

## Review Article

# A Review of the Recent Findings on Ductal Carcinoma In Situ of the Breast (DCIS)

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## Abstract

Ductal Carcinoma in Situ is an entity which bears the risk of progression into Invasive Breast Carcinoma of No Special Type (IBC of NST). Evidence suggests that the malignant potential is already present in the non invasive period. Currently, the tumor microenvironment interaction has gained importance since the genetic and translational modifications of the DCIS lesion itself does not inform about the probability of malignancy sufficient for the risk stratification concerning the prognosis of the entity. Recent evidence underlines the interaction of the surrounding cells as affecting the fate of DCIS. Reproducibility of a diagnosis and grading of DCIS is another problem which is tried to be overcome with the incorporation of deep learning convolutional neural network analyses and various gene expression assays. This summarizes the findings of the recent studies to elucidate the transition of DCIS to IBC of NST regarding the histopathology, molecular biology while reflecting on the current prognostic data of DCIS with the treatment methods that are in application.

**Keywords:** Ductal Carcinoma In Situ; Breast; DCIS

## **Introduction**

Breast Cancer is the leading cause of cancer-related death among women in the US [1]. In 2018 alone, it was responsible for the death of 627,000 [2]. One in seven women in the UK [3] and one in eight women in the US [4] will get a diagnosis of breast cancer in their lifetime. Due to the advancements in early diagnostic measures, breast cancer related mortality has shown a decline over the last 20 years [4].

Implementation of nationwide screening programs had an undeniable role in this trend [2]. Screening methods such as mammography has resulted in an increase in the early detection of breast carcinoma and in situ breast carcinoma [2,3]. The current approach to Ductal Carcinoma in Situ (DCIS) lesions is Breast Conserving Therapy (BCT) followed by radiotherapy [5]. Given that only 20-53% of individuals with DCIS will develop invasive breast cancer [6] and considering the 10-year breast cancer specific survival rate of 98-99% [7]; concerns about overdiagnosis and overtreatment arise [5]. Overtreatment mainly exists due to lack of prognostic markers to detect the subtypes of DCIS which have a higher probability to progress into invasive breast cancer [8].

The objective of this article is to present the challenges in contemporary methods of diagnosis and treatment, while touching upon the history of the disease entity and to the recent data on prognosis.

## **Definition of DCIS, History and Epidemiology**

Ductal Carcinoma in Situ is described as a neoplastic proliferation of epithelial cells in the terminal ductolobular units of the breast with varying degrees of cellular atypia, confined to the mammary ductolobular system [9] and considered to have an inherent but non-obligatory tendency to develop invasive breast carcinoma [10]. The earliest recognition of a DCIS-like lesion could be traced back to 1893, which was then described as comedocarcinoma by Bloodgood [11]. Comedo DCIS, was described by Lewis and Geschikter in 1938 [12], later to be followed by the recognition of Lobular Cancerization in DCIS by Azzopardi [13]. Page and Dupont described cribriform, micropapillary and some other types of non comedo DCIS as high-risk factors for the development of Ipsilateral Invasive Breast Tumor (IIBT) [13]. Ductal Carcinoma in Situ (DCIS) has shown a drastic increase in incidence from 1.87% to 32.5%, over the years of 1974- 2004. In 2017, 83% of the cases of DCIS in the US had been diagnosed in between 2010-2014 [6]. Furthermore, between the years of 1983 and 2006, there was an increase of about 29% of DCIS diagnosis in the population younger than 50 years; a rate that is still increasing [10]. Similar increase in diagnosis of DCIS can be seen in the Netherlands and the UK [3,14]. Despite the increasing incidence of DCIS in the US, the incidence of invasive carcinoma stood relatively stable [6]. Patients who had received a diagnosis of low-grade DCIS, did not have a significant survival benefit from a surgical intervention [15]. Among patients who received BCT, radiotherapy was associated with a reduced risk of ipsilateral tumor recurrence yet did not provide a reduction in the risk of breast cancer related mortality [16]. In autopsy studies, an undiagnosed percentage of about 9% of DCIS cases were observed, which indicates that normal length of life with no complications due to DCIS is a rather common occurrence [17].

Defined risk factors for DCIS are similar to risk factors for developing invasive breast cancer which are: family history, nulliparity, late age at first birth, late menopause, high BMI and increased mammographic breast density [10].

## **Diagnosis, Prognosis and Treatment**

### **Diagnosis**

As 90% of DCIS lesions are asymptomatic, previously it was diagnosed based on detection of a palpable mass before the implementation of mammography screening [6].

The hallmark for detection of DCIS on X-ray mammography is based on the appearance of microcalcifications. Magnetic Resonance Imaging (MRI) has long been considered as less sensitive than X-ray, yet new studies suggest that the addition of MRI to preoperative stage yields less mastectomy and re-excision rates as well as decrease in the requirement for additional biopsy compared to mammography alone. In a study evaluating the accuracy of the assessment of the extent of DCIS by using various imaging techniques; MRI has been found as the most accurate method with a sensitivity of 89% followed by mammography and ultrasound with 55% and 47%, respectively [18]. However, for lower grade lesions, mammography is still considered the more sensitive method [19]. These findings suggest that mammography cannot be substituted by MRI, although its addition to the diagnostic process may reduce unnecessary interventions.

### **Prognosis**

Epidemiological data shows that among women with DCIS; risk of progression into invasive carcinoma nonspecial type (NST) is 1-2.6%, 8 to 10 times higher than that of the general population.

In a retrospective study 20-year breast cancer specific mortality rate was found to be 3.3%, a rate which was higher for women who received the diagnosis of DCIS before the age of 35 [16].

However, prediction of cases with higher predisposition for malignancy and recurrence is a proven challenge. Currently identified factors associated with an increased risk of local recurrence are young age at diagnosis, high nuclear grade, large lesion size, comedo necrosis and positive margin status [20].

Several studies concluded that DCIS lesions with hormone receptor positivity, lack of HER2 amplification and low levels of Ki67 have lower rates of recurrence after excision and also had lower incidence of progression into invasive disease [9].

As an opportunity to target, COX-2 expression has been found to be associated with higher cell proliferation rates, nuclear grade, ER negativity and HER-2 overexpression. COX-2 expression correlated with worse prognosis. Furthermore, utilization of COX-2 inhibitors such as celecoxib, in addition to aromatase inhibitors, reduced Ki-67 expression and reduced tumor cell proliferation; yet concerns about cardiovascular risks of routinely use of COX-2 inhibitors limit its use [21].

The major subtypes of DCIS, which are similar to those of breast cancer, are classified as luminal A, luminal B, HER2, Basal-like and correlates with prognosis and recurrence rate [22].

### **Prognostic Indices for Treatment Decisions**

Prognostic indices have been developed to predict the risk of local recurrence. One of these is the Van Nuys Prognostic Index, which takes the tumor size, margin width, nuclear grade, age, comedonecrosis and expression of HER-2 receptor on the lesion into consideration and aims to aid the decision about postoperative adjuvant therapy.

For the scores in between 4 and 6, the suggested treatment is excision only, whereas for 7 to 9 the suggestion is to add radiation therapy, and for 10 to 12 the suggestion is total mastectomy [20].

Another prognostic index is Oncotype DX12-DCIS, which is an RT-PCR based-assay of gene expression that predicts the risk of recurrence or invasion [9,23].

Oncotype DX-12 uses 12 genes 7 of which are cancer-related (Ki 67, STK15, Survivin, CCNB1, MYBL2, PR, GSTM1) and the rest being reference genes (ACTB, GAPDH, RPLPO, GUS, TFRC) [23]. The risk stratification is made in three categories, as low (DCIS Score <39), intermediate (DCIS Score 39-54) and high (DCIS Score >55). There have been several studies which have attempted to predict the Local Recurrence Risk after treatment with BCS alone, with BCS and additional adjuvant endocrine, or radiotherapy. Rakovitch et al. studied the accuracy of the prediction of LR after BCS of a DCIS lesion based on DCIS scores. The group has identified the DCIS score as an independent predictor of Local Recurrence. Low DCIS scores are significantly associated with lower rates of local recurrence. Yet, as the intermediate DCIS score group demonstrated higher incidence of local recurrence than the high DCIS score group, risk stratification based on gene expression levels of the previously noted 7 genes may be falling short of explaining the invasion or predicting it in higher scores. The results of the group were very similar to those of the E5194 trial [24].

They concluded that DCIS scores may be helpful in making treatment decisions when combined with the patient age, tumor size, age at diagnosis and architectural subtype [24]. Another point to consider is that patients with low-grade DCIS scores had recurrence rates of 11-13%, which is not low enough to justify the omission of radiotherapy [9,25].

When DCIS score is compared with VNPI, prognostic predictions made by USN/VNPI and Oncotype DX were significantly different enough to assign patients into different risk groups for recurrence [26]. Another cause for the scrutiny against OncotypeDX is because the genes that are selected for the assessment of Local Recurrence and Invasion risk, are actually related to cellular proliferation potential rather than invasive potential [27].

Another prediction tool DecisionRT, has recently undergone validation studies. The tool combines molecular, clinical and histopathological markers to develop a biological signature. Molecular markers used in prediction are PR, FOXA1, COX-2, SIAH2, HER2, Ki-67, P16/INK4A, whereas the clinicopathological factors are age, tumor size, margin status and palpability. The tool combines these factors in a non-linear model. The cutoff value between the low risk group and elevated risk group is decided as 10% increased risk for Ipsilateral Breast Events (IBE) and less than 6% increased risk for Ipsilateral Breast Cancer (IBC), which corresponds to a Decision Score(DS) less than or equal to 3 [28].

As a result, the first validation study of DecisionRT demonstrates that DS correlates with 10-year IBC and IBE risks. Furthermore, in the low risk group, they report that there is no significant risk reduction with the use of adjuvant Radiotherapy. On the contrary, the elevated risk group is reported to significantly benefit from adjuvant RT [29].

Even though these findings might be in need of more thorough and long-term studies with a higher number of participants, the integration of the genetic information into prognostic predictability seems to be giving off better results.

Genetic pathways behind the progression of DCIS to IBC are continuing to be revealed and as the interdependencies of various signaling pathways and their effects are being increasingly acknowledged, concepts that are integrating the genetic pathways and the clinicopathological background of the patient seems to be of the most benefit. Moreover, in DCIS, and in most cases of neoplastic progression, the interaction of the microenvironment is another determinant of tumor progression. As a result, in the future development of prognostic markers, associated gene expression profiles of Myoepithelial cells, tumor infiltrating lymphocytes as well as fibroblasts might be incorporated into the prediction analysis.

## **Concepts of Treatment**

### **Surgery**

The surgical treatment options for DCIS are either total mastectomy or Breast Conserving Therapy followed by radiotherapy [5,20]. Yet some studies suggest that patients with low grade DCIS do not benefit from a surgical intervention [15].

One of the most important problems regarding BCT, is the establishment of a consensus regarding the optimal width of negative margin that would benefit from additional radiotherapy when performing BCT. American Society for Radiation Oncology, in their guideline published in 2016, stated that a negative margin of 2mm should be the standard approach, yet negative margins lower than 2mm should not be regarded as candidates for reexcision either, as factors known to impact recurrence rates should be taken into consideration before such a decision. However, positive margins, defined as «ink on DCIS» are associated with increased risk of IBTR even if these patients were to receive Whole Breast Radiation (WBR) therapy [30].

Furthermore, axillary lymph node metastasis is extremely rare in DCIS [20]. Therefore, sentinel lymph node biopsy might be weighted according to its complications such as numbness and edema.

In regards to sentinel lymph node dissection, a study conducted in 2018 demonstrated that surgeons still tend to make rather unnecessary axillary lymph node dissections [31], despite of the results of Z0011 trial in 2011, which showed that in cases of clinically non-detectable sentinel lymph node metastasis, sentinel lymph node biopsy with no further intervention was just as effective as axillary lymph node dissection [32]. As most of the detected metastases in sentinel lymph nodes in series of DCIS cases are micrometastases [20], the decision of an axillary lymph node dissection might be reserved for extensive cases with invasive components.

### **Radiotherapy**

Radiotherapy is found to be associated with associated with lower tumor recurrence rates as the results of a meta-analysis demonstrate a 10.1% 10 year Ipsilateral Breast Tumor Recurrence (IBTR) in adjuvant radiotherapy receivers, compared to 23% of IBTR in the group that had undergone excision without further radiation therapy [30]. Yet most of the studies, except one cohort study done by Giannakeas V, could not establish a significant association between breast cancer associated mortality risk [33,34].

Instead of WBR which is associated with both local and systemic side effects [35] new radiation delivery approaches have been tried. One such technique, Accelerated Partial Breast Irradiation (APBI), focuses radiation directly onto the tumor bed [36] and therefore is associated with fewer side effects [35].

A meta-analysis to assess the long-term survival effects of treatment with Partial Breast Irradiation (PBI) and WBR for early breast cancer, included 9 trials and total of 8720 subjects, concluded that while there is no difference in Breast cancer specific survival, non-breast cancer specific survival was slightly but significantly lower in APBI group [36].

However, lack of evidence for the benefit of APBI on DCIS, was the cause of delay to include the DCIS among indications for APBI [37]. Yet nowadays, increasing evidence shows that the use of APBI in DCIS seems to be a feasible choice [38]. One such evidence came from a trial that assessed the use of Intra Operative Targeted Therapy in DCIS according to the TARGIT-A criteria, of 52 patients treated with BCS and TARGIT for pure DCIS, 4 experienced local recurrences, with a 5-year LRR of 7.7% [38].

### **Endocrine Therapy and Pharmacotherapy**

According to the results of the UK/ANZ DCIS trial, Tamoxifen provided a reduced IBTR, however there was no significant beneficial effect over 15-year cumulative incidence of all-cause mortality and breast cancer mortality. Tamoxifen treatment was associated with a reduction of both ipsilateral and contralateral events. Yet the beneficial effects of tamoxifen were not seen in patients who had received radiotherapy [39]. The trial assessed the effect of radiotherapy on ipsilateral DCIS recurrence too, with 7.1% of IBTR compared to 19.4% in non-radiotherapy receiving group, radiotherapy was associated with a significant reduction of IBTR on the ipsilateral tumor recurrence [39].

The American Society for Radiation Oncology also concluded that adjuvant tamoxifen treatment decreases IBTR in patients with positive margins but does not provide benefit for the patients with negative margins [30].

### **Immunological Therapy/ Vaccination**

Over the years, there has been an interest in immunotherapy and vaccination against tumor specific antigens in the literature. This came about after the discovery of a reduction in the risk of developing cancer after certain autoimmune diseases, or certain viral or febrile illnesses that had been passed in childhood [40].

As the luminal types of Breast cancer are usually associated with estrogen positivity, anti-estrogen therapy is helpful in the prevention of luminal breast carcinomas. On the other hand, there are an undeniable amount of ER(-) Breast cancers, some of which are associated with HER-2 overexpression. Increased Her-2 expression is associated with increased invasive and metastatic potential [41].

In addition, during tumor progression there is a continuous loss of anti-HER2 CD4+ Th-1 cell response, the total absence of which is associated with invasive breast cancer- a phenomenon called “tolerance”. One of the main considerations in treatment of the HER-2 positive pre-cancerous lesions is to maintain the anti-HER-2 response of CD4+ T cells [28].

One of the studies in this area has been conducted by Czerniecki et al, which delivered polarized Dendritic cells with HER-2, accompanied with IL-12 bursts triggered by an exposure to Toll-like receptor (TLR) agonist. By this way, the “rejection” of tumor is accomplished as demonstrated by induction of Th-1 cells, peritumoral lymphatic infiltrates, complement dependent tumor-lytic antibodies as well as reductions in DCIS. In addition, IL-12 bursts by dendritic cells is also associated with increased tumor-killing capacity of CD8 cells, which may further increase the response against HER-2 positive DCIS tissue [28].

There are several molecules and antibodies that interfere with the mechanism of HER2 signaling. One of these is lapatinib, which is used in combination with other HER2 targeted therapy agents such as transtuzumab, a monoclonal antibody against HER2. In 2019, Showalter et al. investigated the effects of Th1 cytokines on the response of HER-expressing breast cancers to lapatinib. They reported that lapatinib potentiates the Th1 cytokines- induced apoptosis of HER2 positive breast cancer. In this in vitro study, the group demonstrated that the presence of Th1 cytokines decrease the amount of lapatinib necessary to achieve the same level of tumor cell killing by several folds. Interestingly, increased Th1 cytokines are also associated with enhanced drug sensitivity to lapatinib in normally lapatinib-resistant tissues [42].

In conclusion, the implementation of immunological treatment was found to be promising.

### **Current Classification and Grading Systems of DCIS**

Lack of reproducibility of the grading to appropriately predict the prognosis of DCIS lesions and predict their invasive potential is by far the most important problem in the treatment decisions of DCIS. Increased detectability of these lesions by Mammography screening came with a need for more detailed classification systems and more categories [43]. The gray border between a diagnosis of atypical ductal hyperplasia and DCIS adds further complexity.

Classification of DCIS used to be done on the basis of the architectural type of the lesions- micropapillary, cribriform, solid and papillary. Nowadays, the routine classification usually integrates the nuclear grade, cell polarization and necrosis. Still, microscopic grading has low reproducibility and high inter-observer variability.

Furthermore, there is currently no known histopathologic feature that accurately predicts the risk for progression into IBC, nor the recurrence possibility after receiving treatment [5, 40].



Current Routine biomarker tests are applied only for ER and PR; as ER positivity and, to a lesser extent, PR positivity yields higher benefit upon tamoxifen treatment [10].

### **To Treat or Not to Treat?**

All in all, years of attempts to provide prognostic factors for DCIS have achieved fewer results than expected.

Results of these studies are as follows [43]:

1. The dogma of linear progression dictating that normal epithelium progress into Usual Ductal Hyperplasia, Atypical Ductal Hyperplasia and then to DCIS or ADH is not always true.
2. FEA, ADH and LGDCIS share a lot of morphologic, molecular and immunohistochemical features.
3. In most cases, low grade DCIS and high-grade DCIS are genetically two distinct disorders leading to different forms of IBC.

The aforementioned results have further complicated the dilemma of treatment based on histopathological grading, but also drew attention to the other side of the coin: biomarkers.

### **An Endless Search: Biomarkers**

#### **Genetic Markers**

In the last 20 years, as genetic approaches have been increasingly adapted into clinical practice after the completion of HGP, along with the insufficiency of the microscopic grading when it comes to treatment decision, studies aiming to identify the genetic markers of progression from DCIS into invasive breast cancer would have been thought to lead to promising results.

Yet, mutations that had been identified in the DCIS with IBC component were not significantly different than that of pure DCIS, suggesting that the mutations responsible for the invasive potential of the DCIS have already been gained in the pre-malignant phase of the lesion. Contributing evidence to this hypothesis comes from the study of Volinia et al, which has identified 66 differentially expressed genes between normal and DCIS, out of which only 9 present conserved differential expression between DCIS and Invasive Breast Carcinoma of No Special Type [8]. Furthermore, the genetic mutations that were identified in low grade DCIS were more similar to ADH than to high grade DCIS, suggesting the two might even arise from different mutations and give rise to different types of IBC, rather than low grade DCIS progressing into high grade DCIS [10]. Several studies have demonstrated that low grade lesions have a loss of 16q and 17p, while high grade lesions usually have more complex genomic alterations such as amplifications of 17q12 and 11q13, and loss of 13q [9].

#### **Micro RNAs**

miRNAs regulate gene expression by associating with RNA transcripts and either targeting them for degradation or inhibiting their translation into peptides [8]. Global downregulation of miRNA is associated with many tumor types [25].



Identification of the miRNA signatures that are associated with progression of DCIS to BC might be of help to identify the silencing of the genes that are associated in the progression of the DCIS to IBC [25].

The cause behind the global downregulation of miRNA might be multifactorial and seems to involve the downregulation of miRNA processing machinery such as DICER and Drosha [44].

Hannafon et al. identified that differentially expressed miRNAs have influences on hormone signaling, cell-cell adhesion, EMT, TGF- $\beta$  signaling, maintenance of cancer stem cells [44]. In total, 29 miRNAs have been found to be altered in between histologically normal mammary tissue and DCIS. One of the important examples of under expressed miRNAs was miR-125b, which is inversely correlated with the expression of Nuclear Receptor Interacting Protein-1, Mediator of Cell motility 1 (MEMO-1). MEMO-1, for example which is upregulated in DCIS due to the under expression of miR-125b, is required for ErbB2/Her2-driven cell motility. In DCIS compared to Histologically Normal Epithelium two of the significantly upregulated miRNAs were miR-182 and miR-183. miR-182 expression is inversely correlated with Docking protein-4 expression, chromobox homolog 7 (CBX7) expression and N-myristoyltransferase 2 expression. DOK4 acts as an inhibitor of Tyrosine kinase and can activate MAPK [44].

Furthermore, knockdown of miR-182 in mice increased the expression of E-cadherin through upregulated expressions of CBX7. miR-183 expression is inversely correlated with Early Growth Response-1 (EGR1) expression, a protein that activates the expression of p53/TP53 as well as TGFB1 and in turn helps prevent tumor formation [44].

Although this study has not compared the miRNA expression between DCIS and Invasive Breast Carcinoma, the sole comparison between the histologically normal breast epithelium and DCIS underlined the fact that some of the important experimentally proven microRNA expression changes occur early in progression from normal breast epithelium to DCIS [44].

Volinia et al have identified 66 differentially expressed micro RNAs between normal and DCIS. Out of these only 9 present conserved differential expression between DCIS and Invasive Breast Carcinoma of No Special Type. The activated ones were: miR-210, let-7d, miR-181a, miR-221; yet miR-10b, miR-126, miR-218, miR-335-5p, and miR-143 were repressed [45]. An important finding was that, let-7d, miR-210, miR-221 expression levels were found to be downregulated in DCIS and upregulated in Invasive Breast Carcinoma of No Special Type.

The group has also identified the prognostic miRNA signatures; miR-127-3p, miR-210, miR-185, miR-143, and let-7b are associated with time to metastasis whereas miR-210, miR-21, miR-221, and miR-652 correlated with the overall survival [45].

Even though there were a handful of genes that are involved in overall survival signature, invasiveness microsignature was only found to be correlated with miR-210 expression. The ability of hypoxia to induce miR-210 expression and consequently regulate the genes involved in tumor initiation is identified as a poor prognostic factor in means of overall survival and time to metastasis. miR-210 expression in progression from DCIS to IBC was found to be correlated with downregulated expressions of BRCA1, FANCD, FANCF, PP2CA, PARP1, NLK, CDH1 (E-Cadherin) and EHMT1 [45].

A recent study aiming to find a differential signature between the exosomal levels of miRNAs, of 47 miRNAs 1 of them, miR-93 was found to be significantly upregulated in DCIS patients compared to healthy women, yet there seemed to be no significant difference between the primary breast cancer and DCIS [46]. As a result it seems obvious that the problem with risk stratification using miRNAs is that most of the miRNA regulation changes occur in the early progression of normal epithelium to DCIS, with relatively few change occurring in the progression from DCIS to IBC [9,25].

### **Long Noncoding RNAs**

One of the earliest findings about the differential lnc-RNA expression of DCIS, came from Iacoangeli et al. [47], namely BC200, seeming to be a prognostic predictor of DCIS as pointed out in the review by Devaux and Herschkowitz [25].

Rao et al.[48], conducted RNA sequencing analysis in Invasive Breast Carcinoma of No Special Type, DCIS and normal tissue samples, with further lncRNA-mRNA co expression network analysis. Their samples included histologically confirmed Invasive Breast Carcinoma of No Special Type (NST) and DCIS, with apparently normal tissues that have been taken from the patients undergoing surgery for non-cancer related causes. They reported 375 lncRNA differentially expressed in Invasive ductal carcinoma of no special type compared with paired and apparent normal samples and 69 differentially expressed lncRNAs in DCIS compared to apparent normal samples as well as 12 differentially expressed lncRNAs between DCIS and Invasive Breast Carcinoma of No Special Type (NST) [48].

Moreover, they report that LINC01614, RP11-490M8.1 and CTB-92J24.3 are the novel lncRNAs reported in the study that had not been associated with breast cancer earlier. They have also concluded that overexpression of WDFY3-AS2 and down regulation of RP11-161M6.2 could be used as early bad prognostic markers that are associated with adverse outcomes [48].

### **Epigenetic Modifications**

The methylation of CpG islands can activate or silence the expression of genes, with dense methylation repressing the transcription by interfering with transcription factor binding.

Increase in global methylation is observed in transition from normal breast epithelium to DCIS yet there is no significant difference between the global methylation of DCIS and IDC samples, except two, CACNA1A and MGMT both of which demonstrate an increase in methylation in progression from DCIS to IDC [9].

In another study, of 641 progression associated CpG loci, regions belonging to 72 genes were found to be differentially methylated. The regions that are enriched belonged to polycomb group gene targets and homeobox genes [22].

A study correlating the methylation with gene expression on normal breast-DCIS-Invasive Breast Carcinoma of No Special Type (NST) axis with integration of patient survival, identified four genes demonstrating increased methylation during progression into Invasive Breast Carcinoma of No Special Type (NST) from normal breast epithelium. These genes were CPA1, CUL7, LRRTM2, POU2AF1 [22,49]. Yet the group also conducted a survival analysis to identify the CpG islands, methylation of which correlated with prognosis. Identification of 18 CpGs, demonstrated association with the expression level of 26 genes none of which were consistently increased methylation from the normal epithelium to Invasive Breast Carcinoma of No Special Type [49].

The change of epigenetically silenced genes throughout the progression from normal epithelium to Invasive Breast Carcinoma is parallel with studies involving miRNA expression, which were previously mentioned, provides a challenge to determine the prognostic risk factors.

Even though most of the methylation changes occur in the earlier stages of Invasive Breast Carcinoma of No Special Type (NST) development, the identification of several genes that are differentially methylated in the DCIS-Invasive Breast Carcinoma of No Special Type (NST) axis are of prognostic value, especially those that are involving the Homeobox(HOX) genes and Polycomb genes.

HOX genes are developmental genes that take part in organogenesis. They upregulate or downregulate several other genes. Their upregulation or downregulation has been implicated in many cancers [50].

Antagonizing that, Polycomb group proteins are special proteins that silence the HOX gene expression that take active role in pluripotent stem cells of the embryo. Their mechanism of action is through induction of covalent post-translational histone modifications which are associated with transcriptional silencing [51].

Recently, the deregulation of PcG genes' role in carcinogenesis has been acknowledged. This deregulation often leads to inappropriate activation of several developmental pathways and inhibiting apoptosis. Furthermore, the absent or aberrant expression of PcG genes have been thought to increase the number of Cancer Stem Cells (CSC) [50].

The enhancement of methylation of Polycomb Repressor Complex (PRC)-2 gene targets as shown in progression of DCIS to Invasive Breast Carcinoma of No Special Type (NST) underlines that epigenetic silencing takes an active role in progression of DCIS. Currently, there is insufficient clinical data to determine the prognostic significance of these modifications.

### **Time to Change Perspective: Microenvironment**

As genetic and epigenetic data of the tumor cells itself have not provided enough information for pathogenesis of invasion and for risk stratification, the new trend is to focus on the surroundings of the tumor.

The microenvironment consists of the surroundings of the tumor cells, namely: immune cells, fibroblasts, and myoepithelial cells.

### **Myoepithelial Cells**

Myoepithelial cells are the surrounding cells of the tumor cells. Their main functions are the deposition of the extracellular matrix and the production of basement membrane proteins such as fibronectin, collagen and laminin [52]. Myoepithelial cells demonstrate significant differences in gene expression between normal and DCIS surroundings, suggesting a pivotal role in the progression [52].

Disruption of Myoepithelial cells is currently regarded as the main indicator of invasion, yet the specificity of the sole use of this evaluation is disputed, as benign conditions of the breast might be associated with absent MEC layer. MEC layer may be absent in locally infiltrative lesions such as microglandular adenosis and infiltrating epitheliosis or in non-infiltrating conditions such as apocrine lesions [52].

Anti-angiogenic, anti-proliferative and anti-invasive properties of the MEC is partly due to their expression of TIMP1, PAI-1 [53], inhibitors of cysteine cathepsins such as stefin-A and stefin-B and expression of MMP-8 to regulate the extracellular matrix [54].

However, myoepithelial cells are also implicated in invasion by their increase in CXCL12 and CXCL14 expression, which are promoters of tumor cell migration and it is also implicated in the overexpression of  $\alpha v\beta 6$  integrin which leads to upregulation of MMP9 via TGF- $\beta$  pathway [54].

Collectively, these results point to the fact that Myoepithelial cells are implicated in both tumor suppression and tumor progression, depending on the progression- making biomarkers a good candidate for progression [8,54].

### **New Findings About Myoepithelial Cells**

#### [Cortisol: Promoter of Invasion? \[55\]](#)

A recent study by Zubeldia et al. has concluded that glucocorticoids fostered the transition from DCIS to IBC by inducing MEC apoptosis and reducing laminin levels in vivo mice models and on isolated human mammary epithelial cells in vitro. The group also has found that all cell lines express glucocorticoid receptors, yet the highest expression was seen in myoepithelial cells.

In MEC, treatment with cortisol caused the arrest of cell cycle in the G1 and G2 stages.

The groups limitation is that their inability to test this effect on in vivo human cohorts, as the primary glucocorticoid of mice (Corticosterone) and humans (Cortisol) differ. Nevertheless, they reported that corticosterone promoted invasiveness through MEC apoptosis just like cortisol did in vitro human cell samples.

This study suggests that the stress levels and concomitant administration of cortisol can be a prognostic factor in progression from DCIS into IBC, as well as the utilization of Glucocorticoid antagonists possibly providing therapeutic benefits.

#### [Stefin A- Suppressor of early Breast Cancer Invasion \[56\]](#)

Cathepsins are a group of proteases that are normally found in lysosomes but are also commonly detected on the surface of or secreted from cancer cells. They are implicated in degradation of ECM proteins and promoting epithelial to mesenchymal transition. Stefin A and Stefin B are the inhibitors of cathepsins.

Duivenvoorden et al. examined the degree of Stefin A expression on formalin fixed paraffin embedded normal breast sections and primary breast carcinoma samples.

Stefin A expression was found to be highly abundant in normal MEC, with progressive loss through higher grades. Yet, Stefin A levels increase in neoplastic cells with an increase in tumor grade. Loss of Stefin A expression in MEC associated with microinvasion could therefore be used as a prognostic marker.

#### **Stromal Cells in DCIS**

##### [Fibroblasts](#)

Fibroblasts are essential components of mammary gland development as they produce collagen and fibronectin which promote epithelial growth and differentiation [54]. By producing several proteases such as MMP-3 and releasing several growth factors, the fibroblasts are also implicated in stromal reorganization.

A study conducted by Morgan et al. Pointed out to the paucity of in vitro cell culture studies taking the effects of microenvironmental cells such as fibroblasts, into account. They also reported that the results of in vivo mice studies should be approached with caution as murine mammary epithelium expresses ER-alpha while human cells do not [57]. They concluded that in the presence of fibroblasts, MCF7 cell line had increased estradiol response, reduced apoptosis.

Another study conducted by Brechbuhl et al. concluded that CD146 expression in Cancer associated fibroblasts (CAF) increased expression of Estrogen receptor and hence, increased the response to tamoxifen treatment. Therefore, CD146 negative fibroblasts were associated with a worse response to tamoxifen [58].

The density of ECM is regulated by fibroblasts through the deposition of collagen, and the changes of ECM properties are thought to have prognostic importance [59]. This hypothesis is supported by the observation of Syndecan-1 expression among stromal fibroblasts found to be correlated with increased vessel density. Conklin et al. conducted a study comparing the collagen alignment and syndecan-1 expression in fibroblasts and collagen alignment around the DCIS compared to normal ductular tissue, in order to evaluate the association of the collagen alignment with the recurrence risk. They concluded that Collagen alignment scores did not predict recurrence. However, the Tumor Associated Collagen Signatures (TCAS) pattern seemed to be more common in DCIS lesions with markers of poor prognosis, including ER, PR, and HER2 status and comedonecrosis [59].

The difference between the cancer associated fibroblasts and normal fibroblasts is that the CAF have increased expression of TGF- $\beta$ 1 and bFGF, important fibroblastic markers that regulate the expression of alpha-SMA [8]. Another evidence for the increase in TGF- $\beta$ 1 signaling in CAF comes from a study conducted in 2010, which demonstrated that the breast cancer microenvironment causes the fibroblasts to downregulate caveolin-1 expression and increase TGF- $\beta$  signaling [60]. These findings have been shown to be held true by the demonstration of loss of stromal expression of Caveolin-1 and increase in stromal expression of MCT4 are associated with DCIS to IBC progression [61].

#### **Crosstalk in the Microenvironment: Who is the boss?**

A study conducted in 2017 by Sameni et al. demonstrated the interplay between Cancer associated fibroblasts (CAF) and Myoepithelial cells (MEC) [53]. They demonstrated that the normal human breast MECs suppressed the previously mentioned proliferative effects of CAFs by inhibiting the IL-6 signaling pathways. Furthermore, MECs also increased the expression of laminin-332 along the DCIS tissue, which is a molecule of basement membrane that is downregulated in several invasive carcinomas such as breast ductal carcinoma, colorectal carcinoma and bladder carcinoma [53].

The group also demonstrated that under co-culture conditions of DCIS, CAF and MECs, DCIS cells and CAF cells migrate towards each other. Yet, approximately 21 days later, the invasive phenotype, which is characterized by the degradation of collagen I and IV, becomes attenuated [54]. This effect is thought to be mainly the result of MECs secretion of Plasminogen Activator Inhibitor (PAI-1), which inhibits uPA, a serine protease that promotes tumorigenesis [53].

The ability of MECs to inhibit the effects of CAF and neoplastic cell crosstalk underlines the importance of MECs in the prevention of metastasis along with demonstrating the presence of dominant cells in the microenvironment that regulate communication.

The revelation of the interactions between the microenvironment and DCIS cells, such as this one, suggests that further research might be conducted on the interference with the interaction pathways of the tumor cells and the microenvironment.

### **High Legumain Expression as a Prognostic Factor [62]**

Legumain, a proteolytic enzyme that is implicated in invasive breast cancer, activates zymogen gelatinase A which then proceeds to degradation of the matrix. The overexpression of the enzyme has a proven association with worse prognosis in several different cancers, including the IBC.

Assessment of legumain expression in malignant epithelial cells and in the fibroblast cells surrounding the DCIS, demonstrated that high legumain expression in DCIS is associated with characteristics of poor prognosis such as high nuclear grade, comedo necrosis, ER and PR negativity, HER-2 positivity, high proliferative index and dense Tumor Infiltrating Lymphocytes. The expression of legumain in TEC cells showed significant increase from 25% in pure DCIS, to 30% in DCIS component mixed component DCIS cases and to 60% in the invasive component of mixed component DCIS cases. Whereas in the surrounding stroma, legumain expression levels range from 5% in pure DCIS, to 70% in the DCIS component of the mixed case and 90% in the invasive component of the lesion.

The fact that legumain is activated under low pH circumstances explains its high expression in the presence of comedo-type necrosis.

Another function of legumain besides its proteolytic effects, is promotion of proliferation probably through increased Calcium influx to the cells.

These findings suggest that legumain expression and its progressive increase might be of help to determine the prognosis. More research with computational analysis to incorporate Legumain expression to the currently known prognostic factors might be of help.

### **Thioredoxine Expression in Fibroblasts in Prediction of the Risk of Invasion [63]**

Thioredoxine is an antioxidant which is regulated by Thioredoxine interacting protein. Loss of TXNIP expression found to be associated with development of tumors. In the light of the previous studies demonstrating loss of TXNIP expression in invasive breast cancer Miligy et al, assessed the TXNIP expression in DCIS tissues as a possible predictor of DCIS progression into IBC. Their findings suggest that DCIS with a mixed invasive component has significantly lower expression of TXNIP compared to pure DCIS. High cytoplasmic expression of TXNIP was also found to be associated with good prognostic factors. Yet, in a small fraction of mixed DCIS cases the assessment of TXNIP has provided conflicting results such as low expression of TXNIP in normal component and higher expression in invasive components. All in all, TXNIP expression seems to be a promising predictor of recurrence and invasion risk of DCIS. Further studies combining the expression of TXNIP with other potential prognostic markers might be beneficial.

### **Immune Cells**

Immune cells depending on the ratio of their subgroups, may act either as a friend or foe. CD4+ Th1 cells are associated with secretion of IFN-gamma which acts as a tumor suppressor, whereas the abundance of Th2 subtypes of CD4+ cells is associated with pro-tumorigenic state [54].



Several studies assessed the immune milieu of DCIS and IBC. Increased neutrophils and decreased CD8/CD4 ratio is found in DCIS and Invasive Breast Carcinoma of No Special Type (NST) compared to normal epithelium [64]. Furthermore, the proportion of Gamma Delta ( $\gamma\delta$ ) T Cells was higher in Invasive Breast Carcinoma of No Special Type (NST) compared to normal epithelium [64]. The observed increase in CD8+ cells in HER2+ Invasive Breast Carcinoma of No Special Type (NST) and DCIS might be due to memory-activation and clonal expansion [64].

Increased Foxp3 levels in high grade DCIS and IBC is thought to be associated with the establishment of an immunosuppressive environment by increasing the Regulatory T cell numbers, which favors metastasis and invasion [54,55].

Bates et al have demonstrated that high CD4+/FOXP3 regulatory T cell infiltrate associated with poor prognosis. Yet, in other studies, this effect was demonstrated only for ER+ invasive breast cancer. For ER(-) breast cancer or HER2(+) breast cancer (regardless of ER positivity) CD4+/FOXP3 regulatory cell infiltrate associated with improved prognosis [65].

Higher grades of DCIS that are associated with poor prognostic factors demonstrate higher levels of tumor infiltrating lymphocytes with heterogenous distribution among the tumor cells [64].

The number of TILs were identified as an independent prognostic variable in DCIS prognosis. Dense TIL infiltration has been shown to be statistically associated with shorter recurrence free intervals and higher nuclear grades. Moreover, dense TIL infiltrates were seen more in estrogen receptor negative cases (72%) than with ER(+) cases (36%). It is important to note that dense TIL is defined by more than 20 cells per DCIS duct in the lesion. The association between dense infiltrating lymphocytes and tumor progression might be due to the fact that tumor cells produce some proteins to evade the host immune system such as PDL-1 [66]. All in all, evidence demonstrates that reporting the density of TILs in the DCIS lesion may contribute to the prediction of prognosis.

Collectively, these findings suggest that not only quantitative but also qualitative analysis of the subtypes of different cells of the immune microenvironment is required. When considered in combination with the dominant chemokines and integrated into other prognostic predictors immune microenvironment represent an undeniable role in progression of DCIS to Invasive Breast Carcinoma of No Special Type (NST) and hence in prediction of prognosis.

### **Creating the Perfect Environment to Better Understand the Microenvironment**

A research group in 2018, sought to find a way to better simulate the mammary duct environment in which the DCIS cells are «trapped in» and developed the microfluidic model that provided better control over the microenvironment and less ethical issues than animal models. The model also provides a chance to assess the effects of hypoxia and lack of glucose diffusion to the DCIS cells [67].

The metabolic analyses conducted on the microfluid model have shown [67] decreased levels of glucose and pyruvate while lactate levels increase, possibly decreasing the pH, creating a favorable environment for legumain expression as discussed before. Decreased Glutathione and Choline levels were also reported. Pathways associated with important functions such as rapid proliferation, glucose metabolism, amino acid metabolism, nucleotide metabolism, ketone body metabolism were found to be altered between normal mammary duct model and DCIS model [67].

The hypoxic and nutrient depleted conditions that were found to be associated with DCIS, were also implicated in activation of matrix metalloproteinase-14 in Tumor Initiating Breast Cancer Cells [68], a known promoter of invasion [8]. The most important observation that was made on the model is the initiation of invasion of the DCIS cells to the surrounding matrix within few days. To assess the vulnerability of the hypoxic cells to the chemotherapy, Tirapazamine had been reported to be caused the death of the cells in the innermost part of the lumen.

Collectively, with these results on microenvironment and molecular interactions, also considering the failure of numerous attempts to provide significant genomic changes between DCIS and Invasive Breast Carcinoma of No Special Type (NST) it could be said that further research could be done on the microenvironment regarding the interactions of MECs with the microenvironment. The model might provide an easier and more realistic way to test the effects of novel molecules on the interaction of tumor and its microenvironment.

### **PPAR $\gamma$ as a Therapeutic Target**

With further elucidation of molecular mechanisms new therapeutic targets are identified. One of these targeted approaches, efatutazone (a PPAR-gamma agonist) treatment have proved to be effective in suppressing the progression of human DCIS cells into IBC *in vivo* [69]. The researchers have found that efatutazone decreased tumoral environment formation and increased the differentiation of tumor initiating cells. They hypothesize that the effect might be due to the loss of Akt activation, which is a serine-threonine kinase that regulates proliferation, survival and apoptosis [70]. Even though the efatutazone failed to eradicate DCIS lesion completely, the molecule might be more effective as a component of a combination to endocrine therapy.

### **Deep Learning: Can it help us?**

Deep Learning algorithms have been increasingly used for data classification and prediction. Bejnordi et al. sought to develop a machine learning algorithm that classifies the tumor associated stroma. Using a convoluted neural network approach, the group has trained the algorithm based on a total of 2387 H&E malignant and benign biopsies. As they point out, currently there is no criteria for the features of tumor associated stroma that might help to classify the DCIS and differentiate it from benign conditions [71,72].

The method of testing their algorithm was randomized assessment of the DCIS cases as training and control. As a result, it yielded an area under Receiver Operator Characteristic Curve (ROC: sensitivity plotted against 1-specificity) of 0.962, implying a high accuracy discrimination. The lesions that were false positively associated with cancers are 10 sclerosing adenosis, two atypical hyperplasia and 11 non-proliferative benign breast disease [71,72].

A study conducted in 2017 was the first of its kind to assess the accuracy of computer enhanced mammographic images at identification of occult invasive disease in DCIS compared with radiologist assessments [73]. The group used 99 patients from 2009-2014, 25 of whom had invasive cancer at the time of definitive surgery [73]. 25 out of 113 features were better than chance at assessment of occult invasive disease with a p value of 0.05 [73]. Overall, computer enhanced images were not inferior to the radiologists assessments but not superior either [73].

The group has developed the idea further with integration of convolutional neural networks as a deep learning algorithm, as a method of unsupervised learning, compared their results with the previous validated results of the computer enhanced imaging [74]. They concluded slightly better yet no significant assessment of occult invasive disease with deep learning was found compared with the computer enhanced mammographic imaging [74].

Even though these two studies did not improve the ability to assess the occult invasion, their importance lies in their demonstration that the failure to detect occult invasive disease was not due to bias or inter-observer variability. Current mammography techniques can achieve Area Under the Curve for Receiver Operating Characteristic values around 0.7 [74].

Bioinformatic approaches such as Convolved Neural Networks have been used for the assessment of expression of myoepithelial cell markers such as calponin and alpha-SMA and association of which with progression of DCIS into Invasive Breast Carcinoma of No Special Type (NST), by possible prediction of myoepithelium discontinuity [75].

Overall, attempts to integrate deep learning into imaging and histopathology have generally yielded non-inferior results to the experienced radiologist or pathologist opinion. Despite of their failed attempt to show better predictability, they might have provided an important insight to the current problem in the prediction of DCIS progression; the current radiologic and histopathological assessment techniques is away from being sufficient in prediction even in absence of interobserver variabilities.

### **Few Words on Communication**

In 2019, new national consensus recommendations for Patient Centered Care (PCC) of DCIS cases have been published based on surveys conducted on women, surgeons, radiation oncologists, medical oncologists, radiologists, nurses and patient navigators [76].

Common recommendations by both clinicians and the patients were on the PCC domains of [76]:

- Fostering patient physician relationship
- Exchanging information
- Responding to emotion
- Managing uncertainty
- Making decisions

- Enabling patient self-management

As the results of this study concluded, when DCIS is referred to as non-invasive breast cancer rather than a “breast lesion” or “abnormal cells”, significantly more women choose surgery, more women preferred mastectomy or bilateral mastectomy and the clinicians tend to overtreat and over diagnose as it “sounds better than doing nothing”. As these results suggest, changing the label for DCIS has been suggested to be helpful in avoiding unnecessary treatment and reducing anxiety [76]. While this approach may decrease the number of overtreated cases, the suggested substitutions for definition of DCIS are less informative than acknowledging the undeniable presence of malignant cells contained within a basement membrane. The proposed change in definition may neglect the inherent tendency of DCIS to progress into Invasive Breast Carcinoma.

Overall, there is a global dissatisfaction among women regarding the patient centered care with DCIS and reports of clinicians underlining the challenges of discussing DCIS with the patients emphasizes the lack of a good communication and informed decision bridge between the patients and clinicians [76].

## **Conclusion**

Ductal Carcinoma in Situ is an entity that bears a potential to evolve into an invasive disease. The problem of having an increasing number of diagnosed pre-invasive cases without seeing a decreasing prevalence of invasive breast cancer prompts a reevaluation of the diagnostic approach to the lesion in terms of treatment efficacy and necessity for every DCIS case.

The most common management method of DCIS lesions is breast conserving surgery and radiotherapy combination. Considering that not all DCIS cases evolve into invasive disease, this approach of treatment may not be suitable for all lesions, given the side effects and economic burden of the treatment.

For risk stratification, the implementation of scoring systems that are based on differential expression and mutation analysis of several genes that are involved in disease progression have provided valuable insights to the risk stratification. Regardless, there remains a gap between known and unknown genetic leading to invasive disease that needs to be filled.

Immune microenvironment and targeted therapy approach to the tumor surroundings seem to be hopeful. The correlation between cortisol concentration and tumor progression in mice is an important clue of the role of immunity in the progression. Combined with the newly discovered changes in the gene expression profile of myoepithelial cells, fibroblasts and immune cells the DCIS microenvironment gains more importance and provides opportunities to target the cell to cell interactions both for treatment and diagnostic purposes. In conjunction with this endeavor new methods that are currently in development to mimic the tumor microenvironment in humans with a higher accuracy might eliminate the unreliability in the results of some studies due to interspecies differences between the humans and mice models.

Deep learning and other sophisticated machine learning approaches might be helpful to eliminate the interobserver variability in some areas and classified the malignancy potential according to the features that are deemed as eligible classifiers according to the test set.

All in all, the approach to diagnosed DCIS lesions require a multidimensional molecular approach correlated with the histopathology and radiology as well as patient history. Patient-tailored approach to each individual case is of utmost importance to prevent unnecessary interventions and to decrease the progression rates to invasive breast carcinoma.

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