Targeting chromosomal instability: Screening and characterization of CIN killers

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Thesis Summary

Chromosomal INstability (CIN), a hallmark of cancer cells, refers to a state in which cells have an increased rate of gain or loss of whole chromosomes or large chromosomal fractions. CIN is linked to the progression of tumours with poor clinical outcomes such as drug resistance and metastasis. Chromosomal instability is mainly caused by defective chromosomal segregation during mitosis and normally prevented by cellular checkpoints. As CIN is not found in normal cells, it offers a cancer-specific target for therapy, which may be particularly valuable because CIN is common in advanced tumours that are resistant to conventional therapy.

In this study, to identify targets which can specifically induce apoptosis in CIN cells, a CIN model was generated by knocking down the spindle assembly checkpoint in *Drosophila*. Defects in the checkpoint lead to high rate of chromosomal segregation defects (lagging chromosomes and chromosome bridges). An RNAi screening approach was used and the set of kinases and phosphatases was screened to identify those candidates that induce apoptosis only in CIN cells. Genes identified include those involved in JNK signaling pathways, mitotic cytoskeletal regulation and metabolic pathways. This screen demonstrates that it is feasible to selectively kill cells with CIN induced by spindle checkpoint defects. It has identified candidates that are currently being pursued as cancer therapy targets (e.g. Nek2: NIMA related kinase 2), confirming that the screen is able to identify promising drug targets of clinical significance.

Further screening and characterization of the JNK pathway demonstrated that signaling through JNK is required to tolerate CIN which is consistent with the fact that many tumours show high levels of JNK expression. JNK signaling is involved in the DNA damage repair process by maintaining an efficient damage repair and anti-oxidant levels which resolves DNA damage before entry into mitosis. Knockdown of the JNK pathway results in unrepaired DNA damage which leads apoptosis only in CIN cells via the caspase dependent pathway, partly independent of p53. Similarly, it was observed that the G2 length, which is required for DNA damage repair is crucial for the survival of CIN cells. These results suggest that JNK is necessary for the proper regulation of the DNA damage induced delay prior to mitotic entry and crucial for the survival of CIN cells, which are already coping with elevated levels of stress.

In addition, CIN can enable tumours to acquire genetic diversity which can provide an advantage in terms of growth and proliferation under stress and also provide resistance against cancer therapies, but this comes at the cost of significant stress to tumour cells. CIN cells evolve their metabolic pathways to increase the ability to tolerate and survive under oxidative and proteotoxic stress, but are still sensitive to these pathways. This study demonstrates the possibility to target both CIN and metabolism for the treatment of highly diverse drug resistant tumours. Further metabolic genes were screened and we demonstrated that CIN cells are particularly sensitive to certain metabolic alterations that do not affect normal cells. These metabolic disruptions lead to high levels of oxidative stress in CIN cells, which are already managing elevated reactive oxygen species (ROS) levels. These potential therapeutic targets are clinically highly desirable because of their potential effects on unstable and highly resistant CIN tumours.

In conclusion, a new *Drosophila* model for CIN was used to demonstrate the principle that it is possible to selectively kill CIN cells. Our RNAi screen identified candidates whose depletion has the potential to kill proliferating CIN cells without affecting their normal counterpart. An efficient DNA damage repair mechanism is required to tolerate CIN and can be used as a target to kill these unstable cells which are already dealing with high levels of DNA damage from ROS. Furthermore, CIN cells are sensitive to metabolic alterations, especially those which are needed to tolerate high levels of proteotoxic and oxidative stress. This study is a significant advance in understanding the target pathways which are involved in CIN tolerance. Further characterization of these pathways may help to identify mechanisms by which cancer cells can tolerate the adverse effects of CIN and aneuploidy which in turn may lead to the identification of novel targets that can specifically kill advanced and drug resistant-CIN tumour cells without harming normal cells.

Declaration

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Zeeshan Shaukat Dated

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List of Publications

- Zeeshan Shaukat, Heidi Wong, Shannon Nicolson, Robert B. Saint, & Stephen L. Gregory*. (2012) A screen for selective killing of cells with chromosomal instability induced by a spindle checkpoint defect. PloS one, 7 (10), e47447.

- Heidi Wong, Zeeshan Shaukat, Jianbin Wang, Robert Saint, & Stephen L. Gregory*. (2014) JNK signaling is needed to tolerate chromosomal instability. *Cell Cycle*, 13 (4), 0-9.

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

Abbreviations

17-AAG	17-N-allylamino-17-demethoxygeldanamycin
AICAR	5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide
AP1	Activator protein 1
APC	Adenomatous polyposis coli
APC/C	Anaphase promoting complex/cyclosome
Asp	Abnormal spindle protein
ATM	Ataxia telangiectasia mutated
Aur	Aurora kinases
AURKA	Aurora kinase A / Aurora A
AURKB	Aurora kinase B / Aurora B
BFB	Breakage-Fusion-bridge
BRCA1	Breast cancer 1
BUB	Budding uninhibited by benzimidazoles
BubR1	Bub1-related protein kinase
CD95	Cluster of differentiation 95 (FAS receptor)
Cdc20	Cell division cycle 20 homologue
CDK1	Cyclin B dependent kinase 1
CDKs	Cyclin dependent kinases
CENP-E	Centromere linked motor protein E
Chk2	Checkpoint kinase 2
CIN	Chromosomal instability
DDR	DNA damage response
DNA	Deoxyribonucleic acid
DSB	Double strand break
FOXO	Forkhead box protein O
Fzr1	Fizzy-related protein homolog 1
G6PD	Glucose-6-phosphate dehydrogenase
HEC1	Highly expressed in cancer protein 1
HIF	Hypoxia-inducible factor
Hippo	Hippo (hpo gene), Salvador/Warts/Hippo (SWH) signaling pathway,
HSP90	Heat shock protein 90
IDH	Isocitrate dehydrogenase
JNK	c-Jun N-terminal kinases
k-MT	kinetochore–microtubule
KRAS	Kirsten rat sarcoma viral oncogene homolog

KSP/Eg5	Kinesin spindle protein
Mad2	Mitotic arrest deficient 2
MAPKKK	Mitogen-activated protein kinase kinase kinase
Mps1	Monopolar Spindle 1
MSI	Microsatellite instability
MT	Microtubules
mTORC1	Mammalian target of rapamycin complex 1
MVA	Mosaic Variegated Aneuploidy
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
Nek2	NIMA-related kinase 2
NHEJ	Non homologous end joining
NIMA	Never In Mitosis Gene A
Notch	Notch proteins: a family of transmembrane proteins
p53	Tumour protein (SDS-PAGE: 53 kDa) p53
PAS	Per Arnt Sim
PASK	PAS domain containing serine/threonine kinase
PI3K	Phosphoinositide 3-kinases
PLK1	Polo-like kinase 1
Plk4	Polo-like kinase 4
PP1	Protein phosphatase 1
PTEN	Phosphatase and tensin homolog
Rad21	RAD21 homolog (S. pombe), kleisin subunits of Cohesin Rad21
Rad54	DNA repair and recombination protein RAD54
Rae1	RNA export 1
Ras	Rat sarcoma
Rb	Retinoblastoma
RNAi	RNA interference
Rod	Rough deal
SAC	Spindle assembly checkpoint
SAK	Snk akin kinase
Scc1	kleisin subunits of Cohesin Scc1
Smc1	Structural Maintenance of Chromosome 1
Smc3	Structural maintenance of chromosomes 3,
SOD1	Superoxide dismutase 1
STAG3	Stromal antigen 3
TCA	Tricarboxylic acid cycle
Trp53	Transformation-related protein 53
Wnt	From: Wingless gene (*Drosophila*), a homolog of int-1
zw10	Zeste white 10, kinetochore associated protein homolog

Thesis outline

Chromosomal instability (CIN) is the hallmark of cancer cells. Chromosomal instability is also linked to tumourigenesis and poor clinical outcomes. CIN cancers are resistant to conventional therapies and require new therapies. CIN is highly tolerated in cancers and provides an opportunity to be specifically targeted. **Chapter 1** of this thesis is a literature review, which describes chromosomal instability, its types and mechanism and its use as a therapeutic target for cancer. **Chapter 2** is based on a published article (Shaukat et al, 2012) from this study which explains the generation of a *Drosophila* CIN model. Chapter 2 also explains the screening of candidates whose knockdown can induce apoptosis in CIN cells without affecting the normal cells. Along with other centrosomal candidates, some JNK pathway candidates were identified which were then further analysed for their role in CIN specific cell death. This characterization is published (Wong et al, 2014) and is a part of this thesis as **chapter 3**.

The initial screening also identified metabolic candidates which are interesting because CIN cells cause metabolic alterations in cancer cells for the adaptation against cellular and external stresses. This CIN specific alteration offers the possibility to target CIN. Further screening of metabolic candidates in our CIN model demonstrated that the CIN cells are sensitive to certain metabolic alterations, especially those which are involved in maintaining the redox potential of the cell as well as oxidative stress response pathway genes. The outcome of this screening and characterization is presented in **chapter 4** of this thesis as a submitted manuscript for publication (under review for Oncogene). All the publication based chapters contain their own introduction, methods, results and discussions. **Chapter 5** includes the combined discussion of the results, their significance, current model and future directions.