ANTICANCER RESEARCH *24*: 4169-4174 (2004)

Fatty Acid Synthase (FAS) is a Marker of Increased Risk of Recurrence in Lung Carcinoma

PAOLO VISCA[2], VALERIA SEBASTIANI[1], CLAUDIO BOTTI[2], MARIA GRAZIA DIODORO[2], ROBERTO PASQUALI LASAGNI[3], FRANCESCA ROMAGNOLI[2], ALESSIA BRENNA[2], BARBARA CAPEZZONE DE JOANNON[1], RAFFAELE PERRONE DONNORSO[2], GIUSEPPE LOMBARDI[1] and PIERO LUIGI ALO[1]

[1]Department of Experimental Medicine and Pathology, University of Rome "La Sapienza", Rome; [2]Department of Pathology and [3]Department of Thoracic Surgery, Regina Elena National Cancer Institute, Rome, Italy

Abstract. *Background: We explored the expression of Fatty Acid Synthase (FAS) in lung carcinomas and its association with clinico-pathological features and prognosis. FAS is a recently discovered molecule involved in the energy supply of normal cells. FAS is also overexpressed in neoplastic tissues because of their increased necessity for energy. Patients and Methods: One hundred and six patients with non-small cell lung carcinoma were followed-up for an average period of 5 years. FAS expression was detected immunohistochemically. Results: FAS staining was observed in 61 out of 106 cases (57.54%). Statistical analysis revealed that FAS had an overall low prognostic value (p=0.14), while FAS-negative expression in stage I patients showed a trend for better survival (p=0.10). PTNM stage (p<0.0001) was the only significant prognostic marker for overall survival. Conclusion: FAS is a reliable marker of low-stage clinically aggressive lung carcinomas. The determination of FAS expression in lung carcinomas may stratify patients and determine therapeutic approaches for their care.*

Lung carcinoma remains the leading cause of death in men and women in the United States (1), mostly affecting people between the ages of 50 and 80 years (2) . Lung carcinoma, in similarity to all tumors, has an increased necessity for energy and, therefore, may develop certain metabolic pathways in order to gain energy, synthesizing, among other sources, endogenous fatty acids. Endogenous fatty acid synthesis is determined by high levels of fatty acid synthase (FAS). FAS is a multifunctional enzyme that has various functions within cells such as energy storage, membrane structure, signal transduction cascades and protein acylation (3). Normally it is expressed at low levels in human tissue due to down-regulation mediated by dietary lipids (4). We and others have shown (5-8) that this enzyme is overexpressed in non-neoplastic, highly proliferative lesions and in aggressive carcinomas with poor outcome. As FAS has a role in the metabolic activities of normal and pathological (*i.e.* neoplastic) tissues, we evaluated, by means of immunohistochemistry, the significance of FAS overexpression in lung carcinomas and its association with clinico-pathological features and prognosis.

Correspondence to: Piero Luigi Alo, Dipartimento di Medicina Sperimentale e Patologia (Anatomia Patologica), Università di Roma "La Sapienza", Viale Regina Elena, 324-00161, Rome, Italy. Tel: 0039-06-4450386, e-mail: pieroluigialo@virgilio.it

Key Words: Fatty acid synthase, lung, carcinoma, prognosis.

Patients and Methods

Patient specimens. We studied 106 patients surgically treated for lung carcinoma at the Regina Elena Cancer Institute of Rome, Italy, between January 1983 and November 1995. Clinical information was obtained from the medical records. The clinical data collected included the patient's name, race, the family and patient's lung cancer history and type of surgery. Ethical approval and informed consent from eligible patients were appropriately acquired. The histopathological data included tumor size, histological subtype (9) and grade (10), evidence of necrosis and stage of the disease according to the TNM classification (11). Control specimens were obtained from 10 patients negative for cancer who underwent lobectomy for bronchiectasis.

Scoring of FAS immunoreactivity. FAS cytoplasmic immunohistochemical expression was performed using a combined scoring system based on the fraction of positive tumor cells and the predominant staining intensity in the tumor. FAS staining intensity was scored blindly, by the primary author (P.V.), in tissue sections identified only by the surgical accession number. FAS staining intensity was scored on a 4-tiered scale: 0=negative, 1=low intensity positive staining, 2=moderate intensity positive staining, 3= strong intensity positive staining. The intensity was determined relative to the intensity of FAS-positive internal controls such as peripheral nerve tissue included in the specimens observed. The fraction of positive tumor cells was estimated using a 4-tiered scale (10%=1, 11-50%=2, 51-80%=3, >80%=4). The overall score in each case was scored as

the product of the staining intensity and the positive fraction score. Each case was scored twice, independently, by each of two pathologists (P.V. and M.G.D.) with subsequent reconciliation of scored values. For statistical purposes, overall scores > 6 were considered positive. Control specimens, prepared in the absence of the primary antibody, confirmed the specificity of the lung epithelial immunoreaction.

Tumor collection and immunohistochemical procedures. We used tissue sections cut from specimens fixed in buffered-formalin and paraffin-embedded at the Department of Pathology of the Regina Elena Cancer Institute of Rome, Italy, in accordance with the institutional guidelines for the use of discarded human tissue. A monoclonal mouse purified antibody specific to FAS (dilution 1:3000) was used. Targeting antibody reaction was performed in an automated immunostainer (DAKO, Carpinteria, CA, USA) using an LSAB2 kit (DAKO) after heat-induced epitope retrieval in citrate buffer, pH 6.0, twice for 5 minutes using a steamer method. 3,3'-diaminobenzidine was the chromogen. Anti-FAS antibody was a gift from F.P. Kuhajda, MD, Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, USA.

Patients' follow-up techniques. Patients were followed postoperatively for a median 5-year period. Disease-free survival (DFS) and overall survival (OS) were calculated from the date of surgery to the date of first recurrence or death with active disease. Recurrence was the evidence of new disease manifesting at or near the original cancer or in a different site. The status of disease at death was determined by autopsy.

Statistical analysis. Cut-off points were decided on the basis of the median value of the immunohistochemical scores observed. Values equal to or greater than the median value were considered positive. Because of the relatively small number of cases available for this study, a strategy to use exact *p*-values in all the statistical analyses was adopted. Groups were compared by using the Fisher exact test. The logistic regression model was used to estimate prognostic factors for 5-year survival rate. In the multivariate logistic regression evaluation only those variables that had a p of <0.10 in univariate analysis were included in the model. All *p*-values were two-sided. Statistics were performed by using the StatXact4 and LogXact4 for Windows (CYTEL Software Corporation,Cambridge,MA,USA).

Results

Histotype. All 106 cases were non-small cell lung carcinomas. Fifty-three cases were squamous cell carcinomas, 41 were adenocarcinomas and 12 were large cell carcinomas. All patients underwent lobectomy (85 cases) or pneumectomy (21 cases) with removal of regional lymph nodes, and were followed-up for 5 years after surgery.

Histological grade. Twenty-five cases were well-differentiated (G1), 33 cases were moderately-differentiated (G2) and 48 cases were poorly-differentiated carcinomas.

Stage. Fifty-six (52.9%) out of 106 cases were Stage I, 26 (24.6%) were Stage II, 20 (18.9%) were Stage IIIA and 4 (3.6%) were Stage IIIB.

Table I. *Impact of clinico-pathological variables and FAS expression after a 5-year follow-up. Univariate analysis.*

Parameter	5-year survival		*P*
	%	95% C.I.	
Sex			
Male	37.7	27.5-47.9	0.35
Female	20.0	3.1-47.5	
Age			
<60 yrs	34.0	20.4-48.1	0.49
>60 yrs	37.4	24.5-50.3	
Histology			
Squamous	33.5	20.3-47.2	0.20
Adenocarcinoma	42.6	26.8-57.6	
Large Cell	25.0	6.1-50.5	
Type of operation			
Pneumonectomy	33.3	13.7-54.5	0.80
Lobectomy	36.4	25.7-47.1	
p-stage			
I	48.2	27.4-55.8	0.00001
II	37.3	18.5-56.2	
IIIa	5.61	3.8-22.6	
IIIb	0	-	
FAS expression			
Negative	47.1	31.7-61.0	0.14
Positive	26.7	15.5-39.2	

Age. Mean age of the patients at the time of surgery was 63 years (57-78), and the cases comprised 96 males and 10 females.

Recurrence. Forty-five patients (42.3%) died of cancer during the follow-up period with a median of 36 months.

Immunohistochemical expression. FAS cytoplasmic staining (Figure 2) was observed in 61 out of 106 cases (57.54%) (30 cases were squamous cell carcinomas, 26 were adenocarcinomas and 5 were large cell carcinomas). Of these 61 cases, 31 were stage I, 14 were stage II, 15 were stage IIIA and 1 case was stage IIIB. No significant correlation was observed between immunohistochemical FAS expression and clinico-pathological variables. As described in Table I, univariate analysis showed that size ($p<0.001$), nodal involvement ($p=0.004$) and stage ($p<0.00001$) were predictors of disease-free survival while FAS had a low prognostic value ($p=0.14$). FAS expression in stage I patients showed a trend for better survival ($p=0.10$) (Figure 1). Concerning overall survival, the only significant prognostic factor was pTNM stage ($p<0.0001$).

Discussion

Most tissues with high cellular turnover appear to utilize circulating lipids for the synthesis of new structural lipids (12), but hyperplastic as well as neoplastic tissues seem to require alternative sources for energy storage. A minor pathway for metabolic accumulation of energy involves the biosynthesis of fatty acids. In mammals and birds, the *de novo* biosynthesis of fatty acids is consolidated into a single protein which is the product of a single gene. This multifunctional enzyme is FAS. FAS is the major enzyme involved in the anabolic conversion of dietary carbohydrates to fatty acids. FAS synthesizes long-chain fatty acids by using acetyl-CoA as a primer, malonyl-CoA as a two carbon donor and NADPH as reductant of the intermediates (13), and mainly synthesizes palmitate (80%), myristate (10%) and stearate (10%). Immunohistochemical expression of FAS has been reported in hyperplastic tissues (14), in proliferative cells of fetal tissues, or cells of adult tissues with high lipid metabolism. FAS mRNA has been reported to be high in human brain, liver, lung and breast tissues (15). Recently Oskovian *et al.* (16) found an increase in the rate of transcription of the FAS gene in neoplastic cells using a breast cancer-derived cell line as a model . The greater abundance of FAS mRNA was found to be responsible for FAS overexpression. FAS expression in normal tissues is regulated by a number of hormonal signals and related to dietary fat intake and metabolism. Moreover, FAS overexpression has been demonstrated in a number of human carcinomas with aggressive features and poor outcome such as ovary (17), prostate (18), thyroid, gastric, bladder (19) and breast carcinomas, some pediatric tumors (20), mesotheliomas (21) and melanomas (22).

In a recently published, paper Takahiro *et al.* (23) examined the expression of FAS in a group of patients with soft tissue sarcomas (STS). They showed that FAS expression is one of the predictive indicators for disease prognosis in STS and that FAS was associated with deep-seated and large-sized tumors. Wang *et al.* (24) found FAS expression in human breast cancer cell culture supernatants and in breast cancer patients. In addition, FAS levels were significantly higher in breast cancer patients with higher clinical stages. FAS seems to be specifically overexpressed in neoplastic cells. Rashid *et al.* (25) evaluated FAS expression in normal and neoplastic colorectal specimens. They observed that FAS faintly-stained native colorectal mucosa, but increased FAS expression was found in adenomas and colorectal carcinomas. The importance of endogenous fatty acid synthesis in human cancers has not yet been elucidated, however it has been postulated that endogenous production of fatty acids may be a necessary energy supply for tumor growth. It is possible that the increased FAS expression of tumor cells is an indirect, associated phenomenon, which occurs to compensate for an insufficiency of dietary fatty acids due to, *e.g.*, lack of angiogenesis.

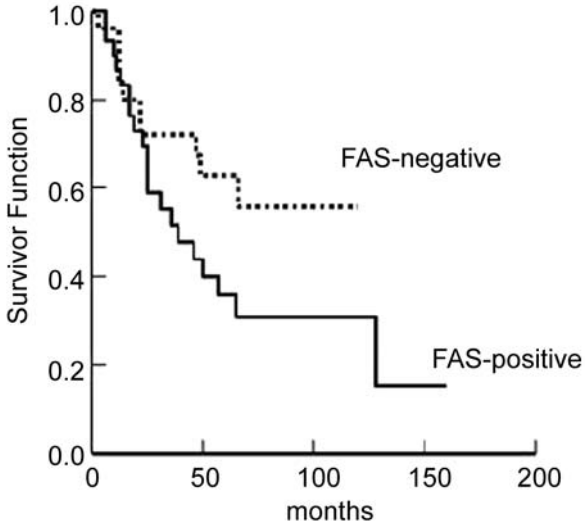

Figure 1. *Survival according to FAS expression (Stage I patients). FAS-negative patients showed a trend for better survival (p=0.10).*

In 1998, Pizer *et al.* (26) evaluated FAS expression in endometrial carcinoma and its correlation with cell proliferation and hormone receptors. They demonstrated that there was a close correlation between FAS and Ki-67 expression and that these two markers were strongly positive in aggressive carcinomas, even in those cases where PR and ER receptors were negative, while well-differentiated tumors showed a weak expression of all four antigens. Being involved in the metabolic activity of lung carcinoma, we decided to study FAS in order to determine if, by itself or in combination, it could be associated with clinico-pathological features or have a prognostic significance. Our data revealed that FAS had a overall low prognostic value, while FAS expression in stage I patients showed a trend for better survival. Concerning overall survival, the only significant prognostic marker was pTNM stage. Other clinico-pathological markers had no prognostic relevance. These data may lead to some conclusions. Lung carcinomas exploit fatty acids for metabolic supplies. In fact, Pihathilake *et al.* (27) observed a significant step-wise increase in fatty acid synthase expression from squamous cell carcinoma-associated uninvolved bronchial epithelium to epithelial hyperplasia to squamous cell carcinoma. Our data are in accord with a recently published work by Wang *et al.* (28), who revealed that FAS expression in stage I lung carcinomas was associated with prognosis, indicating that fatty acids may

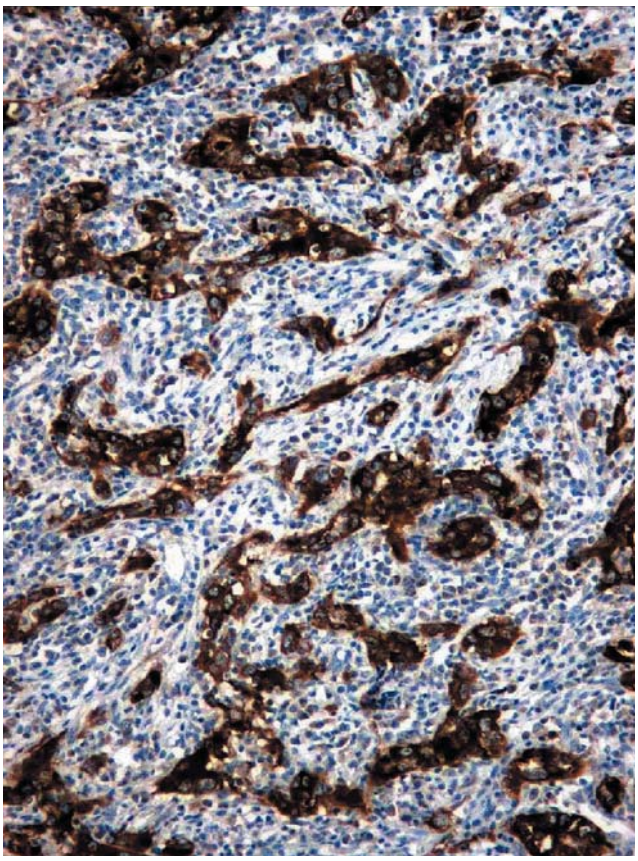

Figure 2. *Lung squamous cell carcinoma. Strong immunohistochemical expression for fatty acid synthase. (DAB x10).*

be a primary source of energy in non-metastatic lung carcinomas. We believe that FAS overexpression in node-negative lung tumors may be a signal of neoplastic aggressiveness, while high-stage tumors may gain energy from sources other than endogenous fatty acids. The knowledge of FAS overexpression in lung carcinomas could have a therapeutic implication. Kuhajda *et al.* (29) reported that inhibition of fatty acid synthesis could be a potential selective target for antineoplastic therapy. Several studies after that (30-32) revealed that cerulenin, a specific noncompetitive inhibitor of FAS, is selectively cytotoxic to cancer cells that have an increased fatty acid synthesis, but not to normal cells. Since cerulenin is chemically instable, Pizer *et al.* (33) recently developed a synthetic small molecule inhibitor of FAS, C75, that has been shown to have a better cytotoxic effect on cancer cells *in vitro*. *In vivo* studies on C75 are ongoing in human cancer xenografts to determine its systemic anti-tumor effects. These results suggest that FAS may be a specific target for pharmacological therapy in a high proportion of human malignancies including for lung carcinomas.

References

1 Kukunoor R, Shah J and Mekhail T: Target therapy for lung cancer. Curr Oncol Rep *5(4)*: 326-333, 2003.

2 Kuo CW, Chen YM, Chao JY, Tsai CM and Perng RP: Non-small lung cancer in very young and very old patients. Chest *117(2)*: 354-357, 2000.

3 Wakil S: Fatty acid synthase: a proficient multifunctional enzyme. Biochemistry *28*: 4523-4530, 1989.

4 Latasa MJ, Moon YS, Kim KH and Sul HS: Nutritional regulation of the fatty acid synthase promoter *in vivo*: sterol regulatory element binding protein functions through an upstream region containing a sterol regulatory element. Proc Natl Acad Sci USA *97(19)*: 10619-10624, 2000.

5 Wilentz RE, Witters LA and Pizer ES: Lipogenic enzymes fatty acid synthase and acetyl-CoA carboxylase are co-expressed with sterol regulatory element binding protein and Ki-67 in fetal tissues. Pediatr Dev Pathol *3(6)*: 525-531, 2000.

6 Alo PL, Visca P, Marci A, Botti C, Mangoni A and Di Tondo U: Expression of fatty acid synthase as a predictor of recurrence in Stage I breast carcinoma patients. Cancer *77*: 474-482, 1996.

7 Epstein JI, Carmichael M and Partin AW: OA-519 (fatty acid synthase) as an independent predictor of pathologic state in adenocarcinoma of the prostate. Urology *45*: 81-86, 1995.

8 Nemoto T, Terashima S, Kogure M, Hoshino Y, Kusakabe T, Suzuki T and Gotoh M: Overexpression of fatty acid synthase in oesophageal squamous cell dysplasia and carcinoma. Pathobiology *69(6)*: 297-303, 2001.

9 World Health Organization: Histological typing of lung tumors. *In*: World Health Organization (second edition), Geneva, 1981.

10 Muller KM: Histological classification and histogenesis of lung cancer. Eur J Resp Dis *65(1)*: 4-19, 1984.

11 Fleming ID, Cooper JS, Henson DE, Hutter RVP, Kennedy BJ, Murphy GP, O'Sullivan B, Sobin LH and Yarbro JW, editors. AJCC Cancer Staging Handbook, fifth edition. Philadelphia-New York: Lippincott-Raven publishers, 1998, pp 117-127.

12 Weiss L, Hoffman GE, Schreiber R, Andres H, Fuchs E, Korber E and Kolb HJ: Fatty acid biosynthesis in man: a pathway of minor importance. Purification, optimal assay conditions and organ distribution of fatty-acid synthase. Biol Chem Hoppe-Seyler *367*: 905-12, 1986.

13 Bresler R and Wakil SJ: Studies on the mechanism of fatty acid synthesis: the conversion of malonyl-CoA to long chain fatty acid. J Biol Chem *236*: 1643-1651, 1961.

14 Alo PL, Visca P, Mazzaferro S, Eleuteri Serpieri D, Mangoni A, Botti C, Monaco S, Carboni M, Zaraca F, Trombetta G and Di Tondo U: Immunohistochemical study of fatty acid synthase, Ki67, proliferating cell nuclear antigen and p53 expression in hyperplastic parathyroids. Ann Diagn Pathol *3(5)*: 287-293, 1999.

15 Chalbos D, Escot C, Joyeux C, Tissot-Carayon MJ, Pages A and Rochefort H: Expression of the progestin-induced fatty acid synthetase in benign mastopathies and breast cancer as measured by RNA *in situ* hybridisation. J Natl Cancer Inst *82*: 602-606, 1990.

16 Oskovian B: Overexpression of fatty acid synthase in SKBR3 breast cancer cells line mediated *via* a transcriptional mechanism. Cancer Lett *149*: 43-51, 2000.

17 Alo PL, Visca P, Framarino ML, Botti C, Monaco S, Sebastiani V, Serpieri DE and Di Tondo U: Immunohistochemical study of fatty acid synthase in ovarian neoplasms. Oncol Rep *7*: 1383-1388, 2000.

18 Swinnen JV, Vanderhoydonc F, Elgamal AA, Eelen M, Vercaeren I, Joniau S, Van Poppel H, Baert L, Goossens K, Heyns W and Verhoeven G: Selective activation of the fatty acid synthetic pathway in human prostate cancer. Int J Cancer *18(2)*: 176-179, 2000.

19 Visca P, Sebastiani V, Pizer ES, Botti C, De Carli P, Filippi S, Monaco S and Alo PL: Immunohistochemical expression and prognostic significance of fatty acid synthase and GLUT1 in bladder carcinoma. Anticancer Res *23*: 335-9, 2003.

20 Camassei FD, Cozza R, Acquaviva A, Jenkner A, Rava L, Gareri R, Donfrancesco A, Bosman C, Vadala P, Hadjistilianou T and Boldrini R: Expression of the lipogenic enzyme fatty acid synthase in retinoblastoma and its correlation with tumor aggressiveness. Invest Opthalmol Vis Sci *44(6)*: 2399-2403, 2003.

21 Gabrielson EW, Pinn ML, Testa JR and Kuhajda FP: Increased fatty acid synthase is a therapeutic target in mesothelioma. Clin Cancer Res *7(1)*: 153-157, 2001.

22 Innocenzi D, Alo PL, Balzani A, Sebastiani V, Silipo V, La Torre G, Ricciardi G, Bosman C and Calvieri S: Fatty acid synthase expression in melanoma. J Cutan Pathol *30(1)*: 23-28, 2003.

23 Takahiro T, Shinichi K and Toshimitsu S: Expression of fatty acid synthase as a prognostic indicator in soft tissue sarcomas. Clin Cancer Res *9*: 2204-2212, 2003.

24 Wang Y, Kuhajda FP, Li JN, Pizer ES, Han WF, Sokoll LJ and Chan DW: Fatty acid synthase expression in human breast cancer cell culture supernatants and in breast cancer patients. Cancer Lett *167(1)*: 99-104, 2001.

25 Rashid A, Pizer ES, Moga M, Milgraum LZ, Zahurak M, Pasternack GR, Kuhajda FP and Hamilton SR: Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. Am J Pathol *150(1)*: 201-208, 1997.

26 Pizer ES, Lax SF, Kuhajda FP, Pasternak GR and Kurman RJ: Fatty acid synthase expression in endometrial carcinoma: correlation with cell proliferation and hormone receptors. Cancer *83(3)*: 528-537, 1998.

27 Piyathilake CJ, Frost AR, Manne U, Bell WC, Weiss H, Heimburger DC and Grizzle WE: The expression of fatty acid synthase is an early event in the development and progression of squamous cell carcinoma of the lung. Human Pathol *31(9)*: 1068-1073, 2000.

28 Wang Y, Zhang X, Tan W, Fu J and Zhang W: Significance of fatty acid synthase expression in non-small lung cancer. Zhonghua Zhong Liu Za Zhi *24(3)*: 271-273, 2002.

29 Kuhajda FP, Jenner K, Wood FD, Hennigar RA, Jacobs LB, Dick JD and Pasternack GR: Fatty acid synthesis: a potential selective target for antineoplastic therapy. Proc Natl Acad Sci USA *91*: 6379-6383, 1994.

30 Vance D, Goldberg I, Mitsuhashi O and Bloch K: Inhibition of fatty acid synthetases by the antibiotic cerulenin. Biochem Biophys Res Commun *48*: 649-56, 1972.

31 Funabashi H, Kawaguchi A, Tomoda H, Omura S, Okuda S and Iwasaki S: Binding site cerulenin in fatty acid synthetase. J Biochem *105*: 751-755, 1989.

32 Slade RF, Hunt DA, Pochet MM, Venema VJ and Hennigar RA: Characterizazion and inhibition of fatty acid synthase in pediatric tumor cell lines. Anticancer Res *23*: 1235-1243, 2003.

33 Pizer ES, Chrest FJ, Di Giuseppe JA and Han WF: Pharmacological inhibitors of mammalian fatty acid synthase suppress DNA replication and induce apoptosis in tumor cell lines. Cancer Res *58*: 4611-4615, 1988.

Received February 19, 2004
Revised June 4, 2004
Accepted August 8, 2004