Genetic Mapping and Allelic Loss Analysis in Mouse Thymic Lymphomas of Helios and Aiolos Belonging to the Ikaros Gene Family

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The Ikaros gene undergoes bi-allelic changes at a high frequency in γ-ray-induced mouse thymic lymphomas, suggesting the relevance of Ikaros to the lymphoma development. Here we test whether Helios and Aiolos, two other members of the Ikaros gene family, are also involved in lymphomagenesis. Genetic mapping showed that Helios is located between D1Mit531 and D1Mit19 on chromosome 1 and Aiolos is between D11Mit222 and D11Mit332 on chromosome 11. Analysis using polymorphic markers around the two regions revealed that neither locus exhibited allelic loss in the 78 lymphomas that were induced in p53 wild-type mice, whereas in 102 p53(KO/+)-mouse-derived lymphomas Helios and Aiolos loci showed allelic loss in 8% (8/102) and 33% (34/102), respectively. However, 33 of the 34 lymphomas showing allelic loss at Aiolos were p53(KO/-) and were accompanied by loss of the p53 wild-type allele on the same chromosome. Homozygous deletion and mutation analyses failed to detect bi-allelic alterations. These results do not suggest any obvious contribution of Helios or Aiolos to oncogenesis of the mouse thymic lymphomas.

Key words: Tumor suppressor gene — Ikaros — Allelic loss (or LOH) analysis — Somatic mutation — γ-Ray-induced thymic lymphoma

The Ikaros gene encodes multiple protein isoforms with zinc finger domains that play an important role in the development of lymphocytes and other hematopoietic cell types.1-4 Studies of Ikaros KO mice suggest that Ikaros participates in proliferation of thymocytes and in an oncogenic process.5 Another gene related to Ikaros was identified by the use of degenerate primers complementary to sequences encoding the carboxy-terminal zinc finger of Ikaros. This gene, designated as Aiolos, exhibits considerable homology to Ikaros and its expression is restricted to the lymphoid lineage, like that of Ikaros.6-8 Helios is a third member of the Ikaros gene family and is expressed primarily in T-cells.7,8 All members of the Ikaros family consist of four N-terminal DNA-binding zinc fingers, a conserved bipartite activation domain and two C-terminal zinc fingers, and bind to similar DNA sequences. They interact physically with each other through the carboxy-terminal zinc fingers, as detected by co-immunoprecipitation and also by nuclear staining with antibodies.5-8

We have previously demonstrated that Ikaros functions as a tumor suppressor gene in the development of γ-ray-induced mouse thymic lymphomas.9 About 20% of the lymphomas examined underwent bi-allelic DNA alterations of the Ikaros gene. As described above, Helios and Aiolos have sequence homologies to Ikaros and show protein–protein interactions, and therefore these two genes might also be involved in lymphoma development. This prompted us to perform genetic mapping and to examine allelic loss in the γ-ray-induced mouse thymic lymphomas. In this paper, we show the chromosomal location of Helios and Aiolos and demonstrate that neither gene shows frequent allelic loss or bi-allelic changes in the thymic lymphomas.

MATERIALS AND METHODS

Mice and lymphomas MSM is an inbred strain derived from Japanese wild mice, Mus musculus molossinus.10 The details of lymphoma induction were described previously.11 In brief, the parental p53-deficient mouse was originally produced by introduction of a neo-gene fragment into the p53 gene locus in the ES cells that had been derived from F1 mouse between C57BL/6(B6) and CBA strains.12 We have developed a congenic MSM mouse carrying a p53-deficient allele by backcrossing this p53-deficient mouse to MSM mice for 13 generations. The male mice (N10 to N13 generation) with the genotype of p53(KO/) were mated with BALB/c female mice. The mice were then subjected to γ-ray irradiation, 2.5 Gy four times at weekly intervals, starting at the age of 4 weeks. Development of thymic lymphoma was diagnosed on the basis of observation of labored breathing.

Polymerase chain reaction (PCR) analysis Isolation of genomic DNA from lymphomas and brain was carried out by using standard protocols. PCR and separation of PCR
products by gel electrophoresis were performed as described previously.12,13) Microsatellite markers were synthesized according to reported sequences.14) One primer (F1-53) located in exon 1 of the p53 gene, a second primer (R1-53) in a region 5' to exon 3, and the remaining one (F2-neo) in the neo gene insert. F1 mice of the Mus m. molossinus subspecies, two distinct alleles originating from different mouse subspecies, Mus m. domesticus and Mus m. molossinus. A primer set amplified a polymorphic region upstream from the transcription start site was synthesized and used for allelic loss analysis of those lymphomas.

RESULTS

Genetic polymorphisms were searched to map Helios and Aiolos genes on the mouse chromosome. Since only the cDNA sequence was available for either gene, we sequenced genomic DNA and BAC clones corresponding to Ikaros exon 7 by aligning their cDNA sequences with cDNA and genomic sequences of Ikaros. As for Helios, a base-substitution between BALB/c and MSM genomes, which creates an AccII restriction enzyme cleavage site, was found and accordingly a set of primers was designed to detect the polymorphism. Gel electrophoresis of AccII digests of PCR products with the primers gave an undigested DNA fragment for BALB/c and two digested fragments for MSM (see Fig. 2). The difference was used for genotyping of 131 intersubspecific backcross mice that were obtained by mating (C57BL/6×MSM)F1 females to MSM males. Likewise, an AccII recognition site polymorphism was found in Aiolos by sequence analysis. A set of primers was synthesized accordingly and used for genotyping. Fig. 1 shows the mapping results of the two genes. The Helios gene is located between markers D1Mit531 and D1Mit19 on chromosome 1, and the Aiolos gene is between D11Mit222 and D11Mit332 on chromosome 11.

Allelic loss analysis of Helios was carried out using the primers used for the Helios mapping and also two markers flanking Helios. Fig. 2A shows gel electrophoretic patterns of AccII digests of the Helios PCR products and the PCR products of D1Mit531 and D1Mit19. Allelic differences were clearly seen. The frequency of allelic loss was 3.9% (7/180) at the D1Mit531 locus, 4.4% (8/180) at the Helios locus and 3.9% (7/180) at the D1Mit19 locus; thus the loss frequency at Helios was higher than those at the other two loci. Fig. 3A shows a compilation of allelic losses at the three loci in individual lymphomas which allows us to classify the lymphomas into four types: type A: the lod scores between D1Mit531 and D1Mit19, between Helios and D1Mit19, between D11Mit222 and Aiolos, and between Aiolos and D11Mit332 are 20.9, 28.7, 33.1 and 30.1, respectively.
A, lymphomas retaining both alleles of all three loci; type B, lymphomas lacking one chromosomal region of MSM that covers all three loci; type C, lymphomas showing allelic losses at the centromeric two loci but retaining both alleles of the telomeric locus; type D, lymphomas retaining both alleles of the centromeric locus but showing allelic losses at the telomeric two loci. There were two lymphomas, one being a type C lymphoma and the other type D, which might suggest the involvement of Helios in lymphomagenesis.

Likewise, allelic loss analysis was done for Aiolos in a similar manner. Fig. 2B shows examples of AccII digests of PCR products, together with PCR products of D11Mit222 and D11Mit332, and Fig. 3B summarizes the results of compilation. There was a lymphoma of type E which exhibited allelic loss only at the Aiolos locus. The frequency of allelic loss was 19% (34/180) at the Aiolos locus, although the loss had a strong preference for the BALB/c allele (see below).

Inactivation of the p53 gene in lymphomas may affect the allelic loss frequency of Helios and Aiolos loci because p53 functions to maintain the genome integrity.16–19 Besides, the p53 and Aiolos genes reside on the same chromosome 11 and hence single allelic loss events at a

Fig. 2. Allelic loss analysis of Helios (A) and Aiolos (B) loci. (A) PCR products for D1Mit531 and D1Mit19 and AccII digests of the Helios PCR products were subjected to gel electrophoresis. The first three lanes on panels represent control DNA of BALB/c, MSM and F1 mice, and the other lanes display lymphoma DNA. The number of lymphomas is given arbitrarily. Type of allelic losses in each lymphoma (see legend to Fig. 3) is indicated at the bottom.

Fig. 3. Chromosomal constitution of lymphomas in the vicinity of the Helios (A) and Aiolos (B) loci. Three loci used for allelic loss analysis are indicated on the left. Triangles marked by C and M indicate BALB/c allele and MSM allele retained, respectively. Two triangles of C and M represent both BALB/c and MSM alleles retained, and one triangle represents one allele lost. Combination of the status of the alleles allows lymphomas to be divided into four types at both loci: type A, lymphomas retaining both alleles of all three loci; type B, lymphomas lacking one chromosomal region of BALB/c or MSM that covers all three loci; type C, lymphomas showing allelic losses at the centromeric two loci but retaining both alleles of the telomeric locus; type D, lymphomas retaining both alleles of the centromeric locus but showing allelic losses at the telomeric loci; type E, lymphomas exhibiting allelic loss only at the Aiolos locus. The number of lymphomas of each type is listed at the bottom.
site of the chromosome may well involve both loci, which can increase the allelic loss frequency of Aiolos. Therefore, the relation between allelic losses of the Helios or Aiolos locus and the p53 locus was investigated. p53 genotyping of lymphomas and the host mice was performed using three sets of primers (see “Materials and Methods”). The typing allowed us to classify 180 lymphomas into four p53 genotypes: [1] (+/+), both wild-type alleles retained; [2] (+/-), one of the two wild-type alleles lost; [3] (KO/+), the KO allele and the wild-type allele retained; [4] (KO/-), the KO allele retained but the wild-type allele lost. The numbers of lymphomas in these four classes were 75, 3, 19, and 83, respectively.

Table I summarizes the status of the Helios and Aiolos alleles in lymphomas of the four different p53 genotypes. It is noteworthy that at either gene locus no allelic loss was found in lymphomas that were induced in p53 wild-type mice. The eight lymphomas showing allelic loss at the Helios locus were of the BALB/c allele. This suggests that almost all of the BALB/c allele(s) retained. In our previous study of Ikaros, we found nine lymphomas with homozygous deletion and six mutations in the zinc finger domain regions. Therefore, analysis of homozygous deletion and mutations was done in a similar manner. For the assay of homozygous deletion of Helios, two pairs of primers on the putative exons 4 and 7 and a primer set for the catenin gene as a positive control were synthesized and used for the eight lymphomas that showed allelic loss. No pattern indicative of homozygous deletion was detected in these samples (data not shown). For mutation analysis of the putative exons 4 and 7, intron-specific primers and primers on the exons were used. We failed to find any mutation in the region. Although preliminary, these results did not provide evidence for involvement of Helios in the development of thymic lymphoma. As for the Aiolos gene, similar studies were carried out for the 34 lymphomas with allelic loss. No result indicating bi-allelic DNA alteration was obtained (data not shown).

**DISCUSSION**

We previously showed that the Ikaros gene undergoes bi-allelic changes at a high frequency in γ-ray-induced mouse thymic lymphomas. In this paper we have tested whether or not Helios and Aiolos, two other members of the Ikaros gene family, are also involved in lymphomagenesis. In contrast to Ikaros, no result was obtained which suggests any obvious contribution of Helios or Aiolos to the development of mouse thymic lymphomas.

The Ikaros family of proteins, Ikaros, Helios and Aiolos, are characterized by the presence of four N-terminal DNA-binding zinc fingers, a conserved bipartite activation domain, and two C-terminal zinc fingers. They can activate transcription of reporter genes driven by the Ikaros consensus binding site, 5′-GGGAA-3′. Gene disruption

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**Table I.** Distribution of Helios and Aiolos Genotypes in Lymphomas of Four Different p53 Genotypes

| Class of host of lymphomas | Helios allele retained
|---------------------------|----------------------|
|                           | C/M  | C&CC  | M&MM  | Total |
| [1] +/+                   | 75   | 0     | 0     | 0/75  |
| [2] +/+                   | 3    | 0     | 0     | 0/3   |
| [3] KO/+                  | 19   | 0     | 0     | 0/19  |
| [4] KO/+ KO/−             | 75   | 6     | 2     | 8/83  |

| Class of host of lymphomas | Aiolos allele retained
|---------------------------|----------------------|
|                           | C/M  | M&MM  | C&CC  | Total |
| [1] +/+                   | 75   | 0     | 0     | 0/75  |
| [2] +/+                   | 3    | 0     | 0     | 0/3   |
| [3] KO/+                  | 18   | 1     | 0     | 0/19  |
| [4] KO/+ KO/−             | 172  | 6     | 2     | 8/180 |

a) Four p53 categories marked on the left include the following lymphomas: [1] +/+ , lymphomas showing no inactivation of the two p53 alleles; [2] +/−, lymphomas exhibiting allelic loss of one p53 allele. Those tumors were induced in p53 wild-type mice. [3] KO/+ , lymphomas inheriting one p53-deficient allele and retaining the wild-type allele; [4] KO/−, lymphomas inheriting one p53-deficient allele and lacking the wild-type allele. KO indicates a p53-deficient allele; + shows a wild-type allele of p53; − represents loss of the p53 wild-type allele.

b) C/M indicates both Helios or Aiolos alleles retained; C&CC, BALB/c allele(s) retained; M&MM, MSM allele(s) retained.
experiments have been carried out for *Ikaros* and *Aiolos* genes. As for *Ikaros*, two distinct strains of Ikaros-KO mice have been produced.\(^5\)\(^{,}\)\(^{21}\) One carries a deletion of the *Ikaros* DNA-binding domain that displays dominant negative effects on transcription through interaction at the C-terminal domain with proteins of the *Ikaros* family (*Ikaros*-DN type), and the other harbors a deletion of the last translated C-terminal exon (*Ikaros*-C type). Interestingly, heterozygotes of the *Ikaros*-DN mouse strain, but not the *Ikaros*-C strain, exhibit defects in the T lineage, which lead to an abnormal accumulation of CD4/CD8 double-positive thymocytes and ultimately result in T cell leukemia and lymphomas. *Ikaros* controls T cell proliferative responses and probably functions as a growth suppressor, and hence loss of the gene function may confer a growth advantage upon these cells which fosters the development of a tumor.\(^5\) On the other hand, mice homozygous for an *Aiolos*-null mutation exhibit a much less severe lymphopoietic defect than Ikaros KO homozygotes do, and the development of B cell lymphomas is frequently seen among aging mutants.\(^2\)\(^{18}\)\(^{,}\)\(^{21}\) The results of gene disruption experiments suggest the involvement of *Ikaros* and possibly *Aiolos* in the development of T cell lymphomas of the mouse. Our previous analysis provided further genetic evidence implicating *Ikaros* in the development of lymphomas in *Ikaros* wild-type mice by showing bi-allelic changes in about 20% of the \(\gamma\)-ray-induced mouse thymic lymphomas.\(^9\)

Immunoprecipitation experiments showed that protein-protein interaction allows *Ikaros* proteins to dimerize with *Helios* or *Aiolos* proteins, as well as themselves. The C-terminal zinc fingers provide a site for these interactions.\(^6\)\(^{,}\)\(^{8}\)\(^{,}\)\(^{21}\)

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