Dimorphic regulation of the MafB gene by sex steroids in hamsters (Mesocricetus auratus)

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Research Article

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Abstract

Background

MafB is a transcription factor that regulates macrophage differentiation. Macrophages are a traditional feature of the hamster Harderian gland (HG); however, studies pertaining to MafB expression in the HG are scant. Here, I cloned and sequenced the full-length cDNA of the MafB gene in adult hamsters.

Results

Molecular characterization revealed that MafB encodes a protein containing 323 amino acids with a DNA-binding domain, a transactivation domain, and a leucine zipper domain. Bioinformatic analysis of the hamster MafB sequence demonstrated 98% consistency with the mammalian group, and quantitative real-time polymerase chain reaction assays indicated that MafB was expressed in different tissues of both sexes. The highest expression levels in endocrine tissues were identified in the pancreas. Gonadectomy in male hamsters was correlated with significantly higher mRNA levels in the HG; replacement with dihydrotestosterone (DHT) restored mRNA expression. The HG in male hamsters contained two-fold more MafB mRNA than the HG of female hamsters. Adrenals revealed similar mRNA expression levels during the estrous cycle. The estrous phase was correlated with higher mRNA levels in the ovary. Significantly up-regulated expression and sexual dimorphism of MafB was found in the pancreas.

Conclusions

MafB in the HG may play an active role in the macrophage differentiation required for phagocytosis activity and intraocular repair. Additionally, sex steroids appear to strongly influence the HG and MafB in the pancreas.

Background

The Maf (musculoaponeurotic fibrosarcoma oncogene) family of proteins are a subgroup of basic region-leucine zipper (bZIP) transcription factors that recognize a long palindromic DNA sequence [TGCTGAC(G)TCAGCA] known as the Maf recognition element (MARE) [1]. The Maf protein B (MafB) consists of NH2-terminal activation or an acidic domain (rich in proline, serine, and threonine residues), a DNA-binding domain, and a bZIP domain in its COOH-terminal region that is necessary for homo- or hetero-dimerization via its leucine-repeats structure [2, 3]. Human and mouse MafB genes contain no introns [4, 5] and encode a protein with 323 amino acids.

In mice, MafB is dispensable for fetal testis morphogenesis and the maintenance of spermatogenesis [6]. MafB is also a transcriptional regulator required for islet β cell maturation [7]. It is furthermore expressed in all developing insulin- and glucagon-producing cells and expressed in a restricted fashion in adult α cells [8]. Likewise, MafB is expressed in podocytes and osteoclasts and plays a key role in their
differentiation and development [9, 10]. In chickens, MafB induction is a specific and essential determinant of the monocytic program in hematopoietic cells, and it is important for macrophage differentiation [11]. Recent studies of MAFB gene mutations have demonstrated an association with multicentric carpotarsal osteolysis (MIM number 166300) and Duane retraction syndrome (MIM number 617041) with focal segmental glomerulosclerosis [12, 13]. Previous reports have shown that MafB is specifically expressed in macrophages at the site of bacterial infections from peripheral blood [14, 15]. In mice, MafB is an androgen-inducible, sexually dimorphic regulator of embryonic urethral masculinization; those attributes suggest an association with human hypospadias [16]. Interestingly, molecular studies have revealed that the expression of the MAFB gene and protein in children with hypospadias is lower than in unaffected children [17]. However, preliminary molecular genetic analyses of patients with hypospadias have reported the absence of MAFB gene mutations; that finding suggests that MAFB might play only a limited role in the formation of the human male urethra [18]. Follow-on studies pertaining to the promoter region of the MAFB gene could contribute to elucidating the transcriptional regulation mechanisms associated with hypospadias.

The Harderian gland (HG) is an exocrine intraorbital gland that is present in most terrestrial vertebrates [19]. Several functions have been ascribed to the mammalian HG, including pheromonal, thermoregulatory, vomeronasal photoreceptive, and orbital lubricatory roles [20]. The HG is additionally characterized by the presence of mast cells, macrophages, melanocytes, and immunocompetent cells. Several studies have noted the presence of macrophages in the rodent HG [21–23]; the HG in hamsters (Mesocricetus auratus) exhibits a marked sexual dimorphism in terms of cell types, and it exhibits dimorphic features in porphyrins, fatty acids, indoleamines, and somatostatin biosynthesis that could be modified by sex steroids [19]. Androgens regulate these sex differences [24–32]. Ovarian steroids are additionally necessary to maintain the structure and activity of the female HG, and androgen administration results in the masculinization of the HG [33–35].

Despite the fact that the hamster HG has been considered to be a localization site of macrophages, the role of transcriptional factor MafB in the hamster HG remains unclear. The aim of this study was to isolate, clone, and sequence the complete cDNA encoding MafB in the hamster HG and investigate its expression in adult hamsters and all methods are reported in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines for the reporting of animal experiments.

**Results**

cDNA cloning of hamster MafB

DNA sequencing analysis revealed that the isolated and cloned cDNA of Mesocricetus auratus MafB consisted of 3066 base pairs (bp) with a 5′-untranslated region (5′-UTR) of 308 bp and a 3′-UTR of 1789 bp. The isolated cDNA clone contained an open reading frame (ORF) of 969 bp encoding 323 amino acids (Fig. 1). The nucleotide and deduced amino acid sequences were submitted to the GenBank database (accession number MZ215994).
Characterization of MafB protein from hamsters

The estimated molecular weight of the deduced peptide was 35731.75 Da, and the theoretical isoelectric point (pI) was 7.17. Hamster MafB was characterized by a high level of consistency (98–99%) with other MafB amino acid sequences from mice (NP_034788.1), rats (NP_062189.1), and humans (NP_005452.2). The protein sequence exhibited all of the features of MafB proteins, including two histidine-rich boxes (131–143 and 158–167 residues) localized in the central region of the sequence. The deduced amino acid sequence predicted a bZIP domain (L$_{266}$, L$_{273}$, L$_{280}$, L$_{287}$, Y$_{294}$, and L$_{301}$) between amino acids 238 and 301. I identified a DNA-binding domain (N$_{248}$XXY$_{251}$A$_{252}$XXC$_{255}$R$_{256}$) and a putative nuclear localization signal (likelihood 0.99; C$_{255}$, R$_{256}$, Y$_{257}$, K$_{258}$, R$_{259}$, V$_{260}$, and Q$_{261}$) at its COOH-terminal end (Fig. 2).

I next analyzed protein and genetic interactions, pathways, co-expression, co-localization, and protein domain similarity using the GeneMANIA server (Fig. 3). Co-expression predictions (Fig. 3a) were identified with ATP-binding cassette, sub-family B, member 4 (Abcb4), nuclear factor, erythroid derived 2,-like 1 (Nfe2l1), FOS-like antigen 2 (Fosl2), FBJ osteosarcoma oncogene B (Fosb), JunB proto-oncogene (Junb), and Jun proto-oncogene (Jun). Physical interactions (Fig. 3b) were predicted using Fos proto-oncogene (Fos), TATA-box binding protein associated factor 5 (Taf5), and paired box 6 (Pax6).

Phylogenetic analysis of MafB proteins in mammals

The multiple sequence alignment of the predicted/modelled and validated/reviewed MafB proteins from mammals was used to construct a phylogenetic tree. To do so, I used Molecular Evolutionary Genetics Analysis (MEGA) X software (https://www.megasoftware.net/). The results of the phylogenetic tree revealed that all of the MafB proteins could be separated into six different groups (groups I–VI; Fig. 4). The proteins were characterized by the orders carnivora, chiroptera, artiodactyla, primates, rodentia, and a small group of prototheria/metatheria, respectively. In some mammalian groups, such as the order chiroptera, the set of MafB proteins was interrupted by proteins of other orders. The hamster MafB protein was included in the rodentia group.

Three-dimensional structure of hamster MafB protein

The 3D structure of MafB was estimated using the Robetta software package and visualized with PyMOL. This model, based on the hamster MafB protein sequence (Fig. 5), revealed several domains: a leucine zipper domain, a nuclear localization signal, a DNA-binding domain, and a transactivation domain.

MafB gene expression in hamsters

Quantitative real-time polymerase chain reaction (qPCR) analysis revealed multiple variations in each Mesocricetus auratus tissue and sex condition. Initially, the MafB transcript was characterized by a broad distribution in adult hamster tissues. The most abundant transcripts were identified in the spleen, gut,
heart, and brain; the least abundant transcripts were identified in the liver, lungs, epididymis, uterus, and hypothalamus (Fig. 6).

qPCR expression values of the \textit{MafB} gene in endocrine tissues revealed that all samples are differentially expressed. Pancreatic tissue from male and female hamsters exhibited the highest average levels of \textit{MafB} gene expression; the transcripts in male and female (metestrus) adrenals were significantly lower than in the testes and ovaries (Fig. 7). Sexual dimorphism was also observed in the gonads; expression in the ovaries (estrus) was significantly higher than in the testes.

In order to gain insights into whether the hamster HG exhibited differential expression patterns as a function of sex steroids and sex condition, I performed MafB mRNA analysis of intact and castrated males. Significantly higher expression of male MafB was detected in the castrated males; the administration of DHT reestablished the transcript levels. In the HG of female hamsters, similar expressions levels were determined in different phases of the estrous cycle. Slightly higher expression was noted during metestrus; however, MafB mRNA did not exhibit a sexually dimorphic expression pattern (Fig. 8).

I next characterized the expression profiles of MafB using qPCR to identify MafB expression in endocrine tissues during the animals’ estrous cycle. Ovarian tissue obtained during estrus exhibited high expression of the MafB transcript; mRNA expression was constant during the other phases (proestrus, metestrus, and diestrus) (Fig. 9). I also compared \textit{MafB} gene expression in the adrenals during the animals’ estrous cycle; no significant changes in MafB expression levels were observed (Fig. 10).

Pancreatic tissue exhibited the highest levels of Mafb expression of all of the endocrine tissues in both males and females in metestrus (Fig. 7). I next focused on MafB expression in the female hamster pancreas. The mRNA gene expression profiles revealed significantly higher MafB transcript levels during metestrus, diestrus, and proestrus. The lowest MafB expression was noted in estrus; significantly higher expression levels were detected in the proestrus pancreas (Fig. 11).

**Discussion**

The transcription factor MafB is expressed in pancreatic α cells, renal podocytes, epidermal keratinocytes, hair follicles, and hematopoietic stem cells; it plays a role in embryonic urethral formation [9, 16, 36–38]. MafB is also expressed in macrophages and regulates their differentiation [39]. In rodents, the HG is an important site of macrophage localization [19]. Even so, the expression and regulation of the \textit{MafB} gene in the hamster HG remains largely unknown.

In this research, I analyzed the influence of sex steroids on MafB mRNA expression in the HG and endocrine tissues of \textit{Mesocricetus auratus}. I first isolated and molecularly characterized hamster MafB; I next identified its gene-expression patterns. This study represents the first time that MafB from hamsters has been isolated and characterized. The results revealed amplification of a cDNA fragment of 3066 bp covering the entire coding region. The ORF was composed of 969 bp, which is consistent with sequences
from other mammalian species [4, 5, 40] and encodes a deduced protein of 323 amino acid residues of 35731.75 Da. The hamster peptide possessed the highly conserved motifs of MafB, such as a DNA-binding domain, a nuclear signal domain, and leucine zipper domain. A phylogenetic tree additionally revealed that hamster MafB has a similar evolutionary line; hamster MafB proteins are closely related to those of the rodentia order. The amino acid sequence of mature hamster MafB peptide exhibited rather high identities and highly conserved structural features. These results suggest that the physiological functions of this peptide are conserved among mammals. In summary, these results pertaining to molecular properties, sequence similarity, conserved domains, phylogenetic relationships, and structural characterization (Fig. 1, 2, 4, and 5) support the assertion that the MafB gene identified in this study is *Mesocricetus auratus* MafB.

The MAfB transcript has been ubiquitously detected in all human tissues examined using Northern blot assays; however, the transcript is expressed predominantly in bone, bone marrow, skeletal muscle, and the heart. Northern blot assays also revealed expression in the spleen, brain, and pancreas [4]. In this study, I used qPCR assays to furthermore identify strong expression in the epididymis, uterus, and hypothalamus (Fig. 6). The pronounced expression of MafB transcription factor in these tissues suggests that this transcription factor participates in cellular maintenance and differentiation, specifically in macrophages, in adult hamsters. I accordingly suggest that MafB functioning may be necessary for macrophage differentiation in spleen cells. On the other hand, MafB mRNA has been reported to exhibit significantly high mRNA levels in goldfish (*Carassius auratus* L.) spleen tissue [41]. However, relatively little is known about the relationship between MafB and splenocytes. Future studies aimed at investigating the inflammatory, infectious, and pathological processes associated with the MafB gene and the spleen will be valuable.

The MafB transcript was principally detected in the pancreas of both male and female hamsters (Fig. 7 and Fig. 11). Sexually dimorphic or oestrous cycle-dependent expression of MafB was not evident in other endocrine tissues (e.g., the testes, ovaries, and adrenals) (Fig. 9 and Fig. 10). However, moderate sexual dimorphism was observed in the gonads, and MafB expression was increased in the animals’ ovaries. Numerous reports have identified MafB transcription factor to be present in human and mouse pancreatic β cells [7, 42–45]. In islet β cells, MafB activity regulates many genes essential to glucose sensing and insulin secretion in a cooperative and sequential manner [7]. Furthermore, MafB is essential to the functional maturation of α and β cells produced by human embryonic stem cell differentiation [7, 43]. In addition to identifying MAFB mRNA expression in hamster pancreas, I also detected sexually dimorphic and sex steroid-dependent expression. These results suggest that sex steroids (likely estrogens and/or progesterone) might be regulating MafB expression and therefore controlling insulin and glucose secretion during the phases of the estrogen cycle. Future studies can contribute to furthering our understanding of how the steroid-receptor complex regulates the expression of the MafB gene in pancreatic tissue in hamsters and how this gene controls the secretion of insulin and glucose.

Finally, the expression of MafB transcription factor has been observed in various types of cells. In chicks, the overexpression of MafB in transformed myeloblasts stimulates the rapid formation of macrophages
In mice, MafB is specifically expressed in macrophages and is a critical regulator of macrophage differentiation [9, 39]. One study showed that nuclear receptor transcription factors are involved in the metabolic and immune activities of macrophages by regulating target genes [46]. The HG is an exocrine intraorbital gland; a cellular feature of the mouse and hamster HG is the presence of macrophages [19]. However, the expression and regulation of the MafB gene in HG macrophages has remained largely unexplored. I have observed somewhat sexually dimorphic expression of MafB in the HG. Even so, our results indicated that MafB expression was increased (Fig. 8) due to the absence of sex steroids (i.e., male gonadectomy). These findings suggest that androgens regulate MafB expression in the hamster HG. Matsushita et al. [47] reported that androgens regulate MafB expression via its 3’UTR during mouse urethral masculinization. Additionally, Vilchis et al. [26] characterized and demonstrated the presence of a specific high-affinity intracellular androgen receptor (AR/NR3C4) in the HG in male hamsters. These results suggest that the androgen-AR complex regulates MafB expression. Therefore, androgen-dependent expression of MafB might influence the phagocytic activity of macrophages in the HG. Due to the intraocular location of the HG, macrophages exist in this tissue to clear cellular and environmental debris [19]. In addition to the AR/NR3C4 transcription factor, several nuclear transcription factors were associated with MafB, such as Nfe2l1, Fosl2, Fosb, Junb, Jun, Fos, Taf5, and Pax6 (Fig. 3). Likewise, gene expression profiles of other transcriptional factors, such as Sox9 and Dax1, have been identified in intraocular tissue [31, 48]. Although I did not examine MafB protein in this intraocular tissue, the expression data suggest that MafB is likely involved in cellular maintenance and differentiation in adult hamsters.

Conclusions

In summary, I have identified and characterized the full-length cDNA sequence of MafB in Mesocricetus auratus. These findings reveal that hamster MafB is similar in sequence, structural homology, and functional domains to other MafB transcription factors reported for different mammalian species. Furthermore, I investigated the tissue-expression profiles of MafB in Mesocricetus auratus. In pancreatic tissue, the MafB gene is expressed in a sexually dimorphic manner; overexpression in females suggests the sex steroid-dependent regulation of α and β cells in the pancreas during the estrous cycle. I also detected somewhat dimorphic expression in the HG. However, androgen-dependent regulation was identified, which could suggest that the Mafb exerts control over the differentiation of Harderian macrophages.

Material and methods

Hamsters

Thirty-five adult hamsters (Mesocricetus auratus) were housed under 12 h/12 h light/dark cycle conditions with water and fed ad libitum (male = 15 and female = 20). The animals were divided into seven groups: intact males (n = 5), males castrated seven days ago (n = 5), males castrated seven days ago and intra-muscularly injected (10 µg) daily with dihydrotestosterone (DHT) or the vehicle alone (50 µl
corn oil) (n = 5). The four stages of the estrous cycle [proestrus (P; n = 5), estrus (E; n = 5), metestrus (M; n = 5), and diestrus (D; n = 5)] were determined in female animals using vaginal smears; groups of 20 animals in each stage were defined. The male hamsters were anaesthetized with ketamine:xylazine (80 mg/kg:8 mg/kg, intramuscularly) prior to gonadectomy and decapitated 24 h after the last injection. A 1.5-cm incision was made at the scrotum, and the testes were exposed, ligated, extracted, and then the wound was sutured closed. Several tissues were immediately removed, frozen on dry ice, and stored at −70°C until the experiments were performed. I used the HGs of the males and females under different endocrine conditions to determine sex steroid-dependent effect on MafB expression. I collected the animals’ HG, lungs, livers, epididymis, hearts, uterus, brains, hypothalamus, guts, spleens, testes, ovaries, adrenals, and pancreas. The Ethics Committee for Research in Animals at Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ, BRE-1930-18-19-1) approved the research for the care and use of animals.

RNA isolation and cDNA synthesis

I used TRIzol reagent (Invitrogen, Carlsbad, CA, USA), as recommended by supplier, to extract total RNA. The concentration and purity of the total RNA was evaluated using a Beckmann (DU 650, Fullerton, CA, USA) spectrophotometer (at 260/280 nm, optical density: 1.8). The integrity of the isolated RNA was assessed by running the RNA samples directly on denaturing formaldehyde-agarose gel (1.2%) stained with ethidium bromide; the RNA was verified by the presence of large and small ribosomal RNA. The first-strand cDNA was synthesized from (1 µg) total RNA using a Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics, Indianapolis, IN, USA) and a Maxima First Strand cDNA Synthesis kit for RT-qPCR (ThermoScientific, Vilnius, Lithuania), according to the manufacturer's guidelines. All the cDNA samples were stored at −20°C until analysis.

Molecular cloning

I designed two primers (forward 5′-gactcatctcaggacctgta-3′ and reverse 5′-cgttctccaggtgatgtttct-3′) to obtain via Polymerase Chain Reaction (PCR) for a partial sequence of hamster MafB cDNA. Primers were designed from the nucleotide sequences of mouse (NM_010658.3), rat (NM_019316.2), and human (NM_005461.5) MafB cDNA. The partial fragment was sequenced. Based on the partial cDNA sequence obtained by PCR sequencing, we designed two gene-specific primers (reverse 5′-cctcagggttctgctgttagtt-3′ for 5′-end and forward 5′-cccagtggtgcaggtataaaacgcgt-3′ for 3′-end) to amplify the 5′-end and 3′-end of MafB using a SMART Rapid Amplification of cDNA Ends (RACE) kit (Clontech, Mountain View, CA, USA), in accordance with the manufacturer's guidelines. These fragments were sequenced and two specific primers (forward 5′-cggttgctccgcgagt-3′ and reverse 5′-acaggacgggagttcagg-3′) for amplifying of complete MafB cDNA were synthesized. The amplified PCR product was purified using electroelution/Amicon ultra-4 10k centrifugal filter devices (Merck Millipore Ltd., Tullagreen, Carrigtwohill Co., Cork, IRL) and cloned using a TOPO-TA Cloning Kit for Sequencing (Invitrogen/ThermoFisher, Waltham, MA, USA). Plasmid cDNA was isolated using a GenElute Five-Minute
Plasmid Miniprep kit (Sigma-Aldrich, St. Louis, MO, USA) and a PureYield Plasmid Maxiprep kit (Promega, Woods Hollow, Madison, WI, USA), according to the manufacturer’s guidelines.

Sequencing and bioinformatic analysis

The nucleotide sequence of full-length cDNA and partial fragments of hamster MafB were determined using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Austin, TX, USA). The thermal cycling conditions included 1 min at 96°C followed by 35 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min (Veriti 96 well Thermal Cycler, Applied Biosystems). The resulting material was then purified using a BigDye XTerminator Purification kit (Applied Biosystems) and run on an ABI-PRISM 310 genetic analyzer Applied Biosystems, Foster City, CA, USA), following the manufacturer’s guidelines. The electrophoresis conditions were as follows: temperature: 50°C; injection voltage: 15 kV; injection time: 5–7 s; 5–8 µA; the run module was KB_310POP6_BDTv3_36Rapid. The sequencing reactions were performed in the forward and reverse directions in two independent experiments. The amino acid MafB sequence was determined using an Expert Protein Analysis System (https://web.expasy.org/translate/). The molecular weight and theoretical isoelectric point of the hamster MafB were predicted using ExPASy ProtParam (https://www.web.expasy.org/protparam/). The multiple sequence alignments with other mammalian MafB proteins were determined using the CLUSTALW program (http://www.genome.jp/tools/clustalw/), and the identity between the amino acid MafB sequences were performed using the Basic Local Alignment Search Tool (BLAST) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The subcellular localization of hamster MafB was identified using DeepLoc-1.0, an eukaryotic protein subcellular localization predictor (http://www.cbs.dtu.dk/services/DeepLoc/).

MafB phylogenetic tree

One hundred and twenty-five amino acid sequences of mammalian MafB were downloaded from the NCBI (https://www.ncbi.nlm.nih.gov/protein) database and aligned with the multiple sequence alignment tool CLUSTALW (https://www.genome.jp/tools-bin/clustalw). The multiple alignment formats were obtained in FASTA file format using the protein database for MafB (https://www.ncbi.nlm.nih.gov/protein/). The molecular evolutionary analyses were performed using Molecular Evolutionary Genetics Analysis (MEGA X) software. The evolutionary history was inferred using the Maximum Likelihood method and a Jones-Taylor-Thornton (JTT) matrix-based model. The tree with the highest log likelihood (-10399.30) is shown in Fig. 4.

Three-dimensional structure prediction

I used the Robetta software package (http://robetta.bakerlab.org) to determine the 3D structure of hamster MafB. I additionally visualized the 3D structure using PyMOL version 2.3 (http://www.pymol.org/).

Gene-expression analysis
The isolation of total RNA was carried out using TRIzol reagent (Invitrogen), according to the manufacturer’s guidelines. The concentration and purity of the total RNA was assessed using a Beckmann spectrophotometer (at 260/280 nm; optical density: 1.8). The integrity of the all of the isolated total RNAs was determined in formaldehyde/MOPS/agarose gels by looking for the presence of ribosomal RNAs. Two µg of total RNA was used for reverse transcription with the Maxima First Strand cDNA Synthesis kit for qPCR (ThermoScientific). Reverse transcription was performed according to the manufacturer’s guidelines. qPCR was carried out in a LightCycler 2.0 system from Roche (Applied Science) with LightCycler TaqMan Master Mix and pre-validated TaqMan hydrolysis probes (Roche Diagnostics, Mannheim, Germany). The relative level of MafB mRNA (sense: 5’-acgctgcagagcttcgac-3’ and antisense 5’-ctgggtacccgtggtgag-3’, 82 bp) was normalized based on the level of hamster β-actin mRNA (sense: 5’-agctatgagctgcctgatgg-3’ and antisense: 5’-caggaaggaaggctggaaa-3’; 82 bp). Transcripts of MafB and β-actin were detected using the universal fluorogenic probes #77 (04–689–003–001) and #9 (04–685–075–001), respectively. The cycling conditions were 95°C for 10 min, 40 cycles of amplification at 95°C for 10 s, 60°C for 30 s, and 72°C for 1 s, and a nal cycle of cooling at 40°C. The qPCR data were analyzed using the relative quantification method provided by LightCycler software, and they are expressed in arbitrary mRNA units as the mean ± standard deviation (SD) of five biological independent replicates for each group.

**Statistical analysis**

Differences in MafB mRNA levels assessed using one-way ANOVA. A P value less than 0.05 was considered to indicate statistical significance.

**Declarations**

**Ethics approval and consent to participate**

The Ethics Committee for Research in Animals at Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ, BRE-1930-18-19-1) approved the research for the care and use of animals. all methods are reported in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines for the reporting of animal experiments)

**Consent for publication**

Not applicable

**Availability of data and materials**


**Competing interests**

The author declares that this study does not have any potential conflicts of interest
Funding

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Authors’ contributions

The author carried out the whole experiments; L.R. statistically analyzed all the data and graphed; L.R. read and approved the final version of the manuscript and consent to its publication.

Authors’ information

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References


**Figures**
Nucleotide and deduced amino acid sequence of *Mesocricetus auratus* MafB. The numbers on the right indicate the positions of the nucleotides and amino acids. The start codon ATG is indicated in bold and the stop codon TGA is indicated with three asterisks. The MafB cDNA contained a 969-bp ORF that encoded a 323-amino acids polypeptide. The nucleotide sequences of the 5’ and 3’ ends of the MafB cDNA are shown.
cDNA consisted of 308 and 1789 bp, respectively. The diagonal lines represent a 3′-UTR of 1203 bp. The GenBank accession number of hamster MafB is MZ215994.

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**Figure 2**

Multiple alignment of amino acid sequences of *Mesocricetus auratus* MafB with those of other mammalian species (*Mus musculus, Rattus norvegicus,* and *Homo sapiens*). Asterisks indicate amino acids consistent across all four sequences. The two histidine-rich boxes are indicated in gray. At the COOH-terminal end, the DNA-binding domain is shown in blue, the nuclear localization signal is underlined, and the bZIP domain appears in yellow. Sequence alignment was performed using ClustalW.
Figure 3

Correlation analysis of co-expression predictions (a) and the physical interaction network (b) of the *MafB* gene by GeneMania. The top nine genes displaying the greatest correlations with *MafB* included *Abcb4*, *Nfe2l1*, *Fosl2*, *Fosb*, *Junb*, *Jun*, *Fos*, *Taf5*, and *Pax6*. 
Figure 4

Phylogenetic analysis of the MafB protein of *Mesocricetus auratus* (blue) and other mammalian species. The phylogenetic tree was assembled using MEGA X and was constructed using the Maximum Likelihood method and a JTT matrix-based model. This evolutionary analysis involved 126 amino acid sequences.
Figure 5

Three-dimensional protein model generated using Robetta software. The domain organization of *Mesocricetus auratus* MafB protein is indicated in different colors, and each domain is labeled with corresponding amino acids. The NH$_2$-terminal end is colored blue, and the COOH-terminal end is colored purple.
Figure 6

Tissue distribution of *Mesocricetus auratus* MafB transcript expression determined using qPCR. The nine examined tissues were obtained from adult hamsters (n = 5 biological independent replicates). Relative gene-expression analysis was performed using the ΔΔCt method, and β-actin mRNA was used as an internal control. The data refer to Mafb mRNA relative expression and are provided as means (bars) ± SD.
Figure 7

Gene-expression analysis of the *Mesocricetus auratus* MafB transcript in endocrine tissues using qPCR. The assessed tissues included a HG of an intact male (HG-IM). The expression levels of the hamster *MafB* gene were normalized by the expression of the β-actin gene. The values represent the mean (bars) ± SD (n = 5 biological independent replicates).
Figure 8

Gene expression levels of the MafB transcript from the *Mesocricetus auratus* HG determined by qPCR. Data from an intact male (HG-IM), a castrated male (HG-CM), a castrated male who received DHT (HG-CM + DHT), a female in proestrus (HG-P), a female in estrus (HG-E), a female in metestrus (HG-M), and a female in diestrus (HG-D) are shown. The expression levels of the *MafB* gene were calculated relative to
the expression of the $\beta$-actin gene. All of the data are expressed as means (bars) ± SD ($n = 5$ biological independent replicates).

**Figure 9**

qPCR quantification of MafB mRNA expression levels from *Mesocricetus auratus* ovaries in proestrus (Ova-P), estrus (Ova-E), metestrus (Ova-M), and diestrus (Ova-D) females. The gene of interest was
normalized to the reference gene (β-actin), and the expression levels were compared using the relative ΔΔCt method. The data are presented as means (bars) ± SD (n = 5 biological independent replicates).

Figure 10

qPCR analysis of MafB transcript expression levels from Mesocricetus auratus adrenals in proestrus (P), estrus (E), metestrus (M), and diestrus (D) females. The assessed tissues included an ovary of an intact
female in proestrus (Ova-P). \( \beta\)-actin was used as a reference gene. The data are presented as means (bars) ± SD (n = 5 biological independent replicates).

**Figure 11**

Relative mRNA expression levels of MafB transcript from *the Mesocricetus auratus* pancreas in proestrus (Pan-P), estrus (Pan-E), metestrus (Pan-M), and diestrus (Pan-D) females. The assessed tissues included
an ovary of an intact female in proestrus (Ova-P). β-actin was used as a reference gene. The relative expression level was calculated using the ΔΔCt method. Each value of MafB mRNA represents means (bars) ± SD (n = 5 biological independent replicates).