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Locally Expressed LHRH Receptors Mediate the Oncostatic and Antimetastatic Activity of LHRH Agonists on Melanoma Cells

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Malignant melanoma is a tumor known for its uncontrollable growth and aggressive metastatic behavior. The mean survival time for patients with a metastatic melanoma is estimated to be less than 6 months, tumor cells being refractory to the conventional chemotherapy. A better understanding of the mechanisms regulating melanoma growth and progression might help increase the number of therapeutic options for this pathology. In this paper, we have shown that LHRH receptors are present in the BLM melanoma cell line, both at mRNA and at protein level; a potent LHRH agonist (LHRH-A; Zoladex) binds to these receptors with high affinity. BLM cells also express the mRNA for LHRH, indicating the presence of an autocrine LHRH-based system in melanoma cells. The treatment of BLM cells with LHRH-A dose-dependently inhibited cell proliferation; this effect was found to be specific because it was completely abrogated by the simultaneous treatment of the cells with a LHRH antagonist. Similar observations could be obtained in another melanoma cell line (Me15392). The activation of LHRH receptors, by means of LHRH-A, also reduced the ability of melanoma cells to invade a reconstituted basement membrane (Matrigel) and to migrate through a Boyden's chamber in response to a chemotactic stimulus. These data represent the first report that 1) LHRH and LHRH receptors are expressed in melanoma tumor cells; and 2) the activation of tumor LHRH receptors reduces both the proliferation and the metastatic potential of melanoma cells. It is suggested that the expression of LHRH receptors might represent a new diagnostic marker for the detection and progression of melanoma. These receptors might also be considered as a possible molecular target for a hormone-based therapeutic approach to this tumor. (*J Clin Endocrinol Metab* 87: 3791–3797, 2002)

MALIGNANT MELANOMA IS a tumor known for its uncontrollable growth (1). The incidence of melanoma, which predominantly occurs in the skin, is increasing dramatically (2). The prognosis of this tumor has improved in the last decades particularly due to early diagnosis, but it remains very poor in advanced cases, when tumor cells acquire a strong potential to disseminate metastases (1). Moreover, advanced melanoma is characterized by an intrinsic resistance to chemotherapy (3–5). A recently introduced new chemotherapeutic drug for metastasized melanoma, temozolomide, failed to offer a significant improvement of mean survival compared with the treatment with the traditional cytotoxic drug DTIC (dacarbazine, Bedford Laboratories, Bedford, OH) (5). Both of these chemotherapeutics induce thrombocytopenia as a classical side effect with considerable risks because metastasis bleeding may occur, especially in cerebral metastases. Due to the ability of melanoma cells to rapidly metastasize, prevention of metastasis formation has been indicated as the main goal in melanoma treatment (6).

Hypothalamic LHRH is the master hormone in reproduction; it controls pituitary-gonadal functions by activating LHRH receptors present on gonadotropin-producing cells of the adenohypophysis (7). LHRH agonists, when given continuously, suppress gonadotropin synthesis and secretion through the down-regulation of these pituitary LHRH receptors (8). This mechanism of action has provided the rationale for the wide and successful use of LHRH agonists in the treatment of hormone-dependent tumors (*e.g.* prostate and breast carcinoma; Refs. 9–11). In addition, it has been shown that these tumors express both LHRH and LHRH receptors. Through the activation of these receptors, locally produced LHRH seems to behave as an autocrine-negative regulator of cancer growth. Moreover, activation of tumor LHRH receptors by exogenous LHRH agonists inhibits the proliferation of tumor cells, both *in vitro* and *in vivo*, indicating an additional and more direct antitumoral activity for these compounds (12).

In a recent paper, van Groeninghen *et al*. (13) reported the expression of LHRH receptors in glioblastoma biopsies, suggesting that these binding sites might represent a diagnostic marker, and possibly a new therapeutical target, for nervous system tumors. On the basis of the observation that malignant gliomas and malignant melanomas are both tumors of neuroectodermal origin, we decided to verifiy whether a LHRH-based system (LHRH and the respective receptors), similar to that previously described in tumors of the reproductive tract, is expressed in melanoma cells. We also investigated whether the activation of this system might affect the proliferation rate as well as the metastatic properties of this tumor.

Materials and Methods

Materials

The LHRH agonist (LHRH-A) Zoladex (D-Ser(tBu)^{6}Aza-Gly^{10}-LHRH) was kindly provided by AstraZeneca Pharmaceuticals, Divi-

Abbreviations: ANT, LHRH antagonist; FBS, fetal bovine serum; LHRH-A, LHRH agonist.

sione Farmaceutici (Milano, Italy). The LHRH-antagonist Antide (ANT, acetyl-β-[2.Naphtyl]-D-Ala-D-p-chloro-Phe-β-[3-pyridyl]-D-Ala-Ser-Nε-[Nicotinoyl]-Lys-Nε-[Nicotinoyl]-D-Lys-Leu-Nε-[Isopropyl]-Lys-Pro-D-Ala-NH_2) was purchased from Sigma Chemical Co. (St. Louis, MO).

Cell cultures

The human melanoma BLM and Me15392 cell lines, both possessing high proliferative and metastatic potential (14), were kindly donated by Dr. Van Muijen (Department of Pathology, University Hospital Nijmegen, The Netherlands) and Dr. Parmiani (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy), respectively. BLM cells were grown in DMEM medium (Seromed, Biochrom KG, Berlin, Germany), whereas Me15392 cells were grown in RPMI medium (Seromed). In both cases, cell culture media were supplemented with 10% fetal bovine serum (FBS, Life Technologies, Inc., Paisley, Scotland), glutamine (1 mM), antibiotics (100 IU/ml penicillin G sodium, 100 μg/ml streptomycin sulfate), and sodium pyruvate (100 mM) in a humidified atmosphere of 5% CO_2/95% air. The human androgen-independent DU 145 prostate cancer cell line was obtained from American Type Culture Collection (Rockville, MD). This cell line has been used as a positive control, because we have previously shown that a LHRH system (LHRH and LHRH receptors) is expressed in these cells (15, 16).

RT-PCR analysis of LHRH and LHRH receptor mRNA

Total RNA from BLM cells (as well as from DU 145 cells, used as positive control) was prepared according to a modification of the guanidium thiocyanate/cesium chloride method (17). RNA (2 μg) was used in a RT reaction. cDNA synthesis was performed using the Gene AMP kit (Perkin Elmer Cetus, Norwalk, CT), with an oligo$(dT)_{16}$ as a primer for the RT. Samples containing cDNAs obtained from prostate tumor cells were then amplified in a 100-μl solution containing PCR buffer (50 mM KCl, 10 mM Tris-HCl), 2 mM $MgCl_2$, 2.5 U Taq polymerase. For LHRH cDNA amplification, PCR was performed for 35 cycles (1-min denaturation at 94 C, 30-sec primer annealing at 60 C, and 2-min primer extension at 72 C) in the presence of the primers: 5′-TGGTGCGTG-GAAGGCTGCTC-3′ (sense, 78/97, 20 pmol) and 5′-CTTCTTCTGC-CCAGTTTCCTC-3′ (antisense, 285/305, 20 pmol; Ref. 18). For the amplification of LHRH receptor cDNA, PCR was performed for 35 cycles (1-min denaturation at 94 C, 1-min primer annealing at 50 C, and 2-min primer extension at 72 C) in the presence of the following primers: 5′-GCTTGAAGCTCTGTCCTGGGA-3′ (sense, −25 to −5, 30 pmol) and 5′-CCTAGGACATAGTAGGG-3′ (antisense, 844–860, 30 pmol; Ref. 19). Both pairs of primers have been used previously in our laboratory to amplify LHRH and LHRH receptor cDNAs in prostate cancer cells (15, 16, 20). The predicted amplified cDNA fragments were 228 and 885 bp for LHRH and LHRH receptor, respectively. After PCR, the amplified cDNA products were separated on 1.5% agarose gels and stained with ethidium bromide. After Southern blotting, blots were hybridized with synthetic ^{32}P-labeled oligonucleotide probes, specific for the LHRH and LHRH receptor cDNAs, as previously described (15, 16).

Western blot analysis of the LHRH receptor

Membrane fractions were prepared from BLM and Me15392 cells (as well as from DU 145 cells, used as positive controls) according to the protocol reported by Limonta *et al.* (16). Samples were homogenized in 10 mM Tris-HCl (pH 7.6) buffer containing 1 mM dithiothreitol on ice. The homogenates were centrifuged two times for 10 min each at 800 × *g* to remove cellular debris, and the resulting supernatants were centrifuged at 18,000 × *g* to pellet down the membrane fractions. The pellets were solubilized in RIPA buffer [50 mM Tris-HCl (pH 7.7), 150 mM NaCl, 0.8% Triton X-100, 0.8% sodium deoxycholate, 0.08% SDS, 10 mM ethylendiamine tetraacetate, 100 μM Na_3VO_4, 50 mM NaF, 0.3 mM phenylmethylsulfonylfluoride, and 5 mM iodoacetic acid] and electrophoresed on 10% polyacrylamide gel under reducing conditions. Proteins were transferred onto a nitrocellulose filter. Filters were probed with the F1G4 monoclonal antibody (15 μg/ml), for 2 h at room temperature. This antibody has been raised against the human pituitary LHRH receptor (kindly provided by Dr. A. A. Karande, Department of Biochemistry, Indian Institute of Science, Bangalore, India). Filters were then incubated for 1 h at room temperatrure, with an antimouse IgG (1:100,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Antibody bound to the LHRH receptor was detected with the ECL-Western blotting detection system after a 5- to 10-min exposure to a Hyperfilm-ECL x-ray film (Amersham Pharmacia Biotech, Milano, Italy) at room temperature (21). The specificity of F1G4 antibody for the human pituitary LHRH receptor has been previously demonstrated (16, 22).

LHRH receptor assay

The binding assay for LHRH receptors in melanoma cells has been performed as previously described (15) by using ^{125}I-LHRH-A (specific activity, 800-1000 μCi/μg) as the specific ligand. Briefly, displacement curves were performed by incubating 100-μl cell membrane aliquots with ^{125}I-LHRH-A (200,000 cpm) in either the absence or the presence of increasing concentrations of unlabeled LHRH-A (10^{-11}-10^{-7} M). Nonspecific binding was determined in the presence of 10^{-5} M unlabeled LHRH-A. Displacement curves allowed the determination of binding characteristics for LHRH receptors (K_d, dissociation constant; B_{max}, maximum concentration of binding sites) on BLM and Me15392 melanoma cells. LHRH receptors have also been analyzed on rat pituitary membranes used as controls. Four displacement curves have been performed for each experimental group. The protein content of each membrane preparation was evaluated according to the method of Bradford (23).

Cell proliferation studies

BLM cells were plated at a density of 700 cells/cm^2 in 10-mm dishes in culture medium. Cells were allowed to attach and start growing for 3 d; the seeding media were then changed. Cells were treated daily (the drug was added to the medium every day), for 7 d with LHRH-A (10^{-11}-10^{-6} M); the medium was changed every 2 d. At the end of the treatment, cells were collected and counted by hemocytometer.

To confirm the specificity of the action of LHRH-A on melanoma cell proliferation, we investigated whether the effects of the LHRH agonist might be counteracted by a potent LHRH antagonist. A preliminary experiment was performed to select the dose of the LHRH anatgonist (ANT) to be used. To this purpose, BLM cells were treated daily with ANT at different doses (10^{-11}-10^{-6} M). Cells were harvested and counted after 7 d of treatment. Subsequently, BLM cells were treated daily, for 7 d, with LHRH-A (10^{-7} M), in either the absence or the presence of ANT (10^{-7} M). Cells were counted 7 d after the beginning of the treatment.

The antiproliferative action of LHRH agonists on melanoma cells has been further investigated in another melanoma cell line (Me15392). These experiments have been performed as described above for BLM cells (same LHRH-A, same doses of the drug, and same length of treatment, *etc.*).

All proliferation experiments were performed in four to six replicates. The data obtained from three independent experiments were analyzed according to the Dunnett's test after one-way ANOVA (24).

Matrigel gel assay

For invasion and migration experiments, the 10^{-6} M dose of LHRH-A has been chosen because it was the most effective in proliferation studies. This dose has also been used in previous papers from our laboratory analyzing the interaction between LHRH agonists and stimulatory growth factors in prostate cancer cells (25, 26).

Subconfluent BLM cells were collected by trypsinization, resuspended in culture medium, and seeded in 20 μl (150,000 cells/drop) on the lid of a culture dish. The lid was then placed on a dish filled with 2 ml of culture medium and incubated at 37 C for 48 h. Matrigel solution (80 μl, 2.7 mg/ml) was pipetted onto the bottom of wells of a 24-well culture dish and left to set at 37 C. Cell aggregates were transferred over the cushion and then overlaid with additional 20 μl of Matrigel. The aggregates into Matrigel were covered with 400-μl culture medium in the absence or in the presence of LHRH-A (10^{-6} M). The aggregates were then observed daily under a light microscope, and at the end of the incubation time phase-contrast pictures of the aggregates were taken.

Chemomigration assay

The assay was performed using a 48-well Boyden's chamber, according to the manufacturer's instruction (Neuroprobe, Cabin John, MD).

Subconfluent BLM cells, grown in culture medium, were pretreated for 5 d with LHRH-A (10^{-6} M) and harvested at the end of the treatment. BLM cell suspensions (10^5 cells/50 μl), resuspended in culture medium deprived of FBS, were placed in the open-bottom wells of the upper compartment of the chamber. Each pair of wells was separated by polyvinylpyrrolidone-free polycarbonate porous membrane (8-μm pores) precoated with gelatin (0.2 mg/ml in PBS). The chemoattractant (FBS 5%) was placed in the lower compartment of the chamber. The chamber was then kept for 4 h in the cell culture incubator. After that, the cells migrated through the pores and adhered to the underside of the membrane; they were fixed, stained (Diff-Quick kit, DADE, Dudingen, Switzerland), and mounted onto glass slides. For quantitative analysis, six random objective fields of stained cells were counted for each well (8 wells/experimental group) and the mean number of migrating cells per square millimeter was calculated. The data obtained from four independent experiments were compared by ANOVA and Dunnett's test (24).

Results

Expression of LHRH and the LHRH receptor in BLM melanoma cells

The expression of LHRH and LHRH receptor mRNA in melanoma BLM cells was investigated by RT-PCR. After PCR, the amplified cDNAs were electrophoresed on a 1.5% agarose gel containing ethidium bromide. With regard to the expression of LHRH, the predicted 228-bp fragment was observed in BLM cells (Fig. 1, *top*, lane 1) as well as in prostate cancer cells used as controls (Fig. 1, *top*, lane 2). No cDNA band was detected in samples without RT (data not shown), ruling out the possibility of genomic DNA contamination. After Southern blotting, the cDNA fragments, obtained from BLM and prostate cancer cells, hybridized with the ^{32}P-labeled oligonucleotide probe specific for LHRH cDNA (Fig. 1, *bottom*, lanes 1 and 2).

In the case of the expression of the LHRH receptor mRNA, the results obtained demonstrated that the predicted 885-bp cDNA fragment could be obtained in BLM (Fig. 2, *top*, lane 1), as well as in prostate cancer cells (Fig. 2, *top*, lane 2). No cDNA band was amplified in samples without RT (data not shown). As expected, the LHRH receptor cDNA bands hybridized with the specific ^{32}P-labeled oligonucleotide probe specific for LHRH receptor cDNA (Fig. 2, *bottom*, lanes 1 and 2).

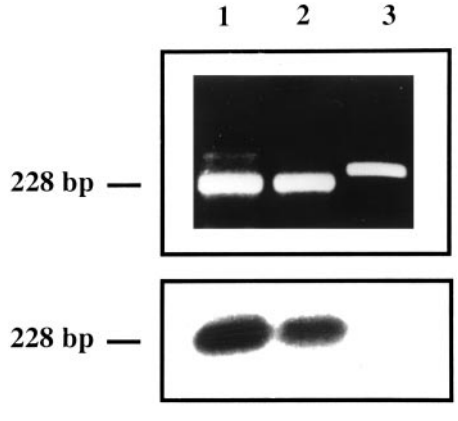

FIG. 1. RT-PCR analysis of the expression of LHRH in BLM cells. *Top,* Ethidium bromide-stained agarose gel of the amplified cDNAs. *Bottom,* Autoradiography of the Southern blot obtained from the gel shown in the *top panel* after hybridization with a ^{32}P-labeled oligonucleotide LHRH cDNA probe. Lane 1, BLM cells; lane 2, prostate cancer cells; lane 3, RT-PCR control (308 bp). One of three experiments performed is reported.

The presence of LHRH receptors in melanoma cells has been further investigated at the protein level by Western blotting technique and by using the F1G4 monoclonal antibody specifically raised against the human pituitary LHRH receptor. As shown in Fig. 3, a major protein band of approximately 64-kDa molecular mass was identified by the antibody in BLM cells (lane 1) as in prostate cancer cells (Fig. 3, lane 2). This molecular mass corresponds to that previously reported for the human pituitary LHRH receptor (27). The level of expression of this receptor was not found to be affected by a 7-d treatment with the LHRH agonist (data not shown).

Effect of LHRH agonists on the proliferation of BLM melanoma cells

The observation that both LHRH and LHRH receptors are expressed in BLM cells prompted us to investigate whether this LHRH-based system might be involved in the local control of melanoma cell growth. To this purpose, BLM cells were treated daily, for 7 d, with the potent LHRH agonist LHRH-A (10^{-11}-10^{-6} M). The treatment resulted in a significant and dose-dependent inhibition of cell proliferation (Fig. 4).

Further studies were performed to evaluate whether the antiproliferative action of LHRH-A on melanoma cells could be antagonized by the simultaneous treatment of the cells

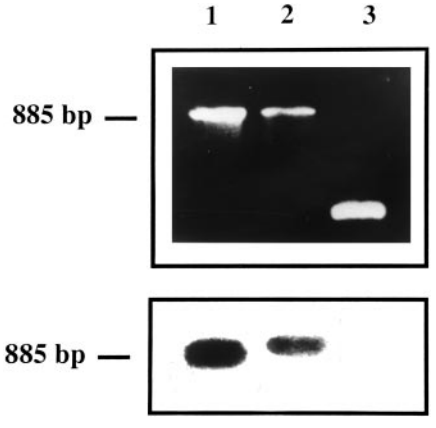

FIG. 2. RT-PCR analysis of the expression of the LHRH receptor in BLM cells. *Top*, Ethidium bromide-stained agarose gel of the amplified cDNAs. *Bottom*, Autoradiography of the Southern blot obtained from the gel shown in the *top panel* after hybridization with a ^{32}P-labeled oligonucleotide LHRH receptor cDNA probe. Lane 1, BLM cells; lane 2, prostate cancer cells; lane 3, RT-PCR control (308 bp). One of three experiments performed is reported.

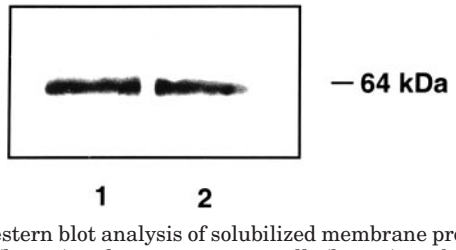

FIG. 3. Western blot analysis of solubilized membrane proteins from BLM cells (lane 1) and prostate cancer cells (lane 2), probed with the F1G4 monoclonal antibody raised against the human pituitary LHRH receptor. One experiment representative of three is reported.

with the LHRH anatagonist ANT. In preliminary experiments, the activity of ANT was evaluated. Figure 5A shows that the antagonist did not affect the proliferation of the cells, when given at the doses 10^{-11}-10^{-7} M. The compound reduced slightly, but not significantly, the growth of BLM cells at the dose of 10^{-6} M. For subsequent experiments, the dose of 10^{-7} M was then selected. Figure 5B confirms that ANT (10^{-7} M), when given alone, has no effect on cell proliferation; on the other hand, ANT totally blocked the antiproliferative action exhibited by LHRH-A.

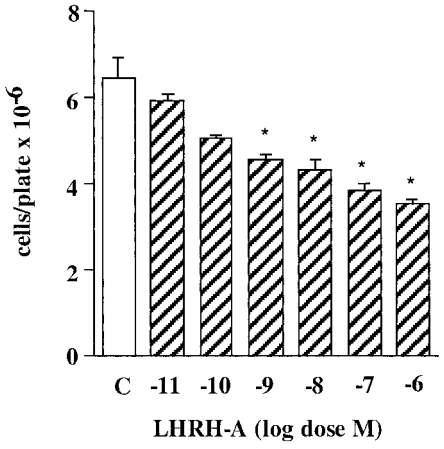

FIG. 4. Effects of the LHRH agonist (LHRH-A) on the proliferation of BLM cells. Results are expressed as mean cell number per plate ± SE. *, $P < 0.05$ *vs.* controls (C).

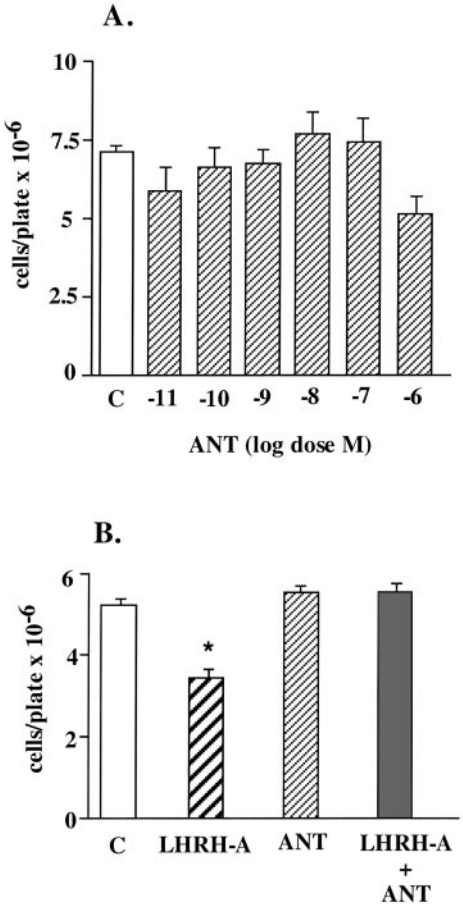

FIG. 5. A, Effects of the LHRH antagonist (ANT) on the proliferation of BLM cells. B, Effects of the LHRH antagonist (ANT, 10^{-7} M) on the inhibition of BLM cell proliferation induced by the LHRH agonist (LHRH-A, 10^{-7} M). Results are expressed as mean cell number per plate ± SE. *, $P < 0.05$ *vs.* controls without drugs (C).

Expression and possible role of LHRH receptors in Me15392 melanoma cells

The presence of LHRH receptors and their possible role in the control of melanoma cell proliferation have been further investigated in an additional melanoma cell line (Me15392). By Western blot analysis and by using the F1G4 monoclonal antibody, we demonstrated that a protein band of 64 kDa was present in membrane preparations from Me15392 cells (Fig. 6A, lane 2). The molecular mass of this band corresponded to that found in BLM cells (Fig. 6A, lane 1).

As in the case of BLM cells, the treatment of Me15392 cells with LHRH-A (10^{-11}-10^{-6} M), for 7 d, resulted in a significant and dose-dependent inhibition of cell proliferation (Fig. 6B).

Binding parameters of LHRH receptors in BLM and Me15392 melanoma cells

LHRH receptors in melanoma cells have been analyzed also in terms of binding parameters. Binding sites for ^{125}I-LHRH-A have been found to be present on the membranes of both BLM and Me15392 cells. Computer analysis of the data obtained from the displacement curves revealed the presence of a single class of high-affinity binding sites (K_d in the nanomolar range) in both melanoma cell lines, as well as in rat pituitaries used as controls (Table 1). This observation agrees with previous data showing the expression of high-affinity LHRH receptors in tumors of the reproductive tract (28, 29).

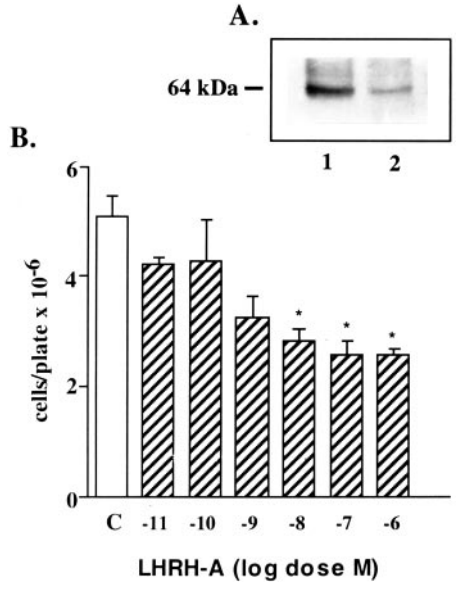

FIG. 6. A, Western blot analysis of the expression of the LHRH receptor in Me15392 cells. Lane 1, BLM cells; lane 2, Me15392 cells. B, Effects of the LHRH agonist (LHRH-A) on the proliferation of Me 15392 cells. Results are expressed as mean cell number per plate ± SE. *, $P < 0.05$ *vs.* controls (C).

Effect of LHRH agonists on the metastatic potential of BLM melanoma cells

These experiments have been performed to verify whether the activation of locally expressed LHRH receptors might affect the metastatic potential of melanoma cells. First, we studied the effects of the LHRH agonist LHRH-A (10^{-6} M) on the ability of BLM cells to invade a matrix of a reconstituted basement membrane (Matrigel). BLM cells spontaneously form cell aggregates in Matrigel, when prepared by the hanging-drop technique. Figure 7 shows that BLM cells actively leave the aggregate and invade the Matrigel preparation at 4, 8, and 12 d. The treatment of BLM cells with Zoladex completely abrogated the migration of the cells through the Matrigel at all time intervals considered (Fig. 7).

We then analyzed whether LHRH agonists might affect the ability of melanoma cells to migrate toward a chemoattractant, using the Boyden's chamber technique and FBS 5% as the chemotactic stimulus. We observed that, when BLM cells were pretreated with Zoladex (10^{-6} M) for 5 d, the number of the cells that migrated in response to the chemoattractant was significantly decreased when compared with control cells (Fig. 8).

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

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

Binding characteristics were evaluated from displacement curves as described in *Materials and Methods*.

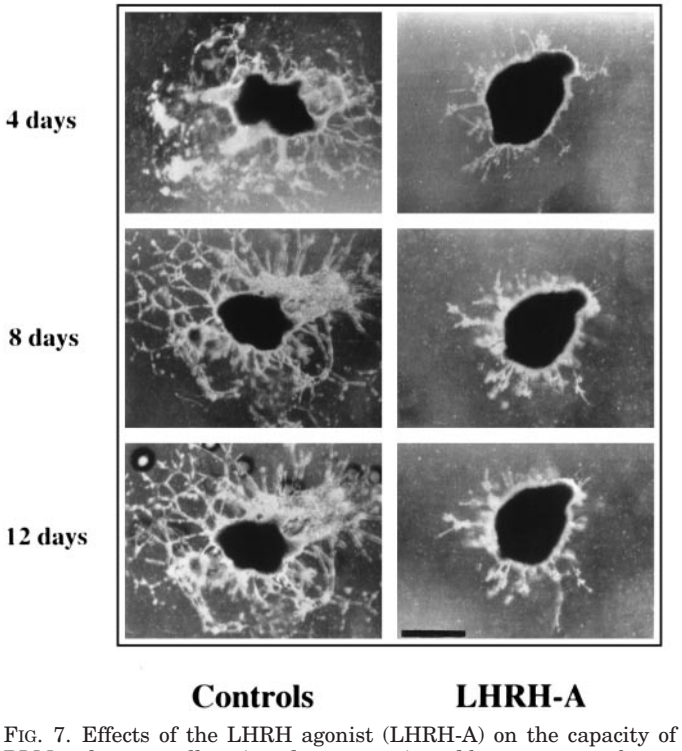

FIG. 7. Effects of the LHRH agonist (LHRH-A) on the capacity of BLM melanoma cells to invade a reconstituted basement membrane after 4, 8, and 12 d of treatment. Results from one of four experiments performed are reported. Scale bar, 700 μm.

Discussion

These results demonstrate that both LHRH and LHRH receptors are expressed in human melanoma cells in culture (BLM and Me15392). This unexpectedly discovered autocrine LHRH system seems to participate in the mechanisms regulating cell proliferation exerting an inhibitory activity, because LHRH receptor activation by an exogenous LHRH agonist brings about a significant decrease of tumor cell proliferation. The inhibitory effect of the LHRH agonist is specific because it is counteracted by the simultaneous treatment of the cells with a LHRH antagonist. It is well known that malignant transformation of normal melanocytes into melanoma cells is characterized, as in other tumors, by a gradual decrease of the dependency on external mitogenic stimuli (30), accompanied by a simultaneous acquisition of an increased expression of multiple growth factors (31, 32). The data reported here indicate that, in addition to growth stimulatory factors, melanoma cells may also express a LHRH-based system, endowed with inhibitory activities. Consequently, melanoma growth and progression may possibly be viewed as the result of the algebraic sum of positive and negative regulators. The activation of the inhibitory system, through the use of LHRH agonists, may reduce tumor growth and interfere with the positive effect of the mitogenic factors.

The mechanism of the antitumor activity of LHRH agonists on melanoma cells is still unclear. Because the treatment of the cells with LHRH-A does not affect the level of expression of LHRH receptors, it seems unlikely that it might be related to a receptor down-regulation. Alternatively, LHRH agonists might act by interfering with the activity of locally produced growth stimulatory factors, as previously described for hormone-related tumors (25, 26, 33).

So far, a direct oncostatic activity of LHRH agonists has been reported only for tumors related to the reproductive system. We have previously demonstrated that LHRH analogs inhibit the growth of prostate cancer cells, both *in vitro* (15, 20, 34) and *in vivo* (35), through the activation of locally expressed LHRH receptors. Similar observations have been

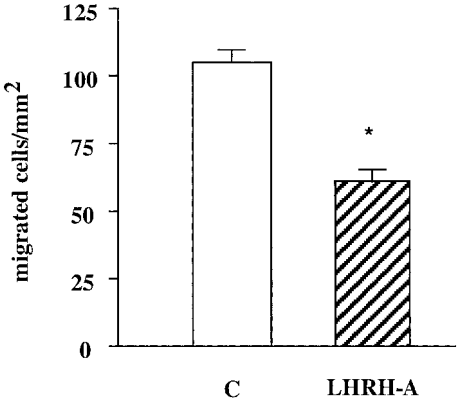

FIG. 8. Effects of the LHRH agonist (LHRH-A) on the ability of BLM melanoma cells to migrate toward a chemotactic stimulus (FBS 5%). *, $P < 0.01$ *vs.* controls (C).

reported for tumors of the female reproductive system (36–42). It is well known that LHRH agonists, given either alone or in combination with antisteroidal agents (9–11), are widely and successfully used for the treatment of hormone-dependent tumors. The anticancer activity of LHRH agonists is mainly based on their ability to suppress the pituitary-gonadal function through the down-regulation of LHRH receptors on gonadotrops. The demonstration of an additional and more direct inhibitory activity of LHRH agonists on steroid-dependent cancer cells has given further support to their clinical application.

The results here reported seem to indicate that LHRH agonists might also exert an antitumor activity in melanoma, a tumor that is not usually included in the list of hormone-related cancers. Another interesting finding of this study is that LHRH agonists significantly reduce the ability of melanoma cells to invade a reconstituted basement membrane and to migrate in response to a chemotactic stimulus. To our knowledge, this is the first time that such an activity is reported for LHRH agonists. It is well known that the main reason for the inefficacy of the clinical treatment of melanoma, in addition to its intrinsic resistance to chemotherapy, is due to its strong ability to rapidly metastasize into target tissues (6). The present results seem to suggest that, in addition to a direct antitumor activity, LHRH agonists might also reduce the metastatic potential of melanoma cells. Further studies are in progress in our laboratory to clarify the mechanism of this antimetastatic activity.

Taken together, the findings here reported might open the way to innovative therapies for skin cancer, aimed at inhibiting both tumor growth and tumor metastastatic behavior. Clinically available LHRH agonists are safe, well tolerated, and have no side effects. However, it must be pointed out that the conventional LHRH agonist preparations have been designed to suppress the pituitary-gonadal functions in which LHRH receptors are present in high concentrations. Therefore, the question of a direct activity of LHRH agonists on tumor cells has been raised. The elucidation of the concentrations of LHRH agonists that might be required in tumor tissues to induce their antiproliferative action might help clarify this issue. This might lead to the development of pharmaceutical formulations or alternative routes of administration to provide the optimal concentration of LHRH agonists at the level of the tumor.

In conclusion, this study represents the first scientific report of a LHRH-based growth inhibitory system present in malignant melanoma cells. This result looks very intriguing for three reasons. First, the finding reveals a new molecular mechanism of autocrine control of melanoma cell proliferation. Second, LHRH receptor activation by means of LHRH agonists significantly inhibits not only the proliferation but also the metastatic potential of melanoma cells. Third, the LHRH receptor might represent a new diagnostic marker for the detection of skin tumors.

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