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Fatty acid synthase gene overexpression and copy number gain in prostate adenocarcinoma☆

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Summary Cancer cells frequently exhibit a significant increase in overexpression and activity of fatty acid synthase (FASN). Elevated FASN pathway activity also occurs in prostate cancer, the second leading cause of cancer-related death in men in the United States. Studies show that genes associated with an increase in protein expression, such as *HER2/neu* in breast cancer, are associated with an increase in gene copy number as well as an increase in transcription. In the present study, we evaluated whether *FASN* follows a similar paradigm in prostate cancer. To date, elevated FASN expression in prostate cancer has not been correlated with gene copy number alterations. Using immunohistochemistry and fluorescence in situ hybridization analysis in paraffin-embedded tissue microarrays, we observed gene copy gain in 24% of all prostate adenocarcinoma specimens examined with concurrent increased FASN protein expression. Immunohistochemistry alone showed 59% of prostate cancer specimens in the same tissue microarray with high FASN expression. Increased *FASN* gene was observed in 53% of all prostate tissues expressing elevated FASN protein levels and in 2 of 5 prostate tumor cell lines tested. These findings suggest that *FASN* gene copy number increases may be involved in the resultant increase in FASN protein expression observed in prostatic disease.

1. Introduction

Fatty acid synthase (FASN), a 250- to 270-kD cytosolic multifunctional polypeptide chain, contributes to the de novo synthesis of fatty acids. Under normal conditions, FASN activity remains minimal due to the supply of endogenous fatty acids by dietary fat [1]. However, in cancer cells, significant overexpression and concurrent increased activity of FASN represents one of

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the most frequently observed phenotypic alterations [2]. Indeed, in prostate cancer (PCa), up-regulation of FASN correlates with progression as well as aggressiveness of the disease [3,4].

Fatty acid biosynthesis occurs in all living organisms and provides essential components for biologic membranes as well as a form of energy storage. Animal FASN is an androgen-regulated multifunctional enzyme that catalyzes the synthesis of long-chain fatty acids via sequential condensation of two-carbon units from malonyl-CoA, an intermediate derived from the carboxylation of acetyl-CoA. Fatty acid synthase is a homodimer of a multifunctional subunit protein that contains 7 distinct activities and a site for the prosthetic group 4′ -phosphopantetheine (acyl carrier protein). Activity and expression of FASN significantly increase in tumorigenic tissue [3,5]. Studies have shown that the highest FASN expression occurs in poorly differentiated and metastatic androgen-independent cancers [6]. These findings suggest that *FASN* represents a potentially important target in aggressive prostate cancer. Furthermore, *FASN* may act as a metabolic oncogene for prostate cancer [2,5]. However, the exact role of *FASN* in prostate cancer progression or onset remains unclear.

Many of the genes coding for the enzymes of the fatty acid biosynthetic pathway reside on human chromosome 17q: *FASN* (17q25), acetyl-CoA carboxylase (17q21) (*ACACA*), and ATP citrate lyase (17q12-21) (*ACLY*). The synthesis of malonyl-CoA is the first committed step of fatty acid synthesis and the enzyme that catalyzes this reaction, acetyl-CoA carboxylase, is the major site of regulation of fatty acid synthesis. Cytosolic ATP-citrate lyase is involved in converting citrate to acetyl-CoA, the precursor for the FASN reactions. This region of chromosome 17 is a common site for amplification of genes, including the *ERBB2* oncogene (*HER2/neu*), as well as for mutations and/or deletions of genes, such as *BRCA1*, metastasis suppressor gene *NME1* (*NM23*), *NGFR* (*p75 NTR*), and *HPC2* [7-11].

Studies have shown that an increase in gene copy number correlates with protein expression in some genes. One such example is the *ERBB2* (*HER2/neu*) oncogene, which is amplified and overexpressed in about 25% to 30% of primary breast cancer cases and is associated with a poor prognosis [12-14]. A strong correlation between HER2/neu protein expression and gene copy number is present. HER2 status is important as a predictive factor to identify breast cancer patients most likely to respond to therapeutic intervention using the HER2/neu antagonist, herceptin.

To date, FASN protein expression and gene copy number have not been correlated in prostate cancer. Thus, to investigate if a correlation is present between FASN expression and gene copy number, we measured FASN protein levels by immunohistochemistry (IHC) and gene copy number by fluorescence in situ hybridization (FISH) assays in prostate cancer tissue and cell lines.

2. Materials and methods

2.1. Cell lines

Androgen-sensitive LNCaP, androgen-independent PC-3, and DU145 human prostate carcinoma cell lines were purchased from American Type Culture Collection (Rockville, Md) and cultured in RPMI 1640 (Gibco BRL, Grand Island, NY) with 10% fetal bovine serum (Hyclone, Logan, Utah) under standard culture conditions. The androgen-independent PPC-1 cell line was obtained from Dr Joel B. Nelson (University of Pittsburgh). A well-characterized human breast cancer cell line, SKBR3 established from the pleural effusion of a hormone-independent human breast cancer (estrogen receptor–negative and progesterone receptor–negative), was obtained from the ATCC. SKBR3 cells were maintained in 75-cm^3 flasks in DMEM (Gibco BRL) supplemented with 10% FBS (Hyclone) and 2 mmol/L glutamine (Sigma Chemical Co, St Louis, Mo).

2.2. Patient material and tissue microarray construction

Prostate cancer specimens from 166 patients were used for this study. The cases were obtained from urologic surgery files of the Urology Department of the University of Pittsburgh, all obtained from 1996 to 1998, and were selected after pathologist review (by Dr Rajiv Dhir) of the hematoxylin and eosin staining to determine Gleason score and grade of cancer. The patients were selected to provide adequate representation of different T stages, and thus, the study included consecutive T2/T3/T4 patients. The PSA failure array was constructed with the 28 PSA failure patients in our repository without positive margins. The array constructed was a "multitumor" array due to the various numbers of cases present on the array.

High-density tissue microarrays (TMAs) were assembled using the manual tissue puncher/array (Beecher Instruments, Silver Springs, Md). Tissue cores were 0.6 mm in diameter and ranged in length from 1.0 to 3.0 mm depending on the depth of tissue in the donor block. Multiple replicate core samples of normal, high-grade PIN, and prostate cancer tissue were acquired from each case. Cores were inserted into a recipient block measuring 45 × 20 × 12 mm and spaced 0.8 mm apart. The TMA set included progression TMAs (88 cases of prostate cancer with different Gleason grades and prostate cancer T stage). The TMAs also contained foci of metastatic prostate cancer, PIN, normal adjacent to tumor (NAT), benign prostatic hyperplasia (BPH), and "true normal" prostatic tissue from organ donors (Table 1).

2.3. FISH assays

The cultured cells were treated with Colcemid (0.1 mg/mL) for 5 hours before harvesting. After mitotic arrest, the cells were processed in accordance with standard cytogenetic