Spleen Tyrosine Kinase Functions as a Tumor Suppressor in Melanoma Cells by Inducing Senescence-like Growth Arrest

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Abstract

Loss of tumor-suppressive pathways that control cellular senescence is a crucial step in malignant transformation. Spleen tyrosine kinase (Syk) is a cytoplasmic tyrosine kinase that has been recently implicated in tumor suppression of melanoma, a deadly skin cancer derived from pigment-producing melanocytes. However, the mechanism by which Syk suppresses melanoma growth remains unclear. Here, we report that reexpression of Syk in melanoma cells induces a p53-dependent expression of the cyclin-dependent kinase (cdk) inhibitor p21 and a senescence program. We first observed that Syk expression is lost in a subset of melanoma cell lines, primarily by DNA methylation–mediated gene silencing and restored after treatment with the demethylating agent 5-aza-2-deoxycytidine. We analyzed the significance of epigenetic inactivation of Syk and found that reintroduction of Syk in melanoma cells dramatically reduces clonogenic survival and three-dimensional tumor spheroid growth and invasion. Remarkably, melanoma cells reexpressing Syk display hallmarks of senescent cells, including reduction of proliferative activity and DNA synthesis, large and flattened morphology, senescence-associated β-galactosidase activity, and heterochromatic foci. This phenotype is accompanied by hypophosphorylated retinoblastoma protein (Rb) and accumulation of p21, which depends on functional p53. Our results highlight a new role for Syk tyrosine kinase in regulating cellular senescence and identify Syk-mediated senescence as a novel tumor suppressor pathway the inactivation of which may contribute to melanoma tumorigenicity. [Cancer Res 2009;69(7):2748–56]

Introduction

Cutaneous melanoma is the leading cause of skin cancer–related mortality in Western societies, owing to its high capability of invasion and rapid metastasis to other organs (1). The first step toward transition to melanoma is uncontrolled proliferation of epidermal melanocytes and dysplasia, which may arise within a preexisting benign lesion (nevus) or more frequently directly from a new site. Subsequently, primary melanoma progresses through the radial growth phase, characterized by horizontal growth of transformed cells within the epidermis and the vertical growth phase, which is associated with depth invasion of cells into dermis and acquisition of metastatic potential (2). Melanoma formation and progression is accompanied by activating mutations in B-RAF, N-RAS, and KIT genes, as well as in cyclin-dependent kinase 4 (CDK4) gene, and loss of tumor suppressor genes, such as CDKN2A, PTEN, and E-cadherin (2, 3). Oncogenic signaling and failure of tumor suppressor mechanisms are believed to contribute to the molecular pathogenesis of melanoma.

Cellular senescence represents a powerful tumor-suppressive process that constrains malignant transformation, as it controls excessive proliferation driven by oncogenic mutations (4–7). B-RAF mutations are the most prevalent oncogenic mutations in human melanocytic skin lesions (8). B-RAF V600E mutation was found in over two-thirds of human melanoma, as well as in a high proportion of benign nevi in which melanocytes were thought to be senescent. Michaloglou and colleagues (9) showed that B-RAF V600E activates a senescence program that induces melanocyte growth arrest and that senescence in nevus cells is triggered by B-RAF V600E signaling. This work supports the model that nevi represent senescent clones of melanocytes and that senescence is a barrier to melanoma progression (10). In this context, senescence was shown to be dependent on p16INK4a expression (11). The B-RAF V600E study, however, has suggested that, besides p16INK4a, another melanoma suppressor(s) contribute to protection against oncogenic B-RAF signaling (9). In this regard, the secreted protein IGFBP7 has been recently proposed to contribute to B-RAF V600E–mediated cell senescence (12).

Spleen tyrosine kinase (Syk) is a nonreceptor tyrosine kinase that is widely expressed in hematopoietic cells. It contains tandem NH2 terminal Src homology 2 (SH2) domains, multiple tyrosine phosphorylation sites, and a COOH terminal tyrosine kinase domain. The SH2 domains bind phosphorylated immunoreceptor tyrosine-based activation motifs (ITAM) and, hence, couple-activated immunoreceptors to multiple downstream signaling pathways. Syk is essential for lymphocyte development and function and signal transduction via a variety of membrane receptors in nonlymphoid cells, such as mast cells or platelets (13). It was believed for years that Syk function was solely linked with hematopoietic cell signaling. However, more recent studies have indicated a ubiquitous pattern of SYK gene expression. Syk is expressed in endothelial cells, fibroblasts, epithelial cells, and neuronal cells, but its function in these cells is not yet completely understood (14).

Accumulating evidence suggests that Syk can function as a tumor suppressor, unlike other tyrosine kinases that generally promote growth-stimulating activity leading to tumorigenicity (15).
Syk inactivation in tumor progression, reexpression of Syk in invasive breast cancer cells was shown to inhibit tumor growth and reduce metastasis in mouse xenografts (16). The tumor-suppressive activity of Syk in breast cancer cells has been associated with abnormal mitotic progression and cell death (18, 19). Epigenetic silencing through hypermethylation of critical CpG islands was proposed to be involved in the loss of Syk in a significant fraction of breast tumors (20). More recently, a similar loss of Syk expression has been documented in melanoma cells. Importantly, reintroduction of Syk was shown to restrict tumor growth and metastasis in vivo (21, 22). However, the molecular events responsible for Syk tumor suppressor effects in melanoma cells have remained completely unknown.

Here, we aimed to investigate the tumor suppressor function of Syk in melanoma cells. We present evidence that Syk exerts its antitumor activity through induction of a senescence-like growth arrest and activation of a p53-dependent pathway, suggesting that loss of Syk may contribute to the senescence bypass generally observed in malignant melanoma. Our findings identify a novel link between the tyrosine kinase Syk, p53, and the senescent program in cancer cells.

Materials and Methods

Reagents and plasmids. The list of antibodies used is provided in Supplementary Materials and Methods. Pifithrin-α (PFT-α) was from Calbiochem. p53 small interfering RNA (siRNA) was purchased from Santa Cruz Biotechnology. Wild-type and kinase-defective (Syk-KD, K395R) porcine Syk cDNAs, as well as SH2 domain-deleted Syk mutant (Syk-ΔSH2), were cloned into the pEF6/V5 vector. These mutants have been described elsewhere (23). The SH2-inactive domain mutant (Syk-SH2D) was produced using a QuikChange site-directed mutagenesis kit supplied by Stratagene. Recombinant adenoviruses expressing wild-type and kinase-defective Syk were kindly provided by Dr. Yamamura (Kobe University) and have been described previously (24).

Cell culture, RNA interference, and adenovirus infection. Human melanoma cell lines were provided by Drs. M. Herlyn (Wistar Institute) and F. Jotereau [Institut National de la Sante et de la Recherche Medicale (INSERM) U601]. Cells were routinely maintained in DMEM supplemented with 7% fetal bovine serum, as described elsewhere (25). Transfection of duplex siRNAs (50 nmol/L) was carried out using Lipofectamine RNAiMAX (Invitrogen). Adenovirus infection was performed, as described previously (26).

Analysis of cell proliferation, colony formation assays, and three-dimensional spheroid growth. For BrdUrd incorporation and DNA content analysis, subconfluent cultures were pulsed with 10 μmol/L BrdUrd for 4 h. Cells were harvested, and DNA was stained with 7-amino-actinomycin D (7-AAD) and a APC-conjugated anti-BrdUrd antibody using the APC BrdUrd flow kit (BD Biosciences). Flow cytometric analyses were performed on a Becton Dickinson FACScanibur machine, and data were analyzed using CellQuest software.

For colony formation assays, cells were transfected by using FuGENE 6 (Roche Diagnostics) with DNA mixtures containing 1 μg of the relevant plasmids plus 0.1 μg of pBabe-Puro vector. After 48 h, cells were selected with 1 μg/mL of puromycin and maintained for 12 to 15 d. Colonies were fixed with 3% paraformaldehyde and stained with 0.4% crystal violet. The number of colonies was scored quantitatively using Image J software.

Melanoma spheroids were generated using the liquid overlay technique as described (27). Briefly, 24-well culture plates were coated with 1.5% agarose prepared in sterile water. Cells from a single-cell suspension were added at 10,000 per well. The plates were gently swirled and incubated at 37°C in 5% CO₂ atmosphere, until spheroid aggregates were formed.

Senescence-associated β-galactosidase staining. Cells were washed in PBS and fixed with 3% formaldehyde for 15 min at room temperature. Staining for senescence-associated β-galactosidase (SA-β-gal) activity was

The role of Syk as candidate tumor suppressor has been well documented in breast cancer. Loss or reduced expression of Syk in human breast cancers was associated with a higher degree of malignancy and poor prognosis (16–18). Consistent with a role for
performed, as described previously (28), using SA-β-gal staining kit (Cell Signaling Technology). Stained cells were visualized by phase-contrast microscopy, photographed, and counted.

**Immunoblotting and immunofluorescence.** Whole-cell lysates were prepared by lysing cells in a radioimmunoprecipitation assay buffer supplemented with 1% NP-40 and subjected to SDS-PAGE. Immunoblotting was accomplished according to standard procedures using enhanced chemiluminescence detection. For immunofluorescence, cells were fixed with 3% paraformaldehyde and then incubated with primary and secondary antibodies, as described elsewhere (25, 29).

**Migration and invasion assays.** Chemotaxis and Matrigel invasion assays were monitored using modified Boyden chambers, as described (25, 29).

**Statistical analysis.** Unless indicated otherwise, experiments shown were representative of at least three independent experiments. Results were expressed as mean ± SD. Where appropriate, the Student’s t test was done and P < 0.001 was considered statistically significant.

**Results**

**Syk expression and Syk promoter methylation status in melanocytes and melanoma cells.** Initially, we examined expression, phosphorylation level, and cellular localization of Syk in melanocytes. A 72-kDa band corresponding to Syk was readily detected by immunoblotting of lysates from different primary human melanocyte cultures (Supplementary Fig. S1). We also observed that Syk is phosphorylated on Tyr352, a site that has been proposed to bind signaling proteins (23, 30). Consistent with previous reports (21, 22), we observed that Syk is absent or nearly undetectable in most human primary and metastatic melanoma cells tested (Supplementary Fig. S2). Methyltransfer-specific PCR studies showed that transcriptional silencing of Syk in Sbcl2, A375, 1205Lu, and WM9 cells was associated with aberrant DNA methylation of the promoter region that we examined. In contrast, no methylation was observed for Syk-positive cell lines and melanocytes. However, we found that in Syk-negative WM793 cells, the promoter is unmethylated, suggesting that mechanism other than methylation of this region may be involved in Syk gene inactivation. Finally, we found that treatment with the demethylating drug 5-azadC led to an increase of Syk mRNA levels, whereas the histone deacetylase inhibitor TSA had no discernible effect on Syk mRNA expression (Supplementary Fig. S2).

**Syk decreases colony formation, tumor growth in three-dimensional spheroids, and chemotaxis.** Having shown that Syk was inactivated in several melanoma cell lines, we examined its potential growth suppressor function in monolayer cultures and three-dimensional spheroid model. We transfected a Syk expression plasmid into melanoma cell lines and colony formation assays were done to evaluate long-term growth on plastic dishes (Fig. 1A). Reintroduction of Syk dramatically reduced colony formation efficiency by at least 70% in Syk-negative A375 and SKmel28 cells. The same experiment was also carried out in 501mel cells expressing endogenous Syk protein levels (Fig. 1A, 501mel lanes; see Supplementary Fig. S2 for Syk blot of 501mel showing endogenous Syk level). In this setting as well, elevated expression of Syk led to inhibition of colony formation. Transient transfection showed that each of the cell lines were able to express comparable levels of exogenous V5 epitope-tagged Syk (Fig. 1B).

We also investigated the effect of Syk reintroduction on tumor spheroid growth using adenoviral delivery system. To exclude the possibility that this effect would be the result of overexpression of Syk, we determined the level of Syk reexpression in melanoma cells and we found that the level of exogenous Syk in lysates made from infected A375 cells was compared with endogenous level seen in melanocytes (see Supplementary Fig. S3). In all subsequent experiments, we infected cells with a multiplicity of infection of 5, which is the dose of virus that induces physiologic level of Syk expression. Culture of WM793 cells under nonadherent conditions...
resulted in the generation of three-dimensional multicellular tumor spheroids, which may be used as an experimental model to study the tumor biology context. We prepared three-dimensional spheroids from cells infected with control or Syk-expressing adenoviruses. After 3 days, there was significant decrease in the diameter of spheroids when cells were infected with Ad-Syk compared with vector control. Expression of adenovirus-delivered Syk was confirmed by immunoblot analysis (Fig. 1B). Together, these observations indicate that Syk has growth-suppressing activity in standard monolayer cultures and three-dimensional spheroids.

Finally, we analyzed the effect of Syk on melanoma cell migration and invasion toward serum using modified Boyden chambers assays. As seen in Fig. 1C, reexpression of Syk markedly decreased A375 chemotactic migration and invasion. Because no further reduction was observed in the invasive capacity of cells, we can conclude that Syk primarily inhibits melanoma cell chemotaxis. Inhibition of tumor cell invasion into collagen I matrix by Syk was also evidenced in three-dimensional cultures (Supplementary Fig. S4).

**Suppression of clonogenic survival by Syk is independent on functional SH2 domains but requires tyrosine kinase activity.** Because A375 had complete methylation and silencing of Syk (Supplementary Fig. S2) and was most sensitive to Syk-induced growth inhibition (Fig. 1A), we analyzed this cell line further. We conducted loss-of-function experiments to define domains of Syk that mediate growth suppression. We took advantage of a kinase-inactive mutant and generated a mutant that harbors inactivating mutations in the SH2 domains, as well as a mutant with a deletion of the two SH2 domains and interdomain A (Fig. 2A). Cells transfected with plasmids encoding these mutants or a wild-type control expressed similar levels of the corresponding proteins 3 days after transfection (Fig. 2B). Subsequently, these same transfections were subjected to colony-forming assay. As shown in Fig. 2C, expression of Syk SH2 mutants inhibited colony formation relative to vector control. Thus, Syk functions independently of its
SH2 domains and interdomain A to suppress colony formation abilities of melanoma cells. However, mutation of the ATP-binding site did not significantly inhibit the ability to form colonies, indicating that enzymatic activity of Syk is required for its effect on growth inhibition.

**Expression of Syk inhibits proliferation by modulating cell cycle progression.** We undertook to determine how Syk reintroduction suppresses melanoma cell growth and survival. A375 were transduced with adenoviral vector expressing Syk, and we analyzed the ability of cells to proliferate by proliferation curves, cell cycle analysis, and BrdUrd incorporation assays. Proliferation curves revealed that Syk reexpression markedly slowed growth of A375 cells (Fig. 3A). Levels of Syk expression in infected cells during the course of the experiment is shown in Fig. 3B. We also examined cell cycle profiles of Syk-expressing and control cells 4 days after infection by flow cytometry (Fig. 3C). Restoration of Syk resulted in accumulation of cells in G0-G1 and decrease in S-phase cells compared with control cells, suggesting that Syk growth suppression results from G1 cell cycle arrest. In support of this, A375 cell population expressing Syk displayed a strong reduction in BrdUrd incorporation compared with vector-transduced cell population (Fig. 3D). Of note, cells transduced with Syk show no increase in sub-G1 phase and no caspase-3 activation, indicating that Syk expression did not result in cell death or apoptosis (Fig. 3A and C; data not shown).

**Figure 4.** Syk triggers senescence-like phenotypes. A, phase-contrast images of A375 cells infected for 6 d with empty vector or with adenovirus encoding Syk. Syk-transduced cells show a large and flat morphology with increased cytoplasmic granularity. A typical representation of the experiment that was performed at least five times is shown. B, induction of SA-β-gal activity in A375 cells by Syk. Cells were fixed and stained for SA-β-gal (pH 6.0) after 6 d of infection. Top, low magnification images; bottom, high magnification images. C, percentage of SA-β-gal–positive cells 7 d after infection. At least 200 cells were scored from multiple randomly selected fields. Columns, mean of three independent measurements; bars, SD. *, P < 0.01. D, Syk induces SAHF. A375 cells were stained by immunofluorescence with anti-Syk (red) and anti-HP1β (green) 6 d after infection and viewed under a fluorescence microscope. The percentage of SAHF-positive cells is indicated in the insert. Numbers given are representative of three independent experiments.

**Expression of Syk promotes senescence-like phenotypes.** We observed that a major population of cells arrested by Syk displayed a flat, enlarged morphology with increased granularity that is characteristic of senescent cells (Fig. 4A). This increase in cell size was also observed by flow cytometry–based assays, wherein Syk-expressing cells showed elevated side scatter, which is a characteristic of senescent cells. Senescence is accompanied by a series of genetic and metabolic changes that include accumulation of SA-β-gal. To confirm that the observed morphologic changes were due to induction of senescence, we assessed SA-β-gal activity 6 days after infection. A blue staining indicative of acidic β-gal activity was observed in the growth-arrested A375 infected with Ad-Syk (Fig. 4B). Notably, ~40% of cells were β-gal-positive in Syk-expressing populations, whereas <2% of A375 cells were positive in vector-infected cultures (Fig. 4C). Lastly, we looked for the presence of senescence-associated heterochromatin foci (SAHF), another feature of senescent cells. Immunofluorescence analysis for HP1β, a marker of SAHF, revealed punctuated regions of DNA corresponding to heterochromatic foci in Syk-expressing cells, but not in control Syk-negative cells (Fig. 4D). All together, these findings indicate that the cell cycle arrest produced by Syk is mainly associated with induction of senescence-like phenotype.

**Expression of Syk up-regulates the p53 signaling pathway.** Next, we sought to understand the molecular pathway(s) involved in senescence-like growth arrest after Syk reintroduction.
Immunoblot analysis was done to investigate expression and phosphorylation levels of specific proteins involved in cell cycle regulation. Increased levels of activating phosphorylation of p53 at Ser15 and induction of the cdk inhibitor p21 were observed in Syk-expressing A375 cells compared with controls (Fig. 5A). We also noticed that Syk expression resulted in modest, but consistent, increase in the levels of total p53. In addition, Syk-expressing cells harbored dephosphorylation of pRb and lost cyclin A expression. Consistent with changes in p53 regulation, immunofluorescence of individual cells showed nuclear p53 accumulation in Syk-positive cells (Fig. 5B). The nuclear accumulation of p53 was similar to the one observed after treatment with the radiomimicking drug bleomycin. These data indicate that Syk expression results in up-regulation of p53/p21 and pRb pathways, which are known to mediate cell cycle arrest and premature senescence.

To substantiate further these observations, we analyzed, by quantitative reverse transcription–PCR, expression level of >90 transcripts involved in p53-dependent cell cycle regulation and survival after Syk reexpression (see Supplementary Information and Supplementary Table S1). Twenty-nine genes seemed to be reproducibly modulated by Syk, and some of them have been validated at protein level (Fig. 5A and Supplementary Fig. S5). Among the 14 genes up-regulated, six were known transcriptional targets of p53 (p21, Gadd45A, Reprimo, MDM2, BAX, and SIVA). DNMT-1 has been recently implicated in p53-dependent repression of Survivin, cdc2/cdk1, and mitotic cdc25C (31). Accordingly, these three genes were found repressed in Syk-expressing cells, as well as other repression targets of p53, such as CHK1 and cyclin B. Some relevant genes also down-regulated by Syk expression included Livin, E2F1, BRCA1, cyclins A2 and E2, and c-Myc. Finally, we found that one of the genes deeply suppressed after Syk expression was...
caspase-2, which is implicated in p53-mediated apoptosis (32). Inhibition of E2F1, caspase-2, and survivin expression was confirmed at protein level (Supplementary Fig. S5). Together, our results indicate that expression of Syk is linked to altered expression of essential cell survival and cell cycle regulatory genes, which consequently results in G₁ cell cycle arrest and inhibition of cell growth.

Inactivation of p53 prevents Syk-induced p21 expression. p21 is a well-characterized cdk inhibitor and a transcriptional target of p53 (33, 34). The p53/p21 axis plays a crucial role in mediating G₁ arrest and senescence in normal and several tumor cells in response to multiple stress insults (4, 5). Our studies show that Syk-induced growth inhibition of melanoma cells is consistently associated with induction of this pathway. Therefore, we next investigated the requirement of p53 in Syk-induced p21 expression. For this, A375 were incubated with the specific p53 inhibitor PFT-α (35). The level of p21 normally seen after Syk expression was significantly lower in PFT-α-treated cells than in control cells (Fig. 6A). Similarly, we observed that induction of p21 by bleomycin was partially suppressed in PFT-α-treated cells. To rule out the possible nonspecific effect of PFT-α, we used siRNA to inhibit p53 expression. Cells were transfected with p53-specific siRNA (or an irrelevant control siRNA) and subsequently infected with adenovirus expressing Syk or empty vector as control. Figure 6B shows that siRNA to p53 led to an efficient knockdown of p53 protein, as well as p21 basal expression. This experiment also revealed that induction of p21 by Syk expression or bleomycin treatment was totally prevented in p53-silenced cells. These data indicate that p53 plays a functional role in mediating Syk-induced p21 expression. According to this, no induction of p21 was seen when Syk is expressed in MeWo cells, which have no functional p53 (Fig. 6C).

Discussion

There is accumulating evidence that the Syk tyrosine kinase is a novel tumor suppressor in melanoma. However, its mechanism of action with respect to tumor suppression is not known. Here, we show that reexpression of Syk in melanoma cells at levels similar to that seen in melanocytes results in suppression of chemotaxis, sustained cell cycle arrest, and senescence accompanied by molecular events associated with activation of the p53 tumor suppressor pathway. Our observations on the antiproliferative effect of Syk support the hypothesis that inactivation of SYK gene confers a specific growth advantage in neoplastic melanocyte cells and promotes migration of tumor cells. Together, our findings agree with recent observations showing that Syk expression in xenografted melanoma cells results in dramatic reduction of tumor growth and metastatic abilities (21, 22).

Consistent with a tumor suppressor function of Syk in melanoma, Syk levels are frequently low or undetectable in melanoma cells and primary tumors. The loss of expression occurs at transcriptional level as a result of DNA hypermethylation (22). Our study identified a similar epigenetic regulation in the majority of melanoma cells analyzed. However, as shown in breast invasive tumors (18), our results suggested that mechanisms other than promoter methylation might account for loss of Syk expression in certain melanoma cells. Nevertheless, our results underscored a potential role of cytosine methylation in SYK gene inactivation in melanoma cells. It now remains to analyze the possible correlation between SYK methylation rate and clinicopathologic parameters in melanoma tumors.

Inhibition of colony-forming activity by Syk provides a model system to study the mechanism by which Syk exerts its growth-suppressive effect. We found that kinase activation of Syk and further downstream signaling are required for Syk-mediated inhibition of colony formation. In contrast, the SH2 domains of Syk are dispensable for the colony growth-suppressive effect. This observation is consistent with the view that the function of Syk in melanoma cells does not require the interaction of the SH2 domains with phosphorylated ITAM tyrosines on putative receptors or adaptor proteins. However, it is possible that Syk activity in melanoma depends on its subcellular localization. Reports have shown that repression of breast tumor cell growth by Syk is
Syk promotes phosphorylation and activation of p53. Phosphorylation of p53 is a major regulator of cell cycle arrest, senescence, or apoptosis (40). Our findings provide the first evidence that tumor growth inhibition, associated with Syk, involves activation of p53, as reflected by phosphorylation of p53, nuclear accumulation, and regulation of various p53-responsive genes. The net effect of these changes tip the balance in favor of proliferative arrest and protects cells from p53-dependent apoptosis. In support of this, expression of caspase-2, a protease implicated in p53-mediated apoptosis (32, 41), was strongly suppressed after Syk reexpression. One of the major contributors of p53-directed cell cycle arrest and senescence is p21 (also known as senescent cell–derived inhibitor; refs. 33, 41, 42), and we provide evidence for a functional requirement of p53 for p21 regulation by Syk. p21 functions by inhibiting cdk activity that results in hypophosphorylation of pRb and repression of E2F target genes, which blocks S-phase entry and mediates cell cycle arrest. According to this, expression of Syk is consistently associated with hypophosphorylation of pRb and inhibition of E2F target genes, such as cyclin A and cyclin B. Importantly, we also found a dramatic loss of E2F1 gene expression that can also account for the proliferative arrest and senescence experienced by melanoma cells expressing Syk.

A similar down-regulation of E2F1 has been recently reported in senescing melanoma cells treated by the anticancer agent diperpene ester (43). At present, we do not know the mechanisms through which Syk promotes phosphorylation and activation of p53. Phosphorylation of p53 may involve ATM/ATR DNA damage signaling pathways. However, it seems unlikely, as we did not observe activation of this pathway in cells expressing Syk. The stress-activated c-jun NH2 terminal kinase might be a candidate for the kinase that phosphorylates p53 (44). In this regard, it is interesting to note that Syk activates this pathway in melanoma cells (Supplementary Fig. S6).

The growth suppression by Syk seems to be mediated through a G1-G2 cell cycle arrest and induction of senescence and did not involve increased apoptosis or cell death. Interestingly, this activity seems specific to melanoma cells compared with breast carcinoma cells, wherein expression of Syk triggered a nonapoptotic cell death through mitotic catastrophe (18, 19). This is consistent with the idea that Syk mediates senescence or death depending on the genetic background of the tumor. At this stage, the mechanism of tumor type–specific activity of Syk remains unclear.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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