Characterization of a novel angiogenic model based on stable, fluorescently labeled endothelial cell lines amenable to scale-up for high content screening.

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Public Summary:
Background. Blood vessel formation is important for many physiological and pathological processes, and is therefore a critical target for drug development. Inhibiting angiogenesis to starve a tumor or promoting "normalization" of tumor blood vessels in order to facilitate delivery of anticancer drugs are both areas of active research. Recapitulation of vessel formation by human cells in vitro allows investigating cell-cell and cell-matrix interactions in a controlled environment, and is thereby a crucial step in developing high content (HC) and high throughput (HT) screening assays to search for modulators of blood vessel formation. Human umbilical vein endothelial cells (HUVECs) exemplify primary cells used in angiogenesis assays. However, primary cells have significant limitations that include phenotypic decay and/or senescence by 6-8 passages in culture, making stable integration of fluorescent markers and large-scale expansion for high throughput screening problematic. To overcome these limitations for HTS, we developed a novel angiogenic model system that employs stable fluorescent endothelial cell lines based on immortalized human microvascular endothelial cells (HMEC-1, hereafter HMECs). We then evaluated HMEC cultures, both alone and co-cultured with an epicardial mesothelial cell (EMC) line that contributes vascular smooth muscle cells, to determine suitability for HTS or HCS. Results. The endothelial and epicardial lines were engineered to express a panel of nuclear- and cytoplasm-localized fluorescent proteins to be mixed and matched to suit particular experimental goals. HMECs retained their angiogenic potential and stably expressed fluorescent proteins for at least 13 passages after transduction. Within 8 hours upon plating on Matrigel, the cells migrated and coalesced into networks of vessel-like structures. If co-cultured with EMCs, the branches formed cylindrical-shaped structures of HMECs surrounded by EMC-derivatives reminiscent of vessels. Network formation measurements revealed responsiveness to media composition and control compounds. Conclusions. HMEC-based lines retain most of the angiogenic features of primary endothelial cells, yet possess long-term stability and ease of culture, making them intriguing candidates for large-scale primary HC and HT screening (of ~10,000-1,000,000 molecules). Furthermore, inclusion of EMCs demonstrates the feasibility of using epicardial-derived cells, which normally contribute to smooth muscle, to model large vessel formation. In summary, the immortalized fluorescent HMEC and EMC lines and straightforward culture conditions will enable assay development for HCS of angiogenesis.

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