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The DREAM complex in anti-tumor activity of imatinib mesylate in gastrointestinal stromal tumors (GISTs)

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Abstract

Purpose of review—Although most gastrointestinal stromal tumors (GISTs) respond well to treatment with the small molecule kinase inhibitor imatinib mesylate (Gleevec), the majority of patients achieve disease stabilization and complete remissions are rare. Furthermore, discontinuation of treatment in the presence of residual tumor mass almost inevitably leads to tumor progression. These observations suggest that a subset of tumor cells not only persists under imatinib treatment, but remains viable. The current article reviews the molecular basis for these findings and explores strategies to exploit them therapeutically.

Recent findings—Although imatinib can induce apoptosis in a subset of GIST cells, it can induce a reversible exit from the cell division cycle and entry into G0, a cell cycle state called quiescence, in the remaining cells. Mechanistically, this process involves the DREAM complex, a newly identified key regulator of quiescence. Interfering with DREAM complex formation either by siRNA-mediated knockdown or by pharmacological inhibition of the regulatory kinase DYRK1A was shown to enhance imatinib-induced GIST cell death.

Summary—Targeting the DREAM complex and imatinib-induced quiescence could provide opportunities for future therapeutic interventions toward more efficient imatinib responses.

Keywords

Gastrointestinal stromal tumor; imatinib mesylate (Gleevec); quiescence; DREAM complex

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract, and recent epidemiologic data suggest that they may belong to the

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CONFLICT OF INTEREST

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most common sarcoma types overall [1]. The majority of GISTs express the KIT receptor tyrosine kinase (RTK) at high levels. Physiologically, KIT is activated by its ligand stem cell factor (SCF) and regulates growth and differentiation of interstitial cells of Cajal (ICC), the potential progenitor cell of GIST. In approximately 80% of GIST, KIT is constitutively activated by an oncogenic mutation [2-4]. Another 5-7% of GISTs, the related RTK *PDGFRA* (platelet-derived growth factor receptor alpha) carry a corresponding activation mutation [5]. Oncogenic mutation of either receptor leads to ligand-independent activation of downstream signaling cascades that drive cellular proliferation, presumably initiate tumor formation and maintain survival [6].

These molecular features underlying GIST pathogenesis were the basis for the implementation of targeted therapy with the small molecule inhibitor imatinib mesylate (Gleevec), a strategy that has revolutionized oncologic therapy in the past decade. Imatinib is an ATP-competitive inhibitor of the KIT, PDGFRA/B and ABL (BCR-ABL) kinases that can lead to dramatic anti-tumor responses in patients with advanced GIST. Several large Phase II and Phase III clinical trials have shown that 83-89% of the patients with non-resectable or metastatic GIST benefit from imatinib [7-9].

The metabolic response of GISTs to imatinib often show a profound decrease as assessed by fluorine-18-fluorodeoxyglucose (¹⁸FDG) uptake and positron emission tomography (PET) [10,11]. Although some patients may achieve substantial tumor shrinkage with imatinib therapy, most objective reductions in tumor size as assessed by computerized tomography (CT) are not as prominent. Only less than 2% of patients experience a complete radiographic regression [8], while most patients show a partial response to imatinib or achieve stable disease [7,8,12]. Although these responses can be quite durable, the residual tumor mass can serve as a source for therapy resistance and disease recurrence. Resistance to imatinib therapy occurs in approximately 50% of patients within the first two years of treatment [7-9].

Pathological examination of residual tumors responding to imatinib therapy confirms this notion. Most samples were found to contain morphologically viable tumor cells remaining [13]. Although the tumor cells undergoing treatment do not show mitotic activity, clinical data suggest that these cells indeed can give rise to tumor recurrence in the event that imatinib therapy is discontinued. Stopping imatinib treatment after three years in patients with previously non-progressing disease resulted in rapidly recurring tumors in almost all of the patients during a median follow-up of 35 months [14]. Progression-free survival in the group of patients that discontinued the treatment was dramatically reduced to 16% from 80% in the individuals that continued on imatinib. It is important to note, however, that most recurring tumors had not lost their sensitivity to imatinib since they responded to imatinib when it was reintroduced [14].

The above observations have been recapitulated in mouse xenograft experiments [15]. After responding to imatinib with a reduction of tumor volume to up to 17% of the baseline level following four weeks of treatment, xenografts immediately returned to their original size or even outgrew it after imatinib had been discontinued for additional four weeks. While a significant fraction of cells underwent apoptosis during the treatment period (up to a 1.8-fold

increase of cleaved caspase 3-positive cells), many xenografts only showed up to 10% of necrosis, myxoid degeneration and/or fibrosis upon histologic examination (equivalent to a grade 1 histologic response [13]) indicating the likelihood that the tumor cells remained viable and eventually gave rise to tumor regrowth [15]. Importantly, another study found that these cells were indeed positive for the cyclin-dependent kinase inhibitor p27^{Kip1}, indicating that they have exited the cell division cycle [16].

Taken together, these clinical and *in vivo* experimental observations suggest that imatinib is not able to fully eradicate all GIST cells and that the remaining subset of cells remains viable and enters a reversible state of quiescence. The present article will review the molecular basis for these observations and discuss strategies for targeting this process therapeutically.

QUIESCENCE AND THE DREAM COMPLEX

Although the general conception of quiescence as being at rest may seem to adequately describe a cell that is not actively engaging in the cell division cycle, quiescence in scientific terms is a molecularly precisely defined cell cycle state [17-19]. Quiescent cells have exited the cell division cycle and entered into G₀, a state that physiologically occurs in non-dividing cells, such as hematopoietic stem cells and adult hepatocytes [20,21]. In contrast to senescence [22], a state of permanent growth arrest [23], cellular quiescence is reversible. The reversibility of quiescence can thereby create a therapeutic challenge when it occurs in malignant cells. Not only do quiescent tumor cells generally not respond well to treatments that target dividing cells [24], they remain viable and capable of forming a reservoir for potential tumor recurrence and therapy resistance.

Experimentally, quiescence can be induced by growth factor withdrawal, contact inhibition or loss of adhesion [19]. On a molecular level, two major signaling pathways have been implicated in this process. The APC^{CDH1}-SKP2-p27^{Kip1} signaling axis is mainly important for reinforcing a prolonged G₁ phase of the cell division cycle [25]. In addition, the pRB retinoblastoma tumor suppressor gene product (RB1) and its “pocket protein” family members p130/RBL2 and p107/RBL1 are regulators of G₀ [26]. Specifically, p130/RBL2 and the repressor E2F transcription factor E2F4 have been identified as major regulators of this process [17]. p130/RBL2 protein levels are elevated in G₀, when it binds to E2F4 and both proteins translocate to the nucleus where they lead to repression of E2F target genes [17,27].

It was not until recently, however, that the regulation of quiescence induction has been elucidated in more detail. Seminal work by Litovchick et al. [28] and Schmit et al. [29] revealed that p130/RBL2 and E2F4 are in fact components of a larger, multi-subunit protein complex, called the DREAM complex. This new key regulator of quiescence is composed of **DP**, p130/**RBL2**, **E2F4** and the mammalian homologs of the *C. elegans* multivulva complex B (**MuvB**) (Fig. 1). The DREAM complex forms upon entry into G₀ when DP/RBL2/E2F4 binds to the MuvB complex and contributes to the repression of more than 800 cell cycle-regulated, E2F-dependent, genes by binding to their promoters, thereby maintaining the quiescent state [28]. It is of note that the MuvB core of the human DREAM complex (LIN9,

LIN37, LIN52, LIN54 and RBBP4) binds to DP/RBL2/E2F4 during G0 and dissociates during in S phase when it binds to B-MYB to promote mitosis and cell division [28-30]. Further insight into the regulation of quiescence comes from a recent study showing that phosphorylation of LIN52 at serine 28 is required for the MuvB core to bind to DP/RBL2/E2F4 and form the DREAM complex [31]. This phosphorylation can be catalyzed by the dual specificity tyrosine phosphorylation-regulated kinase DYRK1A [31].

Since its initial description, several studies have implicated the DREAM complex as a tumor suppressor in human papillomavirus (HPV)-associated cervical carcinoma [32-34]. However, its role in anti-cancer therapy and as a potential therapeutic target is only beginning to emerge [16].

THE DREAM COMPLEX IN THE RESPONSE TO IMATINIB THERAPY

As outlined above, clinical and *in vivo* experimental observations strongly suggest that imatinib treatment of GIST leads to a long-lasting, but reversible cell cycle exit (G0, quiescence). Recent *in vitro* studies clearly support this notion and have contributed significantly to our understanding of the molecular pathways involved in this process. When immortalized cell lines derived from primary, imatinib-sensitive GIST are treated with imatinib, up to 70% of these cells undergo apoptosis with the remaining subpopulation viable. [35-37]. Indeed, it was shown that the GIST cells surviving after imatinib treatment express high nuclear levels of the cyclin-dependent kinase inhibitor p27^{Kip1} indicating that they have exited the cell division cycle [38]. Moreover, two independent studies have shown that these cells are able to re-enter the cell cycle once the drug is removed [16,37]. Cells were able to regrow to full confluency and were able to repopulate the culture dish when replated. These results are illustrated in Figure 2, where GIST cells were treated with imatinib for three days before the drug was removed and cells were followed for another eleven days. Importantly, cells retain sensitivity to imatinib treatment after one cycle of imatinib treatment [16] thus further confirming the clinical observations described above [14].

Recent work further elucidated imatinib-induced quiescence and showed that the DREAM complex is involved in this process [16]. In this study, imatinib treatment of GIST cells led to increased levels of p130/RBL2, which co-localized with E2F4 by immunofluorescence microscopy [39]. This was accompanied by relocalization of both proteins to the nucleus and thereby indicated entry to a quiescent state [27,40-42]. Moreover, p130/RBL2 and E2F4 were shown to physically interact in coimmunoprecipitation experiments. Presence of the DREAM component LIN37 in the coimmunoprecipitation reaction indicated that the DREAM complex formed after imatinib treatment. Similar to studies in other models [31], DREAM formation and quiescence induction in GIST was accompanied by phosphorylation of LIN52 at serine 28 thus indicating regulation of this process by the DYRK1A kinase. Further experiments using small interfering RNA (siRNA) to knockdown DYRK1A and LIN52 mRNA levels confirmed this notion. Importantly, while imatinib treatment increased the levels of the DREAM complex, drug washout experiments showed that DREAM complex formation was reversible, as its disassembly coincided with re-entry into the cell division cycle (as indicated by BrdU uptake and cyclin A upregulation) [16].

To our knowledge, the study outlined above is the first to demonstrate a role of DREAM complex in therapy-induced quiescence of cancer cells. Because this study interrogated a very specific setting (use of the small molecular kinase inhibitor imatinib in GIST), it will be interesting to address similar questions for targeted therapies of other tumors in addition to GIST. Furthermore, it is tempting to speculate whether the DREAM complex also regulates quiescence induction in cancer cells after treatment with chemotherapeutic agents.

TARGETING THE DREAM COMPLEX TO ENHANCE IMATINIB THERAPY

Targeting tumor cell quiescence therapeutically – while being highly desirable clinically – has been virtually impossible in the past because of the lack of targetable regulator proteins. The identification of the DREAM complex and that its formation is mediated by DYRK1A protein kinase may prove to change this notion. Because of its novelty, this topic is only beginning to be addressed experimentally.

The hypothesis that preventing tumor cells from entering quiescence can sensitize them to treatment with anti-neoplastic agents was first tested by targeting the DYRK1A kinase in GIST cells [16]. In this study, the beta-carboline alkaloid harmine was used for DREAM complex inhibition. Harmine has ATP-dependent inhibitory activity towards DYRK1A [43] and has been shown to inhibit LIN52 phosphorylation and DREAM complex formation [31]. When used in conjunction with imatinib in GIST cells, harmine indeed had an enhancing effect on imatinib-induced apoptosis as measured by several experimental readouts [16]. siRNA-mediated inhibition of DREAM complex formation via knockdown of either LIN52 or DYRK1A also led to increased apoptosis when combined with imatinib. Together, these experiments support the hypothesis that it is possible to enhance anti-cancer therapy by simultaneously preventing cells from entering quiescence and establish the DREAM complex as a potential target for enhancing imatinib therapy in GIST. It has to be noted, that DYRK1A has been reported to have many target substrates in addition to LIN52 [44]. Recently, Cyclin D1 levels have been shown to be regulated by DYRK1A contributing to a distinct state of cell cycle arrest [45]. Inhibition of DYRK1A may therefore have many consequences in addition to preventing DREAM complex assembly.

While further pre-clinical studies testing DYRK1A inhibition in GIST are highly warranted, harmine is not suitable for clinical practice. Harmine occurs naturally in several plants, in particular from the Middle East (Syrian rue, *Peganum harmala*) and South America (ayahuasca, *Banisteriopsis caapi*), which have traditionally been used for their psychoactive effects. In addition, harmine inhibits monoamine oxidase A (MAO-A) and it is generally considered unsafe to be used in patients [43]. Fortunately, there is currently a strong interest in developing novel DYRK1A inhibitors with improved pharmacologic properties including harmine derivatives [46,47]. One potential new compound is the synthetic DYRK1A inhibitor INDY, which was found to be active in several *in vitro* and *in vivo* models [48]. A second group of DYRK1A inhibitors currently under investigation are natural compounds derived from the marine sponge *Leucetta microraphis* (“Leucettines”) [49].

Taken together, the studies outlined above have unveiled a new therapeutic option to enhance response to imatinib treatment of GIST in particular and for cancer therapy in general.

CONCLUSION

Cellular quiescence is a common challenge in cancer therapy and has recently been shown to be an inherent problem in imatinib-treated GIST. Elucidation of the molecular mechanisms revealed the DREAM complex and its activating kinase DYRK1A as the key regulators of this process. Recent studies show that it is possible to therapeutically target quiescence and that this can enhance the therapeutic effect of imatinib. Further studies are highly warranted to move this concept into clinical practice.

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ABBREVIATIONS

GIST	gastrointestinal stromal tumor
DREAM	DP, p130/RBL2, E2F4 and MuvB
DYRK1A	dual specificity tyrosine-phosphorylation-regulated kinase 1A

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KEY POINTS

- Imatinib leads to a reversible cell cycle exit (quiescence) in GIST as demonstrated by incomplete remissions, tumor regrowth after therapy discontinuation as well as a number of experimental *in vitro* findings.
- The DREAM complex, a novel key regulator of quiescence/G0, mediates quiescence in imatinib-treated GIST cells.
- It is possible to target quiescence therapeutically via inhibition of the DREAM complex regulatory kinase DYRK1A thereby enhancing the pro-apoptotic effect of imatinib.
- DREAM complex formation and ensuing cellular quiescence is likely to be a universal outcome of kinase inhibitor therapy and possibly anticancer therapy in general.

Figure 1. The mammalian DREAM complex forms in G0 and represses E2F target genes
 The DREAM complex consists of **DP**, p130/**RBL2**, **E2F4** and the mammalian homologs of the *C. elegans* multivulva class B (**MuvB**) gene products LIN9, LIN37, LIN52, LIN54 and RBBP4. Phosphorylation of LIN52 at serine 28 by the dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) is required for DREAM complex formation in G0 and transcriptional repression of E2F target genes.

Figure 2. Imatinib induces a reversible exit from the cell division cycle in GIST

Immunofluorescence microscopic analysis of BrdU incorporation in GIST882 cells. After treatment with imatinib for 72 hours, imatinib-containing tissue culture media was removed and cells were cultured in regular medium for up to 11 days (A, B). A, BrdU incorporation after control DMSO treatment, three days of imatinib treatment or 11 days after washout of the drug. B, Quantification of the percentage of GIST882 cells, showing BrdU incorporation during imatinib treatment for 3 days and after the drug was removed. Nuclei were stained

with DAPI. Scale bar, 50 mm. (Modified from Boichuk et al., Cancer Res. 2013; 73:5120-5129.[16])