

The effect of a VHH cocktail on vascular endothelial growth factor inhibition using a whole-cell enzyme-linked immunosorbent assay (ELISA) method

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Background and Aims: Vascular endothelial growth factor (VEGF) plays an important role in tumor angiogenesis and represents a potential target for anticancer therapy. It is well known that VHHs, variable domains of heavy chain antibodies isolated from Camelidae family, can recognize uncommon or hidden epitopes that are normally not recognized by conventional antibodies. However, using a single VHH may not be effective in neutralization of tumor targets. The purpose of the present study was to examine the effect of a combination of three VHHs (VHH cocktail) on VEGF inhibition using a whole-cell enzyme-linked immunosorbent assay (ELISA) method.

Methods: Human umbilical vein endothelial cells (HUVECs) were cultured in 96-Well Microtiter plate and fixed with methanol. Using our previous selected VHHs, three complementary whole-cell ELISA experiments (A, B, and C) were conducted. In experiment A, VEGF (5 µg/ml) was incubated with cells at room temperature for 1 hour. In experiment B, VEGF was incubated with a single VHH (ZFR-1, ZFR2, or ZFR-5) at room temperature for 1 hour and added to respective wells. In experiment C, VEGF was incubated with a VHH cocktail (ZFR-1:ZFR2 and ZFR-5) at room temperature for 1 hour and added to respective well. The amount of bound VHHs was detected using peroxidase conjugated anti-HA at A450 nm.

Results: Although no single VHH significantly decreased ELISA signal, a VHH cocktail inhibited binding of VEGF to HUVECs and resulted in low ELISA signal.

Conclusions: Our finding suggests that a mixture of VHHs might be useful for complete neutralization of VEGF in vitro and in vivo.

Keywords: VEGF; VHH cocktail; Angiogenesis; Cancer