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MicroRNA as non-invasive biomarkers of breast cancer

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Abstract: Breast cancer is amongst the most frequently recorded malignancies and the leading cause of cancer deaths in women worldwide including Pakistan. During the recent years, an alarming increase in the incidence and mortality rates of breast cancer in Pakistani women especially in the young girls has been observed. Need for rapid, non-invasive and economical diagnostic procedure therefore is enormous for a population marred with lack of awareness, absence of adequate medical check-up facilities and the socio-economic barriers. Small (18-25 nucleotide long), non-protein-coding microRNAs, which are stably present in the bodily fluid may serve as an ideal class of blood-based biomarkers for cancer detection. This is primarily because the expression of microRNAs is frequently dysregulated in various forms of malignancies including breast cancer. This article reviews the potential of microRNAs to serve as molecular marker of cancer diagnosis, prognosis and/or relapse detection.

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INTRODUCTION

Cancer is the leading cause of death and a major public health concern all around the world. Several lines of evidence indicate that the transformation of a normal/healthy cell into malignant tumor and its invasion/metastasis is a multistep process that is accompanied by well-defined genetic, epigenetic and/or molecular abnormalities [1-3]. The uncontrolled, abnormal growth leads to obstruction of blood vessels, ducts or lymphatics, damages the nerves, causes infections and necrosis of nearby surrounding tissues and eventual death of the patient, if it remains undiagnosed or not countered by treatment.

Whereas cancer cells display some unique characteristics, they are also capable of shedding their nucleic acids into the bloodstream, which may be isolated from serum/plasma and analyzed as surrogate source of tumor DNA. Although healthy subjects do have free circulating DNA in their serum/plasma (15-30 ng/ml) but the mean concentration raises several-folds (upto 180 ng/ml) in patients with different forms of neoplasias[3-4]. The mechanism involved in the nucleic acids release is unknown, however, some recent studies have shown interesting results pointing towards the significance of circulating cell-free nucleic acid measurements in early diagnosis of cancers, viz., lung, breast, oral, colon, esophageal and colorectal [3-8].

The potential use of cell-free plasma DNA/RNA for cancer diagnosis is highly attractive as relatively small amount of blood is required for the extraction of nucleic acids and the sample may be drawn repetitively enabling the analyses of disease progression and treatment response in the patients more objectively. Although El-Hefnawy, *et al*. (2004)[9] reported that analysis of extracellular plasma RNA, which is an extremely labile molecule, may not be useful for cancer detection or recurrence monitoring but several others remained successful in exploring the usefulness of gene expression profiling of plasma RNA in differentiating the tumor from the healthy conditions [6-8].

Amongst the circulating nucleic acids, small (18-25 nucleotide long), non-protein coding RNA i.e., microRNA (miRNA) are exceptionally stable in the RNase rich environment of the bloodstream and other bodily fluids and thus may be well-exploited in blood-based screening of cancer including the breast cancer. This mini-review discusses the potential of microRNAs in breast cancer diagnosis, prognosis and/or relapse detection.

Discovery and functions of microRNA

Small, non-coding *lin-4*, which is involved in the regulation of lin-14 protein expression, was the first miRNA that was discovered in *Caenorhabditis elegans* during 1993[10,11]. Few years later, *let-7* (another *mi*RNA), was identified in *C. elegans* [12] and since then several thousand miRNAs have been discovered in different organisms including human beings. The miRBase (www.mirbase.org), a searchable database of miRNAs, contains 28645 published miRNA sequence entries, as per its Release 21 (June 2014) and this

number is increasing with great speed. Interestingly, the studies have shown striking similarities in lin-4 and let-7 from diverse organisms i.e., flies to human beings. This further reflects evolutionary conservation in miRNAs function, which mainly is related to the regulation/control of gene expression in different organisms.

MicroRNAs, like the messenger RNA of higher eukaryotes, are transcribed as long primary-transcripts (pri-miRNA) by type II and type III RNA polymerases, which are processed in the nucleus into relatively small sized (70-100 nucleotides long) precursor miRNAs (pre-miRNA) prior to their export to the cytoplasm where they undergo further processing. Following processing within the cytoplasm, the mature single-stranded miRNAs, in association with / as part of a complex regulatory system (RNA induced silencing complex), play key role in the regulation of gene expression at the post-transcriptional level. Several studies have reported that these interactions with the 'target' may involve direct binding of miRNA to the open reading frames (ORFs) of the target mRNA sequences or the binding of miRNA to the partial-complementary sequences present at the 5′- or the 3′-untranslated regions (UTRs); later being the most common event [10-14].

MicroRNA as biomarkers of breast cancer

During the last decade, several research groups have reported that miRNA impact the cell proliferation as well as apoptotic mechanisms and thus perform fundamental role in tumorigenesis or cancer progression [15-17]. In breast cancer, one of the most prevalent forms of cancer in women worldwide, aberrant expression levels of miRNAs at the different stages of malignancies have been observed. Many different methods were employed for analyzing the expression patterns in these studies which include Northern hybridization (low-throughput technique), microarray, quantitative real-time PCR and next-generation sequencing platforms. By integrating the miRNA expression data with messenger RNA data, new miRNA-gene interactions could be explored which helped in establishing the link between miRNAs

Table 1: Functional microRNAs that are reported to be up- or down-regulated in breast cancer

miRNAs	Status	Functional role in breast cancer	References
hsa-miR-7a	Down-regulated	Suppresses cell proliferation and induces apoptosis	[20] Shi et al., 2015
hsa-miR-10b	Upregulated	Metastasis	[22] Devulapally et al., 2015
hsa-miR-16	Down-regulated	Apoptosis	[24] Ng et al., 2013
Has-miR-20b	Upregulated	Metastasis, cell proliferation	[15] Zhou et al., 2014
hsa-miR-21	Upregulated	Metastasis, cell proliferation	[21] Si et al., 2013
hsa-miR-23b	Upregulated	Post-transcriptional regulation	[25] Fu et al., 2011
hsa-miR-27a	Upregulated	Cell cycle progression, G2-M checkpoint regulation	[26] Heneghan et al., 2010
hsa-miR-34a	Upregulated	DNA damage, proliferation	[27] Blenkiron et al., 2007
has-miR-125	Down-regulated	Inhibits tumor cell growth and cell invasion	[16] Hsieh et al., 2014
hsa-miR-145	Down-regulated	Suppresses cell invasion and metastasis	[27] Blenkiron et al., 2007
hsa-miR-155	Upregulated	TGF-beta signaling	[26] Heneghan et al., 2010
hsa-miR-191	Upregulated	Proliferation and migration	[28] Ahmad et al., 2013
hsa-miR-195	Upregulated	Apoptosis	[26] Heneghan et al., 2010
hsa-miR-200c	Down-regulated	TGF-beta signaling	[29] Kayani et al., 2011
hsa-miR-206	Upregulated	ER signaling	[30] Kondo et al., 2008
hsa-miR-451	Upregulated	Post-transcription regulation	[24] Ng et al., 2013
hsa-miR-491	Down-regulated	Inhibits cell proliferation	[20] Shi et al., 2015
hsa-miR-520c	Upregulated	Metastasis, migration, invasion	[28] Ahmad et al., 2013
hsa-miR-708	Down-regulated	Inhibits metastasis	[19] Ryu et al., 2013
hsa-miR-888	Upregulated	Metastasis	[23] McCubrey et al., 2014

and breast cancer oncogenesis, invasion and/or metastasis [17,18]. On the basis of these results, breast-cancer associated miRNAs have been grouped into two main categories: 1) one that activates cell proliferation/ oncogenesis and hence been referred as 'oncomirs' and 2) the others that inhibit/suppress tumor cell progression. Some of these reported miRNAs along with their functional roles in breast cancer have been summarized in Table. 1.

CONCLUSION

All referenced reports have highlighted the potential of miRNAs in differentiating normal and metastatic tumor cells. Major challenge, however, is to validate the candidate miRNAs in large study cohorts. Moreover, due to the complex nature of cancer/neoplasia, a single miRNA is unlikely to serve as predictive biomarker; instead a panel of such tumor- and stage-specific miRNAs should be engaged in breast cancer screening compliance.

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