Targeting Inflammation in Breast Cancer Pathogenesis

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Risk Factors for Breast Cancer
• Gender
• Age
• Race
• Diet high in fat
• Early onset of menses and late menopause
• Late or no pregnancies
• Family history (BRCA1, BRCA2)
• Dense breast tissue
• Alcohol consumption
• Hormone supplementation


Obesity and Breast Cancer
• Risk factor for development of hormone receptor-positive breast cancer in postmenopausal women.
• Poor prognostic factor for breast cancer patients regardless of menopause or hormone receptor status.
• Altered levels of hormones (estrogen, insulin, IGF-1), adipokines (leptin, adiponectin) and pro-inflammatory mediators (TNFα, IL-1β, PG) contribute to obesity-related breast carcinogenesis.

Obesity, Estrogen and Increased Risk of Postmenopausal Breast Cancer
• After menopause, peripheral aromatization of androgen precursors in adipose tissue yields estrogen.
• Increased risk of ER/PR-positive breast cancer in obese postmenopausal women has been attributed, in part, to elevated levels of circulating estradiol.
• Obesity causes inflammation in visceral and subcutaneous fat. Inflammatory mediators induce aromatase, the rate-limiting enzyme for estrogen biosynthesis.
• Direct link between obesity → breast inflammation → aromatase expression was previously unknown.

Obesity Causes An Inflammatory State

Hypothesis

Obesity induced inflammation will be a/w increased levels of pro-inflammatory mediators (COX-2, TNF-α, IL-1β) leading, in turn, to elevated aromatase expression in breast tissue and visceral fat.

Objectives

• To investigate whether the obesity→inflammation→aromatase axis is deregulated in the mammary gland and visceral fat in mouse models of obesity.

• To elucidate the signal transduction pathway that mediates the increased levels of aromatase in obesity.

• To determine whether obesity is associated with inflammation in the human breast.

Diet Induced Obesity: Experimental Design

C57BL/6J mice (n=40)

4 wks of age (n=20, OVX)

5 wks of age, begin 10-wk treatment with LF [10 kcal%] or HF [60 kcal%] diets

Diet Induced Obesity Causes Focal Inflammation in the Mammary Gland and Visceral Fat

Diet Induced Obesity is Associated with Increased Levels of Pro-inflammatory Mediators

Levels of Aromatase are Increased in Diet Induced Obesity
Obesity Causes Inflammation and Increased Aromatase Levels in the Mammary Gland and Visceral Fat of ob/ob (Leptin Deficient) Mice

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Wt/mouse</th>
<th>ob/ob</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory score</td>
<td>0.0 (0.2)</td>
<td>138 (6.2, 284)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Relative TNF-α expression</td>
<td>0.0 (0.4, 1)</td>
<td>4.9 (1.7-9.7)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Relative IL-1β expression</td>
<td>1.8 (0.4, 2.3)</td>
<td>29 (3.8, 97.3)</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>Relative Cox-2 expression</td>
<td>1.8 (0.4, 2.3)</td>
<td>38 (12.5, 76)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Relative aromatase expression</td>
<td>1.2 (0.2, 5.1)</td>
<td>6.1 (3.4, 76)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Aromatase activity</td>
<td>6.5 (0.12, 210)</td>
<td>15 (1.0, 570)</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
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Diet Induced Obesity Leads to Elevated Levels of Pro-Inflammatory Mediators in the Stromal Vascular Fraction of the Mammary Gland

How Does Obesity Increase Aromatase Levels?

- Lipolysis is increased in obesity.
- Saturated fatty acids released by adipocytes activate the TLR4→NF-κB pathway in macrophages inducing pro-inflammatory mediators: including TNF-α, IL1-β and Cox-2.
- Question: Could saturated fatty acid-mediated induction of pro-inflammatory mediators in macrophages induce aromatase in other cell types via a paracrine mechanism?

Saturated FA-mediated Induction of Pro-inflammatory Mediators in Macrophages Induce Aromatase in Adipocytes

Diet Induced Obesity Causes Elevated Levels of Aromatase and Estrogen-Inducible Target Genes in Adipocytes

Conclusions: Mouse Model

- Obesity caused inflammation in the mammary glands and visceral fat in mice.
- Increased levels of pro-inflammatory mediators (TNF-α, IL1-β, Cox-2), known inducers of aromatase, were found in the stromal vascular fraction (contains macrophages, endothelial cells, fibroblasts) of the mammary gland which includes macrophages.
- Increased aromatase levels in both the SVF and adipocyte fractions of the mammary gland suggests a paracrine mechanism involving cross-talk between activated macrophages and other cell types.
Next Steps - CSL-B in Humans

- In obesity, activation of the TLR4→NF-κB pathway in macrophages is likely to be responsible for increased production of pro-inflammatory mediators leading, in turn, to elevated aromatase expression and estrogen synthesis.
- Hypothesis: Inflammation (CLS-B) occurs in the breast tissue of most overweight/obese women.

Study Design

- Normal breast white adipose tissue was obtained from 30 women who underwent surgery.
- Routine H&E staining and CD68 IHC was performed.

Crown-Like Structures are Common in the Breasts of Overweight and Obese Women

Crown-Like Structures Noted

MSKCC 10-040: Prospective Tissue Acquisition

Severity of Breast Inflammation Correlates with increased BMI
**Conclusions**

- Inflammation (CLS-B) occurs in the breast tissue of most overweight and obese women.

- In both obese women and experimental models of obesity, breast inflammation was paralleled by elevated levels of pro-inflammatory mediators (TNF-α, IL-1β, COX-2, PGE₂).

- In obesity, activation of the TLR4 → NF-κB pathway in macrophages is likely to be responsible for increased production of pro-inflammatory mediators leading, in turn, to elevated aromatase expression and estrogen synthesis.

**Next Steps**

- Further exploration of the obesity → inflammation → aromatase axis to explain the link between obesity and the increased risk of HR-positive breast cancer in postmenopausal women.

- Development of CLS-B as a biomarker of breast cancer risk or poor prognosis.

- Interventions (lifestyle, diet, pharmacological) that disrupt the obesity → inflammation axis may be useful for reducing the risk of breast cancer.