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Stress hormones mediate drug resistance to paclitaxel in human breast cancer cells through a CDK-1-dependent pathway

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Summary Chemotherapy comprises part of successful treatment regimens for breast cancer, however, up to 50% of patients develop resistance. Stress in cancer patients can equate to poor chemotherapeutic responses. We hypothesize that drug resistance may be associated with stress hormone-induced alterations in breast cancer cells. To test this hypothesis, MDA-MB-231 cells were cultured with paclitaxel and/or cortisol, norepinephrine and epinephrine and cytotoxicity, cell cycle analyses, genomic and proteomic analyses were performed. Paclitaxel-mediated cytotoxicity and G2/M cell cycle arrest were reversed significantly by stress hormones. Genomic and proteomic analyses revealed that stress hormones modulated beta-tubulin isotypes and significantly altered genes and proteins involved in regulation of the G2/M transition, including cyclin-dependent kinase-1 (CDK-1). Inhibition of CDK-1 abrogated stress hormone-mediated reversal of paclitaxel-induced cytotoxicity, indicating that the protective effect of stress hormones act through a CDK-1-dependent mechanism. These data demonstrate that stress hormones interfere with paclitaxel efficacy and contribute significantly to drug resistance.

1. Introduction

Breast cancer is one of the most frequently diagnosed malignancies among women in the U.S. and is the leading cause of death worldwide in women between the ages of 40 and 55 years (Jatoi et al., 2005). Although chemotherapy comprises part of a successful regimen for treating breast cancer, as many as 50% of patients fail to benefit due to the development of intrinsic and acquired multiple drug resistance (O'Driscoll and Clynes, 2006). Risk factors associated with

Abbreviations: cort, cortisol; E, epinephrine; NE, norepinephrine.

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onset of a resistant phenotype in breast cancer include; genetic predisposition such as mutations in α and β tubulins, and BRCA1/2; induction of expression of multi-drug resistance (MDR) proteins; alterations in spindle assembly checkpoints, cell cycle proteins and apoptosis [reviewed by (McGrogan et al., 2008)]. Although these factors are known to contribute a risk for chemo-resistance, stress is unfortunately not yet considered as a significant contributing factor.

Drug resistance is an ongoing issue, and studies of additional risk factors that underlie this event have shown that psychological stress can negatively impact chemotherapy efficacy. Recent investigations in rodents have demonstrated that rotational and restraint stress can increase the extent of lung and mammary carcinoma metastasis and that restraint stress specifically can attenuate the anti-tumor effects associated with cyclophosphamide, paclitaxel and doxorubucin (Su et al., 2005; Zorzet et al., 1998). In particular, stress via catecholamines was found to induce MDR-1 in MCF-7 breast cancer cells (Su et al., 2005). The use of the serotonin and NE reuptake inhibitor, nefazodone, was determined to be beneficial in breast cancer in stressed mice infected with the mammary tumor virus (Freire-Garabal et al., 2004). Epidemiological studies in humans have also analyzed the effects of stress and breast cancer risk (Duijts et al., 2003; Lillberg et al., 2001). Furthermore, a phase III clinical trial demonstrated psychological responses, such as depression and anxiety, correlate to breast cancer recurrence (Thornton et al., 2008). When considering the role of stress in breast cancer, evaluations of afflicted patients revealed that approximately 45% experience increased stress and anxiety levels (Bultz and Carlson, 2006; Carlson and Bultz, 2004), with the key psychological stressors noted as being the actual cancer diagnosis and the looming fear of chemotherapy treatment (Herschbach et al., 2008). When taken together, the perceived increases in stress levels reported by breast cancer patients and the recent evidence indicating that stress levels correlate with a decreased response to chemotherapy treatment, it is likely that the stress response may represent an additional factor underlying the onset of chemotherapeutic resistance in breast cancer patients.

The physiological mediators underlying the stress response include the activities of glucocorticoids (GCs) and catecholamine hormones. The GC hormone cortisol and the catecholamines epinephrine (E) and norepinephrine (NE) are produced by the adrenal glands in response to stress and circulate through the bloodstream. Cortisol directly binds to glucocorticoid receptors and catecholamines bind to beta-adrenergic receptors. These receptors are present on the surface of normal, precancerous and cancerous cells and result in activation of signaling cascades that control cell growth and cell cycle regulation (Lutgendorf et al., 2003; Pulaski et al., 2005; Thaker et al., 2006).

This study was designed to elucidate the impact of stress hormones on the phenotype of breast cancer cells treated with the chemotherapeutic agent paclitaxel toward providing insights into the molecular mechanisms which underlie this chemo-resistance. Paclitaxel, a front-line chemotherapeutic agent commonly utilized in the treatment of breast cancer, is a plant alkaloid that disturbs spindle formation during the G2/M phase of the cell cycle (Jordan and Wilson, 2004). Paclitaxel has also been shown to promote apoptosis (Tudor et al., 2000). We elected to use the estrogen receptor-negative and MDR-negative breast cancer cell line, MDA-MB-231, because of its invasive phenotype, its tumorigenic properties and its ability to produce metastases in mice. Similarly to MCF-7 cells, this cell line possesses functional alpha and beta adrenergic receptors (Chiesa et al., 2008; Slotkin et al., 2000) and is glucocorticoid receptor positive. Therefore this cell line provides for a robust initial model system for investigation of the pathways and roles of stress hormones in chemotherapeutic resistance.

The data from the literature provided here supports the hypothesis that development of paclitaxel drug resistance in breast cancer may be attributable to stress hormone-induced alterations in the G2/M cell cycle transition. Co-treatment of the breast cancer cell line MDA-MB-231 with paclitaxel and individual or combinations of cortisol, NE and E showed that stress hormones can decrease cytotoxicity of paclitaxel through reversal of the G2/M phase blockade produced by this agent. Proteomic, genomic and functional analyses further revealed a role for CDK-1 mRNA and protein levels were found to be up-regulated in response to stress hormone treatment. Furthermore, inhibition of CDK-1 activity via co-treatment with the CDK-1 inhibitor abrogated the rescue of paclitaxel-induced cytotoxicity by stress hormones. These data provide evidence to support the need to establish the importance of stress mediators in risk assessment of cancer treating drugs.

2. Materials and methods

2.1. Cell lines

MDA-MB-231$^{ER\text{-}PR\text{-}Her2Neu\text{-}MDR\text{-}Gr+}$ breast cancer cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) and Hams nutrient mixture (F-12) 1:1 with 4 mM L-glutamine and bovine calf serum (10%) in a 37 °C incubator at 5% CO_2. Paclitaxel was dissolved in ethanol and was used to prepare a final concentration of 10^{-3} M in DMEM medium. Cortisol (5×10^{-6} M) was purchased as a stock solution. Norepinephrine, epinephrine, and propranolol (Sigma, St. Louis, MO) were dissolved first in water from which stock solutions (10^{-3} M) were made in DMEM. RU-486 was dissolved in 4% DMSO in water, from which a 10^{-3} M solution in DMEM was made.

2.2. Hormone and chemotherapy studies

MDA-MB-231 cells were incubated for 24 h prior to treatment with paclitaxel (10^{-7} M) and either cortisol (10^{-6} M), NE (10^{-7} M) or E (10^{-7} M), individually, or in combination for the time points indicated. These concentrations mimic the physiological levels of circulating cortisol, NE and E generated during acute stress reported by other researchers (Ruprecht et al., 1997, 1999). As a control for hormone specificity, cells were co-treated with the GC receptor antagonist, RU-486 (10^{-6} M) or beta-adrenergic receptor antagonist, propranolol (10^{-6} M) for 30 min prior to addition of stress hormones.

2.3. Measurement of cell cytotoxicity

Cytotoxicity assays were performed by treatment of MDA-MB-231 cells plated in a 96-well plate with paclitaxel and treat-