Biochemical and Biophysical Research Communications 445 (2014) 58–63

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Guanine nucleotide-binding protein subunit beta-2-like 1, a new Annexin A7 interacting protein

Yue Du [1], Jinyi Meng [1], Yuhong Huang, Jun Wu, Bo Wang, Mohammed M. Ibrahim, Jianwu Tang *

Key Laboratory of Tumor Metastasis of Liaoning Province, Department of Pathology, Dalian Medical University, Dalian 116044, China

ARTICLE INFO

Article history:
Received 16 January 2014
Available online 1 February 2014

Keywords:
Guanine nucleotide-binding protein subunit beta-2-like 1 (Gnb2l1)
Receptor of activated protein kinase C 1 (RACK1)
Annexin A7 (ANXA7)
Lymph node metastasis (LNM)

ABSTRACT

We report for the first time that Guanine nucleotide-binding protein subunit beta-2-like 1 (RACK1) formed a complex with Annexin A7. Hca-F and Hca-P are a pair of syngeneic mouse hepatocarcinoma cell lines established and maintained in our laboratory. Our previous study showed that both Annexin A7 and RACK1 were expressed higher in Hca-F (lymph node metastasis >70%) than Hca-P (lymph node metastasis <30%). Suppression of Annexin A7 expression in Hca-F cells induced decreased migration and invasion ability. In this study, knockdown of RACK1 by RNA interference (RNAi) had the same impact on metastasis potential of Hca-F cells as Annexin A7 down-regulation. Furthermore, by co-immunoprecipitation and double immunofluorescence confocal imaging, we found that RACK1 was in complex with Annexin A7 in control cells, but not in the RACK1-down-regulated cells, indicating the abolishment of RACK1-Annexin A7 interaction in Hca-F cells by RACK1 RNAi. Taken together, these results suggest that RACK1-Annexin A7 interaction may be one of the means by which RACK1 and Annexin A7 influence the metastasis potential of mouse hepatocarcinoma cells in vitro.

1. Introduction

For carcinoma, lymphatic metastasis is a common phenomenon leading to poor prognosis. Metastasis results from alterations of multiple genes and signaling pathways which remain to be characterized. Our series of studies aimed at identifying the signaling pathways and potential protein molecules involved in tumor progression and metastasis. In our previous studies, we choose a pair of syngeneic mouse hepatocarcinoma cell lines, Hca-F and Hca-P, which were established and maintained in our laboratory. When inoculated subcutaneously in Chinese 615 mice, Hca-P showed a low of LNM rate (<30%), whereas Hca-F showed a high LNM rate (>70%). Sharing the same genetic background, these two cell lines are ideal experimental subjects for revealing potential biomarkers related to tumor malignancy [1,2]. Suppressive subtracted hybridization technique, gene chip and quantitative proteomics techniques were used to identify differentially expressed genes and proteins between the two cell lines [3–5]. Guanine nucleotide-binding protein subunit beta-2-like 1 (gene symbol: Gnb2l1), together with Annexin A7, Jun-N-termnal kinase, et al., were expressed higher in Hca-F than Hca-P at both mRNA and protein levels [5–7].

Guanine nucleotide-binding protein subunit beta-2-like 1 was alternatively named Receptor of activated protein kinase C 1 (RACK1). Although RACK1 was early identified as an anchoring protein for protein kinase C (PKC) [8], actually it recruits and binds a variety of signaling molecules such as Integrins, Proto-oncogene tyrosine-protein kinase Src (pp60c-src), Focal adhesion kinase (FAK), et al. [9–11]. Acting as a scaffold protein, RACK1 integrates signals from diverse pathways and contributes to cancer progress. The expression of RACK1 is altered in many cancers. Elevated RACK1 level in pulmonary adenocarcinoma is associated with poor prognosis [12]. In contrast, other reports indicate that RACK1 level is decreased in breast cancer [13]. Several studies have demonstrated that RACK1 may influence migration and proliferation of tumors [14–16], but its underlying mechanisms in hepatocarcinoma remain to be explored further. Therefore, in the current study, we down-regulated RACK1 in mouse hepatocarcinoma cell line Hca-F by RNA interference (RNAi) and assessed its effect on the malignant behaviors. What's more, association between Annexin A7 and RACK1 were explored through Ingenuity Pathway Analysis (IPA) software and protein–protein interaction experiments. These findings offer new perspectives for understanding the way by which RACK1 and Annexin A7 influence malignant behaviors of tumors.

* Corresponding author. Address: Dalian Medical University, Department of Pathology, 9-Western Section, Lvshun Southern Street, 116044 Dalian, Liaoning, China.
E-mail address: jianwutdlmedu@163.com (J. Tang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.01.119
0006-291X/

2. Materials and methods

2.1. Cell culture

Mouse hepatocarcinoma cell line, Hca-F (LNM rate >70%), was established and maintained in our laboratory as previously described [1,2]. The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Gibco, USA) at 37 °C with 5% CO_2.

2.2. Construction of expressing vectors and stable transfection

Five different pairs of Short hairpin RNAs (shRNAs), four targeting the RACK1 mRNA sequence and one with non-targeting sequence to be used as a control, were designed and inserted into pGPU6/GFP/Neo siRNA expression vectors containing neomycin resistance gene for antibiotic selection. All the expression vectors were constructed by Shanghai GenePharma Co., Ltd. (Shanghai, China). Hca-F cells were transferred into a 6-well plate with 8×10^5 cells per well and transfected with 2 μg of shRNA-Gnb2l1 and control-shRNA plasmid respectively by Sofast™ transfection reagent (Sunma Corp., China) according to the manufacture's protocol. The stable cell lines were generated by selecting with G418. The siRNA with the best silencing effect (hairpin sequence: 5′-CCCACTTCGTTAGTGATGTTG-3′) was selected by RT-PCR and Western blot analysis. Cells stably transfected with the most efficient Gnb2l1-specific siRNA were named shRNA-Gnb2l1-Hca-F, whereas cells stably transfected with the negative control were named control-shRNA-Hca-F.

2.3. Detection of Rack1 expression by qRT-PCR and Western blot analysis

Total RNA was isolated using TRIzol (Invitrogen) and reverse-transcripted with PrimeScript®RT reagent kit. The quantification of gene transcripts was carried out with Stratagene Mx3005P (Agilent Tech., USA) using SYBR® Premix Ex TaqTM II under the following conditions: predenaturation for 1 min at 95 °C; 10 s at 95 °C, 1 min at 60 °C, 40 cycles. The sequences of the primers for Gnb2l1 were 5′-AGGGCCACAATGGATGGGTA-3′ (forward) and 5′-CAGCTTCCACATGATGATGGTC-3′ (reverse). Results were normalized to the expression of GAPDH and analyzed with the ΔΔCt relative quantification. Total protein from each group were separated by SDS–PAGE and transferred to PVDF membranes (Millipore Corp., USA). Blocked membranes were incubated with rabbit anti-RACK1 (ab-62735, Abcam) and mouse anti-GAPDH (sc-47724, Santa Cruz) overnight at 4 °C respectively. Dylight 800-conjugated goat anti-rabbit (35571, Thermo) and Dylight 680-conjugated goat anti-mouse (35518, Thermo) secondary antibodies were used in 1:10,000 dilution for 1 h at room temperature. Fluorescence signals on the membrane were detected with the Licor Oddessy imager (Licor, USA).

2.4. Cell proliferation analysis

Cell proliferation was measured using Dojindo's CCK-8 cell proliferation kit (Dojindo, Japan). Briefly, the cell suspensions of shRNA-Gnb2l1-Hca-F, control-shRNA-Hca-F and unmanipulated Hca-F cells at a density of 1×10^4 cells/ml were inoculated into each 96-well plate. We added 10 μl of CCK-8/well at 24, 48, 72 and 96 h and evaluated cell numbers by measuring the absorbancy A450.

2.5. Migration assay

Transwell cell culture inserts was used to determine the effect of Rack1 down-regulation on the migration potential. The upper chambers of the inserts were seeded with 3×10^4 cells in 100 μl serum-free RPMI 1640 whilst the lower chambers were filled with 500 μl RPMI 1640 supplemented with 10% FBS as a chemoattractant except in control wells which contained serum-free RPMI 1640 in both upper and lower chambers. After incubation at 37 °C with 5% CO_2 for 24 h, the filters were stained with 1% crystal violet. Migratory cells were counted under an inverted microscope with magnification of 400×. Five field views were randomly counted and averaged.

2.6. Invasion assay

The inner chambers of the transwell plates were coated with ECM gel (Sigma, USA) and incubated at 37 °C for 1 h to produce an artificial basement membrane. The further steps performed were as described in the migration assay above.

2.7. Annexin A7 Immunoprecipitation and Immunoblotting

Cells were lysed with the commercial Immunoprecipitation (IP) lysis buffer (87787, Thermo) with protease inhibitors (87786, Thermo). Equal Protein concentrations of lysates were incubated with 10 μg of mouse anti-Annexin A7 (sc-17815, Santa Cruz) for 14 h at 4 °C, followed by incubation with magnetic beads coated with Prot G (100.07D, Invitrogen) for 30 min at room temperature. Mouse normal IgG was used as the negative control. In the end, the eluates were submitted to the Immunoblotting. In order to avoid the binding of secondary antibody to the mouse IgG used for Annexin A7 IP, we choose goat anti-rabbit secondary antibody recognizing only the rabbit anti-Annexin A7 (sc-11389, Santa Cruz) and rabbit anti-RACK1 (ab-62735, Abcam) used as primary antibodies in Immunoblotting. The rest steps were carried out similar to the Western blot analysis above.

2.8. Laser confocal cell imaging

Cells seeded on slides were fixed in 4% paraformaldehyde and then incubated in 1%BSA/10% normal goat serum/0.3 M glycine in 0.1% PBS-Tween for 1 h. The cells were then incubated with rabbit anti-RACK1 (ab-62735, Abcam) and mouse anti-Annexin A7 (sc-17815, Santa Cruz) all at a 1:100 dilution overnight at 4 °C. The secondary antibody used were Alexa fluor 555-conjugated goat anti-mouse IgG and Alexa fluor 488-conjugated goat anti-rabbit IgG both at a 1:1000 dilution. DAPI was used to stain the cell nuclei. A Leica laser scanning confocal microscope was applied for the cell imaging.

2.9. Statistical analysis

Each assay was performed three times. Data expressed as the mean ± S.D. were analyzed by SPSS 16 software. *T*-test after ANOVA was used to determine statistical differences between groups. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Confirmation of successful down-regulation

The relative mRNA and protein levels of RACK1 were measured by RT-PCR and Western blot analysis respectively. As shown in Fig. 1, the expression of RACK1 was decreased by about 70% at

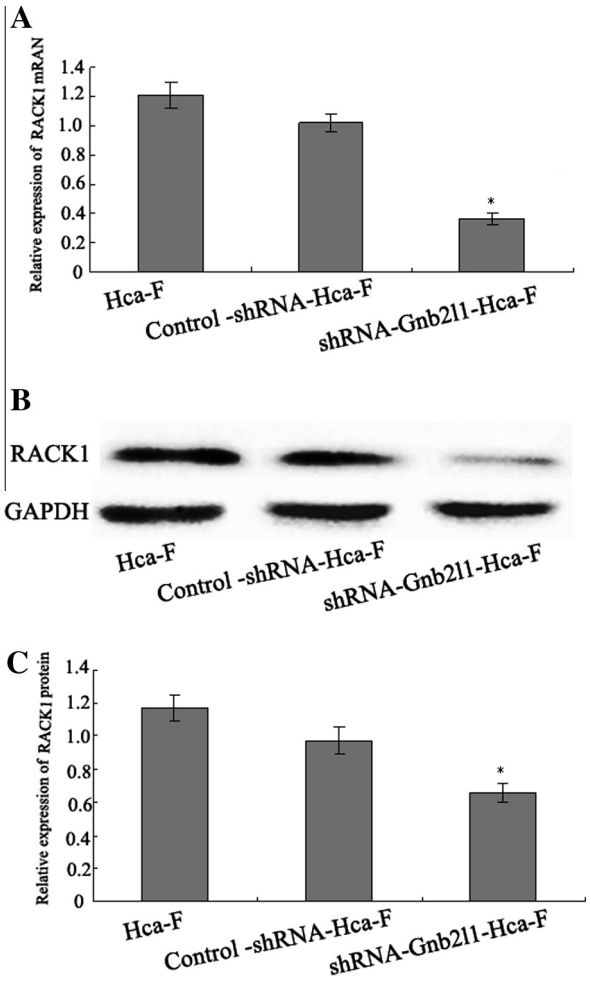

Fig. 1. RACK1 down-regulation confirmed by RT-PCR and Western Blot analysis. (A) Western Blot images of the three cell groups. (B) Graph showing quantification of RACK1 expressions at protein level. (C) Graph showing relative mRNA level of RACK1 expressions. $^{*}P < 0.05$ compared with those of control cells.

mRNA level and 30% at protein level in shRNA-Gnb2l1-Hca-F compared to the control-shRNA-Hca-F cells, confirming that Rack1 was successfully down-regulated.

3.2. RACK1 down-regulation decreased the cell proliferation

To evaluate the effect of the Rack1 silencing on the proliferation potential, we performed CCK-8 cell proliferation test. Data showed significant inhibition of cell proliferation in the shRNA-Gnb2l1-Hca-F cells compared to the two controls. From day 3 of the assay, the percentages of shRNA-Gnb2l1-Hca-F cells were approximately half of the control-shRNA-Hca-F and unmanipulated Hca-F cells, respectively (Fig. 2A).

3.3. RACK1 down-regulation significantly reduced the migration and invasion capacity

We observed an evident difference in the migration and invasion potential between the Rack1-down-regulated group and the control groups using Boyden's transwell chamber with, and without, extracellular matrix coating. No cell migrated or invaded through the filter in the control wells without a chemoattractant. The numbers of cells migrated through the filter were 14 ± 1, 39 ± 3 and 39 ± 6 per field of high magnification in shRNA-Gnb2l1-Hca-F, control-shRNA-Hca-F and unmanipulated Hca-F cells respectively. What's more, the numbers of the cells that invaded through the ECM-coated membrane were 8 ± 1, 21 ± 3 and 22 ± 2 per field of high magnification in the shRNA-Gnb2l1-Hca-F, control-shRNA-Hca-F and unmanipulated Hca-F cells, respectively (Fig. 2B and C).

3.4. Annexin A7 formed a complex with RACK1

By use of Annexin A7 IP followed by Immunoblotting, RACK1 was found to co-immunoprecipitate with Annexin A7 in unmanipulated Hca-F and control-shRNA-Hca-F cells, but not in shRNA-Gnb2l1-Hca-F cells (Fig. 3A). We confirmed specificity of the Annexin A7 IP by use of nonimmune IgG (data not shown). Furthermore, immunofluorescence analysis revealed that the two proteins colocalized in the cytoplasm (Fig. 3B).

4. Discussion

In this study, we first detected the effects of Rack1 knockdown on cell proliferation, migration and invasion capacity of Hca-F cells by RNA interference. CCK-8 test showed that proliferation in the shRNA-Gnb2l1-Hca-F cells was significantly decreased compared to the two controls. The transwell experiments demonstrated the

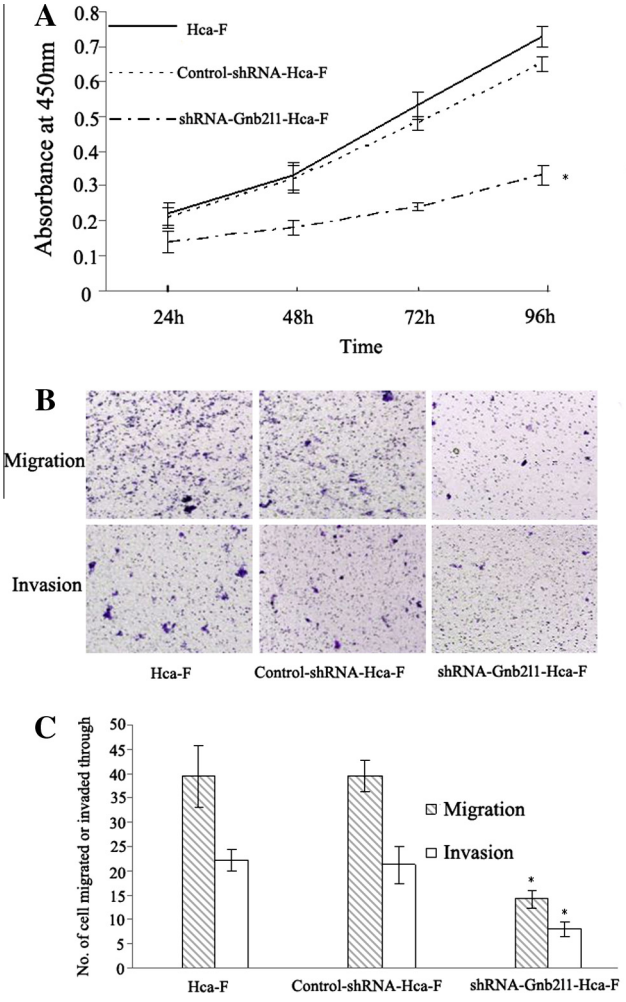

Fig. 2. Effect of RACK1 siRNA on the proliferation, migration and invasion. (A) CCK-8 assay results showed the proliferation of shRNA-Gnb2l1-Hca-F cells lagging behind the controls. (B) Transwell results showing decrease in both migration and invasion in RACK1 down-regulated cells. (C) Graphic representation of both migration and invasion. $^{*}P < 0.05$ compared with those of control cells.

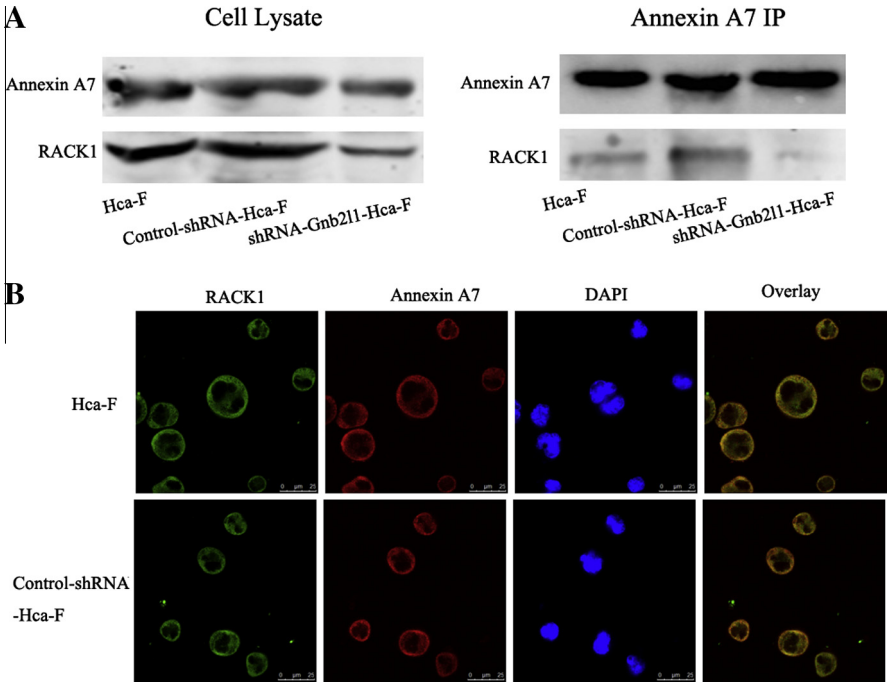

Fig. 3. Interaction of RACK1 with Annexin A7. (A) RACK1 was found to co-immunoprecipitate with Annexin A7 in control cells, but not the RACK1 down-regulated cells. (B) Co-localization of RACK1 and Annexin A7 is shown in the cytoplasma by yellow cellular staining. Scale bars = 25μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

numbers of shRNA-Gnb2l1-Hca-F cells that migrated through the filter and invaded through the ECM-coated membrane were less than half of the two controls. So RACK1 knockdown significantly inhibited these metastatic potentials of Hca-F cells in vitro, implying that RACK1 acts as a promoter of the metastasis potentials in hepatocarcinoma cells.

Retrospectively, we noticed that both Annexin A7 and RACK1 were expressed higher in Hca-F (LNM > 70%) than Hca-P (LNM < 30%) at both mRNA and protein levels. At protein level, RACK1 was about 1.52-fold and Annexin A7 was about 3-fold in Hca-F cells relative to Hca-P cells by mass spectrometry analysis [5,6]. Similar to depletion of RACK1, Annexin A7 knockdown also caused reduction of migration and invasion ability in Hca-F [7].

Annexin A7, a member of the calcium/phospholipid-binding annexin family, serves as a Ca^{2+}-activated GTPase and has been implicated in both exocytotic secretion and control of growth [17,18]. For example, loss of Annexin A7 expression was frequently found in prostate cancer, especially in metastasis and hormone refractory case and ANXA7 gene transfection significantly decreased the proliferation of human prostate tumor cells in vitro [19,20]. In contrast, high levels of Annexin A7 in HER2-negative breast cancers were strongly associated with poor survival of patients and the malignancy of tumor [21]. These results suggested that Annexin A7 has type-dependent impact on cancer development.

Investigation on the interaction proteins provided clues for understanding the role of Annexin A7. For example, Annexin A7 was reported to be phosphorylated by various kinases, including PKC, cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), and pp60c-src, and the selective activation of Annexin A7 by PKC accelerated exocytotic membrane fusion reaction in chromaffin cells [22]. What's more, Annexin A7 was reported to bind The binder of Arl two (BART) in pancreatic cancer cell. BART-ANXA7 complex was transported toward cell protrusions in migrating cells and reduced the PKCα activity [23].

Using Ingenuity Pathway Analysis (IPA) software, we generated connections between Annexin A7 (ANXA7) and RACK1 (GNB2L1). As showed in Fig. 4, Annexin A7 could be the target of pp60c-src and PKC such as PRKCA, PRKCB, et al. On the other hand, RACK1 binds SRC, PKC, Tyrosine-protein kinase Fyn (FYN), et al. All those network references imply that both Annexin A7 and RACK1 are involved in signaling pathways mediated by SRC and PKC although no direct link between the two molecules. Therefore, we probed RACK1-ANXA7 interaction in Hca-F cells. By Annexin A7 IP and double immunofluorescence confocal imaging, RACK1 was identified to be a novel interacting protein of Annexin A7. RACK1-Annexin A7 complex was undetectable in the shRNA-Gnb2l1-Hca-F cells probably due to depletion of RACK1. All these experiment results indicated that Annexin A7 is a new RACK1-associated protein and RACK1 RNAi led to abolishment of RACK1-Annexin A7 interaction in addition to inhibited the metastasis-associated prerequisites such as proliferation, migration and invasion. RACK1-Annexin A7 may play a role in regulating the metastatic potentials of mouse hepatocarcinoma cells.

Studies on RACK1 and its interacting partners help to figure out the role of RACK1. It's now well-known that RACK1 interacts with such a large number of proteins either directly or as part of a larger complex. One of the main functions of RACK1 is to modulate the activity of its binding partners. For example, RACK1 stabilizes the activity of protein phosphatase2A (PP2A) and PKCβII [24–26]. On the contrary, RACK1 binds pp60c-src and FYN to keep them in an inactive state [27–29]. Another key function of RACK1 is to traffic signaling molecules to particular cellular locations. In addition to PKC, RACK1 is reported to mediate the translocation of A Disintegrin And Metalloprotease 12 (ADAM12) to the plasma membrane in hepatic stellate cells [30]. As for RACK1-Annexin A7 complex, much remains unclear. For example, are there any other proteins within this complex? Whether the activity and cellular location of Annexin A7 are changed when it is complexed with RACK1? Therefore it would be of great interest to investigate this further.

In summary, our study is the first to demonstrate Annexin A7 in complex with RACK1. Depletion of RACK1 resulted in the similar phenotype to down-regulation of Annexin A7 in vitro. RACK1-Annexin A7 interaction may contribute to the RACK1 knockdown induced inhibition of metastasis potential in vitro. Therefore,

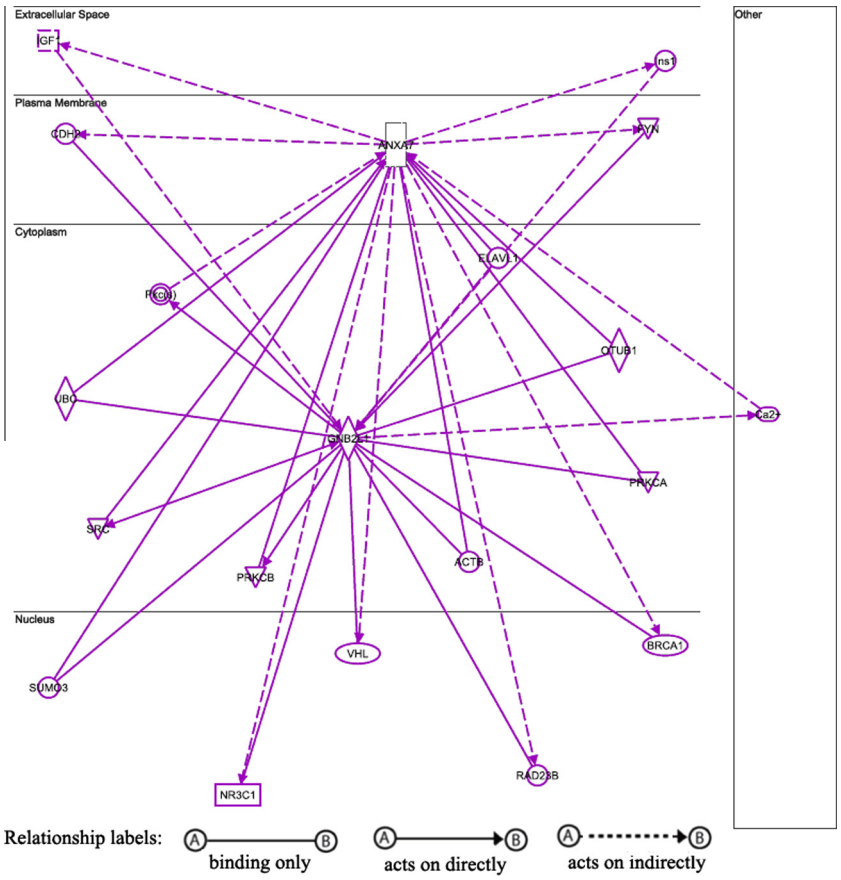

Fig. 4. Ingenuity Pathway Analysis (IPA) generated connections between Annexin A7 (ANXA7) and RACK1 (GNB2L1). No direct link between Annexin A7 and RACK1. RACK1 binds tyrosine-protein kinases including SRC, PKC and FYN, which act on Annexin A7 directly or indirectly. Note: "acts on directly" edge may also include a binding event.

RACK1-Annexin A7 complex may represent a potential target for tumor growth suppression and metastasis prevention.

Acknowledgments

This work was supported by Grants from the Natural Science Foundation of China (Nos. 81071725 and 30772468), the Educational Department of Liaoning Province (No. L20133456) and Grant from the Financial Department of Liaoning Province (No. 20121203). I would like to thank Hongguang Ren from Beijing Institute of Biotechnology for the IPA analysis.

References

[1] M.Y. Ling, M.H. Wang, L.L. Guo, et al., Study of features of lymphatic metastasis associated genes in mouse hepatocarcinoma cell lines: Hca/16A3-F and Hca/A2-P, Chin. Med. J. 75 (1995) 170–171.
[2] H. Li, M. Ling, Y. Xie, et al., Establishment of a lymph node metastatic model of mouse hepatocellular carcinoma Hca-F cells in C3H/Hej mice, Oncol. Res. 10 (1998) 569–573.
[3] X.N. Cui, J.W. Tang, L. Hou, et al., Identification of differentially expressed genes in mouse hepatocarcinoma ascites cell line with low potential of lymphogenous metastasis, World J. Gastroenterol. 12 (2006) 6893–6897.
[4] B. Song, J.W. Tang, B. Wang, et al., Identify lymphatic metastasis associated genes in mouse hepatocarcinoma cell lines using gene chip, World J. Gastroenterol. 14 (2005) 1463–1472.
[5] M.Z. Sun, S. Liu, J.W. Tang, et al., Proteomics analysis of two mice hepatocarcinoma ascites syngeneic cell lines with high and low lymph node metastasis rates provide potential protein markers for tumor malignancy attributes to lymphatic metastasis, Proteomics 9 (2009) 3285–3302.
[6] Y.L. Jin, Z.Q. Wang, H. Qu, et al., Annexin A7 gene is an important factor in the lymphatic metastasis of tumors, Biomed. Pharmacother. 67 (2013) 251–259.
[7] Jun Wu, Jinyi Meng, Yue Du, et al., RACK1 promotes the proliferation, migration and invasion capacity of mouse hepatocellular carcinoma cell line in vitro probably by PI3K/Rac1 signaling pathway, Biomed. Pharmacother. 67 (2013) 313–319.
[8] D. Ron, C.H. Chen, J. Caldwell, et al., Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins, Proc. Natl. Acad. Sci. 91 (1994) 839–843.
[9] J. Liliental, D.D. Chang, RACK1, a receptor for activated protein kinase C, interacts with integrin β subunite, J. Biol. Chem. 273 (1998) 2379–2383.
[10] A.C. Elisabeth, B. David, T.D. Ashley, et al., RACK1 regulates integrin-mediated adhesion, protrusion, and chemotactic cell migration via its Src-binding Site, Mol. Biol. Cell 14 (2003) 658–669.
[11] B. Serrels, E. Sandilands, A. Serrels, et al., A complex between FAK, RACK1, and PDE4D5 controls spreading initiation and cancer cell polarity, Curr. Biol. 20 (2010) 1086–1092.
[12] R. Nagashio, Y. Sato, T. Matsumoto, et al., Expression of RACK1 is a novel biomarker in pulmonary adenocarcinomas, Lung Cancer 69 (2010) 54–59.
[13] P. Seidl, R. Huettinger, R. Knuechel, et al., Three-dimensional fibroblast-tumor cell interaction causes downregulation of RACK1 mRNA expression in breast cancer cells in vitro, Int. J. Cancer 102 (2002) 129–136.
[14] U. Hermanto, C.S. Zong, W. Li, et al., An insulin-like growth factor I (IGF-I) receptor-interacting protein, modulates IGF-I-dependent integrin signaling and promotes cell spreading and contact with extracellular matrix, Mol. Cell Biol. 22 (2002) 2345–2365.
[15] F. Lu, C. Zhang, W.J. Wu, et al., RACK1 downregulation suppresses migration and proliferation of neuroblastoma cell lines, Oncol. Rep. 27 (2012) 1646–1652.
[16] S. Shi, Y.Z. Deng, J.S. Zhao, et al., RACK1 promotes non-small-cell lung cancer tumorigenicity activating sonic hedgehog signaling pathway, J. Biol. Chem. 287 (2012) 7845–7858.
[17] H. Caohuy, M. Srivastava, H.B. Pollard, Membrane fusion protein synexin (Annexin VII) as a Ca2+/GTP sensor in exocytotic secretion, Proc. Natl. Acad. Sci. 93 (1996) 10797–10802.
[18] M. Srivastava, I. Atwater, M. Glasman, et al., Defects in inositol 1,4,5-trisphosphate receptor expression, Ca(2+) signaling, and insulin secretion in the anx7(+/−) knockout mouse, Proc. Natl. Acad. Sci. 13 (1999) 13783–13788.
[19] M. Srivastava, L. Bubendorf, V. Srikantan, et al., ANX7, a candidate tumor suppressor gene for prostate cancer, Proc. Natl. Acad. Sci. 13 (2001) 4575–4580.
[20] Y. Torosyan, O. Simakova, S. Naga, et al., Annexin-A7 protects normal prostate cells and induces distinct patterns of RB-associated cytotoxicity in androgen-sensitive and -resistant prostate cancer cells, Int. J. Cancer 13 (2009) 2528–2539.

[21] M. Srivastava, L. Bubendorf, M. Raffeld, et al., Prognostic impact of ANX7-GTPase in metastatic and HER2-negative breast cancer patients, Clin. Cancer Res. 13 (2004) 2344–2350.
[22] H. Caohuy, H.B. Pollard, Activation of Annexin A7 by protein kinase C in vitro and in vivo, J. Biol. Chem. 276 (2001) 12813–12821.
[23] K. Taniuchi, K. Yokotani, T. Saibara, BART inhibits pancreatic cancer cell invasion by PKCα inactivation through binding to ANX7, PLoS One 7 (2012) e35674.
[24] P.A. Kiely, G.S. Baillie, M.J. Lynch, et al., Tyrosine 302 in RACK1 is essential for insulin-like growth factor-I-mediated competitive binding of PP2A and beta1 integrin and for tumor cell proliferation and migration, J. Biol. Chem. 283 (2008) 22952–22961.
[25] P.A. Kiely, D. O'Gorman, K. Luong, et al., Insulin-like growth factor I controls a mutually exclusive association of RACK1 with protein phosphatase 2A and beta1 integrin to promote cell migration, Mol. Cell Biol. 26 (2006) 4041–4051.
[26] D. Ron, Z. Jiang, L. Yao, et al., Coordinated movement of RACK1 with activated beta II PKC, J. Biol. Chem. 274 (1999) 27039–27046.
[27] R. Yaka, K. Phamluong, D. Ron, Scaffolding of Fyn kinase to the NMDA receptor determines brain region sensitivity to ethanol, J. Neurosci. 23 (2003) 3623–3632.
[28] A.C. Elisabeth, B. David, T.D. Ashley, et al., RACK1 regulates Integrin-mediated adhesion, protrusion, and chemotactic cell migration via its Src-binding site, Mol. Biol. Cell 14 (2003) 658–669.
[29] A.T. Doan, A. Huttenlocher, RACK1 regulates Src activity and modulates paxillin dynamics during cell migration, Exp. Cell Res. 313 (2007) 2667–2679.
[30] K. Bourd-Boittin, H. Le Pabic, D. Bonnier, et al., RACK1, a new ADAM12 interacting protein. Contribution to liver fibrogenesis, J. Biol. Chem. 28 (2008) 26000–26009.