Institute of DNA Medicine
Project Laboratory for Kidney Regeneration

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General Summary

Recently, pluripotent stem cells have been successfully isolated and established from various tissues, which has brought the possibility of using somatic stem cells for organ regeneration one step closer to realization. For stem cell to be used clinically, they must be differentiated into functionally mature forms, and many researchers have attempted to establish individual somatic cell types. However, only very few cell types, such as pancreatic beta cells and cardiac myocytes, have been successfully established thus far. The major reason for this lack of success is that the developmental programs that cause stem cells to differentiate into mature cells consist of numerous factors, some of which are as yet unknown, and contribute to each other temporally and spatially in a tissue-specific manner. Clarifying the developmental program for each cell type one by one is extremely challenging. In this context, we have previously found that bone marrow-derived mesenchymal stem cells (MSCs), but not embryonic stem (ES) cells or induced pluripotent stem (iPS) cells, integrate into the kidney structure and acquire some renal functions when cultured with an immature metanephros *in vitro*. Fetal organs, such as the metanephros, have been suggested to be less immunogenic and more useful for transplantation because: 1) antigen-presenting cells that mediate direct host recognition of alloantigens and xenoantigens would be absent; 2) donor antigens, such as MHC class I and II antigens, might not be expressed by developing organs; and 3) the immune response to transplanted fetal tissue differs from that to adult tissue in terms of eliciting a helper T type 2 cell-biased response when the target organ is of fetal origin. In fact, direct comparison of xenotransplantation clearly shows the immune advantage of developing precursor transplants over developed adult transplants in fully immunocompetent hosts. On the basis of these observations, we speculated that we could use the developmental program of a developing organ by transplanting it into an ectopic site where it continued development *in vivo*. This procedure would facilitate the inward migration of autologous stem cells, which would then be stimulated by the developmental program of the xeno-organ to mature into tissue-specific cells.

In addition, conventional xenotransplantation should require continuous and strong immunosuppression to avoid any humoral rejection that occurs across the xenogeneic barrier, which evokes various adverse effects, including carcinogenicity and severe infection. In contrast, this *in vivo* programming system temporarily uses xeno-organs as the source of the developmental program, and after the tissue of interest has been established, the xenocomponent is no longer needed and can be discarded. Therefore, we introduced a cell fate-regulating system, in which a suicide gene is expressed on demand, and combined this system with the *in vivo* programming system.

Research Activities

This year, we established a xenotransplantation model in which the differentiation of endogenous MSCs into mature erythropoietin-producing tissue is controlled in a niche provided by a developing xenometanephros. Transplantation of rat metanephroi into mouse omentum, and similarly that of pig metanephroi into cat omentum, led to the recruitment of host cells and to erythropoietin production. Erythropoietin-expressing cells were not differentiated from integrating vessels because they did not co-express endothelial markers (Tie-2 and vascular endothelial cadherin). Instead, erythropoietin-expressing cells were shown to be derived from circulating host cells, as indicated by enhanced green fluorescent protein (EGFP) expression in the grown transplants of chimeric mice bearing bone marrow from a transgenic mouse expressing EGFP under the control of the erythropoietin promoter. These results suggest that the recruitment and differentiation of donor cells in a developing xenotransplanted organ is consistent between species. The cells responsible for erythropoietin expression were identified as MSCs by injecting human bone marrow-derived MSCs and endothelial progenitor cells into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Furthermore, using metanephroi from transgenic ER/E2F1 suicide-inducible mice, the xenotissue component could be eliminated, leaving autologous erythropoietin-producing tissue. Our findings might help alleviate adverse effects due to long-term immunosuppression and help mitigate ethical concerns.

Reviews and Books

Yokoo T, Matsumoto K, Yokote S. Potential use of stem cells for kidney regeneration. *Int J Nephrol.* 2011; **2011**: 591731.