The tumor suppressor gene ARHI (DIRAS3) inhibits ovarian cancer cell migration through multiple mechanisms

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ARHI is an imprinted tumor suppressor gene that is downregulated in > 60% of ovarian cancers, associated with decreased progression-free survival. ARHI encodes a 26 kDa GTPase with homology to Ras. Re-expression of ARHI inhibits ovarian cancer growth, initiates autophagy and induces tumor dormancy. Recent studies have demonstrated that ARHI also plays a particularly important role in ovarian cancer cell migration. Re-expression of ARHI decreases motility of IL-6- and EGF-stimulated SKOv3 and Hey ovarian cancer cells, inhibiting both chemotaxis and haptotaxis. ARHI inhibits cell migration by binding and sequestering STAT3 in the cytoplasm, and preventing STAT3 translocation to the nucleus and localization in focal adhesion complexes. Re-expression of ARHI inhibits FAK Y397 phosphorylation, disrupts focal adhesions and blocks FAK-mediated RhoA signaling, resulting in decreased levels of GTP-RhoA. Re-expression of ARHI disrupts formation of actin stress fibers in a FAK- and RhoA-dependent manner. Recent studies indicate that re-expression of ARHI inhibits expression of β1 integrin which may also contribute to inhibition of migration, adhesion and invasion.

Introduction

ARHI is an imprinted tumor suppressor gene that is downregulated in several types of cancer. Decreased ARHI expression in > 60% of ovarian cancers is associated with shortened progression-free survival.1 ARHI encodes a 26 kDa GTPase with homology to Ras, but exhibits a distinctive 34 amino acid N-terminal extension that sets it apart from other Ras family members.1 Re-expression of ARHI inhibits growth, adhesion, and migration in ovarian cancer cells and induces autophagy and tumor dormancy.2-6 Loss of ARHI-mediated inhibition of cell migration may be particularly important for pathogenesis of the disease. Ovarian cancer cells can metastasize through lymphatics and blood vessels, but most often metastasize over the surface of the peritoneum. We previously reported that re-expression of ARHI inhibits ovarian cancer cell motility by interfering with JAK-STAT3 signal transduction, inhibiting the FAK/RhoA signaling pathway and decreasing formation of focal adhesion complexes and stress fibers.2 In this article, we review these data and explore the possibility that re-expression of ARHI also inhibits ovarian cancer cell movement by downregulating integrins required for migration and adhesion. We provide data to support this hypothesis and suggest potential mechanisms by which ARHI could decrease cancer cell motility by regulating integrin/STAT3 and integrin/FAK signaling.

ARHI in Cell Migration and Adhesion

ARHI decreases β1 integrin expression. Integrins are a family of heterodimeric transmembrane receptors composed of α and β subunits.7 As transmembrane receptors, integrins have extracellular, transmembrane and intracellular domains that link the extracellular matrix (ECM) to the cytoskeleton.8,9 Integrins expressed in tumor cells can contribute to tumor cell migration and adhesion.

Keywords: tumor suppressor gene ARHI, migration suppression, STAT3, RhoA GTPase, cytoskeleton, integrin

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progression and metastasis by increasing tumor cell migration and invasion. Integrin adhesion to the ECM provides the traction required for tumor cell invasion. Additionally, integrins also contribute to tumor cell invasion by regulating the localization and activity of matrix-degrading proteases, such as matrix metalloprotease 2 (MMP2) and urokinase-type plasminogen activator (uPA). Integrin-mediated migration is generally required for focal adhesion kinase (FAK) and Src family kinase signaling. Integrin-specific mechanisms also regulate cell motility. Cancer cell invasion during metastasis is a multi-step process that involves cell adhesion to proteolysis of, and migration through the basement membrane. Integrin β1 has a well-established role in cell adhesion and motility in various types of cancers, including ovarian cancer. In ovarian cancer, overexpression of integrin β1 has been found to be associated with higher clinical stage and poor survival. Upregulation of integrin β1 expression promotes ovarian cancer cell migration and matrix cell invasion. Moreover, the integrin β1 subunit has been shown to mediate ovarian cancer adhesion to peritoneal mesothelial cells and to increase peritoneal metastasis. This notion is supported by evidence that overexpression of integrin β1 enhances the invasive properties of ovarian cancer cells. We have recently found that re-expression of ARHI downregulates the expression of integrin β1 in a time-dependent manner (Fig. 1), suggesting that ARHI may play an important role in regulating the several β1 integrin-mediated contributions to cancer cells growth, migration and invasion.

**ARHI decreases STAT3 translocation to the nucleus and STAT3 participation in focal adhesion complexes.** Constitutive activation of the JAK-STAT pathway has been frequently implicated in cancer and persistent activation of STAT3 is described in many solid tumors, including those of ovarian origin. Recent evidence indicates a role for STAT3 in cell motility. In a significant fraction of ovarian cancers, autocrine stimulation of the secreted IL-6 increases cell proliferation and motility. Non-phosphorylated, activated STAT3 is found primarily in the cytosol, whereas phosphorylated, activated STAT3 translocates to the nucleus to induce gene transcription. Nuclear STAT3 has been found in 70% of ovarian cancers, and is associated with a poor prognosis. Our group has demonstrated that re-expression of ARHI at physiological levels in two different ovarian cancer cell lines inhibited nuclear translocation of STAT3 and suppressed motility. This inhibition relates to physical interaction between ARHI and STAT3, resulting in sequestration of STAT3 in the cytoplasm. In addition, re-expression of ARHI eliminates localization of STAT3 at focal adhesions. In focal adhesions, STAT3 may serve as an adaptor protein in integrin-mediated cell adhesion or could function as a sensor of adhesion, becoming activated in focal adhesion and translocating to the nucleus to alter gene expression in response to cell adhesion. Nuclear translocation of activated STAT3 from focal adhesions may induce critical proteins needed for motility. Integrin β1 has a well-established role in cell growth, adhesion and motility in ovarian cancer. Integrin β1 has also been shown to play an important role in IL-6 mediated STAT3 survival signaling in several different types of cancer. Activation of metalloproteinases (MMP-2 and MMP-10) by IL-6 mediated JAK-STAT3-signaling can enhance growth and invasion of pancreatic, brain and lung cancers. Conversely, one recent report indicated that MMP-2 can complex with integrin α5/β1 to upregulate IL-6 mediated STAT3 phosphorylation and recruitment to cyclin D1 and c-Myc promoters. Thus, integrin α5/β1 could contribute to IL-6-mediated constitutive STAT3 activation by directly interacting with MMP-2.
Metalloproteinases require activation and there are also multiple integrins with the β1 subunit uniquely implicated in ovarian cancer metastases. Given the convergence of integrin β1/STAT3 signaling on common downstream signaling targets and their central effect on metastasis-associated entities and the fact of ARHI that inhibits the expression of integrin β1 and STAT3, we hypothesize that ARHI may inhibit cooperation between integrins and STAT3 in both signal transduction and modulation of cell motility during ovarian cancer progression and metastasis.

**ARHI inhibits FAK/Rho signaling.** FAK is activated by a variety of growth factors receptors and integrins and transmits signals downstream to a variety of target molecules to regulate the cycle of focal contact formation and disassembly required for efficient cell movement. Focal adhesion kinase-1 (FAK) is constitutively associated with β-integrin subunits of integrin receptors. The binding to integrins of components of the ECM leads to activation of FAK. The activation of FAK may be further enhanced by the co-stimulation of growth factor receptors by ECM associated growth factors, such as bFGF, EGF or PDGF. FAK is a non-receptor protein tyrosine kinase (NRPTK) that is typically, but not always, associated with supramolecular focal adhesion (FA) complexes (FAC). Focal adhesion complex assembly and disassembly are critical for cell attachment and movement. FAK does not appear to phosphorylate other proteins, however, when FAK is activated it autophosphorylates and binds Src kinase which in turn phosphorylates other sites on FAK and the FAK-binding proteins, Cas and paxillin.

Phosphorylated FAK becomes a docking site within focal adhesion complexes for mediators of multiple signaling events that regulate cell motility. FAK mediates cell motility and adhesion turnover through regulation of the Rho GTPases, especially RhoA, Rac-1 and Cdc42. FAK affects the downregulation of stress fiber formation by activating RhoA activity and upregulates the formation of lamellipodia by activating Rac-1 via a Cas-Crk-DOCK-ELMO complex. Thus, FAK acts as an integrator of cell motility-associated signaling events. Growth factors stimulate cell motility by inducing the phosphorylation of FAK.

Previously we reported that the expression of ARHI inhibited FAKY397 phosphorylation and decreased EGF-induced chemotaxis of SKOv3-ARHI cells indicating that ARHI regulates cell migration by reducing FAK-mediated RhoA activation. Despite these data strongly implicating ARHI inhibition of FAK as a major signaling pathway leading to decreased cell migration, the inhibition of the cooperative effect between integrins and FAK during cell migration is unexplored. Nevertheless, it is attractive to speculate that ARHI inhibition of integrin expression may reduce phosphorylation of FAK and the phosphor-FAK mediated downstream signaling pathway.

**ARHI can inhibit cell migration through several mechanisms.** As re-expression of ARHI downregulates the expression of integrin β1 and FAK, it is possible that ARHI may also regulate cell adhesion. STAT3 has been shown to play a critical role in cell migration through both transcriptional and non-transcriptional mechanisms. The critical target genes transcriptionally regulated by STAT3 include integrin, FN and MMP. Thus, the loss of ARHI-induced blockade of STAT3 signaling may be one critical mechanism regulating motility and invasion, not only in ovarian cancer, but in breast, lung, prostate and pancreatic cancer where ARHI is also downregulated. The engagement of integrin with extracellular matrix proteins results in the regulation of the small GTPase. In addition, the activity of the small GTPases including RhoA regulate the dynamic organization of the actin cytoskeleton, which determines cell morphology and regulates cell migration. Re-expression of ARHI and subsequent downregulation of integrin β1 may significantly decrease the activity of RhoA. In conclusion, ARHI regulation of integrin β1 expression and STAT3 translocation are likely to provide key mechanisms for inhibiting ovarian cancer cell migration.

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