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Review Article

Therapeutic strategies with oral fluoropyrimidine anticancer agent, S-1 against oral cancer☆

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Summary Oral cancer has been recognized as a tumor with low sensitivity to anticancer agents. However, introduction of S-1, an oral cancer agent is improving treatment outcome for patients with oral cancer. In addition, S-1, as a main drug for oral cancer treatment in Japan can be easily available for outpatients. In fact, S-1 exerts high therapeutic effects with acceptable side effects. Moreover, combined chemotherapy with S-1 shows higher efficacy than S-1 alone, and combined chemo-radiotherapy with S-1 exerts remarkable therapeutic effects. Furthermore, we should consider the combined therapy of S-1 and molecular targeting agents right now as these combinations were reportedly useful for oral cancer treatment. Here, we describe our findings related to S-1 that were obtained experimentally and clinically, and favorable therapeutic strategies with S-1 against oral cancer with bibliographic considerations.

Contents

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1. Prolusion

Cancers of the oral cavity accounted for 185,000 cases in 2012, and they were about 2.35 times as more common in men than in women. Also, cancers of the oral cavity is the 12th most common cancer in male and 18th in female in developed countries, and 8th most common cancer in male and 13th in female in developing countries [1]. Although the overall worldwide incidence of oral cancers is gradually decreasing, oral cancers occur in increased frequency in the developing countries compared to the developed countries.

On the other hand, the incidence of oral cancers in Japan is gradually increasing though an accurate nationwide survey on oral cancer has not been conducted. Oral cancers accounted for 2100 cases in 1975 and 6900 cases in 2005. The incidence of oral cancers is expected to continue to increase in step with the aging of the population [2]. Also, the number of head and neck cancer fatalities was 364,872 cases (216,975 in men and 147,897 in women) in 2013 according to the latest survey by National Cancer Center [3]. Above fatalities accounted for 4.3% of all cancer fatalities in Japan, here head and neck cancer ranked as 14th and 18th most common cancer-related fatalities in male and in female respectively. In addition, head and neck cancers have a high percentage (approximately 60%) of advanced cases (stage III and stage IV). Moreover, oral cancer accounts for 35—40% of head and neck cancers, and that approximately 1% of all cancers. Furthermore, oral cancers also have a high percentage of advanced cases [3,4].

Oral cancer is a significant public health problem throughout the world because oral function is very important for breathing, eating and conversation. If oral cancer patients loss their functions by surgical operation, they might suffer from dysfunction all through their life. In spite of recent advances in surgery, radiotherapy, chemotherapy and immunotherapy, the survival rate of oral cancer is unfortunately still below 50% in the advanced stage in Japan [5,6]. Anticancer agents, e.g. 5-fluorouracil (5-FU), Cisplatin (CDDP), Docetaxel (Doc), or Paclitaxel (PXT) have been available for treatment of oral cancer patients in Japan. Therefore, combined therapies with those anticancer agents have been tested for increasing their therapeutic effects [7—10]. In addition, we can currently have new options to choose molecular targeting agent, e.g. Cetuximab (C-mab) in the case of locally advanced, recurrent, or metastatic head and neck cancer [11,12]. However, it was reported that combined therapies of above anticancer agents showed gastrointestinal side effects or myelosuppression markedly. Even though molecular targeting agent has few side effects than anticancer agents, it can develop severe cutaneous manifestation. Briefly, systemic chemotherapy is not often suitable for patients of advanced age or with complications. The enlarged operation may also be inappropriate for those patients. In any case, to improve the prognosis of patients with oral cancer, the development of new, effective chemotherapeutic agents has been expected. S-1 (TS-1®) was approved for the treatment of head and neck cancers on April 2001 under these circumstances as it has high therapeutic effects but low side effects [13].

2. Transition of fluoropyrimidine related anticancer agents

After discovery of 5-FU by Heidelberger in 1957, 5-FU has been used for nearly 60 years as a basic medicine for various cancers [14]. Mechanisms of 5-FU action have been studied intensively in this period, and Giller et al. artificially synthesized tegafur (FT) as the first 5-FU prodrug in 1967 [15]. Fujii et al. could develop oral 5-FU prodrug tegafur/uracil (UFT®) by using this FT in 1976 [16]. Since UFT was approved for marketing in 1984, it has been in heavy usage for cancers of the digestive organs including oral cancer in Japan. Moreover, UFT was applied in a clinical setting worldwide in the 90s, and it was currently approved in more than 60 countries worldwide.

In these years, S-1, a new oral fluoropyrimidine anticancer agent developed by Shirasaka et al. is compared favorably with UFT [13,17]. S-1 was based on the theory of biochemical modulation of 5-FU. S-1 showed the highest response rate among many oral anticancer agents against unresectable advanced carcinomas in phase II studies [18]. S-1 is a novel orally administered anticancer drug that is a combination of tegafur (FT) [15], 5-chloro-2, 4-dihydroxypyridine (CDHP) [19], and oteracil potassium (Oxo) [20] in a 1:0.4:1 molar concentration ratio (Fig. 1). CDHP is a competitive inhibitor of dihydropyrimidine dehydrogenase (DPD), which is involved in the degradation of 5-FU, and

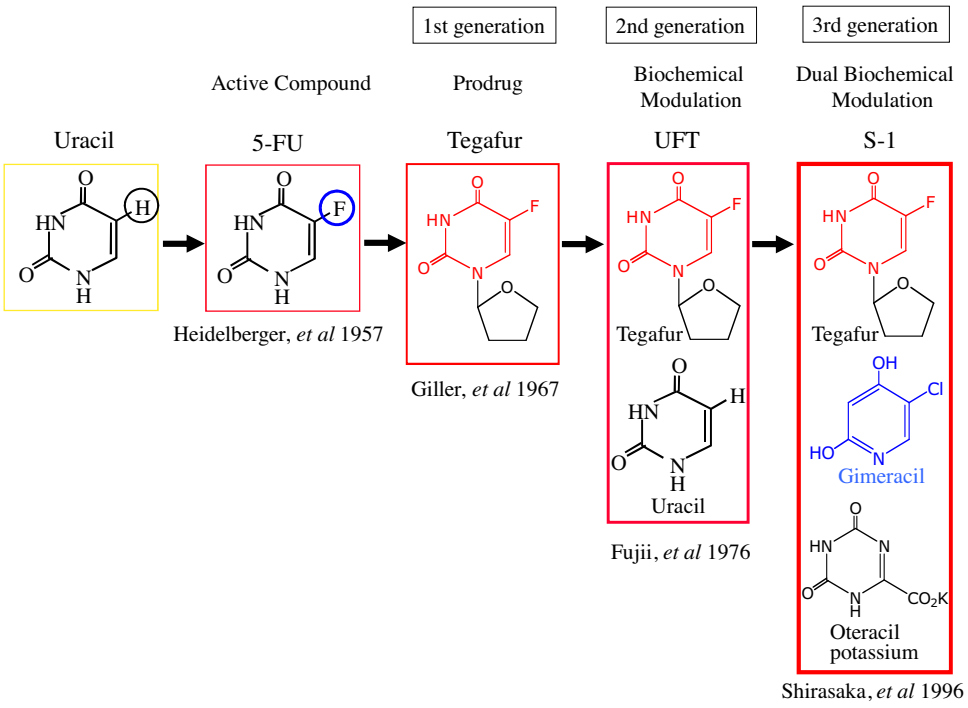

Figure 1 Transition of fluoropyrimidine related anticancer agents.
Heidelberger et al. discovered 5-FU in 1957, and Giller et al. artificially synthesized tegafur (FT) as the first 5-FU prodrug in 1967. In addition, Fujii et al. developed oral 5-FU prodrug tegafur/uracil (UFT®) by using FT in 1976. Moreover, Shirasaka et al. developed a better agent, S-1 in 1996. As policy of S-1 was based on the theory of biochemical modulation of 5-FU, S-1 can maintain efficacious concentrations of 5-FU in plasma by gimeracil, and can reduce the serious gastrointestinal toxicity associated with 5-FU by oteracil potassium.

acts to maintain efficacious concentrations of 5-FU in plasma and tumor tissues. Oxo, a competitive inhibitor of orotate phosphoribosyl transferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract, reducing the serious gastrointestinal toxicity associated with 5-FU. Briefly, S-1 is well thought out agent capable enhancing the anticancer effects while suppressing adverse events.

S-1 showed a high response rate of 44.6% in a phase II trial of gastric cancer as a single agent therapy. In fact, conventional agents have never reached the above high response rate. Therefore, S-1 has been a main medicine for chemotherapy of gastric cancers [21]. Also, its response rates for advanced and recurrent head and neck cancer in the early phase II trial conducted in Japan were 46.2% [22], and S-1 showed a high response rate of 28.8% with acceptable toxicities in the late phase II trial of advanced and recurrent head and neck cancer (59 eligible cases) [23]. This response rate of S-1 is thought to be extremely high because continuous infusion of 5-FU single agent showed a response rate of 13—15% in advanced and recurrent head and neck cancers [24,25]. In the case of other intravenous anticancer drugs against head and neck cancer, the response rates of CDDP [26], carboplatin [27], nedaplatin [28,29] and DOC [30] were 26.0, 20.0, 37.5—39.7, and 22.2%, respectively. Briefly, S-1 has utility similar to other intravenous anticancer agents despite being an oral drug. Chemotherapy plays an important role in patients with advanced oral cancer because oral cancer treatment requires functional preservation and conservation of sensuousness as well as improvement of survival rate than other malignant tumors. S-1 is thought to have been one of main medicines for chemotherapy for oral cancers until now because it shows high therapeutic efficacy and can be prescribed for outpatients.

3. Investigation of mechanisms of S-1 to exert antitumor effect on oral cancer

3.1. Time-dependent effects

The main component of S-1 that exerts antitumor effects on tumor cells is tegafur (FT) which is the prodrug of 5-FU [13—15], and 5-FU is a time-dependent anticancer agent [31—34]. Therefore, the mechanism of action of S-1 could be similar to 5-FU, and also S-1 is thought to show efficacy time-dependently. Skipper et al. could identify the metabolic pathway of 5-FU that somewhat explains how 5-FU exerts antitumor effects (Fig. 2) [35]. Briefly, 5-FU is assimilated and metabolized to 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP) i.e., [(5-FU → FUrd → 5-fluorouridine 5′-monophosphate (FUMP) → 5-fluorouridine 5′-diphosphate (FUDP) → 5-fluoro-2′-deoxyuridine 5′-diphosphate (FdUDP) → (FdUMP)], and FdUMP forms ternary complex [5,10-5,10-methylentetrahydrofolate (CH_2FH_4)-FdUMP-TS] with thymidylate synthase (TS). Interrupting the action of TS through the ternary complex blocks synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication. On the other hand, 5-FU is metabolized to (F)RNA i.e., (5-FU → FUrd → FUMP → FUDP → FUDP → 5-

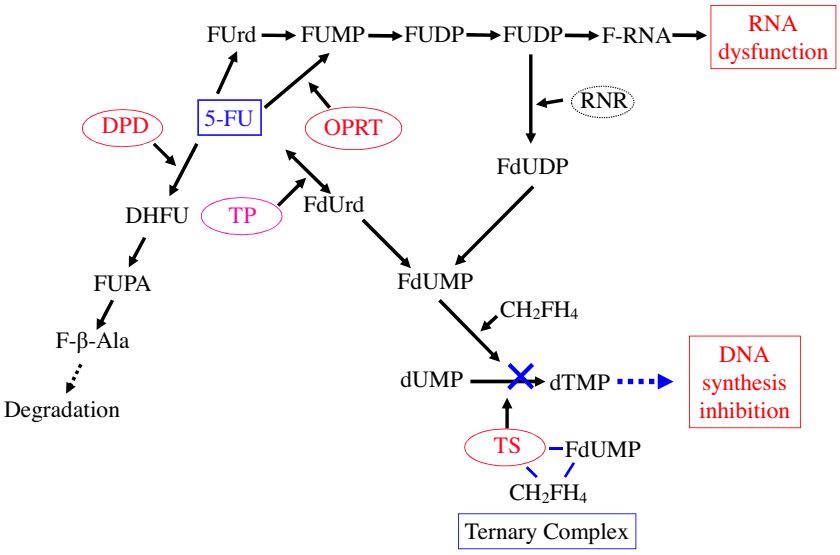

Figure 2 The metabolic pathway of 5-FU and the molecular mechanisms of S-1 that exert antitumor effects.
One of the metabolic pathways of 5-FU begins with the process of phosphorylation by OPRT and further metabolized into (F)RNA. Then, (F)RNA is recognized as an abnormal RNA metabolism, which causes RNA dysfunction. Another process that causes DNA synthesis inhibition starts with phosphorylation of 5-FU by OPRT, then 5-FU is metabolized to FdUMP that forms ternary complex with TS. Also, 5-FU is phosphorylated to FdUrd by TP, TP reversibly converts FdUrd into 5-FU and is involved in the angiogenesis. In addition, DPD can degrade 5-FU in the liver. OPRT, Orotate phosphoribosyl transferase; FUMP, 5-fluorouridine 5′-monophosphate; FUDP, 5-fluorouridine 5′-diphosphate; FUTP, 5-fluorouridine 5′-triphosphate; FdUDP, 5-fluoro-2′-deoxyuridine 5′-diphosphate; FdUMP, 5-fluoro-2′-deoxyuridine 5′-monophosphate; TS, thymidylate synthase; CH_2FH_4, 5,10-methylentetrahydrofolate; DPD, dihydropyrimidine dehydrogenase; TP, Thymidine phosphorylase; FdUrd, 5-fluoro-2′-deoxyuridine.

fluorouridine 5′-triphosphate (FUTP) → (F)RNA). (F)RNA is recognized as an abnormal RNA metabolism, which leads to cause RNA dysfunction. Hence, 5-FU is thought to exert cytotoxicity by DNA synthesis inhibition or RNA dysfunction (Fig. 2). Also, high concentration of 5-FU (1.3—13 μg/ml) is required for induction of RNA dysfunction, whereas low concentration of 5-FU (0.065—0.13 μg/ml) is enough to inhibit DNA synthesis, conversely [36,37]. Therefore, bolus administration of 5-FU raises the circulating concentration of 5-FU transiently, which leads to exert antitumor effects by RNA dysfunction. Inversely, continuous infusion of low dose of 5-FU and S-1 mainly thought to exert antitumor effects by DNA synthesis inhibition [38]. Interestingly, it was reported that continuous infusion of 5-FU for more than 4 weeks have more antitumor effects than bolus administration of 5-FU in these years [39]. Further investigations have revealed that an important factor affecting the therapeutic effect of 5-FU was not dosage amount but cancer cell contact time, as 5-FU efficacy is time-dependent [31—34]. Even high does of 5-FU cannot exert therapeutic effect if 5-FU contacts cancer cells briefly. On the other hand, 5-FU exerts a more potent antitumor effect even if low concentration of 5-FU can contact cancer cells for a long time. In this regard, daily oral administration of S-1 is thought to maintain effective blood concentration of 5-FU easily for long periods.

3.2. Anti-angiogenic property

In order to grow beyond a certain size of tumoral mass under active tumor cell proliferation, blood vessels play a fundamental physiological role in supplying nutrients and oxygen, removing catabolic waste, and circulating cells for immune surveillance. Briefly, Folkman proposed that the growth of solid tumors depends on angiogenesis, and experimental verification has been continued in the past [40]. Also, the basement membrane of formed neovascular vessels is immature, permeable and fragile, which must lead to advance intravasation of tumor cells. In addition, increase in the number of tumor vessels is thought to increase the frequency of metastasis because it is easy for a tumor cell to migrate into the vascular space [41]. Therefore, we can expect to starve cancer cells into surrender by inhibiting feeding vessel of tumor mass in oral cancer treatment if we can inhibit tumor angiogenesis, which is thought to lead to suppress metastasis.

Among the various factors which have been identified as tumor angiogenesis-stimulating factors, vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) are extensively involved in growth, differentiation and migration of vascular endothelial cells, and are directly associated with tumor angiogenesis [42,43]. It has been reported that NF-κB controls VEGF and FGF-2 transcription, and Akt stimulates NF-κB activation [44—46]. Briefly, inhibition of p-Akt can induce suppression of NF-κB activation, which may lead to inhibition of VEGF and FGF-2 production [47]. We have clarified that S-1 exerts anti-angiogenic effects on human oral squamous cell carcinoma (OSCC) cells by suppressing expression of VEGF and FGF2 via inhibition of NF-κB activity, and also by inhibiting p-Akt overexpression in nude mouse tumor, which might lead to antiangiogenesis in tumor cells [47].

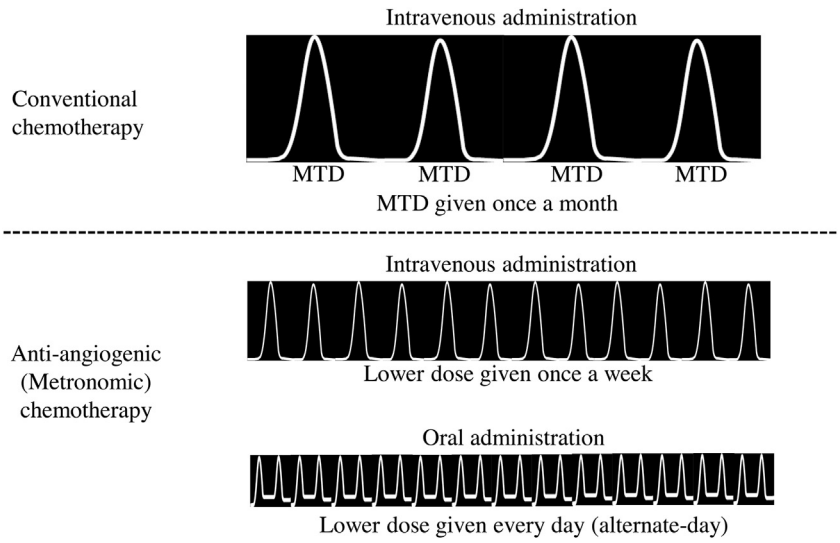

Figure 3 Schedule of cancer chemotherapy.
Maximum tolerable dose (MTD) is administered about once a month in conventional chemotherapy. On the other hand, low-dose than MTD is administered at a close regular interval with no prolonged breaks in metronomic chemotherapy in order to inhibit vascular endothelial cells as well as tumor cells. In addition, metronomic chemotherapy can inhibit feeding vessels of tumor mass, and may lead to starve cancer cells into surrender even when we do not think that the chemotherapeutic agent is effective on the tumor cells.

Metronomic chemotherapy has attracted attention over the years. In contrast to the conventional maximum tolerable dose (MTD) chemotherapy with prolonged breaks, metronomic chemotherapy refers to low-dose chemotherapy administered at a close regular intervals [48] (Fig. 3). This therapy is often compared to metronome, which constantly beats out a rhythm with regularity. Moreover, Kerbel and Folkman reported separately that metronomic chemotherapy can act as an anti-angiogenic therapy almost at the same time. So, metronomic chemotherapy is also called Kerbel—Folkman therapy [48—50]. The rationale for metronomic chemotherapy are as follows: (a) Rapidly multiplying or dividing vascular endothelial cells are sensitive to a variety of chemotherapeutic agents, (b) As host vascular endothelial cells rarely to develop tolerance to chemotherapeutic agents, vascular endothelial cells can continue to be sensitive to the chemotherapeutic agent even if it is ineffective for tumor cells [51]. Briefly, the targets of metronomic chemotherapy are vascular endothelial cells as well as tumor cells. So, metronomic chemotherapy can inhibit feeding vessels of a tumor mass, which may lead to starve cancer cells even when we do not think that the chemotherapeutic agent is not effective on the tumor cells. Especially, Kerbel et al. have an assumption that daily oral administration available UFT or cyclophosphamide (Endoxan®) may fit with the concept of metronomic chemotherapy. In fact, metronomic UFT administration was effective in breast [52,53], gastrointestinal [54], ovarian cancer [55] and lung adenocarcinoma [56]. S-1 also seems to have the potential to fit with the concept of metronomic chemotherapy.

Up-regulation of pro-angiogenic factors and down-regulation of anti-angiogenic factors are essential to promote tumor angiogenesis. As noted above, VEGF and FGF2 are important as a pro-angiogenic factor. On the other hand, a glycoprotein of extracellular matrix family, thrombospondin-1 (TSP-1) is also important as an anti-angiogenic factor. TSP-1 inhibits endothelial cell growth and promotes endothelial cell apoptosis through the intermediary of attaching oneself to the receptor (CD36), which is expressed on vascular endothelial cells [57]. Briefly, up-regulation of VEGF and FGF2, and down-regulation of TSP-1 are considered important for tumor angiogenesis. Therefore, we administered S-1 to oral cancer-bearing nude mice daily, and investigated whether S-1 impact on TSP-1 expression over time. As a result, we clarified S-1 could exert anti-angiogenic effects via up-regulation of TSP-1. Moreover, we also demonstrated that alternate-day administration of S-1 is more useful than consecutive-day administration in terms of anti-angiogenic effects [58]. Our findings suggested that S-1 might fit with the concept of metronomic chemotherapy in oral cancer treatment [58,59] (Fig. 4). Interference with tumor angiogenesis is very important to improve therapeutic effects on oral cancer because oral cancer has high angiogenic capacity [60]. Hence, S-1 is thought to play a crucial role in oral cancer treatment.

3.3. Enhancement of apoptosis and suppression of survival signal

A balance between apoptotic signal and survival signal controls whether cancer cells are alive or dead. Suppression of survival signal as well as promotion of apoptosis is extremely important for efficacious cytocidal effect. Conventional cancer therapy may have focused exclusively on enhancement of apoptosis and ignored suppression of survival signal. Apoptosis is induced via two pathways. One is receptor-mediated pathway and the other is mitochondria-mediated pathway. Caspases play an important role in both pathways. Caspase-8 is activated upon reception of a signal from death receptors. Caspase-9 is activated by cytochrome c released

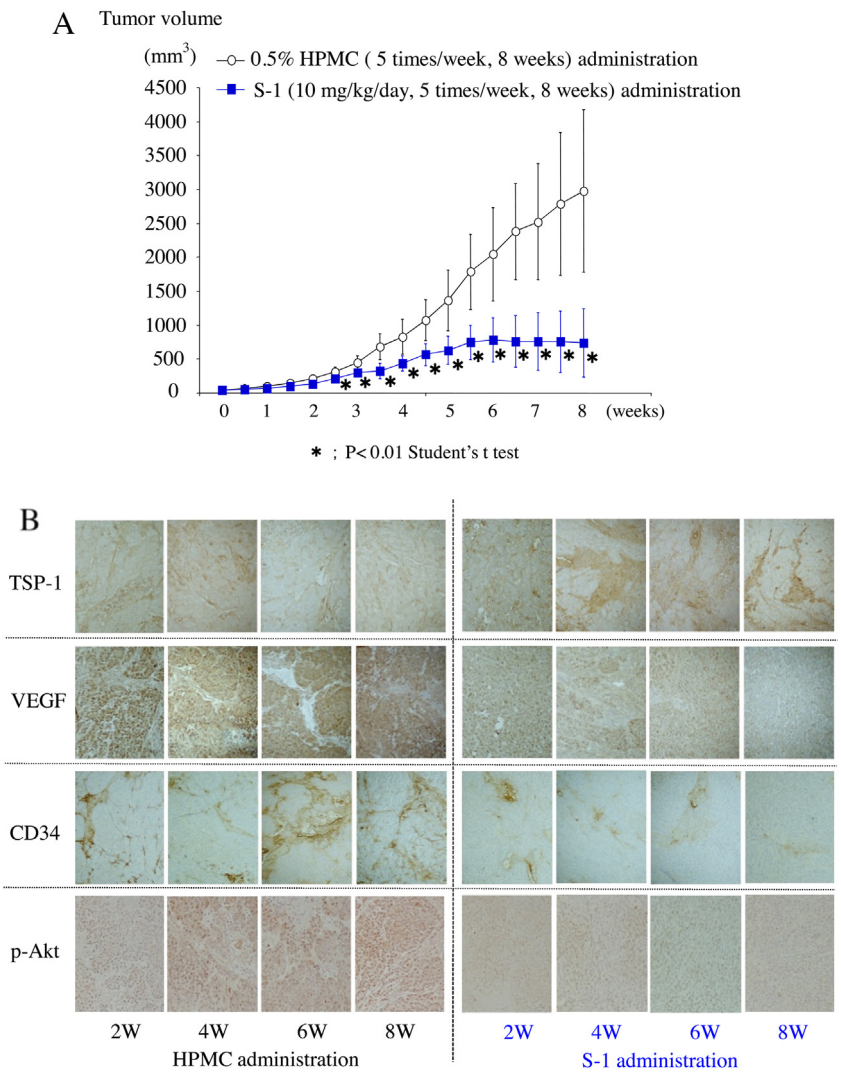

Figure 4 Anti-angiogenic property and suppression of survival signal.
(A) Tumor bearing nude mice was treated orally either with 10.0 mg/kg S-1 five times a week for 8 weeks as the treatment group, or with 0.5% sodium hydroxypropylmethylcellulose (HPMC) five times a week for 8 weeks as the control group. The growth inhibition of S-1 treated tumors was statistically significant when compared to that of HPMC administered tumors. (B) The expression level of VEGF was down-regulated in S-1 treated tumors after the first two week of administration, though VEGF expression was high and was fairly constant over time in HPMC administered tumors. In addition, the expression level of Thrombospondin 1 (TSP-1) was enhanced in S-1 treated tumors after the first four week of administration, although time-dependent change of TSP-1 expression was not that much prominent in HPMC administered tumors. Moreover, the expression level of CD34 and p-Akt was reduced in S-1 treated tumors after the first two week of administration, whereas CD34 and p-Akt expressions were high in HPMC administered tumors.

from mitochondria. Each caspase-8 and caspase-9 communicates a signal to caspase-3 lying downstream of caspase-8 and caspase-9, and then apoptosis is induced [61]. Interestingly, 5-FU is thought to induce caspase-8 and caspase-9 as well as caspase-3 activity, and that eventually induce apoptosis in oral cancer cells [62,63].

Akt/PKB lies downstream of PI3 kinase and plays an important role for apoptosis regulation. Akt/PKB is a serin/threonine kinase, and it is activated to promote the survival of cells [64,65]. Briefly, Akt/PKB is one of the survival signals. It is revealed that Akt/PKB regulates activation of caspases by two-step process, which includes suppression of cytochrome c release from mitochondria into cytoplasm and suppression of caspases activation after cytochrome c release [66]. Therefore, we investigated whether S-1 influence activated form of Akt/PKB (p-Akt) expression. Thus, we clarified S-1 could decrease p-Akt expression (Fig. 5) [67]. Hence, S-1 is thought to enhance anti-tumor efficacy effectively by suppression of survival signal p-Akt expression as well as promotion of apoptotic signal.

3.4. Radiosensitization efficacy

S-1 is thought to have superior radiosensitization efficacy from therapeutic effects of concurrent chemoradiotherapy with S-1 against oral cancer [68]. It is well known fact that

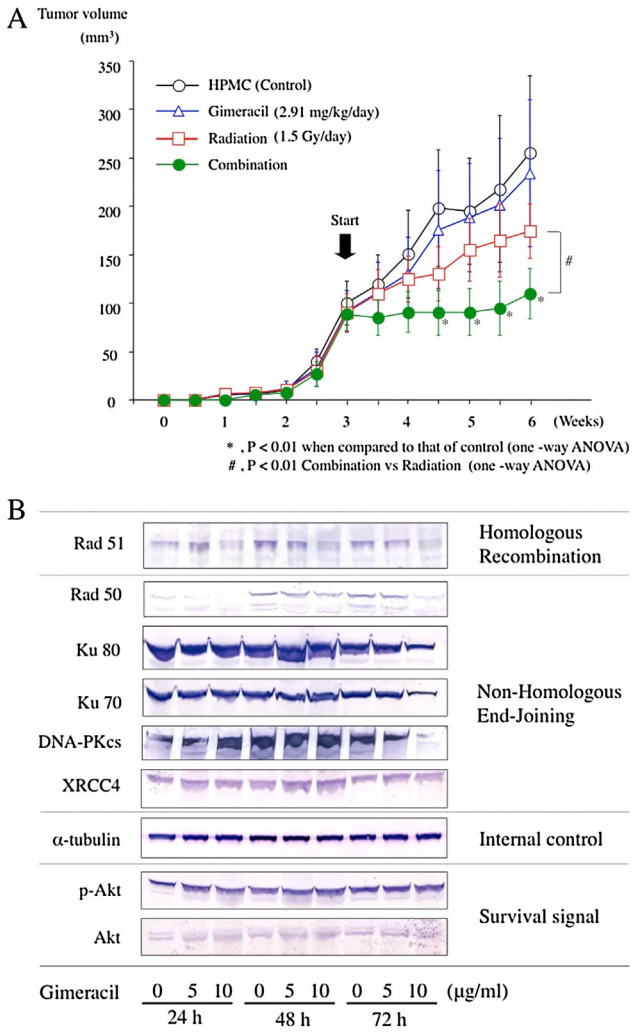

Figure 5 Radiosensitization efficacy of Gimeracil.
(A) One of the component of S-1, Gimeracil or CDHP hardly exerts antitumor effects at all. However, CDHP in combination with radiation exerted a pronounced antitumor effect. (B) CDHP may exert radiosensitization effects by suppression of DNA double strand break repair systems (non-homologous end joining and homologous recombination), and exerts little influence on survival signals.

5-FU has radiosensitization effect [69]. Therefore, chemoradiotherapy with 5-FU has been clinically used for a long time [70—72]. S-1 is thought to act as a radiosensitizer similar to 5-FU as FT, a precursor of 5-FU is one of the components of S-1. In addition, it is reported that 5-FU degradation delays in irradiated cells [73]. As S-1 contains CDHP as the component to slow down the degradation of 5-FU, irradiation may delay 5-FU degradation in S-1-treated cells. Also, CDHP has the potential to exert radiosensitization effects. CDHP is an inhibitor of pyrimidine-metabolizing enzyme, DPD which is responsible for degrading of 5-FU. Moreover, DPD, an enzyme reacts with pyrimidine structures during nucleic-acid metabolism and inhibition of DPD may cause disorder of nucleic-acid metabolism. Briefly, nucleolytic degradation is caused effectively by irradiation due to the presence of CDHP, which may lead to enhance cytocidal effects.

Cell death by irradiation includes mitotic death and interphase death. Mitotic death means cell death with a few cell divisions after irradiation. Briefly, it is caused by loss of infinite proliferative capacity after irradiation. On the other hand, interphase death means direct cell death without cell divisions by irradiation. In addition, it is thought that interphase death is induced by small dose irradiation. Clonogenic assay is frequently used to understand a dose—effect relationship based on interphase death as a quantification method of cell radiosensitivity. It is relatively easy to understand radiosensitization efficacy by using this method. So, we clarified radiosensitization efficacy of S-1 on oral cancer cells using clonogenic assay [74].

Moreover, we investigated radiosensitization effect of CDHP on oral cancer cells in the same way, and demonstrated CDHP could impair the colony forming capacity. Briefly, one of component of S-1, CDHP also has a potential to exert radiosensitization efficacy. Interestingly, it has reported that CDHP may exert radiosensitization effects in various cancer cells by suppression of homologous recombination which is one of DNA double strand break repair systems [75,76]. Radiotherapy with CDHP as well as concurrent chemoradiotherapy with S-1 could exert remarkable anti-tumor effects in our investigation with xenografted mice. Our findings suggested that CDHP might suppress not only non-homologous end joining but also homologous recombination [77] (Fig. 5). As disorder of non-homologous end joining is often seen in oral cancer cells, radiation may have a striking effect on those types of cancer cells. If we can select the responder, radiotherapy with CDHP will be applied in a clinical setting in the future. The component of S-1, CDHP may play an important role in radiotherapy beyond expectation.

In respect of sequential treatment, 5-FU treatment before radiation was more effective than 5-FU treatment after radiation on growth inhibition of oral cancer cells. In addition, cells that received 5-FU treatment before radiation showed significantly lower colony forming capacity than 5-FU treatment after radiation. Moreover, S-1 administration before radiation was more effective than S-1 administration after radiation on growth inhibition of xenograft tumors. Furthermore, S-1 administration before radiation significantly induced apoptosis compared to S-1 administration after radiation [74,78]. Briefly, we should consider maximum drug concentration time, and administer S-1 to patients with oral cancer before radiation. It is thought that we should expose the oral cancer patients to radiation at the same time to reach maximum drug concentration for maximal therapeutic response. This time lag may ensure concurrent chemoradiotherapy with S-1. Combined therapy of radiation and anticancer agent includes neo-adjuvant therapy, adjuvant therapy, concurrent therapy, and alternative therapy thought each therapy has both merits and demerits. However, concurrent therapy is thought to be most suitable for combined therapy of S-1 and radiation because it can exert maximum antitumor effects on radiation site.

3.5. CDDP potentiation

CDDP as well as 5-FU has been one of the main drug for oral cancer treatment, and various combined chemother-

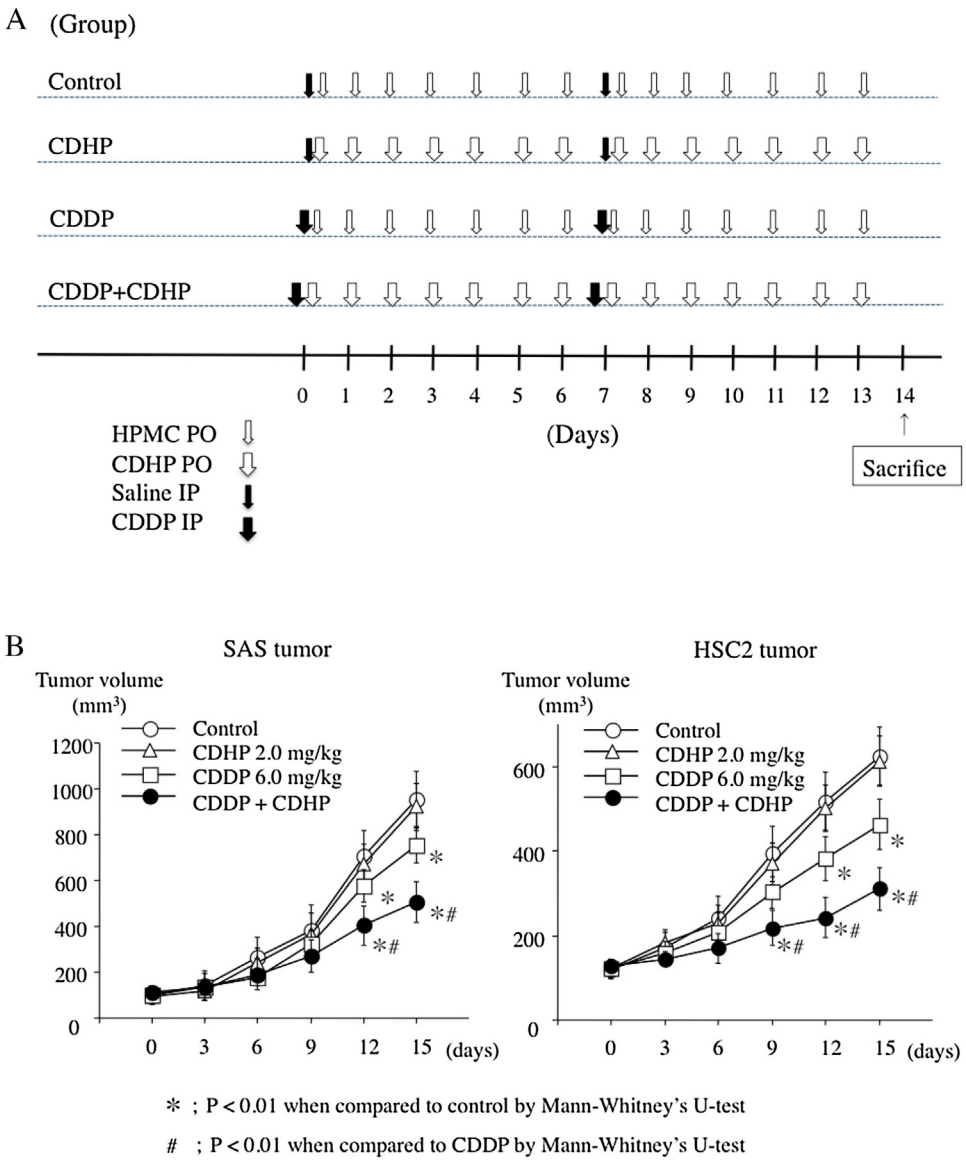

Figure 6 CDDP potentiation by Gimeracil.
(A) We treated xenografted oral tumors with CDHP and/or CDDP. (B) CDHP in combination with CDDP exerted remarkable antitumor effect though CDHP alone hardly exerts antitumor effects at all.

apy with CDDP has been tested against oral cancer. In multidrug chemotherapy, CDDP in combination with 5-FU are often used for the therapy of head and neck cancer including OSCC [79]. However, combined chemotherapy of CDDP and S-1 is used instead of combined chemotherapy of CDDP and 5-FU (CF therapy) to shorten the hospital stay, or to avoid continuous infusion therapy in these years [80,81]. In addition, CF therapy can reduce the expression level of a CDDP resistant factor, glutathione S-transferase π (GSTπ), 5-FU resistant factors, i.e., thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and multidrug resistance-associated protein (MRP) than each agent alone as a mechanism of exerting antitumor effects [82]. Moreover, fractionated administration of low dose CDDP can also enhance the inhibition of DNA synthesis by acceleration of ternary complex (5,10-CH_2FH_4-FdUMP-TS) formation, which leads to increase 5-FU efficacy [83]. Therefore, it is thought that combined chemotherapy of CDDP and S-1 as well as CF therapy exerts synergistic effects in the same mechanism. Recently, we have found that CDHP may enhance CDDP efficacy by suppression of DNA double strand break repair systems [84] (Fig. 6). Also, CDDP in combination with CDHP could exert remarkable antitumor effects in our investigation with the use of xenografts [84] (Fig. 6). Our findings suggested that CDHP might have the potential to draw attention as CDDP sensitizer because CDDP is thought to have lower risk of adverse events.

4. Advantage for adjuvant chemotherapy with S-1

Although the usability of concurrent chemo-radiotherapy after radical surgery against head and neck cancer is gradu-

ally recognized, the advantage for adjuvant chemotherapy against head and neck cancer remains to be clarified [85]. Tsukuda et al. have reported that adjuvant chemotherapy with UFT for one year might suppress the distant metastasis after radical surgery of head and neck carcinoma in a prospective randomized trial [86]. In addition, Kubota et al. have reported that adjuvant chemotherapy with nedaplatin and UFT might improve the loco-regional control rates after concurrent chemoradiotherapy for locally advanced head and neck squamous cell carcinoma [87]. These reports suggest that UFT may be useful for adjuvant chemotherapy against head and neck cancer. Interestingly, Tsukahara et al. have reported that overall survival (OS) was significantly better in the S-1 group than in the UFT group in randomized phase III trial of adjuvant chemotherapy with S-1 after curative treatment in patients with squamous-cell carcinoma of the head and neck (ACTS-HNC) though disease free survival (DFS) did not differ significantly between the groups [88]. Briefly, this clinical trial demonstrates that S-1 may be superior to UFT for adjuvant chemotherapy against advanced head and neck cancer. Hence, S-1 must be one of treatment options after curative therapy for patients with squamous-cell carcinoma of the head and neck.

5. Investigation of S-1 regimen

At first, the four-week administration followed by the two-week rest was recommended as a S-1 regimen because post-marketing survey of S-1 in 3808 gastric cancer patients revealed that median nadir of myelosuppression was 22 days, and of diarrhea or mucositis was 15 days [89]. On the other hand, two-week administration followed by one-week rest also attracted attention because this schedule can rest administration of S-1 duration of the adverse event. Interestingly, Tsukuda et al. conducted a controlled randomized multi-institutional study to define an adequate administration schedule in adjuvant chemotherapy with S-1 for advanced head and neck carcinoma. They clarified that the two-week administration followed by one-week rest was superior to the four-week administration followed by the two-week rest in terms of safety and efficacy [90]. Recently, Arai et al. reported the alternate-day administration of S-1 more useful than consecutive-day administration in gastric cancer cell lines in vitro and in vivo [91]. Turnover of neutrophils are faster than that of tumor cells. So, neutrophils may be able to recover from the damage caused by S-1 after cessation of the drug for one day. However, tumor cells may continue to be damaged by S-1 after cessation of the drug even after the first day of administration. Briefly, alternate-day administration of S-1 may be the method aimed at improving further compliance than the two-week administration followed by one-week rest of S-1.

Also, we have reported that alternate days treatment regimen with S-1 was more useful than four-week treatment and two-week rest or two-week treatment and one-week rest regimen with S-1 as a metronomic chemotherapy [58] (Fig. 7). Briefly, we treated xenograft oral tumors with three different regimens with S-1, given on the four-week treatment and two-week rest, the two-week treatment and one-week rest, or alternate days treatment. The relative tumor growth inhibition was not different between three treated groups. However, body weights were lower in mice with the four-week treatment and two-week rest or the two-week treatment and one-week rest than alternate days treatment during treatment periods. Moreover, reduction of microvessel density, and induction of TSP-1 expression was markedly seen in alternate days treated tumors than in the four-week treatment and two-week rest, the two-week treatment and one-week rest treated tumors. As alternate days treatment of S-1 could exert remarkable anti-angiogenic effects via up-regulation of TSP-1, we believe S-1 may fit with the concept of metronomic chemotherapy in oral cancer treatment [58] (Fig. 7). However, the problem is that whether oral cancer patients can take medication as alternate days treatment regimen in the clinical settings or not.

6. Prediction of S-1 efficacy in oral cancer treatment

TS, DPD, Thymidine phosphorylase (TP), and Orotate phosphoribosyl transferase (OPRT) have attracted attention as predictive factors of 5-FU-related anticancer drug [92]. As described above, continuous infusion of low dose of 5-FU and S-1 is mainly thought to exert antitumor effects by DNA synthesis inhibition [37,38]. This DNA synthesis inhibition is caused by formation of ternary complex (5,10-CH_2FH_4-FdUMP-TS), which leads to interrupting the action of TS. Moreover, inhibition of TS leads to an insufficient supply of dTMP that is essential for DNA synthesis. Therefore, it is thought that the lower expression of TS makes the action of TS interrupted, which leads to an efficiency of 5-FU. Various investigations regarding TS interruption in collected tumors after administration of 5-FU-related anticancer drugs have been conduced, and some researchers reported that inhibitory effect of TS is correlated with an antitumor efficiency [93—96]. However, others reported that therapeutic effect of 5-FU is not always correlated with the inhibitory effect of TS [97—99]. Recently, it has been reported that the expression of TS is directly correlated with malignancy, 5-FU efficacy in digestive organ cancers and prognosis of the disease [100]. On the other hand, several conflicting data have also been reported [97]. So, further investigations must be needed. It is to be noted that uncomplexed TS as a ternary complex (free TS) reduces efficacy of 5-FU-related anticancer drugs. Unfortunately, immunohistochemistry with paraffin sections, PCR with frozen materials, and microarray analysis detects FdUMP-bound TS as well as free TS, which might be the reason of the experimental results showing inverse association between TS expression and efficacy of 5-FU-related anticancer drugs.

DPD, a rate-limiting enzyme in the 5-FU degradation pathway is known as an inactivator of 5-FU. Beck et al. reported that DPD activity in human cultured cells is correlated with sensitivity to 5-FU [101]. Etienne et al. also reported that DPD activity in tumors of head and neck cancer patients might be a predictive factor for chemotherapy with 5-FU [102]. According to above studies, the significance of DPD activity in tumors attracts attention because the lower expression of DPD makes the degradation of 5-FU interrupt, which leads to an efficiency of 5-FU. S-1 may be able to exert antitumor effects against tumors with high DPD activ-

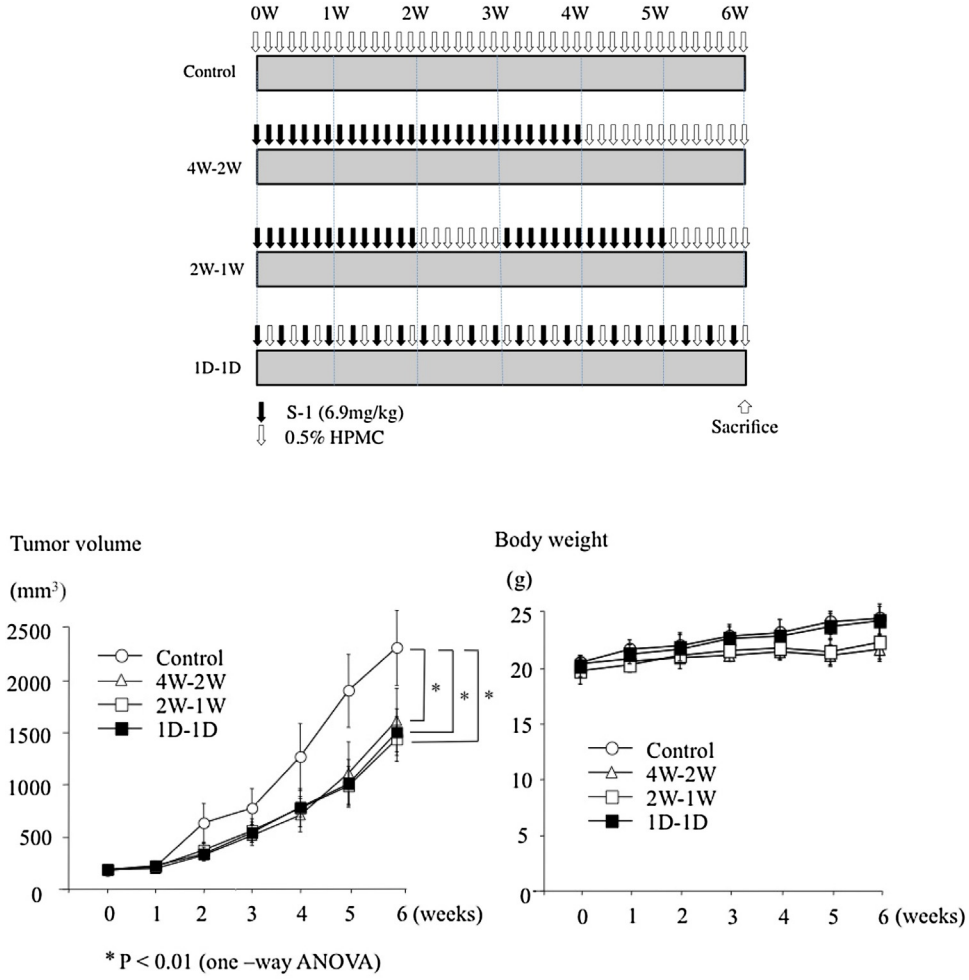

Figure 7 Investigation of S-1 regimen.
(A) We treated xenografted oral tumors with three different regimens with S-1: the four-week treatment and two-week rest (1 cycle), the two-week treatment and one-week rest (2 cycles), or alternate days treatment (6 weeks). (B) Relative tumor growth inhibition was not significantly different between the treated groups. However, body weights were lower in the mice with the four-week treatment and two-week rest or the two-week treatment and one-week rest, than alternate days treatment group.

ity as S-1 includes CDHP of a DPD inhibitor as a component. Briefly, S-1 was developed on the basis of above biochemical modulation theory. S-1 may be effective on 5-FU-resistant tumors. Hence, we have to investigate whether DPD can be a predictive factor for S-1 actively.

TP is an enzyme involved in pyrimidine or nucleotide metabolism. 5-FU isphosphorylated to FdUrd by TP, and TP can act reversibly. So, it is thought that the level of TP expression does not always lead to an increased efficacy of 5-FU. However, it has been thought that enzyme activity of TP is high in tumors as well as in liver and gastrointestinal tract. In addition, TP becomes involved in metabolism of 5′-DFUR, capecitabine, FT as well as above-mentioned FdUrd. Briefly, TP has been thought to play an important role in tumor growth and its regulation. It was revealed that TP has angiogenic activity and was identical to platelet-derived endothelial cell growth factor (PD-ECGF) of angiogenic factor in these years [103]. So, scientists focused on TP for its role on tumor angiogenesis. However, the level of TP does not always enhance 5-FU efficacy directly, and the lower expression of TP makes tumor angiogenesis suppress, which leads to the antitumor efficacy of 5-FU.

OPRT is an enzyme that phosphorylates 5-FU inside the cells, which causes DNA synthesis inhibition or RNA dysfunction subsequently. Also, it is reported that the higher expression of OPRT makes the sensitivity to 5-FU enhance, which leads to an efficiency of 5-FU [104].

It is thought that chemotherapy with 5-FU-related anticancer drugs is effective on tumors with high expression of TS, DPD and TP, and low expression of OPRT. However, we have not reached consensus about TS, DPD, TP and OPRT in oral cancers yet. So, we evaluated the predictive value of the expressions of these genes to clarify the efficacy of S-1 by real-time reverse transcription-PCR in oral cancer patients [105]. We found out that, S-1 also has the tendency to exert antitumor effects on tumors with low expression of TS, DPD and TP, and high expression of OPRT similar to 5-FU. In addition, we found that TS mRNA expression was considered to be a useful prognostic factor in oral cancer patients with S-1 single-agent therapy [106]. In the same

way, we recognized the statistical significance between low levels of OPRT mRNA and treatment effects in oral cancer patients with S-1 based chemo-radiotherapy. Interestingly, chemo-radiotherapy with S-1 could exert antitumor effects on tumors with high expression of TS mRNA or DPD mRNA as well as low expression of TS mRNA. Thus, it was suggested that investigation of TS mRNA level might be very important to select chemotherapy with S-1 because lower expression of TS mRNA make the therapeutic effects to enhance. In addition, we may be able to select chemo-radiotherapy with S-1 for cases with high expression of TS mRNA or DPD mRNA. As number of cases is still small, we should do further investigations with a large number of cases. S-1 may exert different therapeutic effects according to the difference of the expression of TS, DPD, TP or OPRT in tumor cells. Hence, even S-1 single-agent therapy may be able to exert antitumor effects partly on tumors with low expression of TS, it is expected to exert remarkable antitumor effects on tumors that show low TS, DPD and TP and high OPRT expression. Moreover, chemo-radiotherapy with S-1 is thought to be a useful method against tumors with low expression of TS and OPRT, and high expression of DPD and TP. Furthermore, it would be better to select combined chemotherapeutic strategies with molecularly targeted agent and 5-FU-related anticancer drugs or other therapeutic approaches, than 5-FU-related anticancer drugs only for single agent chemotherapy.

7. Investigation of best partner for S-1

As described above, efficacy of S-1 depends on the expression of TS, DPD, TP or OPRT in tumors. So, we investigated whether various agents for oral cancer treatment affect the expression of TS, DPD, TP or OPRT in oral cancer cells or not. As a result, we observed that DOC could downregulate the expression of TS, DPD and TP, and upregulated the expression of OPRT in oral cancer cells, which leads to enhancement of antitumor effects and apoptosis by 5-FU. Similar data has already reported in human gastric cancer cells [107]. In addition, we also clarified that CDDP, Radiation, OK-432 and Lentinan might reduce the expression of TS, which might lead to the enhancement of antitumor effect when combined with S-1 [108]. Moreover, we reported that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) downregulated the expression of TS and DPD, and upregulated the expression of OPRT in oral cancer cells, that in turn enhanced the antitumor effects of S-1 [109]. Furthermore, we examined the effectiveness of combined S-1 and proteasome inhibitor (Bortezomib), and clarified that Bortezomib could enhance apoptosis and reduce autophagy, which leads to increased antitumor efficacy by S-1. Recently, we also have reported that Cetuximab might enhance the effect of S-1 on oral cancers through the down-regulation of TS expressions [110]. Hence, it is possible to conduct combined chemotherapy centering on S-1 without causing much adverse effects, i.e., decreased body weight or severe damage to various organs in an animal study. So, we will be able to identify the best partner for S-1 in near future, and expect that the combined chemotherapy may lead to a new less-invasive therapy against oral cancer patients.

8. Future prospects of therapeutic strategy with S-1 against oral cancer

The standard therapy for oral cancer is operative treatments. Surgical operation is a first-line choice for early stage diseases and advanced cases (Stage I, II). However, it is sometimes preferable to avoid surgical operation if the patient demands functional preservation strongly. For example, a lecturer of an English conversation school may be ambitious to avoid surgical treatment even if he/she has cancer of tongue at an early stage. In these years, a multiple drug therapy and a chemoradiotherapy are often selected to preserve oral functions and sensuousness in advanced cases because a primary doctor tends to decide on courses of treatment in a manner most consistent with the patient's wishes. In oral cancer treatments, we should put emphasis on the alleviation of permanent damage in all stages as well as improvement of survival rate in advance cases. Chemotherapy is essential to achieve these, and sustained efforts for suppression of adverse effects and augmentation of antitumor effects have been made by the development of new anticancer agents and the ingenuity of administration methods. Development of S-1, multiple drug therapy with S-1, and chemoradiation with S-1 may be the results of sustained efforts. The response rate of 67.6% was reported in multi-institutional phase I/II trial of combination therapy with S-1 and CDDP against patients with head and neck cancer [81]. In addition, the response rate of 40.9% was reported in phase II trial of outpatient combination therapy with S-1 and carboplatin against patients with unresectable recurrent/metastatic head and neck cancer [111], whereas the response rate of 50.0% was reported in phase I trial of combination therapy with S-1 and DOC against patients with advanced head and neck cancer [112]. Moreover, the response rate was 100.0% and complete remission (CR) rate was 81.3% in phase I trial of concurrent chemoradiotherapy with S-1 and CDDP in patients with locally advanced head and neck cancer [113]. Various clinical trials incorporating S-1 have been conducted against patients with inextirpable head and neck cancer to develop a highly effective treatment for them. It is essential to conduct clinical trials to evaluate the efficacy of newly developed anticancer drugs. The usability of novel promising novel anticancer agents should be assessed by large multicenter study to establish more appropriate treatment because single-institution study has several limitations in evidence level.

S-1 single-agent therapy only has shown a prominent effect on aged patient with oral cancer, where we often found the low expression of TS and DPD in the oral tumors. We have stated that S-1 has possibility to exert antitumor effects on tumors with low expression of TS, DPD and TP, and high expression of OPRT. If we can identify other useful predictive factor for oral cancer treatment, we may be able to select the most effective course of treatment for the particular case, whether the patient should be treated by S-1 single-agent therapy, multidrug therapy or by chemoradiotherapy. This can lead to tailored therapeutic approaches in oral cancer treatment that might improve the therapeutic effects, reduce adverse effects and require shorter hospitalization period, Moreover, avoidance of oral dysfunction and cosmetic disturbance by the surgical operation.

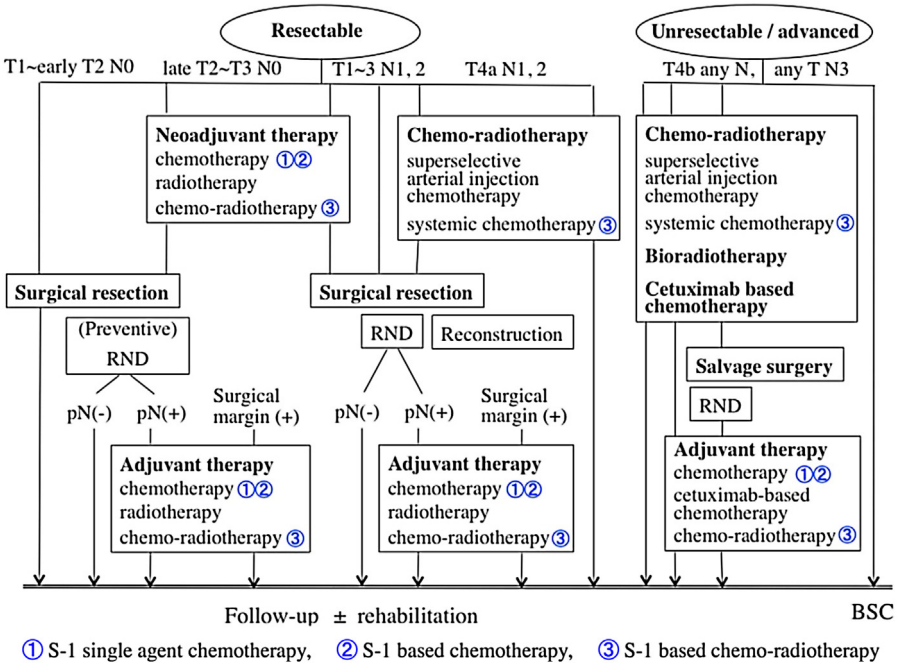

Figure 8 Favorable therapeutic strategies with S-1 against patients with oral cancer.
Cetuximab based chemotherapy has been established as the current standard therapy for platinum-resistant recurrent or metastatic oral cancer. Moreover, cetuximab based bio-radiotherapy may be added to the standard therapy for platinum-resistant recurrent or metastatic oral cancer. However, a triplet bio-chemotherapy consisting of cetuximab, 5-FU, and CDDP is not suitable for patients with renal dysfunction. In addition, cetuximab can cause life-threatening infusion reaction. Therefore, S-1 has great potential in unresectable advanced cases as well as high-risk cases that require adjuvant therapy. Also, S-1 based therapy is found to be useful for functional preservation therapy and neoadjuvant therapy. RND, radical neck dissection; BSC, best supportive care.

We normally decide the therapeutic plan in view of histological analysis of a specific cancer type, general condition, presence or absence of metastasis, and means of salvage when oral cancer was confirmed by biopsy though it is thought to depend on the criteria practiced by each institute. However, after biopsy if it takes another three weeks or so to decide the therapeutic plan, we may as well begin administering S-1 as soon as possible to avoid the risk for local invasion and metastasis through the disseminated cancer cells after undergoing biopsy because of long waiting time for the operation and the examination. As oral cancer has regional characterization that is easily seen by the unaided eye, we can directly confirm an antitumor effect of S-1 on consecutive days. Also, we can predict 30—40% response rate in patients with oral cancer even if S-1 is given for about a few weeks. Depending upon the level of therapeutic effect of S-1 on a particular case, outpatient chemotherapy by S-1 single-agent therapy may be considered one of the options when S-1 single-agent therapy exerts a profound effect. In addition, combination therapy with S-1 and CDDP (or DOC) may also be one of options when S-1 single-agent therapy shows some efficacy. Combination therapy with S-1 and Cetuximab may also be considered as a useful option, though we have to conduct a clinical trial to examine adverse effects. These S-1 based chemotherapy may have the potential to be useful as a neo-adjuvant chemotherapy. Moreover, concurrent chemoradiotherapy with S-1 should be selected when a stronger antitumor effect is required. Furthermore, concurrent chemoradiotherapy with S-1 and CDDP may represent the last option when oral cancer is unresectable and far-advanced (Fig. 8). We paid close attention to the outcome of patient treated with concurrent chemoradiotherapy with S-1 and CDDP whether this regimen can produce prolonged disease-free survival or not because we also have experienced drastic treatment effects (the response rare of 88.9% and CR rate of 66.7%) against unresectable and far-advanced oral cancers. Though we should take particular note of myelosuppression including a neutropenia as the most common adverse event, possible adverse events related to above S-1 therapy can be analyzed by a routine blood test. In addition, oral mucositis invariably recovers about a week after cessation of the therapy with S-1 though oral mucositis could occur in a high frequency. So, we can almost always reinstitute the treatment with S-1. Of course, we should not choose the regimen that ignores safety and efficacy without a clinical trial, and also should not change the regimen without any evidence by just a personal opinion.

9. Closing statement

We summarized the points of favorable therapeutic strategies with S-1 against oral cancer with bibliographic considerations, and also tried to clarify the efficacy of S-1 in oral cancer treatment through basic research about potential of S-1 (Fig. 9). The safety and efficacy of S-1 therapy against oral cancer remains incompletely understood though S-1 has been in existence for 15 years after it got approved for the treatment of head and neck cancers on

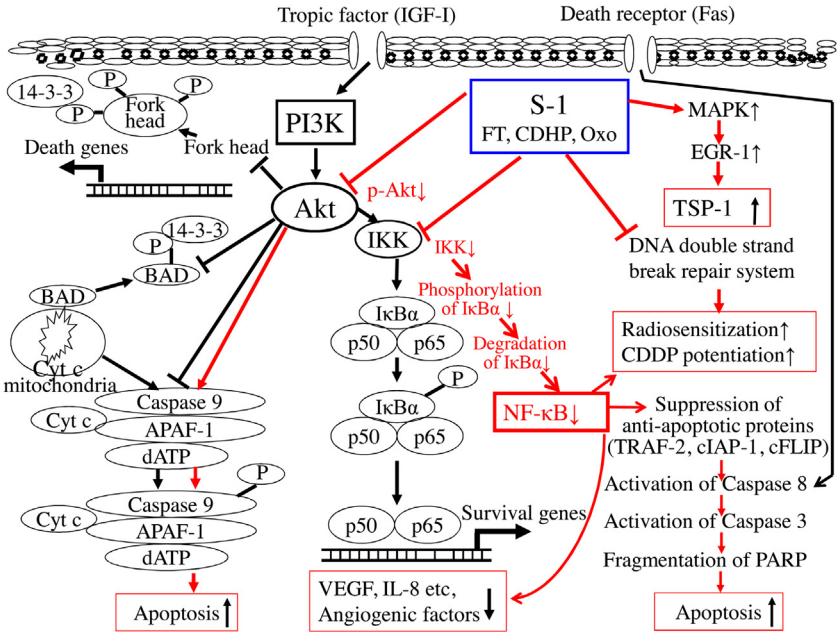

Figure 9 Mechanisms of S-1 to exert antitumor effects.
S-1 has various antitumor effects including antiangiogenic effects, enhacement of apoptosis, suppression of survival signal, radiosensitization efficacy and CDDP potentiation. Briefly, suppression of survival signal such as p-Akt is thought to enhance apoptosis through the activation of caspases. It is thought that inhibition of NF-κB through the IKK suppression may also lead to suppress the expression of angiogenic factors including VEGF and FGF-2 with κB motif in their promoter regions, as well as enhance apoptosis through the activation of caspases. Simultaneously, inhibition of NF-κB can to lead to radiosensitization efficacy and CDDP potentiation. In addition, suppression of DNA double strand break repair systems also may affect radiosensitization efficacy and CDDP potentiation. Moreover, It is thought that enhancement of TSP-1 expression through the MAP kinase and EGR-1 may lead to anti-angiogenesis.

April 2001. However, the oral cancer treatment may appear to progress steadily by the development of new drugs including S-1. Investigations for identifying the best partner for S-1 have been conducted after the approval of S-1. In view of quality of life of oral cancer patients and various problems that beset medical care, S-1 therapy may be provided mainly as outpatient chemotherapy. Therefore, the regimen for S-1 therapy should be proven to be efficacious as well as less toxic and safety. We will have to continue further investigation from now on because we may have the possibilities to get novel tools for oral cancer treatment that includes immunocheckpoint inhibitor (PD-1 antibody/PD-L1 antibody) in the near future. We must take serious responsibilities for selecting and applying S-1 based therapy on cancer patients in order to contribute to the development of S-1, because our country has developed S-1 ahead of leading countries of the West. Here, we discussed therapeutic strategies with S-1 basically as unidirectional characteristics for a new oral cancer treatment, though much remains to be done for the understanding of properties of oral cancer.

Conflict of interest

The authors declare no conflict of interest.

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