

## A Novel Concentrating System of Chicken Stem Cells by Bone Marrow Side Population Cells

Fumitake Usui 1), Yoshiaki Nakamura 1) 2), Yasuhiro Yamamoto 3), Kouichi Tatsumi 1), Kazue Tominari 1), Tamao Ono 1), and Hiroshi Kagami 1)

1) Faculty of Agriculture, Shinshu University, Japan, 2) Animal Breeding and Reproduction Research Team, National Institute of Livestock and Grassland Science (NILGS), Japan, 3) Division of Human Genetics, Department of Integrated Genetics, National Institute of Genetics, Research Organization of Information and Systems, Japan

### **Abstract :**

Numerous studies in mammalian species have recently been reported that many stem cells have an ability to efficiently efflux the vital DNA-binding dye Hoechst 33342, and it is called side population (SP) cells. However, few study have been reported on the avian SP cells. It could be possible that concentration of hematopoietic stem cells (HSCs) in birds since the characteristic of SP cells should be shared in various tissues and species. In this study, we first attempted the isolation of SP cells from chicken bone marrow and the assessment by gene expression and morphologic analyses. Bone marrow cells (BMCs) were flushed from the femurs and tibias of chicks aged at 10 days with PBS. The BMCs were layered on lymphocyte separation medium and centrifuged for excluding the erythrocytes. The separated cells were adjusted to 10<sup>6</sup>/ml in HBSS. Hoechst 33342 were added (1.25 µg/ml) and incubated 60 to 90 minutes at 37°C. Propidium iodide was added (2 µg/ml) to exclude dead cells. The SP cells were isolated with flow cytometer. The sorted cells were stained with May-Gruenwald Giemsa (MG) for morphological analysis and RNA was extracted for gene expression analysis. The avian SP cells which was vanished by addition verapamil could be separated. The percentage of SP cells in chicken bone marrow was about 2.6%. The morphological analysis by MG staining indicated that the SP cells had a larger nuclear and little cytoplasm which were typical characterisation of mouse HSCs. The pattern of gene expressions (CD34, c-Kit, CD4 and CD8) in SP cells also resembled that of the mouse HSCs. These results suggested that the HSCs could be enriched from avian bone marrow cells. Together with these results, it was concluded that SP is one of powerful tools for concentration of avian stem cells.

### **Key Word :**

bone marrow cells, chicken, flow cytometric analysis, hematopoietic stem cells, side population

*Volume 47, Number 1, - 2010, ISSN 1349-0486 (online), ISSN 1346-7395 (print)*