Development and Characterization of an in vitro Co-culture Angiogenesis Assay System Using hTERT Immortalized Cells for High Throughput Drug Screening

Chaozhong Zou, Ph.D., Chengkang (CK) Zhang, Ph.D., Isabela Oliva, M.S., and Metime S. Enuameh, Ph.D.
ATCC Cell Systems, 22 Firstfield Rd, Suite 180, Gaithersburg, MD 20878, UNITED STATES

Abstract

In this study, we established an in vitro co-culture model system using the autocrine endothelial cell line Tel0HAEC (hTERT immortalized human aortic endothelial cell line, ATCC® No. CRL-4502™) and the hTERT immortalized adipose-derived mesenchymal stromal cell line HERT-MSC (ATCC® No. SDRC-A060™). Both cell lines were immortalized by hTERT (human telomerase reverse transcriptase) alone and have been well-characterized showing that the cells retain the most important characteristic of their parental counterparts. Tel0HAECs co-cultured with BJ primary fibroblasts (ATCC® No. CRL-2527™) for 14 days in optimized ATCC angiogenesis medium formed fine tubular structures as shown by staining with CD31 endothelial cell marker. The tubule length elongated with increasing doses of vascular endothelial growth factor (VEGF) and tubule formation can be completely blocked by suramin in a concentration-dependent manner. Next, we introduced GFP into the Tel0HAEC cell line (Tel0HAEC-GFP, ATCC® No. CRL-A404™), allowing for real-time visualization of angiogenesis when co-cultured with BJ fibroblasts, another hTERT immortalized cell line, hERT-MSC, replaced the BJ primary fibroblast in the co-culturing system. It was observed that the new model forms the tubular structures in less than 7 days, and also responds effectively to VEGF and compounds such as suramin. Further, the hTERT-MSC cells which surround the tubular structures have undergone transformation indicated by positive SMA staining (a marker of smooth muscle cells), this indicates that the system has physiological relevance. Therefore, the co-culture models developed by using hTERT-immortalized cell lines described in this report provide a consistent and robust in vitro system for studying vascular biology, drug screening and tissue engineering.

Introduction

Angiogenesis is a multi-step physiological process; it is also involved in a large number of disease states. In vitro angiogenesis models provide very useful tool to study angiogenesis; additionally, this model can be used in drug screening applications.

- Tubes formed in co-culture assays were significantly more heterogeneous and more closely resembled capillaries than Matrigel tubules.
- Few in vitro co-culture models have been developed using primary cells, however, donor variability, low cell quantity per well and shorter lifespan of primary cells limit their usefulness and consistencies.

In this study, we established an in vitro co-culture model system using cell lines that were immortalized by hTERT alone.

A systematic procedure has been employed to validate this co-culture model using VEGF (vascular endothelial growth factor)/pathway-related compounds.

Results

Tel0HAEC retains endothelial cell characteristics

Tel0HAEC-GFP and hTERT-MSC co-culture model represents more close in vivo physiology

Figure 1. Characterization of Tel0HAEC, marker staining and tubulin formation on top of extracellular matrix.
Primary HAEs or Tel0HAECs were seeded on top of an extracellular matrix basement membrane gel (CellMatrix, ATCC® ACS-3035™) at a density of 2x10^5 cells/cm^2. The cells were then stained with A) anti-CD31 antibody (R&D Systems) and Alexa Fluor™ 594 conjugated secondary antibody (Life Technologies), or B) incubated with Alexa Fluor™ 488 conjugated AcLDL (Life Technologies) for 3 hours. Both CD31 and AcLDL makers were positive for more than 90% cells in the Tel0HAEC population. C) Live-cell images of primary HAEs or Tel0HAECs seeded on top of CellMatrix.

Figure 2. Establishment and validation of Tel0HAEC and BJ fibroblast co-culture angiogenesis assay system. Tel0HAEC cells were grown for 14 days with BJ fibroblasts in an optimized angiogenesis medium in the presence or absence of VEGF or suramin at the indicated concentrations and time points. A) and C) The cells were fixed and stained with antibodies directed against CD31. B) and D) Indicate tubule length quantification, performed using Image J software (NIH, Bethesda, MD).

Figure 3. Establishment of Tel0HAEC-GFP and hTERT-MSC co-culture angiogenesis. Tel0HAEC-GFPs co-cultured with hTERT-MSC for 7 days in the optimized angiogenesis medium displayed a long branching organization (A) and exhibited immuno-reactivity to an SMA antibody (Sigma®,) , which co-localized with the Tel0HAEC-GFP/n(D). Phase contrast microscopy indicated the 3-dimensional structure of the tubules(C).

Figure 4. Validation of the Tel0HAEC-GFP and hTERT-MSC co-culture angiogenesis assay system. Tel0HAEC-GFP cells were cocultured with hTERT-MSC as in figure 3 and treated with VEGF or suramin at the indicated concentrations. A) and C) Indicate GFP fluorescence. B) and D) Indicate quantification of the tubule lengths.

Summary

- hTERT immortalized Tel0HAEC cell retains important endothelial markers and tubular formation on cell matrix characteristics.
- An in vitro co-culture angiogenesis assay system was established using Tel0HAEC and BJ or Tel0HAEC/hTERT-MSC (GFP) and hTERT-MSC cells.
- First tubular structures formed in less than 7 days in the Tel0HAEC/Tel0HAEC-GFP/hTERT-MSC system while the Tel0HAEC-BJ system took 14 days.
- The hTERT-MSC cells, which surrounded the tubular structures, expressed a marker of smooth muscle cells which represents a more close in vivo situation.
- The tubular formation efficiency reacts positively to VEGF stimulation and negatively to suramin in a dose-dependent manner in both the Tel0HAEC-BJ and Tel0HAEC/hTERT-MSC in co-culture assay systems.
- This co-culture model is consistent and robust in vitro system for studying vascular biology, tissue engineering, and is useful in drug development.

References


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