
INDEX

ABBREVIATIONS (Page No. 7-8)

ABSTRACT (Page No. 9-11)

Chapter 1 Introduction (Page No. 12-55)

1.1 Wnt Signaling

1.2 Nature of Wnt and secretion

1.3 Importance of Wnt signaling

1.4 Milestones in history of Wnt signaling

1.5 The canonical Wnt signaling / β -catenin cascade

1.6 Structure of β -catenin

1.7 Events during Wnt signaling

1.8 Wnt reception at the membrane

1.9 Post translational regulation of β -catenin by cytoplasmic degradation complex

1.10 β -catenin translocation to the nucleus and transactivation of Wnt responsive genes

1.11 Target genes of canonical Wnt/ β -catenin signaling

1.12 Non-canonical planar cell polarity (PCP) pathway

1.13 Ca^{2+} signaling pathway

1.14 Nuclear pore complex

1.15 Nup358 (or Ran binding protein 2)

1.16 Ran and nucleo-cytoplasmic transport

1.17 APC

1.18 Nuclear export of APC and cancer

1.19 Importance of studying metal toxicity

1.20 Metal carcinogenesis

1.21 Uptake, distribution, and retention of nickel

1.22 Human exposure to nickel and Symptoms

1.23 Molecular mechanisms of action

1.24 Genotoxic effect

-
- 1.25 Epigenetic effects
 - 1.26 Binding of nickel with cellular proteins
 - 1.27 Binding with HIF-1 transcription factor
 - 1.28 Effect of nickel binding with carriers
 - 1.29 Effect of nickel binding with Regulatory proteins
 - 1.30 Effect of nickel binding with structural proteins
 - 1.31 Nickel-induced oxidative damage
 - 1.32 Nickel-induced Protein damage
 - 1.33 DNA damage
 - 1.34 Cross-linking
 - 1.35 Alteration of cell signaling pathways
 - 1.35.1 NF- κ B signaling
 - 1.35.2 p53 pathway
 - 1.35.3 Wnt/ β -catenin Signaling
 - 1.35.4 Oxidative stress signaling

Chapter 2 Materials and Methods (Page No 56-74)

- 2.1 Constructs made in the study
- 2.2 Animals
- 2.3 Medium and Serum
- 2.4 General reagents and plastic wares
- 2.5 Cell culture
- 2.6 Antibodies used in our study
 - 2.6.1 Primary antibodies
 - 2.6.2 Secondary antibodies
- 2.7 Cell transfection
- 2.8 RNA Interference
- 2.9 Generation of polyclonal antibodies against GFP, APC and β -catenin
- 2.10 Immunofluorescence
- 2.11 Immunoprecipitation and western blotting
- 2.12 Luciferase reporter assay
- 2.13 Large scale purification of BPN in CHO cells from pSecTag2 vector

-
- 2.14 Nuclear and cytoplasmic fractionation
 - 2.15 Stripping of Ni-NTA agarose beads
 - 2.16 Ni-NTA pull down
 - 2.17 Luciferase assay in SW480 cells under nickel induced condition
 - 2.18 Immunofluorescence studies in SW80 cells for β -catenin under nickel induced condition
 - 2.19 Expression and purification of recombinant proteins
 - 2.20 Surface plasmon resonance (SPR) measurements
 - 2.21 GST-pull down
 - 2.22 Composition of buffers and solutions
 - 2.23 Nonidet-P40 (NP40) buffer for Ni-NTA pull down
 - 2.24 NiSO₄. 6H₂O stock solutions
 - 2.25 Statistical analysis

Chapter 3 Characterization of interaction between Nup358 and β -catenin (Page No 75-100)

- 3.0 Generation of Rabbit polyclonal antibodies
- 3.1 Generation of polyclonal antibodies against APC in Rabbit
- 3.2 Generation of polyclonal antibodies against β -catenin in Rabbit
- 3.3 Generation of GFP rabbit polyclonal antibody
- 3.4 Nup358 immunoprecipitates endogenous β -catenin
- 3.5 GST- β -catenin pull down endogenous Nup358
- 3.6 β -catenin immunoprecipitates endogenous Nup358
- 3.7 GFP- Full length-Nup358 colocalizes with RFP- β -catenin
- 3.8 RFP- β -catenin colocalizes with BPN
- 3.9 N terminus of Nup358 interacts with the β -catenin
- 3.10 ARM domains of β -catenin interact with Nup358
- 3.11 β -catenin has two sites of interaction in Nup358
- 3.12 The Nup358- β -catenin interaction occurs independent of Ran
- 3.13 Nature of interaction between BPN and β -catenin
- 3.14 Interaction study with urea solubilized His-BPN (143-840 a.a)

-
- 3.15 Nup358 depletion in SW480 cells results in the reduction in Wnt/ β -catenin signaling
 - 3.16 Nup358 depletion in SW480 cells does not lead to change in the level of β -catenin
 - 3.17 Overexpression of GFP-F1-Nup358 in SW480 cells results in increase in Wnt/ β -catenin signaling
 - 3.18 Expression of GFP-BPN and GFP-BPC fragments of Nup358 increases Wnt/ β -catenin signaling significantly
 - 3.19 GFP-BPN and GFP-BPC fragments of Nup358 increases Wnt/ β -catenin signaling significantly without affecting the level of β -catenin
 - 3.20 Nup358 depletion in HeLa S3 (a non-colorectal cancer cell line) results in the reduction in Wnt/ β -catenin signaling
 - 3.21 Nup358 depletion in HeLa S3 leads to the decrease in nuclear β -catenin level
 - 3.22 Nup358 depletion in HeLa S3 leads to the enrichment of β -catenin at the adherens junction
 - 3.23 Discussion

Chapter 4 Characterization of novel metal binding property of β -catenin (Page No. 101-111)

- 4.1 Molecular cloning of β -catenin in pET30a vector
- 4.2 Recombinant β -catenin does not possess 6XHis tag
- 4.3 Nickel binding activity of β -catenin
- 4.4 Reactivity of His antibody against the purified proteins
- 4.5 Endogenous β -catenin from SW480 cell lysate can bind to Ni (II)
- 4.6 Surface plasmon resonance (SPR) studies on β -catenin interaction with Ni (II)
- 4.9 Induced nuclear β -catenin does not alter the Wnt/ β -catenin signaling
- 4.10 Discussion

5. Publications from related work (Page No. 112)

6. Bibliography (Page No. 113-140)

FIGURE INDEX

- Fig 1.1 Canonical Wnt Signaling
- Fig 1.2 Primary structure of β -catenin
- Fig 1.3 β -catenin inside the nucleus
- Fig 1.4 Schematic representation of the non-canonical Wnt signaling pathway
- Fig 1.5 Current NPC model with its major structural components
- Fig 1.6 Schematic representation of the position of the major nucleoporin subcomplexes in metazoans
- Fig 1.7 Schematic representation of human Nup358 (3224 a.a)
- Fig 1.8 Receptor-Mediated nucleo-cytoplasmic Transport
- Fig 1.9 Schematic diagram of full length and one of the C-terminally truncated APC proteins
- Fig 1.10 Schematic representation of the uptake and cellular interactions of Ni (II) derived from nickel compounds
- Fig 2.1 Deletion mutants of β -catenin
- Fig 2.2 Schematic representation of the TOP and FOP luciferase reporter construct
- Fig 3.1 Purified rabbit polyclonal APC antibody recognizes endogenous APC
- Fig 3.2 Affinity-purified rabbit polyclonal antibodies against β -catenin recognizes
- Fig 3.3 Purified rabbit polyclonal antibody against GFP recognizes overexpressed antigen
- Fig 3.4 Nup358 associates with β -catenin in vivo
- Fig 3.5 Nup358 associates with β -catenin in vivo
- Fig 3.6 β -catenin interacts with Nup358
- Fig 3.7 GFP-FL-Nup358 colocalizes with RFP- β -catenin
- Fig 3.8 β -catenin interacts with leucine rich region of Nup358
- Fig 3.9 GFP-BPN and RFP- β -catenin co-localizes on microtubule
- Fig 3.10 ARM domain of β -catenin is required for Nup358 and β -catenin interaction
- Fig 3.11 β -catenin has two sites for interaction in Nup358
- Fig 3.12 Nup358- β -catenin interaction occurs independent of Ran
- Fig 3.13 Expression of pSecTag2BPN in CHO-K1 cells

-
- Fig 3.14 GST pull down assay with purified His-BPN (143-840 a.a)**
- Fig 3.15 Nup358 positively regulates Wnt/ β -catenin signaling**
- Fig 3.16 Nup358 depletion in SW480 cells does not lead to change in the level of β -catenin**
- Fig 3.17 Overexpression of Nup358 increases Wnt/ β -catenin signaling**
- Fig 3.18 BPN and BPC increases Wnt/ β -catenin signaling**
- Fig 3.19 Fragments of Nup358 does not causes detectable change in the level of β -catenin in SW 480 cells (A) (B)**
- Fig 3.20A Nup358 depletion in HeLa S3 results in reduced Wnt/ β -catenin signaling**
- Fig 3.20B Nup358 depletion in HeLa S3 does not alter the level of β -catenin**
- Fig 3.21 Nup358 depletion in HeLa S3 leads to the decrease in nuclear β -catenin level**
- Fig 3.22 Nup358 regulates intracellular localization of β -catenin**
- Fig 4.1 Molecular cloning of β -catenin.**
- Fig 4.2 Induced β -catenin does not have the 6XHis tag.**
- Fig 4.3 β -catenin is a Ni (II) binding protein. (A) (B)**
- Fig 4.5 Endogenous β -catenin binds to Ni (II) specifically**
- Fig 4.6 Binding of β -catenin to Ni (II)**
- Fig 4.7 Binding of GST- β -catenin with Ni (II)**
- Table 4.1 Kinetics and affinity data for interaction of β -catenin and GST- β -catenin with Ni (II)**
- Fig 4.8 Ni (II) induces the nuclear translocation of β -catenin.**
- Fig 4.9 Ni (II) treatment does not change the β -catenin level.**
- Fig 4.10 Ni (II) induced nuclear β -catenin does not alter Wnt/ β -catenin signaling in SW480 cells.**