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Epithelial Wnt Ligands Regulate Pulmonary Vasculature Development

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Commentary on

1. Cornett B, Snowball J, Varisco BM, Lang R, Whitsett J, et al. (2013) Wntless is required for peripheral lung differentiation and pulmonary vascular development. Dev Biol 379: 38-52.
2. Jiang M, Ku WY, Fu J, Offermanns S, Hsu W, et al. (2013) Gpr177 regulates 9 pulmonary vasculature development. Development 140: 3589-3594.

Two recently published papers demonstrated the Wntless gene's critical role in the pulmonary epithelium; the gene functions in the upstream Wnt signaling cassette to dictate Wnt ligand production [1,2]. The phenotypes of the Wntless conditional knockout animals are dramatic; the result is interesting, and it provides useful information on Wnt source in the pulmonary system.

Wnt signaling plays important roles in the development of many organs. Wnt ligands/proteins (such as Wnt7b) bind to the corresponding receptor (Frizzled) and either of the co-receptors (LRP5/6) to activate the "canonical" Wnt signaling pathway, which stabilizes the central molecule β-Catenin to activate downstream targets. Some Wnt ligands (such as Wnt5a) bind to other receptors to activate the "non-canonical" Wnt signaling pathway, which is β-Catenin-independent and activates Wnt/Ca^{2+} or the JNK pathway. However, the fact there are 19 Wnt ligands with complementary functions complicates the investigation on Wnt upstream signal transduction.

Wntless (also known as EVI/GPR177/Sprinter) is a critical chaperone protein required for all Wnt secretion except WntD in Drosophila. By deleting the Wntless gene, different groups have made seminal findings on Wnt secretion in embryogenesis, eyes, skeleton, teeth, hair, and even cancer [3-8]. So far scientists have found that Wntless plays a role only in supporting Wnt secretion. Additional work using Porcupine - a gene that is involved in the lipid modification of Wnt proteins and functions upstream of Wntless for Wnt ligand production - or Porcupine chemical antagonists (such as IWP or C59) will be helpful to confirm Wntless's function in Wnt secretion in a specific tissue or cell type [9,10].

In lungs, Wnt ligands regulate the cell proliferation, proximodistal patterning, and branching morphogenesis [11-14]. According to the literature, two major Wnt ligands from the pulmonary epithelium--Wnt7b and Wnt5--have been proposed to affect lung development. Wnt7b and Wnt5a most highly express in the distal lung bud tip, which has the highest proliferation rate in the embryonic lung. Notably the lung mesenchyme was also Wnt5a positively stained in E12.5 embryos [15]. However other Wnt ligands playing compensatory roles in regulating cell differentiation and morphogenesis were also speculated.

To further decipher the complex interplays among epithelium, mesenchyme and endothelium during lung morphogenesis, these two groups generated Wntless conditional knockout animal models to block the production of all 19 Wnt ligands specifically from the epithelium or the mesenchyme. Basically both groups found that blocking Wnt secretion from epithelium (Shh-cre driven), but not from mesenchyme (Dermo1-cre driven), caused severe defects in pulmonary vasculature development. The mesenchyme-specific Wntless deletion embryos did not survive beyond birth (died at E14.5 in one study, and E15.5 to E17.5 in another); and neither group found any noted phenotype in this strain. A sophisticated study showed that Wnt/β-catenin signaling in intestinal development varied in location and intensity significantly throughout developmental stages [16,17]. This phenomenon may be translatable to other tissues including the lung [18]. So we might have missed some phenotypic changes later on in the mesenchyme-specific Wntless-deficient lungs. However other possibilities remain that could lead to no 16 lung phenotypic change in Dermo1-Cre-driven wls deletion.

Nonetheless, the Shh-Cre-driven Wntless deletion in epithelium caused perinatal death, and showed significant phenotypes before this point. Both studies claimed that the mutant (Shh-Cre) lung hemorrhage 20 phenotype was more severe than Wnt7b-null mutants [19,20], which suggests other Wnt ligands compensated Wnt7b deletion. The epithelium-specific Wntless deficient lungs had impaired differentiation of distal epithelial cells, which led to lung hypoplasia. However both groups found that the proximal to distal patterning at the early stage (E14.5) was normal, which is in contrast to the finding that β-Catenin conditional deletion with the same promoter (shh-Cre) caused absence of trachea and lung [21]. Considering the fact that Wnt ligands are short-distance effectors, we can speculate that mesenchyme-derived Wnts could compensate Wnt abrogation in the epithelium of the Shh-cre-driven Wntless knockout lungs during early stages but not later. In our laboratory we also found that conditionally knocking out Wntless in osteoblasts did not affect bone development until one month of age [6]. Interestingly, blocking epithelial Wnts caused the mesenchymal (but not epithelial) cells proliferated slower in E14.5 lung, which indicates that mesenchyme depends on Wnts from epithelium early on. This is in line with the previous finding that Wnt7b (exclusively expressing in epithelium in the lung) regulates lung vascular smooth muscle integrity through the canonical Wnt signaling pathway [22]. The sensitivities of different tissues to Wnt ligand concentration or Wntless expression may vary. Most of the published data suggest only homozygous Wntless deletion could cause phenotypic change. However heterozygously deleting Wntless in myeloid cells had similar phenotypes with homozygotes in eyes [4]. Which Wnt ligand(s) are involved in pulmonary vasculature development and how the imbalanced regulation functions in epithelial-mesenchymal interaction requires more elucidation.

Finally, both groups tried to look into the molecular mechanism underlining the phenotypes. Corneet et al. showed data that up-regulating non-canonical Wnt signaling could partly rescue endothelial markers in Wntless-deficient lung explants [1]; and Jiang et al. argued that canonical Wnt signaling plays a role in maintaining vascular 1 smooth muscle cells through Klf2 [2]. Since Wntless regulates both canonical and non-canonical Wnt ligands, one would expect both Wnt signaling pathways (canonical and non-canonical) might contribute to the process and multiple Wnt ligands are involved in the fine tuning. There are complex interactions between canonical Wnts and non-canonical Wnts as well. It was proposed that canonical and noncanonical Wnts use a common mechanism to activate completely unrelated coreceptors [23]. Non-canonical Wnt5a could even activate canonical Wnt signaling at the presence of Frizzled4 (Fz4) and Lrp5 (not Lrp6), which is similar to how Norrin (another Wnt-like protein/ligand) stabilizes β-catenin, and so does non-canonical Wnt11 [24-26]. There is an interesting explanation of non-canonical Wnts inducing canonical Wnt signaling: multiple Wnts might act in a combination [27,28]. In fact, the canonical Wnt, Wnt7b was also shown to cooperate with Wnt2 to promote foregut organogenesis [29]. All these complex interactions between Wnt ligands might lead to different observations, in some cases contradictory. For example, morpholino knockdown of β-Catenin resulted in enhanced epithelial branching, while a Wnt antagonist (DKK1) treatment resulted in suppressed branching. The addition of Wnt3a conditioned medium or LiCl strongly repressed growth and proliferation of the lung and lacrimal gland [11,14]. A couple of recent studies using mesenchymal stem cell (MSCs) to treat acute lung injury also showed contradictory results, in terms of canonical Wnt signaling contribution. One claimed that Wnt inhibition encouraged MSCs engraft in vivo, another study showed Wnt activation actually increased MSCs' function [30,31]. So more work is required to further demonstrate the roles of Wnt ligands during pulmonary vasculature and other organ development.

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