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Determining the role of ERbeta in the activation of ERK1/2 by the environmental estrogens, cadmium chloride and sodium arsenite, in human lung adenocarcinoma cells

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Determining the role of ERβ in the activation of ERK1/2 by the environmental estrogens, cadmium chloride and sodium arsenite, in human lung adenocarcinoma cells

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A Senior Honors Thesis Presented in
Partial Fulfillment of the Requirements of the
Bellarmine University Honors Program

Under the Direction of Dr. Mary O. Huff________________________

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ABSTRACT

Estrogen is involved in the proliferation of adenocarcinoma lung cells. However, the proliferative role of two endocrine disrupters, cadmium chloride and sodium arsenite, in lung adenocarcinoma is less clear. Previous studies have shown that these two heavy metals can induce cellular proliferation and activate phosphorylation of ERK1/2 in a human lung adenocarcinoma cell line, NCI-H1793. Induced phosphorylation of ERK1/2 by these compounds is decreased in the presence of an estrogen receptor antagonist, ICI-182,780, suggesting that cadmium and arsenite stimulate these responses, in part, via a non-genomic estrogen signaling pathway. There are three known estrogen receptors, but it is unclear which might be utilized by cadmium or arsenite to activate ERK1/2. The purpose of this study was to determine if estrogen receptor β (ERβ), the predominant estrogen receptor in lung tissue, induces activation of ERK1/2 upon stimulation with cadmium and arsenite. NCI-H1793 adenocarcinoma lung cells were treated with the ERβ antagonist, PHTPP, before cells were treated for ten minutes with nanomolar concentrations of 17β-estradiol, cadmium chloride, or sodium arsenite. Western blot analysis was used to determine phosphorylation levels of ERK1/2. These results support that cadmium chloride and sodium arsenite induce phosphorylation of ERK1/2 as expected. However, overstimulation of ERK1/2 by PHTPP alone made it difficult to determine if ERβ is involved in cadmium and arsenite stimulated ERK1/2 activation.
INTRODUCTION

Lung cancer is one of the most prevalent and deadly human malignancies and is now the leading cause of cancer-related deaths worldwide (Burns & Stabile, 2014). In the US, lung cancer is the second most common form of cancer in both men and women. In 2016, the American Cancer Society estimates 224,390 new cases of lung cancer will be diagnosed, and 158,080 individuals will die due to lung cancer or its effects, resulting in more individuals dying from lung cancer than from colon, breast, or prostate cancer combined. With only a 14% five-year survival rate for stage III non-small cell lung cancer, this disease is currently one of the most difficult cancers to successfully treat and is thus worthy of further investigation in cancer research (Society, 2016).

Histologically, two main forms of lung cancer exist - non-small lung cancer (80-85% of cases) and small cell lung cancer (15-20% of cases). Non-small cell lung cancer (NSCLC) originates from bronchial epithelial cells while small cell lung cancers originate from neuroendocrine cells. Non-small cell lung cancer is further subdivided into squamous cell carcinoma, adenocarcinoma, large-cell carcinoma, bronchoalveolar lung cancer, and mixed histological types. Almost 50% of deaths caused by lung cancer are attributed to the adenocarcinoma subtype (Kadara et al., 2012). Also, it was more recently documented that lung adenocarcinoma incidence rates are increasing while the squamous cell carcinoma subtype incidence rates are declining (Marshall & Christiani, 2013). These data reveal the importance of increased research into the adenocarcinoma subset of non-small cell lung cancers as the most prevalent and deadly form of lung cancer.
Estrogen & Lung Carcinogenesis

In the year 2000, it was estimated that 85% of lung cancers diagnosed in males were due to smoking, while only 47% of female lung cancer cases were attributable to tobacco smoking (Chakraborty et al., 2010). It has also been noted in an epidemiological cohort study that women who undergo hormone replacement therapy (HRT), especially those who have smoked in the past, are at an increased risk of developing lung cancer. Specifically, females who undergo HRT are at an increased risk for developing the adenocarcinoma subtype of lung cancer (Adami et al., 1989). It has also been suggested that a female’s age at which she undergoes menopause is associated with susceptibility to lung cancer. Females who undergo menopause at an age younger than forty-five or who undergo bilateral ovariectomy procedures are at an increased risk for lung cancer development (Koushik et al., 2009). These studies suggested that sex specific hormonal influences may play a role in lung cancer prevalence in women and that estrogen, a key hormone for female sex development, could be involved in this susceptibility (Baik & Eaton, 2012).

Estrogen, a critical growth hormone in females, is now known to play an important role in lung development, pulmonary inflammation, and lung carcinogenesis (Chakraborty et al., 2010; Siegfried, 2014). In the 1980’s, studies established the presence of estrogen receptors (ER) in human lung tissue. The role of these estrogen receptors in lung development was illustrated using an estrogen receptor knockout (-/-) mouse model which showed abnormal lung development and decreased production of surfactant, a critical substance for decreasing surface tension in alveoli (Patrone et al., 2003). Further, lung tumors have shown to exhibit higher levels of estrogen receptors when compared to healthy lung tissue surrounding the tumors, and
these receptors are responsive to estrogen (Beattie et al., 1985). Treatment of the human lung adenocarcinoma cell line, H23, with estrogen showed increased cell proliferation; however, when H23 cells were pre-treated with an estrogen receptor antagonist, fluvestrant (ICI-182,780), a decrease in cell proliferation was observed, suggesting that cellular proliferation is mediated by an estrogen signaling pathway (Pietras et al., 2005).

Types of Estrogen Receptors

Estrogen induces its effects on cell growth through three major estrogen receptors (ERs): ERα, ERβ, and a G-protein linked ER (GPER) (Prossnitz et al., 2008). ERα was the first ER identified and was cloned in 1986. Ten years later, in 1996, a second estrogen receptor, ERβ, was identified and cloned (Lubahn et al., 1993). The α and β versions of the ER are encoded by genes ESR1, located on chromosome 6, and ESR2, located on chromosome 14, respectively (Enmark et al., 1997; Green et al., 1986). Both ERα and ERβ bind 17β-estradiol, the most prevalent form of estrogen in the body, with high affinity (Kuiper et al., 1996) and are found in the cell membrane, cytoplasm, and the nucleus of the cell (Chakraborty et al., 2010; Pedram et al., 2006; Pietras et al., 2005). While similar in structure and cellular location, these receptors may have specific roles in different tissues. ERβ is believed to be the primary and most functional form of the ER found in lung tissue. This theory is supported by observing higher levels of ERβ mRNA compared to ERα mRNA expression in both human fetal lung tissue and the adult mouse lung (Brandenberger et al., 1997). Further, it has been suggested that ERβ is more critical than ERα for proper lung development. In a mouse double ERβ knockout (KO) model, KO mice showed significantly decreased number of alveoli and significant extracellular matrix changes in comparison to wild type (WT) mice (Morani et al., 2006; Patrone et al., 2003).
Conversely, in ERα KO mouse experiments lung development abnormalities were not observed to the same gross extent (Siegfried, 2014).

It has also been suggested that ERα and ERβ may interact with one another in a “ying yang” fashion. In this theory, ERα is responsible for inducing cellular growth while ERβ may serve to inhibit cellular proliferation by preventing ERα from activating transcription. In a microarray analysis of estrogen-induced gene transcription, it was shown that in the absence of ERβ, ERα was able to induce transcription of genes that promote cellular growth. However, when ERβ was present, this upregulation induced by ERα was constrained, suggesting that ERβ inhibits ERα’s activities that promote cell growth (Lindberg et al., 2003).

In 2005, a third estrogen receptor was identified as GPR30, a G-protein linked estrogen receptor (GPER). Due to its more recent discovery, less is known about the GPR30 compared to ERα and ERβ. It has been shown, however, to bind 17β-estradiol (E2) with great affinity. The receptor is also known to mediate similar effects to those of ERα and ERβ in the cell (Ariazi et al., 2010). The GPR30 is localized within the plasma membrane and is known to help mediate and cause non-genomic activities such as activation of MAPK. A report by Prossnitz et al. (2008) showed that in GPER KO mice, decreased tumor growth was observed when treated with 17β-estradiol compared to non-KO mice models, suggesting a relationship between the presence of GPR30 and tumor growth. The lack of information currently available on the GPR30 makes its actions within the cell less specific.

**Mechanisms of Estrogen Action**

When an estrogen receptor in/on the cell has become activated, this steroid receptor mediates a multitude of cellular reactions which ultimately lead to cell proliferation. There are
two cellular pathways believed to be activated when estrogen binds to ERs: the genomic or “classical” pathway involving ERβ (and possibly ERα) and the non-genomic pathway involving ERβ, ERα, and GPR30.

In the genomic pathway, estrogen binds to a cytoplasmic or nuclear ER. Activation of this ER causes its release from an inhibitory complex, known to include Hsp90, and then is followed by receptor dimerization and translocation from the cytoplasm to the nucleus of the cell. The ER can then bind to estrogen response elements found in the promoters of estrogen targeted genes. This activity allows for the formation of a variety of transcription complexes which are believed to help influence and moderate transcriptional activity of ERs (Chakraborty et al., 2010).

In the non-genomic estrogen signaling pathway, (Figure 1), the activation of a membrane-bound ER mediates the association of the signaling proteins Src kinase and P13K to the ER at the cell membrane. This activation has been shown to occur within minutes after treatments of 17β-estradiol. This active protein complex will then activate matrix metallopeptidase 2/9, which will cause breakdown of the extracellular matrix (ECM). This activity by metallopeptidase 2/9 also releases a number of cell surface ligands which can stimulate EGFR, a membrane-bound protein (Marquez-Garban et al., 2007). In turn, EGFR activates Src kinase in the cytoplasm, and Src can directly activate Ras through phosphorylation. Ras then goes on to activate Raf, which induces phosphorylation of MEK. Finally, MEK phosphorylates ERK1/2 (MAPK), which has been shown to be a critical step in cellular growth and anti-apoptotic effects for the cell (Siegfried, 2014).
Environmental Estrogens

There are a number of chemicals found in the environment that can mimic the proliferative effects of estrogen in the cell and are referred to as environmental estrogens. Two known heavy metals, cadmium and arsenite, have been shown to mimic the effects of estrogen as endocrine disrupters by binding to estrogen receptors and stimulating these proliferative cellular pathways (Byrne & al., 2009; Putila & Guo, 2011). Therefore, the mechanisms of these chemicals’ stimulation of ERs and their subsequent signaling pathways should be considered targets of investigation in lung cancer research.

Figure 1. The non-genomic activation of ERK1/2 with a membrane bound estrogen receptor (Stabile, 2016).
Cadmium

Cadmium has been classified as an endocrine disrupter, meaning that it is a compound that can interfere with the body’s endocrine system by binding to hormone receptors that elicit effects in the cell. This binding can cause significant overstimulation of the receptor or can inhibit the proper physiological hormone from binding to its receptor (NIH, 2016). Specifically, cadmium is described as an environmental estrogen due to its ability to mimic effects of estrogen in the cell when bound to ERs (Byrne & al., 2009; Henson & Chedrese, 2004).

Cadmium, a heavy metal and known carcinogen, is most known for its presence in cigarette smoke. However, cadmium can also be ingested from many other environmental sources, including its use in phosphate-based fertilizers. This use of cadmium in fertilizer leads to contamination of foodstuffs and water due to the compound leaking into soil. Thus, many fertilizers potentially expose all humans to low levels of cadmium. In some countries, such as Taiwan, the exposure is more severe, particularly in drinking water (Lu et al., 2007). Other sources of cadmium in the environment include industrial pollution due to its use in galvanizing for metal production, in electrical conductors, and in the production of batteries and plastics (Byrne & al., 2009).

As a known environmental estrogen, it is not surprising that cadmium has been shown to induce tumor growth in a variety of tissues, including lung (Chuang et al., 2000). Ponce et al. (2013) showed that in breast cancer cell lines, acute exposure to cadmium induced ERα mediated transcription factors resulting in increased cellular growth, supporting cadmium’s status as an environmental estrogen. Furthermore, cadmium induced cell proliferation of breast cancer cells was inhibited when cells were treated with an estrogen antagonist. These results
suggest that cadmium does utilize an ER receptor signaling pathway when inducing cellular activation (Byrne & al., 2009).

Arsenite

It has been well documented that ingested inorganic arsenite can be readily metabolized in the liver, converted to a variety of oxidation states, methylated, and excreted in the urine. However, it is possible for deposits of arsenite to accumulate in tissues in the body which can lead to chronic exposure to low levels of this carcinogen and known endocrine disrupter (Tokar et al., 2011). It has been shown that human tissues such as the liver, kidney, skin, lung and prostate are susceptible to its carcinogenic effects (IARC, 1987; Lau et al., 2004).

Arsenite, like cadmium, is a heavy metal and known carcinogen found in cigarette smoke. It is also used in some fertilizers. Due to arsenite’s presence in fertilizers, low levels may contaminate fresh drinking water. This is a particular concern in many countries, including Taiwan, India, and the US. Humans can also be exposed to arsenite from foods such as rice and fish due to soil contamination (NIH, 2016). Arsenite can also be found in the industrial environment due to its use in smelters for the production of insecticides, pesticides, fungicides, pharmaceutical products, and some alcohols - mostly wines (Stoica et al., 2000). Arsenite can also be naturally released into the environment by volcanoes.

Arsenite has been shown to induce lung cancer in a number of previously reported studies. Mice exposed to arsenite in utero showed an increase number of lung tumors during adulthood, suggesting exposure to arsenite during development may play a role in susceptibility to lung cancer in adults (Tokar et al., 2011). Cohort studies on humans have also shown increased susceptibility to bladder and liver cancer from chronic exposure to arsenite (Chen et al., 1992).
Further studies addressing the mechanism of arsenite’s proliferative effects on the cell have shown that it activates a signaling pathway leading to MAPK activation. Lau et al. (2004) demonstrated a rise in ERK1/2 phosphorylation in a rat lung epithelial cell line (LEC) when cells were treated with 2 µM sodium arsenite. Arsenite has also been shown to decrease the expression of ERα in the breast cancer cell line, MCF-7. MCF-7 cells treated with 1 µM arsenite showed a 60% decrease in ERα protein expression with a 40% decrease in ERα mRNA expression. In binding affinity assays, arsenite has also been shown to inhibit the binding of 17β-estradiol to ERα. These results suggested a relationship between ERα’s binding domain and arsenite’s stimulatory effects on the cell (Stoica et al., 2000).

*Previous Studies with Cadmium and Arsenite in a Human Lung Adenocarcinoma Cell Line*

Previous studies in the Huff laboratory at Bellarmine University have shown that nanomolar concentrations of CdCl₂ and NaAsO₂, like 17β-estradiol, induce ERK1/2 phosphorylation in a non-smoker female non-small cell adenocarcinoma lung cell line, NCI-H1793. Further, inhibition studies of specific signaling proteins such as MEK1, Src kinase, and EGFR have suggested that this activation is achieved through the non-genomic pathway. Furthermore, studies showed that activation of MAPK by CdCl₂ and NaAsO₂ is inhibited in the presence of a general estrogen receptor antagonist, ICI-182,780, which targets all estrogen receptor forms. These results suggest that NaAsO₂ and CdCl₂ are utilizing one of the three estrogen receptors to induce activation of ERK1/2 via the estrogen signaling pathway. The purpose of this study was to determine the role of ERβ in the activation of ERK1/2 by cadmium and arsenite. These studies were performed using a specific ERβ antagonist, PHTPP, to inhibit ERβ activation, and ERK1/2 phosphorylation was measured by immunoblot analysis.
METHODS

Cell culture and treatments

The NCI-H1793 human female (non-smoker) non-small cell lung adenocarcinoma cell line from American Type Culture Collection (Manassas, VA) was used in this study and grown according to manufacturer’s directions. Upon reaching 90% confluency, cells were plated at a concentration of 400,000 cells in a final volume of 3 mL in 60 mm³ dishes and allowed to grow in media as defined by ATCC for two days before switching to charcoal-stripped media lacking hormones. After 24 hours, the media was changed and the experiment was performed 24 hours later. Cells were treated with 1 μM PHTPP, the ERβ antagonist, for 6 hours before inducing activation of ERK1/2 by 100 nM cadmium chloride, sodium arsenite, and 17β-estradiol (E₂) for 10 min. For controls, cells were untreated (untx) or treated with 0.1% DMSO.

Protein Collection

To prepare cell lysates, cells were washed first in ice-cold PBS 3x and 60 μL of lysis buffer (1 mL of RIPA Buffer, 1 μL protease inhibitor I, 1 μL phosphatase inhibitor II, 1 μL phosphates inhibitor III, and 10 μL of 100 mM PMSF) was used to collect cells using a cell scraper. The cell lysates were sonicated twice and then centrifuged at 4°C, 12,000 rpm for 10 mins. The supernatants were collected and stored at -80°C.

SDS Gel Electrophoresis and Immunoblot Analysis

To determine protein concentrations, a Bradford assay (BioRad, Hercules, CA) was performed following manufacturer’s directions, and 25 μg of protein was separated on a 10% SDS polyacrylamide gel at 200 V for approximately 1 hour using a running buffer composed of 25 mM Tris pH 8.3, 192mM Glycine, 1% SDS. The proteins were then transferred onto a PVDF membrane with 30V overnight at 4°C in 25 mM Tris, pH 8.3 192 mM glycine, 20% methanol.
The PVDF membrane was rinsed with TTBS (composed of 20 nM Tris pH7.5, 140 mM NaCl and 0.1% Tween 20) multiple times (2 x fast, 1 x 15 min, 3 x 5 mins). The PVDF membrane was then rocked in blocking buffer (5% nonfat dry milk in TTBS) for 1 hour at room temp. The blot was washed again with TTBS as previously described and a primary antibody raised to phosphorylated ERK1/2 from Cell Signaling Technology (Danvers, MA) was added at a 1/1000 dilution in blocking buffer and allowed to incubate overnight at 4 °C with shaking. After washing the blot in TTBS as previously described, the secondary antibody, stabilized peroxidase conjugated goat anti-rabbit (Thermo Scientific, Rockford, IL), was added at a 1/500 dilution in blocking buffer and allowed to incubate for 1 hour at room temp. with shaking. TTBS was then used to wash the blot as previously described.

To visualize the protein bands, chemiluminescent reagents from the Pierce ECL Western Blotting Substrate kit by Thermo Scientific were mixed at equal volumes and added to the blot with shaking for 1 min at room temp. The blot was then exposed to CL-X Posure Film (Clear Blue X Ray Film by Thermo Scientific) and developed. The blot was stripped with Restore Western Blot Stripping Buffer (Thermo Scientific) and the immunoblot analysis was repeated except that the primary antibody used was raised to total ERK1/2 (Cell Signaling Technology) and used at a 1/1000 dilution in blocking buffer. Densitometry using Un-Scan it software (Silk Scientific, Orum, UT) was used to compare phosphorylated MAPK to total MAPK, and the results were reported as relative phosphorylated MAPK to total MAPK.
RESULTS

To determine if ERβ is involved in the activation of ERK1/2 induced by cadmium and arsenite, cells were treated with nanomolar concentrations of cadmium chloride, sodium arsenite, or 17β-estradiol in the presence or absence of the ERβ antagonist, PHTPP. MAPK phosphorylation was examined using immunoblot analysis. Results were analyzed by densitometry and reported as relative phosphorylated MAPK to total MAPK. The experiment was repeated three separate times. The results of the first experiment are shown in Figure 2. As expected, treatment with cadmium and arsenite showed an increase in phosphorylation of ERK1/2 compared to the untreated control. Further, 17β-estradiol alone induce phosphorylation of ERK1/2 when compared to the DMSO control. However, unexpected activation of ERK1/2 was significantly increased in cells treated with PHTPP. This unexpected activation was further observed when cells were treated with cadmium chloride, sodium arsenite, and 17β-estradiol in the presence of PHTPP, making it difficult to form any conclusions.

In the second experiment (Figure 3), there is a significant increase in phosphorylation of ERK1/2 by DMSO. This high level of activation by DMSO was unexpected as it was used as the solvent control for 17β-estradiol and PHTPP. This high level of activation by DMSO does not allow for proper interpretation of 17β-estradiol or the PHTPP treated cells. Densitometry of the western blot revealed no significant changes in phosphorylation of ERK1/2 between PHTPP pretreated cells and cells stimulated in the absence of PHTPP.

In the third replication of the experiment, (Figure 4), DMSO again induced phosphorylation of ERK1/2, which made interpretation difficult to assess. In this experiment, both the treatment with cadmium chloride and sodium arsenite were able to induce phosphorylation of ERK1/2 as compared to the untreated control. There was no significant
decrease in phosphorylation of ERK1/2 for 17β-estradiol, cadmium chloride, or sodium arsenite in the presence of the ERβ antagonist, PHTPP.

To determine the overall pattern of activation of MAPK in the three experiments, the densitometry results from all three studies were averaged, (Figure 5). These results support that cadmium and arsenite alone increase MAPK phosphorylation as compared to the untreated control. However, activation of MAPK by DMSO alone and in the presence of the PHTPP alone made it impossible to determine if MAPK activation by cadmium and arsenite is inhibited by the ERβ antagonist. Therefore, it is difficult to determine if ERβ is involved in activation of ERK1/2 by these compounds.

Figure 2. ERK1/2 activation is induced by the ERβ antagonist, PHTPP. Cells were treated with 100 nM concentrations of E2, CdCl2, and NaAsO2 for 10 mins in the presence or absence of 1 μM PHTPP, and immunoblot analysis was used to measure ERK1/2 phosphorylation. A) Immunoblot analysis; B) Densitometry of western analysis.
Figure 3. Repeated experiment with significant increase in MAPK activation by the control, DMSO. Cells were treated with 100 nM concentrations of E2, CdCl2, and NaAsO2 for 10 mins in the presence or absence of 1 μM PHTPP, and immunoblot analysis was used to measure ERK1/2 phosphorylation. A) Immunoblot analysis; B) Densitometry of western analysis.

Figure 4. The third experiment with increased phosphorylation of ERK1/2 by CdCl2 and NaAsO2 alone and in the presence of PHTPP. Cells were treated with 100 nM concentrations of E2, CdCl2, and NaAsO2 for 10 minutes and immunoblot analysis was used to measure ERK1/2 phosphorylation. A) Immunoblot analysis; B) Densitometry of western analysis.
DISCUSSION

Estrogen is known to induce phosphorylation of ERK1/2 though a membrane-bound estrogen receptor leading to a non-genomic estrogen signaling pathway in the cell (Marquez-Garban et al., 2007). In previous studies, the environmental estrogens, cadmium chloride and sodium arsenite, have also been shown to induce phosphorylation of ERK1/2, and this activation is inhibited by the general estrogen receptor antagonist, ICI-182,780, supporting that these compounds activate ERK1/2 via the estrogen signaling pathway. To determine if cadmium and arsenite induce phosphorylation of ERK1/2 utilizing ERβ, the most prominent estrogen receptor expressed in lung tissue, cells were treated with 100 nM of 17β-estradiol, cadmium chloride, or sodium arsenite in the presence or absence of the ERβ antagonist, PHTPP, and relative phosphorylation levels of ERK1/2 from these treatments were analyzed by immunoblot analysis.

Figure 5. The average densitometry analysis of all three experiments (n=3) revealing no significant decrease in phosphorylation of ERK1/2 when cells were treated with E₂, CdCl₂, or NaAsO₂ in the presence of PHTPP. In each experiment, cells were treated with 100 nM concentrations of E₂, CdCl₂, and NaAsO₂ for 10 minutes and immunoblot analysis was used to measure ERK1/2 phosphorylation.
In these three experiments, cadmium chloride and sodium arsenite induced phosphorylation of ERK1/2, as previously observed, suggesting that these heavy metals activate cellular changes that can promote growth in this adenocarcinoma cell line. Unexpectedly, the DMSO control treatment induced phosphorylation of ERK1/2 significantly in two of these experiments. This high level of MAPK phosphorylation in the presence of DMSO alone, which is the solvent for 17β-estradiol and PHTPP, makes interpretation of any treatments with 17β-estradiol or PHTPP difficult to interpret accurately.

Surprisingly, the results of these experiments showed that the greatest phosphorylation of ERK1/2 was observed when cells were treated with the ERβ antagonist, PHTPP, alone making it difficult to fully establish if ERβ is involved in the induced phosphorylation of MAPK by cadmium and arsenite. Further, this observed activation with the antagonist also questions the effectiveness of PHTPP in these experiments. Since activation of MAPK by PHTPP was observed in all three experiments, it is likely that PHTPP does activates MAPK by some unknown mechanism. Therefore, to address the role of ERβ in future experiments, it may be necessary to utilize a different ERβ antagonist.

While the high level of phosphorylation of MAPK by PHTPP alone was unexpected, it could be speculated that PHTPP induces other responses within the cell unrelated to the estrogen signaling pathway. For example, it might be that activation of MAPK by PHTPP is a type of “shock” response in the cell. MAPK is well known to activate both cell proliferation and apoptosis, depending on the signal being presented (Zhang & Liu, 2002). As stated in the introduction, the Hsp90 has been shown to be a critical protein in the genomic estrogen signaling pathway and is necessary for the release of cytoplasmic ERs for their translocation to the nucleus. If Hsp90 is being activated by PHTPP through some unknown mechanism, this could
cause an increase in translocation of ERs to the nucleus and elicit responses not fully characterized. Possibly, one of these responses may induce a high level of MAPK phosphorylation. Therefore, Hsp90 may be another alternative to understanding why PHTPP induces phosphorylation of ERK1/2.

Although these results are inconclusive, ERβ should still be considered a potential target for cadmium and arsenite. The high level of ERβ mRNA and protein expression in lung tissue and its role in proper lung development provides good rationale that it may play a critical role in cellular proliferation (Brandenberger et al., 1997). Further, it is clear that cadmium and arsenite activate MAPK via an estrogen receptor. Until an experiment can be designed that allows for direct assessment of ERβ in cadmium and arsenite induced activation of MAPK, the importance of this receptor should not be refuted.

Other recent studies in the Huff laboratory at Bellarmine University have supported the role of GPR30 in the activation of MAPK by cadmium and arsenite. In these studies, it was shown that treatment with the GPR30 antagonist, G-15, inhibited MAPK phosphorylation induced by 17β-estradiol, cadmium chloride, and sodium arsenite. As the most newly discovered ER, GPR30’s actions are not yet fully characterized; however, GPR30 has been shown to be particularly important in breast cancer cell development (Filardo et al., 2006). While it is possible that this receptor may be responsible for activation of MAPK by cadmium and arsenite in lung cancer cells, further investigation is needed.

Since there are multiple estrogen receptors in these tissues and the possible role for GPR30 has been established, it is conceivable that a combination of receptors might be utilized to stimulate MAPK by cadmium and arsenite. Therefore, future studies might include combinations of specific antagonists for ERα, ERβ, and GPR30 to see if MAPK phosphorylation
can be inhibited. Specifically addressing the effects of phosphorylation on ERK1/2 in the presence of combined ER antagonists may provide noteworthy results, particularly considering the promising data for the GPR30’s role in phosphorylation of MAPK in lung cancer cells.

Understanding how the endocrine disrupters, cadmium and arsenite, induce cellular proliferation in the cell may lead to tissue specific/personalized pharmaceutical treatments with higher survival outcomes for individuals diagnosed with adenocarcinoma of the lung. It is also possible that it could lead to the development of proactive treatment plans for individuals who reside in areas with contaminated drinking water or who have higher occupational exposure risk to cadmium and arsenite. Improved lung cancer assessment and implementation of personalized medicine would increase the overall quality of life for individuals diagnosed with lung cancer. Therefore, characterization and full descriptions of these estrogen signaling pathways mediated by ERs are key to unraveling the complex mechanism of activation by these environmental estrogens.
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