CHAPTER 5

Investigation of LHRH Receptor Involvement in Melanoma Growth and Progression

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Luteinizing Hormone-Releasing Hormone

The hypothalamic decapeptide luteinizing hormone-releasing hormone (LHRH) is the prime controller of reproductive function (Fink, 1988). It performs this function, after being secreted from the hypothalamus into the hypophysial portal circulation, by binding and activating specific receptors on gonadotrophs (Stojilkovic et al., 1994).

The neurohormone stimulates the synthesis and the release of the two gonadotropins LH and FSH (follicle stimulating hormone), and for this reason, it is also called GnRH (gonadotropin-releasing hormone) (Stojilkovic and Catt, 1995).

Hypothalamic neurosecretory cells release LHRH in a pulsatile way, and LHRH pulses are critical for the maintenance of gonadotropin gene expression and for the physiological pattern of secretion of LH and FSH (Kalra, 1993). The two gonadotropins are themselves secreted in a pulsatile way in the systemic circulation and act on the gonads to regulate gametogenesis and steroid synthesis (Kalra, 1993). Pulsatile administration of LHRH in patients with hypothalamic dysfunction has been shown to induce a regular pattern of gonadotropin secretion, thus restoring the fertility (Conn and Crowley, 1994; Schally, 1994). On the other hand, chronic applications of LHRH or LHRH analogues lead to complete suppression of the reproductive function, due to LHRH receptor down-regulation and desensitization (Stojilkovic and Catt, 1995; Schally, 1994). 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; Schally, 1994).

The human pituitary LHRH receptor (LHRH-r) has been cloned and characterized (Kakar et al., 1992). It encodes a 328-amino-acid protein belonging to the superfamily of seven transmembrane domain receptors. Recently, LHRH and LHRH-r have been consistently shown to be expressed in the brain and in a variety of peripheral organs, both normal and tumoral, where the peptide probably sets in an autocrine/paracrine fashion (Limonta et al., 1992, 1993; Dondi et al., 1994; Imai et al., 1994; Chatzaki et al., 1996). In our laboratory we investigated whether this LHRH-based system might also be present in prostate cancer, and whether it might be involved in the control of tumor growth. These studies were performed in human prostate cancer cell lines, either androgen-dependent (LNCaP) or androgen-independent (DU 145). Our data demonstrated that, in either androgen-dependent or androgen-independent prostate cancer, a local LHRH system is present and might act as a paracrine/autocrine negative regulator of tumor growth (Limonta et al., 1992;
These observations are in agreement with those reported for tumors of the female reproductive tract, such as breast (Kakar et al., 1994; Kottler et al., 1997), endometrial (Imai et al., 1994; Chatzaki et al., 1996), and ovarian cancer (Emons et al., 1993, 2000).

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

LHRH in Melanoma

In a recent paper, it has been reported that binding sites for LHRH are expressed in glioblastoma biopsies; this finding suggests that LHRH receptors might represent a diagnostic marker, and possibly a new therapeutic target, for tumors of the nervous system (van Groeninghen et al., 1998). Since both glial cells and melanocytes share the same neuroectodermal origin, we reasoned that a LHRH-based system (LHRH and LHRH-r), similar to that present in tumors of the reproductive tract, might also be expressed in melanoma cells.

Cutaneous melanoma is one of the most frequent malignant tumors in younger people and is characterized by uncontrollable growth and the 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 past 10 or 20 years, particularly due to early diagnosis, the prognosis 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 normal melanocytes or from potential precursor lesions such as atypical melanocytes or dysplastic or congenital nevi (Herlyn et al., 1987; Albino et al., 1997). The pathology then goes through a radial growth phase, eventually progressing 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 and Herlyn, 1993). Prevention of metastasis is the main goal in melanoma treatment. In current practice, however, this can be achieved only by early detection and excision (Breslow, 1978). A recently introduced new chemotherapeutic drug for metastasized melanoma, temozolomide, failed to offer a significant mean survival improvement compared to the treatment with the traditional cytostatic drug DTGI (decarbazine) (Danny and Wilson, 2000; Hwu, 2000). Both the chemotherapeutics mentioned have thrombocytopenia as the classical side-effect.

The experiments here described were performed to clarify whether an LHRH-based system (LHRH and the respective receptors) is expressed in melanoma cells. We also investigated whether the activation of this system might affect the proliferative rate and the metastatic properties of this tumor. For these studies we used the human melanoma cell line BLM (kindly provided by Dr. Van Muijen, Department of Pathology, University Hospital Nijmegen, The Netherlands). LHRH receptor activation was achieved using a potent LHRH agonist (Zoladex, LHRH-A; provided by AstraZeneca Pharmaceuticals, Divisione Farmaceutici, Milan, Italy).