"The 75th of Stem Cell Biology and Regenerative Medicine Forum"

Date：Nov 27th (Thu) 2014
Time : 18:00 ～ 19:30
Place：8th fl of New Hospital Building

（Internal Speaker）

18:00-18:30 Takashi Ishida（Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, IMSUT, Tokyo, Japan）
A Novel Strategy to Overcome The Cell Dose Barrier to Umbilical Cord Blood Transplants: A Proof of Benefits for Transplantation by Combined Multiple Units of Allogeneic Stem/Progenitor Cells

（External Speaker）

18:30-19:30 Takashi Okada（Department of Biochemistry and Molecular Biology Nippon Medical School）
AAV vector transduction strategy with MSCs-mediated immune-modulation to ameliorate neuromuscular disorders

19:30- Get-Together

Hosted by Center for Stem Cell Biology and Regenerative Medicine

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＊ Please register attendance at the reception desk.
＊ Next forum (the76th) will be held on Dec 19th (Fri) 16:30～ at Auditorium in 1st Building.
＊ Please contact tatsu-m@ims.u-tokyo.ac.jp, for Forum speaker recommendations

A Novel Strategy to Overcome The Cell Dose Barrier to Umbilical Cord Blood Transplants: A Proof of Benefits for Transplantation by Combined Multiple Units of Allogeneic Stem/Progenitor Cells.

Takashi Ishida (Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, IMSUT)

Umbilical cord blood (UCB) currently serves as a suitable donor source in hematopoietic stem cell transplantation. However, the time to engraftment is often delayed due to its low graft cell doses, which may limit its use in the clinical setting, particularly for adult patients. To overcome this cell dose barrier, UCB transplantation (UCBT) using double units has been performed worldwide, but the rapid engraftment has not yet been achieved. Besides, according to the report by Japan Red Cross Society, the majority of UCB units remain unused clinically with their cell doses having failed to meet the minimum standard. Overall, these facts boosted us to look for a new strategy so as to ameliorate UCBT by using multiple (more than three) units.

We have obtained by utilizing mouse transplantation models a proof of concept that combined multiple units of allogeneic hematopoietic stem/progenitor cells (HSPCs) are capable of supporting early hematopoiesis during a bone marrow suppression phase (we call this “bridging effect”). A mixture of allogeneic HSPCs, rendered devoid of mature immune cells, was shown to protect lethally-irradiated recipient mice, and to accelerate hematopoietic recovery when infused additionally with the single-unit congenic graft. Furthermore, we succeeded in enhancement of its “bridging effect” by appropriately manipulating a mixture of allogeneic HSPCs.

The experimental details will be presented with some results regarding manipulation of frozen human cord blood units, with which we are aiming for a proof of concept study for future clinical application.

AAV vector transduction strategy with MSCs-mediated immune-modulation to ameliorate neuromuscular disorders

Takashi Okada（Department of Biochemistry and Molecular Biology Nippon Medical School）

The particular characteristics of adeno-associated virus (AAV)-based vectors with safety and long-term expression have made it an attractive transduction tool for clinical gene therapy. Although host immune reaction against the vector as well as transgene products has been denoted in the clinical studies of neuromuscular gene therapy, there have been various successful observations. As great news for gene therapy community, European Medicines Agency finally recommended first gene therapy medicine for approval to treat LPL deficiency by using AAV vector. However, *in vivo* gene transduction with the AAV-based vectors depends upon laborious procedures for the production of the vector stocks to meet end-product specifications. We developed a method of producing AAV vectors with scalable purification protocol using the high-performance membrane adsorbers for considerable *in vivo* experimentation and clinical investigations.

We have adopted the efficient production system to investigate AAV vector-mediated muscle transduction for the treatment of Duchenne muscular dystrophy (DMD). Cardiac transduction of *mdx* dystrophic mice by the rAAV9-microdystrophin successfully improved EKG abnormalities as well as cardiac dysfunction. In contrast, muscle transduction with rAAV in normal Beagles and canine X-linked muscular dystrophy in Japan ($CXMD_J$) resulted in the transgene expression during a limited period. Although intramuscular rAAV injection showed effective transgene expression, the innate immune response should be considered to improve transduction protocol. Therefore, to investigate therapeutic benefits of the microdystrophin in the canine model, we transduced fetuses of $CXMD_J$ with systemic administration of the rAAV9-microdystrophin. Transduced dogs demonstrated long-term transgene expression with functional improvement. Furthermore, mesenchymal multipotent stromal cells (MSCs) should be effective platform to induce immunotolerance. Interestingly, intravenous injection of MSCs with rAAV9-microdystrophin greatly improved transgene expression as well as locomotive function in $CXMD_J$. These results suggest that long-term transgene expression with therapeutic benefits in neuromuscular

disorders would be achieved by the rAAV-mediated transduction strategy with an adequate regimen to regulate host immure response.