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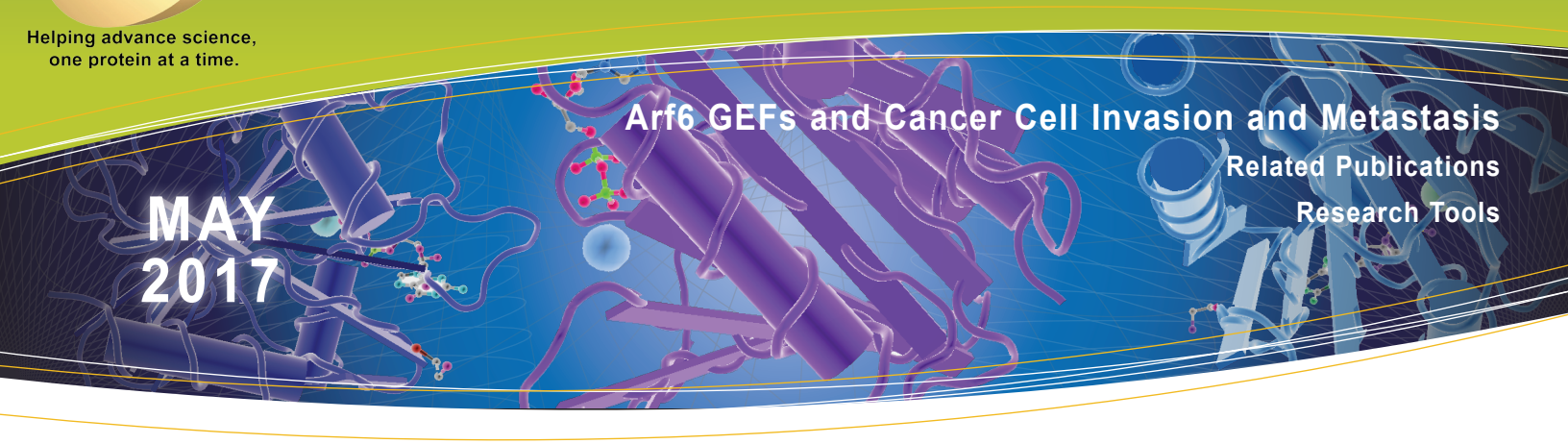

Arf6 GEFs and Cancer Cell Invasion and Metastasis

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Arf6 GEFs and Cancer Cell Invasion and Metastasis

Introduction

The small GTPase ADP-ribosylation factor 6 (Arf6) belongs to the Arf subfamily of Ras superfamily GTPases. Of the three classes of Arf GTPases, Arf6 is the only member of class III and uniquely localizes to the plasma membrane and endosomes, positioning it to regulate cellular processes dependent upon dynamic changes in the actin cytoskeleton, including endocytosis, exocytosis, trafficking/recycling of membrane-localized proteins, and membrane protrusions (e.g., ruffles). These cellular functions underlie physiological and pathological cell motility and intracellular trafficking. Arf6 cycles between an inactive, GDP-bound state and an active, GTP-bound state to act as a molecular switch in the cellular processes listed above. Activation of Arf6 by exchange of GDP for GTP is mediated by guanine exchange factors (GEFs) while inactivation by GTP hydrolysis is mediated by GTPase activating proteins (GAPs)[1-3]. In this newsletter, we discuss the mechanistic roles Arf6 and its GEFs have in cancer cell invasion and metastasis.

Arf6 GEFs and Cancer

Cancer cell invasion and metastasis require dynamic changes in the actin cytoskeleton. Recent work demonstrates that Arf6 has an essential role in tumor angiogenesis and growth, as well as cancer cell invasion and metastasis[4-8]. Moreover, Arf6 GEFs such as BRAG2/GEP100, cytohesin3/Grp1, and EFA6 are also involved in cancer progression[5,7,8]. Prevention of Arf6 activity/cycling inhibits cancer progression[6,7,9].

Of the fourteen known Arf GEFs, eight target Arf6, and of those, five are Arf6-specific. Arf6 GEFs are activated directly through binding with the pleckstrin homology (PH) domain and recruited to the plasma membrane by binding to phosphoinositides produced by phosphatidylinositol 3-kinase (PI3K), as well as a variety of ligand-activated cell surface receptors. There the GEFs' catalytic Sec7 domain activates membrane-localized Arf6. BRAG2/GEP100 and EFA6A-D activate only Arf6 while cytohesin1, cytohesin2/ARNO, and cytohesin3/Grp1 target Arf6, among other Arfs[1-3].

GEP100, the best-characterized Arf6 GEF, promotes the invasion of breast cancer, melanoma, and lung adenocarcinoma cells[5,6,10,11]. In response to epidermal growth factor (EGF) stimulation, GEP100 directly binds the ligand-occupied EGF receptor (EGFR) and activates Arf6 at the plasma membrane[5] (Fig. 1). In turn, GTP-bound Arf6 recruits and activates AMAP1, a downstream effector, which mediates invadopodia formation, the initial step in cancer cell invasion[10]. Activated Her2, human

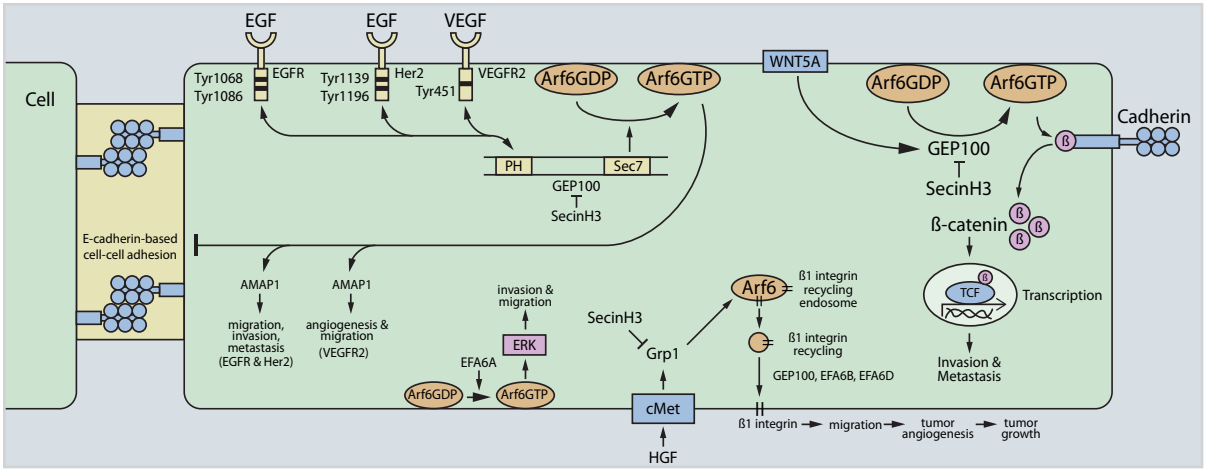

Figure 1. Signaling pathways of Arf6 GEFs and cancer cell invasion and metastasis and tumor angiogenesis.

News

Publications

Research Tools

Continued from Page 1

epidermal growth factor 2, also stimulates GEP100-mediated Arf6 activation, which positively regulates cancer cell invasion[11]. The vascular endothelial growth factor (VEGF) also activates Arf6 via GEP100, stimulating angiogenesis in endothelial cells[12]. GEP100-mediated activation of Arf6 also stimulates cancer cell invasion and metastasis via disassembly of E-cadherin-mediated cell-cell adhesion[10] (Fig. 1). In addition, the Wnt family member 5A (WNT5A) also activates Arf6 through GEP100 in melanoma cells. WNT5A-mediated activation of Arf6 through GEP100 disassembles the β-catenin/cadherin complex. Unbound β-catenin promotes tumor cell invasion and metastasis through β-catenin-mediated transcription and disruption of cell-to-cell adhesions (i.e., adherens junctions)[6] (Fig. 1). These effects are prevented in the presence of SecinH3, a small-molecule inhibitor of GEP100 and the cytohesin family of Arf GEFs[6].

Within the EFA6 family of Arf6 GEFs, a positive or negative regulatory effect is possible, depending on the particular EFA6 GEF isoform and type of cancer. For example, EFA6A positively regulates glioma cell invasion[13], while EFA6B antagonizes breast cancer cell invasion[14]. The EFA6A-mediated effects utilize the extracellular signal-regulated kinase (ERK) signaling cascade[13], which complements an earlier finding that Arf6-induced melanoma cell invasion requires ERK signaling[15]. Complicating matters is the capacity of EFA6A, B, and C isoforms to positively regulate cancer cell invasion, metastasis, and drug resistance[8].

Grp1-mediated activation of Arf6 regulates hepatocyte growth factor (HGF)-dependent tumor angiogenesis via an Arf6-stimulated increase in β1 integrin recycling from the endosomes to the plasma membrane[7] (Fig. 1). β1 integrin recycling to the plasma membrane is necessary for anchoring of cancer cells to allow maturation of newly formed blood vessels. SecinH3 inhibits these effects[7]. Grp1 has also been reported to control breast cancer cell migration[16].

Clinical Significance: Small molecule inhibitors of Arf6

Inhibition of cytohesins and GEP100 is accomplished with the small-molecule Arf GEF inhibitor SecinH3, as discussed above. In animal models, SecinH3 inhibits glioma cell metastasis and angiogenesis of melanoma and lung carcinoma tumors[6,7]; however, treated animals develop hepatic insulin resistance[17]. Another molecule is PIT-1 which inhibits phosphatidylinositol-3,4,5-trisphosphate (PIP3), the lipid product of PI3K and regulator of the Akt cell survival and growth signaling pathway. PIT-1 prevents PIP3 binding to the PH domains of ARNO and Grp1 which results in inhibition of lamellipodia formation and breast cancer cell migration[1,2,16]. In addition, melanoma tumor angiogenesis and metastasis are inhibited. Additional work is required to differentiate the contributions of ARNO and Grp1 to tumor invasion/metastasis and determine if one or both GEFs are targeted in PIT-1's anti-cancer effects.

Conclusions

Recent studies of the Arf6 GTPase and its GEFs demonstrate that these proteins have a clear role in cancer cell invasion and metastasis and tumor angiogenesis. Moreover, some findings suggest that specific cancers are associated with certain GEFs. This observation offers the possibility of very selective, novel anti-cancer treatments targeting Arf6 and/or its GEFs. To assist scientists in these studies, Cytoskeleton Inc., offers Arf6 activation assay kits along with kits for many other Ras superfamily GTPases. The activation assays come in two convenient, easy-to-use formats, the effector bead pull-down assay and the ELISA-based G-LISA assay. In addition, we offer GEF and GAP exchange assay kits and reagents for monitoring changes in F-actin in fixed and live cells. To learn more about these and other tools for measuring GTPase-mediated, dynamic changes in the cytoskeleton, please contact one of our technical support scientists at tservice@cytoskeleton.com.

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Arf Activation Assay Kits

Pull-down or G-LISA	Reactions	Cat. #
Arf1 Activation Assay Biochem Kit	20 assay	BK032-S
Arf6 Activation Assay Biochem Kit	20 assays	BK033-S
G-LISA Arf1 Activation Assay Biochem Kit (colorimetric)	96 assays	BK132
G-LISA Arf6 Activation Assay Biochem Kit (colorimetric)	96 assays	BK133

F-actin Visualization Reagents

Live Cell or Acti-stain Phalloidin	Amount	Cat. #
Spirochrome SiR-Actin Kit	50 nmol	CY-SC001
Spirochrome SiR700-Actin Kit	35 nmol	CY-SC013
Acti-stain™ 488 Phalloidin	300 slides	PHDG1
Acti-stain™ 555 Phalloidin	300 slides	PHDH1
Phalloidin (rhodamine)	500 ul	PHDR1

Other Live Cell Imaging Probes

Probe	Reactions	Cat. #
Spirochrome SiR-DNA Kit	50 nmol	CY-SC007
Spirochrome SiR-Lysosome Kit	50 nmol	CY-SC012