Expression of cox-2 in breast cancer

Immunohistochemical study

Thesis

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INTRODUCTION

Breast cancer ranks the first malignancy affecting females, contributing 30% of all female cancers. Breast cancer is second only to lung cancer as a cause of cancer death in females, almost one of every three affected women will die of the disease. The median age is 50 years, and the majority of patients in the west are postmenopausal **(*El Bolkainy et al., 2005*).**

WHO classified breast cancers into five types; epithelial tumors, mesenchymal, mixed epithelial and mesenchymal, miscellaneous tumors and metastatic tumors. TNM staging was done according to size of primary tumor, regional lymph nodes state and distant metastasis **(*Tavassoli et al., 2003)***.

Prostaglandins are derivatives of fatty acids that are produced in most tissues of the body and have varying physiologic actions. Prostaglandins, thromboxanes and leukotrienes are all classified as members of the eicosanoid class. The first Prostaglandins were so named after their initial isolation from semen in the 1930's because they presumably were added by the prostate. Since then they have been found in most every tissue in the body ***(Needleman et al., 1986***).

Numerous studies have demonstrated that the levels of prostaglandins are greater in various cancers, including breast cancer and colon cancer, than in normal tissues **(*Hwang et al., 1998*).**

Elevated levels of prostaglandin (E_2) have been detected in cultured human breast cancer cells as well as in invasive human breast cancers and are associated with negative hormone receptor status and increased metastatic potential ***(Half et al., 2002)*** .

Cyclo-oxygenases 1 and 2 (COX-1 and COX-2) are the key enzymes in prostaglandin biosynthesis ***(Costa et al., 2002)***.

COX-1 is constitutively expressed in most mammalian tissues and is thought to carry out "housekeeping" functions such as cytoprotection of the gastric mucosa, regulation of renal blood flow, and control of platelet aggregation ***(Zimmermann et al., 1999)*.**

In contrast, expression of COX-2 is not detectable in most healthy tissues but can be induced in response to cell activation by proinflammatory cytokines, growth factors, and tumor promoters, and its role has been connected to inflammation and carcinogenesis ***(Ristimäki et al., 2002)***.

COX-2 overexpression has been observed in colon, head and neck, lung, prostate, stomach, and breast cancer **(*Costa et al., 2002*).**

COX-2 metabolizes the cell membrane fatty acid, arachidonic acid, to yield diffusible PGs. PGE_2 has been found to be the most abundant PG produced by epithelial cells and is released through the basolateral cell compartment into the underlying stroma. In vitro studies have demonstrated that PGE_2

can stimulate epithelial cell proliferation and motility, thereby contributing to neoplastic progression ***(Shim et al., 2003).***

Expression of the COX-2 isoform (but not that of COX-1) is elevated in a variety of human malignancies and in premalignant lesions. Functionally, COX-2-derived prostanoids have been shown to promote angiogenesis, induce invasion, and increase metastasis ***(Ristimäki et al., 2002***).

Cyclooxygenase-2 (COX-2) is overexpressed in breast cancer and may have a role in regulating tumor growth via effects on angiogenesis, cell proliferation, or apoptosis ***(Davies et al., 2003*).**

COX-2, an inducible prostaglandin synthase, has been shown to be important in mammary carcinogenesis, being associated with increased tumor size and unfavorable outcome in breast cancer. Animal studies indicate that COX-2 inhibition is effective in the prevention and treatment of mammary cancers ***(Tan et al., 2004***).

AIM OF THE WORK

The aim of this work is to study the immunohistochemical expression of COX-2 in breast cancer in correlation with clinicopathologic data such as age, histological grading, tumor size, lymph node status, Estrogen /Progesterone receptors status and HER2/neu amplification.

CONTENTS

Anatomical Hints

The breasts are normally paired organs lying on the anterior chest wall between the second rib and seventh rib. The nipples are centrally placed in the midclavicular lines, along which supernumerary nipples may occur. The anterior surface of the breast is the dermis, to which it is attached by fibrous bands called Cooper's ligaments, and the posterior surface is the pectoral fascia. Topographically, the mammary tissue is divided into upper inner, upper outer, lower inner and lower outer quadrants; the subareolar area; and the axillary tail of the upper outer quadrant, which has the axillary and serratus anterior fascia as its posterior surface.

The arterial blood supply is derived from axillary and intercostals arteries and drainage is into the axillary and internal mammary veins. Lymphatic drainage is to the axillary, subclavicular, and the internal mammary lymph node groups. From the upper outer quadrant, drainage is predominantly to axillary lymph nodes and from the inner quadrant it is greater to internal mammary chain of nodes. A high degree of overlap in the lymphatics allows drainage to multiple lymph areas. The nerves are branches of thoracic segmentals ***(Carter, 1996).***

Histologically, the nipple complex is the focal point of the breast. Several collecting ducts connect the lactiferous sinus to the surface of the skin. Characteristically, the skin of the nipple projects 1-2 cm out over the skin of the breasts to form the areola. Numerous sebaceous glands (the glands of Montgomery) are present within the areolar skin. Circular and radiating smooth muscle bundles are present in the dermis and subcutaneous tissue. The lactiferous

sinus is the site of confluence for the twenty ductular units that radiate out into the mammary parenchyma (***Carter, 1996***).

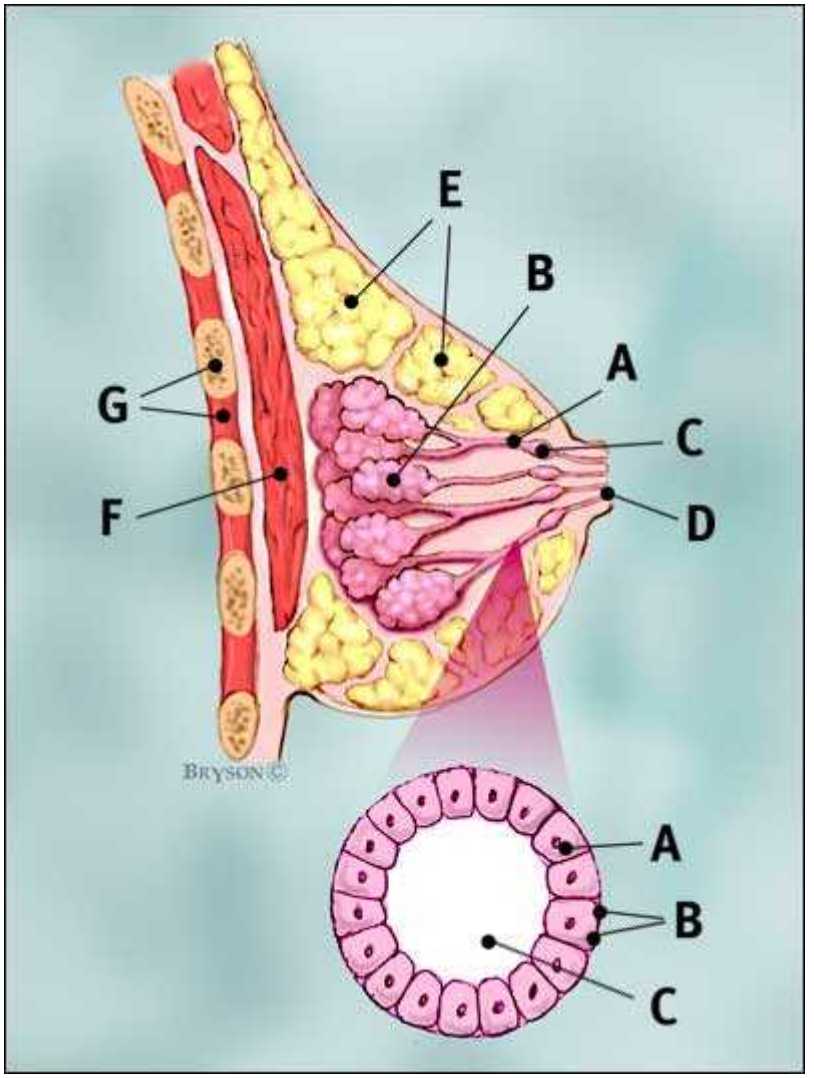

Figure (1): Anatomy of the breast

Breast profile:
A ducts
B lobules
C dilated section of duct to hold milk
D nipple
E fat
F pectoralis major muscle
G chest wall/rib cage

Enlargement:
A normal duct cells
B basement membrane
C lumen (center of duct)

Each ductular unit is made up of large or main duct that progressively branches to form lobules, which are composed of terminal ducts and their supporting stroma. The terminal duct lobular unit was described as the area in which secretion occurs and in which most carcinomas arise. The terminal ducts may be classified as extralobular or intralobular depending on their location relative to lobular stroma (***Wellings, 1980***).

The epithelial elements of the breast are supported by the stroma, which is composed of varying amounts of adipose and fibrous tissues. Before puberty, adipose tissue is dominant. During adolescence, fibrous tissue increases and becomes more cellular as the lobules develop within it. During the postmenopausal period, there is usually a decrease in the cellularity of the stroma, with replacement of the fibrous tissue by an adipose component. The lobules are decreased in both number and size (***Carter, 1996***).

Mammary epithelium is ectodermally derived and in many ways similar to that of sweat glands. It is composed of an inner epithelial cell layer and an outer myoepithelial layer, which separates the epithelial cells from the basement membrane (***Russo and Russo, 1987***).

In the resting breast, the collecting ducts and the lobules are morphologically similar; however ***Rudland, 1993,*** has described subtle immunohistochemical differences between epithelial cells of large ducts, lobular ducts and acini.

By immunohistochemistry, epithelial cells are usually well stained when antibody to epithelial membrane antigen (EMA) is used, whereas the myoepithelial cells are stained when antibodies to smooth muscle (Actin) is used (***Carter, 1996***).

S-100 may be localized to the epithelial cells and/or myoepithelial cells and different molecular weight keratins are variably distributed between the cell types. Keratin, especially low molecular weight, can reliably be found in both benign and malignant duct cells. Cells with apocrine metaplasia characteristically show false-positive intense granular cytoplasmic staining for virtually all antibodies. Antibodies to lactalbumin localize to terminal ducts, especially during lactation (***Carter, 1996***).

RISK FACTORS OF BREAST CANCER

It is almost wrong to think of breast cancer as having a single cause and should be thought as a multi-stage process, which is influenced by different factors **(*Baron et al., 1994*):**

1. Sex incidence:

Breast cancer is rare in male. The ratio female: male is 100:1 (***Boutros et al., 1982)***. Higher incidence in male is recorded in Egypt and this is attributed to altered estrogen metabolism occurring in bilharzial patients; it represents 1:27 (***El-Bolkainy et al., 2005***).

2. Age:

Recent review states that the single most important risk factor after sex is age, with the risk of developing breast cancer at the age 60 being 14 times than the age 30 (***Henderson, 1993***). A bimodal age distribution of the patients with breast carcinoma was described with observation that there are two peaks of age at 40 and again at 65, suggesting that two types of breast cancer may exist. Carcinomas in younger women have a slightly poorer prognosis than those detected in the older age group, primarily because of differences in stage at the time of diagnosis **(*Norris et al., 1970*)**. The median age for breast cancer in Egypt is 46 years, and 60.5% of patients are premenopausal ***(El-Bolkainy et al., 2005*)**. There is a striking increase in the incidence of breast cancer during the perimenopausal period from ages 45 to 49 in particular. It is of interest to note that the increased risk of postmenopausal breast cancer is not seen in the orient ***(Carter, 1996***).

3. Pregnancy and lactation :

Carcinoma of the breast associated with pregnancy or lactation is associated with a higher incidence of axillary lymph node metastases and disseminated disease ***(Anderson et al., 1996)***.

4. Genetic factors:

During the past years, it has become evident that a mutation in a gene BRCA-1 is responsible for an inherited predisposition to breast and ovarian cancer, it has been localized to chromosome17q21 (***Weber et al., 1994***). A similar group of women with breast cancer, especially those developing breast cancer at young age, have been associated with a gene termed BRCA2, which has been localized to chromosome 13q 12-13. Women with BRCA2 mutation are at elevated risk of breast but not ovarian cancer (***Carter, 1996***). The BRCA1 and BRCA2 genes were discovered in 1994 and 1995 respectively, and commercial tests for mutations in these genes are now available. Another genetic mutation associated with heritable breast cancer is found in P53 tumor suppressor gene that is mutated in 25-40% of sporadic breast cancer. Mutation of P53 have been also associated with Li-Fraumeni syndrome which is a susceptibility to breast cancer, sarcomas, and other neoplasms in children and young adults (***Garber,1991***).

5. Risk factors related to steroid hormones:

The marked association of breast cancer with female sex has led investigators to the view that hormones play an important role in breast carcinogenesis. Studies have documented that nulliparous women have twice the risk of developing breast cancer as women who have borne children (***Wooster et al., 1994***).

Women who have borne children before age 19 have half the risk of breast cancer as those who have borne their first child in fourth decade. Women with menarche before age 13 and a late menopause have twice the incidence of carcinoma compared to women with menarche after 13 and an early menopause (***Hulka et al., 1994***).

6. Environmental factors:

A) Infection: It has been shown that transmissible viral agent is associated with high risk of cancer. Viral bodies have been discovered in human milk as well ***(Seman and Dmochowski, 1973***). However, there is no evidence to indicate that human breast cancer is virally induced (***Carter, 1996***).

B) Irradiation: Briefly, the melatonin hypothesis postulates that exposure to electromagnetic field reduces melatonin synthesis, leading to increased concentration of circulating estrogen. Women with the greatest potential for occupational exposure were at higher risk of breast cancer (***Coogan et al., 1996***). Women whose breasts have been exposed to radiation, either therapeutic or diagnostic are at elevated risk (***Carter, 1996***).