FEBS Letters 566 (2004) 178–182 FEBS 28388

Cytosolic sialidase Neu2 upregulation during PC12 cells differentiation

A. Fanzani*, F. Colombo, R. Giuliani, A. Preti, S. Marchesini

Department of Biomedical Sciences and Biotechnology, Unit of Biochemistry, University of Brescia, Viale Europa 11, 25123 Brescia, Italy

Received 30 January 2004; revised 19 March 2004; accepted 31 March 2004

Available online 27 April 2004

Edited by Gerrit van Meer

Abstract The cytosolic sialidase Neu2 is known to be involved in myoblast differentiation. Here, we observed a Neu2 transcriptional induction during nerve growth factor, fibroblast growth factor 2 and epidermal growth factor treatments of PC12 cells, a favored model to study neuronal differentiation. The expression analysis of Neu2 deleted promoter revealed a remarkable increase of luciferase activity in treated PC12 cells, suggesting that in this cell line the Neu2 transcriptional levels are highly regulated.

The enzymatic activity of cytosolic sialidase Neu2 was found to increase transiently only during differentiation, whereas was undetectable in untreated PC12 cells. These data suggest a possible involvement of cytosolic sialidase Neu2 in differentiation of PC12 cells.

Keywords: Neu2; Sialidase; PC12 cell; Growth factor; Differentiation

1. Introduction

Sialidases (EC 3.2.1.18) are exoglicosidases catalyzing the removal of sialic acids from glycoproteins and glycolipids. These enzymes cleave terminal $\alpha2 \rightarrow 3$, $\alpha2 \rightarrow 6$ and $\alpha2 \rightarrow 8$ sialyl linkages and are implicated in biological phenomena such as differentiation, proliferation, signal transduction and cell surface interactions [1]. Alterations in sialylation levels have been observed during malignant transformation correlating with a change of phenotype in terms of metastatic potential and invasiveness [2–4]. Several mammalian sialidases have been purified and cloned, and the classification is based on their subcellular localization [5]. They are lysosomal (Neu1), cytosolic (Neu2) and plasma membrane bound (Neu3). Recently, a new sialidase named Neu4 was cloned from murine brain [6].

Membrane sialidases specifically hydrolyze gangliosides influencing the pathways of transduction and cell–cell interactions [7–10]. The lysosomal sialidase is a glycoprotein that is only active as a part of a molecular multienzymatic complex that contains β-galactosidase and cathepsin A [11,12]. Lysosomal sialidase is involved in two lysosomal storage disorders, sialidosis (OMIM 256550) [13] and galactosialidosis (OMIM 256540) [14], diseases that show a deficiency in sialidase activity. Among the sialidases, the cytosolic form Neu2 normally shows the lower expression level. The cytosolic enzyme is highly expressed in skeletal muscle: in particular, the inhibition of Neu2 translation by a specific oligodeoxyribonucleotide antisense inhibits the myoblasts differentiation [15] and the overexpression of the enzyme in C2C12 myoblasts induces a spontaneous differentiation [16].

Despite its relative abundance in skeletal muscle, cytosolic sialidase was found also in other tissues, such as liver [17], thymus [18] and, although at low levels, brain [19].

In the present work, the expression levels of cytosolic sialidase Neu2 were investigated for the first time in PC12 cells, a favored model to study neuronal differentiation, also generating stable transfected clones overexpressing the rat Neu2 enzyme.

2. Materials and methods

2.1. Cell line

PC12 cells were cultured at 37 °C (in an atmosphere of 5% CO_2) in RPMI medium (Sigma–Aldrich) supplemented with 10% (v/v) horse serum (Sigma–Aldrich), 5% (v/v) fetal bovine serum (Sigma–Aldrich), glutamine 2 mM (Sigma–Aldrich), and 100 μg/ml penicillin/streptomycin (Sigma–Aldrich). To induce differentiation, cells at 50% of density were treated with nerve growth factor (NGF) 100 ng/ml (Alomon) or human fibroblast growth factor 2 (FGF-2) 50 ng/ml (expressed in Chinese hamster ovary cells and purified as described [20]). To induce proliferation, cells were treated with epidermal growth factor (EGF) 50 ng/ml (Gibco).

2.2. Western blot

For the nuclear extracts, cells were rinsed and harvested with lysis buffer (Tris 20 mM, pH 7.4, NaCl 150 mM, EDTA 1 mM, Triton 0.3% (w/v), sodium fluoride 0.1 mM (Sigma–Aldrich), and sodium orthovanadate 0.1 mM (Sigma–Aldrich), added with protease inhibitor mix (Complete Mini Protease Inhibitors, Roche Molecular Biochemicals), vortexed and centrifuged at $12\,000 \times g$ for 10 min at 4 °C.

Protein concentration of the supernatant was assessed by Coomassie assay (Pierce Reagent) according to the manufacturer's instructions. Aliquots (15 μg) of proteins were analyzed by Western blotting using 1:1000 dilution of monoclonal anti-phospho p42/44 MAP Kinase antibody (Santa Cruz Biotechnology). Immunocomplexes were visualized using enhanced chemiluminescence reagent (Pierce) according to the manufacturer's instructions.

2.3. RNA extraction and RT-PCR analysis

Total RNA was obtained as described by Chomczynski and Sacchi [22]. The pellet of RNA was resuspended in RNAase free water, digested with 1 unit of DNAase (DNA-free, Ambion) for 1 h at 37 °C according to the manufacturer's instructions. 2 μg of total RNA was retrotranscribed with 400 units of Moloney murine leukemia virus

* Corresponding author. Fax: +39-30-3701157.
E-mail address: fanzani@med.unibs.it (A. Fanzani).

Abbreviations: NGF, nerve growth factor; FGF-2, fibroblast growth factor 2; EGF, epidermal growth factor; PCR, polymerase chain reaction; 4-MUB-NANA, 4-methylumbelliferyl N-acetylneuraminic acid; MMLV-RT, Moloney murine leukemia virus reverse transcriptase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

0014-5793/$22.00 © 2004 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2004.03.115

reverse transcriptase (MMLV-RT) (Promega) for 1 h at 37 °C and the RT template was used for polymerase chain reaction (PCR) amplification (RT+). Also 2 μg of total RNA was used as template for PCR amplification (RT−) in order to exclude the presence of genomic DNA. For RT-PCR analysis of endogenous rat cytosolic Neu2 sialidase expression, primers 5′-CCGTCCAGGACCTCACAGAG-3′ (sense) and 5′-TCACTGAGCACCATGTACTG-3′ (antisense) were used. Amplification was performed at 61 °C of annealing up to 38 cycles. For the screening of Neu2 transfectants, PCR analysis was performed using the same primers utilized to clone the rat Neu2 cDNA into its expression vector in order to avoid amplification of the endogenous Neu2 mRNA. Amplifications were performed at 65 °C of annealing up to 30 cycles. For RT-PCR analysis of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression, the primers 5′-CGTGGAGTCTACTGGCGTCTTC-3′ (sense) and 5′-GGGAGTTGTCATATTTCTCGTGGTT-3′ (antisense) were used. Amplification was performed at 60 °C of annealing up to 22 cycles.

2.4. Plasmids construction

The -1415/-5 fragment of the Neu2 rat promoter prior to ATG initiation codon [21], i.e., RP1/1410, was obtained by PCR amplification of rat genomic DNA as previously described [16]. Next, the Neu2 rat promoter was cloned into the *Kpn*I site of the luciferase reporter expression vector pGL2-Basic (Promega), thus generating the pGL2-Basic-Neu2 rat promoter vector.

The deletion fragments of the promoter were obtained by PCR amplification of the -1415/-5 fragment using in 3′ the antisense primer 5′-GGGGTACCCC/TGAGATCTGGGCAGAAAGAGAAGAC-3′ and in 5′ internal primers (5′-GGGGTACCCC/CTAAATTTTAGTCTGCTGCATTGTC-3′ for the fragment of 1280 bp, i.e., RP1/1280, 5′-GGGGTACCCC/CAGCCAAGGGTGGTTACTCTGGCTT-3′ for the fragment of 815 bp, i.e., RP1/815, 5′-ATCGGGGTACCCC/TGGAACAGCTTTCTAGGGCTC-3′ for the fragment of 401 bp, i.e., RP1/401, 5′-ATCGGGGTACCCC/TTCGATTCTTGGGGTAGAAGAC-3′ for the fragment of 215 bp, i.e., RP1/215). Amplifications were performed at 65 °C of annealing up to 31 cycles.

The rat Neu2 sialidase cDNA coding sequence was amplified from a PC12 cell cDNA library using the primers 5′-CGGAATTCCG/ATGGAGACCTGCCCCGTCCTCCAGA-3′ (sense) and 5′-CGGAATTCCG/TCACTGAGCACCATGTACTGTGGGA-3′ (antisense) under the following conditions: 95 °C for 2 min, 59 °C for 1 min, 72 °C for 3.5 min followed by 9 cycles of 94 °C for 30 s, 59 °C for 30 s, 72 °C for 3.5 min, followed by 34 cycles of 94 °C for 30 s, 66 °C for 30 s, 72 °C for 3.5 min. Then, the rat Neu2 cDNA was cloned into the *Eco*RI site of pCDNA vector, thus generating the pCDNA-Neu2 expression vector.

2.5. Transient transfection assays

Transfections were performed by Fugene reagent (Roche Molecular Biochemicals). PC12 cells (1×10^5/60 mm dish) were transfected with a mix of 1 μg of plasmids containing the promoter luciferase Reporter (pGL2-Basic-Neu2 promoter) and 10 ng of the transfection control vector pRL-TK-Renilla luciferase. The transfections with the vectors harboring the deleted promoters were performed using identical stoichiometric amounts with respect to the pGL2-Basic-Neu2 promoter, in the same conditions. Following transfection, the medium was added with NGF 100 ng/ml, FGF-2 50 ng/ml or EGF 50 ng/ml. Luciferase activity was measured in the total protein lysates using the Promega Dual luciferase assay system. Data were corrected for transfection efficiency by measuring the Renilla luciferase activity according to the manufacturer's instructions and normalized to 100 μg of proteins.

2.6. Stable transfection

To obtain stable Neu2-overexpressing PC12 cells and the corresponding mock transfectants, cells (1×10^5/60 mm dish) were transfected with pCDNA-Neu2 or pCDNA expression vectors (1 μg/dish), respectively, in Fugene reagent (Roche Molecular Biochemicals) according to the manufacturer's instructions. Stable transfectants were obtained after 25–30 days selection in G418 antibiotic (0.5 mg/ml, Promega).

2.7. Sialidase assay

Cells were washed with phosphate buffer solution and sonicated at 4 °C in nine volumes of 0.25 M sucrose containing 1 mM EDTA and a mix of protease inhibitors for 5 s at an intermediate setting. The mixture was centrifuged at $600 \times g$ for 10 min and the supernatant ultracentrifuged at $105.000 \times g$ for 90 min at 4 °C. The supernatant was used to assay the cytosolic sialidase activity. The assay mixture contained 60 nmoles of the substrate 4-methylumbelliferyl N-acetylneuraminic acid (4-MUB-NANA) (Sigma–Aldrich), 200 μg of bovine serum albumin and aliquots of enzyme fractions (150–200 μg of proteins) in a final volume of 0.2 ml of 50 mM sodium citrate buffer (pH 5.8). After incubation at 37 °C for 4–5 h, the reaction was terminated by addition of 0.8 ml of 0.25 M glycine buffer (pH 10.4), and the amount of 4-methylumbelliferone released was determined fluorometrically with an excitation wavelight of 365 nm and emission of 450 nm.

3. Results

PC12 cells can be induced to differentiate into a neuronal phenotype by NGF or FGF-2 treatments (Fig. 1A, panel a) [23,24] and to proliferate by EGF treatment (Fig. 1A, panel b) [25]. As previously described [26], a prolonged phosphorylation of the extracellular signal-regulated protein kinases 1/2 (ERK 1/2 or p42/44 MAP kinases) is observed during differentiation induced by NGF, whereas a transient activation of ERK 1/2 is observed during proliferation induced by EGF (Fig. 1B). During differentiation or proliferation of PC12 cells, the Neu2 expression analysis was performed. The RT-PCR analysis showed that Neu2 is a rare transcript in untreated PC12 cells, in fact it is undetectable also after 38 cycles of PCR. As shown in Fig. 2, the differentiating treatments with NGF or FGF-2 induced a transient increase in the Neu2 mRNA steady state levels; in particular, NGF treatment showed a stronger and prolonged increase of the Neu2 transcript with respect to FGF-2 treatment. In addition, the proliferating EGF treatment was able to transiently stimulate the Neu2 transcription with a peak at day 2.

To confirm the Neu2 transcriptional induction, a 1.4 kb fragment of rat Neu2 promoter [21] was cloned in a reporter

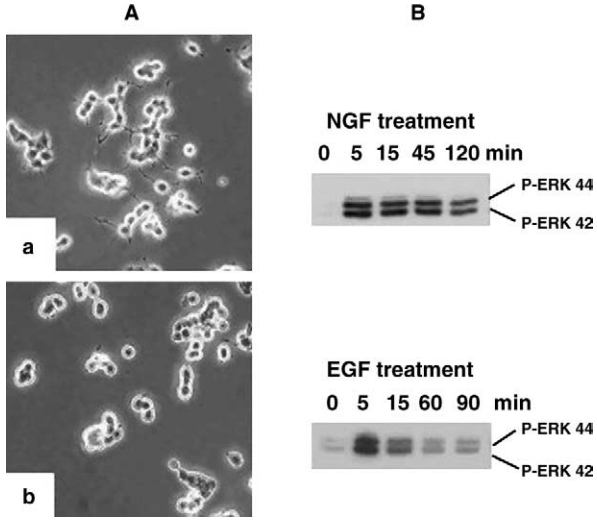

Fig. 1. PC12 cells differentiation and proliferation. (A) Morphology of PC12 cells treated with NGF 100 ng/ml for two days (panel a) and with EGF 50 ng/ml (panel b). The cells were photographed under a phase contrast microscope. Note the neurite outgrowth in PC12 cells differentiated with NGF (panel a). (B) Western blot analysis of ERK 1/2 phosphorylation in PC12 cells treated with NGF or EGF. Cells were serum starved for 12 h and then were added with NGF 100 ng/ml or EGF 50 ng/ml. 15 μg of proteins were probed with monoclonal antiphospho ERK 42/44 antibodies.

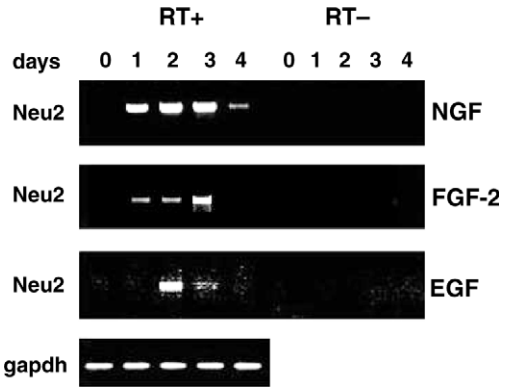

Fig. 2. Neu2 mRNA expression levels in PC12 cells treated with growth factors. PC12 cells were cultured in the presence of NGF (100 ng/ml), FGF-2 (100 ng/ml) or EGF (50 ng/ml) for the indicated periods of time. Neu2 transcript expression was investigated by RT-PCR analysis, as described in Section 2, using gapdh transcript as a control.

luciferase expression vector. To test whether Neu2 rat promoter was responsive to neurotrophic treatments, PC12 cells were transiently transfected with the full-length Neu2 promoter and treated with NGF, FGF-2 or EGF. In agreement with RT-PCR analysis, the treatment with NGF and FGF-2 enhanced the luciferase activity (Fig. 3). As a consequence, in the cells treated up to 72 h with NGF or FGF-2, the luciferase activity reached, respectively, a maximum of ~9 and ~3-fold induction with respect to control cells. By contrast, the treatment of the cells with EGF did not enhance the luciferase expression.

The sequence analysis of Neu2 rat promoter performed using Transfac 4.0 software [27] revealed the presence of responsive elements involved in neuronal differentiation (Fig. 4). In particular, within the promoter region several canonical and non-canonical E-box (–CAxxTG–) are present, sequences known to be signals for the binding of the basic helix–loop–helix transcription factors [28]. In addition, several binding sites for transcriptional factors such as SP1 [29], AP1 [30] and for glucocorticoids receptors [31] were found. Furthermore, typical sequences of muscle regulation such as Myog [32] and

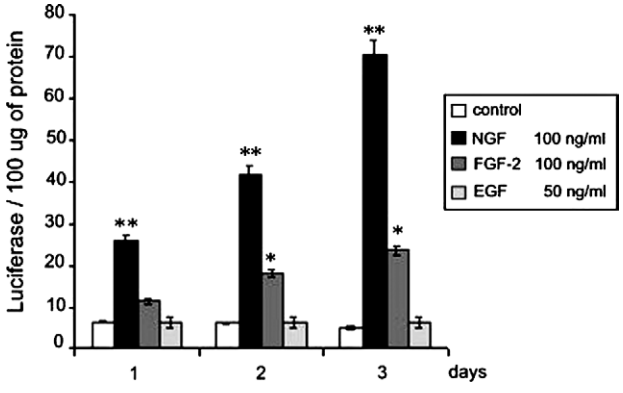

Fig. 3. Neu2 promoter activity in PC12 cells treated with growth factors. Cells were transiently transfected with the expression vector harboring the luciferase reporter gene under the control of the rat Neu2 promoter. The day after, cells were treated with NGF, FGF-2 or EGF and, at indicated times, the luciferase activity was measured in the cell extracts and normalized for proteins content. The results are means of three independent experiments. Statistical analysis was performed by Student's t-test ($n = 3$ for each group). $^{*}P < 0.05$, $^{**}P < 0.01$.

Mef-2 [33] were found, in agreement with higher levels of sialidase Neu2 in the skeletal muscle. Interestingly, in the region from 672 to 776 of promoter a sequence highly similar to a dispersed repetitive element is present. In order to better understand the transcriptional regulation of the Neu2 promoter, four deleted fragments of Neu2 promoter were obtained and used in transient transfections to analyze the luciferase expression (Fig. 4).

The results demonstrated that a progressive deletion of the promoter produced a strong increase of luciferase activity in response to NGF. In particular, two deleted constructs, i.e., RP1/1280 and RP1/815, showed a ~1.2 and ~2.7-fold of luciferase induction with respect to the entire promoter. The transient transfection with the fragment RP1/401 produced a ~11.4-fold induction of luciferase activity, whereas the smaller fragment generated, i.e., RP1/215, revealed a ~8.5-fold induction of luciferase activity.

On the basis of Neu2 transcriptional induction, enzymatic assays were performed. The Neu2 sialidase activity was detected in differentiating PC12 cells, on the contrary no Neu2 activity was found after EGF treatment (data not shown). In particular, the Neu2 activity was detected after NGF treatment at days 1–2 (Fig. 5A), whereas untreated PC12 cells did not reveal the sialidase activity in the cytosolic fraction. As expected, in differentiating PC12 cells the Neu2 activity was found to be low, but strictly correlated with the transcript expression level obtained (Fig. 5A). In agreement, PC12 cells stably transfected with an expression vector harboring the rat cDNA Neu2 sialidase showed high expression of Neu2 transcript and enzymatic activity (Fig. 5B). As a consequence, the enzymatic assays performed at pH of 5.8 revealed that Neu2 transfected sialidase activity was up to 25-times higher than the NGF induced endogenous activity (Fig. 5B).

4. Discussion

The cytosolic sialidase Neu2 is an enzyme particularly expressed in the skeletal muscle [15]. Recently, we demonstrated that its overexpression induces a marked decrement of proliferation and spontaneous differentiation of myoblast C2C12 cells [16]. Although the sialidase Neu2 is expressed in other tissues such as liver [17], thymus [18] and brain [19], its enzymatic activity is often hardly detectable, thus making its study difficult.

In this work, the expression of cytosolic sialidase Neu2 was investigated in PC12 cells, a favored model for the neuronal differentiation. The RT-PCR analysis showed that cytosolic sialidase is a rare transcript in PC12 cells, in fact the Neu2 mRNA was undetectable in untreated cells even after 38 cycles of PCR. This was confirmed on the basis of Northern blot experiments: no Neu2 transcript was detectable also using 5 μg of polyA^{+} RNA of PC12 cells (data not shown). Under differentiating and proliferating conditions the Neu2 transcript increased, suggesting a potential role of cytosolic sialidase in these processes. Using a fragment of 1.4 kb of rat Neu2 promoter in transient transfections of PC12 cells, the transcriptional up-regulation was confirmed only under differentiating conditions. This suggests that the regions able to recognize EGF-dependent nuclear factors could be present in the 5′ upstream of this fragment promoter. The sequence analysis of Neu2 promoter showed the presence of some typical elements

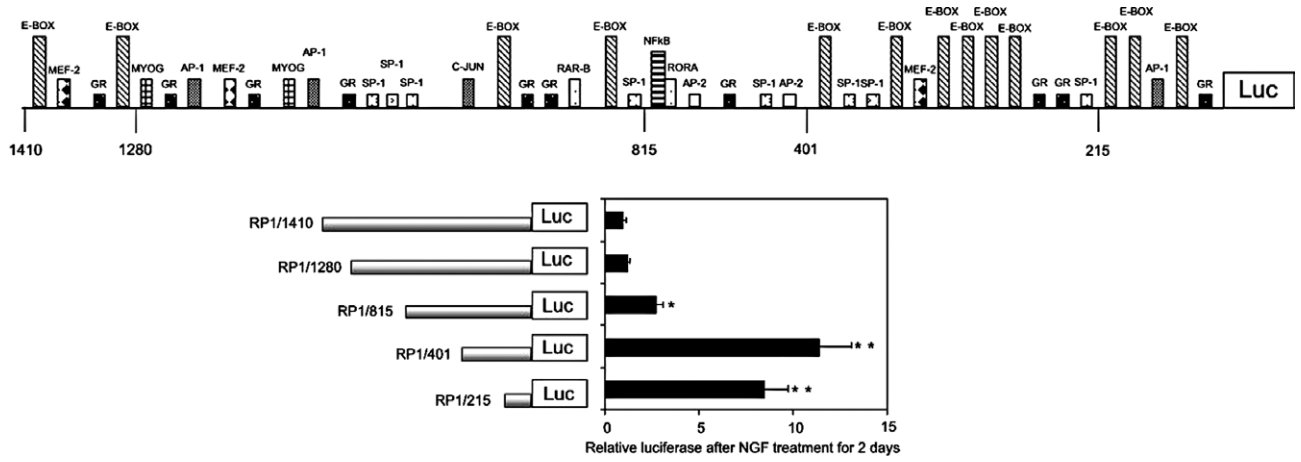

Fig. 4. Sequence analysis of Neu2 promoter and reporter activity of its deleted fragments. The sequence analysis performed using Transfac Promoter software revealed the presence of several E-box (–CAxxTG–), SP1 and AP1 sites. In addition, several binding sites for glucocorticoids receptor (GR) were found. From the position 672 to 776 of promoter, a dispersed repetitive element is present. The reporter vector harboring the entire Neu2 promoter (i.e., RP1/1410) and the different deleted fragments (i.e., RP1/1280, RP1/815, RP1/401 and RP1/215) were transiently transfected in PC12 cells. After NGF treatment, the relative luciferase activity of deleted promoters was calculated as fold-induction number with respect to the activity of entire promoter. The results are means of three independent experiments. Statistical analysis was performed by Student's t-test ($n = 3$ for each group). $^{*}P < 0.05$, $^{**}P < 0.01$.

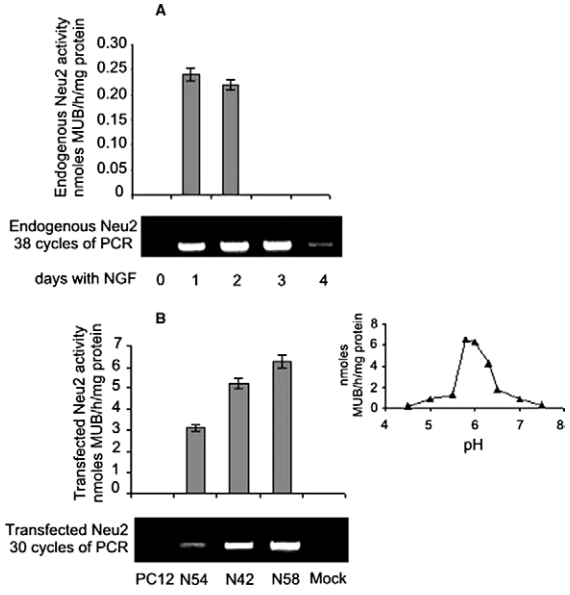

Fig. 5. Enzymatic assays of cytosolic sialidase Neu2. (A) Neu2 endogenous sialidase activity was tested in PC12 cells treated with NGF 100 ng/ml until day four and, in parallel, its transcriptional level was obtained by RT-PCR analysis. (B) Neu2 sialidase activity of PC12 cells stably transfected with the pCDNA-Neu2 vector. After selection, transfectants (i.e., N54, N42 and N58) were compared to parental and mock-transfected cells for rat Neu2 expression by RT-PCR analysis and tested for cytosolic Neu2 sialidase activity. In the upper panel, we report the effect of pH on cytosolic Neu2 activity using the N58 clone.

potentially involved in PC12 cells differentiation, such as binding sites for the nuclear factors SP1 [29] and AP1 [30] and several E-box sequences [28]. Since cooperation between different E boxes present on the same promoter is a common finding in transcriptional regulation of tissue-specific genes [34,35], it is possible that these elements play an important role in Neu2 transcriptional regulation. Interestingly, the remarkable increase of reporter activity obtained by the progressive deletion of Neu2 promoter revealed that within the promoter sequence could exist some regulatory regions that maintain a very low Neu2 expression. In particular, the deletion of the fragment containing a rat dispersed repetitive element caused the major increase of luciferase activity. However, it has been shown that the presence of repetitive sequences in the 5′-untranslated region (5′-UTR) of some genes can influence expression. For example, repetitive elements in the 5′-UTR of a human zinc-finger gene modulate transcription and translation efficiency [36].

The Neu2 enzymatic activity was detectable in PC12 cells at day 1–2 of NGF differentiation, suggesting a potential role of this sialidase in the early steps of neuronal differentiation. The low activity seems to be correlated to its transcriptional levels; in agreement the Neu2 activity in the cytosol of transfectants was found up to 25-times higher than the endogenous activity. The treatment with EGF did not reveal Neu2 activity, but it is possible that a very transient sialidase activity could be present also during EGF induced proliferation.

The role of cytosolic sialidase Neu2 is still unclear, but its reported ability to hydrolyze gangliosides and glycoproteins [37] could influence the sialylation pattern of the glycoconjugates in the cells.

The association of glycosphingolipids to the cytoskeleton in different cell types has been demonstrated [38,39]. As a consequence, the modulation of endogenous gangliosides may represent an early event in cell differentiation preceding neurite outgrowth. In keeping with this hypothesis, Neu2 overexpression has been shown to modulate melanoma cell invasiveness and migration, two processes strictly linked to cytoskeleton functions [40]. Furthermore, the availability of free sialic acids can influence the expression pattern of sialoglycoproteins that play an important role during neuronal processes, in particular during cellular adhesion [41,42]. An increase of free sialic acid at cytoplasmatic and nuclear level, in fact, could represent a potent signal for enhancing the activity of sialyl- and polysialyltransferases. Further studies are in progress in our laboratories in order to better understand the

role of the cytosolic sialidase Neu2 in PC12 cells, also through the characterization of Neu2 transfectants.

Acknowledgements: This work was supported in part by grants from MIUR (Centro di Eccellenza "IDET"), Consorzio Italiano Biotecnologie (CIB) and Centro per lo Studio del Trattamento dello Scompenso Cardiaco (University of Brescia).

References

[1] Reuter, G. and Gabius, H.J. (1996) Biol. Chem. Hoppe Seyler 377, 325–342.
[2] Tokuyama, S., Moriya, S., Taniguchi, S., Yasui, A., Miyazaki, J., Orikasa, S. and Miyagi, T. (1997) Int. J. Cancer 73, 410–415.
[3] Kato, T., Wang, Y., Yamaguchi, K., Milner, C.M., Shineha, R., Satomi, S. and Miyagi, T. (2001) Int. J. Cancer 92, 797–804.
[4] Sawada, M., Moriya, S., Saito, S., Shineha, R., Satomi, S., Yamori, T., Tsuruo, T., Kannagi, R. and Miyagi, T. (2002) Int. J. Cancer 97, 180–185.
[5] Miyagi, T., Sagawa, J., Konno, K. and Tsuiki, S. (1990) J. Biochem. 107, 794–798.
[6] Comelli, E.M., Amado, M., Lustig, S.R. and Paulson, J.C. (2003) Gene 321, 155–161.
[7] Rodriguez, J.A., Piddini, E., Hasegawa, T., Miyagi, T. and Dotti, C.G. (2001) J. Neurosci. 21, 8387–8395.
[8] Proshin, S., Yamaguchi, K., Wada, T. and Miyagi, T. (2002) Neurochem. Res. 27, 841–846.
[9] Sasaki, A., Hata, K., Suzuki, S., Sawada, M., Wada, T., Yamaguchi, K., Obinata, M., Tateno, H., Suzuki, H. and Miyagi, T. (2003) J. Biol. Chem. 278, 27896–27902.
[10] Monti, E., Bassi, M.T., Papini, N., Riboni, M., Manzoni, M., Venerando, B., Croci, G., Preti, A., Ballabio, A., Tettamanti, G. and Borsani, G. (2000) Biochem. J. 349, 343–351.
[11] Bonten, E.J. and d'Azzo, A. (2000) J. Biol. Chem. 275, 37657–37663.
[12] Lukong, K.E., Elsliger, M.A., Chang, Y., Richard, C., Thomas, G., Carey, W., Tylki-Szymanska, A., Czartoryska, B., Buchholz, T., Criado, G.R., Palmeri, S. and Pshezhetsky, A.V. (2000) Hum. Mol. Genet. 9, 1075–1085.
[13] Thomas, G.H. (2001) in: The Metabolic and Molecular Bases of Inherited Disease (Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D., Eds.), 8th ed., Vol. III, pp. 3507–3534, McGraw-Hill, New York.
[14] D'Azzo, A., Andria, G., Strisciuglio, P. and Galjaard, H. (2001) in: The Metabolic and Molecular Bases of Inherited Disease (Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D., Eds.), 8th ed., Vol. III, pp. 3811–3826, McGraw-Hill, New York.
[15] Sato, K. and Miyagi, T. (1996) Bioch. Bioph. Res. Commun. 221, 826–830.
[16] Fanzani, A., Giuliani, R., Colombo, F., Zizioli, D., Presta, M., Preti, A. and Marchesini, S. (2003) FEBS Lett. 547, 183–188.
[17] Miyagi, T. and Tsuiki, S. (1985) J. Biol. Chem. 260, 6710–6716.
[18] Kotani, K., Kuroiwa, A., Saito, T., Matsuda, Y., Koda, T. and Kijimoto-Ochiai, S. (2001) Biochem. Biophys. Res. Commun. 286, 250–258.
[19] Hasegawa, T., Feijoo Carnero, C., Wada, T., Itoyama, Y. and Miyagi, T. (2001) Biochem. Biophys. Res. Commun. 280, 726–732.
[20] Isacchi, A., Statuto, M., Chiesa, R., Bergonzoni, L., Rusnati, M., Sarmientos, P., Ragnotti, G. and Presta, M. (1991) Acad. Sci. USA 88, 2628–2632.
[21] Sato, K. and Miyagi, T. (1995) Glycobiology 5, 511–516.
[22] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[23] Greene, L.A. and Tischler, A.S. (1982) Adv. Cell Neurobiol. 3, 373–415.
[24] Rydel, R.E. and Greene, L.A. (1987) J. Neurosci. 7, 3639–3653.
[25] Huff, K., End, D. and Guroff, G. (1981) J. Cell Biol. 88, 189–198.
[26] Qui, M.S. and Green, S.H. (1992) Neuron. 9, 705–717.
[27] Wingender, E., Chen, X., Hehl, R., Karas, H., Liebich, I., Matys, V., Meinhardt, T., Pruss, M., Reuter, I. and Schacherer, F. (2000) Nucl. Acids Res. 28, 316–319.
[28] Murre, C., Bain, G., van Dijk, M.A., Engel, I., Furnari, B.A., Massari, M.E., Matthews, J.R., Quong, M.W., Rivera, R.R. and Stuiver, M.H. (1994) Biochim. Biophys. Acta 1218, 129–135.
[29] Kadonaga, J.T., Carner, K.R., Masiarz, F.R. and Tjian, R. (1987) Cell 51, 1079–1090.
[30] Leppa, S., Eriksson, M., Saffrich, R., Ansorge, W. and Bohmann, D. (2001) Mol. Cell Biol. 21, 4369–4378.
[31] Strahle, U., Schmid, W. and Schutz, G. (1988) EMBO J. 7, 3389–3395.
[32] Wright, W.E., Sassoon, D.A. and Lin, V.K. (1989) Cell 56, 607–617.
[33] Naya, F.S. and Olson, E. (1999) Curr. Opin. Cell. Biol. 11, 683–688.
[34] Genetta, T., Ruezinsky, D. and Kadesch, T. (1994) Mol. Cell. Biol. 14, 6153–6163.
[35] Weintraub, H., Davis, R., Lockshon, D. and Lassar, A. (1990) Proc. Natl. Acad. Sci. USA 87, 5623–5627.
[36] Landry, J.R., Medstrand, P. and Mager, D.L. (2001) Genomics 76, 110–116.
[37] Tringali, C., Papini, N., Fusi, P., Croci, G., Borsani, G., Preti, A., Tortora, P., Tettamanti, G., Venerando, B. and Monti, E. (2004) J. Biol. Chem. 279, 3169–3179.
[38] Gillard, B.K., Heath, J.P., Thurmon, L.T. and Marcus, D.M. (1991) Exp. Cell. Res. 192, 433–444.
[39] Gillard, B.K., Thurmon, L.T. and Marcus, D.M. (1992) Cell Motil. Cytoskeleton 21, 255–271.
[40] Tokuyama, S., Moriya, S., Taniguchi, S., Yasui, A., Miyazaki, J., Orikasa, S. and Miyagi, T. (1997) Int. J. Cancer 73, 410–415.
[41] Horstkorte, R., Lessner, N., Gerardy-Schahn, R., Lucka, L., Danker, K. and Reutter, W. (1999) Exp. Cell Res. 246, 122–128.
[42] Gallagher, H.C., Odumeru, O.A. and Regan, C.M. (2000) J. Neurosci. Res. 61, 636–645.