Bioinformatic/MS proteomic approach to identify a panel associated with the response to therapy in drug-resistant ovarian cancer tissue.

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Ovarian cancer is the seventh most common cancer and the eighth most common cause of cancer-related death in women. The most frequent subtype is high-grade serous carcinoma. Therapy relies on debulking surgery and platinum-based chemotherapy. Despite the initial high response rates to platinum drugs, most patients relapse, develop resistance to classical chemotherapy, and subsequently die from the disease. Tools for the identification of a sub-population of patients who respond to a specific treatment are needed. In our studies on anticancer drugs targeting the folate pathway, tissue samples collected from patients to whom pemetrexed was administered after failure of the first-line carboplatin-based chemotherapy suggested the possibility of identifying a protein panel to predict the response to pemetrexed-based therapy.

The investigational protein panel was discovered using a mass spectrometry (MS) label-free proteomic relative quantification approach. Statistical metrics of the experimental MS data were combined with a knowledge-based approach that included bioinformatics and a literature review to design a protein set of reference (PSR). The PSR provides feedback for the consistency of MS proteomic data because it includes known validated proteins. The method was applied to three tissue samples from patients with ovarian cancer.

A panel of 24 proteins with levels that were significantly different in pre-treatment samples from patients who responded differently to the pemetrexed treatment was identified. The global interpretation of the MS data was performed using a bioinformatic analysis and was driven by the purposely designed PSR with the aim of providing internal experimental validation.

The final panel should be tested in retrospective clinical trials to validate the ability of the identified tool to predict the response of patients with platinum-resistant ovarian cancer to pemetrexed.

Reference:

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A novel 3′UTR panel to predict axillary lymph node involvement in operable triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) is known for its clinical and molecular heterogeneity. Sentinel lymph node biopsy (SLNB) and axillary lymph node dissection (ALND) are standard surgical staging approaches for operable TNBC. In this study, we developed a novel 3′UTR panel to predict axillary lymph node involvement (ALNI) in TNBC patients with the aim of exempting low-risk patients from the invasive axillary staging surgery.

Methods: We evaluated 3′UTR profiles using currently available microarray data from 327 patients with TNBC. Samples were randomly divided into either a training set (n = 164) or a validation set (n = 163) according to chip batch stratification. We constructed a 15-member 3′UTR panel (consisting of APOL2, IL21R, BID, DGCR14, UST, ADD2, SNN, NRCAM, PKP2, YIPF6, NUMB, KCMF1, TFB2M, OSBPL1A and COL1A1) using an elastic net model to predict the risk of ALNI after initial feature filtering. Receiver operating characteristic (ROC) and logistic analyses were used to assess the predictive power of the panel.

Results: In the training set, ALNI occurred in 50% of high-risk patients (odds ratio [OR] 10.6, 95% CI 4.48-25.1; p<0.001), and in 8.6% of low-risk patients. The panel showed a high distinguishing power with an area under the curves (AUC) of 0.829 (95% CI 0.748-0.909). In the validation set, 37.7% of high-risk patients had nodal involvement (OR 5.01, 95% CI 2.22-11.3; p<0.001) whereas 10.7% of subjects in the low-risk group had an AUC of 0.772 (95% CI 0.684-0.861). After adjustment by clinical factors, the 15-3′UTR-panel retained significant predictive accuracy (OR 8.54, 95% CI 4.56-16.0; p<0.001). A combinatorial analysis of the 3′UTR panel and tumor size increased the AUC to 0.784 (95% CI 0.729-0.839).

Conclusions: This study revealed a 15-3′UTR-panel as a promising predictor for ALNI in operable TNBC. Our results may permit a non-invasive personalized management of ALN staging in TNBC patients.
Circular RNA profile identifies circLMTK2 is upregulated and as a proliferative factor and prognostic marker in gastric cancer

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Background: Despite many advances in the diagnosis and treatment of gastric cancer (GC), the prognosis of patients with GC remains poor. Circular RNAs (circRNAs), a new star of the non-coding RNA network, have been identified as critical regulators in various cancers. There is increasing evidence that circRNAs represent a class of widespread and diverse endogenous RNAs that may regulate gene expression. However, the role of circRNAs in GC remains elusive. Here, we aimed to determine the circRNA expression profile and investigate the functional and prognostic significance of circRNA in GC.

Materials and Methods: In this study, we investigated the expression profile of circRNAs in three GC samples and paired adjacent normal tissues using ribo-minus RNA sequencing and a bioinformatics analysis. Furthermore, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to identify circRNA candidates. Molecular and cellular techniques were used to explore the biological function and mechanism of circRNA in GC cells. The prognostic significance was analyzed using the Kaplan-Meier method and the Cox proportional hazards model.

Results: We first characterized circular RNA transcripts using RNA-seq analysis of ribosomal RNA-depleted total RNA from three paired normal and cancerous gastric tissues. In all, 15623 distinct circRNA candidates were found in these tissues and at least 5500 distinct circRNAs are differently expressed in GC tissues compared with matched normal tissues. We further characterized one abundant circRNA derived from the LMTK2 gene, termed circLMTK2. The expression of circLMTK2 is often upregulated in GC tissues and the silencing of circLMTK2 significantly inhibits gastric cancer cell growth. Furthermore, the level of circLMTK2 was observed as an independent prognostic marker for overall survival and disease-free survival of patients with GC.

Conclusions: Our study revealed the circular RNA profile of GC tissues and characterized a differentially expressed circRNA derived from the LMTK2 gene. circLMTK2 may serve as a new proliferative factor and prognostic marker in gastric cancer.
Figure. (A-C) RNA-seq analysis of circular RNAs in gastric cancer tissues. (D) Clustered heatmap of the differentially expressed circular RNAs. (E) Circos plots showing the differentially expressed circular RNAs and their host genes in GC tissues. (F) The genomic loci of the LMTK2 gene and circLMTK2. (G) qRT-PCR analysis of circLMTK2 in either the cytoplasm or the nucleus. (H) qRT-PCR analysis of circLMTK2 after treatment with Actinomycin D. (I) Schematic representation and target sequences of the siRNAs and qRT-PCR analysis of circLMTK2 expression after treatment with two siRNAs. (J and K) Assessment of proliferation of MGC-803 and AGS cells transfected with control or circLMTK2 siRNAs by CCK-8 assay. (L) circLMTK2 is frequently up-regulated in gastric cancer. (M) Kaplan-Meier analysis of the correlation between circLMTK2 expression and overall survival in gastric cancer patients.
A Novel Splice Variant to NCOR2 as Biomarker for Tamoxifen Resistant Breast Cancer

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More than two-thirds of all breast cancers are estrogen receptor (ER) positive for which Tamoxifen revolutionized their management as adjuvant treatment to prevent cancer recurrence. Almost half of these patients eventually develop resistance. The mechanisms underlying tamoxifen resistance are not yet well understood. There is no robust biomarker to reliably predict those who will be resistant. By the time drug resistance is established, the cancer has already progressed and sometimes metastasized. We have identified a novel splice variant, BQ323636.1 which retains only the N-terminus repression domain 1 of the NCOR2 wild-type protein. Overexpression of BQ conferred resistance to tamoxifen in both in vitro and in orthotopic mouse model. Mechanistically, co-immunoprecipitation showed BQ could bind to NCOR2 and inhibit the formation of co-repressor complex for suppression of the ER signaling. Consistently, BQ overexpression compromised the suppressive role of NCOR2 in regulating estrogen-response element activity and rescued transcriptional suppression of tamoxifen on ER-target genes. We generated a monoclonal antibody specific for BQ used to predict patients’ responses to tamoxifen treatment. Immunohistochemistry was performed on tissue microarray of 355 patients with clinical follow-up data of more than 10 years, who had ER positive primary breast carcinoma and had received adjuvant tamoxifen treatment. Nuclear BQ overexpression was significantly associated with tamoxifen resistance by Chi-square test, \( p = 3.90 \times 10^{-6} \) with a sensitivity of 51.4% and specificity of 72.9%. In tamoxifen treated patients, nuclear BQ overexpression was significantly correlated with cancer metastasis, \( p = 1.72 \times 10^{-6} \), and also significantly associated with disease relapse, \( p = 3.47 \times 10^{-4} \). Consistent with its role in predicting tamoxifen resistance, nuclear BQ was significantly associated with poorer survival by Kaplan-Meier estimate (Log-rank test) \( p = 6.28 \times 10^{-5} \) and \( p = 1.31 \times 10^{-4} \) for overall survival and disease-specific survival respectively. The development of such reliable biomarker would enable appropriate alternative therapy to be given at an early stage, thus saving the patient from the side effects as well as risk of inappropriate treatment by tamoxifen.

Proposed mechanism of how BQ323636.1 may induce tamoxifen resistance in breast cancer
Assessment of the response to neoadjuvant Chemo-Radiation in rectal cancer patients based on a metabolomics approach

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Purpose: We report a metabolomics study to identify potential metabolite biomarkers in predicting pathological response after neoadjuvant chemo-radiation therapy (NCRT) for locally advanced rectal cancer (LARC).

Methods: The ultraperformance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS)–based metabolomics was used to analyze 106 serum samples, which were collected from patients treated with NCRT in Fudan University Shanghai Cancer Center (FUSCC) between July 2014 and January 2016. Differential metabolites were identified between sensitive and resistant patients to NCRT evaluated based on tumor regression grade (TRG) according to univariate analysis and multivariate analysis. The predictive performance was evaluated by area under receiver operating characteristic curve (AUC).

Results: A total of 4810 metabolites were identified and 57 significant metabolites were selected. With the 57 metabolic biomarkers, we were able to differentiate sensitive patients from resistant patients using partial least-squares discriminant analysis (PLS-DA) in a sample set of 56 sensitive patients and 49 resistant controls. The combination of these 57 biomarkers had the AUC value of 0.88, 0.81 and 0.84 in the PLS, Random Forest, and support vector machine prediction model, respectively. The results demonstrate that a panel of metabolite biomarkers is of great potential for the prediction of response after NCRT for LARC. Furthermore, the fifteen highest-ranking significantly metabolites were finally identified. A PLS model constructed with fifteen markers also has an ideal discriminant performance with an AUC of 0.80.

Conclusion: Through a systematic metabolomics analysis, we are able to build a model to predict the response after NCRT for LARC. These results show promise for larger studies that could produce more personalized treatment protocols for LARC.

(a) Fifty seven features are selected (the cutoff values of fold change is set as larger than 1.2 or less than 0.83);
(b) Those 57 features can separate most resistant from most sensitive group sample;
(c) The combination of these 57 biomarkers had the AUC
Oncogenetic key signal RANTES/CCL5 - „Cytokine cross talk“ in tumors and silent inflammation of jawbone

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Background: The importance of the microenvironment surrounding the tumor cells with "silent inflammation" increases. Objective: To check the suspected tumor-relevant inflammatory cytokine sources in fatty-degenerative osteonecrotic jawbone (FDOJ), we analyze these conspicuously altered jawbone areas to assess quantification of cytokine expression.

Material and Method: In 38 tumor patients we determine the levels of cytokines by bead-based Luminex® analysis in samples of FDOJ. Results: Striking is the high content of chemokine RANTES/CCL5 (R/C) in all 38 tissue samples on average at 35 fold higher compared to healthy jawbone.. A single case is reported by high R/C levels in FDOJ sample and simultaneously by metastasizing cells inside the FDOJ sample.

Discussion: R/C interacts on several levels in immune responses and is considered in scientific literature as pathogenetic key point in tumor growth. The study supports a potential mechanism where FDOJ is a mediating link specifically in breast cancer (MaCa) and its metastasis.

Conclusion: The authors conclude from the data of FDOJ analysis that these areas express hyperactivated signal transduction of the chemokine R/C, induce pathogenetic autoimmune processes in tumors, MaCa and its metastasis and serve as a possible cause. It may be suggested to involve FDOJ in an integrative therapy concept for tumor therapy.

Graph shows signaling overexpression of RANTES/CCL5 compared to healthy jawbone. Photographs in left panel shows sample of fatty-degenerative medullary jawbone (FDOJ); right panel shows extent of osteolytic and softened jawbone by contrast medium.
The Expression and Clinical Significance of PERK and p-Elf2α in Pancreatic Ductal Adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest malignancies with a dismal five-year survival rate of 6.0%. Previous studies showed that endoplasmic reticulum (ER) stress plays an important role in PDAC development and chemoresistance. ER stress activates unfolded protein response, which subsequently lead to the phosphorylation of eukaryotic initiation factor 2 alpha (Elf2α) by protein kinase R-like ER kinase (PERK). However the expression and clinical significance of phospho-Elf2α (p-Elf2α) and PERK in PDAC have not been examined.

Materials and methods: We examined the expression of p-Elf2α and PERK in 84 PDAC and their paired normal pancreas samples from patients who underwent surgical resection with curative intent using tissue microarrays and immunohistochemical staining. We also examined PERK and p-Elf2α expression by Western blot using paired frozen samples of normal pancreas and PDAC. The results of p-Elf2α and PERK expression were correlated with clinicopathologic parameters and survival using SPSS Statistics.

Results: The mean H score for PERK expression in PDAC was 140.8 compared to 82.1 in normal pancreas (p<0.001). Using the median H-score in PDAC as a cut off, 42 (50%) PDAC had high level of PERK expression. High level of p-Elf2α expression was present in 47 (56%) PDACs, but only in 5 (7.6%) matched normal pancreas. The expression levels of p-Elf2α and PERK was higher in PDAC than those in normal pancreas, which was validated by Western blots. PERK expression in PDAC correlated significantly with p-Elf2α expression (p=0.008). High levels of PERK and p-Elf2α expression were associated with shorter survival (p=0.048 and p=0.03 respectively). By multivariate analysis, high level of p-Elf2α (p=0.01), positive margin (p=0.002) and lymph node metastasis (p=0.01) were independent predictors for shorter survival in PDAC patients.

Conclusions: Our results show that the expression levels of PERK and p-Elf2α are higher in PDAC than those in normal pancreas. High levels of PERK and p-Elf2α expression are predictors of shorter survival in PDAC patients. Our data suggest that PERK and Elf2α could be promising targets for PDAC.
Assessing tumor heterogeneity and biomarkers of therapeutic resistance using a multi-omics strategy in metastatic colorectal cancer patients

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Background: Colorectal cancer (CRC) is the 2nd leading cause of cancer relating-death in Canada and therapeutic resistance remains a major obstacle in metastatic (m)CRC. Studies aiming at understanding mechanisms of resistance have largely investigated primary tumors. However, selective pressures during therapy can lead to tumor heterogeneity. This highlights the need to characterize the molecular changes of metastasis and plasma over time of treatment and response to decipher tumor evolution and therapeutic resistance mechanisms.

Material and methods: Eighty-eight (88) metastatic liver tissue samples were collected at baseline (pre-biopsies) and at relapse (post-biopsies) in sixty-one (61) responder and non-responder mCRC patients undergoing the same standard first-line treatment. Four (4) patients had multiple post-biopsies, to allow the assessment of intra- and inter-tumor heterogeneity after treatment exposure. Biopsies were profiled using exome and transcriptome sequencing as well as high-density SNP array analysis to capture chromosomal anomalies, loss of heterozygosity and copy number variations (CNV). Serial blood samples were also collected for proteomic and ctDNA analysis.

Results: Exome sequencing allowed the characterization of recurrent mutations and the clonal dynamics over time of treatment. Integrated analysis of matched SNP array analysis and transcriptome datasets of successive pre- and post-biopsies revealed genomic anomalies associated with consistent gene expression changes, allowing the identification of robust candidates. In chemo-naïve biopsies, specific CNV regions have been found significantly associated with patient progression free survival by Kaplan-Meier analysis. Immune gene expression analysis identified a subgroup of patients with putative immune-reactive metastases. Plasma-derived ctDNA analysis was performed to investigate the mutational status during treatment and whether they correlate with their relative levels in biopsies.

Conclusion: Our study, using a multi-omic approach to profile liver metastasis samples and serial liquid biopsies in mCRC patients, constitutes an innovative approach to identify clinical biomarkers and molecular signature of resistance, which may enhance the development of personalized therapy.
P2.10

Comprehensive Genomic Analysis Uncovers Two CTA Components for the Follow Up of Solid Cancers

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We questioned whether the expression dynamics of the entire CTA family in cancer could serve to improve our ability to assess the disease. We employed especially designed computational ranking methods to study the expression of all known CTAs, measured using microarrays and deep sequencing, in close to 15000 samples, including more than 100 types of solid cancers.

CTA provided an expression signature that was consistently sufficient to determine the existence and staging of all solid cancers tested, with close to perfect accuracy. We found two CTA uncorrelated components that coexist in all solid cancers - One component of genes that are part of the mitotic cell cycle appearing in most known cancer prediction signatures, not located on the X chromosome and consistently appearing up regulated in the order of 2 to 5.

The second component, CTA genes mostly residing on chromosome X, aberrantly highly expressed in the order of 5 to 100, and not strongly correlated to other genes.

Treating cell lines, with B-raf and Egfr inhibitors affected the first component. 5-aza-2'-deoxycytidine treatment of cell lines, affected the second component. Residing.