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Original Research Article

Immunohistochemical analysis of caveolin-1 antigen distribution in patients with malign and benign breast disease

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Abstract: Breast cancer is the most common cancer among women and most common cause of cancer related mortality and morbidity. Breast cancer oncogenesis has many pathways involved. Caveolin-1 is a component of the caveolae and is the key molecule involving strategic signalling molecules in the pathogenesis of breast cancer. The aim of the present study is to evaluate the distribution of Caveolin-1 antigen in specimens obtained from benign and malignant breast cancers. On this study tissue samples obtained from benign breast disease (n=203) and malignant breast disease (n=98) was subjected to immunohistochemical staining and positive areas separately for stromal components and epithelial component of the tumour.Caveolin-1 expression was 1746.78±981.89 and 1457.31±1331.91 in the benign and malignant tumour groups respectively. When the benign group was evaluated independently; it was observed that highest expression was observed in the sclerosing adenosis. Among the malignant breast cancer group invasive ductal carcinomas and mucinous carcinoma had the highest expression. Stromal comments were stained predominantly. Caveolin-1 expression loss was seen in malignant lesions. This suggests the tumour suppressor function of this gene for mammary carcinoma oncogenesis.
Keywords: Breast, Malign, Benign, Oncogenesis, Tumour suppressor gene, Caveolin-1, Tumour Stroma, Malign, Benign.

INTRODUCTION

Breast cancer is the most commonly encountered cancer and the leading cause of cancer-related mortality especially among the women [1]. According to the data obtained from the Turkish Statistical Institute; breast cancer constitutes 38.6% of the cancers observed among women. In early cases multimodality treatment provides a chance of cure to the patients however the advanced or recurrent cases have a very poor prognosis [2].

Cancer has a complex microenvironment that is made up of neoplastic epithelial cells, various stromal cells and extracellular matrix (ECM) [3-8]. Tumour microenvironment is currently very popular area of research in terms of its role in tumour development and progression. Sahin *et al.;* emphasized the changes in the tumour microenvironment following oncolytic viral therapy and emphasized the importance of tumour microenvironment in resistance to certain therapeutic strategies [3]. Alien *et al.;* have analysed the differential expression of certain proto-oncogenes in certain components of the stroma and found that myofibroblasts play a central role in the progression from normal tissue to ductal carcinoma in situ and to the invasive carcinoma [4]. Hu *et al.;* have reported that most of the changes in the gene expression have been due to epigenetic changes such as methylation in the target changes [5].Another study by Hu et al have shown that various pathways such as TGFβ, Hedgehog

and SMAD4 pathways were shown to be down regulated in the myofibroblasts leading to reduction in the overall number of myofibroblasts that resulted in progression from in situ to invasive carcinoma in experimental breast cancer models [6]. Tumour-associated stroma also elicits innate drug resistance in tumours and therefore is responsible for the aggressive behaviour of certain types of cancers [7, 8]. This intricate signalling between the tumour cell and reprogrammed tumour associated stroma is a very active area of research and prospectively will lead to development of new the targets for future specific therapies.

Caveola are small invaginations in the plasma membrane. Caveolin 1 is abundantly localized in the Caveola of the tumour cells [9]. Caveolin 1 is expressed in adipocytes, endothelial cells, fibroblasts, certain types of epithelial cells and myoepithelial cells. Caveolin-1 gene is located in the 7q31.1 that encodes a 21-24kDa protein that is located in the caveolar membrane [10]. The major functions of Caveolin 1 are membrane trafficking, gene regulation and signal transduction. It is especially important in the integrins with ERK/RAS pathway during tumorigenesis and progression in breast cancer. Although it is considered as a tumour suppressor gene its role in mammary tumorigenesis is not clearly defined [11].

Caveolin-1 has been postulated to be amplified in breast cancers and have been linked to poor prognosis. However, there are also clinical and preclinical reports that suggest inverse relationship with expression and malignant phenotype [12]. Current literature suggests that Caveolin-1 expression depends on the stage and the type of the tumours. Furthermore, its expression in the tumour stroma has also been investigated and its down regulation in the tumour stroma has been associated with recurrence, metastasis, tamoxifen resistance and therefore poor prognosis [13]. It is also shown to be over-expressed following adjuvant therapy, or aggressive tumour phenotype including those with features of the epithelial to mesenchymal transition [14].

On the other hand, Caveolin 1 has been linked to the tumour metabolism during carcinogenesis [15]. Hence Caveolin-1 increases aerobic glycolysis in the tumour associated fibroblasts and provides fuel for the tumour cells [14, 15]. Down-regulation of the Caveolin-1 has been linked to increased autophagy in the tumour tissue in order to recycle the substrates between the tumour cells. Furthermore, its loss in the tumour-associated stroma may also induce epithelial to mesenchymal transition [16].

Most of the information gathered regarding Caveolin-1 in breast cancer is usually gathered from preclinical models and human studies are very scarce. Therefore; in the present study it's aimed to investigate the Caveolin-1 antigen distribution in paraffin embedded samples from patients with benign and malignant breast tumours.

PATIENTS AND METHODS

Selection of the patients and data collection

The local ethics committee of Yildirim Beyazit University Training and Research Hospital approved the study. The samples were also evaluated retrospectively. Pathological stage of the disease and tumour characteristics is also recorded. Paraffin embedded tissue from patients with 203 benign breast (benign breast tumour group; BG) tumours, 98 breast cancers (malignant breast tumour group; MG) where used to evaluate the antigen distribution using immunohistochemistry staining. Data regarding the patients are summarized in Table 1. All the samples were obtained by informed consent of the patients or primary relatives.

Preparation of the tissues and immunohistochemistry for Caveolin-1

Each tissue block was cut in to 3µm sections y tissue microtome. Heat induced antigen retrieval using Tris EDTA was performed for 30 minutes. Blocking serum using mouse serum (Abcam; MA; USA). Primary antibody for Caveolin-1 (Abcam (ab17052); MA; USA) was used at a dilution of 1/100. The reporter molecule was alkaline phosphatase and the counterstaining was obtained by methyl green. The evaluation of the staining was obtained in ten consecutive 60 times magnified high power field (60Xhpf) with strong positivity areas and mean of all the evaluations were recorded. The same pathologist in the study evaluated all the specimens.

Statistical analysis

The continuous variables in the study were expressed as mean and standard deviation. Continuous variables among the groups were compared among the study groups with Student T test with confidence interval of 95%. Any p value<0.05 was considered significant. All statistical analysis was performed on SPSS version 22 (IBM, NY, USA).

RESULTS

The diagnoses of the patients with benign and

malignant breast tumours are summarized in Table 1. Most common benign breast tumour was fibroadenoma. On the other hand, the most common malignant breast tumour encountered was invasive ductal carcinoma Table 1. Caveolin-1 expression in the benign tumour group was 1746.78±981.89 areas/60Xhpf. The mean Caveolin-1 antigen distribution in the malignant breast tumour group was 1457.31±1331.91areas/60Xhpf lower than the benign breast tumour group. Benign breast tumour group had significantly higher Caveolin-1 antigen distribution when compared to the malignant breast tumour group (Figure 1). The mean Caveolin-1 antigen distribution among the groups is summarized in Table 2.

Table 2 summarizes the mean Caveolin-1 antigen distribution among different benign tumours. Each tumour type in the benign breast tumour group was significantly different than the other tumours in terms of the Caveolin-1 antigen distribution. When the benign group was evaluated independently; it was observed that highest expression was observed in the sclerosing adenosis tumours with a mean Caveolin-1 distribution of 3069.33±1622.36 areas/60Xhpf ($p<0.05$) (Table 2) (Figure 2). Fibroadenoma had the second highest Caveolin-1 antigen distribution which was 2950±472.58 areas/60X hpf (Figure 2).

When the malignant breast tumour group was evaluated independently; invasive ductal carcinomas had the highest Caveolin-1 antigen distribution (4350±1748.99 areas/60Xhpf) when compared other tumour types ($p<0.05$) (Table 2) and (Figure 3). Mucinous carcinoma had the second highest Caveolin-1 antigen distribution with a mean Caveolin-1 antigen distribution of 3750±1767.77 areas/60Xhpf (Figure 3). Mean Caveolin-1 antigen distribution in other malignant tumour types is summarized in Table 2 and Figure 3. Caveolin-1 was found to be mainly expressed in the stroma of the tumours.

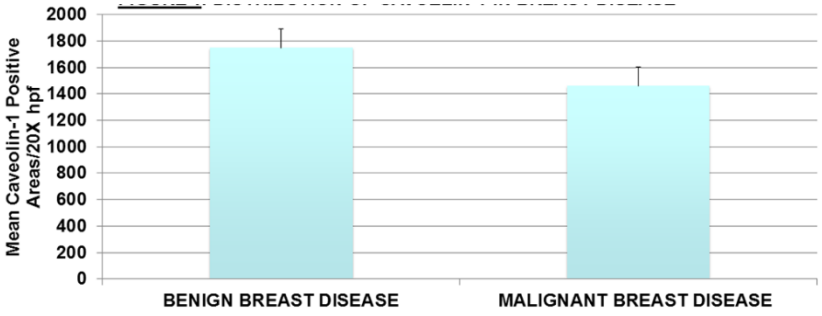

Fig 1: Distribution of cavoelin-1 in breast disease

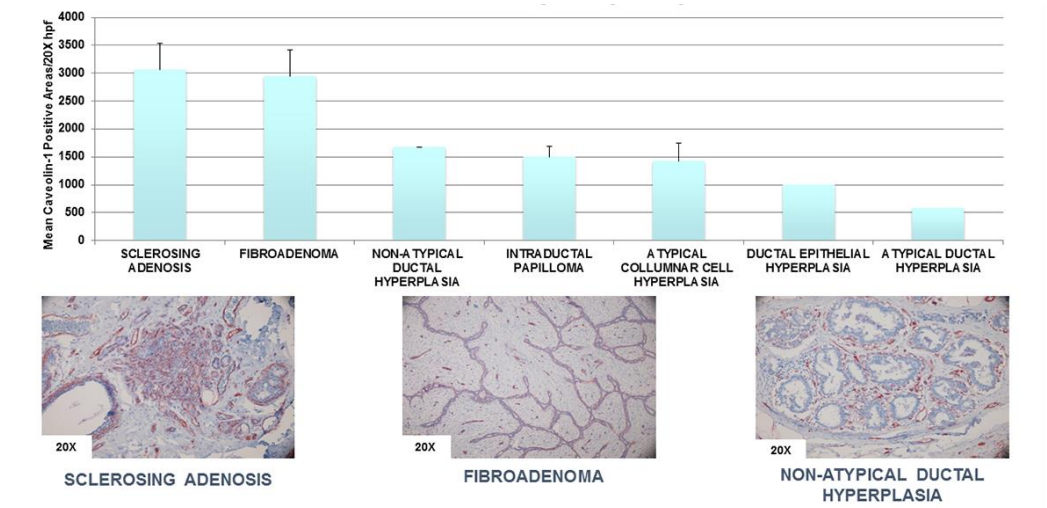

Fig 2: Distribution of Caveolin-1 antigen among the benign breast disease

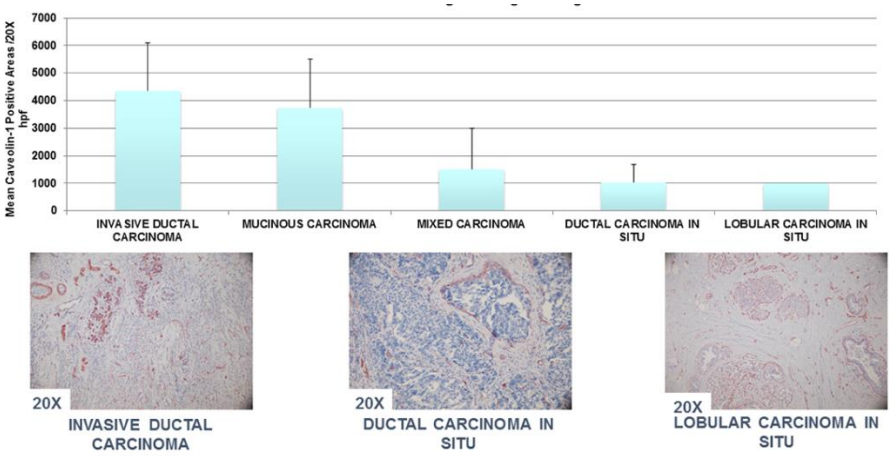

Fig 3: Distribution of Caveolin-1 antigen among the malignant breast disease

Table 1: The histopathologic diagnosis of the patients included in the study

Malignant Breast Disease	**N=98**
Invasive Ductal Carcinoma	26
Mixed Carcinoma	12
Mucinous Carcinoma	8
Cribriform Carcinoma	2
Medullary Carcinoma	2
Tubular Carcinoma	4
Ductal Carcinoma in situ	38
Lobular carcinoma in situ	6
Benign Breast Disease	**N=203**
Fibroadenoma	10
Atypical Ductal Hyperplasia	2
Non-atypical Ductal Carcinoma	78
Intraductal Papilloma	1
SclerosingAdenosis	30
Ductal Epithelial Hyperplasia	36
Atypical Columnar Cell Hyperplasia	46

Table 2: Summary of the Caveolin-1 antigen distribution according to groups

BREAST DISEASE	**MEAN CAVEOLIN-1 POSITIVE AREA /20X hpf**	**STANDARD DEVIATION**
Benign breast disease	1746.78	981.89
Sclerosing adenosis	3069.33	1622.36
Fibroadenoma	2950	472.58
Non-atypical ductal hyperplasia	1671.54	466.36
Intraductal papilloma	1500	0
Atypical columnar cell hyperplasia	1427.83	184.26
Ductal epithelial hyperplasia	994.74	315.93
Atypical ductal hyperplasia	575	35.36
Malignant breast disease	1458.31	1331.91
Invasive ductal carcinoma	4350	1748.99
Mucinous carcinoma	3750	1767.77
Mixed carcinoma	1500	1500
Ductal carcinoma in situ	1042.39	646.25
Lobular carcinoma in situ	993.75	509.51

DISCUSSION

Breast cancer is the most frequent cancer in the females and furthermore it is the most frequent cause of cancer related death among the women. As in any cancer it causes serious physiologic and psychologic disability in the affected individuals [17].

Caveola are membrane invaginations controlling cellular events such as endocytosis, transcytosis, transport of various molecules and cell migration and etc. Caveolins are the family of proteins that are found extensively in the caveolar membrane [18]. Breast carcinogenesis includes multiple pathway of development and one of the pathways involves the deregulated expression of Caveolin 1 in the cancer cells and stromal tissues. Furthermore, re-expression of Caveolin-1 in the cancer cells inhibits oncogenesis and migration [19]. Therefore, the main action of Cavolin-1 seems to be tumour suppressor effect. It is mainly located at 7q31.1 loci and interestingly it is usually found to be deleted in breast cancers [20, 21]. Caveolin is reported to interact with many key pathways that take active part in the breast cancer pathway. Among these pathways are the Src family pf proteins, H-Ras, epidermal growth factor receptor, HER2, oestrogen receptor, MAPK pathway an endothelial nitric oxide synthase [18, 22, 23].

The role of Caveolin-1 has been subject to active debate; usually down regulation of the Caveolin-1 had been linked to enhanced lymph node metastasis [24]. Furthermore; progression in situ carcinoma to invasive ductal carcinoma had been found by down regulation of Caveolin-1 and stromal monocarboxylate transporter 4 (MCT4) in tissues obtained from patients with breast cancers [25].

In accordance to the preclinical studies in the present preliminary study we found the expression of Caveolin-1 was reduced in the breast cancers when compared to benign tumours. Since our study is a preliminary study to determine any targets to further analyse we did not make any further analysis such as quantitative expression analysis of the Caveolin-1 gene. However, this is valuable study for actual patent tissues have been analysed and we have analysed the expression and tissue scatter pattern in the Turkish population. This contradicts with the study performed by Liedtke et al who reported reduced Caveolin-1 expression via tissue microarray in benign diseases such as fibroadenoma, sclerosing adenosis, ductal hyperplasia, radial scar and even ductal carcinoma in situ. However; they reported increased Caveolin- 1 expression in 32 of 109 cases [26]. A study by Williams et al had shown that loss of Caveolin-1 expression hard shown progression of mammary tumours from dysplasia to invasive carcinoma in a transgenic animal model for breast cancer [27].

In the present study the overall Caveolin-1 antigen distribution was more pronounced in the benign breast disease when compared with malignant breast disease. Among the benign breast disease in the present study sclerosing adenosine and fibroadenoma had the highest expression for Caveolin-1. This suggests that Caveolin-1 is especially concentrated in lesions with higher stromal component. In the malignant breast disease invasive ductal carcinoma and mucinous carcinoma had the highest carcinoma. In a study by Savage *et al.;* had found that concurrent Caveolin-1 amplification and over-expression basal-like and metaplastic type breast carcinoma [28].

All the studies report stromal and weak epithelial staining for Caveolin-1 from the humoral tissue [24-28]. In the present study we have found strong myoepithelial staining and negligible epithelial staining. However, since the Caveolin-1 staining was reduced in malignant breast disease in our study; it is suggestive of the tumour suppressor function of the gene. Most probably storm plays a very important role in prevention of progression of malignant process of malignant transformation from the precursor lesions, which are mostly in concordance with current literature [26-28].

CONCLUSION

The study is on-going further evaluating the expression and role in patient survival and prognosis. Furthermore, effect of stage on Caveolin-1 antigen distribution. Hence the results of the present study suggest that its expression seems to be reduced in malignant transformation. Furthermore, its expression is reduced in benign tumours with atypia with respect to tumours without atypia. All these data suggest the tumour suppressor effect of Caveolin-1 in mammary carcinogenesis. However further multi-centric studies with higher patient numbers are needed.

Disclosure of conflict of interest: The authors report no conflict of interest.

REFERENCES

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA: a cancer journal for clinicians. 2010 Sep 1; 60(5):277-300.
2. Place AE, Huh SJ, Polyak K. The microenvironment in breast cancer progression:

biology and implications for treatment. Breast cancer research. 2011 Nov 1; 13(6):227.
3. Sahin TT, Kasuya H, Nomura N, Shikano T, Yamamura K, Gewen T, Kanzaki A, Fujii T, Sugae T, Imai T, Nomoto S. Impact of novel oncolytic virus HF10 on cellular components of the tumor microenviroment in patients with recurrent breast cancer. Cancer gene therapy. 2012 Apr 1; 19(4):229-37.
4. Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S. Molecular characterization of the tumor microenvironment in breast cancer. Cancer cell. 2004 Jul 31; 6(1):17-32.
5. Hu M, Yao J, Cai L, Bachman KE, van den Brûle F, Velculescu V, Polyak K. Distinct epigenetic changes in the stromal cells of breast cancers. Nature genetics. 2005 Aug 1; 37(8):899-905.
6. Hu M, Yao J, Carroll DK, Weremowicz S, Chen H, Carrasco D, Richardson A, Violette S, Nikolskaya T, Nikolsky Y, Bauerlein EL. Regulation of in situ to invasive breast carcinoma transition. Cancer cell. 2008 May 6; 13(5):394-406.
7. Togo S, Polanska UM, Horimoto Y, Orimo A. Carcinoma-associated fibroblasts are a promising therapeutic target. Cancers. 2013 Jan 31; 5(1):149-69.
8. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. cell. 2011 Mar 4; 144(5):646-74.
9. Lamaze C, Torrino S. Caveolae and cancer: A new mechanical perspective. Biomed J. 2015 Sep 1; 38(5):367-79.
10. Popovska SL, Ivanov IN, Ooi A. Caveolin-1 in Tumour Cells is Predominately Expressed in the Basal Subtype of Triple-Negative Breast Carcinoma. Journal of Biomedical and Clinical Research. 2014 Nov 1; 7(1):8-14.
11. Bouras T, Lisanti MP, Pestell RG. Caveolin in breast cancer. Cancer biology & therapy. 2004 Oct 1; 3(10):931-41.
12. Mercier I, Lisanti MP. Caveolin-1 and breast cancer: a new clinical perspective. Caveolins and Caveolae. 2012:83-94.
13. Witkiewicz AK, Dasgupta A, Sotgia F, Mercier I, Pestell RG, Sabel M, Kleer CG, Brody JR, Lisanti MP. An absence of stromal caveolin-1 expression predicts early tumor recurrence and poor clinical outcome in human breast cancers. The American journal of pathology. 2009 Jun 30; 174(6):2023-34.
14. Bailey KM, Liu J. Caveolin-1 up-regulation during epithelial to mesenchymal transition is mediated by focal adhesion kinase. Journal of Biological Chemistry. 2008 May 16; 283(20):13714-24.
15. Sotgia F, Martinez-Outschoorn UE, Howell A, Pestell RG, Pavlides S, Lisanti MP. Caveolin-1 and cancer metabolism in the tumor microenvironment: markers, models, and mechanisms. Annual Review of Pathology: Mechanisms of Disease. 2012 Feb 28; 7:423-67.
16. Pavlides S, Tsirigos A, Migneco G, Whitaker-Menezes D, Chiavarina B, Flomenberg N, Frank PG, Casimiro MC, Wang C, Pestell RG, Martinez-Outschoorn UE. The autophagic tumor stroma model of cancer: Role of oxidative stress and ketone production in fueling tumor cell metabolism. Cell cycle. 2010 Sep 1; 9(17):3485-505.
17. Siegel R, Ma J, Zou Z, Jemal J. Cancer statistics, 2014. CA Cancer J Clin. 2014;64(1):9-29.
18. Kiss AL. Caveolae and the regulation of endocytosis. AdvExp Med Bill. 2012;729:14-28.
19. Anwar SL, Wahyono A, Aryandono T, Haryono SJ. Caveolin-1 in breast cancer: single molecule regulation of multiple key signaling pathways. Asian Pac J Cancer Prev. 2015; 16(16):6803-12.
20. Williams TM, Lisanti MP. Caveolin-1 in oncogenic transformation, cancer, and metastasis. American Journal of Physiology-Cell Physiology. 2005 Mar 1; 288(3):C494-506.
21. Williams TM, Lisanti MP. The caveolin proteins. Genome biology. 2004 Mar 1; 5(3):214.
22. Park JH, Lee MY, Han HJ. A potential role for caveolin-1 in estradiol-17β-induced proliferation of mouse embryonic stem cells: involvement of Src, PI3K/Akt, and MAPKs pathways. The international journal of biochemistry & cell biology. 2009 Mar 31; 41(3):659-65.
23. Gupta R, Toufaily C, Annabi B. Caveolin and cavin family members: dual roles in cancer. Biochimie. 2014 Dec 31; 107:188-202.
24. Folgueira MA, Maistro S, Katayama ML, Roela RA, Mundim FG, Nanogaki S, de Bock GH, Brentani MM. Markers of breast cancer stromal fibroblasts in the primary tumour site associated with lymph node metastasis: a systematic review including our case series. Bioscience reports. 2013 Dec 1; 33(6):e00085.
25. Martins D, Beça F, Sousa B, Baltazar F, Paredes J, Schmitt F. Loss of caveolin-1 and gain of MCT4 expression in the tumor stroma: key events in the progression from an in situ to an invasive breast carcinoma. Cell Cycle. 2013 Aug 15; 12(16):2684-90.
26. Liedtke C, Kersting C, Bürger H, Kiesel L, Wülfing P. Caveolin-1 expression in benign and malignant lesions of the breast. World journal of surgical oncology. 2007 Oct 3; 5(1):110.

27. Williams TM, Cheung MW, Park DS, Razani B, Cohen AW, Muller WJ, Di Vizio D, Chopra NG, Pestell RG, Lisanti MP. Loss of caveolin-1 gene expression accelerates the development of dysplastic mammary lesions in tumor-prone transgenic mice. Molecular biology of the cell. 2003 Mar 1; 14(3):1027-42.
28. Savage K, Lambros MB, Robertson D, Jones RL, Jones C, Mackay A, James M, Hornick JL, Pereira EM, Milanezi F, Fletcher CD. Caveolin 1 is overexpressed and amplified in a subset of basal-like and metaplastic breast carcinomas: a morphologic, ultrastructural, immunohistochemical, and in situ hybridization analysis. Clinical Cancer Research. 2007 Jan 1; 13(1):90-101.