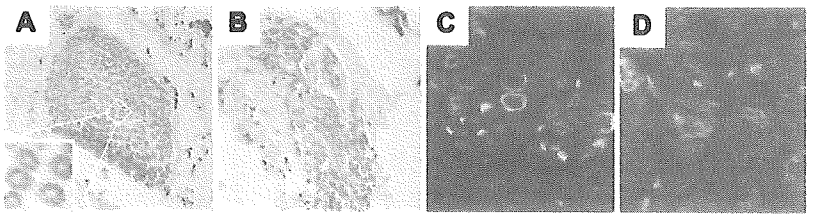

Figure 7. Ectopic implantation of rKS56 cells results in tubule-like structure formation. rKS56 cells marked with black ink were implanted into the peritoneal muscle of SD rats. Implanted cells formed tubule-like structures in peritoneal muscle after 4 weeks (hematoxylin stain, x100, *A, B*). Inset; note the tubule-like structures presumably formed by implanted rKS56 cells (*A*). The tubule-like structures partially expressed AQP-1 (*C*) and AQP-2 (*D*) detected by immunohistochemistry (×200).

sure to ischemia or reagents toxic for tubular epithelial cells. Damaged tubules are quickly repaired and regenerated after a burst of cell proliferation after the injury. However, the origin of the proliferating cells has not been determined yet. We hypothesized that the highly proliferative cell may be near the stem/progenitor cell strain and might exist in the S3 segment of proximal tubules. In this study, we used a microdissection technique and coculture system to obtain the cells possessing a high capacity of proliferation. Previous reports demonstrated the usefulness of cell-sorting technology to harvest hematopoietic, nerve and hepatic stem cells from bone marrow or adult tissue such as liver or brain, by using antibodies against cell-surface markers (26, 27). Since the detailed repertoire of unique cell surface marker proteins expressed on renal stem/progenitor cell is unknown at present, we could not apply a cell-sorting method to isolate renal stem/progenitor cells. In addition, the microdissection technique enabled us to identify the precise origin of proliferating cells. Among cells outgrowing each segment of the nephron, only the S3 segment-derived cells survived through limiting dilution, indicating limited localization of the progenitors with potential to proliferate.

The rKS56 cells expressed Sca-1 and c-kit. Sca-1 and c-kit are well known to be expressed on hematopoietic stem cells (28). The expression of Sca-1 and c-kit suggest that rKS56 cells may have potential to serve as renal stem/progenitor cells. The rKS56 cells exhibited a typical cobblestone appearance with expression of renal epithelial markers such as pan-cytokeratin, Pax-2 and Wnt-4, mesenchymal markers like vimentin and WT1. The expression of epithelial and mesenchymal markers was reported in a metanephric mesenchyme cell line, 7.1.1 cells, containing embryonic renal stem cells (19), and cell lines, mK3 and mK4, derived from SV40-LT transgenic mice (29). Coexpression of Pax-2 and vimentin in regenerative tubular epithelial cells was reported in experimental acute tubular necrosis models (30). rd, rKS56 cells appear to have molecular characteristics in common with metanephric mesenchyme and regenerative tubular cells. Tissue adult stem cells are located in a so-called "niche" where they are protected from environmental injuries (31). Although the turnover time of stem cells residing in the niche is very slow, stem cells undergo rapid multiplication when they are isolated and grown in culture. The rKS56 cells proliferated at a very rapid rate with exponential growth, while the NRK-52E cells grew linearly. The high turnover rate in the rKS56 cells was sustained over 300 days irrespective of immortalization. Neural stem cells obtained with neurosphere methods could be generated from a single primary sphere (21). rKS56 cells have the same character as neural stem cells. These results suggested that rKS56 cells have the potential for self-renewal.

Musashi-1 is a neural RNA binding protein that is specific for neural stem cells and Musashi-1 is required for asymmetric cell division of sensory organ precursor cells (32). Musashi-1 expression was believed to be restricted to neural stem cells, but ectopic expression of the Musashi family has been reported on non-neural origin stem cells, such as mouse intestinal stem and progenitor cells and human breast epithelial stem cells (33–35). We also detected Musashi-1 expression in the rKS56 cells, which is consistent with asymmetric cell division and supports the notion of rKS56 cells having a stem cell phenotype.

Multipotency is another characteristic feature of stem cell. In general, tissue stem cells were believed to have limited lineage-restricted plasticity as compared with multipotent embryonic stem cells or ES cells (36). Recent work has proposed the idea that adult stem cells can differentiate crossing lineage boundaries (5, 8, 37, 38). rKS56 cells had both epithelial and mesenchymal features based on the protein and mRNA expression patterns, and differentiated into mature proximal and distal tubular epithelial cells in vitro, and glomerular epithelial as well as proximal tubular epithelial cells in vivo, suggesting plasticity within renal epithelial cells. Transplantation experiments into extra-renal organs failed to demonstrate multipotency to cross lineage boundaries. Further studies are required to clarify the possibility of multipotency of rKS56.

Alison et al. (37) were the first to demonstrate that bone marrow cells could differentiate into renal tubular cells. Recent work also demonstrated that bone marrow-derived stem cells contributed to the regeneration of renal tubules (39, 40). It is unlikely that bone marrow-derived stem cells in the peripheral blood were contaminants during microdissection methods in our study because the kidney was perfused with saline to wash out retained blood. Even if contamination might have occurred, it was difficult for bone marrow-derived cells to differentiate into renal epithelial cells in vitro. Based on our results, rKS56 was derived from purified renal tubules and shared many characteristics with embryonic renal stem cells, rather than bone marrow-derived stem cells as described above.

We attempted cell therapy to treat acute tubular

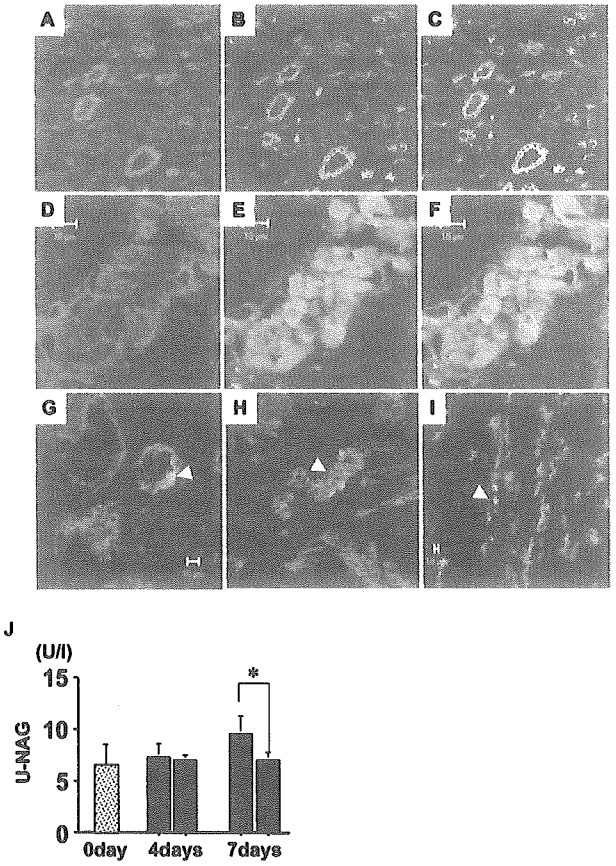

Figure 8. Engraftment of rKS56 cells to injured tubules in renal ischemia-reperfusion model. *A–C)* Double staining of Di-O-labeled rKS56 cells (green) and cytokeratin (red) using kidney sections obtained from rats with renal ischemia-reperfusion and treated with transplantation of rKS56 cells. Representative fluorescent photomicrographs of corticomedullary junction lesion of left kidneys 7 days after cell transplantation are shown. rKS56 cells (green) were incorporated into tubules at corticomedullary junction, and portions of cytokeratin positive cells (red, *A*) were rKS56 cells (green, *B*) as confirmed on the merged image (*C*). *D–F)* Double staining of Di-O-labeled rKs56 cells (green) and AQP-1 (red) using kidney sections obtained from rats with renal ischemia-reperfusion and treated with transplantation of rKS56 cells (day 7). Portions of AQP-1 positive cells (red, *D*) were rKS56 cells (green, *E*) as confirmed on the merged image (F). Di-O positive (rKS56) cells were observed partly in subcortical area (*G*) and medulla (*I*), but mostly in corticomedullary junction lesion (*H*). Implantation of rKS56 cells suppressed U-NAG levels 7 days after I/R (*J*). 0 day: 0 day for control, 4 days: 4 days after I/R, 7 days: 7 days after I/R. Dotted bar: 0 day group, filled bar: vehicle control I/R group, hatched bar: rKS56 cells-implanted group after I/R. $^*P < 0.05$ vs. vehicle control I/R group. Each column consists of means ± SE.

necrosis model using rKS56 cells. Since rKS56 cells migrated, attached and differentiated to mainly tubulointerstitial cells, rKS56 cells implantation might have resulted in the suppression of U-NAG elevation in I/R model. It is possible that direct intrarenal injection of rKS56 cells might have resulted in slightly increased s-Cr and BUN values, and subsequent analysis using various distinct ways of delivery of rKS56 cells such as intravenous, intra-arterial or retrograde ureteral approaches are underway to determine the optimal approach for rKS56 cell therapy.

Oliver et al. reported that renal papilla is a niche for adult kidney stem cells (41). They proved slow cell cycling cells location by BrdU-retaining in embryo kidney. rKS56 cells are harvested from S3 segments, and grew exponentially. So, rKS56 cells might have a progenitor character rather than stem cell character. Stem cell in papilla may migrate to corticomedullary junction, differentiate to progenitor cells and contribute to cell turnover and regeneration.

In conclusion, we were able to isolate renal highly proliferative cells from the S3 segment of proximal tubule in adult rat kidney. The rKS56 cells exhibited self-renewal and multipotency restricted to renal cells in vitro and in vivo. However, the karyotype of rKS56 cells were nearly triploid, suggesting that rKS56 cell is a cell line with triploidy holding highly proliferative potential and possibly having capacity to serve as renal stem/progenitor cells. In this regard, rKS56 cell might not be defined as a physiological renal tissue stem/progenitor cell. Establishment of rKS56 cells will facilitate investigating other characteristics of adult renal stem cells and the regulatory mechanisms of phenotype, which may contribute to the future development of cytotherapy for regeneration of injured kidney. The use of adult tissue stem cells would be advantageous over embryonic tissue stem cells by virtue of the limited source of human embryonic kidneys and the circumvention of ethical issues involving embryos. FJ

A portion of this study was supported by the Health and Labour Sciences Research Grants for Research on Human Genome, Tissue Engineering Food Biotechnology from the Ministry of Health Labour and Welfare of Japan (to H.M.). We are grateful to Dr. H. Nonoguchi (Kumamoto University, Japan) for technical assistance in performing microdissection of nephron segments. We would like to thank Dr. M. Yasui (Johns Hopkins University School of Medicine) for helpful comments on the manuscript.

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Received for publication March 2, 2005.
Accepted for publication June 27, 2005.