

## Review

# Multiple human prolactin receptors and signaling

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Accepted 14 December, 2009

**Human prolactin receptor (PRLR) transcripts and their protein products exhibit heterogenic structures and functions. This multiplicity constitutes a gene regulatory system. Short PRLR might modulate longer PRLR structures and signaling. Here we overviewed 10 forms (including two putative forms) of PRLR structures, signaling and functions and analyzed the possible regulatory system regarding multiple PRLRs. Particularly, we discovered that a mimic of phosphorylated prolactin induced p21 waf1 expression via a short form of PRLR S1b in prostate cancer cells. In addition, an intron retention was discovered in PRLR mRNA transcript via sequence analysis, showing that an intron encodes PRLR once needed. PRLR splicing and intron retention might be a critical modulating system to regulate PRLR structures and functions. Furthermore, PRLR genomic size was extended to 182 kb, versus the former report, 70 kb. Interestingly, dozens of PRLR-linked genes on chromosome 5 might interplay one another. This review fully uncovered the associations in PRLR multiplicity and functions, suggesting that shorter PRLR might modulate long PRLR function via multiple PRLR system in both mRNA and protein levels.**

**Key words:** Prolactin receptor, signaling pathways, intron retention.

## INTRODUCTION

Prolactin is a pituitary-secreted hormone, which functions via binding PRLR and further activates a series of signaling pathways leading to necessary physiological actions in paracrine, autocrine and endocrine ways *in vitro* and *in vivo* (Ben-Jonathan et al., 2008; Goffin et al., 2005; Teilum et al., 2005). PRLR was cloned first in rodents (Davis and Linzer, 1989). A variety of rodent PRLR isoforms were characterized and a large amount of work was done focused on rat PRLR multiplicity (Ben-Jonathan et al., 2008; Goffin et al., 2005; Teilum et al.,

2005). However, little was highlighted on human PRLR multiplicity. Similar to the discovery of multiple growth hormone receptors, by using rodent PRLR cDNA probes, so far at least 10 isoforms (including two putative forms) of human PRLR have been characterized (Boutin et al., 1989; Kline et al., 1999; Kline et al., 2002; Hu ZZ et al., 2001; Trott et al., 2003; Fuh and Wells, 1995; Somers et al., 1994; Laud et al., 2000). These PRLR variants were defined as long form (LF), intermediate form (IF),  $\Delta$ S1 and the other seven short forms on the basis of their molecular weights and structures. The short forms include S1a, S1b,  $\Delta$ 4- SF1b,  $\Delta$ 7/11,  $\Delta$ 4- $\Delta$ 7/11 and two putative soluble forms. Herein we defined them as soluble short forms (SS1 and SS2). In addition, a 32 kD PRL-binding protein (PRLBP) was identified in human serum and milk (Kline and Clevenger, 2001). However, some confusion in terms of PRLR definition in literature was made. Different forms of the PRLR have been designated based upon the length of their intracellular domains (Ormandy et al., 1998), but the  $\Delta$ S1 and S1a are longer than the IF PRLR. In effect, because PRLR has multiple forms with a variety of sizes, it is difficult to define the intermediate form. Via sequence analysis we

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**Abbreviations:** PRLR, Prolactin receptor; LF, long form; IF, intermediate form SF, short form; SS, soluble short forms; PRL, prolactin; PRLBP, PRL-binding protein; UTR, untranslated regions; PRAP, PRL associated protein; GH, growth hormone; GHR, growth hormone receptor; GHRBP, growth hormone binding protein; ECD, extracellular domain; TM, transmembrane domain; FN, fibronectin-like domain; GABA, gamma-aminobutyric acid; AD, antidrug resistance.

noticed that the IF PRLR, S1a, S1b,  $\Delta 4$ -SF1b,  $\Delta 7/11$ ,  $\Delta 4$ - $\Delta 7/11$ , SS1 and SS2 are also mutant forms derived from frame shift. These differential PRLR isoforms originated from either the alternatively spliced transcripts or proteolysis, or both (Boutin et al., 1989; Kline et al., 1999; Kline et al., 2002; Hu ZZ et al., 2001; Trott et al., 2003; Fuh and Wells, 1995; Somers et al., 1994; Laud et al., 2000). These PRLR transcripts come from multiple 5' and 3'-untranslated regions (UTR) and alternatively spliced exons and introns (Hu ZZ et al., 1999; Hu ZZ et al., 2002). By cotransfection with beta-casein promoter-luciferase construct into HEK293 cells, short forms of PRLR S1a and S1b were demonstrated to not induce beta-casein gene expression in response to prolactin (Hu ZZ et al., 2001). Nevertheless, in rodent, some short form PRLR was demonstrated to modulate proliferation and differentiation (Das and Vonderhaar, 1995; Qazi et al., 2006). In our opinion, the formation of short PRLR via premature stop codon might be a regulatory system to modulate the quantity of long PRLRs. Thus, short PRLR might regulate the signaling pathways of long PRLR. This also might result from PRLR heterodimerization or the ligand competition to protect cells from exposure to high levels of lactogen (Edens and Talamantes, 1998; Horseman, 2002). Another possibility is that some short PRLRs work in a unique signaling pathway, such as the signaling via PRL associated protein (PRAP) (Duan et al., 1997).

In reference to soluble growth hormone receptor, some soluble forms of PRLR were assumed to have the following functions: 1) increase prolactin half-life by stabilizing prolactin structure (Baumann, 1993); and 2) serve as a storage reservoir for prolactin and growth hormone (GH) so that they can be released upon requirements (Kopchick and Andry, 2000). Human GH also binds to the extracellular domain of the hPRLR via 1:1 complex (Somers et al., 1994); 3) Serve as a transporter for circulating PRLR and growth hormone (Horseman, 2002). Similar to growth hormone binding protein (GHRBP), these soluble forms of PRLR come from proteolysis and PRLR mRNA alternative splicing (Boutin et al., 1989; Kline et al., 1999; Kline et al., 2002; Hu ZZ et al., 2001; Trott et al., 2003; Fuh and Wells, 1995; Somers et al., 1994; Laud et al., 2000) and 4) They compete ligands with long PRLR to antagonize the effect of the latter (Mannor et al., 1991).

In this review, we also elucidated the gene-gene interaction on this chromosome 5. Evidences show that PRLR gene and many other genes on chromosome 5 have some functional associations in the PRL signaling pathways. Some of them constituting the cytokine receptor superfamily class 1 might originate from the same ancestor gene as a result of evolution (Bole-Feysot et al., 1998). The advantage for those genes to localize on the same chromosome is to maintain the conservation and stability of these genes in genetics (Arden et al., 1990). It is not clear whether the localization is a convenient requirement for their mutual regulation.

## HUMAN PRLR GENE STRUCTURE

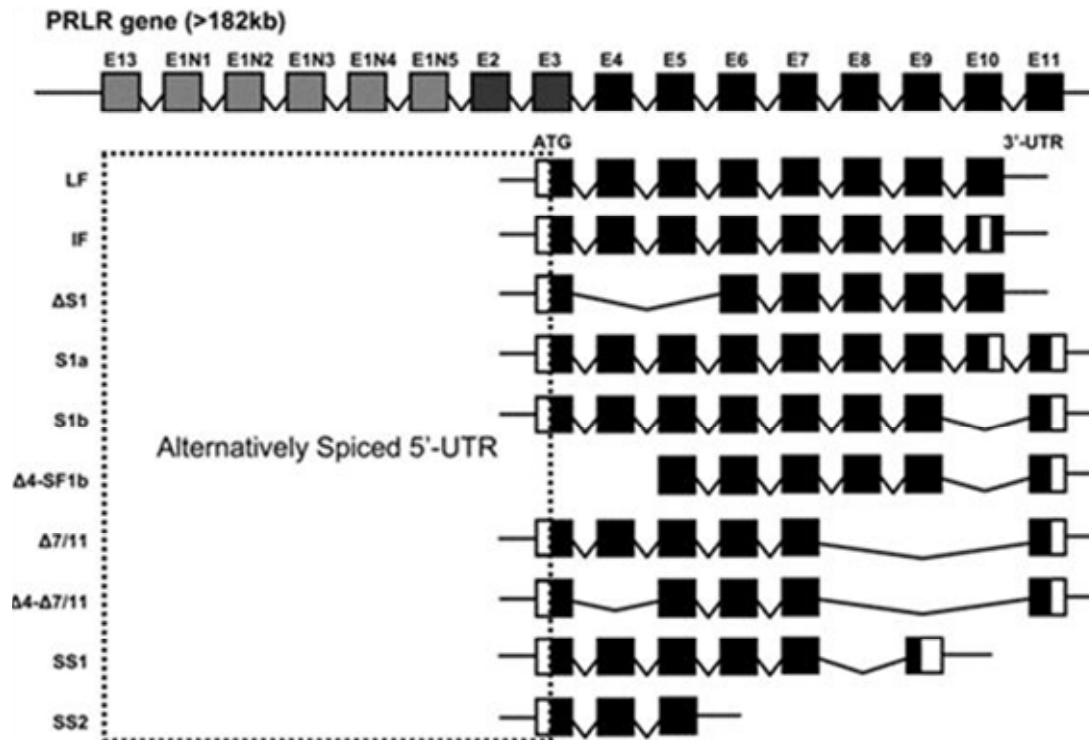
The gene encoding human PRLR was localized on chromosome 5p13-p14 (Bole-Feysot et al., 1998). The analysis of human chromosome 5 clone CTD-2046J7 (accession No: AC010368) and human chromosome 5 genomic contig (accession No: NT\_006679) by NCBI Blast (<http://www.ncbi.nlm.nih.gov/entrez>) revealed that human PRLR gene exceeds 182 kb in length (Figure 1). This is the closest calculation in literature so far. Human PRLR gene contains a multiple promoter region (hPIII and hPN) and at least 11 exons (Hu et al., 1999, 2002; Das and Vonderhaar, 1995). The 5'-UTR of the human PRLR gene contains at least 6 alternative non-coding exon 1 (generic E13, E1N1, E1N2, E1N3, E1N4 and E1N5), a non-coding exon 2 and a non-coding part of exon 3 (Hu et al., 1999, 2002). The partial exon 3, exon 4, 5, 6 and 7 encode the extracellular domain of human PRLR. The exon 8 encodes all transmembrane domains and exon 10 encodes most of the intracellular domains. The first exon E13 is a predominant form in most tissues (Hu et al., 1999, 2002). Promoter hPIII and hPN were demonstrated to use distinct mechanisms to regulate hPRLR transcription (Hu et al., 1999, 2002). S1a and S1b possess different putative poly A signal (AGTAAA) from that (AATAAA) in the long form of PRLR in 3'-UTR (Hu ZZ et al., 1999, 2002; Das and Vonderhaar, 1995).

## HUMAN PRLR TRANSCRIPT HETEROGENEITY

Human multiple PRLR transcripts have been detected in a variety of tissues and cells (Boutin et al., 1989; Kline et al., 1999; Kline et al., 2002; Hu ZZ et al., 2001; Trott et al., 2003; Fuh and Wells, 1995). Multiple forms of 5'-UTR and 3'-UTR of PRLR (including alternative polyadenylation signal) were reported (Hu ZZ et al., 1999, 2002). Heterogeneity of human PRLR transcripts is generated by several types of alternative splicing, including alternative splicing within exons, intron retention, alternative transcription start and termination sites, deletion of partial exon, etc, see Figure 1.

### 5'-UTR heterogeneity

In addition to multiple exon 1, multiple promoters and multiple transcription start sites were discovered in human PRLR genes (Hu ZZ et al., 1999, 2002). Moreover, in some disease tissues, PRLR single nucleotide polymorphisms (SNPs) in 5'-UTR region were reported (Lee et al., 2007). By analysis of human PAC library, two alternative promoters, a generic promoter hPIII common to human and rats and a human specific promoter hPN were cloned and characterized (Hu ZZ et al., 1999, 2002). These different promoters possess transcriptional factor Sp1 and C/EBP binding sites. Human GHR transcripts



**Figure 1.** Prolactin receptor genomic (upper panel) and multiple transcript structures (lower panel). Filled boxes indicate exons, the spaces between exons represent introns. The size interval is not equivalent. PRLR gene is longer than 182 kb, the exon 1 contains E13, E1N1, E1N2, E1N3, E1N4 and E1N5.

have been demonstrated to have 8 alternative 5'-UTR, at least one of them is tissue-specific (Edens and Talamantes, 1998). In addition, in rodents, three tissue-specific PRLR promoters, PI, PII and PIII were cloned; it is believed that the tissue-specific PRLR promoters were utilized in human. Multiple 5'-UTR might involve different levels of transcription and the expression of different transcripts in tissue-specific way.

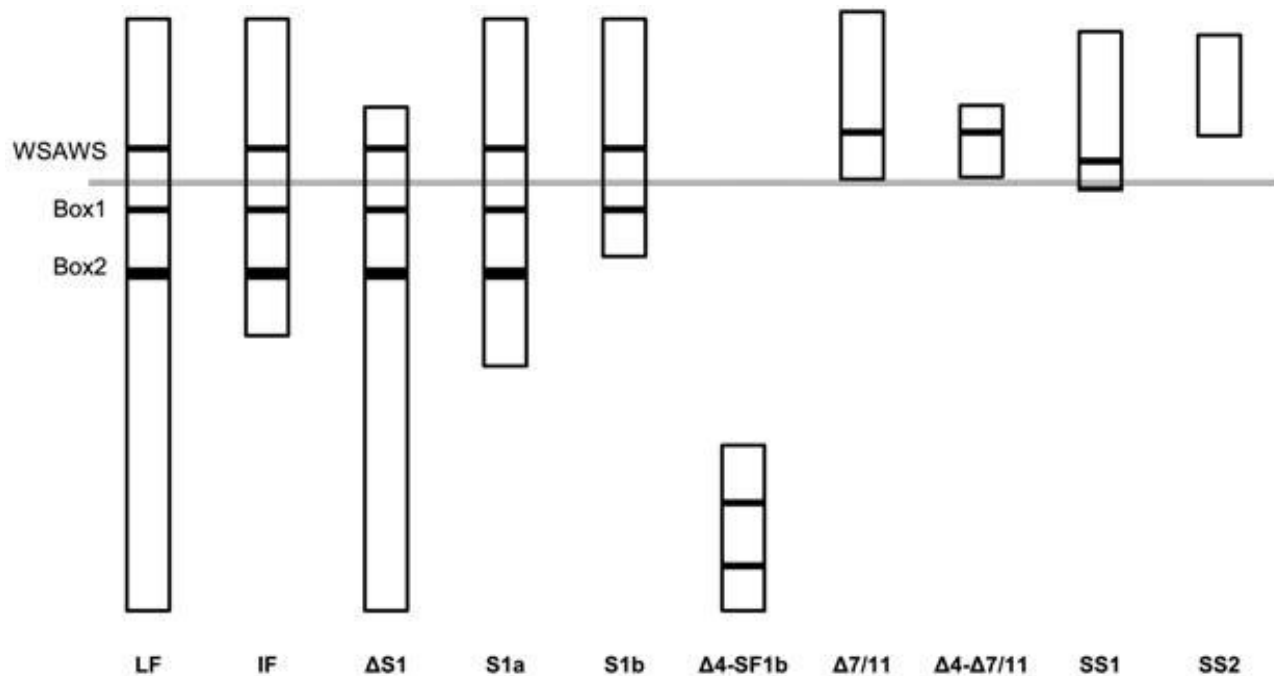
### Deletions in coding region

Several forms of PRLR transcripts were found with deletion and in transmembrane and intracellular domain-encoding regions, see Figure 1. The deletion resulted in the truncated and mutant PRLR proteins. These PRLRs might alter PRL signaling pathways and further lead to different biological properties of PRLR, such as proliferation and differentiation (Das and Vonderhaar, 1995; Horseman, 2002).

1. Extracellular domain (ECD)-encoding mRNA deletion: the receptor  $\Delta$ S1 mRNA was characterized in breast cancer T47D cells with a deletion of whole part of exon 4 and exon 5. Partial exon 3 encodes a 24 amino acid signal peptide (Kline et al., 2002). The SS2 mRNA was characterized by a deletion of exon 6 completely (Hu et al.,

1999).

2. Transmembrane domain (TM)-encoding and intracellular-domain (ICD) encoding mRNA deletion: The receptor  $\Delta$  7/11 mRNA lacks transmembrane-encoding exon 8 and intracellular-domain encoding exon 9, 10, but it has a partial exon 11 (Trott et al., 2003). The receptor  $\Delta$ 4- $\Delta$ 7/11 mRNA lacks both extracellular domain-encoding exon 3, 4, and transmembrane domain-encoding exon 8, and intracellular-domain encoding exon 9, 10, but it has a partial exon 11 (Trott et al., 2003). It is not clear whether  $\Delta$ 4- $\Delta$ 7/11 is a product of  $\Delta$  7/11 proteolysis because the first amino acid for  $\Delta$ 4- $\Delta$ 7/11 is methionine. The IF PRLR mRNA was also discovered in T47D cells with exon 3 and two incomplete parts of exon 10 (Kline et al., 1999). In other words, there is a deletion in exon 10. Also, a frame shift was found in protein encoding. The receptor S1a mRNA comprises exon 3 to exon 11 with a deletion of exon 10. A frame shift was made by alternative splicing of partial exon 10 and exon 11 in the gene product (Hu et al., 2001; Trott et al., 2003). The receptor S1b mRNA contains exons from 3 to 9 plus partial exon 11 with a deletion in the intracellular-domain encoding exon 10 and its coding region has a frame shift (Hu et al., 2001; Trott et al., 2003). The receptor  $\Delta$  4-SF1b mRNA contains all exons in S1b except exon 3 and 4. However, its product might not come from S1b proteolysis because the first amino acid is methionine. The SS1 mRNA contains



**Figure 2.** The structure models of human PRLR on cell membrane and soluble PRLR.

exons 4, 5, 6, 7 and 9. Alternative splicing leads to a frame shift and a deletion of total transmembrane domain and ICD. The SS2 mRNA has a deletion of exon 6 in extracellular domain. The deletion might induce a frame shift and a premature termination codon in the extracellular protein sequence.

### 3'-UTR heterogeneity

By sequence comparison, we made a discovery that long form of PRLR mRNA owns a 3'-UTR which comes from partial intron 10/11. Intron retention was reported in growth hormone receptor (Edens and Talamantes, 1998). Therefore, whether the long form of PRLR is the longest one is still a question. The 3'-UTR for LF PRLR has an AATAA poly A signal. However, the mRNA with exon 11 encoding S1a, S1b, 7/11,  $\Delta 4$ - $\Delta 7/11$ , and  $\Delta 4$ -SF1b has distinct 3'-UTR with poly A signal AGTAA. It is well known that the conserved AATAAA site regulates gene transcription. The removal of this sequence prevents synthesis of mRNA from the transcription unit. Mutation within the AATAAA causes abnormal mRNA formation (Higgs et al., 1983). Therefore, multiple 3'-UTR might modulate diverse mRNA synthesis.

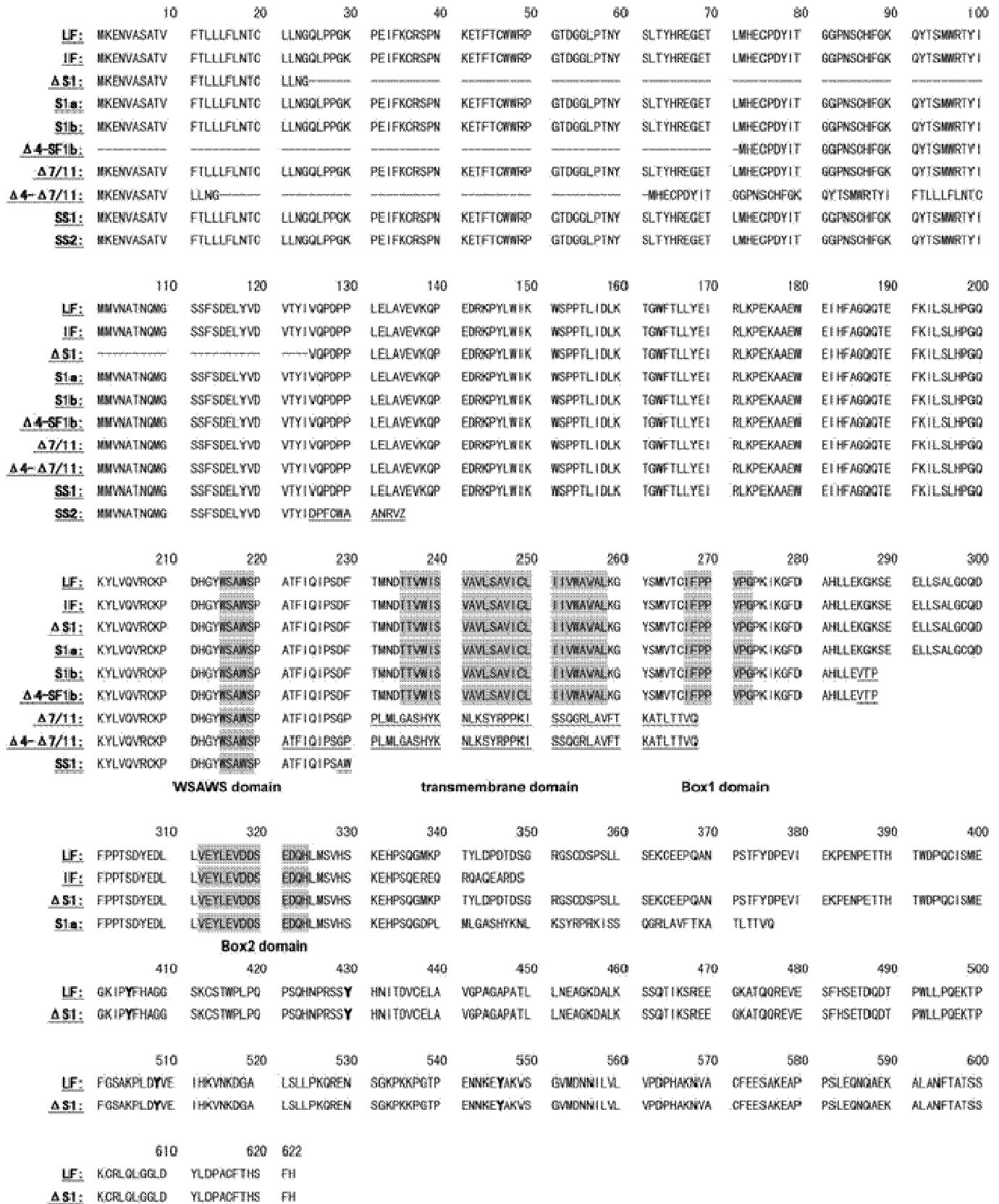
### HUMAN PRLR HETEROGENEITY CONSTITUTES A REGULATORY SYSTEM

Multiple forms of human PRLR were reported in a variety of human tissues (Boutin et al., 1989; Kline et al., 1999;

Kline et al., 2002; Hu et al., 2001; Trott et al., 2003; Fuh and Wells, 1995; Somers et al., 1994; Laud et al., 2000; Peirce and Chen, 2001; Leav et al., 1999; Nevalainen et al., 1997; Ling et al., 2003; Otte et al., 2002) (Figures 2 and 3). They might result from either alternative splicing or proteolysis. The differential forms of PRLR might involve in different PRL signaling pathway and contribute to the distinct functionalities of PRL. Here, we illustrated the structures and functions of 10 forms of human PRLR.

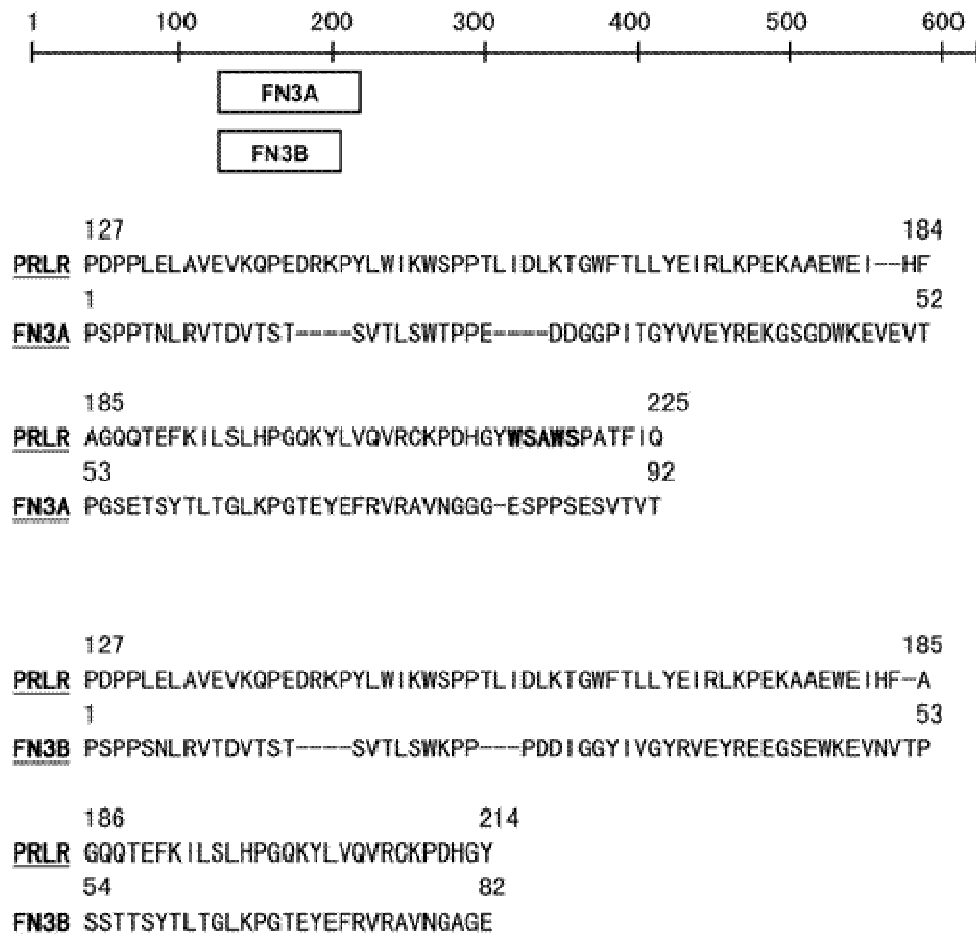
### Long PRLR

Long form of PRLR, a member of the cytokine receptor superfamily, was cloned from hepatoma (Hep G2) and breast cancer (T47D) libraries by using a rat PRL receptor cDNA probe (Davis and Linzer, 1989). The receptor contains an extracellular domain, a transmembrane domain and an intracellular domain. The ECD is composed of the cytokine receptor homology region, which contains two domains, referred to as S1 and S2. The ECD shows the analogies with the type III fibronectin-like domains (see Figure 4). The type III fibronectin domains involve the activation of focal adhesion kinase (FAK), Rho and Rac (Skiyama, 1996). The S1 domain contains most of ligand binding sites, the S2 domain contains conserved motif WSXWS across the cytokine receptor superfamily (Lee et al., 2006). The TM containing 24 amino acids is rarely studied. The PRLR anchorages on cell membrane via some hydrophobic amino acids and works with ICD and TM might regulate internalization (Gadd and Clevenger, 2006). The ICD



**Figure 3.** A sequence alignment comparison of multiple PRLR “—” indicates truncated or deletion parts of PRLR. Blackened “Y” stands for sites for tyrosine phosphorylation. Highlighted boxes indicate WSAWS, Transmembrane, Box1 or Box2 domains as indicated.

## Prolactin Receptor



**Figure 4.** Sequence comparison of PRLR extracellular domain with type III fibronectin domains. The ECD shows the analogies with the type III fibronectin-like domains (FN3A, FN3B). The type III fibronectin domains involve the activation of focal adhesion kinase (FAK), indicating a link of PRL signaling and FAK activation.

contains conserved box 1, box 2 and multiple tyrosine activation sites in the cytokine receptor super family (Bole-Feysot et al., 1998). Box 1 was recognized necessary for activating Janus kinase 2 (Jak2) (Bole-Feysot et al., 1998). The function of box 2 is largely unclear. In rodent studies, the most C-terminal tyrosine residues were dedicated to the activation of signal transducer and activator of transcription 5 (Stat5) and SH2-containing protein tyrosine phosphatase (SHP2) (Lee et al., 2006). The different box of ICD might contribute to activation of different signal molecules involved in MAP kinase, Fyn, FAK, PKC, AKT, PI3K and SOCS pathways (Lee et al., 2006).

### Intermediate PRLR

The intermediate PRLR was found first in T47D cells

(Kline et al., 1999). It is a truncated isoform resulting from a partial deletion of exon 10 and frame shift in protein encoding. The isoform generates a novel 13 amino acid sequence. It has been demonstrated to activate Jak2 in response to PRL, but not to activate the Fyn tyrosine kinase. In addition, the different levels of transcription were revealed in different tissues (Teilum et al., 2005). Hence, intermediate PRLR may work in different signaling pathway from long form PRLR.

### Δ S1

The Δ S1 was characterized in T47D cells (Davis and Linzer, 1989). The receptor was generated from alternative splicing to remove exon 4 and 5 in coding region. The Δ S1 was named for entire loss of S1 domain in ECD. A 24 amino acid signal peptide encoded by partial exon 3

was cleaved by proteolysis. The affinity of the  $\Delta$  S1 for ligand is reduced 7 fold versus long PRLR by radioligand binding analysis. However,  $\Delta$  S1 regulates integrin-associated signaling cascades.  $\Delta$  S1 also activates Jak2 in transfected cells in response to ligand. These indicate that without S1 domain, S2 domain also binds to ligand and lead to signal transduction in response to ligand stimulation (Kline et al., 2002). However, due to loss of S1 domain, ligand binding might regulate receptor confirmation differently from long PRLR and further, trigger different signaling pathways.

### S1a

The short form S1a actually is longer than the intermediate form PRLR (Hu et al., 2001; Trott et al., 2003). This receptor has the domains translated from exon 3 all the way down to exon 11. Partial exon 10 and partial exon 11 were alternatively spliced to produce a premature mRNA with frame shift in protein sequence. Therefore, it in effect produces a mutant protein with box 1 and box 2. The S1a was found in both normal and breast cancer tissues. More, its binding affinity to ligand is equivalent to that in long form PRLR (Hu et al., 2001). Albeit S1a possesses Jak2 and Stat5 contact sites, box 1 and box 2 and activates Jak2, it does not induce beta-casein luciferase expression in response to PRL in HEK293 cells (Hu et al., 2001). A tyrosine residue at or near 382 might be necessary for PRL induced gene transcription (Trott et al., 2003). The S1a contains two tyrosine residues downstream from box 2 at position Y347 and Y353, which might initiate cell signaling.

### S1b

This receptor has the region encoded from exon 3 to exon 9 and partial exon 11 coding region. Alternative splicing from exon 9 to exon 11 produced a frameshift in protein coding. The S1b structurally has a box 1 but no box 2. The S1b also was regarded as a dominant negative form of PRLR in terms of beta-casein promoter luciferase assay in response to PRL. Similar to S1a, the S1b was detected in samples of human breast, placenta, kidney, liver and pancreas (Trott et al., 2003). The S1b has 3-fold higher specific binding to hGH than the long PRLR (Trott et al., 2003).

### $\Delta$ 4-SF1b

The receptor was regarded as an encoded protein without signal peptide and exon 4-translated region. This coded protein was assumed to be a nuclear receptor. Similar to  $\Delta$  4- $\Delta$  7/11, it has no evidence showing that it does not bind to ligand. The  $\Delta$  4-SF1b has box1 but no

box2 and its function remains unknown. It is likely that it dimerizes with long PRLR to regulate the function of the latter.

### $\Delta$ 7/11

The receptor has a deletion in the exons 8, 9, and 10 coding regions. Alternative splicing leads to a frame shift in the junction between exon 7 and exon 11. Thus, it was demonstrated to be a secreted soluble PRL-binding protein. The  $\Delta$  7/11 lacks box 1 and box 2 sites for Jak2 and Stat5 activation (Trott et al., 2003). Differential level of  $\Delta$  7/11 expression was determined in normal breast, colon, placenta, kidney, liver, ovary, pancreas, breast and colon tumors (Trott et al., 2003).

### $\Delta$ 4- $\Delta$ 7/11

Compared with  $\Delta$  7/11 PRLR, the  $\Delta$ 4- $\Delta$  7/11 has an additional deletion for entire exon 3 and exon 4 translated region. It is uncertain whether or not it is a product of  $\Delta$  7/11 PRLR proteolysis (Trott et al., 2003), especially its first amino acid is methionine. Because it does not lack a 24 amino acids signal peptide, it is also a secreted soluble protein. Now no evidence shows that it does not bind to ligand. Similar to the  $\Delta$  S1 PRLR, this receptor also has S2 domain for PRL binding. It is possible that this receptor binds to PRL. Similar to  $\Delta$  4-SF1b, little is reported about  $\Delta$ 4- $\Delta$  7/11 protein information. Although their deduced sequences were reported, it is possible that their mRNA does not encode protein at all.

### SS 1

This is a deduced protein from isolated mRNA sequence in human breast cancer cell line BT-474. Putatively, it is a soluble protein for that it lacks transmembrane domain and has no longer intracellular domain from exon 8. However, it has a S2 binding site and two extra amino acids AW, which comes from alternative splicing by exon 7 and exon 9 (Fuh and Wells, 1995). Theoretically, it does not contact downstream signal. The short PRLR might dimerize LF in mRNA and protein levels.

### SS2

SS2 is so far discovered the shortest PRLR with only partial ECD in T47D and MCF7 cells. The form has a 170 bp deletion generated by alternative exon 6 splicing in PRLR transcripts. The deletion induced a frame shift in protein coding. The protein may be a secreted isoform of PRLR. Due to loss of WSXWS domain, the protein might not bind its ligand. However, long PRLR/SS2 mRNA ratio

is higher in tumoral tissues than normal tissues. This suggests that SS2 mRNA or protein might down-regulate long PRLR mRNA/protein (Laud et al., 2000). Hence, short PRLR mRNA formation might be a regulatory system by blocking the formation of long PRLR mRNA.

### **HUMAN SHORT FORMS OF PRLR IS NOT THE DOMINANT NEGATIVE**

Human and rodent short forms of PRLR were regarded as the dominant negative forms in transfected cells. Due to the lack of box 2 and downstream tyrosine phosphorylated sites, some short PRLRs, such as S1a and S1b, did not induce beta-casein luciferase expression in some cell types. In other words, they are not able to activate Stat5. However, short forms of PRLR, such as ΔS1 might work by the Jak2-Stat5 pathway. Additionally, albeit neither S1a nor S1b did not trigger the Jak2-Stat5 pathway, we recently discovered that S1b dedicated to the inhibition of cancer cell growth (Clevenger et al., 1992). Structurally, both S1a and S1b have box1 site, which is necessary for Jak2 and MAPK activation. The Stat5 activation needs box 2 and downstream phosphorylated tyrosine site. The short PRLR might have proliferative and differentiative function depending on cell types and physiological condition (Das and Vonderhaar, 1995; Halperin et al., 2008; Wu et al., 2005). In normal cells, the formation of short forms of PRLR might be a regulatory system to repress long PRLR. In tumor cells, the short PRLR might downregulate long form signaling by heterodimerization (Qazi et al., 2006). It is conceivable that the long PRLR is related to proliferation whereas the short PRLR is relevant to differentiation (Wu et al., 2003). Therefore, alternative splicing is a means to regulate the ratio of long and short PRLR to ensure appropriate PRLR physiological functions (Meng et al., 2004).

### **GENES COLOCALIZED ON CHROMOSOME 5 MIGHT INTERPLAY**

Gene localization analysis on chromosome 5 via NCBI blast shows that human PRLR gene was closely linked to growth hormone receptor gene (5p13-p12) and interleukin 7 receptor (5p13) gene and co-localized with the genes which express Fyn binding protein (5p13.1), leukemia inhibitory factor receptor (5p13-p12), cyclin B1 (5q12), interferon regulatory factor 1 (5q31.1), interleukin 3,4, 5, 9 (5q31), interleukin 12 B, IL13 (5q31-5q31), IL17B (5q32-5q34) (5q31.3-q33.1), interleukin 6 receptor signal transducing subunit gp130 (5q11), colony stimulating factor 1 receptor (5q33), colony stimulating factor 2 receptor (5q31), glucocorticoid receptor (5q31-q32), dopamine receptor D1A, fibroblast growth factor 1 (5q31.3-q33.2), fibroblast growth factor 4 (5q35), fibroblast growth factor 10 (5p13-5p12), fibroblast growth

factor 18 (5q34-5q35.1), VEGF3 (5q34-5q35), adrenergic alpha 1 B receptor (5q23-q32), adrenergic alpha 1 A receptor (5q32-q34), gamma-aminobutyric acid (GABA) A receptor alpha 1 and alpha 2 (5q34-q35), GABA beta 2 (5q34-5q35), GABA gamma 2 (5q31.1-5q33.1), mitogen-activated protein kinase kinase kinase 1 (MAP3K1) (5q11.2), mitogen-activated protein kinase 9 (5q35), etc. Most products of those genes belong to cytokine superfamily class I, which shares the conserved WSXWS (Trp-Ser-X-Trp-Ser) domain (Bole-Feysot et al., 1998). PRLR and GHR have strong similarity in structure. Alternative splicing produced multiple 5'-UTR, some deleted coding regions and 3'UTR in GH (Hu et al., 2002). More, human GH binds PRLR, indicating that they use the same signaling pathways and may share the similar features. It was demonstrated that soluble truncated growth hormone receptor (GHR) lacking the intracellular domain inhibits the function of LF GHR. This happened probably by the formation of nonproductive dimers or competition of ligands (Hu et al., 2002). PRLR might involve immunoregulation by interacting with IL-7 receptor, LIF receptor, IRF-1, some interleukins (3, 4, 5, 9, 12B, 13, 17B), gp130 CSF1, cyclin B1, etc. PRL has been shown to increase IL-2 receptor and inhibit apoptosis of lymphocytes in rats (Mukherjee et al., 1990; LaVoie and Witorsch, 1995) and PRL increased IRF-1 transcription (Yu-Lee et al., 1990). In the murine T-helper L2 cells, PRL alone induces the expression of IRF-1 but requires co-incubation with IL-2 for inducing cyclin B1 (Clevenger et al., 1992). PRL induces activation of kinase Fyn by activating PRLR (Ali and Ali, 2000). It is well known that PRL activates MAPK pathway via binding PRLR (Clevenger et al., 1994). PRL-PRLR interaction might involve angiogenesis. The proteolytic fragments of native PRL inhibits angiogenesis, whereas intact human PRL has angiogenic activities (Lee et al., 2007), which might be related to the interaction between PRLR and fibroblast growth factors, VEGF. GABA and dopamine inhibit PRL secretion from pituitary (Matsushita et al., 1983). Prolactin and glucocorticoids act in concert to stimulate transcription factors responsible for hormone-dependent milk protein gene expression (Cella et al., 1998). Interaction among the genes, which are localized on the same chromosome, was reported. For example, GR and beta-2 adrenergic receptor genes in close proximity on chromosome 5, which have similar evolutionary features and perhaps functional interaction (Ukkola et al., 2001). GR and ADR genes were shown to be responsible for obesity and fat distribution phenotypes (Ukkola et al., 2001). Gene-gene biological interaction refers to the co-participation of two factors in the same causal mechanism (Yang et al., 1999). The probability of correlations (linkage disequilibrium) between two genes on the same chromosome will increase, especially for those that are physically close to each other (Yang et al., 1999). Thus, PRLR-linked genes on chromosome 5 might interact to keep the stability in heredity and be convenient for mutual modulation.



**Table 1.** Feature comparisons in prolactin receptors.

PRLR	Amino acids	Frame shift	Splicing/Protelysis	Box1/Box2	Jak2/Stat5 Activation	Solubility
LF	622	-	+/-	+/+	+/+	-
IF	349	+	+/-	+/+	+/+	-
ΔS1	521	-	+/+	+/+	+/+	-
S1a	376	+	+/-	+/+	N/D	-
S1b	288	+	+/-	+/-	N/D	-
Δ4-SF1b	217	+	+/+	+/-	N/D	+
Δ7/11	268	+	+	-/-	-/-	+
Δ4-Δ7/11	197	+	+/+	-/-	-/-	+
SS1	206	+	+/-	-/-	-/-	+
SS2	135	+	+/-	-/-	-/-	+

## CONCLUSIONS AND PERSPECTIVE

Multiple structures of the PRLR transcripts and protein reflect the requirements for PRL-regulated function. Hereby, we just reviewed the structure and function and signaling pathways of some cloned and a few putative PRLRs (see Table 1). In fact, the number of prolactin and their transcripts is far more than we herein described. These mRNAs were obtained from a limited number of probes. It is believed that more and more human PRLR forms will be discovered including those, which might result from totally different genes. Tissue-specific promoter in PRLR transcripts will be unveiled. Some PRLR features and functionalities will be further characterized. It has been noted that in PRL triggered response, the influence of growth hormone should not be ignored since growth hormone also binds PRLR. Some short form of PRLR transcripts and protein might play a role in tumor inhibition and differentiation. In summary, short PRLR RNA might regulate the number and structure of long PRLR RNA via heterodimerization or RNAi. Additionally, short PRLR protein might regulate long PRLR or other cytokine receptor superfamily member by forming 'homology zipper' which can open or close so as to be suitable for the requirement of cell function. Based upon homo- and heterodimerization of PRLR, soluble PRLR can be modified to link to a drug for target leukemia or vascular diseases. We demonstrated that S179D PRL upregulates the short PRLR in mouse HC11 cells. Meanwhile, in human short PRLR transfected HEK293 cells, S179D PRL induced AP-1 luciferase expression. Human short forms of PRLR is not the dominant negative, it might use different signaling pathway

## ACKNOWLEDGEMENTS

This study was supported by National Institutes of Health (NIH) grant DK61005. We are very grateful to Dr. Amea Walker (University of California, Riverside) in experimental support. This work was also supported by Science

and Development Plan of Beijing Education Committee (KM200910025008).

## REFERENCES

- Ben-Jonathan N, LaPensee CR, LaPensee EW (2008). What can we learn from rodents about prolactin in humans? *Endocr. Rev.* 29: 1-41.
- Goffin V, Bernichtein S, Touraine P, Kelly PA (2005). Development and potential clinical uses of human prolactin receptor antagonist. *Endocr. Rev.* 26: 400-422.
- Teilum K, Hoch JC, Goffin V, Kinet S, Martial JA, Kragelund BB (2005). Solution structure of human prolactin. *J. Mol. Biol.* 351: 810-823.
- Davis JA, Linzer DIH (1989). Expression of multiple forms of the prolactin receptor in mouse liver. *Mol. Endocrinol.* 3: 674-680.
- Boutin JM, Edery M, Shiota M, Jolicoeur C, Lesueur L, Ali S, Gould D, Djiane J, Kelly PA (1989). Identification of a cDNA encoding a long form of prolactin receptor in human hepatoma and breast cancer cells. *Mol. Endocrinol.* 3: 1455-1461.
- Kline JB, Roehrs H, Clevenger CV (1999). Functional characterization of the intermediate isoform of the human prolactin receptor. *J. Biol. Chem.* 274: 35461-35468.
- Kline JB, Clevenger CV (2001). Identification and characterization of the prolactin binding protein in human serum and milk. *J. Biol. Chem.* 276: 24760-24766.
- Kline JB, Ryczyn MA, Clevenger CV (2002). Characterization of a novel and functional human prolactin receptor isoform (Delta S1 PRLR) containing only one extracellular fibronectin-like domain. *Mol. Endocrinol.* 16: 2310-22.
- Trott JF, Hovey RC, Koduri S, Vonderhaar BK (2003). Alternative splicing to exon 11 of human prolactin receptor gene results in multiple isoforms including a secreted prolactin binding protein. *J. Mol. Endocrinol.* 30: 31-47.
- Fuh G, Wells JA (1995). Prolactin receptor antagonists that inhibit the growth of breast cancer cell lines. *J. Biol. Chem.* 270: 13133-13137.
- Somers W, Ultsch M, De Vos AM, Kossiakoff AA (1994). The x-ray structure of a growth hormone-prolactin receptor complex. *Nature.* 372: 478-481.
- Laud K, Gourdou I, Belair L, Peyrat JP, Djiane J (2000). Characterization and modulation of a prolactin receptor mRNA isoform in normal and tumoral human breast tissues. *Int. J. Cancer* 85: 771-776.
- Ormandy CJ, Binart N, Helloc C, Kelly PA (1998). Mouse prolactin receptor gene: genomic organization reveals alternative promoter usage and generation of isoforms via alternative 3'-exon splicing. *DNA Cell Biol.* 17: 761-770.
- Hu ZZ, Meng JP, Dufau ML (2001). Isolation and characterization of two novel forms of the human prolactin receptor generated by alternative splicing of a newly identified exon 11. *J. Biol. Chem.* 276: 41086-41094.

- Hu ZZ, Zhuang L, Meng JP, Leondires M, Dufau ML (1999). The human prolactin receptor gene structure and alternative promoter utilization: The generic promoter hP111 and a novel human promoter hPN. *J. Clin. Endocrinol. Metab.* 84: 1153-1156.
- Hu ZZ, Zhuang L, Meng JP, Tsai-Morris CH, Dufau ML (2002). Complex 5' genomic structure of the human prolactin receptor: Multiple alternative exons 1 and promoter utilization. *Endocrinology*, 143: 2139-2142.
- Das R, Vonderhaar BK (1995). Transduction of prolactin (PRL) growth signal through both long and short forms of the PRL receptor. *Mol. Endocrinol.* 9: 1750-1759.
- Qazi AM, Tsai-Morris C, Dufau M (2006). Ligand-independent homo- and heterodimerization of human prolactin receptor variants: inhibitory action of the short forms by heterodimerization. *Mol. Endocrinol.* 20: 1912-1923.
- Edens A, Talamantes F (1998). Alternative processing of growth hormone receptor transcripts. *Endocr. Rev.* 19: 559-82.
- Horseman ND (2002). Prolactin receptor diversity in humans: novel isoforms suggest general principles. *Trends Endocrinol. Metab.* 13: 47-8.
- Duan WR, Parmer TG, Albarracin CT, Zhong L, Gibori G (1997). PRAP, a prolactin receptor associated protein: Its gene expression and regulation in the corpus luteum. *Endocrinology*, 138: 3216-3221.
- Baumann G (1993). Growth hormone-binding proteins. *Proc. Soc. Exp. Biol. Med.* 202: 392-400.
- Kopchick JJ, Andry JM (2000). Growth hormone (GH), GH receptor, and signal transduction. *Mol. Genet. Metab.* 71: 293-314.
- Mannor DA, Winer LM, Shaw MA, Baumann G (1991). Plasma growth hormone (GH)-binding proteins: Effect on GH binding to receptors and GH action. *J. Clin. Endocrinol. Metab.* 73: 30-34.
- Bole-Feysoy C, Goffin V, Ederly M, Binart N, Kelly PA (1998). Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr. Rev.* 19: 225-268.
- Arden KC, Boutin JM, Djiane J, Kelly PA, Cavenee WK (1990). The receptors for prolactin and growth hormone are localized in the same region of human chromosome 5. *Cytogenet. Cell Genet.* 53: 161-165.
- Lee KM, Kaneko T (2006). Aida K. Prolactin and prolactin receptor expressions in a marine teleost, *Takifugu rubripes*. *Gen. Comp. Endocrinol.* 146: 318-328.
- Lee SH, Kunz J, Lin SH, Yu-Lee LY (2007). 16-kDa prolactin inhibits endothelial cell migration by down-regulating the Ras-Tiam 1-Pak1 signaling pathway. *Cancer Res.* 67: 11045-53.
- Lee SA, Haiman CA, Burt NP, Pooler LC, Cheng I, Kolonel LN, Pike MC, Altshuler D, Hirschhorn JN, Henderson BE, Stram DO (2007). A comprehensive analysis of common genetic variation in prolactin (PRL) and PRL receptor (PRLR) genes in relation to plasma prolactin levels and breast cancer risk: the multiethnic cohort. *BMC Med. Genet.* 8: 72
- Higgs DR, Goodbourn SE, Lamb J, Clegg JB, Weatherall DJ, Proudfoot NJ (1983). Alpha-thalassaemia caused by a polyadenylation signal mutation. *Nature*, 306: 398-400.
- Peirce SK, Chen WY (2001). Quantification of prolactin receptor mRNA in multiple human tissues and cancer cell lines by real time RT-PCR. *J. Endocrinol.* 71: R1-R4.
- Leav I, MerK FB, Lee KF, Loda M, Mandoki M, McNeal JE, Ho SM (1999). Prolactin receptor expression in the developing human prostate and in hyperplastic dysplastic, and neoplastic lesions. *Am. J. Pathol.* 154: 863-870.
- Nevalainen MT, Valve EM, Ingleton PM, Nurmi M, Martikainen PM, Harkonen PL (1997). Prolactin and prolactin receptors are expressed and functioning in human prostate. *J. Clin. Invest.* 99: 618-627.
- Ling C, Svensson L, Oden B, Weijdegard B, Eden B, Dden S, Billing H (2003). Identification of functional prolactin (PRL) receptor gene expression: PRL inhibits lipoprotein lipase activity in human white adipose tissue. *J. Clin. Endocrinol. Metab.* 88: 1804-1808.
- Otte JM, Otte C, Beckedorf S, Schmitz F, Vonderhaar BK, Folsch UR, Kloehn S, Herzig KH, Monig H (2002). Expression of functional prolactin and its receptor in human colorectal cancer. *Int. J. Colorectal. Dis.* 18: 86-94.
- Skiyama SK (1996). Integrins in cell adhesion and signaling. *Hum. Cell.* 9: 181-186
- Gadd SL, Clevenger CV (2006). Ligand-independent dimerization of the human prolactin receptor isoforms: functional implications. *Mol. Endocrinol.* 20: 2734-2746.
- Halperin J, Devi SY, Elizur S, Stocco C, Shehu A, Rebourcet D, Unterman TG, Leslie ND, Le J, Binart N, Gibori G (2008). Prolactin signaling through the short form of its receptor represses forkhead transcription factor FOXO3 and its target gene *galt* causing a severe ovarian defect. *Mol. Endocrinol.* 22: 513-22.
- Wu W, Ginsburg E, Vonderhaar BK, Walker AM (2005). S179D prolactin increases vitamin D receptor and p21 through up-regulation of short 1b prolactin receptor in human prostate cancer cells. *Cancer Res.* 65: 7509-15.
- Wu W, Coss D, Lorensen MY, Kuo CB, Kuo CB, Xu XL, Walker AM (2003). Differential biological effects of unmodified prolactin and a molecular mimic of phosphorylated prolactin involve different signaling pathways. *Biochemistry*, 42: 7561-7570.
- Meng JP, Tsai-Morris C, Dufau M (2004). Human prolactin receptor variants in breast cancer: low ratio of short forms to the long-form prolactin receptor associated with mammary carcinoma. *Cancer Res.* 64: 5677-5682.
- Mukherjee P, Mastro AM, Hymer WC (1990). Prolactin induction of interleukin -2 receptors on rat splenic lymphocytes. *Endocrinology*, 126: 88-94.
- LaVoie HA, Witorsch RJ (1995). Investigation of intracellular signals mediating the anti-apoptotic action of prolactin in Nb2 lymphoma cells. *Proc. Soc. Exp. Biol. Med.* 209: 257-259.
- Yu-Lee LY, Hrachovy JA, Stevens AM, Schwarz LA (1990). Interferon-regulatory factor-1 is an immediate-early gene under transcriptional regulation by prolactin in Nb2 T cells. *Mol. Cell. Biol.* 10: 3087-3094.
- Clevenger CV, Torigoe T, Reed JC (1994). Prolactin induces rapid phosphorylation and activation of prolactin receptor associated Raf-1 kinase in a T-cell line. *J. Biol. Chem.* 269: 5559-5565.
- Clevenger CV, Sillman AL, Hanley-Hyde J, Prystowsky MB (1992). Requirement for prolactin during cell cycle regulated gene expression in cloned T-lymphocytes. *Endocrinology*, 130: 3216-3222.
- Ali S, Ali S (2000). Recruitment of the protein-tyrosine phosphatase SHP-2 to the C-terminal tyrosine of the prolactin receptor and to the adaptor protein Gab2. *J. Biol. Chem.* 39073-39080.
- Matsushita N, Kato Y, Shimatsu A, Katakami H, Yanaihara N, Imura H (1983). Effects of VIP, TRH, GABA and dompamine on prolactin release from superfused rat anterior pituitary cells. *Life Sci.* 32: 1263-1269.
- Cella N, Groner B, Hynes NE (1998). Characterization of Stat5a and Stat 5b homodimers and heterodimers and their association with the glucocorticoid receptor in mammary cells. *Mol. Cell. Biol.* 18: 1783-1792.
- Ukkola O, Perusse L, Chagnon YC, Despres JP, Bouchard C (2001). Interactions among the glucocorticoid receptor, lipoprotein lipase and adrenergic receptor genes and abdominal fat in the Quebec family study. *Int. J. Obes.* 25: 1332-1339.
- Yang QH, Khoury MJ, Sun FZ, Flanders WD (1999). Case-only design to measure gene-gene interaction. *Epidemiology*, 10: 167-170.