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【Section 1】

NTU principle investigator	
Name (last name, first name)	**Shen, Tang-Long**
Position	**(1) Professor and Chair; (2) Director**
Faculty/Department	**(1) Plant Pathology and Microbiology** **(2) Center for Biotechnology**

KU principle investigator	
Name (last name, first name)	**Harada, Hiroshi**
Position	**(1) Professor; (2) Director**
Faculty/Department	**(1) Graduate School of Biostudies** **(2) Radiation Biology Center**

Type(s) of funding applied
☐ Funding Type 1 (General Funding) only
☐ Funding Type 2 (ECR Funding) only
☑ Both Funding Type 1 (General Funding) and Type 2 (ECR Funding)

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【Section 2】

Project title
Int'l collaboration to elucidate molecular mechanisms underlying distant metastasis of tumors

Period of project	
From dd/mm/yy to dd/mm/yy	**18/02/2024 – 02/03/2024**

Summary of the project (approx. 100 words)
Cancer metastasis, responsible for most cancer deaths, involves complex molecular mechanisms that include hypoxia-enhanced exosome secretion and pre-metastatic niche formation in distant organs. In order to identify a responsible factor for pre-metastatic niche formation, which is incorporated in exosomes, one of Prof. Shen's students (Zeng-Yi Li) in NTU visited Prof. Prof. Harada's laboratory in Kyoto University, cultured cancer cells in a special hypoxic workstation, and harvested culture medium. It was then sent to NTU and subjected to the analysis to identify the responsible factor for pre-metastatic niche formation. This research is expected to deepen the understanding of mechanism behind tumor metastasis. Subsequently, the isolated exosomes derived from differential hypoxic conditions displayed an increased trend in exosome size, number, RNA as well as protein amounts although the experiments are needed to be repeated for a statistical analysis. Furthermore, we also observed the differential mRNA cargos, such as RAD51, RAD52, MLH1, and MALAT1. Currently, we are proceed the proteomics and RNA seq of the varied hypoxia-induced exosomes for deciphering the key molecules in response to different hypoxic conditions. Further experimental designs, including the premetastatic niche formation and genomic instability, resulted from the varied hypoxia-derived exosomes will be conducted in a collaborative way between NTU and KU.

Photographs with captions
*Please submit digital files (such as JPEG or GIF files) of the photographs used in your report as attachments. The size of each image should be at least 4MB, so that it can be used for printed materials. Please ensure that none of the photographs submitted will cause any issues relating to portrait rights.

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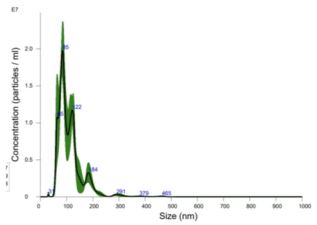
Measurement of Hypoxia, anoxia and normoxia-derived exosomes

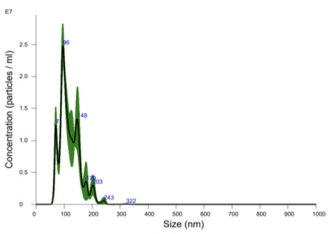
The exosomes derived from different oxygen concentration show different sizes

- Detect by Malvern Panalytical NanoSight NS300®



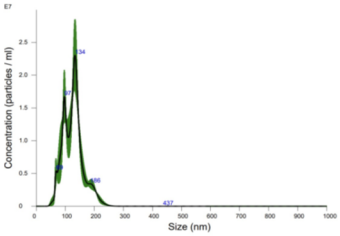
Mean:	112.6 +/- 7.3 nm
Mode:	79.8 +/- 8.2 nm
SD:	48.3 +/- 9.9 nm
D10:	68.9 +/- 4.3 nm
D50:	97.5 +/- 3.3 nm
D90:	171.8 +/- 11.9 nm
Concentration (Upgrade):	1.13e+009 +/- 7.54e+007 particles/ml
	12.5 +/- 0.5 particles/frame
	13.8 +/- 0.5 centres/frame

Normoxia exosome 10x dilution (240328)



Mean:	119.4 +/- 2.0 nm
Mode:	97.3 +/- 2.6 nm
SD:	35.5 +/- 2.7 nm
D10:	77.3 +/- 3.2 nm
D50:	109.7 +/- 1.9 nm
D90:	163.9 +/- 4.0 nm
Concentration (Upgrade):	1.37e+009 +/- 4.84e+007 particles/ml
	15.9 +/- 0.4 particles/frame
	16.3 +/- 0.4 centres/frame

Hypoxia exosome 10x dilution (240320)



Mean:	126.8 +/- 2.8 nm
Mode:	132.3 +/- 1.2 nm
SD:	34.2 +/- 1.3 nm
D10:	85.2 +/- 3.5 nm
D50:	125.5 +/- 2.8 nm
D90:	172.0 +/- 3.8 nm
Concentration (Upgrade):	1.38e+009 +/- 2.20e+007 particles/ml
	18.4 +/- 0.3 particles/frame
	20.0 +/- 0.3 centres/frame

Anoxia exosome 10x dilution (240320)

Differential expression of Hypoxia, anoxia and normoxia-derived exosomes

$3x10^4$ cells (hypoxia, normoxia condition) and $5x10^4$ cells (anoxia condition) were seeded in 100mm plates for 3 days before exosome harvest

Sample Info			NTA					Protein		RNA			
Sample Name	Original volumn (mL)	Final volumn (µL)	Mean particle size (nm)	NTA dilution factor	NTA particle conc. (n/mL)	Particle conc. (n/mL)	Total paticle (n)	Protein conc. (µg/µL)	Total protein (mg)	A260/A280	A260/A230	RNA conc. (ng/µL)	Total RNA (ng)
normoxia exosome	25	500	112.6	10	1.13E+09	1.13E+10	5.65E+09	48	24	1.555	0.19	134.85	67425
hypoxia exosome	25	500	119.4	10	1.37E+09	13700000000	6850000000	83	41.5	1.905	3.29	1703.5	851750
anoxia exosome	25	500	126.8	10	1.38E+09	13800000000	6900000000	71	35.5	1.555	0.135	84.65	42325

URL at which project outcomes can be viewed (Optional)

*E.g. workshop notifications/programs/reports, evidence of academic papers published or otherwise made available, etc.

URL:

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【Section 3】

Visiting ECR*	
Name (last name, first name)	**Li, Zhen-Yi**
Position	**Master student**
Faculty/Department	**Plant Pathology and Microbiology**
Period of Stay (From dd/mm/yy to dd/mm/yy)	**18/02/2024 – 02/03/2024**

Host researcher*	
Name (last name, first name)	**Harada, Hiroshi**
Position	**(1) Professor; (2) Director**
Faculty/Department	**(1) Graduate School of Biostudies** **(2) Radiation Biology Center**

*Please complete if the host researcher is different from the KU principal investigator.

Achievements and outcomes of ECR stay (approx. 100–250 words)

In order to analyze the contents contained in exosomes secreted from cells stimulated by hypoxia, special equipment (hypoxic workstation) is required to culture cells under hypoxic conditions. A member of the NTU laboratory leading exosome research visited the Kyoto University laboratory leading hypoxia research, and conducted international joint research, making it possible for the first time to conduct multidisciplinary research that engage both research fields. By having young NTU researchers take on this role, we could develop a bilingual researcher who expertizes both fields. This initiative was also confirmed to be effective in fostering an international mindset among young researchers in Kyoto University.

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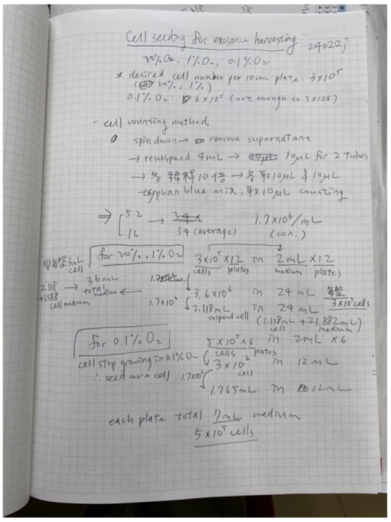
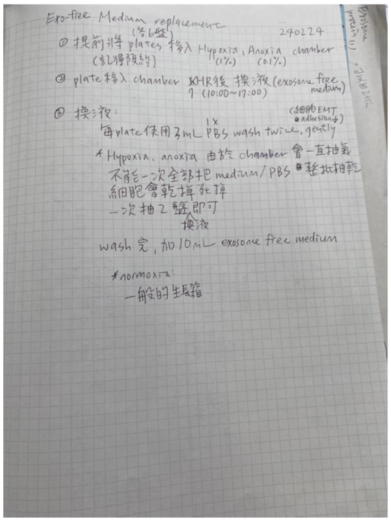
Student's protocols and experimental notes for Hypoxia, anoxia and normoxia exosome isolation





URL at which project outcomes can be viewed (Optional)

*E.g. workshop notifications/programs/reports, evidence of academic papers published or otherwise made available, etc.

URL:

***If there are multiple ECRs, please copy and paste this section and complete them for each ECR.**

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