Fatty Acid Synthase (FASN) Expression Is Strongly Related With Menopause In Early Breast Cancer Patients

Running Title: FASN Expression in Early-Stages of Breast Cancer

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ABSTRACT

OBJECTIVE. Overexpression of fatty acid synthase (FASN), the enzyme involved in *de novo* synthesis of fatty acids, has been reported in several human carcinomas, including breast cancer, and has been related to poor prognosis. Our aim was to analyze the association between the fatty acid synthase tumor tissue expression with clinicopathological and anthropometrical features in early breast cancer patients.

METHODS. We prospectively studied **53** women who were treated for early-stage breast cancer with surgery and post-operative chemotherapy.

RESULTS. Menopause status and age were strongly associated to higher levels of fatty acid synthase tumor expression; $p<0.005$ and $p= 0.038$ respectively. Body mass index (BMI) and pathological stages was also related with fatty acid synthase tumor expression.

CONCLUSION. Our findings suggest that fatty acid synthase could be a ~~molecular marker~~ **potential therapeutic target** in postmenopausal breast cancer patients, **however further studies are needed.**

Key Words: fatty acid synthase; breast cancer; early-stage; menopause

INTRODUCTION

Fatty acid synthase is a multifunctional enzyme that catalyzes the synthesis of long-chain fatty acids by using acetyl-CoA, malonyl-CoA, and NADPH precursors [1]. In most human tissues the diet supplies the needs of fatty acids and FASN expression is low or undetectable. In contrast, in several human carcinomas lipogenic enzymes (mainly FASN) are highly expressed [2-7] and *de novo* fatty acid biosynthesis supplies the needs of long chain fatty acids (LCFA) for energy production, protein acylation, synthesis of biological membranes, DNA synthesis and cell cycle progression among other biological processes, providing an advantage for tumor growth and progression [3-5]. We and others have previously reported that pharmacological and genetic FASN inhibition **(alone or in a combined regimen)** induced apoptosis in several cancer cells **[8-15]** and reduced the growth of human breast cancer xenografts [11, **16, 17**]. In this context, several reports highlight that FASN overexpression in tumor samples could be a putative biomarker and a prognostic factor in several carcinomas, including breast cancer [7, **17-23**]. Other studies indicate that body weight and body fat distribution play an important role in the occurrence of some malignant tumors such as colon, endometrial and breast **[24-25]**. Key lipogenic enzymes (such as FASN) could be relevant to increase adiposity during the menopause in healthy women **[27]** and it could also have a role in postmenopausal breast cancer patients.

Our main objective was to investigate the relationship between FASN tumor expression with several clinicopathological features with prognostic relevance in early stages of breast cancer. We also studied whether FASN tumor expression could be related with anthropometrical features related with body fat distribution and obesity. ~~since FASN could open a novel treatment approach in postmenopausal breast cancer patients.~~

METHODS

We designed a prospective cross sectional study that included all patients diagnosed of invasive early-stage breast cancer from the Catalan Institute of Oncology (ICO)-Girona, between October 2007 and December 2009. ~~All patients were treated with surgery and post-operative chemotherapy~~. Written informed consent was obtained from all patients before surgery. This study was approved by the ethical board of the Dr. Josep Trueta University Hospital. All data were collected in a database specifically created for the study and the identification of patients was encoded. Clinicopathological features **were collected from the clinical records and they included** age, menopausal status, histological type, histological grade, mutifocality by magnetic resonance imaging, tumor size, vascular invasion, lymph nodes metastasis, pathological stage, estrogen and progesterone receptors, HER2 status, Ki-67 levels and p53 mutation. Anthropometrical features included body mass index (BMI), body fat percentage and waist-to-hip ratio.

Histological type and grade, tumor size, vascular permeation and lymph node metastasis were assessed on H&E-stained slides. Estrogen and progesterone receptors, p53 mutation, Ki-67 levels and HER2 status were determined by immunohistochemistry (IHC). HER2 in situ hybridization was done in those tumors expressing moderate levels (2+) of HER2 by IHC. All these procedures were done routinely by the Pathology Department.

Body mass index (BMI) was based on weight/(height)^2. Waist-to-hip ratio was calculated by dividing the waist circumference by the hip circumference in centimeters. We measured the skinfolds of biceps, triceps, subscapular and suprailiac regions by a Harpenden Caliper to estimate the body fat percentage. **All measures were done in triplicates.**

FASN tumor tissue expression was determined by IHC performed on additional sections of 3 μm thick from formalin-fixed paraffin embedded tissue blocks of the primary **breast** tumor. We used Fatty Acid Synthase polyclonal antibody (Assay design, Enzo Life Sciences, Exeter United Kingdom) at a dilution of 1:100 and the detection kit EnVision™ (DAKO) using the AutostainerPlus Link (DAKO). Negative control using mouse IgG at a comparable concentration in place of the primary antibody were included. Also muscular tissue was used as a negative expression of FASN. Expression of FASN was graded from 0 to 3+, meaning 0-1+ normal amounts of FASN protein, 2+ moderate amounts and 3+ highest levels of FASN expression (Fig. 1). The percentage of staining was recorded as: 0-33%, 33-66% and 66-100% (considering all the tissue area). **Results are representative of those obtained from three independent experiments.**

Statistical Analysis

Patient and tumor characteristics were summarized descriptively and assessed for normality before analysis using normal probability plots and Kolmogrov test statistics. Categorical variables were compared by χ^2 or Fisher's exact tests; strength of association among ordinal categorical variables was analyzed by means of Kendall's Tau or Gamma coefficient. If the data were ~~**approximately**~~ **distributed** normal, t-student and ANOVA with Bonferroni correction for multiple comparisons have been used for measuring the difference of continuous variables among groups. If the normality assumption is not warranted, then the Kruskall-Wallis test has been used. All tests were two-tailed; p values below 0.05 were considered significant. Data analysis was performed using the IBM SPSS V20 statistical software and Intercooled Stata 8.0 for windows.

RESULTS

We prospectively analyzed 53 patients with early-stage breast cancer, whose main characteristics analyzed are summarized in Table 1. Briefly, the median age at the time of diagnosis was 49 years old (rage 33-77). Nearly 55 percent of the patients were postmenopausal at the time of the diagnosis. Eighty-three of the tumors were invasive ductal carcinomas. Over 80% of our patients had tumors expressing estrogen or progesterone receptors and 13% overexpressed HER2.

FASN tumors expression was high (3+) in 2**6** patients, moderate (2+) in 25 patients and normal (0-1+) in 2 patients.

Menopause was strongly related to higher levels of FASN tumor expression (p=0.005). Nineteen out of the 28 postmenopausal patients (67.9%) presented tumors overexpressing FASN (3+) (Fig. 1).

Age was also found to be statistically significant related to FASN tumor levels (p=0.038). The mean age of the patients who had tumors with higher levels of FASN (3+) was 54 years, whereas mean age of those patients with tumors expressing 1 or 2 + were 46 and 47.44 years respectively. We also observed a relation between breast cancer stage and the BMI and the FASN tumor tissue levels (p=0.024 and p= 0.018 respectively). No significant relation between FASN tumor levels and waist-to-hip ratio, body fat percentage, multifocality, histological subtype and grade, tumor size, vascular invasion, lymph nodes metastasis, pathological stage, estrogen and progesterone receptors, HER2 status, p53 mutation and Ki-67 levels was found.

In addition, we investigated the relationship between FASN serum levels and HER2 serum levels (data not shown) among patients and no association was found.

DISCUSSION

~~Altered expression of FASN is found in several cancers, including breast cancer [3, 26] Several studies have shown that inhibition of FASN activity inhibits the growth of cancer cells and xenografts [14] and some studies have observed an association between FASN and prognosis in some tumors [18, 27].~~ **Altered expression of FASN has been found in several cancers, including breast cancer [3,26], and it has been proposed as a prognosis biomarker[5,20,28] and a potential therapeutic target[8-17].** ~~Our aim was to assess the association between FASN tumor tissue levels and the clinicopathological features in early-stage breast cancer patients and to find out the association with several anthropometric values regarding obesity and body fat distribution.~~ **We aim to study whether expression levels of FASN in early-breast cancer tumors could be related with clinicopathological characteristics with prognostic relevance. Thus, we designed a prospective cross sectional study.**

We studied a sample of 5**3** early-stage breast cancer patients treated with surgery ~~and postoperatory chemotherapy~~; this sample was well balanced between pre and postmenopausal patients (45.3% versus 54.7%). ~~Our sample is representative of the general population with breast cancer, thus the most frequent clinicopathologic features of this sample are the most common in the general population with breast cancer~~. **Even that this study was performed only with 53 women it is interesting to highlight that the clinicopathologic features [postmenopausal, estrogen (ER) and progesterone receptor (PR) positive and HER2 negative] of these 53 patients are the most frequent in breast cancer population.**

~~In this study~~ We have observed a strong association between FASN tumor levels and the menopausal status and age. These can be explained by the fact that the age and menopause are not independent variables**, however the relationship between menopause and FASN and between age and FASN were analyzed independently.**

As far as we know, this is the first study describing that FASN tumor levels are higher among postmenopausal than in premenopausal early breast cancer patients. This finding is supported by the fact that estrogen therapy (ET) in postmenopausal women decreased the levels of FASN in abdominal adipose tissue [27]. Opposite results have been observed previously by Chalbos *et al.* who observed that the levels of FASN in pre-cancerous breast lesions were significantly higher in premenopausal than in postmenopausal patients and they increased with the grade of tumor differentiation[29].

Furthermore, it is well known that the BMI and the fat body distribution change in postmenopausal women[30] and obesity has been associated with increased risk of postmenopausal breast cancer. The risk of breast cancer increases with increasing body mass index (BMI) and is associated with increased mortality[31]. Interestingly, some studies have shown a relation between the waist-to-hip index and the levels of tumor FASN in endometrial postmenopausal patients[32]. Thus, we **aimed to analyze the relationship between some anthropometriacal characteristics as surrogates of obesity and body fat distribution. We found a positive relationship between FASN tumor levels and BMI suggesting an association between FASN and obesity.** ~~the waist-to-hip ratio and the body fat percentage and the FASN tumor expression but we did not find any relation.~~ **Finally, we also found a relation between FASN tumor expression levels and the tumor pathological stage, however these associations need further investigations given the limited number of patients of our sample.**

Ours is a cross sectional study, that despite its limitations, allows us to generate new hypotesis to be tested with a more definitive design.

CONCLUSION

~~Based on our study we consider it justified to rule out prospective studies with larger number of of postmenopausal with breast cancer especially in advance disease since~~

~~FASN could be a novel molecular target treatment approach in breast cancer patients resistant to anti-hormonal therapy.~~

Based on our study we consider it justified to carry out prospective studies with larger number of breast cancer patients to further investigate the potential relation between FASN tumor expression and breast cancer clinicopathological characteristics, since FASN could be a novel prognosis biomarker and a potential therapeutic target.

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ETHICAL APPROVAL:

Written informed consent was obtained from all patients. The Ethical Committee of the Dr. Josep Trueta University Hospital approved this study.

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FOOTNOTES

~~**Figure 1**. Immunohistochemical staining for FASN (dilution 1:100), 10X magnification. These images show the different intensity of the FASN staining (0-3+). Brown corresponds to FASN staining. **A**. Non-tumor breast tissue (0-1+). **B**. Infiltrating ductal carcinoma (2+). **C**. Infiltrating ductal carcinoma (3+). **D**. Infiltrating lobular carcinoma (3+).~~

Figure 1. Immunohistochemical staining for FASN (dilution 1:100), 10X magnification. These images are the most representative of 53 breast tissue samples stained with FASN (0-3+). Brown corresponds to FASN staining. A. Non-tumor breast tissue (0-1+). B. Infiltrating ductal carcinoma (2+). C. Infiltrating ductal carcinoma (3+). D. Infiltrating lobular carcinoma (3+).