Tumor-released circulating orphan non-coding RNAs reflect treatment response and survival in breast cancer

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Background: Liquid biopsies have emerged as effective diagnostic tools in disease monitoring and minimal residual disease detection. Circulating tumor DNA (ctDNA) was recently shown to be a predictor of poor response and recurrence in breast cancer. However, ctDNA shedding from breast tumors can rapidly decrease during treatment, resulting in reduced sensitivity in measuring early changes in tumor response or residual cancer burden (RCB) after neoadjuvant chemotherapy (NAC). We recently reported the discovery of orphan non-coding RNAs (oncRNAs), a class of small RNAs that are not present in healthy cells, but emerge from cancer cells. Similar to ctDNA, tumor-released oncRNAs can be used to detect the presence of an underlying tumor; however, since they are actively released by cancer cells, their abundance in the cell-free compartment is substantially higher than ctDNA. Therefore, we hypothesized that monitoring circulating oncRNAs in blood permits a more sensitive approach to measuring treatment response (i.e., pathologic complete response, or pCR) and estimating RCB. Patients and Methods: Cell-free RNA (cfRNA) was extracted from ~1 ml sera of 72 breast cancer patients treated in the neoadjuvant I-SPY 2 TRIAL with NAC alone or combined with MK-2206 (AKT inhibitor) treatment. For each patient, treatment-naïve samples (T0) were compared with samples from post-treatment and prior to surgery (T3) time-point. RNA samples were subjected to small RNA sequencing (SMARTer), and the presence and abundance of cell-free oncRNA species were then determined by identifying and counting the reads that map to oncRNA loci across samples. Notably, oncRNAs species were preannotated from the Cancer Genome Atlas (TCGA), and our approach does not require bespoke personalized assays. We used a machine-learning model to compare abundance of cfRNA species before and after treatment (i.e., T3-T0) to predict pCR and RCB. For this, we split our cohort into a training and a testing set (48 and 24) and trained a model to simultaneously learn the presence of residual disease (pCR vs. no pCR) and its extent (RCB). We then measured the performance of our model on the held-out test data and the entire dataset. To confirm the robustness of our model, we also
employed a leave-one-out strategy, whereby pCR and RCBIndex of each patient was predicted using a model that was trained on the other patients in the cohort. Finally, to assess the ability of our oncRNA-based model to risk-stratify patients who fail to achieve pCR (without having been explicitly trained on relapse data), we used the model’s oncRNA score to predict patients at the highest risk of distant recurrence (n=8 out of 36) and performed a multivariate Cox analysis, controlling for HR/Her2 status (median follow-up time was 4.8 years). Results: The model’s accuracy for predicting pCR—based on changes in circulating oncRNA species between T3 and T0—was 85% for the training data and 79% for the held-out test data (positive predictive value of 75% and negative predictive value of 83%) with combined accuracy of 83%; precision 86% and recall 83%; Pearson R=0.5 for RCB. A leave-one-out strategy showed similar performance (area under ROC of 0.77 versus 0.81 in train-test split). Finally, among the patients who failed to achieve pCR, we observed a significantly higher risk of distant recurrence in those with the highest scores (DRFS: hazard-ratio = 8.4, ANOVA P<0.05). Conclusion: In this study, we have shown that the changes in tumor-released oncRNA content of the blood are a significant predictor of clinical outcomes. Our results demonstrate that oncRNA fingerprints are blood-accessible, and allow us to build predictive models of tumor response. We are currently expanding this study to additional cohorts, and we expect to report the results for a longitudinal analysis that includes ~200 patients from I-SPY2.