# mRNA EXPRESSION OF GONADOTROPIN-RELEASING HORMONE (GnRH) AND ITS RECEPTOR IN NORMAL AND NEOPLASTIC RAT PROSTATES

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## Introduction

The hypothalamic gonadotropin-releasing hormone (GnRH or LHRH) is a key hormone in the regulation of male and female reproductive systems through its stimulation of the secretion of gonadotropins, which in turn stimulate the synthesis of sex steroids by the gonads[1]. This peptide hormone is traditionally thought to be produced exclusively by the hypothalamic neurons and functions mainly on the pituitary gonadotrophs through the specific cell surface GnRH receptor. However, a number of recent studies have shown that GnRH or its receptor are also expressed locally in some reproductive organs including prostate gland, mammary gland, ovary and placenta, tumors and cancer cell lines derived from these organs[2-5]. Moreover, it has also been shown that GnRH analogs exert some direct inhibitory effects on the proliferation of human and rat prostate cancer cells, probably mediated by its own specific receptors expressed in these cells[6-9]. In the present study, we studied the mRNA expression of GnRH and its receptor in normal Noble rat prostate gland, and in three rat models of prostate cancer including the sex hormone-induced Noble rat model, an androgen-independent Noble rat prostatic tumor (AIT) and Dunning rat prostatic adenocarcinomas by RT-PCR and Southern blot analyses.

## Methods and Materials

*Noble Rat Model*

Young adult male Noble (Nb) rats were used for RNA extraction and induction of premalignant dysplastic and neoplastic prostates by long-term treatments with androgen and estrogen. For hormonal treatments, the rats were divided into 3 groups: 1) T+$E_2$, 2) T+DES, and 3) control. The rats were implanted with two testosterone- (T) and one 17β-estradiol- ($E_2$) or diethylstilbestrol- (DES) filled

Silastic tubings and treated for 4-9 months[10]. Rats implanted with empty tubings or aged matched rats were used as controls. After treatments or not, individual prostatic lobes (ventral, lateral and dorsal), coagulating gland, seminal vesicle, pituitary gland and hypothalamus were quickly dissected, snap-frozen in liquid nitrogen and stored at –80°C until subsequent RNA extraction. An androgen-independent Nb rat prostatic carcinoma (AIT, kindly provided by Dr. S.M. Ho, University of Massachusetts Medical School) was maintained and grown subcutaneously in castrated Nb rats. This tumor line was originally derived from an estrogen-dependent tumor of the dorsolateral prostate of Nb rats. The tumors were allowed to grow in castrated Nb rats for 2-3 months before harvest.

*Dunning Rat Prostatic Tumor Model*

An androgen-dependent Dunning rat prostatic adenocarcinoma, R3327H (kindly provided by Prof. Y.C. Wong, University of Hong Kong), were maintained and grown subcutaneously in male nude mice, which were supplemented with one T-filled Silastic tubing for 2-3 months before harvest. Two androgen-independent sublines were developed from the original R3327H tumor: DT-AIM subline being grown in castrated male mice and DT-AIF being grown in female mice. The dissected tumors were quickly frozen in liquid nitrogen for subsequent RNA extraction.

*RT-PCR*

Total RNAs, extracted from the homogenized frozen tissues by TRIzol reagent, were reversely transcribed to first strand cDNA using a commercial kit (SuperScript II preamplification system, Life Technology). Two pairs of primers specific for rat GnRH and three pairs of primers for rat GnRH receptor were designed for PCR, based on the published sequence of rat hypothalamic GnRH cDNA[11] and rat pituitary GnRH receptor cDNA[12].

*Cloning and Sequencing Analysis of PCR Products*

PCR products of GnRH cDNA amplified from the normal Nb rat ventral prostate and hypothalamus, Dunning and AIT tumors, and cDNA of GnRH receptor amplified from the rat pituitary gland were subcloned into pBluescript II SK vector and then DNA sequenced. The clones with the correct sequences were used for synthesis of probes for Southern blot analysis.

*Southern Blot Analysis*

The PCR products of GnRH and GnRH receptor amplified in different samples were analyzed by Southern blotting using a rat GnRH cDNA probe (230 bp) cloned from the hypothalamus and a rat GnRH receptor probe (342 bp) cloned from the pituitary gland.

**Results and Discussion**

The results showed that mRNA expression of GnRH was detected in the normal and long term hormone-treated Nb rat prostates, AIT and the three Dunning tumor lines, in addition to its positive

control expression in the hypothalamus. The detection of GnRH mRNA in these prostatic tissues suggests that this peptide hormone could be synthesized locally in these glands and tumors. However, the exact function of the locally produced GnRH in the normal prostate gland, its role in the early development of premalignant lesions and its exact cellular sources are still unclear. *In vitro* studies using GnRH or its analogs have demonstrated that this peptide hormone may exert some direct inhibitory action on the prostatic tumor cell proliferation and counteract the stimulatory effect of some stroma or tumor cell produced growth factors. The expression of GnRH receptor was only detected in the androgen-dependent Dunning R3327H tumor. The detection of both GnRH and its receptor in the androgen-dependent Dunning R3327H tumor tissue suggests that this peptide hormone may have some autocrine and paracrine regulatory functions in this tumor. This regulatory loop formed by GnRH and its receptor is speculated to counteract other stimulatory regulatory loops by EGF or TGFα in the prostate tumors. However, the gene expression of GnRH receptor was not detected in the two androgen-independent Dunning tumor sublines, which were derived from the original R3327H and also the Noble rat prostatic tumor, AIT. The disappearance of the GnRH receptor expression indicates that the growth inhibitory regulatory loop of GnRH, as formed in the androgen-dependent Dunning tumor, is lost or down-regulated when the tumors progress to a hormone-dependent phenotype.

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