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The Role of DOG1 as a Novel Myoepithelial Cell Marker in Breast Lesions: An Immunohistochemical Study

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Abstract

Background: Myoepithelial cells (MECs) can be visualized easily in normal breast ducts and acini, but when these structures dilate or are compressed, it is almost impossible to identify them on hematoxylin and eosin (H&E) stained sections that's why immunohistochemical markers are used to visualize MECs. Many MECs markers are commonly used. DOG1 was initially known as a marker for gastrointestinal stromal tumors (GISTs) and was not studied before as breast MECs marker.

Aim of Study: This study aimingat assessing the immunohistochemical expression of DOG1 in reactive, benign, insitu, and malignant breast lesions to evaluate its usefulness as a novel myoepithelial marker.

Material and Methods: The cohort consisted of 90 cases: Thirty benign lesions, 30 invasive carcinomas (infiltrating duct carcinoma NOS, and infiltrating lobular carcinoma NOS), and 30 noninvasive breast carcinoma (DCIS), as formalin fixed paraffin embedded tissue blocks from archives of Pathology department, Kasr Al-Ainy Faculty of Medicine, Cairo University and Nasr City Health Insurance Hospital, Cairo, inthe period from January 2013 to January 2020. All cases were stained for P63 and SMA as a gold standard comparison.

Results: Were interpreted using H-score (semi quantitative assessment of both the intensity of staining and the percentage of positive cells). Benign cases showed 100% positivity in MECs, carcinoma in situ (DCIS) staining was positive in 100% of cases, however intensity and percentage were variable. All invasive lesions showed no staining.

Conclusion: DOG1 is believed to bea useful marker of breast MECs with excellent sensitivity and specificity, and by adding DOG1 to the MECs identification immunohistochemical panel, this will provide more information when diagnosing is not simple.

Key Words: *DOG1 – Myoepithelial cells – Breast – Duct carcinoma in situ.*

Introduction

THE human breast contains a branching ductal network composed of two cell types: An inner layer of luminal epithelial cells and an outer layer of myoepithelial cells, separated from the surrounding stroma by a laminin-rich layer of basement membrane. The ductal network ends in lobular units which is called the terminal duct lobular units (TDLUs) **[1]**.

As normal ducts, almost all benign breast lesions and insitu component have a peripheral rim of myoepithelial cells (MECs) and basement membrane. Invasion occurs when malignant cells extend beyond the myoepithelial cell layer through the basement membrane causing stromal invasion **[2,3]**.

Because breast cancer arises mainly in the luminal epithelial compartment of the TDLU, little concern has been given to the myoepithelial cell layer **[4]**. Myoepithelial cells, which are present in normal, premalignant breast lesions, and pre invasive in situ carcinomas, rarely transform; however, when they do transform, they generally give rise to tumors of low grade malignancy during progression **[5,6]**.

Earlier investigators used antibodies to basement membrane components such as collagen IV and laminin to discriminate in situ from invasive carcinomas. These trials met with only limited success, as invasive tumor cells are capable of synthesizing basement membrane material **[7]**.

Myoepithelial cells contain smooth muscle-type cytoskeletal proteins that perform the contractile function necessary for milk ejection during lactation. Many of the antibodies used to immunohistochemically detect myoepithelial cells are directed against these components, which are localized to the cytoplasm. Smooth muscle actin (SMA), calponin, and smooth muscle myosin heavy chain are such markers that are commonly used **[8]**.

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In all the diagnostic situations (adenosis, radial scar, sclerosing lesions, versus invasive malignancies, in addition, atypical ductal epithelial hyperplasia (ADH), papillary lesions, and microinvasive carcinoma), it is the presence of myoepithelial cells (MECs) that differentiates between in situ and invasive disease, and between benign pseudo invasive lesions and invasive carcinoma, that's why it's crucial to detect myoepithelial cells **[9,10,11]**.

MECs can be visualized easily in normal breast ducts and acini, but when these structures dilate and fill with proliferating cells or are compressed, it is almost impossible to identify them on hematoxylin and eosin (H&E) stained sections **[12,13]**. Immunohistochemical markers are now used to visualize MECs **[2]**.

The commonly used MECs markers in practice are S 100 protein, high-molecular-weight keratin (HMWK), smooth muscle actin (SMA), calponin, and smooth muscle myosin heavy chain (SMMHC), and they are the most sensitive and specific antibodies to cytoplasmic components of MECs, along with the nuclear marker p63 **[14]**.

DOG1 (discovered on GIST first), also known as TMEM 16A (Tumor-amplified and over expressed sequence 2), ORAOV2 (Oral cancer overexpressed protein 2), and Anoctamin 1, was initially known as a marker for gastrointestinal stromal tumors (GISTs) **[15,16]**.

DOG1 is a calcium-dependent, receptor-activated chloride channel protein. It is believed to be sensitive and specific when detecting GISTs, although expression of DOG1 in other mesenchymal tumors, such as Ewing's sarcoma, angiosarcoma, leiomyosarcoma, and synovial sarcoma, has alsobeen reported **[17]**. Because MECs have myofilaments that have a main function of contraction, DOG1 may be related to the contraction process by regulating cytosolic calcium as a transmembrane anion channel **[18,19]**. It is constantly expressed in myoepithelial cells and to a much-limited extent in luminal epithelial cells in breast tissue. Also, that DOG1 has an advantage over other MECs markers that it shows no immunore activity in stromal or vascular cells **[20]**.

Aim of the work:

In this study, we aimed to assess the immunohistochemical expression of DOG1 in various reactive, benign, insitu, and malignant breast lesions to evaluate its usefulness as a novel myoepithelial marker for discriminating between invasive breast carcinoma and noninvasive breast lesions.

Material and Methods

The cohort consisted of 90 cases: Thirty benign lesions, 30 invasive carcinomas (infiltrating duct carcinoma NST, and infiltrating lobular carcinoma NOS), and 30 noninvasive breast carcinoma (DCIS).

Specimens were collected as formalin fixed paraffin embedded tissueblocks. These were collected from archives of Pathology department, Kasr Al-Ainy Faculty of Medicine, Cairo University and Nasr city health insurance hospital, Cairo, in the period from January 2013 to January 2020.

Each paraffin block was re-cut by rotatory microtome at 5 microns thickness then mounted on glass slides to be stained byhematoxylin & Eosin (H&E) for routine histopathological examination and on charged slides for immunostaining.

Immunohistochemical Staining for DOG1:

Immunostaining was done using Bench Mark XT (Ventana) autostainer with the following steps:

- Deparaffinization by using the EZ-prep solution.
- Cell conditioning (standard cell conditioning CC 1) for 80 minutes.
- Antigen retrieval using reaction buffer (PH 6.0).
- The sections then were incubated with the primary antibody for 1 hour at room temperature. The primary antibody was rabbit polyclonal DOG1 antibody.
- Application of Diaminobenzidine (DAB) as a chromogen.
- (Nex ES iView DAB Detection Kit).
- Counterstaining with Hematoxylin II for 8 minutes.
- Post counter staining with bluing reagent for 4 minutes.
- Slides were cleared in Xylene, and then cover slips were applied.

A section of gastrointestinal stromal tumor (GIST) was used as positive control.

Interpretation:

All available slides were examined, and histopathological subtyping was performed according to the 2019 WHO classification of tumors of the breast.

In order to compare DOG1 staining with the gold standard myoepithelial markers, all cases were stained for P63 and SMA.

Results were collected using H-score which involves a semiquantitative assessment of both the intensity of staining (graded as: 0, no staining; 1, faint; 2, moderate; or 3, strong) and the percentage of positive cells. The range of possible scores was from 0 to 300.

Results

The study included 60 noninvasive breast lesions (30 cases of DCISand 30 benign breast lesions), 25 invasive duct carcinoma and 5 specimens of invasive lobular carcinoma.

Almost all MECs stained positively with DOG1 in the 30 benign lesions (100%), all specimens of DCIS showed DOG1 immunoreactivity in MECs (100%) (Table 1), however, in carcinoma in situ staining intensity and percentage were variable (Table 2 & Fig. 1). All invasive lesions showed no staining.

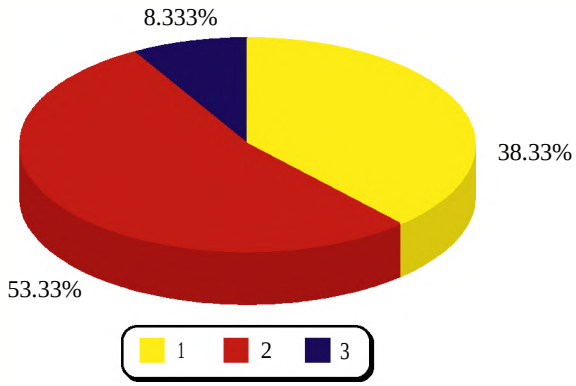

Fig. (1): DOG1 intensity in DCIS.

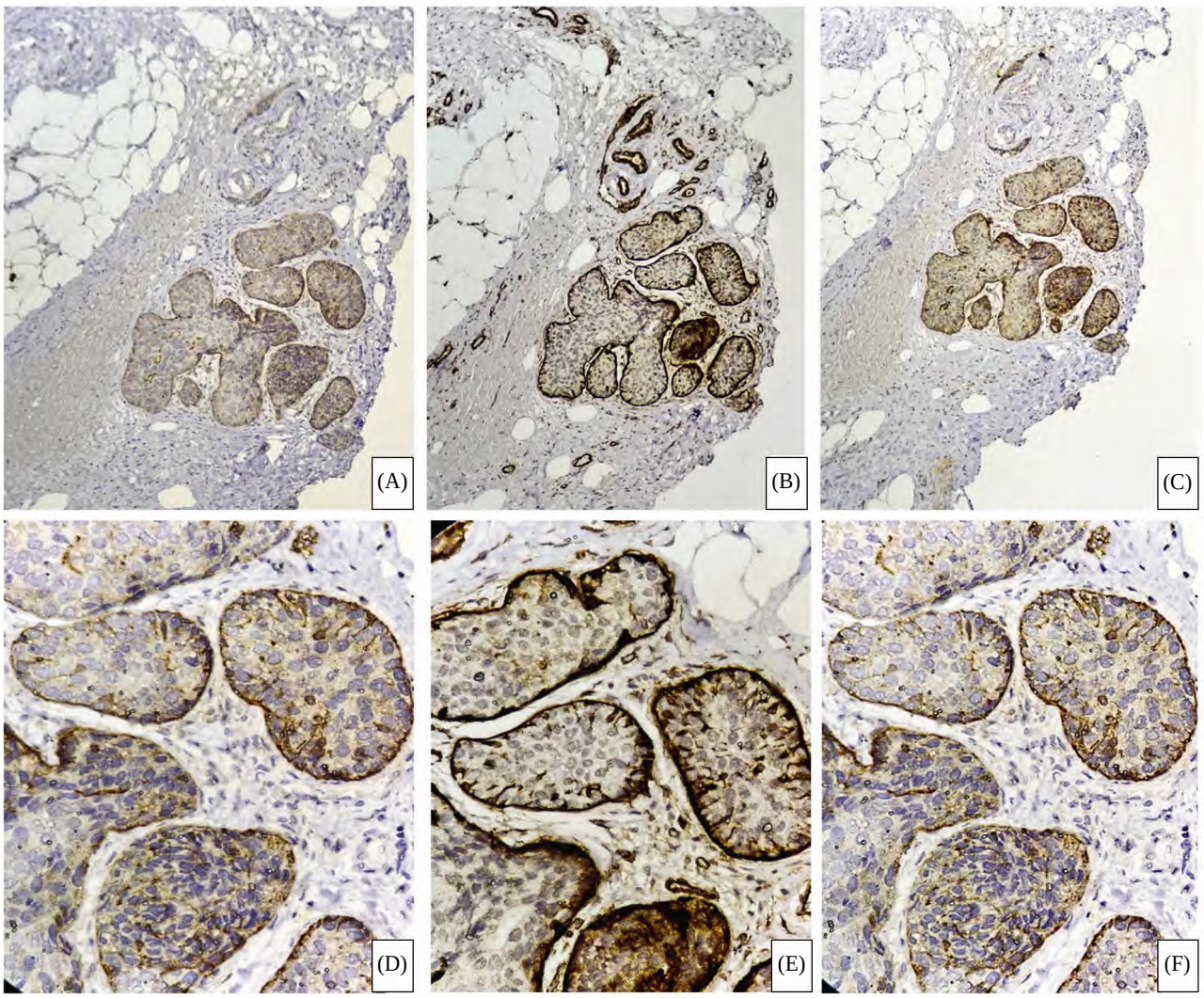

Fig. (2): Photomicrographs showing (A) and (B) DCIS with moderate (2+) DOG1 immunostaining in myoepithelial cells, in comparison with p63 immunostaining in myoepithelial cells (C) and (D), and SMA (E) and (F). [original magnification x40, x100, x40, x100, x40 and x100 respectively].

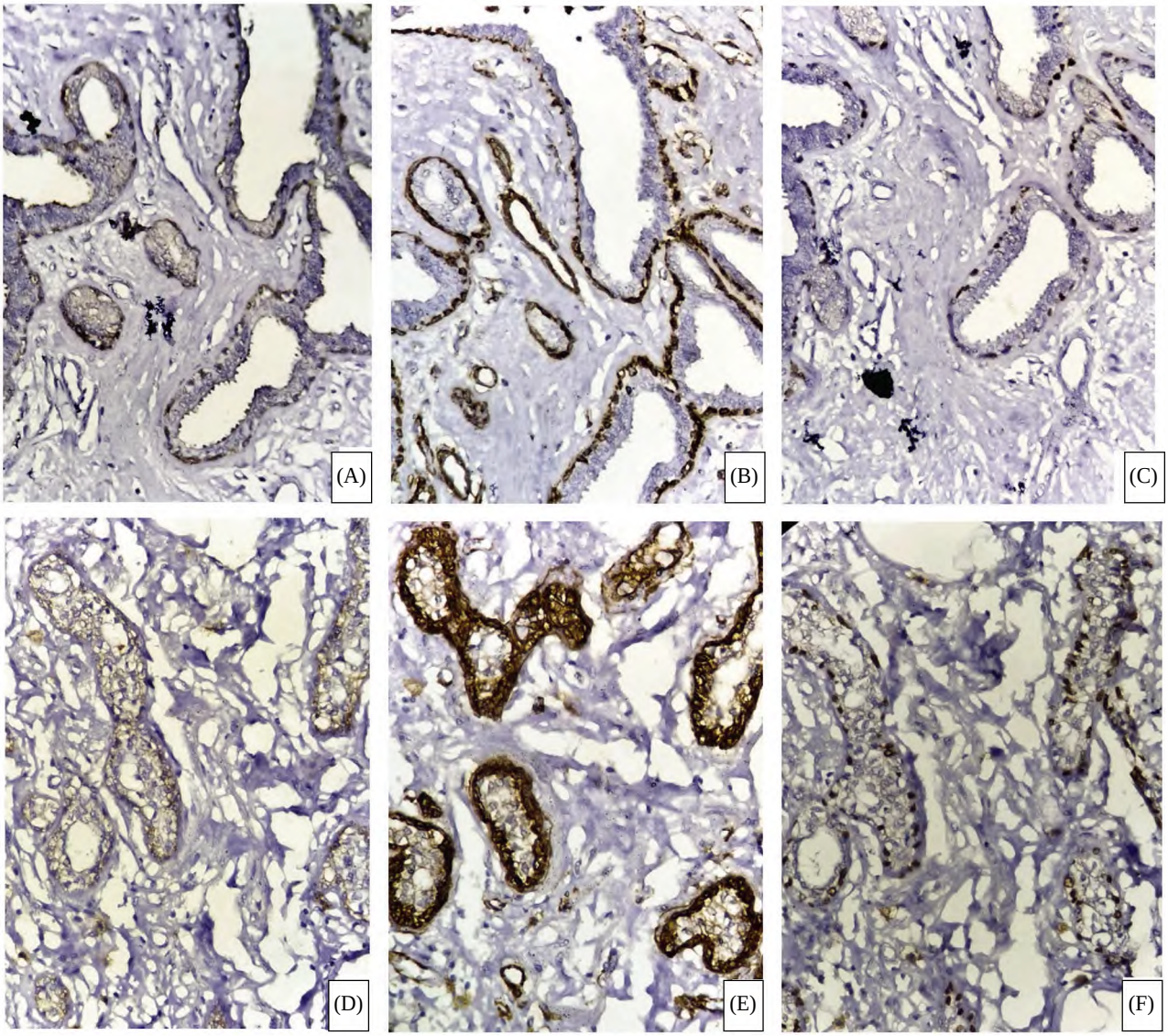

Fig. (3): Photomicrographs showing a case of benign fibrocystic disease / normal ducts with moderate 2+ (A) and faint 1+ (D) DOG1 immunostaining in myoepithelial cells, in comparison with SMA immunostaining in myoepithelial cells (B) and (E), and p63 (C) and (F). [original magnification x100 in all photos].

Table (1): Immunoreactivity of DOG1 in benign breast lesions and DCIS.

	DCIS and benign	Invasive carcinoma
SMA:		
Positive	60	0
Negative	0	30
P63:		
Positive	60	0
Negative	0	30
DOG1:		
Positive	60	0
Negative	0	30

Table (2): Intensity and H score of DOG1 in the Noninvasive (DCIS) and benign breast lesions.

Intensity:	
1	23
2	32
3	5
DOG1 expression	90 (70-90)
Median (IQR)	Range: 50-100
H score	100 (90-180)
Median (IQR)	Range: 50-270

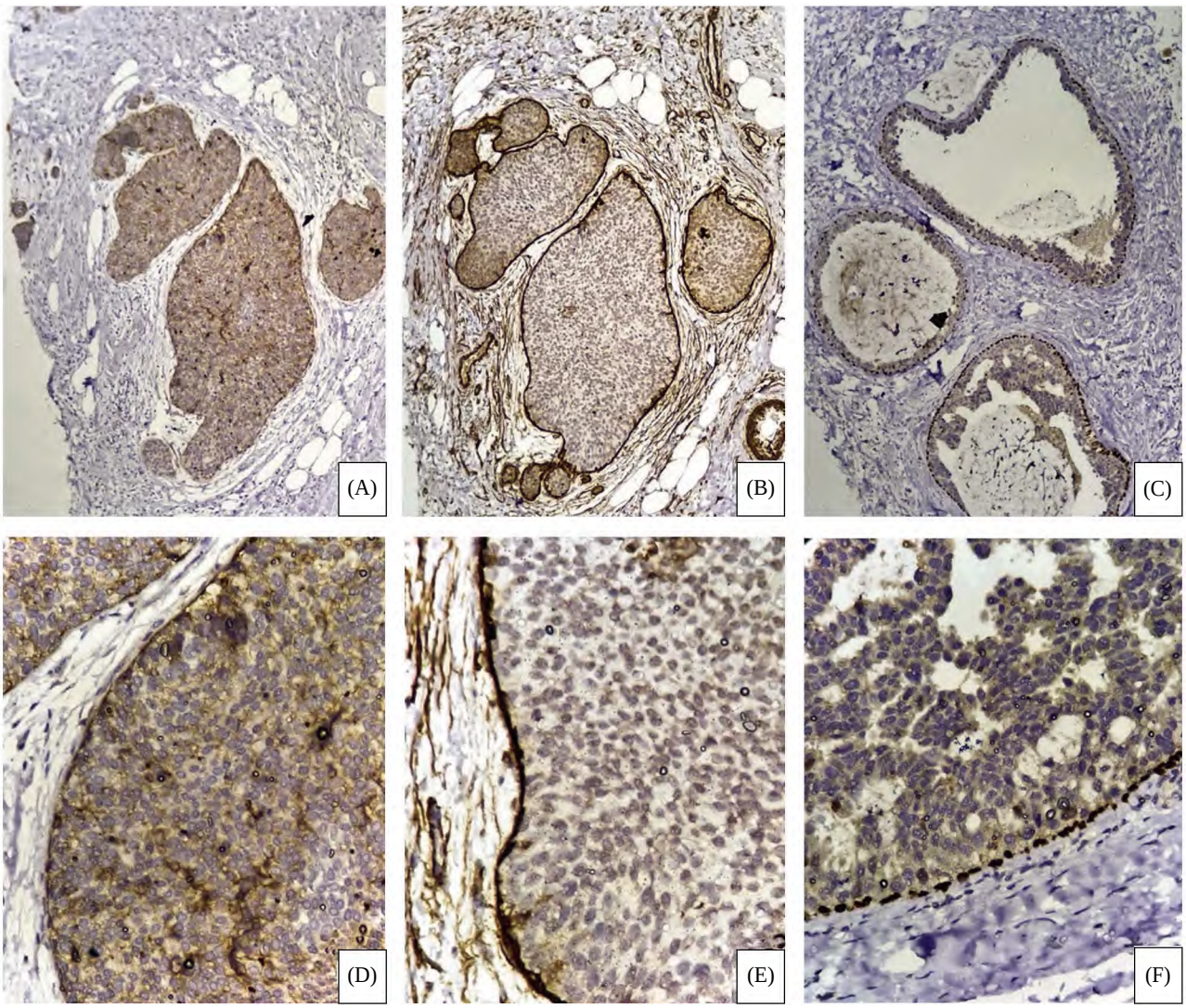

Fig. (4): Photomicrographs showing DCIS with moderate (2+) DOG1 immunostaining in myoepithelial cells (a) and (b), in comparison with SMA immunostaining in myoepithelial cells (C) and (D), and P63 (E) and (F). [original magnification x40, x100, x40, x100, x40 and x100 respectively].

Discussion

DOG1 has been investigated as MECs marker in salivary glands, lungs, and prostate, with little data on MECs of the breast. This study investigates the role of DOG1 as immunohistochemical marker of breast MECs. Ardeleanu C. et al. and Wong NA. discussed DOG1 reactivity in a variety of epithelial cells, including gastrointestinal tract, lung, pancreas, salivary gland, prostate, and kidney **[21,22]**.

Lopes LF. et al., was the first one who used DOG1 in breast lesions in a study reporting that 9 of 11 (81.8%) cases of fibroadenoma showed positive DOG1 staining in MECs **[23]**. Cheˆnevert J. et al., performed a comprehensive study of DOG1 expression in salivary tissue and reported that DOG1 is immunoreactive in both salivary serous acini and salivary tumors with intercalated duct differentiation **[24]**.

Cheng H. et al., with the only published study using DOG1 for differntiation between benign, invasive breast lesions and insitu lesions demonstrated significant differences in DOG1 expression between invasive carcinoma and adenosis or in situ carcinoma (p<0.05) and DOG1 was of great value distinguishing adenosis or intralobular extension of in situ carcinoma from invasive carcinoma or microinvasion, similar to calponin, SM-MHC, and P63 (p> 0.05). This study also reported a significant difference in DOG1 expression between intraductal papillary carcinoma and intraductal papilloma (p<0.05) **[20]**.

Our results showed DOG1 immunoreactivity in all benign breast lesions (30 cases - 100%), all cases of DCIS (30 cases - 100%), while all invasive breast lesions (5 cases of lobular carcinoma and 25 invasive duct carcinoma NOS) were negative to DOG1 staining (100%).

Recommendation:

We recommend testing DOG1 as myoepithelial marker in myoepithelial tumors to overview its confirmatory diagnostic role in benign and malignant myoepithelial cell derived lesions.

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دور DOG1 كعلامة جديدة للخلايا العضلية الظهارية فى أمراض الثدى: دراسة كيميائية مناعية

الخلايا العضلية الظهارية يمكن رؤيتها بسهولة فى قنوات الثدى الطبيعية، ولكن عندما تتوسع هذه الهيا كل أو يتم ضغطها، يكاد يكون من المستحيل التعرف عليها فى شرائح الهيماتوكسيلين والأيوسين وهذا هو سبب استخدام الصبغات المناعية الكيميائية

يتم استخدام العديد من الصبغات المحددة للخلايا العضلية الظهارية بشكل شائع. عُرف DOG1 فى البداية كعلامة لأورام اللحمة المعدية المعوية ولم تتم دراسته من قبل كعلامة للخلايا العضلية الظهارية للثدى.

تهدف هذه الدراسة إلى تقييم التعبير النسيجى المناعى لـ DOG1 فى أمراض الثدى الحميدة والخبيثة غير الغزوية والخبيثة الغزوية لتقييم فائدتها كعلامة جديدة لخلايا الظهارة العضلية.

تكونت هذه الدراسة من ٩٠ حالة : ثلاثون حميدة، ٣٠ سرطانة (سرطان القناة الغازية، تسلل سرطانى مفصص)، و ٣٠ سرطان ثدى غير غزوى.

تم تجميع العينات فى صورة كتل أنسجة مثبتة فى فورمالين وموضوعة فى مكعبات شمع بارافين من أرشيف قسم الباثولوجى، كلية الطب قصر العينى، جامعة القاهرة ومستشفى مدينة نصر للتأمين الصحى بالقاهرة، فى الفترة من يناير ٢٠١٣ إلى يناير ٢٠٢٠.

تم صبغة جميع الحالات بـ P63 و SMA كمقارنة معيارية.

تم تفسير النتائج باستخدام مقياس H (التقييم الدلالى لكل من شدة التلوين ونسبة الخلايا الإيجابية). أظهرت الحالات الحميدة إيجابية بنسبة ١٠٠٪ فى الخلايا العضلية الظهارية، وكان النسبة فى السرطان الموضعى (غير الغزوى) موجباً فى ١٠٠٪، إلا أن شدة التلوين والنسبة المئوية كانت متباينة. جميع الأورام الغازية لم تظهر أى صبغة.

يُعتقد أن DOG1 هو علامة وصبغة مناعية مفيدة للخلايا العضلية الظهارية فى الثدى مع حساسية وخصوصية ممتازة.