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Original Article

Evaluation of annexin A1 expression in lung, breast, colon, and prostatic adenocarcinomas and in tumor microenvironment

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ABSTRACT

Objectives: Annexin A1 (ANXA1) which plays a role in tumor development and metastasis has been reported to be an effective regulator for tumor stroma and interacts with different components in the tumor microenvironment. The role of ANXA1 in tumorigenesis has not been fully understood. One of the main reasons for this is the great variability of ANXA1 expression in malignant tumors across different tumor types.

Materials and Methods: Archived hematoxylin-eosin stained preparations of lung adenocarcinoma, breast invasive ductal carcinoma, colonic adenocarcinoma, and prostatic acinar carcinoma were re-evaluated and tumor regions to be analyzed with the tissue microarray method were determined. The ANXA1 expressions between the tumors and tumor microenvironment were evaluated immunohistochemically.

Results: ANXA1 expression was decreased in the lung, breast, colon, and prostate adenocarcinomas. The most prominent staining was seen in lung adenocarcinoma cases. There was no statistically significant difference between the tumors in terms of ANXA1 staining ($P > 0.05$). ANXA1 was shown to be a more stained tumor microenvironment than in the tumor. Statistically significant staining with ANXA1 between within tumor and tumor microenvironment was observed in breast adenocarcinomas ($P < 0.05$). Our study showed differences between ANXA1 expression in different cancers, in tumor cells, and tumor microenvironment.

Conclusion: Considering the effects of ANXA1 on tumor development and metastasis, a potential use as a biomarker may be suggested. Particularly, in breast adenocarcinomas, the high expression of ANXA1 in the tumor microenvironment supports the notion that it could induce the tumor stroma response.

Keywords: Annexin A1, Breast, Colon, Prostate, Lung adenocarcinomas, Tumor, Tumor microenvironment

INTRODUCTION

Annexins comprise a Ca^{+2}-dependent phospholipid-binding protein family. This family is constituted of 13 members with biological and structural differences. Some members of the annexin family have mediator and regulator roles in extracellular and cellular matrix and function as anti-inflammatory and anticoagulant compounds.[1-3] Annexin A1 (ANXA1) has a molecular weight of 35–440 kDA and can be found in the nucleus, cytoplasm, and plasma membrane of certain cell types. It is known to be involved in the regulation of the extracellular inflammatory response, immune response, and apoptosis, in cell growth and differentiation, and in the pathogenesis of cancer.[4-8] Human tumor studies on the functional role of ANXA1 in cancer biology have reported that ANXA1 plays a tumor suppressor or promoter role depending on the tumor type.[7,8] ANXA1 expression has been investigated in various tumor types, but its role in tumorigenesis could not be clarified. This main cause is the great variability of ANXA1 expression in malignant tumors across different tumor types.[1] ANXA1 is expressed at high rates in certain malignant tumors such as hepatocellular, pancreatic, lung, and breast carcinomas.[6] Some studies have revealed a connection between lower ANXA1 expression and poor prognosis and cancer progression.[1] Previous studies on lung adenocarcinomas have reported high ANXA1 expression to be closely related to short survival times and lymphatic invasion.[6,9] Changes in ANXA1 expression in breast cancer indicate a potential effect on tumorigenesis and metastatic processes.[1] Studies exist that have reported ANXA1 to have varying effects on certain host cell types

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including breast tumor cells, and stromal immune and structural cells, particularly in the context of immune regulation of cancer.[1]

Elevated ANXA1 expression was reported in colorectal carcinomas in comparison to normal colon mucosa, although its role remains unclear. However, this could not be linked to the tumor stage.[5,8] While ANXA1 expression increases in proliferated colorectal carcinoma cells, it remains unchanged in non-proliferating or non-cancerous cells.[8]

Tumors are heterogenous masses that include both malignant tumor cells and stromal cells. The role of the tumor stroma in tumor progression and metastatic ability has gained increasing importance. Tumor stroma consists of the extracellular matrix, which constitutes the supportive tissue surrounding the tumor, fibroblasts, endothelial cells, immune cells, and inflammatory cells.[10] ANXA1 is reported to have a proangiogenic role in vascular endothelial cell sprouting.[10] It was detected in vascular endothelial cells in certain carcinomas.[11] ANXA1, which is involved in tumor growth and metastasis, was described to be an effective regulator of the tumor stroma and to interact with different components in the tumor microenvironment.[10,12]

This study compared levels of ANXA1 expression in tumor cells and the tumor microenvironment in lung, breast, colon, and prostatic adenocarcinomas.

MATERIALS AND METHODS

Patients

An Ethics Committee approval was obtained for the study (2019/42). Report archives of the Pathology Department at Inonu University Faculty of Medicine were scanned, and clinical and histopathological data from patients with lung adenocarcinoma (*n*: 30), breast invasive ductal carcinoma (*n*: 30), colonic adenocarcinoma (*n*: 30), and prostatic acinar carcinoma (*n*: 30) were evaluated.

Histopathological study

Archived hematoxylin-eosin stained preparations of selected patients were re-evaluated and tumor regions to be analyzed with the tissue microarray (TMA) method were determined. Determined regions were extracted from the donor paraffin block using a 3-mm punch (Harris uni-core, catalog no: 69036-30 electron microscopy sciences). TMA grafts extracted from the donor paraffin blocks were transferred to recipient paraffin blocks that had been prepared using the TMA paraffin block apparatus. Slides of 4 µm thickness were obtained from the prepared TMA paraffin blocks (Leica RM 2255 microtome). For immunohistochemical (IHC) analysis, preparations were stained with the ANXA1 antibody (Catalog no 6362. Mouse monoclonal antibody. Bio SB 69 Santa Felicia Dr. Santa Barbara, CA 93117, USA) in a Ventana Benchmark XT IHC staining device. Staining was performed using the Ventana antibody diluent (catalog no: 251–018) and Ventana UltraView Universal DAB detection kit (catalog no: 760-500 Ventana Medical Systems, Inc. A Member of the Roche Group 1910 Innovation Park Dr. Tucson, AZ 85755 USA). Slides were examined using a light microscope (Olympus BX-51; Olympus, Tokyo, Japan). Images were captured using a digital DP70 connected to a BX-51 microscope (Olympus).

In the IHC evaluation, ANXA1 expression was evaluated in external positive control. Slides were evaluated by considering the prevalence of nuclear and cytoplasmic stainings. Each specimen was scored according to the density of staining and the area percentage of staining. Nuclear and cytoplasmic staining density signals in cancer tissue and tumor microenvironment were rated as +3; strong staining, +2; medium staining, +1; weak staining, and 0; no staining. The area percentage of staining was evaluated as follows: 0; no staining of cells in any microscopic fields, 1+;<30% of tissue stained positive, 2+; between 30% and 60% stained positive, and 3+; >60% stained positive. Finally, the staining density score was calculated. The degree of staining density and the percentage of staining were multiplied. A combined staining score (density percentage of staining) of ≤3 was considered to be low expression; a score between 4 and 6 was considered to be moderate expression; whereas a score between 7 and 9 was considered to be high expression.[8,12,13]

Statistical analysis

Data were evaluated using SPSS 25.0 commercial statistics program. The difference in ANXA1 expression among the groups was evaluated using a non-parametric Spearman's correlation test in all groups. The level of significance was accepted as $P < 0.05$.

RESULTS

Cases of lung, breast, colon, and prostatic adenocarcinoma included in the study showed decreased expression of ANXA1 in tumors, and lower staining was observed within the tumor than in the tumor microenvironment. Among tumors, cases of lung adenocarcinoma manifested the most pronounced staining [Figure 1]. Other tumors demonstrated a more marked decrease in expression compared to that in the lung [Figures 2-4]. However, ANXA1 staining in lung adenocarcinomas was not statistically different from that in breast, colon, and prostatic adenocarcinomas [Table 1]. ANXA1 showed less staining in the tumor than in the tumor microenvironment. In many cases, there was a decrease or loss of ANXA1 expression in the tumor while inflammatory and stromal cells were stained in the tumor microenvironment. When the groups were compared, inflammatory and

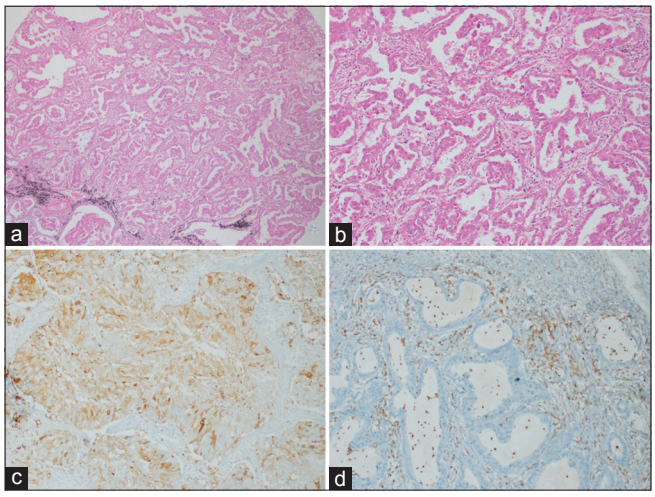

Figure 1: Lung adenocarcinoma and Annexin A1 (ANXA1) immunostaining. (a and b) Hematoxylin and eosin sections, ×40, ×100. (c) ANXA1 expression in lung adenocarcinoma tissues, ×200. (d) Decreased ANXA1 expression in the tumor. Moderate expression in tumor microenvironment in lung adenocarcinoma tissues. Original magnification, ×200.

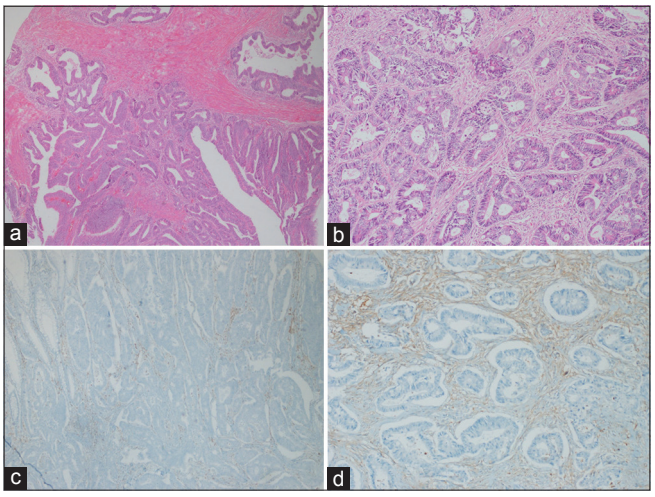

Figure 3: Colon adenocarcinoma and Annexin A1 (ANXA1) immunostaining. (a and b) Hematoxylin and eosin sections, ×40, ×100. (c) Prominent loss of ANXA1 expression in colon adenocarcinoma, ×100 (d) Decreased ANXA1 expression in colon adenocarcinoma, prominent expression in the tumor microenvironment, Original magnification, ×200.

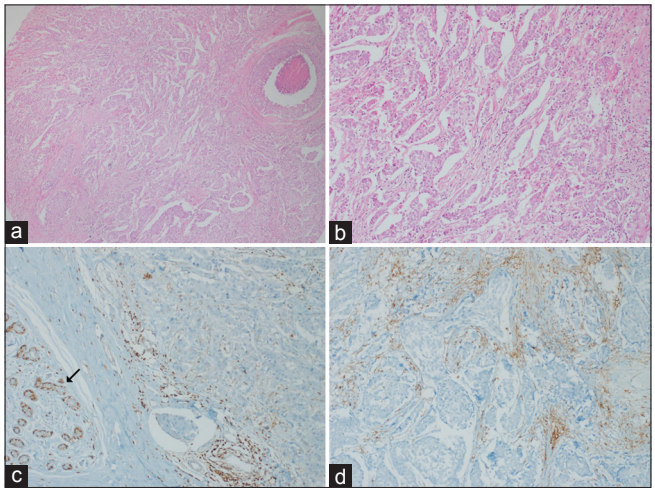

Figure 2: Breast carcinoma and Annexin A1 (ANXA1) immunostaining. (a and b) Hematoxylin and eosin sections, ×40, ×100. (c) ANXA1 expression in benign breast parenchyma (arrow). Decreased ANXA1 expression in breast adenocarcinoma, ×200 (d) Decreased ANXA1 expression in breast adenocarcinoma, prominent expression in the tumor microenvironment. Original magnification, ×200.

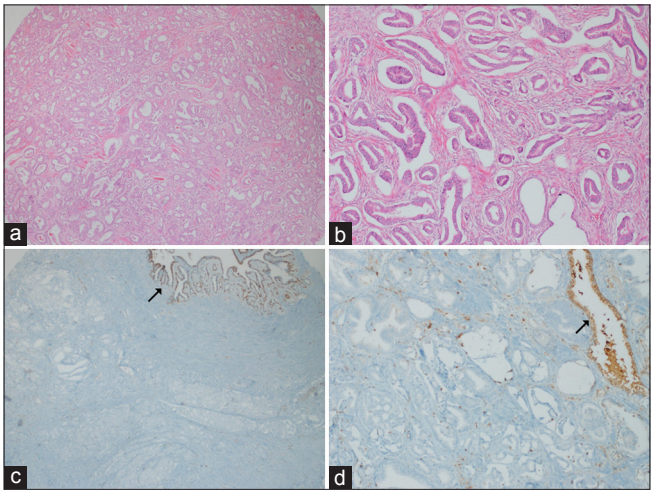

Figure 4: Prostate adenocarcinoma and Annexin A1 (ANXA1) immunostaining. (a and b) Hematoxylin and eosin sections, x40, ×100. (c) ANXA1 expression in benign prostate parenchyma(arrow). Prominent loss of ANXA1 expression in prostatic adenocarcinoma ×200 (d) ANXA1 expression in benign prostate parenchyma (arrow). Decreased ANXA1 expression in prostatic adenocarcinoma and negative ANXA1 immunostaining in the tumor microenvironment, Original magnification, ×200.

stromal responses were prominent in breast and colon adenocarcinomas.

Breast adenocarcinomas showed a statistically significant difference between tumor cells and the microenvironment in terms of the ANXA1 staining score ($P < 0.05$, r = 0.0391). There was no statistically significant difference between tumor cells and the microenvironment in terms of ANXA1 staining in lung, colon, and prostatic adenocarcinomas.

DISCUSSION

Tumor cell behaviors such as migration, invasion, proliferation, and epithelial-mesenchymal transition (EMT) are associated with varying ANXA1 expression levels. Differences between ANXA1 expression within the tumor and in the tumor microenvironment are important for the growth and metastasis of various tumors. ANXA1 expression

Table 1: ANXA1 staining scores for the tumor and the tumor microenvironment in lung, breast, colon, and prostate adenocarcinomas.

Adenocarcinomas	Tumor staining score Mean±SD	Tumor microenvironment staining score Mean±SD	*P* and r values
Lung	1.33±1.155	1.53±0.937	r=0.187 *P*=0.323
Breast	0.13±0.346	1.13±0.9	r=0.391 *P*=0.032*
Colon	0.03±0.183	1.57±0.935	r=0.114 *P*=0.55
Prostat	0.57±0.858	0.97±0.615	r=0.098 *P*=0.607

Spearman's Rank-order correlation, *$P<0.05$. ANXA1: Annexin A1, SD: Standard deviation

shows variability depending on the pathological type of cancer.[7] The expression is downregulated in certain types of cancer, while, in others, it is upregulated. We investigated differences in ANXA1 expression in various carcinomas. ANXA1 expression was determined to be particularly higher in lung adenocarcinomas compared to breast, colon, and prostate carcinomas. Biaoxue *et al*.[14] found that ANXA1 was upregulated in lung cancer patients and proposed that this was linked to an unfavorable prognosis and lymphatic metastasis. Moreover, ANXA1 upregulation in tumor cell cytoplasm was shown to contribute to cancer progression.[14] Our study also showed a difference between ANXA1 expression levels within the tumor and its environment in lung adenocarcinomas. When ANXA1 expressions in tumor cells and environment were compared in lung adenocarcinomas with regard to the degree of staining in stromal cells, greater staining was determined in the tumor environment. ANXA1 was suggested to be involved in signal transmission between tumor cells and stromal cells in the tumor microenvironment.[12] In our study, breast tumors observed a significant difference between levels of ANXA1 expression in epithelial and stromal cells. Tu *et al*.[7] reported that ANXA1 expression in invasive breast carcinomas was decreased in epithelial areas and increased in stromal cells. ANXA1 is an endogenous anti-inflammatory protein that is involved in various cellular functions such as inflammation, phagocytosis, proliferation, and apoptosis.[15] The immune system controls tumor cell production and the escape of tumor cells from the silent state. When homeostasis is disrupted, the immune system is also functionally disrupted, and tumor cells may escape elimination. The role of ANXA1 in the modulation of immune response to tumors has not been clarified.[7] ANXA1 is reported to play a role in the apoptosis of inflammatory cells.[16] However, its effects on immune system cells are still subject to discussion. ANXA1 has been reported to contribute to the proliferation and differentiation of T lymphocytes in some studies,[7,17,18] while ANXA1 suppresses T-cell activation.[19-21] Suppression of dendritic cell activation eliminates the recruitment of CD8+ T-cells, resulting in the development of tumor cells. The presence of ANXA1 in the tumor microenvironment both induces the secretion of tumor toxic mediators from dendritic cells and attenuates interferon-gamma secretion from CD4+ T-cells. When the effects of ANXA1 on macrophages in the tumor immune microenvironment were investigated, ANXA1 was found to induce M2 polarization[22] and inhibit M1 phenotype polarization.[7]

Apart from immune system cells, the tumor microenvironment consists of stromal and endothelial cells. Particularly, cancer-associated fibroblasts (CAF) are known to induce tumor growth and contribute to invasion and metastasis. The effects of ANXA1 on CAFs also remain unclear. Geary *et al*.[23] reported that high ANXA1 levels in prostatic CAFs contributed to EMT by gaining prostatic tumor cells with stem cell-like qualities. In our study, prostatic carcinomas showed greater ANXA1 staining in the tumor microenvironment than in the tumor. While a decrease in ANXA1 expression was observed in prostate cancers, expression was reported in prostatic stromal cells.[10] In accordance with this finding, our study observed a decrease in expression in prostate carcinomas. Patton *et al*.[24] determined differences in ANXA1 expression among the histological grades of prostatic adenocarcinomas. They determined that ANXA1 expression was lower in moderate- and high-grade prostate tumors and, therefore, suggested that it contributed to tumor progression.

CONCLUSION

Our study showed differences between levels of ANXA1 expression in different cancers, in tumor cells, and tumor microenvironments. Considering the effects of ANXA1 on tumor development and metastasis, a potential use as a biomarker may be suggested. Particularly, in breast adenocarcinomas, the high expression of ANXA1 in the tumor microenvironment supports the notion that it could induce the tumor stroma response.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of ınterest.

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