

More Effective Macrophage Apoptosis Induction VEGF165^{HuXp} - Human Cell Expressed



INTRODUCTION

Cytokines are a group of proteins and polypeptides that organisms use as signaling molecules. Most cytokines are glycoproteins less than 30 kDa in size and bind to specific, high-affinity cell surface receptors. Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory and infectious diseases and widely used in research, diagnostics and therapeutics. Cytokines generally alter the gene expression pattern of the target cell which leads to changes in the rate of cell proliferation and/or in the state of cell differentiation. Currently, these proteins are predominantly produced in non-human cells (e.g. E. coli) and therefore lack authenticity due to the absence of physiologically relevant glycosylation. In addition, a number of important cytokines are not commercially available due to inadequate proteolytic processing, protein folding or other post-translational modifications that occur in the non-human cell expression systems.

HumanZyme has developed an efficient human-cell based technology, **HumaXpress™**, for cost-effective and scalable production of human cytokines. We have an expanding range of tag-free cytokines, including difficult-to-express protein members of the TGFβ superfamily. As demonstrated below, HumanZyme's authentic VEGF165^{HuXp} can be used as highly preferred reagents for apoptosis research.

Materials and Methods

VEGF165 is a member of the cysteine-knot growth factor superfamily. This cytokine stimulates proliferation and survival of endothelial cells, and induces apoptosis. Currently, commercially available VEGF165 proteins are produced from non-human cells including E. coli and insect cells. HumanZyme has produced VEGF165^{HuXp} (See

product catalog number HZ-1013 and HZ-1038 at www.humanZyme.com) from engineered human 293 cells. The E. coli expressed protein is a monomer - dimer mixture and has a molecular mass of 18 and 34 kD in SDS-PAGE. This compares with VEGF165^{HuXp} which migrates as a band of 36 kD due to glycosylation and dimerization.

The THP-1 monocytic cell line (ATCC, Manassas, VA) was cultured in RPMI 1640 (Mediatech, Herndon, VA) containing 10% FBS (Atlanta Biologicals, Lawrenceville, GA). THP-1 cells were differentiated into macrophages by incubation with PMA (Phorbol-12-myristate-13-acetate; EMD Biosciences, San Diego, CA) for three days, and were then treated with increasing concentrations of VEGF 165 (E, coli expressed obtained from a US manufacturer specializing in cytokines) and VEGF165^{HuXp} an HEK293 cell expressed cytokine from HumanZyme Inc. (Chicago, IL) (0, 10, 50, 100, 200 ng/ml). The THP-1-derived macrophages were then stained with Annexin V-FITC using the AposcreenTM Annexin V-FITC kit (Southern Biotech, Birmingham, AL), according to manufacturer's instructions. Stained cells were mounted with Vectashield (Vector Laboratories, Burlingame, CA), and were then visualized by fluorescence microscopy, using a Nikon TE300 fluorescence microscope. The number of stained cells present after each treatment was counted in 10 40x fields, and then normalized to the control. Comparisons between treated and untreated groups were performed using GraphPad InStat software. The significance of differences between treatment groups were determined using ANOVA; groups with significant differences were then subjected to the Tukey-Kramer Multiple Comparisons Post Test. Data are shown as fold change relative to the control, mean +/- SD, *P<0.05, ***P<0.001.

Results

VEGF treatment increased the number of cells stained with Annexin V-FITC, as shown by fluorescence microscopy; this effect was dose-dependent, and could be observed in cells treated with VEGF concentrations as low as 10 ng/ml. When comparing the effects of E. coli expressed VEGF versus VEGF165^{HuXP} from HumanZyme, it is apparent that VEGF165^{HuXP} is more effective than E. coli expressed VEGF at similar concentrations for inducing Annexin V-FITC staining, and thus apoptosis. Moreover, the response elicited by E. coli expressed VEGF was quite variable, with large error bars when low VEGF concentrations were used for treatment, such that the effect did not reach statistical significance at 10 ng/ml; the effect was significant at 50 ng/ml ($P < 0.05$), with the most significant effects seen at 100-200 ng/ml ($P < 0.001$). In addition, there were no statistically significant differences among the groups treated with different concentrations of VEGF. In contrast, the human cell expressed VEGF appeared to exert a more consistent and more potent response, as shown by small error bars and a statistically

significant increase in Annexin V-FITC staining at the low concentration of 10 ng/ml ($P < 0.001$). It is noteworthy that the low concentration of 10 ng/ml of VEGF165^{HuXP} significantly induced Annexin V-FITC staining, and thus apoptosis, in THP-1 macrophages, because comparable VEGF levels have been detected under physiological conditions. Furthermore, Annexin V-FITC staining induced by 200 ng/ml VEGF was significantly greater than that seen with 10 and 50 ng/ml VEGF ($P < 0.001$), and staining induced by 100 ng/ml VEGF was significantly greater than that seen with 10 ng/ml VEGF ($P < 0.001$). No significant differences were observed when comparing 10 vs. 50 ng/ml VEGF, 50 vs. 100 ng/ml VEGF, and 100 vs. 200 ng/ml VEGF treatment ($P > 0.05$).

The experiments were performed and the data kindly provided by:

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