions of the KIT gene correspond to different regions of the KIT protein. Mutations in KIT can be found in about 80% of GISTs. However, about 8% of GISTs have a normal KIT gene (wildtype) but show mutations in the gene for PDGFRA . PDGFRA is a protein that is member of the same family as the KIT protein. KIT and PDGFRA are both receptor tyrosine kinases and have a very similar structure. The PDGFRA gene is also very similar to the KIT gene, and PDGFRA mutations have been found in exons 12, 14 and 18 (corresponding to exons 11, 13 and 17 of KIT). Interestingly, the genes coding for KIT and PDGFRA receptors are found in close proximity on chromosome 4ql2. In group of the GISTs 80.9% showed KIT mutations, while 7.1% showed PDGFRA mutations, and 12% were wildtype. The KIT mutant GISTs are 95% KIT-positive. The majority have mutations in exon 1 1 (66.1%) and show spindle cell morphology. About 13% of KIT-mutant GISTs show exon 9 mutations. Mutations in exons 13 and 17 of KIT each occur in around 1% of GISTs or less [5,8]. Epithelioid or mixed cell-type GISTs from non-gastric locations usually show KIT mutations. The KΓ protein is a cell membrane-spanning signaling molecule (receptor tyrosine kinase) that normally functions to trigger cell growth when it is activated by its specific ligand stem cell factor. KIT is normally expressed by the interstitial cells of Cajal (ICC), the “pacemaker cells of the gut” that send nerve signals to propel food along its course through the system via muscle contractions (peristalsis)[2,6,8], KIT is also normally expressed by a few other types of cells in the body such as melanocytes in the skin, mast cells, and hematopoietic stem cells involved in making new blood cells (this is where “stem cell factor).

The expression of KIT(CD117) is the gold standard for diagnosing GISTs. Therefore, a positive result for KIT antibody testing is a strong indication of a GIST diagnosis. GISTs with KIT mutations would stain strongly for KIT(CD117) which was confirmed in our study too. With rare exceptions, KIT is not expressed by other abdominal tumors[2, 3,6].

CD 117 commonly shows strong diffuse cytoplasmic and membrane positivity. The identification of mutations mostly in exon 11 and to a lesser extent in exon 9,13 in the KIT protooncogen coding for CD117 in many GISTs has resulted in a better understanding of their oncogenic mechanism.

Recent data indicate that the response of GIST patients to tyrosine kinase inhibitors (drugs including Gleevec and Sutent) varies by the specific mutation displayed by their tumors. The American Society for Clinical Oncology indicate a stronger and longer-duration response to Gleevec for patients with KIT mutations in exon 11 than for those with mutations in exon 9[5,6,8].

CONCLUSION

Indentification and expression of C-KIT mutation with immunohistochemical method is the gold standard for diagnosing GISTs. KIT is a rare example for protooncogen which is not only a specific marker for histogenesis and diagnostic, but is also a substrate for target therapy with tyrosine kinase inhibitors.

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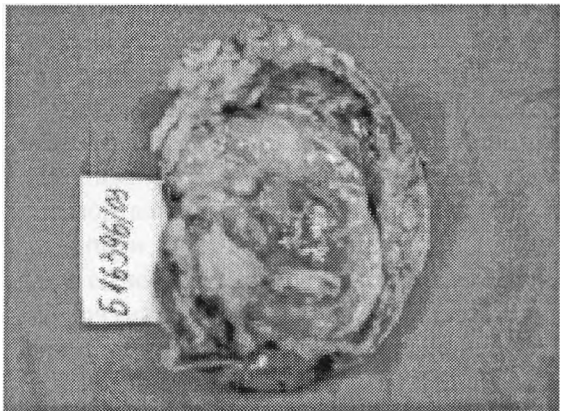

fig.1 Gross appearance of benign GIST omentum

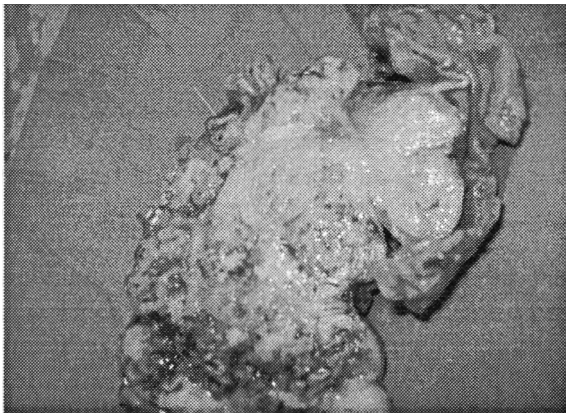

fig.2 Gross appearance of malignant GIST large bowel

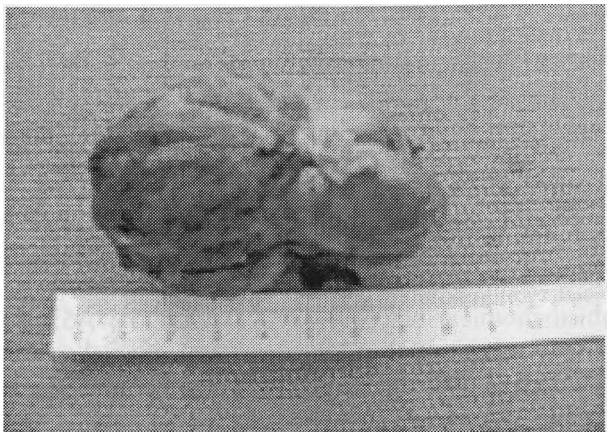

fig.3 Gross appearance of bordeline GIST mesentery

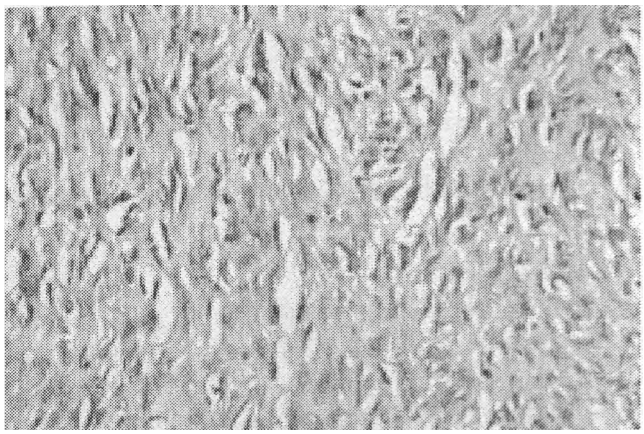

fig.4 GIST-spindle cell pattern(HEX400)

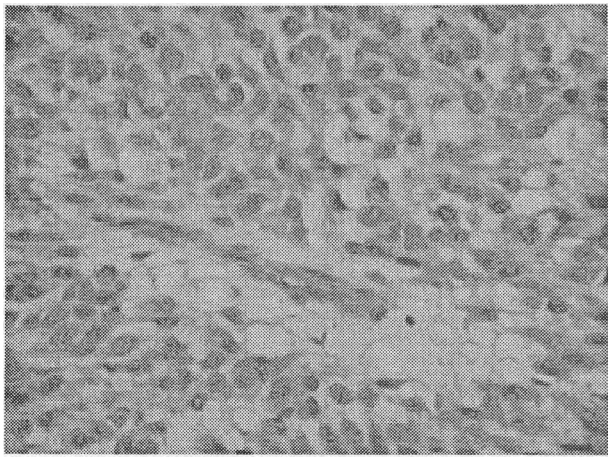

fig.5 GIST-epithelioid cell pattern(HEX400)

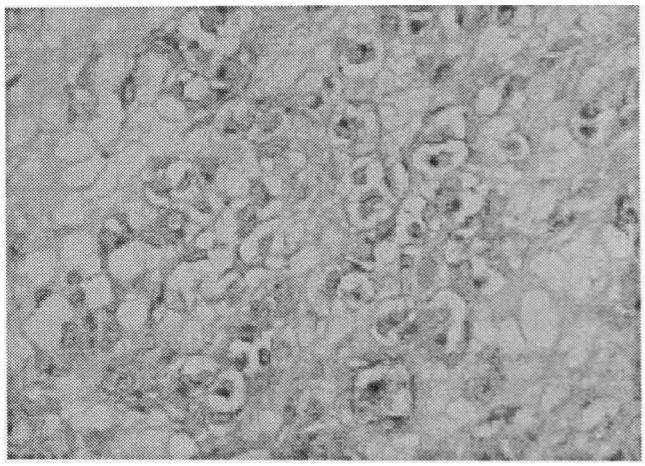

fig.6 GIST stained with a KIT(CD117) antibody(IHHX400)