Molecular Evaluation of Oxidative Stress and Apoptosis in Breast Cancer

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Aim of Work

To investigate the possible role of oxidative stress in pathogenesis of breast cancer and its relation to apoptosis.

Introduction

Breast cancer is a major global problem, with nearly 1 million cases occurring each year. Over the past several decades, the incidence of the disease has risen worldwide, increasing in developing and developed countries. This rise in breast cancer incidence has been attributed to changes in lifestyle and reproductive factors ***(kurian et al., 2009).***

Worldwide, breast cancer is one of the most common neoplasms in women and is a leading cause of cancer related deaths ***(do Val Carneiro et al., 2009*)**.

Reactive oxygen species (ROS) such as superoxide anions ($O2^{-}$) hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) and nitric oxide ($NO^{\cdot}$) are directly or indirectly involved in multistage process of carcinogenesis **(Cejas *et al., 2004***). They are mainly involved in DNA damage leading sometimes to mutations in tumor suppressor genes. They also act as initiator and/or promotor in carcinogenesis. MDA, a by-product of lipid peroxidation, is said to be involved in DNA adduct formations, which are believed to be responsible for carcinogenesis ***(Ray and Husain, 2002)***.

Human tumor cell lines in vitro produce ROS at a far

greater rate than do non-transformed cell lines and markers of constitutive oxidative stress have been detected in samples from in vivo breast carcinomas ***(Tas et al., 2005***). The deleterious actions of oxidants can be countered by antioxidant defense system in humans such as superoxide dismutase (SOD), glutathione peroxidase, and catalase (CAT) ***(Ray and Husain et al., 2002).***

Reactive oxygen species (ROS) and mitochondria play an important role in apoptosis induction under both physiologic and pathologic conditions. Interestingly, mitochondria are both source and target of ROS. Cytochrome C release from mitochondria, that triggers caspase activation, appears to be largely mediated by direct or indirect ROS action **(*Simon et al., 2000).***

It is of interest that accumulating evidence suggests that oxidative stress-induced apoptosis plays an important role in the anti-carcinogenic effect of several chemo preventive agents ***(Sun et al., 2004).*** Although ROS has a suggesting role in initiation and/or progression of breast neoplasia, its production is a mechanism shared by many chemotherapeutic drugs due to their implication in apoptosis control ***(Mobley and Brueggemeier, 2004)***. Thus the status redox is of great

importance for oncogenetic process activation and it is also implicated in tumor susceptibility to specific chemotherapeutic drugs **(Cejas *et al., 2004)*.**

Cellular proliferation, cellular arrest and cellular suicide appear to be modulated by relative concentrations of electronically modified oxygen derivatives. Cautious use of antioxidants may be appropriate for individuals with tumors or pre-neoplastic growths. ROS offer a therapeutic site in the selective killing of neoplastic cells, without causing harm to normal cells. Indeed, the potential of therapeutically increasing ROS levels in combating disease offers the possibility of a promising opportunity ***(Nazarewicz et al., 2007).***

Nitric oxide ($NO^{\bullet}$) is an intra- and extracellular messenger that mediates diverse signaling pathways in target cells and is known to play an important role in many physiological processes including neuronal signaling, immune response, inflammatory response, modulation of ion channels and phagocytic defense mechanism ***(Tuteja et al., 2004).***

It was found that NO• has a controversial effect on apoptosis ***(Brüue et al., 1999).*** This controversial effect was obvious in human breast cancer, where at low concentration it increases proliferation by increasing synthesis of some cells

cycle protein and in higher concentrations it leads to apoptosis by decreasing translation of some cell cycle proteins ***(Pervin et al., 2008a).***

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List of Abbreviations

Apaf-1	**Apoptotic protease activating factor-1**
AJCC	**American joint committee on cancer**
Apo2L/TRAIL	**Apoptosis 2 ligand/TNF related apoptosis inducing ligand**
BAK	**Bcl-2 antagonist killer**
BAX	**Bcl-2 associated x protein**
BCL2	**B-cell lymphoma 2**
$BCLX_L$	**Long form of Bcl-x**
BH3	**Bcl-2 Homology 3**
BRCA1	**Breast cancer antigen 1**
BRCA2	**Breast cancer antigen2**
CA15.3	**Carbohydrate antigen -15.3**
CAT	**Catalase**
CBB	**Coomassie Brilliant Blue**
CEA	**Carcino-embryonic antigen**
cGMP	**Cyclic guanosine monophosphate**
COX	**Cytochrome oxidase**
CT	**Computed tomography**
DISC	**Death inducing signaling complex**
DNA	**Deoxyribonucleic acid**
DPA	**Diphenylamine**
ECD	**Extracellular domain**
EGFR	**Epidermal growth factor receptor**
ER	**Estrogen receptor**
FADD	**Fas-associated death domain**
Fas	**Fibroblast associated factor**
FasL	**Fibroblast associated factor-Ligand**
Fig	**Figure**
GSH	**Reduced glutathione**
H_2O_2	**Hydrogen Peroxide**
HOCL	**Hypochlorous acid**

HER2	**Human epidermal growth factor receptor 2**
IFN-γ	**Interferon –gamma**
IL	**Interleukin**
ILC	**Invasive lobular carcinoma**
iNOS	**Inducible NOS**
LPS	**Lipopolysaccharide**
iNOS	**Inducible NOS**
MAP	**Mitogen activated protein kinase**
MCA	**Mucine- like carcinoma associated antigen**
MDA	**Malondialdehyde**
MPO	**Myeloperoxidase**
MRI	**Magnetic resonance imaging**
mSOD	**Mitochondrial super oxide dismutase**
mtDNA	**Mitochondrial DNA**
mtNOS	**Mitochondrial NOS**
MUC1-PEM	**MUC-polymorphic epithelial mucin**
NF-kB	**Nuclear factor kappa B**
nNOS	**Neuronal NOS**
NO	**Nitric oxide**
NOS	**Nitric oxide synthase enzyme**
$O_2\bullet^-$	**Superoxide anion**
$OH^\bullet$	**Hydroxyl radical**
$ONOO^\bullet$	**Peroxynitrite**
PET	**Positron emission tomography**
pNA	**p-nitroanilide**
PR	**Progesterone receptor**
RNS	**Reactive Nitrogenous Species**
ROC	**Receiver operating characteristics**
ROS	**Reactive oxygen species**
SPSS	**Statistical Package for the Social Sciences**
TAO	**Total antioxidants**
TBA	**Thiobarbituric Acid**
TBARS	**Thiobarbituric acid reactive substances**

TCA	**trichloroacetic acid**
TNF-α	**Tumor necrosis factor –alpha**
TNM	**Tumor-nodes-metastases**
TPA	**Tissue polypeptide antigen**
VEGF	**Vascular endothelial growth factor**

Breast Cancer

Worldwide, breast cancer is the most common neoplasms in women and is a leading cause of cancer related deaths ***(do Val Carneiro et al., 2009)***. Approximately one out of nine to one out of thirteen women who reach age of ninety in the western world could get breast cancer. It is the second fatal cancer in women (after lung cancer), and the number of cases has significantly increased since the 1970s, a phenomenon partly blamed on modern life styles in the western world ***(laurance, 2006).***

Epidemiology of Breast Cancer

Breast cancer is the most common malignancy among women, with a projected incidence of 178,480 in the United States, over 40,000 women died from metastatic disease in 2007 ***(Jemal et al., 2007).***

Carcinoma of the breast is overwhelmingly a disease of females (female to male ratio is approximately 200:1). It accounts for 22% of all female cancers and causes 20% of cancer deaths in women" the second after lung cancer" ***(Cotran et al., 2005).***

In Egypt, carcinoma of the breast is the most prevalent cancer among Egyptian women and constitutes 29% of national cancer institute cases. It also estimated that the median age of breast carcinoma in Egypt at diagnosis is one decade younger than in countries of Europe and North America and most patients are premenopausal ***(Omer et al., 2003*)**. They reported that tumors are relatively advanced at time of presentation. This study also revealed that the majority of tumors are invasive duct subtype and the profile of hormone receptors is positive for estrogen receptors and/or progesterone receptors in less than half of cases.

Breast cancer screening trial has been done in Cairo by ***Boulos et al. (2005*)** they confirmed that breast cancer is usually diagnosed at an advanced stage. High rates of breast cancer were observed in this study and they suggested that many women in the community with early but palpable breast cancer fail to seek medical attention until their cancer is advanced.

However, ***Macmahon and Cole, (2008*),** suggested that the incidence of breast cancer has been declining since the year 2000. This apparent trend may be at least partially explained by