Application Forum

Fluorescent human co-culture angiogenesis assay: analysis of pro-angiogenic growth factors

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Introduction

Angiogenesis, which is the formation of new blood vessels from pre-existing vessels, is mandatory for tumor growth. Angiogenesis is also involved in other pathological disorders such as psoriasis and macular degeneration. The process can be divided into 3 stages: endothelial cell (EC) proliferation, EC migration and vessel morphogenesis. Essen's GFP-AngioKit, an in vitro co-culture assay system, recapitulates these three stages of the angiogenic cascade. The kinetic method described herein is contrasted to currently available methods in that it provides dynamic information not available from end point experiments.

Methods

GFP-AngioKits were prepared by first expressing GFP in HUVECs and then co-culturing them in a 24-well microplate (Orange Scientific) with normal human dermal fibroblasts (NHDF) as previously described by Bishop et al. (1) and in collaboration with TCS CellWorks. The 24-well GFP-AngioKit was fed every 2-3 days and tubule formation was monitored by imaging the cultures every 12 hours in IncuCyte™ FLR. Essen's Angiogenesis Analysis Module was used to quantify tube length, tube area, and branch point formation over the time course of the experiment.

Results

In the presence of VEGF, GFP-labeled HUVECs proliferate and migrate to form cell clusters by day 5 (Figure 1, A and B). Further treatment with VEGF and other pro-angiogenic factors, such as bFGF and EGF, promote tubule formation and lengthening from day 5 to 14 (Figure 1E). VEGF-stimulated cultures are fully mature by day 14 (Figure 1, C and D). Neutralizing antibodies to VEGF blocked the EGF-potential of VEGF, which establishes that EGF is acting via the VEGF pathway (Figure 1F). In the absence of VEGF, there is greatly diminished proliferation, migration, and tubule formation.

Conclusion

Combining GFP-AngioKits with IncuCyte™ FLR enables kinetic and quantitative measurements of both the stimulation and inhibition of microvascular tubule formation. The GFP-AngioKit,

Figure 1. Kinetic analysis of microvascular tubule formation. Panel A and C show IncuCyte FLR images of GFP-labeled tubes taken at day 5 and day 14, respectively. A corresponding computational analysis of tube formation using the Angiogenesis Analysis Module for day 5 and 14 is presented in panels B and D, respectively. The effect of VEGF, bFGF, and EGF on tube length over time is shown in panel E. Anti-VEGF antibody treatment inhibits VEGF-stimulated tube formation, presented in panel F. More information can be found at www.essen-instruments.com/angio.

in conjunction with IncuCyte™ FLR, can be used to screen for drugs affecting all stages of angiogenesis and to dissect signal transduction pathways.

References

1. Bishop, E.T., G.T. Bell, S. Bloor, I.J. Broom, N.F. Hendry, and D.N. Wheatley. 1999. An in vitro model of angiogenesis: basic features. *Angiogenesis*. 3:335-344.