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Levels of IL-1 beta control stimulatory/inhibitory growth of cancer cells

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1. ABSTRACT

Different cellular signaling pathways operate in response to varying levels of IL-1 beta leading to genotoxic damage, cell apoptosis or cell growth. At high levels of IL-1 beta, cells receiving genotoxic insults engage apoptotic pathways. The IL-1 beta over expressing stable MCF7 cell secreting high level of IL-1 beta peptides undergo cell apoptosis. Cotreatment with an inhibitor of IL-1 beta and TNF- alpha synthesis prevented stilbene estrogen-induced lesions. In addition to direct effect of 17 beta-estradiol (E2) on mitochondria and redox cycling of catechol estrogens, E2-induced overexpression of IL-1 beta can produce an increase in the level of ROS. Our recent data showed that MCF7 cell growth and cyclin D1 expression are suppressed by antioxidants and mitochondrial blockers. Stably IL-1 beta transfected cells secreting moderate level of IL-1 beta peptides stimulated the clonal expansion of MCF7 cells. These studies support that in addition to ovarian estrogens, mitogenic signals may also come from TNF- alpha and IL-1 beta-generated $O_2^{\bullet-}$ and hydrogen peroxide. Further validation of this concept that the concentrations of the peptide interleukin-1 beta within the cells determine its stimulatory or inhibitory signals regulating the growth of estrogen-dependent tumors might result in novel preventive strategies.

2. INTRODUCTION

Elevated level of estrogen is a risk factor for cancer in hormone-dependent organs, particularly breast, endometrium, ovary, prostate, and testis (1,2,3,4,5,6). In 1999, the International Agency for Research on Cancer (IARC) had categorized combined oral contraceptives consisting of the steroid hormone estrogen in combination with a progestogen and postmenopausal estrogen therapy as human carcinogens (2). In 2002, the US National Toxicology Program (NTP) listed steroidal estrogens used in estrogen replacement therapy and oral contraceptives as human carcinogens (3). The exact mechanisms of initiation and progression of estrogen-related cancers are not clear.

Increasing evidence indicate that both endogenous and exogenous estrogen-induced genetic instability are critical for the development of estrogen-dependent cancers. Although ER-mediated genomic signaling pathways play a role in the promotion of initiated cells and progression of tumor, these pathways cannot fully explain estrogen-induced genetic alterations observed in estrogen-dependent cancers. Estrogens after aromatic hydroxylation gets converted into catechol estrogens by cytochrome P450/peroxidase enzymes (7,8,9,10). Catalytic oxidation of catecholestrogens gives rise to estrogen-

quinones, which react with DNA to form adducts (7,10). These adducts can either be stable DNA adducts that remain in DNA unless removed by repair or it can form depurinating adducts that are released from DNA by destabilization of the glycosyl bond (11). Quinone and semi-quinone forms of catechol estrogens-induced DNA adducts are found in various target tissues of cancer (11). LC-MS-MS analysis of mammary tissue extract from rat showed the formation of an alkylated depurinating guanine adduct induced by equine estrogen metabolite 4-hydroxyequilenin (12). Estrogen DNA-adducts have been detected in human breast tumor tissue and healthy tissue by combined nano LC-nano ES tandem mass spectrometry (13). Recently, 4-hydroxy catechol estrogen conjugates with glutathione or its hydrolytic products (cysteine and N-acetylcysteine) were detected in picomol amounts in both tumors and hyperplastic mammary tissues from ERKO/Wnt-1 mice demonstrating the formation of CE-3,4-quinones (14). 2-OHE quinone-derived DNA adducts have been shown to be mutagenic generating primarily G --> T and A --> T mutations in simian kidney (COS-7) cells (15). E(2)-3,4-Q induced rapidly-depurinating 4-hydroxy estradiol (4-OHE(2))-1-N3Ade adduct and abundant A to G mutations in H-ras DNA was observed in SENCAR mouse skin treated with estradiol-3,4-quinone (E(2)-3,4-Q) (16). These studies indicate that estrogen metabolites react with DNA to form adducts in both humans and experimental models, and these adducts generate mutations and may contribute to tumor initiation. However, it is unlikely that these base modifications occur immediately after E2 exposure, as E2 would have to be hydroxylated and oxidized to a reactive intermediate in the endoplasmic reticulum prior to its transport to the nucleus. Although this type of adduction may play a role in the generation of mutations, it appears to be a later event.

Mitochondria are significant targets of estrogen (17,18,19). Recently Felty *et al*, 2005 (20) have reported that physiological concentrations of E2 stimulate a rapid production of intracellular reactive oxygen species (ROS), and ROS formation in epithelial cells depends on cell adhesion, the cytoskeleton, and integrins. In our studies of E2-induced ROS generation in MCF7 and other cells, we were not able to find any hydroxylated estrogen metabolites or their adducts immediately after addition of E2, and this rules out the possibility of ROS generation by redox cycling of hydroxylated estrogens. These events occur earlier than ER-mediated genomic actions. E2-stimulated ROS production does not depend on the presence of the ER in breast cancer cells as the ER negative cell line MDA-MB 468 produced ROS equal to that of ER-positive cell lines MCF7, T47D, and ZR75. ROS formation upon E2 exposure can explain oxidative damage to hormone-dependent tumors and subsequent genetic alterations as reported earlier by Malins and others (21,22,23,24). E2-induced ROS generation also provides mechanistic support to the generation of mutations by physiological concentrations of estrogens (25,26).

Our recent data strongly support that estrogen-induced activation of pro-inflammatory cytokines that results in the oxidative damage and mutation of the testicular and uterine genome is involved in the initiation and promotion of testicular and uterine cancers (5). For example, neonatal exposure of Syrian hamsters or CD mice to synthetic stilbene estrogen (diethylstilbestrol) or natural estrogen (17ß-estradiol, E_2) leads to the development of uterine and testicular tumors (5). Neonatal exposure of hamsters to diethylstilbestrol (DES) produces persistent inflammation in the uteri and testes of pubertal animals. This is accompanied by hyperproduction of the proinflammatory cytokines, TNF- alpha and IL-1 beta, in the uteri and testes of pubertal animals (5). Administration of pentoxifylline, an inhibitor of cytokine synthesis, inhibited the effects of neonatal exposure to DES in the puberatal animals. Pentoxifylline, significantly inhibited all indicators of the inflammatory response, *i.e.*, decreased levels of myeloperoxidase (MPO) activity and TNF- alpha and IL-1 beta mRNA, as well as reduced oxidative damage to DNA as indicated by the levels of hydroxydeoxyguanosine (8-OHdG) (5). Our recent study (Sarkar and Roy, Unpublished) showed that 1) IL-1 beta over expressing stable testicular Leydig TM3 cells show significantly higher level of ROS formation compared to non-transfected TM3 cells. IL-1 beta over expressing stable MCF7, TM3 or HELA cells secreting high level of IL-1 beta peptides showed inhibition of their growth compared to nontransfected cells, and elevated level of p53 protein was detected particularly in MCF7 and HELA cells. Whereas stably transfected cells secreting moderate level of IL-1 beta peptides stimulated the clonal expansion of MCF7 cells. This and above studies indicate that IL-1 beta can produce genotoxic effects as well as support the growth of estrogen-dependent tumors.

Thus, it appears that estrogen exposure can produce genetic alterations by several mechanisms. In addition to ER pathways, E2 can support the growth of tumor cells by other alternative pathways. In this review article, we have focused our efforts describing IL-1 beta as an initiator of DNA damage, which in turn, can produce genetic stability, and the level of IL-1 beta determines whether it will promote or inhibit the growth of estrogen-dependent cancers through autocrine and/or paracrine signaling.

3. INFLUENCE OF ESTROGEN ON IL-1B

Interleukin-1 (IL-1) is a type of biological response modifier that stimulates immune system cells that fight disease, and is involved in inflammatory responses (27,28). There are two known forms of IL-1, a membrane-bound IL-1 alpha and secretory IL-1 beta, which are encoded by two distinct genes and share 27% homology (27,28). These forms exert similar biological effects. Both forms of IL-1 are produced by the body, and can also be made in the laboratory. Interleukin-1 beta (IL-1 beta) is expressed by many cells including macrophage, NK cells, monocytes, and neutrophils. Estrogen increases IL-1 synthesis. Ovariectomy leads to decreased levels of IL-1 synthesis, and this effect is reversed by estrogen replacement (29). Treatment of male Wistar rats with E2 results in an up regulation of the IL-1 beta transcripts (30). Estradiol treatment increases the number of peritoneal

macrophages and the peritoneal macrophages became more sensitive to lutenizing hormone (LH) to produce IL-1 in hamsters (31). Human peripheral monocyte IL-1 activity is modulated by physiologic levels of gonadal steroids (32). Low concentrations of both E2 (10^{-9} M-10^{-10} M) and progesterone (P) (10^{-8} M-10^{-9} M) result in maximal IL-1 stimulation (32). At higher concentrations of both E2 (10^{-7} M) and P (10^{-7} M-10^{-5} M) there is a significant reduction in IL-1 activity.

4. EFFECTS OF IL-1B ON GROWTH AND METASTASIS OF ESTROGEN-DEPENDENT TUMORS

There is increasing evidence that cytokines may play an important role in the progression of ovarian cancer (33). Many cytokines are expressed in the normal ovary. Multiple cytokines are produced by ovarian cancer cells. Five different human ovarian epithelial tumor cell lines and tumor cells isolated from the ascitic fluid of four cancer patients have been shown to express IL-1 and IL-1 genes constitutively (34). Ovarian cancer cells also secrete IL-6 and macrophage colony-stimulating factor, constitutively. Serum IL-6 levels were shown to be higher in ovarian cancer patients than in healthy controls. More over, higher serum IL-6 levels have been found in ovarian cancer compared with other gynecological malignancies. Scambia *et al* (35) reported that 53% of primary epithelial ovarian tumors were IL-6 positive, whereas 35% and 10% of endometrial and cervical cancer patients were found to be IL-6 positive, respectively.

Recently, it has been shown that breast tumor cells express IL-1 alpha, IL-1 beta, and IL-1ra in most specimens tested (36). The IL-1 beta content measured in tissues from >200 invasive breast carcinomas and smaller numbers of ductal carcinoma *in situ* (DCIS) and benign lesions showed that significant titers of IL-1 beta are present within the microenvironment of most breast carcinomas and the proportion of invasive tumors with these characteristics was significantly increased in a subgroup of tumors having very high IL-1 beta content (37). Analysis performed with primary breast cancers revealed a positive correlation between increased IL-1 alpha expression and lymph node metastasis (38). The local expression of IL-1 results in the activation of a population of cells within the human breast tumor microenvironment. It is considered that the activation of the IL-1/IL-1R cytokine family leading to a cascade of secondary cytokines induce the expression of numerous protumorigenic activities such as the expression of IL-8, and subsequently contribute to angiogenesis, tumor proliferation, and tumor invasion (37,39).

IL-1 transcripts have been detected in the androgen-insensitive prostate cancer PC-3 cell line (40). Moreover, IL-1 is present in both stromal and epithelial compartments in human prostate carcinoma specimens. Neonatal s.c. single administration of DES to Syrian hamster induces structural abnormalities and tumors in the uterus and testis. The pathological study showed infiltration of neutrophils and macrophages in the testis. Testis inflammation was also indicated by increased activities of MPO. Cotreatment with an inhibitor of synthesis of IL-1 beta and TNF- alpha, pentoxifylline, decreased DES-induced levels of MPO, 8-OHdG formation, and gene mutations, and prevented DES-induced lesions (5). The mechanism by which cytokines enhance tumor cell growth is not full understood. It appears that cytokines can enhance tumor growth directly by functioning as growth factors, promoting metastasis, and by enhancing tumor angiogenesis (33). Cytokines produced by tumors may modulate immune responses that favor tumor progression. The direct effect of IL-1 on tumor cells still remains to be characterized. IL-1 beta is linked with all the major stages of cancer growth, such as proliferation, adhesion of cancer cells, and angiogenesis, and we have discussed it briefly below.

IL-1 beta and cell proliferation

Previous studies have shown contradictory effects of IL-1 beta on the growth of breast tumor cells. For example, some studies have shown stimulatory effect of IL-1 beta on the growth of tumor cells, whereas others have shown that it exerts inhibitory activity. *In vivo* effect of IL-1 beta on the E2 dependent cancers, except ovary, is scarce. IL-1 beta induces proliferation of ovarian, melanoma and leukemic cells (41,42,43). The addition of the cytokine increases the expression of adhesion molecules, tissue factors and mannosylation of cell surface proteins, these effects in turn contribute to cell growth [44]. Some of the growth related signaling proteins induced by IL-1 beta have been identified and are TNF- alpha, IL-6, IL-8 and TGF beta. The proliferation of normal or malignant ovarian epithelial cells is stimulated by IL-1 beta or TNF- alpha (44). Stimulation of proliferation by IL-1 beta is partially blocked by an antibody against TNF- alpha or by soluble TNF- alpha receptor. IL-1 beta stimulated growth of human ovarian carcinoma cells can be prevented in presence of the antisense to TNF- alpha RNA (43). These studies support the role of IL-1 beta in proliferation. The study indicating influence of estrogen on IL-1 beta induced cell proliferation has been very limited and contradictory. IL-1 beta stimulates the activity of aromatase which in turn results in more estrogen production in the cell (45). However, under similar experimental condition IL-1 beta by itself failed to induce growth thereby suggesting that IL-1 beta is able to induce growth only in presence of estrogen (46). In primary cultures of cortical fibroblasts (CFs), obtained from kidney, responded to enhanced DNA upon exposure to IL-1 beta. This increase was also accompanied by increase in transforming growth factor beta-1 (TGF- beta1) partly explaining the cause of growth in these cultured cortical fibroblasts. Thus growth inducing properties IL-1 beta is dependent upon the cellular environment and has to associate with other partners like estrogen or TGF- beta1 (47). IL-6 stimulates the growth of prostate cancer cell lines LNCaP and pcDNA3 cells (48). IL-6 may function as a growth factor for prostatic carcinoma cells either by autocrine and/or paracrine mechanisms (49). Recently, chronic inflammatory lesions in the prostate resulting in hyperactive prostate epithelium and an increase in proliferative rate have been reported (50). Our recent study showed that IL-1 beta over expressing stable MCF7 cells

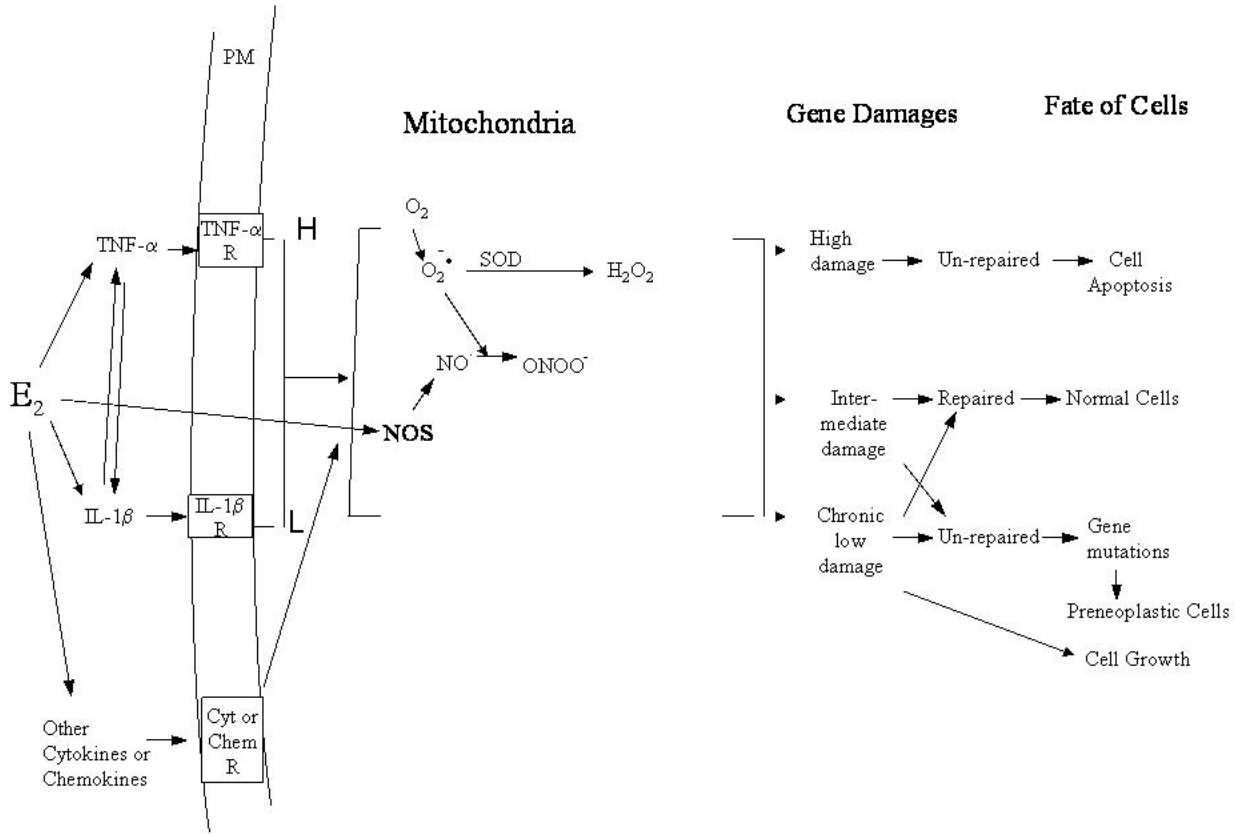

Figure 1. The concentration of the peptide interleukin-1 beta determines its stimulatory or inhibitory growth activity. High concentration of IL-1 beta may produce cell apoptosis, whereas generation of low levels of IL-1 beta in the same cells may stimulate growth of cells.

secreting high level of IL-1 beta peptides compared to showed inhibition of cell growth compared to nontransfected cells, and elevated level of p53 protein was detected in these cells. Whereas stably transfected cells secreting moderate level of IL-1 beta peptides stimulated the clonal expansion of MCF7 cells (Sarkar and Roy, Unpublished). This and other studies indicate that IL-1 beta can support the growth of estrogen-dependent tumors. It is the concentration of the peptide interleukin-1 beta which determines its stimulatory or inhibitory growth activity (Figure 1).

IL-1 beta and angiogenesis

IL-1 beta has been emerging as one of the cytokine assisting tumor cells for angiogenesis. Vascular endothelial growth factor (VEGF) promotes angiogenesis and inflammatory reactions (51,52). IL-1 beta can induce VEGF m-RNA. In human vascular smooth muscle cells treatment with IL-1 beta increased VEGF by transcriptionally up-regulating and stabilizing the m-RNA. The increase in VEGF m-RNA was inhibited by using P38 MAPK inhibitor SB203580 thereby indicating the role of P38 MAPK in the signaling cascade (53). The cells expressing IL-1 beta show generation of more aggressive tumor generation as determined by the metastatic potential of these tumors (51). Treatment of IL-1 beta receptor antagonist shows significantly higher number of capillaries thereby suggesting the potential for inducing vascularization in the cornea (54,55). The inoculation of Lewis lung carcinoma (LLC) cells stably expressing IL-1 beta into C57BL/6 mice showed faster growth of tumors and abundant vasculature as compared to the respective vector control. LLC-IL-1 beta induced tumors showed an increased expression of VEGF and hepatocyte growth factor (HGF) as compared to vector control. Infiltrating cells produced more HGF in response to the over expression of IL-1 beta and that the VEGF induction by IL-1 beta was dependent upon HGF (56). Recently angiogenic property of IL-1 beta has been demonstrated using IL-1 beta knock out mice. In the IL-1 beta knockout mice, the formation of local tumor or lung metastases is abrogated when B16melanoma cells are injected to the mice. In parallel, the wild type mice show invasivness and angiogenesis of the B16 melanoma cells into the lung. This and other studies indicate that IL-1 beta helps in metastasis and therefore it is a key player in the cancer progression (57).

IL-1 beta and cell adhesion

IL-1 beta increases tumor invasiveness and metastasis (58,59,60). IL-1 beta expression at the site of tumor development enhances the expression of adhesion molecules on endothelial and malignant cells, thereby facilitating the invasion of malignant cells into the

circulation and distribute it to remote locations (61,62). It has been demonstrated that tumor cells engineered to secrete IL-1 beta resulted in more severe and invasive pattern of tumor formation as compared to the control. IL-1 beta induces adhesion molecule expression on both malignant and endothelial cells (63). Exogenous IL-1 beta enhances the expression intracellular adhesion molecule (ICAM-1) and matrix metalloproteinase-2 (MMP-2) and both of these molecules have been augmented in cancer progression to metastasis. Recent studies have proposed that endogenous IL-1 beta promotes metastasis of melanoma cells by upregulating tumor-cell binding to the endothelial cells via induction of adhesion molecules such as ICAM-1 (63) and vascular cell adhesion molecule-1 (VCAM-1). IL-1 and TNF- alpha may promote tumor growth by increasing production of IL-6, because .IL-6 enhances ovarian tumor cell growth by promoting tumor cell attachment and migration (33). In an *in vivo* study, lung derived A549 cells engineered to express IL-1 beta showed higher rate of metastasis by enhancement of multiple events governing metastatic cascade (63). This included adhesion coupled with invasion and angiogenesis. The proliferative index of these cells did not show any difference between the control and the IL-1 beta over expressing clone *in vivo* as determined by KI-67 staining. However, vascularization as determined by CD31 staining and infiltrated macrophages as determined by scavenger receptor staining was significantly high. Both these markers support the role of IL-1 beta in cell adhesion. MMP-2 which is responsible for the degradation of the extra cellular matrix is increased upon exposure to IL-1 beta and this protein is crucial to degrade the basement membrane to allow the smooth invasion of the tumor cells.

5. IL-1B-MEDIATED GENERATION OF GENOTOXIC DAMAGES

Exposure to human fibroblast cells to IL-1 beta or TNF- alpha releases significant levels of $O_2^{\bullet-}$ (5 nmol/h/10^6 cells) (64,65). Here, we discuss in brief evidence in support of induction of IL-1 beta can lead to oxidative DNA damages resulting in gene mutations or cell growth depending upon its level in the estrogen-dependent target organ of cancers. In addition to direct effect of E2 leading to mitochondrial generation of ROS (20), and redox cycling of catechol estrogens producing ROS (10), over expression of IL-1 beta can also produce an increase in the level of ROS. There is increasing evidence that inflammation produces genotoxic effects (5). The inflammatory response coupled with increased proliferation may put cells at high risk for DNA damage and development of neoplastic lesion. Oxidants produced by inflammatory cells can produce mutations in both bacterial and mammalian cells *in vitro* (66,67). TNF- alpha exposure of cells has been shown to result in single-strand DNA breaks and other types of DNA damage (68). Increasing evidence shows that estrogen through oxidative stress and/or its metabolic products induce genetic alteration affecting both the structural as well as function of the genes (6). Various types of numerical and structural changes at chromosome level as well as at the gene level and the functional changes of the genes have been documented in the estrogen-related human cancers as well as in estrogen-induced cancers in animal model (6). How estrogen exposure produces these changes in the cells is not clear. Interaction of estrogen-induced oxidants and estrogen metabolites with DNA has been shown to generate mutations in genes (11,10,5,6). Macrophage-secreted cytokines, such as IL-1 beta and TNF- alpha induce significant levels of directed infiltration of leukocytes, which may be due to induction of synthesis of other, more potent chemotactic peptides. Monocytes-macrophages are not simply phagocytic cells of the immune system but also are involved in important endocrine processes during development and express estrogen receptors and the ability to convert androgen to estrogen by aromatase. Our recent studies show that, unlike the mild and transient inflammatory response to hyperstimulation with LH or human chronic gonadotropin (hCG), neonatal DES exposure produces a significant and persistent inflammatory responses in the uterus and testes of hamsters. In many respects, the leukocyte infiltration in the uterus in response to neonatal DES exposure as observed during our preliminary studies is strikingly similar to the inflammation in the uterus elicited by tissue injury or lipopolysaccharide (LPS) (4). In the case of women undergoing menopause, treatment with hormone replacement therapy results in a significant increase in the levels of intracellular activity and MPO-release (69,70). Neonatal exposure of hamsters to DES leads to a several fold induction of myeloperoxidase in the uterus and this was accompanied by hyperproduction of proinflammatory cytokines, TNF- alpha and IL-1 beta in the uterus in pre- and pubertal animals. Our recent data reveal that the co-culture of activated macrophages and endometrial cells leads to increased formation of 8-hydroxy guanosine in DNA compared to unexposed cells. Moreover, exposure of neonatal hamsters to DES produced a 2-fold increase in 8-OHdG levels in uterine and testicular DNA and mutations in the uterine and testicular genome compared to age-matched control uteri. These findings showing ROS formation upon E2 and/or IL-1 beta exposure is not only able to explain oxidative damage to estrogen-dependent breast tumors and subsequent genetic alterations as reported earlier, but also provide mechanistic support to the generation of mutations by physiological concentrations of estrogens (26). Since IL-1 beta can produce ROS, it can serve as initiator of DNA damage (Figure 2). Thus, IL-1 beta can be protumorigenic and may play a role in the initiation of estrogen-dependent cancers.

6. E2 CELL SIGNALING THROUGH ROS

There is a growing body of evidence that cytokines can act normally to stimulate the generation of superoxide anion ($O_2^{\bullet-}$)and hydrogen peroxide (H_2O_2) in their target inflammatory cells (64,65). These reactive oxygen species have been shown to be obligatory for a number of important proliferative responses induced by cytokines. Exogenously added H_2O_2 has been shown to stimulate growth responses in a variety of cell types, including HEK cells (71). Liposomes containing superoxide dismutase (SOD) or catalase inhibited *in vitro* estrogen-induced proliferation of Syrian hamster renal proximal tubular cells (72). The cytokines IL-1 beta and

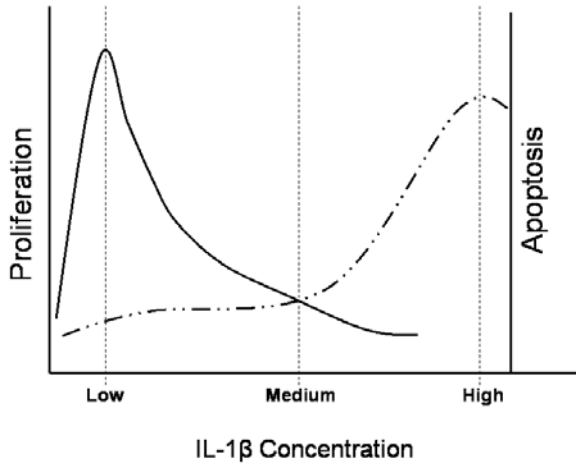

Figure 2. The concentration of the peptide interleukin-1 beta within the cellular microenvironment determines its stimulatory or inhibitory paracrine and/or autocrine signals as well as its genotoxic effects regulating the growth of estrogen-dependent cells. Exposure of toxicological concentration of estrogen to cells may produce compounds, such as proinflammatory cytokines, such as IL-1 beta and TNF- alpha that may be growth inhibitory at high concentrations. High (H) concentrations of these cytokines may be directly toxic to the cells by activating transduction pathways coupled to receptors (Rs), such as [IL-1 beta R, TNF- alpha-R, other cytokine (Cyt) or Chemokine (Chem)] located directly on the membrane producing high levels of reactive oxygen and nitrogen species (RO/NS). Whereas exposure of physiological doses of estrogen to the same cells under this condition resulting in the moderate and low production of proinflammatory cytokines leading to the generation of moderate or low (L) levels of reactive oxygen and nitrogen species (RO/NS) may stimulate growth signals. This may regulate the induction of growth of estrogen-dependent cells. Alternatively, estrogen exposure may also enhance the expression of NOS (nitric oxidase synthase) leading to the formation of NO (nitric oxide). NO after reacting with superoxide resulting in the production of peroxinitrate ($ONOO^-$) may also participate in growth signaling.

TNF- alpha are known to cause the release of $O_2^{\bullet-}$ from human fibroblast cells. Co-treatment with an inhibitor of IL-1 beta and TNF- alpha synthesis, pentoxifylline, prevented estrogen-induced testicular preneoplastic lesions (5). IL-1 beta induced signaling is triggered within a few minutes after binding to its receptor (73,74). The downstream targets of the cascade of IL-1 beta signaling are not well defined and are mostly dependent upon the cell type for its specificity. The biochemical events which are associated with IL-1 beta signaling includes hydrolysis of GTP, phosphotidylcholine, phosphotidylserine or phosphotidylethanolamine (75,76,77) and release of neutral sphingomyelinase (78). Most of these changes can occur within two minutes after the addition of IL-1 beta. One of the early signaling molecules, MAP kinase, is activated immediately after IL-1 beta binds to its receptor. The activation of the MAP kinase leads to phophorylation on serine and threonine residues of epidermal growth factor, heat shock protein p27, myelin basic protein and serine 56 and 156 of beta-casein in IL-1 beta stimulated cells (79,80). Estrogen-induced stimulation of macrophage cells (81) and MCF7 cells in part occurs through ROS(82,20,20). We have also observed inhibition of estrogen-induced MCF7 cell growth by ROS scavengers such as N-acetylcysteine, ebselen, and catalase (82). ROS can modulate effector molecules such as PKC, p53, extracellular regulated kinase (ERK), nuclear factor-κB (NF-κB), and the c-fos/c-jun heterodimer (AP-1); and these effector molecules are known to participate in growth signal transduction (64). Recently we have demonstrated evidence for the involvement of redox signaling with estrogen-induced cell proliferation (82). Physiological concentrations of E2 stimulate a rapid production of intracellular ROS which lead to the phosphorylation of c-jun and CREB; and increased activity of redox sensitive transcription factors Nrf-1, c-jun, and CREB known to be involved in the regulation of cell cycle genes (20). Although direct ER transcription factor effects are required to promote DNA synthesis, our recent data showed that MCF7 cell growth and cyclin D1 expression are suppressed by antioxidants and mitochondrial blockers (82) which supports our novel finding that nongenomic, estrogen-induced mitochondrial ROS modulate the early stage of cell cycle progression.

These studies suggest that in addition to ovarian estrogens, mitogenic signals may also come from TNF-alpha and IL-1 beta-generated $O_2^{\bullet-}$ and H_2O_2 (a schematic representation is shown in Figure 2). This, in turn, would help to fix the genotoxic effect of the estrogen and/or inflammation and the production of mutational changes in the genome. In the absence of mitogenic stimuli, DNA damaged viable cells might undergo senescence or apoptosis (Figure 2). Therefore, G1 arrested cells waiting for the repair of DNA damage may receive pressure from the mitogenic signals produced by estrogens and/or TNF-alpha and IL-1 beta generated low concentrations of super oxide and H_2O_2, which may force cells to exit out of G1 arrest. This mitogenic pressure may allow cells to enter into the S phase and proceed through G1/S checkpoint in order to complete cell division. This would result in an increased rate of fixation of DNA damage leading to mutation (Figure 2). Since estrogen and IL-1 beta exert mitogenic effects in the cells, this may also contribute to the potential for fixation of damage to bases of DNA leading to a probability of higher mutational frequency. These studies suggest that the proinflammatory cytokines, IL-1 beta and TNF- alpha, generated oxidants together with an estrogen-driven increase in epithelial cell proliferation may initiate and promote neoplastic lesions in estrogen-sensitive tissues.

7. SUMMARY

A cancer cell from its genesis to the pathologically advanced stage of metastasis undergoes enormous and rapid changes in both the genome as well as at in the proteins to facilitate its development in the hostile environment of the host. These changes in the genome can not be explained by ER-mediated signaling pathways. To

what extent IL-1 beta may participate in these processes is not very clear. In the pathogenesis of estrogen-dependent cancers, particularly, breast and ovary, the role of IL-1 beta is implicated in protumorigenic insults, cell proliferation, angiogenesis and cell adhesion. It appears that it is the concentration of the peptide interleukin-1 beta which determines its stimulatory or inhibitory paracrine and/or autocrine signals that regulate the growth of estrogen-dependent tumors. Different cellular signaling may operate in response to varying levels of IL-1 beta leading to genotoxic damage and cell apoptosis or cell growth (representative scheme is shown in Figure 2). In summary, further validation of the concept that the concentration of the peptide interleukin-1 beta determines its stimulatory or inhibitory paracrine and/or autocrine signals that regulate the growth of estrogen-dependent tumors might result in novel preventive strategies. Studies are in progress in our laboratory to critically evaluate the influence of IL-1 beta on estrogen-induced carcinogenesis.

8. ACKNOWLEDGEMENT

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9. REFERENCES

1. Roy, D. & J. G. Liehr: Inhibition of estrogen-induced kidney carcinogenesis in Syrian hamsters by modulators of estrogen metabolism. *Carcinogenesis* 11, 567-570 (1990)
2. International Agency for Research on Cancer Working Group Monographs on the evaluation of hormonal contraception and postmenopausal hormonal therapy. 399-530 (1999)
3. National Toxicology Program Federal report on carcinogens. 77283-77285 (2002)
4. NCI Uses of hormones after menopause. *NCI* - (2003)
5. Roy, D. & Q. Cai: Estrogen, immunoactivation, gene damage, and development of breast, endometrial, ovarian, prostate, and testicular cancers. *Recent Res Devel Steroid Biochem Mol Biol* 3, 1-32 (2002)
6. Roy, D. & K. P. Singh: Estrogen-induced genetic alterations and their role in carcinogenicity. *Curr Genomics* 5, 245-257 (2004)
7. Liehr, J. G. & D. Roy: Free radical generation by redox cycling of estrogens. *Free Radic Biol Med* 8, 415-423 (1990)
8. Markides, C. S., D. Roy, & J. G. Liehr: Concentration dependence of prooxidant and antioxidant properties of catecholestrogens. *Arch Biochem Biophys* 360, 105-112 (1998)
9. Roy, D., B. Kalyanaraman, & J. G. Liehr: Xanthine oxidase-catalyzed reduction of estrogen quinones to semiquinones and hydroquinones. *Biochem Pharmacol* 42, 1627-1631 (1991)
10. Roy, D. & J. G. Liehr: Estrogen, DNA damage and mutations. *Mutat Res* 424, 107-115 (1999)
11. Cavalieri, E., K. Frenkel, J. G. Liehr, E. Rogan, & D. Roy: Estrogens as endogenous genotoxic agents--DNA adducts and mutations. *J Natl Cancer Inst Monogr* 75-93 (2000)
12. Zhang, F., S. M. Swanson, R. B. van Breemen, X. Liu, Y. Yang, C. Gu, & J. L. Bolton: Equine estrogen metabolite 4-hydroxyequilenin induces DNA damage in the rat mammary tissues: formation of single-strand breaks, apurinic sites, stable adducts, and oxidized bases. *Chem Res Toxicol* 14, 1654-1659 (2001)
13. Embrechts, J., F. Lemiere, W. Van Dongen, E. L. Esmans, P. Buytaert, E. Van Marck, M. Kockx, & A. Makar: Detection of estrogen DNA-adducts in human breast tumor tissue and healthy tissue by combined nano LC-nano ES tandem mass spectrometry. *J Am Soc Mass Spectrom* 14, 482-491 (2003)
14. Devanesan, P., R. J. Santen, W. P. Bocchinfuso, K. S. Korach, E. G. Rogan, & E. Cavalieri: Catechol estrogen metabolites and conjugates in mammary tumors and hyperplastic tissue from estrogen receptor-alpha knock-out (ERKO)/Wnt-1 mice: implications for initiation of mammary tumors. *Carcinogenesis* 22, 1573-1576 (2001)
15. Terashima, I., N. Suzuki, & S. Shibutani: Mutagenic properties of estrogen quinone-derived DNA adducts in simian kidney cells. *Biochemistry* 40, 166-172 (2001)
16. Chakravarti, D., P. C. Mailander, K. M. Li, S. Higginbotham, H. L. Zhang, M. L. Gross, J. L. Meza, E. L. Cavalieri, & E. G. Rogan: Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-ras gene. *Oncogene* 20, 7945-7953 (2001)
17. Roy D, Parkash J, & Narayan S: Genetics and Bioenergetics of Mitochondria Influencing the Etiology and Pharmacology of Steroidal Hormones. *Current Pharmacogenomics* 2, 379-390 (2005)
18. Felty, Q. & D. Roy: Mitochondrial signals to nucleus regulate estrogen-induced cell growth. *Med Hypotheses* 64, 133-141 (2005)
19. Felty, Q. & D. Roy: Estrogen, mitochondria, and growth of cancer and non-cancer cells. *J Carcinog* 4, 1-(2005)
20. Felty, Q., W. C. Xiong, D. Sun, S. Sarkar, K. P. Singh, J. Parkash, & D. Roy: Estrogen-induced mitochondrial reactive oxygen species as signal-transducing messengers. *Biochemistry* 44, 6900-6909 (2005)
21. Malins, D. C. & R. Haimanot: Major alterations in the nucleotide structure of DNA in cancer of the female breast. *Cancer Res* 51, 5430-5432 (1991)
22. Malins, D. C., N. L. Polissar, K. Nishikida, E. H. Holmes, H. S. Gardner, & S. J. Gunselman: The etiology and prediction of breast cancer. Fourier transform-infrared spectroscopy reveals progressive alterations in breast DNA leading to a cancer-like phenotype in a high proportion of normal women. *Cancer* 75, 503-517 (1995)
23. Musarrat, J., J. Arezina-Wilson, & A. A. Wani: Prognostic and aetiological relevance of 8-hydroxyguanosine in human breast carcinogenesis. *Eur J Cancer* 32A, 1209-1214 (1996)
24. Yamamoto, T., K. Hosokawa, T. Tamura, H. Kanno, M. Urabe, & H. Honjo: Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in women with or without gynecologic cancer. *J Obstet Gynaecol Res* 22, 359-363 (1996)
25. Singh K, Lopez-Guerrero JA, Llombart-Bosch A, & Roy D. Estrogen-induced mutations and its role in the development of tumorigenesis. Hormonal Carcinogenesis

IV, Li, Jonathan J.; Li, Sara A.; Llombart-Bosch, Antonio (Eds.) Springer-Verlag, (2005)
26. Kong, L. Y., P. Szaniszlo, T. Albrecht, & J. G. Liehr: Frequency and molecular analysis of hprt mutations induced by estradiol in Chinese hamster V79 cells. *Int J Oncol* 17, 1141-1149 (2000)
27. Dinarello, C. A. & N. Savage: Interleukin-1 and its receptor. *Crit Rev Immunol* 9, 1-20 (1989)
28. Dinarello, C. A. Biologic basis for interleukin-1 in disease. *Blood* 87, 2095-2147 (1996)
29. Hu, S. K., Y. L. Mitcho, & N. C. Rath: Effect of estradiol on interleukin 1 synthesis by macrophages. *Int J Immunopharmacol* 10, 247-252 (1988)
30. Harris, M. T., R. S. Feldberg, K. M. Lau, N. H. Lazarus, & D. E. Cochrane: Expression of proinflammatory genes during estrogen-induced inflammation of the rat prostate. *Prostate* 44, 19-25 (2000)
31. Yoshida, Y., Y. Nakamura, N. Sugino, K. Shimamura, M. Ono, & H. Kato: Changes in interleukin-1 production of peritoneal macrophages during estrous cycle in golden hamsters. *Endocr J* 43, 151-156 (1996)
32. Polan, M. L., A. Daniele, & A. Kuo: Gonadal steroids modulate human monocyte interleukin-1 (IL-1) activity. *Fertil Steril* 49, 964-968 (1988)
33. Nash, M. A., G. Ferrandina, M. Gordinier, A. Loercher, & R. S. Freedman: The role of cytokines in both the normal and malignant ovary. *Endocr Relat Cancer* 6, 93-107 (1999)
34. Li, B. Y., D. Mohanraj, M. C. Olson, M. Moradi, L. Twiggs, L. F. Carson, & S. Ramakrishnan: Human ovarian epithelial cancer cells cultures *in vitro* express both interleukin 1 alpha and beta genes. *Cancer Res* 52, 2248-2252 (1992)
35. Scambia, G., U. Testa, P. B. Panici, R. Martucci, E. Foti, M. Petrini, M. Amoroso, V. Masciullo, C. Peschle, & S. Mancuso: Interleukin-6 serum levels in patients with gynecological tumors. *Int J Cancer* 57, 318-323 (1994)
36. Miller, L. J., S. H. Kurtzman, K. Anderson, Y. Wang, M. Stankus, M. Renna, R. Lindquist, G. Barrows, & D. L. Kreutzer: Interleukin-1 family expression in human breast cancer: interleukin-1 receptor antagonist. *Cancer Invest* 18, 293-302 (2000)
37. Jin, L., R. Q. Yuan, A. Fuchs, Y. Yao, A. Joseph, R. Schwall, S. J. Schnitt, A. Guida, H. M. Hastings, J. Andres, G. Turkel, P. J. Polverini, I. D. Goldberg, & E. M. Rosen: Expression of interleukin-1beta in human breast carcinoma. *Cancer* 80, 421-434 (1997)
38. Bhat-Nakshatri, P., T. R. Newton, R. Goulet, Jr., & H. Nakshatri: NF-kappaB activation and interleukin 6 production in fibroblasts by estrogen receptor-negative breast cancer cell-derived interleukin 1alpha. *Proc Natl Acad Sci U S A* 95, 6971-6976 (1998)
39. Pantschenko, A. G., I. Pushkar, K. H. Anderson, Y. Wang, L. J. Miller, S. H. Kurtzman, G. Barrows, & D. L. Kreutzer: The interleukin-1 family of cytokines and receptors in human breast cancer: implications for tumor progression. *Int J Oncol* 23, 269-284 (2003)
40. Robinette, C. L. Sex-hormone-induced inflammation and fibromuscular proliferation in the rat lateral prostate. *Prostate* 12, 271-286 (1988)
41. Burrows, F. J., D. O. Haskard, I. R. Hart, J. F. Marshall, S. Selkirk, S. Poole, & P. E. Thorpe: Influence of tumor-derived interleukin 1 on melanoma-endothelial cell interactions *in vitro*. *Cancer Res* 51, 4768-4775 (1991)
42. Vidal-Vanaclocha F, A. A. A. A. A. M. D. C. Juxtacrine activation of liver sinusoidal endothelium by B16 melanoma cell surface-associated IL-1 stimulates on site release of prometastatic factors. 119-123 (1995)
43. Wu, S., W. A. Meeker, J. R. Wiener, A. Berchuck, R. C. Bast, Jr., & C. M. Boyer: Transfection of ovarian cancer cells with tumor necrosis factor-alpha (TNF-alpha) antisense mRNA abolishes the proliferative response to interleukin-1 (IL-1) but not TNF-alpha. *Gynecol Oncol* 53, 59-63 (1994)
44. Wu, S., C. M. Boyer, R. S. Whitaker, A. Berchuck, J. R. Wiener, J. B. Weinberg, & R. C. Bast, Jr.: Tumor necrosis factor alpha as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis factor alpha expression. *Cancer Res* 53, 1939-1944 (1993)
45. Morioka, M., K. Shimodaira, Y. Kuwano, H. Fujikawa, H. Saito, & T. Yanaihara: Effect of interleukin-1beta on aromatase activity and cell proliferation in human osteoblast-like cells (HOS). *Biochem Biophys Res Commun* 268, 60-64 (2000)
46. Honma, S., K. Shimodaira, Y. Shimizu, N. Tsuchiya, H. Saito, T. Yanaihara, & T. Okai: The influence of inflammatory cytokines on estrogen production and cell proliferation in human breast cancer cells. *Endocr J* 49, 371-377 (2002)
47. Vesey, D. A., C. Cheung, L. Cuttle, Z. Endre, G. Gobe, & D. W. Johnson: Interleukin-1beta stimulates human renal fibroblast proliferation and matrix protein production by means of a transforming growth factor-beta-dependent mechanism. *J Lab Clin Med* 140, 342-350 (2002)
48. Stoker, T. E., C. L. Robinette, & R. L. Cooper: Perinatal exposure to estrogenic compounds and the subsequent effects on the prostate of the adult rat: evaluation of inflammation in the ventral and lateral lobes. *Reprod Toxicol* 13, 463-472 (1999)
49. Kawamura, H. & I. Ichihara: Possible microapocrine-type secretion developing in prostatic secretory epithelial cells of older rats in the early stage of castration. *Anat Anz* 163, 289-300 (1987)
50. Hoosein, N. M. Neuroendocrine and immune mediators in prostate cancer progression. *Front Biosci* 3, D1274-D1279 (1998)
51. Tanaka, T., H. Kanai, K. Sekiguchi, Y. Aihara, T. Yokoyama, M. Arai, T. Kanda, R. Nagai, & M. Kurabayashi: Induction of VEGF gene transcription by IL-1 beta is mediated through stress-activated MAP kinases and Sp1 sites in cardiac myocytes. *J Mol Cell Cardiol* 32, 1955-1967 (2000)
52. Jung, Y. D., W. Liu, N. Reinmuth, S. A. Ahmad, F. Fan, G. E. Gallick, & L. M. Ellis: Vascular endothelial growth factor is upregulated by interleukin-1 beta in human vascular smooth muscle cells via the P38 mitogen-activated protein kinase pathway. *Angiogenesis* 4, 155-162 (2001)
53. Guesdon, F., N. Freshney, R. J. Waller, L. Rawlinson, & J. Saklatvala: Interleukin 1 and tumor necrosis factor stimulate two novel protein kinases that phosphorylate the heat shock protein hsp27 and beta-casein. *J Biol Chem* 268, 4236-4243 (1993)
54. Coxon, A., B. Bolon, J. Estrada, S. Kaufman, S. Scully, A. Rattan, D. Duryea, Y. L. Hu, K. Rex, E. Pacheco, G.

Van, D. Zack, & U. Feige: Inhibition of interleukin-1 but not tumor necrosis factor suppresses neovascularization in rat models of corneal angiogenesis and adjuvant arthritis. *Arthritis Rheum* 46, 2604-2612 (2002)
55. Dana, M. R., S. N. Zhu, & J. Yamada: Topical modulation of interleukin-1 activity in corneal neovascularization. *Cornea* 17, 403-409 (1998)
56. Saijo, Y., M. Tanaka, M. Miki, K. Usui, T. Suzuki, M. Maemondo, X. Hong, R. Tazawa, T. Kikuchi, K. Matsushima, & T. Nukiwa: Proinflammatory cytokine IL-1 beta promotes tumor growth of Lewis lung carcinoma by induction of angiogenic factors: *in vivo* analysis of tumor-stromal interaction. *J Immunol* 169, 469-475 (2002)
57. Song, X., E. Voronov, T. Dvorkin, E. Fima, E. Cagnano, D. Benharroch, Y. Shendler, O. Bjorkdahl, S. Segal, C. A. Dinarello, & R. N. Apte: Differential effects of IL-1 alpha and IL-1 beta on tumorigenicity patterns and invasiveness. *J Immunol* 171, 6448-6456 (2003)
58. Hedges, J. C., M. A. Dechert, I. A. Yamboliev, J. L. Martin, E. Hickey, L. A. Weber, & W. T. Gerthoffer: A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. *J Biol Chem* 274, 24211-24219 (1999)
59. Giavazzi, R., A. Garofalo, M. R. Bani, M. Abbate, P. Ghezzi, D. Boraschi, A. Mantovani, & E. Dejana: Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. *Cancer Res* 50, 4771-4775 (1990)
60. Apte, R. N., T. Dvorkin, X. Song, E. Fima, Y. Krelin, A. Yulevitch, R. Gurfinkel, A. Werman, R. M. White, S. Argov, Y. Shendler, O. Bjorkdahl, M. Dohlsten, M. Zoller, S. Segal, & E. Voronov: Opposing effects of IL-1 alpha and IL-1 beta on malignancy patterns. Tumor cell-associated IL-1 alpha potentiates anti-tumor immune responses and tumor regression, whereas IL-1 beta potentiates invasiveness. *Adv Exp Med Biol* 479, 277-288 (2000)
61. Vidal-Vanaclocha, F., G. Fantuzzi, L. Mendoza, A. M. Fuentes, M. J. Anasagasti, J. Martin, T. Carrascal, P. Walsh, L. L. Reznikov, S. H. Kim, D. Novick, M. Rubinstein, & C. A. Dinarello: IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc Natl Acad Sci U S A* 97, 734-739 (2000)
62. Vidal-Vanaclocha, F., C. Amezaga, A. Asumendi, G. Kaplanski, & C. A. Dinarello: Interleukin-1 receptor blockade reduces the number and size of murine B16 melanoma hepatic metastases. *Cancer Res* 54, 2667-2672 (1994)
63. Yano, S., H. Nokihara, A. Yamamoto, H. Goto, H. Ogawa, T. Kanematsu, T. Miki, H. Uehara, Y. Saijo, T. Nukiwa, & S. Sone: Multifunctional interleukin-1beta promotes metastasis of human lung cancer cells in SCID mice via enhanced expression of adhesion-, invasion- and angiogenesis-related molecules. *Cancer Sci* 94, 244-252 (2003)
64. Burdon, R. H. Oxyradicals as signal transducers. 1, 289-322 (1997)
65. Wang, S., S. S. Leonard, V. Castranova, V. Vallyathan, & X. Shi: The role of superoxide radical in TNF-alpha induced NF-kappaB activation. *Ann Clin Lab Sci* 29, 192-199 (1999)
66. Jackson, J. H., E. Gajewski, I. U. Schraufstatter, P. A. Hyslop, A. F. Fuciarelli, C. G. Cochrane, & M. Dizdaroglu: Damage to the bases in DNA induced by stimulated human neutrophils. *J Clin Invest* 84, 1644-1649 (1989)
67. Weitzman, S. A. & T. P. Stossel: Mutation caused by human phagocytes. *Science* 212, 546-547 (1981)
68. Delaney, C. A., D. Pavlovic, A. Hoorens, D. G. Pipeleers, & D. L. Eizirik: Cytokines induce deoxyribonucleic acid strand breaks and apoptosis in human pancreatic islet cells. *Endocrinology* 138, 2610-2614 (1997)
69. Bekesi, G., Z. Magyar, R. Kakucs, D. Sprintz, I. Kocsis, B. Szekacs, & J. Feher: [Changes in the myeloperoxidase activity of human neutrophilic granulocytes and the amount of enzyme deriving from them under the effect of estrogen]. *Orv Hetil* 140, 1625-1630 (1999)
70. Bekesi, G., R. Kakucs, S. Varbiro, J. Feher, T. Pazmany, Z. Magyar, D. Sprintz, & B. Szekacs: Induced myeloperoxidase activity and related superoxide inhibition during hormone replacement therapy. *BJOG* 108, 474-481 (2001)
71. Singh KP & Roy D: Estrogen-induced cell Proliferation of human kidney epithelial cells. *The Toxicologist* 78, - (2004)
72. Oberley, T. D., R. G. Allen, J. L. Schultz, & L. J. Lauchner: Antioxidant enzymes and steroid-induced proliferation of kidney tubular cells. *Free Radic Biol Med* 10, 79-83 (1991)
73. Pantschenko, A. G., I. Pushkar, L. J. Miller, Y. P. Wang, K. Anderson, Z. Peled, S. H. Kurtzman, & D. L. Kreutzer: *In vitro* demonstration of breast cancer tumor cell sub-populations based on interleukin-1/tumor necrosis factor induction of interleukin-8 expression. *Oncol Rep* 10, 1011-1017 (2003)
74. Kuno, K. & K. Matsushima: The IL-1 receptor signaling pathway. *J Leukoc Biol* 56, 542-547 (1994)
75. Rossi, B. IL-1 transduction signals. *Eur Cytokine Netw* 4, 181-187 (1993)
76. O'Neill, L. A., T. A. Bird, & J. Saklatvala: How does interleukin 1 activate cells? Interleukin 1 signal transduction. *Immunol Today* 11, 392-394 (1990)
77. Rosoff, P. M., N. Savage, & C. A. Dinarello: Interleukin-1 stimulates diacylglycerol production in T lymphocytes by a novel mechanism. *Cell* 54, 73-81 (1988)
78. Rosoff, P. M. Characterization of the interleukin-1-stimulated phospholipase C activity in human T lymphocytes. *Lymphokine Res* 8, 407-413 (1989)
79. Andrieu, N., R. Salvayre, & T. Levade: Evidence against involvement of the acid lysosomal sphingomyelinase in the tumor-necrosis-factor- and interleukin-1-induced sphingomyelin cycle and cell proliferation in human fibroblasts. *Biochem J* 303 (Pt 2), 341-345 (1994)
80. Bird, T. A., P. R. Sleath, P. C. deRoos, S. K. Dower, & G. D. Virca: Interleukin-1 represents a new modality for the activation of extracellular signal-regulated kinases/microtubule-associated protein-2 kinases. *J Biol Chem* 266, 22661-22670 (1991)
81. Venkat, S., Q. Felty, & D. Roy: Estrogen-induced stimulation of macrophages leading to the generation of reactive oxygen species in the target organ of cancer. *Toxicologist* 67, 235 (2003)
82. Felty, Q., K. P. Singh, & D. Roy: Estrogen-induced G(1)/S transition of G(0)-arrested estrogen-dependent breast cancer cells is regulated by mitochondrial oxidant signaling. *Oncogene* 24, 4883-4893 (2005)

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