Review Article

Gastrointestinal stromal tumors (GIST): A model for molecule-based diagnosis and treatment of solid tumors

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Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the human gastrointestinal (GI) tract. The c-kit receptor tyrosine kinase (KIT) is expressed by practically all GISTs, and gain-of-function mutations of KIT are present in most GISTs. Interstitial cells of Cajal (ICC) are the pacemaker of the peristaltic movement of the GI tract. Since signals through KIT are essential for development of ICC and since multiple GISTs develop from the hyperplastic lesion of ICCs in familial GIST patients with germline mutations of KIT, GISTs are considered to originate from ICC. Imatinib mesylate, which was developed for treatment of chronic myeloid leukemia (CML), was found to be useful for treatment of GISTs. Imatinib mesylate inhibits BCR-ABL fused tyrosine kinase that causes CML. Imatinib mesylate also inhibits the mutated KIT observed in most GISTs, and this explains the effectiveness of Imatinib mesylate on GISTs. GISTs appear to serve as a model for molecule-based diagnosis and treatment of solid tumors. (Cancer Sci 2003; 94: 315–320)

The gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the human gastrointestinal (GI) tract.[1, 2] GIST used to be a collective term referring to primary mesenchymal tumors of the GI tract,[2, 3] but now it is considered to be a particular tumor that originates from an interstitial cell of Cajal (ICC) or its precursor.[1, 4] ICC is a pacemaker of the peristaltic movement of the GI tract.[5] In 1998, we found the expression of c-kit receptor tyrosine kinase (KIT) in practically all GISTs, and the presence of gain-of-function mutations of KIT in most GISTs.[1] Metastatic GISTs had been resistant to all available chemotherapy.[6] However, soon after our report, Imatinib mesylate (STI-571), which was developed for the treatment of chronic myeloid leukemia (CML), was found to be useful for the treatment of metastatic GISTs as well.[7] Imatinib mesylate inhibits BCR-ABL fused tyrosine kinase and thereby kills CML cells.[8] Imatinib mesylate also inhibits normal KIT[9] as well as the mutated KIT found in most GISTs.[10] GISTs appear to serve as a good model for molecule-based diagnosis and treatment of solid tumors. In the present review, we describe the remarkable progress that has occurred recently in the diagnosis and treatment of GISTs.

KIT and its loss-of-function mutations

Our story about GISTs does not start from the classical histopathology of GI tumors, but from mutant mice that lack mast cells.[11, 12] Through these mutant mice, we became familiar with KIT,[13] then ICC[14] and finally arrived at GISTs.[1] The c-kit is the cellular homologue of the oncogene v-kit of HZ4 feline sarcoma virus, and encodes a receptor tyrosine kinase that is structurally similar to the receptors for macrophage colony-stimulating factor (M-CSF) and platelet-derived growth factor (PDGF).[15] These receptor tyrosine kinases have unique features: an extracellular (EC) domain made up of five immunoglobulin-like repeats, and a tyrosine kinase (TK) domain that is split into two domains by an insert sequence of variable length. The structure and amino acid sequence of KIT are well preserved in humans, mice and rats. For many years after the discovery of v-kit gene, it remained unclear whether KIT played any role in the development of human neoplasms.

The W locus of mice was demonstrated to encode KIT.[16, 17] Many types of loss-of-function mutants have been reported at the W locus. Among them, double heterozygous mice of W/W^v genotype are most frequently used. The W mutant allele encodes a truncated KIT without the transmembane domain; as a result, the extracellular domain is not expressed on the cell surface.[18, 19] The W^v mutant allele is a point mutation at the TK domain, resulting in a remarkable decrease in the TK activity.[18] W/W^v mice show five abnormalities due to the loss of KIT function: 1) anemia due to hypoproduction of erythrocytes, 2) white coat color due to the lack of melanocytes, 3) sterility due to depletion of germ cells, 4) depletion of mast cells,[11] 5) depletion of ICCs.[20] Abnormalities 1 to 3 have been known for a long time. We found the depletion of mast cells in 1978,[11] and Maeda *et al.*[20] found the depletion of ICCs in 1992.

The ligand of KIT was identified and named stem cell factor (SCF).[21] Since SCF is encoded by the Sl locus, homozygous or double heterozygous mutant mice at the W or Sl locus have the same phenotype. The most frequently used mutant mice of the Sl locus are of Sl/Sl^d genotype. Sl/Sl^d mice show anemia, white coat color, sterility, depletion of mast cells and depletion of ICCs.

We found spontaneous development of forestomach papillomas[22] and antral ulcers[23] in W/W^v and Sl/Sl^d mutant mice at around the same time when we discovered depletion of mast cells in these mice.[11, 12] We identified bile reflux from duodenum to stomach as a cause of the stomach lesions.[24] However, the mechanism of the bile reflux remained unclear. Since the depletion of ICCs was identified as the fifth abnormality of W/W^v and Sl/Sl^d mice[20, 25, 26] and since ICCs regulate the peristaltic movement of the GI tract,[5] the bile reflux is now attributed to the depletion of ICCs.

Gain-of-function mutation of KIT as a cause of mast cell neoplasms

Binding of dimerized SCF induces the dimerization of KIT.[27] This leads to autophosphorylation of KIT on tyrosine and to as-

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sociation of KIT with substrates such as phophatidylinositol 3-kinase (PI3K).[27] In human mast cell leukemia cell line HMC-1, KIT was constitutively phosphorylated on tyrosine, activated, and then could associate with PI3K without the addition of SCF. Furitsu *et al.*[28] found that KIT gene of HMC-1 cells was composed of normal, wild-type allele and mutant allele with point mutations resulting in substitution of Val-560 to Gly in the juxtamembrane (JM) domain and Asp-816 to Val in the tyrosine kinase-II (TK-II) domain. Amino acid sequences in the region of the two mutations are completely conserved in mouse, rat and human KIT.

In order to determine the causal role of these mutations in the constitutive activation, mutant KIT gene with Val-560 to Gly mutation in the JM domain or with Asp-816 to Val mutation in the TK-II domain was constructed and expressed in a human embryonic kidney cell line, 293T.[28] In the transfected cells, KIT with either mutation was abundantly phosphorylated on tyrosine and activated in the immune complex kinase reaction without the addition of SCF. Tsujimura *et al.*[29, 30] found the mutation corresponding to Asp-816 to Val of the human HMC-1 cell line in the P-815 mouse mastocytoma cell line (Asp-817 to Tyr) and the RBL-2H3 rat mast cell leukemia cell line (Asp-814 to Tyr). Both P-815 and RBL-2H3 cells showed constitutive activation of KIT without SCF. These KIT mutations were considered to induce the transformation of mast cells. In fact, the Asp-816 to Val mutation has been found in various types of mast cell neoplasms of human patients (Fig. 1).[31, 32] Moreover, in some dog mastocytomas, internal tandem duplication has been found in the JM domain of KIT.[33] Mast cell neoplasms are very rare in humans, but they are the most common malignant tumors in dogs. In some human germ cell tumors, Asp-816 to His mutation has been reported.[34]

To examine the transformation potential of the KIT activation mutation, we used the murine inerleukin-3 (IL-3)-dependent IC-2 mast cell line as a transfectant.[35] The IC-2 cells that had been established by Koyasu *et al.*[36] from murine cultured mast cells did not express KIT on the surface. The Asp-814 to Val or Val-559 to Gly murine type mutant KIT cDNA was introduced into IC-2 cells using a retrovirus vector. The mutant KIT expressed in IC-2 cells was constitutively phosphorylated on tyrosine and demonstrated kinase activity in the absence of SCF. IC-2 cells expressing mutant KIT showed factor-independent growth in suspension culture and produced tumors in nude athymic mice.[35] Introduction of the mutant KIT cDNAs also resulted in the transformation of the IL-3-dependent Ba/F3 murine pro-B cells.[37]

The mechanisms of constitutive activation were different in the cases of Val-559 to Gly JM mutation and Asp-814 to Val TK-II.[47] This was shown by chemical cross-linking analysis. A substantial fraction of phosphorylated KIT with Val-559 to Gly mutation dimerized, whereas phosphorylated KIT with Asp-814 to Val mutation did not. Tsujimura *et al.*[38] found another gain-of-function mutation at the JM domain of the FMA3 murine mastocytoma cell line. The KIT cDNA of FMA3 cells carried an in-frame deletion of 21 base pairs (bp). The FMA3-type KIT cDNA was introduced into IC-2 cells. The FMA3-type KIT was constitutively phosphorylated on tyrosine and activated. IC-2 cells expressing FMA3-type KIT grew in suspension culture without IL-3 and SCF, and became leukemic in nude athymic mice. Although the Val-559 to Gly mutation and the FMA3-type mutation with 21-bp deletion were different in nature, their biological effects were comparable.[37, 38]

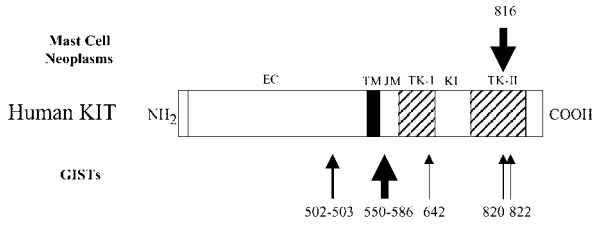

Fig. 1. KIT mutations reported in human mast cell neoplasms and GISTs. EC, extracellular domain; TM, transmembrane domain, JM, juxtamembrane domain; TK-I, tyrosine kinase-I domain; KI, kinase insert; TK-II, tyrosine kinase-II domain. Sizes of arrows roughly parallel the proportion of cases with each mutation. The mutation of the TK-II domain at position 820 has been reported only in a family with familial GISTs.

Sporadic GISTs

Loss-of-function mutation of KIT resulted in depletion of mast cells,[11] while gain-of-function mutation resulted in mast cell neoplasms.[28, 31, 32, 35, 38] Since loss-of-function mutation of KIT caused depletion of ICCs,[20, 25] it seems reasonable that gain-of-function mutation might induce neoplasms of ICCs. However, when we started examining this possibility, the existence of ICC-derived tumors was not known. Therefore, we examined whether any mesenchymal tumors in the human GI tract express KIT, by using immunohistochemistry[1] (Fig. 2). Authentic leiomyomas and schwannomas did not express KIT, but most tumors designated as GISTs did express it.[1] This suggested that GISTs originated from ICCs. Soon after the publication of our paper, Kindblom *et al.*[4] independently reported a similar result. Practically all gastric and intestinal tumors that were previously classified as leiomyomas and leiomyosarcomas express KIT, and consequently they are currently considered to be GISTs.[39]

The whole coding region of KIT was obtained from six GISTs and sequenced. Mutations were observed in 5 out of 6 GISTs (Fig. 1).[1] All mutations were detected in the JM domain, but not at identical sites. We next examined whether the KIT mutations found in the GISTs resulted in constitutive activation, by means of transient introduction of mutant KIT cDNAs into 293T human embryonic kidney cell line. The KIT mutants found in GISTs showed constitutive tyrosine phosphorylation in 293T cells without SCF.[1] The KIT mutants also exhibited constitutive kinase activation in *in vitro* kinase assay. To investigate the biological consequences of mutant KIT, we introduced the KIT mutations found in GISTs into mouse KIT cDNA and then stably transfected it into the IL-3-dependent Ba/F3 murine pro-B cell line. Ba/F3 cells with the mutated murine KIT grew autonomously in nude mice.[1]

Mutations in the JM domain were most common in GISTs (~80%), and therefore were firstly found. Mutations in other

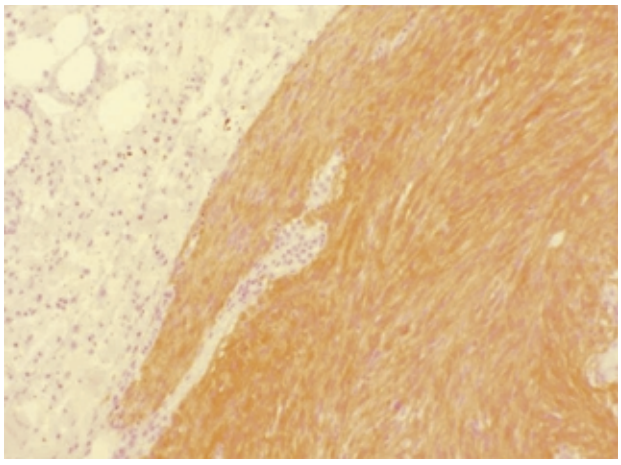

Fig. 2. Expression of KIT in a GIST revealed by immunohistochemistry.

domains were found thereafter; duplication of two particular amino acids in the EC domain (~5%),[40–42] a point mutation at the TK-I domain (~3%),[40, 41] and a point mutation at the TK-II domain (~2%) (Fig. 3).[43] The site of this TK-II mutation was different from that of the Asp-816 to Val mutation, which is found in human mast cell neoplasms (Fig. 1).[31, 32] In approximately 10% of GISTs, no KIT mutations were found even when the whole coding region was examined using fresh materials.[40, 42]

Recently Heinlich *et al.*[44] investigated the cause of GISTs without KIT mutations and found gain-of-function mutations of PDGF receptor α in about one-third of GISTs without KIT mutations. We independently obtained a similar result (Hirota *et al.*, unpublished data). Mutated PDGF receptor α activated not only itself, but also wild-type KIT. Since the signal transduction pathway of PDGF receptor α is similar to that of KIT,[27] gain-of-function mutation of PDGF receptor α by itself may cause transformation of ICCs. Another possibility is that the mutated PDGF receptor α may affect ICCs through activation of the wild-type KIT. These two possibilities are not mutually exclusive (Hirota *et al.*, unpublished data).

Allander *et al.*[45] examined the gene expression profile of KIT mutation-positive GISTs by cDNA microarray and found a remarkably distinct and uniform expression profile. This homogeneity suggests that the molecular pathogenesis of GIST involves expansion of a clone that has acquired an activating mutation in KIT without the extreme genetic instability found in the common epithelial cancers. This is consistent with the results of Corless *et al.*,[46] who found mutated KIT in very small GISTs that were incidentally found at the time of surgical operations for other purposes.

The presence of KIT mutation has been correlated with survival of GIST patients.[47] We extracted DNA samples from paraffin sections of 124 GIST cases.[47] Exon 11 encoding the JM domain and exon 17 encoding the TK-II domain were amplified by PCR and sequenced. Survival was better in patients without KIT mutation than in patients with KIT mutation.[47] However, the mutation rate was rather low (57%) in this study. When the whole KIT coding region was sequenced in fresh materials, the observed mutation rate increased to ~90%.[42] In such studies, the presence of KIT mutation did not significantly correlate with survival.

There is a tendency that GISTs that are small (<2 cm in diameter) and show low mitotic activity (<5/50 high-power fields) have a good prognosis. However, a few GISTs apparently lacking mitotic activity may metastasize.[39] It is difficult to clearly divide GISTs to benign and malignant classes. Therefore, GISTs are practically classified into low-risk, intermediate-risk and high-risk groups.[48] Even when GISTs do metastasize, the sites of metastasis are limited to the liver and peritoneal cavity.

Familial GISTs

Most GISTs are sporadic, but we found three families with germline mutation of KIT and development of multiple GISTs.[49–51] Firstly, we found multiple GISTs in 60-year-old Japanese woman who received two surgical operations due to intestinal obstruction.[49] A nephew of this woman also suffered from multiple GISTs. Analysis of the family pedigree revealed that many family members suffered from intestinal obstruction that may be attributable to multiple GISTs. Although the GISTs of the 60-year-old woman and her nephew were benign, a niece of the woman died of malignant GISTs that disseminated to the peritoneal cavity. DNA was extracted from GISTs and leukocytes of the woman and the nephew. An identical mutation was found in the JM domain of KIT, while this mutation was not detected in leukocytes obtained from other family members in whom GISTs were not observed.[49]

The second family was originally reported by O'Brien *et al.*[52] under the diagnosis of multiple familial gastrointestinal autonomic nerve tumors (GANTs). Multiple GANTs were observed in a mother and a daughter, and at least one GANT metastasized to the liver of the daughter. O'Brien *et al.* kindly made available to us the paraffin blocks of the GANTs and surrounding tissues. An identical JM mutation of KIT was found in both GANTs and the normal surrounding tissues of the mother and the daughter.[50] This showed that the multiple familial GANTs were identical to familial GISTs. Since sporadic GANTs also showed JM mutations of KIT, GANTs in general are now considered to be a subtype of GISTs with particular histological characteristics.[48]

The third family showed a unique symptom. The family members with multiple GISTs claimed dysphagia, a difficulty in swallowing.[51] However, no mechanical obstruction was found, and the esophagus was not remarkably dilated. Endoscopic ultrasonography at the esophagocardiac junction showed a thickened hyperechoic layer between the circular and longitudinal muscle layers, suggesting hyperplasia of ICCs at the myenteric plexus layer. Manometry showed low resting lower esophageal sphincter pressure and abnormal simultaneous contractions of the esophagus without normal peristalsis. These findings indicate that the dysphagia of this family is different

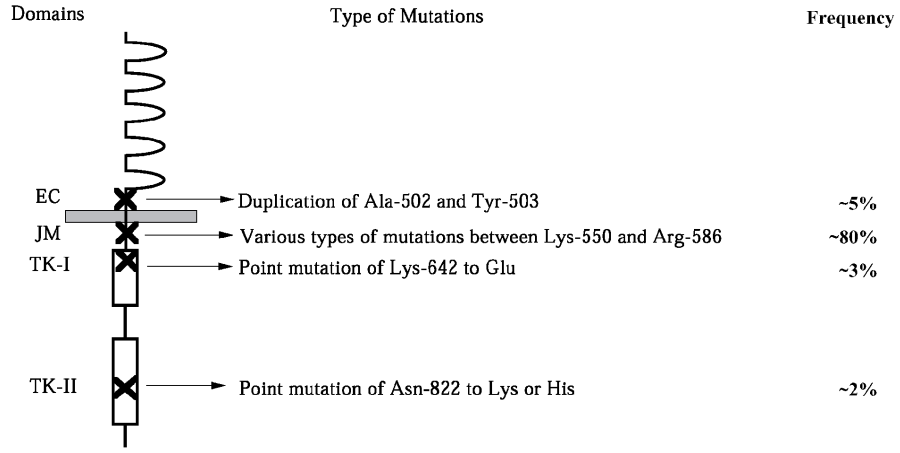

Fig. 3. KIT mutations in sporadic GISTs. KIT mutations were detected in ~90% of GISTs, but not in ~10% of them. The frequency of each mutation in sporadic GISTs is shown. EC, extracellular; JM, juxtamembrane; TK-I, tyrosine kinase-I; TK-II, tyrosine kinase-II.

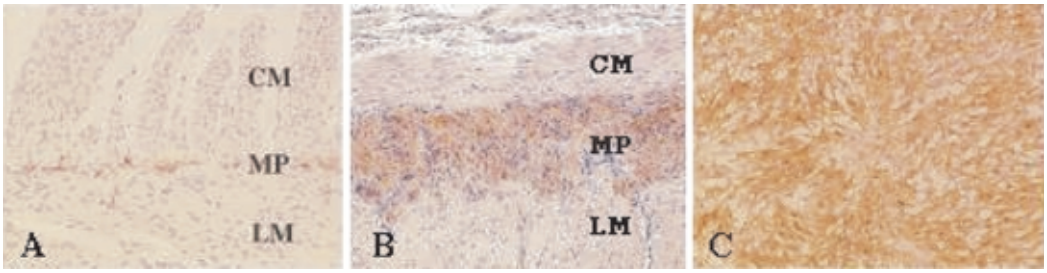

Fig. 4. Hyperplasia of ICCs in a familial GIST patient. A, ICCs in the small intestine of a normal subject. ICC hyperplasia (B) and a GIST (C) in a familial GIST patient with germline mutation of the c-kit gene. Each monoclonal GIST develops from a polyclonal hyperplastic lesion of ICCs. KIT expression is demonstrated by immunohistochemistry. CM, circular muscle layer; MP, myenteric plexus layer; LM, longitudinal muscle layer.

from typical achalasia.[51] The family members with dysphagia showed germline gain-of-function mutation of KIT at the TK-II domain (Asp-820 to Tyr), which is different from the Asp-816 to Val mutation found in mast cell neoplasms (Fig. 1). Although this family is the first case of familial GISTs with the TK-II domain mutation, it was not clear whether the presence of dysphagia was related to this type of KIT mutation.

In addition to the above-mentioned three families, other three families with multiple GISTs and gain-of-function mutation of KIT have been described.[53–55] Two families possessed KIT mutation at the JM domain,[54, 55] and the remaining one family, at the TK-I domain.[53]

In addition to multiple GISTs, hyperpigmentation of the skin[49, 54, 55] and/or mast cell tumors[55] were reported in some families. Most family members with multiple GISTs survive and can have offspring, and moreover, various KIT mutations cause the familiar GISTs. Therefore, this disease entity may be a more common cancer syndrome than is presently supposed.

In familial GISTs, remarkable hyperplasia of ICCs was observed in the small and large intestines.[50, 51, 53, 55] ICCs constitute only one or two cell layers in the normal intestine, whereas hyperplasia with 10 to 20 cell layers was observed in familial GIST patients (Fig. 4). Multiple GISTs developed from the hyperplasia. We examined the clonality of the hyperplastic lesions and GISTs in patients with familial GISTs.[56] To examine the clonality of human samples, random inactivation of one of two female X chromosomes is commonly used. Currently, the polymorphism of the human androgen receptor (HUMARA) locus, which is located on the X chromosome, is most frequently used as it has a highly polymorphic tandem repeat. Samples obtained with a laser capture microdissection system were used. The hyperplastic lesion was polyclonal and each GIST derived from the hyperplastic lesion was monoclonal. This clearly indicated that each GIST was derived from an ICC.[56]

Molecular target therapy of GISTs

Treatment of GISTs has changed dramatically since the introduction of Imatinib mesylate. Since GISTs were not routinely distinguished from other mesenchymal tumors of the peritoneal cavity, it is difficult to determine accurately the response rate of GISTs to conventional chemotherapeutic agents. However, it appears to be less than 10%.[6]

Imatinib mesylate was initially developed at Ciba-Geigy (now Novartis) as a specific inhibitor of the PDGF receptor.[57] However, it was found to be a potent and selective inhibitor of ABL TKs, including BCR-ABL. BCR-ABL is a fusion protein produced by balanced translocation between chromosomes 9 and 22. BCR-ABL has uncontrolled TK activity, which leads to constitutive intracellular signaling and induces the development of CML. Druker and scientists at Novartis showed that Imatinib mesylate selectively blocked BCR-ABL and killed CML cells *in vitro*.[8] They also examined the utility of this finding by means of a clinical trial. Data from the clinical trial were published in early 2001.[58] The US Food and Drug Administration approved Imatinib mesylate as a safe and effective therapy for CML patients in May 2001. The efficacy of Imatinib mesylate is striking, with greater than a 90% complete response rate in chronic-phase CML.[58]

As already mentioned, we reported gain-of-function mutations of KIT in GISTs, in 1998.[1] We speculated that the constitutive activation of KIT functioned as a critical step in the development of GISTs. In addition to the inhibitory effect of Imatinib mesylate on the PDGF receptor and ABL, Buchdunger *et al.*[9] found an inhibitory effect on wild-type KIT. Some reports showed that Imatinib mesylate also inhibited various types of mutated KIT found in GISTs.[10, 59] Before the publication of these data, the treatment of the first GIST patient with Imatinib mesylate started in Finland. The patient's tumor expressed KIT and contained a JM mutation of KIT. The patient had progressive, widely metastatic disease after failure of previous extensive therapy, including multiple surgical procedures, chemotherapy, and even investigational antiangiogenic therapy.[6, 7] Within a few weeks of starting daily oral administration of Imatinib mesylate, the patient exhibited an objective clinical response that was maintained for more than 18 months. This encouraging result was published in 2001.[7] A multicenter trial on advanced GISTs was done and reported in 2002.[60] The US Food and Drug Administration has approved Imatinib mesylate as effective therapy for advanced GIST patients, and the role of Imatinib mesylate in adjuvant therapy is now being evaluated.

The position of KIT mutation appears to be related to the effectiveness of Imatinib mesylate. Since JM domain mutations of KIT are found in 80% of all GISTs,[40, 42] the therapeutic effect of Imatinib mesylate on such GISTs with JM mutations is definitive. In contrast, effectiveness is not so apparent in the case of GISTs without KIT mutations.[6] The proportions of EC, TK-I or TK-II mutations are small, and therefore it is rather difficult to evaluate the effectiveness of Imatinib mesylate against such GISTs with EC, TK-I or TK-II mutation. The Asp-816 to Val TK-II mutation has not been observed in GISTs, but it was frequently found in human mast cell neoplasms.[31, 32] Imatinib mesylate did not inhibit the growth of COS cells, into which KIT cDNA with the Asp-816 to Val mutation had been introduced.[61]

Remaining problems

The definition of GISTs as tumors of ICC origin was established after our findings that most GISTs express KIT and that most GISTs have gain-of-function mutations of KIT.[1] In the 4 years after those findings, the treatment of GISTs has changed dramatically. The primary cause of such rapid progress was the prior existence of Imatinib mesylate as a molecular target drug for CML.[6] GIST and CML appear to have similar pathogenic

mechanisms, each being driven by a single dominant stimulus.[6] On the other hand, most human carcinomas are caused by more complicated mechanisms. If carcinomas can be divided into certain types with definitely identifiable molecular changes, a specific molecule-based therapy might be possible for each type.

In spite of the remarkable progress, there are many problems remaining to be clarified in the pathogenesis and therapy of GISTs. The KIT mutation appears to be an early event in the development of a GIST.[46] Although polyclonal hyperplasia of ICCs was observed throughout the GI tract of familial GIST patients, benign monoclonal GISTs developed within the hyperplasia.[56] In addition to the KIT mutation, other factors appear to be necessary for development of even benign GISTs. Such factors should be identified.

The signal transduction pathways from KIT should be clarified. Frequent development of GISTs in neurofibromatosis (NF)-1 patients is known.[62] Loss-of-function mutation of the NF-1 gene, which causes neurofibromatosis,[62] may be involved in the development of GISTs. The NF-1 protein negatively regulates the signal transduction of RAS.[62] Interaction of the KIT signal with RAS may play an important role. Further investigation of GISTs, which developed in NF-1 patients, may clarify the mechanisms of interaction among KIT, RAS and NF-1.

Although multiple benign GISTs develop in persons with germline mutation of KIT,[49–51, 53–55] most benign GISTs do not become malignant. More than half of such persons with germline mutation of KIT survive to rather old age without development of malignant GISTs. This suggests that KIT mutation alone does not cause malignant transformation of GISTs. This is consistent with data on the prognosis of patients with sporadic GISTs. The proportion of GISTs with KIT mutation reached ~90% when the whole coding region of the KIT gene obtained from fresh materials was sequenced.[40, 42] When prognosis was compared between GISTs with KIT mutation and those without KIT mutation, no significant difference was found.[6] Factors causing the transformation of benign GISTs to malignant GISTs should be examined. If such factors are identified, the selection of patients for adjuvant therapy with Imatinib mesylate will become much easier.

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