VIP Pipes Up: Neuronal Signals Direct Tubulogenesis

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Biological tubes serve as the body's plumbing system, transporting fluids and gases throughout secretory, circulatory, and respiratory organs. In this issue of *Developmental Cell*, Nedvetsky et al. (2014) find that vasoactive intestinal peptide (VIP), secreted by parasympathetic nerves, is a surprising player in directing epithelial tubulogenesis in salivary glands.

All peripheral organs and tissues are innervated by the autonomic nervous system, which sends signals to regulate organ homeostasis. In adult organs, the sympathetic and parasympathetic branches of the autonomic nervous system control diverse physiological processes, including circulation, respiration, body temperature, digestion, and metabolism. The onset of peripheral innervation is coincident with embryonic stages of growth, differentiation, and maturation of innervated organs, yet little is known about the functional contribution of innervation to organogenesis. The emerging view that dysfunction of neuronal elements in peripheral organs may instigate pathogenesis of disease, including diabetes and heart failure (Hasan, 2013; Saravia and Homo-Delarche, 2003), underscores the need to understand the relevance of these contributions. In this issue of *Developmental Cell*, Nedvetsky et al. (2014) provide new insight into the role of innervation in organogenesis.

Tubes are the basic building blocks of a number of organs including the lungs, vascular system, kidneys and exocrine glands. They are the biological pipes that carry vital gases, nutrients, hormones, and waste. Epithelial tubes are established by diverse cellular mechanisms that include budding, wrapping, folding, and invagination of polarized sheets of cells to create hollow lumens (Andrew and Ewald, 2010). After tube formation, lumen size is controlled by changes in cell number, shape, and physical forces of luminal pressure and flow. Epithelial tubes are also surrounded by a stromal compartment that includes mesenchymal cells, endothelial cells, and nerves. However, the role of the neighboring vasculature and nerves in the establishment of functional interconnected tubular networks has received little attention. Nedvetsky et al. (2014) provide evidence that parasympathetic nerves instruct tubulogenesis in embryonic mouse submandibular glands (SMG) and define the neuropeptide vasoactive intestinal peptide (VIP) as a master regulator of lumen formation and expansion.

Explants of embryonic mouse SMG provide a powerful and physiologically relevant model system to study organogenesis in culture while preserving intimate contacts with blood vessels and nerves (Patel et al., 2006). In vivo, the SMG appears as rudimentary epithelial buds at midgestation that then undergo branching to form stalks and terminal buds, generating microlumens that are later joined into a continuous ductal passage. Finally, lumenal expansion allows for passage of liquids through the mature duct. SMG morphogenesis occurs in tandem with the appearance of nerves that originate locally from parasympathetic ganglia and are directed to terminal buds by the epithelium-derived neurotrophic factor, neurturin.

To examine the role of the autonomic nervous system in tubulogenesis, Nedvetsky et al. (2014) used a neurturin function-blocking antibody to deplete innervation and observed that lumen formation is disrupted in explanted SMGs. Similar results were observed in salivary glands in vivo in mice lacking peripheral nerves. The authors initially focused on the parasympathetic neurotransmitter acetylcholine as the potential nerve-derived signal regulating SMG lumen formation. In an earlier study, Knox and colleagues had found that cholinergic signaling through muscarinic (M1) receptors maintains a multipotent progenitor pool during SMG branching (Knox et al., 2010). However, in their current work, the authors observed that ductal formation is not impaired by pharmacological inhibition of muscarinic receptor signaling, despite salivary gland hypoplasia due to a drastic reduction in resident progenitor cells. Thus, the authors reasoned that other neuronal signals must instruct SMG tubulogenesis. They therefore mined an available gene expression data set generated from developing salivary glands, leading to the identification of the neuropeptide VIP as a candidate regulatory factor. VIP is a cotransmitter in the mature autonomic nervous system with well-characterized effects on immune function and release of hormones and catecholamines, although VIP's functions during organogenesis are less clear. Nedvetsky and colleagues found that expression of the VIP receptor (VIPR) is coincident with lumen formation and is restricted to the SMG epithelium. Importantly, a VIPR peptide antagonist disrupted lumen formation, whereas exogenous VIP was sufficient to promote ductal growth in rudimentary SMG explants in the absence of nerves. VIP also functions at later stages in lumen formation, inducing coalescence of microlumens into one continuous duct and rapidly promoting an expansion of luminal cavities, as revealed by live imaging. Finally, exogenous VIP elicited dilation of existing primary duct lumens in SMG explants, implying distinct regulatory effects of VIP on lumen formation and expansion.

How does VIP control the diverse morphogenetic events of lumen initiation, fusion, and expansion? The authors

demonstrate that cyclic AMP (cAMP)/protein kinase A (PKA) signaling acts downstream of VIP and is a shared regulatory mechanism in these processes. A membrane-permeable cAMP analog recapitulated all the effects of VIP, whereas pharmacological inhibition of PKA activity abrogated VIP-dependent lumen formation and expansion. However, the downstream pathways that elicit distinct functional outcomes remain undefined. While the authors show that VIP activates the transcription factor CREB in salivary epithelia, likely promoting the mitogenic events needed for lumen growth, VIP-stimulated luminal expansion occurs on a timescale of 10 min, seemingly precluding transcriptional regulation. Additionally, although apoptosis has been considered a primary mechanism underlying the creation of a hollow lumen (Andrew and Ewald, 2010), the authors observed no evidence of cell death in VIP-treated ducts, and tubulogenesis proceeds normally in mice lacking the proapoptotic factor Bax. Interestingly, the authors provide compelling evidence that cystic fibrosis transmembrane conductance regulator (CFTR) acts downstream of VIP to promote lumen expansion. Lumen expansion is known to rely on chloride ion transport mediated by CFTR. The authors demonstrated that CFTR is highly expressed in SMG ducts during lumenogenesis and that pharmacological inhibition of CFTR effectively blocked VIP-dependent lumen expansion without affecting formation of a contiguous lumen. These results place CFTR downstream of VIP in mediating lumen expansion, but not lumen formation.

After decades of classical studies documenting neurotrophic support of peripheral neurons by their targets, this study adds to emerging evidence showing that neuronal signals reciprocally contribute to organogenesis (Borden et al., 2013; Knox et al., 2010). Intriguingly, neurotransmitters previously thought to only modulate adult organ homeostasis are now being unveiled as instructive cues during organ development. However, several key questions remain. How do nerves and target tissues coordinate the precise timing of their developmental interactions? More broadly, as other epithelial tissues such as pancreas and kidneys innervated by VIP-positive fibers also undergo lumen formation (Kesavan et al., 2009; Yang et al., 2013), is innervation a general regulatory mechanism during tubulation? Additionally, can developmental cues be harnessed to drive adult tissue regeneration or lumen formation in transplanted organs? In support of this idea, restoring innervation promotes regeneration of salivary glands damaged by radiation therapy in head and neck cancers (Knox et al., 2013).

Luminal defects are also associated with several human conditions such as polycystic kidney diseases, hypertension, and epithelial cancers (Andrew and Ewald, 2010). An important question, therefore, is whether altered autonomic innervation may instigate the aberrant tubular architecture associated with these pathological conditions. Altogether, the results reported by Nedvetsky et al., in combination with other recent studies, suggest that the autonomic nervous system not only contributes to tissue homeostasis as classically viewed but also plays a significant role in tissue organogenesis, regeneration, and possibly disease pathogenesis.

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