



New Splicing Variants of the Murine Damaged DNA Binding 2

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Damaged DNA binding (DDB) protein is an important gene in the repair of damaged DNA. DDB is a heterodimer (DDB1 and DDB2) protein, murine *DDB2* has 10 exons about 1.5kb in size (Genbank Accession No. AY027937). Here we identified five *DDB2* variants (M1-M5) from various mouse tissues that are generated by alternative splicing. We used reverse transcription-PCR (RT-PCR) to identify splicing variants and isolated PCR products using an agarose-gel PCR purification kit. All isolated PCR products were cloned and the structure of splicing variants was confirmed by sequencing. The first splicing variant M1 was generated by omission of exon 4. The second splicing variant M2, by omission of exons 4-5. The third variants M3 was generated by omission from the middle of exon 1 to exon 6 and was expressed in the heart. Fourth variants M4 was generated by omission of exon 2 and exons 4-7. M5, the last splicing variant was generated by omission of exons 4-7. M4 and M5 were expressed in the spleen. Analysis of tissue distribution by RT-PCR indicates that M1 is most highly expressed in the mouse brain. These results indicated that murine *DDB2* has five splicing variants and splicing variants expression patterns were different depending on mouse tissue. Further functional studies of each splicing variants will provide more information about the molecular mechanism of *DDB2* function and *DDB2* gene expression regulation.

Key words: DDB2, splicing variant, repair, mouse

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The damaged DNA binding (DDB) protein complex was identified as a heterodimeric complex that binds to the damaged DNA (Keeney *et al.*, 1993). The DDB protein complex is composed of two subunits with molecular masses of 127 and 48 kDa (known as DDB1 and DDB2), and the activity is dependent on the formation of the heterodimeric complex (Fujiwara *et al.*, 1999). DDB strongly binds to damaged DNA, caused by UV light. The DDB binding to damaged DNA might suggest a direct role for DDB in nucleotide excision repair (NER) (Aboussekhra *et al.*, 1995). Recognition of lesions for NER involves two mechanisms; transcription-coupled repair (TCR) and global genomic repair (GGR) (Bohretal., 1985). TCRs repair lesions on the transcribed strand of expressed genes, while GGRs repair lesions from

both non-transcribed genomic DNA and the non-transcribed strand of expressed genes. DDB is thought to be involved in GGR UV-DDB protein recognizes the cyclobutane pyrimidine dimers (CPDs) at the initial step of global genomic repair. *DDB2* also has a critical role for the initiation step of NER (Hwang *et al.*, 1999). Moreover, the *DDB1* has no mutation form, but *DDB2* has several mutation forms in XP patients (Itoh *et al.*, 1999).

Four human *DDB2* variants were identified from HeLa cells. These are generated by alternative splicing and, especially, some splicing variants are dominant negative inhibitors of DNA repair (Inoki *et al.*, 2004). The splicing variants disrupted the complex formation between *DDB1* and *DDB2*. Recent study shows that *DDB2* gene disruption leads to skin tumors and resistance to apoptosis after exposure to UV but not a chemical carcinogen (Itoh *et al.*, 2004). The *DDB2* gene structure and functional studies were very well studied but murine *DDB2* splicing variants are still unknown.

In this study, we identified five splicing variants of *DDB2* from murine tissues. Murine *DDB2* splicing variants were

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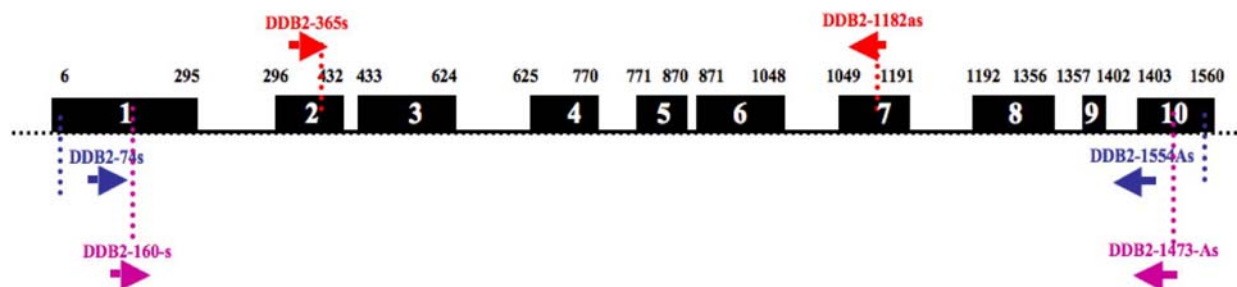


Figure 1. Mouse *DDB2* genomic structure. Exons 1 to 10 are shown as black boxes. The numbers in the black boxes represent exon numbers and the above numbers are nucleotide numbers. Arrows indicated primers.

differently expressed depending on the murine tissue. These results indicated that murine *DDB2* does not function as the same as human *DDB2* variants and requires further study of *DDB2* splicing variants function.

Materials and Methods

RNA preparation and reverse transcriptase polymerase chain reaction

Total RNA was extracted from various mouse tissues used Trizol according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). The cDNAs were synthesized using the Reverse Transcription system (Promega, Madison, WI). Primary 3 software (Whitehead Institute, MIT Center for Genome Research, Cambridge, MA) was used to design specific primers for *DDB2* and *tubulin*. Each forward and reverse primers were as followed. *DDB2*-74-s, 5'-atcgtagtccttctgtt-3'; *DDB2*-160-s, 5'-gagtagctatggctccc-3'; *DDB2*-365-s, 5'-gtagcagcattgtcaggg-3'; *DDB2*-1264-As, 5'-gtgagtgctgaaatgg-3'; *DDB2*-1473-As, 5'-ccatcttcatagtcttcatg-3'; *DDB2*-1554-As, 5'-tacttttggcctattaac-3'.

cDNA cloning and Sequencing

The amplified DNA fragments for wild-type and variants were separated on a 3% agarose gel and purified using GeneClean Turbo kit (Qbiogene, Carlsbad, CA). Cloning was done by the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Sequencing reactions were performed by Macrogen (Seoul, South Korea).

Results and Discussion

The mouse *DDB2* gene has 10 exons and total size is about 1.5 kbp (Figure 1). To determine the *DDB2* gene expression pattern, first, we examined the distribution of *DDB2* gene expression in various mouse tissues (Figure 2A). The reverse transcriptase-PCR (RT-PCR) using *DDB2*-74-s and *DDB2*-1554-As, these primers amplified from exon 1 to exon

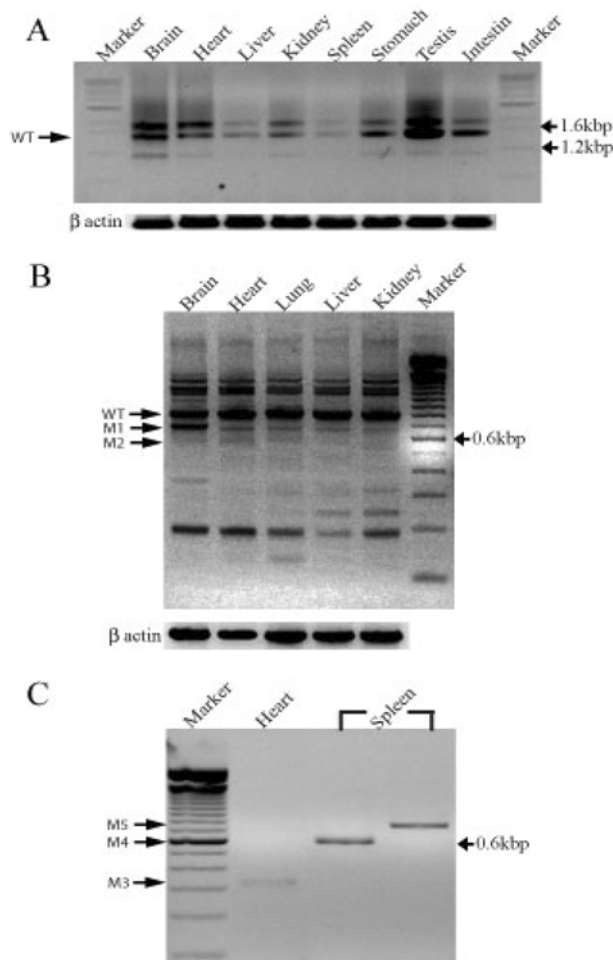


Figure 2. Mouse *DDB2* expression and splicing variants in various mouse tissues. (A) *DDB2* wild type (WT) distribution in various tissues by reverse-transcriptase PCR (RT-PCR). (B) *DDB2* splicing variants (M1 and M2) in various tissues (C) Amplification of *DDB2* splicing variants (M3, M4 and M5) cloned in TOPO vector in the heart and spleen. β -actin was used as an internal control for the PCR.

10. *DDB2* wild-type gene was expressed more in the brain, heart and testes than other tissues. Recently studied, the human *DDB2* splicing variants were more expressed in human brain and heart (Inoki, *et al.*, 2004). We selected mouse

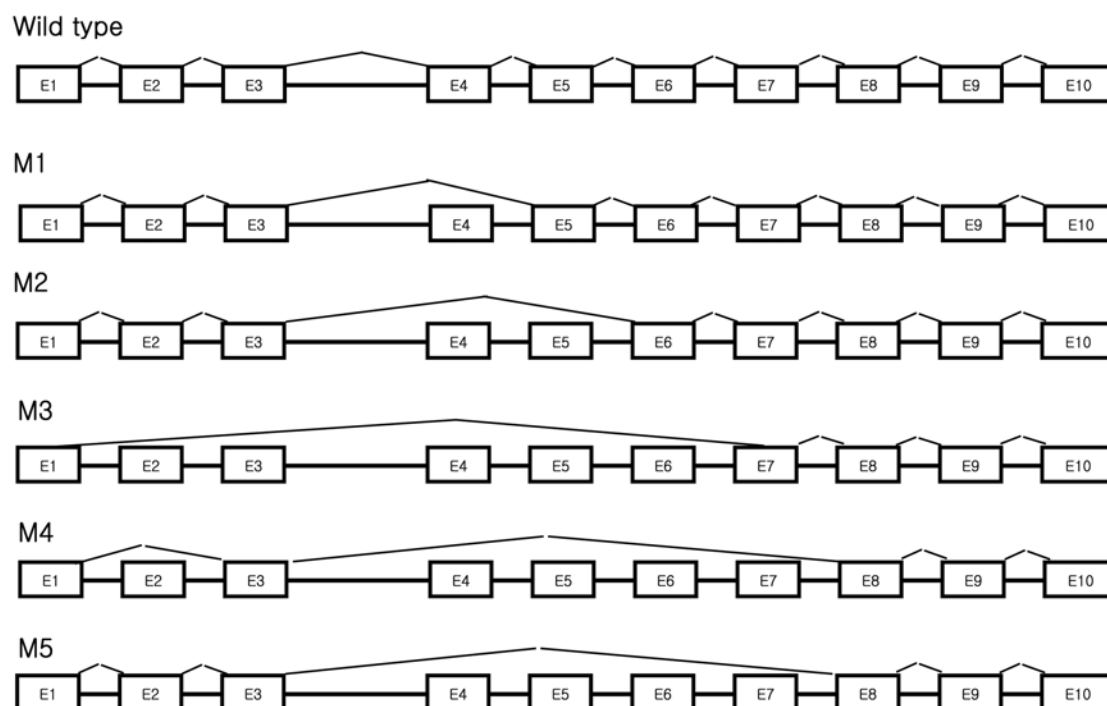


Figure 3. DDB2 wild type and variants gene structure. The structure of splicing variants was confirmed by sequencing. M1, omission of exon 4; M2, omission exons 4-5; M3, omission from the middle of exon 1 to exon 6; M4, omission of exon 2 and exons 4-7; M5, omission of exons 4-7.

brain, heart, lung, liver and kidney for detection of mouse *DDB2* splicing variants, using primer DDB2-365-s and DDB2-1264 which amplified from exon 2 to exon 7 (Figure 2B). The amplified DNA fragments are separated on a 3% agarose gel, purified using agarose-gel purification kit. All fragments were cloned and sequenced. These DNA fragments identified a splicing variant product from the *DDB2* gene and other bands are non-specific band. The wild type *DDB2* fragment was not omitted from any exons, and highly expressed in various tissues. The 146bp fragment was skipped in the splicing variant of *DDB2*, M1, which was generated by omission of exon 4 (Figure 2B). M2, the second splicing variant was generated by omission of exon 4 and 5 (Figure 2B). These two fragments were strongly expressed in the brain (Figure 2B). These results indicated that *DDB2* gene was expressed in various mouse tissues being most highly expressed in the brain.

We investigated whether the other tissues has different splicing variants. Primers, DDB2-160-s and DDB2-1473-As, amplified the *DDB2* gene from exon 1 to exon 10. The heart has one different variant, the splicing variant M3, generated by omission of the middle of exon 1 (starting point of amino acid sequence 179) and exons 2-6. The spleen has two splicing variants M4 and M5. M4 was generated by skipping exons 2 and 4-7. The last variant, M5, was

generated by omitting from exon 4 to exon 7 (Figure 2C). These results indicated that the *DDB2* gene has five different splicing variants and the splicing variants has different expression pattern in various mouse tissues (Figure 3).

Alternative splicing is a crucial mechanism for gene regulation and for generating proteomic diversity. Recent estimates indicate that the expression of nearly 95% of human multi-exon genes involves alternative splicing (Roesser *et al.*, 1993; Itoh *et al.*, 2006; Fedor, 2008). *DDB2* is important for formation of DDB complex dimer and function (Stoyanova *et al.*, 2009). The human *DDB2* splicing variants type 2 is a dominant negative inhibitors of damaged DNA repair (Inoki, *et al.*, 2004). But, numbers and functions of splicing variants of mouse *DDB2* are still unknown. Our results showed that mouse *DDB2* has five splicing variants and that each splicing variant expression pattern was different. We confirmed that mouse brain tissue has two splicing variants, the heart has one variant and the spleen has two variants. Alternative splicing contributes to genomic diversity and tissue specificity (Graveley, 2001). Comprehending tissue-specific alternative splicing requires an understanding of the regulatory network of protein-protein, protein-RNA and RNA-RNA interactions that are involved in this process (Graveley, 2009). Tissue-specific alternative splicing is thought to be controlled by differentially expressed splicing regulators and ubiquitously expressed splicing factors

of different concentrations and/or activity (Black, 2003). Moreover, splicing of individual pre-mRNA is frequently controlled by combinatorial or competitive effects of both activators and inhibitors (Mayeda *et al.*, 1993). Tissue-specific alternative splicing events can be explained in part by tissue-specific expression of splicing factors, and the corresponding regulation of their target mRNA transcripts (David and Manley, 2008; Ohno *et al.*, 2008). Further studies are required in order to define the splicing variants function and to regulation of wild type *DDB2* expression in each tissues. In conclusion, we identified five mouse *DDB2* splicing variants in various mouse tissues. Each tissue shows a different expression pattern. An exploration of *DDB2* variants functional studies should provide new insights into the *DDB2* gene mechanism and regulation.

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