Changes in Thymidine Phosphorylase Gene Expression Related to Treatment of Rectal Cancer

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Abstract. *Background: The enzyme thymidine phosphorylase (TYMP) has tumor-promoting functions and its expression is often elevated in tumors. Patients and Methods: TYMP gene expression in tumorous and mucosal tissues was assessed using real-time polymerase chain reaction, in a study of patients with rectal cancer where chemotherapy and radiotherapy were given sequentially. Results: TYMP levels decreased after chemotherapy. For patients given radiotherapy, there was a significant increase in TYMP expression comparing biopsies before and after radiotherapy. The increase was also observed in the mucosa, although it was less pronounced. Conclusion: Cancer treatment alters gene expression in tumor and adjacent mucosa of patients with rectal cancer. Chemotherapy may cause a decrease in TYMP gene expression, whereas radiotherapy, given as adjuvant treatment, causes a significant increase in expression. These results are of importance when interpreting TYMP expression data in rectal cancer and may be of clinical interest as TYMP participates in the activation of capecitabine.*

Thymidine phosphorylase (TYMP) is an enzyme (E.C. 2.4.2.4) with a regulatory role in the pyrimidine metabolism in the cell. Although expression of TYMP has been found in tumor epithelial cells, it seems to be particularly expressed in the interstitia by stromal cells such as macrophages, monocytes and fibroblasts, as well as in endothelial cells (1, 2). An elevated TYMP level has been found in several solid tumor types, including colorectal carcinomas (3-5). TYMP has several tumor-promoting functions, one of which is related to angiogenesis. This gene has also been correlated to factors such as vascular endothelial growth factor and microvessel density (6-9). In hypoxic environments, TYMP seems to mediate resistance to apoptosis in tumor cells and plays a part in the inflammatory process (10). Indeed, TYMP is increased in several inflammatory diseases, such as inflammatory bowel disease and rheumatoid arthritis (11-13). Inflammatory cytokines such as interleukin-8, tumor necrosis factor-α, and interferon-γ have been shown to up-regulate TYMP (12, 14, 15). In a previous study, we noted that rectal cancer tissue had higher *TYMP* gene expression compared to colon cancer tissue (16). The theory that this in part was irradiation-induced expression is supported by a few other studies (17, 18). A decrease in TYMP, on the other hand, has been noted after start of chemotherapy (19). The aim of the present study was to validate the findings by comparing *TYMP* gene expression in rectal tumor and mucosal tissue of patients before and after preoperative treatment with chemo- and radiotherapy. The hypothesis was that treatment can alter *TYMP* gene expression and that radiotherapy induces an inflammatory response in the affected tissue, leading to an increase in *TYMP* gene expression.

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Patients and Methods

Patients and study design. A prospective phase I/II study on preoperative chemotherapy using pemetrexed (Alimta) was conducted between June 1, 2006 and January 30, 2008, with a total of 37 patients included (20). The inclusion criteria were a confirmed diagnosis of an adenocarcinoma of the rectum and good general condition. Patients who were planned for local excision or palliative surgery were excluded. All included patients received three cycles of intravenous infusion of pemetrexed at 500 mg/m^2 along with vitamin supplementation and dexamethasone pre-operatively. Surgery and, if appropriate, preoperative radiotherapy, were given according to regular guidelines. Radiotherapy was given as 5 Gy for five days, the week before the scheduled operation, when indicated. Magnetic resonance imaging scans were scheduled before and after chemotherapy, and included an assessment of tumor size. Tissue biopsies, from both tumor and adjacent macroscopically normal mucosa (10 cm from tumor), were obtained before treatment (biopsy I), after completion of chemotherapy (and thus before radiotherapy; biopsy II) and at time of surgery (biopsy III). A schematic overview

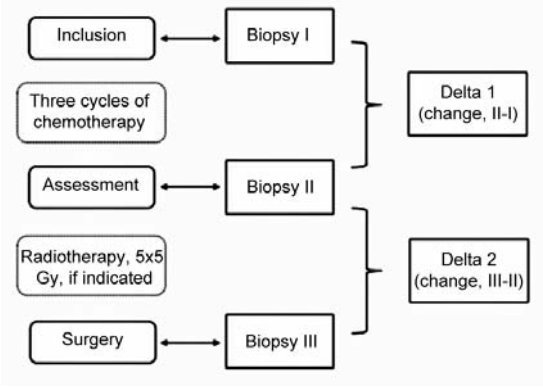

Figure 1. *Overview of the pemetrexed (Alimta) study showing the time of biopsy sampling. Alterations in relative TYMP gene expression measured on two occasions were expressed as delta values.*

of the study is shown in Figure 1. The regional Ethical Review Board approved the study (129-06) and participation was voluntary. For the present study, we included all patients where the biopsy series were complete with three samples each from tumor and mucosa. One patient was excluded due to long-term radiotherapy and in a further eight cases, the samples were incomplete. A common cause for missing samples was difficulty in examination due to tumor stenosis or pain. The tissue samples were assessed by real-time quantitative polymerase chain reaction (PCR) for the relative *TYMP* gene expression as described below. The gene expression was recorded by treatment and samples, as well as by time-point for possible changes during the sampling intervals. Patient charts were reviewed and both clinical and pathology data were recorded. The *TYMP* gene expression was then correlated to the given treatment and to pathological data.

Total RNA extraction, cDNA preparation and real-time quantitative PCR. Tissue samples were snap-frozen in liquid nitrogen and stored at −80˚C until used. Total RNA was isolated from 10-30 mg tissue using the High Pure RNA Tissue Kit (Roche Diagnostics GmbH, Mannheim, Germany). cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and run on a Perkin Elmer Gene Amp PCR System 9600 system (Norwalk, CT). Real-time quantitative PCR was performed using the 7500 Fast Real-Time PCR system (Applied Biosystems). The expression of the target gene, *TYMP*, was quantified using assay-on-demand from Applied Biosystems (Hs00157317_m1). The relative TYMP gene expression was calculated using *β-actin* (*ACTB*) as a reference gene. *β-actin* was also used as an endogenous control to compensate for variation in the amount of RNA and to check the efficiency of the reverse transcription reaction. Primer and probe sequences, and multiplex PCR conditions were as described previously (16).

Statistics. JMP 8.0 statistics software (SAS Inc., Cary, NC, USA) was used for statistical analyzes. Descriptive statistics of demographics and *TYMP* gene expression are shown by median values and ranges. Statistical comparisons were made mainly by t-test/ANOVA, but due to the limited number of patients, also cross-checked by non-parametric tests. The treatment-related changes by

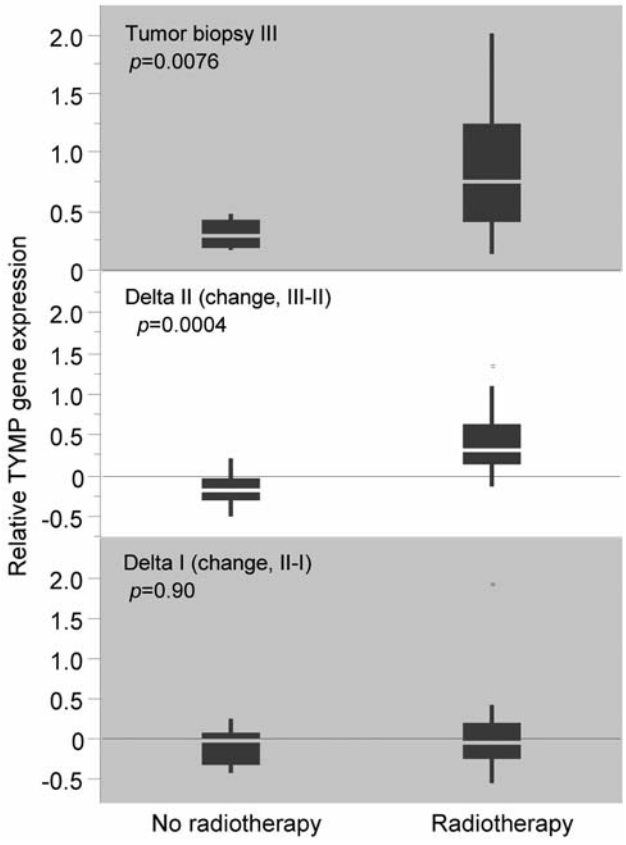

Figure 2. *Effect of radiotherapy on relative TYMP gene expression in rectal tumor tissue. Alterations in TYMP gene expression measured on two occasions were expressed as delta values. Biopsy I was obtained before treatment, biopsy II after completion of chemotherapy (before radiotherapy, if given), and biopsy III at the time of surgery. The distribution of TYMP expression is displayed as box-and-whisker plots with median, 25% and 75% quantiles (box), minimum and maximum (whisker), and outliers (dots). A significant increase in gene expression was only seen after radiotherapy (5X5 Gy), and not after chemotherapy alone.*

both chemotherapy and radiotherapy in *TYMP* expression was also assessed by a non-parametric test in matched-pair analysis. The significance level was set at 95%.

Results

The median age of the patients was 61 (range 34-82) years. There were 20 males and 8 females. Eight patients had stage I disease and 10 each had stage II and III, respectively. There was no statistical correlation between either demographics or staging and *TYMP* expression in tumor or mucosa. The median *TYMP* expression was significantly higher in tumors compared to mucosa, regardless of sampling occasion. Twenty patients were eligible for, and received, radiotherapy while eight were not. *TYMP* gene expression grouped by radiotherapy is shown in Table I. As shown, the *TYMP*

Table I. *The relative median (range) thymidine phosphorylase (TYMP) gene expression, before and after chemotherapy, and at surgery, stratified by treatment with radiotherapy.*

		Median relative TYMP gene expression (range)				
		Biopsy I (Before chemotherapy)	Biopsy II (After chemotherapy)	Biopsy III (At surgery)	Change, biopsy II-I (Delta 1)	Change, biopsy III-II (Delta 2)
No radiotherapy (n=8)	Tumor	0.52 (0.29-0.87)	0.48 (0.26-0.67)	0.30 (0.17-0.48)	–0.020 (–0.42-0.25)	–0.19 (–0.50-0.22)
	Mucosa	0.28 (0.10-0.57)	0.29 (0.14-0.42)	0.20 (0.12-0.30)	0.010 (–0.24-0.16)	–0.075 (–0.25-0.070)
Radiotherapy 5×5 Gy (n=20)	Tumor	0.31 (0.10-1.03)	0.26 (0.12-2.14)	0.74 (0.14-2.01)	–0.040 (–0.54-1.92)	0.30 (–0.13-1.35)
	Mucosa	0.22 (0.14-0.46)	0.19 (0.12-0.63)	0.38 (0.13-1.15)	0.015 (–0.28-0.41)	0.18 (–0.30-0.82)

expression in tumor tissue decreased slightly after chemotherapy. In patients who did not receive radiotherapy, the expression decreased further over time, as seen in tumor biopsy III. The difference in *TYMP* gene expression between biopsies I and III was significant (p=0.046). In patients subjected to radiotherapy, on the other hand, there was a significant increase in *TYMP* gene expression in biopsy III compared to biopsies I and II (p=0.0040 and p=0.0003, respectively). The significant difference was also seen between values for biopsies II and III (Figure 2), when patients were sub-grouped according to radiotherapy (p=0.0004). The increase was also seen in the mucosa, but was not as prominent.

Discussion

The decrease in *TYMP* gene expression seen after chemotherapy is of interest. It concurs with the findings in the Alimta trial of a high degree of tumoral response seen as size reductions (20). The finding also concurs with those of other studies. Both in cell lines, as described by Miyazaki *et al*. (21), and in the clinical setting as reported by Bartsch *et al*. (19), TYMP expression in plasma was affected by treatment. Radiotherapy has previously been shown to induce inflammation and matrix remodeling, thereby also increasing diverse biomarkers such as matrix metalloproteinases (22). Thus, it is plausible that radiotherapy induces an increase in TYMP as part of the inflammatory process. In the present study, we found a significant increase in *TYMP* expression in biopsy III compared to biopsy II for patients given radiotherapy. Of note is also the increased *TYMP* expression in irradiated mucosa, although this was less prominent than the increase in tumor. There are possible challenges when interpreting TYMP as a marker in rectal cancer assessed from single biopsies. TYMP has been shown to have a higher expression in more advanced tumors with a higher degree of lymph node involvement and a worse histological tumor grade (16). Patients with more advanced tumors are also likely to receive pre-operative radiotherapy, with or without the addition of chemotherapy, which could further increase the TYMP level. As a majority of patients with rectal cancer are often given some form of radiotherapy, the overall TYMP level, on group basis, could be interpreted as being higher than in colon cancer. Moreover, any decrease due to chemotherapy would be hidden by the effect of radiotherapy. A potential clinical interest in TYMP emanates from one of its enzymatic functions. It catalyzes the last step in the conversion of the oral pro-drug capecitabine (Xeloda) to 5-fluorouracil, which is still the main agent used in colorectal cancer treatment. The duality of roles in cancer development and treatment has been described in previous publications, including a review by Bronckaers *et al*. (5). A selective generation of 5-FU due to a higher TYMP activity in tumor cells compared to normal cells has been suggested to increase the therapeutic ratio. In a recently published study on stage III colon and rectal cancers, we failed to detect any significant difference in *TYMP* gene expression between tumorous and mucosal tissues (16). In the present study, however, there was a significant difference and, furthermore, irradiation was found to up-regulate *TYMP* expression to a higher level in tumor tissues compared to the adjacent mucosa. These results are in support of a previous study by Sawada *et al*. (18), who found that TYMP protein expression was up-regulated in human cancer xenografts by irradiation. In those experiments, whole-body irradiation also up-regulated TYMP protein expression in tumors, but did not increase the enzyme's levels in the liver. The expression of TYMP protein in response to irradiation was evaluated by Kim *et al*. (17) in tumor tissues from 22 patients with locally advanced rectal cancer before and after irradiation. The expression of TYMP protein was found to be increased in 82% of cases compared to the baseline value after one week of irradiation. Since the best response associated with capecitabine appears to be linked to high expression of TYMP, the use of capecitabine and

irradiation in a pre-operative setting is likely to be a useful treatment strategy for patients with rectal cancer. The present study is based on tissue material gathered in one of few studies on pre-operative chemotherapy. The use of multiple biopsies from the same patients, treated sequentially, first with chemotherapy and then XRT, all accrued in a standardized manner, makes the material almost unique. There are some limitations in the present study, including the low number of patients. Another common limitation, in studying gene expression, is that we do not know if there is a positive correlation between TYMP expression at the gene and protein levels. Still, the study indicates that *TYMP* gene expression can be altered by the given treatment. The findings are of importance for future research in the field. One finding of potential clinical interest, warranting further studies, is the radiotherapy-induced increase in *TYMP,* related to the capacity for activating capecitabine and possible improvement of treatment effect. In conclusion, cancer treatment can alter gene expression in both tumor and the adjacent mucosa of patients with rectal cancer. Chemotherapy may cause a decrease in *TYMP* gene expression, whereas radiotherapy, given as adjuvant treatment, does cause a significant increase in *TYMP* gene expression. Induction of high *TYMP* expression by radiotherapy might sensitize tumors to the effect of capecitabine.

Conflicts of Interest

All Authors report no conflicts of interest in this work.

Acknowledgements

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