



The Progressive Journey from Ductal Carcinoma *in situ* into Invasive Breast Cancer: An Extensive Systematic Literature Review on Biomarkers

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Abstract

Breast Cancer (BC) is the most common cancer in women, and second most cancer worldwide. It is a progressive and fatal disease that affects women of all ages, and is divided into several subtypes with distinct histological, genomic and transcriptomic profiles, outcomes, and responses to therapy. Ductal carcinoma *in situ* (DCIS) is a pre-invasive stage in the development of invasive breast carcinoma. DCIS presents a clinical problem, with high risk of potential progressive disease. A subset of patients with ductal carcinoma *in situ* (DCIS) will develop Invasive Breast Cancer (IBC). Thus, biomarkers are pivotal important to help identify DCIS/IBC genes that might probably lead to potential rational treatment therapies. While biomarkers are defined as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention, cancer biomarkers provide diagnostic, prognostic and therapeutic information about a particular cancer and show their ever-increasing importance in early detection and diagnosis of the disease. Estrogen Receptor (ER), Progesterone Receptor (PR), antigen Ki-67 (Ki67), Her2, and cytokeratin's are among the approved biomarkers by the Food and Drug Administration (FDA) for disease diagnosis, prognosis, and therapy selection. Up to now, little is known/or there is still a lot to know about molecular biomarkers (BM) that may help to determine the likelihood that DCIS identified on diagnostic biopsy would remain contained *in situ* or become invasive. This review elaborates and emphasizes on biomarkers that have the potential to identify cancer progression, and it enlightens research in breast cancer biomarkers related to DCIS and IBC.

Keywords

Breast cancer; Ductal carcinoma *in situ*; Biomarkers; Invasive breast cancer

Introduction

Breast cancer (BC) is a heterogeneous disease associated with diverse biological behaviors and clinical outcome [1]. It is the second most common cancer in the world and, by far the most frequent cancer among women, as 1.6 million cases are diagnosed annually

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[2,3]. According to the 2011 estimation of World Health Organization (WHO), cancer now causes more deaths than all coronary heart disease or stroke. In 2006, about 21% of the world population was covered by Population-Based Cancer Registries (PBCR), with sparse registration in Asia (8% of the total population) and in Africa (11%) [3-6]. In Edward RS's review highlights, it is estimated that in 2011, 508,000 women died of breast cancer worldwide [7]. The same paper stated that in 2017, approximately 41,070 women in United States were projected to die from breast cancer [7]. According to WHO, early detection in order to improve breast cancer outcomes and survival remains the cornerstone of breast cancer control [8]. BC can begin in various areas of the breast (the ducts, the lobules, or in some cases the tissue in between). Therefore, it can be categorized in different types—non-invasive, invasive and metastatic breast cancers, as well as intrinsic or molecular subtypes of breast cancer.

Ductal carcinoma *in situ* (DCIS), also referred to as non-invasive or intra-ductal cancer, is defined as a neoplastic proliferation of epithelial cells confined to the ductal-lobular system and is characterized by subtle to marked cytological atypia as well as an inherent (but not necessary obligate) tendency to progress to IBC (Invasive Breast Cancer) [9,10]. It is the most common type of non-invasive form breast cancer [11]. It includes a heterogeneous group of pre-invasive breast tumors with variable clinical outcome. The incidence of DCIS has remarkably increased. Since the early 1970s, the incidence of DCIS has increased from 1.8 per 100,000 women to 32.5 per 100,000 women in the mid-2000s [12]. Currently, DCIS represents approximately up to 20% of breast cancer diagnosed cases with the help of the mammography. Patients with DCIS have an increased risk of second breast cancer (SBC) recurrence, which can be invasive breast cancer (IBC) or any other type of cancers, and if uncontrolled might eventually lead to death [13-15]. The progression from DCIS to IBC remains controversial, involving many types of cell behaviors, including growth, migration, and invasion through various cell signalling pathways.

Biomarkers are to prevent DCIS progression, and attempt to diagnose at the earliest possible time for better treatment, and identify drug target therapy, in order to maximize the chances of major impact on the management and better outcomes [16]. Here, we review the biological process that likely to play a significant role in the phenomenon of progression from DCIS to invasive disease, the challenges faced by clinicians, pathologists and researchers in order to improve and develop predictive biomarkers; and define the subsets of DCIS to the probability of progression to Invasive Ductal Cancer (also sometimes called Infiltrating Ductal Carcinoma, IDC) [17].

Brief Overview of Breast Cancer

Cancer remains the major devastating disease throughout the world. It is estimated that cancers are responsible for over 6 million lives per year worldwide with an annual 10 million or more new cases [18]. Factors that stimulate the risk of breast cancer include gender, age, family history and additionally alcohol intake, dietary fat, obesity in postmenopausal age, and hormonal stimulations. Remaining the most frequently diagnosed cancer and the leading cause of cancer death among women, breast cancer is accounting for 25% of the total cancer cases (1.68 million) and 15% of the cancer

deaths (520,000) worldwide [19,20]. The disease is localized to the breast at presentation in 61% of cases, regionally advanced in 32%, and metastatic in 7% [21]. When localized or regionally advanced, the disease is potentially curable with local and systemic therapy [22]. According to the Surveillance, Epidemiology, and End Results Program (SEER) of the National Cancer Institute of the United States, while the estimated new cases of breast cancer was the highest among the other common types of cancers, breast cancer was estimated to be the third leading cause of deaths in 2017 [21]. Based on the same national cancer registry, published data show that breast cancer occurs in every race/ethnicity, with predominance in white, black and non-Hispanic women; and its highest prevalence remains women aged 55-64 [21].

The cellular origin of most breast cancers occurs in the normal terminal duct lobular unit (TDLU). The human breast cancer is known as a progressive disease, and the key stages in this progression are called Hyperplastic Enlarged Lobular Units (HELU), Atypical Ductal Hyperplasia (ADH), Ductal carcinoma *in situ* (DCIS), and invasive breast cancer (IBC), as shown in [23]. In DCIS treatment, the key aim is to prevent tumor recurrence and the development of invasive disease. To reduce morbidity and achieve high cure rates, most DCIS patients have been treated by a combination of surgery and postoperative radiation followed by endocrine therapy if the ER is detected by immunohistochemistry [9]. Decades ago, mastectomy with axillary dissection was the first line of treatment for the DCIS patients. Although this approach resulted in a cure rate exceeding 99%, the morbidity and aesthetic aspects forced surgeons to use more conservative options [9]. However, disease recurrence is very probable in patients that exclusively underwent this modality of treatment. Several clinical trials have compared surgery with radiotherapy to surgery without radiotherapy, and findings showed that radiotherapy reduces the rates of recurrences by 50% in patients undergoing breast-conserving therapy [24] (Figure 1).

Clinical and histopathological features of DCIS and IBC

Both *in situ* and invasive breast tumors are comprised of heterogeneous phenotypes, with variations in clinicopathological features such as histological grade, Estrogen Receptor alpha (ER), Progesterone Receptor (PR), Human Epidermal Growth Factor Receptor 2 (HER2/ERBB2), Ki67, Cytokeratins (CK) 5/6, and Epidermal Growth Factor Receptor (EGFR) status [25]. Additionally, tumors of the various intrinsic subtypes are found among both DCIS and IDC [23,26], indicating subtype-specific progression paths. BCs have been categorized into four molecular subtypes: Luminal A, Luminal B, Her2-enriched, and Basal-like/triple-negative (Table 1) [27]. All of these major molecular subtypes which are present in IBC are seen in DCIS, although at different frequencies. Specifically, the frequency of the luminal B and HER2+ phenotypes is significantly higher in DCIS than in IDC, and the frequency of the luminal A phenotype is significantly higher in IDC than in DCIS. Based on the regulators category, key biomarker including HER2, EGFR, FAT-1, survivin and much more others will be discussed in our following parts series.

DCIS is not a single entity, but rather a spectrum of disease; in essence, it refers to malignant change in the ductal epithelium. It is routinely distinguished clinically from the related, numerically less frequent lesion, Lobular Carcinoma *in situ* (LCIS) by light microscopy. The pathology of DCIS can be considered as a spectrum ranging between atypical ductal hyperplasia and invasive disease with features such as grade and necrosis reflecting the likely clinical behavior as well as the presentation on mammography [28]. Previous studies have suggested that clinical and intrinsic subtype, as opposed to disease stage, are the dominant sources of variability among tumor expression profiles [26]. Traditionally, DCIS was classified based on architectural growth pattern of the epithelial proliferation, into comedo subtypes (defined by high-grade cells, prominent central necrosis and associated pleomorphic micro calcifications) and non-comedo subtypes (further subdivided into solid, cribriform, papillary,

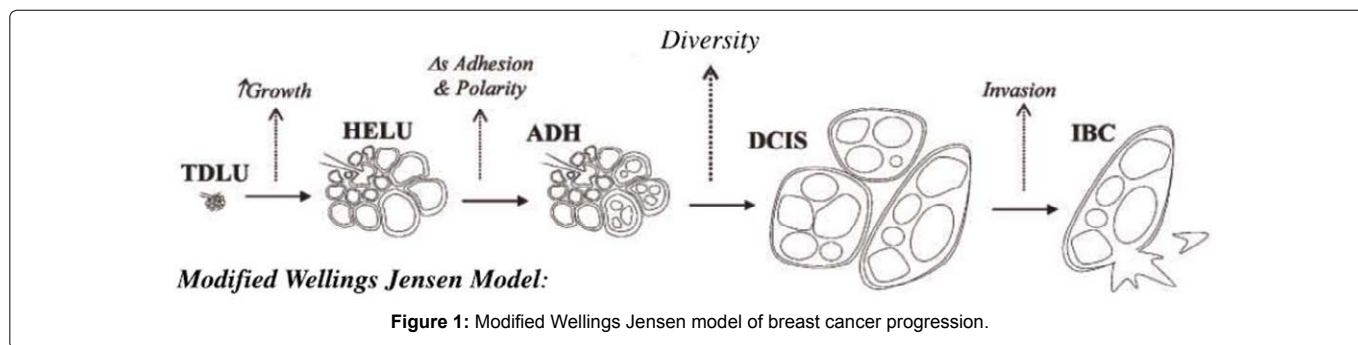


Table 1: Molecular subtypes of breast cancer.

Molecular Subtype	ER	PR	Her2	Ki67	CK5/6	EGFR	Observations
Luminal A	+	+	-	≤14%	Any	Any	This subtype makes up about 40% of all breast cancers, and carries the best prognosis
Luminal B	+	+	-	≥14%	Any	Any	About 20% of breast cancers are luminal B subtype. Compared to luminal A tumors, they tend to lead to a poorer prognosis including poorer tumor grade, larger tumor size, and lymph node metastasis
Her2-enriched	-	-	+	High	Any	Any	This subtype makes up about 10%-15% of breast cancers, and is characterized by high expression of Her2 and proliferative gene cluster. Her2 subtype tumors have a fairly poor prognosis and are prone to early and frequent recurrence and metastases
TN	Basal-like	-	-	High	-	-	They cannot be treated with hormone therapies or trastuzumab (Herceptin) because they are ER (-) and Her2 (-). This subtype of breast cancer is often aggressive and has a poorer prognosis compared to the receptor- positive subtypes
	Non-Basal	-	-	Any	-	-	

ER: Estrogen Receptor; PR: Progesterone Receptor; HER2: Human Growth Factor Receptor 2; CK5/6: Cytokeratin 5/6; EGFR: Epidermal Growth Factor Receptor; TN: Triple-Negative

micro papillary, clinging, apocrine, and mixed subtypes) [29,30]. Currently, based on cytonuclear atypia, DCIS is generally classified in low grade (small, monomorphic, well-polarized cells, with uniform size and regular chromatin pattern and rare mitotic), intermediate grade (similar to those of low grade but with occasional nucleoli, mitotic figures, and coarse chromatin), or high nuclear grade (large size, pleomorphic, and poorly polarized nuclei, with prominent nucleoli, numerous mitotic cells, and presence of necrosis) [9,29].

The ability of DCIS to evolve into invasive carcinoma is supported by similarities in morphology and hormone receptor profiles. Invasive breast cancer includes all the tumors in which stromal invasion are detectable including the micro invasive carcinoma. Based on architectural patterns and cytological features, the two major histological variants of breast cancer are invasive ductal carcinoma (IDC, also called No Otherwise Specified (NOS)) and invasive lobular carcinoma (ILC) representing 70%-80% and 10%-12% of all BCs, respectively [31]. The remainder of the invasive carcinomas are classified as tubular, cribriform, medullary, metaplastic, apocrine, adenoid cystic, mucoepidermoid, polymorphous, mucinous, papillary, micro papillary, inflammatory carcinomas, and some exceptional rare types and variants (including secretory, oncocytic, sebaceous, Lipid-rich cell, glycogen-rich clear cell and acinic cell carcinoma) [32,33]. Although therapeutic approach at equivalent stage does not differ significantly between IDC and ILC, except for stage 0 disease, these two major subtypes of IBC vary in terms of clinic pathological features. The major characteristics of ILC are multicentricity, multimodality, bilaterality, difficulty in detection by mammographic examinations due to ill-defined margins, more frequent hormone receptor positivity, and tendency to metastasize to gastrointestinal organs and peritoneum [34].

Potential biomarkers involved in the progression from DCIS into IBC

Traditionally, DCIS have been classified according to the architectural features of their lesions [35]. However, this classification is of limited clinical value, particularly as individual DCIS lesions often demonstrate architectural heterogeneity [36]. As most breast cancers evolve from precursors, which gradually change over time, identifying biological alterations associated with early precursors before the development of substantial diversity will help scientists to reveal effective strategies for the prevention of the majority of breast cancers, independent of differentiation [23].

All existing DCIS classification schemes, including those focused on nuclear grade are unable to reliably differentiate between DCIS that

will remain stable, regress and those likely to progress to full invasive tumors [37]. Over the past several decades, tremendous efforts have been made to screen and characterize useful cancer biomarkers for the use in clinical practice. According to their clinical use, they currently fall into three major categories: (1) prognostic, (2) predictive, and (3) pharmacodynamics markers [38]. Even though, an individual biomarker may serve more than one purpose and thus can fall into more than one of the above categories. Based on the biomarkers statistics from the Division of Cancer Prevention of the American National Cancer Institute, breast cancer biomarkers represent the third large group of investigated biomarkers by scientists, behind prostate and ovary cancers, respectively, showing that breast cancer remains a major public health concern and therefore it has been the most frequent female neoplasms (Figure 2).

DCIS progression is a complex process involving various types of cells, molecules and genes playing pivotal role at different phases, including growth, migration, and invasion. In many studies regarding the process from a ductal carcinoma *in situ* into invasive breast carcinoma, several researchers have identified some unique gene expression profiles of human DCIS and IBC. The search for biological markers and targeting some of these genes may improve the detection, diagnosis, therapy and progression of DCIS [39]. DNA biomarkers provide useful information on the process of tumor growth; protein biomarkers are also indicator for predictive and prognostic markers.

The steroids receptors

Steroid hormones are critical for the growth and development of breast tissue as well as of breast cancer. estrogen receptor (ER) and progesterone receptor (PR) were the first biologic markers evaluated in breast cancer. Studies have demonstrated that 50% to 75% of DCIS lesions express ER, and ER expression appears to correlate with DCIS grade, as it does with IBC grade [39]. The expression of ER and PR in tumors is associated with better prognosis and sensitivity to endocrine therapy. Apart from its well-known role in developing and maintaining the male phenotype, Androgen Receptor (AR) also plays an important role in female fertility [40].

Estrogen receptor (ER): It is well established that ER plays an important role in the genesis and progression of breast cancer. Approximately 75% of low-grade DCIS lesions express ER compared to only 30% of the high-grade DCIS lesions [41]. ER positivity is found in up to 60 to 70% of invasive breast cancers. Expression of ER in DCIS alone compared to contiguous DCIS associated with invasive carcinoma has been investigated in the past. With its two major variants (ER α and ER β), the level of ER positivity is an important

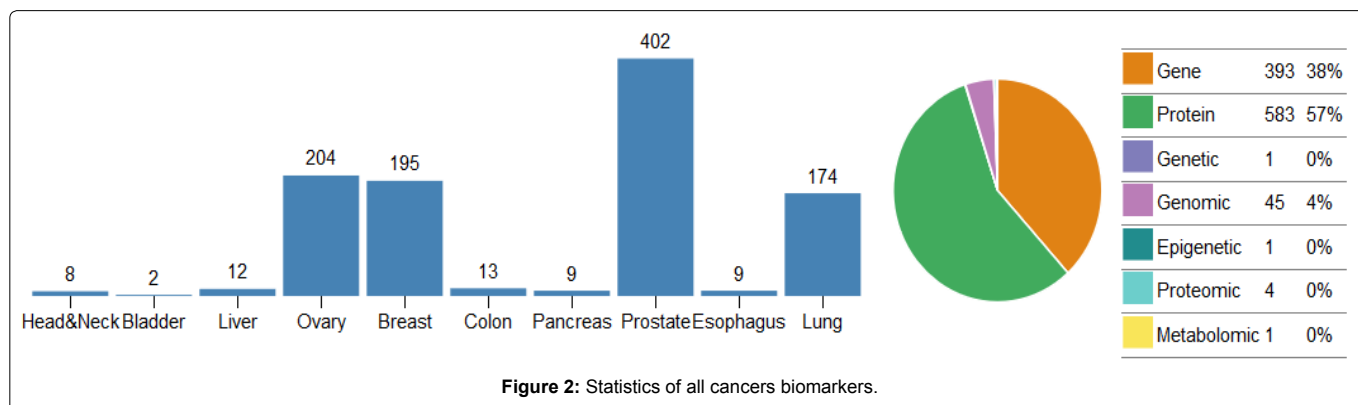


Figure 2: Statistics of all cancers biomarkers.

predictor of response to endocrine therapy as well as a prognosticator. While endocrine therapies mainly seem to target ER α , the clinical significance of ER β is less well known [42]. Several studies have shown the correlation between the expression of ER and the DCIS recurrence/invasive breast cancer. Studies have elaborated and proven the correlation between negative steroid receptor status and positive HER2 status, concluding they were independent predictors of DCIS recurrence after adjustment for tumor grade [43]. Zhang et al. carried out a clinical and histopathological study involving mastectomy specimens of 120 cases containing both DCIS and IDC [44]. The findings suggested that the expression of ER proteins decreased with the tumor progression from DCIS to IDC ($\chi^2=4.034$, $p=0.045$), and furthermore the expression levels of ER were significantly higher in the DCIS than those in the normal mammary glands. Moreover, in contrast HER-2/neu increased in high-grade tumors compared to DCIS [44]. In two nested case-control studies conducted, and respectively, the findings revealed a strong and significant statistical association between ER-negative DCIS and risk of local recurrence and/or subsequent invasive cancer [43,45].

Estradiol (E2) is the principal stimulator of estrogen receptor (ER)-mediated tumor proliferation. Its intratumoral levels are mainly regulated by the aromatase-mediated conversion from androgens and Estrogen Sulfotransferase (EST)-mediated reduction of bioavailability [46]. Studies revealed that stromal aromatase expression is significantly higher in IBC compared to adjacent DCIS components, and epithelial EST levels were found to be significantly down regulated in high-grade DCIS compared to non-high grade cases [46,47]. Concluding that aromatase through local estrogen synthesis in breast tissue plays an important role in breast carcinogenesis, and in tumor cell invasion. Moreover, a study showed that intraductal carcinoma associated with invasive cancer was more frequently ER-positive compared to DCIS without associated invasion [48]. There was a strong concordance of ER/PR expression in contiguous DCIS associated with invasive cancer (98%) with virtually all cases being ER/PR positive. Overall, these findings suggest reduced expression of this steroid hormone is a marker for aggressive clinical behavior in patients with breast carcinoma, indicating that alterations of the molecule are involved in the mechanisms of breast carcinogenesis.

Progesterone receptor (PR): Just as ER, PR is considered to be important in invasive breast cancer. In IBC, expression of PR is weakly prognostic with respect to disease-free survival and a predictor of response to endocrine therapy [49]. PR α and PR β are the 2-variants of PR, which are widely studied, even though most knowledge has been obtained on the first variant [42]. The key role of PR expression regarding the transition from DCIS to IBC has been well studied. Lots of data emphasized that patients with high-grade DCIS were less likely than patients with non-high-grade DCIS to have PR-positive disease [50,51]. Its expression is more common in cribriform DCIS and noncomedo DCIS than in other subtypes. Furthermore, study revealed that PR expression was more common in papillary, solid, cribriform, micropapillary, and comedocarcinoma subtypes than in other subtypes [42].

In their nested case-control study, Provenzano et al. found that PR negativity was independently associated with risk of local-regional recurrence. Patients who developed a local-regional recurrence were more likely than controls to have PR-negative disease (63% vs. 34%) [43]. In another study, the authors found that a cell biological index that included ER and PR negativity, overexpression of HER2, accumulation of p53, and high Ki-67 expression was a strong predictor of recurrence [52].

Androgen receptor (AR): Androgen receptor (AR), similar to ER and progesterone receptor (PR), belongs to the steroid nuclear receptor family. In the recent years, AR has been a newly emerged biomarker and may serve for prognosis and prediction in BC. AR has biological and therapeutic utilization in prostate cancer, but its use in breast cancer treatment is limited because of the widespread and effective use of anti-estrogen hormonal therapies [53]. Its expression is significantly associated with both the stage and the grade, as it has been associated with apocrine differentiation in DCIS [50,54]. Its loss expression is usually associated with early onset, high-grade tumors negative for ER, PR and HER2 [55]. Previous studies have significantly shown that AR expression has the potential to predict disease progression [49,50,54,55]. The findings of a clinical pathological study carried out by Hanley et al, demonstrated that AR expression was higher in high-grade DCIS than in non-high-grade DCIS. Hanley et al. found that unlike expression of the other steroid receptors (ER and PR), expression of AR was slightly higher in high-grade DCIS than in non-high-grade DCIS [50]. In the same study, through a studied pattern of co-expression of ER and PR with AR, the authors reported that 87% of non-high-grade DCIS lesions compared with 30% of high-grade DCIS lesions expressed both AR and ER. The co-expression pattern was similar for PR [50].

Others studies have investigated androgen-regulated tumour suppressor genes and others relevant influential regulatory genes [56]. The findings revealed that Lin28A plays an important activating role in the AR expression *via* c-myc and thereby promotes ER-/Her2+ breast cancer cell proliferation and invasiveness. Overall, the lack of expression of androgen receptor may play a critical role in transformation from *in situ* to invasive basal subtype of high-grade ductal carcinoma of the breast, as pointed [50].

The marker of proliferation-Ki-67

As a key element of progression of the disease, the nuclear antigen Ki-67 is commonly used to assess the proliferation rate of breast cancer tumors [57]. Several studies have investigated the relationship between Ki-67 expression and the prediction of DCIS recurrence, and or progression to IBC [51,58-60]. As High-grade DCIS has been associated with an increased risk of recurrence and progression to invasive carcinoma. In study, the findings indicated that significantly more women in the non-recurrent group had a low (0% to 10%) Ki67 score (a proliferation marker), compared with those women in the recurrent group (87% vs. 50%, respectively; $P=0.002$) [58]. In another study, the authors identified a high Ki67 proliferation index in the majority of cases of their series (70% for pure DCIS and 71.8% for Invasive mammary carcinoma-associated DCIS) [59]. The Ki67 expression rate those series was likely higher than the mean rate described in other studies (10.9% to 15.5%), which included DCIS of different grades [49]. Moreover, in another study, Ki67 expression was significantly higher ($p<0.05$) in IDC cases (64%) versus IDC/DCIS (49.7%) [60]. Ki67 high proliferative activity has been revealed to be comedo DCIS than in DCIS with other architectural patterns [49]. In a co-expression of p16, Ki-67 and Cox-2, found that the phenotype Ki-67+/p16+ and the phenotype Ki-67+/p16+/COX-2+ were associated with subsequent invasive tumor. In addition, Ki-67 was individually associated with DCIS recurrence, and the phenotypes Ki-67+/ER-, Ki-67+/p16+, and Ki-67+/p16+/COX-2+ were also associated with DCIS recurrence. Overall, in various multivariate analyses, the Ki-67+/p16+/COX-2+ phenotype has been statistically and significantly a strong predictor of subsequent invasive recurrence after a DCIS diagnosis [45,59].

The cell cycle regulators—part one

Regulators that control the cell cycle mechanism determine the fate (apoptosis or mitosis) of each cell, either normal or neoplastic. Carcinogenesis is a phenomenon triggered by both increased stimulation of cell growth and loss of cell death [39]. Several genes and proteins playing a pivotal invasive role in breast cancer can be related under this category. In this first part, we present and discuss some of the regulators of cell cycle that are key elements in tumor invasion from non/pre-invasive cancer into invasive breast carcinoma, whereas the second part of these cycle regulators shall be presented and discussed extensively in our second part series.

The cyclins: Cyclins are members, of family proteins, which are considered as markers of proliferation and mitotic activity, which through activating cyclin-dependent kinases (Cdk) enzymes control the progression of cells through the cell cycle [61]. Based on their main activities in the different part of the cell cycle, they are known as Cyclin D, E, A and B.

Cyclin D: Cyclin D is one of the major cyclins produced in terms of its functional importance. So far, Cyclin D1 remains the most investigated in breast cancer as it is known as a marker of invasiveness in breast cancer [61-65]. Cyclin D1 overexpression is present in approximately 20% of DCIS lesions, with recurrence risk [61]. And in approximately 50% of IBC; and 5% to 20% of these tumors have *CCND1* gene amplification [62]. Throughout the progression of breast cancer from DCIS to invasive cancer, cyclin D1 immunostaining pattern was found along with a great than threefold amplification of *CCND1* [64]. Using univariate and multivariate analyses, results suggest that Cyclin D1 is significantly and inversely associated with invasive recurrence, and can be used as a prognostic marker in DCIS [65].

Cyclin A: Cyclin A is the only cyclin that regulates multiple steps of the cell cycle, due to its association with, and thereby activates distinctly Cdk1 and Cdk2 [66]. Few studies have investigated its expression in DCIS. However, according to Millar et al. study, 35% of DCIS lesions expressed Cyclin A [66]. Its expression has been significantly found higher in comedo DCIS than in noncomedo DCIS [61]. Furthermore, in a global proliferation factor study, after multivariate analysis, Cyclin A in combination with Ki-67 and p21, was correlated with recurrence [61].

Cyclin E: Considered as a prognostic marker in BC, the altered expression of Cyclin E increased with the increasing stage and grade of the tumor, and its dysregulation occurs in 18%-22% of BC [67]. A study revealed that 25% of 92 cases of DCIS studied had high Cyclin E expression, but the statistical analysis failed to prove its correlation in disease subsequent invasive/recurrence [65]. Moreover, in a univariate analysis, Pillay et al. found that Cyclin E expression was significantly associated with age, grade, lymph node spread and vascular invasion with distant Metastases Free Survival (MFS) in all invasive carcinomas, and the subgroup of IDC [68]. However, cyclin E provides some prognostic value as there is a direct statistical association with the development of distant metastases.

Cyclin B: One study had put in exert the expression of Cyclin B1 in 43 cases of DCIS [64]. There was no significance difference according to the histologic grade of DCIS; however, high grade-DCIS (HG-DCIS) tends toward a higher PI Cyclin B1 than intermediate or low grade DCIS (LG-DCIS). In the same study, according to the histologic subtype, expression of Cyclin B1 was found higher in comedo DCIS than in noncomedo DCIS but with no statistical difference [64].

p-family proteins

p16: Also known as cyclin-dependent kinase inhibitor 2A or multiple tumor suppressor 1, p16 plays a pivotal role in cell cycle regulation by decelerating cells progression from G1 phase to S phase, and for this reason is now being explored as a prognostic biomarker for various cancers. Several studies have investigated its expression and its impact during the transition from DCIS and IBC [45,59,65,67,69,70]. While some early findings suggested that there is no direct association between p16 and DCIS subsequent recurrence/invasive [65]. Later studies have statistically proven their correlation [69,70]. In a tissue microarray analysis, among other G1/S-regulatory proteins, the authors found that p16 was not associated with local recurrence in DCIS [65]. However, in their study (including 400 cases of DCIS and IDC, and 50 cases of normal control), Shan and al. significantly found that luminal-A cancers expressed the lowest level of p16 among the subtypes in DCIS, and the level of p16 expression was up-regulated in the luminal-A of IDC ($P < 0.008$) [69]. In the same study, luminal lesion types with high p16 expression in DCIS were associated to be more likely to develop into aggressive breast cancers, possibly promoted by p53 dysfunction [69]. Findings of univariate analysis suggested p16 to be associated with subsequent DCIS invasive recurrence [45]. In multivariate analyses, its co-expression in combination with other biomarkers including p16+/COX-2+/Ki-67+[45, 59], p16-/p53+/Ki-67+[71] had been significantly associated to subsequent invasive carcinoma, and p16+/CXO-2-/Ki-67+ [45] predicted higher risk of subsequent recurrent DCIS.

p21: Implicated in a variety of pathways, p21 immunoreactivity has been detected both in benign and malignant epithelium, thus making its role quite complex [70]. As far as DCIS is concerned, several studies have been published highlighting the expression of p21 regarding the tumor recurrence and/or invasive carcinoma [43,72-75]. p21 has been found significantly associated with well-differentiated histologic grade, non-comedo type, ER+/p53- [73,74]. Statistically, patients that presented local regional recurrence were more likely to have p21-positive disease than those who did not (54% vs. 15%) [43]. Furthermore, p-21 activated kinase 1 (PAK1) has been investigated to determine its role regarding progression/invasiveness in breast cancer [76,77]. While CA-PAK1 (its constitutively activated form) increases cells motility and invasiveness in MCF-7 cells, its dominant-negative mutant (DN-PAK1) suppresses cellular motility and invasiveness in MDA-MB-435 and MCF-7 breast cancer cells [76]. Overall, overexpression and activation of PAK1 mediates increase motility and invasiveness of human breast cancer, stimulates cyclin D1 expression, support epithelial-to-mesenchymal transition, and induces an increase in proteolytic activity, which is in consistent with the conversion from pre-invasive lesions (DCIS) to invasive cancers [77].

p27: p27 is a potent inhibitor of cyclin E/Cdk 2 and cyclin A/Cdk 2 and its expression is highest in quiescent cells and decreases upon re-entry into the cell-cycle [78]. Its increase is associated with cell growth arrest, cell differentiation whereas decreased p27 expression is related to increased proliferation and tumorigenesis [79]. Various papers have highlighted its correlation with others biomarker. Its expression is found to be significantly correlated with p16/Cyclin D1, Cyclin D1/PR and/or ER status [66,68,74]. Down-regulation of p27 is likely to be an early event in breast cancer as it has been detected with the same prevalence in small lymph node negative tumours with limited invasion and in larger lymph node positive groups [80]. It has also been suggested that low p27 is a strong and independent

marker of poor clinical outcome [68]. Cell proliferation, hormonal and differentiation characteristics differed in DCIS with respect to IDC, and the main variation in the transition between the two histologic lesions was the decrease in p27 expression and Microvessel Density (MVD) [78]. Conversely, p27 expression and MVD dramatically decreased during the transition from *in situ* to invasive carcinomas, indicating that loss expression of p27 was found to be indicative of IDC relapse [78].

p53: p53 is a tumor suppressor gene with pleotropic functions located on chromosome 17. It has an important role in regulating transcription of many other genes and it is an important component of breast cancer pathophysiology [81]. p53 is the most commonly mutated gene in human cancers [39], and regarding breast cancer, it is mutated or inactive in approximately 20% to 30% of HG-DCIS and IG-DCIS lesions and they have not been identified in normal breast tissue [54,82]. Loss or mutation of p53 is associated with high rates of proliferation and development of malignant cells clones, correlates with loss of bcl-252 and with steroid receptor status [39]. In a correlation analysis between p16 and p53, the aberrant overexpression of p53 (of which the p53 mutation is closely correlated) [83]. It was found to be related to high p16 expression in DCIS and IDC triple-negative subtypes, and of IDC cases the most of the luminal-A subtypes were negative for p53, as were almost half of luminal-B subtypes, the majority of Her-2 subtypes, and almost all triple-negative IDC subtypes [69]. In a cell biological index analysis, p53 in combination with others biomarkers including ER-, PR-HER2+, Bcl-2- and Ki-67+, was found to be predictor of subsequent local recurrence of DCIS/progression [52]. Taken together, these findings suggest that dysfunction (mutation/inactivation) of p53 is a risk factor for subsequent breast carcinogenesis, associated with the progression from DCIS to IDC [69,84].

p63: p63 is also an important regulator of terminal differentiation and polarity of both epidermal and myoepithelial cells, and the disruption of these biological processes was shown to promote progression of DCIS to invasive cancers [85-87]. Several studies have highlighted its role either individually or in combination with others markers, regarding the progression of DCIS into invasive breast cancers. p63 expression is either restricted to myoepithelial cells or is present in both the myoepithelial and luminal areas of DCIS, and an inverse correlation between p63 and CK8/18 expression suggesting that the transition between a basal to a luminal phenotype of breast carcinoma requires the loss of p63 expression [88]. α -Smooth muscle actin (α -SMA), calponin and p63 loss are commonly used to identify DCIS progression into IBC. DCIS negative for p63 were also negative for α -SMA, and both of them were negative in the foci of micro-invasiveness in all cases of DCIS with micro-invasion analyzed [88]. Other published data suggest that sequential loss of expression of p63, calponin, and α -SMA can occur in DCIS-involved ducts before overt loss of the myoepithelium, and this loss occurs in such trends with loss of p63>calponin> α -SMA [87]. In an intricate interaction network analysis involving TGF β , Hedgehog and p63, the loss of p63 in MCFDCIS cells resulted in the loss of myoepithelial cells, and therefore accelerated progression to invasion [89]. Overall, these data support that the transition from DCIS into invasive disease implicates the loss of this critical function, even though p63 is not as highly sensitive as α -SMA in staining myoepithelial cells.

Forkhead box (FOX) proteins

Forkhead box (Fox) proteins are an extensive family of transcription factors, which rely on precise temporal and spatial

controls, to directly play a key role in the regulation of crucial biological processes, including cell proliferation, differentiation, metabolism, tissue homeostasis, senescence, survival, apoptosis, and DNA damage repair [90]. Based on their protein sequence homology, they are divided into 19 subclasses (FoxA to FoxS). However, the best-studied Fox proteins involved in cancer are FoxO3a, FoxM1, and FoxA1 [90,91].

FoxA: FoxA1, FoxA2 and FoxA3 are currently the three known proteins of the FoxA subfamily [91]. Known also as hepatocyte nuclear factor 3 alpha (HNF-3 α), FoxA1 remains the most-studied FoxA proteins involved in breast cancer [92-95]. FoxA1 is a prominent “pioneer factor” with the ability of initiating transcriptional competency and recruiting other transcription factors to target genes. This pioneer function makes it an important factor in breast and prostate cancers as FoxA1 is a key cooperating factor for the nuclear hormone receptors, estrogen receptor- α (ER), and androgen receptor (AR) [96-98]. By univariate and multivariate analyses, FoxA1 expression has been significantly associated as an independent prognostic factor for distant disease-free survival in ER+/HER2-breast cancer [92,94,95]. FoxA1 is the downstream target of GATA binding protein 3 and maintains ER sensitivity [99]. Already overexpressed in ADH (by 32% and 54% with respect to histological-normal tissue), FoxA1 and GATA binding protein 3 (GATA3) expression greatly increased in DCIS (by 93% and 70%, respectively) [93]. Moreover, in a polyoma middle T (PyMT) transgenic mouse model study, the progression of the disease has been associated with phenotypes from ER α +/FoxA1+/GATA-3+ tumors in the early stage to ER α \pm /FoxA1-/GATA-3+ in the late stage [100]. Meanwhile, FoxA1 has been shown to transcriptionally activate p27, inhibited the ER pathway activity and forced expression of this transcription factor in the luminal (MCF7 and SKBR3) and basal (MDA-MB-231) breast cancer cell lines led to a reduction in number and size of surviving colonies of all cell lines compared with empty vector transfected controls [96]. The analysis of FoxA1 expression in breast tissue arrays revealed significantly higher expression in pure DCIS compared to invasive ductal carcinomas (IDC); and in IDC, high expression of FoxA1 was associated with favorable prognostic factors whereas the loss of FoxA1 expression was noted with worsening tumor grade [93,95]. Clarified that those metastatic breast cancer patients whose tissue highly expressed FoxA1 took a long time to relapse compared to low FoxA1 patients, indicating that FoxA1 might be related to late subsequent recurrence [101]. Based on these scientific evidences, findings suggest that the loss of FoxA1 expression is a significant prognostic factor of breast progression from DCIS to IBC.

With a domain nearly identical to that of FoxA1 and FoxA3, the role of FoxA2 (HNF-3 β) in breast cancer invasion remains unclear and least studied [102]. Meanwhile FoxA2 remains an important regulator of glucose and lipid metabolism and organismal energy balance [103]. As its controls the uptake of extracellular lipids for breast cancer growth [104], the Mesenchymal to Epithelial Transition (MET) plays critical roles in the progression of breast cancer metastasis. Findings suggested strong correlation between the expression levels of FoxA2 and the epithelial phenotype of clinical human breast ductal carcinoma samples. With two *in vitro* cell models of epithelial-type or mesenchymal-type breast cancer cells, the authors clarified that FoxA2 prevented EMT of breast cancer cells by stimulating the transcription of epithelial-related E-cadherin and repressing the expression of EMT-related transcription factor ZEB2 [105]. FoxA2 is an independent prognostic factor of subsequent recurrence, and is expressed in Triple-Negative/Basal-like breast

carcinomas [102]. FoxA2 is expressed in normal human mammary epithelial cells, but its expression is down regulated by the increased methylation of the *FoxA2* gene in clinical breast cancer samples, thereby suggesting that FoxA2 affects breast cancer progression [106]. These findings suggest that the overexpression of FoxA2 abolishes metastasis of breast cancer cells whereas its knockdown or aberrant expression is a marker for breast cancer invasion.

FoxC: The FoxC subfamily in humans is composed of two proteins known as FoxC1 and FoxC2 [91]. These two proteins play distinct key roles in development. FoxC1 was associated with Axenfeld-Rieger anomaly of the anterior eye chamber, while FoxC2 was associated with lymphedema-distichiasis [107]. Therefore, presumably they may also exert different roles in breast cancer cells invasion and metastasis.

FoxC1 was initially identified as an important regulator in embryonic development including brain, eye and heart formation [108]. Mounting of data suggests that FoxC1 is a sensitive biomarker for triple negative breast cancers, and particular in Basal-Like Breast Cancer (BLBC) [109]. Furthermore, it is directly linked to tumor metastasis and invasion [108]. Matrix Metalloprotease 7 (MMP7) has been identified as a key downstream effector of FoxC1-mediated invasiveness. The transient overexpression of FoxC1 induces MMP7 expression in non-transformed mammary epithelial cell lines, thus promoting breast cancer invasion [109]. Published data show that FoxC1 may play an important role in the degree of malignancy and drug resistance of relapsing invasive ductal carcinoma [110]. In a series of 27 DCIS, 28 IDCs, 34 mixed cases (invasive tumors with *in situ* components) and 28 normal tissues, carried out a quantitative methylation analysis of FoxC1 and others biomarkers including ABCB1, PPP2R2B and PTEN [111]. FoxC1 displayed a significantly increased methylation levels from normal breast tissue to invasive tumors, the tumors with less methylation tended to be of the basal-like and normal-like breast cancer subtypes [111]. Moreover, both the methylated and unmethylated DCISs and invasive tumors displayed significantly lower levels of FoxC1 gene expression. These data show that frequent aberrant DNA methylation of FoxC1 gene expression in both methylated and unmethylated DCIS, and in IDCs indicates that the loss of its expression is an early event during breast cancer progression.

FoxC2 has been associated to play a key function in promoting cancer cell invasion and metastasis. In breast cancer, expression of FoxC2 is associated with, and causal to chemotherapy resistance and metastasis in triple negative breast cancer (TNBC) [112,113]. FoxC2 is an important regulator of EMT, a key process that is often activated during tumor progression and metastasis. Its overexpression leads to mesenchymal differentiation, as well as the induction of MMP2 and MMP9 expression [113]. FoxC2 indirectly represses expression of the epithelial marker E-cadherin, whose loss is considered a hallmark of EMT [114]. Through our data extraction, we could not find published data relating the clear expression of FoxC2 in association with the transitional invasion from DCIS to IBC. However, these observations indicate that FoxC2 plays a central role in promoting invasion and metastasis in breast cancer.

FoxF: FoxF1 and FoxF2 proteins compose the FoxF subfamily [91]. There is no match published data on their role in breast cancers, almost nothing published regarding their involved function in carcinoma invasion from DCIS to IBC.

Significant loss or down regulation of FoxF1 has been expressed in 9 of 10 breast carcinoma cells compared to normal control. This

expression is aberrantly silenced in through epigenetic mechanisms, demonstrating that FoxF1 exerts tumor suppressor activity [115]. FoxF1 also promotes mesenchymal cell migration by transcriptionally regulating integrin $\beta 3$ [116] and plays an important role in invasion during breast tumor progression. FoxF1 overexpression induces in a dependent-manner breast cancer cells migration/invasion by upregulating Lysyl Oxidase (LOX) and suppressing Smad2/3 signalling [117].

Clinical study carried by Kong et al. revealed that the under-expression of FoxF2 is associated with early-onset metastasis and poor prognosis [118]. It transcriptionally down-regulates FoxC2 and suppresses EMT and multidrug resistance in BLBC cells [119]. Moreover, Lo et al. findings revealed that FoxF2 may play a dual role in tumorigenesis functioning as either a tumor suppressor or an oncogene in a tissue-context- and stage-specific manner, precisely in regulation of DNA replication and the epithelial-mesenchymal transition [120].

FoxK: Studies suggested that FoxK proteins could repress the initiation autophagy programs, and function as transcriptional repressors of autophagy in muscles cells and fibroblasts [121]. FoxK1 has been associated in many programs including skeletal muscle regeneration, breast cancer development and progression. The histopathological analysis of cancer samples revealed that the higher Foxk1 expression was significantly associated with lower clinical stage, ER-positive breast cancer, benign histological type and lower histological grade [122]. Furthermore, the expression of FoxK1 was significantly decreased in MCF-7(ER+) and MDA-MB-231 (Triple-negative) cells, comparing with that in MCF-10A (normal breast cells). Moreover, the *in-vivo* MRI assay demonstrated that Foxk1 overexpression resulted in the progression of noninvasive MCF-7 to a more detectable phenotype, the permeability-surface area product was significantly higher than the vector [122]. Since MRI could reliably differentiate between *in situ* and invasive cancer [123]. Taken together, these findings suggest that the knockdown Foxk1 or loss expression of FoxK1 promotes the invasive capacity of breast cancer.

Mounting of data suggests FoxK2 is required for normal development and is involved in tumor development and progression. Significantly, the expression of FoxK2 is progressively lost during breast cancer progression, and low FoxK2 expression is strongly correlated with higher histologic grades, positive lymph nodes, and triple-negative status [124]. Moreover, FoxK2 is trans-activated by ER α and transrepressed *via* reciprocal successive feedback by HIF1 β /EZH2. In our review, we could not find published data involving FoxK2 expression in tumor invasion from non-invasive type or normal breast cell into invasive carcinomas.

FoxL: FoxL1 has been associated to the regulation of epithelial cell proliferation in gastrointestinal tracts. Its loss expression led to a marked increase in cellular proliferation of intestinal epithelia in mice, thus promoting the distortion in the tissue architecture of the stomach and small intestine [125]. FoxL1 is specifically expressed in low-grade fibromyxoid sarcoma as compared to other morphologically similar tumor type [126]. FoxL1 over-expression is associated with better prognosis and its up-regulation significantly inhibited cell proliferation, migration and invasion in gallbladder cancer tissues and cell lines [127]. High expression of FoxL1 is associated with Normal Breast Epithelial Cell (NBEC) whereas its down-regulation has been observed in breast cancer cells (MDA-MB-231, MCF-7 and BT-474). Furthermore, overexpression of FoxL1 significantly down-regulated the protein expression levels of β -catenin, c-Myc and cyclin

D1 in MDA-MB-231 cells [128]. Taking together, the loss expression or down-regulation of FoxL1 is a potential marker of tumor invasion in breast cancer.

FoxM1: Among the extensive family of the Forkhead box (Fox) proteins, FoxM1 (with FoxO3a and FoxA1) is one of the three best-studied Fox proteins involved in cancer [90]. FoxM1 has a vital role in oncogenesis, angiogenesis, invasion, metastasis, DNA damage repair and the development of chemotherapeutic drug resistance breast diseases [129,130]. Published data revealed that *FoxM1* gene has three distinct isoforms (including FoxM1a, FoxM1b and FoxM1c) and Lam et al. findings support that FoxM1b which is overexpressed in cancer cells has a greater oncogenic potential than FoxM1c [129,131]. Multitude of factors (including cyclin-dependent kinase (CDK), histone deacetylase (HDAC), RUNX, Aurora Kinase A (AURKA), FoxA1, GATA3, MMP2 and MMP9) play important role in the transcription and gene expression of FoxM1 proteins [130,132-135]. Studies revealed that in breast cancers, FoxM1 overexpression is significantly associated with aggressive phenotypes and poor prognosis of ER-positive, and behaves like the oncogenic transcription factor [133,135,136]. Moreover, it is found to be highly expressed in IDC (MDA-MB-231, MCF7, T47D, and SKBR3 cells) versus DCIS, MCF-10A cells or normal tissues [134,137]. Overexpressed in DCIS or in normal tissues, FoxM1 induces invasive breast cancer [134,137]. Co-expression of FoxM1, survivin, and nuclear XIAP was associated with poor outcomes of women with stage III breast cancer with significantly reduced 5- and 10-year survival rates versus women with tumors without these features [138]. In breast cancer cells, FoxM1 over-regulation resulted in increased cell growth, migration and invasion. In contrary the FoxM1 down-regulation inhibited cell growth, clonogenicity, migration, and invasion, as the same time expression of various factors including uPA, uPAR, MMP2, MMP9, ErbB2, and vascular endothelial growth factor (VEGF) [134,139]. ErbB2 is frequently highly expressed in DCIS, showed that activation of ErbB2-dependent signalling results in up regulation of FoxM1, and its transcriptional targets, MMP2. Inhibition of FoxM1 by RNA interference prevented induction of invasion by IR, and overexpression of FoxM1 in MCF10A cells was sufficient to promote IR-induced invasion. Moreover, 14-3-3 ζ was also upregulated by IR in cancer cells in a ROS-dependent manner is required for IR-induced invasion in ErbB2-positive breast cancer cells and together with FoxM1 is sufficient for invasion in ErbB2-negative breast cancer cells. These findings clarified that IR-mediated activation of ErbB2 and induction of 14-3-3 ζ collaborate to regulate FoxM1 and promote invasion of breast cancer cells [134]. Published data have shown that FoxO3a can interact with FoxM1 in the ER α promoter and regulate ER α expression in breast cancer cells. These two Forkhead box (Fox) proteins are often transcriptionally antagonistic. They not only compete for binding to the same DNA motif in target promoters, but also have opposing transcriptional output. Karadedou et al. showed that FoxO3a activation correlates with down-regulation of FoxM1 and VEGF expression [140]. FoxO3a plays a critical tumor-suppressive role in breast cancer [135]. As a result, FoxO3a negatively regulates the transcriptional output of FoxM1, which promote tumorigenesis and cancer progression [136]. Based on these data, FoxM1 promote invasion of breast cancer from DCIS into IDC.

FoxO: FoxO subfamily proteins play important role in the regulation of metabolism, oxidative stress resistance and cell cycle arrest. Under fasting conditions, FoxO transcriptionally activates insulin-responsive genes, which include genes encoding enzymes responsible for gluconeogenesis or glucose homeostasis in the liver

[91,107]. FoxO1, FoxO3, FoxO4 and FoxO6 are the members of this subfamily of proteins. Evidences suggested that in the human body, there is a broad overlap in their expression patterns. They are uniquely enriched in specific tissues: FoxO1 in adipose and liver tissue, FoxO3A in the brain, and FoxO4 in skeletal muscle, while FoxO6 appears to be expressed almost exclusively in adult brain [141-143]. Despite these specific tissues enrichment, clear data clarified the involvement of FoxO proteins in breast cancer angiogenesis and invasion. These proteins, based on the cellular context and the stage of the disease, are not solely tumor suppressors, but also support tumor growth and metastasis by regulating a plethora of cellular processes essential for tumorigenesis [141,143,144]. Activity of FoxO proteins is activated by several kinases pathways including the stress-activated c-Jun-NH2-kinase (JNK), AMP-activated protein kinase (AMPK), in other hand other kinases such as PI3K/AKT, CK1, IKK β and ERK1/2 suppress their activity [143,145]. found that FoxO6, but not FoxO1, 3, and 4, was frequently overexpressed in breast cell lines and tumors compared to normal cells [143].

FoxO3a, a tumor suppressor, is under-expressed in many breast cancer patients [146]. In breast cancer cells, the role of FoxO3 in cell migration and invasion has been linked to the ER α status. Indeed, in ER α + cells, FoxO3 cooperates with 17 β -estradiol to reduce cell invasiveness, while in ER α - cells, FoxO3 tends to increase it [140,147,148]. Thus, its expression is associated with favorable outcome in ER-positive breast cancer specific survival and distant metastasis free interval [136]. Overexpression of FoxO3a induces a decrease in invasiveness, and anchorage-independent growth in ER-positive breast cancer but increases invasion in ER-negative breast tumors [141,147]. Data reported that while FoxO3 promotes migration on cellular invasion by inducing the expression of MMP-9 and MMP-13, FoxO1 induces MMP-1 and, therefore enhances the cellular invasive potential [149,150]. FoxO3a is an important downstream effector of the PI3K/Akt pathway. The silencing of FoxO3a expression in pre-invasive breast cells result in an induction of breast cancer invasion.

Contrary to the others FoxO proteins, FoxO6 is frequently overexpressed in breast cancer cells than in normal mammary cell lines or tissues, suggesting it to be an oncogene in human breast carcinogenesis [143]. This functional difference between FoxO6 and the others proteins of this subfamily can be explained by the major structural differences that exhibits FoxO6, as the activation of the activation of the PI3K-AKT pathway by growth factors inhibits FOXO6 transcriptional activity mainly *via* a mechanism independent of shuttling to the cytosol [141,143].

Overall, these data suggest that the silencing of FoxO1, 3, and 4, and overexpression of FoxO6 in normal or pre-invasive breast cells are important factor for invasive breast cancer.

FoxP: FoxP subfamily is composed of 4 proteins (FoxP1, P2, P3 and P4) that play dramatically different functions. For instance, FoxP1 and FoxP4 play essential parts in cardiac morphogenesis, FoxP2 has been shown to be required for speech acquisition in humans, and FoxP3 is essential for the programming of regulatory T cells [91,107]. Nevertheless, studies' findings have showed their implication in the migration and invasion of breast cancer cells.

FoxP1 has been suggested as a tumor promoter and an oncogene, depending on the cell type, although these observations are primarily based on correlations between mRNA levels and clinical outcomes [151]. Expression of FoxP1 is associated with ER [151-155]. A

systematic review and meta-analysis of prognostic value of decreased FoxP1 protein expression in various tumors revealed the decreased FoxP1 expression was significantly correlated with an unfavourable relapse-free survival [152]. Study revealed that FoxP1 was more highly expressed in familial (hereditary) breast cancers compared with sporadic cancers for luminal and basal, but not HER2 and null phenotypes. Moreover, the absence of FoxP1 expression was associated with a shorter relapse free and overall survival in familial breast cancer [153]. In a clinico-pathological study, while FoxP1 staining was predominantly localized in the nuclei in usual ductal hyperplasia (UDH), the FoxP1 nuclear distribution gradually decreased from ADH, DCIS to IDC, and the cytoplasmic staining increased. These observations strongly indicated that FoxP1 expression might shift from the nucleus to the cytoplasm during breast tumorigenesis, and therefore, cytoplasmic mislocalization of FoxP1 is suggested play an important role in breast cancer progression [154]. Published data suggested that FoxP1 immunoreactivity predicted better relapse-free survival but not OS in breast cancer patients [156,157]. Moreover, FoxP1 immunoreactivity may predict a favorable prognosis for breast cancer patients treated with tamoxifen [155,158].

FoxP2 is the first and most well-studied gene to be implicated in human speech and language skills, and its heterozygous mutations cause a severe speech and language disorder characterized by childhood apraxia of speech (CAS) and accompanied by expressive and receptive language problems [159-161]. Growing information revealed that dysregulation of FoxP2 expression is somehow involved in cancer initiation, maintenance, invasion and progression [160-164]. Aberrant expression of FoxP2 has been detected in several cancer types, including up or down-regulated FoxP2 levels depending on the type of cancer. This suggests that aberrant FoxP2 levels may play the dual role of a pro-oncogenic/deficient tumor-suppressor that may be tissue specific and vary along the progression of cancer [160,162,163]. The expression of FoxP2 is significantly lower in breast cancer (including IDC and ILC) than normal breast tissues [162]. Moreover, FoxP2 was found significantly less expressed in breast cancer tissue compared to adjacent control tissue [162]. In a wound-healing assay and Transwell assay, and significantly showed that the knockdown of FoxP2 could promote cell migration and invasion *in vitro*, and this phenomenon was significantly inhibited after FoxP2 overexpression [162,164]. Furthermore, the authors also found that downregulation of FoxP2 expression induced an up-regulation of TGF β pathway related proteins (including TGF β R1, p-SMAD3, SMAD4 and Snail) [162]. Taken together, these data highlight the tumor suppressor role of FoxP2.

Forkhead Box Protein3 (FoxP3), a member of transcription factor winged-helix family is involved in regulating the immune system development and function [91,165]. FoxP3 up- or down regulates a large number of genes. For instance, *in vitro*, FoxP3 represses the transcription of the HER2, SKP2, MYC, MMP2/9, CXCR4, VEGF, and *uPA* genes and induces the expression of p21 and LATS2 [166-169]. Thus, inhibited cell growth, cell migration, and cell invasion have been observed in various cell lines derived especially from breast cancers that overexpress FoxP3 [166]. The mutation of FoxP3 expression is responsible for X-linked autoimmune diseases in mice (scurfy mice) and humans (Immune dysregulation, polyendopathy, enteropathy, X-linked, IPEX) [170,171]. FoxP3 expression has recently been noted in epithelial cells in both normal and cancerous tissues from the breast and it has been most notable for its role as the master regulator in the function and development of CD4+

CD25hi regulatory T cells (Treg), as Tregs have high level expression of FoxP3 [169,172-176]. The loss of FoxP3 function or its aberrant expression leads to Treg deficiency, resulting in lethal autoaggressive lymphoproliferation, whereas FoxP3 overexpression results in severe immunodeficiency [166,172,176,177].

Several studies have addressed the role of FoxP3 in breast cancer invasion/progression [167,168,178,179]. Gupta et al. have analyzed the intratumoral expression of FoxP3 in IBC and compared it with its level in DCIS and adjacent normal tissue, and its correlation with the levels of TGF- β 1, VEGF and intratumoral micro vessel density (IMD) were also investigated [178]. The authors significantly found that its expression in infiltrating cancers (IBC) was higher than in DCIS, and was approximately two fold higher than in normal tissues ($p < 0.001$) [178]. Moreover, in infiltrating carcinoma, a significant positive correlation between FoxP3 expression and TGF- β 1 expression was noted ($p < 0.001$). Furthermore, a positive correlation between FoxP3 expression with VEGF expression and IMD values was also detected; however, statistically that was non-significant [178]. In another study carried out a quantitative analysis of FoxP3 expression in lymphocytes as well as in epithelial cells in a set of thirty-two breast tumors with synchronous normal epithelium, DCIS, and IDC components [179]. The findings of the study showed that median proportion of FoxP3-expressing CD3 cells significantly increased with malignant progression from normal to DCIS to IDC components (0.005, 0.019 and 0.030, respectively; $p < 0.0001$ for normal vs. IDC and $p = 0.004$ for DCIS vs. IDC) [179]. Moreover, the median intensity of epithelial FoxP3 expression was also increased with invasive progression and most markedly augmented between normal and DCIS components (0.130 vs. 0.175, $p < 0.0001$) [179]. Bates et al. also investigated the prognostic significance of FoxP3-positive Tregs in noninvasive breast cancer, suggesting that Treg accumulation represents a marker of breast cancer progression [174]. Their findings showed the median Treg number differed significantly between normal, DCIS, and IBCs ($p < 0.001$). Moreover, higher numbers of Treg in DCIS patients also indicated a worse relapse-free survival (RFS) [174]. In all three studies, Treg infiltration and epithelial FoxP3 expression were both higher in grade 3 vs. grade 1 tumor [174,178,179]. In a clinical study involving 202 breast cancer patients and 130 normal healthy women, both of Indian origin, the authors found that Foxp3 rs37161548 has a potential to be a polymorphic marker for tumor progression in premenopausal breast cancer patients [180]. Notably, Treg infiltration significantly correlated with epithelial up-regulation of FoxP3 expression. A linear association of intratumoral FoxP3 expression with invasion, size and vascularity suggests a utility of FoxP3, an indicator of Treg activity as a marker of breast cancer invasion, progression and metastasis.

FoxP4, an additional member of the FoxP family, is highly homologous to FoxP1 and has been shown to dimerize with other FoxP proteins. FoxP4 is involved in the development of the central nervous system [181]. It is expressed in both thymocytes and peripheral CD4+ and CD8+ T cells [182]. Compared to other FoxP proteins, there is no much information regarding the involvement of FoxP4 in breast cancer research, especially in terms of the transitory journey from non-invasive into invasive carcinoma of the breast. In addition to the circMYO9B, FoxP4 expression was significantly higher in BC cell lines (BT474, MCF-7, MDA-MB-231, T47D and MDA-MB-453) than that in normal breast epithelial cell line (MCF-10A), whereas its expression was significantly reversed through circMYO9B knockdown in BC cells by MiR-4316 [183]. These data suggest that FoxP4 plays an essential role in regulating BC cell proliferation,

migration and invasion, thus presenting potential oncogenic role of FoxP4.

FoxR: FoxR subfamily consists of FoxR1 and FoxR2. While scientists reported deregulation of some Fox subfamilies genes (including FoxA, FoxC, FoxF, FoxM, FoxP and FoxO) could lead to carcinogenesis and congenital disorders, research in FoxR subfamily is numbered [91,184,185]. The human FoxR2, also known as FoxN6 is an ortholog of FoxR1, which can functionally replace Myc and drive proliferation [185]. Research evidences showed that FoxR2 is expressed in breast cancer cell lines and primary breast cancer tissues [185-187] and upregulated in Hodgkin lymphoma (HL) [188]. Moreover, found that FoxR2 was not expressed in the 3 normal human breast epithelial cell lines (MCF10A, HMLE, and HBL100) but was detectable in breast cancer cell lines (including ZR75.1, T47D, MCF-7, MDA-MB-468, and SUM52PE) [187]. High expression of FoxR2 is significantly associated with clinic pathologic classification of tumor size and Ki-67 status [185]. Up regulation of FoxR2 in MCF10A cells leads to its interaction with Myc, and promotes breast tumor growth and invasion, and conversely FoxR2 knockdown in breast cancer cells leads to diminished cell proliferation [187]. Suggesting FoxR2's role as oncogene to promote proliferation. These observations suggested that FoxR2 might play a role in the tumorigenesis of breast cancer and may function as an oncogene in breast cancer.

Conclusion

Most breast cancers occur in the normal terminal duct lobular unit (TDLU). Known as a progressive disease, the human breast cancer is categorized in 4 key stages: HELU, ADH, DCIS, and IBC. The prognosis, prediction and treatment of breast cancers are complicated by the diverse constellation of causative alterations within multiple biological pathways that lead to this heterogeneous disease.

Ductal carcinoma *in situ* is a premalignant condition involving unlimited growth, angiogenesis, genomic elasticity, invasion and metastasis. DCIS presents a clinical risk of development to invasive. Over century, studies had shown DCIS has a malignant phenotype, prevention of development of invasive, biomarkers play a necessary key component for tumor growth and invasive, the identification of specific markers expressed in DCIS might be useful to identified patients which high risk of recurrence, prognosis and treatment. Initial strategies to treat breast cancer have therefore employed gene specific, tissue-specific as well as whole genome approaches to identify specific signatures related to particular breast cancer types, which can then be exploited to optimize treatment targeting a specific patient's tumors [188,189].

From a clinical standpoint, despite the presumption that early treatment for DCIS would reduce cancer incidence and mortality [190], a small proportion of patients with DCIS ultimately die of breast cancer [191]. While some patients experience an in-breast invasive recurrence prior to death, some women die of breast cancer without first receiving a diagnosis of local invasive disease [192-194]. Therefore, it is unclear to what extent mortality from breast cancer after DCIS is the direct consequence of an invasive recurrence or whether fatal cases of DCIS have high malignant potential from the outset (Table 1).

However, as death from breast cancer after DCIS is too rare to be used as an end point in randomized clinical trials, information on the lethality of DCIS must be indirectly derived from the features of its potential recurrence. Notably, for the vast majority of cases, a unilateral or contralateral recurrence of DCIS has no impact on mortality while an invasive cancer does (18-fold for unilateral and 13-fold for contralateral), leading to the accepted conclusion that nearly all risk depends on whether an invasive disease presents [190].

In this review, we presented the first of a 5-part series of an extensive overview of the investigated molecular markers that might determine the progressive course from DCIS into IBC. Further researches are needed to identify biomarkers genes of DCIS and improve better prognosis and treatment for breast cancer. Regardless the huge numbers of published data on cancer biomarkers, relatively few are widely used in patient management (Table 2). For instance, in breast cancer, only very few biomarkers are FDA-approved, even though there several others being used clinically without FDA clearance to be discussed extensively in our next paper). Failure to properly validate, and to show clinical value (utility) for emerging biomarkers constitutes the main reason for this limited use [195]. The biomarkers discussed in this paper have showed us the glimpse of the complex physiological and signalling pathways that occur during the transitory journey from DCIS to IBC. For instance, biomarkers, even though belonging to the same subfamily can be dysregulated in different way. This tells us that there is a much to study and to understand in terms of the evolution of breast cancer. Currently, there are no effective predictive biomarkers for identifying this subset with worse prognosis whose lesions are essentially indistinguishable histologically from those with favorable outcomes (Table 3).

In future researches, biomarker(s) that correctly predict outcomes in a specific disease stage, and allow physicians and patients to make informed treatment decisions need to be developed. However; let us emphasize that an ideal cancer biomarker should possess all or most of the following properties: a) have an analytically validated assay for its measurement, b) have undergone validation for addressing a specific clinical problem, c) have been shown to have clinical utility

Table 2: Handful list of FDA-approved biomarkers in current clinical use regarding breast cancer.

Biomarker	Clinical use	Specimen	Methodology	Year first approved or cleared
Circulating Tumor Cells (EpCAM, CD45, cytokeratins 8, 18+, 19+)	Prediction of cancer progression and survival	Whole blood	Immunomagnetic capture/ immune-fluorescence	2005
Estrogen receptor (ER)	Prognosis and Prediction	FFPE tissue	Immunohistochemistry	1999
Progesterone receptor (PR)	Prognosis and Prediction	FFPE tissue	Immunohistochemistry	1999
HER-2/neu	Prognosis and Prediction	FFPE tissue	Immunohistochemistry	1998
CA15-3	Monitoring	Serum, plasma	Immunoassay	1997
CA27.29	Monitoring	Serum	Immunoassay	1997
Carcino-embryonic antigen (CEA)	Aid in management and prognosis	Serum, plasma	Immunoassay	1985

HER-2/neu: Receptor Tyrosine-Protein Kinase erbB-2; EpCAM: Epithelial Cell Adhesion Molecule; CD45: CA15-3: Carcinoma Antigen 15-3; CA27.29: Carcinoma Antigen 27.29; CEA: Carcino-Embryonic Antigen

Table 3: Some breast cancer biomarkers clinically used without FDA-clearance.

Biomarker	Clinical use	Biomarker	Clinical use
Collagen XXIII	Prognosis	PAI-1	Recurrence
MMP	Prognosis	Cathepsin B and L	Recurrence
MMP Inhibitors	Prognosis	Cyclin D1	Prognosis, Recurrence
Urokinase-type plasminogen activator (uPA)	Recurrence	Ki67	Prognosis

MMP: Matrix Metalloproteinases; uPA: Urokinase Plasminogen aActivator; PAI-1: Plasminogen Activator Inhibitor-1.

Table 4: Summary of discussed biomarkers potentially involved in the recurrent and progressive journey from DCIS to IBC.

Biomarkers	Transition	References	Biomarkers	Transition	References
Steroids Receptors			Cell Cycle Regulators		
ER	↓	[44]	<i>Forkhead box proteins</i>		
PR	↓	[50-52]	FoxA1	↓	[93,95,96]
AR	↓	[50]	FoxA2	↓	[106]
Marker of Proliferation			FoxC1	↓	[110, 111]
Ki-67	↑	[58,60]	FoxC2	↓	[112,113]
Cell Cycle Regulators			FoxF1	↓	[115-117]
The Cyclins			FoxF2	↑	[118-120]
Cyclin D	↑	[61,62]	FoxK1	↓	[122,123]
Cyclin A	↑	[61]	FoxK2	↓	[124]
Cyclin E	↑	[67,68]	FoxL1	↓	[126-128]
Cyclin B	↑	[64]	FoxM1	↑	[134,137]
p-family proteins			FoxO3a	↓	[141,147]
p16	↑	[45,69]	FoxO6	↑	[143,143]
p21	↑	[43,76]	FoxP1	↓	[154]
p27	↓	[68,78]	FoxP2	↓	[162-164]
p53	↓	[54,82]	FoxP3	↓	[178,179]
p63	↓	[87-89]	FoxP4	↓	[183]
			FoxR2	↑	[187]

ER: Estrogen Receptor; PR: Progesterone Receptor; AR: Androgen Receptor; FOX: Forkhead Box Protein;
 ↓: Loss or down-regulation expression from DCIS into IBC
 ↑: Gain or up-regulation expression from DCIS to IBC
 ↑↓: Down-regulated or up-regulated, and can be either a tumor suppressor or an oncogene in a tissue-context- and stage-specific manner

such as improving patient outcome, enhancing quality of life, or reducing cost of care, d) have a cost-effective assay, and e) be a target for therapy (Table 4) [196-198].

Author’s Contributions

All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting, reviewing, revising and/or editing the manuscript for intellectual content; and (c) final approval of the final manuscript for publication.

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Conflict of Interests

Upon manuscript submission, all the authors declare that they have no conflicts of interest.

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