

Article

Tumor Angiogenic Inhibition Triggered Necrosis (TAITN) in Oral Cancer

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Abstract: CXCR4 is a chemokine receptor crucial in tumor progression, although the angiogenic role of CXCR4 in oral squamous cell carcinoma (OSCC) has not been investigated. Here we show that CXCR4 is crucial for tumor angiogenesis thereby supports tumor survival in OSCC. Immunohistochemistry on human clinical specimens revealed that CXCR4 and a tumor vasculature marker CD34 were co-distributed in tumor vessels in human OSCC specimens. To ask the effects of CXCR4 inhibition, we treated the OSCC-xenografted mice with AMD3100, so-called plerixafor, an antagonist of CXCR4. Notably, we found a unique pathophysiological structure defined as Tumor Angiogenic Inhibition Triggered Necrosis (TAITN), which was induced by the CXCR4 antagonism. Treatment with AMD3100 increased necrotic area with the induction of hypoxia-inducible factor-1 α in the xenografted tumors, suggesting that AMD3100-induced TAITN was involved in hypoxia and ischemia. Taken together, we demonstrated that CXCR4 plays a crucial role in tumor angiogenesis required for OSCC progression, whereas TAITN induced by CXCR4 antagonism could be an effective anti-angiogenic therapeutic strategy in OSCC treatment.

Keywords: tumor blood vessel; Tumor Angiogenic Inhibition Triggered Necrosis (TAITN); CXCR4 antagonist; oral squamous cell carcinoma; hypoxia

1. Introduction

C-X-C motif chemokine receptor (CXCR4) is expressed in various hematopoietic cells such as peripheral blood lymphocytes, unprimed T cells [1], monocytes [2], pre-B cells, and plasma cells [3,4] and also expressed in non-hematopoietic cells such as vascular smooth muscle cells [5], endothelial cells [6,7], intestinal [8] and alveolar epithelial cells [9]. CXCR4 can be bound with the ligand, stromal cell-derived factor-1 (SDF-1) also known as C-X-C motif chemokine ligand 12 (CXCL12) and the SDF-1/CXCR4 signaling axis retains hematopoietic stem cells in the bone marrow [10]. It has been shown that CXCR4 involves proliferation, infiltration, and adhesion of hematopoietic stem cells [11,12]. CXCR4 is essential for the formation of large blood vessels that feed the gastrointestinal tract in the

fetal stage [13]. Notably, it has been shown that CXCR4 was also expressed in various types of cancers including glioblastoma, liver cancer, breast cancer, and esophageal cancer [14–17]. CXCR4-positive tumors often grew faster than CXCR4-negative tumors [18,19]. It has been shown that lymph node metastasis and distant organ metastasis frequently occurred in CXCR4-positive tumor cases [15,17,20] thereby CXCR4 is considered to be a poor prognosis factor in cancer. It was shown that CXCR4 was expressed in 50–60% in OSCC clinical cases [21]. CXCR4-positive OSCC tumors metastasized to lymph nodes and to distant organs more frequently than CXCR4-negative tumor cases [21,22]. Consistently, suppression of CXCR4 reduced tumor volumes and metastases to lymph nodes and distant organs in mice transplanted with OSCC cells [23]. Despite the fact that OSCC cells express a considerably high level of CXCR4, neither proliferation nor survival of the cancer cells was inhibited by CXCR4 antagonists [21,23,24]. These studies suggest that peripheral and adjacent cells around OSCC cells might play important roles in the anti-tumor effect of the CXCR4 antagonist. We therefore hypothesized that CXCR4 antagonism could repress OSCC not by directly targeting cancer cells but by targeting peri-tumoral stromal cells. In the present study, we first aimed to clarify which type of cells could be positive with CXCR4 in OSCC and whereby focused on CXCR4/CD34 double positive tumor vasculatures in clinical specimens of OSCC. CD34 molecule was first characterized as a protein that is expressed by human hematopoietic progenitor cells and has been considered as a definitive marker of angiogenesis in neoplastic lesions [25]. In the presence of pathological angiogenesis, at the sprouting tips of growing vessels, the SDF-1/CXCR4 axis was shown to play a fundamental role in endothelial cell invasion, mobilization/migration, extravasation, directional migration, homing, and cell survival [26–28]. We therefore examined the alteration of CD34-positive blood vessels under the administration of the CXCR4 antagonist. Blood vessels generally supply oxygen and nutrients crucial for tumor growth [29,30]. Such tumor blood vessels could form a favorable tumor microenvironment for tumor growth. We therefore focused on the histological feature of OSCC enriched in blood vessels between the tumor nests. Previous studies of CXCR4 in OSCC have focused on CXCR4 expressed on cancer cells and have not reported CXCR4-positive blood vessels in OSCC.

We here aimed to evaluate (i) the sub-tumor distribution of CXCR4 in human clinical specimens of OSCC and (ii) effects of a CXCR4 antagonist AMD3100 so-called plerixafor on inhibition of tumor angiogenesis and tumor growth in mice xenografted with OSCC cells.

2. Materials and Methods

2-1. Patients samples

Patient-derived OSCC samples were obtained from the Department of Oral Pathology, Okayama University. This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (Approval number: 1608-018). The well-differentiated type of tongue squamous cell carcinoma (10 cases) was examined in the retrospective study (Table 1). All excised tumor tissues were fixed in 4% paraformaldehyde for 12 hours. Tissues were embedded in paraffin wax and sections were prepared.

Table 1. List of patient-derived samples

Sex	Age	T	N	M	Stage
Male	78	2	2b	0	4a
Male	61	1	0	0	1
Female	76	2	0	0	2
Female	39	2	0	0	2
Male	30	1	0	0	1
Male	62	1	0	0	1
Male	52	2	0	0	2
Female	86	2	0	0	2
Male	72	1	0	0	1
Male	76	1	0	0	1

T, assessment of the primary tumor; N, assessment of the regional lymph nodes; M, assessment of distant metastasis; Stage criteria follow the TNM classification defined in Malignant Tumors 8th Edition, published by Union International Cancer Control [31]. The criteria of the stages are shown in Table S1.

2-2. Cell line and cell culture

Human OSCC cell line HSC-2 was obtained from JCRB Cell Bank at National Institutes of Biomedical Innovation, Health and Nutrition (Osaka, Japan). HSC-2 cells were cultured in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal calf serum (FCS) (Thermo Fisher Scientific, Waltham, MA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Sigma-Aldrich) at 37°C in humidified air with 5% CO₂.

2-3. Reagent

AMD3100/plerixafor (ChemScene, NJ) (10 mg) was dissolved in 20 ml of physiological saline and stored at 4°C before use. This concentration is the same as the concentration of the drug used in the oral cancer experiment using mice [21,23].

2-4. Xenograft and administration of AMD3100 to mice

HSC-2 cells were transplanted subcutaneously to backs and heads of 5 nude mice (BALB/c-nu/nu) at 10×10^5 cells per xenograft. A week after the injection, AMD3100 (50 µg/day) was administered intraperitoneally to HSC-2-transplanted mice everyday for 21 days. As a control, 5 nude mice were xenografted by the above-mentioned method and administered intraperitoneally with saline. Mice were fixed by perfusion with 10% neutral buffered formalin. Tumors were excised, fixed by immersion in 10% neutral buffered formalin, dehydrated, and embedded in paraffin. Paraffin sections were prepared for hematoxylin-eosin (HE) staining and IHC and observed with a light microscope.

2-5. Immunohistochemistry

IHC was carried out using anti-CD34 (Histofine, Tokyo, Japan; no dilution), anti-CXCR4 (Abcam, Cambridgeshire, UK; dilution of 1:300), and anti-HIF-1α (Abcam, Cambridgeshire, UK; dilution of 1:200) antibodies. Signals were enhanced by the avidin-biotin complex method (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA). Detection was performed with 3, 3'-diaminobenzidine (DAB), and the staining results were observed with an optical microscope. Tumor tissue maximum section was used for comparison of necrosis area, tumor vessels structure, and HIF-1α-positive area by using ImageJ, an image processing software [32].

For double-fluorescent IHC, the above-mentioned CD34 and CXCR4 antibodies were diluted with Can Get Signal (Toyobo, Osaka, Japan). Anti-mouse IgG Alexa Fluor 488 and anti-rabbit IgG Alexa Fluor 568 (Life Technologies, Carlsbad, CA) were used as secondary antibodies at a dilution of 1:200. After the reactions, the specimens were stained with 0.2 g/mL of DAPI (Dojindo Laboratories, Kumamoto, Japan). The staining results were observed with a fluorescence microscope.

2-6. Statistical analysis

Data were recorded and entered into an electronic database during the course of the evaluation by means of Microsoft Excel (Microsoft, Inc., Redmond, WA). Means and standard deviations were used for normal data distributions. The database was transferred to JMP version 11.2 for Macintosh computers (SAS Institute Inc., Cary, NC) for statistical analysis. Statistical analysis was performed to investigate whether there were significant differences between the mean values of the groups. Wilcoxon's matched-pairs signed rank test was used for non-normally distributed measurements,

and t-test was used for normally distributed measurements. The level of significance was set at $P<0.05$.

3. Results

3.1. CXCR4/CD34 double positive vessels in the stroma of human oral squamous cell carcinoma

To investigate whether CXCR4 expression in vessels could be different between tumor and non-tumor areas in OSCC clinical cases, we first defined the tumor and non-tumor areas in the OSCC specimens. By HE staining, a typical morphology of squamous cell carcinoma was found to be surrounded by a subepithelial connective tissue (Fig. 1a, b; tumor area). The tumor cells containing eosinophilic cytoplasm and nuclear atypia formed large and small tumor nest (Fig. 1b). Abundant blood vessels and fibrous connective tissue were observed between the tumor nest (Fig. 1b).

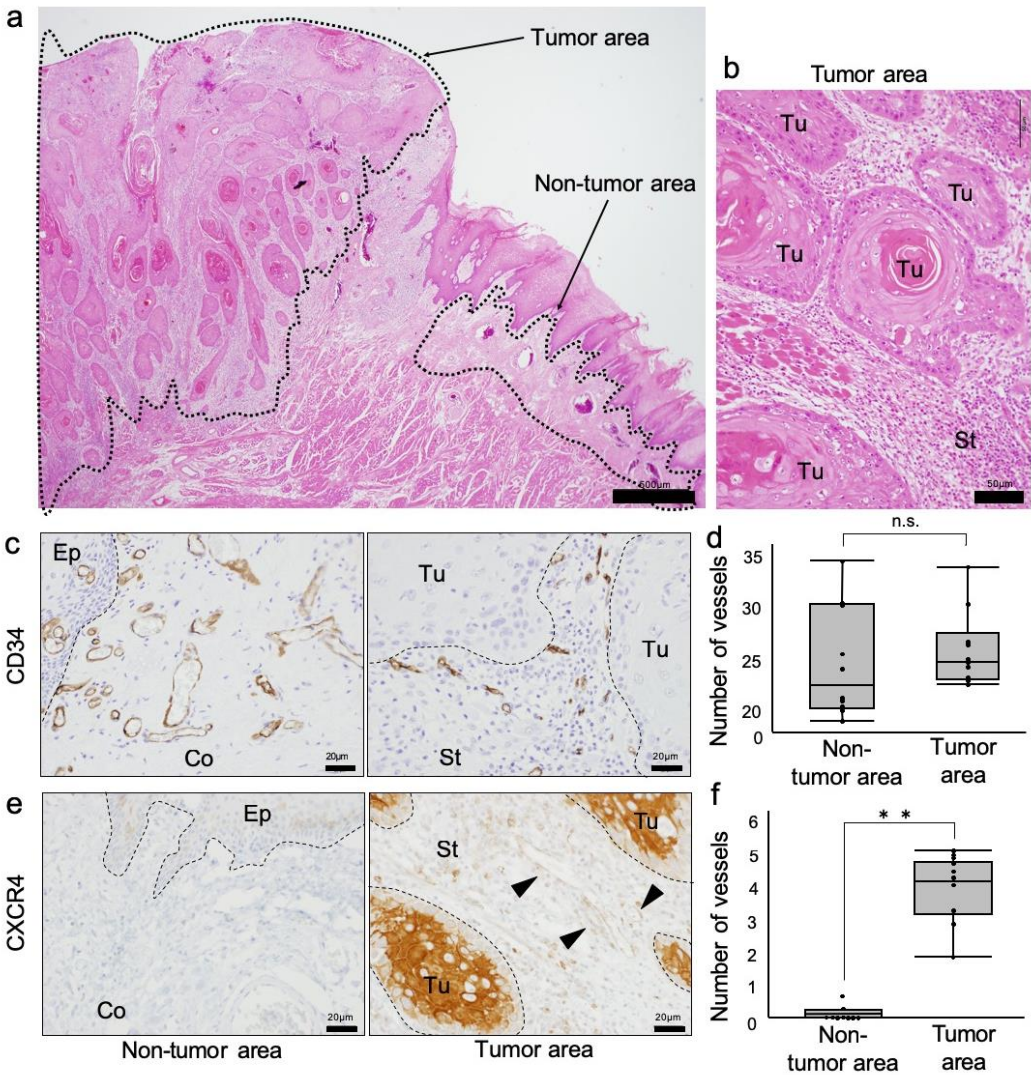


Figure 1. CXCR4/CD34 double positive vessels found in OSCC stroma. (a) HE staining of an OSCC tissue for the definition of the tumor and non-tumor areas. Tumor area and non-tumor area were surrounded by dotted lines. (b) High-power magnification of tumor area stained with HE. Tu: tumor. St: stroma. (c) IHC for CD34 in tumor and non-tumor areas. Borders between epithelia (Ep), connective tissue (Co), tumor (Tu), and stroma (St) were shown with dotted lines. (d) The number of vessels in the tumor and non-tumor areas. $P=0.289$, $N=10$ (e) IHC for CXCR4 in tumor and non-tumor areas. Arrowheads indicate CXCR4-positive vessels specifically existed in the tumor area. (f) The number of CXCR4-positive vessels in the tumor and non-tumor areas. $**P<0.0001$, $N=10$.

We next examined the expression and distribution of CD34 and CXCR4 in the tumor and stroma areas. CD34-positive vascular endothelial cells forming luminal structures were found in the stroma and connective tissues (Fig. 1c). Tumor stroma CD34-positive blood vessels appeared to be smaller in structure than normal, while the number of blood vessels was not different. (Fig. 1d). CXCR4-positive lumen structures were found in the stroma, although CXCR4 was distributed in both tumor and stromal cells (Fig. 1e). CXCR4-positive vessels were significant in the tumor area more abundantly than those in non-tumor areas (Fig. 1f). These findings indicated that CXCR4 was selectively distributed in tumor vessels of OSCC.

To ask whether CXCR4 and CD34 could be co-distributed in the vessels, we next performed double-fluorescent IHC. It was first confirmed that CXCR4 was distributed in both tumor cells and vessel-like structures in the stroma (Fig. 2a). CD34 was distributed in endothelial cells in the stroma but not in tumors (Fig. 2b). CXCR4 / CD34-double positive endothelial cells were found in the stroma (Fig. 2c arrowheads). CXCR4 was not uniformly distributed in all tumor blood vessels, but partially localized at the constricted and branched parts of the blood vessels. Conversely, non-tumor areas' CD34-positive blood vessels were CXCR4-negative (Fig. 2f, arrowheads).

These findings indicated that CXCR4-positive endothelial cells existed in OSCC stromata and prompted us to hypothesize that the CXCR4-positive blood vessels could support tumor progression.

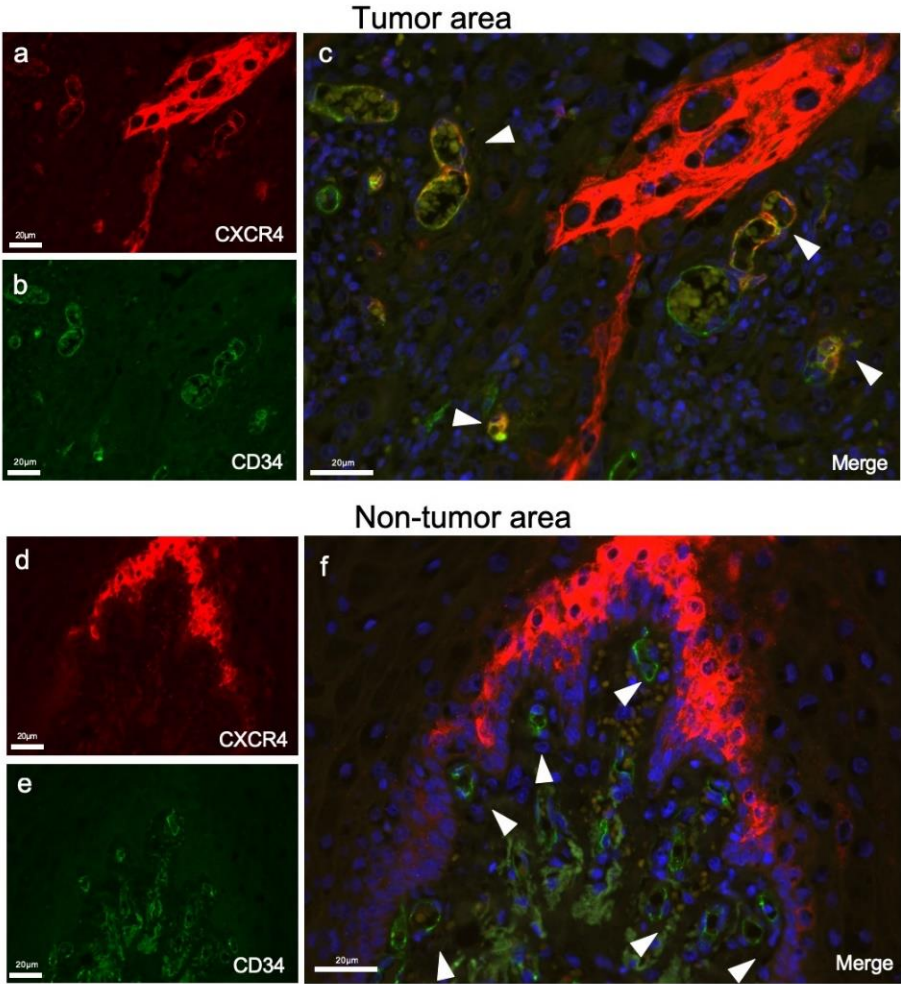


Figure 2. Double-fluorescent IHC for CXCR4 and CD34 in tumor area and non-tumor area. (a) CXCR4 stained in stromal vessels and tumor cells (red). (a) CD34 stained only on vessels in the tumor stroma (green). (c) A merged IHC image of CD34 and CXCR4. Nuclei were stained with DAPI. Arrowheads indicate CXCR4/CD34 double positive tumor vessels in the OSCC stroma. (d) CXCR4 stained in the non-tumor area (red). (e) CD34 stained in the non-tumor area (green). (f) A merged IHC image of CD34 and CXCR4. Nuclei were stained with DAPI. CD34-positive endothelial cells were all negative for CXCR4 (arrowheads).

3.2. A CXCR4 antagonist AMD3100 induced tumor necrosis in OSCC-xenotransplanted mice

We next asked whether a CXCR4 antagonist AMD3100 could alter the tumor status of OSCC using tumor xenograft mouse model. Macroscopically, the size of tumors was not different between the AMD3100 and saline groups. However, surface ulceration of tumors was notably found in the AMD3100 group but not in the control group (Fig. 3a, b). On histological examination, wide necrotic areas were found in the center and periphery of tumors in the AMD3100 group (Fig. 3c). In the necrotic areas of the two groups, nuclei of tumor cells were lost and eosin-stained debris was seen (Fig. 3d). The necrotic areas were significantly enlarged in the AMD3100 group compared to the control group (Fig. 3e). These findings indicated that CXCR4 antagonism induced tumor necrosis in OSCC in vivo.

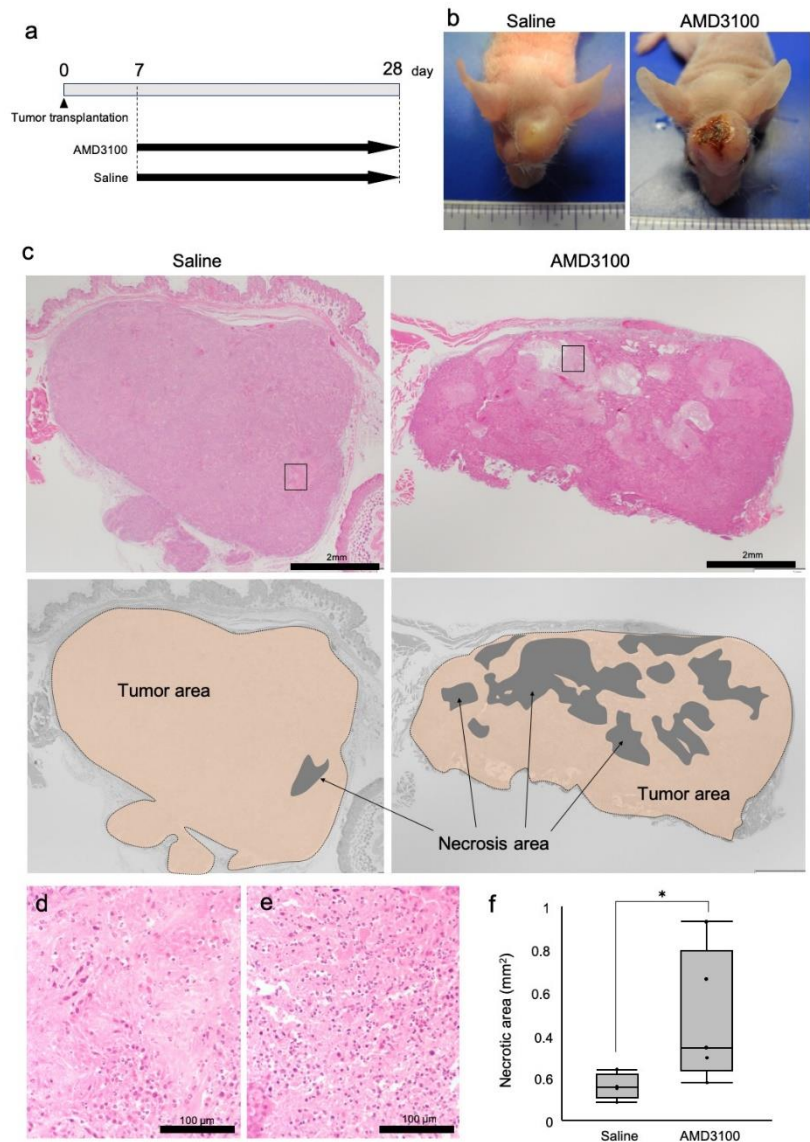


Figure 3. CXCR4 antagonist AMD3100 induced tumor necrosis. Mice were injected with HSC-2 cells subcutaneously in the back and head. Seven days after tumor transplantation, AMD3100 or saline was intraperitoneally administrated everyday for 21 days. (a) A schematic protocol of tumor injection and AMD3100 administration. (b) Representative photographs of subcutaneous tumors formed in mice. Necrosis and bleeding were observed only in the AMD3100 group. (c) Definition of necrotic areas. Top, HE staining. The areas closed with rectangles were enlarged in “d” and “e”. Bottom, diagrams defining necrotic areas in each tumor. (d,e) High power magnification view of tumor necrotic areas in the saline-treated mouse. (d) and AMD3100-treated mouse (e). The places were crossed square in fig. 3c. (f) Box-whisker-dot plot of tumor necrotic areas with or without AMD3100 administration. *P=0.0416, N=5.

3.3. Pathophysiological structure of Tumor Angiogenic Inhibition with Tumor Necrosis induced by the CXCR4 antagonism

To investigate the pathological mechanism of tumor necrosis induced by the CXCR4 antagonist, we next investigated structures of vessels in the necrotic tumor. Tumor tissues were likely islands interspersed with necrotic tissue in the AMD3100 group (Fig. 4a, left). With a higher power magnification, we found that vessel-associated tumor cells survived whereas those away from the vessels were necrotic (Fig. 4a, right). Tumor island-associated vessels were expressing CD34 and thus confirmed to be endothelial cells (Fig. 4b, c).

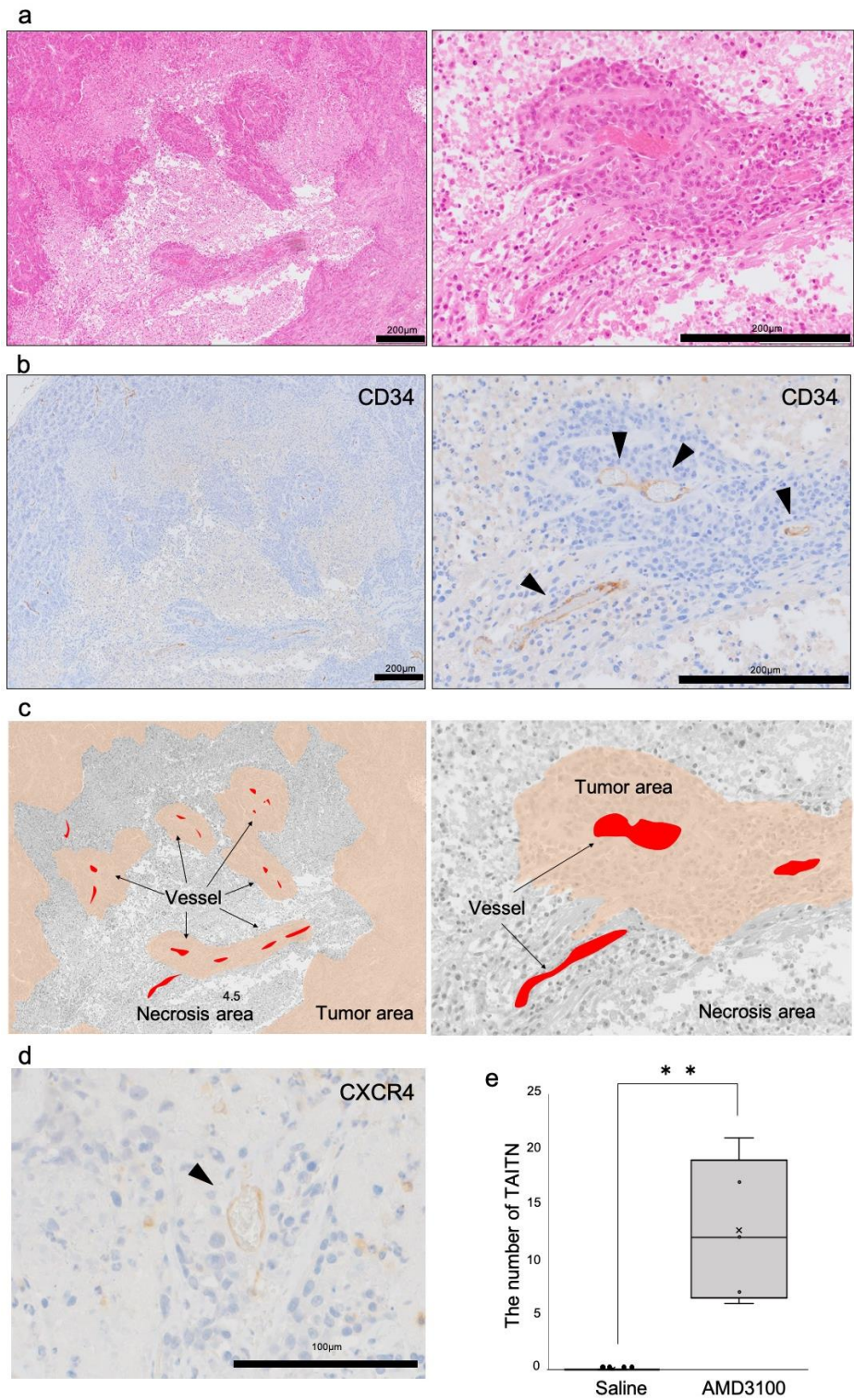


Figure 4. Pathological analyses of Tumor Angiogenic Inhibition with Tumor Necrosis (TAITN) triggered by CXCR4 antagonism. (a) Representative HE staining of tumor necrotic area in an

AMD3100-treated mouse. (b) Representative IHC for CD34 in the tumor necrotic area in an AMD3100-treated mouse. (c) Illustration of the structure of TAITN found in AMD3100-treated mice. Gray, necrotic area. Red, tumor vessels. Brown, tumor area. Islanded tumor areas were surrounded by necrotic area. The center of each tumor island was occupied by vessels. We defined this unique pathophysiological conception as Tumor Angiogenic Inhibition with Tumor Necrosis, so-called TAITN. (d) IHC for CXCR4 in the TAITN. CXCR4-positive blood vessels were observed at the center of TAITN. (e) Number of vessel-tumor islands in the AMD3100 group and saline group. **P=0.0031, N=5.

The histologically unique phenomenon of tumor necrosis associated with disorganized vasculatures was defined as Tumor Angiogenic Inhibition with Tumor Necrosis (TAITN) (Fig. 4a-c). The vessels located at the center of TAITN were CXCR4-positive (Fig. 4d). Any TAITN-like structure was not seen in the saline group. In the saline group, CD34-positive tumor vessels did not infiltrate the necrotic area. TAITN significantly occurred in the AMD3100-treated group but not found in the control tumors (Fig. 4e).

These results indicated that the CXCR4 antagonist triggered TAITN in OSCC tumors.

3.4. Hypoxia-inducible factor-1 α was induced in necrotic tumors under CXCR4 antagonism.

We next hypothesized that the TAITN triggered by CXCR4 antagonism could also induce tumor hypoxia inasmuch as angiogenic inhibition. The major regulator of oxygen tension is hypoxia-inducible factor-1 (HIF-1), a transcription factor complex stabilized under low oxygen tension to mediate cellular responses, which activates and binds to a large number of target genes [33]. HIF-1 complex is composed of alpha and beta subunits. The alpha subunit of HIF-1 (HIF-1 α) is rapidly inactivated and degraded by ubiquitination and subsequent proteasomal pathway, a process that is inhibited under hypoxic conditions [34]. These studies prompted us to ask whether HIF-1 α levels could be altered in tumors treated with or without AMD3100. We found that HIF-1 α -positive tumor area tended to be larger in the AMD3100 group than that in the control group (Fig. 5a). HIF-1 α -positive cells spread in the whole tumor area to the tumor periphery in the CXCR4-inhibited group, whereas HIF-1 α -positive cells were limitedly localized in the central area of tumors in the control group (Fig. 5b). HIF-1 α was increased in cancer cells in the AMD3100-treated mice compared to the untreated mice (Fig. 5c, d). The number of HIF-1 α -positive cells in the tumors were significantly increased in the AMD3100-treated group compared to the control group (Fig. 5d).

These results suggested that intra-tumoral hypoxia was usually limited in the central area of tumors, whereas CXCR4 antagonism enhanced hypoxia occurring in the whole tumor.

3.5. Tumor vessels were disorganized by CXCR4 antagonism.

We next hypothesized that CXCR4 antagonism-induced tumor necrosis and hypoxia could be mediated by disorganization of vessels, whereby oxygen and nutrition were limited. This hypothesis prompted us to prove a concept of TAITN to be Tumor Angiogenic Inhibition Triggered Necrosis. We therefore asked whether and how CXCR4 antagonism could disrupt structures of blood vessels occupied by viable (non-necrotic) tumor cells. Tumor blood vessels in the AMD3100-treated group were fewer than those in the control (Fig. 6a). The tumor blood vessels in the AMD3100-treated group were thinner and shorter and poorly branched than those in the control (Fig. 6b-e, Table 1). The total and average length of blood vessels in the AMD3100 group was significantly shorter than those in the control group (Fig. 6c, d). The ratio of blood vessels longer than 100 μ m was lesser in the AMD3100-treated group (1.8%) compared to the saline-treated group (6.9%) (Fig. 6e, Table 1). The blood vessels shorter than 50 μ m were more abundant in the AMD3100 group (86.2%) compared to the control group (76.7%) (Table 1, Fig. 6e).

These data indicated that the CXCR4 antagonist AMD3100 exerted a powerful anti-angiogenic effect disorganizing blood vessels in OSCC tumors- a proof of concept for Tumor Angiogenic Inhibition Triggered Necrosis (TAITN).

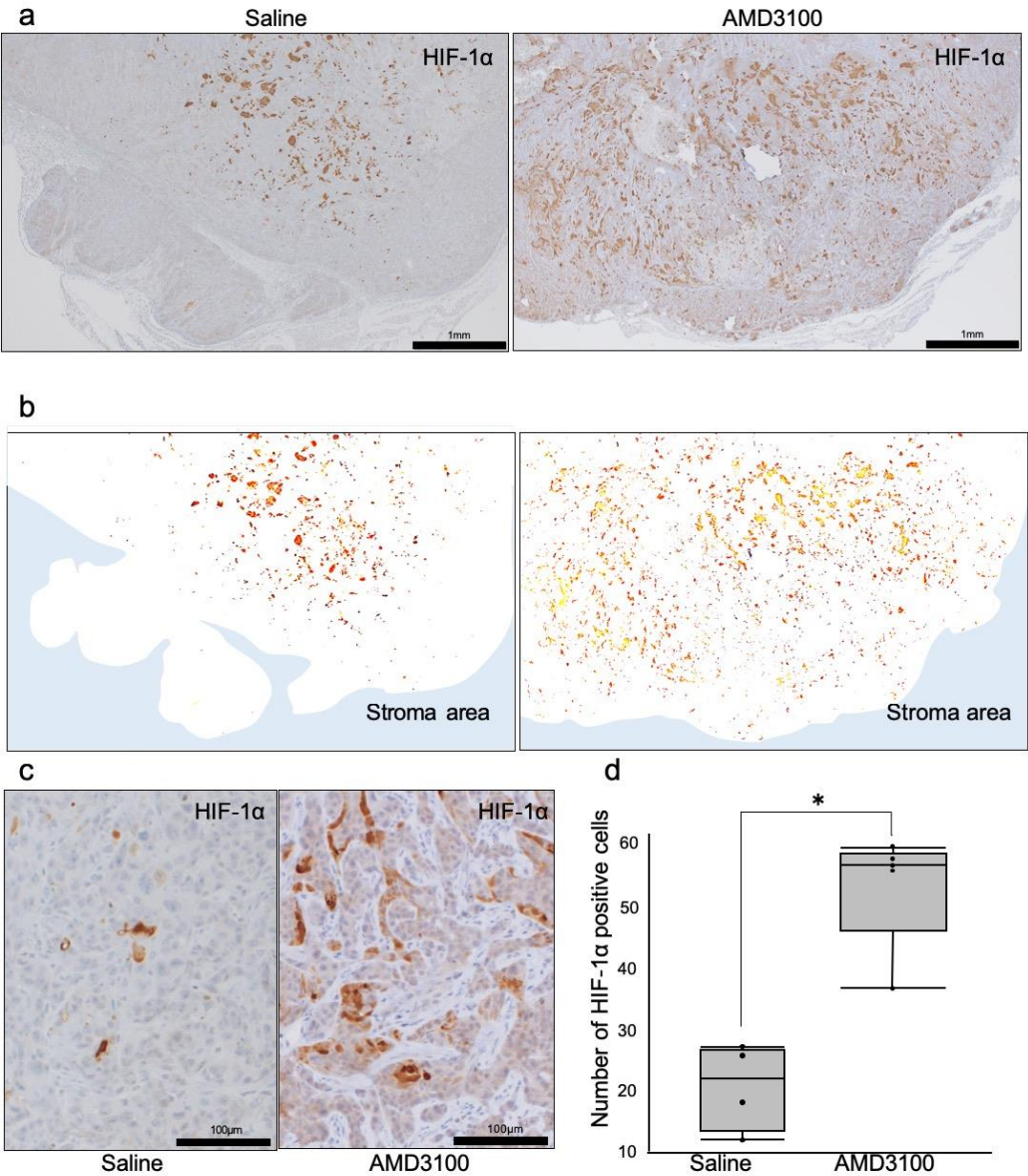


Figure 5. Distribution of hypoxia-inducible factor-1α in tumors treated with or without CXCR4 antagonist AMD3100. (a) IHC for HIF-1α in the tumor area under low-power magnification. (b) Illustration of HIF-1α distribution in tumors. White area, tumor. Blue area, stroma. (c) IHC of HIF-1α under high-power magnification. (d) The number of HIF-1α-positive tumor cells. *P=0.02 N=5.

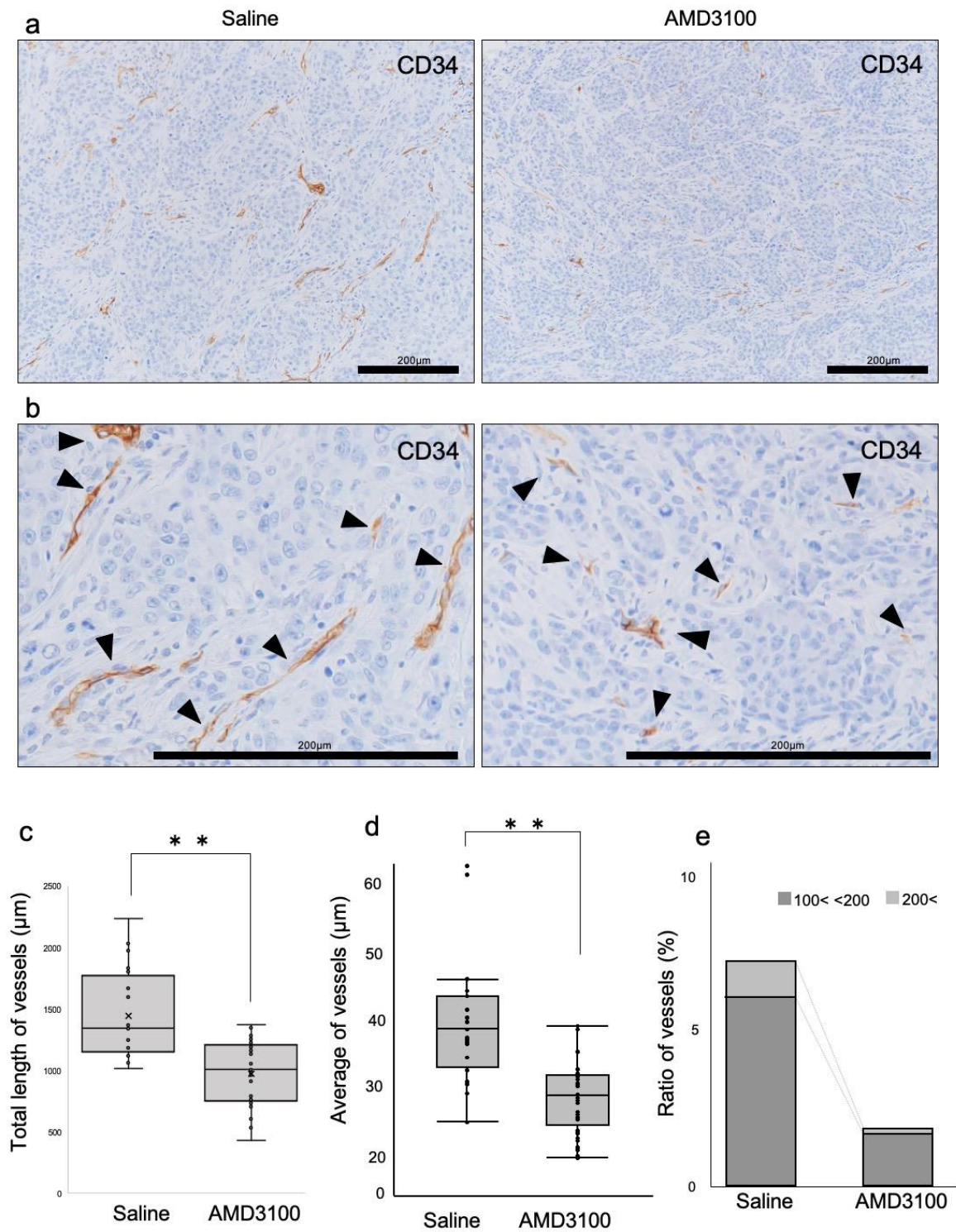


Figure 6. Tumor vessels were disorganized by CXCR4 antagonism. (a) Low-power magnification IHC of CD34. (b) High-power magnification IHC of CD34. (c) The total length of CD34-positive vessels. **P<0.0001, N=25. (d) The average length of CD34-positive vessels. **P<0.0001, N=25. (e) Rate of vessels sized 100~200 μm, and >200 μm.

Table 1: Rate of vessels sized <50 μm, 50~100 μm, 100~200 μm, and >200 μm.

	<50	50≤, <100	100≤, <200	200≤
Saline	76.7%	16.4%	5.8%	1.1%
AMD3100	86.2%	11.9%	1.6%	0.2%

4. Discussion

Our studies revealed that the blood vessels existed in OSCC stroma highly expressed CXCR4 (Fig. 1) and CXCR4 antagonism promoted tumor necrosis in OSCC (Fig. 3). Therefore it was conceivable that CXCR4 highly expressed vessels could support tumor survival by supplying nutrients and oxygen. CXCR4 distributed in the blood vessels in the stroma was thought to be involved in tumor progression and survival, consistent with the absence of CXCR4 in the stromal blood vessels of the non-tumor area (Fig. 2). Consistently, it has been shown that SDF-1/CXCR4 signaling is important in neoangiogenesis as well as infections and immunity [35,36]. Furthermore, the SDF-1/CXCR4 axis plays decisive roles in tumor progression, including the enhancement of migration and invasion, cancer cell–tumor microenvironment interaction, and angiogenesis [37–39]. These studies were consistent with our data evidencing the pro-angiogenic role of CXCR4 in OSCC. Moreover, the participation of the SDF-1/CXCR4 axis in tumor neovascularization through VEGF-dependent and -independent mechanisms have been reported [40]. It was also shown that CXCR4 upregulated VEGF expression through the Akt signaling pathway in breast cancer [40]. In addition, CXCR4 was reported to be upregulated under hypoxic conditions in glioblastoma, via HIF-1 α and VEGF [41]. Several previous studies have demonstrated that HIF-1 α play a crucial role in the expression and regulation of CXCR4 [34,41,42]. It has been reported that hypoxia, particularly HIF-1 α , may promote the expression of CXCR4 and activate SDF-1 α /CXCR4 signaling axis, contributing to tumor cell invasion and metastasis. Although a number of studies have reported HIF-1 to CXCR4 axis, it has not been investigated whether CXCR4 antagonism could alter HIF-1 expression in tumors. Our study indicated that tumor angiogenesis was inhibited by antagonizing CXCR4, although this anti-angiogenesis approach could result in tumor hypoxia inducing HIF-1 α . However, our data indicate that the increases in the HIF-1 α -positive area can be an indicator of tumor hypoxia and TAITN.

It has been shown that AMD3100 acts as a specific antagonist blocking the binding pocket of CXCR4 [43,44]. The first clinical trials with AMD3100 were designed for the treatment of HIV. Notably, an increased amount of white blood cells was observed in healthy volunteers in phase I clinical trials [45]. These findings led to the discovery that AMD3100 mobilizes CD34-positive human hematopoietic stem and progenitor cells from the bone marrow to peripheral blood [46]. AMD3100 was finally approved by the U.S. Food and Drug Administration as a mobilizer of hematopoietic CD34-positive cells from the bone marrow to the circulation [47,48]. However, recent studies applied AMD3100 for cancer treatment [49]. The effective CXCR4-related anti-metastatic therapies may require the daily administration of AMD3100 to continuously prevent the migration of pre-metastatic cells to the metastatic site, which may cause chronic leukocytosis [50]. Our study was performed with the same dose and condition whereby no adverse effect of AMD3100 was observed.

Our data notably define a novel anti-angiogenic pathophysiology so-called TAITN. CXCR4 antagonism disorganized vessel structures and induced necrosis in the tumor xenograft mouse model (Fig. 3,4). Tumor necrosis generally begins at the center of tumor tissue due to intratumoral hypoxia and malnutrition. Such hypoxia and malnutrition occur depending on the tumor-vessel distance, which inhibits neovascularization keeping up with tumor growth [51,52]. In our model, CXCR4 antagonism induced tumor necrosis along with hemorrhage (Fig. 3). The structure of TAITN was particularly found in the CXCR4-antagonized group but not in the control group (Fig. 4). We define TAITN as a pivotal indicator of disorganized branching and angiogenesis of tumor blood vessels. TAITN was induced by CXCR4 antagonism and resulting in hypoxia and enhanced necrosis in tumors.

In this study, suppression of CXCR4 inhibited the formation of tumor blood vessels through reducing the thickness and length of tumor blood vessels (Fig. 6). In addition, CXCR4 expression site in tumor blood vessels was limited to a bifurcation or constricted part of the blood vessels (Fig. 2). These results suggest that CXCR4 is involved in tumor blood vessel angiogenesis and branching. As a proof of concept of TAITN, the inhibition of CXCR4 caused angiogenic inhibition whereby the surrounding tumor tissue was induced with hypoxia and became necrotic. TAITN is a novel distinctive subtype of necrosis inasmuch as marked suppression of tumor vasculature resulted in the

retention of an island-like tumor mass confined by inhibited vessels in the necrotic tissue (Fig. 7). Hence, these data could open up CXCR4-targeted personalized therapeutics targeting CXCR4-hi tumor blood vessels.

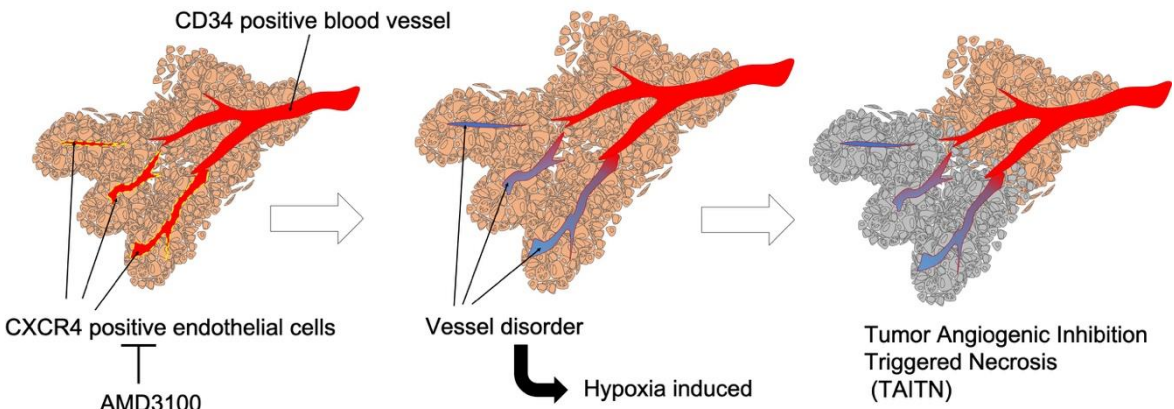


Figure 7. Graphical abstract. Inhibition of CXCR4 induced tumor necrosis through vessel disorder.

5. Conclusions

CXCR4 plays a crucial role in tumor angiogenesis required for OSCC progression, whereby TAITN induced by the CXCR4 antagonist could be an effective anti-angiogenic therapeutic strategy in OSCC treatment.

Author Contributions: conceptualization, H.K.; methodology, H.K., K.N., K.T.; formal analysis, S.S., S.Y.; investigation, S.Y., H.K., M.W.O., C.A.; data curation, S.Y., H.K.; writing—original draft preparation, S.Y., H.K.; visualization, S.Y., H.K.; project administration, H.K.; funding, H.K., S.Y.; resources, H.N., H.K.; discussion and supervision, H.N., K.O., T.E.; writing—reviewing and editing the manuscript, T.E.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CXCR4	C-X-C motif chemokine receptor 4
HIF-1α	Hypoxia-inducible factor 1 alpha
IHC	Immunohistochemistry
OSCC	Oral squamous cell carcinoma
SDF-1	Stem cell-derived factor-1
TAITN	Tumor Angiogenic Inhibition Triggered Necrosis

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