The genetic and biochemical analysis of *Drosophila* Wwox protein function

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Declaration

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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Abbreviations

%:	percentage
°C:	degrees celsius
µg:	microgram
µl:	microliter
µM:	micromolar
1°:	primary
1-DE:	one-dimensional electrophoresis
2°:	secondary
2-DE:	two-dimensional electrophoresis
2D:	two dimensional
3D:	three dimensional
aa:	amino acid
ACN:	acetonitrile
ATP:	adenosine triphosphate
bp:	base pair
BSA:	bovine serum albumin
BVA:	biological variation module
CHAPS:	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CID:	collision-induced dissociation
cM	centimetre
Cy:	Cyanine
Da:	Dalton
da:	*daughterless*
DIGE:	direct in gel analysis
DNA:	deoxyribonucleic acid
dNTP:	deoxynucleoside triphosphate
DTT:	dithiothreitol
EDA:	extended data analysis
EDTA:	ethylenediaminetetraacetic acid
ELISA:	enzyme-linked immunosorbent assay
emPAI:	exponentially modified protein abundance index
ESI:	electro spray ionisation
EtOH:	ethanol

FA:	formic acid
GE:	General Electric
H_2O:	water
HCA:	hierarchical clustering analysis
HCCA:	α-cyano-4-hydroxycinnamic acid
HPLC:	high performance liquid chromatography
IEF:	isoelectric focusing
IMVS:	Institute of Medical and Vetenary Science
IPG:	immobilised pH gradient
KCl:	potassium chloride
kDa:	kilodalton
LC:	liquid chromatography
M:	molar
mA:	milliampere
MALDI:	matrix assisted laser desorption ionisation
ml:	millilitre
mm:	millimetre
mM:	millimolar
MQ:	MilliQ
mRNA:	messenger RNA
MS:	mass spectrometry
MS/MS:	tandem mass spectrometry
m/z:	mass-to-charge
N:	number of replicates
NaC:l	sodium chloride
$NaPO_4$:	sodium phosphate
NCBI:	National Centre for Biotechnology Information
ng:	nanogram
nl:	nanolitre
ORF:	open reading frame
p:	pico
PAGE:	polyacrylamide gel electrophoresis
PAI:	protein abundance index
PBS:	phosphate buffered saline
PC1:	first principal component
PC2:	second principal component

PCA:	principal component analysis
PCR:	polymerase chain reaction
pH:	hydrogen ion concentration
pI:	isoelectric point
ppm:	parts per million
PTM	post-translational modification
RNA:	ribonucleic acid
RO:	reverse osmosis
rpm:	revolutions per minute
SDS:	sodium dodecyl sulfate
SDR:	short-chain dehydrogenase reductase
S/N:	signal to noise
SOC:	Super Optimal broth plus glucose (originally for Catabolite repression
TBE:	Tris/boric acid / EDTA buffer
TBST:	Tris-buffered saline Tween-20
TFA:	trifluoroacetic acid
TOF:	time of flight
Tris:	Tris (hydroxymethyl) aminomethane
U:	units
UAST:	upstream activation sequence
UV:	ultraviolet
V:	volts
v/v:	volume per volume
w/v:	weight per volume
w/w:	weight per weight
WWOX / Wwox:	WW domain containing oxidoreductase
X-Gal	X-galactoside (5-bromo-4-chloro-3-indolyl-β-D-galactoside; BCIG)

Abstract

WWOX (WW domain-containing oxidoreductase) is a candidate tumor suppressor gene that has been shown to be involved in various cancers including breast, lung, prostate, gastric and hepatic. The *Drosophila* ortholog *Wwox* was identified and subjected to targeted 'loss of function' mutagenesis. The resulting mutants were found to be viable when homozygous with no obvious defects in the adult fly. As *Wwox* mutant flies were found to exhibit an increased sensitivity to ionising radiation (IR), a number of Wwox proteins specifically deleted or mutated at positions consisting of conserved functional protein motifs, or regions that are highly conserved among WWOX / Wwox homologs. The Wwox variants were tested for their ability to modify the IR sensitivity phenotype. In the course of this study, it was found that background mutations introduced during the generation of the mutant flies was responsible for the IR sensitivity phenotype. As a result, proteomic alterations resulting from changes in Wwox protein levels in *Drosophila* were investigated in order to ascertain the possible molecular functions of the Wwox protein. 2D-DIGE analysis was conducted on a number of different fly genotypes expressing differing levels of Wwox protein in both adult and embryonic flies. The proteomic changes resulting from lack of Wwox function as well as Wwox over-expression were detected with the proteins of interest identified by mass spectrometry (MS) using both MALDI-TOF/TOF-MS and LC-ESI-MS/MS. Label free quantitative MS analysis was also performed in order to determine the most abundant protein(s) in those spots found to contain multiple proteins. These proteomic studies identified changes in a wide variety of proteins with a significant number of metabolic proteins as well as proteins involved in oxidative stress response as a result of different levels of *Wwox* expression. Of particular interest, consistent changes in different isoforms of superoxide dismutase 1 (Sod1) were identified. Due to the known roles these proteins play in pro and anti-apoptotic pathways, it is possible that Sod1 and Wwox may work in concert to regulate the delicate balance of defence mechanisms in response to environmental stresses, particularly oxidative stress. The protein/gene targets identified in this work therefore offer some insights into normal Wwox function.