**TITLE OF THESIS**

# CAS-L WAS OVER-EXPRESSED IN IMATINIB RESISTANT GASTROINTESTINAL STROMAL TUMOR CELLS

( Cas-L はイマチニブ耐性の消化管間質腫瘍細胞に過剰発現していた)

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**Abstract:** Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors in the gastrointestinal tract, and they have been refractory to conventional chemotherapy with a median survival of only 12 to 19 months in patients with unresectable or metastatic diseases. Most GISTs express KIT, a receptor tyrosine kinase encoded by proto-oncogene *KIT*. Approximately 90% of GISTs have somatic gain-of-function mutations of the *KIT* and half of GISTs without *KIT* mutations have been demonstrated to have gain-of-function mutations in the *PDGFRA* (platelet-derived growth factor receptor-α) that encodes another receptor tyrosine kinase. Imatinib mesylate (imatinib) is a specific tyrosine kinase inhibitor that acts with BCR-ABL, PDGFRA, KIT, and has been used to treat GISTs that have constitutive activating mutations in KIT. Although more than 80% of inoperable GISTs patients have dramatic clinical benefits from imatinib, most of these patients will eventually progress. The effect of imatinib is different in various types of *KIT* and *PDGFRA* mutations, and the secondary resistance against imatinib is often acquired by the secondary mutation or amplification of the *KIT* or *PDGFRA*. Since only about half of GISTs with secondary resistance to imatinib have secondary mutations in *KIT* or *PDGFRA*, the mechanisms of the remaining secondary resistance have not yet been fully elucidated.

To explore additional mechanisms of imatinib-resistant GISTs, I generated resistant cells from imatinib-sensitive GIST-T1 cells with heterozygous del(V560-Y578) in exon 11 by exposing them to increasing concentrations of imatinib for 6 months. The resultant cell line, GIST-T1 IR showed resistant to imatinib in vitro with $IC_{50}$ 5-7 μM of imatinib, and phosphorylated KIT and its downstream intermediates such as AKT and JAK2 with the presence of 1 μM imatinib (Figure 1). To see whether there were any new mutations occurring in *KIT*, *PDGFRA, PKCθ,* and *JAK2*, I sequenced all exons of these genes of GIST-T1 IR cells; however, I did not find any new mutation. I next used DNA micro-

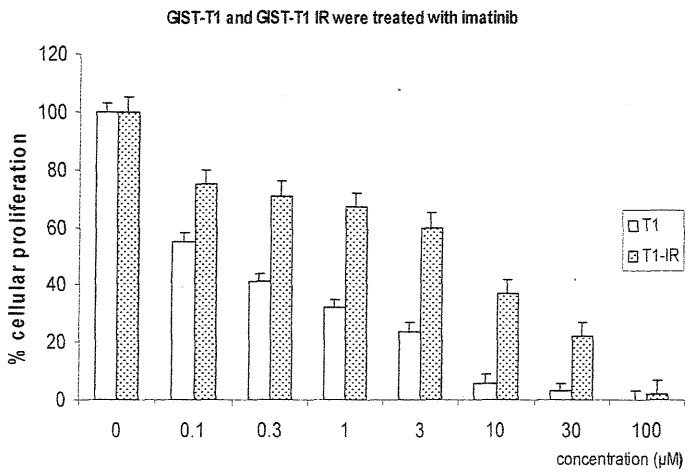
array to check the expression profile of GIST-T1 IR cells, I found over-expression of *Cas-L* in the resistant cells with 513 fold higher than that in the parental cells. The over-expression of Cas-L was also observed in GIST-T1 IR cells at protein level (Figure 2). Cas-L is a scaffolding protein at focal adhesion sites; its action involves regulated assembly of protein complexes. The interaction of Cas-L, FAK (focal adhesion kinase) and SRC kinases plays an important role at adhesion sites, over-expression or hyper-activation of one of these proteins leads to the activation of the others. I therefore checked the activations of SRC and FAK in GIST-T1 IR cells. As expected, I found hyper-activation of SRC and FAK in GIST-T1 IR cells comparing with those in GIST-T1 cells (Figure 2).

To verify whether the increased SRC signaling underlies the acquired resistance to imatinib of GIST-T1 IR cells, I used MTT assay to examine the effect of PP1, a SRC inhibitor, on cellular proliferation of GIST-T1 IR cells. PP1 (10 μM) or imatinib (1 μM) alone could suppress the proliferation of GIST-T1 IR by 40% and 33%, respectively. However, the combination of them showed strong inhibitory effect on the growth of the resistant cells by suppressing 84% of cellular proliferation. Additionally, the combination of 10 μM PP1 and 1 μM imatinib drastically suppressed the activation of KIT and SRC kinases in GIST-T1 IR cells (Figure 3).

Tyr568, a SRC binding site of KIT, was deleted in the parental and the resistant cells and it is likely that SRC kinase did not have a crucial function in the parental cells, but it turned out to be activated in the resistant cells. The next important question is whether or not the SRC signaling activities are dependent on KIT signaling pathway. In order to examine the relation between KIT, Cas-L and SRC kinases, I transfected *KIT siRNA* and *Cas-L siRNA* into GIST-T1 IR cells. The expression of Cas-L and the phosphorylation of SRC were almost vanished in *KIT siRNA* transfected GIST-T1 IR cells, suggesting that the expression of Cas-L and the activation of SRC kinase were dependent on KIT signaling. Additionally, the phosphorylation of SRC was decreased in corresponding to the decreased expression of Cas-L, suggesting that Cas-L expression was important for SRC activation. To scrutinize more the important role of Cas-L and SRC kinase in inducing imatinib-resistance of GIST-T1 IR cells, I next checked the effect of imatinib on GIST-T1 IR cells transfected with *Cas-L*

*siRNA*. As expected, *Cas-L siRNA* transfected GIST-T1 IR cells turned out to become again sensitive to imatinib with $IC_{50}$ < 0.1 μM (Figure 4)

I herein reported for the first time the over-expression of Cas-L and its important role in imatinib-resistance GISTs. This should be of interests to oncologists to further investigate signaling pathways related to Cas-L/SRC in GISTs.



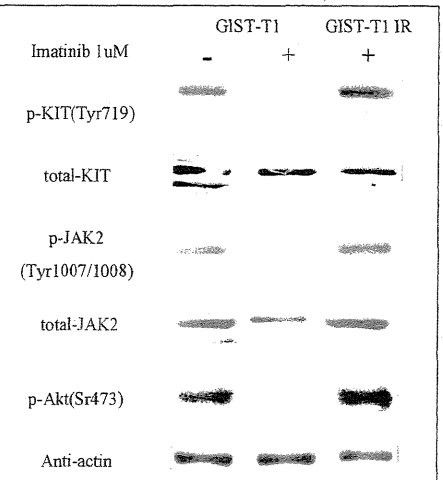




***Figure 1**: GIST-T1 IR cells showed resistant to imatinib. The phosphorylated of KIT and its downstream intermediates remained activated in the presence of 1 μM imatinib.*

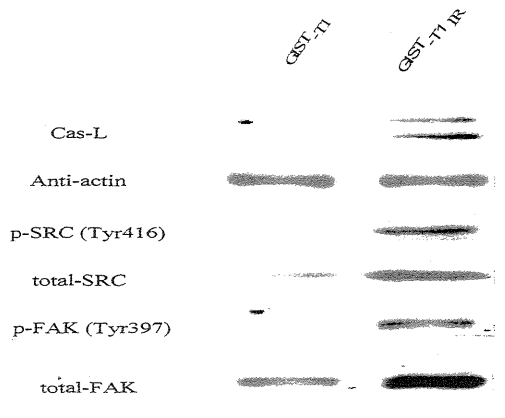



***Figure 2:** Cas-L was over-expressed; SRC and FAK were hyper-activated in GIST-T1 IR cells*

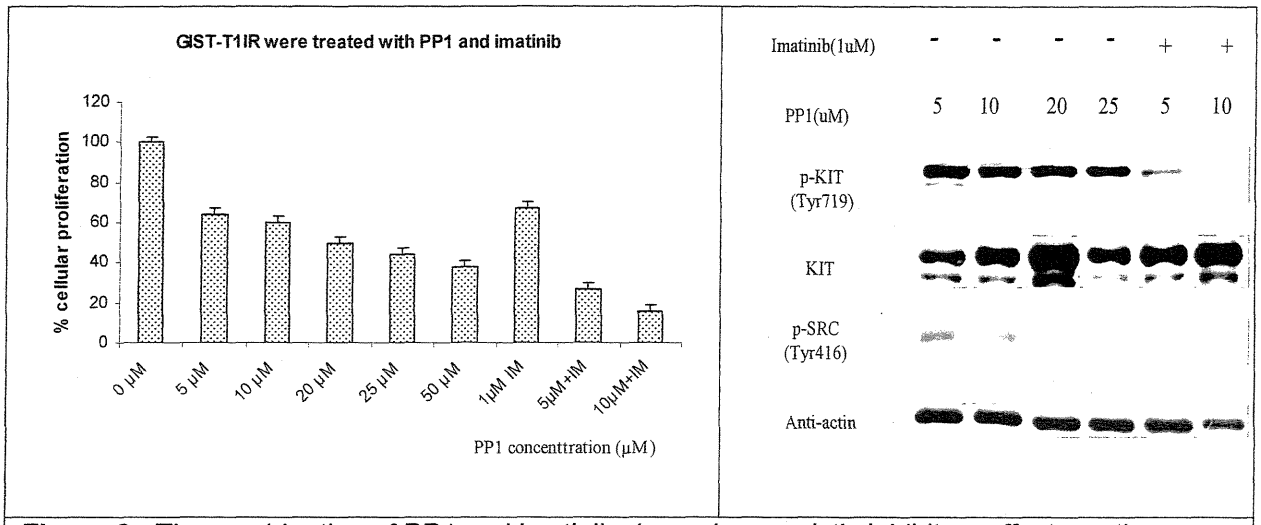



***Figure 3**: The combination of PP1 and imatinib showed synergistic inhibitory effects on the cellular proliferation as well as the phosphorylation of KIT and SRC in GIST-T1 IR cells*

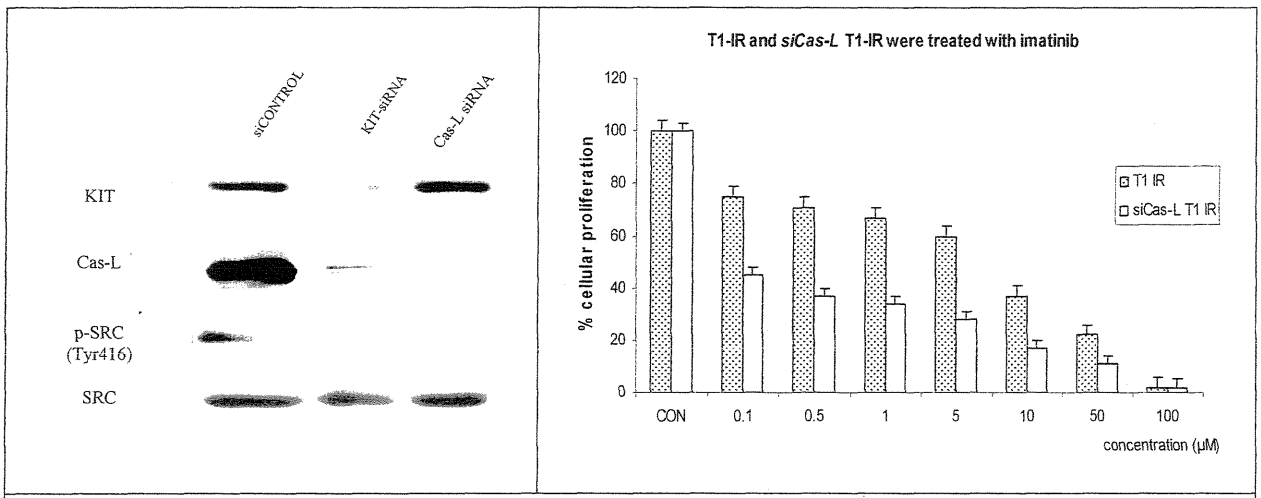



***Figure 4**: Cas-L and SRC were dependent on KIT signaling. Cas-L depletion sensitized the resistant GIST-T1 IR cells to imatinib.*