Journal of Cellular Biochemistry 94:1–4 (2005)

VIEWPOINT

In Support of Fatty Acid Synthase (FAS) as a Metabolic Oncogene: Extracellular Acidosis Acts in an Epigenetic Fashion Activating FAS Gene Expression in Cancer Cells

Javier A. Menendez,[1,2]* Josiah P. Decker,[1] and Ruth Lupu[1,2]*

[1]Department of Medicine, Breast Cancer Translational Research Laboratory, Evanston Northwestern Healthcare Research Institute, Evanston, Illinois
[2]Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois

Abstract Relatively little information exists on the ultimate molecular mechanisms by which the lipogenic enzyme Fatty Acid Synthase (FAS) is differentially overexpressed in a biologically aggressive subset of human malignancies. Since the microenvironment of solid tumors contains regions of poor oxygenation and high acidity, it has recently been suggested that cancer-associated FAS is a novel metabolic oncogene conferring a selective growth advantage upon stresses such as hypoxia and/or low pH. Here, we performed transient transfection studies with a 178-bp FAS promoter fragment harboring a complex Sterol Regulatory Element Binding Proteins (SREBP)-binding site to evaluate whether extracellular low pH and/or hypoxia may act in an epigenetic fashion by inducing changes in the transcriptional activation of FAS gene in cancer cells. First, MCF-7 breast cancer cells cultured in acidosis (pH 6.5), but not under hypoxia or in the presence of hypoxia mimetics, demonstrated a more than two-fold increase in the transcriptional activity of FAS promoter-reporter constructs compared with control cells grown under standard culture conditions (pH 7.4). Second, the up-regulatory effect of extracellular acidosis on the transcriptional activation of FAS gene was not observed when the FAS promoter was truncated at the SREBP-binding site. Third, MCF-7 cells engineered to overexpress the Her-2/*neu* (*erb*B-2) oncogene exhibited a SREBP-dependent activation of the FAS promoter-reporter construct up to three-fold higher than that found in wild-type MCF-7 cells, while extracellular acidosis resulted only in a marginal increase of Her-2/*neu*-promoted activation of FAS gene. This study reveals for the first time that extracellular acidosis can work in an epigenetic fashion by up-regulating the transcriptional expression of FAS gene in breast cancer cells, a stimulatory effect that is equally mimicked by well-characterized oncogenic stimuli such as Her-2/*neu*. These findings, altogether, support the ''metabolic oncogene'' theory for FAS overexpression in cancer cells. J. Cell. Biochem. 94: 1–4, 2005.

Key words: fatty acid synthase; oncogene; acidosis; pH; SREBP; cancer

In the 1950s, some studies showed that endogenous fatty acid biosynthesis in tumor tissues occur at very high rates [Medes et al., 1953]. In the mid-1980s, it was established that de novo synthesis of fatty acids accounts for almost all fatty acids in the tumor cells, despite adequate nutritional supply [Ookhtens et al., 1984]. However, the endogenous fatty acid synthesis pathway did not become a focus of clinical interest as a means to treat or diagnose human cancer until the early 1990s. In 1994, Kuhajda and colleagues unambiguously identified through sequence homology and enzymology the oncogenic antigen-519 (OA-519)—a prognostic molecule found in tumor cells from breast cancer patients with markedly worsened prognosis—as fatty acid synthase (FAS), the key metabolic multi-enzyme that is responsible for the terminal catalytic step in the de novo fatty acid biosynthesis [Kuhajda et al., 1994]. After numerous clinical and basic research studies, it now appears that human cancers constitutively express high levels of FAS and undergo significant endogenous fatty acid biosynthesis. Moreover, FAS up-regulation also appears to be linked to malignant transformation,

Grant sponsor: The Auxiliary of Evanston Northwestern Healthcare.

*Correspondence to: Javier A. Menendez, PhD and Ruth Lupu, PhD, Evanston Northwestern Healthcare Research Institute, 1001 University Place, Evanston, IL 60201.
E-mail: javiermenendez72@yahoo.com; r-lupu@northwestern.edu

Received 22 August 2004; Accepted 24 August 2004
DOI 10.1002/jcb.20310

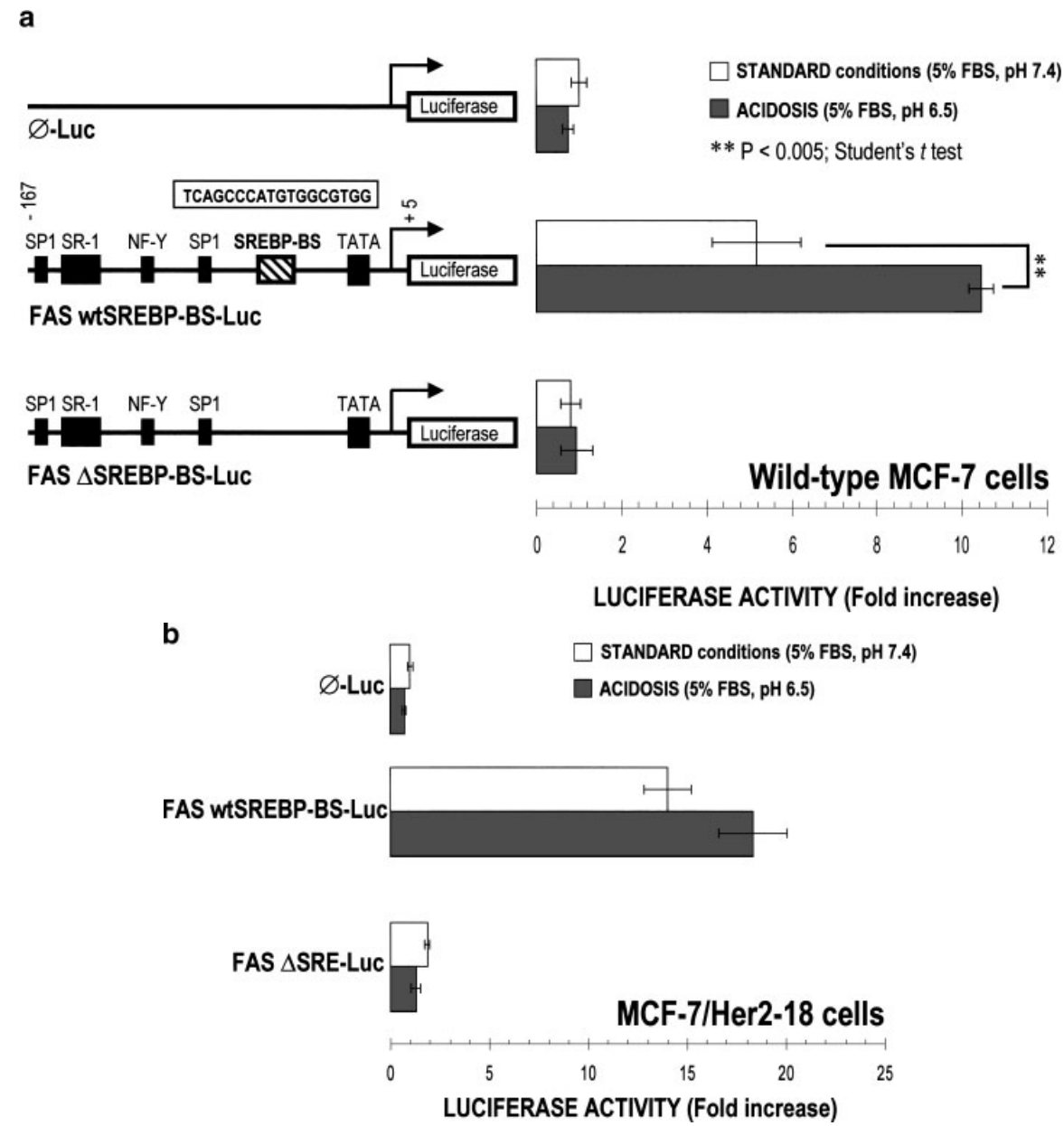

Fig. 1. Effects of extracellular acidosis on FAS gene activation in cancer cells. MCF-7 (**panel a**) and MCF-7/Her-2 (clone 18; **panel b**) cancer cells were routinely grown in phenol red-containing improved MEM (IMEM, Biosource International, Camarillo, CA) supplemented with 5% (v/v)-heat-inactivated fetal bovine serum (FBS) and 2 mM L-glutamine, and maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Using FuGENE 6 transfection reagent (Roche Biochemicals, Indianapolis, IN) as directed by the manufacturer, overnight serum-starved cells seeded into 24-well plates ($\sim 5 \times 10^4$ cells/well) were transfected in low-serum (0.1% FBS) media with 300 ng/well of the pGL3-luciferase (Promega, Madison, WI) construct containing a luciferase reporter gene driven by either an intact (FAS wtSREBP-BS-Luc) or damaged (FAS ΔSREBP-BS-Luc) 178-bp FAS promoter fragment along with 30 ng/well of the internal control plasmid pRL-CMV, which was used to correct for transfection efficiency. After 18 h, the transfected cells were washed and then incubated upon standard (5% FBS, pH 7.4) or acidic conditions (5% FBS, pH 6.5). Culture medium was acidified by supplementing the regular medium with 25 mM 4-morpholinepropanesulfonic acid (Sigma, St. Louis, MO). The acidity of the medium was adjusted to a final pH of 6.5 with 1N NaOH. Approximately 24 h after treatments, luciferase activity from cell extracts was detected using a Luciferase Assay System (Promega, Madison, WI) according to the protocol specified by the manufacturer using a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). The magnitude of activation in FAS promoter-Luciferase-transfected cells was determined after normalization to the luciferase activity in cells co-transfected with equivalent amounts of the empty pGL3-luciferase vector lacking the FAS promoter (∅-Luc) and the internal control plasmid pRL-CMV, which was taken as 1.0-fold. This control value was used to calculate the relative (fold) change in transcriptional activities of FAS promoter-Luciferase-transfected cells in response to treatments after normalization to pRL-CMV activity, and the data are shown as the means ± SD from three separate experiments (performed in triplicate).

since high levels of FAS expression and activity have been identified in pre-neoplastic lesions [Kuhajda, 2000].

Although tumor-associated FAS, seems to play a vital role in cancer cell survival and proliferation, and it may be directly involved in maintenance or enhancement of the malignant phenotype, relatively little information exists on the ultimate molecular mechanisms by which this lipogenic enzyme is constitutively up-regulated in cancer cells. A very attractive theory to explain cancer-associated FAS overexpression has recently been put forth by Baron et al. [2004]. Since the microenvironment of solid tumors contains regions of poor oxygenation and high acidity, they proposed, in agreement with Hochachka et al. [2002], FAS as a putative metabolic oncogene conferring a selective growth advantage upon stresses such as hypoxia, low pH, and/or nutritional deprivation. We recently performed a preliminary in vitro approach to test this hypothesis.

The first condition to embrace the "metabolic oncogene" theory for FAS overexpression in cancer cells is that extracellular low pH and/or hypoxia should act in an epigenetic fashion by inducing changes in the transcriptional activation of FAS gene. To evaluate this option, the non-aggressive breast cancer cell line MCF-7 was transiently transfected with a 178-bp FAS promoter–reporter construct (FAS-Luc) that harbors all the elements necessary for high level expression in cancer cells [Swinnen et al., 1997]. Figure 1a shows that MCF-7 cells cultured in acidosis (pH 6.5) demonstrated a more than two-fold increase in reporter activity, compared with control cells grown under standard culture conditions (pH 7.4). Remarkably, we failed to observe any transcriptional activation of FAS gene in response to hypoxic conditions or hypoxia mimetics, such as deferoxamine mesylate and $CoCl_2$ (data not shown).

The second condition to support Baron's proposition is that a common up-stream control system should operate for the simultaneous transcriptional up-regulation of all the genetic components required for the fatty acid synthesis pathway. Chief amongst these transcription factors are sterol regulatory element binding proteins (SREBPs), which bind to sterol regulatory elements (SREs) in promoter regions of target genes. To examine whether the effects of acidosis on FAS gene activation were mediated by the SBREP-binding sites present in the proximal FAS promoter, MCF-7 cells were transiently transfected with a truncated construct in which the region responsible for SREBP binding is deleted. Interestingly, the up-regulatory effect of extracellular acidosis on the transcriptional activation of FAS gene was not observed when the FAS promoter was truncated at the SREBP-binding site (Fig. 1a).

Finally, well-characterized oncogenic stimuli should mimic the up-regulatory effects of microenvironmental stresses on FAS gene expression. Likewise, a striking picture emerged when the above experiments were carried out in MCF-7 cells engineered to overexpress the Her-2/neu (erbB-2) oncogene, a master player in the etiology and aggressive behavior of several human malignancies molecularly related to FAS [Kumar-Sinha et al., 2003; Menendez et al., 2004]. Upon standard conditions, MCF-7/Her2-18 cells exhibited a SREBP-dependent FAS gene activation that was up to three-fold higher than that found in wild-type MCF-7 cells. Interestingly, extracellular acidosis resulted only in a marginal increase of Her-2/neu-promoted hyperactivation of FAS gene (Fig. 1b).

These findings, altogether, reveal for the first time that extracellular acidosis, but not hypoxia, can work in an epigenetic fashion by up-regulating the expression of the cancer-associated FAS gene, a stimulatory effect that is equally mimicked by well-characterized oncogenes such as Her-2/neu. Therefore, it is reasonable to suggest that FAS overexpression is a very early and necessary molecular event of the malignant cell phenotype, thus supporting the role of FAS as a novel "metabolic oncogene" in cancer cells.

ACKNOWLEDGMENTS

The authors thank Johannes Swinnen (Catholic University of Leuven, Leuven, Belgium) for the FAS promoter–reporter constructs and Mien-Chie Hung (The University of Texas MD Anderson Cancer Center, Houston, TX) for the MCF-7/Her-2 (clone 18) cells. This work was supported by The Auxiliary of Evanston Northwestern Healthcare. Javier A. Menendez is the recipient of a Translational Research Pilot Project (PP2) from the Specialized Program of Research Excellence (SPORE) in Breast Cancer (Robert H. Lurie Comprehensive Cancer Center, Chicago, IL), a Basic, Clinical and Translational Award (BRCTR0403141) from the Susan G. Komen Breast Cancer Foundation, and a

Breast Cancer Concept Award (BC033538) from the Department of Defense (DOD).

REFERENCES

Baron A, Migita T, Tang D, Loda M. 2004. Fatty Acid Synthase: A metabolic oncogene in prostate cancer? J Cell Biochem 91:47–53.

Hochachka PW, Rupert JL, Goldenberg L, Gleave M, Kozlowski P. 2002. Going malignant: The hypoxia-cancer connection in the prostate. Bioessays 24:749–757.

Kuhajda FP. 2000. Fatty-acid synthase and human cancer: New perspectives on its role in tumor biology. Nutrition 16:202–208.

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

Kumar-Sinha C, Ignatoski KW, Lippman ME, Ethier SP, Chinnaiyan AM. 2003. Transcriptome analysis of HER2 reveals a molecular connection to fatty acid synthesis. Cancer Res 63:132–139.

Medes G, Thomas A, Weinhouse S. 1953. Metabolism of neoplastic tissue. IV. A study of lipid synthesis in neoplastic tissue slices in vitro. Cancer Res 13: 27–29.

Menendez JA, Vellon L, Mehmi I, Oza BP, Ropero S, Colomer R, Lupu R. 2004. Inhibition of fatty acid synthase (FAS) suppresses HER2/neu (erbB-2) oncogene overexpression in cancer cells. Proc Natl Acad Sci USA 101:10715–10720.

Ookhtens M, Kannan R, Lyon I, Baker N. 1984. Liver and adipose tissue contributions to newly formed fatty acids in an ascites tumor. Am J Physiol 247:R146–R153.

Swinnen JV, Ulrix W, Heyns W, Verhoeven G. 1997. Coordinate regulation of lipogenic gene expression by androgens: Evidence for a cascade mechanism involving sterol regulatory element binding proteins. Proc Natl Acad Sci USA 94:12975–12980.