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CASE REPORTS

Clear Cell Carcinoma in the Oral Cavity with Three Novel Types of *EWSR1-ATF1* Translocation: A Case Report

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Abstract

Clear cell carcinoma (CCC) is a rare epithelial malignant tumor of the salivary glands. It is characterized by tumor cells with clear cytoplasm, hyalinized stroma, and most importantly the fusion genes *EWSR1-ATF1*, *EWSR1-CREM*, and *EWSR1-PLAG1*. Break-apart FISH has been performed for multiple CCC cases, but direct sequencing analysis has been performed in relatively few. Herein, we report an interesting case of CCC harboring three *EWSR1-ATF1* translocations: *EWSR1 exon 8-ATF1 exon 4*, *EWSR1 exon 7-ATF1 exon 4*, and *EWSR1 exon 7-ATF1 exon 5*. This case indicates the possibility of independent *EWSR1-ATF1* gene translocations, and could provide insight into CCC tumorgenesis.

Keywords Clear cell carcinoma · Hyalinizing clear cell carcinoma · Salivary duct tumor · EWSR1 · ATF1

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Introduction

Clear cell carcinoma (CCC) is a low-grade malignant salivary gland tumor of epithelial origin, which is composed of malignant cells with clear cytoplasm and often surrounded by hyalinizing stroma [1]. It most frequently occurs at salivary gland sites in the oral and oropharyngeal cavities.

Ewing sarcoma breakpoint region 1 (*EWSR1*)*–activating transcription factor 1* (*ATF1*) is a specific fusion gene in CCC [2]. The significance of this chimeric gene in the pathogenesis of CCC has been demonstrated. Shah et al. performed *EWSR1* break-apart fluorescence in situ hybridization (FISH) in a variety of salivary gland tumors and detected *EWSR1* rearrangement in 87% of CCCs [3]. In addition, sequence analyses have been performed in five CCC cases to date; *EWSR1* exon11-*ATF1* exon 3 chimeric type was identified in three cases [2, 4, 5]. *EWSR1* exon15-*ATF1 exon 5* [6] and *EWSR1* exon8-*ATF1* exon 4 [5] were identified in one case each.

The fusion gene *EWSR1–cAMP response element modulator* (*CREM*) has been identified in one case [7]. Moreover, the novel fusion gene *EWSR1–pleomorphic adenoma gene 1* (*PLAG1*) was discovered via retrospective analyses of clear cell myoepithelial carcinoma by Skálová et al. [8].

EWSR1 gene rearrangement is involved in other tumors, including clear cell odontogenic carcinoma (CCOC), clear cell sarcoma (CCS), and clear cell sarcoma-like tumor of the gastrointestinal tract (CCSLTGT). FISH for *EWSR1* demonstrated that 62.5% of CCOCs show *EWSR1* rearrangement [9]. *EWSR1-ATF1 and EWSR1-CREB1* chimeric transcripts have been identified in soft parts of CCS [10]. Both *EWSR1-ATF1* and *EWSR1–cAMP responsive element binding protein 1* (*CREB1*) fusion genes have been reported in CCSLTGT [11, 12]. Therefore, translocations in *EWSR1* are crucial in the pathogenesis of various tumors, particularly those with a clear-cell phenotype.

We describe an interesting case of CCC with *EWSR1 exon 8-ATF1 exon 4*, *EWSR1 exon 7-ATF1 exon 4*, and *EWSR1 exon 7-ATF1 exon 5* and review the relevant literature.

Case Report

A 54-year-old Japanese male developed a mouth ulcer of the right oral mucosa after biting his cheek. The ulcer persisted and he became aware of swelling and contact pain in the right lower lip. Intraoral examination revealed an elastic, hard swelling with ulceration, approximately 33 × 31 mm in size. Computed tomography (CT) revealed an ill-defined heterogeneous mass with exophytic growth (Fig. 1A). T1-weighted (T1w) magnetic resonance imaging (MRI) showed that lesion intensity was approximately equivalent to surrounding muscles, but the lesion showed heterogeneous high intensity in T2-weighted (T2w) MRI (Fig. 1B, C). About 9 months after first awareness of symptoms, an incisional biopsy was performed under a provisional diagnosis of malignant tumor originating from the minor salivary gland or an appendage of the skin.

Histopathological examination of a biopsy specimen showed the proliferation of atypical epithelial cells presenting a cord- or nest-like structure (Fig. 2A, B). Some of the atypical cells had small round nuclei and clear to eosinophilic cytoplasm (Fig. 2C). Fibrous stroma was often seen between nests of atypical cells. Immunohistochemistry demonstrated that atypical cells were positive for cytokeratin (CK) AE1 + AE3 (Fig. 2D), and focally positive for CK5/6, EMA, and p63 (Fig. 2E–G). By contrast, atypical cells were negative for smooth muscle actin (SMA) and glial fibrillary acidic protein (GFAP) (Fig. 2H, I). Therefore, the lesion

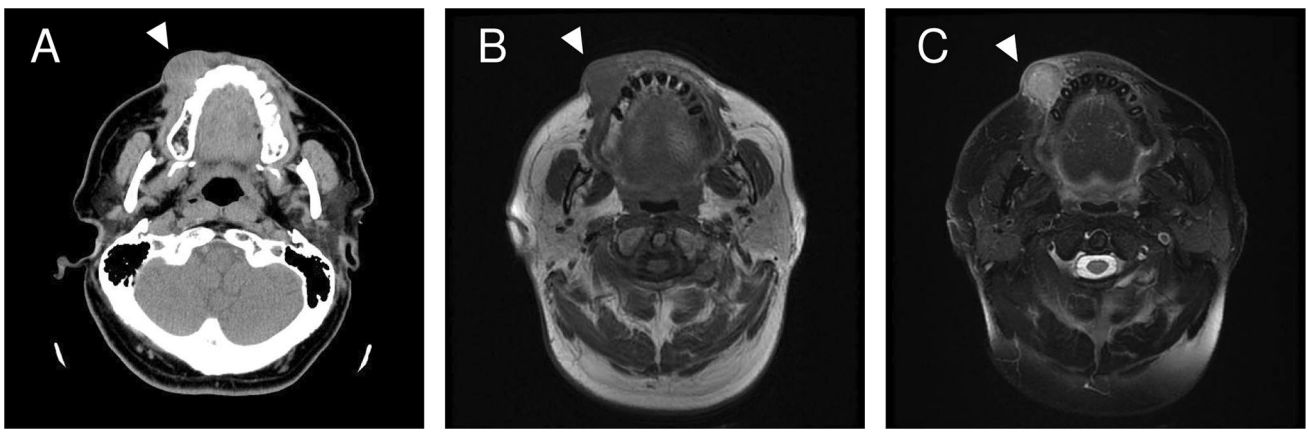

Fig. 1 **A** Computed tomography (CT) image in horizontal view. Arrowhead indicates tumor mass lesion. **B** T1-weighted (T1w) magnetic resonance imaging (MRI) scan of the tumor lesion. **C** T2-weighted (T2w) MRI scan of the tumor lesion

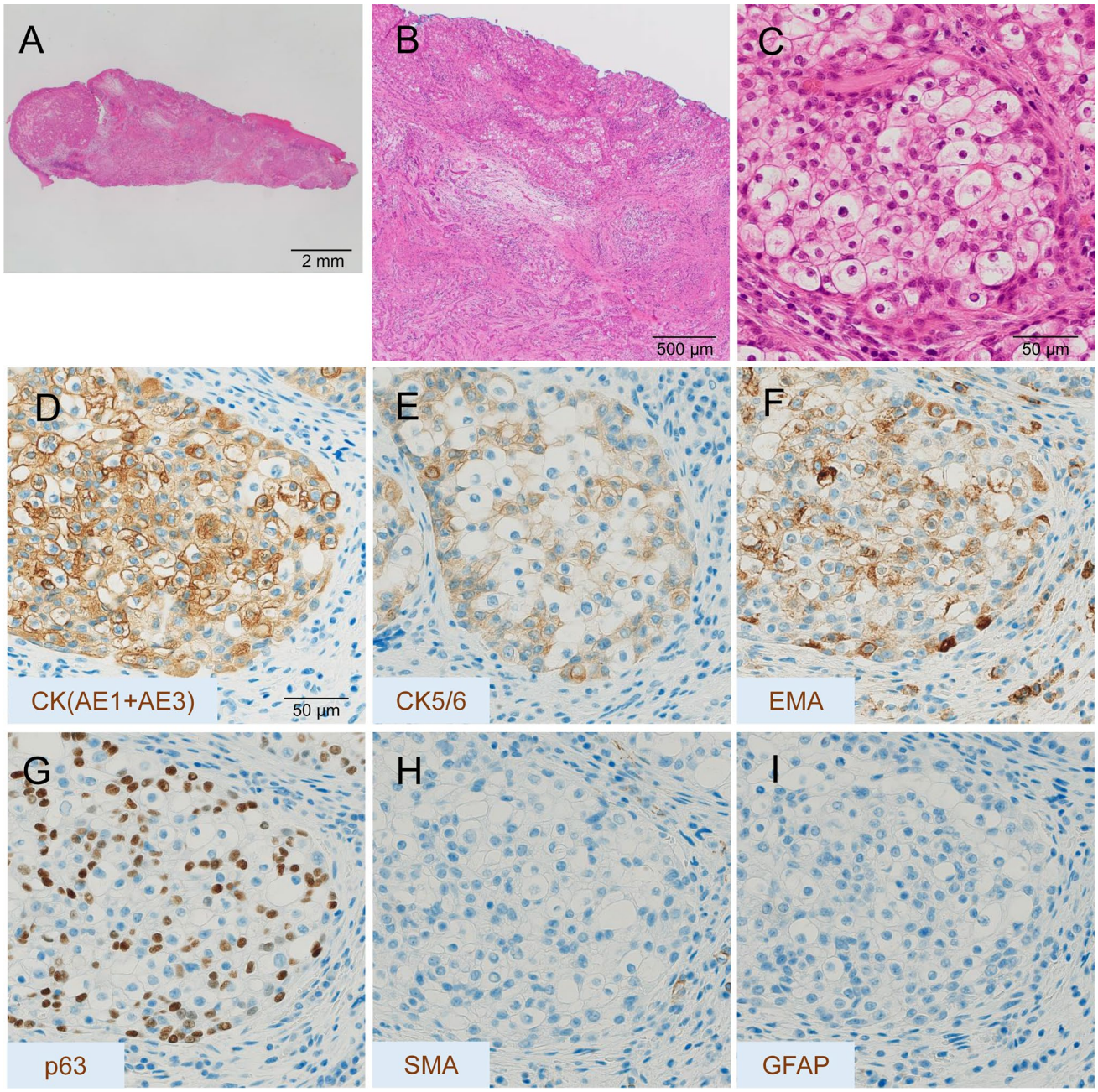

Fig. 2 **A**–**C** Histological findings of the biopsy specimen. **D**–**I** Immunohistological findings for cytokeratin (CK) AE1 + AE3, CK5/6, EMA, p63, smooth muscle actin (SMA), and glial fibrillary acidic protein (GFAP)

was diagnosed as malignant epithelial tumor and a surgical tumorectomy was performed.

The tumor lesion in the surgical specimen presented as a grayish-white solid mass occupying space between the skin and oral mucosa (Fig. 3A, B). Also in histopathological evaluation, the tumor presented as a nodular lesion occupying nearly the whole of this space. Margin of the tumor lesion did not show continuity with epidermis, skin appendages, or oral mucosa (Fig. 3C–E). Histopathological examination of the tumor showed proliferation of atypical cells with clear to eosinophilic cytoplasm in a nest, solid sheet, or trabecular pattern (Fig. 3F, G). Hyalinized or sclerotic stroma surrounding tumor nests was often seen. These atypical cells were positive for CK5/6, EMA, and p63 in immunohistochemistry (Fig. 3H–J). In addition, a small part of the tumor lesion was composed of spindle-shaped atypical cells suggesting poor differentiation (Fig. 3K, L). Perineural invasion was also observed (Fig. 3M). Ki-67 labeling index (LI)

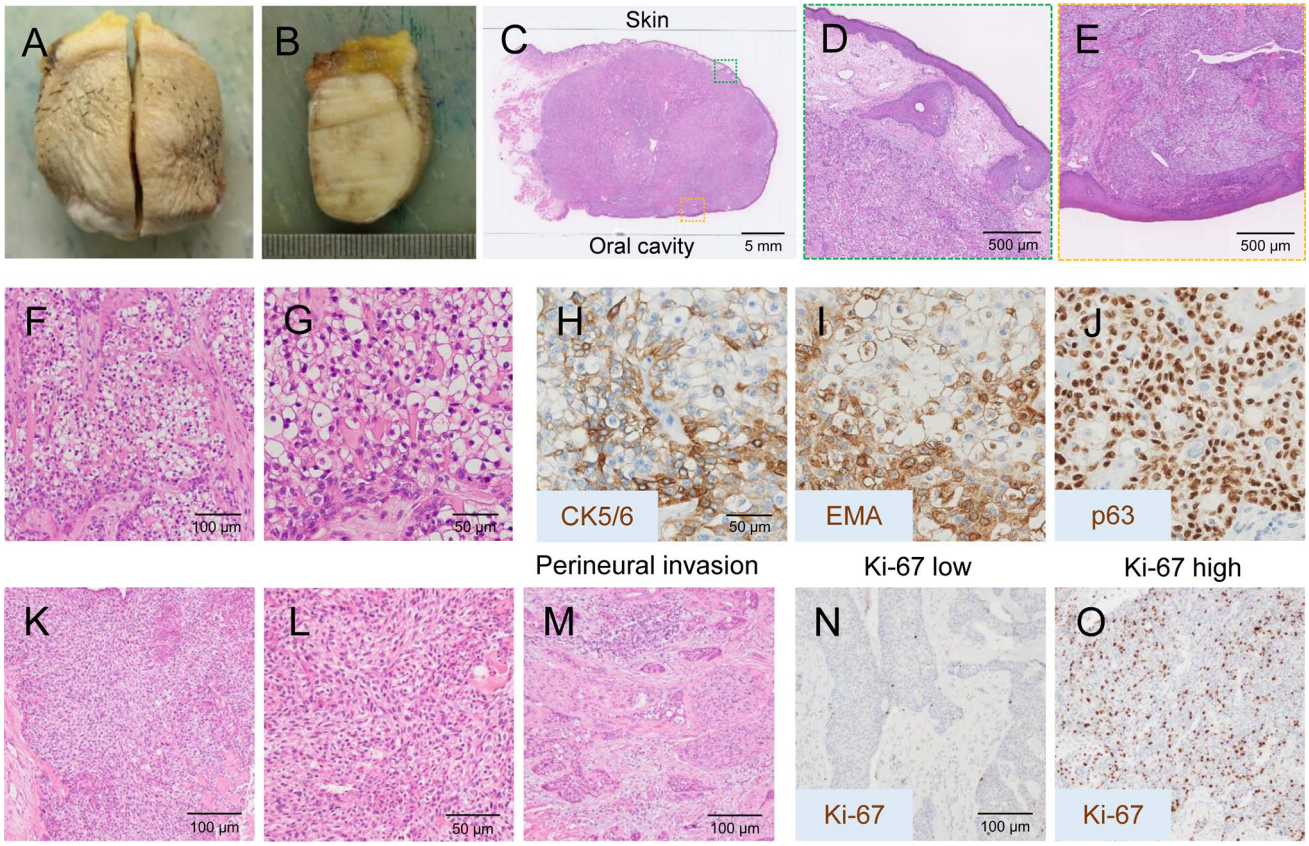

Fig. 3 **A**, **B** Gross appearance of the tumor lesion in the surgical specimen. **C**–**G** Histological findings of the surgical specimen. **H**–**J** Immunohistological findings for CK5/6, EMA, and p63. **K**, **L** Histological findings of poorly differentiated component. **M** Histological finding of perineural invasion. **N**, **O** Immunohistological findings for Ki-67 with low- or high-labeling indices

was approximately 30% in the highest region whereas most tumor cells showed less than 1% of Ki-67 LI (Fig. 3N, O).

Genetic analysis using the tissue from this surgical specimen revealed that the tumor harbored the *EWSR1-ATF1* fusion gene. Three types of fusion gene were identified by direct sequence analysis: *EWSR1 exon 8-ATF1 exon 4*, *EWSR1 exon 7-ATF1 exon 4*, and *EWSR1 exon 7-ATF1 exon 5* (Fig. 4A, B). The *CRTC1-MAML2* and *CRTC3-MAML2* fusion genes were not identified. Therefore, we made a final diagnosis of CCC derived from the minor salivary gland. At 20 months after surgical resection, there was no evidence of recurrence.

Discussion

CCC is a low-grade salivary gland carcinoma that is consistently associated with a *EWSR1-ATF1* fusion gene. Sequence analysis of *EWSR1*-associated fusion genes has been performed in several cases [2, 4–7]. The *EWSR1* exon 11-*ATF1* exon 3 fusion type has been reported in three cases [2, 4, 5]. Hirose et al. described the *EWSR1* exon 15-*ATF1 exon 5* fusion gene [6]. Heft Neal et al. reported a case of CCC harboring *EWSR1 exon 8-ATF1 exon 4* [5]. In addition, various *EWSR1-CREM* fusion genes (*EWSR1* exon 14 with *CREM* exon 6, *EWSR1* exon 14 with *CREM exon 5*, or *EWSR1* exon 14 with *CREM* exon 6, *EWSR1* exon 11 with *CREM* exon 6) were identified by Chapman et al. via Sanger sequencing [7].

EWSR1 plays a crucial role in development and is involved in the pathogenesis of various soft tissue tumors. This gene consists of 17 exons, the first 7 of which encode the N-terminal transactivation domain. The C-terminal exons of *EWSR1* encode the putative RNA-binding domain and the domains encoded by these exons, particularly exons 15–17, repress its N-terminal activation domain [13, 14]. *ATF1* encodes an activating transcription factor protein of the ATF subfamily and the basic-region leucine zipper (bZIP) family. ATF1 regulates cell growth, survival, and other activities [15]. Exon 3 in *ATF1* encodes an activation domain and phosphorylation at Ser63 in this exon is essential for regulating cell proliferation [16]. Although the mechanisms by which *EWSR1-ATF1* gene translocation contributes to oncogenesis are unclear, the EWSR1 N-terminal domain likely constitutively activates the *ATF1*

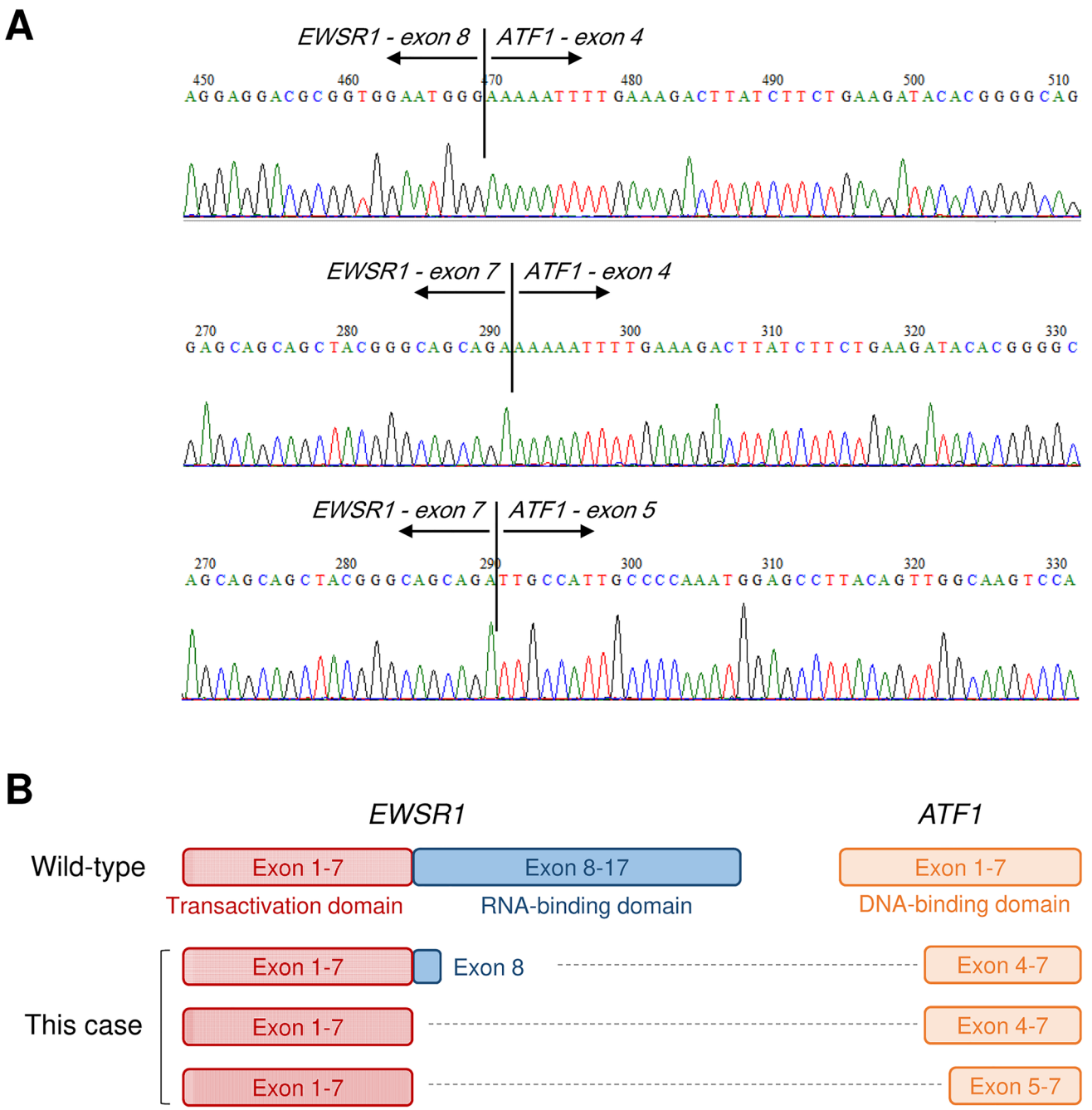

Fig. 4 **A** Genetic analysis using the surgical specimen. Direct sequencing showed three types of *EWSR1-ATF1* fusion gene. **B** Diagram of the *EWSR1-ATF1* fusion gene variants in this case

DNA-binding domain in fusion transcripts, and loss of the EWSR1 C-terminal domain could enhance this inappropriate transactivation. Indeed, CCC with the *EWSR1* exon 11-*ATF1* exon 3 fusion gene typically has a good prognosis, whereas CCSs harboring the *EWSR1 exon 8-ATF1 exon 4* fusion gene show aggressive clinical behavior [10]. A study using integrative sequencing demonstrated that CCCs with the *EWSR1-ATF1* fusion show a gene signature enriched with the ATF1-motif [5].

In the present case, the *EWSR1 exon 8-ATF1 exon 4*, *EWSR1 exon 7-ATF1 exon 4*, and *EWSR1 exon 7-ATF1 exon 5* fusion genes were identified in CCC. This suggests that the tumor cells have a triplet chromosome harboring the *EWSR1-ATF1* fusion. This is the first case of a single

CCC lesion harboring three types of *EWSR1-ATF1* fusion gene. This result further suggests that at least one of the translocations must have occurred after duplication of the chromosome. Previous studies with molecular cell biology demonstrate that aneuploidy promotes chromosomal aberrations and tumorigenesis [17]. Carcinogenesis in this case might depend on such mechanisms. Among the fusion genes in this case, *EWSR1 exon 8-ATF1 exon 4* are almost always found in CCS but were recently identified in a case of CCC [5]. Both *EWSR1 exon 7-ATF1 exon 4* and *EWSR1 exon 7-ATF1 exon 5* are found only in cases of CCS, and this is the first report of these types of fusion genes in CCC.

Notably, such types of fusion gene were found in a malignant tumor of epithelial phenotype. Interestingly, Tsukamoto et al. reported that four types of *EWSR-ATF1* fusions were detected in the metastatic site of CCS, whereas only single *EWSR1-ATF1* fusion was found in the primary site [18]. Coexistence of multiple *EWSR1-ATF1* fusion genes might cause increased metastatic ability of tumor cells. If this hypothesis is applicable to CCC, careful follow-up for metastasis will be needed. From the viewpoint of molecular mechanisms, because the transcripts encoded by the three types of *EWSR1-ATF1* translocations contain only a part of the suppressive EWSR1 C-terminal domain, this case might have a poor prognosis. However, there was no recurrence at 20 months after resection.

In summary, we report a case of CCC with a novel pattern of *EWSR1-ATF1* gene translocations. This is the first case in which a single tumor lesion harbors three different types of *EWSR1-ATF1* fusion gene, all of which are reported mainly in CCSs. Further studies of the gene translocations and their inter-relationships will provide insight into the carcinogenesis of CCC.

Authors Contribution EM designed this study. SN, HH, KH, SIN, KO, YF, ST, and EM interpreted histological and immmunohistochemical findings. SN also interpreted genetic findings and wrote the manuscript. MK performed direct sequence analysis. HK, YN, KM, NU, and MF contributed to patient management. All authors read and approved the final manuscript.

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Data Availability The data in this study are available from the corresponding author, Eiichi Morii, upon reasonable request.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval The study in this case report was performed in accordance with the ethics committee requirement of Osaka University and with Declaration of Helsinki.

Informed Consent Informed consent for publication of clinical details and images was obtained from the patient.

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