

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 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.

References

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 pulmonary vasculature development. *Development* 140: 3589-3594.
3. Fu J, Jiang M, Mirando AJ, Yu HM, Hsu W (2009) Reciprocal regulation of Wnt and Gpr177/mouse Wntless is required for embryonic axis formation. *Proc Natl Acad Sci U S A* 106: 18598-18603.
4. Stefater JA 3rd, Lewkowich I, Rao S, Mariggi G, Carpenter AC, et al. (2011) Regulation of angiogenesis by a non-canonical Wnt-Flt1 pathway in myeloid cells. *Nature* 474: 511-515.
5. Zhu X, Zhu H, Zhang L, Huang S, Cao J, et al. (2012) Wls-mediated Wnts differentially regulate distal limb patterning and tissue morphogenesis. *Dev Biol* 365: 328-338.
6. Zhong Z, Zylstra-Diegel CR, Schumacher CA, Baker JJ, Carpenter AC, et al. (2012) Wntless functions in mature osteoblasts to regulate bone mass. *Proc Natl Acad Sci U S A* 109: E2197-2204.
7. Myung PS, Takeo M, Ito M, Atit RP (2013) Epithelial Wnt ligand secretion is required for adult hair follicle growth and regeneration. *J Invest Dermatol* 133: 31-41.
8. Augustin I, Goidts V, Bongers A, Kerr G, Vollert G, et al. (2012) The Wnt secretion protein Evi/Gpr177 promotes glioma tumorigenesis. *EMBO Mol Med* 4: 38-51.
9. Proffitt KD, Madan B, Ke Z, Pendharkar V, Ding L, et al. (2013) Pharmacological inhibition of the Wnt acyltransferase PORCN prevents growth of WNT-driven mammary cancer. *Cancer Res* 73: 502-507.
10. Chen B, Dodge ME, Tang W, Lu J, Ma Z, et al. (2009) Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. *Nat Chem Biol* 5: 100-107.
11. De Langhe SP, Sala FG, Del Moral PM, Fairbanks TJ, Yamada KM, et al. (2005) Dickkopf-1 (DKK1) reveals that fibronectin is a major target of Wnt signaling in branching morphogenesis of the mouse embryonic lung. *Dev Biol* 277: 316-331.
12. Mucenski ML, Wert SE, Nation JM, Loudy DE, Huelsken J, et al. (2003) beta-Catenin is required for specification of proximal/distal cell fate during lung morphogenesis. *J Biol Chem* 278: 40231-40238.
13. Shu W, Guttentag S, Wang Z, Andl T, Ballard P, et al. (2005) Wnt/beta-catenin signaling acts upstream of N-myc, BMP4, and FGF signaling to regulate proximal-distal patterning in the lung. *Dev Biol* 283: 226-239.
14. Dean CH, Miller LA, Smith AN, Dufort D, Lang RA, et al. (2005) Canonical Wnt signaling negatively regulates branching morphogenesis of the lung and lacrimal gland. *Dev Biol* 286: 270-286.
15. Li C, Xiao J, Hormi K, Borok Z, Minoo P (2002) Wnt5a participates in distal lung morphogenesis. *Dev Biol* 248: 68-81.
16. Kim BM, Mao J, Taketo MM, Shivdasani RA (2007) Phases of canonical Wnt signaling during the development of mouse intestinal epithelium. *Gastroenterology* 133: 529-538.
17. Kapoor A, Li HJ, Leiter AB (2007) Intestinal development: the many faces of Wnt signaling. *Gastroenterology* 133: 710-712.
18. Tebar M, Destrée O, de Vree WJ, Ten Have-Opbroek AA (2001) Expression of Tcf/Lef and sFrp and localization of beta-catenin in the developing mouse lung. *Mech Dev* 109: 437-440.
19. Rajagopal J, Carroll TJ, Guseh JS, Bores SA, Blank LJ, et al. (2008) Wnt7b stimulates embryonic lung growth by coordinately increasing the replication of epithelium and mesenchyme. *Development* 135: 1625-1634.
20. Shu W, Jiang YQ, Lu MM, Morrisey EE (2002) Wnt7b regulates mesenchymal proliferation and vascular development in the lung. *Development* 129: 4831-4842.
21. Harris-Johnson KS, Domyan ET, Vezina CM, Sun X (2009) beta-Catenin promotes respiratory progenitor identity in mouse foregut. *Proc Natl Acad Sci U S A* 106: 16287-16292.
22. Cohen ED, Ihida-Stansbury K, Lu MM, Panettieri RA, Jones PL, et al. (2009) Wnt signaling regulates smooth muscle precursor development in the mouse lung via a tenascin C/PDGFR pathway. *J Clin Invest* 119: 2538-2549.
23. Grumolato L, Liu G, Mong P, Mudbhary R, Biswas R, et al. (2010) Canonical and noncanonical Wnts use a common mechanism to activate completely unrelated coreceptors. *Genes Dev* 24: 2517-2530.
24. Mikels AJ, Nusse R (2006) Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. *PLoS Biol* 4: e115.
25. Tao Q, Yokota C, Puck H, Kofron M, Birsoy B, et al. (2005) Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell* 120: 857-871.
26. van Amerongen R, Fuerer C, Mizutani M, Nusse R (2012) Wnt5a can both activate and repress Wnt/ β -catenin signaling during mouse embryonic development. *Dev Biol* 369: 101-114.
27. Cha SW, Tadjuidje E, Tao Q, Wylie C, Heasman J (2008) Wnt5a and Wnt11 interact in a maternal Dkk1-regulated fashion to activate both canonical and non-canonical signaling in *Xenopus* axis formation. *Development* 135: 3719-3729.
28. Cha SW, Tadjuidje E, White J, Wells J, Mayhew C, et al. (2009) Wnt11/5a complex formation caused by tyrosine sulfation increases canonical signaling activity. *Curr Biol* 19: 1573-1580.
29. Miller MF, Cohen ED, Baggs JE, Lu MM, Hogenesch JB, et al. (2012) Wnt ligands signal in a cooperative manner to promote foregut organogenesis. *Proc Natl Acad Sci U S A* 109: 15348-15353.

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30. Sun Z, Gong X, Zhu H, Wang C, Xu X, et al. (2014) Inhibition of Wnt/ β -catenin signaling promotes engraftment of mesenchymal stem cells to repair lung injury. *J Cell Physiol* 229: 213-224.
31. Ai-ran Liu, Le Liu, Song Chen, Yi Yang, Hong-jie Zhao, et al. (2013) Activation of canonical wnt pathway promotes differentiation of mouse bone marrow-derived MSCs into type II alveolar epithelial cells, confers resistance to oxidative stress, and promotes their migration to injured lung 37 tissue in vitro. *J Cell Physiol* 228: 1270-1283.