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Cytokines and Genes in Pituitary Tumorigenesis: RSUME Role in Cell Biology

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Abstract

Cytokines of the IL-6 or gp130 family regulate many cellular responses and play regulatory roles in numerous tissues, and are placed as auto-paracrine regulators of pituitary function acting in normal and tumoral anterior pituitary cells. Especially, IL-6 has a regulatory role in the hormone secretion and growth of the anterior pituitary and is involved in adenoma pathogenesis. Recently, IL-6 has been shown to mediate oncogene-induced senescence (OIS). IL-6 might participate in such a process in adenomas pituitary as well. From pituitary tumoral gp130 overexpressing cells, an unknown protein, RSUME, has been cloned. RSUME is induced by hypoxia in pituitary tumors and regulate pathways involved in angiogenic and tumorigenic processes (NF-kB/IkB and HIF-1α pathways). Thus, it could have an important role in the development of the pituitary tumors.

The gp130 Cytokines and Their Role in Pituitary

Cytokines exhibit functional pleiotrophy and redundancy, a particular cytokine may have a wide variety of biologic functions on various tissues and cells, and several different cytokines exert similar and overlapping functions on a certain cell. Cytokines that belong to the gp130 cytokine family (or the IL-6 cytokine family) have been shown to be synthesized in numerous organ systems such as hematopoietic tissues, reproductive tissues, thymus, heart, liver, pituitary, and the nervous system and are found to play roles in the regulation of cell differentiation, proliferation, cell survival, hormone secretion and inflammatory response [1]. These cytokines have also been shown to be involved in the development and growth of different tumors [2].

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The IL-6 cytokine family is composed by IL-6, leukemia inhibitory factor (LIF), IL-11, ciliary neurotrophic factor (CNTF), oncostatin M, cardiotrophin-1 [3], neuropoietin [4] and cardiotrophin-like cytokine, also known as stimulating neurotrophin-1/B cell-stimulating factor-3 [5]. The binding of these cytokines to their specific receptors trigger the association of their alpha subunits with the membrane glycoprotein gp130, which functions as an initial cellular signal transducer [3]. Thus, this cytokine group is also named the gp130 cytokine family.

Considering that gp130 is ubiquitously expressed, the time and place at which gp130 functions in vivo appears to be determined by spatially and chronologically regulated expression of specific cytokine-binding receptor chains or cytokines themselves [2, 6, 7].

In the pituitary gland, many studies have demonstrated not only the expression of gp130 mRNA [8] but also the expression of specific receptors for IL-6 [9], LIF [10], IL-11 [11, 12], and CNTF [12]. Also, the synthesis of gp130 cytokines in different types of pituitary cells, such as LIF in developing human fetal pituitary and in normal and adenomatous human adult tissue [13], IL-11 in pituitary folliculostellate (FS), lactosomatotrophic and corticotrophic cells [11, 12] and CNTF in FS and lactosomatotrophic cells [12], has been shown. Particularly, several groups have demonstrated IL-6 production localized in FS cells of normal pituitary tissue [14], and in the tumor cells of pituitary adenomas [6, 15–19], and the presence of IL-6 mRNA in anterior pituitary cells [14, 15]. In addition, the JAK/STAT/SOCS-3 pathway, involved in the signal transduction of gp130 cytokines [3, 20], has also been described in pituitary cells [11, 21]. Therefore, the gp130 cytokines should be considered as auto-paracrine regulators of pituitary function in physiological and pathophysiological conditions, mainly in hormone secretion, cell growth, maintenance of homeostasis and tumorigenesis.

For example, the role of the cytokine transducer gp130 during the tumorigenic process in pituitary was demonstrated in a study where stable rat lactosomatotropic GH3 clones expressing different gp130 levels were generated and injected in athymic nude mice [22]. In contrast to mice injected with cells overexpressing gp130, those injected with cells expressing low gp130 levels showed a severely impaired in vivo tumor development. Furthermore, this clones with low gp130 levels showed reduced proliferation and hormone secretion (GH and prolactin) in response to gp130 cytokines. In contrast, the overexpression of gp130 did not significantly modify the cellular behavior, indicating that pituitary gp130 endogenous levels fulfill for a normal functional cellular response [22].

In addition, reduced levels of gp130 protein in MtT/S (pituitary somatotrophic cell line) cells stably transfected with gp130 antisense cDNA blocked cell growth and hormone secretion stimulated by CNTF and led to severely impaired in vivo tumor development in athymic nude mice [23]. These data, together, provide in vivo evidence supporting a link between gp130 and pituitary abnormal growth, and between gp130 and hormone secretion.

IL-6 Action in Pituitary Adenoma Growth

As stated above, the IL-6 cytokine and its receptor are produced in the pituitary gland, and regulate synthesis of anterior pituitary hormones, consistent with a paracrine or autocrine model. IL-6 directly regulates pituitary cell growth [6, 16, 17, 24]. In the normal pituitary, both paracrine and autocrine-derived IL-6 [6, 16] inhibit pituitary trophic growth. In contrast, in several tumor types (ACTH, PRL-secreting, GH-secreting and nonfunctioning adenomas), IL-6 exhibited either inhibitory or stimulatory effects not associated with tumor type or size [25].

Recently, it was reported that oncogene-induced senescence (OIS) is mediated by IL-6, activated in response to oncogenic stress [26]. This cytokine is required for both induction and maintenance of OIS, and acts in a cell-autonomous fashion to enable OIS. Experimental evidence suggests that IL-6 acts in an autocrine manner to regulate OIS as this signaling cascade is blocked by siIL-6 mRNA and requires an intact IL-6R. Thus, a cell-autonomous pool of IL-6 produced by senescent cells and acting in an autocrine and paracrine fashion mediates OIS [26].

Such a response to oncogenic stress, which restrains proliferation but allows the cell to remain viable and perform its physiological function, may be acting in the pituitary gland and thus favor vital functioning for homeostasis control. Pituitary adenoma senescence has been recently described [27, 28].

Given its role in pituitary growth, it is tempting to postulate the involvement of endogenous IL-6 in development of pituitary adenoma OIS, which, may explain the benign nature of these abundant tumors.

Cloning of Genes in Pituitary by mRNA Differential Display

Higher organisms contain many different genes, of which only a small fraction, perhaps 15%, are expressed in any individual cell. It is the choice of which genes are expressed that determines all life processes – development and differentiation, homeostasis, response to insults, cell cycle regulation, aging, and even programmed cell death. Altered gene expression lies at the heart of the regulatory mechanisms that control cell biology. Comparisons of gene expression in different cell types provide the underlying information we need to analyze the biological processes that control our lives [29].

mRNA differential display is potentially a powerful method for identifying genes that are over- or underexpressed in one mammalian cell type or tissue relative to another in distinct situations such as a specific developmental stage, certain time points or after an in vitro treatment. In this technique, the general strategy is to amplify partial cDNA sequences from subsets of mRNAs by reverse transcription and the polymerase chain reaction (PCR) [29, 30].

This approach resulted effective in the pituitary. First, a comparison of the gene expression pattern between normal anterior pituitaries and prolactinomas using, as a

model of this latter, female dopamine D2-receptor-deficient mice (D2R–/–), allowed the identification of a band present only in the normal pituitary tissue as noggin, a specific inhibitor of bone morphogenetic protein 4 (BMP-4) [31]. BMP4 was further showed to stimulate lactotrophic and inhibit corticotrophic cell growth, having an opposite effect in both cell types [31, 32].

Recently, this cloning technique also was used for compared one stable clone from the rat pituitary lactosomatotrophic tumor cell line GH3 overexpressing gp130, that showed increased tumorigenic and angiogenic potential when injected into nude mice [22], to a control GH3 clone stable for the empty vector. In a GH3 clone that overexpressed gp130 appeared a differential band corresponding to a previously uncharacterized mRNA that was called RSUME [33].

RSUME Characterization and Function

The unknown gene cloned from the pituitary cell line, RSUME, has two splicing variants in humans of 195 or 267 amino acids, coding for a small RWD domain-containing protein. Its name is based on its structure and function (RSUME for RWD-domain-containing sumoylation enhancer). This new protein is highly conserved in higher vertebrates, does not seem to belong to any previously known protein family. Also has been demonstrated by confocal microscopy that RSUME protein is located in the cytoplasm and nucleus, despite lacking a nuclear localization signal [33].

RSUME is expressed in various tissues, but the higher expression levels were found in cerebellum, pituitary, heart, kidney, liver, stomach, pancreas, prostate, and spleen. It was upregulated by cellular stress stimuli such as hypoxia and heat shock. Particularly, in pituitary tumors RSUME expression was increased by hypoxia [33].

RSUME enhances protein sumoylation through a direct interaction with Ubc9, a SUMO conjugase. Moreover, the correct folding of its RWD domain is essential for RSUME activity. RSUME has its strongest effect in the step of forming the Ubc9-SUMO-1 thioester, but also acted in the transference of SUMO-1 from the thioester to a substrate [33].

RSUME increased sumoylation and protein stability of IkB, resulting in inhibition of NF-kB transcriptional activity and, consequently, suppressing the inflammatory process mediated by NF-kB targets such as interleukin-8 (IL-8) and cyclooxigenase-2 (Cox-2). Furthermore, RSUME plays a role in the cell's response to hypoxic stress because it increased hypoxia inducible factor-1α (HIF-1α) protein levels and enhanced it sumoylation and stability, thus regulating adaptive responses to changes in oxygen tension and angiogenesis in mammalian cells [33].

Given the fact that the actions of RSUME in sumoylation have functional implications on the NF-kB/IkB pathway and on the regulation of HIF-1α, both involved in angiogenic and tumorigenic processes, its expression and induction by hipoxia

in pituitary tumors and its cloning from gp130 overexpressing pituitary tumor cells, RSUME mechanism of participation in adenoma pathogenesis is under study.

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References

1 Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, Graeve L: Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J 1998;334:297–314.

2 Carbia-Nagashima A, Arzt E: Intracellular proteins and mechanisms involved in the control of gp130/JAK/STAT cytokine signaling. IUBMB Life 2004;56:83–88.

3 Kishimoto T, Akira S, Narazaki M, Taga T: Interleukin-6 family of cytokines and gp130. Blood 1995;86:1243–1254.

4 Derouet D, Rousseau F, Alfonsi F, Froger J, Hermann J, Barbier F, Perret D, Diveu C, Guillet C, Preisser L, Dumont A, Barbado M, Morel A, de Lapeyriere O, Gascan H, Chevalier S: Neuropoietin, a new IL-6-related cytokine signaling through the ciliary neurotrophic factor receptor. PNAS 2004;101:4827–4832.

5 Senaldi G, Varnum BC, Sarmiento U, Starnes C, Lile J, Scully S, Guo J, Elliott G, McNinch J, Shaklee CL, Freeman D, Manu F, Simonet WS, Boone T, Chang MS: Novel neurotrophin-1/B cell-stimulating factor-3: a cytokine of the IL-6 family. Proc Natl Acad Sci USA 1999;96:11458–11463.

6 Arzt E, Páez-Pereda M, Perez-Castro C, Pagotto U, Renner U, Stalla GK: Pathophysiological role of the cytokine network in the anterior pituitary gland. Front Neuroendocrinol 1999;20:71–95.

7 Arzt E, Stalla GK: Cytokines: autocrine and paracrine roles in the anterior pituitary. Neuroimmunomodulation 1996;3:28–34.

8 Shimon I, Yan X, Ray D, Melmed S: Cytokine-dependent gp130 receptor subunit regulates human fetal pituitary adrenocorticotropin hormone and growth hormone secretion. J Clin Invest 1997;100:357–363.

9 Ohmichi M, Hirota K, Koike K, Kurachi H, Ohtsuka S, Matsuzaki N, Yamaguchi M, Miyake A, Tanizawa O: Binding sites for interleukin-6 in the anterior pituitary gland. Neuroendocrinology 1992;55:199–203.

10 Akita S, Webster J, Ren SG, Takino H, Said J, Zand O, Melmed S: Human and murine pituitary expression of leukemia inhibitory factor: novel intrapituitary regulation of adrenocorticotropin hormone synthesis and secretion. J Clin Invest 1995;95:1288–1298.

11 Auernhammer CJ, Melmed S: Interleukin-11 stimulates proopiomelanocortin gene expression and adrenocorticotropin secretion in corticotroph cells: evidence for a redundant cytokine network in the hypothalamo-pituitary-adrenal axis. Endocrinology 1999;140:1559–1566.

12 Perez-Castro C, Carbia-Nagashima A, Páez-Pereda M, Goldberg V, Chervin A, Largen P, Renner U, Stalla GK, Arzt E: The gp130 cytokines interleukin-11 and ciliary neurotropic factor regulate through specific receptors the function and growth of lactosomatotropic and folliculostellate pituitary cell lines. Endocrinology 2000;141:1746–1753.

13 Akita S, Conn PM, Melmed S: Leukemia inhibitory factor (LIF) induces acute adrenocorticotrophic hormone (ACTH) secretion in fetal rhesus macaque primates: a novel dynamic test of pituitary function. J Clin Endocrinol Metab 1996;81:4170–4178.

14 Vankelecom H, Carmeliet P, Van Damme J, Billiau A, Denef C: Production of interleukin-6 by folliculo-stellate cells of the anterior pituitary gland in a histiotypic cell aggregate culture system. Neuroendocrinology 1989;49:102–106.

15 Spangelo BL, Gorospe WC: Role of the cytokines in the neuroendocrine-immune system axis. Front Neuroendocrinol 1995;16:1–12.

16 Arzt E: gp130 cytokine signaling in the pituitary gland: a paradigm for cytokine-neuro-endocrine pathways. J Clin Invest 2001;108:1729–1733.

17 Arzt E, Buric R, Stelzer G, Stalla J, Sauer J, Renner U, Stalla GK: Interleukin involvement in anterior pituitary cell growth regulation: effects of IL-2 and IL-6. Endocrinology 1993;132:459–467.

18 Renner U, Gloddek J, Páez-Pereda M, Arzt E, Stalla GK: Regulation and role of intrapituitary IL-6 production by folliculostellate cells. Domestic Anim Endocrinol 1998;51:353–362.
19 Jones TH, Daniels M, James RA, Justice SK, McCorkle R, Price A, Kendall-Taylor P, Weetman AP: Production of bioactive and immunoreactive interleukin-6 (IL-6) and expression of IL-6 messenger ribonucleic acid by human pituitary adenomas. J Clin Endocrinol Metab 1994;78:180–187.
20 Levy DE, Darnell JE: Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol 2002;3: 651–662.
21 Busquet C, Zatelli MC, Melmed S: Direct regulation of pituitary proopiomelanocortin by STAT3 provides a novel mechanism for immunoneuroendocrine interfacing. J Clin Invest 2000;106:1417–1425.
22 Perez-Castro C, Giacomini D, Carbia-Nagashima AC, Onofri C, Graciarena M, Kobayashi K, Páez-Pereda M, Renner U, Stalla GK, Arzt E: Reduced expression of the cytokine transducer gp130 inhibits hormone secretion, cell growth, and tumor development of pituitary lactosomatotrophic GH3 cells. Endocrinology 2003;144:693–700.
23 Graciarena M, Carbia-Nagashima A, Onofri C, Perez-Castro C, Giacomini D, Renner U, Stalla GK, Arzt E: Involvement of the gp130 cytokine transducer in MtT/S pituitary somatotroph tumour development in an autocrine-paracrine model. Eur J Endocrinol 2004;151:595–604.
24 Arzt E, Sauer J, Buric R, Stalla J, Renner U, Stalla GK: Characterization of Interleukin-2 (IL-2) receptor expression and action of IL-2 and IL-6 on normal anterior pituitary cell growth. Endocrine 1995; 3:113–119.
25 Páez-Pereda M, Goldberg V, Chervín A, Carrizo G, Molina A, Andrada J, Sauer J, Renner U, Stalla GK, Arzt E: Interleukin-2 (IL-2) and IL-6 regulate c-fos proto-oncogene expression in human pituitary adenoma explants. Mol Cell Endocrinol 1996;124: 33–42.
26 Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, Aarden LA, Mooi WJ, Peeper DS: Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell 2008;133:1019–1031.
27 Chesnokova V, Zonis S, Rubinek T, Yu R, Ben-Shlomo A, Kovacs K, Wawrowsky K, Melmed S: Senescence mediates pituitary hypoplasia and restrains pituitary tumor growth. Cancer Res 2007;67: 10564–10572.
28 Chesnokova V, Zonis S, Kovacs K, Ben-Shlomo A, Wawrowsky K, Bannykh S, Melmed S: p21(Cip1) restrains pituitary tumor growth. Proc Natl Acad Sci USA 2008;105:17498–17503.
29 Liang P, Pardee AB: Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 1992;257:967–971.
30 Sompayrac L, Jane S, Burn TC, Tenen DG, Danna KJ: Overcoming limitations of the mRNA differential display technique. Nucl Acids Res 1995;23: 4738–4739.
31 Páez-Pereda M, Giacomini D, Refojo D, Nagashima AC, Hopfner U, Grubler Y, Chervin A, Goldberg V, Goya R, Hentges ST, Low MJ, Holsboer F, Stalla GK, Arzt E: Involvement of bone morphogenetic protein-4 in pituitary prolactinoma pathogenesis through a Smad/estrogen receptor crosstalk. Proc Natl Acad Sci USA 2003;100:1034–1039.
32 Giacomini D, Páez-Pereda M, Theodoropoulou M, Labeur M, Refojo D, Gerez J, Chervin A, Berner S, Losa M, Buchfelder M, Renner U, Stalla GK, Arzt E: Bone morphogenetic protein-4 inhibits corticotroph tumor cells: involvement in the retinoic acid inhibitory action. Endocrinology 2006;147:247–256.
33 Carbia-Nagashima A, Gerez J, Perez-Castro C, Páez-Pereda M, Silberstein S, Stalla GK, Holsboer F, Arzt E: RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1α during hypoxia. Cell 2007;131:309–323.

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