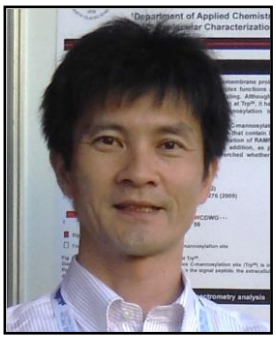
Principal Investigator: Siro Simizu
Grant Title: Identification of Novel *C*-mannosyltransferase in Human Cells
Abstract

Objectives:



C-mannosylation is a mysterious protein glycosylation, because there are two reasons; i) the number of substrates is still about 30 proteins and ii) only DPY19 family is responsible *C*-mannosyltransferase so far. To identify novel *C*-mannosyltransferase, we sought to find unique substrate proteins, and searched for novel biological role of *C*-mannosylation in cultured cell lines.

Methods:

We have already established the protocol for purification of recombinant protein from HT1080 cells, a human fibrosarcoma cell line. We obtained several cDNAs encoding candidates as *C*-mannosylated proteins, transfected and established proteins of interest-overexpressing cells. Moreover, we tried to clarify the role of *C*-mannosylation for differentiation, we examined whether *C*-mannosylation is required for the myogenic differentiation by knock-out of *DPY19L3* gene.

Results:

We could identify several novel *C*-mannosylated proteins, such as Isthmin-1, ADAMTS4, RAMP1, PMEL and Vmo1. Isthmin-1 and ADAMTS4 are *C*-mannosylated in the TSR domain. The *C*-mannosylation positively regulates for secretion of both Isthmin-1 and ADAMTS4. Interestingly, RAMP1 was *C*-mannosylated by DPY19 family-independent manner, suggesting that there is another *C*-mannosyltransferase(s) in human cells.

Furthermore, we deleted DPY19L3 gene from C2C12 cells, and evaluated whether DPY19L3 regulates myogenic differentiation. Knock-out of *DPY19L3* gene resulted in suppression of MEK/ERK phosphorylation levels, expressions of differentiation markers and myogenic differentiation, indicating that DPY19L3-mediated *C*-mannosylation (*C*-mannosylated protein(s)) is important for the differentiation.

References:

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