Urinary micro RNA pattern as marker for early breast cancer diagnosis

Non-invasive, hazard-free detection of early breast cancer in urine

Technology

Breast cancer (BC) represents the most frequent malignant disease in women worldwide. In Germany 72.000 cases are diagnosed each year (worldwide appr. 1.2 million women). Detection of BC is based on clinical symptoms (e.g. lumps) or established screening programs for detection of early disease by mammography. Early detection is crucial for improved curation rates and minimization of therapeutical procedures (surgery, chemotherapy). However, screening with X-rays is controversially discussed and participation rates are suboptimal due to the exposure to radiation doses and their potential harmful biological effects.
MicroRNAs (miRNAs) are small (< 30 basepairs) non-coding RNAs with multiple crucial functions also in breast cancer as tumor promoting as well as tumor suppressing factors. We developed an unique urine-based miRNA assay (9 specific miRNAs) to detect early, primary breast cancer.

Innovation

- Quantification of 9 miRNAs in midstream urine (5ml) with a specific expression pattern to detect early breast cancer
- High sensitivity and specificity (detection rate: > 90%)
- Non-invasive and hazard-free

Application

- Screening for breast cancer as part of an innovative multiple step concept to avoid unnecessary diagnostic work up and radiation exposure
- Tool for follow-up after breast cancer treatment for detection of recurrences
- Monitoring of treatment response in the neoadjuvant and metastatic setting

Developmental Status

- Assay fully developed
- For immediate application
- Only standard equipment and technology (realtime PCR) required

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Diagnostic

Patent Status

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Feasibility of urinary microRNA detection in breast cancer patients and its potential as an innovative non-invasive biomarker

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Background

Since recent studies revealed the feasibility to detect blood-based microRNAs (miRNAs, miR) in breast cancer (BC) patients a new field has been opened for circulating miRNAs as potential biomarkers in BC. In this pilot study, we evaluated to our knowledge for the first time whether distinct pattern of urinary miRNAs might be also applicable as innovative biomarkers for BC detection.

Methods

Urinary miRNA expression levels of nine BC-related miRNAs (miR-21, miR-34a, miR-125b, miR-155, miR-195, miR-200b, miR-200c, miR-375, miR-451) from 24 untreated, primary BC patients and 24 healthy controls were quantified by realtime-PCR. The receiver operating characteristic analyses (ROC) and logistic regression were calculated to assess discriminatory accuracy.

Results

1

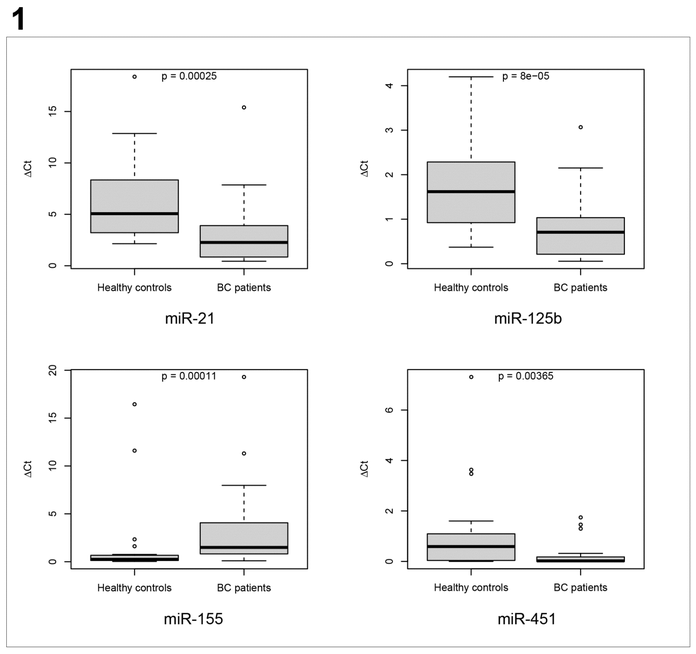

Figure 1: Box plots **of ΔCt-values of significant urinary miRNAs in breast cancer patients compared to healthy controls**
Median urinary expression levels of miR-21 (2.27 vs. 5.07; p<0.001), miR-125b (0.72 vs. 1.62; p<0.001), and miR-451 (0.02 vs. 0.590; p=0.004) were significantly decreased in BC patients compared to healthy controls, respectively. Urinary miRNA-155 expression was signifi-cantly increased in BC patients compared to healthy controls (1.49 vs. 0.25; p<0.001). Median ΔCt-value and interquartile range of duplicate experiments. Thick lines: median (50% percentile); gray boxes: 25% to 75% percentile; thin lines: minimal and maximal value,[0]: moderate outlier,. Mann Withney-U test. Quantitative realtime-PCR.

Significant differences were found in the expression of four BC-associated miRNAs quantified as median miRNA expression levels. Urinary miR-155 levels were significantly higher in BC patients compared to healthy controls (1.49vs.0.25; p<0.001). In contrast, compared to healthy controls, BC patients exhibited significantly lower urinary expression levels of miR-21 (2.27 vs.5.07; p<0.001), miR-125b (0.71vs.1.62; p<0.001), and miR-451 (0.02vs.0.59 p=0.004), respectively. The ROC including all miRNAs as well as the group of the four significant deregulated miRNAs separated BC patients from healthy controls with a very high (area under the receiver operating characteristic curve [AUC], 0.932) and high accuracy (AUC=0.887), respectively.

2

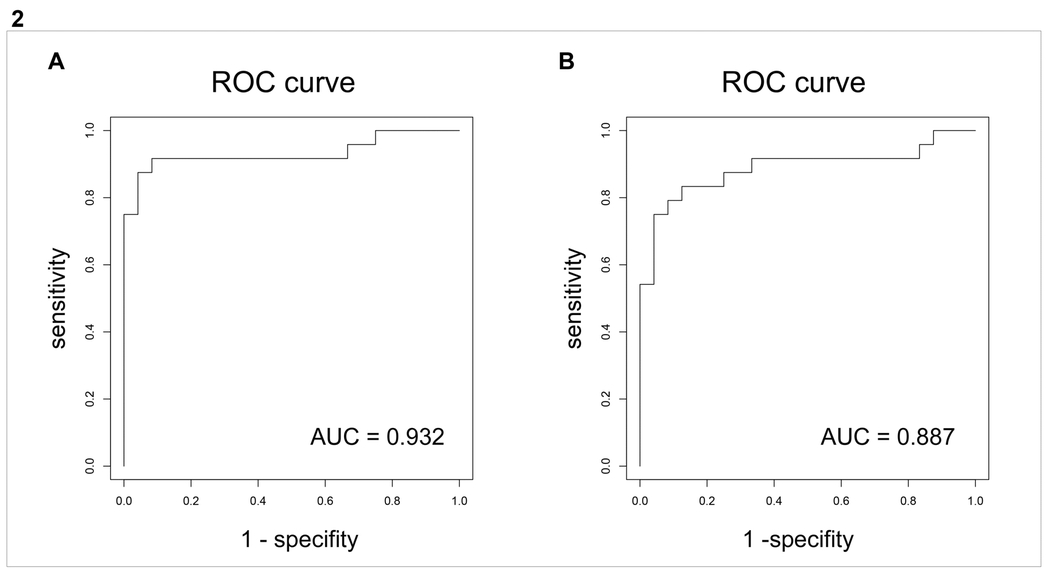

Figure 2: ROC (receiver operating characteristic) curve of (A) all miRNAs for the score combined from all miRNA (miR-21, miR-34a, miR-125b, miR-155, miR-195, miR-200b, miR-200c, miR-375, miR-451) in discrimination between BC patients and healthy controls. A combined ROC (receiver operating characteristic) curve of all miRNAs showed the excellent AUC (area under the curve) of 0.932 and a optimal sensitivity of 0.917 (95%-CI [0.812; 1.000]) and specifity of 0.917 (95%-CI [0.686; 0.978]), respectively. **(B)** ROC curve of the four significantly deregulated miRNAs (miR-21, miR-125b, miR-155, miR-451) was performed and showed high diagnostic accuracy with an AUC of 0.887 and a sensitivity of 0.833 (95%-CI [0.697; 0.997]) and specifity of 0.875 (95%-CI [0.640; 0.957]), respectively.

3

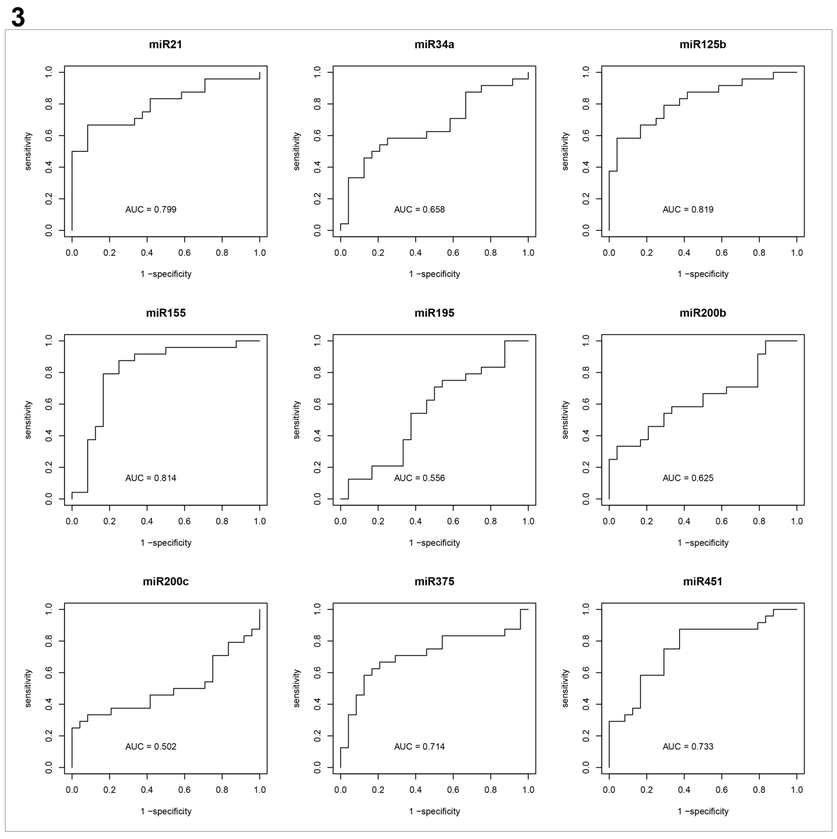

Figure 3: ROC curves of the diagnostic potential of the individual urinary miRNAs (miR-21, miR-34a, miR-125b, miR-155, miR-195, miR-200b, miR-200c, miR-375, miR-451) in discri-mination between BC patients and healthy controls.

The AUC values ranged from 0.502 to 0.819, respectively.

Conclusion

We were able to demonstrate for the first time the feasibility to detect distinct BC-dependent urinary miRNA profiles. The expression levels of four urinary miRNAs were specifically altered in our cohort of BC patients compared to healthy controls. This distinct pattern offers the possibility for a specific discrimination between healthy women and primary BC patients. This sustains the potential role of urinary miRNAs as non-invasive innovative urine-based biomarkers for BC detection.

Reference
Erbes et al. BMC Cancer (2015) 15:1939