Induction of interdigitating cell processes in podocyte culture

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Highly organized cell processes characterize glomerular podocytes *in vivo*. However, podocytes in culture have a simple morphology lacking cell processes, especially upon reaching confluence. Here, we aimed to establish culture conditions under which cultured podocytes extend cell processes at confluence. Among various culture conditions that could possibly cause phenotypic changes in podocytes, we examined the effects of heparin, all-*trans* retinoic acid, fetal bovine serum, and extracellular matrices on the morphology of podocytes in rat primary culture. Consequently, long arborized cell processes were observed to radiate extensively from the cell body only when cells were cultured in the presence of heparin and all-*trans* retinoic acid on laminin-coated dishes with decreasing concentrations of fetal bovine serum. Primary processes branching repeatedly into terminal processes and cell process insertion under adjacent cell bodies were evident by electron microscopy–based analysis. Immunostaining for podocin showed conspicuous elongations of intercellular junctions. Under these conditions, the expression levels of podocyte-specific proteins and genes were markedly upregulated. Thus, we succeeded in establishing culture conditions in which the cultured podocytes exhibit phenotypes similar to those under *in vivo* conditions.

Kidney International (2018) **93,** 519–524; http://dx.doi.org/10.1016/j.kint.2017.06.031

KEYWORDS: cell process; confluence; culture; podocin; podocyte

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Received 2 May 2017; revised 13 June 2017; accepted 22 June 2017; published online 8 September 2017

Cultured podocytes have been used to understand the biology of podocytes *in vivo*.[1] However, there are tangible differences in the morphology and gene expression between cultured podocytes and their *in vivo* counterparts.[2] Podocytes *in vivo* project elaborate cell processes referred to as primary processes and foot processes.[3] These processes interdigitate with each other. Cultured podocytes also form elongate cell processes when there is cell-free space around cells.[4–6] However, they lose such cell processes when they reach a confluent density similar to the cell density *in vivo*.[2] Some culture conditions have been reported to upregulate podocyte-specific gene expressions, but no studies have shown the active extension of cell processes inserted beneath adjacent cells like those present *in vivo*.

In this study, we tried to establish culture conditions under which cultured cells exhibit a morphology similar to that exhibited *in vivo*. Various culture conditions have been reported to cause phenotypic changes in podocytes. Heparin, all-*trans* retinoic acid (ATRA), and decreasing concentrations of fetal bovine serum (FBS) are known to upregulate the podocyte-specific gene expressions.[7,8] Laminin accelerates the podocyte process formation.[5] We combined these conditions and succeeded in inducing arborized cell processes at confluence in culture.

RESULTS AND DISCUSSION

When cultured on laminin-coated dishes at confluence, a notable difference was evident between cell morphologies in the basal media containing 5% FBS without heparin or ATRA (Figure 1a) and in inducing media containing heparin and ATRA but without or with only 0.5% FBS (Figure 1b); morphology was simple in the former case with few cell processes, and in the latter, it was arborized, with extended cell processes. The cell processes from adjacent cells were often mutually interlocked, showing interdigitation or forming a mazelike configuration (Figure 1b). Although cell process formation was observed in the range of 20 to 80 U/ml heparin and 0.1 to 1 μM ATRA, experiments were carried out in inducing media containing 0.5% FBS, 40 U/ml heparin, and 0.1 or 0.2 μM ATRA because the formation was sometimes poor at higher concentrations of heparin or ATRA. The formation of cell processes reached completion at ∼5 days after subculture and persisted until day 10 (Supplementary Figure S1 online). All these culture conditions were necessary for extensive formation of cell processes (Supplementary Figures S2–S4 online).

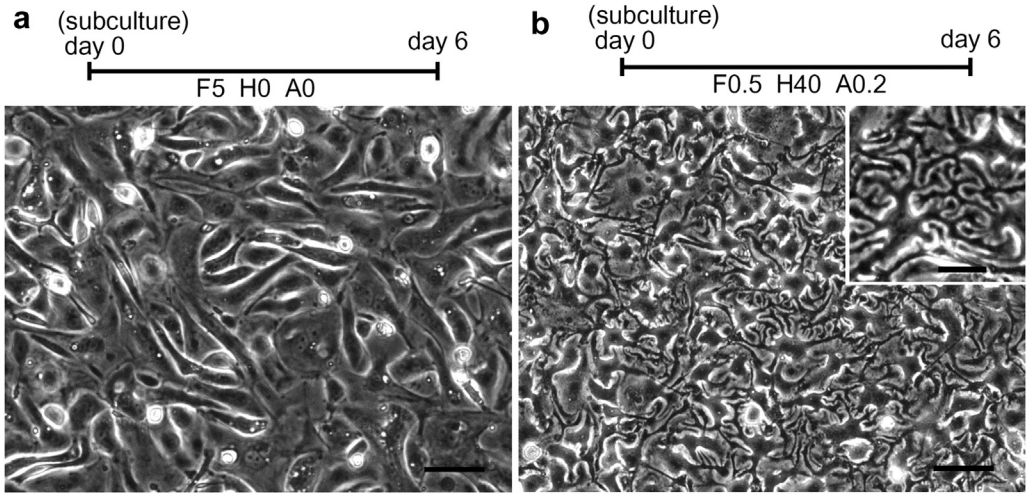

Figure 1 | Phase-contrast microscopy of cells cultured on laminin-coated dishes in basal (a) and inducing (b) media 6 days after subculture. F, H, and A denote fetal bovine serum (FBS), heparin, and all-*trans* retinoic acid (ATRA), respectively. For example, F0.5 H40 A0.2 in (**b**) indicates 0.5% FBS, 40 U/ml heparin, and 0.2 μM ATRA. Bar = 50 μm in (**a**) and (**b**) and 20 μm in inset. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

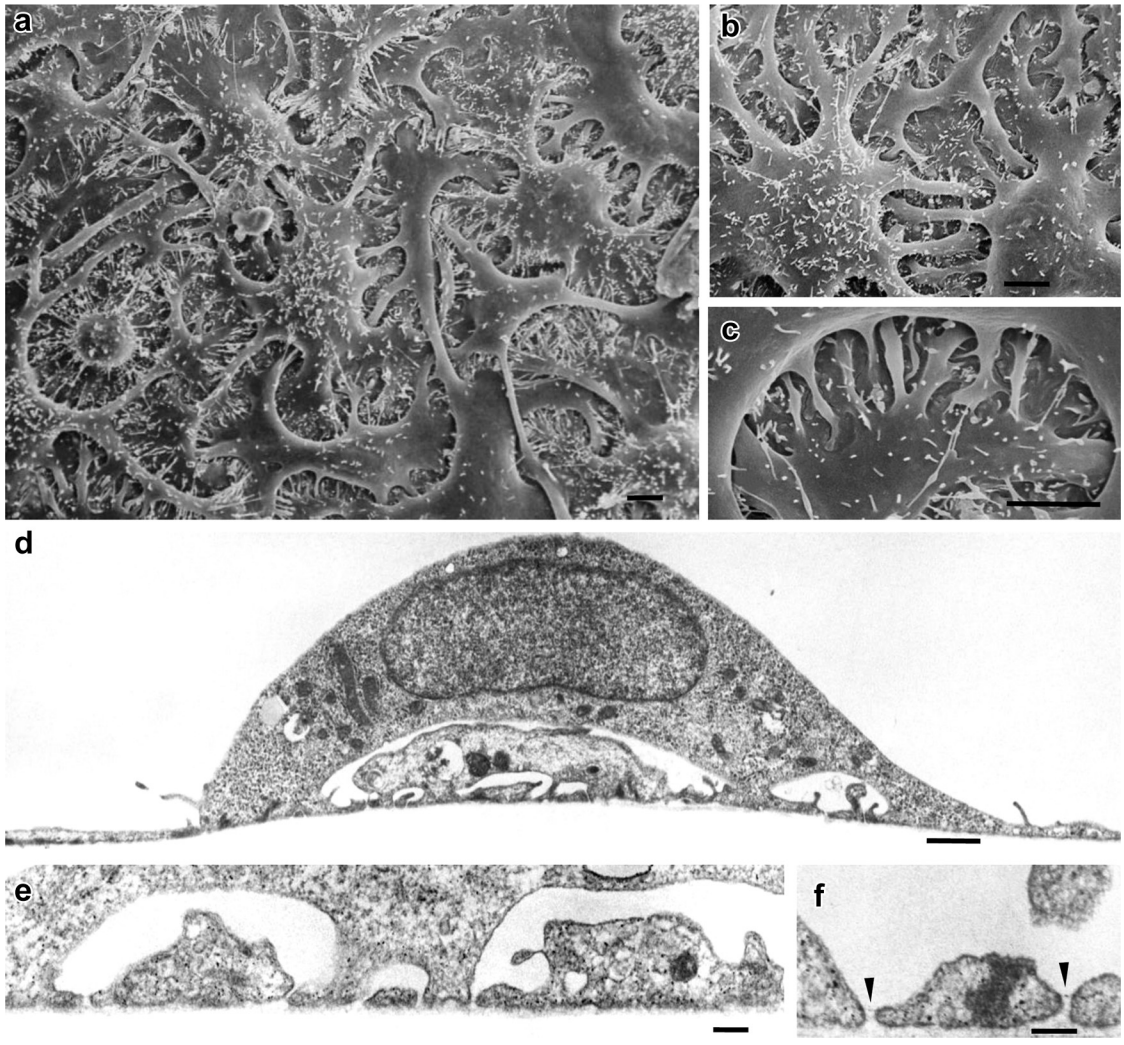

Figure 2 | Scanning (a–c) and transmission electron microscopy (d–f) of cells cultured on laminin-coated dishes in inducing media for 7 days after subculture. (**a**) Cells with microvilli showed multiple elongated cell processes and covered the culture dish without space. (**b**,**c**) Primary processes branched repeatedly into terminal processes and interdigitated. (**d**,**e**) Vertical sections of the cells show that terminal processes were inserted under adjacent cell bodies. (**f**) Slit diaphragm–like structures (arrowheads) were observed between terminal processes. Bar = 5 μm (**a**–**c**), 1 μm (**d**), and 200 nm (**e**,**f**). To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.