REVIEW ARTICLE

Role of MicroRNA in Breast Cancer

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ABSTRACT:
Breast Cancer is the commonest cancer affecting women worldwide especially in Asia. Several proteinaceous,genetic and epigenetic biomarkers areallied with the disease but their efficacy as vigorous and robust indicators of disease remains uncertain. The need to detectand differentiate aggressive from non-aggressive breast tumors at cellular level is being investigated. MicroRNAs seem to be a promising marker to identify the disease before it reaches aggressive level. MicroRNAs (miRNAs) are small 18-24 nucleotide RNAs which regulate the expression of approximately 30% of human genes. Their expression is frequently deregulated in cancers. .miRNAshave been found in significantly large copy numbers in serum/plasma of cancer patients. The stability of the serum miRNAs is not compromised even if the samples are treated with RNase or incubated at room temperature over prolong periods or subjected to repeated freeze-thaw cycles. miRNAs that are breast cancer-specific can therefore be employed as disease predicting biomarkers.
Keywords: Breast cancer, Micro RNA, Role, Serum, Stability, Biomarkers.

INTRODUCTION:

MicroRNAs (miRNAs) are described as non-coding ribonucleic acids with 18-22 nucleotide bases. Expression of miRNAscan be transformed in breast cancer when compared to healthy breast tissue.There may also be difference in expression in breast cancer subtypes. Their mode of action maybe as either oncogene or tumor suppressors; this shows that their expression is deregulated in cancers. MicroRNAs are also used during development stages and have been found to be involved in processes such as proliferation, differentiation and apoptosis. Breast cancers are controlled by cancer-restricted signaling pathways.[1]Methods to diagnose breast cancer are many, the most common being mammography which is utilized for screening and diagnosing purposes. But scanning is not the gold standard for diagnosis as confirmation of cancers is by means of biopsy which is an invasive procedure. This procedure is performed when the patient complains of lumps in the mammary gland.[1,2] Breast Cancer originates at cellular level but diagnosis can only take place once the signs and symptoms appear. Multiple genetic based procedures have been determined to mark the genetic signatures causing this cancer.In comparison to other continents, Asia has the highest rates of breast cancer; it is considered as the most frequently occurring cancer of origin in women in this region. Pakistan's leading cancer to date in women is also breast cancer.[2,3] Till to date no robust and precise method has been determined to predict the occurrence of this deadly disease. Hence there is a need to identify sensitive biomarkers that will be beneficial for discovering breast cancers and differentiate between aggressive versus non-aggressive tumors.[4,5,6] The association of manifold miRNAs with breast cancer is becoming a promising method for diagnosing this cancer at cellular level which may lead to diminishing prevalence of the disease. Therefore there is a dire need to identify miRNA and label them as diagnostic factors. They may also be useful in prognosis of the disease, as well as possible remedy targets.[7, 8, 9] Literature search was performed by using database of PubMed. The keywords used were microRNA (52 searches) and breast cancer (169 searches). PERN was used by database of Bahria University. This included literature and articles from international sources; local literature on this topic was not available. Out of these, 35 articles were shortlisted which discussed relation of microRNA genetic expression in breast cancer. These articles were consulted for this review.

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LIEERATURE REVIEW:

MicroRNAs (miRNAs) are RNAs of 18-22 nucleotides (nt) in extent which are found profusely in plants, animals and also viruses.These small genes are formed by a sequence of events first in the nucleus and then in the cytoplasm. In the nucleus they are originally transcribed as primary-mi-RNA (pri-miRNA) due to the presence of RNase polymerase II which is 100-1000 nucleotide in length. This is followed by the process of capping and polyadenylation. Further this pri-miRNA is cut by RNase III, DROSHA and its co-factor DGCR8 into smaller70 nucleotide stem loops called as pre-RNA.[7,8] This pre-RNA journey`s from nucleus to cytoplasm by means of exportin-5. The loop region of pre-RNA is removed by DICER (RNase III) and its binding partner TRBP. A mature miRNA-miRNA* duplex is released. [9] The single dominant strand is incorporated with RISC (RNA induced silencing complex) to finally regulate gene expression by complementary-base pair interaction resulting in interference with translational ability and stability of target mRNA or it may result in its degradation.[10,11] Multiple miRNAsare linked with breast cancer and it is a fact now that most of these post transcript structures may transform complex functional

networks of mRNAs. Diagnostic tools are available but more genetic markers which would allow the prevention of the disease are required. This is where the importance of identifying miRNAs as potential biomarkers is of great importance to scientists.An additional advantage related tomiRNAs in oncology is that they are remarkably stable and are notably detectable in serum and plasma. Triple negative breast cancer which is aggressive in nature shows strong linkage to various miRNAs. Five microRNA (miRNA)clusters which include miR-17-92 at 13q 31.3, miR-183-182 at 7q32.2, miR-200-429 at 1p 36.33, miR-301b-130b at 22q 11.21, and miR-532-502 at xp 11.23 were upregulated in triple negative breast cancer. This research is a promising research in clearly identifying global miRNAs regulatory characteristics in ER–ve, PR-ve and HER2 –ve breast cancer.[3]Studies related to gene expression have proven that estrogen and progesterone receptors (positive/-negative) are expressed as distinct disease at molecular level. Human epidermal growth factor receptor 2(HER2) if tested significantly affirmative promotes the growth of cancer cells and is normally involved in functions such as signal transduction pathways influencing cell growth and differentiation.[4] Huang GI et al has reported that ER/ PR negative cancers show high expression of miR-21. ER, PR and HER2 negative (triple negative tissue) have exhibited very high expression of some miRNAs e.g. miR-21, miR-155, miR-145, miR-16.[5] This finding is consistence with several other reports indicating the aggressive nature of tumor with negative receptor proteins. Metastasis of cancer is also linked to triple negative tumors and have poor prognosis and do not respond properly to chemotherapy. Several current studies have shown the importance of detecting blood based miRNAs in breast cancer and this has revealed new biomarkers. A pilot study shows that the presence of urinary miRNAs may also be used to diagnose breast cancer. It was shown that urinary miR-155 levels were significantly higher in breast cancer patients as compared to healthy controls. This urinary miRNA was quantified by real time PCR.[6]

A greater proportion of miRNAs which are around 600 in number, have been identified already which participate in development as well as regulate developmental sequences in a growing fetus.[12,13,14] This includes mechanism such as increase in the cell number of organs, their differentiations to form body structures and these genes may also control the process of apoptosis and cellular metabolism.[15] A substantial number of genes and their expression are controlled by miRNAs impact. It is likely that any changes in their expression or DNA sequence are likely to culminate in a diseased state. A study on Hispanic women showed a different pattern in women after pregnancy who are diagnosed with breast cancer in less than 5 year of pregnancy. This postpartum development of breast cancer showed two-fold or higher difference in expression with miR-138, miR-660, miR-31, miR-135b, miR-135b, miR-17, miR-454 and miR-934 being overexpressed. This study forecasts that miRNAs may have different pattern depending upon the ethnicity and condition of women.[15, 16]The miRNomes of several cancers have been studied and such studies have identified miRNAs whose expression levels are robustly compromised. In biopsies obtained from breast cancer patients such miRNA profiling exercise revealed 29 miRNAs whose expression was down-regulated in tumors.[1] A recent study revealed specific miRNA signatures that were unique for several benign and malignant tumors, some of the common organs include the breast, prostrate, lung and the intestines. In advanced breast cancer, metastasis to the brain is a cause of death in such patients. The phenomena behind this is that cancer cells migrate through blood brain barrier, however the molecular mechanism still remains to be investigated. It has been shown that the delivery of these cancer cells maybe via miRNAs which maybe involved in the breakdown of the blood brain barrier. The identified miRNA which may promote this event is miRNA-181c.This identified miRNA causes destruction of blood brain barrier through abnormal localization acting via down regulations of its target gene PDPK1.[17, 18] Some of the highly expressed miRNAs that were found in this study were miRNAs 155, 17-5p, 20a, 21, 92, and 106a. Functional genomic validation of some of the miRNAs identified through the gene array screens proved that miRNAshave a definite action as oncogenes or suppressor genes implying that any mutations or un-programmed changes in their expression levels has the potential to trigger uncontrolled cellular proliferation miRNAs that are metastasis promoters as well as suppressors have also been identified.[19, 20] In this decade several studies carried out on cell lines derived from breast cancers as well on primary tumors have been performed in an effort to identify miRNAs which are unique to these types of cancers and which are abnormally expressed. Profiles obtained from 76 breast cancer tissues which were primary in nature as compared to 34 non malignant counterparts led to the identification of 5 different miRNAs ,that were deregulated. Among the given cases 3 showed that miR-10b,-125b, and 145 were most significantly down-regulated. In addition 2miRNAs were found to be up-regulated (miR21, and miR155).[1] In another study, miRNA profiles were obtained from primary breast cancers, in which 5were normal breast samples and 21 samples included breast cancer tissue cell lines.On further experimentation of these samples derived from breast cancer individuals,exposed conspicuous variances in miRNA expression when ER-ve and ER+ve tumors were studied. The investigators also showed that they were able to discriminate between HER2+ and HER2– cancers based on miRNA signature.[3]

Recently, Literature search revealed that Mitchell et al has studied the presence of miRNA in human plasma which are unusually stable culminating to the fact that they are able to resist degradation even if exposed to freeze thaw cycles repeatedly. These micro genes also have the capability tosurvive prolonged incubations at room temperature. This experiment demonstrated that miRNAs are secure from endogenous RNase performance in the plasma of human beings thus prposing that they may be considered as a portion of complexes

or may be associated with exosomal vesicles. Although inconclusive, this study also found that mir-141 can serve as a biomarker.[21]

Studies related to gene expression have proven that estrogen and progesterone receptors (positive/negative) are expressed as distinct disease at molecular level. Human epidermal growth factor receptor 2(HER2) if shown to be present promotes the growth of cancer cells and is normally involved in functions such as signal transduction pathways influencing cell growth and differentiation. Correlation between clinico-pathological factors and miRNAs concentration is very important especially for treatment purposes. Multi variant analysis of breast cancer in various stages show very high concentration of some circulating miRNAs but this may be independent of ER, PR and HER2. Henegham reportednumber of miR-21 in a greater quantity in the circulation in ER negative disease as compared to those individuals whose biopsy showed ER positive breast cancer.[22] They also tried to establish a relationship between this circulating gene, type of breast cancer which maybe of in situ type but may also be invasive, the subtype and HER2 status but no significant relation could be established.[2]

In a study on Asian Indians and Pakistani, the receptor status in relation to age was analyzed showing that in ages between 40-50 years an increased percentage of ER/PR negative disease was noted as compared to the younger group. However over time pathologically assessed cancers show that triple negative receptors are more common in this part of the world which may result in recurrences, resistance to treatment and metastasis in brain and spinal cord.[21,23]

Pakistan presently is considered to have the uppermost rates of breast cancer in Asia; it has also been proved that breast cancer is the most common cancer among other gynecological cancers. India and Pakistan have reported a significant rise in the incidence of breast cancer. ER, PR negative breast cancer are more common according to several reported studies.[2] In a tertiary cancer care hospital in Karachi, a study of over a span of nine yearswas conducted which showed that breast cancer was the most frequently occurring cancerin females and consisted of 38.2% of the entirecancer cases inducted in that institution, this was proved to be highest in Asia.[23]

For breast cancer treatment to be attainable we must first identify biomarkers which will robustly reduce universal morbidity and mortality due to this disease. Research also shows that there is a difference in the expression of miRNAs in various diseases. There is indication that there may also be different expression in various populations. Hence the importance of identifying these micro genes in a sample of Pakistani population will be of benefit as we will be able to differentiate it with the western data. [16] This will however be possible with larger sample size studies with participants from all parts of the country. Smaller studies should also be conducted as they may provide cues for further studies.Therefore identification and the quantity of tumor-derived miRNAs in the circulation are an essential methodology for blood based exposure of human cancer which will lead to (1)diagnosis and (2) prognosis of cancer by non-invasive methods and will (3) determine their relation with protein receptors in tissue biopsy of breast cancer and level of aggressiveness of the breast tumor.[24, 25]

As developing confirmations highlight the significance of miRNAs in diagnosis and prognosis, the usefulness of miRNAs based breast cancer therapy is also being explored. Many miRNAs have been associated in numerous cancers, including breast cancer. They are known to control cell cycle and developmental processes. Therefore it is likely that miRNAs are beneficial targets for exploring in anti-cancer treatments.[25,26]The therapeutic strategies based on miRNAs suggest a substitute for targeting multiple gene networks that are controlled by a single miRNA. These approaches can be expressed by either antagonizing or reinstating the functions of miRNAs[27,28,29] Anti-miRNA 2-O-methyl or blocked nucleic acid oligonucleotides used to inactivate oncomiRNAs such as miR-21 in breast cancers may taper down tumor growth. Anti-miR-21induced decrease in cancer growth was revealed to be potentiated by the addition of topotecan (a chemotherapeutic agent), this is an inhibitor of DNA topoisomerase I.[29,30,31] Such novel event highly recommends that suppression of the oncogenic miR-21 could sensitize tumor cells to anti-cancer therapy.This is a promising prospect for patients displaying a poor response to initial stages of chemotherapy.[32,33,34,35] Another point of interest shows the capability of miRNA 34a to inhibit proliferation and migration of breast cancer through down regulation of Bcl-2 and SIRT1[36,37,38,39,40]

CONCLUSION:

It is important to check the contributory factors (genetic and non-genetic) since incidence rates are on such a rapid rise. As with many cancers, early detection of breast cancers has been inadequate and methods for prognosis and diagnosing the disease are limited to invasive procedures. Due to advances in understanding the cancer cell at molecular level, development of several targeted therapies are progressing and have also lead to advancement in the treatment. To achieve such individualized treatment appropriate targets must be identified.

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