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JAK2, STAT3 and SOCS3 gene expression in women with and without breast cancer

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ABSTRACT

Introduction: Breast cancer is a disease that arises from the accumulation of alterations in the genome of cells that make up the mammary gland. The Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway has been reported to participate in the development of breast cancer and is activated by adipocytokines such as leptin, which are elevated in obesity. In contrast, alterations in suppressor of cytokine signaling 3 (SOCS3) gene expression have been found in patients with decreased breast cancer metastasis.
Objective: The current study sought to identify whether JAK2, STAT3 and SOCS3 gene expression is associated with body mass index (BMI) and breast cancer.
Methods: This was a cross-sectional prospective study. JAK2, STAT3 and SOCS3 gene expression levels were determined using RT-qPCR from the biopsies of 26 patients with breast cancer and 43 patients with benign breast lesions. We compared the expression of these genes, relative to the housekeeping genes, ACTB and GAPDH, against BMI, clinical stage and immunohistochemistry.
Results: STAT3 gene expression was increased in breast cancer patients ($p \leq 0.001$; AUC = 0.65; AUC 95% CI: 0.5–0.8), and SOCS3 expression was decreased in obese patients with benign breast lesions ($p \leq 0.001$; AUC = 0.51; AUC 95% CI: 0.36–0.65). With regard to the clinical stage, there were significant differences in STAT3 gene expression between stage II and III ($p \leq 0.011$) and stage II and IV ($p \leq 0.033$) breast cancers. Among all women, there was a positive correlation between JAK2 and STAT3 expression ($R = 0.493$, $p = 0.000$). In addition, breast cancers that were negative for HER2 were associated with JAK2 and SOCS3 ($R = 0.645$, $p = 0.003$).
Conclusion: High levels of STAT3 expression were associated with early stages of breast cancer development and patients in the control group with obesity showed higher expression of SOCS3 regarding overweight.

1. Introduction

Breast cancer is a disease that arises from the accumulation of alterations in the genome of cells that make up the mammary gland. Breast cancer is the most common type of cancer among women, with an estimated 1.38 million new cases of cancer diagnosed in 2008 (23% of all cancers), and the second most common type of cancer overall (10.9% of all cancers). In Mexico in 2008, there were 13,939 cases of breast cancer (27.2 per 100,000 individuals) and 5217 deaths as a result of breast cancer, resulting in a mortality of 10.1 per 100,000 individuals in the population (Del Socorro Romero-Figueroa et al., 2010; Knaul et al., 2009; WHO, 2008).

Although most studies of premenopausal women have not found a relationship between breast cancer and obesity, the prognosis for both pre- and postmenopausal breast cancer is substantially worse among obese compared to normal-weight individuals. Increasing evidence suggests that these associations may be mechanistically related to sex hormones, insulin and certain adipokines (Amadou et al., 2013). Obesity, defined as the presence of a body mass index (BMI) 30 kg/m^2, is another primary cause of mortality in Mexico, affecting 60–70% of the adult population. The incidence of both breast cancer and obesity has risen sharply over the past 20 years. An analysis of trends in BMI categories

Abbreviations: ACTB, beta actin; AUC, area under the curve; BMI, body mass index; CT, threshold cycle; EPO, erythropoietin; ER, estrogen receptor; ERK1/2, extracellular signal-related kinase 1/2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GH, growth hormone; GM-CSF, granulocyte macrophage colony-stimulating factor; HER2, human epidermal growth factor receptor 2; IL, interleukin; JAK2, Janus kinase 2; MAPK, mitogen-activated protein kinases; PI3K, phosphoinositol 3-kinase; PR, progesterone receptor; ROC, receiver–operator characteristic; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3; TPO, thrombopoietin; TYK2, tyrosine kinase 2.

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in women 20–49 years old developed in Mexico by the National Health Survey (ENSANUT) showed that, from 1988 to 2006, the prevalence of the overweight condition increased by 41.2% and that of obesity increased by 270.5%. While the trend of the overweight condition decreased 5.1% between 2006 and 2012, the prevalence of obesity increased by 2.9% during this time (ENSANUT, 2012; Villaseñor, 2011).

Obesity increases the risk of breast cancer development and progression, and several reports indicate that the adipokine leptin, whose synthesis and plasma levels increase with obesity, may play an important role in modulating the breast cancer cell phenotype (Giordano et al., 2013). Furthermore, the binding of leptin to its receptor (Ob-R) induces the activation of signaling cascades, including the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) (mainly JAK2/STAT3), extracellular signal-related kinase 1/2 (ERK1/2), mitogen-activated protein kinases (MAPK) and phosphoinositol 3-kinase (PI3K) pathways (Amitabha and Cleary, 2012).

The mammalian family of JAKs is composed of JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2), which selectively associate with the cytoplasmic domains of various cytokine receptors (O'Shea et al., 2013). In particular, JAK2 has been shown to be associated with breast cancer. JAK2 proteins are also associated with membrane receptors and possess enzymatic activity to phosphorylate tyrosine residues and participate in the signal transduction of erythropoietin (EPO), thrombopoietin (TPO), interleukin (IL)-3, IL-5, leptin, granulocyte macrophage colony-stimulating factor (GM-CSF), prolactin and growth hormone (GH). As a result, these proteins are involved in processes such as erythropoiesis, thrombopoiesis, mammary gland development, lactation and immunity. In various studies, constitutive activation and/or mutations of JAK2 have been linked to several pathologies, such as polycythemia vera, cancer and inflammatory diseases, and therefore represent novel therapeutic targets for treating such diseases (Haricharan and Li, 2013; Smirnova et al., 2007; Wagner and Hallgeri, 2008; Wagner and Schmidt, 2011).

The phosphorylated tyrosines on JAKs and their receptors recruit several signaling substrates, the most prominent of which are members of the STAT family. These proteins were numbered based on their order of discovery and include STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6 (Haricharan and Li, 2013; Wagner and Schmidt, 2011). Depending on the cell type and ligand receptor complex involved, each JAK activates one or more of the seven STAT proteins identified in mammalian cells.

Following JAK-mediated phosphorylation, STAT proteins dimerize and translocate to the nucleus, where they regulate gene expression (Constantinescu et al., 2007). Constitutive STAT3 (Pensa et al., 2009) and STAT5 activation has been associated with numerous cancers (O'Shea et al., 2013; Seavey and Dobrzansky, 2012), and disturbances to the state of equilibrium of these JAK/STAT pathways (in particular JAK2/STAT2 and JAK1/STAT3 signaling) lead to developmental defects and contribute to mammary carcinogenesis (Wagner and Schmidt, 2011).

Several studies have performed cell culture and immunohistochemistry and have shown that STAT3 and STAT5 proteins are found in greater abundance and are activated in different types of cancer; therefore, these proteins have been proposed to be associated with carcinogenesis. STAT3 is considered a proto-oncogene because a mutation that leads to a constitutive activation of STAT3 is sufficient for malignant cell transformation, activating diverse genes such as c-myc, cyclin D1, p21 waf1, c-jun, junB, erg-1 and Bcl-2, which are heavily involved in cell survival and proliferation (Cirillo et al., 2008; Walker et al., 2013).

The suppressors of cytokine signaling (SOCS) proteins are negative regulators of the JAK/STAT pathway and inhibit cytokine signaling. SOCS proteins are thought to attenuate this signaling by inhibiting JAK activity or by promoting protein degradation. Moreover, SOCS3 has been identified as an inducible suppressor of leptin signaling, and it has been proposed that prolonged Ob-Rb stimulation can attenuate leptin signaling via SOCS3 in human embryonic kidney cells and in the mouse hypothalamus. In breast cancer cells, SOCS3 overexpression has been shown to decrease proliferation and anchorage-independent growth (Palianopoulou et al., 2011). SOCS3 gene expression is stimulated by various cytokines, including GH, prolactin, ILs and insulin, but is inhibited by glucocorticoids. The growth and function of the mammary gland are regulated by cytokines and modulated by SOCS proteins. STAT3 and potentially STAT1 and STAT5 can stimulate SOCS3 gene transcription (Le Provost et al., 2005) and also possess negative regulatory effects on JAK signaling, and this suppressive action is known to block the signaling mediated by cytokine receptors in a classical feedback loop (Pijet et al., 2013). Thus, one potential mechanism for leptin resistance is the increased hypothalamic expression of SOCS3, a feedback inhibitor of the JAK-STAT pathway that prevents STAT3 activation. Ample studies have confirmed the important role of SOCS3 in leptin resistance and obesity. However, the degree to which SOCS3 participates in the regulation of energy homeostasis in non-obese conditions remains largely undetermined (Matarazzo et al., 2012).

The purpose of this study was to measure the expression of JAK2, STAT3 and SOCS3 in breast cancer patients and to determine the associations between the expression of these genes and BMI.

2. Materials and methods

2.1. Study population

This study was a cross-sectional prospective study conducted at the Maternal–Perinatal Hospital "MónicaPretelini" (HMPMP), Health Institute of the State of Mexico (ISEM), Toluca, Mexico, from January to December 2011. The identified women as potentially having a breast tumor via mammogram were referred to the Imagenology Service of the HMPMP for a Tru-Cut biopsy to determine whether their breast lesions were malignant or benign. Patients undergoing antineoplastic or hormonal therapy were excluded from this study.

2.2. Clinical measurements

We measured the weight (kg), height (m; Seca, GmbH, Germany) and waist circumference (cm) of all participants. BMI was calculated as weight (kg) divided by height (m) squared. Women were classified as (a) normal weight (BMI <24.9 kg/m^2), (b) overweight (24.9 kg/m^2 < BMI < 29.9 kg/m^2) or (c) obese (BMI >30 kg/m^2). The participating women were also categorized according to the Breast Imaging Reporting and Data System (BI-RADS) score and the St. Gallen criteria, the cutoffs/criteria used to determine whether women had cancerous tumors or benign lesions were 4 to 6. The biopsies were taken under anesthesia using the ultrasound-guided (Voluson E8, GE Healthcare,USA) Tru-Cut (Angiotech Pharmaceuticals, Inc., Canada) biopsy technique. One sample from each biopsy was stored frozen at −80 °C until processing, and a second sample was placed in saline solution for immediate histopathological analysis.

2.3. Pathology

Hematoxylin and eosin (HE) staining was performed on the paraffin-embedded biopsied tissue sections. Immunohistochemical analyses of the cancer tissues were conducted at the Pathology Service of the Oncological Cancer Center (COE), ISSEMYM, Toluca, Mexico. If the tissue samples were determined to be positive for cancer, an immunohistochemical analysis (VentanaBenchMark, Ventana Medical Systems Inc., Tucson, AZ) was performed. The tissue sections (4 μm) were formalin-fixed (fixation time 6–8 h), paraffin-embedded and analyzed for the presence of estrogen receptor (ER; anti-ER, 1DS clone), progesterone receptor (PR; anti-PgR, RBT22 clone) and HER-2/neu (c-erb-2 clone, her-2/neu).