Form 1-5-2

Japan-U.S. Brain Research Cooperation Program
Researchers Dispatched to the U.S. FY2009: Report

Field: ④

1. Researcher
Name: Hirokazu Yagi
Title: Assistant Professor
Affiliation: Graduate School of Pharmaceutical Sciences, Nagoya City University

2. Research Title:
The functional significance of the N-glycans in the differentiation of neural stem cells

3. U.S. Joint Researchers/Institutes
Please give the name, title and affiliation.
Name: Robert K. Yu
Title: Professor
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4. Research Period, from/to (mm/dd/yyyy):
From 07/01/2009 to 03/25/2010

5. Abstract, Results, and Research Significance (300 Words):

Neural stem cells (NSCs) are undifferentiated neural cells characterized by their high proliferative potential and the capacity for self-renewal with retention of multipotency to differentiate into brain forming cells, such as neurons and glia. Environmental factors of NSCs, such as growth factors, extracellular matrice (ECMs), and cell-adhesion molecules, are important in maintaining stem cell population throughout specific cell-lineage pathways. Glycoconjugates, including glycoproteins, proteoglycans, and glycolipids, are mainly expressed on the cell surface as ECM, and they are known to regulate cell to cell communications. So we hypothesized that the carbohydrates in NSCs might be regulated to maintain stemness and their differentiation.

In this study, we found that two glycotopes (Lewis X and HNK-1) were expressed in embryonic mouse NSCs and that this expression was lost during the process of differentiation.

Lewis X has been well known a carbohydrate antigenic epitope of undifferentiated cells as a stage-specific embryonic antigen-1 (SSEA-1). However, the exact nature of the carrier proteins has not been fully characterized. Using proteomics analyses, we demonstrated that a lysosomal protein, LAMP-1, is a major carrier protein of SSEA-1 in NSCs, despite the common belief that SSEA-1 is mainly expressed on the cell surface and constitutes a component of the extracellular matrix. This result has been accepted in a scientific journal, *Glycobiology*.

Furthermore it was revealed that the HNK-1 epitopes were almost exclusively displayed on an extracellular matrix protein, Tenascin-C (TNC). Furthermore, the HNK-1 epitope was found to present only on the largest isoform of the TNC molecules. In addition, the expression of HNK-1 was regulated by expression of the largest TNC variant but not by enzymes involved in the biosynthesis of HNK-1. By knocking down HNK-1 sulfotransferase or TNC by small interfering RNA, we further demonstrated that HNK-1 on TNC was associated with proliferation of NSCs.

6. Other (Research concerns, particular points of note):

*Please attach any reference materials as necessary.