Metabolomic Studies of a Prostate Cancer Cell Line Following RNA Interference-Mediated Silencing of the Fatty Acid Synthase Gene

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Introduction: Fatty acid synthase (FASE) is the enzyme that catalyzes the terminal steps in the synthesis of saturated fatty acid (FA). FASE expression is low in normal human tissues because the bulk of required lipids are obtained from diet. Over-expression of FASE has been found in a variety of human cancers and is associated with poor prognosis. As such FASE is an attractive therapeutic target for developing novel anticancer drugs. Current FASE inhibitors have several shortcomings which include non-specific inhibition. A new approach based on selective gene silencing by RNA interference (RNAi) has therefore been developed to interfere with FASE activity and induce growth arrest and apoptosis in cancer cells [1]. RNAi is a cellular process resulting in enzymatic cleavage and breakdown of mRNA guided by sequence-specific double-stranded RNA oligonucleotides (siRNA) [2].

Aims: To obtain and examine the metabolic profiles and changes that are associated with silencing of the FASE gene. This information will be useful in the development of pharmacodynamic markers for FASE inhibitors.

Experimental: The human prostatic cancer cell line LNCaP was used in this study. The LNCaP cells were plated at a density of $5x10^5$/6cm dish and cultured in RPMI 1640 supplemented with fetal calf serum and glutamine at 37°C in 5% CO_2/95% air. The next day, cells were transfected with siRNA-targeting FASE (n = 7) or luciferase (a nonspecific siRNA control, n = 5) [1] and further incubated for 72 hours. Cells were then extracted using a dual-phase (in chloroform/methanol/water) extraction procedure [3]. Cell lipid and water-soluble metabolite extracts were reconstituted in $CDCl_3/CD_3OD$ (2:1) and D_2O, respectively. Sodium 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate was added to the samples for chemical shift calibration and quantitation. High resolution ^1H NMR spectroscopy was performed on the cell extracts using a 600MHz Bruker system.

Results: Significant changes in lipids and water-soluble metabolites were observed following siRNA inhibition of FASE when compared with controls. Data are summarised in Table 1 and 2, respectively. No change in valine, alanine, acetate, glutamine, glutamate, succinate, choline, glycerophosphocholine, myo-inositol, creatine and tyrosine level was observed.

Table 1: Significant* changes in lipid levels following siRNA of FASE

	Saturated fatty acids					Unsaturated fatty acids			
	-CH_3	**-$(CH_2)_n$-**	CH_2-**CH_2**-$(CH_2)_n$	$(CH_2)_n$-**CH_2**-CH	CH_2-**CH_2**-CO_2	=CH-**CH_2**-CH=	**-CH=CH-**	Peth	PGly
Control (n = 5)	5.20 ±0.32	16.33 ± 0.77	1.45 ±0.06	0.668 ±0.028	0.896 ±0.023	0.144 ±0.002	0.516 ±0.014	0.035 ±0.004	0.157 ±0.002
FASE (n = 7)	6.71 ±0.49	19.13 ± 1.03	1.76 ±0.10	0.744 ±0.017	0.995 ±0.036	0.203 ±0.006	0.591 ±0.014	0.020 ±0.003	0.169 ±0.005

Concentration in mmol/g protein. Data expressed as mean ± s.e.m. PEth: phosphatidylethanolamine; PGly: phosphatidylglycerol. In all cases, *P<0.05 when compared to control.

Table 2: Significant* changes in water-soluble metabolites following siRNA of FASE.

	Leu	Ile	Lac	Glucose	PC	Gly	PCr	ATP+ADP	UTP+UDP	NADH	Formate
Control (n = 5)	23.35 ±1.30	16.43 ±1.35	381.18 ±22.05	67.44 ± 6.41	52.07 ± 1.13	80.65 ± 2.49	56.56 ± 0.85	26.68 ± 0.81	84.72 ± 2.67	5.67 ±0.27	8.99 ±0.45
FASE (n = 7)	18.58 ±0.66	12.75 ±0.78	250.26 ±13.18	47.32 ± 3.31	78.79 ± 2.13	134.16 ± 5.33	76.17 ± 2.38	39.81 ± 1.34	102.72 ± 6.17	8.01 ±0.64	10.90 ±0.64

Concentration in µmol/g protein. Data expressed as mean ± s.e.m. Leu: leucine; Ile: isoleucine; Lac: lactate; PC: phosphocholine; Gly: glycine; PCr: phosphocreatine; ATP+ADP: adenosine triphosphate and adenosine diphosphate; UPT+UDP: uridine triphosphate and uridine diphosphate; NADH: nicotinamide adenine dinucleotide. In all cases, *P<0.05 when compared to control.

Discussion: In agreement with previous observations [1], silencing FASE, the principal enzyme of FA synthesis, significantly decreased phosphatidylethanolamine. However, several other results were unexpected. Paradoxically, saturated FA signals *increased* when FASE was inhibited, as did phosphocreatine and the nucleotides ATP+ADP and UTP+UDP. One possible explanation for the saturated FA increase could be that the normal oxidation of the FA absorbed from the culture medium was inhibited by accumulation of malonyl-CoA, the substrate of FASE. Another consequence of inhibited FA oxidation would be reliance on oxidation of lactate and amino acids; this could explain the fall in lactate, isoleucine and leucine. However the rise in glycine is not easily explained on this basis. An explanation for the rise in unsaturated FAs could be induction of apoptosis, since inhibition of FASE has been shown to induce apoptosis in cancer cells [1] and apoptosis is associated with increased unsaturated FAs [4].

1) E DeSchrijver et al., Cancer Research 63; 3799-3804 (2003). 2) SM Elbashir et al., Nature 411; 494-498 (2001). 3) RK Tyagi et al., Magn Reson Med 35; 194-200 (1996). 4) JL Griffin et al., Cancer Research 63; 3195-3201 (2003). This work is supported by Cancer Research UK.