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Commentary

MicroRNAs: Emerging as Highly Promising Biomarkers for Early Breast Cancer Screening

Karl Krupp[1*], Jennifer M Segar[2], Juan Lewis Fernández-Martínez[3], Purnima Madhivanan[4]

[1]Department of Public Health Practice, Policy, and Translational Research, University of Arizona, Phoenix, Arizona, USA; [2]Department of Medicine, University of Arizona Cancer Center, Tucson, Arizona, USA; [3]Department of Applied Mathematics, University of Oviedo, Oviedo, Spain; [4]Department of Health Promotion Sciences, University of Arizona, Tucson, Arizona, USA

DESCRIPTION

The excitement about the possibilities of cell-free tumor DNA (ctDNA) for early diagnosis of Breast Cancer (BC) has waned as doubts emerge about its utility as a biomarker [1]. In BC patients, tumor DNA levels are variable in plasma and their estimated half-life of 114 minutes make their measurement impractical for most applications [2,3]. Studies examining ctDNA in patients diagnosed with BC report high specificity (99%) but low sensitivity (≤ 43%) in women with stage 1 disease, the primary target for screening programs [4,5].

Tumor Deoxyribonucleic Acid (DNA) may have more promise as a prognostic biomarker, but even there, it has limitations. In a recent systematic review and meta-analysis, patients with pretreatment plasma detection of ctDNA had 3.3 higher odds of reduced disease-free survival [6]. Post treatment sampling for ctDNA however, failed to achieve statistical significance. Other studies have found no association between circulating ctDNA levels prior to treatment, and Time To Recurrence (TTR) or pathologic Complete Response (pCR) [7,8]. Mixed results may be related to the variability of ctDNA detection [9]. Agassi, et al. reported that circulating ctDNA expression in BC patients varied widely by tumor size, nodal involvement, and cancer stage [10]. Bettegowda and colleagues showed that ctDNA was found in only about 50% of breast adenocarcinomas [3].

Circulating micro Ribonucleic Acid (RNAs) have been emerging as an alternative to ctDNA, for detection of breast cancer. Short non-coding RNAs are involved in post-transcriptional gene expression [11,12], and microRNAs are remarkably stable and feature in various cancer-related processes including cell division, proliferation, differentiation, cell apoptosis and angiogenesis, making them attractive biomarkers for cancer [12]. Other appealing qualities include detection in most peripheral fluids, and upregulation in early BC stages [13,14]. A 2023 systematic review and meta-analysis found 34 microRNAs substantially dysregulated in early-stage BC that have potential as biomarkers [15]. Other research demonstrates the utility of combining multiple microRNAs into panels for early BC diagnosis. Sharifi, et al. reported that three microRNAs (miR-92a-3p, miR-23b-3p and miR-191-5p) discriminated between patients with BC and healthy controls with an 89% sensitivity and a 96% specificity [16]. Another multi-marker panel developed by Sadeghi and colleagues that contained hsa-miR-106b-5p, -126-3p, -140-3p, -193a-5p, and -10b-5p, detected early-stage BC with 79%sensitivity, 86% specificity and 82% accuracy [17]. These are only a few of the growing number of studies showing the utility of microRNAs as a biomarker in early-stage BC [18].

Equally exciting is research showing that microRNA expression may guide treatment decisions at almost every stage of BC [19]. In a systematic review by Zografos, et al. 110 dysregulated microRNAs were reportedly associated with BC progression [20]. As biomarkers, they are stable for up to four days and resist degradation over multiple freeze-thaw cycles [21,22]. Growing evidence also shows that they operate in multiple pathways regulating BC initiation, progression, and recurrence [23]. The miR-200 family for instance, is associated with tumor initiation, cancer cell stemness, regulation of drug resistance, evasion of immune response, and regulation of genomic instability and mutations [23,24]. Research shows that more than 39 microRNAs are associated with BC recurrence or disease-free survival [25].

What are the challenges moving forward with microRNA-based diagnostics? Perhaps the biggest obstacle to translating current research is the lack of consensus on the methods, samples and platforms used to study microRNA expression [26]. For instance, researchers have assessed microRNA dysregulation in cell lines, serum, plasma, extracellular vesicles, and saliva [27]. Researchers have also used quantitative Polymerase Chain Reaction (PCR), digital PCR, *in situ* hybridization microarrays, next-generation sequencing, and northern blot hybridization to measure microRNA expression just to name a few [28,29]. Even preanalytical and analytical methods are a subject of debate [30,31]. How do we sort through all these evolving issues? Research on the accuracy and inter-comparability of different platforms for microRNA expression have demonstrated that

Correspondence to: Dr. Karl Krupp, Department of Public Health Practice, Policy, and Translational Research, University of Arizona, Phoenix, Arizona, USA, E-mail: kkrupp@arizona.edu

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quantitative PCR is the most accurate and comparable method presently, although this may change [32,33]. As with all research findings, microRNA studies should also come with a disclaimer: empirical research requires extensive replication before results should be trusted [34].

We should also ask whether microRNA diagnostics will eventually reach people that don't have access to large tertiary hospitals. Here, there is also growing and hopeful research. Quantitative PCR multiplex kits for microRNA are already on the market and these will likely be the modality in which the initial diagnostics could be implemented [35]. More exciting yet is research on colorimetric nano-biosensors that will not require sophisticated equipment for cancer detection [36-38]. Other technologies being explored are too numerous to mention here, but they are moving ahead at breakneck speed that will likely match the pace of work on microRNA diagnostics [39].

In summary, circulating ctDNA is not there yet but there are newer and more promising biomarkers that may take its place. As we ponder the future of blood-based diagnostics for cancer, microRNAs may have an exciting place in our future diagnostic armamentarium.

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