The DREAM Complex Mediates GIST Cell Quiescence and Is a Novel Therapeutic Target to Enhance Imatinib-Induced Apoptosis

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Abstract

Gastrointestinal stromal tumors (GIST) can be successfully treated with imatinib mesylate (Gleevec); however, complete remissions are rare and patients frequently achieve disease stabilization in the presence of residual tumor masses. The clinical observation that discontinuation of treatment can lead to tumor progression suggests that residual tumor cells are, in fact, quiescent and, therefore, able to re-enter the cell division cycle. In line with this notion, we have previously shown that imatinib induces GIST cell quiescence in vitro through the APC\(\text{CDH1}\)-SKP2\(\text{p27kip1}\) signaling axis. Here, we provide evidence that imatinib induces GIST cell quiescence in vivo and that this process also involves the DREAM complex, a multisubunit complex that has recently been identified as an additional key regulator of quiescence. Importantly, inhibition of DREAM complex formation by depletion of the DREAM regulatory kinase DYRK1A or its target LIN52 was found to enhance imatinib-induced cell death. Our results show that imatinib induces apoptosis in a fraction of GIST cells while, at the same time, a subset of cells undergoes quiescence involving the DREAM complex. Inhibition of this process enhances imatinib-induced apoptosis, which opens the opportunity for future therapeutic interventions to target the DREAM complex for more efficient imatinib responses. Cancer Res; 73(16); 5120–9. ©2013 AACR.

Introduction

The majority of gastrointestinal stromal tumors (GIST), the most common mesenchymal tumor of the gastrointestinal tract, are characterized by oncogenic mutations in the \(\text{KIT}\) or platelet-derived growth factor receptor-\(\alpha\) (PDGFR\(\alpha\)) receptor tyrosine kinase (1–3). Because these oncogenic mutations lead to constitutive, ligand-independent activation of the receptor, GISTs can be successfully treated with the small-molecule kinase inhibitor imatinib mesylate (Gleevec; ref. 4). Although approximately 85% of patients with advanced or metastatic disease benefit from imatinib treatment, complete responses are rare and the majority of patients only achieve partial response or stable disease with remaining tumor load (5).

Successful cancer therapy is often hampered by the occurrence of tumor cell quiescence, because quiescent cells remain viable and, therefore, are a reservoir for tumor progression (6–8). This reversible exit from the cell division cycle and entry into G\(_0\) has previously been shown to involve the anaphase-promoting complex (APC\(\text{CDH1}\)-SKP2\(\text{p27kip1}\) signaling axis (9–13). In this pathway, the APC, together with its activator CDH1, promotes the polyubiquitylation and subsequent degradation of SKP2, a substrate adaptor component of the SCF (SKP1–Cullin–F-box) complex. SKP2 loss results in the accumulation of its target, the CDK inhibitor p\(_{27}\)kip1, and the reinforcement of a quiescent state. In a previous study, we could show that this process is active in imatinib-treated GIST cells (14).

A second major group of proteins that negatively regulate the cell cycle are the retinoblastoma tumor suppressor protein (pR\(_{B}\)) and the related ‘pocket protein’ family members p107 (RBL1) and p130 (RBL2; refs. 15, 16). p130 has been shown to accumulate in G\(_{0}\) when it interacts with E2F4 to repress E2F-dependent gene transcription (17–19). p130 protein levels are also regulated by SKP2 (20), which emphasizes that quiescence involves multiple interconnected pathway components.

In line with this notion, it has been shown that p130/E2F4 are part of a larger, multisubunit protein complex, the mammalian DREAM complex (21). This complex consists of DP, RBL2 (p130), E2F4 and the mammalian homologs of the Caenorhabditis elegans (C. elegans) synthetic multivulva class...
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B (synMuvB) gene products LIN9, LIN37, LIN52, LIN53/RBBP4, and LIN54 (21). The DREAM complex forms in G0 and represses E2F target genes. In humans, LIN9, LIN37, LIN52, LIN53/RBBP4, and LIN54 have been shown to form a stable complex (the MuvB core) that dissociates from p130 and E2F4/DP in S phase and, instead, binds to B-MYB, as it is referred to as the MYB–MuvB (MMB) complex (21–23). In a recent study, phosphorylation of the DREAM component LIN52 at Ser28 was shown to regulate complex formation in G0 (24).

Here, we show that imatinib induces GIST cell quiescence in vivo and in vitro and that this process involves the DREAM complex as evidenced by upregulation of p130, increased p130/E2F4/LIN37 complex formation, and enhanced phosphorylation of the DREAM subunit LIN52. Importantly, abrogation of quiescence by siRNA-mediated knock-down of LIN52 or the DYRK1A kinase were both found to significantly increase imatinib-induced GIST cell apoptosis. Therefore, interference with DREAM-mediated quiescence can enhance imatinib-induced anti-GIST cell activity, which emphasizes the relevance of the DREAM complex as novel drug target warranting further preclinical and clinical investigations.

Materials and Methods

Cell culture, inhibitor treatments, and transfections

The human GIST cell line GIST882 (a generous gift from Jonathan A. Fletcher, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA) was derived from an untreated metastatic GIST and maintained in RPMI-1640 supplemented with 15% FBS (Gemini BioProducts), 1% L-glutamine, 50 U/mL penicillin (Cambrex), and 50 μg/mL streptomycin (Cambrex) as described previously (25).

Cells were incubated in imatinib mesylate [1 μmol/L in dimethyl sulfoxide (DMSO); LC Laboratories], harmine (10 μmol/L in DMSO; Sigma-Aldrich), or mock-treated with 0.1% DMSO for up to 72 hours or as indicated.

For siRNA experiments, single RNA duplexes were used to reduce protein expression of members of the DREAM complex (Qiagen) and DYRK1A (Dharmacon/Thermo Fisher Scientific). Briefly, GIST882 cells were trypsinized and 3 × 10^6 cells were transfected with 5 μL of 10 μmol/L annealed RNA duplexes using nucleofection (Amaxa/Lonza). For dual knockdown experiments, 2.5 μL of 20 μmol/L of each annealed RNA duplexes was used. Cells were then transferred to 35 mm tissue culture dishes with 2 mL RPMI-1640 free of antibiotics. Cells were either incubated for 72 hours without further manipulation before further processing or treated with 1 μmol/L imatinib of 0.1% DMSO at 24 hours after transfection and incubated for another 48 hours (72 hours total). Knock-down efficiency was monitored by immunoblotting or quantitative real-time reverse transcriptase PCR (qRT-PCR) for LIN54 only.

Immunologic and cell-staining methods

Protein lysates of cells growing as monolayer were prepared by scraping cells into lysis buffer (1% NP-40, 50 mmol/L Tris-HCl pH 8.0, 100 mmol/L sodium fluoride, 30 mmol/L sodium pyrophosphate, 2 mmol/L sodium molybdate, 5 mmol/L EDTA, 2 mmol/L sodium orthovanadate) containing protease inhibitors (10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 μmol/L phenylmethylsulfonyl fluoride). Lysates were incubated for 1 hour with shaking at 4°C and then cleared by centrifugation for 30 minutes at 14,000 rpm at 4°C. Protein concentrations were determined by the Bradford assay (Bio-Rad). Thereafter, 30 μg of protein was loaded on a 4% to 12% Bis–Tris gel (Invitrogen) and blotted onto a nitrocellulose membrane. Commmunoprecipitation experiments were carried out using TrueBlot IP/ Western reagents (Rockland Immunochemicals) according to the manufacturer’s instructions.

For immunofluorescence analysis, cells grown on coverslips were briefly washed in PBS and fixed in 4% paraformaldehyde in PBS for 15 minutes at room temperature. Cells were then washed in PBS and permeabilized with 1% Triton X-100 in PBS for 15 minutes at room temperature followed by washing in PBS and blocking with 10% normal donkey serum (Jackson ImmunoResearch) for 15 minutes at room temperature. Cells were then incubated with primary antibodies overnight at 4°C in a humidified chamber and incubated for another 3 hours at 37°C the next morning. After a brief wash in PBS, cells were incubated with fluorescein isothiocyanate (FITC)-anti-mouse secondary antibodies (Jackson ImmunoResearch) for at least 2 hours at 37°C, washed with PBS, and counterstained with 4′, 6-diamidino-2-phenylindole (DAPI; Vector Laboratories). Cells were analyzed using an Olympus AX70 epifluorescence microscope equipped with a SpotRT digital camera.

Immunofluorescence microscopic staining of paraffin-embedded sections was done as described previously (26). In brief, slides were deparaffinized in xylene and rehydrated in a graded ethanol series. After microwaving in 0.01 mol/L citrate buffer (pH 6.0) for 10 minutes, slides were washed in distilled water and PBS before continuing with staining as described earlier.

Primary antibodies used for immunoblotting and immunofluorescence were actin (Sigma); bromodeoxyuridine (BrdUrd; Roche Applied Science); cleaved caspase 3 (Cell Signaling Technology); cyclin A (Novocastra); DYRK1A and E2F4 (both from Santa Cruz Biotechnology); pKIT Y719 (Cell Signaling Technology); KIT (DakoCytomation); LIN9, LIN37, LIN52, pLIN52 S28, and LIN54 (all from Litovchick and colleagues, refs. 21, 24); p27Kip1 (BD Biosciences Pharmingen), p27Kip1 (Invitrogen/Zymed Laboratories and Becton Dickinson); p130 (RBL2, Santa Cruz Biotechnology); and PARP and SKP2 (both from Invitrogen/Zymed Laboratories).

BrdUrd assay

To assess the proliferating cell fraction, GIST882 cells were incubated in BrdUrd labeling medium (Roche Applied Science) for 2 hours. Cells were then washed in PBS and fixed in ice-cold methanol for 10 minutes. After another wash in PBS, DNA was denatured by incubation in 2 N HCl for 1 hour at 37°C. The HCl was neutralized by immersion in 0.1 mol/L borate buffer, pH 8.5, before incubation with a mouse monoclonal anti-BrdUrd antibody (Roche) overnight at 4°C in a humidified...
chamber. Detection and analysis was carried out as described previously (14).

**Senescence-associated β-galactosidase activity**
Senescence-associated β-galactosidase activity was assessed using the Senescence β-Galactosidase Staining Kit (Cell Signaling Technology) according to the manufacturer’s instructions.

**Quantitative real-time reverse transcriptase PCR**
For qRT-PCR, cells were transfected with control siRNA or siRNA duplexes targeting LIN54 and RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol. RNA was treated with DNase I enzyme (Fermentas) to remove any contaminating traces of genomic DNA, and cDNA was transcribed by RT-PCR using random primers and the Maxima First Strand cDNA Synthesis Kit (Fermentas). LIN54 and β-actin cDNA was amplified using exon-overlapping, mRNA/cDNA-specific primers to LIN54 (forward: 5’-ATTGCTAAGAAGCCTCGAAC-3’, reverse: 5’-TGGTGAAAC-TTGAGTTGCTTCGTC-3’; IDT, Leuen, Belgium) and β-actin (forward: 5’-CCAAGGCCAACCGAGAAGATGAC-3’, reverse: 5’-AGGGTGACATGGTGGTGCCGACGAC-3’) and measured using the SsoFast EvaGreen Kit (Bio-Rad). Cycling conditions were 95°C (30 seconds, activation), 95°C (5 seconds, denaturation), 55°C (10 seconds, annealing/extension) for 40 cycles on a Bio-Rad CFX96 Real-Time System run on a C1000 Thermal Cycler platform (Bio-Rad). β-actin served as the reference gene for relative quantification.

**Cell-cycle analysis**
Cell-cycle analysis was conducted by measuring the amount of propidium iodide (PI)-labeled DNA in ethanol-fixed cells. In brief, cells were harvested by trypsinization, washed twice with prechilled PBS (1% FBS), and fixed with ice-cold 70% ethanol. After the fixation step, the cells were washed with PBS/1% FBS, resuspended in PI (Sigma-Aldrich)/RNase staining solution (50 μg/mL PI, 10 mmol/L Tris pH 7.5, 5 mmol/L MgCl₂, 10 μg/mL RNase) and incubated at 37°C in the dark for 30 minutes. The analysis was conducted in the University of Pittsburgh Cancer Institute Flow Cytometry Core Facility using a Gallios Flow Cytometer (Beckman Coulter) and the Kaluza 5 acquisition software.

**GIST xenograft models**
For mouse xenograft models, GIST882 cells [carrying a KIT p.K642E (exon 13 mutation)] or tumors originating from the biopsies of two patients [bearing KIT p.V650D (exon 11) or KIT p.A502_Y503dup (exon 9) mutations, respectively] were implanted in both flanks of two mice as described previously (27). Second-passage xenografts were generated by explanting established xenografts and implanting them into the flanks of a second set of mice. When tumors were 1 cm in diameter, mice were treated with placebo or imatinib (100 mg/kg, orally daily) for 12 days. Tumor volume, weight, and general health of the mice were recorded as previously described (27). After the mice were sacrificed, tumors were excised and processed for histopathologic examination. The animal experiment was approved by the Ethics Committee of KU Leuven (Leuven, Belgium).

**Results**
**Imatinib induces GIST cell quiescence in vivo and in vitro**
To analyze imatinib-induced quiescence in vivo, several cell line- and patient-derived GIST xenograft models were analyzed (Fig. 1; ref. 27). Mice were treated with placebo or imatinib and tumor tissues were stained for the quiescence marker p27<sup>Kip1</sup>. Imatinib treatment led to a significant increase of tumor cells exhibiting nuclear p27<sup>Kip1</sup> staining in GIST882 xenografts (Fig. 1A) and patient-derived xenografts carrying a mutation in KIT exon 11 (Fig. 1B) thereby confirming the ability of imatinib to induce quiescence in GIST cells in vivo. A xenograft derived from a tumor with a KIT exon 9 mutation showed only a minimal increase in nuclear p27<sup>Kip1</sup> staining. This was not due to differences in the expression of its upstream regulator SKP2 (Supplementary Fig. S1), indicating that baseline SKP2 levels do not determine levels of p27<sup>Kip1</sup> after imatinib treatment. Remarkably, KIT exon 9 mutations are known to be associated with a reduced sensitivity to imatinib in patients with GIST (28), but there are currently no GIST cell line models available to dissect the precise underlying molecular mechanisms.

A main characteristic of quiescence is the fact that it is reversible, in contrast to cellular senescence, which is largely irreversible (29). To further corroborate the fact that imatinib treatment leads to quiescence and not senescence in GIST cells, GIST882 cells were treated with imatinib for 72 hours (3 days), after which the drug-containing media was removed and cells were cultured without drug for up to 11 days. The percentage of cells in S-phase was measured by BrdUrd incorporation and was found to decrease significantly during imatinib treatment (from 26.9% to 1.3%; Fig. 1C), with a subset of cells showing morphologic signs of apoptosis (Supplementary Fig. S2). However, 5 days after drug washout, a significant increase of cells in S-phase was observed and cellular proliferation was restored to pretreatment levels at 7 to 9 days after imatinib removal (Fig. 1C). These findings were corroborated by immunoblot analyses (Fig. 2A), showing that inhibition of KIT phosphorylation by imatinib is completely reversible after imatinib washout. KIT reactivation was associated with a cell-cycle reentry (as measured by expression levels of SKP2, p27<sup>Kip1</sup>, and cyclin A) at day 5 after drug removal. These results confirm clinical findings showing that metastatic or inoperable GISTs resume growth when imatinib treatment is discontinued (30).

The same study (30) reported that GISTS, which progress after discontinuation of imatinib, usually do retain their responsiveness to imatinib during further treatment. To recapitulate these findings in vitro and to test whether there would be any detectable differences between imatinib-naive and imatinib-pretreated cells, we re-exposed GIST cells that had completed one imatinib washout cycle (i.e., 11 days after removal of drug) to imatinib in comparison to imatinib-naive GIST cells. As shown in Fig. 2B, prior imatinib-induced quiescence had no effect on inhibition of KIT phosphorylation, induction of apoptosis, or induction of cell-cycle arrest when cells were re-exposed to the drug. There was, in fact, a more
pronounced PARP cleavage band that most likely reflects cell synchronization. These results provide in vitro evidence that pretreated GIST cells retain their responsiveness to antineoplastic activities of imatinib as suggested by previous clinical reports.

The results shown above imply that GIST cells either undergo apoptosis or enter quiescence after imatinib treatment and, furthermore, that imatinib does not lead to a senescent phenotype, that is, permanent cell-cycle exit. To corroborate the latter notion, we stained imatinib-treated GIST882 cells for senescence-associated \( \beta \)-galactosidase (SA-\( \beta \)-gal) activity (Supplementary Fig. S3A). There was a low (<5%) baseline level of SA-\( \beta \)-gal-positive multinucleated cells, but no change in the percentage of SA-\( \beta \)-gal-positive cells was detected when imatinib-treated cells were compared with control-treated cells. Furthermore, immunofluorescence microscopic staining of the senescence-associated marker p16\(^{INK4A} \) (CDKN2A) of imatinib-treated GIST882 cell populations did not reveal any changes in total protein levels (Supplementary Fig. S3B). Taken together, these results indicate that indeed most, if not all, GIST cells undergo either apoptosis or quiescence when treated with imatinib and that imatinib does not induce a senescent phenotype in these cells.

### The DREAM complex is involved in imatinib-induced quiescence

The multiprotein DREAM complex was recently identified as one of the key regulators of cellular quiescence in addition to the \( APC^{CDH1-SKP1-p27Kip1} \) signaling axis (21). We therefore tested whether imatinib-induced quiescence in GIST cells involves DREAM complex-mediated mechanisms.

To test this hypothesis, we initially focused on the core members of the DREAM complex, p130 (RBL2) and the mammalian MuvB homolog LIN37. As expected, imatinib induced a time-dependent increase of p130 protein expression (Fig. 3A) indicating cellular quiescence. Conversely, p130 expression decreased when imatinib was removed, indicating exit from quiescence and cell-cycle reentry (Fig. 3B). At the same time,
Knockdown of single DREAM complex subunits (p130, E2F4, LIN9, LIN37, or LIN54) did not result in a significant increase of imatinib-induced GIST cell apoptosis (Supplementary Fig. S4A) indicating a certain redundancy of DREAM complex components as previously suggested (21).

A combined knockdown of E2F4 and LIN54, however, was able to enhance GIST cell apoptosis from 36.6% in control siRNA-transfected and imatinib-treated cells to 53.1% in E2F4/LIN54 siRNA-transfected and imatinib-treated cells (P < 0.001; Supplementary Fig. S4B). E2F4/LIN54 knockdown lead to an increased DNA synthesis in comparison with control siRNA-transfected cells as measured by BrdUrd incorporation (30.0% vs. 21.0% BrdUrd-positive cells, respectively), suggesting that inhibition of efficient DREAM complex formation resulted in increased baseline proliferation in GIST cells (P < 0.001; Supplementary Fig. S4C).

Phosphorylation of DREAM complex component LIN52 at serine 28 (S28) by the DYRK1A protein kinase has previously been reported to play an important role in DREAM complex assembly and activity (24). We, therefore, asked whether this process also plays a role in imatinib-induced GIST cell quiescence. pLIN52 S28 indeed, increased in imatinib-treated GIST cells as shown by immunoblotting (Fig. 4A) whereas basal expression levels of DYRK1A and LIN52 remained unchanged, which is line with a previous report (24). LIN52 phosphorylation and, therefore, DREAM complex formation were reversible because pLIN52 S28 expression was undetectable after removal of imatinib (Fig. 4B).

We then tested whether interference with DYRK1A can also modulate GIST cell apoptosis in response to imatinib. Because DYRK1A is a protein kinase, it is possible to inhibit its activity with pharmacologic agents such as harmine. Harmine is a dimethylamino-β-carboline alkaloid with ATP-competitive activity toward DYRK1A (32). We treated GIST882 cells with harmine and found a statistically significant, 19.5-fold increase of apoptosis from 2.64% in DMSO-treated, LIN52-depleted cells to 37.51% in imatinib-treated, LIN52-depleted GIST cells (Fig. 4D and E; P < 0.0001).

We then tested whether interference with DYRK1A can also modulate GIST cell apoptosis in response to imatinib. Because DYRK1A is a protein kinase, it is possible to inhibit its activity with pharmacologic agents such as harmine. Harmine is a β-carboline alkaloid with ATP-competitive activity toward DYRK1A (32). We treated GIST882 cells with harmine and found an enhanced cell death in combination with imatinib when compared with imatinib as a single agent (Fig. 5A and B). The abrogation of imatinib-induced quiescence by harmine is shown by the reduction of p130 protein expression levels (Fig. 5A). We next used siRNA to knockdown DYRK1A expression and found an induction of apoptosis in DYRK1A-depleted GIST cells (Fig. 5C). When DYRK1A-depleted cells were treated with imatinib, a statistically significant, 24-fold increase of apoptosis was detected (Fig. 5C and D; \( P < 0.0001 \)).

Collectively, these results show that abrogation of DREAM complex components can significantly increase the apoptotic response of GIST cells to imatinib.

**Discussion**

GISTs can successfully be treated with imatinib, but complete remissions are rare and most patients with advanced or
metastatic tumors only experience disease stabilization or a partial remission. It has been shown that the remaining tumor mass contains viable GIST cells even while still responding to imatinib, and that these cells resume proliferation when imatinib treatment is discontinued (14, 30, 33).

In the present report, we corroborate that imatinib induces GIST cell quiescence not only \textit{in vitro} but also \textit{in vivo} using cell line- and patient-derived mouse xenografts. In line with clinical observations, we find that drug removal leads to reactivation of cellular proliferation, yet does not affect the sensitivity of GIST cells when rechallenged with imatinib. We have previously shown that imatinib-induced quiescence involves the APCCDH1–SKP2–p27Kip1 axis (14).

Here, we provide evidence for a second pathway that contributes critically to imatinib-induced quiescence in GIST cells that involves the DREAM complex. This multisubunit complex consists of the pRB-related protein p130, E2F4, DP, LIN9, LIN37, LIN52, LIN53/RBBP4, and LIN54 (21, 22). DREAM complex formation occurs in G0 to repress E2F target genes in a process that involves phosphorylation of DREAM component LIN52 at S28 by the DYRK1A protein kinase. Our results are in full agreement with this model because we show increased p130 expression and enhanced p130 nuclear translocation, p130/E2F4/LIN37 complex formation, and increased pLIN52 S28 phosphorylation in imatinib-treated GIST cells. It is noteworthy that SKP2 provides a link between the two quiescence-associated signaling pathways as it also regulates p130 levels (20).

One of the key findings of the present study is that abrogation of quiescence by either siRNA or a pharmacologic agent that inhibits DYRK1A kinase activity can significantly enhance imatinib-induced GIST cell apoptosis. Although the precise mechanism warrants further investigation, in the most simplistic model, GIST cells that are prevented from entering a state of quiescence now undergo apoptosis when exposed to imatinib. We have previously shown that imatinib-induced cell death involves upregulation of core histone variant H2AX, followed by chromatin condensation and a transcriptional block (25). Although H2AX mRNA is synthesized in all phases of the cell division cycle (34), more than 90% of total histone synthesis takes place in S-phase (35). It is conceivable that cells that are unable to enter quiescence and continue to proliferate are more vulnerable to imatinib-induced H2AX upregulation and subsequent cell death than cells that have exited the cell cycle and have a reduced or no H2AX expression.

The precise mechanism of imatinib-induced quiescence, including p130 upregulation and enhanced phosphorylation of LIN52 leading to DREAM complex activation, remains to be determined. Whereas the former event could be related to reduced SKP2 expression leading to p130 accumulation, it is unlikely that DYRK1A activity is acutely regulated on the level of gene expression or protein abundance, because its levels did not change significantly in imatinib-treated GIST cells (20).

Figure 3. The DREAM complex mediates imatinib-induced quiescence in GIST cells. A, immunoblot analysis of GIST882 cells treated with imatinib for the indicated times and probed for DREAM complex members p130 and LIN37. Immunoblot for actin is shown to display equal loading. Note the upregulation of p130 following imatinib treatment. B, immunoblot analysis of GIST882 cells for p130 and LIN37 expression in GIST882 cells treated with imatinib for 72 hours and cultured for up to 11 days after removal of the drug. Note the changes in p130 expression, indicating entry into and exit from quiescence. C, immunofluorescence microscopic analysis of p130 expression and subcellular localization in GIST882 cells treated with DMSO or imatinib for 48 hours (left). Quantification of the percentage of GIST882 cells, showing nuclear p130 after imatinib treatment (right). Nuclei were stained with DAPI. Scale bar, 50 μm. *P < 0.0015, Student t test for independent samples. D, interactions among p130, E2F4, and LIN37 proteins in GIST cells as shown by coimmunoprecipitation (IP)–immunoblot of GIST882 cells treated with DMSO or imatinib for 72 hours. Coimmunoprecipitation was conducted with either anti-p130 (left) or anti-E2F4 antibodies (right) followed by Western blotting for the proteins indicated. Whole-cell lysates were used as a positive control to show an equal input.
not change after imatinib treatment or during the cell cycle (24). In contrast, stimulation of DYRK1A likely involves activity through modulation of feedback loops, such as LATS2 activity (36) or interaction with SPRED1 (37) or 14-3-3 (38). These possibilities warrant further experimentation.

The fact that formation of the DREAM complex is regulated by phosphorylation of its subunit LIN52 offers a window of opportunity for therapeutic interventions. The plant alkaloid harmine, which was used in the current study, displays specificity for DYRK1A (39), but its strong inhibitory effect on monoamine oxidase A and hallucinogenic properties unfortunately limit its use in patients (40). A recently described synthetic inhibitor of DYRK1A, INDY, was found to be active in several in vitro and in vivo models and may show an improved toxicity profile (41). Further support for the concept of quiescence inhibition as a strategy for cancer therapy stems from a previous study in ovarian cancer (42). This report focused on DYRK1A family member DYRK1B, which is overexpressed in ovarian carcinoma cells and has also been shown to be capable of phosphorylating LIN52 (24). Inhibition of DYRK1B by RO5454948, a compound that also inhibits DYRK1A, led to escape from quiescence and an increased apoptotic response (42). Importantly, treatment with RO5454948 did not affect the viability of normal ovarian epithelial cells.

Results shown here lend further support to the notion that induction of tumor cell quiescence can negatively affect anti-neoplastic therapies. It is remarkable that imatinib itself is able
to stimulate quiescence in a subset of GIST cells, thereby intrinsically limiting its own effectiveness. Our findings, therefore, emphasize the need to exploit synthetic lethal approaches such as KIT and DYRK1A inhibition as shown here to increase antitumor efficacy.

One of the key remaining questions is what determines whether GIST cells undergo apoptosis or quiescence when treated with imatinib. It is possible that the cell-cycle stage at the time of treatment plays a role, but other mechanisms that can modulate the switch between cell death and survival merit further exploration. It is possible that whole genome or transcriptome analyses may allow a stratification of GISTS as being more prone to apoptosis or quiescence. In addition, it will be important to determine whether E2F4 target genes, loss of the molecular components of the DREAM complex, or members of the APC<sup>CTD110</sup>–SKP2–p27<sup>kip1</sup> signaling axis may play a role. Thus far, mining of available databases did not yield any insights into these open questions that warrant more detailed investigations.

Taken together, we show here that the DREAM complex is a critical mediator of imatinib-induced GIST cell quiescence and that abrogation of quiescence enhances the proapoptotic activity of imatinib. The DREAM complex is, therefore, a promising therapeutic target to make imatinib treatment more effective with an aim toward more complete patient responses.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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