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〈Review〉

Gonadotropin-releasing Hormone and Its Receptor as a Therapeutic Concept in the Progression of Epithelial Ovarian Cancer

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ABSTRACT

Ovarian cancer is a significant cause of cancer-related death in women, but the main biological causes remain open questions. Hormonal factors have been considered to be an important determinant causing ovarian cancer. Recent studies have shown that gonadotropin-releasing hormone (GnRH)-I and its analogs have clinically therapeutic value in the treatment of ovarian cancer. In addition, numerous studies have shown that the potential of GnRH-Ⅱ in normal reproductive system or reproductive disorder. GnRH-Ⅰ receptors have been detected in approximately 80% of ovarian cancer biopsy specimens as well as normal ovarian epithelial cells and immortalized ovarian surface epithelium cells. GnRH-Ⅱ receptors have also been found to be more widely expressed than GnRH-Ⅰ receptors in mammals, suggesting that GnRH receptors may have additional functions in reproductive system including ovarian cancer. The signal transduction pathway following the binding of GnRH to GnRH receptor has been extensively studied. The activation of protein kinase A/C (PKA/PKC) pathway is involved in the GnRH-Ⅰ induced anti-proliferative effect in ovarian cancer cells. In addition, GnRH-Ⅰ induced mitogen-activated protein kinase (MAPK) activation plays a role in anti-proliferative effect and apoptosis in ovarian cancer cells and the activation of transcriptional factors related to cellular responses. However, the role of GnRH-Ⅰ and Ⅱ receptors, there are discrepancies between previous reports. In this review, the role of GnRH in ovarian cancer and the mechanisms to induce anti-proliferation were evaluated.

(Key words : GnRH, GnRH receptors, ovarian cancer, signaling pathways)

HORMONAL FACTORS IN THE ETIOLOGY OF OVARIAN CANCER

Ovarian cancer is a significant cause of cancer-related death in women, following breast, lung, and colorectal cancer (Schally *et al.*, 2001). Because of the absence of symptoms in early-stages and location in the pelvis, ovarian cancer is difficult to detect in early stages (Herbst, 1994) and has a high fatality and low five-year survival rate compared with other gynecologic cancers.

A few possibilities including endocrine factors that induce ovarian tumors have been suggested (Risch, 1998; Shoham, 1994). Hormonal factors have been implicated in the etiology of ovarian cancer, according to the incessant ovulation theory (Risch, 1998; Shoham, 1994). Women who have frequent ovulation in their twenties and have a family history of ovarian cancer may contribute to high risk of ovarian cancer, suggesting that ovarian cancer is essentially a hereditary genetic disease (Godwin *et al.*, 1992; Negri *et al.*, 2003; Purdie *et al.*, 2003). Hormonal factors have also been considered to be an important determinant causing ovarian cancer. On the other hand, taking oral contraceptives and multiparity might reduce the risk of ovarian cancer, also suggesting that hormonal factors are important in ovarian cancer development (Risch, 1998).

GONADOTROPIN-RELEASING HORMONE (GnRH) AND GnRH RECEPTORS

1. Physiological Role of GnRH-Ⅰ and GnRH-Ⅱ

Gonadotropin-releasing hormone (GnRH) is a central regulator of the mammalian reproductive system. It is well documented that GnRH is secreted from the hypothalamus and intermittently binds to its receptor in the anterior pituitary to stimulate the synthesis and release of gonadotropins such as folli-

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cle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. Since the amino acid sequence of GnRH was reported in the 1970s (Burgus *et al.*, 1972; Matsuo *et al.*, 1971), the expression of GnRH and GnRH receptors are considered to be important for gonadal steroidogenesis and for the maintenance of pregnancy and efforts have focused on its molecular biology. Exogenously administered GnRH agonists action desensitizes, and down-regulates GnRH receptors in the pituitary and decreases LH and FSH production, which induces the subsequent decrease in the circulating sex steroid levels (Neill, 2002). In the ovary, stimulatory effects of GnRH include augmentation of steroidogenic and ovulatory processes but GnRH has an inhibitory effect on ovarian function to reduce gonadotropin receptor biosynthesis, steroidogenesis, and follicular development.

Three structural variants of GnRH were determined in non-mammalian vertebrates. These GnRH variants have almost similar amino acid sequences but different functions in the regulation of reproduction (Sealfon *et al.*, 1997; Sherwood *et al.*, 1993). One of these GnRH variants is GnRH-Ⅱ (also called chicken GnRH-Ⅱ), which is totally conserved in structure from fish to mammals. GnRH-Ⅱ was sequenced and its gene expression was identified in human (Neill, 2002). Although the normal physiologic function of GnRH-Ⅱ is poorly understood, it has been observed that GnRH-Ⅱ induced the secretion of human chorionic gonadotropin (hCG) in cytotrophoblastic cells (Islami *et al.*, 2001). Chicken GnRH-Ⅱ is less likely to regulate the gonadotropin secretion than chicken GnRH-Ⅰ in hens (Sharp *et al.*, 1990). Interestingly, the expression of GnRH-Ⅱ was detected at higher levels (up to 30 times than GnRH-Ⅰ) in kidney, bone marrow, and prostate (White *et al.*, 1998) and the role of GnRH-Ⅱ in these organs remain to be determined.

2. Roles of GnRH in Cancer Cells

In addition to its important role in the reproductive system, GnRH-Ⅰ and its analogs are known to have therapeutic value in the treatment of reproductive disorders such as infertility, polycystic ovarian disease, and precocious puberty (Heger *et al.*, 2006; Homburg, 2003). GnRH is also clinically valuable in the treatment of prostate, breast, and ovarian cancers (Harrison *et al.*, 2004). GnRH-Ⅰ showed inhibitory effects on human mammary, ovarian, endometrial, and prostatic tumor growth, and has been implicated as an anti-proliferative regulator of gynecologic cancers (Santen *et al.*, 1990; Savino *et al.*, 1992; Schally, 1999; Schally *et al.*, 2001). The general principle of GnRH therapy is to suppress the function of the hypothalamus-pituitary-gonadal axis, called 'chemical castration' (Florio *et al.*, 2002). Moreover, the detection of GnRH-Ⅰ and its receptor in breast, placenta, ovary, and prostate tissues implies that GnRH-Ⅰ may have direct effects at peripheral targets (Kim *et al.*, 2004; Kleinman *et al.*, 1994; Motomura, 1998; Schally *et al.*, 2001). In ovarian cancer cells, it has been reported that GnRH-Ⅰ induces apoptosis and has an autocrine/paracrine action (Kang *et al.*, 2000b; Motomura, 1998), suggesting that treatment with GnRH-Ⅰ is important for the direct suppression of proliferation and the induction of apoptosis in gynecologic cancers.

In addition to GnRH-Ⅰ, the expression and potential anti-proliferative effect of GnRH-Ⅱ indicate that GnRH-Ⅱ, similar to GnRH-Ⅰ, may have a growth-regulatory effect in normal and neoplastic ovarian surface epithelial cells (Choi *et al.*, 2001). Furthermore, a recent study demonstrated that GnRH-Ⅱ has an anti-proliferative effect in gynecologic tumors and may exert a stronger anti-proliferative effect than GnRH-Ⅰ in ovarian cancer cells (Grundker *et al.*, 2002; Kim *et al.*, 2004), suggesting that GnRH-Ⅱ could be considered asa novel target for anti-proliferative therapeutic approaches. However, in contrast to GnRH-Ⅰ, the biological mechanism of GnRH-Ⅱ remains obscure.

3. GnRH-Ⅰ Receptor

Three types of GnRH receptors have been reported and the structure of mammalian GnRH-Ⅰ receptorwas determined by sequencing of a cDNA isolated from an immortalized murine gonadotroph cell line (αT3-1) (Reinhart *et al.*, 1992; Tsutsumi *et al.*, 1992). The GnRH-Ⅰ receptor is a member of the G protein-coupled receptor (GPCR) family (Kraus *et al.*, 2001) and a member of the 7 transmembrane receptor superfamily that transduce an extracellular signal into an intracellular signal (G protein activation). The signaling of GnRH-Ⅰ might then be passed onto the nucleus eliciting protein phosphorylation or dephosphorylation and eventually transcriptional activation or inactivation. The signal transduction pathway following the binding of GnRH-Ⅰ to GnRH-Ⅰ receptor has been extensively studied (Millar *et al.*, 2004). Intracellular communication of the mammalian GnRH-Ⅰ receptor is unique because it lacks the common carboxyl-terminal cytoplasmic domain and possesses a relatively short intracellular third loop among GPCRs (Reinhart *et al.*, 1992). Understanding of the GnRH receptor structure can lay the foundation for the design of a new generation of GnRH analogs for the treatment of cancer and reproductive

disorders. In pituitary cells, GnRH-Ⅰ receptors have been identified (Bourne *et al.*, 1980; Clayton and Catt, 1980; Clayton *et al.*, 1979; Clayton *et al.*, 1980; Naor *et al.*, 1980; Pal *et al.*, 1992; Schulz *et al.*, 1993; Weil *et al.*, 1992; Wormald *et al.*, 1985). In extrapituitary tissues, the gene of GnRH and its receptors are expressed in gonads (Currie *et al.*, 1981; Iwashita *et al.*, 1986), and placenta (Emons *et al.*, 1992; Miller *et al.*, 1985) but not in liver and spleen (Kakar *et al.*, 1992). In neoplastic tissues, GnRH-Ⅰ receptors have been identified in human epithelial ovarian carcinoma (Emons *et al.*, 1992; Irmer *et al.*, 1995; Pahwa *et al.*, 1989), human breast cancer tissue (Fekete *et al.*, 1989; Miller *et al.*, 1985), endometrial cancer (Furui *et al.*, 2002), and prostate tumors (Hong *et al.*, 1998; Qayum *et al.*, 1990).

GnRH-Ⅰ and its receptor have been detected in approximately 80% of ovarian cancer biopsy specimens as well as normal ovarian epithelial cells and immortalized ovarian surface epithelium cells (Choi *et al.*, 2001; Irmer *et al.*, 1995). OVCAR-3 and SKOV-3 cells, ovarian adenocarcinoma cell lines, express the GnRH-Ⅰ receptor protein. Both cell lines were derived from serous carcinoma patient's ascites, which accounts for most part of ovarian cancer patients. The each shape of OVCAR-3 and SKOV-3 cells implies that OVCAR-3 is more likely undifferentiated than SKOV-3 cell because OVCAR-3 has cuboidal shape which takes after normal ovarian surface epithelia cells but SKOV-3 cells has enlongated shape. Although it has been known that GnRH stimulates the release of the gonadotropins, FSH and LH, the functions of GnRH-Ⅰ in other tissues are not well understood. Nonetheless, the presence of GnRH-Ⅰ and its receptor indicate that it may have a functional role in the reproductive system. How GnRH recognizes and activates its receptor is considered important for elucidating the regulation of its reproductive function and treatment in cancer therapy.

4. GnRH-Ⅱ Receptor

In most vertebrates, several structural variants of GnRH exist, suggesting that additional receptors for GnRH in mammalian may exist in some tissues and organisms. Indeed, three distinct types of GnRH receptor were determined in the bullfrog (Wang *et al.*, 2001) and a novel chicken pituitary GnRH receptor has been cloned (Sun *et al.*, 2001). Neill *et al.* observed that GnRH-II receptor gene is present in humans (Neill *et al.*, 2001). GnRH-Ⅱ receptors were identified in mammals and found more widely than GnRH-Ⅰ receptors in the body including the brain (Kang *et al.*, 2000b; Millar *et al.*, 1999; Millar *et al.*, 2001), suggesting that GnRH-Ⅱ may have more functions than GnRH-Ⅰ.

Nevertheless, to date, direct evidence demonstrating the existence of full-length, functional GnRH-Ⅱ receptor RNA transcript in human tissues is lacking. Although a full length GnRH-Ⅱ receptor has not been cloned or sequenced, Grundker *et al.* suggested the anti-proliferative effect of GnRH-Ⅱ in endometrial and ovarian cancer cells (Grundker *et al.*, 2002).

As to the role of GnRH-Ⅰ and II receptors, there are discrepancies between previous reports. It has been suggested that the signal transduction pathways coupled to the GnRH-Ⅱ receptor may be different from those triggered by activation of the GnRH-Ⅰ receptor (Millar *et al.*, 2001; Neill *et al.*, 2001). Enomoto *et al.* showed that GnRH-Ⅱ receptor is necessary to mediate the effect of GnRH-Ⅱ (Enomoto *et al.*, 2004) and Grundker *et al.* reported that the anti-proliferative effect induced by GnRH-Ⅱ is not mediated through GnRH-Ⅰ receptor (Grundker *et al.*, 2004). However, a human GnRH-Ⅱ receptor protein has not been identified since the human GnRH-Ⅱ receptor transcript has a frame-shift resulting in a premature stop codon (Morgan *et al.*, 2003) and the mechanisms of antiproliferative action of GnRH-Ⅰ and GnRH-Ⅱ is not clearly understood. Thus, the issue of whether this transcript encodes a functional receptor protein in human tissues and the potential roles of the GnRH-Ⅱ receptor in mediating the effects of GnRH-Ⅰ and II remains obscure. In addition, a recent study showed that GnRH-Ⅱ receptor inhibits the expression of GnRH-Ⅰ receptor, indicating that GnRH-Ⅰ receptor may be a common receptor that mediates the effects of both GnRH-Ⅰ and GnRH-Ⅱ in ovarian cancer cell lines (Pawson *et al.*, 2005). Therefore, the exact mechanism mediated by these treatments needs to be elucidated.

GnRH SIGNALING PATHWAYS

1. G Protein-coupled Receptor (GPCR)

The signaling of GnRH is transferred to cytoplasmic signaling pathway to induce the cellular biological effect. After GnRH-Ⅰ binds to cell membrane receptors, GnRH receptors become internalized and undergo lysosomal degradation and/or undergo receptor recycling (Hazum and Conn, 1988). Binding of GnRH to the GnRH receptor leads to conformational changes in the receptor. As stated, the GnRH-Ⅰ receptor belongs to the GPCRs family and GnRH-Ⅰ transmits extracellular signals into the intracellular milieu via heterotrimeric (α,

β, and γ subunits) GTP-binding proteins (G-proteins) (Birnbaumer, 1992).

The α-subunit has a binding site for GTP or GDP and carries the GTPase activity. The β, and γ subunits exist as a complex and are active in this form. Although the $\beta\gamma$ subunit-complex is involved in signal transmission, the majority of G proteins-related signaling occurs via the α-subunit. G_α-proteins can be divided into four families based on amino acid sequence, $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q}$ and G_{12} (Downes and Gautam, 1999). A characteristic of the members of the $G_{\alpha s}$ subfamily is that they are inhibited by cholera toxin. Cholera toxin catalyzes the ADP-ribosylation of an Arg201 in $G_{\alpha s}$, which interferes with its function and inactivates the GTPase activity leading to the blockage of the intrinsic deactivation mechanism of the $G_{\alpha s}$-protein. Members of the $G_{\alpha i}$ subfamily have inhibitory effects on adenylyl cyclase and are sensitive to pertussis toxin which catalyzes the ADP-ribosylation and subsequent inhibition of the $G_{\alpha i}$-subunit. Mastoparan isolated from wasp venoms is able to stimulate the GDP-GTP exchange of $G_{i/o}$ type G-proteins (Higashijima *et al.*, 1990; Hirai *et al.*, 1979; Tanaka *et al.*, 1998). The next signal protein in the reaction sequence of G protein is phospholipase C (Drissi *et al.*, 1998; Hubbard and Hepler, 2006; Kuhn and Gudermann, 1999; Zhu and Birnbaumer, 1996). Members of the $G_{\alpha q}$ subfamily are not modified by cholera toxin or pertussis toxin (PTX). The functions of the G_{12} subfamily have been implicated as the activation of c-Jun N-terminal protein kinase, reorganization of the cytoskeleton, and stimulation of Na^+/H^+ exchange (Gohla *et al.*, 1999; Lin *et al.*, 1999; Nagao *et al.*, 1999). $G_{\alpha i}$ is involved in GnRH signaling in ovarian carcinoma and uterine leiomyosarcomas (Imai *et al.*, 1996). The anti-proliferative effect was mediated through the PTX-sensitive $G_{\alpha i}$ protein in these cell lines (Grundker *et al.*, 2001b). However, it has also been reported that the G-proteins involved in GnRH-Ⅰ signaling differ depending on cell type (Fang *et al.*, 2002; Grundker *et al.*, 2001b; Kraus *et al.*, 2001) and it is assumed that the individual subtypes of G proteins have specific roles in different cell compartments, cells and tissues. The mechanisms involved in the activation of G-protein signaling pathways by GnRH have yet to be fully elucidated.

2. Protein Kinase C and GnRH Signaling

The GPCRs can be coupled to the $G_{\alpha q/11}$ protein that activates phospholipase Cβ, leading to the activation of protein kinase C (PKC) and various downstream signal transduction cascades, including the mitogen-activated protein kinase (MAPK) pathways (Harris *et al.*, 1997). In addition, the activation of the PKC pathway has been well documented in response to GnRH-Ⅰ stimulation. GnRH-Ⅰ induces the activation of ERK1/2 through PKC, which may participate in gonadotropin release or synthesis in pituitary cells (Shacham *et al.*, 2001). A diversity of GPCRs can activate extracellular signal-regulated kinase 1 and 2 (ERK1/2) and the activation of PKC is one of the important signaling pathways in the activation of ERK1/2 by GnRH in pituitary cells (Andrews and Conn, 1986; Zheng *et al.*, 1994). The G-protein involved in GnRH-Ⅰ signaling pathway in the pituitary gland cells is not $G_{\alpha i}$ but might be $G_{q/11}$, which activates phospholipase C (PLCβ) to mediate inositol 1,4,5-triphosphate (IP_3), and diacylglycerol (DAG) production (Anderson *et al.*, 1993; Hsieh and Martin, 1992). IP_3 releases calcium from intracellular stores (Stojilkovic *et al.*, 1994; Tse *et al.*, 1997) and DAG stimulates the PKC pathway in the pituitary gonadotrophs. Activated PKC might be involved in the Ca^{2+} influx and might up-regulate GnRH-Ⅰ receptors (Naor, 1990). The activation of PKC with an increase of Ca^{2+} concentration in cytoplasm is important for mediating GnRH-Ⅰ action for gonadotropin secretion in the pituitary gland. In granulosa cells, PKC is known as a major component in activating ERK signaling from GnRH-Ⅰ receptor (Kang *et al.*, 2001). Activation of PKC appears to be an important second messenger mediating GnRH-Ⅰ-induced ERK activation in pituitary cells (Harris *et al.*, 1997) and ovarian cancer cells (Chamson-Reig *et al.*, 2003). However, unlike pituitary cells, it was reported that ERK activation of GnRH-Ⅰ might be mediated by $G_{\beta\gamma}$ complex, neither by G_α subunit, PKC pathway, nor extracellular Ca^{2+}. In addition, the activation of ERK is involved in the anti-proliferative effect of GnRH-Ⅰ in the Caov-3, human ovarian cancer cell line (Kimura *et al.*, 1999).

3. Mitogen-activated Protein Kinase (MAPK)

The family of MAPK consists of both mitogen-activated protein kinase and stress-activated protein kinase. Every MAPK has dual phosphorylation motif (Thr-Xaa-Tyr) and is activated by upstream kinases called MAPK kinases (MEKs or MKKs). MAPK family members are directly regulated by kinases known as MAPK kinases (MAPKKs), which activate MAPKs by the phosphorylation of tyrosine and threonine residues (Cobb and Goldsmith, 1995; Robinson and Cobb, 1997). Although 12 different MAPKs have been identified in mammalian cells to date, ERK1/2, c-Jun N-terminal protein kinase/Stress activated

protein kinase 1 (JNK/SAPK1) and p38/SAPK2 are three of the best-characterized MAPK moieties (Cobb and Goldsmith, 1995). Biological roles of other MAPKs (ERK3, 4, and 5, four p38-like kinases, and p57 MAPK, etc) have not been elucidated. ERK1 (p44 MAPK) and ERK2 (p42 MAPK) are activated by mitogenic stimuli and represent a group of the most extensively studied members. In contrast, JNK/SAPK1 and p38 MAPK are activated in response to stress such as heat shock, osmotic shock, cytokines, protein synthesis inhibitors, antioxidants, ultra-violet, and DNA-damaging agents (Cobb and Goldsmith, 1995; Garrington and Johnson, 1999; Kennedy and Davis, 2003; Lin, 2003). Although ERK is proposed to contribute to protecting against UV-induced damage, it has been reported that ERK1/2 is involved in cell cycle arrest and the inhibition of growth as well as cell survival and differentiation (Alblas *et al.*, 1998; Herskowitz, 1995; Yen *et al.*, 1998). The activation of ERK1/2 by GnRH is relatively sustained in alpha T3-1 and HEK293 cells but it is transient in GT1-7 neurons (Shah *et al.*, 2003). It has been proposed that gene expression and other specific intracellular responses may vary according to the duration and the magnitude of MAPK activation in individual cell types (Shah *et al.*, 2003) and the effect of the activation of MAPK seems to vary in many cell types (Alblas *et al.*, 1998; Mansour *et al.*, 1994; Yen *et al.*, 1998).

GnRH-Ⅰ might activate diverse cytoplasmic proteins to transfer its signal into the nucleus, and MAPK is considered to be one of the important pathways in GnRH-Ⅰ signaling pathway (Kraus *et al.*, 2001; Naor *et al.*, 2000). MAPK cascades are activated via two distinct classes of cell surface receptors, receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs). Signals transmitted through these cascades induce the activation of diverse molecules which regulate cell growth, survival and differentiation (Naor *et al.*, 2000). In pituitary αT3-1 cells, the GnRH receptor signals are mediated via all four major MAPK cascades including ERK1/2, JNK, p38 MAPK and BMK1/ERK5 (Kraus *et al.*, 2001; Naor *et al.*, 2000). Four subtypes of G (G_s, G_q, G_i, and G_{12}) might activate MAPK family and $G_{\beta\gamma}$ dimer of G-protein also activates MAPK through PI3K (Jiang *et al.*, 1998). G12/13 exerts its action by stimulating protein tyrosine kinases (PTKs) (Dikic and Blaukat, 1999). Furthermore, GPCRs have cross talk with RTKs and PTK can convey GPCRs signals to regulate proliferation, migration, and adhesion, suggesting that the MAPK cascade is activated by RTKs and GPCRs (Cobb and Goldsmith, 1995). Although it is difficult to define each of the mechanisms involved in the regulation of MAPKs in response to external stimuli, it is important to clarify the specific signaling pathways utilized by GnRH. Thus, it is important to study the MAPK pathway involved in GnRH-Ⅰ and GnRH-Ⅱ signaling pathway to elucidate the mechanism of anti-proliferative effect.

GnRH-Ⅰ revealed distinct differences in signaling pathways between cell types. The signaling mechanism mediating the activation of MAPKs by GnRH-Ⅰ also seems to be significantly different in cell types. In pituitary cells, it has been reported that GnRH-Ⅰ activates ERK 1/2, JNK and p38 in αT3 and LβT2 cells (Levi *et al.*, 1998; Liu *et al.*, 2002; Reiss *et al.*, 1997; Roberson *et al.*, 1999). GnRH-Ⅰ induces the activation of ERK1/2 through protein kinase C (PKC), which may participate in gonadotropin synthesis or release in pituitary cells.

The role of the MAPK family in the anti-proliferative effect of GnRH in CaOV-3 has been demonstrated (Kimura *et al.*, 1999). In our previous reports, we demonstrated that follicle-stimulating hormone (FSH) stimulated the activation of the ERK1/2 cascade and induced the phosphorylation of Elk-1 in neoplastic ovarian surface epithelial cells (Choi *et al.*, 2002) and that the p38 MAPK pathway is involved in the anti-proliferative effect of GnRH-Ⅱ in ovarian cancer cells (Kim *et al.*, 2004). The sequential transcriptional cascade, which is followed by GnRH-Ⅰ induced MAPK activation, should be characterized. Studies conducted in COS7 cells showed that the ant-proliferative effect of GnRH-Ⅰ agonist was mediated through G_i (Kraus *et al.*, 2001). Furthermore, GnRH-Ⅰ had no effect in the activation of JNK in this cell line. Following binding to its own receptors, the activation of PKC is involved in the activation of ERK 1/2 directly through Raf-1, Ras and dynamine, which are downstream of Src in αT3 cells. However, it was shown that the $G_{\beta\gamma}$ complex, receptor tyrosine kinase, and focal adhesion kinase (FAK) did not play a role in ERK1/2 activation (Benard *et al.*, 2001; Sundaresan *et al.*, 1996). In granulosa cells, PKC is a major component in the activation of ERK from GnRH-Ⅰ receptor (Kang *et al.*, 2001). However, the effect of GnRH-Ⅱ on MAPKs is poorly understood.

4. Transcription Factors Involved in the GnRH Response

GnRH-Ⅰ stimulates the activation of transcriptional factors-such as Elk-1 by the activation of MAPK in αT3 cells (Roberson *et al.*, 1995). GnRH-Ⅰ also increases immediate early response gene (ERG) mRNA such as c-*fos* and c-*jun* in this cell line (Cesnjaj *et al.*, 1995). The p38 MAPK might be in-

volved in GnRH-Ⅰ integration of c-*fos* promoter activity (Roberson *et al.*, 1999). It has been reported that GnRH-Ⅰ analog activates AP-1, a transcriptional factor in human endometrial cancer cells (Grundker *et al.*, 2001a). In our previous reports, we demonstrated that follicle-stimulating hormone (FSH) stimulates the activation of the ERK1/2 cascade and phosphorylates Elk-1 in neoplastic ovarian surface epithelial cells (Choi *et al.*, 2002). However, despite these observations, the exact mechanism of the signaling pathway between MAPK and transcriptional factors by GnRH-Ⅰ and GnRH-Ⅱ in ovarian cancer cells remains to be investigated.

It has been demonstrated that MAPKs associate with transcription factors such as c-Myc, Elk-1, c-Jun, and ATF-2, (Garrington and Johnson, 1999; Johnson and Lapadat, 2002; Wasylyk *et al.*, 1998). ERK, JNK and p38 activate Elk-1 that binds to serum response element (SRE) and enhances SRE-dependent c-*fos* expression (Cavigelli *et al.*, 1995; Gille *et al.*, 1995a; Gille *et al.*, 1995b; Raingeaud *et al.*, 1996; Whitmarsh *et al.*, 1995). JNK phosphorylates c-*jun* and ATF-2 (Derijard *et al.*, 1994; Gupta *et al.*, 1995), and p38 activates ATF-2 (Raingeaud *et al.*, 1995; Raingeaud *et al.*, 1996). Both ATF-2 and c-*jun* form heterodimers, which bind to TPA-response elements (TREs) resulting in enhancing c-*jun* expression (van Dam *et al.*, 1995). c-Fos and c-Jun form AP-1, which induce a cellular response following binding to AP-1 response elements in gene promoter. It is suggested that AP-1 is involved in a protective function against the effects of UV-induced cell damage. JNK and p38 are involved in apoptosis via AP-1 (Verheij *et al.*, 1996; Xia *et al.*, 1995). MAPK contributes to the induction of AP-1 activity through activating transcription factor 2 (ATF-2) (Raingeaud *et al.*, 1995).

5. Interaction between the GnRH and EGF Signaling pathway

The EGF receptor family (EGFR, also known as type I receptor tyrosine kinases or ErbB tyrosine kinase receptors) is the best studied growth factor receptor system. This family is comprised of four homologous receptors: the epidermal growth factor receptor (ErbB1/EGFr/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4) (Jorissen *et al.*, 2003; Klapper *et al.*, 2000). This receptor family regulates the intracellular effects of ligands such as EGF and transforming growth factor-α (TGFα) (Wells, 1999; Yarden and Sliwkowski, 2001). It has been observed that EGFRs are markedly overexpressed on a number of various epithelial cancers including ovarian cancers (Salomon *et al.*, 1995). Therefore, it has been hypothesized that the binding of EGF to its receptor could be blocked to prevent receptor activation and thereby inhibit cell proliferation. They might be candidates for the development of novel therapeutics for ovarian cancer treatment (Maihle *et al.*, 2002).

The EGF signaling pathway is also considered to be an important mechanism for GnRH-Ⅰ signaling because GnRH-Ⅰ is known to reverse the effects of EGF by the activation of tyrosine phosphatases (Lee *et al.*, 1991). Alpha T3-1 cells express EGF receptor and respond to EGF stimulation with significant but transient ERK1/2 phosphorylation (Shah *et al.*, 2003). In addition to the anti-proliferative effect of GnRH-Ⅰ by interfering with the EGF pathway mediating the mitogenic action in prostatic cancer cells (Dondi *et al.*, 1996), the binding of GnRH-Ⅰ agonists and antagonists to GnRH-Ⅰ receptors inhibits the activation of MAPK and the expression of c-*fos* by EGF in gynecologic cancer cell lines (Grundker *et al.*, 2000). Furthermore, GnRH-Ⅰ inhibits MAPK activity induced by EGF in endometrial and ovarian cancer cell lines (Emons *et al.*, 1998).

Genistein, a protein tyrosine kinase (PTK) inhibitor, inhibits GnRH-Ⅰnduced FSH release and reduces GnRH-Ⅰ-analog-stimulated MAPK activity in αT3-cells, suggesting that PTK signaling may be involved in GnRH action in the pituitary (Johnson *et al.*, 1995; Reiss *et al.*, 1997). However, the role of PTK remains to be determined in other tissues.

CLINICAL APPLICATION OF GnRH IN CANCER TREATMENT

Despite continueing efforts for ovarian cancer therapy, the techniques for early diagnosis and treatment by surgery and chemotherapy are not very effective. In addition, the responses have been variable and the treatments are not particularly effective. A number of treatment strategies are still in development.

Recent findings suggest that hormone treatment including gonadotropin-releasing hormone (GnRH) has potential benefits for patients with ovarian cancer that is unresponsive to chemotherapy (Rzepka-Gorska *et al.*, 2003). GnRH analogs produced favorable results when conventional therapy was supplemented for late-stage ovarian cancer treatment (Zidan *et al.*, 2002). Increasing our understanding of GnRH signaling pathways may improve the efficacy of chemotherapy in using these agonists in ovarian cancer treatment.

Apoptosis is a natural cellular process which directs programmed cell death to achieve homeostasis. Apoptosis is accom-

panied by changes in cell morphology such as chromatin condensation, the degradation of DNA, cell shrinkage and fragmentation of the cell nucleus, which distinguish apoptosis from the another form of cell death known as necrotic cell death. Cancer is considered as a disease of abnormal cell growth (Chao and Korsmeyer, 1998; Minn *et al.*, 1998). It has been shown that treatment with GnRH-Ⅰ induces apoptosis in pituitary and prostate cancer cells (Kraus *et al.*, 2006; Rose *et al.*, 2004). In addition, it has been reported that GnRH-Ⅰ analogs induce apoptosis in GnRH receptor-bearing gynecologic cancers (Grundker *et al.*, 2000; Kang *et al.*, 2000a; Kim *et al.*, 1999; Kleinman *et al.*, 1994; Kraus *et al.*, 2006; Motomura, 1998; Thompson, 1995). However, the effect of GnRH-Ⅰ analogs on apoptosis is still controversial (Grundker *et al.*, 2000; Kim *et al.*, 1999; Kim *et al.*, 2004; Motomura, 1998) and it might be due to the different cell lines. It has been demonstrated that GnRH-Ⅰ increased Fas ligand (FasL) expression in reproductive tract tumors and GnRH-Ⅰ analogs had no effect on the cell growth in Fas-negative cells (Imai *et al.*, 1998a; Imai *et al.*, 1998b). This accounts for the anti-proliferative effect through the Fas/FasL complex. However, different cell types display various sensitivity for Fas-induced apoptosis (Schulze-Osthoff *et al.*, 1998). Although GnRH-Ⅰ also increased the expression of Fas/FasL in cultured uterine leiomyoma cells, the effect of GnRH on the expression of Fas/FasL is still controversial (Huang *et al.*, 2002a; Huang *et al.*, 2002b; Wang *et al.*, 2002). The caspases, existing as procaspases in cells, are also important mediators for apoptosis as well as Fas. These procaspases are activated by the cell death signals such as Fas activation. The two main apoptotic signals are mediated by a death receptor (caspase-8) and mitochondria (caspase-9), and they initiate to activate downstream caspases. Caspase 12 in endoplasmic reticulum (ER) can be activated by ER stress such as disruption of Ca^{2+} homeostasis (Mehmet, 2000; Nakagawa *et al.*, 2000). Caspase 8 can be activated by Fas or TNF receptor activation. Alternatively, caspase 9 can be activated by the release of cytochrome c from mitochondria by apopototic triggers such as mutagens or ionizing radiation. Caspases 8 and 9 activate downstream capases such as caspases 3, 6, and 7 (Mehmet, 2000; Salvesen and Dixit, 1997).

CONCLUSION

Recently, GnRH has been implicated as an anti-proliferative regulator of gynecologic cancers and considered as a clinically valuable treatment in prostate, breast, and ovarian cancers. Moreover, the detection of GnRH and its receptor in these tissues implies that GnRH may have direct effects at peripheral targets. In ovarian cancer cells, treatment with GnRH-Ⅰ is important for the anti-proliferative effect and the induction of apoptosis. In addition, the expression and potential anti-proliferative effect of GnRH-Ⅱ indicate that GnRH-Ⅱ may have a growth-regulatory effect in normal and neoplastic ovarian surface epithelial cells, suggesting that GnRH-Ⅱ could be considered as a novel target for anti-proliferative therapeutic approaches. PKC and MAPK pathways seem to mainly contribute to the GnRH signaling pathway to induce the anti-proliferative effect on ovarian cancer. Although the biological mechanism of GnRH-Ⅱ still remains obscure and there are discrepancies between previous reports to propose the role of GnRH-Ⅰ and Ⅱ in ovarian cancer, understanding of the GnRH receptor structure can lay the foundation for the design of a new generation of GnRH analogs for the treatment of ovarian cancer and further research will provide a basis for promising new therapeutic approaches for ovarian cancer.

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