Case Study
HTG EdgeSeq
miRNA Whole Transcriptome Assay

Using the HTG EdgeSeq miRNA Whole Transcriptome Assay to research the differences between benign and malignant breast cancer in liquid biopsies

Problem: Mammograms are prone to false positives and confirmation requires invasive biopsies

Traditional mammograms have limited sensitivity. To confirm diagnoses after the identification of abnormal mammograms, patients are subjected to invasive and costly procedures—a core needle biopsy is often required. Further, in those women recalled for additional testing following an abnormal mammogram, only 1.5% are ultimately diagnosed with breast cancer[1]. Beyond initial diagnosis, differentiating between benign and malignant breast cancer is also difficult, and it is expected that only 30-50% of biopsied samples will be found malignant[2]. The degree of uncertainty involved in the traditional diagnostic approach thus translates to hardship for the patient in terms of cost, unnecessary treatment, and other associated complications.

Recently, there has been a surge of interest in research of miRNAs as biomarkers for cancer identification. miRNAs are post-transcriptional gene regulators that can act as oncogenes or tumor suppressors, blocking access to mRNA or triggering degradation. In biopsied tissue, altered regulation of 29 miRNAs was shown to be associated with breast cancer[3]. Using a set of 15 of these miRNAs researchers were able to correctly differentiate between normal breast tissue and tumors with 100% accuracy[3]. miRNAs are therefore promising biomarkers for breast cancer. Until recently, however, research in miRNA from breast cancer patients was limited to the testing of biopsied tissue and not focused on circulating miRNAs.

Solution: Predicting the likelihood of malignancy with circulating miRNA biomarkers

Recently, The National Medical Research Council of Singapore supported a study aimed at determining if circulating miRNAs could also serve as biomarkers for stratifying malignant breast cancer patients. Using the HTG EdgeSeq miRNA Whole Transcriptome Assay, Loke *et al.* investigated the expression of 2,083 miRNAs from serum samples of 125 patients with either malignant or benign breast cancer[4]. Eight miRNAs were identified that were significantly upregulated in patients with malignant tumors *(Table 1)*.

Table 1. *Using the HTG EdgeSeq miRNA Whole Transcriptome Assay, Loke et al. identified eight miRNAs that were significantly upregulated in patients with malignant tumors versus benign controls. Adapted from Loke et al.[4]*

miRNA	Fold Change (log_2)	Adjusted *p*-Value
miR-3162-5p	2.2134	9.12×10^{-25}
miR-6869-5p	1.7624	1.97×10^{-21}
miR-6781-5p	1.5745	1.97×10^{-21}
miR-1249	1.6705	1.75×10^{-20}
miR-7108-5p	1.7253	2.69×10^{-17}
miR-6804-3p	1.2225	1.87×10^{-14}
let-7e-3p	1.4523	2.26×10^{-12}
miR-1306-5p	1.1950	7.17×10^{-12}

Loke *et al.* then created a Bayesian logistic regression algorithm based on the eight miRNAs. The resulting model was capable of predicting the difference between patients with benign and malignant tumors in a separate test cohort at up to a 95.42% confidence interval *(Table 2)*[4].

Table 2. *According to Receiver Operating Characteristic (ROC) analysis, the training model generated by Loke et al. based on upregulated miRNA identified using the HTG EdgeSeq miRNA Whole Transcriptome Assay differentiated patients with malignant verses benign tumors with an area under the curve (AUC) of over 95%. Recall, precision, and balanced accuracy were all also over 90% for both training and test sets. Adapted from Loke et al.*[4]

	Eight-miRNA Signature Model	
Performance Metrics	**Training Set**	**Test Set**
AUC (95% CI)	0.9889 (0.9772, 1.0000)	0.9542 (0.8832, 1.0000)
Recall	0.9625	0.9412
Precision	0.9506	0.9412
Balanced Accuracy	0.9368	0.9150

Significance: Further research into miRNA profiling could help reduce the need for invasive procedures in breast cancer

Loke *et al.* used the HTG EdgeSeq miRNA Whole Transcriptome Assay to demonstrate, for the first time, that circulating miRNAs can act as biomarkers to differentiate patients with benign and malignant breast cancer in liquid biopsies.

In conclusion, the HTG EdgeSeq miRNA Whole Transcriptome Assay is an important tool for identifying cancer biomarkers from blood tests. In the future, the HTG EdgeSeq miRNA Whole Transcriptome Assay could be used to research circulating miRNA biomarkers in a variety of additional cancers[8,9].

References

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Rev 2: 29-APR-2021