Alterations of the Tumor Suppressor Gene *ARLTS1* in Ovarian Cancer

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Abstract

***ARLTS1* is a tumor suppressor gene initially described as a low-penetrance cancer gene: a truncated Trp149Stop (MUT) polymorphism is associated with general familial cancer aggregation and, particularly, high-risk familial breast cancer. DNA hypermethylation has been identified as a mechanism of *ARLTS1* expression down-regulation in lung carcinomas and B-cell chronic lymphocytic leukemia. We found that, in the majority of ovarian carcinomas (61.5%) and in a significant proportion of ovarian and breast cancer cell lines (45%), *ARLTS1* is strongly down-regulated due to DNA methylation in its promoter region. After *ARLTS1* restoration by adenoviral transduction, only the negative TOV-112 and the homozygously mutated (MUT) MCF7 cells, but not the OV-90 cells expressing a normal *ARLTS1* product, underwent apoptosis and inhibition of cell growth. Furthermore, *ARLTS1* reexpression significantly reduced the tumorigenic potential of TOV-112 in nude mice. On the contrary, the *ARLTS1*-MUT induced significantly lower levels of apoptosis in infected cells and reduced *in vivo* tumorigenesis only partially, supporting the hypothesis that Trp149Stop polymorphism is retained in the general population and predisposes to cancer because of a reduction, but not full loss, of normal *ARLTS1* function.** (Cancer Res 2006; 66(21): 10287-91)

Introduction

ARLTS1 [ADP ribosylation factor-like 11 (ARL11)] is a recently identified tumor suppressor gene described in association with familial cancers: a Trp149Stop (G446A) variant (MUT) leading to premature termination of translation was identified in cancer kindreds displaying various combinations of breast, ovarian, prostate, gastric, and lung carcinomas, melanomas, and B-cell chronic lymphocytic leukemias (B-CLL; ref. 1). However, the Trp149Stop variant frequency is similar in control cases and patients with sporadic cancers (1), and in fact, it was shown that no association with general risk of CLL (2) or various types of sporadic cancers (3) could be identified. *ARLTS1* is located at chromosome 13q14.3, a genomic region frequently homozygously or heterozygously deleted in various types of solid and hematologic malignancies, including CLL and prostate, breast, and colon carcinomas (4–6). Furthermore, down-regulation by promoter hypermethylation of *ARLTS1* was reported in sporadic cases of B-CLL and lung cancers (1). After the initial description, an independent group linked *ARLTS1* Trp149Stop to both familial risk of breast cancer in *BRCA1*-negative and *BRCA2*-negative families (7) and risk of bilateral breast cancer (8). Moreover, they showed that another variant, the Cys148Arg, revealed a significant association with high-risk familial breast cancer (8) and melanomas (9).

Ovarian cancer is the most lethal of gynecologic malignancies in the United States with ~23,000 new cases and >15,000 deaths estimated for 2006 (10), and hereditary breast and ovarian cancers are among the most commonly encountered adult genetic diseases (11). Therefore, we decided to investigate the roles of *ARLTS1* in ovarian cancer. Herein, we showed that *ARLTS1* is frequently down-regulated in ovarian primary tumors and cell lines and restoration of its expression by adenoviral *ARLTS1* or by the demethylating agent 5-AZA-2-deoxycytidine (5-AZA) effectively induced apoptosis *in vitro* and suppressed ovarian cancer tumorigenicity in nude mice. No similar effects on ovarian cancer cells constitutively expressing the *ARLTS1* protein have been observed. In addition, we showed that *ARLTS1* Trp149Stop polymorphism greatly reduced the protein apoptotic function in ovarian and breast cancer cell lines.

Materials and Methods

Tumor samples and cell lines. Sixteen ovarian tumor samples, including 13 malignant, 2 borderline, and 1 benign tumor, were obtained from Fox Chase Cancer Center (Philadelphia, PA) according to institutional guidelines. The clinical data are presented in Supplementary Table S1. A portion of the tissue specimens were assessed for tumor content by histology, and only tissues with >60% tumor cells were used. A set of 11 ovarian and breast cell lines, including TOV-112, OV-90, OV-3, MB-453, DU-4475, MB-436, Sk-Br3, MCF7, BT-474, BT-549, and MB-361 cancer cell lines, was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained according to ATCC protocols. As controls, multiple normal total RNAs from ovary (three samples) and breast (three samples) were used (Ambion, Austin, TX and Stratagene, La Jolla, CA).

***ARLTS1* expression analysis.** Total RNA from ovarian and breast cancer samples was processed as described previously (1). The relative expression of *ARLTS1* normalized with β-actin in each sample was quantified using the GelDoc software (Bio-Rad, Hercules, CA).

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi:10.1158/0008-5472.CAN-06-2289

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Recombinant adenoviruses and *in vitro* transduction. Wild-type *ARLTS1* and mutant (G446A) *ARLTS1* full-length cDNAs were cloned into Adenovator-CMV5(CuO)-IRES-*GFP* transfer vector (Qbiogene, Irvine, CA). This vector allows transgene expression driven by the cumate-inducible CMV5(CuO) promoter. An internal ribosome entry site sequence ensures coexpression of *GFP*. Cells were transduced with recombinant adenoviruses at multiplicity of infection (MOI) 75, and transduction efficiency was determined by visualization of *GFP*-expressing cells.

Cell growth and cell cycle kinetics. Cells (2×10^5) were infected at MOIs of 75 and they were harvested at 48-hour intervals for 6 days, stained with trypan blue, and counted (ViCell counter, Beckman Coulter, Fullerton, CA). Number of viable cells was assayed using CCK-8 test (Dojindo, Gaithersburg, MD) as per the manufacturer's protocol. For flow cytometry by EPICS XL scan (Beckman Coulter), cells were harvested 2, 4, and 6 days after infection, fixed in cold methanol, RNase treated, and stained with propidium iodide (50 μg/mL). All analyses were done in duplicate.

Western blot analysis. Protein extraction and immunoblot analysis were done as described in ref. 12. The following primary antisera were used: rabbit polyclonal anti-caspase-3 (1:1,000; Cell Signaling Technology, Beverly, MA), rabbit polyclonal anti-caspase-9 (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-caspase-8 (1:200; Chemicon, Temecula, CA), rabbit monoclonal anti-Bcl-2 (1:500; Santa Cruz Biotechnology), and mouse monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:10,000; Santa Cruz Biotechnology).

DNA methylation studies. TOV-112 cells were treated with 5 μmol/L 5-AZA (Sigma, St. Louis, MO) and 1 μmol/L trichostatin A (TSA). Maximal *ARLTS1* reexpression was observed by treating 1×10^6 cells with 5 μmol/L 5-AZA on days 2 and 5. After the 5th day, medium was replaced with medium containing 1 μmol/L TSA and incubation was continued for 24 hours and cells were collected for use. RNA was extracted and reverse transcription-PCR (RT-PCR) was done as described (1).

Caspase inhibition assay. The caspase inhibitors z-VAD-FMK (general), z-IETD-FMK (caspase-8), and z-LEHD-FMK (caspase-9) were purchased from BD Biosciences (San Jose, CA). TOV cells were seeded (10^4 per well) in 96-well culture plates and the infection was done using MOI 75. The cells were incubated for 2, 4, and 6 days with different caspase inhibitors (50 μmol/L), and the media with the inhibitors were replaced daily. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell proliferation assay (CCK8) was done as recommended by the manufacturer (Dojindo).

RNA interference. TOV-112 cells were transfected with ARL11 SMARTpool (Dharmacon, Lafayette, CO) at different concentrations (10, 50, and 100 nmol/L). We used siPORT NeoFX Transfection Reagent (Ambion) as per the manufacturer's protocol. Twenty-four hours after transfection, cells were treated with 5-AZA only as described above, and *ARLTS1* reexpression was assayed at 2, 4, and 6 days by RT-PCR.

***In vivo* studies.** Animal studies were done according to institutional guidelines. TOV-112 cells were infected *in vitro* with Ad-*ARLTS1* or Ad-*ARLTS1*-MUT or Ad-*GFP* or mock infected (MOI 75). Alternatively, they were treated *in vitro* with 5 μmol/L 5-AZA. At 24 hours after infection/treatment, 10^7 viable cells were injected s.c. into left flanks of 6-week-old female nude mice (Charles River Breeding Laboratories, Wilmington, MA), five mice per infected/treated or control group. Tumorigenic controls were untreated TOV-112. Tumor diameters were measured every 5 days, and tumors were weighed after necropsy. Tumor volumes were calculated by using the equation V (in mm^3) = $a \times b^2 / 2$, where a is the largest diameter and b is the perpendicular diameter.

Statistical analysis. Results of *in vitro* and *in vivo* experiments were expressed as mean ± SD. Student's two-sided t test was used to compare values of test and control samples. $P < 0.05$ indicated significant difference.

Results

***ARLTS1* is down-regulated in ovarian cancers.** To identify a potential role of *ARLTS1* in ovarian cancer, we analyzed its expression by semiquantitative RT-PCR in a set of 19 ovarian samples, consisting of 13 malignant primary tumors, 2 borderline primary tumors, 1 benign tumor, and 3 normal ovarian tissues (Supplementary Table S2). Eight malignant tumors (8 of 13, 61.5%), but none of the normal tissues, displayed a reduction to <33%, with four (25%) ovarian cancer showing a reduction to <10% of the normal expression, respectively (Fig. 1*A*). *ARLTS1* was highly down-regulated in the only benign ovarian tumor that we analyzed (#89, Fig. 1*A*) suggesting that, at least in this case, down-regulation of *ARLTS1* level can be an early event in ovarian tumorigenesis. Because the majority of primary malignant tumors showed consistent reduction in *ARLTS1* expression, we analyzed a second panel of 11 ovarian and breast cancer cell lines. In five cell lines (TOV-112, BT-549, BT-474, MB-436, and MB-453), a reduction to <10% of normal expression was found (Fig. 1*B*). Globally, 14 of 27 (52%) tumor samples (primary tumors and cell lines combined) and none of 6 normal samples showed reduction in *ARLTS1* expression ($P < 0.05$, Fisher's exact test). Therefore, *ARLTS1* is highly down-regulated in about half of primary ovarian cancer and in a similar proportion of ovarian and breast cancer cell lines.

***ARLTS1* inhibits proliferation in ovarian and breast cancer cells.** To assess the effect of *ARLTS1* restoration in ovary and breast cancer cell lines, we selected an *ARLTS1*-negative ovarian cell line (TOV-112), an *ARLTS1*-positive ovarian cell line (OV-90), and the MCF7 breast cancer cell line expressing only the truncated form (MUT) of *ARLTS1* (1). Of note, both ovarian cell lines harbor mutations in TP53, although MCF7 is TP53 wild-type.

Gene reexpression was achieved through adenoviral transduction of either the wild-type (Ad-*ARLTS1*-WT) or mutated G446A (Ad-*ARLTS1*-MUT) forms of *ARLTS1*. The expression was confirmed by RT-PCR (Supplementary Fig. S1). Infection with MOI 75 was sufficient to restore gene expression in >90% of infected cells as estimated by the percentage of fluorescent cells. Infection with control vector (Ad-GFP) showed no significant toxic effect at MOI 75.

Ninety-six hours after Ad-*ARLTS1*-WT infection, both TOV-112 and MCF7 cell lines showed markedly decreased proliferative activity and higher cell death as determined by cell count and viability (CCK-8) assay (Supplementary Fig. S2A and B). Specifically, at 96 hours after Ad-*ARLTS1*-WT infection, a 76% cell growth inhibition in TOV-112 cells compared with the untreated cells ($P < 0.001$) was found, whereas at 144 hours the cell growth inhibition increased to 86% ($P < 0.001$). MCF7 cells, which express only the truncated form of *ARLTS1*, showed a 63% cell growth

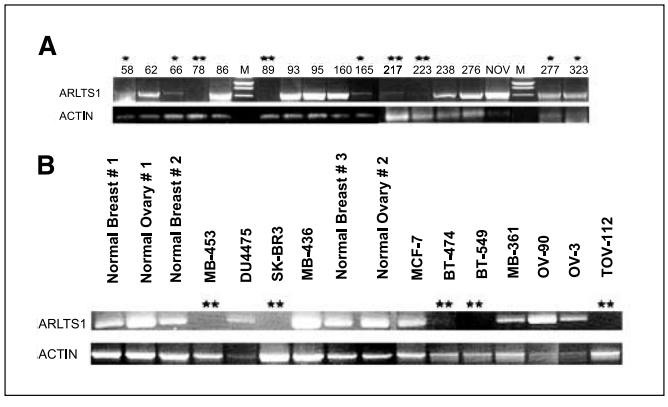

Figure 1. *ARLTS1* expression in various normal and malignant samples. *A,* expression in ovarian carcinomas by RT-PCR. *B,* expression in ovarian and breast cancer cell lines by RT-PCR. *One asterisk,* reduction by at least 66%; *two asterisks,* reduction with >90% from that in normal samples as measured with the GelDoc quantification software. *NOV,* normal ovary (Ambion sample 2); *M,* 1 kb marker.

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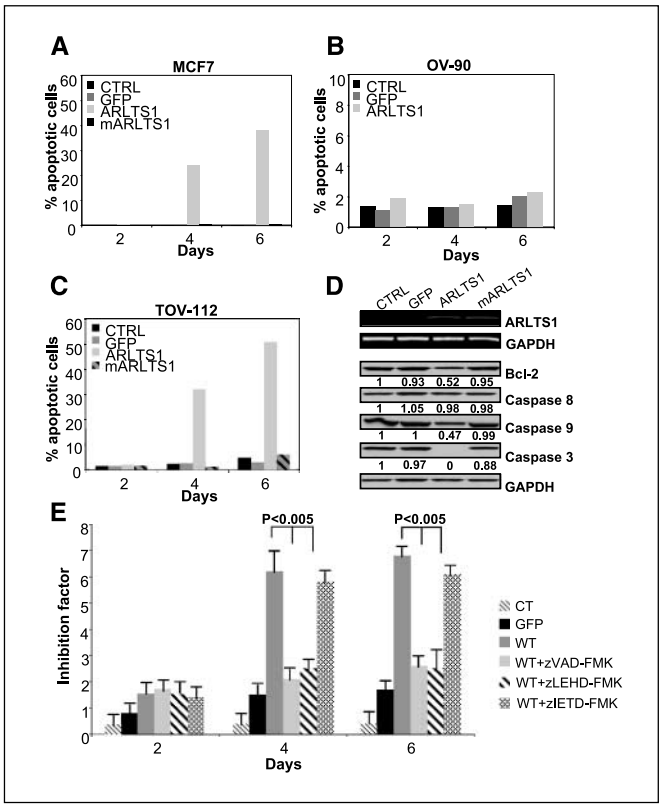

Figure 2. Flow cytometry analysis of untreated, Ad-GFP-infected, Ad-*ARLTS1*-WT-infected, and Ad-*ARLTS1*-MUT-infected cells. *A,* restoration of wild-type *ARLTS1* induces apoptosis (36.21%) in MCF7 cells after 6 days. *B, ARLTS1* reexpression does not induce cell death in OV-90 cells. *C,* wild-type (*ARLTS1= WT*), but not mutant (*mARLTS1*), induces apoptosis (50.75%) in TOV-112 cells after 6 days. *D,* apoptotic pathway induced by *ARLTS1*-WT restoration in TOV-112 cells. Antiapoptotic protein Bcl-2 is reduced (50% reduction) after *ARLTS1* restoration, and the intrinsic caspase pathway is activated by activation of the initiator caspase-9 (50% reduction of procaspase-9) and complete cleavage of the effector caspase-3. Caspase-8 levels (extrinsic pathway) are not affected by *ARLTS1* activation. Numbers represent relative expression to control (CTRL). *E,* kinetics of caspase inhibition assay in TOV cells by CCK-8 cell viability assay. Effects of the general caspase inhibitor z-VAD-FMK and inhibitors of caspase-8 (z-IETD-FMK) and caspase-9 (z-LEHD-FMK) on *ARLTS1*-transduced and untransduced TOV cells at 2, 4, and 6 days after infection. *Columns,* mean in quadruplicate; *bars,* SD. *P* presented when statistically significant. At 4 and 6 days, pan-caspase and caspase-9 inhibitors blocked ($P = 0.001$) whereas caspase-8 ($P = 0.31$) did not affect TOV-induced apoptosis.

inhibition ($P < 0.001$) after 144 hours of treatment with wild-type *ARLTS1*. There was no statistically significant difference in the growth of OV-90 cells after *ARLTS1* gene restoration, suggesting that exogenous *ARLTS1* has no effect in cells where its expression is normal, whereas it has significant effects in cells either lacking *ARLTS1* expression or expressing only the truncated form.

On the other hand, infection with Ad-*ARLTS1*-MUT had a minimal effect on cell proliferation and viability (Fig. 2), showing that the truncated ARLTS1 protein has no significant activity *in vitro*. Specifically, restoration of mutant *ARLTS1* form in TOV-112 cells did not affect the cell growth significantly ($P = 0.12$). Moreover, the strong antiproliferative effect (65% cell growth inhibition; $P < 0.001$) of Ad-*ARLTS1*-WT in the MCF7 cell line confirms that the endogenous mutated form expressed in this cell line has no significant biological activity.

Restoration of *ARLTS1* induces apoptosis in ovarian and breast cancer cells. To fully understand the nature of the antiproliferative response to *ARLTS1* restoration, we studied cell cycle kinetics and we observed an evident apoptotic peak 96 hours after infection in TOV-112 (50.75% in sub-G_1 phase) and MCF7 (36.21% in sub-G_1 phase) cell lines infected with Ad-*ARLTS1*-WT (Fig. 2*A* and *B*; Supplementary Fig. S3). Accordingly, with growth curves and CCK-8 assay, the apoptotic program was not activated in OV-90 cell line (Fig. 2*C*) or in any cell line infected with Ad-*ARLTS1*-MUT, confirming its lack of significant function *in vitro*.

Western blot analysis revealed that Ad-*ARLTS1*-WT activates the intrinsic apoptotic pathway as shown by the cleavage of the initiator caspase-9 and the effector caspase-3. In addition, down-regulation of the antiapoptotic protein Bcl-2 was detected (Fig. 2*D*). To validate that *ARLTS1*-induced apoptosis is caspase dependent, we infected TOV cells with *ARLTS1* and treated them with different caspase inhibitors (Fig. 2*E*). Treatment with the pan-caspase and caspase-9 inhibitors blocked the *ARLTS1*-induced apoptosis, confirming that *ARLTS1* induced apoptosis through the intrinsic (mitochondrial) caspase pathway.

Treatment with 5-AZA restores *ARLTS1* expression in ovarian cancer cells and inhibits their proliferation. Because hypermethylation had already been described as a potential mechanism of *ARLTS1* down-regulation (1), we decided to study if *ARLTS1* expression could be restored in TOV-112 cells using either DNA methyltransferase inhibitor 5-AZA or the histone deacetylase inhibitor TSA or both. RT-PCR on total RNA extracted from treated cells showed that only 5-AZA was able to restore ARTLS1 expression in TOV-112 (Fig. 3*A*), confirming that DNA hypermethylation, but not histone modifications, regulates *ARLTS1* expression in this cell line.

To assess whether an antiproliferative effect of demethylation on TOV-112 was associated with *ARLTS1* restoration, we transfected cells with small interfering RNA (siRNA) targeting *ARLTS1*, and after 24 hours, we treated them with 5-AZA. Treatment with 100 nmol/L siRNA against *ARLTS1* was able to dramatically down-regulate its expression (Fig. 3*B*). TOV-112 showed a 4-fold decreased proliferation rate ($P < 0.001$) after treatment with 5-AZA *in vitro* (Fig. 3*C*). Notably, cells in which *ARLTS1* expression was silenced by siRNA showed decreased sensibility to 5-AZA treatment, suggesting that *ARLTS1* has an effective role in response to demethylating agents (Fig. 3*D*).

***Ex vivo ARLTS1* gene restoration suppresses tumor growth *in vivo*.** To verify the *in vivo* effect of *ARLTS1* restoration in ovarian cancer cells, we did xenografts of *ex vivo*–treated TOV-112 cells in nude mice. At 30 days after injection, tumor growth was completely suppressed in mice inoculated with TOV-112 cells infected with Ad-*ARLTS1*-WT (Fig. 4*A*). The average tumor weights for controls (Ad-*GFP* and untreated TOV-112 cells) at day 30 were 1.39 ± 0.85 g and 1.53 ± 0.29 g, respectively. At 30 days, four of five mice inoculated with Ad-*ARLTS1*-infected TOV-112 cells showed no tumors, and tumor weight of the only mouse who developed tumor was 0.15 g, significantly lower ($P < 0.001$) than tumors of Ad-*GFP*-infected TOV-112 cells (1.53 ± 0.29 g) and mock-treated TOV-112 cells (1.39 ± 0.85 g; Fig. 4*B*). In the five mice injected with 5-AZA-treated TOV-112 cells, only one mouse developed a small tumor (weight of 0.21 g).

Notably, *ARLTS1*-MUT retained partial activity *in vivo* because two of five mice showed no tumor after 30 days and the average tumor weight in mice that developed tumors was 0.68 ± 0.35 g (Fig. 4). This is at least four times higher as the only Ad-*ARLTS1*-WT developed tumor and thrice higher as the 5-AZA-treated cells induced tumors but also about twice as smaller as in the two

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control groups. These *in vivo* data support the idea that the Trp149Stop is retained in the general population and predisposes to cancer because of a reduction, but not full loss, of normal *ARLTS1* function.

Discussion

In the present study, we showed the tumor suppressor function of *ARLTS1* in ovarian cancer and we investigated the biological importance of the Trp149Stop polymorphism in both ovarian and breast cancer models. We previously described the methylation of a predicted promoter as the cause of reduced or absent *ARLTS1* expression (1). Here, we proved that the same mechanism is involved in the silencing of *ARLTS1* in TOV-112 cells: treatment with demethylating agent 5-AZA, but not with the histone deacetylase inhibitor TSA, induced its reexpression.

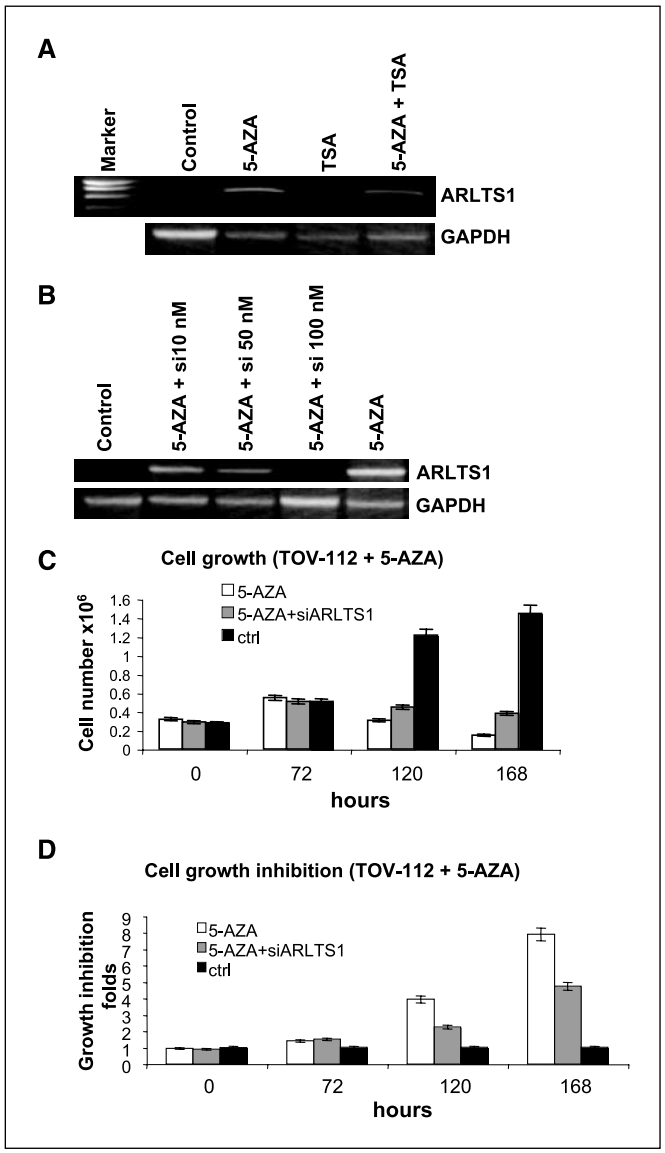
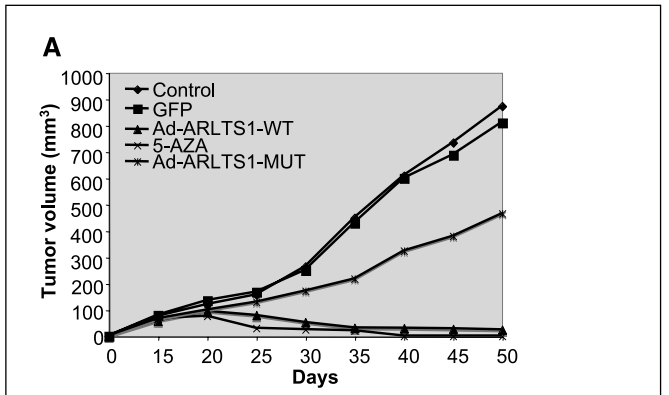


Figure 3. Reexpression of *ARLTS1* in TOV-112 cells after treatment with the demethylating agent 5-AZA. *A,* RT-PCR of untreated (control) and 5-AZA- or TSA-treated cells. GAPDH levels were used as a loading control. 5-AZA treatment restored *ARLTS1* mRNA expression, whereas TSA treatment did not. *B,* down-regulation of *ARLTS1* levels (6 days after transfection) by siRNA treatment. siRNA treatment (100 nmol/L) against *ARLTS1* was able to inhibit the expression of *ARLTS1* induced by 5-AZA treatment. *C,* cell growth analysis of untreated, 5-AZA, and combination 5-AZA + *ARLTS1* siRNA treatment of TOV-112 cells. *D,* 5-AZA treatment suppressed TOV-112 cell growth, whereas 5-AZA + *ARLTS1* siRNA treatment decreased the 5-AZA inhibitory effect by 50% ($P < 0.001$).



B

Treatment	Tumor incidence	Tumor weight (g) ± SD
CTRL	5/5 mice	1.53 ± 0.29
GFP	5/5 mice	1.39 ± 0.85
ARLTS1 WT	1/5 mice	0.15
ARLTS1 MUT	3/5 mice	0.68 ± 0.35
5-AZA	1/5 mice	0.21

Figure 4. Effect of *ARLTS1* expression on tumorigenicity of TOV-112 ovarian cancer cells. *A,* tumor volume of untreated, Ad-GFP-treated, Ad-*ARLTS1*-treated, Ad-*ARLTS1*-MUT-treated, and 5-AZA-treated cells. Restoration of *ARLTS1* expression by adenovirus or 5-AZA treatment *in vitro* suppressed significantly ($P < 0.001$) tumor growth in nude mice. *B,* tumor weight of tumors in untreated, Ad-GFP-treated, Ad-*ARLTS1*-treated, Ad-*ARLTS1*-MUT-treated, and 5-AZA-treated nude mice. Tumor weight of Ad-*ARLTS1*-treated and 5-AZA-treated mice was significantly different compared with the control ($P < 0.0001$).

We adopted two different methods to restore gene expression: adenoviral transduction (in cancer models with *ARLTS1* deletion and/or mutation) and use of demethylating agents (in cancer models with *ARLTS1* hypermethylation). In both cases, the apoptotic effects are evident in *ARLTS1*-negative ovarian cancer cell line TOV-112. On the other hand, overexpression of *ARLTS1* in positive ovarian cancer cell lines OV-90 has no effect, suggesting that *ARLTS1* selectively restores apoptotic programs only in negative cancer cells, where lack of expression is selected during tumor evolution. Restoration of wild-type *ARLTS1* expression in MCF7 breast cancer cell line, which expresses only the truncated *ARLTS1*, determines a strong apoptotic response. Exogenous expression of the variant Trp149Stop has no significant *in vitro* antiproliferative effect in any of the cell lines we analyzed; however, *ARLTS1* variant Trp149Stop retained some antiproliferative function *in vivo*. The same partial effect was proved also in A549, a lung carcinoma cell line with very low expression of *ARLTS1* (1).

Another polymorphism in position Cys148Arg has been described to be associated with increased risk of familial breast cancers (8), and therefore, it is very likely that a functional domain important in cancer predisposition resides in this region of the protein. Of note, a mutation in ARL1 (Asp151Gly), a close member of the same ADP ribosylation-like family of genes as *ARLTS1* that corresponds to Asp146 in human *ARLTS1*, has been proven to inhibit the autophagic cell death in yeast consequent to a defect in

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vacuole formation (13). Both Trp149Stop and Cys148Arg are located in the vicinity of this position. In *ARLTS1*-negative lung A549 and ovarian TOV-112 cells, reexpression induced by transfection of an *ARLTS1* expression vector in A549 or infection with an Ad-*ARLTS1* in TOV-112 induced apoptosis characterized by activation of caspase-3 and caspase-9 but not caspase-8. It was shown that chloroquine-induced neuronal cell death is characterized by autophagosome accumulation, caspase-3 activation, and cell death (14), a mechanism resembling the autophagic cell death. As *ARLTS1* is a member of the ADP ribosylation factor/ADP ribosylation-like subfamily of GTP-binding proteins (15) involved in membrane trafficking, it is possible that reexpression of *ARLTS1* in negative cancer cells induces a type of cell death resembling the autophagic apoptosis, and the Trp149Stop is significantly reducing this specific function of the wild-type protein, with subsequent increase in various types of cancer predisposition.

Our experiments showed that a single treatment of TOV-112 cells with either Ad-*ARLTS1* or 5-AZA *ex vivo* is sufficient to restore *ARLTS1* expression and to inhibit tumor growth. The tumor suppressor effect in cells treated with 5-AZA might be due to multiple genes whose expression is restored on demethylation. However, we showed that silencing *ARLTS1* by siRNA *in vitro* partially reduced response of TOV-112 cells to 5-AZA, suggesting that *ARLTS1* plays an effective role in response to this drug. In addition, it would be valuable to study an eventual association between *ARLTS1* down-regulation and resistance to specific therapeutic agents like decitabine.

In conclusion, we have shown that *ARLTS1* is a tumor suppressor gene down-regulated in a large proportion of ovarian cancers. Restoration of expression by adenoviral infection in negative cells induced high levels of apoptosis, whereas introduction of the truncated form of *ARLTS1* associated with familial predisposition to breast cancer has no effect. Breast cancer cells endogenously expressing the Trp149Stop variant are sensitive to apoptosis induced by the wild-type protein, supporting the idea that the truncated variant is maintained in the general population and is predisposing to cancer because of a reduction in normal function.

Acknowledgments

Received 6/23/2006; revised 8/18/2006; accepted 9/18/2006.

Grant support: National Cancer Institute Program Project grants P01CA76259 and P01CA81534 (C.M. Croce), Kimmel Scholar Award (G.A. Calin), Department of Defense Predoctoral Award grant BC043090 (D. Iliopoulos), Italian Ministry of Public Health, Italian Ministry of University Research, and Italian Association for Cancer Research (M. Negrini).

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