Astrocytoma and B-cell Lymphoma Development in a Man with a p53 Germline Mutation

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We report a case with a germline mutation of the p53 gene developing both a non-Hodgkin's lymphoma and an astrocytoma. The astrocytoma could be cured by two operations and combined chemotherapy but 33 months after the onset of the disease, he suffered from a diffuse, large cell centroblastic malignant lymphoma of B-cell lineage. In spite of clear rearranged fragments observed with IgH and c-MYC gene probes, we could not diagnose a Burkitt's lymphoma morphologically. The malignant lymphoma was chemoresistant and the patient died of multi-organ failure. He was confirmed to have a germline mutation of the p53 gene by analysis of c-DNA from peripheral lymphocytes and loss of heterozygosity (LOH) of p53 was evident in the lymphoma. The results were suggestive of the Li-Fraumeni syndrome (LFS), a rare autosomal dominantly inherited syndrome with a germline mutation of p53 gene and diverse malignancies, but this could not be confirmed in the present case. Alternatively, a de novo mutation could have been involved.

Key words: germline mutation of p53 gene – non-Hodgkin’s lymphoma – astrocytoma – Li-Fraumeni syndrome

INTRODUCTION

It is well known that the p53 is a tumor suppressor gene, often inactivated by deletion or point mutation in many types of solid tumors and blood malignancies (1–8). A strong correlation has been reported between p53 mutations and loss of chemosensitivity (9–11). A germline mutation of the p53 gene has been reported in a cancer-prone family, described as the Li–Fraumeni syndrome (LFS) (12–16). Here, we report a case of a male patient with a p53 gene germline mutation developing both an astrocytoma and a non-Hodgkin’s lymphoma.

CASE REPORT

The patient was a 30-year-old Japanese male. He was operated on for a brain tumor in September 1993, this being histologically diagnosed as an anaplastic astrocytoma. Subsequently, he received anticancer chemotherapy (interferon-β, nimustine), but recurrence was found in February 1994 and a second operation was performed. Chemotherapy with cisplatin and etoposide was then continued with no recurrence of the brain tumor.

Thirty-three months after the onset of astrocytoma, he complained of epigastric discomfort and abdominal fullness in March 1996. He was diagnosed as suffering from a diffuse, large cell centroblastic variant (WHO) malignant lymphoma of B-cell lineage from examination of biopsy material from the peritoneal tumor.

After the diagnosis, he received chemotherapy. At first, a VEPA regimen (adriamycin, cyclophosphamide, vincristine, prednisolone) was tried but tumor regression was transient and therefore irinotecan and a CNOP regimen (cyclophosphamide, mitoxantrone, vincristine, prednisolone) were applied. However, these therapies were not effective and finally he died of multi-organ failure (Fig. 1).

MAGNETIC RESONANCE IMAGING (MRI) OF THE BRAIN

MRI revealed a cystic brain tumor (6 cm in diameter) in the left temporal lobe with a mural nodule that was strongly enhanced by
Malignancies with a p53 germline mutation

Figure 1. Clinical course: ADR, Adriamycin; VCR, vincristine; CPM, cyclophosphamide; PSL, prednisolone; CPT-11, irinotecan; MIT, mitoxantrone; Ara-C, cytosine arabinoside.

Figure 2. Magnetic resonance imaging (MRI) findings. (a) At the time of diagnosis. The brain tumor is apparent as a cystic mass (6 cm in diameter) in the left temporal lobe with a mural nodule that is strongly enhanced by gadolinium. (b) After two operations and chemotherapy. There is no apparent brain tumor.

gadolinium (Fig. 2). After the second operation, no recurrence of the brain tumor was detected (Fig. 2).

Histopathological Finding of the Brain Tumor

In the specimens obtained at the first resection, the tumor was richly supplied by blood vessels and composed of thickly clustered cells and spindle-shaped cells (Fig. 3). These tumor cells showed nuclear atypism and high mitotic activity and were immunoreactive for glial fibrillary acidic protein (GFAP). Thus the tumor was diagnosed as an anaplastic astrocytoma. In the tissue removed at the second operation, cellular pleomorphism with multinucleated giant cells containing hyperchromatic irregular-shaped nuclei was prominent (Fig. 3). Nuclear atypism might have been caused by the previous radiation therapy.

Abdominal CT, Positron Emission Tomography (PET) and Gallium Scintigraphy (Ga-scinti)

Abdominal CT showed massive ascites and giant mass of convoluted mesenterium (Fig. 4). PET showed uptake of fluorodeoxyglucose in the tumor (Fig. 4) and Ga-scinti showed a hot lesion broadly disseminated in the abdomen (Fig. 4).

Characterization of the Ascites

In samples of ascites, lymphoblast-like large cells were apparent (Fig. 5) and by flow cytometry these showed CD10+ and CD19+.
Figure 3. Histopathology of the brain tumor. (a) First operation. The tumor is composed of thickly clustered cells around these vessels and spindle cells. (Hematoxylin-eosin stain, x200.) (b) Second operation. Cellular pleomorphism with multinucleated giant cells containing hyperchromatic irregular-shaped nuclei and vascular proliferation are more prominent. (Hematoxylin-eosin stain, x200.)

Figure 4. (a) Abdominal CT in March 1996, showing a mass in convoluted mesenterium. (b) Positron emission tomography (PET) findings at about the same time, demonstrating uptake of fluorodeoxyglucose. (c) Gallium scintigraphy. A hot lesion is broadly disseminated in the abdomen.

and a lymphoid malignancy was strongly suggested (Fig. 5). The lymphoma cells showed various chromosomal abnormalities. Of 20 cells, three had 43, 12 had 44, two had 45 and only two had the normal chromosome count. Common abnormalities were −4, add(7) (q22), add(14) (q32), −15, add(17) (p11) and add(18) (q23) (Fig. 6).
CHARACTERIZATION OF THE PERITONEAL TUMOR

To obtain a definite diagnosis, a biopsy was taken of the peritoneal tumor. The tumor cells were round-shaped, ranging from 12 to 15 μm in diameter and had polymorphic nuclei with a few distinct nucleoli and relatively abundant cytoplasm. Cytoplasmic droplets were rarely seen on Giemsa staining (Fig. 7). Electron microscopic examination clearly defined these as lipid (Fig. 7). The tumor cells showed CD10+, CD19+, CD20+, CD22+, CD38+, CD45+, IgG+ and IgLκ+ by both flow cytometry and immunohistochemistry of frozen sections. Rearranged bands were detected by Southern blot analysis with probes for the IgH and kappa light chain genes as well as the c-MYC gene. Other probes used showed germline configurations. In spite of the CD10 positivity and clear rearranged fragments with c-MYC probe, we could not diagnose this patient as having a 'Burkitt's lymphoma' because of the atypical proliferation pattern and cellular morphology. He was diagnosed as suffering from a diffuse, large cell centroblastic variant (WHO) malignant lymphoma of B-cell lineage.

EXAMINATION OF p53

We first analysed p53 cDNA from the patient's lymphocytes using a yeast-based functional assay (FASAY) which has been used to detect heterozygous p53 mutations (17). As shown in Fig. 8, only 60% of transformants showed a His+ phenotype (control lymphocytes: 96%), indicating the presence of a heterozygous mutation of the p53 gene. Sequence analysis demonstrated a functionally inactivating heterozygous missense mutation, C242Y (Fig. 8) in the lymphocytes. To confirm inactivation of both alleles of p53 in the tumor, polymerase chain reaction–restriction length polymorphism analysis (PCR–RFLP) was performed using a set of primers (see caption to Fig. 8) which generated a 105 bp fragment containing a novel PstI site at codons 242 and 243. As shown in Fig. 6, the PCR product from the patient's lymphocytes was partially digested by PstI, generating both a digested fragment (82 bp) and an undigested fragment (105 bp) because of the heterozygous mutation at codon 242 (lane 2). The PCR product derived from his lymphoma tissue retained the mutant p53 allele. Whereas that from his mother's lymphocytes was completely digested by PstI, indicating the lack of any germline p53 mutation (lane 1). We also examined 17 autopsy specimens including cerebrum, cerebellum, spinal cord, skin, tongue, esophagus, stomach, small intestine, lung, heart, liver, kidney, adrenal gland, bone marrow, aorta, muscle and adipose tissue by PCR–RFLP analysis and confirmed the heterozygous p53 mutation in all cases (data not shown).

AUTOPSY FINDINGS

There was thickening and fibrous adhesion of the whole peritoneum, 2–8 cm in thickness, involving the entire intestines, liver and urinary bladder. Swelling of the mesentral and parapancreatic lymph nodes was observed up to 1 cm in diameter. No recurrence of the astrocytoma in the left temporal lobe of the cerebrum was apparent.

DISCUSSION

In the present case, the patient suffered from astrocytoma and malignant lymphoma at a young age and he had a germline mutation of p53 gene and LOH of p53 was shown in the
Figure 6. Results of chromosome analysis of lymphoma cells (ascites). 44, XY, add(1) (q32), -4, add(7) (q22), dup(8) (q13q22), add(11) (q23), add(14) (q32), -15, add(17) (p11), add(18) (q23) are prevalent (12/20 cells).

Figure 7. (a) Histopathology of the peritoneal tumor. Light microscopic features of the biopsied material. The lymphoma cells are proliferating diffusely and are 12-15 μm in diameter. Polymorphic nuclei have two or three distinct nucleoli. (Hematoxylin–eosin stain, x1000.) (b) Electron microscopic appearance of lymphoma cells. Note the lipid droplets in their cytoplasm (x4800).
lymphoma tissue. This finding suggests a strong link between a germline mutation of the p53 gene and the astrocytoma and non-Hodgkin’s lymphoma development.

Germline mutations of the p53 gene have been reported in cancer-prone families. The Li-Fraumeni syndrome (LFS) is a rare autosomal dominantly inherited syndrome with the following familial characteristics: a proband with either acute lymphocytic leukemia, sarcoma, breast cancer, brain tumor and/or adrenocortical carcinoma before the age of 45; a first-degree relative with a cancer in this group; and a first- or second-degree relative with sarcoma at any age or any cancer before age 45. The affected individuals have a germline mutation of p53 (12–16). The prevalence of a germline p53 mutation is approximately 0.01% in the general population, but is 5–10% among young patients with multiple cancers. For example, in one study examining 59 children and young adults who would not otherwise be considered as having HFS, but who had developed two malignancies, the overall frequency of germline p53 mutations was ~7% (18,19).

In this case, his mother suffered from breast cancer at 36 years of age but her germline p53 was wild type. The p53 status of the father was unknown but he was free of neoplasia. As far as we could determine, there was no other family history of malignancies except for the mother’s brother (rectal cancer). We could not diagnose him as LFS, if he had not died at a young age, he might have been shown to be a LFS proband.

It is well known that the p53 is a tumor suppressor gene often inactivated in many types of solid tumors and blood malignancies (1–8). Several studies have shown that p53 can mediate apoptotic cell death and that it is required for efficient activation of apoptosis following irradiation or chemotherapy (9–11). There is a clear link between p53 gene mutation and aggressive tumor behavior and a poor prognosis, in some cases associated with activation of the MDR1 (multidrug resistance) gene (20). With regard to hematological malignancies, a strong correlation has been found between p53 mutations and chemosensitivity in AML, MDS and CLL (21). It has recently been reported that non-Hodgkin’s lymphomas with a p53 abnormality are more likely to be drug resistant but that does not correlate with any other particular clinical characteristic (22). A similar situation appears to be the case for some types of B-cell lymphomas (23,24).

Regarding brain tumors, p53 gene mutations have been examined in astrocytomas, glioblastomas and some other tumors (25–28). In anaplastic astrocytomas and glioblastomas, almost 30–40% were found to be positive (26,28). In our survey of p53 germline mutations in brain tumors, we found a case of a yolk sac carcinoma that demonstrated an additional mutation, known to lead to functional alteration of p53 protein (29).

It has been reported that the presence of both p53 inactivation and c-MYC oncogene activation may be important in the pathogenesis of Burkitt’s lymphoma (30), along with t(8:14)
involving translocation of the IgH gene at 14q32 and the c-MYC gene at 8q24. Actually, p53 alterations in Burkitt’s lymphoma are present in about 40% of cases. In the present study, chromosome analysis of ascites revealed various abnormalities, but no t(8:14). While rearranged bands were obtained by Southern blot analysis with probes for the IgH and c-MYC genes, a diagnosis of ‘Burkitt’s lymphoma’ was impossible on morphological grounds.

In this case, CA-125 was very high (1279.5 U/ml; normal <35.0 U/ml) and on chemotherapy it rapidly decreased. While this is typical of mediastinal B-cell lymphomas with sclerosis, no intratumoral CA-125 could be demonstrated by immunohistochemical staining, so aspecific secretion by extratumor tissues was concluded (31).

The present case of a p53 germline mutation in an individual developing both an astrocytoma and a non-Hodgkin’s lymphoma appears not to represent LFS but rather a de novo change in the gene.

References