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RhoGDI2 suppresses bladder cancer metastasis via reduction of inflammation in the tumor microenvironment

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Low expression of RhoGDI2 is associated with poor outcome in cancer patients. In animal models, RhoGDI2 suppresses lung metastasis by reducing the expression of the proteoglycan versican, whose levels portend poor patient prognosis. Versican promotes metastasis through enhanced tumor migration and creation of an inflammatory lung environment involving macrophages and the CCL2/CCR2 signaling axis. Targeting this mechanism may provide novel adjuvant strategies for delaying the appearance of clinical metastasis.

Half of patients with muscle-invasive urothelial cancer of the bladder develop distant metastases, even after radical surgery of the primary tumor. Clinical data from human disease as well as experimental rodent models of carcinogenesis and metastasis revealed that bladder cancer metastasizes mainly to regional lymph nodes and to the lungs.[1,2] The high incidence of pulmonary metastases in cancer patients was initially believed to be a random process, however, previous data from our group indicates that the development of lung metastasis is likely to be an active, highly selective process instigated by tumor cells and strongly influenced by their interactions with host cells in the tumor microenvironments.[1,2]

A decade ago, we identified Rho GDP dissociation inhibitor (GDI) β (ARHGDIB, RhoGDI2, Ly-GDI, GDID4) as a metastasis suppressor in human bladder cancer cell lines,[3] and we showed that its expression is inversely associated with clinical outcome after treatment of muscle-invasive tumors.[4] Independently, in comparative gene expression profiling of invasive bladder cancer cell lines and human muscle-invasive urothelial cancer samples, we discovered that versican (VCAN), also known as chondroitin sulfate proteoglycan 2 (CSPG2), is highly expressed in invasive and metastatic cancers.[5]

Versican is a complex and versatile extracellular matrix (ECM) molecule that is indispensable for life.[6] Not only it functions as a scaffold or substrate to be consumed during tumor-cell invasion, but also represents a central component of cancer-related inflammation as it can bind multiple types of cell adhesion molecules/receptors, growth factors/their receptors and chemokines to provide a complex set of environmental cues to inflammatory and cancer cells in versican-rich sites.[6,7]

In a recent report,[8] using comparative gene expression profiling of bladder cancer models and cohorts of human bladder cancer patients, we showed a correlation between low RhoGDI2 expression, high versican expression and poor clinical outcomes. Experiments with human and murine xenografts in the context of pharmacologic and genetic manipulations (transgenic mice) suggested that both macrophages and the CCL2/CCR2 axis were necessary for versican to exert its metastasis promoting role (**Fig. 1**). Several reports have implicated this chemokine in myriad of activities that impact cancer progression and metastasis.[9] Thus, tumor-derived CCL2 has been involved in the recruitment of $CCR2^+$ myeloid cells not only to the primary tumor but also to prospective metastatic sites as well as in their differentiation into an inflammatory phenotype that fosters extravasation, seeding and persistent growth of tumor cells.[9,10] This highly inflammatory microenvironment induces high versican expression, with increased macrophage infiltration. In turn, macrophage infiltration exacerbates versican overexpression along with the secretion of other cytokines and inflammatory mediators. Ultimately, this "inflammatory storm" causes uncontrolled, prolonged inflammation. A vicious cycle between versican expression and the inflammatory cytokines inevitably occurs and further contributes to progressive cancer cell colonization of the lung. Within this context, versican appears to mediate a dialog between inflammatory cells, cytokines and cancer cells in the tumor microenvironment. Hence, we propose that versican could be the first step in the amplification of inflammation.[8]

Recent data demonstrate that targeting CCL2 with blocking antibodies inhibits lung and bone metastases in vivo, perhaps representing a novel approach to cancer treatment.[8-10] Our report shows

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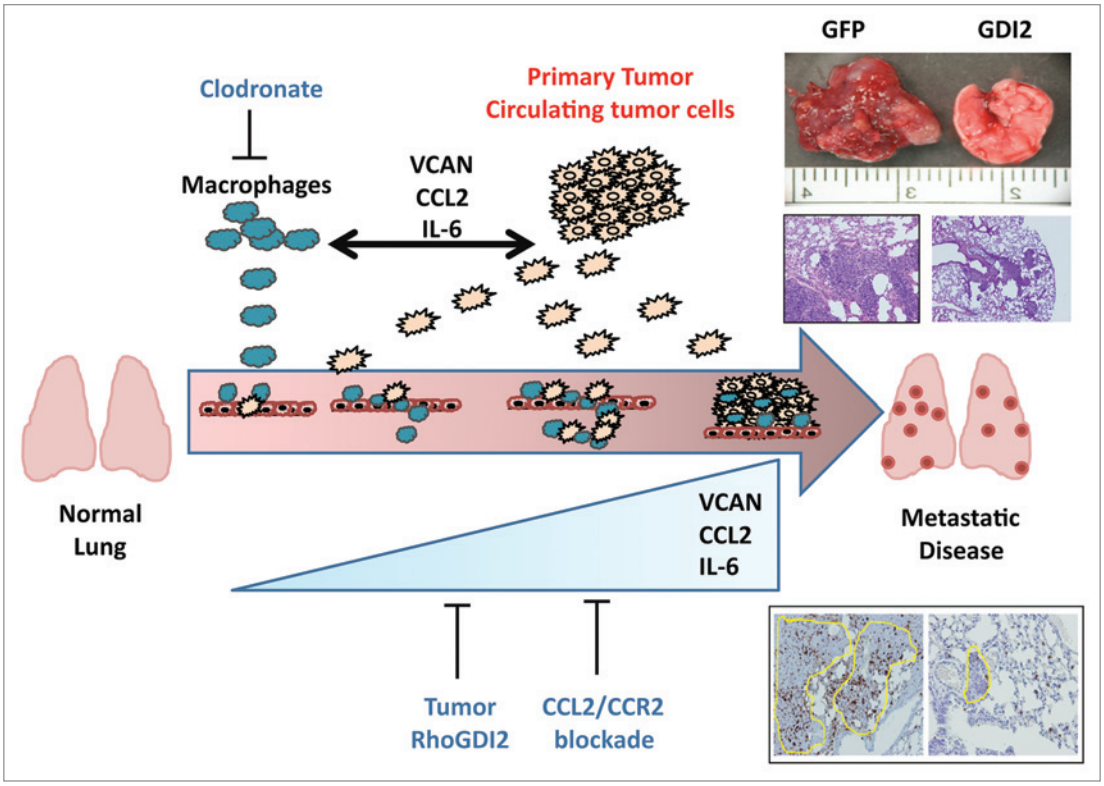

Figure 1. Proposed model for the mechanism of metastasis suppressor effect of RhoGDI2. Invasive primary tumors and shed circulating tumor cells that lack or express low levels of RhoGDI2 secrete soluble factors including versican, CCL2 and IL-6 that stimulate macrophage recruitment and retention in the pre-metastatic lungs. In turn macrophages acquire an inflammatory phenotype and in turn contribute to the progressive increase in the levels of versican, CCL2 and IL-6 in the pre-metastatic lungs with subsequent tumor cell extravasation, colonization and formation of micro- then macro-metastases. Targeting macrophages by clodronate-encapsulated liposomes or blocking the CCL2/CCR2 axis phenocopies the metastasis suppressor effect of RhoGDI2 and inhibit versican-mediated metastasis.

for the first time that the host CCL2/CCR2 axis is necessary for versican-driven metastasis, suggesting its utility for patient stratification. Thus, one could speculate that factors in the tumor microenvironment trigger signaling intermediates that would induce both versican and CCL2. Given that of the pro-metastasis effect of versican is dependent on host CCL2, such parallel induction would confer an advantage to a tumor by maximally promoting metastatic colonization.

Our work is the first demonstration of a metastasis suppressor that blocks the pro-metastatic inflammatory host response in a distant organ, thus highlighting the therapeutic potential of anticancer strategies targeting both malignant and host derived components of the tumor microenvironment.

In conclusion, we argue that since versican is a complex structural molecule that is also important for the outgrowth of disseminated cancer cells, targeting versican or blocking the cytokines that are required for its function such as CCL2 may provide a therapeutic strategy for delaying the clinical evolution of metastatic disease from microscopic deposits. Specific antibodies or small molecule antagonists against CCR2 hence appears as a promising anticancer strategy that can be selectively targeted to high versican-expressing tumors, optimizing the chance for a clinical response.

References

1. Said N, Smith S, Sanchez-Carbayo M, Theodorescu D. Tumor endothelin-1 enhances metastatic colonization of the lung in mouse xenograft models of bladder cancer. J Clin Invest 2011; 121:132-47; PMID:21183790; http://dx.doi.org/10.1172/JCI42912.
2. Said N, Theodorescu D. Pathways of metastasis suppression in bladder cancer. Cancer Metastasis Rev 2009; 28:327-33; PMID:20013033; http://dx.doi.org/10.1007/s10555-009-9197-4.
3. Gildea JJ, Seraj MJ, Oxford G, Harding MA, Hampton GM, Moskaluk CA, et al. RhoGDI2 is an invasion and metastasis suppressor gene in human cancer. Cancer Res 2002; 62:6418-23; PMID:12438227.
4. Theodorescu D, Sapinoso LM, Conaway MR, Oxford G, Hampton GM, Frierson HF Jr. Reduced expression of metastasis suppressor RhoGDI2 is associated with decreased survival for patients with bladder cancer. Clin Cancer Res 2004; 10:3800-6; PMID:15173088; http://dx.doi.org/10.1158/1078-0432.CCR-03-0653.
5. Wu Y, Siadaty MS, Berens ME, Hampton GM, Theodorescu D. Overlapping gene expression profiles of cell migration and tumor invasion in human bladder cancer identify metallothionein 1E and nicotinamide N-methyltransferase as novel regulators of cell migration. Oncogene 2008; 27:6679-89; PMID:18724390; http://dx.doi.org/10.1038/onc.2008.264.
6. Wight TN. Versican: a versatile extracellular matrix proteoglycan in cell biology. Curr Opin Cell Biol 2002; 14:617-23; PMID:12231358; http://dx.doi.org/10.1016/S0955-0674(02)00375-7.
7. Serra M, Miquel L, Domenzain C, Docampo MJ, Fabra A, Wight TN, et al. V3 versican isoform expression alters the phenotype of melanoma cells and their tumorigenic potential. Int J Cancer 2005; 114:879-86; PMID:15645431; http://dx.doi.org/10.1002/ijc.20813.

8. Said N, Sanchez-Carbayo M, Smith SC, Theodorescu D. RhoGDI2 suppresses lung metastasis in mice by reducing tumor versican expression and macrophage infiltration. J Clin Invest 2012; 122:1503-18; PMID:22406535; http://dx.doi.org/10.1172/JCI61392.

9. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature 2011; 475:222-5; PMID:21654748; http://dx.doi.org/10.1038/nature10138.

10. Zhang J, Lu Y, Pienta KJ. Multiple roles of chemokine (C-C motif) ligand 2 in promoting prostate cancer growth. J Natl Cancer Inst 2010; 102:522-8; PMID:20233997; http://dx.doi.org/10.1093/jnci/djq044.