ISSN 2225-6016

ВЕСТНИК

Смоленской государственной медицинской академии

Том 19, №1

2020

УДК 16-01/09 14.03.03 Патологическая физиология

THE ROLE OF SIALIDASES IN REVERSION OF MALIGNANT PHENOTYPE OF TUMOUR CELLS DERIVED FROM MUSCLE AND NEURAL TISSUE

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Abstract

Objective. To elucidate the role of lysosomal and plasma-membrane ganglioside sialidases for tumours derived from muscle and neural tissues.

Methods. The treatment of human neuroblastoma NB-1 cells by db-cAMP induced the high frequency of neural cells bearing neurite as well as more than two-fold elevated activity of sialidase associated with plasma-membrane ganglioside sialidase (PMGS). The elevated silaidase activity was accompanied by increasing the quantity of mRNA for that enzyme as was estimated by RT-PCR. The metastatic potential of tumour cells of rat rhabdomyosarcoma was estimated by inoculation tumour cell into rats. Then tumour cell clones were estimated by molecular and genetic techniques for sialidase activity.

Results. The neural cells with elevated PMGS activity and developed neurite were shown to have high activity of another enzyme (acetylcholinesterase) that is strongly considered as a biochemical marker of neural cell differentiation. The lysosomal sialidase activity has been shown to be associated with inhibition of metastatic potential of tumour cells of rat rhabdomyosarcoma. The low metastatic ability of rhabdomyosarcoma was also associated with elevated frequency of nuclear anomalies of tumour cells like internuclear bridging. The PMGS was not shown to influence the rat rhabdomyosarcoma metastasis.

Conclusion. The results presented in this paper strengthen an idea that sialidases hydrolyzing sialoconjugates including unique glycosphingolipids as gangliosides can influence directly and/or indirectly on cell pathophysiology and morphology.

Keywords: sialidases, neural cells, rhabdomyosarcoma

ЗНАЧЕНИЕ СИАЛИДАЗ В РЕВЕРСИИ ЗЛОКАЧЕСТВЕННОГО ФЕНОТИПА ОПУХОЛЕВЫХ КЛЕТОК, ПРОИСХОДЯЩИХ ИЗ МЫШЕЧНОЙ И НЕЙРАЛЬНОЙ ТКАНИ

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Резюме

Цель. Изучить значение лизосомальной сиалидазы и сиалидазы, ассоциированной с плазматической мембраной, для опухолей, имеющих мышечное и нейральное тканевое происхождение.

Методика. Клетки человеческой нейробластомы NB-1 обрабатывались дибутирил-цАМФ, после чего в клеточной культуре оценивалась длина нейральных отростков и сиалидазная активность молекулярно-генетическими методом. Метастатический потенциал клеток перевивной рабдомиосаркомы крыс оценивали с помощью инокуляции опухолевых клеток внутривенно.

После чего подсчитывали частоту опухолевых клонов в лёгких и исследовали эти клоны на сиалидазную активность молекулярно-генетическим методом.

Результаты. После обработки клеток человеческой нейробластомы NB-1 дибутирил-цАМФ в опухолевых клетках резко возрастала активность сиалидазы, ассоциированной с плазматической мембраной, что было ассоциировано с увеличением длины нейральных отростков и повышением ацетилхолинэстеразной активности, что свидетельствует о дифференцировке опухолевых клеток. Повышение лизосомальной активности опухолевых клеток перевивной рабдомиосаркомы крыс сопровождалось снижением метастатического потенциала. Снижение метастатического потенциала этой опухоли было ассоциировано с повышением частоты аномалий ядер опухолевых клеток.

Заключение. На основании полученных результатов можно сделать вывод, что повышение сиалидазной активности опухолевых клеток нейрального и мышечного происхождения ассоциировано с их морфологической и функциональной дифференцировкой.

Introduction

It is well-known fact that neural and muscle tissues differ in their histogenetic origin. Meanwhile those tissues are functionally related. It has been suggested that existence of neural tissue is unreasonable without muscle tissue [15, 16]. Neuronal network controls muscle cells (tissue) But there is feed-back regulation between muscle and neural tissues [1]. It may result in various movement activities of beings. The diversity in specialization of neural and muscle cells caused the certain distinction in complexity of specific molecules and factors which are responsible for structural and functional features of these cells. It has been proved that neural tissue is particularly enriched in specific glycosphingolipids which are gangliosides as compared to striated muscle cells [8, 38]. The enrichment of neural tissue by gangliosides may be basis for high plasticity of neurons and neural tissue as a whole. Meanwhile the gangliosides themselves can serve as a substrate for certain type of enzymes hydrolyzing sialic acids [22]. Sialidases were recognized as enzymes which are particularly relevant to hydrolyzing glycolconjugates bearing sialic acids. At present time several types of mammalian sialidases have been cloned – lysosomal sialidase (LS), cytosolic sialidase (CS) and plasma-membrane ganglioside sialidase (PMGS) [2, 9, 14, 43]. Biochemical approach cleared those sialidases to possess distinct substrate specificity. LS and CS hydrolyze preferentially sialic acids in oligosaccharides whereas PMGS prefers as a substrate gangliosides [20]. Noteworthy PMGS appears to be more active against gangliosides GD1a, GM3, and GD3 [42]. Apparently the location of sialic acids in structure of these unique glycosphingolipids eases PMS the accessibility to the substrate. It has been shown that distinct types of sialidases influence on cell physiology and developmental processes in different manner [17]. CS accompanies differentiation of muscle cells which appears as tube formation [36, 37]. PMGS is one of key factor for axonogenesis and strongly suggested to be a crucial enzyme in a chain of events causing regeneration of damaged axons [33]. It is considered that the change in activity of sialidases could accompany various pathological processes. For example the permanently high activity of PMGS changes an activity of receptor for EGF. In its turn it accompanies development of diabetes mellitus type II [11]. Elevated activity LS is associated with metastasis of marine melanoma B16 [13]. Probably LS affects the glycozilation profile of adhesive molecules by hydrolyzing sialic acid residues on glycolproteins of plasma membrane [24, 25, 26]. Thus it becomes evidently that various types of sialidases changing quantitative and somewhat important qualitative composition of glycolconjugates including gangliosides can be the key factors in regulating intrinsic mechanisms of cell physiology [7, 23].

The clarification of a role of sialidases in pathology of neural and muscle tissues which are histogenetically distinct but functionally relevant is of great interest. In this context the rhabdomyosarcoma of rat and human neuroblastoma NB-1 cells were studied.

The aim of work was to elucidate the role of lysosomal and plasma-membrane ganglioside sialidases for tumours derived from muscle and neural tissues.

Methods

Rhabdomyosarcoma metastases. Rhabdomyosarcoma of rats was induced by 20-methylcholanthrene and assimilated by passages every three weeks for 20 years to grow in lung tissue [40]. The standard scheme

has been used to make each other passage of tumor cells in Wistar rats (males of three months of age) (fig. 1).

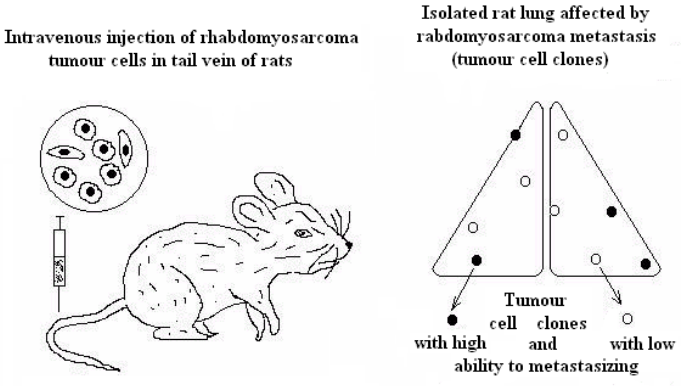

Figure 1. Scheme of experiment to process metastasizes of rhabdomyosarcoma cells in rats

The tumor cell clones can be visualized on the surface of lung as spheres in diameter of 2-3 mm of slight gray colour plunged a bit little in lung tissue. The clones are accurately isolated out of lung tissue and consequently processed with scissor and through syringe. The obtained suspension was filtered through nylon tissue and centrifuged for 10 min at 1000 g. The pallet of tumor cells is resuspended and diluted in appropriate concentration in 199 medium. The viability of cells was estimated Neubauer-improved chamber. To get metastases of rhabdomyosarcoma of rats the tumour cells are injected in concentration of 60.000 cells per 0.2 ml. Afterwords the experimental animals (rats) were sacrificed as to 25 days of experiment and tumour clones (metastases) had accurately been isolated. For ordinary experiment the metastases are halved. One part of tumour cell clone is preserved in medium 199 (Russia) for a day at +4°C and another is promptly processed for cytological analysis and biochemical assay (sialidase activity). Viability of tumour cells after storing under above-mentioned conditions was not less than 15%. Viability was assayed using solution of 0.4% Trypan Blue (Aeroc Organics, USA). Rhabdomyosarcoma cells were processed on to slides, fixed by ethanol, stained by fluorescent dye Chechst-33258, and investigated for the frequency of an anomaly of cell nucleus as interphase (internuclei) bridges under microscope Olympus Vanox-T.

Metastatic potential of rhabdomyosarcoma. After assaying cell nuclear anomalies (interphase bridges) rhabdomyosarcoma cell clones (that means first half of a clone) with high and with low frequency of interphase bridges were tested for metastatic potential as described elsewhere [29]. The tumour cells were intravenously injected in concentration of 60.000 per 0.2 ml. The 25 days later the experimental animals have been sacrificed and the quantity of metastases were scored.

Cell culture. NB-1 cells (Health Science Research Bank) were grown in 45 % RPMI-1640 and 45 % Eagle's minimum essential medium containing 10 % FBS on poly-L-lysine-coated dishes (Falcon). Cells were then serum-starved for 24 h and 48 h with or without 2 mM of db-cAMP, and harvested for biochemical purposes (sialidase and acetylcholinesterase activity assays). To determine neuritogenesis of NB-1 cells, the neurite length of the cells was measured from the cell body to the growth cone [30]. More than 20 arbitrarily chosen fields were imaged under an inverted phase-contrast microscope (Nikon), and over 1200 cells were examined for each experimental condition. The cells bearing neurites were subdivided into four groups: those with neurite length less than 12 μm (ultrashort), 12-24μm (short), 25-36 μm (intermediate), and longer than 36 μm (long).

Quantitative RT-PCR. The human PMGS mRNA level was evaluated by quantitative RT-PCR with a cDNA competitor prepared by NcoI digestion of Bluescript vectors containing the entire open reading frame of human PMS. The primers used were: sense (5'-GACAGAGGGATTACCTACCGGATC-3', nucleotides 55-78 from the start codon) and antisense (5'-GAGCCATGATTCTGACGGTGTT-3', nucleotides 966-987). Total RNA was extracted from the cells by the acid guanidium-phenol-chloroform extraction procedure. First-strand cDNAs were synthesized from the total RNA by revers transcription and then used as templates for PCR under the following conditions: 1 min at 94°C, 1 min at 60°C, and 72°C for 40 cycles, followed by 10 min at 72°C. To normalize for sample variation, the expression of β-actin was measured using a β-actin competitor.

Sialidase and acetylcholinesterase assay. Cells collected were sonicated on ice in ice-cold phosphate-buffered saline containing 10 mM phosphate buffer (pH 7.0), 1 mM EDTA, 0.5 mM phenylmethanesulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml pepstatin (Labsonic M, Sartorius). After centrifugation at 1000 x g for 10 min at 4°C, the cell homogenates were assayed for PMgS activity with bovine brain mixed gangliosides as the substrate in the presence of 0.1 % Triton X-100. After incubation

at 37°C for 1-2 h, released sialic acid was determined by the thiobarbituric acid method of Aminoff [41]. LS activity was determined by fluorometric measurements using 4-methylumbellyferone-α-D-*N*-acetylneuraminic acid as substrate [28]. Acetylcholinesterase activity was assayed according to the spectrophotometric procedure by Ellman et al. [29]. Neural cell homogenate were assayed with acetylthiocholine iodide as the substrate in the presence of 5,5'-dithiobis-(2-nitrobenzoic acid).

Results

Human neuroblastoma NB-1 cells. Spontaneous formation of neurites could be determined in neural cells without db-cAMP treatment. Spontaneous formation of neurites by neural cells is not significant under ordinary conditions if cells are culturing for a day. However more long incubation of neural cells in complete medium can accompany more prominent neurite formation. Due to this fact it was reasonable to design experiment under serum starved conditions. In a day of culturing in the presence of db-cAMP the frequency of cells with neurites of intermediate and long lengths has significantly been elevated (fig. 2).

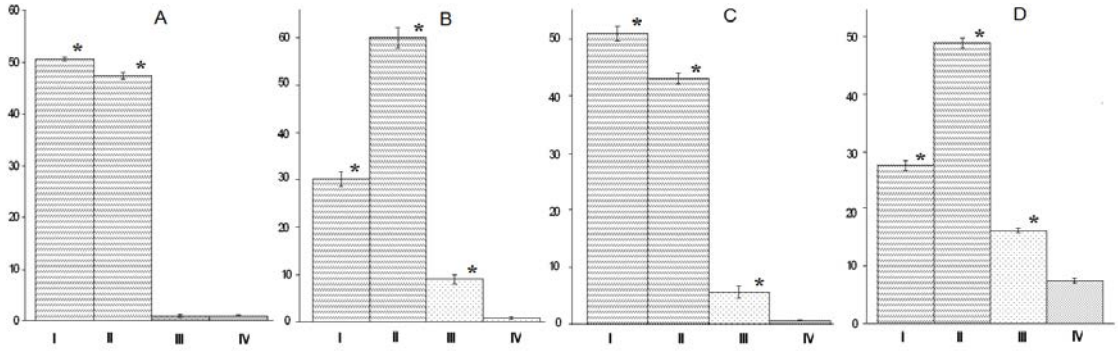

Figure 2. The neurite length in human neuroblastoma NB-1 cells. A – A day of incubation without db-cAMP,.B – A day of incubation with db-cAMP. C – Two days of incubation without db-cAMP. D – Two day of incubation with db-cAMP Serum-starved condition. The neural cell types according to the length of neurites: I – <12 μm; II – 12-24 μm; III – 25-36 μm; IV – >36 μm. y-axis – %. Significantly different from neural cell types III and IV (*, $p<0.001$ evaluated by Student's t-test)

The frequency of neural cells with neurites length of which exceeded more than 24 μm has achieved 9% (type III). Whereas neural cells without incubation in the presence of synthetic analog of cyclic-adenosine-monophosphate showed slight development of intermediate neurites. The culturing neural cells in the presence of db-cAMP for 48 h let elucidate more significant changes in neurite length as compared to the culture without db-cAMP. Dibutyryl-cyclic-adenosine-monophosphate stimulated neural cells to develop neurites length of which exceeded 36 μm. The frequency of those cells was being achieved 7.4±0.4 % (fig. 3). At the same time the paired culture but without db-cAMP contained less than 1% of cells of type IV.

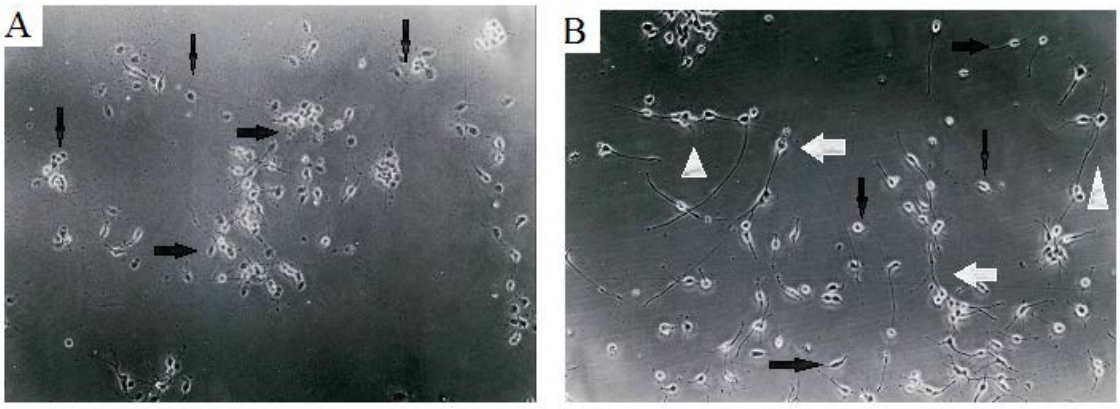

Figure 3. The development of neurites in human neuroblastoma NB-1 cells. A – without db-cAMP treatment and B – db-cAMP added in serum-starved medium (two days of experiment). The neural cells of types I, II, III, and IV are depicted by thin, black thick, white thick, and pyramidal arrows, respectively. Phase-contrast microscope, magnification ×250

Sialidase activity assay of neural cell homogenate with gangliosides was shown to be higher in cultures treated by db-cAMP (fig. 4). In those cells the sialidase activity achieved 10.1±1.7 units/mg protein in the presence of db-cAMP in a day. At the same time the enzyme activity was two–fold lower in neural cells in the absence db-cAMP and did not significantly differ from the value assayed for parental culture. It is noteworthy that the sialidase activity tended to decrease in neural cells which had been cultured in the presence of db-cAMP within 48 hours of experiment. But the activity of enzyme was still elevated. Sialidase activity assay of neural cell homogenate in the presence of 4-methylumbellyferone-α-D-*N*-acetylneuraminic acid showed no difference between cells cultured either in the presence or in the absence of db-cAMP (Table 1).

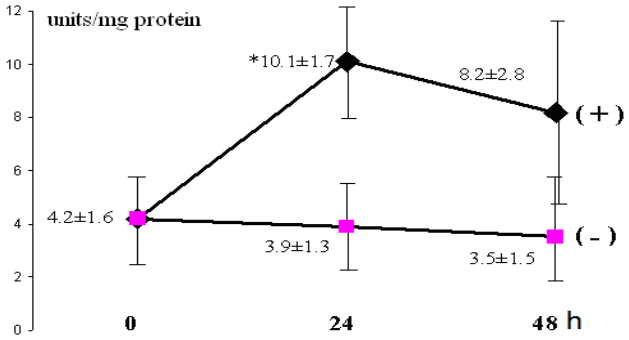

Figure 4. Plasma membrane sialidase activity in human neuroblastoma NB-1 cells. Incubation of neural cells in presence (+) or absence (–) of db-cAMP (* – $p<0.05$ evaluated by Student't-test)

Table 1. Lysosomal sialidase activity (units/mg protein) in human neuroblastoma NB-1 cell

Incubation time, h	Db-cAMP	
	–	+
24	0.896±0.177	0.905±0.097
48	1.114±0.215	0.931±0.121

Note. In parental cell culture (0 h) LS activity showed 0.884±0.139 units/mg protein

High activity of PMGS was also accompanied by the elevation of mRNA quantity for that sialidase (fig. 5). All neural cells treated by db-cAMP as compared to neural cells without exposition to db-cAMP showed significant elevation in amount of mRNA. It is of interest to note that the treatment of neural cells had not only been accompanied by development of neurites but also resulted in an increase in the activity of well-proved biochemical marker of neural cell differentiation as acetylcholinesterase (fig. 6). The increase in enzyme activity was not detected in the culture of neural cells without exposure to db-cAMP.

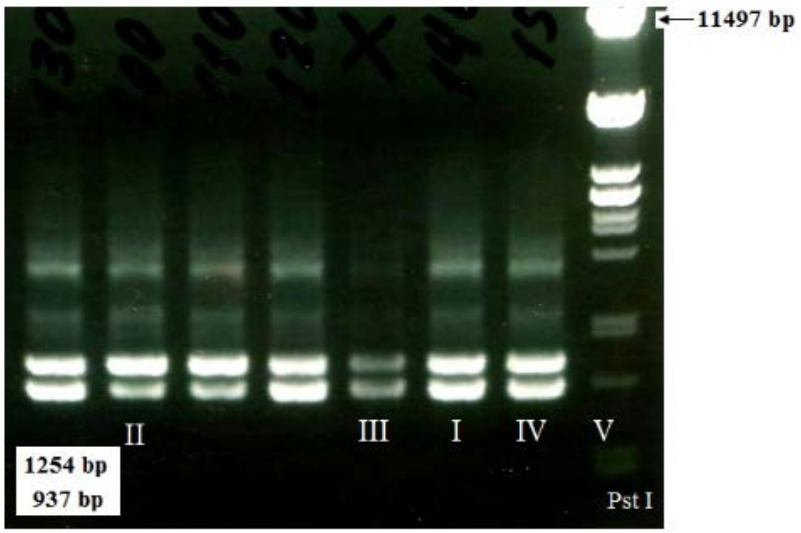

Figure. 5. The mRNA level for PMGS in human neuroblastoma NB-1 cells determined by quantitative RT-PCR using β-actin as an internal control. I – 93.5 fg (control); II – 400.7 fg (db-cAMP + , 24 h); III – 108.7 fg (db-cAMP –, 24 h); IV – 387.8 fg (db-cAMP + , 48 h); V – 96.2 fg (db-cAMP, 48 h)

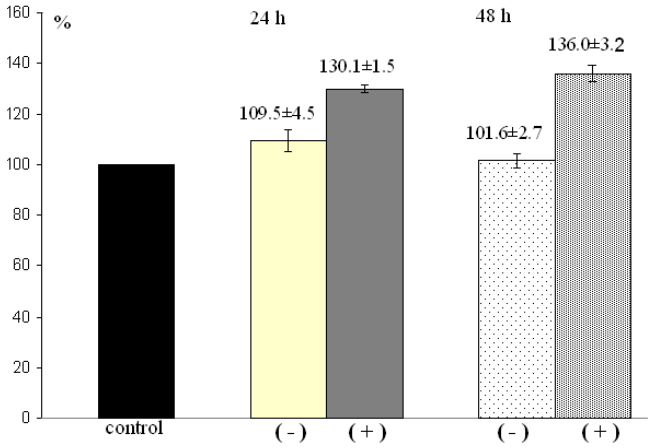

Figure 6. Acetylcholinesterase activity in human neuroblastoma NB-1 cells. Incubation of neural cells in presence (+) or absence (–) of db-cAMP

Rhabdomyosarcoma of rats. The studying tumour cells from metastases (tumour clones) of rhabdomyosarcoma showed that the frequency of nuclear anomalies like internuclear bridges can vary among tumour clones (fig. 7). Tumour cells with low frequency of internucler bridges tend to produce more metastases and vice versa (table 2). If the mean frequency of cells with internuclear bridges per tumour clone does not exceed 0.5% the quantity of rhabdomyosarcoma metastases formed in lung a rat can be not less than 100. Otherwise the clones with tumour cells found to be enriched by internuclear bridges show clearly low ability to form metastases.

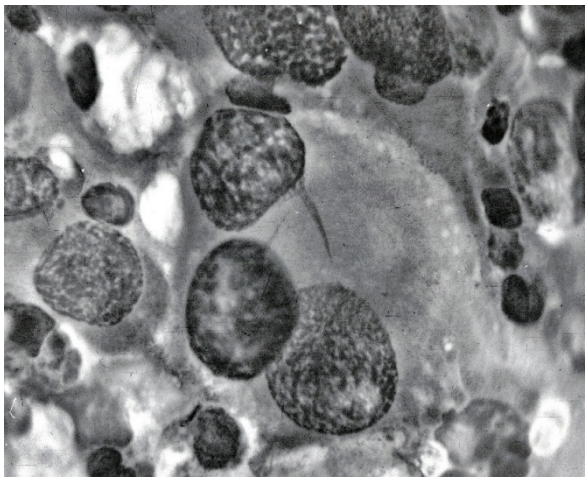

Figure 7. Tumour cell from rhabdomyosarcoma metastasis with internuclear bridges. Chechst 33258 staining. Magnification ×1000

Due to distinct ability to form metastases it is of interest to elucidate if ability to metastasizing is associated with activity of various types of sialidases. As it is known LS and PMGS are distinct in their substrate specificity changing preferentially composition of oligosaccharides and gangliosides, respectively. It can in its turn change structural composition and functional features plasma cell membrane and affect ability of tumour cells to metastasizing and invasion. Connection this high and low metastatic tumour cells from respective metastases (see Table 2) have been assayed for sialidase activity with different substrates. As follows from table 3 the metastases originated from low metastatic tumour cells showed lysosomal sialidase activity in the crude extract as of 1.100±0.284 units/mg protein on the average. LS activity in the metastases from high metastatic tumour cells determined under the same conditions was determined as mean value of 0.818±0.271 units/mg protein (Tabl. 3). Then the enzyme assay of particulate fraction showed the metastases formed by low and high metastatic tumour cells to have been more different in LS activity. The mean values exhibited 2.70±0.29 and 1.48±0.12, respectively. These values were found to differ significantly ($p<0.01$). Sialidase activity assay against gangliosides of tumour cell homogenates of rhabdomyosarcoma metastases resulted from tumour cells with low and high metastatic ability did not show them to have prominent difference (Table 4).

Table 2. Ability of rhabdomyosarcoma cells to form metastases and internuclear bridging

Tumour cell clones	Mean frequency of internuclear bridges, %	The quantity of metastases per animal
low frequency of tumour cells with internuclear bridges	0.5	107.1±23.9 (* N=15)
high frequency of tumour cells with internuclear bridges	18.5	28.6±7.5 (N=15)

Note. * N – the number of animals in each group

Table 3. Lysosomal sialidase activity of rhabdomyosarcoma metastases resulted from tumour cells with high and low ability to metastasis

Metastases		Lysosomal sialidase activity, units/mg protein	
		Crude extract	Particulate fraction
Tumour cell clones with high metastatic ability	D 1	0.602	1.38
	D 2	0.905	1.49
	D 3	0.950	1.57
Mean value		0.818±0.271	1.48±0.12
Tumour cell clones with low metastatic ability	C 1	0.903	2.81
	C 2	1.297	2.44
	C 3	1.101	2.86
Mean value		1.100±0.284	2.70±0.29 *

Note. * – $p<0.01$ (evaluated by Student's t-test)

Table 4. Plasma membrane ganglioside sialidase activity of rhabdomyosarcoma metastases resulted from tumour cells with high and low ability to metastasis

Metastases		Plasma membrane ganglioside sialidase activity, units/mg protein
Tumour cell clones with high metastatic ability	D 1	0.96
	D 2	1.32
	D 3	0.84
Mean value		1.040±0.187
Tumour cell clones with low metastatic ability	C 1	1.04
	C 2	0.91
	C 3	0.84
Mean value		0.930±0.167

Discussion

The previous results have elucidated that neural cells can develop prominent neurites in response to various stimuli. The significant neurite formation of Neuro2A cells was accompanied by elevating PMS activity in response to 5-bromodeoxyuridine treatment. In the experiment with BrdUrd the frequency of cells developing neurites have achieved 44 %. At the same time in control less than 31 % had neurites. The accelerated neurite formation of marine neural cells of Neuro 2A was accompanied by marked elevation of acetylcholinesterase activity [10].

Cyclic-adenosine-monophosphate has previously been proved as a factor of choice to induce differentiation in neural cells. Cell line NG108-15 has been shown to develop neurites in response to db-cAMP application [18]. In this paper human neuroblastoma NB-1 cells have clearly been shown to be responsive to cyclic-adenosine-monophosphate. Cyclic-adenosine-monophosphate well-known as a second messenger is certainly involved in molecular signaling [39]. A-kinase is strong considered to be a

key factor in cAMP-signaling pathway. It has already been shown that both A-kinase and PMGS are associated in rafts in the plasma membrane [12, 31, 32]. It can strongly be suggested that exposition of neural cells to db-cAMP induces activation of A-kinase which in its turn can directly or indirectly transactivate PMGS. It has earlier been shown that PMGS in its primary structure possesses a phosphorylation site for A-kinase [11, 27]. PMGS in its turn activates factors involved in neurite formation by neural cells and an axon by neurons. The later event could particularly be important in relation to axonogenesis. Most likely PMGS changing quantitative and somewhat important qualitative composition of gangliosides in plasma membrane can induce polarization of neurons which get started developing axons. According to this it is reasonable to make a suggestion that PMS appears as real candidate for induction of axonogenesis.

The study of primary hippocampal neurons derived from rat embryos clearly showed that an increase in PMGS activity not only efficiently accelerated axonogenesis but also evidently resulted in the regeneration capacity of the initial axon growth. Otherwise the inhibition of neuronal PMGS activity diminished neurite growth. Hippocampal neurons which had high activity of PMGS developed axons longer as compared to ones which had had basal level of that activity. The regeneration of damaged axons took place in those neurons whose PMGS was high [33]. In this paper the exposition of human neuroblastoma NB-1 cells to db-cAMP did not resulted in elevation of lysosomal sialidase activity. This observation clearly shows that the regulatory role in neurite formation belongs to PMGS but not LS. The incubation of human neuroblastoma NB-1 cells under serum starved condition underlines that serum depletion itself does not possess stimulatory effect on neurite formation and is not associated with elevation in activity of either PMGS or LS. Thus it can be concluded from this investigation that the differentiation of tumour cells derived from neural tissue is positively related with high PMGS activity and has no relationship with changing in activity of LS.

In this context it is of interest to discuss the effects of sialidase activities on the malignant phenotype of cancer cells derived from muscle tissue. Because it is well-known fact that differentiation of skeletal muscle cells are associated with glycosylation events as elucidated by studies of β-D-galactosyl specific lectins [6, 3, 19]. Cytosolic sialidase has been proved to be associated with differentiation of rat L6 myogenic cells. The myotube formation in myogenic cells was accompanied the increase in activity of this type of sialidases. Further studies on sialidases have clearly showed that not only cytosolic type but also lysosomal type can closely be associated with regulation of malignant phenotype of tumour cells. In this context it is of particular interest the suppression of pulmonary metastasis in murine B16 melanoma cells by overexpression of both cytosolic and lysosomal types of sialidase. The high expression of sialidases in parental cells resulted in drastic diminishes of experimental pulmonary metastasis and tumour progression. The levels of sialidase activity which had been assayed in transformed rat 3Y1 cells of different metastatic potential clearly showed inverse correlation of sialidase activities with metastatic potential [40].

The metastatic potential of tumour cells is appeared to be dependent on motility, attachment to substrate, and others. Those events are closely related to cytoskeleton component remodeling. Due to this fact the nuclear anomalies in rat rhabdomyosarcoma cells are not only consequence of genome instability. Internuclear bridging in malignant cells could be dependant on and supported by cytoskeleton remodeling. Although a relationship between changing in sialidase activity and nuclear anomalies as a hallmark of cytoskeleton remodeling remains to be proved, there is a row of data supporting significance of sialoconjugates in cytoskeleton-related functions. In this paper the inverse relationship was found between LS activity and metastatic potential, and direct correlation between LS activity and internuclear bridging in rat rhabdomyosarcoma cells. In spite of the fact that sialoconjugates including gangliosides are abundant constituents of plasma membrane there is a body of evidence that those molecules can be localized intracellular. For example monosialoganglioside GM3 is strongly supposed to be intracellular associated with the cytoskeleton. Desialylation of GM3 by sialidase is considered as an event in the regulation of cytoskeleton function. It is still discussable if any sialidase activity can present in nuclear membrane. But the research in this way is in progress [5, 34, 35].

The results presented in this paper strengthen an idea that sialidases hydrolyzing sialoconjugates including unique glycosphingolipids as gangliosides can influence directly and/or indirectly on cell physiology (pathophysiology) and morphology. The research scale of the paper is limited by studies of two malignant tumours and the data obtained here should be considered as preliminary. However the study would be useful in searching of molecular targets to combat those tumours. The differentiation of human neural cells that is accompanied by elevation in plasma membrane ganglioside sialidase and the decrease in metastatic capacity of rat rhabdomyosarcoma cells that is associated with the increase in activity of lysosomal sialidase may indicate the distinct role of these two types of sialidase in reversion of malignant phenotype of tumours derived from neural and muscle tissues.

Conclusion

The results presented in this paper strengthen an idea that sialidases hydrolyzing sialoconjugates including unique glycosphingolipids as gangliosides can influence directly and/or indirectly on cell physiology (pathophysiology) and morphology. The research scale of the paper is limited by studies of two malignant tumours and the data obtained here should be considered as preliminary. However the study would be useful in searching of molecular targets to combat those tumours. The differentiation of human neural cells that is accompanied by elevation in plasma membrane ganglioside sialidase and the decrease in metastatic capacity of rat rhabdomyosarcoma cells that is associated with the increase in activity of lysosomal sialidase may indicate the distinct role of these 2 types of sialidase in reversion of malignant phenotype of tumours derived from neural and muscle tissues.

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