

Review

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Role of gonadotropin-releasing hormone (GnRH) in ovarian cancer

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Abstract

The expression of GnRH (GnRH-I, LHRH) and its receptor as a part of an autocrine regulatory system of cell proliferation has been demonstrated in a number of human malignant tumors, including cancers of the ovary. The proliferation of human ovarian cancer cell lines is time- and dose-dependently reduced by GnRH and its superagonistic analogs. The classical GnRH receptor signal-transduction mechanisms, known to operate in the pituitary, are not involved in the mediation of antiproliferative effects of GnRH analogs in these cancer cells. The GnRH receptor rather interacts with the mitogenic signal transduction of growth-factor receptors and related oncogene products associated with tyrosine kinase activity via activation of a phosphotyrosine phosphatase resulting in downregulation of cancer cell proliferation. In addition GnRH activates nucleus factor κ B (NF κ B) and protects the cancer cells from apoptosis. Furthermore GnRH induces activation of the c-Jun N-terminal kinase/activator protein-1 (JNK/AP-1) pathway independent of the known AP-1 activators, protein kinase (PKC) or mitogen activated protein kinase (MAPK/ERK).

Recently it was shown that human ovarian cancer cells express a putative second GnRH receptor specific for GnRH type II (GnRH-II). The proliferation of these cells is dose- and time-dependently reduced by GnRH-II in a greater extent than by GnRH-I (GnRH, LHRH) superagonists. In previous studies we have demonstrated that in ovarian cancer cell lines except for the EFO-27 cell line GnRH-I antagonist Cetrorelix has comparable antiproliferative effects as GnRH-I agonists indicating that the dichotomy of GnRH-I agonists and antagonists might not apply to the GnRH-I system in cancer cells. After GnRH-I receptor knock down the antiproliferative effects of GnRH-I agonist Triptorelin were abrogated while the effects of GnRH-I antagonist Cetrorelix and GnRH-II were still existing. In addition, in the ovarian cancer cell line EFO-27 GnRH-I receptor but not putative GnRH-II receptor expression was found. These data suggest that in ovarian cancer cells the antiproliferative effects of GnRH-I antagonist Cetrorelix and GnRH-II are not mediated through the GnRH-I receptor.

Introduction

The hypothalamic decapeptide gonadotropin releasing hormone (GnRH, GnRH-I), also called luteinizing hormone releasing hormone (LHRH), plays a key role in the regulation of mammalian reproduction [1-3]. It is

released from the hypothalamus in a pulsatile manner and stimulates the synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). In addition to this classic hypophysiotropic actions, GnRH functions as a modulator of the activity of diverse

systems in the brain and many peripheral organs [for review see [4]]. An autocrine/paracrine function of GnRH has been suggested to exist, for instance, in the placenta, granulosa cells, myometrium, and lymphoid cells [for review see [4,5]]. In addition, it is probable that such GnRH-I-based autocrine systems are present in a number of human malignant tumors including cancers of the ovary, endometrium, breast and prostate [for review see [4,5]].

The GnRH-I system in human ovarian cancers

In earlier studies the expression of GnRH-I and its receptor (GnRH-I receptor) as well as direct antiproliferative effects of GnRH-I and its analogs have been demonstrated in a number of malignant human tumors, including cancers of the ovary [6–14]. Data available today suggest that approximately 80% of ovarian cancers express high-affinity GnRH-I receptors [4,5,15]. These findings suggested the presence of a local regulatory system based on GnRH-I. The same situation was found in endometrial, breast and prostate cancer cells [4,5,15,16].

The *in vitro* proliferation of a variety of human tumor cell lines, including those from ovarian cancers can be inhibited by GnRH-I and its agonistic analogs in a dose- and time-dependent manner [4,9,11,12,17–20]. In most human ovarian cancer cells except for the ovarian cancer cell line EFO-27 GnRH-I antagonists act like agonists indicating that the dichotomy of GnRH-I agonists and antagonists does not exist in tumor cells [5,9]. Using human ovarian cancer cell line OV-1063 xenografted into nude mice, Yano et al. [12] demonstrated a significant inhibition of tumor growth by chronic treatment with the GnRH-I antagonist Cetrorelix but not with the GnRH-I agonist Triptorelin. As both GnRH-I analogs induced a comparable suppression of the pituitary-gonadal axis, the authors speculated that *in vivo* anti tumor effects of Cetrorelix were exerted directly on GnRH-I receptors in tumors [8]. The findings on direct anti tumor effects of GnRH-I analogs in ovarian and endometrial cancer reported by several other groups are completely or partly in agreement with the results described earlier [4,8,21,22]. In contrast, other investigators failed to detect direct anti tumor effects of GnRH-I analogs in human ovarian and endometrial cancer cell lines or observed them only at extremely high GnRH-I analog concentrations [23–25]. These discrepancies might be due to the fact that probably the majority of the cell lines used by these authors did not express high-affinity GnRH-I receptors [4,26]. Alternatively, differences in culture or experimental conditions as well as in the types of GnRH-I analogs used might be responsible for the observed variance. In the case of prostate cancer, several groups reported direct antiproliferative effects of GnRH-I analogs *in vitro* and *in animal in vivo*

models, which could be mediated through specific GnRH-I-binding sites [1,27–32].

The proliferation of human ovarian cancer cells was significantly increased after treatment with an antiserum to GnRH-I, suggesting that GnRH-I produced by human ovarian cancer cells acts as a negative autocrine regulator of proliferation [33]. In contrast, Arencibia and Schally [34] have recently reported that in ES-2 human ovarian cancer cells GnRH-I agonist Triptorelin at 10 ng/ml stimulated the proliferation *in vitro* after 48 h, but was inhibitory after 72 h and at concentrations of 1000 ng/ml. GnRH-I antagonist Cetrorelix inhibited growth of ES-2 cell line only at 1000 ng/ml. The incubation of ES-2 ovarian cancer cells *in vitro* with an GnRH-I antibody inhibited cell proliferation in a time and concentration-dependent manner. These results suggest that GnRH-I may function as an autocrine growth factor in this ovarian cancer cell line [34]. Differences between tumor cell lines e.g. variances in G-protein coupling and signaling might explain these discrepancies.

Signaling mechanisms mediating the direct anti-tumor effects of GnRH-I

During the last ten years, the signaling mechanisms mediating the antiproliferative effects of GnRH-I analogs in ovarian, endometrial and breast cancer cells have been elucidated. The signaling mechanism of GnRH-I receptor in human cancers is quite different from that in pituitary gonadotrophs, where GnRH-I receptors couple to G-protein α_q and activate phospholipase C (PLC), protein kinase C (PKC), and adenylyl cyclase (AC) [reviewed in [4]]. Although we could clearly demonstrate the activation of PLC, PKC, and AC in these tumor cells by pharmacological stimuli [35], the signaling pathways induced by GnRH-I in pituitary gonadotrophs were not activated by GnRH-I agonist Triptorelin in ovarian, endometrial and breast cancer cell lines [35,36]. We found, however, that after binding of its ligand, the GnRH-I receptor in these cancers couples to G-protein α_i and activates a phosphotyrosine phosphatase (PTP) [35–40] (Fig. 1A). This PTP dephosphorylates EGF receptors [36]. As a result, mitogenic signaling induced by EGF binding to its receptor is abrogated leading to a suppression of EGF-induced activation of mitogen-activated protein kinase (MAPK) [35], *c-fos* expression [41], and EGF-induced proliferation [35] (Fig. 1A). These findings are in accord with reports that GnRH-I analogs reduce expression of growth factor receptors and their mRNA [12,42,43] (Fig. 1B) and/or growth factor induced tyrosine kinase activity [35,37–39,42,44–46]. The reason for the differences of the GnRH-I receptor signaling between pituitary gonadotrophs and tumor cells remains unclear, as we could not find mutations or splice variants in the tumor GnRH-I receptor which might explain this phenomenon.

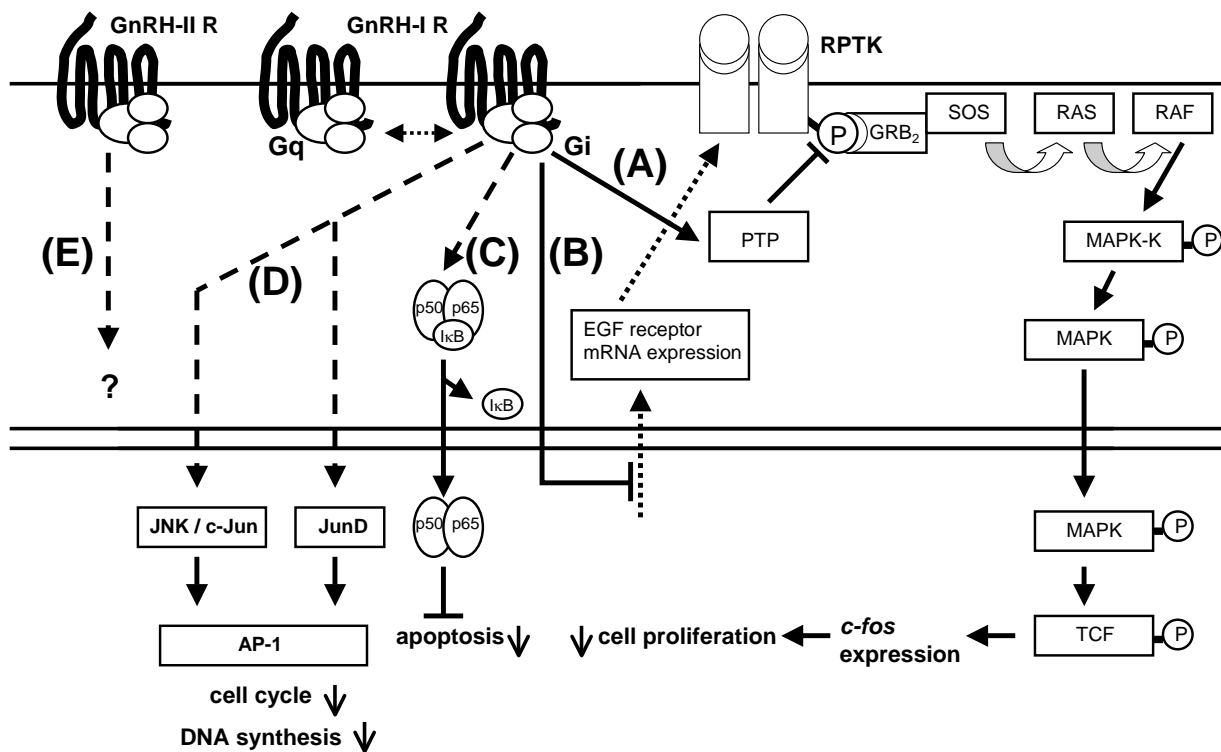


Figure 1

GnRH-I and GnRH-II signaling in human gynecological cancer cells: **A)** GnRH-I activates a phosphotyrosine phosphatase (PTP) inhibiting the mitogenic signal transduction of growth factor receptors resulting in downregulation of cell proliferation. **B)** GnRH-I downregulates epidermal growth factor (EGF) receptor mRNA expression. **C)** Activated GnRH-I receptor induces nucleus factor κ B (NF κ B) activation and nuclear translocation of activated NF κ B. Activated NF κ B now couples to κ B DNA binding sites and induces expression of anti-apoptotic mechanisms. **D)** GnRH-I activates c-Jun N-terminal kinase (JNK), induces JunD-DNA binding and stimulates activator protein (AP-1) activity, resulting in reduced proliferation as indicated by increased $G_{0/1}$ phase of cell cycle and decreased DNA synthesis. **E)** Unknown signal transduction of a putative human GnRH-II receptor. In human gynecological cancer cells GnRH-I analogs mediate antiproliferative actions via inhibition of growth factor-induced mitogenic signal transduction. In addition GnRH-I induces growth factor receptor downregulation. GnRH-I protects the cancer cells from apoptosis via activation of NF κ B, stimulates AP-1 activity and extends cell cycle. PPTK, receptor protein tyrosine kinase; GRB₂, adaptor protein; SOS, guanine nucleotide exchange factor; RAS, small GTPase; RAF, a protein-serine/threonine kinase; MAPK-K, mitogen activated kinase kinase; MAPK, mitogen activated kinase; TCF, transcription factor; I κ B, inhibitory κ B; Gq, G-protein α q; Gi, G-protein α i; p50, p65, NF κ B subunits.

Recently, it was speculated that induction of apoptosis might be involved in the antiproliferative activity of GnRH-I and its analogs [47–49]. However, though we have tried to show induction of apoptosis by GnRH-I analogs, we have found it only in one (Ca-Ov-3) of nine (EFO-21, EFO-27, OVCAR-3, AN-3-CA, Ca-Ov-3, SK-OV-3) ovarian cancer cell lines [50] and unpublished results.

In contrast, we found that GnRH-I agonist Triptorelin reduced apoptosis induced by the cytotoxic agent doxorubicin. Since Triptorelin-induced reduction of Doxorubicin-induced apoptosis was blocked by inhibition of nucleus factor kappa B (NF κ B) translocation into the nucleus and Triptorelin was shown to induce NF κ B activation (Fig. 1C), we concluded that GnRH-I has an antiap-

optotic effect mediated through NF κ B activation in these human ovarian cancer cells [50]. This possibility to protect ovarian cancer cells from programmed cell death is a new and important feature in GnRH-I signaling in ovarian tumors apart from the inhibitory interference with the mitogenic pathway.

Recently, it became evident that it is not only mitogenic signaling of growth factor receptors that is modulated by GnRH-I in human cancers. In human ovarian and endometrial cancer cells GnRH-I agonist Triptorelin stimulates the activity of activator protein-1 (AP-1) mediated through pertussis toxin-sensitive G-protein α i (Fig. 1D). In addition, Triptorelin activates JNK, known to activate AP-1 [51] (Fig. 1D). In earlier investigations we have shown that Triptorelin does not activate phospholipase C (PLC) and protein kinase C (PKC) in endometrial and ovarian cancer cells [35]. In addition, it has been demonstrated that Triptorelin inhibits growth factor-induced mitogen activated protein kinase (MAPK, ERK) activity [35]. Thus Triptorelin-induced activation of the JNK/AP-1 pathway in endometrial cancer cells is independent of the known AP-1 activators, PKC or MAPK (ERK) (Fig. 1D).

In ovarian and endometrial cancer cells GnRH-I analogs mediate antiproliferative actions via inhibition of growth factor-induced mitogenic signal transduction. GnRH-I agonist Triptorelin protects the cancer cells from apoptosis via activation of NF κ B, and Triptorelin stimulates AP-1 and JNK activity. Recently Yamauchi et al. [52] found that JNK is involved in inhibition of cell proliferation induced by α 1B-adrenergic receptor in human embryonic kidney cells. In a study in rats, *c-jun* mRNA depression and endometrial epithelial cell proliferation were suggested to be linked [53]. In UT-OC-3 ovarian cancer cells cytokines have inhibitory effects on cell proliferation and activate AP-1 and NF κ B [54]. Since the antiproliferative GnRH-I agonist Triptorelin activates the JNK/*c-jun* pathway and JNK/*c-jun* was found to be involved in downregulation of cell proliferation in different systems, it seems reasonable to speculate that the JNK/*c-jun* pathway is involved in the antiproliferative actions of the GnRH agonist Triptorelin. In addition, we have shown that GnRH-I agonist Triptorelin induces JunD-DNA binding, resulting in reduced proliferation as indicated by increased G_{0/1} phase of cell cycle and decreased DNA synthesis (Fig. 1D). Since GnRH-I activates NF κ B and protects ovarian cancer cells from Doxorubicin-induced apoptosis and JunD is shown to decrease cell cycle and cell proliferation, we propose that JunD activated by GnRH-I acts as a modulator of cell proliferation and cooperates with the anti-apoptotic and anti-mitogenic functions of GnRH-I [55].

GnRH-II and its receptor

In non-mammalian vertebrates it became evident that three structural variants of GnRH were present in individual species [56,57]. A similar situation seems to exist in mammals. One of these GnRH variants is GnRH-II, which is totally conserved in structure in the evolution from fish to mammals [58,59]. In human granulosa-luteal cells expression of GnRH-II was found [60]. In these cells GnRH-I agonists exerted a biphasic effect on GnRH-I receptor density, while GnRH-II agonists induced a down-regulation of GnRH-I receptor expression and of GnRH-II itself [60]. Recently Millar et al. cloned a type II GnRH receptor from the marmoset monkey which is highly selective for GnRH-II [61]. At the same time Neill et al. cloned the GnRH-II receptor from the rhesus monkey [62]. Only 41% (marmoset GnRH-II receptor) and 39% (rhesus monkey GnRH-II receptor) identities with the GnRH-I receptor have been reported [61,62]. In contrast to the GnRH-I receptor the GnRH-II receptor in the marmoset and rhesus monkey has a C-terminal, cytoplasmic tail resulting in a more rapid internalization [61,62].

Using RT-PCR and Southern blot analysis we could recently show that human ovarian and endometrial cancer cells express a putative second GnRH receptor specific for GnRH-II [63] (Fig. 1E). The proliferation of these cell lines was reduced in a dose- and time-dependent manner by native GnRH-II. These effects were significantly higher than the antiproliferative effects of equimolar doses of GnRH-I agonist Triptorelin [63]. In the GnRH-II receptor mRNA positive but GnRH-I receptor negative ovarian cancer cell line SK-OV-3 native GnRH-II but not GnRH-I agonist Triptorelin had antiproliferative effects [63]. In previous studies we have demonstrated that in ovarian cancer cell lines except for the EFO-27 cell line GnRH-I antagonist Cetrorelix has comparable antiproliferative effects as GnRH-I agonists indicating that the dichotomy of GnRH-I agonists and antagonists might not apply to the GnRH-I system in cancer cells [9] After GnRH-I receptor knock down in EFO-21 and OVCAR-3 human ovarian cancer cell lines the antiproliferative effects of GnRH-I agonist Triptorelin were abrogated while the effects of GnRH-I antagonist Cetrorelix and GnRH-II were still existing [unpublished results]. In addition, in the ovarian cancer cell line EFO-27 GnRH-I receptor but not putative GnRH-II receptor expression was found [unpublished results]. These data suggest that in ovarian and endometrial cancer cells the antiproliferative effects of GnRH-I antagonist Cetrorelix and GnRH-II are not mediated through the GnRH-I receptor. It is possible that these antiproliferative effects are mediated through a putative GnRH-II receptor. However, the human GnRH-II receptor is expressed as a variety of splice variants [64] and a functional human GnRH-II receptor transcript has not been found until now. Further investigations are required to

determine whether these GnRH-II receptor splice variants translate to functional proteins.

Gonadotropin biosynthesis and secretion by GnRH-I can be mediated by activation of MAP kinases. Therefore, Millar et al. [61] assessed the capacity of both human GnRH-I receptor and marmoset GnRH-II receptor to activate the MAP kinases ERK2, JNK, and p38 α in COS-7 cells transfected with either the human GnRH-I receptor or the marmoset GnRH-II receptor. At the GnRH-I receptor, GnRH-I was considerably more potent than GnRH-II in activating ERK2 whereas at the GnRH-II receptor, GnRH-II was markedly more potent than GnRH-I [61]. Neither GnRH-I receptor nor GnRH-II receptor stimulation resulted in activation of JNK [61]. Activation of p38 α was detected on stimulation of GnRH-II receptor with GnRH-II but not with stimulation of GnRH-I receptor with GnRH-I [61]. These data suggest that there are distinct differences in the signal transduction by the two GnRH receptors. However, the signal transduction mechanisms mediating the antiproliferative activity of GnRH-II in human ovarian cancer cells are not known (Fig. 1E).

As the antiproliferative activity of native GnRH-II is significantly superior to that of the GnRH-I superagonists, superactive agonists of GnRH-II might become efficacious drugs for the therapy of human cancers. However, the more widely distributed expression pattern of GnRH-II receptor [61] could limit the effectiveness of GnRH-II agonists.

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