SMGroup

Expression Profiling of mRNA and Micro RNA in Detection of Breast Cancer

D Devananda[1]*

[1]Chan Re Diagnostic Laboratory, India

***Corresponding author:** Nandini Manoli, Department of Pathology, JSS Medical College, a constituent of JSS University, Mysore, Karnataka, India, Email: nandinimanoli65@gmail.com

Published Date: April 30, 3016

INTRODUCTION

Breast cancer is the second most common cancer diagnosed and leading cause of death in women worldwide. [1-3]. In 2015, an estimated 231,840 new cases of breast cancer were diagnosed in the United States and it was estimated that breast cancer accounts for 27% of all new cancer cases and 15% of cancer-related mortality in women [4]. Breast cancer is a complicated disease involving many morphological, clinical, and molecular characteristics. Incidence and mortality rates vary greatly around the world with higher incidence presenting in developed countries than in developing counties [1]. Even though, the aetiology of breast cancer is not clear, many factors have been described previously as being linked with increased or decreased risk of cancer development. These risk elements have been classified as: hereditary, endocrine, reproductive, environmental and life-style elements (e.g., diet, physical activity, toxic substance exposure) [5].

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

For many years, breast cancer has been diagnosed based on conventional parameters, such as histological type, grade, tumor size, lymph node involvement, vascular invasion and biomarkers such as estrogen receptor, progesterone receptor, and epidermal growth factor receptor 2, which are the most common proficiencies used for the patient management. With advanced imaging techniques like digital mammography, tomosynthesis, ultrasonography, magnetic resonance imaging, nuclear medicine, and genomic techniques, such as microarrays and real-time RT-PCR, flowcytometry, breast cancer diagnostics is undergoing a significant evolution. Newer molecular techniques have both altered our understanding of the basic biology of breast cancer and allowed the basis for new methods of "personalized" prognostic and predictive testing [6]. Imaging technologies have advanced breast cancer diagnosis, helping in treatment and early detection of primary or metastatic lesions, differentiating benign from malignant lesions and promoting intra operative surgical guidance, postoperative specimen evaluation and survival rates. Advancement in Genomic and transcriptomic technologies help in the analysis of gene expression signatures and mutation status possible so that tumors may be differentiated more accurately with respect to diagnosis and prognosis.

For many years, breast cancer has been divided based on clinical and pathological criteria such as histological features, grade, tumor size, lymph node involvement, and vascular invasion. However, the predictive power of these criteria for selection of the optimal therapeutic approach is limited. In recent years, with substantial advances in genomic and proteomic technologies researchers are better able to interpret the biological characteristics of tumour cell and identify new biological biomarkers involved in multiple signaling pathways that can advance general clinical practice like early detection, prognosis, diagnosis, detection of recurrence after therapy, risk assessment, identification of targets for therapy, prediction of response to therapies (prediction), monitoring clinical outcomes of therapies, and imaging disease processes.

Advancement of new molecular techniques, such as QPCR and microarrays, have allowed testing of numerous biomarkers and a more detailed classification of breast cancer, contributing to a personalized prognostic and predictive approach of management.

This Book Chapter Provides an Overview of the Following

The advent of Molecular diagnostics and pharmacogenomics, into the current quest against cancer has sparked a great deal of excitement among researchers and clinicians alike. Of late, the discovery of oncogenes, tumour-suppressor genes and RNA interference, along with the diagnostic applications of Molecular techniques like the microarray, Real time - PCR and various other biomarker assays, promise a better understanding of cancer and offer an even better chance of winning the fight against it. In this chapter, the techniques mentioned above are discussed concisely to help a better understanding of concepts.

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

MICRO ARRAY

The microarray technique, based on the principles of complementary base pairing and hybridization, is a 2D array on a solid substrate, usually a Glass slide or Silicon thin film, that assays large amounts of biological material using high throughput screening, miniaturized and multiplexed with parallel processing and detection.

The various types of Microarray include

1) DNA Microarrays, used to analyze codas, Oligonucletides, Bacterial Artificial Chromosomes **(BAC)** and Single Nucleotide Polymorphisms **(SNP)**.

2) MMChips, for the surveillance of microRNA populations.

3) Protein Microarrays, to validate the presence of particular proteins.

4) Peptide microarrays, for detailed analyses of Protein-Protein interactions.

5) Tissue Microarrays, to facilitate Multiplex Histological Analyses.

6) Cellular Microarrays, for the interrogation of the living cells and analyzing their behaviours at particular situations.

7) Antibody Microarrays, for the purpose of detection of Antigens and Protein expressions.

8) Carbohydrate Microarrays, called Glycoarrays, used for large scale screening of samples for the presence of a particular Carbohydrate.

9) Phenotype Microarrays, an approach for High throughput phenotyping of cells and genomes.

10) Reverse Phase Protein Microarrays, for estimating the levels of protein expression in biological samples.

11) Interferometric Reflectance Imaging Sensor **(IRIS)**, for accurate multiplexing of Protein-Protein, Protein-DNA and DNA-DNA interactions, without the use of fluorescent labels.

Evolved from Southern Blotting, the DNA Microarrays are a collection of microscopic DNA spots attached to a solid surface, mainly for the analysis of gene expression profiling. The technique, also known as the DNA chip or the Biochip, uses the complementary base pairing principle to simultaneously measure the expression levels of a large number of genes, or to analyze the genetic make-up of multiple regions of a genome, a cell, an organism, or an individual (Figure 1).

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

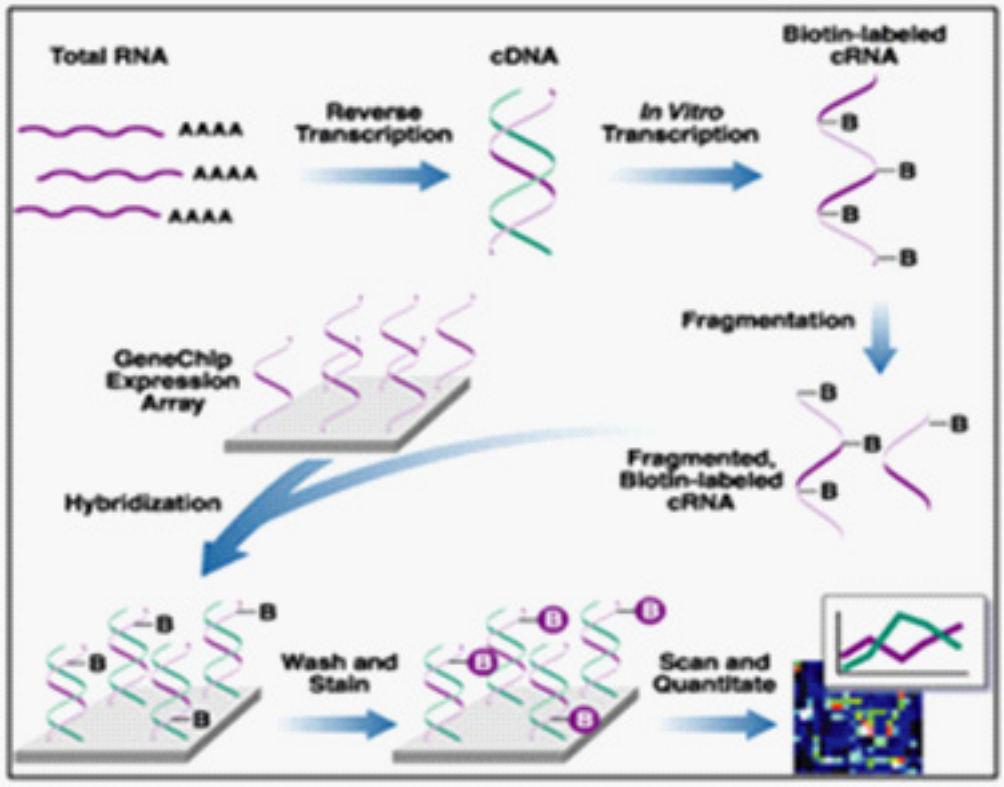

Figure 1: DNA chip or the BioChip, uses the complementary base pairing principle to simultaneously measure the expression levels of a large number of genes. (Source: dkfz. German Cancer Research Centre, Affymetrix Page.)

The ease of understanding the implications of non-hereditary mutations, particularly in the case of a specific cancer and ascertaining the distinct variations in the genotype of the disease and the diseased, makes DNA microarray, clinically and scientifically more preferred a technique in diagnosing cancer. As a matter of fact, Breast cancer has been among the earliest and most intensely studied diseases using this technique. This concept of Molecular profiling has not only enriched understanding breast cancer heterogeneity but also has facilitated a new prognostic and predictive information that could enhance the treatment efficacy [7] (Figure 2).

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

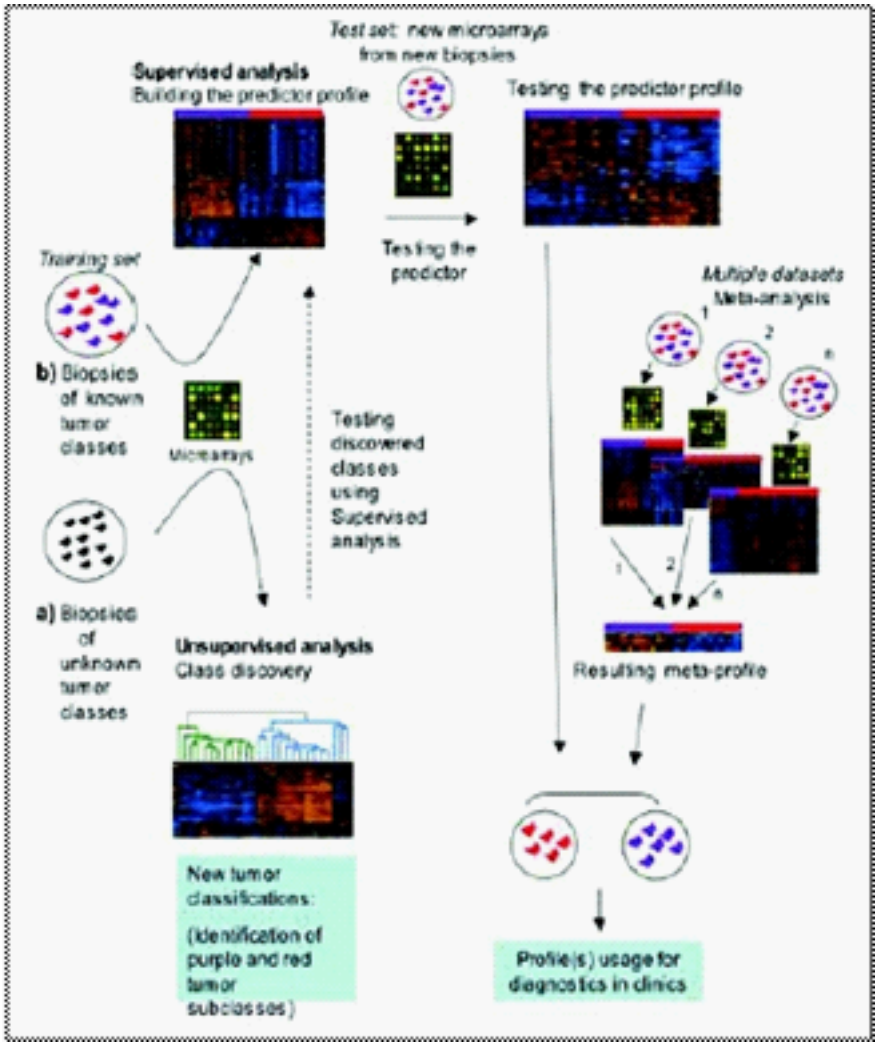

Figure 2: DNA microarray, clinically and scientifically more preferred a technique in diagnosing cancer. Analysis of microarray data to discover new tumor classification and to build gene predictors for cancer diagnosis. a) For class discovery, biopsies, which we hypothesize belong to different tumor classes (black biopsies), are analyzed by unsupervised methods to see the global similarities and differences between them at the molecular level. In this case, the biopsies formed two main clusters (green and blue) depending on their gene expression, which means that there are indeed 2 different classes within our samples. b) To build a gene predictor profile for cancer diagnosis we analyze samples that belong to the 2 classes we want to distinguish (purple and red). The supervised analysis will give the list of genes that are differentially regulated in one class with respect to the other. This list of genes will form the predictor profile. A new group of samples of known classes (test set) is analyzed to test the validity of the predictor. For this, the gene predictor profile is used to assign test set samples to one of the classes. Gene profiles

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

created in different laboratories can be combined in a meta-profile. Resulting profiles are applied for diagnostics. (Source: Madame Curie Bioscience Database).

Over time, the microarray data for cancer diagnosis has been refined to predominantly identify the differences at the molecular levels. As of now, there are 3 different ways in which the microarray technique could help:

1) **Class Comparison:** To identify the differences, at molecular levels, within a known group or class of cancer.

2) **Class Prediction:** To diagnose or 'Predict', the group or class of cancer, to which a new tumor sample belongs.

3) **Class Discovery:** To verify and confirm the possibility of emergence of a new group or class of cancer, from a new tumor sample procured.

REAL TIME - POLYMERASE CHAIN REACTION

The process of amplification of specific fragments of DNA, called the Genes of Interest, is known as the Polymerase Chain Reaction **(PCR)**. Real time-PCR is a specialized technique that allows a PCR reaction to be visualized "in Real Time", as the reaction progresses. This quantitative PCR method is used extensively to determine the number of complementary PCR templates, such as DNA or cDNA, in a PCR reaction, which in turn would significantly improve detection of cancer by the virtue of its sensitivity and high-throughput capacity.

The RT-PCR is of 2 Major Types, Depending on the Type of Dye used for the Analysis. They are:

Probe Based RT-PCR

In this type, the use of hydrolytic probes, designed to increase the specificity of quantification, is seen. The probes used are fluorogenic, containing a reporter fluorescent dye, called the Fluorophore, and a quencher dye. While the fluorophores, attached to the 5' end of the primer, detect only the probe sequence containing DNA, the quencher molecule, seen attached at the 3' end of the primer, controls the fluorescence emitted by the former. This inhibitory property of the quencher is observed only when it is in proximity to the fluorophore. Upon completion of the hybridization, the quencher molecule is separated from the fluorophore and thus allows the necessary fluorescence to be emitted for the purposes of detection. The reporter is specific only to the probe sequence containing DNA and thus enables a better data resolution even in the event of non-specific DNA amplification. The principle of the entire probe based assay relies upon the 5' to 3' exonuclease activity, of the Taq Polymerase, to cleave a dual labelled probe during hybridization. TaqMan® probe is one of the commercially available RT-PCR probes [8] (Figure 3).

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

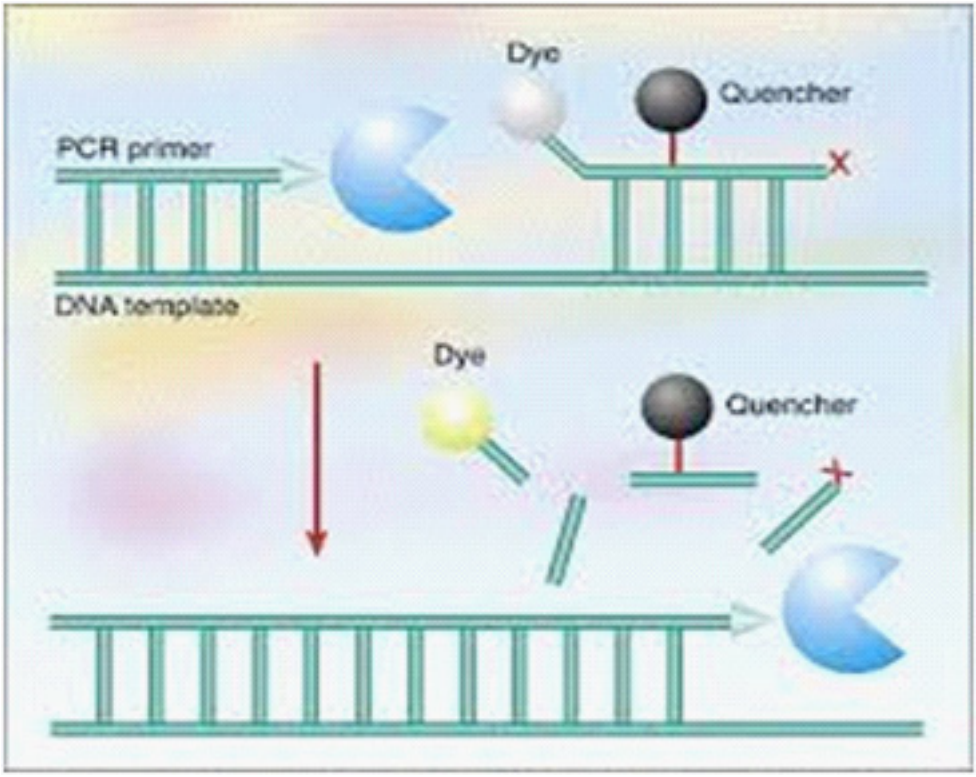

Figure 3: Dyes & Fluorescence detection chemistry in qPCR-Biosearch technologies. (Image courtesy: Dyes & Fluorescence detection chemistry in qPCR-Biosearch technologies).

The probe based assays are widely used in Gene Expression assays, Pharmacogenomics, Human Leukocyte Antigen **(HLA)** genotyping, Viral Load determination, Bacterial identification, DNA quantification, SNP Genotyping and Verification of microarray results [9].

Intercalator-Based RT-PCR

Use of dyes that preferentially bind to the double stranded DNA is practiced in this flavour of RT-PCR. The resulting DNA-Dye complex is excited upon absorption of light at a particular wavelength and emits fluorescence. The light emitted is detected and used in quantitation of the DNA amplified. Due to their low specificity to single stranded DNA, RNA and compared to the probe based assays, intercalator-based dyes are less relied upon and used only in Gene expression, DNA quantitation and Chromatin Immunoprecipitation **(ChIP)** assays. The most common intercalator-based probe commercially available is SYBR Green, an asymmetrical Cyanine dye.

As well, RT-PCR is used in the validation of the microarray. Although there would be occurrence of difference in the results of both, due to a variation in the normalization procedure in the Amplification assay, when compared to the Chip based assay (Figure 4).

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

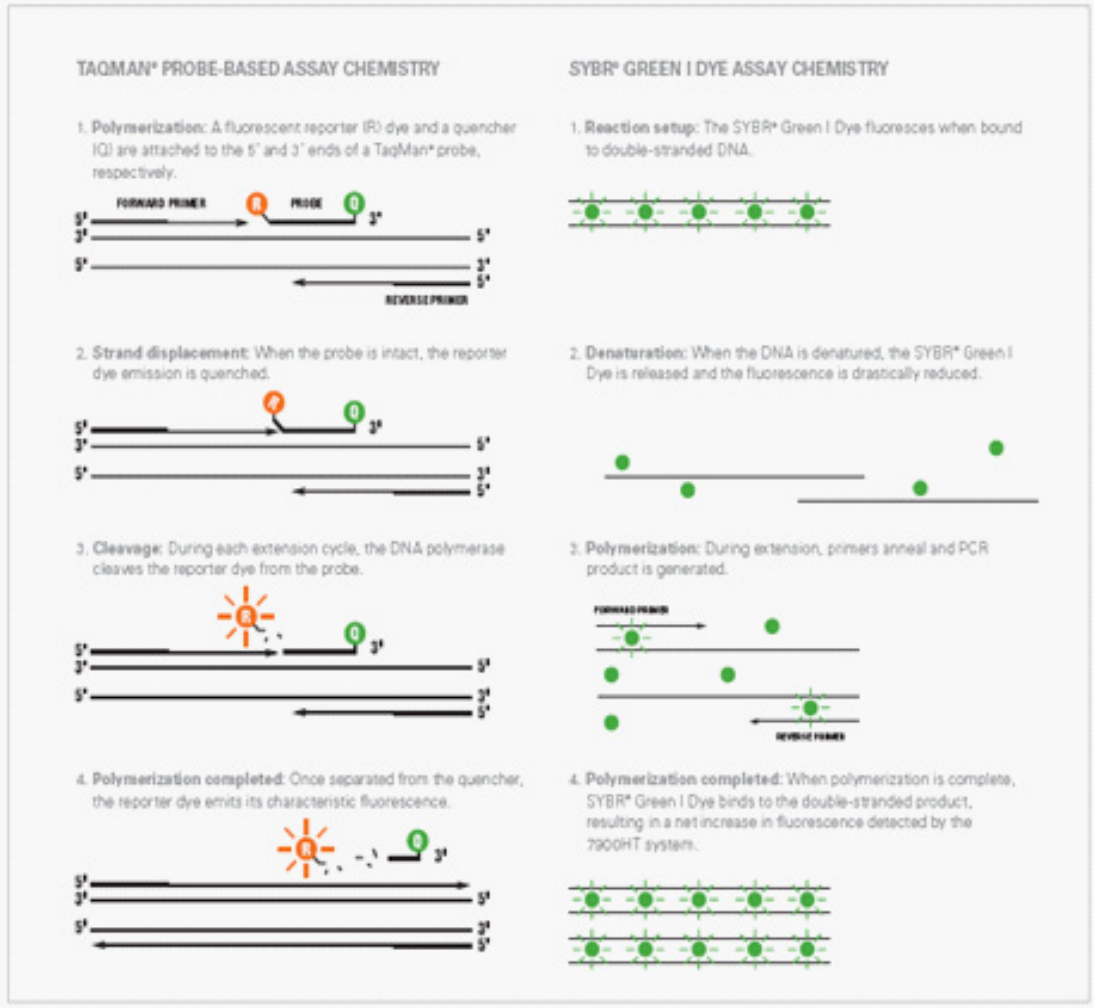

Figure 4: Real Time - Polymerase Chain Reaction assay of both Taqman probe based assay chemistry and Sybr green based assay chemistry. (Source: http://www.biosyn.com/tew/taqman-vs-sybr-green-chemistries.aspx)

RNA INTERFERENCE

Also known as Co-suppression, Post-transcriptional Gene Silencing **(PTGS)** or Quelling, RNA interference **(RNAi)** is a conserved biological response in which Gene expression is inhibited by RNA molecules, typically by promoting the destruction of the specific mRNA molecules, with the help of the RNA Induced Silencing Complex **(RISC)**. The first well understood work on RNAi, by Craig Mello and Andrew Fire, won them the Nobel Prize in Physiology or Medicine for the year 2006.

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

There are 2 types of RNA Molecules that Contribute to the PTGS Phenomenon. They are:

Small Interfering RNA (siRNA)

The double stranded RNA with about 20 to 25 base pairs, with a Phosphorylated 5' end and a Hydroxylated 3' end. The siRNA are exogenous, mostly of viral origin, are fully complementary to the targeted mRNA molecules, as they are designed and introduced into the organism based on the requirement of the study being conducted.

Micro RNA (miRNA)

These gene silencing RNA molecules, containing 20 nucleotides, are usually of an endogenous origin. Found in Plants, Animals and Viruses, miRNAs have remained a part of the organism over evolution. The miRNAs, as a natural component of cell, are partially or fully complementary to the target messenger molecules and are responsible for the natural gene regulation processes occurring in the organism.

Chronic Lymphocytic Leukaemia was the first disease known to be associated with miRNA dysregulation. Dysregulation of certain miRNAs, better known as the Oncomirs, has been associated with specific cancer forming events and many different oncomirs have now been identified in numerous types of cancers. Associated with carcinogenesis, malignant transformation and metastasis, some oncomir genes are oncogenes, while a few other oncomir genes are tumor suppressors in normal cells. As a result, under expression of the latter or over expression of the former would ultimately lead to the same result, Cancer. Various studies have been performed to evaluate the effectiveness of miRNAs as potential markers for Breast cancer. miR-21, miR-155, miR-205 and miR-569 are a few such miRNAs found in abundance in the event of Breast cancer. While, miR-200A, miR-200B, miR-200c, miR-141, miR-429 are found in lesser than normal quantities.

The reliability on miRNAs as diagnostic markers can be attributed to their stability and specificity, and validating the presence of a specific miRNA and quantitation of the same can be done by a low-throughput, yet standardized method like Northern blotting or by Oligonucleotide miRNA microarray and reverse transcriptase RT-PCR for a high-throughput screening. RT-PCR is said to be more sensitive compared to the other assays, yet an increase in the number of miRNA could make the technique less practical than microarray [10].

Tumor suppressor mi RNAS

These suppress oncogenic expression and are expressed at a lower level in cancer cells. These are downregulated in breast cancer [11]. The effects of these tumor suppressor miRNAs are shown in the table below [12] (Table 1).

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

Table 1: Effects of tumor suppressor miRNAs.

MiRNA	Target	Action
miR- 125b	EPO.EPOR	Inhibits cell proliferation and differentiation[13-15]
miR -205	HMGB3	Inhibits cell proliferation and differentiation[16-17]
miR- 206	CYCLIN D2,CX4	Decreases migration,invasion and metastasis[18,19]
miR- 31	RhoA,WAVES	Reduces cancer progression and metastasis[20-22]

Micro RNAs as Biomarkers

Micro RNAs have high tissue specificity, great stability and aberrant expression in different tumor types and are useful as biomarkers helping in diagnostic, predictive and prognostic potential.

Diagnostic biomarkers help differentiate between normal tissue and tumors. These act as early stage markers and help in classifying tumors to various subtypes.

miR-1, miR-133a, miR-133b, miR-92a are up regulated in breast cancer. miR-145 is down regulated in breast cancer. miR-18a, 181a and 222 are over expressed in cancer patients when compared to normal controls [23-27].

Predictive Biomarkers Helps in Assessing the Response to Therapy

miR-30C increased levels sensitized breast cancer cell lines to paclitaxel and doxorubicin [28], mirR-21 up regulation resulted in taxol resistance [29]. Low levels of miR-34a resulted in increased resistance of the breast cancer cells to radiotherapy [30]

Prognostic Biomarkers Helps Assess the Course and Outcome of the Disease

Let7b and mirR-205 have positive prognostic value. Let7b targets the oncogenesis RAS and HMGA2 and is deregulated early in the development of breast cancer [31-34]. miR-375 inhibits cell invasion and migration by inhibiting JAK [35].

miR associated with negative prognosis are miR-122(relapse) [36]; miR-27b3p inhibits the tumour suppressor FOXO1, its level is increased in breast cancer [37,38]. miR-21 is over expressed in breast cancer [39].

Detection of Micro RNAs

Many detection platforms are used for the detection of micro RNAs. The most widely used ones are:

1. Northern Blotting [40]
2. Oligonucleotide Microarray [41]
3. Reverse Transcriptase polymerase chain reaction [42]
4. Bead based Flow Cytometry [43]
5. Insitu Hybridization [44]

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

BIOMARKERS AND AUTO-ANTIBODIES

The Biomarkers are measureable indicators of the presence or severity of a diseased state. A cancer biomarker, indicative of the presence of cancer, may be a biomolecule secreted by the cancerous cells or by the body in specific response to the diseased condition. In other words, they are the over-expressed gene products or responsive elements produced, respectively, to promote or inhibit the tumor growth.

Breast cancer diagnosis is a combination of both clinical and physical examinations. Evaluation of biological fluids along with appropriate histopathological examinations would help diagnose the class specificity of the tumor and in turn develop a suitable therapy for the condition.

Of late, alongside the over-expressed protein biomarkers, many auto-antibodies have been identified to a number of different intracellular and surface antigens in patients with breast cancer, thus demonstrating the humoral immune response to the cancer in humans. The immune response shown by the immune system is tumor specific and in the case of breast cancer, is directed against oncoproteins, such as MUC-1C, mutated proteins such as p53 or other aberrantly expressed proteins such as p16. Although it is still not clear about the benefits of such antibodies, they can be definitely helpful in early detection of breast cancer [45].

The Current Promising Biomarkers for Breast Cancer Include

1) Auto-antibodies such as RS/DJ-1, p53, HSP60, HSP90 and Mucin-related antibodies.
2) Serum Proteins likes the CA-15-3, RS/DJ-1 and HER-2/neu.
3) Ductal Proteins such as α-2-HS-glycoprotein, Lipophilin B, β-globin, Hemopexin and Vitamin D Binding Proteins.

Biomarker Detection can be Carried Out Using

1) Genomic approaches like Northern Blotting, Gene Expression assays, Serial Analysis of Gene Expression **(SAGE)** and DNA Microarray.
2) The Proteomic approaches of Western blotting, 2D-Electrophoresis, Tandem Mass Spectroscopy, Surface-Enhanced Laser Desorption/Ionization–Time of Flight, Matrix-Assisted Laser Desorption/Ionization–Time of Flight, Antibody array and Tissue Microarray.
3) Metabolomic profiling of the organism to analyse the metabolic response and the resulting products, due to the activation or deactivation of metabolic pathways, in a diseased condition.
4) The analysis of lipids, also known as Lipidomics, for the variations in Lipid metabolism, on the occurrence of breast cancer would as well seem a reliable and promising assay due to the established links between obesity and Breast cancer.

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

5) A consideration non-invasive imaging technique for the identification of biomarkers is a growing trend lately. Magnetic Resonance Imaging **(MRI)** Optical Coherence Tomography **(OCT)** and Infrared spectroscopy are a few examples for the same.

It would be noteworthy that, from a diagnostic point of view, serum cancer markers are even more important than tissue markers because of their ease of procurement for large screenings for early cancer diagnosis. However, the search for serum markers is the more challenging. In fact, a candidate for serum marker, selected from gene expression profiles, should not only be over-expressed locally in the cancer microenvironment, but also codes for a protein that is secreted to the periphery in sufficient levels to be detected in blood. In this situation, bioinformatics tools like Gene Ontologies are helpful to choose genes with the characteristics of interest (e.g., secreted molecule) among the huge amount of differentially expressed genes [46].

CONCLUSION

In conclusion, the discovery of tumor suppressor genes and oncogenes and the advent of newer molecular techniques like RNA interference, Micro arrays, Real time PCR will help in the better understanding of breast cancer. In addition these techniques offer a great promise in the treatment of breast cancer.

SUMMARY

The incidence of breast cancer is on the increase worldwide. Earlier breast cancer was diagnosed and treated based on conventional methods like histology, lymph node involvement, vascular invasion and biomarkers like ER, HER2 etc. The advent of newer molecular and imaging techniques has changed the basic understanding of breast cancer diagnostics and therapy.

The microarray technique has made the analysis of a large amount of biological material simpler. The DNA micro array helps detect non hereditary mutations and made personalized treatment a near reality. It also helps in classifying tumors by comparing them with the already known tumor classes.

Real time PCR helps quantitate the DNA or cDNA, will help in better detection of breast cancer due to its high sensitivity and high throughput capacity.

Micro RNAs due to their high specificity and sensitivity are proving to be very effective diagnostic biomarkers and also aid in assessing response to therapy.

The presence of auto antibodies to a number of intracellular and surface antigens has proved to be a boon in early detection of breast cancer.

These newer techniques when used in combination with traditional approaches help in better understanding of breast cancer, its diagnosis and treatment.

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

References

1. Parkin DM, Bray F, Ferlay J, Pasani P. Global Cancer Statistics, 2002. CA Cancer J Clin. 2005; 55: 74-108.
2. Benson JR, Jatoi I. The global breast cancer burden. Future Oncol. 2012; 8: 697-702.
3. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27sites in the adult population in 2008. Int J Cancer. 2013; 132: 1133-1145.
4. Rebecca LS, Kimberly DM, Ahmedin J. Cancer Statistics, 2015. CA Cancer J Clin. 2015; 65: 5-29.
5. Garcia-Solis P, Aceves C. Study of nutritional factors related to breast cancer prevention. Importance of animal models approaches. Arch Latinoam Nutr. 2005; 55: 211-225.
6. Allison KH. Molecular pathology of breast cancer: what a pathologist needs to know. Am J Clin Pathol. 2012; 138: 770-780.
7. Kumar R, Sharma A, Tiwari RK. Application of microarray in breast cancer: An overview. J Pharm Bioallied Sci. 2012; 4: 21-26.
8. Leutenegger CM. The Real-Time TaqMan PCR and Applications in Veterinary Medicine. Veterinary Sciences Tomorrow. 2001.
9. Lowery A, Lemetre C, Ball G, Kerin M. Microarray Technology - Expression Profiling of MRNA and MicroRNA in Breast Cancer. Computational Biology and Applied Bioinformatics. 2011.
10. Bandres E, Cubedo E, Agirre X, Malumbres R, Zarate R, Ramirez N, et al. Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Mol cancer. 2006; 5: 29.
11. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. Dev Biol. 2007; 302: 1-12.
12. van Schooneveld E, Wildiers H, Vergote I, Vermeulen PB, Dirix LY, van Laere SJ. Dysregulation of microRNAs in breast cancer and their potential role as prognostic and predictive biomarkers in patient management. Breast Cancer Res. 2015; 17: 21.
13. Gabriely G, Teplyuk NM, Krichevsky AM. Context effect: microRNA-10b in cancer cell proliferation, spread and death. Autophagy. 2011; 7: 1384-1386.
14. Huang TH, Wu F, Loeb GB, Hsu R, Heidersbach A, Brincat A, et al. Up-regulation of miR-21 by HER2/neu signaling promotes cell invasion. J Biol Chem. 2009; 284: 18515-18524.
15. Han M, Wang Y, Liu M, Bi X, Bao J, Zeng N, et al. MiR-21 regulates epithelial-mesenchymal transition phenotype and hypoxia-inducible factor-1α expression in third-sphere forming breast cancer stem cell-like cells. Cancer Sci. 2012; 103: 1058-1064.
16. Qi L, Bart J, Tan LP, Platteel I, Sluis TV, Huitema S, et al. Expression of miR-21 and its targets (PTEN, PDCD4, TM1) in flat epithelial atypia of the breast in relation to ductal carcinoma in situ and invasive carcinoma. BMC Cancer. 2009; 9: 163.
17. Song B, Wang C, Liu J, Wang X, Lv L, Wei L, et al. MicroRNA-21 regulates breast cancer invasion partly by targeting tissue inhibitor of metalloproteinase 3 expression. J Exp Clin Cancer Res. 2010; 29: 29.
18. Li Y, Hong F, Yu Z. Decreased expression of microRNA-206 in breast cancer and its association with disease characteristics and patient survival. J Int Med Res. 2013; 41: 596-602.
19. Fu Y, Jiang BQ, Wu Y, Li ZD, Zhuang ZG. Hsa-miR-206 inhibits the migration and invasion of breast cancer by targeting Cx43. Zhonghua Yi Xue Za Zhi. 2013; 93: 2890-2894.
20. Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szasz AM, Wang ZC, et al. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell. 2009; 137: 1032-1046.
21. Augoff K, McCue B, Plow EF, Sossey-Alaoui K. miR-31 and its host gene lncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer. Mol Cancer. 2012; 11: 5.
22. Sossey-Alaoui K, Downs-Kelly E, Das M, Izem L, Tubbs R, Plow EF. WAVE3, an actin remodeling protein, is regulated by the metastasis suppressor microRNA, miR-31, during the invasion-metastasis cascade. Int J Cancer.2011; 129: 1331-1343.
23. Kodahl AR, Lyng MB, Binder H, Cold S, Gravgaard K, Knoop AS, et al. Novel circulating microRNA signature as a potential non-invasive multi-marker test in ER-positive early-stage breast cancer: a case control study. Mol Oncol. 2014; 8: 874-883.
24. Wang F, Hou J, Jin W, Li J, Yue Y, Jin H, et al. Increased circulating microRNA-155 as a potential biomarker for breast cancer screening: a meta-analysis. Molecules. 2014; 19: 6282-6293.
25. Chan M, Liaw CS, Ji SM, Tan HH, Wong CY, Thike AA, et al. Identification of circulating microRNA signatures for breast cancer detection. Clin Cancer Res. 2013; 19: 4477-4487.
26. Cuk K, Zucknick M, Heil J, Madhavan D, Schott S, Turchinovich A, et al. Circulating microRNAs in plasma as early detection markers for breast cancer. Int J Cancer. 2013; 132: 1602-1612.
27. Ng EK, Li R, Shin VY, Jin HC, Leung CP, Ma ES, et al. Circulating microRNAs as specific biomarkers for breast cancer detection. PLoS One. 2013; 8: e53141.

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.

28. Godfrey AC, Xu Z, Weinberg CR, Getts RC, Wade PA, DeRoo LA, et al. Serum microRNA expression as an early marker for breast cancer risk in prospectively collected samples from the Sister Study cohort. Breast Cancer Res. 2013; 15: R42.

29. Mei M, Ren Y, Zhou X, Yuan XB, Han L, Wang GX, et al. Downregulation of miR-21 enhances chemotherapeutic effect of taxol in breast carcinoma cells. Technol Cancer Res Treat. 2010; 9: 77-86.

30. Dong J, Zhao YP, Zhou L, Zhang TP, Chen G. Bcl-2 Upregulation induced by miR-21 via a direct interaction is associated with apoptosis and chemoresistance in MIA PaCa-2 pancreatic cancer cells. Arch Med Res. 2011; 42: 8-14.

31. Stankevicins L, Almeida da Silva AP, Ventura Dos Passos F, Dos Santos Ferreira E, Menks Ribeiro MC, G David M, et al. MiR-34a is up-regulated in response to low dose, low energy X-ray induced DNA damage in breast cells. Radiat Oncol. 2013; 8: 231.

32. Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, et al. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. Genome Biol. 2007; 8: R214.

33. Sempere LF, Christensen M, Silahtaroglu A, Bak M, Heath CV, Schwartz G, et al. Altered microRNA expression confined to specific epithelial cell subpopulations in breast cancer. Cancer Res. 2007; 67: 11612-11620.

34. Dangi-Garimella S, Yun J, Eves EM, Newman M, Erkeland SJ, Hammond SM, et al. Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7. EMBO J. 2009; 28: 347-358.

35. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, et al. Let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell. 2007; 131: 1109-1123.

36. Wu X, Somlo G, Yu Y, Palomares MR, Li AX, Zhou W, et al. De novo sequencing of circulating miRNAs identifies novel markers predicting clinical outcome of locally advanced breast cancer. J Transl Med. 2012; 10: 42.

37. Guttilla IK, White BA. Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. J Biol Chem. 2009; 284: 23204-23216.

38. Shen S, Sun Q, Liang Z, Cui X, Ren X, Chen H, et al. A prognostic model of triple-negative breast cancer based on miR-27b-3p and node status. PLoS One. 2014; 9: e100664.

39. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. Oncogene.2007; 26: 2799-2803.

40. Varallyay É, Burgyan J, Havelda Z. Micro RNA detection by northern blotting using locked nucleic acid probes. Nat Protoc. 2008; 3: 190-196.

41. Hammond SM. microRNA detection comes of age. Nat Methods. 2006; 3: 12-13.

42. Balcells I, Cirera S, Busk PK. Specific and sensitive quantitative RT-PCR of miRNAs with DNA primers. BMC Biotechnol.2011; 11: 70.

43. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature. 2005; 435: 834-838.

44. Li W, Ruan K. MicroRNA detection by microarray. Anal Bioanal Chem. 2009; 394: 1117-1124.

45. Lacombe CDJ, Eat A, Thierry, Maudelonde J. SOLASSOL. Autoantibodies and early diagnosis of cancers. Med Sci (Paris). 2011; 27: 633-638.

46. Perez-Diez A, Morgun A, Shulzhenko N. Microarrays for cancer diagnosis and classification. Adv Exp Med Biol. 2007; 593: 74-85.

Copyright © Manoli N.This book chapter is open access distributed under the Creative Commons Attribution 4.0 International License, which allows users to download, copy and build upon published articles even for commercial purposes, as long as the author and publisher are properly credited.