RGC Ref.: X-HKU702/14
(please insert ref. above)

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

(Please attach a copy of the completion report submitted to the Scottish Funding Council by the Scottish researcher)

Part A: The Project and Investigator(s)

1. Project Title

In vivo delivery and expression of shRNA targeting SK1 and SK2 by *Salmonella* to tumours

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

	Hong Kong Team	Scottish Team
Name of Principal Investigator *(with title)*	Jiandong Huang (Professor)	Nigel Pyne (Professor)
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Unit / Department / Institution	The University of Hong Kong	University of Strathclyde
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Co-investigator(s) *(with title and Institution)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	01-JAN-2015		
Project Completion date	31-DEC-2015		
Duration *(in month)*	12		
Deadline for Submission of Completion Report			

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

With the identification of sphingosine kinases (SK1 and SK2) as potential novel cancer therapeutic targets, we aim to:

1. use optimized conditions for bacterial delivery of shRNA constructs (for SK1 and SK2) to tumours in vivo and to determine the effect on regression of the tumour. (performed in HK)

2. optimize hypoxic-dependent bacterial delivery of shRNA constructs (for SK1 and SK2) to eliminate these enzymes from cancer cells in vitro and to determine the effect on cancer cell survival. (performed in Scotland)

If successful, this strategy represents a novel therapeutic approach to treating cancer with potential increased efficacy and reduced side-effects.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

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

Summary of significant discoveries:

1. YB1 bacterial delivery system enabled shRNA construct transportation with higher efficiency compared to chemical transfection reagents (lipofectamine 2000), but RNAi against targeted Sphingosine Kinases was absent.

2. Co-transfection of SK1 and SK2 targeting shRNA constructs ruled out potential synergistic compensation between two types of SKs that might mask RNAi effect, and attributed the knockdown compromise to the T7 RNAP cassettes in the shRNA constructs.

3. Lack of functional EGFP when MDA-MB-231 cells were transfected with pIT7S-EGFP construct confirmed that function of the second T7 promoter for shRNA expression appeared compromised, and a detected read through effect that produced super long transcript could contribute to the incompetency.

By showing that YB1 bacterial delivery system could achieve a more rapid and robust cargo delivery when compared to standard chemical transfection reagents, this study provided "proof of concept" in terms of developing new anti-cancer therapeutics with optimized efficiency. On the other hand, due to its robustness and normal functioning in both prokaryotic and mammalian cells, T7 RNAP systems are broadly used for various goals. Their detailed dynamics in cells are therefore essential to designing efficient and proper circuits and avoiding miscellaneous unexpected effects. By uncovering the read-through effect and contextual property of T7 RNAP in this project, our studies hinted that strong termination signal was required to assure correct processing of transcripts, and over-expression of cargo genes could not be achieved by simply involving contextual positive feedback.

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

In further proceeding with our study, we have proposed a new construct design, in which we will relocated the shRNA cassette to upstream of the T7 RNAP positive feedback cassette in an opposite position, aiming to eliminate mutual influence of these two robust T7 promoters. Additionally, a class II T7 terminator (VSV terminator) will be added to the end of the original terminator, hopefully to increase termination efficiency to 90% or above.

Once we have efficient expression of shRNAs for SK1 and SK2, we will re-evaluate knockdown on cancer cell survival. Moreover, with the pIT7S-IRES-EGFP plasmid, we will study the dynamics between the shunt cassette and the positive feedback loop, expecting to understand more about the T7 RNAP contextual effect and hopefully facilitate construction of useful synthetic circuits. The Pyne lab observed that exosomal release of S1P2 receptor in MDA-MB-231 cells likely involves SK1/SK2 driving fibroblast activation in the tumour microenvironment. This may serve as a biomarker/phenotypic assay for further characterisation of the constructs designed to knockdown SK1/SK2.

7. The Layman's Summary

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

This research programme provides a means to confer specificity in targeting SK1 and SK2 only to anaerobic regions of the tumour, thereby avoiding indiscriminate targeting of these enzymes in aerobic healthy tissue and thus, avoiding side-effects. S1P released from tumour cells also stimulates neovasculaturisation, thereby correcting the oxygen insufficiency to the tumour. Therefore, shRNA knockdown of SK1 or SK2 is predicted to inhibit neovascularisation and to reduce the survival/growth of cancer cells. The expertise in Scotland concerns the role of sphingosine kinase and sphingosine 1-phosphate in cancer cell biology, while the expertise in Hong Kong is focused on the development of bacterial

systems to deliver therapeutic payloads to anaerobic/hypoxic regions of the tumour. The expertise of the two groups are complementary and combining these provides a novel translational programme in the development of new cancer therapeutics. Our studies have by far identified possible interference of two transcription units in the shRNA expression construct, and provided insights on how to enhance efficacy of treatment by high efficiency transfection and knockdown of SK expression, resulting in cancer cell death with the bacterial delivery system.

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)*						

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)*

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
Mr. Zhendong Feng	PhD	01-Sep-2013	30-Nov-2017