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Amphiregulin contributes to tumor progression in lung adenocarcinoma

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Background: Amphiregulin(AREG), a ligand of EGFR, is associated with shortened survival of patients with NSCLC and poor prognosis. However, the ability of amphiregulin to mediate distinct function in lung metastasis remains unknown.
Material and Methods: PC9 cells (human pulmonary adenocarcinoma cell line harboring EGFR exon 19 deletion) were stably transfected with a vector harboring AREG cDNA and colonies were selected. AREG protein was measured by ELISA analysis. Anoikis was induced in P03 (cell transfected with AREG-expressing clones) and PA3 cell lines(cell transfected with the empty vector) with the use of poly-HEMA. Apoptosis assay was performed by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The cells were cultured in soft agar to evaluate anchorage-independent colony formation. P03 or PA3 cell lines were injected intravenously into BALB/c nude mice. 6 weeks later, lungs were excised, and the number of nodules formed on all lobe surfaces was counted to assess the importance of AREG as mediator of lung colonization. Immunohistochemistry for COX-2, CD31, VEGF-D, MMP-1, $P21^{WAF1/Cipl}$ were performed on lung cancer tissues. Vessel number and vessel size were quantified with the computer program ImageJ. Five to six independent tumors were analysed and at least three sections per tumor were quantified.
Results: Greater anoikis resistance was associated with higher levels of AREG expression.PA3 cell line resulted in a larger and more colonies in a cologenic assay. The expressions of CD31, VEGF-D, MMP-1, $P21^{WAF1/Cipl}$ were increased in AREG transfected lung cancer tissues. Not only the tumour size was increased, but also the vessel density and the vessel size were increased.
Conclusions: AREG may contribute to its metastatic capacity and tumor angiogenesis, providing a possible explanation for the aggressive nature of lung cancer that overexpress AREG.

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Design of improved calixarene-based anti-tumor agents

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Galectin-1 (gal-1), which binds β-galactoside groups on various cell surface receptors, is crucial to cell adhesion and migration, and is found to be elevated on endothelial cells and stroma in several cancers. Previously, we identified gal-1 as the target for the designer peptide anginex, a potent antiangiogenic and anti-tumor agent. Here, we report on the development of new agents that target gal-1 and thereby inhibit tumor growth in mice. Since small molecules have pharmacological advantages over therapeutic peptides, we designed non-peptide mimetics to approximate the molecular dimensions, amphipathicity, and cationic topology of the β-sheet-folded anginex peptide. Since calix[4]arene mimics the overall backbone dimensions of the active entity within anginex, we chose calix[4]arene as the scaffold. Earlier, we reported on calixarene-based compounds 0118 and 1097 that are potent anti-angiogenic and anti-tumor agents (Dings RPM, et al. JNCI 2006;98(13):932−6). Using a structure-based design approach, we chemically modified the hydrophobic and hydrophilic faces of these amphipathic anginex mimetics, which resulted in the discovery of new compounds that possess *in vitro* and *in vivo* activities that are greatly improved over 0118 or 1097. In the syngeneic B16F10 melanoma tumor mouse model, the best new compound inhibits tumor growth about 20-fold better than e.g. 0118. Overall, this research contributes to the design of peptidomimetics as novel therapeutics that inhibit tumor growth, potentially in the clinical setting.
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Serum levels of placental growth factor correlate with survival in patients with neuroendocrine tumors of the pancreas

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Background: Genetic and pharmacological studies have recently established the proangiogenic role of placental growth factor (PlGF), a VEGF homolog, and its receptor Flt1 in tumor angiogenesis. Dense vascularization is a characteristic of neuroendocrine tumors of the pancreas (pNET) and correlates with an unfavorable course of disease. Here, we evaluated the role of PlGF and its soluble receptor sFlt1 as prognostic parameters and potential therapeutic targets in pNET.
Methods: PlGF and sFlt1 serum levels in 101 patients with histological proof of pNET and 96 healthy control subjects were determined using the Roche Elecsys® assay and correlated with clinicopathologic parameters, including tumor grading and patients' survival. Immunhistochemical studies further allowed to determine the expression of PlGF in tumor specimens of 30 patients with pNET compared to healthy pancreatic control tissues.
Results: As compared to healthy controls, serum levels of PlGF and sFlt1 were found to be significantly elevated in patients with pNET (19,3 vs 12,9 pg/ml and 83,6 vs 72,4 pg/ml; $P < 0{,}0001$). Moreover, compared to patients with PlGF levels below median, PlGF levels above median were associated with poor prognosis and reduction of tumor related survival (log-rank test, $P = 0{,}041$), thus indicating that PlGF serum levels represent a prognostic marker in pNET disease. Accordingly, we observed a significant correlation of PlGF serum levels with the grade of tumor differentiation based on Ki-67 labeling. Finally, in line with elevated PlGF serum levels, immunhistochemical studies revealed an increased tumor PlGF expression in human specimens of pNET as compared to healthy pancreatic control tissues (28% vs. 3%; $P < 0{,}05$).
Conclusions: These data provide first evidence that PlGF represents an independent prognostic parameter in patients with pNET and most probably plays a pivotal role in the tumor biology of pNET. Thus, further studies are warranted to evaluate the potential of PlGF as a target molecule to optimize therapeutic strategies and to improve the prognosis of this tumor entity.

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INK128, a potent and selective TORC1/2 inhibitor, demonstrates anti-tumor activity in preclinical models of renal cell carcinoma by a distinct mechanism

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Background: Renal cell carcinoma (RCC) is characterized by mutations or silencing of the von Hippel–Lindau gene leading to an accumulation of HIF1alpha (HIF1a), a key mediator of hypoxia-triggered neo-angiogenesis. The mammalian target of rapamycin (mTOR) is upstream of HIF1a and downstream of the VEGF pathway. mTOR kinase operates in two distinct intra-cellular multi-protein complexes, TORC1 and TORC2, that together regulate growth, metabolism, angiogenesis and survival by integrating nutrient and hormonal environmental signals. Pharmaceutical derivatives of rapamycin, a partial allosteric inhibitor of TORC1, provide clinical proof of concept for the therapeutic value of targeting mTOR in RCC, but also provided insights into how mechanistically novel TORC1/2 inhibitors might demonstrate greater efficacy. Through rational drug design we have discovered INK128, a potent and selective TORC1/2 inhibitor with excellent drug-like properties. We investigated the mechanism of action, pharamcokinetc pharmacodynamic (PK/PD) correlation and efficacy of INK128 in preclinical *in vitro* and *in vivo* models of RCC.
Methods: mTOR and PI3K-isoform kinase IC_{50}'s were generated in a homogeneous time-resolved fluorescence (FRET) screen using commercially available reagents from Invitrogen (mTOR) and Millipore (PI3K isoforms). A range of tumor cells were treated with various concentrations of INK128 for 2 hours and subsequently lysed in cell lysis buffer. Lysates were subjected to SDS-PAGE followed by Western blot analysis to detect downstream signaling markers. Cell proliferation was performed using the CellTiter-Glo® Luminescent Cell Viability Assay Kit (Promega). Anti-tumor activity of INK128 was assessed in mouse xenograft models. INK128 was administered orally as a solution at doses of 0.3 mg/kg (QD), 1 mg/kg (QD), and 3 mg/kg (Q2D). Tumors were extracted at 2 hours post last dose (n = 3/time point). Pathway inhibition was determined by Western blot analysis of tumor lysates and immunohistochemistry (IHC) analysis of formalin fixed paraffin embedded tumor tissue.
Results: *In vitro*, INK128 inhibits phosphorylation of S6 and 4EBP1, downstream substrates of TORC1, as well as NDRG1 and AKT,