ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science **2014** Vol. 24. No. 5. 588~593 DOI : http://dx.doi.org/10.5352/JLS.2014.24.5.588

- Review -

RalA-binding Protein 1 is an Important Regulator of Tumor Angiogenesis

Seunghyung Lee*

Department of Anatomy & Cell Biology, The Sol Sherry Thrombosis Research Center, Temple University School of Medicine, Philadelphia, PA 19140, USA

Received March 4, 2014 / Revised May 7, 2014 / Accepted May 9, 2014

Tumor angiogenesis is important in tumorigenesis and therapeutic interventions in cancer. To know inhibitor and effector of tumor angiogenesis in cancer, the specific gene of tumor and angiogenesis may develop the mechanisms of cancer suppression and therapy. Recently, we described the role of RalA-binding protein 1 (RLIP76) in tumor angiogenesis. Tumor vascular volumes were diminished, and vessels were fewer in number, shorter, and narrower in RLIP76 knockout mice than in wild-type mice. Moreover, angiogenesis in basement membrane matrix plugs was blunted in the knockout mice in the absence of tumor cells, with endothelial cells isolated from the lungs of these animals exhibiting defects in migration, proliferation, and cord formation *in vitro*. RLIP76 is expressed in most human tissues and is overexpressed in many tumor types. In addition, the protein regulates tumorigenesis and angiogenesis *in vivo* and *in vitro*. As the export of chemotherapy agents is a prominent cellular function of RLIP76, it is a major factor in mechanisms of drug resistance. Moreover, RLIP76 acts as a selective effector of the small GTPase, R-Ras, and regulates R-Ras signaling, leading to cell spreading and migration. Furthermore, in skin carcinogenesis, RLIP76 knockout mice are resistant, with tumors that form showing diminished angiogenesis. Thus, RLIP76 is required for efficient endothelial cell function and angiogenesis in solid tumors.

Key words : Cancer, Endothelial cell, RalBP1/RLIP76, Tumor angiogenesis, Tumorigenesis

Introduction

RalA-binding protein 1 (RLIP76) plays an important role in cancer, such as melanoma regression, regression of lung and colon cancer, colorectal cancer, breast cancer, and carcinoma cancer [21, 24, 32, 35, 36]. RLIP76 knockout mice are highly resistant to chemical carcinogenesis and are even resistant to the growth of subcutaneously implanted cancer cells [20, 21, 32, 33, 36, 37, 41]. Recently, we have been published in RLIP76 regulates tumor angiogenesis *in vivo* and *in vitro*, these studies suggest that suppression of RLIP76 can inhibit vascular growth and/or angiogenesis for tumor angiogenesis.

Tumor angiogenesis is important for tumor growth and therapeutic intervention in cancer. These primarily consist of the release of angiogenic factors, activation of metalloproteases to break down extracellular matrix, and subsequent remodeling. Also angiogenic stimulants induced in tumor cells and produced, and endothelial cells are responded in proliferation, migration, spreading, and angiogenesis to tumor. To know inhibitor and/or its effector of tumor angiogenesis in cancer, the specific gene of tumor and angiogenesis may develop the mechanisms of cancer suppression and therapy. This review will focus on the function and role of RLIP76 in tumor angiogenesis and cancer.

***Corresponding author**
Tel : +1-215-707-8015, Fax : +1-215-707-6499
E-mail : s.lee@temple.edu

Functions of RLIP76

RLIP76 is a modular, multifunctional protein of 655 amino acids, harboring an N-terminal putative helical domain of poorly characterized function, a central RhoGAP domain, and a conserved Ral-binding domain (RalBD) near the C-terminus (Fig. 1). Like all Ras superfamily small G proteins, Ral proteins are signal transducers that become activated upon release of guanine diphosphate (GDP) and binding to guanine triphosphate (GTP), upon which Ral undergoes a conformational shift to expose high affinity binding sites for signaling effectors. Thus RLIP76 is a unique Ral effector, connecting upstream activation of Ral to downstream molecular and cellular events.

Also the Ral effector property of RLIP76 was described as linking Ral to Rho GTPase pathways through the

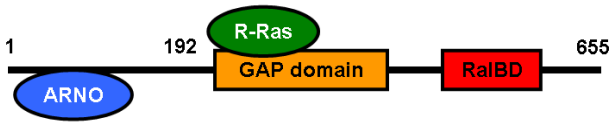

Fig. 1. Schematic diagram of the RLIP76.

RhoGAP domain [9, 17, 44]. The Rho subfamily of Ras small G proteins, most prominently RhoA, Rac and Cdc42, are, like Ras and Ral, regulated by guanine nucleotide exchange, such that RhoGAPs facilitate conversion from the GTP-bound, active state to the GDP-bound, inactive state. Thus, an important cellular and physiological function of RLIP76 is to couple Ral effector function with Rho signaling and actin cytoskeletal remodeling, promoting altered cell morphologies.

Moreover, our group identified RLIP76 as a selective effector of the small GTPase R-Ras, and found that RLIP76 regulates R-Ras signaling leading to cell spreading and migration [8, 11]. These effects are the result of RLIP76 adapter function, whereby it recruits ARNO, a small GTPase guanine exchange factor, to R-Ras at recycling endosomes to regulate vesicular trafficking necessary for spreading and migration [8]. RLIP76 also potentiates Ral-mediated cell spreading, potentially through similar signaling pathways [7]. Thus, RLIP76 regulates a broad spectrum of molecular, cellular and physiological processes, many of which stem from its function as a molecular adapter in various cellular locales (Fig. 2).

RLIP76 in cancer

RLIP76 is expressed in most human tissues including liver, heart, ovary, lung, muscle, and kidney as well as in most human tumor cell lines, and is over-expressed in multiple cancers, such as lung and ovarian carcinomas and melanomas [3, 4, 6, 28]. Since a prominent cellular function of

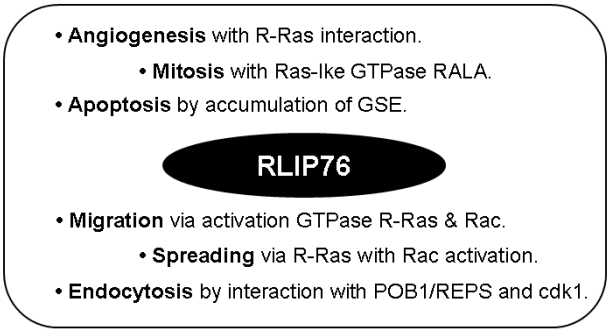

Fig. 2. Summary of RLIP76 functions in cells.

RLIP76 is export of chemotherapy agents, it is a major factor in the mechanisms of drug resistance. Moreover, blockade of RLIP76 with targeting antibodies or antisense has been shown to greatly increase sensitivity to radiation and chemotherapy and lead to pronounced tumor regression in multiple types of solid tumors in mice, including xenografted tumors of cancer cells [32, 33, 36].

Of the many functions of RLIP76 related to cancer initiation and progression, the most thoroughly characterized is as a molecular transporter of glutathione-electrophile conjugates (GS-E). GS-Es form by thioether conjugation of glutathione (GSH, between 1–10 mM cytolosic concentration in cells), an electron donor and thus a reducing agent, with electrophilic or oxidant chemicals that are derived both endogenously (endobiotics) and from the environment (xenobiotics). In this way, GSH acts as a scavenger for alkylating agents and other electrophiles [15]. GS-E conjugates become trapped in cells, and require energy-dependent transport for their removal to prevent toxicity both by excessive GS-E and by impairing the overall process of reduction of electrophilic toxins [16, 42]. Some of this transport function is carried out by ATP-binding cassette (ABC) transporters [1, 10]. Thus, in addition to removal from cells of toxic endobiotics such as 4-hydroxynonenal (4-HNE), GS-E transport is also essential for protection from xenobiotics [16, 43]. Multi-drug resistance (MDR), particularly for alkylating chemotherapeutic drugs, is very often the result of a failure of transport in the target cells; hence, transporters such as ABC type are classified as MDR proteins, which have long been pursued as therapeutic targets to inhibit drug resistance in cancer cells [19, 29].

RLIP76 is a novel, non-ABC type transporter, which utilizes both of its two ATP binding sites - in the N-terminal domain (aa 69-74) and adjacent to the RalBD (418-425) - for ATPase and transport activity, but lacks the canonical Walker domain of ABC-type transporters [25]. Interestingly, PKC-mediated phosphorylation of RLIP76 increases its transport activity [27]. A wealth of subsequent studies showed that RLIP76 is the major transporter for a wide range of structurally distinct endobiotics and xenobiotic chemotherapy agents, including GS-E, doxorubicin, sulfates, leukotriene C4, vinorelbine, glucorinides, colchicine, and other organic anions and cations [2, 5, 27, 30, 34, 36, 38, 39]. Due to this broad spectrum of transport targets, RLIP76 plays important roles in resistance to apoptosis due to heat shock and oxidative stress (in part through interaction with

HSF-1), radiation sensitivity, and perhaps most prominently, to multi-drug resistance in cancer therapy [22, 37, 40].

RLIP76 regulates tumor angiogenesis

RLIP76 has unique functions in endothelial cells, specialized squamous epithelia that form the inner core of all blood vessels, and are the sole cellular components of the microvasculature [14, 21]. We have recently found a role for RLIP76 in tumor angiogenesis (Fig. 3). Angiogenesis, the outgrowth of new blood vessels from existing ones, is required for progression of tumor growth and metastasis. Solid tumors require a nutrient blood supply to grow beyond ~1 mm diameter, and inhibiting tumor angiogenesis has long been pursued as an approach to preventing tumor growth and subsequent metastasis [23]. Angiogenesis is a complex process resulting from combined simultaneous

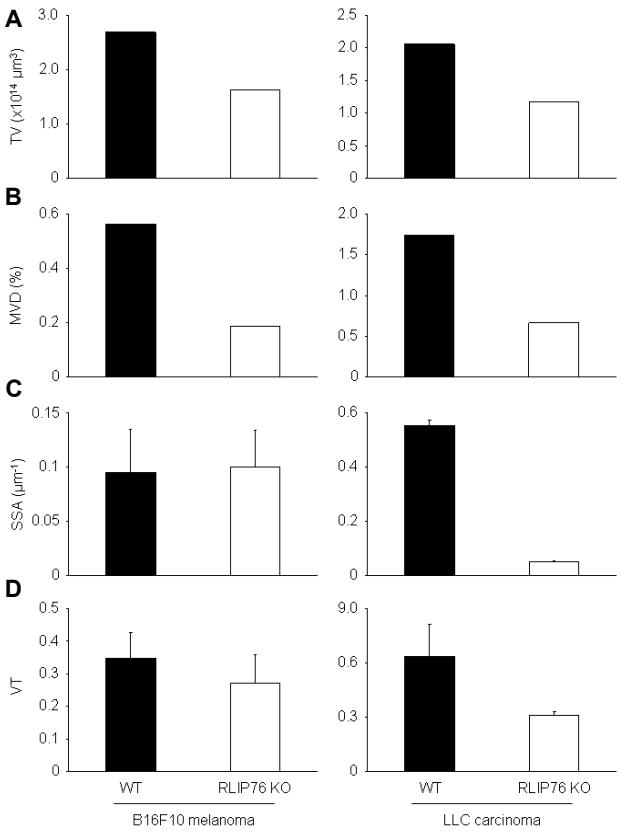

Fig. 3. Quantification of tumor vasculature in wild type and RLIP76 knock-out mice. B16F10 melanoma and LLC carcinoma tumor cells were cultured, and injected into flank of wild type and RLIP76 knock-out mice. Collected solid tumors scanned by micro-CT, and analyzed total volume (A), micro-vascular density (B), specific surface area (C), and vessel tortuosity (D) using micro-CT software program and analyze 10.0.

up-regulation of proliferation and migration in endothelial cells. During initial stages of angiogenesis, proliferating and migrating endothelial cells convert to a spindle-shaped morphology and organize into branched capillary networks, which differentiate into fully-formed lumenal vessels carrying blood from the source vasculature to the new sites such as into solid tumors [23].

Based on the ability of RLIP76 to interact with R-Ras – a modulator of tumor angiogenesis – as well as with other proteins important for endothelial function, we recently investigated a potential physiological role for RLIP76 in angiogenesis in solid tumors xenografted in mice [18]. Tumor growth from B16F10 melanoma or Lewis lung carcinoma cells xenografted into the flanks of C57Bl/6 wild type (WT) mice was blunted in isogenic RLIP76$^{-/-}$ mice. We used X-ray micro-computed tomography to reconstruct tumor vascular structures in 3D in resected tumors from WT and RLIP76 knock-out mice, and found defects in both the extent and form of tumor angiogenesis in RLIP76 knock-out mice. Specifically, tumor vascular volumes were diminished and vessels were fewer in number, shorter, and narrower in RLIP76 knock-out mice than in WT mice. Moreover, we found that angiogenesis in basement membrane matrix plugs was blunted in the knockout mice in the absence of tumor cells, with endothelial cells isolated from the lungs of these animals exhibiting defects in migration, proliferation and cord formation *in vitro* [14]. Furthermore, in a model of induced skin carcinogenesis, to which RLIP76 knock-out mice are already resistant [13], the tumors that did form showed diminished angiogenesis; conversely, liposome-mediated reconstitution of RLIP76 expression restored tumor growth and tumor angiogenesis in this model [14]. Thus, in addition to regulating tumor cell growth, RLIP76 is required for efficient endothelial cell function and angiogenesis in solid tumors.

Conclusions

In tumor angiogenesis, effects of RLIP76 targeting in tumor xenografts are the result of combinatorial effects in tumor cells and stromal cells. The prevention of xenografted tumor growth and synergy with inhibition of tumor angiogenesis in RLIP76 knockout mice, and conversely the enhanced tumor angiogenesis observed following ectopic application of RLIP76 in the knockout mice, strongly supports the idea of a one-two punch attack on growth of solid tumors, by blocking RLIP76 function in tumor cells and the

tumor vasculature.

Acknowledgment

This work was supported by American Heart Association Grant 12POST12040257 to S.L.

References

1. Ambudkar, S. V., Dey, S., Hrycyna, C. A., Ramachandra, M., Pastan, I. and Gottesman, M. M. 1999. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu Rev Pharmacol Toxicol* **39**, 361-398.
2. Awasthi, S., Sharma, R., Singhal, S. S., Zimniak, P. and Awasthi, Y. C. 2002. RLIP76, a novel transporter catalyzing ATP-dependent efflux of xenobiotics. *Drug Metab Dispos* **30**, 1300-1310.
3. Awasthi, S., Singhal, S. S., Awasthi, Y. C., Martin, B., Woo, J. H., Cunningham, C. C. and Frankel, A. E. 2008. RLIP76 and Cancer. *Clin Cancer Res* **14**, 4372-4377.
4. Awasthi, S., Singhal, S. S., Srivastava, S. K., Zimniak, P., Bajpai, K. K., Saxena, M., Sharma, R., Ziller, S. A., Frenkel, E. P. and Singh, S. V. 1994. Adenosine triphosphate-dependent transport of doxorubicin, daunomycin, and vinblastine in human tissues by a mechanism distinct from the P-glycoprotein. *J Clin Invest* **939**, 58-965.
5. Awasthi, S., Singhal, S. S., Yadav, S., Singhal, J., Drake, K., Nadkar, A., Zajac, E., Wickramarachchi, D., Rowe, N., Yacoub, A., Boor, P., Dwivedi, S., Dent, P., Jarman, W. E., John, B. and Awasthi, Y. C. 2005. RLIP76 is a major determinant of radiation sensitivity. *Cancer Res* **65**, 6022-6028.
6. Awasthi, Y. C., Singhal, S. S., Gupta, S., Ahmad, H., Zimniak, P., Radominska, A., Lester, R. and Sharma, R. 1991. Purification and characterization of an ATPase from human liver which catalyzes ATP hydrolysis in the presence of the conjugates of bilirubin bile acids and glutathione. *Biochem Biophys Res Commun* **175**, 1090-1096.
7. Awasthi, Y. C., Ansari, G. A. and Awasthi, S. 2005. Regulation of 4-hydroxynonenal mediated signaling by glutathione S-transferases. *Methods Enzymol* **401**, 379-407.
8. Awasthi, Y. C., Chaudhary, P., Vatsyayan, R., Sharma, A., Awasthi, S. and Sharma, R. 2009. Physiological and pharmacological significance of glutathione-conjugate transport. *J Toxicol Environ Health B Crit Rev* **12**, 540-551.
9. Bauer, B., Mirey, G., Vetter, I. R., Garcia-Ranea, J. A., Valencia, A., Wittinghofer, A., Camonis, J. H. and Cool, R. H. 1999. Effector recognition by the small GTP-binding proteins Ras and Ral. *J Biol Chem* **274**, 17763-17770.
10. Borst, P., Evers, R., Kool, M. and Wijnholds, J. 2000. A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst* **92**, 1295-1302.
11. Cheng, J. Z., Sharma, R., Yang, Y., Singhal, S. S., Sharma, A., Saini, M. K., Singh, S. V., Zimniak, P., Awasthi, S. and Awasthi, Y. C. 2001. Accelerated metabolism and exclusion of 4-hydroxynonenal through induction of RLIP76 and hGST5.8 is an early adaptive response of cells to heat and oxidative stress. *J Biol Chem* **276**, 41213-41223.
12. Downey, C. M., Singla, A. K., Villemaire, M. L., Buie, H. R., Boyd, S. K. and Jirik, F. R. 2012. Quantitative ex-vivo micro-computed tomographic imaging of blood vessels and necrotic regions within tumors. *PLoS One* **7**, e41685.
13. Goldfinger, L. E., Ptak, C., Jeffery, E. D., Shabanowitz, J., Han, J., Haling, J. R., Sherman, N. E., Fox, J. W., Hunt, D. F. and Ginsberg, M. H. 2007. An experimentally derived database of candidate Ras-interacting proteins. *J Proteome Res* **6**, 1806-1811.
14. Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T. and Munzel, T. 2001. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* **104**, 2673-2678.
15. Ishikawa, T. 1992. The ATP-dependent glutathione S-conjugate export pump. *Trends Biochem Sci* **17**, 463-468.
16. Jedlitschky, G., Leier, I., Buchholz, U., Barnouin, K., Kurz, G. and Keppler, D. 1996. Transport of glutathione, glucuronate, and sulfate conjugates by the MRP gene-encoded conjugate export pump. *Cancer Res* **56**, 988-994.
17. Jullien-Flores, V., Dorseuil, O., Romero, F., Letourneur, F., Saragosti, S., Berger, R., Tavitian, A., Gacon, G. and Camonis, J. H. 1995. Bridging Ral GTPase to Rho pathways. RLIP76, a Ral effector with CDC42/Rac GTPase-activating protein activity. *J Biol Chem* **270**, 22473-22477.
18. Komatsu, M. and Ruoslahti, E. 2005. R-Ras is a global regulator of vascular regeneration that suppresses intimal hyperplasia and tumor angiogenesis. *Nat Med* **11**, 1346-1350.
19. LaBelle, E. F., Singh, S. V., Srivastava, S. K. and Awasthi, Y. C. 1986. Dinitrophenyl glutathione efflux from human erythrocytes is primary active ATP-dependent transport. *Biochem J* **238**, 443-449.
20. Leake, K., Singhal, J., Nagaprashantha, L. D., Awasthi, S. and Singhal, S. S. 2012. RLIP76 regulates PI3K/Akt signaling and chemo-radiotherapy resistance in pancreatic cancer. *PLoS One* **7**, e34582.
21. Lee, S., Wurtzel, J. G., Singhal, S. S., Awasthi, S. and Goldfinger, L. E. 2012. RALBP1/RLIP76 depletion in mice suppresses tumor growth by inhibiting tumor neovascularization. *Cancer Res* **72**, 5165-5173.
22. Margutti, P., Matarrese, P., Conti, F., Colasanti, T., Delunardo, F., Capozzi, A., Garofalo, T., Profumo, E., Rigano, R., Siracusano, A., Alessandri, C., Salvati, B., Valesini, G., Malorni, W., Sorice, M. and Ortona, E. 2008. Autoantibodies to the C-terminal subunit of RLIP76 induce oxidative stress and endothelial cell apoptosis in immune-mediated vascular diseases and atherosclerosis. *Blood* **111**, 4559-4570.
23. Matarrese, P., Colasanti, T., Ascione, B., Margutti, P., Franconi, F., Alessandri, C., Conti, F., Riccieri, V., Rosano, G., Ortona, E. and Malorni, W. 2011. Gender disparity in susceptibility to oxidative stress and apoptosis induced by autoantibodies specific to RLIP76 in vascular cells. *Antioxid Redox Signal* **15**, 2825-2836.
24. Mollberg, N. M., Steinert, G., Aigner, M., Hamm, A., Lin,

F. J., Elbers, H., Reissfelder, C., Weitz, J., Buchler, M. W. and Koch, M. 2012. Overexpression of RalBP1 in colorectal cancer is an independent predictor of poor survival and early tumor relapse. *Cancer Biol Ther* **13**, 694-700.
25. Nakashima, S., Morinaka, K., Koyama, S., Ikeda, M., Kishida, M., Okawa, K., Iwamatsu, A., Kishida, S. and Kikuchi, A. 1999. Small G protein Ral and its downstream molecules regulate endocytosis of EGF and insulin receptors. *EMBO J* **18**, 3629-3642.
26. Savai, R., Langheinrich, A. C., Schermuly, R. T., Pullamsetti, S. S., Dumitrascu, R., Traupe, H., Rau, W. S., Seeger, W., Grimminger, F. and Banat, G. A. 2009. Evaluation of angiogenesis using micro-computed tomography in a xenograft mouse model of lung cancer. *Neoplasia* **11**, 48-56.
27. Saxena, M., Singhal, S. S., Awasthi, S., Singh, S. V., Labelle, E. F., Zimniak, P. and Awasthi, Y. C. 1992. Dinitrophenyl S-glutathione ATPase purified from human muscle catalyzes ATP hydrolysis in the presence of leukotrienes. *Arch Biochem Biophys* **298**, 231-237.
28. Sharma, R., Gupta, S., Singh, S. V., Medh, R. D., Ahmad, H., LaBelle, E. F. and Awasthi, Y. C. 1990. Purification and characterization of dinitrophenylglutathione ATPase of human erythrocytes and its expression in other tissues. *Biochem Biophys Res Commun* **171**, 155-161.
29. Sharma, R., Singhal, S. S., Cheng, J., Yang, Y., Sharma, A., Zimniak, P., Awasthi, S. and Awasthi, Y. C. 2001. RLIP76 is the major ATP-dependent transporter of glutathione-conjugates and doxorubicin in human erythrocytes. *Arch Biochem Biophys* **391**, 171-179.
30. Sharma, R., Singhal, S. S., Wickramarachchi, D., Awasthi, Y. C. and Awasthi, S. 2004. RLIP76-mediated transport of leukotriene C4 (LTC4) in cancer cells: implications in drug resistance. *Int J Cancer* **112**, 934-942.
31. Shojaei, F., Wu, X., Zhong, C., Yu, L., Liang, X. H., Yao, J., Blanchard, D., Bais, C., Peale, F. V., van Bruggen, N., Ho, C., Ross, J., Tan, M., Carano, R. A., Meng, Y. G. and Ferrara, N. 2007. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature* **450**, 825-831.
32. Singhal, S. S., Awasthi, Y. C. and Awasthi, S. 2006. Regression of melanoma in a murine model by RLIP76 depletion. *Cancer Res* **66**, 2354-2360.
33. Singhal, S. S., Roth, C., Leake, K., Singhal, J., Yadav, S. and Awasthi, S. 2009. Regression of prostate cancer xenografts by RLIP76 depletion. *Biochem Pharmacol* **77**, 1074-1083.
34. Singhal, S. S., Sharma, R., Gupta, S., Ahmad, H., Zimniak, P., Radominska, A., Lester, R. and Awasthi, Y. C. 1991. The anionic conjugates of bilirubin and bile acids stimulate ATP hydrolysis by S-(dinitrophenyl) glutathione ATPase of human erythrocyte. *FEBS Lett* **281**, 255-257.
35. Singhal, S. S., Singhal, J., Nair, M. P., Lacko, A. G., Awasthi, Y. C. and Awasthi, S. 2007 Doxorubicin transport by RALBP1 and ABCG2 in lung and breast cancer. *Int J Oncol* **30**, 717-725.
36. Singhal, S. S., Singhal, J., Yadav, S., Dwivedi, S., Boor, P. J., Awasthi, Y. C. and Awasthi, S. 2007. Regression of lung and colon cancer xenografts by depleting or inhibiting RLIP76 (Ral-binding protein 1). *Cancer Res* **67**, 4382-4389.
37. Singhal, S. S., Singhal, J., Yadav, S., Sahu, M., Awasthi, Y. C. and Awasthi, S. 2009. RLIP76: a target for kidney cancer therapy. *Cancer Res* **69**, 4244-4251.
38. Singhal, S. S., Yadav, S., Drake, K., Singhal, J. and Awasthi, S. 2008. Hsf-1 and POB1 induce drug sensitivity and apoptosis by inhibiting Ralbp1. *J Biol Chem* **283**, 19714-19729.
39. Singhal, S. S., Yadav, S., Roth, C. and Singhal, J. 2009. RLIP76: a novel glutathione-conjugate and multi-drug transporter. *Biochem Pharmacol* **77**, 761-769.
40. Singhal, S. S., Yadav, S., Singhal, J., Zajac, E., Awasthi, Y. C. and Awasthi, S. 2005. Depletion of RLIP76 sensitizes lung cancer cells to doxorubicin. *Biochem Pharmacol* **70**, 481-488.
41. Singhal, S. S., Wickramarachchi, D., Yadav, S., Singhal, J., Leake, K., Vatsyayan, R., Chaudhary, P., Lelsani, P., Suzuki, S., Yang, S., Awasthi, Y. C. and Awasthi, S. 2011. Glutathione-conjugate transport by RLIP76 is required for clathrin-dependent endocytosis and chemical carcinogenesis. *Mol Cancer Ther* **10**, 16-28.
42. Srivastava, S. K., Hu, X., Xia, H., Bleicher, R. J., Zaren, H. A., Orchard, J. L., Awasthi, S. and Singh, S. V. 1998. ATP-dependent transport of glutathione conjugate of 7beta, 8alpha-dihydroxy-9alpha,10alpha-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene in murine hepatic canalicular plasma membrane vesicles. *Biochem J* **332**, 799-805.
43. Stuckler, D., Singhal, J., Singhal, S. S., Yadav, S., Awasthi, Y. C. and Awasthi, S. 2005. RLIP76 transports vinorelbine and mediates drug resistance in non-small cell lung cancer. *Cancer Res* **65**, 991-998.
44. Vatsyayan, R., Lelsani, P. C., Awasthi, S. and Singhal, S. S. 2010. RLIP76: a versatile transporter and an emerging target for cancer therapy. *Biochem Pharmacol* **79**, 1699-1705.
45. Walls, J. R., Coultas, L., Rossant, J. and Henkelman, R. M. 2008. Three-dimensional analysis of vascular development in the mouse embryo. *PLoS One* **3**, e2853.
46. Yang, J., Yu, L. X., Rennie, M. Y., Sled, J. G. and Henkelman, R. M. 2010. Comparative structural and hemodynamic analysis of vascular trees. *Am J Physiol Heart Circ Physiol* **298**, H1249-1259.

초록 : Tumor angiogenesis에 있어서 RLIP76의 중요성

이승형*
(템플대학교 의과대학 해부 & 세포 생물학, 솔 세리 혈전증 연구 센터)

본 논문은 RLIP76 단백질이 암, 종양 혈관 신생 및 그 치료에 미치는 중요성을 보고함에 있다. 암의 연구에 있어서, 종양의 혈관 신생을 억제시키는 인자와 영향을 끼치는 인자를 밝혀내는 것은 암의 억제와 치료를 위한 분자 생물학적 기전에 중대한 영향을 미친다. 최근 연구에서, RLIP76 단백질이 혈관 신생에 영향을 끼치는 역할을 발견하였다. RLIP76 제거 마우스의 종양은 일반 종양과 비교하여 혈관의 크기가 작으며, 가늘고, 그 혈관의 수가 적고 길이가 짧은 것으로 보고되고 있다. 게다가, Matrigel plugs을 이용한 혈관 신생 실험에서, RLIP76이 제거된 마우스에서는 혈관 생성이 억제 되었으나, 일반 마우스에서는 혈관이 생성되었다. 또한, 혈관세포를 이용한 *in vi-tro* 실험에 있어서, proliferation, migration 및 cord formation 모두가 RLIP76에 의해서 조절되었다. 일반적으로 RLIP76은 대부분의 인간 조직과 종양에서 발현되며, 약의 저항 기전 연구에 이용되고 있기도 한다. 또한, 이 RLIP76은 small GTPase R-Ras와 상호작용을 통하여 세포 spreading 및 migration에 관여하고 있다. 이러한 결과는 RLIP76와 암 연구의 중요성을 보고하고 있으며, 혈관 세포의 기능의 기전 및 종양의 혈관 신생을 위한 RLIP76 단백질의 중요성을 알리고 있고, RLIP76의 추가적인 연구를 통하여 종양의 혈관 신생의 기전을 밝히는 것이 필요함을 제안하는 바이다.