Brief Report

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Intratumor dihydropyrimidine dehydrogenase mRNA expression levels are decreased in extramammary Paget's disease

Yuki Nakamura, Ikko Kajihara*, Saori Yamada-Kanazawa, Saki Maeda-Otsuka, Takamitsu Johno, Jun Aoi, Toshikatsu Igata, Shinichi Masuguchi, Satoshi Fukushima, Masatoshi Jinnin, Hironobu Ihn

Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Honjo, Kumamoto, Japan.

Summary **S-1, a 5-fluorouracil (5-FU)-based anti-cancer agent, is an important drug for treating metastatic extramammary Paget's disease (EMPD). Although intratumor expression levels of 5-FU metabolism enzymes have been studied widely in many solid tumors, no studies have examined on the expression levels of thymidylate synthase (TS), orotate phosphoribosyl-transferase (OPRT) or dihydropyrimidine dehydrogenase (DPD) in skin cancers. The aim of this study was to estimate the intratumoral mRNA expression levels of these genes in EMPD by real time PCR. Intratumoral DPD mRNA levels were decreased in EMPD compared to those in normal skin, but its intratumoral DPD mRNA expression levels were not correlated with clinical manifestations. Intratumoral DPD mRNA levels were positively correlated with OPRT mRNA levels in EMPD. Based on these results, low expression of intratumoral DPD mRNA in EMPD may contribute to the pathogenesis of this disease.**

Keywords: S-1, extramammary Paget's disease, mRNA, real time PCR, thymidylate synthase, orotate phosphoribosyl-transferase, dihydropyrimidine dehydrogenase

1. Introduction

Extramammary Paget's disease (EMPD) is a rare skin cancer that shows erosive erythema and nodules in pubic or axillary lesions. Although the prognosis of EMPD with distant metastasis is poor, S-1 monotherapy (*1*) or S-1/docetaxel therapy (*2,3*) is an effective treatment. In cutaneous malignancy except for EMPD, S-1 based chemotherapy has also been reported to be a promising treatment for advanced squamous cell carcinoma (*4*) and angiosarcoma (*5*).

S-1 is an oral anti-tumor drug containing tegafur, potassium oxonate and 5-chloro-2,4-dihydroxypyrimidine (CDHP) and tegafur is a prodrug of 5-fluorouracil (5-FU) (*6*). The main enzymes responsible for the effect of S-1 are thymidylate synthase (TS), orotate phosphoribosyl-transferase (OPRT) and dihydropyrimidine dehydrogenase (DPD) (*7*). Fluorodeoxyuridine monophosphate, a 5-FU metabolite, inhibits TS which is responsible the DNA synthesis (*8*). OPRT converts 5-FU into 5-fluorouridine monophosphate, leading to inhibition of RNA synthesis (*9*). CDHP inhibits DPD which plays an important role in the inactivation of 5-FU (*6*).

The intratumor expression levels of these enzymes have been investigated in many solid tumors. TS and DPD activity in gastric and non-small lung cancer tissues are higher than those in normal tissues (*10*). In metastatic colon-rectal cancer, high TS and DPD mRNA expression levels in cancer tissues are negatively correlated with survival time (*11,12*). In metastatic gastric cancer patients, low TS and DPD mRNA expression levels were found associated with good response to S-1 (*13*).

However, to our knowledge, there have been no studies on the TS, DPD and OPRT expression levels in skin cancer. The aim of this study was to evaluate the mRNA expression levels of TS, DPD and OPRT in EMPD.

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**Address correspondence to:*
Dr. Ikko Kajihara, Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto, 860-8556, Japan.
E-mail: kajiderma@gmail.com

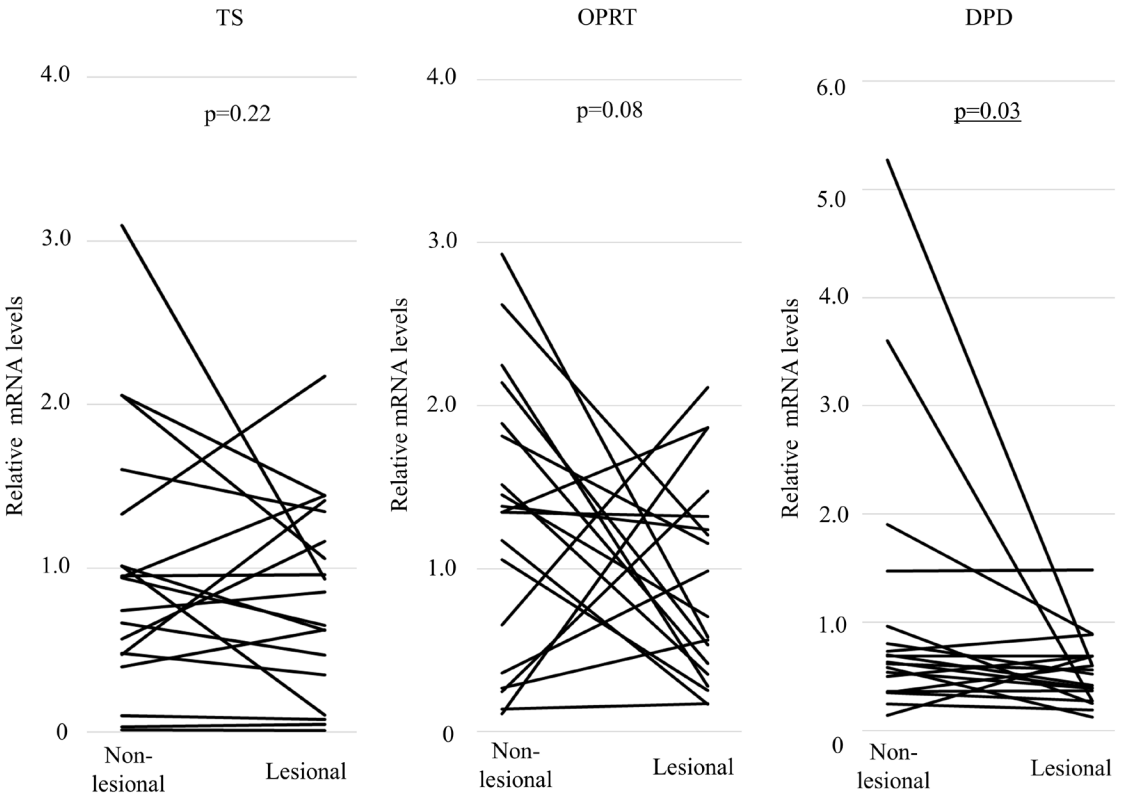

Figure 1. Relative intratumoral TS, OPRT and DPD mRNA expression levels in EMPD compared to those in normal tissues. TS: thymidylate synthase, OPRT: orotate phosphoribosyl-transferase, DPD: dihydropyrimidine dehydrogenase (DPD), EMPD: extramammary Paget's disease.

2. Materials and Methods

2.1. *Patients*

All patients were diagnosed with EMPD at Kumamoto University Hospital between November 2008 and August 2014. Eligible patients fulfilled the following criteria: histological diagnosis of extramammary Paget's disease, enforcement of mapping biopsy using punch biopsy before operation and sufficient tissue available in paraffin blocks for the assessments by real time polymerase chain reaction (PCR). Lesional or non-lesional skin was assessed by hematoxylin and eosin staining. Institutional review board approval and written informed consent for this study were obtained according to the Declaration of Helsinki.

2.2. *RNA isolation from tissue, cDNA synthesis and real time PCR analysis*

RNA was isolated from paraffin sections of skin samples using the RNeasy FFPE kit (Qiagen, Hilden, Germany). cDNA was synthesized using the first-strand cDNA using the RT^2 First Strand Kit (SABiosciences, Frederick, MD, USA). Quantitative real-time PCR was performed as previously described (*14*). Primer sets for TS, OPRT, DPD and GAPDH were obtained from SABiosciences. DNA was amplified for 50 cycles of denaturation for 15 seconds at 95°C and 35 seconds at 55°C, and annealing for 30 seconds at 72°C. Each transcript level was normalized to that of GAPDH.

2.3. *Statistical analysis*

Statistical analyses were performed using the Wilcoxon signed-rank test to compare matched mRNA expression levels in lesional and non-lesional skin. Correlations were assessed according to Fisher's correlation coefficient. A *p*-value of < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. *DPD mRNA levels in tumor tissue were decreased compared to in normal skin*

We evaluated the mRNA expression levels of 5-FU metabolism in EMPD using real time PCR. Although there were no differences in the TS and OPRT mRNA levels between paired tumor and normal sections, DPD mRNA levels in tumors were significantly lower than (less than 50%) those in non-lesional skin (Figure 1). Next, we examined the correlations between up/down-regulated DPD mRNA levels in tumor tissues and clinical manifestations (sex, age, degree of invasiveness and dying of EMPD). Intratumoral DPD mRNA expression levels in EMPD were not correlated with

Table 1. Correlation of DPD mRNA expression levels and clinical features

DPD mRNA	< 1 (n = 12)	≥ 1 (n = 7)	p-values
Age	79.7 ± 8.9	69.4 ± 6.2	0.06
Sex			
Male	9	4	0.62
Female	3	3	
Degree of invasiveness			
in situ	11	5	0.52
microinvasion/carcinoma	1	2	
Death from cancer	1	1	1.00

Relative DPD mRNA levels in EMPD tissues were lower (< 1) or higher (≥ 1) than those in normal tissues.

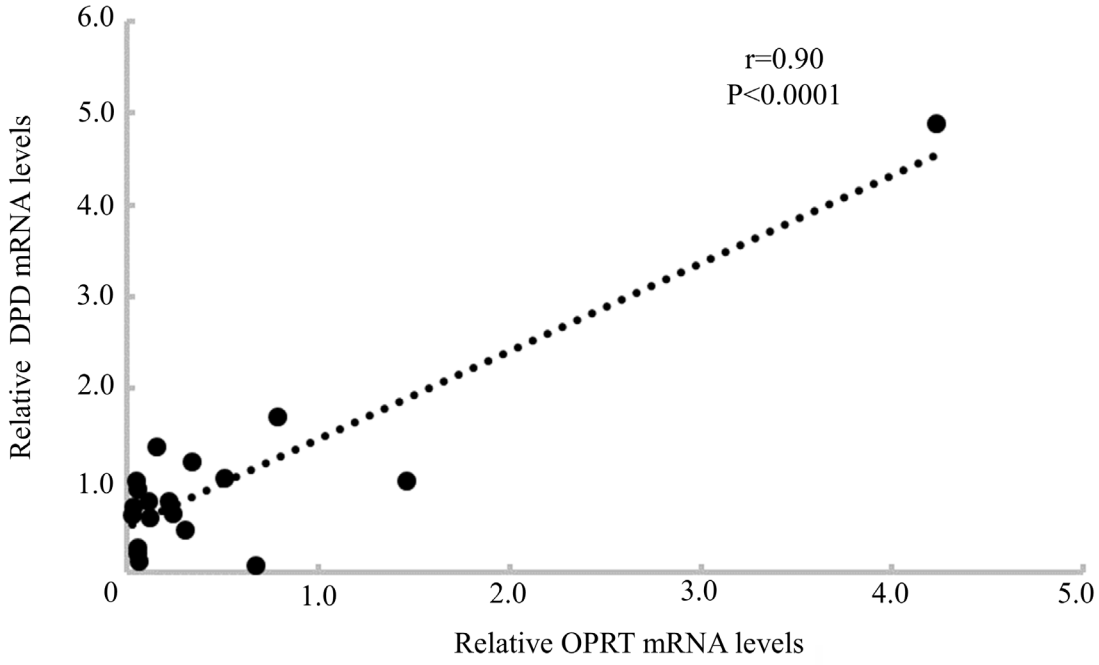

Figure 2. Correlation between intratumoral OPRT and DPD mRNA expression levels in EMPD.

any clinical information (Table 1).

3.2. *Intratumoral DPD mRNA levels positively correlated with OPRT mRNA levels*

We analyzed the relationship among intratumoral TS/OPRT/DPD mRNA expression levels compared to those in non-lesional tissues in EMPD. Although there were no differences in the correlation between TS/DPD (r < 0.01) or TS/OPRT (r = 0.05) mRNA levels, there was statistical correlation between OPRT and DPD mRNA expression levels (r = 0.90, p < 0.0001) (Figure 2).

We have investigated the intratumoral mRNA expression levels of 5-FU metabolism in EMPD and revealed three major findings. First, intratumoral DPD mRNA expression levels in EMPD were significantly lower compared to those in normal skin. Although the correlation between expression levels of 5-FU metabolism and the clinical efficacy of S-1 based therapy has been investigated, few studies have compared these expression levels in paired lesional and non-lesional tissues. Intratumoral DPD mRNA levels in colorectal cancers are higher compared with normal tissues, while those in gastric cancers are lower (*15*). This suggests that the mRNA expression levels of 5-FU metabolism differ in different for various types of malignant tumors.

Second, intratumoral DPD mRNA expression levels were not correlated with clinical features including prognosis. This result may be because of the small sample size. Using immunohistochemistry, it was determined that patients with DPD-positive tumors have significantly poorer prognosis than those with DPD-negative tumors in breast cancer (*16*). In several studies, lower intratumoral DPD mRNA levels were found to be correlated with good response to 5-FU (*17,18*). Additionally, in non-small cell lung cancer, low DPD protein expression level was correlated with longer survival and positive response to S-1/carboplatin therapy (*19*). There is no report about the connection between intratumoral DPD mRNA expression levels

and therapeutic response to S-1 in EMPD. To clarify this important subject, the accumulation of clinical case study is necessary. Taken together, a large sample size may confirm that EMPD is associated with good prognosis and efficacy following 5-FU-based chemotherapy because intratumoral DPD mRNA levels are decreased in EMPD.

Finally, DPD mRNA levels were positively correlated with OPRT mRNA levels although intratumoral OPRT mRNA expression levels were not increased. This result may be an outlier, with remarkably increased DPD and OPRT levels. In large-scale population analysis, the DPD/OPRT ratio varies in several cancers (*15*). Further investigations are needed to be clarify this.

In conclusion, we found that intratumoral DPD mRNA levels are overexpressed in EMPD. However, our results are limited because of this retrospective study evaluated a small sample size.

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