Abstract

Cell polarization is crucial during development and tissue homeostasis and is regulated by conserved proteins of the Scribble, Crumbs, and Par complexes. The partitioning defective (Par) complex consists of Par3, Par6 and aPKC. Using a Ras-driven skin tumorigenesis model, our lab recently revealed a dual function of Par3: epidermal Par3 deletion resulted in strongly reduced numbers and growth of papillomas, unraveling pro-oncogenic functions of Par3. However, it predisposed mice to the formation of keratoacanthoma, highlighting also context-dependent tumor-suppressive functions of Par3. Interestingly, besides affecting epithelial tumorigenesis, epidermal Par3 deletion resulted in melanocytic hyperplasia and increased melanomagenesis, indicating that epidermal polarity proteins modulate the cross-talk of keratinocytes (KCs) and melanocytes (MCs).

The aim of this study was to understand how loss of polarity proteins such as Par3 impairs MC homeostasis and metastatic melanoma. By combining *in vitro* coculture systems and *in vivo* mouse melanoma models, the role of epidermal Par3 in heterologous communication between KCs and MCs was investigated. Indeed, in a genetic melanoma mouse model, loss of epidermal Par3 resulted in increased melanoma multiplicity and lung metastasis, demonstrating that epidermal Par3 expression is crucial to control MC homeostasis and suppresses melanomagenesis. Diverse coculture experiments revealed increased MC proliferation, survival and less-differentiated morphology when exposed to Par3 KO KCs as compared to control KCs, phenotypes mainly driven by calcium dependent direct KC-MC communication. Loss of Par3 led to improved P-cadherin stability on surface of KCs and increased P-cadherin enrichment in heterologous KC-MC contacts. Loss of function confirmed that P-cadherin is required to stimulate melanocytic expansion and proliferation upon loss of keratinocytic Par3. Coculture experiments using P-cadherin overexpressing CHO cells and other gain-of function experiments revealed that P-cadherin is sufficient to drive MC hyperplasia, implicating pro-oncogenic functions of abnormal P-cadherin-mediated KC-MC communication in melanoma. Similarly, analysis of human PAR3 and P-Cadherin in skin of melanoma patients uncovered reduced epidermal PAR3 expression in areas adjacent to melanoma, negatively correlating with melanoma progression. P-Cadherin instead robustly localized to heterologous KC-MC adhesions, indicating that P-Cadherin may contribute to KC-MC cross-talk also in the context of human disease. Interestingly, blocking studies of direct cocultures revealed that proper interactions of junctional adhesion molecules (JAMs) might be important in regulating melanocyte homeostasis downstream of Par3.

Par3 complex proteins are also robustly expressed intrinsically in MCs and melanoma, and Par3 co-localized with ZO-1 and N-cadherin at sites of intercellular adhesions. Par3 expression positively correlated with melanoma proliferation rate and aggressiveness, whereas loss of Par3 or aPKCζ/λ led to reduced melanoma cell growth suggesting that intrinsic Par3/aPKC expression promotes melanoma progression. Furthermore, loss of Par3 led to reduced dendricity whereas loss of aPKCζ/λ as well as aPKC kinase inhibition resulted in increased dendricity, demonstrating next to similar pro-oncogenic functions also distinct roles of Par3 and aPKCζ/λ in melanoma cell architecture.

Together, this study unravels yet unknown functions of Par complex proteins in MC homeostasis and melanoma, and therefore highlights that an intact polarity machinery is not only relevant in the context of epithelial pathologies but also a key factor that contributes to growth and progression of non-epithelial malignancies.