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⌬ Hedgehog and WNT Signaling Hubs in Tracheal Morphogenesis

The trachea provides a direct conduit from the respiratory system to
the external environment. In mammals, the trachea is surrounded by
cartilaginous rings that perform several duties, including providing
structural support and keeping the trachea patent. The development of
the trachea has been explored using genetic mouse models that reveal
the importance of multiple paracrine signaling pathways, such as the
SHH (sonic hedgehog) and WNT (Wingless-related integration site)
pathways (1–4). In humans, tracheal defects are common in the
pediatric patient population and include defects such as
tracheomalacia and the rarer disease, complete tracheal ring
deformity (CTRD) (5, 6). In CTRD, the cartilaginous rings
surrounding the trachea are complete and lack a dorsal gap filled in
with the trachealis muscle. As with other orphan diseases, the
pathogenic mechanisms of CTRD have eluded investigators given the
paucity of tissue and representative animal models of these diseases.
As such, it is frequently unclear whether disease-causing mutations in
humans follow molecular pathways similar to those discovered in
genetic mouse models. In this issue of the *Journal*, Sinner and
colleagues (pp. 1267–1281) uncover multiple human genetic lesions
that lead to CTRD, including mutations in genes within the SHH
pathway and mutations in multiple genes within the WNT pathway,
including *ROR2* (receptor tyrosine kinase–like orphan receptor 2) (7)
(Figure 1). This study highlights the close relationship between mouse
models of respiratory development and human congenital tracheal
abnormalities, and it provides new target genes and pathways that
play an important role in tracheal development in humans.

The trachea initially develops from the anterior foregut.
Subsequently, mesenchymal–epithelial cross-talk sets up a
ventral–dorsal gradient of WNT and BMP (bone morphogenetic
protein) signaling to promote the proper specification, patterning,
and separation of the trachea and esophagus, respectively (1, 2, 8,
9). Along with WNT and BMP signaling, SHH signaling has been
show to play important roles in separation of the trachea and
esophagus, as well as in promoting proper differentiation of
mesenchymal derivatives in the trachea and other regions within
the respiratory system (3, 4, 10). After separation occurs, the
cartilaginous progenitors within the trachea are denoted by
expression of the transcription factor SOX9, which is both a marker
and functional regulator of cartilage development (11). Defects in
the development of these progenitors can lead to failure to form the
tracheal cartilaginous rings surrounding the trachea. Sinner and
colleagues performed a trio analysis with whole-exome sequencing
of patients with CTRD along with their parents to identify novel
mutations (7). Remarkably, many of the mutations implicated
either the WNT or SHH pathway. These included both heritable
and spontaneous mutations. The mutations in the WNT pathway,
including *ROR2* and *LRRC7* (leucine rich repeat containing 7),
involve both β-catenin–dependent (*LRRC7*) and β-catenin–
independent (*ROR2*) pathways. *De novo* mutations were found
in *SHH*, and compound heterozygous mutations were observed
in *HSPG2*, an extracellular heparin sulfate proteoglycan that is
known to modulate SHH and other paracrine pathways (12).

Previous studies in mice have demonstrated important roles for
WNT and SHH in the development of multiple tissues within the
respiratory system, including the trachea. Loss of SHH also leads to
failure of tracheal cartilage formation (10). As SHH is expressed
exclusively within the developing respiratory endoderm, this
suggests that SHH acts in a paracrine manner to drive cartilage
formation, possibly by regulating the differentiation of SOX9$^+$
progenitors. This is consistent with the authors' finding of
heterozygous gene variants in the downstream transcriptional
effectors of SHH signaling (*GLI1* [GLI family zinc finger 1], *GLI2*,
and *GLI3*) in several patients with CTRD. The role of WNT
signaling in tracheal cartilage formation was directly tested by the
authors through gene deletion of the essential component of WNT
ligand secretion, *Wls* (Wntless). Loss of *Wls* in the developing
respiratory endoderm resulted in loss of tracheal cartilage
formation and a reduction in SOX9$^+$ cartilage progenitors. This
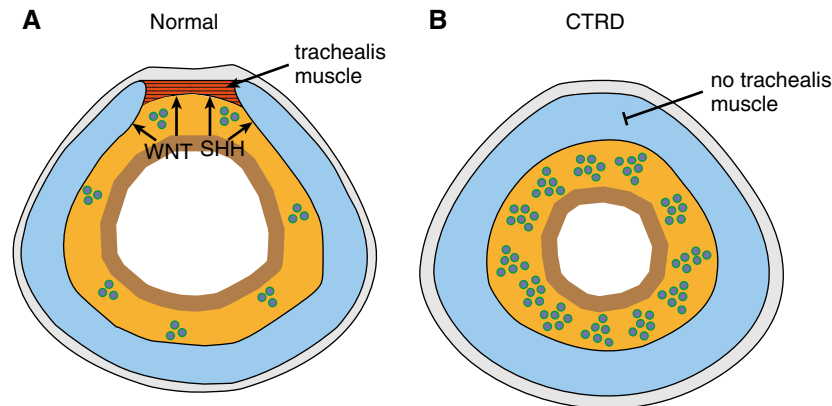correlated with a general loss of WNT signaling throughout the

Figure 1. Disruption in SHH or WNT signaling leads to complete tracheal ring deformity. (*A*) Normal tracheal development results in incomplete cartilage rings surrounding the trachea with the trachealis muscle on the dorsal side. (*B*) Complete tracheal ring deformity (CTRD) resulting from mutations in genes within the WNT and SHH pathways generates complete cartilage rings and an absence of the trachealis muscle.

lung, as noted by decreased expression of the WNT target gene *Axin2*, and decreased WNT reporter activity. Although the authors found that multiple aspects of WNT signaling were disrupted, including downregulation of the ligand *Wnt5a*, there are additional important ligands expressed in the developing lung endoderm (e.g., *Wnt7b*) that may contribute to the phenotype (13). The finding that loss of *Wls* led to the almost complete loss of cartilage formation in the trachea as compared with mutations in human *WLS* exhibiting complete cartilaginous ring phenotype, indicates differences either in the role of WNT signaling in human tracheal development or differences in how the human mutations affect WNT signaling versus a complete loss due to deletion of *Wls*.

One of the more interesting findings of this study is that many of the patients with CTRD also had associated cardiac defects, including defects in pulmonary artery development. Both the heart and lung develop in a coordinated fashion to establish proper functioning of the cardiopulmonary system. Previous work has demonstrated a common cardiopulmonary progenitor (CPP) that can generate mesenchymal derivatives in both the developing lungs and heart (14). CPPs express the WNT2 ligand and their function is regulated by SHH activity. It is tempting to speculate that the defects in SHH and WNT signaling that lead to CTRD may involve defective CPP development in humans.

Although there are many signaling pathways that play critical roles in embryonic morphogenesis, some pathways, such as the WNT and SHH signaling pathways, appear to play a central role in many tissues, indicating that they act as hubs of cell–cell interactions during tissue development. Moreover, both the WNT and SHH signaling pathways are active during normal adult respiratory homeostasis and play important roles in repair and regeneration of multiple cell types in the respiratory tract (15, 16). Such a reiterative use of a core set of signaling pathways throughout the lifespan of mammals underscores the impact these pathways have on both genome stability and the inherent plasticity that is necessary to rapidly engage specific progenitor cells in the adult after injury.

The findings in this report will help advance our understanding of both the genetic causes of tracheal defects in humans and the cell–cell interactions that are important for mesenchymal development in the airways. Such interactions have become a hallmark of organ development as well as repair and regeneration in the respiratory

tract and other tissues. Providing valuable human correlates in this area of research will both support and enhance model organism studies, which remain a necessary workhorse of mechanistic science. ∎

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Should We Tip Our CAPs to Statins?

Community-acquired pneumonia (CAP) exerts a high burden of mortality and morbidity in older patients, even when antibiotics covering the most likely pathogen(s) are appropriately prescribed. There is a pressing need to improve outcomes for this patient population. The intriguing article by Sapey and colleagues (pp. 1282–1293) in this issue of the *Journal* provides cautious optimism in this setting (1).

On the basis of the pivotal role of neutrophils in the pathogenesis of CAP, and armed with preliminary data demonstrating that statins reverse aberrant function in neutrophils from older patients with CAP, the group performed an exploratory, single-center, randomized placebo-controlled trial of simvastatin (80 mg once daily for 7 d) in 62 older patients with CAP and sepsis who were managed outside the ICU.

The authors should be commended for focusing on a patient group with real unmet clinical need, which is understudied (because such research is so challenging). They have produced tantalizing data suggesting statins can beneficially modulate key neutrophil functions associated with CAP, such as impaired chemotaxis, extracellular release of human neutrophil elastase, and the generation of neutrophil extracellular traps (NETs) in response to *in vitro* stimulation. Within the rigor of a randomized controlled trial, these consistent signals strongly suggest the observed effects of statins on neutrophil function are "real."

However, perhaps the most fascinating and clinically relevant data pertain to the significant reduction in Sequential Organ Failure Assessment score and "hospitalization-free survival" associated with simvastatin. The improvement in both hints that the (nonsignificant) reduction in mortality in the statin group deserves

further study. This is supported by the acceptable safety profile, and particularly the suggestion that concomitant use of macrolides that inhibit cytochrome P450 3A4 (CYP3A4) was well tolerated.

If statins genuinely improve outcomes in older patients with CAP, are the changes in neutrophil function responsible or do they reflect improvement generated through another mechanism(s)? Although biologically plausible, there is insufficient evidence that neutrophils mediate the improvement associated with statins. The effects on neutrophil functions observed were small, and their clinical relevance is far from certain. Whether effects of statins are maintained when circulating neutrophils adhere to vascular endothelium or extravasate to enter the alveoli remains unknown. Data from other lung conditions suggest markedly different phenotypes for circulating and alveolar neutrophils (2). If an immunomodulatory effect of statins is responsible, we must keep in mind that statins influence other immune cells, particularly lymphocytes (3), and these were not studied by Sapey and colleagues (1).

A more likely explanation might lie within cardiovascular effects of statins. CAP is associated with excess major cardiovascular events, both in the short and medium term (4, 5). Periprocedural use of statins for cardiac interventions has yielded varying results (6, 7). An undefined number of patients in the trial by Sapey and colleagues (1) were taking maintenance, lower-dose statins, and statin "reloading" has been associated with beneficial outcomes after percutaneous coronary intervention (8). However, whether prior use of statins has a beneficial effect on outcomes after CAP remains contentious (9, 10), and acute use of statins for acute coronary syndrome (analogous to application in the trial considered here) does not improve outcomes (11).

The possibility remains that subtle effects on vascular tone and perfusion in key tissue beds underlie potential benefits. Statins have various positive effects on endothelial function, particularly around nitric oxide bioavailability (12, 13).

Whatever the mechanism(s) by which simvastatin appears to have improved outcomes in the study, further trials to validate these