GENE EXPRESSION PROFILING AFTER ANGIOGENESIS INHIBITOR TREATMENT

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Abstract

The angiogenic process can be summarized as cell activation by a lack of oxygen releases angiogenic molecules that attract inflammatory and endothelial cells and promote their proliferation. Several protein fragments produced by the digestion of the blood-vessel walls intensify the proliferative activity of endothelial cells. Acetyl salicylic acid is often used as an analgesic drug to relieve minor aches and pains, a drug with antitumour activity and an anti-inflammatory medication. In our experiments we propose using the angiogenesis model and photodynamic therapy (PDT) for observing changes in angiogenesis after treatment, and also for increasing the effect of PDT by addition of angiogenesis inhibitors.

Keywords

angiogenesis, photodynamic therapy, acetyl salicylic acid

Introduction

The vascular network regulates the body homeostasis by transporting oxygen, liquids, nutrients, cells and signaling molecules to all part of the body and disposing of waste from the tissues [1].

Normal blood vessel formation takes place in two main ways by: vasculogenesis and angiogenesis [2, 3].

Vasculogenesis is the differentiation *de novo* of vascular endothelial cells from precursor cells known as angioblasts during embryonic development [4]. The endothelial cell lattice created by vasculogenesis then serves as a scaffold for abiogenesis [3].

Angiogenesis is the formation of new capillary sprouts from pre-existing blood vessels [5]. After the primary capillary plexus is formed, it is remodeled by the sprouting and branching of new vessels from preexisting ones. The normal mechanism of angiogenesis depends on the coordination of several independent processes, for example removal of pericytes from the endothelium and destabilization of the vessel by angiopoietin-2 (Ang2) and shift of

endothelial cells from a stable, growth-arrested state to a plastic, proliferative phenotype [3].

VEGF is other candidate as a potential regulator of angiogenesis. This emanates from the fact that VEGF is a secreted protein, its binding sites are selectively expressed in endothelial cells in vitro and in vivo and the expression of its mRNA correlates with blood vessel growth. Vascular endothelial growth factor (VEGF, VEGF-A) is a major regulator of physiological and pathological angiogenesis. Several VEGF inhibitors have been approved by the FDA for the treatment of advanced cancer and neovascular age-related macular degeneration [6]. Further, vascular endothelial growth factor (VEGF)-induced hyperper-meability allows for local extravasation of proteases and matrix components from the bloodstream [3].

Angiogenesis is also stimulated by fibroblast growth factor (FGF) [4]. **FGF** is synthesized and secreted by human adipocytes and the concentration of bFGF correlates with the BMI in blood samples [7]. Additionally, bFGF is a critical component of human embryonic stem cell culture medium; the growth factor is necessary for the cells to remain in an undifferentiated state, although the mechanisms by which it does this are poorly defined [8].

It must be pointed out, that the remodelling and growth of the vasculature is a normal bodily process, particularly in wound healing, in pathological disease processes such as cancer and in the female menstrual cycle and hence in certain cases it may be important that the effects of gene therapy, once delivered, are strictly controlled [3, 4, 9].

One potentially useful toxic gene product is the tumour necrosis factor-α (TNF-α). TNF-α is toxic for proliferating, but not quiescent, microvascular endothelium. TNF-α is by itself active and therefore needs to be regulated at a transcriptional level. However, its wide-ranging toxicity against tumours make it well worth further investigation [9].

Another interesting gene platelet-derived growth factor (PDGF) is released in high concentrations during tissue repair and inflammatory processes, first from platelet alpha granules and subsequently by activated macrophages. Other peptide growth factors that have been shown to be actively released from platelets and activated macrophages, together with PDGF, include epidermal growth factor (EGF), transforming growth factor (TGF)-alpha, TGF-beta, and basic fibroblast growth factor (bFGF) [10].

Experiments

The study aimed to investigate the use of acetyl salicylic acid (ASA) for inhibiting angiogenesis (angiogenesis model in vitro was designed by a scientific team from FICAM). The experiments were carried out with co-culture of two types of cells: hASC (human adipose stem cells, 22 000 cells/well) and HUVEC (human umbilical vein endothelial cells, 4 000 cells/well). Cells were treated with 1mM ASA in concentrations 2, 1, 0.5, 0.25 and 0.125 mmol/l.

Second, the isolation of RNA was performed using RNeasy mini kit (Qiagen). Consequently RNA was transcribed to cDNA (RT2 Prolifer PCR Array). Finally, real-time PCR was carried out using 96-well array: 84 target genes, 5 reference genes and 7 control genes. The expression of genes was assessed using the program BIO-RAD CFX Manager.

Graphs 1–7 show the first results of real-time PCR for selected interesting genes only. All results represent samples treated by ASA. The experiments were performed using 96-well arrays.

Graphs 1, 2 and 3 show the the curves of three selected reference genes Beta-2-microglobulin, very often used Glyceraldehyde-3-phosphate dehydrogenace and Hypoxanthine phosphoribosyltransferase.

The amplification curve (graph 4) is for Fibroblast growth factor 2 (FGF2) which mediates the formation of new blood vessels (angiogenesis) during wound healing of normal tissues and tumour development. It is an important mediator responsible for induction of endothelial precursor cells from the mesoderm [11]. It is apparent that the FGF2 amplification curve began to increase several cycles after reference curves but it is still very early to draw any conclusions.

Angiopoietin 1 (graph 5) is an important protein because of its role in vascular development and angiogenesis. Its amplification curve predicates very high and early expression. It plays a critical role in mediating reciprocal interactions between the endothelium and surrounding matrix and mesenchyme [12, 13].

Platelet-derived growth factor alpha (graph 6) polypeptide is the factor for cells of mesenchymal origin.

Transforming growth factor alpha (TGFα) (graph 7) is a ligand for the epidermal growth factor receptor and activates a signaling pathway for cell proliferation, differentiation and development [14].

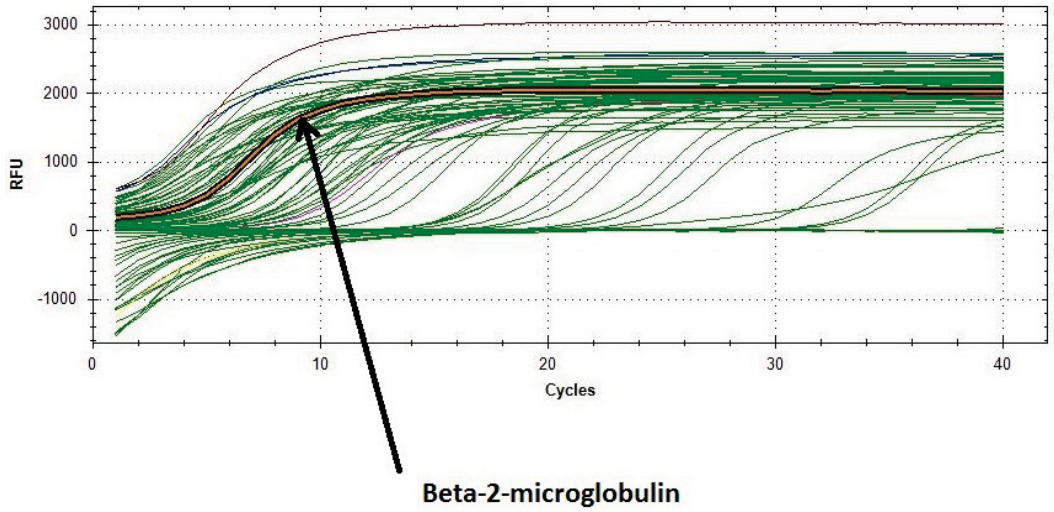

Fig. 1: Curve of Beta-2-microglobulin.

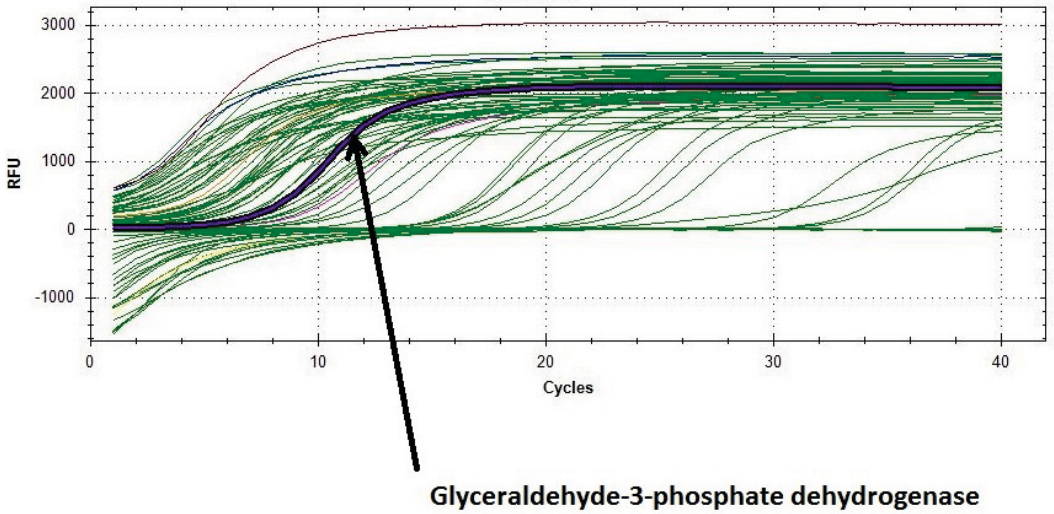

Fig. 2: The curve of Glyceraldehyde-3-phosphate dehydrogenace.

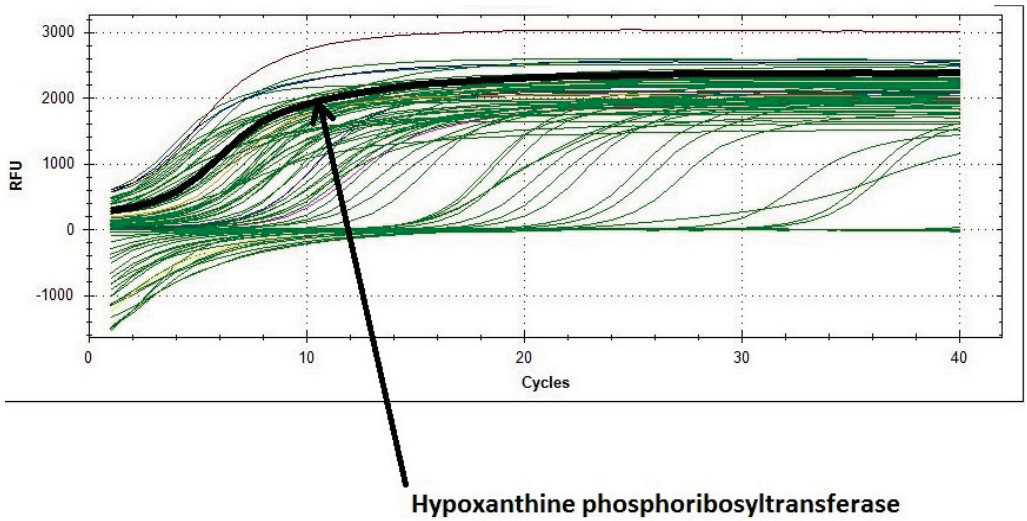

Fig. 3: The curve of Hypoxanthine phosphoribosyltransferase.

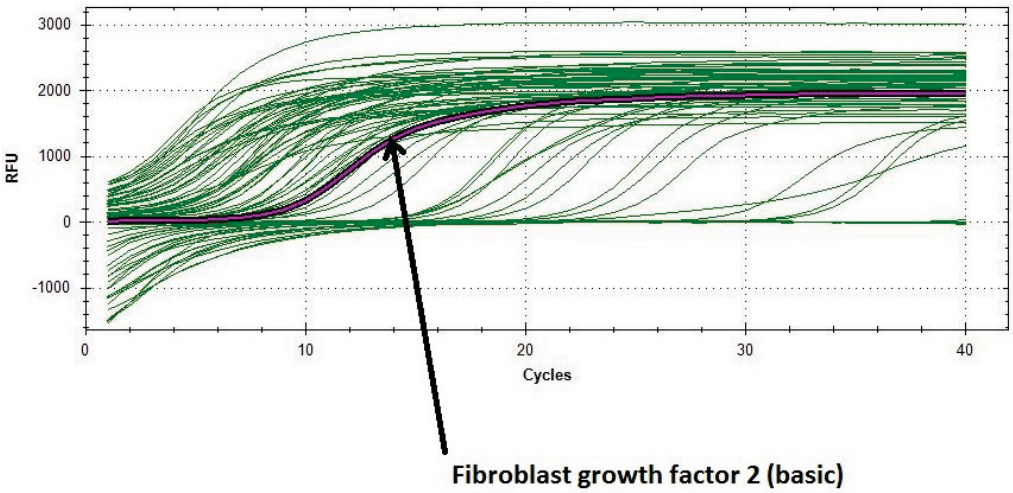

Fig. 4: The curve of Fibroblast growth factor 2 (basic).

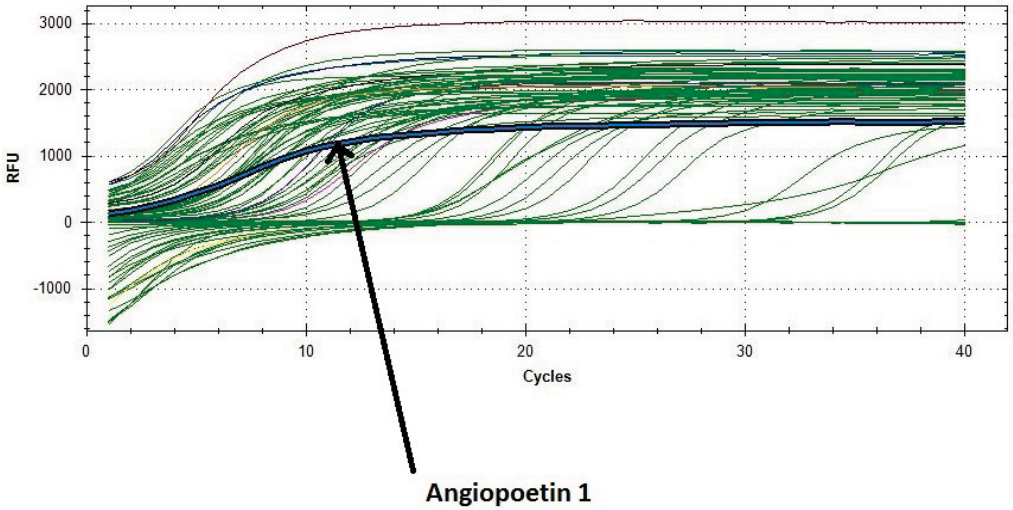

Fig. 5: Curve of Angiopoetin 1.

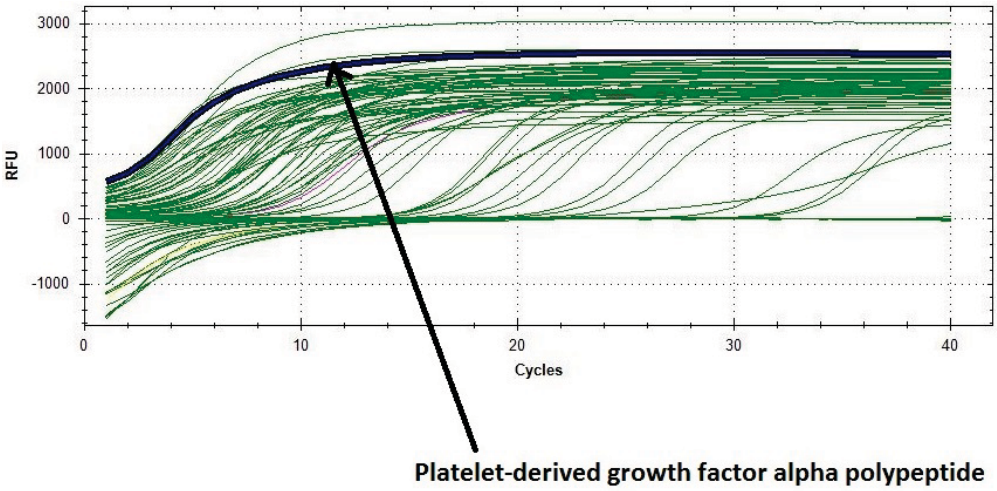

Fig. 6: The curve of Platelet-derived growth factor alpha polypeptide.

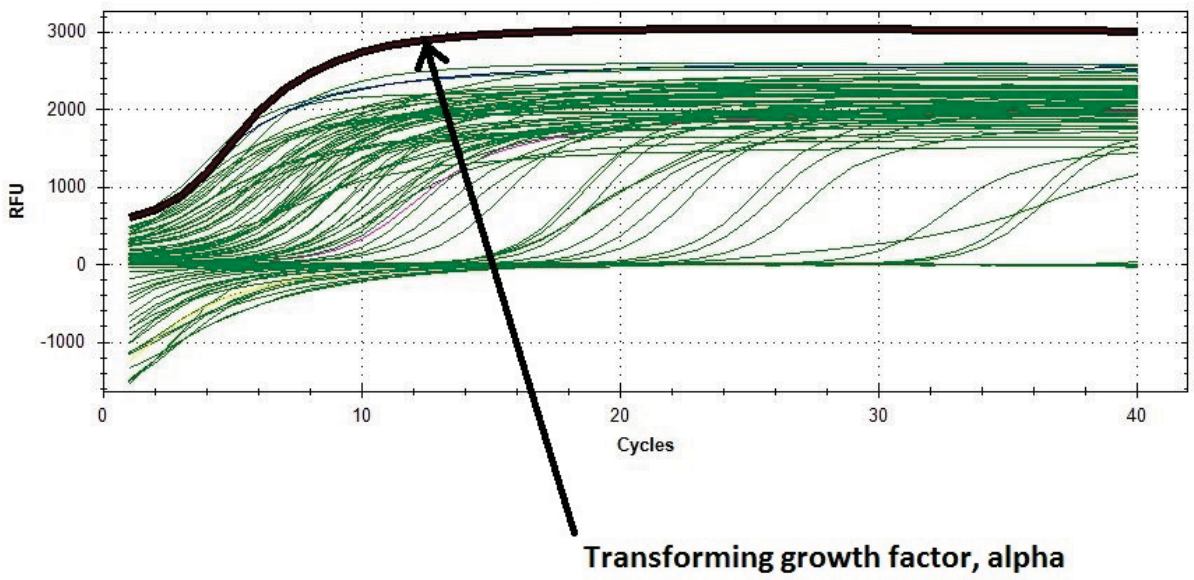

Fig. 7: The curve of Transforming growth factor alpha (TGFα).

Conclusion

Our research laboratory is focused on photodynamic therapy (PDT) *in vitro*. *This* combinates visible light of an appropriately wavelength and non-toxic chemical agent called photosensitiser. This combination is used to destroy cancer cells or to eradicate bacterial cells [15-25].

Currently new strategies and alternatives are needed to increase the options for treatment of cancer. Photodynamic therapy (PDT) has become progressively established as a mode of treatment of malignant as well as non-malignant diseases which are characterized by the occurrence of unwanted or harmful cells [26].

Combining angiogenic inhibitors with chemotherapy and radiotherapy has demonstrated the potential to be an attractive and effective approach to cancer treatment. Ferrario and colleagues first demonstrated that PDT efficacy could be enhanced by combining angiogenic inhibitors with the treatment regimen [27].

Acetylsalicylic acid (ASA, aspirin) appears to be a significant inhibitor of angiogenesis. It has remained the most commonly-used drug for relieving pain, inflammatory symptoms, and fever. Recently, ASA has been shown to reduce the risk for colorectal cancer by as much as 40%, a property that is shared with other nonsteroidal anti-inflammatory drugs (NSAIDS) [28].

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