Institute of DNA Medicine
Project Laboratory for Kidney Regeneration

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General Summary

Many efforts are being made to apply regenerative medicine to the treatment of renal diseases. Renal diseases in which renal structure is maintained might be treated with infusions of stem cells isolated from the bone marrow or adult kidney. However such cell-based therapy cannot be used to treat chronic renal diseases, in which renal structures, including the kidney scaffold, are completely disrupted. Therefore, absolute kidney regeneration is needed to rebuild an entire functional kidney de novo and eliminate the need for dialysis. However, owing to the anatomical complexity of the kidney and the need for communication between cells to fulfill renal function, the kidney has been considered the most difficult organ to regenerate. Only a few groups are investigating the potential for reconstructing an organized and functional kidney structure, and, among them, we are using the developing xenoembryo as an organ factory for this purpose.

Research Activities

Use of the E2F1 transgenic suicide-inducible mice permits regeneration of complete human kidneys

We have established the transgenic estrogen receptor (ER)-E2F1 suicide-inducible mice that express the ER-E2F1 fusion protein. E2F1 is a transcription factor that regulates cell proliferation, and its ectopic expression induces apoptosis in differentiated cells; therefore, cells from ER-E2F1 mouse can be eliminated on demand by administering tamoxifen. Metanephroi from ER-E2F1 mice (E2F1 group) and C57BL/6 mice (control group) were cultured with or without tamoxifen for 14 days. The fate of cells was observed with fluorescence microscopy. Metanephroi were transplanted to Sprague-Dawley rats (N＝10 in both groups), and FK506 and tamoxifen were administered daily for 2 weeks. The average weight of the grown transplants was recorded, and any fibrotic changes were observed histologically. The expression of erythropoietin was assayed with the quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) using species-specific primers.

Results: *In vitro* tamoxifen treatment of metanephros cells derived from ER-E2F1 mice successfully eliminated these cells, whereas wild-type cells remained viable. The percentage of the grown transplants *in vivo* was 32% in the E2F1 group versus 56% in control group ($P=0.045$). The final weight of transplants was 2.4 ± 0.8 mg in the E2F1 group and 8.7 ± 1.3 mg in the control group ($P=0.035$). Histological analysis showed that kidney tissue is replaced by fibrous tissue in the E2F1 group. Quantitative RT-PCR revealed that rat erythropoietin was produced from grown metanephroi in both

groups and that there was no significant difference between the groups.
Conclusion: Metanephroi derived from ER-E2F1 mice are a powerful tool to regenerate complete human-derived kidney tissue.

Publications

Fukui A, Yokoo T, Matsumoto K, Kawamura T, Hosoya T, Okabe M. Integration of human mesenchymal stem cells into the Wolffian duct in chicken embryos. *Biochem Biophys Res Commun* 2009; **385:** 330–5.

Reviews and Books

Yokoo T, Kawamura T. Xenobiotic kidney organogenesis: a new avenue for renal transplantation. *J Nephrol* 2009; **22:** 312–7.
Yokoo T, Fukui A, Kawamura T, Kobayashi E. Kidney development and regeneration. In: Singh SR, Mishra PK, Hou SX, editors. Stem Cell: Organogenesis and Cancer. India: Research Signpost Press, 2009. p. 57–76.