

Received: 2004.08.04
Accepted: 2004.09.21
Published: 2004.12.15

Fatty acid synthase-catalyzed *de novo* fatty acid biosynthesis: from anabolic-energy-storage pathway in normal tissues to jack-of-all-trades in cancer cells

Javier A. Menendez^{1, 2} and Ruth Lupu^{1, 2}

¹ Department of Medicine, Evanston Northwestern Healthcare Research Institute, Evanston, IL, USA

² Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Source of support: 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, USA), of a Basic, Clinical and Translational Award (BRCTR0403141) from the Susan G. Komen Breast Cancer Foundation (USA), and of a Breast Cancer Concept Award (BC033538) from the Department of Defense (DOD, USA).

Summary

In 1994, Kuhajda and colleagues unambiguously identified the oncogenic antigen-519, a prognostic molecule found in breast cancer patients with markedly worsened prognosis, as fatty acid synthase (FAS), the key enzyme for the *de novo* fatty acid biosynthesis. It now appears that human carcinomas and their pre-neoplastic lesions constitutively over-express FAS and undergo significant endogenous fatty acid biosynthesis. Moreover, FAS blockade specifically induces apoptotic cancer cell death and prolongs survival of cancer xenograft hosts. Therefore, FAS signaling seems to play a central role in the maintenance of the malignant phenotype by enhancing cancer cell survival and proliferation. This review documents the rapidly changing perspectives on the function of FAS in cancer biology. First, we describe molecular mechanism by which aberrant transduction cascades driven by oncogenic changes subvert the down-regulatory effects of dietary fatty acids, resulting in tumor-associated FAS insensitivity to nutritional signals. Second, we speculate on the putative function that hypoxia can play as the epigenetic factor that triggers and maintains FAS overexpression in cancer cells by inducing changes in gene expression and in metabolism for survival. Third, we explore the role that FAS exhibits in cancer evolution by specifically regulating cancer-related proteins such as Her-2/*neu* oncogene and estrogen receptor. Finally, we reveal previously unrecognized functions of FAS on the response of cancer cells to chemo-, endocrine-, and immuno-therapies. These findings, all together, should ultimately enhance our understanding of how FAS-dependent endogenous fatty acid metabolism, once considered a minor anabolic-energy-storage pathway in normal cells, has become a jack-of-all-trades in cancer cells.

Key words: fatty acid synthase • fatty acids • oncogene • cancer • metabolism

Full-text PDF: http://www.aite-online/pdf/vol_52/no_6/6563.pdf

Author's address: Ruth Lupu, Ph.D., Evanston Northwestern Healthcare Research Institute, 1001 University Pl., Evanston, IL 60201, USA, tel: +1 847 570-7671, fax: +1 847 570-8022, e-mail: r-lupu@northwestern.edu

BRIEF OVERVIEW OF THE FAS AND FAS-DEPENDENT FATTY ACID SYNTHETIC PATHWAY

Fatty acid synthase (FAS) is a complex multifunctional enzyme that contains 7 catalytic domains and a 4'-phosphopantetheine prosthetic group on a single 260 kDa polypeptide that plays a central role in endogenous lipogenesis in mammals. Palmitate, a saturated 16-carbon fatty acid, is the predominant product of FAS synthesized *de novo* from the substrates acetyl-CoA, malonyl-CoA, and NADPH. The FAS enzymatic complex is situated as a head-to-tail dimer with the ketoacyl synthase and malonyl/acetyl transferase domains of one monomer working together with the dehydratase, enoyl reductase, ketoacyl reductase, acyl carrier protein, and thioesterase domains on the adjacent monomer (Fig. 1). These enzymatic domains act sequentially to condense acetyl-CoA with malonyl-CoA to form a 4-carbon intermediate. Six additional turns of the enzyme's cycle convert this intermediate to palmitate, which is then liberated from FAS by the action of the thioesterase domain^{18, 61,72, 83, 100}.

In highly lipogenic tissues such as liver, lactating breast, and adipose tissue, the FAS-dependent fatty acid synthetic pathway has 3 primary functions: a) storage of excess energy intake, such as fat, b) synthesis of fat from carbohydrate or protein when the diet is low

in fat, or c) synthesis of fat for lactation. In well-nourished adults, FAS is responsible primarily for energy storage by converting excess carbohydrate to fatty acids that are then esterified to store triacylglycerols. It normally functions in the liver to make lipids for export to metabolically active tissues of storage in adipose tissue^{85, 107, 112}. FAS has specialized physiological functions, including the production of milk lipids in lactating breast tissue and surfactant in infant lung, but it is minimally expressed in most normal adult tissues^{99, 111, 114}. Indeed, most normal tissues, even those with high cellular turnover, seem to utilize circulating lipids for the synthesis of new structural lipids¹¹¹. However, 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 biosynthesis⁴⁹. Importantly, tumor-associated FAS overexpression and hyperactivity seem to be independent of the regulatory signals that down-regulate fatty acid synthesis in normal cells.

TUMOR-ASSOCIATED FAS (ONCOGENIC ANTIGEN-519) IS UNRESPONSIVE TO NUTRITIONAL REGULATION

Under normal physiological conditions any increase in FAS expression in human tissues is tightly regulated by a number of environmental, hormonal, and nutritional signals. Although FAS activity is not

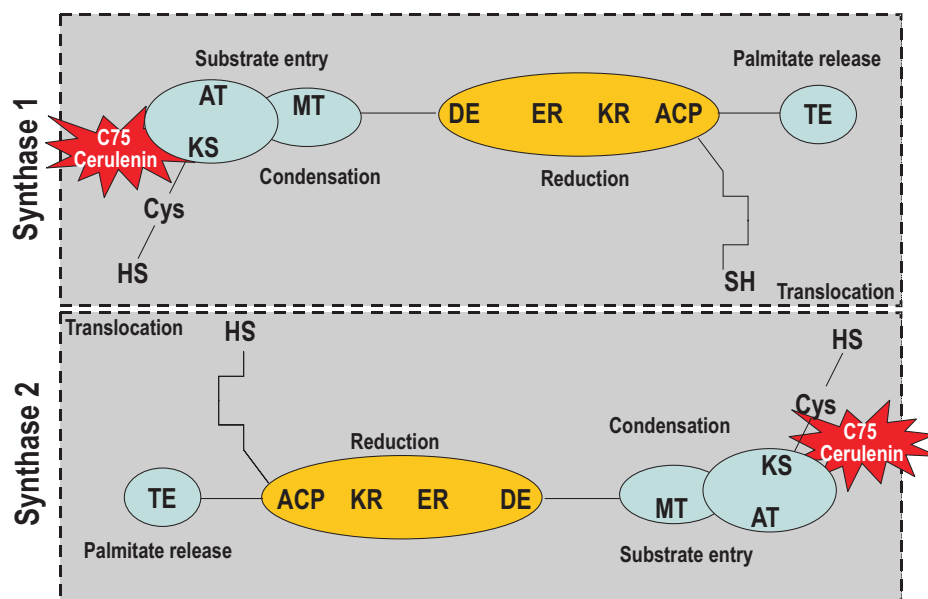


Figure 1. Schematic representation of FAS enzymatic complex and target sites of chemical FAS blockers cerulenin and C75. FAS enzymatic complex contains seven separate enzymatic pockets situated as a head-to-tail dimer with the β -ketoacyl synthase (KS) and malonyl (MT)/acetyl transferase (AT) domains of one monomer working together with the dehydratase (DE), enoyl reductase (ER), β -ketoacyl reductase (KR), acyl carrier protein (ACP), and thioesterase (TE) domains on the adjacent monomer. These enzymatic domains act sequentially to condense acetyl-CoA with malonyl-CoA to form a four-carbon intermediate. Six additional turns of the enzyme's cycle convert this intermediate to palmitate, which is then liberated from FAS by the action of the thioesterase domain. Because FAS functions as head-to-tail dimer, targeted inhibition of one of the enzymatic domains of FAS can ablate the activity of one or both FAS subunits. Cerulenin, a natural mycotoxin, is an antagonist of the ketoacyl synthase domain (the condensing enzyme) of FAS and functions by covalently modifying the active site cysteine, resulting in dead-end inhibition. C75, a synthetic analog of cerulenin, also targets the condensing enzyme and inhibits fatty acid synthesis.

known to be regulated by allosteric effectors or covalent modification, the expression of the FAS gene, as for many other lipogenic and glycolytic genes involved in maintenance of energy balance, is highly dependent on nutritional conditions in lipogenic tissues, liver, and adipose tissue⁹². Indeed, endogenous fatty acid biosynthesis in liver and adipose tissue is suppressed by the presence of small amounts of fatty acids in the diet^{20, 21, 29, 43}. In other words, *de novo* fatty acid biosynthesis and FAS expression occur constitutively at very low levels, since the requirement for fatty acids is sufficiently met by dietary intake under normal conditions¹¹¹. Surprisingly, immunohistochemical studies have reported that FAS protein is overexpressed in the majority of human malignancies and their pre-neoplastic lesions, including cancer of the prostate, colorectum, ovary, bladder, esophagus, stomach, lung, oral tongue, endometrium, and breast. Increased FAS protein expression is also found in nephroblastoma, mesothelioma, melanoma, and soft tissue sarcomas, thus suggesting that FAS overexpression is one of the most common molecular changes in cancer cells^{3-5, 14, 15, 25, 30, 31, 38, 39, 48, 51, 52, 56, 70, 72, 75, 79, 84, 88, 95, 98, 104, 105}. Importantly, FAS hyperactivity and overexpression preferentially occur in the aggressive varieties of these cancers and serves as an independent prognostic indicator of adverse clinical outcome.

We recently examined whether FAS activity and expression in cancer cells occur independently of the dietary signals that down-regulate endogenous fatty acid synthesis in lipogenic tissues. We demonstrated that physiological concentrations of linolenic acid (18:2n-6) and arachidonic acid (20:4n-6), the most potent dietary fatty acids suppressing FAS expression in both cultured adipocytes and hepatocytes, failed to repress tumor-associated FAS activity and expression in cancer cells⁶⁶. Therefore, the mechanism of control of tumor-associated FAS by exogenously derived fatty acids apparently differs from the dietary fatty acid-mediated FAS suppression in lipogenic tissues. Indeed, our findings demonstrating that tumor-associated FAS is resistant to linoleic acid- and arachidonic acid-induced suppression raise the possibility that fundamental differences in the ability of the FAS gene to respond to normal dietary fatty acids' regulatory actions may account, at least in part, for the observed high levels of FAS in a biologically aggressive subset of human cancers.

FAS HYPERACTIVITY IS NECESSARY FOR CANCER CELL SURVIVAL

The importance of FAS activation in cancer cells is highlighted by the fact that the natural product cerulenin [(2R, 3S), 2-3-epoxy-4-oxo-7, 10-trans, trans-

-dodecadienamide], a specific noncompetitive inhibitor of the β -ketoacyl synthase activity of FAS^{19, 71, 73, 101}, is selectively cytotoxic for cancer cells *in vitro*^{50, 76, 77, 81}. *In vivo* treatment with cerulenin or a novel small-molecule inhibitor of FAS activity, C75, a α -methylene- γ -butyrolactone with comparable inhibitory effects on endogenous fatty acid biosynthesis^{53, 57, 80, 120}, results in significantly increased survival in human cancer xenografts^{80, 81}. Furthermore, silencing of the FAS gene by the potent and highly sequence-specific mechanism of RNA interference (RNAi) significantly inhibits cancer cell growth and ultimately results in induction of apoptosis²³.

The fact that specific blockade of FAS activity significantly attenuates the growth and proliferation of tumor cells strongly suggests that tumor-associated FAS hyperactivity is vital for the survival of human cancer cells and further confirms FAS as a target pathway for chemotherapy development. However, it was unresolved whether a metabolic adaptation of cancer cells to the ambient concentration of dietary fatty acids may be relevant to the goal of utilizing FAS inhibition as an interventional molecular target. We recently evaluated the protective effects of exogenous dietary fatty acids on FAS inhibition-induced tumor cytotoxicity⁶⁵. Exogenous addition of supraphysiological concentrations of ω -3 (α -linolenic acid, docosahexaenoic acid, and eicosapentaenoic acid) and ω -6 (linoleic acid and arachidonic acid) dietary polyunsaturated fatty acids was unable to reverse the cytotoxic effects following pharmacological blockade of FAS activity. On the other hand, addition of oleic acid (a monounsaturated ω -9 fatty acid synthesized from palmitate) significantly ameliorated tumor cytotoxicity induced by chemical FAS blockers. In previous reports, replenishment of media with palmitate (the primary end-product of FAS), also succeeded in "rescuing" cancer cells from the growth inhibitory effects of FAS blockers^{50, 82}. There are various implications for these data. The ability to partially reverse chemical FAS inhibitors' effects on tumor cell viability by oleic acid, a FAS-derived metabolic product, but not by other dietary fatty acids implicates a role for endogenous fatty acid metabolism in tumor cell proliferation. In fact, oleic acid-induced reduction of FAS blockade-induced cytotoxicity was significant for 72 h in cancer cells expressing moderate amounts of tumor-associated FAS, such as MCF-7 breast cancer cells, but only within 24 h in SK-Br3 breast cancer cells, which express enormous levels of tumor-associated FAS⁶⁵. Therefore it does appear that for unclear reasons some cancer cells have an apparently obligatory requirement for FAS-catalyzed endogenous fatty acid biosynthesis for their proliferation, despite the

presence of physiological or supraphysiological levels of exogenous fatty acids in their microenvironment. Importantly, that only supraphysiological amounts of free oleic acid were effective in modulating chemical FAS blockers-induced cytotoxicity *in vitro* implies that tumors should be sensitive to the inhibition of FAS regardless of access to circulating plasma lipids.

FAS ACTIVATION IN PRE-NEOPLASTIC LESIONS: THE HYPOXIA-CANCER CONNECTION

Although it has become clearer that cancer cell proliferation and survival are dependent on FAS-catalyzed endogenous fatty acid synthesis, and elevated levels of FAS associate with poorer prognosis in a subset of human carcinomas, one of the main questions that arise from the above studies is related to the ultimate molecular mechanisms underlying the early up-regulation of FAS in pre-malignant lesions of numerous malignancies. Another important question that remains to be addressed is whether activation of FAS in neoplastic cells is a mere manifestation of an early and common dysregulation of up-stream regulatory pathways or if it actively contributes to the cancer phenotype. All the current notions plead for an epigenetic basis of increased FAS expression in cancer cells and suggest that changes in up-stream regulatory circuits (hormones-hormone receptors, growth factors-growth factor receptors → lipogenic transcription factors → lipogenic genes) lie (at least in part) at the basis of this phenomenon. In this scenario, tumor-associated FAS appears to be part of a more general change in the genetic program controlling lipogenesis. A very attractive hypothesis is that the early activation of FAS in pre-malignant cells represents a necessary survival strategy which occurs to compensate for an insufficiency of both oxygen and dietary fatty acids due to, for example, lack of angiogenesis. Focal areas of low-oxygen tension ($\leq 2.0\% \text{ O}_2$) are inherent to biological processes such as carcinogenesis. Indeed, oxygen availability is known to play a key role in the growth-regulatory process underlying carcinogenesis¹³. Interestingly, cancer cells have an unusual tolerance to limiting O_2 availability, with an unusual carbohydrate metabolism displaying excessive lactate production by aerobic glycolysis¹¹⁰. In many hypoxia-tolerant organisms, the need for more oxidizing power when O_2 is limiting is satisfied by using the FAS pathway as a means for balancing redox through its ability to consume reducing equivalents (i.e. NADPH) as part of its normal function³³. Therefore it is reasonable to suggest that the physiological function of FAS activation in pre-malignant lesions, and probably also in advanced carcinomas, is to make up for the shortfall in oxidizing power. This extremely

attractive theory to explain FAS overexpression has recently been put forth by Baron et al.⁶ and Hochachka et al.³⁴ in prostate cancer cells. Under hypoxic conditions, excessive lactate is produced by anaerobic glycolysis, which, in turn, limits the respiratory chain and results in limited oxidizing power. In this metabolic situation, cancer cells favor synthesis and chain elongation of endogenous fatty acids because they supply oxidizing power for key oxidative steps. Therefore, FAS overexpression and hyperactivity would result in a significant improvement in redox balance despite surrounding hypoxic conditions.

Although it cannot be excluded that increased FAS expression is intrinsic for highly proliferative cells (including cancer cells), it is plausible to suggest further that constitutive FAS overexpression and hyperactivity occur not only for improving redox balance despite conditions of extreme hypoxia, but also to compensate for a concomitant insufficiency of dietary (exogenous) fatty acids. Thus, FAS expression may not be essential even in highly proliferative tumor cells in the presence of enough fatty acids. Interestingly, our group has shown that the cell death of breast cancer cells treated with pharmacological inhibitors of FAS activity is only rescued by adding supraphysiological amounts of dietary fatty acids, such as oleic acid, one of the most abundant circulating fatty acid⁶⁵. This very simple observation strongly implies that breast cancer cells are dependent on FAS hyperactivity for their proliferation and survival when an insufficient amount of exogenous fatty acids is present in their microenvironment. Altogether, the evidence supports the notion that the unusual metabolic situation in the hypoxic microenvironment of pre-malignant cancer stages may convert FAS, an anabolic-energy-storage pathway largely considered of minor importance in humans, into a “metabolic oncogene”. Moreover, it is reasonable to assume that only the tumor cells capable of developing a FAS-dependent tolerance to limiting oxygen and dietary fatty acid availability, which continue to occur as the malignancy advances, can survive. Indeed, the early and “obligatory acquisition” of an activated FAS-dependent signaling may explain why tumor cells bearing high levels of tumor-associated FAS overexpression exhibit a selective growth advantage.

WHY DOES TUMOR-ASSOCIATED FAS IGNORE DIETARY FATTY ACIDS?

The involvement of the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK1/2) and phosphatidylinositol-3'-kinase (PI-3'K)/protein kinase B (AKT) signaling cascades, two

pathways frequently contributing to tumor cell proliferation and virulence, in the overexpression of FAS in MCF10 normal breast epithelial cells transformed with the oncogene *H-ras*¹¹⁶ and LNCaP prostate cancer cells¹⁰² has been demonstrated. Recently, a transcriptome analysis of *Her-2/neu* (*erbB-2*) oncogene revealed a molecular connection to FAS in human breast cancer cells through a PI-3'K/AKT-dependent signaling⁵⁴. Similarly, the active involvement of *erbB-1* (EGFR) and its downstream effectors MAPK ERK1/2 and PI-3'K/AKT pathways in the overexpression of FAS has been demonstrated in prostate cancer cells⁹⁴. A remarkable aspect of these results is the fact that insulin-regulated stimulation of FAS expression in normal adipose tissue cells is also mediated by the PI-3'K pathway, with AKT being involved as a downstream effector¹⁰⁸. Moreover, tumor-associated FAS overexpression occurs through modulation of the transcription factor sterol regulatory element-binding protein (SREBP)-1c, the major factor involved in the regulation of FAS in liver and adipose tissue^{32, 45, 58, 59, 87, 96, 108, 117}.

The above evidence reveals that the signal transduction pathways regulating FAS expression in normal and cancer cells seem to share identical downstream elements. However, the upstream mechanisms that control FAS expression in cancer cells must be dif-

ferent from those in adipose or liver tissues, since tumor-associated FAS expression seems to be insensitive to nutritional signals. Therefore it is reasonable to suggest the following hypothetical model to explain the differential regulation of FAS in normal and cancer cells. In lipogenic cells such as hepatocytes and adipocytes, FAS-catalyzed *de novo* fatty acid biosynthesis is stimulated by a high carbohydrate diet, whereas it is inhibited by dietary fatty acids and by fasting⁴⁴. These effects are partially mediated by hormones, which stimulate (insulin) or inhibit (leptin) FAS-dependent lipogenesis. Nutritional and hormonal regulation of FAS expression in lipogenic cells occur through a modulation of the SREBP-1c, which is known to be regulated by MAPK ERK1/2 and PI-3'K/AKT signaling cascades^{28, 47} (Fig. 2, left panel). In cancer cells, SREBP-1c expression will be driven by a constitutive activation of MAPK and PI-3'K/AKT signaling pathways in response to oncogenic changes, including overexpression of growth factors (e.g. EGF, heregulin), and/or overexpression of growth factor receptors (e.g. *Her-2/neu*, EGFR), rather than physiological regulators (e.g. insulin, leptin). The constitutive activation of oncogenic cascades would spoil the ability of FAS gene to respond to a normal fatty acid's down-regulatory actions, thus resulting in the observed gigantic levels of FAS in cancer cells (Fig. 2, right panel).

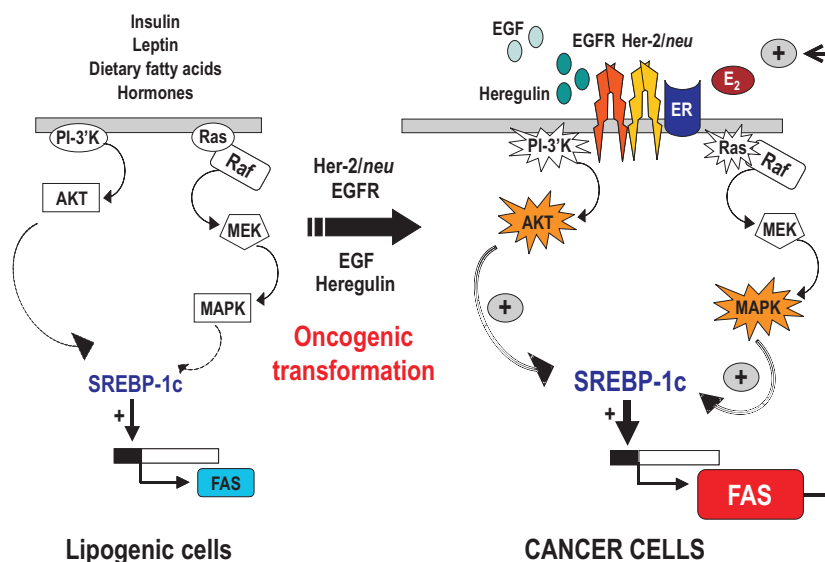


Figure 2. Hypothetical model for FAS hyperactivity and overexpression in cancer cells. **Left panel** – regulation of FAS expression in hepatocytes and adipocytes. FAS-dependent lipogenesis is stimulated by a high carbohydrate diet, whereas it is inhibited by exogenously derived (dietary) fatty acids and by fasting. These effects are partly mediated by hormones, which stimulate (insulin) or inhibit (leptin) FAS expression and/or activity through SREBP-1c, a critical intermediate in the pro- and anti-lipogenic action of several hormones and nutrients. **Right panel** – working model for FAS as a metabolic oncogene in cancer cells. FAS regulation in neoplastic cells seems to occur also through a modulation of SREBP-1c, which is driven by a constitutive hyperactivation of up-stream oncogenic cascades (EGFR, *Her-2/neu* → Ras/Raf/MEK/MAPK; PI-3'K/AKT) thus resulting in nutritional insensitivity of tumor-associated FAS activity and expression. In addition, the bidirectional nature of the cross-talk between tumor-associated FAS and cancer-regulating proteins such as *Her-2/neu* and ER strongly suggest that FAS is a novel metabolic oncogene that actively contributes to the enhancement and maintenance of cancer phenotype. Abbreviations: SREBP – sterol-regulatory element-binding protein, EGF – epidermal growth factor, EGFR – epidermal growth factor receptor, MEK – mitogen-activated or extracellular signal-regulated protein kinase, MAPK – mitogen activated protein kinase, PI-3'K – phosphatidylinositol-3'-OH-kinase, AKT – protein kinase B.

DOES TUMOR-ASSOCIATED FAS IGNORE ALL THE DIETARY FATTY ACIDS?

In the above theoretical model, normal nutritional signals from elevated levels of dietary fatty acids such as linoleic acid (18:2n-6) and/or arachidonic acid (20:4n-6), the most potent fatty acids suppressing FAS expression in cultured adipocytes and hepatocytes, should be incapable of repressing FAS activity and/or expression in cancer cells, as we previously demonstrated. Accordingly, supraphysiological concentrations of linoleic acid and arachidonic had no significant inhibitory effects on the activity and expression of tumor-associated FAS in SK-Br3 breast cancer cells, an experimental paradigm of FAS-overexpressing tumor cells in which FAS constitutes up to 28%, by weight, of the cytosolic proteins⁶⁶. However, a strong inhibitory effect was observed in the presence of γ -linolenic (18:3n-6) and α -linolenic (18:3n-3) fatty acids. Interestingly, it was recently noted that fatty acids such as γ -linolenic and α -linolenic fatty acids modulate SREBP-1c expression in rat hepatocytes, whereas palmitic (16:0) or oleic acid (18:1n-9) had no inhibitory effect²⁴. γ -Linolenic- and α -linolenic-induced inhibition of SREBP-1c expression may contribute to their ability to suppress FAS expression in cancer cells, although the specific mechanisms by which these fatty acids can molecularly target tumor associated-FAS certainly merit further investigation. Interestingly, γ -linolenic and α -linolenic fatty acids have raised interest as novel anti-cancer treatments as they demonstrate selective toxic actions on malignant cells^{9-11, 35-37, 40}, thereby introducing the concept that a higher level of γ -linolenic and/or α -linolenic fatty acids in cancer tissues could provide an effective means of influencing the outcome of FAS-overexpressing carcinomas, a subset of carcinomas with poor prognosis. Interestingly, there is evidence that a decreased level of α -linolenic acid (an essential ω -3 polyunsaturated fatty acid present in green vegetables and in soy and linseed oils) in adipose tissue of breast cancer patients associates with breast cancer risk and subsequent development of visceral metastases^{12, 46, 60}. Although the ultimate mechanisms by which specific dietary fatty acids may modulate tumor development are unclear, it will be of interest to evaluate whether the balance between specific exogenously derived fatty acids and FAS-catalyzed endogenous fatty acid biosyntheses coordinately regulates the etiology and progression of human malignancies. Moreover, the fact that the main end-products of FAS palmitic and oleic fatty acids are also the most abundant circulating fatty acids, along with their incapability to modulate SREBP-1c²⁴, strongly suggests that fundamental differences in the ability of lipogenic genes (acetyl-CoA

carboxylase, FAS, etc.) to respond to normal fatty acids' down-regulatory actions could synergistically interact with oncogenic signals to constitutively maintain an elevated FAS-dependent *de novo* fatty acid biogenesis in cancer cells.

TUMOR-ASSOCIATED FAS: A NEW METABOLIC ONCOGENE

The early and nearly universal up-regulation of FAS in many human cancers and its association with poor clinical outcome both strengthen the hypothesis that FAS is involved in the development, maintenance, and enhancement of the malignant phenotype. However, FAS overexpression in tumor cells appears to be part of a more general change in the genetic program controlling lipogenesis, as evidenced by the concomitant increase of other enzymes of the same lipogenic pathway^{70, 96, 97}. Interestingly, our more recent data are compatible with the hypothesis that tumor-associated FAS is not only necessary to integrate a number of signaling pathways that regulate metabolism, proliferation, and survival in cancer cells, but plays a further active role in cancer evolution by regulating oncogenic proteins closely related to malignant transformation.

Tumor-associated FAS regulates Her-2/neu (erbB-2) oncogene expression in cancer cells

We recently identified a molecular link between FAS and Her-2/neu (erbB-2) oncogene, a marker for poor prognosis that is overexpressed in 30% of breast and ovarian cancers^{86, 89, 90}. First, we provided evidence that a high level of both FAS expression and activity positively correlates with Her-2/neu oncogene amplification and/or overexpression in a wide panel of human breast cancer cell lines⁶⁵. Since Her-2/neu mediates tumorigenic activities by regulating key genes, our results suggested that FAS may be a novel downstream effector of Her-2/neu-promoted tumorigenicity and cancer progression. Accordingly, a recent transcriptome analysis of Her-2/neu revealed that FAS was one of the genes differentially regulated by Her-2/neu in human breast epithelial cells⁵⁴. Lately, the pharmacological FAS inhibitors cerulenin and C75 were found to suppress Her-2/neu oncoprotein expression in breast and ovarian Her-2/neu overexpressors⁶⁷. Similarly, Her-2/neu expression was dramatically down-regulated when FAS gene expression was silenced by using the highly sequence-specific mechanism of RNAi⁶⁷. We identified that pharmacological and RNAi-mediated inhibition of FAS represses Her-2/neu expression at the transcriptional level, concomitantly up-regulating the expression of PEA3, an *Ets* factor that specifically reverses the *in vitro* transformed phenotype of Her-2/neu-overexpressing cancer cells

through the attenuation of the *Her-2/neu* oncogene promoter activity^{109, 113}. Therefore, FAS blockade indirectly suppresses *Her-2/neu* overexpression through the ability of PEA3 to repress *Her-2/neu* promoter activity. These results demonstrate that tumor cell response to metabolic stress after perturbation of FAS activity is accompanied by the specific suppression of *Her-2/neu* oncogene, one of the most important oncogenes in human cancer. Although these findings may solely delineate a previously undescribed mechanism of action for chemical FAS inhibitors in cancer cells, the specific down-regulation of *Her-2/neu* that follows RNAi-mediated silencing of the FAS gene further reveals that *Her-2/neu* could act as a molecular sensor of energy imbalance that participates actively in the maintenance of an abnormally elevated endogenous fatty acid metabolism in cancer cells (Fig. 3). Moreover, it is reasonable to suggest that a molecular bi-directional cross-talk between the FAS and *Her-2/neu* signalings at the levels of transcription, translation, and activity is taking place in cancer cells.

Tumor-associated FAS regulates estrogen receptor expression and activity in cancer cells

Although FAS is minimally expressed in most other normal human tissues because they appear to utilize preferentially circulating lipids for the synthesis of

new structural lipids, under normal conditions, FAS is highly expressed in hormone-sensitive cells⁵⁵. During the menstrual cycle, the expression of FAS in the human endometrium is closely linked to the expression of the proliferation antigen Ki-67, estrogen receptor (ER), and progesterone receptor (PR), suggesting a connection between FAS and the ER-dependent signaling in endometrial cell proliferation. Thus, FAS expression increases in endometrial glands and stromal cells from the proliferative to the early secretory phase, and after cessation of cell proliferation in the mid- to the late-secretory phase, the endometrial tissues become FAS negative^{26, 78}. Interestingly, it has been shown that estradiol (E₂), progesterone, and androgens have a role in FAS regulation in hormonally responsive tumors. Thus, FAS expression is part of the E₂-driven cellular response that leads to proliferation in hormone-dependent endometrial carcinoma cells and is associated with higher endometrial tumor grades⁷⁹. E₂, progesterone, and synthetic progestins also stimulate cell growth and concomitant FAS expression in hormone-dependent human breast cancer cells such as MCF-7 and T47-D^{17, 41, 42}. In the androgen-dependent prostate cancer cell line LNCaP, androgens have been shown to stimulate coordinate expression of FAS and enzymes involved in cholesterol synthesis^{32, 93}. Moreover, the recent identification of a novel FAS-ER

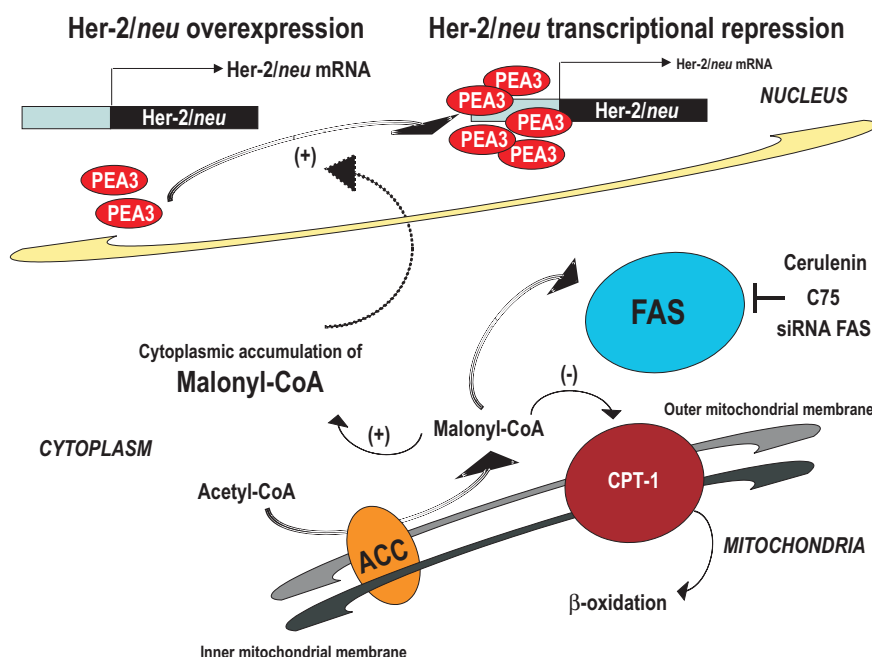


Figure 3. Blockade of tumor-associated FAS transcriptionally represses *Her-2/neu* oncogene expression in cancer cells. FAS produces the 16-carbon fatty acid palmitate through successive NADPH-dependent condensations of acetyl-CoA with malonyl-CoA. As a result of FAS blockade, high levels of malonyl-CoA continue to be generated by acetyl-CoA carboxylase (ACC), the rate-limiting enzyme of the fatty acid synthesis pathway. Concomitantly, FAS inhibition up-regulates the expression of polyomavirus enhancer activator 3 (PEA3), an erythroblast transformation specific (*Ets*) transcription factor that attenuates the promoter activity of the *Her-2/neu* oncogene. It is reasonable to suggest that malonyl-CoA accumulation is the molecular trigger linking FAS blockade and transcriptional repression of *Her-2/neu* oncogene through the ability of PEA3 to repress the promoter activity of *Her-2/neu* gene. (CPT-I – carnitine palmitoyltransferase I).

fusion transcript expressed in a variety of human cancer cell lines including prostate, breast, cervical, and bladder cancer cells further suggests a close linkage between FAS and the ER signaling pathway¹¹⁸. We recently examined the effects of FAS inhibition on E₂-stimulated ER-driven molecular responses in Ishikawa cells, an *in vitro* model of well-differentiated human endometrial carcinoma⁶⁹. First, we evaluated the effects of FAS inhibition on E₂-induced ER transcriptional activity by using transient cotransfection assays with an estrogen-response element reporter construct (ERE-Luciferase). Anti-estrogenic effects of the chemical FAS blockers cerulenin and C75 were observed by dose-dependent inhibition of E₂-stimulated ERE-dependent transcription, whereas FAS inhibitors did not significantly increase the levels of ERE transcriptional activity in the absence of E₂. To address the reliability of transient transfection assays, the effects of FAS inhibitors on E₂-inducible gene products were evaluated. FAS blockade induced a dose-dependent decrease in both E₂-inducible alkaline phosphatase activity and E₂-stimulated accumulation of PR. FAS inhibition also resulted in a marked down-regulation of E₂-stimulated ER expression and noticeably impaired E₂-induced ER nuclear accumulation. To rule out non-FAS cerulenin- and C75-related effects, we finally monitored ER signaling after silencing of FAS gene expression using the highly sequence-specific mechanism of RNAi. Interestingly, the concentrations of E₂ inducing half-maximal ERE activity (EC₅₀) dramatically increased (>100 times) in FAS RNAi-transfected Ishikawa cells. Moreover, depletion of FAS by RNAi also caused loss of ER expression and down-regulation of PR in E₂-stimulated Ishikawa cells⁶⁹.

Remarkably, our current studies provide evidence that tumor-associated FAS actively regulates genomic and non-genomic ER activities in breast cancer cells⁶⁸. Thus, pharmacological inhibition of breast cancer-associated FAS activity induced a dramatic augmentation of E₂-stimulated ER-driven transcriptional activity (ER genomic activity) and synergistically enhanced E₂-mediated down-regulation of ER protein and mRNA expression. Mechanistically, FAS inhibition was found to enhance ER genomic activity through a non-genomic cross-talk between ER and the downstream MAPK ERK1/2. Accordingly, co-treatment with the pure anti-estrogen ICI 182,780 and the MAPK inhibitor U0126 completely abolished the synergistic action of FAS inhibition on E₂-stimulated ER transcriptional activity. Of note, FAS inhibition drastically inactivated the anti-apoptotic protein kinase AKT, and completely abrogated E₂-dependent anchorage-independent growth of ER-positive breast cancer cells, which is mediated by

a non-genomic ER-AKT cross-talk. Thus our working model postulates that the ability of tumor-associated FAS to differentially regulate genomic and non-genomic ER activities determines why its blockade induces hypersensitivity to E₂-stimulated ER transcriptional activity but does not enhance E₂-dependent breast cancer cell growth-promoting actions.

By definition, selective estrogen receptor modulators (SERMs) such as tamoxifen exert agonist action in some target tissues while acting as estrogen antagonists in others²⁷. Previously we demonstrated that FAS blockade works as a potent antagonist of E₂-dependent agonist transactivation of ER in human endometrial adenocarcinoma cells⁶⁹. Although early, these findings together not only reveal a new molecular mechanism through which chemical FAS inhibitors may exert their antitumor activities in cancer cells by acting as SERMs, but further support the notion that a complex molecular connection between tumor-associated FAS hyperactivity and ER-dependent signaling is taking place in hormone-responsive cancer cells.

Tumor-associated FAS regulates cancer sensitivity to chemotherapeutic agents

Although the tumoricidal activity of pharmacological inhibitors of FAS activity has begun to emerge, the relationship between tumor-associated FAS hyperactivity and the efficacy of chemotherapy has not been studied. Interestingly, pharmacological inhibition of FAS preferentially was recently found to preferentially induce apoptosis of breast epithelial cells engineered to overexpress Her-2/*neu* relative to matched vector control cells⁵⁴. This result strongly suggested that an active FAS signaling may be necessary in the maintenance and/or enhancement of Her-2/*neu*-promoted cell proliferation and survival. On the basis of these findings, and considering that Her-2/*neu* overexpression confers resistance to certain chemotherapeutic regimens^{1, 119}, we recently hypothesized that pharmacological inhibition of FAS activity in cancer cells might prove useful in combination with conventional anticancer therapy. Likewise, we found that pharmacological blockade of FAS activity synergistically sensitizes Her-2/*neu*-overexpressing breast cancer cells to docetaxel- and paclitaxel-induced cytotoxicity and apoptotic cell death, two cytotoxic drugs belonging to the taxanes family of anti-microtubule agents^{62, 64}. Similarly, FAS blockade synergistically enhanced the cytotoxic and apoptotic activity of vinorelbine, a member of the vinca alkaloids family of anti-mitotics⁶³. It could be argue that this improved sensitization must be attributed, at least in part, to the ability of chemical FAS blockers to reduce Her-

-2/*neu* expression, and therefore confined to Her-2/*neu*-related chemoresistance breast cancer phenotypes. Interestingly, we previously described that anthracycline (doxorubicin) resistance on multidrug P-glycoprotein-overexpressing (MDR1)-MCF-7/AdrR cells is synergistically reversed in the presence of chemical FAS blockers¹⁰³. Although additional studies are needed to determine whether the sensitization to chemotherapy that follows exposure to chemical FAS blockers is exclusively dependent on FAS activity and/or on Her-2/*neu* depletion and whether FAS overexpression predicts chemotherapy efficacy in cancer patients, to the best of our knowledge these are the first studies demonstrating that tumor-associated FAS is playing an active role regulating cancer chemosensitivity. If chemically stable FAS inhibitors or cell-selective vector systems able to deliver RNAi targeting FAS gene demonstrate systemic anticancer effects *in vivo*, our results render FAS as a novel molecular approach to enhance the cytotoxic effects of existing chemotherapeutic agents toward human tumors.

Tumor-associated FAS regulates cancer sensitivity to novel therapeutic strategies: monoclonal antibodies

Trastuzumab (Herceptin®) is a recombinant anti-Her-2/*neu* monoclonal antibody directed at the p185^{Her-2/*neu*} ectodomain that is active against tumor cells that overexpress Her-2/*neu* oncogene^{8, 7, 16, 106, 115}. However, not all Her-2/*neu*-overexpressing cancer cells respond to treatment with trastuzumab. Moreover, its clinical benefit is limited by the fact that most breast cancers become resistant to trastuzumab in less than 12 months^{2, 22, 91}. We hypothesized that cancer-associated FAS hyperactivity may play a role determining the efficacy of trastuzumab against Her-2/*neu*-overexpressors. For this purpose we first analyzed the cytotoxic interactions between chemical FAS blockers and trastuzumab in a panel of cancer cell lines expressing high, moderate, and low levels of Her-2/*neu* oncogene⁶⁷. Remarkably, pharmacological blockade of FAS activity dramatically enhanced the efficacy of trastuzumab in a dose-dependent manner. The most significant changes were seen in Her-2/*neu*- and FAS-overexpressing SK-Br3 breast cancer cells, in which co-exposure to FAS blocker cerulenin sensitized cancer cells to trastuzumab by 200-fold. Similarly, concurrent RNAi-induced attenuation of FAS and Her-2/*neu* genes expression was found to synergistically induce apoptotic cell death in SK-Br3 breast cancer cells⁶⁷. Taken

together, these results demonstrate that a synergistic augmentation of the trastuzumab efficacy following blockade of FAS activity occurs in Her-2/*neu*-overexpressing cancer cells through the ability of tumor-associated FAS to specifically regulate Her-2/*neu* expression.

CLINICAL PERSPECTIVES

Increased expression of FAS gene and of FAS catalytic activity, likely along with genes for other enzyme components in fatty acid biosyntheses, may play a central role in neoplastic transformation through their ability to compensate for an insufficiency of both oxygen and dietary fatty acids due to lack of angiogenesis in the early stages of cancer development. Thereafter, this epigenetically programmed FAS up-regulation is maintained in coordination with increased demand for fatty acid metabolism and/or membrane synthesis in response to cancer-related overexpression of growth factors and/or mitogens (e.g. EGF, heregulin, estradiol) and/or growth factor receptors and/or hormone receptors (e.g. EGFR, Her-2/*neu*, ER). The aberrant MAPK and PI-3'K/AKT transduction cascades driven by these oncogenic changes, through a constitutive activation of SBREP-1c, may subvert the down-regulatory effects of physiological concentrations of dietary fatty acids, thus resulting in a cancer-associated FAS insensitivity to nutritional signals. This working model provides a mechanistic rationale for future research and treatment approaches in several malignancies. Antibodies and/or small-molecular inhibitors directed against growth factor receptors EGFR (*erbB-1*) and Her-2/*neu* (*erbB-2*) or small-molecule blockers of MAPK and/or PI-3'K/AKT activities may abrogate the expression of tumor-associated FAS, a molecular marker closely linked to malignant transformation and to tumor virulence in population studies of human cancer. On the other hand, our more recent results support the notion that tumor-associated FAS is not only a mere manifestation of early and common cancer-associated epigenetic changes, but actively contributes further to the cancer phenotype by specifically regulating malignant transformation. The unexpected bidirectional nature of the cross-talk between FAS and cancer-regulating proteins such as Her-2/*neu* and ER (Fig. 2, right panel) should ultimately highlight a previously unrecognized role of endogenous fatty acid metabolism in tumor biology and may provide new diagnostic and therapeutic moieties for patient care.

REFERENCES

- Alaoui-Jamali M. A., Paterson J., Al Moustafa A. E. and Yen L. (1997): The role of ErbB-2 tyrosine kinase receptor in cellular intrinsic chemoresistance: mechanisms and implications. *Biochem. Cell Biol.*, 75, 315–325.
- Albanell J. and Baselga J. (2001): Unrevealing resistance to trastuzumab (Herceptin): insulin-like factor-1 receptor, a new suspect. *J. Natl. Cancer Inst.*, 93, 1830–1832.
- Alo P. L., Visca P., Botti C., Galati G. M., Sebastiani V., Andreano T., Di Tondo U. and Pizer E. S. (2001): Immunohistochemical expression of human erythrocyte glucose transporter and fatty acid synthase in infiltrating breast carcinomas and adjacent typical/atypical hyperplastic or normal breast tissue. *Am. J. Clin. Pathol.*, 116, 129–134.
- Alo P. L., Visca P., Marci A., Mangoni A., Botti C. and Di Tondo U. (1996): Expression of fatty acid synthase (FAS) as a predictor of recurrence in stage I breast carcinoma patients. *Cancer*, 77, 474–482.
- Alo P. L., Visca P., Trombetta G., Mangoni A., Lenti L., Monaco S., Botti C., Serpieri D. E. and Di Tondo U. (1999): Fatty acid synthase (FAS) predictive strength in poorly differentiated early breast carcinomas. *Tumori*, 85, 35–40.
- Baron A., Migita T., Tang D. and Loda M. (2004): Fatty acid synthase: a metabolic oncogene in prostate cancer? *J. Cell. Biochem.*, 91, 47–53.
- Baselga J. (2001): Clinical trials of Herceptin^(R) (trastuzumab). *Eur. J. Cancer*, 37, 18–24.
- Baselga J., Norton L., Albanell J., Kim Y. M. and Mendelshon J. (1998): Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2-neu overexpressing human breast cancer xenografts. *Cancer Res.*, 58, 2825–2831.
- Begin M. E., Das U. N. and Ells G. (1986): Cyto-toxic effects of essential fatty acids (EFA) in mixed cultures of normal and malignant human cells. *Prog. Lipid Res.*, 25, 573–576.
- Begin M. E., Das U. N. and Horrobin D. F. (1986): Differential killing of human carcinoma cells supplemented with n-3 and n-6 polyunsaturated fatty acids. *J. Natl. Cancer Inst.*, 77, 1053–1062.
- Begin M. E., Ells G. and Horrobin D. F. (1988): Polyunsaturated fatty acid-induced cytotoxicity against tumor cells and its relationship to lipid peroxidation. *J. Natl. Cancer Inst.*, 80, 188–194.
- Bougnoux P., Koscielny S., Chajes V., Descamps P., Couet C. and Calais G. (1994): α -Linolenic acid content of adipose breast tissue: a host determinant of the risk of early metastasis in breast cancer. *Br. J. Cancer*, 70, 330–334.
- Brown J. M. and Giaccia A. J. (1998): The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res.*, 58, 1408–1416.
- Bull J. H., Ellison G., Patel A., Muir G., Walker M., Underwood M., Khan F. and Paskins L. (2001): Identification of potential diagnostic markers of prostate cancer and prostatic intraepithelial neoplasia using cDNA microarray. *Br. J. Cancer*, 84, 1512–1519.
- Camassei F. D., Jenkner A., Rava L., Bosman C., Francalanci P., Donfrancesco A., Alo P. L. and Boldrini R. (2003): Expression of the lipogenic enzyme fatty acid synthase (FAS) as a predictor of poor outcome in nephroblastoma: an interinstitutional study. *Med. Pediatr. Oncol.*, 40, 302–308.
- Carter P., Presta L., Gorman C. M., Ridgway J. B., Henner D., Wong W. L., Rowland A. M., Kotts C., Carver M. E. and Shepard H. M. (1992): Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc. Natl. Acad. Sci. USA*, 89, 4285–4289.
- Chalbos D., Joyeux C., Galtier F. and Rochefort H. (1992): Progesterin-induced fatty acid synthetase in human mammary tumors: from molecular to clinical studies. *Steroid Biochem. Mol. Biol.*, 43, 223–228.
- Chirala S. S., Jayakumar A., Gu Z. W. and Wakil S. (2001): Human fatty acid synthase: role of interdomain in the formation of catalytically active synthase dimer. *Proc. Natl. Acad. Sci. USA*, 98, 3104–3108.
- Christie W. W., Hunter M. L. and Clegg R. A. (1981): The effects of cerulenin on lipid metabolism *in vitro* in cellular preparation from the rat. *Biochim. Biophys. Acta*, 666, 284–290.
- Clarke B. A., Armstrong M. K. and Jump D. B. (1990): Nutritional control of rat liver fatty acid synthase and S14 mRNA abundance. *J. Nutr.*, 120, 218–224.
- Clarke S. D. (1993): Regulation of fatty acid synthase gene expression: an approach for reducing fat accumulation. *J. Anim. Sci.*, 71, 1957–1965.
- Cobleigh M. A., Vogel C. L., Tripathy D., Robert N. J., Scholl S., Fehrenbacher L., Wolter J. M., Paton V., Shak S., Lieberman G. and Slamon D. J. (1999): Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER-2 overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.*, 17, 2639–2648.
- De Schrijver E., Brusselmans K., Heyns W., Verhoeven G. and Swinnen J. V. (2003): RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. *Cancer Res.*, 63, 3799–3804.
- Deng X., Cagen L. M., Wilcox H. G., Park E. A., Raghow R. and Elam M. B. (2002): Regulation of the rat SREBP-1c promoter in primary rat hepatocytes. *Biochem. Biophys. Res. Commun.*, 290, 256–262.
- Epstein J. I., Carmichael A. and Partin A. W. (1995): OA-519 (fatty acid synthase) as an independent predictor of pathologic stage in adenocarcinoma of the prostate. *Urology*, 45, 81–86.
- Escot C., Joyeux C., Mathieu M., Maudelonde T., Pages A., Rochefort H. and Chalbos D. (1990): Regulation of fatty acid synthetase ribonucleic acid in the human endometrium during the menstrual cycle. *J. Clin. Endocrinol. Metab.*, 70, 1319–1324.
- Favoni R. E. and de Cupis A. (1998): Steroidal and nonsteroidal estrogen antagonists in breast cancer: basic and clinical appraisal. *Trends Pharmacol. Sci.*, 19, 406–415.
- Fleischmann M. and Iynedjian P. B. (2000): Regulation of sterol regulatory-element binding protein 1 gene expression in liver: role of insulin and protein kinase B/cAkt. *Biochem. J.*, 349, 13–17.
- Fukuda H., Iritani N., Sugimoto T. and Ikeda H. (1999): Transcriptional regulation of fatty acid synthase by insulin/glucose, polyunsaturated fatty acids and leptin in hepatocytes and adipocytes in normal and genetically obese rats. *Eur. J. Biochem.*, 260, 505–511.
- Gabrielson E. W., Pinn M. L., Testa J. R. and Kuhajda F. P. (2001): Increased fatty acid synthase is a therapeutic target in mesothelioma. *Clin. Cancer Res.*, 7, 153–157.
- Gansler T. S., Hardman W. 3rd, Hunt D. A., Schaffel S. and Hennigar R. A. (1997): Increased expression of fatty acid synthase (OA-519) in ovarian neoplasms predicts shorter survival. *Hum. Pathol.*, 28, 686–692.
- Heemers H., Maes B., Fougelle F., Heyns W., Verhoeven G. and Swinnen J. V. (2001): Androgens stimulate lipogenic gene expression in prostate cancer cells by activation of the sterol regulatory element-binding protein cleavage activating protein/sterol regulatory element-binding protein pathway. *Mol. Endocrinol.*, 15, 1817–1828.
- Hochachka P. W. (1980): *Living without oxygen*. Harvard University Press, Cambridge, MA, USA, 1–181.
- Hochachka P. W., Rupert J. L., Goldenberg L., Gleave M. and Kozlowski P. (2002): Going malignant: the hypoxia-cancer connection in the prostate. *Bioessays*, 24, 749–757.
- Horrobin D. F. (1989): Polyunsaturated fatty acids and human cancer. In Abraham S. (ed.): *Carcinogenesis and dietary fat*. Kluwer Academic Publishers, Boston, 247–262.
- Horrobin D. F. (1990): Essential fatty acids, lipid peroxidation, and cancer. In Horrobin D. F. (ed.): *Omega-6 essential fatty acids*. Wiley-Liss, New York, 351–378.

37. Horrobin D. F. (1994): Unsaturated lipids and cancer. In Horrobin D. F. (ed.): New approaches to cancer treatment. Churchill, Edinburgh, 1–29.
38. Innocenzi D., Alo P. L., Balzani A., Sebastiani V., Silipo V., La Torre G., Ricciardi G., Bosman C. and Calvieri S. (2003): Fatty acid synthase expression in melanoma. *J. Cutan. Pathol.*, 30, 23–28.
39. Jensen V., Ladekari M., Holm-Nielsen P., Flemming B. S. and Fleming M. (1995): The prognostic value of oncogenic antigen 519 (OA-519) expression and proliferative activity detected by antibody MIB-1 in node negative breast cancer. *J. Pathol.*, 176, 343–352.
40. Jiang W. G., Bryce R. P. and Horrobin D. F. (1998): Essential fatty acids: molecular basis of their anti-cancer action and clinical implications. *Crit. Rev. Oncol. Hematol.*, 27, 179–209.
41. Joyeux C., Rochefort H. and Chalbos D. (1989): Progesterin increases gene transcription and messenger ribonucleic acid stability of fatty acid synthetase in breast cancer cells. *Mol. Endocrinol.*, 3, 681–686.
42. Kalkhoven E., Kwakkenbos-Isbrucker L., de Laat S. W., van der Saag P. T. and van der Burg B. (1994): Synthetic progestins induce proliferation of breast tumor cell lines via the progesterone or estrogen receptor. *Mol. Cell. Endocrinol.*, 102, 45–52.
43. Katsurada A., Iritani N., Fukuda H., Matsumura Y., Nishimoto N., Noguchi T. and Tanaka T. (1990): Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of fatty acid synthase in rat liver. *Eur. J. Biochem.*, 190, 427–433.
44. Kersten S. (2001): Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep.*, 2, 282–286.
45. Kim J. B., Sarraf P., Wright M., Yao K. M., Mueller E., Solanes G., Lowell B. B. and Spiegelman B. M. (1998): Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J. Clin. Invest.*, 101, 1–9.
46. Klein V., Chajes V., Germain E., Schulgen G., Pinault M., Malvy D., Lefrancq T., Fignon A., Le Floch O., Lhuillery C. and Bougnoux P. (2000): Low α -linolenic acid content of adipose breast tissue is associated with an increased risk of breast cancer. *Eur. J. Cancer*, 36, 335–340.
47. Kotzka J., Muller-Wieland D., Roth G., Kremer L., Munck M., Schurmann S., Knebel B. and Krone W. (2000): Sterol regulatory element binding proteins (SREBP)-1a and SREBP-2 are linked to the MAP-kinase cascade. *J. Lipid Res.*, 41, 99–108.
48. Krontiras H., Roye G. D., Beenken S. E., Myers R. B., Mayo M. S., Peters G. E. and Grizzle W. E. (1999): Fatty acid synthase expression is increased in neoplastic lesions of the oral tongue. *Head Neck*, 21, 325–329.
49. Kuhajda F. P. (2000): Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition*, 16, 202–208.
50. Kuhajda F. P., Jenner K., Wood F. D., Hennigar R. A., Jacobs L. B., Dick J. D. and Pasternack G. R. (1994): Fatty acid synthesis: a potential selective target for antineoplastic therapy. *Proc. Natl. Acad. Sci. USA*, 91, 6379–6383.
51. Kuhajda F. P., Katumuluwa A. I. and Pasternack G. R. (1989): Expression of haptoglobin-related protein and its potential as a tumor antigen. *Proc. Natl. Acad. Sci. USA*, 86, 1188–1192.
52. Kuhajda F. P., Piantadosi S. and Pasternack G. P. (1989): Haptoglobin-related protein (Hpr) epitopes in breast cancer as a predictor of recurrence of the disease. *N. Engl. J. Med.*, 321, 636–641.
53. Kuhajda F. P., Pizer E. S., Li J. N., Mani N. S., Frehywot G. L. and Townsend C. A. (2000): Synthesis and anti-tumor activity of a novel inhibitor of fatty acid synthase. *Proc. Natl. Acad. Sci. USA*, 97, 3450–3454.
54. Kumar-Sinha C., Ignatowski K. W., Lippman M. E., Ethier S. P. and Chinnaiyan A. M. (2003): Transcriptome analysis of HER2 reveals a molecular connection to fatty acid synthesis. *Cancer Res.*, 63, 132–139.
55. Kusakabe T., Maeda M., Hoshi N., Sugino T., Watanabe K., Fukuda T. and Suzuki T. (2000): Fatty acid synthase is expressed mainly in adult hormone-sensitive cells or cells with high lipid metabolism and in proliferating fetal cells. *J. Histochem. Cytochem.*, 48, 613–622.
56. Kusakabe T., Nashimoto A., Honma K. and Suzuki T. (2002): Fatty acid synthase is highly expressed in carcinoma, adenoma and in regenerative epithelium and intestinal metaplasia of the stomach. *Histopathology*, 40, 71–79.
57. Li J. N., Gorospe M., Chrest F. J., Kumaravel T. S., Evans M. K., Han W. F. and Pizer E. S. (2001): Pharmacological inhibition of fatty acid synthase activity produces both cytostatic and cytotoxic effects modulated by p53. *Cancer Res.*, 61, 1493–1499.
58. Li J. N., Mahmoud M. A., Han W. F., Ripple M. and Pizer E. S. (2000): Sterol regulatory element-binding protein-1 participates in the regulation of fatty acid synthase expression in colorectal neoplasia. *Exp. Cell Res.*, 261, 159–165.
59. Magana M. M. and Osborne T. F. (1996): Two tandem binding sites for sterol regulatory element binding proteins are required for sterol regulation of fatty-acid synthase promoter. *J. Biol. Chem.*, 271, 32689–32694.
60. Maillard V., Bougnoux P., Ferrari P., Jourdan M. L., Pinault M., Lavillonniere F., Body F., Le Floch O. and Chajes V. (2002): N-3 and N-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. *Int. J. Cancer*, 98, 78–83.
61. Mattick J. S., Nickless J., Mizugaki M., Yang C. Y., Uchiyama S. and Wakil S. J. (1983): The architecture of the animal fatty acid synthetase. I. Proteolytic dissection and peptide mapping. *J. Biol. Chem.*, 258, 15300–15304.
62. Menendez J. A., Colomer R., Cortes-Funes H. and Lupu R. (2004): Tumor-associated fatty acid synthase hyperactivity regulates breast cancer cell sensitivity to paclitaxel (Taxol)-induced apoptosis. *Proc. Am. Assoc. Cancer Res.*, 45, LB-283.
63. Menendez J. A., Colomer R. and Lupu R. (2004): Inhibition of tumor-associated fatty acid synthase activity enhances vinorelbine (Navelbine[®])-induced cytotoxicity and apoptotic cell death in human breast cancer cells. *Oncol. Rep.*, 12, 411–422.
64. Menendez J. A., Lupu R. and Colomer R. (2004): Inhibition of tumor-associated fatty acid synthase hyperactivity induces synergistic chemosensitization of HER-2/neu-overexpressing human breast cancer cells to docetaxel (taxotere). *Breast Cancer Res. Treat.*, 84, 183–195.
65. Menendez J. A., Mehmi I., Atlas E., Colomer R. and Lupu R. (2004): Novel signaling molecules implicated in tumor-associated fatty acid synthase-dependent breast cancer cell proliferation and survival: role of exogenous dietary fatty acids, p53-p21WAF1/CIP1, ERK1/2 MAPK, p27KIP1, BRCA1, and NF- κ B. *Int. J. Oncol.*, 24, 591–608.
66. Menendez J. A., Ropero S., Mehmi I., Atlas E., Colomer R. and Lupu R. (2004): Overexpression and hyperactivity of breast cancer-associated fatty acid synthase (oncogenic antigen-519) is insensitive to normal arachidonic fatty acid-induced suppression in lipogenic tissues but it is selectively inhibited by tumoricidal α -linolenic and γ -linolenic fatty acids: a novel mechanism by which dietary fat can alter mammary tumorigenesis. *Int J Oncol.*, 24, 1369–1383.
67. Menendez J. A., Vellon L., Mehmi I., Oza B. P., Ropero S., Colomer R. and 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.
68. Menendez J., Oza B. P., Atlas E., Vazquez-Martin A., Colomer R. and Lupu R. (2004): The anorectic fatty acid synthase (FAS) inhibitor C75 regulates genomic and non-genomic estrogen receptor- α activities in breast cancer cells. *Proc. Am. Assoc. Cancer Res.*, 45, LB-269.
69. Menendez J. A., Oza B. P., Atlas E., Verma V. A., Mehmi I. and Lupu R. (2004): Inhibition of tumor-associated fatty acid synthase activity antagonizes estradiol- and tamoxifen-induced agonist transactivation of estrogen receptor (ER) in human endometrial adenocarcinoma cells. *Oncogene*, 23, 4945–4958.
70. Milgraum L. Z., Witters L. A., Pasternack G. R. and Kuhajda F. P. (1997): Enzymes of the fatty acid synthesis pathway are highly expressed in *in situ* breast carcinoma. *Clin. Cancer Res.*, 3, 2115–2120.
71. Moche M., Schneider G., Edwards P., Dehesh K. and Lindqvist Y. (1999): Structure of the complex between the antibiotic cerulenin and its target, β -ketoacyl carrier protein synthase. *J. Biol. Chem.*, 274, 6031–6034.

72. Nemoto T., Terashima S., Kogure M., Hoshino Y., Kusakabe T., Suzuki T. and Gotoh M. (2001): Overexpression of fatty acid synthase in oesophageal squamous cell dysplasia. *Pathobiology*, 69, 297–303.
73. Omura S. (1976): The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. *Bacteriol. Rev.*, 40, 681–697.
74. Oshi A. K., Witkowski A. and Smith S. (1997): Mapping of functional interactions between domains of the animal fatty acid synthase by mutant complementation *in vitro*. *Biochemistry*, 36, 2316–2322.
75. Piyathilake C. J., Frost A. R., Manne U., Bell W. C., Weiss H., Heimburger D. C. and Grizzle W. E. (2000): The expression of fatty acid synthase (FAS) is an early event in the development and progression of squamous cell carcinoma of the lung. *Hum. Pathol.*, 31, 1068–1073.
76. Pizer E. S., Chrest F. J., DiGiuseppe J. A. and Han W. F. (1998): Pharmacological inhibition of mammalian fatty acid synthase suppress DNA replication and induce apoptosis in tumor cell lines. *Cancer Res.*, 58, 4611–4615.
77. Pizer E. S., Jackisch C., Wood F. D., Pasternack G. R., Davidson N. E. and Kuhajda F. P. (1996): Inhibition of fatty acid synthase induces programmed cell death in human breast cancer cells. *Cancer Res.*, 56, 2745–2747.
78. Pizer E. S., Kurman R. J., Pasternack G. R. and Kuhajda F. P. (1997): Expression of fatty acid synthase is closely linked to proliferation and stromal decidualization in cycling endometrium. *Int. J. Gynecol. Pathol.*, 16, 45–51.
79. Pizer E. S., Lax S. F., Kuhajda F. P., Pasternack G. R. and Kurman R. J. (1998): Fatty acid synthase expression in endometrial carcinoma: correlation with cell proliferation and hormone receptors. *Cancer*, 83, 528–537.
80. Pizer E. S., Thupari J., Han W. F., Pinn M. L., Chrest F. J., Frehywot G. L., Townsend C. A. and Kuhajda F. P. (2000): Malonyl-coenzyme-A is a potential mediator of cytotoxicity induced by fatty-acid synthase inhibition in human breast cancer cells and xenografts. *Cancer Res.*, 60, 213–218.
81. Pizer E. S., Wood F. D., Heine H. S., Romantsev F. E., Pasternack G. R. and Kuhajda F. P. (1996): Inhibition of fatty acid synthase delays disease progression in a xenograft model of ovarian cancer. *Cancer Res.*, 56, 1189–1193.
82. Pizer E. S., Wood F. D., Pasternack G. R. and Kuhajda F. P. (1996): Fatty acid synthase (FAS): a target for cytotoxic antimetabolites in HL60 promyelocytic leukemia cells. *Cancer Res.*, 56, 745–751.
83. Rangan V. S., Joshi A. K. and Smith S. (2001): Mapping the functional topology of the animal fatty acid synthase by mutant complementation *in vitro*. *Biochem.*, 40, 10792–10799.
84. Rashid A., Pizer ES, Moga M., Milgraum LZ, Zahurak M, Pasternack GR, Kuhajda FP and Hamilton S. R. (1997): Elevation of fatty acid synthase expression and fatty acid synthetic activity in colorectal neoplasia. *Am. J. Pathol.*, 150, 201–208.
85. Roncari D. A. K. (1974): Mammalian fatty acid synthase. I. Purification and properties of human liver complex. *Can. J. Biochem.*, 52, 221–230.
86. Ross J. S. and Fletcher J. A. (1998): The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. *Stem Cells*, 16, 413–428.
87. Shimano H., Yahagi N., Amemiya-Kudo M., Hasty A. H., Osuga J., Tamura Y., Shionoiri F., Iizuka Y., Ohashi K., Harada K., Gotoda T., Ishibashi S. and Yamada N. (1999): Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J. Biol. Chem.*, 274, 35832–35839.
88. Shurbaji M. S., Kuhajda F. P., Pasternack G. R. and Thurmond T. S. (1992): Expression of oncogenic antigen 519 (OA-519) in prostate cancer is a potential prognostic indicator. *Am. J. Clin. Pathol.*, 97, 686–691.
89. Slamon D. J., Clark G. M., Wong S. J., Levin W. J., Ullrich A. and McGuire W. L. (1987): Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, 235, 177–182.
90. Slamon D. J., Godolphin W., Jones L. A., Holt J. A., Wong S. G., Keith D. E., Levin W. J., Stuart S. G., Udove J. and Ullrich A. (1989): Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science*, 244, 707–712.
91. Slamon D. J., Leyland-Jones B., Shak S., Fuchs H., Paton V., Bajamonde A., Fleming T., Eiermann W., Wolter J., Pegram M., Baselga J. and Norton L. (2001): Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.*, 344, 783–779.
92. Sul H. S. and Wang D. (1998): Nutritional and hormonal regulation of enzymes in fat synthesis: studies of fatty acid synthase and mitochondrial glycerol-3-phosphate acyltransferase gene transcription. *Annu. Rev. Nutr.*, 18, 331–351.
93. Swinnen J. V., Esquet M., Goossens K., Heyns W. and Verhoeven G. (1997): Androgens stimulate fatty acid synthase in the human prostate cancer cell line LNCaP. *Cancer Res.*, 57, 1086–1090.
94. Swinnen J. V., Heemers H., Deboel L., Foulfelle F., Heyns W. and Verhoeven G. (2000): Stimulation of tumor-associated fatty acid synthase expression by growth factor activation of the sterol regulatory element-binding protein pathway. *Oncogene*, 19, 5173–5181.
95. Swinnen J. V., Roskams T., Joniau S., Poppel H. V., Oyen R., Baert L., Heyns W. and Verhoeven G. (2002): Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. *Int. J. Cancer*, 98, 19–22.
96. Swinnen J. V., Ulrix W., Heyns W. and 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.
97. Swinnen J. V., Vanderhoydonc F., Elgamel A. A., Eelen M., Vercaeren I., Joniau S., Van Poppel H., Baert L., Goossens K., Heyns W. and Verhoeven G. (2000): Selective activation of the fatty acid synthesis pathway in human prostate cancer. *Int. J. Cancer.*, 88, 176–179.
98. Takahiro T., Shinichi K. and Toshimitsu S. (2003): Expression of fatty acid synthase as a prognostic indicator in soft tissue sarcomas. *Clin. Cancer Res.*, 9, 2204–2212.
99. Thompson, B. J. and Smith S. (1985): Biosynthesis of fatty acids by lactating human breast epithelial cells: an evaluation of the contribution of the overall composition of human milk fat. *Pediatr. Res.*, 19, 139–143.
100. Tsukamoto Y., Wong H., Mattick J. S. and Wakil S. J. (1983): The architecture of the animal fatty acid synthetase complex. IV. Mapping of active centers and model for the mechanism of action. *J. Biol. Chem.*, 258, 15312–15322.
101. Vance D., Goldberg I., Mitsuhashi O. and Bloch K. (1972): Inhibition of fatty acid synthetases by the antibiotic cerulenin. *Biochem. Biophys. Res. Commun.*, 48, 549–565.
102. Van de Sande T., De Schrijver E., Heyns W., Verhoeven G. and Swinnen J. V. (2002): Role of the phosphatidylinositol 3'-kinase/PTEN/Akt kinase pathway in the overexpression of fatty acid synthase in LNCaP prostate cancer cells. *Cancer Res.*, 62, 642–646.
103. Vazquez-Martin A., Menendez J. A., Ropero S., Montero S., Cortes-Funes H. and Colomer R. (2002): Anthracycline resistance on breast cancer cells is reversed by inhibition of fatty acid synthase. *Proc. Am. Assoc. Cancer Res.*, 43, #4709, 951.
104. Visca P., Alo P. L., Del Nonno F., Bottie C., Trombetta G., Marandino F., Filippi S., Di Tondo U. and Donnorso R. P. (1999): Immunohistochemical expression of fatty acid synthase, apoptotic-regulating genes, proliferating factors, and ras protein product in colorectal adenomas, carcinomas, and adjacent non-neoplastic mucosa. *Clin. Cancer Res.*, 5, 4111–4118.
105. Visca P., Sebastiani V., Pizer E. S., Botti C., De Carli P., Filippi S., Monaco S. and Alo P. L. (2003): Immunohistochemical expression and prognostic significance of FAS and GLUT1 in bladder carcinoma. *Anticancer Res.*, 23, 335–339.
106. Vogel C. L., Cobleigh M. A., Tripathy D., Gutheil J. C., Harris L. N., Fehrenbacher L., Slamon D. J., Murphy M., Novotny W. F., Burchmore M., Shak S., Stewart S. J. and Press M. (2002): Efficacy and safety of trastuzumab as a single agent in first-line

- treatment of HER2-overexpressing metastatic breast cancer. *J. Clin. Oncol.*, 20, 719–726.
107. Wakil S. (1989): Fatty acid synthase, a proficient multifunctional enzyme. *Biochem. J.*, 28, 4523–4530.
108. Wang D. and Sul H. S. (1998): Insulin stimulation of the fatty acid synthase promoter is mediated by the phosphatidylinositol 3-kinase pathway. Involvement of protein kinase B/Akt. *J. Biol. Chem.*, 273, 25420–25426.
109. Wang S. C. and Hung M. C. (2000): Transcriptional targeting of the HER-2/neu oncogene. *Drugs Today*, 36, 835–843.
110. Warburg O. (1956): On the origin of cancer cells. *Science*, 123, 309–314.
111. Weiss L., Hoffmann G. E., Schreiber R., Andres H., Fuchs E., Korber E. and Kolb H. J. (1986): Fatty-acid biosynthesis in man, a pathway of minor importance. Purification, optimal assay conditions, and organ distribution of fatty-acid synthase. *Biol. Chem.*, 367, 905–912.
112. Witkowski A., Rangan V. S., Randhawa Z. I., Amy C. M. and Smith S. (1991): Structural organization of the multifunctional animal fatty acid synthase. *Int. J. Biochem.*, 198, 571–579.
113. Xing X., Wang S. C., Xia W., Zou Y., Shao R., Kwong K. Y., Yu Z., Zhang S., Miller S., Huang L. and Hung M. C. (2000): The ets protein PEA3 suppresses HER-2/neu overexpression and inhibits tumorigenesis. *Nat. Med.*, 6, 189–195.
114. Xu Z.-X., Stenzel W., Sasic S. M., Smart D. A. and Rooney S. A. (1993): Glucocorticoid regulation of fatty acid synthase gene expression in fetal rat lung. *Am. J. Physiol.*, 265, L140–L147.
115. Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S and Arteaga C. L. (2002): Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. *Cancer Res.*, 62, 4132–4141.
116. Yang Y. A., Han W. F., Morin P. J., Chrest F. J. and Pizer E. S. (2002): Activation of fatty acid synthesis during neoplastic transformation: role of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. *Exp. Cell Res.*, 279, 80–90.
117. Yang Y. A., Morin P. J., Han W. F., Chen T., Bornman D. M., Gabrielson E. W. and Pizer E. S. (2003): Regulation of fatty acid synthase expression in breast cancer by sterol regulatory element binding protein-1c. *Exp. Cell Res.*, 282, 132–137.
118. Ye Q., Chung L. W., Li S. and Zhau H. E. (2000): Identification of a novel FAS/ER- α fusion transcript expressed in human cancer cells. *Biochim. Biophys. Acta.*, 1493, 373–377.
119. Yu D. and Hung M.-C. (2000): Role of erbB2 in breast cancer chemosensitivity. *Bioessays*, 22, 673–680.
120. Zhou W., Simpson P. J., McFadden J. M., Townsend C. A., Medghalchi S. M., Vadlamudi A., Pinn M. L., Ronnett G. V. and Kuhajda F. P. (2003): Fatty acid synthase inhibition triggers apoptosis during S phase in human cancer cells. *Cancer Res.*, 63, 7330–7337.