KRAS Q61H Mutation Confers Cancer Cells with Acquired Resistance to SHP2 Inhibition

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Cancer cells with varied KRAS mutations exhibit different sensitivity to SHP2 inhibition. A recent work published in *Nature Communications* revealed the underlying drug resistance mechanism of cancer cells harboring KRAS Q61H mutation to SHP2 inhibitors (SHP2i).[1] This work showed that KRAS Q61H mutation renders cancer cells resistant to SHP2i via decoupling KRAS from SHP2-mediated upstream nucleotide exchange factors for (guanine nucleotide exchange factor [GEF])/GTPase activating protein (GAP) regulation, providing new insights into treating cancers with KRAS Q61H mutations.

KRAS, the most frequently mutated RAS isoform, is a proto-oncogene that encodes small GTPase transductor protein. In response to upstream signals, KRAS can switch between inactive guanosine diphosphate (GDP) state and active guanosine triphosphate (GTP) state by GEFs, such as Son of Sevenless (SOS), or GAPs.[2] KRAS mutations, primarily at codons 12, 13, or 61, account for 86% of RAS mutations. In particular, glutamine 61 plays a direct role in the catalytic process by positioning the attacking water molecule and stabilizing the transition state of the hydrolysis reaction.[3,4] Conventionally, mutant KRAS can result in accumulation of active GTP-bound KRAS by affecting GAP-mediated GTP hydrolysis, leading to hyperactivation of the RAS–RAF–MEK–ERK pathway accompanied by uncontrolled cell proliferation.[4] KRAS mutations are frequently observed in numerous human cancers, especially in pancreatic cancer, non-small-cell lung cancers and colorectal cancer. It is worth mentioning that specific KRAS mutations may cause different prognosis and therapeutic responses of tumor-bearing patients. Thus, KRAS mutations have posed challenges to researchers of cancer therapy.[2,4,5] Historically, KRAS has been considered as an "undruggable" drug target, as it does not contain a classical druggable binding pocket for small molecules.[6] Indirect and direct approaches have been developed for anticancer drug development by shutting down the oncogenic KRAS network.[6,7] The druggable pocket below the switch-II loop region in the KRAS-G12C variant has provided binding sites for pan-KRAS inhibitors and irreversible covalent inhibitors such as clinical candidates AMG 510 and MRTX849. However, inhibiting the enzymatic function of KRAS directly is still frustrated as for the possible off-target toxicity. Indirect KRAS suppression strategies include inhibiting upstream signaling molecules (e.g., EGRF, SOS1, and SHP2) or downstream effectors (e.g., RAF, MEK, ERK, and PI3K), inhibiting KRAS expression or processing processes, or suppressing related downstream processes (e.g., glycolysis, autophagy, and immunosuppression), proving novel directions for treating KRAS-driven cancer. These indirect approaches provide guidance for designing combination therapies to overcome drug resistance, whereas the combined therapies may cause increased toxicity.[7] Accordingly, full characterization of underlying pathogenenic mechanisms of mutant KRAS is vital for treating KRAS-driven cancers.

SHP2, encoded by proto-oncogene *PTPN11*, is a protein tyrosine phosphatase that serves as a converge node in different signaling pathways. As an important upstream regulator, SHP2 participates in several RAS-mediated signaling pathways including the RAS–RAF–MEK–ERK and PI3K–AKT–mTOR to regulate cell survival, proliferation, and differentiation.[8–11] As an adaptor protein, SHP2 provides binding sites for the recruitment of Grb2/SOS complex and dephosphorylates p120-Ras-GAP. Thereby, SHP2 modulates the KRAS GTPase cycle through promoting GEF and restraining GAP activity, favoring KRAS activation. Additionally, SHP2 directly reverses Src phosphorylation of KRAS and then enhances KRAS-binding affinity for its effector proteins.[9,12] Evidence has shown that SHP2i alone or in combination with MEK, ERK, or ALK can suppress cell growth and induce death of KRAS-dysregulated cell lines.[9,13–15] Several studies also suggested that distinct
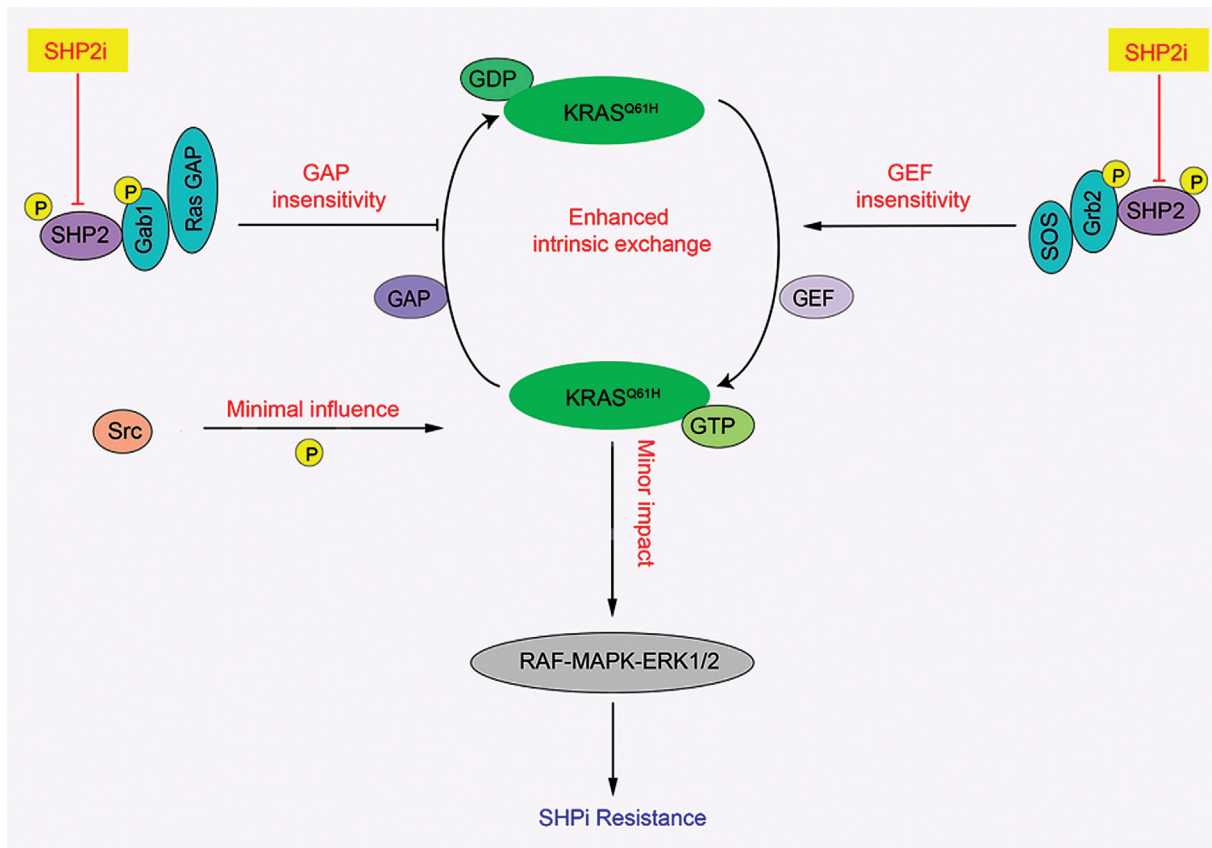
Fig. 1 Resistance mechanism of KRAS Q61H mutation to SHP2 inhibition.

KRAS-mutant or BRAF-mutant cancer cell lines were insensitive to SHP2i. Thus, it is necessary to elucidate detailed mechanisms for the different sensitivity of KRAS mutants to SHP2i.[1]

Gebregiworgis et al reported that pancreatic ductal adenocarcinoma cells with KRAS Q61H mutation were insensitive to both allosteric and orthosteric SHP2i, which is a unique feature for Q61H mutant.[1] Q61H mutation was insensitive to SOS-mediated nucleotide exchange as for the unstable KRAS–SOS complex. Given the important role of Gln61 in hydrolysis, the GTPase cycle in KRAS Q61H mutation was also severely decoupled from regulation by GEF and GAP activities of SOS1 and RASA1. Further phosphorylation profiling of KRAS showed that Q61H mutation did not affect KRAS phosphorylation by Src or dephosphorylation by SHP2. Therefore, the resistance to SHP2i was not caused by differential phosphorylation of mutants. However, Src phosphorylation of Q61H promoted the intrinsic exchange rate of KRAS, leading to the accumulation of active GTP-bound KRAS and activation of downstream signaling. It was caused by structural perturbations of KRAS switch I and II residue regions for coordinating nucleotides GAPs and GEFs. Moreover, the structural alternation of the Q61H switch I region also interfered the interaction with effector proteins (e.g., RAF). Thereby, the Src phosphorylation of KRAS Q61H had little impact on the binding of RAF, which was different from the reduced binding affinity to RAF in WT or G12V mutant KRAS. This result indicated that KRAS

Q61H was resistant to phosphorylation-dependent regulation of MAPK signaling. In summary, distinct biochemical properties of each KRAS mutant may confer varied sensitivity of cancer cells with specific KRAS mutations to SHP2i. The specific characteristic of Q61H mutation disturbs the intrinsic nucleotide change of KRAS but does not evade phosphorylation by Src to confer resistance to SHP2 inhibition (►**Fig. 1**).

In view of the conserved catalytic domains of all RAS GAPs and GEFs, Q61H mutation may also confer resistance to the regulation of other RAS GAP and GEF activities. For example, KRAS Q61H mutation is one of the main causes of acquired drug resistance to estimated glomerular filtration rate inhibitors in both lung and colorectal cancers.[1] Hence, targeting the upstream effectors such as RTKs, SHP2, and SOS may be ineffective in KRAS Q61H-mutated tumor, whereas targeting the downstream signaling molecules of KRAS (e.g., RAF, MEK, ERK, PI3K, AKT, mTOR) may be a feasible approach for the treatment of KRAS Q61H-driven cancers. Moreover, combination of KRAS inhibitor and downstream target inhibitors may show therapeutic promise in tumors harboring KRAS Q61H mutation.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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