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SPERMIDINE ACTIVATES AUTHOPHAGY BUT DOES NOT RESCUE HUMAN NEUROBLASTOMA SH-SY5Y CELLS FROM EFFECTS OF ARGININE STARVATION

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Background. Neuroblastoma is a malignant tumor of the sympathetic nervous system common in early childhood. Autophagy is manifested in neuroblastoma cells at basal levels, but is often upregulated in cells of the aggressive neuroblastoma forms.

The aim of the study was to evaluate effects of polyamine spermidine and deficiency of arginine on cell viability and autophagy regulation in cells of human neuroblastoma.

Materials and Methods. The human neuroblastoma SH-SY5Y cell line was an experimental model for the MTT assay of metabolic activity and cell viability upon exposure to different concentrations of spermidine in complete and arginine-free media. Assessing autophagy induction under arginine deficiency and spermidine treatment was carried out using fluorescence microscopy of neuroblastoma cells labeled with autophagic lysosomes-staining dye monodancylcadaverine.

Results and Discussion. It was found that arginine withdrawal abrogates proliferation of SH-SY5Y cells. In the presence of arginine precursor, citrulline, in arginine-free medium, SH-SY5Y cells could not proliferate and, therefore, were auxotrophic for arginine. SH-SY5Y cells were more sensitive to arginine starvation than to starvation for indispensible amino acids lysine or leucine. It was also revealed that spermidine at low concentrations of 5–10 μM did not affect SH-SY5Y cells viability irrespective of arginine availability. However, at 50 μM and higher concentrations this polyamine was highly cytotoxic in arginine-sufficient or deficient media. Analysis of autophagy induction by spermidine and under arginine starvation revealed an increase in the number of autophagic lysosomes in SH-SY5Y cells and additive effect of the two stimuli.

Conclusion. The described experiments revealed that arginine deprivation abrogated proliferation led to a decrease in cell viability and induced autophagy in human neuroblastoma SH-SY5Y cells. Spermidine at the concentrations of 5–10 μM, while upinducing autophagy, did not improve the survival of SH-SY5Y cells under arginine deprivation, whereas at a concentration above 50 μM, spermidine had a strong cytotoxic effect. The main observation of this study is that autophagy can be readily manipulated in neuroblastoma cells by simultaneous deprivation for arginine and spermidine treatment.

Keywords: cancer, neuroblastoma, SH-SY5Y, autophagy, spermidine.

INTRODUCTION

Autophagy is a multifaceted vital process in cells that depending on external stimuli can either support cells viability or stimulate cell death. To maintain normal cellular homeostasis, autophagy should occur at a low basal level. The main housekeeping function of autophagy is to destroy and recycle misfolded proteins aggregates or impaired organelles (Ghosh *et al.*, 2020). Such stimuli as nutrient deprivation, hypoxia, oxidative stress, infection, etc. up-induce the formation of intracellular vesicles with double membrane – autophagosomes. Such vesicles surround or fuse with cellular proteins or organelles for their transfer to the lysosomes to produce low-molecular weight compounds such as amino acids, nucleotides and others. However, once this degradation process turns into excessive, it can serve as a mechanism of programmed cell death (Mizushima *et al.*, 2010).

Autophagy usually functions as a main suppressor or inhibitor of tumorogenesis in the early stages of malignant cell transformation. Nevertheless, as soon as the tumor has adapted to its environment, autophagy may act as a defense mechanism used by malignant cells to overcome metabolic stresses, as well as those induced by medical treatments. It was therefore proposed that inhibition of autophagy in certain cancer cells may lead to the programmed cell death and tumor reduction (Belounis *et al.*, 2016). When certain stimuli trigger both apoptosis and autophagy, autophagy is often observed before apoptosis induction as a prosurvival anti-stress mechanism (Shen & Codogno, 2011). In this case, autophagy counteracts apoptosis (Boya *et al.*, 2005). On the other hand, apoptosis can also inhibit autophagy by cleavage of autophagy-related proteins ATG3, BECN1, or AMBRA1 (Luo & Rubinsztein, 2010; Oral *et al.*, 2012; Wirawan *et al.*, 2010). The physiological significance of autophagy-associated cell death in mammals is still under debate, since it does not have clear ontological effects, but it was implicated into drug-induced cell death in some cancer cells (Fazi *et al.*, 2008; Shimizu *et al.*, 2004). The consequences of autophagy activation on the other hand depend on the degree of its induction, duration, and cellular context. Although the numerous roles of autophagy in cancer require further elucidation, it is clear that it is the key process in many malignant cells physiological responses to stress (Tian *et al.*, 2021).

It has been previously reported that autophagy occurs in neuroblastoma cells at the basal level (Belounis *et al.*, 2016). Other data revealed augmented autophagic turnover in aggressive human neuroblastomas associated with enhanced proliferation rate and MYCN oncogene amplification (Samardzija *et al.*, 2016). However, the role of autophagy in malignant transformations leading to neuroblastoma remains obscure.

Neuroblastoma is a neoplasm of the sympathetic nervous system with neuroendocrine nature of development. This type of cancer is one of the most common in early childhood, but can also occur in adulthood (Nakagawara *et al.*, 2018). Neuroblastomas are caused by numerous abnormalities at the different structural levels. One of the cellular mechanisms that can be disturbed due to malignant changes in the genome, epigenome, transcriptome, proteome, etc. is autophagy. Inhibition of autophagy in neuroblastoma cells (especially high-risk neuroblastomas) often leads to inhibition of proliferation and activation of apoptosis. Thus, modulating autophagy in the treatment of neuroblastoma seems to be a reasonable approach (Huang & Gu, 2020; Silva *et al.*, 2020).

Although many chemotherapeutic drugs evoke apoptotic (or in some instances necroptotic) cell death, it is not excluded that therapeutic overinduction of the already disregulated autophagy in many tumors may lead to an effective cellular catastrophic death, specifically for malignant cells. Such a direction of research is of importance not only for our basic understanding of cross-regulation of cell death pathways, but also of practical importance in case triggers of autophagy are physiologically applicable *in vivo*.

It has been proven that autophagy can be induced by nutritional, pharmacological or genetic interventions. This includes caloric restriction or intermediate fasting, application of mTOR inhibitor rapamycin, sirtuin-1 activator resveratrol, polyamine spermidine, or genetic ablation of p53 (Pietrocola *et al.*, 2015).

It has been reported that cellular polyamines can modulate the survival of normal and cancer cells. Polyamines regulate gene activities, post-translational modifications of proteins, processes of cell proliferation, autophagy, and exhibit a pronounced cytoprotective effect. In turn, the depletion of polyamine pools activates cellular control points that limit proliferation or induce apoptosis (Evageliou & Hogarty, 2009). Excessive accumulation or depletion of the intracellular polyamine spermidine can affect many cellular functions (Tian *et al.*, 2021). For instance, spermidine belongs to low-molecular-weight autophagy regulators (Eisenberg *et al.*, 2009) and can be potentially used *in vivo* to hyperactivate this process in cancer cells (Chen *et al.*, 2018). It induces autophagy by decreasing the activity of a specific acetyltransferase, E1A-binding protein p300 (EP300) that acts as a sensor of nutrient-dependent acetyl-CoA levels (Mariño *et al.*, 2014). EP300 in turn inhibits autophagy by acetylation of a number of key autophagy-related proteins LC3, ATG5, ATG7 and ATG12 (Lee & Finkel, 2009; Ghosh *et al.*, 2020). It was proposed that autophagy-repressive activity of EP300 may not only depend on the intracellular pool of acetyl-CoA, but also be regulated by spermidine and likely other polyamines (Pietrocola *et al.*, 2015).

Arginine is a semi-essential amino acid that, in addition to protein synthesis, is involved in numerous metabolic and signaling pathways necessary for normal cell functioning. These include, in particular, the synthesis of polyamines (**Fig. 1**) and NO, and regulation of mTOR and autophagy (Zou *et al.*, 2019). It has been previously established that arginine deficiency strongly inhibits proliferation of many malignant neoplasms *in vitro* and *in vivo* (Stasyk *et al.*, 2015; Zhang *et al.*, 2021). For instance, auxotrophic for arginine tumors (melanomas, lymphomas, hepatocarcinomas, malignant pleural mesotheliomas, prostate, ovarian, colon, pancreatic cancers and some others) are highly sensitive to arginine limitation. This property applies even to a subset of tumors that are not auxotrophic for this amino acid (Stasyk *et al.*, 2015).

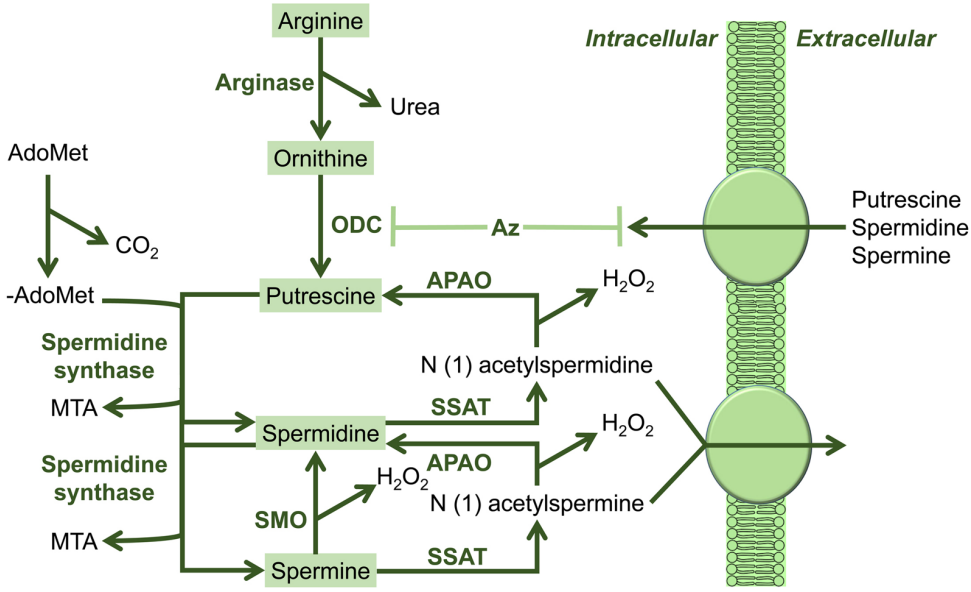

Fig. 1. Polyamine metabolism in mammals. *Legend*: ODC – ornithine decarboxylase (EC 4.1.1.17); AdoMetDC – S-adenosylmethionine decarboxylase (EC 4.1.1.50); SSAT – spermidine/spermine-N(1)-acetyltransferase (EC 2.3.1.57); APAO – FAD-dependent N (1)-acetyl polyamine oxidase (EC1.5.3.11); SMO – spermine oxidase (EC 1.5.3.3); Az – protein Az; MTA – 5′-deoxy-5′-methylthioadenosine; AdoMet – S-adenosylmethionine; DC-AdoMet – decarboxylated S-adenosylmethionine (adapted from Huang *et al.*, 2009)

The aim of this study was to evaluate for the first time the effects of spermidine and arginine starvation on proficiency of autophagy and cell viability on the model of human neuroblastoma cells.

MATERIALS AND METHODS

Cell culture. The human neuroblastoma SH-SY5Y cell line was kindly provided by Prof. J. Redowicz (Nencki institute of experimental biology, PAN, Warsaw, Poland), and was originally derived from a metastatic bone tumor biopsy (Ross, Spengler, & Biedler). Cells were cultivated in complete medium (CM) that contained Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, Germany) with 10% fetal bovine serum (FBS; Sigma-Aldrich, Germany), 2 mmol/L glutamine, and 40 mg/L gentamycin and maintained in the incubator at 37 °C with 5% CO_2. Individual amino acid-free media were formulated based on synthetic CM and dialyzed human serum. Arginine-free medium (AFM) was also prepared by treating regular CM with recombinant human Arginase I in final concentration of 2 U/mL.

MTT-assay. Metabolic activity as a function of cell viability under tested experimental conditions was determined using the MTT-assay. Cells were seeded in a 96-well plate with seeding density of 20,000 cells per well. MTT-assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan crystals by NAD(P)H-dependent oxidoreductase which is

present only in metabolically active cells (Vistica *et al.*, 1991). The MTT reagent was prepared as follows: 5 mg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was dissolved in 1 mL of 10 mM PBS. 200 mL of CM medium with the addition of 1% MTT reagent was added to wells previously washed from the experimental medium with PBS. The plates were incubated at a temperature of 37 °C for three hours. DMSO was used as a solvent for formazan crystals formed during the reaction. To fully dissolve formazan crystals, plates were incubated for 5 minutes at a temperature of 37 °C. The concentration of formazan in the wells was determined by the spectrophotometric method on the "Plate Reader BioTek" device by optical absorption at a wavelength of 490 nm. The number of living cells was determined by the ratio of optical densities (in percent). As an indicator of 100% of living cells in the culture, the index of extinction (λ = 490 nm) of the reaction mixture containing the MMT reagent obtained for cells cultivated for 24 h on the CM has been taken.

Assessing autophagy induction by fluorescence microscopy. Monodansylcadaverine (MDC) is an autofluorescent weak base. This quality of MDC enables accumulation of MDC in acidic autophagic lysosomes (Klionsky *et al.*, 2012). To monitor autophagy, SH-SY5Y cells were pre-attached to glass coverslips. Coverslip glasses with cells were incubated with 0.05 mM MDC (Sigma-Aldrich) in PBS at 37 °C for 10 min. After incubation, cells were washed three times with PBS and immediately examined with a fluorescence microscope (ZEISS, Axio Imager A1) equipped with Axio Vision Software (v. 4.6.3). Cells were observed under 400× magnification. For fluorescence microscopy we used DAPI filter and shutter speed of 50 ms. Images were captured with a CCD camera and imported into Photoshop. Quantification of cell fluorescence was conducted using ImageJ 1.48v Software.

IC_{50} values. IC_{50} values for SH-SY5Y cells cultivated in CM and AFM with addition of spermidine was calculated using online tool "IC_{50} Calculator" (https://www.aatbio.com/tools/ic50-calculator).

Statistical analysis. Statistical analysis of the results was performed using Microsoft Excel 2016. The calculation of the main statistical indicators was performed on the basis of the direct quantitative data (arithmetic mean value – M; arithmetic mean standard error – m). To assess the probability of the difference between the statistical characteristics of the three alternative data sets (data of three independent experiments), the Student's ratio was calculated. The difference in the indications of probability $p \geq 0.95$ (significance level) was considered probable at $P < 0.05$ after calculating *t* according to the table of Student's *t*-distribution.

RESULTS AND DISCUSSION

The effect of arginine deficiency on the viability of human neuroblastoma SH-SY5Y cells. The first task of our study was to analyze the effect of arginine deficiency on proliferation and viability of neuroblastoma SH-SY5Y cells. We used several defined cultivation media, such as complete medium (CM), arginine- (AFM), lysine- (KFM) and leucine- (LFM) free media, as well as citrulline-containing (AFM + Cit) medium. CM provided control condition for the normal growth; limitation of indispensable amino acids lysine or leucine was used as a control for comparison with arginine deprivation; and supplementation of citrulline into arginine-free medium served as a control for cells ability to

synthesize arginine from its anabolic precursor. The MTT test was used to determine the proportion of metabolically active cells cultivated at different conditions as described in Materials and Methods.

It was found that restriction of the exogenous supply of arginine from the cultural medium abrogated proliferation of neuroblastoma cells and reduced the fraction of viable cells to 48% after 72 h of incubation in AFM (**Fig. 2**). The residual survival of SH-SY5Y cells in KFM and LFM was 58% and 89% respectively, suggesting that neuroblastoma cells were the most sensitive specifically to arginine deprivation. Similar antiproliferative effect of arginine limitation was observed for neuronal-derivative human glioblastoma cell lines U251 MG and U87 MG (Pavlyk *et al.*, 2015). In the latter study it was also established that the growth of glioblastoma cells in AFM was not rescued by arginine precursor, ornithine, but was partially restored by another precursor, citrulline. For neuroblastoma SH-SY5Y cells, exogenous 400 μM citrulline was not able to rescue growth or cell viability (**Fig. 2**), suggesting that SH-SY5Y cells are deficient in argininosuccinate synthetase (ASS), the key enzyme of urea cycle that converts citrulline to the immediate arginine precursor argininosuccinate. Thus, these data confirm that SH-SY5Y cells are auxotrophic for arginine.

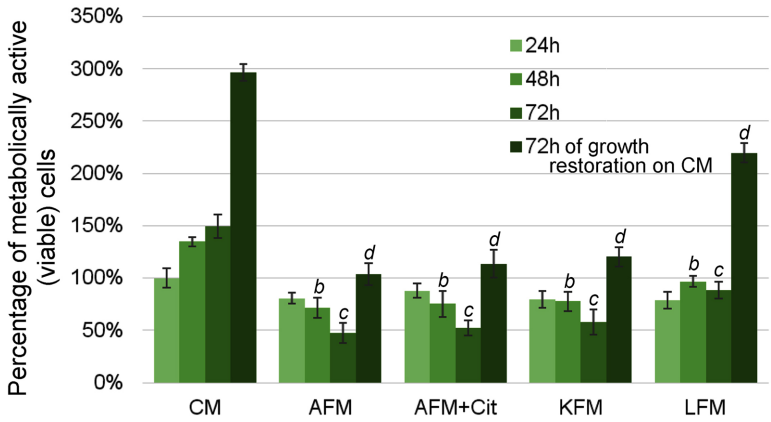

Fig. 2. The effect of single amino acid deprivation on human neuroblastoma SH-SY5Y cells. The viability of cells cultivated in the complete medium (CM), arginine-free medium (AFM), arginine-free medium with citrulline (AFM + Cit), or lysine-free (KFM) and leucine-free medium (LFM) for 24, 48, 72 h, and at 72 h after re-supplementing the corresponding amino acid (complete CM) (***a*** – the difference is significant as compared to the cells grown in the CM on 24; ***b*** – on 48 h; ***c*** – on 72 h; ***d*** – on 72 of growth restoration; $P < 0.05$)

However, we observed that after the replacement of arginine-free medium with CM containing arginine, i.e., re-supplementing the amino acid, SH-SY5Y cells were able to resume growth, similarly to cells starved for lysine or leucine (**Fig. 2**). These data indicate that the cytostatic effects of arginine deprivation in human neuroblastoma SH-SY5Y cells are reversible. Essentially a similar effect was observed for human glioblastoma cells (Pavlyk *et al.*, 2015), suggesting that transformed cells of human neuronal origin are relatively resistant to a single amino acid, in particular arginine, deprivation.

The effect of spermidine on the viability of SH-SY5Y cells starved for arginine. Since arginine is a precursor in polyamine biosynthesis, deficiency of this amino acid can potentially cause polyamine deficiency at least in some cancer cell types. It is also known

that cells are capable of compensating the lack of certain nutrients, including amino acids, by inducing the mechanism of autophagy. We addressed the question whether polyamine spermidine, a known cytoprotective compound and autophagy modulator, would influence the viability and residual proliferation potential of neuroblastoma cells starved for arginine.

To analyze its effects, spermidine was introduced into the CM and AFM media at the final concentrations of 5, 10, 50 and 100 μM. It was observed that 5 and 10 μM spermidine did not significantly affect SH-SY5Y cells viability, whereas 50 and 100 μM concentrations of this polyamine were cytotoxic and caused apparent cell death in both media, i.e., irrespective of exogenous arginine presence (**Fig. 3**).

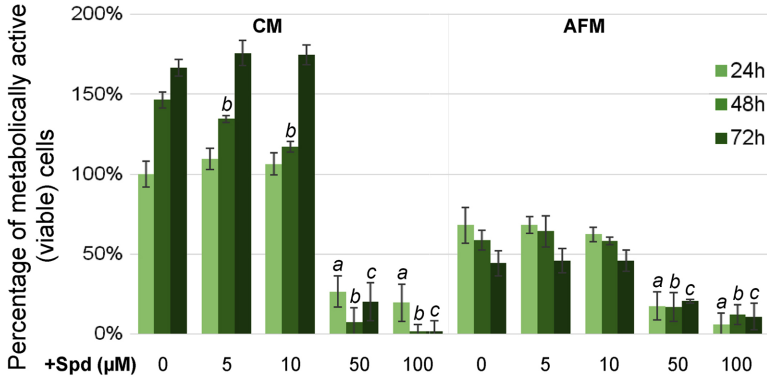

Fig. 3. The effect of spermidine on the viability of human neuroblastoma SH-SY5Y cells. The viability of cells cultivated in the complete medium (CM), arginine-free medium (AFM), and these media supplemented with spermidine (CM + Spd, AFM + Spd). Spermidine was added in concentrations of 5, 10, 50, and 100 μM and the cells were analyzed at 24, 48 and 72 h of cultivation (***a*** – the difference is significant as compared to the cells grown in the CM/AFM on 24; ***b*** – on 48 h; ***c*** – on 72 h; $P < 0.05$)

In particular, addition of spermidine to cultural media in higher concentrations of 50 and 100 μM rapidly decreased the percentage of metabolically active cells to approximately 20% already after 24 h of incubation. No significant changes in the percentage of living cells were observed during further extended cultivation for 48 or 72 h. Therefore, spermidine's IC_{50} values for SH-SY5Y cells cultivated in CM were similar for different incubation time points: 24.5 μM on 24 h, 18.1 μM on 48 h and 39,5 μM on 72 h (**Fig. 4*A***). The IC_{50} values for spermidine for SH-SY5Y cells cultivated in AFM were also between 20.1 and 47.4 μM (**Fig. 4*B***). Thus, for the tested neuroblastoma cells, the effects of spermidine are not dependent on exogenous arginine levels and cells' proliferative status.

Our data obtained with cultured SH-SY5Y neuroblastoma cells conform to experiments performed on human skin fibroblasts, a non-malignant cell model, for which spermidine showed a growth-promoting effect. There the exposure of fibroblasts to 10 and 100 nmol spermidine in CM for 24 or 48 h increased the cell number compared to untreated cells by approximately 30%. However, higher spermidine concentrations (1 μM – 10 mM) resulted in the progressive reduction of the stimulatory effect, with the higher doses evoking linear dose-dependent growth-inhibiting effect (Ghisalberti *et al.*, 2013). Thus it can be argued that the effects of spermidine on both normal human skin fibroblast and malignantly transformed neuroblastoma cells are essentially similar.

The effect of spermidine treatment and arginine starvation on autophagy activation in SH-SY5Y neuroblastoma cells. It was previously observed that 20 μM spermidine treatment of mouse embryonic fibroblasts (MEF) was very effective in activating autophagy (Chrisam *et al.*, 2015). In another study, it was found that in MEF cells treated with low 20 nM spermidine exhibitted an increased autolysosomal pool, a morphological indicator of activated autophagic flux (du Toit *et al.*, 2018).

Thereby, our next task was to analyze the effect of spermidine on the autophagic lysosome pool in SH-SY5Y cells cultivated in the CM versus those cultivated in AFM. The process of autophagy was monitored by fluorescence microscopy using monodansylcadaverine (MDC) as a fluorescent dye due to its ability to selectively label lumen of autophagic acidified lysosomes, contrary early or late endosomes (Munafó & Colombo, 2001; Vázquez & Colombo, 2009).

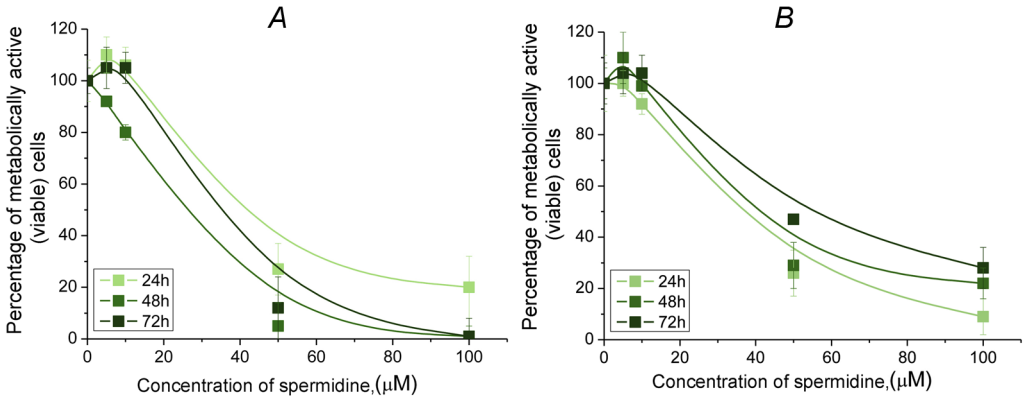

Fig. 4. Viability of human neuroblastoma SH-SY5Y cells at the spermidine treatment. Percentage of metabolically active cells cultivated in CM supplemented with different concentrations of spermidine (***A***), percentage of metabolically active of cells cultivated in AFM supplemented with different concentrations of spermidine (***B***). Results of MTT assay are presented as M±m

Based on our cell viability test (see **Fig. 3**) and literature data, we have chosen 10 μM spermidine as a working experimental concentration, which is not cytotoxic toward SH-SY5Y cells and below IC_{50} values under our experimental conditions. It was found that spermidine evidently increased the number of MDC-labeled fluorescent (autophagic) vacuoles in SH-SY5Y cells cultivated in CM for 6 h (**Fig. 5**). For comparison, autophagolysosomes were detected only at the basal level in the cells cultivated in the control complete medium. At the same time, the formation of autophagolysosomes in the cells cultivated in AFM was observed already after 2 h of incubation, suggesting that arginine starvation alone activates autophagy in neuroblastoma cells. 10 μM spermidine augmented this effect for AFM-treated cells (**Fig. 5**). However, such an inducing effect did not alter cell viability (see **Fig. 3**).

Taken together, the results of fluorescence microscopy suggest that at low micromolar concentrations (e.g., 10 μM) spermidine is able to co-activate autophagy producing an apparent additive effect under arginine starvation. It remains to be elucidated whether higher spermidine concentrations (e.g., in the range of 50–100 μM) may lead to hyperactivation of autophagy.

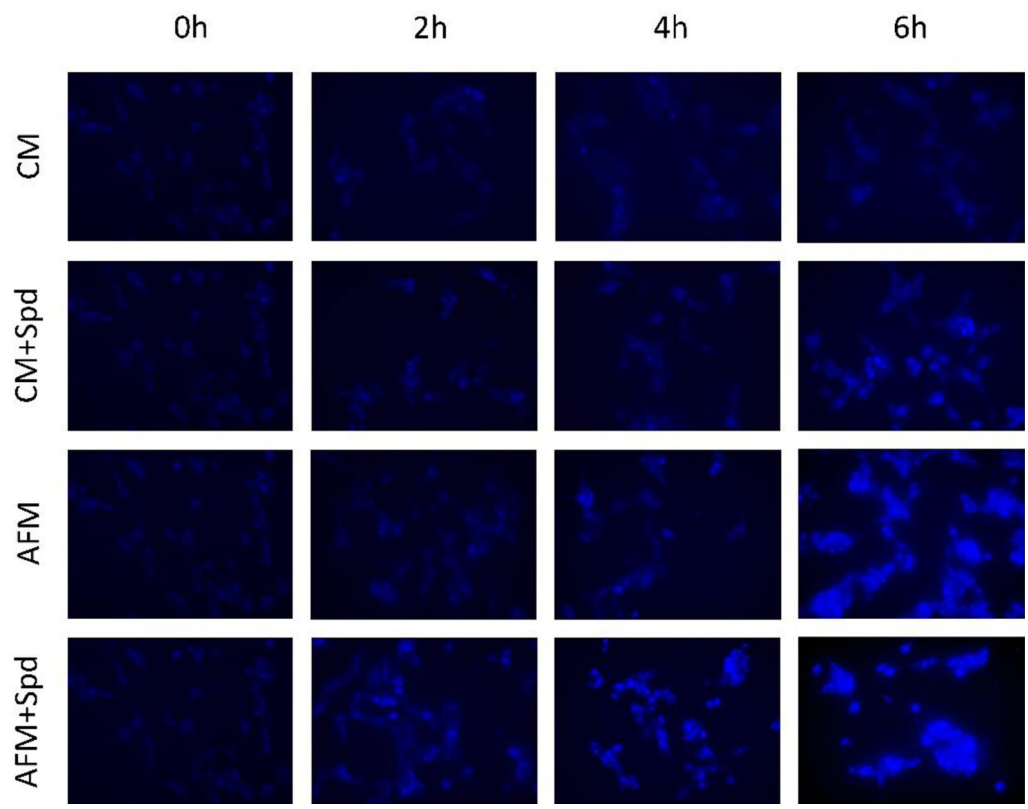

Fig. 5. Microscopic visualization of MDC-labelled autophagic vacuoles in human neuroblastoma SH-SY5Y cells. Spermidine was suplemented into CM and AFM at the final concentration of 10 μM. Fluorescence filter for DAPI and duration of exposure for 50 ms were applied. Magnification 400×

CONCLUSION

The described experiments revealed that i) human neuroblastoma SH-SY5Y cells are arginine auxotrophs and arginine deprivation abrogates their proliferation; ii) sensitivity of SH-SY5Y cells to arginine deprivation is higher, i.e. they exhibit faster drop in viability, relative to starvation for other essential amino acids, lysine and leucine; iii) elevated concentrations of polyamine spermidine (50–100 μM) were cytotoxic for SH-SY5Y cells irrespective of the presence of exogenous arginine; iv) spermidine at low micromolar concentrations further up-induced autophagic flux in arginine-starved SH-SY5Y cells, but was neither cytoprotective nor cytotoxic; v) arginine deprivation bears potential as an approach of metabolic anticancer therapy against human neuroblastoma.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by the any of the authors.

Animal studies: All international, national and institutional guidelines for the care and use of laboratory animals were followed.

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AUTHOR CONTRIBUTIONS

Conceptualization, [O.G.S., O.V.S.]; methodology, [O.G.S., Y.V.N.]; validation, [O.G.S., O.V.S.]; formal analysis, [O.G.S., O.V.S., Y.V.N.].; investigation, [O.G.S., O.V.S., Y.V.N.]; resources, [O.G.S., O.V.S., Y.V.N.]; data curation, [O.G.S., O.V.S.]; writing – original draft preparation, [Y.V.N., O.G.S.]; writing – review and editing, [Y.V.N., O.G.S., O.V.S.]; visualization, [Y.V.N., O.G.S.] supervision, [O.G.S., O.V.S.].

All authors have read and agreed to the published version of the manuscript.

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СПЕРМІДИН АКТИВУЄ МЕХАНІЗМ АВТОФАГІЇ, ПРОТЕ НЕ ПІДВИЩУЄ ЖИТТЄЗДАТНІСТЬ КЛІТИН НЕЙРОБЛАСТОМИ ЛЮДИНИ SH-SY5Y ЗА ДЕФІЦИТУ АРГІНІНУ

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Вступ. Нейробластома є злоякісною пухлиною симпатичної нервової системи, поширеною у ранньому дитячому віці. Автофагія у клітинах нейробластоми відбувається на базальному рівні, проте для агресивних форм нейробластоми людини характерною є її наіндукція. Метою дослідження було встановити ефекти поліаміну спермідину та дефіциту аргініну на життєздатність і регуляцію автофагії в клітинах нейробластоми людини.

Матеріали та методи. Клітинна лінія нейробластоми людини SH-SY5Y була експериментальною моделлю для МТТ аналізу метаболічної активності клітин під впливом різних концентрацій спермідину під час культивування у повному та безаргініновому середовищах. Моніторинг автофагії за дефіциту аргініну та за дії спермідину проводили за допомогою флуоресцентної мікроскопії клітин нейробластоми зі специфічно міченими монодансилкадаверином автофагійними лізосомами.

Результати. Було виявлено, що дефіцит аргініну блокує проліферацію клітин SH-SY5Y. За наявності попередника аргініну цитруліну в безаргініновому середовищі клітини SH-SY5Y були нездатні до проліферації, а отже, є ауксотрофами за аргініном. Клітини нейробластоми були також більш чутливими до голодування за аргініном, порівняно з голодуванням за іншими незамінними амінокислотами, лізином і лейцином. Також виявлено, що спермідин у низьких концентраціях 5–10 мкМ не впливав на життєздатність клітин SH-SY5Y під час культивування у повноцінному чи безаргініновому середовищі, але 50–100 мкМ концентрації спермідину в обох середовищах були високоцитотоксичними. За дії спермідину та за дефіциту аргініну виявлено значне збільшення кількості автофагійних лізосом у клітинах SH-SY5Y, що вказує на активуючий вплив обох чинників на процес автофагії.

Висновки. Встановлено, що голодування за аргініном призводило до зупинки проліферації та зниження життєздатності клітин, а також індукції автофагії у клітинах нейробластоми людини SH-SY5Y. Спермідин у концентраціях 5–10 мкМ хоч індукував автофагію у клітинах SH-SY5Y, проте не покращував їхнє виживання за дефіциту аргініну; водночас у концентраціях понад 50 мкМ спермідин виявляв потужну цитотоксичну дію. Отже, встановлено можливість ефективної маніпуляції механізмом автофагії у клітинах нейробластоми за одночасної дії дефіциту аргініну та поліаміну спермідину.

Ключові слова: злоякісні клітини, нейробластома, SH-SY5Y, автофагія, спермідин

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