OSTEOPROTEGERIN AS A PROGNOSTIC MARKER IN PATIENTS WITH BREAST CANCER

Thesis

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Abstract

Bone is a common site for metastasis in breast cancer; approximately 75% of women with advanced breast cancer will develop bone metastases. Bone metastases associated with breast cancer are predominately osteolytic. OPG overexpression by breast cancer cells enhances osseous tumor growth. OPG promote tumor cell survival and protect them from TRAIL induced apoptosis.

The present study aimed to assess the relevance of OPG to other prognostic factors in breast cancer patients with and without bone metastases.

The study was conducted on eighty females who were divided into four groups: group I (n=20) healthy females as a control group, group II (n=20) non-metastatic breast cancer patients, group III a (n=20) breast cancer patients with bone metastases not receiving Bisphosphonates treatment and group III b (n=20) breast cancer patients with bone metastases and were receiving Bisphosphonates treatment.

All participants were subjected to a thorough clinical assessment, and estimation of blood levels of fasting glucose, ALT, AST, alkaline phosphatase, urea, creatinine, bilirubin, and OPG.

The serum levels of OPG showed a significant increase in breast cancer patients (with and without bone metastasis) compared to the control group. Also, a significant increase in OPG level was found in breast cancer patients with bone metastasis with Bisphosphonates treatment compared to those without Bisphosphonates treatment. Also, a significantly positive correlation was found between OPG and each of tumor size and lymph node involvement.

This means that, OPG may be used as a prognostic marker in breast cancer patients.

Key words: Breast cancer- OPG- Bone metastasis.

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ALT	Alanine transaminase
ALP	Alkaline phosphatase
ApppI	Triphosphoric acid 1adenosine
AST	Aspartate transaminase
BP	Bisphosphonates
BMSCs	Bone marrow stromal cells
BMD	Bone mineral density
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CRP	C- Reactive protein
[NFAT] c1	Calcineurin/nuclear factor of activated T cells
Cbfa1	Core binding factor alpha 1
DDH	Death domain homologous
DR	Death receptor
DC	Dendritic Cells
DCIS	Ductal carcinoma in situ
ELISA	Enzyme Linked Immuno- Sorbent Assay technique
ESR	Erythrocyte Sedimentation Rate
ER	Estrogen receptor
ECM	Extracellular Matrix
ERK	Extracellular signal-related kinase
FPP synthase	Farnesyl diphosphonate synthase
FBS	Fasting blood sugar
FNAC	Fine Needle Aspiration and Cytology
GGPP	Geranyl geranyl diphosphate
Grb-2	Growth factor receptor binding protein-2

HRT	Hormone replacement therapy
HER2	Human epidermal growth factor receptor 2
IGF-1	Insulin growth factor-1
IL	Interleukin
IPP	Isopentenyl diphosphate
LCIS	Lobular carcinoma in situ
M-CSF	Macrophage- colony stimulating factor
MMP-9	Matrix metalloproteinase-9
mRNA	Messenger RNA
MITF	Microphthalmia transcription factor
MAPKs	Mitogen-activated protein kinases
NBR2	Near breast cancer gene 2
N-BPs	Nitrogen containing bisphosphonates
Non NBPs	Non-Nitrogen containing bisphosphonates
OPGL	OPG ligand
ODAR	Osteoclast differentiation and activation receptor
OCPs	Osteoclast precursors
OCIF	Osteoclastogenesis inhibitory factor
OPG	Osteoprotegerin
p53	Tumor protein 53
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone related peptide
PI3K	Phosphatidyl Inositol-3-kinase
PUFAs	Polyunsaturated fatty acids
PR	Progesterone receptor
ROS	Reactive oxygen species
SHBG	Sex hormone binding globulin
SRE	Skeletal related event

sRANKL	Soluble RANKL
RANK	The receptor activator for nuclear factor κβ
RANKL	The receptor activator for nuclear factor κβ ligand
TRAFs	TNF receptor associated factors
TR-1	TNF receptor-like molecule 1
TNFSF	TNF superfamily
TGFα	Transforming growth factor alpha
TGF-β	Transforming growth factor-β
TNF	Tumor necrosis factor
TRAIL	Tumor necrosis factor related apoptosis inducing ligand
TNM	Tumor-Lymph node-Metastasis
VAB	Vacuum-assisted breast biopsy
VEGF	Vascular endothelial growth factor
ZOL	Zoledronic acid

Introduction

Breast cancer is the third most common cancer in the world, and in 65% to 75% of patients, with progressive disease, bone metastases are common ***(Lipton et al., 2007).***

Breast cancer displays a high predilection for metastasis to bone, which results in pathological bone fractures, hypercalcemia, spinal cord compression, as well as significant pain burden ***(Mundy, 2002).*** The formation of distant metastases is a multi-step process that includes local tumor migration, intravasation, survival in circulation, extravasation, and the ability to thrive in the metastatic site ***(Chambers et al., 2002).***

Bone remodeling in adults occurs by removal of old bone (resorption) by osteoclasts, followed by new bone formation by osteoblasts. Osteoprotegerin (OPG) and Receptor activator of nuclear factor-kappa β ligand (RANKL) are dominant regulators of bone resorption ***(Paul, 2005)***.

OPG is a 380-amino acid protein that belongs to the tumor necrosis factor receptor family (TNFRs) and is mainly produced by various mesenchyme derived cells such as osteoblasts ***(Woo et al., 2002)*** and bone marrow stromal cells ***(Kondo et al., 2004).*** It differs from the other TNFRs (as RANK and RANKL) due to the lack of a trans-membrane domain, making this a soluble molecule with the ability to bind a number of different ligands. OPG has a wide tissue distribution; it is found in vascular tissues, bone, prostate, testis, kidney, liver, lung, heart and a range of other tissues. Three main species of OPG have been identified,

the most abundant being the 2.2–3 kb species, with two minor splice variant forms of 4.2–4.4 and 6.5–6.6 kb ***(Ingunn and Claire, 2006).***

Expression of OPG and RANKL is controlled by various cytokines, hormones and growth factors such as transforming growth factor-β (TGF-β) ***(Thirunvakkarasu et al., 2001)*** and insulin-like growth factor-1 (IGF-1) ***(Rubin et al., 2002)***, and it is the ratio of OPG/RANKL that determines the pool size of active osteoclasts ***(Hofbauer et al., 2000).***

RANKL has an important role at various stages of osteoclast differentiation and function. The fusion of osteoclast precursors to form multinucleated cells, their differentiation into mature osteoclasts and the attachment of osteoclasts to bone and activation to resorb bone are all influenced by RANKL ***(Lacey et al., 1998)***. OPG is a soluble decoy receptor for RANKL that can inhibit the osteoclastogenic interaction between RANKL and RANK ***(Blair et al., 2007).***

Osteoclastogenesis (as the first step of establishment of micro-metastasis) occurs when tumor cells chemotactically stimulate osteoclast precursor cells (pre-osteoclasts) to fuse and form mature osteoclasts. This osteoclastogenesis process is mediated via the OPG /RANK/ RANKL system and leads to osteoclast-mediated bone resorption. Osteolysis leads to the release of growth factors, resulting to tumor progression ***(Mundy, 2002).*** A study of ***Fisher et al. (2006)*** revealed that OPG overexpression by breast cancer cells enhances osseous tumor growth.

In addition to being central to regulating RANK/RANKL interactions in bone metabolism, OPG can also stimulate cell survival by acting as a receptor for TNF-related apoptosis-inducing ligand (TRAIL) ***(Emery et***

al., 1998). OPG acts as a soluble decoy receptor, binding TRAIL and preventing its interaction with the functional death receptors, thus allowing cells to escape cell death.OPG may be involved in survival of a number of tumor cell types via this mechanism ***(Holenet al., 2002; Shipman and Croucher, 2003and Neville-Webbe et al., 2004)***.

Bisphosphonates are important inhibitors of bone resorption widely used clinically to treat orthopedic disorders, it reduce skeletal morbidity rate by about 25% -40% in patients who present with breast cancer metastasized to bone ***(Rosen et al., 2003).***

There is evidence suggests that bisphosphonates modulate OPG expression and preventing RANK activation**.** Also it increase osteoblast proliferation and up-regulate expression of genes involved in new bone formation ***(Viereck et al., 2002)***

Objectives: (Aim of the work)

The aim of this study was to assess the relevance of OPG to other prognostic factors in breast cancer patients with and without bone metastases.

Breast Cancer

Worldwide, breast cancer is the most frequently diagnosed life-threatening cancer in women and the leading cause of cancer death among women ***(Siegel et al., 2010)***

Anatomy:

The breasts of an adult woman are milk-producing glands situated on the front of the chest wall. They rest on the pectoralis major muscle and are supported by and attached to the front of the chest wall on either side of the sternum by ligaments. Each breast contains 15-20 lobes arranged in a circular fashion. The fat that covers the lobes gives the breast its size and shape. Each lobe comprises many lobules, at the ends of which are glands where milk is produced in response to hormones ***(American Cancer Society, 2009-2010).***

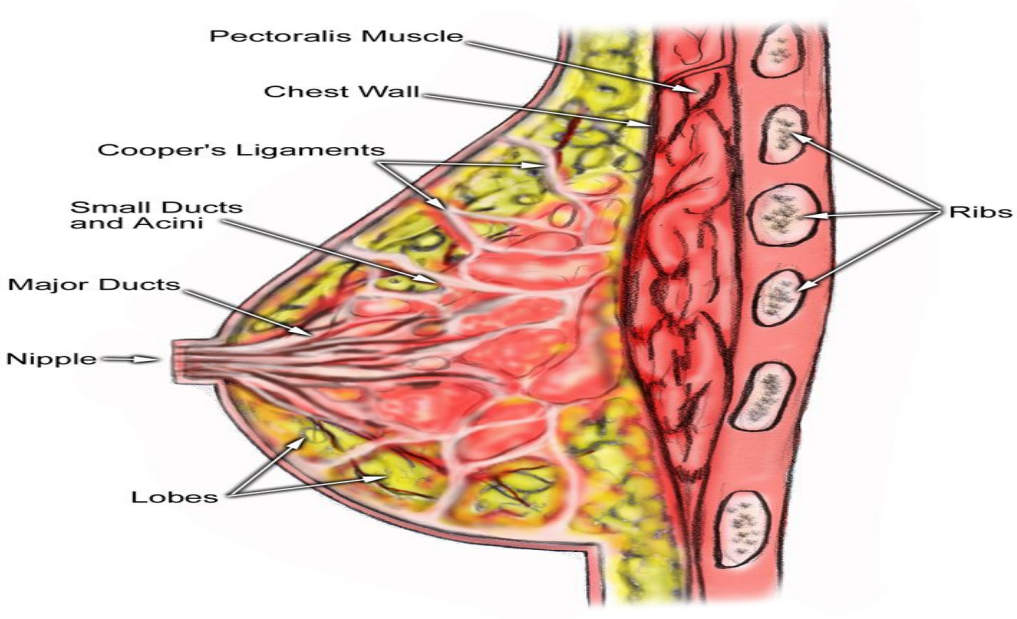

Figure (1): Anatomy of an adult woman breast

Pathophysiology

Breast cancer, like other cancers, occurs because of an interaction between the environment and a defective gene. The mutations known to cause cancer, such as p53 (tumor protein 53), BRCA1 (breast cancer gene 1) and BRCA2 (breast cancer gene 2), occur in the error-correcting mechanisms. These mutations are either inherited or acquired after birth. Presumably, they allow the other mutations, which allow uncontrolled division, lack of attachment, and metastasis to distant organs ***(Clemens et al., 2002)***. Normal cells will commit cell suicide (apoptosis) when they are no longer needed. Until then, they are protected from cell suicide by several protein clusters and pathways. One of the protective pathways is the PI3K/AKT(phosphatidylinositol-3-kinase) pathway; another is the RAS/MEK/ERK(mitogen-activated protein kinase-ERK kinase) pathway. Sometimes the genes along these protective pathways are mutated in a way that turns them permanently "on", rendering the cell incapable of committing suicide when it is no longer needed ***(McCubrey et al., 2007)***. Mutations that can lead to breast cancer have been experimentally linked to estrogen exposure ***(Cavalieri et al., 2006)*** or failure of immune surveillance (the removal of malignant cells throughout one's life by the immune system) ***(Farlex, 2008).*** Also, abnormal growth factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth ***(Wiseman and Werb, 2002 and Haslam and Woodward, 2003).***

Risk factors for breast cancer

Breast cancer, like other forms of cancer, is considered to result from multiple environmental and hereditary risk factors.