The Role of Hyaluronan in Angiogenesis Through RHAMM and CD44 Receptors in Human Liver Micro-Vessel Endothelial Cells

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Abstract

Angiogenesis occurs in tissue development and in pathological conditions such as tumour growth. Oligosaccharides of hyaluronan (o-HA; 3-10 disaccharides) are pro-angiogenic and act through the receptors CD44 and RHAMM (receptor for hyaluronan-mediated motility). HA is over-produced by tumour stroma and HA receptors might be implicated in cancer metastasis in liver. Utilizing human liver micro-vessel derived endothelial cells (H_LMVEC), we have investigated the role of CD44 and RHAMM in o-HA-induced angiogenic signalling pathways. H_LMVEC were characterized by immunohistochemistry with antibodies directed against the endothelial cell markers CD31, von Willebrand factor (vWF), CD105 and CD34. Stimulation of H_LMVEC with o-HA enhanced their proliferation, migration and tube formation. To investigate the respective role of the o-HA receptors, we knocked-down CD44 and RHAMM expression by small interference RNA (siRNA) technology. At 48 hours post-transfection and demonstrated by RT-PCR analysis, CD44 and RHAMM gene expression was respectively decreased by ~70% and ~90%, compared to un-transfected and negative control (NC)-siRNA treated cells. At 72 hours post-transfection and demonstrated by Western blotting analysis, CD44 and RHAMM protein expression was decreased by ~90% compared to un-transfected and NC siRNA-treated cells. After CD44 and RHAMM knock-down, transfected cells were examined in in vitro angiogenesis assays, in the presence or in the absence of o-HA. A total decrease of o-HA-induced cell proliferation and migration was observed after CD44 and RHAMM knock-down showing their requirement in the mitogenic and chemotactic activities of o-HA. Surprisingly after CD44 and RHAMM knock-down, a significant increase of o-HA induced tube formation was demonstrated using Matrigel assay. The knock-down of CD44 and RHAMM significantly decreased the phosphorylation of extracellular-signal regulated kinase (ERK) which is the last step of key proteins involved in the signalling pathways resulting in both cell proliferation and migration. Further experiments are in progress to investigate the respective roles of RHAMM and CD44 in o-HA-induced angiogenic signalling pathways. This study may be used to select potential targets to modify wound healing and recovery following acute stroke and tumour angiogenesis.

1. Introduction

Angiogenesis, the formation of new blood vessels from a pre-existing vascular network, is essential for some physiological situations such as tissue development, female reproductive cycle and wound repair and for some pathological conditions such as metastases, tumour progression [1]. The normal blood vessel wall is lined by a quiescent endothelial cell monolayer, which surrounds the lumen. By their key position in the blood vessel structure, endothelial cells (EC) are the main cells involved in angiogenesis. Angiogenesis is a complex process tightly regulated by local balance between pro-angiogenic and anti-angiogenic factors [2]. Under the effect of a pro-angiogenic factor, EC undergo metabolic modifications including stimulation of migration, proliferation to increase the vessel length then differentiation into sprouts with a lumen formation and the production of basement membrane proteins

leading to formation of new blood vessels. Major component of the extracellular matrix, hyaluronan (HA) is a large non-sulphated glycosaminoglycan consisting of simple repeated disaccharide units of D-glucuronic acid and N-acetylglucosamine ([-β (1, 4)-GlcUA-β (1, 3)-GlcNAc] $_n$). Native HA has a molecular weight up to 10^7 kDa and inhibits angiogenesis whereas oligosaccharides of hyaluronan (o-HA) comprising 3-10 disaccharides units are pro-angiogenic and were shown to stimulate angiogenesis *in vivo*. Recently o-HA have been demonstrated to stimulate EC proliferation and migration by activation of downstream intracellular signalling pathways through its main receptors CD44 [3] and the receptor for Hyaluronan-Mediated Motility (RHAMM) [4]. In this paper we tested the effect of o-HA in human normal liver micro-vessel endothelial cells (H_LMVEC) from collaboration with Dr G Alessandri to investigate the roles of CD44 and RHAMM in o-HA-induced angiogenesis. To demonstrate the respective roles, we knocked-down CD44 and RHAMM gene expression by using small interference RNA (siRNA) technology.

2. Experimental procedures

2.1. Reagents

Endothelial basal medium (EBM) and foetal bovine serum (FBS) were provided by BioWhittaker (Cambrex Bioscience, Verviers, Belgium). Glutamine, antibiotics (penicillin and streptomycin), gelatine, bovine serum albumin (BSA), paraformaldehyde (PFA), ethylene diamine tetra-acetic acid (EDTA), trypsin-EDTA, sodium orthovanadate, sodium deoxycholate, ethylene glycol tetra-acetic acid (EGTA), phenylmethylsulfonyl fluoride (PMSF), digitonin, leupeptin, pepstatin, hydrocortisone, and heparin were purchased from Sigma-Aldrich (St Louis, MO), phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} from Oxoid (Hampshire, England) and Tris-hydroxychloride (Tris-HCl), sodium dodecyl sulfate (SDS), Triton X-100 from BDH (Poole, England). Growth factor-reduced Matrigel was provided by Becton Dickinson (BD Biosciences, San Jose, CA). Bovine recombinant basic fibroblast growth factor (FGF-2) and recombinant human epidermal growth factor (EGF) were bought from R&D systems (Minneapolis, MN). Oligosaccharides of hyaluronan were prepared as previously described [3].

2.2. Antibodies

The following antibodies against human surface receptors and intracellular proteins were used: anti-mouse/human CD44 monoclonal antibody (rat IgG_{2b}, κ, clone IM7) purified and goat anti-RHAMM polyclonal antibody (E-19) were purchased from BioLegend (San Diego, CA) and Santa Cruz Biotechnology (Heidelberg, Germany), respectively. Goat anti-human CD105, rabbit anti-human von Willebrand factor (vWF), and mouse anti-human CD31 antibodies were provided from DAKO (Copenhagen Denmark).

2.3. Human micro-vessel endothelial cell culture

Human liver-derived micro-vessel endothelial cells (H_LMVEC) were isolated by our collaborator. The cells were plated onto T-75 culture dishes, previously coated with 1μg/cm type I collagen and 1μg/cm fibronectin for 12 hours in 37^0C in humidified atmosphere of 5% CO_2 The cells were cultured in EBM growth medium, composed of endothelial basal medium (EBM) supplemented with: 15% foetal bovine serum (FBS), 1 μg/ml hydrocortisone, 100 μg/ml heparin, 2 ng/ml basic fibroblast growth factor (FGF-2), 10 ng/ml epidermal growth factor (EGF), 10 μg/ml bovine brain extract (ECGF) and 0.5 ml of antibiotic (25 mg/ml gentamicin and 50 μg/ml amphotericin B). Morphologically normal liver EC presented larger cells, cytoplasmic elongations and much lower density on reaching confluence. Throughout this study, H_LMVEC were used at passage 10-14.

2.4. Immunohistochemistry staining

H_LMVEC were seeded at 10^5cells/ml in EBM growth medium on chamber slides previously coated with type I collagen and fibronectin. After 24-48 hours of incubation at 37^0C, the cells were fixed in cold-ice solution of acetone/methanol (v/v) for 20 min at -20^0C. Washed twice in PBS and incubated in 0.5% hydrogen peroxide for 30 min, the cells were washed again with PBS for 5 min. Non-specific sites were saturated with blocking serum (1% bovine serum albumin, BSA or horse serum, provided from Vectastain Kit) for 20 minutes. Diluted primary antibodies directed against von Willebrand factor ($_V$WF; 1:50), CD31 (PECAM-1; 1:50), and CD105 (1:50) were incubated for 1 hr at room temperature. The cells were washed 3 times in PBS and the secondary antibodies conjugated to biotin (1:50) were added for 30 min incubation. After washing with PBS, avidin-D conjugated with horseradish

peroxidase (1:200) was added for 30 min incubation at room temperature. Then the cells were washed with PBS. The DAB Substrate chromogen solution from Vectastain Kit was added and incubated for 5-10 min at room temperature until suitable staining was developed and the slides, well rinsed in distilled water. The cells were counterstained with heresy hematoxyline for 2 min then the slides were washed with running water and finally mounted with aqueous mounting medium.

2.5. Short-Interfering RNA targeting to CD44 and RHAMM

Short-interfering RNA targeting human CD44 (CD44 siRNA) or RHAMM (RHAMM siRNA) hyaluronan receptors were selected online from MWG biotech (MWG-biotech.com) from human gene sequences encoding CD44 (GenBank access number: NM_001001390, NM_001001391) and RHAMM (GenBank access number: (NM_012484, NM_012484). Twenty-one nucleotide RNA sequences were chemically synthesized by GenePharma (Shanghai, China). We designed 3' overhangs of 2'-deoxythymidine in all siRNAs used in this study. The siRNA sequences of human CD44 and RHAMM corresponded to the coding regions 494-643 and 1236-1767 relative to the first nucleotide of the start codon, respectively shows the sequences of each siRNA pair used (Table 1). The duplexes of siRNA were reconstituted in 140 µl of H_LMVEC media only to a final concentration of 20 µM. Negative control (NC) siRNA duplexes were included in all experiments. Selected specific sequences were subjected to a BLAST search to ensure there was no significant homology with other genes. To examine the transfection efficiency, NC-FAM (a fluorescein derivative) siRNA was transfected into H_LMVEC, and the following day, cells were washed with PBS, fixed with 4% PFA and visualized with a Zeiss fluorescent microscope. For mRNA 'knock-down', the reverse transfection method was applied. Briefly, the siRNA duplexes were transfected into 70-80% confluent HLMVEC cultured in 24-well plates at a final concentration of 50 nM. Cells were cultured for a further 48-72 hrs according to the assays used.

2.6. RNA extraction and RT-PCR

Extraction of total RNA was performed using a modified protocol combining the cell lysis solution from SuperScript III Cellsdirect cDNA Synthesis System – Invitrogen kit (Invitrogen, Carlsbad, CA) with DNase I from Cells-to-cDNA II-Ambion kit (Ambion, Cambridgeshire, UK). The amount of RNA from each condition was normalised to the number of cells.

Briefly, 2 µl of cell suspension in PBS (8 000 cells/µl) were lysed with 1 µl of lysis enhancer and 10 µl of lysis buffer (Invitrogen kit) for 10 min at 75°C. Cell lysates were kept on ice for 5 minutes then briefly vortexed. RNA samples were obtained after enzymatic degradation of DNA with DNase I (Ambion) for 15 min at 37°C followed by 5 minutes at 75°C then briefly vortexed. RT-PCR was used to monitor human CD44 and RHAMM mRNA. All RT-PCR reagents were acquired from Promega (Southampton, UK). Complementary DNA (cDNA) was produced from total RNA extracts in two steps. First, total RNA samples were mixed with oligo$(dT)_{15}$ primers and 10 mM dNTP mix for 5 min at 70°C. After 3 min on ice, reverse transcriptase, ribonuclease inhibitor, 5X reaction buffer, $MgCl_2$ and nuclease-free water were added to the RNA samples. cDNA was polymerised at 50°C for 50 min followed by inactivation of the reaction at 85°C for 5 min. All gene-specific primer pairs used for PCR were individually optimized for cycle number (CD44 and S14 = 35 and RHAMM = 40 cycles) and t_m (60°C). Primer pairs (Invitrogen) were selected by software Primer3 and derived from human gene the sequences are shown (Table 2). Briefly, for each PCR reaction, 1 µl of cDNA from reverse transcriptase reactions was mixed with 1 µl of gene specific primers (forward and reverse each at 1 µM), 5 µl of 2X Master Mix and 3 µl of nuclease-free water. Amplification conditions were 10 min at 94°C and 35 (for CD44 or S14) or 40 (for RHAMM) cycles of 1 min at 94°C/ 1 min at 60°C/1 min 30 at 72°C then 10 min at 72°C. PCR products were analysed by 1.5% agarose gel electrophoresis. Human ribosomal protein S14 expression was also used as a cDNA loading control.

Table 1. SiRNA sequences used for human CD44 and RHAMM knock-down gene expression.

Human Gene (assession number)	siRNA sense sequence (5' → 3')	siRNA anti-sense sequence(5' → 3')
CD44-1(NM_001001390)	UGA CAA CGC AGC AGA GUA AdTdT	AA| UGA CAA CGC AGC AGA GUA A|UUdTdT
CD44-2(NM_001001391)	UGA CCA ACU GUU AUU GUU CdTdT	AA| UGA CCA CUG UUA UUG UUA C|UUdTdT
RHAMM-1(NM_012484)	GUG GCG UCU CCU CUA UGA AdTdT	AA|GUG GCG UCU CCU CUA UG|AAdTdT
RHAMM-2(NM_012484)	GGA GUC UCG AAG AGU CUC AdTdT	AA|GGA GUC UCG AAG AGU CUUCA|AGdTdT

Table 2. Human specific primer sequences and expected size of amplified products

Primers	Sequences	Size (bp)
CD44f	5'-GTGGGCCAACAAAGAACACT-3'	218
CD44r	5'-TGGAGCAGGCCCAAATATAG-3'	218
RHAMMf	5'-GAAAGGGAAGGAGGCTGAAC-3'	154
RHAMMr	5'-TGCCAAAATCTGATGCTGAAA-3'	154
S14f	5'-CAGATTTGCGGTCAACAAAGA-3'	180
S14r	5'-CCATGTAGCCCTCTCATCTGC-3'	180

2.7. In vitro angiogenesis assays

2.7.1. Cell proliferation assay

Un-tansfected cells or cells previously transfected with 50 nM CD44, RHAMM or NC siRNA for 48 hrs were seeded in complete medium at a concentration of 2 x 10^4 cells/ml for 2ml/well in 24-well plates (Nunc). After 4 hrs of incubation, the cultured medium was replaced with serum poor medium (SPM) supplemented with 2.5% FBS (in which the cells grew at a significantly reduced rate) with or without o-HA (1 µg/ml). After 72 hrs of incubation, the medium was discarded from each well and the H_LMVECs were washed with 1 ml of PBS without Ca^{2+} and Mg^{2+}, detached with 500 µl of trypsin/EDTA. Cells were checked for their detachment by microscopy. The cells were re-suspended with a syringe then diluted in 10 ml of isotonic solution and counted on a Coulter counter (Coulter Electronics, Hialeah, FL).

2.7.2. Cell migration assay

Un-transfected cells or cells previously transfected with 50 nM CD44, RHAMM or NC siRNA for 72 hrs were seeded (100 µl, 7.3 x 10^4 cells/ml) on the porous membrane of the Boyden chamber with or without o-HA (0.1 µg/ml). After 18 hrs incubation, non-migrated cells were removed with a cotton swab soaked with PBS and migrated cells were fixed with 4% PFA for 15 min then left dried before staining with Giemsa. The cells were counted by using phase-contrast microscopy in eight fields taken in picture with a digital camera. This experiment was performed three times and in duplicate.

2.7.3. Tube formation assay

Un-transfected cells or cells previously transfected with 50 nM CD44, RHAMM or NC siRNA for 72 hrs were mixed (40 µl, 1.5 x 10^6 cells/ml) in equal volume with 40 µl of growth factor-reduced Matrigel (10 mg/ml) with or without o-HA (0.5 µg/ml). Forty µl from the mixture (cells/Matrigel with or without pro-angiogenic molecule) was poured in duplicate into each well and the mixture left to polymerize for 1 hour at 37°C in the incubator. After polymerization, 500 µl of complete culture medium was added to cover each cell/Matrigel spot. After 24 hrs of incubation, cells were fixed with 4% PFA for 15 min. Tube formation was examined by using phase-contrast microscopy in eight fields taken in picture with a digital camera. Tube formation was quantified by counting the total number of closed areas in each well [5].

2.8. Western blot analysis

Cells were seeded in 24-well plates at a concentration of 2.5 x 10^5 cells/450 µl and transfected with 50 µl of CD44, RHAMM or NC siRNA (50 nM in final concentration). After 48 hrs incubation, the medium was replaced with SPM for 48 h incubation then 1 µg/ml o-HA was added to the cells for 7 min stimulation at 37°C in the incubator. After washing in cold PBS, cells were lysed at 4°C for 1 min in 150 µl/well of ice-cold radioimmunoprecipitation (RIPA) buffer (pH 7.5) containing 15 mM Tris-HCl, 150 mM NaCl, 0.5% sodium deoxycholate, 0.5% SDS, 1 mM EDTA, 1 mM sodium orthovanadate, 1 mM PMSF, 1% Triton X-100 and 1 µM leupeptin. The buffer containing the total cell lysates was collected and stored at -20°C. Protein concentration of cell lysated were determining using Biorad protein assay and equal amount of proteins was mixed with 2X Laemmli buffer and boiled in a water bath for 15 min. Samples were separated along with pre-stained molecular weight markers (27 – 180 kDa) by 12% SDS-PAGE. The proteins were electroblotted (Hoefer, Scientific instruments) onto nitrocellulose membranes at 90mA/gel for 1 hr. Membranes were blocked with 1% BSA in TBS-Tween for 1h at RT, and stained overnight with diluted primary antibody directed against α-actin (1:1 000), CD44 (1:250), RHAMM (1:500), ERK (1:1 000) and phosphorylated ERK (^{P}ERK, 1:1 000) in blocking buffer at 4^{o}C on a rotating mixer. Anti-α-actin and anti-ERK were used to show equality of protein loading. Membranes were washed in TBS-Tween (5 x 5min) and stained with the appropriate peroxidase-conjugated secondary antibody (diluted in 5% milk in TBS-Tween) for 1h at RT, then washed in TBS-Tween (5 x 5min). Blots were developed using the ECL or ECL^{plus} Western blotting detection systems. The relative amounts of proteins were semi-quantitatively estimated by measuring the density of the bands on the X-ray film in a laser scanning densitometer or by analysis with camera connected with G:BOX image analysis software.

3. Results

3.1. Characterization of human micro-vessel endothelial cells

By immunohistochemistry staining, H_LMVECs were characterized using antibodies directed against von Willebrand factor (VWF) (showing a cytoplasmic

staining) and CD31 (PECAM-1) (showing an inter-membrane staining) which are known EC markers (data not shown).

3.2. Knock-down of CD44 and RHAMM gene/protein expression

The transfection experiments using the reverse method were optimized by the efficacy of the transfection reagent for siRNA duplexes taken up by H_LMVECs. The knock-down of CD44 and RHAMM gene expression was demonstrated by RT-PCR analysis and by immunofluorescent staining and Western blotting, for protein expression. The efficiency of siRNA transfection using FAM fluorescein-conjugated negative control (NC) siRNA was evaluated. The transfection efficiency was determined by counting the fluorescent cells at 24 h post-transfection and almost 100% of H_LMVECs were transfected with FAM-NC siRNA duplexes, visualized by fluorescence microscopy in the cytoplasm of the cells (Figure 2).

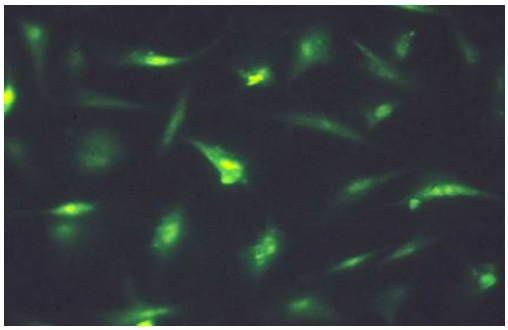

Figure 2. Uptaken of 100 nM NC siRNA-FAM by H_LMVEC (original magnification X400)

By RT-PCR, we demonstrated after 48 hrs incubation a significant decrease of about 70% and 90% of CD44 and RHAMM gene expression, respectively compared to un-transfected control cells and NC siRNA-treated cells (data not shown). At 72 hrs post-transfection, cells transfected with CD44 or RHAMM siRNA showed a decrease by about 90% in both CD44 and RHAMM expression, compared to un-transfected cells and NC siRNA-treated cells (Figure 3).

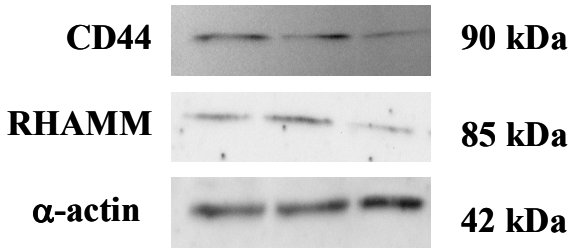

Figure 3. Knock-down of CD44 and RHAMM after 72 hrs transfection with CD44 and RHAMM siRNA

3.3. Both CD44 and RHAMM knock-down inhibited o-HA-induced H_LMVEC proliferation

After 72 hrs incubation, o-HA significantly increased by 2-fold ($p < 0.001$) the proliferation of H_LMVEC, compared to the control (Figure 4). After the knock-down of CD44 and RHAMM gene expression, a total decrease of o-HA-induced H_LMVEC proliferation was observed, compared to o-HA alone whereas o-HA kept its mitogenic effect in NC siRNA-treated cells.
In the absence of o-HA and from transfected cells, a significant decrease of the cell number was observed after RHAMM knock-down, compared to un-transfected control cells and NC siRNA-treated cells.

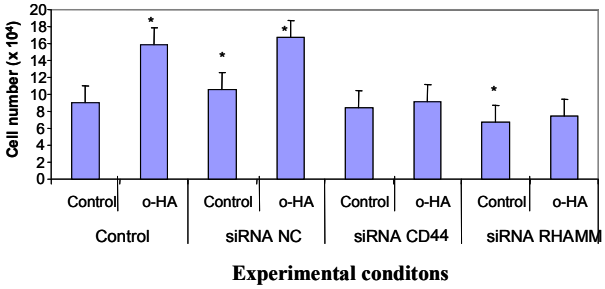

Figure 4. Inhibition of o-HA-induced ed H_LMVEC proliferation after CD44 and RHAMM knock-down

3.4. Both CD44 and RHAMM knock-down inhibited o-HA-induced H_LMVEC migration

In Migration assays and after 18 hrs incubation, o-HA significantly increased by 4-fold ($p < 0.01$) the migration of H_LMVEC, compared to the control (Figure 5). After RHAMM and CD44 knock-down, a 5-fold significant decrease of o-HA-induced cell migration was observed, compared to o-HA effect in un-

transfected control cells and NC siRNA-treated cells (Figure 5).

3.5. Increase of o-HA-induced tube formation after CD44 and RHAMM knock-down

In Matrigel assays, o-HA significantly induced increased EC tube formation (4-fold; $p < 0.01$), compared to the control (Figure 6). After the knock-down of CD44 and RHAMM expression, we observed an increase in o-HA-induced tube formation for both CD44 and RHAMM (2-fold; $p < 0.01$), compared to the effect of o-HA in NC siRNA-treated cells and un-transfected control cells (Figure 6).

3.6. Both CD44 and RHAMM knock-down inhibited o-HA-induced ERK phosphorylation

We showed a decrease of ^{p}ERK expression which is the last step of key protein involved in angiogenic signalling pathways after both CD44 and RHAMM knock-down (data not shown).

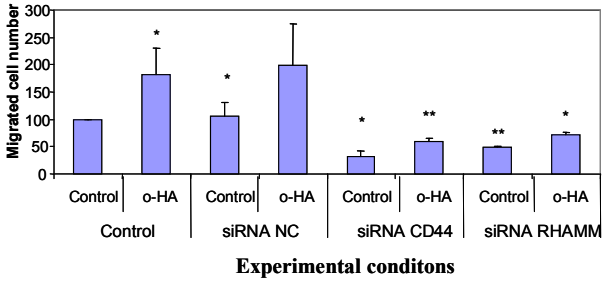

Figure 5. Inhibition of o-HA-induced ed H_LMVEC migration after CD44 and RHAMM knock-down

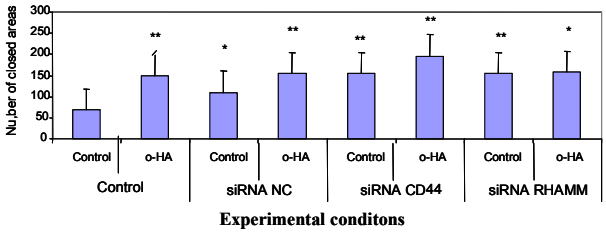

Figure 6. Inhibition of o-HA-induced ed H_LMVEC tube for,ation after CD44 and RHAMM knock-down

3. Discussion

Hyaluronan, a major component of the extracellular matrix, is an important regulator of angiogenesis. Its degradation products, oligosaccharides of hyaluronan (o-HA), have been shown to stimulate EC proliferation and migration by activation of downstream intracellular signalling pathways through its main receptor CD44 [3] and receptor for hyaluronan-Mediated Motility (RHAMM). In this paper, we have shown the involvement of CD44 and RHAMM in the mediation of o-HA's pro-angiogenic effects including H_LMVEC proliferation, migration and tube formation and we have investigated the associated signalling pathways. To delineate roles of o-HA receptors, we knocked-down the gene expression of CD44 and RHAMM using specific siRNA to degrade their respective messenger RNA and we confirmed it by using *in vitro* angiogenesis. Recently using siRNA technology, high molecular weight HA has been reported to induce vascular smooth muscle cell migration through RHAMM activation but not through CD44 with an involvement of phosphatidylinositol 3-Kinase (PI3K)-dependent Rac activation [7]. Our study showed that CD44 or RHAMM knock-down inhibited o-HA induced EC proliferation and lost its mitogenic effects. In contrast to our results, Savani et al. [6] showed a role for CD44 but not RHAMM in HUVEC proliferation using blocking antibodies. Our results showed that CD44 or RHAMM knock-down decreased o-HA induced EC migration and lost its chemotactic effect with a stronger inhibition in RHAMM siRNA-treated cells. RHAMM functions as a cytoskeletal accessory protein and not as a surface receptor for HA. Recently, a key role of RHAMM as a cell growth regulator was reported in mitosis following binding to mitotic spindles. In agreement with our results, other reports showed an inhibition of glioma cell migration on a surface pre-coated with HA by an anti-RHAMM antibody but not by anti-CD44.We determined increases in o-HA-induced tube formation after CD44 or RHAMM knock-down. Although CD44 knock-down had significant decreased effect on EC proliferation and migration, surprisingly it resulted in an increase of tube-like structures formation in Matrigel. Compared to our study with EC down-regulated CD44 expression, others demonstrated the involvement of endothelial CD44 in *in vivo* angiogenesis. These results demonstrate the important and central role of CD44 and RHAMM in o-HA-induced angiogenesis. In our study, we have highlighted key proteins involved in o-HA-induced signalling pathways through CD44 or RHAMM after their knock-down by siRNA.We showed a decrease of ^{p}ERK expression which is the last step of key protein involved in signaling pathways resulting in both cell proliferation and differentiation for both CD44 and RHAMM whereas CD44 is less involved in cell migration in o-HA-induced ^{p}Erk over-expression. This study has identified the receptor-signal transduction pathways through which o-HA stimulates neo-vascularization. In summary, the loss of CD44 and

RHAMM receptor expression by siRNA technology on ECs induced loss of the o-HA's pro-angiogenic effect on cell proliferation and migration. RHAMM appears to play a central role in mediating o-HA-induced *in vitro* angiogenesis. A better understanding of RHAMM in the formation of vessels *in vivo* may provide insights into RHAMM inhibition as a therapeutic target for drug discovery.This study may be used to define potential selective targets for modifying endothelial cell function in order to intervene in different angiogenesis processes associated with wound healing, recovery following acute stroke and tumour angiogenesis.

4. References

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