Dharanija Madhavan
Dr. sc. hum

Investigation of Circulating microRNAs and Circulating DNA as Diagnostic and Prognostic Markers in Metastatic Breast Cancer

Promotionsfach: Humangenetik
Doktormutter: Prof. Barbara Burwinkel

Breast cancer is an ever-increasing global health problem, and metastatic spread of the disease is especially debilitating. Improving outcome and quality of life for metastatic breast cancer patients can be achieved by the use of biomarkers. However, there is a paucity of diagnostic and prognostic biomarkers for metastatic breast cancer with high sensitivity and specificity, and which can be applied to all breast cancer subtypes. Circulating nucleic acids, which includes circulating microRNA (miRNA) and circulating DNA, have been explored for their use as a biomarker in cancer and other diseases. Access by minimally invasive procedures, possibility of repeated sampling and periodic monitoring, high stability, and evaluation by relatively inexpensive methods make them attractive candidates for biomarker development. In this study here, the potential of circulating miRNAs and circulating DNA as diagnostic and prognostic markers in metastatic breast cancer has been explored and established.

Circulating miRNA profiling and subsequent validation in metastatic breast cancer patients and healthy controls led to the identification of 9 miRNAs that were differentially represented between cases and controls. Specifically, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-210, miR-375 and miR-801 was increased with fold changes ranging from 1.5 to 43 ($P < 0.03$), and miR-768-3p was decreased in cases with fold change of 0.6 ($P = 0.06$). The metastatic breast cancer patients consisted of patients with circulating tumour cells (CTC-positive) and those without (CTC-negative), and miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-210, miR-375, and miR-801 were differentially present between these two patient groups (fold change from 3 to 24, $P < 10−6$), and could thus differentiate metastatic breast cancer patients based on their CTC status. Multivariable regression analysis engendered a panel of miRNAs chosen from the above 9 miRNAs that was most informative and least redundant for discriminating the three groups of study subjects with AUC > 0.7.

A second independent profiling of circulating miRNAs was carried out to identify miRNAs of prognostic value in plasma of metastatic breast cancer patients. After validation, sixteen miRNAs were found to be significantly correlated to overall survival: miR-141, miR-144, miR-193b, miR-200a, miR-200b, miR-200c, miR-203, miR-210, miR-215, miR-365, miR-375, miR-429, miR-486-5p, miR-801, miR-1260, and miR-1274a (hazard ratio > 2, $P < 0.05$).

Of these, miR-141, miR-144, miR-193b, miR-200a, miR-200b, miR-200c, miR-203, miR-215, miR- 375, miR-429, miR-801, and miR-1274a were also associated with progression-free survival (hazard ratio > 1.4, $P < 0.04$). The prognostic value of majority of these miRNAs was valid even when measured in plasma samples collected from the patients after undergoing one cycle of therapy. miRNA signatures were built by LASSO Cox regression analysis to predict survival. The developed miRNA signatures performed significantly better than the currently established FDA-approved prognostic marker, CTC status, in predicting overall survival since the integrated prediction error of the miRNA model was 1.347 as opposed to that of CTC status, which was 1.457. The miRNAs identified by us were found to be independent prognostic markers. The correlation of miR-144, miR-200a, miR-200b, miR-200c, miR-210, miR-215, and miR-486-5p to overall survival was validated in a second independent cohort (hazard ratio > 1.4, $P < 0.03$). Additionally, miR-200a, miR-200b, miR-200c, miR-210, miR-215, and miR-486-5p could also predict prospectively the onset of metastasis in breast cancer patients up to two years prior to event. Majority of the here-identified miRNAs have previously been shown to have functional roles in cancer and metastasis development, thus underscoring a mechanistic basis for their observed differential levels in circulation.

Lastly, the use of circulating DNA integrity and concentration as biomarkers in breast cancer was evaluated. Circulating DNA integrity, a measure of how fragmented the DNA is, and circulating DNA concentration were estimated by measuring the repetitive elements, Alu and LINE1. Circulating DNA integrity was significantly decreased in breast cancer patients compared to healthy individuals ($P < 0.048$). Within breast cancer patients it decreased from primary to metastatic breast cancer, which in turn had a decreased integrity in CTC-positive patients in comparison to CTC-negative patients ($P < 10-6$). Circulating DNA concentration evinced the opposite trend ($P < 10-7$). Combination of the circulating DNA parameters measured here could clearly differentiate the breast cancer cases from controls, and also demarcate the type of breast cancer cases with AUC > 0.71. Apart from their diagnostic properties, circulating DNA integrity and concentration could also predict progression-free (hazard ratio of 0.65 for ALU and 0.81 for LINE1) and overall survival (hazard ratio of 0.35 for ALU and 0.47 for LINE1) in metastatic breast cancer patients, while outperforming CTC status. Thus, they also have prognostic capabilities.

Overall, we have identified circulating miRNAs and defined miRNA signatures in plasma, along with establishing circulating DNA integrity and concentration that can be used as diagnostic and prognostic markers in metastatic breast cancer. This would be of significant translational relevance since they can be used in a clinical setting either as a stand-alone or in combination with other established markers.