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Annexin A2 interacting with ELMO1 regulates HCC chemotaxis and metastasis

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ABSTRACT

Aims: SDF-1α induced chemotaxis plays an important role in hepatocellular carcinoma metastasis. CXCR4 stimulated by SDF-1α/CXCL12 triggers heterotrimeric G proteins activation, which regulate migration and chemotaxis of hepatocellular carcinoma cells. The pathways linking the chemokine GPCR/Gi signaling to actin polymerization for migration of cancer cells are not known.

Materials and methods: Through would healing assay, chemotaxis assay, F-actin polymerization assay, confocal assay, immunohistochemical assay, protein identification and coimmunoprecipitation assay, we detected the role and mechanisms of Annexin A2 in hepatocellular carcinoma.

Key findings: In the present study, we firstly investigated the role of Annexin A2 in HepG2 cell chemotaxis and metastasis. Immunohistochemical analysis showed that Annexin A2 was highly expressed in hepatocellular carcinoma tissues. Its expression was closely associated with lymph node and distant metastasis. Knockdown Annexin A2 impaired cancer cell chemotaxis. Co-immunoprecipitation results showed an interaction between Annexin A2 and ELMO1. CXCL12 triggers an ELMO1-dependent membrane translocation of Annexin A2.

Significance: Taken together, our results indicated an important role of Annexin A2 in hepatocellular carcinoma tissues metastasis and revealed a novel molecular mechanism of its activation.

1. Introduction

Nowadays, hepatocellular carcinoma (HCC) with increasing prevalence rates, becomes the sixth most common cancer. A half of hepatocellular carcinoma patients happened in China [1–3]. However, the patients with HCC were diagnosed mostly as metastasis stages [4,5]. Although there are more developments and advances in disease management and medical techniques have been performed, recurrence and metastasis of HCC patients remains of the high incidence [5,6]. In a word, it will be significant to focus on the researches of novel biomarkers and molecular mechanisms for HCC migration and metastasis.

Metastasis is the transmission of cancerous cells from an original site to other selective organs elsewhere in the body. Chemotaxis is the process of the cells directly moving by the gradient concentration of chemokines. Moreover, chemotaxis, determining the specificity of metastasis to target organs, is also one of the significant mechanisms of cancer metastasis [7–9]. Chemokines are small molecules, as extracellular signals detected by chemokine receptors, a subfamily of G-protein-coupled receptors (GPCRs). It has been researched that when chemokine receptors were inhibited, the metastasis of cancer was blocked [7,10,11]. However, the mechanisms of chemokines binding with their receptors are still unknown on regulating the actin cytoskeleton resulting in cancer migrations and metastasis.

Activating a chemokine receptor promotes heterotrimeric G-proteins dissociation, which regulates signal transduction pathways that finally effect the actin cytoskeleton organization to drive cell migration and metastasis [12–16]. In Dictyostelium discoideum, *Caenorhabditis elegans*, mouse and human, ELMO/Dock180 complexes, functioning as GEFs for Rac molecular, evolutionarily control actin cytoskeleton [17–20]. Previous research illustrated GPCR activates an interaction between Gβγ subunits and the ELMO/Dock complex regulating chemotaxis [7,19]. Nowadays, it has been reported that ELMOs, as a new family protein, play a significant role in the different kinds of cancer. In the human glioma, it is the first time that ELMO1 and Dock180 stimulate glioma cell migration and invasion [21]. In neutrophils, both PI3K- and Src-ELMO-Dock2 dependent pathways work in parallel to activate Rac2 and modulate chemotaxis in response to a CXCL8 gradient [22]. In cervical carcinoma, the impact of ELMO1 on the

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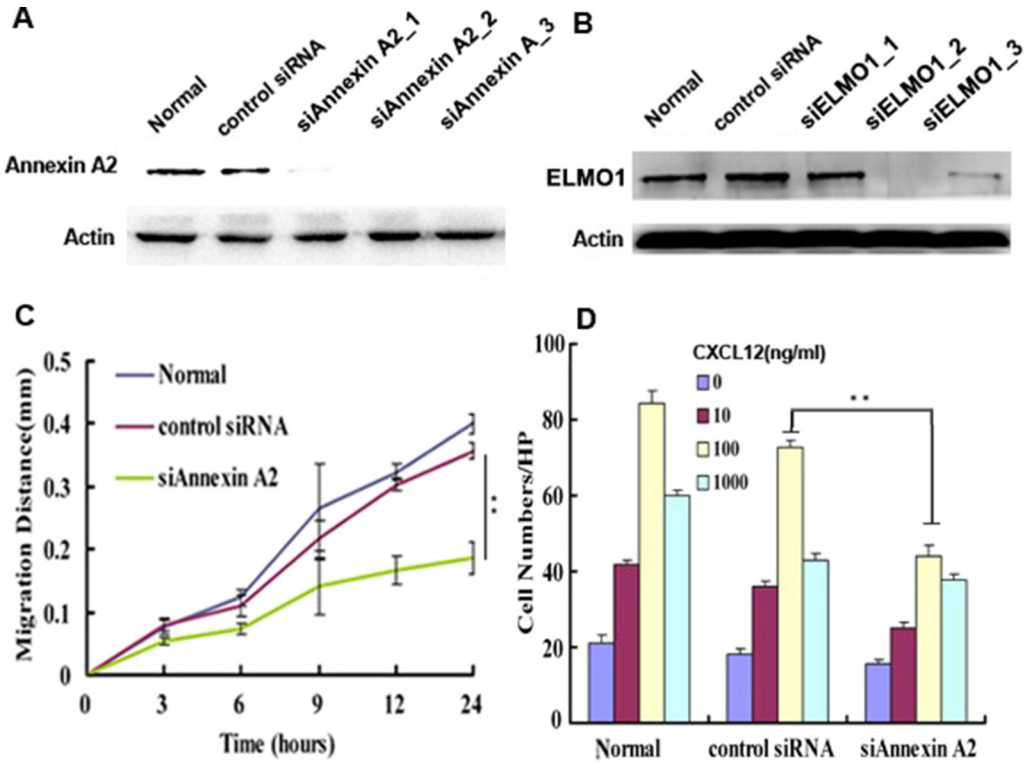


Fig. 1. siAnnexin A2 inhibited cell migration and chemotaxis in the HCC cells. A. Annexin A2 was obviously knocked down in the HCC cells. B. ELMO1was obviously knocked down in the HCC cells. C. Wound healing assay: HCC cells were plated in six-well plates and formed a fluent monolayer. The gap distance was measured at 0, 3, 6, 9, 12 and 24 h (points = mean of three independent experiments; two-way ANOVA, **P < 0.001). D. Chemotaxis analysis of Annexin A2 knockdown cells. Western blotting analysis of ELMO1 expression in HCC cells (points = mean of three independent experiments; two-way ANOVA, **P < 0.001).

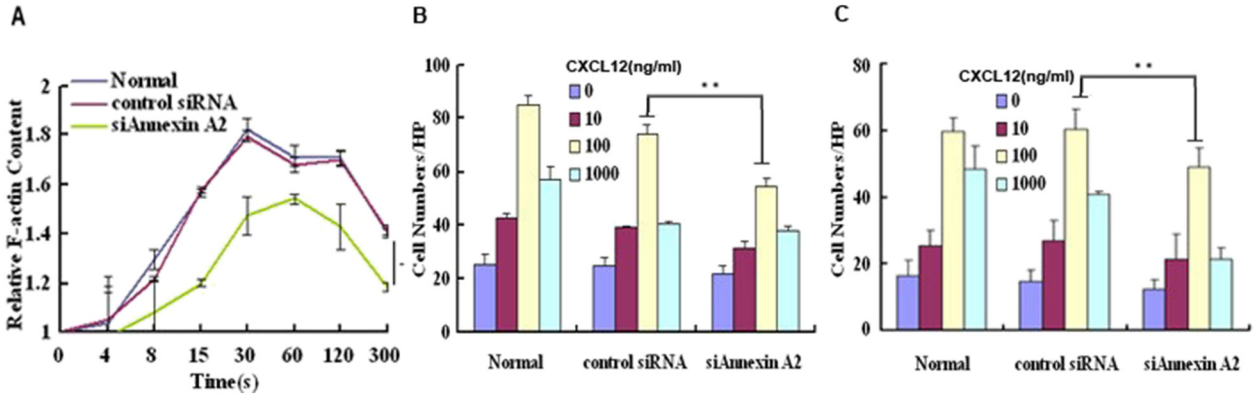


Fig. 2. Annexin A2 functions in HCC F-actin polymerization and invasion. A. Actin polymerization in siAnnexin A2 cells was decreased. Time course of relative F-actin content in normal, control, siAnnexin A2 cells followed by CXCL12 stimulation. The value was measured at 0, 4, 8, 15, 30, 60, 120 and 300 s (points = mean of three independent experiments; one-way ANOVA, *P < 0.05). B. The reduction of Annexin A2 impaired adhesion ability of HCC cells (points = mean of three independent experiments; two-way ANOVA, **P < 0.001). C. The reduction of Annexin A2 impaired invasion of HCC cells, (points = mean of three independent experiments; two-way ANOVA, **P < 0.001).

morphology of HeLa cells grown and cell elongation [23]. Moreover, our previous data also explain that ELMO1 interacting with Gαi2/Dock180 regulate actin polymerization and chemotaxis in breast cancer cells. Thus, a considerable amount of additional work is required before we can fully understand how ELMO1 act concomitantly to regulate the mechanisms of chemotaxis and cancer metastasis.

12 annexins are expressed in humans, annexins A1-A11 and A13. Annexin A2, a 36 kDa protein [24], is located on chromosome15q22.2 [25]. Annexin A2 is arguably the most extensively investigated with respect to health and disease, for example, cardiovascular disease, nasopharyngeal carcinoma, and ovarian cancer [26–28]. In cancer cells, endothelial cells, macrophages, and mononuclear cells, Annexin A2 is detected and expressed. There are three distinct functional regions: the N terminal region, the C-terminal region, and the core region. Annexin A2, functioning as a tumor-associated protein, promotes cancer progression including proliferation, invasion, and metastasis in various cancers [28–33].

In a word, we illustrate that Annexin A2 associates with ELMO1/Dock180 regulate migration and actin polymerization in hepatocellular carcinoma cells. Our study reveals a novel pathway, Annexin A2 interacting with ELMO1/Dock180, which has an important role in CXCL12 mediated chemotaxis and metastasis of HCC cells. In the future, Annexin A2 may be a potential prognostic factor, a potential diagnostic factor for cancer, and a therapeutic target for new drug development after analysis in large scale, well controlled, prospective clinical trials.

2. Methods

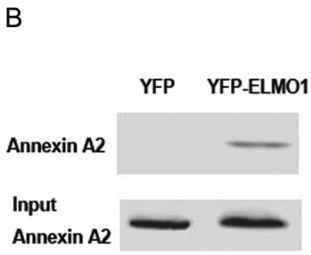
2.1. Cell and transfection

HepG2 cells was purchased from ATCC; cultured with RPMI-1640

A

Mass spectrometry analysis

Protein Name	Protein Mass	No. of Peptide	Control
Annexin A2	38779.88	8	0
ELMO1-YFP	84516	34	0
DOCK 180	216207	2	0



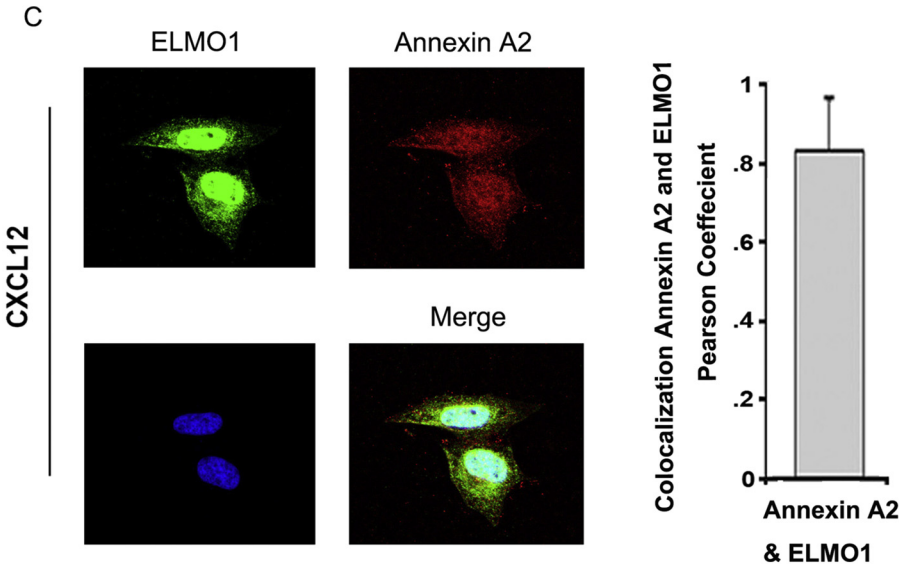


Fig. 3. Annexin A2 interacts with ELMO1. A. Mass spectrometry analysis. B. Co-IP assay. The eluted proteins were separated by SDS-PAGE and were probed with antibodies. C. CXCL12-induced ELMO1 colocalization with Annexin A2 on the plasma membrane by confocal microscopy analysis. Colocalization efficiency was calculated through Image J software.

medium added 10% FBS from Hyclone. Cells were ready and transfected with Lipofectamine 2000 according to instructions. Human ELMO1 siRNA sequences: 5′-UGACAAGCAUGAGUACUGU-3′, 5′-GAAC UCGCUU UCUCCAUCU-3′, 5′-GCAUUACGGAGACUUAGAA-3′. Annexin A2 siRNA sequences were 5′-GGTCTGAATTCAAGAGAAA-3′, 5′-GCCA AAGAAATGAACATTC-3′, 5′-GCTCAGAGAACAATTCTAG-3′. Control siRNA was 5′-UUCUCCGAACGUGUCACGUTT-3′. For the knockdown of Annexin A2, the methods were the same [19].

2.2. Protein identification and coimmunoprecipitation

The immunoprecipitation was performed as the instruction of the μMACS GFP isolation kit. The proteins were eluted and separated by SDS-PAGE. Then the samples were blocked with anti-Annexin A2. YFP were used as control.

2.3. Wound healing assay

Cells were scratched with a 10-ml pipette tip. At 0, 3, 6, 9, 12 and 24 h, the distance of the wounds was measured under a light microscope (×100).

2.4. Chemotaxis assay

Briefly, chemokine CXCL12 was added to the lower while the cell suspension was into the upper. Then incubated at 37 °C for 3 h. At last, membrane was stained and counted with light microscopy (×400).

2.5. F-actin polymerization assay

Stimulation with 100 ng/ml CXCL12, cells were incubated as the time points, fixed and permeabilized. The cells were stained with Alexa Fluor 568-phalloidin. Lastly, fluorescence value was tested by a microplate reader. The values were counted as F-actin t / F-actin 0¼(fluorescence t / mg ml^{-1}) / (fluorescence t0 / mg ml^{-1}).

2.6. Confocal assay

The assay was performed as bellow. Cells were starved and stimulation with 100 ng/ml CXCL12, then fixed and permeabilized. Cells were blocked with primary antibody, after that stained with secondary antibody Alexa Fluor 488-conjugated or 546-conjugated. Lastly visualized with confocal laser scanning microscopy (Olympus FV1000). Twenty-five images were analyzed.

2.7. Immunohistochemical assay

The entire sample (91 HCC tissues and 8 normal liver tissues) were from the Department of Liver Cancer, the Beijing 302 Hospital. According to the staining standard, intensity and the percentage of positive cells were measured with semiquantitative analysis. The antibodies Annexin A2 (1:100), and Polink-2 plus Polymer HRP System was detected for assay. Statistics method was χ^2-statistics.

3. Results

3.1. Annexin A2 functions in hepatocellular carcinoma cell migration and chemotaxis

To determine whether Annexin A2 was involved in the HCC cells migration, we first examined the expression level of Annexin A2 in HCC cells. In cancer, it is necessary that the migration and invasion activated

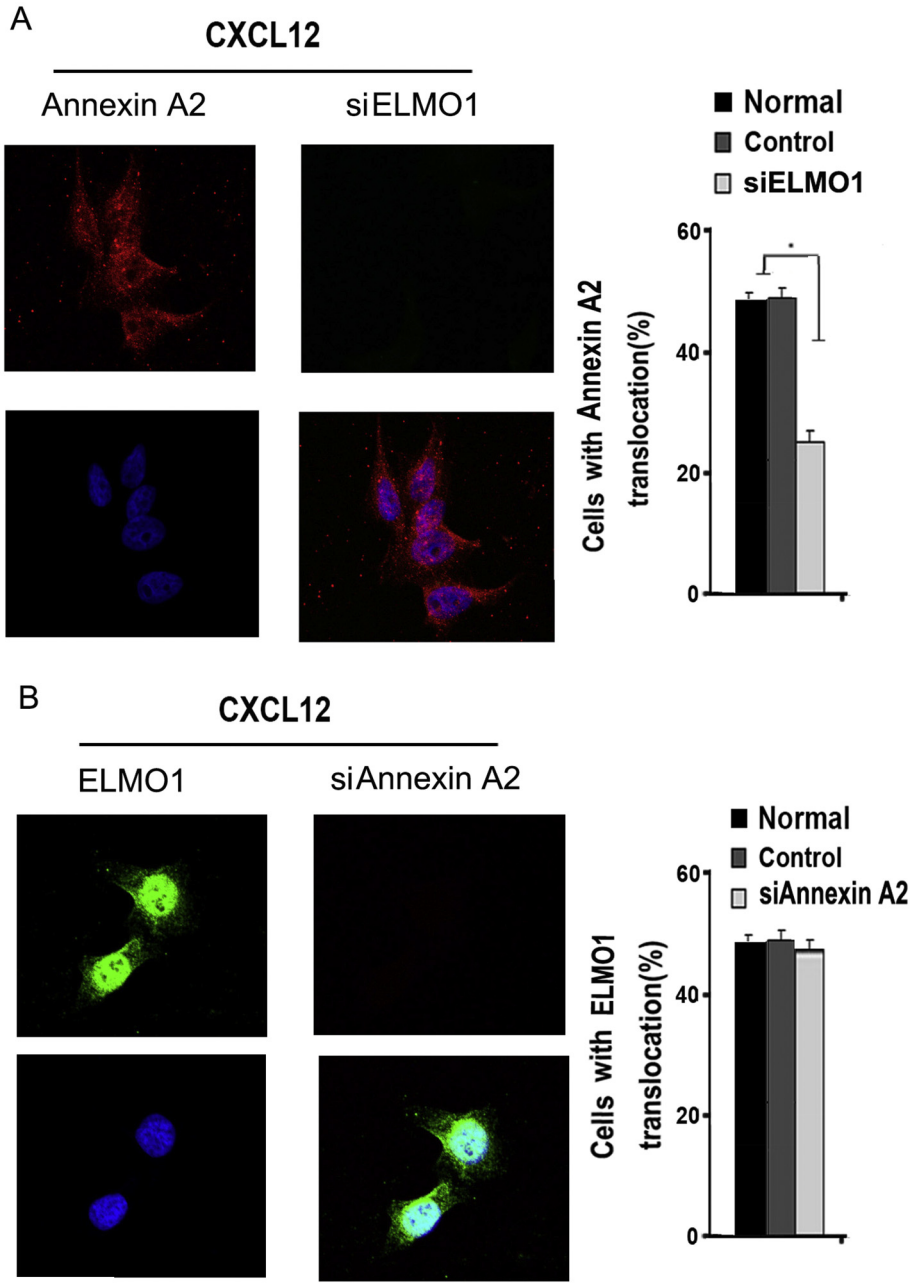


Fig. 4. CXCL12 triggers a ELMO1-dependent membrane translocation of Annexin A2. A. Knockdown of ELMO1 impaired CXCL12-induced membrane translocation of Annexin A2. Twenty-five images were analyzed by ImageJ software. One-way ANOVA, *P < 0.05 B. Knockdown of Annexin A2 did not impair CXCL12-induced membrane translocation of ELMO1. One-way ANOVA, P > 0.05.

by CXCL12 was one of the mechanisms of cancer metastasis. For detecting the role of Annexin A2 in the hepatocellular carcinoma cells migration, we specifically knocked down Annexin A2 or ELMO1 expression in HCC cells, using a small interference RNA (siRNA) method (Fig. 1A, B). Through chemotaxis assay, it was demonstrated that CXCL12 triggered chemotaxis of HCC cells was inhibited in siAnnexin A2 group (Fig. 1D). A migration assay showed that when Annexin A2 was knocked down, ability of migration in siAnnexin A2 cells was decreased (Fig. 1C).

3.2. siAnnexin A2 inhibits F-actin polymerization and invasion in HCC cells

Through an invasion assay, we found that siAnnexin A2 cells were inhibited in CXCL12-mediated invasion (Fig. 2C). It is essential for cancer cell migration and invasion that chemokine binding to receptors activates actin polymerization in cancer cells. Consistent with previous studies [19], CXCL12 induced a transient F-actin level increase (about 1.7-fold) in HCC cells (Fig. 2A), whereas the level was decreased in siAnnexin A2 cells within 30 s. We also examined the role of Annexin A2 in the adhesion of HCC (Fig. 2B) and found that siAnnexin A2 cells exhibited significant reduction of cell adhesion. Taken together, our results indicated Annexin A2 was involved in CXCL12-mediated migration and invasion of hepatocellular carcinoma cells.

3.3. Annexin A2 interacts with ELMO1

The proteins that interacted with Annexin A2 were identified through mass spectroscopy assay for potential molecular function with Annexin A 2 in HCC. We overexpressed ELMO1-YFP in HCC cells stimulated by CXCL12. Then elutes from the purified lysates were

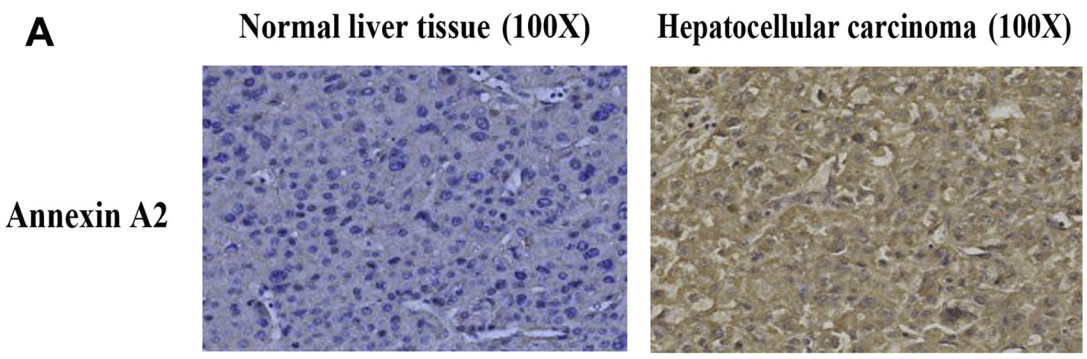


Table 1. Annexin A2 expression in liver tissue.

		Annexin A2 expression		
	total	positive	negative	P-value
Normal liver tissue	8	1	7	0.0049
Hepatocellular carcinoma	91	62	29	

Table 2. Correlation of Annexin A2 expression with clinical pathologic parameters and other biomarkers.

		Annexin A2 expression		
Parameters/markers	total	positive	negative	P-value
Age (years)				
<50	33	21	12	0.8156
≥50	58	41	17	
Tumour size (cm)				
<5	25	16	9	0.5987
≥5	66	46	20	
Lymph node status				
Positive	56	48	8	0.0002
Negative	35	14	21	
Distance metastasis				
Positive	31	26	5	0.0148
Negative	60	36	24	

Fig. 5. Annexin A2 has a role in HCC metastasis. A. Immunohistochemical analysis of Annexin A2 expression in hepatocellular carcinoma tissues and normal liver tissues. The entire sample was blocked for 1 h. The antibodies and the dilution factors were as follows: Annexin A2 (1:100). Table 1. Annexin A2 expression in liver tissues. Table 2. Correlation of Annexin A2 expression with clinical pathologic parameters and other biomarkers.

subjected to SDS gel electrophoresis (Fig. 3A). ELMO1-YFP, Dock180 and Annexin A2 were analyzed from the identified proteins (Fig. 3A). It has been reported that Dock180 and ELMO1 functioning as a GEF for small G-protein Rac regulated actin polymerization to induce cell migration [17]. We also confirmed Annexin A2 associated with ELMO1 by immunoprecipitation analyses (Fig. 3B). The results showed that Annexin A2 was coimmunoprecipitated with ELMO1-YFP but not in the control. The Dock180/ELMO1/Annexin A2 pathway may regulate chemokine receptor-mediated migration and chemotaxis in hepatocellular carcinoma cells.

3.4. Annexin A2 membrane translocation under CXCL12 stimulation

Under CXCL12 stimulation, ELMO1 was translocated to membrane. Though immunostaining assay, we detected subcellular localization of Annexin A2 and ELMO1 (Fig. 3C). Upon CXCL12 stimulation, we found Annexin A2 could colocalize with ELMO1 on cell membrane (Fig. 3C). Moreover, when ELMO1 was knock down in HCC cells, Annexin A2 membrane translocation was inhibited by CXCL12 stimulation (Fig. 4A). However, ELMO1 membrane enrichment was detected in Annexin A2 knockdown cells (Fig. 4B). Therefore, our data demonstrated Annexin A2 translocated to membrane was depended on ELMO1 under CXCR4 chemokine receptor activations.

3.5. Annexin A2 has a role in hepatocellular carcinoma chemotactic responses

For detecting the roles of Annexin A2 on the metastasis of hepatocellular carcinoma in vivo, we first evaluated Annexin A2 expression in 91 HCC tissue specimens and 8 healthy controls through an immunohistochemical analysis (Fig. 5A, Table 1). Annexin A2 was stained as positive in 62 patient samples while 1 healthy control. Meanwhile, 48 out of 56 HCC patients samples with lymph nodes metastasis was stained as positive for Annexin A2, however, only 14 out of 35 lymph nodes without metastases stained as positive ($P = 0.0002$, χ^2-statistics, Table 2). Furthermore, Annexin A2 analyses were detected in 26 out of 31 with lung distant metastasis cases, whereas in 36 out of 60 without lung distance metastasis samples (Table 2). These results demonstrated that Annexin A2 expressions were closely associated with migration and metastasis of hepatocellular carcinoma. In a word, Annexin A2 is significantly required for hepatocellular carcinoma metastasis in vivo.

4. Discussion

500 million years ago, the annexins is a traditional family of > 60 highly conserved, Ca^{2+}-regulated, phospholipid-binding proteins [34]. Moreover, Annexin A2 are involved in many processes including cancer progression, including, cell migration, invasion, adhesion, proliferation and angiogenesis [35–38].

According to our results, in cancer cells we have found and demonstrated that a signaling pathway induced by chemokine receptor, including Gαi2, the ELMO1/Dock180 complex, Annexin A2, Rac1 and Rac2, activating actin polymerization. Our results illustrated that the function of Annexin A2 interacting with ELMO1 promotes the migration and chemotaxis under CXCL12 stimulation in the hepatocellular carcinoma cells. Through mass spectrometry and coimmunoprecipitation analyses, our results showed that Annexin A2 interacts with ELMO1, associating with Dock180. Moreover, Annexin A2 translocated to membrane depending on ELMO1 under CXCL12 stimulation.

Together, our results demonstrated that when CXCL12 bind to its receptor Annexin A2 was activated. Then, Annexin A2 translocated from cytosol to membrane, interacting with the ELMO1, so that promoted actin polymerization in the HCC cells. Knockdown of Annexin A2 impairs actin polymerization. Though mass spectrometry analysis, our data has showed Annexin A2 was associated with ELMO1. When ELMO1 was knocked down in HCC cells, Annexin A2 translocation was decreased by CXCL12 stimulation. It showed that the function of ELMO1 is irreplaceable. We also have demonstrated that the Annexin A2 has a critical role on CXCL12-induced migration and chemotaxis of HCC cells in vitro and hepatocellular carcinoma metastasis in vivo. In vivo, from Annexin A2 expression analyses, it showed a relatively high level in human HCC samples, associating with lymph node and lung distant metastasis. Therefore, it would be useful to establish the value of other signaling components in these pathways. Moreover, Annexin A2, will be a novel biomarker for the diagnosis, treatment and prognosis of hepatocellular carcinoma metastasis.

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Declaration of conflicting interests

The authors have no conflicts of interest to declare.

References

[1] F. Sipos, O. Galamb, Epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions in the colon, World J. Gastroenterol. 18 (7) (2012) 601–608.
[2] L. Kikuchi, C.P. Oliveira, F.J. Carrilho, Nonalcoholic fatty liver disease and hepatocellular carcinoma, Biomed. Res. Int. 2014 (2014) 106–247.
[3] M. Nakanishi, D.W. Rosenberg, Roles of cPLA2alpha and arachidonic acid in cancer, Biochim. Biophys. Acta 1761 (11) (2006) 1335–1343.
[4] S. Lamouille, J. Xu, R. Derynck, Molecular mechanisms of epithelial-mesenchymal transition, Nat Rev Mol Cell Biol 15 (3) (2014) 178–196.
[5] J.M. Lee, S. Dedhar, R. Kalluri, E.W. Thompson, The epithelial-mesenchymal transition: new insights in signaling, development, and disease, J. Cell Biol. 172 (7) (2006) 973–981.
[6] Qin L, Yin YT, Zheng FJ, et al. WNT5A promotes stemness characteristics in nasopharyngeal carcinoma cells leading to metastasis and tumorigenesis. Oncotarget. 2015;6(12):10239–10252.
[7] Muller, A. et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001; 410: 50–56.
[8] A. Zlotnik, Involvement of chemokine receptors in organ-specific metastasis, Contrib. Microbiol. 13 (2006) 191–199.
[9] G.L. Nicolson, Paracrine and autocrine growth mechanisms in tumor metastasis to specific sites with particular emphasis on brain and lung metastasis, Cancer Metastasis Rev. 12 (1993) 325–343.
[10] T. Jin, X. Xu, D. Hereld, Chemotaxis, chemokine receptors and human disease, Cytokine 44 (2008) 1–8.
[11] L. Hernandez, M.A. Magalhaes, S.J. Coniglio, J.S. Condeelis, J.E. Segall, Opposing roles of CXCR4 and CXCR7 in breast cancer metastasis, Breast Cancer Res. 13 (2011) R128.
[12] J.F. Cote, K. Vuori, GEF what? Dock180 and related proteins help Rac to polarize cells in new ways, Trends Cell Biol. 17 (2007) 383–393.
[13] Dong, X. et al. P-Rex1 is a primary Rac2 guanine nucleotide exchange factor in mouse neutrophils. Curr. Biol. 2005; 15: 1874–1879.
[14] N. Isik, J.A. Brzostowski, T. Jin, An Elmo-like protein associated with myosin II restricts spurious F-actin events to coordinate phagocytosis and chemotaxis, Dev. Cell 15 (2008) 590–602.
[15] Li, Z. et al. Directional sensing requires G beta gamma-mediated PAK1 and PIX alpha-dependent activation of Cdc42. Cell. 2003; 114: 215–227.
[16] P.J. Van Haastert, P.N. Devreotes, Chemotaxis: signalling the way forward, Nat. Rev. Mol. Cell Biol. 5 (2004) 626–634.
[17] P.W. Reddien, H.R. Horvitz, The engulfment process of programmed cell death in Caenorhabditis elegans, Annu. Rev. Cell Dev. Biol. 20 (2004) 193–221.
[18] J.F. Cote, A.B. Motoyama, J.A. Bush, K. Vuori, A novel and evolutionarily conserved PtdIns(3,4,5)P3-binding domain is necessary for DOCK180 signalling, Nat. Cell Biol. 7 (2005) 797–807.
[19] Yan, J. et al. A Gbetagamma effector, ElmoE, transduces GPCR signaling to the actin network during chemotaxis. Dev. Cell 2012; 22: 92–103.
[20] Grimsley, C. M. et al. Dock180 and ELMO1 proteins cooperate to promote evolutionarily conserved Rac-dependent cell migration. J. Biol. Chem. 2004; 279: 6087–6097.
[21] Jarzynka, N.J., et al., ELMO1 and Dock180, a bipartite Rac1 guanine nucleotide exchange factor, promote human glioma cell invasion. Cancer Res., 2007. 67(15): p. 7203–7211.
[22] Sai, J.Q., et al., Parallel phosphatidylinositol 3-kinase (PI3K)-dependent and Src-dependent pathways lead to CXCL8-mediated Rac2 activation and chemotaxis. J. Biol. Chem., 2008. 283(39): p. 26538–26547.
[23] Patel, M., et al., An evolutionarily conserved autoinhibitory molecular switch in ELMO proteins regulates Rac signaling. Curr. Biol., 2010. 20(22): p. 2021–2027.
[24] K.A. Hajjar, S. Krishnan, Annexin II: a mediator of the plasmin/plasminogen activator system, Trends in Cardiovascular Medicine 9 (5) (1999) 128–138.
[25] K. Huebner, L. A. Cannizzaro, A. L. Frey et al., "Chromosomal localization of the human genes for lipocortin I and lipocortin II," Oncogene Research. 1988; 2(4): 299–310.
[26] Hedhli N, Falcone DJ, Huang, B, et al. The annexin A2/S100A10 system in health and disease: emerging paradigms. J Biomed Biotechnol. 2012; 40:6273–6286.
[27] M. Luo, K.A. Hajjar, Annexin A2 system in human biology: cell surface and beyond, Semin. Thromb. Hemost. 39 (2013) 338–346.
[28] Chen CY, Lin YS, Chen CL, Chao PZ, Chiou JF, Kuo CC, Lee FP, Lin YF, Sung YH, Lin YT, et al. Targeting annexin A2 reduces tumorigenesis and therapeutic resistance of nasopharyngeal carcinoma. Oncotarget. 2015; 6(29):26946–59.
[29] N.A. Lokman, A.S.F. Elder, M.P. Ween, C.E. Pyragius, P. Hoffmann, M.K. Oehler, C. Ricciardelli, Annexin A2 is regulated by ovarian cancer-peritoneal cell interactions and promotes metastasis, Oncotarget 4 (8) (2013) 1199–1211.
[30] H. Zhai, S. Acharya, I. Gravanis, S. Mehmood, R.J. Seidman, K.R. Shroyer, K.A. Hajjar, S.E. Tsirka, Annexin A2 promotes glioma cell invasion and tumor progression, J. Neurosci. 31 (40) (2011) 14346–14360.
[31] H.J. Zhang, D.F. Yao, M. Yao, H. Huang, L. Wang, M.J. Yan, X.D. Yan, X. Gu, W. Wu, S.L. Lu, Annexin A2 silencing inhibits invasion, migration, and tumorigenic potential of hepatoma cells, World J. Gastroenterol. 19 (24) (2013) 3792–3801.
[32] Zheng L, Foley K, Huang LQ, Leubner A, Mo GL, Olino K, Edil BH, Mizuma M, Sharma R, Le DT, et al. Tyrosine 23 phosphorylation-dependent cell-surface localization of Annexin A2 is required for invasion and metastases of pancreatic Cancer. PLoS One. 2011;6(4).
[33] M.R. Sharma, L. Koltowski, R.T. Ownbey, G.P. Tuszynski, M.C. Sharma, Angiogenesis-associated protein annexin II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression, Exp.

Mol. Pathol. 81 (2) (2006) 146–156.
[34] S.E. Moss, R.O. Morgan, The annexins, Genome Biol. 5 (2004) 1–8.
[35] C.Y. Wang, C.F. Lin, Annexin A2: its molecular regulation and cellular expression in cancer development, Dis. Markers (2014) (2014) 308976.
[36] N.A. Lokman, M.P. Ween, M.K. Oehler, C. Ricciardelli, The role of annexin A2 in tumorigenesis and cancer progression, Cancer Microenviron. 4 (2011) 199–208.
[37] X. Zhang, S. Liu, C. Guo, J. Zong, M.Z. Sun, The association of annexin A2 and cancers, Clin. Transl. Oncol. 14 (2012) 634–640.
[38] X.H. Xu, W. Pan, L.H. Kang, H. Feng, Y.Q. Song, Association of annexin A2 with cancer development, Oncol. Rep. 33 (2015) 2121–2128.