ABSTRACT

Wnt signaling has been shown to control a variety of developmental processes including cell fate specification, cell proliferation, cell polarity and cell migration. Spatio-temporal misregulation of Wnt signaling is a cause for many cancers including colorectal cancer. Therefore, understanding the molecular details of Wnt signaling is a prerequisite for the development of novel strategies for therapeutic intervention of the disease.

Wnt pathway is known to function mainly in two ways: 1) Canonical (β-catenin-dependent) pathway and 2) Non Canonical (β-catenin independent) pathway. 1) Canonical pathway: Binding of canonical Wnt such as Wnt3a to its cognate receptors causes inhibition of the degradation complex and accumulation of β-catenin in the cytoplasm and nucleus. In the nucleus, β-catenin interacts with transcription factors such as lymphoid enhancer-binding factor 1 (LEF-1)/ T cell specific transcription factor (TCF) to transactivate the expression of a set of genes responsible for cell proliferation. 2) Non-Canonical pathway: The non-canonical Wnt signaling pathway, mainly regulates cell movements and planar cell polarity, and is mediated by the PDZ and DEP domains of Dvl. At the level of Dishevelled (Dvl), two independent and parallel pathways lead downstream to the activation of the small GTPases Rho and Rac. Recently, however, a third branch has emerged in non-canonical pathway, which suggests that Dvl regulates microtubules and localization of adenomatous polyposis coli (APC) to the leading edge of migrating cells. Dishevelled governs microtubule stability in neurons via GSK3β and Axin. Furthermore, it is shown that Wnt5a, a non-canonical Wnt, cooperates with Cdc42 to regulate APC localization and cell polarity through a mechanism involving Dvl and Axin.
Nup358 (also called RanBP2) is localized to the cytoplasmic side of the Nuclear Pore Complex (NPC). The Nup358 gene encodes large 358 KDa nuclear pore protein, with four Ran-binding domains (RanBD) Nup358, being a Ran-binding protein, is believed to be involved in different Ran-dependent cellular processes. Nup358 also functions as SUMO E3 ligase for various proteins such as topoisomerase II. However, not much is known about cytoplasmic role of Nup358.

In the present study, we show that the nuclear pore protein Nup358 (also known as RanBP2) plays an essential role in Wnt signaling. Asymmetric localization of APC to the ends of a subset of microtubules located in the leading edges is essential for the establishment of front-rear polarity during cell migration. We identified Nup358 as a novel interactor of APC and regulator of its localization. Moreover, we found that Nup358 associates with Kinesin2 motor protein and Dishevelled (Dvl), a critical player of Wnt pathway, and regulates polarized accumulation of APC, thereby promoting cell polarity. Collectively, our results suggest that Nup358, Dvl and APC cooperate to mediate Wnt5a-mediated cell polarization during migration.

A novel interaction between Nup358 and APC was identified through immuno-precipitation. Furthermore, immunofluorescence studies showed that the exogenous expression of APC alone is sufficient to recruit endogenous Nup358 at the leading edges. We also found that Nup358 interacts with the middle domain of APC (APC-M), which primarily contains β-catenin and Axin binding sites. However, further characterization suggested that the middle region of APC interacts with Nup358 in a β-catenin- and Axin-independent manner.

APC is known to interact with microtubules in three different ways: through its Arm domain, which interacts with Kinesin-2, through its basic domain, which directly binds to microtubules and through its extreme C terminus, which interacts with the microtubule plus end binding protein EB1. Interestingly, it was found that APC has a novel microtubule plus end binding domain in its middle region, which is
sufficient to recruit endogenous Nup358 to the plus ends of microtubules. Further, results indicated that both APC-M localization and Nup358 targeting to plus ends of microtubules are independent of EB1. It is possible that other plus end binding proteins may play a role in this process. However, results from this and other studies indicate that, at least, EB1 might not be directly involved in this regulation.

Interaction of Nup358 with APC and Kinesin-2 suggest that they exist in a complex. Our data and results from another study from Akiyama’s lab suggested that association of APC with either Kinesin-2 or Nup358 alone is not sufficient for its targeting to cell cortex; rather interaction with both the proteins might be required for polarized localization of APC.

We have tried to identify the region of Nup358 that interacts with APC using deletion analysis; however, we could not delineate the interaction domain(s). This indicates that the interaction may require additional proteins or that the interaction may involve different regions of Nup358. However, we found that overexpression of the N-terminal region (BPN) of Nup358 to a great extent, and C-terminal region (BPC) of Nup358 to a lesser extent, interfered with the accumulation of APC at cell cortex and cell polarization. Although these results unequivocally establish a critical role for Nup358 in APC localization, how these fragments acted in a dominant negative fashion is still not clear.

Our study has also identified Dvl as a novel in vivo binding partner of Nup358. Dvl has three domains: N-terminal DIX domain, middle PDZ domain and C-terminal DEP domain. Our results show that DIX domain plays a negative role in the interaction of Dvl2 with both APC and Nup358. Our extensive studies on domain analysis suggest that Nup358 requires its Leucine-rich region for interaction with Dvl, whereas Dvl requires region encompassing the PDZ and DEP, with PDZ being the region significantly contributing to the interaction with Nup358. Similarly, in APC, the N terminus, which contains Armadillo domain, is
essential for interaction with Dvl2, whereas Dvl2 requires both PDZ and DEP containing regions for interaction with APC.

Furthermore, this study highlights the inhibitory role played by DIX domain on the localization of Nup358 and APC inside the cell. Results suggest that overexpression of full length Dvl2 acts in a dominant negative fashion and sequesters both BPN and APC into punctate structures within the cytoplasm, whereas removal of DIX domain (Dvl2-Δ-DIX) facilitates the in vivo functioning of Nup358 and APC in terms of their microtubule binding ability.

Through siRNA mediated depletion of KAP3 and Nup358, we also showed that these two players are required for the accumulation of APC at the cell cortex. The study also suggests that exogenous expression of APC-M results in reduced targeting of endogenous APC to the leading edge of cell cortex, indicating that the fragment functions in a dominant negative manner. Further, we also used RNA interference of Nup358 and expression of dominant negative constructs of Dvl to interfere Wnt5a induced polarity. Results indeed suggested that Nup358 and Dvl are critical players functioning downstream to Wnt5a in polarized cell migration.

Also, our results indicated that Nup358 cooperates with Kinesin-2 to regulate the localization of APC to the cell cortex through a nuclear-transport-independent mechanism. Our results also suggested that Nup358 functions along with Dvl and APC to regulate Wnt5a-mediated cell polarization. Thus, these results revealed a more active role for structural nucleoporins in regulating fundamental cellular processes than previously anticipated.