Luteinizing hormone-releasing hormone (LHRH) receptor agonists vs antagonists: a matter of the receptors?

Yuri Tolkach, Steven Joniau* and Hendrik Van Poppel*

*Urology Clinic, Military Medical Academy, Saint-Petersburg, Russia, and *Department of Urology, University Hospital Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium*

Luteinizing hormone-releasing hormone (LHRH) agonists and antagonists are commonly used androgen deprivation therapies prescribed for patients with advanced prostate cancer (PCa). Both types of agent target the receptor for LHRH but differ in their mode of action: agonists, via pituitary LRHR receptors (LHRH-Rs), cause an initial surge in luteinizing hormone (LH), follicle-stimulating hormone (FSH) and, subsequently, testosterone. Continued overstimulation of LHRH-R down-regulates the production of LH and leads to castrate levels of testosterone. LHRH antagonists, however, block LHRH-R signalling causing a rapid and sustained inhibition of testosterone, LH and FSH. The discovery and validation of the presence of functional LHRH-R in the prostate has led to much work investigating the role of LHRH signalling in the normal prostate as well as in the treatment of PCa with LHRH agonists and antagonists. In this review we discuss the expression and function of LHRH-R, as well as LH/human chorionic gonadotropin receptors and FSH receptors and relate this to the differential clinical responses to agonists and antagonists used in the hormonal manipulation of PCa.

Keywords

androgen deprivation therapy, LHRH agonist, GnRH antagonist, LHRH receptor, prostate cancer

Introduction

The most common type of treatment prescribed for patients with advanced prostate cancer (PCa) is LHRH agonists and these are increasingly being used in patients with non-metastatic disease or recurrent disease after attempted curative treatment [1]. Eventually, the disease will progress in every patient despite the persistence of castrate levels of androgens (<1.73 nmol/L or 50 ng/dL) and yet may remain hormone-sensitive. In this situation the recommended option is to perform secondary hormonal manipulations such as adding or withdrawing an antiandrogen, changing antiandrogen, adding an oestrogen compound or changing agonist [1].

The suppression of testosterone can also be achieved with the use of a LHRH antagonist and the clinical efficacy of these agents is well established [2–5]. Furthermore, LHRH antagonists may be associated with improved disease control compared with LHRH agonists [6]. This benefit could be explained by the different action of agonists and antagonists on the LHRH receptor (LHRH-R), the mechanism of which has been intensively investigated for the last 30 years. LHRH agonists, by continually stimulating the LHRH-R, down-regulate receptor expression in the pituitary leading to decreased levels of LH, and to a lesser extent, FSH. Before this inhibition occurs, however, there is an initial increase in LH, FSH and testosterone levels. LHRH antagonists, by contrast, directly block the effect of LHRH on the pituitary, causing a rapid and sustained inhibition of testosterone, LH and FSH. The present review considers the actions of agonists and antagonists on the different types of receptors present on tumour cells and their potential clinical implications.

LHRH-R Expression

The LHRH-R was originally shown to be expressed primarily in the pituitary and to be responsible for eliciting the actions of LHRH released from the hypothalamus; pituitary tissues have a ~190-fold higher expression level of LHRH-R than normal prostate tissue [7]. It is now known that LHRH-R is relatively highly expressed in the pituitary, breast, prostate, kidney, thymus and in lymphocytes [8–10] and at lower levels in a variety of other organs [9]. The

detection of LHRH-R in these tissues suggests that LHRH agonists and antagonists may also have direct actions on peripheral targets; however, the precise role of LHRH-R in extrapituitary tissues and whether it is responsible for the progression of prostate tumours which may also express LHRH-R and produce LHRH for autoregulation is not known [8].

LHRH-R Expression in the Prostate

Several studies have shown that normal prostatic tissue expresses LHRH-R [7,9,10], but at lower levels compared with PCa cells [9] and there is strong evidence, elicited from multiple studies using human cancer specimens, cell lines and animal experimental models, that PCa cells express LHRH-R on their surface [11–18]. Overall, the expression of LHRH-R has been found on cancer cells in up to 100% of patients with PCa [13]. The main limitation of these studies is that, although they detect the presence of LHRH-R (e.g. by PCR, ligand binding or immunolabelling) the potential physiological impact of receptor signalling has not been assessed. It is important to outline that BPH cells also show substantial expression of LHRH-R, which is detectable in up to 95–100% of patients [13,16]. Consequently, the functional role of LHRH and the LHRH-R in the normal prostate and BPH gland is still under active investigation.

Expression of LHRH-R by Tumour Grade, Stage and Agonist or Antagonist Pretreatment

Tumour grade can have a substantial influence on the density of LHRH-R expression [12,13]. This means that more aggressive tumours, not treated using androgen withdrawal strategies, were more likely to have a low LHRH-R density on their surface which could partly explain the worse efficacy of LHRH agonists in these patients. Another way to describe the density of a receptor's expression is to assess binding capacity. Biochemical analysis of tumour samples has shown that a higher Gleason score correlates with a higher LHRH-R binding affinity (greater intermolecular force between the ligand and receptor) but a lower expression level and binding capacity [12,13]. High-affinity binding of a ligand to its receptor could be physiologically important, resulting in altered activity of an associated ion channel or enzyme. Another important consideration is that high-affinity binding could be used for targeting these cells with cytotoxic agents [12].

Several studies have gathered clinical data [19,20] to assess the concept that LHRH-R expression is related to the pathological stage of the tumour. In hormone-naïve patients with tumour detected histologically in the lymph nodes, <16% expressed LHRH-R mRNA [20]. This is substantially lower than ever identified in the primary tumour and raises some concerns regarding the methodology of study, although this low expression could be one of the changes in tumour cells leading to metastatic behaviour.

Assessment of LHRH-R expression on tumour cells of patients with hormone-refractory PCa after palliative TURP has shown that LHRH-R mRNA was detectable in all hormone-refractory PCa samples, although there was no significant correlation with other clinical data [19]. This compares with 46% of hormone-naïve PCa samples expressing LHRH-R in the same study. These results correlate with those from *in vitro* measurements, which showed high receptor expression in both androgen-dependent and -independent cell lines [21–24]; however, another clinical study showed that LHRH-R expression was independent of the tumour pathological stage [12] but was dependent on tumour grade. This could mean that cellular changes drive LHRH-R down-regulation which could further enhance effective tumour spread. Treatment with both LHRH agonists and antagonists has been shown to down-regulate the expression of LHRH-R in experimental [23,25,26] and clinical settings [12,15].

These studies suggest that short-term exposure to an LHRH agonist could lead to up-regulation of LHRH-R, independently of the androgen-sensitivity of the tumour, whereas long-term treatment will probably result in down-regulation of expression. For the moment there is no precise explanation for this phenomenon, but one idea is that LHRH-R mRNA levels, measured in many studies, do not necessary reflect actual LHRH-R expression. Furthermore, a decrease in the binding affinity of LHRH-R could regulate receptor function without significant changes in overall expression.

An important insight was gained into the control of LHRH-R expression in the absence of PCa by a study on rats. Castration or treatment with agonist or antagonist for 28 days showed that LHRH-R over-expression in these three situations seems to be a reaction to the reduced testosterone levels and related epithelial atrophy, i.e. the protective pathway of cell maintenance in a compromised environment [7]. Otherwise, it is difficult to explain why prostatic cells express LHRH-R in the presence of elevated LHRH. Interestingly, it has been suggested that the presence of LHRH-R on PCa cells could be an indicator of good outcome in the advanced stage of disease, while the absence of this receptor could suggest a worse prognosis [27].

In summary, cell changes occurring during the natural course of PCa progression could drive changes in LHRH-R expression or function which are currently poorly

understood and may be further complicated by the use of agonists and antagonists. Understanding the expression pattern is only the first step and further investigation of receptor functionality at different stages of the tumour life-cycle is warranted.

Clinical Implications of LHRH-R Function in Models of PCA

Role of Endogenous LHRH

One of the most important concepts regarding LHRH-R in extrapituitary tissues is that it is part of local autocrine/paracrine loop which regulates growth and proliferation [21,26,28,29]. This conclusion was reached as a result of multiple studies which were able to identify mRNA or protein of LHRH type I and II or LHRH-like peptides in PCa samples, the culture medium of cell lines (DU-145, LNCaP and PC-3) [22,26,28,30,31] as well as in both androgen-dependent and -independent rat models of PCa [32]. These ligands were also present in BPH tissue, which confirms the natural origin of this regulatory loop [29,32,33]. Interestingly, Azad et al. [33] showed that 2 weeks after castration of healthy rats LHRH mRNA levels increased thirteenfold, leading to a substantial increase in the concentration of LHRH in the prostate. It is also possible that the tumour micro-environment contains a pool of LHRH precursor, which could be converted to mature LHRH immediately after castration [33]. By using these two mechanisms the tumour could possibly regulate its internal concentration of LHRH to effectively maintain growth potential and protect itself.

Role of LHRH Agonists and Antagonists

It is likely that agonists and antagonists act directly on normal and cancerous cells of the prostate as it is almost universally recognized that both express LHRH-R. To confirm this, it has been demonstrated that LHRH-R expressed on PCa cells are functional. Evidence for this includes inhibitory studies in cell lines, experimental animal models of PCa and the failure of the LHRH-R-induced antiproliferative effect when silencing RNA is introduced to cells.

Multiple *in vitro* studies have shown that LHRH agonists exert an antiproliferative effect in a dose-dependent manner on PCa cells [26,34–37]. For example, one investigation showed that by blocking LHRH-R expression with silencing RNA, the antiproliferative effect of leuprorelin was inhibited, confirming that effects on proliferation are mediated via the LHRH-R pathway [38].

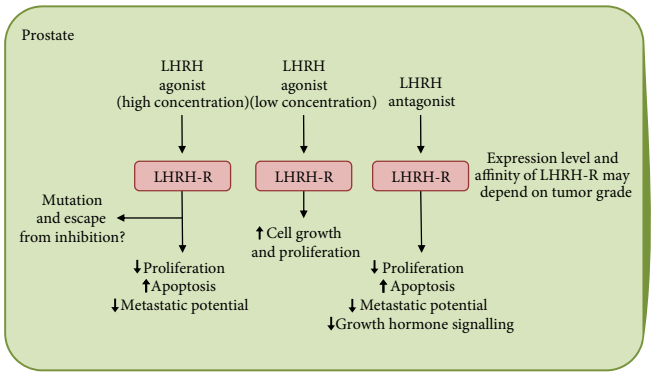
The antiproliferative effect of LHRH agonists, however, is not as straightforward as it might first appear. A study by Qayum et al. [26] showed that in androgen-sensitive cells the antiproliferative effect of agonist (buserelin) was only evident at high concentrations, while in androgen-insensitive cells buserelin elicited no effect at all, which the authors explained as being attributable to the presence of low-affinity LHRH-R on the latter cell line. Moreover, the same study showed that buserelin treatment of androgen-sensitive cells was biphasic; a low concentration was stimulatory for cell growth (up to 40% compared with controls), whereas a high concentration inhibited growth (by up to 35%). Adding a hundredfold excess concentration of antagonist to cell cultures partially blocked the stimulatory effect and almost completely blocked the inhibitory effect of high concentrations of buserelin. This corresponds with the findings of Ravenna et al. [39] who showed that inhibition of growth of androgen-sensitive but not insensitive cells was evident while on triptorelin treatment and that the inhibition of proliferation was blocked by addition of the antagonist cetrorelix. GnRH antagonists also exhibit an antiproliferative effect on tumour cells; several studies have reported that cetrorelix also inhibits growth of androgen-insensitive cells in culture and xenografts in nude mice [25,36,40,41].

Proposed Antiproliferative Mechanisms

Numerous studies have investigated potential mechanisms underlying the antiproliferative effect of the LHRH-R pathway, and the results fall broadly into three categories: induction of apoptosis; inhibition of growth factor pathways; or decreased metastatic potential. Induction of apoptosis has been reported for agonists via increased expression and phosphorylation of p53 [42] and altered expression of *bcl-2, bax* and *c-myc* [43] and for both agonists and antagonists by changes in the ratio between neurotrophin receptors TrkA and p75 [37] and activation of the protein kinase C (PKC)/mitogen-activated protein kinase (MAPK) pathways [44].

There is a body of evidence showing the possible attenuation of growth factor-mediated mitogenic signalling through high-affinity LHRH-R [8,35,45,46]. Several articles showed that treatment with different antagonists, but not agonists, led to a significant decrease in tumour volume *in vivo*. This was accompanied by a substantial decrease in the expression of high-affinity epithelial growth factor (EGF) receptor [40,41,47] and EGF itself [47] after antagonist treatment and is evidence of a possible link between the LHRH-R and EGF receptor pathways [40,41,47,48]. One study has reported a small but significant decrease in the mRNA of EGF receptor whilst on antagonist treatment [25]. Several studies have shown that antagonists could also exhibit a negative influence on the expression and function of the IGF-I receptor and IGF type II production by tumour tissue [48–51].

Fig. 1 Possible direct effects of LHRH agonists and LHRH antagonists on the prostate. LHRH agonists may have a biphasic effect in the prostate; a low concentration may drive proliferation while high concentrations may produce antiproliferative effects. There is also some evidence that prolonged stimulation with LHRH agonists can drive genetic mutations in the signalling pathway allowing escape from inhibition. LHRH antagonists do not stimulate signalling, leading to reduced proliferation, increased apoptosis and reduced metastatic potential. It is also possible that antagonists inhibit the growth factor pathways stimulated by EGF and IGF, providing another mechanism of reducing cell proliferation.



The metastatic potential of the tumour may be further reduced by the slowed degradation of the extracellular matrix [34], up-regulation of cell adhesion molecules (E-cadherin, β- and γ-catenin) [52], antagonism of testosterone activation of androgen receptors [53,54] and suppression of the extracellular signal-regulated kinases (ERK) cascade by LHRH agonists [45] as well as by inhibition of the plasminogen activator system by both LHRH agonists and antagonists [34]. The direct effects of agonists and antagonists on the prostate discussed above are shown in Fig. 1.

Differentiating between Agonist and Antagonist Effects on the LHRH Pathway

Recently, with the adoption of LHRH antagonists as an alternative therapy for patients in need of androgen suppression, potential differences in efficacy compared with agonists have been under investigation. An exploratory analysis of one recent study showed that, in patients with baseline PSA > 20 ng/mL, the PSA failure rate during the first year of treatment was lower on degarelix than on leuprolide [6].

An important fact to consider is that the affinity (potentially the key factor of its functional behaviour), capacity or density of expression of LHRH-R can change according to conditions. It seems that an initial high tumour grade and tumour de-differentiation in the natural course of disease or under LHRH agonist treatment leads to decreased affinity (desensitization) and decreased density of LHRH-R expression. Some tumours completely lose their LHRH-R, but it is not clear which tumours will shed their receptors, although it seems to be the consequence of early alterations because this state is possible even in low-grade tumours. It is also unclear which factor is more important for receptor down-regulation: initial tumour grade or LHRH agonist treatment.

As it has been shown that low concentrations of LHRH agonists may be stimulatory for prostate cell growth [26], the regulation of the expression of LHRH-R within an advanced tumour may allow the switching of agonists to a stimulatory route. It could even be hypothesized that, at an advanced stage, it becomes a 'closed' system and cells become sensitive only to LHRH-like peptides synthesized within the tumour, possibly also accompanied by LHRH-R mutation (although this has never been shown experimentally). This partly corresponds to the data on stimulation of androgen receptors by the autocrine androgens in the castration-refractory tumours shown by de Bono et al. [55].

For antagonists, however, there is evidence of a direct antiproliferative effect but little evidence for stimulation of tumour growth. Indeed, they have been shown to block the stimulatory activity of agonists on tumour growth under various circumstances [26,39]. This could be interpreted as an important rationale for antagonist treatment in patients with advanced disease when the tumour becomes a 'closed' system, particularly as antagonists have a higher affinity for the LHRH-R than agonists, and possibly than autocrine LHRH-peptides synthesized by the tumour itself. Thus, antagonists would not exhibit the biphasic activity reported for agonists. Evidence for the efficacy of LHRH antagonists as second-line agents is limited, however, with studies showing responses in 10 and 17% of patients when treatment with an LHRH agonist was changed to abarelix or degarelix, respectively [56,57].

A final consideration is that constant stimulation of this antiproliferative mechanism via the LHRH-R can eventually exhaust it and lead to additional mutations allowing the tumour to escape from this down-modulation. In theory, an antagonist should not drive tumour mutations in this pathway as they block signalling through the LHRH-R and therefore reduce stimulation of this and other associated pathways, such as EGF and IGF.

LHRH-R Summary

It is well understood that non-clinical studies are limited in that they try to investigate the positive anti-tumour function of LHRH-R pathway in artificial situations. For example, using cell cultures or isolated tissue removes the influence of the pituitary, hypothalamus and gonads, when using xenografts the interaction with the normal host could be compromised and finally, data from experimental

animal models do not always match that of clinical studies. Unsurprisingly, therefore, the results of these studies raise further questions. If LHRH-R is only an antiproliferative pathway, why is the receptor overexpressed in normal prostate tissue after castration or with an LHRH agonist or antagonist treatment? Furthermore, why does this occur in parallel with prostatic atrophy on a background of documented testosterone depletion and, importantly, increased LHRH concentration? Part of the answer is that this could be the 'initial' response, because studies of LHRH agonist treatment with longer follow-up show down-regulation of the receptor. Moreover, it appears that the LHRH-R pathway is not solely antiproliferative, but a more complicated defence mechanism, helping to maintain cell growth in critical situations and compromised environments, so measuring the expression of LHRH-R is only the beginning of the story. Further functional studies are warranted to investigate autocrine/paracrine regulation within the tumour, possible interconnections of the LHRH-R pathway and, most importantly, the functional behaviour of the LHRH-R in conditions as close to those found in the human body as possible, especially in the changing environment of hormonal treatment.

The LHRH and its receptor should be thought of as part of a multifaceted pathway which includes interactions with growth factor pathways, the androgen receptor pathway and even with the systems of cell adhesion and plasminogen activation. PCa cells, in contrast to other tumours, are often exposed to agonist pressure for extended periods of time, explaining why mutations accumulate to counteract the inhibition of the LHRH pathway. Its multifaceted nature, therefore, could make a substantial contribution to tumour autonomy and insensitivity to standard treatment after eventual escape from LHRH agonist.

Expression and Function of the LH Receptor and FSH Receptor

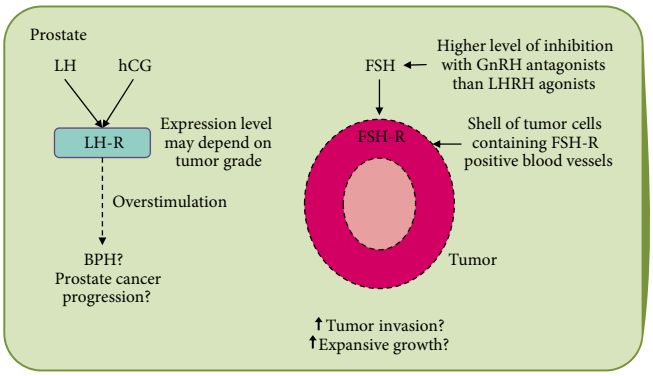
Besides the LHRH-R, other receptors such as the LH receptor (LH-R) and FSH receptor (FSH-R) may be involved in the response to LHRH agonist and antagonist activity. Several studies have shown expression of the high-affinity LH-R in human prostate epithelial cells, BPH epithelial cells and stroma and the epithelia of seminal vesicles [58–61]. The highest levels of expression are seen in the peripheral regions of the central zone of the normal prostate [58] and the lowest levels in atrophic glands [60]. This means that the LH-R pathway is probably used by epithelial cells under normal conditions.

Some studies have also shown that PCa cells express LH-R, but at a lower level than in normal prostate tissue and in BPH [60]. Immunostaining showed that tumour grade correlated negatively with the density of receptor expression. Thus, high grade tumours showed weak expression, while low grade tumours expressed LH-R more intensely. Expression was also shown at the protein level for the androgen-sensitive and -insensitive cell lines, with higher expression in the former [60,62].

Importantly, there are several studies indicating that prostate cells contain mRNA for LH, hCG and LH-R, suggesting the presence of an autocrine/paracrine regulatory loop with the primary function of promoting cell growth [62,63]. Excessive activation of the LH-R pathway could be one of the causes of BPH [63]. Moreover, stimulation of the LH-R pathway may up-regulate steroidogenesis within the tumour, making cells more viable and resistant to unfavourable conditions [62], although it is unclear if the LH-R pathway activation is relevant to PCa progression (Fig. 2). Nevertheless, the only current treatment which would prevent this activation is an LHRH antagonist, as clinically a pulse or constant increase in LH production is always observed while on LHRH agonist treatment or after surgical castration.

It is thought that FSH and its receptor could play an important role in the progression of PCa as an autocrine or paracrine factor [63–67] and/or as a result of extraprostatic FSH stimulation of FSH-R on tumour blood vessels [3]. Evidence of FSH-R expression is mixed; reports have shown that FSH-R is expressed by both androgen-sensitive and -insensitive cell lines and by samples of human prostate adenocarcinoma [68]. By contrast, another study

Fig. 2 Possible impact of LH-R or FSH-R stimulation in the abnormal prostate. High affinity LH-R is expressed in the normal and diseased prostate and prostate cells may also express LH and hCG, forming an autocrine or paracrine loop promoting cell growth. It is unclear if the LH-R pathway is relevant to the development of BPH or PCa progression. FSH, produced by the pituitary or prostate itself, stimulates FSH-R expressed by tumour blood vessels in a shell on the periphery of the tumour. The location of FSH-R has been proposed to be related to a possible role in the expansive growth or invasive properties of the tumour. Currently, the impact of LHRH agonists and LHRH antagonists on these receptor pathways is not fully understood.



showed that androgen-insensitive cells expressed FSH-R, while sensitive cells did not [69]. The response of androgen-insensitive cell lines to FSH-R stimulation suggests FSH-R could have a proliferative function in the castrate-refractory tumour state. In human hormone-naïve PCa samples, FSH-R expression was found in 21 out of 30 cases (70%) compared with 69% of normal prostate cases and 53% BPH cases. In general, PCa samples expressed FSH-R at a higher level than that found in normal prostate tissue and BPH glands [69].

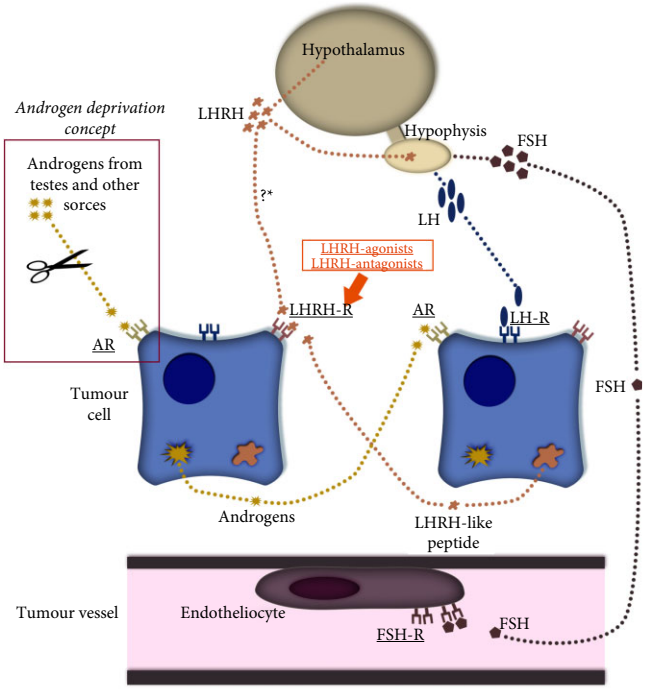
The presence of FSH-R on the surface of tumour vessel endothelial cells in patients with PCa has been confirmed by Radu et al. [64]. FSH-R-positive vessels were located on the periphery of the tumour in a shell 7–15 mm in thickness, extending toward the central part of the tumour and normal tissue by a few mm. FSH-R was not expressed in normal tissue located more than 10 mm from the tumours. Also, tumour lymphatic vessels did not express FSH-R. All prostatic carcinomas tested expressed FSH-R with this distribution pattern in contrast to only 20% of cases in patients with BPH (where expression was more diffuse throughout the hyperplastic areas). Radu et al. suggest that FSH-R distribution could be linked with tumour invasion and expansive growth at the tumour periphery (Fig. 2).

A recent study by Klotz et al. [2] showed that in patients receiving an LHRH agonist (leuprolide) as primary treatment, FSH serum concentration is significantly higher than that of those patients receiving LHRH antagonist (degarelix) after 1 year of treatment. This is an interesting finding in the light of the higher PSA failure rate in some patients treated with agonist. This effect on FSH has also been shown for the antagonist abarelix [70]; thus blocking the FSH-R pathway could be beneficial for tumour control. One study by Beer et al. [71] showed 31% of patients who received abarelix as second-line treatment for PSA progression after castration experienced reductions in PSA level ranging from 9 to 32% on the background of the declined FSH concentration (although they had not met criteria for PSA response, defined as a 50% reduction

In conclusion, the FSH/FSH-R axis may play an important role in tumour progression; blocking this pathway could be beneficial for tumour control in patients progressing on an agonist and more studies are warranted to investigate this important hypothesis. Importantly, these studies need to account for both incomplete suppression of LHRH production when switching agonists and the effect of performing surgical castration after PSA failure on agonist treatment which previously produced a substantial PSA response [72,73].

Besides the accepted mechanism of inhibiting the pituitary axis, LHRH agonists and antagonists act directly on LHRH-R found on PCa cells. This important concept is supported by a plethora of data showing that prostate tumours express an abundance of LHRH, FSH and LH/hCG receptors which can be activated by ligands originating from both extraprostatic sources as well as from autocrine/paracrine pathways within the tumour, ultimately playing an important role in tumour growth (Fig. 3). These findings could explain the differences in the effects of the most commonly prescribed LHRH agonists and antagonists in patients with advanced disease. Furthermore, the action of this receptor network may ultimately explain the different clinical profiles of agonists and antagonists under different disease conditions. Clearly, more studies are necessary to fully characterize the direct action of agonists

Fig. 3 Summary of the potential roles of LHRH-R, LH-R and FSH-R in the growth and expansion of prostate tumours. LHRH-R is highly expressed in the pituitary but is also found in other tissues including the prostate and on PCa cells. PCa cells may also express the AR and LH-R. Since these cells can produce LHRH and/or LHRH-like peptides and androgens, the potential exists for a strong autocrine/paracrine stimulatory growth pathway. LHRH agonists and antagonists probably act directly on PCa cells, potentially inhibiting tumour growth via a number of proposed mechanisms. This is complicated by variations in LHRH-R expression, affinity and receptor shedding at different tumour grades and stages and in response to agonist or antagonist treatment. The FSH-R exhibits an expression pattern restricted to tumour vessels in a shell around the tumour, leading to the suggestion that the FSH/FSH-R axis is important for tumour invasion and growth into surrounding tissue. AR, androgen receptor. *It is unclear whether LHRH synthesized in the hypothalamus interacts with the LHRH-R expressed by tumour cells.



and antagonists on PCa cells and the possible implications for clinical practice.

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Conflict of Interest

None declared.

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Correspondence: Yuri Tolkach, Urology Clinic, Military Medical Academy, Ushinskogo str. 15-3-10, 195267 Saint-Petersburg, Russia.

e-mail: yuri.tolkach@gmail.com

Abbreviations: PCa, prostate cancer; LHRH-R, LHRH-receptor; EGF, epithelial growth factor; LH-R, LH receptor; FSH-R, FSH receptor.

