Expression of the Messenger RNAs for Luteinizing Hormone-releasing Hormone (LHRH) and Its Receptor in Human Ovarian Epithelial Carcinoma

Gabriele Irmer, Christiane Bürger, Rolf Müller, Olaf Ortmann, Ursula Peter, Sham S. Kakar, Jimmy D. Neill, Klaus-Dieter Schulz, and Günter Emons

Department of Obstetrics and Gynecology, Philipps-University, Marburg, Germany, and Department of Physiology and Biophysics [S. K., J. D. N.], University of Alabama at Birmingham, Birmingham, Alabama 35294

ABSTRACT

Recently we reported the presence of specific high affinity binding sites for luteinizing hormone-releasing hormone (LHRH) and its analogues (Kₐ = 1.5 or 1.7 nM) in the human epithelial ovarian cancer cell line EFO-21 and EFO-27. The proliferation of these cell lines was inhibited by nanomolar concentrations of a LHRH agonist. This study was performed to ascertain whether these ovarian cancer cell lines produce LHRH and whether the high affinity LHRH binding site found previously was identical to the pituitary LHRH receptor. Significant amounts of immunoreactive LHRH were found in the extracts of both the EFO-21 cell line (449 ± 56 fmol/10⁶ cells) and the EFO-27 line (409 ± 76 fmol/10⁶ cells). LHRH bioactivity of these extracts, assessed in terms of release of luteinizing hormone by rat pituitary cells, was comparable to that of authentic LHRH. EFO-21 and EFO-27 cells expressed the mRNAs for both human LHRH and human LHRH receptor as assessed by reverse transcriptase-PCR using oligonucleotide primers according to published sequences. In addition, in eight of eight biopsy samples of human epithelial ovarian cancers we detected mRNA for LHRH, six of these specimens expressed the mRNA representing the LHRH receptor. These data support the concept that human epithelial ovarian cancers might have a local system to regulate cell proliferation. It is still obscure at present whether LHRH produced locally has a stimulatory, inhibitory, or no effect on the proliferation of ovarian cancer cells. However, exogenous LHRH agonists seem to have a clear antiproliferative activity, probably mediated through the LHRH receptors. This finding might provide the basis for novel approaches in the therapy of epithelial ovarian cancer.

INTRODUCTION

Searching for new approaches for the endocrine therapy of epithelial ovarian cancer, we demonstrated that approximately 80% of these tumors express specific binding sites for LHRH⁵ and its agonistic and antagonistic analogues (1, 2). Thompson et al. (3) observed that in vitro proliferation of the human ovarian epithelial cancer cell line 2774 was retarded by high concentrations (1 μM, 100 μM) of the LHRH agonist leuprolide. We recently reported the presence of specific high affinity binding sites for LHRH and its analogues (Kₐ = 1.5 or 1.7 nM) in the human epithelial ovarian cancer cell lines EFO-21 and EFO-27 (4). The proliferation of both cell lines was time- and dose-dependent inhibited by the LHRH agonist [D-Trp⁶]LHRH and in the case of EFO-27 also by the two modern LHRH antagonists cetrorelix and ramorelix (4). In the EFO-27 cell line, both LHRH antagonists tested at concentrations from 10 pm to 10 μM had no effect on cell proliferation. Cetrorelix, however, partially antagonized the antiproliferative effect of the agonist [D-Trp⁶]LHRH in a dose-dependent manner in the EFO-27 cell line (4). Because the antiproliferative effects of LHRH analogues were significant at nm concentrations, we thought it reasonable to assume that they were mediated through the high affinity LHRH binding sites (4). The exact mechanism of action of this antiproliferative effect is still obscure. It is not known whether a putative endogenous ligand stimulates proliferation of the ovarian cancer cells through the high affinity binding site, which might be down-regulated by continuous treatment with a potent LHRH agonist or competitively blocked by LHRH antagonists. The findings obtained in the EFO-21 cell line uphold this view (4). An alternative hypothesis is that the putative LHRH receptor mediates direct antiproliferative effects of LHRH agonists. This speculation is supported by the findings in the EFO-27 line, where the agonistic LHRH analogue inhibited proliferation, while both LHRH antagonists alone were ineffective and cetrorelix partially antagonized the antiproliferative effect of [D-Trp⁶]LHRH (4).

Furthermore, it remained to be elucidated whether the high affinity LHRH binding site found in the EFO-21 and EFO-27 ovarian cancer cell lines is identical with the human pituitary LHRH receptor. The present study was undertaken to assess whether or not the ovarian cancer cell lines EFO-21 and EFO-27 express LHRH activity and the mRNAs for LHRH and its receptor. In addition, in order to extend the results obtained in cell lines to primary cancers, biopsy samples of human epithelial ovarian carcinoma obtained at surgery were checked for the expression of the respective mRNAs and LHRH binding sites.

MATERIALS AND METHODS

Cell Lines. The human ovarian cancer cell lines used were derived from a poorly differentiated serous adenocarcinoma (EFO-21; Ref. 10) or a mucinous papillary adenocarcinoma of intermediate differentiation (EFO-27; Ref. 11). Cells were cultured as described in detail previously (4).

Determination of LHRH Immunoreactivity. Confluent cells (32–42 × 10⁶) from either the EFO-21 or EFO-27 cell line were lysed by incubating with 5 ml of 0.1 M sodium hydroxide for 20 min at 20°C. After adjusting the pH to 7.4 with 1 M HCl, we removed cellular debris by centrifugation at 400 × g. Supernatants were analyzed in a double-antibody RIA for LHRH, using antibody K-29 (Ferring, Kiel, Germany) which is specific for the 6–10 sequence of the LHRH decapeptide at a final dilution of 1:18,000. 125I-labeled LHRH (specific activity, 200 Ci/mmole) was obtained from Amersham Corp. (Braunschweig, Germany) and LHRH came from Sigma (Deisenhofen, Germany). The second antibody (donkey anti-rabbit IgG) was purchased from IBL (Hamburg, Germany). The sensitivity of this RIA was 2 pg/tube and intraassay and interassay variances were 3 or 15%, respectively. Recovery of authentic LHRH added to lysates of fibroblasts, which expressed no LHRH, was 80–90%.

Determination of LHRH Bioactivity. Primary cultures of pituitary cells from adult female rats prepared and cultured as described previously (12, 13) were pretreated with 1 nm estradiol for 48 h to improve their sensitivity to LHRH (14) and then incubated for 3 h in the absence or presence of increasing concentrations of authentic LHRH (10 pm-10 nm) or different dilutions of the extracts of ovarian cancer cell lines EFO-21 or EFO-27 (see above). LH release from the gonadotrophs was measured by specific RIA using the reagents (reference preparation RP-3) and instructions kindly provided by the National...
Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (13, 14). These experiments were repeated with nearly identical results with extracts of three different passages of the ovarian cancer cell lines in three different rat pituitary cell preparations.

**Isolation of RNA.** Total RNA was prepared from cells or tissue samples by homogenizing in 4 ml guanidinoisothiocyanate and subsequent CsCl gradient centrifugation (15). The RNA pellets were suspended in 200 μl Tris (10 mM)-EDTA (1 mM) buffer (pH 7.5) and precipitated by adding 20 μl of 3 M sodium acetate (pH 6.0) and 440 μl ethanol.

**Reverse Transcription and PCR Amplification.** First-strand cDNA was obtained from 4 μg total RNA using p(dT)12 primers (Boehringer Mannheim, Mannheim, Germany) with Moloney murine leukemia virus reverse transcriptase according to the instructions of the manufacturer (GIBCO, Eggenstein, Germany). The cDNAs (~2 ng) were amplified in a 50 μl reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, 0.1 mg/ml gelatin, 200 μM of each of the deoxyribonucleoside triphosphates, and 1 μM of the appropriate primers in a Perkin Elmer/Cetus DNA thermal cycler 480 (Norwalk, CT). The primers used were: (a) for LHRH: sense 5'-GCAAGCCAGCAAGCTGTCG-3' (base pairs 904-924) and antisense 5'-GCAACTTGTGTAAGGAT-3' (base pairs 1478-1461) according to the sequence published for human LHRH (16); and (b) for the LHRH receptor: sense 5'-GCTTGAGCCCTCCCTGCGGA-3' (base pairs 31-51) and antisense 5'-CTAGGACATAGTAGGG-3' (base pairs 915-899) according to the sequence published for the human LHRH receptor (6). Thirty-five cycles of amplification were carried out: denaturation at 94°C for 1 min, annealing at 54°C for 1 min, followed by extension at 72°C for 1 min. The respective DNA products were run on 1 or 1.2% agarose gels and bands were visualized by ethidium bromide staining on an UV transilluminator.

**Restriction Enzyme Analysis.** PCR products were digested with the restriction endonucleases Drai and TaqI (for LHRH) and BamHI and PstI (for the LHRH receptor) under the conditions recommended by the manufacturer (Boehringer Mannheim). The digested products along with untreated aliquots of each PCR sample were then fractionated on 1.2% agarose gels and stained.

**Southern Blot and Hybridization.** PCR products, separated on 1-1.2% agarose gels, were visualized as described above and then transferred to Hybond-N+ membranes (Amersham Corp.) according to Southern (17). The blots were then hybridized with the established cDNA for human LHRH (base pairs 973-1353) (16) or the established cDNA for the human pituitary LHRH receptor (base pairs 1-1560) (6) using the ECL-direct nucleic acid labeling and detection system RPN 3000 (Amersham) according to the instructions of the manufacturer.

**LH Radioreceptor Assay.** Plasma membranes were prepared from biopsy specimens of human epithelial ovarian cancers or other intraabdominal tumors obtained at surgery according to (1, 2, 4) and then subjected to a radioreceptor assay using [125I]-LHRH as radioligand, as recently described (see "Materials and Methods") yielding the expected 574-base pair product (Fig. 3). As a positive control, the human breast cancer cell line MCF-7 was used. LHRH mRNA was also found in the abdominal metastasis of a solid undifferentiated breast cancer (sample 8), whereas no signal was obtained from a benign ovarian cystadenoma (sample 10) (Fig. 3). PCR amplification of the cDNAs derived from the two ovarian cancer cell lines and the eight samples from epithelial ovarian cancers using the oligonucleotide primers for the human LHRH receptor (see "Materials and Methods") yielded the expected 884-base pair product in both cell lines and in six of the tumor samples analyzed (Fig. 4). As a positive control, the human breast cancer cell line MCF-7 was used (6). The breast cancer metastasis studied (sample 8) also expressed the mRNA for the human LHRH receptor, whereas no signal was found in the benign ovarian cystadenoma (sample 4). Cleavage of the 574-base pair PCR product (LHRH) with restriction enzymes yielded the expected fragments of 447 base pairs and 127 base pairs (Drai) and 352 base pairs and 222 base pairs (TaqI) (data not shown). Cleavage of the 884-base pair PCR product (LHRH receptor) with restriction endonucleases yielded the expected fragments of 517 base pairs and 367 base pairs (BamHI) and 625 base pairs and 259 base pairs (PstI) (data not shown). The sequence of the PCR products was further confirmed by Southern blotting and subsequent hybridization with the established cDNAs for human LHRH (16) and for human pituitary LHRH receptor (Ref. 6; Figs. 3 and 4).

**RESULTS**

Human ovarian cancer cells of the EFO-21 line contained 591 ± 73 pg (449 ± 56 fmol) LHRH immunoreactivity/10⁶ cells, while those of the EFO-27 line contained 538 ± 99 pg (409 ± 76 fmol)/10⁶ cells (mean ± SE of 4 experiments in 4 different passages of the cell lines). Serial dilutions of the extracts of EFO-21 or EFO-27 cells produced inhibition curves of binding of [125I]-labeled LHRH to the LHRH antisem which were parallel to the standard curve obtained with authentic LHRH (Fig. 1). The biological activity of the LHRH immunoreactive extracts from EFO-21 or EFO-27 cells was assessed in the rat pituitary cell culture system, which responds to LHRH by a dose-dependent release of LH (12, 14). Exposure of pituitary cell cultures to increasing amounts of extracts of EFO-21 or EFO-27 ovarian cancer cells resulted in a dose-dependent release of LH by the pituitary cells (Fig. 2). In these experiments, we found the bioactivity of the immunoreactive LHRH material in the extracts of EFO-21 and EFO-27 to be comparable to that of authentic LHRH (Fig. 2).

PCR amplification of cDNA from EFO-21 and EFO-27 cell lines as well as from eight biopsy samples from human epithelial ovarian cancers (Table 1) with the oligonucleotide primers for human LHRH (see "Materials and Methods") yielded the expected 574-base pair product (Fig. 3). As a positive control, human term placenta was used. LHRH mRNA was also found in the abdominal metastasis of a solid undifferentiated breast cancer (sample 8), whereas no signal was obtained from a benign ovarian cystadenoma (sample 10) (Fig. 3). PCR amplification of the cDNAs derived from the two ovarian cancer cell lines and the eight samples from epithelial ovarian cancers using the oligonucleotide primers for the human LHRH receptor (see "Materials and Methods") yielded the expected 884-base pair product in both cell lines and in six of the tumor samples analyzed (Fig. 4). As a positive control, the human breast cancer cell line MCF-7 was used (6). The breast cancer metastasis studied (sample 8) also expressed the mRNA for the human LHRH receptor, whereas no signal was found in the benign ovarian cystadenoma (Fig. 4). Cleavage of the 574-base pair PCR product (LHRH) with restriction enzymes yielded the expected fragments of 447 base pairs and 127 base pairs (Drai) and 352 base pairs and 222 base pairs (TaqI) (data not shown). Cleavage of the 884-base pair PCR product (LHRH receptor) with restriction endonucleases yielded the expected fragments of 517 base pairs and 367 base pairs (BamHI) and 625 base pairs and 259 base pairs (PstI) (data not shown). The sequence of the PCR products was further confirmed by Southern blotting and subsequent hybridization with the established cDNAs for human LHRH (16) and for human pituitary LHRH receptor (Ref. 6; Figs. 3 and 4).
DISCUSSION

Our data demonstrate that human ovarian cancer cell lines EFO-21 and EFO-27, the proliferation of which is inhibited by LHRH agonists (4), probably through high affinity binding sites (4), express LHRH immunoactivity and bioactivity as well as the mRNA for LHRH. Also eight of eight randomly collected ovarian cancer biopsies expressed the mRNA for LHRH. The finding that no LHRH mRNA was expressed by a benign ovarian cystadenoma supports the specificity of our methods. It is true that our data on LHRH immunoactivity and bioactivity only permit the conclusion that LHRH-like activity is produced by the EFO-21 and EFO-27 cell lines. Further purification and the elucidation of the amino acid sequence are necessary to provide a conclusive evidence on the peptide level. But taken together with our findings on LHRH mRNA expression, our data suggest that LHRH is expressed by the ovarian cancer cell lines studied. This assumption is further corroborated by a recent report by Ohno et al. (19). Using a different LHRH antibody and a different LHRH bioassay (LHRH induced inositol phosphate production in rat granulosa cells), these authors demonstrated LHRH immunoactivity and bioactivity in the SK-OV3 human ovarian cancer cell line and in two ovarian cancer biopsy samples (19). Using oligonucleotide primers different from ours, Ohno et al. (19) also detected mRNA for LHRH. Thus far, however, to the best of our knowledge, no data on LHRH receptors or antiproliferative effects of LHRH analogues in ovarian cancer have been published by this group.

Our results on the expression of the mRNA for the human LHRH receptor support the concept that the high affinity LHRH binding site previously found in the EFO-21 and EFO-27 cell lines might be identical to or at least closely similar to the human pituitary LHRH receptor. This assumption is further corroborated by the finding that, with one exception, those ovarian cancer biopsy samples expressing the mRNA for the LHRH receptor also had high affinity binding sites for LHRH. Similarly the two samples not expressing LHRH receptor mRNA also exhibited no specific binding of the labeled LHRH agonist. The latter observation and the result that the benign cystadenoma expressed no mRNA for the LHRH receptor supports the notion that most, but not all ovarian cancers express LHRH receptors and that our methods are specific. To the best of our knowledge, this is the first demonstration of the expression of mRNA for the human LHRH receptor in ovarian cancer cell lines and biopsy samples.

It is of interest that also the abdominal breast cancer metastasis expressed the mRNAs for both, LHRH and its receptor, and exhibited high affinity LHRH binding, which is in agreement with the assumption that a large percentage of breast cancers might have a local regulatory system based on LHRH (5–7, 20).

In the present and in our previous article (4) we have demonstrated the existence of several components essential for a local regulatory system based on LHRH in the human ovarian cancer cell lines EFO-21 and EFO-27: expression of LHRH, LHRH receptors, and antiproliferative activity of LHRH agonists. Several points, however, have to be elucidated, before the existence of an LHRH based autocrine-paracrine regulatory system can be accepted:

(a) One would expect to find LHRH immunoactivity or bioactivity in the culture media of the ovarian cancer cells. Despite several efforts we were not able to detect LHRH activity in the media conditioned by EFO-21 or EFO-27 ovarian cancer cells (data not shown). Also Ohno et al. (19) reported LHRH activity only in extracts of the SK-OV3 cells but not in conditioned media. In contrast, Qayyum et al. (21) detected LHRH immunoactivity in the media, conditioned by the prostatic cancer cell lines DU 145 and LNCaP, which, however, was present only in very low amounts (8–12 fmol/million cells). One reason for the inability of Ohno’s (19) and our group to detect LHRH

Table 1 Characteristics of the epithelial ovarian cancers and other intraabdominal tumors analyzed

<table>
<thead>
<tr>
<th>Sample</th>
<th>Histology</th>
<th>Grade</th>
<th>Specific binding of [125I]-labeled [D-Trp6]LHRH (Kd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Partly cystic, partly papillary ovarian carcinoma</td>
<td>2</td>
<td>No binding</td>
</tr>
<tr>
<td>2</td>
<td>Solid, papillary ovarian carcinoma</td>
<td>3</td>
<td>88 nM</td>
</tr>
<tr>
<td>3</td>
<td>Partly papillary ovarian carcinoma</td>
<td>2</td>
<td>2.7 nM</td>
</tr>
<tr>
<td>4</td>
<td>Serous papillary ovarian carcinoma</td>
<td>2</td>
<td>1.8 nM</td>
</tr>
<tr>
<td>5</td>
<td>Papillary ovarian carcinoma</td>
<td>2</td>
<td>410 nM</td>
</tr>
<tr>
<td>6</td>
<td>Papillary ovarian carcinoma</td>
<td>1</td>
<td>47 nM</td>
</tr>
<tr>
<td>7</td>
<td>Partially solid, partly papillary ovarian carcinoma</td>
<td>3</td>
<td>No binding</td>
</tr>
<tr>
<td>8</td>
<td>Omental metastasis of an invasive ductal mammary carcinoma</td>
<td>3</td>
<td>1.3 nM</td>
</tr>
<tr>
<td>9</td>
<td>Papillary ovarian adenocarcinoma</td>
<td>2</td>
<td>No material for binding assay</td>
</tr>
<tr>
<td>10</td>
<td>Serous cystadenoma of the ovary</td>
<td></td>
<td>No material for binding assay</td>
</tr>
</tbody>
</table>

*Representative portions of the tumors were snap frozen at —72°C immediately after their surgical removal and stored at —80°C until analysis.

*Specific binding of [125I]-labeled [D-Trp6]LHRH was determined in crude membranes prepared from homogenized tumor samples by centrifugation at 70,000×g in the absence or presence of unlabeled [D-Trp6]LHRH (10 pm–100 pm) as previously described in detail (4). Binding data were analyzed with the Ligand program (18).
imunoactivity in the culture media of the human ovarian cancer cell lines SK-OV3, EFO-21, and EFO-27 might be the lack of sensitivity of our RIA systems and an insufficient concentration procedure used by us. Assuming that 1,000,000 cells of the EFO-21 or EFO-27 line, respectively, which contain approximately 400 fmol of LHRH immunoactivity, secrete only 8–12 fmol of this activity into the culture medium as described by Qayum et al. (21) for the prostate cancer cell lines, we would need at least a 40-fold concentration of the culture media before a reliable measurement of immunoactivity and bioactivity becomes possible. Limonta et al. (9) have recently speculated that LHRH or LHRH-like activity might be released by the LNCaP prostatic cancer cell line only under certain culture conditions, i.e., in the absence of steroids (9). Systematic studies with the EFO-21 and EFO-27 cell lines should clarify whether different culture conditions (absence or presence of fetal serum, phenol red, defined growth factors, steroids, etc.) have an influence on the secretion of LHRH.
activity. It might also be speculated that LHRH activity is secreted by the ovarian cancer cells periodically and rapidly degraded in the culture medium, thus escaping detection by the present approach. Furthermore, taking into account that all eight ovarian cancer biopsy samples studied by us and the two samples described by Ohno et al. (19) express LHRH mRNA, one might hypothesize that in vivo ovarian cancer cells produce and secrete LHRH activity, while the ovarian cancer cell lines studied thus far in vitro produce LHRH but do not secrete it. Therefore it is necessary to study other established ovarian cancer cell lines and primary cultures of ovarian cancer cells which might have retained the capacity of secreting significant amounts of LHRH activity.

(b) It remains obscure whether the LHRH activity produced by ovarian cancers has stimulatory or inhibitory or no effects on the proliferation of these tumors. If LHRH produced by the ovarian cancer cells had a stimulatory effect on their proliferation, it could be expected that low concentrations of an LHRH agonist might also be mitogenic and only higher concentrations of this analogue have antiproliferative effects mediated through receptor down-regulation. In our previous study we found that concentrations of ≥1 nm of the LHRH agonist [D-Trp⁶]LHRH significantly inhibited the proliferation of the ovarian cancer cell lines EFO-21 and EFO-27 (4). At a concentration of 10 pm this agonist slightly, but significantly inhibited the proliferation of the EFO-21 cells and had no or a marginal inhibitory effect on the proliferation of the EFO-27 line (4). These findings might be interpreted as an evidence for a general antiproliferative activity of LHRH in the ovarian cancer cell lines studied. However, it is also possible that even 10 pm concentrations of the potent LHRH agonist [D-Trp⁶]LHRH lead to a down-regulation of LHRH receptors under the experimental conditions used (5 days of culture). Studies using short incubation periods with low concentrations (10 fm-1 nm) of native LHRH are presently performed in our laboratory to address this problem. Having demonstrated the expression of mRNA for the LHRH receptor in the EFO-21 and EFO-27 cell lines, we shall also investigate the regulation of LHRH receptor expression by different treatment protocols with native LHRH and agonistic and antagonistic (see below) analogues.

Limonata et al. (9) have recently shown that the proliferation of the prostatic cancer cell line LNCaP was stimulated by long-term treatment (7-15 days) with a LHRH antagonist (Nal-Arg-LHRH, 10 nm). They interpreted this finding as an antagonization of the antiproliferative effect of the LHRH activity produced by these tumor cells and as possible evidence for the hypothesis that LHRH produced locally by the LNCaP cells inhibits their proliferation (9). In our previous study (4), we found that two potent LHRH antagonists (cetrorelix, ramorelix) had no stimulatory, but clear inhibitory effects on the proliferation of the EFO-21 cell line. These antiproliferative effects were dose dependent and significant at agonist concentrations ≥10 pm (4). These findings might be interpreted as evidence for a stimulatory effect of locally produced LHRH. It cannot be ruled out, however, that the LHRH binding sites of the EFO-21 cells mediate direct antiproliferative effects of LHRH antagonists. This means that the LHRH antagonists behaved as an agonist in the antiproliferative function. This hypothesis is supported by the results of Levy et al. (22) that LHRH antagonists can also stimulate increases of intracellular Ca²⁺ and inositol phospholipid turnover in rat pituitary and human pituitary adenomas. In two endometrial cancer cell lines (HEC-1A and Ishikawa), we recently found similar dose-dependent antiproliferative effects of both the LHRH antagonist cetrorelix and the LHRH agonist [D-Trp⁶]LHRH (23), a result supporting the above hypothesis.

In contrast, in the EFO-27 ovarian cancer cell line the LHRH antagonists cetrorelix and ramorelix had no effects on proliferation at the wide range of concentrations tested (10 pm-10 μM) while the LHRH agonist [D-Trp⁶]LHRH exhibited dose-dependent antiproliferative effects, which could be partially antagonized by the LHRH antagonist cetrorelix (4). These data might be interpreted as evidence against a functional role of locally produced LHRH in the regulation of proliferation of this cell line, indicating that only exogenous LHRH agonists are able to exert antiproliferative effects.

In the prostatic cancer cell line LNCaP, Limonta et al. (8) originally described antiproliferative activity of high concentrations (1 μM) of the LHRH antagonist [D-Trp⁶]LHRH, a finding which is in contrast to the stimulatory effects of this antagonist on the proliferation of this cell line observed in a later study by this group (9). As mentioned above, Limonta et al. (9) explain this discrepancy by different culture conditions (absence or presence of steroids). In addition, in several human breast cancer cell lines, dose-dependent antiproliferative effects of LHRH antagonists have been observed (for review, see Refs. 5 and 7). It appears probable that the mechanism of action of LHRH antagonists is not uniform, but that individual response patterns exist in different cancer cell lines and under different culture conditions. Therefore results obtained with LHRH antagonists have to be interpreted cautiously and in our opinion give no clear answer to the question whether LHRH produced locally in tumor cells stimulates or inhibits cell proliferation.

As mentioned above, experiments using lower concentrations of native LHRH and different incubation times might clarify this issue. Also the use of antibodies to LHRH should be helpful to answer this question. Respective experiments are presently performed in our laboratory.

Even if LHRH expressed by the ovarian cancer cell lines EFO-21 and EFO-27 should have no functional importance in the regulation of proliferation of these cells, exogenous LHRH agonists clearly inhibit their proliferation (4). The present demonstration that these cell lines express the mRNA for the human LHRH receptor supports the concept that these antiproliferative effects are mediated through the high affinity LHRH binding sites (4) which now might deserve the term "LHRH receptor." In addition, the majority of the ovarian cancer biopsies studied expressed high affinity LHRH binding and mRNA for the human LHRH receptor. Therefore, LHRH agonists could theoretically also reduce the proliferation of ovarian cancer in vivo. A further elucidation of the mechanism through which LHRH analogues reduce proliferation of ovarian cancer cells is important for the clinical use of these drugs since a better understanding of their mechanism of action would accelerate the development of an efficacious therapeutic regimen, exploiting their direct antitumor effects (7). Clinical trials, evaluating the impact of suppression of endogenous gonadotrophins by conventional LHRH agonists have thus far only shown marginal activity of this approach in ovarian cancer (for review, see Ref. 7). Therefore basic and clinical research on the direct antiproliferative effects of LHRH analogues on ovarian cancer should be intensified. Since present methods for therapy of ovarian cancer are unsatisfactory (for review, see Ref. 24), a successful development of a nontoxic endocrine therapy based on LHRH analogues would represent a relevant advance.

It might be argued that the data obtained in ovarian cancer cell lines 2774 by Thompson et al. (Ref. 3, antiproliferative effect of LHRH agonist), SK-OV3 by Ohno et al. (Ref. 19, expression of LHRH activity and LHRH mRNA) EFO-21 and EFO-27 by our group (Ref. 4 and present article, high affinity LHRH binding sites, production of LHRH activity, expression of the respective mRNAs, antiproliferative effects of LHRH agonist) are not representative for ovarian cancer in vivo. Since the above data were independently obtained in three different laboratories using different cell lines and experimental conditions, their basic congruence rather supports the concept of a possible role of LHRH in the
regulation of proliferation of ovarian cancer, at least of a pharmacological antiproliferative activity of LHRH analogues. In addition, the fact that Ohno et al. (19) and we (present article) have demonstrated expression of LHRH activity and LHRH mRNA in a variety of ovarian cancer biopsies, and our present demonstration of high affinity LHRH binding sites and expression of mRNA for the human LHRH receptor in the majority of our ovarian cancer samples suggests that the data obtained in ovarian cancer cell lines might be of relevance for the in vivo situation.

In the present article and in a recent article (4) we demonstrated that the human ovarian cancer cell lines EFO-21 and EFO-27 express LHRH immunoactivity and bioactivity, the mRNA for LHRH, high affinity LHRH binding sites as well as the mRNA for the human LHRH receptor. The proliferation of these cell lines is dose-dependent inhibited by a LHRH agonist. Although many questions remain to be answered, it might be assumed that in these ovarian cancer cell lines exists a local regulatory system based on LHRH. In addition, we could demonstrate for the first time the expression of mRNA for the human LHRH receptor and corresponding high affinity LHRH binding sites in ovarian cancer biopsies. Taking into account a recent report by Ohno et al. (19) on the expression of LHRH immunoactivity and bioactivity as well as of LHRH mRNA in two ovarian cancer biopsies, a finding which has now been confirmed by us in another eight ovarian carcinoma samples, it seems reasonable to speculate that LHRH might act as a local regulator of epithelial ovarian cancer.

ACKNOWLEDGMENTS

We thank Dr. P. H. Seeburg, Zentrum für Molekularbiologie, Heidelberg, Germany, for the gift of the cDNA for human LHRH. We are grateful to Ferring Arzneimittel, Kid, Germany, for supplying the LHRH antiserum and Dr. M. Krause, Institute for Molecular Biology and Tumor Research, Marburg, Germany, for the preparation of the oligonucleotide primers.

REFERENCES

Expression of the Messenger RNAs for Luteinizing Hormone-releasing Hormone (LHRH) and Its Receptor in Human Ovarian Epithelial Carcinoma

Gabriele Irmer, Christiane Bürger, Rolf Müller, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/55/4/817