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Article

Yap Tunes Airway Epithelial Size and Architecture by Regulating the Identity, Maintenance, and Self-Renewal of Stem Cells

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SUMMARY

Our understanding of how stem cells are regulated to maintain appropriate tissue size and architecture is incomplete. We show that Yap (Yes-associated protein 1) is required for the actual maintenance of an adult mammalian stem cell. Without Yap, adult airway basal stem cells are lost through their unrestrained differentiation, resulting in the simplification of a pseudostratified epithelium into a columnar one. Conversely, Yap overexpression increases stem cell self-renewal and blocks terminal differentiation, resulting in epithelial hyperplasia and stratification. Yap overexpression in differentiated secretory cells causes them to partially reprogram and adopt a stem cell-like identity. In contrast, *Yap* knockdown prevents the dedifferentiation of secretory cells into stem cells. We then show that Yap functionally interacts with p63, the cardinal transcription factor associated with myriad epithelial basal stem cells. In aggregate, we show that Yap regulates all of the cardinal behaviors of airway epithelial stem cells and determines epithelial architecture.

INTRODUCTION

How adult tissues maintain their proper size and architecture is poorly understood. Here we explore how the regulation of adult stem cells is linked to epithelial architecture using the airway epithelium as a model system. Epithelial tissues are generally classified as simple, pseudostratified, or stratified. The murine tracheobronchial airway epithelium represents a model of pseudostratified epithelium intermediate between a simple single-layered epithelium and a multilayered stratified epithelium. Airway basal stem cells directly and broadly abut the basement membrane. In contrast, differentiated suprabasal secretory and ciliated cells have smaller zones of contact with the basement membrane and possess extensive luminal surfaces with their nuclei displaced toward the lumen. This arrangement of cells essentially creates a two-layered epithelium (Morrisey and Hogan, 2010; Rock et al., 2009). Theoretically, disturbances in the regulation of basal stem cells could, on the one hand, lead to a hypertrophic epithelium characterized by basal stem cell excess and stratified squamous metaplasia, as is frequently observed in conditions such as chronic obstructive pulmonary disease. Conversely, decreased stem cell numbers would be predicted to result in epithelial hypoplasia, which is thought to play a role in conditions such as bronchiolitis obliterans and airway fibrosis (O'Koren et al., 2013; Rock et al., 2010). Thus, tightly controlled mechanisms to regulate basal stem cell maintenance, proliferation, and differentiation must exist to properly police epithelial size and architecture.

Yap (Yes-associated protein 1) is a transcriptional coactivator in the conserved Hippo kinase cascade that has been shown to be involved in growth control as well as the regulation of stem and progenitors cells (Barry and Camargo, 2013; Halder and Johnson, 2011; Pan, 2007, 2010; Ramos and Camargo, 2012; Zhao et al., 2011). In epithelia, Yap modulation has diverse consequences on stem and progenitor cell behaviors (Ramos and Camargo, 2012; Zhao et al., 2011). In the embryonic neuroepithelium, Yap loss leads to decreased progenitor cell survival (Cao et al., 2008), whereas in the embryonic epidermis, Yap loss leads to decreased progenitor cell proliferation (Schlegelmilch et al., 2011). In contrast, Yap activation leads to the same phenotype in both of these tissues, namely increased progenitor and stem cell replication (Cao et al., 2008; Schlegelmilch et al., 2011; Zhang et al., 2011). Unexpectedly, Yap loss throughout the intestinal epithelium results in no obvious phenotype but causes hyperplasia and increased stem cell replication after injury (Barry et al., 2013). Surprisingly, Yap overexpression leads to a loss rather than a gain of intestinal stem cells (Barry et al., 2013). Thus, Yap acts in a tissue-, cell-, and context-dependent manner, even within epithelia.

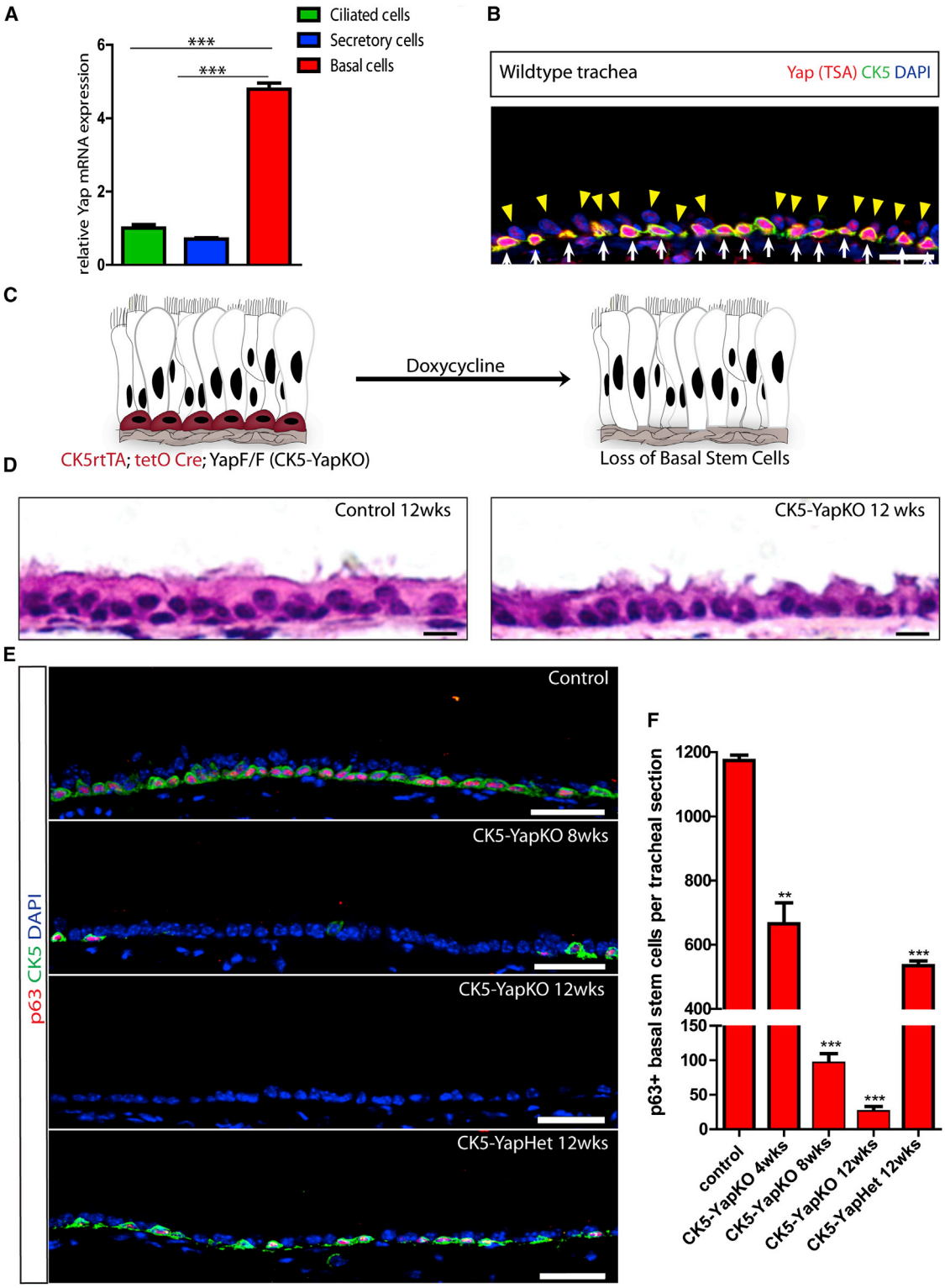

Figure 1. Yap Is Required for the Maintenance of Adult Airway Basal Stem Cells and *Yap* Loss Results in the Simplification of a Pseudostratified Epithelium into a Columnar Epithelium

(A) Expression of *Yap* mRNA in basal and secretory cells relative to that in ciliated cells.

(B) Immunostaining for Yap (red) and the basal stem cell marker cytokeratin 5 (CK5, green). Yap protein is highly enriched in the nuclei of basal stem cells (white arrows) as compared to differentiated cells (yellow arrowheads).

(C) A schematic of the strategy and phenotypic outcome of stem cell-specific *Yap* deletion.

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