SHORT COMMUNICATION

Aggressive Squamous Cell Carcinoma in a Case of Epidermodysplasia Verruciformis Carrying a TMC6 Splice-site Mutation

Kazunori YOKOI[1], Noriko ARASE[1], Takashi SHIMBO[2], Manabu FUJIMOTO[1] and Atsushi TANEMURA[1]
[1]Department of Dermatology, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita-shi, Osaka 565-0871 and [2]Department of Stem Cell Therapy Science, Graduate School of Medicine, Osaka University, Suita, Japan. E-mail: kyokoi@derma.med.osaka-u.ac.jp
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Epidermodysplasia verruciformis (EV; MIM #226400) is a rare inherited skin disease, first described by Lewandowsky & Lutz in 1922 (1). The disease is caused by abnormal susceptibility of the skin to β-human papillomavirus (HPV) (2). In particular, human papilloma virus (HPV)-5 and HPV-8 were classified as "possibly carcinogenic" in patients with epidermodysplasia verruciformis (EV)" by the WHO (3). Approximately half of patients with EV develop non-melanoma skin cancers (NMSC), mainly on sun-exposed areas by around their 40s (4). EV is classified into a genetic type and an acquired type as a result of immunosuppression caused by HIV or solid-organ transplantation (5). Genetic EV is classified into classical EV associated with *TMC6* or *TMC8* gene mutations and non-classical EV associated with non-*TMC* gene mutations, such as *RHOH*, *MST-1* and *CORO1A* (5). Classical EV and most non-classical EV are inherited in an autosomal recessive manner. On the other hand, cases of X-chromosome recessive or autosomal dominant inheritance have been reported with unspecified loci (5). Approximately half of all patients with EV have been classified as classical EV, with mutations in *TMC6* or *TMC8* (5).

We describe here a case of EV with an aggressive squamous cell carcinoma. The c.892-2A>T mutation of the *TMC6* gene was detected in the patient. We report, for the first time, that the c.892-2A>T mutation is estimated to induce an amino acid mutation of p.Gly298*.

CASE REPORT

The patient was a 50-year-old man, who presented with small pale-red spots, which had been present on his limbs and trunk in childhood. Keratotic lesions of various sizes, including seborrhoeic keratosis, actinic keratosis and verruca vulgaris, were found on his neck, trunk, and limbs. A large erythema with keratosis and partial ulceration was found on the left side of his chest (**Fig. 1**a). The lesion on his chest was diagnosed as squamous cell carcinoma (SCC) from a biopsy (**Fig. 1**b). Extended resection and left lymph node dissection were performed for the SCC. He received postoperative adjuvant therapy with peplomycin sulphate. Six months later, a large metastatic right lymph node was found on positron emission tomography–computed tomography (PET-CT) (**Fig. 1**c). Right lymph node dissection was performed (**Fig. 1**d). Since then, Bowen's disease, 3 SCCs and a sebaceous carcinoma were found within 15 years, none of which showed evidence of metastasis. Histopathological examination of the SCC on his back revealed koilocytosis, which suggested viral infection (**Fig. 1**e–h). His older sister, who was deceased, had experienced similar skin symptoms. His parents, who were also deceased, and his 2 surviving daughters showed no symptoms. His maternal grandfather and paternal grandmother were related to each other, as shown in the family tree (**Fig. 1**i). The patient gave his informed consent to provide a blood sample and tissue biopsy specimens of the skin and lymph node. This research was approved by Osaka University ethics committee (number 683).

Genomic DNA and total RNA were extracted from the blood of the patient and a healthy control. Sequencing analyses of *TMC6* were performed. Details were shown in Appendix S1.

Genotyping of β-HPV was performed with the previously reported method using the single tube nested "hanging droplet" PCR (6).

RESULTS

Genomic DNA sequencing found a c.892-2A>T mutation on the intron 8 of *TMC6* (Fig. S1a). Sequence analysis of cDNA reverse transcribed from the mRNA found that c.892_932 of *TMC6* (the first 41 base pairs of exon 9) was deleted. This splicing abnormality was estimated to induce the 298th amino acid to become a stop codon (p.Gly298*), suggesting that the amino acids after exon 9 were not translated (Fig. S1b). This splice site mutation may be caused by the sequence of c.930_931AG, which was estimated to function as a novel end of intron 8 instead of c.892-2_892-1AG (Fig. S1c). HPV-5 was detected in the SCC on the patient's back (Fig. S2).

DISCUSSION

To date, various types of *TMC6* mutations have been reported in classical EV (7–15) (**Table I**). In the current study, a c.892-2A>T mutation was detected. This is not a novel mutation of *TMC6* in EV; 2 cases with this mutation have been reported from Japan. One showed compound heterozygous mutation of c.744C>T and c.892-2A>T (9) in *TMC6*. The other showed homozygous mutation of c.892-2A>T (10). Although c.892-2A>T was estimated to be a splice site mutation, and c.892-2A>T (VCV000662928.2) has already been reported as probably pathogenic in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) (accessed 3 June 2022), its actual effect is unknown. We report here, for the first time, that c.892-2A>T induced an amino acid mutation of p.Gly298*. Nonsense mutations, such as p.Gln393* (VCV001437451.1) and p.Ser678* (VCV001456110.1), which are located downstream of the 298th amino acid,

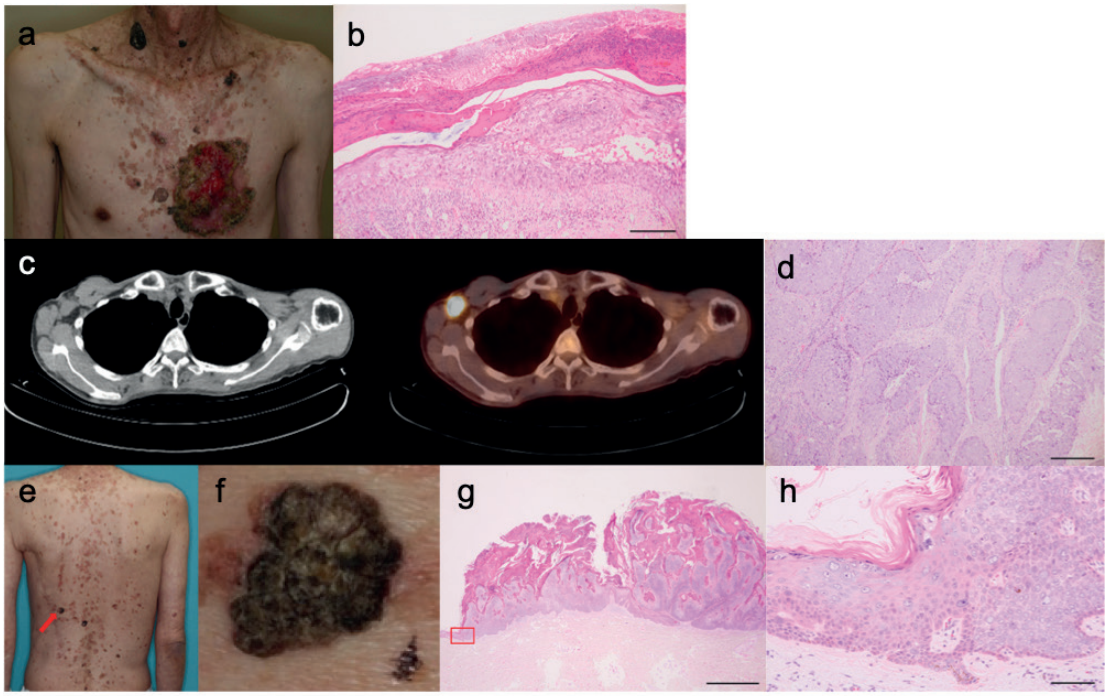

Fig. 1. Clinical features of the patient with epidermodysplasia verruciformis (EV) in this report. (a) Clinical symptoms of the case. A large squamous cell carcinoma (SCC) was present on the patient's chest. Various sized keratotic lesions, including seborrhoeic keratosis, actinic keratosis, and verruca vulgaris, were observed on his neck and trunk. *Bar* = 100 μm. (b) Biopsy specimen from the SCC. Atypical keratinocytes proliferated in the epidermis. (c) Positron emission tomography – computed tomography (PET-CT) images. A metastatic right axillary lymph node was found. (d) Biopsy specimen from the lymph node. Lymphoid follicles were destroyed and replaced by atypical tumour cells. *Bar* = 100 μm. (e) Clinical photograph of the SCC on his back (*red arrow*). (f) Enlarged image of the SCC. (g) Biopsy specimen of SCC on the patient's back. *Bar* = 2 mm. (h) Magnified image of the base of the tumour (*framed by red lines*) shows koilocytosis, suggesting viral infection of the keratinocytes. *Bar* = 100 μm. (i) Family tree of the current case. His maternal grandfathers and paternal grandmothers (*arrows*) were related.

have already been reported as pathogenic mutations in ClinVar. Therefore, c.892-2A>T, which was found in the current patient, was considered to be a pathogenic mutation. This splice site mutation was caused by the sequence of c.930_931AG, which was estimated to function as a novel splice site instead of c.892-2_892-1AG. In comparison with the previously reported patients with EV with c.892-2A>T mutation, the current case presented very severe clinical symptoms with rapidly progressing SCC with lymph node metastasis. The case reported by Tate et al. (9) was a 65-year-old woman who developed Bowen's disease on her hands at 51, 52 and 64 years of age. An SCC and Bowen's disease were found on her face at 65 years of age. In this case, neither HPV-5 nor HPV-8 was detected. The case reported by Sunohara et al. (10) was a 53-year-old man who developed Bowen's disease on his face several times. HPV-3, HPV-14 and HPV-38 were detected in this case. Neither case was associated with advanced cutaneous malignancies that metastasized to other sites. HPV-5, which has been classified as "possibly carcinogenic" in patients with EV, might contribute to the aggravation of SCC in the current case.

Table I. *TMC6* mutations identified in patient with epidermodysplasia verruciformis (EV)

TMC6 mutation	Type	Proteins	Origin	Reference
c.[220C>T];[220C>T]	Nonsense	p.[(Gln74*)];[(Gln74*)]	Japan	(7)
c.[280C>T];[280C>T]	Nonsense	p.[(Arg94*));[(Arg94*)]	Algeria	(8)
c.[280C>T];[280C>T]	Nonsense	p.[(Arg94*)];[(Arg94*)]	Algeria	(8)
c.[744C>A];[892-2A>T]	Nonsense & splice site	p.[(Tyr248*)];[?]	Japan	(9)
c.[892-2A>T];[892-2A>T]	Splice site	p.[?];[?]	Japan	(10)
c.[892-2A>T];[892-2A>T]	Splice site	p.[(Gly298*)];[(Gly298*)]	Japan	Present study
c.[916_917insCATGT];[916_917insCATGT]	Frameshift	p.[(Tyr306fs)];[(Tyr306fs)]	China	(11)
c.[968delT];[968delT]	Frameshift	p.[(Leu323fs)];[(Leu323fs)]	Pakistan	(12)
c.[1110C>G];[1110C>G]	Nonsense	p.[(Tyr370*)];[(Tyr370*)]	France	(13)
c.[1110C>G];[1110C>G]	Nonsense	p.[(Tyr370*)];[(Tyr370*)]	Mexico	(14)
c.[1726G>T];[1726G>T]	Nonsense	p.[(Glu576*)];[(Glu576*)]	Colombia	(8)
c.[2278-2A>G];[2278-2A>G]	Frameshift & nonsense	p.[(Glu760Glyfs*17)];[(Glu760Glyfs*17)]	China	(15)

Mutations of c.892-2A>T have only been reported from Japan. It was found, for the first time, that c.892-2A>T mutation caused amino acid change of p.Gly298*.

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The authors have no conflicts of interest to declare.

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