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Transcript variants and expression profiles analysis of *Mitf* gene in minipigs

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Abstract

Object: To identify transcript variants and expression patterns of porcine *Mitf*.
Materials and methods: A pairwise BLAST search at NCBI database was performed to deduce the structure of porcine *Mitf* gene. Subsequently, 5′ RACE and fluorescent quantitative RT-PCR were used to analyze the expression pattern of porcine *Mitf* in different tissues.
Results: Four transcript variants of porcine *Mitf*, MITF-A, MITF-H, MITF-M and MITF-SUS were identified, all sharing high homology with those in humans, except Mitf-SUS.
Conclusion: The sequence of porcine *Mitf* appear highly homologous to human *MITF*. However, only 4 transcript variants of porcine *Mitf* were identified in these minipigs, less than the 9 transcript variants in human *MITF*.

Keywords: Minipigs; MITF/Mitf gene; Transcript variants

1. Introduction

Microphthalmia-associated transcription factor (*MITF*) plays significant roles in the proliferation, development and differentiation of neural crest-derived melanocytes and the formation of melanin (Fuse et al., 1999; Hornyak et al., 2001). However, as a key regulator gene for melanocyte survival, the regulation mechanism of *MITF*/*Mitf* still remains unclear. Recently, an increasing number of studies have found that mutations of *Mitf* in minipigs can lead to a variety of coat color phenotypes, hereditary hearing loss and primary melanoma (Tachibana et al., 1996;Nishimura et al., 2005). Considering its proximity with humans in evolution, the minipig becomes an ideal animal model in study human diseases (Guo et al., 2015a,b). To establish a suitable animal model for further studies into *MITF*/*Mitf* functions, the current study aims to identify alternatively spliced transcript variants of porcine *Mitf* and analyze their expression in different tissues.

2. Materials and methods

2.1. Animals

Minipigs were obtained from China Agricultural University (CAU), Zhuozhou, Hebei Province, China.

2.2. Gene annotation of porcine Mitf

As already reported, human *MITF* expresses several isoforms, but the transcript profile of porcine *Mitf* has not been described explicitly. To characterize the structure of porcine *Mitf*, we compared the sequence of porcine *Mitf* (obtained from the NCBI UniGene database) with the sequence of human *MITF* (obtained from the Genbank database). The

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Table 1
Primer sequences for porcine *Mitf* isoforms.

Primer	Sequence	Location
E1F1	cacagctccaaagtaagaacagag	Exon 1A
E1F2	agagcccaaaacctattacgaact	Exon 1A
E4F1	cttgcagaacaccttaaaggaaaa	Exon 1H
E4F2	tgccagaactaactttgactttca	Exon 1H
E5R1	gtgatgtcatactggaggagctta	Exon 1B
E5R2	tagcaagatgcgtgatgtcatact	Exon 1B
E6R1	cgatttgtagactggcatagagaa	Exon 1M
E6R2	caatgagaaatggtggactattca	Exon 1M
CDS1F	atagtccaccatttctcattggat	Exon 1M
CDS1R	gccctgttttgttcttcaaactta	Exon 3
CDS2R	tctgtctgttttcaagctcttttg	Exon 8

structure characterization of porcine *Mitf* loci and the distribution among coding sequence were demonstrated by performing a pairwise BLAST search at the gene annotation database of NCBI.

2.3. Isoform-specific reverse transcription-polymerase chain reaction (RT-PCR)

To detect porcine *Mitf* expression, primers for isoform-specific RT-PCR were designed using the Primer Premier 5.0. The key principles in primer designing were followed, i.e. 1) sequences of primers and templates must be strictly complementary; 2) complementarity must not exit in primer itself and between primers to avoid formation of primer dimers and hairpin structures; and 3) mispairing must not happen at target sites of DNA templates. In our study, the forward primers located at upstream of specific isoforms M, H, and A, respectively. Primer sequences for porcine *Mitf* isoforms are listed in Table 1.

2.4. 5′ Rapid amplification of cDNA ends (5′ RACE)

Total RNA was extracted from the inner ear and skin tissues of normal minipigs, which was used to generate cDNA with reverse transcriptase. Subsequently, the cDNA was synthesized to analyze the expression of each isoform by 5′ RACE according to the manufacturers' instructions.

3. Results

3.1. Characterization of porcine Mitf cDNA coding sequence

The whole porcine *Mitf* sequence was scanned to deduce the homologous regions of *MITF* exons using BLAST at the NCBI database. All the 15 exons reported in human *MITF* were found in homologous regions in porcine *Mitf* with similar lengths and above 95% similarity. Longer than the 229 kb length of human *MITF*, the porcine *Mitf* gene spanned 310 kb and located at chr.13:56308861-56618423. The indentified 15 exons, arranging differently from human, were Exon1A, Exon1O, Exon1C, Exon1H, Exon6, Exon5, Exon4, Exon3, Exon3X, Exon2, Exon1M, Exon9, Exon1B, Exon7 and Exon8 successively. However, the sequences of 8 exons coded reversely and 16 gaps were found in the 310 kb-sequence, which might have resulted from splicing errors. The details for structural characterization of the *MITF*/*Mitf* coding sequence are showed in Table 2.

3.2. Identification of alternative splice variants of porcine Mitf

Since multiple transcript variants, differing in their initial exons spliced onto the later part, are common both in human and in murine *MITF/Mitf*, primers specific to the widest expressed variants in human, including Transcript4 (NM_000248.3), Transcript3 (NM_006722.2), Transcript1 (NM_198159.2) and Transcript2 (NM_198177.2), were designed to identify putative corresponding variants in minipigs.

RT-PCR was performed to amplify transcript variants expressed in cDNA isolated from skin and inner ear tissues.

Table 2
Structural characterization of *MITF*/*Mitf* coding sequence.

Exon No.	*Sus scrofa* (Pigs)					*Homo sapiens* (Human)				
	Length	Ori	Chr.	Start	End	Length	Ori	Chr.	Start	End
Exon1A	268	+	13	56308861	56309128	267	+	3	69788586	69788852
Exon1O	138	+	13	56331501	56331638	137	+	3	69812707	69812843
Exon1C	134	+	13	56331751	56331884	132	+	3	69812962	69813093
Exon1H	125	+	13	56442743	56442867	123	+	3	69915375	69915497
Exon1B	248	+	13	56596334	56596581	250	+	3	69928285	69928534
Exon1M	153	−	13	56475071	56475223	156	+	3	69985751	69985906
Exon2	231	−	13	56473795	56474025	228	+	3	69986973	69987200
Exon3X	708	−	13	56472778	56473485	714	+	3	69987503	69988216
Exon3	86	−	13	56472662	56472747	84	+	3	69988249	69988332
Exon4	99	−	13	56470136	56470234	96	+	3	69990387	69990482
Exon5	112	−	13	56462127	56462238	118	+	3	69998202	69998319
Exon6	58	−	13	56459059	56459116	57	+	3	70000981	70001037
Exon7	76	+	13	56614705	56614780	76	+	3	70005606	70005681
Exon8	149	+	13	56618275	56618423	148	+	3	70008424	70008571
Exon9	3586	−	13	56590670	56594255	3491	+	3	70013998	70017488

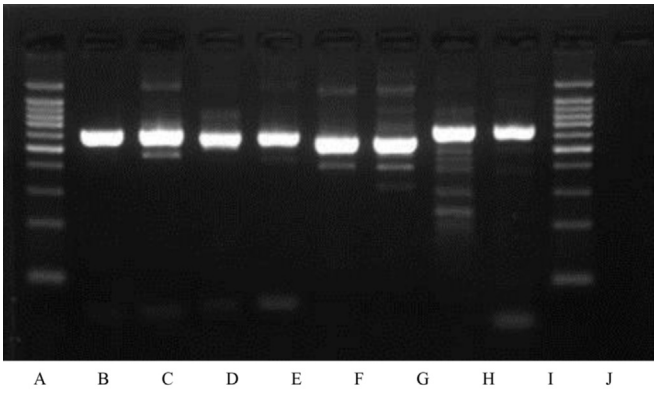

Fig. 1. RT-PCR gel photograph of porcine *Mitf* transcript variants. A: 100 bp DNA ladder; B: E4F2+CDS1R; C:E4F2+CDS1R; D:E4F1+CDS1R; E:E4F1+CDS1R; F:E1F2+CDS1R; G:E1F2+CDS1R; H:E1F1+CDS1R; I:E1F1+CDS1R; J:100 bp DNA ladder.

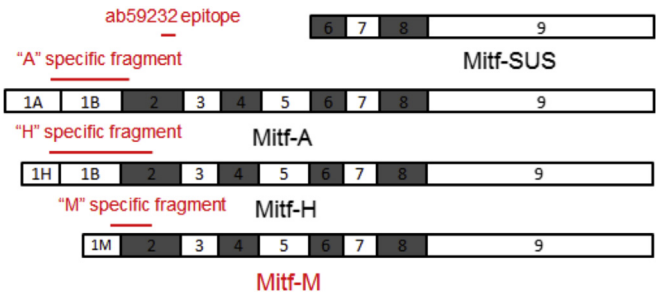

Fig. 2. Expression patterns of porcine *Mitf* transcript variants.

Only 4 transcript variants were detected by the four primer pairs: E1F1+CDS1R, E1F2+CDS1R, E4F1+CDS1R and E4F2+CDS1R (Fig.1).

Subsequently, the amplifications were recycled and cloned into DH5α E. coli. After sequencing the plasmid DNA extracted from the E. coli, we detected three homologous isoforms, corresponding to human MITF-A, MITF-H and MITF-M, respectively.

In minipigs, the isoform Mitf-A, transcribed by primer pair E1F1+CDS1R, begins with first exon1A and is spliced successively to Exon1B, Exon2 and Exon3. Meanwhile, the isoform Mitf-H, transcribed by primer pair E4F2+E5R2, begins with first exon1H and is spliced successively to Exon1B, Exon2 and Exon3. Another isoform Mitf-M, transcribed by primer pair CDS1F+CDS1R, begins with first exon1M and is spliced successively to Exon2 and Exon3.

3.3. Isoform-specific expression patterns of porcine Mitf

5′ RACE was used to determine if there were any alternative transcription initiation sites. The results proved that no exons existed in upstream of the determined first exons. Furthermore, a novel truncated porcine *Mitf* isoform was detected, which started from Exon6 and was spliced to Exon7 and Exon8. As this has never been reported and is first found in minipigs, this truncated transcript is recorded as Mitf-SUS.

In total, four porcine *Mitf* isoforms were found in our study, sharing high level homology with those of humans, except Mitf-SUS. Their expression patterns are described in Fig.2.

3.4. Expression of porcine Mitf isoforms in different tissues

Via fluorescent quantitative RT-PCR, expression of Mitf-A and Mitf-M isoforms in the heart, kidney, skeletal muscle, skin and inner ear was analyzed with GAPDH as an equal loading control. The expression of two isoforms varied with one accord in these tissues and both of them expressed at the highest level in skin and at the lowest level in kidney. Notably, Mitf-M in skin expressed two magnitudes higher than in other tissues, which might have resulted from the great amount of melanocytes in skin and hair follicles. Otherwise, Mitf-A expressed at a similar level in heart, skeletal muscle and skin (see Fig.3).

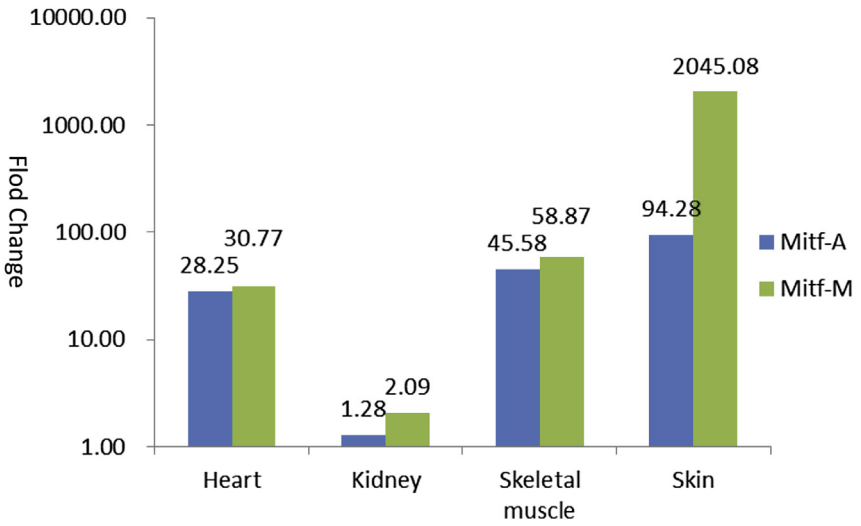

Fig. 3. Fluorescent quantitative RT-PCR for expression of porcine *Mitf* isoforms in different tissues.

4. Discussion

Based on the pig genome database (Sus 10.2), only four exons of porcine *Mitf* have been described with no experimental evidence. In our study, a pairwise BLAST search against the human cDNA at NCBI databases was performed to complement the analysis of porcine *Mitf*. Consequently, the structures and expression patterns of porcine *Mitf* were demonstrated in details.

According to previous studies, human *MITF* gene is expressed as a series of isoforms differing in their specific promoters and first exons. So far, at least nine transcript variants has been described, which start with specific promoters and initial exons and then are spliced to the common exons2–9 (Hershey and Fisher, 2005; Widlund and Fisher, 2003). At GenBank database, 8 transcript variants (NM_000248.3, NM_001184968.1, NM_006722.2, NM_198158.2, NM_198159.2, NM_198177.2, NM_198178.2, NM_001184967.1) are found and comprise of 15 exons. All these 15 exons are found in homologous regions in porcine *Mitf* with proximate length and above 95% similarity via the BLAST search, thus 15 exons of the porcine *Mitf* gene have been deduced.

To our knowledge, isoforms MITF-A, MITF-H and MITF-B express in all cell lines, MITF-M expresses only in melanocytes, while MITF-C expresses in all cell lines but melanocytes in human (Widlund et al., 2002; Udono et al., 2000). As there is no previous experimental evidence or report on the expression of porcine *Mitf*, the expression pattern in tissues of minipigs was described in our study by performing fluorescent quantitative RT-PCR, from which a porcine *Mitf* gene database was established.

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References

Fuse, N., Yasumoto, K., Takeda, K., Amae, S., Yoshizawa, M., Udono, T., Takahashi, K., Tamai, M., Tomita, Y., Tachibana, M., Shibahara, S., 1999. Molecular cloning of cDNA encoding a novel microphthalmia-associated transcription factor isoform with a distinct amino-terminus. J. Biochem. 126, 1043–1051.

Guo, W., et al., 2015a. The morphological and functional development of the stria vascularis in miniature pigs. Reprod. Fertil. Dev.

Guo, W., et al., 2015b Mar. The morphology and electrophysiology of the cochlea of the miniature pig. Anat. Rec. 298 (3), 494–500. http://dx.doi.org/10.1071/RD15183.

Hershey, C.L., Fisher, D.E., 2005. Genomic analysis of theMicrophthalmia locus and identification of the MITF-J/Mitf-Jisoform. Gene 347, 73–82.

Hornyak, T.J., et al., 2001. Transcription factors in melanocyte development: distinct roles for Pax-3 and Mitf. Mech. Dev. 101, 47–59.

Nishimura, E.K., et al., 2005. Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science 307, 720–724.

Tachibana, M., Takeda, K., Nobukuni, Y., Urabe, K., Long, J.E., Meyers, K.A., Aaronson, S.A., Miki, T., 1996. Ectopic expression of MITF, a gene for Waardenburg syndrome type 2, converts fibroblasts to cells with melanocytes characteristics. Nat. Genet. 14, 50–54.

Udono, T., Yasumoto, K., Takeda, K., Amae, S., Watanabe, K., Saito, H., Fuse, N., Tachibana, M., Takahashi, K., Tamai, M., Shibahara, S., 2000. Structural organization of the human microphthalmia-associated transcription factor gene containing four alternative promoters. Biochim. Biophys. Acta 1491, 205–219.

Widlund, H.R., Fisher, D.E., 2003. Microphthalamia-associated transcription factor: a critical regulator of pigment cell development and survival. Oncogene 22, 3035–3041.

Widlund, H.R., et al., 2002. Beta-catenin-induced melanoma growth requires the downstream target microphthalmia-associated transcription factor. J. Cell Biol. 158, 1079–1087.