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

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Angiogenesis is a multi-step physiological process which is involved in a large number of normal and disease state processes; in vitro angiogenesis models provide very useful tools to study these processes, one of which is the analysis of tubule formation. Tubules formed in co-culture assays were significantly more heterogeneous and more closely resembled capillaries than Matrigel® tubules. Current co-culture models using primary cells have donor variability and inconsistent results due to lot to lot variation. In this study, we established an in vitro co-culture model system consisting of an assay ready mixture of an aortic endothelial cell line TeloHAEC-GFP (hTERT immortalized human aortic endothelial cell line) and a hTERT immortalized adipose-derived mesenchymal stem cell line hTERT-MSC in a common specially formulated medium, the Angio-Ready™ angiogenesis medium with VEGF supplement. 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. The new co-culture system forms functional tubular structures in less than 7 days, and in addition, the hTERT-MSC cells which surround the tubular structures have undergone transformation indicated by elevated positive αSMA staining (a marker of smooth muscle cells), indicating that the system has physiological relevance. Next, we tested the new system with compounds that impact angiogenesis, results demonstrated that the angiogenesis system responds positively to elevated doses of VEGF and negatively to increasing concentrations of suramin; more importantly, the tubular formation efficiency is reduced or blocked by well-known cancer drugs such as Sunitinib (SUTENT®) and Bevacizumab (Avastin®), both of which target the VEGF pathway. Notably, our results showed the co-culture system has
minimal lot-to-lot variation indicated by the treatment of three lots of the Angio-
Ready™ system with the cancer drug, Ramucirumab (Cyramza®), which also
targets the VEGF pathway. Finally, we used the Angio-Ready™ system validated
4 HIF-1(hypoxia inducible factors-1) inhibitors which have anti-angiogenic
properties identified by high-throughput screening methods; data showed the
results of the new system match with other screening methods, what’s more,
Angio-Ready™ system screening time can be as short as 3 days. Therefore, the
coculture models developed by using hTERT-immortalized cell lines described in
this report provide a consistent and robust in vitro system for studying
cardiovascular biology, drug screening and tissue engineering.

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