UNIVERSITI PUTRA MALAYSIA

HUMAN HEPATITIS B VIRAL PROTEINS HBX AND HBE: ROLES IN HepG2 CELL LINE SURVIVAL AND CELL DEATH

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HUMAN HEPATITIS B VIRAL PROTEINS HBX AND HBE: ROLES IN HepG2 CELL LINE SURVIVAL AND CELL DEATH

By

ANDREA LISA HOLME

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree
of Doctor of Philosophy

January 2004

DEDICATION

In Loving Memory

Of

Elizabeth Christie Holme

Abstract of this thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

HUMAN HEPATITIS B VIRAL PROTEINS HBX AND HBE: ROLES IN CELL SURVIVAL AND CELL DEATH

By

Andrea Lisa Holme

January 2004

Chairman: **Professor Datin Farida Jamal, M.B.B.S., M.Sc., M.R.C. Path.**

Faculty: **Medicine and Health Sciences**

Existing reports of viral hepatitis, resulting in liver cell death have not been fully explained with regards to the mechanism of the viral proteins involved. The objective of the study is to determine if any of the Hepatitis B viral proteins cause changes in the survival of human hepatocytes and if so by what means. The two main candidates for inducing survival changes were the precore proteins (HBE) and HBX, both of which have been reported to accumulate in the liver of patients and to trigger an immune response. The human liver HepG2 cell line was chosen to study the effect of these proteins during transient expression. The results from this study show that both viral proteins can induce cell death by an apoptotic mechanism via caspases. HBX appears to trigger more cell death than HBE, while HBE-induced an initial proliferation of the cell culture followed by cell death. HBX-induced apoptosis appears to involve both extrinsic and intrinsic cell death systems through the Fas

system and the mitochondria, respectively. There is also a total loss of the PI3K/Akt pathway surivial signals. The HBE-induced apoptosis appears to be through DNA damage triggering an intrinsic cell death program, coupled with a partial loss of the PI3K/Akt pathway that allows GSK3β to be activated, while keeping FHKR inactive. In both cases, the viral cell death can be prevented using the correct dosage of IL-6 stimulation, while loss of serum or the addition of ethanol can have an overall positive effect on the viability of HBX and HBE transfected cells. The deaths can also be prevented in varing degress by the inhibition of MEK1 and PP1A/2A suggesting these pathways are involved probably by cross talking.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai syarat memenuhi keperluan untuk Ijazah Doktor Falsafah

PROTIN HBX DAN HBE VIRUS HEPATITIS B MANUSIA: PERANAN DALAM KEHIDUPAN SEL DAN KEMATIAN SEL

Oleh

Andrea Lisa Holme

Januari 2004

Pengerusi: Profesor Datin Faridah Jamal, M.B.B.S., M.Sc., M.R.C. Path.

Fakulti: Perubatan Dan Sains Kesihatan

Laporan tentang virus hepatitis yang mengakibatkan kerosakan hati belum lagi dijelaskan dengan sepenuhnya dalam aspek mekanisma dan peranan protin virus yang terlibat. Projek ini bertujuan menyiasat sebarang protin virus hepittitis B yang mempengaruhi kehidupan sel hati manusia dan, jika ada bagaimana protin tersebut berfungsi. Dua protin yang memainkan peranan penting adalah protin precore (HBE) dan HBX. Kedua- dua protin tersebut telah dilaporkan terkumpul di dalam hati pesakit dan akan merangsangkan respon keimunan. Sel kanser hepatoblastoma, HepG2, telah dipilih untuk menyiasat kesan protin tersebut semasa transcient ekspesi. Keputusan menunjukan kedua-dua protin virus itu dapat merangsangkan kematian sel melalui mekanisma yang bergantung kepada caspase. HBX didapati merangsangkan kematian sel yang banyak berbanding dengan HBE. Manakala, HBE merangsangkan fasa awal pembahagian sel diikuti dengan kematian sel. Perangsangan apoptosis oleh HBX melibatkan sistem

kematian sel ekstrinsik dan intrinsik melalui sistem Fas dan mitokondria masing-masing. Terdapat juga kehilangkan isyarat kehidupan bagi perjalanan PI3K/Akt. Kematian sel akibat daripada HBE adalah disebabkan oleh kerosakan DNA yang seterusnya merangsangkan program kematian sel intrinsik. Bersamaan kejadian tersebut, terdapat kehilangan separa dalam perjalanan PI3K/Akt yang membolehkan keaktifan GSK3β tanpa mengaktifkan FHKR. Kesan kematian sel akibat daripada kedua-dua protin virus ini dapat diterbalikkan dengan sukatan IL-6 tertentu. Manakala, kehilangan serum atau penambahan etanol boleh membawa kesan positif ke atas viabiliti sel yang dijangkiti HBX dan HBE. Darjah penyongsangan kematian sel dipengaruhi oleh penyahaktifan MEK 1 dan PP1A/2A. Kesimpulannya, kedua-dua protin virus tersebut berkerjasama merangsangkan kematian sel yang dapat dipengaruhi oleh factor-faktor luaran.

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I certify that an Examination Committee met on 5th January 2004 to conduct the final examination of Andrea Lisa Holme on her Doctor of Philosophy thesis entitled "Human Hepatitis B Viral Proteins HBX and HBE: A Role in Cell Survival and Cell Death" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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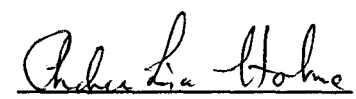
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



ANDREA LISA HOLME

Date: 5th January 2004

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4E-BP	eIF-4E binding protein
Abl	Ableson protein tyrosine kinase
AKT	Cellular homolog of the v-akt oncogene, an S/T protein kinase
Apaf-1	Apoptotic protease activating factor-1
ASK	Apoptosis signal-regulating kinase
Bcl	B cell leukemia oncogene
Caspase	Cysteine proteases with aspartate specificity
CBP	CREB binding protein
CDK	Cyclin-dependent kinase
c-Raf	Raf proto-oncogene S/T protein kinase
CREB	cAMP response element-binding protein, CREB1
DAG	Diacylglycerol
DAPI	4', 6-Diamidino-2-phenyindole
DED	Death Effector Domain
DR	Death receptor
E2F	Transcription factor family including E2F- and DP-like subunits
eEF	Eukaryotic elongation factor
eIF	Eukaryotic initiation factor
ELK1	Ets domain protein
ERK	Extracellular signal-regulated kinase, MAPK
FADD	Fas-associated protein with death domain
FAK	Focal adhesion kinase

FasL	Fas Ligand
FasR	Fas Receptor
FKHR	Forkhead in rhabdomyosarcoma
FLIPs	FLICE (Caspase 8) inhibitory protein
GSK-3β	Glycogen synthase kinase-3β
HBE	all precursor protein forms of Hepatitis B virus
HBeAG	secreted precursor protein
HepG2-HBE	HepG2 transfected cells with HBEpTARGET™ vector
HepG2-HBX	HepG2 transfected cells with HBXpTARGET™ vector
IAP	Inhibitor of apoptosis
ICAD	Inhibitor of caspase-activated deoxyribonuclease
IkB	Inhibitor of NF-kB
IKK	IkB kinase
INK4	Inhibitor of CDK 4
IRS	Insulin receptor substrate
ISRE	Interferon-stimulating response element
Jak	Janus-family tyrosine kinase
JNK	Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MEK	MAPK/ERK kinase, MAPKK
MEKK	MEK kinase
MLK	Mixed lineage kinase
MTT	Methylthiazoletetrazolium
NF-kB	Nuclear factor kappa B

NIK	NF-kB Induced kinase
NOS	Nitric oxide Synthase
p53	Tumour suppressor protein that protects from DNA damage
PDK	3-phosphoinositide-dependent protein kinase
PH	Pleckstin homology domain
PI3K	Phosphoinositide-3 kinase
PIAS	Protein inhibitors of activated STATs
PKA	Protein kinase A
PKC	Protein kinase C
PKR	dsRNA-dependent serine/threonine protein kinase
PP1	Phosphoprotein phosphatase 1
PP2A	Phosphoprotein phosphatase 2A
PP2B	Phosphoprotein phosphatase 2B
PYK2	Proline-rich tyrosine kinase-2
PCR	Polymerase Chain Reaction
RAIDD	RIP-associated ICH/CED-3-homologous protein with a death domain
RIP	Receptor-interacting protein
SAPK	Stress-activated protein kinase
Shc	SH2-containing collagen-related proteins
Smad	Contraction of Sma and Mad (Mothers against decapentaplegic)
TEN	Phosphatase and tensin homolog deleted on chromosome ten
TNF	Tumor necrosis factor
TRADD	TNF receptor-1-associated death domain protein