GnRH signaling in intrauterine tissues

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Abstract

Type I GnRH (GnRH-I, GNRH1) and type II GnRH (GnRH-II, GNRH2), each encoded by separate genes, have been identified in humans. The tissue distribution and functional regulation of GnRH-I and GnRH-II clearly differ despite their comparable cDNA and genomic structures. These hormones exert their effects by binding to cell surface transmembrane G protein coupled receptors and stimulating the Gq/11 subfamily of G proteins. The hypothalamus and pituitary are the main origin and target sites of GnRH, but numerous studies have demonstrated that extra-hypothalamic GnRH and extra-pituitary GnRH receptors exist in different reproductive tissues such as the ovary, endometrium, placenta, and endometrial cancer cells. In addition to endocrine regulation, GnRH is also known to act in an autocrine and paracrine manner to suppress cell proliferation and activate apoptosis in the endometrium and endometrial cancer cells through several mechanisms. Both GnRH-I and GnRH-II exhibit regulatory roles in tissue remodelling during embryo implantation and placentation, which suggests that these hormones may have important roles in embryo implantation and early pregnancy. The presence of varied GnRH and GnRH receptor systems demonstrate their different roles in distinct tissues using dissimilar mechanisms. These may result in the generation of new GnRH analogues used for several hormone-related diseases.

Introduction

GnRH, a hypothalamic neuronal secretory decapptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2), is essential for human reproduction. GnRH is processed in specialized hypothalamic neurons and is released in a pulsatile manner into the hypothalamo-hypophyseal portal circulation before being transported to the gonadotrope cells in the anterior pituitary. GnRH stimulates the biosynthesis and secretion of LH and FSH from pituitary gonadotropes after binding to its cognate receptor (GnRH receptor). Both LH and FSH are released in asynchronous pattern of responses to changing GnRH pulse frequency, and in turn regulates gonadal steroidogenesis and gametogenesis (Fink 1988). Since its discovery, ~1000 GnRH analogues have been identified and widely studied (Conn & Crowley 1994, Cheung et al. 2006). Besides its well-known endocrine function, GnRH may directly regulate some extra-pituitary reproductive tissues such as endometrium, ovary and placenta (Islami et al. 2001, Chou et al. 2003, Grundker et al. 2004, Kim et al. 2006). Recent studies revealed that both GnRH and GnRH receptors are expressed in the human endometrium and endometrial cancer. Functional studies have also demonstrated that GnRH regulates cell proliferation, apoptosis, and tissue remodeling. Autocrine or paracrine regulation results in varying responses depending on physiological conditions and the endometrial tissue. Consequently, the extra-pituitary roles of GnRH have attracted interest in the fields of reproductive biology, clinical reproductive medicine and tumor biology. Here, we will summarize the scientific literature regarding the extra-pituitary GnRH and GnRH receptor system in endometrium and endometrial cancer.

Applications of GnRH analogues to uterine related diseases

GnRH analogues are used in the treatment of many hormone-related diseases, uterine related diseases especially (Table 1). Prolonged use of GnRH analogue and tibolone as add-back therapy acted efficiently for the treatment of endometrial hyperplasia (Agorastos et al. 2004). GnRH analogues seem to be suitable drugs for an efficacious and less toxic endocrine therapy for endometrial cancer (Fister et al. 2007). The effect of GnRH analogues on cervical cancer risk is difficult to predict, but the reduced steroid concentrations are likely to result...
in less proliferation of the cervix than normal ovulatory cycles and hence reduces the risk of cervical cancer (Pike & Spicer 2000). Desensitization and thereby reduction of steroid hormones are widely used in clinical medicine to treat many hormone-related diseases (Millar et al. 1987, Moghissi 1992, Emons & Schally 1994). Non-peptide orally-active GnRH antagonists are possible substitutes for agonists as they avoid the undesirable stimulation that leads to desensitization (Millar et al. 2000). The GnRH peptide antagonists compete with endogenous GnRH, and then inhibit the reproductive system (Griesinger et al. 2006). The numerous clinical applications of GnRH analogues in uterine related diseases have prompted many studies of cellular and molecular hormone function which have increased the understanding of the hormone regulation system and optimal applications.

**GnRH isoforms in humans**

Because it was first isolated and sequenced in mammals, hypothalamic GnRH is often referred to as type I mammalian GnRH (mGnRH) or GnRH-I (GNRH1; Millar et al. 2004). GnRH-I is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) that has a key role in the process of reproduction. It is produced by hypothalamic neurosecretory cells and released in a pulsatile manner into the hypothalamo-hypophyseal portal circulation, through which the hormone is transported to the anterior pituitary gland (Cheng & Leung 2005). The human GNRH1 gene is composed of four exons separated by three introns and is present as a single gene copy on chromosome 8p11.2–p21 (Yang-Feng et al. 1986, Radovick et al. 1990). The function for GnRH has been highly conserved in vertebrate evolution regardless of the variation of its amino acids sequence (Kasten et al. 1996).

The second type, type II GnRH (GnRH-II, GNRH2) or midbrain GnRH, is expressed in the midbrain, hippocampus, and discrete nuclei of the hypothalamus. GnRH-II differs from GnRH-I by three amino acid residues at positions 5, 7, and 8 (His⁵Trp⁷Tyr⁸GnRH-I) and is conserved from primitive fish to humans (White et al. 1998, Millar 2003). The human GNRH2 gene has been cloned and mapped to chromosome 20p13 by fluorescence in situ hybridization. GNRH2 also comprises four exons interrupted by three introns, and the predicted GnRH-II preprohormone is organized identically to the GnRH-I precursor. The GnRH-II hormone is expressed at significantly higher levels in extra-brain tissues such as the reproductive tissues (White et al. 1998). Researchers have made substantial effort to elucidate the roles of varying GnRH-II expression in the human reproductive system.

### Table 1 Application of GnRH analogues in uterine related diseases.

<table>
<thead>
<tr>
<th>Benign tumors</th>
<th>Malignant tumors</th>
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<tbody>
<tr>
<td>Endometrial hyperplasia</td>
<td>Endometrial cancer</td>
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<td>Uterine leiomyoma</td>
<td>Cervical cancer</td>
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<td>Adenomyosis</td>
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<td>Endometriosis</td>
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**GnRH receptors**

The GnRH receptor is a member of the rhodopsin-like G protein-coupled receptor (GPCR) superfamily, and contains a characteristic seven-transmembrane (TM)-domain structure (Cui et al. 2000, Cheng & Leung 2005). However, a unique feature of the GnRH receptor is its lack of a carboxy-terminal cytoplasmic tail, which is known to contribute to various aspects of GPCR regulation by desensitizing and internalizing a network of receptor-associated proteins (Bockaert et al. 2003). The classical GPCR internalization pathway involves GPCR kinases, β-arrestin, clathrin-coated pits, and the GTPase dynamin (Millar et al. 2004). The finding of GnRH-II in humans and cloning of its receptor from fishes, amphibians, and primates have increased the likelihood of this receptor in humans (Neill et al. 2001). Primate studies reveal that GnRH-II receptor cDNA encodes a 379-amino acid G protein-coupled/seven-TM receptor having a C-terminal cytoplasmic tail not found in the GnRH-I receptor. The existence of a C-terminal tail is a characteristic of GPCR, which modulates receptor desensitization following serial ligand stimulation. This phenomenon implies that these receptors are coupled to different signal transduction pathways and may play distinct roles in the reproductive functions. The GnRH-II receptor is shown to be highly selective for GnRH-II and is widely expressed in reproductive tissues and in the central nervous system (Millar 2003). In addition, GnRH-II receptor is expressed in the majority of gonadotropes, suggesting its role in the regulation of gonadotropin secretion.

**Binding of GnRH to GnRH receptor**

The binding interaction between the ligand and the receptor is a basic mechanism of receptor function. The GnRH and GnRH receptor interaction initiates intra-cellular signaling through the receptor. Ligand binding is the first step in receptor activation or receptor-mediated transduction of hormone signals across the cell membrane. The GnRH and GnRH receptor interaction was elucidated by analyzing the molecular mechanisms of receptor conformation and receptor activation. The active conformation is associated with a triple network of ligand, receptor, and G-protein. This system has one initial binding step common to both agonist and antagonist and one transition step leading to formation of the ternary network. This system also exhibits...
formation of receptor-G-protein complex with a high affinity for agonistic ligands. Ligand-stabilized and ligand-induced conformation changes are required for receptor activation. Studies of the GnRH receptor have improved understanding of the molecular mechanism of receptor activation. Several GnRH receptor active conformations are specific for GnRH analogues and intracellular signaling pathways (Millar et al. 2004).

**Interaction of GnRH and GnRH-R in reproductive tissues**

Expression of GnRH mRNA has been demonstrated in human reproductive tissues, including human endometrium. The GnRH receptor mRNA has also been recognized in human ovary (Raga et al. 1999, Kang et al. 2000). Two homologous genes for GnRH-II receptor are present in the human genome, one on chromosome 14 and the other on chromosome 1. The chromosome 14 gene lacks exon 1, required to encode a full-length receptor. In comparison, the chromosome 1 gene contains all the three exons. The exon 1-containing transcripts of GnRH-II receptor have been identified in mature human sperm. This suggests a relationship between GnRH and male reproduction such as spermatogenesis, sperm maturation, and fertilization (van Biljon et al. 2002). The growing evidence of endogenous GnRH and GnRH receptors in reproductive tissues has prompted researchers to examine autocrine and paracrine mechanisms in reproductive tissues (Hsueh & Jones 1981).

The GnRH receptor mRNA is expressed by both normal and neoplastic endometrial cells, including those derived from stromal and ectopic endometrial tissues. Two classes of GnRH-I binding sites are found in endometrial carcinoma cell lines, but only one class of high-affinity binding sites can be detected in endometrial cancer cells and normal endometrial tissue. Sequence analysis reveals no mutations or alternative splicing patterns throughout the entire coding region of endometrial GnRH receptor RNA transcripts. (Borroni et al. 2000, Grundker et al. 2001b).

**Ligand receptor-mediated intracellular signal transduction in pituitary and extra-pituitary tissues**

Guanyl nucleotide binding protein (G-protein) has an important role in GnRH-mediated action. Increased GTP induces inositol phosphate production with a resultant decrease in GnRH binding affinity to GnRH receptor (Perrin et al. 1989). The Gq/11α is the major G-protein coupled to GnRH receptor. In human female reproductive tissues and cells, GnRH receptor is coupled to a 41 kDa Gqα protein. The G-proteins have a general sequence for palmitoylation, and receptor-mediated palmitoylation of G-proteins is a common specific phenomenon acting in a time- and dose-dependent manner (Stanislaus et al. 1998). The second and third intracellular loops are important for receptor-G protein interaction and signaling functions in GPCRs. Differences in the levels of GnRH receptor affect the GnRH-stimulated production of inositol phosphate within cells and contribute to the cell type-specific effects of GnRH (Morgan et al. 2008). A specific alanine residue is involved in certain GPCRs, the equivalent of which is Ala-261 in the GnRH receptor. Ala on the human GnRH receptor cannot be involved in ligand binding but is critical for coupling of the receptor to its cognate G protein. Mutation of Ala to Pro or Val partially uncouples G protein, whereas Lys, Leu, Glu or Ile substitutions completely block GnRH-mediated IP production. This suggests that GnRH receptor can couple to multiple G proteins at the second and third intracellular loops. For instance, GnRH agonists have poor activation of Gq, and some antagonists can activate Gi. Therefore, the activation of G-proteins in different cells induces several intracellular signaling pathways through GnRH and GnRH receptor. This pathway is also affected by the ligands, indicating ligand receptor-dependent signaling (Chabre et al. 1994, Myburgh et al. 1998).

Intracellular transmission of extra-cellular signals is partially activated by some groups of sequentially activated protein kinases such as the MAPK cascade (Fig. 1). The MAPKs play an important role in GPCR-mediated intracellular signaling (Luttrel 2002). The GnRH receptor activates MAPK cascades, including ERK1/2, c-Jun amino-terminal kinase (JNK), p38 MAPK, and big MAPK (BMK1/ERK5) to different levels via a tyrosine kinase-, Ca²⁺- and protein kinase C (PKC)-dependent mechanism (Johnson & Lapadat 2002). The ERK is the key protein kinase in the signaling of growth factors. The ERK1/2 controls the intracellular signaling pathway which regulates the cellular growth and differentiation. The activation of ERK1/2 is mainly PKC-dependent and involves two different pathways that meet at RAF1. Constitutive localization of the GnRH receptor to low-density membrane microdomains is necessary for GnRH signaling to ERK (Navratil et al. 2003). The GnRH activates ERK1/2 by phosphorylating Sos and Shc through a Gi-protein coupled pathway. The GnRH receptor can couple with either Gq/11- or Gi/o-mediated activation of the MAPK cascade and activate the MAPK cascade by mechanisms similar to that of the other GPCRs (Kimura et al. 1999). The actual mechanisms of MAPK cascade activation by GnRH receptor and the regulation of intracellular loops in coupling to MAPK cascades, however, is still undetermined. Activation of JNK is highly dependent on cytosolic Ca²⁺ and is regulated through the pathway by serial stimulation of PKC, c-Src, CDC42/RAC1, and MAPK kinase (MEK)K1 (Mulvaney & Roberson 2000, Weston & Davis 2007). Although JNK may affect the survival, proliferation, apoptosis, and invasion of cancer cells, its physiological
and genetic mechanisms are not well understood. Activated p38 MAPK is involved in the PKC-dependent cascade. The p38 MAPK kinase participates in the activation of biological effects in cell cultures in response to varying stimuli. These effects depend on the particular stimuli and cell types. The p38 MAPK kinase is known to induce apoptosis in some cells but prevent apoptosis in others. Similarly, opposing effects of the kinase have been observed in cell cycle regulation (Bradham & McClay 2006). The GnRH-I receptor in extra-pituitary tissues resembles that in the pituitary gonadotrophs, but GnRH signaling in extra-pituitary tissues and tumors may differ from those in the pituitary gonadotrophs.

In extra-pituitary cells, activation of MAPK cascades by the GnRH receptor has been investigated. The intracellular mechanisms seemed to involve transactivation of the epidermal growth factor (EGF) receptor (Grundker et al. 2002b, Shah et al. 2003). The GnRH analogues reduce the expression of growth factor receptors and growth factor-induced tyrosine kinase activity. Growth factor-induced tyrosine phosphorylation is likely neutralized by GnRH analogues via activation of the phosphotyrosine phosphatase (PTP), which is likely coupled to the GnRH receptor through a G-protein in human gynecological tumors (Imai et al. 1996). In endometrial and ovarian cancer cells, the GnRH receptor stimulates the PTP, which neutralizes EGF-induced tyrosine phosphorylation of the EGF receptor with a resultant downregulation of mitogenic signal transduction and cell growth. However, this phenomenon can be negated by pertussis toxin, which suggests the involvement of pertussis toxin-sensitive G-proteins in GnRH-induced PTP activity (Grundker et al. 2001b). However, whether GnRH signaling in extra-pituitary tissues differs from that in pituitary gonadotroph is still unresolved. Differing GnRH signaling between pituitary gonadotrophs and extra-pituitary tissues may result from varied coupling to G-proteins. In human endometrial cancer cells, GnRH agonist activates the activator protein-1 (AP-1) mediated through the pertussis toxin-sensitive G-protein. Furthermore, GnRH agonist activates AP-1 by simulating JNK (Grundker et al. 2001a; Fig. 1). The GnRH agonist suppresses growth factor-induced MAPK activity and does not activate phospholipase C and PKC in endometrial cancer cells (Grundker et al. 2002b). Therefore, PKC and MAPK may not be involved in the GnRH agonist-induced activation of the JNK/AP-1 pathway. Further studies are needed to elucidate GnRH agonist-induced AP-1 activity related to the antiproliferative action of GnRH analogues through the control of cell cycle.

The c-fos proto-oncogene is a key regulator of normal cell growth and differentiation. Extra-cellular stimuli, including several mitogens and steroid hormones, rapidly induce a c-fos response (Kovacs 1998). Transcriptional regulation of c-fos partly depends on the interactions of nuclear proteins with multiple cis-elements in the c-fos gene promoter. Serum response element (SRE) is one of the cis-elements. The SRE is essential for c-fos induction, which activates MAPK pathways by extra-cellular stimuli (Karin 1994). Several studies have shown that estrogen receptor z (ERz, ESR1) mediates 17β-estradiol (E2)-activated expression of c-fos, which is induced as an immediate early-response gene.

**Figure 1** GnRH-I and GnRH-II signaling in endometrial cancer cell: (A) GnRH induces apoptosis through Gαi and activation of Fas ligand. (B) GnRH-II induces apoptosis through Gαi and activation of p38 and AP-1. (C) GnRH arrests cell cycle by activating JNK, increasing AP-1 activity and the binding of JunD-DNA. (D) Gβγ subunit activates ERK and mediates GnRH-induced growth inhibition. (E) Through Gαi, GnRH analogs activate PTP to dephosphorylate EGFR and abolish EGF-induced ERK activation, c-fos expression, and proliferation. Dashed arrows represent EGF-stimulated mitogenic signaling pathway.
in ESR1-positive cancer cell lines such as endometrial cancer (Bonapace et al. 1996, Duan et al. 1998). The GnRH agonists counteract EGF-induced proliferation and c-fos gene expression via Ras/MAPK signaling. The transcriptional activation of SRE by E2 is due to ESR1 activation of the MAPK pathways. The pathway is blocked by GnRH with a resulting reduction of E2-induced SRE activation and consequent reduction of E2-induced c-fos expression. This activity then supresses E2-induced cell proliferation in endometrial cancer (Grundker et al. 2004).

GnRH and apoptosis


In cancer cells, the role of GnRH-stimulated apoptosis is still uncertain. GnRH treatment in vitro induces a dose-dependent stimulation of Fas ligand expression in several reproductive cancer cell lines and cells. Fas is usually expressed in GnRH receptor-positive tumors, which suggests that GnRH is an autocrine proapoptotic factor in Fas-positive tumors and that this proapoptotic property may partially reflect its antitumor effect (Imai et al. 1998). The GnRH-I analogues have two possible counteracting effects, antiapoptotic and antiproliferative, mediated by two different signaling cascades but triggered by the same Gz protein in some ovarian cancer cells (Cheung et al. 2006). In the uterus, GnRH-I analogues in vivo or in vitro can suppress myometrium cell growth by activating apoptosis (Wang et al. 2002). Conversely, GnRH-I analogues can suppress the expression of some proapoptotic factors while upregulating the in vivo antiapoptotic effects of BCL2 protein (Huang et al. 2002). Numerous studies consistently show that GnRH-I analogues can induce apoptotic cell death in endometriotic cells in vitro (Meresman et al. 2003, Bilotis et al. 2007; Table 2). Different responses to GnRH analogues occur in some cell lines. The possibility is that differences in the levels of GnRH receptor expression exist during cell passage in vitro or as a result of varied culture conditions. Consequently, differences in levels of GnRH receptor and signaling pathway individually contribute to the induction of apoptosis and play an important role in the regulation of GnRH on cell type-specific growth (Morgan et al. 2008).

Endometrial cancer

Endometrial cancer is one of the most common gynecological cancers in the world and each year accounts for ~50,000 deaths worldwide. The disease occurs primarily in post-menopausal women although 25% of patients are premenopausal, and 5% are younger than 40-years old (Parkin et al. 2005). Prolonged exposure to endogenous or exogenous estrogens is one of the risk factors for endometrial cancer. Adjuvant treatment with tamoxifen for breast cancer is associated with increased incidence of endometrial cancer. Medical treatment for endometrial cancer includes aromatase inhibitors, progestational agents, and tamoxifen. The effectiveness of aromatase inhibitors for treating endometrial cancer remains unclear, but some reports have documented a lower response rate than that for progestagens (Rose et al. 2000). Tamoxifen, which clearly enhances survival and reduces recurrence in hormone-sensitive breast cancer is a widely used therapeutic antiestrogenic agent in endometrial cancer. However, many reports reveal that tamoxifen may worsen the endometrial cancer related to ERβ (ESR2) expression and hormone-resistant phenotype (Wilder et al. 2004). GnRH is an important molecule of the hypothalamus–pituitary–gonadal axis related to estrogen steroidogenesis is in vertebrates. The GnRH agonists and antagonists targeting the hypothalamic–pituitary–ovarian axis may have an antiproliferative role in endometrial cancer (Jeyarajah et al. 1996).

Endocrine effects of GnRH analogues on human endometrial cancer

Most endometrial cancers depend on the estrogen for development and growth. GnRH analogues offer an alternative treatment for endometrial cancer through estrogen deprivation by inhibiting the hypothalamic–pituitary–ovarian axis and thereby inhibiting estrogen production (Kullander 1992). GnRH analogues may be

<table>
<thead>
<tr>
<th>Target site</th>
<th>Extra-pituitary effect</th>
<th>Nature of GnRH-I and/or GnRH-II</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometriotic cell</td>
<td>Inhibition of proliferation</td>
<td></td>
<td>Leuprolide</td>
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<tr>
<td>Endometriotic cell</td>
<td>Activation of apoptosis</td>
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<td>Buserelin</td>
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<tr>
<td>Decidual stromal cell</td>
<td>Activation of PAI-1 expression</td>
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<td>Native GnRH-I</td>
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<tr>
<td>Decidual stromal cell</td>
<td>Suppression of PAI-1 expression</td>
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<td>Native GnRH-II</td>
</tr>
<tr>
<td>Endometrial epithelial cell</td>
<td>Activation of apoptosis</td>
<td></td>
<td>Leuprolide</td>
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<td>Endometrial stromal cell</td>
<td>Inhibition of proliferation</td>
<td></td>
<td>Native GnRH-II</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Inhibition of proliferation</td>
<td></td>
<td>Triptorelin/native GnRH-II</td>
</tr>
</tbody>
</table>

PAI, plasminogen activator inhibitor.

Table 2 Summary of extra-pituitary effects of type I GnRH (GnRH-I) and type II GnRH (GnRH-II) in human endometrium and endometrial cancer.
used for conservative treatment of endometrial cancer. Therefore, the cancer stage and histopathology result should be the main consideration for GnRH analogue treatment. More studies are necessary to elucidate the endocrine role of GnRH analogues in endometrial cancer.

**Autocrine and paracrine effects of GnRH analogues on human endometrial cancer**

Specific low affinity and high-capacity binding sites for GnRH analogues were described in studies of endometrial cancer almost 20 years ago, which demonstrates the specific binding of GnRH in membrane preparations from endometrial cancer (Srlakovic et al. 1990). The GnRH agonist and antagonist exert time- and dose-dependent inhibitory effects on the proliferation of endometrial cancer cell lines. The GnRH may play an autocrine or paracrine regulatory role in the growth of reproductive tissue tumors. Many studies have clearly demonstrated the participation of GnRH in endometrial cancer. Researchers suggest that the autocrine regulatory system stimulates cancer development and growth through direct effects on the endometrium based on GnRH receptor expression and GnRH peptide generation in endometrial cancers (Furui et al. 2002, Eicke et al. 2006). A second GnRH system has been described in primates. The antiproliferative effects of GnRH-I or GnRH-II agonists have been demonstrated in endometrial cancer cell lines positively expressing GnRH-II receptor mRNA. The antiproliferation of GnRH-II receptor positive endometrial cancer cell lines is induced by GnRH-II in a time- and dose-dependent manner, and the antiproliferative effects are significantly greater than those of GnRH-I agonist (Grundker et al. 2002a, Volker et al. 2002). However, the underlying signal transduction mechanisms of GnRH-II system are still unknown. Recent studies reveal that the mitogenic effects of growth factors in endometrial cancer cell lines are counteracted by GnRH-II agonist, implying an interaction with the mitogenic signal transduction. The GnRH-II hormone may reduce EGF-induced tyrosine auto-phosphorylation of EGF-receptors by activating PTP, and EGF-induced activation of MAP kinase is obstructed in cells treated with GnRH-II. The EGF-induced expression of the immediate early gene c-fos is suppressed by GnRH-II treatment. The GnRH-II agonist still stimulates PTP and suppresses the EGF-induced mitogenic signal transduction following the knock-out of GnRH-I receptor expression, which suggests that the effects of GnRH-II may be independent of GnRH-I receptor (Eicke et al. 2006).

**GnRH and endometriosis**

Endometriosis is one of the most common diseases in women of reproductive age. The disease is characterized by the presence of endometrial tissue outside the uterine cavity. The prevalence of endometriosis is 6–10% in women in the general population and 20% in women who have undergone laparoscopy for infertility or pelvic pain (Eskenazi & Warner 1997). The pathogenetic mechanisms of endometriosis remain unclear. Endocrine, genetic, immune, and environmental factors are believed to participate in the pathogenesis of endometriosis. The hypothesis that peritoneal endometriosis is due to menstrual dissemination of endometrial tissue into the peritoneal cavity is presently the most widely accepted. Endometriosis is highly related to infertility, probably resulting from poor folliculogenesis, oogenesis, fertilization, embryo development, and implantation. Endometriosis is known to be an estrogen-dependent disease. The development and maintenance of endometriosis mainly depends on deranged generation of estrogen from endometriotic stromal cells. Several proteins such as 17β-hydroxysteroid dehydrogenase (HSD17B) type I, HSD3B, P450 aromatase, STAR protein, and P450 side-chain cleavage enzyme (P450scc) required for synthesizing estrogen occur in endometriotic stromal cells (Tsai et al. 2001). Long term expression of GnRH mRNA has been demonstrated in human endometrium. The expression of GnRH mRNA isolated from endometrial cells reveals a dynamic pattern such as significantly elevated GnRH mRNA expression detected in the secretory phase of the menstrual cycle. Furthermore, GnRH-I immunoreactivity has been demonstrated in various endometrial cells with the strongest staining observable in the luteal phase (Dong et al. 1998, Raga et al. 1998). The expression of GnRH-II has been explored in human endometrium throughout the entire reproductive cycle, and two splice variants of GNRH2 mRNA are expressed with the shorter transcript carrying a 21-bp deletion. Dynamic expression of GnRH-II has been demonstrated in all endometrial cell types with stronger immunoreactivity in the early and midsecretory phases, which is similar to GnRH-I and suggests that GnRH-II may have an important role in human embryo implantation (Cheon et al. 2001). The GnRH-I and GnRH-II hormones are known to activate urokinase-type plasminogen activator in human extra-villous cytotrophoblasts and decidual stroma cells in vitro, indicating that the hormones may have a possible role in regulating the proteolytic degradation of the extracellular matrix of the endometrial stroma, an essential process for decidualization and trophoblast invasion (Paria et al. 2002, Chou et al. 2003). Taken together, clinical studies of GnRH-I and GnRH-II indicate that these hormones may have a critical role in autocrine and paracrine regulation in human embryo implantation and early pregnancy.
administration of GnRH analogues is applied to obtain a condition equivalent to hypogonadotropic hypogonadism by downregulating pituitary GnRH receptors with a resulting suppression of gonadotropin production. Because of its unique functions, analogues of GnRH have been applied for medical treatment of many hormone-related diseases such as endometriosis. Furthermore, some studies report that the GnRH system may play a role of autocrine and paracrine regulation in endometriosis since GnRH and GnRH receptor mRNA have been detected in human eutopic and ectopic endometrium (Imai et al. 1994, Borroni et al. 2000). Concerning the apoptosis in endometriosis induced by GnRH analogues, it was demonstrated that GnRH analogues may have a direct effect by enhancing the apoptotic index, decreasing the secretion of pro-mitogenic cytokines such as interleukin-1β and VEGF, increasing the expression of the pro-apoptotic proteins BAX and Fas ligand, decreasing the expression of the anti-apoptotic protein BCL2 and suppressing cell proliferation in endometriosis (Meresman et al. 2003, Bilotas et al. 2007). The GnRH-II peptide also has shown antiproliferative and anti-inflammatory effects in endometrial stromal cells. The decreased expression of GnRH-II on eutopic and ectopic endometrium of women with endometriosis implies that endogenous cytostatic regulation of GnRH-II may be weakened in the development of endometriosis (Morimoto et al. 2005).

Conclusion
Estrogen deprivation induced by GnRH analogues is now considered as a conservative treatment for some hormone-related diseases such as endometrial cancer and endometriosis through the effects of endocrine regulation. Additionally, direct inhibition of growth induced by GnRH analogues has also been demonstrated in vitro in endometrial cancer and endometriosis through different mechanisms. Different GnRH isoforms have been demonstrated in mammals. These may play important roles in reproductive and non-reproductive tissues, since GnRH exists in varied tissues. The emergence of different GnRH systems in widely distinct tissues reveals the varied mechanisms of action and the likely resultant evolution of a new generation GnRH analogues. Specific GnRH analogues binding sites occur in hormone-related diseases such as endometrial cancer. The evolution and clinical evaluation of GnRH analogues may play a role in targeted chemotherapy with better efficacy and lower systemic toxicity than traditional chemotherapy. Future studies will elucidate the connection of the GnRH–GnRH receptor system, histopathology and other markers, as well as the roles of the various GnRH–GnRH receptor systems in endometrium and endometrial cancer.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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