

Evaluation of Vitamin D Receptor Gene Methylation in Hepatocellular Carcinoma

Thesis

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By

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ABSTRACT

Liver cancer in adult men is the fifth most frequently diagnosed cancer worldwide, and is the second leading cause of cancer-related death in the world. The genomic actions of vitamin D are mediated through its binding to the Vitamin D Receptor (VDR). In the current study we planned to evaluate the methylation of vitamin D receptor for the first time in hepatocellular carcinoma, to investigate the possibility that methylation-induced silencing of VDR in Hepatocellular carcinoma might account for the Vitamin D insensitivity.

Subjects were categorized into three groups, Hepatocellular carcinoma, chronic liver lesions diagnosed after exclusion of malignancy by pathology and apparently normal liver. Subjects were recruited from referrals of biopsies. Methylation specific PCR was done on all samples using methylated and unmethylated VDR primers. There was a highly significant difference between the three groups as regard Vitamin D Receptor Gene Promoter methylation percentage, $p < 0.01$; demonstrating VDR gene promoter methylation as a potential diagnostic biomarker to differentiate between Hepatocellular Carcinoma and other non malignant lesions.

KEYWORDS: Hepatocellular Carcinoma, Vitamin D Receptor Gene, Methylation, Diagnostic.

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List of Abbreviations

χ^2	Chi-Square
$\mu\text{g/ml}$	Microgram/millilitre
μl	Microliter
1,25(OH)₂D	1 α ,25-dihydroxyvitamin D
AE	Elution Buffer
AF	Activation Function
AFB1	Aflatoxin B1
AFP	α -fetoprotein
AL	Lysis Buffer
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AUC	Area Under Curve
AW	Washing Buffer
AZA	5'deoxy-Azacytidine
BCL2	B Cell Lymphoma 2 gene
BCLC	Barcelona Clinic Liver Cancer
BCL-X_L	B Cell Lymphoma extra large
Ca²⁺	Calcium
cAMP	Cyclic Adenosine Monophosphate

CDH13	Cadherin 13 gene
CDKN1A	Cyclin Dependant Kinase N1A
CLD	Chronic Liver Disease
CLIP	Cancer of the Liver Italian Program
CpG	Cytosine Guanine (linear dinucleotide)
CT	Computerised Tomography
CYP24A1	Cytochrome P450 family 24 subfamily A member 1
CYP27B1	Cytochrome P450 family 27 subfamily B member 1
dATP	Deoxyadenosine Triphosphate
DBD	DNA Binding Domain
dCTP	Deoxycytosine Triphosphate
DEPC	Diethylpyrocarbonate
dGTP	Deoxyguanosine Triphosphate
DMH	Dimethylhydrazine
DNA	Deoxyribo Nucleic Acid
dNTP	Deoxynucleotide Triphosphate
DR3	Death Receptor 3
dTTP	Deoxythymidine Triphosphate
FGF23	Fibroblast Growth Factor 23
FN	False Negative
FP	False Positive

GADD45	Growth Arrest and DNA Damage 45
GSTP1	Glutathione S-transferase P 1 gene
HAI	Histology Activity Index
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HCV Ab	Hepatitis C Virus Antibody
HM	Hypermethylated
hMLH1	Human mutL homologue 1
IL8	Interleukin 8
IM	Intermediately Methylated
INK4	Inhibitor of Cyclin dependant Kinase 4
kbp	Kilo base pair
kDa	Kilo Dalton
LBD	Ligand Binding Domain
M	Methylated
M₀	Mock(no enzyme)
M_s	Methylation Sensitive Restriction Enzyme
M_d	Methylation Dependant Restriction Enzyme
M_{sd}	Double Restriction Endonuclease Digestion
MDRE	Methylation Dependant Restriction Endonuclease
mg	Milligram

MGMT	Methylguanine Methyl Transferase
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribo Nucleic Acid
MSRE	Methylation Sensitive Restriction Endonuclease
MYC	Myelocytomatosis viral oncogene homolog
ng/ml	Nanogram/millilitre
nm	Nanometer
NPV	Negative Predictive Value
NR	Nuclear Receptor
OD	Optical Density
p	Significance
PCR	Polymerase Chain Reaction
PEI	Percutaneous Ethanol Injection
PH	Portal Hypertension
P_i	Inorganic Phosphate
PI3K	Phosphatidylinositol 3 Kinase
PPV	Positive Predictive Value
PS	Performance Status
PT	Prothrombin Time
PTH	Parathyroid Hormone
r	Correlation Coefficient
RARβ	Retinoic Acid Receptor beta
RASSF1A	Ras association domain family 1A gene

RFA	Radiofrequency Ablation
ROC	Receiver Operating Characteristics
rpm	Revolutions per minute
RXR	Retinoic X Receptor
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
TACE	Transarterial Chemoembolisation
TERT	Telomerase Reverse Transcriptase
THBS1	Thrombospondin 1
TK1	Thymidine Kinase 1
TN	True Negative
TP	True Positive
TYMS	Thymidylate Synthetase
UM	Unmethylated
US	Ultrasound
VDR	Vitamin D Receptor
VDRE	Vitamin D Response Element
VEGF	Vascular Endothelial Growth Factor
Xg	Relative centrifugal force (to Earth's gravity)
ΔCT	Comparative threshold cycle

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