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Novel circulating biomarker of MET addiction and susceptibility to MET inhibitors

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Background: MET inhibitors are promising therapeutic agents for cancer cells exhibiting addiction to MET oncogene. MET-targeted therapies in cancer appear to be effective exclusively for tumor types whose survival depends on MET, meaning that a precise diagnosis of MET addiction will be the key to the success of such therapies. Non-invasive assessment of MET addiction by circulating biomarker might be particularly useful in determining the therapeutic options for patients with inoperable tumors. *MET* gene amplification was identified as a sole molecular marker of MET addiction and susceptibility to MET inhibitors. However, current methods for measurement of *MET* amplification require surgical specimens, resulting in resource limitation for inoperable patients. Thus, we explore non-invasive biomarker of MET addiction and susceptibility to MET inhibitors.
Material and Methods: We used several non-small-cell lung cancer (NSCLC) and gastric cancer cell lines with and without *MET* gene amplification. Each cell lysate and conditioned medium was investigated with antibodies against different moieties of MET in immunoblot analysis. To examine whether the identified marker could be detected in serum specimens, we generated tumor-bearing mice by xenografting nude mice with cells with *MET* gene amplification. The level of the biomarker in mouse serum was quantified with ELISA.
Results: NSCLC and gastric cancer cell lines with *MET* gene amplification exhibted MET addiction and high susceptibility to MET inhibitor. We found that fragmented MET was detected specifically in conditioned medium from these cancer cells exhibiting MET addiction, whereas the expression of intact MET was observed even in lysates from cells without *MET* amplification. In tumor xenografted mouse model, the fragmented MET was found to be specifically secreted from MET-addicted cancer cells into serum, and the level of the fragmented MET in serum was highly correlated with the size of xenografted tumors.
Conclusions: Fragmented MET secreted from cells seems to be a promising novel circulating biomarker for MET addiction, while the expression of intact MET within cells was not a good predictor for the addiction. Detecting fragmented MET in body fluid may be a cost-effective and non-invasive manner which can identify a subset of patients with inoperable tumors appropriate for clinical trials of targeted therapy using MET inhibitors.

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Tivozanib biomarker identifies tumor infiltrating myeloid cells contributing to tivozanib resistance in both preclinical models and human renal cell carcinoma

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Background: To identify biomarkers associated with tivozanib response, a population-based, genetically engineered breast tumor model comprising 107 tumors was developed, characterized, and used to test the efficacy of tivozanib, a VEGFR-1, 2, and 3 kinase inhibitor that has shown clinical activity in RCC [ASCO 2009, abstract #5032].
Results: Twenty-five tumors from the archive were treated with tivozanib revealing both responding and non-responding tumor (40% responders, 60% resistant). Bioinformatics analysis of RNA microarray expression profiles of pretreatment tumors identified a set of 200 genes that were significantly associated with resistance. A novel coherence-based bioinformatics approach incorporating multiple human tumor datasets led to a 42 gene resistance signature representing components of hematopoietic gene expression. IHC quantitation of myeloid markers in the tumors identified the presence of infiltrating myeloid cells, whose percentage composition in the tumor correlated with both the 42 gene signature and resistance to tivozanib. Examination of both the signature and myeloid infiltration in human tumor microarray datasets indicated that this resistant phenotype is present in a significant subset of all seven human tumor types examined, including human kidney cancer. The preclinically derived IHC marker was then used to retrospectively examine the relationship between myeloid infiltration and maximum tumor shrinkage achieved in available patient samples from a phase 2 study of tivozanib in renal cell carcinoma [ASCO 2009, abstract #5032]. IHC analysis of infiltrating myeloid cells in 21 patient samples demonstrated a significant correlation between the percent myeloid cell composition in the tumors and maximum tumor shrinkage by RECIST.
Conclusions: These observations (a) reveal the presence of tivozanib-sensitive and insensitive angiogenesis mechanisms in both murine and human solid tumors, (b) provide a candidate response biomarker for tivozanib and (c) demonstrate the utility of this population-based preclinical model in predicting response in humans.

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Integrative analysis of DNA methylation and gene expression identifies a DNA methylation signature associated with erlotinib resistance in EGFR wild type non-small cell lung cancer cells

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Background: While EGFR mutation status dictates sensitivity to EGFR Tyrosine Kinase Inhibitors (TKI) in non-small cell lung cancers (NSCLC), the molecular pathways responsible for sensitivity or resistance to TKIs in EGFR wild type cells are relatively unknown. We sought to determine if there is a DNA methylation profile that can help predict for TKI response in a panel of NSCLC cell lines.
Methods: As a screen to find genes whose gene expression is strongly regulated by DNA methylation, we performed integrative analysis between DNA methylation profiles of 74 NSCLC cell lines using the Infinium HumanMethylation27 beadchip and gene expression changes in a subset of these cells using both Affymatrix U133 and the Illumina Expression Beadchip (WG6-v2).microarray platforms. To find the gene signature that correlates with responsiveness to TKIs, we performed two sample t-test for each gene and applied Beta Uniform Mixture modeling for adjusting multiple comparison. Using appropriate False Discovery Rate (FDR) cutoffs, we identified gene signature that are associated with erlotinib responsiveness in EGFR wild type cells.
Results: Using FDR = 0.5 % (corresponding $p < 0.0001$) and Spearman correlation (rho) cutoff of −0.65, we identified 147 unique genes that are significantly inversely correlated with the degree of methylation. We then correlated these genes with Erlotinib resistance. We found the DNA methylation of a subset of these genes to be highly related to the Epithelial-to-Mesenchymal Transition (EMT) phenotype of these cells based on the expression of E-Cadherin and β-catenin proteins using Reverse-Phase Protein Arrays. We independently confirmed these results using the Illumina WG6-v2 microarray platform.
Conclusions: We have found a gene panel whose expression is largely regulated by DNA methylation in a panel of NSCLC cell lines. By analyzing the degree of methylation of these genes with drug responsiveness to erlotinib in EGFR wild type cells, we have discovered a DNA methylation signature for EMT that predicts for TKI responsiveness. This could serve as a useful biomarker to identify TKI resistance in lung cancer patients.

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Perioperative cancer cell dissemination is not associated with worse prognosis in pancreatic cancer

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Background: Perioperative cancer cell dissemination (CCD) has hardly been studied in pancreatic ductal adenocarcinoma (PDAC). Epithelial cell adhesion molecule (EpCAM) is over-expressed on the surface of tumor cells in many epithelial cancers and may therefore serve as a surrogate marker for circulating tumor cells (CTC). Our aim was to study the prognostic value of perioperatice detection of tumor cells with a real-time RT-PCR for EpCAM in the peripheral blood and peritoneal cavity of patients undergoing pancreatic resection for PDAC.
Methods: From 40 patients who underwent curative pancreatic resection (PR) for PDAC, 10 patients undergoing PR for benign disease and 3 healthy subjects 10ml of EDTA-treated venous blood was drawn preoperatively (n = 40), immediately postoperatively (n = 40) and postoperative days 1, 7 (POD1, n = 35; POD7, n = 39) and after 6 weeks (n = 34). Of all patients undergoing PR a sample of 40ml peritoneal lavage fluid was taken after laparotomy prior to resection (n = 39) and after PR prior to abdominal closure (n = 39). A real-time RT-PCR assay (TaqMan, ABI Prism 7700) was developed for the detection of EpCAM mRNA. Beta-glucuronidase (GUS) was used as the control gene. To discriminate between EpCAM-positive and negative samples a cut-off strategy was used. Median postoperative follow-up was 24.0 months (range: 0.7−41.3).