Spectroscopy 18 (2004) 123–132
IOS Press

Monitoring intracellular metabolites in neuroblastoma with ^{1}H NMR spectroscopy: effects of growth factor withdrawal and modulation of lipid metabolism

Magnus Lindskog [a,*], Jüri Jarvet [b], Astrid Graslund [b] and Per Kogner [a]

[a] *Childhood Cancer Research Unit, Department of Woman and Child Health, Karolinska Institutet, Karolinska Hospital, Stockholm, Sweden*

[b] *Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

Abstract. ^{1}H NMR spectroscopy has previously been employed to detect and monitor changes in the lipid metabolism of neuroblastoma cells upon cytotoxic treatment. Here, we addressed the question whether altered growth conditions, by presence or absence of serum, would impact on the metabolites detectable with ^{1}H NMR spectroscopy. Chronic serum deprivation of SH-SY5Y human neuroblastoma cells resulted in a decrease in the intracellular content of several metabolites, in particular total choline. This metabolic effect was paralleled by significant growth inhibition. In addition, we investigated the potential functional origin of intracellular ^{1}H NMR visible lipids in SH-SY5Y cells. A drop in lipid methylene protons could be observed shortly after serum-withdrawal. Contrary, removal of lipoproteins from the serum led to a pronounced increase in intracellular lipids, as did inhibition of *de novo* sterol synthesis by lovastatin. In conclusion, we demonstrate that intracellular total choline in neuroblastoma cells *in vitro* is highly dependent on the availability of growth factors. Furthermore, we show that ^{1}H NMR visible lipids decrease upon serum-withdrawal but are accumulated when cholesterol supply is abrogated. The biological and potential clinical implications of these findings are discussed.

1. Introduction

Continuous supply of biomolecules is required to enable rapid cell division, one of the hallmarks of cancer. Metabolic processes could therefore be suitable targets for molecular imaging of cancer biology as well as for innovative therapeutic strategies. In this respect lipids and phospholipids are of particular interest due to their abundance in membrane structures as well as their key roles in intracellular signal transduction [1,2]. ^{1}H nuclear magnetic resonance (NMR) spectroscopy enables non-invasive monitoring of the lipid and phospholipid metabolism of living cells. From an oncological perspective, the strength of this technique lies in its ability to non-invasively assess biological activity of cancer tumours, by means of conventional magnetic resonance imaging equipment [3].

*Corresponding author: Magnus Lindskog, Childhood Cancer Research Unit, Q6:05, Astrid Lindgren Children's Hospital, Karolinska Hospital, S-171 76 Stockholm, Sweden. Tel.: +46 70 539 00 69; Fax: +46 8 5177 3475; E-mail: Magnus.Lindskog @kbh.ki.se.

Neuroblastoma, which is the most common extracranial solid tumour in children, often has a poor prognosis despite highly intensive treatment [4]. Methods to more accurately monitor effects of novel therapies are therefore of great interest for this group of patients.

Choline-containing compounds and mobile lipids are frequently detected in ^{1}H NMR spectra of human tumour cells [5,6]. High choline content in tumour cells have been correlated to rapid cell proliferation [7,8]. Upon challenge with chemotherapeutic agents, ^{1}H NMR spectroscopy commonly shows a shift from choline dominance (exponential tumour growth) to extensive accumulation of highly mobile lipids [9–11]. Polyunsaturated fatty acids (PUFA), in particular, seem to indicate cytotoxicity [12]. We have previously demonstrated that SH-SY5Y neuroblastoma cells undergo similar metabolic changes as described for other tumour types, with increasing lipid/choline ratios in drug sensitive but not in resistant cells, when treated with cytotoxic agents [13]. Using a rat model of neuroblastoma we could also confirm these findings *in vivo* [14]. The biological significance of ^{1}H NMR detectable lipids and phospholipids in living cells is, however, complex and not sufficiently understood [15]. For example, it was shown that activation of certain cell types is associated with intracellular mobile lipid accumulation [16,17]. In some cell types phosphocholine and glycerophosphocholine may accumulate during the early stages of growth arrest [18].

Moreover, a functional mechanistic understanding of these lipid signals in cancer cells is lacking. The respective roles played by lipases, *de novo* lipid synthesis or, uptake of either albumin-bound free fatty acids and/or lipoproteins, remain to be explored.

The aim of the present study was to investigate the relation between growth rate of neuroblastoma cells and intracellular metabolites detectable with ^{1}H NMR spectroscopy. This was achieved by experimental manipulation of signalling from paracrine growth factors (serum). In addition, experiments to gain insight in the functional origin of ^{1}H NMR detectable lipid signals in neuroblastoma were conducted.

2. Methods

2.1. Chemicals

Lovastatin (Mevinolin from *Aspergillus sp*) (a kind gift from Dr Jerker Olsson, Huddinge Hospital, Karolinska Institutet) was dissolved in ethanol at a concentration of 10 mg/ml and stored in light-protected vials at -20°C. Human lipoprotein deficient serum (LPDS; density $>$ 1.215 g/ml) (kindly provided by Dr Michelle Masquelier, Clinical Pharmacology, Karolinska Hospital) was isolated from serum of healthy blood donors by sequential ultracentrifugation. The purity of LDL and LPDS preparations was examined by agarose gel electrophoresis, and the absence of cholesterol in LPDS was confirmed by enzymatic cholesterol analysis (Merck) [19].

2.2. Cell culture

The human neuroblastoma cell line SH-SY5Y (kindly provided by Dr. June Biedler, New York) [20] was used for all experiments described. Cells were cultured at 37°C in a humidified 95% air/5% CO_2 atmosphere. Dulbecco's modified Eagle's minimal essential medium (DMEM) was supplemented with 10% fetal bovine serum (FBS), L-glutamine 2 mM, penicillin G 100 IU/ml and streptomycin 100 μg/ml (Gibco BRL, Paisley, Scotland, UK). The medium was changed twice weekly and confluent cultures were subcultivated after treatment with 0.5 g/l trypsin and 0.2 g/l EDTA (Gibco BRL). Cultures were free from mycoplasma as verified by DNA staining. The viability and exact cell concentration were assessed by trypan blue dye exclusion using a hematocytometer.

2.3. Growth inhibition and cell death assays

Proliferation of neuroblastoma cells was measured using the tetrazolium salt-based colorimetric assay (MTT) [21]. In short, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) is cleaved by mitochondrial dehyrogenases in living cells and is converted to formazan. Cells were plated in 96-well plates (10–40 $\times$ 10^3 cells/well, depending on experimental setup) and allowed to attach overnight. Medium was changed and fresh medium, when indicated with the addition of chemicals, was added. After the desired incubation period (48 hours or 96 hours), the medium was removed from the wells and MTT (5 mg/ml) was added for further incubation for 3 hours in dark before formazan-crystals were dissolved by adding 150 μl isopropanol with HCl (3.3 ml 37% HCl per litre isopropanol). The absorbance was measured spectrophotometrically at 595 nm. The absorbance from empty wells (without cells) was used to calculate the background.

2.4. ^{1}H NMR spectroscopy

Prior to preparation of samples for ^{1}H NMR spectroscopy, cells were counted under a microscope and the number of viable cells was determined by their ability to exclude trypan blue. 5 mm Shigemi tubes were used and typically 5 $\times$ 10^7 cells were enough to fill the active volume. Cells were washed twice with PBS, suspended in 600 μl PBS with 10% D_2O, and placed on ice until data acquisition. Samples were analyzed on a 500 MHz Bruker spectrometer at 25°C. The residual H_2O signal at $\approx$4.75 ppm was suppressed by low-power presaturation. The acquisition parameters included: 90° pulse, repetition time 1.5 s, 256 or 512 scans (depending on desired signal to noise), 8k points, spectral width of 5 kHz. Following acquisition, spectra were Fourier transformed, phased and baseline corrected. Signal intensities were then integrated using XWINNMR software (version 3.1; Bruker). Changes in cell viability during spectroscopic analysis were negligible, as confirmed by rescanning cell suspensions after 4 hours of storage on ice (data not shown).

2.5. Statistical analysis

Student's unpaired two-sided t-test was used for comparison between experimental groups. $P < 0.05$ was considered to be significant.

3. Results

3.1. Effects of serum-withdrawal on ^{1}H NMR detectable metabolites in neuroblastoma cells

Serum contains a wide variety of growth factors and cytokines that enhance the growth of cells in culture. SH-SY5Y cells are able to survive under serum-free conditions although their proliferation is impaired [22,23]. In order to investigate whether the availability of paracrine growth factors would impact on the (phospho)lipid metabolism of SH-SY5Y cells, ^{1}H NMR spectroscopic analysis of chronically serum-starved and control cells, respectively, was performed (Fig. 1A). Chronic (96 hours) serum deprivation was associated with a drop in total choline (Cho) compared to the other metabolites (Fig. 1A,B). In addition, the lipid (–CH_2–) signals resonating at 1.2–1.5 p.p.m. and, the resonances from multiple molecules at 2–2.5 p.p.m. appeared to decrease upon serum-starvation (Fig. 1A). A sharp signal resonating at 1.9 p.p.m. was detectable in serum-fed cells, but became more dominant under serum-free conditions (Fig. 1A). Chronic serum starvation was associated with 40% inhibition of proliferation (Fig. 1C), but did not lead to cellular detachment or membrane collapse (data not shown).

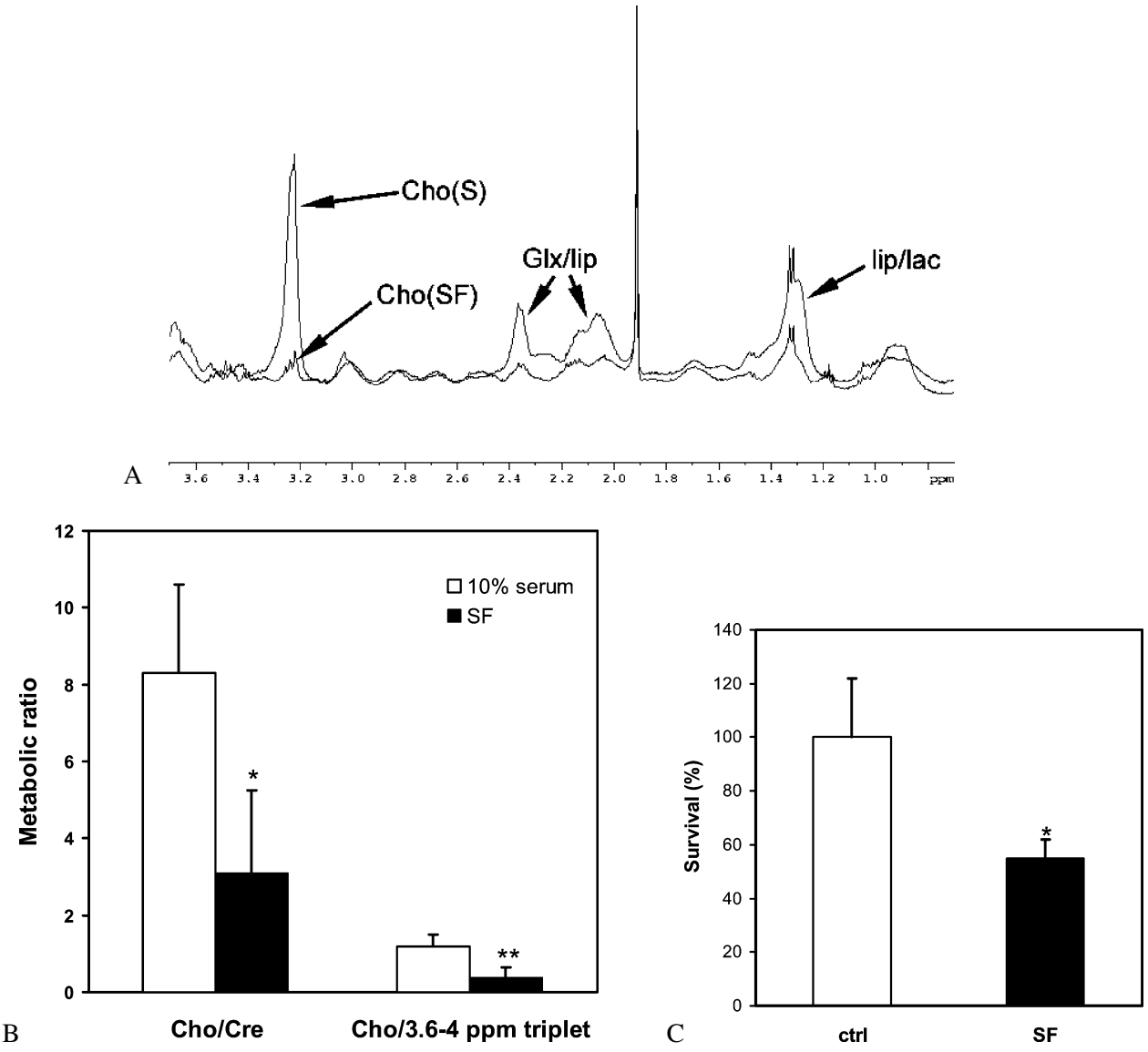

Fig. 1. Metabolic and cytostatic effects of serum deprivation. A, Overlay of two ^{1}H NMR spectra (500 MHz, 25°C) from control (10% FBS, *S*) and serum-deprived (*SF*) SH-SY5Y neuroblastoma cells. Spectroscopy was performed after 96 hours incubation. NMR parameters included a presaturation pulse for water suppression, a 90° pulse, 256 scans. Serum-deprived cells were characterized by a relative decreased in: total choline (Cho, 3.22 p.p.m.), lipids/lactate (lip/lac, 1.2–1.4 p.p.m.) and glutamine/glutamate/lipids (Glx/lip, 2–2.4 p.p.m.). Arrows, indicating signals from total choline (Cho) in the control (S) and serum-deprived (SF) spectra, highlight the pronounced differences in Cho content depending on serum availability. The experiment was performed in duplicate and was repeated on three separate occasions with consistent results. B, Metabolic ratios obtained from ^{1}H NMR spectra of control and serum-deprived SH-SY5Y cells. *Cho* = total choline, *Cre* = total creatine. Compared to other metabolites Cho was significantly lower in serum-deprived cells. $^*p = 0.03$, $^{**}p = 0.01$. Bars indicate standard deviations. C, Effects of serum availability on neuroblastoma proliferation. SH-SY5Y cells were seeded in a microtiter plate (2×10^7 cells per well, 8 replicates) and allowed to attach overnight. The cells were washed with PBS and the medium was changed to medium containing 10% FBS (*ctrl*) or, serum-free medium (*SF*), respectively. The proliferation was assessed with MTT after 96 hours of incubation. The experiment was repeated on three separate occasions. The data are expressed as percent compared to control. Bars indicate standard deviations. $^*p < 0.001$.

3.2. ^{1}H NMR effects in response to manipulation of lipid availability

Together with total choline mobile lipids dominate in ^{1}H NMR spectra of neuroblastoma cells [13]. This phenomenon, commonly observed in malignant cells, could be caused by perturbations in lipid trafficking, such as excess liberation of fatty acids from intracellular stores, *de novo* synthesis, uptake from outside the cell or hindered secretion of cellular triglycerides [15]. To gain insight in the mechanisms contributing to ^{1}H NMR visible lipids in SH-SY5Y cells, we performed spectroscopic analyses of cells after manipulating various aspects of lipid trafficking.

In the first set of experiments the metabolic effects of acute serum withdrawal was assessed (Fig. 2A,B). Removal of serum was associated with an acute drop in methylene lipid signals (1.2–1.5 p.p.m.), exposing the lactate doublet at ≈1.31 p.p.m., and, with increased intensity of the signal at 1.9 p.p.m (Fig. 2A,B). No significant inhibition of cell proliferation was observed at these time points (data not shown).

To investigate the potential contribution to ^{1}H NMR signals of cellular lipoprotein import, we cultured SH-SY5Y cells in medium prepared with lipoprotein deficient serum (LPDS) [19]. Intriguingly, spectroscopy detected a significant increase in lipid resonances at 1.2–1.5 p.p.m., 2.8 p.p.m. and 5.35 p.p.m. in cells grown with LPDS (Fig. 2C). To test whether abrogating *de novo* synthesis of sterols would, simi-

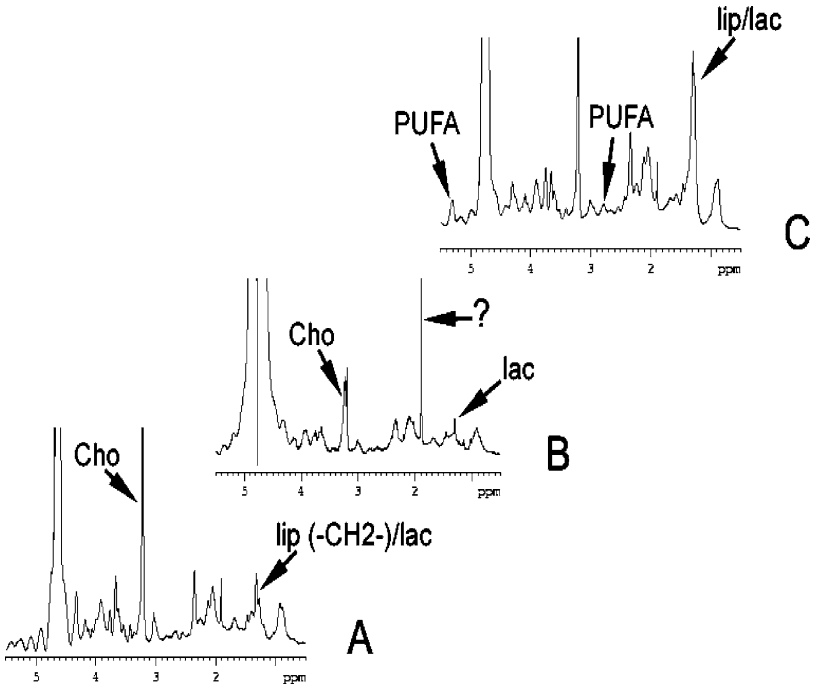

Fig. 2. Analyzing neuroblastoma lipid uptake with ^{1}H NMR spectroscopy. SH-SY5Y cells were seeded in culture flasks and allowed to attach overnight. The serum-rich (10% FBS) medium was removed, the cells were washed with PBS, followed by the addition of fresh, serum-free medium. Spectroscopy was performed after 24 and 48 hours. A, Representative spectrum of exponentially growing SH-SY5Y cells cultured in the presence of 10% FBS after 48 hours. B, Spectrum of SH-SY5Y cells deprived of serum for 48 hours. The lipid (methylene, $-CH_2-$) resonance at 1.2–1.4 p.p.m. has decreased, exposing lactate (lac, 1.31 p.p.m.) (*lip/lac*). An unassigned signal at approximately 1.9 p.p.m. has increased in intensity compared to control cells. C, SH-SY5Y cells incubated for 48 hours in the presence of lipoprotein-deficient serum (LPDS). Relative to other resonances the lipid signals at 1.2–1.4 p.p.m. (*lip/lac*) and, in particular, signals from polyunsaturated fatty acids (*PUFA*, 2.8 and 5.35 p.p.m.) are increased.

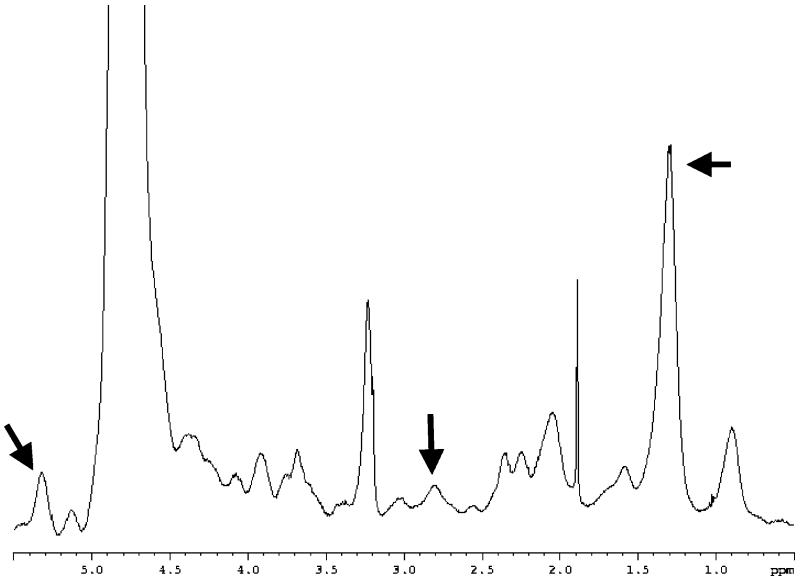

Fig. 3. Abrogated cholesterol supply results in intracellular fatty acid accumulation. Spectrum of SH-SY5Y cells incubated for 48 hours in lipoprotein-deficient serum (LPDS) with the addition of lovastatin (10 μM). Lipid signals (arrows), in particular PUFA resonances (2.8 p.p.m.; 5.35 p.p.m.) are markedly increased compared to controls.

larly, affect the levels ^{1}H NMR visible lipids SH-SY5Y cells were treated with lovastatin, an inhibitor of HMG-CoA-reductase, the rate limiting enzyme in sterol *de novo* synthesis [24]. Spectra from lovastatin-treated cells demonstrated increased levels of ^{1}H NMR visible lipids, similar to the findings with LPDS. The lipid increase upon treatment with lovastatin was not due to increased uptake of lipids from outside the cells, since it also occurred under serum-free conditions (data not shown). As previously reported for neuroblastoma cell lines, lovastatin potently inhibited the proliferation of SH-SY5Y cells (data not shown). Notably, addition of lovastatin to medium with LPDS was associated with further increase in the levels of ^{1}H NMR visible lipids (Fig. 3).

4. Discussion

^{1}H NMR spectroscopy is widely recognized as an excellent method to study biophysical properties of peptides and membrane preparations *in vitro*. In addition, this technique is now rapidly emerging as a highly interesting tool in the field of biomedicine due to its ability to provide biochemical information from most tissues of the human body [25]. In this context, the method is commonly referred to as proton magnetic resonance spectroscopy (^{1}H MRS). From an oncological perspective, ^{1}H MRS is a particularly attractive method since it permits non-invasive monitoring of biological activity in cancer tumours. Currently available standard radiological techniques, including magnetic resonance imaging, lack the ability to accurately separate viable (biologically active) tumor tissue from necrotic or fibrotic remnants.

The often poor prognosis of the common childhood cancer neuroblastoma has not improved substantially over the last decades, despite innovative biological research and collaborative clinical trials [4]. For high-risk neuroblastoma patients experimental treatment within clinical trials is therefore always

considered. However, the lack of methods to monitor treatment effect in a sensitive and non-invasive manner so far hampers early assessment of therapeutic effect.

Employing ^{1}H NMR spectroscopic techniques, we have previously demonstrated that successful treatment of experimental neuroblastoma is associated with a shift in intra-tumoral metabolites with an increase in mobile lipids (ML) and decrease in total choline (Cho), both *in vitro* and *in vivo*. The ML/Cho-ratio was found to be inversely correlated to the overall viability of the tumor tissue [13,14].

In the present study, we investigated whether the availability of growth factors (serum) impacts on the intracellular metabolites that can be detected with ^{1}H NMR spectroscopy in neuroblastoma cells. This is of relevance since tumour growth is known to be dependent on growth factors and, since novel molecularly targeting anticancer therapies commonly interact with growth factor signalling [26]. Our findings demonstrate that the intracellular level of choline is in fact highly dependent on the availability of growth factors in the microenvironment of SH-SY5Y neuroblastoma cells. The drop in choline upon chronic serum starvation was paralleled by a significant inhibition of cell proliferation (Fig. 1A,B), but not with loss of cell viability (not shown). This apparently cytostatic, but not cytotoxic, effect could possibly explain the finding that signals (mostly lipids) resonating at approximately 2 p.p.m. and 1.3 p.p.m., previously shown to increase during early phases of cell death [10–12], here seemed to decrease in response to serum-deprivation (Figs 1 and 2). Choline, which is used by all mammalian cells for synthesis of phosphatidylcholine acts as an intermediate in cellular metabolism [27]. Malignant cells typically have an increased content of total choline and high tumour content of total choline has been associated with rapid cell proliferation in several types of cancer [28–30]. The findings of the present study suggest that such an association exists also for neuroblastoma cells, choline levels declining upon growth inhibition caused by serum-withdrawal. Insulin and insulin-like growth factors are known to be important serum components that enhance the proliferation of neuroblastoma cells [22]. Intracellular phosphocholine can promote the mitogenic effects of insulin and IGF-IIn breast cancer cells [31]. In experimental breast tumours phosphocholine (PCho) was found to be particularly high during DNA synthesis (S-phase) while it dropped with cell cycle arrest [18]. Activation of the oncogenic protein Ras was associated with increased PCho and stimulation of tumour cell proliferation, which could be prevented with Ras inhibitors [32].

Uptake of choline from outside the cell rather than *de novo* synthesis is considered to be the main source of intracellular choline [33]. Inside the cell choline can be phosphorylated (phosphocholine) and linked to phospholipid backbones [31]. Since the culture medium employed in this study contains choline chloride the decrease in total choline that we observed in chronically serum-starved cells can not be attributed to a lack of choline supply.

The mechanistic reasons underlying intracellular accumulation of mobile lipids in cancer cells remain poorly understood. Several possible explanations, including lipid uptake from outside the cell, *de novo* lipid synthesis, or membrane degradation, have been suggested [15]. In the present study, we demonstrate that in exponentially growing SH-SY5Y neuroblastoma cells the ^{1}H NMR detectable lipid resonances depend, at least in part, on uptake of lipids from the microenvironment. This conclusion is based on the observation that serum-removal was associated with an early drop in, particularly, lipid methylene protons (Figs 1A, 2B). We can not completely exclude that this is a result of growth factor deficiency (serum is rich in various growth factors), although the observation of decreased lipid resonances at time points when cell proliferation was not yet affected by the serum-removal (24 and 48 hours) does not support such a hypothesis. Nevertheless, the low lipid content observed in chronically serum-deprived SH-SY5Y cells (Fig. 1B) suggests that a combination of these two reasons could underlie the drop in intracellular lipid methylene protons observed.

In order to further understand the role of lipid import, we cultured SH-SY5Y cells in medium prepared with lipoprotein-deficient serum (LPDS), thus removing cholesterol, cholesteryl esters and triglycerides from the tumour microenvironment. Counter intuitively, this approach led to a prominent increase in the lipid methylene signal (1.2–1.5 p.p.m.) as well as of PUFA resonances at 2.8 and 5.35 p.p.m. in SH-SY5Y cells (Fig. 2C). Thus, although import of lipoproteins may possibly contribute to ^{1}H NMR visible lipids in SH-SY5Y cells, lipoproteins are not required for intracellular accumulation of these biomolecules. Consistent with this finding, treatment of SH-SY5Y cells growing in lipoprotein-deficient medium with lovastatin, which prevents sterol biosynthesis by inhibiting the rate-limiting enzyme HMG-CoA-reductase [24], led to an even further increase in intracellular lipids as observed with ^{1}H NMR spectroscopy (Fig. 3). The addition of lovastatin was associated with a pronounced cytotoxicity (data not shown), as previously reported [34]. These observations are in agreement with a previous report by Galli and colleagues who demonstrated that the biosynthesis of long chain-polyunsaturated fatty acids (LC-PUFA) in SK-N-BE neuroblastoma cells could be enhanced by treatment with statins [35]. Thus, several lines of evidence suggest the existence of an inverse relationship between intracellular cholesterol and LC-PUFA in neuroblastoma cell lines. This is of interest in view of the recently reported finding that PUFA may prevent growth factor signalling and induce apoptosis of tumour cells, while cholesterol-rich microdomains near the plasma membrane appear crucial in enabling a cellular response to growth factors, as well as for protecting tumour cells from apoptosis [15,19,36]. In agreement with this, we have observed that cholesterol depletion sensitizes neuroblastoma cells to cytotoxic agents (unpublished observation).

5. Conclusion

Three main findings emerged from this study:

(1) A marked decrease in intracellular levels of total choline, as detected with ^{1}H NMR spectroscopy, is characteristic of chronically serum starved, growth-inhibited neuroblastoma cells. This suggests that monitoring of intratumoural choline with ^{1}H MRS *in vivo* might be of value for non-invasive assessment of tumour response to novel cytostatic compounds in neuroblastoma patients.
(2) Baseline levels of intracellular ^{1}H NMR visible lipids in neuroblastoma cells are, at least in part, dependent on lipid uptake from serum, since acute serum-withdrawal results in decreased levels of intracellular mobile lipids.
(3) Abrogation of cholesterol supply to neuroblastoma cells, either by removing lipoproteins from the culture medium or by inhibition of sterol *de novo* synthesis, results in intracellular accumulation of fatty acids and/or triglycerides.

Acknowledgements

This work was supported in part by grants from The Swedish Children's Cancer Foundation, and Mary Béve Foundation.

The authors wish to express their gratitude to Mrs Michelle Masquelier and Dr Jerker Olsson for kindly supplying lipoprotein-deficient serum and lovastatin, respectively.

References

[1] F. Galbiati, B. Razani and M.P. Lisanti, Emerging themes in lipid rafts and caveolae, *Cell* **106** (2001), 403–411.
[2] F. Podo, Tumour phospholipid metabolism, *NMR Biomed.* **12** (1999), 413–439.
[3] M.O. Leach, Magnetic resonance spectroscopy applied to clinical oncology, *Technol. Health Care* **2** (1994), 235–246.
[4] G.M. Brodeur, Neuroblastoma: biological insights into a clinical enigma, *Nat. Rev. Cancer* **3** (2003), 203–216.
[5] E. Ackerstaff, B.R. Pflug, J.B. Nelson and Z.M. Bhujwalla, Detection of increased choline compounds with proton nuclear magnetic resonance spectroscopy subsequent to malignant transformation of human prostatic epithelial cells, *Cancer Res.* **61** (2001), 3599–3603.
[6] R. Katz-Brull, D. Seger, D. Rivenson-Segal, E. Rushkin and H. Degani, Metabolic markers of breast cancer: enhanced choline metabolism and reduced choline-ether-phospholipid synthesis, *Cancer Res.* **62** (2002), 1966–1970.
[7] H. Shimizu, T. Kumabe, R. Shirane and T. Yoshimoto, Correlation between choline level measured by proton MR spectroscopy and Ki-67 labeling index in gliomas, *AJNR Am. J. Neuroradiol.* **21** (2000), 659–665.
[8] G. Tedeschi, N. Lundbom, R. Raman, S. Bonavita, J.H. Duyn, J.R. Alger and G. Di Chiro, Increased choline signal coinciding with malignant degeneration of cerebral gliomas: a serial proton magnetic resonance spectroscopy imaging study, *Neurosurgery* **87** (1997), 516–524.
[9] J.A. Lazareff, R.K. Gupta and J. Alger, Variation of post-treatment H-MRSI choline intensity in pediatric gliomas, *J. Neurooncol.* **41** (1999), 291–298.
[10] F.G. Blankenberg, R.W. Storrs, L. Naumovski, T. Goralski and D. Spielman, Detection of apoptotic cell death by proton nuclear magnetic resonance spectroscopy, *Blood* **87** (1996), 1951–1956.
[11] T. Bezabeh, M.R. Mowat, L. Jarolim, A.H. Greenberg and I.C. Smith, Detection of drug-induced apoptosis and necrosis in human cervical carcinoma cells using (1)H NMR spectroscopy, *Cell Death Differ.* **8** (2001), 219–224.
[12] J.M. Hakumaki, H. Poptani, A.M. Sandmair, S. Yla-Herttuala and R.A. Kauppinen, 1H MRS detects polyunsaturated fatty acid accumulation during gene therapy of glioma: implications for the in vivo detection of apoptosis, *Nat. Med.* **5** (1999), 1323–1327.
[13] M. Lindskog, C. Spenger, J. Jarvet, A. Graslund and P. Kogner, Functional imaging using magnetic resonance spectroscopy (^{1}H MRS) provides significant information on treatment response in neuroblastoma in vitro and in vivo, *Med. Ped. Oncol.* **41** (2003), 293.
[14] M. Lindskog, P. Kogner, F. Ponthan, P. Schweinhardt, B. Sandstedt, T. Heiden, G. Helms and C. Spenger, Noninvasive estimation of tumour viability in a xenograft model of human neuroblastoma with proton magnetic resonance spectroscopy (^{1}H MRS), *Br. J. Cancer* **88** (2003), 478–485.
[15] J.M. Hakumaki and R.A. Kauppinen, 1H NMR visible lipids in the life and death of cells, *Trends Biochem. Sci.* **25** (2000), 357–362.
[16] R.E. Whatley, K. Satoh, G.A. Zimmerman, T.M. McIntyre and S.M. Prescott, Proliferation-dependent changes in release of arachidonic acid from endothelial cells, *J. Clin. Invest.* **94** (1994), 1889–1900.
[17] P.F. Weller, S.W. Ryeom, S.T. Picard, S.J. Ackerman and A.M. Dvorak, Cytoplasmic lipid bodies of neutrophils: formation induced by cis-unsaturated fatty acids and mediated by protein kinase C, *J. Cell Biol.* **113** (1991), 137–146.
[18] T.A. Smith, S. Eccles, M.G. Ormerod, A.J. Tombs, J.C. Titley and M.O. Leach, The phosphocholine and glycerophosphocholine content of an oestrogen-sensitive rat mammary tumour correlates strongly with growth rate, *Br. J. Cancer* **64** (1991), 821–826.
[19] L. Tatidis, M. Masquelier and S. Vitols, Elevated uptake of low density lipoprotein by drug resistant human leukemic cell lines, *Biochem. Pharmacol.* **63** (2002), 2169–2180.
[20] J.L. Biedler, L. Helson and B.A. Spengler, Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture, *Cancer Res.* **33** (1973), 2643–2652.
[21] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods* **65** (1983), 55–63.
[22] J.R. Singleton, A.E. Randolph and E.L. Feldman, Insulin-like growth factor I receptor prevents apoptosis and enhances neuroblastoma tumorigenesis, *Cancer Res.* **56** (1996), 522–529.
[23] M.A. Meghani, D.M. Martin, J.R. Singleton and E.L. Feldman, Effects of serum and insulin-like growth factors on human neuroblastoma cell growth, *Regul. Pept.* **48** (1993), 217–224.
[24] R. Hampton, D. Dimster-Denk and J. Rine, The biology of HMG-CoA reductase: the pros of contra-regulation, *Trends Biochem. Sci.* **21** (1996), 140–145.
[25] I.J. Cox, Development and applications of in vivo clinical magnetic resonance spectroscopy, *Prog. Biophys. Mol. Biol.* **65** (1996), 45–81.
[26] S.P. Ethier, Signal transduction pathways: the molecular basis for targeted therapies, *Semin. Radiat. Oncol.* **12** (2002), 3–10.
[27] A. Lykidis and S. Jackowski, Regulation of mammalian cell membrane biosynthesis, *Prog. Nucleic Acid Res. Mol. Biol.* **65** (2001), 361–393.

[28] E.O. Aboagye and Z.M. Bhujwalla, Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells, *Cancer Res.* **59** (1999), 80–84.
[29] S. Herminghaus, U. Pilatus, W. Moller-Hartmann, P. Raab, H. Lanfermann, W. Schlote and F.E. Zanella, Increased choline levels coincide with enhanced proliferative activity of human neuroepithelial brain tumors, *NMR Biomed.* **15** (2002), 385–392.
[30] T. Tamiya, K. Kinoshita, Y. Ono, K. Matsumoto, T. Furuta and T. Ohmoto, Proton magnetic resonance spectroscopy reflects cellular proliferative activity in astrocytomas, *Neuroradiology* **42** (2000), 333–338.
[31] Z. Kiss, Regulation of mitogenesis by water-soluble phospholipid intermediates, *Cell Signal.* **11** (1999), 149–157.
[32] S.M. Ronen, L.E. Jackson, M. Beloueche and M.O. Leach, Magnetic resonance detects changes in phosphocholine associated with Ras activation and inhibition in NIH 3T3 cells, *Br. J. Cancer* **84** (2001), 691–696.
[33] R. Massarelli, J. Ciesielski-Treska, A. Ebel and P. Mandel, Choline uptake in glial cell cultures, *Brain Res.* **81** (1974), 361–363.
[34] J. Dimitroulakos, L.Y. Ye, M. Benzaquen, M.J. Moore, S. Kamel-Reid, M.H. Freedman, H. Yeger and L.Z. Penn, Differential sensitivity of various pediatric cancers and squamous cell carcinomas to lovastatin-induced apoptosis: therapeutic implications, *Clin. Cancer Res.* **7** (2001), 158–167.
[35] C. Galli, P. Rise, F. Marangoni, A. Petroni and F. Visioli, Manipulation of the fate of long chain polyunsaturated fatty acids in cultured cells, *Prostaglandins Leukot. Essent. Fatty Acids* **57** (1997), 23–26.
[36] L. Zhuang, J. Lin, M.L. Lu, K.R. Solomon and M.R. Freeman, Cholesterol-rich lipid rafts mediate akt-regulated survival in prostate cancer cells, *Cancer Res.* **62** (2002), 2227–2231.