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Protein tyrosine phosphatase receptor type E (PTPRE) regulates the activation of wild-type KIT and KIT mutants differently

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ABSTRACT

Activation of receptor tyrosine kinases needs tight control by tyrosine phosphatases to keep their normal function. In this study, we investigated the regulation of activation of the type III receptor tyrosine kinase KIT by protein tyrosine phosphatase receptor type E (PTPRE). We found that PTPRE can associate with wild-type KIT and inhibit KIT activation in a dose-dependent manner, although the activation of wild-type KIT is dramatically inhibited even when PTPRE is expressed at low level. The D816V mutation of KIT is the most frequently found oncogenic mutation in mastocytosis, and we found that PTPRE can associate and inhibit the activation of KIT/D816V in a dose dependent manner, but the inhibition is much weaker compared with wild-type KIT. Similar to mastocytosis, KIT mutations are the main oncogenic mutations in gastrointestinal stromal tumors (GISTs) although GISTs carry different types of KIT mutations. We further studied the regulation of the activation of GISTs-type KIT mutants and other mastocytosis-type KIT mutants by PTPRE. Indeed, PTPRE can almost block the activation of GISTs-type KIT mutants, while the activation of mastocytosis-type KIT mutants is more resistant to the inhibition of PTPRE. Taken together, our results suggest that PTPRE can associate with KIT, and inhibit the activation of both wild-type KIT and GISTs-type KIT mutants, while the activation of mastocytosis-type KIT mutants is more resistant to PTPRE.

1. Introduction

Receptor tyrosine kinase KIT is a member of the type III family of receptor tyrosine kinases. Similar to other family members (PDGFR, FLT3, and CSF-1R), KIT has five immunoglobulin-like extracellular domains, a transmembrane domain, and a kinase domain. Upon the binding of its ligand stem cell factor (SCF), two KIT monomers dimerize and their kinase activity is activated, certain tyrosine residues are phosphorylated and serve as docking sites for downstream signaling molecules, leading to activation of signals that mediate cellular responses such as cell survival, proliferation and differentiation [1,2].

Oncogenic mutations of KIT have been identified in malignancies such as mastocytosis, gastrointestinal stromal tumors (GISTs), and acute myeloid leukemia. Mastocytosis is characterized by excessive mast cell infiltration in tissues, gene sequencing showed that KIT mutations are dominant mutations in mastocytosis with the D816V mutation in exon 17 of KIT most commonly occuring [3–6]. Similar as mastocytosis, GISTs are dominated by KIT mutations as well although they harbor different types of KIT mutations. In GISTs, KIT mutations mainly map to exon 11 of KIT, followed by exon 9, 13 and 14 [7–12]. The reason why mastocytosis and GISTs carry different types of KIT mutations is not well understood yet. Targeted therapies against KIT have been approved and successfully used in clinic. KIT inhibitors Imatinib, sunitinib and regorafenib respectively, are used as first, second and third-line targeted therapy of GISTs [13–15] while no KIT inhibitor has been approved for mastocytosis treatment yet due to the poor response of KIT mutants to these inhibitors in mastocytosis.

Phosphatases can dephosphorylate their targets and play important roles in the regulation of signal transduction activated by kinases. PTPRE is a receptor type tyrosine phosphatase, it has a transmembrane domain and it is expressed at cell surface. Previous studies have shown that PTPRE can associate with the insulin receptor and inhibit its

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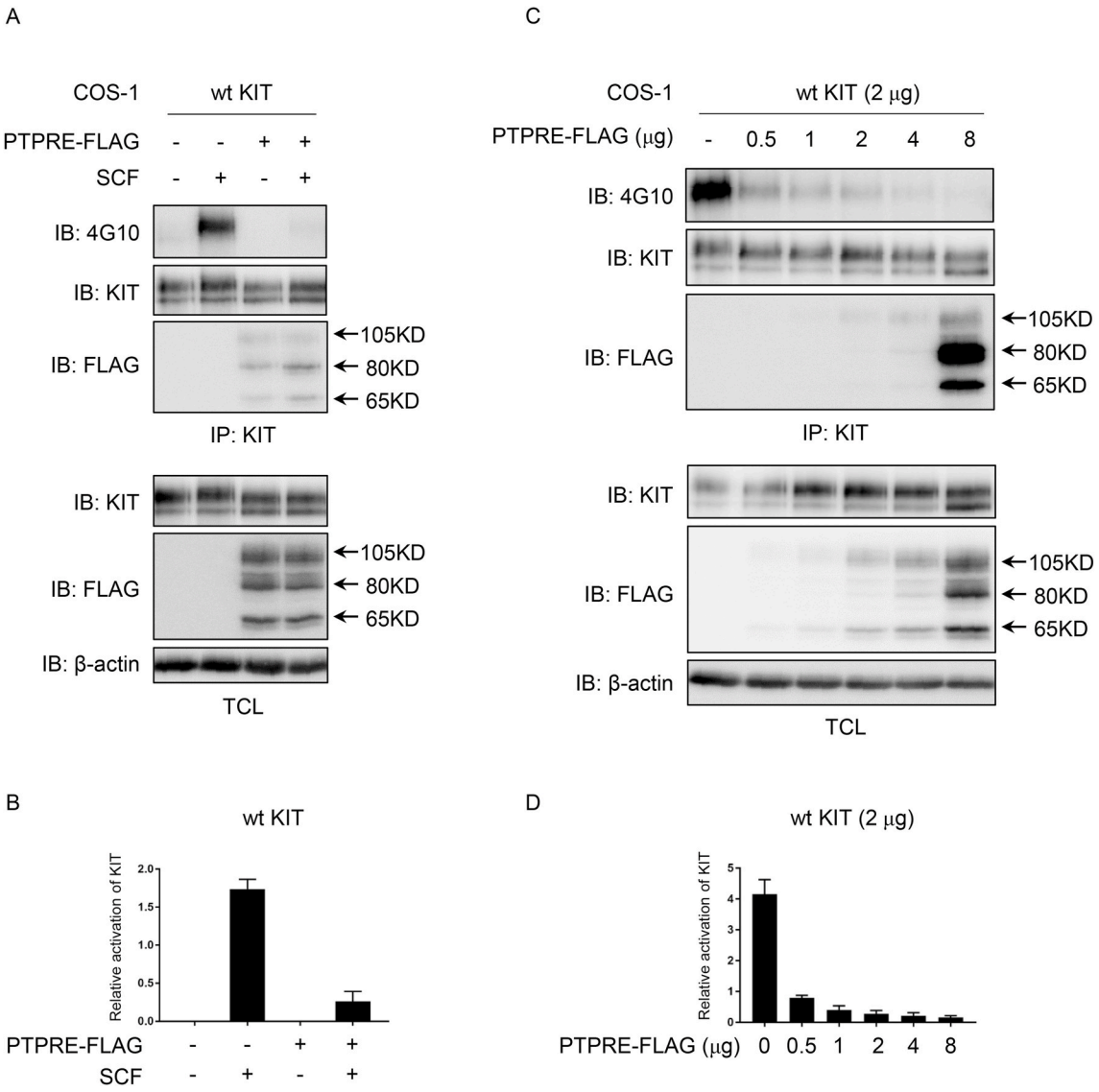

Fig. 1. PTPRE associates with wild-type KIT and inhibits its activation, A. Wild-type KIT expressing plasmid (2 μg) and PTPRE expressing plasmid (2 μg) or empty vector as control were transfected into COS-1 cells, in the next day, cells were starved overnight and lysed after stimulation with SCF for 5 min. KIT was pulled down using a KIT antibody to detect its activation and association with PTPRE. B. Quantification of the wild-type KIT activation shown in A. C. Wild-type KIT expressing plasmid (2 μg) and the indicated amount of PTPRE expressing plasmid were transfected into COS-1 cells, cells were stimulated with SCF for 5 min and treated as described in A. D. Quantification of the KIT activation shown in C.

activation [16], and inhibit PDGFRB signaling [17]. In this study, we investigated the regulation of KIT activation by PTPRE. We found the different response of wild-type KIT, GIST-type KIT mutants and mastocytosis-type KIT mutants to PTPRE. Our results suggested the differential activation features of wild-type KIT and different types of KIT mutants, which may contribute to the understanding of tissue specific distribution patterns of KIT mutations, and better design of drugs for the treatment of malignancies carrying KIT mutants.

2. Materials and methods

2.1. Reagents and plasmids

Phosphor-tyrosine antibody 4G10 was purchased from Millipore (Billerica, MA), KIT antibody was described previously [18], HRP conjugated β-actin antibody, HRP conjugated goat anti-mouse IgG antibody, and HRP conjugated goat anti-rabbit antibody were from Santa Cruz Biotechnology (Dallas, TX), FLAG antibody was from Sigma (St. Louis, MO). Lipofectamine 2000 and protein G-sepharose beads were from Thermofisher scientific (Waltham, MA), chemiluminescent HRP substrate was from Millipore (Billerica, MA). Human KIT cDNA was described previously [19], human PTPRE cDNA in pcDNA3.1 with FLAG tag at 3′ end was ordered from FenghuiBio (Changsha, China). QuikChange mutagenesis kit was purchased from Agilent (La Jolla, CA) and used according to the manufacturer's instructions. Recombinant human stem cell factor (SCF) was from ORF genetics (Kópavogur, Iceland).

2.2. Cell culture

COS-1 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) plus 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. Cells at 60–80% confluence were transfected with KIT and/or PTPRE expressing plasmids using lipofectamine 2000 according to the manufacturer's instructions.

2.3. Cell stimulation, immunoprecipitation (IP) and western blotting

COS-1 cells were washed with PBS for three times to remove serum,

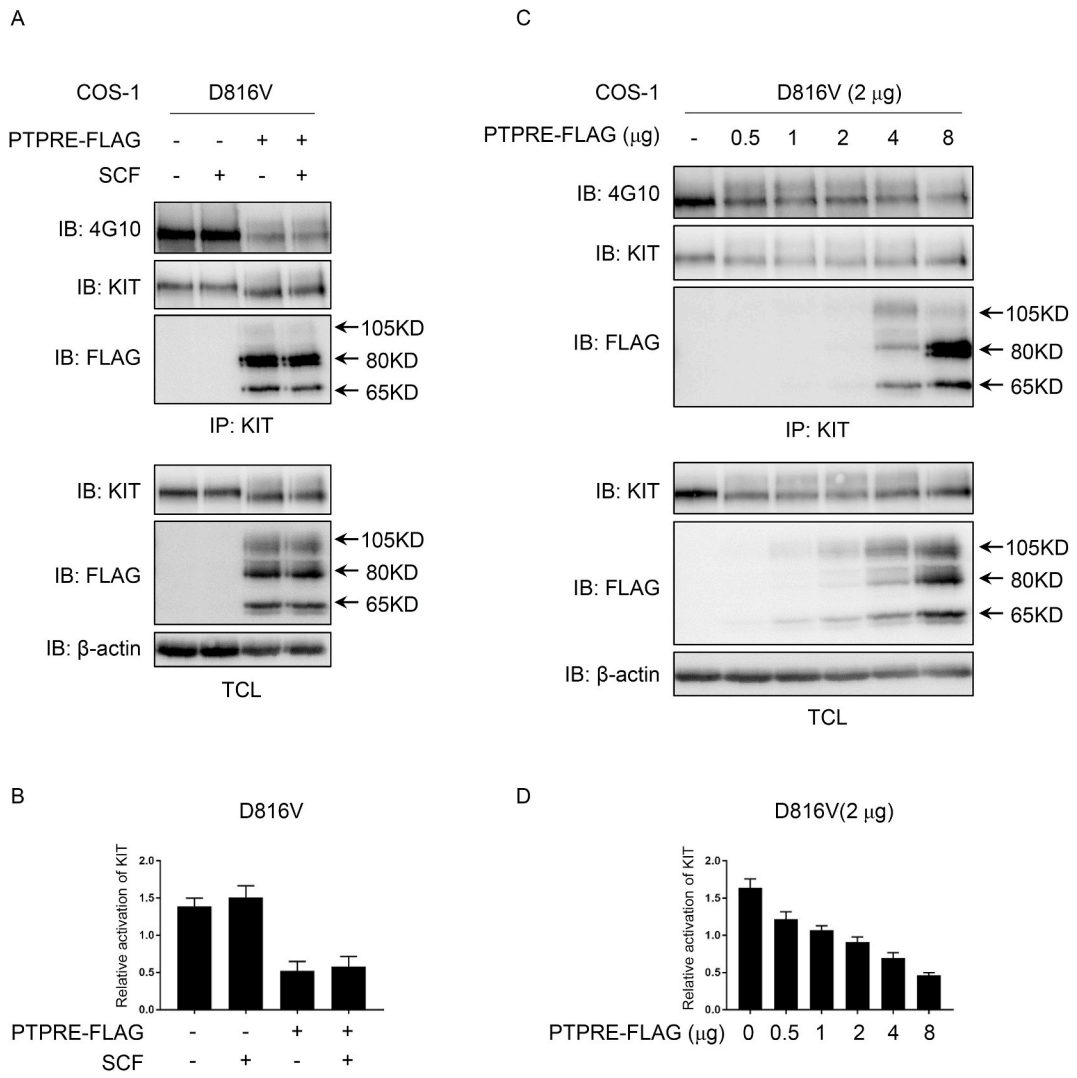

Fig. 2. The activation of KIT/D816V is more resistant to PTPRE, A. KIT/D816V expressing plasmid (2 μg) and PTPRE (2 μg) expressing plasmid or empty vector as control were transfected into COS-1 cells, in the next day, cells were starved overnight and lysed. KIT was pulled down using a KIT antibody to detect its activation and association with PTPRE. B. The activation of KIT/D816V were quantified. C. KIT/D816V expressing plasmid (2 μg) and the indicated amount of PTPRE expressing plasmid were transfected into COS-1 cells, cells were treated as described in A. D. KIT/D816V activation was quantified.

and starved overnight in DMEM without serum. Cells were then stimulated with 100 ng/ml SCF for 5 min at 37°, after washing with cold PBS, cells were lysed in a lysis buffer containing 1% Triton X-100, 25 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 2 mg/ml Trasylol, 1 mM Na_3VO_4 and 1 mM phenylmethylsulfonyl fluoride. Cell lysates were centrifuged for 5 min and supernatants were proceeded for Western blot or immunoprecipitation. For Western blot, cell lysates were separated by SDS-PAGE and transferred into PVDF membrane. After blocking, membranes were incubated with indicated antibody followed by washing and incubation with HRP conjugated secondary antibody. After washing, membranes were developed with chemiluminescent HRP substrate, and signals were captured by Bio-rad instrument. For immunoprecipitation, cell lysates were incubated end-over-end with indicated antibody for 1 h at 4°, followed by incubation with protein G-sepharose beads. After washing, the immunoprecipitated proteins were separated by SDS-PAGE and examined by Western blot.

3. Results

3.1. PTPRE associates with wild-type KIT and inhibits its activation in a dose-dependent manner

PTPRE is a phosphatase that can catalyze the removal of the phosphate group from tyrosine residues in its substrates, it has been shown that PTPRE can inhibit the activation of receptor tyrosine kinases such as insulin receptor and PDGFRB [16,17]. In order to know that whether PTPRE regulates the activation of KIT, we co-expressed wild-type KIT and PTPRE in COS-1 cells. By immunoprecipitation with KIT antibody, PTPRE was pulled down together with KIT (Fig. 1A), suggesting the physical association of PTPRE with KIT. Detection with phosphor-tyrosine antibody further showed that KIT phosphorylation is almost blocked in the presence of PTPRE (Fig. 1A and B), indicating a strong inhibition of KIT activation by PTPRE. We next asked whether the inhibition of KIT activation by PTPRE was dependent on the dose of PTPRE. A constant dose of wild-type KIT was co-expressed with various doses of PTPRE. The results showed that PTPRE strongly inhibits KIT activation even at low dose and increased amount of PTPRE further inhibits the activation of KIT (Fig. 1C and D).

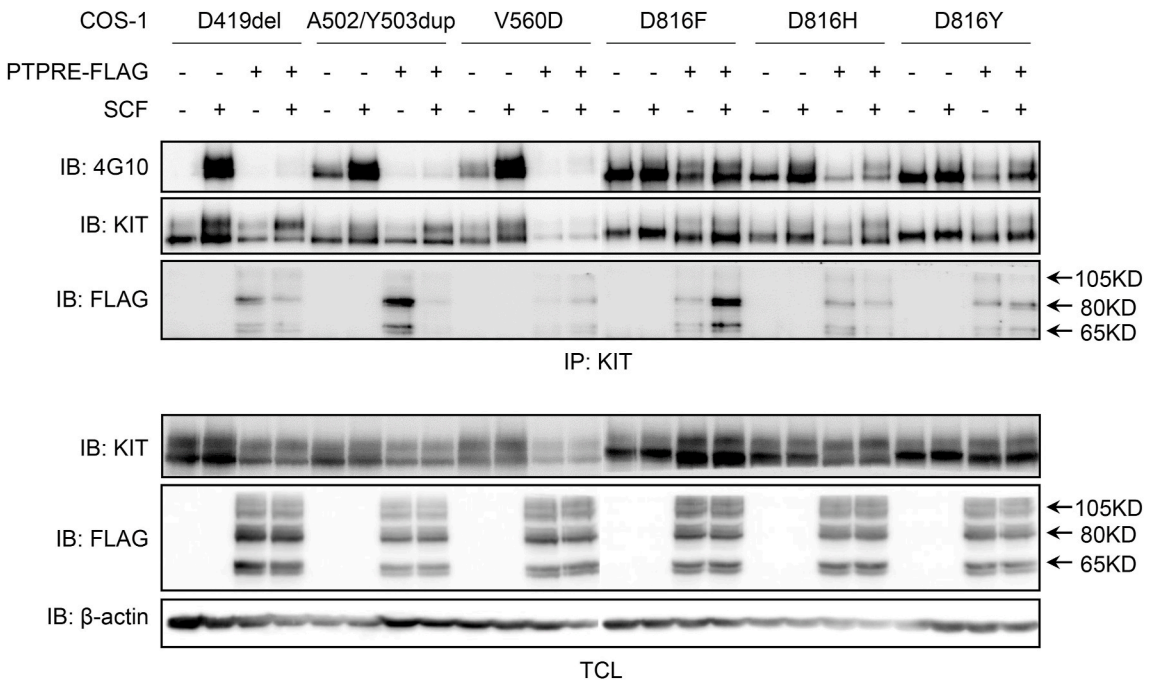

Fig. 3. PTPRE inhibits the activation of GISTs-type KIT mutants and mastocytosis-type KIT mutants differently, 2 μg KIT/D419del, KIT/A502/Y503dup, KIT/V560D, KIT/D816F, KIT/D816H, or KIT/D816Y expressing plasmid and 2 μg PTPRE expressing plasmid or empty vector as control were transfected into COS-1 cells, followed by cell starvation on the next day. Cells were lysed after stimulation with SCF for 5 min. KIT was pulled down using a KIT antibody to detect its activation and association with PTPRE.

3.2. The activation of KIT/D816V is less sensitive to the inhibition of PTPRE

Oncogenic KIT mutations often occur in malignancies such as GISTs and mastocytosis [3,7], and our previous studies suggested that KIT mutants mediated distinct signaling pathways in addition to the ligand-independent activation [20–22]. In order to know whether PTPRE attenuates the activation of KIT mutants similarly as wild-type KIT, we further studied the function of PTPRE on the activation of KIT mutation D816V which is the most often happened mutation in mastocytosis. Similar to the wild-type KIT, KIT/D816V associates with PTPRE as well (Fig. 2A). However, unlike the strong inhibition of wild-type KIT activation by PTPRE, the activation of KIT/D816V is only partially inhibited (Fig. 2A and B), indicating the less sensitivity of KIT/D816V to the inhibition of PTPRE. Expression of KIT/D816V with various amounts of PTPRE further showed dose-dependent inhibition of KIT/D816V activation by PTPRE, which is similar as wild-type KIT, but much less sensitive. The activation of KIT/D816V retained strong even at high dose of PTPRE (Fig. 2C and D). These results suggested that KIT/D816V is much less sensitive to the inhibition of PTPRE compared with wild-type KIT.

3.3. The activation of GISTs-type KIT mutants and mastocytosis-type KIT mutants shows different sensitivity to PTPRE

KIT mutations are the main driver mutations in both GISTs and mastocytosis, however, these two malignancies carry totally different types of KIT mutants. Exon 11 is the hot spot of KIT mutation in GISTs followed by exon 9, 13 and 14 [8–12], while the D816V mutation in exon 17 is the main KIT mutation in mastocytosis [5,6]. The reason for the different mutation pattern of KIT in these two malignancies is still unknown. Since wild-type KIT and KIT/D816V respond differently to the inhibition of PTPRE, we wanted to know whether the difference applies to all KIT mutations in GISTs and mastocytosis. The results showed that, unlike KIT/D816V, the activation of GISTs-type mutants are almost blocked by PTPRE regardless of ligand stimulation, which is similar as wild-type KIT. While the activation of mastocytosis–type KIT mutants was more resistant to the inhibition of PTPRE, which is similar as KIT/D816V (Fig. 3). These results suggested that GISTs-type KIT mutants and mastocytosis-type KIT mutants respond differently to PTPRE although both of them are oncogenic mutants.

4. Discussion

PTPRE is a protein tyrosine phosphatase that can regulate the function of its targets by catalysis of the removal of phosphate. Previous studies have showed that it can regulate the activation of receptor tyrosine kinases such as insulin receptor and PDGFRB [16,17]. In this study, we found that PTPRE can associate with both wild-type KIT and various KIT mutants, and attenuate the activation of KIT, suggesting an important role of PTPRE in KIT signaling.

KIT is a receptor tyrosine kinase that plays an important role in hematopoiesis, gametogenesis and melanogensis, and oncogenic KIT mutations have been identified in GISTs, mastocytosis, acute myeloid leukemia, melanoma and others [1–3,7,23,24]. Signaling studies of wild-type KIT and KIT mutants have showed that KIT mutants have different signaling properties in addition to the ligand independent activation which was considered as the main cause of cell transformation. For example, SRC family kinases are important for the activation of wild-type KIT and its downstream signaling while KIT mutants circumvent the requirement of SRC family kinases [20]. Furthermore, we have previously found that KIT/D816V can phosphorylate p110delta of PI3 kinase, SLAP and MITF which contribute to the cell transformation mediated by KIT/D816V [22,25,26]. All these results suggested a difference in signaling between wild-type KIT and KIT mutants which might provide new clues for the development of novel treatment of malignancies carrying KIT mutations. In this study we found that GISTs-type KIT mutants and mastocytosis-type KIT mutants respond differently to PTPRE, which further showed the different signaling property between different types of KIT mutants. The less sensitivity of mastocytosis-type KIT mutants to PTPRE might give them less control by PTPRE and therefore stronger ability to transform host cells. The

difference in the response to PTPRE between the GISTs-type KIT mutants and mastocytosis-type KIT mutants might due to that mastocytosis-type KIT mutants are stronger than GISTs-type KIT mutants. Ligand stimulation to KIT contributes to the oncogenesis of GISTs, since it has been found that GISTs produce SCF which can promote the activation of KIT through autocrine/paracrine loop [27–29].

Mutations of KIT are the major causes of GISTs and mastocytosis, and the difference of KIT mutants in both malignancies has been well delineated by gene sequencing. Mutations of KIT in GISTs are mainly located in exon 11, and in exon 9, 13 and 14 but to a lesser extent [8–12], while mastocytosis mainly carry KIT mutation in exon 17 [5,6]. Although both GISTs-type KIT mutants and mastocytosis-type KIT mutants can be ligand-independently activated, they showed a different response to the currently approved KIT inhibitors in the clinic. The first-line targeted therapy drug Imatinib can inhibit the activation of most GISTs-type KIT mutants [30] while the main mastocytosis-type KIT mutation D816V is resistant to Imatinib. Currently, there is still no KIT inhibitor approved for the treatment of mastocytosis carrying KIT/D816V yet. Our results maybe give more clues about the difference in the regulation of the activation of these two types of KIT mutants, and contribute to the better design of treatment drugs.

Due to the important functions of KIT in normal situations such as hematopoiesis, gametogenesis and melanogenesis, and the frequent mutations of KIT in malignancies such as GISTs and mastocytosis, KIT signaling have been widely studied. Currently, we have known part about KIT activation and its regulation, and treatment against oncogenic mutations of KIT have been developed and approved in clinic. However, there are still questions to be answered about KIT, for example, why GISTs and mastocytosis carry different type of KIT mutants, why these two types of KIT mutants respond so differently to their inhibitors and so on. Further work is still needed to investigate KIT signaling in order to discover more details about KIT signaling, therefore will benefit the treatment of KIT related diseases.

Conflicts of interest

The authors declare that they have no conflict of interest.

Author statement

Shaoting Zhang: run experiments and data analysis. Liangying Zhang: run experiments. Zongying Jiang: run experiments. Yue Guo: data analysis. Hui Zhao: drafting the manuscript. Jianmin Sun: project design, drafting the manuscript and revision.

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