Defining the role(s) of non-classical tumour suppressor Wwox in cellular function using Drosophila melanogaster genetic modelling

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by

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Abbreviations

°C – degrees Celsius

% – percentage

μg – micrograms

μL – microlitre

A – adenosine (in context of DNA)

A – alanine (in context of amino acid)

Akt – v-akt murine thymoma viral oncogene homolog/protein kinase B

ATP – adenosine triphosphate

bp – base pairs

C – cytosine

CFS – common fragile site

CDD – conserved domain database

cDNA – complementary DNA

CIN – chromosomal instability

CoVa – cytochrome c oxidase subunit Va

CoVb – cytochrome c oxidase subunit Vb

da – daughterless

DEPC – diethyl pyrocarbonate

DNA – deoxyribonucleic acid

dNTP – deoxyribonuecleoside triphosphate

DMSO – dimethyl sulfoxide

EDTA – ethylene diamine tetra-acetic acid

en - engrailed

ETC – electron transport chain

EV – empty vector

ey – eyeless

F – phenylalanine

FLP – flippase

Foxo – forkhead box, sub-group O

FRT – flippase recognition target

G – guanosine

GFP – green fluorescent protein

GSH – glutathione

GWAS – genome wide association studies

HDL-C – high density lipoprotein-cholesterol

hh – hedgehog

HIF1α - hypoxia inducible factor 1α

IDH – isocitrate dehydrogenase

IMS – intermembrane space

kb – kilobase

kDa – kilodalton

L – lysine

LB – Luria Broth

LiCl – lithium chloride

LOH – loss of heterozygosity

M – Molar

MARCM – mosaic analysis with a repressible cell marker

mg – milligram

ml – millilter

mM – millimolar

mRNA – messenger RNA

N – asparagine

NAD^+ – nicotinamide adenine dinucleotide (oxidised)

NADH – nicotinamide adenine dinucleotide (reduced)

$NAD(P)^+$ - nicotinamide adenine dinucleotide phosphate (oxidised)

NAD(P)H - nicotinamide adenine dinucleotide phosphate (reduced)

ND23 – NADH:ubiquinone reductase 23kD subunit precursor

ND42 – NADH:ubiquinone reductase 42kD subunit precursor

ND75 – NADH:ubiquinone reductase 75kD subunit precursor

NLS – nuclear localisation sequence

ng – nanograms

ORF – open reading frame

P – proline

PBS – phosphate buffered saline

PBST – PBS + Tween

PCR – polymerase chain reaction

pmol – picomole

QTL – quantitative trait loci

R – arginine

RNA – ribonucleic acid

RNAi – RNA interference

ROS – reactive oxygen species

Rp49 – Ribosomal protein 49

rcf – relative centrifugal force

Scrib – scribbled planar cell polarity protein

SDR – short-chain dehydrogenase reductase

SDS – sodium dodecyl sulfate

Sima - similar

SOC – super-optimal broth with catabolite repression

SOD – superoxide dismutase

T – thymine (in context of DNA)

T – threonine (in context of amino acid)

Tgo - tango

TCA – tricarboxylic acid

TMRE – tetramethylrhodamine, ethyl ester

TNFα - tumor necrosis factor α

Tub – tubulin

U – uracil

UAS – upstream activator sequence

UTR – untranslated region

V – volts

VDRC - Vienna Drosophila Resource Centre

W – tryptophan

WW1 – 1st WW domain of WWOX

WW2 – 2nd WW domain of WWOX

WNP –WWOX mutant line carrying a triple mutation in the tryptophan 58 (W58F), asparagine 81 (N81A) and proline 84 (P84A) residues

WWOX – WW domain-containing oxidoreductase

Y – tyrosine

***Drosophila* nomenclature**

The *Drosophila* nomenclature used is according to conventional notation as stated on the *Drosophila* database, Flybase (www.flybase.org). Genes are represented by italicised text (e.g. *Wwox*) and proteins are represented by non-italicised text (e.g. Wwox).

Abstract

The *WWOX* gene has been identified as the gene that spans the *FRA16D* common chromosomal fragile site (CFS), which is a frequent site of DNA instability in cancer. Perturbation of the *WWOX* gene has been reported in various cancers, with low *WWOX* levels correlating with poorer prognosis. Individuals who inherit a non-functional copy of *WWOX* have also been found to be at greater risk of developing cancer. WWOX has been implicated in various cellular pathways, however the role of WWOX in tumourigenesis is not yet fully defined. There is therefore a need to determine the normal function(s) of WWOX and how perturbation of these roles is likely to contribute to cancer. A model was previously established to examine the cellular function of the *Drosophila* orthologue, Wwox and to identify novel functional interactors. Loss of Wwox in *Drosophila* was not found to result in any obvious cellular dysfunction that manifested as a phenotype. The aim of this study was to identify the types of cellular dysfunction brought about by other genes that could be modulated by Wwox. As Wwox has previously been implicated in metabolic processes, particularly aerobic metabolism and redox homeostasis, an RNA interference (RNAi) screen was performed to identify the types of metabolic stress that can be modulated by altered Wwox levels. Wwox was found to regulate cellular homeostasis in cells with mitochondrial dysfunction, with a requirement for the active site of its short-chain dehydrogenase/reductase (SDR) enzyme. Other genetic effectors of the mitochondrial dysfunction were also identified as candidates for further investigation into the pathway(s) in which Wwox participates. The contributions of Wwox to two other models of cellular dysfunction were also examined. Wwox was found to have a role in a *Drosophila* model of intrinsic tumour suppression. In addition, Wwox was also shown to affect cells with chromosomal instability (CIN), with loss of Wwox resulting in oxidative stress, DNA damage and subsequently apoptosis of CIN cells. This study has identified roles for Wwox in three different novel models of cellular dysfunction. These findings provide further insight into the tumourigenic potential of WWOX and could contribute to the ultimate aim of designing therapeutics for treatment of cancers with low WWOX levels.