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Developmental Characteristics of Mice Lacking the DNA Excision Repair Gene XPG

Xue-Zhi SUN,[1] Yoshi-Nobu HARADA,[2] Chun CUI,[3] Rui ZHANG,[3] Sentaro TAKAHASHI[1]
Yoshihiro Fukui[3] and Yoshiharu MURATA[4]
[1] Environmental and Toxicological Sciences Research Group
[2] Frontier Research Center
National Institute of Radiological Sciences, Chiba 263-8555, Japan
[3] Department of Anatomy and Developmental Neurobiology
School of Medicine, Tokushima University, Tokushima 770-8503, Japan
[4] Department of Molecular and Cellular Adaptation
Research Institute of Environmental Medicine
Nagoya University, Nagoya 464-8601, Japan

Abstract: A new mutant mice that carried the nonfunctional xeroderma pigmentosum group G gene (the mouse counterpart of the human XPG gene) alleles have been generated through a gene-targeting and embryonic stem cell technology. The developmental characteristics of the -/- homozygous mice identified by PCR and Northern blotting were studied. Body size of mutants was clearly smaller than normal littermates from the age of 6 days. Such postnatal growth failure became more and more obvious with developmental proceeding. By postnatal day 23, all of the mutants died after showing great weakness and emaciation. These symptoms in the mutants were similar to the clinical phenotypes of Cockayne syndrome. Moreover, some progressive neurological signs also appeared in the homozygous mice around 2 weeks after birth. When compared development of brains at postnatal day 19, both cerebrum and cerebellum of the xpg-mutants were smaller and significant difference from the wild-types. Their weights only accounted for 79.5% and 66.9% of those in the wild-types, respectively. Such microcephaly and progressive neurological signs mimicked the clinical phenotype of the patients with XPG. We believe that the xpg null mice will be an animal model for studying mechanisms concerning the clinic symptoms and Cockayne syndrome in XPG patients.

Key words: cerebellum, cerebrum, Cockayne syndrome, xeroderma pigmentosum

Xeroderma pigmentosum (XP) is a rare aurtosomal recessive disease characterized by hypersensitivity of the skin to sunlight and an increased risk of skin cancer in sun-exposed parts of the body, and defective DNA repair has been reported in this disease.[1,2] Individuals with this disease can be divided into eight complementation groups: A-G and an XP-variant form according to cell fusion studies.[3] Each represents a different genetic defect in DNA repair. Complementation group G (XPG) is one of the pathophysiologically heterogeneous forms of this autosomal disease. XP patients in group G show various clinical symptoms, ranging from very mild cutaneous abnormalities to severe dermatological impairments, and a few cases are also associated with the neurological degeneration and growth retardation of Cockayne syndrome. However, possible mechanisms in these clinical symptoms are still unknown.

To study such mechanisms, we have generated mice carrying the nonfunctional xpg (the mouse counterpart of the human XPG gene) alleles by using gene-targeting and embryonic stem cell technology. The present study described the generation and developmental characteristics of the mice with the nonfunctional xpg gene.

Materials and Methods

1. xpg-mutant mice

1.1. xpg targeting vector

Mouse genomic clones containing several exons of the xpg were isolated from a lambda DASH II (Stratagene) phage library constructed with genomic DNA from the mouse strain 129/Sv-derived ES cell line D3. An insertional mutation at the mouse xpg locus was showed in Fig.1. A 4.8-kb Bam Hi-Bg/II fragment obtained from one of the genomic clones was subcloned into pUC118 and was used to generate the targeting vector. Since above fragment contained exon 3, that just corresponds to nucleotide residues 264 to 380 downstream of the ATG start cordon of the xpg cDNA.[4,5] An insertional mutation was generated by inserting the 1.1-kb XhoI-BamHI neo cassette from pMC1neo (Stratagene) into the XhoI site of exon 3 in the same transcriptional orientation. A 3.5-kb BamHi-EcoRI fragment of the herpes simplex virus thymidine kinase (HSV-TK) cassette was positioned at the 3' end of the construct for negative selection. The targeting vector constructed was designated as pMER5/TV.

Author for correspondence: Dr. Xue-Zhi SUN at the above address. Tel: 043-206-3159 Fax: 043-2514853 E-mail: sun_s@nirs.go.jp

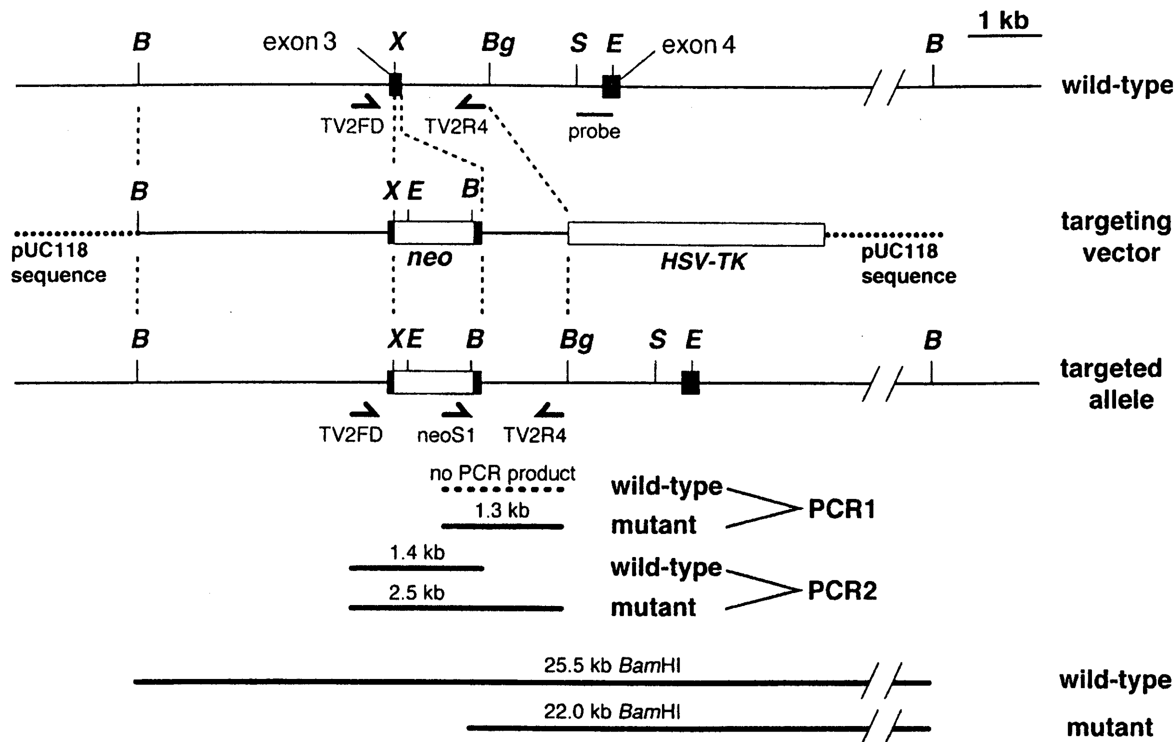

Fig. 1 Showing gene targeting at the xpg locus.
Black boxes represent two exons (3 and 4) of the xpg gene. Arrows indicate polymerase chain reaction (PCR) primers. A solid bar corresponding to the S-E fragment on the wild-type map on the top shows the 3' external probe used for Southern blot analysis. Solid lines on the bottom show the diagnostic fragments of 22.0 and 25.5 kb. B, BamHi; X, XhoI; Bg, Bg/II; E, EcoRI; S, a probe containing a 0.5-kb SphI-EcoRI fragment.

1.2. Gene targeting in ES cells

ES cell line D3 derived from mouse strain 129/Sv was maintained in Dulbecco's modified Eagle medium (Nissui Seiyaku Co., Ltd, Tokyo, Japan). The cell were typsinized, resuspended at a concentration of 10^7 cells/ml in phosphate buffer saline, and electroporated with 50 μg of SalI-linearized vector DNA at 2,000 V/ml for 1 s with an electroporation (somatic hybridized SSH, Shimadzu Co., Ltd., Kyoto, Japan). Twenty-four hours after cells were transferred onto culture dishes coated with 0.1% gelatin in the medium described above; these cells were cultured with a selection medium coating 200 μg of G418 per ml (GIBCO) and 2 μM ganciclovir (Syntex Research). The targeting event occurred at a frequency of 15.4%.

1.3. Generation of xpg homozygous mutant mice

Chimeras were constructed by injection of targeted ES cells into C57BL/6J balstocysts collected at day 3.5 postcoitum. About 10 to15 ES cells from each cell clone with a normal karyotype and carrying the homologous recombination were microinjected into the recipient blastocysts, and five embryos were transferred into each uterine horn of ICR pseudoprefnant foster mothers. Five chimeras derived from two independent ES clones were crossed with C57BL/6J females to transmit the mutant allele through the germ line. All five chimeric males generated offspring with pigmentation, which is the hallmark of derivation from D3-derived germ cells.

2. Examination of development of xpg homozygous mutant mice

Development of the xpg -/- homozygous mutant mice were observed since postnatal day 0. Mice types were identified by polymerase chain reaction (PCR) and Northern blotting (Figs not shown). Out of 163 pups born, 39 mice showed wild type (+/+), 89 mice were heterozygous (+/-), and 35 mice were homozygous (-/-). Although the heterozygous mice did not exhibit differences of development from wild-type mice, we chose the wild-type mice as controls in the present study. Around 3 week of age, all of the xpg homozygous mice died, so the bodies of the offspring were weighted during postnatal days 0 to 23, and their brains were removed and subsequently weighted at postnatal day 19. Cerebrum and cerebellum were weighted separately before fixing with 4% buffered formaldehyde. About 8-35 offspring were examined at each experimental period in each group. The data were analyzed using the Student's t-test, and p values of less than 0.05 were considered significant.

Results and Discussion

Fig. 1 summarized in a simplified manner gene targeting at the xpg locus. As a targeting vector, the pMER5/TV2 was designed to generate an insertional mutation in the 3rd exon of the mouse xpg gene. The capable of transmitting the mutant allele to F1 offspring from the targeted ES cells injected into C57BL/6J blastocysts was identified by both PCR and Southern blot analyses. The predicted PCR products were PCR1 with the neoS1 and TV2R4 primers and PCR2 with the TV2FD and TV2R4 primers. The restriction fragments were detected in the fragments of 22.0 and 25.5 kb. These results indicated that the xpg gene was inserted successfully.

Gross observation revealed no obvious differences in the body size between -/- homozygous mice and +/+ wild-type mice within 4 days after birth. However, body sizes of mutants were clearly smaller than normal littermates from the age of 6 days by their lower body weights. Such postnatal growth failure in the xpg homozygotes became more and more obvious with developmental proceeding. The average of body weight in the mutant mice only accounted for near half of that in the normal littermates at postnatal day 23 (Fig. 2). The life span of the homozygotes was shown in Fig. 3. At age of 16 days, number of survivors was only the half of total mice. By postnatal day 23, all of the mutant mice died after showing great weakness and emaciation. To eliminate the reason of suckling, we also observed that mutant pups sucked mother's milk. No any obvious physical changes or abnormal behaviors involving suckling were found. These mutant homozygous pups seemed to cling normally to the teats of their dams. Therefore, developmental characteristics like postnatal growth retardation and premature death in mutant mice should result from introducing the nonfunctional xpg gene. These symptoms in xpg mutants were similar to the clinical phenotypes of Cockayne syndrome.[6,7]

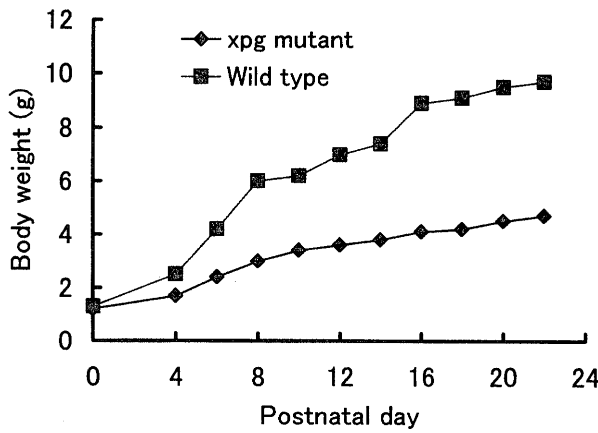

Fig. 2 Development of body of the wild-type mice and the xpg-mutant mice.
Body weights in the mutant mice at the age of 6 days and thereafter are significant different from that in control mice ($P<0.001$; n = 8 ~ 25).

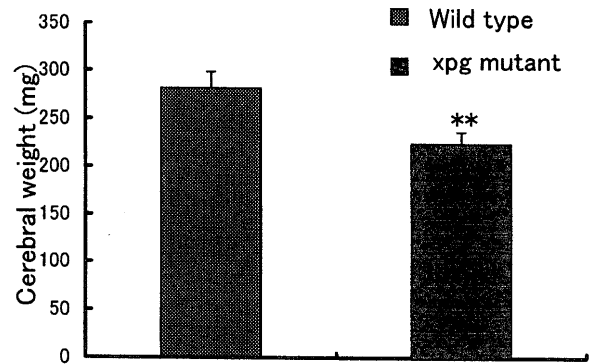

Fig. 4 Development of cerebrum of the wild type mice and the xpg mutant mice at the postnatal day 19.
Significant difference of the cerebral weight of the mutants from the wild-type mice is indicated by asterisks. **: $P<0.001$, n = 9.

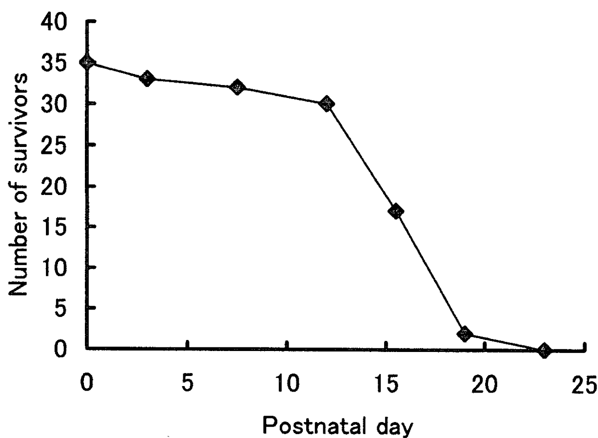

Fig. 3 Life span of the xpg mutant mice.
At age of 16 days, number of survivors was only the half of total mice. All of the xpg mutant mice died at the postnatal day 23.

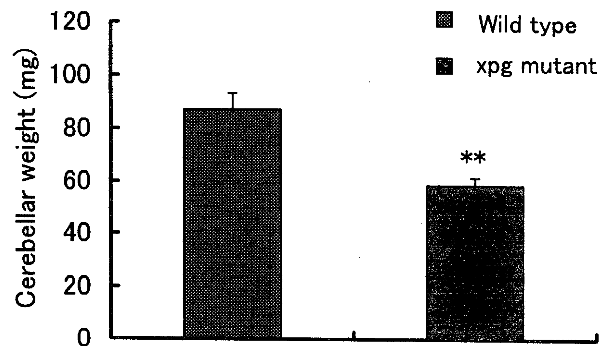

Fig. 5 Development of cerebellum of the wild type mice and the xpg mutant mice at the postnatal day 19.
Significant difference of the cerebellar weight of the mutants from the wild-type mice is indicated by asterisks. **: $P<0.001$, n = 9.

On the other hand, some progressive neurological signs in the xpg mutant mice were also observed. Their level of activity and response to environmental stimuli appeared to be low around 2 weeks after birth and a progressive ataxia also appeared in some mutant mice at this developing stage. When compared development of brains between mutants and controls at postnatal day 19, we found that brain sizes including cerebrum size and cerebellum size were smaller than that of the wild type mice of the same age. T-test result indicated that both cerebrum and cerebellum of the mutant mice were significant difference from the controls (Figs. 4, 5). The average of cerebral weight and cerebellar weight in the homozygous mice only accounted for 79.5% and 66.9% of those in the wild-types, respectively. Such small size brain (microcephaly), brain developmental retardation, and progressive neurological signs mimicked the clinical phenotype of the patients with XPG.[8,9]

The findings in the present study revealed characteristic development mice carrying the nonfunctional xpg. We believe that the xpg null mice we have generated will be an animal model for studying mechanisms concerning the clinic symptoms and Cockayne syndrome in XPG patients. The precise studies in the cellular and molecular levels are hopeful to understand these mechanisms.

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