

## Review Article

# Induction of Cisplatin Resistance by Hormones in Breast Cancer

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## Abstract

Cisplatin is an effective anti-cancer drug which has been successfully utilized in the treatment of many cancer types. However, cisplatin has shown only limited efficacy in the majority of breast tumors, and is not generally included as a monotherapy in this disease. The potential role of hormones in chemoresistance in breast cancer has received little attention. This review focuses on two classes of hormones: a) protein hormones that include prolactin, growth hormone and leptin, all of which act via class 1 cytokine receptors and activate the Jak2/stat signaling pathway, and b) steroid hormones that include estrogens, xenoestrogens, and glucocorticoids, which generally act via ligand-regulated nuclear receptors. Accumulating evidence indicate that each of these hormone antagonizes cisplatin cytotoxicity in breast cancer cells by a variety of mechanisms. The implications of chemoresistance by these hormones to patients with hormone-sensitive breast cancer, and the potential benefits of increasing chemosensitivity by using anti-hormone treatment regimens are discussed.

## Keywords

- Resistance to cisplatin
- Breast cancer
- Protein hormones
- Steroids

## ABBREVIATIONS

BCC: Breast Cancer Cells; BPA: Bisphenol A; Dex: Dexamethasone; E2: 17 $\beta$  Estradiol; Era: Estrogen Receptor Alpha; Er $\beta$ : Estrogen Receptor Beta; ERE: Estrogen Response Elements; GH: Growth Hormone; GHR: Growth Hormone Receptor; GRE: Glucocorticoid Response Element; GPR30: G-Protein Coupled Receptor 30 (Also Named GPER); GST: Glutathione-S-Transferase; Jak2: Janus Kinase 2; KLF5: Krüppel-Like Factor 5; MAPK: Mitogen-Activated Protein Kinases; Ob-R: Leptin Receptor; PLC: Phospholipase C; PRL: Prolactin; PRLR: Prolactin Receptor; ROS: Reactive Oxygen Species; SIRT1: Sirtuin 1; Tg $\alpha$ : Transforming Growth Factor Alpha; Tg $\beta$ : Transforming Growth Factor Beta; UCP2: Uncoupling Protein 2

## INTRODUCTION

Cisplatin and carboplatin serve as the first line anti-cancer treatment in ovarian, testicular, and lung cancers. However, they have only limited efficacy in triple-negative breast cancer, and are largely ineffective in the majority of breast tumors, which are hormone-sensitive [1,2]. Yet, the potential role of hormones as contributing factors to cisplatin resistance in this disease has received little attention. Poor responsiveness to cisplatin can result from several causes, including diminished intracellular drug accumulation due to drug efflux or metabolic inactivation, increased anti-apoptotic proteins, and enhanced DNA damage repair [3].

This short review focuses on two classes of hormones that antagonize cisplatin cytotoxicity in breast cancer: a) protein hormones such as prolactin (PRL), growth hormone (GH) and

leptin, all of which bind to class 1 cytokinemembrane receptors and activate the Jak2/Stat signaling pathway, and b) steroid hormones, such as estrogens, xenoestrogens and glucocorticoids, all of which bind primarily to nuclear hormone receptors that act as transcription factors. Below we outline some features of these hormones and their receptors, and review the data demonstrating their ability to reduce cisplatin efficacy. We also discuss the implications of this resistance to patients with breast cancer, and the potential benefits of developing anti-hormonal treatment regimens to improve cisplatin efficacy.

## Resistance to cisplatin by selected protein hormones

**Prolactin:** PRL is a 23 kDa multifunctional protein hormone that is produced by the pituitary gland as well as by many extra pituitary sites [4]. Within the human breast, PRL is produced by both compartments: the epithelium, where tumors developed, and the surrounding stroma, composed of PRL-producing adipocytes, fibroblasts, endothelial cells, and immune cells. Consequently, breast tumors are exposed to PRL from the circulation (endocrine regulation), as well as from local sources (paracrine and autocrine regulation). The PRL receptor (PRLR) is a single-pass membrane protein that belongs to class 1 cytokinereceptor superfamily. These receptors are characterized by a tripartite structure: an extracellular ligand binding domain, a short transmembrane domain, and an intracellular domain that couples to a variety of signaling molecules [5]. Alternative splicing of the PRLR generates long, intermediate, and several short isoforms, which differ in the length of their intracellular domains. Jak2/Stat5a/b is the main, albeit not the exclusive,

signaling pathway that is activated by ligand binding to a dimerized long PRLR.

The involvement of PRL in breast tumorigenesis is well established. For example, in rodents, PRL stimulates the growth of mammary tumors [6]. In women, elevated serum PRL levels increase the risk of developing breast cancer [7]. In cultured human breast cancer cells (BCC), PRL increases the proliferation, survival, invasiveness, and clonogenic growth [8,9].

Studies from our laboratory revealed that pretreatment of several BCC with PRL significantly reduced cytotoxicity by cisplatin [10]. This antagonism was ascribed to PRL-induced activation of glutathione-S-transferase (GST), a phase II detoxification enzyme which conjugates cisplatin to glutathione, thereby enabling its expulsion from the cells. This observation was indirectly supported by the report that inhibition of glutathione synthesis reverses Bcl-2-mediated cisplatin resistance in BCC [11]. Indeed, upregulation of the anti-apoptotic protein Bcl-2 is another mechanism by which PRL enhances resistance to cisplatin [12]. In addition to cisplatin, PRL increases resistance to chemotherapeutic agents representing different classes, including doxorubicin, taxol and vinblastine [10], establishing the action of PRL as an anti-apoptotic hormone which provides survival advantage to cancer cells. Notably, another study reported that autocrine PRL protects T47D and MCF-7 cells from cisplatin cytotoxicity [13]. This notion was based on the enhancement of cisplatin-induced apoptosis by co-treatment with a hPRL antagonist. The mechanism by which the antagonist induced apoptosis was attributed to TGF $\alpha$  downregulation, TGF $\beta$  upregulation, and caspase 3 activation.

**Growth hormone:** GH is a 23 kDa pituitary hormone with a similar structure to, and some overlapping functions with, PRL. Likewise, GH is also expressed in many extrapituitary sites [14]. In addition to acting via its cognate receptor (GHR), a class 1 cytokine receptor, human GH (hGH) binds to and activates the hPRLR. Consequently, GH may affect breast cancer by acting on GHR, PRLR or both. Several lines of evidence indicate that GH contributes to the development and progression of breast cancer [15]. As discussed in a recent review, GH increases chemoresistance in breast cancer by altering drug efflux, oxidative stress response, and apoptosis [16]. Although we found no reports that specifically examined the effects of GH on cisplatin, in all likelihood its actions resemble those of PRL. Indeed, in endometrial adenocarcinoma cells, GH increases resistance to cisplatin by markedly blunting the cisplatin-induced activation of caspase 3/7 [17].

**Leptin:** Leptin is a 16kDa protein hormone synthesized in white adipose tissue, and serves as a key regulator of the nutritional status and energy metabolism. Leptin acts through the leptin receptor (Ob-R), a class 1 cytokine receptor with a prototypical tripartite structure but a more complex extracellular moiety. The receptor exists as six isoforms that differ in the length of the intracellular domains [18]. Similar to PRL and GH, leptin signals primarily through the Jak2/Stat pathway. In obesity, elevated serum leptin levels play significant roles in breast tumor initiation, growth, invasion, and metastatic progression [19]. Chronic treatment of MCF-7 cells with leptin counteracted cisplatin-induced cytotoxicity [20]. The antagonistic effect of

leptin was attributed to a reduction in cisplatin-induced ROS (reactive oxygen species) generation, resulting from decreased activity of mitochondrial uncoupling protein 2 (UCP-2), which prevents ROS production, and increased activity of SIRT1, which is involved in the adaptive response against oxidative stress. Others reported that treatment of BCC with a leptin antagonist coupled to nanoparticles augmented the apoptotic effects of cisplatin, indicating that leptin is a survival factor in these cells [21].

## Resistance to cisplatin by steroid hormones

Estrogens are physiologically defined by their ability to develop and maintain the female reproductive system. This category includes naturally occurring steroids i.e., 17 $\beta$  estradiol (E2), estrone and estriol, as well as a variety of non-steroidal compounds from the environment, which mimic the effects of estrogens and are termed xenoestrogens or endocrine disruptors.

**Natural estrogens:** The breast is exposed to natural estrogens from two sources: the circulation and local production. Both breast tumors and the surrounding fat express sex-steroid synthesizing enzymes that include aromatase, 17 $\beta$ -hydroxysteroid dehydrogenase, and steroid sulfatase [22]. Estrogens bind to several receptors of diverse structure that are localized in the membrane, cytoplasm and nucleus. The two 'classical' receptors, ER $\alpha$  and ER $\beta$ , are the products of distinct genes and differ primarily in their ligand binding domains [23]. Following ligand binding, the ERs dimerize, translocate to the nucleus and bind to estrogen response elements (EREs) in the promoters of target genes. In many cell types, a subpopulation of ER $\alpha$  is tethered to the cell membrane through palmitoylation. Activation of the membrane-associated ERs by estrogens results in rapid, non-genomic actions. Another class of estrogen-binding receptors is represented by GPR30 (or GPER), a seven-transmembrane domain receptor that signals through G-proteins [24]. Binding of estradiol to GPR30 stimulates the cAMP pathway, but directly or indirectly also activate other pathways such as MAPK, PI3K and PLC.

Studies from our laboratory revealed that estradiol abrogated cisplatin toxicity in several BCC by increasing cell proliferation and by decreasing apoptosis [25]. Such protection by estrogen occurred in the presence of ER $\alpha$  and ER $\beta$  antagonists, in ER $\alpha$ -negative cells, and in cells with ER $\beta$  knockdown, suggesting an action that is independent of classical ERs. Unlike PRL, E2 does not reduce cisplatin entry into the nucleus, indicating that it acts downstream of DNA damage. E2 increases the expression of Bcl-2 in T47D cells, both in the presence and absence of cisplatin. Given that a potent Bcl-2 inhibitor only partially abrogated protection by E2, other mediators may be involved [25].

**Xenoestrogens:** A wide variety of industrial chemicals, pesticides, pharmaceuticals, and plant-derived compounds (phytoestrogens) mimic or antagonize endogenous estrogens [26]. Human exposure to these compounds occurs through the food and water supply, household goods, some dental composites and medical devices. One of the best studied endocrine disruptors is bisphenol A (BPA), a component of polycarbonate plastics and epoxy resins, which are prevalent in many consumer products. In BCC, BPA affects cell proliferation and activates certain sets

of genes, not all of which also respond to estradiol [27]. In addition, BPA rapidly activates non-genomic signaling, causing phosphorylation of MAPK and Akt within 10 min of exposure.

Studies from our laboratory found that similar to the actions of PRL and E2, BPA antagonizes multiple anti-cancer drugs, showing equimolar potency with estradiol in opposing cisplatin toxicity [25]. BPA alone or in combination with doxorubicin [25], or cisplatin [28], increased Bcl-2 expression. Treatment with a Bcl-2 inhibitor completely blocks the BPA-induced antagonism of cisplatin, but only partially abrogates protection by E2. This suggests that BPA and estrogen exert protection against drug cytotoxicity by somewhat different mechanisms. Indeed, analysis of BrdU incorporation showed that BPA alone increases cell survival while estrogen alone increased cell proliferation [28]. Whether BPA binds to classical ERs or activates as yet an unidentified receptor remains to be determined. This notion is not unprecedented, as exemplified by opioid and cannabinoid receptors which were discovered many years after the bioactivity of their exogenous ligands was recognized. There are still hundreds of membranes, cytoplasmic and nuclear receptors without an identified endogenous ligand.

**Glucocorticoids:** Cortisol, produced and secreted by the adrenal cortex, is the most abundant glucocorticoid in humans. Cortisol is essential for life, and it regulates or supports cardiovascular, metabolic, immunologic and homeostatic functions. Synthetic glucocorticoids are widely utilized in medical practice as replacement therapy in glucocorticoid deficiency, or to suppress the immune system. The glucocorticoid receptors (GR) are members of the nuclear receptor superfamily. These are typical hormone-dependent transcription factors that regulate gene transcription by binding to GREs (glucocorticoid response element) in target genes. Dexamethasone (Dex), a potent glucocorticoid analog, is used in the pretreatment of cancer patients undergoing chemotherapy to prevent allergic reactions, to alleviate chemotherapy-induced nausea and vomiting, and to improve appetite. Krüppel-like factor 5 (KLF5) is a transcription factor that is highly expressed in triple-negative breast cancer and promotes cell proliferation, survival, tumorigenesis and chemoresistance. Acting by increasing KLF5 transcription, Dex antagonized the apoptotic effects of both docetaxel and cisplatin *in vitro* and *in vivo* [29].

## CONCLUSIONS AND CLINICAL IMPLICATIONS

Only few studies have focused on the potential roles of hormones in cisplatin resistance in breast cancer. One reason for this oversight is the multiple sites of origin of these hormones, which reach breast tumors from the circulation, from the adjacent stroma, and from cancer cells themselves. Consequently, analysis of blood levels of lactogens or estrogens does not reveal the full extent of tumor exposure to these hormones. Unfortunately, logistical problems limit the clinical ability to assess the true exposure level of breast tumors to these hormones within the microenvironment of the intact human breast. A second confounding issue is receptor promiscuity, with estrogen receptors capable of binding both steroidal and non-steroidal compounds, while the prolactin receptor capable of binding two distinct hormones, PRL and GH, which are classified as lactogens. This is further complicated by the presence of multiple receptor

isoforms, with estrogens binding to both nuclear and membrane receptors, and lactogens binding to different PRLR isoforms that are coupled to several signaling pathways. Another issue is the inability to assess the extent of exposure to xenoestrogens which likely contribute to cisplatin resistance. Leptin, whose circulating levels are in direct proportion to adipose tissue mass, represent the contributions of obesity to chemoresistance. Dexamethasone, a synthetic glucocorticoid which is prescribed to patients undergoing chemotherapy to alleviate unpleasant side effects, may also impact on chemoresistance. Addressing some of the above issues by basic researchers would require non-conventional thinking. In addition, clinicians should consider implementation of combinatorial treatments, i.e., anti-lactogens and anti-estrogens, as a means for improving cisplatin efficacy, as well as finding substitutes for synthetic glucocorticoids.

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## REFERENCES

1. Decatris MP, Sundar S, O'Byrne KJ. Platinum-based chemotherapy in metastatic breast cancer: current status. *Cancer Treat Rev.* 2004; 30: 53-81.
2. Petrelli F, Coinu A, Borgonovo K, Cabiddu M, Ghilardi M, Lonati V, et al. The value of platinum agents as neoadjuvant chemotherapy in triple-negative breast cancers: a systematic review and meta-analysis. *Breast Cancer Res Treat.* 2014; 144: 223-232.
3. Amable L. Cisplatin resistance and opportunities for precision medicine. *Pharmacol Res.* 2016; 106: 27-36.
4. Ben-Jonathan N, Mershon JL, Allen DL, Steinmetz RW. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocr Rev.* 1996; 17: 639-669.
5. Swaminathan G, Varghese B, Fuchs SY. Regulation of prolactin receptor levels and activity in breast cancer. *J Mammary Gland Biol Neoplasia.* 2008; 13: 81-91.
6. Arendt LM, Schuler LA. Transgenic models to study actions of prolactin in mammary neoplasia. *J Mammary Gland Biol Neoplasia.* 2008; 13: 29-40.
7. Tworoger SS, Hankinson SE. Prolactin and breast cancer etiology: an epidemiologic perspective. *J Mammary Gland Biol Neoplasia.* 2008; 13: 41-53.
8. Clevenger CV, Gadd SL, Zheng J. New mechanisms for PRLr action in breast cancer. *Trends Endocrinol Metab.* 2009; 20: 223-229.
9. LaPensee EW, Ben-Jonathan N. Novel roles of prolactin and estrogens in breast cancer: resistance to chemotherapy. *Endocr Relat Cancer.* 2010; 17: R91-107.
10. LaPensee EW, Schwemberger SJ, LaPensee CR, Bahassi eM, Afton SE, Ben-Jonathan N. Prolactin confers resistance against cisplatin in breast cancer cells by activating glutathione-S-transferase. *Carcinogenesis.* 2009; 30: 1298-1304.
11. Rudin CM, Yang Z, Schumaker LM, VanderWeele DJ, Newkirk K, Egorin MJ, Zuhowski EG, Cullen KJ. Inhibition of glutathione synthesis reverses Bcl-2-mediated cisplatin resistance. *Cancer Res.* 2003; 63: 312-318.

12. Beck MT, Peirce SK, Chen WY. Regulation of bcl-2 gene expression in human breast cancer cells by prolactin and its antagonist, hPRL-G129R. *Oncogene*. 2002; 21: 5047-5055.
13. Ramamoorthy P, Sticca R, Wagner TE, Chen WY. In vitro studies of a prolactin antagonist, hPRL-G129R in human breast cancer cells. *Int J Oncol*. 2001; 18: 25-32.
14. Perez-Ibave DC, Rodriguez-Sanchez IP, Garza-Rodriguez ML, Barrera-Saldana HA. Extra pituitary growth hormone synthesis in humans. *Growth Horm IGF Res*. 2014; 24: 47-53.
15. Perry JK, Mohankumar KM, Emerald BS, Mertani HC, Lobie PE. The contribution of growth hormone to mammary neoplasia. *J Mammary Gland Biol Neoplasia*. 2008; 13: 131-145.
16. Subramani R, Nandy S, Pedroza DA, Lakshmanaswamy R. Role of Growth Hormone in Breast Cancer. *Endocrinology*. 2017.
17. Gentilin E, Minoia M, Bondanelli M, Tagliati F, Degli Uberti EC, Zatelli MC. Growth Hormone differentially modulates chemoresistance in human endometrial adenocarcinoma cell lines. *Endocrine*. 2016.
18. Carpenter B, Hemsworth GR, Wu Z, Maamra M, Strasburger CJ, Ross RJ, et al. Structure of the human obesity receptor leptin-binding domain reveals the mechanism of leptin antagonism by a monoclonal antibody. *Structure*. 2012; 20: 487-497.
19. Barone I, Giordano C, Bonofiglio D, Andò S, Catalano S. Leptin, obesity and breast cancer: progress to understanding the molecular connections. *Curr Opin Pharmacol*. 2016; 31: 83-89.
20. Nadal-Serrano M, Sastre-Serra J, Valle A, Roca P, Oliver J. Chronic-leptin attenuates Cisplatin cytotoxicity in MCF-7 breast cancer cell line. *Cell Physiol Biochem*. 2015; 36: 221-232.
21. Harmon T, Harbuzariu A, Lanier V, Lipsey CC, Kirilin W, Yang L, et al. Nanoparticle-linked antagonist for leptin signaling inhibition in breast cancer. *World J Clin Oncol*. 2017; 8: 54-66.
22. Foster PA. Steroid metabolism in breast cancer. *Minerva Endocrinol*. 2008; 33: 27-37.
23. Huang B, Warner M, Gustafsson JÅ. Estrogen receptors in breast carcinogenesis and endocrine therapy. *Mol Cell Endocrinol*. 2015; 418: 240-244.
24. Prossnitz ER, Barton M. Estrogen biology: new insights into GPER function and clinical opportunities. *Mol Cell Endocrinol*. 2014; 389: 71-83.
25. Lapensee EW, Tuttle TR, Fox SR, Ben-Jonathan N. Bisphenol A at low nanomolar doses confers chemoresistance in estrogen receptor-alpha-positive and -negative breast cancer cells. *Environ Health Perspect*. 2009; 117: 175-180.
26. Soto AM, Sonnenschein C. Environmental causes of cancer: endocrine disruptors as carcinogens. *Nat Rev Endocrinol*. 2010; 6: 363-370.
27. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocr Rev*. 2009; 30: 75-95.
28. Lapensee EW, Lapensee CR, Fox S, Schwemberger S, Afton S, Ben-Jonathan N. Bisphenol A and estradiol are equipotent in antagonizing cisplatin-induced cytotoxicity in breast cancer cells. *Cancer Lett*. 2010; 290: 167-173.
29. Li Z, Dong J, Zou T, Du C, Li S, Chen C, et al. Dexamethasone induces docetaxel and cisplatin resistance partially through up-regulating Kruppel-like factor 5 in triple-negative breast cancer. *Oncotarget*. 2017; 8: 11555-11565.

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