FRIGID ZONE **MEDICINE**
Original Article • DOI: 10.1515/fzm-2024-0024

The function and effect on prognosis of ANXA2 in gastric cancer peritoneal metastasis patients in cold region

Shiyang Jin[1#], Longxu Liang[2#], Xiaoyi Huang[2*], Kuan Wang[1*]

Abstract

Objective: Heilongjiang Province is part of the northern cold areas of China, where gastric cancer is one of the most common gastrointestinal malignancies. Peritoneal metastases (PM) are the leading cause of mortality among patients. This study conducted bioinformatics and basic research on the gene ANXA2 (Annexin A2), which may influence the prognosis of patients. **Methods:** Genome sequencing was performed on patients from Heilongjiang to identify potential genes impacting survival time. The function of ANXA2 in gastric cancer was analyzed using multiple bioinformatics databases, focusing on its pathways and mechanisms. ANXA2-knockout gastric cancer cell lines were constructed, and *in vitro* assays, including CCK-8, flow cytometry, scratch, and Transwell experiments, were conducted. A nude mouse tumorigenesis model was also developed to analyze *in vivo* effects. **Results:** ANXA2 was found to be expressed at higher levels in gastric cancer tissue than in normal gastric tissue, and its mRNA levels were associated with short overall survival (OS). Enrichment analysis indicated that ANXA2 is primarily localized on the cell membrane and primarily influences the PI3K-AKT signaling pathway. Cytological experiments demonstrated that knockdown of ANXA2 suppresses the growth and migration of gastric carcinoma cells, an effect that was also observed *in vivo*. **Conclusions:** ANXA2 is essential for gastric cancer growth and may represent a potential risk factor affecting the survival probability of patients in cold regions.

Keywords

Gastric cancer; ANXA2; prognosis survival; bioinformatics; cold region

Received 27 August 2024, accepted 17 September 2024

[1]*Department of Gastrointestinal Surgery, Harbin Medical University Cancer Hospital, Harbin 150081, China*
[2]*Heilongjiang Cancer Biotherapy Center, Harbin Medical University Cancer Hospital, Harbin 150081, China*

**Corresponding author Xiaoyi Huang, E-mail: xyhuang@hrbmu.edu.cn; Kuan Wang, E-mail: 0696@hrbmu.edu.cn*
[#]These authors contributed equally to this work.

1 Introduction

Gastric cancer is one of the most common malignant tumors. In recent years, its incidence has gradually decreased due to improved living conditions. In Heilongjiang Province, one of the coldest regions in China, the incidence of gastric cancer has dropped to sixth place. However, high rates of alcohol consumption and lifestyle habits, such as a preference for pickled foods, have contributed to a mortality rate that remains the fourth highest among all cancers in the province. Peritoneal metastasis is common in gastric cancer patients, with its prevalence increasing as the disease progresses.

Through exome sequencing of patients with peritoneal metastases who underwent tumor subtraction, we identified ANXA2 (Annexin A2, also known as Annexin II) as a calcium-regulated phospholipid-binding protein that plays a crucial role in various biological processes[1-4], including key tumor-related mechanisms such as cell proliferation, apoptosis, migration and invasion . The role of ANXA2 in tumors has been extensively studied, revealing that its expression level is often elevated in tumor cells and correlates with tumor progression and prognosis[5].

ANXA2 is implicated in the regulation of cell proliferation and growth by activating multiple signaling pathways[6-11]. Moreover, it plays a crucial role in invasion and metastasis[12-19], as well as tumor angiogenesis. ANXA2-activated fibrinolytic enzymes can initiate neovascularization, providing nutrients to tumor cells[15-16,19-21], which may promote the formation of peritoneal

metastases. Furthermore, ANXA2 is closely associated with drug resistance in tumor cells[22-26], posing significant challenges for cancer treatment.

ANXA2 has been shown to be aberrantly expressed in various tumor tissues, with approximately 30% of gastric cancer patients exhibiting high levels of ANXA2 protein[27]. Elevated levels of ANXA2 DNA have also been detected in the peripheral blood of some gastric cancer patients[28]. At the cellular level, studies indicate that ANXA2 expression promotes gastric cancer metastasis and increases resistance to chemotherapy[26,29]. Moreover, abnormal phosphorylation of ANXA2 has been found to significantly influence tumor growth[30]. However, research on ANXA2 in gastric cancer specific to cold regions remains limited, primarily focusing on cellular mechanisms without robust clinical evidence, particularly regarding its role in peritoneal implantation and prognostic data in patient populations. Therefore, this study aims to comprehensively analyze the role of ANXA2 in gastric cancer using bioinformatics and basic research approaches, as well as to evaluate the impact of ANXA2 expression on the prognosis of gastric cancer patients.

2 Materials and Methods

2.1 Specimen collection

We conducted comprehensive preoperative assessments and gathered baseline clinical data. Then, we performed laparoscopic exploration and peritoneal lavage, including patients with peritoneal metastases identified during the procedure. We collected primary tumor tissue during the initial laparoscopic intervention. The tissue samples were promptly frozen and stored at -80°C. All participating patients provided informed consent and the study procedures adhered to medical ethics requirements (2023-200-QX).

2.2 Bioinformatics analyses

Analysis of The Cancer Genome Atlas (TCGA) database *via* The University of Alabama at Birmingham (UALCAN) showed ANXA2 mRNA expression between gastric cancer and normal tissues, with gene expression analyzed by cancer stage using *t*-tests ($P < 0.05$)[31-37]. The Kaplan-Meier plotter was employed to assess the prognostic value of overall survival (OS) related to ANXA2 in gastric cancer[32]. The Human Protein Atlas (HPA) provided immunohistochemistry images comparing ANXA2 protein expression in normal human gastric tissues and gastric cancer tissue[33]. The Gene Expression Profiling Interactive Analysis (GEPIA) 2 platform identified the top 100 genes closely associated with ANXA2[34].Multi-protein analysis using the Search Tool for the Retrieval of Interaction Gene/Proteins (STRING) platform, with a minimum interaction score of 0.4, identified 10 proteins interacting with ANXA2 in the primary group[35]. Metascape and R 4.1.3 enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) pathways to elucidate the biological roles of ANXA2 co-expressed genes, focusing on GO terms (biological process, cellular component, molecular function) and KEGG pathways ($P < 0.05$)[36]. The Starbase database (https://rnasysu.com/encori/) was used to assess the impact of predicted miRNAs and TFs on ANXA2 expression and gastric cancer prognosis[37].

2.3 *In vitro* and *in vivo* experiments

The gastric cancer cell lines HGC27 and MKN45 were cultured in RPMI1640 medium. All cells were maintained in a constant temperature and humidity incubator at 37°C with 5% CO_2. For these gastric cancer cell lines, shRNA lentivirus transfection was performed (Table 1).

QRT-PCR and Western Blot experiments were performed to verify gene expression. Cell proliferation was assessed using the CCK-8 assay, while flow cytometry was employed to analyze cell cycle changes. Cell invasion was evaluated using Transwell and Scratch assays. *In vivo* tumor proliferation and peritoneal metastasis were assessed using a subcutaneous tumor and peritoneal implantation model in nude mice. ANXA2 expression was verified using immunohistochemistry in various tissues.

2.4 Statistical analyses

We used the Mann-Whitney test for continuous variable data and Fisher's exact test for categorical variable data. The chi-square test was used to analyze the relationship between mRNA expression and immunohistochemical expression characteristics. For survival analysis, we conducted the log-rank test and Kaplan-Meier analysis. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox regression models. Spearman correlation analysis was used to assess the correlation between

Table 1 ShRNA sequence of NC and ANXA2

ShRNA	Strand
ShNC Top	GATCCGTTCTCCGAACGTGTCACGTAATTCAAGAGATTACGTGACACGTTCGGAGAATTTTT
ShNC Bottom	AATTGAAAAAATTCTCCGAACGTGTCACGTAATCTCTTGAATTACGTGACACGTTCGGAGAA
ShANXA2 Top	CCGGCGGGATGCTTTGAACATTGAATTCAAGAGATTCAATGTTCAAAGCATCCCGTTTTTTG
ShANXA2 Bottom	AATTCAAAAAACGGGATGCTTTGAACATTGAATCTCTTGAATTCAATGTTCAAAGCATCCCG

two continuously related variables. All statistical analyses were performed using SPSS 22.0 (USA), with *P*-values < 0.05 considered statistically significant.

3 Results

3.1 Whole-exome sequencing and bioinformatics analyses

After conducting whole-exome sequencing and variant screening, we found that patients with poorer prognosis had a significantly higher number of mutations in the ANXA2 gene. Fig. 1 demonstrates that the mRNA expression level of ANXA2 in gastric cancer samples was markedly higher than that in healthy samples, with expression levels of ANXA2 showing an increasing trend correlating with clinical stage. The HPA results suggest that ANXA2 protein levels are also elevated in gastric cancer, although the findings were not statistically significant. Additionally, the KM plotter database revealed that patients with high ANXA2 mRNA expression had significantly shorter survival times compared to those with low expression ($P < 0.001$).

The enriched pathways for GO and KEGG analyses are shown in Fig. 2. The most relevant KEGG-enriched pathway identified is the PI3K-AKT signaling pathway. GO analysis indicated that ANXA2 is primarily localized to the cell membrane and is mainly involved in protein synthesis functions. We identified the 10 proteins most associated with ANXA2, which include DYSF, AHNAK, S100A11, S100A10, PLAT, PLG, CTSB, S100A4, SNAP23, and SLP1. In addition, the expression of CD274, the gene coding the PD-L1 protein, was found to be closely related to ANXA2, revealing a positive correlation between the two groups (Fig. 2).

The results show that four miRNAs may have a regulatory relationship with ANXA2. Validation using the Starbase database revealed that two of these miRNAs, miR-1-3p and miR-9-5p, exhibited a low expression in tumors, with their levels inversely correlating with ANXA2 mRNA expression. For transcription factor prediction, PPARG and SMAD3 were both highly expressed in tumors, showing a proportional relationship with ANXA2 expression. However, further prognostic analyses revealed that only SMAD3 overexpression was associated with poor patient prognosis (Fig. 3).

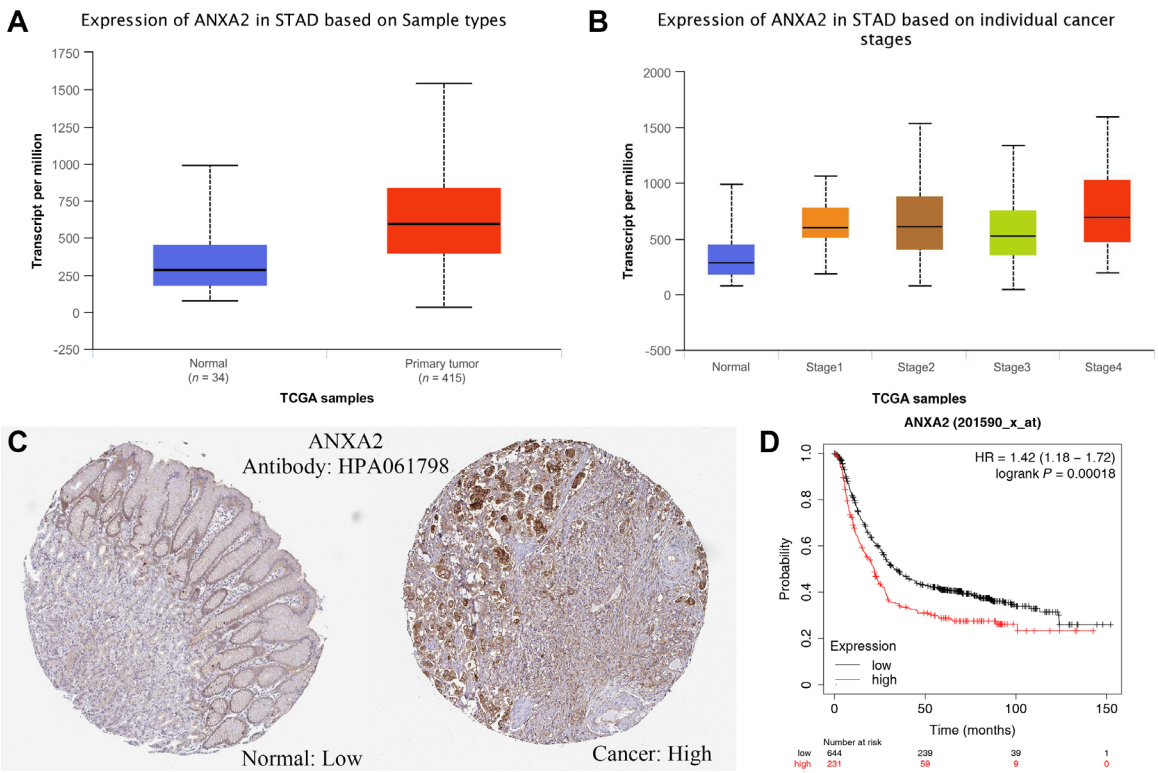

Fig. 1 Bioinformatics results of ANXA2

(A) Changes of ANXA2 mRNA expression levels in gastric cancer and normal gastric tissues; (B) Expression levels of ANXA2 mRNA in the tissues of gastric cancer patients at different clinical stages; (C) Immunohistochemical analysis of ANXA2 protein expression in gastric cancer and normal tissues; (D) Relationship between ANXA2 mRNA levels and the survival of gastric cancer patients.

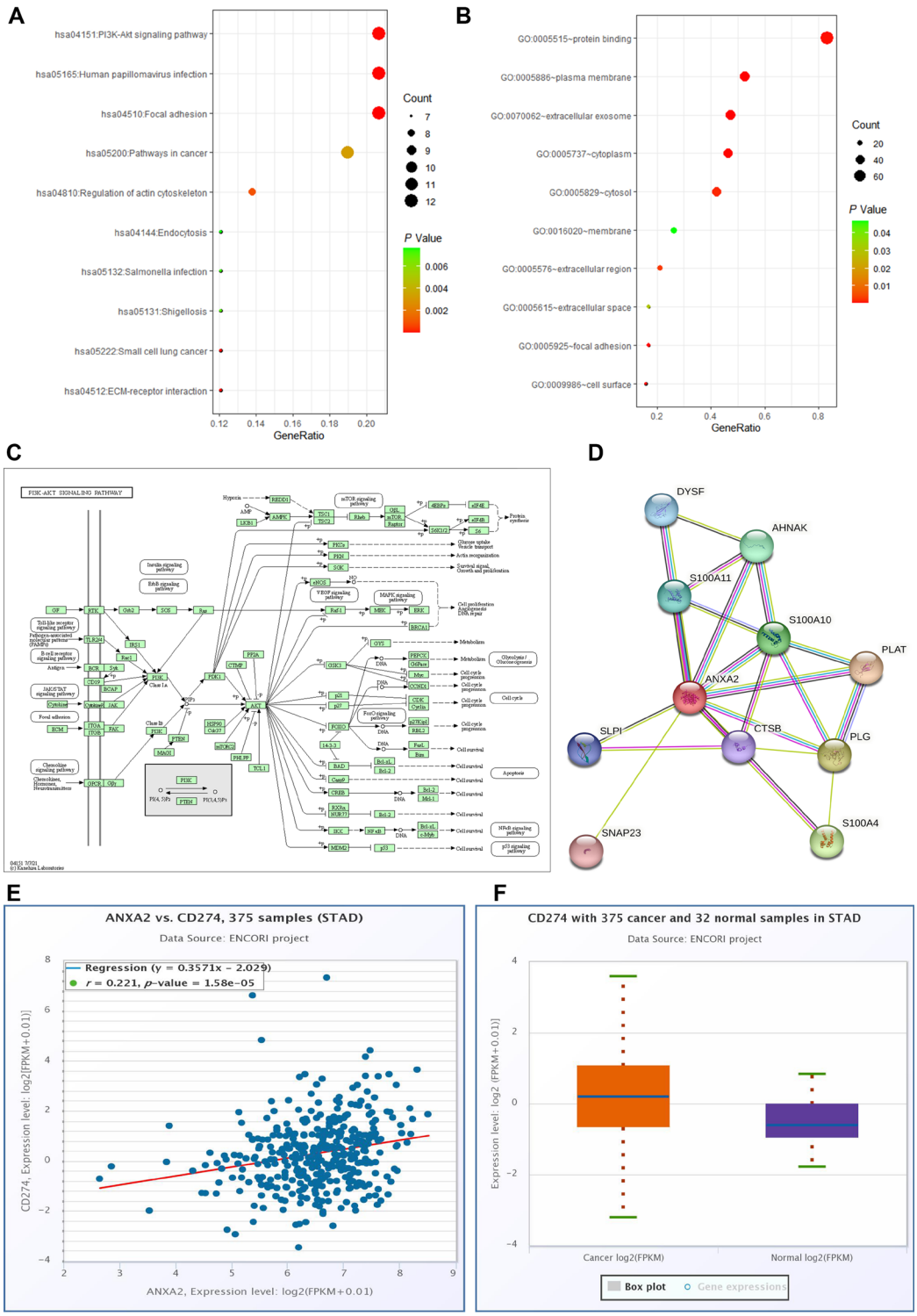

Fig. 2 Enrichment results and relationship with PDL1 and ANXA2

(A-B) Enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) related to ANXA2; (C) The PI3K-AKT pathway in KEGG database; (D) Protein interaction network of ANXA2; (E) Relationship between mRNA expression of ANXA2 and PDL1; (F) mRNA expression of PDL1 in gastric cancer and normal tissues. (E) Relationship between mRNA expression of ANXA2 and PDL1; (F) Expression of PDL1 mRNA in gastric cancer and normal tissues.

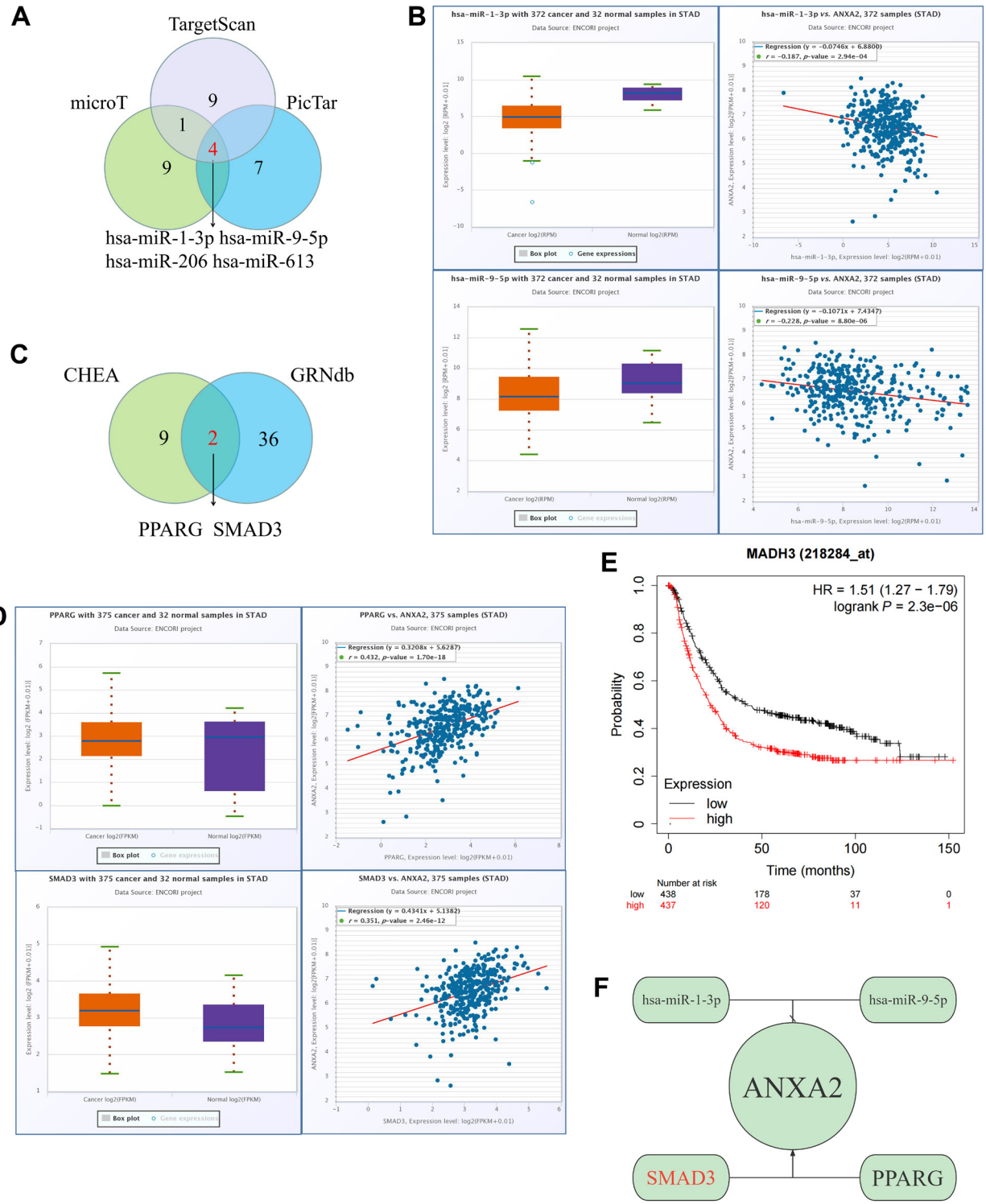

Fig. 3 miRNAs and TF of ANXA2

(A) Predicted Wayne diagram results of miRNAs; (B) Predicted miRNA expression in gastric cancer and normal tissues and the correlation with ANXA2 expression; (C) Predicted Wayne diagram results of transcription factors; (D) Predicted transcription factor expression in gastric cancer and normal tissues and the correlation with ANXA2 expression; (E) mRNA of SMAD3 expression in relation to the prognosis of gastric cancer patients; (F) Overall relationship among various factors.

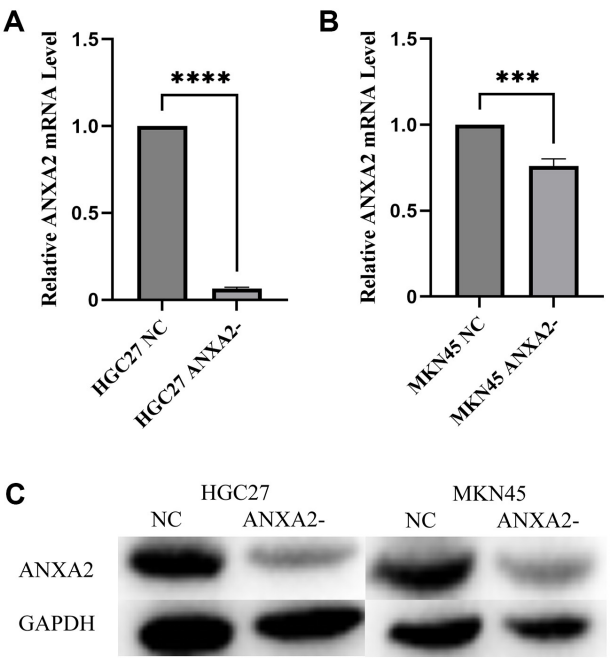

Fig. 4 ANXA2 expresson levels in different cell lines

(A) ANXA2 mRNA expression levels in HGC27 cell line; (B) ANXA2 mRNA expression levels in MKN45 cell line; (C) ANXA2 protein levels in different cell lines. $^{****}P < 0.0001$, $^{***}P < 0.001$.

3.2 *In vitro* and *in vivo* results

shANXA2 was transfected into HGC27 and MKN45 cells where it markedly downregulated ANXA2 expression (Fig. 4).

The CCK-8 results showed that at 24 h, 48 h, and 72 h, the proliferative ability of both ANXA2 knockdown cell lines was inhibited to different degrees (Fig. 5A, B). The flow cytometry experiments results showed a significant increase in the percentage of G2/M phase cells in the two knockdown cell lines, and the difference was statistically significant (Fig. 5C-H).

The results from the Transwell and scratch assays demonstrated that the metastatic ability of tumors was significantly decreased in ANXA2 knockdown cell lines compared with the negative controls ($P < 0.001$) (Fig. 6).

The results indicated that the tumor formation in mice implanted with HGC27 cells with ANXA2 knockdown was slower, with both tumor volume and weight significantly smaller than those in the negative control group. Proteins extracted from the tumor tissues were analyzed using Western blotting, revealing a significant increase in E-cadherin, a protein associated with epithelial-mesenchymal transition, alongside a marked decrease in N-cadherin with the reduction of ANXA2. Additionally, we constructed an

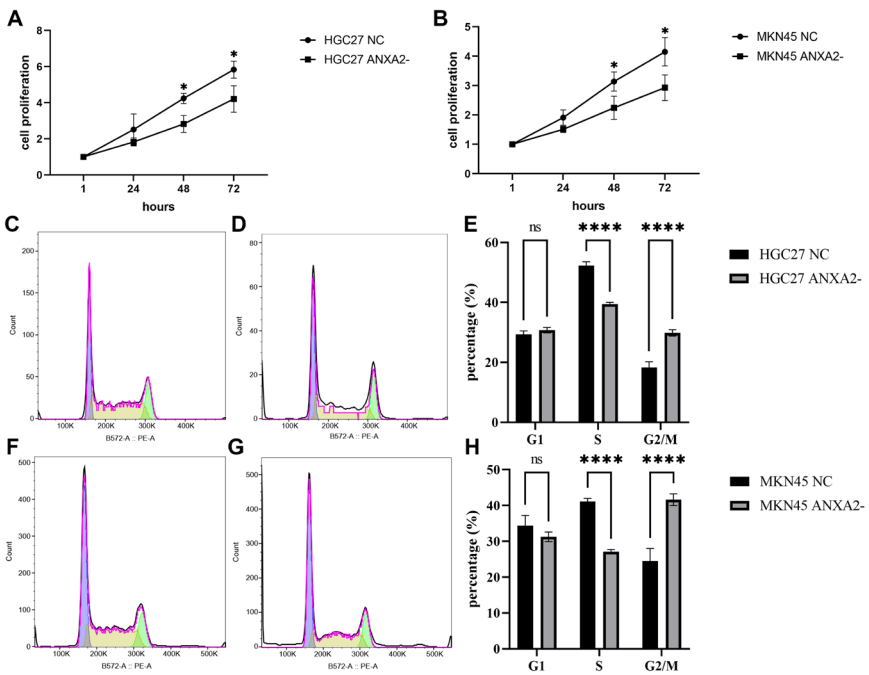

Fig. 5 Cell proliferation and Flowcytometric results of different cell lines

(A) Cell proliferation in the HGC27 cell line; (B) Cell proliferation in the MKN45 cell line; (C) Flow cytometric analysis of cell cycle in HGC27 NC cells; (D) Flow cytometric analysis of cell cycle in ANXA2- HGC27 cells; (E) Flow cytometric analysis of cell cycle in HGC27 cell line; (F) Flow cytometric analysis of cell cycle in MKN45 NC cells; (G) Plot of flow cytometric analysis of cell cycle of ANXA2- MKN45 cells; (H) Proportion of various cell cycles in MKN45 cells. $^{*}P < 0.05$, $^{****}P < 0.0001$.

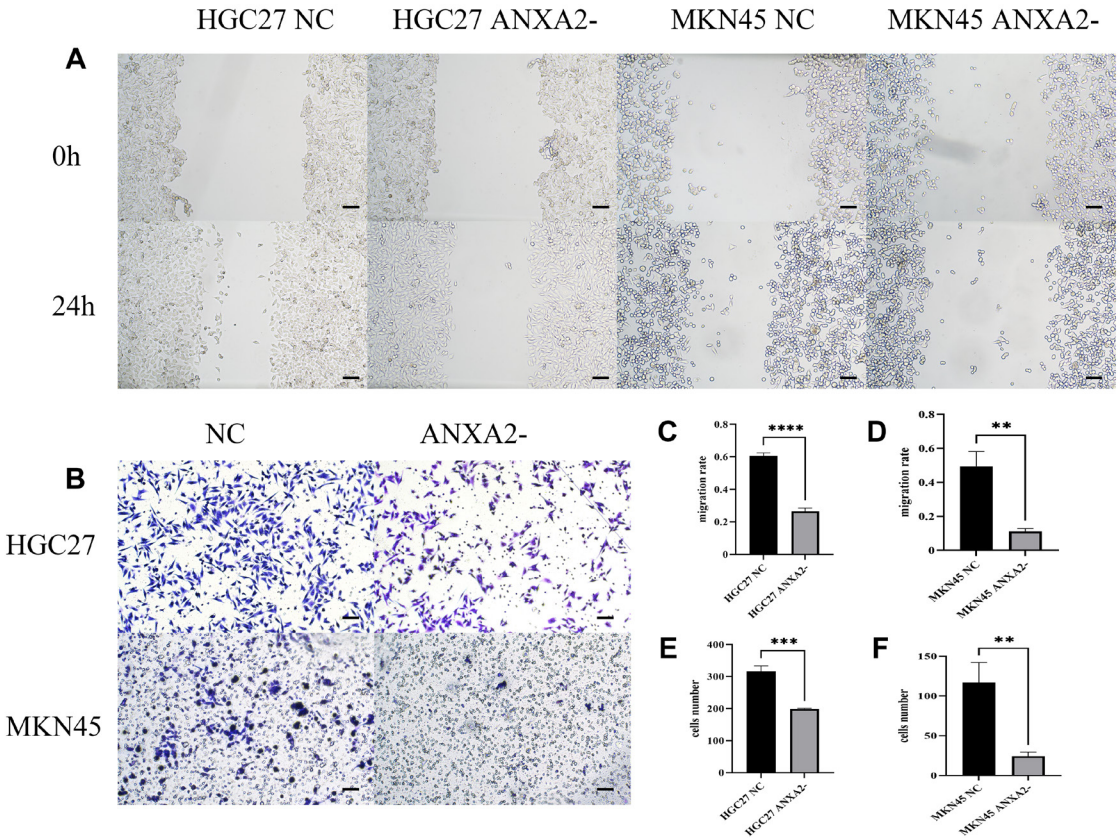

Fig. 6 Metastatic ability of different cell lines

(A) Images of scratch experiments (100×) for different cell lines; (B) Representative images showing the results from Transwell experiments for various cell lines as specified; (C-D) Bar graphs of statistical results from 24-hour scratch experiments; (E-F) Bar graphs of statistical results from Transwell experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

intraperitoneal implantation model of gastric cancer in mice. Tumor metastasis was monitored using an IVIS (*In vivo* imaging system) imaging system, and it was found that five days after tumor injection, the mice in the knockdown group exhibited lower abdominal luminescence and fewer metastatic foci of abdominal implantation compared to those in the negative control group, with the difference being statistically significant (Fig. 7).

4 Discussion

The family of calcium-dependent membrane-bound proteins plays an important role in various cellular processes, with ANXA2 being a significant member of this family. Previous studies have suggested that ANXA2 contributes to multiple tumor types. Our analysis of sequencing results suggests that ANXA2 has potential prognostic implications for patients with peritoneal metastasis of gastric cancer, prompting us to focus our study on this protein.

Initially, we conducted bioinformatics analyses to explore the multifaceted role of ANXA2. Our findings indicate that ANXA2 is critically involved in gastric cancer with its RNA expression levels closely correlating with patient prognosis, aligning with earlier studies. Enrichment analysis of ANXA2-related genes confirmed its pivotal role in the PI3K-AKT signaling pathway, which is essential for tumor proliferation, invasion, metastasis, and immune evasion. Notably, our study highlighted ANXA2's potential to regulate PD-L1 expression *via* this pathway, which could influence tumor responses to immunotherapy.

Next, we investigated potential upstream regulators of ANXA2. Through the prediction of miRNAs and TFs, we identified six candidates, with SMAD3 emerging as the most likely regulator affecting ANXA2 expression. Previous research has shown that SMAD3 plays a dual role in oncogenesis, underscoring its connection to ANXA2 as a critical pathway in promoting cancer progression. Future studies will further investigate this interaction to deepen our understanding of its implications in cancer development.

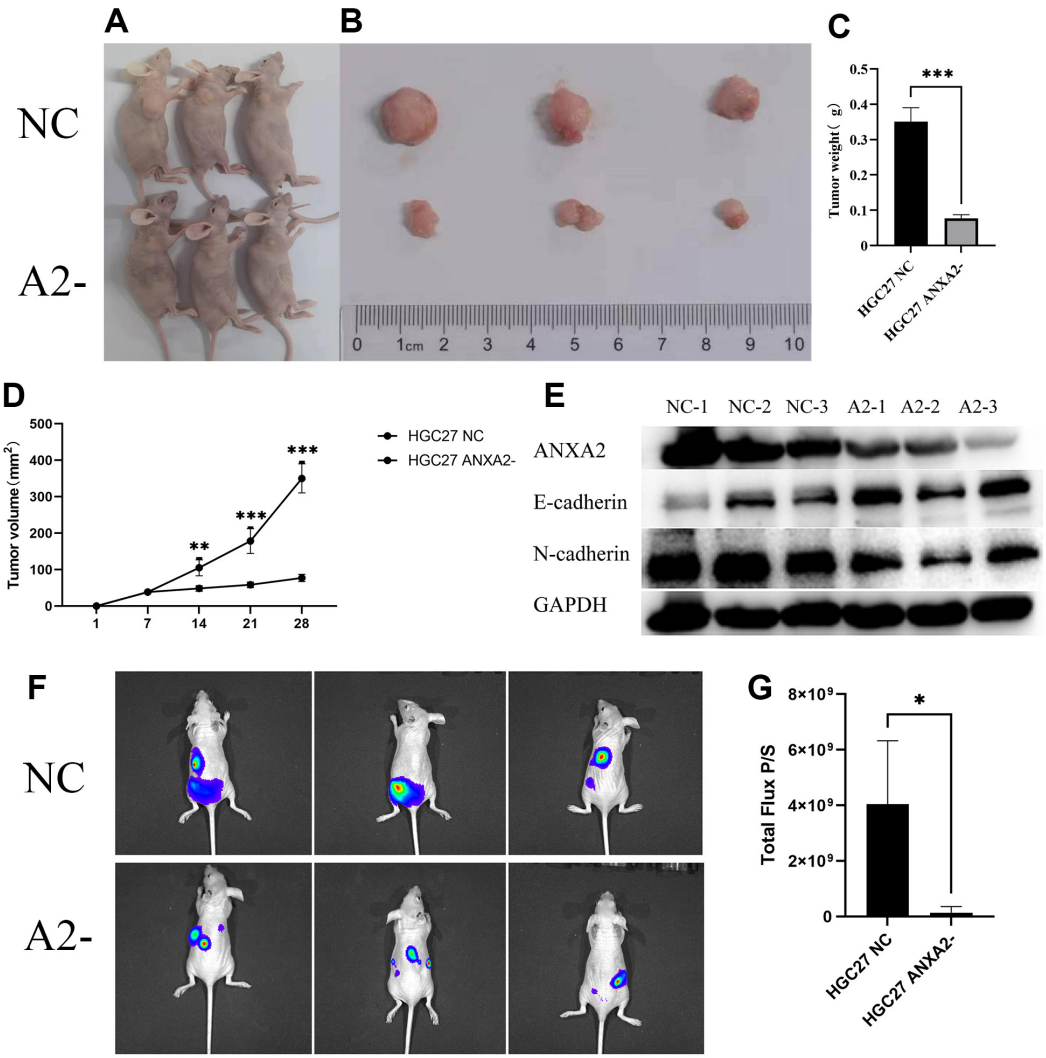

Fig. 7 *In vivo* results of different cell lines

(A) Photographs of HGC27 cell-implanted mice and the control littermate; (B) Photographs showing the relative tumor sizes between HGC27 cell-implanted mice and the control counterparts; (C) Statistical results of tumor weight between the two groups; (D) Time-dependent changes of tumor volume between the two groups; (E) Relative protein levels in tumor tissues; (F) Luminescence images showing abdominal implantation in mice; (G) Statistical data of luminescence intensity on abdominal implantation tumors. $^{*}P < 0.05$, $^{***}P < 0.001$.

To confirm the role of ANXA2 in gastric cancer, particularly in peritoneal metastasis, we initiated basic research. While earlier studies have established ANXA2's pro-cancer role at the cellular level, *in vitro* experiments have provided limited evidence. Therefore, we innovatively constructed a mouse intraperitoneal implantation model using cell lines with varying ANXA2 expression levels. The results demonstrated that ANXA2 not only significantly influences tumor formation but also promotes peritoneal metastatic implantation of gastric cancer. Several companies have developed targeted inhibitors against ANXA2, and we hope these can be actively translated into clinical in the near future, facilitating early intervention for patients with gastric cancer, particularly in cold regions.

5 Conclusion

ANXA2 plays a crucial role in the development of gastric cancer, primarily through the PI3K-AKT signaling pathway. Reduced expression of ANXA2 can inhibit peritoneal implantation metastasis of gastric cancer. These findings suggest that ANXA2 may be a potential risk factor affecting the survival probability of gastric cancer patients, particularly in cold regions.

Author contributions

Wang K and Huang X Y searched the literature and conceived the article design. Jin S Y performed the manuscript preparation and finished *in vitro* and *in vivo* experiments. Liang L X collected and analyzed data and constructed the Figures. All authors read and approved the final manuscript for submission.

Source of funding

We are grateful to Haiyan Fund, Harbin Medical University Cancer Hospital (JJED2016-02) for the financial support. The funding source had no role in the design, practice or analysis of this study.

Ethical approval

The recruitment of participants for this survey was conducted in accordance with the Helsinki Declaration, and the study was approvedby the Human Research Ethics Committee of Harbin Medical University (2023-200-QX).

Informed consent

Written informed consent was obtained from all participant.

Conflict of interest

All authors declare that they have no conflict of interest.

Data availability statement

The data are available from the corresponding author on reasonable request.

References

[1] Hajjar K A, Krishnan S. Annexin II: a mediator of the plasmin/plasminogen activator system. Trends Cardiovasc Med, 1999; 9(5): 128-138.
[2] Morel E, Gruenberg J. Annexin A2 binding to endosomes and functions in endosomal transport are regulated by tyrosine 23 phosphorylation. J Biol Chem, 2009; 284(3): 1604-1611.
[3] Deora A B, Kreitzer G, Jacovina A T, *et al*. An annexin 2 phosphorylation switch mediates p11-dependent translocation of annexin 2 to the cell surface. J Biol Chem, 2004; 279(42): 43411-43418.
[4] de Graauw M, Tijdens I, Smeets M B, *et al*. Annexin A2 phosphorylation mediates cell scattering and branching morphogenesis *via* cofilin Activation. Mol Cell Biol, 2008; 28(3): 1029-1040.
[5] Shiozawa Y, Havens A M, Jung Y, *et al.* Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. J Cell Biochem, 2008; 105(2): 370-380.
[6] Vishwanatha J K, Jindal H K, Davis R G. The role of primer recognition proteins in DNA replication: association with nuclear matrix in HeLa cells. J Cell Sci, 1992; 101 (Pt 1): 25-34.
[7] Kazami T, Nie H, Satoh M, *et al.* Nuclear accumulation of annexin A2 contributes to chromosomal instability by coilin-mediated centromere damage. Oncogene, 2015; 34(32): 417741-417789.
[8] Castaldo S A, Ajime T, Serrão G, *et al.* Annexin A2 regulates AKT upon H_2O_2-dependent signaling activation in cancer cells. Cancers (Basel), 2019; 11(4): 492.
[9] de Graauw M, Cao L, Winkel L, *et al.* Annexin A2 depletion delays EGFR endocytic trafficking *via* cofilin activation and enhances EGFR signaling and metastasis formation. Oncogene, 2014; 33(20): 2610-2619.
[10] Sarkar S, Swiercz R, Kantara C, *et al*. Annexin A2 mediates up-regulation of NF-κB, β-catenin, and stem cell in response to progastrin in mice and HEK-293 cells. Gastroenterology, 2011; 140(2): 583-595.e4.
[11] Feng X, Liu H, Zhang Z, *et al.* Annexin A2 contributes to cisplatin resistance by activation of JNK-p53 pathway in non-small cell lung cancer cells. J Exp Clin Cancer Res, 2017; 36(1): 123.
[12] Zhang HJ, Yao D F, Yao M, *et al*. Annexin A2 silencing inhibits invasion, migration, and tumorigenic potential of hepatoma cells. World J Gastroenterol, 2013; 19(24): 3792-3801.
[13] Wang Y X, Lv H, Li Z X, *et al.* Effect of shRNA mediated down-regulation of Annexin A2 on biological behavior of human lung adencarcinoma cells A549. Pathol Oncol Res, 2012; 18(2): 183-190.
[14] Sharma M R, Rothman V, Tuszynski G P, *et al.* Antibody-directed targeting of angiostatin's receptor annexin II inhibits Lewis Lung Carcinoma tumor growth *via* blocking of plasminogen activation: possible biochemical mechanism of angiostatin's action. Exp Mol Pathol, 2006; 81(2): 136-145.
[15] Ling Q, Jacovina A T, Deora A, *et al.* Annexin II regulates fibrin homeostasis and neoangiogenesis *in vivo*. J Clin Invest, 2004; 113(1): 38-48.
[16] Zhai H, Acharya S, Gravanis I, *et al.* Annexin A2 promotes glioma cell invasion and tumor progression. J Neurosci, 2011; 31(40): 14346-14360.
[17] Liu Y, Li H, Ban Z, *et al.* Annexin A2 inhibition suppresses ovarian cancer progression *via* regulating β-catenin/EMT. Oncol Rep, 2017; 37(6): 3643-3650.
[18] Rocha M R, Barcellos-de-Souza P, Sousa-Squiavinato A, *et al*. Annexin A2 overexpression associates with colorectal cancer invasiveness and TGF-ß induced epithelial mesenchymal transition *via* Src/ANXA2/STAT3. Sci Rep, 2018; 8(1): 11285.
[19] Atkinson S J, Gontarczyk A M, Alghamdi A A, *et al.* The β3-integrin endothelial adhesome regulates microtubule-dependent cell migration. EMBO Rep, 2018; 19(7): e44578.
[20] Wu Y, Cai F, Lu Y, *et al.* lncRNA RP11-531A24.3 inhibits the migration and proliferation of vascular smooth muscle cells by downregulating ANXA2 expression. Exp Ther Med, 2021; 22(6): 1439.
[21] Zhou S, Yi T, Liu R, *et al.* Proteomics identification of annexin A2 as a

key mediator in the metastasis and proangiogenesis of endometrial cells in human adenomyosis. Mol Cell Proteomics, 2012; 11(7): M112.017988.
[22] Chen C Y, Lin Y S, Chen C H, *et al.* Annexin A2-mediated cancer progression and therapeutic resistance in nasopharyngeal carcinoma. J Biomed Sci, 2018; 25(1): 30.
[23] Wu B, Zhang F, Yu M, *et al.* Up-regulation of Anxa2 gene promotes proliferation and invasion of breast cancer MCF-7 cells. Cell Prolif, 2012; 45(3): 189-198.
[24] Yao H, Zhang Z, Xiao Z, *et al.* Identification of metastasis associated proteins in human lung squamous carcinoma using two-dimensional difference gel electrophoresis and laser capture microdissection. Lung Cancer, 2009; 65(1): 41-48.
[25] Zhang F, Zhang H, Wang Z, *et al.* P-glycoprotein associates with Anxa2 and promotes invasion in multidrug resistant breast cancer cells. Biochem Pharmacol, 2014; 87(2): 292-302.
[26] Zhang Z D, Li Y, Fan Q, *et al.* Annexin A2 is implicated in multi-drug-resistance in gastric cancer through p38MAPK and AKT pathway. Neoplasma, 2014; 61(6): 627-637.
[27] Emoto K, Yamada Y, Sawada H, *et al.* Annexin II overexpression correlates with stromal tenascin-C overexpression: a prognostic marker in colorectal carcinoma. Cancer, 2001; 92(6): 1419-1426.
[28] Tas F, Tilgen Yasasever C, Karabulut S, *et al.* Circulating annexin A2 as a biomarker in gastric cancer patients: correlation with clinical variables. Biomed Pharmacother, 2015; 69: 237-241.
[29] Xie R, Liu J, Yu X, *et al.* ANXA2 Silencing inhibits proliferation, invasion, and migration in gastric cancer cells. J Oncol, 2019; 2019: 4035460.
[30] Mao L, Yuan W, Cai K, *et al.* EphA2-YES1-ANXA2 pathway promotes gastric cancer progression and metastasis. Oncogene, 2021; 40(20): 3610-3623.
[31] Chandrashekar D S, Bashel B, Balasubramanya S, *et al.* UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia, 2017; 19(8): 649-658.
[32] Lánczky A, Győrffy B. Web-based survival analysis tool tailored for medical research (KMplot): development and implementation. J Med Internet Res, 2021; 23(7): e27633.
[33] Asplund A, Edqvist P H, Schwenk J M, *et al.* Antibodies for profiling the human proteome-the human protein atlas as a resource for cancer research. Proteomics, 2012; 12(13): 2067-2077.
[34] Modhukur V, Iljasenko T, Metsalu T, *et al.* MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data. Epigenomics, 2018; 10(3): 277-288.
[35] Szklarczyk D, Kirsch R, Koutrouli M, *et al.* The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res, 2023; 51(D1): D638-D646.
[36] Zhou Y, Zhou B, Pache L, *et al.* Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun, 2019; 10(1): 1523.
[37] Li J H, Liu S, Zhou H, *et al.* starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res, 2014; 42(Database issue): D92-D97.