ABI3 ectopic expression reduces in vitro and in vivo cell growth properties while inducing senescence

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Abstract

Background: Mounting evidence has indicated that ABI3 (ABI family member 3) function as a tumor suppressor gene, although the molecular mechanism by which ABI3 acts remains largely unknown.

Methods: The present study investigated ABI3 expression in a large panel of benign and malignant thyroid tumors and explored a correlation between the expression of ABI3 and its potential partner ABI3-binding protein (ABI3BP). We next explored the biological effects of ABI3 ectopic expression in thyroid and colon carcinoma cell lines, in which its expression was reduced or absent.

Results: We not only observed that ABI3 expression is reduced or lost in most carcinomas but also that there is a positive correlation between ABI3 and ABI3BP expression. Ectopic expression of ABI3 was sufficient to lead to a lower transforming activity, reduced tumor in vitro growth properties, suppressed in vitro anchorage-independent growth and in vivo tumor formation while, cellular senescence increased. These responses were accompanied by the up-regulation of the cell cycle inhibitor p21^WAF1 and reduced ERK phosphorylation and E2F1 expression.

Conclusions: Our result links ABI3 to the pathogenesis and progression of some cancers and suggests that ABI3 or its pathway might have interest as therapeutic target. These results also suggest that the pathways through which ABI3 works should be further characterized.
also demonstrated that ABI3BP is potentially associated with pathogenesis of colon, ovary and thyroid, as its expression was reduced in primary tumors compared to paired normal samples [9].

Our hypothesis is that, similar to ABI3BP, ABI3 expression might be reduced in thyroid carcinomas and possibly plays a functional role in the pathogenesis and/or progression of thyroid tumors as well as other cancers.

To test this hypothesis, we investigated the expression of ABI3 in thyroid benign and malignant lesions. We found a decreased expression of ABI3 in thyroid carcinomas. We next explored the biological role of ABI3 in thyroid and colon carcinoma cells. We showed that ABI3 suppressed the in vitro and in vivo transformation, induced senescence and inhibited the oncogenic signaling. These findings demonstrate the tumor suppressing activity of ABI3 and suggest that it may be a target for therapy.

Methods

Tissue samples

A total of 81 thyroid tissue specimens obtained from patients undergoing thyroid surgery for thyroid disease at Hospital São Paulo, Federal University of São Paulo, Brazil, were used for this study. Samples were frozen immediately after surgical biopsy and stored at -80°C. The samples included 7 normal thyroid tissues, 21 follicular thyroid adenomas, 14 Hürthle cell adenomas, 15 follicular thyroid carcinomas, 6 Hürthle cell carcinomas and 18 papillary thyroid carcinomas. All tissue samples were obtained with informed consent according to established Human Studies Protocols at Federal University of São Paulo. The study of patient materials was conducted according to the principles expressed in the Declaration of Helsinki.

RNA extraction, cDNA synthesis and quantitative PCR (qPCR)

To investigate the level of ABI3 expression in thyroid tumors, total RNA and cDNA synthesis was performed as previously described [10]. An aliquot of cDNA was used in 20 μl PCR reactions containing TaqMan universal PCR master mix, 10 μM of each specific primer and FAM-labeled probes for the target gene (ABI3) and VIC-labeled probe as the reference gene (S8) (TaqMan®Gene Assays on Demand; Applied Biosystems, Foster City, CA). Gene expression was normalized to the average of S8 expression and relative expression was calculated as described earlier [11,12].

Correlation of ABI3 and ABI3BP expression in thyroid tumors

The level of ABI3 expression was correlated with the level of ABI3BP, which was previously investigated in this set of samples [8].

Cell Culture

A follicular thyroid carcinoma cell line (WRO) and a colon cancer-derived HT-29 cell line (ARO) [13] were grown in DMEM (Invitrogen Corp., Carlsbad, CA) supplemented with 10% FBS (Invitrogen Corp.), 100 units/mL of penicillin and 100 μg/mL streptomycin in a humidified incubator containing 5% CO2 at 37°C [14,15].

Generation of stable transfected clones Expressing of ABI3

Plasmid encoding the full-length cDNA of human ABI3 was kindly donated by Dr. Satoru Matsuda (Nagoya University School of Medicine, Nagoya, Japan). To establish cell lines expressing ABI3, 10 μg of DNA construct were transfected into WRO and ARO cells by electroporation using a Gene Pulser II (Bio-Rad Laboratories Inc., Hercules, CA). ARO and WRO cells transfected with pcDNA3.1 vector were used as the negative controls. Clones were isolated after 3 weeks of selection with G418 (800 μg/mL). At least six G418-resistant clones from each transfection were isolated, expanded, maintained on G418 (400 μg/mL) and tested for ABI3 expression by qPCR. To this end, total RNA extracted from each clone was used for cDNA synthesis as described [8]. An aliquot of cDNA was used in a 20 μl PCR reaction containing SYBR Green PCR Master Mix (Applied Biosystems) and 200 nM of each primer for target or reference genes. qPCR was performed in triplicates and the threshold cycle (Ct) was averaged (SD ≤1). Primer sequences for ABI3 and S8 (internal control) were as follows: ABI3 sense 5'-CAGGTG-GAAGCCCCGTGTAAG-3' and antisense 5'-AGTGGC-TAAGGTGCCCAGTCG-3', yielding a product of 89 bp; S8 sense 5'-TGAAAAAGGAAAAAGAATGCCAAAA-3' and antisense 5'-CAGGTG-GAAGCCCCGTGTAAG-3', yielding a product of 96 bp. Gene expression was normalized to the average of S8 and relative expression was calculated as described [11,12]. For each cell line, two independently isolated clones that expressed ABI3 at similar levels and two pcDNA3.1 clones were used for further in vitro and in vivo experiments.

Transformation assay

About 5 × 10⁶ WRO cells were transfected with 10 μg of the ABI3 DNA construct as described above. Control plates were transfected with pcDNA3.1. After 3 weeks of selection with G418 (800 μg/mL), cells were fixed in 10% acetic acid and 10% of methanol and stained with 1% crystal violet. G418-selected colonies were counted. Each experiment was performed in triplicate.

Proliferation Assay

Stably transfected clones for ARO and WRO were analyzed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay as described [8,16].
In brief, 2 × 10⁴ cells were seeded in 35-mm plates on day 0. Cell growth was measured from day 1 to 5 by adding 0.5 mg/mL of MTT (Sigma-Aldrich, St. Louis, MO) to the medium at 37°C for 3 hours. The medium was removed and purple formazan crystals were dissolved by adding acid isopropanol. The absorbance of the supernatant was measured at 560 nm.

Quantification of apoptotic cells by annexin-V labeling

To test whether ectopic expression of ABI3 induces apoptosis, 2 × 10⁴ cells were seeded in 35-mm plates and double-stained with Annexin V and Nexin 7-AAD according to the manufacturer’s recommendations (Guava Nexin method; Guava Technologies). Cell-associated fluorescence was analyzed by the Guava PCA flow cytometer (Guava Technologies). Results are expressed as the percentage of apoptotic positive cells. Both early apoptotic (annexin V-positive) and late apoptotic (annexin V- and 7 AAD-positive) cells were included in the analysis. Experiments were performed in quintuplicates.

Cell viability assay

ARO Cells (2 × 10⁴) were seeded in 35-mm plates. Cells were mixed with Guava ViaCount Reagent and allowed to stain for 10 minutes (Guava Technologies, Hayward, CA). Viable cells were quantified using a Guava Personal Analyzer (PCA) flow cytometer (Guava Technologies). Data represents mean of an experiment performed in quintuplicates.

Cell cycle analysis

ARO cells (2 × 10⁵) were seeded in 35-mm dishes. After synchronization of the cells by serum starvation for 24 hours, cells were replaced with DMEM medium supplemented with 10% FBS for 24 hours. Cells were fixed in 70% ethanol for 1 hour, labeled with Guava Cell Cycle Assay reagent and analyzed using Guava PCA flow cytometer (Guava Technologies) following the manufacturer’s specifications. Experiments were performed in quintuplicates.

Expression of p21WAF1 and E2F1 by qPCR

The transcript levels of p21WAF1 and E2F1 were tested in stably expressing ABI3 ARO and WRO cells and controls, as described [8].

Western blot analysis

Western blot analysis was performed as described [8]. Briefly, membranes were blocked and incubated overnight at 4°C with anti-phospho-ERK (pERK; dilution 1:1000), anti-phospho-AKT (pAKT; dilution 1:400) and anti-α Tubulin (dilution 1:1000). Detection was carried out using the SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL, USA).

Cellular senescence

Senescence-associated (SA) β-gal staining was performed as described [17]. Briefly, ARO and WRO cells (2 × 10⁴) were seeded in 35-mm plates. Cells were washed twice with PBS, fixed for 15 minutes and stained with 1 mg/mL 5-bromo-4-chloro-3-indolyl-b-D-galactoside (X-gal) in buffer (dimethylformamide, 40 mM citric acid/sodium phosphate pH 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl and 2 mM MgCl₂). Cells were incubated at 37°C in 5% CO₂ for 18 hours and washed twice with PBS. Cells were examined using a light microscope and counted in 5 optical fields (100×). Data represents mean of an experiment performed in quintuplicates.

Matrigel invasion assay

Cell invasion was analyzed using BioCoat Matrigel Invasion Chamber according to the manufacturer’s recommendation (Becton Dickinson, Bedford, MA). WRO cell clones were added to the invasion or control chambers at a density of 2.5 × 10⁴ and, after 24 hours, cells remaining above the insert membrane were removed by gentle scraping with a sterile cotton swab. FBS was used as chemotactant. Cells that had invaded through the Matrigel to the bottom of the insert were fixed and stained with rapid panoptic LB (Laborclin, Brazil) and mounted. Cells were examined using a light microscope and counted in 3 optical fields (100×). Experimental and control groups were performed in triplicates. The percentage of invasion cells was determined by the mean of cells invading through Matrigel insert membrane divided by the mean of cells migrating through control insert membrane X100.

Cell Migration from spheroids

Because high migration capacity might be correlated with cell spreading and metastasis in vivo, migration from spheroids was assayed as previously described [8]. Briefly, spheroids were prepared by seeding WRO cells in DMEM supplemented with 10% FBS, onto 35-mm tissue culture dishes coated with 0.75% Noble agar. Cells were cultured until spheroids were formed and 2 mM MgCl₂. Cells were incubated at 37°C in 5% CO₂ for 18 hours and washed twice with PBS. Cells were examined using a light microscope and counted in 5 optical fields (100×). Data represents mean of an experiment performed in quintuplicates.
medium and 0.35% agar and seeded in triplicate over a bottom layer of solidified agar. The dishes were incubated at 37°C in 5% CO₂. After 3 weeks, colonies greater than 20 μm in diameter were counted. Colony formation rate was calculated as percentage of total seeded cells. Two independent experiments were performed.

**Nude mouse xenograft model**

Four to five week old male athymic nude (nu/nu) mice were maintained according to the guidelines of the Division of Animal Resources at the Federal University of São Paulo. ARO stable cell clones were suspended in sterile PBS to 2 × 10⁶/200 μL and injected subcutaneously into the flank of mice. Mice were then monitored biweekly during three weeks. Tumor volume was calculated by the rotational ellipsoid formula: \( V = A \times B^2/2 \) (A = axial diameter; B = rotational diameter). Tumor tissues were collected and embedded in paraffin for conventional histology or were stored at -80°C.

**Statistical analysis**

The relative expression values were log transformed before the application of statistical analysis. Pearson correlation coefficient was used to verify the correlation between \( ABI3 \) and \( ABI3BP \) expression. *In vitro* results were log transformed and analyzed by a Student's t test. *In vivo* results were analyzed by the Wilcoxon test. Significance is presented as p value of <0.05 (*), < 0.01 (**) and < 0.001 (***)

**Results**

**\( ABI3 \) expression is reduced in malignant thyroid lesions**

To test the possibility that \( ABI3 \) expression is associated with thyroid tumor malignancy, we examined mRNA expression in a panel of thyroid tumors specimens and normal thyroid. As demonstrated by qPCR, \( ABI3 \) expression was reduced in a high percentage of thyroid carcinomas while it was expressed in most of benign lesions and normal thyroid (p≤0.001; Figure 1A). Since we previously investigated the level of \( ABI3BP \) in the aforementioned set of samples [8], we next correlated the expression of \( ABI3 \) with the expression observed for \( ABI3BP \). We found a medium positive correlation between reduced expression of \( ABI3 \) and \( ABI3BP \) (\( r = 0.346; \ p = 0.019 \); Figure 1B). However, a large positive correlation was observed in malignant lesions (\( r = 0.564; \ p = 0.003 \); Figure 1B). These findings and those reported from two-hybrid system suggest that \( ABI3 \) and \( ABI3BP \) may act through a common signaling pathway, although biological evidences are still needed to demonstrate this hypothesis.

**Ectopic expression of \( ABI3 \) in human carcinoma cell lines**

To investigate a functional role of \( ABI3 \) in cancer development, \( ABI3 \) expression was tested in a panel of cell lines derived from human cancers. A thyroid follicular cell line (WRO) and a colon cancer cell line (ARO), which did not express or expressed at very low levels, were chosen (Figure 1C). A construct expressing \( ABI3 \) and an empty vector (control) were transfected into ARO and WRO cell lines. Seven transfected clones from each cell line were subsequently tested by qPCR. Two clones with similar level of \( ABI3 \) expression and two clones from control group were chosen for *in vitro* and *in vivo* studies (p < 0.01; Figure 1C).

**Expression of \( ABI3 \) suppresses focus formation**

To determine the effects of the \( ABI3 \) on transformation of human cancer cells we first determined the effects of \( ABI3 \) on cells growth in a focus formation assay. WRO cells were stably transfected with vector expressing \( ABI3 \). Control transfections were also performed with empty vector. G418-selected colonies were counted after two weeks. WRO cells transfected with empty vector formed numerous foci (32.70 foci/μg of plasmid DNA). In contrast, ectopic expression of \( ABI3 \) reduced the number of colonies formed (0.63 foci/μg of plasmid DNA) (p < 0.001; Figure 1D). This finding suggests that \( ABI3 \) has a strong inhibited foci formation.

**The ectopic expression of \( ABI3 \) reduces cell proliferation**

To confirm the effects of the \( ABI3 \) on malignant transformation, we next examined the effects of ectopic expression of \( ABI3 \) in growth rate. \( ABI3 \) induced a growth inhibitory effect in the two cell lines as assessed by MTT assays, mainly at day 5 (Figure 2A). The data represents the mean ± SD of two experiments performed in triplicates.

**\( ABI3 \) expression increases the percentage of cells in G0/G1 phase and reduces cell viability but did not induce apoptosis on carcinoma cells**

The ability of \( ABI3 \) to inhibit cell growth could be due to the cell cycle arrest and/or apoptosis. Therefore we investigated the effect of \( ABI3 \) on apoptosis. Although we observed a trend toward increased apoptosis in WRO cells expressing \( ABI3 \) when compared to control cells, the growth attenuation was, however, not accompanied by a corresponding increase in apoptotic cells (Figure 2B). The \( ABI3 \)-induced growth suppression was further studied by flow cytometric analysis, which revealed a decrease in cell viability (Figure 2C) and cell cycle arrest at the G0/G1 phase (p < 0.05, Figure 2D).

**\( ABI3 \) induces the expression of p21<sup>WAF1</sup> and reduces E2F1 expression and phosphorylation of ERK**

Since p21<sup>WAF1</sup> inhibit and E2F1 promotes cell cycle progression, we tested the transcripts levels of expression of p21<sup>WAF1</sup> and E2F1 following \( ABI3 \) expression by quantitative PCR. Stable expression of \( ABI3 \) in WRO cells
Figure 1 Status of ABI3 expression in thyroid tumors and in carcinomas cell lines. (A) ABI3 expression is reduced in most thyroid malignant tumors (p < 0.001). (B) A positive correlation was observed between levels of ABI3 and ABI3BP expression in the panel of thyroid samples, mainly in malignant tumors. (C) ABI3 expression in control cells (cells transfected with empty vector) or clones expressing ABI3 (two of each cell line). (D) ABI3 ectopic expression reduced focus formation. The data represents the mean ± SD of the experiment performed in triplicates. FTA, Follicular thyroid adenomas; HCA, Hürthle cell adenomas; FTC, follicular thyroid carcinomas; HCC, Hürthle cell carcinomas; PTC, papillary thyroid carcinomas. *p < 0.05, **p < 0.01, ***p ≤ 0.001.
induced $p21^{WAF1}$ while reduced $E2F1$ expression at days 3 and 5 post-seeding ($p < 0.05$; Figure 3A).

Although at lower levels, the expression of $p21^{WAF1}$ was induced and $E2F1$ was reduced in ARO cells expressing $ABI3$. We next tested phosphorylation of ERK, which is known to play a pivotal role in cell proliferation. We observed a decrease of ERK phosphorylation in $ABI3$-expressing WRO cells compared to control cells. Two clones from each group are shown independently (Figure 3B). No effect was observed in AKT phosphorylation (data not shown).

$ABI3$ induces senescence in carcinoma cells

The number of β-Gal positive cells was higher in ARO and WRO cells expressing $ABI3$ at days 3 and 5 post-seeding (Figure 4A and 4B). Representative results are
Figure 3 ABI3 induced p21WAF1 expression while reducing ERK phosphorylation and E2F1 expression in carcinoma cell lines. (A, two first panels - two upper panels) An increase in p21WAF1 and decrease in E2F1 expression was observed following ABI3 ectopic expression. Black bars correspond to control cells and white bars correspond to ABI3 expressing cells. Graphs show mean ± SD of two clones for each transfectant. (B, third panel-bottom) ABI3 ectopic expression decreases phosphorylated ERK (p-ERK) in WRO cells. Results from two selected clones are shown. C1 and C2 (controls clones) and ABI3 1 and ABI3 2 (clones expressing ABI3). α-Tubulin was used as internal control. *p < 0.05.
shown on Figure 4C and 4D. As predicted from cell proliferation and cell cycle assays, the effect was higher in WRO cells (p < 0.01) than in ARO cells (p < 0.05).

**ABI3 expression effects on cells migration**

Given that ABI3 was previously associated with a marked reduction in cell motility, we here tested using spheroid assay. Expression of ABI3 reduced cell migration and invasion of WRO cells (Figure 5A and 5B), although it was not considered statistically significant. ARO cells did not form spheroids.

**ABI3 reduces tumor growth in nude mice**

ARO cell line was selected for in vivo assay based on the fact that ARO cells form large tumors in nude mice [18], while WRO form smaller tumors with a long latent period. ARO cells expressing ABI3 did not form tumors in nude mice (n = 3) or formed a very small tumor (0.65 ± 1.17 cm³; n = 5). In contrast, control mice had extensive tumors (3.62 ± 2.84 cm³; n = 8; Figure 6A). The results are graphically represented as mean of tumor volume (p = 0.027; Figure 6B). Tumors were processed for routine histology and immunohistochemical analysis. H&E staining revealed no histological differences among the tumors. Neither lung nor lymph node metastases were found in any mice.

**ABI3 affects anchorage-independent cell growth**

Since WRO cells had a very low colony-forming efficiency in agar, ARO cells were selected for its ability to

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**Figure 4** ABI3 ectopic expression induced senescence in carcinoma cell lines. The percentage of β-galactosidase positive cells was higher in ARO (A) and WRO (B) cells expressing ABI3 than in control cells. Representative results illustrate increased senescence-associated β-galactosidase (SA-β-gal) staining in ARO (C) and WRO (D) cells following ABI3 expression on days 3 and 5. *p < 0.05 and **p < 0.01.
growth in soft agar [18]. Following ABI3 expression, a significant reduction of the ability of the cells to grow in semi-solid media was observed (p < 0.05; Figure 6C).

**Discussion**

We have previously shown that ABI3BP expression is lost in most malignant carcinomas. We also provided evidence that ectopic expression of ABI3BP in ABI3BP-deficient carcinoma cell lines inhibited in vitro and in vivo cell growth [8]. Based on our previous findings [6,8] and on the fact that ABI3BP was originally described as an ABI3-SH3-binding protein isolated by yeast two-hybrid technique [19], we investigated the expression of ABI3 in thyroid lesions.

![Figure 5](image_url)  
**Figure 5** Effect of ABI3 expression in invasion and migration. (A) WRO cells expressing ABI3 had a lower ability to migrate. The graphs display the mean ± SD of at least 12 spheroids of each clone. (B) The percentage of invasive cells through the Matrigel assay was lower in WRO cells expressing ABI3. Black bars correspond to control clones (n = 2) and white bars correspond to ABI3 expressing clones (n = 2).

![Figure 6](image_url)  
**Figure 6** ABI3 reduced tumor formation in nude mice and the ability to growth in semi-solid medium. (A) Representative animals that received either control cells (left panel) or cells expressing ABI3 (right panel) are shown. The arrows pointed out the formed tumors. (B) Tumorigenicity experiments are summarized in the graph showing mean ± SD of the volume of tumors after inoculation of ARO cells. (C) The percentage of colony formation in soft agar is significantly decreased in ARO cells expressing ABI3 in comparison with control cells. Experiments were performed in triplicates. *p < 0.05.
In this study, we observed that ABI3 expression is lost or reduced in most malignant samples, compared to benign thyroid samples. Since both genes had similar patterns of expression, we subsequently investigated whether the expression of ABI3 and ABI3BP correlated in thyroid samples. We observed a positive correlation between ABI3 and ABI3BP expression, which was more evident in malignant lesions.

Our findings, in association with the fact that ABI3 expression is frequently lost in invasive cancer cell lines, and that ABI3 re-expression markedly inhibited cell motility and significantly reduced the formation of tumor metastasis in vivo [3], suggest that ABI3 loss of expression may play an important role in the pathogenesis and/or progression of several tumors subtypes.

Herein, we sought to investigate the consequences of stable expression of ABI3 on diverse steps of carcinogenesis including proliferation, transformation, survival, migration, and invasion in vitro and in vivo. To this end, we stably transfected ABI3 into a thyroid and a colon carcinoma cell lines [13].

We first demonstrated that ABI3 ectopic expression reduced cell transformation and suppressed proliferation of carcinoma cell lines, mainly in a follicular thyroid carcinoma cell line. The undetectable basal expression and high fold induction of ABI3 in the follicular thyroid carcinoma cell line could provide a potential explanation for the observed inhibitory effects of ABI3 on the cell proliferation. The different genetic background may also be responsible for the phenotypic differences.

Moreover, ABI3 expression was able to delay cell cycle progression and reduce cell viability at significant levels. Additionally, we found that ABI3 expression induces senescence, determined by positive results of SA-β-gal staining, which is a specific cellular senescence marker.

Even though it is not completely understood in detail how ABI3 promotes cell cycle arrest, reduces proliferation and induces senescence, our findings indicate that the ABI3-induced response was mediated by an increase in p21WAF1 expression, and down regulation of E2F1 expression.

Interestingly, p21WAF1 is a major player in cell cycle control. Various mechanisms exist to regulate the levels of p21WAF1. Although tumor suppressor p53 is a major transcription factor involved in the regulation of p21WAF1, other factors including TGFβ, p73, Sp1, Rac1, Rho, are known to induce the expression of p21WAF1. Therefore, the induction of p21WAF1 expression could also occur in a p53-independent manner [20-22]. Once activated, p21WAF1 exerts a negative effect on cell cycle progression by preventing the CDK2/cyclin E complex formation, leading to dephosphorylation (activation) of Rb and, thereby, preventing E2F-mediated transcriptional activation [21]. In addition to its role and cell cycle progression, previous studies provided evidences that p21WAF1 can trigger senescence either in a p53-dependent and p53-independent manner [23].

In the present study, the effect of ABI3 expression on cell senescence, coupled with inhibition of cell cycle progression, up regulation of p21WAF1 and down regulation of E2F1 expression, occurred in cancer cells in which p53 function is disrupted [24]. Therefore, in our model, p53 is unlikely to promote the ABI3-induced p21WAF1 expression.

Although further analysis is needed to identify the underlying mechanism by which ABI3 induces p21WAF1 expression, our findings indicate that increase in p21WAF1 may mediate cell cycle arrest and senescence by blocking the CDk/Rb/E2F axis. It will be of interest to assess the effect of ABI3 expression on total Rb levels and its phosphorylation status.

In agreement with our findings, it was recently demonstrated that the apoptotic effect of iodine, in these cell lines, was mediated by mitochondrial pathway that involved p21WAF1 accumulation in a p53-independent mechanism. The authors suggested that p21WAF1 is believe to be an important molecule in drug induced tumor suppression, given that the block of p21WAF1 significantly diminishes iodine-mediated apoptosis [25]. Up-regulation of p21WAF1 has been reported to enhance apoptosis induced by antitumor agent in thyroid cancer cells in a p53-independent manner [26].

Although we did not observe changes in AKT phosphorylation when ABI3 was re-expressed, our findings corroborate with previous studies which demonstrated that ABI3 re-expression had no effect on AKT phosphorylation in v-Src transformed NIH3T3 and U87 MG cell lines [3].

In addition to increased growth rate, malignant transformation requires the acquisition of a number of tumor features. Although no significant differences were observed in migration and invasion assays, here we observed a direct correlation between ABI3 expression and anchorage-independent growth. Additionally, ABI3 significantly decreased xenograft growth in mice.

Interestingly, it has been previously demonstrated that ABI3 is a suppressive molecule in malignant cells [3]. The authors showed that ABI3 expression reduces cell motility and metastatic dissemination of a highly metastatic murine fibroblast transformed by v-Src (SRD) and the human glioblastoma cell line (U87 MG), while it did not interfere with cellular growth [3]. To examine molecular mechanism underlying ABI3-mediated effects in cell motility, the authors investigated whether the expression of Cdc42, Ras, Rac and Rho GTPases was affected by ABI3 expression. Neither significant activation, nor suppression was found in Cdc42, Rac, Ras and Rho. Interestingly, a marked reduction in phosphorylation of PAK2 was observed following expression of ABI3.
Furthermore, the authors demonstrated that ABI3 and PAK2 colocalized at the leading edge of the cells [3]. These findings corroborate with our hypothesis that ABI3 loss could be common to other cancer types and suggest that, similar to other ABI-family members [1], ABI3 seems to function in a highly context-dependent way. Additional studies will be required to verify whether PAK2 and/or Rho and Rac small GTPases are affected by ABI3 expression in other cancer subtypes and to identify other mediator of cell motility.

In summary, our results indicate that ABI3 expression plays an important role in suppressing tumor growth and progression, given that its expression was significantly lower in malignant specimens compared to benign lesions and ectopic expression reduced the transforming phenotype of both cell lines. The identification of molecular events in the ABI3 pathway that control processes such as senescence, migration and invasion may suggest new therapeutic strategies for cancer.

Conclusion

Our results indicate that ABI3 expression plays an important role in the pathogenesis and the progression of several cancers. A more detailed understanding of the pathway by which ABI3 contribute to senescence may lead to the development of novel agents that can suppress tumor development.

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Authors’ contributions

FRL contributed to assay design, interpretation of the data, statistical analysis and drafted the manuscript. JPH performed in vivo assays, interpretation of the data and final art design. BF contributed to acquisition of the data, analysis and interpretation of the data. GO contributed to assay design and interpretation of the data. GJR participated in the design of the study and helped drafted and edited the manuscript. JMC directed the design and coordination of the study and contributed drafted the manuscript, responded to reviewers and interpreted the results. All the authors have read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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