EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 1 (January-2015) pp. 4-15

www.ijcrbp.com

Original Research Article

Investigating the Expression of Hepatocyte Growth Factor and Macrophage Stimulating Protein Genes and their Receptors in Human Scalp Hair Follicles

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Abstract

The study was aimed to investigate whether the mRNA for hepatocyte growth factor (HGF), macrophage stimulating protein (MSP), and their receptors, c-Met and RON, are expressed by human scalp hair follicles. Scalp skin from healthy individuals undergoing cosmetic surgery was transported in RNA*later* for molecular biological investigations or growth medium for cell culture. Human scalp hair follicles were microdissected and PCR was carried out. Gene identity was confirmed by sequencing. Further investigation was done at the level of hair bulb components including the dermal sheath, epithelial matrix and dermal papilla to refine the location of these molecules within the hair follicles. In addition, dermal papilla cells were cultured to examine whether its cells express the genes for these molecules. This study demonstrated the expression of HGF, MSP and their receptors in human scalp hair follicles. Results showed that the dermal papilla and dermal sheath of non-balding scalp hair follicles express HGF, MSP, c-Met and RON, while the matrix cells only produce MSP and RON. The results also showed that the relative expression levels of HGF and MSP being higher than that of their receptors, and the expression levels of MSP were significantly higher than that of HGF. This suggests that HGF and MSP may play a role as possible paracrine factors produced by the dermal papilla cells and therefore modulate the hair growth.

Keywords

c-Met

Gene expression

Hair follicle

Hepatocyte growth factor

Macrophage stimulating protein

RON

Introduction

Hepatocyte growth factor (HGF) is a multifunctional peptide which acts as a mitogen (Sonnenberg et al., 1993), motogen (Stoker et al., 1987; Weidner et al., 1990) and morphogen (Montesano et al., 1991), and has effects on various epithelial and endothelial cells. HGF interacts with responsive cells by binding to the cell surface receptor, c-Met, activating a tyrosine kinase signaling cascade (Carpenter and Liao, 2009) leading to regulation of cell growth, cell proliferation, cell motility, and morphogenesis (Medico et al., 1996).

HGF may act as a paracrine factor secreted by mesenchymal-derived cells in a variety of organs and acts primarily upon epithelial cells and endothelial cells (Rubin et al., 1991; Matsumoto and Nakamura, 1992; Sulpice et al., 2009). HGF also plays an important role in embryonic organ development and in adult organ regeneration (Stern et al., 1990), for example in lung formation and repair (Ohmichi et al., 1998; Mizuno et al., 2005; Lassus et al., 2006), the early development of the kidney and kidney repair (Woolf et al., 1995; Van Adelsberg et al., 2001; Baer and Geiger, 2006), and angiogenesis and vascular repair (Montesano et al., 1991; Rosen et al., 1991; McKinnon et al., 2006). HGF may interact with other paracrine factors. Based on *in vitro* studies, basic fibroblast growth factor (bFGF), which plays a role in embryonic development and wound repair, has been found to stimulate HGF secretion in many of mesenchymal-derived cell lines in human beings (Roletto et al., 1996).

HGF has been implicated in hair growth control. It has been observed to stimulate growth of human scalp hair follicles (Jindo et al., 1995) as well as mouse vibrissae (Jindo et al., 1994) in organ culture. Human recombinant HGF can also stimulate the growth of mouse pelage hair follicles *in vivo* and retards murine hair follicles regression into catagen (Jindo et al., 1998). When recombinant mouse HGF was injected under the back skin of mice, the result showed that HGF significantly delayed catagen development during both early and late stages of hair follicle regression *in vivo* (Lindner et al., 2000). The gene for HGF was strongly expressed in rat anagen tissue and slightly in telogen tissue, and HGF mRNA was detected in rat cultured dermal papilla cells, using RT-PCR (Yamazaki et al., 1999). HGF has also been observed to localise to the dermal papilla cells of mouse pelage anagen follicles using immunohistoreactivity (Lindner et al., 2000). These results suggest that HGF can regulate rodent hair follicles and is produced by their dermal papilla. Another study showed that HGF can increase DNA synthesis in cultured keratinocytes derived from human scalp hair bulb in a dose-dependent manner *in vitro* (Shimaoka et al., 1995). In addition, HGF stimulated the proliferation of rat vibrissae dermal papilla cells with maximal effect at 50ng/ml in a concentration-dependent manner, whereas there was minimal effect on cultured fibroblasts (Yu et al., 2004).

Cultured dermal papilla cells of human scalp hair follicles were found to express HGF, which has stimulatory effects on human scalp hair follicle growth *in vitro* (Shimaoka et al., 1995). No effects were noticed in HGF gene expression when balding, non-balding scalp and beard cultured dermal papilla cells were cultured with testosterone (Merrick, 2000). However, HGF gene expression was very low in balding scalp cells whereas beard cells showed a greater expression than non-balding scalp cells. This differential expression suggests that HGF may play a role in maintaining large follicles and the levels of HGF in androgen dependent follicles may be changed by *in vivo* androgen exposure (Randall et al., 2001). The gene for the HGF receptor, c-Met, was expressed in human scalp and rat vibrissae cultured dermal papilla cells as observed by the RT-PCR technique, and it was also found in rat vibrissae follicular matrix keratinocytes by in situ hybridization (Yu et al., 2004). c-Met was also prominently localised in the inner root sheath and outer root sheath in mouse pelage anagen follicles using immunohistochemistry (Lindner et al., 2000).

Another member of hepatocyte growth factor family also implicated in hair follicle growth is macrophage stimulating protein (MSP). It was initially considered as a serum factor which stimulated chemotaxis of peritoneal resident macrophages (Brunelleschi et al., 2001). MSP exerts its actions on target cells by binding to a cell surface receptor called RON (Recepture d'origin Nantaise) in human beings (Camp et al., 2007), also known as macrophage stimulating 1 receptor (MST1R). RON belongs to the Met proto-oncogene family, a distinct subfamily of th receptor tyrosine kinase family (Wang et al., 2003).

Several functions for MSP have been determined, including the ability to act as an inflammatory cytokine to activate macrophages (Skeel et al., 1991), promote bone resorption (Kurihara et al., 1996; 1998), induce cell motility (Santoro et al., 1996), promote cellular proliferation (Gandino et al., 1994), stimulate liver cell morphogenesis and motogenesis (Medico et al., 1996), promote the growth and motility of keratinocytes (Wang et al., 1996b), stimulate maturation of megakaryocytes (Banu et al., 1996), and prevent cellular apoptosis (Iwama et al., 1996) of epithelial cells separated from the extracellular matrix (Danilkovitch and Leonard, 2001).

MSP induced telogen follicles to enter the anagen growth phase *in vivo* (McElwee et al., 2004). In addition, exposure to different concentrations of MSP *in vitro*, gave an increase in human hair follicle length in organ culture. Using immunohistochemistry, the MSP receptor, RON, was localised in human hair follicles, with higher intensity in the outer and inner root sheath, and hair matrix, to a lesser extent in the dermal papilla (McElwee et al., 2004). Therefore MSP may also play a role as a paracrine modulator of hair growth.

Materials and methods

Biological materials

Human skin samples from non-balding individuals were obtained from healthy donors undergoing elective cosmetic dermatological surgeries, with full written donor consent. For molecular biological investigations, tissues were collected from occipital regions of three men (aged 30, 29, and 37 year) and two women (aged 32 and 37 year) were placed individually into sterile universal tubes (10 ml) containing RNA stabilization solution, RNA*later* (Sigma-Aldrich Ltd., UK), to inhibit RNases. The samples were stored in the fridge at 4°C overnight to allow the RNA*later* to penetrate the tissues. For cell culture investigations, two women (aged 34 and 37 year) donated skin. Samples were collected into sterile universal tubes containing RPMI 1640 growth medium (Gibco, Paisly, UK).

Human anagen hair follicles from non-balding scalp were microdissected individually from each skin samples under a leica MZ8 dissecting microscope (Wetzlar, Germany) using sterile equipment and plastic ware. Each skin sample was transferred to a petri dish containing RNA*later* for molecular biological investigations or PBS (Oxoid, Basingstoke, UK) for cell culture. The skin sample was cut at the junction between the epidermis and dermis using a sterile scalpel blade. The hair follicles were pulled from the skin gently using fine forceps and then transferred into another petri dish containing fresh RNA*later* or PBS kept on ice. The isolated hair follicles were cleaned of any dermis or fat debris under a higher magnification using sterile syringe needles (27G1/2 tuberculin syringe; Sigma).

To localize the gene expression in the hair bulb, bulb components, the dermal sheath, dermal papilla, and epithelial hair matrix, were microdissected from 150 follicles from each individual before separate total RNA isolation. Hair bulb components from three individuals were analyzed separately.

Cell culture procedure

The isolated dermal papilla cells were placed into a polystyrene rectangular canted-neck cell culture flask with a surface area of 25 cm² (Thomas Scientific, USA). Each flask contained 10 ml RPMI 1640 growth medium (Gibco). The growth media was routinely supplemented with penicillin (10 units/ml), streptomycin (100 µg/ml), fungizone (2.5 ng/ml), 2 mM L-glutamine and fetal calf serum (20%, v/v). The flasks were placed in the incubator for 24 hours to allow the cells to attach to the surface of the flasks. Cell culture was carried out in a humidified atmosphere at 37ºC in 95% air and 5% CO2 using a Heraeus B5060 EK incubator. The medium was changed every 3 days. The maintenance of the dermal papilla cells was performed in a Class II MDH laminar flow cabinet. Growing cultures were viewed daily using an inverted, phase contrast microscope (Leitz, Wetzlar, Germany) to observe general morphology and growth conditions. Photographs of cultures were taken under a phase contrast microscope (Leitz).

Passaging was carried out when cell cultures reached approximately 90% confluence. Trypsinisation was performed to detach cells from the flask by proteolysis. The medium was emptied from the flasks and the cells were rinsed three times in a suitable amount of sterile PBS (10-15 ml), to remove any excess serum protein which could possibly inactivate the trypsin. An appropriate volume of trypsin/EDTA, approximately 500 µl, was added to each cell culture flask (25 cm²) and the flasks were replaced in the incubator for approximately 2-3 min until the cells had rounded up and detached. The cell detaching was viewed under a phase contrast microscope (Leitz) until all cells had detached from the surface of the flask. The cells of each flask were transferred and divided between three 25 cm² flasks containing 10 ml of fresh growth media. The flasks were placed back in the incubator and the media changed every 3 days. When cultured dermal papilla cells reached confluence, they were collected from the flasks using a cell scraper (Bioscience Technology, USA) and transferred to universal tubes. The tubes were

spun down in a Sanyo Harrier 15/80 centrifuge (Jepson Bolton & Co Ltd, UK) at 1200 rpm for about 10 min until the cell pellets were formed. The supernatant was carefully removed to avoid disruption of the cell pellets. The pellets were resuspended in 2 ml of stabilization reagent, RNA*later*. The resulting pellets were used for RNA isolation and later cDNA synthesis.

Molecular biological investigations

Total RNA isolation: Total RNA was extracted from scalp anagen follicles or isolated follicular components from each individual immediately after microdissection using the GenElute Mammalian Total RNA kit (Sigma) or RNeasy Mini Kit (Qiagen, Crawly, UK). The extraction process was performed in an area cleaned before use with 70% (v/v) ethanol and RNase Zap solution (Sigma). The quality of total RNA was checked by agarose gel electrophoresis 1.5% (w/v) before further purification to isolate poly (A) RNA (i.e., mRNA) using GenElute mRNA Miniprep Kit (Sigma) following the manufacturer's instructions.

RT-PCR: RT-PCR was used to investigate the expression of mRNA for HGF, MSP, and their receptors; c-Met and RON in anagen scalp hair follicles. In order to ensure that the RNA samples to be used for cDNA synthesis were free of any contaminating DNA, the sample was treated with the DNA amplification Grade I Kit (Invitrogen Ltd., UK). cDNA synthesis was carried out by using an Avian Myloblastosis Virus (AMV) reverse transcription system (Promega, Southampton, UK) to produce single strand cDNA from DNase-treated poly (A) RNA. The cDNA was aliquoted into 10 µl cDNA portions for storage at -20°C until required.

PCR ampilification was performed using 5 µl of cDNA in 50 µl reaction volume containing 0.5 µM concentrations of forward and reverse primers (Sigma-Genosys Ltd., Pamisford, UK), 5 µl of 10X PCR reaction buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl; Invitrogen), 200 µM concentrations of each dNTP (Promega), 1.5 µM (HGF and RON) or 2.5 mM (β-actin, MSP, and c-Met) MgCl2 depending upon primer set, and 0.5 µl of Taq DNA polymerase (5 units/ µl; Invitrogen). For each PCR reaction, a negative control was set up replacing the cDNA with nuclease free water. To prevent evaporation of the reaction mixture, one drop of mineral oil (Sigma) was added on the top of the mixture. The primer sequences used for RT-PCR had been previously optimised and used previously by other workers. The primers were as follows:

β-actin: (Davies et al., 2005)

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Forward
5'-TCTGGCACCACACCTTCTACAATGAGCTGCG-3'
Reverse
5'-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3'
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HGF: (Shimaoka et al., 1995)

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Forward
5'-TTCACAACCAATCCAGAGGTACGC-3'
Reverse
5'-GAGGGTCAAGAGTATAGCACCATG-3'
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MSP: (Shorter et al., 2008)

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Forward
5'-AGGAGGATGTG GCAGATGC-3'
Reverse
5'-GA TTTGATGCCGCAGCTCT-3'
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RON: (Matsuzaki et al., 2005)

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Forward
5'-TCAACTCCCACATCACCATCTG-3'
Reverse
5'-AGTGTAAAGCCAGCAGCTCCAT-3'
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c-Met: (Imaizumi et al., 2003)

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Forward
5'-ACTCCCCCTGAAAACCAAAGCC-3'
Reverse
5'-GGCTTACACTTCGG GCACTTAC-3'
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Initial denaturation of cDNA at 95°C for 5 min was followed by 35 cycles of PCR ampilification. Cycling conditions were as follows: for β-actin and RON, 35 cycles of 95°C for 1 min, annealing at 56°C for 1 min, and 72°C for 1 min; for HGF, 94°C for 1 min, 63°C for 1 min and 72°C for 60 sec for 45 cycles; for MSP, 35 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 60 sec; for c-Met, 94°C for 1 min, 60°C for 1 min and 70°C for 60 min for 30 cycles. Final extension period of 11 min at 72°C completed the thermocycling before cooling at 4°C. The PCR products were analyzed by gel electrophoresis on 1.5% Tris-Acetate EDTA (TAE) agarose gel (Invitrogen). PCR products were visualised using the Uvitec gel documentation system (Uvitec Limited, Cambridge, UK) at 312 nm wavelength and the image captured.

DNA Sequencing of PCR products: To confirm the identity of PCR products, the PCR process was repeated with thin-walled PCR tubes (VWRInternational, Poole, UK). The PCR products were separated by using a low melting point agarose gel to allow the use of a low temperature for dissolving the gel and to facilitate the isolation of DNA fragments from the gel. The DNA fragments were extracted from the gel using a MinElute Gel Extraction Kit (Qiagen), following the manufacturer's instructions. The purified sample of PCR product was sent to Complement Genomics (Sunderland, UK) for sequencing. The sequencing data were compared to the previously identified gene sequences using the NCBI BLAST programme (http:www.ncbi.nlm.nih.gov/blastlb12seq/wblast2.cgi). The chromatogram of the sequencing data was produced using the Chromas Lite software (version 2.0) available from http://www.technelysium.com.au/.

Real-time PCR: Real-time PCR was performed using the MyiQ™ single-colour real-time PCR detection system (Bio-Rad, UK) and SYBER® Green PCR Master Mix (Applied Biosystem, USA). For each real-time PCR reaction, the following reaction mix was prepared and used: SYBER® Green PCR Master Mix (12.5 µl), the forward and reverse primers of the target gene (1 µl each), the cDNA template (1 µl) and the mixture were brought to 25 µl by adding nuclease-free water (9.5 µl). The reaction mixture was then transferred into an optical 96-well reaction plate (Applied Biosystem). Each well of the reaction plate was also supplied with 25 µl of the appropriate PCR master mix. Each well was tightly covered using specific optical caps. The highly expressed housekeeping protein, GAPDH, was used as an endogenous control. The plate was then placed into the real-time PCR machine.

Real-time PCR was performed using specific forward and reverse primers for each cDNA target sequence. The annealing temperature for each target primer set was initially optimized using the cDNA template, synthesized from the universal human reference RNA (Stratagene, UK) which composed of total RNA isolated from 10 cell lines representing different human tissues which were chosen to ensure a standard broad coverage of human genes. The real-time PCR was performed under the following cycling conditions: 94ºC for 3 min, followed by denaturing at 94ºC for 15 seconds, annealing (gradient) of 55ºC to 63ºC for 30 seconds, followed by 72ºC for 15 seconds; this was repeated for 40 cycles. Real-time PCR data and the differences between samples and controls were calculated using the Genex database software based on the comparative (ΔΔCt) equitation method (Livak and Schmittgen, 2001) to calculate relative quantities of a nucleic acid sequence. The Ct is the threshold cycle during which a reaction emits the threshold level of fluorescence. The detectable amount of fluorescence when a signal is significantly greater than background is known as the threshold. Data was normalized to the corresponding values of an endogenous control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Results

Checking the quality of cDNA

To determine whether human scalp hair follicles actually produce the hepatocyte growth family member genes, the gene expression for HGF, MSP, and their receptors c-Met and RON was investigated using RT-PCR. Total and poly (A) RNA (i.e mRNA) was successfully isolated from microdissected scalp hair follicles (Fig. 1). Prior to investigating the gene expression, the quality of cDNA was initially confirmed by PCR using primers specific for the positive control, housekeeping gene, β-actin, a highly expressed cytoskeletal protein.

The detection of β-actin as a housekeeping gene would denote that the isolated RNA from all experimental samples was of sufficient quality for reverse transcriptase PCR to be performed successfully. The expression of the β-actin sequence which amplified by RT-PCR in all five individual samples were similar in size to that expected from the human sequence which is 838 bp (Fig. 2). The identity of the β-actin PCR products was verified by sequence analysis.

Scalp hair follicles express the genes for HGF, MSP and their receptors

This study demonstrated the expression of hepatocyte growth factor family member genes and their receptors in human scalp hair follicles using PCR. All five human hair follicle cDNA samples produced PCR products of the expected size 262 bp, 415 bp, 536 bp, and 217 bp for the genes of HGF, MSP, and their receptors, c-Met and RON

respectively (Fig. 2). The negative control, in which cDNA was omitted from the PCR reaction mix and replaced with nuclease free water, was clear of any bands. This demonstrated that all PCR products were originated from the amplification of the cDNA synthesis from the mRNA samples and demonstrated an absence of any contamination. Sequence analysis was used to ascertain the identity of the PCR products. The sequenced PCR product of each gene was compared to the known expected human sequence. Thus, sequencing verified all genes against their relevant human sequences in GeneBank.

Fig. 1: Isolated human scalp hair follicle and its bulb. (A) Scalp skin, showing hair follicles and skin layers. (B) Isolated lower human scalp anagen hair follicle. (C) Isolated human hair bulb shows the different parts of the hair follicle including the dermal sheath, hair matrix and dermal papilla. The hair follicles were microdissected for the purpose of total RNA isolation and later investiged for the expression of specific molecules using RT-PCR.

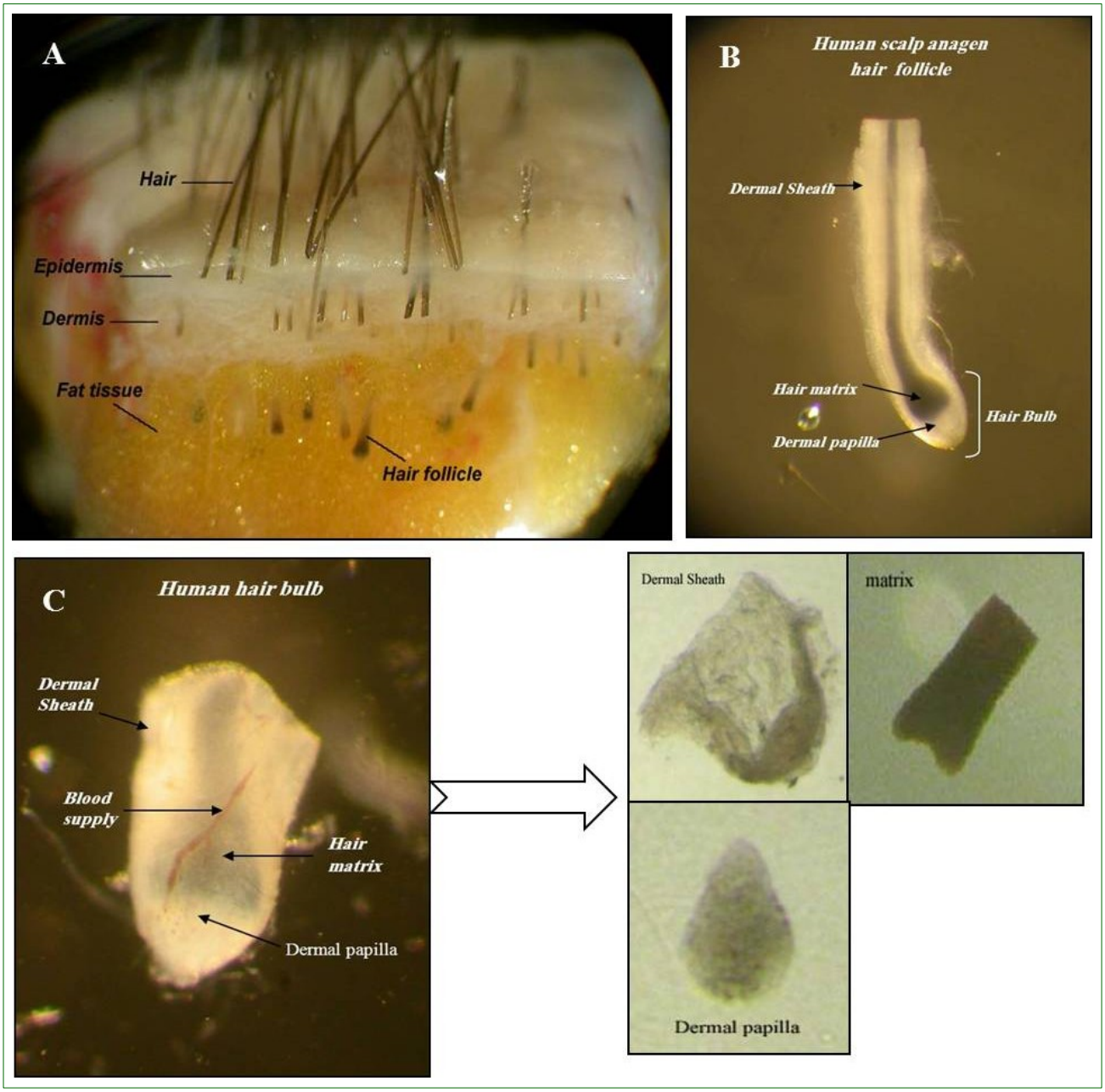


Fig. 2: Expression of HGF, MSP and their receptors in non-balding scalp hair follicles. Reverse transcriptase PCR demonstrated expression of HGF, MSP and their receptors c-Met and RON in mRNA from five human hair follicle samples. Hair follicle cDNA samples were taken from non-balding scalp of five individuals. RT-PCR was performed using specific primers for target genes. The housekeeping gene, β-actin, was used as a positive control. PCR products were applied to 1.5% agarose gel for electrophoresis with ethidium bromide staining. DNA was visualized with UV illumination. Lane 1 denotes 100bp DNA molecular size marker (range from 100-1,500), Lane 2-6 human hair follicle PCR products. Lane 7 contained the negative control in which nuclease free water was used as a template instead of cDNA. The cDNA amplification products were predicted to be in length as indicated.

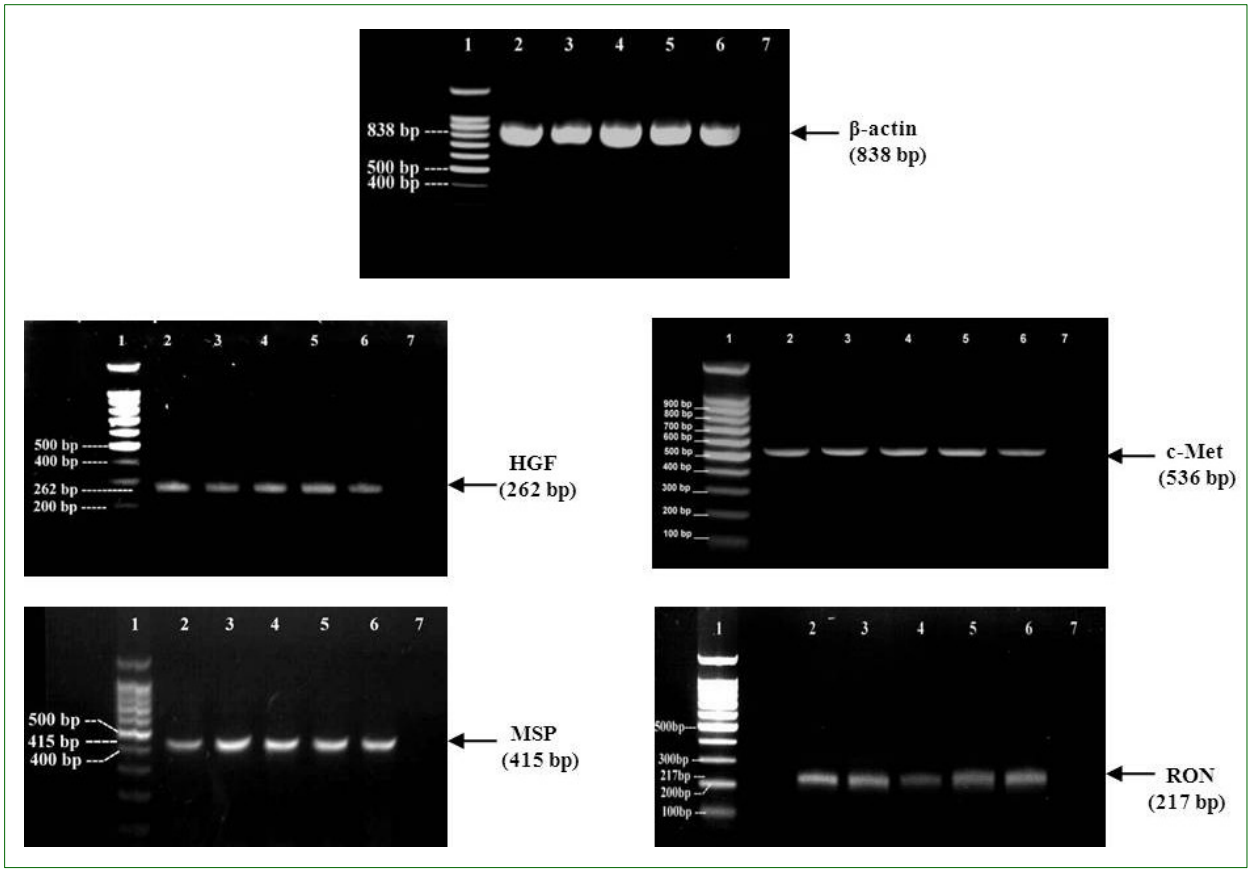


Table 1. Localization of HGF, MSP, and their receptors c-Met and RON in hair bulb components. Hair follicle components were microdissected, and their expression of hepatocyte growth factor family member genes was investigated by RT-PCR. Analysis was performed on the dermal sheath, the hair matrix, and the dermal papilla microdissected from 150 scalp follicles from each of 3 individuals separately. Hair bulb components from all three individuals gave the same pattern of expression. √, expressed; x, not expressed.

Gene	Dermal sheath	Hair matrix	Dermal papilla
HGF	√√√	×××	√√√
MSP	√√√	√√√	√√√
c-Met	√√√	×××	√√√
RON	√√√	√√√	√√√

Localization of HGF, MSP and their receptors in scalp hair bulb tissues

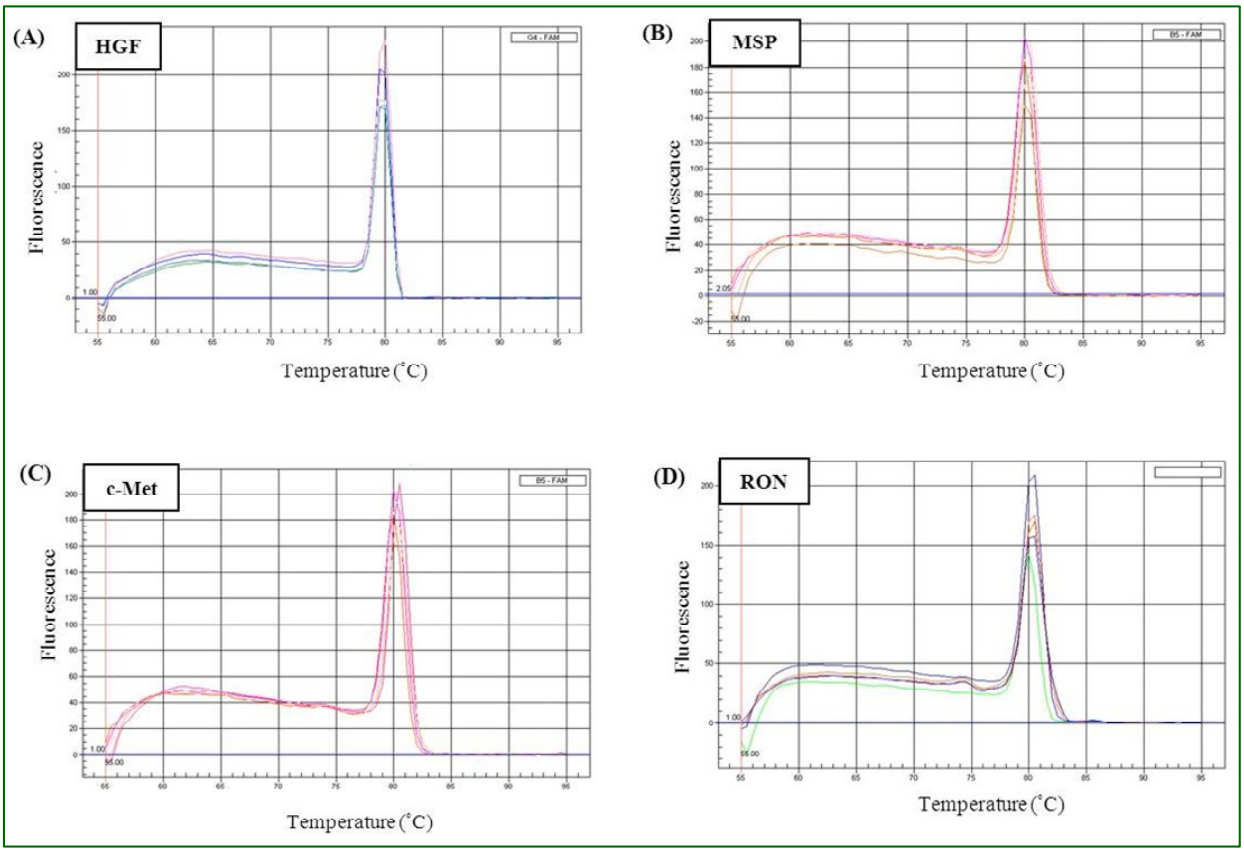
The expression of HGF, MSP and their receptors in human hair follicles was further clarified by the investigation of its expression in the three cell types of the hair bulb using the RT-PCR. Microdissection of the hair follicles bulb components proved to be extremely difficult and time consuming, requiring patience and dedication (Fig. 1). A total of 150 hair follicles were isolated from each individual. Equal numbers of the three cell types of the hair bulb were collected separately from each individual, and sufficient good quality cDNA was obtained. The dermal papilla cells gave the lowest yield of cDNA. The dermal sheath and dermal papilla samples all expressed HGF, MSP and their receptors c-Met and RON (Table 1).

In contrast, the hair matrix samples expressed only MSP and its receptor RON (Table 1). In addition, cultured dermal papilla cells also expressed all four genes (Fig. 3).

The relative expression levels for HGF, MSP and their receptors in scalp follicles

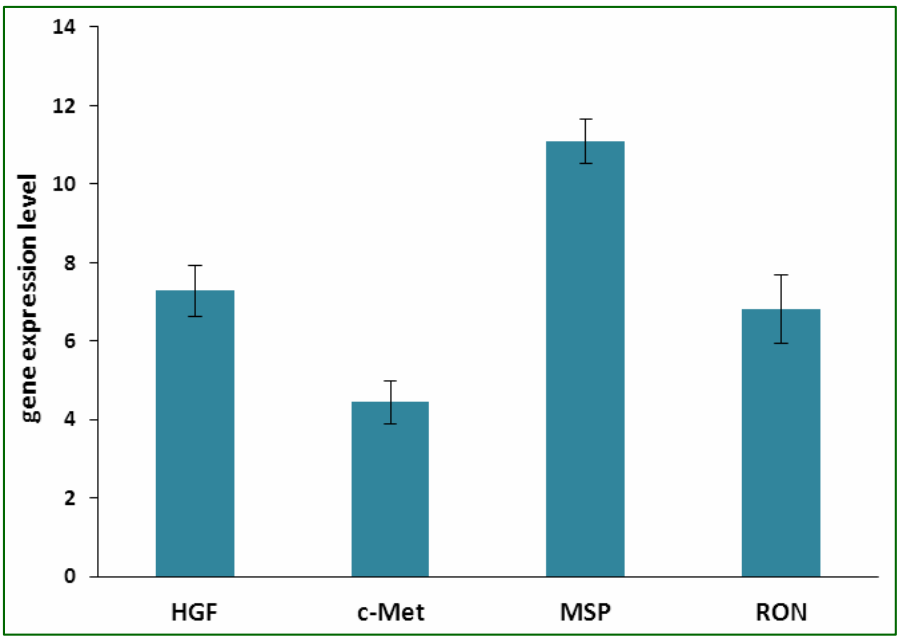
The relative expression levels of HGF, MSP, c-Met, and RON were investigated using relative quantitative real-time PCR. Since SYBR Green binds to any double stranded DNA, it is necessary to examine the specificity of the resulting PCR products of each gene. Melt-curve analysis allows the identification of any non-specific product which may be amplified with these genes such as genomic DNA contamination and primer-dimers, as the presence of a non-specific product would show up as an additional peak in the melt-curve. The melt-curves for all examined genes contained only single peaks indicating that these reactions generated only one product for each gene in each of the five samples used, and no contaminating products were present (Fig. 4). It is apparent from the graphs that the melting temperature (the inflection point) occurred around 80˚C in all investigated genes. Data from all five individual hair follicle samples were collected as cycle threshold (Ct) and the gene expression levels were calculated by normalizing the data against those of the endogenous control GAPDH in each sample.

Fig. 4: Melt-curve analysis for HGF, MSP and their receptors, c-Met and RON. Melt-curves were generated by real-time PCR for HGF (A), MSP (B), c-Met (C) and RON (D) from five different non-balding hair follicle samples. The melt-curves for all genes contain only one peak indicating that no contaminating products are present in these reactions and the reactions in (A), (B), (C) and (D), all generated only one product.



All five non-balding hair follicle samples expressed HGF, MSP and the receptors c-Met and RON, with the relative expression levels of HGF (7.282 ± 0.657) being higher than that of its receptor, c-Met (4.442 ± 0.537) (p<0.0001) and with expression levels of MSP (11.081 ± 0.560) being higher than that of its receptor, RON (6.814 ± 0.862) (p<0.001). In addition, the relative gene expression levels of MSP were significantly higher (p<0.0005) than that of HGF and the relative expression levels of RON were significantly higher (p<0.01) than that of c-Met (Fig. 5).

Fig. 5: Relative expression levels of HGF, MSP and their receptors in non-balding hair follicles. Relative quantitative real-time PCR was performed to analyze the relative expression levels of HGF, MSP, c-Met and RON in human non-balding scalp hair follicles. Expression levels were calculated by normalizing the values against those of the endogenous control (GAPDH). Data are the mean values ± SEM from five different individuals.



Discussion

The expression of HGF and its receptor, c-Met, was investigated in isolated non-balding scalp hair follicles, to examine the suggestion that HGF is a possible paracrine factor involved in hair growth. It has been revealed that HGF may act as a powerful modulator of hair growth and be involved in morphogenesis and cycling (Jindo et al., 1995; Lindner et al., 2000). In a variety of organs, HGF is known to exert its effect as a paracrine factor secreted by mesenchyme- derived cells acting on neighbouring epithelial or endothelial cells (Peus and Pittelkow, 1996; Young-Ran et al., 2001).

The results of this study indicated that the dermal papilla and dermal sheath have the capacity to synthesise HGF and c-Met indicating their ability to produce c-Met which allow them to respond to HGF. It has been observed that the gene for HGF was strongly expressed in rat anagen, and slightly in telogen, follicles using cDNA extracted from skin sections (Yamazaki et al., 1999) and localised to the dermal papilla cells of mouse pelage anagen follicles (Lindner et al., 2000). Yu et al. (2004) reported c-Met expression in human scalp cultured dermal papilla cells and also in rat follicular matrix keratinocytes.

Since the dermal papilla is believed to be the main regulator of the hair follicle and can determine what sort of hair is produced (Jahoda et al., 2001; Randall, 2008), cultured dermal papilla cells are used extensively as a model system (Itami et al., 1995; Jahoda et al., 2001; Inamatsu et al., 2006; Hamada and Randall, 2006). Therefore, the expression of HGF and c-Met, was examined also in human cultured dermal papilla cells of non-balding scalp hair follicles. The detection of genes for both HGF and its receptor, c-Met, in the dermal papilla cells would enable this signalling system to work as an autocrine regulator. Dermal papilla cells have been shown to secrete autocrine regulatory factors which alter the growth of the dermal papilla cells *in vitro* (Thornton et al., 1998; Hamada and Randall, 2006). Since the size of the dermal papilla and number of cells it contains are directly related to the size of the hair produced by the follicles (Van Scott and Ekel, 1958; Elliott et. al., 1999), alteration in such factors could be important in alteration in hair follicle and hair size.

The findings of this investigation also indicated that the dermal papilla, the matrix and the dermal sheath have the capacity to synthesise the MSP receptor, RON, which allows them to respond to MSP. Since the earlier studies detected MSP in the dermal

papilla and dermal sheath of human occipital scalp follicles (McElwee et al., 2004; Shorter, 2007) and also the bulb matrix (Shorter et al., 2008), this would suggests that this signalling pathway works as paracrine signalling system. Since the dermal papilla can express both MSP and the receptor RON, this could be an autocrine response, as suggested earlier in the discussion of HGF and its receptor. On the other hand it could be the more normal paracrine route with the signal being produced by either the dermal papilla or the dermal sheath or the matrix cells and received by the receptors in one of the other tissues. Therefore, MSP and its receptor RON seem good candidates for a paracrine role in alteration of hair growth.

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