The diverse roles of integrin α3β1 in cancer: Lessons learned from skin and breast carcinogenesis

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APPENDICES

SUMMARY

Cells in epithelial tissues, such as skin, are tightly bound to each other and to the underlying extracellular matrix. A family of proteins that plays a crucial role in this process is integrins, transmembrane proteins that connect extracellular environment (they are ligated by the extracellular matrix proteins) with the intracellular cytoskeleton (e.g. actin). As such they play a crucial role in maintenance of the integrity of epithelial tissues. However, the roles of integrins expand beyond a simple adhesion – they can translate mechanical signals into biochemical, and thus modulate mechanisms driving numerous aspects of the cell behavior, ranging from cell migration and spreading to cell survival and proliferation. Considering this, it is not unexpected that integrins play a role in development and progression of numerous types of cancers.

Our group has previously demonstrated the prominent role of integrin α3β1, which is ligated by extracellular matrix proteins laminins-332 and -551, in development of non-melanoma skin tumorigenesis in mice, exposed to pro-tumorigenic DMBA/TPA treatment. Interestingly, whereas α3β1 was crucial for the development of benign skin tumors, its expression inhibited tumor progression in later stages of skin carcinogenesis. Such diverse and even opposing roles of α3β1 have been reported before, indicating that there is a need for a better understanding of the function of this integrin in well-defined histopathological types and stages of cancer. In this thesis we uncover the mechanisms behind the role of integrin α3β1 during the initiation of non-melanoma skin tumorigenesis and explore its role in HER2-driven breast carcinogenesis.

In **chapter 1** we briefly introduce the topic and the scope of the thesis. We focus on the non-melanoma skin and HER2-driven breast cancer and highlight the available knowledge on the role of α3β1 in both cancer types.

In **chapter 2** we summarize the main findings on the roles that laminin-binding integrins, especially α3β1 can have in cancer. We dive into the diversity of the functions of α3β1 in different stages and types of the disease and provide insight into the underlying mechanism and the role of the cell environment the mechanisms and the cell environment that defines them.

Chapters 3 and **4** are focused on the mechanisms behind the tumor-promoting role of α3β1 in the DMBA/TPA-mediated non-melanoma skin tumorigenesis. In **chapter 3** we start by building on the hypothesis, formed by the previous members of our group, which suggests that the absence of the formation of skin tumors in mice, lacking α3β1 in epidermis is due to the changes in the behavior of the main hair follicle stem cell

population – hair bulge stem cells (HB SCs). They suggested that HB SCs represent the main population of the tumor cells-of-origin and that that they egress from their niche in the absence of α3β1. This leads to their loss due to the terminal squamous differentiation before they could outgrow as tumors. We test this hypothesis by generating mice with HB SC-specific deletion of α3β1 and by tracking α3β1-deficient HB SCs over time. Our findings reject the original hypothesis: HB SCs remain confined within their niche regardless and hardly contribute to the tumor mass regardless of the α3β1 expression. However, a deletion of α3β1 in HB SCs nonetheless reduces the number of tumors in mice, suggesting that the contribution of HB SCs to tumorigenesis is indirect, via the promotion of a tumor permissive environment. We uncover that α3β1 can modulate the expression of connective tissue growth factor (CCN2) in HB SCs and propose that the secretion of CCN2 promotes tumorigenic potential and tumor growth of transformed keratinocytes.

As the deletion of α3β1 in HB SCs resulted in only moderate reduction of tumorigenesis, we re-examined the mechanisms behind the essential role of epidermal α3β1 in DMBA/TPA-driven tumorigenesis in **chapter 4.** We uncovered that during the initiation of tumorigenesis, α3β1 plays a central role in promoting the activation FAK/Src, Akt and Stat3 – all members of signaling pathways that are crucial for tumor formation in skin. Furthermore, α3β1 together with its binding tetraspanin CD151 regulates signaling molecules that control the survival of differentiating keratinocytes.

In **chapter 5** we investigate whether α3β1 plays a role also in HER2-driven breast cancer. We uncover that the downregulation of α3β1 in HER2-driven mouse model and in HER2-enriched human mammary carcinoma cells promotes tumor progression and invasiveness of the cells. We show that the role of α3β1 in cell invasion depends on the environmental factors, especially extracellular matrix composition and the interstitial fluid flow conditions. By comparing the role of α3β1 in HER2-positive and HER2-negative human mammary carcinoma cells we reaffirm that the role of α3β1 is cancer-type dependent.

In **chapter 6** we discuss the remaining questions, address the potential future research and provide future perspectives through the preliminary data based on human skin biopsies and human colorectal organoids.

In summary, this thesis highlights that the role of α3β1 in cancer depends on time and place: the nature of the cell environment (such as extracellular matrix composition), type of cancer and its driving mechanism, as well as the stage of the disease. We provide a new insight into the mechanisms behind the role of α3β1 in HER2-driven breast cancer and in DMBA/TPA-induced non-melanoma skin tumorigenesis.