Chapter

Tumor Angiogenesis in Pituitary Adenoma

Daizo Yoshida and Akira Teramoto

Abstract

The role of angiogenesis in pituitary tumor development used to be questioned, since pituitary tumors have been usually found to be less vascularized than the normal pituitary tissue. Nevertheless, a significantly higher degree of vasculature has been shown in invasive or macropituitary prolactinomas when compared to noninvasive and microprolactinomas. We should know VEGF was found firstly in pituitary anterior lobe, then tumor angiogenesis must occur. Meanwhile the vascular arrangement raised by VEGF is irregular, that sometimes lead to pituitary apoplexy. In this chapter, hypoxia inducible factors (HIF), transcription factors regulating expression of several genes related to oxygen homeostasis are in response to hypoxic stress. We focus on tumor angiogenesis regulated by the signaling cascade in tumor angiogenesis in pituitary tumor.

Keywords: hypoxia inducible factors, tumor angiogenesis, pituitary adenoma

1. Introduction

Hypoxia is critical for the life. Autonomic nerves system responds to the hypoxia regulating circulatory and respiratory organs to ensure adequate oxygen delivery. Separately, cellular responses to hypoxia are mainly regulated by the activation of transcription factors called hypoxia-inducible factors (HIFs). HIFs affect hypoxia and stress response signaling pathways that influence development, metabolism, inflammation, and circulatory and respiratory physiology [1–5]. Hypoxia-inducible factors are also associated with many diseases in the circulatory system, mainly via VEGF. Copper is a co-factor of bFGF, accumulated in malignant glioma, the chelation inhibits glioma growth and angiogenesis in murine model. HIF pathways are triggered by hypoxia. The hypoxia regulates both in the cell signal level and in the circulatory and respiratory system by autonomic nerves. Hence, compromised response to ischemia is crucial. Inhibition of angiogenesis by reducing the HIF pathway can be a rational method in patients with ischemic diseases. Investigation regarding hypoxia mediated by intracellular signaling have been emerged as new targets focusing on the related genes or protein delivery to stabilize HIFs, but not yet accomplished. Oxygen tension is markedly below physiological levels in solid tumors also in pituitary adenoma. In fact, solid tumors contain severely hypoxic regions, in which pO_2 values are <10 mmHg [6, 7]. Tumor vessels raised by VEGF are regularly lacking tight junction, we consider that it leads pituitary apoplexy, hemorrhagic infarction.

In this chapter, we focus on the current understanding of the relationship between HIFs and pituitary adenoma in tumor angiogenesis.

2. Discussion

Endocan is known as endothelial cell-specific molecule-1 (ESM-1) that has a 50 kDa polypeptide with a single dermatan sulfate [8, 9]. After secreted from endothelial cell, endocan interacts between leukocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1). Recent studies have shown that endocan mRNA expression in endothelial cells is specific to several angiogenic factors and cytokine, such as VEGF and TNF. Herein, function of endocan has been emerged in tumor hypoxia context. Endocan overexpress stimulates tumor progression in mouse models of human tumor xenografts. Anyway, these studies demonstrated that endocan can be a biomarker of tumor progression, and a potentially therapeutic target for cancer. Despite general immunotherapeutic therapy to cancer is not satisfactory, antibodies against endocan be still promising cancer treatment. Both plasma endocan and VEGF-A levels are elevated in patients with invasive tumor. Cornellius showed that, pituitary adenoma cells expressed endocan, though it was not observed in all normal pituitary [10]. Microvessels revealed significantly greater mean vessel areas in subgroups of tumors with endothelial endocan expression. Thus, endocan in endothelial cells may be a relevant marker of aggressiveness in pituitary tumors.

Two p53 binding sites are present in the promoter sequence of the gene encoding cathepsin D [11, 12] suggesting a direct relationship between cathepsin D and the induction of apoptosis. Cathepsin D is activated by an intracellular acid-dependent autoactivation mechanism. It has been reported that cathepsin D secreted by prostate carcinoma cells is responsible for the generation of angiostatin, an endogenous inhibitor of angiogenesis that is produced by the tumor-mediated proteolysis of plasminogen. Clinically, cathepsin D overexpression has been studied in several malignant tumor types [11] although most research has been focused on breast cancer, in which cathepsin D expression correlates with poor prognosis. Expression of cathepsin D is also significantly higher in malignant than in benign ovarian tumors [12]. In colon cancer cells, cathepsin D is upregulated by HIF 1α under hypoxic conditions, perhaps counteracting the effects of VEGF via angiostatin regulation [13]. Angiogenesis is a major mechanism by which oxygen supply is increased in tumors. Hypoxia has been found to regulate angiogenesis activators and may some- times downregulate angiogenesis inhibitors. In the mouse pituitary adenoma cell line GH4C, secretion of cathepsin D was inhibited under hypoxic conditions, suggesting that hypoxia acts directly on pituitary lactotrophs to inhibit PRL expression. In addition, cathepsin D can promote tumor invasiveness by acting as an autocrine growth factor within the pituitary to stimulate cell growth. The hormonal moiety in the hypoxia-responsive motif, however, has not yet been established.

In pituitary adenomas, regional oxygen saturation is lower than in normal pituitary lobes. VEGF and HIF-1α are also expressed in several pituitary adenomas; however, the role of HIF-1α and the relationship between HIF-1α and VEGF has been emerged Vidal et al. reported that HIF-1α was expressed in all types of pituitary adenoma and that the expression level in GH-producing pituitary adenomas and pituitary carcinomas was higher than in the other adenomas. We detected HIF-1α mRNA and protein in several pituitary adenoma types. Our statistical analysis confirmed earlier results that there was no significant correlation between HIF-1α expression and

patient age, gender, and tumor size. GH-producing adenomas exhibited the highest, and ACTH-producing adenomas the lowest expression levels of HIF-1α; however, the difference was not statistically significant, possibly due to the small number of available samples. Our study confirmed earlier reports that VEGF was expressed in all types of pituitary adenoma [14, 15]. According to Lloyd et al., VEGF expression was high in GH-producing adenomas, corticotrophs, silent corticotrophs, silent subtype 3 tumors, non-oncocytic null-cell adenomas, and pituitary carcinomas [16]. However, between normal tissue and adenomas or tumors of different histotypes, there was no statistically significant difference with respect to VEGF expression. We also found no significant difference among the different adenoma types we examined.

We performed quantitative assessment of the expression of HIF-1α and VEGF in pituitary adenomas and examined the co-expression of HIF-1α and VEGF. Our results suggest that VEGF may be regulated not only by HIF-1α but by a different mechanism mediated by several cytokines and growth factors. In normal pituitary cells, pituitary adenylate cyclase-activating polypeptide (PACAP) and IL-6 can stimulate VEGF expression in vitro, whereas glucocorticoid has inhibitory action. In pituitary adenoma cells, VEGF expression was increased by TGF-α, PACAP, estradiol, IL-6, IGF-I, and pituitary tumor transforming gene (PTTG), and was inhibited by dexamethasone. Moreover, VEGF was co-localized with various pituitary hormones, suggesting that hypothalamic factors may play a role in the regulation of pituitary VEGF release. Therefore, the regulation of VEGF in pituitary tumors may not depend primarily on HIF-1α expression.

Our study also demonstrated that stromal cell-derived factor (SDF)-1 expression was positively correlated to microvascular density (MVD), strongly obvious in macroadenomas. Intensity for immunoreactivity for SDF-1 was not related. Given by these results we consider, abnormal blood vessels in the pituitary adenoma tissue may be not be able to supply the normal oxygen concentration like the normal vessels. Both SDF-1 mRNA and protein expression were firmly upregulated in hypoxia, and then regulate tumor angiogenesis in pituitary adenoma.

SDF-1 (CXCL12) is expressed both in embryo and cancer cell lines, and is an ELR-CXC chemokine that has angiogenic activity, role of the capillary-like formation stimulating human vascular endothelial cells also in pituitary adenoma [17]. Meanwhile CD34 is a cell-surface marker of hematopoietic stem cells (HSCs), mature vascular endothelial cells also express a receptor for SDF-1, CXCR4. CD34 cell migration is stimulate by CXCR4 via SDF-1 in vitro and could be a key factor for trafficking HSC between the peripheral blood and the bone marrow, named a homing effect. During embryogenesis, primitive blood vessels are shaped newly by the angioblasts aggregation, which is termed vasculogenesis.

In embryo, when the vasculogenesis starts mainly fibroblast growth factors (FGFs) cause some cells in the mesoderm differentiated into endothelial progenitors. SDF-1/CXCR4 axis has an initial role in all of hematopoiesis, vascular development, and cardiogenesis [18–20], whiles also in adults, homing of HSCs to the bone marrow and CD34 progenitor cell proliferation is regulated by SDF-1 [17]. Various organs, such as the liver, brain, and lymphoid organs widely expressed SDF-1. In particular, human ovarian cancer was firstly discuss to express high levels of SDF-1, and subsequently has been reported in glioblastoma.

Recently, several studies have focused on pituitary adenoma. Some showed that SDF-1 and its receptor, CXCR4, were expressed in rat pituitary adenomas, but they did not discuss the relationship between SDF-1 expression and angiogenesis [21–23]. Both prolactin and GH in the GH4C1 are regulated in cell proliferation and the release

by CXCR4 activation, plausibly through complicated intracellular signals. However, discussion of exogenous SDF-1 has not yet clearly disclosed, because pituitary adenoma cells express CXCR4 but not SDF-1. Barbieri et al. analyzed the expression of both SDF-1 and CXCR4 in human pituitary adenomas, compared with normal hypophyses. They elucidated first the SDF-1 and CXCR4 expression in normal and adenomatous human pituitary and revealed that overexpression occurs in adenomas comparing normal-related pituitary cells, then indicating that this profile may contribute to the increasing proliferation [24].

Invasive pituitary adenoma has a complicated mechanism and interacts with the nerve-endocrine-immune network. It is affect DDR1 ligand combined with DDR1 can promote the DDR1 signaling pathway. DDR1 promotes MMP-2/9 expression, leading to ECM reconstruction and tumor invasion [25–27]. Cell apoptosis, change tumor cell invasiveness, and regulation of energy metabolism is mediated by hypoxic condition. Herein, discoidin domain receptor (DDR)-1 expression and its effect on pituitary adenoma under hypoxia still need further investigation. Our study confirmed that DDR1 mRNA and protein are elevated in primary pituitary adenoma cells along with hypoxia. Elevated DDR1 expression can regulated expression of MMP-2 and MMP-9 expression in supernatant, thereby promoting cell proliferation and invasion of pituitary adenoma. Nilotinib administration can diminish DDR1 expression and further reduce MMP-2 and -9 expression to reduce pituitary adenoma cells proliferation and invasion.

The above-mentioned factors have been discussed much few in pituitary adenoma. Cornelius et al. investigated that endocan, secreted by endothelial cells, associated with an aggressive behavior in pituitary tumors. The study by immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR) in patients operated for a pituitary adenoma, comparing normal post-mortem pituitaries. In normal pituitaries, endocan was never observed in vessels but was detectable in adenoma cells. In adenoma tissue, a significant relation between endocan immunoreactivity in endothelial cells and progression, tumor size, mitotic count, and p53 expression were demonstrated. The immunohistological study of endocan in endothelial cells therefore can be a new marker of aggressive behavior in pituitary tumors [28].

Cathepsin B expressed in invasive pituitary adenoma and is an important functional protein in apoptosis. One might hypothesize that shifting the balance between mediators of cell death could result in changes in pituitary tumor behavior [29].

Pituitary adenoma is considered to be benign, accounting 20% of intracranial tumors generally, that is the third most common intracranial tumor. But approximately 30% of pituitary adenomas are invasive. It can be said the already-established molecular mechanisms of the pituitary adenomas invasion, turning out mainly HIF-1α, pituitary tumor transforming gene, FGF-2, VEGF, and MMPs (mainly MMP-2, and MMP-9) are core signaling. These molecules have the ability to create a suitable microenvironment within the tumor. Together, they have a complicated interaction [30].

Nonfunctioning pituitary adenoma is sometime hard for surgery. However, there is no established conservative treatment. MicroRNA-134 (miR-134) may be promised that suppress tumor cell proliferation and invasion. Therefore, the effect of miR-134 on improving non-functioning pituitary tumor cells expansion is considered to be challenging. The molecular mechanism of the SDF-1α/miR-134/VEGFA axis is representative a novel mechanism in the pathogenesis of NF-PitNETs and may serve as a potential therapeutic target for the treatment of NF-PitNETs [31].

Study with flow cytometry show that the rates of CXCR4- and CXCL12-positive cells in invasive pituitary adenomas was significantly elevated in the cell

suspensions than those in non-invasive pituitary adenomas. Immunohistochemical study unveiled that CXCR4 and CXCL12 staining index of the invasive pituitary adenomas were clearly higher than those of the non-invasive pituitary adenomas. Meanwhile, none of flow cytometry and immunohistochemistry could disclose significant difference between CD44 and CD147 expression, respectively. Then, CXCR4 and CXCL12 may potentially can be powerful biomarkers to detect early stage of pituitary adenomas [32].

Recently, Nilotinib has been highlighted to reduce DDR1 expression, decrease MMP-2 and MMP-9 expression, and inhibit pituitary adenoma cells proliferation and invasion [33].

Conclusively further investigations are required to elucidate the mechanisms underlying the invasiveness of pituitary adenoma-related phenomena is a new horizon in the field of neuro-oncology.

Conflict of interest

The authors declare no conflicts of interest.

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