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

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INTRODUCTION AND SCOPE OF THE THESIS

Half of the century has passed since we discerned that the survival of normal epithelial cells depends on their adhesion to the extracellular matrix and that this limitation can be overcome by transformed cells during the progression of carcinogenesis [1,2]. Moving two decades forward, integrins, a family of transmembrane proteins that mediate cell-extracellular matrix adhesion, emerged as the main regulators of this process [3]. Since then, our knowledge on integrin-mediated adhesion and signaling in normal and cancer cells has grown by leaps and bounds with novel roles of integrins beyond cell-matrix adhesion emerging [4,5].

The groundwork for this thesis was laid by former graduate student Norman Sachs and by postdoctoral researcher Pablo Secades, both working as my predecessors in the research group of prof. dr. Arnoud Sonnenberg. They were the first to observe the dramatic effect of the epithelial deletion of the laminin-binding integrin α3β1 on skin carcinogenesis: mice, lacking α3β1 in the skin were almost completely protected against tumor formation induced by two-stage chemical carcinogenesis protocol. Their research also reinforced the notion that the roles of integrin α3β1 in cancer can be diverse and even opposing at different stages of the disease: when α3β1 was absent from the epidermis during the progression of cutaneous carcinogenesis, the invasive potential and the malignant grade of carcinomas increased [6].

In the light of the crucial but often diverse roles that integrin α3β1 can have in cancer (discussed in depth in chapter 2), we reasoned that there is a need for a better understanding of the function of this integrin in well-defined histopathological types and stages of cancer. This research thesis was launched with the goal to uncover the mechanism behind the previously demonstrated essential role of integrin α3β1 during the initiation of non-melanoma skin tumorigenesis. I was fortunate enough to expand our objective to HER2-driven breast carcinogenesis, common epithelial cancer in which the role of α3β1 has not been thoroughly investigated before. Here, we briefly introduce both cancer types and present the scope of this thesis.

NON-MELANOMA SKIN CANCER

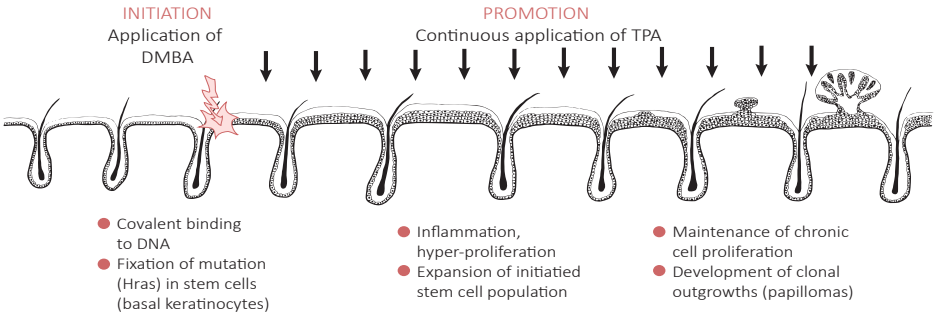

Figure 1: Two-stage chemical carcinogenesis model (DMBA/TPA treatment).

Non-melanoma skin cancer is the 5th most common cancer, with a rough estimate of one million diagnoses worldwide in 2018. Although the first stages of the disease can be successfully treated, late detection of invasive non-melanoma skin cancer often offers poor prognosis [7]. To better understand the molecular changes, driving different stages of the disease, a mouse skin model of multi-stage chemical carcinogenesis (also called DMBA/TPA treatment) has been developed over 60 years ago and since then extensively studied on various transgenic mouse models [8]. The first stage of the model, i.e. initiation, consists of a single application of carcinogen 7,12-dimethylbenz[a]-anthracene (DMBA), which causes an activating mutation in *Hras* gene. Although this event is irreversible, second, promotion stage needs to take place for the outgrowth of benign tumors called papillomas. The common promotion agent is phorbol ester, 12-Otetradecanoylphorbol-13-acetate (TPA), which is applied bi-weekly for 20 weeks for full two-stage chemical carcinogenesis protocol (**Fig. 1**). If the TPA-treatment is continued, some of the papillomas eventually will progress into invasive squamous carcinomas and metastasize [8]. Pro-inflammatory TPA-treatment causes activation of several growth factor signaling pathways, leading to hyperproliferation of the skin and the expansion of DMBA-initiated keratinocytes into papillomas. Decades of the research on transgenic mouse models helped us to identify the main signaling pathways that play a central role in this tumorigenesis (**Fig. 2**).

As DMBA-initiated keratinocytes have to persist in epidermis sufficiently long to accumulate additional mutations before they can outgrow into clonal papillomas, the target cell population for tumor initiation has long been determined as epidermal stem cells: slow-cycling keratinocytes, located in the basal layer of epidermis [11]. However, the contributions of specific epidermal stem cell populations to DMBA/TPA-initiated

tumorigenesis has remained a controversial topic, with hair bulge stem cells often suggested to be the main reservoir for tumor-initiating cells [12].

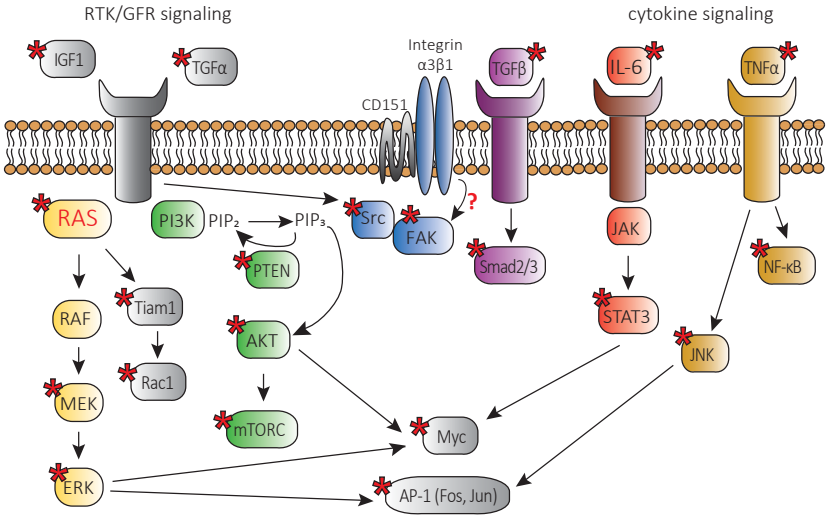

Figure 2: Simplified scheme of the main signaling pathways that have been shown to play a central promoting role in the DMBA/TPA-driven tumor formation.
Asterisk: signaling components have been directly investigated for their *in vivo* functions during skin tumor development using transgenic mouse models. RTK: receptor tyrosine kinase. GFR: growth factor receptor. Adjusted from: [9,10].

Even though our group has demonstrated the indispensable role of integrin α3β1 during the initiation of DMBA/TPA-induced tumorigenesis, the mechanism behind it remains largely speculative and fails to resolve the relation of α3β1 with known key oncogenic signaling pathways of this model. The mice with epidermal deletion of α3β1 exhibited an increased epidermal turnover and the loss of slow-cycling keratinocytes, residing in the hair follicles and in interfollicular epidermis. These observations coincided with the miss-localization of keratinocytes expressing keratin 15, a marker of hair bulge stem cells. Thus, the hair bulge stem cells were suggested to be the main reservoir for tumor-initiating cells in our mouse model and the mechanism behind the absence of tumor formation upon α3β1 deletion was proposed to be their loss by premature efflux and terminal differentiation [6].

HER2-DRIVEN BREAST CANCER

Breast cancer is a heterogeneous disease, which can be categorized into several subtypes. Majority of breast cancers are carcinomas, i.e. they arise from epithelial cells, and can be further divided into at least six distinct "intrinsic" subtypes based on global gene expression analyses: luminal A, luminal B, HER2-enriched, basal-like (i.e. triple-negative) and claudin-low tumors, as well as a normal breast-like group [13]. HER2-enriched subtype is defined by gene amplification and/or overexpression of a member of the epidermal growth factor receptor family, epidermal growth factor receptor 2 (HER2), leading to activation of PI3K/Akt and MAPK/ERK signaling pathways [14]. Roughly 20-25% of breast cancers are classified as HER2-enriched and even though their treatment strategy has come far with several HER2-targeting therapies available, HER2-positive advanced cancer still remains an aggressive disease, associated with a poor prognosis and survival outcome [15].

Consistent with the heterogeneity of the breast cancer, the role of α3β1 in this disease strongly varies depending on the study and/or the disease model (described in depth in chapters 2 and 5). Whereas the pro-survival and pro-proliferative role of α3β1 in basal-like breast cancer types has been quite established [16,17], the role of α3β1 in HER2-enriched mammary cancer remains to be investigated *in vivo*.

SCOPE OF THE THESIS

In this thesis, we aim to shed light on the diverse and often opposing roles of integrin α3β1 in cancer. In **chapter 2**, we provide an overview of current literature on two major laminin-binding integrins in epidermal cells: α3β1 and α6β4, both of which are known to act as promoters and suppressors of tumorigenesis and tumor progression, depending on the cell type and context. We speculate the conditions that define the nature of their role in cancer and we establish the importance of determining their function in well-defined tumor types and cancer stages. In **chapters 3 and 4**, we focus on the role of integrin α3β1 in the first stages of non-melanoma skin cancer using the two-stage chemical carcinogenesis model. In **chapter 3**, we uncover that α3β1 in hair bulge stem cells contributes only moderately to the formation of papillomas and that this contribution is indirect, via the promotion of a tumor permissive environment. We refute the original hypothesis that the dramatic effect of epidermal *Itga3* deletion on tumor formation is due to an efflux of hair bulge stem cells, thus reopening the question of the mechanism behind the essential role of α3β1 in DMBA/TPA-driven tumorigenesis. We address this in **chapter 4**, where we uncover that α3β1 plays a central role in promoting the activation of several pro-tumorigenic signaling pathways and together

with the tetraspanin CD151 regulates signaling molecules that control the survival of differentiating keratinocytes. In **chapter 5**, we extended our research to breast cancer and uncovered 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 environmental factors and reaffirm that this role is specific for HER2-enriched cell-type. 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.

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