Beeke Wienert

v-Myb proteins and their oncogenic potential

A study on how two point mutations affect the interaction of v-Myb with other proteins

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Abbreviations

α	anti- (antibody against)
aa	amino acid(s)
AMV	avian myeloblastosis virus
APS	ammonium peroxodisulphate
ATP	adenosine triphosphate
β-Gal	β-galactosidase
bp	base pairs
CARM1	coactivator-associated arginine methyltransferase 1
C/EBP	CCAAT/enhancer-binding protein
CBB	Coomassie Brilliant Blue
CBP	CREB-binding protein
cDNA	complementary DNA
CMV	cytomegalovirus
DBD	DNA-binding domain
DHS	DNase I hypersensitive site
DNA	desoxyribonucleic acid
dNTP	desoxynucleotide
DTT	1,4-dithio-DL-threitol
E26	avian leukaemia virus
ECL	enhanced chemiluminescence
E. coli	*Escherichia coli*
EDTA	ethylene diamine tetraacetat
ELB	egg lysis buffer
FL	full length
g	gram
GFP	Green fluorescent protein
GRP78	Glucose regulated protein 78
GST	glutathione-S-transferase
h	hour(s)
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIPK2	Homeodomain-interacting protein kinase 2

I	isoleucine
IPTG	isopropyl-β-D-1-thiogalactopyranoside
kb	kilo base pairs
kDa	kilodalton
L	litre
LB	Luria broth
M	mol/L
MBS	Myb binding site
MCS	multiple cloning site
mim-1	*myb-inducible myelomonocytic 1*
min	minute
MRE	Myb-responsive element
NP-40	Nonidet 40 (octylphenoxypolyethoxyethanol)
NRD	negative regulatory domain
OD	optical density
PAA	polyacrylamide
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Pdcd4	Programmed cell death 4
PMSF	phenylmethylsulfonyl fluoride
PRMT4	Protein arginine methyltransferase 4
R	arginine
rpm	revolutions per minute
RT	room temperature
SBM	SUMO binding motif
SDS	sodium dodecyl sulphate
SIM	SUMO interacting motif
SUMO	Small ubiquitin-related modifier
TAD	transactivation domain
TAE	Tris acetic acid EDTA
Taq	*thermus aquaticus*

TE	Tris EDTA
TEMED	tetramethylethylenediamine
UPR	unfolded protein response
UV	ultra violet
V	valine
(v/v)	volume percentage per total volume
WB	Western Blot
wt	wild type
(w/v)	weight per volume
X	any amino acid
YFP	Yellow fluorescent protein

1 Abstract

The oncogene *v-myb* of the retroviruses AMV (avian myeloblastosis virus) and E26 (avian leukaemia virus) encodes a transcription factor (v-Myb) which is a truncated homolog of its cellular progenitor c-Myb. c-Myb plays an essential role in the development of haematopoietic cells and is known to be a regulator for many target genes. v-Myb AMV is responsible for the transformation of myelomonocytic cells and arresting them in an immature stage, presumably because of deregulation the expression of specific target genes. In addition to truncation of the coding region a number of amino acid substitutions are responsible for the high oncogenicity of v-Myb AMV. Due to the amino acid substitutions v-Myb AMV and v-Myb E26 differ in their target gene spectrum. The chicken *mim-1* gene is activated by v-Myb E26 and c-Myb but not by v-Myb AMV. The gene consists of two *cis*-regulatory regions, a Myb responsive promoter and cell-specific Myb-inducible enhancer. Recently it was shown that two amino acid substitutions in a hydrophobic patch in the transactivation domain of v-Myb AMV are sufficient to disrupt its ability to stimulate the enhancer.

This work focused on the consequences of these amino acid substitutions by investigating protein-protein interactions of the hydrophobic region of v-Myb AMV in comparison to v-Myb E26. Previous experiments identified GRP78 as an interaction partner of v-Myb. In this study a cytosolic variant of GRP78, GRP78va, was confirmed to interact with both v-Myb proteins. It was shown that its interaction site is limited to a very small region of v-Myb preceding the hydrophobic patch. Additionally, it was shown that GRP78va associated with all other members of the Myb-family and also with C/EBPβ and HIPK2 suggesting a non-sequence-specific binding of GRP78va. Furthermore, reporter gene experiments demonstrated a repressing effect of GRP78va on the transactivation potential of v-Myb E26.

In addition, GST-pull down assays and co-immunoprecipitation experiments were used to precipitate endogenous proteins that could represent potential interaction partners of v-Myb. SDS-PAGE analysis revealed candidate bands but mass spectrometry analysis failed to identify any proteins relevant for interaction with v-Myb.

Two other proteins were tested for their interaction with the hydrophobic patch of v-Myb. Co-immunoprecipitation experiments confirmed that C/EBPβ interacts with the hydrophobic region of v-Myb and that the amino acid substitutions seem to affect the interaction in a

negative way. Furthermore, PRMT4 was identified as an interaction partner of v-Myb Mapping experiments showed the interaction to be mediated by hydrophobic region. The point mutations in v-Myb AMV appear to positively influence the affinity for PRMT4. The fact that a SUMO binding motif is located in the same region might suggest a potential involvement of SUMO in the interaction of PRMT4 and v-Myb.