Possible angiogenic roles for claudin-4 in ovarian cancer

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Abstract

Claudin proteins are frequently overexpressed in various tumors such as breast, prostate and ovarian cancer. While their functions in cancer have not been completely elucidated, roles in survival, adhesion, and invasion have been suggested. In order to clarify the roles of claudins in ovarian cancer, we have performed gene expression profiling of ovarian surface epithelial cells overexpressing claudin-4 and compared the expression patterns to the parental, non-expressing cells. Claudin-4 expression leads to the differential expression of several genes, including many that have previously been implicated in angiogenesis. In particular, angiogenic cytokines, such as IL-8, were found elevated while genes of the angiostatic interferon pathway were found down-regulated. *In vitro* assays show that claudin-4-expressing cells produce factors that can stimulate angiogenesis as measured by tube formation and migration in HUVEC cells. In addition, an *in vivo* mouse dorsal skinfold assay confirms that cells expressing claudin-4 secrete factors that can mediate angiogenesis in the dorsal skin of mice. Our data suggest a novel function for claudin-4 in cancer and provide an additional rationale for its common overexpression in human tumors.

Keywords

Ovarian Cancer; expression profiling; claudin; interleukin; interferon; angiogenesis

Introduction

Ovarian cancer is the fifth cause of cancer-related deaths in women in the US.[1] Advanced ovarian cancer has a particularly poor prognosis and, unfortunately, the lack of obvious symptoms and screening strategies results in late diagnosis for most ovarian cancer patients. While the exact molecular pathways involved in ovarian tumorigenesis have not been completely elucidated, recent work in the field has begun to shed some light on the fundamental molecular mechanisms involved in this disease.[2,3] Molecular genetics evidence suggest a two-pathway model for ovarian tumorigenesis with one pathway involved in the development of

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low grade tumors (Type I Pathway), and the other in the generation of serous high grade tumors (Type II pathway).[2] Epithelial ovarian tumors are believed to arise from ovarian surface epithelial (OSE) cells covering the ovary [4-6] and the relatively undifferentiated OSE cells can undergo metaplastic transformation, possibly through aberrant activation of certain HOX genes, to give rise to the various histological subtypes of ovarian carcinoma.[7] In addition to the changes important in histological differentiation, pathways important for increased growth, evasion from apoptosis and senescence, as well as events related to angiogenesis and metastasis have been shown aberrantly regulated in ovarian cancer.[3]

Among the molecular features observed in ovarian cancer is the frequent overexpression of several members of the claudin family of tight junction proteins.[8] In particular, we and others have shown that claudin-3 and claudin-4 are frequently elevated in ovarian cancer.[9-18] Certain claudins have been suggested as prognostic markers in various cancers and, in ovarian cancer, claudin-3 and claudin-7 levels are inversely correlated with survival.[17,18] Claudin-3 and claudin-4 expression has been shown to be associated with poor clinical outcome in endometrial cancer [19] and renal cell carcinoma.[20]

While claudins play crucial roles in the formation and function of tight junction in normal epithelial and endothelial cells,[21] the functional consequences of overexpressed claudin-3 and -4 in ovarian cancer have not been completely elucidated. Several lines of evidence suggest that these proteins may be important for invasion, motility, and survival.[22-27] Consistent with these findings, other claudins have also been associated with invasiveness and motility in cancer.[28,29]

In this report, we have used microarray analysis to identify molecular changes that occur in claudin-4-overexpressing cells. We find that several genes that have previously been implicated in the regulation of angiogenesis are altered following claudin-4 overexpression. Importantly, we show that these genes may be functionally relevant, since cells expressing claudin-4 exhibit elevated angiogenesis properties as measured by both *in vitro* and *in vivo* assays. Our data suggest a novel function for overexpressed claudin-4 in cancer and may explain the frequent presence of this protein in a number of human cancers.

Results

Gene expression alterations in response to claudin-4 expression

The HOSE-B cells were generated from immortalization of normal ovarian surface epithelial cells [30] and lack claudin-4 expression. Because we have previously observed changes in the behavior of HOSE-B cells following forced claudin-4 expression,[23] we hypothesized that claudin-4 expression may lead to significant changes in gene expression. In order to test this hypothesis, the Human Illumina Expression BeadChip system was used to perform gene expression profiling on claudin-4-overexpressing cell lines HOSE-CLDN4-1 and HOSE-CLDN4-2, as well as two non-expressing control lines. These cell lines have been described previously.[23] Microarray analysis revealed that a large number of genes were altered following expression of claudin-4 (Figure 1). Overall, the expression patterns in the two independent claudin-4-expressing clones were very similar and the vast majority of genes altered were decreased (Figure 1A). Genes up-regulated more than 2-fold (Figure 1B) or downregulated more than 3-fold (Figure 1C) were identified and clustered. Table 1 and Table 2 list the top 25 most upregulated and downregulated genes, respectively, in cells overexpressing claudin-4.

Several of the genes identified have previously been implicated in proliferation/survival (*SPOCK1*, *CAMK2*, *DNER*), migration (*MMP1*, *MMP3*), or angiogenesis (*CXCL8* (*IL-8*), *IL1B*, *CXCL1*, *CXCL2*, *POSTN*). The upregulation of several genes known to encode cytokines that promote angiogenesis was particularly intriguing. In addition, several of the downregulated

genes are involved in interferon signaling (Figure 1C, Table 2). For example, interferon inducible genes *G1P2*, *G1P3*, *IFIT2*, *IFITM1*, *IFIT1*, *OASL*, and *ISG20* were all among the top 25 most highly downregulated genes in claudin-4 expressing HOSE-B cells. Interferons and some of their targets have previously been shown to exhibit angiostatic activity.[31-33]

Interestingly the most highly elevated gene in claudin-4 expressing HOSE-B cells was *MMP1* (Table 1). This is consistent with previous work showing that claudins may be involved in invasion/motility and that MMPs may play a role in this process. [23,26,29,34] Moreover, MMPs may have important roles in angiogenesis.[35] In order to validate our finding that *MMP1* mRNA is elevated in claudin-4 expressing cells, we investigated the levels of MMP1 protein in HOSE-EV1 and HOSE-CLDN4 cells by immunoblotting (Figure 2A). We found that MMP1 protein was greatly increased in both the cell lysates and the media of claudin-4 expressing cells.

Because many of the genes identified as altered in claudin-4 expressing cells are cytokines that have previously been implicated in inflammation-induced angiogenesis, we used the RayBio human angiogenesis antibody arrays to investigate the expression of several proteins involved in angiogenesis (Figure 2B). This experiment confirmed that several angiogenesis proteins were elevated in claudin-4 expressing HOSE-B cells compared to non-expressors. Indeed, the GRO, IL-8, uPAR, and MMP-1 were all shown to be elevated in HOSE-CLDN4 media using the antibody arrays.

Claudin-4 expression increases angiogenesis in HUVEC assays

Because the claudin-4-mediated expression pattern changes suggested a possible angiogenic role for this protein, we next sought to functionally test this hypothesis. We first investigated whether claudin-4 overexpression could have angiogenic consequences using an *in vitro* HUVEC model system. HUVECs grown in the presence of conditioned media obtained from cells expressing claudin-4 exhibited an increase in tube formation compared to HUVECs grown in the presence of control cells (Figure 3A). In fact, the numbers of tubes formed was almost doubled when media from claudin-4 expressing cells added to the HUVEC (Figure 3B). Similarly, the claudin-4 media significantly increased HUVEC cell migration (Figure 3C), another indication that claudin-4 expression could promote angiogenesis.

In vivo angiogenesis is increased by claudin-4 expression

To extend the results of the *in vitro* angiogenesis studies to *in vivo* conditions, we performed angiogenesis experiments using the mouse dorsal skinfold assay. This assay allows the measurements of neovasculature formed in the skin of mice in response to factors secreted by the cells present in an implanted chamber.[36] In the presence of HOSE-B cells expressing claudin-4, we observed a significant increase in skin blood vessels compared to what was observed in the presence of cells that do not express this protein (Figure 4A). When the length of the vasculature was quantitated, both claudin-4-expressing HOSE-B clones exhibited more than five-fold increase in neovasculature compared to skin that had been exposed to non-expressing cells (Figure 4B).

Discussion

The aberrant expression of claudin proteins in cancer has been well documented but the exact functions of these proteins in tumorigenesis remain under investigation. Based on experiments involving the forced expression or knockdown of specific claudin proteins, we and others have suggested that these proteins may have roles in survival, invasion, and motility.[22-27] These roles would be consistent with claudin contributing to the emergence of metastasis in advanced tumors. Because, the changes in claudin levels leads to significant alteration in the behavior of the cells, we hypothesized that claudin overexpression may lead to measurable changes in

the global patterns of gene expression. We further hypothesized that knowledge of these changes may suggest pathways by which claudins may influence cell behavior in cancer. We therefore used our cell line model consisting of overexpression of claudin-4 in immortalized normal ovarian HOSE-B cells in order to identify changes in gene expression. Overexpression of claudins in these cells may mimic the appearance of these proteins during ovarian cancer progression as the majority of epithelial ovarian cancers express claudin-4.

Genes expression profiling was performed on two HOSE-B clones overexpressing claudin-4 and both the clones exhibited a remarkable similarity in gene expression (Figure 1A). Many of the genes identified as altered in these clones have been implicated in cell growth, survival, and invasion, consistent with previous functional studies suggesting roles for claudins in these processes. For example, *SPOCK*, *CAMK2*, and *DNER* have been implicated in cell proliferation and survival. Similarly, the overexpression of MMPs may be related to the ability of claudins to affect invasion and motility.[23,26,29] However, our attention was drawn to a number of genes and pathways that have previously been implicated in angiogenesis. Indeed, several cytokines, (IL-8, IL-1B, CXCL1, CXCL2) which have previously been implicated in angiogenesis [37] were found elevated in claudin-4 expressing cells. MMPs have also been implicated in promoting angiogenesis.[35] Conversely, several genes of the interferon pathway, which has been shown to inhibit angiogenesis,[31,33] were found downregulated by claudin-4 expression. The gene expression changes following claudin-4 expression therefore suggest specific alterations in pathways that would favor a more angiogenic environment.

Among the several pro-angiogenic cytokines found elevated in claudin-4-expressing cells, IL-8 is particularly interesting, as it has been implicated in angiogenesis and other tumorigenic processes in several cancers, including ovarian cancer.[38-43] IL-8 binding to its receptors (CXCR1/2) has been shown to result in downstream signaling that promotes cell survival, proliferation, invasion, and angiogenesis.[44] In particular, it is believed that IL-8 production by the cancer cells can result in significant changes in the microenvironment, leading to increases in proliferation and survival of the cancer cells through autocrine mechanisms, as well as increases in angiogenesis through IL-8 action on neighboring endothelial cells. Interestingly, it has been reported that the presence of IL-8 can lead to EGFR activation in endothelial cells. [45] The EGFR downstream signaling cascade, which includes the MAPK pathway, is known to be crucial in the angiogenic response of endothelial cells.[46] In addition, IL-8 was recently shown to induce endothelial cells permeability through VEGFR2 activation in a VEGF-independent fashion.[47] This suggests that IL-8 may, in certain cases, be able to substitute for some VEGF functions, which may explain why we do not see significant changes in VEGF in our system (data not shown). Overall our finding that IL-8, together with other pro-angiogenic cytokines (such as IL-1B, CXCL1, and CXCL2) are highly elevated following claudin-4 expression supports our hypothesis that claudin-4 may promote angiogenesis in ovarian tumors.

IL-8 is overexpressed in a number of malignancies and is often associated with poor clinical outcome.[40] In particular, in melanoma, IL-8 expression was shown to be associated with increased microvessel density and an IL-8 antibody could reduce tumor growth and metastasis in a mouse model of melanoma.[41] In ovarian cancer, IL-8 expression is associated with poor clinical outcome.[43] IL-8 knockdown using siRNA showed a significant therapeutic benefit in terms of tumor size in three different ovarian xenograft mouse models.[43] Importantly, tumors treated with IL-8 siRNA had significantly lower microvessel density compared to control treatment. In addition, MMP2 and MMP9 were reduced following IL-8 silencing. This is interesting considering the fact that we previously showed that claudin-3 and claudin-4 overexpression led to an increase in these MMPs.[23] In addition to their roles in invasion and metastasis, these MMPS have been shown to promote angiogenesis. Overall, our findings suggest that IL-8 overexpression may be linked to claudin proteins. The mechanism by which

claudin-4 overexpression leads to the expression of IL-8 and other pro-angiogenic cytokines is currently under investigation.

Remarkably, several IFN-regulated genes are downregulated in the claudin-4 expressing cells, implying a decrease in IFN production or signaling by these cells. IFNs have long been known to inhibit angiogenesis, and in fact, were the first angiogenesis inhibitors identified.[31,32,48] The antiangiogenic effects of IFNs have been demonstrated both in animal models and in the clinic. [49] The strong effect of IFNs on angiogenesis likely stems from their ability to modulate the tumor cell angiogenic signal [31] as well as their direct effects on endothelial cells.[48,50] The effects of IFNs on endothelial cells are believed to be mediated through inhibition of bFGF and other FGF family members.[49,51] In combination with the increase in pro-angiogenic cytokines described above, the apparent decrease in anti-angiogenic IFNs following claudin-4 expression suggests a role for this protein in the regulation of the balance between angiogenic and angiostatic cytokines.

In order to functionally investigate the significance of these changes in gene expression, we performed *in vitro* and *in vivo* angiogenesis assays. Consistent with our hypothesis that claudin-4 may alter cytokine secretion from the tumor cells to favor a more angiogenic environment, we found that media from claudin-4 expressing cells was much more efficient at mediating tube formation in HUVEC than media from control cells (Figure 3). Interestingly, IL-8 is known to be sufficient to mediate angiogenesis phenotypes in HUVECs and HMECs cells.[52-54] However, the changes in expression of multiple cytokines in our system, as well as the complex interactions between the angiogenic and angiostatic factors may explain why we were unsuccessful at inhibiting the angiogenesis effects of the media using an anti-IL-8 antibody (data not shown). We are currently attempting to identify the exact combination of factors that may be sufficient to mediate tumor angiogenesis following claudin-4 overexpression. Similarly, *in vivo* angiogenesis assays using a mouse skinfold approach clearly demonstrated that claudin-4 expressing cells represented a more pro-angiogenic environment compared to non-expressing cells (Figure 4).

Overall, our data show that claudin-4 expression leads to significant changes in gene expression, including changes in a number of soluble factors that are known to mediate angiogenesis. We were able to show that these changes in the environment of claudin-4 expressing cells were accompanied by functional changes in angiogenesis using both *in vitro* and *in vivo* assays. Claudin-4 is overexpressed in several tumors and has been suggested to have roles in cell proliferation, motility, invasion, and survival. The current data suggest yet another role for claudin-4 in cancer through its ability to reorganize the cellular environment to favor angiogenesis

Materials and Methods

Cell Culture

HOSE-B cell clones stably expressing claudin-4 (HOSE-CLDN4-1, HOSE-CLDN4-2) as well as corresponding non-expressing vector-transfected cell lines (HOSE-EV1 and HOSE-EV2) were previously generated in our laboratory.[23] These cell lines were cultured in RPMI medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum, 5 ng/ml epidermal growth factor, antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin), 300 μg/ml G418, and 350 μg/ml Zeocin.

For the preparation of lysates, the cells were cultured to 80% confluency, washed with HBSS (Invitrogen), and whole cell lysates were made using lysis buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS). Protein concentration was determined using the BCA assay kit (Pierce, Rockford, IL). For the preparation of conditioned culture media, cells were grown to 80%

confluency and then incubated with medium without fetal bovine serum for 24 hours. The culture media were collected and centrifuged at 1,000 rpm for 10 min to remove cells/debris, and were further concentrated by Centriplus YM-10 (Millipore, Billerica, MA).

Immunoblotting

Fifteen μg of lysate proteins or 10 μl of concentrated culture media were separated by 10-20% SDS-PAGE (Tris-Glycine gels, Invitrogen Life Technologies, Carlsbad, CA), and transferred to PVDF membranes (Millipore, Bedford, MA). The membranes were blocked with 5% nonfat dry milk, washed in TBST buffer and probed with the primary antibody. The primary antibodies used for this work are: claudin-4 (Invitrogen), MMP1 (R&D Systems), and GAPDH (Abcam Inc.). After washing in TBST, membranes were incubated with HRP-conjugated secondary antibody (anti-mouse IgG: 1:10,000; Amersham Biosciences Corp, Piscataway, NJ). Detection was carried out using the ECL plus kit (Amersham Biosciences Corp).

RNA isolation for microarrays

The claudin-4-expressing cell lines HOSE-CLDN4-1 and HOSE-CLDN4-2, as well as the two non-expressing control lines (HOSE-EV1 and HOSE-EV2) were cultured to 50% confluence in duplicate. Total RNA was then isolated using the RNAeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's recommendation. A total of 8 RNAs (4 cell lines in duplicate) were prepared for microarray analysis. The RNA quality and quantity was assessed using an Agilent 2100 bio-analyzer and the RNA 6000 Nano Chip kit.

Illumina oligonucleotide microarray

Total RNA was used to generate biotin labeled cRNA using the Illumina TotalPrep RNA Amplification Kit (Ambion; Austin, TX, cat # IL1791). Briefly, 0.5 μg of total RNA was first converted into single-stranded cDNA with reverse transcriptase using an oligo-dT primer containing the T7 RNA polymerase promoter site and then copied to produce double-stranded cDNA molecules. The double stranded cDNA was purified and concentrated with the supplied columns and used in an overnight *in vitro* transcription reaction where single-stranded RNA (cRNA) was generated and labeled by incorporation of biotin-16-UTP. A total of 0.75 μg of biotin-labeled cRNA was hybridized at 58 degrees C for 16 hours to Illumina's Sentrix HumanRef-8 Expression BeadChips (Illumina, San Diego, CA). Each BeadChip has 24,000 well-annotated RefSeq transcripts with an average of 30-fold redundancy. The arrays were washed, blocked and the labeled cRNA was detected by staining with streptavidin-Cy3. The arrays were scanned using an Illumina BeadStation 500X Genetic Analysis Systems scanner and the image data extracted using Illumina BeadStudio software, version 3.0. Gene expression data was transferred to an excel spreadsheet and analyzed using the Z-score method.[55] Changes in gene expression were compared for each individual gene by comparing array results for the HOSE-EV and HOSE-CLDN4 clones. Clustering analysis and gene expression visualization were done with the Dchip software.[56]

Angiogenesis Antibody Membrane Arrays

RayBio human angiogenesis antibody membrane array I and II (RayBiotech, Inc., Norcross, GA) were used to screen for angiogenic factors induced by claudin-4 expression in HOSE-B cells. The screen was conducted according to the manufacturer's recommendations. In brief, the array membranes were sequentially incubated with blocking buffer, 1 ml conditioned media, biotin-conjugated primary antibody, and HRP-conjugated streptavidin. The positive protein spots were detected by chemiluminescence (Amersham ECL) according to the manufacturer's protocol.

In Vitro Angiogenesis Assay

The assay was performed using the Chemicon International Inc (Billerica, MA) *In Vitro* Angiogenesis Assay Kit. Briefly, ECMatrix gel (50 μl) was added to a 96-well plate and incubated at 37°C for 1.5 hours to allow the gel solution to solidify. Human umbilical vein endothelial cells (HUVECs) were starved for 4 hours and 1×10^4 cells were seeded onto the ECMatrix in 100 μl of McCoy 5A medium containing 0.2% BSA. Conditioned media from HOSE cells with or without claudin-4 expression were concentrated 10-fold and 50 μl added to HUVECs. After 16 hours incubation, the tube-like structures were quantified by counting branch points in whole microscope fields.

Migration Assay

HUVECs were grown to about 80% confluency, serum-starved for 3 hours, and labeled with 5 μM calcein AM for 1 hour in serum-free medium. Cells were then trypsinized and 5×10^4 cells were added to the upper chamber of a fluoroblok insert (8 μm pore size and 24-well format; BD Biosciences) in 0.2% BSA-McCoy 5A medium. Conditioned media from HOSE-B cells expressing claudin-4 or without expression (cells transfected with empty vector) were added to the lower chamber. The assay plate was incubated at 37°C for 1, 2, 3, or 4 hours and the migrated cells were measured by a Cytofluor-4000. Experiments were repeated three separate times in triplicate.

In vivo Dorsal Skinfold Angiogenesis assay

Dorsal skinfolds of nude mice were exposed to the factors secreted by claudin-expressing cells by placing the claudin-expressing (or control) cells in diffusion chambers and implanting them to skinfolds as described previously.[36,57,58] Briefly, the diffusion chambers were prepared by cementing 0.45-μm membranes on both sides of the rim of an "O" ring (Millipore, Bedford, MA). The chambers were then sterilized by UV radiation for 20 minutes and wetted with sterile PBS. HOSE-EV1, HOSE-CLDN4-1, or HOSE-CLDN4-2 cells (2×10^6 cells per 125 μL of sterile PBS) were injected individually into each chamber. A dorsal air sac in an anesthetized mouse was prepared by injecting 10 ml of air in the skinfold. The air sac was then opened to implant the chamber underneath the skin, and the incision was sutured. After 10 days, the animals were euthanized, and carefully skinned around the implanted chambers. The skinfolds covering the chambers were carefully removed, stretched on a glass slide and photographed under visible light microscope (20X). The length of newly formed vessels was measured and plotted against a PC-3M variant cell lines. Sterile small-animal surgical techniques were followed during the entire procedure. The protocol was approved by the institutional Animal Care and Use Committee.

Statistics

The Z-score method was used for the analysis of microarray data. [55] One-way ANOVA and Newman-Keuls tests were used to determine significance of the changes in blood vessel lengths in the mouse skinfolds. Student's t-tests were used to estimate statistical significance of the *in vitro* angiogenesis (tube formation) changes as well as the changes in HUVEC migration.

Acknowledgments

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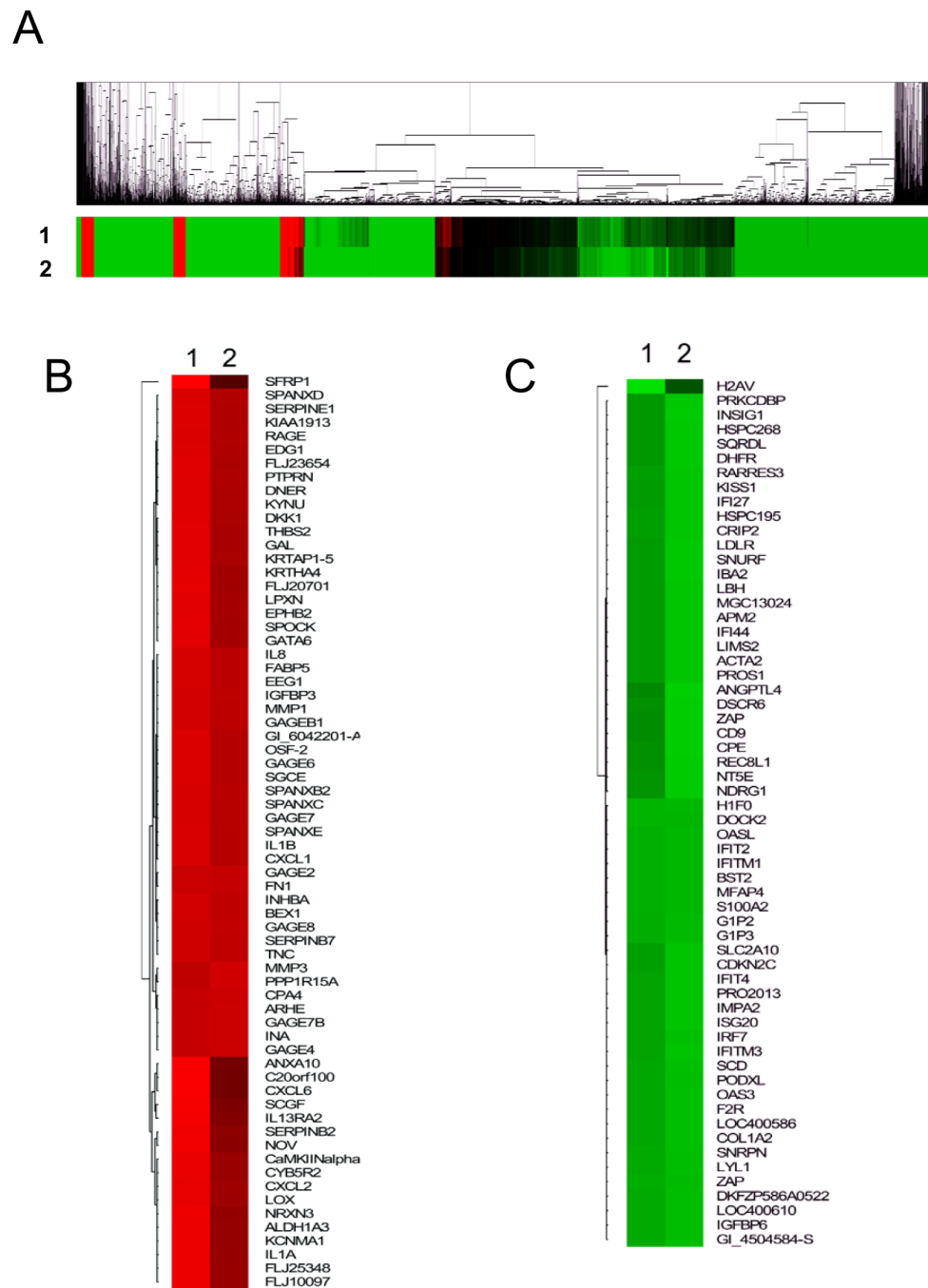
Figure 1.
Clustering analysis of gene expression following claudin-4 overexpression. Gene expression patterns for claudin-4 overexpressing clones HOSE-CLDN4-1 (1) and HOSE-CLDN4-2 (2) were obtained using the Illumina Sentrix Beadchip microarray. Gene expression levels are shown relative to HOSE-EV1 and HOSE-EV2, two cell lines lacking claudin-4 expression. A. All genes expressed above background levels (a total of 8,793 genes) were used for clustering analysis and the results shown as a heat map. B. Clustering analysis is shown for a subgroup of genes elevated more than 3-fold in claudin-4 expressing cells. C. A similar analysis is shown for genes downregulated more than 3.5 fold in claudin-4 expressing cells. In the heat maps,

the red-coded areas correspond to elevated genes while the green color represents decreased genes.
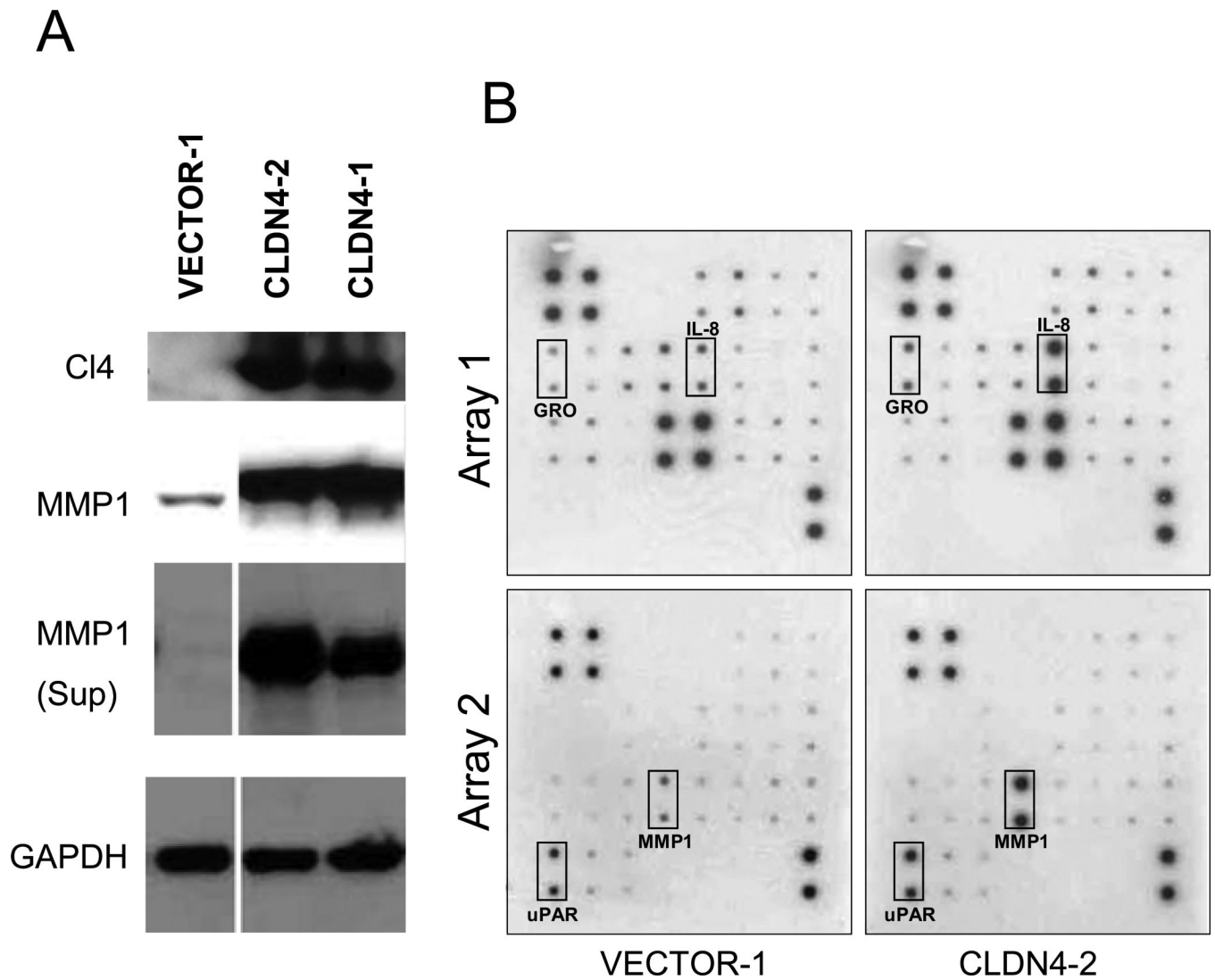
Figure 2.
Validation of Illumina array results by Western analysis and antibody arrays. A. Two cell lines overexpressing claudin-4 (CLDN4-1 and CLDN4-2) were assayed by Western for MMP1 expression. MMP1 was found elevated in both the cell lysates and the supernatants of claudin-4 expressing cells. B. Conditioned media from claudin-4-expressing HOSE-B cells (CLDN4-2) were used for angiogenesis antibody arrays. IL-8, GRO (CXCL1), uPAR, and MMP1 expression were increased significantly in claudin-4 expressing cells.
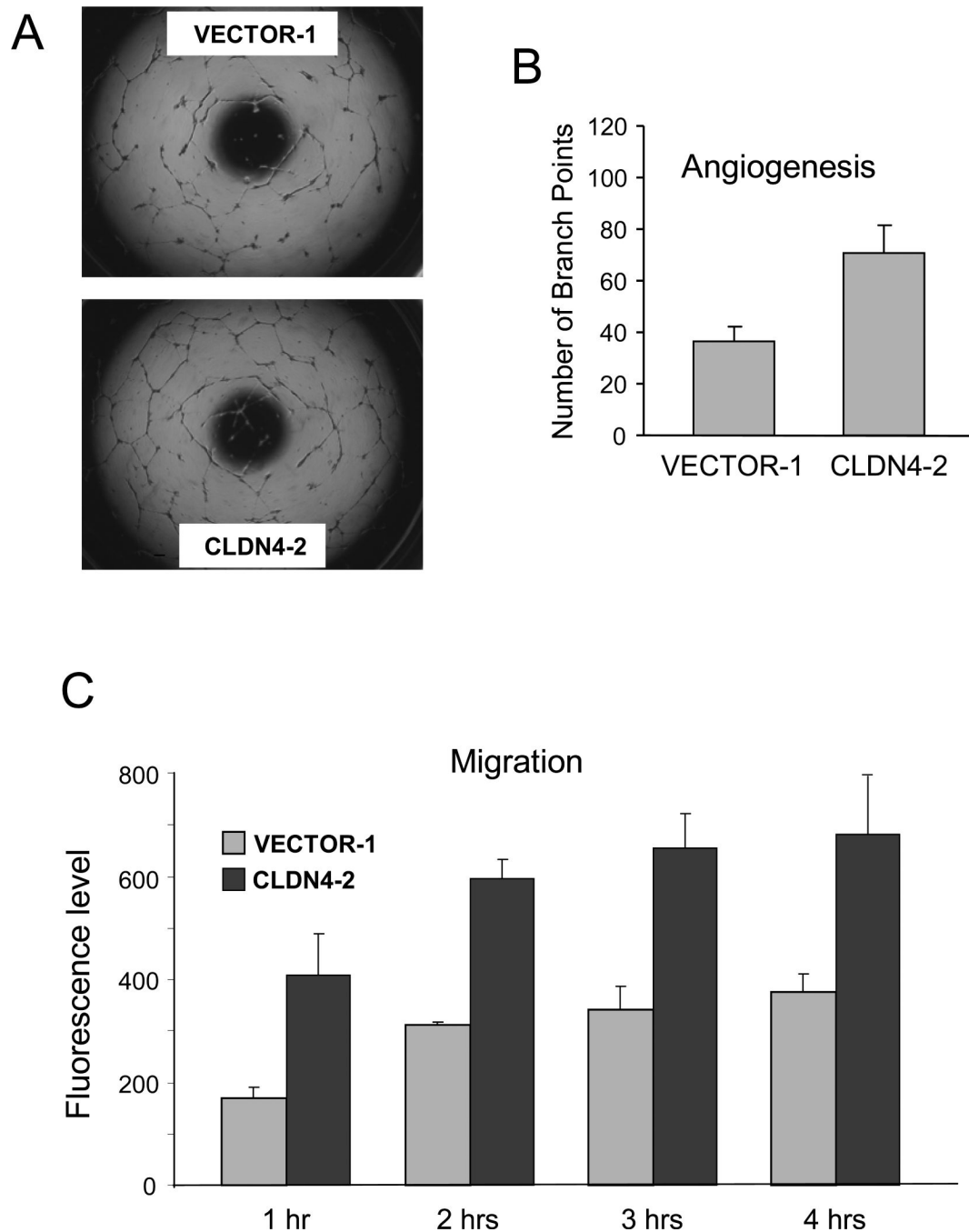
Figure 3.
Claudin-4 promotes HUVEC angiogenesis. A. Pictures show HUVEC cultures following incubation with conditioned media from HOSE-B cells expressing claudin-4 (CLDN4-2) or non expressing control-tranfected cells (VECTOR-1). HUVEC incubated with claudin-4 expressing cells show an increase in tube formation. B. Tube formation was quantitated by counting the number of branch points in a given frame. The graph shows the average of 3 experiments. C. Effects of claudin-4 expression on HUVEC migration. The graph shows the migration (fluorescence levels) following claudin-4 expression at four different time points. The data shown is the average of 3 independent experiments. Media from claudin-4 expressing cells lead to a significant increase in HUVEC migration.
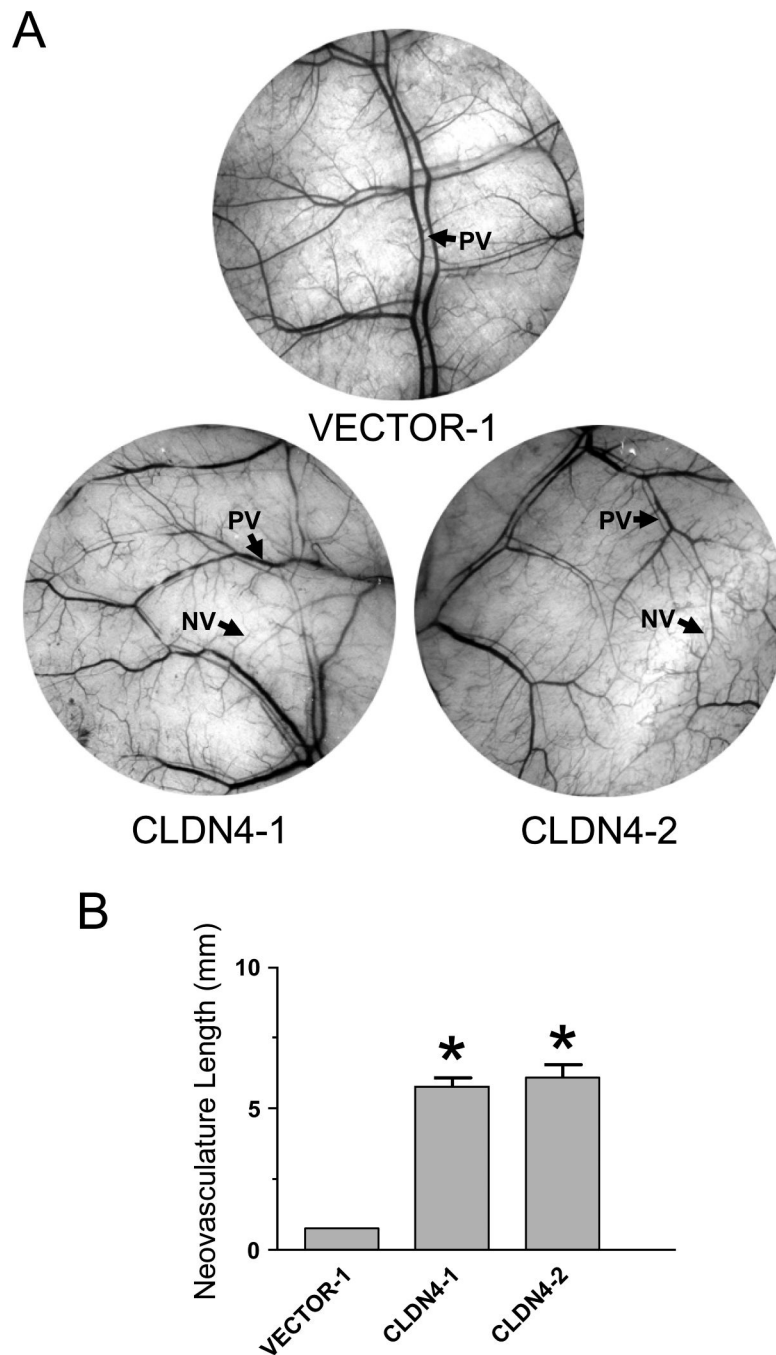
Figure 4.
Claudin-4 promote angiogenesis *in vivo*. Cells expressing claudin-4 (CLDN4-1 and CLDN4-2) or lacking claudin expression (VECTOR-1) were implanted in mouse skin and the resulting changes in vasculature observed. A. Representative pictures of mouse skin showing blood vessels 10 days after the implantation of the chamber containing the indicated cells. The presence of claudin-4-expressing cells (CLDN4-1 and CLDN4-2) leads to an increase in neovasculature (NV). Preexisting vasculature is indicated (PV). B. Changes in neovasculature resulting from exposure to the different cell lines were quantitated by evaluating the length of new blood vessels in each microscope field. Results shown are the average of 3 mice.

Table 1

Genes elevated in claudin-4-expressing cells

Gene Symbol	Accession	Gene Names	Fold in CLDN4-1	Fold in CLDN4-2	Av. fold change
MMP1	NM_002421.2	Matrix metalloproteinase 1 (interstitial collagenase)	93.0	95.1	94.0
MMP3	NM_002422.2	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)	32.1	36.9	34.5
IL1B	NM_000576.2	Interleukin 1, beta	24.8	24.8	24.8
GAGEB1	NM_003785.2	G antigen, family B, 1	18.9	19.4	19.1
CXCL8 (IL8)	NM_000584.2	C-X-C chemokine-interleukin 8 (IL-8)	18.0	18.3	18.2
SPANXE	NM_145665.1	SPANX family, member E	11.6	11.7	11.7
FABP5	NM_001444.1	Fatty acid binding protein 5	10.9	11.1	11.0
CXCL1	NM_001511.1	C-X-C chemokine-growth-related oncogene alpha (GROα)	9.6	9.6	9.6
DNER	NM_139072.2	Delta-notch-like EGF repeat-containing transmembrane	9.3	9.0	9.1
SPANXB2	NM_145664.1	SPANX family, member B2	8.6	8.7	8.7
GAGE6	NM_001476.1	G antigen 6	8.4	8.4	8.4
SERPINB2	NM_002575.1	Serine (or cysteine) proteinase inhibitor, clade B 2	8.5	7.3	7.9
POSTN	NM_006475.1	Periostin	7.0	7.0	7.0
KYNU	NM_003937.1	Kynureninase	6.6	6.5	6.5
INHBA	NM_002192.1	Inhibin, beta A (activin A)	5.7	6.0	5.8
CAMK2A	NM_018584.4	Calcium/calmodulin-dependent protein kinase II alpha	6.0	5.4	5.7
CXCL2	NM_002089.1	C-X-C chemokine-growth-related oncogene beta (GROβ)	5.8	5.4	5.6
PTPRN	NM_002846.2	Protein tyrosine phosphatase, receptor type, N	5.2	5.1	5.2
SPOCK1	NM_004598.2	Sparc/osteonectin, cwcv and kazal-like proteoglycan	5.0	4.7	4.8
TMEM200A	NM_052913.2	Transmembrane protein 200A	4.8	4.8	4.8
SPANXD	NM_032417.2	SPANX family, member D	4.6	4.6	4.6
GAGE7B	NM_001477.1	G antigen 7B	4.3	4.8	4.6
SPANXC	NM_022661.2	SPANX family, member C	4.4	4.6	4.5
KRTHA4	NM_021013.3	Keratin, hair, acidic, 4	4.6	4.3	4.5
ALDH1A3	NM_000693.1	Aldehyde dehydrogenase 1 family, member A3	4.5	4.1	4.3

Table 2

Genes decreased in claudin-4-expressing cells

Gene Symbol	Accession	Gene Names	Fold in CLDN4-1	Fold in CLDN4-2	Av fold change
H1F0	NM_005318.2	H1 histone family, member 0	12.5	12.3	12.4
G1P2	NM_005101.1	Interferon, alpha-inducible protein	7.0	7.3	7.1
OASL	NM_198213.1	2'-5'-oligoadenylate synthetase-like	6.5	6.6	6.5
DOCK2	NM_004946.1	Dedicator of cytokinesis 2	5.9	5.5	5.7
G1P3	NM_002038.2	Interferon, alpha-inducible protein	5.6	5.7	5.6
MFAP4	NM_002404.1	Microfibrillar-associated protein 4	5.2	5.1	5.1
IFIT2	NM_001547.3	Interferon-induced prot. with tetratricopeptide repeats 2	4.8	4.7	4.8
S100A2	NM_005978.3	S100 calcium binding protein A2	4.5	4.4	4.4
F2R	NM_001992.2	Coagulation factor II (thrombin) receptor	4.0	4.4	4.2
SCD	NM_005063.3	Stearoyl-CoA desaturase (delta-9-desaturase)	4.0	4.4	4.2
LOC400586	XM_375424.1	Similar to stearoyl-CoA desaturase	3.9	4.2	4.0
COL1A2	NM_000089.2	Collagen, type I, alpha 2	3.8	4.0	3.9
IFITM1	NM_003641.2	Interferon induced transmembrane protein 1 (9-27)	3.8	3.7	3.7
SQRDL	NM_021199.1	Sulfide quinone reductase-like	3.4	4.2	3.7
LOC400610	XM_375478.1	Similar to RIKEN cDNA 1100001G20	3.7	3.7	3.7
IGFBP6	NM_002178.1	Insulin-like growth factor binding protein 6	3.7	3.7	3.7
NT5E	NM_002526.1	Ecto-5-prime-nucleotidase	3.2	4.0	3.6
PODXL	NM_005397.2	Podocalyxin-like	3.5	3.6	3.5
ZC3HAV1	NM_024625.3	Zinc finger CCCH type, antiviral Protein 1	3.5	3.4	3.5
IFIT1	NM_001548.1	Interferon-induced prot. with tetratricopeptide repeats 1	3.4	3.5	3.5
BST2	NM_004335.2	Bone marrow stromal cell antigen 2	3.5	3.3	3.4
APM2	NM_006829.1	Adipose specific 2	3.2	3.6	3.4
CXXC5	NM_016463.5	CXXC finger 5	3.2	3.5	3.3
ISG20	NM_002201.4	Interferon stimulated gene 20kDa	3.1	3.3	3.2
KISS1	NM_002256.2	KISS-1 metastasis-suppressor	3.0	3.4	3.2