International Congress on Hormonal Steroids and Hormones and Cancer

Inhibitory activity of luteinizing hormone-releasing hormone on tumor growth and progression

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Abstract

Luteinizing hormone-releasing hormone (LHRH) is the key hormone in the control of reproductive functions. In recent years, it has become evident that LHRH might act as a growth modulatory factor in tumors of the reproductive system. We have shown that in prostate cancer cells LHRH is expressed, together with its receptors, to negatively regulate cell proliferation. In these cells, LHRH acts as an antimitogenic factor through the activation of the Gi-cAMP intracellular signaling pathway. More recently, we investigated whether an LHRH-based autocrine system might also be expressed in tumors that are not classically related to the reproductive tract, such as melanoma. Malignant melanoma is known to be characterized not only by a high proliferation rate, but also by an aggressive metastatic behavior. We have demonstrated that both LHRH and LHRH receptors are expressed in human melanoma cells (BLM and Me15392). Activation of LHRH receptors by means of a potent LHRH agonist (Zoladex) significantly inhibited cell proliferation. The LHRH agonist also reduced the ability of melanoma cells to invade a reconstituted basement membrane (Matrigel) and to migrate in response to a chemotactic stimulus. These data indicate that: (a) in prostate cancer cells the LHRH receptor is coupled to a Gi-cAMP signal transduction pathway; (b) LHRH and LHRH receptors are also expressed in tumors that are not classically related to the reproductive system, such as melanoma; in melanoma cells, LHRH might act as an inhibitory factor on both cell proliferation and metastatic behavior. It is suggested that, in melanoma, LHRH receptors might represent a diagnostic marker and a possible molecular target for new therapeutic approaches for this pathology.

Endocrine-Related Cancer (2003) **10** 161–167

Introduction

Luteinizing hormone-releasing hormone (LHRH) is the hypophysiotropic decapeptide synthesized in the hypothalamus that plays a crucial role in the control of reproductive functions (Fink 1988, Shupnik 1996). The human LHRH gene, which is organized into four exons and three introns, is located on chromosome 8 (Fernald & White 1999). The peptide is synthesized in neurons of the hypothalamus as a pre-pro-hormone before being processed to the mature hormone which is then released into the portal system of the median eminence. Through the portal vessels, the hormone reaches the anterior pituitary where it binds to specific receptors on the gonadotropes to regulate gonadotropin synthesis and secretion (Fink 1988, Shupnik 1996). LHRH and the two

gonadotropins (luteinizing hormone, LH; follicle stimulating hormone, FSH) are secreted in a pulsatile way, and this pulsatility is crucial for the maintenance of the functions of the pituitary–gonadal axis (Kalra 1993). Pulsatile administration of LHRH in patients with hypothalamic dysfunctions has been shown to induce a regular pattern of LH and FSH secretion, thus restoring the fertility capacity (Conn & Crowley 1994, Schally 1994). On the other hand, chronic applications of LHRH or LHRH analogs lead to a complete suppression of reproductive function, due to LHRH receptor down-regulation and desensitization (Schally 1994, Stojilkovic & Catt 1995). This mechanism of action represents the molecular basis for the treatment of hormone-dependent pathologies such as breast and prostate cancer

(Crawford *et al.* 1995, Lippman 1998, Green & Furr 1999, Manni 1999).

The pituitary LHRH receptor has been cloned and characterized (Kakar *et al.* 1992). In humans, its gene is located on chromosome 4 and is organized into three exons and two introns (Kakar *et al.* 1992). This gene encodes a 328 amino acid protein belonging to the superfamily of seven transmembrane domain receptors (Kakar *et al.* 1992, Stojilkovic *et al.* 1994). The series of events that follows the binding of the neurohormone to its receptor has been widely investigated. It is now clear that the pituitary LHRH receptor is coupled to a Gq/11 protein (Naor *et al.* 1998, Kraus *et al.* 2001). Phospholipase (PL) Cβ is the primary effector in the intracellular cascade of events triggered by LHRH; the generated second messengers inositol trisphosphate and diacylglycerol lead to the activation of protein kinase C and to the mobilization of intracellular pools of Ca^{2+} (Naor *et al.* 1998). LHRH also activates PLA_2, PLD, and the MAP kinase cascade, which provides an important link for the transmission of signals from the cell surface to the nucleus and plays a role in the regulation of gonadotropin transcription (Naor *et al.* 1998, Kraus *et al.* 2001).

Recently, a gene coding for a second form of LHRH (which is called LHRH-II) has been identified in several placental mammals, including humans (White *et al.* 1998). The LHRH-II peptide is identified as [His^5-Trp^7-Tyr^8]-LHRH, indicating that the amino acid sequence presents three substitutions in these three positions. LHRH-II is mainly present in the midbrain, but it is also widely expressed in peripheral tissues such as prostate, bone marrow, and kidney (White *et al.* 1998).

The demonstration of the presence of two forms of LHRH suggests specific functions for each peptide. This observation prompted different authors to investigate the possible existence of multiple LHRH receptors. These studies led to the identification of an LHRH-type II receptor in monkeys. This receptor is 96% identical with a putative human LHRH-type II receptor, which is composed of 379 amino acids (Neill 2002). The functions of LHRH-II and its putative receptor are still under investigation (White *et al.* 1998, Neill 2002).

LHRH in prostate cancer

LHRH and LHRH receptors have been reported to be expressed also in some peripheral tissues, particularly in those related to the endocrine system. In our laboratory we investigated whether LHRH and LHRH receptors might be present in prostate cancer, and whether they might be involved in the control of tumor growth. These studies have been performed in either androgen-dependent (LNCaP) or androgen-independent (DU 145) human prostate cancer cell lines.

By RT-PCR we have shown first that the mRNA for LHRH is expressed in both LNCaP and DU 145 cells (Limonta *et al.* 1993, Dondi *et al.* 1994). In these cell lines we have also demonstrated the presence of the mRNA coding for the LHRH receptor, which appeared to be identical in its sequence to that found in the pituitary (Dondi *et al.* 1994). By Western blot analysis and by using a specific monoclonal antibody raised against the human pituitary receptor (Karande *et al.* 1995), we have further been able to demonstrate that a protein band of approximately 64 kDa is present in membrane preparations of prostate cancer cells. This molecular weight corresponds to that previously reported for the LHRH receptor at the pituitary level in humans (Karande *et al.* 1995).

Taken together, these data demonstrated that an LHRH-based system is expressed in prostate cancer, which might participate in the local regulation of tumor growth. This hypothesis has been confirmed by showing that the activation of locally expressed LHRH receptors by means of potent LHRH superagonists significantly reduces the proliferation of prostate cancer cells both *in vitro* (Limonta *et al.* 1992, Dondi *et al.* 1994) and *in vivo* (Dondi *et al.* 1998).

On the basis of these results we have concluded that, in either androgen-dependent or androgen-independent prostate cancer, the local LHRH system might act as a paracrine/autocrine negative regulator of tumor growth. These observations are in agreement with those reported for tumors of the female reproductive tract, such as breast (Keri *et al.* 1991, Kakar *et al.* 1994, Kottler *et al.* 1997), endometrial (Imai *et al.* 1994, Chatzaki *et al.* 1996), and ovarian (Yin *et al.* 1998, Emons *et al.* 2000) cancer.

These data seem to suggest that, when utilized for the treatment of hormone-related tumors, LHRH agonists might also exert a direct inhibitory action at the level of the tumor tissue. Moreover, LHRH analogs should be considered for a possible treatment of prostate cancer, in its androgen-independent stage.

Mechanism of action of LHRH

At the pituitary level, LHRH receptors mediate the stimulatory action of the neuropeptide on gonadotropin synthesis and secretion. As mentioned in the Introduction, these receptors are coupled to the Gq-PLC signal transduction pathway. In cancer cells and tissues, activation of LHRH receptors brings about a significant inhibition of cell proliferation. Therefore, we hypothesized that the signal transduction pathway coupled to the LHRH receptor in prostate cancer might be different to that described for the pituitary receptor.

In preliminary experiments, we observed that the treatment of prostate cancer cells, either androgen-dependent or androgen-independent, with LHRH agonists does not affect phosphoinositide turnover or intracellular Ca^{2+} levels (RM Moretti, M Montagnani Marelli & P Limonta, unpublished

observations). This suggested to us that, in these cells, LHRH receptors might not be coupled to the Gq/11-PLC system. Therefore, we considered that the antiproliferative action of LHRH agonists might be mediated by the Gi-cAMP signal transduction pathway. It is well known that pertussis toxin (PTX), through ADP-ribosylation of Gi proteins, impairs the receptor–effector interaction in this pathway (Ui & Katada 1990). Therefore, we investigated whether PTX might interfere with the antimitogenic action of a potent LHRH agonist (LHRH-A; Zoladex (AstraZeneca)). As expected, LHRH-A significantly inhibited prostate cancer cell growth; the antiproliferative action of the LHRH agonist was completely prevented by PTX, thus suggesting that the receptor might be coupled to a Gi protein. To verify this hypothesis, we analyzed whether LHRH-A might affect PTX-induced ADP-ribosylation of this protein. Incubation of prostate cancer cell membranes with PTX, in the presence of ^{32}P-NAD, brought about ADP-ribosylation of a 41-kDa Gi protein. LHRH-A substantially counteracted the transfer of ^{32}P-ADP-ribose to this protein. To definitely confirm that in prostate cancer cells LHRH receptors might be coupled to Gi proteins, we studied the effects of LHRH-A on forskolin-induced cAMP accumulation; it is actually well known that the Gi subunit is negatively correlated with cAMP production. We found that, in tumor cells, LHRH-A did not affect cAMP levels when given alone. However, the LHRH analog completely counteracted the increase in cAMP levels induced by forskolin.

Taken together, these results indicate that, in prostate cancer cells, the LHRH receptor is linked to a Gi protein which, through inhibition of cAMP accumulation, may mediate the antiproliferative action of the peptide. These observations are in line with those reported for the LHRH receptor in ovarian and endometrial carcinoma (Imai *et al.* 1996, Imai & Tamaya 2000).

The molecular mechanisms which follow Gi activation to lead to the inhibition of cancer cell proliferation are still poorly understood. In previous studies, we have reported that LHRH agonists counteract the mitogenic stimuli of growth factors, such as epidermal growth factor or insulin-like growth factor (Moretti *et al.* 1996, Montagnani Marelli *et al.* 1999). These LHRH agonists seem to interfere with some of the intracellular events activated by the growth factors, such as receptor expression or receptor tyrosine phosphorylation (Moretti *et al.* 1996, Montagnani Marelli *et al.* 1999). It is then possible that, after the activation of the Gi protein, the reduced levels of cAMP might directly affect the intracellular mechanisms which mediate mitogenic stimuli in cancer cells (Fig. 1).

LHRH in melanoma

In a recent paper, it has been reported that LHRH binding sites are present in glioblastoma biopsies and it has been suggested that they might represent a diagnostic marker, and
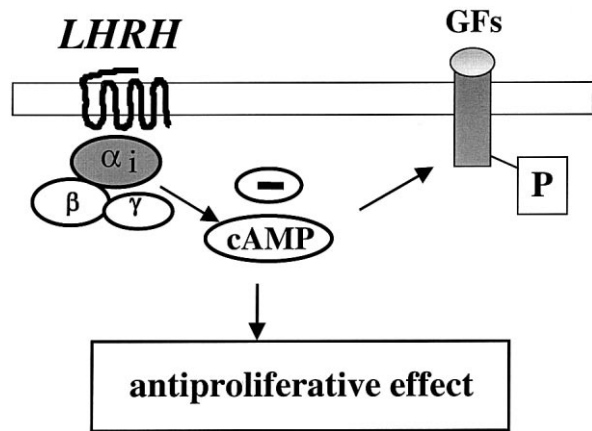

Figure 1 Schematic representation of the suggested intracellular transduction pathway linked to the LHRH receptor in tumor cells. GFs, growth factors.

possibly a new therapeutic target, for nervous system tumors (van Groeninghen *et al.* 1998). Since both glial cells and melanocytes share the same neuroectodermal origin, we reasoned that an LHRH-based system (LHRH and LHRH receptors) might also be expressed in melanoma cells.

Cutaneous melanoma is a tumor known for its uncontrollable growth and for its ability to give rise to metastases (MacKie 1998). The incidence of this tumor is increasing dramatically (Parkin *et al.* 1999) and although its prognosis has improved in the last decades particularly due to early diagnosis, this remains very poor in advanced cases when tumor cells acquire a strong potential to disseminate metastases (MacKie 1998). Moreover, advanced melanoma is a multistep process, which starts from the initial transformation of melanocytes, then goes through a radial growth phase, to eventually progress to the vertical growth phase (Lazar-Molnar *et al.* 2000). It is particularly in this phase that tumor cells start giving rise to metastases (Shih & Herlyn 1993).

The molecular mechanisms that are involved in the growth and progression of melanoma are still poorly understood. The experiments here described have been performed to clarify whether an LHRH-based system (LHRH and the respecitve receptors) is expressed in melanoma cells. We have also investigated whether the activation of this system might affect the proliferative rate as well as the metastatic properties of this tumor. For these studies we utilized two human melanoma cell lines: BLM and Me15392 (kindly provided by Dr Van Muijen, Department of Pathology, University Hospital, Nijmegen, The Netherlands and by Dr Parmiani, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy respectively). LHRH receptor activation has been achieved by using the potent LHRH-A Zoladex.

Table 1 Characteristics of ^{125}I-LHRH-A binding to human melanoma cell membranes

	Dissociation constant (K_d) (nM)	^{125}I-LHRH-A binding capacity (fmoles/mg protein)
BLM cells	0.7–1.1	150–200
Me15392 cells	0.1–0.6	200–250
Rat pituitaries	1.5–2.0	70–100

Expression of LHRH and LHRH receptors

The expression of both LHRH and LHRH receptors in melanoma cells has been investigated by RT-PCR, as described above for the studies performed in prostate cancer cells. With regard to the expression of LHRH, according to the sequence of the oligonucleotide primers used, the predicted fragment of 228 bp was observed in both BLM and Me15392 cells. After Southern blotting, the cDNA fragments hybridized with the ^{32}P-labeled oligonucleotide probe specific for the LHRH cDNA (Oikawa *et al*. 1990). In the case of the expression of the LHRH receptor mRNA, the results obtained demonstrate that the predicted 885-bp cDNA fragment is present both in BLM and in Me15392 cells; this band hybridized with the ^{32}P-labeled probe specific for the LHRH receptor cDNA (Kakar *et al*. 1994). The presence of LHRH receptors in melanoma cells has been further investigated at the protein level by Western blot and by using the specific F1G4 antibody raised against the human pituitary receptor (Karande *et al*. 1995). The results obtained demonstrate that a protein of approximately 64 kDa molecular mass is present in membrane preparations from both BLM and Me15392 cells. This molecular weight corresponds to that previously reported for the human pituitary receptor (Wormald *et al*. 1985). Finally,

LHRH receptors in melanoma cells have been analyzed also in terms of binding parameters. Radioreceptor assays have been performed using ^{125}I-LHRH-A as the specific ligand (Limonta *et al*. 1992, Dondi *et al*. 1994). The assays demonstrated that binding sites for ^{125}I-LHRH-A are present on the membranes of both BLM and Me15392 cells. Computer analysis of the data obtained from displacement curves indicated the presence of a single class of high affinity binding sites (K_d in the nM range) in both melanoma cell lines, as well as in rat pituitaries used as controls (Table 1).

So far, divergent results have been reported for the binding characteristics of LHRH receptors in cancers related to the reproductive tract (Limonta *et al*. 1992, Emons *et al*. 1993, Dondi *et al*. 1994, Emons & Schally 1994, Imai *et al*. 1994). The data here described indicate that, in melanoma cells, LHRH receptors can bind LHRH analogs with a high affinity.

Antiproliferative activity of LHRH

The observation that both LHRH and LHRH receptors are expressed in melanoma cells prompted us to investigate the possible role played by this LHRH-based system in the
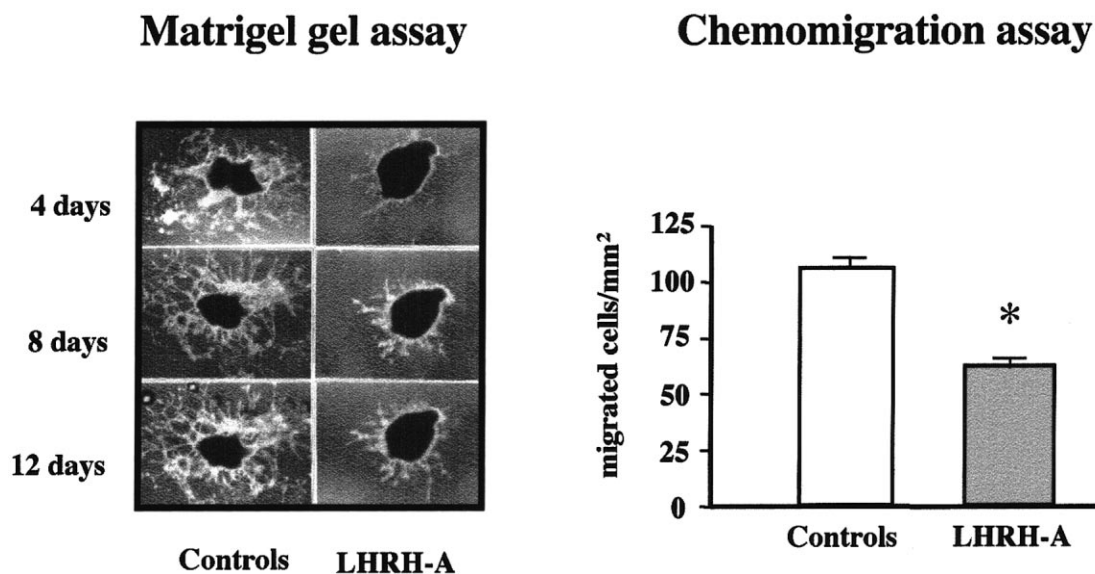

Figure 2 Effects of the LHRH agonist (LHRH-A) Zoladex on the invasive (Matrigel assay) and on the migratory (Chemomigration assay) properties of melanoma cells. *$P < 0.05$ compared with Controls.

control of melanoma growth. Melanoma cells have been treated daily, for 7 days, with LHRH-A (10^{-11}–10^{-6} M). The treatment resulted in a significant and dose-dependent inhibition of cell proliferation, with an EC_{50} dose in the nM range. This antimitogenic activity was found to be specific since it was completely counteracted by the simultaneous treatment of the cells with an LHRH antagonist (Antide).

As in the case of other tumors, the development of melanoma has been found to be related to a decreased dependency on external mitogenic stimuli (Halaban 1996) as well as to an increased expression of locally produced growth factors (Lu & Kerbel 1994, Shih & Herlyn 1994). The data here reported indicate that, in addition to stimulatory growth factors, melanoma cells might express an LHRH-based inhibitory system. Activation of this system by means of LHRH agonists may reduce tumor growth, possibly by interfering with the remaining positive effect of the mitogenic stimuli.

Antimetastatic activity of LHRH

Experiments have been performed to verify whether the activation of locally expressed LHRH receptors might also affect the metastatic behavior of melanoma cells. In a first series of experiments, we evaluated the effects of LHRH-A on the ability of BLM cells to migrate towards a chemoattractant (fetal bovine serum 5%), using the Boyden's chamber technique. We found that treatment of BLM cells with LHRH-A (10^{-6} M for 5 days) significantly reduced the ability of the cells to migrate in response to the chemoattractant (Fig. 2). We then analyzed the effects of LHRH-A (10^{-6} M) on the ability of melanoma cells to invade a reconstituted basement membrane (Matrigel). BLM cells spontaneously form cell aggregates in Matrigel when prepared by the hanging-drop technique. For these experiments, the aggregates in Matrigel were covered with culture medium in the absence or in the presence of LHRH-A (10^{-6} M) for 4, 8, or 12 days. The results obtained show that BLM cells actively leave the aggregate by degrading the Matrigel preparation. The treatment of the cells with LHRH-A completely counteracted the migration of the cells at all the time intervals considered.

Taken together, these data indicate that the activation of locally expressed LHRH receptors significantly reduces the ability of melanoma cells to migrate in response to a chemotactic stimulus and to degrade extracellular matrix components. To the authors' knowledge, this is the first report of a possible antimetastatic activity of LHRH in tumors. Preliminary data obtained in our laboratory seem to indicate that LHRH exerts this antimetastatic activity by reducing the expression of cell adhesion molecules (integrins) as well as the expression and the activity of enzymes able to degrade the extracellular matrix (matrix metalloproteinases, MMPs). Both integrins and MMPs have been shown to play a crucial role in the molecular mechanisms leading to melanoma progression (Natali *et al.* 1993, Hofmann *et al.* 2000).
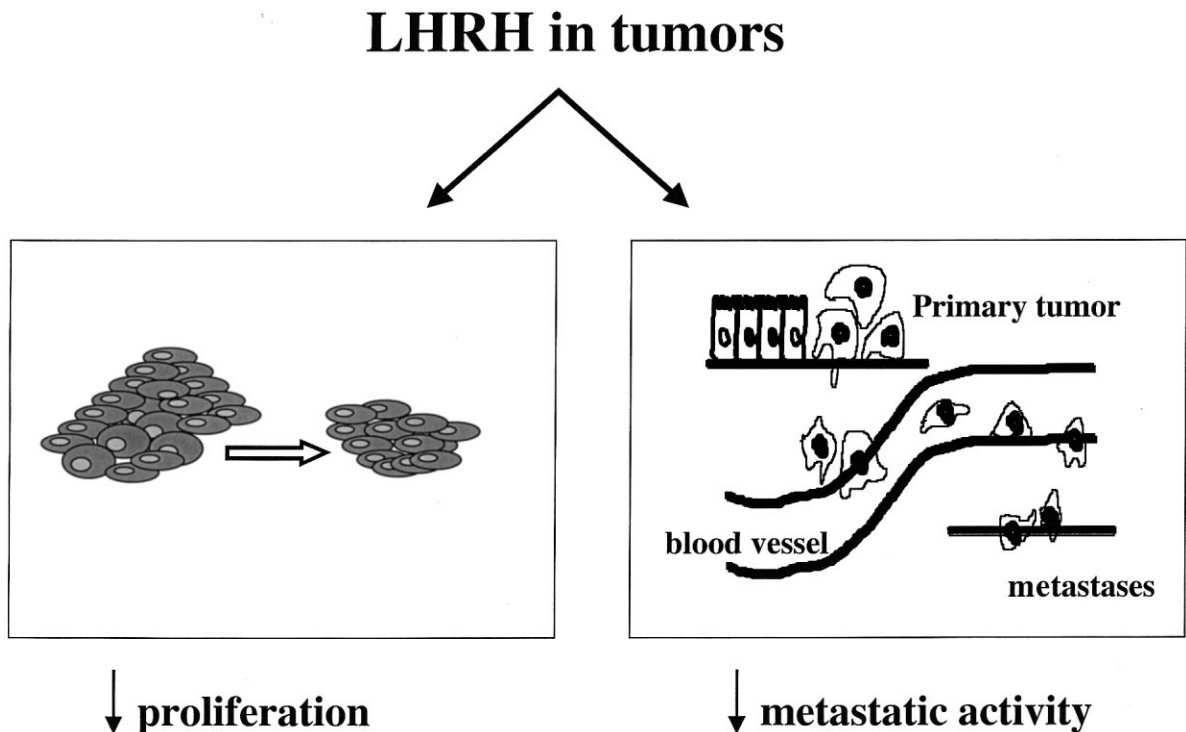

Figure 3 Schematic representation of the antiproliferative and antimetastatic properties of LHRH in tumor cells.

As pointed out above, the clinical options for advanced melanoma are still limited, due to the intrinsic resistance of the tumor to standard chemotherapy, but also to its ability to give rise to metastases (Meyer & Hart 1998). The data here reported seem to suggest that LHRH agonists might reduce not only the proliferation rate, but also the metastatic potential of melanoma cells (see Fig. 3).

The intracellular signaling pathways that are triggered after the activation of LHRH receptors in melanoma cells are still unclear. In preliminary experiments, we have observed that LHRH-A does not affect forskolin-induced cAMP levels in BLM cells (RM Moretti, M Montagnani Marelli & P Limonta, unpublished observations). This seems to suggest that the intracellular signaling pathway coupled to the LHRH receptor in melanoma is different from that found in prostate cancer. Experiments are in progress in our laboratory to clarify this issue.

Conclusions

LHRH is expressed, together with its receptors, in hormone-related tumors such as prostate cancer, to act as a local autocrine/paracrine growth inhibitory factor. In prostate cancer cells, the LHRH receptor is coupled to the Gi-cAMP pathway to inhibit cell proliferation. This is at variance with the LHRH receptor at the pituitary level which is coupled to the Gq-PLC pathway to stimulate gonadotropin synthesis and secretion.

LHRH and LHRH receptors are expressed also in tumors that are not classically related to the endocrine system, such as melanoma. In melanoma cells, the locally expressed LHRH-based system negatively regulates the proliferation of the cells as well as their ability to migrate towards chemotactic stimuli and to invade a reconstituted basement membrane. Therefore, in melanoma, the activation of LHRH receptors might reduce not only tumor growth but also its metastatic potential. Moreover, the LHRH receptor might represent a new diagnostic (and possibly prognostic) marker for the detection of skin tumors.

Acknowledgements

References

Chatzaki E, Bax CMR, Eidne KA, Anderson L, Grudzinskas JG & Gallagher CJ 1996 The expression of gonadotropin releasing hormone and its receptor in endometrial cancer and its relevance as an autocrine growth factor. *Cancer Research* **56** 2055–2065.

Conn PM & Crowley WF Jr 1994 Gonadotropin-releasing hormone and its analogs. *Annual Review of Medicine* **45** 391–405.

Crawford ED, De Antonio EP, Labrie F, Schroder FH & Geller J 1995 Endocrine therapy of prostatic cancer: optimal form and appropriate timing. *Journal of Clinical Endocrinology and Metabolism* **80** 1062–1078.

Dondi D, Limonta P, Moretti RM, Montagnani Marelli M, Garattini E & Motta M 1994 Antiproliferative effects of luteinizing hormone-releasing hormone (LHRH) agonists on human androgen-independent prostate cancer cell line DU 145: evidence for an autocrine-inhibitory loop. *Cancer Research* **54** 4091–4095.

Dondi D, Moretti RM, Montagnani Marelli M, Pratesi G, Polizzi D, Milani M, Motta M & Limonta P 1998 Growth inhibitory effects of luteinizing hormone-releasing hormone (LHRH) agonists on xenografts of the DU 145 human androgen-independent prostate cancer cell line in nude mice. *International Journal of Cancer* **76** 506–511.

Emons G & Schally AV 1994 The use of luteinizing hormone-releasing hormone agonists and antagonists in gynecological cancers. *Human Reproduction* **9** 1364–1379.

Emons G, Ortmann O, Becker M, Irmer G, Springer B, Laun R, Holzel F, Schulz KD & Schally AV 1993 High affinity binding and direct antiproliferative effects of LHRH analogues in human ovarian cancer cell lines. *Cancer Research* **54** 5439–5446.

Emons G, Weiss S, Ortmann O, Grundker C & Schulz K-D 2000 LHRH might act as a negative autocrine regulator of proliferation of human ovarian cancer. *European Journal of Endocrinology* **142** 665–670.

Fernald RD & White RB 1999 Gonadotropin-releasing hormone genes: phylogeny, structure and functions. *Frontiers in Neuroendocrinology* **20** 224–240.

Fink G 1988 Gonadotropin secretion and its control. In *The Physiology of Reproduction*, pp 1349–1377. Eds E Knobil & JD Kneill. New York: Raven Press.

Green S & Furr B 1999 Prospects for the treatment of endocrine-responsive tumours. *Endocrine-Related Cancer* **6** 349–371.

van Groeninghen JC, Kiesel L, Winkler D & Zwirner M 1998 Effects of luteinizing hormone-releasing hormone on nervous-system tumours. *Lancet* **352** 372–373.

Halaban R 1996 Growth factors and melanoma. *Seminars in Oncology* **23** 673–681.

Hofmann UB, Westphal JR, van Muijen GNP & Ruiter DJ 2000 Matrix metalloproteinases in human melanoma. *Journal of Investigatve Dermatology* **115** 337–344.

Imai A & Tamaya T 2000 GnRH receptor and apoptotic signaling. *Vitamins and Hormones* **59** 1–33.

Imai A, Ohno T, Iida K, Fuseya T, Furui T & Tamaya T 1994 Gonadotropin-releasing hormone receptors in gynecological tumors. *Cancer* **74** 2555–2561.

Imai A, Horibe S, Takagi H, Fuseya T & Tamaya T 1996 Signal transduction of GnRH receptor in the reproductive tract tumor. *Endocrine Journal* **43** 249–260.

Kakar SS, Musgrove LC, Devor DC, Sellers JC & Neill JD 1992 Cloning, sequencing and expression of human gonadotropin-releasing hormone (GnRH) receptor. *Biochemical and Biophysical Research Communications* **189** 289–295.

Kakar SS, Grizzle WE & Neill JD 1994 The nucleotide sequences of human GnRH receptors in breast and ovarian tumors are identical with that found in pituitary. *Molecular and Cellular Endocrinology* **106** 145–149.

Kalra SP 1993 Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge. *Endocrine Reviews* **14** 507–538.

Karande AA, Rajeshwari K, Schol DJ & Hilgers JHM 1995 Establishment of immunological probes to study human gonadotropin-releasing hormone receptors. *Molecular and Cellular Endocrinology* **114** 51–56.

Keri G, Balogh A, Szoke B, Teplan I & Csuka O 1991 Gonadotropin-releasing hormone analogues inhibit cell proliferation and activate signal transduction pathways in MDA-MB-231 human breast cancer cell line. *Tumour Biology* **12** 61–67.

Kottler ML, Starzec A, Carre MC, Lagarde JP, Martin A & Counis R 1997 The genes for gonadotropin-releasing hormone and its receptor are expressed in human breast with fibrocystic disease and cancer. *International Journal of Cancer* **71** 595–599.

Kraus S, Naor Z & Seger R 2001 Intracellular signaling pathways mediated by the gonadotropin-releasing hormone (GnRH) receptor. *Archives of Medical Research* **32** 499–509.

Lazar-Molnar E, Hegyesi H, Toth S & Falus A 2000 Autocrine and paracrine regulation by cytokines and growth factors in melanoma. *Cytokine* **12** 547–554.

Limonta P, Dondi D, Moretti RM, Maggi R & Motta M 1992 Antiproliferative effects of luteinizing hormone-releasing hormone agonists on the human prostatic cancer cell line LNCaP. *Journal of Clinical Endocrinology and Metabolism* **75** 207–212.

Limonta P, Dondi D, Moretti RM, Fermo D, Garattini E & Motta M 1993 Expression of luteinizing hormone-releasing hormone mRNA in the human prostatic cancer cell line LNCaP. *Journal of Clinical Endocrinology and Metabolism* **76** 797–800.

Lippman ME 1998 Endocrine-responsive cancer. In *Williams Textbook of Endocrinology*, pp 1675–1692. Eds JD Wilson, DW Foster, HM Kronenberg & PR Larsen. Philadelphia: WB Saunders Co.

Lu C & Kerbel RS 1994 Cytokines, growth factors and the loss of negative growth controls in the progression of human cutaneous malignant melanoma. *Current Opinion in Oncology* **6** 212–220.

MacKie RM 1998 Incidence, risk factors and prevention of melanoma. *European Journal of Cancer* **34** S3–S6.

Manni A 1999 Hormonal approaches to the chemoprevention of endocrine-dependent tumors. *Endocrine-Related Cancer* **6** 483–485.

Meyer T & Hart IR 1998 Mechanisms of tumor metastasis. *European Journal of Cancer* **34** 214–221.

Montagnani Marelli M, Moretti RM, Dondi D, Motta M & Limonta P 1999 Luteinizing hormone-releasing hormone agonists interfere with the mitogenic activity of the insulin-like growth factor system in androgen-independent prostate cancer cells. *Endocrinology* **140** 329–334.

Moretti RM, Montagnani Marelli M, Dondi D, Poletti A, Martini L, Motta M & Limonta P 1996 Luteinizing hormone-releasing hormone agonists interfere with the stimulatory actions of epidermal growth factor in human prostatic cancer cell lines, LNCaP and DU 145. *Journal of Clinical Endocrinology and Metabolism* **81** 3930–3937.

Naor Z, Harris D & Shacham S 1998 Mechanism of GnRH receptor signaling: combinatorial cross-talk of Ca^{2+} and protein kinase C. *Frontiers in Neuroendocrinology* **19** 1–19.

Natali PG, Nicotra MR, Bartolazzi A, Cavaliere R & Bigotti A 1993 Integrin expression in cutaneous malignant melanoma: association of the alpha3/beta1 heterodimer with tumor progression. *International Journal of Cancer* **54** 68–72.

Neill JD 2002 GnRH and GnRH receptor genes in the human genome. *Endocrinology* **143** 737–743.

Oikawa M, Dargan C, Ny T & Hsueh AJW 1990 Expression of gonadotropin-releasing hormone and prothymosin-messenger ribonucleic acid in the ovary. *Endocrinology* **127** 2350–2356.

Parkin DM, Pisani P & Ferlay J 1999 Global cancer statistics. *CA Cancer Journal of Clinics* **49** 33–64.

Schally AV 1994 Hypothalamic hormones from neuroendocrinology to cancer therapy. *Anticancer Drugs* **5** 115–130.

Shih IM & Herlyn M 1993 Role of growth factors and their receptors in the development and progression of melanoma. *Journal of Investigative Dermatology* **100** S196–S203.

Shih IM & Herlyn M 1994 Autocrine and paracrine roles for growth factors in melanoma. *In Vivo* **8** 113–123.

Shupnik MA 1996 Gonadotropin gene modulation by steroids and gonadotropin-releasing hormone. *Biology of Reproduction* **54** 279–286.

Stojilkovic SS & Catt KJ 1995 Expression and signal transduction pathways of gonadotropin releasing hormone receptors. *Recent Progress in Hormone Research* **30** 161–205.

Stojilkovic SS, Reinhart J & Catt KJ 1994 Gonadotropin-releasing hormone receptors. Structure and signal transduction pathways. *Endocrine Reviews* **15** 462–499.

Ui M & Katada T 1990 Bacterial toxins as probe for receptor-G coupling. In *The Biology and Medicine of Signal Transductions*, pp 63–69. Ed Y Nishizuka. New York: Raven Press.

White RB, Eisen JA, Kasten TL & Fernald RD 1998 Second gene for gonadotropin-releasing hormones in humans. *PNAS* **95** 305–309.

Wormald PJ, Eidne KA & Millar RP 1985 Gonadotropin-releasing hormone receptors in human pituitary: ligand structural requirements, molecular size, and cationic effects. *Journal of Clinical Endocrinology and Metabolism* **61** 1190–1194.

Yin H, Cheng K, Hwa H, Peng C, Auersperg N & Leung P 1998 Expression of the messenger RNA for gonadotropin-releasing hormone and its receptor in human cancer cell lines. *Life Sciences* **62** 2015–2023.