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## Feather on the Cap of Medicine

Cathleen Chiu<sup>1</sup> and Cheng Ming Chuong<sup>1,2,3,4,\*</sup>

<sup>1</sup>Department of Pathology, University of Southern California, 2011 Zonal Avenue, Los Angeles, California 90033, USA

<sup>2</sup>Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University

<sup>3</sup>Laboratory for Wound Repair and Regeneration, Cheng Kung University, Tainan, Taiwan

<sup>4</sup>Integrative Evolutionary Galliform Genomics Center, Chung-Hsing University. Taichung, Taiwan

### SUMMARY

Through cyclic regeneration, feather stem cells are molded into different shapes under different physiological states. With its distinct morphology, context-dependent growth and experimental manipulability, the feather provides a rich model to study growth control, regeneration and morphogenesis *in vivo*. Recent examples include novel insights revealed by transient perturbation with chemotherapeutic reagents and irradiation during feather growth.

### Keywords

Evo-Devo; regeneration; hormone; tumor environment; animal model; skin

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Researchers utilize testable models to address specific physiological and pathological processes in hopes of extracting clinically applicable information. Physiological complexities contribute to the difficulty of biomedical research and the importance of models that best represent *in vivo* reality. While the mouse is a very useful model offering strength in genetics, other complementary animal models, such as the chicken provide insights into context-dependent growth. For example the chicken permits analysis of spatial variance in region-specific feather morphology and temporal variance in age-dependent feather types (Chen et al., 2014a). Here we show some examples related to tumorigenesis, hormone-dependent growth, and keratin biology.

One classical example highlighting the importance of the *in vivo* model is the context-dependent behavior of Rous sarcoma virus (RSV) transformed cells. RSV was shown to transform chicken embryo fibroblast in culture and can cause sarcomatous tumors when

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\*Corresponding author: Cheng Ming Chuong, M.D., Ph.D., Professor of Pathology, Director, Laboratory of Tissue Development and Regeneration, Chair of Pathology Graduate Committee, University of Southern California, HMR 313B, 2011 Zonal Avenue, LA, CA 90033, TEL 323 442 1296, cmchuong@usc.edu.

### CONFLICT OF INTEREST

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injected into hatched chicks. But, when infected into early chicken embryos, RSV infected cells are incorporated to become part of the normal feather epithelia and mesenchyme (Stoker et al., 1990). RSV Injections into chickens cause tumor formation in the wounded sites. Subsequently, a favorable environment for tumorigenesis was found to be caused by wound induced TGF- $\beta$  expression (Sieweke *et al.* 1990). These studies demonstrate the effect of different micro-environments on the tumorigenic behavior of transformed cells, and highlight new insights that an in vivo model can provide.

A recent unique study addressed a cancer-related clinical issue by perturbing feather growth with chemotherapeutic agents (Xie *et al.* in this issue). Cyclophosphamide (CYP) has been shown to cause chemotherapy-induced alopecia (CIA) in mice and humans. Unfortunately, once hairs are lost, it is difficult to conduct further investigations. Unlike hairs, the regenerating feather suffers only transient disruption. The strengths of the feather model lies in the derivation of its 4-dimensional platform (Fig. 1). Isochronic zones, representing cells generated in the same time period, are aligned along the main axis of the feather (rachis) linearly from the proximal (formed later) to the distal end (formed earlier) (Fig. 1, Chen *et al.* 2014a). At the base of the feather, under the skin surface, is the follicle which contains a ring of stem cells lying a short distance above the dermal papilla. As transiently amplifying (TA) cells supply new cells, more mature cells are displaced distally, where they undergo differentiation and contribute to the formation of barbs (in the lateral and posterior sides). The helical growth of the barb ridges and their later fusion forms the rachis (located in the anterior) as a result of BMP/Noggin, Shh, and Wnt signaling. This occurs along the anterior-posterior axis and provides an observable morphological gradient.

Treating regenerating feather follicles with CYP causes an asymmetric response. CYP disrupted the pattern of the *Shh*-expressing branching epithelium and induced apoptosis but left the rachis unaffected. Branching epithelium exhibited a loss of *Shh* expression. Indeed, down-regulation of *Shh* expression was coincident with the hair loss in mice after chemotherapy. Thus, the feather model made it possible to identify *Shh*-regulated proliferation in distinct epithelial cells were involved in CIA and exposed a possible target for managing chemotherapy-induced tissue damage. Though causality was not established in the loss of *Shh* expression and CIA, the finding is the first step towards further investigating *Shh* signaling in chemotherapy-damaged human hair follicles and identifying the upstream factors responsible for initiating CIA. Interestingly, different follicle regions respond differently to CYP, providing another example of context-dependent responses toward the same growth perturbing agent. The mechanism of this complex growth control in different types of TA cells (rachis versus barb ridges, with different levels of *Shh* expression) remains to be investigated.

In this same vein, the Yue group conducted a parallel study exploring ionizing radiation (IR) and its wide-ranging impact on normal tissue to determine the specific cause of tissue damage (Chen *et al.* 2014b). It is known that IR can induce cytokine expression, which in turn may have a local or general impact. Possible outcomes include inflammation, tissue fibrosis, DNA damage, cell cycle arrest, apoptosis, and perturbation of the stem cell niche, to name a few. Thus, it is important to gain a better understanding of the resulting damage in order to better manage and ameliorate unwanted side effects in patients receiving IR

treatments. The study showed that p53 activation, DNA damage and repair, cell cycle arrest, and apoptosis were activated in a dose-dependent response to IR exposure (Chen *et al.* 2014b). The accessibility of the feather model allowed them to deliver agarose beads coated in IL-1, IFN-gamma and TNF-alpha to further evaluate the contribution of cytokines to the observed disruption of feather branching morphogenesis. They found that the Jak/Stat1 pathway is important in transducing the cytokine signal and, thus, identified a target for rescuing the IR-induced defect. This finding may contribute to new clinical strategies to reduce IR-induced tissue damage.

The feather model can be used to address physiological regeneration and integument regional specificity in a hormonal-context dependent morphogenesis (Chuong *et al.*, 2012). Indeed, feathers and mammary glands are both skin appendages that undergo sex hormonemodulated cyclic activation of progenitors. The glandular secretory mammary organ develops by invaginating into the dermis and form branches. The feather, on the other hand, evaginates and branches out of the body surface to build sexually dimorphic structures (Chuong *et al.* 2014). While mammary and prostate cancers represent hormone-regulated abnormal growth, feathers represent regulated new growth (Mayer *et al.* 2004). By exploring the fundamental mechanism of hormone-dependent regulated new growth, we may learn new strategies to control hormone-dependent abnormal growth.

Hormones also induce abnormal hair growth in humans. Disorders such as androgenic alopecia and hirsutism are examples. How the same androgen can lead to opposite effects in hairs from different regions remains a mystery. The mouse is not an ideal model to investigate this issue. Unlike humans and birds, mice sense their environment more by olfactory rather than visual stimuli, which may explain why they show few clearly visible sexually dimorphic characteristics. In contrast, birds show easily recognizable sexually-dimorphic morphology. This dimorphism is regulated at several different levels in the hormone metabolic pathways. For example, henny feathering is associated with increased aromatase activity in the skin, which converts androgen to estrogen (George & Wilson 1980). Thus, the feather organ provides an *in vivo* model to study the biological principles of sex hormone-dependent appendage growth to help us understand the mechanism(s) that controls the distribution and growth of human hairs.

Many chicken variants are bred by domesticated bird enthusiasts for their unique appearance (Chen *et al.*, 2014a). They show exceptional feather distributions, morphologies and colors. The feathers of the Frizzled chicken possess an altered rachis causing all contour feathers to curl outward and upward. The cause of this curvature was found to be due to a mutation in KRT75, an alpha-keratin (Ng *et al.* 2012). It turns out that KRT75 is a major component of the rachis and the curved feather is due to defective rachis of this breed. KRT75 mutant mice showed a similar defect in hair filament integrity (Chen *et al.*, 2008). KRT75 defects also underlie the cause of pseudofolliculitis barbae in humans (Winter *et al.*, 2004). The avian study into Frizzled chickens led to new understanding of keratin biology and appendage mechanics. Genomic organization study showed avian  $\alpha$ -keratins diversify less than mammalian  $\alpha$ -keratins, probably due to the evolution of  $\beta$ -keratins. With  $\alpha$  and  $\beta$ -keratins, keratinocytes can build more complex architectures. It seems likely that other integumentary

variants will also lead to more new insights in molecular pathways involved in different aspects of skin appendage morphogenesis.

Birds in the wild also have many unexpected features. An interesting recent example identified that birds in the *Pitohui* and *Ifrita* genus possess the ability to carry neurotoxins in their skin and feathers (Menon & Dumbacher 2014). These birds accumulate the neurotoxin in their skin and feathers by ingesting neurotoxin-containing insects, similar to poison dart frogs. In addition, the epidermis of these birds is permeable, which facilitates the sequestration of the toxins.

With just a few examples shown here, we can appreciate that the feather model offers a great complement to our pursuit of knowledge in biomedical research. The evolution and diversification of feathers make it a multi-disciplinary model (Xu et al., 2014) that can inspire many novel bio-mimetic approaches.

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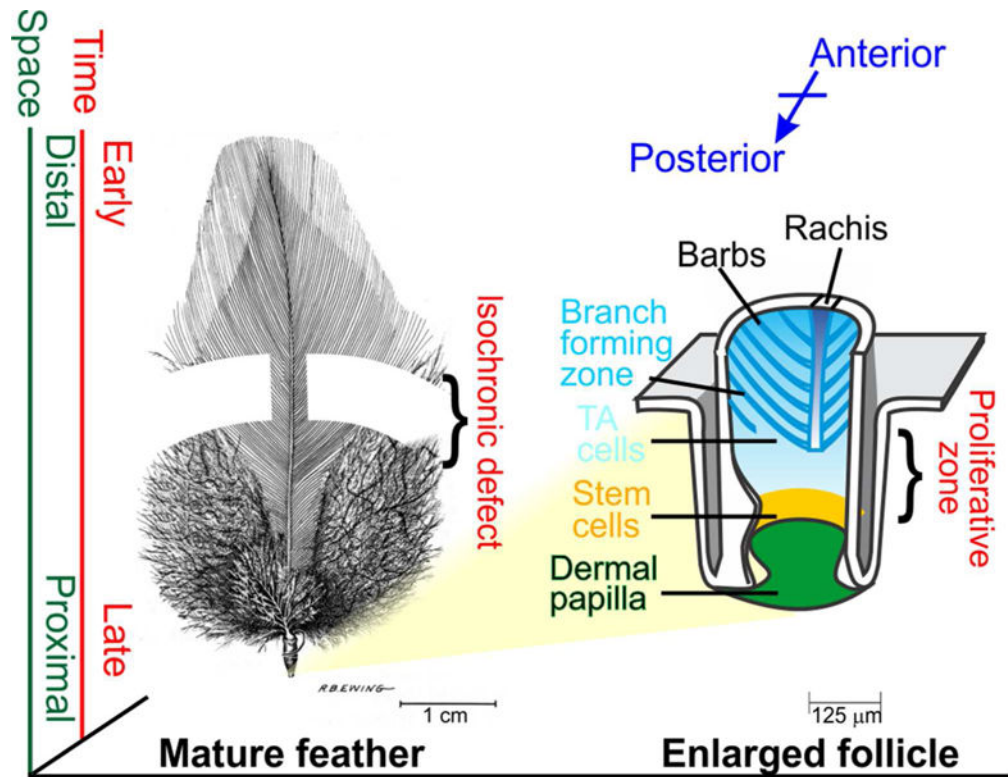
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**CLINICAL RELEVANCE**

- Feathers provide a four dimensional platform to evaluate the effect of chemotherapeutic agents and irradiation, aiming to deter tumor growth while preserving normal growth.
- The feather offers a model for context –dependent new growth, to analyze how appendage progenitors respond under different hormone status, with implications for hormone-dependent tumorigenesis, androgenic alopecia, hirsutism, etc.
- Opening doors to bio-inspired designs for a wide range of issues.



**Fig. 1. The temporal-spatial axis of a mature feather and the enlarged follicle in growth phase**  
 The proximal-distal growth axis is generated by stem cells that supply transiently amplifying (TA) cells at the proximal end that differentiate as they travel distally. Hence younger cells are at the proximal end and older cells are at the distal end. The proximal region of the mature feather is enlarged in the cartoon on the right (about 50×, light yellow shade). Regions within the proximal feather are indicated. An anterior-posterior axis is generated by the helical growth of the barb ridges and their fusion to form a rachis. Transient disruption by growth-perturbing chemotherapeutic agents or irradiation leads to a segments of phenotypic abnormalities with regions proximal and distal to it showing phenotypic normalcy and a dose-responsive interface. Isochronic zone represents the feather region made by keratinocytes “born” in the same time period. In this case, they are all subject to suppression of chemotherapeutic agents and therefore a segment of defective structure. Rachis (anterior) and barb (posterior) regions of the feather filament exhibit differential response to chemotherapeutic agents, thus providing a four dimensional platform for analyzing the effect of growth perturbing agents. Left panel is modified from Lucas and Stettenheim, 1972, Avian Anatomy-Integument.