RGC Ref.: N_HKBU215/13 NSFC Ref. : 313191026
(please insert ref. above)

The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Dynamic Regulation of the p53 Pathway and Its Control over Cell Fate at the Single-cell Level
p53信号網絡在單細胞水平的動態響應及其對細胞的調控机制

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Dr. Jue SHI 史珏博士	Prof. Feng LIU 劉鋒教授
Post	Associate Professor	Professor
Unit / Department / Institution	Department of Physics, Hong Kong Baptist University	School of Physics, Nanjing University
Contact Information	jshi@hkbu.edu.hk	fliu@nju.edu.cn
Co-investigator(s) *(with title and institution)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/2014		
Project Completion date	31/12/2017		
Duration *(in month)*	48		
Deadline for Submission of Completion Report			

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Measure dynamics of key components of the p53 pathway in response to variable DNA damage strength at the single-cell level.

2. Quantify the cell-to-cell variability in both the damage-induced pathway dynamics and cell fate.

3. Based on the experimental data, develop a quantitative model for the p53 pathway that delineates the cellular responses to variable damage signals. Characterize the pathway dynamics, its control over cell fate and the variability between individual cells.

4. Conduct computational analysis to identify pathway components/feedbacks that are crucial for the p53 pathway control and the associated single-cell variability. Validate the theoretical predictions by experiments or use the experimental data to refine the mode

5.2 Revised Objectives: **N.A.**

Date of approval from the RGC: _____

Reasons for the change: _____

_____

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

By combining quantitative single-cell microscopy assays, ensemble profiling and mathematical modeling, in this collaborative study we elucidated a novel dynamic mechanism that governs differential cell fate outcome in response to variable DNA damage. While cancer cell lines activated similar p53 oscillation followed by cell-cycle arrest in response to low dose of DNA damage, they switched in a bimodal manner to monotonic or extended pulse dynamics at high damage. Cell lines with monotonically increasing p53 underwent rapid and extensive drug-induced apoptosis, while those exhibiting an extended p53 pulse mostly went into cell-cycle arrest. The variable dynamics were further characterized to arise from differential output of a four-component regulatory module involving ATM, p53, Mdm2 and Wip1, which generates bimodal p53 dynamics through coupled feed-forward and feedback. We also found that the inhibitory strength between ATM and Mdm2 determined the differential output observed in distinct cancer cell lines. Given that DNA damaging chemotherapeutics exert their anticancer effect mainly through p53 pathway-mediated DNA damage response in p53 wild-type tumors, our results on cell fate control by bimodal p53 dynamics revealed a new dynamic mechanism of chemoresistance to drug-induced cell death. Overall, our data not only point to p53 pulsing as a potentially druggable mechanism that mediates resistance to cytotoxic chemotherapy, but also identified that combinatorial inhibition of Mdm2 and Wip1 was an effective strategy to alter p53 dynamics in resistant cancer cells and sensitize their apoptotic response.

The above results have been published in Science Advances (Yang et al, Sci. Adv. 2018, 4, eaat5077) and constituted a major part of the PhD thesis of Ruizhen Yang (PhD defense expected in May 2019). In addition, one of the reviewers of our grant proposal pointed out that a weakness of our proposed study is that the p53 pathway that we considered using ODE modeling was relatively small. He/she suggested us to consider expanding the dynamic analysis of DNA

damage-induced cell fate control to a larger p53 network using alternative systemic modeling strategy. Taking the reviewer's advice, we extended the project in collaboration with Prof. KH Cho's group at KAIST and employed the attractor landscape method developed by the Cho group to examine the dynamic response of a larger p53 network. The acquired results revealed additional synergistic and antagonistic interactions in the p53 network that modulated DNA damage response induced by DNA damaging chemotherapeutics, and the results have been published in Nature Communications (Choi et al, Nat Commun. 2017, 8(1):1940). Please refer to Part C for details of the research outcome.

Potential for further development of the research and the proposed course of action
(maximum half a page)

Although the four-component module can largely recapitulate the dynamic response of p53 pathway in response to saturating low and high level of DNA damage, this simple model fell short of explaining the complex p53 dynamics that we observed at intermediate levels of DNA damage. Simulations of the four-component module predicted that at intermediate level of DNA damage, p53 would exhibit transitional dynamics with p53 oscillation at an increasing level. However, we did not observe such transitional dynamics. All cell lines that we studied showed a sharp switch of p53 dynamic mode from periodic pulsing to either monotonic induction or an extended large pulse. This result suggested that additional regulatory component, such as positive feedback loop, was present and key to promote the sharp, bimodal dynamic switch. In the follow-up study, we will further investigate the intriguing transitional dynamics of p53 at intermediate DNA damage level by considering other regulatory component(s) in the p53 signaling network. We will also investigate how other stress stimuli, such as ribosomal stress and hypoxia, activate differential p53 pathway dynamics for cell fate control.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

The cellular machinery, consisting of multiple networks/pathways, is highly robust in response to genetic and environmental changes, and a living cell is capable of efficiently adjusting collective pathway activities to different cellular states and stress stimuli. However, there is still a large gap in our understanding of how complex dynamical responses are modulated and how they vary between different cell types. In this collaborative project, by combining quantitative single-cell microscopy assays, ensemble profiling and computational modeling, we uncovered a novel dynamic mechanism underlying differential cellular response to variable DNA damage mediated by the p53 pathway. p53 is an important mediator of cellular responses to DNA damaging chemotherapeutics, but even p53-wild-type cells vary in drug sensitivity for unclear reasons. The dynamic mechanism that we identified in this study provided novel insight of phenotypic mechanism that can cause chemoresistance. And our work not only elucidated the molecular basis of differential p53 pathway dynamics in response to DNA damage but also identified a strategy to re-sensitize resistant cells to combat chemotherapy resistance.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) ***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
2018				**Yang R, Huang B, Zhu Y, Li Y, Liu F*, Shi J***	Cell type -dependent bimodal p53 activation engenders a dynamic mechanism of chemoresistance. ***Sci. Adv.*** 4, eaat5077.	No	Yes	Yes	Yes
2017				Choi M+, **Shi J**+, **Zhu Y, Yang R**, Cho K* (+: co-first author)	Network dynamics-based stratification of cancer panel for systemic prediction of anticancer drug response. ***Nat Commun.*** 8(1), 1940.	No	Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
01/2014/ Hong Kong	Unraveling cell fate control by differential pathway dynamics at the single cell level	Frontiers of Soft Matter Physics	No	Yes	Yes	No
04/2018/ Germany	Cell-type dependent p53 dynamics in cell fate control	EMBO workshop	No	Yes	Yes	No

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Ruizhen YANG	PhD	09/2015	Expected on 05/2019

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

N.A.