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p63 Expression in Ductal Carcinoma In Situ (DCIS) of the Breast and Its Correlation with Histopathological Grading and Morphological Variants

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ABSTRACT

Background: Ductal carcinoma in situ (DCIS) is a non-invasive breast cancer with varying potential for progression to invasive carcinoma. Myoepithelial cells (MECs) play a role in preventing this progression, and their absence is a hallmark of invasive disease. The p63 protein is a myoepithelial marker that can be assessed by immunohistochemistry (IHC). This study aimed to evaluate the relationship between p63 expression in MECs, the grade of DCIS, and the morphological subtype of DCIS. **Methods:** A cross-sectional study was conducted on 35 cases of DCIS diagnosed at the Anatomical Pathology Laboratory of Dr. M. Djamil General Hospital Padang. Paraffin blocks were collected, and Hematoxylin and Eosin (H&E) slides were reviewed to confirm the diagnosis and determine the histopathological grading (low, intermediate, and high) and morphological variants (comedo and non-comedo) of DCIS. Paraffin blocks were re-cut for p63 immunohistochemical staining. The extent of p63 expression was classified as complete or incomplete. **Results:** The majority of DCIS cases were high grade (54.3%) and of the non-comedo subtype (68.4%). All cases with complete p63 expression were of low histologic grade, while all cases with incomplete p63 expression were of high histologic grade. The results of the Chi-square test showed a statistically significant relationship between p63 expression and histopathological grading ($p < 0.001$). There was no statistically significant relationship between p63 expression and morphological variant. **Conclusion:** The absence of p63 expression in DCIS is associated with high histologic grade. This finding suggests that p63 IHC may be a useful adjunct in evaluating DCIS.

1. Introduction

Breast cancer remains a significant global health challenge, representing a prevalent malignancy among women and a leading cause of cancer-related mortality. In 2020, the International Agency for Research on Cancer (IARC) estimated approximately 2.3 million new cases of breast cancer diagnosed worldwide, underscoring the substantial burden of this disease. Ductal carcinoma in situ (DCIS) constitutes a non-invasive form of breast cancer characterized by the proliferation of malignant epithelial cells confined within the breast ducts. Although DCIS represents a precursor lesion to

invasive breast cancer, it exhibits a heterogeneous nature with varying potential for progression. The accurate assessment of DCIS is critical for determining appropriate treatment strategies and preventing progression to invasive disease.^{1,2}

Myoepithelial cells (MECs) are specialized contractile cells that form a continuous layer surrounding the epithelial cells of the breast ducts and lobules. These cells play a crucial role in maintaining the structural integrity of the mammary gland and are thought to contribute to the suppression of tumor growth and invasion. The presence of an intact layer of MECs is a hallmark of

DCIS, distinguishing it from invasive ductal carcinoma (IDC), where the MEC layer is disrupted. The disruption of the MEC layer is associated with an increased risk of progression to invasive disease, highlighting the importance of MECs in the pathogenesis of breast cancer.³⁻⁵

The p63 protein, a member of the p53 family of transcription factors, is a well-established marker of MECs. It plays a critical role in the differentiation and maintenance of MECs and is commonly used in immunohistochemical (IHC) studies to assess the presence and integrity of the MEC layer in breast lesions. The loss of p63 expression has been observed in various cancers, including breast cancer, and is often associated with a more aggressive phenotype and a poorer prognosis. In the context of DCIS, the loss of p63 expression may indicate a higher risk of progression to invasive disease, underscoring the potential utility of p63 IHC as a prognostic marker.^{6,7}

Several studies have investigated the role of p63 expression in DCIS and its correlation with histopathological features. Some studies have reported a significant association between the loss of p63 expression and high-grade DCIS, suggesting that p63 may be a useful marker for identifying more aggressive lesions. However, other studies have found no significant correlation between p63 expression and DCIS grade or morphological subtype. These conflicting findings highlight the need for further research to clarify the role of p63 in DCIS and its potential clinical implications.⁸⁻¹⁰ This study aimed to evaluate the relationship between p63 expression in MECs, the grade of DCIS, and the morphological subtype of DCIS.

2. Methods

This study employed a cross-sectional design, utilizing observational data collected from cases diagnosed at the Anatomical Pathology Laboratory of Dr. M. Djamil General Hospital Padang. The study population encompassed all cases of breast DCIS diagnosed within this facility during the period spanning January 2019 to December 2023. A total of

35 cases met the inclusion criteria and were incorporated into the final analysis. The primary data for this investigation consisted of histopathological assessments derived from Hematoxylin and Eosin (H&E) stained slides and immunohistochemical (IHC) staining for the p63 protein. H&E slides were reviewed to confirm the diagnosis of DCIS and to ascertain the histopathological grading and morphological variants. Paraffin blocks were re-cut to produce additional sections for IHC staining.

The histopathological grading of DCIS was determined based on the degree of nuclear atypia, a well-established prognostic factor in DCIS. The grading system employed in this study adhered to the World Health Organization (WHO) classification of tumors of the breast, which categorizes DCIS into three grades: low, intermediate, and high. Low-grade DCIS is characterized by the presence of small, uniform cells with minimal nuclear pleomorphism and infrequent mitotic figures. Intermediate-grade DCIS exhibits greater nuclear pleomorphism and a higher mitotic rate than low-grade DCIS. High-grade DCIS is characterized by marked nuclear pleomorphism, prominent nucleoli, and frequent mitotic figures. The morphological variants of DCIS were also assessed according to the WHO classification. This classification recognizes two main subtypes: comedo and non-comedo. The comedo subtype is characterized by the presence of central necrosis within the DCIS lesion, often accompanied by calcification. The non-comedo subtype encompasses a variety of patterns, including solid, cribriform, papillary, and micropapillary. The distinction between comedo and non-comedo subtypes is relevant, as the comedo subtype is generally associated with a higher risk of progression to invasive carcinoma.

Immunohistochemical staining for the p63 protein was performed to assess the presence and integrity of the MEC layer in DCIS lesions. The p63 protein is a reliable marker of MECs and is commonly used to distinguish between DCIS and IDC. In DCIS, an intact layer of p63-positive MECs is typically observed surrounding the lesion, whereas in IDC, the MEC layer

is disrupted or absent. The IHC staining procedure was conducted using the streptavidin-biotin complex (SBC) method, a widely used technique for detecting antigens in tissue sections. This method involves the sequential application of primary and secondary antibodies, followed by a streptavidin-enzyme conjugate and a chromogenic substrate. The primary antibody binds specifically to the antigen of interest (in this case, p63), while the secondary antibody binds to the primary antibody. The streptavidin-enzyme conjugate then binds to the secondary antibody, and the chromogenic substrate is converted by the enzyme to produce a visible precipitate at the site of antigen localization. In this study, the primary antibody used was a monoclonal antibody against p63, and the chromogenic substrate was diaminobenzidine (DAB). The stained sections were then examined under a light microscope to assess the pattern and extent of p63 expression. The extent of p63 expression was categorized as either complete or incomplete. Complete expression was defined as the presence of a continuous layer of p63-positive MECs surrounding the DCIS lesion, while incomplete expression was defined as the absence of a continuous layer of p63-positive MECs.

The data collected in this study were analyzed using SPSS, version 24.0. Descriptive statistics were used to summarize the data, including the frequency and percentage of cases with different histopathological grades, morphological variants, and p63 expression patterns. A statistically significant result ($p\text{-value} < 0.05$) indicates that there is a significant association between the variables. Fisher's exact test, another statistical test used for analyzing categorical data, was used to evaluate the relationship between p63 expression and morphological variant. This test is particularly useful for small sample sizes, where the Chi-square test may not be appropriate. Similar to the Chi-square test, a statistically

significant result ($p\text{-value} < 0.05$) indicates a significant association between the variables. The results of the statistical analyses were presented in tabular and narrative form, with p-values reported to indicate the level of statistical significance.

3. Results

Table 1 presents the clinicopathological characteristics of the 35 ductal carcinoma in situ (DCIS) cases included in this study; Age Distribution: The most common age group was 41-50 years, accounting for 43.9% of the cases. The age range spanned from 31 to 70 years, with an average age of 46 years. This aligns with general trends of breast cancer incidence, where the risk increases with age and peaks in the pre-menopausal and perimenopausal years; Histopathological Grade: High-grade DCIS was slightly more prevalent (54.3%) than low-grade DCIS (45.7%). This finding suggests a potentially aggressive nature of DCIS in this cohort, as high-grade DCIS is associated with a greater risk of recurrence and progression to invasive cancer; Histopathological Morphological Variants: The non-comedo subtype was predominant (68.4%) compared to the comedo subtype (31.6%). Non-comedo subtypes include various patterns like solid, cribriform, papillary, and micropapillary, each with potentially different biological behaviors. While comedo DCIS is often considered more aggressive, the higher proportion of non-comedo subtypes in this study may reflect the diversity of DCIS presentations; p63 Expression: The distribution of complete and incomplete p63 expression was almost equal, with 45.7% showing complete expression and 54.3% showing incomplete expression. This finding highlights the variability of p63 expression in DCIS and its potential role in differentiating between cases with varying levels of aggressiveness.

Table 1. Clinicopathological characteristics of ductal carcinoma in situ of the breast.

Variable	Frequency	Percentage (%)
Age (n=41)		
31-40 years	9	21.9
41-50 years	18	43.9
51-60 years	10	24.4
61-70 years	4	9.8
Histopathological grade (n=35)		
Low grade	16	45.7
High grade	19	54.3
Histopathological morphological variants (n=19)		
Comedo	6	31.6
Non-comedo	13	68.4
p63 expression (n=35)		
Complete	16	45.7
Incomplete	19	54.3

Figure 1 provides a visual representation of the microscopic features of Ductal Carcinoma In Situ (DCIS) at different grades and with varying morphologies; A & B: Low-Grade DCIS. A (200x) shows a relatively uniform population of cells with small, regular nuclei. This indicates a lower degree of cellular atypia, suggesting a less aggressive form of DCIS. B (400x) highlights the presence of myoepithelial cells (arrow) and an intact basement membrane (arrowhead). These features are crucial in distinguishing DCIS (non-invasive) from invasive carcinoma; C & D: High-Grade DCIS with Comedo Necrosis. C (200x) demonstrates the characteristic comedo necrosis (arrow), a central area of cell death within the duct. This feature is often associated with more aggressive behavior. D (400x) shows significant

cellular atypia with pleomorphic nuclei (varied sizes and shapes), coarse chromatin (darkly stained genetic material), and prominent nucleoli (arrowhead). Mitotic figures (double arrow), indicative of active cell division, are also present. Despite these aggressive features, the basement membrane and myoepithelial cells (double arrowhead) appear intact, confirming the non-invasive nature of this DCIS; E & F: High-Grade Non-Comedo DCIS. E (200x) displays a proliferation of cells with significant atypia, similar to the high-grade comedo DCIS. However, there is no central necrosis. F (400x) emphasizes the cellular atypia with pleomorphic nuclei, coarse chromatin, and prominent nucleoli (arrow). Mitotic figures (double arrow) are also evident. The basement membrane and myoepithelial cells (double arrow) remain intact, confirming it as DCIS.

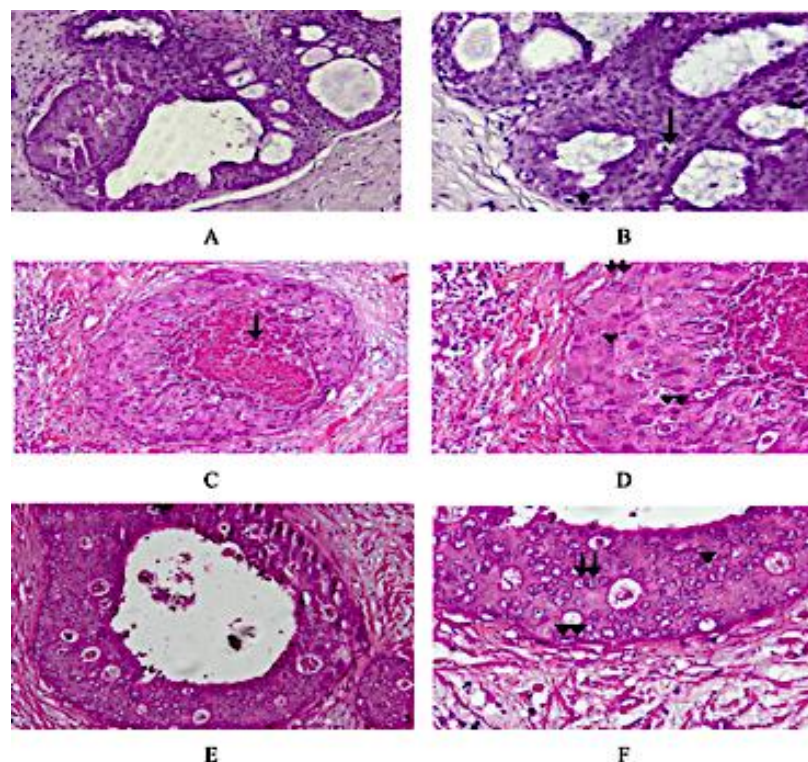


Figure 1. Microscopic features of Ductal Carcinoma in Situ (DCIS). A. Low Grade with H&E staining, this image displays a proliferation of epithelial cells characterized by small, monomorphic nuclei with homogeneous chromatin and inconspicuous nucleoli. (Magnification 200x). B. Low Grade with H&E staining, this image demonstrates the presence of myoepithelial cells (arrow) and an intact basement membrane (arrowhead). (Magnification 400x). C. High-grade DCIS with comedo necrosis (H&E stain). The image shows a proliferation of pleomorphic epithelial cells with a central area of necrosis within the ductal lumen (arrow) (200x magnification). D. High-grade DCIS with comedo necrosis (H&E stain). The image displays a proliferation of epithelial cells with pleomorphic nuclei, coarse chromatin, and prominent nucleoli (arrowhead). Mitotic figures are also observed (double arrow). The basement membrane and myoepithelial cells appear intact (double arrowhead) (400x magnification). E. High-grade non-comedo DCIS with H&E staining. This image shows a proliferation of pleomorphic epithelial cells (200x magnification). F. High-grade non-comedo DCIS with H&E staining. This image demonstrates a proliferation of epithelial cells with pleomorphic nuclei, coarse chromatin, and prominent nucleoli (arrow). Mitotic figures are also observed (double arrow). The basement membrane and myoepithelial cells appear intact (double arrow) (400x magnification).

Table 2 presents the association between p63 expression and histopathological grade in ductal carcinoma in situ (DCIS) of the breast. The table demonstrates a very strong correlation between p63 expression and the grade of DCIS. This is evident in the stark contrast between complete and incomplete p63 expression across the grades. All cases (100%) with complete p63 expression were classified as low-grade DCIS. This suggests that the presence of a continuous layer of myoepithelial cells, as indicated by complete p63 expression, is strongly associated with

less aggressive DCIS. Conversely, all cases (100%) with incomplete p63 expression were high-grade DCIS. This indicates that the loss or disruption of the myoepithelial cell layer, as indicated by incomplete p63 expression, is strongly associated with more aggressive, higher-grade DCIS. The p-value of <0.001 further emphasizes the statistical significance of this association. This very low p-value indicates that the observed relationship between p63 expression and DCIS grade is highly unlikely to be due to chance.

Table 2. Association between p63 expression and histopathological grade in ductal carcinoma in situ of the breast.

p63 expression	Histopathological grade		Total	p-value
	Low Grade	High Grade		
	f (%)	f (%)	f (%)	
Complete	16 (100%)	0 (0%)	16 (45.7%)	<0.001
Incomplete	0 (0%)	19 (100%)	19 (54.3%)	-

Figure 2 provides a visual representation of the immunohistochemical (IHC) staining for p63 in ductal carcinoma in situ (DCIS), highlighting the differences in expression patterns between low-grade and high-grade lesions; A & B: Complete p63 Expression in Low-Grade DCIS. A (200x) shows strong and continuous brown staining for p63 around the entire circumference of the duct involved by low-grade DCIS. This indicates the presence of an intact layer of myoepithelial cells, a characteristic feature of less aggressive DCIS. B (400x) highlights the brown staining (arrow) in the nuclei of myoepithelial cells, confirming positive p63 expression. The staining pattern appears complete and continuous, forming an uninterrupted circle around the duct. This reinforces

the observation of an intact myoepithelial cell layer, which acts as a barrier against invasion; C & D: Incomplete p63 Expression in High-Grade DCIS. C (200x) demonstrates a high-grade DCIS lesion where the p63 staining is discontinuous along the periphery of the duct. This indicates incomplete expression of p63, suggesting a disruption or loss of the myoepithelial cell layer. D (400x) shows positive p63 staining (brown, arrowhead) in some myoepithelial cell nuclei, but also highlights areas where p63 staining is absent (circled). This confirms the incomplete expression pattern and further suggests a compromised myoepithelial cell layer in high-grade DCIS.

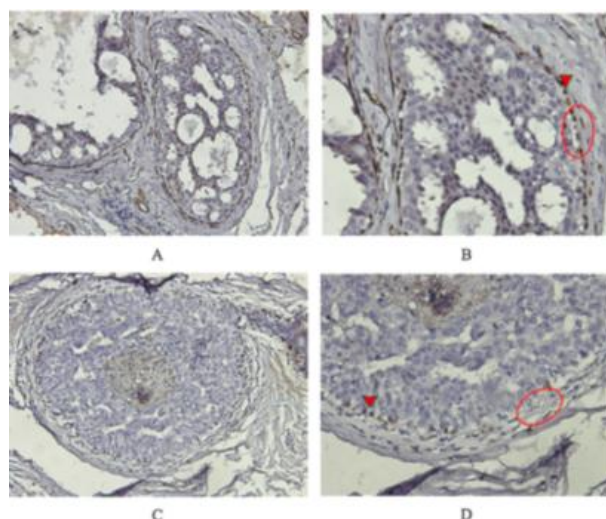


Figure 2. A. Complete p63 expression in the lumen of low-grade ductal carcinoma in situ (200x magnification). This image shows strong and continuous staining for p63 around the entire circumference of the duct involved by low-grade DCIS. B. Positive p63 staining in the nuclei of myoepithelial cells (400x magnification). This image highlights the brown staining (arrow) that indicates positive p63 expression. The staining pattern appears complete and continuous, forming an uninterrupted circle around the duct, which is characteristic of an intact myoepithelial cell layer. C. Incomplete p63 expression in the lumen of high-grade ductal carcinoma in situ (200x magnification). This image shows a ductal carcinoma in situ lesion where the p63 staining is not continuous along the periphery of the duct, indicating incomplete expression. D. Positive p63 staining in the nuclei of myoepithelial cells (brown, arrowhead) with areas of incomplete staining (circle) (400x magnification). This higher magnification image demonstrates the nuclear localization of p63 staining within the myoepithelial cells (the brown stain). It also highlights the areas where p63 staining is absent (circled), confirming the incomplete expression pattern.

Table 3 explores the relationship between p63 immunoexpression distribution and histopathological morphological variants (comedo vs. non-comedo) in ductal carcinoma in situ (DCIS) of the breast. The table categorizes p63 expression into three levels based on the percentage of immunopositive cells; +3 (>50%): More than 50% of cells express p63; +2 (25-50%): 25-50% of cells express p63; +1 (<25%): Less than 25% of cells express p63. There were no cases in this study with more than 50% of cells expressing p63. This suggests that strong, widespread p63 expression might be uncommon in DCIS. All 3 cases (100%) with 25-50% p63 expression were of the non-comedo subtype. This hints at a potential association between moderate p63 expression and this less aggressive

morphological variant. However, the small sample size warrants caution in drawing definitive conclusions. The majority of cases (16, or 84.2%) had less than 25% p63 expression. These cases were distributed across both comedo (31.6%) and non-comedo (68.4%) subtypes. This suggests that low p63 expression may be present in both morphological variants, and might not be a strong discriminator between them. The p-value of 0.2 indicates that there was no statistically significant association between p63 immunoexpression distribution and the morphological variants of DCIS. This implies that, in this study, the level of p63 expression did not reliably predict whether a DCIS lesion would be of the comedo or non-comedo subtype.

Table 3. Association between p63 expression and histopathological morphological variants in ductal carcinoma in situ of the breast.

p63 immunoexpression distribution (%)	Histopathological morphological variants		n (%)	p-value
	Comedo	Non-Comedo		
	f (%)	f (%)		
+3 (>50%)	0 (0.0)	0 (0.0)	0 (0.0)	0.2
+2 (25-50%)	0 (0.0)	3 (100.0)	3 (15.8)	-
+1 (<25%)	6 (31.6)	10 (68.4)	16 (84.2)	-

Figure 3 provides a direct visual comparison of p63 expression in comedo-type and non-comedo-type ductal carcinoma in situ (DCIS); A: Comedo-type DCIS. Brown staining indicates the presence of p63 protein in the nuclei of myoepithelial cells. This confirms that even in comedo-type DCIS, some myoepithelial cells are still present. The circled area shows a region where p63 staining is absent, indicating a loss of myoepithelial cells. This focal loss is consistent with the more aggressive nature of comedo-type DCIS, where myoepithelial cell layers can

be disrupted or discontinuous; B: Non-comedo-type DCIS. Similar to image A, brown staining highlights p63 expression in the nuclei of myoepithelial cells. This confirms the presence of these cells in non-comedo DCIS as well. The circled area shows a region with no p63 staining, indicating a focal absence of myoepithelial cells. While non-comedo DCIS is generally considered less aggressive, this image demonstrates that focal disruptions in the myoepithelial cell layer can still occur.

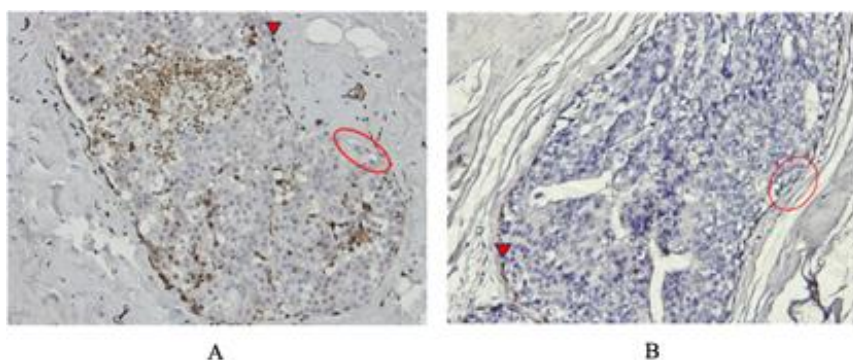


Figure 3. A. p63 expression in comedo-type ductal carcinoma in situ. p63 staining is observed in the nuclei of myoepithelial cells (arrowhead), with an area lacking myoepithelial cells (circle). B. p63 expression in non-comedo-type ductal carcinoma in situ. p63 staining is present in the nuclei of myoepithelial cells (arrowhead), with an area showing absence of myoepithelial cells (circle).

4. Discussion

The significant correlation between p63 expression and the histopathological grade of DCIS is a striking finding that warrants an in-depth discussion. The observation that all cases with complete p63 expression were low-grade DCIS, while those with incomplete expression were high-grade, underscores the critical role of MECs in DCIS progression. This finding aligns with a growing body of evidence that links the loss of MECs to a more aggressive disease phenotype. Myoepithelial cells (MECs) are essential components of the breast ductal system, providing a protective layer surrounding the epithelial cells. Their presence is vital not only for maintaining the structural integrity of breast tissue but also for actively participating in tumor suppression. In the context of ductal carcinoma in situ (DCIS), MECs serve as a crucial barrier against the progression to invasive carcinoma. MECs are contractile cells that contribute to the architectural integrity of the breast ductal system. They help maintain the shape and organization of the ducts, ensuring proper function and milk flow during lactation. Their presence is crucial for the overall health and proper functioning of breast tissue. MECs play a role in regulating the normal function of breast ducts. They interact with epithelial cells and influence their behavior, ensuring proper fluid and electrolyte transport, as well as maintaining the balance of cell growth and

differentiation within the ducts. Beyond their structural and functional roles, MECs actively participate in tumor suppression. They act as a natural defense mechanism against cancer development and progression. Their presence helps to create a microenvironment that is less conducive to tumor growth and invasion. In DCIS, MECs form a critical barrier that prevents the malignant epithelial cells from breaking through the basement membrane and invading the surrounding stroma. MECs secrete protease inhibitors, which are molecules that neutralize enzymes capable of degrading the extracellular matrix (ECM). The ECM is a complex network of proteins and other molecules that provide structural support to tissues and regulate cell behavior. By inhibiting proteases, MECs help preserve the integrity of the basement membrane, a specialized layer of the ECM that separates the epithelium from the underlying stroma. This intact basement membrane acts as a physical obstacle, preventing DCIS cells from penetrating and invading the surrounding tissues. Matrix metalloproteinases (MMPs) are another class of enzymes involved in ECM degradation. MECs can suppress the expression of MMPs, further reinforcing the basement membrane and hindering the invasive potential of DCIS cells. By keeping MMP activity in check, MECs contribute to maintaining tissue integrity and preventing the spread of cancer cells. MECs produce a variety of tumor

suppressor proteins, including maspin, a protein that can inhibit cell motility and induce apoptosis (programmed cell death) in cancer cells, and p63, a transcription factor that regulates the expression of various genes involved in cell cycle control, DNA repair, and apoptosis. By producing these tumor suppressor proteins, MECs actively contribute to the anti-cancer defense mechanisms within the breast ductal system. The study's findings highlight the significance of MECs in the grading of DCIS. The strong correlation between the absence of p63 expression, a marker of MECs, and high-grade DCIS suggests that the loss of MECs is associated with a more aggressive disease phenotype. High-grade DCIS is characterized by a greater degree of cellular atypia and a higher proliferative rate, indicating a greater potential for invasion and progression to invasive carcinoma. The loss of MECs in high-grade DCIS may further contribute to its invasive potential by disrupting the protective mechanisms described earlier. When the MEC layer is compromised, the basement membrane becomes vulnerable to degradation, and the tumor suppressor functions of MECs are diminished. This creates an environment that favors invasion, where DCIS cells can more easily break through the weakened barrier and infiltrate the surrounding stroma. The loss of myoepithelial cells (MECs) is a critical event in the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer. MECs, which form a protective layer around the breast ducts, play a crucial role in maintaining tissue integrity and suppressing tumor growth. However, when MECs are lost or their function is compromised, the risk of DCIS progression increases significantly. MECs employ several mechanisms to prevent DCIS cells from invading the surrounding stroma. MECs secrete protease inhibitors, which neutralize enzymes that can degrade the extracellular matrix (ECM). The ECM provides structural support to tissues and acts as a barrier against cell invasion. By inhibiting proteases, MECs help preserve the integrity of the basement membrane, a specialized layer of the ECM that separates the epithelium from the underlying

stroma. Matrix metalloproteinases (MMPs) are another class of enzymes involved in ECM degradation. MECs can suppress the expression of MMPs, further reinforcing the basement membrane and hindering the invasive potential of DCIS cells. MECs produce various tumor suppressor proteins, such as maspin and p63, which can inhibit the growth and invasion of cancer cells. The loss of MECs, as indicated by the absence or reduction of p63 expression, can disrupt these protective mechanisms, increasing the likelihood of DCIS progression. When the MEC layer is compromised, the basement membrane becomes vulnerable to degradation, and the tumor suppressor functions of MECs are diminished. This creates an environment conducive to invasion, where DCIS cells can break through the weakened barrier and infiltrate the surrounding stroma. The strong association between incomplete p63 expression and high-grade DCIS observed in this study underscores the importance of MECs in DCIS progression. High-grade DCIS is characterized by a greater degree of cellular atypia and a higher proliferative rate, indicating a more aggressive phenotype. The loss of MECs in these lesions may further contribute to their invasive potential. High-grade DCIS cells often exhibit altered expression of adhesion molecules, which are proteins that help cells stick together and to the ECM. These alterations can disrupt cell-cell and cell-ECM interactions, promoting cell motility and invasion. Additionally, high-grade DCIS cells may secrete growth factors and cytokines that stimulate angiogenesis (the formation of new blood vessels), providing the tumor with the nutrients and oxygen it needs to grow and spread. The loss of MECs can also contribute to a process called epithelial-mesenchymal transition (EMT), which is associated with increased invasiveness in cancer cells. During EMT, epithelial cells lose their polarity and cell-cell adhesion, and acquire mesenchymal characteristics, such as increased motility and invasiveness. This transition is often driven by signaling pathways that are activated in response to changes in the tumor microenvironment, including the loss of MECs. The

loss of MECs and the associated increase in invasive potential have significant clinical implications for the management of DCIS. Patients with high-grade DCIS and/or evidence of MEC loss may benefit from more aggressive treatment strategies to prevent progression to invasive carcinoma. Mastectomy is the surgical removal of the entire breast. It may be considered for patients with high-risk DCIS, such as those with large or multifocal lesions, high-grade disease, or a strong family history of breast cancer. Radiotherapy uses high-energy rays to kill cancer cells. Adjuvant radiotherapy, which is given after surgery, may be recommended for patients with high-risk DCIS to reduce the risk of local recurrence. Hormonal therapy, such as tamoxifen, can block the effects of estrogen on breast tissue. It may be considered for patients with estrogen receptor-positive DCIS to reduce the risk of recurrence. The findings of this study suggest that p63 immunohistochemistry (IHC) could be a valuable tool in the diagnostic and prognostic evaluation of ductal carcinoma in situ (DCIS). By assessing p63 expression, pathologists can gain insights into the integrity of the myoepithelial cell (MEC) layer, which can help predict the likelihood of progression to invasive disease. This information can aid clinicians in making informed treatment decisions, tailoring therapies to the individual risk profiles of patients. p63 IHC can help differentiate between DCIS and invasive ductal carcinoma (IDC). In DCIS, an intact layer of p63-positive MECs typically surrounds the lesion, while in IDC, the MEC layer is disrupted or absent. This distinction is critical for accurate diagnosis and appropriate treatment planning. In some cases, DCIS may contain small, microscopic foci of invasion that are difficult to detect on routine hematoxylin and eosin (H&E) staining. p63 IHC can help identify these foci by highlighting areas where the MEC layer is disrupted, allowing for more accurate staging and risk assessment. p63 IHC can assist in delineating the extent of DCIS within a breast specimen. By staining for p63, pathologists can more clearly visualize the boundaries of the lesion and identify any areas of extension into surrounding ducts

or lobules. This information is crucial for ensuring complete surgical excision and reducing the risk of local recurrence. The study's findings suggest that p63 expression is a strong predictor of DCIS grade, which is a well-established prognostic factor. Complete p63 expression is associated with low-grade DCIS and a lower risk of progression, while incomplete expression is linked to high-grade DCIS and a higher risk of invasion. p63 IHC can help identify high-risk DCIS lesions that may benefit from more aggressive treatment strategies. Patients with incomplete p63 expression, indicating a disrupted MEC layer, may be candidates for mastectomy or adjuvant radiotherapy to reduce the risk of recurrence and progression to invasive disease. The prognostic information provided by p63 IHC can aid clinicians in making informed treatment decisions. Patients with complete p63 expression and low-grade DCIS may be suitable for less aggressive treatment approaches, such as breast-conserving surgery followed by close monitoring. Conversely, patients with incomplete p63 expression and high-grade DCIS may require more aggressive therapies to minimize the risk of recurrence and progression. Incorporating p63 IHC into the routine assessment of DCIS could lead to more personalized treatment strategies. By providing valuable information about the integrity of the MEC layer and the risk of progression, p63 IHC can help tailor therapies to the individual needs of patients. p63 IHC can enhance risk stratification in DCIS, allowing clinicians to identify patients who are most likely to benefit from specific treatments. This can help avoid overtreatment in low-risk patients and ensure that high-risk patients receive the appropriate level of care. The prognostic information provided by p63 IHC can facilitate the development of personalized treatment plans. By considering p63 expression alongside other clinicopathological factors, clinicians can tailor therapies to the individual risk profiles of patients, optimizing treatment outcomes and minimizing side effects. Ultimately, the integration of p63 IHC into the routine evaluation of DCIS has the potential to enhance patient care. By providing more accurate

diagnoses, predicting the risk of progression, and guiding treatment decisions, p63 IHC can contribute to improved outcomes and a better quality of life for patients with DCIS.¹¹⁻¹⁴

While this study did not find a statistically significant association between p63 expression and the morphological variants of DCIS (comedo vs. non-comedo), it's crucial to understand the nuances of this finding and its implications in the broader context of DCIS progression. The initial hypothesis that p63 expression would differ significantly between comedo and non-comedo DCIS subtypes was not supported by the statistical analysis. This suggests that p63 expression may not be a strong independent predictor of DCIS morphology. The study included a relatively small sample size, which may have limited the power to detect subtle differences in p63 expression between the morphological variants. Larger studies with greater statistical power are needed to confirm these findings. Even within individual DCIS lesions, p63 expression can be heterogeneous, with areas of complete and incomplete staining. This heterogeneity may contribute to the lack of a clear association between p63 expression and morphological subtype. Despite the lack of a statistically significant association, the study did observe focal absence of MECs in both comedo and non-comedo DCIS lesions. This suggests that the loss of MECs, even in a focal manner, may be a more universal phenomenon in DCIS progression, regardless of the specific morphological subtype. The comedo subtype of DCIS is characterized by central necrosis, a feature often associated with more aggressive behavior and a higher risk of progression to invasive carcinoma. While this study did not find a direct link between p63 expression and comedo necrosis, the presence of focal MEC loss in comedo lesions suggests that MEC disruption may still contribute to their aggressive potential. Comedo necrosis is thought to arise from the rapid proliferation of DCIS cells within the confined space of the breast duct. This rapid growth can outpace the blood supply, leading to cell death and the formation of necrotic areas. The disruption of tissue architecture

and the release of cellular debris associated with comedo necrosis may create an environment that favors invasion, even in the presence of some residual MECs. Non-comedo DCIS encompasses various growth patterns, including solid, cribriform, papillary, and micropapillary. These subtypes are generally considered less aggressive than comedo DCIS, but they can still progress to invasive carcinoma. The observation of focal MEC loss in non-comedo lesions in this study suggests that MEC disruption may also play a role in their progression. The mechanisms underlying MEC loss in non-comedo DCIS may differ from those in comedo DCIS. While rapid proliferation and necrosis may drive MEC loss in comedo lesions, other factors, such as genetic alterations or microenvironmental changes, may contribute to MEC disruption in non-comedo subtypes. The findings of this study have clinical implications for the management of DCIS, even in the absence of a statistically significant association between p63 expression and morphological variants. The study emphasizes the importance of a comprehensive evaluation of DCIS, considering not only morphology but also grade and MEC integrity. While morphology provides valuable information, it should not be the sole determinant of treatment decisions. p63 IHC can serve as a valuable adjunct to H&E staining in assessing DCIS. By highlighting the presence or absence of MECs, p63 IHC can provide additional information about the risk of progression, particularly in cases with ambiguous morphology. The findings support the use of personalized treatment strategies for DCIS. Patients with high-grade DCIS or evidence of MEC loss, regardless of morphology, may benefit from more aggressive treatment approaches to minimize the risk of recurrence and progression.¹⁵⁻¹⁷

The findings of this study have potential clinical implications for the management of ductal carcinoma in situ (DCIS). The strong association between incomplete p63 expression and high-grade DCIS suggests that p63 immunohistochemistry (IHC) could be a valuable tool in risk stratification. By identifying patients with high-risk DCIS lesions, clinicians can

make more informed decisions regarding treatment options, such as the use of adjuvant radiotherapy or tamoxifen. Currently, the standard treatment for DCIS is surgical excision, often followed by radiotherapy. However, there is ongoing debate regarding the optimal management of low-grade DCIS, as some studies suggest that these lesions may not require aggressive treatment. The use of p63 IHC could help identify low-grade DCIS lesions that are unlikely to progress, potentially sparing patients from unnecessary treatment and its associated side effects. The heterogeneity of DCIS necessitates a personalized approach to treatment. p63 IHC can aid in risk stratification by identifying patients with high-risk lesions who may benefit from more aggressive therapies. Conversely, it can also identify patients with low-risk lesions who may be candidates for less aggressive treatment or even active surveillance. Patients with incomplete p63 expression, indicating a disrupted myoepithelial cell (MEC) layer, are at a higher risk of progression to invasive carcinoma. These patients may benefit from mastectomy or adjuvant radiotherapy to reduce the risk of recurrence and progression. Patients with complete p63 expression, suggesting an intact MEC layer, are at a lower risk of progression. These patients may be suitable for breast-conserving surgery (BCS) followed by close monitoring or even active surveillance, in which treatment is deferred until there is evidence of progression. The decision to pursue a particular treatment strategy should be made on a case-by-case basis, considering various factors such as the patient's age, overall health, tumor characteristics, and personal preferences. p63 IHC can provide valuable information to guide these decisions, but it should not be the sole determinant of treatment. Mastectomy is the surgical removal of the entire breast. It may be considered for patients with high-risk DCIS, such as those with large or multifocal lesions, high-grade disease, or a strong family history of breast cancer. BCS involves the removal of the tumor along with a margin of healthy tissue. It is often followed by radiotherapy to reduce the risk of local

recurrence. BCS may be an option for patients with low-risk DCIS, particularly those with complete p63 expression. Radiotherapy uses high-energy rays to kill cancer cells. Adjuvant radiotherapy, which is given after surgery, may be recommended for patients with high-risk DCIS to reduce the risk of local recurrence. Hormonal therapy, such as tamoxifen or aromatase inhibitors, can block the effects of estrogen on breast tissue. It may be considered for patients with estrogen receptor-positive DCIS to reduce the risk of recurrence. Active surveillance involves close monitoring of the DCIS lesion without immediate treatment. This approach may be appropriate for select patients with low-risk DCIS, such as those with small, low-grade lesions and complete p63 expression. The use of p63 IHC could play a crucial role in the selection of patients for active surveillance. By identifying patients with low-risk DCIS and an intact MEC layer, p63 IHC could help ensure that only those patients who are unlikely to experience progression are placed on active surveillance. This could potentially spare many patients from unnecessary treatment and its associated side effects.¹⁸⁻²⁰

5. Conclusion

The study reveals a strong association between the absence of p63 expression and high-grade DCIS, suggesting a potential role for p63 immunohistochemistry in assessing the risk of progression in this disease. Contrary to our initial hypothesis, no statistically significant association was found between p63 expression and the morphological variants of DCIS (comedo vs. non-comedo). This suggests that p63 expression may not be a strong independent predictor of DCIS morphology. The results of this study have potential clinical implications for the management of DCIS. The strong association between incomplete p63 expression and high-grade DCIS suggests that p63 IHC could be a valuable tool in risk stratification.

6. References

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