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Mutational analysis of epithelial ovarian cancer using OncoMap

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Background: Epithelial ovarian cancer is the most lethal of all of the gynecologic malignancies, and new treatments are needed for both newly diagnosed patients as well as patients with recurrent cancer. The success of conventional chemotherapy has reached a plateau, and new means of characterizing ovarian cancer so that treatment can be "personalized" are needed. Activating point mutations in proto-oncogenes have been observed in many human cancers, and such mutations often confer 'oncogene addiction' upon the relevant cancer cells.
Materials and Methods: The Center for Cancer Genome Discovery (CCGD) Program at the Dana-Farber Cancer Institute (DFCI) has adapted a high-throughput genotyping platform to determine the mutation status of a large panel of known cancer genes. The mutation detection protocol, termed OncoMap, uses mass spectrometric-based genotyping technology (Sequenom) to identify specific oncogenic mutations. This methodology has been expanded to detect more than 1100 mutations in 114 oncogenes and tumor suppressor genes in both fresh frozen and formalin-fixed paraffin-embedded (FFPE) tissue samples. We performed a pilot OncoMap study using our latest version of OncoMap (v3) on a set of 40 FFPE advanced ovarian cancer specimens. All samples were advanced stage (stage III or IV), high grade serous histology in patients without known germline *BRCA* mutations. We isolated genomic DNA from these samples, and after a battery of quality assurance tests, ran each of these samples on the OncoMap v3 platform.
Results: In a sample of high grade serous ovarian cancers, 85% (34/40) of these cancers harbored candidate mutations. In total, 76 candidate mutation calls were made across 26 different genes. This work identified mutations genes previously identified by the Sanger Institute (COSMIC), specifically, *KRAS, BRAF, CTNNB1* and *PIK3CA; KRAS* and *PIK3CA* were amongst the most frequent genes containing mutations in high grade serous cancers (>25% frequency) and were confirmed by hME. *BRAF* mutations were rare as expected (<5%). In addition, we identified novel mutations in *EGFR, SRC,* and *FGFR3*.
Conclusions: Sequenom analysis using OncoMap using DNA extracted from FFPE ovarian cancer samples is feasible and leads to the detection of potentially druggable mutations. Our group is now genotyping all new ovarian cancers diagnosed at our institution and expanding our recurrent cancer cases to include non-serous tumors as well.

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Development and validation of a novel multiplexed PCR-coupled liquid bead array system for gene expression in circulating tumor cells

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Introduction: The aim of our study was to develop and validate a multiplexed PCR-coupled liquid bead array to detect the expression of multiple genes in Circulating Tumor Cells (CTCs), and application of this assay in peripheral blood samples of breast cancer patients.
Patients and method: We developed a novel multiplexed PCR-coupled liquid bead array-assay for gene expression in CTCs. The assay consist of: (a) in silico designed gene-specific primers and capture probes for *CK-19, HER-2, Mammaglobin (hMAM), MAGE-A3, TWIST-1* and *PBGD*, (b)-RNA isolation from immunomagnetically enriched CTCs and PBMC fraction, (c) multiplex RT-PCR, (d) sequence hybridization array, (e) fluorescence detection in the Luminex bead array platform. We performed extensive optimization experiments using the SKBR3 and MDA-MB-231 cell lines as positive controls, to maximize analytical sensitivity and specificity. We further validated the performance of this assay by cross reactivity and intra and inter-assay precision studies. Finally, we applied the developed methodology in peripheral blood samples of 64 patients with operable breast cancer, 25 patients with verified metastasis and 17 healthy individuals.
Results: The analytical performance of the developed liquid bead array was evaluated in tumor cell lines in respect to analytical sensitivity and specificity. Cross reaction studies have shown that the assay is highly specific for each gene in complex multiplexed formats, is highly sensitive, since it can detect the expression of each individual gene at one SKBR3 cell level. Validation experiments included within day and between-days precision studies. None of the genes tested was detected in the CTC fraction of healthy donors while in patients with verified metastasis, *CK-19* was detected in 65%, *HER-2* in 20%, *MAGE-A3* in 30%, *hMAM* in 20% and *TWIST-1* in 20%. In operable breast cancer patients, *CK-19* was detected in 26.6%, *HER-2* in 12.5%, *TWIST-1* in 31.2%, *MAGE-A3* in 18.7% while *hMAM* was detected in 10.9% of the patients.
Conclusions: The developed multiplexed PCR liquid bead array can be successfully used to study individual gene expression in CTCs. This may form an efficient basis for a multiplex approach to measure multiple genes (up to 100) in the same sample, thus saving sample volume and reducing the total cost and time of analysis. This is the first time that the Luminex technology is used for gene expression studies in CTCs.

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An epithelial to mesenchymal transition (EMT) gene expression signature identifies Axl as an EMT marker in non-small cell lung cancer (NSCLC) and head and neck cancer (HNC) lines and predicts response to erlotinib

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Background: Epithelial/mesenchymal transition (EMT) is associated with loss of cell adhesion molecules such as E-cadherin and increased invasion, migration, and proliferation in epithelial cancers. In non-small cell lung cancer (NSCLC), EMT is associated with worse prognosis and resistance to EGFR inhibitors. Despite the clinical implications, no gold standard exists for classifying a cancer as epithelial or mesenchymal. The goal of this study was to develop a robust EMT gene expression signature and test its correlation with drug response.
Materials/Methods: The EMT signature was derived in 54 DNA finger-printed NSCLC cell lines profiled on Affymetrix U133A, B, and Plus2.0 arrays and tested on the Illumina WGv2 and WGv3 platforms and in an independent set of head and neck cancer lines (HNC). E-cadherin and other protein levels were quantified by reverse phase protein array and correlated with the first principal component of the EMT signature. IC50s were determined for NSCLC cell lines by MTS assay.
Results: Expression of 76 genes (the EMT signature) correlated with mRNA expression of known EMT markers E-cadherin, vimentin, N-cadherin, or fibronectin 1 and was bimodally distributed across the NSCLC panel. Classification of the NSCLC lines as epithelial or mesnchymal by the EMT signature agreed for 51/52 cell lines tested on both Affymetrix and Illumina platforms. In an independent validation set of 62 HNC lines, the signature identified a subset of six mesenchymal cell lines. The EMT signature score correlated well with E-cadherin protein levels in NSCLC ($r = 0.90$) and HNC ($r = 0.73$). mRNA levels for Axl, a tyrosine kinase receptor associated with EMT in breast cancer, had the most negative correlation with E-cadherin ($r = -0.45$) of any signature gene after ZEB1 and vimentin and was positively correlated with vimentin ($r = 0.60$) and N-cadherin ($r = 0.54$) expression. Higher Axl total protein was confirmed in NSCLC and HNC mesenchymal-like cell lines. Mesenchymal phenotype (classified by the EMT signature) was more strongly correlated with NSCLC erlotinib resistance ($p = 0.028$) than E-cadherin mRNA or protein level.
Conclusions: An EMT gene expression signature accurately classifies cell lines as epithelial or mesenchymal-like across three microarray platforms and two cancer types and identifies Axl as a novel EMT marker in NSCLC and HNC. The EMT signature was a better predictor of erlotinib resistance than single mRNA or protein markers such as E-cadherin.