ISSN 2706-6819 Vol.5, Issue 3: 1-7, DOI: 10.25236/IJFM.2023.050301

Pan-carcinogenic analysis of Annexin A1 gene in human tumors: A bioinformatics study

Yuepeng Wang[1,a], Su Yang[1,b], Pengfei Han[2,c], Shilei Qin[3,d], Yunfeng Xu[3,e,*]

[1]Department of Graduate School, Graduate Student Department of Changzhi Medical College, Changzhi, China
[2]Department of Orthopaedics, Heping Hospital Affiliated to Changzhi Medical College, Changzhi, China
[3]Department of Orthopaedics, Changzhi Yunfeng Hospital, Changzhi, China
[a]wangypys@163.com, [b]y13214058593@163.com, [c]18035526520@163.com, [d]hero21005555@sina.com, [e]yunfeng.yiyuan@163.com
**Corresponding author*

Abstract: *Background: Annexin A1 (ANXA1) and cancer appear to be related, based on emerging cell-based or animal research, but pan-cancer analysis is lacking. Objective: By using bioinformatics, we will examine the expression characteristics of the ANXA1 gene in pan-cancers. Methods: In collaboration with TCGA and GEO database, ANXA1 gene was analyzed pan-cancer, and through gene expression analysis, survival analysis, gene variation, immune infiltration, and enrichment analysis, ANXA1 played a role in the pathogenesis of different tumors. Results: A significant correlation has been found between ANXA1 expression and prognosis in some patients with tumors. The expression level of ANXA1 was correlated with the pathological stage of head and neck squamous cell carcinoma (HNSC) and thyroid carcinoma (THCA). The survival rates are related to the expression level of ANXA1 in different tumors. There is a strong correlation between the expression of ANXA1 and immune cell infiltration in some tumors and patients with sarcoma (SARC) and uterine corpus endometrial carcinoma (UCEC) have a higher mutation and amplification rate for the ANXA1 gene. ANXA1 may participate in the occurrence and development of tumor through "proteoglycans in cancer" signal pathways. Conclusion: The ANXA1 gene is involved in the pathogenesis of multiple kinds of tumors, and the study of its pathogenic mechanism may be an effective approach to treating tumors.*

Keywords: *ANXA1; bioinformatics; tumor; gene expression; immune infiltration*

1. Introduction

In view of the complexity of tumorigenesis, it is important to analyze the pan-cancer expression of any gene of interest and to evaluate its correlation with clinical prognosis and potential molecular mechanism. Some studies have found that the content of Annexin in cells is relatively high (accounting for 0.5% of cell protein content). Its main function is to participate in membrane transport and a series of calmodulin-dependent activities on the membrane surface, including vesicle transport, membrane fusion during exocytosis, DNA replication, signal transduction, cell proliferation, apoptosis and ion channel formation [1-4]. The Annexin A1 gene (ANXA1) is the first member of the Annexin family, and It is also involved in many cellular life activities, such as phagocytosis and clearance of apoptotic cells, regulation of cell differentiation and proliferation, and inflammatory response [5-7]. ANXA 1 is abnormally expressed in many kinds of tumors and is closely related to the prognosis of tumor patients [8-10]. However, there is still no evidence of pan-cancer to show the relationship between ANXA1 and various tumor types, based on a large number of clinical data. The existing TCGA and GEO databases contain functional genomics data sets for different tumors, thus allowing us to conduct pan-cancer analysis.

In our study, pan-cancer analysis of ANXA1 was performed using TCGA and GEO databases, included a set of factors, such as gene expression, survival analysis, gene variation, immune infiltration and enrichment analysis, to explore the potential molecular mechanisms of ANXA1 in different cancer pathogenesis or clinical prognosis.

ISSN 2706-6819 Vol.5, Issue 3: 1-7, DOI: 10.25236/IJFM.2023.050301

2. Materials and methods

2.1. Gene expression

Enter ANXA1 into the "Gene DE" module of Exploration in TIMER2 (http://timer.cistrome.org/) website and observe the difference of ANXA1 expression between tumors of different tumors or specific tumor subtypes and adjacent normal tissues in TCGA database. The correlation between the expression of ANXA1 and the clinicopathological stage of the tumor was further explored by using the "Stage Plot" module in the "Expression DIY" part of GEPIA2. The expression level of ANXA1 is expressed by $Log_{2}^{(TPM+1)}$.

2.2. Survival analysis

The "Survival Analysis" module of GEPIA2 website was used to obtain the effects of different ANXA1 on tumor patients' overall survival time (overall survival, OS) and disease-free survival time (disease-free survival, DFS) in TCGA database. The results were represented by Kaplan-Meier curve analysis.

2.3. Genetic variation analysis

Using the "Query" section of the cBioPortal website (https://www.cbioportal.org/), TCGA pan-cancer map analysis was selected to mine the variation of ANXA1 in the TCGA database, including mutation frequency, mutation type and copy number changes. The information of ANXA1 gene mutation site is displayed by clicking on the "Mutation" module.

2.4. Immune infiltration analysis

The TIMER2 tool "Immune" module was used to explore the correlation between the expression of ANXA1 and immune infiltration in TCGA database. Macrophage, monocyte, T cell regulatory, cancer-associated fibroblast (CAF) and endothelial cell were selected as the research objects. Different algorithms such as MCPCOUNTER, CIBERSORT, QUANTISEQ, XCELL, EPIC and TIDE were used to evaluate immune infiltration, and the results were represented by heat map.

2.5. In enrichment analysis

50 ANXA1-binding proteins were screened by STRING (https://string-db.org/) database. In the "Protein by name" section, input "ANXA1", species selection "Homo species". In the "settings" section, active interaction sources only select "Experiments", and minimum required interaction score select "low confidence (0.150)". Max number of interactors to show is no more than 50 interactors. The network display options only select "disable structure previews inside network bubbles", and finally, select "UPDATE". The first 100 ANXA1-related targeting genes were screened by the "Similar Genes Detection" module in the "Expression Analysis" section of GEPIA2 database, and 50 ANXA1 binding proteins and the first 100 ANXA1-related targeting genes were cross-analyzed by Jvenn (http://bioinformatics.psb.ugent.be/webtools/Venn/) web. R4.1.3 software was used to analyze the KEGG pathway, and the results were represented by a bubble diagram.

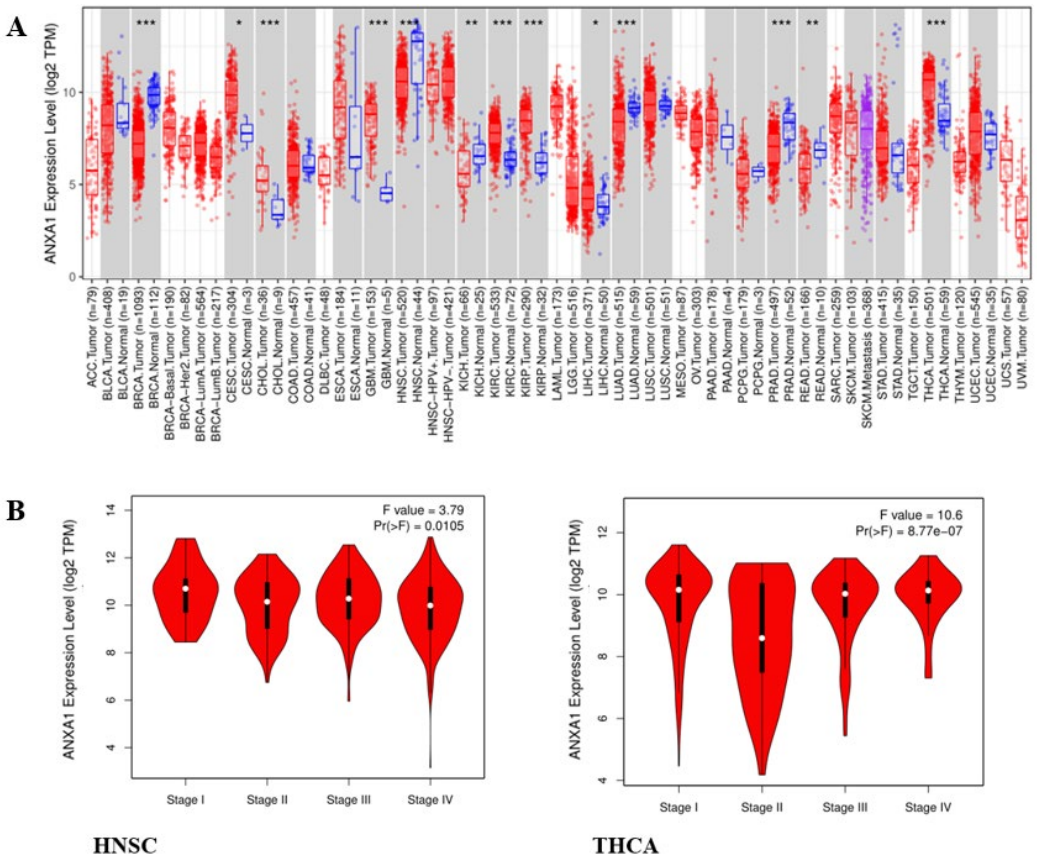
3. Result

3.1. Gene expression

Gene Compared with normal tissues, the expression of ANXA1 was significantly higher in cervical squamous cell carcinoma and endometrial adenocarcinoma (CESC), cholangitis carcinoma (CHOL), glioblastoma multiform (GBM), kidney Chromophobe (KIRC), kidney renal clear cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC) and thyroid carcinoma (THCA). However, the expression was significantly decreased in invasive breast invasive carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney Chromophobe (KICH), lung adenocarcinoma (LUAD), prostate adenocarcinoma (PRAD) and rectum adenocarcinoma (READ) (Figure 1A). Using GEPIA2 database to analyze the relationship between the expression level of ANXA1 in different tumor tissues and the clinicopathological stage of tumor patients. We found that the expression of ANXA1 protein was closely

ISSN 2706-6819 Vol.5, Issue 3: 1-7, DOI: 10.25236/IJFM.2023.050301

related to the pathological stages of head and neck squamous cell carcinoma (HNSC) and THCA, but there is no obvious correlation to other tumors (Figure 1B).



*:P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001, Compared with normal tissue.*

Figure 1: A. Expression of ANXA1mRNA in different tumors and paracancerous tissues. B. Relationship between the expression of ANXA14 and different clinical stages of tumor.

3.2. Survival analysis

According to the expression level of ANXA1, the patients were divided into high expression group and low expression group to further explore the relationship between the expression of ANXA1 and the prognosis of tumor patients. We found that the high expression of ANXA1 was closely related to the poor prognosis of overall survival (OS) in patients with bladder urogenital carcinoma (BLCA) (P=0.0023), Brain lower grade glioma (LGG)(P=2.1e-07) and pancreatic adenocarcinoma (PAAD) (P=0.008), while the low expression of ANXA1 was closely related to the poor prognosis of OS in patients with skin cutaneous melanoma (SKCM) (P=0.002) and THCA (P=0.0099) (Figure 2A). The disease free survival (DFS) data analysis showed that high expression of ANXA1 was associated with poor prognosis in patients with LGG (P=7.9e-05) and PAAD (P=0.0063) and uveal melanoma (UVM) (P=0.035), while low expression of ANXA1 was associated with poor prognosis in patients with CESC (P=0.002) (Figure 2B).

ISSN 2706-6819 Vol.5, Issue 3: 1-7, DOI: 10.25236/IJFM.2023.050301

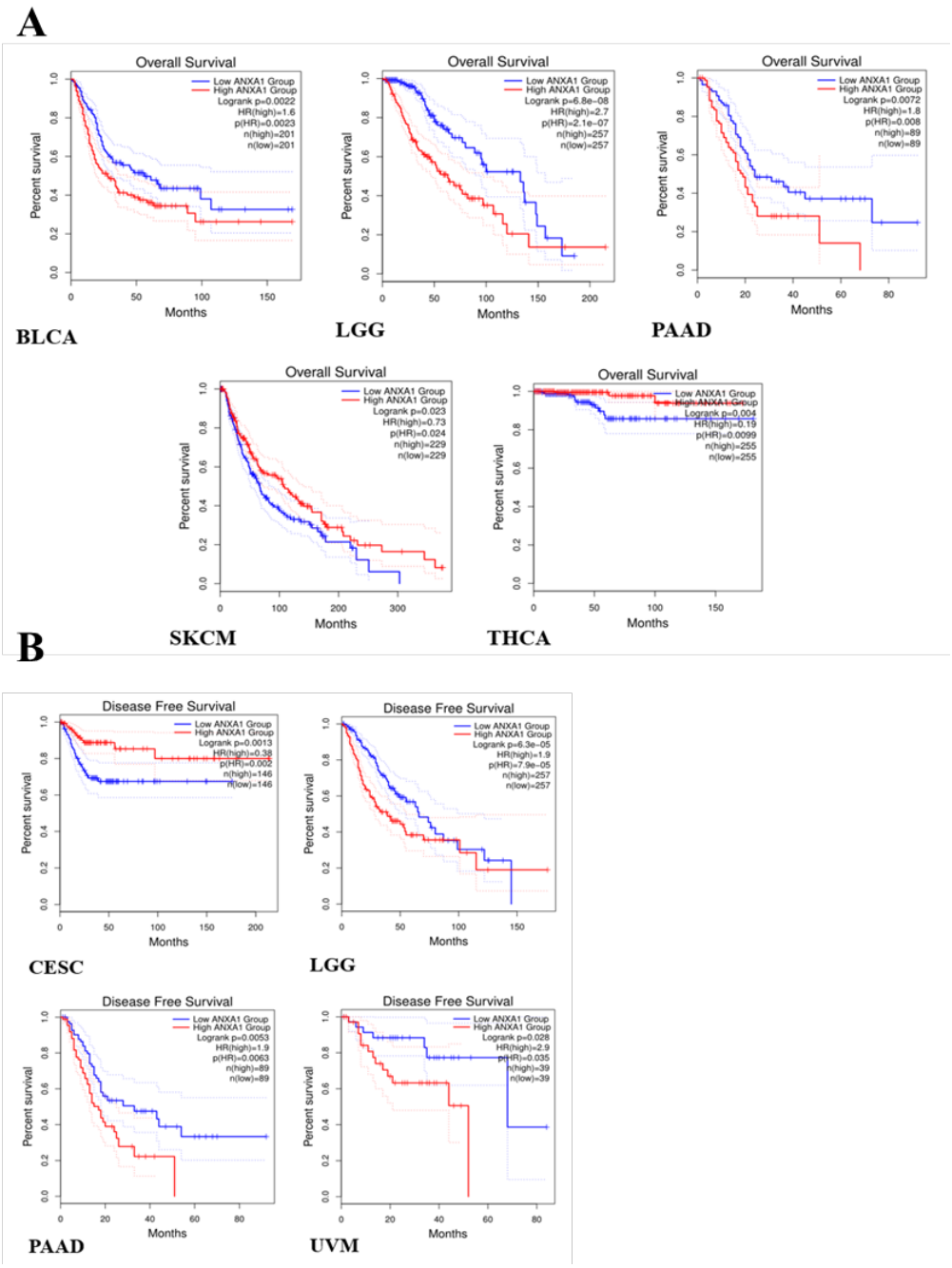


Figure 2: A. Correlation between expression of ANXA1 gene and overall survival of patients with different tumors. B. Correlation between expression of ANXA1 gene and disease free survival of patients with different tumors.

3.3. Genetic variation analysis

We found that the mutation frequency of ANXA1 gene is relatively high in patients with SARC and uterine corpus endometrial carcinoma (UCEC) with ANXA1 mutation and amplification as the main mutation type. ANXA1 mutations are found in patients with SKCM, KICH, KIRC and LGG). ANXA1 is deeply deleted in patients with lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), thymoma (THYM), acute myeloid leukemia (LAML), and THCA (Figure3A). The type, locus and number of cases of ANXA1 genetic variation are further displayed. ANXA1 missense mutations were the main type of genetic variation, and R188Q alterations were detected in 1 SKCM, 1 colon adenocarcinoma (COAD), 1 BRCA, and 1 Colorectal Adenocarcinoma (COADREAD) patient (Figure3B).

ISSN 2706-6819 Vol.5, Issue 3: 1-7, DOI: 10.25236/IJFM.2023.050301

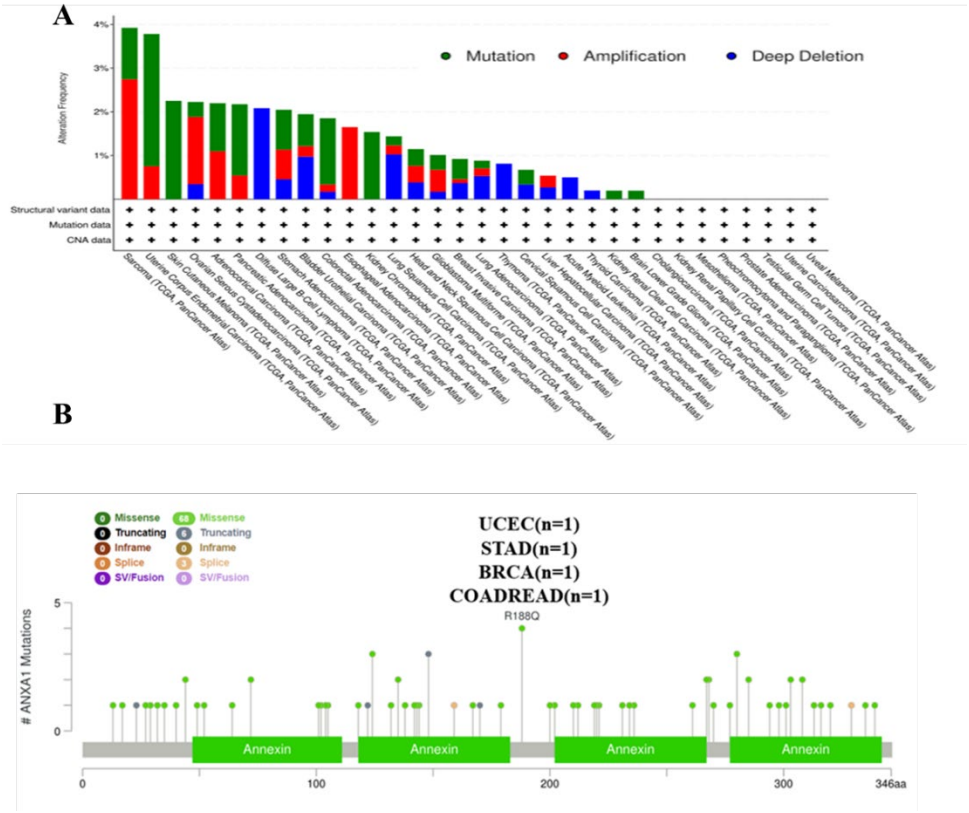


Figure 3: A. Variation types of ANXA1 in tumors. B. The change frequency of ANXA1 mutation site.

3.4. Immune infiltration analysis

Immune infiltration analysis We studied the relationship between tumor immune cell infiltration and ANXA1 gene expression in TCGA database, and found that in testicular germ cell tumors (TGCT), there was a positive correlation between monocyte immune infiltration and ANXA1 expression and the difference was statistically significant. In THCA, the expression of ANXA1 was positively correlated with the immune infiltration of T cell regulatory, and the difference was statistically significant. In BRCA, BRCA-LumA, BRCA-LumB, COAD, LGG, LIHC, PAAD, pheochromocytoma and paraganglioma (PCPG), READ, stomach adenocarcinoma (STAD), TGCT and THYM, the expression of ANXA1 was positively correlated with CAF, but negatively correlated with CAF infiltration in HNSC, HNSC-HPV- and lung squamous cell carcinoma (LUSC). Similarly, in BRCA, BRCA-LumA, COAD, LGG, PCPG and TGCT, ANXA1 was positively correlated with endothelial cell infiltration, but negatively correlated with endothelial cell infiltration in esophageal carcinoma (ESCA) and THCA (Figure 4A).

3.5. In enrichment analysis

Through the STRING tool, we obtained a total of 50 experimentally verified ANXA1-binding proteins (Figure 4B). We used the Venn website to intersect 50 binding proteins with the first 100 related target genes and screened two common members (S100A8Magi S100A9), as showed in Figure 4C. Finally, results of KEGG enrichment analysis showed that "oncoproteoglycan" (proteoglicansincancer) may be involved in the role of ANXA1 in tumorigenesis (Figure 4D).

ISSN 2706-6819 Vol.5, Issue 3: 1-7, DOI: 10.25236/IJFM.2023.050301

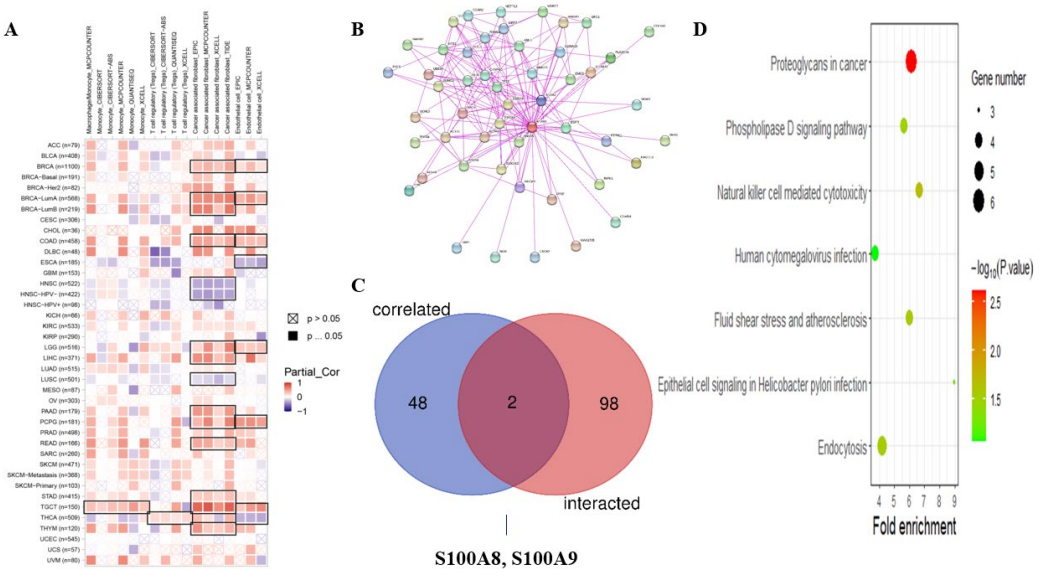


Figure 4: A. Correlation between ANXA1 expression and immune cell infiltration. B. 50 ANXA1-binding proteins verified by experiments. C. Cross analysis of two sets of data. D. Analysis of KEGG pathway ofANXA1-related genes.

4. Discussion

ANXA1 is a member of the calcium-dependent phospholipid binding protein family, which is widely expressed in different human tissues. As a danger-associated molecular pattern (DAMP) ANXA1 alerts the innate immune system to tissue disturbances [11,12]. It is an endogenous anti-inflammatory protein that mediates the anti-inflammatory effect of glucocorticoids and involved in a variety of physiological processes, such as extracellular and extracellular signal transduction, cell differentiation, proliferation and apoptosis, as well as inflammatory and immune responses. It has been found that ANXA1 is differentially expressed in different tissue types of tumors, which is closely related to the occurrence, development, invasion and metastasis of tumors[13,14].

In this study, we found that the expression of ANXA1 was different in different tumors. For example, ANXA1 is up-regulated in cervical squamous cell carcinoma and intracervical carcinoma (CESC), cholangiocarcinoma (CHOL), pleomorphic glioma (GBM), renal clear cell carcinoma (KIRC), renal papillary cell carcinoma (KIRP), hepatocellular carcinoma (LIHC), and thyroid carcinoma (THCA). It was down-regulated in invasive breast carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), renal chromophobe cell carcinoma (KICH), lung cancer (LUAD), prostate cancer (PRAD) and rectal cancer (READ). It is reported that the expression of ANXA1 in normal tissues was compared with that in normal tissues. It was found that tissues with high expression of ANXA1 showed varying degrees of loss of ANXA1 after carcinogenesis, while tissues with no or very low expression of ANXA1 showed high expression. This not only suggests that the difference in tissue distribution of ANXA1 may be related to the expression of ANXA1 in different tissues during malignant transformation, but also indicates that the up-regulation or down-regulation of ANXA1 expression in different tumor tissues is closely related to the occurrence and development of tumors.

In view of the fact that ANXA1 is expressed abnormally in a variety of tumors, we explored in depth its variation characteristics. Among the types of variation we found, mutation and amplification are most common. Tumor infiltrating immune cells are an important part of complex tumor microenvironment. Our study found that in some specific tumors, there is a close correlation between ANXA1 expression and monocyte, T cell regulatory, monocytes, endothelial cells and CAF. It is suggested that because of the difference in cell types, it may regulate the pathogenic process of the tumor. The results of KEGG enrichment analysis showed that proteoglycans in cancer may be involved in the role of ANXA1 in tumorigenesis. The growth of cancer cells is driven by abnormal signals and metabolic reprogramming, and oncogenes are closely related to metabolic changes in cancer cells. Metabolic reprogramming is usually mediated by a variety of carcinogenic signals and controls the metabolism of cancer cells by changing the expression and / or activity of some key metabolic enzymes [15-17]. mTOR pathway is related to the occurrence and development of tumor. It is the downstream molecule of multiple signal pathways,

ISSN 2706-6819 Vol.5, Issue 3: 1-7, DOI: 10.25236/IJFM.2023.050301

which is over-activated by upstream molecules such as EGFR, which promotes the progression of tumor. The inhibitory effect of ANXA1 on cell proliferation is closely related to EGF through MAPK/ERK pathway, and it can also be used as the substrate of EGFR tyrosine kinase [18-21].

To sum up, the pan-cancerous analysis of ANXA1 shows that ANXA1 is abnormally expressed in many tumors and is related to clinical prognosis, gene variation and immune cell infiltration. These findings provide a preliminary understanding of the biological function of ANXA1, but further experiments are needed to confirm its specific mechanisms and effects.

References

[1] Iseki Y, Imoto A, Okazaki T, Harigae H, Takahashi S. Identification of annexin 1 as a PU.1 target gene in leukemia cells [J]. Leuk Res. 2009; 33(12):1658-1663.
[2] Li YZ, Wang YY, Huang L, Zhao YY, Chen LH, Zhang C. Annexin A protein family in atherosclerosis [J]. Clin Chim Acta. 2022; 531:406-417.
[3] Rescher U, Gerke V, Lim LHK, Jaiswal JK. Special Issue "Recent Developments in Annexin Biology"[J]. Cells. 2020; 9(11):2477.
[4] Xi Y, Ju R, Wang Y. Roles of Annexin A protein family in autophagy regulation and therapy [J]. Biomed Pharmacother. 2020; 130:110591.
[5] Guan X, Fang Y, Long J, Zhang Y. Annexin 1-nuclear factor-κB-microRNA-26a regulatory pathway in the metastasis of non-small cell lung cancer [J]. Thorac Cancer. 2019; 10(4):665-675.
[6] McArthur S, Juban G, Gobbetti T, et al. Annexin A1 drives macrophage skewing to accelerate muscle regeneration through AMPK activation[J]. J Clin Invest. 2020; 130(3):1156-1167.
[7] Chen R, Chen C, Han N, et al. Annexin-1 is an oncogene in glioblastoma and causes tumour immune escape through the indirect upregulation of interleukin-8[J]. J Cell Mol Med. 2022; 26(15):4343-4356.
[8] Bai F, Zhang P, Fu Y, et al. Targeting ANXA1 abrogates Treg-mediated immune suppression in triple-negative breast cancer [J]. J Immunother Cancer. 2020; 8(1): e000169.
[9] Prates J, Moreli JB, Gimenes AD, et al. Cisplatin treatment modulates Annexin A1 and inhibitor of differentiation to DNA 1 expression in cervical cancer cells[J]. Biomed Pharmacother. 2020; 129:110331.
[10] Yamanoi M, Yamanoi K, Fujii C, Fukuda MN, Nakayama J. Annexin A1 expression is correlated with malignant potential of renal cell carcinoma [J]. Int J Urol. 2019; 26(2):284-290.
[11] Juban G, Mounier R. Réparation. Tissue repair: Key role of annexin A1 in the control of inflammatory response [J]. Med Sci (Paris). 2021; 37(4):324-326.
[12] Baracco EE, Petrazzuolo A, Kroemer G. Assessment of annexin A1 release during immunogenic cell death [J]. Methods Enzymol. 2019; 629:71-79.
[13] Costa MB, Mimura KKO, Freitas AA, et al. Mast cell heterogeneity and anti-inflammatory annexin A1 expression in leprosy skin lesions[J]. Microb Pathog. 2018; 118:277-284.
[14] Shijo M, Hamasaki H, Honda H, et al. Upregulation of Annexin A1 in Reactive Astrocytes and Its Subtle Induction in Microglia at the Boundaries of Human Brain Infarcts[J]. J Neuropathol Exp Neurol. 2019; 78(10):961-970.
[15] Li X, Xia Q, Mao M, et al. Annexin-A1 SUMOylation regulates microglial polarization after cerebral ischemia by modulating IKKα stability via selective autophagy [J]. Sci Adv. 2021;7(4): eabc5539.
[16] Li P, Li L, Li Z, et al. Annexin A1 promotes the progression of bladder cancer via regulating EGFR signaling pathway [J]. Cancer Cell Int. 2022; 22(1):7.
[17] Kotepui KU, Obchoei S, Vaeteewoottacharn K, Okada S, Wongkham S, Sawanyawisuth K. Annexin A1 Is a Potential Prognostic Marker for, and Enhances the Metastasis of, Cholangiocarcinoma[J]. Asian Pac J Cancer Prev. 2022; 23(2):715-721.
[18] Mossmann D, Park S, Hall MN. mTOR signalling and cellular metabolism are mutual determinants in cancer[J]. Nat Rev Cancer. 2018;18(12):744-757.
[19] Xu X, Gao W, Li L, et al. Annexin A1 protects against cerebral ischemia-reperfusion injury by modulating microglia/macrophage polarization via FPR2/ALX-dependent AMPK-mTOR pathway [J]. J Neuroinflammation. 2021; 18(1):119.
[20] Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges [J]. Cell Biosci. 2020; 10:31.
[21] Duval AP, Jeanneret C, Santoro T, Dormond O. mTOR and Tumor Cachexia[J]. Int J Mol Sci. 2018; 19(8):2225.