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Using gene expression profiling to identify the molecular basis of the synergistic actions of hepatocyte growth factor and vascular endothelial growth factor in human endothelial cells

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Hepatocyte growth factor (HGF) and vascular endothelial cell growth factor (VEGF) are two potent endothelial mitogens with demonstrated angiogenic activities in animal models of therapeutic angiogenesis. Several recent studies suggest that these growth factors may act synergistically, although the mechanism of this interaction is not understood. Changes in the gene expression profile of human umbilical vein endothelial cells treated with HGF, VEGF or the combination of the two were analyzed with high-density oligonucleotide arrays, representing approximately 22,000 genes. Notably, the genes significantly up- and downregulated by VEGF *versus* HGF exhibited very little overlap, indicating distinct signal transduction pathways. The combination of HGF and VEGF markedly increased the number of significantly up- and downregulated genes. At 4 h, the combination of the two growth factors induced a number of chemokine and cytokines and their receptors (IL-8, IL-6, IL-11, CCR6, CXCR1,CXC1 and IL17RC), numerous genes involved in growth factor signal transduction (egr-1, fosB, grb10, grb14,MAP2K3,MAP3K8, MAPKAP2,MPK3, DUSP4 and DUSP6), as well as a number of other growth factors (PDGFA, BMP2, Hb-EGF, FGF16, heuregulin beta 1, c-kit ligand, angiopoietin 2 and angiopoietin 4 and VEGFC). In addition, the VEGF receptors neuropilin-1 and flt-1 were also upregulated. At 24 h, a clear 'cell cycle' signature is noted, with the upregulated expression of various cell cycle control proteins and gene involved in the regulation of mitosis and mitotic spindle assembly. The receptor for HGF, c-met, is also upregulated. These data are consistent with the hypothesis that the combination of HGF and VEGF results in the cooperative upregulation of a number of different molecular pathways leading to a more robust proliferative response, that is, growth factor(s), receptors, molecules involved in growth factor signal transduction, as well as, at later time points, upregulation of the necessary cellular proteins required for cells to escape cell cycle arrest and enter the cell cycle.
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Abbreviations: FGF, fibroblast growth factor; HGF, hepatocyte growth factor; HUVEC, human umbilical vein endothelial cells; PAI-1, plasminogen activator inhibitor-1; VEGF, vascular endothelial growth factor

Introduction

There is considerable interest in the use of various growth factors, administered either as proteins or a genes, to induce angiogenesis to treat ischemic coronary and peripheral vascular disease. Growth factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) have been evaluated by many investigators, and at least in animal models of ischemic vascular disease showed promising effects (Baffour *et al*., 1992; 2000; Chleboun & Martins, 1994; Asahara *et al*., 1995; Baumgartner & Isner, 1998; Bush *et al*., 1998; Stark *et al*., 1998; Ferrara & Alitalo, 1999; Hayashi *et al*., 1999; Lazarous *et al*., 2000; Yang & Feng, 2000). Thus far, however, clinical trials using either intravascular or extravascular means of protein delivery have been disappointing, with only, at best, modest improvements in perfusion or clinical outcome (Henry *et al*., 2003; Khan *et al*., 2003).

While numerous studies evaluating the potential of gene-based therapy to deliver growth factors are now underway, the majority of these are evaluating the effects of a single factor. However, there are several studies that indicate that a combination of two or more growth factors may be far more effective than either factor alone (Pepper *et al*., 1992; Van Belle *et al*., 1998; Xin *et al*., 2001).

HGF and VEGF are potent endothelial mitogens, mitogens, and morphogens (Morimoto *et al*., 1991; Bussolino *et al*., 1992; Nakamura *et al*., 1996; Rosen *et al*., 1997; Ferrara, 1999). Data from several laboratories have shown that *in vitro*, the combination of HGF and VEGF results in a much more robust proliferative and chemotactic response than either growth factor alone (Van Belle *et al*., 1998; Xin *et al*., 2001). In three-dimensional collagen gels, neither HGF nor VEGF alone are sufficient to induce human endothelial cell survival and tubulogenesis, yet the combination of the two growth factors

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will (Xin *et al*., 2001). *In vivo* studies also suggest that combining HGF and VEGF can induce a more robust angiogenic response (Van Belle *et al*., 1998; Xin *et al*., 2001) than either growth factor alone.

The mechanism for the synergistic interactions of HGF and VEGF remains unclear. Van Belle *et al*. (1998) suggested that one of the effects of HGF *in vivo* was to induce VEGF production by surrounding smooth muscle cells. However, Sengupta *et al*. (2003) reported that HGF induced angiogenesis *in vivo* independently of VEGF. Wojta *et al*. (1999) found that HGF also increased the expression of VEGF and PAI-1 in human keratinocytes, and the VEGF receptor KDR (flk-1) in human endothelial cells.

Gene expression profiling offers the opportunity to assess rapidly the molecular pathways activated by growth factors, cytokines, and other stimuli. The effects of VEGF on endothelial gene expression have been described by several groups. However, the effects of HGF, or the combination of HGF and VEGF on endothelial gene expression are not well defined.

To address the molecular interactions of the HGF and VEGF signaling pathways, we evaluated endothelial mRNA expression using Affymetrix oligonucleotide arrays, examing the expression of over 20,000 genes at 4 and 24 h.

Methods

Cell culture

Human umbilical endothelial cells (HUVEC) were obtained from Clonetics (Cambrex Bioscience Walkersville, MD, U.S.A.). For the present study, three independent cell lots, each derived from three different pools (3–4 donors per pool) of umbilical cords, were used for replicates of each experimental condition. All cells were cultured under rigidly standardized conditions, with great care taken to ensure that the identical lot numbers of media, serum, growth factors, other supplements, and tissue culture plastic were used. The profiling experiments were performed on nearly confluent (95%) HUVEC that had been incubated in starvation medium: M199 containing $1\times$ ITS (insulin–transferrin–selenium-A), 2 mM glutamine, 100 U ml^{-1} pennicillin and 100 μg ml^{-1} streptomycin (all from Invitrogen Corp.; Carlsbad, CA, U.S.A.), and 1% fetal bovine serum (Tissue Culture Biologicals; Tulare, CA, U.S.A.) for 18 h. (This enables identification of growth factor-induced genes without the background caused by high concentrations of fetal bovine serum.) At the initiation of the experiments, the media was changed to fresh starvation medium with or without addition of VEGF (100 ng ml^{-1}), HGF (100 ng ml^{-1}), or a combination of both, and the cells incubated for either an additional 4 or 24 h. The concentrations of VEGF and HGF used were maximal effective doses (based on preliminary proliferation assays); that is, addition of higher concentrations of VEGF or HGF did not elicit any additional biological responses. At the termination of the experiments, 15 ml of Trizol (Invitrogen) was added and samples stored frozen at -80°C. RNA was subsequently extracted following the manufacturer's instructions. Total RNA isolated in this way further purified using RNeasy Mini kits as described in the product manual (Qiagen Inc.; Valencia, CA, U.S.A.).

mRNA extraction, affymetrix microarrays, and data analysis

DNAse-treated total RNA, 5 μg, was converted to cRNA and fragmented cRNA was hybridized to arrays (U133A) as per the manufacturer's suggested protocol (Affymetrix, Santa Clara, CA, U.S.A.). Data were analyzed with the MASv5 (Affymetrix) and Rosetta Resolver (Rosetta Biosoftware). Array results that met manufacturer's (Affymetrix) recommended quality criteria were imported into Rosetta Resolver (Roberts *et al*., 2000). Replicate hybridizations or profiles were combined to create ratio experiments using the Rosetta Resolver system as described (Stoughton & Dai, 2002). System processing consists of interchip normalization and nonlinear error correction (Schadt, 2002, #978). Ratios are calculated from the combined replicate profiles.

Results

There is very little overlap in the genes up- or downregulated by HGF and VEGF

To identify the genes up- and downregulated by HGF and VEGF, the data from the three independent experiments were combined and ratios of HGF *versus* basal and VEGF *versus* basal at 4 and 24 h were generated (Table 1). Significantly regulated (both up- and downregulated) probesets with a log ratio of 0.17 (corresponding to 1.5-fold change) and a *P*-value $\leqslant 0.1$ were identified in the experimental ratios (time-matched basal conditions *versus* treated). These somewhat conservative and 'arbitrary' cuts based on both the fold and significance of the change in expression are useful methods to analyze genes of interest rapidly. Unexpectedly, there was minimal overlap in the profiles of gene expression elicited by the two growth factors. For example, at 4 h, VEGF treatment resulted in the upregulated total of 607 different probe sets, which represent 432 different genes (since several genes were represented by multiple probesets). HGF upregulated 356 probe sets. However, only 107 different probe sets were common to the two treatment paradigms. Similar conclusions are derived from the 24 h plot of upregulated genes and the 4- and 24-h plot of downregulated genes (Figure 1).

Table 1 Number of significantly regulated probesets (log ratio $\geqslant 0.15$, *P*-value $\leqslant 0.1$) in HUVEC treated with HGF, VEGF or the combination of the two growth factors

Treatment	*4 h upregulated*	*4 h downregulated*	*24 h upregulated*	*24 h downregulated*
HGF	356	259	109	105
VEGF	607	274	463	310
HGF + VEGF	566	323	716	142

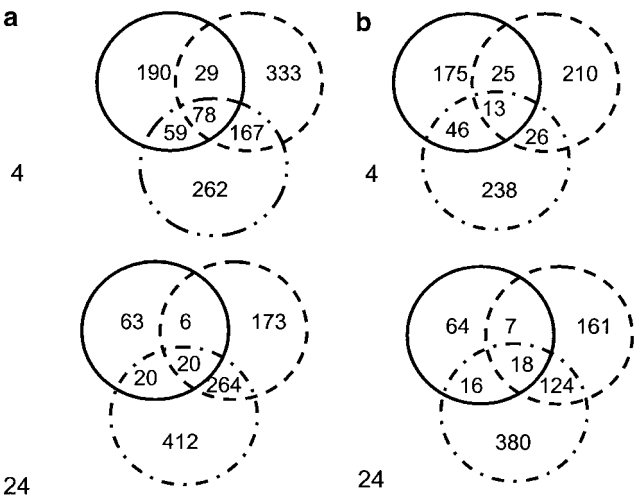

Figure 1 Venn diagram representation of those genes significantly upregulated (a) or downregulated (b) by HGF (solid circle), VEGF (dashed circle) or the combination of the two growth factors (circle with dashes and dots) at 4 h (top) and 24 h (bottom).

There are a number of genes that demonstrate additivity or synergy in expression levels when HGF and VEGF are combined

Figure 1 also illustrates the additive to synergistic interactions of HGF and VEGF. For example, at 4 h, the combination of HGF and VEGF resulted in the identification of 566 significantly upregulated probesets, 262 of which were unique to the combination of the two growth factors. In Figure 2, the expression data associated with the 566 probesets upregulated by the combination of HGF and VEGF (at 4 h) are compared to their regulation in the presence of HGF and VEGF individually *versus* basal ratio experiments. There are almost twice as many genes regulated by the combination treatment of HGF and VEGF together than either one alone.

Figure 3 shows an agglomerative cluster of the different probe sets. The 'black' bars denote 'no data' and represent probesets that did not meet the statistical cutoff of $P<0.1$. This figure illustrates the differences in the global gene expression profiles elicited by HGF *versus* VEGF; moreover,

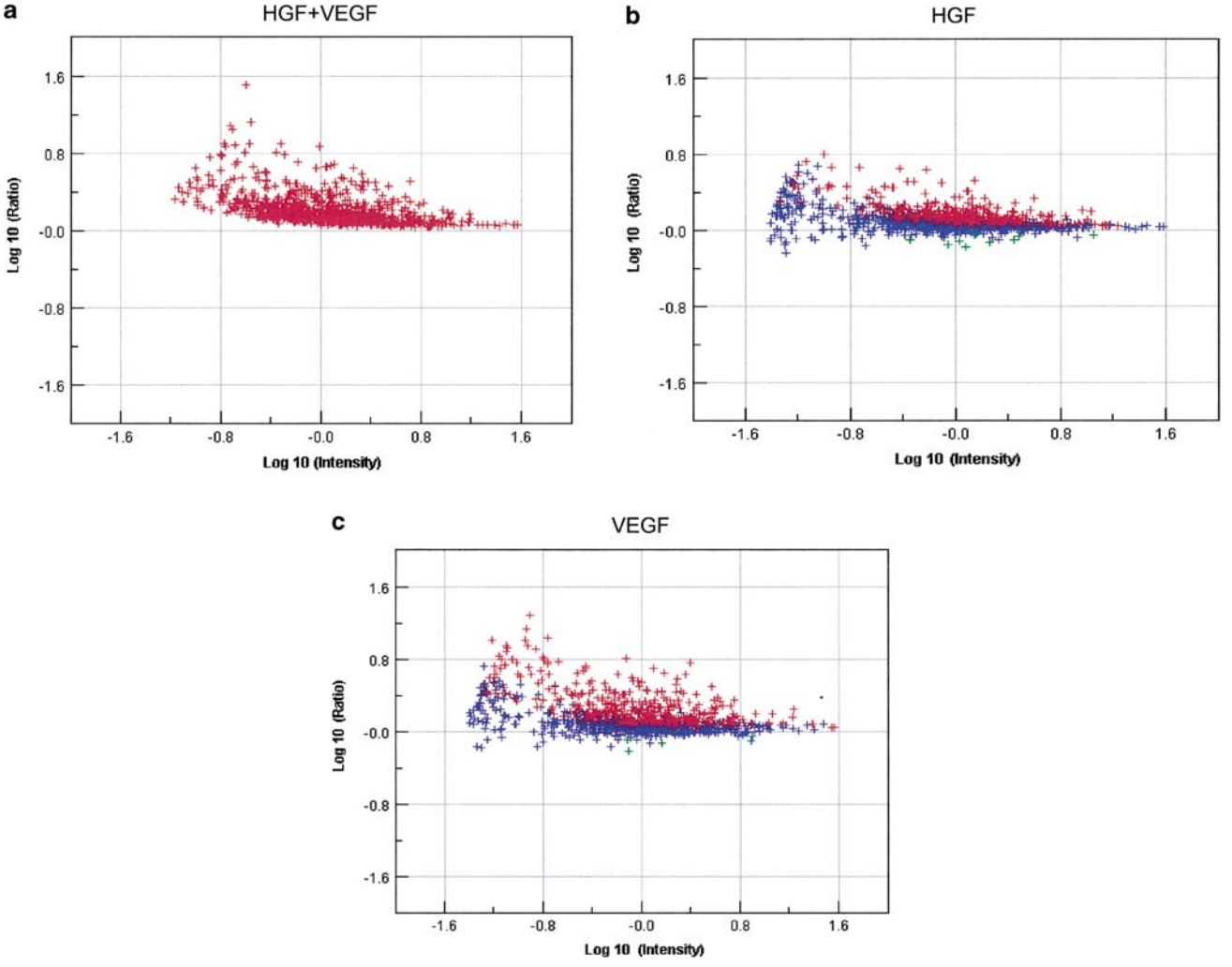

Figure 2 Dot plot representation of the expression of 566 probes upregulated by more than 1.5-fold, $P<0.1$ at 4 h, in the presence of the combination of HGF and VEGF at 4 h. The same probesets were compared to the expression data for HGF alone (b) and VEGF alone (a) at 4 h. Blue denotes not significantly changed from basal and red, significantly upregulated over basal expression.

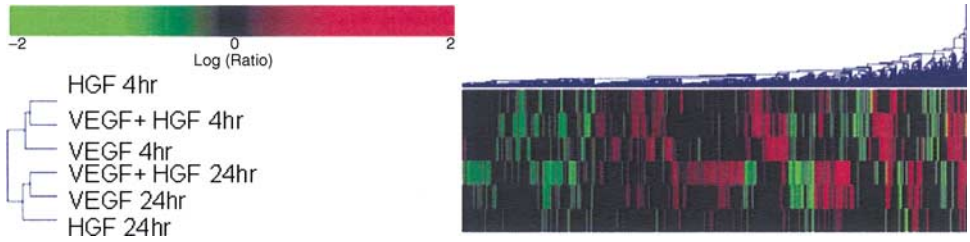

Figure 3 Agglomerative cluster of genes significantly upregulated or downregulated by HGF, VEGF, or the combination of the two growth factors at 4 h and 24 h. Black indicates 'not detected' or did not reach statistical significance ($P > 0.1$); green represents downregulated, and red, upregulated.

it also suggests that the effects of HGF predominate at 4 h and those of VEGF predominate at 24 h, when cells are treated with the combination of the two growth factors.

What are the major pathways induced by the combination of HGF and VEGF?

Individual genes are represented by 'probe sets' on the Affymetrix oligonucleotide array. Details on the probe design and sequence information, reproducibility, and oligonucleotide array analysis are available on the manufacturer's web site (www.affymetrix.com). A 'probe set' consists of 11 perfect match and 11 mismatch 25 mers representing each transcript. For each probe designed to be perfectly complementary to a target sequence, a partner probe is generated that is identical except for a single base mismatch in its center. These probe pairs, called the perfect match probe (PM) and the mismatch probe (MM), allow the quantitation and subtraction of signals caused by nonspecific cross-hybridization. The difference in hybridization signals between the partners, as well as their intensity ratios, serves as indicators of specific target abundance. The probe sets are selected based on their predicted hybridization properties, and filtered for specificity to reduce the potential for cross-hybridizing with similar, but unrelated sequences. To obtain a complete picture of a gene's activity, some probes are selected from regions shared by multiple splice or polyadenylation variants. In other cases, unique probes that distinguish between variants are favored. Interprobe distance is also factored into the selection process. Probes are 3′-biased to match the target generation characteristics of the amplification method, but are also widely spaced to sample various regions of each transcript and provide robustness of detection.

There are various approaches to identify pathways or cellular processes activated by cytokines and growth factors when using genome scale profiling. One method is group or cluster genes based on their patterns of expression. Another method is to group or cluster genes based on their proposed functions or interactions with other genes. As is evident in Tables 2 and 3, a number of genes are represented several times by different probe sets, and the probe sets were designed from different Genbank or REFSEQ accession numbers. Using the Netaffyx web site (www.affymetrix.com), probe subsets can be readily generated based on the annotation associated with each probeset (e.g. Kegg pathways, Gene Ontology, PRO domains, etc.). To obtain further molecular insights into the possible mechanism(s) of VEGF/HGF interactions, a union subset of probesets was generated from those identified as significantly upregulated when HGF and VEGF were combined (note this is the total set, not the set exclusive to the combination of the two growth factors). The keywords used were 'cell proliferation'; 'apoptosis', 'receptors', and 'growth factors', and the intersection of these probe sets with the subset of genes identified as upregulated from the combination of HGF and VEGF determined at 4 and 24 h determined.

The resulting subsets of genes for the 4 and 24 h time points, respectively, are provided in Tables 2, and 3 with annotation of the Genbank accession number and gene abbreviation, and corresponding ratio and *P*-values, for the HGF *versus* basal, VEGF *versus* basal, and HGF plus VEGF *versus* basal experiments.

While there are a number of the same genes represented on both the 4 and 24 h lists, careful perusal of the lists indicates significant differences in the ongoing molecular events at these time points. For example, the 4 h list contains a number of chemokines and chemokine receptors (IL-8, CCR6, CXCR4, and CXC1), and cytokines and cytokine receptors (IL-6, -11, and IL17RC). Another notable feature of the 4 h list is the upregulated expression of a number of genes playing an important role in growth factor signal transduction, including egr-1, fos B, grb10, grb14, MAP2K3, MAP3K8, MAPKAP2, MPK3, DUSP4 and 6, which may play a role in amplifying the growth factor response. A number of genes with some relationship to calcium homeostasis are also upregulated, including stanniocalcin 1, calcitonin gene-related peptide, and parathyroid hormone-related protein precursor. Another notable signature are the number of G-protein-coupled receptors (in addition to the chemokine receptors) and related signaling proteins, which are also upregulated, including "G-protein-coupled receptor-induced protein GIG2" (C8FW), RGS2, D(3) dopamine receptor, serotonin receptor 7, GPR4, GPR37, thromboxane A2 receptor, and lectomedin 1. Several growth factors including PDGF A chain, BMP2, Hb-EGF, FGF-16, heuregulin-beta 1, and c-kit ligand are induced, as well as the potent endothelial mitogen, VEGF-C, and an inhibitor of endothelial cell proliferation, VEGI (TNFSF15). ADAMTS1 (which is upregulated to a similar extent by both HGF and VEGF and is not further upregulated by the combination) is known to inhibit endothelial proliferation by binding and sequestering VEGF. Interestingly, the ligand 'cardiotrophin like cytokine' and its receptor 'ciliary neurotrophic factor receptor alpha precursor' show very similar regulation and suggest a potential role for this pathway in the angiogenesis induced by HGF and VEGF. Angiopoietin 2, which is believed to play a modulatory role in angiogenesis and lymphangiogenesis (Kim *et al*., 2000; Veikkola & Alitalo, 2002; Satchell & Mathieson, 2003), is upregulated by both VEGF and HGF, although there appears to be no additive interaction

Table 2 Genes significantly upregulated (>1.5-fold, $P<0.1$) by the combination of HGF and VEGF at 4 h

Sequence derived from	*Gene symbol*	*Gene description*	*Ratio of HGF+VEGF basal*	P-*value*	*Ratio of VEGF basal*	P-*value*	*Ratio of HGF basal*	P-*value*
AK023795.1	ADAMTS1	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	4.6	<0.01	4.8	<0.01	0.9	0.69
AB003476.1	AKAP12	A kinase (PRKA) anchor protein (gravin) 12	1.6	<0.01	1.2	0.31	1.6	<0.01
M90360.1	AKAP13	A kinase (PRKA) anchor protein 13	1.6	0.04	2.0	<0.01	1.3	0.35
NM_001147.1	ANGPT2	Angiopoietin 2	3.2	<0.01	5.1	<0.01	2.8	<0.01
AF187858.1	ANGPT2	Angiopoietin 2	3.2	<0.01	4.6	<0.01	2.7	<0.01
NM_015985.1	ANGPT4	Angiopoietin 4	3.0	0.08	0.9	0.85	0.7	0.52
NM_016109.1	ANGPTL4	Angiopoietin-like 4	7.6	<0.01	4.6	<0.01	1.7	0.41
NM_012099.1	ASE-1	CD3-epsilon-associated protein; antisense to ERCC-1	1.8	0.02	1.3	0.35	1.7	0.06
NM_001673.1	ASNS	Asparagine synthetase	1.5	0.02	1.3	0.25	1.5	0.07
AF095192.1	BAG2	BCL2-associated athanogene 2	1.7	<0.01	1.1	0.8	1.4	0.23
NM_004049.1	BCL2A1	BCL2-related protein A1	6.3	<0.01	2.0	0.23	2.8	0.09
AA583044	BMP2	Bone morphogenetic protein 2	2.0	<0.01	2.1	<0.01	1.7	<0.01
NM_001200.1	BMP2	Bone morphogenetic protein 2	1.9	<0.01	1.7	<0.01	1.4	<0.01
NM_001717.1	BNC	Basonuclin	1.9	0.09	1.6	0.32	1.6	0.37
NM_025195.1	C8FW	Phosphoprotein regulated by mitogenic pathways	2.1	<0.01	1.5	0.08	1.5	<0.01
NM_005795.1	CALCRL	Calcitonin receptor-like	1.5	<0.01	1.2	0.17	1.0	0.89
U17473.1	CALCRL	Calcitonin receptor-like	1.5	0.01	1.2	0.32	1.1	0.71
NM_016557.1	CCRL1	Chemokine (C–C motif) receptor-like 1	7.2	<0.01	5.8	<0.01	1.8	0.01
AV700298	CD44	CD44 antigen (homing function and Indian blood group system)	2.0	0.06	2.3	0.06	1.9	0.09
NM_003672.1	CDC14A	CDC14 cell division cycle 14 homolog A (*S. cerevisiae*)	1.9	0.01	2.2	0.01	1.8	0.1
AF115544.1	CDKN2A	Cyclin-dependent kinase inhibitor 2A(melanoma, p16, inhibits CDK4)	4.3	0.07	3.5	0.16	1.4	0.81
BC000059.1	CELSR1	Cadherin, EGF LAG seven-pass G-typereceptor 1 (flamingo homolog, Drosophila)	2.5	0.05	2.5	0.14	1.7	0.52
NM_021797.1	CHIA	Eosinophil chemotactic cytokine	3.0	0.01	1.0	0.93	2.2	0.38
NM_000748.1	CHRNB2	Cholinergic receptor, nicotinic, beta polypeptide 2 (neuronal)	6.8	0.03	2.1	0.7	3.0	0.6
NM_013246.1	CLC	Cardiotrophin-like cytokine; neurotrophin-1/B-cell-stimulating factor-3	2.6	0.1	1.5	0.58	1.9	0.29
NM_001842.1	CNTFR	Ciliary neurotrophic factor receptor	2.8	0.02	2.3	0.08	1.9	0.63
NM_004750.1	CRLF1	Cytokine receptor-like factor 1	5.6	0.03	0.7	0.71	3.1	0.44
D83702.1	CRY1	Cryptochrome 1 (photolyase-like)	1.9	<0.01	1.9	<0.01	1.1	0.42
BC005921.1	CSH1	Chorionic somatomammotropin hormone 1 (placental lactogen)	4.0	0.01	2.0	0.5	3.2	0.13
U83410.1	CUL2	Cullin 2	2.0	0.02	1.7	0.26	2.0	0.16
L01639.1	CXCR4	Chemokine (C–X–C motif) receptor 4	3.0	<0.01	2.8	<0.01	4.2	<0.01
AJ224869	CXCR4	Chemokine (C–X–C motif) receptor 4	2.4	<0.01	2.5	<0.01	3.0	<0.01
AF348491.1	CXCR4	Chemokine (C–X–C motif) receptor 4	1.9	<0.01	1.7	0.04	2.2	0.02
BC003637.1	DDIT3	DNA-damage-inducible transcript 3	1.9	<0.01	3.3	<0.01	1.6	0.1
AL050069.1	DOK5	Docking protein 5	3.1	<0.0	1.5	0.16	1.0	0.81
NM_000796.1	DRD3	Dopamine receptor D3	2.6	0.02	1.5	0.38	2.5	0.13
M60278	DTR	Diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	2.6	<0.01	3.8	<0.01	0.7	<0.01
NM_001945.1	DTR	Diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	2.2	<0.01	3.1	<0.01	0.7	0.04
BC002671.1	DUSP4	Dual specificity phosphatase 4	9.3	<0.01	8.5	<0.01	3.8	0.11
NM_001394.2	DUSP4	Dual specificity phosphatase 4	5.8	<0.01	6.5	<0.01	2.8	0.03
BC005047.1	DUSP6	Dual specificity phosphatase 6	7.2	<0.01	6.3	<0.01	3.2	0.03
BC003143.1	DUSP6	Dual specificity phosphatase 6	3.1	<0.01	2.5	<0.01	1.7	<0.01
BC003143.1	DUSP6	Dual specificity phosphatase 6	2.9	<0.01	2.7	<0.01	1.7	<0.01
NM_001964.1	EGR1	Early growth response 1	4.7	<0.01	5.8	<0.01	4.4	<0.01
NM_012153.1	EHF	ets homologous factor	2.3	0.05	1.1	0.88	1.4	0.64
BF445047	EMP1	Epithelial membrane protein 1	3.2	<0.01	1.9	<0.01	1.9	<0.01

Table 2 (Continued)

Sequence derived from	*Gene symbol*	*Gene description*	*Ratio of HGF+VEGF basal*	P-*value*	*Ratio of VEGF basal*	P-*value*	*Ratio of HGF basal*	P-*value*
NM_001423.1	EMP1	Epithelial membrane protein 1	2.1	<0.01	1.5	<0.01	1.6	<0.01
NM_001423.1	EMP1	Epithelial membrane protein 1	1.7	<0.01	1.5	<0.01	1.4	<0.01
NM_004438.1	EPHA4	EphA4	3.1	0.06	1.8	0.2	2.2	0.18
NM_001993.2	F3	Coagulation factor III (thromboplastin, tissue factor)	7.8	<0.01	19.1	<0.01	0.8	0.84
NM_012306.1	FAIM2	Fas apoptotic inhibitory molecule 2	3.2	0.02	2.5	0.05	3.0	<0.01
NM_003868.1	FGF16	Fibroblast growth factor 16	2.1	0.03	1.6	0.12	1.1	0.57
U01134.1	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	13.5	<0.01	4.3	<0.01	2.7	0.01
NM_006732.1	FOSB	FBJ murine osteosarcoma viral oncogene homolog B	7.4	<0.01	1.2	0.9	1.0	1
BG251266	FOSL1	FOS-like antigen 1	1.9	<0.01	1.4	<0.01	1.3	0.02
NM_005261.1	GEM	GTP binding protein overexpressed in skeletal muscle	1.5	0.01	1.3	0.34	1.0	0.99
U35399.1	GPR4	G protein-coupled receptor 4	1.7	0.08	1.4	0.37	1.8	0.04
AL554008	GPR56	G protein-coupled receptor 56	1.9	<0.01	1.1	0.68	1.5	0.03
U66065.1	GRB10	Growth factor receptor-bound protein 10	1.7	<0.01	1.2	0.01	1.6	<0.01
D86962.1	GRB10	Growth factor receptor-bound protein 10	1.7	<0.01	1.3	0.03	1.5	<0.01
NM_004490.1	GRB14	Growth factor receptor-bound protein 14	1.6	<0.01	1.1	0.47	1.7	<0.01
NM_021643.1	GS3955	GS3955 protein	1.9	0.09	1.8	0.08	0.8	0.58
AI761561	HK2	Hexokinase 2	3.5	<0.01	1.5	0.06	2.6	<0.01
NM_000872.2	HTR7	5-Hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled)	2.6	0.1	2.1	0.14	1.7	0.49
AA284705	ICAM1	Intercellular adhesion molecule 1 (CD54), Human rhinovirus receptor	1.8	0.07	2.0	0.03	1.5	0.31
NM_000201.1	ICAM1	Intercellular adhesion molecule 1 (CD54), Human rhinovirus receptor	1.8	<0.01	2.6	<0.01	1.1	0.22
AI608725	ICAM1	Intercellular adhesion molecule 1 (CD54), Human rhinovirus receptor	1.7	<0.01	1.8	<0.01	1.0	0.86
M31159.1	IGFBP3	Insulin-like growth factor binding protein 3	5.5	<0.01	2.5	<0.01	0.8	0.65
NM_000641.1	IL11	Interleukin 11	3.4	<0.01	2.2	0.04	1.7	0.22
BF112057	IL-17RC	Interleukin 17 receptor C	4.5	0.06	2.3	0.44	2.1	0.5
NM_000600.1	IL6	Interleukin 6 (interferon, beta 2)	1.7	<0.01	3.1	<0.01	1.1	0.59
AF043337.1	IL8	Interleukin 8	2.3	<0.01	3.2	<0.01	0.9	0.68
NM_000584.1	IL8	Interleukin 8	1.9	<0.01	2.1	0.02	1.0	0.88
NM_002192.1	INHBA	Inhibin, beta A (activin A, activin AB alpha polypeptide)	1.6	0.04	2.2	0.04	1.0	0.94
NM_002201.2	ISG20	Interferon-stimulated gene 20 k Da	6.8	0.03	3.3	0.35	1.9	0.65
U88964	ISG20	Interferon-stimulated gene 20 k Da	1.9	<0.01	1.2	0.47	1.4	0.18
NM_002203.2	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	1.9	<0.01	1.5	0.14	1.7	<0.01
AV733308	ITGA6	integrin, alpha 6	1.6	<0.01	1.3	0.11	1.1	0.41
AA215854	ITGB1	Integrin, beta 1 (Fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	2.6	0.04	3.3	0.04	2.2	0.11
NM_002214.1	ITGB8	Integrin, beta 8	4.1	<0.01	2.6	0.04	2.8	<0.01
BC002630.1	ITGB8	Integrin, beta 8	1.7	0.08	0.9	0.82	1.1	0.86
L38019.1	ITPR1	Inositol 1,4,5-triphosphate receptor, type 1	4.5	0.04	4.9	<0.01	2.4	0.35
NM_002224.1	ITPR3	Inositol 1,4,5-triphosphate receptor, type 3	1.7	0.03	0.9	0.64	1.1	0.57
AL137000	KIAA0970	KIAA0970 protein	1.7	0.05	1.9	<0.01	1.1	0.81
AF119835.1	KITLG	KIT ligand	4.3	<0.01	2.2	0.1	1.4	0.53
NM_000899.1	KITLG	KIT ligand	2.8	<0.01	1.8	0.01	1.3	0.15
NM_012302.1	LPHH1	Latrophilin 1	2.5	<0.01	2.1	<0.01	1.7	<0.01
NM_017522.1	LRP8	Low-density lipoprotein receptor-related protein 8, apolipoprotein e receptor	1.9	0.02	1.0	0.97	1.4	0.42
AA780381	MAP2K3	Mitogen-activated protein kinase kinase 3	5.9	<0.01	3.5	<0.01	3.2	<0.01
AA780381	MAP2K3	Mitogen-activated protein kinase kinase 3	2.6	<0.01	1.6	<0.01	1.5	<0.01
NM_002756.1	MAP2K3	Mitogen-activated protein kinase kinase 3	2.5	<0.01	1.5	0.02	1.5	<0.01
NM_005204.1	MAP3K8	Mitogen-activated protein kinase kinase kinase 8	2.4	0.03	3.8	<0.01	0.9	0.73
NM_004759.1	MAPKAPK2	Mitogen-activated protein kinase activated protein kinase 2	1.5	<0.01	1.4	<0.01	1.0	0.7

NM_000381.1	MID1	Midline 1 (Opitz/BBB syndrome)	1.5	<0.01	1.7	<0.01	1.2	0.3
AL545921	MPHOSPH10	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)	1.5	0.02	1.5	0.02	1.1	0.44
NM_002467.1	MYC	V-myc myelocytomatosis viral oncogene homolog (avian)	1.5	<0.01	1.0	0.67	1.2	0.07
BF337329	NAB2	NGFI-A binding protein 2 (EGR1 binding protein 2)	10.0	<0.01	7.4	<0.01	5.1	0.01
U08015.1	NFATC1	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	1.7	<0.01	1.1	0.19	0.9	0.58
AW027545	NFATC1	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	1.7	0.07	1.2	0.57	0.9	0.73
M55643.1	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	1.5	0.01	1.3	0.23	1.1	0.26
NM_006170.1	NOL1	Nucleolar protein1, 120 k Da	1.6	<0.01	1.1	0.59	1.3	<0.01
D21262.1	NOLC1	Nucleolar and coiled-body phosphoprotein 1	2.3	<0.01	1.4	0.01	1.9	<0.01
NM_000271.1	NPC1	Niemann–Pick disease, type C1	1.6	<0.01	1.0	0.76	1.3	0.05
D49728.1	NR4A1	Nuclear receptor subfamily 4, group A, member 1	7.9	<0.01	8.1	<0.01	1.6	0.79
NM_002135.1	NR4A1	Nuclear receptor subfamily 4, group A, member 1	6.6	<0.01	8.5	0.03	1.0	0.98
S77154.1	NR4A2	Nuclear receptor subfamily 4, group A, member 2	3.6	<0.01	2.6	0.02	0.8	0.63
NM_006186.1	NR4A2	Nuclear receptor subfamily 4, group A, member 2	2.4	0.01	2.3	0.06	1.0	0.95
U12767.1	NR4A3	Nuclear receptor subfamily 4, group A,member 3	3.1	<0.01	3.5	0.26	1.3	0.84
AF146343.1	NR5A2	Nuclear receptor subfamily 5, group A, member 2	4.3	<0.01	4.2	<0.01	2.6	<0.01
AF228413.1	NR5A2	Nuclear receptor subfamily 5, group A, member 2	3.2	<0.01	2.6	0.09	2.4	0.07
NM_003822.1	NR5A2	Nuclear receptor subfamily 5, group A, member 2	2.4	0.09	2.2	0.11	1.9	0.18
NM_005010.1	NRCAM	Neuronal cell adhesion molecule	1.9	<0.01	1.7	<0.01	1.7	<0.01
NM_013960.1	NRG1	Neuregulin 1	1.5	0.08	1.0	0.93	1.2	0.59
NM_012377.1	OR7C2	Olfactory receptor, family 7, subfamily C, member 2	5.4	0.04	1.5	0.58	1.3	0.42
X03795.1	PDGFA	Platelet-derived growth factor alpha polypeptide	4.1	0.03	1.4	0.61	1.7	0.44
NM_002607.1	PDGFA	Platelet-derived growth factor alpha polypeptide	3.0	<0.01	1.1	0.44	1.8	<0.01
NM_007169.1	PEMT	Phosphatidylethanolamine *N*-methyltransferase	1.7	0.1	1.3	0.67	1.5	0.4
AA576961	PHLDA1	Pleckstrin homology-like domain, family A, member 1	1.7	<0.01	1.3	0.17	1.1	0.56
NM_006875.1	PIM2	pim-2 oncogene	1.7	0.02	1.6	<0.01	1.0	0.85
NM_002658.1	PLAU	Plasminogen activator, urokinase	1.8	<0.01	1.3	0.05	0.7	<0.01
K03226.1	PLAU	Plasminogen activator, urokinase	1.7	<0.01	1.1	0.53	0.6	<0.01
AY029180.1	PLAUR	Plasminogen activator, urokinase receptor	7.8	<0.01	3.3	0.03	2.8	0.03
U08839.1	PLAUR	Plasminogen activator, urokinase receptor	3.3	<0.01	1.7	<0.01	1.6	<0.01
NM_025179.1	PLXNA2	Plexin A2	1.8	0.01	1.7	<0.01	1.3	0.17
AI688418	PLXNA2	Plexin A2	1.6	<0.01	1.4	<0.01	1.2	<0.01
NM_002674.1	PMCH	Pro-melanin-concentrating hormone	17.0	<0.01	5.6	<0.01	2.1	0.47
L03203.1	PMP22	Peripheral myelin protein 22	2.8	<0.01	2.0	<0.01	1.6	<0.01
NM_003967.1	PNR	Putative neurotransmitter receptor	3.5	0.1	1.1	0.97	0.4	0.58
BC000535.1	PPAN	Peter pan homolog (Drosophila)	2.3	0.08	1.3	0.49	1.3	0.49
AF014403.1	PPAP2A	Phosphatidic acid phosphatase type 2A	1.8	<0.01	2.8	<0.01	1.1	0.34
AB000888.1	PPAP2A	Phosphatidic acid phosphatase type 2A	1.8	<0.01	2.5	<0.01	1.1	0.62
AB000889.1	PPAP2B	Phosphatidic acid phosphatase type 2B	6.3	<0.01	4.8	<0.01	1.7	<0.01
AL576654	PPAP2B	Phosphatidic acid phosphatase type 2B	4.4	<0.01	3.5	<0.01	1.5	<0.01
BC005961.1	PTHLH	Parathyroid hormone-like hormone	13.2	<0.01	2.8	0.07	6.0	<0.01
NM_002820.1	PTHLH	Parathyroid hormone-like hormone	7.8	<0.01	1.7	0.28	2.9	<0.01
J03580.1	PTHLH	Parathyroid hormone-like hormone	3.6	0.01	1.0	0.96	1.7	0.32
NM_002849.1	PTPRR	Protein tyrosine phosphatase, receptor type, R	2.3	0.05	1.4	0.63	1.7	0.41
BE615277	PVR	Poliovirus receptor	1.5	<0.01	1.3	0.29	1.1	0.59
NM_000319.1	PXR1	Peroxisome receptor 1	1.5	0.05	1.2	0.34	1.2	0.2
AI817041	RDC1	G protein-coupled receptor	1.7	<0.01	1.5	0.12	0.9	0.45
NM_002923.1	RGS2	Regulator of G-protein signalling 2, 24 k Da	4.5	<0.01	2.1	<0.01	1.5	<0.01
BF062629	RIS1	Ras-induced senescence 1	7.6	<0.01	1.8	0.26	2.8	0.02
NM_002575.1	SERPINB2	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	3.5	<0.01	1.5	0.13	2.5	<0.01
NM_003012.2	SFRP1	Secreted frizzled-related protein 1	2.0	0.02	1.2	0.51	1.3	0.37
AF017987.1	SFRP1	Secreted frizzled-related protein 1	1.7	0.05	1.1	0.8	1.0	0.84
NM_005627.1	SGK	Serum/glucocorticoid regulated kinase	2.0	<0.01	2.1	<0.01	1.2	0.24
AF153330.1	SLC19A2	Solute carrier family 19 (thiamine transporter), member 2	1.7	0.01	1.3	0.11	1.4	0.09
NM_005415.2	SLC20A1	Solute carrier family 20 (phosphate transporter), member 1	2.8	<0.01	1.4	<0.01	1.9	<0.01

Table 2 (Continued)

Sequence derived from	*Gene symbol*	*Gene description*	*Ratio of HGF+VEGF basal*	P-*value*	*Ratio of VEGF basal*	P-*value*	*Ratio of HGF basal*	P-*value*
AW452623	SLC7A1	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	2.1	<0.01	2.1	0.01	1.2	0.51
NM_003045.1	SLC7A1	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	2.0	0.02	1.9	0.08	0.8	0.37
NM_014720.1	SLK	Ste20-related serine/threonine kinase	1.5	0.04	1.3	0.08	1.1	0.36
AB004903.1	SOCS2	Suppressor of cytokine signaling 2	2.3	0.05	1.4	0.55	1.7	0.29
NM_003877.1	SOCS2	Suppressor of cytokine signaling 2	1.8	0.06	1.1	0.74	0.9	0.77
NM_003155.1	STC1	Stanniocalcin 1	30.9	<0.01	7.8	<0.01	1.4	0.44
AI300520	STC1	Stanniocalcin1	12.9	<0.01	3.6	0.03	0.8	0.47
U46768.1	STC1	Stanniocalcin 1	11.0	<0.01	4.3	<0.01	1.1	0.87
NM_005990.1	STK10	Serine/threonine kinase 10	1.9	0.01	1.9	0.08	1.1	0.68
BE550452	SYN47	Homer, neuronal immediate early gene, 1B	2.6	0.09	2.5	0.12	2.5	0.07
NM_015727.1	TACR1	Tachykinin receptor 1	3.4	0.1	2.7	0.21	3.4	0.05
J04152	TACSTD2	Tumor-associated calcium signal transducer 2	1.9	<0.01	1.1	0.5	1.1	0.55
D38081	TBXA2R	Thromboxane A2 receptor	1.5	0.04	0.9	0.55	1.3	0.1
NM_030751.1	TCF8	Transcription factor 8 (represses interleukin 2 expression)	1.9	0.02	1.5	0.15	1.6	0.14
NM_000361.1	THBD	Thrombomodulin	4.9	<0.01	3.4	<0.01	1.4	0.04
NM_000361.1	THBD	Thrombomodulin	4.9	<0.01	3.1	0.06	1.3	0.7
NM_003254.1	TIMP1	Tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)	1.7	<0.01	1.2	0.1	1.4	<0.01
NM_003823.1	TNFRSF6B	Tumor necrosis factor receptor superfamily, member 6b, decoy	1.6	<0.01	0.9	0.77	1.3	0.21
NM_005118.1	TNFSF15	Tumor necrosis factor (ligand) superfamily, member 15	2.2	<0.01	1.6	0.16	1.3	0.5
NM_016179.1	TRPC4	Transient receptor potential cation channel, subfamily C, member 4	2.6	0.09	2.5	0.09	3.2	<0.01
AF001294.1	TSSC3	Tumor suppressing subtransferable candidate 3	1.6	<0.01	1.0	0.84	1.5	0.04
U58111.1	VEGFC	Vascular endothelial growth factor C	1.7	<0.01	1.4	0.01	1.3	0.11
AI983115	WSX1	Class I cytokine receptor	3.8	0.02	1.9	0.43	3.1	0.06
NM_004843.1	WSX1	Class I cytokine receptor	2.2	0.09	1.0	0.94	1.4	0.44
NM_005283.1	XCR1	Chemokine (C motif) receptor 1	3.2	0.03	2.8	0.27	3.2	0.15
NM_016164.2	XLKD1	Extracellular link domain containing 1	2.2	0.06	2.8	0.07	0.6	0.58
U87460.1		Putative endothelin receptor type B-like protein [Homo sapiens], mRNA sequence	5.1	<0.01	4.2	<0.01	2.4	0.19
NM_004367.1		Chemokine (C–C motif) receptor 6; chemokine (C–C) receptor 6; G proteincoupled receptor 29; seven-transmembrane receptor, lymphocyte, 22; chemokine receptor-like 3 [Homo sapiens], mRNA sequence	2.3	0.09	1.7	0.3	1.4	0.6
AA058828		Soluble vascular endothelial cell growth factor receptor (A49636)	1.7	0.03	2.6	0.01	1.5	0.02

Table 3 Genes significantly upregulated (>1.5-fold, $P<0.1$) by the combination of HGF and VEGF at 24 h

Sequence derived from	*Gene symbol*	*Description*	*Ratio of HGF–VEGF/basal*	P-*value*	*Ratio of VEGF/basal*	P-*value*	*Ratio of HGF/basal*	P-*value*
NM_000014.3	A2M	Alpha-2-macroglobulin	28.2	<0.01	19.5	<0.01	2.5	0.29
NM_000675.2	ADORA2A	Adenosine A2a receptor	2.2	<0.01	1.5	0.16	1.3	0.29
NM_001621.2	AHR	Aryl hydrocarbon receptor	1.7	<0.01	1.3	0.07	1.3	<0.01
AF187858.1	ANGPT2	Angiopoietin 2	5.9	<0.01	3.8	<0.01	1.9	<0.01
NM_001147.1	ANGPT2	Angiopoietin 2	6.0	<0.01	4.1	<0.01	1.7	0.05
AF007150.1	ANGPTL2	Angiopoietin-like 2	1.9	<0.01	1.9	<0.01	1.1	0.33
NM_012098.1	ANGPTL2	Angiopoietin-like 2	2.2	0.04	1.6	0.23	1.4	0.45
AF007150.1	ANGPTL2	Angiopoietin-like 2	1.6	<0.01	1.4	0.11	1.0	0.79
NM_006305.1	ANP32A	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	1.5	0.01	1.0	0.87	1.3	0.15
AF149794.1	APAF1	Apoptotic protease activating factor	1.5	<0.01	1.0	0.86	1.0	0.93
AB000815.1	ARNTL	Aryl hydrocarbon receptor nuclear translocator-like	1.6	0.06	1.3	0.72	1.6	0.35
NM_006716.1	ASK	Activator of S phase kinase	1.8	0.03	1.4	0.18	1.1	0.79
AI735391	BIKE	BMP-2-inducible kinase	2.4	0.08	2.1	0.09	1.8	0.29
AB028869.1	BIRC5	Baculoviral IAP repeat-containing 5 (survivin)	1.8	<0.01	1.3	0.14	1.2	0.11
AA648913	BIRC5	Baculoviral IAP repeat-containing 5 (survivin)	2.6	<0.01	1.9	0.08	1.5	0.16
NM_001168.1	BIRC5	Baculoviral IAP repeat-containing 5 (survivin)	2.2	<0.01	1.7	<0.01	1.3	0.17
NM_016098.1	BRP44L	Brain protein 44-like	1.8	<0.01	1.5	0.01	1.1	0.22
AF043294.2	BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	2.3	<0.01	1.9	<0.01	1.3	0.17
NM_001211.2	BUB1B	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	2.0	<0.01	1.7	<0.01	1.2	0.21
W72082	C1QR1	Complement component 1 q subcomponent receptor 1	1.5	0.08	1.3	0.32	1.1	0.72
AF098158.1	C20orf1	Chromosome 20 open reading frame 1	2.1	<0.01	1.6	0.04	1.3	0.2
U17473.1	CALCRL	Calcitonin receptor-like	1.8	<0.01	1.1	0.56	1.4	<0.01
NM_005795.1	CALCRL	Calcitonin receptor-like	1.5	<0.01	1.0	0.87	1.3	0.05
N25325	CALM1	Calmodulin 1 (phosphorylase kinase delta)	1.6	<0.01	1.3	0.15	1.0	0.96
BF439983	CASP8	Caspase 8 apoptosis-related cysteine protease	1.9	0.06	1.8	0.04	1.6	0.24
NM_004166.1	CCL14	Chemokine (C–C motif) ligand 14	2.6	<0.01	1.5	0.03	1.9	<0.01
NM_006273.2	CCL7	Chemokine (C–C motif) ligand 7	3.2	0.08	1.0	0.99	1.0	1
AF112857.1	CCNE2	Cyclin E2	2.2	<0.01	1.6	0.08	1.0	0.88
U17105.1	CCNF	Cyclin F	2.8	0.01	1.4	0.48	1.0	0.97
AF064103.1	CDC14A	CDC14 cell division cycle 14 homolog a (*S. cerevisiae*)	2.5	<0.01	1.9	0.13	1.8	0.19
AF064103.1	CDC14A	CDC14 cell division cycle 14 homolog a (*S. cerevisiae*)	3.5	<0.01	2.5	0.16	1.6	0.55
NM_003672.1	CDC14A	CDC14 cell division cycle 14 homolog A (*S. cerevisiae*)	2.2	<0.01	1.7	0.02	1.1	0.75
AL524035	CDC2	Cell division cycle 2 G1 to S and G2 to M	2.5	<0.01	2.0	<0.01	1.2	0.07
NM_001786.1	CDC2	Cell division cycle 2 G1 to S and G2 to M	2.5	<0.01	1.9	<0.01	1.2	0.26
D88357.1	CDC2	Cell division cycle 2 G1 to S and G2 to M	3.0	<0.01	2.1	<0.01	1.3	0.32
NM_001255.1	CDC20	CDC20 cell division cycle 20 homolog (*S. cerevisiae*)	1.9	<0.01	1.5	0.04	1.5	0.06
AI343459	CDC25A	Cell division cycle 25A	3.4	<0.01	3.1	0.1	1.3	0.68
NM_021873.1	CDC25B	Cell division cycle 25B	1.6	<0.01	1.5	0.01	1.1	0.39
NM_001790.2	CDC25C	Cell division cycle 25C	1.6	<0.01	1.4	0.07	1.1	0.59
NM_003504.1	CDC45L	CDC45 cell division cycle 45-like (*S. cerevisiae*)	2.1	<0.01	2.1	0.02	1.0	0.94
U77949.1	CDC6	CDC6 cell division cycle 6 homolog (*S. cerevisiae*)	2.0	<0.01	1.6	0.14	0.9	0.55
NM_001254.1	CDC6	CDC6 cell division cycle 6 homolog (*S. cerevisiae*)	1.9	<0.01	1.8	<0.01	1.0	0.9
AB012305.1	CDK2	Cyclin-dependent kinase 2	1.9	<0.01	1.3	0.45	1.3	0.51
U17074.1	CDKN2C	Cyclin-dependent kinase inhibitor 2C (p18 inhibits CDK4)	2.6	0.08	1.8	0.58	1.9	0.32
NM_001262.1	CDKN2C	Cyclin-dependent kinase inhibitor 2C (p18 inhibits CDK4)	1.8	0.01	1.4	0.18	1.3	0.42
AF213033.1	CDKN3	Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	1.6	0.03	1.3	0.3	1.3	0.25
U30872.1	CENPF	Centromere protein F, 350/400 ka (mitosin)	1.6	<0.01	1.3	0.06	1.3	0.08
NM_005196.1	CENPF	Centromere protein F, 350/400 ka (mitosin)	1.9	<0.01	1.5	0.03	1.2	0.28
AF041461.1	CFLAR	CASP8 and FADD-like apoptosis regulator	1.7	0.01	0.9	0.51	1.1	0.49
NM_003879.1	CFLAR	CASP8 and FADD-like apoptosis regulator	1.5	<0.01	1.0	0.98	1.0	0.83

Table 3 (Continued)

Sequence derived from	*Gene symbol*	*Description*	*Ratio of HGF–VEGF/basal*	P-*value*	*Ratio of VEGF/basal*	P-*value*	*Ratio of HGF/basal*	P-*value*
AF009619.1	CFLAR	CASP8 and FADD-like apoptosis regulator	1.5	0.02	0.9	0.66	1.0	0.88
X06130.1	CHC1	Chromosome condensation 1	1.7	0.01	1.0	0.9	1.2	0.45
NM_001826.1	CKS1B	CDC28 protein kinase regulatory subunit 1B	1.9	<0.01	1.5	<0.01	1.3	0.14
AF053640.1	CSE1L	CSE1 chromosome segregation 1-like (yeast)	1.7	<0.01	1.5	0.01	1.2	0.14
AF053641.1	CSE1L	CSE1 chromosome segregation 1-like (yeast)	1.5	<0.01	1.3	0.05	1.1	0.26
NM_022646.1	CSH2	Chorionic somatomammotropin hormone 2	3.3	0.09	1.7	0.63	1.6	0.64
AJ224869	CXCR4	Chemokine (C–X–C motif) receptor 4	3.1	<0.01	2.7	<0.01	1.4	<0.01
L01639.1	CXCR4	Chemokine (C–X–C motif) receptor 4	4.5	<0.01	3.2	<0.01	1.6	<0.01
AF348491.1	CXCR4	Chemokine (C–X–C motif) receptor 4	4.1	<0.01	2.8	<0.01	1.4	<0.01
U33833.1	DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, *S. cerevisiae*)	2.0	<0.01	1.4	0.42	0.7	0.65
NM_004399.1	DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, *S. cerevisiae*)	1.5	0.04	1.9	0.03	1.0	0.73
R60068	DDX3	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3	1.5	<0.01	0.9	0.73	1.3	0.09
NM_001930.2	DHPS	Deoxyhypusine synthase	1.6	0.05	1.8	0.13	1.4	0.3
NM_007309.1	DIAPH2	Diaphanous homolog 2 (Drosophila)	2.0	<0.01	2.3	<0.01	1.1	0.47
NM_006729.1	DIAPH2	Diaphanous homolog 2 (Drosophila)	1.9	<0.01	2.3	<0.01	1.0	0.83
AJ010395	DKC1	Dyskeratosis congenita 1, dyskerin	1.6	0.1	1.3	0.48	1.2	0.64
BC003541.1	DOK4	Docking protein 4	1.5	0.08	1.8	0.11	1.4	0.32
NM_012145.1	DTYMK	Deoxythymidylate kinase (thymidylate kinase)	2.5	0.03	2.2	0.13	1.3	0.5
NM_001394.2	DUSP4	Dual specificity phosphatase 4	3.5	<0.01	2.9	0.03	1.4	0.3
BC002671.1	DUSP4	Dual specificity phosphatase 4	3.4	<0.01	2.3	0.11	1.5	0.31
BC003143.1	DUSP6	Dual specificity phosphatase 6	1.7	<0.01	1.2	0.15	1.0	0.66
BC005047.1	DUSP6	Dual specificity phosphatase 6	2.3	<0.01	1.3	0.23	1.1	0.69
BC003143.1	DUSP6	Dual specificity phosphatase 6	1.5	<0.01	1.2	0.03	1.0	0.96
AV702405	EBP	Emopamil binding protein (sterol isomerase)	1.7	<0.01	1.3	0.15	1.2	0.14
NM_006579.1	EBP	Emopamil binding protein (sterol isomerase)	1.7	<0.01	1.5	0.01	1.1	0.59
AF061192.1	ED1	Ectodermal dysplasia 1, anhidrotic	3.6	0.08	1.9	0.59	1.5	0.76
NM_001964.1	EGR1	Early growth response 1	1.5	0.01	1.7	0.08	1.4	0.18
BF445047	EMP1	Epithelial membrane protein 1	1.7	<0.01	1.6	0.04	1.0	0.97
NM_001424.1	EMP2	Epithelial membrane protein 2	1.7	<0.01	1.6	0.02	1.1	0.72
NM_001776.1	ENTPD1	Ectonucleoside triphosphate diphosphohydrolase 1	7.1	<0.01	5.9	<0.01	2.4	0.08
U87967.1	ENTPD1	Ectonucleoside triphosphate diphosphohydrolase 1	8.1	<0.01	4.4	0.03	2.0	0.43
AV717590	ENTPD1	Ectonucleoside triphosphate diphosphohydrolase 1	2.6	<0.01	1.8	0.08	1.1	0.77
NM_007036.2	ESM1	Endothelial cell-specific molecule 1	2.1	<0.01	2.4	<0.01	1.0	0.43
NM_005238.1	ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	1.6	0.01	1.1	0.82	1.1	0.76
NM_004629.1	FANCG	Fanconi anemia, complementation group G	1.7	<0.01	1.7	<0.01	1.2	0.24
NM_004117.1	FKBP5	FK506 binding protein 5	6.9	<0.01	3.3	0.07	3.7	0.03
U01134.1	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	2.6	0.01	0.9	0.85	1.1	0.76
NM_021953.1	FOXM1	Forkhead box M1	3.0	<0.01	2.2	<0.01	1.3	0.34
NM_002039.1	GAB1	GRB2-associated binding protein 1	1.6	0.02	1.3	0.45	1.0	1
NM_022560.1	GH1	Growth hormone 1	3.0	0.1	3.5	0.03	3.5	0.1
NM_022559.1	GH1	Growth hormone 1	4.1	0.1	2.7	0.22	2.8	0.31
NM_022561.1	GH1	Growth hormone 1	4.6	0.04	2.8	0.36	1.1	0.83
NM_015895.1	GMNN	Geminin DNA replication inhibitor	1.8	<0.01	1.8	<0.01	1.0	0.81
NM_006572.1	GNA13	Guanine nucleotide binding protein (G protein) alpha 13	2.6	<0.01	1.3	0.55	2.1	<0.01
AB018301.1	GPR116	G protein-coupled receptor 116	3.3	<0.01	5.4	<0.01	1.6	0.35
AB018301.1	GPR116	G protein-coupled receptor 116	1.9	0.1	2.2	0.07	1.2	0.59
NM_005282.1	GPR4	G protein-coupled receptor 4	2.4	<0.01	3.1	0.03	0.9	0.82

U35399.1	GPR4	G protein-coupled receptor 4	2.0	0.01	1.8	0.17	1.0	0.98
NM_005308.1	GPRK5	G protein-coupled receptor kinase 5	1.9	<0.01	1.3	0.35	1.3	0.31
W93728	GUCY1B3	Guanylate cyclase 1 soluble, beta 3	2.8	<0.01	1.4	0.26	1.4	0.13
AF020340.1	GUCY1B3	Guanylate cyclase 1 soluble, beta 3	2.4	<0.01	1.3	0.34	1.1	0.77
NM_006101.1	HEC	Highly expressed in cancer, rich in leucine heptad repeats	2.2	<0.01	1.9	0.05	1.4	0.18
NM_018063.1	HELLS	Helicase lymphoid-specific	4.1	<0.01	3.0	0.09	0.8	0.83
NM_012485.1	HMMR	Hyaluronan-mediated motility receptor (RHAMM)	2.2	<0.01	1.8	0.01	1.5	0.1
U29343.1	HMMR	Hyaluronan-mediated motility receptor (RHAMM)	1.9	<0.01	1.4	0.02	1.3	0.14
NM_002158.1		HTLF human T-cell leukemia virus enhancer factor	1.9	0.06	1.7	0.22	1.6	0.36
BE964655	HUMGT198 A	GT198 complete ORF	2.1	0.02	2.7	0.04	1.1	0.84
NM_001552.1	IGFBP4	Insulin-like growth factor binding protein 4	2.1	<0.01	1.9	<0.01	1.2	0.45
NM_002189.1	IL15RA	Interleukin 15 receptor alpha	1.7	0.03	1.9	0.06	1.1	0.76
NM_002183.1	IL3RA	Interleukin 3 receptor alpha (low affinity)	6.8	0.06	8.7	<0.01	1.1	0.95
M96651.1	IL5RA	Interleukin 5 receptor alpha	2.8	0.01	2.5	0.04	1.3	0.6
NM_002184.1	IL6ST	Interleukin 6 signal transducer (gp130 oncostatin M receptor)	2.1	<0.01	1.3	0.42	1.5	<0.01
BE300521	INSIG1	Insulin-induced gene 1	1.6	<0.01	1.0	0.87	1.2	0.16
BE300521	INSIG1	Insulin-induced gene 1	1.6	0.02	0.8	0.63	1.3	0.28
NM_005542.1	INSIG1	Insulin-induced gene 1	1.5	0.02	0.9	0.8	1.1	0.68
AI247494	IRS3L	Insulin receptor substrate 3-like	2.0	0.01	1.8	<0.01	1.3	0.19
AV733308	ITGA6	Integrin alpha 6	4.0	<0.01	2.2	<0.01	1.3	<0.01
NM_000210.1	ITGA6	Integrin alpha 6	2.6	<0.01	2.1	<0.01	1.2	0.21
NM_014288.1	ITGB3BP	Integrin beta 3 binding protein (beta3-endonexin)	1.5	<0.01	1.4	0.07	1.1	0.37
AF002256.1	KIR2DL4	Killer cell immunoglobulinlike receptor two domains, long cytoplasmic tail 4	1.7	<0.01	1.4	0.41	1.5	0.23
AF119835.1	KITLG	KIT ligand	3.9	0.02	2.8	0.06	2.9	0.02
NM_004523.2	KNSL1	Kinesin-like 1	2.4	<0.01	1.7	<0.01	1.3	0.1
AC002301	KNSL4	Kinesin-like 4	2.2	<0.01	1.4	0.43	1.3	0.36
AY026505.1	KNSL6	Kinesin-like 6 (mitotic centromere-associated kinesin)	1.8	<0.01	1.3	0.11	1.2	0.24
U63743.1	KNSL6	Kinesin-like 6 (mitotic centromere-associated kinesin)	1.8	<0.01	1.5	<0.01	1.1	0.39
NM_020242.1	KNSL7	Kinesin-like 7	2.0	0.02	1.7	0.17	1.1	0.73
NM_004690.2	LATS1	LATS large tumor suppressor homolog 1 (Drosophila)	1.5	0.03	1.4	0.11	1.3	0.51
S70123.1	LDLR	Low-density lipoprotein receptor (familial hypercholesterolemia)	2.3	<0.01	1.1	0.82	1.2	0.38
AI861942	LDLR	Low-density lipoprotein receptor (familial hypercholesterolemia)	2.1	0.02	1.1	0.9	1.1	0.73
NM_013296.1	LGN	LGN protein	5.8	<0.01	3.3	0.18	2.7	0.3
W05463	LOC51275	Apoptosis-related protein PNAS-1	1.5	0.02	1.3	0.27	1.1	0.62
NM_017522.1	LRP8	Low-density lipoprotein receptor-related protein 8, apolipoprotein e receptor	1.7	0.02	1.3	0.29	1.0	0.83
NM_002349.1	LY75	Lymphocyte antigen 75	3.7	<0.01	3.8	<0.01	1.5	0.56
NM_002358.2	MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	2.0	<0.01	1.6	<0.01	1.2	0.21
NM_005903.1	MADH5	MAD mothers against decapentaplegic homolog 5 (Drosophila)	1.7	0.09	1.6	0.32	1.4	0.54
AA780381	MAP2K3	Mitogen-activated protein kinase kinase 3	1.7	0.04	3.4	0.02	0.9	0.84
Z25432.1	MAPK14	Mitogen-activated protein kinase 14	1.6	0.06	0.8	0.65	1.3	0.43
NM_021960.1	MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	1.6	<0.01	1.0	0.9	1.3	0.1
NM_004526.1	MCM2	MCM2 minichromosome maintenance deficient 2, mitotin (*S. cerevisiae*)	1.7	<0.01	1.6	<0.01	1.0	0.9
AA807529	MCM5	MCM5 minichromosome maintenance deficient 5 cell division cycle 46 (*S. cerevisiae*)	2.0	<0.01	2.3	<0.01	1.1	0.65
NM_006739.1	MCM5	MCM5 minichromosome maintenance deficient 5 cell division cycle 46 (*S. cerevisiae*)	2.1	<0.01	1.7	0.1	1.0	0.83
NM_002389.1	MCP	Membrane cofactor protein (CD46 trophoblastlymphocyte cross-reactive antigen)	1.7	<0.01	1.1	0.74	1.2	0.01
D84105.1	MCP	Membrane cofactor protein (CD46 trophoblastlymphocyte cross-reactive antigen)	1.8	<0.01	1.0	0.98	1.2	0.11
NM_014791.1	MELK	Maternal embryonic leucine zipper kinase	1.8	<0.01	1.8	<0.01	1.0	0.79
NM_005924.1	MEOX2	Mesenchyme homeo box 2 (growth arrest-specific homeo box)	3.3	<0.01	2.1	0.23	1.6	0.47
BG170541	MET	Met proto-oncogene (hepatocyte growth factor receptor)	1.7	<0.01	1.4	0.27	1.3	0.25
X54559.1	MET	Met proto-oncogene (hepatocyte growth factor receptor)	1.6	<0.01	1.0	0.84	1.2	0.39
BC005043.1	MGC31957	Hypothetical protein MGC31957	2.2	0.02	1.3	0.57	1.4	0.22

Table 3 (Continued)

Sequence derived from	*Gene symbol*	*Description*	*Ratio of HGF–VEGF/basal*	P-*value*	*Ratio of VEGF/basal*	P-*value*	*Ratio of HGF/basal*	P-*value*
BF001806	MKI67	Antigen identified by monoclonal antibody Ki-67	2.1	<0.01	1.7	0.01	1.0	0.76
Z69744	MLL	Myeloid/lymphoid or mixedlineage leukemia (trithorax homolog Drosophila)	1.7	<0.01	1.2	0.44	1.4	0.03
NM_016195.1	MPHOSPH1	M-phase phosphoprotein 1	1.9	0.01	1.8	0.2	1.2	0.58
NM_006540.1	NCOA2	Nuclear receptor coactivator 2	2.0	0.05	2.0	0.3	2.1	0.01
NM_002497.1	NEK2	NIMA (never in mitosis gene a)-related kinase 2	1.6	0.08	1.5	0.16	1.3	0.44
Z25425.1	NEK2	NIMA (never in mitosis gene a)-related kinase 2	1.7	0.03	1.3	0.53	1.3	0.54
Z25434.1	NEK3	NIMA (never in mitosis gene a)-related kinase 3	1.7	0.01	1.3	0.34	1.3	0.3
NM_007361.1	NID2	Nidogen 2 (osteonidogen)	2.1	<0.01	2.4	<0.01	1.0	0.83
AF146343.1	NR5A2	Nuclear receptor subfamily 5, group a member 2	3.2	0.08	3.1	0.06	1.7	0.27
AF228413.1	NR5A2	Nuclear receptor subfamily 5, group a member 2	4.3	<0.01	3.0	0.07	1.4	0.62
NM_003822.1	NR5A2	Nuclear receptor subfamily 5, group a member 2	1.9	0.08	1.5	0.38	1.1	0.84
AF145712.1	NRP1	Neuropilin 1	2.0	<0.01	1.1	0.77	1.5	0.09
NM_003999.1	OSMR	Oncostatin M receptor	1.7	0.07	1.3	0.49	1.2	0.59
NM_005746.1	PBEF	Pre-B-cell colony-enhancing factor	1.6	<0.01	1.2	0.17	1.2	0.05
BF575514	PBEF	Pre-B-cell colony-enhancing factor	1.5	<0.01	1.2	0.12	1.1	0.1
BC001422.1	PGF	Placental growth factor, vascular endothelial growth factor-related protein	1.5	<0.01	1.7	<0.01	1.2	0.06
AA805318	PIK3CB	Phosphoinositide-3-kinase, catalytic beta polypeptide	1.7	0.09	1.1	0.73	1.4	0.3
NM_004203.1	PKMYT1	Membrane-associated tyrosine- and threoninespecific cdc2-inhibitory kinase	1.7	0.03	1.7	0.1	1.0	0.97
NM_005030.1	PLK	Polo-like kinase (Drosophila)	1.6	0.02	1.3	0.21	1.1	0.82
NM_025179.1	PLXNA2	Plexin A2	1.6	0.04	0.9	0.8	1.0	0.94
NM_002674.1	PMCH	Pro-melanin-concentrating hormone	10.7	<0.01	6.6	<0.01	0.5	0.46
BC002715.1	PPARD	Peroxisome proliferative activated receptor delta	2.7	0.06	2.1	0.21	0.8	0.79
NM_003981.1	PRC1	Protein regulator of cytokinesis 1	2.4	<0.01	2.0	<0.01	1.3	0.24
AF100763.1	PRKAA1	Protein kinase AMPactivated, alpha 1 catalytic subunit	1.5	<0.01	1.0	0.98	1.0	0.76
NM_000315.1	PTH	Parathyroid hormone	1.8	0.08	1.9	0.07	2.2	0.07
AF074979.1	RGS20	Regulator of G-protein signalling 20	2.6	<0.01	2.7	<0.01	1.2	0.59
BF062629	RIS1	RAS-induced senescence 1	5.9	<0.01	10.5	<0.01	3.2	0.27
NM_003035.1	SIL	TAL1 (SCL) interrupting locus	1.5	0.03	1.5	0.05	1.0	0.99
BC001441.1	SKP2	S-phase kinase-associated protein 2 (p45)	1.7	<0.01	1.1	0.74	1.3	0.2
NM_005983.1	SKP2	S-phase kinase-associated protein 2 (p45)	3.7	0.05	1.1	0.76	0.9	0.89
NM_006444.1	SMC2L1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	1.9	<0.01	1.5	<0.01	1.2	0.11
AU154486	SMC2L1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	2.2	<0.01	1.7	0.16	1.3	0.25
BG035761	SOCS3	Suppressor of cytokine signaling 3	3.0	0.07	2.5	0.26	0.7	0.71
NM_001049.1	SSTR1	Somatostatin receptor 1	3.1	0.05	2.0	0.39	1.1	0.88
NM_003155.1	STC1	Stanniocalcin 1	22.8	<0.01	2.6	0.02	0.8	0.53
NM_005990.1	STK10	Serine/threonine kinase 10	2.3	<0.01	2.4	<0.01	1.0	0.84
AB015718	STK10	Serine/threonine kinase 10	1.5	<0.01	1.7	<0.01	1.0	0.9
AB011446.1	STK12	Serine/threonine kinase 12	1.9	<0.01	1.6	0.06	1.1	0.51
AL043646	STK18	Serine/threonine kinase 18	5.0	<0.01	4.9	<0.01	2.9	0.11
NM_014264.1	STK18	Serine/threonine kinase 18	3.0	<0.01	2.1	0.05	1.2	0.66
NM_003158.1	STK6	Serine/threonine kinase 6	1.8	<0.01	1.4	0.14	1.2	0.32
NM_003600.1	STK6	Serine/threonine kinase 6	1.7	<0.01	1.4	0.1	1.2	0.36
NM_003242.1	TGFBR2	Transforming growth factor, beta receptor II (70/80 k Da)	1.5	0.02	0.9	0.61	1.1	0.28
NM_000361.1	THBD	Thrombomodulin	2.8	<0.01	2.6	<0.01	1.1	0.78
NM_000361.1	THBD	Thrombomodulin	5.6	<0.01	5.4	<0.01	1.2	0.79
AF153687.1	TNFRSF10B	Tumor necrosis factor receptor superfamily member 10b	1.8	<0.01	0.9	0.58	1.2	0.17
AF012536.1	TNFRSF10C	Tumor necrosis factor receptor superfamily member 10c, decoy without an intracellular domain	2.2	<0.01	1.5	0.16	1.3	0.36
NM_003841.1	TNFRSF10C	Tumor necrosis factor receptor superfamily member 10c, decoy without an intracellular domain	2.1	<0.01	1.4	0.09	1.1	0.44

BF664114	TNFRSF5	Tumor necrosis factor receptor superfamily member 5	2.3	0.01	1.9	0.03	0.8	0.49
NM_001250.1	TNFRSF5	Tumor necrosis factor receptor superfamily member 5	4.7	<0.01	2.8	0.02	1.1	0.65
M15565.1	TRA	T cell receptor alpha locus	1.9	0.07	1.7	0.02	1.0	0.98
NM_005879.1	TRIP	TRAF interacting protein	1.7	0.09	2.0	0.02	1.2	0.66
NM_004237.1	TRIP13	Thyroid hormone receptor interactor 13	1.7	<0.01	1.6	0.06	1.1	0.7
NM_003318.1	TTK	TTK protein kinase	2.1	<0.01	1.5	0.08	1.3	0.19
AJ003062.1	TUBGCP3	Tubulin gamma complex-associated protein 3	1.6	0.03	1.3	0.39	1.2	0.65
NM_007019.1	UBE2C	Ubiquitin-conjugating enzyme E2C	1.7	<0.01	1.4	0.04	1.1	0.57
NM_007063.1	VRP	Vascular Rab-GAP/TBC containing	2.0	<0.01	1.7	0.01	1.2	0.12
AI983115	WSX1	Class I cytokine receptor	3.9	<0.01	2.7	0.12	2.3	0.16
NM_016164.2	XLKD1	Extracellular link domain containing 1	3.4	0.01	1.6	0.47	1.2	0.81
Z25433.1		Protein-serine/threonine kinase (*Homo sapiens*), mRNA sequence	4.5	<0.01	2.3	0.32	2.5	0.06
X07868		*Homo sapiens* cDNA:FLJ22066 fis clone HEP10611 mRNA sequence	2.9	<0.01	3.8	<0.01	1.4	0.23
AA058828		Soluble vascular endothelial cell growth factor receptor (A49636)	1.9	<0.01	1.6	0.03	1.1	0.45
AA351360		KIAA0585 protein (*Homo sapiens*) (AB011157)	2.0	<0.01	1.4	0.74	1.2	0.86

between the two growth factors on the expression of this gene. The related angiopoietin 4 is also upregulated at 4 h, and a recent report suggests this factor may also have angiogenic activities (Zhu *et al*., 2002). The VEGF receptors, neuropilin-1 and flt-1 are also upregulated. Also notable is the upregulated expression of a number of orphan nuclear receptors (NURR1/NR4A2, NR4A3, NR5A2).

Several of the genes in the HGF + VEGF subset are involved in the regulation of hematopoietic precursor cells, which may be related to the recruitment and survival of angiopoietic precursor cells to sites of ongoing angiogenesis. For example, kit ligand is able to augment the proliferation of both myeloid and lymphoid hematopoietic progenitors in bone marrow cells (Smith *et al*., 2001; Duarte & Franf, 2002; Heike & Nakahata, 2002), and can also mediate cell adhesion (Pesce *et al*., 1997; Bendall *et al*., 1998; Ashman, 1999; Mitsunari *et al*., 1999; Shimizu *et al*., 2001). Interleukin 11 stimulates the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells. (Turner *et al*., 1996; Nandurkar *et al*., 1998; Lazzari *et al*., 2001; Momose *et al*., 2002). The cellular adhesion molecules ICAM-1 and CD44 may also play a role in the recruitment of progenitor cells.

A review of the list of genes identified as upregulated at 24 h reveals a strong 'cell cycle' signature, with the upregulation of a number of genes involved in the regulation of the cell cycle, including the cyclins E2 and F, the dual specificity phosphatase CDC14A, the protein kinase CDC2, as well as other related cell cycle control proteins including CDC20, CDC25a,b, and c, CDC6, CDK2, CKS1b, and CDKN2C, and genes involved in the regulation of mitosis including BUB1 (mitotic checkpoint serine–threonine-protein kinase), mitotic spindle assembly checkpoint protein (MAD2L1), DNA replication licensing factor (MCM5), proliferating cell nuclear antigen KI67, and the kinases PLK1, STK 6,12, and 18. Related to this control of the cell cycle 'theme' there is also upregulation of genes involved in the regulation of apoptosis, including CSE1l, BIRC5 (survivin), apoptotic protease-activating factor (APAF1), and procaspase 8 (CASP8). At 24 h, there is also upregulation of the HGF receptor, c-met, and the flt-1 ligand, placental growth factor.

These data are consistent with the hypothesis that the combination of HGF and VEGF provides a strong push to move cells from quiescence into the cell cycle. At earlier time points, a number of important steps in receptor tyrosine kinase signaling and downstream activation of mitogen-activated protein kinase pathways are upregulated, as well as the mRNA for a number of important growth factors and receptors with a potential role in the proliferative response to the two growth factors. At a later time point (24 h), the sequelae of these early events are apparent with the clear signal that the cells have progressed from cell cycle arrest and are actively undergoing mitosis. These data are also consistent with previous published observations that the combination of HGF and VEGF are additive to synergistic on endothelial cell proliferation (Van Belle *et al*., 1998).

To validate the expression of some of the mRNAs identified in this study, we performed independent analysis of HGF- and VEGF-treated HUVEC mRNA (from three different endothelial isolates (i.e. different from those used for the array studies)) using real-time PCR (Taqman) analysis as previously described (Kahn *et al*., 2000; Gerritsen *et al*., 2002; Yang *et al*., 2002). Data were normalized to the housekeeping gene

Table 4 Taqman validation

Gene	*Ratio of fold change/cyclophilin*	*Ratio of fold change/cyclophilin*
	4 h	24 h
ANGPT2	15.91±1.83	18.73±0.88
CXCR4	1.02±1.00	10.50±1.95
NID2	1.89±0.76	9.62±1.64
STC1	11.00±11.50	60.71±4.93

cyclophilin. As shown in Table 4, all four of the genes evaluated exhibited alterations in gene expression, consistent with the data obtained from the oligonucleotide array analysis. In addition, we measured the protein levels by ELISA of one of the more highly regulated, and 'synergistic' genes, that is, the secreted protein STC1. At 24 h, the levels of STC-1 in basal, HGF- or VEGF-treated cell supernatants were below the level of detection ($<0.02\,ng\,ml^{-1}$). However, when cells were treated with the combination of HGF and VEGF, STC-1 levels were markedly increased ($2.8\pm0.2\,ng\,ml^{-1}$).

Discussion

Using rigorous and tightly controlled experimental conditions, tightly controlled biological replicates, and multiple comparisons, gene expression profiling using the Affymetrix oligonucleotide technology combined with software analysis packages can yield reliable, highly validated results. For example, using a similar approach, Gerritsen and co-workers identified over 1000 differentially expressed genes as regulated in a three-dimensional collagen gel model of endothelial differentiation (Gerritsen *et al*., 2002; 2003). Several hundred of these genes were selected for further evaluation by an independent method (RT–PCR, Taqman) and greater than 95% of the genes identified were shown to be regulated in a manner suggested by the results from the oligonucleotide array technology.

In the present study, we have identified discrete subsets of genes that were upregulated by HGF, VEGF, and the combination of the two growth factors. Examination of the list of genes upregulated by VEGF provided further confirmation of the method, since many of the genes identified (e.g. DUSP6, stanniocalcin, CXCR4, FGF16, angiopoeitin 2, Flt-1, Nurr44, nidogen2, melanin concentrating hormone, stanniocalcin-1) have been reported in earlier studies by Yang *et al*., Bell and co-workers, Abe and others (Abe & Sato, 2001; Bell *et al*., 2001; Yang *et al*., 2002). Similarly, although the literature on HGF-induced endothelial cell gene expression is limited; upregulated expression of ets-1, CD44, thymosin B4, and downregulated expression of occludin 1 has been previously noted (Jiang *et al*., 1999; Oh *et al*., 2002; Recio & Merling, 2003; Tomita *et al*., 2003). We found that probe sets corresponding to these same genes were also identified as 'significantly' regulated. The present study shows for the first time, the additive to synergistic interactions of HGF with VEGF on endothelial gene expression, the differential effects of HGF *versus* VEGF on endothelial cell gene expression, and moreover, provides the first large-scale gene expression analysis of HGF-induced gene expression in endothelial cells.

There are known differences in the actions of HGF *versus* VEGF. For example, in addition to eliciting endothelial proliferation, VEGF also induces increased vascular permeability *in vivo*, and increased expression of fenestrae *in vitro*. HGF does not demonstrate these activities. HGF was originally called 'scatter factor' based on its ability to include scattering of polarized epithelial cells, an activity not shared by VEGF. The effects of VEGF are primarily restricted to endothelial cells, due to the limited expression of the receptors KDR and flt-1 (although the expression of flt-1 has been described on cells of monocytic lineage and on smooth muscle cells). In contrast, the c-met receptor is expressed in epithelial cells, various tumor cells, keratinocytes, smooth muscle cells, hepatocytes, and endothelial cells.

This study clearly demonstrates that HGF and VEGF signal through independent pathways in endothelial cells. Since both HGF and VEGF are upregulated at sites of pathological angiogenesis (e.g. tumors, rheumatoid arthritis, diabetic retinopathy), this raises the possibility that successful anti-angiogenic therapies may require antagonism of multiple pathways of angiogenic growth factor signaling. These observations also suggest that further evaluation of growth factor combinations in 'therapeutic angiogenesis' indications should be encouraged.

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