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Prolactin as an autocrine/paracrine growth factor in human cancer

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Prolactin (PRL) has a dual function – as a circulating hormone and as a cytokine. This understanding is based on PRL production and distinct regulation in extrapituitary sites, its binding to membrane receptors of the cytokine receptor superfamily, and activation of signaling pathways that promote cell growth and survival. There is increasing evidence that PRL plays a role in several types of cancer in reproductive and non-reproductive tissues via local production or accumulation. The expression of both PRL and its receptor in human cancer cell lines of diverse origin lends further support to its action as an autocrine/paracrine growth factor. Establishment of PRL as an active participant in tumorigenesis should inspire the development of novel therapies aimed at reducing tumor growth by suppressing PRL production or by blocking its receptors.

Published online: 5 June 2002

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Carcinogenesis results from a progressive loss of cellular control mechanisms and is affected by genetic, environmental, dietary and hormonal factors. Although hormones do not function as *bona fide* carcinogens, they can promote growth of transformed cells by interacting with growth factors and oncogenes. The role of gonadal steroids in cancers of reproductive tissues is well established, whereas the contribution of prolactin (PRL), an accessory reproductive hormone, is controversial. In addition to its function as a circulating hormone of pituitary origin, PRL shares many properties with cytokines. These include multiple sites of synthesis, ubiquitous receptor distribution, homologous receptor structure and similar signal transduction pathways. Here, we outline salient features of PRL as a cytokine/growth factor and present emerging evidence for its involvement in the growth of several reproductive and non-reproductive tumors.

Features of PRL as a cytokine/growth factor

PRL is a 23-kDa protein comprising 199 amino acids in four antiparallel α helices with three disulfide loops. The location of the loops is conserved but the primary sequence varies among species. Post-translational modifications, such as glycosylation, phosphorylation, cleavage and polymerization, generate molecular heterogeneity [1]. Human PRL (hPRL) is *N*-glycosylated on Asp^{31}, with both glycosylated and non-glycosylated forms circulating at variable ratios. Glycosylated PRL has a lower binding affinity to the PRL receptor and a reduced activity in some bioassays, whereas phosphorylated PRL binds well to the receptor but might act as an antagonist [2]. A cleaved form of PRL (16K PRL) has antiangiogenic properties [3]. Polymerization and conjugation to IgG can form large molecular species; 'big' PRL (50–60 kDa) and macro-PRL (150–170 kDa) are present in serum of patients with hyperprolactinemia. hPRL, but not PRL or growth hormone (GH) from other species, binds to heparin [4]. Binding of growth factors to the extracellular matrix (ECM) provides protection from inactivation and facilitates receptor binding.

Hormones are produced by defined endocrine glands, whereas growth factors are made by many cell types. Indeed, PRL is synthesized in multiple extrapituitary sites, including the decidua, myometrium, breast, prostate, brain and immune cells [5]. Uptake and retention from the circulation is another distinct feature of PRL. Uptake can be used for transporting PRL into fluid compartments such as

 1043-2760/02/$ – see front matter PII: S1043-2760(02)00603-3

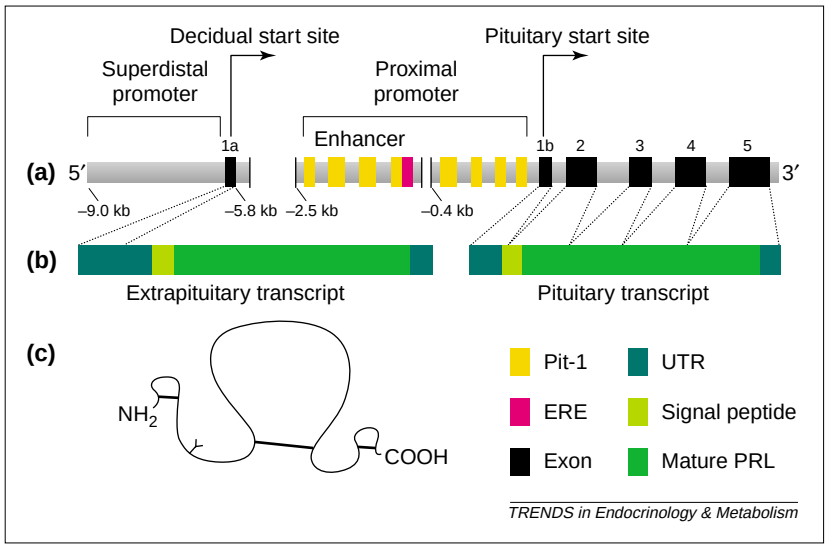

Fig. 1. Comparison of pituitary and extrapituitary prolactin (PRL). (a) Diagram of the gene encoding human PRL illustrating the proximal and superdistal promoter regions; (b) pituitary and extrapituitary transcripts; (c) PRL protein and the location of the three disulfide bridges. Note the use of exon 1a as the transcription start site for extrapituitary PRL and the longer 5′-untranslated region. The site of *N*-glycosylation (Y-Asp[31]) is shown.

cerebrospinal fluid and milk [6], whereas PRL retention by the ECM can increase its concentration in the vicinity of responsive cells. PRL is also internalized within target cells [7], although the exact function(s) of intracellular PRL or the potential for its recycling and exocytosis are unclear.

The PRL receptor and its signaling pathways

The PRL receptor comprises a single transmembrane region that divides the receptor into an extracellular ligand-binding domain and an intracellular domain [8]. The extracellular domain has two disulfide bonds and a Trp-Ser-X-Trp-Ser motif. The cytoplasmic domain has a proline-rich motif ('box 1') that couples to protein kinase signaling molecules. An intermediate receptor isoform with a deleted intracellular segment [9] in addition to two alternatively spliced short isoforms [10] have been identified in human tissues and cancers. A soluble isoform, containing only the extracellular domain, is present in human serum and milk. It serves as a PRL-binding protein, and could either provide a stable pool of PRL that extends its biological activity or render it less accessible for receptor binding [11].

PRL binding induces sequential receptor dimerization. Two sites on PRL (site 1 made of helices 1 and 4, and site 2 made of helices 1 and 3) are involved in receptor homo-dimerization and the formation of an active trimeric complex [8]. Similar to many cytokines that induce receptor dimerization, PRL exhibits an inverse U-shaped curve rather than a linear dose–response relationship [12]. Hence, at very high doses, PRL can be less effective than at physiological concentrations. Another complication is activation of the PRL receptor by placental lactogens and human GH (hGH). The presence of lactogenic hormones in serum additives (e.g. fetal bovine serum) often obscures the mitogenic effects of exogenous PRL [13]. In addition, the hPRL receptor has a lower binding affinity to PRL from other species than to the homologous hormone.

The PRL receptor is devoid of intrinsic tyrosine kinase activity, utilizing instead the Jak–Stat pathway as its main signaling cascade. Jak2 (Janus kinase 2), a protein tyrosine kinase, which is constitutively associated with 'box 1', is rapidly phosphorylated upon receptor dimerization and induces phosphorylation of the receptor and Stat (signal transducers and activators of transcription) proteins. Among the Stat proteins, Stats 1, 3 and 5 are activated by PRL, with Stats 5a and 5b serving as the primary mediators [8]. Activated Stats dimerize, translocate into the nucleus, and bind to specific sequences on target genes. The ras/raf/mitogen-activated protein kinase cascade and fyn, a member of the Src kinase family, are also activated by PRL but appear to be of lesser importance than the Jak–Stat pathway for transducing the effects of PRL.

Dissimilar regulation of pituitary and extrapituitary PRL

Extrapituitary PRL protein is identical to pituitary PRL. In spite of the similarity of the mature proteins, PRL is differentially regulated in pituitary and extrapituitary sites [5]. As shown in Fig. 1, pituitary PRL is controlled by a proximal promoter, which requires the Pit-1 transcription factor for *trans*-activation. The promoter is divided into a proximal region and a distal enhancer, both of which are necessary for optimal pituitary-specific expression. The pituitary-type promoter and its regulation by dopamine, estrogens, neuropeptides and some growth factors have been well characterized [14].

The synthesis of extrapituitary PRL is driven by a superdistal promoter, located 5.8 kb upstream of the pituitary start site (Fig. 1). This promoter is silenced in the pituitary, does not bind Pit-1 and is not affected by dopamine or estrogens [15]. Exon 1a, serving as the alternative transcriptional start site, is spliced into exon 1b, yielding an identical coding region to the pituitary transcript, except for a longer 5′-untranslated region. The superdistal promoter contains binding sites for several transcription factors but its regulation is poorly understood [16]. The dissimilar control of PRL among the various tissues is exemplified by progesterone, which increases PRL synthesis in the endometrium, decreases it in the myometrium, and has no effect on pituitary PRL (Table 1). Extrapituitary PRL is not stored in secretory granules and is not subjected to Ca^{2+}-dependent exocytosis, underscoring another point of divergence from pituitary PRL.

Mitogenic/antiapoptotic actions of PRL

Tumors result from cellular transformation leading to an inappropriate increase in cell number. As is becoming increasingly clear, cell-cycle progression and apoptosis are intertwined. Impaired apoptosis

Table 1. Differential regulation of PRL synthesis and release in the pituitary and uterus[a,b]

Substance	Pituitary[c]	Endometrium[d]	Refs	Myometrium[e]	Refs
Peptides/neurotransmitters					
Endothelin 1	↓↓	↓↓	[48]	nd	
TRH	↑↑↑	=	[48]	=	[56]
VIP	↑↑	nd		=	[54]
Dopamine	↓↓↓	=	[48]	nd	
Steroids					
Estrogen	↑↑	=	[50,53]	↑	[50,53]
Progesterone	=	↑↑↑	[50,53]	↓↓	[50,53]
Growth/hematopoietic factors					
EGF	↑	nd		↓	[54]
IGF-I	↑↑	↑↑	[48]	=	[56]
IL-1	↓	↓↓	[48]	nd	
IL-4	nd	nd		↓↓	[54]
TNF-α	nd	↓↓	[48]	nd	
Protein hormones					
hCG	nd	nd		↑↑	[55]
Insulin	↑	↑↑	[48]	↑	[56]
Lipocortin 1	nd	↓↓↓	[48]	nd	
Relaxin	↑	↑↑	[50]	nd	

[a]Abbreviations: EGF, epidermal growth factor; hCG, human chorionic gonadotropin; IGF-I, insulin-like growth factor I; IL, interleukin; nd, not determined; PRL, prolactin; TNF-α, transforming growth factor-α; TRH, thyrotropin-releasing hormone; VIP, vasoactive intestinal peptide. [b]Changes: ↑, stimulation; ↓, inhibition; =, no change. [c]Data obtained from rat pituitary cells, reviewed in [14]. [d]Includes either endometrial or decidual explants/cells. [e]Includes either normal myometrium or leiomyomas.

augments tumor progression, because apoptosis eliminates cells with increased malignant potential. PRL can act as a survival (antiapoptotic) factor or as a mitogen. Indeed, PRL prolongs the lifespan of the lobuloalveoli in the lactating mammary gland, which undergo apoptosis upon cessation of suckling and PRL withdrawal [17]. PRL increases the expression of the antiapoptotic protein Bcl-2 in Nb2 cells [18], whilst acting as a mitogen in glia [19] and breast cancer cells [13]. The gene promoters of both cyclin D1 (which regulates cell-cycle progression) and the antiapoptotic factor Bcl-x are targeted by Stat 5 proteins [20]. There are also interactions between PRL and oncogenic factors, such as Her-2 [21] and BRCA1 [22].

Table 2. Expression of PRL and the PRL receptor in human breast and prostate cell lines[a]

Cell type	PRL				PRL receptor			
	mRNA	Refs	Protein	Refs	mRNA	Refs	Protein	Refs
Breast								
BT-20	–	[13]	nd		+	[13]	+	[12]
Hs578t	+	[13]	nd		+	[12,29,70]	nd	
MCF-7	+	[2,13,30]	nd		+	[12,29,70]	+	[12,70,71]
MCF-10	+	[13,30]	nd		+	[13]	nd	
MDA-MB-231	+	[30]	nd		–	[29,70]		
MDA-MB-468	+	[13]	nd		+	[13,29]	nd	
SK-BR3	+	[13]	nd		+	[13]	+	[12]
T47D	+	[13,30]	+	[12]	+	[13]	+	[12,29,71]
ZR75-1	+	[13]	nd		+	[13,70]	+	[70]
Prostate								
DU145	+	[2]	+	[2]	nd		+	[44]
LNCap	+	[2]	nd		+	[29,43]	nd	
PC-3	+	[2]	nd		+	[29,43]	nd	

[a]Abbreviations: nd, not determined; PRL, prolactin; –, not detectable; +, detectable.

Breast cancer

PRL affects cellular growth and differentiation in the breast and is obligatory for milk production. Because the breast is the main target of PRL, it follows that PRL is also involved in its aberrant growth. This is well established in rodents, where hyperprolactinemia correlates with increased mammary tumorigenesis. PRL administration increases the incidence, size and number of spontaneous and virus-induced mammary tumors, and sustains carcinogen-induced tumor growth [23]. Moreover, transgenic mice overexpressing the gene encoding PRL develop mammary tumors [24]. Yet, an association between the circulating levels of PRL and breast cancer in humans is unclear. Although there is some correlation between increased serum PRL levels and risk of breast cancer in postmenopausal women [25], treatment of patients with bromocriptine and somatostatin (to suppress both PRL and GH) does not reduce morbidity or mortality [26].

A renewed interest in PRL and growth dysregulation in the breast came with the recognition that 80–90% of breast carcinomas express the PRL receptor, with a higher expression in neoplastic than adjacent tissue [27]. There is increasing evidence that breast PRL acts as a local growth factor. PRL and PRL receptor expression have been detected in normal and malignant breast tissues and in many breast-derived cell lines (Table 2) [13,28,29]. The PRL transcript in the breast originates from the superdistal promoter; that is, it contains exon 1a [30]. This suggests that breast PRL is regulated differently from pituitary PRL, providing a plausible explanation for the failure of dopamine agonists to suppress breast PRL and affect PRL-dependent tumors in patients. Notably, breast carcinomas overexpressing the oncogene *HER2* (official symbol *ERBB2*) have higher proliferative and metastatic indices if they also produce autocrine PRL [21].

The mitogenic activity of locally produced PRL has been supported by the suppression of T47D breast cancer cell proliferation by PRL antisense oligonucleotides [13], anti-PRL antibodies [31], or PRL antagonists [27,32]. Increased proliferation of breast cancer cells by exogenous PRL becomes apparent only when lactogenic hormones are removed from the culture media [13]. Tumors derived from T47D breast cancer cells inoculated into nude mice grow larger upon treatment with hPRL, whereas treatment with a PRL antagonist inhibits tumor growth [33]. The complexity of PRL action is underscored by a recent report on upregulation of the *BRCA1* susceptibility gene in breast cancer cells by PRL [22]. Because BRCA1 is a tumor suppressor, its induction by PRL might antagonize the mitogenic

effects of PRL. PRL also causes a rapid, dose-dependent phosphorylation of both FAK and paxillin [34], suggesting activation of pathways involved in cellular adhesion. Induction of breast cancer cell motility by PRL provides further support for its potential role in metastasis [35].

Prostatic hyperplasia and neoplasia

Prostate cancer and breast cancer have similar lifetime risks, mortality rates and dependence on hormones. Although the effects of androgens on prostate growth and tumorigenesis are undisputed, PRL is one of several cytokines with tropic effects on the prostate. In rodents, PRL participates in prostate organogenesis, secretory activity and hyperplasia [36]. PRL increases prostate weight and nuclear androgen receptor content and promotes development of dysplasia and adenocarcinoma of the dorsolateral lobes of the rat prostate, a region analogous to the human prostate [37]. Mice overexpressing PRL have a massive increase in prostatic weight [38], whereas targeted disruption of the gene encoding PRL [39] causes a reduction in prostate size. Both PRL receptor expression [40] and PRL production [41] by rat prostatic epithelium have been reported.
Prostate PRL is stored in secretory granules and its synthesis is androgen dependent. Incubation of androgen-deprived rat prostate explants with PRL inhibits apoptosis, establishing PRL as a survival factor for epithelial cells of the dorsal and lateral, but not the ventral, lobes [41].

There is little correlation between hyperprolactinemia and increased risk of prostate cancer in men [42]. However, emerging evidence suggests that prostate-produced PRL acts locally. The PRL receptor is expressed in fetal, prepubertal and adult human prostate epithelial cells [43]. It is also detected in benign prostatic hyperplasia and its expression is higher in dysplasia but lower in higher-grade carcinomas. This suggests that PRL might contribute to early carcinogenesis, whereas advanced cancer could be independent of its actions. The human prostate produces PRL, expresses the PRL receptor, and PRL added to human prostates in organ culture increases DNA synthesis and alters epithelial morphology [44]. Among cancer cell lines, the PRL receptor is expressed by both the androgen-dependent LNCaP cells and the androgen-independent PC-3 cells (Table 2) [29,43]. At low doses, PRL stimulates proliferation of both DU145 and PC3 cells but has no effects on LNCaP cells [45]. Bioactive PRL is released by DU145 cells, and phosphorylated PRL, presumably acting as an antagonist, suppresses their proliferation [2].

Tumors of the female reproductive tract

PRL is produced by the endometrium, myometrium and cervix. During the late luteal phase of the menstrual cycle, endometrial stromal cells differentiate under the effects of progesterone and start to express PRL [46], which is believed to play a role in trophoblast implantation and invasion [47]. Throughout pregnancy, the decidua produces large amounts of PRL, which is transported to the amniotic fluid and reaches peak levels at midgestation [48]; the function(s) of this PRL is unclear. Cultured decidual explants or endometrial stromal cells release significant amounts of PRL, and the rate of production increases over time, suggesting removal from inhibition [49]. Estrogen, progesterone and relaxin increase PRL release from decidualized endometrial cells (Table 1) [48,50]. Little is known about PRL and endometrial neoplasms. Serum PRL is elevated in a subpopulation of women with endometriosis [51] but is unchanged in patients with endometrial cancer. However, PRL synthesis by endometrial cancer should be re-evaluated, given that an immortalized human endometrial stromal cell line, N5, expresses PRL and responds to estrogen and progesterone [52].

The myometrium shares a common embryonic origin with the stroma of the endometrium. Explants of normal myometrium release PRL, which is similar to pituitary PRL by all criteria [53]. PRL release from myometrial explants is inhibited by progesterone and interleukin 4 (IL-4) and is stimulated by human placental conditioned medium [54] and human chorionic gonadotropin (hCG) [55] (Table 1).
Uterine leiomyomas, the most common pelvic tumors in women, which rarely become malignant, also produce PRL and respond to hCG [56]. Incubation of myometrial or leiomyoma cells with anti-PRL antibodies causes a significant decrease in cell number, supporting a role for PRL as a paracrine/autocrine growth factor [57]. PRL has also been detected in preterm cervical mucus [58] and in about 50% of uterine cervical carcinomas [59], but there is currently no information about PRL synthesis by cervical carcinoma cell lines.

The hematopoietic system

The importance of PRL as an immune regulator has been questioned after finding that transgenic mice lacking either PRL or its receptor have no discernible immune deficiencies [60]. It has been proposed that PRL is either non-essential for proper immune function or plays a role only under stress. In humans, elevated serum PRL levels are occasionally seen in patients with systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis and AIDS [61], but leukemias and lymphomas are not associated with increased serum PRL levels [62].
The potential for an autocrine/paracrine action of PRL in hematopoietic cells is supported by several lines of evidence. A B-lymphoblastoid cell line, IM-9-P, produces relatively high levels of PRL, and has been used to characterize the superdistal PRL promoter [15]. A myeloid leukemic cell line and myeloblasts from patients with acute leukemia produce PRL [63], as do several non-Hodgkin's lymphoma cell lines [64].

Table 3. Expression of PRL and PRL receptor in human hematopoietic cell lines[a]

Cell type	PRL				PRL receptor			
	mRNA	Refs	Protein	Refs	mRNA	Refs	Protein	Refs
B cells								
AS283	+	[64]	+	[64]	nd		–	[64]
CA46	–	[64]	–	[64]	nd		+	[64]
Daudi	+	[64]	+	[64]	nd		+	[64]
H-BL1	+	[64,65]	nd		+	[64]	nd	
IM9-P3	+	[64,67]	+	[64,67]	nd		nd	
Ramos	+	[64]	+	[64]	nd		nd	
U937	nd		nd		+	[65]	+	[65]
T cells								
HUT78	+	[65]	nd		+	[65]	nd	
Jurkat	+	[65]	+	[68]	+	[65]	+	[66]
Molt4	–	[65]			+	[65]	nd	
Other							+	
EoL-1	+	[63]	+	[63]	nd		nd	
HL60	nd		nd		+	[65]	+	[67]
K562	nd		nd		+	[65]	+	[67]
YT	nd		+	[63]	+	[65]	+	[67]

[a]Abbreviations: nd, not determined; PRL, prolactin; –, not detectable; +, detectable.

The PRL receptor is expressed by most human immune cells [64–66] and PRL production is evident in normal [65] and malignant [63,64,67,68] cells (Table 3). Whereas the PRL receptor is expressed by most classes of immune cells, PRL is produced primarily by T cells [65]. The precise function of PRL in immune cells is unclear. Although the rat Nb2 lymphoma cell line depends on PRL for growth, a similar obligate role for PRL in most human immune cells has not been demonstrated. Rather, PRL has been reported to act as a co-mitogen, especially by inducing IL-2 receptor expression and promoting IL-2-stimulated proliferation [69]. One exception is the Jurkat T-leukemic cell line, which does not constitutively express IL-2 or the IL-2 receptor, whereby PRL acts as an autocrine mitogen [68].

Summary and conclusions

PRL is an extremely versatile molecule, affecting over 100 different functions across vertebrates. These include development of the mammary gland, initiation and maintenance of lactation, immune modulation, osmoregulation and behavioral modification. At the cellular level, PRL affects mitogenic, morphogenic or secretory activities. The diversity of PRL actions is derived from several components: structural polymorphism, local production and processing, receptor isoforms and divergent intracellular signaling pathways and target genes.

Neither the full spectrum of PRL functions in humans nor its involvement in carcinogenesis is well understood. The common view is that PRL is essential for lactation, is deleterious to reproduction when produced in excess, but has no distinct functions in non-pregnant, non-lactating women or in normal men. This view can be challenged by the following. First, although the human fetus is exposed to unusually high levels of PRL, potential morphogenic or regulatory functions of PRL during fetal development are unknown because human fetuses are inaccessible for experimentation and there are no comparable animal models. Second, unlike GH, dysfunctional mutations in either PRL or its receptor have not been identified in humans. Third, the consequences of PRL absence in adults are unclear because hypophysectomy or panhypopituitarism do not eliminate extrapituitary PRL. Fourth, GH can bind to PRL receptors and compensate for some of the functions of PRL in cases of severe PRL deficiency. Finally, because of significant species differences in PRL actions, extrapolation from studies with transgenic mice to humans should be interpreted with caution.

Future research should focus on local PRL and its actions in a variety of human cancers. The presence of PRL at any given site should not be considered evidence for local synthesis. In fact, there is often a significant discrepancy between PRL detection by antibody-based methods and those measuring gene expression. In addition to local production, PRL can be delivered to tumors by binding proteins, infiltrating lymphocytes or migratory macrophages, and both normal and tumor cells can also internalize PRL. Whether resulting from local synthesis or from accumulation, tumor PRL represents a novel molecular target in the search for the etiology and treatment of human cancer.

Acknowledgements
This work was supported by NIH grants ES10154, ES09555, and CA80920, NSF grant 9986713 and a grant from the Pardee Foundation.

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