Differential Expression of Annexin A1 Protein in Colorectal, Lung, and Liver Cancer Tissues

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Abstract

Background: Annexin A1 (ANXA1) is a phospholipid-binding protein involved in mediating the anti-inflammatory effects of glucocorticoids. Its role in inflammation regulation and its association with various diseases, including cancer, have been extensively studied. ANXA1 is expressed in multiple tissues and plays a crucial role in cell growth, differentiation, and apoptosis regulation. Dysregulation of ANXA1 expression has been observed in several cancer types, highlighting its potential as a diagnostic and prognostic marker. Methods: This study aimed to evaluate the expression of ANXA1 in colorectal, lung, and liver cancer tissues using immunohistochemical analysis. Tissue samples were obtained from 29 newly diagnosed cancer patients undergoing tumor removal. Immunohistochemical staining was performed using ANXA1 antibody, and the staining protocol followed established procedures. Results and Discussion: Immunohistochemical analysis revealed a significant overexpression of ANXA1 in colorectal, lung, and liver cancer tissues compared to standard diagnostic markers. In colorectal cancer, ANXA1 showed higher efficiency in diagnosis than CK20, while in lung cancer, it exhibited higher expression compared to CK7. Previous studies have also linked elevated ANXA1 expression with tumor aggressiveness and poor prognosis in various cancers, including lung and liver cancer. These findings suggest the potential of ANXA1 as a diagnostic marker and prognostic indicator in cancer. Conclusion: ANXA1 antibody staining can serve as a valuable tool for immunohistological diagnosis, indicating tumor spread. Furthermore, the differential expression of ANXA1 across different cancer types underscores its potential utility in cancer diagnosis and prognosis. The observed higher expression of ANXA1 in lung cancer tissues compared to liver and colon cancer tissues highlights its significance in specific cancer types.

Keywords: Annexin A1, Cancer, Immunohistochemistry, Tumor markers, Diagnostic tool

Introduction

Cancer is a multifaceted disease characterized by uncontrolled cell growth and the potential to invade or spread to other parts of the body. Early and accurate diagnosis is critical for effective treatment and improved prognosis. Immunohistochemistry (IHC) has emerged as a vital tool in cancer diagnosis, enabling the detection and localization of specific proteins within tissue sections. Among the numerous biomarkers used in IHC, Annexin A1 (ANXA1) has garnered significant attention due to its involvement in various cellular processes and its potential as a diagnostic and prognostic marker in multiple cancers.

Annexin A1 (ANXA1) is a phospholipid-binding protein with a molecular weight of 37 kilodaltons, expressed in several cell types

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Significance | ANXA1's differential expression in colorectal, lung, and liver cancers could aid precise diagnosis and prognostication, guiding treatment decisions.

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including leukocytes, lymphocytes, epithelial cells, and endothelial cells (Gerke & Moss, 2002). First identified in the early 1980s, ANXA1 has since been recognized for its role in mediating the anti-inflammatory effects of glucocorticoids. It exerts these effects by inhibiting the synthesis of pro-inflammatory mediators and promoting the resolution of inflammation. ANXA1 activates the formyl peptide receptor 2 (FPR2), a pro-inflammatory receptor, underscoring its complex role in immune regulation (Gerke & Moss, 2002).

In addition to its anti-inflammatory properties, ANXA1 plays a crucial role in various cellular processes, including cell growth, differentiation, migration, and apoptosis. Its expression and secretion are regulated through multiple mechanisms, including phosphorylation, interaction with ATP-binding cassette (ABC) transporters, and release from intracellular granules upon cellular stimulation (Perretti & Solito, 2004; Alldridge & Bryant, 2003; Williams et al., 2010). Notably, ANXA1 can be released into the bloodstream through autocrine and paracrine signaling pathways, further influencing its systemic effects.

ANXA1 expression is modulated by glucocorticoids, with cortisol levels impacting its expression in different pathological states. For instance, reduced cortisol production in Addison's disease is associated with decreased ANXA1 levels in leukocytes, whereas excessive cortisol in Cushing's syndrome leads to elevated ANXA1 levels (Seaton & Dedman, 1998; Clark et al., 2012). These variations in expression highlight the sensitivity of ANXA1 to hormonal regulation and its potential role as a biomarker in endocrine disorders.

Recent studies have highlighted the overexpression of ANXA1 in various cancers, including colorectal, lung, pancreatic, liver, glioma, cervical, thyroid, laryngeal, prostate, head and neck, stomach, breast, esophageal, and bile duct cancers (Su et al., 2010; Liu et al., 2011; Bai et al., 2004; Cheng et al., 2013; Wang et al., 2008; Silistino-Souza et al., 2007; Patton et al., 2005; Jorge et al., 2013; Han et al., 2014). In these malignancies, ANXA1 is often associated with aggressive tumor behavior, poor

prognosis, and advanced disease stages. For example, high levels of ANXA1 in lung cancer tissues correlate with malignant behavior and worse clinical outcomes, making it a potential marker for prognosis and therapeutic targeting (Rong et al., 2014; Min-Chun Chuang et al., 2021).

In colorectal cancer, ANXA1 enhances the invasion of cancer cells by activating the ERK/2 ITGB1BP1 pathways, thereby promoting tumor progression (Xi Wang et al., 2023). Similarly, in hepatocellular carcinoma (HCC), elevated ANXA1 expression is linked to higher tumor grades and poor prognosis, indicating its role in tumor aggressiveness and potential as a therapeutic target (Suo et al., 2021; Lin et al., 2014).

This study aimed to investigate the expression of ANXA1 in colorectal, lung, and liver cancers and compare its diagnostic efficiency with other established markers such as CK20, CK7, and CK8. By analyzing the immunohistochemical expression of ANXA1 in these cancer types, we seek to elucidate its potential as a reliable biomarker for early diagnosis, prognosis, and therapeutic targeting in oncology.

2. Materials and Methods

This study included 29 newly diagnosed patients with cancers of the liver (9 patients), colon (11 patients), and lung (9 patients) who were undergoing tumor removal in the hospital. A biopsy, the only definitive method for diagnosing cancer, was used for each patient.

Immunohistochemistry

The sample preparation followed the method described by Hai-Tao Luo et al. (2017). The tissue sections, fixed in formalin for less than two hours, were loaded onto positively charged slides using Mayr's albumen. The samples were then transferred to an oven at 60°C for 60 minutes. They were treated with two changes of xylene and then passed through a descending series of ethyl alcohol for five minutes each, followed by a rinse with phosphate-buffered saline (PBS) for 10 minutes.

The slides were then transferred to a glass container containing antigen retrieval solution, pre-heated in a microwave at 93°C for 20 minutes. After cooling, the slides were stained with primary antibodies and incubated in the dark at 40°C overnight. Following incubation, the slides were rinsed with PBS and treated with the color generator 3,3'-diaminobenzidine (DAB) for 3 to 20 minutes. The slides were counterstained with Mayer's haematoxylin, passed through an ascending series of ethyl alcohol, and treated with two changes of xylene. The slides were left to air dry for 20 minutes and then covered with DPX mountant and a coverslip.

Preparation of ANXA1

The ANXA1 IgG antibody, manufactured by Fine Test, was used at a dilution of 1:100. Preparation of Annexin A1 (ANXA1) involves several steps, ranging from recombinant protein expression to purification. First, the ANXA1 gene is inserted into an expression vector, such as the pET vector system, with appropriate tags like a His-tag for purification. This plasmid is then transformed into competent E. coli cells, typically BL21(DE3), using a heat shock or electroporation method. Transformed cells are selected by plating on LB agar plates containing the appropriate antibiotic, such as ampicillin or kanamycin, and incubating overnight at 37°C. For expression, a single colony is inoculated into LB broth with the antibiotic and incubated overnight at 37°C with shaking. The culture is then scaled up to a larger volume and grown until the OD600 reaches 0.6-0.8, at which point IPTG is added to induce ANXA1 expression, and the incubation continues for 4-6 hours at

37°C or overnight at a lower temperature (16-20°C) to enhance protein solubility.

Cells are harvested by centrifugation and resuspended in a lysis buffer containing Tris-HCl, NaCl, imidazole, and protease inhibitors. The cells are lysed using sonication or a French press, and the lysate is clarified by centrifugation to remove cell debris. For purification, the clarified lysate is loaded onto a Ni-NTA agarose column pre-equilibrated with lysis buffer. The column is washed with a buffer to remove non-specifically bound proteins, and the ANXA1 protein is eluted with an imidazole-containing buffer. The elution buffer is then exchanged to a suitable storage buffer, such as PBS, using dialysis or a desalting column, and the protein is concentrated if necessary using centrifugal concentrators. If higher purity is required, the ANXA1 protein can undergo further purification using size-exclusion chromatography. The purified protein is verified using SDS-PAGE and Western blotting, and its concentration is determined using a suitable method, such as the BCA assay. Finally, the purified ANXA1 protein is aliquoted and stored at -80°C for long-term storage.

3. Results and Discussion

The immunohistochemical analysis of ANXA1 expression across colorectal, lung, and liver cancer tissues revealed significant findings. This section details the observed expression patterns and their implications in the diagnosis and prognosis of these cancer types.

Colorectal Cancer

In colorectal cancer tissues, ANXA1 exhibited a markedly higher expression compared to CK20, the commonly used diagnostic marker. As shown in Figure 1 and 2, the intensity and extent of ANXA1 staining were significantly greater, indicating its robust presence in cancerous cells. The overexpression of ANXA1 in colorectal cancer aligns with its known role in enhancing the invasion of cancer cells through the activation of the ERK/2 ITGB1BP1 pathways . This pathway is crucial for cell migration and invasion, suggesting that ANXA1 not only serves as a diagnostic marker but also plays a functional role in tumor progression. These findings support the potential utility of ANXA1 as a more efficient marker than CK20 for the immunohistopathological diagnosis of colorectal cancer.

Lung Cancer

Similarly, ANXA1 was found to be significantly overexpressed in lung cancer tissues compared to CK7, as illustrated in Figure 3 and 4. The enhanced expression of ANXA1 in lung cancer corroborates previous studies that have linked elevated ANXA1 levels to aggressive tumor behavior and poor clinical outcomes . ANXA1's high expression in lung cancer tissues is associated with its role in promoting malignant phenotypes and facilitating tumor spread to lymph nodes. This overexpression underscores the potential of ANXA1 as a diagnostic and prognostic marker in lung cancer, offering higher sensitivity and specificity compared to CK7. The ability of ANXA1 to predict poor prognosis and advanced disease stages makes it a valuable tool in the clinical management of lung cancer patients.

Liver Cancer

In liver cancer tissues, ANXA1 also showed higher expression levels compared to CK8, as depicted in Figure 5 and 6. This observation is consistent with previous research indicating that ANXA1 is upregulated in hepatocellular carcinoma (HCC) and is associated with higher tumor grades and poor prognosis . The elevated ANXA1 expression in liver cancer highlights its role in tumor aggressiveness and its potential as a therapeutic target. The significant presence of ANXA1 in liver cancer tissues suggests that it can be utilized as a reliable biomarker for early diagnosis and prognosis, providing insights into the malignancy and potential therapeutic strategies for HCC.

Comparative Analysis

The comparative analysis across colorectal, lung, and liver cancers revealed that ANXA1 expression was most pronounced in lung cancer tissues, followed by liver and colorectal cancers. This differential expression suggests that while ANXA1 is a valuable diagnostic and prognostic marker across multiple cancer types, its expression levels and diagnostic efficiency vary depending on the cancer type. The higher expression in lung cancer emphasizes the need for tailored diagnostic approaches and the potential for ANXA1-targeted therapies in specific cancers.

Clinical Implications

The findings from this study underscore the potential of ANXA1 as a superior biomarker for cancer diagnosis and prognosis. Its higher expression in colorectal, lung, and liver cancers compared to traditional markers (CK20, CK7, and CK8) suggests that ANXA1 can enhance diagnostic accuracy and provide better prognostic information. The use of ANXA1 in immunohistochemistry can improve the detection and characterization of cancer, leading to more precise treatment decisions and potentially better clinical outcomes for patients.

Conclusion

This research demonstrated that ANXA1 protein is highly expressed in colorectal, lung, and liver cancer tissues, making it a valuable marker in the immunohistopathological diagnosis of these cancers. In colorectal cancer, ANXA1 showed a significantly higher expression compared to CK20, underscoring its role in enhancing the invasion of gastrointestinal cancer cells through the ERK/2 ITGB1BP1 pathways. This suggests that ANXA1 is an efficient marker for diagnosing colon cancer.
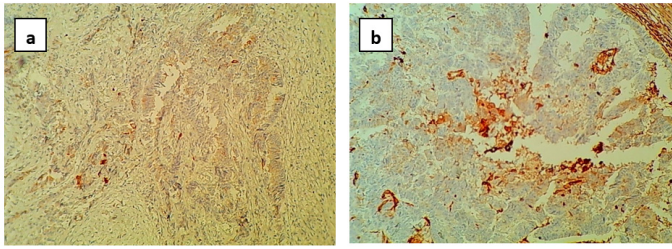
Figure 1. Expression of the cytokeratin protein CK20 of IHC immunohistological diagnosis of colorectal cancer, (a: 100x, b: 400x).
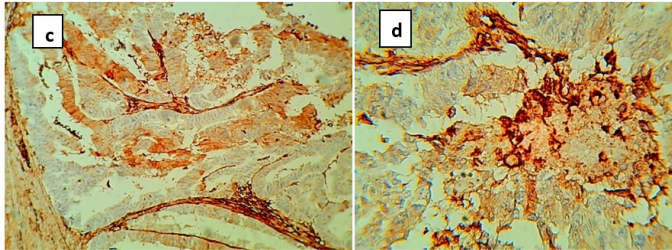

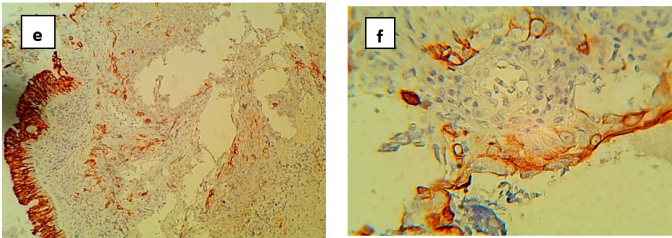**Figure 2.** High ANAX A1 protein expression in colorectal cancer using IHC (c: 100x, d: 400x).

Figure 3. The expression of the cytokeratin protein CK7 in IHC immunohistochemical diagnosis of bronchogeniccarcinoma, (e: 100x, f: 400x).
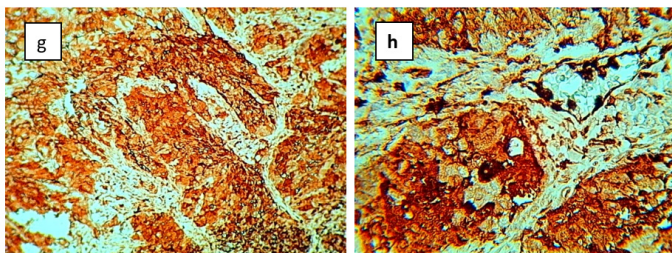

Figure 4. High ANAX A1 protein expression in Bronchogenic carcinoma using IHC (g: 100x, h: 400x).
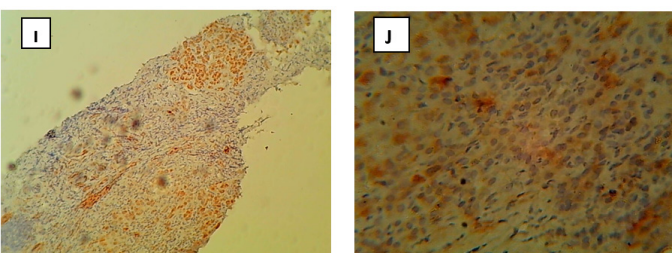

Figure 5. The expression of the cellular keratin protein CK8 in the IHC immunohistological diagnosis of liver cancer, (I: 100x, J: 400x).
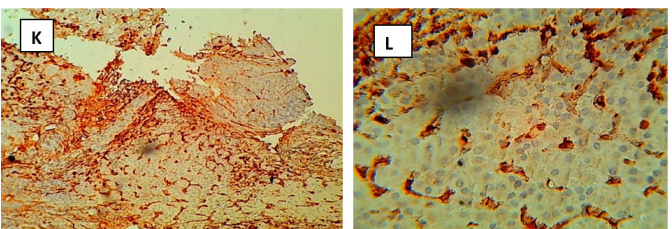

Figure 6. High ANAX A1 protein expression in Liver Cancer using IHC (K: 100x, L: 400x).

In lung cancer, ANXA1 expression was notably elevated compared to CK7, confirming previous findings that link high ANXA1 levels to malignant behavior and poor prognosis. The excessive expression of ANXA1 in lung cancer tissues and its association with tumor spread in lymph nodes and advanced stages highlight its potential as a diagnostic tool.

Similarly, ANXA1 exhibited high expression in liver cancer tissues compared to CK8. This aligns with previous studies that associate elevated ANXA1 levels with higher tumor grades and poor prognosis in hepatocellular carcinoma.

Overall, the findings suggest that ANXA1 can be used as a reliable marker in immunohistological diagnosis, providing valuable insights into tumor spread and malignancy. The study also indicates that ANXA1 expression is more pronounced in lung cancer tissues than in liver and colon cancer tissues, emphasizing its potential as a diagnostic and prognostic tool across different types of cancers.

Author contributions

H.Y.K., N.K., B.A.S.A. developed the Study design, and wrote, reviewed, and edited the paper.

Competing financial interests

The authors have no conflict of interest.

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