the presence of foci in multiple organs was assessed using fluorescence microscopy. Incidence of metastasis decreased in the ovary and uterine horn, while incidence in all other organs was unaffected by CTCE-9908 treatment, regardless of injection site or treatment. Lung metastases from tail vein injections decreased only marginally by about 30% with CTCE-9908 treatment. After intracardiac injection, the number and size of the foci decreased in most organs with treatment. The number of foci per femur increased upon treatment with the CXCR4 inhibitor, but the size of foci was greatly decreased. The large metastases in the untreated animals likely obscured the small foci observed in the CTCE-9908 treated animals. Foci in the lung and heart were significantly decreased in number and size after CTCE-9908 treatment. Decreases in the number of foci, although not significant, were also noted in the liver, ribs, kidneys, pancreas and spleen. While treatment with CTCE-9908 did not decrease the incidence of metastasis as hypothesized, it decreased the metastatic burden in all organs examined. Animal survival was not measured but an increase in survival could be predicted as a result of the overall decrease in disease burden. The possible mechanisms of this decrease include changes in apoptosis, proliferation and angiogenesis as well as 'homing' of cells to the secondary sites. All of these will require further examination to understand fully the effect of CTCE-9908 on breast cancer metastasis. Preliminary results from a Phase I/II clinical trial with CTCE-9908 was presented in 2007. Final results are expected to be presented this year.

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Novel therapeutic efficacy of E7080 for controlling experimental metastases of human lung cancer cells in natural killer cell-depleted severe combined immunodeficient mice

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Background: Lung cancer is often characterized by rapid growth and metastatic spread. Because tumor growth and metastases are angiogenesis dependent, there is great interest in therapeutic strategies that aim to inhibit tumor angiogenesis.
Materials and Methods: The therapeutic efficacy of E7080, an orally available multiple tyrosine kinase inhibitor which inhibits VEGFR1−3, FGFR1−4, PDGFRs, RET etc., was examined in experimental multiple-organ metastasis models with human lung cancer cell lines (SBC-5, H1048 and PC14PE6) in natural killer cell-depleted severe combined immunodeficient mice.
Results: E7080 did not inhibit the proliferation of three human lung cancer cell lines (IC50 >1 microM), whereas it inhibited that of human microvascular endothelial cells induced by VEGF (IC50 0.3 nM) and bFGF (IC50 100 nM) in vitro. The large, medium and few amounts of VEGF were detected in the culture supernatant of PC14PE6, SBC-5 and H1048 cells, respectively. Intravenously inoculated human small cell lung cancer SBC-5 cells produced experimental metastases in the liver, lung, and bone on day 28, whereas H1048 cells produced the metastases in the liver, systemic lymph nodes, kidneys and bone on day 56. Human adenocarcinoma PC14PE6 cells yielded massive pleural effusion and lung metastases 28-days after intravenous inoculation. Daily treatment with E7080 (1, 3 and 10 mg/kg), started on day 14 (after the establishment of micrometastases), significantly reduced the amount of pleural effusion and the number of large (>2 mm) metastatic colonies (in the liver, lymph nodes and the lungs) and osteolytic bone lesions. E7080 treatment did not significantly reduce the number of small (<2 mm) metastatic lesions found in the lungs (SBC-5) or kidneys (H1048), consistent with an antiangiogenic mechanism of action. No significant adverse events of E7080 treatment, such as body weight loss were observed in these in vivo experiments. Histochemical analysis of metastatic deposits in the liver showed conspicuous necrosis, indicating that E7080 treatment inhibited angiogenesis in vivo.
Conclusions: These results suggest that E7080 may be of potential therapeutic value in inhibiting the growth of metastatic lung cancer in humans.

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Inhibitors of mitochondrial ATP synthesis show preferential cytotoxicity to pancreatic cancer cells under glucose-deprived conditions

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Background: The tumor microenvironment exerts an important influence on cancer progression. Because of the disorganized vascular systems in tumors, large areas of tissues are exposed to nutrient starvation and hypoxic conditions. Even under these severe growth conditions, certain cancers, such as a pancreatic cancer, which is characterized as hypovascular tumors, show an inherent ability to tolerate such severe conditions. Since chronic deprivation of nutrients seldom occurs in normal tissue, targeting nutrient-deprived cancer cells might be a promising strategy for the development of anticancer agents. The purpose of our study is to identify cytotoxic agents that function preferentially under nutrient-deprived conditions.
Materials and Methods: Human pancreatic cancer PANC-1 cells were cultured in nutrient-rich and nutrient-limited media. The cell survival was determined by the MTT method.
Results: Through screening cultured media of microorganisms and chemical compounds, we found that the NADH-ubiquinone reductase (complex I) inhibitor rotenone, the succinate-ubiquinone reductase (complex II) inhibitor atpenin A5, the ubiquinone-cytochrome c (complex III) inhibitor antimycin A3 and the F1F0-ATPase inhibitor (complex V) oligomycin exhibited preferential cytotoxicity to PANC-1 cells under nutrient-deprived conditions, but exhibited minimal cytotoxicity under nutrient-rich conditions. These compounds preferentially caused cell death under glucose-limiting condition, irrespective of the presence or absence of amino acids and/or serum. Although PANC-1 cells survived nutrient starvation even after 24 h, the intracellular ATP concentrations were markedly decreased. Therefore, inhibitors of mitochondrial ATP synthesis could exert preferential cytotoxicity under nutrient-deprived conditions.
Conclusions: These data indicate that inhibitors of mitochondrial ATP synthesis show preferential cytotoxicity to human pancreatic cancer PANC-1 cells under nutrient-deprived conditions. Therefore, these inhibitors may be useful for anticancer therapy and microenvironment-oriented therapeutic approaches could be a promising strategy for anticancer therapy.

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The EGFR-GEP100-Arf6 pathway in breast cancer invasion and metastasis

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Background: Expression of epidermal growth factor receptor (EGFR) is highly implicated in tumor malignancy. However, it awaits to be clarified whether there exist signaling pathways downstream of EGFR, that are specifically used for tumor invasion and metastasis though not generally used in normal cells. We have shown previously that a small GTPase Arf6 and its downstream effector AMAP1 are both highly overexpressed in invasive breast cancer cells and plays essential roles for their invasion and metastasis. Here, we identify a mechanism by which Arf6 is activated to induce tumor invasion and metastasis.
Material and Methods: We conducted siRNA-mediated knockdown of ArfGEFs expressed in highly invasive breast cancer MDA-MB-231 cells and examined their effects on their Matrigel chemoinvasion activities, in order to identify candidate GEFs responsible for invasion. Lung metastasis were assessed by use of mouse breast cancer 4T1/luc cells, by injecting them into fadpad of Balb/c mice.
Results: There are 16 genes encoded by human genome, bearing the Sec7 (ArfGEF) domain. We found that MDA-MB-231 cells express 10 different types of ArfGEFs and knockdown of GEP100, but not other ArfGEFs, blocked the Matrigel invasion activity. shRNA-mediated suppression of GEP100 also very effectively blocked invasion and metastasis of 4T1/luc cells in vivo. GEP100, via its PH domain, bound directly to phosphorylated Tyr1068 and Tyr1086 sites of EGFR to activate Arf6. Overexpression of GEP100, together with Arf6, caused non-invasive MCF7 cells to become invasive, which was dependent on EGF stimulation.

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