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Gonadotropin-releasing hormone receptors as molecular therapeutic targets in prostate cancer: Current options and emerging strategies

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ABSTRACT

Prostate cancer is androgen-dependent in its early stages and androgen deprivation therapy represents the most effective first-line therapeutic approach. However, after an initial remission, prostate cancer progresses towards the castration resistant prostate cancer (CRPC) stage, with increased malignancy and resistance to conventional chemotherapy.

Pituitary gonadotropin-releasing hormone receptors (GnRH-Rs) represent the most effective molecular target for the treatment of steroid-dependent prostate cancer. GnRH agonists (through GnRH-Rs desensitization) suppress the pituitary–testicular axis and, therefore, represent the treatment of choice for prostate cancer patients.

GnRH-Rs are also expressed in prostate cancer, even when the tumor has reached the CRPC stage, and are endowed with antitumor activity, supporting the notion that they might represent a molecular target for GnRH analog-based therapeutic strategies. In addition to GnRH agonists and antagonists, GnRH-based bioconjugates (cytotoxic GnRH bioconjugates, GnRH-conjugated lytic peptides and GnRH-toxin bioconjugates) have been developed and are now undergoing intensive investigations; some of them (*i.e.*, AN-152, Dox-[D-Lys^6]-GnRH) have entered clinical trials. The advantage of these treatments is the specific delivery of cytotoxic agents to cancer cells. Interestingly, other isoforms of the peptide have been identified. One of them is GnRH-III, which was isolated from sea lamprey. GnRH-III specifically binds to GnRH-Rs in cancer cells and exerts antiproliferative effects; on the other hand, its endocrine effects at pituitary level are insignificant, supporting its selective antitumor activity. Based on these observations, different cytotoxic GnRH-III bioconjugates have recently been synthesized; preliminary *in vitro* studies suggest that these compounds might represent a new promising treatment strategy for prostate cancer.

Introduction

Prostate cancer still represents a major medical burden, being the most common cancer in men and the second leading cause of cancer-related deaths among men in Western countries.[1] Most prostate cancers are dependent on androgens for growth and survival. Thus, even if the majority of patients are effectively cured with definitive primary treatments, such as radical prostatectomy or radiation therapy, for high-risk locally advanced or metastatic prostate cancers, the most effective treatment is represented by androgen ablation therapy, aimed at blocking androgen secretion/activity.[2,3] This therapy includes chemical castration, which can be achieved either by gonadotropin-releasing hormone (GnRH) agonist monotherapy or by a GnRH agonist in combination with a pure antiandrogen (combined androgen blockade, CAB).[4–12] Combined androgen blockade, avoiding the initial flare and providing long-term maximal androgen deprivation, demonstrated an overall survival advantage compared with GnRH agonist monotherapy.[2,4–6,12] More recently, GnRH antagonists have been introduced in the clinical setting based on their ability to avoid testosterone surge and clinical flare, while improving testosterone control and prostate-specific antigen (PSA) progression-free survival.[13]

Unfortunately, despite an excellent initial response, in approximately 2–3 years most prostate cancer will progress to castration resistant prostate cancer (CRPC) stage with increased growth, invasion and malignancy.[14–17] Once the stage of CRPC is reached,

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prostate cancer has historically been considered refractory to chemotherapy. Initially, mitoxantrone-based chemotherapy plus prednisone was introduced for treating CRPC patients in order to reduce the duration and intensity of pain, although it did not improve survival.[18] Later, the agents docetaxel and cabazitaxel were reported to provide a modest survival benefit (nearly 2–3 months) in CRPC patients as first- and second-line chemotherapy, respectively; however, the majority of treated patients progressed within a few months and required additional treatments.[19–21] Efforts are now being made in order to elucidate the molecular mechanisms underlying metastatic CRPC progression; these include: multidrug resistance mechanisms, specifically by escape from apoptosis protecting cancer cells against cytotoxic drugs; change in the balance between pro- and antiapoptotic factors; reactivation of the androgen receptor axis (receptor amplification, mutations, transactivation); activation of growth factor signaling pathways; development of immunologic tolerance together with the failure of the body's immune system to elicit antitumor effects.[16,17,22–24] These efforts have led to the development and clinical evaluation of new drugs, such as cabazitaxel, sipuleucel-T, abiraterone, MDV3100, etc.[25–31]

More recently, targeted therapy has become an attractive innovative strategy to overcome tumor growth. This strategy is based on the rationale that ligands of receptors highly expressed on tumor cells may target anticancer drugs directly to the tumor, thus enhancing the specificity of these compounds, while decreasing their toxicity. To this purpose, it must be recalled that receptors for the decapeptide GnRH are expressed in prostate cancer cells, and their expression persists after prolonged GnRH analog treatment, suggesting the potential therapeutic utility to target the receptor even when the tumor has become castration resistant.[32]

This review focuses on the role of pituitary and cancer GnRH receptors as molecular targets in the treatment of prostate cancer: current options as well as emerging strategies will be addressed.

GnRHs and GnRH-Rs

GnRH (also designated GnRH-I)

GnRH was first identified as the hypothalamic decapeptide (Table 1) that plays a key role in the control of the reproductive functions.[33–38] It is synthesized in a small number of hypothalamic neurons and released in a pulsatile manner into the hypophyseal portal circulation through which it reaches the anterior pituitary. By binding to specific receptors (GnRH-Rs) on pituitary gonadotropes, the decapeptide stimulates the synthesis and the release of the two gonadotropins (luteinizing hormone, LH and follicle stimulating hormone, FSH), thus regulating gonadal steroidogenesis in both sexes.[34,39,40]

In humans, the GnRH gene is composed of four exons separated by three introns and resides as a single gene copy on chromosome 8.[41,42] This gene encodes a precursor protein of 92 amino acids, consisting of a signal sequence (23 amino acids) sequentially followed by two serine residues, the GnRH decapeptide, a GKR sequence and a 56-amino acid peptide called GAP (GnRH-associated peptide).[34–37]

Table 1
Amino acid sequences of natural GnRH isoforms.

GnRH (also designated GnRH-I)	Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
GnRH-II	Glp-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2
GnRH-III	Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2

Glp: pyroglutamic acid.

The human pituitary GnRH-R is a 328-amino acid protein encoded by a gene located on chromosome 4. This receptor belongs to the family of rhodopsin-like G protein coupled receptors (GPCR) containing seven transmembrane domains and an extracellular 35-amino acid amino-terminal domain with two putative glycosylation sites.[35,43–48] Interestingly, this receptor is characterized by the lack of a carboxy-terminal cytoplasmic tail; therefore, it internalizes relatively slowly and it does not rapidly desensitize.[49–53] Upon hormone binding, pituitary GnRH-Rs couple to a $G_{\alpha q/11}$ protein stimulating the activity of phospholipase Cβ (PLCβ). As a consequence, intracellular levels of inositol triphosphate (IP_3) and diacylglycerol (DAG) increase, leading to intracellular Ca^{2+} mobilization and protein kinase C (PKC) activation, respectively. Downstream GnRH-Rs activate other important signaling pathways operating within the mitogen-activated protein kinase (MAPK) cascades, such as ERK, JNK and p38 kinase. Finally, phospholipase D and phospholipase A2 are also sequentially activated.[35,37,43,53–57] All these pathways are important for GnRH-mediated effects on gonadotropin synthesis and secretion.

Pituitary GnRH-Rs require pulsatile stimulation by GnRH to activate their intracellular signaling pathways and ultimately to synthesize and release LH and FSH. Native GnRH is administered in a pulsatile delivery pattern to restore gonadal functions in patients with hypogonadotropic hypogonadism.[58,59] On the other hand, sustained stimulation of these receptors by natural GnRH or agonists, after the initial flare event, desensitizes gonadotrope cells with a consequent decrease in serum gonadotropin levels; this leads to a decline in gonadal functions, thus resulting in medical castration.[11,34,36,37,60] Sustained, and at high doses, the administration of GnRH agonists is currently used in endocrine disorders, such as endometriosis, adenomyosis, uterine leiomyomas, polycystic ovarian disease and precocious puberty. GnRH agonists are also used to suppress gonadal function in *in vitro* fertilization/assisted reproductive technologies.[61–63]

GnRH antagonists bind to pituitary GnRH-Rs competing with the endogenous GnRH, thus immediately suppressing the pituitary–gonadal functions in the absence of the initial flare. Based on this mechanism of action, these compounds are utilized for the treatment of endometriosis, benign prostatic hyperplasia and for *in vitro* fertilization.[64–66] Currently, both GnRH agonists and antagonists are indicated for the treatment of steroid-dependent malignancies, such as breast, ovarian, endometrial and prostate cancer.[4,12,38,67–73]

During the last 20 years, it has become increasingly clear that GnRH and GnRH-Rs are also expressed in several tumors, both related and unrelated to the reproductive system. In these tumors, GnRH behaves as a paracrine–autocrine growth factor endowed with a strong antitumor activity. These findings support the concept that tumor GnRH-Rs might represent a direct target for GnRH analog-based anticancer strategies (see below).[36–38,67–69,74–77]

GnRH-II

At present, it is known that, in addition to the hypothalamic GnRH, several other isoforms of the decapeptide exist in vertebrates. All these isoforms are characterized by the conservation of the length of the peptide as well as by the amino acid sequences of the N-terminal (Glp-His-Trp-Ser) and C-terminal (Pro-Gly-NH_2) domains, indicating that these molecular features are critical for the binding to, and the activation of, the receptor. In particular, a second form of GnRH was first isolated from chicken brain, and its amino acid sequence was shown to be highly conserved from fish to mammals[35,36,43,78–84] (Table 1). This form has been designated GnRH-II, while the classical hypophysiotropic form is often designated GnRH-I. In humans, the decapeptide GnRH-II is encoded by a gene located on chromosome 20; it is characterized