Gene profiling of human VEGF signaling pathways in human endothelial and retinal pigment epithelial cells after anti VEGF treatment

Shani Golan1*, Michal Entin-Meer2, Yonathan Semo2, Sofia Maysel-Auslender2, Daphna Mezad-Koursh1, Gad Keren2, Anat Loewenstein1 and Adiel Barak1

Abstract

Background: Ranibizumab (Lucentis®) is a Fab-antibody fragment developed from Bevacizumab, a full-length anti-VEGF antibody. Both compounds are used for treating age-related macular degeneration (AMD). The influence of bevacizumab and ranibizumab on genes involved in signal transduction and cell signaling downstream of VEGF were compared in order to detect possible differences in their mode of action, which are not related to their Fab-antibody fragments.

Methods: Human umbilical vein cell lines (EA.hy926) and retinal pigment epithelial cells (ARP-19) were exposed to oxidative stress. The cells were treated with therapeutic concentrations of bevacizumab (0.25 mg/mL) and ranibizumab (125 mg/mL) for 24 hours prior to all experiments, and their effects on gene expressions were determined by RT-PCR.

Results: After exposure to bevacizumab, more genes in the endothelial cells were up-regulated (KDR, NFATc2) and down-regulated (Pla2g12a, Rac2, HgdC, PRKCG) compared to non-treated controls. After exposure to ranibizumab, fewer genes were up-regulated (PTGS2) and down-regulated (NOS3) compared to controls. In comparison between drugs, more genes were up-regulated (NFATc2 and KDR) and more were down-regulated (Pla2g12a, Pla2g1b, Ppp3r2, Rac2) by bevacizumab than by ranibizumab. In RPE cells, NOS3 and PGF were up-regulated and Pla2g12b was down-regulated after exposure to ranibizumab, while PIK3CG was up-regulated and FIGF was down-regulated after exposure to bevacizumab, but the differences in gene expression were minor between drugs (PIK3CGand PGF were down-regulated more by ranibizumab than by bevacizumab).

Conclusions: The different gene expressions after exposure to ranibizumab and bevacizumab in endothelial and RPE cells may indicate a somewhat different biological activity of the two compounds.

Keywords: Bevacizumab, Ranibizumab, Gene expressions, VEGF, PCR

Background

Age-related macular degeneration (AMD) is the leading cause for legal blindness among the elderly in the industrialized world. Pathological angiogenesis is the underlying cause of the exudative form of AMD.

VEGF-A is a potent endothelial cell mitogen, and recent evidence indicates that it acts as an autocrine growth and survival factor in VEGF-A-producing cells [1,2]. Several studies suggest that it is a major mediator of angiogenesis and vascular leakage in exudative AMD [3,4]. Inhibition of VEGF-A activity has been a central theme in many therapies under investigation. Several inhibitors have been developed and are now used clinically.

In 2006, ranibizumab (Lucentis; Genentech, Inc., San Francisco, CA, USA), an antibody fragment developed against all fragments of VEGF, was approved by the FDA for treating AMD following the MARINA [5] and the ANCHOR [6] studies, which showed that ranibizumab had a stabilizing effect in 90% of the patients and a beneficial effect in 30%.
Ranibizumab is highly effective but considerably expensive. In contrast, bevacizumab, a full-length anti-VEGF antibody approved for use in colon cancer [7], costs much less than ranibizumab.

Ocular use of bevacizumab is currently not FDA approved; nevertheless, it is widely used for treating AMD [8-11]. Bevacizumab has been described in case reports [8-10], retrospective studies [10,11], and controlled prospective studies (CATT and IVAN trials) as effective and well tolerated. To date, only limited experimental data and studies comparing the effects of bevacizumab and ranibizumab have been published. The comparative trials CATT and IVAN evaluated the therapeutic efficacy of bevacizumab compared with ranibizumab, concluding that the former was not inferior to the latter [12,13].

Despite the fact that ranibizumab and bevacizumab share the same Fab-fragment blockage properties, they are actually different molecules. Bevacizumab is produced in Chinese hamster ovary (CHO) cells from the expression plasmids VID5.ID.LLnspeV.xvegf36HC.LC. The resulting antibody is produced from the G7 clone as a 149-kDa full-length IgG1 antibody composed of two 214-residue light chains and two 453-residue heavy chains. Each light chain is covalently linked to a heavy chain and the two heavy chains are covalently bonded. The resulting bevacizumab antibody contains 93% human amino acid sequence [14,15].

Ranibizumab was developed in an effort to obtain higher affinity variants of Fab-12 with improved potency and efficacy [16]. Although bevacizumab was derived from Fab-12, ranibizumab was derived from an in vitro CDR mutation from a different humanized anti-VEGF Fab variant, known as MB1.6 [17,18]. Ranibizumab is produced as a 48 kDa Fab in *Escherichia coli* from the expression plasmid pyO317. The heavy and light chains fold into their native conformation following secretion into the bacteria’s periplasmic space and are covalently linked. The resulting Fab-Y0317 is known as ranibizumab [19,20].

It has been shown previously that the two molecules act differently and possess' different pathway activities which may be unrelated to their anti-VEGF activities [21-23].

As bevacizumab and ranibizumab differ in their molecular composition and physiologic properties, the present study compared VEGF inhibitors in terms of their effects on genes involved in signal transduction and cellular damage resulting from oxidative stress in both RPE and endothelial cells.

Cellular damage resulting from oxidative stress in both endothelial and RPE cells plays a causative role in AMD [3]. Oxidative stress-induced RPE cell apoptosis has been proposed as a major pathophysiological mechanism of AMD [3,22-24]. In particular, RPE cell apoptosis is an important feature of the advanced form of dry AMD [3,25]. Thus, oxidative stress induces VEGF-A expression from the RPE and also RPE death [3,22], suggesting a role for such stress in both neovascular and advanced dry AMD.

The effects on gene expression were examined using a model of ischemia (12 hours in a hypoxic chamber) to mimic significant stress imposed upon the cells in neovascular AMD in real time.

### Methods

#### Cell culture

EA.hy926 cells (a human umbilical vein cell line) were seeded at 100,000/cm² in T-75 cm² flasks containing DMEM with 15 Mm Hepes buffer, 10% fetal bovine serum, 2 mM L-glutamine solution and 10% pen-strep at 37°C for 1 week. Serum was withdrawn in DMEM + 1% bovine serum albumin for 3 days to make the cells quiescent. ARPE-19 cells were seeded on 1*10⁶/10 cm plates containing DMEM with 10% fetal bovine serum, 1% L-glutamine solution, and 10% pen-strep at 37°C for 1 week, and serum was withdrawn in DMEM + 1% bovine serum albumin for 3 days to make the cells quiescent.

#### Exposure to bevacizumab and ranibizumab

Before all experiments, both cell lines were treated for 12 hours in a hypoxic chamber (exposure to less than 2% oxygen in the chamber). Therapeutic dosages of both bevacizumab and ranibizumab (0.25 mg/mL and 0.125 mg/mL, respectively) were then added to the cell lines. These concentrations were prepared using serial dilutions of the drug in the respective serum-free culture medium. The solution of the drug mixed with media was then directly added to the cells in order to obtain a uniform concentration of drug throughout the well of the tissue culture plate. In addition to bevacizumab and ranibizumab, the cells were also treated with 10 ng/ml hVEGF (PeprotechInc, Rocky Hill, NJ, USA).

#### Control groups

All experiments were compared to controls. Controls were cells that had been treated with human VEGF (hVEGF) alone and no bevacizumab or ranibizumab.

#### RNA production

After 48 hours of exposure to either ranibizumab or bevacizumab, the total cellular RNA was isolated from the cells by a QiagenRNeasy® Mini Kit (Catalog # 74104) according to the manufacturer’s instructions. RNA samples
underwent DNase treatment and removal (QiagenRNase®
Mini Kit, Catalog # 74104). RNA quantification was per-
fomed with spectrophotometry (ND-1000; NanoDrop
Products, Thermo Fisher Scientific, Wilmington, DE), after
which 250 ng of total RNA was analyzed by agarose gel
electrophoresis to confirm integrity. The resultant RNA
was stored at -80°C.

The RNA was reverse transcribed using the RT [2] First
Strand Kit (Qiagen).

Real-time quantitative (q) RT-polymerase chain reaction (PCR)
A SAB biosciences RT [2] Profiler PCR Array assay
(Qiagen) was performed according to the manufacturer’s
instructions, using syber green [26].

The RT array included five controls for housekeeping
genes, one control for genomic DNA and three reverse
transcription controls (no RNA loaded).three positive RNA
controls were also present. PCR was performed on an ABI
Prism 7700 Sequence Detector (©2008 Applied Biosystems.
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were analyzed using the web-based software RT2 Profiler
PCR Array data analysis tool, (Qiagen). The ΔΔCt method
was used for data analysis. Specifically, fold-changes for
each gene were calculated as difference in gene expression
between bevacizumab exposure and control, and between
ranibizumab exposure and control. A positive value indi-
cated gene up-regulation and a negative value indicated
gene down-regulation.

Statistical analysis
Each experiment was independently repeated at least
twice (as recommended by the manufacturers guidelines
for statistical significance). Genes with greater than 2.5
fold change in expression compared to control were
identified as significant (p < 0.05).Only results that showed
a relatively high (>30) average threshold cycle of a gene in
either the control or the test sample, and a reasonably low
(<30) average threshold cycle in the other sample were in-
cluded. Data that demonstrated the gene’s expression was
relatively low in one sample and reasonably detected in
the other sample suggested that the actual fold-change
value was at least as large as the calculated and reported
fold-change result. The results that fitted this formula
were graded “A”. Results that were graded “B” indicated
that the gene’s average threshold cycle was relatively high
(>30), implying that its expression level was relatively low
in both the control and test samples, and that the P value
for the fold-change was relatively high (P > 0.05). All ex-
periments were repeated at least twice. The list of genes
examined is summarized in Table 1.

Results
A comparison of the various gene expression patterns of
RPE cells after exposure to both anti-VEGF agents and
exposure solely to hVEGF (control) are presented in
Tables 2,3 and 4 and Figure 1A–C. Several genes of
RPE cells that were treated with bevacizumab were
over-expressed compared to controls (MAPK genes,
the SPHK gene and the VEGFA gene), while others
(the KDR, NOS, PIK3R and PLA2G genes) were under-
expressed. When the RPE cells were treated with ranibizu-
mb, the NFATC, MAPK, SPHK and VEGFA were up-regulated, whereas the KDR and NOS3 genes were
under-expressed compared to controls. Only the PGF and
PIK3CG genes were down-regulated in the RPE cells that
had been treated with bevacizumab compared to when
they had been treated with ranibizumab.

Comparisons of the various gene expressions of endo-
thalial cells after exposure to bevacizumab, ranibizumab,
and controls are shown in Table 5 and Figure 1D. After
exposure to ranibizumab, PTGS2 was up-regulated com-
pared to controls (hVEGF), while NOS3 expressions were
down-regulated.

After exposure to bevacizumab, different genes were
up- and down-regulated compared to controls (Table 6
and Figure 1E, F). The KDR gene was over-expressed
and the FIGF gene was under-expressed. However, in cells
were under-expressed compared to controls. Only the PGF and
PIK3CG genes were down-regulated in the RPE cells that
had been treated with bevacizumab compared to when
they had been treated with ranibizumab.

Discussion
The results of the present study suggest that gene expres-
sions differ after exposure to ranibizumab and bevacizumab
in RPE and endothelial cell lines. This finding may indicate
that although they both block the VEGF signaling pathway,
they probably do so in a somewhat different biological
mechanism, or through different transmitters.

RPE cells exposed to either bevacizumab or ranibizumab
exhibit different gene expression as compared to controls.
Mainly MAPK and VEGFA are over-expressed when
treated with either bevacizumab or ranibizumab compared
to controls, and the expression of KDR and NOS genes was
down-regulated. However, when the two treatment arms
were compared (bevacizumab compared to ranibizumab),
no major differences in gene expression are noted
(besides PGF and PIK3CG). Both gene expressions play a role in the VEGF receptor dimerization and au-
tophosphorylation, and play a crucial role in the VEGFA
mitogenic signaling pathway.

In endothelial cells, the situation was somewhat different.
No major gene expression was noted when comparing both
treatment arms to control; however, when comparing beva-
cizumab to ranibizumab, major differences in gene expres-
sions were noted (KDR and NOS3 were up-regulated and
NFATc2 was down regulated with bevacizumab. NFATc2 is a calcineurin/nuclear factor of activated T cells c2 (NFATc2) pathway and has displayed an anti-apoptotic role in melanoma cells [27].

VEGF is a heparin-binding homodimeric glycoprotein that acts via endothelial-specific receptor tyrosine kinases, VEGFR1 (Flt1), VEGFR2 (KDR/Flk1), and VEGFR3 (Flt4) [28]. In addition to VEGFA, the VEGF family of growth factors contains five other known members, namely placenta growth factor (PIGF), VEGFB, VEGFC, VEGF-D and viral VEGF homologs. Disruption of the genes encoding either VEGF or any of the three receptors of the VEGF family results in embryonic lethality due to failure of blood vessel development.

VEGFR2 is the main signal transducing VEGF receptor for angiogenesis and mitogenesis of endothelial cells. After receptor dimerization and autophosphorylation, several other signal transduction molecules are activated either directly or by indirect mechanisms [29].

NFATc2 was down regulated with bevacizumab. NFATc2 is a calcineurin/nuclear factor of activated T cells c2 (NFATc2) pathway and has displayed an anti-apoptotic role in melanoma cells [27].

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**Table 1 Positions of genes and genes symbol**

| Position | Symbol | B11 | MAPK12 |
|----------|--------|-----|--------|
| A01      | AKT1   | B12 | MAPK13 |
| A02      | AKT2   | C01 | MAPK14 |
| A03      | AKT3   | C02 | MAPK3  |
| A04      | ARNT   | C03 | MAPKAPK2 |
| A05      | BAD    | C04 | MAPKAPK3 |
| A06      | CASP9  | C05 | NFAT5  |
| A07      | CAV1   | C06 | NFATC1 |
| A08      | CDC42  | C07 | NFATC2 |
| A09      | FIGF   | C08 | NFATC3 |
| A10      | FLT1   | C09 | NFATC4 |
| A11      | FLT4   | C10 | NOS3   |
| A12      | GRB2   | C11 | NRA5   |
| B01      | HIF1A  | C12 | NRP1   |
| B02      | HRS    | D01 | NRP2   |
| B03      | HSP90AA1 | D02 | PDGFC  |
| B04      | HSPB1  | D03 | PGF    |
| B05      | KDR    | D04 | PIK3CA |
| B06      | KRA5   | D05 | PIK3C3 |
| B07      | MAP2K1 | D06 | PIK3C3D|
| B08      | MAP2K2 | D07 | PIK3C3G|
| B09      | MAPK1  | D08 | PIK3R1 |
| B10      | MAPK11 | D09 | PIK3R2 |
| D10      | PIK3R3 | F10 | PRKCG  |
| D11      | PIK3R5 | F11 | PTGS2  |
| D12      | PLAG1G10 | F12 | PTK2   |
| E01      | PLAG1G12A | G01 | PXN    |
| E02      | PLAG1G12B | G02 | RAC1   |
| E03      | PLAG1G1B  | G03 | RAC2   |
| E04      | PLAG2G2A | G04 | RNF1   |
| E05      | PLAG2G2D | G05 | SH2D2A |
| E06      | PLAG2G2E | G06 | SHC2   |
| E07      | PLAG2G2F | G07 | SIK1   |
| E08      | PLAG2G3  | G08 | SHK2   |
| E09      | PLAG2G4A | G09 | SRC    |
| E10      | PLAG2G4B | G10 | VEGFA  |
| E11      | PLAG2G5  | G11 | VEGFB  |
| E12      | PLAG2G6  | G12 | VEGFC  |
| F01      | PLCG1   | H01 | B2M    |
| F02      | PLCG2   | H02 | HPR1   |
| F03      | PPP3CA  | H03 | RPL13A |
| F04      | PPP3CB  | H04 | GAPDH  |
| F05      | PPP3CC  | H05 | ACTB   |
| F06      | PPP3R1  | H06 | HGDC   |

**Table 2 Fold change of gene expression in retinal pigment epithelial cells exposed to bevacizumab compared to human vascular endothelial growth factor-treated control**

| Gene symbol | Gene name | Bevacizumab/ control | Grade |
|-------------|-----------|----------------------|-------|
| MAPK3       | Mitogen-activated protein kinase 3 | 19.7066 | A |
| NFATC2      | Nuclear factor of activated T cells | 5.2333 | A |
| NRP1        | Neuropilin 1 | 7.0536 | A |
| SIK1        | Sphingosine kinase 1 | 22.0982 | A |
| SRC         | Vascular endothelial growth factor A | 61.0411 | A |
| SH2D2A      | Nitric oxide synthase | -170.2534 | A |
| SHC2        | Neuritin | -6.2684 | A |
| PKR1        | Phosphoinositide-3-kinase, regulatory subunit 3 (gamma) | -4.0004 | A |
| RPL13A      | Phosphoinositide-3-kinase, regulatory subunit 5 | -14.6532 | B |
| GAPDH       | | | |
| ACTB        | | | |
| HGDC        | | | |
| B2M         | | | |
| HPR1        | | | |
| RPL13A      | | | |
| GAPDH       | | | |
| ACTB        | | | |
| HGDC        | | | |
VEGFA and MAPK were both up-regulated in RPE cells treated with either bevacizumab or ranibizumab. A differential involvement of mitogen activated protein kinases (MAPK) has been previously shown to be involved in VEGF expression and regulation [22], as P38 is involved in constitutive and oxidative stress regulated VEGF expression. This pathway conveys the VEGF signal to microfilaments inducing rearrangements of the actin cytoskeleton that regulate endothelial cell migration by modulating the activation of MAPK2/3 (MAP kinase activated protein kinase-2/3) and phosphorylation of the F-actin polymerization modulator, HSP27 (heat shock protein-27).

KDR and NOS genes were both down-regulated in RPE cells exposed to both anti-VEGF agents. Both genes have key roles in mediating VEGF activity in increasing the proliferation and permeability of capillary endothelial cells. Increasing the proliferation and permeability of endothelial cells may produce unwanted side effects, such as tumor angiogenesis, vascular leakage, edema, and inflammation [30]. In endothelial cells, the VEGF-Flk1/KDR signal system is a very important generator of NO (nitric oxide) through the activation of its downstream effectors PI3K, Akt kinase and eNOS (endothelial NO synthase).

Their role in RPE cells has also been previously investigated. KDR has been proposed to transmit protective signaling against oxidant induced cell death in both normal conditions and disease states such as AMD [31]. It was also suggested to be involved in autocrine VEGF regulation [32].

Both genes were statistically significantly down-regulated after treatment with both anti-VEGF agents in RPE cell, perhaps indicating that both anti-VEGF agents block the angiogenesis and vascular leakage through these two genes.

Interestingly, both KDR and NOS3 genes were up-regulated in endothelial cells treated with bevacizumab compared to ranibizumab. This finding may suggest that there is a somewhat different mechanism of action between the two compounds in endothelial cells specifically.

Early preclinical [33,34] and clinical [35-37] studies have implicated VEGF as a major factor in the pathogenesis of neovascular eye diseases, supporting the rationale for further investigation of the therapeutic potential of VEGF-targeted agents. VEGF-based therapies for a variety of human pathological situations that are associated with aberrant endothelial proliferation and aberrant neovascularization include the use of neutralizing antibodies against VEGFA or VEGFR2, antisense oligonucleotides, negative regulatory peptides, soluble receptors, and ATP analogs to inhibit the kinase activity of VEGFR [17]. Interference with VEGF function has, therefore, become a subject of major interest for drug development to block angiogenesis, and the targeting of the VEGF signaling pathway may be of therapeutic importance for many diseases.

**Conclusions**

The present study describes the preliminary results of altered gene expression downstream of the VEGF signal pathway when comparing ranibizumab treatment to bevacizumab treatment in both RPE and endothelial cell lines. Our results indicate a somewhat different biological activity of the two compounds that need to be further explored.

This study has several limitations; first of being only based on RNA analysis (no Confirmation of results at the protein level) and results at the RNA level are often not replicated at the protein level.

The second limitation is that the study relies on a commercial custom-based Q-PCR assay, and the test genes were all genes in the VEGF pathway that were present on the kit. It could be that other more complex involvement of genes expression is involved and was not investigated.

**Table 3** Fold change of gene expression in retinal pigment epithelial cells exposed to ranibizumab compared to human vascular endothelial growth factor-treated control

| Gene symbol | Gene name                          | Ranibizumab/control | Grade |
|-------------|------------------------------------|---------------------|-------|
| NOS3        | Nitric oxide synthase              | -293.5515           | A     |
| KDR         | Kinase insert domain receptor      | -5.0041             | A     |
| MAPK3       | Mitogen-activated protein kinase 3 | 20.0674             | A     |
| VEGFA       | Vascular endothelial growth factor | 73.4215             | A     |
| PIK3CG      | Phosphoinositide-3-kinase, catalytic, gamma polypeptide | 7.1785 | A |
| SPHK1       | Sphingosine kinase 1              | 28.9047             | A     |
| PLA2G2E     | Phospholipase A2, group IIE        | -26.829             | B     |
| PLA2G2F     | Phospholipase A2, group IIF        | 125.5516            | B     |
| PLA2G2A     | Phospholipase A2, group IIA (platelets, synovial fluid) | -12.4228 | B |
| PLA2G1B     | Phospholipase A2, group IB         | -89.4568            | B     |

**Table 4** Fold change of gene expression in retinal pigment epithelial cells exposed to bevacizumab compared to ranibizumab

| Gene symbol | Gene name                          | Ranibizumab/bevacizumab | Grade |
|-------------|------------------------------------|-------------------------|-------|
| Pgf         | Placental growth factor            | -4.2627                 | A     |
| Pik3cg      | Phosphoinositide-3-kinase, catalytic, gamma polypeptide | -13.5561 | A |
The third limitation is the control groups chosen; because we have examined AMD model, and because of the complexity of the analysis of gene array, we have chosen not to further complex our statistics by adding non-starved and non-hypoxic cells.

Another major limitation of the study is the non-conventional use of statistical analysis. We were unable to perform column analysis like t-testing or one way ANOVA because of the small number of samples obtained and the lack of normal distribution. In further studies, our results must be validated when using a larger scale analysis.

Table 5 Fold change of gene expression in endothelial cells exposed to Bevacizumab or Ranibizumab compared to control

| Gene symbol | Gene name                      | Bevacizumab/control | Grade |
|-------------|--------------------------------|---------------------|-------|
| KDR         | kinase insert domain receptor  | +6.3173             | A     |
| FIGF        | C-fos induced growth factor (vascular endothelial growth factor D) | -3.1746             | A     |
| PLA2G5      | Phospholipase A2, group V     | -3.928              | B     |
| PRKCG       | Protein kinase C gamma        | -5.0676             | B     |

Table 6 Fold change of gene expression in endothelial cells exposed to bevacizumab compared to endothelial cells exposed to ranibizumab

| Gene symbol | Gene name                      | Bevacizumab/ranibizumab | Grade |
|-------------|--------------------------------|-------------------------|-------|
| KDR         | kinase insert domain receptor  | +5.547                  | A     |
| NOS3        | Nitric oxide synthase 3 (endothelial cell) | +86.3228              | A     |
| NFATC2      | Nuclear factor of activated T-cells | -3.3708               | A     |
| PLA2G2E     | Phospholipase A2, group IIE    | -11.1207               | B     |
| PRKCG       | Protein kinase C, gamma        | -5.3751                | B     |
Competing interests
The authors report no competing interests. The authors alone are responsible for the content and writing of the paper.

Authors’ contributions
All authors carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. All authors read and approved the final manuscript.

Author details
1Departments of Ophthalmology, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv 64239, Israel. 2Departments of Cardiology, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel.

Received: 17 May 2013 Accepted: 27 August 2014 Published: 8 September 2014

References
1. Foroozghan F, Das B. Anti-angiogenic effects of ribonucleic acid interference targeting vascular endothelial growth factor and hypoxia-inducible factor-1alpha. Br J Ophthalmol 2007, 194:761–769.
2. Brussels K, Bono F, Collen D, Herbst JM, Carmelet P, Deweern CH: A novel role for vascular endothelial growth factor as an autocrine survival factor for embryonic stem cells during hypoxia. J Biol Chem 2005, 280:3493–3499.
3. Winkle RS, Boulton ME, Gotsch JD, Stemberg P: Oxidative damage and age-related macular degeneration. Mol Vis 1999, 5:32.
4. Kliemann M, Sharma HS, Mooy CM, de Jong PT: Increased expression of angiogenic growth factors in age-related maculopathy. Br J Ophthalmol 1997, 81:154–162.
5. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chong CY, Kim RY, MARINA study group: Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 2006, 355:1410–1413.
6. Kaiser PK, Brown DM, Zhang K, Hudson HL, Holz FG, Shapiro H, Schneider S, Acharya NR: Ranibizumab for predominantly classic neovascular age-related macular degeneration: subgroup analysis of first-year ANCHOR results. Am J Ophthalmol 2007, 144:850–857.
7. Kramer I, Lipp HP: Bevacizumab, a humanized anti-angiogenic monoclonal antibody for the treatment of colorectal cancer. J Clin Pharm Ther 2002, 32:11–14.
8. Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ: Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Am J Ophthalmol 2006, 142:2375–2378.
9. Perotti V, Baldassari P, Bersani I, Molla A, Vegetti C, Tassi E, Dal Col J, Dolcetti R, Anichini A, Mortarini R: NFATc2 is a potential therapeutic target in human melanoma. J Invest Dermatol 2012, 132(1):2652–2656.
10. Endo A, Nagashima K, Kurose H, Mochizuki S, Matsuda M, Mochizuki N: Sphinogonin 1-phosphate induces membrane ruffling and increases motility of human umbilical vein endothelial cells via vascular endothelial growth factor receptor and CrkII. J Biol Chem 2007, 282:23747–23754.
11. Matsumoto T, Caesens-Welsh L: VEGF receptor signal transduction. Sci STKE 2001, 2001.: RE 21.
12. Cai JK, Kim I, Lim ST, Chung MJ, Kim WH, Kim HG, Ko JK, Koy GY: Co-administration of angiopoietin-1 and vascular endothelial growth factor enhances collateral vascularization. Anesthesiology Thromb Vasc Biol 2000, 20:2573–2578.
13. Saint-Geneix M, Maharaj ASR, Walsho TE, Tucker BA, Sekiyama E, Kurthar T, Darland DC, Young MJ, D’Amore PA: Endogenous VEGF is required for visual function: Evidence for a survival role on Muller cells and photoreceptors. PLoS One 2008, 3:ee3554.
14. Kettner A, Westhues D, Lassen J, Bartsch S, Roeder J: Regulation of constitutive vascular endothelial growth factor secretion in retinal pigment epithelium/chorioid organ cultures: p38, nuclear factor kappaB, and the vascular endothelial growth factor receptor-2/phosphatidylinositol 3 kinase pathway. Mol Vis 2013, 19:281–291.
15. Adams PA, Shima DT, Tolentino MJ, Gragoudas ES, Ferrara N, Folkman J, D’Amore PA, Miller JW: Inhibition of vascular endothelial growth factor prevents retinal ischemia-associated iris neovascularization in a nonhuman primate. Arch Ophthalmol 1996, 114:66–71.
16. Kozutko MG, Alshari MA, Adams AP, Gaudreau J, Gragoudas ES, Michauda NA, Kozutko MG, Alshari MA, Adams AP, Gaudreau J, Gragoudas ES, Michauda NA: Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment. Arch Ophthalmol 2002, 120:338–346.
17. Reik LM, Lambert V, Devy L, Luttun A, Carmelet P, Glaes C, Nguyen L, Foidart JM, Noél A, Munaut C: Placental growth factor, a member of the
VEGF family, contributes to the development of choroidal neovascularization. Invest Ophthalmol Vis Sci 2003, 44:3186–3193.

36. Wells JA, Murthy R, Chibber R, Nunn A, Molinatti PA, Kohner EM, Gregor ZJ: Levels of vascular endothelial growth factor are elevated in the vitreous of patients with subretinal neovascularisation. Br J Ophthalmol 1996, 80:363–366.

37. Adamis AP, Miller JW, Bernal MT, D’Amico DJ, Folkman J, Yeo TK, Yeo KT: Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol 1994, 118:445–450.

Cite this article as: Golan et al.: Gene profiling of human VEGF signaling pathways in human endothelial and retinal pigment epithelial cells after anti VEGF treatment. BMC Research Notes 2014, 7:617.