Persistent Activation of the Fyn/ERK Kinase Signaling Axis Mediates Imatinib Resistance in Chronic Myelogenous Leukemia Cells through Uprogulation of Intracellular SPARC

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Abstract

SPARC is an extracellular matrix protein that exerts pleiotropic effects on extracellular matrix organization, growth factor availability, cell adhesion, differentiation, and immunity in cancer. Chronic myelogenous leukemia (CML) cells resistant to the BCR-ABL inhibitor imatinib (IM-R cells) were found to overexpress SPARC mRNA. In this study, we show that imatinib triggers SPARC accumulation in a variety of tyrosine kinase inhibitor (TKI)–resistant CML cell lines. SPARC silencing in IM-R cells restored imatinib sensitivity, whereas enforced SPARC expression in imatinib-sensitive cells promoted viability as well as protection against imatinib-mediated apoptosis. Notably, we found that the protective effect of SPARC required intracellular retention inside cells.

Introduction

Chronic myelogenous leukemia (CML) is a hematopoietic stem cell disease due to the t(9; 22) translocation that encodes p210BCR-ABL (1), which activates several signaling pathways including ERK and Src kinases (2, 3). Imatinib is the first line treatment option for patients with CML and has proven efficacy in all stages of the disease (4, 5). However, resistance to imatinib occurs in all phases of the disease and second-generation inhibitors, such as dasatinib (6, 7) and nilotinib (8), have been developed to circumvent these resistances. Although resistance to imatinib generally involved point mutations in the kinase domain of BCR-ABL (9), other modes of resistance have also been reported including overexpression of Src kinases (10, 11). We recently isolated IM-R K562 cell clones that displayed Fyn overexpression as a possible mechanism of resistance (12). Accordingly, pharmacologic inhibition of Fyn in IM-R cells circumvented imatinib resistance. Also, using a pangenomic approach, we established that unlike their imatinib-sensitive (IM-S) counterpart, IM-R cells expressed a high level of SPARC mRNA (12).

SPARC, a matricellular protein, is recognized as an important modulator of cell–matrix interactions (13–15). It is associated with remodeling tissues and pathologic responses to injury and tumorigenesis (16). It is also involved in osteogenesis, immune modulation, cell proliferation, adhesion, and migration (13, 14). These biological activities are mediated through interaction with adhesion receptors and numerous components of the extracellular matrix in a cell-type specific and cellular context–dependent manner. Besides its well-characterized extracellular and pericellular functions, emerging data suggest that SPARC may also function inside cells (17). Indeed, intracellular SPARC is associated with tubulin in ciliated epithelial cells (18) and is found in the nuclei of ciliated epithelial and lens epithelial cells (19, 20). Together, these reports indicate that SPARC might function intracellularly to influence diverse cellular activities.
SPARC has been linked to the progression and invasive abilities of many solid tumors, such as prostate and breast cancers, and melanoma (21–24), whereas it appears to have tumor-suppressing properties in ovarian cancers and certain myeloid malignancies (25, 26).

Regarding hematopoietic malignancies, the human SPARC gene maps to the commonly deleted region in the 5q–myelodysplastic syndromes (MDS) and its insufficiency has been implicated in the pathogenesis of MDS (27, 28). Furthermore, SPARC-null mice show hematologic abnormalities that are consistent with a potential role in MDS. In addition, lower levels of SPARC expression are found in acute myelogenous leukemia (AML) cells from patients with MLL translocations (25). However, the role of SPARC in other myeloid malignancies, such as CML, is not known.

Using pangenomic profiling, we identified SPARC as an upregulated gene in IM-R K562 cells (12). Here we aimed to investigate the functional contribution of SPARC accumulation to imatinib resistance. We demonstrate that SPARC silencing, using specific siRNAs in IM-R cells, circumvents imatinib resistance. Alternatively, overexpression of SPARC in IM-S cells promotes protection against imatinib-mediated cell death. Interestingly, the protective effect of SPARC seems to depend on its intracellular localization. In addition, specific SPARC accumulation in resistant cells requires persistent activation of an Fyn/ERK signaling pathway that is constitutively activated in IM-R CML cells.

In conclusion, we describe an original mechanism of resistance linking constitutive activation of Fyn and ERK to SPARC accumulation, thereby promoting imatinib resistance via inhibition of CML cell apoptosis. Our study suggests that upregulation of SPARC may participate in the clinical behavior of imatinib-resistant disease.

Materials and Methods

Cell culture and reagents

Human CML cell lines K562, LAMA, and JURL-MK1 were maintained as described previously (12). IM-R and PD166-R K562 clones have been described elsewhere (12, 29–31). Nilotinib-resistant (Nilo-R) K562 cells and imatinib- or nilotinib-resistant LAMA cells were established as described earlier (32). Imatinib-resistant JURL-MK1 cells were obtained by the addition in the culture medium of increasing concentrations of imatinib, as described for IM-R cells. The human melanoma cell line A375 has been described previously (24).

The list of antibodies used in immunoblotting is provided in Supplementary informations. Ac-DEVD-AMC was from Alexis Biochemicals and U0126 and PP2 from Calbiochem. Primers and culture reagents were obtained from Invitrogen. All other chemicals were obtained from Sigma.

Primary cell isolation and real-time quantitative PCR analysis

Blood samples were collected from 14 patients newly diagnosed with CML and after 1 year of imatinib treatment. All patients were part of an institutional protocol and had a complete cytogenetic but not molecular response at 1 year. Peripheral blood mononuclear cells were isolated by density centrifugation (Ficoll-Paque Plus), and total RNA was extracted using TRIzol reagent (Invitrogen) and reverse transcribed using MultiScribe reverse transcriptase (Applied Biosystems). Quantitative PCR (qPCR) analysis was carried out from 2 μg of cDNA in an ABI Biosystems 7900HT Sequence Detector System (Applied Biosystems), using the SYBR Green detection protocol, as outlined by the manufacturer. For each patient, relative expression level of SPARC mRNA was normalized using ABL as an invariant control. Statistical analysis was performed using the Student’s test with P < 0.05 deemed as statistically significant.

Plasmid generation and expression of SPARC constructs

The cDNA encoding human SPARC (nucleotides 1–912) was cloned into the pcDNA3/Myc-His vector (Invitrogen). SPARC–ΔS (nucleotides 55–912) and SPARC–ΔEC (nucleotides 1–486) were constructed by PCR amplification from the full-length SPARC. K562 cells were transiently transfected as described previously (33). One day after transfection, cells were treated before the determination of cell viability, caspase activity, and cell-cycle analysis.

RNA interference

Stealth small interfering RNAs (siRNA) targeting SPARC or Fyn were purchased from Invitrogen. K562 cells were transfected as described previously (33). Four days after transfection, cells were harvested for protein expression analysis. For other experiments, cells were treated with indicated effectors 2 days after transfection.

Western blot

After stimulation, cells were harvested and lysed in buffer containing 1% Triton X-100 and supplemented with protease and phosphatase inhibitors (Roche Diagnostics). Lysates were pelleted, and 50 μg of proteins were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, as described previously (34).

Cell viability assay

Cells were incubated in a 96-well plate with different effectors, and cell viability was analyzed by XTT assay, as described earlier (35).

Colony formation assay

Two days after transfection, imatinib was added to K562 cell lines growing in semisolid methylcellulose medium (MethoCult H4100 for cell lines; StemCell Technologies Inc.). Colony formation assays have been reported elsewhere (33).

Caspase activity measurement

Caspase assays have been described in detail elsewhere (35).

Cell-cycle analysis by flow cytometry

Cells were washed, fixed in citrate buffer, and incubated at ~20°C for 1 hour. They were next stained in buffer containing 40 μg/mL of propidium iodide for 1 hour at 4°C. Cell-cycle
profiles were collected using a FACScan instrument and analyzed with the CellQuest software (Becton-Dickinson).

**Results**

**SPARC is overexpressed in several CML cell lines and CML cells from imatinib-treated patients**

We have reported earlier that IM-R K562 cells expressed high levels of SPARC mRNA (12). Accordingly, SPARC protein was detected in IM-R K562 and LAMA cells but not in their IM-S counterpart (Fig. 1A). Interestingly, SPARC expression was further increased by imatinib in IM-R K562 and LAMA-R cells (Fig. 1A). Although overexpressed in Nilo-R LAMA cells (Fig. 1B, bottom) and PD166-R K562 cells (Fig. 1C), the amount of SPARC was modified by neither nilotinib nor PD166326. In contrast to IM-R cells, SPARC was expressed neither in parental K562 cells nor in their Nilo-R counterpart (Fig. 1B, top). The dual BCR-ABL and Src kinase

![Figure 1](image-url)

**Figure 1.** Increased expression of SPARC in IM-R K562 and LAMA cells. A–C, TKI-resistant CML cells, IM-R, Nilo-R, or PD166-R, were stimulated with increasing concentrations of imatinib (A), nilotinib (B), or PD166326 (C) for 48 hours. SPARC and HSP60 (loading control) levels were visualized by immunoblotting. D, IM-R cells were incubated for various times with 1 μmol/L of imatinib. SPARC level was detected by immunoblotting. expo, exposure. E, qPCR analysis of SPARC mRNA level from 14 patients diagnosed with CML before and after a year of therapy with imatinib. *, P < 0.05 (Student’s t test). CT, control; Ima, imatinib; Nilo, nilotinib.
inhibitor PD166326 failed to increase SPARC expression in IM-R cells (Supplementary Fig. S1A). In addition, imatinib did not modulate SPARC expression in PD166-R or Nilo-R cells (Supplementary Fig. S1B and C). Induction of SPARC expression was detected rapidly after imatinib addition and was maximal after 24 hours (Fig. 1D). Finally, increased SPARC expression was detected in 4 different IM-R K562 cell clones, ruling out the possibility of a clonal effect (Supplementary Fig. S1D).

SPARC is deleted in MDS with 5q31 deletion (28), but whether, and how, SPARC affects the pathogenesis of CML is not known. As IM-R cells have been selected following long-term incubation with imatinib, we were wondering whether such an accumulation of SPARC mRNA occurred in CML patients treated for long periods of time with imatinib. qPCR analysis of SPARC mRNA expression was thus carried out on PBMC from 14 CML patients at diagnosis or after 12 months of imatinib treatment. As shown in Figure 1E, all except 1 patient treated with imatinib displayed a statistically significant increase in SPARC transcript levels at 12 months.

Hypermethylation of the SPARC promoter has been associated with low SPARC expression in epithelial tumors (36). A bisulfite treatment of genomic DNA isolated from IM-S and IM-R K562 cells followed by a methylation-specific PCR demonstrated that the lack of SPARC expression in IM-S cells, but not in IM-R cells, is associated with complete methylation of its promoter (Supplementary Fig. S2A). SPARC mRNA expression could be reinduced in IM-S cells by 5-aza-2'-deoxycytidine, a DNA methylation inhibitor (Supplementary Fig. S2B).

Inhibition of SPARC expression in IM-R cells circumvents imatinib resistance

To investigate whether SPARC participates in imatinib resistance, we next knocked down SPARC by using 2 distinct siRNAs. Both siRNAs abolished SPARC expression in IM-R cells (Fig. 2A). Inhibition of SPARC expression was found to
circumvent imatinib resistance as judged by the significant decrease in cell viability in IM-R cells depleted in SPARC (Fig. 2A). This result was also confirmed in clonogenic assays (Fig. 2B) and strongly suggested that increased expression of SPARC in IM-R cells contributes to imatinib resistance.

As imatinib disrupted the cell-cycle distribution of IM-S cells, but not that of their IM-R resistant counterpart (12), we next analyzed the effect of SPARC silencing in IM-R cells treated with TKI. As expected, imatinib induced accumulation of IM-S cells in the G0/G1 phase of the cell cycle 24 hours after drug addition (Fig. 2C). At 48 hours, there was also an increase in the percentage of cells that accumulates in the sub-G1 phase of the cell cycle, reflecting increased apoptosis. Importantly, knockdown of SPARC failed to alter cell cycle and apoptosis in IM-S cells that did not express SPARC. Finally, inhibition of SPARC expression, using specific siRNAs, significantly increased the accumulation of IM-R cells in the G0/G1 phase of the cell cycle at 24 hours and apoptosis at 48 hours.

Forced expression of SPARC in IM-S cells mitigates imatinib-mediated apoptosis

If SPARC is involved in imatinib resistance, its ectopic expression in IM-S cells should mitigate the effect of imatinib on both cell cycle and viability. When IM-S cells were transiently transfected with a plasmid encoding the full-length SPARC, significant levels of SPARC protein were detected after transfection (Fig. 3A). SPARC expression drastically reduced the proportion of IM-S cells in the G0/G1 and sub-G1 phases of the cell cycle at 24 and 48 hours of imatinib treatment (Fig. 3B).

SPARC is a secretory glycoprotein composed of 3 domains that can function independently to influence cellular activities (Fig. 3C). Of note, the EC domain binds components of the extracellular matrix and is responsible for mediating many of SPARC`s extracellular effects (24). However, there is compelling evidence that SPARC might also exert intracellular functions (20, 37). Given the significant role of SPARC in the resistance of IM-R cells, we investigated which domain of

Figure 3. Forced expression of SPARC protects IM-S cells from cell death induced by imatinib. A and B, IM-S K562 cells were transfected with the indicated vectors for 2 days before imatinib treatment (1 μmol/L). A, SPARC level was analyzed by immunoblotting, using an anti-Myc tag antibody. B, either 24 or 48 hours after imatinib treatment, cell-cycle profiles were analyzed by flow cytometry of propidium iodide–stained cells. Histograms represent the percentage of cells in each phase of the cell cycle. C, top, schematic representation of SPARC constructs. SPARC-WT and 2 mutants, SPARC-ΔS (lacking the signal peptide required for secretion) and SPARC-ΔEC (lacking the biological active domain interacting with extracellular components). C and D, IM-S K562 cells were transfected with different plasmids for 2 days and then treated with imatinib (1 μmol/L). C, bottom, for control of SPARC expression (D) 2 days after imatinib treatment, cell viability was assessed by XTT assay and caspase 3 activity was measured, using z-DEVD-AMC as a substrate. Results are expressed in percentage of control for 4 independent experiments. A.U., arbitrary unit; CT, control; Ima, imatinib.
the protein is needed for this activity. We used different SPARC mutants to discriminate between an intracellular or extracellular action of this protein. The full-length SPARC (SPARC-WT), a mutant lacking the signal peptide (SPARC-ΔS) that cannot be secreted, and a mutant deleted for the EC domain (SPARC-ΔEC) were transfected in SPARC-negative IM-S cells. We first checked that all constructs were produced in K562 cells (Fig. 3C). Next, we evaluated their effects on viability and caspase 3 activity following imatinib treatment. In contrast to SPARC-ΔEC, both mature SPARC-WT and nonsecreted SPARC-ΔS protected IM-S cells from imatinib-induced loss of viability and apoptosis (Fig. 3D). Therefore, SPARC-mediated resistance to imatinib in IM-S cells is likely due to an intracellular action.

### SPARC is not secreted in IM-R cells

SPARC-ΔS and SPARC-ΔEC were produced at identical levels in IM-S and A375 melanoma cells, used as a positive control of SPARC secretion (Fig. 4A). Interestingly, neither SPARC-WT nor SPARC-ΔS or SPARC-ΔEC was secreted in IM-S cells, whereas, as expected, SPARC-WT and SPARC-ΔEC were detected in the culture medium of melanoma cells (23).

Therefore, the defect of SPARC secretion seems to be inherent in the K562 cell line. In contrast to A375 cells, neither exogenous nor endogenous SPARC was secreted in IM-S and IM-R cells (Fig. 4B). Ectopically expressed SPARC was detected only in the microsomal fraction of IM-S cells (Supplementary Fig. S3). Moreover, localization of endogenous SPARC in IM-R cells was identical to that of transfected cells. Specific siRNA directed against SPARC abolished microsomal SPARC expression (Supplementary Fig. S3). Taken together, our findings show that SPARC, both endogenous and exogenous, is present in the same subcellular compartments and that SPARC is retained in the microsomal fraction of IM-R cells.

### Increase in SPARC expression is due to permanent activation of the Fyn/ERK module

Imatinib-mediated increase in SPARC expression correlated with permanent phosphorylation of ERK1/2, mTOR, and S6 ribosomal protein in IM-R cells (Fig. 5A). Activation of these pathways was independent of BCR-ABL because imatinib could still reduce BCR-ABL phosphorylation in IM-R cells. Importantly, using an anti-phospho-Src antibody, we detected an increased phosphorylation of p59Fyn, together with a concomitant reduction in p53/p65lyn phosphorylation in imatinib-treated IM-R cells. Finally, the overall profile of tyrosine phosphorylation was only moderately altered by imatinib in IM-R cells compared with their IM-S counterpart (Fig. 5A).

To gain insights into this new mechanism of resistance, we next investigated the hierarchy of kinase activation leading to SPARC accumulation in IM-R cells, using a pharmacologic approach. First, rapamycin failed to alter SPARC expression in identical conditions, ruling out an implication of the mTOR pathway in SPARC regulation (Supplementary Fig. S4). U0126 (a MEK1 inhibitor) abrogated constitutive activation of ERK1/2 and consequently blocked both basal SPARC expression and imatinib-mediated accumulation of SPARC (Fig. 5B). This finding demonstrated that SPARC expression is controlled specifically by the ERK1/2 pathway.

In addition, cotreatment of IM-R cells with PP2 (a broad-range inhibitor of Src kinases) and imatinib phenotyped the effect of U0126 on SPARC expression and also inhibited ERK1/2 phosphorylation (Fig. 5C), suggesting that Src kinases, and most likely p59Fyn, act upstream of ERK1/2 in IM-R cells to promote SPARC accumulation.

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**Figure 4.** SPARC is not secreted in IM-S and IM-R cells. A and B, K562 cells and A375 melanoma cells were transiently transfected with indicated vectors for 48 hours. Levels of intracellular (in lysates) and secreted (in culture media) SPARC were analyzed by immunoblotting in transfected IM-S cells (A) and IM-R cells (B) compared with A375 cells. HSP60 was used as a loading control of lysate and fibronectin as a loading control of the medium. endo, endogenous.
To investigate further the role of Fyn in ERK1/2 and SPARC regulation, we knocked down Fyn with a specific siRNA. Both Fyn silencing and imatinib treatment abrogated constitutive ERK1/2 activation and SPARC accumulation in IM-R cells (Fig. 5D). Therefore, increased expression and activity of p59Fyn in IM-R cells likely contribute to persistent ERK1/2 activation, which, in turn, promotes SPARC accumulation. This mode of regulation was observed in different IM-R cell clones (Supplementary Fig. S5).

**Fyn governs overexpression of SPARC in CML-R cells**

As Fyn is important to transduce the signals leading to SPARC accumulation in IM-R cells, we next investigated signal transduction in different TKI-resistant CML cell lines. Nilo-R
K562 and IM-R JURL-MK1 cells, both of which overexpressed active Lyn, but not Fyn, exhibited a low level of phosphorylated ERK1/2 and were negative for SPARC expression (Fig. 6A and B). Conversely, SPARC-positive IM-R LAMA cells, which expressed a high level of active Fyn, but not Lyn, had an elevated level of phosphorylated ERK1/2 especially in the presence of imatinib (Fig. 6C). All together, these findings confirmed the implication of Fyn in SPARC accumulation in different TKI-resistant CML cell lines. Finally, we sought to determine how imatinib upregulates SPARC expression in IM-R cells. The possibility of a transcriptional control of SPARC by imatinib was not investigated, as this TKI failed to impair SPARC mRNA accumulation (Supplementary Fig. S6).

Inhibition of ERK circumvents imatinib resistance

On the basis of the previous results, it was of importance to investigate whether inhibition of MEK by U0126 might also resensitize IM-R cells to imatinib-mediated cell death. Both U0126 and imatinib were found to decrease IM-S cell viability, and the combination of both effectors exerted an additive effect (Fig. 7A). As expected, imatinib failed to alter cell viability in IM-R cells. In contrast, U0126 strongly decreased cell viability in IM-R cells to approximately the same extent as that of the combination of imatinib and U0126 in IM-S cells. Loss of imatinib- or U0126-mediated cell viability was accompanied with increased caspase 3 activity (Fig. 7B). Finally, knockdown of SPARC with specific siRNA did not in turn alter ERK1/2 phosphorylation in imatinib-treated IM-R cells (Fig. 7C).

Discussion

Resistance to TKI, which is a major drawback in patient with CML, can be linked to increased expression of Src kinases (10, 38). We have recently isolated IM-R K562 clones that overexpressed the Fyn tyrosine kinase as a new mechanism of resistance (12). Accordingly, targeting Fyn with specific Src kinase inhibitors (including PP2 and SU6656) resensitized
IM-R cells to imatinib (12). However, downstream targets or comediators of Fyn implicated in imatinib resistance of IM-R cells are unknown. Searching for genes modulated in IM-S versus IM-R cells led us to the identification of SPARC mRNA upregulation. Here, we investigated the possibility that increased expression and activity of Fyn and accumulation of SPARC were interconnected events. We established that increased SPARC mRNA and protein expression are due to permanent activation of the Fyn/ERK module. Interestingly, increased SPARC expression was not a unique feature of imatinib in IM-R K562 or LAMA cells, as SPARC overexpression was found in other TKI-resistant CML. In addition, we showed that SPARC does contribute to imatinib resistance because silencing of SPARC with specific siRNAs decreases IM-R cell viability and clonogenic potential only in the presence of this drug. Importantly, SPARC siRNAs had no effect, by themselves, on cell viability and apoptosis, supporting the notion that overexpression of SPARC is linked specifically to imatinib resistance. Moreover, SPARC silencing also restored G0/G1 arrest and apoptosis induced by imatinib in IM-R cells. In contrast, ectopic overexpression of SPARC in SPARC-negative IM-S cells conferred protection against imatinib-mediated loss of viability and caspase 3 activity.

Study of SPARC in hematopoietic malignancies has led to conflicting reports regarding its role as a tumor promoter or suppressor. For instance, in 5q- MDS, SPARC is recognized as a tumor suppressor, the deletion of which is associated with the pathogenesis of the disease (28). Accordingly, in patients with low-risk MDS carrying a 5q31 deletion, lenalidomide both inhibited growth of 5q- erythroblasts and upregulated SPARC, suggesting a role of this protein as a tumor suppressor (27).

In the same line, low SPARC expression is associated with AML patients with MLL gene rearrangement, suggesting that it also acts as a tumor suppressor in this disease. In contrast, SPARC is overexpressed in extramedullary plasmacytoma (39). These findings underline the dual role of SPARC in various hematopoietic malignancies.

Our results provide the first demonstration that SPARC is overexpressed in imatinib-resistant CML cell lines. Together with the observation that SPARC expression is also significantly increased in patients treated for 12 months with imatinib, we point out an important and unexpected role of SPARC as a potential cause of resistance to TKI in CML cells.

Alterations in SPARC expression have been associated with remodeling tissues and sites of high cellular turnover during
embryogenesis, wound healing, and tumorigenesis. Of note, loss of SPARC expression in solid tumors and in some AMLs may be linked to hypermethylation of its promoter (25, 40). We found that the SPARC promoter is hypermethylated in IM-S cells and that 5aza-dC, a demethylating agent, could increase SPARC expression in these cells through demethylation of its promoter.

Our data also suggest that overexpression of SPARC in IM-R cells is due to constitutive activation of the ERK pathway, as basal or imatinib-mediated SPARC upregulation is abrogated by U0126, an MEK1 inhibitor. To investigate the link between Fyn and ERK activation in IM-R cells, we carried out additional experiments on a set of resistant CML cell lines. We confirmed the crucial role of p59Fyn, but not Lyn (a kinase involved in resistance to TKI in CML cells), in the regulation of ERK activation and SPARC expression. This is consistent with our previous observations showing an increased expression and activity of Fyn and a decreased activity of Lyn in IM-R cells (12).

Interestingly, we also established that Fyn knockdown by siRNA or inhibition of Fyn activity using PP2 could abrogate ERK activation and basal SPARC expression.

An attractive hypothesis to explain this observation is that both BCR-ABL inhibition by imatinib and Fyn downregulation are required to mediate a complete inactivation of ERK and consequently a decrease of SPARC in IM-R cells. Therefore, despite BCR-ABL inhibition by imatinib, hyperactivation of Fyn maintains ERK activity and SPARC accumulation in IM-R cells. Further work is needed to define the mechanisms underlying Fyn/ERK-dependent SPARC overexpression in IM-R CML cells, even though our present findings clearly show that epigenetic mechanisms are important.

The notion that SPARC may trigger part of its biological effects intracellularly is an emerging one. Our data extend this recent paradigm regarding SPARC, its cellular localization, and activity. Indeed, an intriguing observation is that despite its high level of expression in IM-R cells, SPARC is not secreted into the medium but is rather found in an intracellular compartment. Subfractionation experiments showed that SPARC accumulates in the microsomal fraction of IM-R cells. This uncommon expression of SPARC is not specific to IM-R cells, as forced expression of full-length SPARC in IM-S K562 cells also leads to its retention inside the cells. In contrast, overexpression of SPARC in A375 melanoma cells is accompanied by accumulation in the culture medium (23). Thus, intracellular SPARC retention seems inherent in K562 cells. Our results support the notion that SPARC mediates its protective effect in IM-R cells from inside the cells. Accordingly, expression of a nonsecreted form of SPARC (mutant SPARC-L5) is as efficient as wild-type SPARC to promote cell survival and inhibition of apoptosis.

We do not know at present how intracellular SPARC may protect IM-R cells from cell death. It has been reported that SPARC may bind procaspase 8, leading to increased apoptosis and potentiation of colorectal cancer cells to chemotherapy (37). These findings are in contrast with our own data, showing that SPARC functions as an inhibitor of apoptosis in imatinib-resistant CML cells. SPARC has been shown to activate Akt in human glioma cells, an effect that favors tumor progression by enabling cancer cells to survive stress conditions (41).

This is consistent with the prosurvival and antiapoptotic roles of SPARC described herein. However, the mechanism underlying SPARC effects in promoting CML cell survival is probably different, as it does not require Akt (not shown) but rather relies on permanent Fyn/ERK activation.

Many clinical studies have reported a correlation between SPARC expression, malignant progression, and patient outcome. The ability of SPARC to affect tumor biology has been attributed to its capacity to modulate cell–matrix interactions, integrin-dependent pathways, as well as growth factors and chemokine availability. One possibility that arises from our study is that the divergent action of SPARC might also depend on whether it is secreted and associated with the extracellular matrix or retained inside the cells. This view supports the idea that cellular localization of SPARC might represent a significant determinant in predicting its activity. Nevertheless, how SPARC may affect signal transduction inside the cells remains a conundrum and an opened question that warrants further studies.

Overcoming resistance to chemotherapy is a major challenge to cure cancer. This is particularly true in CML in which resistance is an important drawback for a small group of patients who are refractory to TKI. The role of SPARC in the resistance to chemotherapy is poorly documented. Tai and colleagues reported that the chemotherapy-resistant MIP101 colorectal cancer cells, which expressed a low level of SPARC, are refractory to the combination of 5-fluorouracil and irinotecan and that the addition of exogenous SPARC and/or reexpression of SPARC in MIP101 tumor xenografts restored their sensitivity to these drugs (42). As opposed to this situation in which SPARC reverses therapy resistance, our study strongly supports the notion that it confers imatinib resistance in CML cells. Finally, as also shown here, SPARC overexpression is not only involved in the resistance to TKI in vitro but also linked to TKI treatment in vivo.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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