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Review Article

Review: The Contribution of Technological Advancements in Breast Cancer from Next Generation Sequencing to Mass-Spectrophotometer

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Abstract

Breast Cancer has been recognized as a global health problem and a complex disease entity. When considering all cancer types, breast cancer is becoming the most frequent type of cancer affecting women of all ages and across the whole world. Era before next generation sequencing, diagnostic approaches employing genetic analysis were focused on individual factors and were unable to illustrate the comprehensive network of biological complexity encountering this aggressive disease. The introduction of next generation sequencing has facilitated the acquisition of high resolution whole genome, exome and transcriptome sequencing data. This previously unattainable data enabled health professionals to gain a global view of breast cancer genomes and the full spectrum of its involvement. With the utilization of Next Generation Sequencing data from large number of breast cancer patients, it is expected that the exact signaling pathways, leading to the oncogenic transformation of breast tissue, become more understood. Next Generation Sequencing promises to revolutionize breast cancer research, therapy and diagnosis. Combining the recent technological advances and the ability to integrate data from the areas of genomics, transcriptomics and proteomics; scientists have a greater opportunity to further investigate tumor evolution, gene expression and protein involvements. In this article, we have reviewed the recent technological advancements in breast cancer research and highlighted the contributions of Next Generation Sequencing technologies to such advancements.

Keywords: Breast Cancer; Mass Spectrophotometer; NGS and Technology

Introduction

Cancer has been recognized as a global health problem and a complex disease entity. Cancer, from a pathophysiological point of view, is a collective predisposition of multiple genetic and environmental factors working their respective parts within a biological and developmental context. According to GLOBCAN statistics has shown 2,088,849 of new cases were reported and 626,679 deaths of Breast Cancer worldwide in 2018. Breast cancer is found to be affecting one in eight females and almost a quarter of cancer cases in women. However, breast cancer is mainly caused by several dynamic changes in the cellular lining of ducts or lobules in the breast tissue. Breast cancer is the leading cause of death among women

in low- and middle-income countries [1,2]. Earlier diagnostic approaches employing genetic analysis were focused on individual factors and were unable to illustrate the comprehensive network of biological complexity encountering this aggressive disease. This can be mainly attributed to the limitations in scalability, throughput, and interpretability of available data from genetic analysis. Breast cancer consists of many biologically entities with different pathological features and clinical implications. Thus, in curing and managing cancer patients, the one size fits all strategies are failing in various percentages of different diseases including breast cancer [2,3]. Thus, it requires different treatment responses and different therapeutic strategies [4]. Indeed, the personalize therapies would demand an understanding at a molecular level for identifying underlining disease in each patient or a group of patients. Technology has succeeded in resolving many human problems and provided a better lifestyle in every day's life of today. Of course, a big development and advances are also taking place in medicine in the last few decades.

Certainly, the incidence of breast cancer in the developing countries is rising due to the changes in reproductive factors, lifestyle and increased life expectancy. The high fatality rate in developing countries can be referred to the inequities in early detection and access to treatment [5]. The attainable evidence of stage, at the time of diagnosis reveals that a high proportion of cases are detected in their late stage in developing countries [11]. The main points of importance in diagnosing breast cancer disease at an early stage and similarly identifying the molecular basis of its progression, metastasis and treatment options are the only effective strategy for handling the complexity of the disease. In fact breast cancer become more obvious as a heterogenic disease when first was subclassified by Perou and colleagues after identifying various patterns of gene expressions to four main intrinsic subtypes (Luminal, EGFR-2 enriched, basal-like and normal breast like) [6]. Traditional diagnostic methods like tissue biopsy and Sanger sequencing contributed to reveal some biological insights about the disease, but the diagnostic utility is confined to "regions of interest" and failed to develop a comprehensive genetic alterations map to the level of a complete genome up to date. Cancer is now described as the disease of the genome among which is the breast cancer. New Generation Sequencing (NGS) is the way forward to further understanding disease diagnosis, prognosis and treatment options. NGS is a rapid revolution innovative platform sequencing processes attained by using instrumental technology which has high through output data analysis tool. This technology is biologically meaningful and gives insight genome structure and clinically relevant with potential applicable benefits for our patients. The utility of NGS-based gene panel would provide a great breakthrough for the oncologist in treating the patients of tomorrow and that cannot be saved today [7]. Initial human genome sequencing was initially completed and published in 2001 and then further in 2003 with high costs of both time and money. Since then the technology advancement and furthered knowledge obtained has improved quality and made tremendous decrease in costs that still continue to drop, see selected components of work in humans genome sequencing progress time-map (Figure 1).

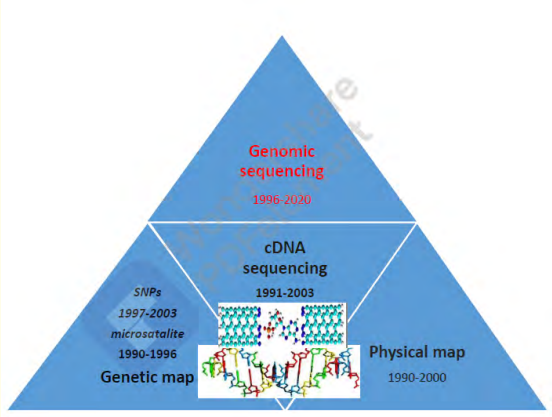

Figure 1: The human genome sequencing progress time-map.

(Work from 1990 on Human model until completion in 2003 based on data from NIH webpage and data published on Nature 2001).

Microarrays has played a significant role in breast cancer diagnosis, which helped the interrogation of cancer genome for changes in DNA-copy numbers and loss of heterozygosity events along with entire cancer transcriptomes for changes in gene expression levels [8]. This has helped in turn to a better understanding the biology of its molecular classification, refinement in prognosis and in identification of predictive markers for the response to commonly administered anticancer treatments [9]. A microarray has also identified many novel susceptibility loci for the disease, but our

understanding of the functional role of these loci in the context of etiology and pathogenesis needs to be investigated further [10,11].

Next generation sequencing (NGS) in breast cancer

NGS platforms have become widely available in the recent years with numerous advantages than sanger sequencing as it is massively parallel large scale sequencing with built in scalability, ultra-high throughput and has the comprehensive ability to uncover the complex maps of genetic information with robust sensitivity in a cost effective manner [12,13].

Cancer research in the past decade has witnessed remarkable advances that can be attributed to NGS technologies and the data that the technology provides. This has not only helped to understand cancer biology in a broader perspective but also helped to elucidate the interplay between cancer genome, epigenome and transcriptome [14,18]. In 2008, a cancer genome study was published using exome sequencing in which tumor cells from acute myeloid leukemia (AML) were compared with normal cells [15]. This study reported more than eight new genes that helped to identify novel mutations associated with cancer thus it has led to many similar analyses of cancer types being published [16]. NGS provides quantitative information and finer assessment of the genome at base pair level leaving great technological promise for cancer research in general and breast cancer in Particular [17].

NGS is currently used extensively in both clinical and research settings and has already boosted our knowledge in breast cancer molecular pathology. This surely will pave the way for the development of advanced and more efficient clinical protocols [14,17]. In 2012, a series of landmark papers resulted in an explosion in the field of breast cancer genomics that resulted in unravelling the mutational landscape in small and large sets of breast tumors by using NGS [18,19]. NGS also have the edge in identifying low level mutations using high-depth sequencing that are prevalent in certain solid tumours [20]. Friedman., *et al.* 2015 has reported for the first time the case of low-level, multiple tissue, constitutional mosaicism in BRCA1 gene. The study also highlights the need for using deep sequencing in clinically suspected individuals whom are suspected of having predisposition to cancer when their tumors displays a BRCA mutation [21].

Stephens., *et al.* 2012, using exome sequencing has reported the identification of 7,241 somatic mutations in 100 ER+ and ER- primary tumors and have further confirmed the presence of mutations in driver genes such as AKT1, BRCA1, CDH1 and GATA3 that were previously involved in the development of breast cancer [18,22]. Nadine Tung and co-workers have reported that around 10.7% of women were identified to have germ line mutations within a gene from a panel of 25 predisposition genes that predispose women to breast or ovarian cancer [23]. They also significantly observed that the frequency of mutations in non -BRCA1/BRCA2 genes was 4.3% in their 25-gene panel [24].

Also Lin., *et al.* came up with sequencing panel consists of 68 genes and subsequently discovered alterations in RAD50, TP53, ATM, BRIP1, FANCI, MSH2, MUTYH, and RAD51C which can be better used for empirical breast cancer risk assessment [25]. Lhota., *et al.* identified 127 truncated variants from an NGS experiment consists of 581 genes in 325 breast cancer patients previously tested as negative from BRCA1/BRCA2/PALB2 diagnosis [26]. Nik-Zainal., *et al.* has remarkably illustrated the mutational processes underlying breast cancer by sequencing 21 breast cancers and by comparing with normal tissues has identified 183,916 somatically acquired base substitutions [27]. Interestingly, Schenkel., *et al.* systematically demonstrated using a combined technical evaluation that NGS pipeline outperforms the coupled gene panel analysis using Sanger sequencing and Multiplex Ligation-Dependent probe amplification (MLPA) [28]. Finally, the NGS era of breast cancer testing has broadened the mutational spectrum of breast cancer genes and revealed the complex genetic variation landscape that coexists with the disease-causing mutation. This warrants the successful implementation of an NGS based gene panel strategy in clinical setting to do a systematic risk assessment and identify the causative mutation. This approach is started in hereditary cancer testing and has contributed to diagnostic quality as the mutation detection rate is increased and the understanding about the disease has enhanced.

Transcriptomics in breast cancer

Apart from the genomic alterations, acquired genomic aberrations and inherited genetic variations, the process in the progression to breast cancer can also be influenced due to changes in splicing events, transcriptome and the resulting protein functions [29,30]. The diversity and flexibility of genomic processes that make a cancer cell to "tailor-make" specific functional units from the exons of the gene has been reported recently from transcrip-

tome profiling studies [31-34]. For each relevant cancer gene, its complete transcript annotations for the human genome is still far from complete as this is driven by multiple processes such as alternative pre-RNAs, promoter usage, splicing, polyadenylation that modify protein coding regions and subsequently, the function of the resulting proteins [35-39]. Identifying the transcript variants and tissue specific splice variants generated due to natural transcriptomics dynamics in cancer will be challenging and important for the better understanding of cancer specific splicing of genes [40-44].

Yi-Rong Liu., *et al.* 2016, has reported a novel Triple-negative breast cancer (TNBC) classification system by combining the expression profiles of mRNAs and lncRNAs in a large TNBC cohort. Based on the study, TNBC can be classified into four subtypes such as immunomodulatory (IM), luminal androgen receptor (LAR), mesenchymal-like (MES) and basal-like and immune-suppressed (BLIS). The authors have also determined subtype-specific lncRNAs that can be used as potential biomarkers and targets [45].

John., *et al.* in 2016 demonstrated that Studying of the epigenome, transcriptome, and proteome can also identify target genes and have the potential to predict novel therapeutic approaches [46]. Fe Chen., *et al.* reported nine genes (FSIP1, ADCY5, FSD1, HMSD, CMTM5, AFF3, CYP2A7, ATP1A2, and C11orf86) were significantly linked with the prognosis of TNBC patients, but three of them (ADCY5, CYP2A7 and ATP1A2) were involved in the hormone-related pathways [47].

In Breast cancer also micro RNAs (miRNAs), e.g. miRNA-91 is well studied and findings show that it targets the AIBI oncogene (amplified), Insulin-Like Growth Factor-1 (IGF-1), interlukine-8 (IL-8), CyclinD, and that it can also inhibit anchorage-independent growth as well as substantiating miRNA-91 as a tumor and metastasis suppressor [48-50]. While this evidence is out, other researchers are stating that miRNA-91 is invariably a tumor suppressor and have brought in some evidence to support it promotion of tumor development [51]. However, different studies have reported that different miRNAs have interfered in the pathogenesis or prognosis of breast cancer. A single miRNA might not provide a precise answer, a multi marker might provide a more reliable diagnostic or prognostic marker and help in understanding the ncRNAs role in this type of cancer. Studies using multi markers have proven more consistent and sensitive markers in diagnosing breast cancer [52]. Introducing stronger miRNA panels that share relatively similar roles in such disease can improve sensitivity and reliability. A research group that worked on a multi-marker of seven miRNA panels (miR-127-3p, miR376a, miR- 652, miR-148b, miR-376c, miR-409-3p, and miR-801) suggested that this would be considerably reliable pre-screening panel, thus showing that the markers are routinely elevated in breast cancer [52]. However, despite the various studies carried out by Cuks' group for the purpose of identifying an ideal screening panel for breast cancer, finding one does not appear to be that easy. A review article by Hamam., *et al*. concluded that there are no current successful panels of miRNAs to be employed in oncology practice, but they are very optimistic that diagnostic, prognostic, or predictive biomarkers will be brought to clinical practice from these free molecules in breast cancer patients [52].

Furthermore, ncRNAs have shown to be involved in some cancer drug resistance, such as Tamoxifen, ectopic expression of miR-221/222 resistance due to targeting the p27 apoptosis pathway [53]. It is likely that miR-342 in Tamoxifen as well as Trastuzumab (anti Her2/neu drug) increase drug resistance as it targets miR-375 in both drugs [54,55]. The mysteries of ncRNAs continue and the more we understand about them, the more there is to be revealed for future RNA-based therapies in various human diseases.

Proteogenomics in breast cancer

Large scale genomics study has helped in identifying dysfunctional transcriptome harbouring large numbers of non-synonymous single nucleotide variants (SNVs), insertions and deletions (indels), aberrant gene fusions, alternative splicing variants and aberrations in copy-numbers [56,57]. However, proteins are central to cellular functions and malfunctioning proteins stimulates tumour initiation, progression and response to treatment [58]. Nonetheless, the proteomic data has been rarely used to model these phenotypes comparing to the usage of genomics data [58]. Though, the partial correlation between protein and mRNA abundance has already been established to be very poor, flow of information from genome to proteome in tumour remains to be unexplored in cancer biology [59,60]. It is thus very important to have high throughput methods in exploring cancer proteome in order to study the changes in signalling pathways, protein isoforms and post-translational modifications [58]. Mass spectrometry (MS) with its recent technological and methodological advances provides wide proteome cov-

erage with increased precision comparing to other methods such as reverse phase protein arrays and ribosomal profiling [61]. The current technological advancement in deep sequencing and proteomics to effectively define human proteomes in association with multiplexed targeted proteomic assays to measure proteins panels that are involved in biological pathways has made remarkable progress in our understanding of cancer at its molecular level [62]. With the current focus of proteomics in quantifying changes in proteins and its interacting partners, post-translational modifications (PTMs) and isoforms in response to a particular stimuli and further linking this to high throughput genomics and transcriptomics data, will result in novel and complementary information that can be garnered from this multi-dimensional omics data thus helping us to have a better understanding of cancer biology as a whole system [62].

Philipp Mertins., *et al.* have reported proteogenomic analysis of 77 high quality breast cancer data. Their analysis has provided insights of somatic cancer genome including the consequences of chromosomal loss, such as the *5q* deletion characteristic of basal-like breast cancer. The study has confirmed stromal-enriched group of proteins in addition to basal and luminal clusters. The pathway analysis of the phosphoproteome in this study has led to the identification of G-protein-coupled receptor cluster that was not identified at the mRNA level. This study has nicely illustrated the use of technological and analytical approaches that can be used in cancer research to provide new opportunity in linking the genome to the proteome [63].

Conclusion

In conclusion, the NGS era of breast cancer research has broadened the mutational spectrum of breast cancer genes and revealed the complex genetic variation landscape that coexists with the disease-causing mutations. This warrants the successful implementation of an NGS based gene panel strategy in clinical setting in the near future. Indeed, this will lead to a systematic risk assessment and the identification of the causative variants in breast cancer patients at an individual level. Hereditary breast cancer testing has already been established and contributed to diagnostic quality of today. A key challenge is to establish biological link between a genetic variant and the etiological mechanisms of the disease to involve a range of biochemical intermediates including coding and non-coding RNA proteins and metabolites. The integration of the new technologies we have today is a great potential to identify the emergent behaviours that cannot be found by studying mRNA, proteins or metabolites as separate entities or in isolation, as they do not act on their own in biological systems, but through complex interactions with each other. However challenges in analysis and interpretation of huge data sets will still remain. But this is definitely a huge opportunity for advanced assessment of the disease and a better future for clinical management.

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