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Author Response: Effects of Fibroblastic and Endothelial Extracellular Matrices on Corneal Endothelial Cells

We thank Dr. Baum for his kind, encouraging words and for pointing out his very interesting article on how matrix influences HCEC morphology.[1] His work is of great importance to the field of corneal endothelial research. It had a major impact on our earlier work on establishing and optimizing cell cultivation and transplantation of HCECs—namely, his article, "Mass Culture of Human Corneal Endothelial Cells,"[2,3] which we cited in one of our most important papers.[3]

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Bevacizumab Suppression of Establishment of Micrometastases in Experimental Ocular Melanoma

We read with great interest the article entitled, "Bevacizumab Suppression of Establishment of Micrometastases in Experimental Ocular Melanoma" by Yang et al.,[1] published in the June issue. We congratulate the authors for this informative study. However, we have a few concerns about the study.

1. In Figure 1, the authors demonstrate that bevacizumab reduced VEGF secretion. However, it is more likely that bevacizumab neutralized the secreted VEGF, rather than suppressed it.

2. It is not clear why, even though bevacizumab may have neutralized the secreted VEGF, the neutralized VEGF could not be measured by ELISA. The epitopes for antibodies used in ELISAs (27-191 amino acids) are likely to be against a different region of VEGF than are the functional epitopes bound by bevacizumab.

3. The authors state that VEGF secretion was slightly suppressed with 10 μg/mL bevacizumab ($P = 0.0046$) and greatly reduced with 100 μg/mL ($P = 0.0091$) in B16LS9 cells, compared with the effect of the IgG1 control treatment. However, they also state that the levels of VEGF after 10 and 100 μg/mL bevacizumab were 8.51 and 15.99 pg/mL, respectively. Thus, the levels were higher in cultures treated with a higher dose of bevacizumab (100 μg/mL). Also, in Figure 1C, it does not appear that the VEGF levels were decreased significantly by bevacizumab.

4. Several earlier studies (e.g., Ferrara et al.[2]) reported that bevacizumab does not neutralize mouse VEGF, raising the question of how bevacizumab may have reduced the VEGF levels.

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Author Response: Bevacizumab Suppression of Establishment of Micrometastases in Experimental Ocular Melanoma

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biological activity of human vascular endothelial growth factor (VEGF) in in vitro and in vivo assay systems. It contains human framework regions and the complementarity-determining regions of a murine antibody that binds to VEGF.

When comparing the sequence of human VEGF protein with that of mouse VEGF protein, we found that 86% of the protein sequence in mouse VEGF164 is similar to that of human VEGF165.[1] We believe that it is possible for bevacizumab to block human and mouse VEGF. A blocking antibody is defined as an antibody that does not have a reaction when combined with an antigen, but prevents other antibodies from combining with that antigen.[2,3] We believe that bevacizumab fits this definition and explains why the neutralized VEGF is not detected by ELISA. The human or mouse VEGF immunoassay (Quantikine; R&D Systems, Minneapolis, MN) that we used in our experiment can detect human VEGF165 and VEGF121 or mouse VEGF164 and VEGF120, but does not detect VEGF189 and VEGF206. VEGF121 and VEGF165 are diffusible proteins that are secreted into the medium. VEGF189 and VEGF206 have high affinity for heparin and