MUTATIONAL ANALYSIS OF THE HUMAN FACTOR IX PROMOTER

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Although haemophilia provides for an expansive area of research, I hope that this topic will not fall to the wayside of more politically favoured or "trendy" subjects as researchers struggle to attain funding.

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Abstract

Haemophilia B is a rare congenital bleeding disorder that affects 1 in 30,000 males. It is caused by a functional deficiency in the blood coagulation protein, factor IX, which is expressed primarily within the liver. Patients suffering from the Haemophilia B Leyden phenotype show a distinct pattern of factor IX expression that is characterised by severe to moderate haemophilia within children, which gradually ameliorates after puberty. Such deficiencies in factor IX are created by mutations that occur within the -22 to +13 region of the factor IX promoter. These mutations are responsible for down-regulating factor IX transcription leading to factor IX deficiency by disrupting the binding sites of transcription factors critical for factor IX gene expression. Three specific transcription factors, C/EBP, DBP and HNF4 are thought to be required for constitutive promoter expression.

The aim of this thesis was to analyse the roles of these three transcription factors in the regulation of the factor IX promoter. The current studies were focused on two regions (-220 to -202 and +20 to +45) of the factor IX promoter which have been implicated in transcriptional activation. Reporter gene assays using the human hepatoma cell line, Alexander, were carried out on both normal and mutant promoter constructs. Recognition sites for each of the three transcription factors were disrupted by oligonucleotide-directed PCR mutagenesis. The mutated promoter inserts were subsequently inserted into the luciferase reporter gene expression vector, pGL2 Basic (Promega). These constructs were then expressed within the Alexander cell line to compare the extent of transcriptional disruption created by each mutation. EMSA studies were also used to analyse the binding ability of the HNF4 transcription factor to the -6 region of the factor IX promoter.

Mutations within the -220 to +45 region of the factor IX promoter downregulated transcription from the promoter to different extents. This suggested that each transcription factor may play a different role in regulating the factor IX promoter. An increase in promoter expression observed with mutant constructs in the presence of exogenous HNF4 confirmed previous experiments which suggested that the HNF4

transcription factor could also act as an activator of promoter expression. Furthermore, the transactivation of the promoter constructs containing a mutation within the main HNF4 site at region -15 to -30 with exogenous HNF4, indicated that a second HNF4 site may be present within the factor IX promoter.

Abbreviations

A	adenine
AR	androgen receptor
ARE	androgen receptor element
bp	base pair
BRL	Bethesda research laboratories
C	cytosine
CAT	chloroamphenicol acetyl transferase
C/EBP	CCAAT enhancer binding protein
DBP	D-site binding protein
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DNase I	deoxyribonuclease I
dNTP	deoxynucleotide triphosphate
DTT	dithiothreitol
E.coli	*Esherichia coli*
EDTA	ethylene diamine tetra-acetate
EMSA	electrophoretic mobility shift assay
G	guanine
HNF4	hepatocyte nuclear factor 4
IL-6	interleukin-6
kb	kilobase
kDa	kilodalton
ng	nanogram
MEM	minimal essential media
MPC	magnetic particle concentrator
mRNA	messenger ribonucleic acid
NF-1	nuclear factor 1
ONPG	o-Nitrophenol B-D-Galacto-pyranoside
PBS	phosphate buffered saline

PBSE	phosphate buffered saline EDTA
PCR	polymerase chain reaction
Pfu	*Pyrococcus furiosus*
PIC	pre-initiation complex
poly (dI-dC)	poly (dI-dC) poly (dI-dC)
RLU	relative light units
SDS	sodium dodecylsulphate
T	thymine
TAE	Tris acetate EDTA
Taq	*Thermus aquaticus*
TBE	Tris Boric acid EDTA
TEMED	N, N, N', N'-Tetramethylethylenediamine
TFIIA	transcription factor II A
TFIIB	transcription factor II B
TFIID	transcription factor II D
TFIIE	transcription factor II E
TFIIF	transcription factor II F
Tris	Tris-(hydroxymethyl) aminomethane
UV	ultraviolet
Xq27	chromosome X, long arm band 27

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