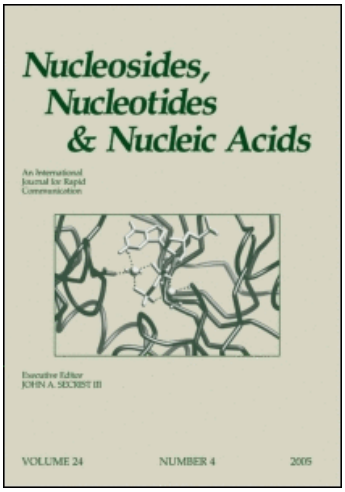

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Upregulation of Platelet Derived Endothelial Cell Growth Factor/Thymidine Phosphorylase by Interferon Alpha

M. de Bruin[a]; K. Smid[a]; M. A. van der Ent[a]; M. Fukushima[b]; H. M. Pinedo[a]; G. J. Peters[a]
[a] Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands [b] Taiho Pharmaceutical Co. Ltd., Saitama, Japan

Online publication date: 27 October 2004

To cite this Article de Bruin, M. , Smid, K. , van der Ent, M. A. , Fukushima, M. , Pinedo, H. M. and Peters, G. J.(2004) 'Upregulation of Platelet Derived Endothelial Cell Growth Factor/Thymidine Phosphorylase by Interferon Alpha', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1367 — 1370
To link to this Article: DOI: 10.1081/NCN-200027622
URL: http://dx.doi.org/10.1081/NCN-200027622

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1367–1370, 2004

Upregulation of Platelet Derived Endothelial Cell Growth Factor/Thymidine Phosphorylase by Interferon Alpha

M. de Bruin,[1,*] K. Smid,[1] M. A van der Ent,[1] M. Fukushima,[2] H. M. Pinedo,[1] and G. J. Peters[1]

[1]Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands
[2]Taiho Pharmaceutical Co. Ltd., Saitama, Japan

ABSTRACT

Thymidine phosphorylase (TP) catalyzes the phosphorolytic cleavage of thymidine to thymine and deoxyribose-1-phosphate. TP, which is overexpressed in a wide variety of solid tumors, is involved in the activation and inactivation of fluoropyrimidines. TP is known to be regulated by several cytokines and interferons. In our HT29 cell line the TP mRNA and activity expression increased 2–3 fold after treatment with interferon alpha.

Key Words: Platelet derived endothelial cell growth factor; Thymidine phosphorylase; Interferon-gamma.

INTRODUCTION

TP is assumed to be identical to the angiogenic factor platelet derived endothelial cell growth factor, and its expression has been studied extensively in numerous immunohistochemical studies. It has been shown that TP is overexpressed in a wide

*Correspondence: M. de Bruin, Department of Medical Oncology, VU University Medical Center, 1081 HV Amsterdam, The Netherlands.

DOI: 10.1081/NCN-200027622
1525-7770 (Print); 1532-2335 (Online)

variety of solid tumors and has been described as proangiogenic.[1] TP catalyzes the phosphorolytic cleavage of thymidine to thymine and deoxyribose-1-phosphate, besides this natural substrate it can use other substrates among which are several fluoropyrimidines. It can activate 5′-deoxyfluorouridine to its active form 5-fluorouracil. It is known to be regulated by cytokines and interferons (IFN) such as TNF-α, IL1-α IFN-α, β and γ.[2,3] Since we studied the potential regulation of TP by some of its fluoropyrimidine substrates with or without a specific inhibitor,[4] we used the induction of TP by IFN-α as a positive control for our experiments. In these experiments we could not find a uniform regulation by any of the substrates used, but we found an increasing effect of IFN-α on the colon cancer cell line tested.

MATERIALS AND METHODS

HT29 colon cancer cells were incubated for 24 hours with 500 units IFN-α/ml prior to being harvested. Cells were stored at −80°C prior to use.

Competitive Template RT-PCR to Determine TP mRNA Expression Levels

The quantitative RT-PCR technique is based on the co-amplification of a competitive template (CT), functioning as an internal standard designed specifically for each different target. The principles have been described in detail elsewhere.[5–7] PCR was used for co-amplification of the cDNA samples with CTs to ensure accurate quantification of the native target (NT). PCR products were separated by electrophoresis and measured by densitometry. The relative expression of TP mRNA was given as the ratio of the concentration NT of TP versus NT of β-actin.

Thymidine Phosphorylase Activity

The TP activity was determined using an assay previously described.[8] Activity was measured using [^{14}C-]thymidine as a substrate by calculating its conversion to thymine.

Statistics

The one-tailed paired Student's t-test was used to study the differences in activity and mRNA content between the treated and untreated cells.

RESULTS AND DISCUSSION

Treatment of HT29 cells with IFN-α resulted in an 2.18 fold increase in mRNA and was significantly different (Fig. 1A). As shown in Fig. 1B, activity increased 2.2 fold (135 to 270 pmol/hr/10^6 cells for HT 29 and HT29 IFN-α treated, respectively).

The increase in mRNA and activity was somewhat lower as described by Schwarz et al.,[9] approximately 4.5 and 5.5, respectively, but in a similar range as described by Laurent et al.[10] Fukushima et al.[11] described that the increase was dependent on the

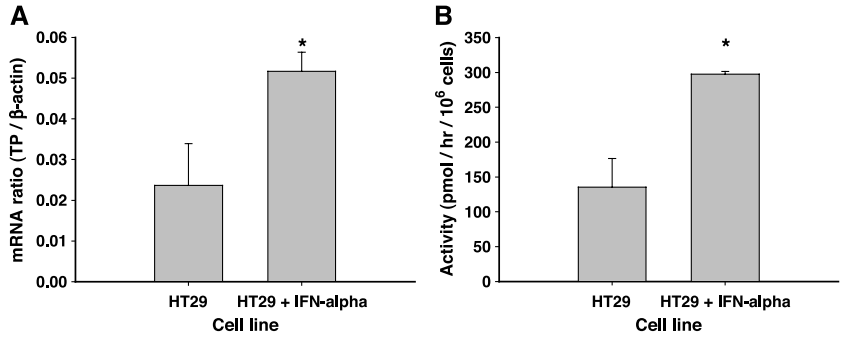

Figure 1. A) TP mRNA levels as measured with CT-RT-PCR in HT29 and HT29 IFN-α treated cells. B) TP activity in HT29 and IFN-α treated cells. In both the mRNA and activity measurements the treatment with IFN-α resulted in significant increases ($P < 0.05$).

initial TP expression: cells with low expression could be stimulated, whereas others with high expression could not. This regulation appeared to related to the STAT1 expression. STATs are a family of transcription factors involved in gene regulation and may also play a role in the upregulation of other genes activated by IFN-α. The results demonstrate that HT29 cells can be used as a model for TP activation. IFN-α has been used in the past to modulate 5FU sensitivity, with varying results. Its mechanism was possibly related to an increase in FdUMP formation and DNA damage. However the extent of these effects varied very much between cell lines and were often negligible, possibly explaining the lack of enhanced 5FU cytotoxicity in most cell lines.[12]

REFERENCES

1. Morita, T.; Matsuzaki, A.; Suzuki, K.; Tokue, A. Role of thymidine phosphorylase in biomodulation of fluoropyrimidines. Curr. Pharm. Biotechnol. **2001**, *2*, 257–267.
2. Eda, H.; Fujimoto, K.; Watanabe, S.; Ura, M.; Hino, A.; Tanaka, Y.; Wada, K.; Ishitsuka, H. Cytokines induce thymidine phosphorylase expression in tumor cells and make them more susceptible to 5′-deoxy-5-fluorouridine. Cancer Chemother. Pharmacol. **1993**, *32*, 333–338.
3. Schwartz, E.L.; Hoffman, M.; O'Connor, C.J.; Wadler, S. Stimulation of 5-fluorouracil metabolic activation by interferon-alpha in human colon carcinoma cells. Biochem. Biophys. Res. Commun. **1992**, *182*, 1232–1239.
4. De Bruin, M.; Van Capel, T.; Smid, K.; Van der Born, K.; Fukushima, M.; Hoekman, K.; Pinedo, H.M.; Peters, G.J. Role of platelet derived endothelial cell growth factor/thymidine phosphorylase in fluoropyrimidine sensitivity and potential role of deoxyribose-1-phosphate. Nucleosides Nucleotides Nucleic Acids **2003**. *in press*.
5. Willey, J.C.; Crawford, E.L.; Jackson, C.M.; Weaver, D.A.; Hoban, J.C.; Khuder, S.A.; DeMuth, J.P. Expression measurement of many genes simultaneously by quantitative RT-PCR using standardized mixtures of competitive templates. Am. J. Respir. Cell Mol. Biol. **1998**, *19*, 6–17.

6. Rots, M.G.; Willey, J.C.; Jansen, G.; Van Zantwijk, C.H.; Noordhuis, P.; DeMuth, J.P.; Kuiper, E.; Veerman, A.J.; Pieters, R.; Peters, G.J. mRNA expression levels of methotrexate resistance-related proteins in childhood leukemia as determined by a standardized competitive template-based RT-PCR method. Leukemia **2000**, *14*, 2166–2175.
7. De Bruin, M.; Van Capel, T.; Van der Born, K.; Kruyt, F.A.; Fukushima, M.; Hoekman, K.; Pinedo, H.M.; Peters, G.J. Role of platelet-derived endothelial cell growth factor/thymidine phosphorylase in fluoropyrimidine sensitivity. Br. J. Cancer **2003**, *88*, 957–964.
8. Laurensse, E.J.; Pinedo, H.M.; Peters, G.J. A sensitive non-radioactive assay for pyrimidine nucleoside phosphorylase using reversed-phase high performance liquid chromatography. Clin. Chim. Acta **1988**, *178*, 71–78.
9. Schwartz, E.L.; Wan, E.; Wang, F.S.; Baptiste, N. Regulation of expression of thymidine phosphorylase/platelet-derived endothelial cell growth factor in human colon carcinoma cells. Cancer Res. **1998**, *58*, 1551–1557.
10. Laurent, P.L.; Tevaearai, H.T.; Eliason, J.F.; Givel, J.C.; Odartchenko, N. Interferon alpha and 5′-deoxy-5-fluorouridine in colon cancer: effects as single agents and in combination on growth of xenograft tumours. Eur. J. Cancer **1994**, *30A*, 1859–1865.
11. Fukushima, M.; Okabe, H.; Takechi, T.; Ichikawa, W.; Hirayama, R. Induction of thymidine phosphorylase by interferon and taxanes occurs only in human cancer cells with low thymidine phosphorylase activity. Cancer Lett. **2002**, *187*, 103–110.
12. Van der Wilt, C.L.; Smid, K.; Aherne, G.W.; Noordhuis, P.; Peters, G.J. Biochemical mechanisms of interferon modulation of 5-fluorouracil activity in colon cancer cells. Eur. J. Cancer **1997**, *33*, 471–478.