GENOMIC STRUCTURE OF MOUSE TBX2 AND DETECTION OF EXPRESSION OF TBX2 IN NORMAL AND MALIGNANCE MELANOPHORE BY RT-PCR

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ABSTRACT

Objective: Sequencing of mouse Tbx2 gene and observing the expression of Tbx2 gene in normal and malignant melanophore. Methods: The PCR products of Tbx2 cDNA were cloned into pUC18 vector and sequenced. The normal and malignant melanocytes were used to extract total RNA. The expression of Tbx2 gene was detected by RT-PCR. Results: The Tbx2 genome is composed of seven exons and six introns. No expression of Tbx2 gene in the normal melanocytes was noted, but all malignant melanocytes showed expression of Tbx2 gene. Conclusion: The observation showed the analysis of the genomic structure of mouse Tbx2. Tbx2 plays a critical role during the development of the malignant melanophore.

Key words: Gene amplification, Sequence analysis, Genom, Gene expression, Melanophore, Tbx2

T-box genes represent a recently identified family of highly evolutionarily conserved genes that encode proteins ranging in size from ~400 to >900 amino acids.[1,2] Members of this gene family share a motif-200 amino acids which in the T gene product has been shown to exhibit sequence-specific, DNA-binding activity.[3] Chapman and associates[4] have shown that six murine T-box genes exhibit overlapping but unique patterns of expression during embryogenesis, indicating that expression of these gene is temporally and spatially regulated. This suggests that, similar to Pax gene, T-box genes play important roles in development. Recent mutation in two of the human T-box gene, Tbx3 and Tbx5, have been shown to be responsible for two human dysmorphoric syndromes, Holt Oram[5] and lunar-mammary syndrome.[6] Tbx2 is a member of T-box gene family of transcription factors.[7] Little is known about the structure and function of mouse Tbx2. In this study, we present the complete genomic structure of mouse Tbx2 and confirmed the relation between function of mouse Tbx2 genes and tumors.

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MATERIALS AND METHODS

Reagents

The enzymes of AMV, Taq DNA polymerase and dNTPs were purchased from Promega CO. T4 DNA ligases and T4 DNA polymerase was purchased from Biolabs CO. The endonuclease BamH I, Hind III, EcoR I, Nco I and XhoI were purchased from Promega C0.

Cell Lines, Bacterial and Plasmid

The normal melanophore line K1735 and malignant melanophore lines Mela I, Mela II, Mel c, HM96 and B16 were maintained in RPMI 1640 medium containing 10% fetal bovine serum, penicillin and streptomycin in a humidified 37° incubator with 5%CO2. Escherichia coli DH 5 α strain was used in molecular cloning. The plasmid pUC18 was a gift from Dr. CR Goding at Marie Curie Cancer Research Institute in the UK.

RNA Isolation, Reverse Transcription and PCR

Isolation of total cellular RNA procedures was as described.[8] For the RT-PCR isolation of the Tbx2 cDNA, total cell RNA was subjected to reverse transcription with AMV reverse transcriptase followed by a first strand cDNA synthesis. All PCR of primers (Mouse Tbx2 primers and G3PDH primer as positive control) were designed according to the published

sequence of Gene Bank (MMU15566) and produced from Marie Curie Cancer Research Institute. PCR was performed in the volume of 100μl containing 500 ng upstream and downstream primer each, 1.5mM MgC12, and 4 Taq DNA polymerase. The parameters of PCR amplification of RT-PCR and cloning PCR for sequencing were first cycle: 95°C 5 min, when 70°C, Taq was added, and then 50°C 45" and 72°C 1min and other cycles: 95°C 40", 50°C 45" and 72°C 1min from 2 to thirty cycles. After thirty cycles of amplification, the PCR products (10μl) were subjected to electrophoresis on 1.5% agarose gel and the product was followed by ethidium bromide staining.

Cloning of the Mouse Tbx2 PCR Product

PCR products were received by sucrose gradient centrifugation. The amplified PCR fragment was inserted into the sequencing vector pUC18 after BamH I and Xho I T4 DNA ligases was added to the mixture for ligation at 21°C for six hours. The reaction products were transformed into competent E coli DH5 α cell that was previously prepared with CaC12. The transforming mixture was selected on LB agar plates with ampicillin 100mg/L. The transformants were screened by plasmid minipreparation. Constriction mapping and DNA amplification further identified all recombinant clones.

Sequencing of Mouse Tbx2

Sybersyn CO performed sequence analysis after Sephrose 4B purified all recombinant clones. The complete genomic structure of human Tbx2 have been described[9] and compared with mouse Tbx2 genomic structure.

RESULTS

The Expression of Mouse Tbx2 in Normal and Malignance Melanophore by RT-PCR

Total cellular RNA of the normal melanophore line K1735 and malignant melanophore lines Mela I, Mela II, Mel c, HM96 and B16 were extracted and the expression of mouse Tbx2 was examined with RT-PCR technique. No expression of Tbx2 gene in the normal melanocyte was noted, but all malignant melanocytes showed expression of Tbx2 gene. These data are present in Figure 1.

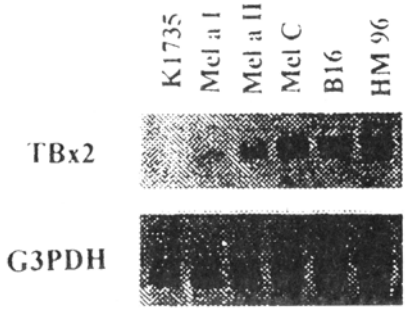

Fig. 1. The expression of mouse Tbx2 in normal and malignance melanophore by RT-PCR

Cloning of the Mouse Tbx2 PCR Product

The PCR products were subcloned into the BamH I/Xho I sites of the pUC18. Briefly, the cloning products were transformed into competent E coli DH5 α cell and then were selected on LB agar plates with ampicillin 100mg mg/L. The transformants were screened by plasmid minipreparation. Restriction mapping and DNA amplification further identified all recombinant clones. It was suggested that the fragments by restriction mapping and DNA amplification were the fragments of prediction.

Sequencing of Mouse Tbx2

The Tbx2 genom is composed of seven exons and six introns according to sequencing of PCR product. The structure of the mouse Tbx2 genom was similar to human Tbx2 genom, but exon/intron boundaries between mouse and human Tbx2 were completely different. These data are present in figure 2 and 3.

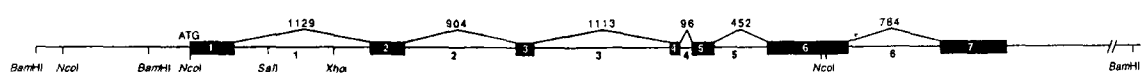

Fig. 2. Genomic structure of mouse Tbx2

DISCUSSION

Almost 70 years ago, a mutant mouse having a short blunt-ended tail was recovered in the progeny from an X-ray-induced mutagenesis study.[10] The mutation responsible for this phenotype was named Brtachyury (short tail), or simply T for tail. In homozygotes, the T mutation was found to express a

more severe phenotype resulting in embryonic death during midgestation with abnormalities in mesoderm-derived tissue. The cloning of genes characterized by embryonic lethal alleles has often been disappointing in terms of providing an understanding of development because most essential genes will not be

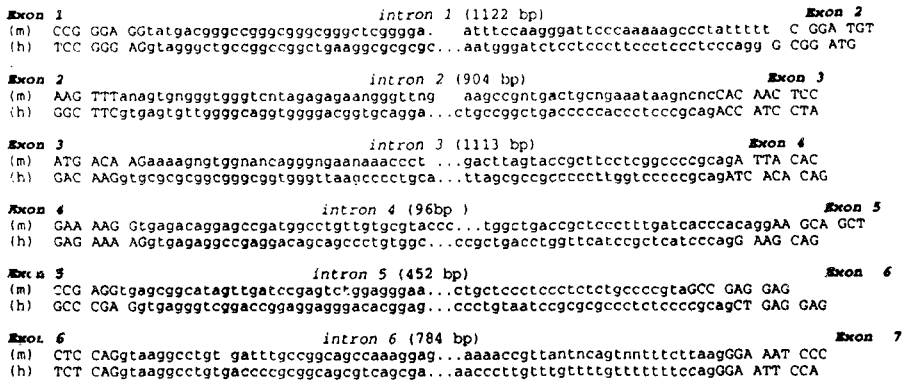

Fig. 3. Results of genomic structure between Tbx2 mouse and human

directly involved to be an exciting exception to this general rule.[11] The expression pattern of the T gene during normal mouse development corresponds directly with the tissues affected in embryoshomozygous for the null mutation.[12] The T gene product has been shown to have sequence-specific DNA binding properties,[13] and studies in both the mouse and zebrafish have shown a nuclear localization of the T gene product.[14] Its role as a transcription factor is supported by the characterization of 2 dominant negative mutations (T^c and T^{wis}) that produce truncated protein products which retain the N-terminal DNA-binding domain but not the C-terminal region.[15] Recently the T protein has been shown to activate expression of a reporter gene through binding to the T consensus sequence, confirming its role as a transcription factor.[15] The cloning and characterization of T gene homology in a variety of other vertebrate species provides strong evidence for the conservation of its function as a transcriptional regulator for a set of as yet unknown target genes involved in mesoderm development. Tbx2 is a member of a recently discovered gene family of transcription factors that contain the T-box domain and have been named T-box 1–6 (Tbx1 through Tbx6). Up to date, no research report was found in the literature on the relation of mouse Tbx2 between the structure and function. It has been reported that Tbx2 expression is evident in the hindlimb and forelimb, rib cartilage during early mouse development ect. The study by Carreira, et al. indicated that the brachyury-related transcription factors is a repressor of the melanocyte-specific TRP-1 promoter.[16] Our data has demonstrated the complete genomic structure of mouse Tbx2 and showed that Tbx2 plays a critical role during the development of the malignant melanophore.

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