Molecular analysis of a new splice variant of the human melanocortin-1 receptor

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Abstract The primary hormonal regulator of pigmentation is melanocyte stimulating hormone derived from proopiomelanocortin by proteolytic processing. The melanocortin-1 receptor serves a key role in the regulation of pigmentation. We describe the identification of the first intron within a melanocortin receptor. A new melanocortin-1 receptor isoform, generated by alternative mRNA splicing, encodes an additional 65 amino acids at the predicted intracellular, C-terminal tail of the melanocortin-1 receptor. When expressed in heterologous cells, the new spliced form of the melanocortin-1 receptor (melanocortin-1 receptor B) appears pharmacologically similar to the non-spliced melanocortin-1 receptor. Melanocortin-1 receptor B is expressed in testis, fetal heart and melanomas.

Key words: Melanocortin; G-protein-coupled receptor; Pigmentation

1. Introduction

The melanin pigments, black eumelanin and red phaeomelanin, are synthesized from tyrosine. Upon exposure to ultraviolet radiation, eumelanin is protective while phaeomelanin generates free radicals causing damage in type I (always burn, never tan) and type II (always burn, slight tan) skin [1–5]. Light-skinned, red-haired individuals fail to tan and are at risk for ultraviolet radiation damage. These individuals have predominantly phaeomelanin in their skin and hair with little eumelanin.

Five melanocortin receptors (MC-1R through 5) have been described belonging to the G-protein-coupled receptor (GPC-R) superfamily [6]. Melanocyte stimulating hormone (MSH) stimulates pigment cells by binding to the MC-1R causing an elevation of intracellular cyclic AMP (cAMP) which regulates the tyrosinase activity [7,8]. An increased tyrosinase activity and subsequent eumelanin synthesis is caused by activation of MC-1R while a decreased tyrosinase activity and reduced MC-1R activation produces phaeomelanin [9,10].

We describe the molecular analysis of a new MC-1R isoform (MC-1RB) generated by alternative mRNA splicing. The discovery of MC-1RB adds additional diversity to the MC-R family and has implications for MC-1R-mediated signalling events in humans.

2. Materials and methods

2.1. Expressed sequence tag (EST) identification

GenBank databases [11] were monitored daily using the TFASTA search program [12] with amino acid sequences from the human MC-4R.

2.2. Cloning of the MC-R spliced variant (MC-1RB) from human genomic DNA

Touchdown PCR was performed with sheared human genomic DNA (0.5 μg: Clontech, Palo Alto, CA, USA) in a GeneAmp 9700 PCR system (Perkin Elmer, Foster City, CA, USA). Two sense primers, MC-1R- 5′for1 (5′-TCTCACACTCATCGTCCTCTGCCC-3′) and MC-1R-5′for2 (5′-CATCGCCTACTACGACCACGTGGC-3′), were designed based on the published sequence of human MC-1R [13,14,18]. The anti-sense primers, MC-1R-3′rev1 (5′-CGCTGCAAGGCTGTTGGATGAAGC-3′) and MC-1R-3′rev2 (5′-GTGGGAGTAGCTCTTGGCACACAC), were derived from EST AI123000. An Advantage cDNA PCR kit (Clontech, Palo Alto, CA, USA) was used in the PCR reactions essentially following the manufacturer's instructions. Two exceptions were the addition of 5% DMSO to the PCR reactions and PCR cycling as described below: (1) 94°C for 1 min, (2) five cycles of 94°C for 30 s, 72°C for 3 min, (3) five cycles of 94°C for 30 s, 70°C for 3 min, (4) 20 cycles of 94°C for 30 s, 68°C for 3 min. DNA sequencing was performed using Big Dye terminator cycle ready sequencing reactions (ABI-Perkin Elmer, Foster City, CA, USA) on a ABI model 377 instrument and analyzed using the Sequencher 3.0 program (Gene Codes, Ann Arbor, MI, USA).

2.3. Cloning of the MC-R spliced variant (MC-1RB) from human testis mRNA

Reverse transcriptase PCR (RT-PCR) using 1 μg of testis mRNA (pool of 25 male caucasians) was performed using the Advantage RT for PCR kit with MMLV reverse transcriptase (Clontech, Palo Alto, CA, USA) essentially following the manufacturer's instructions. PCR was conducted with the Advantage cDNA PCR kit (Clontech, Palo Alto, CA, USA) essentially following the manufacturer's instructions with the exception of adding 5% DMSO to the PCR reaction (cycling conditions: 94°C for 1 min, 60°C for 2 min, 72°C for 2 min, 72°C for 10 min. The forward sense primer utilized (appending *Eco*RI restriction enzyme site and optimized initiation sequence based on Kozak rules) was 5′-GATCGAATTCGCCGCCATGGCTGTGCAGGGATCCCAGAGAAG-3′ while the reverse antisense primers were 5′-GATCGAATTCCTAGGGGGGCTCCTGCAAACCTG-3′ or 5′-GATCGAATTCGTGCCCAGTCTGAGCCTTAGAACCG-3′. Amplified products were agarose gel-purified, digested with *Eco*RI and ligated to the mammalian expression vector pcDNA-3.1 (−) (Invitrogen, Carlsbad, CA, USA).

2.4. Human MC-1RB stable cell line development

All cell culture reagents were purchased from Gibco-BRL, Life Technologies. CHO cells were grown in complete media (Iscove's modified Dulbecco's medium, with L-glutamine and 25 mM HEPES buffer supplemented with HT (0.1 mM sodium hypoxanthine, 0.016 mM thymidine), penicillin-streptomycin (100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate in 0.85% saline/water), 2 mM L-glutamine and 10% heat-inactivated fetal bovine serum). Cells were transfected with SuperFect transfection reagent (Qiagen) as per manufacturer's recommendations. At 24 h post-transfection, the cells were

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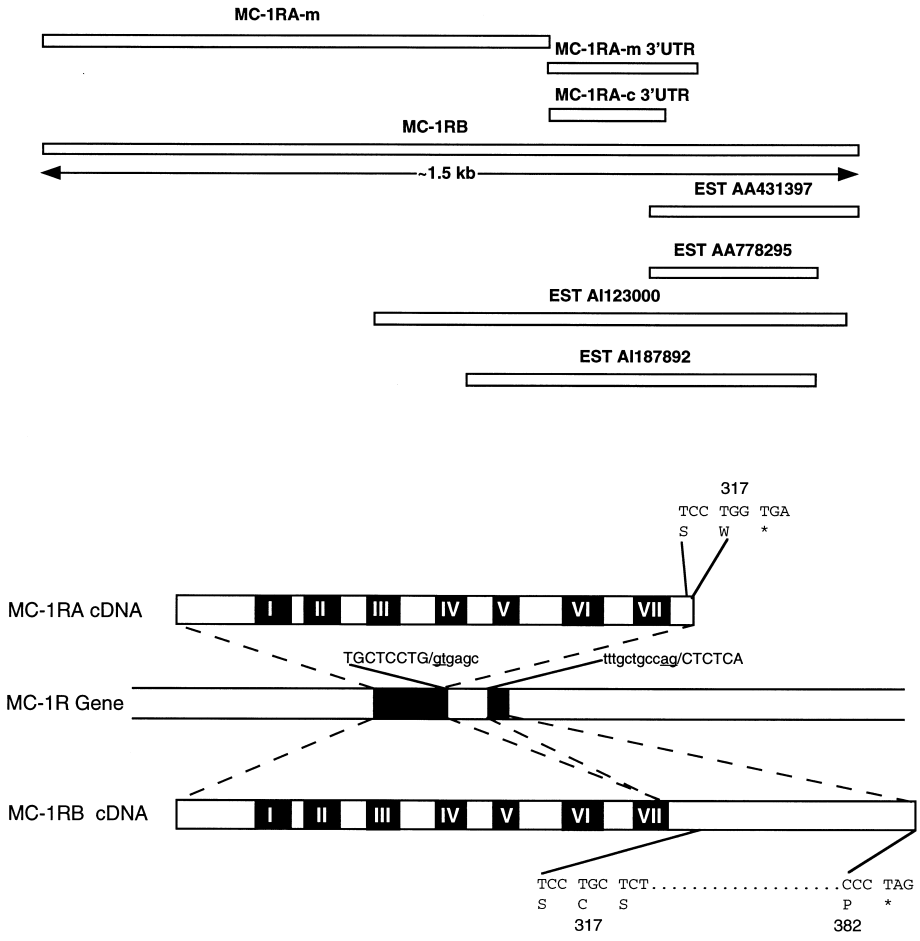

Fig. 1. (Top panel) Graphical comparison of MC-1R sequences. The approximate overlap of various nucleotide MC-1R sequences is shown. GenBank accession numbers are used for dbEST sequences. MC-1RA-m refers to [15] and MC-1RA-c refers to [14]. (Bottom panel) Schematic representation of the human MC-1R gene. Splice donor and acceptor sites are shown. The gray boxes are representative of the seven transmembrane domains denoted by roman numerals. The black boxes represent the two exons separated by an intron and flanked by an untranslated sequence. The nucleotide sequence of the intron is shown in lower case.

trypsinized and plated as a 2-fold dilution series in 100 cm dishes containing complete media plus 1 mg/ml geneticin (G418). Resistant colonies were identified in about 2 weeks, expanded and assayed for binding and functional responses to MSH peptides.

2.5. Cell membrane preparation

Cells were rinsed with phosphate buffered saline (PBS) lacking $CaCl_2$ and $MgCl_2$ (Life Technologies, Gaithersburg, MD, USA) and then detached with enzyme-free dissociation media (Specialty Media, Lavellette, NJ, USA). Cells were pelleted at $2800\times g$ for 10 min. The cell pellet was resuspended in membrane buffer (20 mM Tris pH 7.2, 5 mM EDTA) with 5 μg/ml leupeptin, 5 μg/ml aprotinin, 40 μg/ml bacitracin, 25 μg/ml Pefabloc (Boehringer Mannheim) and homogenized with 10 strokes using a motor-driven teflon-coated pestle in a glass dounce homogenizer at low speed. The resulting cell suspension was centrifuged at $4100\times g$, 4°C, for 20 min. The pellet was resuspended in fresh membrane buffer with protease inhibitors, aliquoted, snap-frozen in liquid nitrogen and stored at −80°C. The resulting crude membranes were titrated to determine the optimal level necessary for performing binding studies (see below).

2.6. Melanocortin (MC) radioligand binding assay

Binding reactions (total volume = 250 μl) contained MBB (50 mM Tris pH 7.2, 2 mM $CaCl_2$, 1 mM $MgCl_2$), 0.1% BSA, crude membranes prepared from CHO cells expressing the MC-1RB, 200 pM [^{125}I]NDP αMSH (Amersham, Arlington Heights, IL, USA) and increasing concentrations of unlabelled test compounds dissolved in DMSO (DMSO final concentration = 2%). Reactions were incubated for 1 h without shaking and then filtered through 96 well filter plates (Packard, Meriden, CT, USA). Filters were washed three times with TNE buffer (50 mM Tris pH 7.4, 5 mM EDTA, 150 mM NaCl), dried and counted using Microscint-20 in a Topcount scintillation counter (Packard, Meriden, CT, USA). Non-specific binding was determined in the presence of 2 μM unlabelled NDP αMSH (Peninsula, Belmont, CA, USA).

2.7. cAMP functional receptor assay

Receptor-mediated stimulation of cAMP formation was assayed in stably transfected CHO expressing MC-1RB. Cells expressing MC-1RB were dissociated from tissue culture flasks by PBS lacking $CaCl_2$ and $MgCl_2$ (Life Technologies, Gaithersburg, MD, USA) and detached following 5 min incubation with enzyme-free dissociation buffer (Specialty Media, Lavellette, NJ, USA). Cells were collected by centrifugation and resuspended in Earle's Balanced Salt Solution (EBSS) (Life Technologies, Gaithersburg, MD, USA) with additions of 10 mM HEPES pH 7.5, 5 mM $MgCl_2$, 1 mM glutamine and 1 mg/ml bovine serum albumin. Cells were counted and diluted to $2–4\times10^6$/ml). The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine was added at a concentration of 0.6 mM. Test peptides were diluted in EBSS with the above additions and 10% DMSO, 0.1 volume of compounds added to 0.9 volume of cells. After room temperature incubation for 40 min, cells are lysed by incubation at 100°C for 5 min to release accumulated cAMP. cAMP was measured in an aliquot of the cell lysate with a cAMP detection assay kit (Amersham, Arlington Heights, IL, USA). The amount of cAMP production which results from an unknown compound is compared to that amount of cAMP produced in response to αMSH which is defined as a 100% agonist.

2.8. Northern analysis

Northern blots purchased from Clontech (Palo Alto, CA, USA) were pre-hybridized (50% formamide, 4×Denhardt's solution, 5×SSPE, 0.1% SDS, 30 μg/ml sheared salmon sperm DNA) at 32°C for 2 h followed by hybridization (50% formamide, 10% dextran sulfate, 4×Denhardt's solution, 5×SSPE, 0.1% SDS, 30 μg/ml sheared salmon sperm DNA) at 32°C overnight. The probes used were a MC-1RA/MC-1RB exon junction probe which included 100 bp of MC-1RA sequence 5′ to the exon boundary and the entire MC-1RB tail sequence. In a separate probing, the MC-1RB tail probe (exon II only) was used. The filters were washed at high stringency (0.1% SSC, 0.1% SDS, 65°C) and exposed to film (Kodak X-Omat) for 1 week at 8−0°C.

3. Results and discussion

An EST (dEST database accession # AI123000, deposited 8/18/97) derived from five normalized and pooled cDNA libraries was identified with a significant homology score to the MC-4R amino acid search sequence. However, FASTA searches against GenBank revealed that EST AI123000 exhibited the highest sequence identity (>90% DNA level) to the 3′-end of the gene for the human MC-1R. Two additional ESTs were subsequently deposited (accession # AI187892 from human testis, deposited 10/13/98; accession #AI379405 from melanocyte, fetal heart, uterus normalized and pooled cDNA) with a similar high sequence identity to the 3′-end of the human MC-1R gene. Retrospective searching of the dbEST subset of GenBank identified two other human ESTs with a high sequence identity to the human MC-1R: AA43197 (deposited 5/22/97, from human testis mRNA) and AA778295 (deposited 2/5/98, from human fetal heart mRNA). DNA sequence verification of EST AI123000 confirmed that it represented an alternatively spliced form of the human MC-1R gene, herein termed MC-1RB encoding 382 amino acids. PCR using human genomic DNA as a template showed the presence a cryptic 381 bp intron immediately upstream (at the C-terminal Trp-317 residue) of the TGA stop codon of the human MC-1R gene (Fig. 1). The nucleotide boundaries describing this intron conformed to the consensus splice junction sequences. A conserved consensus splice donor site (A/C)AG/gt (nucleotides 950-2) was found which forms the first two

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  1 ATG GCT GTG CAG GGA TCC CAG AGA AGA CTT CTG GGC TCC CTC AAC TCC ACC CCC ACA GCC   60
  1 M   A   V   Q   G   S   Q   R   R   L   L   G   S   L   N   S   T   P   T   A    20
                                                                        I
 61 ATC CCC CAG CTG GGG CTG GCT GCC AAC CAG ACA GGA GCC CGG TGC CTG GAG GTG TCC ATC   120
 21 I   P   Q   L   G   L   A   A   N   Q   T   G   A   R   C   L   E   V   S   I    40

121 TCT GAC GGG CTC TTC CTC AGC CTG GGG CTG GTG AGC TTG GTG GAG AAC GCG CTG GTG GTG   180
 41 S   D   G   L   F   L   S   L   G   L   V   S   L   V   E   N   A   L   V   V    60
                                                   II
181 GCC ACC ATC GCC AAG AAC CGG AAC CTG CAC TCA CCC ATG TAC TGC TTC ATC TGC TGC CTG   240
 61 A   T   I   A   K   N   R   N   L   H   S   P   M   Y   C   F   I   C   C   L    80

241 GCC TTG TCG GAC CTG CTG GTG AGC GGG AGC AAC GTG CTG GAG ACG GCC GTC ATC CTC CTG   300
 81 A   L   S   D   L   L   V   S   G   S   N   V   L   E   T   A   V   I   L   L   100
                                                                    III
301 CTG GAG GCC GGT GCA CTG GTG GCC CGG GCT GCG GTG CTG CAG CAG CTG GAC AAT GTC ATT   360
101 L   E   A   G   A   L   V   A   R   A   A   V   L   Q   Q   L   D   N   V   I   120

361 GAC GTG ATC ACC TGC AGC TCC ATG CTG TCC AGC CTC TGC TTC CTG GGC GCC ATC GCC GTG   420
121 D   V   I   T   C   S   S   M   L   S   S   L   C   F   L   G   A   I   A   V   140

421 GAC CGC TAC ATC TCC ATC TTC TAC GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG   480
141 D   R   Y   I   S   I   F   Y   A   L   R   Y   H   S   I   V   T   L   P   R   160
            IV
481 GCG CGG CGA GCC GTT GCG GCC CTC TGG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC   540
161 A   R   R   A   V   A   A   L   W   V   A   S   V   V   F   S   T   L   F   I   180
                                                    V
541 GCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC CTG GCT ATG CTG   600
181 A   Y   Y   D   H   V   A   V   L   L   C   L   V   V   F   F   L   A   M   L   200

601 GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC CGG GCC TGC CAG CAC GCC CAG GGC   660
201 V   L   M   A   V   L   Y   V   H   M   L   A   R   A   C   Q   H   A   Q   G   220
                                                                            VI
661 ATC GCC CGG CTC CAC AAG AGG CAG CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT   720
221 I   A   R   L   H   K   R   Q   R   P   V   H   Q   G   F   G   L   K   G   A   240

721 GTC ACC CCC ACC ATC CTG CTG GGC ATT TTC TTC CTC TGC TGG GGC CCC TTC TTC CTG CAT   780
241 V   T   P   T   I   L   L   G   I   F   F   L   C   W   G   P   F   F   L   H   260
                                                                    VII
781 CTC ACA CTC ATC GTC CTC TGC CCC GAG CAC CCC ACG TGC GGC TGC ATC TTC AAG AAC TTC   840
261 L   T   L   I   V   L   C   P   E   H   P   T   C   G   C   I   F   K   N   F   280

841 AAC CTC TTT CTC GCC CTC ATC ATC TGC AAT GCC TTC ATC GAC CCC CTC ATC TAC GCC TTC   900
281 N   L   F   L   A   L   I   I   C   N   A   F   I   D   P   L   I   Y   A   F   300

901 CAC AGC CAG GAG CTC CGC AGG ACG CTC AAG GAG GTG CTG ACA TGC TCC TG/gt...ag/CT
301 H   S   Q   E   L   R   R   T   L   K   E   V   L   T   C   S                   316
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MC-1R form A

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    TGG TGA
317 W   *
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MC-1R form B

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    TGC TCT CAG GAC CGT GCC CTC GTC AGC TGG GAT GTG AAG TCT CTG GGT GGA AGT GTG TGC
317 C   S   Q   D   R   A   L   V   S   W   D   V   K   S   L   G   G   S   V   C

    CAA GAG CTA CTC CCA CAG CAG CCC CAG GAG AAG GGG CTT TGT GAC CAG AAA GCT TCA TCC
337 Q   E   L   L   P   Q   Q   P   Q   E   K   G   L   C   D   Q   K   A   S   S

    ACA GCC TTG CAG CGG CTC CTG CAA AAG GAG GTG AAA TCC CTG CCT CAG GCC AAG GGA CCA
357 T   A   L   Q   R   L   L   Q   K   E   V   K   S   L   P   Q   A   K   G   P

    GGT TTG CAG GAG CCC CCC TAG
377 G   L   Q   E   P   P   *   382
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Fig. 2. Nucleotide and deduced amino acid sequence of the MC-1RA and the alternative splice form MC-1RB. The predicted transmembrane domains are underlined and numbered from 1 to 7.

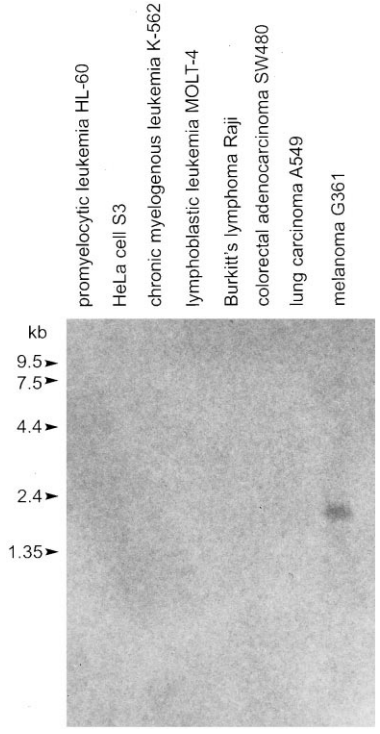

Fig. 3. Northern blot analysis of MC-1RB expression. Northern blots purchased from Clontech with 1–2 μg poly(A)$^+$ RNA per lane were hybridized with a MC-1R probe which is non-selective for both MC-1RA and MC-1RB. The same filters were subsequently stripped of the non-selective probe (blots were checked for any residual signal by autoradiographic exposure) and re-hybridized with a MC-1RB-specific probe. Following each probing, the blots were washed at high stringency and exposed to a X-ray film for a week at −80°C. A representative Northern blot is shown in which the only positive signal occurs in melanoma cells. Both hybridization probes gave the same hybridization profile. Poly(A)$^+$ mRNAs that tested negative are amygdala, caudate nucleus, corpus collossum, hippocampus, whole brain, substantia nigra, thalamus, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, peripheral blood leukocytes, pancreas, adrenal medulla, thyroid, adrenal cortex, testis, thymus and stomach.

bases of the Trp triplet codon (Fig. 1) [13]. Also noted was a conserved consensus splice acceptor site cag/R (nucleotides 1330-2). Formation of this splice junction results from the donor supplying TG and the acceptor supplying C to form the triplet codon for Cys (instead of the C-terminal amino Trp of the MC-1RA). Extended encoding sequence results in a reading frame encoding an additional 65 amino acids (not including the Trp-317 to Cys substitution) (Figs. 1 and 2). To document that the full-length open reading frame (ORF) encoding MC-1RB was expressed, RT-PCR was conducted from human testis poly(A)$^+$ which resulted in the cloning of the complete ORF, as shown in Fig. 2. The 65 amino acid tail does not significantly match any known protein sequence (Swiss-Prot Database). This is the first description of an intron and and alternatively spliced mRNA form of a MC-R. The splice donor site, CTG/gt, is conserved in all 14 species whose MC-1R sequence is present in the GenBank database. Further experiments will be necessary to determine if the splice variant is unique to humans.

Numerous potential polymorphisms have been reported for the human MC-1RA, generally clustered in TM-1, TM-2, the second intracellular loop and TM-7 [14–20]. From both cDNA and genomic templates, we obtained a nucleotide sequence which matched the genomic encoding sequence for MC-1RA as reported in the GenBank data base entry by Chhajlani and Wikberg [14] through amino acid 316 (exon 1). It is worthy to note that we have found that exon 1 and exon 2 (containing the 65 amino acid extension) of the human MC-1R gene are prone to the introduction of PCR artefacts as both genomic and cDNA templates yielded sequence nucleotide differences using several polymerases. With the exception of the original papers [14,15] describing the MC-1RA cloning from a cDNA and genomic library, determination of the nucleotide sequence of shorter PCR fragments has been used to characterize potential MC-1RA variants. Using nucleotide sequence data reported in [14] for exon 1 and dbEST submissions AI123000 and AI187892 for exon 2 as benchmarks, we confirmed both exon nucleotide sequences from cDNA and genomic templates and then used a MC-1RB construct devoid of any amino acid changes for expression studies.

Preliminary studies using MC-1RB DNA transiently transfected into COS-7 cells indicated that this receptor is able to bind MC peptides with a high affinity and that this binding resulted in signalling via cAMP formation (data not shown). To fully characterize this receptor, we established cell lines stably expressing the MC-1RB in CHO cells (CHO 1bX). Cell line 1bX exhibited specific increases in intracellular cAMP formation evoked by several MC agonists or mixed agonists/antagonists, including αMSH, MT-II, SHU-9119, γ_2MSH, NDP-αMSH and ACTH (Table 2). In addition, several MC-derived peptides potently displaced the binding of [^{125}I]NDP-αMSH indicating the presence of a high affinity binding site conferred by MC-1RB expression. Previous studies [14,15] have documented that activation of the MC-1RA isoform by MC agonists results in an elevation of the intra-

Table 1
Human MC1-R allelic variants

Mountjoy (MC-1RA)	Chhajlani (MC-1RA)		Tan (MC-1RA)	Valverde (MC-1RA)	Box (MC-1RA)
	D	M			
T90	S90	S90	S90	A64S, F76Y, D84E, V92M, T95M, V97I, A103V, L106Q, D294H	V60L, K65N, R67V, V93L, R142H, R151C, R160W, A299T
P162	R162	R162	R162		
R163	Q163	Q163	Q163		
A164	A164	R164	R164		

Listing of alleles in the literature according to the first publication on a particular variant [14–16,20]. Amino acid changes for a given residue are shown as X-residue number-Y. For reference, both Valverde et al. and Box et al. use the Chhajlani et al. GenBank database entry (given as D above) as their wild-type allele with the exception that A164 is R. M refers to the sequence presented in reference [14]. As noted in the text, MC1-RB matches Chhajlani's database entry through amino acid 316.

Table 2
Pharmacology of MC-1RB

Peptide	IC_{50} (nM)		EC_{50} (nM)	
	MC-1RA	MC-1RB	MC-1RA	MC-1RB
MT-II	0.6	0.3	0.2	0.39 (0.07)
SHU-9119	0.7	0.6	NA	0.73 (0.14)
ACTH	2.5	4.8	8	9.1 (1.0)
αMSH	0.2	2.2	2	3.3 (0.09)
NDP-αMSH	0.1	0.7	0.03	0.9 (0.05)
γ_2MSH	11.2	463	>100	12.7 (4.6)

IC_{50} values were calculated from displacement curves of [^{125}I]NDP-αMSH by linear regression analysis using Prism 2.0 (Graphpad software, San Diego, CA, USA). EC_{50} values were calculated from cAMP dose-response curves as above. Values are an average of two (IC_{50}) or four (EC_{50}; S.E.M. is shown in brackets) experiments. Data for MC-1RA are taken from [23–26]. Peptide sequences: α-MSH (SYSMEHFRWGKPV), MT-II (nLDHdFRWK; [26]), SHU-9119 (nLDHdFRWK; [27]), γ-MSH (YVMGHFRWDRFG), NDP-αMSH (SYSnLEHdFRWGKPV) and β-MSH (AEKKDEGPYRMEHFRWGSPPKD).

cellular cAMP production through the coupling of G-proteins to activation of membrane-bound adenylate cyclase. The rank order of potency of the above peptides in eliciting the cAMP response was MT-II ∼ SHU9119 ∼ NDP-α-MSH > αMSH ∼ ACTH > γ_2MSH. For comparison, literature values are also given for the human MC-1RA suggesting that the 65 amino acid C-terminal extension does not significantly alter the binding or activation rank order of potency. The above results indicate that MC-1RB encodes a functional receptor for MC ligands with similar pharmacological properties to the MC-1R-A isoform.

To map the expression pattern of MC-1RB, Northern blots were hybridized separately under high stringency conditions with a MC-1RB-specific probe and a second hybridization probe which should detect both MC-1RA and MC-1RB transcripts. The results were identical with only a single hybridizing species exclusively observed in melanoma cells (Fig. 3), even after prolonged autoradiographic exposure. MC-1RB is also expressed in testis and fetal heart, albeit at a lower level than melanoma cells (RT-PCR results and random cDNA library screening for the EST database clones). Human MC-1RA mRNA has been shown to be most highly expressed in melanocytes [14,15] and is present in the testis using RT-PCR [21].

There are numerous common polymorphisms in the encoding sequence of the MC-1RA, some of which are correlated with variation in pigmentation in humans and other mammals [14–17,20]. It is therefore possible that allelic variation at the MC-1R locus may affect the pigmentation profile. By example, Valverde et al. [17] correlated nine amino acid substitutions on the receptor to varying shades of red hair and fair skin, while Box et al. [20] showed that MC-1R polymorphisms are necessary but not always sufficient for red hair production by studying an additional 11 sites of allelic variation (Table 1) on red-haired twins. The functional significance of altered amino acids due to allelic variation at the human MC-1R locus is presently unclear. A V92 to M variant was shown to have a ∼5-fold reduced potency for binding radiolabelled αNDP-MSH [18] but showed no difference from the wild-type receptor when assessed for functional coupling to cAMP elevation [22]. Further studies are necessary to determine the role of the MC-1RB in MC-1R signalling and pigmentation.

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