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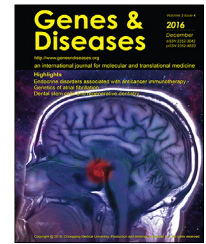

SHORT COMMUNICATION

Differential alterations of positive and negative regulators of beta catenin enhance endogenous expression and activity of beta catenin in A549 non small cell lung cancer (NSCLC) cells

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Abstract Beta catenin has been well documented in previous studies to be involved in non small cell lung cancer (NSCLC). Beta catenin abundance and transcriptional activity are significantly regulated by several factors. Though it is well known that Akt and Gsk3 beta are respective positive and negative regulators of beta catenin, however, no single study has so far documented how the expression and activity of both positive as well as negative regulators play favorable role on beta catenin expression and activity in NSCLC. In this study, we compared expression and activity of beta catenin and its regulators in normal lung cell WI38 and NSCLC cell A549 by western blot, qRT-PCR and luciferase assay. We observed that beta catenin positive regulators (Akt and Hsp90) and negative regulators (Gsk3 beta and microRNA-214) have differential expression and/or activity in NSCLC cell A549. However the differentially altered statuses of both the positive and negative regulators rendered cumulative positive effect on beta catenin expression and activity in A549. Our study thus suggests that chemotherapeutic modulations of regulating factors are crucial when abrogation and/or inhibition of key oncogenic proteins are necessary for cancer chemotherapy.
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Abbreviations: NSCLC, non small cell lung cancer; SCLC, small cell lung cancer; miRNA, microRNA; snRNA, small nuclear RNA.

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Introduction

Non small cell lung cancer (NSCLC) is a group of lung cancers that are named for the types of cells affected and their morphology under the microscope. NSCLCs account for 85% of all the cases of lung cancers.[1] NSCLCs are relatively chemo-resistant, with respect to small cell lung cancer (SCLC).

Beta catenin is an oncogenic protein that is pivotal for the expression of Wnt responsive genes[2–6] which regulate proliferation, cell division, anti-apoptosis, angiogenesis, metastasis and is consequently necessary for carcinogenesis and cancer progression. In NSCLC, beta catenin mutations are extremely uncommon.[7,8] But, the relationship between aberrant beta catenin expression and NSCLC poor prognosis is well documented.[9,10] Beta catenin was previously shown to be important for proliferation, migration and clonogenic ability of A549 NSCLC cell.[11] Hence, beta catenin status in terms of its expression and activity is of immense importance in NSCLC.

Cellular abundance and transcriptional activity of beta catenin are regulated by multiple positive and negative regulators. Since, beta catenin has been demonstrated to be involved in NSCLC, the expressions and activities of key beta catenin regulators may have some crucial contribution in NSCLC. So far no single study has shown how the expression and activity of positive as well as negative regulators of beta catenin are altered in NSCLC and how they have overall effect on beta catenin. Hence, we studied whether the expression and activity of known regulators of beta catenin are modulated in NSCLC cell that could be beneficial for beta catenin abundance and transcriptional activity. We observed that, besides beta catenin upregulation, well known positive regulators of beta catenin like Akt & Hsp90 and negative regulators like Gsk3 beta and microRNA-214 (miR-214) are differentially modulated in A549 NSCLC cell that had overall positive effect on beta catenin expression and transcriptional activity.

Materials and methods

Antibodies and chemicals

A list of antibodies and other chemicals used in this study is given in Supplementary Table S1.

Cell culture, drug treatment and transfection

Normal lung cell WI38 (Gifted by Dr. Mahadeb Pal, Bose Institute, India) and NSCLC lung cancer cell A549 (obtained from National Centre For Cell Science, India) were cultured in DMEM supplemented with 10% Fetal Bovine Serum and incubated at 37 °C in a humidified incubator supplied with 5% CO_2. Cells were treated with 500 nM 17AAG or vehicle (DMSO) and incubated for 48 h. For transfected cells to be used in western blot or real time qRT-PCR, cells were split in 60 mm dishes and were transfected with 3 μg pRNA-U61/Hygro empty vector (empty U61) control or 3 μg pRNA-U61-pre-miR-214 (miR-214-U61) and incubated for 72 h. 10 μl Lipofectamine 2000 was used per dish as transfection reagent. Throughout all the experiments passage number varied between 5th to 26th for WI38 cells and between 3rd to 18th for A549 cells. For every experiment it was ensured that cells had been passaged at least thrice after thawing.

Sub-cellular extraction

Both cytosolic and nuclear extractions were performed as described.[12] Cells were fractionated after 48 h of plating when they reached ~90% confluency.

Western blot

All western blotting experiments were done in this study as per our previous work.[13] Untreated/untransfected cells were harvested at 48 h after plating when they reached ~90% confluency. 17-AAG treated and empty-U61 or miR-214-U61 transfected cells were harvested after 48 h of incubation.

RNA isolation, cDNA preparation and real-time quantitative PCR

Cells were harvested at ~48 h after plating when they reached ~90% confluency or harvested at 48 h after transfection with empty U61 and miR-214-U61. To study the expression of mRNAs viz. CTNNB1, GSK3B, AKT1, MYC, CCND1, JUN, FOSL1, BIRC5, ACTB (reference gene), the same reagents and same RNA isolation, cDNA preparation, real time qPCR techniques were used as per our previous work.[13] For miRNA expression study, the use of U6 snRNA as reference gene has been well validated.[14] 100 ng of RNA was reverse transcribed into cDNA with MuLV-Reverse Transcriptase and stem-loop primer for miR-214 and U6 snRNA and then subjected to real time qPCR using SYBR green mastermix on Mastercycler Ep Realplex (Eppendorf, Germany) using the forward primers of miR-214, U6 snRNA and universal reverse primer. Ct value of target (miR-214) was normalized to Ct value of reference (U6 snRNA). $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change in miR-214 expression. A list of sequences of the primers used in this study is added in Supplementary Table S2.

Luciferase assay

Luciferase assays were performed with same principle and method as described previously by us.[13] Briefly, 200 ng TOPFLASH TCF-reporter plasmid was transfected in cells grown in 24 well plates and then incubated for 24 h and 48 h respectively and then harvested for experiments. 5 μg lysates from incubated samples were subjected to luciferase reporter assay as described.[13]

Statistical analysis

All values were expressed as mean ± SD. Significant difference between experimental groups were determined by two tailed Student's t-test using GraphPad QuickCals, an online tool.

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