GEP100 was expressed in more than 80% of invasive ductal carcinomas (n = 32), and in about 60% of ductal carcinomas in situ (n = 70) in which GEP100 was preferentially coexpressed with EGFR in their malignant cases.
Conclusion: We conclude that GEP100 links EGFR signaling to Arf6 activation to induce invasion and metastasis of some breast cancer cells. Since Arf6 is not overexpressed in non-invasive breast cancer cells as well as in normal mammary epithelial cells, this EGFR-GEP100-Arf6 pathway appears to constitute a signaling specifically used in some breast cancer cells for their invasion and metastasis. Our results reveal an aspect of the precise molecular mechanism of cancer invasion and metastasis, in which full invasiveness is not acquired just by alternations of cancer cells themselves, but their microenvironments or EGF may also play pivotal roles.

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Endothelin A receptor/beta-arrestin signaling is critical for ovarian cancer metastasis: novel molecular therapeutic applications

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Metastatic relapses remain a major challenge in the management of ovarian cancer. In this tumor, activation of the endothelin A receptor (ET_AR) by endothelin-1 (ET-1) promotes epithelial to mesenchymal transition (EMT), a metastatic early event. In search of downstream mediators in ET-1-induced EMT, we focused on β-arrestin, as an adaptor protein of G-protein coupled receptors. Here, we identify a new mechanism whereby β-arrestin is a novel interaction partner of ET_AR to transactivate the epidermal growth factor receptor (EGFR), forming a trimeric signaling complex with c-Src. Z-stack analyses of HEY cells by confocal microscopy together with immunoprecipitation and Western blotting analysis revealed that ET-1 induced the membrane translocation of β-arrestin, facilitating c-Src activation and causing the assembly of ET_AR/β-arrestin/c-Src signaling complex ('signalplex'). By expressing wild-type or mutant S412D-β-arrestin-1, which contains a point mutation at Ser412 that mimics the phosphorylated form causing a loss of c-Src binding, we showed that this signalplex was crucial for EGFR transactivation, which, in turn, controlled β-catenin stabilization by affecting its tyrosine (Y) phosphorylation. The Y-phospho β-catenin translocated to the nucleus and bound the TCF4 transcription factor, thus representing a transcriptional active pool. At the functional level, β-arrestin siRNA inhibited β-catenin/TCF4 transcriptional activity and cell invasion, delineating previously unknown biological functions of b-arrestin in EMT-related signaling. ZD4054, a specific ET_AR antagonist, prevented the engagement of β-arrestin in the interplay between the ET_AR and EGFR pathways in invasive signaling. In an intraperitoneal metastasis model of ovarian cancer, ZD4054 treatment significantly inhibited tumor burden and metastatic nodules, which were maximally impaired by combination of ZD4054 with gefitinib, an EGFR inhibitor. Interestingly, HEY cells that express the S412D-β-arrestin-1 mutant metastasized at a reduced rate, highlighting the importance of β-arrestin-mediated EGFR signaling in metastasis formation. Our results demonstrate that β-arrestin links the ET-1 axis to β-catenin signaling, indicating that new therapeutic opportunities for ovarian cancer may require combined regimens targeting the ET_AR and EGFR. Supported by AIRC, Ministero della Salute and AstraZeneca.

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PI3K/Akt pathway regulates Shh/Gli-mediated EMT and invasion of gastric cancer cells

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Background: It is known that the activation of Sonic hedgehog (Shh) signaling is involved in the progression and invasion of various tumors, including gastric carcinoma. Epithelial-mesenchymal transition (EMT) is a complex process that converts epithelia into migratory mesenchymal cells. Generally, increased motility and invasion are positively correlated with EMT. In this study, we investigated the impact of phosphoinositide 3-kinase (PI3K)/Akt pathway on the Shh/Gli-mediated EMT and invasion of gastric cancer cells.
Material and Methods: The proliferation, migration, and invasion of gastric cancer cells in response to Shh N-terminal peptide (N-Shh) for various times were investigated using MTT, wound healing, and Matrigel invasion assay, respectively. The morphologic changes of gastric cancer cells through the EMT process were monitored by immunofluorescence staining and Western blot assay for EMT markers E-cadherin and Slug. To investigate the functional relationship between Shh/Gli-induced EMT and PI3K/Akt pathway, we performed these assays using cells either transfected with constitutively active AktMyr or kinase-dead Akt (AktK179M) or treated with LY294002.
Results: We found that stimulation of N-Shh in gastric cancer cells enhanced cellular motility and invasiveness and induced a full EMT process characterized by Snail induction, E-cadherin down-regulation, and up-regulation of mesenchymal and invasiveness markers. Meanwhile, blockade of Shh/Gli signaling by KAAD-Cyclopamine (a Shh signaling inhibitor), anti-Shh neutralizing antibodies, or Gli siRNA also restored these changes of EMT markers and inhibited N-Shh-induced invasiveness of gastric cancer cells. The phosphorylation of Akt was also enhanced by treatment with N-Shh, but not KAAD-cyclopamine, anti-Shh neutralizing antibodies, or Gli siRNA. The cells transfected with constitutively active AktMyr enhanced Shh/Gli-induced EMT and invasiveness by treatment with N-Shh. However, blockade of the Akt kinase using kinase-dead Akt, Akt siRNA, or LY294002 in the presence of N-Shh significantly inhibited the Shh-induced EMT and invasiveness. Immunohistochemistry on gastric tumor biopsies showed that the levels of Gli, E-cadherin, and phosph-Akt expression were enhanced in cases of metastatic gastric cancer than in cases of primary gastric cancer. Moreover, the strong correlation between Gli and E-cadherin or phospho-Akt expression was also observed in lymph node metastasis specimens.
Conclusion: These data indicate that Shh/Gli signaling pathway promotes EMT and invasiveness of gastric cancer cells through activation of PI3K/Akt pathway. Additionally, our findings suggest a role and mechanism for Shh/Gli – PI3K/Akt signaling as it relates to EMT and the metastatic potential of gastric cancer, which indicates it has the potential to be a therapeutic molecular target to decrease metastasis.

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PI3K/Akt pathway regulates BMP2-mediated EMT and invasion of gastric cancer cells

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Background: Up-regulation of BMPs and their receptors by tumor is an important hallmark in cancer progression, as it contributes through autocrine and paracrine mechanisms to tumor development, invasion, and metastasis. Generally, increased motility and invasion are positively correlated with epithelial-mesenchymal transition (EMT). Herein, we investigated the involvement of phosphatidylinositol 3-kinase (PI3K)/Akt pathways by BMP-2 stimulation in the modulation of this EMT and invasive process in gastric cancer cells.
Material and Methods: To investigate the effects of BMP2 on proliferation, migration, and invasion of gastric cancer cells, we performed BrdU labeling, wound healing, and Matrigel invasion assays. The morphologic changes and induction of the EMT process by BMP2 stimulation were monitored by immunofluorescence staining and Western blot assay for EMT markers E-cadherin and Snail. To investigate the functional relationship between BMP2-induced EMT and PI3K/Akt pathways, we performed these assays using cells either transfected with constitutively active AktMyr or kinase-dead Akt (AktK179M) or treated with LY294002.
Results: An increased concentration of BMP2 strongly enhanced motility and invasiveness in gastric cancer cells, whereas no increase was observed in cells treated with either Noggin (a BMP2 inhibitor) or BMP2 siRNA. A morphologic change of the BMP2-treated cells from epithelial-like shape to a spindle, fibroblastic-like appearance is accompanied by a decrease or loss of E-cadherin and a gain of Snail. Blocking of BMP2 signaling by Noggin or BMP2 siRNA restored these changes of EMT markers. The phosphorylation of Akt was also suppressed by treatment with BMP2, but not Noggin or BMP2 siRNA. Blockade of the Akt kinase using kinase-dead Akt or LY294002 in the presence of BMP2 significantly enhanced the BMP2-induced EMT and cell motility/invasiveness. However, the cells transfected with AktMyr inhibited BMP2-induced EMT and migration/invasiveness by treatment with BMP2. Immunohistochemistry on gastric tumor biopsies showed that the levels of BMP2 and E-cadherin were enhanced in cases of metastatic gastric cancer than in cases of primary gastric cancer. Moreover, the inverse correlation between BMP2