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ANXA2 REMODELS THE MICROSTRUCTURES OF CACO2 CELLS

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Abstract

ANXA2 was reported as a multiple tumors relevant gene expressed excessively in many tumor tissue types, especially in the cancers from digestion system, and its aberrant expression enhances the malignant properties of cancer cells. We suppose that the microstructure heterogeneity is important to maintain the malignancy of cancer cells, and excessive *ANXA2* expression enhance the malignancy by remodeling the microstructures of cancer cells. To validate the proposal, the *ANXA2*$^{-/-}$caco2 cell line was generated and the changes of the microstructures in the ANXA2 deleted and wild type caco2 cells were observed under fluorescence microscope, laser scanning confocal microscope and electron microscope. We found that ANXA2 deletion induced the pseudopodia shorted and spared, non-stained areas increased, mitochondria decreased, and the expression and polymerization of F-actin and β-tubulin changed. By the findings above, it is firstly reported in this paper that the *ANXA2* excessive expression induces the significant changes of the microstructures in cancer cells. Combining our previous data together, our results indicate that *ANXA2* excessive expression enhances the malignancy of cancers partially by remodeling the cell microstructures.

Key words: Cell microstructure, ANXA2, colorectal carcinoma, gene function.

Article information

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INTRODUCTION

ANXA2 (Annexin A2) is a 36-kDa member of the annexin family that is Ca^{2+} binding phospholipid protein family involved in many diseases and tumor development (4). Published data (8, 17, 19, 21) and our previous data (9) indicate that *ANXA2* is excessively expressed in numerous tumor tissue types and enhances the malignancy of cancers for the proliferation, invasion, and metastasis of cancer cells.

The special appearances and structures, especially microstructures of cancer cells are usually changed as some morphological heterogeneity compared with normal cells. These changes are the basics for the malignant development of cancers. The activation of the F-actin filament reorganization is one of the key events in the cancer cells with enhanced motility, and needs the interaction among the actin-binding proteins, regulatory proteins and actin cytoskeleton (1). ANXA2 mediates the F-actin reorganization by its binding to F-actin, thus ANXA2 is important to stabilize the membrane-associated protein complexes in actin cytoskeleton (6). ANXA2 functions as a platform so as to maintain the expression of membrane-associated actin cytoskeleton and promotes cell migration through the interaction with F-actin, and ANXA2 regulates F-actin polymerization by its affinity to the barbed end of actin microfilaments (16). Döppler et al reported that F-actin is important to pseudopodia formation and action, and increased reorganization of the actin cytoskeleton can enhance cell motility (5). Yu et al suggested that F-actin is crucial for extension of invasive pseudopods and for providing the correct cytoskeletal framework to couple matrix remodeling with protrusive invasion (20). The results introduced as above imply that the excessive *ANXA2* expression may contribute to the enhancement of cancer malignant development by its interaction with F-actin. Microtubule may be also important to enhance the microstructure heterogeneity of cancer cells. The enhanced and stably reorganized microtubules promoted the microtentacles formation of cancer cells, and consequently facilitated cancer cells' aggregation and reattachment *in vitro* (2, 13), so tubulin expression and its reassembly may be a key event to maintain the malignancy of cancer cells. We proposed that the excessive *ANXA2* expression may enhance the malignancy of cancer cells by its regulation for the expression and polymerization of F-actin and β-tubulin to remodel the microstructure heterogeneity of cancer cells.

To remove the effects from the remained expression of the target gene, the correlation of the excessive *ANXA2* expression with the cell microstructure remodeling needs to be further studied in such an experimental environment without any *ANXA2* expression. To explore the roles ANXA2 played to remodel cell microstructure objectively, in our study, *ANXA2*$^{-/-}$caco2 cell line was successfully generated, and the differences of cell malignancy relevant microstructures were observed and analyzed in ANXA2 deleted and wild type caco2 cells.

MATERIALS AND METHODS

Generation of ANXA2-/-caco2 cell line

Cell culture and medium

Caco2 cells purchased from ATCC (Rockville, MD) were cultured with DMEM medium supplemented with 10% fetal bovine serum at 37℃ and in 5% CO_2.

ANXA2 knockout recombinant construction

pPNT vector (Fig. 1A) was kindly obtained from Dr. Ye-Shih Ho (Institution of Environmental Health Sciences, Wayne State University, Detroit, MI). The designated locus for human *ANXA2* knockout is shown as Fig. 1B. The primers used for the recombinant (*pPNT/ANXA2/*

Table 1. The primers used the knockout recombinant construction and cell clone identification.

Primer names	Sequences (5'→3')	Lengths of amplified products
Upper I 1 *Not I* Lower I 1 *Xho I/SgrA I/Asc I*	ATATATGCGGCCGCTTTGGGAAAACTACTTCCTTG ATTACAC AGGCTCGAGCACCGGTGAAAGGCGCGCCCTGGTT GGTTCTGGAGCAGATGATCTCAATG	Insertion fragment 1 from genome (intron 5-6 + exon 6) 3542bp
Upper I 2 *Asc I* Lower I 2 *SgrA I*	ATATATGGCGCGCCCGCCACCATGGTGAGCAAG GCGCGCCACCGGTGTTATCTAGATCCGGTGGATCC	Insertion fragment 2 for EGFP 833bp
Upper I 3 *Kpn I* Lower I 3 *EcoR I*	GAGAGAGGTACCGAGCTGCAGGAAATTAACAGAG GTGCGCGAATTCTTCTAATAAACATTGCTGTCAG	Insertion fragment 3 from genome (exon 6 + intron 6-7) 3524bp
Discover human ANXA2 Upper Discover human ANXA2 Lower	GAAGTAGGCTAATGGTGGGGAAGC AGGCAATGAGGAGGTGAAGGAGAG	ANXA2 +/+ cells: 335bp ANXA2 +/- cells: 335/2988bp ANXA2 -/- cells: 2988bp

EGFP) construction and positive cell clone identification are shown as Tab. 1.

Two genomic fragments (3.5kb) of *ANXA2* were amplified from caco2 cell genomic DNA. The fragment 1 was inserted into the *Not* I/*Xho* I site at the upstream of neo^R gene of pPNT. The fragment 3 was inserted into the *Kpn* I/*EcoR* I site at the downstream of neo^R gene of pPNT. The fragment 2 amplified from *pEGFP-C1* plasmid was inserted into the *Asc* I/*SgrA* I site rebuilt in fragment 1. The discover primers would be used to identify cell clones. All primers were synthesized by Sangon Biotech (Shanghai, China).

Caco2 cell transfection

The recombinant (*pPNT/ANXA2/EGFP*) was digested by *Not I* to be linearized. Linearized recombinant was introduced into caco2 cells by using a Bio-Rad Xcell-Electroporator (Bio-Rad China, Shanghai, China) and following the manufacturer's instruction book.

Selection for $ANXA2^{-/-}$caco2 cell clone

Two days post transfection, add G418 (Calbiochem, German) into the transfected well and in another well that wild type caco2 cells were cultured with same density and conditions (G418 working concentration: 700μg/ml). Two weeks later, when wild type cells died all most, continue to culture the cells with G418 (700μg/ml) for four weeks. Then, clone the cells using limited dilution method: Make cell suspension in DMEM medium plus 20% FBS at 2.5 cells/ml concentration, pipette the suspension and add into 96-well plates (0.3ml/well). Check cell number for each well under a low resolution microscope and mark the wells containing single cell as "single clone". When the cells in the wells marked as "single clone" grown up over 50% confluence, transfer them into the wells of 6-well plates to make expanded culture.

Identification of $ANXA2^{-/-}$caco2 cell clones

Identification by PCR

Each cell clone was identified using the previously designated specific PCR (by using discover primers). Product lengths: 335bp for $ANXA2^{+/+}$ cells; 335bp plus 2988bp for $ANXA2^{+/-}$ cells; 2988bp for the positive clone, $ANXA2^{-/-}$ cell.

Identification by western blot

The PCR identified positive clones were confirmed using western blot. When the cell confluency was about 90%, the cell lysate was collected to run a 10% SDS-PAGE and nitrocellulose membrane (PALL, CA) transfer. The transferred membrane was incubated with polyclonal rabbit-anti human ANXA2 antibody (1:1000, Santa Cruz Biotech, CA) first, then with HRP labeled goat-anti rabbit antibody (1:3000, Biosynthesis Biotech, Beijing, China). The membrane was developed using an ECL Kit (Pierce, Shanghai, China) following the manufacture's instruction. Experiment was repeated for three times independently.

Observation for the microstructure changes

Observation under scanning electron microscope (SEM)

The ANXA2 deleted and wild type caco2 cells were cultured on the slide cover-glasses in the wells of 6-wells plate for 24 hours. The cover-glass was rinsed using PBS and fixed for 1 hour at 4℃ using 2.5% Glutaraldehyde (Sigma, St. Louis, NY). The fixed cells were dehydrated using gradient diluted ethanol solutions (30%, 50%, 70%, 80%×2, 90%×2, 95%×2, 100%×2, 15mins/each). The dehydrated cells were further treated following the instruction book of the environmental scanning electron microscope (Quanta2000, Philips-FEI, Netherlands) and observed under this microscope.

Observation under transmission electron microscope (TEM)

The cell grouping, fixing and dehydrating are same as the above. The cell samples were treated and observed following the instruction book of the transmission electron microscope (JEM-2100, Japan).

Observation for the microstructure associated proteins

F-actin

The ANXA2 deleted and wild type caco2 cells were cultured on the slide cover-glasses in the wells of 6-wells plate for 24 hours. The cover-glass was rinsed using PBS for three times, fixed using 3.7% paraformaldehyde for 10 minutes at 37℃ and treated with 0.1% TritonX-100 for 10 minutes at 37℃. The cover-glass was firstly incubated with 10% normal goat serum (Ding-Guo Biotech, Beijing, China) for 45 minutes at room temperature, and then with FITC-labeled phalloidine (2μg/ml) (Ding-Guo Biotech, Beijing, China) for 60 minutes at room temperature. The cells were observed to check the F-actin changes under a fluorescence microscope (Leica, Germany) and laser scanning confocal microscope (TCS SP5, Leica, Germany).

β-tubulin

The steps before antibody incubation are same with the steps before FITC-labeled phalloidine incubation in F-actin staining. After the normal goat serum was removed, the cover-glass was incubated with rabbit-anti-human β-tubulin polyclonal antibody (1:50) (Ding-Guo Biotech, Beijing, China) at 4℃ for overnight, and then with Cy3-labeled goat-anti-rabbit IgG (1:100) at 4℃ for overnight. The cells were observed to check β-tubulin distribution under the fluorescence

RESULTS

Generation of the ANXA2-/-caco2 cell line

Insertion fragment1 was inserted into *Not I /Xho I* site of pPNT, insertion fragment3 was inserted into *Kpn I /EcoR I* site of pPNT, and insertion fragment2 was inserted into the *Asc I /SgrA I* site rebuilt in fragment1. The recombinant, *pPNT/ANXA2/EGFP*, was successfully constructed, and the success was confirmed by sequencing the recombinant.

The recombinant plasmid, *pPNT/ANXA2/EGFP*, was linearized by the digestion with *Not I*. The linearized plasmid was induced into caco2 cells by using an electroporator, and the transfection efficiency was over 80%. The transfected cells and wild type cells were continually cultured with G418 (700μg/ml) together for 6 weeks when the wild type cells died completely. The transfected and G418 selected caco2 cells were clonally cultured using a limitation dilution method to culture the single cell in each well of 96-well plates, and the grown up cells from each clone were transferred into each well of 6-wells plate for continual culture. The cell clone, 12B8, was identified as the positive clone, *ANXA2*$^{-/-}$caco2 clone, by the result of the previously designated PCR (Fig. 1C), and confirmed by the result of western blot assay (Fig. 1D). The positive clone was expanded and stored as the ANXA2 deleted caco2 cell line in our lab.

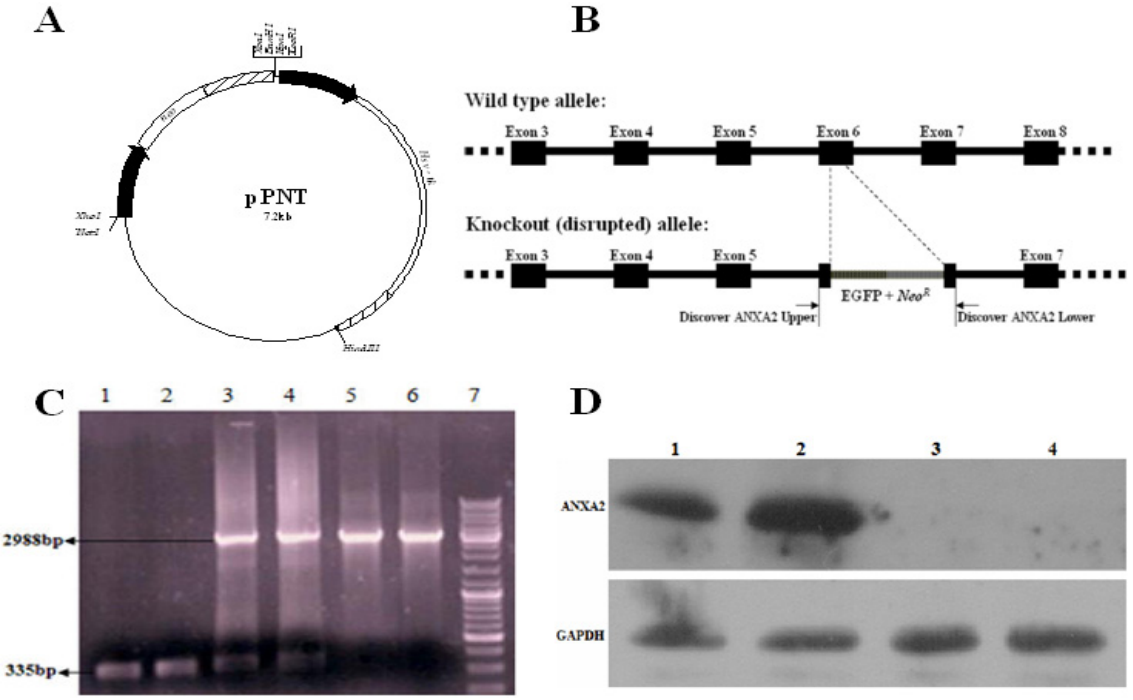


Figure 1. The generation of ANXA2-/-caco2 cell line. **A:** The map of vector; **B:** The illustration of ANXA2 knockout approach; **C:** The ANXA2 deleted cell clone identification using specific PCR (1, 2: ANXA2$^{+/+}$caco2 cells; 3, 4: ANXA2$^{+/-}$caco cells; 5, 6: ANXA2$^{-/-}$caco2 cells; 7: DNA ladder); **D:** The ANXA2 deleted cell clone confirmation using western blot (1, 2: ANXA2+/+caco2 cells; 3, 4: ANXA2$^{-/-}$ caco2 cells).

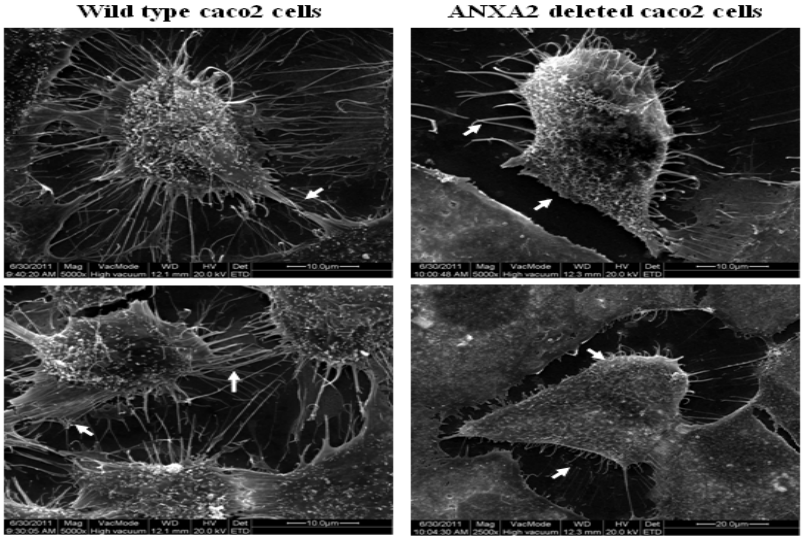


Figure 2. The cultured cells under scanning electron microscope.The pseudopodia/filopodia of wild type caco2 cells is much more vigorous than those of ANXA2 deleted caco2 cells. The contact inhibition reappears in ANXA2 deleted cells.

The cell structure changes after ANXA2 deletion

In order to explore the correlation of ANXA2 with cancer cell motility, the microstructures of pseudopodia/filopodia in ANXA2 deleted and wild type caco2 cells were observed under scanning electron microscope and transmission electron microscope. The pseudopodia/filopodia of wild type caco2 cells were much more vigorous than those of ANXA2 deleted caco2 cells, and the contact inhibition among cells reappeared in ANXA2 deleted cells (Fig. 2).Much more cytosol transparent bubbles could be observed in ANXA2 deleted cells than in wild type caco2 cells (Fig. 3). These findings indicate that *ANXA2* expression is important to maintain the enhanced cell motility for the invasion and metastasis of cancers and the unlimited proliferation of cancer cells.

The F-actin changes after ANXA2 deletion

Any effect on F-actin bundles assembly or disassembly may be important to regulate the motility, invasion and metastasis of cancer cells. We compared F-actin expression in wild type and ANXA2 deleted caco2 cells by displaying F-actin using FITC-labeled phalloidine staining. F-actin expression was significantly enhanced and vigorously bundled along the inner face of membrane in wild type caco2 cells, but distinctly inhibited and disassembled in ANXA2 deleted caco2 cells (Fig. 4). The changes above indicate a close correlation between ANXA2 expression and F-actin changes in caco2 cells.

The β-tubulin changes after ANXA2 deletion

By the enhanced microtubule stability and assembly, cancer cells present an increased deformability to conquer the deforming force during their metastasis through microcapillary vessels system. Tubulin is important to increase cell deformability and motility. Using β-tubulin immunofluorescence staining assay, the significantly inhibited β-tubulin expression and polymerization was observed in ANXA2 deleted caco2 cells comparing with wild type caco2 cells (Fig. 5). This result implies that caco2 cells may utilize ANXA2 to sustain their microtubule assembly for microtentacles formation and enhance their metastatic potential like what described by Matrone et al (13) and Chen et al (3).

DISCUSSION

ANXA2 is excessively expressed usually in malignant tumors and may play roles in cancer invasion and metastasis (9, 10). The enhanced motility is necessary for the cancer cells with powerful migration behavior, and pseudopodia/filopodia are essential structures for such a cell type. Pseudopodium is the thin, sheet-like protrusion filled with polymerized actin meshwork, and filopodia is the finger-like protrusion filled with tight bundles of filamentous actin (14). Filopodia promotes cell motility directly, so abundant filopodia bundles enhance cancer cells migration and metastasis (18). We observed much weaker and poorer developed pseudopodia/filopodia in ANXA2 deleted caco2 cells than in wild type caco2 cells, the contact inhibition among cells reappeared in ANXA2 deleted cells (Fig. 2), and much more cytosol transparent bubbles in ANXA2 deleted caco2 cells than in wild type caco2 cells (Fig. 3). These findings indicate that ANXA2 is necessary to maintain the enhanced cell motility for the invasion and metastasis of cancers. F-actin expression and its polymerization are essential for the development of pseudopodia/filopodia (12), we hypothesize that ANXA2 may increase the F-actin polymerization when the pseudopodia/filopodia sprouting and elongating is enhanced. ANXA2 deletion inhibits F-actin polymerization and causes the poor development of pseudopodia/filopodia, and finally results in weak motility of cancer cells.

ANXA2-S100A10 heterotetramer enhances F-actin bundles *in vitro* (11) and orients F-actin to lipid rafts of cell

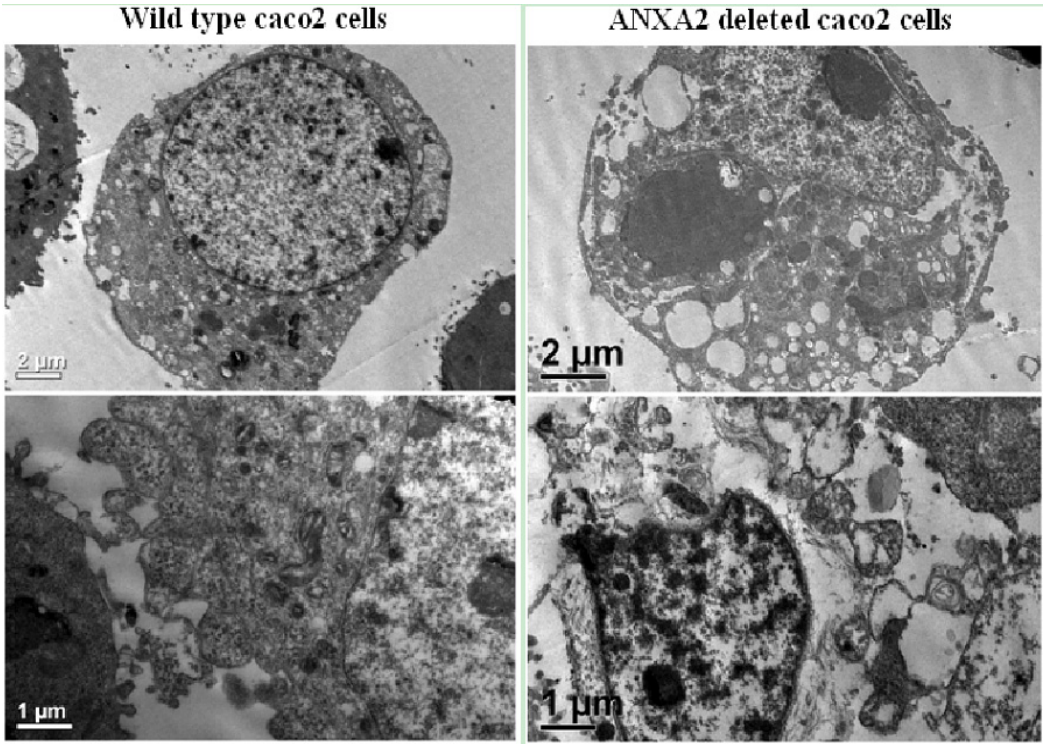
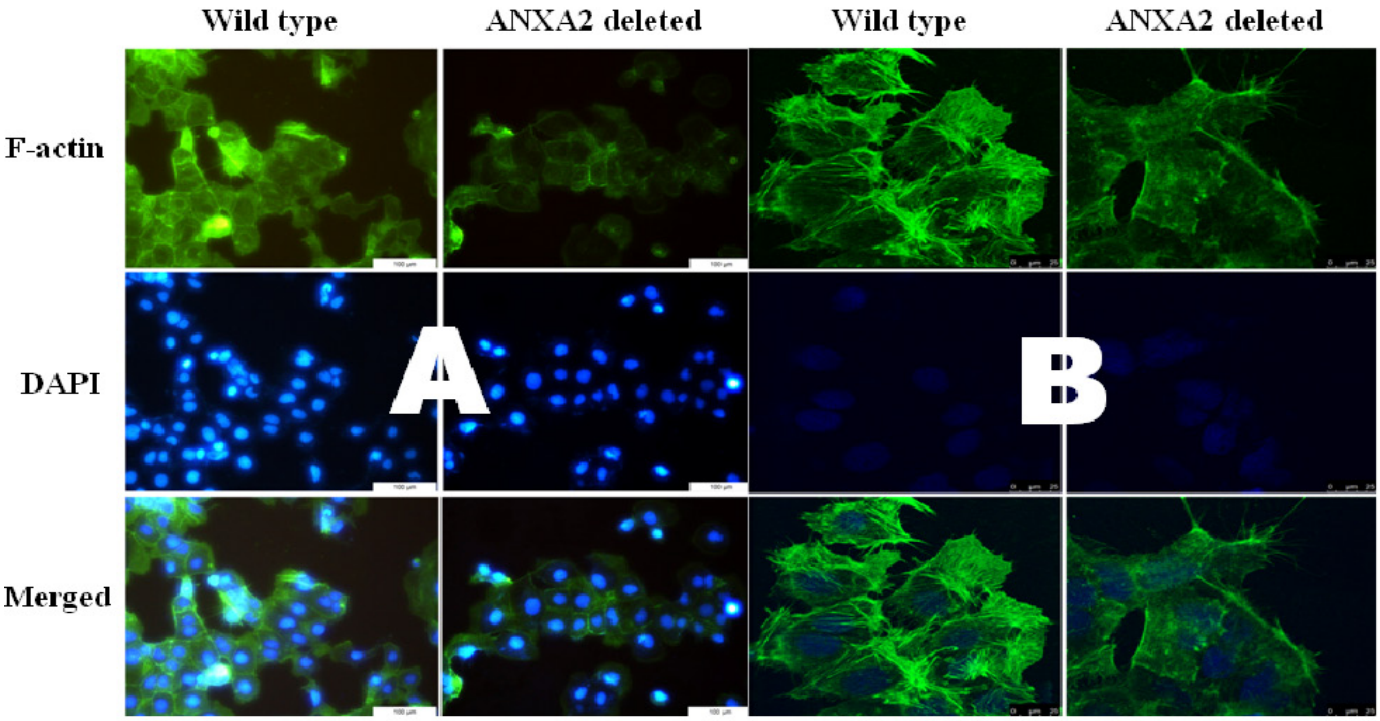


Figure 3. The observation for cultured cells under transmission electron microscope. Much more cytosol transparent bubbles are formed in ANXA2 deleted cells than in wild type caco2 cells.



Fiure 4. Immunofluorescence staining assay for F-actin. **A:** Under fluorescence microscope; **B:** Under laser confocal microscope DAPI staining is for nuclei. F-actin expression is significantly enhanced and vigorously bundled in wild type caco2 cells, and distinctly inhibited and disassembled in ANXA2 deleted caco2 cells.

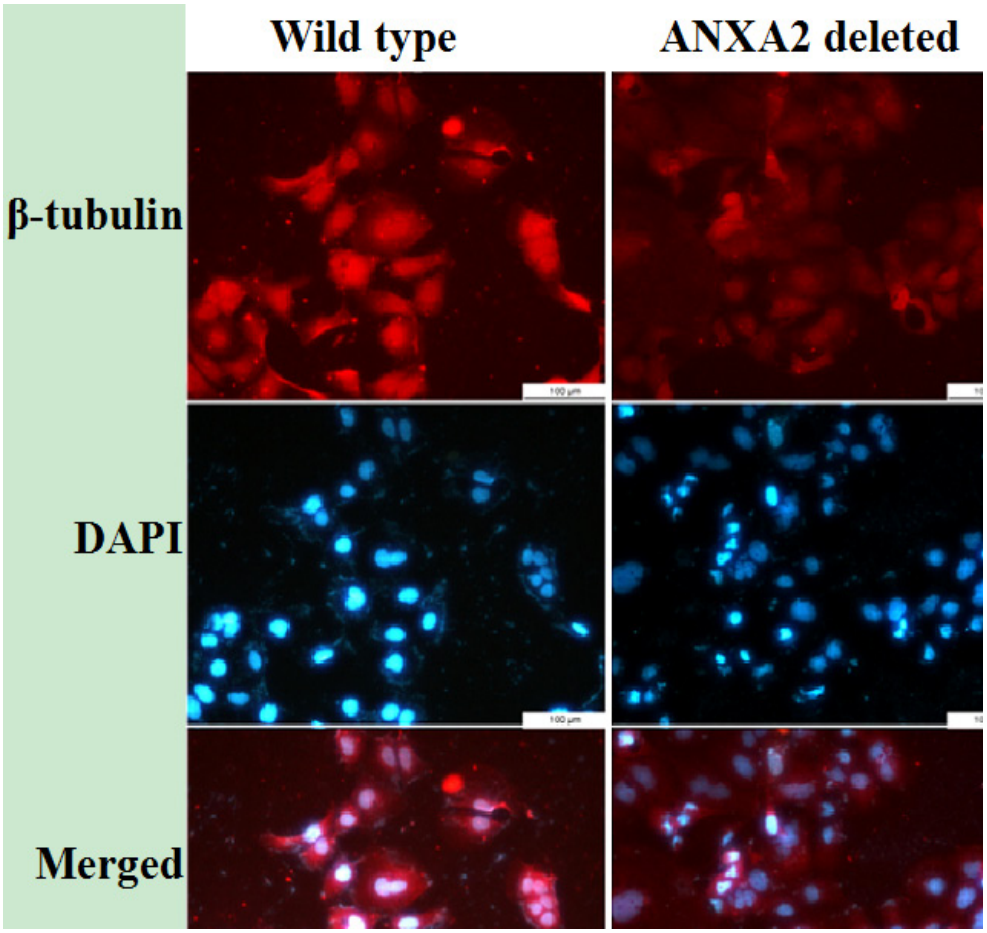


Figure 5. Immunofluorescence staining assay for β-tubulin**.** DAPI staining is for nuclei. β-tubulin expression is significantly enhanced and vigorously bundled in wild type caco2 cells, and distinctly inhibited and disassembled in ANXA2 deleted caco2 cells.

membrane (22). Our results indicate that following ANXA2 deletion, F-actin polymerization was decreased in ANXA2 deleted cells (Fig. 4). This result indicates that ANXA2 is necessary for F-actin polymerization. Other factors, such as Rho GTPase (1), may function with ANXA2 together to enhance F-actin polymerization. ANXA2 orients signal molecules to specific membrane domain where actins will be polymerized (7), but the orientation is disrupted in ANXA2 deleted cells. It is easy to be understood that ANXA2 interacts with actins to maintain the enhanced motility so as to promote the invasion and metastasis of cancer cells.

Microtubules are the essential elements to form the active microtentacles (McTNs) (13) that facilitate the motility of cancer cells and enhance the malignancy of cancers consequently. Cancer cells are more deformable than normal cells depending on the enhanced stability of microtubules (3), and the increased deformability facilitates cancer metastasis. Fig. 5 shows that ANXA2 deletion induced *β-tubulin* expression decreased and microtubules disassembled. The enhanced microtubule disassembly may result in cell lamellipodia formation inhibited, cell motility decreased, and cell proliferation disrupted. Reported data explored that the microtubule disassembly destabilizes vascular vessels, and inhibits angiogenesis and *VEGFR2* expression (15), so the disassembly suppresses the invasion and metastasis of cancer. Our results indicate that the expression and polymerization of F-actin and β-tubulin were evidently disrupted in ANXA2 deleted caco2 cells, and ANXA2 may play important roles for the expression and polymerization of F-actin and β-tubulin to enhance the motility, angiogenesis, invasion, and metastasis of cancer cells.

In summary, we constructed *ANXA2*$^{-/-}$caco2 cell line successfully, observed the changes of the pseudopodia/filopodia microstructures and the expression and polymerization of F-actin and β-tubulin after ANXA2 deletion. We found that the ANXA2 deletion resulted in the pseudopodia/filopodia weakened, loosed, and non-stained areas increased, the contact inhibition among cells reappeared, and caused the expression of *F-actin* and *β-tubulin* inhibited, and induced the polymerized F-actin and β-tubulin disassembled. These findings indicate that ANXA2 plays important roles to maintain the enhanced motility and suppressed contact inhibition of cancer cells by increasing the expressions of *F-actin* and *β-tubulin*, and assembling the bundles of F-actin and β-tubulin. By the roles above, the motility of cancer cells can be vigorously enhanced, consequently, the invasion and metastasis of cancers can be promoted. In conclusion, ANXA2 enhances the invasion and metastasis of cancers by increasing the expression and polymerization of F-actin and β-tubulin, and suppressing cell contact inhibition, consequently, promotes the invasion and metastasis of cancers. *ANXA2* can be considered as an important candidate to be used as a targeting gene for the gene therapy of cancers.

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REFERENCES

1. Babbin, B.A., Parkos, C.A., Mandell, K.J., Winfree, L.M., Laur, O., Ivanov, A.I. and Nusrat, A., Annexin 2 regulates intestinal epithelial cell spreading and wound closure through Rho-related signaling. *Am. J. Pathol.* 2007, **170:** 951-966.

2. Balzer, E.M., Whipple, R.A., Cho, E.H., Matrone, M.A. and Martin, S.S., Antimitotic chemotherapeutics promote adhesive responses in detached and circulating tumor cells. *Breast. Cancer. Res. Treat.* 2010, **121:** 65-78.

3. Chen, J., Kanai, Y., Cowan, N.J. and Hirokawa, N., Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. *Nature*. 1992, **360:** 674-677.

4. Diaz, V.M., Hurtado, M., Thomson, T.M., Reventós, J. and Paciucci, R., Specific interaction of tissue-type plasminogen activator (t-PA) with annexin II on the membrane of pancreatic cancer cells activates plasminogen and promotes invasion in vitro. *Gut.* 2004, **53:** 993-1000.

5. Döppler, H., Bastea, L.I., Eiseler, T. and Storz, P., Neuregulin Mediates F-actin-driven Cell Migration through Inhibition of Protein Kinase D1 via Rac1 Protein. *J. Biol. Chem.* 2013, **288:** 455-465.

6. Gerke, V. and Moss, S.E., Annexins: from structure to function. *Physiol. Rev.* 2002, **82:** 331-371.

7. Hansen, M.D., Ehrlich, J.S. and Nelson, W.J., Molecular mechanism for orienting membrane and actin dynamics to nascent cell-cell contacts in epithelial cells. *J. Biol. Chem.* 2002, **277:** 45371-45376.

8. Hedhli, N., Falcone, D.J., Huang, B., Cesarman-Maus, G., Kraemer, R., Zhai, H., Tsirka, S.E., Santambrogio, L. and Hajjar, K.A., The annexin A2/S100A10 system in health and disease: emerging paradigms. *J. Biomed. Biotechnol.* 2012, Epub ahead of print

9. Hou, Y., Hou, Y., He, S. and Xing, R., The Novel Insights into Spatiotemporal Cell Biology and Its Schematic Frame, Triple W. *J. Cell. Physiol.* 2012, **227:** 1787 – 1790.

10. Hou, Y., Yang, L., Mou, M., Hou, Y., Zhang, A., Pan, N., Qiang, R., Wei, L. and Zhang, N., Annexin A2 regulates the levels of plasminogen, S100A10 and fascin in L5178Y cells. *Cancer. Invest.* 2008, **26:** 809–815.

11. Hubaishy, I., Jones, P.G., Bjorge, J., Bellagamba, C., Fitzpatrick, S., Fujita, D.J. and Waisman, D.M., Modulation of annexin II tetramer by tyrosine phosphorylation. *Biochemistry.* 1995, **34:** 14527-14534.

12. Mallavarapu, A. and Mitchison, T., Regulated actin cytoskeleton assembly at filopodium tips controls their extension and retraction. *J. Cell. Biol.* 1999, **146:** 1097-1106.

13. Matrone, M.A., Whipple, R.A., Thompson, K., Cho, E.H., Vitolo, M.I., Balzer, E.M., Yoon, J.R., Ioffe, O.B., Tuttle, K.C., Tan, M. and Martin, S.S., Metastatic breast tumors express increased tau, which promotes microtentacle formation and the reattachment of detached breast tumor cells. *Oncogene.* 2010, **29:** 3217-3227.

14. Mattila, P.K. and Lappalainen, P., Filopodia: molecular architecture and cellular functions. *Nat. Rev. Mol. Cell. Biol.* 2008, **9:** 446-454.

15. Meissner, M., Pinter, A., Michailidou, D., Hrgovic, I., Kaprolat, N., Stein, M., Holtmeier, W., Kaufmann, R. and Gille, J., Microtubule-targeted drugs inhibit VEGF receptor-2 expression by both transcriptional and post-transcriptional mechanisms. *J. Invest. Dermatol.* 2008, **128:** 2084-2091.

16. Ozorowski, G., Ryan, C.M., Whitelegge, J.P. and Luecke, H., Withaferin A binds covalently to the N-terminal domain of annexin A2. *Biol. Chem.* 2012, **393:** 1151-1163.

17. Shetty, P.K., Thamake, S.I., Biswas, S., Johansson, S.L. and Vishwanatha, J.K., Reciprocal regulation of annexin A2 and EGFR with Her-2 in Her-2 negative and herceptin-resistant breast cancer. *PLoS. One.* 2012, **7:** e44299.

18. Vignjevic, D., Schoumacher, M., Gavert, N., Janssen, K.P., Jih, G., Laé, M., Louvard, D., Ben-Ze'ev, A and Robine, S., Fascin, a novel target of beta-catenin-TCF signaling, is expressed at the invasive front of human colon cancer. *Cancer. Res.* 2007, **67:** 6844-6853.

19. Wang, C.Y., Chen, C.L., Tseng, Y.L., Fang, Y.T., Lin, Y.S., Su, W.C., Chen, C.C., Chang, K.C., Wang, Y.C. and Lin, C.F., Annexin A2 silencing induces G2 arrest of non-small cell lung cancer cells through p53-dependent and -independent mechanisms. *J. Biol. Chem.* 2012, **287:** 32512-32524.

20. Yu, X., Zech, T., McDonald, L., Gonzalez, E.G., Li, A., Macpherson, I., Schwarz, J.P., Spence, H., Futó, K., Timpson, P., Nixon, C., Ma, Y., Anton, I.M., Visegrády, B., Insall, R.H., Oien, K., Blyth, K., Norman, J.C. and Machesky, L.M., N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. *J. Cell. Biol.* 2012, **199:** 527-544.

21. Zhang, H.J., Yao, D.F., Yao, M., Huang, H., Wu, W., Yan, M.J., Yan, X.D. and Chen, J., Expression characteristics and diagnostic value of

annexin A2 in hepatocellular carcinoma. *World. J. Gastroenterol.* 2012, **18:** 5897-5904.
22. Zhao, P., Zhang, W., Tang, J., Ma, X.K., Dai, J.Y., Li, Y., Jiang, J.L., Zhang, S.H. and Chen, Z.N., Annexin II promotes invasion and migration of human hepatocellular carcinoma cells in vitro via its interaction with HAb18G/CD147. *Cancer. Sci.* 2010, **101:** 387-395.