An Investigation of Mutant p53 Function

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ABSTRACT

The *TP53* tumour suppressor gene is mutated in approximately 50% of all human cancers. The majority of these mutations are missense mutations resulting in the expression of a mutated form of the full-length p53 protein. This mutant protein exhibits a loss of tumour suppressive activity, dominant-negative activity to inactivate functional p53 and gain-of-function properties to drive tumour progression and metastasis. Investigation into mutant p53-mediated oncogenic pathways and the mechanisms through which they are controlled plays an integral role in identifying new therapeutic targets for a range of mutant p53-expressing tumours.

To model the initial events that occur in cancer following sporadic p53 mutation, an isogenic panel of cell lines was established in the p53 null, H1299 lung cancer cell line, expressing wild-type or various p53 hotspot mutants under the control of an inducible promoter. These cell lines were harnessed to investigate a range of wild-type and mutant p53 functions. The induced wild-type p53 protein is demonstrated to be transcriptionally and biologically active, and its function can be further mediated by DNA damaging agents or expression of regulatory proteins. Conversely, induced mutant p53 exhibits a loss of the majority of the normal wild-type transcriptional activity while mediating gain-of-function, oncogenic phenotypes in H1299 cells. This system is demonstrated to provide an important platform with which to investigate both wild-type and mutant p53 function.

Mutant p53 is reported to function as an aberrant transcription factor, re-programming the cellular transcriptome to enhance oncogenic pathways. The mechanisms

underlying this were specifically examined through expression microarray analysis, which identified a number of mutant p53-regulated targets. Surprisingly, these targets were predominately also direct targets of wild-type p53. A novel mechanism for mutant p53 activity is subsequently suggested, whereby mutant p53 is recruited to the DNA through its interaction with p63.

A key function of mutant p53 is its ability to drive tumourigenesis through the initiation of a range of oncogenic pathways. Through utilising the inducible system, mutant p53 is demonstrated to influence mitotic pathways, resulting in multinucleation, and enhance the invasive and migratory properties of cancer cells. Importantly, an endogenous protein, ANKRD11, is identified with the capacity to suppress the oncogenic properties of mutant p53 and provide a potential target for the development of new cancer therapeutics.

The role of mutant p53 in driving the invasive and metastatic potential of breast cancer cells was further explored and a relationship between mutant p53 and a micro-RNA (miR-155) established. Mutant p53 expression is shown to correlate with miR-155 expression, with miR-155 target genes involved in invasive pathways. ZNF652 is specifically identified as a target of miR-155 and loss of ZNF-652 is correlated with increased invasion and poor prognosis in breast cancer.

Collectively, these studies identify key mechanisms through which mutant p53 functions to enhance tumourigenesis and importantly identify novel targets, ANKRD11, miR-155 and ZNF652, for the development of cancer therapies.

DECLARATION

I, Jacqueline Elise Noll, certify that this work contains no material which 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.

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LIST OF PUBLICATIONS

Noll JE, Jeffery J, Al-Ejeh F, Kumar R, Khanna KK, Callen DF and Neilsen PM. (2011) Mutant p53 drives multinucleation and invasion through a process that is suppressed by ANKRD11. *Oncogene.* Submitted. Second Revision.

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Chee JLY, Saidin S, Lane DP, Leong SM, Phua YT, **Noll JE**, Neilsen PM, Gabra H and Lim TM. (2011) Wild type and mutant p53 mediate cisplatin resistance through interaction and inhibition of caspase-9. *Carcinogenesis.* Submitted

Neilsen PM*, **Noll JE***, Tay BS, Bracken C, Schulz R, Lim S, Gregory P, Kumar R, Goodall G and Callen DF. (2011) Mutant p53 drives invasion in breast tumors through a pathway involving miR-155 and ZNF652. *Text in Manuscript*

* These authors contributed equally to this work

ABBREVIATIONS

ANK – Ankyrin

ANKRD11 – Ankyrin repeat domain 11

BCA – Bicinchoninic acid

CBP – CREB binding protein

CIP – Calf intestinal phosphatase

cDNA – Complimentary DNA

ChIP – Chromatin immunoprecipitation

DBD – DNA binding domain

DMEM – Dulbecco's modified eagle medium

DMSO – Dimethyl sulfoxide

DN – Dominant negative

DNA – Deoxyribonucleic acid

ECL – Enhanced Chemiluminescence

EDTA – Ethylenediaminetetraacetic acid

EI – Ecdysone inducible

EMT – Epithelial to mesenchymal transition

FACS – Fluorescence-activated cell sorting

FBS – Fetal bovine serum

GFP – Green fluorescent protein

GOF – Gain of function

HRP – Horseradish peroxidase

LOF – Loss of function

MDM2 – Murine double minute 2

mRNA – Messenger RNA

ORF – Open reading frame

PBS – Phosphate buffered saline

PCR – Polymerase chain reaction

PonA – Ponasterone A

P/CAF – p300/CBP associated factor

RE – Response element

RNA – Ribonucleic acid

RT – Room temperature

RT-PCR – Reverse transcription PCR

SDS – Sodium dodecyl sulfate

SDS-PAGE – Sodium dodecyl sulfate polyacrylamide gel electrophoresis

shRNA – Short hairpin RNA

SNP – Single nucleotide polymorphism

ssDNA – Salmon sperm DNA

SV40 – Simian virus 40

UTR – Untranslated region

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