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Investigation of circulating microRNAs as minimally invasive markers for the early detection of breast cancer

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Breast cancer is the most common type of cancer and the leading cause of cancer-related death among women worldwide. Its early detection remains one of the major challenges and holds promise of a more favourable disease outcome. Therefore, the aim of this study was to investigate the potential of circulating miRNAs to serve as minimally invasive, early detection markers of breast cancer. In the initial array-based approach plasma miRNA profiles of stage I and II breast cancer patients were compared to those of healthy individuals. As a result 38 miRNAs were identified as potentially deregulated in the plasma of early stage breast cancer patients. After the application of stringent selection criteria nine marker candidates were chosen for further large-scale validation.

Seven circulating miRNAs were confirmed as present at higher levels in the plasma of breast cancer patients compared to healthy controls after the analysis of more than 400 plasma samples from two validation cohorts. These miRNAs were: miR-127-3p, miR-148b, miR-376a, miR-376c, miR-409-3p, miR-652 and miR-801. To investigate whether they also show alterations in patients with benign breast tumors, samples from women with such lesions were included in the second validation cohort. Subsequently, circulating miR-148b, miR-652 and miR-801 were found to be elevated in the plasma of women with benign breast tumors when compared to healthy individuals.

In terms of breast cancer (early) detection, circulating miR-127-3p, miR-148b, miR-376a, miR-376c, miR-409-3p, miR-652 and miR-801 have shown great potential in discriminating between plasma samples from healthy individuals and women with benign and/or malignant breast tumors. ROC curve analysis revealed that a combination of all seven circulating miRNAs resulted in the highest discriminatory power. In the first validation cohort this panel of seven circulating miRNAs has reached reasonable breast cancer detection accuracy with an AUC value of 0.70, which was superior in younger women (up to 50 years of age) for whom the AUC equalled 0.75. For a fixed sensitivity of 70% the median specificity was 62%. In the second validation cohort the accuracy for the identification of women with benign and/or malignant breast tumors could be tested. It was observed that both benign and malignant breast tumors could be detected with identical accuracy (AUC=0.81). And more importantly, for younger women the discriminatory power was again superior and exceeded an AUC value

of 0.85, which is indicative of a good and useful marker. Here, also the sensitivity and specificity improved as for sensitivity fixed at 80% the specificity was slightly above 70% representing a good trade-off between these two marker characteristics. The increase of accuracy, sensitivity and specificity of the proposed miRNA panel in women up to the age of 50 years is especially important in light of the fact that mammography, the current gold standard breast cancer screening and detection method, seems to be less specific for younger women. Therefore, they would probably benefit the most from such an alternative method of breast cancer screening and detection.

Circulating miR-801 levels correlated with the tumor proliferation marker Ki-67 and the tumor grading, whereas miR-148b and miR-652 correlated with the tumor marker p53, pointing to the possibility of these circulating miRNAs being prognostic markers as well. Among the seven identified circulating miRNAs, there was an enrichment of miRNAs derived from the chromosomal region 14q32, underscoring the importance of this genomic region for breast cancer. Furthermore, plasma levels of the four 14q32-located miRNAs (miR-127-3p, miR-376a, miR-376c and miR-409-3p) correlated strongly to each other.

The analysis of tissue samples revealed that, contrary to the findings in plasma, miR-127-3p, miR-148b, miR-376a, miR-376c, miR-409-3p and miR-652 levels were decreased in malignant when compared to benign breast tissue, whereas miR-801 did not show any significant difference. In post-operative plasma samples collected 4-10 weeks after surgery the plasma levels of none of these miRNAs decreased. MiR-409-3p even displayed increased plasma levels after surgery. Taken together these results indicate that tumor cells are not the only source of circulating miRNAs.

Experiments comparing miRNA levels of plasma and serum samples prepared by a one- or a two-step (double-spin) centrifugation protocol, showed that blood cells can cause contamination of plasma and serum if the samples are not prepared properly. In cases where only samples obtained by a one-spin protocol are available, serum samples seem to be a better option than plasma. Finally, plasma prepared by the double-spin protocol seems to be the best choice for marker discovery and development outperforming serum processed by the double-spin protocol.

In conclusion, seven miRNAs have been identified and validated as elevated in the plasma of breast cancer patients compared to control individuals. These circulating miRNAs have shown great potential for the discrimination of healthy women from those with benign breast tumors and malignant breast cancer. Therefore, they could be used alone as a screening tool or in combination with other markers to complement and improve existing screening and early detection methods for breast cancer. These findings are especially relevant for younger women, for whom the proposed miRNA marker panel exhibited the highest detection accuracy.