Reversible transdifferentiation of blood vascular endothelial cells to a lymphatic-like phenotype in vitro

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Accepted 16 July 2010
Journal of Cell Science 123, 3808-3816
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doi:10.1242/jcs.064279

Summary
Blood vascular cells and lymphatic endothelial cells (BECs and LECs, respectively) form two separate vascular systems and are functionally distinct cell types or lineages with characteristic gene expression profiles. Interconversion between these cell types has not been reported. Here, we show that in conventional in vitro angiogenesis assays, human BECs of fetal or adult origin show altered gene expression that is indicative of transition to a lymphatic-like phenotype. This change occurs in BECs undergoing tubulogenesis in fibrin, collagen or Matrigel assays, but is independent of tube formation per se, because it is not inhibited by a metalloproteinase inhibitor that blocks tubulogenesis. It is also reversible, since cells removed from 3D tubules revert to a BEC expression profile upon monolayer culture. Induction of the lymphatic-like phenotype is partially inhibited by co-culture of HUVECs with perivascular cells. These data reveal an unexpected plasticity in endothelial phenotype, which is regulated by contact with the ECM environment and/or cues from supporting cells.

Key words: Angiogenesis, Endothelial cell, Blood vascular, Lymphatic, Prox-1, LYVE-1, Pericyte

Introduction
Higher animals possess two separate circulatory networks, the blood and lymphatic vascular systems, which have distinct but interdependent functions. In the developing mammalian embryo, the lymphatic vasculature appears after the formation of the blood vasculature and is believed to be of venous origin. In fact, blood vascular cells and lymphatic endothelial cells (BECs and LECs, respectively) share a common progenitor (Jain and Padera, 2003; Kubo and Alitalo, 2003). A subset of cells in the embryonic cardinal vein transdifferentiate and take on a lymphatic identity via the stepwise expression of lymphatic regulatory genes (Oliver and Harvey, 2002; Wigle et al., 2002). The Sox18 transcription factor specifies target cells in which expression of the Prox-1 transcription factor is induced (Francois et al., 2008), which, in turn, commits cells to differentiation into the lymphatic lineage such that this subset of cells gives rise to the entire lymphatic vascular network. This view is supported by data from mouse knockout studies, which show that the absence of Prox-1 expression leads to a failure to differentiate into the lymphatic endothelial cell lineage (Johnson et al., 2008). Once the two endothelial cell lineages are established, BECs and LECs are considered to be mature, non-interchangeable cell types that display characteristic profiles of gene expression (Hirakawa et al., 2003; Kriehuber et al., 2001; Podgrabinska et al., 2002).

In this study, we have identified an unexpected plasticity in the phenotype of human endothelial cells when analysed in several conventional in vitro models of angiogenesis. We show that blood vascular endothelial cells are able to adopt an expression profile resembling that of lymphatic endothelial cells in response to particular in vitro ECM environments, and this behaviour is reversible and independent of tubulogenesis. These studies shed new light on the environmental cues that regulate endothelial cell differentiation and lineage maintenance.

Results
Human umbilical vein endothelial cells adopt a ‘lymphatic’ gene expression profile when cultured in 3D matrices
Endothelial cells form tube-like structures when cultured in 3D matrix environments and stimulated with pro-angiogenic growth factors (Fig. 1A–C). We performed a microarray analysis to compare gene expression in human umbilical vein endothelial cells (HUVECs) undergoing tube formation in a 3D fibrin matrix (Fig. 1B) with monolayer cultures (Fig. 1A). This analysis identified 169 genes that were upregulated and 554 genes that were downregulated during tubulogenesis (supplementary material Table S1). Unexpectedly, we observed altered expression of a number of genes that had been previously identified to be mainly or exclusively expressed in either BECs or LECs (referred to henceforth as BEC or LEC markers). A number of marker genes were examined further using quantitative real-time RT-PCR (qRT-PCR). These included the LEC markers: lymphatic vascular endothelial hyaluronan receptor (LYVE1) (Banerji et al., 1999); PROX1 (Hong et al., 2002; Wigle and Oliver, 1999); vascular endothelial growth factor-3 (FTL4) (Adams and Alitalo, 2007; Kaipainen et al., 1995); integrin α9 (ITGA9) (Huang et al., 2002; Mishima et al., 2007) and podoplanin (PDPN) (Hirakawa et al., 2003; Kriehuber et al., 2001; Petrova et al., 2002; Schacht et al., 2003). The BEC markers selected were: Lamin-B1 (LMNB1) (Podgrabinska et al., 2002), VEGFC (Hirakawa et al., 2003; Kriehuber et al., 2001; Makinen et al., 2001) and CD44 (Cao et al., 2006; Hirakawa et al., 2003). Expression profiles of the markers were assessed in HUVECs undergoing tubulogenesis in either type I collagen or fibrin 3D
angiogenesis assays, compared with HUVECs in standard monolayer culture conditions on plates coated with type I collagen. In both types of 3D matrix environment, the LEC markers LYVE1, PROX1 and FLT4 were upregulated, and the BEC markers LMNB1, VEGFC and CD44 were downregulated compared with monolayer culture (Fig. 1D–G). Another LEC marker, ITGA9, was upregulated in 3D collagen cultures, whereas no significant changes in expression were detected in 3D fibrin gels (data not shown). In contrast to the other LEC markers, expression of PDPN was not detected in either assay. The mRNA expression data were supported by the appearance of LYVE-1 protein consistently throughout the entire tubular network in HUVECs undergoing tube formation in 3D collagen gels, whereas only a few isolated cells were weakly positive for LYVE-1 in HUVECs cultured as a monolayer (Fig. 1H and supplementary material Fig. S1). We also observed VEGFR3 and nuclear Prox-1 staining in HUVECs that formed tubes (supplementary material Figs S2 and S5), and detected downregulation of Lamin-B1 by western blot (supplementary material Fig. S3). These data demonstrate that mature blood vascular endothelial cells alter their gene expression profiles upon culture in 3D matrices, where they transdifferentiate to a ‘lymphatic-like’ phenotype.
Transdifferentiation is not restricted to endothelial cells of fetal origin

We tested whether endothelial cells derived from other tissue sources showed gene expression changes in 3D culture. Committed adult human aortic endothelial cells (HAECs) showed similar changes in BEC or LEC marker gene expression during tubulogenesis in type I collagen as those observed in HUVECs (Fig. 2A,B). Once again, expression of the LEC marker podoplanin was not induced. Similar data were obtained with adult human dermal microvascular endothelial cells (HDMECs; data not shown), with the exception that podoplanin was expressed by these cells. However, data from these cells must be considered with caution because HDMEC preparations are known to contain variable proportions of LECs and BECs (Makiné et al., 2001). The finding that mature adult blood vascular endothelial cells are capable of adopting a lymphatic-like gene expression profile indicates that this plasticity is a property of many endothelial cell types.

Adoption of a ‘lymphatic-like’ phenotype is not dependent on a 3D environment

Induction of the lymphatic-like phenotype occurred in 3D culture in both matrix types tested, but not in cells in monolayer. We therefore investigated whether a 3D environment is essential for this process using the 2D Matrigel assay. In this assay, endothelial cells seeded on top of Matrigel migrated and aligned to form a network of cord-like structures within 24 hours (Fig. 2C). Analysis of expression of BEC and LEC marker genes showed that, similarly to the 3D tubulogenesis assays, HUVECs had upregulated expression of mRNA encoding LYVE-1, Prox-1, VEGFR3 (and integrin α9, data not shown) paralleled by downregulation of mRNA encoding lamin-B1, VEGF-C and CD44 over the course of network formation (Fig. 2D–E). Comparison of BEC and LEC marker expression in HUVECs cultured within 3D Matrigel versus cells seeded on Matrigel revealed similar patterns; indeed, induction of Prox-1 was strongest in cells forming alignments on top of this matrix. These data suggest that a 3D environment per se is not...
obligatory for induction of the ‘lymphatic’ phenotype, but the nature of the ECM is critically important.

Based on the results obtained when endothelial cells were grown on Matrigel, we next asked whether similar results could be obtained with cells grown in monolayer on top of collagen or fibrin gels. We observed that when seeded on top of collagen gels, HUVECs formed alignments in a manner comparable with cells seeded on Matrigel (Fig. 3A). By contrast, cells seeded on fibrin gels retained a cobblestone appearance resembling that of cells in standard culture (Fig. 3B,C). Expression analysis showed that cells seeded on collagen gels adopted the ‘LEC’ profile of gene expression, whereas cells seeded on fibrin retained the same profile as the standard 2D cells (Fig. 3D,E). The ‘LEC’ phenotype was induced more effectively when the cells were cultured in 3D collagen rather than on top of the same gel matrix (data not shown). These data provide confirmation that a 3D environment is not essential for the BEC to LEC transition, but they emphasise that the nature of the matrix is critical, because although monolayer growth on a dense collagen gel is permissive for the phenotypic change, growth on fibrin is not.

Adoption of a ‘lymphatic-like’ phenotype is independent of tubulogenesis and is reversible

We asked whether the ‘LEC’ phenotype was functionally linked with tubulogenesis by analysing marker expression in HUVECs cultured in 3D collagen in the presence of the broad-spectrum matrix metalloproteinase inhibitor BB-94 (Fig. 4A–D). This inhibitor blocks tube formation by inhibition of key enzymes required for ECM digestion and remodelling, such as MT1-MMP (Hiraoka et al., 1998; Lafleur et al., 2002). We observed that upregulation of expression of LEC markers and downregulation of BEC markers was largely unaffected in cultures in which tubulogenesis was inhibited, although a reduction in the level of FLT4 induction was detected. These data show that HUVECs undergo the transition to a ‘lymphatic’ phenotype when cultured in conditions conducive to tube formation, even when the tubulogenesis is inhibited.

We also tested whether induction of the ‘LEC’ expression profile might be reversed after transfer of the cells from a 3D environment back to monolayer culture. HUVECs were suspended within 3D collagen gels for 24 hours and then the gels were digested with collagenase and the cells returned to monolayer cultures and maintained for a further 48 hours. As previously observed, 3D culture led to induction of expression of the LEC markers LYVE1, PROX1 and FLT4, and the downregulation of the BEC markers LMNB1 and VEGFC. Following replating in monolayer culture, expression of each of these markers demonstrated full reversibility, returning to levels that were comparable with those observed in control monolayer samples at the assay end point (Fig. 4E,F). These data demonstrate that the pattern of BEC and LEC marker gene expression is directly regulated by the extracellular environment of the cells.

Adoption of the ‘lymphatic-like’ phenotype is partially inhibited by co-culture with perivascular cells

Blood vessels in vivo have an extensive basal lamina and are in close contact with smooth muscle cells or pericytes, whereas
lymphatic vessels have little basement membrane and largely lack pericyte coverage (Pepper and Skobe, 2003). We hypothesised that the culture of isolated BECs in direct contact with the ECM in the absence of perivascular cells might create a microenvironment more similar to that associated with lymphatic vessels and thus encourage the cells to take on LEC-type behaviour; conversely, co-culture of HUVECs with perivascular cells might favour the maintenance of the ‘BEC’ phenotype. We therefore compared

Fig. 4. Change in LEC and BEC marker gene profile is not dependent on tube formation, and is reversible upon transfer of endothelial cells from a 3D to a monolayer environment. (A–D) Effect of BB94 on LEC-BEC gene expression changes in HUVECs cultured within collagen gels. HUVECs were grown as previously and BB94 (BB94, 5 mM) or vehicle control (CON) were added where indicated both to the culture medium, and in the gel in the 3D cultures, at the beginning of the experiment. Images (×20 magnification) show cells in the presence of vehicle control (A) or 5 μM BB94 (B). The gels were incubated for 2 days and RNA was analysed by qRT-PCR. Expression levels were determined for (C) LEC markers LYVE1, PROX1 and FLT4 and (D) BEC markers LMNB1 and VEGFC. Each data point is the average of triplicates ± s.e.m. Three-way ANOVA was used to determine alterations of expression level according to time point, 2D or 3D culture, and presence of BB94 vs vehicle control. For all genes, expression differed significantly according to 2D or 3D culture (P<0.001), but FLT4 is the only gene for which expression varied according to presence or absence of BB94 (P=0.01). This variation occurred only at the 48 hour time point (P=0.002). (E,F) BEC and LEC expression transitions are reversible and dependent on environment. TaqMan RT-PCR on HUVECs cultured either within 3D collagen matrices (3D) or on 2D collagen coated surfaces (2D) for 2 days in standard growth medium. HUVECs were then released from the gels with collagenase or trypsinised to release them from their 2D substrate and each culture replated separately onto collagen-coated dishes and grown as a monolayer for a further 2 days. Total RNA was analysed as previously for (E) LEC markers LYVE1, PROX1 and FLT4 and (F) BEC markers LMNB1 and VEGFC. Each data point is the average of triplicate samples ± s.e.m.
marker gene expression levels in HUVECs cultured in 3D collagen gels either as monocultures or co-cultured with murine perivascular cells (Brachvogel et al., 2005; Brachvogel et al., 2007). By using species-specific qRT-PCR probes we were able to determine the expression levels in the human cells only. HUVECs cultured in the presence of perivascular cells showed markedly reduced expression of mRNA encoding LYVE-1 and Prox-1 compared with monocultures, whereas VEGFC mRNA expression increased (Fig. 5A,B). These results demonstrate a reversal of the expression of several BEC and LEC marker genes compared with the pattern seen upon monoculture in 3D matrices, such that induction of the ‘lymphatic’ phenotype was at least partially inhibited. LYVE-1 protein was clearly expressed throughout the tubular networks in HUVEC monocultures, and strongly downregulated in HUVEC and perivascular cell co-cultures (Fig. 5A,B; supplementary material Fig. S4). However, not all the BEC and LEC markers followed the same pattern, because co-culture with perivascular cells resulted in increased expression of FLT4 (Fig. 5B; supplementary material Fig. S5), and decreased expression of LMNB1 (Fig. 5B) compared with levels in HUVEC monocultures, suggesting that these genes respond to the matrix environment rather than the presence of pericytes. Interestingly, we saw a similar overall trend in the effects on BEC and LEC marker expression (namely, decreased LYVE1 and increased VEGFC and FLT4) when we used siRNA to reduce induction of PROX1 expression in endothelial cells seeded on Matrigel (supplementary material Fig. S6).

Discussion

The culture of endothelial cells within or on top of 3D matrices such as type I collagen, fibrin and Matrigel provides simple, popular models of angiogenesis (Auerbach et al., 2003; Goodwin, 2007; Phung and Dass, 2006; Ucuzian and Greisler, 2007). The present work reports that when blood vascular endothelial cells such as HUVECs and HAECs are cultured under such conditions, they show gene expression changes that are characteristic of a shift to a lymphatic phenotype. The lymphatic-like phenotype was independent of tubulogenesis and fully reversible upon transfer of cells from 3D to monolayer culture on collagen-coated tissue culture plastic, suggesting that interactions between endothelial cells and matrix account for the alterations in gene expression observed. This was further reinforced by the observation that HUVECs adopt the lymphatic-like phenotype when cultured on top of Matrigel or a dense collagen gel, but not when grown on top of a fibrin gel. Also, co-culture of HUVECs with pericytes partially

Fig. 5. Co-culture of HUVECs with pericytes partially suppresses induction of the LEC phenotype. (A) HUVECs were cultured in 3D type I collagen gels either as monocultures (top panel) or co-cultured with murine perivascular cells (lower panel) in a 5:1 (HUVEC to PVC ratio) in the presence of VEGF (10 ng/ml) and PDGF-BB (10 ng/ml). After 72 hours, cultures were fixed and stained for LYVE-1 (green), the endothelial cell marker CD31 (red), and counterstained with DAPI. Negative controls are shown in supplementary material Fig. S4. Scale bars: 200 \( \mu \text{m} \) (top), and 100 \( \mu \text{m} \) (bottom). (B,C) RNA was extracted and pooled from quadruplicate samples of monocultures and triplicate samples of co-cultures, from three separate experiments. Data show TaqMan analysis of LYVE1, PROX1 and FLT4 (B) and LMNB1 and VEGFC (C). Each data point is the average of nine (co-culture) or 12 (monoculture) samples ± s.e.m.
prevented the development of the LEC-like phenotype. These findings indicate that the concept of LECs and BECs as committed, differentiated lineages needs to be revised, and demonstrate the importance of contact with different extracellular matrices and perivascular cells in regulating endothelial cell phenotype and lineage specification.

Endothelial cells of different tissue origins represent different functional cell types, with characteristic gene expression profiles (Chi et al., 2003). We considered the possibility that, because HUVECs are of fetal origin, they might show increased phenotypic plasticity compared with adult endothelial cells. However, blood vascular cells of adult origin such as HAECs and HDMECs showed the same induced changes in gene expression, indicating that ability to adopt a lymphatic-like phenotype is a common property of cultured BECs. We also considered it possible that the HUVECs used in our initial experiments might contain a subset of LECs, which came to be the dominant cell type in the culture conditions we used. However, lymphatic vessels are absent from the umbilical cord, and moreover, the lack of expression of podoplanin in all our angiogenesis models using HUVECs and HAECs confirms the absence of a fully differentiated LEC subpopulation. By contrast, HDMECs that are known to contain an LEC subpopulation (Makinen et al., 2001) did indeed express podoplanin. Rather, these data, and in particular the induction of expression of Prox-1, imply an ability of BECs in 3D culture as a whole to undergo a type of transdifferentiation in which cells adopt a more lymphatic-like phenotype. But it is also clear from the absence of podoplanin expression in HUVECs undergoing 3D tubulogenesis that the cells do not undergo full transdifferentiation from a BEC to an LEC phenotype. This might imply that the cells adopt an intermediate phenotype that shows characteristics of both LECs and BECs, or it might reflect the fact that podoplanin expression is a late marker of lymphangiogenesis, which is consistent with the proposed role of podoplanin in later stages of lymphatic vascular patterning (Schacht et al., 2003). It is possible that HUVECs commence BEC-to-LEC transdifferentiation in the 3D culture models, but the process is not complete in the timeframe of the assays.

Adoption of the ‘LEC’ phenotype could be correlated with the tendency of the endothelial cells to form tube or cord structures. However, our findings show that induction of the ‘LEC’ phenotype is neither a consequence of tubulogenesis per se, nor dependent upon it. Rather, the nature of the cell’s extracellular environment determines the induction of the ‘lymphatic’ phenotype. Importantly, the phenotype is reversible upon returning cells from 3D to standard monolayer culture. This reversibility shows that the ‘LEC’ expression profile is not the result of simple culture-induced de-differentiation, a possibility that needs to be considered because cultured endothelial cells are known to lose their lineage specificity (Amatschek et al., 2007), and low-level LYVE-1 expression has been reported in late-passage HUVECs (Banerji et al., 1999).

One of the most interesting observations from the present study is that inclusion of pericytes in the HUVEC tubulogenesis cultures blocked the changes in expression of key LEC and BEC markers such as LYVE-1, Prox-1 and VEGF-C. This indicates that signals provided by perivascular supporting cells help to reinforce the BEC phenotype. It is interesting that co-culture of HUVECs with perivascular cells resulted in a largely similar effect on marker expression to that observed in cells following knockdown of Prox-1. This suggests that signals elicited by interactions between endothelial cells and pericytes act via Prox-1 to regulate endothelial cell lineage specification. The converse might also apply, namely that Prox-1 expression in endothelial cells influences endothelial cell to pericyte communication. Supporting this notion, ectopic expression of Prox-1 in isolated blood vascular endothelial cells has been shown to convert the transcriptional programme of the cells toward the lymphatic phenotype by upregulating expression of LEC-specific marker genes, as well as downregulating expression of a large number of BEC-specific transcripts (Mishima et al., 2007; Petrova et al., 2002). Furthermore, constitutive Prox-1 expression is required for maintenance of LEC lineage specificity because conditional inactivation of Prox-1 in mice resulted in loss of phenotypic identity in lymphatic vessels and adoption of blood vessel characteristics, including abnormal coverage by pericytes (Johnson et al., 2008).

An unexpected result of both co-culture of HUVECs with pericytes and knockdown of Prox-1 was the marked induction of VEGFR3 expression. This is surprising given that VEGFR3 is directly regulated by Prox-1 (Hong et al., 2002; Mishima et al., 2007; Petrova et al., 2002). However, VEGFR3 is expressed in the absence of Prox-1 before lymphatic vascular formation (Kaipainen et al., 1995) and is re-expressed in blood vascular endothelium in some conditions (Tammela et al., 2008; Valtola et al., 1999). Furthermore, in our system, in both Prox1 siRNA-treated samples and under co-culture conditions, expression of the VEGFR3 ligand VEGF-C was increased relative to controls, which could create an autocrine stimulatory circuit.

Recently, the concept of endothelial plasticity, and lymphatic endothelial plasticity in particular, is receiving increasing attention (Bixel and Adams, 2008). Our findings add a novel and unexpected aspect to this discussion because we show that normal, committed blood vascular cells have the capacity to undergo aspects of a BEC to LEC transition in response to their ECM environment or the presence of perivascular supporting cells. The effects that we have observed on the expression profiles of endothelial cells grown in specific matrix environments strongly indicate a role for adhesion molecules, particularly integrins, in the control of the endothelial cell phenotype. Mechanotransduction of forces applied to cells by ECM environments of differing stiffness is known to have a profound impact on the specification of cell identity (Butcher et al., 2009), and it will thus be interesting to explore the effects of matrix density on the BEC and LEC expression profiles.

The question arises as to whether the artificial in vitro ECM conditions that we have studied relate to environments that EC experience in vivo. The 3D fibrin assay models an early phase of capillary formation in vivo, when the parent vessel dilates and extravasated fibrinogen is converted to fibrin, forming a provisional scaffold for assembly of the new vessel (Senger, 1996). The type I collagen model is more representative of later stages when EC in vivo migrate away from the parent vessel invading collagen-rich tissue stroma. By contrast, Matrigel might reflect the complex matrix environments of pathologies such as cancer and thus be relevant to the sprouting of tumour neovessels. The BEC-LEC plasticity that we have seen might be reflected in other recent observations from both in vitro and in vivo systems. For instance, expression of lymphatic marker genes in BEC has been seen in vitro in response to treatment with inflammatory cytokines such as interleukin-3 (Groger et al., 2004) or lysophosphatidic acid (Lin et al., 2008). Another recent paper described the presence of a subset of dermal blood capillaries expressing LYVE-1 and podoplanin in chronically inflamed skin (Groger et al., 2007). Likewise, the LEC marker VEGFR3 is seen on tumour neovessels and its inhibition attenuates blood vessel formation (Tammela et al., 2008).
In conclusion, our study has revealed an unexpected plasticity in the phenotype of endothelial cells when cultured in ‘traditional’, well-established in vitro angiogenesis assays, which suggests that these types of monoculture assays might in fact represent processes involved in lymphangiogenesis, rather than blood vessel formation.

Materials and Methods

ECM components, angiogenic factors, and other reagents
Plasminogen-depleted fibrinogen was obtained from Calbiochem (Merck). Growth-factor-depleted Matrigel (BD Biosciences) and high concentration rat-tail type-I collagen solutions were purchased from BD Biosciences. Calf skin type-I collagen for coating tissue culture surfaces was purchased from Sigma. VEGF and basic FGF were purchased from PeproTech EC. Antibodies used were a polyclonal goat anti-human LYVE-1 [R&D Systems (AF2089)], polyclonal rabbit anti-human antibodies to VEGFR3 (Abcam, ab27728), Prox-1 (Abcam, ab19411 and Lamin-B1 (Abcam ab6048), and a mouse anti-human PECAM/CD31 (555444, BD Pharrmingen). Polyclonal anti-GAPDH was from Cell Signaling Technology (Danvers, MA). Secondary antibodies included a FITC-conjugated polyclonal donkey-anti goat secondary antibody (Abcam), a goat anti-mouse Alexa-Fluor-488-conjugated secondary antibody (Invitrogen Technologies). Alexa Fluor-488 (1:2000 dilution) was from Invitrogen (Carlsbad, CA). Antibodies used were a polyclonal goat anti-human LYVE-1 (Abcam, ab27728), Prox-1 (Abcam, ab19411 and Lamin-B1 (Abcam ab6048), and a mouse anti-human PECAM/CD31 (555444, BD Pharrmingen). Polyclonal anti-GAPDH was from Cell Signaling Technology (Danvers, MA). Secondary antibodies included a FITC-conjugated polyclonal donkey-anti goat secondary antibody (Abcam), a goat anti-mouse Alexa-Fluor-488-conjugated secondary antibody (Invitrogen Technologies). Alexa Fluor-488 (1:2000 dilution) was from Invitrogen (Carlsbad, CA).

Cells and cell culture

Primary human umbilical vein endothelial cells (HUVECs), primary adult human aortic endothelial cells (HAECs), and adult human dermal microvascular endothelial cells (HDMECs) were obtained from TCS Cellworks, except for the gene array studies for which the HUVECs were purchased from Clonetics. Cells were grown in 2D cultures on plastic coated with 0.1% gelatin and 0.1% fibronectin (Sigma). Tissue culture flasks and plates were coated with 0.1% gelatin and 0.1% fibronectin (Sigma) before addition of endothelial cell culture medium (ECM). HUVECs, HAECs, and HDMECs were cultured in serum-containing endothelial cell culture medium (ECM), supplemented with 25 ng/ml VEGF and FGF-2.

Cell isolation

Murine MII perivascular cells have been described previously (Brachvogel et al., 2005). The cells were identified from mice carrying a targeted insertion in the Alox15 gene and were purified from heterozygous mice carrying the Alox5-lacZ fusion gene (Brachvogel et al., 2005; Brachvogel et al., 2007). The cells were originally identified from mice carrying a targeted insertion in Alox15 gene and were purified from heterozygous mice carrying the Alox5-lacZ fusion gene (Brachvogel et al., 2005; Brachvogel et al., 2007). The cells were routinely maintained on tissue culture plastic in Dulbecco’s Modified Eagle’s Medium (high glucose with GlutaMAX, Invitrogen), supplemented with 10% fetal bovine serum at 37°C and 5% (v/v) CO2. Pericytes were used between passages 35 and 40.

Gene expression analysis

Microarray analysis of differential gene expression during HUVEC tubulogenesis

HUVECs were embedded in 3D fibrin matrices and cultured as described above. For 2D cultures, 2.5×10^5 HUVECs were seeded on top of 1 ml fibrin gels (prepared as described above). For 3D cultures, a mixture of four 300 μl gels were pooled and homogenised per sample. The cDNA for gene array analysis was prepared from 20 μg of total RNA. For each sample, two cDNA preparations were made and coupled to either Cy3 or Cy5 dyes (Amersham Biosciences, Little Chalfont, UK) in 0.1 M Na2CO3, pH 9.0 for 1 hour at room temperature. The coupling reaction was then quenched with 1 M hydroxylamine for 15 minutes and the labelled cDNA purified with Amicon Microcon-30 concentrators. Quantification of labelled cDNA was performed with an ND-1000 nanodrop spectrophotometer with readings at A260/A280. 25 pmol of dye labelled cDNA was used for each microarray probe.

Human 19K glass slide ‘oligo arrays’ were obtained from the Australian Genome Research Facility (AGRF, Parkville, Australia). Slides were first blocked in 0.1% SDS at 95°C for 1 hour, followed by a 1 minute wash in 5% ethanol and a further 1 minute wash in distilled H2O, and then dried. Slides were then rehydrated in 0.1% SDS at 37°C for 10 minutes by washing in 0.2× SSC, 0.2% SDS, for 10 minutes in 0.1× SSC, and then for 10 minutes in 0.2× SSC, and glass slides were then dried by centrifugation at 500 g for 3 minutes and scanned with a GenePix 4000B scanner (Molecular Dynamics, Amersham). Gene array images were analysed using the GenePix Pro 4.0 software. Local background correction was performed and data normalised according to the median of ratios. A gene was considered differentially regulated if the ratio between sample pairs A and B was >1.5 or <0.67.

Quantitative analysis

RNA extraction

Total RNA was extracted from endothelial cells using High Pure RNA extraction kit (Roche). RNAeasy mini columns (Qiagen), or TRIzol reagent (Life Technologies), all as per manufacturer’s instructions and quantified using an ND-1000 spectrophotometer (Nanodrop Technologies). 0.1–1 μg total RNA per sample was reverse transcribed to cDNA using random hexamers (Amersham Pharmacia), Superscript II reverse transcriptase (Life Technologies), dNTP mix (Roche) and RNase inhibitor (Promega) as per manufacturer’s instructions.

Protein expression analysis by western blotting

Monolayer cultures were harvested by rinsing cells and scraping into 1 ml of PBS. Cells were harvested from Matrigel by digestion using Dispase, as described above. To obtain sufficient protein for western blotting, cells harvested from ten separate 100 mm dishes were combined into one sample. Total protein was extracted by centrifugation and pellets were resuspended in lysis buffer (10 mM Tris-HCl, pH 7.6, 10 mM NaCl, 3 mM MgCl2, 1% NP40, supplemented with Roche Complete Mini EDTA-free protease inhibitor tablets. Samples were left on ice for 1 hour with occasional mixing. Cellular debris was pelleted by centrifugation, and the protein content of the soluble fraction was measured using a BCA protein assay kit according to the manufacturer’s instructions (Thermo Scientific, Rockford, IL). For western blotting, samples separated by SDS−PAGE on a 10% resolving gel were transferred to PVDF (polyvinylidene difluoride) membranes (Bio-Rad). Protein bands were detected by incubation with the appropriate primary antibody followed by horseshadish peroxidase (HRP)-conjugated secondary antibody (DAKO).

Microarray and qPCR analysis

qPCR procedures were carried out as described previously (Nuttall et al., 2004). The primer and probe sequences used are listed in supplementary Table S2.

Cell counting

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added into the samples incubated in blocking buffer overnight at 4°C and then washed else with TBS-T (TBS, 0.1% Tween 20). Corresponding secondary antibodies conjugated with Cy2 or Cy3 (Jackson Immunoresearch) were incubated in blocking buffer overnight at 4°C and washed as described above. For secondary antibody control, there were no primary antibodies added before incubation with same secondary antibodies. After nuclei staining, samples were mounted in Gelvatol and examined by fluorescence microscopy (StReto LumarV12 and Axiosplan2, Carl Zeiss, Germany). Pictures were captured with Axiovision software (version 4.5). Antibodies used in double staining procedures have been tested for minimal cross reactivity and spectral overlap.

siRNA
Two pre-designed siRNA duplexes targeting PROX1 and a negative control siRNA duplex were purchased from Ambion (UK). HUVECs were seeded in collagen-coated six-well plates and transfected the following day with the various siRNAs at a concentration of 50 nM. After 1 h of incubation at 37°C, the medium was replaced with fresh medium containing 2% FBS. Cells were grown for a further 24 h. The following day, the medium was replaced with fresh medium containing 2% FBS, and cells were cultured for a further 24 hours at 37°C and 5% (v/v) CO2. Samples were harvested as described above at the indicated time points.

Statistical analyses
TaqMan expression data were analysed using one-, two- or three-way ANOVA tests as described in the text. P<0.05 was considered significant.

This work was supported by funding from the Biotechnology and Biological Sciences Research Council (BBBSC), the Big C, Norfolk Fundraisers and the EU Framework Programme 6 Cancerdegradation Project LSCH-CT-2003-503297. L.S.C. was a recipient of a BBaRC PhD studentship. Z.Z. and E.P. were supported by the British Heart Foundation, Project PG/06/071/21115T. E.W.T. and M.A.L. were supported by the Victorian Breast Cancer Research Consortium, Australia.

Supplementary material available online at http://jcs.biologists.org/cgi/content/full/123/1/3808/DC1

References


The figure shows a gel electrophoresis analysis comparing 2D and 3D conditions. The molecular weight markers are indicated in kDa:

- LaminB1: 75 kDa
- GAPDH: 37 kDa
Lyve-1/CD-31/DAPI

Object 10

Object 20

Object 40
Prox-1

LYVE-1

VEGFR3

LaminB1

VEGF-C

% relative to control

T0 4hr 24hr

% relative to CTR

T0 4hr 24hr

% relative to CTR

T0 4hr 24hr

% relative to CTR

T0 4hr 24hr

% relative to CTR

T0 4hr 24hr
Table S1. Genes downregulated during HUVEC tubulogenesis in 3D fibrin matrices compared with monolayer culture in more than two of eight experiments.

Genes in *italics* were identified in one of eight experiments, and are more likely to represent false positives. Genes whose expression patterns were confirmed using TaqMan RT-PCR are highlighted in yellow.

**Growth factors, cytokines, hormones, chemokines, ligands and their receptors**

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<th>Gene ID</th>
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16D10; Homo sapiens chemokine (C-C motif) receptor 9 (CCR9), mRNA; NM_006641; Hs.225946; chemotaxis [0006935]
20B20; Homo sapiens platelet-derived growth factor alpha polypeptide (PDGFA), mRNA; NM_002607; Hs.37040; cell proliferation [0008283]

20F18; Homo sapiens vascular endothelial growth factor C (VEGFC) mRNA; NM_005429; Hs.79141; positive control of cell proliferation [0008284]

1018; Homo sapiens angiopoietin 2 (ANGPT2) mRNA; NM_001147; Hs.115181; cell growth and/or maintenance [0008151]

**Signal transduction molecules**

24P16; Homo sapiens AXL receptor tyrosine kinase (AXL) mRNA; NM_001699; Hs.83341; signal transduction [0007165]

25P18; Homo sapiens Leman coiled-coil protein (LCCP), mRNA; NM_016201; Hs.92186; signal transduction [0007165]

9P19; Homo sapiens serum/glucocorticoid regulated kinase (SGK) mRNA; NM_005627; Hs.296323; protein amino acid phosphorylation [0006468]

26I11; Homo sapiens hypothetical protein FLJ20185 (FLJ20185), mRNA; NM_017701; Hs.272972; signal transduction [0007165] aka Rho GTPase activating protein 8 (ARHGAP8)

24H12; Homo sapiens RAN binding protein 1 (RANBP1), mRNA; NM_002882; Hs.24763; signal transduction [0007165]

9J19; Homo sapiens KIAA0175 gene product (KIAA0175), mRNA; NM_014791; Hs.184339; protein amino acid phosphorylation [0006468] aka maternal embryonic leucine zipper kinase (MELK)

24N1; Homo sapiens protein kinase, cAMP-dependent, catalytic, beta (PRKACB), mRNA; NM_002731; Hs.87773; signal transduction [0007165]

24E2; Homo sapiens PDZ-binding kinase; T-cell originated protein kinase (TOPK), mRNA; NM_018492; Hs.104741; signal transduction [0007165]

26L10; Homo sapiens regulator of G-protein signalling 5 (RGS5) mRNA; NM_003617; Hs.24950; regulation of G-protein coupled receptor protein signaling pathway [0008277]

24L24; Homo sapiens growth factor receptor-bound protein 14 (GRB14), mRNA; NM_004490; Hs.83070; signal transduction [0007165]

26H6; Homo sapiens A kinase (PRKA) anchor protein (gravin) 12 (AKAP12), mRNA; NM_005100; Hs.788; G-protein coupled receptor protein signaling pathway [0007186]

50P11; Homo sapiens sprouty (Drosophila) homolog 2 (SPRY2), mRNA; NM_005842; Hs.18676; histogenesis and organogenesis [0007397]

24L1; Homo sapiens Ran GTPase activating protein 1 (RANGAP1), mRNA; NM_002883; Hs.183800; signal transduction [0007165]

25I13; Homo sapiens MCF-2 cell line derived transforming sequence (MCF2), mRNA; NM_005369; Hs.89543; signal transduction [0007165]

26L14; Homo sapiens G-protein gamma-12 subunit (LOC55970), mRNA; NM_018841; Hs.118520; regulation of G-protein coupled receptor protein signaling pathway [0008277]

9L21; H.sapiens protein-serine/threonine kinase gene, complete CDS; Z25433; protein amino acid phosphorylation [0006468]

20M2; Homo sapiens Ras homolog enriched in brain 2 (RHEB2), mRNA; NM_005614; Hs.279903; GTPase [0003924]

17L16; Homo sapiens Semaphorin IV (SEMA4), mRNA; NM_004186; Hs.32981; immune response [0006955]

18H22; Homo sapiens SWAP-70 homolog mRNA, complete cds; AF134894; Hs.153026; cytoskeleton organization and biogenesis [0007010]

20A10; Homo sapiens v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1 (YES1), mRNA; NM_005433; Hs.194148; protein tyrosine kinase [0004713]
19L6: Homo sapiens protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform (PPP2CB) mRNA; NM_004156; Hs.80350; spindle assembly [0007051]

15N18: Homo sapiens ADP-ribosylation factor 4 (ARF4), mRNA; NM_001660; Hs.75290; intracellular protein transport [0006886]

11A18: Homo sapiens COP9 complex subunit 4 (LOC51138), mRNA; NM_016129; Hs.6671; ubiquitin-dependent protein degradation [0006511]

24B12: Homo sapiens Rag C mRNA, complete cds; AF272035; Hs.110950; signal transduction [0007165]

26J22: Human RGP4 mRNA, complete cds; U27768; Hs.227571; regulation of G-protein coupled receptor protein signaling pathway [0008277]

24H22: Homo sapiens hedgehog-interacting protein mRNA, complete cds; +D490; Hs.72116; signal transduction [0007165]

21I6: Homo sapiens dual specificity phosphatase 1 (DUSP1), mRNA; NM_004417; Hs.171695; cell cycle [0007049]

30F20: Homo sapiens endoglin (Osler-Rendu-Weber syndrome 1) (ENG) mRNA; NM_000118; Hs.76753; circulation [0008015]

28F15: Homo sapiens calmodulin 2 (phosphorylase kinase, delta) (CALM2) mRNA; NM_001743; Hs.182278; developmental processes [0007275]

29G15: Homo sapiens dickkopf (Xenopus laevis) homolog 1 (DKK1), mRNA; NM_012242; Hs.40499; embryogenesis and morphogenesis [0007345]

28N10: Homo sapiens dickkopf (Xenopus laevis) homolog 3 (DKK3), mRNA; NM_013253; Hs.4909; embryogenesis and morphogenesis [0007345]

28B16: Homo sapiens TRAF4 associated factor 1 mRNA, partial cds; U81002; Hs.181466; spermatogenesis [0007283]

27K22: Homo sapiens Ras-GTPase activating protein SH3 domain-binding protein 2 (KIAA0660), mRNA; NM_012297; Hs.6727; RAS protein signal transduction [0007265]

27O23: Homo sapiens tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ) mRNA; NM_003406; Hs.75103; intracellular signaling cascade [0007242]

30K17: Homo sapiens caveolin 2 (CAV2) mRNA; NM_001233; Hs.139851; muscle development [0007517]

24H4: Homo sapiens mitogen-activated protein kinase kinase 1 (MAP2K1), mRNA; NM_002755; Hs.3446; signal transduction [0007165]

18O14: Homo sapiens LIM protein (similar to rat protein kinase+D317 C-binding enigma) (LIM), mRNA; NM_006457; Hs.154103; signal transduction [0007165]

25N11: Homo sapiens cornichon-like (CNIL) mRNA; NM_005776; Hs.201673; signal transduction [0007165]

**ECM molecules and their receptors**

23I17: Homo sapiens sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) (SPOCK) mRNA; NM_004598; Hs.93029; cell adhesion [0007155]

23M23: Homo sapiens thrombospondin 1 (THBS1) mRNA; NM_003246; Hs.87409; cell adhesion [0007155]

23E24: Homo sapiens beta-netrin mRNA, complete cds; AF278532; Hs.102541; cell adhesion [0007155]

18L1: Homo sapiens human chromosome-associated polypeptide (bamacan) (HACP) mRNA; NM_005445; Hs.24485; chromosome organization and biogenesis (sensu Eukarya) [0007001] and a proteoglycan in the ECM when secreted

31A21: Homo sapiens EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1), transcript variant 1, mRNA; NM_004105; Hs.76224; vision [0007601]

23K16: Homo sapiens integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor) (ITGA2) mRNA; NM_002203; Hs.271986; cell adhesion [0007155]

23N1: Homo sapiens integrin, alpha 5 (fibronectin receptor, alpha polypeptide) (ITGA5) mRNA; NM_002205; Hs.149609; cell adhesion [0007155]
24G13; Homo sapiens integrin, alpha 6 (ITGA6) mRNA; NM_000210; Hs.227730; cell-matrix adhesion [0007160]

23D15; Human mRNA for collagen VI alpha-1 C-terminal globular domain; X15880; Hs.108885; cell adhesion [0007155]

23E21; Homo sapiens collagen, type XIII, alpha 1 (COL13A1), mRNA; NM_005203; Hs.211933; cell adhesion [0007155]

**Cell adhesion molecules/gap junctions/membrane fusion**

13O12; Homo sapiens endomucin-1 (LOC51169), mRNA; NM_016241; Hs.272558; cholesterol catabolism [0006707]

17K11; Homo sapiens fer-1 (C. elegans)-like 3 (FER1L3), mRNA; NM_013451; Hs.234680; muscle contraction [0006936]

44M19; Homo sapiens gap junction protein, alpha 5, 40kD (connexin 40) (GJA5), mRNA; NM_005266; Hs.247926; biological process unknown [0000004]

31G20; Homo sapiens partial mRNA for Claudin-11 protein (CLDN11 gene); AJ245902; biological process unknown [0000004]

23J7; Homo sapiens activated leucocyte cell adhesion molecule (ALCAM) mRNA; NM_001627; Hs.10247; cell adhesion [0007155]

23P3; Human cell adhesion molecule (CD44) mRNA, complete cds; M59040; Hs.169610; cell adhesion [0007155]

22O13; Homo sapiens poliovirus receptor (PVR), mRNA; NM_006505; Hs.321018; invasive growth [0007125]

23G14; Homo sapiens melanoma adhesion molecule (MCAM) mRNA; NM_006500; Hs.211579; cell adhesion [0007155]

18H15; Homo sapiens Friedreich ataxia region gene X104 (tight junction protein ZO-2) (ZO-2), mRNA; NM_004817; Hs.75608; intercellular junction assembly [0007043]

23N7; Homo sapiens KIAA1070 protein (KIAA1070), mRNA; NM_014932; Hs.71132; cell adhesion [0007155] aka neuroligin-1

23P21; Homo sapiens oligodendrocyte transmembrane protein (OTM) mRNA; NM_005602; Hs.153297; cell adhesion [0007155] aka claudin-11

23L8; Homo sapiens cadherin 2, N-cadherin (neuronal) (CDH2) mRNA; +D150; Hs.161; cell adhesion [0007155]

23K8; Homo sapiens mRNA for cadherin FIB1, partial cds; AB000895; cell adhesion [0007155]

14P3; Homo sapiens podocalyxin-like (PODXL) mRNA; NM_005397; Hs.16426; cation transport [0006812]

23H10; Homo sapiens hypothetical protein (FLJ20798), mRNA; NM_019055; Hs.111518; cell adhesion [0007155] aka magic roundabout

**Cytoskeleton**

22O17; Homo sapiens actinin, alpha 1 (ACTN1) mRNA; NM_001102; Hs.119000; invasive growth [0007125]

22L19; Homo sapiens catenin (cadherin-associated protein), alpha-like 1 (CTNNAL1) mRNA; NM_003798; Hs.58488; apoptosis [0006915]

19D24; Homo sapiens clone 24464 beta-tubulin mRNA, complete cds; AF070600; Hs.179661; microtubule-based process [0007017]

22C24; Homo sapiens tubulin, alpha, ubiquitous (K-ALPHA-1) mRNA; NM_006082; Hs.334842; cell shape and cell size control [0007148]

9H14; Homo sapiens protein tyrosine kinase 9 (PTK9) mRNA; NM_002822; Hs.82643; protein amino acid phosphorylation [0006468]

50P16; Homo sapiens caldesmon 1 (CALD1) mRNA; NM_004342; Hs.325474; muscle development [0007151]

23P22; Homo sapiens ras homolog gene family, member E (ARHE) mRNA; NM_005168; Hs.6838; cell adhesion [0007155]

19D5; Homo sapiens plastin 3 (T isoform) (PLS3), mRNA; NM_005032; Hs.4114; cytoskeleton organization and biogenesis [0007010]
17G19; Human nonmuscle myosin heavy chain-B (MYH10) mRNA, partial cds; M69181; muscle contraction [0006936]
19N1; Homo sapiens radixin (RDX), mRNA; NM_002906; Hs.250613; cytoskeletal anchoring [0007016]
20D2; Homo sapiens breast cancer anti-estrogen resistance 1 (BCAR1), mRNA; NM_014567; Hs.273219; cell proliferation [0008283]
19H9; Homo sapiens Arp2/3 protein complex subunit p16 (ARC16) mRNA; NM_005717; Hs.82425; structural constituent of cytoskeleton [0005200]
18D16; Homo sapiens novel retinal pigment epithelial cell protein (NORPEG) mRNA, complete cds; AF155135; Hs.15165; cytoskeleton organization and biogenesis [0007010]
19O23; Homo sapiens syndecan binding protein (syntenin) (SDCP) mRNA; NM_005625; Hs.8180; cytoskeleton organization and biogenesis [0007010]
13K12; Homo sapiens trophinin associated protein (tastin) (TROAP), mRNA; NM_005480; Hs.171955; cholesterol catabolism [0006707]
22K6; Homo sapiens vinculin (VCL), transcript variant meta-VCL, mRNA; NM_014000; Hs.75350; cell shape and cell size control [0007148]
36B14; Homo sapiens tubulin, beta, 2 (TUBB2) mRNA; NM_006088; Hs.251653; biological process unknown [0000004]
19H10; Homo sapiens tubulin, gamma 2 (TUBG2), mRNA; NM_016437; Hs.279669; microtubule cytoskeleton organization and biogenesis [0002226]
16H5; Homo sapiens myristoylated alanine-rich protein kinase C substrate (MARCKS, 80K-L) (MACS) mRNA; NM_002356; Hs.75607; cell motility [0006928]

Proteases and protease inhibitors
18G13; Homo sapiens protease inhibitor 9 (ovalbumin type) (PI9) mRNA; NM_004155; Hs.104879; acute-phase response [0006953]
10O6; Human cathepsin-L-like (CTSL2) mRNA; L25628; proteolysis and peptidolysis [0006508]
11G5; Homo sapiens cathepsin C (CTSC) mRNA; NM_001814; Hs.10029; proteolysis and peptidolysis [0006508]
17I22; Homo sapiens chitinase 1 (CHIT1) mRNA; NM_003465; Hs.91093; response to bacteria [0009617]
27C4; Homo sapiens a disintegrin and metalloproteinase domain 9 (meltrin gamma) (ADAM9) mRNA; NM_003816; Hs.2442; protein kinase cascade [0007243]
11L19; Homo sapiens disintegrin metalloproteinase with thrombospondin repeats (ADAMTS9), mRNA; NM_020249; Hs.126855; glycoprotein degradation [0006516]
15J6; Homo sapiens alpha-2-macroglobulin (A2M), mRNA; NM_000014; Hs.74561; intracellular protein transport [0006886]
10P2; Homo sapiens matrix metalloproteinase 1 (interstitial collagenase) (MMP1) mRNA; NM_002421; Hs.83169; proteolysis and peptidolysis [0006508]
18I17; Homo sapiens plasminogen activator inhibitor, type II (arginine-serpin) (PAI2) mRNA; NM_002575; Hs.75716; acute-phase response [0006953]
18I9; Human plasminogen activator inhibitor-1 (PAI-1) mRNA, complete cds; M16006; Hs.82085; acute-phase response [0006953]

Transcription factors/transcription regulation
4E18; Homo sapiens high-mobility group (nonhistone chromosomal) protein 1 (HMG1) mRNA; NM_002128; Hs.274472; DNA packaging [0006323]
4H22; Homo sapiens FOS-like antigen-1 (FOSL1), mRNA; NM_005438; Hs.283565; transcription regulation [0006355]
17G20; Homo sapiens aryl hydrocarbon receptor (AHR) mRNA; NM_001621; Hs.170087; stress response [0006950]
4N4; Homo sapiens nuclear receptor subfamily 2, group F, member 1 (NR2F1), mRNA; NM_005654; Hs.144630; transcription regulation [0006355]
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<td>Homo sapiens pleomorphic adenoma gene-like 1 (PLAGL1), splice variant 2, mRNA</td>
<td>NM_006718</td>
<td>Hs.75825</td>
<td>induction of apoptosis [0006917]</td>
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<td>17P4</td>
<td>Homo sapiens stimulated trans-acting factor (50 kDa) (STAF50) mRNA</td>
<td>NM_006074</td>
<td>Hs.318501</td>
<td>immune response [0006955]</td>
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<td>4B16</td>
<td>Homo sapiens clone L3-5 zinc finger protein mRNA, partial cds; AF024698</td>
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<td>transcription regulation [0006355]</td>
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<td>21B16</td>
<td>Homo sapiens transcription factor 19 (SC1) (TCF19), mRNA</td>
<td>NM_007109</td>
<td>Hs.249184</td>
<td>cell cycle control [0000074]</td>
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<td>21F4</td>
<td>Homo sapiens transcription factor Dp-1 (TFDP1), mRNA</td>
<td>NM_007111</td>
<td>Hs.79353</td>
<td>cell cycle control [0000074]</td>
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<td>4H11</td>
<td>Homo sapiens polymerase (RNA) II (DNA directed) polypeptide B (140kD) (POLR2B), mRNA</td>
<td>NM_009398</td>
<td>Hs.296014</td>
<td>transcription [0006350]</td>
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<td>5I8</td>
<td>Homo sapiens zinc finger protein 205 (ZNF205) mRNA, and translated products; NM_003456</td>
<td>Hs.13128</td>
<td>transcription regulation [0006355]</td>
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<td>5N17</td>
<td>Human Kox19 mRNA for zinc finger protein, partial; X52350</td>
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<td>transcription regulation [0006355]</td>
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<td>5A5</td>
<td>Homo sapiens Friend leukemia virus integration 1 (FLI1), mRNA</td>
<td>NM_002017</td>
<td>Hs.108043</td>
<td>transcription regulation [0006355]</td>
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<td>7M23</td>
<td>Homo sapiens nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) mRNA</td>
<td>NM_006164</td>
<td>Hs.155396</td>
<td>transcription, from Pol II promoter [0006366]</td>
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<td>20I17</td>
<td>Homo sapiens fusion, derived from t(12;16) malignant liposarcoma (FUS) mRNA, NM_004960</td>
<td>Hs.99969</td>
<td>RNA binding [0003723]</td>
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<td>7M12</td>
<td>Homo sapiens SKI-INTERACTING PROTEIN (SNW1), mRNA</td>
<td>NM_012245</td>
<td>Hs.79008</td>
<td>mRNA splicing [0006371]</td>
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<td>17K12</td>
<td>Homo sapiens nuclease sensitive element binding protein 1 (NSEP1), mRNA</td>
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<td>Hs.74497</td>
<td>transcription regulation [0006355]</td>
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<td>6D2</td>
<td>Homo sapiens cardiac ankyrin repeat protein (CARP), mRNA</td>
<td>NM_001255</td>
<td>Hs.31432</td>
<td>transcription regulation, from Pol II promoter [0006357]</td>
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<td>31A6</td>
<td>Homo sapiens transcription factor BMAL2 (LOC56938), mRNA</td>
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<td>circadian rhythm [0007623]</td>
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<td>17A7</td>
<td>Homo sapiens acidic 82 kDa protein mRNA</td>
<td>NM_014597</td>
<td>Hs.85769</td>
<td>muscle contraction [0006936]</td>
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<td>aka estrogen receptor bining protein (ERBP)</td>
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**Cell cycle control**

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<thead>
<tr>
<th>Gene ID</th>
<th>Description</th>
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<th>Reference</th>
<th>Function</th>
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<tr>
<td>21N15</td>
<td>Homo sapiens cell division cycle 20, S.cerevisiae homolog (CDC20) mRNA</td>
<td>NM_001255</td>
<td>Hs.82906</td>
<td>cell cycle control [0000074]</td>
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<td>19L12</td>
<td>Homo sapiens protein regulator of cytokinesis 1 (PRC1) mRNA</td>
<td>NM_003981</td>
<td>Hs.5101</td>
<td>motor [0003774]</td>
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17G24; Homo sapiens cell growth regulatory with ring finger domain (CGR19) mRNA; NM_006568; Hs.59106; stress response [0006950]
27P15; Homo sapiens KIAA0008 gene product (KIAA0008), mRNA; NM_014750; Hs.77695; synaptic transmission [0007268]
21H20; Homo sapiens cell cycle progression 2 protein (CPR2) mRNA; NM_004749; Hs.333534; cell cycle arrest [0007050]
22A19; Homo sapiens activator of S phase kinase (ASK) mRNA; NM_006716; Hs.152759; G1/S transition of mitotic cell cycle [0000082]
22D16; Homo sapiens (E2F-1) pRB-binding protein mRNA, complete cds; M96577; Hs.96055; apoptosis [0006915]
21C4; Homo sapiens deoxymethylidate kinase (DTYMK), mRNA; NM_012145; Hs.79006; cell cycle [0007049]
21N16; Homo sapiens cell division cycle 25A (CDC25A) mRNA; NM_001789; Hs.1634; control of mitosis [0007088]
9D22; Homo sapiens NIMA (never in mitosis gene a)-related kinase (NEK2) mRNA; NM_002497; Hs.153704; protein amino acid phosphorylation [0006468]
9P1; Homo sapiens putative serine-threonine protein kinase (SID6-1512), mRNA; NM_014397; Hs.9625; protein amino acid phosphorylation [0006468]
9N19; Homo sapiens TTK protein kinase (TTK) mRNA; NM_003318; Hs.169840; protein amino acid phosphorylation [0006468]
5M13; Homo sapiens retinoblastoma-like 1 (p107) (RBL1) mRNA; NM_002895; Hs.87; transcription regulation [0006355]
22G15; Homo sapiens kinesin-like protein 2 (hklp2), mRNA; NM_020242; Hs.150587; mitosis [0007067]
20J4; Homo sapiens prohibitin (PHB) mRNA; NM_002634; Hs.75323; negative control of cell proliferation [0008285]
3A16; Homo sapiens Cdc7 (CDC7) mRNA, complete cds; AF015592; Hs.28853; DNA replication initiation [0006270]
21N17; Homo sapiens polo (Drosophila)-like kinase (PLK) mRNA; NM_005030; Hs.77597; cell cycle control [0000074]
21L24; Homo sapiens cdk inhibitor p21 binding protein (TOK-1), mRNA; NM_016567; Hs.279862; regulation of CDK activity [0000079]
21M12; Homo sapiens cell division cycle 2, G1 to S and G2 to M (CDC2) mRNA; NM_001786; Hs.184572; cell cycle [0007049]
21P19; Homo sapiens cyclin A2 (CCNA2) mRNA; NM_001237; Hs.85137; cell cycle control [0000074]
21B6; Human cyclin B mRNA, 3' end; M25753; Hs.23960; cell cycle control [0000074]
21D22; Homo sapiens cyclin B2 (CCNB2) mRNA; NM_004701; Hs.194698; cell cycle control [0000074]
21H16; Homo sapiens cyclin D1 (PRAD1: parathyroid adenomatosis 1) (CCND1) mRNA; NM_001758; Hs.82932; cell cycle control [0000074]
21N4; Homo sapiens cyclin E2 (CCNE2) mRNA; NM_004702; Hs.30464; regulation of CDK activity [0000079]
21B22; Homo sapiens cyclin F (CCNF) mRNA; NM_001761; Hs.1973; cell cycle control [0000074]

**DNA replication/repair/topology**

3O21; Homo sapiens topoisomerase (DNA) II alpha (170kD) (TOP2A) mRNA; NM_001067; Hs.156346; DNA topological change [0006265]
3O3; Homo sapiens ribonucleotide reductase M2 polypeptide (RRM2) mRNA; NM_001033; Hs.75319; DNA replication [0006260]
3I5; Homo sapiens ribonucleotide reductase M1 polypeptide (RRM1) mRNA; NM_001033; Hs.2934; DNA replication [0006260]

17G17; Homo sapiens centromere protein F (400kD) (CENPF) mRNA; NM_005196; Hs.77204; muscle contraction [0006936]
3K13; Human mRNA for P1cdc47, complete cds; D55716; Hs.77152; DNA replication [0006260]
Mitotic spindle control/chromosome segregation/chromosome maintenance

20013; Homo sapiens pituitary tumor transforming gene protein 2 (PTTG2) mRNA, complete cd; AF095288; Hs.211506; transcription factor [0003700]

20011; Homo sapiens pituitary tumor-transforming 1 (PTTG1) mRNA; NM_004219; Hs.252587; transcription factor [0003700]

4G18; Homo sapiens nucleolar protein p40 (P40), mRNA; NM_006824; Hs.74407; chromatin assembly/disassembly [0006333]

20015; Homo sapiens pituitary tumor transforming gene protein 3 (PTTG3) mRNA, complete cd; AF095289; Hs.247762; transcription factor [0003700]

21L19; Homo sapiens highly expressed in cancer, rich in leucine heptad repeats (HEC) mRNA; NM_006101; Hs.58169; mitotic chromosome segregation [0000070]

18L3; Homo sapiens chromosome-associated polypeptide C (CAP-C) mRNA; NM_005496; Hs.50758; chromosome organization and biogenesis (sensu Eukarya) [0007001]

21L5; Homo sapiens D229 mRNA, complete cd; AF235023; Hs.193602; mitotic chromosome condensation [0007076]

22C19; Homo sapiens M-phase phosphoprotein 1 (MPHOSPH1) mRNA; NM_016195; Hs.240; M phase of mitotic cell cycle [0000087]

21P16; Homo sapiens BUB3 (budding uninhibited by benzimidazoles 3, yeast) homolog (BUB3) mRNA; NM_004725; Hs.40323; mitotic spindle checkpoint [0007049]

2G13; Homo sapiens budding uninhibited by benzimidazoles 1 (yeast homolog), beta (BUB1B) mRNA; NM_001211; Hs.36708; mitosis [0007067]

19B16; Homo sapiens kinesin-like 4 (KNSL4) mRNA; NM_007317; Hs.119324; microtubule-based process [0007017]
21P18; Homo sapiens budding uninhibited by benzimidazoles 1 (yeast homolog) (BUB1) mRNA; NM_004336; Hs.98658; mitotic spindle checkpoint [0007094]

22K1; Homo sapiens anaphase-promoting complex subunit 5 (APC5), mRNA; NM_016237; Hs.7101; mitotic anaphase [000090]

21P8; Homo sapiens protein phosphatase 1, catalytic subunit, gamma isoform (PPP1CC) mRNA; NM_002710; Hs.79081; mitotic spindle checkpoint [0007094]

20K21; Homo sapiens microtubule-associated protein, RP/EB family, member 1 (MAPRE1), mRNA; NM_012325; Hs.234279; protein C-terminus binding [0008022]

22G19; Homo sapiens kinesin-like 1 (KNSL1) mRNA; NM_004523; Hs.8878; mitosis [0007067]

19H1; Homo sapiens ZW10 interactor (ZWINT), mRNA; NM_007057; Hs.42650; cytoskeleton organization and biogenesis [0007010]

Histones/DNA packaging

4C20; Homo sapiens sir2-related protein type 6 (SIRT6), mRNA; NM_016539; Hs.105463; DNA packaging [0006323]

4E6; Homo sapiens histone acetyltransferase 1 (HAT1) mRNA; NM_003642; Hs.13340; DNA packaging [0006323]

7D2; Homo sapiens Hairpin binding protein, histone (HBP), mRNA; ^D361; Hs.75257; histone mRNA 3'-end processing [0006398]

4K20; Homo sapiens nucleosome assembly protein 1-like 4 (NAP1L4) mRNA; NM_005969; Hs.78103; nucleosome assembly [0006334]

4K17; Homo sapiens high-mobility group (nonhistone chromosomal) protein 14 (HMG14) mRNA; NM_004966; Hs.251064; DNA packaging [0006323]

33D11; Homo sapiens H1 histone family, member 2 (H1F2) mRNA; NM_005319; Hs.7644; biological_process unknown [0000004]

34H6; Homo sapiens H1 histone family, member 3 (H1F3), mRNA; NM_005320; Hs.136857; biological_process unknown [0000004]

36H5; Homo sapiens H1 histone family, member 4 (H1F4), mRNA; NM_005321; Hs.248133; biological_process unknown [0000004]

36H7; Homo sapiens H1 histone family, member 5 (H1F5) mRNA; NM_005322; Hs.131956; biological_process unknown [0000004]

34F5; Homo sapiens H2A histone family, member I (H2AFI) mRNA; NM_003511; Hs.233568; biological_process unknown [0000004]

4K4; Homo sapiens histone H2A.1b mRNA, complete cds; L19778; Hs.51011; nucleosome assembly [0006334]

4F21; Homo sapiens H2A histone family, member M (H2AFM), mRNA; NM_003513; Hs.248174; biological_process unknown [0000004]

4I24; Homo sapiens H2A histone family, member X (H2AFX) mRNA; NM_002105; Hs.147097; nucleosome assembly [0006334]

38I16; Human mRNA for histone H2A.Z, 5'UTR (sequence from the 5'cap to the start codon); D28450; biological_process unknown [0000004]

32J14; Homo sapiens H2B histone family, member D (H2BFD) mRNA; NM_003520; Hs.154576; biological_process unknown [0000004]

34H11; Homo sapiens H2B histone family, member E (H2BFE) mRNA; NM_003521; Hs.182432; biological_process unknown [0000004]

33P18; Homo sapiens H2B histone family, member G (H2BFG) mRNA; NM_003522; Hs.182137; biological_process unknown [0000004]

34H13; Homo sapiens H2B histone family, member H (H2BFH) mRNA; NM_003523; Hs.182138; biological_process unknown [0000004]

31N8; Homo sapiens H2B histone family, member J (H2BFJ) mRNA; NM_003524; Hs.249216; biological_process unknown [0000004]
Small nucleolar RNA and associated proteins/RNA splicing/RNA processing/nucleases

27L10; Homo sapiens DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68kD) (DDX5), mRNA; NM_004396; Hs.76053; developmental processes [0007275] RNA helicase

7D5; Homo sapiens splicing factor, arginine/serine-rich 2 (SFRS2) mRNA; NM_003016; Hs.73965; RNA processing [0006396]

7D19; Homo sapiens heterogeneous nuclear ribonucleoprotein H1 (H) (HNRPH1) mRNA; NM_005520; Hs.245710; RNA processing [0006396]

20P1; Homo sapiens dyskeratosis congenita 1, dyskerin (DKC1) mRNA, and translated products; NM_001363; Hs.4747; cell proliferation [0008283]

7F8; Human Gu protein mRNA, partial cds; U41387; Hs.169531; 35S primary transcript processing [0006365]

4H15; Homo sapiens nucleolar protein NOP5/NOP58 (NOP5/NOP58), mRNA; +D132; Hs.119908; transcription [0006350] 28K6; Homo sapiens DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 1 (DDX1) mRNA; NM_004939; Hs.76053; developmental processes [0007275]

7D7; Homo sapiens heterogeneous nuclear ribonucleoprotein A1 (HNRPA1) mRNA; NM_002136; Hs.249495; RNA processing [0006396]

7F1; Homo sapiens RNA binding motif, single stranded interacting protein 2 (RBMS2), mRNA; NM_002898; Hs.20938; RNA processing [0006396]

8E7; Homo sapiens CGI-110 protein (LOC51639), mRNA; NM_016047; Hs.177861; mRNA splice site selection [0006376] 7L20; Homo sapiens splicing factor proline/glutamine rich (polypyrimidine tract-binding protein-associated) (SFPQ) mRNA; NM_005066; Hs.180610; mRNA splicing [0006371]

3G2; Homo sapiens CGI-97 protein (LOC51119), mRNA; NM_016038; Hs.110445; leading strand elongation [0006272] aka Shwachman-Bodian-Diamond syndrome (SBDS)

7H15; Homo sapiens heterogeneous nuclear ribonucleoprotein F (HNRPF) mRNA; NM_004966; Hs.808; RNA processing [0006396]
7D3; Homo sapiens hnRNp 2H9B mRNA, complete cds; NM_006924; mRNA processing [0006396]
7P9; Homo sapiens splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor) (SFRS1), mRNA; NM_006386; mRNA processing [0006397]
7P3; Homo sapiens GAP-associated tyrosine phosphoprotein p62 (Sam68) (SAM68) mRNA; NM_006559; mRNA processing [0006397]
7L3; Homo sapiens DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide (72kD) (P72) mRNA; NM_006386; mRNA processing [0006397]
44H19; Homo sapiens RNA transcript from U17 small nuclear RNA host gene, variant U17HG-AB; NM_006835; mRNA processing [0006396]
7D22; Homo sapiens nucleolar phosphoprotein p130 (P130) mRNA; +D307; mRNA processing [0006364]
7N6; Homo sapiens small nuclear ribonucleoprotein D1 polypeptide (16kD) (SNRPD1), mRNA; +D424; mRNA processing [0006371]
7P16; Homo sapiens U2 small nuclear ribonucleoprotein auxiliary factor (65kD) (U2AF65), mRNA; NM_004593; mRNA processing [0006396]
8C17; Homo sapiens splicing factor, arginine/serine-rich 3 (SFRS3) mRNA; NM_003017; mRNA splice site selection [0006376]
7K24; Homo sapiens splicing factor, arginine/serine-rich (transformer 2 Drosophila homolog) 10 (SFRS10) mRNA; NM_004593; mRNA processing [0006396]
7H23; Homo sapiens heterogeneous nuclear ribonucleoprotein A2/B1 (HNRPA2B1) mRNA; NM_002137; mRNA processing [0006396]
7B9; Homo sapiens heterogeneous nuclear ribonucleoprotein complex K [human, mRNA, 2302 nt]; S74678; mRNA processing [0006396]
7H23; Homo sapiens heterogeneous nuclear ribonucleoprotein complex K [human, mRNA, 2302 nt]; S74678; mRNA processing [0006396]
3A4; Homo sapiens polymyositis/scleroderma autoantigen 1 (75kD) (PMSCL1) mRNA; NM_005033; mRNA processing [0006396]
4E21; Homo sapiens rad2 nuclease family member, homolog of S. cerevisiae exonuclease 1 (HEX1) mRNA; NM_003400; mRNA processing [0006396]

Nuclear lamina

22C11; Homo sapiens lamin B1 (LMNB1) mRNA; NM_005573; mRNA processing [0000115]
22G20; Human lamin A mRNA, 3'end; NM_005573; mRNA processing [0000115]

Nuclear/cytoplasmic transport

20P11; Homo sapiens chromosome segregation 1 (yeast homolog)-like (CSE1L) mRNA; NM_001316; mRNA processing [00008283]
16M22; Homo sapiens nucleoporin 155kD (NUP155) mRNA; NM_004298; mRNA processing [0006913]
16M18; Homo sapiens nucleoporin 153kD (NUP153) mRNA; NM_005124; mRNA processing [0006913]
16D22; Homo sapiens exportin 1 (CRM1, yeast, homolog) (XPO1), mRNA; NM_003400; mRNA processing [0006913]
26M15; Homo sapiens RAN binding protein 7 (RANBP7), mRNA; NM_006391; mRNA processing [0006913]
16M10; Homo sapiens exportin, tRNA (nuclear export receptor for tRNAs) (XPOT), mRNA; NM_007235; mRNA processing [0006913]

Amino acid/nucleoside transporters

50C8; Homo sapiens amino acid transporter system A1 mRNA, complete cds; AF271070; mRNA processing [0006865]
15I2; Homo sapiens amino acid transporter 2 (KIAA1382), mRNA; NM_018976; mRNA processing [0006865]
20P5; Homo sapiens NBMPR-insensitive nucleoside transporter 2 (ENT2), mRNA, complete cds; AF034102; Hs.32951; cell proliferation [0008283]

18F17; Homo sapiens membrane nucleoside transporter (MBNT), mRNA; NM_014713; Hs.111894; vacuolar transport [0007034]

**Metal ion transport/homeostasis/heavy metal metabolism**

14B8; Homo sapiens metallothionein 1L (MT1L), mRNA; NM_002450; Hs.94360; heavy metal ion transport [0006823]

15F3; Homo sapiens metallothionein 2A (MT2A), mRNA; +D80; Hs.118786; copper homeostasis [0006878]

18O8; H.sapiens mRNA for metallothionein isoform 1R; X97261; Hs.94360; heavy metal sensitivity/resistance [0009634]

14B10; H.sapiens mRNA for metallothionein; X64177; Hs.2667; heavy metal ion transport [0006823]

32E13; metallothionein MT-1g isoform [human, monocytes, mRNA Partial, 93 nt]; S68954; biological_process unknown [0000004]

15I11; Homo sapiens transferrin receptor (p90, CD71), (TFRC), mRNA; NM_003234; Hs.77356; iron transport [0006826]

15F5; Human hemopexin mRNA, 3' end; J03048; Hs.1504; iron homeostasis [0006879]

**Redox function/antioxidants**

13H22; Homo sapiens NAD(P)H menadione oxidoreductase 1, dioxin-inducible (NMOR1) mRNA; NM_000903; Hs.80706; xenobiotic metabolism [0006805]

20P3; Homo sapiens peroxiredoxin 1 (PRDX1), mRNA; NM_002574; Hs.180909; cell proliferation [0008283]

18G8; Homo sapiens peroxiredoxin 3 (PRDX3), nuclear gene encoding mitochondrial protein, mRNA; NM_006793; Hs.75454; oxidative stress response [0006979]

13H10; Homo sapiens 15 kDa selenoprotein (SEP15) mRNA; NM_004261; Hs.90606; peroxidase reaction [0006804]

17K24; Homo sapiens thioredoxin reductase beta (TR), mRNA; NM_006440; Hs.12971; response to pest/pathogen/parasite [0009613]

**Membrane channels**

30K20; Homo sapiens chloride intracellular channel 4 like (CLIC4L), mRNA; NM_013993; Hs.25035; pregnancy [0007565]

**Heat shock proteins/response**

17G22; Homo sapiens heat shock cognate 40 (HSC40), mRNA; NM_012266; Hs.237506; stress response [0006950]

8P2; Homo sapiens ER-associated DNAJ; ER-associated Hsp40 co-chaperone; hDj9; ERj3 (LOC51726), mRNA; NM_016306; Hs.278605; protein folding [0006457]

18M14; Homo sapiens mRNA for heat shock protein apg-2, complete cds; AB023420; Hs.90093; heat shock response [0006951]

8L16; Homo sapiens heat shock protein, DNAJ-like 2 (HSJ2), mRNA; NM_001539; Hs.94; protein folding [0006457]

18I18; Homo sapiens DNAJ-like heat shock protein 40 (HLJ1), mRNA; NM_007034; Hs.41693; response to heat [0009408]

18K22; Homo sapiens heat shock 105kD (HSP105B), mRNA; NM_006644; Hs.36927; heat shock response [0006951]

18I24; Homo sapiens heat shock 90kD protein 1, beta (HSPCB), mRNA; NM_007355; Hs.74335; heat shock response [0006951]

18M2; Homo sapiens heat shock 70kD protein 10 (HSC71) (HSPA10), mRNA; NM_006597; Hs.180414; heat shock response [0006951]
18M4; Homo sapiens endoplasmic reticulum luminal Ca2+ binding protein grp78 mRNA, complete cds; AF216292; Hs.75410; heat shock response [0006951]

**Protein synthesis and modification**

9G6; Homo sapiens Rab geranylgeranyltransferase, beta subunit (RABGGTB) mRNA; NM_004582; Hs.78948; protein modification [0006464]

8N5; Homo sapiens asparaginyl-tRNA synthetase (NARS), mRNA; NM_004539; Hs.18131; amino acid activation [0006418]

10M12; Homo sapiens N-myristoyltransferase 2 (NMT2), mRNA; NM_004808; Hs.122647; protein-lipoylation [0009249]

13I1; Homo sapiens signal sequence receptor, gamma (translocon-associated protein gamma) (SSR3), mRNA; NM_007107; Hs.28707; mannose-inositol-P-ceramide (MIPC) metabolism [0006675]

9M19; Homo sapiens lysyl oxidase (LOX) mRNA; NM_002317; Hs.102267; protein modification [0006464]

8O3; Homo sapiens methionine-tRNA synthetase (MARS), mRNA; NM_004990; Hs.279946; protein biosynthesis [0006412]

9G16; Homo sapiens lysyl oxidase-like 2 (LOXL2) mRNA; NM_002318; Hs.83354; protein modification [0006464]

9I19; Homo sapiens ribophorin II (RPN2), mRNA; NM_002951; Hs.75722; translational regulation, elongation [0006448]

10M15; Homo sapiens SUMO-1 activating enzyme subunit 2 (UBA2) mRNA; +D483; Hs.4311; protein sumoylation [0016925]

10K21; Homo sapiens protein-L-isoaspartate (D-aspartate) O-methyltransferase (PCMT1) mRNA; NM_005389; Hs.79137; protein amino acid methylation [0006479]

8H17; Homo sapiens G1 to S phase transition 1 (GSPT1) mRNA; NM_002094; Hs.2707; protein synthesis elongation [0006414]

12E3; Homo sapiens thyroid hormone sflotransferase (ST1B2), mRNA; NM_014465; Hs.129742; biogenic amine metabolism [0006576]

**Ubiquitin-dependent protein degradation**

11D9; Homo sapiens ubiquitin E3 ligase SMURF2 (SMURF2) mRNA, complete cds; AF301463; Hs.194477; polyubiquitylation [0006209]

11O18; Homo sapiens ubiquitin carrier protein E2-C (UBCH10), mRNA; NM_007019; Hs.93002; ubiquitin cycle [006512]

10M24; Homo sapiens F-box only protein 5 (FBXO5), mRNA; NM_012177; Hs.272027; proteolysis and peptidolysis [006508] ubiquitination

11F11; Homo sapiens ubiquitin-specific protease 1 (USP1), mRNA; NM_003368; Hs.35086; deubiquitylation [0006514]

11A14; Homo sapiens ubiquitin specific protease 18 (USP18), mRNA; NM_017414; Hs.38260; ubiquitin-dependent protein degradation [006511]

11O12; Homo sapiens ubiquitin-conjugating enzyme E2D 3 (homologous to yeast UBC4/5) (UBE2D3) mRNA; NM_003340; Hs.118797; ubiquitin cycle [006512]

11C14; Homo sapiens 26S proteasome-associated pad1 homolog (POH1) mRNA; NM_005805; Hs.178761; ubiquitin-dependent protein degradation [006511]

15N16; Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 2 (PSMC2) mRNA; NM_002803; Hs.61153; intracellular protein transport [006886]

11M10; Homo sapiens proteasome (prosome, macropain) 26S subunit, non-ATPase, 13 (PSMD13) mRNA; NM_002817; Hs.279554; ubiquitin-dependent protein degradation [006511]

4L9; Homo sapiens proteasome (prosome, macropain) 26S subunit, non-ATPase, 7 (Mov34 homolog) (PSMD7) mRNA; NM_002811; Hs.155543; transcription [006350]
Intracellular transport/vesicular transport/trafficking/ER associated proteins

39G5: Homo sapiens GTP-binding protein (RAB1A) mRNA, 3' untranslated region; AF170935; biological process unknown [0000004]
15P24: Homo sapiens RAB1, member RAS oncogene family (RAB1) mRNA; NM_004161; Hs.3642; vesicle-mediated transport [0016192]
19F8: Homo sapiens RAB6 interacting, kinesin-like (RAB6KIFL) mRNA; NM_005733; Hs.73625; microtubule-based process [0007017]
15P16: Homo sapiens SEC24 (S. cerevisiae) related gene family, member D (SEC24D), mRNA; NM_014822; Hs.19822; vesicle-mediated transport [0016192]
15P2: Homo sapiens transmembrane trafficking protein (TMP21), mRNA; NM_006827; Hs.74137; intracellular protein transport [0006886]
16C21: Homo sapiens SEC14 (S. cerevisiae)-like 1 (SEC14L1), mRNA; NM_003003; Hs.75232; non-selective vesicle transport [0006899]
16O3: Homo sapiens RAB2, member RAS oncogene family (RAB2) mRNA; NM_002865; Hs.78305; ER to Golgi transport [0006888]
15F2: Homo sapiens coated vesicle membrane protein (RNP24), mRNA; NM_006815; Hs.323378; intracellular protein transport [0006886]
16I9: Homo sapiens transmembrane protein (63kD), endoplasmic reticulum/Golgi intermediate compartment (P63), mRNA; NM_006825; Hs.74368; non-selective vesicle transport [0006899]
27J18: Homo sapiens reticulocalbin 2, EF-hand calcium binding domain (RCN2), mRNA; NM_002902; Hs.79088; developmental processes [0007275]
21K6: Human leucine-rich protein mRNA, complete cds; M92439; cell cycle [0007049]
23P7: Homo sapiens PTD009 protein (PTD009), mRNA; NM_016146; Hs.279901; cell adhesion [0007155] aka trafficking protein particle complex 4 or synbindin
16M19: Homo sapiens vesicle trafficking protein sec22b (SEC22B) mRNA; NM_004892; Hs.50785; ER to Golgi transport [0006888]
16M7: Homo sapiens coatomer protein complex, subunit beta 2 (beta prime) (COPB2) mRNA; NM_004766; Hs.75724; exocytosis [0006887]
16G10: Homo sapiens KIAA0171 gene product (KIAA0171), mRNA; NM_014666; Hs.132853; endocytosis [0006897] lathrin mediated endocytosis
29I15: Homo sapiens clathrin, heavy polypeptide (Hc) (CLTC), mRNA; NM_004859; Hs.178710; embryogenesis and morphogenesis [0007345]

Biosynthesis/metabolic function related enzymes

14A5: Homo sapiens carbonyl reductase 1 (CBR1), mRNA; NM_001757; Hs.88778; nitrogen metabolism [0006807]
29P22: Homo sapiens 3-prime-phosphoadenosine 5-prime-phosphosulfate synthase 2 (PAPSS2) mRNA; NM_004670; Hs.274230; skeletal development [0001501]
2K21: Homo sapiens UDP-N-acetylglucosamine pyrophosphorylase 1; Sperm associated antigen 2 (UAP1) mRNA; NM_003115; Hs.21293; UDP-N-acetylglucosamine biosynthesis [0006048]
11H24: Homo sapiens dimethylarginine dimethylaminohydrolase 1 (DDAH1), mRNA; NM_012137; Hs.303180; arginine catabolism [0006527]
2I10; Homo sapiens M2 mitochondrial autoantigen dihydrolipoamide acetyltransferase mRNA, complete cds; J03866; Hs.115285; main pathways of carbohydrate metabolism [0006092]

17K23; Homo sapiens aspartate beta-hydroxylase (ASPH) mRNA; NM_004318; Hs.283664; muscle contraction [0006936]

11L20; Homo sapiens glutamate-cysteine ligase (gamma-glutamylcysteine synthetase), regulatory (30.8kD) (GLCLR) mRNA; NM_002061; Hs.89709; cysteine metabolism [0006534]

11L10; Homo sapiens gamma-glutamyl hydrolase (conjugase, foly/polygammaglutamyl hydrolase)(GGH) mRNA; NM_003878; Hs.78619; glutamine metabolism [0006541]

1F20; Homo sapiens hypothetical protein FLJ10983 (FLJ10983), mRNA; NM_018290; Hs.23363; carbohydrate metabolism aka phosphoglucomutase 2 (PGM2)

13K11; Homo sapiens microsomal glutathione S-transferase 1 (MGST1), mRNA; NM_020300; Hs.790; prostaglandin metabolism [0006936]

20C1; Homo sapiens phenylalanine-tRNA synthetase beta-subunit (PheHB) mRNA; NM_005687; Hs.9081; phenylalanine-tRNA ligase [0004826]

2J14; Homo sapiens multifunctional polypeptide similar to SAICAR synthetase and AIR carboxylase (ADE2H1) mRNA; NM_006452; Hs.117950; purine base biosynthesis [0009113]

12G23; Homo sapiens S-adenosylmethionine decarboxylase 1 (AMD1) mRNA; NM_001634; Hs.262476; polypamine biosynthesis [0006596]

11P6; Homo sapiens phosphoserine aminotransferase (PSA) mRNA, complete cds; AF113132; Hs.286049; methionine metabolism [0006555]

11J6; Homo sapiens asparagine synthetase (ASNS) mRNA; NM_001673; Hs.75692; asparagine biosynthesis [0006529]

34K20; Homo sapiens mannosyl (alpha-1,6-)glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT2) mRNA; NM_002408; Hs.172195; biological process unknown [0000004]

13H19; Homo sapiens sepiapterin reductase (7,8-dihydrobiopterin:NADP+ oxidoreductase) (SPR) mRNA; NM_003124; Hs.301540; tetrahydrobiopterin biosynthesis [0006729]

2O19; Homo sapiens aldehyde dehydrogenase 1, soluble (ALDH1) mRNA, and translated products; NM_006899; Hs.76392; aldehyde metabolism [0006081]

8N13; Homo sapiens arginyl-tRNA synthetase (RARS), mRNA; NM_002887; Hs.334651; arginyl-tRNA aminoaacylation [0006420]

13J1; Homo sapiens trans-prenyltransferase (TPT), mRNA; NM_014317; Hs.279865; coenzymes and prosthetic group metabolism [0006731]

10M7; Homo sapiens isoprenylcysteine carboxyl methyltransferase (ICMT), mRNA; NM_012405; Hs.183212; C-terminal protein amino acid methylation [0006481]

11H20; Homo sapiens ornithine aminotransferase (gyrate atrophy) (OAT), nuclear gene encoding mitochondrial protein, mRNA; NM_002744; Hs.75485; arginine biosynthesis [0006526]

1N13; Homo sapiens UDP-glucose dehydrogenase (UGDH) mRNA, and translated products; NM_003359; Hs.28309; carbohydrate metabolism [0005975]

1P7; Homo sapiens glyoxalase I (GLO1) mRNA, complete cds; NM_006708; Hs.75207; carbohydrate metabolism [0006092]

11L8; Homo sapiens guanine-monophosphate synthetase (GMPS), mRNA; NM_003875; Hs.5398; glutamine metabolism [0006541]

2O14; Homo sapiens phosphoglycerate kinase 1 (PGK1) mRNA; NM_000291; Hs.78771; glycolysis [0006096]

2K20; Homo sapiens lactate dehydrogenase B (LDHB), mRNA; NM_002300; Hs.234489; glycolysis [0006096]

2J2; Homo sapiens 5-aminimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) mRNA; NM_004044; Hs.90280; nucleobase, nucleoside, nucleotide and nucleic acid metabolism [0006139]

12G15; Homo sapiens spermine synthase (SMS) mRNA; NM_004595; Hs.89718; polyamine metabolism [0006595]
1P22; Homo sapiens transketolase (Wernicke-Korsakoff syndrome) (TKT) mRNA; NM_001064; Hs.89643; glucose catabolism [0006007]

1L4; Homo sapiens glutamate dehydrogenase 1 (GLUD1) mRNA; NM_005271; Hs.77508; glutamate catabolism [0006538]

12G17; Homo sapiens ornithine decarboxylase 1 (ODC1) mRNA; NM_002539; Hs.75212; polyamine biosynthesis [0006596]

27F16; Homo sapiens GTP cyclohydrolase 1 (dopa-responsive dystonia) (GCH1) mRNA; NM_000161; Hs.86724; neurotransmitter synthesis and storage [0001506]

3A11; Homo sapiens thymidine kinase 1, soluble (TK1) mRNA; NM_003258; Hs.105097; DNA metabolism [0006259]

2P23; Homo sapiens non-metastatic cells 1, protein (NM23A) expressed in (NME1) mRNA; NM_000269; Hs.118638; nucleobase, nucleoside, nucleotide and nucleic acid metabolism [0006139]

Lipid biosynthesis/metabolism, cholesterol synthesis/metabolism

12N9; Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) (HMGCS1) mRNA; NM_002130; Hs.77910; lipid metabolism [0006629]

13D7; Homo sapiens squalene epoxidase (SQLE), mRNA; NM_003129; Hs.71465; ergosterol biosynthesis [0006696]

13D17; Homo sapiens sterol-C4-methyl oxidase-like (SC4MOL), mRNA; NM_006745; Hs.239926; ergosterol biosynthesis [0006696]

13G12; Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCR) mRNA; NM_000859; Hs.11899; cholesterol biosynthesis [0006695]

35O24; Homo sapiens insulin induced gene 1 (INSIG1), mRNA; NM_005542; Hs.56205; biological_process unknown [0000004]

13E22; Homo sapiens CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 1 (CD36L1) mRNA; NM_005505; Hs.180616; cholesterol metabolism [0008203]

13G16; Homo sapiens 7-dehydrocholesterol reductase (DHCR7) mRNA, and translated products; NM_001360; Hs.11806; cholesterol biosynthesis [0006695]

12F13; Homo sapiens acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase) (ACAT2) mRNA; NM_005891; Hs.278544; lipid metabolism [0006629]

12L10; Homo sapiens delta-6 fatty acid desaturase (FADS6) mRNA; NM_004265; Hs.184641; fatty acid desaturation [0006636]

12F11; Homo sapiens lysophospholipase-like (HU-K5), mRNA; NM_007283; Hs.6721; lipid metabolism [0006629]

13O9; Homo sapiens fatty-acid-Coenzyme A ligase, long-chain 4 (FACL4) mRNA; NM_004458; Hs.81452; learning and memory [0007611]

12F20; Homo sapiens peroxisomal D3,D2-enoyl-CoA isomerase (PECI) mRNA; NM_006117; Hs.15250; fatty acid metabolism [0006631]

12H6; Homo sapiens fatty-acid-Coenzyme A ligase, long-chain 3 (FACL3) mRNA; NM_004457; Hs.268012; fatty acid metabolism regulation [0006632]

12B17; Homo sapiens lipase, endothelial (LIPG), mRNA; NM_006033; Hs.65370; lipid metabolism [0006629]

13A6; Homo sapiens cytochrome P450, 51 (lanosterol 14-alpha-demethylase) (CYP51) mRNA; NM_000786; Hs.226213; steroid biosynthesis [0006694]

13A20; Homo sapiens farnesyl-diphosphate farnesyltransferase 1 (FDFT1) mRNA; NM_004462; Hs.48876; steroid biosynthesis [0006694]

Mitochondrial associated

2H3; Homo sapiens mRNA for cytochrome c, partial cds; D00265; Hs.169248; electron transport [0006118]

2G16; Homo sapiens SCO (cytochrome oxidase deficient, yeast) homolog 1 (SCO1), nuclear gene encoding mitochondrial protein, mRNA; NM_004589; Hs.14511; energy pathways [0006091]
19K16; Homo sapiens inner membrane protein, mitochondrial (mitofilin) (IMMT), mRNA; NM_006839; Hs.78504; cytoskeleton organization and biogenesis [0007010]

2B17; Homo sapiens malate dehydrogenase 1, NAD (soluble) (MDH1) mRNA; NM_004077; Hs.239760; main pathways of carbohydrate metabolism [0006092]

2I16; Homo sapiens citrate synthase (CS), nuclear gene encoding mitochondrial protein, mRNA; NM_006099; Hs.75187; protein targeting [0006605]

Protein folding

9A11; Homo sapiens FK506-binding protein 4 (59kD) (FKBP4) mRNA; NM_002014; Hs.848; protein folding [0006457]

8P14; Homo sapiens tetratricopeptide repeat domain 1 (TTC1) mRNA; NM_003314; Hs.7733; protein folding [0006457]

8L2; Homo sapiens chaperonin containing T-complex subunit 6 (CCT6) mRNA; NM_001762; Hs.82916; protein folding [0006457]

9A17; Homo sapiens chaperonin containing TCP1, subunit 8 (theta) (CCT8), mRNA; NM_006585; Hs.15071; protein folding [0006457]

8L10; Homo sapiens FK506 binding protein precursor (LOC51303), mRNA; NM_016594; Hs.24048; protein folding [0006457]

Apoptosis

9O20; Homo sapiens death-associated protein kinase-related 1 (DRAK1) mRNA; NM_004760; Hs.9075; protein amino acid phosphorylation [0006468]

22L10; Homo sapiens death-associated protein (DAP), mRNA; NM_004394; Hs.75189; induction of apoptosis by extracellular signals [0008624]

22J15; Homo sapiens myeloid cell leukemia sequence 1 (MCL1) mRNA, complete cds; AF118124; Hs.86386; apoptosis [0006915]

Immune/inflammatory response

17H7; Homo sapiens pentaxin-related gene, rapidly induced by IL-1 beta (PTX3) mRNA; NM_002852; Hs.2050; inflammatory response [0006954]

17O22; H.sapiens mRNA for C-reactive protein; X56692; Hs.76452; inflammatory response [0006954]

17M22; Homo sapiens transmembrane protein B7-H2 ICOS ligand mRNA, complete cds; AF289028; Hs.14155; defense response [0006952]

17P6; Human beta-thromboglobulin-like protein mRNA, complete cds; M17017; Hs.624; immune response [0006955]

18K3; Human membrane cofactor protein (MCP) mRNA, complete cds; M58050; Hs.83532; complement activation [0006956]

12O20; Homo sapiens encoding Polymeric immunoglobulin receptor, X73079; Hs.288579; protein secretion [0009306]

30F2; Homo sapiens annexin A5 (ANXA5), mRNA; NM_001154; Hs.300711; blood coagulation [0007596]

12P21; Homo sapiens annexin A1 (ANXA1) mRNA; NM_000700; Hs.78225; lipid metabolism [0006629]

Miscellaneous

14H2; Homo sapiens ATPase, H+ transporting, lysosomal (vacuolar proton pump) 31kD; Vacuolar proton-ATPase, subunit E; V-ATPase, subunit E (ATP6E), mRNA; NM_001696; Hs.77805; proton transport [0015992]

1H6; Homo sapiens seladin-1 mRNA, complete cds; AF261758; Hs.75616; carbohydrate metabolism [0005975]

40G24; Homo sapiens six transmembrane epithelial antigen of the prostate (NOTE: non-standard symbol and name) (STEAP), mRNA; NM_012449; Hs.61635; phenylalanine metabolism [0006558]
20C20; Homo sapiens transforming acidic coiled-coil containing protein 3 (TACC3), mRNA; NM_006342; Hs.104019; actin binding [0003779]

7C24; Homo sapiens HPV16 E1 protein binding protein mRNA, complete cds; U96131; Hs.6566; transcription, from Pol II promoter [0006366]

25G19; Homo sapiens nucleolar GTPase (HUMAUNTAG), mRNA; NM_013285; Hs.75528; signal transduction [0007165]

17A19; Homo sapiens leucine zipper protein FKSG14 (FKSG14) mRNA, complete cds; AY009151; Hs.192843; muscle contraction [0006936]

20E13; Homo sapiens sushi-repeat protein (SRPUL), mRNA; NM_014467; Hs.126782; cell adhesion molecule [0005194]

9O3; Homo sapiens hepatocellular carcinoma-associated protein HCA10 mRNA, complete cds; AF320070; Hs.55058; protein modification [0006464]

29P3; Homo sapiens fasciculation and elongation protein zeta 2 (zyggin II) (FEZ2), mRNA; NM_005102; Hs.103419; axon guidance [0007411]

30H5; Homo sapiens synuclein, alpha (non A4 component of amyloid precursor) (SNCA), transcript variant NACP140, mRNA; NM_000345; Hs.76930; pathogenesis [0009405]

10J7; Homo sapiens gis5 mRNA for glucocorticoid-inducible protein, complete cds; AB050716; Hs.173508; proteolysis and peptidolysis [0006508]

26N1; Homo sapiens FSH primary response (LRPR1, rat) homolog 1 (FSHPRH1), mRNA; NM_006733; Hs.123122; G-protein coupled receptor protein signaling pathway [0007186]

25J7; Homo sapiens AND-1 protein (AND-1), mRNA; NM_007086; Hs.72160; signal transduction [0007165]

10I14; Human transposon-like element mRNA; M23161; Hs.84775; N-terminal protein myristoylation [0006499]

8D21; Homo sapiens density regulated protein drp1 mRNA, partial cds; AF038554; Hs.22393; protein synthesis initiation [0006413]

14L15; Homo sapiens progesterone membrane binding protein (PMBP) mRNA; NM_006320; Hs.9071; cation transport [0006812]

21J21; Homo sapiens sudD (suppressor of bimD6, Aspergillus nidulans) homolog (SUDD) mRNA; NM_003831; Hs.209061; chromosome condensation [0000068]

12K16; Homo sapiens SURF-4 mRNA, complete cds; AF078866; Hs.284296; mitochondrial processing [0006627]

27A10; Homo sapiens epithelial membrane protein 1 (EMP1) mRNA; NM_001423; Hs.79368; ionic insulation of neurons by glial cells [0007272]

8F17; Homo sapiens homolog of rat elongation factor p18 (P18) mRNA; NM_004280; Hs.298581; protein synthesis elongation [0006414]

21B5; Homo sapiens serum deprivation response (phosphatidylerine-binding protein) (SDPR) mRNA; NM_004657; Hs.26530; cell cycle [0007049]

22M5; Homo sapiens core binding factor beta isoform PEBP2B mRNA, complete cds; AF294326; Hs.179881; asymmetric cytokinesis [0008356]

28P1; Homo sapiens nuclear autoantigenic sperm protein autosomal variant mRNA, partial cds; AF035191; Hs.243886; spermatogenesis [0007283]

28J13; Homo sapiens reticulocabin precursor mRNA, complete cds; AF183423; Hs.39619; developmental processes [0007275]

31L2; Homo sapiens Ewing sarcoma breakpoint region 1 (EWSR1), transcript variant EWS-b; mRNA; NM_013986; Hs.129953; biological process unknown [0000004]

50O21; Homo sapiens Down syndrome critical region gene 2 (DSCR2), mRNA; NM_003720; Hs.5198; biological process unknown [0000004]

Unknown
19D6; Homo sapiens clone HQ0310 PRO0310p1 (LOC51203), mRNA; NM_016359; Hs.279905; microtubule-based process [0007017]

48B13; Homo sapiens mRNA; cDNA DKFZp761F1924 (from clone DKFZp761F1924); AL137433; biological_process unknown [0000004]

49I22; Homo sapiens cDNA FLJ10500 fis, clone NT2RP2000369; AK001362; Hs.173374; biological_process unknown [0000004]

19D8; Homo sapiens cDNA FLJ10433 fis, clone NT2RP1000478, highly similar to TUBULIN BETA-5 CHAIN; AK001295; Hs.274398; microtubule-based process [0007017]

19C4; Homo sapiens hypothetical protein FLJ20364 (FLJ20364), mRNA; NM_017785; Hs.32471; cytoskeleton organization and biogenesis [0007010]

13K6; Homo sapiens KIAA0680 gene product (KIAA0680), mRNA; NM_014721; Hs.102471; cholesterol catabolism [0006707]

22N18; Homo sapiens cDNA: FLJ22009 fis, clone HEP07114; AK025662; Hs.123253; necrosis [0008220]

38N20; Homo sapiens hypothetical protein FLJ11029 (FLJ11029), mRNA; NM_018304; Hs.274448; biological_process unknown [0000004]

22D1; Homo sapiens cDNA: FLJ23360 fis, clone HEP15172; AK027013; Hs.161279; cell death [0008219]

11G19; Human mRNA for unknown product, partial cds; D29810; Hs.153445; proteolysis and peptidolysis [0006508]

15F9; Homo sapiens clone 23914 mRNA sequence; AF038186; Hs.177776; iron homeostasis [0006879]

18P17; Homo sapiens hypothetical protein FLJ10517 (FLJ10517), mRNA; NM_018123; Hs.279797; cytoskeleton organization and biogenesis [0007010]

27O18; Homo sapiens KIAA0013 gene product (KIAA0013), mRNA; NM_014783; Hs.172652; RHO protein signal transduction [0007266]

14H1; Homo sapiens cDNA: FLJ20995 fis, clone CAE02480; AK024648; ion transport [0006811]

22D20; Homo sapiens CGI-115 protein (LOC51018), mRNA; NM_016052; Hs.56043; apoptosis [0006915]

19G19; Homo sapiens cDNA: FLJ23468 fis, clone HSI11603; AK027121; Hs.38178; cytoskeleton organization and biogenesis [0007010]

19K21; Homo sapiens hypothetical protein FLJ10540 (FLJ10540), mRNA; NM_018131; Hs.14559; cytoskeleton organization and biogenesis [0007010]

11B21; Homo sapiens mRNA; cDNA DKFZp761J182 (from clone DKFZp761J182); AL359623; Hs.306516; monoubiquitylation [0006513]

23J4; Homo sapiens hypothetical protein PRO1855 (PRO1855), mRNA; NM_018509; Hs.283558; cell adhesion [0007155]

11A17; Homo sapiens mRNA for KIAA1301 protein, partial cds; AB037722; Hs.8707; proteolysis and peptidolysis [0006508]

9C11; Homo sapiens PNAS-102 mRNA, complete cds; AF275798; Hs.1600; protein folding [0006457]

7B12; Homo sapiens KIAA0020 gene product (KIAA0020), mRNA; NM_014878; Hs.2471; mRNA processing [0006397]

36O2; Human HepG2 partial cDNA, clone hmd2c09m5; D16988; calcium binding [0005509]

34M9; Human velo-cardio-facial syndrome 22q11 region mRNA sequence; U84526; biological_process unknown [0000004]

50G16; Homo sapiens mRNA for KIAA0836 protein, partial cds; AB020643; Hs.183006; leading strand elongation [0006272]

28C5; Homo sapiens cDNA FLJ13194 fis, clone NT2RP3004378, weakly similar to Drosophila melanogaster separation anxiety protein (san) mRNA; AK023256; Hs.288932; developmental processes [0007275]

28H12; Homo sapiens mRNA; cDNA DKFZp434C0931 (from clone DKFZp434C0931); partial cds; AL137718; Hs.122164; oogenesis [0007029]

22H23; Human mRNA for KIAA0367 gene, partial cds; AB002365; Hs.23311; apoptosis [0006915]
35K7; Homo sapiens DC2 (DC2) mRNA, complete cds; AF201937; Hs.103180; biological_process unknown [0000004]
36P12; Homo sapiens M-phase phosphoprotein 6 (MPP-6), mRNA; NM_005792; Hs.152720; biological_process unknown [0000004]
31I21; Homo sapiens mRNA HTPCRH03 for olfactory receptor; X64975; olfaction [0007608]
6N24; Human probable zinc finger protein H101 mRNA, partial cds; U81557; repression of transcription, from Pol II promoter [0000122]
12A24; Homo sapiens KIAA0057 gene product; TRAM-like protein (KIAA0057), mRNA; NM_012288; Hs.153954; co-translational membrane targeting [0006613]
11I18; Homo sapiens HSPC125 protein (HSPC125), mRNA; NM_014165; Hs.5232; cell growth and/or maintenance [0008151]
15N12; Homo sapiens CGI-120 protein (LOC51644), mRNA; NM_016057; Hs.181271; intracellular protein transport [0006886]
7J12; Homo sapiens CGI-74 protein (LOC51631), mRNA; NM_016019; Hs.7194; RNA splicing [0008380]
2L6; Homo sapiens DKFZP566E144 protein (DKFZP566E144), mRNA; NM_015523; Hs.7527; nucleotide metabolism [0009117]
17G16; Homo sapiens cDNA: FLJ21353 ffs, clone COL02771; AK025006; Hs.6101; stress response [0006950]
16I17; Homo sapiens hypothetical protein (DKFZp761K102), mRNA; NM_016451; Hs.3059; non-selective vesicle transport [0006899]
31I7; Homo sapiens hypothetical protein PRO2249 (PRO2249), mRNA; NM_018518; Hs.198363; DNA replication [0006260]
17O5; Homo sapiens small acidic protein (IMAGE145052), mRNA; NM_014267; Hs.78050; muscle contraction regulation [0006937]
11G4; Homo sapiens PRO2000 protein (PRO2000), mRNA; NM_014109; Hs.46677; ubiquitin-dependent protein degradation [0006511]
10I16; Homo sapiens cDNA: FLJ20931 ffs, clone ADSE01282; AK024584; Hs.203076; cell growth and/or maintenance [0008151]
11D10; Homo sapiens KIAA0618 gene product (KIAA0618), mRNA; NM_014833; Hs.295112; amino acid metabolism [0006520]
18H9; Homo sapiens cDNA: FLJ23548 ffs, clone LNG08487; AK027201; Hs.22895; lysosomal transport [0007041]
18M8; Human HepG2 3’ region cDNA, clone hmd2e07; D16892; heat shock response [0006951]
5N14; Homo sapiens mRNA; cDNA DKFZp434P072 (from clone DKFZp434P072); partial cds; AL137421; Hs.154583; transcription regulation [0006355]
15F8; Homo sapiens cDNA FLJ13045 ffs, clone NT2RP3001356; AK023107; Hs.108115; intracellular protein transport [0006886]
7P14; Homo sapiens hypothetical protein (HSPC068), mRNA; NM_016389; Hs.197298; mRNA splicing [0006371]
21E13; Homo sapiens PRO1848 protein (PRO1848), mRNA; NM_014102; negative control of cell proliferation [0008285]
4E19; Human mRNA for KIAA0039 gene, partial cds; D26018; Hs.82502; DNA recombination [0006310]
12N7; Homo sapiens CGI-82 protein (LOC51109), mRNA; NM_016026; Hs.179817; lipid metabolism [0006629]
21K1; Homo sapiens cDNA: FLJ22618 ffs, clone HSI05382; AK026271; Hs.5152; cell cycle [0007049]
1G19; Homo sapiens hypothetical protein FLJ20425 (FLJ20425), mRNA; NM_017816; Hs.71040; cell growth and/or maintenance [0008151]
<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer or probe sequence</th>
<th>Accession No.</th>
</tr>
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</table>
| LYVE-1   | Forward primer: GAACCAGCACCTGAAATTTCACA  
Reverse primer: TGACCACGAATCTCCATCTCACA  
Probe: AGCCCTGAAAGCTAGCTTTGAAACTTGCAGCTAT | NM05324       |
| Prox-1   | Forward primer: TCCCAGCTCAAATATGCTGAA  
Reverse primer: TGTAGTAAAAACATCACCGAAATTTGC1AA  
Probe: T1CTC5CGACGTAAAGTCACACGATGC1ACC | NM002763      |
| VEGFR3   | Forward primer: CTCTTACGTT TG1TG1GAGAGAC1T1  
Reverse primer: TCACAGCGCGATGCCATGA1G  
Probe: ATCAACAGCCTGACACGCTC1TGGTTC | NM182925.4    |
| Integrin α9 | TaqMan Gene Expression assay code Hs00174408_m1 (Applied Biosystems).  
Selected by A.B. |
| Podoplanin| Forward primer: AAAGTCCAAGCGCCACAG  
Reverse primer: TGTCCTG1TG1GCTCCACATCCAC  
Probe: Universal Probe Library: #39, Cat. no. 04688104001 | NM006474      |
| LaminB1  | Forward primer: CAGATCGAGCTGGGCAAGG1  
Reverse primer: TGATCTGGGCGCCATTAAG  
Probe: ACACGACGCTGTCCTCACCACATGCT | NM005573      |
| VEGF-C   | Forward primer: ACTTCTGCGATCGATGTC1  
Reverse primer: AGX1GTGATATGTCATCTCGTC  
Probe: AGACGTGTCCTGCGACCACACACACACACACAC | NM005429.2    |
| CD44     | TaqMan Gene Expression assay code Hs011075682_m1 (Applied Biosystems).  
Selected by A.B. |
| Human β2-microglobulin | Forward primer: TTCTGGCCTGGAGGCTATC  
Reverse primer: TCAGGAAATTTGACTTCCATTC  
Probe: Universal Probe Library probe: #42, Cat. no. 04688015001 | NM004048.2    |