

Redox sensitive Pyk2 as a target for therapeutics in breast cancer

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1. ABSTRACT

Breast cancer progression is dependent on the formation of new blood vessels that not only help the tumor by supplying additional nutrients, but also allow cancer cells to spread from the breast to distant sites in the body. Several studies suggest a positive correlation between new vessel formation and estrogens. Estrogenic environmental chemicals such as PCBs have been shown to increase the expression of factors known to promote vessel formation in breast tumors. These studies highlight a growing concern that women exposed to estrogenic environmental compounds may be more susceptible to either aggressive metastatic tumors or a high recurrence of breast cancer. Our concept offers a fundamental new understanding of the way the environment contributes to breast cancer progression. This review will be focused on a highly novel Pyk2 signaling complex as a target for therapy of estrogen dependent breast tumor angiogenesis. A better understanding of the role of Pyk2 signaling in estrogen dependent tumor vascularization may lead to the development of a new therapy against aggressive breast cancer using small molecule inhibitors of Pyk2.

2. INTRODUCTION

Estrogen is a known growth factor for endothelial cells (ECs) and we have shown the inhibitory effect of antioxidants on estrogen-induced growth of human vascular ECs. Our previous studies have shown that physiological concentrations of estradiol induce the formation of reactive oxygen species (ROS) involved in signaling cell proliferation and vascularization (1;2). Since estrogen is a known mitogen of endothelial cells, we postulate that aggressive breast cancer growth is a consequence of excess or unopposed estrogen exposure. Estrogen receptor (ER)-mediated signaling pathways are considered to support cell proliferation; however, discrepancies between the binding affinity of various estrogens to the ER and their growth potency both *in vitro* and *in vivo* have been reported (3;4). Although selective ER modulators such as tamoxifen and antiestrogens such as ICI 182,780 prevent the growth of estrogen sensitive tissue, the contribution of other mechanisms cannot be ruled out as these compounds also block metabolism and redox cycling of estrogen, and are free radical scavengers (5). In this review, we will challenge the prevailing dogma that resistance to ER signaling is responsible for mediating aggressive breast

Table 1. Environmental exposures are risk factors for tumor angiogenesis

Environment	Angiogenic Gene	Response
Arsenic	↑ VEGF, NOS3, bFGF	↑ Endothelial cell proliferation and tube formation ↑ VEGF secretion and NO production (69)
Arsenic+ Ethanol	↑ VEGF, IGF-1	Co-exposure ↑ Endothelial cell tube formation (25)
Cigarette Smoke	↑ VEGF, MCP-1 ↑ PIGF	Second hand smoke ↑ tumor vessel density in xenograft model (70) Cigarette smoke extract ↑ secretion of placental growth factor (71)
Estrogen Therapy	↑ VEGF ↑ SDF-1	↑ Angiogenesis under hypoxic and normoxic conditions (72) Promoted myocardial angiogenesis (73)
Ethanol	↑ VEGF	↑ Tumor microvessel density ↑ Tumor growth (74)
Human cytomegalovirus	↑ Various growth factors (i.e. TGF-β, VEGF)	↑ Endothelial cell tube formation (75)
Hypoxia	↑ VEGF	Inhalational hypoxia ↑ VEGF protein expression in rat myocardium (76) Chronic systemic hypoxia ↑ angiogenesis of adult rat retina (77)
Nicotine	↑ VEGF	↑ Tumor vessel density in xenograft model (78)
Polychlorinated biphenyl 104	↑ VEGF	↑ Endothelial hyperpermeability (79)
Roxarsone A poultry feed additive	↑ HGF	↑ Endothelial cell tube formation greater than inorganic As ^{III} (80) ↑ NOS3 activity
Soluble manganese [Mn(II)]	↑ VEGF, VEGFR-1, HIF-α subunits	Subchronic inhalation ↑ angiogenic gene expression in mouse lung (81)
Xenoestrogens (i.e. BPA, OP, dieldrin, phthalates)	↑ VEGF	Potential for ↑ breast tumor angiogenesis (19)

cancer growth by summarizing the current knowledge of estrogen-induced redox signaling focused on focal adhesion protein Pyk2. We also discuss how new therapy using small molecule inhibitors of Pyk2 can be used as a new therapy for metastatic breast cancer.

3. BREAST TUMOR ANGIOGENESIS AND ESTROGEN

3.1. Estrogen and angiogenesis

Like most solid tumors, breast cancers require new blood vessel growth (angiogenesis) if they are to grow beyond a few millimeters in diameter (6). The new vessels not only help the tumor by supplying additional nutrients, but also provide routes for tumor dissemination and metastasis. A growing body of evidence suggests that estrogen directly modulates angiogenesis via effects on endothelial cells (7). In pathological circumstances, such as breast cancer, solid human tumors cannot continue growing without an adequate network of blood vessels from normal tissue to supply oxygen and nutrients and to remove waste products. Continued growth of a malignant tumor beyond a certain size is dependent on the tumor's ability to attract and develop a network of blood vessels that serve to provide nutrients to the expanding mass of cells. Several lines of evidence have shown a clear association between estrogen, ER expression by endothelial cells, angiogenic activity, and/or tumor invasiveness (7). Not surprisingly, poor breast cancer prognosis has been shown to correlate with increasing microvascular density with factors that stimulate new vessel growth (8). Estradiol has been shown to enhance endothelial cell activities important in neovascularization and suggests a promoting influence of estrogens on angiogenesis (9). For instance, human umbilical vein endothelial cell (HUVEC) proliferation increased 3- to 5-fold, respectively, in the presence of estradiol. Estradiol also enhanced the ability of HUVECs to organize into tubular networks when plated on Matrigel. *In vivo*, capillary endothelial cells in rat breast cancer have been shown to be estrogen dependent, and the observed tumor regression induced by decreased estrogen-level was attributed to necrosis from capillary insufficiency and

anoxia (10). These findings suggest that pathological vessel formation is associated with an effect of estrogens on endothelial cell angiogenic activity.

3.2. Environmental estrogens and angiogenesis

There is a growing body of evidence establishing exposure to environmental agents as a risk factor for tumor angiogenesis. As shown in Table 1, angiogenic gene expression has been reported after exposure to environmental agents in both humans and animals. Over the past 20 years, a great deal of attention has focused on the impact of endocrine disruptors released in the environment on animal and human health. In general, these so called endocrine disruptors which include PCBs, such as PCB153, have been reported to possess estrogenic activity (11-13). PCBs are a class of polychlorinated aromatic hydrocarbons composed of 209 discrete congeners. Due to their high lipophilicity and structural stability, PCBs are among the most extensively investigated persistent environmental pollutants that bioaccumulate in the food chain and are concentrated in fatty tissues (14). At least 24 studies of human populations show a possible link between PCBs and breast cancer while more than 50 additional laboratory studies illustrate in animals or cell cultures how PCBs may cause or promote breast cancer (15). Chronic exposure to PCBs produces a variety of effects including pro-inflammatory effects, carcinogenesis as well as tumor-promoting effects (16-18). Recently, estrogenic environmental chemicals, *i.e.* PCBs, bisphenol A, octylphenol, have been shown to significantly increase VEGF expression in breast cancer cells (19). This is of particular interest because VEGF is a stimulator of angiogenesis/vascular permeability *in vivo* and acts as an autocrine growth factor for mammary cancer cells (20). The relevance of an increase in angiogenic factors by estrogenic PCB exposure to breast tumor angiogenesis is not clear. Retrospective studies on the association of VEGF with relapse-free survival and overall survival have reported that patients with early-stage breast cancer who have tumors with increased concentrations of VEGF have a higher likelihood of recurrence or death than patients with low VEGF producing tumors (21). Taken together these

findings highlight a growing concern that women exposed to estrogenic environmental compounds such as PCBs are more susceptible to angiogenesis that consequently leads to either aggressive metastatic tumors or a high recurrence of breast cancer.

3.3. Estrogen and oxidative stress

High concentrations [10 micromolar] of 17 beta-estradiol (E2) have been shown to act as antioxidants *in vitro* (22). In contrast, we have demonstrated that physiological concentrations of E2 (100pg/ml to 1ng/ml) which do not act as antioxidants actually induce the formation of ROS (1;23;24). Evidence in support of the role of ROS signaling endothelial cell proliferation comes from our studies showing that antioxidants block E2-induced DNA synthesis and endothelial vessel formation (1;2). The conventional paradigm of estrogen action is based on binding to its receptors, ER alpha/beta, which initiates transcription by binding to estrogen response elements of genes involved in cell growth. Discrepancies between the binding affinity of various estrogens to the ER and their growth potency both *in vitro* and *in vivo* have been reported (3;4). Furthermore, we have shown that antioxidants N-acetylcysteine and ebselen, which are not ER antagonist, prevented E2—induced DNA synthesis and vessel formation. This suggests that this signaling mechanism does not solely rely on ER genomic signaling, but rather an oxidative stress sensitive signaling pathway. Although selective ER modulators such as tamoxifen and antiestrogens such as ICI 182,780 prevent the growth of estrogen dependent cells, the contribution of other mechanisms cannot be ruled out as these compounds also block metabolism and redox cycling of estrogen, and are free radical scavengers (5).

Many environmental contaminants exhibit their adverse effects via redox signaling. For instance, environmentally relevant concentrations of arsenic have been shown to induce oxidant formation that increased DNA synthesis and endothelial cell proliferation. Arsenic has also been shown to activate the focal adhesion kinase Pyk2 as well as increased the expression of angiogenic genes and vascular formation in human endothelial cells (25). Interestingly, Pyk2 is a redox sensitive kinase (26) that we have shown to be activated by estrogen-induced ROS (24). Therefore we propose that estrogenic PCB-induced redox signaling via Pyk2 mediates angiogenesis. Oxidative stress is considered a major mechanism involved in the pathogenesis of endothelial cell dysfunction, the initiation and progression of angiogenesis, and its adverse events. Endothelial cells acquire a proliferative and invasive phenotype in the process of angiogenesis. PCBs, such as PCB77, have been reported to increase endothelial cell permeability, increase oxidative stress, decrease cellular antioxidants, and activate redox-regulated transcription factors (16;27). There is some evidence that aggressive breast tumor growth exploit this proliferative and invasive phenotype. PCB-induced oxidants may activate an additional redox signaling pathway that is different from the classical ER-signaling pathway.

3.4 Estrogen and paracrine effects in angiogenesis

Poor breast cancer prognosis has been shown to correlate with increasing microvascular density with factors

that stimulate new vessel growth (8). Accordingly, an extensive body of research has focused on identifying the factors in the tumor microenvironment that promote and support angiogenesis, with the hope of limiting angiogenesis and ultimately tumor growth and metastasis. Carcinomas of the breast are composed of not only tumor epithelial cells but also of infiltrating endothelial cells, fibroblasts, and macrophages. Tumor angiogenesis depends on the paracrine interaction between these cells, e.g., paracrine stimulation of endothelial proliferation by tumor epithelium. For instance, production of vascular endothelial growth factor (VEGF) by both fibroblasts and inflammatory cells leads to microvascular permeability and the recruitment of endothelial cells to the tumor (28). Tumor associated macrophages (TAMs) are widespread in human breast carcinomas and it has been suggested that they may play an important role in the regulation of tumor angiogenesis (29). *In vitro* studies have shown that TAMs secrete growth factors that are mitogenic for both tumor cells and endothelial cells (30). Tumor epithelial cells are proposed to mediate angiogenesis by secreting soluble factors that enhance endothelial cell proliferation, migration and tube formation as well as by direct cellular interactions with endothelial cells (31). Evaluation of the role of angiogenesis in cancer, as well as the identification of suitable antiangiogenic therapies, requires a thorough understanding of EC response to angiogenic-promoting factors.

Although a number of paracrine effects by estrogen have been reported, the direct role of estrogen-induced ROS in angiogenesis remains to be defined. Our studies provide strong support for the concept that ROS is a paracrine signaling molecule involved in estrogen-induced angiogenesis. We showed that co-cultured HUVEC exposed to estrogen showed a greater angiogenic phenotype compared to mono-cultured HUVEC; and the estrogen-induced endothelial tube formation was dramatically inhibited by antioxidant co-treatment. Many environmental contaminants exhibit their adverse effects via redox signaling. We have provided evidence for the role of redox signaling in the aggressive angiogenic phenotype of endothelial cells. Thus, we postulate that estrogen exposed metastatic breast tumors release ROS that stimulates an aggressive angiogenic phenotype in neighboring endothelial cells (Figure 1).

3.5. Environmental compounds, redox signaling, and breast cancer

There is a growing concern that estrogenic environmental compounds may contribute to tumor angiogenesis or vascular lesions. Estrogen is a known mitogen of ECs and we have shown the inhibitory effect of antioxidants on E2-induced DNA synthesis and proliferation of vascular ECs (1). PCB congeners, PCB153 and PCB77, have been reported to possess estrogenic activity in ECs and induce oxidative stress. Since PCBs have been identified in human adipose tissue, blood, and milk; further research is warranted to better understand the role of estrogenic chemicals in breast tumor angiogenesis. The angiogenic phenotype is typically associated with aggressive and metastatic tumors. Silencing of secreted

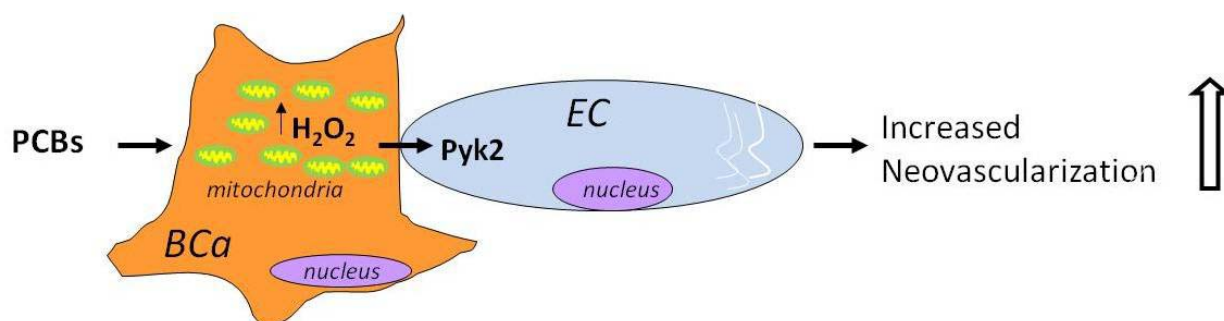


Figure 1. Proposed signaling pathway. Metastatic breast cancer cells (BCa) exposed to estrogenic polychlorinated biphenyls (PCBs) release the paracrine signaling molecule hydrogen peroxide (H_2O_2). The redox sensitive kinase Pyk2 in neighboring endothelial cells (EC) is activated by H_2O_2 . Consequently, Pyk2 signaling in neighboring endothelial cells leads to increased neovascularization that exacerbates breast tumor growth and invasiveness.

paracrine factors from metastatic MDA-MB-231 cells has been shown to inhibit endothelial cell vessel formation (32). However the mechanism by which metastatic cells support the angiogenic phenotype of endothelial cells is not clear. High concentrations [10 micromolar] of E2 have been shown to act as antioxidants *in vitro* (22). In contrast, we have demonstrated that physiological concentrations of E2 which do not act as antioxidants actually induce the formation of ROS in human breast cancer cell lines: MCF7, T47D, ZR751, and MDA-MB-468 (1;23;24). Similarly, exposure to PCBs, i.e. PCB153 and PCB126, has been shown to induce a concentration-dependent increase in the formation of ROS (most likely hydrogen peroxide) in ER α (+)/T47D and ER α (-)/MDA-MB-231 human breast cancer cells (33). Furthermore, this study reported that highly metastatic breast carcinoma cell line MDA-MB-231 produced a significantly greater amount of ROS compared to its low metastatic counterpart T47D when exposed to PCBs. The relevance of PCB-induced oxidative stress to breast tumor angiogenesis is not clear, however, it has been shown that free-radical mediated oxidative stress is, at least partly, associated with organochlorine pesticide residues in human breast tumors (34).

Oxygen radicals have been shown to increase tumor cell production of angiogenic factors such as VEGF (35). Although a number of paracrine effects by estrogen have been reported, the direct role of estrogen-induced ROS in angiogenesis remains to be defined. ROS vary in their inherent reactivity, stability, chemistry, and diffusibility. Hydrogen peroxide (H_2O_2), in most biological contexts, is generally less reactive and more long-lived than either superoxide radical or hydroxyl radical; moreover, H_2O_2 is lipid-soluble and can diffuse across biological membranes (36). We have previously shown that the induction of ROS formation by estrogen occurs in both ER alpha positive and ER alpha negative breast cancer cell lines and originates from the mitochondria (24;37). Metastatic breast cancer cell lines have been reported to have a higher level of mitochondria compared to non-metastatic cells (38); and this may explain why estrogenic PCB-exposed metastatic breast cancer cells produce more ROS than low metastatic cells (33). Stimulation of endothelial cells by tumor cells establishes an endothelial

phenotype consistent with the initial stages of angiogenesis. Taken together, these studies suggest that environmental estrogen exposed metastatic breast tumors release H_2O_2 that stimulates an aggressive angiogenic phenotype in neighboring endothelial cells. In summary, these findings provide a new mechanism by which high ROS production by environmental estrogen exposed metastatic breast cancer cells mediate an aggressive angiogenic phenotype, and thereby may regulate tumor growth and invasiveness.

3.6. Pyk2 as a biomarker for breast cancer

Pyk2 (Proline-rich tyrosine kinase 2) is a member of the FAK (focal adhesion kinase) family. The molecular structure of Pyk2 and its physical association with cytoskeletal proteins suggest that it might play a pivotal role in breast cancer metastasis. Pyk2 is involved in several cellular functions, such as adhesion, motility, cell proliferation, and the G1 to S phase transition of the cell cycle (39). Interestingly, we have shown that estrogen-induced ROS control the G1 to S phase progression in breast cancer cells which suggests that the redox sensitive Pyk2 may play a pivotal role in breast cancer growth (1;23;24). We have previously shown that E2-induced ROS production depends on cell adhesion (24). Another important novel biological consequence observed in our previous study deals with the contribution of E2 to cell adhesion as shown by the increase in the total level of cdc42, a marker of cell adhesion, and increase in Pyk2 activation. Based on our findings, it appears that Pyk2 activation is a late event in the E2-induced ROS signal pathway which could help in cell adhesion of serum starved non-adherent cells. A recent study that showed ROS increased cell adhesion by the activation of focal adhesion kinases supports this idea (40).

Genomic instability is a hallmark of breast cancer, and specific sub-chromosomal copy number changes may result in addition/deletion of one or both alleles of members of the focal adhesion kinase family. Breast cancer cell lines grown in monolayer culture frequently express constitutively activated FAK, whereas normal mammary epithelial cells grown under similar conditions do not (41). Copy number gains of FAK have been shown in cell lines derived from invasive epithelial tumors (42). Pyk2 was recently shown to be a cancer

related kinase sensitive to copy number changes in breast cancer (43). Thus, there is potential for Pyk2 to serve as a biomarker in the prevention and treatment of breast cancer.

3.7. Pyk2 redox signaling in aggressive breast cancer

Since some breast cancers are more aggressive than others, there is a quest for new, more effective therapy for these aggressive cancers. Many aggressive, ER positive breast cancers, show either *de novo* resistance or rapidly acquired resistance to antiestrogen treatments. We showed that both ER positive and negative breast cancer cells rapidly produce high levels of oxidants upon exposure to estrogens (24). We have shown that ER-mediated target gene activation is a late event when compared to the rapid formation of E2-induced oxidants. Our data indicate that this rapid E2-stimulated oxidant production is a critical early signal for initiation of breast cancer and endothelial cell growth (1;23).

The observation that inhibition of FAK in breast tumor cells results in small avascular tumors in mice (44) suggests that pharmacological inhibitors of FAK related proteins may function both as potent anti-tumor growth and anti-vascular agents. Pyk2 is a novel FAK related protein that mediates breast cancer cell migration and invasion. Overexpression of wild-type Pyk2 increases MDA-MB-435 and MCF-7 breast cancer cell invasion, while overexpression of a kinase-dead Pyk2 did not (45). Moreover, the Pyk2-specific inhibitor, tyrphostin A9, was shown to block chemotaxis by nearly 50% and chemoinvasion by about 40% in the aggressive estrogen-independent breast cancer cell line MDA-MB-231 (46); which is known to form extensive and well-vascularized metastatic lesions in nude mice. The novel focal adhesion kinase Pyk2 may be an important target in epithelial cells based on the following: (i) overexpression of wild-type Pyk2 significantly increases MDA-MB-435 and MCF-7 breast cancer cell invasion (45), (ii) the Pyk2-specific inhibitor, tyrphostin A9, was shown to block chemotaxis by nearly 50% and chemoinvasion by about 40% in the aggressive breast cancer cell line MDA-MB-231 (46); and (iii) inhibition of focal adhesion kinase results in small avascular breast tumors in mice (44). Together, these findings suggest that small molecule inhibitors of Pyk2 may function as potent anti-tumor growth and anti-vascular agents. Our previous study showed that E2-induced Pyk2 activation depends on oxidants in MCF7 cells (24). Furthermore, we showed a distinct preference for E2 activation of Pyk2 while the structurally similar FAK showed no change which suggests that Pyk2 is involved in a distinct redox signaling pathway. Although there is evidence that Pyk2 plays a role in endothelial cell motility necessary for angiogenesis (47), its contribution to aggressive breast cancer growth via E2-induced tumor vascularization is not known and merits investigation.

3.8. Novel Pyk2 protein-protein interactions and breast tumor angiogenesis

Pyk2 interacts with many of the same proteins as FAK, but the functions of these protein interactions are poorly understood. Pyk2 represents a novel member of the focal adhesion kinase family thought to act as a key

component in vasculogenesis (48); however, its role in breast tumor angiogenesis has yet to be studied. Understanding the molecular mechanism by which the Pyk2 protein-protein interactions signal endothelial cells to undergo angiogenesis will lead to the development of new breast cancer therapies that work by blocking angiogenesis thereby starving the tumor. Our concept challenges the prevailing dogma that the ER is responsible for signaling the angiogenic effects of estrogen. Although the ER is required for the growth of cells, we consider ER actions as a late event when compared to the rapid formation of estrogen-induced oxidants that we have previously shown initiates the early signal for endothelial cell growth (1;23;24). Our preliminary previous study showed that E2 exposure activates Pyk2 that showed a distinct preference for estrogen activation of Pyk2 while the structurally similar FAK showed no change (24). This unique finding suggests that Pyk2 is involved in a different signaling pathway that may be specific to estrogen-induced angiogenesis. Thus, E2 may also activate an additional signal transduction pathway that is different from the classical ER-signaling pathway

G protein coupled receptors (GPCRs) have been implicated in the pathogenesis of vascular cell proliferation, leading to the development of vascular lesions (49). Pyk2 plays an important role in coupling GPCRs and growth factor receptors to classic MAPK pathway activation in a number of cell types. Several lines of evidence demonstrate that stimulation of GPCRs leads to the formation of protein complexes. For instance, activation of the G protein-coupled m1 muscarinic acetylcholine receptor has been shown to form a Pyk2/c-Src/Grb2 protein complex (50). Interestingly, many of these complexes are composed of oxidant sensitive proteins such as Pyk2 (26), c-src (51), Vav1, and Grb2 (52). Since the activation of GPCRs is known to produce oxidative stress, these protein complexes may be a result of redox signaling. Several estrogenic environmental chemicals including PCB have been shown to bind to the novel membrane estrogen receptor, GPR30, to activate alternative estrogen signaling pathways (53). In patients undergoing coronary artery bypass graft surgery, GPR30 expression was shown in both arteries and veins (54). GPR30 expression has also been shown in HUVECs and may play an important role in the regulation of endothelial function (55). Since GPR30 has been shown to signal estrogen-induced cell proliferation and DNA synthesis (56), it is biologically plausible that GPR30 could play a role in estrogen-induced angiogenesis. Pyk2 has also been shown to associate with the cytoskeletal protein Vav1 upon GPCR activation (57). Vav proteins participate in the reorganization of the actin cytoskeleton which is critical to processes such as cell division, growth, and adhesion. Overexpression of wild-type Vav1 has been shown to cause uncontrolled proliferation of cells (58). More recently, tyrosine phosphorylation of Pyk2 has been shown to activate Vav1 (59). Currently, we have not found any published reports that show the formation of a Pyk2/GPR30/Vav1 protein complex when stimulated by estrogen. PCBs have been shown to: generate oxidative stress (76), possess estrogenic activity (20;22), and bind the estrogen membrane receptor GPR30 (77). Since the

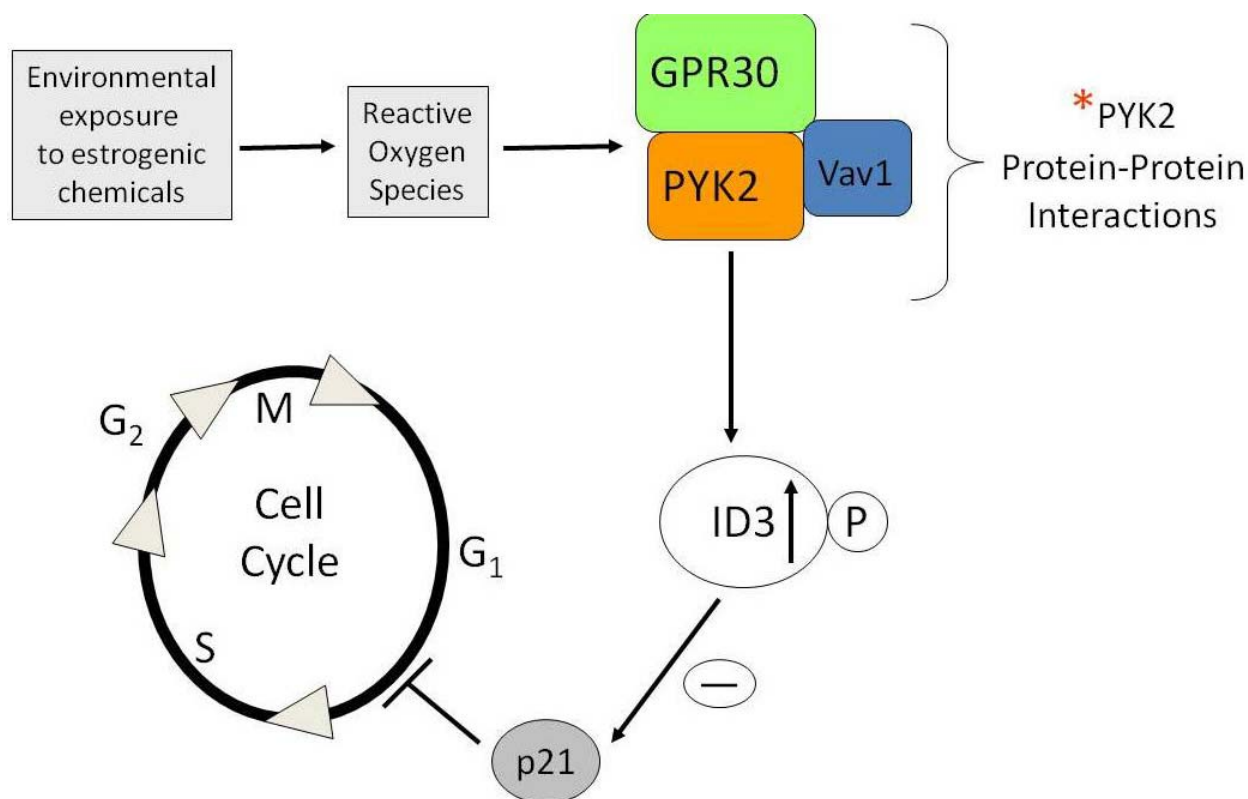


Figure 2. Novel Pyk2 protein-protein interactions proposed to signal tumor vascularization by estrogenic environmental chemicals. Estrogenic PCB exposure has been reported to stimulate blood vessel formation. We propose a novel Pyk2 signaling complex is recruited via reactive oxygen species from the exposure to estrogenic compounds. Estrogen-induced Pyk2 signaling may then activate a downstream redox sensitive molecule such as the transcription factor Id3. Subsequently, Id3 represses p21^{Cip1} gene transcription leading to permissive conditions for G1 to S cell cycle progression and cell proliferation. A new line of research targeting estrogen-dependent redox sensitive molecules such as the focal adhesion kinase Pyk2 and the transcription factor Id3 may serve as a new class of targets for therapeutics in cancer treatment.

cytoskeleton-associated protein Vav1 regulates actin cytoskeleton reorganization (58;60), we postulate that estrogen-induced Pyk2 protein-protein interactions signal the cytoskeleton by way of Vav1 resulting in the abnormal growth of vascular lesions (Figure 2). In turn, this increase in tumor vascularization will lead to the aggressive growth of breast cancer.

3.9. Estrogen redox signaling and cell cycle progression

The level of the cyclin dependent kinase inhibitor (CDKI), p21^{Cip1}, is elevated in quiescent cells where it acts as an inhibitor of cell proliferation (61). The estrogenic PCB153 has been shown to regulate p21^{Cip1} protein expression (62). Estrogen treatment has been shown to repress p21^{Cip1} gene transcription leading to permissive conditions for the progression through the G1 to S transition and proliferation (63). Since the maintenance of appropriate levels of p21^{Cip1} during the cell cycle is sensitive to the oxidative status of the cell (64), it is plausible that PCB-induced redox signaling regulates p21^{Cip1} expression. Focal adhesion kinase was shown to signal the loss of p21^{Cip1} protein in HUVEC. We postulate that redox signaling via Pyk2 may control estrogen-induced expression of p21^{Cip1}. Basic helix-loop-helix (bHLH)

transcription factors called E proteins have been shown to bind the E-box sequence (CANNTG) in the p21^{Cip1} promoter and activate transcription (65). Id (Inhibitor of DNA binding) proteins are important determinants of mitogen induced G1to S cell cycle progression (66). Id3 disrupts DNA binding by these E-box proteins and thereby blocks transcriptional activation by these factors. Simply stated, Id3 mediates its mitogenic effect via inhibition of p21^{Cip1} expression, subsequently increasing DNA synthesis and proliferation. In vascular cells, serine phosphorylated Id3 has been shown to inhibit E-box proteins from activating the p21^{Cip1} promoter (67). Our studies have shown that E2-induced serine phosphorylation of Id3 was redox sensitive in HUVECs and Id3 RNAi inhibited E2—induced vascular formation (2). Since focal adhesion kinase was shown to signal the loss of p21^{Cip1} protein in HUVEC (68), we postulate that estrogen-induced Pyk2 signaling may activate redox sensitive protein Id3 to block p21^{Cip1} expression.

4. SUMMARY AND PERSPECTIVE

Since the spread of aggressive breast carcinoma depends on tumor-infiltrating blood vessels, a more

effective therapy would be to disrupt two cell mechanisms in combination. A Phase III trial for patients with metastatic breast cancer showed that those who received Avastin®, an inhibitor of the formation and growth of new blood vessels, in combination with chemotherapeutic Taxol® survived twice as long as patients who received chemotherapy alone. These positive results serve as proof of concept for using a vascular targeting agent designed to find and destroy blood vessels within cancerous tumors in conjunction with inhibitors of cancer growth. Several studies suggest a positive correlation between new vessel formation and estrogens. Recently, estrogenic environmental chemicals such as PCBs have been shown to increase the expression of factors known to promote vessel formation in breast cancer. Since PCBs have been identified in human fatty tissue, blood, and milk; these studies highlight a growing concern that women exposed to estrogenic environmental compounds may be more susceptible to the formation of new blood vessels in breast tumors that consequently leads to either aggressive metastatic tumors or a high recurrence of breast cancer. Our research offers a fundamental new understanding of the way the environment contributes to breast cancer progression. PCB153 have been reported to possess estrogenic activity in ECs and induce oxidative stress. PCBs have been identified in human adipose tissue, blood, and milk; with a reported concentration range in human serum of PCB153 to be 0.60-1.63ng/ml. Further research is warranted to better understand the role of estrogenic chemicals in the growth of aggressive metastatic tumors. In this review, we propose a new line of research targeting estrogen-dependent redox sensitive molecules such as the focal adhesion kinase Pyk2 as a new class of targets for the development of new therapeutics in cancer treatment.

5. ACKNOWLEDGEMENTS

This work was supported in part by a grant from Florida Department of Health, Bankhead-Coley Research Program NIR Award 09BN-06 (Felty, Q.)

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Abbreviations: polychlorinated biphenyls (PCBs), 17 β -estradiol (E2), proline rich tyrosine kinase 2 (Pyk2), focal adhesion kinase (FAK), human umbilical vein endothelial cells (HUVEC), reactive oxygen species (ROS), estrogen receptor (ER), vascular endothelial growth factor (VEGF), tumor associated macrophages (TAMs), endothelial cells (ECs), G-protein coupled receptors (GPCRs), cyclin dependent kinase inhibitor (CDKI), hydrogen peroxide (H₂O₂), basic helix-loop-helix (bHLH), inhibitor of DNA binding protein 3 (Id3)

Key Words: Pyk2, PCBs, Estrogen, Breast Cancer, Angiogenesis, Metastasis, Redox Signaling, Review

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