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Altered gene expression profiles and higher frequency of spontaneous DNA strand breaks in APEX2-null thymus

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ABSTRACT

A second class II AP endonuclease, APEX2, possesses strong 3′–5′ exonuclease and 3′-phosphodiesterase activities but only very weak AP-endonuclease activity. APEX2 associates with proliferating cell nuclear antigen (PCNA), and the progression of S phase of the cell cycle is accompanied by its expression. APEX2-null mice exhibit severe dyslymphopoiesis in thymus as well as moderate dyshematopoiesis and growth retardation. Comparative gene expression profiling of wild-type and APEX2-null mice using an oligonucleotide microarray revealed that APEX2-null thymus has significantly altered gene expression profiles, reflecting its altered populations of thymocytes. Beyond these altered populations, APEX2-null thymus exhibits significant alterations in expression of genes involved in DNA replication, recombination and repair, including *Apex1*, *Exo1* and *Fen1* as well as master genes for the DNA damage response, such as *E2f1*, *Chek1*, and proapoptotic genes. We therefore examined the extent of DNA strand breakage, and found that both of single-strand breaks detected as comets and double-strand breaks detected as γH2AX foci were significantly higher in frequency in most APEX2-null thymocytes compared to wild-type thymocytes. This higher frequency of DNA breaks was accompanied by increased expression of PCNA and increased phosphorylation of p53 at Ser23 and to a lesser extent, at Ser18. The present study clearly demonstrates that APEX2-null lymphocytes have a higher frequency of DNA breaks, indicating that APEX2 may play an important role(s) during their generation and/or repair.

1. Introduction

Oxidation or other chemical modifications of nucleotides and genomic DNA is a major threat to living organisms because the damage inflicted may cause alterations in base pairs or block progression of DNA replication and transcription [1,2]. Base excision repair (BER) is one of the major cellular defense mechanisms for eliminating damaged bases in genomic DNA. DNA glycosylases catalyze the first step of BER by excising the damaged bases, and leaving apurinic/apyrimidinic (AP) sites. Among the various mammalian DNA glycosylases, uracil DNA glycosylase (UNG) and MutY homolog (MUTYH) excise misincorporated bases during DNA replication, and both enzymes associate with PCNA, a scaffold protein for DNA replication

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machinery, thus both can initiate replication-associated BER [3,4]. APEX2/APE2, a minor mammalian AP endonuclease [5], is known to associate with PCNA through its PCNA binding motif whereas the major AP endonuclease, APEX1/APE1, lacks this motif [6].

We previously reported that APEX2 is highly expressed in mouse thymus, spleen and bone marrow, and that disruption of the mouse *Apex2* gene results in significant abnormalities in these organs accompanied by a reduction in body weight [7]. Thymus glands of APEX2-null mice exhibited significant atrophy and the total number of thymocytes in APEX2-null thymus decreased to one fifth of that in the wild type. The number of peripheral T and B cells was also significantly reduced in APEX2-null mice, although the numbers of red blood cells, monocytes/granulocytes and natural killer cells were only slightly reduced. Furthermore, we reported that a significant accumulation of both thymocytes and mitogen-stimulated splenocytes in G2/M phase was seen in APEX2-null mice compared to the wild type, indicating that APEX2 is required for proper cell cycle progression of proliferating lymphocytes.

The major AP endonuclease, APEX1, is also highly expressed in tissues [8] whose functions are largely impaired by an APEX2 deficiency, indicating that APEX1 and APEX2 play different roles in these tissues. In the present study, we performed comprehensive analyses of gene expression profiles in various tissues compared to those in wild-type mice, and found that APEX2 deficiency results in a selective alteration of gene expression profiles in thymus which is likely to be caused by a higher frequency of spontaneous strand breaks in nuclear DNA.

2. Materials and methods

2.1. *APEX2-null mice*

Apex2-disrupted mice were established as described previously [7,9]. Genotypes were analyzed by PCR using mouse tail DNA. PCR primers used to detect the wild-type allele were U796M (5′GCAAGGCATCTCAACTATGGCTC3′) and L1321 (5′CTTCTCATCTTTGGACTCTGG3′). To detect the mutated allele, Mp2-5 (5′CTACGCATCGGTAATGAAGG3′) and L1321 were used. Heterozygous female (*Apex2*$^{+/-}$) mice were backcrossed with C57BL/6J males (*Apex2*$^{+/Y}$) (Clea Japan Inc., Tokyo, Japan). N13 female mice were mated with C57BL/6J males, and the male offspring (*Apex2*$^{+/Y}$ *and* *Apex2*$^{-/Y}$) were used in the experiments. All animals were maintained in an air-conditioned, light-time-controlled, specific-pathogen-free room. The handling and sacrificing of all animals were carried out under pentobarbital anesthesia (75 mg/kg) in accordance with nationally prescribed guidelines, and ethical approval for the studies was granted by the Animal Care and Use Committee of Kyushu University.

2.2. *Body and thymus weight analysis*

The body weight of each mouse was measured once a week from 4 to 12 weeks after birth without anesthesia and the mean value was calculated. To measure thymus weights, mice at 4 weeks of age were dissected and abdominal vessels were cut for blood drainage. Each thymus was carefully removed and its weight was measured immediately. The number of thymocytes was then determined as described previously [7].

2.3. *Analysis of blood cells*

Peripheral blood was collected from an axillary artery at 12 weeks of age, and promptly diluted with the same volume of PBS supplemented with 0.2% of EDTA-3K. Densities of white blood cells, red blood cells and platelets in peripheral blood were analyzed using a K-4500 hematology analyzer (Sysmex, Kobe, Japan).

2.4. *Sample preparation for microarray analyses*

Most of the tissues (muscle, liver, hippocampus, cerebral cortex and kidney) were isolated from 12-week-old male mice ($n = 5$), while thymus glands were isolated from 4-week-old males ($n = 4$). Each tissue (30–50 mg) was rinsed in PBS to remove blood and treated with 1 ml of RNAlater (Takara Biochemicals, Kyoto, Japan) at 4 °C overnight according to the manufacturer's instructions, and stored at −80 °C until RNA preparation. Total RNA was purified from mouse tissues with an RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA quality was examined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA), and concentrations were determined by UV spectrometry.

2.5. *Microarray hybridization and data analysis*

Total RNA from each tissue was used for biotin-labeled cRNA synthesis using one-cycle target labeling and control reagents (Affymetrix, Santa Clara, CA), and the labeled cRNA was subjected to hybridization with a GeneChip Mouse Genome 430 2.0 Array (Affymetrix), a high-density oligonucleotide array comprised of 45,101 probe sets representing over 34,000 well-substantiated mouse genes, according to the manufacturer's instructions. Hybridization and scanning of the arrays were performed using a standard procedure. The expression value (average difference) for each gene was determined by calculating the average of differences in intensity (perfect match intensity minus mismatch intensity) between its probe pairs. The expression value for each gene was calculated with MAS 5.0 (Affymetrix). In all samples, probe sets called "Absent" were then removed and the resulting list of expressed probes was imported into GeneSpring GX 7.3.1 (Agilent Technologies Inc.) for further data characterization. Briefly, a new experiment was generated after importing raw data and further normalized based on the default order of (1) data transformation: set measurements less than 0.01–0.01; and (2) per chip, normalized to the 50th percentile. Lists were made of genes that were altered more than 1.5-fold between the wild-type and APEX2-null groups, and whose raw values in the higher group were larger than 50. A *t*-test with the Benjamini and Hochberg false discovery rate [10] was applied to the lists, and genes with a *p*-value <0.05 were selected. The selected genes were further analyzed for their functions and networks using Ingenuity