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Identification and characterization of novel alternative splice variants of human *SAMD11*

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ABSTRACT

Sterile alpha motif domain-containing 11 (*SAMD11*) is evolutionarily conserved from zebrafish to human. Mouse *Samd11* is predominantly expressed in developing retinal photoreceptors and the adult pineal gland, and its transcription is directly regulated by the cone–rod homeodomain protein Crx. However, there has been little research on human *SAMD11*. To investigate the function of human *SAMD11*, we first cloned its coding sequence (CDS) and identified up to 45 novel alternative splice variants (ASVs). Mouse *Samd11* ASVs were also identified by aligning the mouse *Samd11* expressed sequence tags (ESTs) with the annotated sequence. However, the range of expression and transcriptional regulation of *SAMD11* differs between human and mouse. Human *SAMD11* was found to be widely expressed in many cell lines and ocular tissues and its transcription was not regulated by CRX, OTX2 or NR2E3 proteins. Furthermore, functional analysis indicated that human SAMD11 could promote cell proliferation slightly. In conclusion, this study elucidated the basic characteristics of human *SAMD11* and revealed that, although the occurrence of alternative splicing of *SAMD11* was conserved, the function of *SAMD11* may vary in different species.

1. Introduction

In the developing vertebrate retina, progenitors differentiate into photoreceptor cells in a conserved order (Swaroop et al., 2010). Many transcription factors are implicated in photoreceptor cell fate determination. Some transcription factors are responsible for eye formation and maintain the retinogenic potential of progenitor cells, such as PAX6 (paired box 6), SIX3 (SIX homeobox 3) and SIX6 (SIX homeobox 6) (Li et al., 2002; Liu et al., 2010; Shaham et al., 2012). Other transcription factors determine rod and cone cell fate, such as OTX2 (orthodenticle homeobox 2), NRL (neural retina leucine zipper), CRX (cone–rod homeobox) and NR2E3 (nuclear receptor subfamily 2, group E, member 3) (Glubrecht et al., 2009; Hennig et al., 2008). CRX is expressed early in photoreceptor precursors and enhances the expression of photoreceptor-specific genes (Mitton et al., 2000; Swaroop et al., 2010). OTX2 is expressed during final mitosis in retinal progenitors and early precursors, and is committed to photoreceptor cell fate (Chen et al., 1997; Koike et al., 2007). NR2E3 primarily suppresses the expression of cone genes and also serves as a coactivator of rod genes (Swaroop et al., 2010). The temporal–spatial expression of specific genes regulated by transcription factors guarantees the normal development of retina.

Sterile alpha motif domain-containing 11 (*SAMD11*), which has a SAM domain near its C-terminus, is evolutionarily conserved from zebrafish to human (Inoue et al., 2006). Human *SAMD11* is homologous to mouse *Samd11*, which is predominantly expressed in developing retinal photoreceptors and the adult pineal gland (Inoue et al., 2006). Genome-wide sequencing has identified the full-length human *SAMD11* sequence (FL-*SAMD11*) (GenBank ID: NM_152486) and two complete coding sequences (CDSs) with alternative promoters (alternative promoter *SAMD11*, AP-*SAMD11*) (GenBank ID: BC033213, BC024295) (Fig. 1). One alternative splicing (AS) event occurred in BC024295 (AP-*SAMD11*-ASV). There is no functional research that has been performed on human *SAMD11* to date.

To elucidate the function of human *SAMD11*, we first cloned its full-length CDS and identified up to 45 novel alternative splice variants (ASVs). Next, we examined the expression and transcriptional

Abbreviations: *SAMD11*, sterile alpha motif domain containing 11; SAM, sterile alpha motif; CDS, coding sequence; AS(V), alternative splicing (variant); EST(s), expressed sequence tag(s); PAX6, paired box 6; SIX3, SIX homeobox 3; SIX6, SIX homeobox 6; OTX2, orthodenticle homeobox 2; NRL, neural retina leucine zipper; CRX, cone-rod homeobox; NR2E3, nuclear receptor subfamily 2, group E, member 3; FL-*SAMD11*, full-length human *SAMD11*; AP-*SAMD11*, alternative promoter *SAMD11*; RPE, retinal pigment epithelium; HCE, human corneal epithelium; HREC, human retinal microvascular endothelial cell; PBS, phosphate buffered saline; (real-time) RT-PCR, (real-time) reverse transcription-polymerase chain reaction; PTC, premature translation termination codons; NMD, nonsense-mediated mRNA decay.

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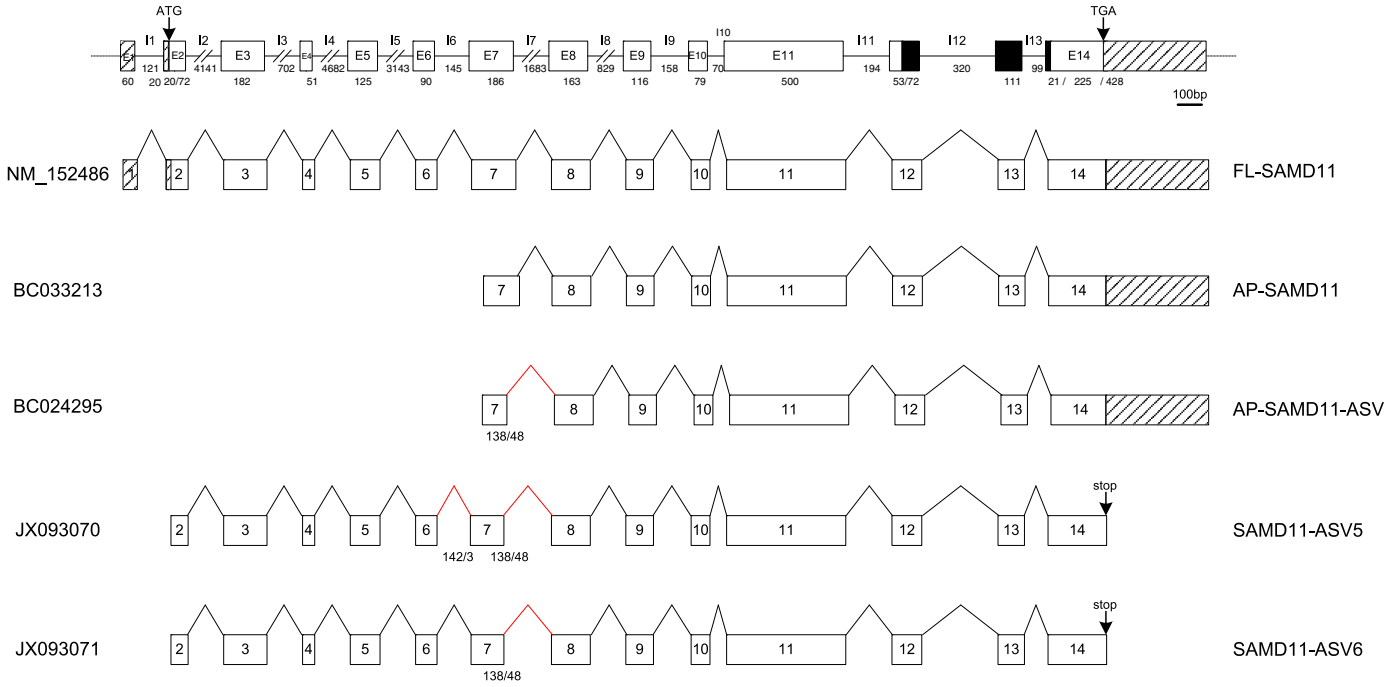

Fig. 1. Schematic representation of splicing patterns of human *SAMD11*. The first and second lines represent human *SAMD11* gene structure and constitutive splicing, respectively. The third and fourth lines denote AP-*SAMD11* and AP-*SAMD11*-ASV, respectively, which are approximately three-quarters of the length of FL-*SAMD11*. The fifth and sixth lines represent *SAMD11*, *ASV5* and *ASV6*, respectively. The numbers under the frames and solid lines indicate the base pairs of the corresponding exons and introns, respectively. Frames with shadows indicate the UTR. Solid black boxes indicate the SAM domain. The numbers under the segments of frames indicate the specific positions of AS sites on the relative exons or introns.

regulation of human *SAMD11*. Finally, we determined the effect of human SAMD11 on cell proliferation.

2. Materials and methods

2.1. Tissues, plasmids and antibodies

Ocular tissues were obtained from cornea donors who had no history of ocular diseases. Informed consent adhering to the tenets of the Declaration of Helsinki was obtained from the cornea donors. This study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center.

FL-*SAMD11* and AP-*SAMD11* were subcloned into pOZFHNP (Li et al., 2013). The full-length CDSs of CRX, OTX2, and NR2E3 were subcloned into pFLAG-CMV-2 (Sigma, USA); 2.0-kb, 1.5-kb, 1.0-kb and 0.5-kb upstream genomic sequences of *SAMD11* (Pro-2.0 k, Pro-1.5 k, Pro-1.0 k, Pro-0.5 k) were subcloned into pGL3-Basic (Promega, USA). The pGL3-Basic vector subcloned with mouse *Samd11* promoter (*Samd11*-Pro-1.2 k) was kindly provided by Tatsuya Inoue (Osaka Bioscience Institute, Japan).

Three antibodies were used: anti-SAMD11 (1:200, Abmart, Shanghai, China), anti-TUBB (1:1000, Chengdu Zen Bioscience, Chengdu, China) and anti-FLAG (1:1000, Abmart, Shanghai, China).

2.2. Cell culture and proliferation assay

Y79 (a retinoblastoma cell line), RPE (retinal pigment epithelium), HCE (human corneal epithelium), HREC (human retinal microvascular endothelial cell), 293T, HL-60 and SHI-1 (both human promyelocytic leukemia cell lines) cells were cultured as described previously (Wu et al., 2000).

For the proliferation assay, stable cell lines were established by introducing FL-*SAMD11*, AP-*SAMD11* or the pOZFHNP vector into 293T and RPE cells using the pOZFHN retroviral system. These cells were cultured in 12-well plates and trypsinized for cell counting on the subsequent three days.

2.3. Cloning and PCR

For cloning, the full-length CDS of human *SAMD11* was amplified using the forward primer 5′-ATGTCCAAGGGGATCCTGCAGG-3′ and the reverse primer 5′-TCAACACAGAGGCTGGGAAGGG-3′. For PCR, six pairs of human *SAMD11* primers and a pair of *GAPDH* primers were designed. The sequences of the primers are detailed in Table 2.

2.4. Western blot analysis

Cultured cells were washed with ice-cold PBS and lysed with lysis buffer. Ocular tissues were flash frozen in liquid nitrogen, homogenized by milling and lysed with lysis buffer. The composition of the lysis buffer and the Western blot procedures were as described previously (Wu et al., 2000).

2.5. Luciferase assay

Cells were plated in 6-well plates and transfected with luciferase reporters driven by *SAMD11* proximal promoter regions of different lengths (Pro-2.0 k-luc, Pro-1.5 k-luc, Pro-1.0 k-luc and Pro-0.5 k-luc) and transcription factors. Cells transfected with mouse *Samd11* promoter fused to the luciferase gene (*Samd11*-Pro-1.2 k-luc) and CRX expression vector were used as a positive control. Luciferase assay was performed as described previously (Chen et al., 2012; Li et al., 2013; Shen et al., 2006).

2.6. Statistical analysis

Results were expressed as mean ± sd. In the luciferase assay, statistical differences were evaluated by one-way analysis of variance (ANOVA) followed by LSD multiple comparisons tests using