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NRCPD-OUAVM Joint Research Report

Date: _____

Project no: _____

1. Principal investigator

Name: **Chia-Kwung Fan**

Position: **Professor & Chairman**

Affiliation: **Department of Molecular Parasitology and Tropical Diseases, School of Medicine Center for International Tropical Medicine, College of Medicine Taipei Medical University, Taiwan**

2. Project title: **Molecular pathogenesis of inflammasome and carcinogenesis induced by *T. vaginalis* on human normal prostate epithelial cell (PZ-HPV-7)**

3. Collaborating research group members at NRCPD

Name: **Shin-ichiro Kawazu**

Position: **NRCPD, Obihiro University, Japan**

4. Research period (in mm/dd/yyyy, and total number of years)

April 1,2016 – March 31, 2017

5. Purposes and objectives

Prostate cancer (PC) is the second most incident cancer in men worldwide. In 2008 there were 903,500 estimated new cases and 258,400 estimated deaths worldwide, resulting as the sixth cause of death by cancer in men. Recently, scientists found a significantly increased PC risk in men having had sexually transmitted diseases (STD). Due to high incidence of both STD and PC worldwide, prevention of STD may help preventing a considerable number of PC cases. *Trichomonas vaginalis*, a motile protozoan, is the most common curable STD worldwide, with an estimated 174 million new cases a year, of which 154 million occur in resource-limited settings. In addition, *T. vaginalis* has been detected in prostate tissue sections from men with trichomonosis, interestingly near areas of inflammation and focal epithelial hyperplasia, which were hypothesized to be the result of trichomonal infection. Potential interactions of *T. vaginalis* in its

prostatic habitat may be investigated with respect to their possible contribution to the inflammatory pathogenesis of prostatic tissue, since inflammatory cytokines have been shown to sustain prostatic hyperplastic growth. Chronic *T. vaginalis* infections may result in inflammation and cell proliferation, thus triggering pathways that contribute to the promotion and progression of PC.

Recent studies have shown that *T. vaginalis* macrophage migration inhibitory factor (TvMIF) can act as a molecular mimic of human macrophage migration inhibitory factor (HuMIF) and binding to the human CD74 receptor activates extracellular signal-regulated kinases (ERK)1/2 and Akt protein kinase/proapoptotic Bcl-2 associated death promoter (BAD) pathways as well as secretion of proinflammatory IL-8 from monocytes, reduces monocyte migration, and increases growth and invasiveness of benign prostate hyperplasia (BPH-1) and prostate cancer (PC3) cells. Together, *T. vaginalis* may increase IL-1β expression in human prostate epithelium through activation of ROS, ERK, and NF-kB, and this in turn may induce the migration of neutrophils and monocytes and lead to an inflammatory response.

RNA-seq has become a common technique in the detection of transcriptome. It is a sequencing technique with high-throughput ability to sequence cDNA library which is transcribed from all RNAs in tissues or cells, and can be used to quantify or discover RNA transcripts by sequence reads. It has been widely used in biological, medical, clinical and pharmaceutical research.

We intend to undertake RNA-seq analysis of inflammatory and carcinogenic mechanism induced by *T. vaginalis* on human normal prostate epithelial cell (PZ-HPV-7). PZ-HPV-7 is an in vitro model of carcinogenesis of the prostate induced by *T. vaginalis* infection. The outcomes from RNA-seq experiments will be confirmed by RT-PCR and RNAi experiments for which we are expecting collaboration with NRCPD. In addition, a comparison in pathogenicity will be made between different strains of *T. vaginalis* obtained from Taiwanese patients and the other *T. vaginalis* from different geographic districts in the world. These results may not only provide advanced information concerning RNA levels on how *T. vaginalis* is involved in regulation to induce inflammatory and carcinogenic developments in PZ-HPV-7, but also the molecular mechanism of RNA level regulation between host and parasite will be disclosed.

6. Outline of research process

a. Co-culture PZ-HPV-7 with *T. vaginalis* (TMU).

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b. Establish transcriptome of PZ-HPV-7 and *T. vaginalis* by using RNA-seq analysis (TMU).

c. Using Real-Time PCR to confirm the quantity and significance of individual Unigene involved in PZ-HPV-7 and *T. vaginalis* expression which participate in the pathway of Prostate Cancer. (NRCPD)

d. mRNA expression of inflammatory factors (IL-1beta, CCL2 and CXCL8) and carcinogenic genes (GSTP1, NKX3.1, PTEN and AR) will be assessed by using RNA interference (RNAi) to transfect PZ-HPV-7 and *T. vaginalis* to further insight into molecular mechanisms of RNA levels of inflammatory and carcinogenic factors. (TMU and NRCPD)

e. A comparison in pathogenicity will be made between *T. vaginalis* obtained from Taiwanese patients and the other *T. vaginalis* from different geographic districts in the world (TMU).

7. Outline of research achievements

a. Our project is sequenced on the platform of Illumina Hiseq 2000. 5968814760nt bases are generated totally. In the results of assembly, 38645 unigenes are detected, total length for unigenes is 24996552 nt, average length is 647 nt，N50 is 890 nt. For function annotation analysis, we get 37318, 36548, 13338, 11720, 9338, 13154 unigenes which annotate to the NR, NT, Swiss-Prot, KEGG, COG, GO database, respectively, the total annotation unigenes are 37492. For protein coding region prediction analysis, the number of CDS that mapped to the protein database is 37159, the number of predicted CDS is 789. The total number of CDS is 37948. SSR is 946.

Output statistics of sequencing

Samples	Total Raw Reads	Total Clean Reads	Total Clean Nucleotides (nt)	Q20 percentage	N percentage	GC percentage
T_vaginalis_trophozoite	74,532,944	66,320,164	5,968,814,760	97.93%	0.00%	46.47%

Statistics of assembly quality

	Sample	Total Number	Total Length (nt)	Mean Length (nt)	N50	Total Consensus Sequences	Distinct Clusters	Distinct Singletons
Contig	T_vaginalis_trophozoite	56,652	26,999,372	477	766	-	-	-
Unigene	T_vaginalis_trophozoite	38,645	24,996,552	647	890	38,645	2,591	36,054

b. Unigene annotation provides information of expression and functional annotation of Unigene. Information of functional annotation gives protein functional annotation, COG functional annotation and Gene Ontology (GO) functional annotation of Unigenes. Unigene sequences are firstly aligned to protein databases like NR, Swiss-Prot, KEGG and COG (e-value<0.00001) by

blastx, and nucleotide database NT (e-value<0.00001) by blastn, retrieving proteins with the highest sequence similarity with the given Unigenes along with their protein functional annotations, the results about this are inculded in the folder annotation. Unigenes were annotated with the databases of NR, NT, Swiss-Prot, KEGG, COG and GO. Then counted the number of Unigenes annotated with each database

Statistics of annotation results

Sequence File	NR	NT	Swiss-Prot	KEGG	COG	GO	ALL
T_vaginalis_trophozoite-Unigene.fa	37,318	36,548	13,338	11,720	9,338	13,154	37,492

c. KEGG is a database that is able to analyze gene product during metabolism process and related gene function in the cellular processes. With the help of KEGG database, we can further study genes' biological complex behaviors, and by KEGG annotation we can get pathway annotation for Unigenes.

KEGG Pathway is divided three levels, level 3 owns the detailed pathway gragh and response gene,we annotate the unigenes to each level 3 pathway gragh throught mapping, and the pathway graghs are maked as html format, in order to skipping conveniently.

d. A total of 239 unigenes of *T. vaginalis* participated in Prostate Cancer Carcinogenesis pathway.

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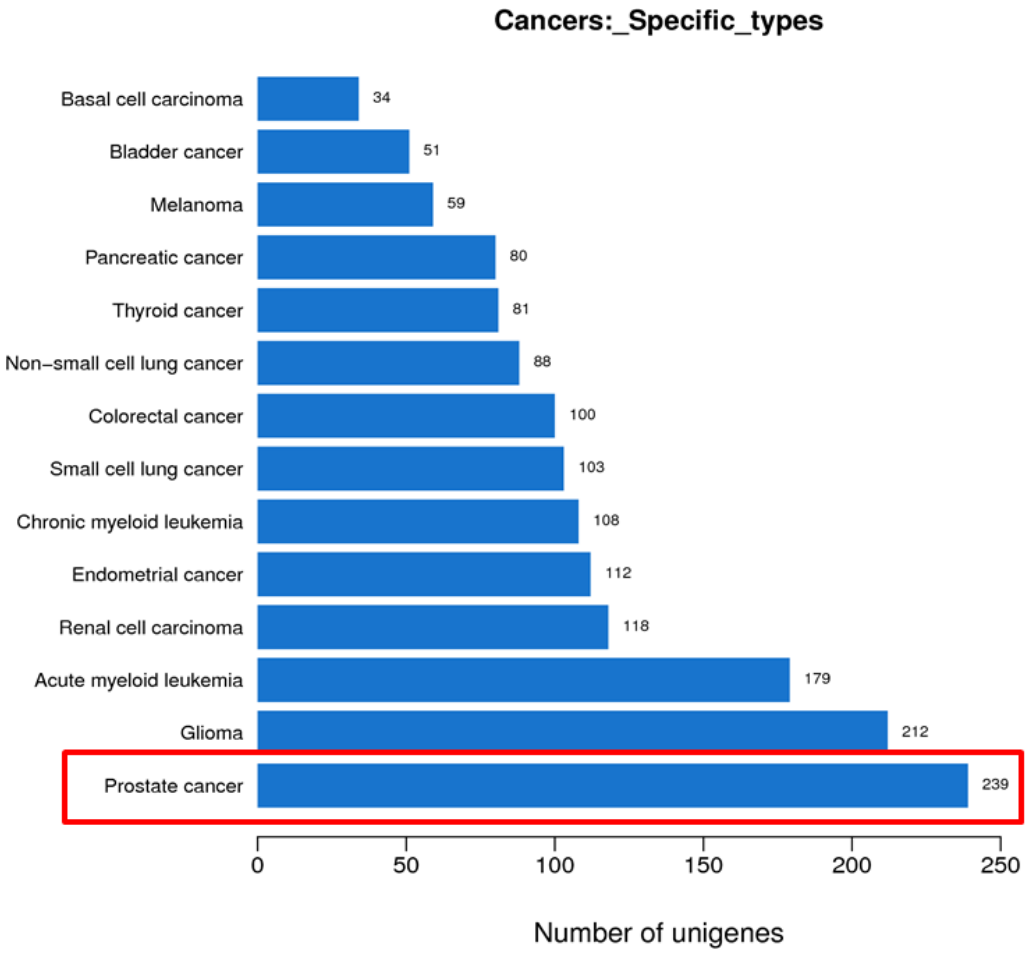

f. A total of 91 unigenes of *T. vaginalis* participated in JAK-STAT signaling pathway

g. A total of 377 unigenes of *T. vaginalis* participated in MAPK signaling pathway

h. A total of 245 unigenes of *T. vaginalis* participated in Chemokine signaling pathway (IL-1beta, CCL2 and CXCL8)

8. Publication of research achievements

Attach reference materials as necessary.