



iJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 10 Issue: V Month of publication: May 2022

DOI: <https://doi.org/10.22214/ijraset.2022.43624>

www.ijraset.com

Call:  08813907089

E-mail ID: ijraset@gmail.com

“Genetic Profiling of Breast Cancer using cDNA Microarray.”

Anirudh Modak¹, Dr. Tanaya Ghosh², Shamayita Basu³

¹Student, Department of Medical Biotechnology, Sikkim Manipal University

²Former Faculty, Department of Medical Biotechnology, Sikkim Manipal University

³Senior Research Scholar, Kalyani University

Abstract: *In defining the grade, stage, and residual determination of Breast Cancer and its subtypes, the molecular basis of analysis has become increasingly important. Vast-scale research studies have discovered a large number of genetic changes that cause the development and progression of breast cancer subtypes. Microarray and biotechnological approaches of hybridization-based analysis and microtiter level evaluation have come a long way in determining the expression level of mutant or hereditary genes. For Genomic Research and Diagnostics, DNA Microarray analysis allows for the simultaneous investigation of thousands of DNA sequences. Breast cancer subtypes are distinguished by clusters of co-expressed genes discovered by the manipulation of mammary epithelial cells. The efficacy of a treatment is determined by the prognostic development of an individual patient. A comparison of gene expression levels in the Pre-treatment and Post-treatment stages has evolved into a concept that has the potential to change genetic dissection, drug development, disease diagnostics, and individualised therapeutic solutions. The goal of this review was to look into how to diagnose breast carcinomas using changes in gene expression patterns derived from cDNA microarrays and how to link tumour characteristics to clinical outcomes.*

Keywords: *Breast Cancer, Genes, Mutations, Gene Expression, DNA Microarray, Microarray-Analysis*

I. INTRODUCTION

For decades, evidence of cancer's genetic origins has been accumulating. However, technological improvements in DNA sequencing and other approaches that allow the genome-wide study of cancer cells have only recently enabled a thorough accounting of the degree of these genetic abnormalities. Although the intricacy of these data is intimidating, and the messages concealed within them have yet to be fully decoded, some "genomic motifs" have arisen that are likely to be relevant to all cancers.

Carcinogenesis is driven by nonlethal genetic damage. The initial damage (or mutation) may be induced by environmental exposures, inherited in the germline, or spontaneous and random, and so fall into the category of "poor luck." Exogenous agents, such as viruses or environmental chemicals, or endogenous products of cellular metabolism that have the ability to damage DNA or modify gene expression through epigenetic mechanisms are referred to as environmental in this context.

Microarray technology is an excellent tool for biological research. For genomic research and diagnostic applications, microarrays allow the simultaneous study of thousands of DNA sequences. This technology represents the most recent and interesting advancement in the biological sciences in the use of hybridization-based methodologies for analysis. (1) Gene expression profiling of cancers represents the largest research category using microarrays and appears to be the most robust approach for molecular characterization of cancers (2). It is becoming recognized that microarray technology will be a fundamental tool for future genomic research. Soon after microarrays were introduced, many researchers realized that the technique could be used to find new subclasses in disease states (3,4) and identify biological markers (biomarkers) associated with disease (5) and that even the expression patterns of the genes could be used to distinguish subclasses of disease. (6–9)

II. WHAT IS BREAST CANCER?

Breast cancer is caused by a mix of an external (environmental) component and a genetically susceptible host, just like other malignancies. Normal cells divide for as long as they need to before stopping. They attach themselves to other cells and stay in tissues. Cells become cancerous when they lose their ability to stop dividing, adhere to other cells, stay put, and die at the appropriate time. Experiments have linked oestrogen exposure to breast cancer-causing mutations. (10) G-protein coupled oestrogen receptors have also been related to breast cancer and other female reproductive system cancers. (11)

Malignant cell proliferation can be aided by abnormal growth factor signalling in the interaction between stromal cells and epithelial cells. (12,13) In breast adipose tissue, overexpression of leptin leads to increased cell proliferation and cancer. (14)

Genetics is believed to be the primary cause of 5–10% of all cases. (15) Women whose mother was diagnosed before 50 have an increased risk of 1.7 and those whose mother was diagnosed at age 50 or after have an increased risk of 1.4. (16) In those with zero, one or two affected relatives, the risk of breast cancer before the age of 80 is 7.8%, 13.3%, and 21.1% with a subsequent mortality from the disease of 2.3%, 4.2%, and 7.6% respectively. (17) In those with a first degree relative with the disease, the risk of breast cancer between the age of 40 and 50 is double that of the general population. (18)

In less than 5% of cases, genetics plays a more significant role in causing a hereditary breast-ovarian cancer syndrome (19). This includes those who carry the *BRCA1* and *BRCA2* gene mutation (19). These mutations account for up to 90% of the total genetic influence with a risk of breast cancer of 60–80% in those affected. (15) Other significant mutations include *p53* (Li–Fraumeni syndrome), *PTEN* (Cowden syndrome), *STK11* (Peutz–Jeghers syndrome), *CHEK2*, *ATM*, *BRIP1*, and *PALB2* (15) In 2012, researchers said that there are four genetically distinct types of the breast cancer and that in each type, hallmark genetic changes lead to many cancers. (20)

III. GENES INVOLVED IN BREAST CANCER

A. High Penetrance Genes

BRCA1, which is found on chromosome 17, was the first major gene linked to hereditary breast cancer. In 1990, linkage analysis in families with suggestive pedigrees led to the discovery of this gene. (21) *BRCA2* was mapped to chromosome 13, in 1994. (22) Breast and other cancers are more likely if you have a *BRCA1* or *BRCA2* mutation. Large rearrangements and deletions in *BRCA1* or *BRCA2* can potentially affect *BRCA* function, resulting in a clinical condition similar to that seen in *BRCA* mutant carriers. The Hereditary Breast Ovarian Cancer (HBOC) syndrome is the clinical picture seen in *BRCA* mutation carriers, while there are people with this clinical picture who are determined to be negative for mutations in both *BRCA1* and *BRCA2*. (23)

BRCA1 and *BRCA2* mutations are autosomal dominant but act recessively on the cellular level as tumour suppressor genes involved in dsDNA break repair. (24) Female carriers of mutations in *BRCA1* or *BRCA2* have a lifetime risk of breast cancer of 50-85%. (25,26) Male carriers of *BRCA1* have an increased risk of breast cancer, though to a lesser degree than carriers of *BRCA2* who have an estimated 5-10% lifetime risk. (10)

B. Moderate Penetrance Gene

Additional replicable loci for highly penetrant genes leading to breast cancer have not been discovered by linkage studies. (27) it should be highlighted, however, that these studies may not be sufficiently powerful to uncover extremely rare high penetrance genes. This has encouraged new research directions in the study of breast cancer's hereditary origins. Several studies have looked at genes that have been proposed to enhance the risk of breast cancer based on their known biological functions in families with breast cancer pedigrees. Several more DNA repair genes that interact with *BRCA1*, *BRCA2*, and/or the *BRCA* pathways and confer a twofold increase in breast cancer risk have been discovered in studies, including *CHEK2* (28), *BRIP1* (*BACH1*) (29), *ATM* (30), and *PALB2* (31). *CHEK2*1100delC* is the most common mutation, seen in up to 1-2% of the population; it is found in higher numbers in breast cancer patients, especially those with a family history or those who had negative *BRCA1* and *BRCA2* testing, where the prevalence may be as high as 5%. (28) *CHEK2* is a protein kinase involved in cell cycle regulation at G2 that is rapidly phosphorylated in response to DNA damage. Activated *CHEK2* interacts with *BRCA1* and stabilises *p53*. The *CHEK2*1100delC* mutation increases female breast cancer by around twofold and male breast cancer by tenfold. Additional rare *CHEK2* mutations have been discovered in the Ashkenazi Jewish population, suggesting a founder impact. (32) Co-carriers of the *CHEK2* and *BRCA1* or *BRCA2* mutations have no increased risk, probably due to an overlapping effect on DNA repair. (28) Again, there is no documented biallelic phenotype for *CHEK2*, implying that it is embryonic lethal. (24)

C. Low Penetrance Genes

A modest number of polymorphisms in breast cancer-related genes have been linked to an elevated risk of the disease. In premenopausal women, a Pro919Ser polymorphism in *BRIP1* has an odds ratio of 1.39 ($p=0.002$) but was not linked to an elevated risk of breast cancer in the general population. Low penetrance SNPs are frequently found in non-coding areas of the genome (e.g., 2q35, 8q24), making finding a related gene more difficult. Increased cancer risk could be caused by the activation of growth-promoting genes rather than the inactivation of DNA repair genes, which is the most typical mechanism for genes with moderate or high penetrance. On average, each allele raises risk just slightly and is additive rather than multiplicative, with odds ratios indicating a 1.26-fold rise in risk for heterozygotes and a 1.65-fold increase in risk for homozygotes. (24)

IV. DNA MICROARRAY – BACKGROUND

A. Methodology

Microarrays allow for massively parallel investigation of gene expression, DNA sequence variation, protein levels, tissues, cells, and other biological and chemical substances. Microarray manufacturing, hybridization, detection, and data analysis technologies are robust, allowing inexperienced users to quickly adapt to this intriguing technology while more experienced users push the limits of discovery. (33)

High-density microscopic array elements, planar glass substrates, low reaction volumes, multicolour fluorescent labelling, high binding specificity, high-speed apparatus for production and detection, and sophisticated software for data processing and modelling are all used in microarrays. The array of elements reacts with tagged mixtures in a precise way, generating signals that indicate the identification and concentration of each marked species in the solution. (33)

These characteristics enable the exploration of any organism on a genomic scale using tiny biological experiments. Similar to recombinant DNA, microarray analysis (34) and the polymerase chain reaction (PCR) (35), is a foundational technology with broad applications in areas including genetic screening, proteomics, safety assessment and diagnostics.

B. Sample Collection transformation and Data Representation

Gene-specific probes that represent thousands of individual genes are used in the microarray approach. The probes are arranged on an inert substrate, and gene expression levels in a target biologic sample are measured (Fig. 2). RNA is taken from target tissues, labelled with a detectable marker (usually a fluorescent dye), and allowed to hybridise into arrays. On the array, messenger RNA (mRNA) samples hybridise with complementary gene-specific probes.

The relative fluorescence intensity of each gene-specific probe is a measure of the degree of expression of the particular gene; images are created using confocal laser scanning. The stronger the signal, the greater the degree of hybridization, reflecting a higher relative level of expression.

Microarray data can be generated in one of two ways. Two samples of RNA, each labelled with a different dye, are hybridised into a two-colour array at the same time (Fig. 3). The query sample (for example, a sample of breast cancer) is labelled with one dye, while the reference sample (for example, normal breast tissue) is tagged with a different dye; the two samples are combined in an approximate 1:1 ratio based on the dye incorporation. The logarithm of the ratio of RNA in a query sample to that in a control sample is reported as an expression in this assay, which compares paired samples. Single-colour arrays, such as the GeneChip (Affymetrix), are ideal, each sample is labelled and individually incubated with an array (Fig. 3). The level of expression of each gene is presented as a single fluorescence intensity that represents an estimated level of gene expression after non-hybridized material in the sample is removed by washing. The data used in all future studies, regardless of the strategy or technique, are expression measures for each gene in each sample. (36)

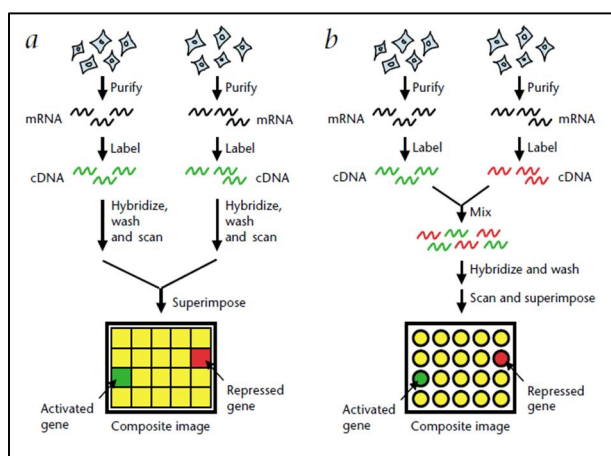


Figure 1: Expression analysis by microarray. a. One-color expression analysis uses a single fluorescent label (green wavy lines) and two chips to generate expression profiles for two or more cell samples. Activated and repressed genes (green and red squares, respectively) are obtained by superimposing images obtained from different chips. **b.** Two-colour expression analysis uses two different fluorescent labels (green and red wavy lines) and a single chip to generate expression profiles for two different cell samples. Activated and repressed genes (green and red spots, respectively) are obtained by superimposing images generated in different channels on a single microarray. Genes expressed equally in the two samples appear as yellow squares or spots in the two analyses. (33)

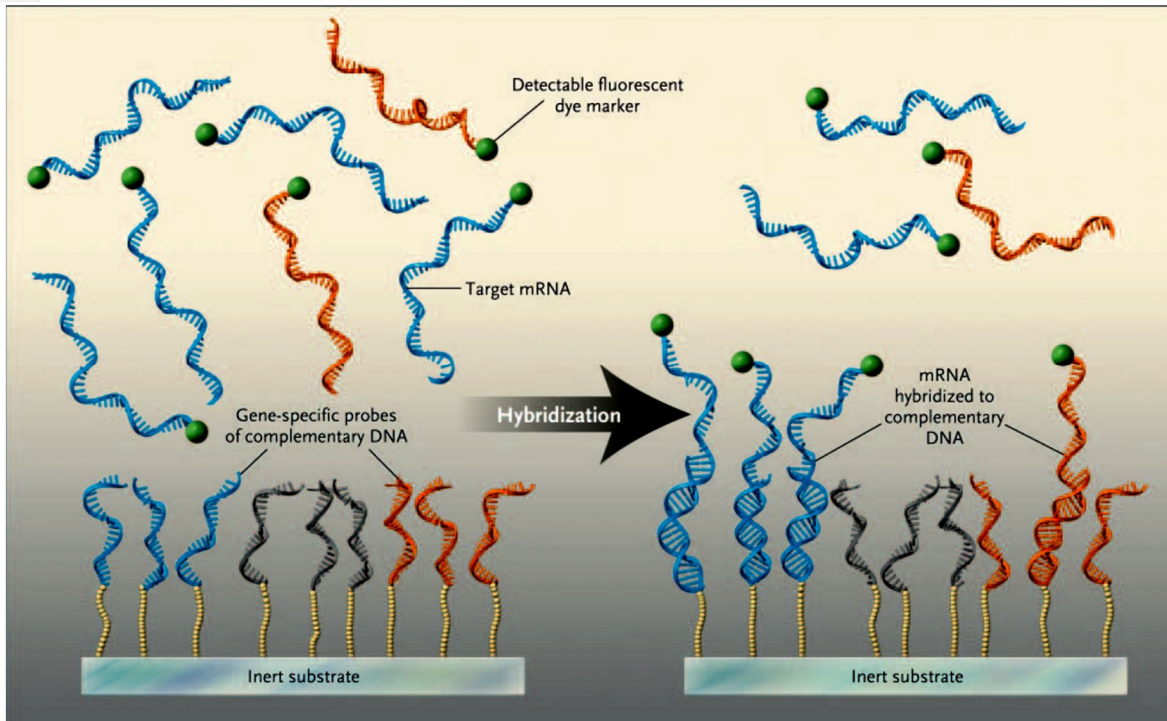


Figure 2: Hybridization with Gene Elements on a Microarray. (36)

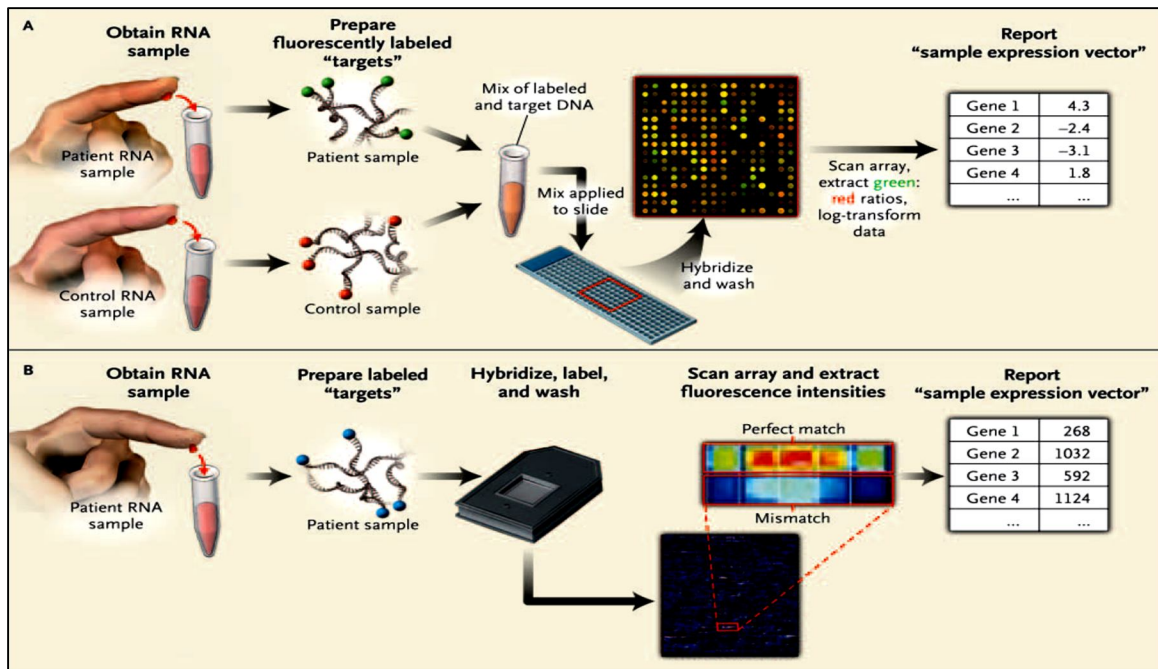


Figure 3: Overview of DNA Microarray Analysis. (36)

The data is frequently standardised after collection to make comparisons across different hybridization experiments easier. Differences in labelling, hybridization and detection procedures are compensated for using normalisation. There are numerous techniques for data normalisation; the most appropriate strategy will be determined by the type of array and assumptions about data biases. (36–40). Following that, the data is frequently filtered using objective criteria (e.g., eliminating genes with low variation in the samples) or statistical studies to pick genes with expression levels that correlate with specific groups of samples.

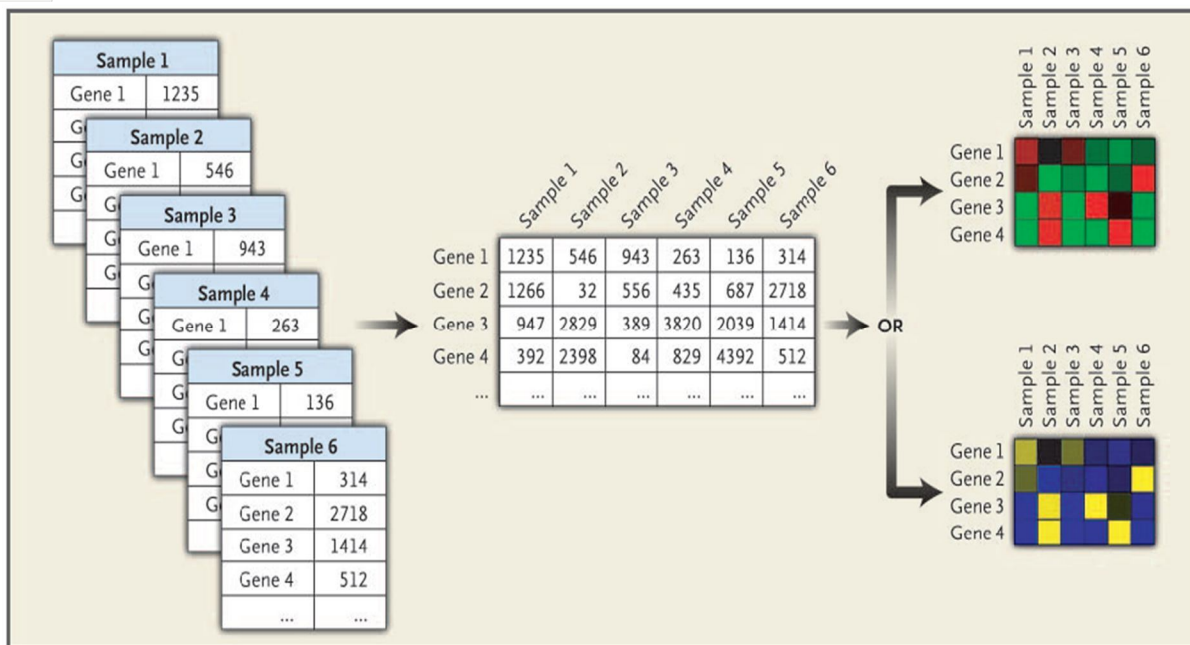


Figure 4: Development of an Expression Matrix. (36)

V. cDNA MICROARRAY IN BREAST CANCER DIAGNOSTICS

This multistep process may manifest as a series of pathologically defined phases in the development of breast cancer. Breast cancer is thought to start with the premalignant stage of atypical ductal hyperplasia (ADH), proceed to the preinvasive stage of ductal carcinoma in situ (DCIS), and finally to the potentially fatal stage of invasive ductal carcinoma (IDC) (41). The use of detection tools like mammography in the hopes of diagnosing and treating breast cancer at an earlier clinical stage has been justified by this linear model of breast cancer progression. (42). However, both within a tumour and among individual tumours, the stages of DCIS and IDC are variable in terms of mitotic activity and cellular differentiation. Several tumour-grading methods have been developed to better characterise DCIS and IDC in terms of heterogeneity. Clinically, such methods are used to divide DCIS and IDC stages into three tumour grades, with grade I, II, and III lesions corresponding to well, moderately, and poorly differentiated breast tumours, respectively. (43,44). Poorly differentiated, high-grade DCIS or IDC lesions are associated with much worse clinical outcomes, making tumour grade a highly helpful predictive indicator for breast cancer. (43–45)

VI. CONCLUSION

Breast carcinomas can be investigated using mRNA expression profiling. Similar arrays for analysing DNA and protein expression profiles are in the works. mRNA arrays have been utilised for predicting prognosis and responsiveness to therapy, assessing tumour changes following therapy, and defining hereditary carcinomas in addition to detecting tumour kinds, as in this case. Although transcriptome profiling may not be viable in every clinical case of breast cancer, this research will yield data that will lead to improved diagnostic, prognostic, and therapeutic testing for all patients. As malignancies originate from the accumulation of various genetic and epigenetic alterations, microarray technologies are becoming more essential in cancer research. Microarrays are increasingly being employed for cancer diagnostic categorization. In cancer research, comprehensive and high-throughput genomic analysis is an unavoidable research tool. However, there are certain disadvantages to using microarrays regularly. Microarray experiments are expensive, and experimental processes must be more reliable. Microarray and experimental protocol standardisation are also significant for comparing data amongst research organisations. Tools and procedures for data analysis must also be developed. Despite these drawbacks, it is apparent that microarray technology will become a standard tool in cancer research in the future. Gene expression profiling may be used to predict prognosis following chemo- or radiation, and oligonucleotide microarrays will be used to make an early cancer diagnosis. Gene expression analysis employing microarrays can assist researchers to find meaningful answers to cancer-related problems using conventional histopathology data.

REFERENCES

- [1] Schena M. Microarrays: biotechnology's discovery platform for functional genomics. *Trends in Biotechnology*. 1998 Jul 1;16(7):301–6.
- [2] Russo G, Zegar C, Giordano A. Advantages and limitations of microarray technology in human cancer. *Oncogene*. 2003 Sep 29;22(42):6497–507.
- [3] Alon U, Barkai N, Notterman DA, Gish K, Ybarra S, Mack D, et al. Broad patterns of gene expression revealed by clustering analysis of tumour and normal colon tissues probed by oligonucleotide arrays. *Proceedings of the National Academy of Sciences*. 1999 Jun 8;96(12):6745–50.
- [4] Perou CM, Jeffrey SS, van de Rijn M, Rees CA, Eisen MB, Ross DT, et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proceedings of the National Academy of Sciences*. 1999 Aug 3;96(16):9212–7.
- [5] Moch H, Schraml P, Bubendorf L, Mirlacher M, Kononen J, Gasser T, et al. [Identification of prognostic parameters for renal cell carcinoma by cDNA arrays and cell chips]. *Verhandlungen der Deutschen Gesellschaft für Pathologie*. 1999;83:225–32.
- [6] J Khan, R Simon, Y Chen, M Bittner, S B Leighton, T Pohida, et al. Gene expression profiling of alveolar rhabdomyosarcoma with cDNA microarrays. *Cancer Research*. 1998 Nov 15;58(22):5009–13.
- [7] Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, et al. Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring. *Science (1979)*. 1999 Oct 15;286(5439):531–7.
- [8] Bloom G, Yang I v., Boulware D, Kwong KY, Coppola D, Eschrich S, et al. Multi-Platform, Multi-Site, Microarray-Based Human Tumor Classification. *The American Journal of Pathology*. 2004 Jan;164(1):9–16.
- [9] Eschrich S, Yang I, Bloom G, Kwong KY, Boulware D, Cantor A, et al. Molecular Staging for Survival Prediction of Colorectal Cancer Patients. *Journal of Clinical Oncology*. 2005 May 20;23(15):3526–35.
- [10] Liede A, Karlan BY, Narod SA. Cancer Risks for Male Carriers of Germline Mutations in BRCA1 or BRCA2 : A Review of the Literature. *Journal of Clinical Oncology*. 2004 Feb 15;22(4):735–42.
- [11] Filardo EJ. A role for G-protein coupled estrogen receptor (GPER) in estrogen-induced carcinogenesis: Dysregulated glandular homeostasis, survival and metastasis. *The Journal of Steroid Biochemistry and Molecular Biology*. 2018 Feb;176:38–48.
- [12] Haslam SZ, Woodward TL. Host microenvironment in breast cancer development: Epithelial-cell–stromal-cell interactions and steroid hormone action in normal and cancerous mammary gland. *Breast Cancer Research*. 2003 Aug 1;5(4):208.
- [13] Wiseman BS, Werb Z. Stromal Effects on Mammary Gland Development and Breast Cancer. *Science (1979)*. 2002 May 10;296(5570):1046–9.
- [14] Jardé T, Perrier S, Vasson MP, Caldefie-Chézet F. Molecular mechanisms of leptin and adiponectin in breast cancer. *European Journal of Cancer*. 2011 Jan;47(1):33–43.
- [15] Gage M, Wattendorf D, Henry LR. Translational advances regarding hereditary breast cancer syndromes. *Journal of Surgical Oncology*. 2012 Apr 1;105(5):444–51.
- [16] Colditz GA, Kaphingst KA, Hankinson SE, Rosner B. Family history and risk of breast cancer: nurses' health study. *Breast Cancer Research and Treatment*. 2012 Jun 19;133(3):1097–104.
- [17] Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58 209 women with breast cancer and 101 986 women without the disease. *The Lancet*. 2001 Oct;358(9291):1389–99.
- [18] Nelson HD. Risk Factors for Breast Cancer for Women Aged 40 to 49 Years. *Annals of Internal Medicine*. 2012 May 1;156(9):635.
- [19] Pasche B, editor. *Cancer Genetics*. Vol. 155. Boston, MA: Springer US; 2010.
- [20] Rahimi M, Talebi Kakroodi S, Tajvidi M. The Importance of RTK Signaling Genes and their Inhibitors in Breast Cancer. *Journal of Obstetrics, Gynecology and Cancer Research*. 2022 Jul 1;7(4):258–71.
- [21] Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, et al. Linkage of Early-Onset Familial Breast Cancer to Chromosome 17q21. *Science (1979)*. 1990 Dec 21;250(4988):1684–9.
- [22] Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, et al. Localization of a Breast Cancer Susceptibility Gene, BRCA2 , to Chromosome 13q12-13. *Science (1979)*. 1994 Sep 30;265(5181):2088–90.
- [23] Lakhani SR, van de Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, et al. The Pathology of Familial Breast Cancer: Predictive Value of Immunohistochemical Markers Estrogen Receptor, Progesterone Receptor, HER-2, and p53 in Patients With Mutations in BRCA1 and BRCA2. *Journal of Clinical Oncology*. 2002 May 1;20(9):2310–8.
- [24] Stratton MR, Rahman N. The emerging landscape of breast cancer susceptibility. *Nature Genetics*. 2008 Jan 27;40(1):17–22.
- [25] King MC, Marks JH, Mandell JB. Breast and Ovarian Cancer Risks Due to Inherited Mutations in BRCA1 and BRCA2. *Science (1979)*. 2003 Oct 24;302(5645):643–6.
- [26] Lindor NM, McMaster ML, Lindor CJ, Greene MH. *Concise Handbook of Familial Cancer Susceptibility Syndromes - Second Edition*. JNCI Monographs. 2008 Jun 1;2008(38):3–93.
- [27] Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. *Molecular Oncology*. 2010 Jun;4(3):174–91.
- [28] Low-penetrance susceptibility to breast cancer due to CHEK2*1100delC in noncarriers of BRCA1 or BRCA2 mutations. *Nature Genetics*. 2002 May 22;31(1):55–9.
- [29] Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, et al. Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. *Nature Genetics*. 2006 Nov 8;38(11):1239–41.
- [30] Renwick A, Thompson D, Seal S, Kelly P, Chagtai T, Ahmed M, et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nature Genetics*. 2006 Aug 9;38(8):873–5.
- [31] Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, et al. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nature Genetics*. 2007 Feb 31;39(2):165–7.
- [32] Shaag A, Walsh T, Renbaum P, Kirchoff T, Nafa K, Shiovitz S, et al. Functional and genomic approaches reveal an ancient CHEK2 allele associated with breast cancer in the Ashkenazi Jewish population. *Human Molecular Genetics*. 2005 Feb 15;14(4):555–63.



- [33] Stears RL, Martinsky T, Schena M. Trends in microarray analysis. *Nature Medicine*. 2003 Jan;9(1):140–5.
- [34] Jackson DA, Symons RH, Berg P. Biochemical Method for Inserting New Genetic Information into DNA of Simian Virus 40: Circular SV40 DNA Molecules Containing Lambda Phage Genes and the Galactose Operon of Escherichia coli. *Proceedings of the National Academy of Sciences*. 1972 Oct;69(10):2904–9.
- [35] Mullis KB, Faloona FA. [21] Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. In 1987. p. 335–50.
- [36] Quackenbush J. Microarray data normalization and transformation. *Nature Genetics*. 2002 Dec;32(S4):496–501.
- [37] Irizarry RA. Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Research*. 2003 Feb 15;31(4):15e–15.
- [38] Schadt EE, Li C, Ellis B, Wong WH. Feature extraction and normalization algorithms for high-density oligonucleotide gene expression array data. *Journal of Cellular Biochemistry*. 2001;84(S37):120–5.
- [39] Vyang I v, Chen E, Hasseman JP, Liang W, Frank BC, Wang S, et al. Within the fold: assessing differential expression measures and reproducibility in microarray assays . *Genome Biology*. 2002;3(11):research0062.1.
- [40] Yang YH. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Research*. 2002 Feb 15;30(4):15e–15.
- [41] Allred DC, Mohsin SK, Fuqua SA. Histological and biological evolution of human premalignant breast disease. *Endocr Relat Cancer*. 2001 Mar;47–61.
- [42] Tabár L, Dean PB, Kaufman CS, Duffy SW, Chen HH. A new era in the diagnosis of breast cancer. *Surg Oncol Clin N Am*. 2000 Apr;9(2):233–77.
- [43] Dalton LW, Pinder SE, Path MRC, Elston CE, Path FRC, Ellis IO, et al. Histologic Grading of Breast Cancer: Linkage of Patient Outcome with Level of Pathologist Agreement. 2000.
- [44] Holland R, Peterse JL, Millis RR, Eusebi V, Faverly D, van de Vijver MJ, et al. Ductal carcinoma in situ: a proposal for a new classification. *Semin Diagn Pathol*. 1994 Aug;11(3):167–80.
- [45] Page DL, Gray R, Allred DC, Dressler LG, Hatfield AK, Martino S, et al. Prediction of Node-Negative Breast Cancer Outcome by Histologic Grading and S-Phase Analysis by Flow Cytometry. *American Journal of Clinical Oncology: Cancer Clinical Trials*. 2001 Feb;24(1):10–8.



10.22214/IJRASET



45.98



IMPACT FACTOR:
7.129



IMPACT FACTOR:
7.429



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089  (24*7 Support on Whatsapp)