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Mini Review

Immunohistochemical Surrogate Markers for Breast Cancer Classification: A Prognostic and Predictive Perspective

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Abstract

Breast cancer (BC) is the most frequent malignancy with heterogeneous disease pattern of female. The rational integration of routinely evaluated clinico-pathological parameters through biological markers such as proliferative index, inflammatory conditions and pathological status provides an important guide towards diagnosis as well as for systematic therapy decision. The aim of this assessment is to highlight the importance and advantage of immunohistochemical (IHC) surrogate markers towards classification and prognosis. The current diagnosis and therapies are incomplete, due to lack of accuracy in identifying relapsed disease. The cancer genome charts stated diverse genetic variations associated with molecular intrinsic subtypes. Gene expression profiling, is an advanced technique, it would generate multi-variate molecular signatures providing diagnostic, prognostic predictive information. Additional value of such molecular information for the management is still in evaluation through clinical trials. There is intense competition towards identification of molecular markers through advanced genome based techniques, where the gene expression profiling delineates an aggressive subtype of BC. In this case, expanding the utilization of IHC surrogate markers for clinical classification, diagnosis can be manageable even in low resource settings it emphasize this technique as cost effective, quality assurance and it avoid variation in pathological grading.

Keywords: *Breast Cancer; Immunohistochemical; Biomarkers; Gene Expression; Diagnosis; Prognosis*

Introduction

Breast cancer (BC) is one among the utmost common malignant tumours of female in the Asian population [1]. It is a heterogenous complex disease with diverse clinical behaviour, understanding of its broader term is necessary. Where, the largest group of ductal cancer have the highest heterogeneity. Indian Council of Medical Research (ICMR) survey showed incidence of BC has doubled, and it occurs at younger pre-menopausal age. The age group was observed to be between 15-34 years. The incidence is rare in women younger than 25yrs and it increases rapidly after 30yrs. Among 100,000 women for Delhi, the frequency rate was found to be high as 41, followed by 37.9 in Chennai, Bangalore (34.4) and Thiruvananthapuram as 33.7 [2]. Though, there was an decreased rate of mortality through advanced technologies in early diagnosis and treatment modalities [3]. Beside early detection and advances in therapy, metastatic breast cancer is hopeless and is responsible for about 90% of cancer associated deaths [4].

The BC aetiology is multifactorial, through the interplay between genetic and environmental factors (Figure 1) [5]. The sturdiest risk factor of BC is family history of the disease, with few or several risk susceptibility genes from high to moderate, and multiple low-risk modifier genes implicated. In specific, women with one or two first-degree relatives with positive history are likely two to three-fold increased risk. Even the risk still higher when there are more number of relatives are affected, history of BC before menopause and it was bilateral. In such circumstances, the familial predisposition to BC endured unidentified until the identification of susceptibility genes known as BRCA1. The transcriptional genes in BC was being up-regulated or down-regulated in larger groups, this way of molecular biomarkers have revealed through high sensitive gene expression profiling [6]. This review aimed to identify the associations between the novel immunohistochemical markers that can be endorsed in clinical applications.

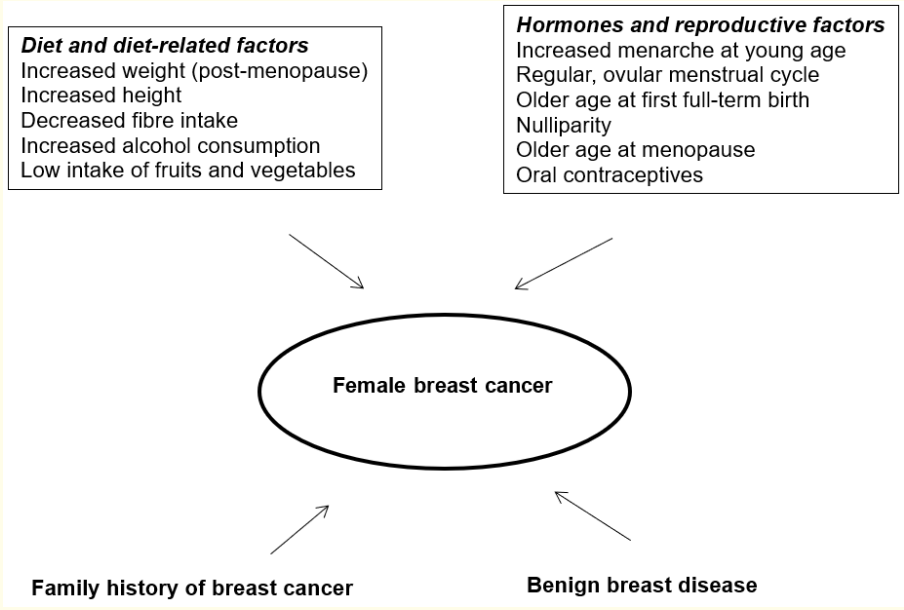

Figure 1: *Aetiological factors involved in the development of breast cancer.*

Evolution of breast cancer

Over a period of time, the BC exceeded lung cancer (11.4%) with 2.3 million new cases (11.7%) and 6.9% of new deaths of women with BC [7]. About 70% of all BC constitute non-specific invasive ductal carcinoma, difference within a single tumor (intra) or morphologically similar tumor type (intertumoral) is accepted, hence, the clinicians would attempt new systems through pathologist to screen their patients recovering. Traditionally, the evaluation of such pathological interpretation is determined through evaluation of the degree of tubular differentiation, nuclear pleomorphism and mitosis rate. Over the past decades, due to advancement in analytical methods applied to tissue based screening technologies have helped in determining the prognostic and predictive cancer biomarkers. The introduction of microarray applications has been very beneficial towards identifying the genomic expression profiling for the classification based on tumor biology instead of morphological features. Because, this supports the notion that BC is a molecularly heterogeneous disease having different pattern of gene expressions that influence prognosis. The terminology "Molecular Classification" was proposed by Perou and Sorlie have shown the evidence difference in gene expression pattern through comprehensive study [6]. Based on different gene expression pattern the BC has been delineated by genotypic-phenotypic correlations, whereby two distinct pathways has significant role in

evolution of low- and high-grade invasive carcinomas. In this case, it consistently shows oestrogen receptor (ER) and progesterone receptor (PgR) positivity and 16q loss. The changes in E-cadherin expression play an distinct role through distinguishing molecular evolution between high/low-grade ductal and lobular carcinomas [8].

Advancement of BC arises from the terminal ductal lobular units (TDLUs) of the breast through sequential changes that are predictable as pre-invasive lesions. These wounds are characterised by propagation of epithelial cells that endure within the basement membrane and are limited in the breast ductal structure [9]. Most frequently, the columnar cell lesions (CCL), atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), and lobular carcinoma in situ (LCIS); with 'ductal' and 'lobular' breast cancers are recognized precursor lesions. In an unique model, the development of BC evolve in a stepwise manner, beginning as hyperplastic benign lesions (hyperplasia of usual type) that progress to atypical hyperplasia. Consecutively, the invasive cancer penetrates the basement membrane and invades the local stroma. This progression was distinct for ductal and lobular types of cancer. Empathetic of this idea has advanced over a period of time with more sophisticated techniques (e.g. sequencing, loss-of-heterozygosity (LOH) and comparative genomic hybridization (GGH) analyses). At this consequence it is understandable as a complexed model. A significant finding in favour of this multistep BC progression in both DCIS and invasive was due to LOH at 16q and 17p. This also seen at a related incidence in ADH [9]. Common hyperplasia lesions and its types are no longer considered as clonal precursor lesions, however between normal epithelium and atypical hyperplasia the columnar cell lesions are the apparent and preliminary morphological stage [10]. Essentially, these detected lesions (pre-invasive) at initial investigations are considered as sign of risk for invasive breast cancer [11].

Types of breast cancer

For the past few years there has been much progress in understanding the pathology and molecular biology of BC, although, classification of BC defined molecularly have not yet arrived. The studies are incorporating from molecular and genetic data into the morphologically defined system [12]. Morphologically, it must be clearly understood that the tumor is limited in epithelial components of breast otherwise invaded the surrounding stroma, and this tumor mass appeared in the mammary ducts or lobes [12,13]. Basically, the BC is of three types' ductal carcinoma, inflammatory BC and lobular carcinoma. Nevertheless, in histopathological practice, representative features of cell, type of secretion, immunohistochemical profile and architectural characteristics determine whether the tumor is ductal or lobular. Essentially, the breast cancers are of two different types invasive and non-invasive. In which, the non-invasive breast cancer are classified as two types based on inner lining of ducts called ductal carcinoma and lobules-milk producing glands (Lobular carcinoma) [6,11]. These non-invasive cancers are called carcinoma in situ and are referred as pre-cancers as given in figure for the types and sub-types of BC (Figure 2). DCIS is the most common type of non-invasive breast cancer; it initiates in the milk ducts and it hasn't spread to other organs. DCIS isn't life threatening, but it increases the chances of developing from non-invasive to an invasive BC at later stage.

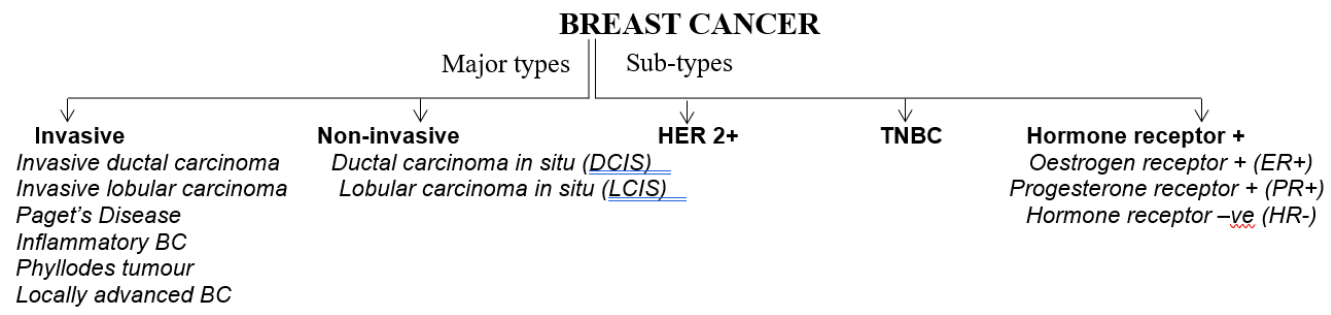

Figure 2: *Breast cancer types and subtypes.*

In case of invasive BC, there are seven types that have spread in surrounding tissues either in outside the ducts or in lobules. Initially, the BC refers to the cancer that is limited to breast and it would spread only in surrounding lymph nodes in the breast or armpit (axilla), nonetheless not in elsewhere. Invasive ductal carcinoma (IDC) is the first most common type of breast cancer (80%), where it start from the milk ducts and it spread into the surrounding breast tissue after broken through the lining of the milk duct. IDC has 'no specific type' because the tumor mass did not show any morphological features and they can also be recognised as a 'special type' when it show sufficient cellular and molecular behaviour characteristics.

Over a period of time, this invasive ductal BC could spread essentially to the lymph nodes and possibly to various parts of the body [14]. Invasive lobular carcinoma (ILC) initiated in the milk producing lobules of the breast and it broke through the lining of the lobule to metastasize around the breast tissue. ILC is the second most common type of BC. Sequentially, the rare form of BC is Paget's disease (PD) where the tumour cells grow in the nipple or the areola (the area around the nipple) become scaly, red, and itchy. Mostly, individuals with Paget's disease also have either non-invasive (DCIS) or invasive BC and it is identified through the unfamiliar alterations in the nipple and areola as a crucial signs. Followed by, the rare and aggressive form of invasive BC is inflammatory BC (IBC) which targets the blood vessels in the skin and/or lymphatic vessels of the breast. Phyllodes tumour (PT) of the breast are mostly benign (not cancerous) and rarely malignant (cancerous). Wherein, the cancerous cells propagate rapidly through metastasis in external region of the breast. Morphologically, the other invasive type of BC includes locally advanced BC (LABC) which is large and spread to other nearby areas like skin, chest wall muscle and may have extensive involvement of local lymph node. In other distinct type of BC is metastatic breast cancer, which is also known as advanced stage of BC (stage 4). It could spread to other major organs of the body such as the bones, liver, lungs or brain.

Molecular subtyping based on immunohistochemical surrogate markers

About 2/3rd of the BC are hormone receptor positive, these molecular subtypes are characterized based on activity of female hormones like oestrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor-2 (HER-2) (Figure 3). Based on hormonal expressions, the BC is characterized into 4 groups and these surrogate IHC markers (ER, PR and HER-2) could identify molecular subtypes of BC [15].

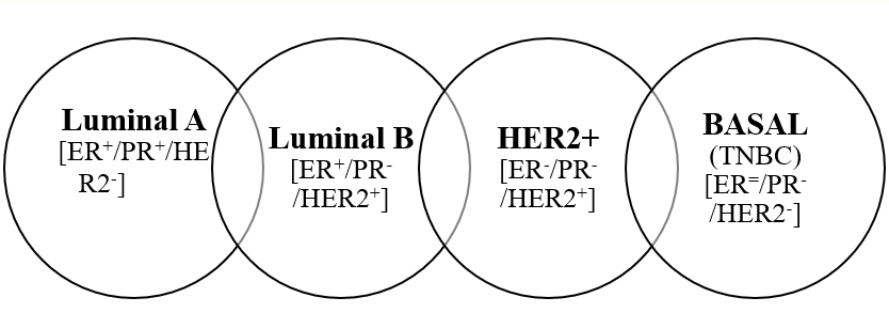

Figure 3: *Classification of BC based on hormones.*

For Luminal A, the positivity of ER, PR and HER2-ve identified through IHC staining (Figure 4). Most cases with lobular carcinoma in situ identified through molecular profile portray as luminal A cancers [16]. Uninterruptedly, the majority (56 - 61%) of invasive lobular carcinomas have identified as characteristic of luminal and they tend to have long-term survival [17]. The genes found in this type of BC are typically expressed in luminal epithelium that lines the ducts [16,18]. In addition to the variation in hormonal level, the involvement

of Ki67 either low (luminal A) or high (luminal B) could distinguish the pathological significance. HER2 and TNBC are considered as non-luminal group.

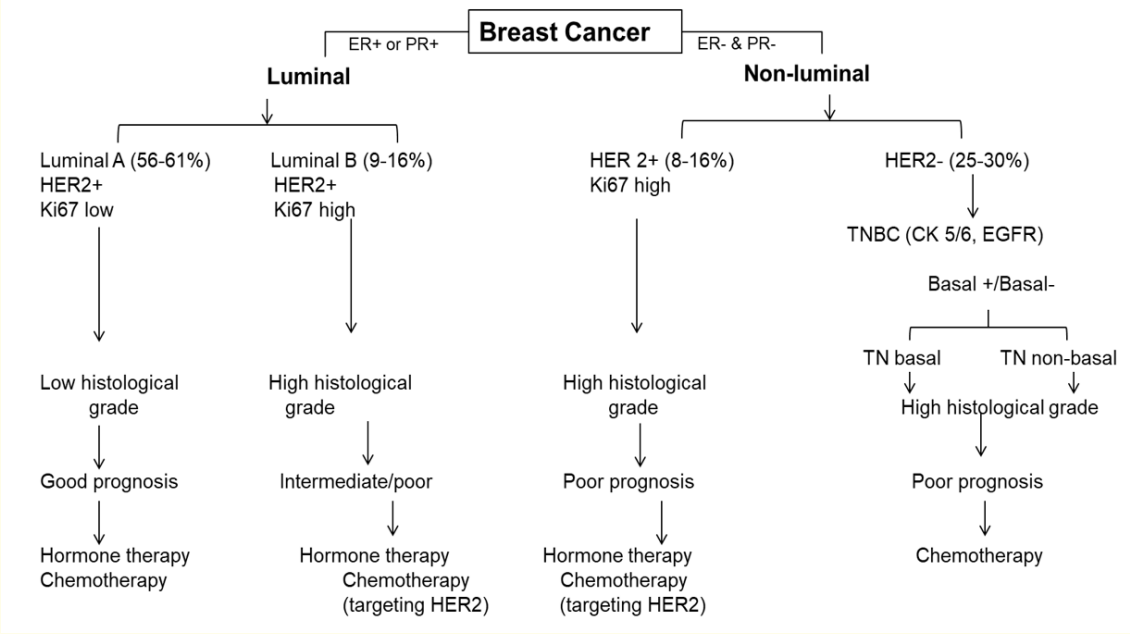

Figure 4: *Classification of BC based on IHC molecular markers.*

Similarly, the positivity of ER and HER2 expressions with absence of PR characterized as Luminal B and it includes 9-16% of most aggressive cases compared to luminal A. In case of Luminal B breast cancers, the high grade tumours have reduced survival [17]. Originally, this subtype (Ki-67) was not included in defining this subtype [15]. Rendering to modern amendments, the surrogate classification of intrinsic BC in to two parts as, luminal B (HER2 negative) and luminal B (HER2 positive). In both the cases, the IHC markers ER and/or PR positivity, HER2 negativity and high Ki-67, but luminal B (HER2 positive) subtype includes breast cancer cases with positive ER and/or PR in connection with positive HER2 and any Ki-67 level [19]. Based on the involvement of these surrogate IHC markers (ER, PR, HER2, Ki67) and basal markers (CKs 5/6, 14 and EGFR) can mimic molecular classification of breast cancer.

Based on HER2+ and TN expression status, the non-luminal group was further divided through the frequency of HER2 positive subtype (18-16%). This is characterised by lack of ER and PR expression by immunohistochemistry technique, while HER2 overexpression or HER2/neu gene detected through fluorescent in situ hybridisation (FISH). The HER2 positive subtype contains two subtypes, ER-negative that cluster near the basal-like tumours (HER2 positive ER negative subtype), and ER (may also express PR) positive as in luminal B subtype, both the types are based on ER expression [18]. Mostly, the p53 is not expressed and the expression of CK 8/18 is heterogeneous and moderate. When it is positive, the response for EGFR is crucial and restricted to less than 5% of tumour cell population. HER2 type is often related with ductal carcinoma insitu (DCIS), many cases have high grade and are characterized by poor prognosis [17,18].

The high proliferative potential of basal cells represents more differentiated luminal cells. Based on clinical investigation, the basal-like cancers designates molecular phenotype identified through advanced techniques lacking ER, PR and HER2 expressions [20]. Though,

most triple negative BC cluster within the basal-like subgroup, there is almost 30% discordance between these two groups and the terms are not synonymous [21,22], but detected the expression pattern of CK 5/6 and/or EGFR through microarray or by immunohistochemistry techniques [20].

While most triple negative BC cluster originate within the basal-like subgroup, these terms are not synonymous and about 30% consistency amongst the two groups [21]. However, there is no precise hallmark landscapes on repetitive histopathological slides that guide to identify these tumours. However, few morphologies such as solid architecture, pushing borders, prominent lymphocyte infiltration, scant stroma, high grade, high nuclear/ cytoplasmic ratio, high mitotic index and occurrence of necrosis for basal-like cancer will be more recurrent in premenopausal patients [23,24]. Appearance of high rate of p53 mutations are widely seen in basal-like cancer and are common among BRCA1 mutation carriers [18]. In which, the progressive pattern of EGFR, CK 5/6, CK 14, CK 17, and p63 shows metaplastic features in about 90.8% of basal-like BC [25]. On the other side the advantage of immunohistochemical panel have identify 93.8% metaplastic breast cancer as basal-like tumours. Through genetic and immunohistochemical investigation, medullary carcinoma identified as basal-like sub type through triple negative character and CK 5/6 expression [26].

Author	Patients	Biomarker_IHC staining	Major findings	References
Majima *et al*., 1987	112 patients of BC	Estrogen, progesterone, carcinoembryonic antigen (CEA)	Beneficial for the predicting of patients with a risk of early recurrence.	[36]
Inoue *et al.,* 1998	Metastatic-13, primary-5	Cathepsin-D	Strong expression of Cathepsin D an aspartic proteinase found to be useful as an adjunct to a panel of IHC stains in detrmining the primary site of origin of metastatic cancer in the skin.	[37]
Marchetti *et al*., 1993	Invasive BC -148	p53	TP53 mutation+, nuclear staining for p53, Ki67	[38]
Al-Joudi *et al*., 2008	382 cases of invasive ductal breast carcinoma	p53	grade; lymph node status; tumour size; side of tumour, expression of ER / PR.	[14]
Anan *et al.,* 1998	44 patients of BC	VEGF,CD31, c-erbB2	VEGF mRNA+ expression positively correlated with degree of angiogenesis as quantitated by IHC staining with a CD31. Increased expression of c-erbB2 in lymph node metastasis.	[39]
Bidard *et al*., 2008	293 patients diagnosed for BC	ER, PR, HER2 and p53.	Logistic regression for multivariate analysis of predictors for pathological complete response.	[40]
Kim *et al*., 2010	125 patients of BC	p53 and BCL2 expression	p53 and Bcl-2 expression are useful molecular markers predicting loco-regional relapse-free and distant metastasis-free survival.	[41]
Millar *et al*., 2011	498 Invasive BC	ER, PR, Ki-67, p53, HER2.	Distant metastasis-free survival; breast cancer-specific survival.	[42]
Dookeran., *et al.,* 2012	331 African American and 203 non-African American	p53 protein. Cox regression model.	occurrence of triple negative subtype; mortality due to all causes	[43]
Lundgren *et al*., 2012	1155 patients of BC	cyclin D1.	CCND1 amplification and low nuclear expression of cyclin D1 predicted poor clinical outcome in postmenopausal breast cancer patients undergone chemotherapy.	[44]

Table 1: *Characteristics of specific biomarkers from selected studies identified using IHC.*

The involvement of P-cadherin (75%) expression considered as good additional marker for basal-like DCIS, it would differentiate form invasive basal-like carcinoma for early in situ precursor lesion [18]. In association of DCIS with basal cancer it has solid, flat or micro papillary structure, high grade necrosis. Wherein, the rapid growth of tumour was better explained through absence of atypical ductal hyperplasia and small quantities of DCIS [27]. For triple negative BC phenotype, the molecular and IHC analyses showed the lack of ER, PR, HER2, CK5/6 and EGFR expressions. Among all BCs, TNBC represents about 10 - 17% in which 15-23% fall under the age of 40, 16-30% for the patients aged 40 - 49 and from 11 - 54% are aged above 50 years [28,29]. The assessment of the molecular profile in many studies has established that triple negative tumours as basal-like and unclassified tumours. In this case, the three markers (ER, PR and HER2) used regularly has major advantage towards diagnosis and to guide therapeutic strategy. The diagnosis of these tumours has the advantage that these three stains (ER, PR and HER2) are already routinely used in immunohistochemistry to guide the therapeutic strategy. The destructive appeal of this category of cancer is established through relapses that ensue between 1 and 3 years, and most of the deaths happens at first 5 years after therapy [17]. About 77-96.8% of the cases are unfavourable prognosis and it is predominantly of grade 3, histologically [17,28,29]. TNBC form a dissimilar group, in which about 56 to 84% of them express CK 5/6 and EGFR. There is an overlap (80%) between triple negative and intrinsic basal-like subtype, but TNBC includes medullary and adenoid cystic carcinoma with low risk of distant recurrence. [19].

Impending directions on prognostic and predictive perspectives of breast cancer

Clinically, the management of existing monotonous breast cancer depend on the accessibility of healthy clinical and pathological prognostic and predictive factors in making supportive decisions for the suitable treatment options [30]. The treatment of BC has brought up numerous variations over the past decades; this is owing to the detection of target specific prognostic and predictive biomarkers that allow the application of more personalized treatments. This ensured specific differences in respect to clinical behaviour pattern. The morphological variances had insufficient prognostic and predictive power towards classification of BC. The effective investigation about the gene expression patterns and its correlation with precise phenotypes had changed the way of classifying BC at the molecular level. The hierarchical clustering of gene patterns would help in "Molecular portraits" of human breast tumours.

For few decades, the studies on proliferative markers have been evaluated for both prognostic and predictive significance towards identification of early stage of BC patients. Many such studies have reported few markers that often show contradictory results due to uncertainty about the value of these markers. Based on critical reviews on proliferative markers like thymidine kinase, Ki-67, S phase, Cyclin E, cyclin D, cyclic inhibitors p27 and p21, as well as topoisomerase IIa. Through high-end multivariate analysis, the diagnostic and prognostic value was analysed separately. However, unfortunately there are several technical concerns prevented from including any one of these proliferative markers among the standard factors [31].

The revolution for the identification of initial identification of BC with improved methods and screening programs highlight the need of new biomarkers for quantifying the patient's residual risks. The molecular summary of six biomarkers appears to be the utmost valuable tool and this group of classification is selectively applied only in the clinic because of the high cost and difficulties connected with understanding the data. Because of this goal, the application of molecular signatures into the clinic has been insufficient, in this case high resolution molecular profiling has been extremely supportive in understanding the biology of breast cancer. Nevertheless, the prognostication is still likely relying on surrogate IHC markers. This is mainly due to the trials connected with practical investigation in analysis of gene signature pattern in a diagnostic setting. The molecular testing remains all the time more vital for the prevention, diagnosis and treatment of breast cancer. Regardless of the massive extent of effort that has been supported to progress molecular classification of BC, it is still evolving. Genetic array testing has defined the subtypes of BC in approximation to immunohistochemistry [32,33]. The identified subtypes would have different natural histories, epidemiological risk factors and response to therapies [34,35]. The variations in diagnostic markers indicate that clinicians managing BC should consider cases within the distinct subtypes in order to arrive at appropriate therapeutic conclusion.

Conclusion

On other side, the immunophenotyping an active area of research, their application in clinical setting is done carefully through various phase of investigation and unique outcome of their application extensively instigate from educational laboratories that have been technically authenticated. The fundamental to understanding tumour initiation, progression and identification of more efficient biomarkers for the increased expression of such markers would determine individual cell fate. Beside the advantage of advanced screening methods, such as NGS, the probability of detecting these IHC surrogate markers would open our eyes to the possibility of targeted therapy for BC subtypes. As highlighted in this review, the application of IHC and the knowledge of BC pathogenesis has led to remarkable success towards early detection, prognosis and it holds promise for the cure of BC. Over the past few decades, there are various biomarkers that have been reported to stratify risk estimates based on traditional variables of BC.

Molecular profiling primarily used to identify basal-like breast cancer, but are not extensively accessible in daily practice, currently such testing platforms lack the robustness and cost-efficiency for routine clinical use. Identifying the surrogate IHC markers has adequate sensitivity and specificity in both research and medical communities. Construction on the clinical triple negative phenotype (ER/PR/HER2), basal cytokeratin definitions and the combined immunopanel has pronounced biomarkers for various types of BC. Authentication of biomarkers beside a gold standard technique is essential to define the intrinsic molecular subtypes of breast cancer by immunohistochemistry. Authentication of biomarkers beside a gold standard technique is essential to define the intrinsic molecular subtypes of breast cancer by immunohistochemistry.

Beside traditional practise of identifying the disease pathogenesis, together comprehensive morphological analysis and molecular characterization pattern would be better translated into clinical diagnosis and management.

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