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The follicle-stimulating hormone receptor: A novel target in genitourinary malignancies

Benjamin A. Gartrell, M.D.*, **Che-kai Tsao, M.D.**, and **Matthew D. Galsky, M.D.**

Division of Hematology and Medical Oncology, The Tisch Cancer Institute, Mount Sinai School of Medicine, New York, NY 10029, USA

Abstract

Follicle-stimulating hormone (FSH) is a central hormone in mammalian reproductive biology. The FSH receptor (FSHR), which was previously believed to be expressed primarily in the ovary and testis, was recently found to be expressed in the tumor blood vessels of many solid tumor types, including prostate adenocarcinoma, urothelial carcinoma, and renal cell carcinoma. While the biologic significance of FSHR in tumor blood vessels has yet to be elucidated, FSHR may contribute to neoangiogenesis. FSHR has been reported to be expressed by prostate cancer cells and, thus, targeting FSHR in prostate cancer may be of particular utility. In this report, we discuss the finding of FSHR in tumor blood vessels and review the literature concerning FSHR in genitourinary malignancy. We also discuss the features that make FSHR an appealing target for therapeutic and imaging purposes and the potential utility of FSHR as a prognostic and/or predictive biomarker in genitourinary cancers. © 2013 Elsevier Inc. All rights reserved.

Keywords

Follicle-stimulating hormone receptor; Targeted therapeutics; Neoangiogenesis

1. Introduction

The follicle-stimulating hormone receptor (FSHR) was recently found to be expressed in the vascular endothelium of a wide range of solid tumors, including prostate cancer (CaP), urothelial carcinoma, and renal cell carcinoma [1]. FSHR is a glycosylated transmembrane protein that belongs to the G protein-coupled receptor family. Upon binding follicle stimulating hormone (FSH), FSHR transduces signal primarily via the adenylyl cyclase-cAMP-protein kinase A pathway. FSH, a central hormone of mammalian reproduction, is produced by the gonadotroph cells in the anterior pituitary gland. The target organs for FSH are the ovary and testis.

FSHR is expressed by ovarian granulosa cells and is critical for follicular maturation during the menstrual cycle and in the production of estradiol through aromatization of androgens [2]. FSHR is also expressed by ovarian epithelial cells and the endometrium [3,4]. In males, testicular Sertoli cells express FSHR, which is essential for Sertoli cell proliferation in

*Corresponding author. Tel.: +1-646-283-5572; fax: +1-212-659-5599. benjamin.gartrell@mssm.edu (B.A. Gartrell).

immature testis and maintenance of qualitatively and quantitatively normal spermatogenesis in adults [5]. FSHR is also expressed in human osteoclasts and monocytes [6]. In the vasculature of nonmalignant tissues, FSHR is only expressed in placental endothelial cells [1] and, to a lesser extent, in the endothelium of ovaries [7] and testis [8].

2. FSHR is expressed in tumor blood vessels

FSHR is expressed in the endothelial cells associated with a diverse range of solid tumors. In the initial report demonstrating FSHR expression in the tumor endothelium, 1,336 primary solid tumors representing 11 tumor types were evaluated utilizing immunohistochemistry and immunoblotting with several FSHR-specific monoclonal antibodies [1]. In situ hybridization was also used to identify FSHR in tumor blood vessels. Genitourinary malignancies were strongly represented in this study and included 773 prostate adenocarcinomas, 203 urothelial carcinomas (126 in situ carcinomas), 64 renal cell carcinomas, and 5 seminomas. Remarkably, FSHR was identified in the neovasculature of every tumor evaluated. FSHR expression was identified almost exclusively at the tumor periphery, in a region that extended <1 cm inside or outside of the tumor. One exception to this distribution was renal carcinoma in which approximately 30% of samples had equal expression of FSHR in the endothelium located throughout the tumor.

Importantly, FSHR expression was largely limited to neoplastic tissue. Though 20% of samples from patients with benign prostatic hyperplasia (BPH) demonstrated FSHR expression in the endothelium, FSHR expression was not observed in the blood vessels of nonmalignant tissues, including conditions such as rheumatoid arthritis, chronic pancreatitis, Crohn's disease, and normal wound healing. Uninvolved normal tissues adjacent to tumor served as a negative control and, beyond several millimeters from the tumor, were always negative for FSHR.

A mouse xenograft model was used to show that FSHR can be targeted by circulating ligands. Lymph node carcinoma of the prostate (LNCaP) tumors were grown on nude mice, and the mice were subsequently sacrificed and perfused with anti-FSHR antibody that had been coupled to colloidal gold. Upon evaluation with electron microscopy, the conjugated gold particles could be seen adhering to the neovasculature. These particles were internalized into endothelial cells through coated vesicles, endosomes, and multivesicular bodies. These findings confirm that FSHR is expressed on the luminal endothelial surface and can bind to, and internalize, targeted ligands, characteristics critical to the development of drug- or radio-immunoconjugates and imaging agents targeting FSHR.

3. Functional characterization of FSHR in the neovasculature

While the biologic function of FSHR in the neovasculature remains to be elucidated, the ubiquitous nature of FSHR in tumor blood vessels across 11 tumor types suggests that FSHR is of biologic significance in human solid tumors. The presence of FSHR on tumor blood vessels raises the possibility that FSHR contributes to neoangiogenesis. FSH has been shown to induce vascular endothelial growth factor (VEGF) in granulosa cells and conceivably, a similar role of FSH-FSHR signaling could exist in the tumor endothelium [9].

FSHR may also contribute to the development of meta-static disease. The position of FSHR on the luminal endothelial surface suggests a potential role in tumor intravasation. Intravasation is a key component of the metastatic process in which malignant cells penetrate the endothelium and enter the circulation. The molecular mechanisms of this process are poorly understood. The expression of FSHR at the periphery of tumors, where the tumor interacts with stroma, further suggests that FSHR may be of relevance to the metastatic process. The epithelial-to-mesenchymal transition (EMT) is believed to be critical for the formation of metastases [10], and stromal elements interacting with tumor cells at the tumor periphery are thought to contribute to EMT [11,12]. Whether FSHR expression may be induced by stromal elements, and may be related to EMT, requires further study.

4. FSHR in prostate cancer

It should be noted that the bulk of the literature with regard to FSH and FSHR in genitourinary malignancies concerns CaP. This is a reflection of the significance of the hypothalamic-pituitary-testicular axis in the production of testosterone and of the depletion of testosterone in the first line of therapy for advanced disease. Gonadotropin-releasing hormone (GnRH) agonists and antagonists inhibit the secretion of the gonadotropins luteinizing hormone (LH) and FSH, ultimately suppressing testosterone production. There has also been interest in a direct role of FSH and FSHR in CaP for over a decade. The first reports of FSH expression by normal prostate, BPH, and CaP came out over 20 years ago [13,14]. Since that time, several reports have also described the expression of FSHR in normal prostate, BPH, and CaP [15–17].

In 1998, Dirnhofer et al. used techniques including RT-PCR to demonstrate that FSH and FSHR are expressed in BPH and CaP [15]. The following year, Ben-Josef et al. also reported that FSH and FSHR are expressed by several CaP cell lines and in human CaP specimens. FSHR was also found to be present, but to a lesser extent, in normal prostate tissue and in BPH. Interestingly, FSH was shown to induce proliferation of a CaP cell line [16]. A similar report in 2006 identified FSHR expression in a CaP cell line. Exposure to FSH was shown to increase the intracellular concentration of cAMP, which confirmed that FSHR induced signaling upon ligand binding. In agreement with the earlier report [16], FSHR was found to also be expressed in normal prostate tissue and in BPH, but at significantly lower levels than in CaP [17].

5. Potential clinical evidence of activity in targeting FSH/FSHR in prostate cancer

5.1. GnRH antagonists

One small clinical trial consisting of 16 patients investigated the use of abarelix, a GnRH antagonist, in CaP patients who developed castration-resistant disease following orchiectomy [18]. As no further reduction in androgens would be expected with a GnRH antagonist in the post-orchiectomy setting, this trial allowed the benefit of a reduction in gonadotropins to be evaluated independent of suppression of androgens. Of the 16 patients treated, none met the predefined definition of a prostatic specific antigen (PSA) response (a decrease by 50% of pretreatment value). However, 5 patients (31%) had reductions in PSA,

which ranged from 9.3% to 31.8%. After 6 months of treatment, 6 patients had stable disease. The median time to progression was 12 weeks (95% CI 6–18). The mean FSH concentration before beginning abarelix was 45.1 IU/l, which decreased to an average of 3.6 IU/l after 20 weeks of treatment, and 13/16 patients achieved an FSH level of <5 IU/l on treatment. This small study supports the hypothesis that depleting FSH may have a therapeutic role in castration-resistant CaP.

A phase III randomized study (CS21) comparing degarelix, a GnRH antagonist, to leuprolide, a GnRH agonist, in the treatment of CaP has been performed [19]. While the suppression of testosterone was similar in patients treated with degarelix and leuprolide, degarelix suppressed FSH further (89% vs. 55%). In an extension study that is currently ongoing, patients on leuprolide were randomized to continue with this therapy vs. switching to degarelix. An interim report showed that patients randomized to degarelix had a significantly reduced PSA progression-free survival hazard rate [20].

These data demonstrate that GnRH antagonists may have antineoplastic activity beyond their testosterone-reducing properties. Whether the clinical benefits of degarelix are mediated by an impact on FSHR expressing CaP cells or an anti-angiogenic effect on FSHR expressing tumor endothelium cannot be determined based on the available data and requires further evaluation.

5.2. Suramin

Suramin is an antiparasitic agent that has been in clinical use for nearly 100 years. Suramin was also found to be a reverse transcriptase inhibitor and was used in an effort to treat HIV in the 1980s. An antineoplastic effect was noted in both HIV-associated lymphoma and in Kaposi's sarcoma [21]. The antineoplastic benefit of suramin was attributed to the inhibition of signaling via several peptide hormones, including platelet-derived growth factor (PDGF) [22], epidermal growth factor (EGF) [23], and TGF- β [24]. Later studies demonstrated that suramin inhibited testosterone production and FSHR signaling [25,26]. A randomized phase III trial compared suramin plus hydrocortisone ($n = 229$) to placebo with hydrocortisone ($n = 231$) in symptomatic patients with castration-resistant CaP [27]. Pain control was better in the suramin-treated group. Post-treatment PSA declines of 50% were achieved in 33% of the suramin group and 16% of the placebo group ($P = 0.01$). Despite a statistically significant decrease in progression rate favoring the suramin group, there was no significant difference in overall survival (286 days in the suramin group and 279 days in the placebo group). However, crossover was permitted and >70% of the placebo group eventually received suramin. Neither the number of patients who had previously undergone orchiectomy nor the post-treatment changes in FSH were reported. Thus, suramin, an FSHR inhibitor, had activity in castration-resistant CaP, but whether this activity was related to FSHR inhibition is unclear.

6. FSHR as a potential biomarker in genitourinary (GU) malignancies

A recent study has demonstrated that FSHR expression in the tumor endothelium of renal carcinoma correlates with response to sunitinib [28]. Fifty patients who had undergone radical nephrectomy for advanced clear-cell renal carcinoma and had subsequently been

treated with sunitinib in the first-line setting were identified. Given the previous observation that FSHR is expressed at the leading edge of the tumor, tissue was evaluated only 5 mm from the tumor-stroma interface. The percentage of vessels expressing FSHR in this area was recorded. Patients were divided into those who had an objective response ($n = 15$) following 3 months of sunitinib, those with stable disease ($n = 18$), and those with progressive disease ($n = 17$). Whereas there was no difference in the total vessel density in these 3 groups, patients achieving an objective response to sunitinib were found to have a higher density of FSHR staining vessels ($56.8\% \pm 5.4\%$) than those with stable ($11.4\% \pm 2.0\%$) or progressive disease ($7.3\% \pm 0.7\%$). The difference in the density of FSHR staining vessels between groups reached statistical significance in each instance. We are currently further exploring FSHR expression in the neovasculature of renal carcinoma as a potential predictive and prognostic biomarker.

Prognostic and predictive biomarkers are also urgently needed in bladder cancer. For example, neoadjuvant chemotherapy improves survival in patients with muscle-invasive bladder cancer [29], but the absolute survival benefit is modest and methods to refine patient selection could potentially spare patients from unnecessary chemotherapy while increasing the curability of those at highest risk of recurrence. Our group is currently evaluating the prognostic significance of FSHR expression in the bladder cancer neo-vasculature from radical cystectomy specimens.

7. Therapeutics targeting FSHR

As FSHR plays a central role in human reproductive biology, efforts have been made to target the receptor with small molecules. Several classes of compounds that antagonize FSHR signaling have been identified. In general, these molecules are negative allosteric modulators of FSHR [30–35]. Allosteric modulators do not compete with ligand for interaction with the receptor but, instead, associate with the receptor in such a way that signaling may be positively or negatively modulated. A group of thiazolidinone analogs were shown to be allosteric modulators of FSHR [32]. Modifications in the chemical structure of the thiazolidinone backbone allowed for generation of positive, mixed, and negative allosteric modulators of FSHR.

Several reports have explored the use of FSHR allosteric modulators *in vivo*. A negative allosteric modulator of FSHR was shown to inhibit ovulation in rats. However, there was no decrease in number or appearance of follicles, suggesting that inhibition of ovulation may have been due to off target effects (ovarian inflammation was noted) [30]. More recently, a study of ADX61623, another negative allosteric modulator of FSHR, was reported. ADX61623 was shown to inhibit some aspects of FSHR signaling (cAMP production and progesterone synthesis) while failing to inhibit estradiol synthesis [35]. *In vivo*, ADX61623 did not reliably inhibit FSH-induced follicular maturation. If these, or similar, molecules antagonize FSHR in tumor blood vessels, they may have a role in targeting FSHR in malignancy.

8. Conclusions

FSHR is a newly discovered marker of tumor endothelium in solid tumors. FSHR may be a useful molecular biomarker, which could be informative in clinical decision making and patient counseling. A variety of characteristics suggest that FSHR in the neovasculature is a promising novel target for cancer therapy and imaging (Table 1). The FSH-FSHR signaling axis can be targeted with therapeutics in several ways. FSH levels can be decreased by the use of currently available drugs, such as GnRH antagonists. The development and use of neutralizing antibodies against FSH could potentially lead to an even greater reduction in FSH. FSHR may be targeted by the use of small molecules, antibodies, and with drug- or radio-immunoconjugates. Targeting FSHR may be of particular interest in CaP, where FSHR may also be expressed by CaP cells. Given the well-established role of anti-angiogenics in renal cell carcinoma and of the reported correlation of FSHR expression with response to sunitinib in this disease, targeting FSHR deserves evaluation in renal cell carcinoma. There is reason for optimism, but much work remains to fully exploit this exciting new target for the treatment of genitourinary cancers.

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Table 1

Key characteristics of FSHR as an attractive therapeutic target in genitourinary malignancies

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- Expression in the neovasculature of all genitourinary cancer specimens analyzed to date
 - Limited expression of FSHR in normal tissues
 - Potential functional role in angiogenesis and in the development of metastatic disease
 - Accessible to circulating ligands and internalization in endothelial cells upon ligand binding
 - Possible expression by prostate cancer cells
-