ArgBP2-Dependent Signaling Regulates Pancreatic Cell Migration, Adhesion, and Tumorigenicity

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Abstract

The poor prognosis of pancreatic cancer is due to rapid locoregional invasion, the early development of metastases, and the limited efficacy of current therapies. To date, none of the identified oncogenes and suppressors involved in this disease have led to efficient treatments. Here, we describe that the scaffold protein ArgBP2 is repressed during oncogenic transformation of the pancreas. We could show, using a pancreatic cancer cell line model, that this repression of ArgBP2 participates in the progression of this disease. Interestingly, in vitro analyses revealed that the antitumoral potential of ArgBP2 is linked to the control of cell adhesion and migration rather than to the regulation of cell proliferation or sensitivity to apoptosis. Moreover, we could detail part of the molecular mechanism responsible by identifying new ArgBP2-interacting proteins, and show that this function is partly achieved by the control of a WAVE/PTP-PEST/c-Abl signaling complex. These findings point to a new mechanism of pancreatic cancer progression leading to invasion and metastasis and suggest that the ArgBP2 signaling pathway could represent a new target for cancer therapy. [Cancer Res 2008;68(12):4588–96]

Introduction

Over the past two decades, research aimed at understanding and fighting the complex mechanisms of cellular transformation which lead to tumor development and the establishment of metastases has helped to improve the detection and treatment of many cancers. However, cancer in Western countries is still a critical health problem (1), and some tumors have not benefited from these advances. Among them, tumors from the pancreas, which are rapidly invasive, metastatic, and resistant to standard therapies (2), remain the type of cancer with the worst clinical outcome—having a median survival of 6 months (3). Many molecular markers have been identified, including the activation of known oncogenes and the inactivation of tumor suppressor genes (4). To date, their help in refining prognosis and as therapeutic targets has proved very limited, but some of these molecules are still under evaluation (4).

ArgBP2 is a multi-adaptor protein first identified as a new binding protein and substrate for two members of the Abl kinase family, c-Abl and c-Arg (5). ArgBP2 contains a SoHo (sorbin homology) domain in its amino-terminal part and three SH3 (Src homology 3) domains in its carboxyl-terminal part. It is the third member of the SoHo family of proteins, besides CAP (Cbl-associated protein)/ponsin and vinexin, which display the same structural organization and present overlapping functions (6). A longer brain-specific variant of ArgBP2, named nArgBP2, is generated by an alternative splicing that inserts a central zinc finger domain (7, 8). The SoHo domain is known to bind the lipid raft–resident protein flotillin (9) and α2-spectrin (8), a component of the membrane-associated actin cytoskeleton. The three COOH-terminal SH3 domains engage proline motifs contained in an increasing number of identified ArgBP2-associated proteins, involving ArgBP2 in distinct cellular processes. The interaction of ArgBP2 with the ubiquitin ligase c-Cbl was shown to regulate the function of the tyrosine kinases c-Abl and Pyk2 (10, 11). By binding both Akt and PAK1, ArgBP2γ (another spliced isoform of ArgBP2) is involved in cell survival (12). Several of these ArgBP2-binding proteins have pointed at a central function for ArgBP2 in coordinating signals and actin cytoskeleton-dependent cellular processes such as cell adhesion, motility, and vesicle transport in neuronal cells (7, 8), or structural organization of cardiomyocyte Z-discs (13).

We report here that ArgBP2 expression in the pancreas, which is high in normal tissue, is often lost during oncogenic transformation. We could further show that ArgBP2 plays an antitumoral role in vivo, probably due to its negative regulation of processes that are usually enhanced in cancer cells such as cell adhesion and migration. The identification of new ArgBP2-interacting proteins, and the study of their interplay with ArgBP2, shed new light on the molecular mechanisms by which ArgBP2 controls these cancerous cell properties. Also, our findings strongly suggest that cell migration is an important component of the aggressiveness of pancreatic tumors, which points to new putative therapeutic strategies to fight this cancer.

Materials and Methods

Cell lines. The pancreatic cancer–derived cell line MiaPaCa2, Capan-2 and BxPC3 cells, and HEK-293T cells were manipulated following American Type Culture Collection recommendations. To generate a stable ponasterone-inducible ArgBP2-expressing MiaPaCa2 cell line, cells were first transfected with pVgRXR vector and selected using zeocin at 0.4 mg/mL. A stable clone expressing retinoid X receptor was selected and transfected with pVgRXR and selected using zeocin at 0.4 mg/mL. Clones expressing the protein of interest, when stimulated by ponasterone at 5 μg/mL, were selected and, except during experiments, were maintained in standard medium containing 0.2 mg/mL of zeocin and 0.2 mg/mL of geneticin.

Plasmids and transfection. Myc-ArgBP2 and c-Abl–expressing vectors have been described previously (10), and Myc-tagged WAVE1 in pRK5 vector was provided by P. Aspenström (Ludwig Institute for Cancer Research, Uppsala, Sweden). The generation of other expressing constructs is described in the Supplementary data. HEK-293T cells were transiently transfected using Lipofectamine 2000 reagent (Invitrogen) following the
manufacturer's instructions. Briefly, 60% to 80% confluent 293T cells in six-well plates were transfected using a mix of 2 μg of DNA and 6 μl of Lipofectamine per well for 4 h. Cells were lysed 24 h posttransfection. For short interfering RNA (siRNA) experiments, 60% to 80% confluent MiaPaCa-ArgBP2, Capan-2, or BxPC3 cells in 25 cm² flasks were transfected with a mix of 0.5 nmol PTP-PEST or ArgBP2 siRNA and 20 μl of Effectene transfection reagents (Qiagen) in a 2.6 ml final volume of OptiMEM for 4 h. The PTP-PEST–specific siRNA used was the He_PTPN12_7 HP–validated siRNA from Qiagen and the control siRNA was from the same source. The ArgBP2–specific siRNA was from Eurogentec (UCCGGAUACCCCGACGAA).

**In vivo experiments.** MiaPaCa-ArgBP2 cells (10⁶) in 100 μl of serum-free DMEM were injected s.c. or into the pancreas of 6-week-old athymic NUDE mice. Each group of mice (10 mice per group) were implanted s.c. with ponasterone or placebo-releasing pellets (100 mg/pellet, 21-day or 60-day release; Innovative Research of America) according to the manufacturer's recommendations. Subcutaneous tumors were allowed to develop for 6 weeks whereas intrapancreatic tumors were allowed to develop for 2 months postimplantation. Subcutaneous tumors were stained for ArgBP2 using standard immunohistochemistry protocols with the anti-ArgBP2 monoclonal antibody C1. Analyses and scoring of samples (Supplementary Fig. SI) were done by an anatomicopathologist and a 2 test was used for statistical purposes. Details of the patient groups are shown in Table S1 of the Supplementary information.

**Immunohistochemistry.** Human pancreatic tumor samples, alone or displayed on a tissue microarray, were stained for ArgBP2 using standard immunohistochemistry protocols with the anti-ArgBP2 monoclonal antibody C1. Analyses and scoring of samples (Supplementary Fig. S1) were done by an anatomicopathologist and a 2 test was used for statistical purposes. Details of the patient groups are shown in Table S1 of the Supplementary information.

**Antibodies and reagents.** The following antibodies and serum were used: rabbit polyclonal anti-ArgBP2 serum (ref. 10; ArgBP2-3H3), mouse monoclonal anti-Flag (M2, Sigma), mouse monoclonal anti-myc (9E10, Santa Cruz Biotechnology), mouse monoclonal anti-ArgBP2 (clone C1), mouse monoclonal antiphosphotyrosine (PY99, Santa Cruz Biotechnology), mouse monoclonal anti-HA (12CA5, Roche), mouse monoclonal anti-myeloid differentiation primary response 8 (MyD88, Santa Cruz Biotechnology), mouse monoclonal anti-Flag (M2, Sigma) and mouse monoclonal anti–PTP-PEST (AG25, Sigma) antibodies, goat polyclonal anti–glutathione S-transferase (GST; Pharmacia), rabbit polyclonal anti-Abl (K12, Santa Cruz Biotechnology), and rabbit polyclonal anti-WAVE (H-180, Santa Cruz Biotechnology) antibodies.

**Immunoprecipitation, GST pull-down, Western blotting.** Twenty-four hours posttransfection, cells were lysed in lysis buffer and centrifuged at 15 min at 4°C. Cleared lysates with adjusted protein concentrations (protein assay, Bio-Rad) were used for immunoprecipitation and GST pull-down assays as described in the Supplementary data. Western blot analyses were performed as previously described (10).

**PTPase assays.** Lysates from 293T cells transfected with ArgBP2 and c-Abl or WAVE1 and c-Abl expression vectors were subjected to immunoprecipitation with anti-ArgBP2 and anti-WAVE1 antibodies, respectively. Immunoprecipitates were subjected to PTPase assays as described in the Supplementary data.

**Interference reflection microscopy.** MiaPaCa-ArgBP2 cells in standard culture medium treated or not with ponasterone, were allowed to attach to fibronectin-coated coverslips for 30 min. Slides were deposited on an Axiovert 135 inverted microscope (Zeiss) equipped with a heating stage (TRZ 3700) set at 37°C and interference reflection microscopy was performed with an antiflex objective (×63 magnification, 1.25 numerical aperture). Student's test was used for statistics.

**Cell spreading.** Twenty-four–well plates were coated overnight at 4°C with 250 μl of fibronectin at 10 μg/ml. Coated wells were blocked with 0.5% bovine serum albumin in PBS for 30 min, then washed twice with 0.1% PBS. Single cell suspensions (25,000 cells/0.5 ml) were seeded in substratum-coated wells and allowed to adhere for 2 h at 37°C. Cell areas were measured microscopically using Metaview software and Student's test was used for statistics.

**Migration assays.** The effect of ArgBP2 expression on MiaPaCa cell migration was determined using modified Boyden chambers (NeuroProbe, Inc.) with 8-μm pore polycarbonate Nucleopore membranes (Costar). The undersurface of the membrane was precoated with 10 μg/ml of fibronectin. The lower tank was filled with DMEM containing 0.1% bovine serum albumin and the membrane was placed in the chamber. Ponasterone-treated or untreated cells (50 × 10⁵) were seeded on the top side of perforated filters. Following incubation (5 h), nonmigratory cells on the upper surface of the filter were wiped with a cotton swab. Cells that migrated to the lower surface of the filter were fixed and stained with Coomassie blue. Haptotaxis was determined by counting cells in 10 microscopic fields (magnification, ×320) per well. Migration results are expressed at the average number of cells per microscopic field. This experiment was conducted in standard conditions and in the presence of phorbol 12-myristate 13-acetate.

**Results**

The expression of ArgBP2 is decreased in malignant pancreatic tumors. The tissue distribution of ArgBP2 expression in the mouse was partially described by monitoring the mRNA levels in different organs (data not shown). In order to obtain a more detailed profile of expression in humans, we used mouse monoclonal and rabbit polyclonal antibodies on tissue microarrays covering most tissues (normal and cancerous). We detected ArgBP2 in the normal pancreas. Pancreatic expression was mainly localized to acinar cells, duct cells, and all cell types in islets (Fig. 1A). Interestingly, ArgBP2 localized preferentially to the apical/secretion pole of the acinar cells, suggesting that it could play a role in the secretion process. Furthermore, whereas ArgBP2 was still present in benign pancreatic tumors such as intraductal papillary mucinous tumors, its expression seemed to be strongly repressed in undifferentiated adenocarcinomas. To validate these data, we have monitored ArgBP2 expression in a large number of pancreatic cancer samples of different types and at different stages of the disease by using a tissue microarray comprised of 135 different samples. The quantitative analysis of scoring showed that the expression of ArgBP2 was lost or at least significantly decreased in ~50% of adenocarcinomas and metastases (Fig. 1B). The statistical analysis of this data confirmed the association between pancreatic cancer progression and down-regulation of ArgBP2.

**ArgBP2 has an antitumoral potential.** To test whether the loss of ArgBP2 during pancreatic carcinogenesis could be a critical step towards increased aggressiveness of cancer cells, we have evaluated the effect of ArgBP2 expression on the tumorigenicity of a pancreatic cancer cell line. We have used MiaPaCa2 cells, highly transformed and devoid of endogenous ArgBP2 expression, to create a transgenic cancerous pancreatic cell line in which the expression of ArgBP2 is induced by the insect-derived hormone ponasterone (see Materials and Methods and Supplementary Fig. S2). We have used this model to study how ArgBP2 expression could influence the tumorigenicity of these cells injected into NUDE mice. Subcutaneous injection of these cells resulted in tumor formation in 60% of mice implanted with a placebo-releasing pellet (Fig. 2A), whereas no tumor could develop in mice implanted with a ponasterone-releasing pellet. Moreover, when...
ArgBP2 inhibits adhesion and migration of pancreatic cells. In order to explain the *in vivo* reduction of tumorigenicity induced by ArgBP2, we have performed *in vitro* studies of its effect upon pancreatic cancer cell phenotype. Surprisingly, both cell proliferation and apoptosis were not modulated by ArgBP2 (Supplementary Fig. S4). However, we could observe that ArgBP2 colocalized with vinculin and filamentous actin at focal adhesions and lamellipodia of pancreatic cells (Supplementary Fig. S5). Considering that proteins from the SoHo family act at the interface between integrins and the actin cytoskeleton (6, 8), and because cell adhesion and motility are profoundly affected during cell transformation (15), we suspected that ArgBP2 could modulate these processes in pancreatic cancer cells. We could observe that β1 and α3 integrin subunits were the main extracellular matrix receptors present at the plasma membrane of these cells (Supplementary Fig. S6). As β1 integrin, also known as fibronectin receptor, is involved in a number of pathologic situations (16), we have studied the influence of ArgBP2 on the behavior of MiaPaCa2 cells when plated onto fibronectin. We could observe that ArgBP2 expression reduced cell spreading by almost 50% (Fig. 3A). Moreover, we have studied the effect of ArgBP2 on cell adhesion by using interference reflection microscopy (17), and found that ArgBP2 significantly delayed cell adhesion onto fibronectin (Fig. 3B). This negative regulation of cell adhesion by ArgBP2 has been confirmed in Capan-2 and BxPC3 pancreatic cancer cells (Supplementary Fig. S7). Among the panel of cell behaviors possibly modulated by adhesion, cell migration is one of importance for cancer cells (18). Hence, we have monitored the influence of ArgBP2 on single cell migration and found that expression of ArgBP2 reduced the number of migrating cells by >95% (Fig. 3C). The addition of phorbol 12-myristate 13-acetate, an activator of protein kinase C, during the experiment strongly enhanced the migration of both ponasterone-treated and ponasterone-untreated cells, however, ArgBP2 could still efficiently block the migration of these cells. We have shown that the highly deleterious effect of ArgBP2 on cell migration was not an artifact due to ponasterone itself (Supplementary Fig. S8). Importantly, we were able to confirm this last finding in two other cell types because inhibition of ArgBP2 expression strongly enhanced the migratory potential of both Capan-2 and BxPC3 pancreatic cancer cells (Fig. 3D). Taken together, these data show that ArgBP2 has the potential to regulate the adhesion and migration of pancreatic cancer cells independently of their proliferation and survival abilities and that these characteristics might be of importance for tumor progression.

ArgBP2 interacts with regulators of actin dynamics. In order to elucidate the molecular mechanisms responsible for ArgBP2 functions, we have sought to identify new ArgBP2-associated proteins. To this end, we have used the CytoTrap yeast two-hybrid system, with either the NH2-terminal or the COOH-terminal part of ArgBP2 as bait. Screening of a HeLa cell library with the COOH-terminal part of ArgBP2 revealed approximately 20 new putative ArgBP2-interacting proteins including WAVE1 (WASP family verprolin-homologous protein) and the protein tyrosine phosphatase PTP-PEST (data not shown). Interestingly, these two proteins are well known to be involved in the regulation of the actin structures necessary for cell adhesion and migration (19, 20). To study the interaction of ArgBP2 with WAVE1 and PTP-PEST, we have carried out a pull-down assay with ArgBP2 protein–expressing MiaPaCa2 cell line (14). These findings show that ArgBP2 expression antagonized the development of such pancreatic tumors.

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**Figure 1.** ArgBP2 protein expression is decreased in pancreatic carcinomas. A, immunohistochemical analysis of ArgBP2 expression in normal pancreatic samples (Acini, Ducts, and Islet) and in cancerous pancreatic samples (IPMT, intraductal papillary mucinous tumors; ADC, adenocarcinomas). Higher magnifications of acini and ducts show basal nucleus (yellow arrow) and apical localization of ArgBP2 (red arrow). B, to bypass tumor heterogeneity, the expression of ArgBP2 was assessed in a tissue microarray composed of pancreatic tissue samples from 135 patients (5-μm-thick sections), and scored as follows: 0, null; +, low staining or sparse staining; ++, strong and dense staining (see Supplementary Fig. S1). Columns, relative percentages of each scoring for nontumoral tissue (Normal; n = 4), cystadenomas (CysAd; n = 4), intraductal papillary mucinous tumors (IPMT; n = 13), adenocarcinomas (ADC; n = 94), and adenocarcinoma metastases (Met; n = 20); *, P < 0.0001, different from normal.
proteins, we have performed GST pull-down experiments with either the native COOH terminus of ArgBP2 or single SH3 domains fused to GST. We could observe that only SH3 A and B of ArgBP2 mediated the binding to WAVE1, whereas all three SH3s of ArgBP2 could independently bind PTP-PEST proteins (Fig. 4A).

We next confirmed that these interactions take place in the cell and performed coimmunoprecipitation experiments of transfected proteins (Fig. 4B). It seems that both WAVE1 and PTP-PEST are able to specifically bind to ArgBP2. Importantly, we could observe the interaction of ArgBP2 with endogenous WAVE and PTP-PEST proteins in MiaPaCa-ArgBP2 cells (Fig. 4C), indicating the validity of this pathway in our pancreatic cancer cell model. Finally, we could show that the expression of ArgBP2 in MiaPaCa2 cells mediated the partial translocation of WAVE1 and PTP-PEST proteins to focal adhesion structures containing both ArgBP2 and filamentous actin at leading edges of the cell (Fig. 4D).

PTP-PEST regulates ArgBP2’s phosphorylation. ArgBP2 being both an interactor and substrate for the c-Abl kinase (5), we were interested in evaluating the consequence(s) of the interaction with PTP-PEST regarding the phosphorylation of ArgBP2 by c-Abl. As expected, when PTP-PEST was coexpressed with ArgBP2, phosphorylation by c-Abl was strongly impaired (Fig. 5A), suggesting that PTP-PEST can inhibit the phosphorylation-dependent functions of ArgBP2. Furthermore, as the tyrosine phosphorylation of ArgBP2 was shown to inhibit some of its interactions (10), we have used PerVanadate treatment of the cells to block the phosphatase activity of PTP-PEST and so, to increase the phosphorylation of ArgBP2 (Fig. 5B). The study of ArgBP2/PTP-PEST interaction under these conditions showed that the binding between these two proteins is impaired by ArgBP2’s phosphorylation (Fig. 5B). Then, by dephosphorylating ArgBP2, PTP-PEST also increases its binding to ArgBP2. As PTP-PEST can directly dephosphorylate and inactivate c-Abl (21), we have verified the direct dephosphorylation of ArgBP2 in vitro by using a purified PTP-PEST phosphatase (Fig. 5C).

ArgBP2/PTP-PEST complex negatively regulates WAVE1 function. The c-Abl kinase has recently been shown to phosphorylate WAVE proteins and to regulate their activity (22, 23). Therefore, we have evaluated the effect of ArgBP2 and PTP-PEST expressions on the c-Abl–mediated phosphorylation of WAVE1. We could observe that the phosphorylation of WAVE1 induced by the overexpression of c-Abl was greatly enhanced in the presence of ArgBP2 (Fig. 6A), suggesting that by binding

Figure 2. ArgBP2 expression reduces pancreatic tumor development in mice. A, athymic NUDE mice were injected subcutaneously or intrapancreatically with 10^7 transgenic MiaPaCa-ArgBP2 in the presence or absence of ponasterone. Columns, the percentage of positive subcutaneous xenografts. For intrapancreatic injections, the number of intrapancreatic tumors was determined for each mouse and the average number of tumors per mouse is represented. *, **, P < 0.05, significantly different from placebo. B, representative overviews of intrapancreatic tumors. Note the large tumor volume in the absence of ArgBP2 expression (Placebo). Histologically, tumors were undifferentiated in both groups of animals. The limits between tumor and host tissues (yellow) and the surfaces of all selected areas (black) were quantified by pixel counting. Columns, mean surface of the largest sections of each tumor, from ponasterone-treated and nontreated groups. *, P < 0.05, significantly different from placebo.
both WAVE1 and c-Abl, ArgBP2 facilitates their mutual interaction and hence the phosphorylation of WAVE1. This finding is supported by the increased amount of c-Abl that coprecipitates with WAVE1 when ArgBP2 is expressed. By contrast, the overexpression of PTP-PEST completely abolished the c-Abl-mediated phosphorylation of WAVE1, even in the presence of ArgBP2 (Fig. 6A). This result was confirmed in MiaPaCa-ArgBP2 cells (Fig. 6A) and suggests that PTP-PEST does not

![Figure 3. ArgBP2 regulates pancreatic cancer cell adhesion and migration.](image)

A, spreading of MiaPaCa-ArgBP2 cells, treated or not with ponasterone, was evaluated by seeding cells on fibronectin for 5 h. Columns, average surface area of spreading. *, $P < 0.05$, significantly different from control. B, the effect of ArgBP2 expression on cell adhesion was evaluated by interference reflection studies of ponasterone-treated and nontreated MiaPaCa-ArgBP2 cells seeded on fibronectin-coated glass slides. Columns, the percentage of cells that are either highly adherent ++, poorly adherent +/-, or nonadherent + - after 30 min of plating. *, $P < 0.01$; **, $P < 0.05$, ponasterone-treated cells significantly different from untreated cells. C, MiaPaCa-ArgBP2 cells treated or not with ponasterone and phorbol 12-myristate 13-acetate (PMA) were allowed to migrate through a perforated filter coated with fibronectin. After 5 h, filters were fixed and cells stained with trypan blue. Representative fields (top); columns, quantification of migration expressed as the average number of migrating cells per field; bars, SD (bottom). D, Capan-2 and BxPC3 cells were transfected with ArgBP2-specific siRNA or control siRNA, and 24 h later, allowed to migrate as in C. Columns, average number of migrating cells; bars, SD. The efficacy of the siRNA was verified by reverse transcription-PCR.
regulate the function of ArgBP2 only via the dephosphorylation of ArgBP2 but also via the dephosphorylation of some of its associated proteins. Using an *in vitro* dephosphorylation assay, we could observe that PTP-PEST was extremely efficient in dephosphorylating WAVE1 as 2 minutes of reaction were enough to almost completely dephosphorylate WAVE1 (Fig. 6B). Importantly, we could show that PTP-PEST efficiently bound to WAVE1 protein and that this interaction was strongly enhanced in the presence of ArgBP2 (Fig. 6C). Intriguingly, as the catalytically inactive PTP-PEST mut bound to WAVE1 with less affinity than PTP-PEST WT (Fig. 6C), it seemed that the dephosphorylation of PTP-PEST-associated proteins can enhance their mutual interaction.

Hence, by interacting with both WAVE1 and PTP-PEST, ArgBP2 could facilitate the interaction between these two proteins and therefore promote the dephosphorylation of WAVE1. Therefore, the negative regulation of cell migration by ArgBP2 should be at least partially dependent on its interaction with this phosphatase. To test this hypothesis, we have evaluated whether PTP-PEST activity is required for this particularly important function of ArgBP2. We have used specific siRNAs to knock-down endogenous PTP-PEST expression prior to migration assays. As expected, inhibition of MiaPaCa2 cell migration by ArgBP2 was markedly impaired upon siRNA treatment (Fig. 6D). Then, the PTP-PEST phosphatase activity was indeed part of the mechanism required for the ArgBP2-mediated inhibition of cell migration.
Discussion

A number of potential biological markers for pancreatic cancer have been described, from tumor suppressor genes such as p53 which is inactivated in ~60% of patients, to oncogenes such as K-ras which is activated in 80% of cases (4). However, most of those already evaluated have limited sensitivity and specificity. Importantly, as many of these proteins are involved in the control of major cell functions such as proliferation or apoptosis, they also represent potential molecular targets for the development of new therapeutic drugs. Several molecules have already been developed and entered into clinical trials (24), but none of them have proved really efficient yet.

These proteins are principal actors of major cellular pathways, and because of that, they can only interfere with the outcome of the pathway they are involved in. Along with the major pathways, scaffold proteins represent a growing population of proteins that act by modulating the activity of one or several members of one or several pathways. Hence, some of these proteins might modulate several cellular behaviors that are altered in cancer cells such as proliferation, apoptosis, or migration. The observation that ArgBP2 is repressed in approximately half of advanced pancreatic tumors (Fig. 1) indicates that it could be one of them. Consistent with a scaffold function, ArgBP2 might act by modulating the activity of one or several members of one or several pathways. Hence, the loss of ArgBP2 expression observed in mice could account, at least in part, for the reduction of malignancy observed in mice.

Immunohistochemical studies of normal pancreatic tissue revealed the apical localization of ArgBP2, suggesting that it could be involved in the organization of the secretory apparatus present in differentiated cells. Such cytoskeleton organizational functions of ArgBP2 have been previously suggested for the sarcomeric Z-disc of cardiomyocytes (13) or for neuronal synapses (7). Altogether, these observations suggest that, in various cell types, ArgBP2 organizes and stabilizes the actin cytoskeleton required for a functional phenotype. Hence, the loss of ArgBP2 expression observed during oncogenic transformation of the pancreas would lead to actin disorganization, cell dedifferentiation, and eventually, to abnormal cell migration and invasion.

Figure 5. PTP-PEST mediates the dephosphorylation of ArgBP2. A, lysates from 293T cells expressing combinations of ArgBP2, c-Abl kinase, and PTP-PEST as indicated were subjected to immunoprecipitation (IP) with an anti-ArgBP2 antibody. The amount of precipitated ArgBP2 and of coprecipitated c-Abl was also verified by blotting with specific antibodies. Expression of all constructs was also verified in TCL. B, to determine the influence of ArgBP2 phosphorylation upon its interaction with PTP-PEST, transfected 293T cells were treated with 0.3 mmol/L of PerVanadate (PVd) for the indicated times prior to lysis. Lysates were subjected to immunoprecipitation with an anti-Flag (PTP-PEST) followed by blotting with an anti-ArgBP2 antibody, or immunoprecipitation with an anti-ArgBP2 antibody followed by blotting with an antiphosphotyrosine antibody. Levels of ArgBP2 and PTP-PEST have been verified. C, dephosphorylation assay of ArgBP2. Lysate from 293T cells expressing ArgBP2 in combination with c-Abl was subjected to immunoprecipitation with an anti-ArgBP2 antibody. Immunocomplexes were subjected to PTP assay, as described in Materials and Methods for the indicated times. Phosphorylation of ArgBP2 was revealed by blotting with antiphosphotyrosine (PY) antibody at the level of ArgBP2. Filter was then blotted for ArgBP2 and PTP-PEST to confirm equal amounts of both proteins.
Despite the possibility that ArgBP2 could modulate the translocation of integrin-containing vesicles to the plasma membrane, we did not detect any significant alteration of the expression at the cell surface of these extracellular matrix receptors (Supplementary Fig. S6). Our data suggest that ArgBP2 acts downstream from integrin receptors by modulating the dynamic of actin-based mechanisms responsible for cell spreading, remodeling, and migration. The interaction of ArgBP2 with WAVE1, PTP-PEST, and c-Abl—all of these proteins playing a role in actin-related processes—supports this hypothesis. Indeed, the proteins of the WASP family, which are directly involved in the regulation of actin polymerization, play a central role in cancer cell migration (18). The c-Abl kinase, well known for its oncogenic potential (26), is involved at different levels in the control of actin filament dynamics regulating cell shape, cell adhesion, and cell migration (27), and the tyrosine phosphatase PTP-PEST has been implicated in the regulation of cell adhesion and migration (19, 28, 29). Therefore, by interacting with a kinase and a phosphatase, we
found a dual role for ArgBP2 which could then control the activity of WAVE1 protein by enhancing either its phosphorylation or its dephosphorylation (Fig. 6A and B). We have previously described a similar mechanism for ArgBP2 which could, on the one hand, enhance the activity of c-Abl, and on the other hand, end this signal by mediating the ubiquitination-dependent degradation of c-Abl (10). In our cellular model (MiaPaCa2), significant expression of WAVE1 and of both c-Abl and PTP-PEST mRNAs was detected by microarray analysis (data not shown). Therefore, in these cells, inhibition of cell adhesion and cell migration by ArgBP2 could be mediated, at least in part, by PTP-PEST-dependent functions. This would result in the dephosphorylation of, among other targets, WAVE1 protein (Fig. 6A). Importantly, the in vivo relevance of this hypothesis is supported by the fact that in the absence of PTP-PEST, ArgBP2 was impaired in blocking MiaPaCa2 cell migration (Fig. 6D).

Our findings suggest that targeting the ArgBP2/WAVE/PTP-PEST/c-Abl axis might change the fate of pancreatic tumor development by restricting their invasive/metastatic potential. PEST/c-Abl axis might change the fate of pancreatic tumor development by restricting their invasive/metastatic potential.

**References**


**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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