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

M. Hanibuchi[1], H. Ogino[1], K. Ikuta[1], S. Kakiuchi[1], H. Uehara[2], A. Tsuruoka[3], T. Uenaka[3], Y. Nishioka[1], S. Yano[4], S. Sone[1]. *[1]University of Tokushima, Respiratory Medicine and Rheumatology, Tokushima, Japan; [2]University of Tokushima, Molecular and Environmental Pathology, Tokushima, Japan; [3]Eisai Co. Ltd., Tsukuba Research Laboratories, Tsukuba, Japan; [4]Kanazawa University, Medical Oncology, Kanazawa, Japan*

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

I. Momose[1], D. Tatsuda[1], M. Kawada[1], D. Ikeda[1]. *[1]Microbial Chemistry Research Center, Numazu Bio-Medical Research Institute, Shizuoka, Japan*

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

S. Hashimoto[1], M. Morishige[2], E. Ogawa[3], Y. Toda[4], H. Kotani[5], M. Hirose[6], A. Hashimoto[1], Y. Nio[7], H. Wada[3], H. Sabe[1]. *[1]Osaka Bioscience Institute, Molecular Biology, Osaka, Japan; [2]School of Medicine Oita University, Neurosurgery, Oita, Japan; [3]Faculty of Medicine Kyoto University, Thoracic Surgery, Kyoto, Japan; [4]Graduate School of Medicine Kyoto University, Center for Anatomical Studies, Kyoto, Japan; [5]Kyoto University Hospital, Diagnostic Pathology, Kyoto, Japan; [6]Institute for Protein Research Osaka University, Supramolecular Crystallography, Osaka, Japan; [7]Kodama Breast Clinic, Diagnostic Pathology, Kyoto, Japan*

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.