| RGC Ref.: N_HKU740/14<br>NSFC Ref. : 31461163002 |
|---|
| *(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

Nur77: new insights in signaling and mechanism of activation of epithelial-mesenchymal transition and tumor metastasis

Nur77誘導上皮間質轉化和腫瘤轉移的新機制

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

| | Hong Kong Team | Mainland Team |
|---|---|---|
| Name of Principal Investigator *(with title)* | Dr. Wong Alice Sze-Tsai<br>黃思齊博士 | Dr. Zeng Jin-Zhang<br>曾錦章博士 |
| Post | Professor<br>教授 | Deputy Dean & Professor<br>教授兼副院長 |
| Unit / Department / Institution | School of Biological Sciences, University of Hong Kong<br>香港大學生物科學學院 | School of Pharmaceutical Sciences, Xiamen University<br>厦門大學药學院 |
| Contact Information | | |
| Co-investigator(s) *(with title and institution)* | | |

### 3. Project Duration

| | Original | Revised | Date of RGC/ Institution Approval |
|---|---|---|---|

| | | | *( must be quoted*) |
|---|---|---|---|
| Project Start date | 1/1/2015 | | |
| Project Completion date | 31/12/2018 | | |
| Duration *(in month)* | 48 | | |
| Deadline for Submission of Completion Report | 31/12/2019 | | |

## Part B: The Completion Report

## 5. Project Objectives

### 5.1 Objectives as per original application

*1.* To investigate Nur77, FKBP38, Rheb, and mTORC1 interactions in the postulated crosstalk events, and the subsequent activation and signaling mechanisms;

*2.* To determine the cellular effects mediated by the Nur77-mTORC1 signaling axis on EMT and tumor metastasis; and

3. To elucidate the role of honokiol and its derivatives in the regulation of the Nur77-FKBP38/Rheb-mTORC1 axis, and with the use of animal models to explore potential clinical 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)*

Metastasis is the major cause of cancer deaths. Epithelial-mesenchymal transformation (EMT) is the initiating and key rate-limiting step of cancer progression and metastasis. The core of this project is to study the pro-survival role of Nur77 in tumor, especially on how Nur77 activates and regulates mTORC1 under hypoxia and TNFα stimulation. In this project, emphases have been made on the following research work: role of Nur77-dependent mTORC1 activity in EMT and metastasis of cancer; the molecular mechanism of Nur77 activating mTORC1 (FKBP38 and Rheb); and the identification of compounds targeting Nur77-mTORC1 and their anticancer mechanisms. Through these studies, we identified the potential targeting value of Nur77-mTORC1 signaling pathway.

The main achievements include: 1) Nur77 is the key regulator of TNFα activation and the maintenance of mTORC1. The pro-survival function of Nur77 depends on the stimulation of TNFα, and Nur77-dependent mTORC1 activity has important role in the TNFα-promoted EMT and migration and metastasis of cancer cells. 2) Mechanistically, Nur77 activation of mTORC1 was due to its up-regulation Rheb, which could be modulated by direct interaction with FKBP38. 3) We identified several compounds including NAM446-1 that could directly bind Nur77 and reverse the pro-EMT and proliferative effect of TNFα on colon and breast cancer by targeting Nur77-mTORC1 pathway. 4) Interestingly, another compound N446-7S that induces and binds Nur77 could strongly induce the degradation of FKBP38 and Bcl-2, leading to inhibition of EMT and metastasis of colon cancer. In contrast, the derivative N446-7W that could only marginally induce Nur77 expression and had weak affinity on Nur77 could not inhibit mTORC1 activity. Both XMU and HKU contribute to generating above data (see the Chinese version contained original data which have been submitted to NSFC, which are under preparation for publication). 5) We also disclose a new role of Nur77 that regulates mTORC1 activity through modulating let-7i-5p biogenesis that directly targets the 3'UTR of p110α mRNA, which action mediates the cell survival under hypoxia (Part C Ref. #7). 6) Based on this new role of Nur77, we found another compound ginsenoside derivative compound K that directly targets Nur77 could release its inhibitory effect on microRNA Let7i-5p and thus downregulating PI3K/AKT/mTORC1, which led to the inhibition on EMT of colon cancer (Part C Ref. #6, 7). 7) Finally, we found that honokiol derivative HK could target Nur77 and mediate the interaction between Nur77-dependent AMPK and mTORC1 and then inhibited EMT in colon cancer.

Therefore, this project has systematically elucidated the role of Nur77 in promoting EMT of cancer and revealed the potential of Nur77-mTORC1 as a therapeutic target. Importantly, we have successfully identified several lead compounds including NAM446-1, N446-7s, Honokiol and compound K that could target Nur77 and modulate Nur77-dependent mTORC1 activity. Some of them are under modification and optimization for further application. Under the support of this project, 6 academic papers have been published and 3 patents were applied and authorized. We attended 3 international meetings, and trained 2 postdoctoral fellows, 2 PhD students and 2 MPhil students. One of postdoctoral fellows was hired as an assistant professor by Xiamen University. The principle investigators of this project were awarded the leading scientific and technology innovative talents of Fujian "Shuangbai Project", and Fellow of Royal Society of Biology.

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

This project is basic exploratory research work, and the transformation of the results has not been carried out yet. However, some compounds which target Nur77 identified in this project, such as NAM446-1, N446-7s, honokiol and compound K, have the potential of further optimization and modification.

## 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)*

Malignant tumors pose a serious threat to human health. Metastasis is the main characteristic of malignant tumors and the main cause of cancer death. Up to 90% of cancer patients eventually die of cancer metastasis. The initial step of metastasis is EMT. Interestingly, EMT is reversible, which provides an excellent direction for the development of anti- metastasis cancer therapies. Many growths signaling pathways are involved in the regulation of EMT. mTORC1 is a growth factor and energy metabolism sensing pathway. It is often found overactivated in many tumors and plays an important role in EMT. Nur77 is an orphan nuclear receptor, which is an early gene with dual functions of survival and death. The abnormal expression and function of Nur77 is related to tumorigenesis and development. This project revealed that Nur77 was a key regulatory protein in hypoxia and inflammatory microenvironment of tumor. Nur77 could indirectly or directly regulate mTORC1 activity and inhibit EMT and metastasis of cancer cells. Based on these novel regulatory mechanisms, the compounds identified in this project which target Nur77, such as NAM446-1, N446-7s, honokiol and compound K, are worth further optimization and modification.

# 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) | Title and Journal/ Book | Submitted to RGC | Attached to this report | Acknowledged the support of this Joint Research Scheme | Accessible from the institutional repository |
|---|---|---|---|---|---|---|---|---|---|
| Year of publication | Year of Acceptance *(For paper accepted but not yet published)* | Under Review | Under Preparation *(optional)* | ***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)* | *(with the volume, pages and other necessary publishing details specified)* | *(indicate the year ending of the relevant progress report)* | *(Yes or No)* | *(Yes or No)* | *(Yes or No)* |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 2017 | | | | Wang PY, Zeng WJ, Liu J, Wu YL, Ma Y, Zeng Z, Pang JY, Zhang XK, Yan X, **Wong AS***, **Zeng JZ*** | TRC4, an improved triptolide derivative, specifically targets to truncated form of retinoid X receptor-alpha in cancer cells. ***Biochemical Pharmacology*** 124, 19-28 | 31/12/2016 | Yes | Yes | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 2017 | | | | Zeng W, Zhang C, Cheng H, Wu YL, Liu J, Chen Z, Huang JG, Ericksen RE, Chen L, Zhang H, **Wong AS***, Zhang XK, Han W and **Zeng JZ*** | Targeting to the non-genomic activity of retinoic acid receptor-gamma by acacetin in hepatocellular carcinoma. ***Scientific Reports*** 7, 348 | 31/12/2016 | Yes | Yes | Yes |
| 2017 | | | | Liu J, Wang H, Chen G, Yang M, Wu Z, Ericksen RE, **Wong AS***, Han W, and **Zeng JZ*** | The synergy of helicobacter pylori and lipid metabolic disorders in induction of Th17-related cytokines in human gastric cancer. ***J. Cancer Metastasis and Treatment*** 3, 169-176 | No | Yes | Yes | Yes |
| 2017 | | | | Hu H, Lin C, Ao M, Ji Y, Tang B, Zhou X, Fang M, **Zeng JZ***, Wu Zhen | Synthesis and biological evaluation of 1-(2-(adamantane-1-yl)-1H-indol-5-yl)-3-substituted urea/thiourea derivatives as anticancer agents. ***RSC Advances*** 7, 51640-51651 | No | Yes | Yes | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 2020 | 2019 (in press) | | | Zhang S, Gao W, Tang J, Zhang H, Zhou Y, Liu J, Chen K, Liu F, Li W, To SK, **Wong AS***, Zhang X, Zhou H, and **Zeng JZ*** | The roles of GSK-3β in regulation of retinoid signaling and sorafenib treatment response in hepatocellular carcinoma. ***Theranostics*** 10, 1230-1244 | No | Yes | Yes | Yes |
| 2019 | | | | Shi ZY, **Zeng JZ***, and **Wong AS*** | Chemical structures and pharmacological profiles of ginseng saponin**s.** ***Molecules*** 24, E2443 | No | Yes | Yes | Yes |
| | | 2019 | | Deng S, To SK, Shi Z, Zhang S, Tang J, **Zeng JZ***, and **Wong AS*** | Hypoxia-induced Nur77 activates PI3K/Akt signaling via suppression of Dicer/Let-7i-5p to induce epithelial-to-mesenchymal transition. ***Science Signaling*** (in revision) | 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)* |
|---|---|---|---|---|---|---|
| Dec 2016/London, UK | Nur77 regulates hypoxia-induced epithelial-to-mesenchymal transition in colon cancer cells | Pharmacology 2016, British Pharmacological Society | 31/12/2016 | Yes | Yes | Yes |
| Nov 2017/Xiamen, China | Targeting β-catenin signaling for anti-metastasis therapies | Asian Federation of Pharmaceutic Sciences Conference | No | Yes | Yes | Yes |
| Mar-Apr 2019/Atlanta, GA, USA | Hypoxia-induced Nur77 activates PI3K-p110〈/Akt/mTOR via suppression of Dicer/Let-7i-5p to induce epithelial-to-mesenchymal transition in colon cancer cells | AACR Annual Meeting 2019 | No | Yes | Yes | Yes |

**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 |
|---|---|---|---|
| Deng Shan | PhD | 2014 | July 2018 |

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

The PIs would like to thank NSFC/RGC for its support. In addition to publication in top-tier journals, other impact includes:

**Patents:**

(1) US Provisional Application No. 62/426,937 (by Deng Shan and **Alice S. T. Wong** on treating cancer by ginsenoside and its analogs).

(2) 中国专利, 吴云龙; 陈晓东; **曾锦章**; 张晓坤, 细胞牵引力显微镜及其在抗癌药物药效及药理检测中的方法，授权, 2018.7.10-2035.2.17，201410538575.9

(3) 中国专利, 吴振; 方美娟; **曾锦章**; 胡鸿雨; 林春蓉; 敖名涛; 钱宇卿, 1-(2-(金刚烷-1-基)-1H-吲哚-5-基)-3-取代脲衍生物及制备和用途，授权, 2018.4.10-2036.2，201510749625.2

**Awards:**

**Alice S. T. Wong**: Fellow, Royal Society of Biology, UK (2019).

**Jin-Zhang Zeng**: leading scientific and technology innovative talents of Fujian "Shuangbai Project"