RARE DISEASES
2016, VOL. 4, NO. 1, e1165909 (8 pages)
http://dx.doi.org/10.1080/21675511.2016.1165909

Taylor & Francis
Taylor & Francis Group

MINI-REVIEW

OPEN ACCESS

Orotate phosphoribosyltransferase is overexpressed in malignant pleural mesothelioma: Dramatically responds one case in high OPRT expression

Yoichiro Hamamoto[a], Shinjiro Takeoka[a], Atsuto Mouri[a], Munehisa Fukusumi[a], Kazushige Wakuda[a], Tatsuya Ibe[a], Chie Honma[a], Yoshihito Arimoto[a], Kazuaki Yamada[b], Miyuki Wagatsuma[b], Akito Tashiro[c], Shingo Kamoshida[c], and Mitsuhiro Kamimura[a]

[a]Respiratory Department, National Hospital Organization Disaster Medical Center, Tachikawa, Tokyo, Japan; [b]Department of Pathology, National Hospital Organization Disaster Medical Center, Tachikawa, Tokyo, Japan; [c]Department of Medical Biophysics, Laboratory of Pathology, Kobe University Graduate School of Health Sciences, Kobe, Hyogo, Japan

ABSTRACT

Objective: Malignant pleural mesothelioma (MPM) is a rare and aggressive, treatment-resistant cancer. Pemetrexed, an inhibitor of thymidylate synthase (TS), is used worldwide for MPM as a first-line chemotherapy regimen. However, there is little consensus for a second-line chemotherapy. S-1, a highly effective dihydropyrimidine dehydrogenase (DPD)-inhibitory fluoropyrimidine, mainly acts via a TS inhibitory mechanism similar to pemetrexed. Orotate phosphoribosyltransferase (OPRT) is a key enzyme related to the first step activation of 5-fluorouracil (5-FU) for inhibiting RNA synthesis. We investigated 5-FU related-metabolism proteins, especially focusing on OPRT expression, in MPM Methods and Patients: Fifteen MPM patients who were diagnosed between July 2004 and December 2013 were enrolled. We examined the protein levels of 5-FU metabolism-related enzymes (TS, DPD, OPRT, and thymidine phosphorylase [TP]) in 14 cases Results: High TS, DPD, OPRT, and TP expressions were seen in 28.6%, 71.4%, 85.7%, and 35.7% of patients, respectively. We found that OPRT expression was extremely high in MPM tissue. We experienced one remarkable case of highly effective S-1 combined therapy for pemetrexed refractory MPM. This case also showed high OPRT protein expression Conclusion: The present study suggests that OPRT expression is high in MPM tumors. Although pemetrexed is mainly used for MPM chemotherapy as a TS inhibitor, S-1 has potential as an anticancer drug not only as a TS inhibitor but also inhibiting RNA synthesis through the OPRT pathway. This is the first report investigating OPRT protein expressions in MPM.

ARTICLE HISTORY
Received 2 December 2015
Revised 16 February 2016
Accepted 7 March 2016

KEYWORDS
dihydropyrimidine dehydrogenase (DPD); mesothelioma; orotate phosphoribosyltransferase (OPRT); S-1; thymidylate synthase (TS)

Introduction

MPM is a rare malignancy that is mainly localized to the pleura. Asbestos exposure is the dominant etiological agent, with a latency period of 20-40 y. Recently in Japan, the incidence of MPM has been increasing. This is a reflection of the use of asbestos during the 1970s to the mid-1990s. The maximum number of MPM cases is expected to reach approximately 1700 per year by 2010 to 2015.[1] A previous clinical trial suggested that treatment with pemetrexed plus cisplatin and vitamin supplementation resulted in superior survival in first-line treatment for patients with MPM.[2,3] Numerous targeted agents have been tried in small studies in salvage settings, but none have been granted approval.[3,4] There are no randomized trials showing any survival benefit beyond the 2nd line setting, and the definitive optional regimen is not known.[5]

Among potential candidates, S-1 (TS-1; Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) is an anticancer agent for TS inhibition. S-1 is an oral fluoropyrimidine agent that consists of tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate in a molar ratio of 1:0.4:1.[6,7] There have been a few large clinical trials for S-1 for lung cancer. Okamoto et al. reported noninferiority of carboplatin and S-1 compared with carboplatin and paclitaxel in terms of overall survival.[8,9] There have been no S-1 trials reported for MPM.

However, pemetrexed, an antifolate anticancer drug, is widely used for MPM treatment. Both S-1 and

CONTACT Yoichiro Hamamoto hamamoto32@gmail.com Respiratory Department, National Hospital Organization Disaster Medical Center, 3256 Midoricho, Tachikawa, Tokyo 190-0014, Japan.

pemetrexed exhibit their main anti-tumor activity through TS inhibition via a similar mechanism. However, an OPRT-related pathway that leads to RNA synthesis inhibition is also thought to be an important mechanism for S-1.[10-12]

The aim of this retrospective study was to assess the OPRT expression which is a potential prognostic indicator of S-1 treatment, for MPM. During this study, we experienced one pemetrexed-refractory MPM case that dramatically responded to S-1 treatment, which also showed high OPRT expression.

Materials and methods

Patients' characteristics

The study included all patients who were diagnosed with MPM at National Hospital Organization National Disaster Medical Center in Tokyo, Japan between July 2004 and December 2013. Analysis of tumor samples, which were available after routine diagnostic histopathological workup, provided adequate pretreatment biopsies from 15 patients. The study protocol was approved by the institutional ethics committee (No. TDMC2013-8/2013).

The characteristics of the study population are summarized in Table 1. The age of the patients ranged from 41 to 87 y (median age, 68). Disease staging was assessed according to International Mesothelioma Interesting Group Tumor Node Metastasis (IMIG TNM) staging criteria.[13] All tumors were assessed using the modified Southwest Oncology Group (SWOG) criteria.[14] According to IMIG TNM classification, 11 were in stage III/IV and two were stage I. Of these patients, 13 received pemetrexed-based chemotherapy. Three underwent surgical treatment: two received EPP (extrapleural pneumonectomy) and the other P/D (pleurectomy/decortication). These surgical patients also received pemetrexed-based chemotherapy after recurrence. One patient was excluded from further studies because tissue specimens were not available. For the pathological type,[15] five were epithelioid mesothelioma type, four were sarcomatoid type mesothelioma (all four were diagnosed as desmoplastic type), and three were biphasic type mesothelioma. The remaining three cases were diagnosed only as MPM because of difficulty to classify.

Fifteen patients with MPM were treated with chemotherapy; nine received pemetrexed plus carboplatin, two received pemetrexed plus cisplatin, one received gemcitabine plus carboplatin, and one received pemetrexed alone. Two patients requiring palliative care did not receive chemotherapy.

Immunohistochemical staining

Immunohistochemical staining was performed according to the procedure described in previous reports.[16,17] The following antibodies were used: mouse monoclonal antibody against TS (Anti-TS Mouse IgG MoAb→; Immuno-Biological Laboratories Co., Ltd., Gunma, Japan); mouse monoclonal antibody against DPD (Anti-DPD Mouse IgG MoAb→; Immuno-Biological Laboratories Co., Ltd.); rabbit antibody against OPRT (Anti-OPRT Rabbit IgG Affinity Purify→ Immuno-Biological Laboratories Co., Ltd.); and mouse monoclonal antibody against TP (Anti-TP Mouse IgG MoAb→; Immuno-Biological Laboratories Co., Ltd.). Positive

Table 1. Individual patient data on malignant pleural mesothelioma.

No.	Age	Sex	Histology	Asbestos history	S.I.(pack/year)	Stage	Surgical history	TS	DPD	OPRT	TP	Initial treatment	Survival time(days)
1	70	F	Epithelioid mesothelioma	—	unknown	I	P/D	1	5	5	5	CBDCA+Pem	381*
2	87	M	Biphasic mesothelioma	—	40	IV	—	4	1	4	1	Pem	72
3	66	M	Desmoplastic mesothelioma	unknown	unknown	IV	—	NA	NA	NA	NA	CBDCA+Pem	562*
4	41	M	Mesothelioma, malignant	—	Never	NA	—	1	5	4	4	CBDCA+GEM	190
5	63	M	Mesothelioma, malignant	—	unknown	NA	—	3	1	2	1	BSC	23
6	71	M	Metothelioma, malignant	+	69	IV	—	1	5	5	1	CDDP+Pem	1492*
7	60	M	Biphasic mesothelioma	+	30	III	EPP	3	4	2	1	CBDCA+Pem	591
8	63	M	Biphasic mesothelioma	—	80	IV	—	4	5	5	4	CDDP+Pem	80
9	73	M	Epithelioid mesothelioma	—	Never	III	EPP	1	5	5	1	CBDCA+Pem	960*
10	66	M	Epithelioid mesothelioma	+	24	IV	—	2	4	3	3	CBDCA+Pem	928*
11	68	M	Desmoplastic mesothelioma	unknown	Never	IV	—	1	1	5	1	CBDCA+Pem	485
12	66	M	Desmoplastic mesothelioma	—	22	III	—	1	1	5	2	CBDCA+Pem	177
13	68	M	Epithelioid mesothelioma	unknown	45	IV	—	2	4	4	5	BSC	33
14	78	M	Epithelioid mesothelioma	—	Never	I	—	1	4	5	5	CBDCA+Pem	318*
15	83	M	Desmoplastic mesothelioma	—	20	III	—	1	4	5	3	CBDCA+Pem	262*

Note.*As of December 2014, alive at the time of evaluation.

controls were sections of colonic adenocarcinoma for TS, lung adenocarcinoma for DPD, squamous cell lung carcinoma for OPRT, and breast ductal carcinoma for TP.

Two investigators (A.T. and S.K.) with no previous knowledge of the clinicopathological characteristics assessed immunostained sections. Positive expression of TS, OPRT, DPD, and TP was identified if nuclear or cytoplasmic staining was present. The percentage of positive tumor cells was analyzed using a semiquantitative score as follows: 1, <10 %; 2, 10–25%; 3, 26–50%; 4, 51–75%; 5, >75 %.[18] Tumors in which stained cells made up more than 25% of the tumor were graded as highly positive.

Statistical analysis

One-way ANOVA followed by Dunnett's multiple comparison tests was used for statistical analysis to determine the immunohistochemical staining score. The Kaplan-Meier method was used to estimate survival as a function of time, and survival differences were analyzed by the log-rank test. Overall survival (OS) was defined as the time between the start of chemotherapy and death from any cause. Statistical analysis was performed using JMP 10 (SAS, Institute Inc., Cary, NC, USA) for Mac and statistical significance was set a *P* value of 0.05.

Results

Between July 2004 and December 2013, 15 patients consecutively diagnosed for MPM were enrolled.

Immunohistochemical analysis

We evaluated immunohistochemical staining using a semi-quantitative scoring method. Of 15 patients, 14 samples were available for 5-FU-related metabolic enzyme staining and analysis. TS, OPRT, and TP were expressed in the nuclei and/or cytoplasm of the tumor cells, and DPD consistently showed cytoplasmic staining (Fig. 1).

Average scores of TS, DPD, OPRT, and TP were 1.9 (standard error = 0.3), 3.5 (0.5), 4.2 (0.3), and 2.6 (0.5), respectively (Table 1). OPRT protein expression was much higher compared to the other 5-FU metabolism-related proteins in MPM. As compared to TS protein expression, OPRT was more than twice as high.

High expression (defined as >25 % stained cells) was seen for TS, DPD, OPRT, and TP in 28.6%(4/14), 71.4% (10/14), 85.7%(12/14), and 35.7%(5/14) of tumors, respectively. Positive rates of expression of each protein according to clinicopathological factors are shown in Table 2. TS protein tended to be expressed more strongly for older patients. Low TS expression patients (n = 10) all had high OPRT protein expression. For high DPD protein expression (n = 10), 9 patients tended to have high OPRT protein expression (Table 2).

Survival analysis for pemetrexed-based treatment

Of 15 patients, 14 had adequate samples available for TS, DPD, OPRT, and TP analysis. Twelve patients were treated with pemetrexed-based chemotherapy. Using the semi-quantitative scoring method, samples were classified as having "high" or "low" TS, DPD, OPRT, or TP protein expression (Table 2). We found a significant association between high and low TS and DPD expression and survival time ($P = 0.029$, $P = 0.0067$ respectively). However, we did not reveal any other significant associations between other parameters.

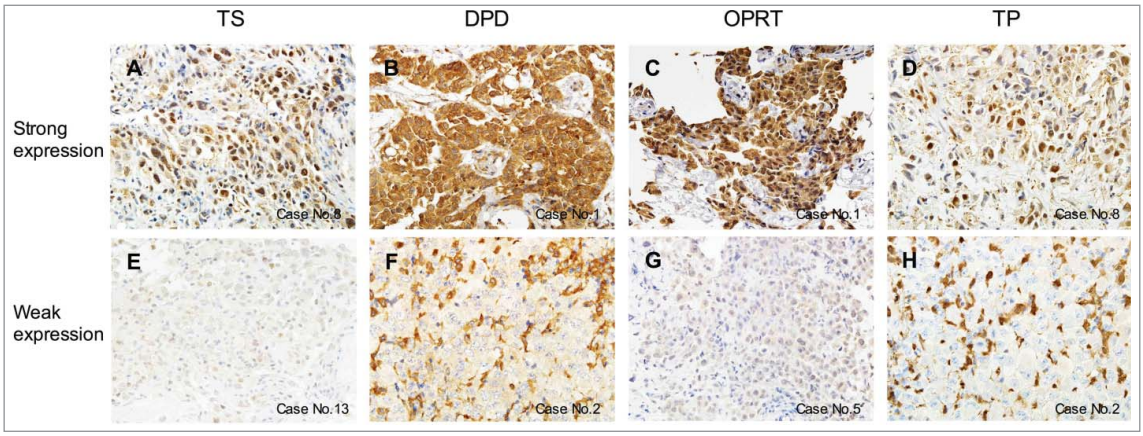

Figure 1. Examples of immunohistochemical staining of MPM.

Table 2. Positive rates of TS, DPD, OPRT, and TP according to clinicopathological factors.

	TS		DPD		OPRT		TP	
Parameter	High (n = 4)	Low (n = 10)	High (n = 10)	Low (n = 4)	High (n = 12)	Low (n = 2)	High (n = 5)	Low (n = 9)
Age (≤65 />65 years)	1/3	1/9	7/3	3/1	10/2	0/2	3/2	7/2
Gender (Male/female)	4/0	9/1	9/1	4/0	11/1	2/0	4/1	9/0
Histology (Epithelioid/Desmoplastic/Biphasic/unknown)	0/0/3/1	5/3/0/2	5/1/2/2	0/2/1/1	5/3/2/2	0/0/1/1	3/0/1/1	2/3/2/2
TS (High/Low)	—	—	2/8	2/2	2/10	2/0	1/4	3/6
DPD (Hight/Low)	2/2	8/2	—	—	9/3	1/1	5/0	5/4
OPRT (High/Low)	2/2	10/0	9/1	3/1	—	—	5/0	7/2
TP (High/Low)	1/3	4/6	5/5	0/4	5/7	0/2	—	—

Dramatic response to S-1 treatment of an MPM case and OPRT protein expression

A 66-year-old male with unresectable MPM was admitted to our hospital for fourth-line treatment. The patient was diagnosed with MPM on September 2009. A chest computed tomography scan at the initial time revealed a mass extending to the pleura with pleural effusion that was predominantly on the upper mediastinal side. Under thoracoscopy, a pleural biopsy of a pleural thickening mass was pathologically compatible for MPM. Immunohistochemical analysis showed that the tumor cells were positive for calretinin. The findings were consistent with a diagnosis of MPM, stage IV.

The patient was treated with multiple chemotherapy regimens according to the modified Southwest Oncology Group (SWOG) criteria. The first-line chemotherapy was four cycles of cisplatin plus pemetrexed. The tumor regrew after the first treatment, necessitating additional treatment. A similar four cycles of carboplatin plus pemetrexed was chosen, and 15 cycles of pemetrexed maintenance was applied after combination therapy. However, the tumor regrew again and the patient was treated with carboplatin and gemcitabine as a third-line chemotherapy. Three months after the third-line chemotherapy, pleural thickening was again found. Therefore, S-1 and carboplatin salvage combination therapy was administered. We obtained informed consent. The patient received carboplatin (AUC,5) on day 1 plus oral S-1 (40 mg/m^2 twice per day) on days 1 to 14. Chemotherapy was repeated every 3 weeks for a maximum of six cycles unless there was earlier evidence of disease progression or treatment intolerance. In regards to tumor response, the pleural thickening dramatically shrank in size (Fig. 2). Immunohistochemical staining of TS, OPRT, DPD, and TP showed 1+, 5+, 5+, and 1+ scores, respectively.

Grade 4 anemia appeared, which required transfusion since the patient had undergone many courses of

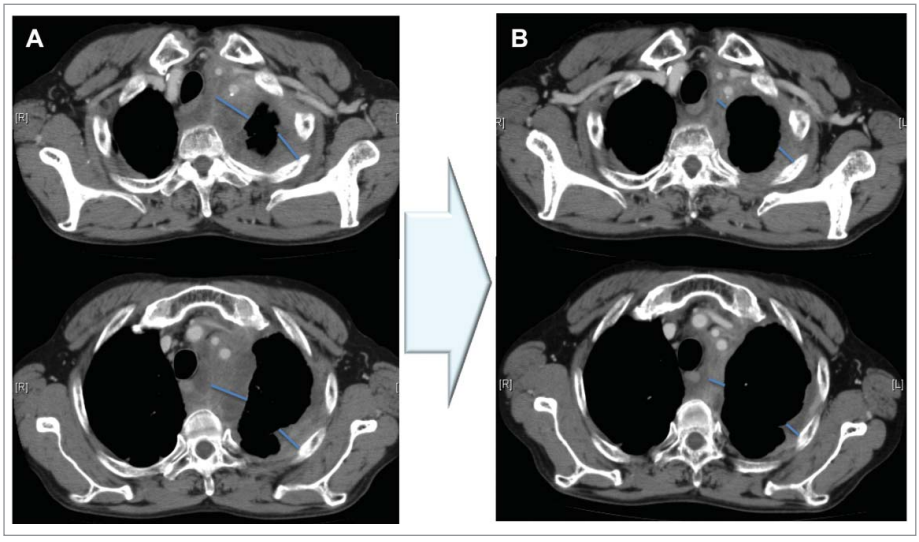

Figure 2. Computed tomography shows S-1+CBDCA treatment course.

chemotherapy. At present, 5 months after the start of treatment, the patient remains well and maintains a partial response status.

Discussion

To our knowledge, this is the first study evaluating the presence of OPRT protein expression in MPM. We found that OPRT protein in mesothelioma tumor tissue was significantly higher compared to other 5-FU metabolic-related enzymes.

MPM is an aggressive, treatment-resistant cancer that is increasing in frequency throughout the world. Median survival is now 12 months from diagnosis.[19] The current standard of care for first-line systemic therapy in patients with unresectable MPM is combination chemotherapy with pemetrexed and cisplatin.[2,3] However, there is no current standard of care for second-line chemotherapy due to insufficient evidence. We documented a remarkable response with S-1 combination therapy for pemetrexed-refractory MPM in one patient.

S-1 has been reported to be effective in the treatment of various solid tumors, including gastric cancer, colon cancer, and non-small cell lung cancer.[8,20] We previously reported successful S-1 treatment for refractory thymic carcinoma with high OPRT expression.[10] The anticancer activity of 5-FU has been reported to be closely related to the intratumoral expression of TS and DPD in lung cancer.[11] We have also hypothesized that strong OPRT expression may be a predictive biomarker for S-1 therapy. There are a few papers describing TS and DPD expression in MPM.[21-23] Therefore, we took interest in 5-FU related-metabolic proteins, especially OPRT, in mesothelioma. As a result, we found high OPRT expression in MPM cells, which might be a predictive biomarker for S-1 treatment.

5-FU exerts its antitumor effect primarily through the inhibition of DNA synthesis. However, the mechanism of its anti-tumor effect also occurs through dysfunction of RNA synthesis. The hypothesized mechanism underlying its inhibition of DNA and RNA is shown in Figure 3.

The first mechanism of action is to inhibit DNA synthesis. *In vivo*, 5-FU is converted to its active form, 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP).[6] Then, with reduced folic acid as a coenzyme, it strongly binds with TS, which catalyzes the synthesis of thymidine necessary for DNA synthesis, forming a tripartite complex.[18] This reduces the activity of TS, thereby inhibiting DNA synthesis. However, when TS is highly expressed in tumor tissues, DNA synthesis occurs due to the residual excess TS, and the antitumor effect of 5-FU is attenuated. TS expression in the tumor tissues of various solid cancers has been investigated and has been found to be lower in lung cancer than in other cancers.[20] In view of the above, though 5-FU should be effective against most lung cancers based on its expression patterns, its efficacy is actually poor. A proposed reason for this is that, although 5-FU is activated *in vivo*, it is rapidly broken down by DPD, a 5-FU-degrading enzyme.[24] Furthermore, in cancer types with high intratumoral levels of DPD activity, 5-FU is rapidly degraded and its efficacy is decreased. DPD activity has been reported to be at least two-fold higher in lung carcinoma than in gastric and colon cancers.[20] Therefore, when 5-FU is used to treat carcinoma types with high DPD activity levels, inhibition of DPD is considered to be essential, and this realization led to the development of S-1, which contains gimeracil (CDHP; 5-chloro-2,4-dihydroxypyridine). We also found high DPD expression in mesothelioma tissues, but theologically, S-1, which contains CHDP, a DPD inhibitor, may have an anticancer effect in tumors with high DPD expression.

The second mechanism of action of 5-FU is the inhibition of RNA function, which is mediated by the conversion of OPRT to fluorouridine monophosphate (FUMP) by a phosphorylating enzyme. Investigation of OPRT expression in lung cancer has shown that it is at least two-fold higher in squamous cell carcinoma than in adenocarcinoma.[20] It can be surmised that S-1 exerts an antitumor effect on squamous cell carcinoma, mainly by inhibiting RNA function. In the present study, we found high OPRT expression in MPM tumor tissues. Therefore, we presumed that MPM would have the potential to respond more to S-1 treatment.

In contrast, although pemetrexed inhibits multiple enzymes involved in pyrimidine and purine synthesis,[25] its major target enzyme is TS (Fig. 3). After cellular uptake, pemetrexed is converted into more effective polyglutamated forms by folylpoly-γ-glutamate synthetase (FPGS). There is no pathway related to OPRT in its mode of action. Overall, like in squamous lung cancer and thymic carcinoma,[10] when combined with TS inhibition, cancer tissue that have high OPRT protein expression have the potential to

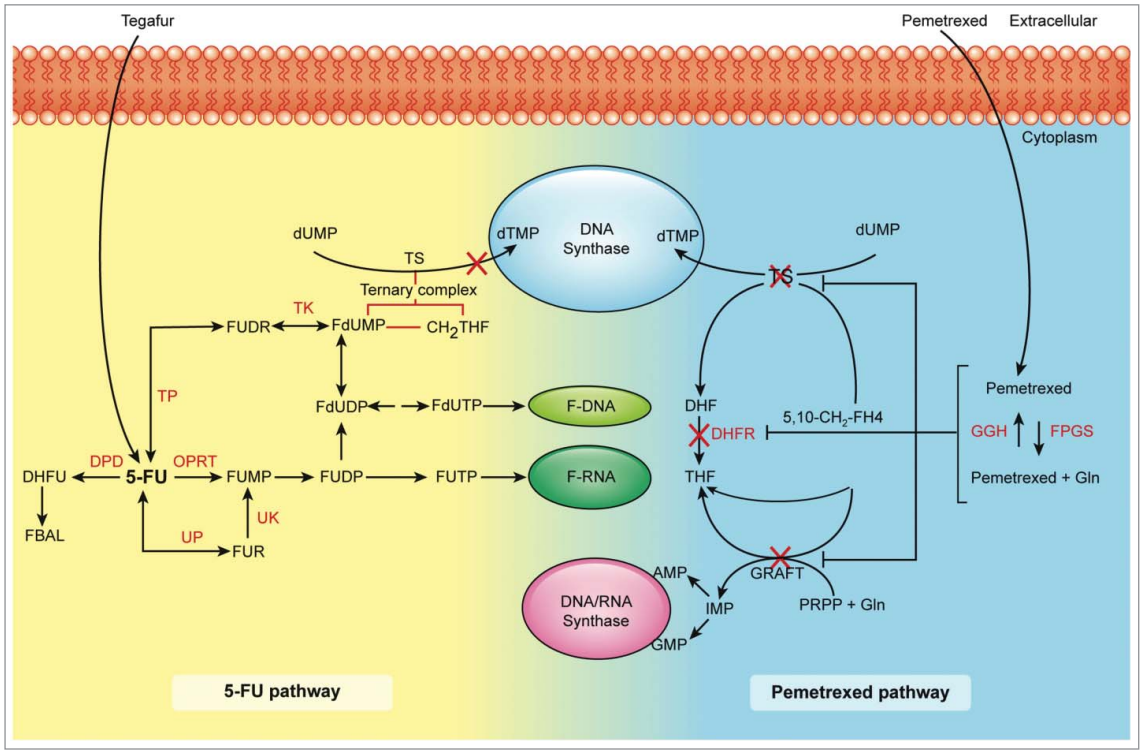

Figure 3. Mechanism of TS inhibitors (5-FU and Pemetrexed).

be treated with S-1 through the inhibition for RNA synthease.

There are a few limitation of this study that were the small sample size, single institution and retrospective case control study. Twelve of 15 patients were treated with pemetrexed-based chemotherapy. Though we could find statistical significant between TS expression and overall survival, in 2013 Lustgarten et al. for 85 MPM patients[26] and Mairinger et al. for 63 MPM patients[27] reported that TS expression might not be a marker of pemetrexed efficacy. Given this, TS expression might not be important in we will follow-up on patients in the current study.

Another limitation is that we observed only one effective case of S-1 treatment. For this patient, we administrated a combination of CBDCA plus S-1, so the efficacy of S-1 was not determined in isolation. Theoretically, however, the high OPRT expression should have affected S-1 treatment.

Finally, there are side effects of S-1 therapy. In this present case, the patient was treated with S-1 after 17 courses of pemetrexed and gemcitabine. There were thus hematological side effects (grade 4 anemia requiring blood transfusion). Rare treatments of this kind are difficult to generalize, so we will require more prospective studies.

In conclusion, in MPM patients, we found high OPRT expression (12/14 cases) compared to other 5-FU metabolic-related proteins. And we experienced that for TS inhibition, pemetrexed as well as S-1 respond to refractory MPM patient. Theoretically, high OPRT expression could be expected to be more effective for S-1 treatment. Further clinical studies of S-1 with MPM are needed.

Abbreviations

EPP	extrapleural pneumonectomy
P/D	pleurectomy/decortication
PS	performance status
mut	mutation
PFS	progression free
BSC	best supportive care
NA	Not applicable
CBDCA	carboplatin
Pem	pemetrexed
GEM	gemcitabine
TS	thymidylate synthase
DPD	dihydropyrimidine dehydrogenase
OPRT	orotate phosphoribosyl transferase
TP	thymidine phosphorylase
S.I.	smoking index

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Inai K. Pathology of Mesothelioma. Japanese J Lung Cancer 2007; 47:223–232.

[2] Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003; 21:2636-44; PMID:12860938; http://dx.doi.org/10.1200/JCO.2003.11.136

[3] Remon J, Lianes P, Martinez S, Velasco M, Querol R, Zanui M. Malignant mesothelioma: new insights into a rare disease. Cancer Treat Rev 2013; 39:584-91; PMID:23276688; http://dx.doi.org/10.1016/j.ctrv.2012.12.005

[4] Tsao AS. Mesothelioma. J Thorac Oncol 2012; 7:S399-400; PMID:23160333; http://dx.doi.org/10.1097/JTO.0b013e31826df254

[5] Ceresoli GL, Zucali PA, Gianoncelli L, Lorenzi E, Santoro A. Second-line treatment for malignant pleural mesothelioma. Cancer Treat Rev 2010; 36:24-32; PMID:19879055; http://dx.doi.org/10.1016/j.ctrv.2009.09.003

[6] Okamoto I, Fukuoka M. S-1: a new oral fluoropyrimidine in the treatment of patients with advanced non-small-cell lung cancer. Clin Lung Cancer 2009; 10:290-4; PMID:19632949; http://dx.doi.org/10.3816/CLC.2009.n.040

[7] Shirasaka T, Nakano K, Takechi T, Satake H, Uchida J, Fujioka A, Saito H, Okabe H, Oyama K, Takeda S, et al. Antitumor activity of 1 M tegafur-0.4 M 5-chloro-2,4-dihydroxypyridine-1 M potassium oxonate (S-1) against human colon carcinoma orthotopically implanted into nude rats. Cancer Res 1996; 56:2602-6; PMID:8653704

[8] Okamoto I, Yoshioka H, Morita S, Ando M, Takeda K, Seto T, Yamamoto N, Saka H, Asami K, Hirashima T, et al. Phase III trial comparing oral S-1 plus carboplatin with paclitaxel plus carboplatin in chemotherapy-naive patients with advanced non-small-cell lung cancer: results of a west Japan oncology group study. J Clin Oncol 2010; 28:5240-6; PMID:21079147; http://dx.doi.org/10.1200/JCO.2010.31.0326

[9] Yoshioka H, Okamoto I, Morita S, Ando M, Takeda K, Seto T, Yamamoto N, Saka H, Atagi S, Hirashima T, et al. Efficacy and safety analysis according to histology for S-1 in combination with carboplatin as first-line chemotherapy in patients with advanced non-small-cell lung cancer: updated results of the West Japan Oncology Group LETS study. Annals Oncol 2013; 24:1326-31; http://dx.doi.org/10.1093/annonc/mds629

[10] Hamamoto Y. Better efficacy of S-1 treatment for thymic carcinoma: case report and review of the literature. Int Cancer Conference J 2013; 2:17–21; http://dx.doi.org/10.1007/s13691-012-0056-8

[11] Kaira K, Ohde Y, Nakagawa K, Okumura T, Murakami H, Takahashi T, Kondo H, Nakajima T, Endo M, Yamamoto N. Thymidylate synthase expression is closely associated with outcome in patients with pulmonary adenocarcinoma. Medical Oncol 2012; 29:1663-72; http://dx.doi.org/10.1007/s12032-011-0069-8

[12] Okuma Y, Shimokawa T, Takagi Y, Hosomi Y, Iguchi M, Okamura T, Shibuya M. S-1 is an active anticancer agent for advanced thymic carcinoma. Lung cancer (Amsterdam, Netherlands) 2010; 70:357-63; PMID:20951466; http://dx.doi.org/10.1016/j.lungcan.2010.09.004

[13] Rusch VW. A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. Chest 1995; 108:1122-8; PMID:7555126; http://dx.doi.org/10.1378/chest.108.4.1122

[14] Byrne MJ, Nowak AK. Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. Annals Oncol 2004; 15:257-60; http://dx.doi.org/10.1093/annonc/mdh059

[15] Inai K. Pathology of mesothelioma. Environmental Health Preventive Med 2008; 13:60-4; http://dx.doi.org/10.1007/s12199-007-0017-6

[16] Nakano J, Huang C, Liu D, Masuya D, Nakashima T, Yokomise H, Ueno M, Wada H, Fukushima M. Evaluations of biomarkers associated with 5-FU sensitivity for non-small-cell lung cancer patients postoperatively treated with UFT. Br J Cancer 2006; 95:607-15; PMID:16880781; http://dx.doi.org/10.1038/sj.bjc.6603297

[17] Kaira K, Endo M, Abe M, Nakagawa K, Ohde Y, Okumura T, Takahashi T, Murakami H, Tsuya A, Nakamura Y, et al. Biologic correlation of 2-[18F]-fluoro-2-deoxy-D-glucose uptake on positron emission tomography in thymic epithelial tumors. J Clin Oncol 2010; 28:3746-53; PMID:20625125; http://dx.doi.org/10.1200/JCO.2009.27.4662

[18] Kaira K, Serizawa M, Koh Y, Miura S, Kaira R, Abe M, Nakagawa K, Ohde Y, Okumura T, Murakami H, et al. Expression of thymidylate synthase, orotate phosphoribosyltransferase and dihydropyrimidine dehydrogenase in thymic epithelial tumors. Lung Cancer 2011; 74:419-25; PMID:21550686; http://dx.doi.org/10.1016/j.lungcan.2011.04.001

[19] Robinson BW, Musk AW, Lake RA. Malignant mesothelioma. Lancet 2005; 366:397-408; PMID:16054941; http://dx.doi.org/10.1016/S0140-6736(05)67025-0

[20] Fukui Y, Oka T, Nagayama S, Danenberg PV, Danenberg KD, Fukushima M. Thymidylate synthase, dihydropyrimidine dehydrogenase, orotate phosphoribosyltransferase mRNA and protein expression levels in solid tumors in large scale population analysis. Int J Mol Med 2008; 22:709-16; PMID:19020767

[21] Van TT, Hanibuchi M, Kakiuchi S, Sato S, Kuramoto T, Goto H, Mitsuhashi A, Nishioka Y, Akiyama S, Sone S. The therapeutic efficacy of S-1 against orthotopically implanted human pleural mesothelioma cells in severe combined immunodeficient mice. Cancer Chemotherapy Pharmacol 2011; 68:497-504; http://dx.doi.org/10.1007/s00280-010-1503-x

[22] Christoph DC, Asuncion BR, Mascaux C, Tran C, Lu X, Wynes MW, Gauler TC, Wohlschlaeger J, Theegarten D, Neumann V, et al. Folylpoly-glutamate synthetase expression is associated with tumor response and outcome from pemetrexed-based chemotherapy in malignant pleural mesothelioma. J Thorac Oncol 2012; 7:1440-8; PMID:22895141; http://dx.doi.org/10.1097/JTO.0b013e318260deaa

[23] Sakamoto E, Nagase H, Kobunai T, Oie S, Oka T, Fukushima M, Oka T. Orotate phosphoribosyltransferase expression level in tumors is a potential determinant of the efficacy of 5-fluorouracil. Biochem Biophysical Res Communications 2007; 363:216-22; http://dx.doi.org/10.1016/j.bbrc.2007.08.164

[24] Diasio RB. The role of dihydropyrimidine dehydrogenase (DPD) modulation in 5-FU pharmacology. Oncology 1998; 12:23-7; PMID:9830621

[25] Calvert AH. Biochemical Pharmacology of Pemetrexed. Oncology 2004; 18:13-7; PMID:15655931

[26] Lustgarten DE, Deshpande C, Aggarwal C, Wang LC, Saloura V, Vachani A, Wang LP, Litzky L, Feldman M, Creaney J, et al. Thymidylate synthase and folylpolyglutamate synthase are not clinically useful markers of response to pemetrexed in patients with malignant pleural mesothelioma. J Thorac Oncol 2013; 8:469-77; PMID:23486267; http://dx.doi.org/10.1097/JTO.0b013e318283da3e

[27] Mairinger F, Vollbrecht C, Halbwedl I, Hatz M, Stacher E, Gully C, Quehenberger F, Stephan-Falkenau S, Kollmeier J, Roth A, et al. Reduced folate carrier and folylpolyglutamate synthetase, but not thymidylate synthase predict survival in pemetrexed-treated patients suffering from malignant pleural mesothelioma. J Thorac Oncol 2013; 8:644-53; PMID:23449276; http://dx.doi.org/10.1097/JTO.0b013e318287c224