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Merlin interacts with LRP6 to block initiation of Wnt/β-catenin signaling

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Abbreviation: NF2, Neurofibromatosis type II; Wnt-CM, Wnt3a-conditioned media; PAK, p21 activated kinase; dnPAK1, dominant-negative form of PAK1, PIP_2, phosphatidylinositol 4,5 bisphosphate

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Merlin, encoded by the NF2 gene, is a tumor suppressor that exerts its function via inhibition of mitogenic receptors at the plasma membrane. Although multiple mutations in Merlin have been identified in Neurofibromatosis type II (NF2) disease, its molecular mechanism is not fully understood. Here, we showed that Merlin interacts with LRP6 and inhibits LRP6 phosphorylation, which is a critical step for the initiation of Wnt signaling. We found that treatment of Wnt3a causes phosphorylation of Merlin by PAK1, which leads to the detachment of Merlin from LRP6 and allows the initiation of Wnt/β-catenin signaling. A higher level of β-catenin was exhibited in tissues from NF2 patients. Enhanced proliferation and migration caused by knockdown of Merlin in glioblastoma cells were inhibited by the suppression of β-catenin. Conclusively, these results suggest that sustained Wnt/β-catenin signaling activity induced by the abrogation of Merlin-mediated inhibition of LRP6 phosphorylation may be a cause of NF2 disease.

Merlin, a tumor-suppressive protein encoded by NF2 gene, is mainly localized to the plasma membrane and interacts with a number of membrane proteins. Merlin exerts its tumor suppressive effects on multiple mitogenic signaling pathways via modulating the interaction with growth factor receptors. Furthermore, Merlin activates the Hippo pathway and turns off YAP/TAZ mediated expression of genes that are involved in proliferation and anti-apoptosis. Finally, recent studies suggest that Merlin inhibits Wnt/β-catenin signaling through inhibiting phosphorylation of β-catenin, which blocks the translocation of β-catenin from membrane to nucleus by inhibiting dissociation of β-catenin from adherens junction. Although the

functional relationship between growth factor receptor and Merlin has been extensively studied, the role of Merlin in Wnt/β-catenin signaling at the level of receptor has not been demonstrated. Our study has shown that active Merlin, a de-phosphorylated form of Merlin on Ser 518, is a binding partner of Wnt co-receptor LRP6. Interaction between Merlin and LRP6 was inhibited by incubation with Wnt-conditioned media (Wnt-CM). We also showed that the knockdown of Merlin enhances Wnt reporter activity and overexpression of Merlin inhibits Wnt reporter activity. Further, a secondary axis, which was induced by the injection of a constitutive active form of LRP6 into ventral side of *Xenopus* embryos, was significantly reduced by the co-injection of Merlin. Overall, these data suggest that Merlin inhibits Wnt/β-catenin signaling via interaction with LRP6.

Except for Merlin, mutations in components of Hippo pathway are uncommon in human cancer. It seems that the increase of the level of YAP/TAZ alone may not be enough, and additional mutations are required to cause human cancer. Our results show that not the level of YAP, a terminal regulator of Hippo pathway, but the β-catenin has been significantly increased in all schwannomas isolated from NF2 patients compared with normal adjacent tissues. We found that enhanced glioblastoma cell growth and migration induced by the suppression of Merlin were restored by the depletion of β-catenin. Thus, our data illustrate that NF2 disease, which has mutations in Merlin, may be caused mainly by the activation of Wnt/β-catenin signaling in addition to the enhanced YAP/TAZ signaling.

LRP6 phosphorylation is critical for activation of Wnt/β-catenin signaling. We showed that Merlin interacts with LRP6 and suppresses Wnt/β-catenin signaling upstream of β-catenin by blocking phosphorylation of LRP6. As we previously demonstrated that formation of PIP_2 by Arf1, a small G protein, is necessary for the phosphorylation of LRP6 (Kim et al. 2013), our current finding reveals that PIP_2 is used as a docking site for PAK1 (p21 activated kinase). In

the presence of Wnt3a-CM, activated PAK1 (p21 activated kinase) binds to PIP_2 and phosphorylates Merlin on Ser 518, which induces the detachment of Merlin from LRP6 and allows phosphorylation of LRP6 for the initiation of Wnt/β-catenin signaling (Figure 1).

Overall, our current study reveals that active Merlin binds to LRP6 and inhibits initiation of Wnt/β-catenin signaling, which may block non-specific activation of that signaling until the level of Wnt ligand is above certain threshold. While we showed that the detachment of Merlin from LRP6 allows activation of Wnt/β-catenin signaling, the role of detached Merlin from LRP6 in the regulation of Hippo signaling needs to be further studied in the future. Specific regulation of Wnt/β-catenin signaling with minimal influence of other signaling pathways is extremely valuable in therapeutic purposes. The small molecules that may control the interaction between Merlin and LRP6 will be very specific modulators of Wnt/β-catenin signaling.

Reference

Kim et al. 2013. *Oncogene* 32(28): 3390-6.

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