Alternative Cell Fate in Response to DNA Damage Regulated by Differential p53 Pathway Dynamics

CHEN XI

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Philosophy

Principal Supervisor: Dr. SHI Jue

Hong Kong Baptist University

June 2012
Abstract

The tumor suppressor protein, p53, and its downstream effectors play a crucial role in mediating proper cellular process of repair or death in response to a wide variety of stress stimuli. Cell fate varies, depending on the type of stress, its level and the genetic background of individual cell types. In this study we quantify the phenotypic response to DNA damage, and investigate how the response process is differentially regulated by the p53 pathway dynamics. We induced DNA damage in selected cultured mammalian cell lines by using a DNA-damaging drug, etoposide. By using quantitative time-lapse microscopy, we tracked dynamics of individual cells in real time, acquiring information with respect to protein kinetics and cellular processes that are beyond ensemble approaches. Our results showed that when being treated with low dosage of etoposide, the majority of cells exhibited continuous oscillation of p53 followed by cell-cycle arrest, while at high dosage cells tended to die rapidly with monotonic elevation of p53. In contrast to common hypothesis, we found the bimodal p53 dynamics did not control phenotypic response by modulating p53’s transcriptional activation as DNA damage increased. Our data so far showed that the differential p53 dynamics regulate the alternative cell fate of arrest vs. death mainly by modulating p53’s direct pro-death activity in the cytoplasm in a damage-dose dependent manner.
# Table of Contents

Declaration ......................................................................................................................... i

Abstract .............................................................................................................................. ii

Acknowledgements.............................................................................................................. iii

Table of Contents................................................................................................................ iv

List of Figures....................................................................................................................... vii

List of Abbreviations .......................................................................................................... viii

Chapter 1  Introduction ...................................................................................................... 1

1.1 DNA damage responses ................................................................................................. 1

1.2 Function and regulation of p53 ..................................................................................... 2

1.3 Regulation of p53 in response to double strand breaks .............................................. 5

1.4 Cell fate response regulated by p53 ............................................................................. 6

1.5 Aim of study .................................................................................................................. 8

Chapter 2  Materials and methods .................................................................................... 9

2.1 Cell culture ................................................................................................................... 9

2.1.1 Cell lines ..................................................................................................................... 9

2.1.2 Generation of fluorescent reporter cell line .............................................................. 9

2.1.3 Selection of isogenic clone ......................................................................................... 11

2.1.4 Gene knockdown by RNA interference (RNAi) ....................................................... 11

2.2 Chemicals .................................................................................................................... 12

2.3 Protein analysis and detection ..................................................................................... 12

2.3.1 Cell harvest and lysis ............................................................................................... 12

2.3.2 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis ............................. 13
2.3.3 Western Blot (protein gel blot) ................................................................. 13
2.3.4 Antibodies ................................................................................................. 14
2.4 Subcellular fractionation ............................................................................... 15
2.5 Time-lapse microscopy .................................................................................. 16

Chapter 3 p53 dynamics and cell fate response to DNA-damaging agent, etoposide ...................................................................................................................... 17
3.1 Differential p53 dynamics in response to various doses of DNA damage .... 17
3.2 Correlation between single-cell p53 dynamics and cell fate .................... 22
3.3 Cellular mechanisms of the bimodal dynamics of p53 ............................. 24
3.4 Discussion ........................................................................................................ 30

Chapter 4 Differential regulation of p53’s dual function by bimodal p53 dynamics ....................................................................................................................... 32
4.1 Introduction ....................................................................................................... 32
4.2 Results .............................................................................................................. 33
  4.2.1 Role of p53’s transcriptional activity in modulating differential cell fate .................................................................................................................. 33
  4.2.2 Role of p53’s direct pro-death activity in modulating differential cell fate .................................................................................................................. 37
4.3 Discussion ........................................................................................................ 39

Chapter 5 Cell-line variation in p53 pathway-mediated DNA damage response ............................................................ 41
5.1 Introduction ....................................................................................................... 41
5.2 Results .............................................................................................................. 42
5.2.1 Cell line comparison of response to high and low concentration of etoposide in U2OS and A549 cells ............................................................ 42

5.2.2 Expression profile of p53 pathway proteins in A549, HeLa, RPE, OVCAR5 and HCT116 under high and low dosage of etoposide ........................................ 45

5.3 Discussion........................................................................................................ 50

List of References .................................................................................................. 51

Curriculum Vitae .................................................................................................... 54