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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

Role of TRPC5 channels in multidrug resistance in adriamycin-resistant breast cancer cells

瞬時受體通道在乳腺癌多藥耐藥中的作用

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Xiaoqiang Yao	Prof. Jian Jin
Post	Professor	Professor and Dean
Unit / Department / Institution	School of Biomedical Sciences, Chinese University of Hong Kong	School of Pharmaceutical Science, Jiang-Nan University
Contact Information	yao2068@cuhk.edu.hk	jinjian31@126.com
Co-investigator(s) *(with title and institution)*	Prof. FL Chan, Chinese University of Hong Kong	

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	Jan 1, 2014		
Project Completion date	Dec 31, 2017		
Duration *(in month)*	48 months		
Deadline for Submission of Completion Report	Dec 31, 2018		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To examine whether microvesicle-mediated transfer of TRPC5 and/or NFATc3 is indeed important for recipient cells to acquire long-lasting multidrug resistance.
2. To determine the role of TRPC5 in angiogenesis in drug resistant tumors

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)

6.1. We found that after the incubation with microvesicles from drug-resistant donor cells, the recipient cells acquired TRPC5 and P-gp. The recipient cells also had demonstrated an increased nuclear transfer of NFATc3. We developed a hypothesis that drug resistance property can be transferred from drug-resistant cancer cells to non-resistant cells via microvesicle-mediated transfer" of TRPC5.

6.2. TrpC5-containing circulating EVs were found in peripheral blood from patients who underwent chemotherapy but not patients without chemotherapy. We propose that TRPC5-containing EVs could be a potential diagnostic biomarker for chemoresistant breast cancer.

6.3. We found that the number of blood vessels is much more in drug-resistant tumor than in drug-sensitive tumor, and that suppressing TRPC5 with TRPC5-siRNA could reduce the number of blood vessels in adriamycin-resistant tumor. The mechanism may involve TRPC5-Ca^{2+}-HIFα signaling pathway. We also found that TRPC5 can promote endothelial tube formation and endothelial cell migration. Suppression of TRPC5 inhibits this type of endothelial tube formation and migration.

6.4. We uncovered another mechanism of TRPC5-mediated drug resistance of human breast cancer. Here we found that TRPC5 promotes autophagy via CaMKKβ/AMPKα/mTOR pathway. This confers the breast cancer cells with the properties of drug resistance.

In summary, most of the above-mentioned data are published in Ma X, et al., 2014 *Proc Natl Acad Sci USA.* 111:6389-6394; Zhu Y, et al., 2015 *Pharmacol Res.* 93:36-42; Meng H, 2014 M.Phil Thesis, Chinese University of Honf Kong; Zhang P et al., 2017 *Sci Rep.* 7(1):3158. The funding from this grant also enabled multiple TRP channel-related functional studies, which are published in nine other papers, including those in Nature Communication (Lau OC. *Nature Commun* 7:11947), Cell Death Differentiation (Sun L et al., 2018, *Cell Death Differ* 25:368-79) and Stem Cells (Wang Y et al., 2015 *Stem cells* 33(10):2973-84; Lu J et al., 2018 *Stem Cells* 36(4):501-513).

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

Further development of this research will be at several directions

1) During the process of studying TRPC5 in breast cancer chemoresistance, we identified another protein, named TM9SF4, that is crucial for breast cancer chemoresistance. We are planning to further explore whether TM4SF4 could be another potential target for overcoming chemoresistance of breast cancer.
2) In translational direction, we are in the process of developing a diagnostic kit for potential clinical usage. This diagnostic kit is based on TrpC5-containing circulating EVs.

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)

Development of multidrug resistance in tumor cells is a serious problem in cancer chemotherapy. Furthermore, the property of drug resistance can be transferred from drug-resistant tumor cells to drug-sensitive tumor cells via microvesicles. A key protein for drug resistance is P-glycoprotein (P-gp). P-gp pumps cytotoxic drugs from tumor cells, making the tumor cells resistant to chemotherapeutic drugs. Previous studies from us demonstrated that, in drug-resistant breast cancer cells, a Ca2+-permeable ion channel TRPC5 is upregulated to promote P-gp production, which renders breast cancer cells with drug-resistant property. Through the study of the present grant, we found TRPC5 and NFATc3 are transferred from drug-resistant tumor cells to drug-sensitive tumor cells via microvesicles. This allows the drug-sensitive tumor cells to acquire the drug resistance property. Furthermore, we also found that TRPC5 promotes angiogenesis via TRPC5-Ca^{2+}-HIFα signaling pathway. The results from this study validated a crucial functional role of TRPC5 in drug-resistance of breast cancer. It is possible to target TRPC5 and/or its associated signaling molecules as a treatment strategy for drug-resistant breast cancer.

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)*						
2014				**Ma X*, Chen Z,** Hua D, He D, Wang L, **Zhang P,** Wang J, **Cai Y,** Gao C, **Zhang X, Zhang F,** Wang T**,** Hong T, Jin L, Qi X, **Chen S, Gu X, Yang D, Pan Q, Zhu Y, Chen Y,** Chen D, Jiang L, Han X, Zhang Y, **Jin J*, Yao X**	Essential role for TrpC5-containing extracellular vesicles in breast cancer with chemotherapeutic resistance. *The Proceedings of the National Academy of Sciences, U.S.A.* 111:6389-6394.	2016	Yes	Yes	Yes
2015				**Zhu Y, Pan Q, Meng H, Jiang Y, Mao A,** Wang T, Hua D, **Yao X, Jin J*, Ma X***	Enhancement of vascular endothelial growth factor release in long-term drug-treated breast cancer via transient receptor potential channel 5-Ca^{2+}-hypoxia-inducible factor 1α pathway. *Pharmacol Res.* 93:36-42.	2016	Yes	Yes	Yes
2015				**Qi Y, Li ZC,** Kong CW, Tang NL, Huang Y, Li RA, **Yao X***	Uniaxial cyclic stretch stimulates TRPV4 to induce realignment of human embryonic stemcell-derived cardiomyocytes. *J Mol Cell Cardiol* 87:65-73.	2016	Yes	Yes	Yes
2015				**Wang Y, Li ZC, Zhang P,** Poon E, Kong CW, Boheler KR, Huang Y, Li RA, **Yao X***	Nitric oxide-cGMP- PKG pathway acts on Orai1 to inhibit the hypertrophy of human embryonic stem cell-derived cardiomyocyte. *Stem Cells* 33:2973-84.	2016	Yes	Yes	Yes

2016				**Lau OC, Shen B, Wong CO, Tjong YW, Lo CY,** Wang HC, Huang Y, Yung WH, Chen YC, Fung ML, Rudd JA, **Yao X***	TRPC5 channels participate in pressure-sensing in aortic baroreceptors. *Nature Commun* 7:11947		Yes	Yes	Yes
2016				**Lau EOC, Lo CY**, Yao Y, Mak AFT, Jiang LW, Huang Y, **Yao X***	Aortic baroreceptors display higher mechanosensitivity than carotid baroreceptors. *Front Physiol* 7:384.		Yes	Yes	Yes
2017				**Zhang P, Liu X, Li H, Chen Z, Yao X, Jin J*, Ma X***	TRPC5-induced autophagy promotes drug resistance in breast carcinoma via CaMKKβ/AMPKα/mTOR pathway. *Sci Rep.* 7(1):3158.		Yes	Yes	Yes
2017				**Zheng CB,** Zhong M, Qi Z, Shen F, **Zhao Q,** Wu L, Huang Y, Tsang SY, **Yao X***	Histone deacetylase (HDAC) inhibitors relax mouse aorta partly through their inhibitory action on L-type Ca^{2+} channels. *J Pharmacol Exp Ther* 363(2):211-220.		Yes	Yes	Yes
2017				**Zheng CB, Lo CY, Meng Z, Li Z, Zhong M**, **Zhang P, Lu J,** Yang Z, **Yan F, Zhang Y, Huang Y, Yao X***	Gastrodin inhibits store-operated Ca^{2+} entry and alleviates cardiac hypertrophy. *Front Pharmacol* 8:222.		Yes	Yes	Yes
2017				**Lu J**, Lee YK, Ran X, Lai WH, Li RA, Keung W, Tse K, Tse HF, **Yao X***	An abnormal TRPV4-related cytosolic Ca^{2+} rise in response to uniaxial stretch in induced pluripotent stem cells-derived cardiomyocytes from dilated cardiomyopathy patients. *Biochim Biophys Acta - Mol Basis Disease* 1863:2964-2972.		Yes	Yes	Yes
2018				**Sun L, Meng Z, Zhu Y, Lu J, Li Z, Zhao Q,** Huang Y, Jiang LW, **Yao X***	TM9SF4 is a novel factor promoting autophagic flux under amino acid starvation. *Cell Death Differ* 25:368-79.		Yes	Yes	Yes
2018				**Lu J,** Boheler KR, Jiang LW, Chan CW, Tse WW, Keung W, Poon ENY, Li RA, **Yao X***	Polycystin-2 plays an essential role in glucose starvation-induced autophagy in human embryonic stem cell derived cardiomyocytes. *Stem Cells* 36(4):501-513.		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)*
May/2015/ Hong Kong	Role of TRPC5 in multidrug resistance of breast cancer cells	ASCEPT(Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists)-British Pharmacological Society (BPS) Joint Scientific Meeting	2016	Yes	Yes/acknowledged verbally in the speech	No
May/2016/ Isparta, Turkey	Role of TRPC5 in multidrug resistance	6th Oxidative Stress, Calcium Signaling and TRP Channel World Congress.		Yes	Yes/acknowledged in the abstract and ppt	No
December/ 2017/Hong Kong	Role of TRPC5 in multidrug resistance of breast cancer.	Cardiovascular Calcium Signalling Pathway: Role in Health and Disease.		Yes	Yes/acknowledged in the abstract	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
Meng, Huan	M.Phil	August 2012	July 2014
Wang, Yan	Ph.D.	September 2011	October 2014

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