Regulation of Topoisomerase IIα Expression in Humans

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Abstract

In mammalian cells, the loss or down-regulation of tumour-suppressor genes and/or the mutation or overexpression of proto-oncogenes, whose products promote unregulated proliferation in cells, characterise the process of malignant transformation. This generates mitogenic signals that promote abnormal cell growth resulting in tumour progression. Topoisomerase IIα (topo IIα) is an enzyme present in elevated concentrations in highly proliferating cells due to the requirement for untwisting and unknotting of the DNA which is essential for replication. Because of this requirement, a number of anti-cancer drugs have been designed with topo IIα as their primary target. The effectiveness of these drugs however is limited by the development of resistance. One factor linked to drug resistance is the down-regulation of topo IIα at the transcription level. Expression of topo IIα appears to be regulated through various transcription factors with members of the Sp1 family having a major contribution.

Previous work has shown down regulation of topo IIα can occur at the level of transcription. Nucleotide sequencing of the topo IIα promoter in drug-resistant cell lines has not revealed any mutations thus far. Three known proteins and one uncharacterised protein are capable of interacting with the proximal topo IIα promoter region. The uncharacterised protein may act as a co-activator or a co-repressor depending on the complement of transcription factors associated with the DNA in this region. Because drug resistant cell lines showed modulated expression of these transcription factors, it is important to identify the unknown protein and characterise its role in regulating topo IIα expression.

This research aimed to identify the minimal binding site and DNA elements required for the uncharacterised protein to bind, as well as introduce mutations into this proximal region and examine their functional significance. The results of this study could provide insights into the molecular mechanisms responsible for the development of drug resistance, contributing to more efficient and effective methods for the treatment of cancer.

Abbreviations

Amp	Ampicillin
ATP	Adenosine triphosphate
β-gal	β-galactosidase
bp	Base pairs (DNA)
BSA	Bovine serum albumin
CDE	Cell-cycle dependent element
cpm	counts per minute
CTD	C-terminal domain
DMSO	Dimethyl sulfoxide
DNA	deoxyribose nucleic acid
dNTP	deoxynucleoside triphosphate (dATP, dTTP, dGTP, dCTP)
EDTA	Ethylene diamine tetra-acetic acid
EMSA	Electrophoretic mobility shift assay
FCS	Fetal calf serum
GC1	GGGCGGG box
GC2	GGGGCGGGG box
GCG	Genetics computer group
GFP	Green fluorescent protein
G segment	Gated segment (DNA)
HAT	Histone acetyl transferases
HeLa	Human cervical carcinoma cells (Helen Lane)
HTETOP	Human fibrosarcoma cell line HT180, tTA-expressing topo IIα
ICB	Inverted CCAAT box
kb	kilobases
LB	Luria Bertani bacteriological media
MCS	Multiple cloning site
MDR1	Multidrug resistance gene
MEM	Eagle's minimal essential media
mt	mutated/mutant

NES	nuclear export signal
NLS	nuclear localisation signal
NF-Y	nuclear factor Y
ONPG	o-Nitrophenyl β-D-Galacto-pyranoside
PAGE	Polyacrylimide gel electrophoresis
p53	Tumour suppressor protein
PBS	Phosphate buffered saline
PBSE	Phosphate buffered saline plus EDTA
PCR	Polymerase chain reaction
pGL3B	pGL3 Basic vector
PIC	preinitiation complex
RNase	Ribonuclease
RT	Room temperature
Sp1	Specificty protein 1
Sp3	Specificity protein 3
STET	Sucrose, Tris, EDTA and triton-X buffer
SV40	Simian virus 40
T segment	Transport segment (DNA)
TAE	Tris acetate EDTA buffer
TAFs	TBP associated factors
TATA	TATA box; conserved A/T rich septameter transcriptional sequence
TBE	Tris borate EDTA
TBP	TATA binding protein
TE	Tris-EDTA buffer
TEMED	N,N,N',N'-Tetramethylethylenediamine
TEN	Tris-EDTA buffer with sodium
TIFs	Transcription initiation factors
TFIID	Transcription initiation factor complex; TBP and TAFs
TF	Transcription factor
UV	Ultra-violet light
wt	wild type

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