death. The genomic characteristics of CTC and DTC have to be considered in order to better understand the biological properties and refine the clinical implications of these cells.
Material and Methods: CTC or DTC from metastatic breast cancer patients were identified by immunocytochemical (ICC) staining followed by light microscopy evaluation. Single cells were isolated by micromanipulation or micro dissection, including verification of the single cell end product. The DNA from each single cell was amplified by whole genome amplification (WGA) and the amplified product was applied to Agilent 44k or 244k Comparative Genomic Hybridization arrays.
Results: We adapted and established methods for micromanipulation, amplification and Single Cell array CGH (SCaCGH) at different sites.
Reproducibility and sensitivity of the methods were tested by analyzing the breast cancer cell line SKBR3. Genomic profiles of the different CTC and DTC were compared in relation to each other and among the patients. Our preliminary results show that genomic profiles of DTC/CTC present common breast cancer tumour aberrations, like gains and deletions at chromosome 1, 8, 11 and 17. Further, the profiles exhibit a high degree of concordance within the same patients and discordance among different patients.
Conclusions: Genomic analysis of single tumour cells is possible through micromanipulation, amplification and SCaCGH. This provides us with a powerful tool for in-depth studies of CTC and DTC in localized breast cancer, for identification of aberrations relevant for their metastatic potential and/or therapeutic susceptibility.
Acknowledgements: This work has received research funding by the Communities Sixth Framework programme as part of the international DISMAL collaboration for research into disseminated epithelial malignancies and The Research Council of Norway.

368 The molecular switch NHERF1 induces tumour phenotypic changes associated with distinct metastatic organotropism in breast cancer via its PDZ domains

M.R. Greco[1], N. Rucci[2], M. Capulli[2], A. Teti[2], G. Busco[1], V. Casavola[1], E. Antelmi[1], S.J. Reshkin[1], R.A. Cardone[1]. *[1]University of Bari, Department of General and Environmental Physiology, Bari, Italy, [2]University of l'Aquila, Department of Experimental Medicine, l'Aquila, Italy*

Background: The mechanisms allowing disseminated cancer cells to colonize specific targets organs (organotropism) are unknown. However, organotropism is thought to emerge via acquisition of distinct sets of cellular capabilities, which are controlled by finely regulated signal transduction modules and result in specific tumour phenotypes. The PDZ domain-containing scaffolding protein NHERF1 recruits signaling protein partners and directs proteins to specific cellular locations thus regulating a range cellular behaviors. Moreover, NHERF1 is overexpressed in human breast cancer and its overexpresssion is associated with aggressive clinical characteristics and poor prognosis.
Material and Methods: To gain insight into the mechanisms and role of NHERF1 in tumour phenotypes and metastasis, we stably transfected a metastatic breast cell line, MDA-MB-231, with the pcDNA 3.1/Higro empty vector, with wildtype (wt) NHERF1 or with NHERF1 mutated in either the PDZ1 (HRF1) or PDZ2 (HRF2) domains and tested these clones both *in vitro* for their biological activities of invasion, migration, anchorage independent growth, invadopodia/podosome activation program, vasculogenic mimicry and angiogenic properties and *in vivo* for their tumourigenic ability and metastatic organotropism.
Results: Anchorage-independent growth and *in vivo* xenograft formation are reduced in wt-NHERF1 and HRF2-NHERF1 and increased in HRF1-NHERF1 with respect to pcDNA 3.1. Only HRF2-NHERF1 inhibited invadopodium formation and ECM degradation while podosomes, vasculogenic mimicry and neoangiogenesis were inhibited in wt-NHERF1 and HRF1-NHERF1. Intracardiac injection of BALB/c-nu/nu mice demonstrated that HRF1-NHERF1 produced visceral metastases and HRF2-NHERF1 favored bone metastasis. Therefore, NHERF1 overexpression can either (i) via its PDZ1 domain reduce tumour growth but increase *in vivo* osteotropism by inducing podosomes and vasculogenic mimicry in the tumour cells and neoangiogenesis in endothelial cells or (ii) via its PDZ2 domain promote invasion and *in vivo* visceral metastases by inducing invadopodia formation and ECM proteolysis.
Conclusions: We conclude that NHERF1 can differently reprogram the neoplastic phenotype and metastatic organotrophism by specific alteration of its PDZ domain function.

369 Dihydroartemisinin is a hypoxia active anticancer drug

T. Ontikatze[1], R. Handrick[1], F. Grimm[1], G. Henke[2], P.T. Daniel[3], C. Belka[2], V. Jendrossek[1]. *[1]University Hospital Duisburg Essen, Cell Biology, Tumour Research, Essen, Germany, [2]University of Tübingen, Radiation Oncology, Tübingen, Germany, [3]Charité, Universitätsmedizin Berlin, Hematology Oncology and Tumour Immunology, Berlin, Germany*

Background: Major aim of cancer therapy is the eradication of clonogenic tumour cells. Unfortunately, the microenvironment of solid tumours is mostly characterized by regions of acute or chronic hypoxia which are known to decrease tumour cell sensitivity to cell death induction by classical genotoxic treatments. Here, we propose a novel strategy to overcome therapy resistance of tumour cells under acute hypoxia by using the radical-forming endoperoxide Dihydroartemisinin (DHA). Aim of the present study was to evaluate the antineoplastic potential of DHA in colon carcinoma cells focusing on the role of hypoxia for its cytotoxic effects.
Methods: Sensitivity of colon cancer cells to cell death induction by DHA (12.5−50 μ?) was analyzed by fluorescence microscopy (cytochrome c release and Hoechst33342/propidium iodide-staining, PI), flow cytometry ($\Delta\psi$m, DNA fragmentation) and immunoblotting (caspase-activation, PARP-cleavage). To investigate the molecular mechanisms under normoxia (21% O_2) and hypoxia (0.2% O_2), HCT116 wild type (wt) and subclones with defined defects in apoptosis signalling (Bax−/−, Bak−/− or Bax/Bak−/−) were used. Clonogenic death was tested by colony formation assays. In addition, HCT116 wt xenograft experiments were performed with NMRI nu/nu mice.
Results: DHA induced concentration-dependent apoptosis in colon cancer cells under normoxic conditions. HCT116 wt cells were also highly sensitive to DHA-induced apoptosis under conditions of strong hypoxia (0.2% O_2), although absolute apoptosis levels were decreased compared to normoxia. Loss of Bax, Bak or Bax and Bak largely decreased DHA-induced apoptosis in normoxia and hypoxia. However, eradication of clonogenic tumour cells was only reduced in the Bax, Bak, or Bax/Bak-deficient HCT116 cells when treatment was performed under normoxic conditions; in contrast, the response of the different HCT116 subclones in strong hypoxia was almost similar. Finally, our preliminary data indicate *in vivo* activity of DHA in a HCT116 wt xenograft model.
Conclusions: DHA efficiently induces apoptosis in colon cancer cells under normoxic and hypoxic conditions in a Bax and Bak dependent manner. In contrast, loss of these two main effectors of apoptosis execution affected DHA-induced eradication of clonogenic tumour cells only in normoxia. Our findings suggest that DHA may be of particular value for the treatment of human solid tumours characterized by high levels of tissue hypoxia and apoptosis resistance.

370 The metastasis-promoting protein, S100A4, regulates mammary branching morphogenesis

K. Andersen[1], H. Mori[2], J.E. Fata[3], T. Oyjord[1], G.M. Malandsmo[1], M.J. Bissell[2]. *[1]Norwegian Radium Hospital, Tumour Biology, Oslo, Norway, [2]Lawrence Berkeley Laboratory, Life Sciences Division, Berkeley, USA, [3]City University of New York, Department of Biology, New York, USA*

High levels of the S100 calcium binding protein A4 (S100A4), also called fibroblast specific protein 1 (FSP1), has been established as an inducer of metastasis and indicator of poor prognosis in breast cancer. The mechanism by which S100A4 leads to increased cancer aggressiveness has yet to be established; moreover, the function of this protein in normal mammary gland biology has not been investigated. To address the role of S100A4 in normal mammary gland, its spatial and temporal expression patterns and possible function in branching morphogenesis were investigated. We show that the protein is expressed mainly in cells of the stromal compartment during active ductal development, in pregnancy and in involution. In 3D culture models, topical addition of S1004 induced significant increase of the branching phenotype and a concomitant increase in expression of a previously identified branching morphogen, metalloproteinase-3 (MMP-3). These events were found to be dependent on MEK activation. Down-regulation of S100A4, using shRNA, significantly reduced TGFa induced branching and altered E-cadherin localization. These findings provide evidence that S100A4 is developmentally regulated and that it plays a functional role in mammary gland development by activating MMP-3, and in concert with MMP-3, acts as a morphogen required for invasion into the fat pad during branching. We suggest that S100A4-mediated effects during branching morphogenesis provide a plausible mechanism for how it may function in breast cancer progression.

371 Differences in the stroma of human ovarian carcinoma xenografts endowed with different angiogenic phenotypes

A. Silini[1], G. Chiorino[2], C. Ghilardi[1], R. Dahse[3], R.B. Pedley[4], R. Giavazzi[1], M.R. Bani[1]. *[1]Mario Negri Institute for Pharmacological Research, Oncology, Milan, Italy, [2]Fondo Edo Tempia, Laboratory of Cancer Genomics, Biella, Italy, [3]HELIOS Clinics Institute of Pathology, Pathology, Erfurt, Germany, [4]University College London Cancer Institute, Tumour Biology, London, United Kingdom*

Background: A fundamental characteristic of malignant cancers is the ability to surmount environmental controls by the host and induce changes in the neighboring tissue to favor local tumour growth, invasion, metastatic spreading, and perhaps contribute to drug response.
The over-expression of vascular endothelial growth factor (VEGF) is associated to poor prognosis and malignant progression. Importantly, VEGF, a major