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Lipidomic analysis reveals a radiosensitizing role of gamma-linolenic acid in glioma cells

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ABSTRACT

Previous studies have demonstrated that gamma-linolenic acid (GLA) is effective against glioma cells under both in vitro and in vivo conditions. In the present study we determined how GLA alone or in combination with irradiation alters the fatty acid (FA) and lipid profiles, the lipid droplet (LD) content, the lipid biosynthetic gene expression and the apoptosis of glioma cells. In GLA-treated cells direct correlations were found between the levels of various FAs and the expression of the corresponding FA biosynthetic genes. The total levels of saturated and monosaturated FAs decreased in concert with the down-regulation of FASN and SCD1 gene expression. Similarly, decreased FADS1 gene expression was paralleled by lowered arachidonic acid (20:4 n−6) and eicosapentaenoic acid (20:5 n−3) contents, while the down-regulation of FADS2 expression was accompanied by a diminished docosahexaenoic acid (22:6 n−3) content. Detailed mass spectrometric analyses revealed that individual treatments gave rise to distinct lipidomic fingerprints. Following uptake, GLA was subjected to elongation, resulting in dihomo-gamma-linolenic acid (20:3 n−6, DGLA), which was used for the synthesis of the LD constituent triacylglycerols and cholesteryl esters. Accordingly, an increased number of LDs were observed in response to GLA administration after irradiation. GLA increased the radioresponsiveness of U87 MG cells, as demonstrated by an increase in the number of apoptotic cells determined by FACS analysis. In conclusion, treatment with GLA increased the apoptosis of irradiated glioma cells, and GLA might therefore increase the therapeutic efficacy of irradiation in the treatment of gliomas.

1. Introduction

Numerous studies have demonstrated that polyunsaturated fatty acids (PUFAs) have anti-cancer activities and can be used as cancer chemopreventive agents. The application of PUFAs has been confirmed to exert beneficial effects against glioma under both in vitro and in vivo conditions [1–9]. However, the exact mechanisms underlying the anti-tumor effects of PUFAs are complex and remain poorly understood. PUFAs induce oxidative stress through the generation of reactive oxygen species (ROS) which results in enhanced lipid peroxidation and decreased cell survival. This may be one of the main indirect causes of the cytotoxicity of PUFAs [10]. Additionally, ROS can induce a dysfunction of the mitochondria, which can lead to apoptosis [11]. Interestingly, PUFA treatment can enhance the radiosensitivity of cancerous cells, while it has proved to be radioprotective to normal cells [12,13]. This phenomenon has been attributed to the possible differences in sensitivity of cancer and normal cells in combating lipid peroxidation products [13].

It was shown earlier that cyclooxygenase inhibitors diminish the gamma-linolenic acid (GLA)-induced radiosensitivity of astrocytoma cells, and PUFAs may therefore induce radioresponsiveness through prostanoid synthesis, though their cytotoxicity may not be related to this effect [9]. There have been efforts to find radiosensitizers to intensify radiotherapy for glioma, but none have resulted in improved survival [14].

PUFA availability is known to alter the composition of the membranes, their fluidity and the way microdomains organize [15]. This causes the release of heat shock signals, which lead to heat shock protein (HSP) expression [15]. Membrane modulations, signal

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pathways from membranes to hsp genes and the HSPs themselves play essential roles in the etiology of several diseases, including cancer [16].

Of the various PUFAs, the most extensive studies have been performed with GLA in glioma treatment [1–3]. GLA enhances the effects of radiotherapy on cancerous cells, at the same time exerting a cytoprotective effect on normal cells [2,3]. In clinical experiments, the infusion of GLA increased the length of survival [3]. Our previous studies of the mechanisms by which GLA and other PUFAs in combination with irradiation exert their effects on glioma cells revealed the additive effect of GLA treatment and irradiation on cell proliferation inhibition, as confirmed by the lactate dehydrogenase activity and gene expression profiling [4].

The balance between fatty acid (FA) alimentation, synthesis, storage and apoptosis influences cancer development. In comparison with normal cells, de novo FA synthesis is activated during carcinogenesis [17,18]. In consequence of their continuous proliferation, cancer cells need increased FA biosynthesis to support lipoprotein synthesis and cell membrane biogenesis [18,19]. Chajès et al. [17] found that FA synthesis for cell proliferation is dispensable when FAs are available in the medium. Furthermore, in normal cells, FA synthesis is inhibited by exogenous FAs, while in cancerous cells the synthesis is intensely active [19]. Although the correlation between the lipid metabolism and cancer development has not yet been deciphered, alterations in the lipid metabolism and the accumulation of lipids in lipid droplets (LDs) have been implicated in carcinogenesis [13]. LD accumulation occurs in prenecrotic cancer tissues in vivo, and LDs can therefore, serve as in vivo markers of cancer [20]. Moreover, small-molecule drugs that target the LDs proved to induce endoplasmic reticulum and oxidative stress and to exert cytotoxic effects in diverse cancer cell lines [21], and to suppress hepatocellular carcinoma in vivo [22].

The possible application of GLA as an adjuvant in clinical experiments on the treatment of glioma necessitates a better understanding of the overall effects of co-exposure to irradiation and GLA. In this study we set out to investigate the dynamics of the FA and lipid metabolism and storage and the rate of apoptosis by performing experiments on the U87 MG glioblastoma cell line exposed to irradiation and/or GLA. FA and lipid profiles were recorded by means of mass spectrometric techniques and the expression of several genes present in the biosynthetic pathway of FAs was evaluated, including genes coding enzymes responsible for de novo synthesis of saturated and monounsaturated FAs (*FASN*, *SCD1* and *SCD5*) and desaturases and elongases that participate in the synthesis of PUFAs (*FADS1*, *FADS2*, *ELOVL1*, *ELOVL2* and *ELOVL5*). We additionally measured the expression of several heat shock genes, monitored the LD content by fluorescence microscopy, and determined the rate of apoptosis by FACS analysis.

2. Results

2.1. Effects of GLA treatment and irradiation on the rate of apoptosis

To confirm the apoptosis-inducing effects of GLA and irradiation, FACS analysis was performed. As shown in Fig. 1, dose-dependent increases in apoptotic cells occurred in response to 5 Gy or 10 Gy of irradiation. Similarly, GLA alone significantly increased the number of apoptotic cells and, in parallel, decreased the total number of living cells. When GLA was used in combination with irradiation, moderate increases in apoptotic cell number were registered. As expected, the most pronounced effects on cell survival and apoptosis were seen in samples treated with 10 Gy irradiation and GLA.

2.2. Total FA analysis of GLA-treated cells by gas chromatography–mass spectrometry

To determine how GLA treatment influences the total FA profile of U87 MG glioma cells, we used GC–MS (Fig. 2). GLA-treated cells exhibited decreases in the total saturated and monounsaturated FAs (SFAs and MUFAs, respectively; Fig. 2, inset). As expected, the level of GLA increased on GLA treatment. The analysis also revealed a very significant, over 7-fold increase in the amount of the elongated derivative dihomo-gamma-linolenic acid (20:3 n−6; DGLA). In parallel, the contents of arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decreased.

2.3. Effects of irradiation, GLA and their combination on the lipid molecular species profile

To reveal the changes elicited by irradiation and/or GLA treatment in the lipidomes of U87 MG cells, high-sensitivity, high-resolution shotgun profiling was carried out on an Orbitrap Elite hybrid tandem mass spectrometer. Altogether almost 250 lipid molecular species were identified and quantified, each of which accounted for more than 0.4% within its lipid class (Supplementary Table 1S). The data generated (i.e. 248 molecular species × 2 × 3 different treatments × 3 independent replicates) served as a basis for partial least squares discriminant analysis (PLS-DA) to test for possible lipid differences caused by the various treatment conditions and also to assess overall experimental variation. PLS-DA is a supervised multivariate classification method that is useful for reducing multidimensional data to lower dimensions, thereby simplifying the visualization of complex datasets for exploratory analysis. It was confirmed by cross-validation and permutation test that the achieved good separation (Fig. 3) is based on real signals. The PLS-DA analysis indicated that the two highest ranking components, components 1 and 2, accounted for 58% and 9%, of the total variance, respectively. It clearly emerged that component 1 captured most of the variations

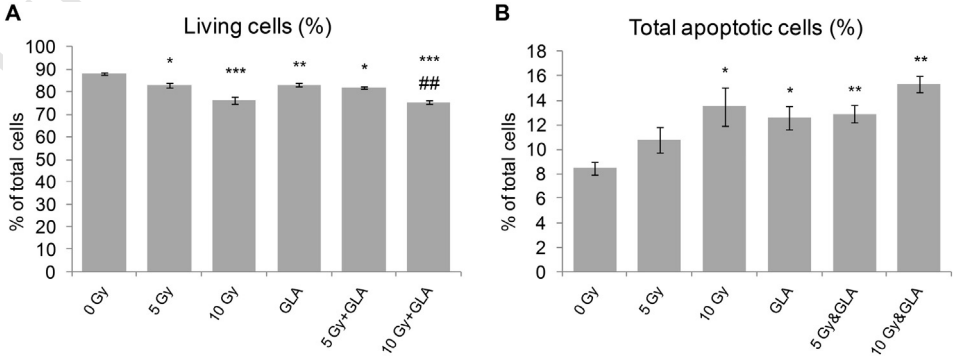

Fig. 1. Rate of apoptosis of GLA-treated and/or irradiated U87 MG cells after a 48 h incubation. Means ± S.E. values are shown (n = 3), $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control cells, $^{\#\#}p < 0.01$ compared with GLA-treated cells.

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