432e Human Glioma Cells Exhibit Marked Reductions in 13c NMR-Detected Tca Cycle Activity and Oxygen Consumption during Late-Stage Apoptosis

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Introduction: Most cancer chemotherapeutics are designed to interact with DNA in a manner that eventually induces apoptosis. Clinical methods that specifically detect apoptosis could very valuable for monitoring chemotherapeutic efficacy. One of the early changes that occurs during apoptosis is release of cytochrome-c from the space between the inner and outer mitochondrial membranes (1). In the cytoplasm, cytochrome-c serves as a critical signaling molecule in the apoptotic cascade. However, it can no longer perform its normal functions in oxidative phosphorylation. Other changes are known to occur in mitochondria during apoptosis, which may also alter aerobic metabolism (1). We hypothesized that human glioma cells treated with temozolomide (TMZ), a new DNA methylating for treatment of primary brain tumors, would exhibit disrupted oxidative metabolism that could be detected with ^{13}C NMR. To examine this hypothesis, cells were studied with an artificial tumor method developed in our lab, which allows apoptosis to be monitored under well-defined conditions (2).

Materials and Methods: SF188 cells (human glioma grade 4, Brain Tumor Research Center, UCSF, San Francisco, CA) were grown with DMEM medium (supplemented with 10% serum and 50 mg/ml gentamicin) in porous collagen microcarriers (Hyclone, Logan, UT). These microcarriers had a mean diameter of 200 mm when fully hydrated and were used in combination with solid polystyrene spheres (1:1 volume ratio) inside a 20-mm NMR tube (2). The cells were sustained at physiologic conditions (37 °C, pH = 7.2, dissolved oxygen = 0.2 mM) with a system constructed in our laboratory (3). NMR spectra were acquired with a 9.4T spectrometer (Varian, Palo Alto, CA). ^{31}P spectral parameters were: 60° pulse width, 1000 ms repetition time, 4096 points, and 15000 Hz spectral width. ^{13}C spectra were acquired with 60° pulses, a repetition time of 1200 ms, 4096 points, 25000 Hz spectral width and ^{1}H bi-level WALTZ-16 decoupling. Cells were initially fed DMEM with 10 mM un-enriched glucose while background spectra were acquired. Subsequently, the un-enriched medium was completely replaced with DMEM containing 10 mM [1,6-$^{13}C_2$] glucose (Cambridge Isotopes, Andover, MA). Absolute intracellular concentrations were calculated as described previously (3). Oxygen consumption was determined continuously with polarographic oxygen probes located upstream and downstream of the tumor (4). ^{13}C measurements were performed for three independently prepared artificial tumors, before and 4 days after TMZ treatment. In parallel studies, cells grown inside microcarriers were treated with TMZ and examined with a standard TUNEL assay for apoptosis (BD Biosciences, Palo Alto, CA). With this assay, endonuclease-cleaved DNA was labeled with fluorescein and detected with fluorescence confocal microscopy (Biorad, Hercules, CA).

Results: Approximately 12 days after microcarrier inoculation, the total viable cell number (estimated from ^{31}P NMR-detected NTP levels (4)) in the artificial tumor was ~8 x 10^8. ^{13}C spectra of the tumor exhibited resonances for C-3 of lactate and alanine, C-2, C-3 and C-4 of glutamate, C-1 and C-6 of fructose-1,6-diphosphate (F-1,6-DP), and –CH_2- groups of fatty acids. The C-3 glutamate resonance was a triplet due to coupling with C-4 of glutamate (3), which demonstrates that complete TCA cycle activity is present in these cells. Cells in the tumor were treated twice with TMZ (125 mg/ml with a delay of 24 h between treatments). NTP levels and oxygen consumption began to decline approximately 12 hours after the second treatment. The decline in oxygen consumption preceded the decline in NTP. Approximately 4 days after the second TMZ treatment, significant levels of NTP remained and the cells continued to produce lactate; however, oxygen consumption was undetectable. The tumor was again perfused with medium containing 10 mM [1,6-$^{13}C_2$] glucose. No labeling was detected for any intracellular resonances except a very small amount at C-4 of glutamate. On an NTP normalized basis,

the rate of incorporation of ^{13}C label into C-4 glutamate by 85%. TUNEL analysis demonstrated that 2 days after the second treatment, ~10% of cells in the microcarriers contained endonuclease cleaved DNA. This value increased to ~30% 3 days post-treatment.

Discussion: Glutamate is a key reporter molecule for TCA cycle labeling (5). The marked reduction in labeling for glutamate and the lack of oxygen consumption indicate that mitochondrial function was disrupted sub-acutely by TMZ. Because endonuclease cleavage of DNA occurs relatively late in apoptosis, the finding that only 30% of cells were stained in the TUNEL assay was not surprising. Many cells may have released their cytochrome-c into the cytoplasm, but had not yet initiated DNA cleavage. Direct detection of cytochrome-c release will be studied in future work to elucidate the time course for this important change following TMZ treatment.

Conclusions: Our results indicate that ^{13}C NMR can be used to detect changes in oxidative metabolism of human glioma cells following treatment with TMZ. This finding may help to advance the use of ^{13}C MRS methods (e.g. ^{1}H-detected ^{13}C MRS) for clinical monitoring of apoptosis in human gliomas.

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References:

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