

In Vitro Tubulogenesis from Cell Lines Derived from Embryonic Kidney Modulated by Extracellular Matrices and Growth Factors

Xing Zhang, Rongshu Wang, Wei Wu, Travis Smith, Kevin T Bush, Sanjay K. Nigam

University of California, San Diego

Statement of Purpose: Tubulogenesis and branching morphogenesis, fundamental processes involved in epithelial tissue development, are usually modulated by different extracellular matrices and growth factors. In kidney development, such processes include formation of the collecting duct system from the ureteric bud and formation of the proximal through distal tubules from cells of metanephric mesenchymal origin. In vitro cell culture systems have been used to study mechanisms of tubulogenesis and branching morphogenesis in the development of urinary collecting duct system [1-3], given the complexity of in vivo and in vitro organ culture systems. However, cell culture systems for study of formation of proximal and distal tubules from cells of metanephric mesenchymal origin have not yet been reported. In this investigation, two cell lines, BSN and RIMM-18, derived from metanephric mesenchymal cells isolated from embryonic kidneys, were used for 3D tubulogenesis. The effect of different extracellular matrices such as Matrigel, Type I collagen, Type IV collagen, and laminin and growth factors, including hepatocyte growth factor (HGF), epidermal growth factor (EGF) on tubulogenesis have been studied. Conditioned media from ureteric bud cells was used for tubulogenesis assays. In vitro cell culture models established herein can be used for study of tubulogenesis mechanisms as well as for disease and drug discovery studies.

Methods:

RIMM-18 and BSN cell lines. RIMM-18 cell line, obtained from Dr. Perantoni's lab (National Cancer Center, NIH), was established by transfection of primary mesenchymal cells isolated from embryonic kidneys of 13-day postcoitum (dpc) rat embryos with an E1A-ER fusion construct subcloned into pEF1 α -neo [4]. BSN cell line was obtained from mesenchymal cells isolated from embryonic kidneys of 11.5-day dpc mouse embryos transgenic for the early region of SV40 [2].

3D tubulogenesis assay. Cells were cultured in 96-well plates in a sandwich method for the tubulogenesis assay. 30 μ l growth factor reduced Matrigel was first placed in the wells as a bottom coating layer. Cells were trypsinized, and isolated cells were resuspended in Matrigel at a density of 10^5 cells/ml, then added to the coated wells as a middle layer (60 μ l each well) with a culture medium (60 μ l each well) placed on top.

Preparation of Type I collagen gel. Type I collagen gels were prepared by mixing eight parts of sterile collagen solution (type I collagen from rat tail, BD) with one part of 10x concentrated essential minimum Egel's medium (EMEM) and one part of sterile 1 M Heps solution. The pH of the final solution was adjusted to 7.4 with NaOH solution (1M), and the collagen solution (~2.9 mg/ml) was kept on ice to prevent gelation. Type I collagen solution was used either as an extracellular matrix alone

instead of Matrigel or in a mixture of Matrigel for tubulogenesis assays.

Conditioned media from ureteric bud cells. To obtain UB cell conditioned medium (UB-CM), a confluent UB cell monolayer was first washed with PBS, followed by application of serum-free DMEM/F12 medium and incubation for 3-5 days. The collected UB-CM was centrifuged at a low speed to remove cell debris, and further concentrated with Amicon[®] ultra centrifuge filters in DMEM/F12 medium.

Results: As shown in Fig. 1, RIMM-18 cells formed cysts when cultured in Matrigel with DMEM/F12 medium with 10% FBS (Fig. 1A), while forming tubules with lumens for cultured in the presence of UB-CM with or without growth factors (Fig. 1B-1D).

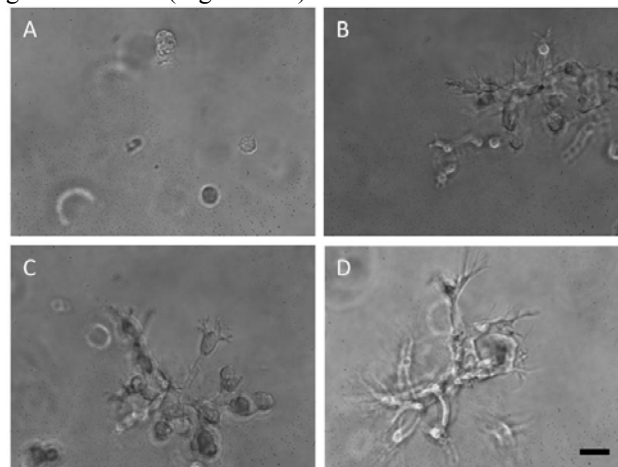


Fig. 1. RIMM-18 cultured in Matrigel for 3 days with different culture medium (with 10% FBS): A. DMEM/F12; B. UB-CM; C. UB-CM with 100 ng/ml HGF; UB-CM with 100 ng/ml EGF. Scale bar = 400 μ m.

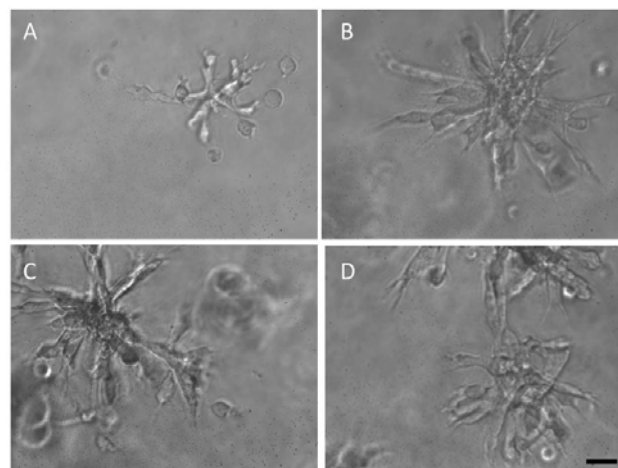


Fig. 2. BSN cells cultured in Matrigel for 3 days with different culture medium (with 5% FBS): A. DMEM/F12; B. UB-CM; C. UB-CM with 100 ng/ml HGF; UB-CM with 100 ng/ml EGF. Scale bar = 400 μ m.

BSN cells formed tubules in both DMEM/F12 medium (Fig. 2A) and UB-CM (Fig. 2B) with 5% FBS, although more tubulogenesis was evident in the cultures with UB-CM than with DMEM/F12. Supplementation with growth factors such as HGF (Fig. 2C) and EGF (Fig. 2D) resulted in more prominent tubules than in cultures without growth factors.

Both BSN cells (Fig. 3A) and RIMM-18 cells (Fig. 3C) formed cysts with no tubules found when cultured in Type I collagen (which was then used as a base matrix for studying the effect of other extracellular matrices). By adding Matrigel into type I collagen (in a volume ratio of 1:1), both BSN cells (Fig. 3B) and RIMM-18 cells (Fig. 3D) formed tubules after being cultured for 3 days. In addition, tubular structures also formed by culturing RIMM-18 cells in a mixture of type I collagen and type IV collagen (Fig. 3E) or laminin (Fig. 3F).

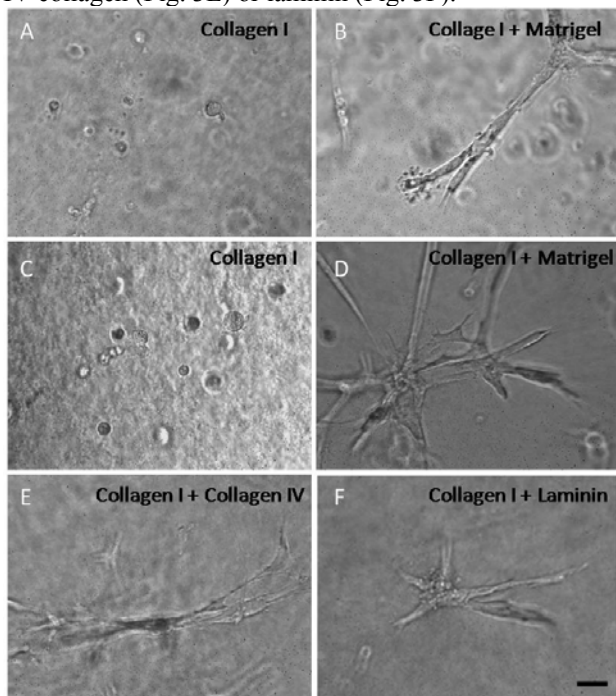


Fig. 3. BSN cells cultured with UB-CM containing 100 ng/ml HGF in type I collagen alone (A) or in a mixture of type I collagen and Matrigel (B). RIMM-18 cells cultured with UB-CM containing 100 ng/ml HGF in type I collagen alone (C) or in a mixture of Type I collagen and Matrigel (D), type IV collagen (E) or Laminin (F). Scale bar = 400 μ m.

Conclusions: Tubulogenesis from BSN and RIMM-18 cells, derived from metanephric mesenchymal cells from embryonic kidneys, are modulated by both extracellular matrices and growth factors.

For RIMM-18 cells, UB-CM plays an essential role in their tubulogenesis, indicating some factors in UB-CM alone or in combination are necessary for tubule formation. Growth factors such as HGF and EGF facilitate the tubulogenesis with EGF having the best effect. RIMM-18 cells formed large tubular structures

with lumens in Matrigel alone, but did not in type I collagen. Mixing of the extracellular matrices, Matrigel, type IV collagen or laminin into type I collagen, also induced tubulogenesis of these cells.

BSN cells formed tubules in Matrigel when supplied with sufficient concentrations of FBS (e.g. $\geq 5\%$) and HGF or EGF (100 ng/ml), indicating UB-CM is not necessary for tubule formation in BSN cells. However, many more tubules were found in cultures with UB-CM compared with those without UB-CM, showing that UB-CM does provide factors which promote tubule formation from BSN cells. BSN cells did not form tubules in type I collagen alone, while they did form tubules in a mixture of Matrigel and type I collagen.

References:

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