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13. ABSTRACT (Maximum 200 Words)

Breast cancer is one of the leading causes of death in women. BRCA1 has an important role in DNA repair. Chemotherapeutic agents used in the treatment of breast cancer produce their cytotoxic effects by creating DNA damage. Estrogen (ER) and retinoid acid receptors (RAR) are members of a family of ligand dependent transcription factors. ER, RAT, and BRCA require CREB binding protein (CVP) to activate target gene transcription. The application proposed a new mechanism by which ER and RAR regulate BRCA1 mediated DNA repair via CBP. In the first year of the project, we determined that CBP is recruited to ligand bound ER and RAR in breast cancer cells. This recruitment is accompanied by decreased association of CBP with BRCA1. E2 and RA treatment of human breast cancer cell lines did not directly alter expression of DNA repair proteins with the exception of MSH2. We determined that RA increased apoptosis induced by etoposide, but that E2 protected ER+ breast cancer cells against apoptosis induced by etoposide alone or the combination of etoposide and RA. E2 failed to protect ER+ cells from cisplatin induced apoptosis, and treatment with the E2/RA combination resulted in the highest levels of cisplatin induced apoptosis.

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INTRODUCTION

BRCA1, DNA Repair, and Breast Cancer. Breast cancer is one of the leading causes of death in women. The disease and its consequences are a significant cause of morbidity and mortality (for review see Russo et al., 2000). Surgical removal of the tumor followed by radiotherapy is the therapeutic mainstay for early disease; however mastectomy with axillary lymph node dissection and chemotherapy may be required for disseminated breast cancer. Inactivating mutations in the tumor suppressor BRCA1 have been discovered in familial forms of the disease and are associated with significantly increased risk of developing breast cancer (Yang and Lippman, 1999). The BRCA1 gene encodes a protein of 1863 amino acids and shows no similarity to previously described proteins with the exception of a RING zinc finger motif in the amino terminus and two BRCT (BRCA1 carboxyl terminus) repeats (for review see Bertwistle and Ashworth, 1998). The BRCT repeat is found in a range of proteins involved in DNA repair (Koonin et al., 1996; Callebaut and Mornon, 1997). BRCA1 has been shown to induce expression of the DNA damage response gene GADD45 (MacLachlan et al., 2000). Additionally, BRCA1 functionally associates with Rad51 protein which is involved in double strand break repair (Scully et al., 1997). This evidence suggests an important role for BRCA1 in DNA repair and maintaining genome integrity (Kinzler and Vogelstein, 1997; Brugarolas and Jacks, 1997). In support of this hypothesis, BRCA1 is involved in repair of double strand breaks induced by chemotherapy drugs (Husain et al., 1998). A number of chemotherapeutic agents used in the treatment of breast cancer produce their cytotoxic effects by creating DNA damage (for review see Hoeijmakers, 2001).

Nuclear Hormone Receptors, Coactivators, and BRCA1. Among the most important nuclear hormone receptors expressed by breast cancer cells are those for estrogen and retinoic acid (Russo and Russo, 1998). For many years, potent estrogens such as 17-β-estradiol (E2) have been shown to dramatically enhance proliferation of mammary gland epithelium (Huseby et al., 1984). In contrast, a variety of natural and synthetic retinoids have been shown to inhibit proliferation of these cells and have therefore been used as chemotherapy drugs in the treatment of breast cancer (Dawson et al., 1995; Li et al, 1999). Estrogen receptors (ER) and retinoic acid receptors (RAR) are members of a family of ligand dependent transcription factors that include steroid, thyroid, and vitamin D receptors (for review see Mangelsdorf et al., 1995). Both ER and RAR have functional domains for DNA binding, ligand binding and dimerization with other factors, and transcriptional activation. ER and RAR require coactivator proteins such as CREB binding protein (CBP) to activate target gene transcription. CBP interacts with ER and RAR in their ligand bound conformation to induce gene expression (Chakravarti et al., 1996). CBP has histone acetyltransferase activity, allowing for histone disassembly and transcriptional activation (Ogryzko et al., 1996). CBP has been shown to interact with and enhance the function of BRCA1 (Pao et al., 2000).

A New Role for Estradiol and Retinoic Acid in BRCA1 Mediated DNA Repair. While the effects of E2 and RA on proliferation of human breast cancer cells have been known for many years, no studies have suggested a role for these hormones in DNA repair. The original application proposed a new mechanism by which ER and RAR regulate BRCA1 mediated DNA repair via CBP. This model may ultimately predict which breast cancers will respond to inclusion of retinoids in the chemotherapy regimen, ultimately providing better treatment outcomes for women with this disease.

BODY OF REPORT

In the first year of the funded application (April 2002-April 2003), we have made considerable progress towards accomplishing Task 1 as outlined in the Statement of Work. In Task 1a, we have immunoprecipitated CBP from E2 and RA treated breast cancer cell lines (MCF7, T47D, MDA-MB-213, and MDA-MB-468) and probed for interaction of ERα, RARα, and BRCA1 by western blotting. We have determined that CBP interacts with ERα in a ligand dependent manner, with maximum interaction occurring between 1 and 2 hours after E2 addition to the culture medium. This interaction was not detectable in ER negative breast cancer cell lines. CBP was not immunoprecipitated with a preimmune IgG control antibody. Increased interaction of CBP with RARα was observed beginning at 1 hour after addition of all trans RA or the RAR selective ligand Am80. Conversely, both E2 and RA treatment inhibited interaction of CBP with BRCA1. This decreased association occurred later in the time course of treatment, beginning at 4 hours after ligand addition to the culture medium. We concluded that E2 and RA treatment of ER+ human breast cancer cell lines recruited CBP to their respective receptors, producing inhibition of interaction with BRCA1.

As part of Task 1b, we examined E2 and RA dependent regulation of DNA repair protein expression. These proteins may interact directly or indirectly with CBP or BRCA1. We determined that the four human breast cancer cell lines expressed double strand break repair proteins of the Rad and XRCC groups in addition to Nibrin and Mre11. These proteins repair DNA damage due to ionizing radiation and certain drugs such as etoposide. These cell lines also expressed MLH1, MSH2, and XPA proteins which are involved in repair of DNA adducts due to chemotherapy agents such as cisplatin. We examined regulation of expression of these proteins in cultured human breast cancer cell lines treated with E2 or RA. Hormone dependent induction or repression of these protein levels may have an important effect on BRCA1 mediated DNA repair. With one exception, expression of DNA repair proteins were not significantly affected by E2 or RA treatment of human breast cancer cells. Both E2 and RA suppressed expression of MSH2 in ER+ cell lines which correlated with increased apoptosis following cisplatin treatment (see below). These results indicate that E2 and RA do not regulate DNA damage response primarily through modulation of repair protein expression in human breast cancer cell lines. Rather, we hypothesize that repair protein activity may be regulated by interaction with BRCA1 and CBP whose availability is modulated by ligand bound ER and RAR.

In accomplishing Task 1c, we determined the levels of DNA damage induced cell death in human breast cancer lines treated with E2 or RA followed by chemotherapeutic agents. We used 30 μg/ml etoposide (an inhibitor of topoisomerase) to induce double strand DNA breaks and 10 μg/ml cisplatin as a control drug which induces a different form of DNA damage (adduct formation). These two types of DNA damage are repaired by different sets of proteins. Etoposide treatment resulted in 60% apoptotic cells by 24 hours after addition to the culture medium. Pre-treatment with 100 nM to 1 μM RA increased the fraction of apoptotic cells in etoposide treated cultures to 80%. These results were observed in both ER+ and ER- human breast cancer cell lines. In contrast, E2 treatment decreased the fraction of apoptotic cells in etoposide treated cultures of ER+ positive breast cancer lines to 50%. This effect was specific for ER+ cells, since no decrease in apoptotic cells due to E2 was observed in etoposide treated ER- breast cancer lines. E2 exerted its protective effect over etoposide induced DNA damage

even in the presence of RA; ER+ cells pretreated with a combination of E2 and RA followed by etoposide showed apoptotic cell fractions similar to those of etoposide alone (60%). No apoptotic cell death was observed by E2 or RA treatment alone. We concluded that E2 protected ER+ human breast cancer lines from cell death induced by double strand breaks. In contrast RA increased the fraction of apoptotic cells induced by DNA damage in both ER+ and ER- lines.

In contrast to our results with double strand break DNA damage, E2 treatment of human breast cancer cell lines with cisplatin (which induces bulky adduct formation) did not show a protective effect against apoptosis induced by this drug. Cisplatin was as effective as etoposide at inducing apoptosis in cultured human breast cancer cells (60% dead cells after 24 hours). E2 treatment produced increased apoptosis in ER+ cell lines (75% dead cells after 24 hours) but had no effect on ER- lines. These results were predicted by our model system which was proposed in the funded application. RA treatment alone had minimal effect on apoptosis in cisplatin treated cultures, but combined treatment with E2 and RA increased the number of dead cells to 85% in ER+ cells but not ER- lines as predicted by our model. We concluded that E2 can increase the apoptotic effect of cisplatin in ER+ but not ER- human breast cancer cell lines.

KEY RESEARCH ACCOMPLISHMENTS

Task 1a

1. CBP interacts with ERα in a ligand dependent manner, with maximum interaction occurring between 1 and 2 hours after E2 addition to the culture medium.
2. Increased interaction of CBP with RARα was observed beginning at 1 hour after addition of all trans RA or the RAR selective ligand Am80.
3. Both E2 and RA treatment inhibited interaction of CBP with BRCA1. This decreased association occurred later in the time course of treatment, beginning at 4 hours after ligand addition to the culture medium.

Task 1b

1. Four human breast cancer cell lines (MCF7, T47D, MDA-MB-231, MDA-MB-468) expressed double strand break repair proteins of the Rad and XRCC groups in addition to Nibrin and Mre11.
2. These cell lines also expressed MLH1, MSH2, and XPA proteins.
3. Both E2 and RA suppressed expression of MSH2 in ER+ cell lines which correlated with increased apoptosis following cisplatin treatment.

Task 1c

1. Etoposide treatment resulted in 60% apoptotic cells by 24 hours after addition to the culture medium.
2. Pre-treatment with 100 nM to 1 μM RA increased the fraction of apoptotic cells in etoposide treated cultures to 80%. These results were observed in both ER+ and ER- human breast cancer cell lines.
3. E2 treatment decreased the fraction of apoptotic cells in etoposide treated cultures of ER+ positive breast cancer lines to 50%. This effect was specific for ER+ cells, since no decrease in apoptotic cells due to E2 was observed in etoposide treated ER- breast cancer lines.
4. E2 exerted its protective effect over etoposide induced DNA damage even in the presence of RA; ER+ cells pretreated with a combination of E2 and RA followed by etoposide showed apoptotic cell fractions similar to those of etoposide alone (60%).
5. No apoptotic cell death was observed by E2 or RA treatment alone.
6. Cisplatin was as effective as etoposide at inducing apoptosis in cultured human breast cancer cells (60% dead cells after 24 hours).
7. E2 treatment produced increased apoptosis in ER+ cell lines (75% dead cells after 24 hours) but had no effect on ER- lines.
8. RA treatment alone had minimal effect on apoptosis in cisplatin treated cultures; combined treatment with E2 and RA increased the number of dead cells to 85% in ER+ cells but not ER- lines.

REPORTABLE OUTCOMES

Not applicable

CONCLUSIONS

In the first year of the funded application we have demonstrated that CBP is recruited to ligand bound ER and RAR in human breast cancer cell lines. This recruitment is accompanied by decreased association of CBP with BRCA1 later in the time course of treatment. These data indicate that the affinity of CBP for its various protein partners is variable, and that temporal changes in gene expression in response to hormones may be a consequence of this differential association. We also examined the expression of a large number of DNA repair proteins in human breast cancer cells and how levels of these proteins are regulated by hormones. In general, we determined that E2 and RA treatment of human breast cancer cell lines did not directly alter expression of DNA repair proteins with the exception of MSH2. Levels of this protein were repressed by both E2 and RA which correlated with increased apoptosis following cisplatin treatment of ER+ breast cancer cell lines. These data indicate that functional interaction between nuclear hormone receptors, CBP, BRCA1, and DNA repair proteins rather than alteration of gene expression may be important in regulating the cellular response to DNA damage in human breast cancer lines. We also examined hormonal regulation of apoptosis in response to DNA damage by two different agents, namely etoposide and cisplatin. We determined that RA increased apoptosis induced by etoposide, which produces double strand breaks. E2 protected ER+ breast cancer cells against apoptosis induced by etoposide alone or by the combination of etoposide and RA. These effects were not observed when cells were treated with cisplatin, which results in DNA adduct formation. E2 failed to protect ER+ cells from cisplatin induced apoptosis, and treatment with the E2/RA combination resulted in the highest levels of cisplatin induced apoptosis. These results indicate that hormones may regulate the DNA damage response of breast cancer cells differently depending on the nature of the DNA damage.

The key finding from the first year of the funded application is that hormones present in the microenvironment of breast epithelium can control the cellular response to DNA damage. The effects of nuclear hormones such as E2 and RA on proliferation of breast epithelium have been documented over many years. However for the first time, our preliminary results point to an important role for estrogens and retinoids in controlling cellular response to DNA damage. When DNA damage is detected, cells undergo growth arrest at specific cell cycle checkpoints until repair can be completed. If the levels of DNA damage are too extensive, the cell undergoes apoptotic cell death. Our results indicate that E2 and combinations of E2 and RA increase the apoptotic response to DNA adduct formation. However, while RA increased the cellular apoptotic response to a different form of DNA damage (double strand breaks), E2 protected ER+ cells from etoposide induced apoptosis. At the molecular level, it will be important to determine whether E2 and RA can regulate cell cycle checkpoints in response to DNA damage. These results have potential clinical implications for breast cancer patients in that anti-estrogen therapy may be more effective when combined with certain therapies (radiation) than with other types of treatment (chemotherapy).

In the second year of the funded application, we will begin Task 2 by altering the expression and activity of ER, RAR, and BRCA1 to assess the role of these proteins in mediating ligand dependent regulation of DNA damage in breast cancer cells. The career development activities detailed in the funded application will also continue.

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