Review Article

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# Role of p63 in Breast Carcinoma - A Review

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## Introduction

Invasive breast carcinoma accounts for 22% of all female cancers worldwide, thereby making it the most common carcinoma in women. [1] Adenocarcinomas, considered to be derived from cells of the terminal ductal lobular units, are reported to be the most common among these tumors. A wide range of morphological phenotypes of breast carcinomas are known, which have particular prognostic significance. [2]

The morphologic distinction between benign and malignant (in situ and invasive) diseases of the breast can be problematic, particularly in the setting of core needle biopsies. Although morphology alone can diagnose majority of breast biopsies and excisions, considerable disagreement for interpretation of difficult lesions based on histology has been documented. [3] The functional glandular and ductal elements of the breast are embedded in fibro fatty tissue, which forms most of the mammary gland. The relative proportion of fat and collagenous stroma varies greatly among individuals and with age and is influenced by physiologic and hormonal factors. Cells that form the duct epithelium are of two types. Majority are columnar or cuboidal cells lining the lumen. They have cytoplasm endowed with abundant organelles that are involved in secretion. Myoepithelial cells lie between the epithelial layer and the basal lamina, where they form a network of slender processes investing the overlying epithelial cells. Spindle-shaped ductal myoepithelial cells lie parallel to the long axis of the duct and form a continuous layer. Contraction of myoepithelial cells in lobules and around ducts contributes to the flow of milk during lactation. The histologic appearance and immunoreactivity of myoepithelial cells are variable, especially in pathologic conditions, and depend on the degree to which the myoid or epithelial phenotype is accentuated in a particular situation.

The retention of myoepithelial cells (MCs) in most benign lesions and loss in malignancy, aid in diagnosis. [4] Invasive carcinoma arising from Ductal carcinoma in situ is diagnosed once a small cluster of atypical cells breaches the myoepithelial layer of the ductal lobular unit. [5]

Because the identification of myoepithelial cells may be difficult in routinely stained sections, several immunocytochemical markers have been introduced in the daily practice to highlight these cells. Owing to the epithelial and contractile nature of myoepithelial cell, [6] a number of immunocytochemical markers related to smooth muscle-related antigens are available globally. [7]

p63, a recently identified member of the *p53* gene family, is located on chromosome 3 q27. p63 is necessary to maintain a stem cell epithelial population, and is expressed in the basal cells of several epithelia. [8] It is utilized primarily as a marker of squamous, myoepithelial (MEC), prostate basal and urothelial cells [9] in current surgical pathology practice. Although its biologic functions are postulated to include maintenance of epithelium-specific stem cells, diagnostic applications of its expression include the identification of poorly differentiated carcinomas as squamous or transitional cells. [10] Recently, p63 and other cell markers have been reported in matrix producing and metaplastic carcinomas of the breast, suggesting that these tumors share a myoepithelial cell differentiation. [11,12] p63 is documented to be specific for metaplastic carcinomas with spindle and/or squamous areas. This nuclear marker has a strong and diffuse staining pattern that is easily detectable, hence used in the diagnostic workup of challenging spindle cell tumors of the breast. [13]

## Isoforms of P63

*TP53* (which encodes p53) is a well-known tumor suppressor gene whose functions are well elucidated. However, much research needs to be done regarding the tumor suppressive functions of p63 and p73. [14] It has been widely accepted now that there are certain isoforms of p63 and p73 that have tumor suppressive roles. [15] [16]

There are basically two categories of isoforms encoded by TP63. One is the acidic transactivation domain, known as transactivation (TA) isoforms and the other is the ΔN isoforms which lack the transactivation domain. [17] p53 can induce apoptosis and cell cycle arrest in response to DNA damage by activating BCL-2-associated Xprotein (*BAX*), BCL-2 binding component 3 (*BBC3*), *PERP* and cyclin-dependent kinase inhibitor 1A (*CDKN1A*; which encodes

**Table -1**

| Year | Authors | Study Conducted |
|---|---|---|
| 2013 | Xiaohua Su, Deepavali Chakravarti [17] | p63 plays a crucial role in the suppression of tumorigenesis and metastasis |
| 2013 | Shekhar et al [31] | Comedo-DCIS co expressing p63/Her2neu - a precursor lesion for basal-like breast carcinoma: |
| 2014 | Ivana et al [27] | p63 transcriptionally regulates the expression of matrix metalloproteinase |
| 2014 | Sang Kyum et al [34] | To note the expression of p40 (ΔNp63) in breast disease and its correlation with p63 immunohistochemistry |
| 2015 | Rohilla et al [22] | Myoepithelial cells in DCIS –phenotypically and functionally different from normal counterparts |
| 2015 | Russell et al [21] | Progressive loss of myoepithelial differentiation markers like p6, calponin and alpha smooth muscle actin in DCIS |
| | Ginter et al [32] | The minimal carcinoma triple stain is superior to commercially available multiplex immunohistochemical stains: breast triple stain and LC/DC breast cocktail. |
| 2015 | Yang et al [33] | The significance of combined CK5/6 and p63 immunohistochemistry in predicting the risks of subsequent carcinoma development in intraductal papilloma of the breast |
| 2016 | Lodilinsky et al [29] | p63/MT1-MMP axis is required for in situ to invasive transition in basal-like breast cancer |

p21).[17] TAp63 isoform is a transactivator of the above mentioned p53 target genes like MDM2 and BAX gene and is therefore functionally and structurally homologous to p53.However numerous in vitro experiments have provided evidence that the ΔN isoforms of *TP63* have the ability to antagonize tumor suppressors like p53,TAp63and TAp73,inhibit apoptosis and hence have oncogenic potential.[17]

**Role of p63 in Progression of DCIS to IDC**

DCIS (ductal carcinoma in situ), characterised by proliferation of tumor cells in the tubulolobular unit, considered a precursor of IDC (invasive ductal carcinoma). [18] Transition from normal to DCIS involves many tumor cell gene expression changes as proven by genomic data analyses with only a few gene expression changes occurring in the transition from in situ to invasive ductal carcinoma. [19] Myoepithelial cells have a tumor suppressive function by secreting protease inhibitors and producing tumor suppressive proteins [18]. Transition of ductal carcinoma in situ (DCIS) to invasive ductal carcinoma involves the loss of this tumor suppressive function of myoepithelial cells. [20]

Russell et al have reported a progressive loss of myoepithelial cell differentiation markers like p63, calponin and alpha smooth muscle actin in ductal carcinoma in situ (DCIS) involved ducts in animal model. [21] Three markers such as alpha smooth muscle actin, Calponin and p 63 were used to study myoepithelial expression. Uniform myoepithelial expression of these markers was found in non-tumor bearing murine ducts whereas DCIS involved ducts showed variable expression of these myoepithelial markers. Murine ducts showing DCIS with micro invasion showed an additional loss of these markers.This study concluded that myoepithelial cell layer was compromised before DCIS progressed to overt carcinoma. The earliest marker to be lost was p63 compared to other two. [21]

In a study reported by Rohilla et al, only 3.3 % of DCIS associated myoepithelial cells showed p 63 expression compared to 70% of normal myoepithelial cells showing expression. This study concludes that DCIS associated myoepithelial cells were phenotypically different from their normal counterparts. [22]

**P63 –activator of matrix metallopeptidase**

Human matrix Metallopeptidase 13 (MMP13) is a proteolytic enzyme that shows high activity towards fibrillary collagens. [23] MMP 13 has many physiological functions like skeletal development [24] and proper functioning of synovial membranes. [25] Human diseases where excessive collagen degradation is involved, MMPs are generally considered important targets. [26]

Different type of cancers osteoarthritis and rheumatoid arthritis are the pathological states associated with MMP13 up regulation. [27] MMP13 contributes to the high invasion capacity of cancer cells by playing a role in degrading the basement membrane which is thought to be an early event in tumour metastasis. [28] Ivana et al in their murine experimental model identified MMP13 to be a direct transcriptional target of p63. [27] p63 can transcriptionally control the expression of MMP13 by binding its promoter gene and promoting its function. [27]

## P63/MT1-MMP AXIS IN BREAST CARCINOMA

Murine experimental models have proven that the capacity of DCIS tumor xenografts to progress into infiltrating lesions is impaired by silencing MT1-MMp expression. [29]

Lodilinsky et al have reported a positive association between N isoform of p63 and MT1 –MMP particularly in hormone negative epithelial cell lines as supported by RT-PCR analysis of Np63and MT1-MMP in a panel of breast cell lines. Increased expression of N isoform of p63 led to increased expression of MT1-MMP expression while silencing of ΔNp63 led to diminished MT1-MMP Protein and mRNA levels expression. [29]

## ESTABLISHING LINK BETWEEN COMEDO DCIS AND BASAL –LIKE BREAST CANCER

Basal breast cancer which represent high grade lesions with an aggressive clinical course express p63, cytokeratin 5, Cytokeratin 6, cytokeratin 14 and cytokeratin 17 antigens characteristic of the myoepithelial lineage. This type of breast cancer generally lacks Her2/neu and hormone receptor expression. However, data on the precursor lesions which can give rise to basal like breast carcinomas is still limited. [30]

Shekhar et al revealed two patterns of p63 and Her2 neu expression in a commercial cell line induced comedo DCIS. In one type p63 was limited to myoepithelial cells along with no expression of Her2 neu, whereas the second type showed a co expression for both markers in the microinvasive cancer adjacent to comedo form. This co expressing group formed the precursor link between comedo DCIS and basal –like breast cancer. [31]

## USE IN MINIMAL CARCINOMA TRIPLE STAIN

Breast triple stain (cocktail of cytokeratin CK5, p63 and CK8/18) and LCDC (cocktail of E –cadherin and p120) are two dual colour chromogen immunostains used to solve diagnostic dilemmas in breast pathology. While Breast Triple Stain (BTS) is used to distinguish invasive carcinoma from in situ carcinoma, LCDC is used to distinguish ductal breast proliferations from lobular ones. [32] Ginter et al developed a minimal carcinoma triple stain which is a trichromogen immunostain using brown,blue and red colours and stains against antibodies to E –cadherin,CK-7 and p63 which identify membrane ,cytoplasm and nucleus respectively. This stain serves the dual purpose of being utilised to distinguish in situ from invasive carcinomas and identifying ductal and lobular ductal proliferations in a single focus. [32]

MC triple stain was easier to interpret, particularly for myoepithelial identification as a blue chromogen was utilised in MC triple stain. In comparison, Brown chromogen was utilised in BTS which localised to the cytoplasm and nucleus. [32] MTC is used in specific situations and cannot replace single antibodies which can sufficiently clarify doubts. The MC Triple Stain is particularly useful in evaluating small foci of carcinoma or small sized needle core biopsies, particularly when both the histologic type and extent of disease (in situ vs invasive) cannot be discerned with ease. Also because of the ease in interpretation, it is useful for the inexperienced pathologists. [32]

## PREDICTING CANCER DEVELOPMENT IN INTRADUCTAL PAPILLOMAS

Cytokeratin 5/6 [CK5/6] and p63 markers have been proposed to predict the subsequent risk of cancer development in intraductal papillomas but its standardization has also remained controversial. Yang et al [33] conducted a study of 17 patients who were initially diagnosed as intraductal papillomas and later developed breast cancer. Yang et al documented a positive association between p63 and CK 5/6 positive patients and subsequent development of breast cancer. 17.9 % of CK5/6 positive patients developed breast cancer while 8.6 % of p63/CK5/6 double positive patients developed cancer. Thus, a significantly lower incidence of subsequent carcinoma was observed in p63/CK5/6 double positive status patients, which indicates a more accurate prognostic utility. The authors proposed the use of immunohistochemistry (IHC) markers like CK 5/6 and p63 to predict subsequent development of breast cancer in patients diagnosed with intraductal papilloma. [33]

## P40– NOVEL MYOEPITHELIAL MARKER

Sang et al investigated the expression patterns of the pan-p63 (TP63, 4A4, Dako, 1:700), p40 antibody [CalBiochem Biosciences, (CB)], and p40 antibody [polyclonal, Diagnostic BioSystems, (DB)] in various forms of breast disease. p63 and p40 (DB) expression in myoepithelial cells was similar in majority of cases unlike p40 (CB). p40 (CB) was more sensitive (99.0%) but less specific (85.8%), and p63 was less sensitive (93.8%) in adenosis, IP, and DCIS. In IDCs, while p63 and p40 (DB) had similar expression in cancer cells; p40 (CB) expression was different. p 40 must be carefully interpreted in cancer cells due to their low specificity. Kovari et al concluded that both p40 and p63 can be used to

identify myoepithelial cells. However, these markers may show differences in expression in a subset of tumor cells in triple negative breast carcinomas. [34]

## ROLE OF P63 IN DISTINGUISHING CUTANEOUS ADNEXAL CARCINOMAS FROM BREAST CARCINOMA METASTATIC TO SKIN

As has been stated above several studies showed that p63 is a selective nuclear marker of

myo epithelial cells in breast and has significant diagnostic value in differentiating between in situ and invasive carcinoma. However, an interesting observation is the fact that primary or metastatic adenocarcinomas which are derived from glandular epithelia like those of breast and prostate lose expression of p63. [(35)] Cutaneous metastasis can be seen in 0.7 to 1 % of patients with visceral malignancies and may even be the first manifestation of an internal malignancy. Studies by Di Como CJ et al and Reiss Feilho JS et al have concluded that p63 has detected in the epidermal and adnexal basal/myoepithelial cells and that p63 might be used as a diagnostic marker for primary epidermal or adnexal tumors in the skin. (36,37)

Thus, p63 is one of the most valuable markers to distinguish cutaneous adnexal carcinomas from breast or any other visceral carcinomas metastatic to skin.

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