



Keratinocytes in Normal Tissue and in Tumours

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The epidermis consists of a multilayered epithelium, the interfollicular epidermis, and associated hair follicles, sweat glands and sebaceous glands. All of the different lineages within the epidermis are maintained through proliferation of stem cells and differentiation of their progeny. By investigating how stem cell renewal and differentiation are controlled in normal tissue, we hope to identify new approaches to preventing and controlling tumours of the epidermis and other stratified squamous epithelia.

Stem cell renewal and lineage selection

One of the key pathways that regulates epidermal lineage selection is the canonical Wnt pathway (Watt and Collins, *CSHL Symposium on Quantitative Biology* 2008; doi:10.1101/sqb.2008.73.011). *In vivo* the level of β -catenin signalling determines whether keratinocytes differentiate into hair follicles or into interfollicular epidermis and sebocytes. β -catenin not only activates transcription via interaction with TCF/Lef transcription factors, but also regulates TCF/Lef independent genes by binding to the vitamin D receptor (VDR). In epidermis lacking the VDR hair follicles degenerate – this is not due to stem cell depletion but is correlated with a cell migration defect (Pálmer et al., *J. Invest.*

Dermatol. 2008; 128:2113). We have recently shown that VDR is a TCF/Lef independent transcriptional effector of the Wnt pathway in adult epidermis and that vitamin D analogues can prevent formation of hair follicle tumours that arise through inappropriate activation of Wnt signalling (Pálmer et al., *PLoS ONE* 2008; 3:e1483).

One of the other signalling pathways that interacts with Wnt to regulate epidermal differentiation is the Notch pathway. We have continued our studies of how Notch regulates differentiation and have begun to explore how Notch and Wnt activation can affect vitamin A responsiveness (Collins and Watt, *Dev. Biol.* 2008; 324:55). Our studies of epidermis lacking the Notch ligand Dll1 indicate a selective role for Dll1 in interfollicular epidermal differentiation (Estrach et al., *J. Invest. Dermatol.* 2008; 128:825). We have also made the observation that Dll1-null epidermis develops spontaneous tumours, consistent with the concept that Notch acts as a tumour suppressor gene in the skin (Watt et al., *Curr. Opin. Cell Biol.* 2008; 20:171).

In parallel with our work on mouse models (Giangreco et al., *Aging Cell* 2008; 7:250) we are performing *in vitro* studies of stem cell behaviour, using primary cultures of human epidermal stem cells (Watt et al., *Nat. Rev. Cancer* 2008; 8:234). We have demonstrated that Akt activation occurs on commitment to terminal differentiation (Janes et al., *Cell Res.* 2008; doi:10.1038/cr.2008.281) and are currently developing methods to visualise Akt activity in living cells. We have found that kazrin, a protein that is involved in desmosomal junction maintenance, also regulates proliferation and terminal differentiation (Sevilla et al., *Dev. Dyn.* 2008; 237:1718; Sevilla et al., *J. Cell Sci.* 2008; 121:3561). This has led us to

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propose a model in which upregulation of kazrin in cells in the epidermal basal layer coordinates initiation of terminal differentiation with the junctional remodelling that is required for migration upwards into the suprabasal layers.

Human epidermal stem cells in culture generally differentiate exclusively into interfollicular epidermis. However we have recently developed an *in vitro* model in which we can examine how human epidermal cells select between the interfollicular and sebocyte lineages at the onset of terminal differentiation (Lo Celso et al., *Stem Cells* 2008; 26:1241). This has already revealed contrasting roles of Myc and β -catenin and will be useful for further studies of how lineage choice is determined.

Cancer models

We have now developed mouse models of a range of different epidermal tumour types, including papillomas, squamous cell carcinomas, trichofolliculomas and sebaceous adenomas (Watt and Collins, *CSHL Symposium on Quantitative Biology* 2008; doi:10.1101/sqb.2008.73.011). Different tumour types are associated with aberrant signalling events. Sustained high level activation of β -catenin has previously been shown to result in formation of trichofolliculomas. However, we have recently found that in the absence of the VDR the tumours that arise in response to β -catenin resemble basal cell carcinomas. We observe high levels of nuclear β -catenin and low levels of VDR in human infiltrative basal cell carcinomas, suggesting that the same signalling pathways are important in mouse and human skin tumours (Pálmer et al., *PLoS ONE* 2008; 3:e1483).

While it is most likely that stem cells drive tumour development in stratified squamous epithelia, nondividing, terminally differentiating cells can profoundly affect the course of the disease (Janes and Watt, *Nat. Rev. Cancer* 2006; 6:175). We are currently investigating the underlying mechanisms and have evidence that both cell-cell adhesion and production of secreted factors are involved. We have generated transgenic mice in which a constitutively activated form of MEK1 is expressed under the control of the involucrin promoter to activate Erk in the suprabasal epidermal layers and found that they develop papillomas at sites of wounding (Hobbs et al., *J. Invest. Dermatol.* 2004; 123:503). We are currently using MEK1 transgenic mice to investigate the role of inflammation in epidermal tumour formation. We are also comparing the stromal compartment of tumours with different subpopulations of fibroblasts in normal skin (Figure 1).

We previously identified a panel of new human epidermal stem cell markers by generating single cell cDNA libraries. We have now gone on to examine expression of the markers in human squamous cell carcinomas (Jensen et al.,

Cancer Lett. 2008; 272:23). We find that co-expression of the markers is lost in the tumours. It appears that tumour cells hijack the homeostatic control pathways that operate in normal stem cells and either maintain or eliminate them, depending on whether or not they confer a growth advantage. We are currently developing xenograft models of human squamous cell carcinomas to enable us to define the tumour stem cell compartment further.

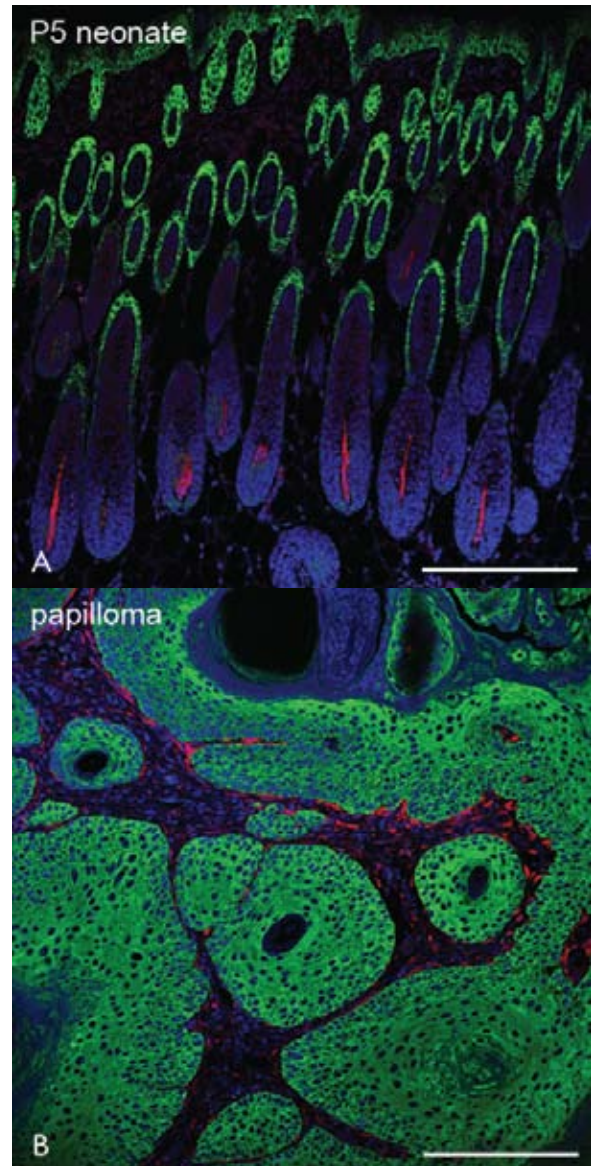


Figure 1. In normal skin (A) the retinoic acid binding protein CRABP1 is expressed in the specialised mesenchymal cells at the base of the hair follicle, known as the dermal papilla. In skin tumours (B) CRABP1 is widely expressed in the stroma. Red: CRABP1; green: keratin 14. See Collins and Watt, *Dev. Biol.* 2008; 324:55.

Scale bars: 200 μ m

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