ABSTRACT

The Wnt signaling pathway controls a variety of processes during homeostasis and development of multicellular organisms including cell polarity, cell proliferation, cell fate decision, axis formation, and organ development. 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.

Currently, three different pathways are believed to be activated upon Wnt receptor activation a) the canonical Wnt/β-catenin cascade b) the noncanonical β-catenin independent pathway, that is further divided into two distinct branches, the Planar Cell Polarity pathway and the Wnt/Ca^{2+} pathway.

β-catenin, the effector molecule of canonical Wnt signaling is a nucleo-cytoplasmic shuttling protein. Shuttling activity of this signaling molecule may help in relaying the Wnt signal in an effective manner. Canonical Wnts have been shown to regulate the nucleo-cytoplasmic distribution of β-catenin.

Upon Wnt induction non-phosphorylated form of β-catenin translocates into the nucleus and brings about the transcription of various Wnt responsive genes such as c-Myc and Cyclin D1, in association with transcription factors such as T cell factor (TCF) or lymphocyte enhancer binding factor (LEF).

Transport of β-catenin across the nuclear membrane is critical for the control of Wnt signaling, many attempts have been made to discern the mechanism of β-catenin transport across the nuclear membrane. But still a clear picture is lacking as to how the transport of β-catenin is precisely regulated.

The nucleoporin Nup358, also called Ran binding protein2 (RanBP2), is a giant nuclear pore protein with a molecular weight of 358 KDa, localizes to the cytoplasmic side of the nuclear pore complex (NPC). It is a modular protein with well-defined structural modules such as a four RanBP1-like Ran binding domains (RanBD), leucine-rich region (LRR), zinc finger domains (ZnFs), a cyclophilin-homology domain (CHD). Nup358 functions as a SUMO E3 ligase. Nup358, being a Ran binding protein and nuclear pore protein, is believed to regulate nucleo-cytoplasmic transport.

In the present study, we demonstrate the possible role of Nup358 in regulating Wnt/β-catenin signaling, which is expected to shed more light on the mechanistic aspects of this important evolutionary conserved developmental signaling pathway.

A novel interaction between Nup358 and β-catenin was identified through reciprocal immunoprecipitations, further; the β-catenin interaction domain was narrowed down to the N terminus of the Nup358. Moreover, full length Nup358 and N terminus of it were found to be co-localizing with β-catenin. In our study, we also show that ARM domain of β-catenin could mediate its interaction with Nup358. In order to check if the interaction of β-catenin and N terminus of Nup358 (BPN) is direct, we tried several methods to get the soluble BPN as starting material for in vitro pull down assays, but, we could not succeed to get the protein soluble. Further, Nup358, being a Ran binding protein, we also examined the possibility of Ran being involved in the modulation of interaction. However, we could not find any effect of RanGTP or RanGDP on the interaction between Nup358 and β-catenin.

We investigated the functional significance of this interaction in normal cells (non-colorectal cancer cell line), by using a TCF based reporter assay. After siRNA mediated depletion of Nup358, we found a decrease in the β-catenin signaling in cells devoid of Nup358, as compared to control siRNA treated cells, suggesting a positive role of Nup358 in Wnt signaling. Similar results were also found in the case of SW480 cells (a colorectal cancer cell line with truncation in APC), suggesting that Nup358 mediated regulation of β-catenin signaling may occur independent of APC.

Nucleo-cytoplasmic fractionation studies of control siRNA treated and Nup358 depleted cells suggested that Nup358 plays an important role in the nuclear accumulation of β-catenin. Interestingly, Nup358 depletion in HeLa cells led to an increased accumulation of β-catenin at the cell-cell junctions as compared to the control cells.

Taken together, our result identifies a novel interaction between the nucleoporin Nup358 and β-catenin, and a positive role for Nup358 in the regulation of Wnt/β-catenin signaling.

During the characterization of Nup358 and β-catenin interaction, we came across an interesting observation. We found that β-catenin possesses the ability to interact with metal ions such as nickel and cobalt. We demonstrated

that untagged version of recombinant β-catenin expressed in bacteria could be specifically purified by Ni-NTA but not NTA beads. Similarly, endogenous β-catenin from SW480 cells lysate could be specifically pulled down with Ni-NTA beads. These results suggested that β-catenin is a nickel binding protein. Further, to study the kinetics of this interaction, we performed SPR analyses with purified, untagged and GST-tagged β-catenin protein. The studies further confirmed that the specific interaction of β-catenin with nickel, with an association / dissociation constant of 1.08×10^{-5} and 3.8×10^{3}.

Effect of the nickel β-catenin interaction on the Wnt signaling was investigated by treating the cells with nickel and examined the localization of β-catenin using immunofluorescence analysis and its activity using the TCF-based luciferase reporter assay. Our study suggested that nickel induced nuclear localization of β-catenin; however, we could not detect any effect on β-catenin signaling.

Our study identifies a novel interaction of β-catenin with nickel, which may provide more mechanistic details of β-catenin function and a better understanding of metal induced carcinogenesis.