NWO/RGC JOINT RESEARCH SCHEME
COMPLETION REPORT

Project Reference Number

D-HK005/11T

Project Title

Functional analysis of the dyslexia candidate protein (KIAA0319L) using baculovirus expression vectors for expression in insect cells

Particulars

	Hong Kong team				Dutch team
Name of Principal Investigator (with title)	English: Prof Dr Mary M.Y. Waye Chinese : 韋妙宜				Prof Dr Just M. Vlak
Name of Co-Investigator (if any)	English: Chinese:				Dr Monique M. van Oers
Institution or Institutional affiliation		CityU		HKU	Laboratory of Virology, Wageningen University
	✓	CUHK		HKUST	
		HKBU		LU	
		HKIEd		PolyU	
Other project team members (if any)					

Funding Period

	1st year	2nd year (if applicable)
Start Date	July 1, 2011	NA
Completion Date	June 30, 2012	NA

Objective(s) as per original application

1. To sub-clone the cDNA of human KIAA0319L into recombinant baculovirus plasmid vectors
2. To express the recombinant protein KIAA0319L in insect cells

[Please attach relevant document(s)]

i) Outline of proposed research and results obtained

The laboratory in Wageningen has been involved in the design and development of the baculovirus insect cell system and has produced many proteins that are biological active, such as those for vaccines and diagnostics. The Laboratory of Dr Waye has shown that the candidate dyslexia related protein KIAA0319L can interact with the Nogo Receptor (Poon et al, 2010). The biological significance of such interaction is not clear; however since the Nogo Receptor is an important axon guidance molecule involved in inhibition of axon regeneration in the central nervous system, we speculate that the binding of KIAA0319L might also affect the function of Nogo Receptor.

To further study the biological function of KIAA0319L high level production of a functionally active Molecule is needed that is suitable for *in vitro* work. Since the baculovirus insect cell expression system has a suitable glycosylation pattern, we planned to design and make appropriate recombinant plasmid and viral constructs for the expression of KIAA0319L using this expression system. We aimed at expressing membrane bound and secreted forms of this protein.

We constructed four transfer vectors to make recombinant bacmids, baculovirus genomes that can be amplified in *E. coli*. From there we produced four baculovirus recombinant viruses that encode the KIAA0139-lik protein. The constructs vary in signal peptide (human or insect derived) and whether the construct does have a C-terminal transmembrane region or not. All 4 viruses produced the KIAA00319L protein during virus infection of insect cells as seen with antibodies against the attached HA tag, and specific antibodies against the recombinant protein. All 4 proteins are not secreted, probably due to the fact that there is also a hydrophobic region, more towards the N-terminus of the protein (i.e. amino acid no. 30-50).

ii) Significance of research results

The results showed that we can indeed express this protein in insect cells and also demonstrated that making a secreted form is more complicated than we thought before. Meanwhile new data about processing of a related protein KIAA0319 became available, and this gives clues for further research for KIAA0319L.

iii) Research output

Four recombinant viruses and recombinant protein expression in insect cells of Ha tagged KIAA0319L protein. Viruses and cells have been transferred to Hong Kong to introduce the technology there. A thesis report has been written by a Wageningen BSc student (see attachment). A manuscript is in preparation for publication titled "Expression of KIAA0319L protein in baculovirus vectors".

iv) Potential for or impact on further research collaboration

The protein can now be produced in larger quantities for purification and functionality tests. Adding the protein in roughly purified form to measure wound healing ability of neuronal cells (a measure for neuronal migration), test the interaction with the NOGO-receptor in detail and see how downstream effectors respond to binding of this protein. During our collaboration also many new ideas came up like efficiently delivering the KIAA0319L gene, as well as its relative the KIAA0319 protein, with baculovirus vectors into human cells. The absence of the secreted form in the supernatant (despite of many attempts to try to purify the secreted form) might be due to the fact that the protein becomes trapped in the extracellular matrix. The potential role of this protein as a signaling molecular in the extracellular matrix would be another area for further collaborative research.