Background: Previously we showed that oncogene Ha-Ras stimulates the metastatic activity of both spontaneously transformed and v-src-transformed Syrian hamster fibroblasts in vivo. We revealed that Ha-Ras/RalGDS/Ral-associated signalling pathway was the most important for metastasis among the variety of Ha-Ras associated signaling cascades.
Methods: Retroviral infection, molecular cloning, spontaneous metastatic activity, gelatine zymography, RT-PCR, cell viability assay, peroxide decomposition activity assay.
Results: We showed that both RalA and RalB stimulate the metastatic potential of cells, but RalB is less efficient than RalA. To understand what Ral interacting proteins contribute to this phenomenon we compared the level of metastatic potential of the cell lines expressing RalA and RalB effecter domain mutants. The search revealed that among the three best-studied Ral downstream partners RalA-PLD1 and RalB-RalBP1/RalB-Sec5 interactions were essential. The introduction of active form of RalA (RalA+ cells), unlike RalB, downregulates the secretion of MMP1, MMP2 and MMP9 in comparison to parental cells, although the cocultivation of RalA+ cells with hamster embryo fibroblasts leaded to stimulation of MMPs secretion suggesting that RalA+ cells can it in stromal cells. The highly metastatic cells acquired the peroxide resistance as well as peroxide decomposition activity and this viability may serve as a protection against the host immune system.
Conclusion: RalB can stimulate metastatic ability of transformed cells, but less than RalA. These two closely-related proteins probably stimulate metastasis through distinct downstream signaling pathways. MMPs secretion is involved in Ral-dependent metastasis, but RalA and RalB utilize different cellular mechanisms.

doi:10.1016/j.ejcsup.2006.04.073

P14. ADAM-17: A MEDIATOR OF BREAST CANCER PROGRESSION?

Patricia McGowan[a], Brid Ryan[a], Arnold Hill[b], Enda McDermott[b], Niall O'Higgins[b], Michael J. Duffy[a,b]. [a]*UCD School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Dublin 4, Ireland;* [b]*Education and Research Centre, St. Vincent's University Hospital, Dublin 4, Ireland.*

Background: The ADAMs are a family of transmembrane, multidomain proteins, involved in both cellular adhesion and proteolysis. Multiple studies from model systems suggest that specific ADAMs are involved in cancer progression. The aim of this investigation was to see if ADAM-17 was involved in the progression of human breast cancer.
Materials, Methods and Results: ADAM-17 protein was measured by both Western blotting and ELISA. ADAM-17 protein was found to exist in two main forms in human breast tissue, a 120 kDa precursor and 100 kDa active protein. Both forms of ADAM-17 protein were found to be upregulated in primary breast carcinomas compared to normal breast tissue (Mann–Whitney *U*-test: $p = 0.005$, $p = 0.0003$, respectively). The ratio of 100 kDa protein to 120 kDa protein increased with disease progression from normal breast tissue to axillary node metastases (Kruskal–Wallis statistical test: $p = 0.002$), indicating an increase in processing from precursor to active protein in malignant breast tissue. A moderate, but significant correlation was found between the 100 kDa form of ADAM-17 (measured by Western blotting) and ADAM-17 levels determined by ELISA (Spearman Rank: $p = 0.0006$, $r = 0.405$). No significant relationship was found between the 120 kDa protein form and levels measured by ELISA. ADAM-17 protein levels, as measured by ELISA, were significantly higher in grade 3 tumours as compared to tumours classed as grades 1 and 2 (Mann–Whitney *U*-test: $p = 0.03$), and in node-positive compared to node-negative cancers (Mann–Whitney *U*-test: $p = 0.04$). Furthermore, both forms of ADAM-17 correlated with cell proliferation, as measured by PCNA (Spearman Rank: $r = 0.524$, $p < 0.0001$; $r = 0.365$, $p = 0.002$, respectively) and with metastatic potential, as measured by uPA (Spearman Rank: $r = 0.246$, $p = 0.032$; $r = 0.428$, $p = 0.0001$, respectively).
Conclusion: We conclude that ADAM-17 protein exists in two main forms in breast cancer. Furthermore, our results suggest that ADAM-17 is involved in breast cancer progression.

doi:10.1016/j.ejcsup.2006.04.074

P15. PROFILING OF NEOPLASMS OF THE PANCREAS USING MICROARRAY-TECHNOLOGY

Andrea Bauer[a], Melanie Bier[a], Martin Wirtz[b], Jörg Kleeff[b], Helmut Friess[b], Jörg D. Hoheisel[a]. [a]*Division of Functional Genome Analysis, Deutsches Krebsforschungszentrum, Heidelberg, Germany;* [b]*Department of General Surgery, University of Heidelberg, Germany.*

Background: Pancreatic cancer is the fifth most frequent cause of cancer-related deaths in industrialized countries. The diagnosis of ductal adenocarcinoma of the pancreas is associated with a poor prognosis, an increasing incidence and no or only ineffective means of treatment. Cystic pancreatic neoplasms account only approximately 5% of primary malignancies of the pancreas and may be benign, pre-malignant or malignant. In this study, we are developing and evaluating a DNA-diagnostic-chip with about 3500 human genes known to be differentially transcribed in pancreatic cancer cells and thus expected to show a representative expression pattern in both ductal adenocarcinoma and cystic lesions.
Methods: cDNAs representing different human genes were PCR-amplified, purified and robotically arrayed onto slides with an epoxy surface. Fluorescently labelled cDNA samples were prepared from total RNA isolated from cells of human pancreas tissue by incorporation of labelled dCTPs during reverse transcription.
Results: Microarray experiments on various samples are being performed and analysed allowing classification of different types of pancreas lesions and the identification of potential targets as a means of eventually developing new modes of treatment.
Conclusion: The resulting diagnostic DNA-chip will be of significant clinical utility to detect cancer cells in tissues from patients with different types of pancreatic carcinoma and to draw prognostic conclusions based on their molecular appearance. Furthermore, comparative studies on transcriptional profiling and actual protein expression by means of complex DNA- and antibody microarrays are under way.

doi:10.1016/j.ejcsup.2006.04.075