Significance of WNT molecules expression in Egyptian acute leukemia Patients

THESIS

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بسم الله الرحمن الرحيم

قَالُوْا سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا
إِنَّكَ أَنْتَ الْعَلِيْمُ الْحَكِيْم. البقرة(٣٢)

Abstract

Wnt proteins, derived from the names of two genes; *Drosophila Wingless* (Wg) and the *mouse Int-1 genes*, This novel family of proteins are intimately involved in cellular signaling pathways that play a role in a variety of processes that involve embryonic cell patterning, proliferation, differentiation, orientation, adhesion, survival, and apoptosis.

Several secreted protein families antagonize or modulate Wnt/β-catenin signaling, two of the most important antagonists are SFRP1& DKK3.

Functional loss of Wnt antagonists by promoter hypermethylation can contribute to activation of the Wnt pathway and results in carcinogenesis.

This study aimed to assess the role and the frequency of epigenetic silencing of the Wnt antagonists SFRP1 and DKK3 by promoter hypermethylation in B-ALL and AML patients by a method of methylation specific PCR.

Results: we found a significantly higher frequency of methylation in B-ALL and AML compared to control.

SFRP1 and DKK3 silencing, by promoter hypermethylation, is an early and a common event in the evolution of acute leukemia.

Patients with aberrant methylation phenotype may benefit from treatment with demethylating agents as this line of management expected to damp down the Wnt signaling activity and hence the activity of the disease.

This study also offers a preliminary basis for further studies to monitor hypermethylation of Wnt antagonists as a marker for minimal residual disease.

Key Words:

Wnt proteins - SFRP1 - DKK3 .

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List of abbreviations

AIDS	Acquired Immuno Deficiency Syndrome
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
APC	Adenomatous Polyposis Coli
APL	Acute Promyelocytic Leukemia
ATRA	all- trans. Retinoic Acid
BM	Bone Marrow
Bmp	Bone Morphogenic Protein
CBFs	Core Binding Factors
c- DNA	Cloned DNA
CGH	Comparative Genomic Hybridization
CGALB	Cancer And Leukemia Group B
CIMP	CPG island Methylator Phenotype
CK1	Casein Kinase 1
CML	Chronic Myeloid Leukemia
CMV	Cyto Megalo Virus
CNS	Central Nervous System
CR1	First Clinical Remission
CVAD	Cyclophosphamide, Vincristine, Adriamycine
DFz	Dorsophila- Frizzled
DIC	Disseminated Intravascular Coagulation
DKK	Dickkopf form german dick (thick), kopf (head)

DS	Down Syndrome
Dvl	Disheveled
EBV	Epstein- Barr virus
ER	Endoplasmic Reticulum
ET	Essential Thrombcythemia
FAB	French American British
FC	Flow Cytometry
FEVR	Familial Exudative Vitero-retinopathy
FGF	Fibroblast Growth Factor
FISH	Fleurescent In Situ Hybridization
Fz	Frizzled
GMALL	German Multicenter ALL
GSK3	Glycogen Kinase Synthase 3
HBM	High Bone Mass
HDAC	Histone Deacetylase
HLA-DR	Human Leucocyte Antigen – DR
IGFBP-4	Insulin like Growth Factor Binding Protein-4
IGH	Immunoglobulin Histochemisrty
IHC	Immunohistochemistry
Krm	Kremen
LDL	Low Density Lipoprotein
LOH	Loss Of Heterozygosity
LRP5/6	LDL- Receptor- related protein 5 and6
LSC	Leukemia Stem Cells
MDR1	Multi Drug Resistance protein1

MDS	Myelodysplastic Syndrome
miRs	micro RNAs
MLL	Mixed Lineage Leukemia
MPN	Myeloproliferative Neoplasm
MPO	Myelo Peroxidase
MRD	Minimal Residual Disease
M-DKK3	Methylated-DKK3
M-SFRP1	Methylated-SFRP1
NK	Natural Killer
NOS	Not Otherwise Specified
OPPG	Osteoporosis Pseudo Glioma
PAS	Periodic Acid Schiff
PCP	Planer Cell Polarity
PORCN	Porcupine
PMF	Primary Myelo Fibrosis
PML	Promyelocytic Leukemia
PPPSPxs	P; proline, S; serine or threonine, x; available residue
PV	Polycythemia rubra Vera
RGS	Regulator of G protein Signaling
REIC	Reduced Expression In Cancer
RQ- PCR	Reverse transcriptase Polymerase Chain Reaction
Rspo	R- spondin
SCT	Stem Cell Transplantation
SFRPs	Secreted Frizzled Proteins
SNP	Single Neucleotide Polymorphism

(t)-AML	Treatment- related AML
TCF/LEF	T cell Factor/ Lymphoid Enhancer Factor
TdT	Terminal deoxynucleotidyl Transferase
TLS-ERG	Translocation Liposarcoma gene fused to ETS- related gene
t- MDS	Treatment –related MDS
UKALL	United Kingdom ALL
UN-DKK3	Unmethylated-DKK3
UN-SFRP1	Unmethylated-SFRP1
WBC	White Blood Cell
WHO	World Health Organization
3' UTR	3' Untranslated

Introduction and aim of the work

Introduction:

Wnt proteins, derived from Drosophila Wingless (Wg) and the mouse Int-1 genes, represent a large family of secreted cysteine-rich glycosylated proteins. This novel family of proteins is intimately involved in cellular signaling pathways that play a role in a variety of processes that involve embryonic cell patterning, proliferation, differentiation, orientation, adhesion, survival, and apoptosis (**Nelson and Nusse, 2004).** Convincing evidence has established a crucial role for Wnt signaling in the maintenance and self-renewal of hematopoietic stem cells (HSC) **(Nemeth and Bodine, 2007)**.

Constitutive activation of the Wnt pathway has been found in solid tumors as well as haematopoietic malignancies **(Clevers, 2004; Sansom et al, 2004)**. Over activation of Wnt signaling cascade have been demonstrated to have a role in leukemia pathogenesis; and dysregulation of the pathway seems to lead to a gain of self-renewal capacity of progenitor cells, resulting in the promotion of different forms of leukemia **(Khan and Bendal, 2006; Deshpande and Buske, 2007; Zhao, et al., 2007)**.

WNT signaling is controlled by a number of natural Wnt antagonists that interfere with the ligand-receptor interaction, including members of the Dikkopf (DKK) family and the secreted frizzled-related protein (SFRP) family **(Reya et al., 2003)**.

The human Dkk-3 gene, located on chromosome 11p15.1 is a recently found mortalisation-related gene, It has been determined that Dkk-3 possesses an antiproliferative activity against tumour cells, suggesting that Dkk- 3 may function as a tumour suppressor, and that its effect seems to be mediated by its ability to antagonize Wnt signaling **(Taipale and Beachy, 2001 and Tsuji et al, 2001)**. DKK-3 expression is largely attenuated in many immortalized and tumour derived cell lines **(Tsuji et al, 2000)**.

The family of SFRPs belongs to a group of proteins antagonizing the Wnt signaling pathway by interaction with the Wnt receptor. The functional role of SFRPs in normal and malignant haematopoiesis has not yet been

systematically investigated. Four of the five known SFRP genes are characterized by a CpG island in the promoter region.

A loss of function of tumour suppressor genes can result from mutations, chromosomal deletions or epigenetic dysregulation **(Jost et al, 2006).** The best studied epigenetic mechanism for silencing of cancer-related genes is hypermethylation of CpG islands in the promoter region of genes. CpG island hypermethylation of tumour suppressor genes has been described in almost all solid and haematopoietic malignancies **(Herman and Baylin, 2003; Galm et al, 2006).**

SFRP promoter hypermethylation is a frequent event in solid tumours, as was shown especially for SFRP1 and SFRP2 in colorectal cancer cells **(Suzuki et al, 2004)**. Some studies proposed the epigenetic silencing of negative regulators of the Wnt signaling pathway may affect the Wnt regulatory proteins; (DKK) and (SFRPs) **(Chim et al, 2006; Roman-Gomez et al, 2007).**

Functional loss of Wnt antagonists can contribute to activation of the Wnt pathway and result in carcinogenesis through deregulation of cell proliferation and differentiation. Recent studies have shown that impaired activation of Wnt antagonists such as sFRP1 and DKK3 by promoter hypermethylation is present in several human malignancies **(Mazieres et al., 2004 and Batra et al., 2006)**.

However, little is known about the potential role of promotor hypermethylation of Wnt antagonists (SFRP1, DKK3) in leukemia.

Aim of the work:

This study aimed to analyze the frequency and the possible impact of epigenetic hypermethylation of the SFRP1 and DKK3 genes in acute leukemia patients.