

Phenotype-Based High-Content Chemical Library Screening Identifies Small Molecules That Enhance Endothelial Cell Branching and Tubulogenesis

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The goal of therapeutic angiogenesis is enhancing new vessel growth that can effectively provide blood supply to ischemic tissues. The endothelial cellular process such as establishment of new connections, branching and tubulogenesis are essential aspects in the growing of a functional vascular network. With the aim of identifying novel small molecule enhancers of these aspects during angiogenesis, we used endothelial tube formation assay to develop an automated assay that enables high-throughput/content screening of small molecule libraries.

Methods: The assay is automated and miniaturized into a 384-well format enabling to profile a library of 1280 Pharmacologically Active Compounds (LOPAC1280) for their angiogenic activity. The screen was conducted in duplicate at a final compound concentration of 10 μ M using primary Human Umbilical Vein Endothelial Cells. Following cell fixation and staining, the plates were imaged using a high content microscopy system (Operetta). The images were processed and analyzed using fully-automated Metamorph image analysis software.

Results: The screen resulted in an initial active compound list of 74 inhibitors and 14 enhancers. Hit characterization revealed 8 enhancer small molecules that reproducibly increased significantly both branching points and total tubule length in a dose dependent manner with low micromolar range ($EC_{50} < 10 \mu$ M). Among enhancer hits, there were: GBR-12909 (dopamine reuptake inhibitor), SB 202190 (p38 MAP kinase inhibitor), and Y-27632 (ROCK inhibitor). Moreover, the effect of enhancer hits was examined on multiple cellular aspects of angiogenesis: cell adhesion, proliferation, motility and 3D endothelial sprouting.

Conclusions: Our findings suggest that high-throughput/content approach provides a powerful tool to identify new potential targets or to prioritize therapeutic agent candidates for in vivo and clinical testing of pro-angiogenic therapies for ischemic diseases.