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Gastrointestinal stromal tumor transition to KIT-independence is enabled by a Cyclin D1 dependent proliferation program

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the gastrointestinal tract. GISTs generally contain oncogenic gain-of-function KIT (~85%) or PDGFRA (~5-10%) mutations which are crucial for GIST proliferation and survival, as underscored by the dramatic clinical responses to targeted KIT/PDGFRA-inhibition in patients with advanced GIST. Oncogenic KIT or PDGFRA tyrosine kinase mutations are compelling therapeutic targets in most GISTs. Most patients with inoperable GIST patients treated with the KIT/PDGFRA small molecule drugs such as the first line-imatinib, second line-sunitinib, or third line-regorafenib, has achieved disease stabilization. However, a substantial proportion of patients develop resistance to these inhibitors over time, eventually experience disease progression.

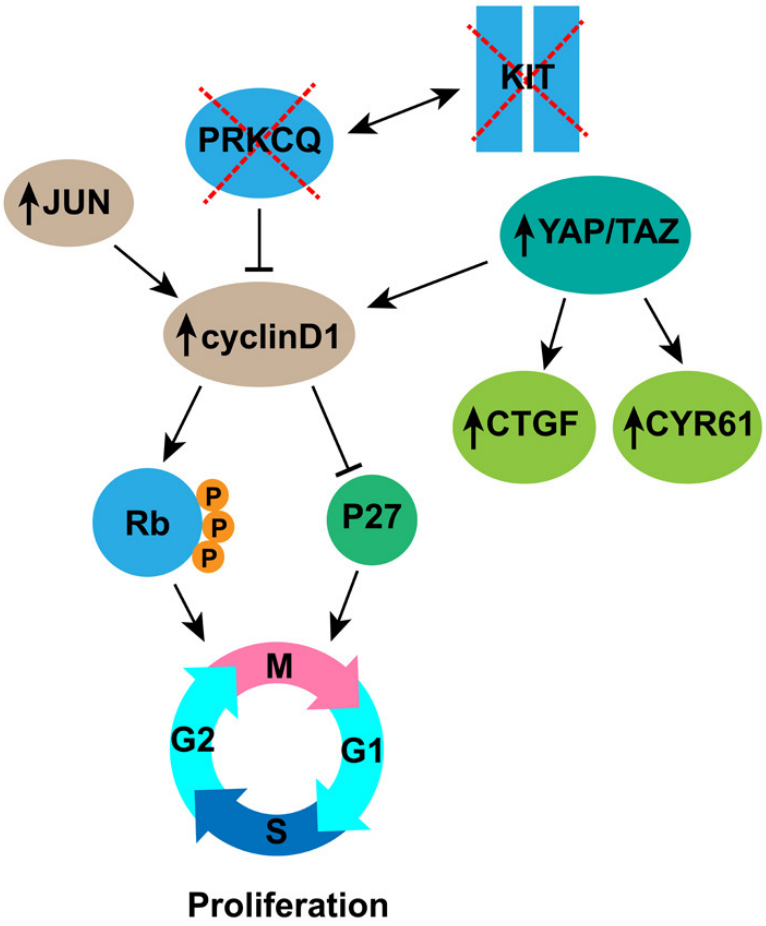

Fig. 1. Constitutive KIT oncogenic signaling is supplanted by a cyclin D1 dependent program in KIT-independent GISTs. Loss of KIT protein expression accompanies with PRKCQ expression silencing. Cyclin D1 induction is coordinately regulated by PRKCQ, JUN, and, the Hippo pathway/YAP-TAZ, resulting in cell proliferation by Rb inactivation and p27 inhibition.

These clinically progressing GIST subclones often remain KIT/PDGFRA-dependent, due to imatinib-resistant secondary KIT/PDGFRA kinase domain mutations, *KIT* genomic amplification, or activation of an alternate kinase oncoprotein. However, a particularly challenging subset of imatinib-resistant GIST (~10%) are newly KIT-independent, as evidenced by *KIT* transcriptional silencing, even in continued presence of the original *KIT* genomic mutation.

We identify an overarching transforming mechanism, unique to the KIT-independent subset of GIST, in which constitutive KIT/PDGFRA oncogenic signaling is supplanted by cyclin D1 dependent programs, which are coordinately regulated by PRKCQ, JUN, and the Hippo pathway (Fig. 1). We firstly developed isogenic GIST lines in which the parental forms were KIT oncoprotein-dependent, whereas sublines had loss of KIT oncoprotein expression, accompanied by markedly downregulated expression of the GIST biomarker, protein kinase C-θ (PRKCQ). PRKCQ is a diagnostic biomarker in GIST. Importantly, PRKCQ is tyrosine phosphorylated by KIT, PRKCQ positively regulates KIT expression, and PRKCQ and KIT co-expresses in GIST cells. Loss of KIT protein expression is accompanied with PRKCQ expression silencing. Whole transcriptome sequencing demonstrated extremely low *CCND1* transcripts in KIT-dependent GIST cell lines, whereas *CCND1* was expressed abundantly in KIT-negative, KIT-independent sublines. qRT-PCR and immunoblotting demonstrated overexpression of CCND1 and JUN, and downregulation of KIT and PRKCQ in the KIT-independent GISTs, compared to the KIT-dependent parental GISTs. Cyclin D1 inhibition in KIT-independent GISTs was accompanied by anti-proliferative effects, and these anti-proliferative responses were associated with Rb activation and p27 CDK inhibitor upregulation. By contrast, in KIT-dependent GISTs, cyclin D1 silencing had little or no effect on Rb phosphorylation and no impact on cell proliferation or apoptosis. In addition, JUN inhibition markedly inhibited expression of cyclin D1 and the GIST proliferation in KIT-independent GISTs, but not in KIT-dependent GISTs. The transcriptome profiles in the KIT-independent GISTs also showed marked upregulation of Hippo pathway effectors, including CCND1, CYR61, CTGF, AXL, ANKRD1 and DKK1. Further studies, by YAP and TAZ knockdowns and using the Hippo-inhibitor, verteporfin, showed that the Hippo pathway regulated cyclin D1 expression and cell growth in KIT-independent GIST cell lines (Fig. 1).

Altogether, our results support the model depicted in Figure 1. These novel findings highlight the roles of PRKCQ, JUN, Hippo and cyclin D1 as oncogenic mediators in GISTs that have converted, during TKI-therapy, to a KIT-independent state. Inhibitors of these pathways could be effective therapeutically for these now untreatable tumors.

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Publication

Cyclin D1 is a mediator of gastrointestinal stromal tumor KIT-independence
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