Published in final edited form as:

Trends Endocrinol Metab. 2010 November ; 21(11): 691–698. doi:10.1016/j.tem.2010.08.004.

Unexploited Therapies in Breast and Prostate Cancer: Blockade of the Prolactin Receptor

Eric M. Jacobson¹, Eric R. Hugo², Traci R. Tuttle², Ruben Papoian³, and Nira Ben-Jonathan²

¹ Division of Endocrinology, Diabetes and Metabolism, University of Cincinnati, Cincinnati OH 45267-0521, USA

² Department of Cancer and Cell Biology, University of Cincinnati, Cincinnati OH 45267-0521, USA

³ Drug Discovery Center, University of Cincinnati, Cincinnati OH 45267-0521, USA

Abstract

Breast and prostate cancers are hormone-sensitive malignancies that afflict millions of women and men. While prolactin (PRL) is known as a survival factor that supports tumor growth and confers chemoresistance in both cancers, its precise role in these tumors has been understudied. Growth hormone and placental lactogen also bind to the PRL receptor (PRLR) and mimic some of PRL actions. Blockade of the PRLR represents a novel treatment for patients with advanced breast or prostate cancer with limited therapeutic options. This review discusses different approaches for generating PRLR antagonists. Emphasis is placed on technological advances which enable high throughput screening for small molecule inhibitors of PRLR signaling that can serve as oral medications.

Parallels between breast and prostate cancer

Breast and prostate cancers afflict millions of women and men worldwide and have similar life time risk profiles. Despite differences in their respective etiologies, these tumors share many properties, especially in their responsiveness to hormones and disease progression [1]. Although hormones do not typically initiate tumors, they can promote growth of transformed cells by interacting with growth factors and oncogenes. Like estrogens in breast cancer, the essential function of androgens in prostate cancer is undisputed. In both cancers, steroidal deprivation or receptor blockade can suppress growth of receptor-positive tumors. Unfortunately, tumors often escape regulation by steroids, rendering hormonally-based therapies ineffective. Patients with advanced disease have limited therapeutic options, as their tumors are resistant not only to hormonal intervention, but also to most chemotherapeutic agents [1]. This provides a major incentive for developing novel treatments aimed at reducing morbidity and increasing survival in these patients. Box 1 (breast cancer) and Box 2 (prostate cancer) describe selected characteristics of each disease.

Corresponding Author: Ben-Jonathan, N. (Nira.Ben-Jonathan@uc.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Box 1**Salient Features of Breast Cancer****Epidemiology**

breast cancer represents ~15% of all cancer cases in women, and is the fifth most common cause of cancer death. The overall lifetime risk for a woman is 12%. Risk factors include: early menarche, late menopause, delayed childbearing, nulliparity, age, obesity, benign breast disease, and a family history of breast or ovarian cancer (e.g., mutated *BRCA1* and *BRCA2*).

Classification

tumors are classified by stage (size and invasiveness), pathology (ductal or lobular), grade (cell differentiation), and receptor status (estrogen receptors, progesterone receptors and HER2/neu).

Diagnosis

this is commonly done by a 'triple test' which constitutes physical examination, mammography, and fine needle aspiration and/or core biopsy.

Treatment

The most common curative treatment is surgery, entailing lumpectomy or mastectomy with or without lymph node removal. Depending upon tumor stage and spread, surgery is accompanied by radiotherapy, adjuvant therapy or both. Hormone-insensitive tumors usually represent a more severe form of the disease.

Hormonal and Chemical Therapies

these consist of three main groups of medications: hormone blocking therapy (e.g., tamoxifen and aromatase inhibitors), chemotherapy (e.g., doxorubicin, cyclophosphamide, and taxanes), and humanized monoclonal antibodies against HER2 (e.g., herceptin).

Metastasis

the common sites for metastases are bone, lung, liver and brain.

Prognosis and Survival

the 10-year disease-free survival varies widely from 98% to 10%, depending on tumor staging, spread, age, and patient responsiveness to the various therapies. Breast cancer in premenopausal women often has poorer prognosis than that in postmenopausal women.

Box 2**Salient Features of Prostate Cancer****Epidemiology**

prostate cancer represents ~25% of all cancer cases in men, and is the ninth most common cancer. The average age of onset is > 65 years. Risk factors include: age, androgen, ionizing radiation, lack of exercise, diet, and pattern of sexual behavior. Prostate cancer in the USA is most common among black men and least common among Asian men. Genetic components for prostate cancer have not been well defined.

Classification

prostate tumors are classified by a four-stage system that evaluates: size, local invasiveness, number of involved lymph nodes, and presence of other metastases.

Diagnosis

this can be done by blood test for prostate-specific antigen (PSA), digital rectal exam, cystoscopy, and trans-rectal ultrasonography. The most definitive diagnosis is done by biopsy.

Treatment

primary treatment options include surgery, radiation therapy, and proton therapy. Other localized treatments involve cryosurgery and high intensity focused ultrasound. Androgen-independent tumors usually represent a more severe form of the disease.

Hormonal and chemical therapies

hormone therapy (androgen ablation and GnRH agonists) is effective in early-stage tumors that express the androgen receptor. Chemotherapy is primarily used as a palliative treatment in advanced metastatic disease.

Metastasis

the common sites of metastases are bone, lymph nodes, rectum and bladder.

Prognosis and Survival

the Gleason Score is based on an extensive microscopic evaluation of the tumors. Cancers with high Gleason score are more aggressive and have worse prognosis. The overall 5-year survival rate is ~95%. Given the late onset and slow growth of prostate cancer, most patients die from other causes.

This review focuses on the role of PRL as a survival factor which supports tumor growth and confers chemoresistance in breast and prostate cancers. We also discuss other lactogens, i.e., growth hormone (hGH) and placental lactogen (hPL), which mimic many of PRL actions and likely do so via the PRL receptor (PRLR). Finally, we review current strategies for blocking the PRL signaling cascade, emphasizing technological advances that enable screening for small molecule inhibitors of the PRLR that can serve as a new class of oral medications.

The PRLR is targeted by all three lactogens

The three human lactogens share many structural and functional characteristics. They are composed of 191–199 primary residues that form a four anti-parallel helical bundle and have a similar three-dimensional configuration (Figure 1a). hPRL is a multifunctional hormone whose main target is the breast, where it exerts secretory, mitogenic and anti-apoptotic actions. hGH regulates growth and metabolism, with the liver serving as its main target. hPL, the product of the placenta, exerts GH-like metabolic actions during pregnancy (reviewed in [2]).

The PRLR is a member of the cytokine receptor superfamily, characterized by a tripartite structure: an extracellular ligand binding domain (ECD), a short transmembrane domain, and an intracellular domain (ICD) which couples to signaling pathways, including JAK2/STAT5, MAPK and PI3K [3]. Activation of the PRLR is contingent upon the presence of two binding sites on the ligand: a high affinity site 1 that binds one receptor, and a lower affinity site 2 that binds another receptor, forming a ternary complex comprised of one hormone molecule and two receptors. As determined by surface plasmon resonance, the K_d 's for recombinant human PRL ranged from 6.5 to 62 nM for site 1 and 32.9 μ M for site 2 [4,5]. Recombinant human pituitary GH has a K_d of 5.76 μ M to site 1, which increased to 388 nM in the presence of zinc, while human placental growth hormone, the product of the *GH2* gene, does not bind to the

PRLR [6]. In the presence of zinc, recombinant human placental lactogen binds to the PRLR at 1.49 nM [5]. Since a unique receptor for hPL has not been identified, hPL is believed to bind solely to the PRLR.

As depicted in Figure 1b, the three human lactogens bind to the PRLR, while hGH also binds to GHR, its cognate receptor. The lactogens are equipotent in stimulating the growth of Nb2 cells (Figure 1c), a rat lymphocyte cell line which depends on ligand-activated PRLR for survival. Given their exquisite sensitivity to PRL, Nb2 cells serve as the most common bioassay for PRL detection, even though they also respond to other lactogens and to IL-2. Crystallographic and functional studies on ligand-receptor interactions provide only partial explanation for the promiscuity of the PRLR [3,7,8], and there is no ready explanation for some of the dissimilarities in the actions of the three lactogens.

PRL as a survival and chemoresistance agent in breast cancer

Elevated serum PRL levels are associated with higher risk of breast cancer [9], as well as with increased treatment failure and worse survival in patients with advanced disease [10]. However, PRL reaches the tumors not only from the circulation but also from local sources, i.e., both the stromal (adipose) and epithelial compartments of the breast [11]. Autocrine/paracrine PRL stimulates tumor growth, as evident by the development of mammary hyperplasia and invasive carcinomas in transgenic mice overexpressing hPRL in their mammary gland [12,13]. In addition, PRL-overexpressing breast cancer cells implanted in nude mice developed faster growing tumors that were characterized by upregulation of the PRLR and the anti-apoptotic protein Bcl-2 [14]. Table 1 summarizes the data on the expression of the three lactogens, PRLR and GHR in human breast tissue and cell lines.

Consistent with its actions as a survival factor in breast cancer cells, PRL antagonizes cytotoxicity by chemotherapeutic agents which include the DNA-damaging drugs cisplatin and doxorubicin, and the mitotic inhibitor taxol [15]. One mechanism by which PRL reduces drug efficacy is by activating glutathione-S-transferase, a detoxification enzyme which conjugates electrophilic drugs to glutathione and facilitates their extrusion [15,16]. Another mechanism is by increased expression of the anti-apoptotic protein Bcl-2 [17]. Antagonism of drug toxicity by PRL is further supported by a clinical study in which hyperprolactinemic women with metastatic breast cancer were less responsive to taxol than those with normal serum PRL levels [18].

Cross-talk between the PRL signaling cascade and other pro-cancer pathways is an important aspect of breast cancer. One example is HER-2/neu, a receptor tyrosine kinase which is overexpressed in many breast carcinomas and is associated with a shorter relapse time and decreased patient survival [19]. PRL induced tyrosine phosphorylation of HER2 and increased the proliferation of breast cancer cells that overexpress HER-2 [20]. Furthermore, the simultaneous inhibition of the PRLR and HER-2 was more effective than inhibiting HER-2 alone in suppressing cell proliferation [21]. The PRL pathway also increases the activity of the IGF-1 [22] and EGF [23] pathways. Finally, PRL increased the serine-118 phosphorylation of estrogen receptor α (ER α), and enhanced estrogen-induced cell proliferation in T47D breast cancer cells, supporting cross-talk between PRLR and ER α [24].

The significance of the PRL/PRLR axis in prostate cancer

Epidemiological data have not shown an association between circulating PRL levels and prostate cancer [25], whereas increasing evidence indicates a strong role for locally-produced PRL. As a proof of concept, transgenic mice overexpressing PRL in their prostates developed a dramatic enlargement of the gland with diminished apoptosis [26]. As depicted in Table 2, both PRL and the PRLR are expressed in normal and malignant human prostates. An autocrine

loop for PRL was revealed by the reduced cell viability in response to hPRL antagonists in the androgen-independent 22Rv1 cells [27], and the androgen-sensitive LnCaP cells [28]. Additionally, PRL initiated a distinct temporal phosphorylation pattern of ERK1/2, JAK2 and STAT5 in cultured human prostate explants [29].

Immunohistochemical analysis of prostate cancer samples showed a strong link between PRL and its signaling and prostate cancer pathology. For example, 50% of 183 hormone-insensitive prostate cancer specimens, and 63% of prostate cancer metastases, stained for PRL [27]. The PRL-positive tumors showed a correlation with activated STAT5 and a high Gleason score (high grade, hormone refractory and metastatic disease), which portends a much greater mortality than those with a low score. A recent gene expression array study found a 6.7-fold increase in *PRLR* mRNA in ductal vs acinar prostate carcinomas [30]; ductal adenocarcinoma presents a more aggressive clinical picture than acinar carcinoma.

The strongest evidence linking the PRL pathway to prostate cancer comes from studies on STAT5, which was found to be constitutively active in human prostate cancer and a prognosticator of early disease recurrence [31,32]. Indeed, STAT5 promotes metastatic behavior of human prostate cancer cells *in vitro* [33] and is essential for growth of human prostate cancer xenografts in nude mice [27]. Indeed, inhibition of STAT5 by siRNA induced a massive death of human prostate cancer cells *in vitro* and in nude mice [34].

The oncogenic potential of GH in breast and prostate cancer

Elevated serum GH levels are associated with increased risk of both breast [35] and prostate [36] cancer, while GH deficiency results in the opposite effects. Although most attention has focused on the GH-IGF-1 axis, a direct role for GH has also been demonstrated. As depicted in Tables 1&2, both hGH and the GHR are expressed in breast and prostate tissues and in some cell lines [37–39]. The involvement of local hGH in breast cancer has been extensively studied by Lobie *et al*, who used MCF-7 cells stably transfected with intact or mutated hGH genes (reviewed in [35]). They found that autocrine hGH promoted cell proliferation and survival, increased epithelial-mesenchymal transition and invasion, and upregulated genes associated with chemo- or radio-resistance. Based on the blockade of some of these effects by a GHR antagonist, they concluded that autocrine hGH acts via the GHR [40]. However, the concurrent expression of the *PRLR* in breast cancer cells and the ability of hGH to activate this receptor raise the possibility that some of GH actions in breast cancer are mediated through the *PRLR*.

hGH actions have also been reported in prostate cancer. In LnCaP prostate cancer cells, hGH induced rapid and dose-dependent phosphorylation of the GHR itself, JAK2, STAT5A, MAPK and Akt/PKB [39]. Although GH did not alter basal or androgen-induced cell proliferation or PSA secretion, it caused a rapid and transient increase in immunoreactive androgen receptor (AR) levels. This suggested that GH regulates functions other than proliferation in prostate cancer cells, and that it also interacts with the AR. Mediation of hGH actions by GHR, *PRLR* or both in prostate cancer remains to be clarified.

Role of placental lactogen in hormone-responsive cancers

The placenta is a transient organ which accommodates the growing fetus within the maternal organism. hPL is produced by the trophoblast and is first detected in the maternal circulation at 6 weeks of gestation. The trophoblast has been defined as a ‘pseudo-malignant’ tissue [41], given its very high proliferation rate, lack of cell-contact inhibition, migratory and invasive properties, increased angiogenesis, and the capacity to escape immune rejection.

Ectopic hormone production by malignant tissues is not uncommon. Another placental hormone, chorionic gonadotropin (hCG), is expressed not only in choriocarcinomas, but also

in tumors of the ovary, breast, prostate, kidney, and pancreas [42]. Notably, ectopic production of hPL in breast cancer has been reported several decades ago (Table 1). For example, hPL was detected by immunocytochemistry in 42/50 breast carcinomas but not in 12 benign tumors [43]. A 1977 report found detectable serum hPL levels in 10/72 patients with breast carcinomas but not in sera from 13 patients with benign breast disease, 20 normal women or 20 normal men [44]. A retrospective study of 233 patients with invasive breast carcinomas found that 31% and 25% of invasive ductal and lobular carcinomas, respectively, stained positively for hPL [45], while non-neoplastic breast epithelium was negative for this antigen. More recently, hPL was detected by Western blotting in several breast cancer cell lines [46]. Whereas we did not find previous reports on hPL expression in the human prostate, this issue warrants further investigation.

Various methods for blocking the PRL signaling cascade

Early clinical trials attempting to benefit breast cancer patients by suppressing pituitary PRL release with bromocriptine have been unsuccessful [47]. This failure is commonly explained by the fact that expression of pituitary and extrapituitary PRL is driven by different promoters with dissimilar regulators [48]. This, together with the potential contribution by other lactogens, suggest that blockade of the PRLR or further downstream, rather than suppression of individual lactogens, should comprise a more efficacious treatment for patients with breast or prostate cancers.

Recognizing the PRLR as an excellent therapeutic target, several groups have generated PRLR antagonists by mutating the PRL molecule [49–51]. One such antagonist is S179D hPRL which mimics phosphorylated PRL, while another is G129R hPRL which disrupts receptor dimerization. The most promising of the bioengineered PRL molecules is Δ 1-9-G129R-hPRL, reported to act as a pure antagonist by decreasing site 2 binding by an order of magnitude, while not affecting binding to site 1 [4]. In spite of good antagonistic activities of engineered PRL molecules *in vitro* and in rodent models, their use in patients has several limitations. These include the need to deliver protein-based therapies by injections, short half-life in the circulation, and the difficulty in achieving sustained therapeutic levels. On the other hand, small molecule antagonists of the PRLR should have better delivery and pharmacodynamic properties.

To date, there is only one report on non-protein molecules that bind the PRLR [52]. After screening a library of small cyclic peptides on a solid support, 20 ‘hits’ from a 1.2×10^7 member library were identified. Although some of these had binding constants in the 2–3 μ M range, their antagonistic activity in live cells was not demonstrated. Whereas cyclic peptides should be more resistant to proteolysis than polypeptides, the molecular weight for even a small cyclic peptide is considerably greater than that of a typical therapeutic small molecule, limiting its use as an oral medication.

Strategy for discovering small molecule inhibitors

Small molecule candidates are typically discovered by using ‘libraries’ of various chemicals and high throughput screening (HTS). HTS affords an automated, simultaneous testing of tens of thousands of compounds, with the ultimate goal of identifying those molecules that are both effective and specific for a given target. HTS has become the mainstay of the pharmaceutical industry, resulting in many marketed drugs [53]. Compounds with molecular weights of 500 or below have proven especially valuable for treating diseases, and most oral medicines marketed today belong to this class of compounds [54].

The design of a small molecule library is usually based upon the ‘Lipinski’s rules of 5’ [55] which maximizes the chance that an identified compound would be orally active in humans.

The rules entail the following: no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular weight of less than 500 daltons, and an octanol-water partition coefficient log P of less than 5. A diversity oriented library should occupy a vast chemical space, while maximizing chirality and molecular rigidity, two factors that are important in the design of pharmaceuticals [56].

Structure-based computer modeling and the selection of readouts for HTS

As outlined in Figure 2, small molecule antagonists of the PRL signaling can be identified by a combination of molecular modeling, availability of a large and diversity-oriented library, and a judicious selection of cell-based readouts. For example, the ‘glycine cavity’ that surrounds G129 and is critical for PRLR binding to site 2 on PRL [7] could be interrogated *in silico* using computer modeling. A virtual screen of a small molecule database, taking into considerations steric and energetic criteria [57], could select those compounds whose fit within this cavity is predicted to prevent receptor docking by the lactogens. While attractive, this approach does not take into account other site interactions between the ligand(s) and the receptor, or between the receptor and downstream effectors (i.e., JAK2). Therefore, an effective screening must include a large set of diverse compounds that are not directly related to receptor docking.

A cell-based readout can either be responsive to a specific signaling pathway, or be sensitive to changes in cell phenotype. STAT5 activation constitutes an excellent endpoint of the PRLR pathway. To this end, an engineered cell line with a stable copy of a STAT5-driven luciferase promoter can be used. PRL was recently reported to cause a ~40 fold induction of the CISH (cytokine-inducible SH2-containing protein) promoter in T47D cells [58]. As an alternative to luciferase reading, a proximity-based sandwich immunoassay in a 384-well format can be used to quantify activated STAT5 [59]. However, this approach disregards other signaling pathways which are activated by the lactogens.

Cell proliferation and/or survival should comprise a highly effective readout. For example, Nb2 cells, given their exquisite sensitivity and responsiveness to the three lactogens (see Figure 1c), could constitute an excellent cellular model. Important advantages of cell-based readouts include their sensitivity to cytotoxic effects or to membrane permeability of the candidate molecules. In addition, cell-based approaches would be more likely than *in vitro* or *in silico* methodologies to translate observed activities into future therapeutics.

Validation and confirmation of HTS ‘hits’

The goal of the primary screening is to find many compounds with significant potency, and to identify structurally inactive compounds for establishing an initial Structure-Activity Relationship (SAR). Hits identified by HTS must be validated in a secondary assay as PRLR antagonists by their ability to abrogate growth or anti-cytotoxic properties of lactogens in breast and prostate cancer cells (Figure 2). In addition, the specificity of putative antagonists must be verified in PRLR-deficient cells. A post-screening stage should assess which antagonists have the highest likelihood of progressing to the pre-clinical development. The goals are to have the strongest initial SAR, to select compounds with good drug-like properties, and to progress with those compounds where future modifications by medicinal chemistry are most feasible. This goal notwithstanding, few compounds identified at early screening stages may serve as valuable probes for studying the biological ramifications of PRLR signaling disruption. Ultimately, extensive validation of candidate molecules in animal models, followed by clinical trials will be needed in order to gain acceptance as a treatment modality.

Which patients are most likely to benefit from PRLR blockade?

A therapy, no matter how well-designed or intentioned, is often effective only in a certain percentage of patients. One mechanism which can predict the course of therapeutic outcomes and prognoses is the presence of single nucleotide polymorphisms (SNPs) in relevant genes. To date, two SNP variants of the PRLR, which cause a sequence change of a single amino acid of the molecule (non-synonymous SNPs), have been characterized. The PRLR polymorphism I146L was found to be associated with follicular breast adenoma [60,61], and approached a significance in a study with a limited patient size (38 breast cancer patients vs. 100 healthy controls) [62]. Importantly, when transfected into MCF-7 cells, this receptor variant showed constitutive activation of STAT5 and MAPK, highlighting another potential mechanism for PRLR activation independent of circulating PRL [60]. A second non-synonymous SNP, I76V, approached a significant association in benign breast disease, was not tested in breast cancer, and like I146L, exhibited constitutive activation, albeit to a lesser extent [61]. There is a clinical need to conduct association studies of PRLR polymorphisms on a larger scale.

Ostensibly, patients harboring a PRLR variant which is constitutively active or more sensitive to lactogens would benefit most from PRL pathway antagonists. Nonetheless, such antagonists should be effective in reducing tumor growth and increasing sensitivity to chemotherapy in patients with wild-type PRLR. Furthermore, PRLR inhibition need not be a stand-alone treatment, with maximal clinical benefit obtained when applied in tandem with inhibitors of other pathways which cross-talk with the PRLR.

Conclusions and clinical implications

Although lactogens exhibit only modest mitogenic activity, they can support tumor growth by suppressing apoptosis. Upregulation of anti-apoptotic proteins is a hallmark of aggressive breast and prostate cancer. Chronic exposure of breast and prostate tumors and their metastases to circulating and/or locally produced lactogens likely activate anti-apoptotic mechanisms, resulting in sustained tumor growth and desensitization to many anticancer drugs. Consequently, an effective blockade of the PRLR activity should improve outcome in patients with advanced disease by increasing the repertoire as well as the efficacy of existing therapeutic agents. By tapping into the immense potential of HTS, novel small molecule modulators of the PRL signaling pathway can be unveiled and brought to the clinic. To date, targeting the PRLR has not been a high priority objective for drug discovery by the pharmaceutical industry. Such an oversight should be reconsidered.

Acknowledgments

This work was supported by NIH grants CA096613 and ES016803, Department of Defense grant BC05725, Susan G. Komen for the Cure grant BCRT87406, and a grant from the Elsa U Pardee Foundation (to NBJ). EMJ was supported by a pilot grant (P30 ES06096) by the University of Cincinnati Center of Environmental Genetics.

Reference List

1. Lopez-Otin C, Diamandis EP. Breast and prostate cancer: an analysis of common epidemiological, genetic, and biochemical features. *Endocr Rev* 1998;19:365–396. [PubMed: 9715372]
2. Ben-Jonathan N, et al. What can we learn from rodents about prolactin in humans? *Endocr Rev* 2008;29:1–41. [PubMed: 18057139]
3. Svensson LA, et al. Crystal structure of a prolactin receptor antagonist bound to the extracellular domain of the prolactin receptor. *J Biol Chem* 2008;283:19085–19094. [PubMed: 18467331]
4. Jomain JB, et al. Structural and thermodynamic bases for the design of pure prolactin receptor antagonists: X-ray structure of Del1-9-G129R-hPRL. *J Biol Chem* 2007;282:33118–33131. [PubMed: 17785459]

5. Voorhees JL, Brooks CL. Obligate ordered binding of human lactogenic cytokines. *J Biol Chem* 2010;285:20022–20030. [PubMed: 20427283]
6. Solomon G, et al. Large-scale preparation and in vitro characterization of biologically active human placental (20 and 22K) and pituitary (20K) growth hormones: placental growth hormones have no lactogenic activity in humans. *Growth Horm IGF Res* 2006;16:297–307. [PubMed: 17010651]
7. Broutin I, et al. Crystal structure of an affinity-matured prolactin complexed to its dimerized receptor reveals the topology of hormone binding site 2. *J Biol Chem* 2010;285:8422–8433. [PubMed: 20053995]
8. Elkins PA, et al. Ternary complex between placental lactogen and the extracellular domain of the prolactin receptor. *Nat Struct Biol* 2000;7:808–815. [PubMed: 10966654]
9. Tworoger SS, et al. A prospective study of plasma prolactin concentrations and risk of premenopausal and postmenopausal breast cancer. *J Clin Oncol* 2007;25:1482–1488. [PubMed: 17372279]
10. Tworoger SS, Hankinson SE. Prolactin and breast cancer etiology: an epidemiologic perspective. *J Mammary Gland Biol Neoplasia* 2008;13:41–53. [PubMed: 18246319]
11. Zinger M, et al. Prolactin expression and secretion by human breast glandular and adipose tissue explants. *J Clin Endocrinol Metab* 2003;88:689–696. [PubMed: 12574200]
12. Rose-Hellekant TA, et al. Prolactin induces ERalpha-positive and ERalpha-negative mammary cancer in transgenic mice. *Oncogene* 2003;22:4664–4674. [PubMed: 12879011]
13. Wennbo H, et al. Activation of the prolactin receptor but not the growth hormone receptor is important for induction of mammary tumors in transgenic mice. *J Clin Invest* 1997;100:2744–2751. [PubMed: 9389738]
14. Liby K, et al. Prolactin overexpression by MDA-MB-435 human breast cancer cells accelerates tumor growth. *Breast Cancer Res Treat* 2003;79:241–252. [PubMed: 12825859]
15. Lapensee EW, et al. Prolactin confers resistance against cisplatin in breast cancer cells by activating glutathione-S-transferase. *Carcinogenesis* 2009;30:1298–1304. [PubMed: 19443905]
16. Lapensee EW, Ben-Jonathan N. Novel roles of prolactin and estrogens in breast cancer: resistance to chemotherapy. *Endocr Relat Cancer* 2010;17:R91–107. [PubMed: 20071456]
17. Beck MT, et al. Regulation of bcl-2 gene expression in human breast cancer cells by prolactin and its antagonist, hPRL-G129R. *Oncogene* 2002;21:5047–5055. [PubMed: 12140755]
18. Lissoni P, et al. A clinical study of taxotere versus taxotere plus the antiprolactinemic agent bromocriptine in metastatic breast cancer pretreated with anthracyclines. *Anticancer Res* 2002;22:1131–1134. [PubMed: 12168912]
19. Engel RH, Kaklamani VG. HER2-positive breast cancer: current and future treatment strategies. *Drugs* 2007;67:1329–1341. [PubMed: 17547474]
20. Yamauchi T, et al. Constitutive tyrosine phosphorylation of ErbB-2 via Jak2 by autocrine secretion of prolactin in human breast cancer. *J Biol Chem* 2000;275:33937–33944. [PubMed: 10938266]
21. Scotti ML, et al. Additive effects of a prolactin receptor antagonist, G129R, and herceptin on inhibition of HER2-overexpressing breast cancer cells. *Breast Cancer Res Treat* 2008;111:241–250. [PubMed: 17955362]
22. Carver KC, et al. Prolactin enhances insulin-like growth factor I receptor phosphorylation by decreasing its association with the tyrosine phosphatase SHP-2 in MCF-7 breast cancer cells. *J Biol Chem* 2010;285:8003–8012. [PubMed: 20080972]
23. Huang Y, et al. Prolactin modulates phosphorylation, signaling and trafficking of epidermal growth factor receptor in human T47D breast cancer cells. *Oncogene* 2006;25:7565–7576. [PubMed: 16785991]
24. Chen Y, et al. Prolactin and estradiol utilize distinct mechanisms to increase serine-118 phosphorylation and decrease levels of estrogen receptor alpha in T47D breast cancer cells. *Breast Cancer Res Treat* 2010;120:369–377. [PubMed: 19377875]
25. Stattin P, et al. Plasma prolactin and prostate cancer risk: A prospective study. *Int J Cancer* 2001;92:463–465. [PubMed: 11291087]
26. Dillner K, et al. Gene expression analysis of prostate hyperplasia in mice overexpressing the prolactin gene specifically in the prostate. *Endocrinology* 2003;144:4955–4966. [PubMed: 12960074]

27. Dagvadorj A, et al. Autocrine prolactin promotes prostate cancer cell growth via Janus kinase-2-signal transducer and activator of transcription-5a/b signaling pathway. *Endocrinology* 2007;148:3089–3101. [PubMed: 17412813]
28. Xu X, et al. A molecular mimic of phosphorylated prolactin markedly reduced tumor incidence and size when DU145 human prostate cancer cells were grown in nude mice. *Cancer Res* 2001;61:6098–6104. [PubMed: 11507059]
29. Hair WM, et al. Prolactin receptor expression in human testis and accessory tissues: localization and function. *Mol Hum Reprod* 2002;8:606–611. [PubMed: 12087074]
30. Sanati S, et al. Gene expression profiles of ductal versus acinar adenocarcinoma of the prostate. *Mod Pathol* 2009;22:1273–1279. [PubMed: 19633648]
31. Li H, et al. Activation of signal transducer and activator of transcription-5 in prostate cancer predicts early recurrence. *Clin Cancer Res* 2005;11:5863–5868. [PubMed: 16115927]
32. Tan SH, et al. Transcription factor Stat5 synergizes with androgen receptor in prostate cancer cells. *Cancer Res* 2008;68:236–248. [PubMed: 18172316]
33. Gu L, et al. Stat5 promotes metastatic behavior of human prostate cancer cells in vitro and in vivo. *Endocr Relat Cancer* 2010;17:481–493. [PubMed: 20233708]
34. Gu L, et al. Transcription factor Stat3 stimulates metastatic behavior of human prostate cancer cells in vivo, whereas Stat5b has a preferential role in the promotion of prostate cancer cell viability and tumor growth. *Am J Pathol* 2010;176:1959–1972. [PubMed: 20167868]
35. Perry JK, et al. The contribution of growth hormone to mammary neoplasia. *J Mammary Gland Biol Neoplasia* 2008;13:131–145. [PubMed: 18253708]
36. Fuhrman B, et al. Basal growth hormone concentrations in blood and the risk for prostate cancer: a case-control study. *Prostate* 2005;64:109–115. [PubMed: 15666390]
37. Chopin LK, et al. Co-expression of GH and GHR isoforms in prostate cancer cell lines. *Growth Horm IGF Res* 2002;12:126–136. [PubMed: 12175650]
38. Gebre-Medhin M, et al. Growth hormone receptor is expressed in human breast cancer. *Am J Pathol* 2001;158:1217–1222. [PubMed: 11290538]
39. Weiss-Messer E, et al. Growth hormone (GH) receptors in prostate cancer: gene expression in human tissues and cell lines and characterization, GH signaling and androgen receptor regulation in LNCaP cells. *Mol Cell Endocrinol* 2004;220:109–123. [PubMed: 15196705]
40. Kaulsay KK, et al. The effects of autocrine human growth hormone (hGH) on human mammary carcinoma cell behavior are mediated via the hGH receptor. *Endocrinology* 2001;142:767–777. [PubMed: 11159849]
41. Ferretti C, et al. Molecular circuits shared by placental and cancer cells, and their implications in the proliferative, invasive and migratory capacities of trophoblasts. *Hum Reprod Update* 2007;13:121–141. [PubMed: 17068222]
42. Butler SA, Iles RK. Ectopic human chorionic gonadotropin beta secretion by epithelial tumors and human chorionic gonadotropin beta-induced apoptosis in Kaposi's sarcoma: is there a connection? *Clin Cancer Res* 2003;9:4666–4673. [PubMed: 14581335]
43. Horne CH, et al. Prognostic significance of inappropriate production of pregnancy proteins by breast cancers. *Lancet* 1976;2:279–282. [PubMed: 59853]
44. Sheth NA, et al. Ectopic production of human placental lactogen by human breast tumors. *Cancer* 1977;39:1693–1699. [PubMed: 856451]
45. Lee AK, et al. Tumor marker expression in breast carcinomas and relationship to prognosis. An immunohistochemical study. *Am J Clin Pathol* 1985;84:687–696. [PubMed: 3878077]
46. Polgar N, et al. Lysyl oxidase interacts with hormone placental lactogen and synergistically promotes breast epithelial cell proliferation and migration. *J Biol Chem* 2007;282:3262–3272. [PubMed: 17130123]
47. Bonnetterre J, et al. Tamoxifen plus bromocriptine versus tamoxifen plus placebo in advanced breast cancer: results of a double blind multicentre clinical trial. *Eur J Cancer Clin Oncol* 1988;24:1851–1853. [PubMed: 3065085]
48. Ben-Jonathan N, et al. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocr Rev* 1996;17:639–669. [PubMed: 8969972]

49. Chen WY, et al. A human prolactin antagonist, hPRL-G129R, inhibits breast cancer cell proliferation through induction of apoptosis. *Clin Cancer Res* 1999;5:3583–3593. [PubMed: 10589775]
50. Goffin V, et al. Drug Insight: prolactin-receptor antagonists, a novel approach to treatment of unresolved systemic and local hyperprolactinemia? *Nat Clin Pract Endocrinol Metab* 2006;2:571–581. [PubMed: 17024156]
51. Walker AM. Therapeutic potential of S179D prolactin--from prostate cancer to angioproliferative disorders: the first selective prolactin receptor modulator. *Expert Opin Investig Drugs* 2006;15:1257–1267.
52. Liu T, et al. Synthesis and screening of a cyclic peptide library: discovery of small-molecule ligands against human prolactin receptor. *Bioorg Med Chem* 2009;17:1026–1033. [PubMed: 18234500]
53. Fox S, et al. High-throughput screening: update on practices and success. *J Biomol Screen* 2006;11:864–869. [PubMed: 16973922]
54. Bleicher KH, et al. Hit and lead generation: beyond high-throughput screening. *Nat Rev Drug Discov* 2003;2:369–378. [PubMed: 12750740]
55. Lipinski CA, et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 2001;46:3–26. [PubMed: 11259830]
56. Neumann H, Neumann-Staubitz P. Synthetic biology approaches in drug discovery and pharmaceutical biotechnology. *Appl Microbiol Biotechnol* 2010;87:75–86. [PubMed: 20396881]
57. Mobley DL, Dill KA. Binding of small-molecule ligands to proteins: “what you see” is not always “what you get”. *Structure* 2009;17:489–498. [PubMed: 19368882]
58. Fang F, et al. Quantification of PRL/Stat5 signaling with a novel pGL4-CISH reporter. *BMC Biotechnol* 2008;8:11. [PubMed: 18254957]
59. Osmond RI, et al. Development of cell-based assays for cytokine receptor signaling, using an AlphaScreen SureFire assay format. *Anal Biochem* 2010;403:94–101. [PubMed: 20382104]
60. Bogorad RL, et al. Identification of a gain-of-function mutation of the prolactin receptor in women with benign breast tumors. *Proc Natl Acad Sci USA* 2008;105:14533–14538. [PubMed: 18779591]
61. Courtillot C, et al. Characterization of two constitutively active prolactin receptor variants in a cohort of 95 women with multiple breast fibroadenomas. *J Clin Endocrinol Metab* 2010;95:271–279. [PubMed: 19897676]
62. Canbay E, et al. Could prolactin receptor gene polymorphism play a role in pathogenesis of breast carcinoma? *Curr Med Res Opin* 2004;20:533–540. [PubMed: 15119991]
63. Fields K, et al. Detection of prolactin messenger RNA in mammary and other normal and neoplastic tissues by polymerase chain reaction. *Lab Invest* 1993;68:354–360. [PubMed: 8450651]
64. Shaw-Bruha CM, et al. Expression of the prolactin gene in normal and neoplastic human breast tissues and human mammary cell lines: promoter usage and alternative mRNA splicing. *Breast Cancer Res Treat* 1997;44:243–253. [PubMed: 9266104]
65. Reynolds C, et al. Expression of prolactin and its receptor in human breast carcinoma. *Endocrinology* 1997;138:5555–5560. [PubMed: 9389544]
66. Mol JA, et al. Expression of the gene encoding growth hormone in the human mammary gland. *J Clin Endocrinol Metab* 1995;80:3094–3096. [PubMed: 7559904]
67. Touraine P, et al. Increased expression of prolactin receptor gene assessed by quantitative polymerase chain reaction in human breast tumors versus normal breast tissues. *J Clin Endocrinol Metab* 1998;83:667–674. [PubMed: 9467590]
68. Mertani HC, et al. Cellular expression of growth hormone and prolactin receptors in human breast disorders. *Int J Cancer* 1998;79:202–211. [PubMed: 9583737]
69. Bhatavdekar JM, et al. Prognostic significance of immunohistochemically localized biomarkers in stage II and stage III breast cancer: a multivariate analysis. *Ann Surg Oncol* 2000;7:305–311. [PubMed: 10819372]
70. Ormandy CJ, et al. Coexpression and cross-regulation of the prolactin receptor and sex steroid hormone receptors in breast cancer. *J Clin Endocrinol Metab* 1997;82:3692–3699. [PubMed: 9360527]

71. Ginsburg E, Vonderhaar BK. Prolactin synthesis and secretion by human breast cancer cells. *Cancer Res* 1995;55:2591–2595. [PubMed: 7780973]
72. Howell SJ, et al. Prolactin receptor antagonism reduces the clonogenic capacity of breast cancer cells and potentiates doxorubicin and paclitaxel cytotoxicity. *Breast Cancer Res* 2008;10:R68. [PubMed: 18681966]
73. van GE, Schalken JA. Morphogenic and tumorigenic potentials of the mammary growth hormone/growth hormone receptor system. *Mol Cell Endocrinol* 2002;197:153–165. [PubMed: 12431808]
74. Peirce SK, et al. Quantification of prolactin receptor mRNA in multiple human tissues and cancer cell lines by real time RT-PCR. *J Endocrinol* 2001;171:R1–R4. [PubMed: 11572805]
75. Leav I, et al. Prolactin receptor expression in the developing human prostate and in hyperplastic, dysplastic, and neoplastic lesions. *Am J Pathol* 1999;154:863–870. [PubMed: 10079264]
76. Decouvelaere C, et al. Presence of the two growth hormone receptor messenger RNA isoforms in human breast cancer. *Cell Growth Differ* 1995;6:477–483. [PubMed: 7794815]
77. Baudhuin A, et al. Transcription of the human prolactin gene in mammary cells. *Ann NY Acad Sci* 2002;973:454–458. [PubMed: 12485910]
78. Nevalainen MT, et al. Prolactin and prolactin receptors are expressed and functioning in human prostate. *J Clin Invest* 1997;99:618–627. [PubMed: 9045863]
79. Wang Y, et al. MMDB: annotating protein sequences with Entrez's 3D-structure database. *Nucleic Acids Res* 2007;35:D298–D300. [PubMed: 17135201]

Figure 1.

Structural characteristics and bioactivity of the three human lactogens. (a) Comparison of the 3D structures of recombinant human placental lactogen (rhPL), prolactin (rhPRL), and growth hormone (rhGH), redrawn from Entrez 3D-structure data according to [79]. (b) The PRL receptor (PRLR) binds all three lactogens; hGH also binds to GHR, its cognate receptor. (c) The three human lactogens are equipotent in stimulating the survival of Nb2 rat lymphocytes which are used as the most common bioassay for the lactogens. RFU – relative fluorescence units.

Figure 2.

Flow diagram of a screening strategy for identifying small molecule inhibitors of the PRLR. First, computer modeling of crystallographic data of the PRLR and its ligands is used to screen virtual libraries of small molecules and identifies those that may bind to the receptor, followed by the selection of a set of compounds of related chemistry and a set of chemically diverse compounds from a small molecule compound library. Next, primary (e.g., Nb2 cells) and secondary (e.g., luciferase-reporter) cell-based readouts are adapted for high throughput screening (HTS). After optimizing assay accuracy, reproducibility, and signal to background ratio, HTS is performed with as many molecules as feasible, and retesting 'hits' to yield EC₅₀ values. Finally, antagonist activity and selectivity of 'hits' are verified using functional endpoints in breast and prostate cancer cells.

Table 1

Expression of PRL, GH, PL, PRLR and GHR in human breast tissue and selected breast cancer cell lines at the mRNA and protein levels.

	PRL	GH	PL	PRLR	GHR
Tissue					
Normal	mRNA [63,64] <i>in situ</i> [65] IC [65] Bioassay [11]	mRNA [66] IC [66]		mRNA [67] <i>in situ</i> [65] IC [65,67]	mRNA [38,68] IC [68]
Malignant	mRNA [63,64] <i>in situ</i> [65] IC [69]	mRNA [66]	IC [43,45]	mRNA[67,70] IC [65,67]	mRNA [38,68] <i>in situ</i> [68] WB [38] IC [38,68]
Cell lines					
MCF-7	mRNA [71] WB [72]	mRNA [73]	WB [46]	mRNA [70,74] WB [75]	mRNA [73,76]
T47D	mRNA [71] WB [71]		WB [46]	mRNA [70,74]	mRNA [76]
SK-BR-3	mRNA [64,72]			mRNA [70]	
MDA-MB-231	mRNA [64]		WB [46]	mRNA [70,74]	mRNA [73,76]
ZR-75-1	mRNA [77]			mRNA [70]	
BT-474	mRNA [77]			mRNA [70,74]	

mRNA – determined by RT-PCR

in situ – determined by *in situ* hybridization

IC – determined by immunohistochemistry

WB – determined by Western blotting

Table 2
Expression of PRL, GH, PL, PRLR and GHR in prostate tissue and selected cell lines at the mRNA and protein levels.

	PRL	GH	PL	PRLR	GHR
Tissue					
Normal	mRNA [78] IC [78]			mRNA [78] <i>in situ</i> [75] IC [75,78]	mRNA [39]
Malignant	IC [27]			mRNA <i>in situ</i> [75] IC [27,75]	mRNA [39] WB [39]
Cell lines					
LnCaP	mRNA [27,28]	mRNA [37,73]		mRNA [28] WB [75]	mRNA [37,39]
PC3	mRNA [27,28]	mRNA [37,73]		mRNA [28] WB [75]	mRNA [37,39] IC [37]
DU145	mRNA [27,28]	mRNA [37,73]		mRNA [28]	mRNA [37,39]

mRNA – determined by RT-PCR

in situ – determined by *in situ* hybridization

IC – determined by immunohistochemistry

WB – determined by Western blotting