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RAPID COMMUNICATION

LncRNA MIR205HG expression predicts efficacy of neoadjuvant chemotherapy for patients with locally advanced breast cancer

To the Editor,

Recent studies reported that lncRNA MIR205HG expression is associated with sensitivity to anti-epidermal growth factor receptor (EGFR) drug in lung cancer cells. However, few clinical studies reported the role of this molecule in breast cancer, particularly in the neoadjuvant setting. In this study, we explored the clinical significance of MIR205HG expression with its predictive and prognostic value for patients with locally advanced breast cancer. It turned out that MIR205HG is a downregulated lncRNA in breast cancer compared with adjacent nontumor tissues. While it's positively associated with estrogen receptor (ER) and progesterone receptor (PR), it's reversely related to human epidermal growth factor receptor 2 (HER2) and Ki67 index. Importantly, MIR205HG expression level could serve as an independent predictive factor of pathological complete response (pCR) and an independent prognostic factor of relapse-free survival (RFS) for the neoadjuvant cohort. Analysis of public databases suggested that MIR205HG expression is associated with better survival outcomes. Furthermore, pathway analyses revealed the potential function of MIR205HG in transcriptional misregulation in cancer. Therefore, MIR205HG might be a promising novel biomarker of pCR and survival outcomes for patients with locally advanced breast cancer receiving neoadjuvant chemotherapy.

Patients initially diagnosed with locally advanced breast cancer are recommended with neoadjuvant chemotherapy as standard management. Multiple studies have shown that pathological complete response (pCR) is associated with significant improvements in both disease-free survival and overall survival (OS). However, a majority of patients still have residual disease after completing neoadjuvant chemotherapy, not to mention the fact that not all patients have the same expected long-term outcomes after achieving pCR.[1] Taken together, it highlights a demand in biomarkers for predicting individual chemosensitivity and survival outcomes before neoadjuvant treatment.

Long non-coding ribonucleic acids (lncRNAs) are transcripts longer than 200 nucleotides in length without translating into proteins. They function as key regulators of transcriptional interference, alternative splicing patterns, modulating protein activity, etc., which give them nature as cancer biomarkers. Nowadays accumulating evidence indicates that lncRNAs are highly relevant to drug metabolism and resistance. MIR205 host gene (MIR205HG, also identified as ENSG00000230937 and LINC00510), is an intergenic lncRNA and the host gene for miR-205. While miR-205 has been widely studied in cancers, the role of MIR205HG is little investigated. Vishnubalaji et al revealed MIR205HG is downregulated in lesion and associated with improved OS in patients with breast cancer.[2] Nath et al identified the association of MIR205HG with increased response to the anti-EGFR drug erlotinib in lung cancer cells.[3] However, it remains elusive about the predictive and prognostic value of MIR205HG expression for breast cancer receiving neoadjuvant chemotherapy. Given the background, we aimed to explore whether MIR205HG expression could serve as a predictive biomarker for neoadjuvant chemosensitivity and a prognostic biomarker for locally advanced breast cancer.

To be brief for the methods, 10 pairs of fresh primary cancer and adjacent nontumor tissues were surgically resected from patients with breast cancer at Department of Breast Surgery, Renji Hospital, School of Medicine, Shanghai Jiao Tong University between 2015 and 2017. Patients who had ever been treated with preoperative therapy were

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excluded from this cohort. For the neoadjuvant cohort, fresh primary cancer tissues were obtained through core needle biopsy before neoadjuvant treatment from the patients participating in our prospective trials SHPD001 (ClinicalTrials.gov identifier: NCT02199418) and SHPD002 (ClinicalTrials.gov identifier: NCT02221999). In total, 112 patients enrolled between April 2014 and October 2018 with qualified and adequate tissue samples were included in this study. Total RNA was extracted from tissues using TRIzol reagent (Molecular Research Center, USA) and reversely transcribed to complementary DNA using PrimerScript™ RT Master Mix (Takara, Japan). Obtained cDNAs were quantified with RT-qPCR assay labeled with ChamQ SYBR Color qPCR Master Mix (Vazyme Biotech Co., Ltd, China) on LightCycler® 96 (Roche, Germany). The gene-specific primers used were listed in Table S1. All statistical analyses were performed by R v3.5.1. The P value < 0.05 was considered as statistical significance. More details are described in Supplementary Materials and Methods.

It turned out that MIR205HG expression was significantly lower in breast cancer than their matched adjacent non-tumor tissues in the internal cohort ($P = 0.017$; Fig. S1A). This was verified by the external GEPIA dataset ($P < 0.05$; Fig. S1B). For the neoadjuvant cohort, no correlation was found between clinicopathological features and MIR205HG expression as categorical variables (Table S2), while MIR205HG expression was reversely related to Ki67 index as continuous variables (Spearman's $r = -0.25$, $P = 0.009$; Fig. S2). Besides, the TCGA breast cancer data revealed positive correlations of MIR205HG with ER ($P = 0.002$) and PR ($P < 0.001$) and a reverse correlation with HER2 ($P = 0.003$; Fig. S3). Reportedly, Vishnubalaji et al analyzed TANRIC database and found the downregulation of MIR205HG in invasive breast cancer.[2] Such evidence altogether indicates the protective role of MIR205HG regarding to breast. However, *in vivo* and *in vitro* experiments are required to elucidate the role of MIR205HG in breast cancer.

For the neoadjuvant cohort, patients with higher MIR205HG expression achieved a lower pCR rate of 38.1%, compared with 63.6% for those with lower expression (Fig. S4), although the difference was not significant (OR = 0.359, 95% CI 0.099—1.309, $P = 0.121$). However, the multivariate analysis suggested that MIR205HG was independently predictive of pCR (OR = 0.180, 95% CI 0.038—0.858, $P = 0.031$; Table 1). In the subgroup analysis (Fig. S5), the predictive value of MIR205HG expression was verified by the multivariable analysis in postmenopausal (OR = 0.106, 95% CI 0.013—0.843, $P = 0.034$; Table S3), node-positive (OR = 0.184, 95% CI 0.039—0.874, $P = 0.033$; Table S4), and HorR-positive subgroups (OR = 0.110, 95% CI 0.015—0.832, $P = 0.033$; Table S5). Till date, few clinical studies reported the predictive value of MIR205HG for benefit from chemotherapy. Nath et al highlighted that MIR205HG is an indicator of improved sensitivity to anti-EGFR therapy in lung cancer cells.[3] Nevertheless, chemotherapeutic drugs differ from anti-EGFR drug in terms of therapeutic mechanisms. Some basic researches indirectly support our results. Reportedly, MIR205HG could bind to and inhibit SRSF1 in cervical cancer cell lines.[4] Interestingly, SRSF1 increased daunorubicin-induced apoptosis by regulating the alternative splicing of caspase 9 in lung cancer cell lines.[5] In addition, we performed a series of bioinformatics analysis of its predicted RBPs based on our clinical observations. The key GO biological processes were enriched including viral translation termination-reinitiation, miRNA catabolic and metabolic process, pre-miRNA processing, 3′-UTR-mediated mRNA destabilization and stabilization, negative regulation of posttranscriptional gene silencing, etc (Fig. S6A, B). The critical KEGG pathways were spliceosome, RNA transport, IL-17 signaling pathway, transcriptional misregulation in cancer, etc (Fig. S6C, D). Besides, the interactions between the RBPs might give more insight into the role of MIR205HG (Fig. S6E). Notably, several RBPs including IGF2BP3 and FUS play critical roles in cellular response to chemotherapy. Therefore, MIR205HG might induce chemoresistance by modulating SRSF1, IGF2BP3, FUS, etc., which requires future work to validate.

For patients who received neoadjuvant chemotherapy, the MIR205HG-high group showed a significantly better RFS compared with the MIR205HG-low group (log-rank $P = 0.038$; adjusted HR = 0.051, 95% CI 0.008—0.343, $P = 0.002$; Fig. 1A). Besides, MIR205HG was independently prognostic for postmenopausal (log-rank $P = 0.310$; adjusted HR = 0.058, 95% CI 0.005—0.625; $P = 0.019$; Fig. 1B) and node-positive patients (log-rank $P = 0.064$; adjusted HR = 0.056, 95% CI 0.008—0.375, $P = 0.003$; Fig. 1C). The improved prognosis with higher MIR205HG

Table 1 Univariate and multivariate analysis for predictive factors of pCR.

Variables	Comparison for OR	Univariate analysis ($n = 112$)				Multivariate analysis ($n = 112$)			
		OR	95% CI		P	OR	95% CI		P
MIR205HG expression	High *vs.* low	0.359	0.099	1.309	0.121	0.180	0.038	0.858	**0.031**
Age (years)	40 *vs.* <40	0.667	0.201	2.214	0.508	0.454	0.115	1.785	0.258
Clinical tumor stage	T4 *vs.* T2-3	0.426	0.176	1.031	0.058	0.200	0.064	0.629	**0.006**
HorR status	Positive *vs.* negative	0.418	0.178	0.981	**0.045**	0.302	0.112	0.818	**0.018**
HER2 status	Positive *vs.* negative	2.990	1.365	6.552	**0.006**	5.838	2.187	15.579	**<0.001**
Ki67 index	20% *vs.* <20%	3.034	0.614	15.004	0.173	2.102	0.349	12.671	0.418

Abbreviations: pCR, pathological complete response; OR, odds ratio; CI, confidence interval; HorR, hormone receptor; HER2, human epidermal growth factor receptor 2.

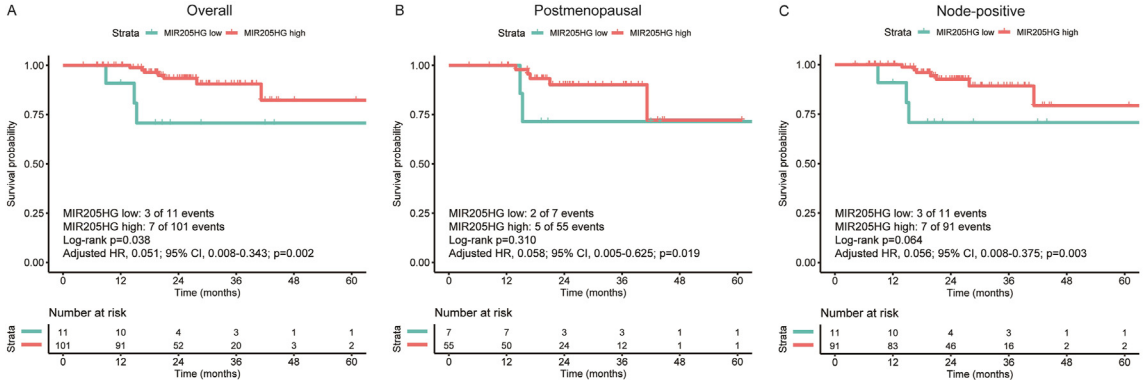

Figure 1 Kaplan–Meier estimates of relapse-free survival according to MIR205HG expression levels. **Notes**: Kaplan–Meier estimates of relapse-free survival in total **(A)**, postmenopausal **(B)**, and node-positive patients **(C)**. **Abbreviations**: HR, hazard ratio; CI, confidence interval.

expression is supported by its tumor suppressor role, and validated by the results of external cohorts. Based on Kaplan–Meier Plotter data, RFS was significantly superior in patients with higher MIR205HG expression to the low-expression counterpart (Fig. S7A), especially in node-positive (Fig. S7B) and ER-positive women (Fig. S7C). The TCGA data showed that patients with higher MIR205HG expression derived prolonged OS compared with those with lower expression (Fig. S8A), especially in postmenopausal (Fig. S8B) and HorR-positive patients (Fig. S8C). Vishnubalaji et al analyzed TCGA breast cancer datasets and also screened out MIR205HG for its correlation with better OS,[2] which is concordant with our data.

This study has several limitations. First, the sample size was small. However, it did reveal the potential underlying rules as a retrospective study of prospective trials. Second, the follow-up period was too short to analyze OS in our cohort. Nevertheless, we concluded the prognostic value of MIR205HG for RFS in our cohort and for OS in public databases. Follow up will be continued in future study.

In summary, our data suggested that MIR205HG could be a promising biomarker for pCR and survival outcomes for breast cancer treated with neoadjuvant chemotherapy. It may help distinguish candidate responders and improve treatment strategy. Further research is warranted to elucidate the mechanisms.

Author contributions

JS Lu and WJ Yin designed and conducted the study. YH Wang, YP Lin, LH Zhou, J Zhang, WJ Yin, and JY Ma collected the clinical data. YQ Xu, CW Yuan, and J Peng carried out RNA extraction and RT-qPCR. YQ Xu performed data analysis and drafted the manuscript. JS Lu and WJ Yin revised the manuscript. All authors have read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interest.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2021.10.001.

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