New mechanistic insight of Wnt5a inhibition involving ROR1

Md Kamrul Hasan. Ph.D.
Department of Medicine, University of California San Diego, 9500 Gilman Dr., La Jolla, CA 92093, USA. **E-mail:** k0hasan@ucsd.edu

Abstract:
Wnt5a is one of the most extensively studied proteins of the Wnt family and is known to play an important role in cellular motility and proliferation. Wnt5a is a ligand of the receptor tyrosine kinase-like orphan receptor 1 (ROR1), an onco-embryonic protein expressed on chronic lymphocytic leukemia (CLL) B cells, and on a variety of solid tumors, but not on normal adult tissues. This mini-review summarizes recent results regarding the inhibition of effects of Wnt5a signaling by targeting ROR1.
Keywords: Wnt5a, ROR1, Cancer, Cell growth, Migration

In humans, Wnt family proteins consist of 19 members that have important roles in the regulation of diverse processes including cell proliferation, survival, migration and metastasis[1-3]. Three Wnt signaling pathways have been characterized[4-6];

(i) canonical Wnt pathway: Also known as the Wnt/β-catenin pathway, which causes an accumulation of β-catenin in the cytoplasm and its eventual translocation into the nucleus to act as a transcriptional coactivator of transcription factors that belong to the TCF/LEF (T-cell factor/lymphoid enhancing factor) family.

(ii) noncanonical planar cell polarity (PCP) pathway: The PCP pathway is activated via the binding of Wnt to a Frizzled (FZD) receptor. The receptor then recruits Dishevelled (Dsh), which forms a complex with Dishevelled-associated activator of morphogenesis 1 (DAAM1) that activates the small G-protein Rho through a guanine exchange factor.

(iii) noncanonical Wnt/calcium pathway: This pathway regulates calcium release from the endoplasmic reticulum (ER), in order to control intracellular calcium levels. Upon ligand binding, the activated Frizzled (FZD) receptor directly interacts with and stimulates Dishevelled (Dsh), as well as trimeric G-protein, which can lead to the activation of Phospholipase C *(*PLC), that in turn cleaves the plasma membrane component PIP2 (Phosphatidylinositol 4,5-bisphosphate) into DAG (diacylglycerol) and IP3 (Inositol trisphosphate). When IP3 binds its receptor on the ER, calcium is released. Increased concentrations of calcium and DAG can activate Cdc42 (Cell division control protein 42 homolog) through PKC (Protein kinase C).

Wnt signaling pathways are potential therapeutic targets in cancer[6]. Wnt5a, one of the members of the Wnt family, has been shown to have a crucial role in cellular proliferation and motility[7,8]. Altered expression of Wnt5a is significantly related to poor clinical outcome in chronic lymphocytic leukemia (CLL), ovarian and gastric cancer[9-11]. Fukuda *et al.* showed that Wnt5a is a ligand of receptor tyrosine kinase-like orphan receptor 1 (ROR1)[12].

ROR1 is a transmembrane protein, comprised of an extracellular domain consisting of an immunoglobulin-like motif, a cysteine-rich frizzled domain, a kringle domain, a cytoplasmic tyrosine kinase domain, two serine/threonine-rich domains and a proline-rich domain[13]. This protein is expressed during embryogenesis, playing a key role in skeletal and neural organogenesis. ROR1 expression attenuates

during fetal development, and with a few exceptions[14], is not expressed in normal post-partum tissues[12]. As a receptor for Wnt5a, ROR1 can induce non-canonical signaling to promote such oncogenic activities. ROR1 is expressed on CLL B cells, as well as on several solid tumors[15-18], and its expression is associated with markers of epithelial-mesenchymal transition (EMT), cancer-cell motility/invasiveness, metastatic disease, and poor prognosis[19,20].

Recent studies described that Wnt5a induces cellular motility and proliferation by enhancing hetero-oligomerization of ROR1 with ROR2, which then recruits guanine nucleotide exchange factors (GEFs) that in turn activate downstream GTPases RhoA and Rac1 in chronic lymphocytic leukemia (CLL) cells[21]. The extracellular kringle domain of ROR1 is important for ROR1/ROR2 hetero-oligomerization and the cysteine-rich domain or intracellular proline-rich domain is necessary for Wnt5a-induced recruitment of GEFs to ROR1/ROR2. Treatment with the humanized anti-ROR1 monoclonal antibody Cirmtuzumab (UC-961) can block such effects and inhibit engraftment of MEC1 cells expressing ROR1, as well as ROR1×TCL1 leukemia cells and ovarian cancer cells[21,22].

Moreover, pre-clinical studies have shown that UC-961 has a potential specificity and safety[23]. In this study, Choi *et al.* performed a Good Laboratory Practice-compliant human tissue cross-reactivity study using UC-961, and identified ROR1 in pancreatic cancer cells, but not in normal postpartum tissues, including the pancreas or adipose tissue. The authors also conducted rodent and primate studies to assess for off-target or non ROR1-specific activity, and found UC-961 was well tolerated and no adverse effects were observed. They also performed studies in cynomolgus monkeys using UC-961, and observed no changes in body weight, clinical chemistry values, or hematologic parameters, including absolute numbers of T or B cells.

Collectively, these results indicate that UC-961 may have important applications in the treatment of patients with CLL, in addition to the treatment of patients with solid-tumor malignancies that express ROR1.

Acknowledgement
I would like to thank reviewers for their constructive critiques and suggestions.

References

1. Masckauchan TNH. et al. (2005) Wnt/beta-catenin signaling induces proliferation, survival and interleukin-8 in human endothelial cells. Angiogenesis 8(1):43-51. http://dx.doi.org/10.1007/s10456-005-5612-9 PMid:16132617

2. Luga V. et al. (2012) Exosomes Mediate Stromal Mobilization of Autocrine Wnt-PCP Signaling in Breast Cancer Cell Migration. Cell 151(7): 1542-1556. http://dx.doi.org/10.1016/j.cell.2012.11.024 PMid:23260141

3. Schlessinger K. et al. (2009) Wnt signaling pathways meet Rho GTPases. Genes & Dev. 23: 265-277.http://dx.doi.org/10.1101/gad.1760809 PMid:19204114

4. Komiya Y. & Habas R. (2008) Wnt signal transduction pathways. Organogenesis 4(2): 68–75. http://dx.doi.org/10.4161/org.4.2.5851

5. Klaus A. & Birchmeier W. (2008) Wnt signalling and its impact on development and cancer. Nature Reviews Cancer 8: 387-

398.
http://dx.doi.org/10.1038/nrc2389 PMid:18432252

6. Anastas JN. & Moon RT. (2013) WNT signalling pathways as therapeutic targets in cancer. Nature reviews Cancer 13(1):11-26. http://dx.doi.org/10.1038/nrc3419 PMid:23258168

7. Nestor H T. et al. (2006) Wnt5a Signaling Induces Proliferation and Survival of Endothelial Cells In Vitro and Expression of MMP-1 and Tie-2. Mol Biol Cell 17(12): 5163–5172.
http://dx.doi.org/10.1091/mbc.E06-04-0320 PMid:17035633 PMCid:PMC1679681

8. Weeraratna AT. et al. (2002) Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. Cancer Cell 1(3): 279-288. http://dx.doi.org/10.1016/S1535-6108(02)00045-4

9. Janovska P. et al. (2016) Autocrine Signaling by Wnt-5a Deregulates Chemotaxis of Leukemic Cells and Predicts Clinical Outcome in Chronic Lymphocytic Leukemia. Clinical cancer research : an official journal of the American Association for Cancer Research 22(2):459-469. http://dx.doi.org/10.1158/1078-0432.CCR-15-0154 PMid:26240275

10. Peng C. et al. (2011) Wnt5a as a Predictor in Poor Clinical Outcome of Patients and a Mediator in Chemoresistance of Ovarian Cancer. International Journal of Gynecological Cancer 21 (2): 280-288. http://dx.doi.org/10.1097/IGC.0b013e31820aaadb PMid:21270611

11. Kurayoshi M. et al. (2006) Expression of Wnt-5a Is Correlated with Aggressiveness of Gastric Cancer by Stimulating Cell Migration and Invasion. Cancer Res 66(21): 10439-48. http://dx.doi.org/10.1158/0008-5472.CAN-06-2359 PMid:17079465

12. Fukuda T. et al. (2008) Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a. Proc. Natl. Acad. Sci. USA. 105, 3047–3052. http://dx.doi.org/10.1073/pnas.0712148105 PMid:18287027 PMCid:PMC2268582

13. Masiakowski P. & Carroll RD. (1992) A novel family of cell surface receptors with tyrosine kinase-like domain. The Journal of biological chemistry 267(36):26181-26190. PMid:1334494

14. Broome HE. et al. (2011) ROR1 is expressed on hematogones (non-neoplastic human B-lymphocyte precursors) and a minority of precursor-B acute lymphoblastic leukemia. Leukemia research 35(10):1390-1394.
http://dx.doi.org/10.1016/j.leukres.2011.06.021PMid:21813176 PMCid:PMC3163753

15. Baskar S. et al. (2008) Unique cell surface expression of receptor tyrosine kinase ROR1 in human B-cell chronic lymphocytic leukemia. Clin. Cancer Res. 14, 396-404.
http://dx.doi.org/10.1158/1078-0432.CCR-07-1823 PMid:18223214

16. Zhang S. et al. (2012) ROR1 is expressed in human breast cancer and associated with enhanced tumor-cell growth. PloS one 7(3):e31127.
http://dx.doi.org/10.1371/journal.pone.0031127

17. Zhang S. et al. (2012) The onco-embryonic antigen ROR1 is expressed by a variety of human cancers. The American journal of pathology 181(6):1903-1910. http://dx.doi.org/10.1016/j.ajpath.2012.08.024 PMid:23041612 PMCid:PMC3509760

18. Zhang S. & Kipps TJ. (2014) The pathogenesis of chronic lymphocytic

leukemia. Annual review of pathology 9:103-118. http://dx.doi.org/10.1146/annurev-pathol-020712-163955 PMid:23987584 PMCid:PMC4144790

19. Cui B. et al. (2013) Targeting ROR1 inhibits epithelial-mesenchymal transition and metastasis. Cancer research 73(12):3649-3660. http://dx.doi.org/10.1158/0008-5472.CAN-12-3832 PMid:23771907 PMCid:PMC3832210

20. Widhopf GF, 2nd. et al. (2014) ROR1 can interact with TCL1 and enhance leukemogenesis in Emu-TCL1 transgenic mice. Proceedings of the National Academy of Sciences of the United States of America 111(2):793-798. http://dx.doi.org/10.1073/pnas.1308374111 PMid:24379361 PMCid:PMC3896194

21. Yu J. et al. (2015) Wnt5a induces ROR1/ROR2 heterooligomerization to enhance leukemia chemotaxis and proliferation. The Journal of clinical investigation. http://dx.doi.org/10.1172/JCI83535

22. Zhang S. et al. (2014) Ovarian cancer stem cells express ROR1, which can be targeted for anti-cancer-stem-cell therapy. Proc. Natl. Acad. Sci. USA. 111, 17266–17271. http://dx.doi.org/10.1073/pnas.1419599111 PMid:25411317 PMCid:PMC4260559

23. Choi MY. et al. (2015) Pre-clinical specificity and safety of UC-961, a first-in-class monoclonal antibody targeting ROR1. Clin. Lymphoma Myeloma Leuk. 15, S167-9. http://dx.doi.org/10.1016/j.clml.2015.02.010 PMid:2629727