

Fatty acid synthase: a novel target for anti glioma therapy

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High levels of fatty acid synthase (FAS) expression have been observed in several cancers, including breast, prostate, colon and lung carcinoma, compared with their respective normal tissue. We present data that show high levels of FAS protein in human and rat glioma cell lines and human glioma tissue samples, as compared to normal rat astrocytes and normal human brain. Incubating glioma cells with the FAS inhibitor cerulenin decreased endogenous fatty acid synthesis by approximately 50%. Cell cycle analysis demonstrated a time- and dose-dependent increase in S-phase cell arrest following cerulenin treatment for 24 h. Further, treatment with cerulenin resulted in time- and dose-dependent decreases in glioma cell viability, as well as reduced clonogenic survival. Increased apoptotic cell death and PARP cleavage were observed in U251 and 5NH-19 cells treated with cerulenin, which was independent of the death receptor pathway. Overexpressing Bcl-2 inhibited cerulenin-mediated cell death. In contrast, primary rat astrocytes appeared unaffected. Finally, RNAi-mediated knockdown of FAS leading to reduced FAS enzymatic activity was associated with decreased glioma cell viability. These findings suggest that FAS might be a novel target for anti glioma therapy.

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In 2006, 18,820 individuals living in the US will be diagnosed with primary brain cancer (Jemal *et al.*, 2006). The most common and most lethal primary brain tumour in adults is glioblastoma multiforme. Glioblastoma multiforme represents one of the most challenging of all cancers to treat successfully, characterised not only by aggressive proliferation and expansion but also by inexcusable tumour invasion into distant brain tissue (Kew and Lewis, 2003). Improvements in multimodality treatments including surgery, radiotherapy, and the recent addition of temozolomide have improved median survival, but this is still only approximately 14 months (Stupp *et al.*, 2005) and long-term survival is dismal. The development of novel biological therapies for primary brain tumours remains an urgent need. Current cancer treatment is rapidly evolving in parallel with an understanding of tumour biology (Weiss, 2000; Karpas and Nalbantoglu, 2003). Genetic abnormalities and differential gene expression between normal and cancer cells can provide novel targets for anticancer therapy (Lang *et al.*, 1999; Bansal and Engelhard, 2000; Grill *et al.*, 2001).

Fatty acid synthase (FAS) is a 270 kDa cytosolic multifunctional polypeptide and is the primary enzyme required for catalysing the conversion of dietary carbohydrates to fatty acids (Wakil, 1989). Normal cells preferentially use circulating dietary fatty acids for the synthesis of new structural lipids. Thus, FAS expression is generally low to undetectable in normal human tissues, other than the liver and adipose tissue. In contrast, FAS is overexpressed in many human tumours, including carcinoma of the breast (Milgram *et al.*, 1997; Wang *et al.*, 2001b), prostate (Epstein

et al., 1995; Swinnen *et al.*, 2002), colon (Rashid *et al.*, 1997), ovary (Gander *et al.*, 1997), endometrium (Pizer *et al.*, 1998b), mesothelium (Gabrielson *et al.*, 2001), lung (Piyathilake *et al.*, 2000), thyroid (Vlad *et al.*, 1999), and stomach (Kusaka *et al.*, 2002). Abnormally active endogenous fatty acid metabolism appears to be important for cancer cell proliferation and survival (Ookhtens *et al.*, 1984). Moreover, overexpression of FAS in breast, prostate, and thyroid cancers has been associated with more aggressive malignancies (Epstein *et al.*, 1995; Gander *et al.*, 1997; Vlad *et al.*, 1999; Swinnen *et al.*, 2002). The preferential expression of FAS in cancer cells suggests that FAS could be a promising target for antitumour therapy (Kuhajda, 2006).

Inhibitors of FAS have been used to study the loss of FAS function in tumour cells. The first identified 'specific' inactivator of FAS, cerulenin, (2R, 3S)-2,3-epoxy-4-oxo-7,10-trans,trans-dodecadienamide, is a natural antibiotic product of the fungus *Cephalosporium ceruleans* (Omura, 1976). Cerulenin irreversibly inhibits FAS by binding covalently to the active site cysteine thiol in the β -ketoacyl-synthase domain (Funabashi *et al.*, 1989). Cerulenin is selectively cytotoxic to a number of established human cancer cell lines, including breast (Gabrielson *et al.*, 2001), colon (Pizer *et al.*, 1998a), and prostate (Purys *et al.*, 1997; Pizer *et al.*, 2001). Kuhajda *et al.* (2000) reported that cerulenin inhibited fatty acid synthesis in tumour cells in a dose-dependent manner; the cytotoxic effect of cerulenin to human breast tumour cells generally paralleled the level of endogenous fatty acid synthesis. Fatty acid synthase inhibition by cerulenin leads to apoptotic cell death in breast, prostate, and colon cancer cells (Purys *et al.*, 1997; Huang *et al.*, 2000; Li *et al.*, 2001). However, cerulenin's chemical instability renders it inappropriate as a systemic anticancer agent. C75, a potent derivative of cerulenin and a more stable form of FAS inhibitor, has been tested recently for its anti-breast tumour effects

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