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

and phospho-Akt expression was also observed in lymph node metastasis specimens.
Conclusion: Overall, our studies suggest that invasion in BMP2-induced EMT is mediated through down-regulation of PI-3 Kinase/Akt pathway.

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Integrin LFA-1 expression regulates angiogenesis-stimulating potential of colorectal carcinoma cells at premetastatic niches in the liver

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The recruitment of vascular stromal and endothelial cells is an early pre-angiogenic event of premetastatic niches, but how the microenvironment created by avascular three-dimensional (3D) growth contributes to activation of the angiogenesis-stimulating potential in cancer cells is unclear. Herein, the proangiogenic profile of CT26 colon carcinoma cells was studied in seven-day cultured 3D-spheroids of <300 μm in diameter, produced by the hanging-drop method to mimic the microenvironment of premetastatic niches prior to hypoxia. Spheroid-derived CT26 cells increased VEGF secretion by 70%, which in turn increased in vitro endothelial cell migration by 2-fold. More importantly, spheroid-derived CT26 cells increased LFA-1-expressing cell fraction by 3-fold, and soluble ICAM-1, given to spheroid-cultured CT26 cells, further increased VEGF secretion by 90% via cyclooxygenase (COX)-2-dependent mechanism. Consistent with these findings, CT26 cancer cells also significantly increased LFA-1 expression at premetastatic niches within hepatic lobules. Angiogenesis also markedly increased in both subcutaneous tumors and hepatic metastases produced by spheroid-derived CT26 cells. Finally, two-dimensional electrophoresis plus mass spectrometry revealed that three-dimensional growth of CT26 cells led to the development of a VEGF-secreting cancer cell subset expressing a markedly proangiogenic protein profile, including 60S acidic ribosomal protein, ferritin heavy chain, phosphoglycerate kinase-1, estrogen-related receptor, vimentin and 14−3-3 epsilon alpha. Therefore, three-dimensional growth of cancer cells enriched the proangiogenic cancer cell phenotype needed for metastasis progression. The role of integrin LFA-1 and COX-2 in the microenvironmental activation of angiogenesis-stimulating potential of colorectal carcinoma cells potentially represents a new target combination for therapeutic strategies to block colorectal hepatic metastasis at premetastatic niches.

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Tumor-induced liver nerve growth factor (NGF): a new target for stromal cell inhibition during metastatic colorectal carcinoma growth

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Besides its contribution to differentiation and survival of neurons, nerve growth factor (NGF) also plays a role in cancer progression. In the liver, expression of NGF is increased during tissue regeneration and hepatocellular carcinoma development, but its role during hepatic metastasis is not well understood. Herein, we investigated NGF and neurotrophin receptor expression by cancer and host cells in the hepatic metastasis microenvironment of murine and human systems. NGF immunostaining of metastatic colon cancer cells only occurred in 2 out of 24 patients with hepatic metastases, while around 80% of studied patients had hepatic metastases with NGF-expressing stromal cells. Not statistically significant correlation was demonstrated between NGF immunostaining of tumor-infiltrated stromal cells and cancer cell staining with anti-ki67 antibodies, suggesting that NGF was not involved in cancer cell proliferation. Hepatocytes and hepatic sinusoidal cells showed weak NGF immunostaining, while cholangiocytes had a high immunostaining in the hepatic tissue unaffected by cancer. Hepatic CT26 murine colorectal carcinoma metastases had an intense NGF immunostaining in those hepatocytes and myofibroblast-type stromal cells located at the invasion front of metastases. High NGF-expressing hepatocytes were preferentially located among cancer cells and had phenotypic features suggesting epithelial-to-mesenchymal transition. CT26 cancer cells did neither express in situ nor secrete in vitro NFG. p75-NTR had a low expression level in normal hepatic tissue, but it significantly increased in hepatocytes and HSCs located around and within hepatic metastases, while CT26 cancer cells were negative. Consistent with these in situ findings, NGF significantly increased by 3-fold in the hepatic blood obtained from livers affected by CT26 colorectal carcinoma metastases. NGF concentration was also 7 times higher in the supernatants from primary cultured HSCs than in those from hepatocytes, and it significantly increased in the supernatant of HSCs given C26 cancer cell-conditioned medium, and in those from cultured hepatocytes given tumor-activated HSC-conditioned medium. Recombinant murine NGF dose-dependent increased chemotactic migration, but not proliferation, of HSCs and some cancer cell lines in vitro. Moreover, HSC migration-stimulating activity induced by VEGF was NGF-dependent in vitro. Our results demonstrate for first time that hepatocytes and sinusoidal stellate cells express neutrotrophin receptor p75 and secrete NGF in response to specific stimulating factors released by cancer cell in the hepatic metastasis microenvironment of human and rodent colorectal carcinoma. Tumor-induced liver NGF contributed to intratumor stromal cell recruitment and potentially represents a promising target for tumor-activated stromal cells during metastatic colorectal carcinoma growth.

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Arf6-AMAP1 pathway in invasion of lung cancer and malignant mesothelioma cell lines

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Backgrounds: Distant metastses are the major problem in cancer therapeutics. For most carcinomas, metastases begin with invasion of cancer cells into the basement membrane or the stromal environment. We have shown that a small GTPase Arf6 and its effector AMAP1 play pivotal roles in invasion and metastasis of significant populations of breast cancers. It has been well documented that lung cancers show even more invasive and metastatic tendency clinically than breast cancers.
Tumor cells, including those of epithelial origin, exhibit two distinct phenotypes for their invasion, namely mesenchymal type and amoeboid-like type. The former requires activities of matrix proteases and calpain, while the latter ROCK, a Rho-dependent kinase. Here we examine whether Arf6 and AMAP1 are involved in invasive activities of lung cancer cells and mesothelioma cells, together with analysis on what types of invasiveness each of these cells exhibits.
Materials and Methods: Non-small cell lung cancer cell lines (H1299, Lu99, H460, A549, PC9, PC14, H1650, H441, H522, H1975 and H520 (and malignant mesothelioma cell lines (211H, H2052 and H28) were used.
To examine types of the invasiveness, we used ALLN (a calpain inhibitor) and Y27632 (a ROCK inhibitor). We also used a cocktail of protease inhibitors, which contains GM6001 (a multi metalloprotease inhibitor), E-64 (a cystein inhibitor), pepstatin A, leupeptin, and aprotinin. We performed a matrigel chemoinvasion assay to measure invasive activities, using Biocoat Matrigel chambers (Becton Dickinson). Protein knock-down was done by the siRNA technique using RNAiMAX (Invitrogen). Cell viability was measured using Cell Countin Kit-8 (Dojindo Molecular Technologies).
Results: Six of 14 cell lines we examined (H1299, Lu99, PC9, PC14, 211H and H2052) showed appreciable matrigel invasive activities in vitro, while other three cell lines (H460, A549 and H1650) also exhibit less but detectable levels of invasive activities. Among the former 6 cell lines, we found that siRNA-mediated knockdown of Arf6 and AMAP1 both significantly inhibits invasion of H1299, Lu99, PC14 and 211H. On the other hand, AMAP1 knockdown, but not Arf6 knockdown, inhibited invasion of PC9 and H2052. We moreover found that H1299, Lu99, PC9 and H2052 exhibit the typical mesenchymal-type of invasion, while PC14 and 211H are not.
Conclusions: Consistent with previous studies, our results also suggest that invasive phenotypes are highly diversified among different lung cancer cells and mesothelioma cells. Still then, one can consider Arf6 and/or AMAP1 as molecular targets for the adjuvant therapy of some lung cancers and mesotheliomas.

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Epigenetic changes of tumor suppressor genes and therapeutic implications in glioblastoma

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Background: Glioblastoma is the most common and aggressive type of primary brain tumor and there have been little improvements of its poor survival rate during the last decades. Aberrant DNA methylation, including