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Regulation of matrix metalloproteinase 3 by tumor-associated mutant E-cadherin variants

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Background: Tumor progression is characterized by loss of cell adhesion and increase of invasion and metastasis. The cell adhesion molecule E-cadherin is frequently down-regulated or mutated in tumors. In addition to down-regulation of cell adhesion, degradation of the extracellular matrix by matrix metalloproteinases is necessary for tumor cell spread. To investigate a possible link between E-cadherin and matrix metalloproteinase 3 (MMP-3), we examined expression of MMP-3 in human MDA-MB-435S cells stably transfected with wild-type (*wt*) or three different tumor-associated mutant E-cadherin variants with alterations in exons 8 or 9, originally identified in gastric carcinoma patients.
Materials and Methods: MMP-3 expression in lysates and supernatants of MDA-MB-435S transfectants was determined by Western blot analysis. The effect of NNGH or MMP-3 specific siRNA on cell motility was assayed by time lapse laser scanning microscopy.
Results: In the presence of *wt* E-cadherin, the MMP-3 protein level was decreased in cellular lysates and in the supernatant where a secreted form of the protein is detectable. Down-regulation of MMP-3 was not found in MDA-MB-435S transfectants expressing mutant E-cadherin variants which indicates that E-cadherin mutations interfere with the MMP-3 suppressing function of E-cadherin. We have previously found that cell motility is enhanced by expression of the mutant E-cadherin variants used in this study. Here, we found that application of the synthetic inhibitor of MMP-3 NNGH and small interfering RNA (siRNA) directed against MMP-3 reduce mutant E-cadherin-enhanced cell motility.
Conclusions: Taken together, our results point to a functional link between MMP-3 and E-cadherin. MMP-3 is differentially regulated by expression of *wt* or mutant E-cadherin. On the other hand, MMP-3 plays a role in the enhancement of cell motility by mutant E-cadherin. Both observations may be highly relevant for tumor progression and metastasis since they concern degradation of the extracellular matrix and tumor cell spread.

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Clinical significance of insulin-like growth factor-binding protein-3 expression in advanced gastric cancer

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Insulin-like growth factor (IGF) family proteins play a pivotal role in regulating cell growth and apoptosis in normal and tumor tissues, and their m itogenic and antiapoptotic activities are mainly modulated by a family of high-affinity insulin-like growth factor binding proteins (IGFBPs). Among them, IGFBP-3 is the major binding protein of IGFs and modulates the bioactivity of IGFs. Even though it is well known that IGFBP-3 plays an important role in cell proliferation, the expression of IGFBP-3 and its significance in advanced gastric cancer (AGC) samples are unknown. This study explored IGFBP-3 expression in tumor samples using tissue microarray technique from 227 patients to determine if the expression status of IGFBP-3 influences the prognosis of patients with AGC who underwent curative resection followed by adjuvant chemoradiation therapy (Park et. al., *Ann Oncol* 14:1373–7, 2003). Two-sided statistical analyses were performed to correlate the clinical parameters and the prognostic effect with the IGFBP-3 expression level in this cohort. Reduced IGFBP-3 expression was found in 121 (53.3%) of 227 samples, and it was more frequent in diffuse type than in intestinal type (P=0.01). However, down-expression of IGFBP-3 was not associated with the other clinicopathological parameters tested, such as age, sex, histology, Bormann type, tumor location and staging. In a univariate analysis, patients with decreased IGFBP-3 expression had shorter overall (P=0.03 by log-rank test) and disease free (P=0.05 by log-rank test) survival rates than did patients with normal IGFBP-3 expression. In subgroup analysis, s ignificant statistical correlation between IGFBP-3 expression and disease-specific survival was noted on ly in patients with stage I or II disease. This study demonstrates that reduced expression of IGFBP-3 is a frequent event in advanced gastric cancer and correlates with the disease-specific survival probability of patients, especially patients with stage I or II disease. These results suggest that IGFBP-3 functions as a tumor suppressor and plays an important role in determining biological aggressiveness especially in early stage AGC. Thus, IGFBP-3 is a good target to develop the new treatment strategies for AGC.

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Potent antiproliferative effects of Src kinase inhibition in a model of neuropeptide-induced androgen-independent prostate cancer

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Background: Cells with neuroendocrine (NE) differentiation are found in cancer of the prostate (CaP) and may facilitate the transition to androgen independence by supplying alternate growth factors. Androgen deprivation further induces a subpopulation of androgen-independent NE cells by transdifferentiation. Androgen receptor (AR)- and Src kinase-mediated signalling may participate in this NE differentiation.
Materials and Methods: The neuropeptide gastrin-releasing peptide (GRP) was minimally expressed in androgen-sensitive LNCaP cells. LNCaP-GRP clones demonstrated androgen- and anchorage-independent growth, developed orthotopic tumours in castrated nude mice and activated prostate-specific antigen (PSA) with nuclear localisation of AR. Mouse xenografts were recultured (LNCaP-Pro) and grown in androgen-free soft agar or tissue culture either alone, with synthetic androgen R1881 or with inhibitors including: GRP monoclonal antibody 2A11, 10 μM antiandrogen bicalutamide (bic), Src kinase inhibitors AZM475271, AZD0530 (AstraZeneca, 5 μM) and PP2 (EMD Biosciences, 10 μM).
Results: Growth of LNCaP-GRP cells in castrated mice with activation of PSA and nuclear localisation of AR suggested GRP activation of AR. Androgen-depleted soft agar colony counts are presented. Compared with controls LNCaP-Pro colony formation was not stimulated by R1881. Incubation of LNCaP-Pro cells with GRP 2A11 antibody or bic caused partial inhibition of colony formation; however, when both were used in combination, significant growth reduction ($p<0.05$) resulted compared with control. R1881 mostly reversed the GRP 2A11 inhibition of colony formation. The Src kinase inhibitors AZM475271 and PP2 showed greatest colony formation inhibition ($p<0.05$) [Figure]. To date, AZD0530 has only been tested *in vitro*, but has shown significant growth inhibition compared with untreated or bic-treated cells.
Conclusions: Our model of neuropeptide-mediated androgen-independent CaP growth is dependent on both GRP and AR. Importantly, we find a role for Src kinase inhibition in this model, which may have therapeutic implications for patients with androgen-independent CaP.
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Reduction of in vitro metastatic potential of tamoxifen-resistant breast cancer cells following inhibition of Src kinase activity by AZD0530

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Background: Src kinase plays a central role in many growth factor signalling pathways regulating a diverse array of cellular functions including proliferation, metastasis, invasion and cell survival. Recent studies have demonstrated that Src activity is frequently elevated in human tumours and appears to correlate with disease stage. We have previously shown that, upon acquisition of tamoxifen resistance, MCF7 cells display increased levels of EGFR and a more aggressive phenotype *in vitro*. Since tumours with elevated EGFR signalling have been reported to have elevated levels of Src activity, we investigated the expression and role of Src in our MCF7 tumour model of endocrine resistance (Tam-R cells).
Materials and Methods: Src expression was measured by RT-PCR and Western blotting; Src activity was determined by Western blotting using a phospho-specific Src antibody. To obtain the optical density (OD), Western blots were scanned and analysed using a densitometer. Modulation of Src activity in Tam-R cells was achieved using the potent and selective Src kinase inhibitor AZD0530 (AstraZeneca). Cellular invasion was assessed by seeding 5×10^4 cells onto a Matrigel-coated porous membrane in the presence or absence of 1 μM AZD0530. After 3 days, cells that had migrated to the underside of the membrane were fixed, stained with DAPI and counted using a fluorescence microscope. Cell migration was determined by seeding cells onto a fibronectin-coated, porous surface and allowing migration to occur for 24 h. Migrated cells were then visualised by staining with crystal violet and counted. Changes in FAK activity were determined by Western blotting following treatment of cells with or without AZD0530.