Institute of DNA Medicine
Project Laboratory for Kidney Regeneration

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General Summary

The critical shortage of organs has driven new technologies, such as tissue engineering and regenerative medicine, to achieve functional kidney replacement. Our previous studies have shown that a xenobiotic developmental process for growing xenoembryos allows exogenous human mesenchymal stem cells (MSCs) to undergo epithelial conversion and form a nephron that produces urine and erythropoietin. These findings suggest that MSCs can be a source of cells for future renal regeneration. Furthermore, MSCs are easy to obtain in large numbers and are not costly to establish.
Previously, we used bone marrow-derived MSCs from healthy volunteers, although whether these MSCs differ from MSCs from patients undergoing dialysis is unclear. This uncertainty is due to patients with end-stage renal failure having been exposed over long periods to uremic toxins, which may affect the viability and regenerative capacity of their MSCs and make them unsuitable for kidney regeneration. This year, we have demonstrated differences in the gene and protein expression of MSCs from patients with end-stage kidney disease (ESKD) and healthy individuals using a polymerase chain reaction array and Western blot analysis. We found that long-term uremic conditions lead to persistent and systematic downregulation of *in-vitro* gene and protein expression of P300/cAMP response element-binding protein (CREB)-binding protein (CBP)-associated factor (PCAF) and low angiogenesis activation of *in vivo* assay in the MSCs of patients with ESKD. Furthermore, we demonstrated that the hypoxic responses of PCAF, hypoxia-inducible factor-1α (HIF-1α), and vascular endothelial growth factor (VEGF) were significantly blunted in MSCs from patients with ESKD. We propose that the transcriptional regulation by low levels of PCAF is inappropriately controlled by environmental factors representing long-term ESKD. Low expression of PCAF induced by long-term ESKD may lead to down-regulation under hypoxia of HIF-1α and VEGF in MSCs from patients with ESKD undergoing long-term dialysis (KD-MSCs). These findings should help to elucidate the mechanisms of the effects of uremic toxins. Further studies are needed to clarify the relationship of CKD and the down-regulation of PCAF.

Research Activities

We have previously demonstrated that MSCs differentiate into functional kidney cells capable of urine and erythropoietin production and can, therefore, be used for kidney regeneration. However, the viability of MSCs from patients undergoing dialysis might be affected under uremic conditions. In this study, we isolated KD-MSCs (mean duration of dialysis: 72.3 months) and MSCs from healthy control subjects (HC-MSCs) to compare their viability. The KD-MSCs and HC-MSCs were assessed for their prolifera-

tion potential, senescence, and capacity to differentiate into adipocytes, osteoblasts, and chondrocytes. Gene expression of stem cell-specific transcription factors was analyzed with polymerase chain reaction array and confirmed with Western blot analysis at the protein level. No significant differences in proliferation potential, senescence, or differentiation capacity were observed between KD-MSCs and HC-MSCs. However, gene and protein expression of PCAF was significantly suppressed in KD-MSCs. Because PCAF is a histone acetyltransferase that mediates regulation of HIF-1α, we examined the hypoxic response in MSCs. The HC-MSCs but not KD-MSCs showed up-regulation of PCAF protein expression under hypoxia. Similarly, expression of HIF-1α and VEGF did not increase under hypoxia in KD-MSCs but did increase in HC-MSCs. Additionally, a direct *in vivo* angiogenesis assay showed decreased angiogenesis in KD-MSCs. In conclusion, long-term uremia leads to persistent and systematic down-regulation of *PCAF* gene and PCAF protein expression and low angiogenesis activation in MSCs from patients with ESKD. Furthermore, expression of PCAF, HIF-1α, and VEGF are not up-regulated by hypoxic stimulation of KD-MSCs. These results suggest that the hypoxic response may be blunted in MSCs from patients with ESKD.

Publications

Yamada A, Yokoo T, Yokote S, Yamanaka S, Izuhara L, Katsuoka Y, Shimada Y, Shukuya A, Okano HJ, Ohashi T, Ida H. Comparison of multipotency and molecular profile of MSCs between CKD and healthy rats. *Hum Cell.* 2014; **27:** 59-67. Epub 2014 Feb 5.

Hara S[1], Umeyama K[2], Yokoo T, Nagashima H[2], Nagata M[1] ([1]Univ Tsukuba, [2]Meiji Univ). Diffuse glomerular nodular lesion in diabetic pigs carrying a dominant-negative mutant hepatocyte nuclear factor 1-alpha, an inheritant diabetic gene in humans. *PLoS One.* 2014; **9:** e92219.

Reviews and Books

Yokote S, Yokoo T. Organogenesis for kidney regeneration. *Curr Opin Organ Transplant.* 2013; **18:** 186-90.