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## **Human chorionic gonadotropin (HCG) induction of apoptosis in breast cancer**

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**Background:** Apoptotic induction in cancer cells may improve the efficacy of local or systemic therapy. Human Chorionic Gonadotropin (HCG) injection directly into Kaposi's sarcoma or melanoma has been shown to increase the apoptotic index in these tumor types. The rapid induction of apoptosis in breast cancer immediately preceding local or systemic therapy may improve the response to therapy or local control. We hypothesized that HCG would rapidly increase the apoptotic index in breast cancer after intratumoral injection. The primary objective of this preclinical trial was to determine if intratumoral injection of HCG would significantly increase the apoptotic index in breast cancer xenografts. **Methods:** Using a human breast cancer xenograft model in Nude mice,  $5 \times 10^6$  SK-BR3 human breast cancer cells were injected subcutaneously into each flank of nu/nu mice. When the tumors reached 6 mm, 50  $\mu$ L (100 U/mL) of non-recombinant, naturally occurring HCG (A.P.L., Wyeth) or saline vehicle control was injected directly into the xenograft tumors. After 24 hrs, the tumors were harvested, and the xenografts tested for proliferation (Ki-67) and apoptosis (by TUNEL assay). The apoptotic index was calculated (apoptosis/proliferation) and statistical analysis performed using paired T-test. **Results:** Seven pairs of SK-Br3 xenografts were tested. There were no differences in proliferation by Ki-67 determination between control or treated xenografts. Apoptosis increased in HCG treated xenografts compared to vehicle controls. Apoptosis increased from a mean of 5% (range 1–20%) in control xenografts to a mean of 28% (range 1–70%) in HCG treated tumors ( $p = 0.038$ ). **Conclusions:** Naturally-derived HCG induces apoptosis in human breast cancer xenografts after intratumoral injection. Substantial induction of apoptosis may improve the efficacy of local and systemic therapy in breast cancer. Injection of HCG into breast tumors immediately prior to surgical, radiation, or systemic therapy has the potential to improve local control or response to treatment. Further elucidation of potential synergy with therapeutic modalities is justified.

# Purified human chorionic gonadotropin induces apoptosis in breast cancer

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## Abstract

Agents that induce apoptosis in breast cancer cells have great potential to facilitate chemotherapeutic intervention and improve patient outcomes. In this study, the effects of injecting purified human chorionic gonadotropin (hCG) directly into human breast cancer xenografts grown in nude mice were examined. It was shown that intratumoral injection of purified hCG increased the apoptotic index in breast cancer xenografts. These results were supported by the findings that exposure of breast cancer cells to purified hCG decreased cell viability in five different breast cancer cell lines. In some of these cell lines, the effects of hCG in cell viability appear to correlate with activation/expression of the hCG/luteinizing hormone receptor. Preoperative apoptotic induction by factors such as purified hCG may improve local control or work synergistically with neoadjuvant chemotherapy to improve complete pathologic response of locally advanced breast cancer. [Mol Cancer Ther 2008;7(9):2837–8]

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## Human Chorionic Gonadotropin Decreases Proliferation and Invasion of Breast Cancer MCF-7 Cells by Inhibiting NF- $\kappa$ B and AP-1 Activation\*

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The epidemiological data suggest that breast cancer risk decreases in women who complete full-term pregnancy at a young age. Studies on a rat breast cancer model indicate that human chorionic gonadotropin (hCG), a hormone that is present in very high levels during pregnancy, could be responsible for this decrease. These findings, as well as those demonstrating the presence of functional luteinizing hormone (LH)/hCG receptors

in human breast cells, prompted us to investigate the anti-proliferative and anti-invasive effects of hCG in human breast cancer MCF-7 cells by down-regulating NF- $\kappa$ B and AP-1 transcription factors. Treatment of MCF-7 cells with highly purified hCG resulted in a modest dose-dependent and hormone-specific decrease in cell proliferation. hCG treatment also decreased cell invasion, which was more dramatic than the decrease in cell proliferation. These hCG actions were abrogated when receptor synthesis was inhibited by treatment with antisense hCG/LH receptor phosphorothioate oligodeoxynucleotide. hCG treatment prevented the tumor necrosis factor-dependent NF- $\kappa$ B and AP-1 activation, which paralleled a decrease in the phosphorylation and degradation of I $\kappa$ B $\alpha$ . The findings that hCG treatment increased cAMP synthesis and activated cAMP-dependent protein kinase, dibutyryl cAMP mimicked hCG in preventing NF- $\kappa$ B activation, and dideoxyadenosine, an adenylate cyclase inhibitor, prevented the hCG effect on NF- $\kappa$ B suggested that the hCG actions are mediated via the cAMP-dependent protein kinase A signaling pathway. In summary, our results demonstrate that hCG has anti-proliferative and anti-invasive effects in MCF-7 cells by down-regulating NF- $\kappa$ B and AP-1. These findings support the premise that hCG could be responsible for the pregnancy-induced protection against breast cancer in women.

## **A pregnancy hormone shows promise for preventing breast cancer in women, according to Philadelphia researchers.**

In animal studies, human chorionic gonadotropin (HCG) activated tumor suppressor genes, stopped [cancer cell](#) growth, and induced other genetic changes that indicate an anticancer effect, says Irma H. Russo, MD, chief of molecular endocrinology at Fox Chase Cancer Center.

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*Irma H. Russo, MD, chief of molecular endocrinology, Fox Chase Cancer Center, Philadelphia. Timothy Richard Rebbeck, PhD, professor of epidemiology, University of Pennsylvania, Philadelphia.*

# **Treatment with human chorionic gonadotropin and risk of breast cancer**

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Studies of the induction of mammary tumors by 7,12-dimethylbenz(a)anthracene in a rat model show that human chorionic gonadotropin (hCG) administration reduces tumor incidence in a manner comparable to that of a completed pregnancy. On the basis of their studies, Russo and Russo (*Cancer Epidemiol., Biomarkers & Prev.*, 3: 353-364, 1994) have proposed that hCG treatment of young nulliparous women would reduce their breast cancer risk in a manner similar to that of a term pregnancy. As part of a population-based, case-control study of breast cancer among women ages 40 years or younger, we asked women whether they had received hCG injection as part of a weight loss regimen or as a component of infertility treatment. Participants in this study were 744 women newly diagnosed with breast cancer between July 1983 and December 1988 and 744 controls individually matched on birthdate (within 36 months), race (white), parity (nulliparous/parous), and neighborhood of residence. Forty-five cases and 65 controls reported exposure to hCG (multivariate odds ratio = 0.77, 95% confidence interval = 0.50-1.19). Risk was reduced significantly among women whose maximum nonpregnant body mass index was less than 27.5 kg/m<sup>2</sup> but no reduction in risk was observed among more obese women. Although the odds ratios were reduced substantially for both nulliparous and parous women with maximum nonpregnant body mass indices less than 27.5, only the result for nulliparous women was statistically significant. These results are consistent with the effects proposed by Russo and Russo based on their animal model. Although not definitive, these results suggest that hCG may be a means for reducing breast cancer risk.

**hCG, in combination with radiation and lovastatin, may represent a novel approach to kill prostate cancer**

cells.<http://molpharm.aspetjournals.org/cgi/content/abstract/mol.106.031153v1>

## **Risk of Breast Cancer After Exposure to Fertility Drugs: Results from a Large Danish Cohort Study**

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**Background:** Few epidemiologic studies have examined the association between fertility drugs and breast cancer risk, and results have been contradicting. Using data from the largest cohort of infertile women to date, the aim of this study was to examine the effects of fertility drugs on breast cancer risk overall and according to histologic subtypes.

**Method:** A cohort of 54,362 women with infertility problems referred to all Danish fertility clinics between 1963 and 1998 was established. A detailed data collection, including information of type and amount of treatment, was conducted. We used case-cohort techniques to calculate rate ratios (RR) of breast cancer associated with use of five groups of fertility drugs, after adjustment for parity status.

**Results:** Three hundred thirty-one invasive breast cancers were identified in the cohort during follow-up through 1998. Analyses within cohort showed no overall increased breast cancer risk after use of gonadotrophins, clomiphene, human chorionic gonadotrophin, or gonadotrophin-releasing hormone, whereas use of progesterone increased breast cancer risk (RR, 3.36; 95% confidence interval, 1.3-8.6). For all groups of fertility drugs, no relationships with number of cycles of use or years since first use of fertility drug were found. However, gonadotrophins may have a stronger effect on breast cancer risk among nulliparous women (RR, 1.69; 95% confidence interval, 1.03-2.77). Similar risk patterns were present for ductal, lobular, and tumors of other histologies, indicating identical etiologies.

**Conclusion:** The results showed no strong association between breast cancer risk and use of fertility drugs. Follow-up is, however, needed to assess long-term breast cancer risk after use of progesterone and among nulliparous women exposed to gonadotrophins. (*Cancer Epidemiol Biomarkers Prev* 2007;16(7):1400-7)

# Human chorionic gonadotropin (hCG) and prevention of breast cancer

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## Abstract

Animal and ‘in vitro’ experiences learned that human chorionic gonadotropin (hCG) is capable to protect from breast cancer. Receptors for hCG/luteinizing hormone (LH) are present on human female and male breast cancer cells. hCG decreases proliferation and invasion of breast cancer MCF-7 cells by inhibiting NF-kappa B, AP-1 activation and other genes. Doxorubicin toxicity is enhanced by conjugation with beta-hCG in MCF-7 cells. All these pieces of evidence suggest that hCG is active in human breast cancer. Direct proof however is missing. We performed a pilot study phase I trial for testing the inhibitory effects of recombinant hCG (rhCG) on primary breast cancer. Twenty-five postmenopausal women with newly diagnosed breast cancers of more than 1.5 cm were biopsied before randomization to receive either 500 µg rhCG (*n* = 20) or placebo. After 2 weeks, surgery was done and tissues were analysed with regard to morphological, immunohistochemical and biochemical changes in tissues and plasma. rhCG reduces significantly the proliferative index and the expression of both the oestrogen receptor and progesterone receptor. rhCG does not modify the hormonal level of estradiol, progesterone, inhibin and follicle stimulating hormone (FSH) but increases significantly the level of LH. In a second pilot study, we tested the clinical efficacy through an open-label single centre study in 13 postmenopausal women with metastatic breast cancer. A 500 µg rhCG once every 2 days shows activity in postmenopausal metastatic breast cancer. The time to progression is relatively short. Response to previous hormonal treatment is indicative for rhCG activity. Given the data in primary and metastatic breast cancer rhCG further large scale investigation is highly warranted. rhCG can be an realistic option in (chemo-) prevention trials.

## Recent Advances and Future Directions in Endocrine Manipulation of Breast Cancer

## Breast Differentiation and Its Implication in Cancer Prevention

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Sporadic breast cancer is a fatal disease most frequently diagnosed in American women from all ethnic groups, suggesting that primary prevention should be the ultimate goal for breast cancer control. We have developed a novel paradigm for breast cancer prevention arising from the well-established knowledge that an early first full-term pregnancy protects the breast against neoplastic transformation, as well as from our studies of the biological principle underlying this protection. We have shown experimentally that the first pregnancy induces the expression of a specific genomic signature in the breast that results from the completion of a cycle in this organ's differentiation driven by the reproductive process. This signature, in turn, is a biomarker associated with a possible overall lifetime decrease in breast cancer risk. We have shown in an experimental model that a short treatment with human chorionic gonadotropin, a placental hormone secreted during pregnancy, induces the same genomic signature that occurs in pregnancy, inhibiting not only the initiation but also the progression of mammary carcinomas, and stopping the development of early lesions such as intraductal proliferations and carcinoma *in situ*. These observations indicate that human chorionic gonadotropin given for a very short period, only until this genomic signature is acquired, has significant potential as a chemopreventive agent, protecting the normal cell from becoming malignant. This is a novel concept which challenges the current knowledge that a chemopreventive agent needs to be given for a long period of time to suppress a metabolic pathway or abrogate the function of an organ.

## Direct hCG Actions Afford Protection Against Breast Cancer.

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Breast cancer, the fatal disease most frequently diagnosed in American women, is steadily increasing in incidence in most westernized societies. Although the prevention of this disease has been hindered by the lack of identification of an etiologic agent, the epidemiological observations that breast cancer risk is higher in nulliparous women than in women that have completed one or more pregnancies at a young age provide physiological basis for intervention. These findings highlight the role of endocrinological and reproductive events in breast cancer prevention. This phenomenon has been experimentally confirmed through the demonstration that in rats, one pregnancy or a 21-day treatment with human chorionic gonadotropin (hCG) before administration of a chemical carcinogen

significantly reduce the incidence of mammary tumors. In addition, hCG administered to tumor bearing animals, is therapeutic, since it inhibits tumor progression. The *in vivo* cancer protective effects of hCG are in great part mediated by its stimulatory effect on the ovary through the G protein-coupled lutropin-choriogonadotropin-receptor (LH-CG-R) present in the granulosa and luteal cells of the ovary. This novel hormonal milieu induces mammary gland differentiation, inhibition of cell proliferation, and activation of tumor suppressor genes like inhibin and apoptotic genes, i.e., TRPM2, ICE, p53, c-myc, WAF-1/CIP-1, bcl-XS, and p53. Rat gene microarray analyses, real time RT-PCR, and cluster analysis performed at different time points of hCG treatment have revealed that the genomic profile of the mammary gland varies as a function of the length of treatment and correlates with the stage of development or regression of the organ. A direct effect of hCG on human breast epithelial cells has been demonstrated *in vitro* in the normal immortalized MCF-10F cells and in its derived chemically transformed cell lines. In these cells a 24-hour treatment activated apoptotic genes, a phenomenon preceded by induction of the CAMPPKA, p53, and TGF-beta pathways, for acting on their target gene p21 *WAF1/CIP1*, before proceeding towards cell cycle arrest. HCG inhibits tumor growth in a dose dependent manner in Balb/c nude mice (*nu/nu*) injected with the breast carcinoma cell line MCF-7. In post-menopausal women with primary operable breast cancer diagnosed by needle core biopsy, a 2-week treatment with recombinant hCG prior to therapeutic surgery significantly reduced the tumor's proliferative activity (Ki67 index) and the percentage of steroid hormone receptor positive cells; it also increased the synthesis of inhibin, all effects independent of ovarian function and of the estrogen and progesterone receptor status of the host tissue. Future studies are geared towards the elucidation the genetic and epigenetic pathways through which hCG inhibits cancer, knowledge necessary for unfolding the potential of this model for the prevention and treatment of breast cancer based on physiological mechanisms of gene expression regulation.

## **hCG therapy for the treatment of breast cancer**

Document Type and Number:

United States Patent 7183251

Abstract:

This invention relates to the field of cancer therapy. More particularly, the invention relates to the treatment of mammary tumor, clinically manifest mammary tumor (breast cancer) and metastatic mammary tumor by administration of human Chorionic Gonadotropin (hCG). The treatment preferably comprises the administration of hCG in conjunction with an antiestrogen and/or a Type I Interferon.