

Case Report

IDH2 and TP53 mutations are correlated with gliomagenesis in a patient with Maffucci syndrome

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Key words

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Enchondromas are benign cartilage-forming tumors within the phalanges, metacarpals and metatarsals.⁽¹⁾ Individuals with enchondromatosis syndrome, which encompasses seven major subtypes, develop multiple enchondromas. The most common subtypes are non-hereditary diseases, including Ollier disease (subtype 1) and Maffucci syndrome (subtype 2); the latter is distinguished by multiple cavernous hemangiomas that occur in addition to the enchondromas.⁽¹⁾ Both conditions generally present during childhood and can result in severe deformity of the affected tissues, depending on the extent of skeletal involvement. Malignant degeneration in patients with Ollier disease is estimated to be approximately 25% at the age of 40 years, but is uncertain in patients who have Maffucci syndrome.^(2,3) Moreover, patients with Ollier disease and Maffucci syndrome are predisposed to develop visceral and brain tumors, such as astrocytoma, acute myeloid leukemias, and pancreatic, liver, or breast cancer.⁽²⁾

Isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) are metabolic enzymes that interconvert isocitrate and α -ketoglutarate (α -KG).

In contrast, cancer-associated point mutations in IDH1 and IDH2 confer a neomorphic activity that allows reduction of α -KG to the oncometabolite R-2-hydroxyglutarate (2-HG). Mutations in IDH1/2 lead to not only a “gain of function” to catalyze the NADPH-dependent reduction of α -KG to 2-HG but also to a “loss of function” to reduce α -KG production.^(4,5) The IDH1 and IDH2 mutations are remarkably specific to single codons: the conserved and functionally important Arg132 residue in IDH1 and the Arg172 residue in IDH2.⁽⁶⁾

A recent genome-wide mutational analysis identifies IDH1/2 mutations as early and frequent genetic alterations in astrocytomas, oligodendrogliomas and oligoastrocytomas, as well as in secondary glioblastomas.⁽⁶⁾ Recently, it was reported that Ollier disease and Maffucci syndrome are the result of somatic mosaic IDH1 and IDH2 mutations.^(7,8) Here, we report on a 24-year-old female patient who was diagnosed as having Maffucci syndrome with anaplastic astrocytoma. We analyzed the IDH1 and IDH2 mutations in

the enchondroma, hemangioma and anaplastic astrocytoma tissues, and the same IDH2 mutation (c.516G>T encoding R172S) was detected in all tissues examined. Interestingly, we also detected a TP53 mutation in the anaplastic astrocytoma tissues, but not in the enchondroma or hemangioma tissues.

Case Report

A 24-year-old Japanese woman presented with an approximate 1-month history of headache. Her medical history included Maffucci syndrome with multiple enchondromas of several phalanges and metatarsal bones of the left hand (Fig. 1a,b), combined with hemangiomas of soft tissue in the right foot (Fig. 1c). She had skeletal deformities caused by enchondromas of the phalanges of the left fingers, particularly her index finger, appearing at 10 years of age, and hemangiomas on her

right foot, appearing at 16 years of age. The left-hand deformities gradually worsened, and she underwent surgical excision of the proximal phalanx of the left index finger and complete removal of the hemangiomas in her right foot at 21 years of age. Pathological analysis revealed mesodermal dysplasia manifesting as a combination of enchondromatosis and hemangiomatosis. The enchondromas had a multinodular architecture characterized by islands of cartilage (Fig. 1d).

At the time of presentation the patient was conscious but had a severe headache. Motor function was almost normal, and her remaining cranial nerve function was otherwise intact. There were no abnormal findings on the laboratory blood tests. Computed tomography (CT) and MRI showed a 40 × 30-mm mass located in the posterior fossa, which showed a low signal on T1-weighted images (Fig. 1e) and a high signal on T2-weighted images (Fig. 1f,g). The mass did not show an enhancement following gadolinium infusion. No distant metastatic lesion was observed on CT. Radiologically, the preoperative diagnosis was low-grade glioma. The tumor extended from the middle cerebellar peduncle to the cerebellar vermis, and the intraoperative pathological examination revealed diffuse astrocytoma; therefore, partial removal was performed to avoid cerebellar ataxia or cerebellar mutism. Pathological examination by HE staining demonstrated a mild focal increase in cellularity and a lack of vascular proliferation and necrosis within the sampled tissue (Fig. 1h). The final diagnosis was anaplastic astrocytoma. Given the patient's consent, the relative lack of symptoms and the diffuse nature of the pathology, the patient was administered local radiotherapy with a radiation boost to the residual tumor (50 Gy in total). Chemotherapy was not administered. The cerebellar ataxia appeared at 29 years of age. MRI showed a 16 × 7-mm lesion in the right cerebellar hemisphere. The patient underwent endoscopic tumor resection, followed by stereotactic radiotherapy (peripheral dose 2 Gy, total 20 Gy) and temozolomide (TMZ). TMZ was prescribed at a dosage of 150 mg/m²/day for 5 days every 28 days, but the patient died at 31 years of age.

Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue sections extracted by surgery using Mighty-Amp for FFPE (Takara Bio, Shiga, Japan) according to the manufacturer's instructions and with the informed consent of the patient. We performed mutation analyses of IDH1, IDH2 and TP53 genes (Data S1). We identified the IDH2 mutation (c.516G>T encoding R172S) in the enchondroma, hemangioma and anaplastic astrocytoma tissues, and the same IDH2-R172S mutation was detected in all these tissues (Fig. 2a). Subsequently, the PCR products were subcloned into pCR4-TOPO vectors, and 14 clones (enchondroma), 17 clones (hemangioma) and 69 clones (astrocytoma) were sequenced to confirm the IDH2-R172S mutations (Data S1). As a result, 28.6% (4/14), 11.8% (2/17) and 2.9% (2/69) of each tissue, respectively, were shown to carry the IDH2-R172S mutation, although these percentages do not necessarily imply those of IDH2-R172S-possessing tumor cells (Fig. 2b). Furthermore, missense mutations (562C>A encoding L188M and 1118A>G encoding K373R) of the TP53 gene were identified in the astrocytoma tissues, but not in the enchondroma or hemangioma tissues (Table 1, Fig. S1).

Discussion

We identified the same IDH2 (c.516G>T encoding R172S) mutation in enchondroma, hemangioma and anaplastic astrocytoma tissues and TP53 mutation in astrocytoma taken from a

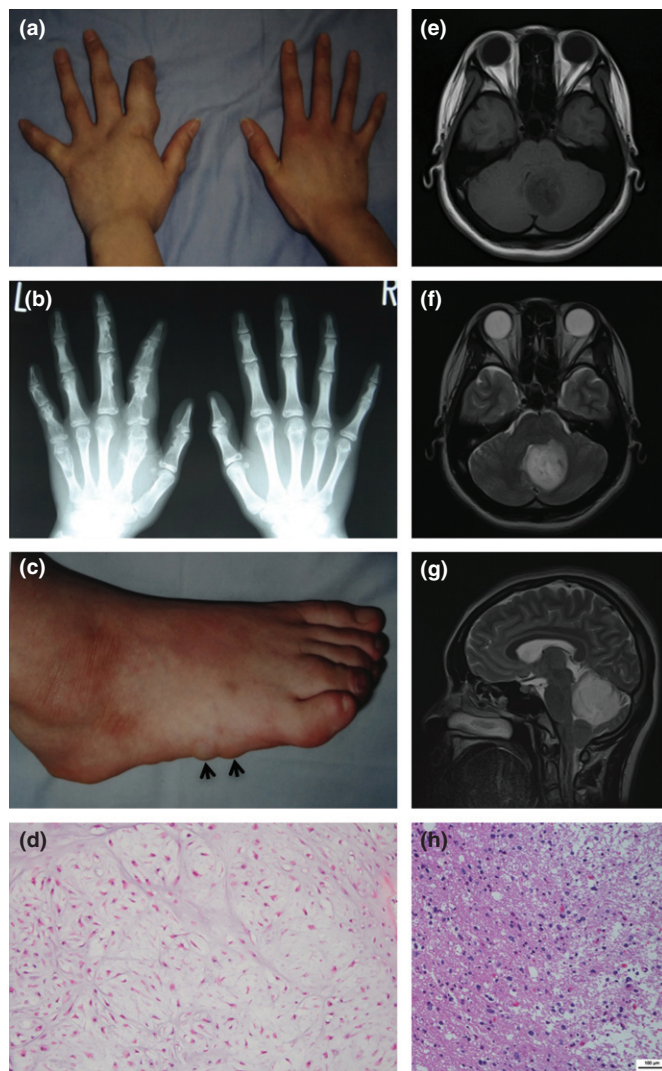


Fig. 1. Clinical features of the patient with Maffucci syndrome. (a) Deformities of the left hand caused by cartilaginous tumors. (b) Plain X-ray image showing multiple cartilaginous tumors in many phalanges and metacarpal bones of the left hand. (c) Hemangiomas at the right lateral foot (arrows). (d) HE staining of cartilaginous tumors of the left hand. (e) T1-weighted MRI imaging of brain tumor. (f) T2-weighted MRI imaging of brain tumor. (g) Sagittal T2-weighted MRI imaging of brain tumor. (h) HE staining of brain tissues.

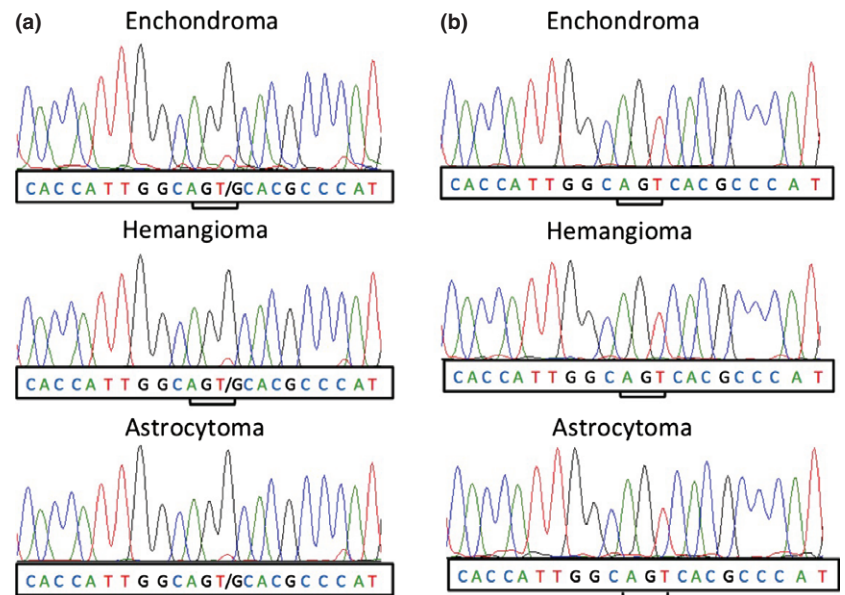


Fig. 2. Mutational analysis of IDH2. (a) Direct DNA sequencing of IDH2 was performed in the enchondroma, hemangioma and astrocytoma tissues. (b) PCR products of IDH2 were subcloned into pCR4-TOPO vectors, and each clone was sequenced to confirm the heterogeneous mutation of IDH2.

Table 1. Detection of IDH1/2 and p53 mutations in Maffucci syndrome

	Sense primer	Antisense primer	Enchondroma	Hemangioma	Astrocytoma
IDH1/2					
IDH1	cggcttcagagaagccatt	gcaaaatcacattattgccaac	Wild type	Wild type	Wild type
IDH2	caagctgaagaagatgtggaa	cagagacaagaggatggcta	R172S	R172S	R172S
p53					
Exon1	ctgcttcgggctcactg	gccaaccttgccttac	Wild type	Wild type	Wild type
Exon2	ctgcttcgggctcactg	gccaaccttgccttac	Wild type	Wild type	Wild type
Exon3	tgactgctttccaccatc	ccctcagggcaactgac	ND	Wild type	Wild type
Exon4	ctgtctctctctctctacag	agcctgtctctctccag	Wild type	Wild type	Wild type
Exon5	tgattctcactgattgctctt	gagacccagtgcaaacc	Wild type	Wild type	L188M
Exon6	ttggcctgtgtatctct	gtggcaagtggctcctga	Wild type	Wild type	Wild type
Exon7	ttgcttctttctatcctga	gcttctgtcctgcttct	Wild type	Wild type	Wild type
Exon8	tttatcaccttctctgctct	ttgataagaggccaagactta	Wild type	Wild type	Wild type
Exon9	tcccctctctgtgct	gaaggggctgaggtcactc	Wild type	Wild type	Wild type
Exon10	tcctgctctgtctctaca	ggggaacaagaagtggagaa	Wild type	Wild type	K373R

ND, not detected.

patient with Maffucci syndrome. IDH mutations may be a common genetic background in both gliomas and other tumors, and TP53 mutation may have triggered gliomagenesis in our patient with Maffucci syndrome. Bathla *et al.* report IDH1 mutations in a case of low grade glioma with Ollier disease; however, they did not perform mutation analyses of TP53.⁽⁹⁾ In contrast, our case suggests that an additional acquired TP53 mutation might induce the malignant transformation in patients with Maffucci syndrome. It is interesting to speculate that our patient with Maffucci syndrome, who developed an anaplastic astrocytoma and other benign tumors with an IDH2 mutation, represents a case of early post-zygotic genetic events, which may have initiated the disease process. Appropriate diagnoses of Ollier disease and Maffucci syndrome are crucial because these diseases are associated with a significant risk of brain tumors, as in our case, and other malignant tumors.^(10,11) Patients with Ollier disease and Maffucci syndrome have better prognoses if the patients are diagnosed with and treated for malignant tumors in the early stages.

Recently, we revealed that an anti-IDH1/2 monoclonal antibody (mAb, clone MsMab-1), which was developed against IDH1-R132G, cross-reacts with IDH2-R172S.^(12,13,14) Using MsMab-1, we performed immunohistochemistry against anaplastic astrocytoma tissues. Although the reactivity was very low, heterogeneity of IDH2-R172S was observed (Fig. S2). There might be several problems about the low detection level of IDH2-R172S in immunohistochemistry: (i) very low sensitivity of MsMab-1; (ii) low frequency of the IDH2-R172S expression; or (iii) IDH-R172S protein degradation in a paraffin section. Although further studies are required to determine the molecular pathogenesis of astrocytic tumors, our case is unique and supports the IDH-dependent genetic pathway and second-hit model for gliomagenesis.

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Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Data S1. Materials and methods.

Fig. S1. Mutational analysis of TP53. Direct DNA sequencings of TP53 exon5 (a) and exon10 (b) were performed in the astrocytoma tissues.

Fig. S2. Immunohistochemical analyses against anaplastic astrocytoma tissues using MsMab-1. Mutated IDH2-R172S protein expression was determined immunohistochemically in paraffin-embedded tumor specimens. Briefly, 4- μ m-thick histologic sections were deparaffinized in xylene and rehydrated. Then, they were autoclaved in citrate buffer (pH 6.0; Dako) for 20 min. Sections were incubated with 5 μ g/ml of MsMab-1 (A) or control (B) overnight at 4°C, followed by treatment with an LSAB kit (Dako). Color was developed using 3, 3'-diaminobenzidine tetrahydrochloride (DAB; Dako) for 10 min, and counterstained with hematoxylin. Insets show that MsMab-1 stained cytoplasm. Magnification: \times 200.