A possible role of thymidine phosphorylase expression and 5-fluorouracil increased sensitivity in oropharyngeal cancer patients

G. Ranieri a, b, L. Grammatica c, R. Patruno d, A.F. Zito e, P. Valerio d, S. Iacobellis d, C. Gadaleta b, G. Gasparini f, D. Ribatti g *

a Department of Experimental Oncology, National Cancer Institute of Bari, Bari, Italy
b Unit of Interventional Radiology, Department of Critical Area and Surgery, National Cancer Institute of Bari, Bari, Italy
c Department of Oncological Surgery, Head and Neck Section, National Cancer Institute of Bari, Bari, Italy
d Division of Surgery, Di Venere Hospital, Bari, Italy
e Unit of Pathology, National Cancer Institute of Bari, Bari, Italy
f Division of Medical Oncology, Azienda Ospedaliera San Filippo Neri, Roma, Italy
g Department of Human Anatomy and Histology, University of Bari Medical School, Bari, Italy

Received: September 21, 2006; Accepted: December 4, 2006

Abstract

Thymidine P₁ deoxyribosyltransferase (TP) is an enzyme involved in DNA synthesis up-regulated in tumours and it is also a pro-angiogenic factor. TP cannot activate capecitabine, because capecitabine first needs conversion by carboxylesterase and cytidine deaminase into 5’-deoxy-fluorouridine. This compound can be activated by TP to 5-fluorouracil (5-FU). Although TP is not necessary for 5-FU toxicity, experimental data suggest that high levels of TP correlate with an enhanced response to 5-FU therapy. In this study, we have analysed by immunohistochemistry CD34, CD68 and TP positive cells in biopic samples from 53 patients with T₁–₃ N₀–₁ M₀ oropharyngeal squamous cell carcinoma (OSC) and from 24 patients with non-dysplastic oropharyngeal leukoplakia (NDOLP). Results showed that the mean of TP-positive cells, CD68 positive macrophages and CD34 positive endothelial cells evaluated as microvessel density (MVD) was significantly higher in OSC than in NDOLP. Moreover, at a median follow-up of 19 months, patients with TP expression and higher MVD showed a better survival rate as compared to those with low MVD, probably as a consequence of 5-FU-based therapy. We hypothesized a role for TP in oropharyngeal tumourigenesis and 5-FU activation in the adjuvant setting of OSC patients.

Keywords: capecitabine • 5-fluorouracil • leukoplakia • oropharyngeal squamous carcinoma • platelet derived-endothelial cell growth factor • thymidine phosphorylase

Introduction

Thymidine P₁ deoxyribosyltransferase (TP) is an enzyme involved in nucleic acid homoeostasis [1–3]. The mean function of TP in the cells is and the phosphorolytic cleavage of thymidine into thymine and 2-deoxyribose-1-phosphate. The reverse reaction is possible, but depends on the availability of the co-substrate 2-deoxyribose-1-phosphate [4–6]. Accordingly, TP is not important for 5-fluorouracil (5-FU) toxicity.

Activation of 5-FU by other pathways, such as uridine phosphorylase and orotate phosphoribosyl-
transferase, is much more important [7]. However, experimental studies suggest that high levels of TP correlate with an enhanced response to the fluoropyrimidine [8, 9]. *In vitro* data indicated that the transfection of TP cDNA into cancer cells increased their sensitivity to 5-FU [10] and the expression of TP is useful for predicting the efficacy and survival of fluoropyrimidine chemotherapy [11]. Accordingly, when 5-FU is administered it is converted to 5-fluoro-2-deoxyuridine by TP, which in turn is converted to 5-fluoro-2-deoxyuridine monophosphate (FdUMP) by thymidine kinase. Finally, FdUMP binds to and inhibits thymidylate synthase (TS) in the presence of 5,10-methylene tetrahydrofolate. Inhibition of TS disrupts intracellular nucleotide pools necessary for DNA synthesis and it is considered one of the principal mechanisms of 5-FU toxicity [7].

TP plays also a key role in the activation of N\(^4\)-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine, commonly called capecitabine (CAP), generating 5-FU at the tumour site. After oral administration, CAP first is converted by hepatic carboxylesterase and cytokine deaminase into 5'-deoxy-5-fluorouridine, which in turn is converted to 5-FU by TP [12].

A putative role of TP in human malignancies has been suggested since the early 1960s and an increased epithelial TP expression has been demonstrated in breast, colon, lung and oral cancer [13–17]. Immunohistochemical expression of TP has been recognized in human epithelial cells, endothelial cells, macrophages and mast-cells [18]. On the other hand, TP corresponds to platelet-derived endothelial cell growth factor (PDECGF), which is involved in stimulation of endothelial cell migration [19, 20].

High TP tumour cell expression is a poor prognostic indicator in oral and oropharyngeal carcinoma [21–23]. However, little information is available on the correlation between microvessel density (MVD) and TP with fluoropyrimidine therapy or clinical outcome, on TP expression in non malignant and malignant oropharyngeal tissue and on the potential role of TP activated 5-FU in oropharyngeal squamous cell carcinoma (OSC) [24–26].

Here, we have analysed by means of immunohistochemistry and morphometry the density of epithelial cells positive to TP, macrophages positive to CD68, and endothelial cells positive to CD34 in OSC and in non-dysplastic oropharyngeal leukoplakia (NDOLP) and we have correlated TP positivity to clinical outcome.

### Patients and methods

#### Patients

Formalin-fixed paraffin-embedded samples of oropharyngeal mucosa from 24 patients with NDOLP and 53 patients with OSC (T\(_{1−3}\) N\(_{0−1}\) M\(_{0}\), treated at the National Cancer Institute of Bari, were selected for the study. NDOLP was histopathologically defined by hyperparakeratosis and papillary hyperplasia, but not by dysplasia. Clinical-pathological parameters of patients with OSC are shown in Table 1. Primary tumour size and lymph node status were classified according to UICC criteria [27]. After surgery, all the patients were evaluated for an adjuvant clinical trial based on a sequential chemoradiotherapy. Patients with either infiltrated tumour resection margins (39 cases) or pathological lymph node involvement (29 cases) or perineural/perivascular invasion (28 cases) received adjuvant treatment. Briefly, 8 cases (T\(_{1}\)N\(_0\) with negative resection margins and no perineural/perivascular invasion) underwent tumour resection only, while 45 cases underwent tumour resection followed by three course of 5-FU plus cisplatin (SF-U 1000 mg/m\(^2\) i.v. over 96 hrs days 1–4, and cisplatin 100 mg/m\(^2\) i.v. on days 1–3).
i.v. day 1 every 28 days at first, fifth and ninth week followed by fractionated radiotherapy (five fraction/week) total dose 60 Gy. A 5-year follow-up was conducted in the Outpatient Clinic of the Institute and updated in December 2003, the median time of follow-up was 19 months.

Immunohistochemical assay

Six-micrometre thick serial sections of formalin-fixed, paraffin-embedded OSC and NDOLP tissue were deparaffinized by the xylene-ethanol sequence. For antigen retrieval, the sections were microwaved at 500 W for 10 min., after which endogenous peroxidase activity was blocked with 3% hydrogen peroxide solution. Adjacent sections were incubated with monoclonal antibodies anti-TP (P-GF.44C Neo-Markers, Fremont, CA) diluted 1:100 for 1 hr at room temperature; anti-CD34 (QB-END 10; Bio-Optica, Milan, Italy) pan-endothelial marker diluted 1:50 for 1 hr at room temperature; anti CD68 (KP1; Dako) macrophage marker diluted 1:100 for 1 hr at room temperature [22, 28]. The bound antibody was visualized using biotinylated secondary antibody, avidin-biotin peroxidase complex, and 3-aminobenzidine. Nuclear counterstaining was performed with Gill's haematoxylin number 2 (Polysciences, Warrington, PA) [18]. Primary antibody was omitted in negative controls.

TP expression was determined in five 400 × fields by the image analysis system (Quantimet 500 Leica) and TP positivity was evaluated on the basis of stained epithelial, macrophages and endothelial cells in terms of MVD [18, 24, 29]. Endothelial cells were identified as CD34- and TP-positive cells and MVD was evaluated in terms of both TP and CD34 immunostained vessels accordingly to Weidner’s method with slight modifications [29]. Macrophages were identified as CD68- and TP-positive cells. Mean values ± standard deviation of epithelial cells, macrophages and MVD in both NDOLP and OSC was determined for each section and group of samples. Median value of epithelial cells, macrophages and MVD in OSC positive to TP was determined for each section and group of sample and was used as a cut-off to distinguish between high and low TP reactivity.

Statistical analysis

The association between TP expression and histological diagnosis (NDOLP versus OSC) was evaluated by Student’s t-test. The correlation between TP expression and the clinical pathological characteristics of OSC was analysed by chi-square test. Survival curves were traced according to Kaplan-Meyer and the differences analysed with the log-rank test (level of significance <0.05). No adjustment for confounding factors was performed in survival analysis. TP expression was determined independently by two investigators (GR and AFZ). Good interobserver reproducibility of the determination of TP expression was guaranteed by a coefficient of concordance >0.90 (K) for all the variables evaluated. Calculations were performed using the SPSS Package (SPSS, Inc., Chicago, IL).

Results

TP immunoreactivity was observed in normal epithelial cells (Fig. 1A), cancer cells (Figs 1B and 3B), macrophages (Fig. 2B) and endothelial cells (Fig. 3B). In the epithelial cells the pattern of TP positive staining was either or only cytoplasmic (Fig. 1A and B) or mixed
cytoplasmic and nuclear (Fig. 3B). Macrophages positive to TP showed a thin cytoplasmic pattern and occasionally a mixed cytoplasmic and nuclear pattern (Fig. 2B), while the pattern of TP positivity in endothelial cells was both nuclear and cytoplasmic (Fig. 3B). Endothelial cells were positive to CD34 (Fig. 3A) and macrophages to CD68 (Fig. 2A). The mean values of epithelial cells, macrophages and endothelial cells positivity were significantly higher in OSC specimens than in NDOLP (Table 2).

The association between different TP expressing cells and the main clinical-pathological characteristics was also analysed. The percentage of tumours with high TP expression was similar irrespective of age, sex, among pT1, pT2 and of pT3 tumours, in N0 and N1 nodes and in tumours with different cytopathological grade.

As concerns the margins of the surgical specimens, due to tumour size and in particular tumour location at oropharyngeal sites, radical surgery was obtained in 14 cases, while 39 patients presented positive resection margins. At a median follow-up of 19 months, a significant better overall survival (OS) was found for the OSC patients with the higher MVD positive to TP as compared to the OSC patients with the lower MVD positive to TP (75% and 45%, respectively; P = 0.03 log-rank test) (Fig. 4).

Discussion

TP has multifunctional roles being an enzyme involved in nucleotide salvage and associated with 5-fluorouracil activation [3, 30–32]. It is also a main pro-angiogenic factor and in several studies increased TP expression was found in highly vascularized tumours [24, 33–36]. MVD has been previously evaluated using pan-endothelial markers such as anti-CD34, anti-CD31 or antifactor-VIII antibodies, and never as endothelial cells positive to TP [18, 37–39]. Here, we evaluated MVD in terms of endothelial cells positive to anti-CD34 and anti-TP antibodies and we demonstrated a significantly higher MVD in TP-positive specimens in patients affected by OSC as compared to those affected by NDOLP.

TP overexpression in epithelial cells and macrophages has been reported in pre-malignant lesions such as gynecological cervical cancer and in oropharyngeal precancerous lesions, as well as in several solid tumours, such as breast, colorectal, bladder, stomach, lung, oral squamous carcinoma [13–17] and oropharyngeal carcinoma, where TP-positivity has been correlated to tumour proliferating cell nuclear antigen positivity, suggesting that TP expression reflected cell proliferation, as a consequence of carcinogenesis in leukoplakia [21].

Here, we have demonstrated a significant increase in positivity of epithelial cells to TP and of macrophages to both CD68 and TP antibodies and these data suggests that TP expression increases in parallel with tumourigenesis of oropharyngeal mucosa. As concerns the clinical outcome, several prognostic studies have been published on TP expression in differently tumour type and no correlation between TP expression and clinical outcome in breast, bladder and gastric cancer has been reported [19, 38].
On the contrary, a correlation between TP expression and a poor prognosis has been described in colorectal cancer [14]. In oral and oropharyngeal cancer, literature data suggested that TP is a poor prognostic indicator [22, 23], while our data do not establish a correlation between TP expression and the main clinical-pathological parameters such as sex, age, cytohistological grade, tumour size or nodal status. However, we found that patients with OSC with the higher MVD showed a better overall survival as compared to the patients with the lower MVD, probably as a consequence of 5-FU adjuvant therapy. However, in the subgroup of 8 N0 patients affected by OSC with negative surgical margins and no perineural/perivascular invasion that not received adjuvant 5-FU, 3 presented a high MVD with a better overall survival, while 5 presented a low MVD with a worse overall survival. Literature data, showing that oral and oropharyngeal cancer with high TP expression are more responsive to 5-FU cytotoxicity than those with low TP expression, supported our results [15, 40].

Overall, our data suggest a role for TP in oropharyngeal tumorigenesis and 5-FU based therapy seems to be a promising adjuvant therapy in OSC patients with high TP expression.

| Oropharyngeal squamous carcinoma n = 53 | Epithelial TP expression (percentage of positive cells at 400 x 0.19 mm² area) | Macrophages CD-68 expression (number of positive cells at 400 x 0.19 mm² area) | Macrophages TP expression (number of positive cells at 400 x 0.19 mm² area) | MVD CD-34 expression (number of positive vessels at 400 x 0.19 mm² area) | MVD TP expression (number of positive vessels at 400 x 0.19 mm² area) |
|---------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|
| 64 ± 29%*                             | 15 ± 8*                                                                         | 13 ± 7*                                                                         | 36 ± 15*                                                                       | 33 ± 14*                                                        |
| Non-dysplastic oropharyngeal leukoplakia n = 24 | 19 ± 14%*                                                                      | 8 ± 4*                                                                          | 7 ± 4*                                                                         | 18 ± 13*                                                       | 16 ± 11*                                                        |

*Mean ± standard deviation.

Table 2  Epithelial cells positive to TP; macrophages positive to CD68 and TP; MVD positive to CD-34 and TP

Fig. 3  (A) Arrows indicate CD34 positive endothelial cells in OSC. (B) Arrows indicate TP positive endothelial cells in OSC. Malignant epithelial cells are positive to TP with mixed nuclear and cytoplasmic immunoreactivity. Original magnification (A, B) × 400.
References

1. Brown NS, Bicknell R. Thymidine phosphorylase, 2-deoxy-d-ribose and angiogenesis. Biochem J. 1998; 334: 1–8.
2. Friedkin M, Roberts DW. The enzymatic synthesis of nucleosides. I. Thymidine phosphorylase in mammalian tissue. J Biol Chem. 1954; 207: 245–6.
3. Friedkin M, Roberts D. The enzymatic synthesis of nucleosides. II. Thymidine and related pyrimidine nucleosides. J Biol Chem. 1954; 207: 257–66.
4. Blank JG, Hoffee PA. Purification and properties of thymidine phosphorylase from Salmonella typhimurium. Arch Biochem Biophys. 1975; 168: 259–65.
5. Kubilus J, Lee LD, Baden HP. Purification of thymidine phosphorylase from human amniochorion. Biochim Biophys Acta. 1978; 527: 221–8.
6. Voytek P. Purification of thymidine phosphorylase from Escherichia coli and its photoactivation in the presence of thymine, thymidine, and some halogenated analogs. J Biol Chem. 1975; 250: 3660–5.
7. Grem JL. 5-Fluoropyrimidines. In: Chabner Longo DL, editor. Cancer chemotherapy and biotherapy. 3rd ed. Philadelphia: Lippincott Raven; 2001. p. 186–264.
8. Evrard A, Cuq P, Robert B, Vian L, Pelegrin A, Cano JP. Enhancement of 5-fluorouracil cytotoxicity by human thymidine-phosphorylase expression in cancer cells: in vitro and in vivo study. Int J Cancer. 1999; 80: 465–70.
9. Ciccolini J, Cuq P, Evrard A, Giacometti S, Pelegrin A, Aubert C, Cano JP, Illiadis A. Combination of thymidine phosphorylase gene transfer and deoxynosine treatment greatly enhances 5-fluorouracil antitumour activity in vitro and in vivo. Mol Cancer Ther. 2001; 1: 133–9.
10. Marchetti S, Chazal M, Dubreuil A, Fischel JL, Etienne MC, Milano G. Impact of thymidine phosphorylase overexpression on fluoropyrimidine activity and on tumour angiogenesis. Br J Cancer. 2001; 85: 439–45.
11. Saito H, Tsujitani S, Oka S, Kondo A, Ikeguchi M, Maeta M, Kaibara N. The expression of thymidine phosphorylase correlates with angiogenesis and the efficacy of chemotheraputic using fluorouracil derivatives in advanced gastric carcinoma. Br J Cancer. 1999; 81: 484–9.
12. Martino MM, Martino R. Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): a review. Oncologist. 2002; 7: 288–323.
13. Fox SB, Westwood M, Moghaddam A, Comley M, Turley H, Whitehouse RM, Bicknell R, Gatter KC, Harris AL. The angiogenic factor platelet-derived endothelial cell growth factor/thymidine phosphorylase is up-regulated in breast cancer epithelium and endothelium. Br J Cancer. 1996; 73: 275–80.
14. Takebayashi Y, Akiyama S, Akiba S, Yamada K, Miyadera K, Sumizawa T, Yamada Y, Murata F, Aikou T. Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma. J Natl Cancer Inst. 1996; 88: 1110–7.
15. Fujieda S, Sunaga H, Tsuzuki H, Tanaka N, Saito H. Expression of platelet-derived endothelial cell growth factor in oral and oropharyngeal carcinoma. Clin Cancer Res. 1998; 4: 1583–90.
16. Giatromanolaki A, Koukourakis, MI, Comley M, Kaklamanis L, Turley H, O’Byrne K, Harris AL, Gatter KC. Platelet-derived endothelial cell growth factor (thymidine phosphorylase) expression in lung cancer. J Pathol. 1997; 181: 196–9.
17. Maeda K, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T, Onoda N, Kato Y, Sowa M. Thymidine phosphorylase/platelet-derived endothelial cell growth factor expression associated with hepatic metastasis in gastric carcinoma. Br J Cancer. 1996; 73: 884–8.
18. Ranieri G, Gasparini G. Surrogate markers of angiogenesis and metastasis. In: Brooks S, editor. Metastasis research protocols. Oxford: Oxford University Press; 2001. pp. 94–114.
19. Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, Usuki K, Takaku F, Risau W, Heldin CH. Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature. 1989; 338: 557–62.
20. Moghaddam A, Zhang HT, Fan TPD, Hu DE, Lees VC, Turley H, Fox SB, Gatter KC, Harris AL, Bicknell R. Thymidine phosphorylase is angiogenic and promotes tumor growth. Proc Natl Acad Sci USA. 1995; 92: 998–1002.
21. Sunaga H, Fujieda S, Tsuzuki H, Asamoto K, Fukuda M, Saito H. Expression of granulocyte colony-stimulating factor receptor and platelet-derived endothelial cell
growth factor in oral and oropharyngeal precancerous lesions. Anticancer Res. 2001; 21: 2901–6.

22. Tsuzuki H, Sunaga H, Ito T, Narita N, Sugimoto C, Fujieda S. Reliability of platelet-derived endothelial cell growth factor as a prognostic factor for oral and oropharyngeal carcinomas. Arch Otolaryngol Head Neck Surg. 2005; 131: 1071–8.

23. Fujieda S, Sunaga H, Tsuzuki H, Fan GK, Saito H. IL-10 expression is associated with the expression of platelet-derived endothelial cell growth factor and prognosis in oral and oropharyngeal carcinoma. Cancer Lett. 1999; 8: 136; 1–9.

24. Ranieri G, Labriola A, Achille G, Florio G, Zito AF, Grammatica L, Paradiso A. Angiogenesis, thymidine phosphorylase, and resistance of squamous cell head and neck cancer to cytotoxic and radiation therapy. Clin Cancer Res. 2000; 6: 381–9.

25. Koukourakis MI, Giatromanolaki A, Fountzilas G, Sivridis E, Gatter KC, Harris AL. Angiogenesis, thymidine phosphorylase, and resistance of squamous cell head and neck cancer to cytotoxic and radiation therapy. Clin Cancer Res. 1999; 8: 136; 1–9.

26. Zatterstrom UK, Brun E, Willen R, Kjellen E, Wennnerberg J. Tumor angiogenesis and prognosis in squamous cell carcinoma of the head and neck. Head Neck. 1995; 17: 312–23.

27. Sobin LH, Fleming ID. TNM classification of malignant tumours, fifth edition. Union Internationale Contre le Cancer and the American Joint Committee on Cancer. Cancer. 1997; 80: 1803–4.

28. Zatterstrom UK, Brun E, Willen R, Kjellen E, Wennnerberg J. Tumor angiogenesis and prognosis in squamous cell carcinoma of the head and neck. Head Neck. 1995; 17: 312–23.

29. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis correlation in invasive breast carcinoma. N Engl J Med. 1991; 324: 1–8.

30. Morita T, Matsuzaki A, Suzuki K, Tokue A. Role of thymidine phosphorylase in biomodulation of fluoropyrimidines. Curr Pharm Biotechnol. 2001; 2: 257–67.

31. Birnie GD, Kroeger H, Heidelberger C. Studies of fluorinated pyrimidines. XVIII. The degradation of 5-fluoro-2'-deoxyuridine and related compounds by nucleoside phosphorylase. Biochemistry. 1963; 2: 566–72.

32. Tokunaga Y, Takahashi K, Saito T. Clinical role of thymidine phosphorylase and dihydroxyuridine dehydrogenase in colorectal cancer treated with postoperative fluoropyrimidine. Hepatogastroenterology. 2005; 52: 1715–21.

33. Nagaoka H, Hino Y, Takei H, Morishita Y. Platelet-derived endothelial cell growth factor/thymidine phosphorylase expression in macrophages correlates with tumor angiogenesis and prognosis invasive breast cancer. Int J Oncol. 1998; 13: 449–54.

34. Engels K, Fox SB, Whitehead RM, Gatter KC, Harris AL. Up-regulation of thymidine phosphorylase expression is associated with a discrete pattern of angiogenesis in ductal carcinomas in situ of the breast. J Pathol. 1997; 182: 414–20.

35. Takebayashi Y, Yamada K, Miyadera K, Sumizawa T, Furukawa T, Kinoshita F, Aoki D, Okumura H, Yamada Y, Akizawa S, Aikou T. The activity and expression of thymidine phosphorylase in human solid tumours. Eur J Cancer. 1996; 32: 1227–32.

36. O'Brien TS, Fox SB, Dickinson AJ, Turley H, Westwood M, Moghaddam A, Gatter KC, Bicknell R, Harris AL. Expression of the angiogenic factor thymidine phosphorylase/platelet-derived endothelial cell growth factor in primary bladder cancers. Cancer Res. 1996; 56: 4799–804.

37. Ranieri G, Passantino L, Patruno R, Passantino G, Jirillo F, Catino A, Mattioli V, Gadaleta C, Ribatti D. The dog mast cell tumour as a model to study the relationship between angiogenesis, mast cell density and tumour malignancy. Oncol Rep. 2003; 10: 1189–93.

38. Ranieri G, Patruno R, Fiore G, Saponaro G, Paradiso A, Grammatica L. Thymidine phosphorylase overexpression in oral squamous carcinoma tissue as a potential target of capecitabine. Lett Drug Design Disc. 2004; 1: 45–9.

39. Gasparini G, Weidner N, Maluta S, Testolin A, Pozza F, Bevilacqua P. Intratumoral microvessel density and p53 protein: correlation with metastasis in head and neck squamous cell carcinoma. Int J Cancer. 1993; 55: 739–44.

40. de Bruin M, van Capel T, Smid K, van der Born K, Fukushima M, Hoekman K, Pinedo HM, Peters GJ. Role of platelet derived endothelial cell growth factor/thymidine phosphorylase in fluoropyrimidine sensitivity and potential role of deoxyribose-1-phosphate. Nucleosides Nucleotides Nucleic. 2004; 23: 1485–90.