Genetics and Breast Cancer – Oncologists Perspectives

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Abstract Breast cancer is the most common cancer in women worldwide. The clinical outcomes of which, have improved in the past decade, primarily due early diagnosis and multimodal management. Understanding of the disease biology with findings from omics-based research and molecular genetic characterization of the disease has been an important component of the therapy in the past 10 years. There is a need to understand the variations in individuals at the molecular level to enable in sub-classification of the different disease phenotypes and if possible to tailor the treatment to the patient. This article attempts to review the beneficial role of genetics in various facets of breast cancer management, in modern scientific medicine.

Keywords

Background

Breast cancer is the most common cancer in women worldwide; with 10 % lifetime risk in general population. As per GLOBOCAN 2012 [1], the incidence rate of breast cancer has increased by 20 % and mortality by 14 % in the last five years. Clinical outcomes in breast cancer (measured as Disease free survival, DFS and Overall survival, OS) has improved in the past decade, primarily contributed by the understanding of the

disease biology with findings from omics-based research. However, there is a continuous challenge for improving the treatment protocols in human breast cancer management.

Molecular genetic characterization of the disease has shown to be an important component of the multimodal disease management in the past 5 years. These are independent of other classical features like age, stage, hormonal status and Ki67 status. The three major aspects of molecular workup includes, understanding the alterations at DNA, RNA and the protein functional changes. Beyond this, regulatory molecules such as siRNA, snRNA, miRNA and epigenetic modifications also significantly contribute to oncogenesis. Hormone receptor ER/PR and *Her2* pathway are the major biomarkers, used at present to make clinical decisions. These are characterized at the protein level by immunohistochemistry, Classifying breast cancer into 4 histo-molecular subtypes. The course of treatment is decided by the molecular classification as well as other clinical parameters such as menopausal status, performance status, stage of the disease and so on. This tailored treatment based approach on the abovementioned molecular classification has some limitations with a significant subset of non-responders, adding to increase in morbidity & mortality. In view of significant differences in the outcome in similar patients there must be factors which are different in subgroups. Therefore, the need to understand the variations in such individuals at the molecular level could shed light in further classification of the different disease phenotypes.

Several research groups across the world joined together to form consortia and have shared their findings in different breast cancer subtypes. Breast cancer, like any other subtype of cancer, is highly heterogeneous often requiring multiple combinatorial therapy options, surgery, and radiotherapy, systemic chemotherapy which includes hormonal and targeted therapies [2, 3]. Retrospective analysis, elucidating the number of unique pathways involved in breast carcinogenesis, and

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correlations with the disease phenotype in various clinical trials has led to a paradigm shift in breast cancer management.

Companion Molecular Diagnostics and Targeted/Personalized Therapy

In this era of precision medicine, inherent genetic makeup together with tumor genotype and phenotype play an important role in deciding systemic therapy tailored for each individual.

Characterization of gene expression signatures such as estrogen and progesterone receptors is, one of the early discoveries, in the field of personalized therapy in breast cancers. Anti-estrogens and their downstream signals have evolved significantly contributing to several targeted therapy related drugs in breast cancer management today. For example: A potential therapeutic target in clinical trials is 17 β hydroxyl steroid dehydrogenase type I which promotes breast cancer progression upon interacting with dihydrotestosterone in addition to producing active estrogen estradiol [4, 5]. ER positive metastatic breast cancers progressing on hormone therapy were found to have over expression of MTOR pathway which is downstream of ER. MTOR inhibitors like everolimus combined with second line aromatase inhibitors have good activity and FDA approved in these patients.

Her2 positive breast cancer patients derive significant benefit from use of anti Her2 therapies especially from Herceptin in all stages of cancer including early, locally advanced and metastatic ones. But there are subsets of Her2 positive patients who do not get benefit from Herceptin and lot of bio research is going on in identifying alterations in the downstream molecules, like PI3K. Adjuvant chemotherapy which includes doxorubicin plus cyclophosphamide followed by paclitaxel is a common regimen in majority of breast cancers. However, this leads to significant cardiac toxicity in a certain group of individuals. The tumor phenotype which is characterized by increased expression of carbonic anhydrase IX and Top2A expression is responsible for resistance to doxorubicin and in this subgroup of patients other regimens can be considered [6].

Molecular Diagnostics

Cancer involves multiple mutations. Understanding the clonal evolution of cancer cells is one of the primary objectives. Molecular screening involves point mutations, deletions, amplifications, epigenetic modifications and differential gene expression signatures. All these together help the pathologists in providing a reasonably accurate prognostication of the breast cancer.

Among these, gene expression signatures stand out to be the most robust and universally adopted classification. There

are four major subtypes viz., Luminal A which are ER, PR positive and Her2 negative; these tumors have a low Ki67 expression, which is the marker for rapid cell division or the aggressive nature of cancer. Luminal B which are ER, PR positive and Her2 positive or ER PR Positive and fast growing. These tumors have a high ki67 index, and hence, are very aggressive in behavior requiring more aggressive therapy. The third subtype, triple negative which includes ER, PR and Her2 negative tumors that are poorly differentiated and have a basal like phenotype. The fourth subtype is Her2 positive breast cancers which are highly aggressive with high risk of relapse but with the use of anti Her2 therapy are curable in certain percentage of patients. Another co-existing prognostic factor is presence of TP53 mutations which relates to poor prognosis and high risk of relapse. Among the four subtypes Luminal A subtype is known to have good prognosis and are usually highly sensitive to hormonal therapy [7–11].

Clinical Staging and Monitoring

Evolution, by acquiring mutations, is a hallmark of the cancer cell. This is also one of the features which could complement the imaging information to provide accurate clinical staging. Tracer molecules which are tagged to antibodies highly specific for the Her2 receptors are now being developed [12]. They could provide accurate staging information in Her2 positive breast cancers. These tracers could also be used, particularly, to evaluate the response during the course of treatment; and also as part of post treatment surveillance.

CTC and CtDNA

Cell-free tumor DNA (CtDNA) is the spillage DNA from dead and dying cancer cells into the bloodstream. Circulating tumor cells are those cells which are continuously dislodged from the primary or metastatic site of cancer into the lymphatics/blood stream. Some of the cancer cells develop ability to intra-vasate and form circulation tumor cells. Some of these cells develop the homing potential to get to other tissues in the body to continue with its proliferation. Hence CTC are considered as a measure for the tumors' potential to develop metastases.

The latest addition to clinical staging is characterization of Circulating tumor DNA (CtDNA) and the number of Circulating tumor cells (CTC) at the time of diagnosis. This provides an edge over conventional imaging techniques such as CT/PET-CT for accurate clinical staging. Several studies have shown that presence of more than five circulatory tumor cells in metastatic setting and even a single cell in adjuvant setting is a poor prognostic factor. In addition, these tools are also useful to predict the chances of relapse/oligometastases and aggressive nature of the disease, thus helping the physicians to change the chemo/radiotherapy regimen. Further,

routine monitoring of the patient's circulating DNA during the treatment course, will reflect the effectiveness of the clinical treatment regimen advised. This may also help in deciding upon improvements such as more aggressive chemo/radio therapy in case of increasing levels of CtDNA during the course. In the laboratory front, recording changes in the peripheral blood parameters like CtDNA/CTC offers to be the least minimally invasive and a highly cost effective method which could be implemented more frequently than other modes of surveillance tests. Further, as the site of recurrence is not evidenced by imaging when the patient is in early relapse, frequent monitoring of CTC/CtDNA is evolving to become the standard of care in the near future and is defined as minimal residual disease (MRD) testing. This feature of MRD in solid tumors has shown promising results in breast [13, 14] as well as other cancers such as lymphoma (DLBCL) [15] by tracking the tumors' genetic status thus predicting survival and informed treatment decisions. In patients where enough tissue is not available to get ER PR and Her2 circulating tumor cells can be useful as a liquid biopsy. Genetic mutations seen in circulating tumor cells may be more accurately representative than the tissue biopsy as it is more monitored in real time.

Role of miRNA in Breast Cancer Management

MicroRNAs (miRNA) have been extensively studied and its clinical value, in the context of diagnosis and management, is beyond doubt. miRNA are short fragments of non-coding RNA of the size of 21–23 nucleotides. They bind to the 3 untranslated regions of mRNA and thus regulate the gene expression in multiple pathways such as proliferation, apoptosis and migration. This feature of miRNA makes it a suitable tool to inhibit protein translation. In particular, miR-21 and miR-34, are considered as most promising targets for RNA-based therapy in non-invasive and invasive breast cancer. Different strategies such as nucleic acid mimics, miRNA sponges, and conjugated drug based miRNA are used for nanodelivery of the drugs to the targeted site. Beyond this, miRNA is also being studied as a clinical biomarker in diagnosis and prognosis. Though still clinically not proven this approach has tremendous clinical potential. [16].

Application of NGS and Array CGH in Clinical Practice

In last two decades, there has been a surge in evolution of new technologies, to address changes in human cells at molecular level. Today, beyond histopathology, molecular testing for DNA/RNA/Protein is being considered useful in clinical laboratories and even in clinics.

Next-Generation Sequencing (NGS)

This technology allows to screen for mutations in DNA found uniquely in cancer cell in the biopsies, surgical resections as well as in cell-free plasma of a patient's blood. Most commonly detected mutations are

- Single nucleotide polymorphism which could be missense mutation (protein sequence altering event) or a non-sense mutation (stop codon generating event) in the coding sequence.
- Short insertions/deletions (less than 15-20 nt stretch), and
- Epigenetic modifications in the tumor and host genome.

Annotation for these molecular alterations in correlation with clinical response is known to add value to our understanding of the tumor genome.

Put together, NGS and Array CGH which provides information on copy number variations, gene duplications, single nucleotide polymorphisms, balanced translocations and large deletions, have greatly contributed our understanding of the molecular alterations that define cancer in the past decade. This evolution has led to addition of novel tumor-specific targets and drugs to block these targets in the field of cancer medicine. Thus the concept called "Genome medicine" was introduced in clinical oncology. However, in order to endorse these findings directing specific therapies based on laboratory hypotheses, extensive clinical testing of the hypotheses to show utility for these potential predictive claims is recommended. Redefining the protocols for trial designs to incorporate the tumor genotype status would make this feasible. Clinical evidence for molecular findings is highly challenging due to the rapid evolution of new information and methods to assess and validate the genetic findings.

RNA Based Sequencing

Sequencing of coding and noncoding RNA which represents the complete transcriptome using NGS platform is called Transcriptome sequencing. Transcriptome sequencing provides information on:

- Gene expression levels (YES/NO information),
- Identification of expressed fusion transcripts including chimeric fusion transcripts which are part of chromosomal aberrations,
- Alternative splicing and RNA editing events and
- Single nucleotide polymorphisms.
- It is more complex than DNA sequencing but more accurate as some of the DNA mutations may not be transcribed and translated. RNA changes are true representation of genetic change.

Genes Involved in Hereditary Breast Cancer and Other Syndromes

A syndrome is a genetic condition wherein a group of diseases co-exist in the same family of related individuals. Most commonly characterized syndromes associated with breast cancer are *Hereditary breast and ovarian cancer syndrome*, *Lynch syndrome*, *Li-Fraumeni syndrome*, *Cowden syndrome*, and *Peutz-Jeghers syndrome*.

There has been substantial progress in our understanding of the genetic component of hereditary breast cancer in the last five years, ever since the first breast cancer susceptibility gene was discovered 20 years ago. Nevertheless, the challenge in management of this subgroup of individuals remains to be explored to a greater extent. As against a 10 % average risk of developing breast cancer for women in a general population; those women with pathogenic mutations in the BRCA1 or BRCA2 genes inherited from their parents, have a risk of up to 85 % in developing breast cancer during their lifetimes [13], and also possibility of developing at an early age. Risk reduction measures which are part of routine clinical monitoring in these patients include intense radiological screening, prophylactic surgery or chemoprevention. Inclusive of BRCA1 and BRCA2, there are other rare susceptibility genes such as TP53, STK11, PTEN, ATM and CHEK2 which also contribute to a significant risk for breast cancer, as part of familial/hereditary breast cancer. In addition, findings from genome wide association studies (GWAS) identified a group of low-risk genetic variants in 90 loci which were recurring and associated with heritability of breast cancer. With the changing paradigm of genetic testing evaluation in clinics, massively parallel sequencing using multi-gene panel testing which includes predictive, prognostic and predisposing genes, is now gaining primary importance in the decision making process of breast cancer management [17].

BRCA Genes and Others: Screening and Risk Assessment

Breast cancer being the most common cancer in women with a substantial disease burden worldwide, a comprehensive screening for women, which would also include a genetic test that predicts the inherent susceptibility for cancer is of utmost importance. This test must be acceptable to patients and society in terms of convenience, comfort, risk, and cost. Early detection and personalized treatment has been shown to improve the disease free survival.

Reducing the incidence of new cancers with the goal of reducing cancer-related morbidity and mortality is the goal of cancer prevention screening camps. Some other examples for the same, may include, smoking cessation, reduced exposure to sunlight, prophylactic surgeries to remove the targeted

risk organ before the cancer onsets, and use of medication to extend the time of onset.

Genetic testing to understand the susceptibility to develop cancer at an early age requires extensive clinical validation. The risk prediction is complimented by several other co-existing factors such as environmental changes and lifestyle which may affect the penetrance of the mutation.

Thus, the clinical validity of genetic testing is known to vary in the context of different populations. Some of the major sources where guidelines for genetic testing are defined as part of breast cancer screening are from American Cancer Society, US Preventive services task force and American College of Obstetricians and Gynecologists.

Brca-1 and Brca-2 gene mutations are not only indicative of occurrence of cancer in the individual. It also helps to an extent in selecting therapy for cancer patient. Generally women with Brca-1 mutation develop triple negative breast cancers and those with Brca-2 mutations have higher chances of developing ER positive breast cancers. In Brca-1 mutated breast cancers the ability to repair damaged DNA is minimal. Hence these patients respond very well to alkylating agents and platinum. A new group of drugs called PARP inhibitors have been FDA approved in Brca mutated ovarian and breast cancer patients.

Breast Cancer Genetics and Survival Variation

The racial survival differences for a given clinical regimen is primarily contributed to variations in the biology of tumor onset and the subsequent genomic evolution. Hence, the clinical interventions are subjected to modifications based on social, ethnic and geographic location of the patient, which guides the clinicians to take informed decisions for these individuals. For example, the incidence rates of breast cancer is more in Caucasian women as compared to African American women, however the mortality rates are more in the latter than the former. Likewise in India, the incidence of triple negative breast cancers is higher than that of western population [18]. Prior interventions have been too narrowly focused on the patient rather than addressing the system and improving care across the continuum of breast cancer evaluation and treatment.

Randomized clinical studies which include information on the nature of the population, geographic distribution, duration of the observation, are some of the critical factors which determine the prevalence of genetic variations, role of genetics in inherited syndromes and the clinical validation of the testing methods. This reinforces the concept of mapping out local genetic variations in our population [18].

Achievements and Future

The gene studies have stratified patients more scientifically and precisely. The prognostication of the patients has improved by the use of these markers and also circulating tumor cells. Newer agents targeting these genes and their products have revolutionized the therapy in breast cancer. Anti- Her2 therapies, MTOR inhibitors and cytotoxic drug conjugates are some of the examples. Drugs working on cell cycles and immune checkpoint are in advanced clinical trial and likely to be in clinical use shortly. Avoiding unnecessary exposure of a drug in patients unlikely to benefit from these expensive drugs is also an active area of investigation. In the near future the complete genomics of cancer may become a necessary tool in classifying prognosticating and treating breast cancer patients which will tailor the treatment to the patient with minimum toxicity and avoid cost.

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