

Integrin $\beta 4$ synergizes with E-and P-selectin to regulate tumor growth: Unravelling the molecular mechanisms

Sandra Hanika^{a,*}, Vera Labitzky^a, Florian Gebauer^b, Daniel Wicklein^a, Hanna Maar^a, Tobias Gosau^a, Jennifer Schröder-Schwarz^a, Stefan Linder^c, Kristoffer Riecken^d, Udo Schumacher^a, Tobias Lange^a

^a Institute of Anatomy and Experimental Morphology, Hamburg, Germany

^b Department of General, Visceral and Thoracic Surgery, Hamburg, Germany

^c Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany

^d Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, Hamburg, Germany

* Corresponding author, email: sa.hanika@uke.de

© 2018 Sandra Hanika; licensee Infinite Science Publishing

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Keywords: metastasis, cancer cell adhesion, ITGB4, selectins

Purpose/Objectives: Metastases are responsible for 90% of cancer-related deaths. Scientific evidence has accumulated showing that adhesion molecules including selectins and integrins play a crucial role in the process of cancer metastasis. In particular, upregulation of integrin $\beta 4$ (ITGB4) is associated with tumor progression in various tumor entities. Thus, we aimed at detecting a potential crosstalk between selectins and integrins in tumor formation and metastatic spread.

Material/Methods: Knockdown of ITGB4 in PC3 (prostate cancer), PaCa5061 (pancreatic cancer) and SKOV3 (ovarian cancer) cells using shRNA, proliferation and colony forming assays, ELISA, WB, 3D chemotaxis assays with human macrophages, tumor initiation assays *in vivo*, immunohistochemistry

Results: Knockdown of ITGB4 affected prostatic PC3, pancreatic PaCa5061 and ovarian SKOV3 cancer xenografts already at the primary tumor site. In particular, tumor growth was strongly inhibited when the ITGB4-knockdown was combined with E-/P-selectin deficiency of mice while selectin-knockout alone had no effect. In this study we wanted to investigate the molecular mechanisms underlying this synergistic growth retardation. Delayed tumor growth was associated with increased apoptosis. Immunostainings further revealed an enhanced immune cell infiltration in ITGB4-knockdown tumors grown in immunodeficient mice. On the contrary, leukocytes were mainly located at the outer tumor periphery of both ITGB4-knockdown and control tumors in E-/P-selectin-knockout mice. The increased leukocyte infiltration in ITGB4-knockdown tumors in control mice seemed to result from a

greater chemotactic potential of ITGB4-knockdown cells. CCL2, 5 and 20 were identified as potential chemotactic factors upregulated upon ITGB4-knockdown in PaCa5061 and SKOV3 cells while the chemoattractant playing a role in ITGB4-knockdown PC3 cells remains to be determined.

Conclusion: Taken together, our results suggest that ITGB4-knockdown tumor initiation depends on pro-tumoral signals from attracted tumor-associated leukocytes. These leukocytes appear to depend on endothelial selectins for infiltration of the tumor stroma. Investigation of how leukocytes actually stabilize the survival of ITGB4-knockdown xenografts is the subject of ongoing experiments.