648 Molecular characterization of breast cancer subtypes derived from joint analysis of high throughput miRNA and mRNA data

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Background: Deregulation of micro-RNAs (miRNAs) has been increasingly implicated in cancer. Several miRNAs have aberrant expression profiles in breast cancer and the expression of some has been correlated to specific clinical features of breast cancer. miRNA dependent regulation is mediated through changes in mRNA levels and function, and miRNA/mRNA interaction in the context of breast cancer highlights clinically relevant pathways. To search for such interactions we performed a comprehensive joint analysis of whole genome miRNA and mRNA data.
Material and Methods: We have performed expression profiling of 799 miRNAs along with genome-wide matched mRNA profiling in a cohort of 101 human primary breast tumour samples with extensive clinical information. The miRNA/mRNA interdependencies were examined using correlation and statistical enrichment methods. Profiling was performed using microarrays and validated by qPCR. The effect of miRNAs on proliferation was validated using high-throughput transfection arrays in breast cancer cell lines.
Results: Statistically significant differentially expressed miRNAs were found in examining different molecular subtypes, extracellular matrix (ECM) classes, TP53 mutation status, proliferation status, and survival. Taking a systems biology approach to study the relationship between miRNA and mRNAs, we identified several cellular processes, such as proliferation, cell adhesion and immune response, which were significantly enriched among genes with strong negative or positive correlation to the expression pattern of certain miRNAs or groups of miRNAs (miRNA-GO association networks). Functional validation assays identified 13 miRNAs that affect proliferation in both tumours and cell lines. Furthermore a group of miRNAs associated to disease free survival was identified.
Conclusions: We introduce a dataset of mRNA and miRNA expression profiles measured in a well studied patient cohort. We show that miRNAs can distinctly differentiate between tumour subtypes, various clinical subclassifications and survival characteristics. In addition, we present functional validation linking some miRNAs to proliferation. Finally, we show that miRNAs can act as reliable proxies to the activity of known biological processes related to breast cancer progression such as cell-cycle, immune response and cell adhesion.

649 Regulation of p53 and cell proliferation by the nucleolar factors

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Background: The nucleolus, one of the subnuclear organelles, has been regarded as a factory of ribosome biogenesis. But, recent works revealed that the nucleolus also plays roles in various cellular events, including regulation of the cell cycle, DNA repair, apoptosis and so on. It is also reported that some nucleolar proteins are involved in the regulation of tumour suppressor, p53 that is a key factor of checkpoint control and apoptosis.
We focused on the regulation of the cell cycle and apoptosis by nucleolar proteins in cancer cells. To identify the proteins involved in these processes, we treated human breast cancer cells with an siRNA library for nucleolar proteins.
Materials and Methods: We screened for nucleolar proteins involved in cell cycle and cell death using a siRNA library. Briefly, MCF-7 cells, the breast cancer cell line, were treated with siRNA (stealth RNA, invitrogen) for about 400 kinds of nucleolar proteins. Then we examined cell proliferation by MTT assay, cell cycle distribution by flow cytometry (Guava cell cycle Reagent, Millipore), and apoptosis by TUNEL assay (DeadEnd Fluorometric TUNEL system, Promega).
Result and Conclusion: We identified several nucleolar proteins whose knockdown suppressed cell growth and induced cell death. Among them, we focused on NOL1/NOP2/Sun domain family, member 1 (NOL1) that is known as a marker of cellular proliferation. We found that NOL1 knockdown induced G1 arrest and apoptosis. Given that p53 regulates G1 checkpoint and apoptosis, we examined the p53 expression level. As expected, we found that NOL1 knockdown resulted in the increase of p53 protein, followed by the enhancement of its target gene products, Cdk-inhibitor, p21 and proapoptotic factor, PUMA. These phenomena were not observed in p53 deficient cells, indicating that the effect of NOL1 knockdown depends on the p53 pathway.
In addition, we found that there were many nucleolar factors, knockdown of which resulted in the accumulation of p53. We have also analyzed the p53 modification, expression level of p53 downstream genes, and cellular phenotypes by the knockdown. Furthermore, we are trying to classify these factors into several categories according to the modification status of p53 and/or cellular phenotypes, which are then compared with the reported functions of the nucleolar factors. We believe that this study will provide a new insight into the relationship between nucleolar functions and p53 regulation mechanisms.

650 Use of the blood based, 96-assay set for breast cancer detection

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Background: Increased survival from breast cancer is achieved by increasing the number of cancers detected at an early stage. Approximately one third of breast cancers in the US are detected at stage 2 or later. Detection of breast cancer at stage 0 or 1 would allow more treatment options and increased survival. Mammography is the primary modality for detecting cancer but has shortcomings, particularly in younger women where breast density is more common. Breast density interferes with mammograms reducing the sensitivity of the test. In addition, mammography has low sensitivity for the detection of lobular cancer which has a diffuse growth pattern and often lacks microcalcifications. We have developed a blood-based gene expression test for the detection of breast cancer. Whole genome array analysis was performed for assay selection and through a series of studies a commercially available 96-assay set has been verified and validated (BCtect®). Gene expression was measured using reverse-transcriptase real-time PCR to determine mRNA levels in whole blood. An algorithm was developed in a calibration study to distinguish between BC and non-BC patients. The current study reports the results from an independent validation of the gene expression test (BCtect®).
Materials and Methods: In a multicentre-study, blood samples were collected from women from 3 groups (1) Stage 0−III BC, (2) benign breast lesions, or (3) negative mammograms. Blood samples were collected in PAXgene™ tubes and shipped on dry ice to a central laboratory for RNA extraction. Quality control of RNA was performed using the Agilent 2100 BioAnalyzer and Nanodrop ND-1000. Gene expression analysis was performed using real time RT-PCR (AB7900 HT) with a microfluidic card containing the BC-specific 96 assay signature. The test software provided a test score for each subject in the independent validation cohort. A positive test score classified a subject as positive for BC, whilst a negative score classified a subject as negative for BC.
Results: The model correctly predicted the class of 78 of the 109 validation samples (overall accuracy 72%). Performance was similar for early and late stage cancer with a sensitivity of 74% for stage 0/1 breast cancer (stage 0 = in situ cancer, and stage 1 = T1N0M0; staging defined by AJCC 2002). The test performed equally well in pre- and post-menopausal women. Lobular cancers were predicted with 72% sensitivity. The test gave a positive result in a cohort of 20 pregnant women suggesting the test measures a biological feature common to both breast cancer and pregnancy. The assays code for proteins that have a biological function in immune response, signal transduction, and cellular metabolism amongst others.
Conclusions: The blood-based gene expression test showed efficacy for the detection of early breast cancer in both pre- and post-menopausal women suggesting the test may be a valuable tool for young women, a group in which mammography has poor performance. Additional validation is ongoing.

651 Trop-2 is a general cancer growth stimulator through ubiquitous tetraspanin platforms

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Background: Trop-2 is a transmembrane calcium signal transducer, first identified in trophoblast and subsequently found in diverse transformed cells. The expression of Trop-2 has been associated with biological aggressiveness and poor prognosis of pancreatic, gastric, oral, ovarian and colorectal cancers, suggesting a potential role in tumour progression. However, a comprehensive scenario of Trop-2 expression is still missing and the function of Trop 2 is as yet unknown.
Material and Methods: Human tumours and normal tissues were analysed for the expression of the *TROP2* gene by DNA microarray, EST, SAGE, RT-PCR, Northern blot analysis. Expression of the Trop-2 protein and of downstream signal transducers was investigated by immunohistochemistry, flow cytometry, high-throughput Western blotting, proteomic array, 2D gels/mass spectrometry and dynamic multi-color imaging. Induction of expression and somatic knockdown of relevant targets was utilized for function analysis, together with protein-protein, protein-gene network interaction analysis.