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Up-regulation of human PNPase mRNA by β-interferon has no effect on protein level in melanoma cell lines

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Human mitochondrial polynucleotide phosphorylase (hPNPase) is an exoribonuclease localized in mitochondria. The exact physiological function of this enzyme is unknown. Recent studies have revealed the existence of a relationship between induction of hPNPase mRNA and both cellular senescence and growth arrest of melanoma cells following β-interferon treatment. The aim of this study was to verify whether the augmented hPNPase mRNA level results in increase of the protein level. In several cell lines established from five metastatic melanoma patients we did not find any such correlation. However, an elevated level of hPNPase protein was observed in interferon-induced HeLa and Jurkat cells. This increase was correlated with a slight shortening of poly(A) tails of mitochondrial ND3 transcript.

Keywords: PNPase, beta interferon, melanoma, poly(A) tails, human mitochondria, polynucleotide phosphorylase

Human polynucleotide phosphorylase (hPNPase) is a nuclear-encoded enzyme that localizes to mitochondria (Piwowarski *et al.*, 2003). It displays 3′-5′ exoribonuclease (Leszczyniecka *et al.*, 2002) and poly(A) polymerase activities *in vitro* (Nagaike *et al.*, 2005). Although the physiological function of the protein remains elusive to date, some reports indicate that it may participate in the regulation of mitochondrial RNA metabolism (Tomecki *et al.*, 2004; Nagaike *et al.*, 2005).

Human hPNPase (hPNPase old-35) was identified by the group of Paul Fisher in the screen for up-regulated transcripts in human melanoma cells that were induced to terminal differentiation by treatment with β-interferon and mezerein, and in primary human progeroid fibroblasts, which entered the state of cellular senescence. β-Interferon treatment of HO-1 melanoma cell line resulted in growth arrest and the induction of apoptosis (Leszczyniecka *et al.*, 2002). The phenomenon of growth-arrest arising from hPNPase up-regulation was further confirmed in HO-1 cells that overexpressed the protein from an adenoviral vector (Sarkar *et al.*, 2003). The induction of hPNPase overexpression in human melanoma cells treated with β-interferon and the subsequent growth arrest were suggested to be significant for the conceivable gene therapy of melanoma and for the elucidation of the processes underlying inflammation and aging (Sarkar *et al.*, 2004).

The mechanism linking the up-regulation of hPNPase with the cell growth inhibition is not clear yet. Of the two models proposed by Fisher's group, the first one assumes specific degradation of *c-myc* mRNA by the exoribonucleolytic activity of hPNPase (Sarkar *et al.*, 2003), while the second one suggests that the overexpression of hPNPase may lead to the increased level of reactive oxygen species (ROS) and consequently to the growth arrest of melanoma cells (Sarkar *et al.*, 2004). Interferon treatment has been also shown to correlate with the reduction of

Abbreviations: hPNPase, human polynucleotide phosphorylase; PBS, phosphate-buffered saline; TBE, tris-borate-EDTA buffer; wt, wild type.

mitochondrial protein synthesis and the steady-state level of mitochondrial mRNA transcripts (Kortsaris *et al.*, 1976; Shan *et al.*, 1990; Matarrese *et al.*, 2002). The mechanism of this phenomenon is not known but up-regulation of hPNPase could be responsible for lowering the mitochondrial transcript levels.

It is well known that melanoma cell lines vary in their response to β-interferon treatment (Chawla-Sarkar *et al.*, 2001; Leaman *et al.*, 2002) and only a small percentage of patients with metastatic melanoma are sensitive to interferon therapy (Worm *et al.*, 2001; Wysocki *et al.*, 2002; Soengas & Lowe, 2003). Thus, identifying those that are susceptible to β-interferon action might be significant for determining the optimal therapy.

The published data on the induction of hPNPase in melanoma cells by β-interferon were based on experiments conducted in a few established human melanoma cell lines only at the level of mRNA (Leszczyniecka *et al.*, 2002). We decided to examine the induction of hPNPase in different melanoma cell lines obtained from metastatic tumors, to check whether the induction of hPNPase expression at the mRNA level results in the increased level of hPNPase protein itself and to correlate that with apoptosis. Such a correlation might prove to be useful in applying the hPNPase mRNA level as a marker of interferon susceptibility of human melanomas.

METHODS

Cell cultures and interferon treatment. Human melanoma cell lines established from Polish patients with metastatic melanoma were obtained from the collection available at the Cancer Centre — Institute of Oncology in Warsaw (Poland). HeLa, Jurkat, HEK 293 and five melanoma cell lines were from early passages: MeW151p21, MeW152p9, MeW155p11, MeW164p13, MeW165p10 and four from distant passages: MeW151p152, MeW152p158, MeW155p150, MeW164p161. Most of the melanoma cell lines were characterized for the presence of molecular tumour markers as tyrosinase, MUC-18, MAGE-3 and MART 1 (Kulik *et al.*, 2001). Cells were grown at 37ºC in 5% CO_2 humidified atmosphere in DMEM (Sigma) or Eagle's (Biomed) medium supplemented with 10% fetal bovine serum (Gibco), 0.2 mM glutamine and penicillin and streptomycin (Sigma). Cells were grown on plates as monolayers and after reaching 50% confluence they were treated with 1000 u/ml of β-interferon (PBL Biomedical Laboratories). Cells were harvested after 24 and 72 h of interferon treatment for RNA/protein purification and for apoptosis analyses, respectively.

Stable cell line. The cell line stably expressing hPNPase from its cDNA cloned into appropriate vector under the control of CMV promoter (HEK-PNP) was made using The Flp-In™ System (Invitrogene) in the Flp-In™ HEK 293 Host Cell Line according to the manufacturer's instructions. This cell line was grown in DMEM supplemented with 100 μg/ml hygromycin.

RNA isolation and cDNA synthesis. Total RNA was isolated using QIAshredder (Qiagen) and RNeasy mini kit (Qiagen) or TRI Reagent (Sigma). Following the use of TRI Reagent, RNA was treated with DNase I (Fermentas). Reverse transcription was performed with 2.5 μg DNA-free RNA, oligo-$(dT)_{18}$ primer and RevertAid™ M-MuLV Reverse Transcriptase (Fermentas). All steps were performed according to the manufacturer's instructions.

Semiquantitative RT-PCR. RT-PCR was carried out to assess the expression of hPNPase mRNA employing β-actin as an internal control. 5 μl of the 10× diluted cDNA were added to 20 μl of PCR reaction mixture containing buffer with 2.5 mM $MgCl_2$, 0.5 mM deoxynucleotide triphosphates, 1 unit of *Taq* DNA polymerase (Fermentas) and 1 μl of Assays-on-Demand™ Gene Expression Assay Mix (Applied Biosystem catalog number for hPNPase: Hs00396733_g1 and for β-actin: Hs99999903_m1) as the primer pair. Preliminary experiments were performed in order to determine the exponential phase of the PCR amplification. PCR reaction proceeded as follows: denaturation at 95°C for 5 min and 29 cycles for hPNPase or 23 cycles for β-actin of 95°C (15 s), 57°C (30 s), and 72°C (15 s). Products were separated in 2.5% agarose gels containing ethidium bromide and visualized with the use of Gene Genius Bio Imaging System (Syngen). The signal intensities of the PCR products were calculated by using GeneTools software (Syngen) and the standard curve was based on the co-amplification of various amounts of the given cDNA.

Real-time PCR. 5 μl of the 10-fold diluted cDNA were used in the real-time PCR reaction, which was carried out using LightCycler (Roche) and QuantiTect SYBR Green PCR Kit (Qiagen). Primers for β-actin described in (Vandesompele *et al.*, 2002) were applied for standardization. Primers PNP-RT-For (5′-CCATTACACCAGTATCTCTG-3′) and PNP-RT-Rev (5′-GGCAAGAGACTTCATTACTG-3′) were used for the quantification of hPNPase mRNA levels relative to the β-actin and/or $β_2$ microglobulin standardization. PCR reaction proceeded as follows: 95°C (15 min) enzyme activation and 40 cycles of 95°C (15 s), 53°C (30 s, slope 2°C/s), and 72°C (15 s), followed by the melting curve.

High resolution Northern analysis. High resolution Northern blots were prepared as described in (Tomecki *et al.*, 2004). In brief, 5 μg of total RNA in 50% formamide were run in 5% denaturing acrylamide/urea gel in 1×TBE. RNA was blotted onto

Nytran-N filter by electrotransfer in 0.5× TBE buffer using Trans-Blot Cell apparatus (Bio-Rad). Hybridizations were performed in PerfectHyb^{TM} Plus buffer (Sigma). PCR product corresponding to the internal region of ND3 transcript was labeled with [α-^{32}P]-dATP using HexaLabel DNA Labeling Kit (Fermentas) and used as a probe. Filters were exposed to PhosphorImager screens (Sigma). Results were obtained by scanning the PhosphorImager screens using Storm Scanner (Molecular Dynamics), and analyzed using ImageQuant.

Western blot analysis. Total proteins were separated in the 8% SDS/polyacrylamide gel and electroblotted onto the Protran® nitrocellulose membrane (Bioscience) using TRANS-BLOT® (Bio-Rad). The filters were blocked for 3 h in 10% milk powder in PBS containing 0.1% Tween-20, and then incubated overnight with the rabbit polyclonal antibodies against hPNPase (described previously in Piwowarski *et al.*, 2003), diluted 200-fold in 10% milk powder in PBS with 1% Tween-20. The filters were subsequently washed three times using PBS with 1% Tween-20. Afterwards, the membranes were incubated for 3 h with the mouse monoclonal anti-rabbit immunoglobulins–peroxidase conjugate (Sigma) diluted 2000-fold in 5% milk powder in PBS with 0.1% Tween-20. The blots were washed three times in PBS with 0.1% Tween-20. Finally, horseradish peroxidase conjugates were visualized by enhanced chemiluminescence system (ECL: 50 µl of 250 mM luminol (Fluka), 25 µl of 90 mM coumaric acid (Sigma) and 3 µl of 30% H_2O_2 were dissolved in 10 ml of 100 mM Tris/Cl, pH=8.5) and the exposure to X-ray films (AGFA), which were then developed with the use of CURIX60 and scanned using SNAPSCAN system (AGFA). The resulting digital images were analyzed using ImageQuant v5.2 sofware. The protein loading was evaluated on the base of Ponceau S Red staining of the membranes following semi-dry electrotransfer.

Analysis of apoptosis. Melanoma cells for apoptosis investigation were grown to 30–50% confluence and then treated with β-interferon (1000 u/ml) for 72 h, to reach 80–90% confluence. After this time cells were gently trypsinized and suspended in PBS. Apoptosis levels were investigated using FACS Calibur cytofluorimeter (Becton Dickinson) and Annexin-V-FLUOS Staining Kit (Roche). The test was performed on 10 000 cells.

***In silico* analysis.** hPNPase gene sequences available in the databases (accession numbers: NM_003109 — positions (–) 3052535-3110385 in chromosome 2, contig NT_005375 — positions (–) 311036-3111407) were subjected to *in silico* analysis, which was performed on Genomatix server (http://www.genomatix.de) using MatInspector tool for the recognition of transcriptional elements.

RESULTS

β-interferon increases the level of hPNPase mRNA

In order to correlate the response to interferon treatment with hPNPase mRNA levels we examined different melanoma cell lines obtained from metastatic tumors that were removed surgically from patients of the Cancer Centre — Institute of Oncology in Warsaw (Poland). Five melanoma cell lines were from early passages (9–21) and four from distant passages (150–161).

Using semiquantitative RT-PCR and β-actin as an internal control we measured the hPNPase mRNA levels for the melanoma cell lines as well as for Jurkat, HEK and HeLa cells as controls. In the case of three samples, namely the line MeW151p21, Jurkat, and HeLa cells we have additionally carried out real-time PCR assays. Since the correlation between semiquantitative PCR and real-time PCR quantification in the preliminary experiments was good (not shown), we assumed that the first method is sufficient for the examination of hPNPase induction in our study: all results from melanomas are for semiquantitative RT-PCR, results for other cell lines are from real-time PCR.

The results revealed that in 8 out of 9 melanoma cell lines tested there was only a moderate increase (1.2- to 2.8-fold) in the level of hPNPase mRNA after β-interferon treatment. A similar observation was made for Jurkat and HEK cells. Only one melanoma cell line: MeW151p21 displayed more significant induction of hPNPase expression at the level of mRNA (5-fold), as compared to the untreated control. Induction was also observed in HeLa cells — 3-fold up-regulation of hPNPase mRNA in this cell line is comparable to what was observed by Leszczyniecka *et al.* (2003). As an additional control, we used HEK cells overexpressing hPNPase (HEK-PNP), where the mRNA level was found to be 3.3-fold up-regulated as compared to wt HEK cell line (Fig. 1).

Protein level of hPNPase are not affected by interferon treatment in melanoma cell lines

The changes in the level of a given mRNA within the cell do not always correlate with the alterations in the level of the respective protein (Scacheri *et al.*, 2004; Le Roch *et al.*, 2004; Schrauwen *et al.*, 2005). Therefore, we were interested whether the increased levels of hPNPase mRNA that we observed in some melanoma cell lines and in different cells like HeLa, Jurkat, HEK and HEK-PNP result in elevated levels of protein. The amount of hPNPase protein was measured by Western blot technique employ-

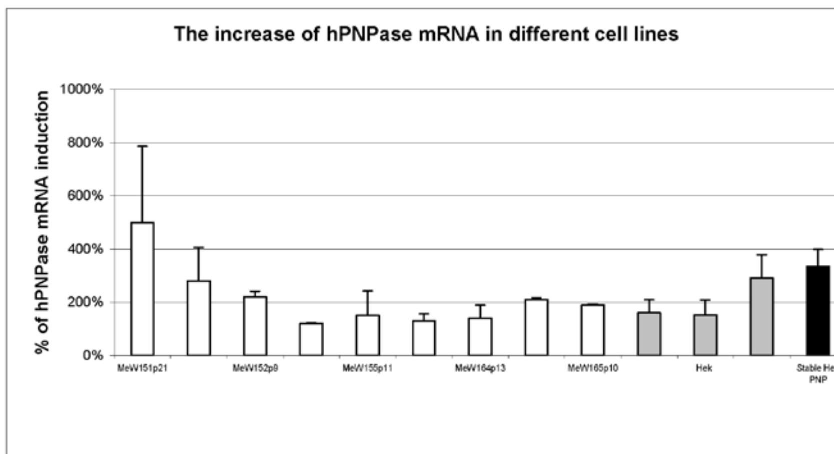

Figure 1. The hPNPase mRNA level was investigated using semiquantitative RT-PCR and/or real-time PCR at least twice for all samples.
Standardization was performed using β-actin and/or B2M transcripts. Melanoma cell lines (white) and Jurkat, HEK and HeLa cells (gray) were treated with β-interferon for 24 h. The level of hPNPase mRNA was compared with the level in untreated cells. The hPNPase mRNA level for a stable cell line overexpressing hPNPase under the control of CMV promoter (marked in black) was compared with wt HEK.

ing anti-hPNPase polyclonal antibodies (Piwowarski *et al*., 2003). We used exactly the same cells that had been previously used for hPNPase mRNA level assays (half of the cell volume was used for RNA and half for protein isolation). The results of the respective Western blots are presented in Fig. 2. For all melanoma cells, almost no difference between interferon-treated and untreated cells was found. Conversely, we have found that in HeLa and HEK cells subjected to β-interferon treatment the expression of hPNPase at the protein level was more than twice up-regulated, in Jurkat cells the hPNPase protein level was increased by about 50%, and in the stable HEK-PNP cell line there were no differences in hPNPase protein level in comparison with wt HEK cells.

Apoptosis in melanoma cell lines is not induced by interferon-β treatment

As reported previously, interferon treatment of melanoma cell lines resulted in apoptosis (Sarkar *et al*., 2003). In our studies, apoptosis was investigated using annexin staining method in the cells treated with β-interferon for 72 h. As shown in Fig. 3, no significant differences in the level of apoptotic cells were found between β-interferon treated cells and untreated melanoma cells. The cell line MeW151p21, exhibiting a highest level of hPNPase mRNA, shows the lowest level of apoptosis in both the β-interferon treated and untreated cells. In MeW165p10 cell line the level of apoptosis was the highest but reached only about 4% both in the treated and untreated cells.

Mitochondrial ND3 transcripts has shortened poly(A) tails after β-interferon treatment in Jurkat and HeLa cells

hPNPase is involved in mRNA metabolism in human mitochondria (Tomecki *et al*., 2004; Nagaike *et al*., 2005). In HeLa cells after RNAi against hPNPase mitochondrial mRNA poly(A) tails become extended by about 20 adenines in comparison with the wild type cells (Nagaike *et al*., 2005). We decided to investigate the length of mitochondrial mRNAs tails after overexpression of hPNPase. Previously we estimated the length of mitochondrial oligo(A) tails as approx. 5 nt and poly(A) tails as approx. 50 nt (Tomecki *et al*., 2004). Two cell lines in which hPNPase is inducible by interferon treatment (HeLa and Jurkat) were chosen for this experiment. After 24 h of β-interferon treatment total RNA was isolated and separated in a 5% denaturing acrylamide gel. In both cell lines poly(A) tails of ND3 transcripts were shortened. In Jurkat cells where hPNPase is about 1.5-times up-regulated after interferon treatment we

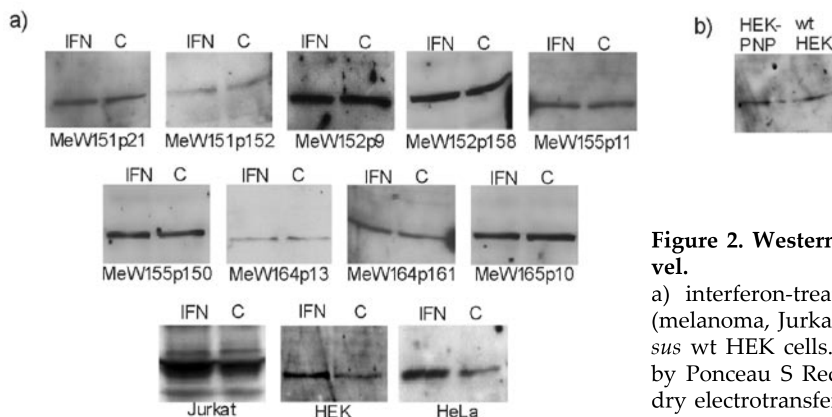

Figure 2. Western-blot analysis of hPNPase protein level.
a) interferon-treated (INF) ***versus*** untreated (C) cells (melanoma, Jurkat, HEK and HeLa) or b) HEK-PNP *versus* wt HEK cells. Equal protein loading was monitored by Ponceau S Red staining of the blots following semi-dry electrotransfer (not shown).

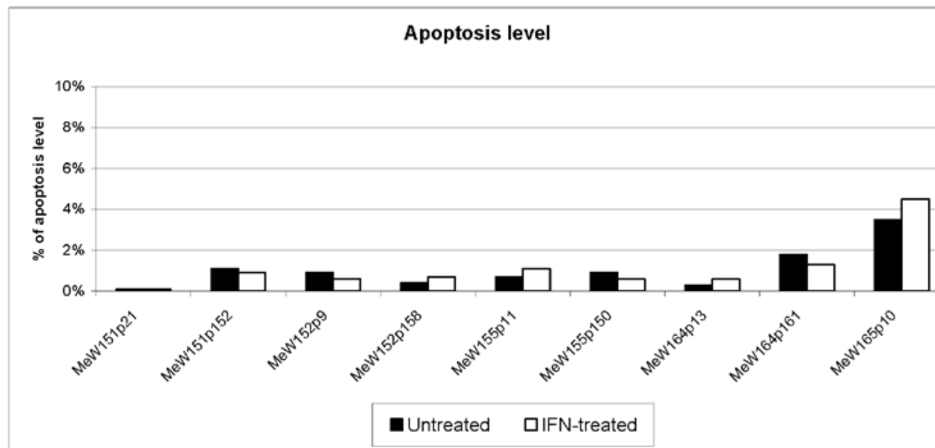

Figure 3. The level of apoptosis in melanoma cells was investigated 72 h after β-interferon treatment using Annexin-V-FLUOS Staining Kit and FACS analysis. Black and white bars corresponded to control and interferon-treated cells, respectively.

estimate that the poly(A) tails of ND3 transcripts were shortened by approx. 5 nt. In HeLa cells where hPNPase is about 3-times up-regulated poly(A) tails were shortened by approx. 10–15 nt (Fig. 4).

In silico analysis of the hPNPase promoter region

We performed an extensive *in silico* analysis of the hPNPase promoter in search for potential transcriptional elements in this region. The analysis, which was done with the use of MatInspector software led to the identification of 17 different binding sites for various transcription factors (Fig. 5 and Table 1). They can be divided into four groups: housekeeping, interferon responsive, developmental and cell cycle. Most of the motifs that were found in this study belong to the first two groups. A similar approach had been used previously for the analysis of hPNPase promoter by Leszczyniecka *et al*. (2003), nevertheless our results indicate the existence of many more putative regulatory sites. Among the newly discovered transcriptional elements were: a) housekeeping: *ATF, CAAT, NFY, TAXCREB, NF1*; b) interferon responsive: *HMGIY, IRF7*; c) all the developmental and cell cycle sites.

DISCUSSION

Responsiveness of patients with melanoma to interferon therapy is rather low (Worm *et al.*, 2001; Wysocki *et al.*, 2002; Soengas & Lowe, 2003). Moreover, studies on cell lines isolated from melanomas indicated that β-interferon induces apoptosis in only a fraction of the cell lines. On the other hand, Leszczyniecka *et al.* (2002) have suggested that there is a correlation between the induction of mitochondrial hPNPase by β-interferon and cell death.

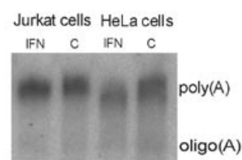

Figure 4. High-resolution Northern blot analysis of mitochondrial ND3 mRNA in control and interferon-treated Jurkat and HeLa cells.
Positions of polyadenylated (poly(A)) and oligoadenylated (oligo(A)) fractions are indicated.

Since the studies linking hPNPase induction with melanoma cell death were conducted only on the HO-1 cell line (Leszczyniecka *et al.*, 2002), we decided to test the possible correlation using several different cell lines established from metastatic melanomas. We decided to use β-interferon as it induces hPNPase mRNA more strongly than α-interferon (Leszczyniecka *et al.*, 2002) and has a stronger cell growth-inhibitory effect (Borden *et al.*, 1982; Schiller *et al.*, 1986; Rosenblum *et al.*, 1990; Johns *et al.*, 1992). We investigated the induction of mitochondrial polynucleotide phosphorylase (hPNPase), both at the mRNA and protein levels, in the investigated melanoma cell lines which were subjected to β-interferon treatment under the same conditions as employed by Fisher's group. hPNPase mRNA levels in 8 cell lines were very moderately induced: ranging from 1.2 to 2.8-fold increase. Only one melanoma cell line was much more sensitive to β-interferon induction, exhibiting a 5-fold increase of hPNPase mRNA level. HeLa, HEK and Jurkat cell lines were found to differ in their response to β-interferon: we observed a relatively strong — about 3-fold induction in HeLa and HEK cells, while low induction was found in the case of Jurkat cells.

The important observation is that the changes in hPNPase mRNA levels did not correlate with the levels of the respective protein. Although the induction of hPNPase mRNA levels varied from 1.2- to 5-fold, depending on the cell line, in all tested melanoma cell lines the level of hPNPase protein remained unchanged after interferon treatment. Also in HEK-PNP cell line the over 3-fold overexpression of hPNPase at the mRNA level did not affect the protein level. In contrast, in HeLa, HEK and Jurkat cells β-interferon treatment led to the

Table 1. The function of the putative transcriptional factors that might be involved in the regulation of hPNPase expression

	Factor's name (family)	Function
HOUSEKEEPING, MITOCHONDRIA (CREB)	ATF(CREB)	Activating transcription factor (Rooney *et al.*, 1990).
	CAAT(PCAT)	Cellular and viral CAAT-box. Transcriptional factor connected with CAAT-box (Benoist *et al.*, 1980); possible interaction with NFY.
	E4BP4(CREB)	Transcriptional repressor (Cowell *et al.*, 1992); interaction with OCT1P.
	GC(SP1F)*	Transcriptional factor connected with GC-islands and housekeeping gene expression (Kadonaga *et al.*, 1986).
	NFY(ECAT)	Nuclear factor Y (Y-box binding factor), transcriptional factor connected with CAAT-box (Dorn *et al.*, 1987); possible interaction with CAAT0.1.
	TAXCREB(CREB)	cAMP response element binding protein, connected with signal transduction by cAMP (Paca-Uccaralertkun *et al.*, 1994).
	NF1F(NF1)	Nuclear factor 1 is a family of activators and sometimes repressors of transcription. NF1 have been implicated in tissue-specific expression of various genes (Bedford *et al.*, 1998).
INTERFERON RESPONSE	HMGIY(SORY)	High-mobility-group protein I (Y). Enhancer of different transcriptional factors, for example IRF1. Its interaction was discovered in many cytokine promoters like IFN-β. This factor is necessary for viral induction of IFN-β gene (Yie *et al.*, 1997); possible interaction with transcriptional factors from POU (OCT) group.
	IRF1(IRFF)*	Interferon regulatory factor 1. Interferon-induced factor involved in gene expression during interferon response (Harada *et al.*, 1989).
	IRF7 (IRFF)	Interferon regulatory factor 7. Interferon-induced factor involved in gene expression during interferon response (Zhang & Pagano, 1997).
	ISRE (IRFF)*	Interferon-stimulated response element. IRF1 and ISGF3 binding site connected with interferon response (Levy *et al.*, 1988).
	STAT1 (STAT)*	Signal transducers and activators of transcription. Ligand-activated transcriptional factor and signal transducer involved in apoptosis (Horvath *et al.*, 1995).
DEVELOPMENT	GLI1(GLIF)	Transcriptional regulator involved in cell growth and tissues development, connected with human hedgehog homolog proteins (Pearse *et al.*, 1999).
	OCT1P(OCTP)	Octamer binding factor 1. Transcriptional factor involved in development and homeotic gene expression regulation (Verrijzer *et al.*, 1992); interacting with E4BP4, and possibly with HMGIY.01
	OCT1(OCT)	Oct1 functions as an activator of a wide range of genes required for development, differentiation and homeostasis (Reece-Hoyes *et al.*, 2005).
CELL CYCLE	E2F3(E2FF)	Transcriptional repressor responsible for gene silencing during G_1 to S phase transition (Wu *et al.*, 2001).
	FAST1(FAST)	Activin signal transducer. Gene expression activator in the presence of TGF-β (Zhou *et al.*, 1998).

*Previously described in (Leszczyniecka *et al.*, 2003).

increase in both mRNA and protein levels. Thus, our data confirm the observations of Leszczyniecka *et al.* (2002), that β-interferon treatment can increase hPNPase mRNA levels in human melanoma cells. We showed, however, that the relative increase of the mRNA level varied greatly among melanoma cell lines and that the up-regulation of hPNPase mRNA did not result in the increase of the protein level.

We measured apoptosis in all melanoma cell lines treated with β-interferon. The level of apoptosis was very low (around 1% in 8 cases) and no significant correlation between treated and untreated cells could be observed, as well as between melanoma cell lines in the early and late stage of growth *in vitro*. In one cell line (MeW165p10) the level of apoptosis reached 4% but the difference between treated and untreated cells was also negligible. Thus, poor

-1500 cacggatcaacattttaattttttttttttttttttttttttttttagatggagtctcggtctggagtgca **-1431**

E2F3(E2FF)

-1430 **gtggcgcgatctcgg**ctcactgcaacctccgcctcctggattcaagcgattctcatgcctcagcctctgg **-1361**

-1360 agtagctgggattacaggcacgcgccaccacaccgagctaatttttgtatttttagtagagacgcggttt **-1291**

NF1F(NF1)

-1290 cagca**tgttggccaggatgccctgg**gtctcttgacctcgtgatccacccgcctctgcctcccagcgtgct **-1221**

-1220 gggattacaggcatgagccactgcgcccggacttttttttttttttttttttttttttttttgagaccg **-1151**

IRF1(IRFF) ATF(CREB)

-1150 ag**tttcactcttgttgcaatgacgcgatctcggc**tcactgcaacctccgcctcccgggttcaagcgattc **-1081**

-1080 ccctgcctcagcctcccgagtagctgggattacaggcataagccaccatgcccggctagttttgtatttt **-1111**

-1010 tagtagagacggggtttctctgtgttggtcaggctggtctcgaactcaggtgatccgcccgcctcggcct **-941**

-940 cccaaagtgctgggattacaggcgtgagccacagcgtccagccaacgtttttaattcttaaaactgtgtt **-871**

HMGIY (SORY)

-870 tgaatgagtgttgggtttttgttgtttggttttctttggggtaaggcggttatcaccttac**acaaaaaat** **-801**

STAT1(STAT)

-800 **ttacatca**taatatgaaaatgcccattg**gttcattcaaggaaaaaca**taaagaatttctaacagaatgga **-731**

IRF1.01(IRFF)

-730 ttaatggggtggaagaaatttatccattctctaaaaatttttcaaatcccaaaaactttt**tttttcatt** **-661**

IRF7.01(IRFF)

-660 **ttaca**aaatatttacttcaaacagtagctggcattttgcatttaagagttcgaccaaaaggga**cagaaat** **-591**

ISRE(IRFF)

- 590 **ataaattg**ctaattattagcaat**ggaaatgaaaatg**aatcagaagtgacaataaagatttctaaaattac **-521**

FAST1(FAST)

-520 aagtcgatgaaaaatgaatt**tactgtaaattggt**taacacaaatcgctgtagtaacatatctgctatgtg **-451**

GLI1(GLIF)

-450 aaatttttaaaataaaaaccagggcggagaaggggagaaggagatctctttccaagg**cccaccacccaaa** **-381**

-380 ttctaattcttagttcacagatccaaataataataatgtgttgttctatgacttctagtattaaaagtaa **-311**

OCT1P(OCTP)//E4BP4(CREB) *OCT1(OCT)*

-310 atataaatttac**tctgcatattaaacatttaca**ttattctaggacttctcct**ttaaattacga**tagtaca **-241**

-240 cacacaaaaaccttatttatctaattgcctttcgttggcacataaagacaaagatcgtttgcaacataat **-171**

TAXCREB(CREB)

-170 cgcgttctgataggtttgaccctctctagtcttcgtagaag**gtggccaagg**tttccgttaccccggcgtc **-101**

ISRE(IRFF) *GC(SP1F)* CAAT- box

-100 cctcgggcacc**gcggaaacgaaactc**cat**caggctccgcccca**cggtctgcggagtgagCAATcagggc **-31**

-30 acagcctgcgttgaccgcgtgccgggtgtcATG

Figure 5. Human hPNPase promoter sequence with the potential binding sites for transcription factors.
Italics was used when the respective site is localized on the (–) strand.

response of hPNPase protein to interferon treatment correlates with the lack of apoptosis.

We have studied the hPNPase promoter using an *in silico* analysis and we have found numerous sites which have not been shown in the previously published data. The hPNPase promoter differs significantly from other promoters of genes coding proteins involved in mitochondrial RNA metabolism. Two examples of such genes: hSUV3 and hmtPAP encoding RNA/DNA helicase and poly(A) polymerase, respectively, contain a very limited set of regulatory protein binding sites, characteristic for housekeeping genes (Minczuk *et al.*, 2005; our unpublished data). In contrast to that, the hPNPase promoter displays a wide variety of binding sites. Among them, of special interest are interferon responsive elements and cell cycle-related elements. Such a complex structure of the hPNPase promoter may indicate the important function of polynucleotide phosphorylase in controlling cell cycle events. The experiments of Leszczyniecka *et*

al. (2002) showing the significant up-regulation of hPNPase mRNA in progeroid fibroblasts entering senescent status is a further proof of the importance of hPNPase regulation in cell cycle events. In spite of these clues, the molecular mechanism of hPNPase function remains obscure.

The only phenotype effect of up-regulation of hPNPase was found by us in HeLa and Jurkat cells. After interferon induction of hPNPase the mitochondrial ND3 poly(A) tails were shortened. This effect is opposite to the results of hPNPase silencing (Nagaike *et al.*, 2005; and our unpublished data), where knock-down of hPNPase expression resulted in the increased length of mitochondrial poly(A) tails.

There is a lot of evidence that after interferon treatment the level of mitochondrial RNA is decreased (Shan *et al.*, 1990; Inagaki *et al.*, 1997; Le Roy *et al.*, 2001). The mechanism of this phenomenon is not clear yet but few hypotheses have been proposed. One of them suggests a decrease of mitochondrial transcription caused by reduction of mitochondrial transcription factor (Inagaki *et al.*, 1997). The second one is based on the shortened half-life of RNA caused by mitochondrial RNase L which inhibitor is reduced by interferon (Le Roy *et al.*, 2001). Our results indicate a third possibility that the decrease of mitochondrial RNA level could be caused by shortening of their poly(A) tails by hPNPase.

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