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Research Paper

Prognostic Value of a BCSC-associated MicroRNA Signature in Hormone Receptor-Positive HER2-Negative Breast Cancer

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ABSTRACT

Purpose: Breast cancer patients with high proportion of cancer stem cells (BCSCs) have unfavorable clinical outcomes. MicroRNAs (miRNAs) regulate key features of BCSCs. We hypothesized that a biology-driven model based on BCSC-associated miRNAs could predict prognosis for the most common subtype, hormone receptor (HR)-positive, HER2-negative breast cancer patients.
Patients and Methods: After screening candidate miRNAs based on literature review and a pilot study, we built a miRNA-based classifier using LASSO Cox regression method in the training group ($n = 202$) and validated its prognostic accuracy in an internal ($n = 101$) and two external validation groups ($n = 308$).
Results: In this multicenter study, a 10-miRNA classifier incorporating miR-21, miR-30c, miR-181a, miR-181c, miR-125b, miR-7, miR-200a, miR-135b, miR-22 and miR-200c was developed to predict distant relapse free survival (DRFS). With this classifier, HR + HER2 − patients were scored and classified into high-risk and low-risk disease recurrence, which was significantly associated with 5-year DRFS of the patients. Moreover, this classifier outperformed traditional clinicopathological risk factors, IHC4 scoring and 21-gene Recurrence Score (RS). The patients with high-risk recurrence determined by this classifier benefit more from chemotherapy.
Conclusions: Our 10-miRNA-based classifier provides a reliable prognostic model for disease recurrence in HR + HER2 − breast cancer patients. This model may facilitate personalized therapy-decision making for HR + HER2 − individuals.

Abbreviations: BCSCs, Breast cancer stem cells; miRNAs, MicroRNAs; DRFS, Distant relapse free survival; IHC, Immunohistochemistry; ER, Estrogen receptor; HR, Hormone receptor; CSCs, Cancer stem cells; RS, Recurrence score; FFPE, Formalin-fixed paraffin-embedded; SYSMH, Sun Yat-sen Memorial Hospital; IRB, Institutional review board; LASSO, Least Absolute Shrinkage and Selection Operator; ROC, Receiver operating characteristic; AUC, Area under curve; EMT, Epithelial-mesenchymal transition; PR, Progesterone receptor; HER2, Human epidermal growth factor receptor 2; BCS, Breast conserving surgery; ET, Endocrine therapy; TAM → AI, Tamoxifen followed by aromatase inhibitor.

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1. Introduction

Hormone receptor (HR)-positive, HER2-negative breast cancer accounts for approximately 60% of all primary breast cancer cases (Sorlie et al., 2003), and around 20% of early-stage estrogen receptor (ER)-positive patients may develop local or distant recurrences after treatment. HR-positive, HER2-negative breast cancer patients usually have a lower risk of tumor recurrence, and thus some of them cannot benefit from cytotoxic chemotherapies (Ring et al., 2004) leading to overtreatment of the patients. Therefore, a prognostic and predictive model for HR-positive, HER2-negative breast cancer is needed in clinical practice. To meet this end, gene assays, such as Oncotype Dx and PAM50, have been developed and validated in multiple clinical trial. However, these models employed computer-based algorithms to select candidate genes for investigation without considering their biological rationales. A hypothesis-driven approach involving factors in DNA repair pathways has been used in a scoring system for ovarian cancer patients treated with platinum-based chemotherapies, which effectively predicted their clinical outcomes (Kang et al., 2012; Paik et al., 2004). Thus, selecting target genes for molecular signatures of prognosis and therapeutic prediction by weighing their biological rationales emerges as an effective and economic approach.

Cancer stem cells (CSCs) are a subpopulation of tumor cells with stem cell like features in solid malignancies, which play an important role in cancer recurrence and metastasis. Breast cancer patients with an elevated proportion of cancer stem cells identified by immunostaining for markers of breast cancer stem cell (BCSC), including ALDH1 and $CD44^{high}CD24^{low}$, reveal unfavorable clinical outcome and poor survival(Dai et al., 2012; Ginestier et al., 2007; Gong et al., 2010). However, the lack of specificity and limited number of BCSC markers restrict their application as effective biomarkers in clinical practice. Moreover, most of these markers do not actually reflect the functional features of BCSCs. On the other hand, our previous study demonstrated that BCSCs express a unique profile of microRNAs (miRNAs), and the deregulated miRNAs play a crucial role in governing BCSC biology (Dalerba and Clarke, 2013; Dirks, 2009). These BCSC-associated miRNAs may function as oncogenes or tumor suppressor genes to regulate self-renewal, anti-apoptosis, invasiveness, transdifferentiation into vascular endothelial cells and chemotherapeutic resistance of BCSCs, and thus contribute to progression and recurrence of

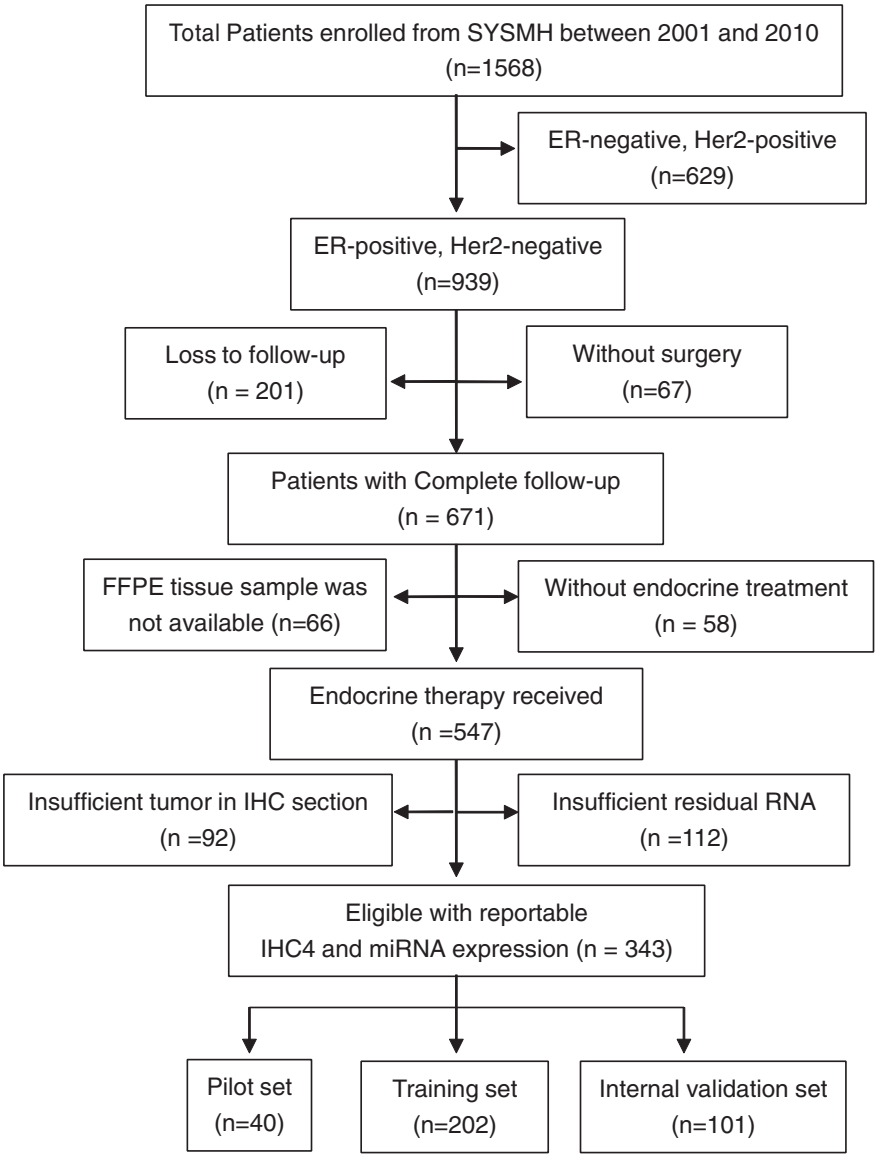

Fig. 1. Consort diagram for the availability of samples for analysis. SYSMH = Sun Yat-sen Memorial Hospital; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2; and IHC = immunohistochemistry.

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