The Investigation of Signalling Pathways in Response to Chromosomal Instability

THE UNIVERSITY of ADELAIDE

Submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

By

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September 2015

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I would like to dedicate my thesis to my beloved father

Shiyong Liu

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Acknowledgments

I would like to thank all the people who helped me in some way during my PhD study. Foremost, I would like to express my sincere gratitude to my supervisor and my mentor Stephen Gregory for his tremendous support, great patience, immense knowledge and persistent encouragement throughout my study. It is my great honour to be under his supervision, indeed, his wisdom and personality have influenced me in many ways as I look the world. I would also like to thank my co-supervisor Louise O'Keefe for her insightful ideas, comments, encouragement and continuous support.

Beside my supervisors, I would like to thank David Adelson, Frank Grützner, Robert Saint and Robert Richards for their great support during my PhD candidature. I would also like to acknowledge the China Scholarship Council for the financial support which has allowed me to undertake my studies.

It would be impossible for me to write this doctoral thesis without the help and support from my lab members and colleagues Zeeshan Shaukat, Rashid Hussain, Mahwish Khan, Sonia Dayan, Donna Denton, Zhipeng Qu, Dong Wang, Amanda Choo, Cheng Shoo Lee, Tianqi Xu, Clare van Eyk, Saumya Samaraweera, Dani Fornarino, Seyyed Hani Moussavi Nik, Kynan Lawlor, and Shannon Nicolson.

I would also like to express my extremely gratitude to my parents, sister, wife and my beloved son for all their unconditional love and support.

List of Publications

- Dawei Liu, Zeeshan Shaukat, Tianqi Xu, Donna Denton, Robert Saint and Stephen L. Gregory*. Autophagy regulates the survival of cells with chromosomal instability. (Manuscript submitted).

- Dawei Liu, Zeeshan Shaukat, Robert Saint and Stephen L. Gregory* (2015). Chromosomal instability triggers cell death via local signalling through the innate immnue receptor Toll. ***Oncotarget***, doi: 10.18632/oncotarget.6035.

- Zeeshan Shaukat, Dawei Liu and Stephen Gregory* (2015). Sterile Inflammation in *Drosophila*. ***Mediators of Inflammation***, doi: 10.1155/2015/369286.

- Zeeshan Shaukat, Dawei Liu, Rashid Hussain, Mahwish Khan and Stephen Gregory* (2015). "The Role of JNK Signalling in Responses to Oxidative DNA Damage." ***Curr Drug Targets***, Volume 16 (E-pub ahead of print).

- Zeeshan Shaukat, Dawei Liu, Amanda Choo, Rashid Hussain, Louise O'Keefe, Robert Richards, Robert Saint and Stephen L. Gregory* (2015). Chromosomal Instability Causes Sensitivity to Metabolic Stress. ***Oncogene***, 34, 4044-4055.

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Abbreviations

5-FU	5-Flurourocil
ACT	Adoptive cell transfer
Ambra1	Activating molecule in BECN1-regulated autophagy protein 1
APC/C	Anaphase promoting complex/cyclosome
Atg1	Autophagy-related 1
Atg4c	Autophagy-related 4c
Atg5	Autophagy-related 5
Atg7	Autophagy-related 7
Atg18	Autophagy-related 18
ATM	Ataxia telangiectasia mutated
Aurora A	Aurora kinase A
Aurora B	Aurora kinase B
BRCA1	Breast cancer 1
BUB	Budding uninhibited by benzimidazoles
BubR1	Bub1-related protein kinase
Cdc20	Cell division cycle 20 homologue
CENPE	Centromere linked motor protein E
CIN	Chromosomal instability
CTLA4	Cytotoxic T-lymphocyte-associated antigen 4
DAMPs	Danger-associated molecular patterns
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
GIN	Genomic instability
HIF	Hypoxia-inducible factor
HMGB1	High mobility group box 1
IL-2	Interleukin 2
JNK	c-Jun N-terminal kinases
KSP/Eg5	Kinesin spindle protein
Mad1	Mitotic arrest deficient 1
Mad2	Mitotic arrest deficient 2
MCAK	Mitotic centromere-associated kinesin
MCC	Mitotic checkpoint complex
Mmp1	Matrix metalloproteinase 1
mTor	Mechanistic target of rapamycin
NDC80	Kinetochore protein NDC80 homolog
Nek2	NIMA-related kinase 2

NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
p53	Tumour protein (SDS-PAGE: 53 kDa) p53
p62	Sequestosome 1
PCM	Pericentriolar material
PCS	Premature chromatid separation
PI3K	Phosphoinositide 3-kinases
PLKs	Polo-like kinases
Rad21	RAD21 homolog (S. pombe), kleisin subunits of Cohesin Rad21
Ras	Rat sarcoma
Rb	Retinoblastoma
RNA	Ribonucleic acid
RNAi	RNA interference
ROS	Reactive oxygen species
SAC	Spindle assembly checkpoint
TCA	Tricarboxylic acid cycle
TLRs	Toll-like receptors
TNFα	Tumour necrosis factor alpha

Abstract

Most human solid cancers show Chromosomal Instability (CIN) in which cancer cells show a higher rate of gain or loss of whole chromosomes or large chromosomal fragments. CIN is associated with the progression of tumorigenesis, the development of cancer drug resistance and the poor prognosis. Since CIN is a hallmark of cancers and not common in normal cells, it has been proposed that CIN is targetable for cancer therapy. In order to target CIN for cancer treatment, there is a need to determine the signalling pathways which enable cells to tolerate CIN.

The aim of this study is to identify signalling pathways activated in response to CIN which could potentially be targeted to specifically kill CIN cells. Using *Drosophila* as the model organism to study CIN **(Chapter 2)**, we found that CIN cells are specifically sensitive to metabolic disruption as the depletion of metabolic genes involved in glycolysis, tricarboxylic acid cycle and oxidative stress response led to high levels of oxidative stress, DNA damage and apoptosis only in CIN cells **(Chapter 3)**. Consistent with its role in stress responses, in the subsequent study, we found that the autophagy pathway is robustly activated in CIN cells and autophagy inhibition can specifically kill CIN cells. We also found that autophagy activation removes defective mitochondria in CIN cell which gives tolerance to CIN in proliferating cells **(Chapter 4)**.

We also found a systemic immune signalling activation in *Drosophila* larvae when CIN was induced in the engrailed region of wing discs. Moreover, we found that the immune signalling Toll pathway is also activated within CIN cells and manipulation of Toll pathway could affect the survival of CIN cells. We proposed that signals released from CIN cells such as reactive oxygen species (ROS) could trigger a local Toll pathway activation in CIN tissue which in turn recruits *Drosophila* blood cells (hemocytes) to the surface of the CIN tissues. These recruited hemocytes then initiate apoptosis in the CIN cell through the TNFα/JNK pathway **(Chapter 5)**.

In conclusion, our studies demonstrated that CIN leads to a variety of consequences in cells: several signalling pathways including metabolic pathways, autophagy and Toll signalling are activated in response to CIN stresses. Understanding the mechanisms of these pathways responding to CIN will provide insights into designing cancer specific drug targets and ultimately contribute to cancer treatment.