Technology and Development Team for BioSignal Program
Subteam for Manipulation of Cell Fate

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Goal

Stem cells are defined as undifferentiated cells capable of making identical copies of themselves and giving rise to specialized cells that make up the tissues and organs of the body. The potential use of stem cells for regenerative therapy is enormous, but before they can be used clinically, the mechanisms that regulate their proliferation and differentiation must be clarified. We are studying to identify the signals that control hematopoietic stem cell fate and hope to develop technologies for manipulating stem cells *in vitro*.

Activities

1. Molecular basis of hematopoietic stem cell proliferation and differentiation (Figure 1)
2. Studies on the plasticity and reprogramming of hematopoietic stem cells (Figure 1)
3. Development of lentiviral vectors for stem cell biology (Figure 2)

Members

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Specific aim

1. Gene expression analysis of hematopoietic stem cells

Gene expression profiling of hematopoietic stem cells (HSCs) would be worthwhile to elucidate regulatory mechanisms of self-renewal and preservation of multi-lineage differentiation potential of HSCs. We have carried out gene expression profiling by microarray in rigorously FACS purified long-term self-renewing HSCs, short-term self-renewing HSCs, and differentiated hematopoietic cells (B cells, T cells, granulocytes, macrophages, erythroblasts) (Figure 1). OP9 and PA6 mouse stromal cell lines were shown to support HSCs *in vitro*, and we have utilized HSC-nonsupportive PA6 subcell lines for microarray to determine differential expression of genes involved in the maintenance of the stem cell state. Based on the microarray analysis, we have chosen about 200 genes that are expected to play an important role in the proliferation and maintenance of HSCs. Now we are analyzing selected candidate genes by transferring cDNA or siRNA (small interfering RNA) of corresponding genes into HSCs or PA6 cells using lentiviral vectors.

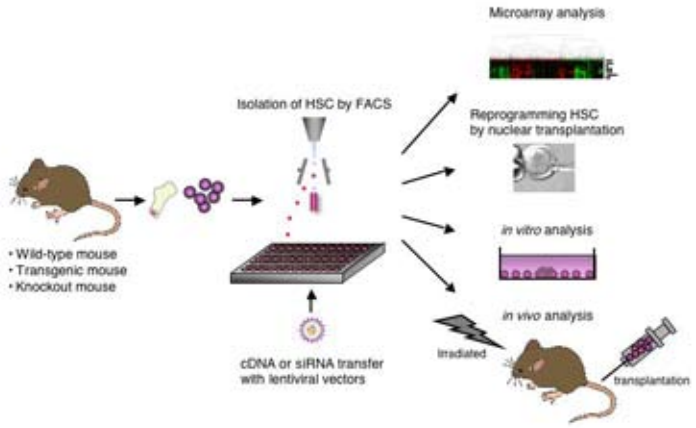

Figure 1.
Studies on hematopoietic stem cell proliferation and differentiation.

2. Homing capability of hematopoietic stem cells

After bone marrow (BM) transplantation via intravenous (IV) injection, HSCs circulate though blood, recognize and extravasate through BM vasculature, and migrate to a supportive microenvironment where HSCs are capable of proliferating and differentiating. However, how HSCs reach BM niches, a process referred to as homing, is poorly understood. To evaluate homing capability, HSCs were transplanted into lethally irradiated mice by direct injection into the BM cavity (IBM) or IV injection. HSCs rapidly proliferated within the injected tibia at 2-3 weeks after transplantation, although there is no significant differences in the final overall contribution of transplanted HSCs to hematopoietic chimerism in IBM versus IV injected mice. At 10 minutes or 1 hour after IBM injection, less than 10% of injected cells were remained in the injected tibia. These observations support the idea that the homing efficiency of HSCs is extremely high.

3. Plasticity of hematopoietic stem cells

In many adult tissues, self-renewing multipotent stem cells are maintained and serve to replace cells that have a limited life span or to regenerate cells after injury. Such somatic stem cells were believed to be limited to generate the specific types of cells present in the tissue in which the stem cell resides. However many recent reports have suggested that somatic stem cells can transdifferentiate into other cell types. To determine whether HSCs have plasticity, a single HSC from GFP transgenic mice was transplanted into lethally irradiated non-transgenic mice and analyzed various tissues from engrafted recipients. GFP-positive cells were hardly detected in all nonhematopoietic cells analyzed. Our results indicate that transdifferentiation of HSCs into nonhematopoietic cells is extremely rare event even if HSCs have plasticity.

Although a significant number of studies also reported a failure to detect BM or HSC contributions to nonhematopoietic tissues, cell fusion recently has been implicated in contribution of transplanted BM cells to regenerating liver hepatocytes in a mouse model of tyrosinaemia type 1, resulting in the cure of metabolic liver disease. This raised the possibility that fusion by BM cells can be used in reparative cell therapy. Therefore, we have evaluated therapeutic effect of BM or HSC transplantation on renal dysfunction of mice carrying mutant mitochondrial DNA. The results showed a significant delay in the onset of renal failure, but no donor-derived cells except hematopoietic cells were found in recipient kidney. The mechanism by which BM transplantation confers a significant survival advantage is under investigation.

4. Reprogramming of somatic stem cells by nuclear transfer

Somatic cell cloning holds great promise for the biomedical application. However, the efficiency of the successful cloning is quite low (< 5%), suggesting incomplete or inappropriate epigenetic reprogramming of the donor nucleus. In the meantime, embryonic stem (ES) cells, compared to differentiated cells, proved to be the most effective cell type for somatic cell cloning. This raised the question whether nuclei from somatic stem cells are also more effectively reprogrammed following nuclear transfer because of the undifferentiated state of their genome. We evaluated the cloning efficiency of HSCs, neural stem cells (NSCs), and mesenchymal stem cells (MSCs) in collaboration with Bioresource Engineering Division at RIKEN BRC (Figure 1). The majority (about 90%) of embryos reconstructed with HSCs developed to the two-cell stage probably because HSCs are predominantly in the G0 phase of the cell cycle. However, only 9% of embryos reached the morula/blastocyst stage *in vitro*, and only 0-0.7% developed to term after embryo transfer (47% and 1.5% for cumulus clones, respectively). Gene expression analysis of two-cell HSC clone embryos

revealed that they failed to activate five out of six zygotically activated genes examined. Importantly, comparison with the results from cumulus clones suggested that HDAC1 (histone deacetylase-1), a key gene for the subsequent embryonic gene activation, was specifically repressed in HSC clones. The superior cloning efficiency was obtained with NSCs (1.6%) though this was similar to that of cumulus. In contrast, embryos reconstructed with MSCs did not develop into live offspring. The failure of embryonic development is almost certainly due to chromosomal abnormality occurred during establishment of MSCs. These results suggest that the genome of somatic stem cells has less plasticity than we expected, at least in terms of the reprogrammability in the ooplasm after nuclear transfer.

5. Development of lentiviral vectors for basic research

Lentiviral vectors have been developed for gene therapy targeting nondividing cells. We have made several modifications to lentiviral vectors for using basic research (Figure 2). The use of Venus instead of GFP as a reporter gene in lentiviral vectors allowed easy detection of fluorescence signal, especially in the case of the IRES (internal ribosomal entry site) vectors. We have introduced the Gateway Technology (Invitrogen), a site-specific recombination system, into lentiviral vectors to facilitate transfer of DNA insert including cDNA library. In collaboration with many other laboratories, we have shown that lentiviral vectors are capable of efficient gene transfer into a wide variety of nondividing cells including primary T cells, B cells, NK cells, monocytes, and HSCs. Lentiviral vectors were also used for functional analysis of certain genes, and the use of the expression cloning system resulted in isolation of genes that prevent cells from HIV-1-induced cell death.

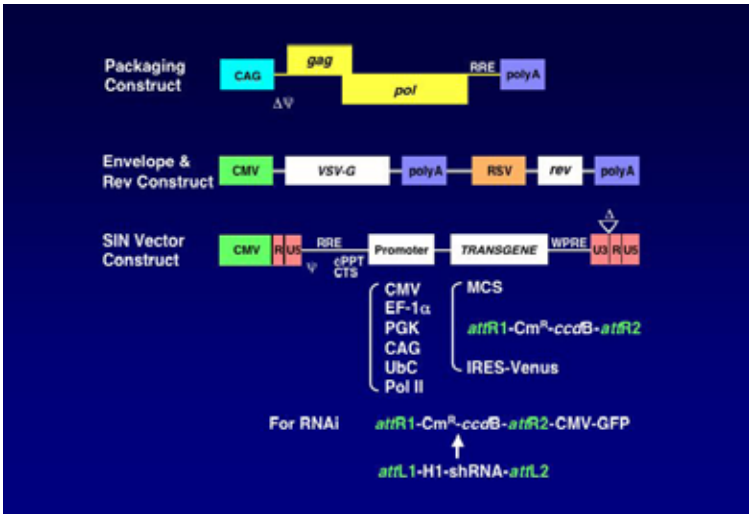

Figure 2.
Current lentiviral vector system.

It is important to choose optimal promoter for transgene expression. In collaboration with Cell Engineering Division at RIKEN BRC, we have shown that lentiviral vectors are capable of efficient gene transfer into mouse and monkey ES cells and the EF-1α promoter facilitates efficient expression of the GFP transgene. We have generated transgenic mice by injecting lentiviral vectors with several promoters into perivitelline space of single-cell embryos, and analysis of each promoter activity in various tissues is now in progress.

Using siRNA expressing lentiviral vectors, we have shown that downregulating PPARγ gene expression resulted in inhibition of preadipocyte-to-adipocyte differentiation. To facilitate tetracycline-regulated expression of siRNA, we have constructed lentiviral vectors containing the tetracycline operator site in the H1 promoter and the tetracycline repressor gene (Figure 3).

6. Functional analysis of Fcα/μ receptor in IgA nephropathy

Fcα/μR, which binds both IgM and IgA, is expressed on the majority of immune cells and is involved in

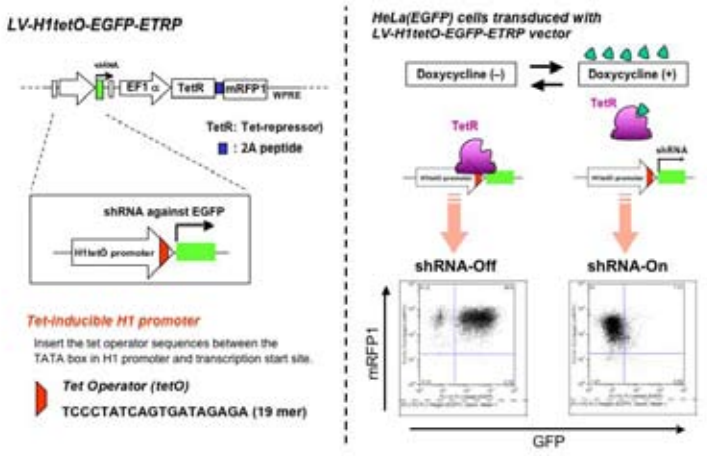

Figure 3.
Inducible shRNA expression lentiviral vector.

the primary stages of the immune response to microbes. Therefore, Fcα/μR may play an important role in the initial stages of immunity. Fcα/μR is also detected nonhematopoietic organs including kidney. IgA nephropathy is the most common form of glomerulonephritis, leading to progressive renal failure in almost one third of the patients. The disease is characterized by mesangial deposits of IgA, though the pathogenesis of IgA nephropathy remains unknown. Accordingly, we examined the potential function of Fcα/μR leading to IgA nephropathy. First, we identified isoforms of Fcα/μR, which may have several functions in kidney. Subsequently, we have generated mice lacking the Fcα/μR gene, and histological and molecular analysis of kidney in Fcα/μR-deficient mice is now in progress.

Publications

(*Peer reviewed Journal)

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1. Miyoshi, H. Evaluation of transdifferentiation potential of hematopoietic stem cells. *The 2nd Annual Congress of the Japanese Society for Regenerative Medicine*, Kobe, Mar. (2003)

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