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Yeast-2-Hybrid screen identifies Rab-interacting proteins as interacting prey targets for the OPCML tumour suppressor, highlighting a general theme of membrane trafficking

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To identify proteins which interact with the OPCML ovarian cancer tumour suppressor (1), we undertook a yeast-2-hybrid (Y2H) screen using the Hybrigenics custom screening facility.

A highly representative brain library containing 10 million independent clones were screened to saturation thanks to their Cell-to-Cell mating protocol. This protocol allows for testing an average of 97 million interactions per screen ensuring the exhaustivity of the screen and likelihood of identifying rare interactions (from the rarest transcripts). The depth of the screening procedure substantially impacts on the false-negative rate. Hybrigenics' bioinformatic analysis offers a comprehensive coverage and reproducibility rate of 90% (2). A common theme associated with Y2H screens is the occurrence of false-positive interactions. However, the high reproducibility of the Hybrigenics screening strategy allows the development of a systematic statistical approach in assigning the confidence score to each clone; the Predicted Biological Score (PBS).

The Hybrigenics screen identified 71 clones interacting the extracellular domains of OPCML as bait which included multiple, interacting clones for 3 known proteins given a high PBS confidence score and 9 single cDNA clones for known proteins of moderate PBS score. The two highest scoring candidate interacting proteins (RIM2 and Synaptotagmin1 (SYT1)) were found to be involved in the same functional process namely Ca2+-dependent vesicle fusion. Three further candidates from the lower PBS values involved in this process were also identified and thus a theme appears of Ca2+- dependent membrane trafficking between cellular compartments; a process known to be important for Receptor Tyrosine Kinase (RTK) signal regulation. Thus, from the Hybrigenics bioinformatics we have a supporting evidence that OPCML may interact with at least 2 Rab-interacting proteins involved in membrane trafficking with RIM2 having the potential to cluster other signaling molecules to membrane locations. Emerging evidence implicates alterations of the Rab proteins and their associated regulatory proteins and effectors in multiple human diseases including ovarian and breast cancer.

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Sanguinarine sensitizes tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant gastric cancer cells though downregulation of Akt and activation caspase-3

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Sanguinarine, a benzophenanthridine alkaloid derived from the root of Sanguinaria canadendid, has been shown to possess anti-proliferative, anti-inflammatory, and anti-oxidant properties. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anticancer agent that induces apoptosis in multiple tumor cell types while sparing most normal cells. However, the cytotoxic effect of the TRAIL limited in some cancer cells, including AGS gastric adenocarcinoma cells. In the present study, we suggested that sanguinarine exerted a synergistic effect leading to apoptosis rates unachievable by incubation with TRAIL alone. Combined treatment with sanguinarine and TRAIL significantly enhanced apoptosis and the apoptosis induction was associated with up-regulation of pro-apoptotic Bax and down-regulation of anti-apoptotic Bcl-2, loss of mitochondrial membrane potential (MMP), activation of caspase-3, degradation of poly-(ADP-ribose) polymerase(PARP) and β-catenin. However, combined treatment-induced apoptosis were significantly inhibited by z-DEVD-fmk, a caspase-3 specific inhibitor, indicating the important role of caspase-3 in the observed cytotoxic effect. In addition, pre-treatment of LY294002, a PI3K/Akt inhibitor, significantly increased combined treatment-induced apoptosis in AGS cells. The present results indicate that caspase-3 is a key regulator of apoptosis in response to combined sanguinarine and TRAIL in human gastric adenocarcinoma AGS cells through inactivation of Akt and mitochondrial dysfunction. Furthermore, the use of TRAIL in combination with subtoxic doses of sanguinarine may provide an effective therapeutic strategy for safely treating some resistant gastric cancer cells.

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Prognostic factors in multiple myeloma

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Introduction: Multiple myeloma is a malignant neoplasm originating in plasma cells. It is frequently associated with poor prognosis and characterized by the production of a monoclonal immunoglobulin called the M component. Multiple myeloma (MM) is connected to a numer of chromosomal abnormalities, in most cases IgH translocation with FGFR3, CCND1, CCND2, CCND3, c-MAF in early stages. Further chromosomal changes appear with the progression of the disease the most frequent of which are monoallelic deletion or monosomy of chromosome 13, trisomies of chromosome 8, 9, 15 and many others. It has also been revealed that some proteins controlling cell the cycle and apoptosis (p53, p16, FGFR3, cyclin D 1, 2, 3, Bcl-2, caspase 3, 8, 9) seem to play an important role during MM pathogenesis and progression. However, there are no reliable data on their prognostic significance in the various stages of disease (monoclonal gammapathy of uncertain significance – MGUS, smouldering MM and advanced MM). Therefore the aim of this pilot study was analysis of expression of these proteins in various stages of MM and potentially extend the panel of prognostic markers which allows differentiation of the above-mentioned disease stages.

Materials and methods: Bone marrow from 35 patients treated by the same chemotherapy protocol (VAD) and autologous transplantation were used. Standard indirect immunohistochemistry on formalin fixed, paraffin-embedded sections was used for the detection of p53, p16, FGFR3, cyclin D 1, 2, 3, Bcl-2, caspase 3, 8, 9 using a high temperature epitope retrieval technique. Immunohistochemical staining was evaluated by a semi-quantitative method using a histoscore which is the multiplication of positivity by intensity of staining. Intensity of staining was scored as weak (1), moderate (2) or strong (3) while positivity of staining was assessed as percentage of tumour cells.

Results: Bone marrow samples of patients in advanced stages of MM showed high expression of Bcl-2 in tumor cells, in contrast to those in remission which showed weak or no positivity for Bcl-2. p53 and p16 were completely negative. Caspase 8 was negative in most cases. However, we detected a few caspase 8 positive cells in patients in remission. Caspase 9 was negative in biopsies taken before treatment but we detected several caspase 9 positive cells in patients after treatment.

Conclusions: Based on decreased BCl-2 expression in patients in remission, these preliminary data suggest that detection of low BCl-2 expression in bioptic samples of MM might be used as positive prognostic factor. Negativity of p16 can be explained by hypermethylation which leads to p16 deactivation. Mutation of p53 is probably infrequent in multiple myeloma but the mechanism of its impaired function needs further study.

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Up-regulation of thymosin β4 is a determinant of the transformed phenotype and invasiveness of mouse fibrosarcoma cells

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Background: Understanding the mechanisms of tumor cell invasion is essential to prevent cancer deaths. S-adenosylmethionine decarboxylase (AdoMetDC), a key enzyme in the synthesis of polyamines, induces transformation of murine fibroblasts upon over-expression. As these transformed cells form highly invasive fibrosarcomas in nude mice, they provide a valuable model to study cell invasion. Materials and methods: We analyzed by DNA microarrays the transformation-related gene expression changes in AdoMetDC-transformed cell lines in comparison to normal NIH3T3 cells, and further studied the roles of the identified genes, in two- and three-dimensional cell cultures (collagen I and Matrigel), using antisense RNA expression, siRNAs, and a sponge toxin latrunculin A. Results: We found marked up-regulation of thymosin β4 to be the most prominent change in these invasive fibrosarcoma cells, and it was further confirmed at the protein level. Thymosin β4 is a major actin sequestering protein, forming 1:1 complex with monomeric actin. Interestingly, we found a sponge toxin latrunculin A, which inhibits the binding of thymosin β4 to actin, to profoundly affect the morphology and proliferation of the AdoMetDC-transformants and to block their invasion/migration in three-dimensional Matrigel. In addition, we detected the up-regulation of thymosin β4 also in ras-transformed mouse fibroblasts and metastatic human melanoma cells. Conclusions: Elevated thymosin β4-expression appears to be related to increased tumorigenicity and metastatic potential, consistent with other studies on fibrosarcoma, melanoma, and colon carcinoma cells. Our results encourage testing latrunculin A-like and other