UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SILENCING DISHEVELLED 1, 2 and 3 mRNA EXPRESSION ON MDA-MB-231 CELL MIGRATION AND INVASION USING siRNAs

SAMUEL KHOO LEON JUAN

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By

SAMUEL KHOO LEON JUAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

May 2015

DEDICATION

Specially dedicated to,

My loving parents, my awesome friends, dear supervisors,

For their invaluable love, understanding, encouragement and patience.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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May 2015

Chairman : Professor Seow Heng Fong, PhD
Institute : Bioscience

The Wnt signalling pathway plays an important role in embryonic development, generation of cell polarity and specification. Previous studies have shown that both canonical and non-canonical Wnt signaling pathways are involved in breast cancer metastasis. Dishevelled, one of the components of both canonical and non-canonical Wnt signaling pathway is involved in proliferation, polarity, terminal differentiation and self-renewal of cells. It is well known that Dishevelled interacting with Axin plays a role in dissociating the β-catenin destruction complex in the canonical Wnt signaling pathway. Although the involvement of Dishevelled in the non-canonical Wnt signaling pathway is less well studied compared to the canonical pathway, the role of Dishevelled is also important for cytoskeletal reorganization in planar cell polarity pathway and promoting tissue separation as well as ventral axis identity in *Xenopus* via the Wnt Calcium pathway. Our hypothesis is that silencing of human Dishevelled 1,2 and 3 isoforms results in inhibition of both canonical and non-canonical Wnt signaling pathway leading to reduced β-catenin interaction with TCF (T-cell Factor) and LEF (Lymphoid Enhancer Factor), reduced cell proliferation, migration and also invasion regardless of canonical or non-canonical Wnt signaling pathway. MDA-MB-231 is the breast cancer cell line used in this study. The objectives in this study include (1) to confirm the silencing of Dishevelled 1,2 and 3 by reverse-transcription quantitative PCR and Western Blotting. (2) to determine the changes in cell viability, migration, invasion, TCF-LEF mediated transcriptional activation after gene silencing using siRNA targeting Dishevelled isoforms. (3) to identify the changes in β-catenin, Glycogen Synthase Kinase-3β (GSK3β) protein, Epidermal Growth Factor Receptor (EGFR) and Extracellular signal-Regulated Kinases (ERK) expression in cells transfected with siRNAs targeting Dishevelled 1, 2 and 3. This study resulted in a few interesting findings: (1) A significant reduction of Dishevelled 1,2 and 3 mRNA levels in cells transfected with siRNA based on reverse transcription quantitative PCR was observed. However, we were not able to detect Dishevelled 1 isoform at the protein

level using the commercially available anti-Dishevelled 1 antibody; (2) different phenotypical changes were observed in cells transfected with siRNA targeting Dishevelled 1 and Dishevelled 2. This occurred with siRNA D1_9 or siRNA D2_4 where cells transfected with both siRNAs showed reduced cell viability. However, there was no significant change in cell viability, migration and invasion with other siRNAs for Dishevelled 1 and Dishevelled 2 siRNA (D1_6 and D2_8, respectively) as compared to control. Further studies showed that both siRNAs D1_9 and D2_4 resulted in reduced cell invasion and migration that were statistically significant. This indicates that there is possibility of target non-specificity by both siRNAs and for siRNA D2_4 or it could also be due to a higher degree of Dishevelled 2 knockdown. (3) Dishevelled 3 plays a role in mediating Wnt3a-stimulated TCF-LEF transcriptional activity. Reduced Wnt3a-stimulated TCF-LEF transcriptional activation was observed in MDA-MB-231 cell line transfected with siRNAs targeting Dishevelled 3 especially on cells transfected with siRNA D3_7 which was found to be statistically significant. (4) both densitometric results of phospho-GSK3β- Serine 9 expression (relative to total GSK3β) and phospho-β-catenin expression (relative to total β-catenin) for cells transfected with Dishevelled 3 siRNAs shows that a higher expression of phospho-GSK3β-Serine 9, which is indicative of inactive GSK3β, can result in reduced β-catenin degradation (phospho-β-catenin). However, the level of β-catenin degradation (relative to total β-catenin) expression in Dishevelled 3 siRNA transfected cells was comparable to Control. The basal phospho-β-catenin in Dishevelled 3 siRNA transfected cells was not related to GSK3β activity in this study. (5) by relating both phospho-β-catenin expression (relative to total β-catenin) on densitometric analysis and Luciferase assay, reduced β-catenin degradation results in a higher TCF-LEF activity for cells transfected with siRNA targeting Dishevelled 1 and 2. However, reduced TCF/LEF mediated transcriptional activity in cells transfected with Dishevelled 3 siRNAs did not show an increase in phospho-β-catenin expression (relative to total β-catenin) as compared to control. Silencing of Dishevelled 3 expression maintains expression of basal phospho-β-catenin, which is sufficient for degradation or dissociation of β-catenin destruction complex resulting in the reduced TCF/LEF mediated transcriptional activity. It is possible that disruption of the interaction between Dishevelled 3 but not Dishevelled 1 and 2 with Axin interferes with formation of β-catenin destruction complex formation resulting in reduced TCF/LEF-sensitive transcriptional activity by Wnt3a in MDA-MB-231 cells or interference at the promoter binding site for β-catenin/ TCF-LEF complex. (6) densitometric analysis of Western Blot involving components along EGFR-ERK pathway shows that Wnt3a stimulation does not result in activation of EGFR-ERK pathway and knockdown of Dishevelled isoforms do not result in reduction of EGFR-ERK pathway under Wnt3a stimulation. In conclusion, Dishevelled 3 is involved in canonical Wnt β-catenin pathway in MDA-MB-231 breast cancer cells, playing a role in inhibiting proteosomal degradation of β-catenin. It is possible that knockdown of Dishevelled 3 expression disrupted the interaction with Axin, reduced β-catenin accumulation and ultimately reduced Wnt3a stimulated TCF/LEF-mediated transcriptional activity. It is possible that silencing of Dishevelled 3 expression interfered with β-catenin/TCF-LEF-mediated transcriptional activity. On the contrary, non-reduction in β-catenin/ TCF-LEF-mediated transcriptional activity and low phospho-β-catenin expression relative to total β-catenin in Dishevelled 1 and Dishevelled 2 siRNA transfected cells suggest that Dishevelled 1 and 2 isoforms are not involved in canonical Wnt β-catenin pathway on MDA-MB-231 cell line.

Abstrak tesis yang dikemukakan kepada Senat Univerisiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN PENYEYAPAN PENYATAAN mRNA DISHEVELLED 1,2 DAN 3 KE ATAS PENGHIJRAHAN DAN SERANGAN SEL MDA-MB-231 DENGAN MENGGUNAKAN siRNA

Oleh

SAMUEL KHOO LEON JUAN

Mei 2015

Pengerusi : Profesor Seow Heng Fong, PhD
Institute : Biosains

Isyarat laluan Wnt memainkan peranan penting dalam perkembangan embrio, penjanaan kekutuban sel dan juga penspesiesan sel. Kajian sebelum ini telah menunjukkan penglibatan isyarat laluan Wnt, sama ada yang berkanun atau isyarat laluan Wnt tidak berkanun dalam perebakan sel. Dishevelled, salah satu komponen yang terkandung dalam isyarat laluan Wnt berkanun dan tidak berkanun, memainkan peranan penting dalam pembahagian, kekutuban, pembezaan terminal dan pembaharuan sendiri dalam sel. Interaksi antara Dishevelled dan Axin memainkan peranan penting dalam pemisahan kompleks pemusnahan β-catenin dalam isyarat laluan Wnt berkanun. Walaupun kajian penglibatan Dishevelled dalam isyarat laluan Wnt tidak berkanun adalah lebih kurang berbanding dengan laluan berkanun, Dishevelled juga memainkan peranan yang penting bagi penyusunan semula perangkaan sel dalam laluan kekutuban satah sel ,mendorong pemisahan tisu dan juga mendoring identiti paksi ventral dalam Xenopus melalui isyarat kalsium Wnt. Hipotesis kami adalah penyenyapan isoform Dishevelled 1,2 dan 3 mengakibatkan perencatan kedua-dua isyarat laluan Wnt berkanun dan tidak berkanun berikutan dengan pengurangan interaksi antara β-catenin dengan TCF dan LEF, pengurangan pembahagian sel, penghijrahan dan juga serangan dalam isyarat laluan Wnt berkanun dan tidak berkanun. Sel MDA-MB-231, modal sel kanser bagi kanser payu dara telah digunakan dalam kajian ini. Tujuan kajian ini termasuk (1) untuk mengesahkan kesan penyenyapan Dishevelled 1,2 dan 3 melalui reverse trankripsi PCR berkuantitatif dan Western Blot. (2) untuk menentukan perubahan dalam kesan kemandirian, penghijrahan, serangan, dan pengaktifan transkribsi yang diantarakan oleh TCF -LEF selepas penyenyapan dengan menggunakan siRNA. (3) Mengenal pasti perubahan β-catenin, Glycogen Synthase Kinase 3β, Faktor Pertumbuhan Epiderma (EGFR) dan Pengawalan Isyarat Luar sellular Kinase (ERK), dalam cell yang dijangkit oleh siRNA yang menyasar Dishevelled 1,2 dan 3. Terdapat beberapa keputusan yang menarik yang dalam kajian ini: (1) terdapat pengurangan yang ketara dalam aras mRNA DIshevelled 1,2 dan 3 berdasarkan keputusan reverse transkribsi quantitative PCR.

Namun, kami tidak dapat mengesan penyataan isoform DIshevelled 1 dalam aras protein dengan mengunakan antibodi anti-Dishevelled 1. (2) terdapat kesan perubahan fenotipik yang dapat diperhatikan di antara siRNA Dishevelled 1 dan Dishevelled 2 . Perkara ini berlaku di antara siRNA D1_9 dan siRNA D2_4 di mana terdapat pengurangan dari segi kemandirian sel yang dijangkiti oleh kedua-dua siRNA tersebut. Namun, tiada perubahan yang ketara dari segi kemandirian , penghijrahan dan serangan sel oleh siRNA yang lain yang juga menyasar Dishevelled 1 dan Dishevelled 2 (siRNA D1_6 dan siRNA D2_8). Kajian yang seterusnya menunjukkan bahawa siRNA D1_9 dan D2_4 mengakibatkan pengurangan serangan dan penghijrahan sel yang ketara dari analisa statistik. Ini menunjukkan bahawa terdapat kemungkinan dari segi ketidak pengkhususan dari segi penyasaran bagi kedua-dua siRNA tersebut dan juga berkemungkinan bahawa kesan penyataan yang lebih tinggi bagi menjatuhkan penyataan Dishevelled 2 bagi siRNA D2_4. (3) Dishevelled 3 memainkan peranan yang penting dalam aktiviti transkripsi TCF-LEF yang diantara oleh Wnt3a. Pengurangan aktiviti transkripsi TCF-LEF yang diantara oleh Wnt3a yang diperhatikan dalam sel MDA-MB-231 yang dijangkiti oleh siRNA yang menyasar Dishevelled 3 terutamanya bagi sel yang dijangkiti old siRNA D3_7 yang mengakibatkan pengurangan yang ketara secara statistik. (4) Keputusan desitometrik oleh fosfo-GSK3β-Serine9 (relative dengan jumlah GSK3β) dan fosfo-β-catenin (relative kepada jumlah β-catenin) bagi sel yang dijangkiti oleh siRNA Dishevelled 3 menunjukkan penyataan fosfo-GSK3β-Serine9 yang lebih tinggi, ini menandakan kecenderungan GSK3β yang tidak aktif. Penyataan fosfo-GSK3β-Serine9 boleh mengakibatkan pengurangan kecenderungan untuk penguraian β-catenin (fosfo-β-catenin). Namun, penyataan aras penguraian β-catenin (relative kepada jumlah β-catenin) oleh sel yang dijangkit oleh siRNA Dishevelled 3 hanya menunjukkan aras yang sama berbanding dengan kawalan. Aras fosfo-β-catenin (relative kepada jumlah β-catenin) yang asas sebagaimana yang ditunjukkan oleh sel yang dijangkit oleh siRNA Dishevelled 3 tidak menunjukkan sebarang kaitan dengan aktiviti GSK3β dalam kajian ini. (5) Dengan mengaitkan penyataaan fosfo-β-catenin (relative kepada jumlah β-catenin) dalam analysis densitometrik dengan keputusan Luciferase assay, kurangnya kecendarungan dalam penguraian β-catenin (relative kepada jumlah β-catenin) mengakibatkan aras aktiviti TCF-LEF yang lebih tinggi bagi sel yang dijangkit oleh Dishevelled 1 dan Dishevelled 2. Namun, bagi sel yang dijangkiti oleh siRNA Dishevelled 3, tiada pertambahan panyataan fosfo-β-catenin (relatif kepada jumlah β-catenin) , meskipun terdapat kekurangan dalam aktiviti transkribsi TCF-LEF. Penyenyapan penyataan Dishevelled 3 hanya menunjukkan penyataan asas fosfo-β-catenin jika dibandingkan dengan kawalan. Penyenyapan penyataan Dishevelled 3 mengekalkan penyataan aras yang asas bagi fosfo-β-catenin dan pengekalan penyataan ini adalah memadai untuk penguraian dan pemisahan complex pemusnahan β-catenin yang mengakibatkan pengurangan aktiviti transkripsi yang diantara oleh TCF-LEF. Keputusan ini menyatakan kemungkinan bahawa gangguan interaksi antara Dishevelled 3 dengan kompleks pemusnah β-catenin dan bukannya Dishevelled 1 dan Dishevelled 2 yang mengakibatkan kurangnya aktiviti transcrpsi TCF-LEF yang diantara oleh Wnt3a. Ada juga kemungkinan bahawa terdapat gangguan oleh tapak promoter diantara β-catenin dan TCF-LEF oleh sel yang dijangkit oleh Dishevelled 3 siRNA. (6) Analisa densitometrik oleh Western Blot yang mengaitkan komponen laluan EGFR-ERK menunjukkan bahawa tiada sebarang pengaktifan laluan EGFR-ERK berlaku semasa stimulasi Wnt3a dan kejatuhan penyataan isoform Dishevelled todak mengakibat kurangnya aktiviti EGFR-ERK semasa stimulasi Wnt3a. Kesimpulannya, Dishevelled 3 terlibat dalam isyarat laluan Wnt berkanun dalam sel

kanser payu dara MDA-MB-231 dan memainkan peranan yang penting dalam merencat penguraian β-catenin. Terdapat kemungkinan bahawa jatuhnya penyataan Dishevelled 3 mengganggu interaksi dengan Axin, dan mengakibatkan kurangnya pengumpulan β-catenin dan akhirnya mengurangkan aktiviti transkripsi TCF-LEF. Terdapat juga kemungkinan bahawa penyenyapan Dishevelled 3 mengganggu aktiviti transkripsi TCF –LEF secara langsung. Sebaliknya, tiadanya kesan pengurangan aktiviti transkripsi TCF-LEF yang diantara oleh β-catenin dan juga kurangnya penyataan fosfo-β-catenin relatif kepada jumlah β-catenin bagi sel yang dijangkiti oleh siRNA Dishevelled 1 dan Dishevelled 2. Ini menunjukkan bahawa tiadanya penglibatan isoform Dishevelled 1 dan Dishevelled 2 dalam isyarat laluan Wnt berkanun dalam sel MDA-MB-231.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Seow Heng Fong, PhD
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Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Yip Wai Kien, PhD
Postdoctoral Researcher
Faculty of Medicine and Health Science
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School of Graduate Studies
Universiti Putra Malaysia

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

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Signature: _____
Name of Member of Supervisory Committee: Dr. Yip Wai Kien

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%	Percentages
°C	Degree Celsius
Akt	V-akt murine thymoma vral oncogene homolog 1
ANOVA	Analysis of Variance
APC	Adenomatous Polyposis Coli
BCL	B-cell lymphoma
b.p	base pairs
BRCA1	Breast Cancer 1, early onset
BRCA2	Breast Cancer 2, early onset
BSA	Bovine Serum Albumin
CAMKII	Ca^{2+}/calmodulin-dependent protein kinase
CBP	CREB Binding Protein
cDNA	complimentary deoxyribonucleic acid
CO_2	Carbon dioxide
Daam1	Dishevelled associate activator of morphogenesis 1
DAPI	4',6-diamidino-2-phenylindole
DEP	*Dishevelled*, Egl-10 and Pleckstrin domain
DEPC	Diethylpyrocarbonate
DIX	Dishevelled/Axin domain
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid.
dNTP	Deoxy-nucleotide-triphosphates
DSH	Dishevelled (short form for *Drosophila sp.*)
DTT	Dithiothreitol

DVL	Dishevelled (short form for *homo sapiens*).
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ER	Estrogen Receptor
ERK	Extracellular signal-regulated kinase
et. al	And all.
FBS	Fetal Bovine Serum
FZD	Frizzled
GSK3β	Glycogen Synthase Kinase 3 β
HCl	Hydrochloric acid
HER2	Human Epidermal Growth Receptor 2
HMGA2	High-mobility group AT-hook 2
HRP	Horse Radish Peroxidase
HRT	Hormone Replacement Therapy
IC	Inhibitory Concentration
IDC	Invasive Ductal Carcinoma
IGF	Insulin Growth Factor
JNK	c-Jun N-terminal Kinase
KCl	Potassium Chloride
LEF	Lymphoid Enhancer Factor
LiCl	Lithium Chloride
M	$moldm^{-3}$
MAPK	Mitogen Activated Protein Kinase
mRNA	Messenger ribomucleic acid
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4 sulfophenyl)-2H-tetrazolium

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NLK	Nemo-like kinase
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PAI	Plasminogen activator inhibitor
PARP	Poly ADP Ribose Polymerase
PBS	Phosphate buffered saline
PCP	Planar cell polarity
PCR	Polymerase chain reaction
PDZ	Postsynaptic Sensity as, disc large, zonula occludens
PEC	Polyelectrolyte complex
p-EGFR	Phosphorylated epidermal growth factor receptor
p-ERK	Phosphorylated extracellular signal-regulated kinase
p-GSK3β-Ser9	Phosphorylate glycogen synthase Kinase 3 beta serine 9
PI	Propidium iodide
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PR	Progesterone receptor
PVDF	Polyvinylidene difluoride
qPCR	Quantitative Polymerase Chain Reaction.
S.D	Standard Deviation
SH3	Src homology 3
siRNA	Small interfering ribonucleic acid.
TCF	T-cell factor
TGF-β	Transforming growth factor beta

TKNS	Tankyrase
TNBC	Triple negative breast cancer
VEGF	Vaso-endothelial growth factor
Wnt	Wingless-type MMTV integration site family

CHAPTER 1

INTRODUCTION

Introduction Breast cancer is the most common cancer amongst women which can result in death (Greenlee *et al.*, 2000). In the year 2013, 32,340 new cases of invasive breast cancer and 39,620 breast cancer deaths were reported in United States (DeSantis *et al.*, 2014). Current treatment options for breast cancer include mastectomy, radiotherapy and chemotherapy (Maughan *et al.*, 2010)

Wnt signaling pathway is a signal transduction pathway which plays an important role in embryonic development, generation of cell polarity and cell specification (Logan *et al.*, 2004). Aberrant activation of Wnt pathway leads to tumorigenesis (Giles *et al.*, 2003). The Wnt signaling pathway consists of canonical signaling pathway and non-canonical signaling pathway. The Wnt non-canonical signaling pathway can be further divided into Wnt planar cell polarity pathway (Wang, 2009) and Wnt calcium pathway (Kühl, 2004).

Wnt was discovered as an oncogene which contributes to mouse mammary tumorigenesis in mouse models of breast cancer. Aberrant activation of Wnt signaling pathway is also linked to tumorigenesis on human cancers (Brown, 2001) which caused by mutations of certain key components that are involved in Wnt signaling pathway. Mutation of certain components can be seen in colorectal cancer, namely APC (Adenomatous Polyposis Coli), a component of the canonical Wnt β-Catenin signaling pathway which plays an important role in β-catenin degradation (Axelrod *et al.*, 1998). Mutations of APC result in resistance of β-catenin to undergo the process of ubiquitinylation and phosphorylation (Bienz *et al.*, 2000). This leads to β-catenin accumulation in the cytoplasm which further results in activation of downstream pathways after T cell Factor (TCF) and Lymphoid Enhancer Factor (LEF) transcriptional activation in the nucleus (Morin *et al.*, 1997). Mutation of other components such as β-catenin itself and Axin also contributes in aberrant activation of canonical Wnt β-catenin pathway (Lammi *et al.*, 2004).

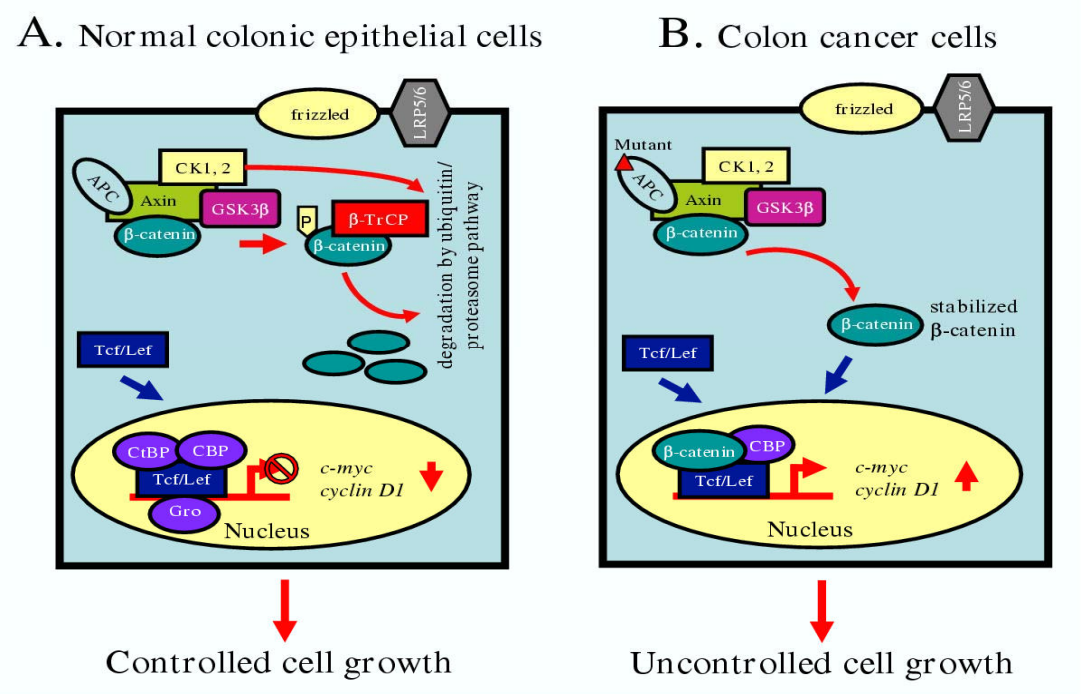

Figure 1 : Mutation of APC in Wnt Signaling Pathway. Mutation of APC resulted in the ability of APC to be involved in β-catenin degration complex (Narayan *et al.*, 2003). Increased stabilized β-catenin resulted in accumulation of β-catenin and translocation into the nucleus, where β-catenin forms a complex with TCF and LEF to activate TCF-LEF mediated transcriptional activation of downstream of Wnt target genes such as c-myc and cyclin D1.

Both canonical and non-canonical Wnt signaling pathways play a role in the pathogenesis of breast cancer metastasis (Dey *et al.*, 2013; Jiang *et al.*, 2013; Zhu *et al.*, 2012). Increased cytoplasmic and nuclear β-catenin levels contributes to an aberrant activation of canonical Wnt Signaling Pathway. Increased expression of cytoplasmic and nuclear β-catenin levels were found in breast cancer samples which indicate an aberrant canonical wnt signaling pathway activation (Prasad *et al.*, 2007). Further studies also showed that upregulation of Wnt β-catenin pathway correlates with elevated expression of Cyclin D1, which is one of the downstream targets of Wnt β-catenin pathway, contributing towards increased cell proliferation (Prasad *et al.*, 2007). On the other hand, β-catenin levels were not observed in normal breast tissues (Prasad *et al.*, 2007). Thus, this provides evidence that stabilized β-catenin also contributes in Wnt β-catenin pathway which results in tumorigenesis of breast cancer.

There are different ways to study the role of Wnt Signaling in breast cancer tumorigenesis by blocking different components that are involved in the pathway. One of the approaches used in previous studies is to block Wnt/FZD (Frizzled) interaction, which is located upstream of the Wnt signaling pathway (Matsuda *et al.*, 2009; Yang *et al.*, 2011). Blocking of this interaction caused a reduction in cell invasion and also in cell proliferation (Matsuda *et al.*, 2009; Yang *et al.*, 2011). Also, blockade of Wnt

signaling is also performed by increasing the expression of certain components involved in β-catenin degradation complex, which can facilitate in reduction of β-catenin levels.

Dishevelled is one of the components which is normally located in the cytoplasm. It is involved in both canonical and non-canonical Wnt signaling pathway. Dishevelled has been shown to play a role in the canonical and non-canonical Wnt signaling pathway in breast cancer cells by influencing cell proliferation, polarity, terminal differentiation and self renewal ability of stem cells (Zhao *et al.*, 2010; Zhu *et al.*, 2012). The expression of Dishevelled isoforms has been found in the cytoplasm and also in the nucleus of invasive ductal carcinomas (Prasad *et al.*, 2007).

In this study, human Dishevelled 1, 2 and 3 isoforms expression was silenced by using siRNA (small interfering RNA) in order to block the canonical or non-caninocal Wnt signaling pathway at the cytoplamic level. This ribonucleic acid which contains 21-23 nucleotide bases plays a role in RNA interference by binding specifically to mRNA (messenger RNA) in order to cleave a specific mRNA. The cleaved mRNA product results in further degradation by cytoplasmic nucleases. This reduces the mRNA levels in siRNA transfected cells which ultimately leads to reduced translation of target protein.

Our hypothesis is that silencing of human Dishevelled 1,2 and 3 mRNA expression leads to blockade in activation of canonical Wnt signaling pathway leading to reduced β-catenin interaction with TCF and LEF, reduced cell proliferation, migration and also in invasion regardless of canonical or non-canonical Wnt signaling pathway.

The overall objective of this study is to determine the role of Dishevelled 1, 2 and 3 in canonical Wnt signaling pathway in breast cancer using the gene silencing approach. The specific objectives are as follows:

1. To confirm the silencing of Dishevelled 1, 2 and 3 by reverse-transcription Quantitative PCR and Western Blotting.
2. To determine the changes in cell viability, migration, invasion, TCF-LEF mediated transcriptional activation after gene silencing using siRNA targeting Dishevelled isoforms.
3. To identify the changes in β-catenin, Glycogen Synthase Kinase 3β (GSK3 β) , Epidermal Growth Factor Receptor (EGFR) and Extracellular signal-Regulated Kinases (ERK) expression in cells transfected with siRNAs targeting Dishevelled 1, 2 and 3.

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