ArgBP2, a member of the SoHo family of adapter proteins, is a regulator of actin-dependent processes such as cell adhesion and migration. Recent data from our lab revealed that by regulating adhesion and migration of pancreatic cancer cells, ArgBP2 is endowed with an anti-tumoral function. We could show that part of the molecular mechanism involved the interaction of ArgBP2 with the Arp2/3 activator WAVE1, the tyrosine phosphatase PTP-PEST, and the tyrosine kinase c-Abl. As ArgBP2 shares common structural organization and overlapping functions with the two other members of this protein family, CAP and Vinexin, it raises the question whether these two other proteins could also be involved in cancer diseases. The control of cell migration being an important issue in tumor treatment, these recent findings suggest that ArgBP2 family-dependent signaling pathways represents potential targets for the development of therapeutic strategies, and highlight the importance of elucidating their molecular mechanisms of cytoskeletal regulation.

Acquisition of migration properties by cancerous cells is a crucial feature for cancer cell invasion and metastasis and, therefore, the control of cell migration is an important issue in tumor treatment. As molecular mechanisms governing cell motility are common to non-neoplastic cells and cancer cells, the elucidation of these mechanisms is crucial in order to better understand how the loss of control of cell adhesion, migration and cytoskeletal organization participate to tumorigenesis.

The Adapter Protein ArgBP2 is an Important Regulator of Actin Cytoskeleton and Cell Motility

ArgBP2 (Arg-binding protein 2) belongs to a family of adapter proteins containing two other members, Vinexin and CAP (c-Cbl associated protein)/Ponsin (Table 1). They all share the same structural organization with a SoHo (Sorbin Homology) domain in their N-terminal part and three SH3 domains in their C-terminal part. ArgBP2 is expressed in many different human tissues but is extremely abundant in the heart. ArgBP2’s SH3 domains are very similar to those found in proteins regulating the actin cytoskeleton, and they actually mediate the binding of ArgBP2 to a significant number of proteins that are directly or indirectly involved in the regulation of actin dynamic. Consequently, ArgBP2 has been observed in most actin based intracellular structures. It is found in focal adhesions where it interacts with important components of cell adhesion sites such as vinculin and afadin. It also localizes at stress fibers of epithelial cells and fibroblasts and in the Z-discs of sarcomeres in cardiac muscle cells.

Several studies have implicated ArgBP2 in the control of cell morphology and membrane protrusion necessary for cell migration. The knock-down of ArgBP2 induces the elongation of neural cells with the formation of peripheral actin ruffles and the partial dissociation of focal adhesion markers, such as FAK (Focal adhesion kinase) and Paxillin, from adhesion foci. ArgBP2-dependent recruitment of Pyk2 and Cbl to lipid rafts, following growth factor stimulation, induces membrane ruffling and formation of lamellipodia at the tip of neurites. This ArgBP2-dependent modulation of cell morphology is surely mediated by its interaction with proteins playing a role in the generation of cell ruffles and lamellipodia such as dynamins, WAVE proteins or synaptojanins. ArgBP2 interacts with and is a substrate for the non-receptor tyrosine kinase c-Abl that relays signals from cell surface growth factor and adhesion receptors to promote cytoskeletal rearrangements by phosphorylating several known regulatory proteins. We previously showed that ArgBP2 is able to enhance the c-Abl kinase activity but also to negatively regulates the c-Abl function by promoting its ubiquitination by the ubiquitin ligase Cbl and its subsequent degradation by the proteasome.

ArgBP2 is a Potential Target for Anti-Tumor Treatment

Results from our lab pointed at an anti-tumoral potential of ArgBP2 in pancreatic cancer, a new function for this protein associated with actin cytoskeleton related signaling. ArgBP2, which is highly expressed in the normal human pancreas, is strongly repressed during the progression of pancreatic cancer. ArgBP2 is still present in benign pancreatic tumors such as intraductal papillary mucinous tumors but is lost or strongly repressed in 50% of pancreatic adenocarcinoma and metastases. This repression of ArgBP2 expression seems to play an essential role regarding the progression of the tumor since restoration of ArgBP2 expression in highly transformed pancreatic cancerous MiaPaCa2 cells reduced their ability to form tumors in vivo when injected into nude mice. We were surprised...
Table 1  The SoHo family of protein

| Member  | Known Isoforms | Structures | Interactions | Functions |
|---------|----------------|------------|--------------|-----------|
| A       |                | SoHo 666aa | c-Abl, c-Arg, c-Cbl, Pyk2, Flotillin, Vinculin, SAPAP, Afadin, spectrin, synaptotigin-1/2b, dynamin1/2, palladin, alpha-actinin, Akt, PAK1, WAVE1/2, PTP-PEST | Actin signaling and organization, Cell adhesion/migration, c-Abl/c-Arg signaling, Akt signaling, Pyk2 signaling and neurites extension, synapse organization |
| B       |                | SH3 640aa  |              |           |
| N-ArgBP2|                |            |              |           |
| gamma   |                | SH3 573aa  |              |           |
| ArgBP2  | isoform 1      | SH3 684aa  | c-Cbl, c-Abl, Insulin Receptor, SOS, Vinculin, Afadin, Flotillin, Grb4, Ataxin-7, paxillin, filamin C, sGCbeta1, tenasin-1, APS | Insulin signaling, glucose transport, macrophage mediated insulin resistance, Erk signaling, muscle differentiation and integrity, cardiomyocytes, cytoskeletal remodeling |
| CAP/Ponsin | isoform 2   | SH3 816aa  |              |           |
| alpha   |                | SH3 671aa  |              |           |
| beta    |                | SH3 329aa  |              |           |
| gamma   |                | SH3 680aa  |              |           |
| Vinexin |                | SH3 680aa  |              |           |

Table presenting the main isoforms of the tree SoHo family members, their relative structural organization, their known interacting proteins, and the functions they were shown to modulate.

Figure 1. Oncogenic interactions of ArgBP2. Schematic representation of the known ArgBP2 containing molecular complexes and their established or probable role in tumorigenic process.
to observe that the in vitro cell proliferation and apoptosis were not affected by ArgBP2 in this cell line. But, owing to the role of ArgBP2 in regulating cytoskeletal functions, and to the fact that cell motility is profoundly altered during cell transformation, we wondered if the anti-tumoral function of ArgBP2 could be linked to an ability to regulate cell adhesion and migration of pancreatic cancer cells. By re-establishing the expression of ArgBP2 in MiaPaCa2 cells, we could demonstrate that ArgBP2 efficiently blocks their migration and adhesion. Accordingly, inhibition of its expression in less aggressive cancerous pancreatic cells, in which ArgBP2 is still expressed, promotes their adhesion and their migration.

In order to understand the molecular mechanisms that mediate the ArgBP2-dependent inhibition of cell migration, we have used a yeast-two-hybrid approach to identify new putative ArgBP2 associated proteins. Some of them were well known to play a role in actin-related processes and the study of their interaction with ArgBP2 suggested that ArgBP2 acts downstream of integrin receptors by modulating the dynamic of actin-dependent mechanisms responsible for cell spreading, remodeling and migration. One of these proteins, WAVE1, belongs to the WASP family of proteins whose members link extra cellular stimuli to actin re-organization and regulate cell migration through the induction of membrane protrusion at the leading edge.11 WAVE1 is directly involved the Arp2/3-mediated actin polymerization and mediates membrane ruffling induced by activated Rac1.12 Since these membrane ruffles were shown to be crucial for cell migration, as they induce degradation of the extra-cellular matrix,13 we have studied the regulation of WAVE1 functions by ArgBP2. We could show that ArgBP2 controls the activity of WAVE1 by promoting its phosphorylation by c-Abl or its dephosphorylation by another interesting new ArgBP2 partner, the phosphatase PTP-PEST (Figure 1). PTP-PEST plays an important role in the control of cell spreading and motility by regulating the turn-over of focal adhesions.14 Interestingly, we have revealed that ArgBP2 is able to inhibit the Abl-dependent phosphorylation of WAVE1 by promoting its association with the phosphatase PTP-PEST and its subsequent de-phosphorylation.10 We hypothesized that inhibition of cell adhesion and migration could be partly mediated by the ArgBP2/PTP-PEST-dependent control of WAVE1 function in our pancreatic cancer cell model. Convincingly, we observed that in the absence of PTP-PEST, ArgBP2 was unable to inhibit migration of MiaPaCa2 cells.10

Cancer cells use various modes of migration to invade tissues and vessels surrounding the tumor but all types of migration require re-organization of the actin cytoskeleton.15 Thus, therapeutic strategies targeting the actin cytoskeleton should have a great impact on the survival of cancer patients by reducing the dissemination of cancer cells.16 ArgBP2 having a central role in the regulation of the actin cytoskeleton reorganization, the modulation of ArgBP2-dependent pathway could represent one of these strategies to limit cancer cell motility. Our data suggest that targeting the ArgBP2/WAVE1/PTP-PEST signaling pathway could be effective in the pancreatic cancer treatment by limiting the invasive and metastatic potential of cancer cells.10 The study of the increasing number of ArgBP2 partners playing a role in cell motility will lead to a better understanding of the mechanisms of ArgBP2-dependent control of cell motility and may reveal new targets for pancreatic cancer therapies as well as perhaps for other types of cancer.

**CAP and Vinexin are also Regulators of Cell Motility**

The two other members of the SoHo family, CAP and Vinexin, have their tissue distribution highly similar to those of ArgBP2.2 Studies of Vinexin suggested that, like ArgBP2, this protein is an important regulator of cytoskeletal organization and of cell adhesion. Vinexin, first isolated as a vinculin binding protein, localizes at inter-cellular junctions and at focal adhesion sites where it is recruited by vinculin.17 Transient expression of Vinexin induces actin cytoskeletal rearrangement in fibroblasts, and promotes cell spreading in myoblasts.17 Vinexin is involved not only in the regulation of cell adhesion/cytoskeletal organization, but also in the regulation of signal transduction. Vinexin interacts with the extracellular signal-regulated kinases (ERK) and c-Raf, an upstream activator of ERK. Phosphorylation of Vinexin by extracellular signal-regulated kinase (ERK) regulates cell spreading, migration and anchorage-independant growth.18 It has been shown recently that, like ArgBP2, Vinexin is also a substrate for c-Abl kinase and that both proteins colocalize at membrane ruffles in rat astrocytes,19 confirming that proteins from SoHo family shares overlapping functions.

CAP is well known to be one of the major players regulating the insulin signaling pathway. CAP enhances insulin-induced phosphorylation of Cbl and recruits Cbl to a lipid raft domain. The recruitment of the CAP/Cbl complex to lipid rafts stimulates the translocation of the insulin-responsive glucose transporter GLUT4 to the plasma membrane.20 Several studies tend to implicate CAP in the control of cell motility. CAP localizes at cell/ECM (Extra Cellular Matrix) and cell/cell adhesion sites where it binds to vinculin, paxillin and afadin.21-23 Exogenous expression of CAP in fibroblasts stimulates formation of actin stress fibers and focal adhesions.21 Overexpression of CAP induces the aggregation of paxillin, vinculin and actin at cell-ECM adhesion sites and inhibits adhesion-dependant processes such as cell spreading and focal adhesion turnover in fibroblasts. Moreover, depletion of CAP by siRNA leads to enhanced cell spreading, migration and the activation of the PAK/MEK/ERK pathway.24 These studies indicate that, similarly to ArgBP2, Vinexin and CAP are important regulators of cell adhesion and motility and therefore, potentially involved in some cancers. The similarities and discrepancies between the three sister proteins have to be further defined in order to better understand how this family of proteins controls cytoskeletal functions.

**SoHo Family of Protein in Oncogenic Processes**

Prior to our findings, little data was available regarding the role of ArgBP2 in oncogenic processes. It has been found fused to MLL in a case of infant M5 acute myeloid leukemia involving 4q35 and 11q23.24 In a Burkitt lymphoma cell line (Elijah), a deleted segment of 251 kb in 4q35.1 between the ARGBP2 and SNX25 genes was identified. As a consequence of this deletion, ArgBP2 lost a C-terminal SH3 domain that may interfere with its binding to its associated proteins.25 A splicing variant of ArgBP2, named ArgBP2gamma was found to interact with Akt and PAK1 (p21-activated protein kinase) thus modulating this cell survival pathway.26 Despite their potential as molecular machines involved in cancer progression, the two other members of the SoHo protein family have been only indirectly and poorly associated with some of these pathologies. For instance, CAP gene has been described to be methylated in a small percentage
(<10%) of testicular germ cell tumors,27 and it has been identified as a new gene induced by p53 in a colon carcinoma cell line model.28 Also, as it has been described for ArgBP2, it seems that these adapter proteins may have distinct functions (sometime opposite) depending on the cellular context or population of their molecular partners. For example, Vinexin was shown to prone survival of prostate cancer cells,29 but to be one of the genes under expressed in imatinib (a tyrosine kinase inhibitor designed to block BCR-Abl activity) resistant chronic myeloid leukemia.30 Then, it is most probable that, like ArgBP2, CAP and Vinexin could be describe, in a near future, as directly involved in the tumoral progression of some cancers.

Concluding Remarks

Elucidating the mechanisms of acquisition of migration properties by cancer cells and establishing therapeutic strategies targeting cell motility are an important challenge in the prevention and treatment of cell invasion and metastasis. Signaling pathways regulated by the SoHo family of adapter proteins constitute a potential target for such therapeutic strategies and further studies are required to elucidate their mechanisms of action.

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