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PROGRAMMED CELL SENESCENCE: IL-6 ROLE IN THE PITUITARY

Melanie Sapochnik[1], Mariana Fuertes[1] and Eduardo Arzt[1,2].

[1]Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA)-CONICET-Partner Institute of the Max Planck Society, Buenos Aires, C1425FQD, Argentina.

[2]Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, C1428EGA, Argentina.

Corresponding author

Correspondence should be addressed to Dr. E. Arzt.

Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA)-CONICET-Partner Institute of the Max Planck Society, Buenos Aires, Argentina. Phone: 54-11-4899-5500.

email: earzt@ibioba-mpsp-conicet.gov.ar

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ABSTRACT

IL-6 is a pleiotropic cytokine with multiple pathophysiological functions. As a key factor of the senescence secretome, it can promote tumorigenesis and cell proliferation but also exert tumor suppressive functions, depending on the cellular context. IL-6, as do other cytokines, plays important roles in function, growth and neuroendocrine responses of the anterior pituitary gland. The multiple actions of IL-6 on normal and adenomatous pituitary function, cell proliferation, angiogenesis and extracellular matrix remodeling indicate its importance in the regulation of the anterior pituitary. Pituitary tumors are mostly benign adenomas with low mitotic index and rarely became malignant. Premature senescence occurs in slow growing benign tumors, like pituitary adenomas. The dual role of IL-6 in senescence and tumorigenesis is well represented in pituitary tumor development, as it has been demonstrated that paracrine IL-6 effects may allow initial pituitary cell growth, while autocrine IL-6 in the same tumor triggers senescence and restrains aggressive growth and malignant transformation. IL-6 is instrumental in promotion and maintenance of the senescence program in pituitary adenomas.

INTRODUCTION

Cytokines perform essential roles during infection, cancer and inflammation where they regulate cellular proliferation, differentiation and survival or death (Dinarello 2007; Dranoff 2004).

In particular, interleukin 6 (IL-6) is a multifunctional cytokine that has been implicated in the pathogenesis of a variety of diseases, including cancer (Hunter & Jones 2015; Yao, et al. 2014). In addition, together with other cytokines and factors, IL-6 has been identified in senescence secretome. However, not all the components of the secretome seem to contribute to the antitumor effects of oncogene-induced senescence (OIS). In fact, the presence of functional protumorigenic and prometastatic factors in the secretome of some senescent cells indicates that they may contribute to tumor progression in a cell nonautonomous manner (Coppe, et al. 2008a).

The dichotomous role of IL-6 in senescence and tumorigenesis is well represented in pituitary tumor development. Pituitary tumorigenesis appears to be regulated by extrinsic and intrinsic factors. It has been demonstrated that paracrine IL-6 effects may allow initial pituitary cell growth (required for senescence bypass) (Arzt, et al. 1999; Arzt 2001; Graciarena, et al. 2004), while autocrine IL-6 in the same tumor triggers senescence and restrains aggressive growth and malignant transformation (Sapochnik, et al. 2016).

This review provides an insight into the current understanding of the role of IL-6 in the regulation of pituitary pathogenesis, focusing in the autocrine action of IL-6. Pituitary cell growth regulation by IL-6 reinforces the role of cytokines as factors controlling pituitary cell division, and the findings of the IL-6 role in OIS suggest that endogenous IL-6 might be involved in development of pituitary adenoma senescence, which may contribute to explain the benign nature of these frequent tumors.

BIOLOGY AND FUNCTIONS OF IL-6

IL-6 was first characterized according to its ability to promote the population expansion and activation of T cells, the maturation of B cells into antibody-producing cells, and regulation of the acute-phase response (Andus, et al. 1987; Hirano, et al. 1986; Hirano 2014; Klimpel 1980; Woloski & Fuller 1985; Yasukawa, et al. 1987; Yoshizaki, et al. 1984). However, it is now known that IL-6 affects vascular disease, lipid metabolism, insulin resistance, mitochondrial activities, the neuroendocrine system and neurophysiological behavior (Bethin, et al. 2000; Hodes, et al. 2014; Jones, et al. 2011; Kraakman, et al. 2015; McInnes & Schett 2007; Rohleder, et al. 2012; Schett, et al. 2013). Accordingly, IL-6 is a pleiotropic cytokine with multiple physiological and pathological functions, produced by almost all stromal cells and cells of the immune system.

The expression of IL-6 is controlled at multiple levels to prevent overshooting systemic conditions. Several factors have been described as regulators of IL-6 mRNA either at transcriptional, as the IL-6 promoter contain motifs for the binding of AP-1, cyclic AMP, C/EBPβ, Sp1, CREB, STAT3 and NF-κB (Gerlo, et al. 2008; Kishimoto 2005; Lee, et al. 1987; Matsusaka, et al. 1993; Spooren, et al. 2010), or post-transcriptional level. This last includes Arid 5a (Masuda, et al. 2013), TNFα and IL-1β (Gruys, et al. 2005) as positive regulators, and regnase-I (Iwasaki, et al. 2011), bromodomain-containing protein 4 (BRD4) (Barrett, et al. 2014), micro RNAs (miR)-26a (Yang, et al. 2013), -142 (Sun, et al. 2013), -146a (He, et al. 2014), -146b (Xiang, et al. 2014), -187 (Rossato, et al. 2012), -200s (Dou, et al. 2013) and -329 (Garg, et al. 2013) as negative regulators (Figure 1).

IL-6 is a glycosylated secreted protein of nearly 25KDa, which varies depending on different N-linked glycosylation and species. Although not necessary for its function, IL-6 glycosylation might be important for stability or half-life of the protein. It has a characteristic structure made up of four long alpha-helices, which are arranged in a way that leads to an up-down-down topology found in all IL-6 type cytokines (Scheller, et al. 2011).

The secretion and availability of IL-6 is ubiquitous and it can bind to various types of cells in different tissues. IL-6 acts on cells as a dimer by binding to a specific IL-6 receptor (IL-6R) complex composed of two IL-6Rα chains (also known as IL-6Rα, gp80 or CD126) and the resultant IL-6/IL-6Rα complex associates with two signal-generating receptor beta chain subunits, named gp130 (also known as IL-6Rβ or CD130), at three distinct receptor-binding sites (Kojima, et al. 2013). In contrast to gp130, IL-6Rα is only expressed on a limited number of cell types, which actually facilitates the selective activation of several target cells (Rose-John, et al. 2006; Scheller & Rose-John 2006). Upon binding to the receptor and gp130, IL-6 induces various functions by activating cell signaling events (Mihara, et al. 2012). IL-6 triggers signal transduction via two different pathways (Kumari, et al. 2016) (Figure 1). The classic signaling, in which IL-6 binds to its transmembrane 80kDa receptor IL-6Rα, and the trans-signaling in which IL-6 binds to the soluble secretory form of IL-6Rα (sIL-6Rα) to form a complex that increases the circulating half-life of IL-6 and promotes its bioavailability (Peters, et al. 1996; Rose-John & Heinrich 1994). In both cases, once IL-6 binds to the receptor (with the same affinity), the complex binds to transmembrane gp130. Since gp130 is ubiquitously expressed, IL-6R expression determines whether a cell is responsive to classic signaling or trans-signaling. Although most soluble receptors are antagonist and compete with their transmembrane receptor, sIL-6Rα is an agonist of IL-6Rα (Wolf, et al. 2014). Classical IL-6R signaling seems to control central homeostatic processes (regulation of the neuroendocrine system), activates anti-inflammatory pathways and promotes the regeneration of tissue, while IL-6 trans-signaling activates pro-inflammatory pathways and plays an important role in many diseases and cancer (Kumari et al. 2016; Wolf et al. 2014). sIL-6Rα is generated by alternative splicing of IL-6 mRNA or by “shedding”, a limited proteolysis of extracellular region of the membrane-bound IL-6R carried out by transmembrane zinc-dependent proteases ADAM17 and ADAM10 (Chalaris, et al. 2011; Jones, et al. 2001; Jones et al. 2011; Yoshida, et al. 1996). Like sIL-6Rα, a soluble form of gp130 (sgp130) is also present in circulation at relative high concentrations during inflammation and cancer (Kovacs 2001; McFarland-

Mancini, et al. 2010; Rose-John 2012). Although classic signaling is not affected by sgp130, trans-signaling is inhibited by sgp130 binding to the IL-6-sIL-6R complex.

Once IL-6-IL-6R complex is formed, JAK kinases go through a conformational change, bringing the two JAKs close enough to phosphorylate each other and became activated. Signal transducer and activator of transcription 3 (STAT3) and STAT1 are recruited to the phosphorylated YXXQ motifs in gp130 and phosphorylated by JAK kinases, at the Y705 and Y701 tyrosine residues, for STAT3 and STAT1 respectively (Hirano, et al. 1997; Hirano, et al. 2000). The activated STAT3 and STAT1 dimerize with each other, making STAT3 or STAT1 homodimers and STAT3/STAT1 heterodimers. These activated STAT dimers enter the nucleus and bind to the specific DNA sequences in the regulatory regions of their target genes (Darnell 1997). STAT3 plays multiple roles depending on the cell context. It is well described the involvement of STAT3 in proliferation and cell survival by activating *c-myc*, *cyclin D1*, *bcl2*, *bclxl* or *mcl1* (Hirano et al. 1997; Hirano et al. 2000), in tumorigenesis (Bowman, et al. 2000; Yu, et al. 2009) and in growth arrest and differentiation (Hirano et al. 1997; Hirano et al. 2000; Nakajima, et al. 1996). To prevent overstimulation, the mechanism to turn off cytokine-mediated signal transduction involves Src-homology 2 domain-containing phosphatase (SHP2), which induce desphophorylation of JAK, gp130 and STATs (Lehmann, et al. 2003); protein inhibitors of activated STATs (PIAS) which inhibits STAT1 signaling by the interaction with the DNA binding of activated STAT1 (Liu, et al. 1998); and suppressor of cytokine signaling (SOCS), which act as classical feedback inhibitors acting on the JAKs and thereby inhibit the phosphorylation of gp130, STATs and JAKs themselves (Naka, et al. 1997; Starr, et al. 1997). Although JAK/STAT is the most described IL-6 signaling pathway, there are two other major pathways activated by IL-6: mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) and phosphatidyl-inositol-3-kinase (PI3K)-AKT pathways (Heinrich, et al. 2003) (Figure 1).

THE DUAL ACTION OF IL-6: TUMOR VS SENESCENCE

Cellular senescence is now recognized as a potent tumor suppressive mechanism that arrests the growth of cells at risk for malignant transformation (Braig, et al. 2005; Chen, et al. 2005; Collado, et al. 2005; Courtois-Cox, et al. 2006; Michaloglou, et al. 2005; Narita & Lowe 2005; Ventura, et al. 2007; Xue, et al. 2007). Recent studies show that senescent cells develop altered secretory activities, i.e. secrete proinflammatory cytokines, proteases and other proteins, that may induce changes in the tissue microenvironment, relaxing its control over cell behavior and promoting tumorigenesis (Acosta, et al. 2008; Coppe et al. 2008a; Coppe, et al. 2008b; Green 2008; Krtolica, et al. 2001; Kuilman, et al. 2008).

The senescent phenotype is not limited to an arrest of cell proliferation. In fact, a senescent cell is a potentially persisting cell that is metabolically active and has undergone widespread changes in protein expression and secretion, ultimately developing the senescence-associated secretory phenotype (SASP). Proliferating cells enter senescence in response to physiological signals during embryonic patterning and organogenesis, pathophysiological signals related to ageing or imminent malignant transformations, or exogenous causes of damage (Muñoz-Espín & Serrano 2014). The SASP includes several families of soluble and insoluble factors. These factors can affect surrounding cells by activating various cell-surface receptors and corresponding signal transduction pathways that may lead to multiple pathologies, including cancer. However, SASP role in tumor progression remains unclear and can be beneficial or deleterious, depending on the biological context (Lecot, et al. 2016). Senescence is a delayed stress response involving multiple effector mechanisms and has been recently described not only as a static endpoint, but also as a dynamic process of phenotypic establishment (Baker & Sedivy 2013; Young, et al. 2013). This distinction becomes more relevant in acute types of senescence, such as OIS, where the initial phenotype of OIS is a highly proliferative state, which mimics transformation, but this mitotic burst is gradually replaced by senescence (Young, et al. 2009).

In particular, it has been shown that OIS is specifically linked to the activation of an

inflammatory transcriptome, including pleiotropic cytokine IL-6 (Coppe et al. 2008a; Coppe, et al. 2010; Kuilman & Peeper 2009). IL-6 has been identified as a key component of the senescence secretome, which enables senescent cells to communicate with their microenvironment. The role of IL-6 and other SASP factors could support tumorigenesis and cell proliferation, but also may exert tumor suppressive functions and trigger an immune response, thereby favoring tumor cell clearance and cancer regression (Cichowski & Hahn 2008). Certainly, the secretory profile and function of the SASP are highly dependent from the cell type and context. Besides its paracrine mitogenic action, IL-6 was shown to actively contribute to the senescence process by reinforcing cell cycle arrest in an autocrine feedback loop: is required for the execution of OIS in a cell-autonomous mode (Kuilman et al. 2008; Sapochnik et al. 2016). IL-6 depletion causes the inflammatory network to collapse and abolishes senescence entry and maintenance. This may suggest that IL-6 pools required for OIS and for promoting oncogenicity or cell proliferation (Ancrile, et al. 2007; Sparmann & Bar-Sagi 2004) are inherently different.

It was suggested that the nature of the IL-6 target cell decides whether IL-6 acts as tumor suppressor or promoter (Kuilman et al. 2008; Yun, et al. 2012). The genetic makeup of the IL-6 target cell, whether normal or transformed, could contribute to specifying the biological response to IL-6.

PATHOPHYSIOLOGICAL ROLE OF IL-6 IN THE PITUITARY

In the adenohypophysis, hypothalamic stimulatory and inhibitory factors, together with feedback signals derived from target organs, converge with the auto-/paracrine factors, to induce transcriptional regulation, translation, and secretion of the pituitary hormones. Collectively, these regulatory mechanisms manage an accurate and dynamic gland homeostatic process (Perez-Castro, et al. 2012).

The physiological importance of the role that cytokines play in modulating the neuroendocrine-immune interconnection is extensively reflected in the anterior pituitary gland (Arzt et al. 1999;

196 Perez-Castro et al. 2012). The gp130 cytokines of the IL-6 family constitute a well-known
197 example, since they play important roles in function, growth and neuroendocrine responses of
198 the gland. The expression of specific receptors for the different gp130 cytokines, as well as
199 the cytokines themselves, are expressed in the anterior pituitary cells, providing basis for the
200 regulation of hormone secretion and cell growth. During acute or chronic inflammation or
201 infection, systemic, hypothalamic, or hypophyseal gp130 cytokines may act on anterior
202 pituitary cells, integrating the neuro-endocrine response. Elevated levels of cytokines alter the
203 physiological hormone production to adapt the endocrine system to the needs of the organism
204 to respond adequately to pathogens.
205 Pituitary tumors are mostly benign, non-metastatic and monoclonal neoplasms constituted by
206 cells of the adeno-pituitary gland, which generally cause small lesions and present a slow
207 growth (Dworakowska & Grossman 2009; Kopczak, et al. 2014; Lake, et al. 2013; Melmed
208 2011, 2015; Scheithauer, et al. 2006). The pathophysiological consequences of a pituitary
209 adenoma are related to over-production of particular pituitary hormones or due to tumor
210 compression and damage to the normal pituitary and vital structures surrounding it (Yu &
211 Melmed 2010).
212 Multiple extracellular and intracellular signals determine pituitary cell proliferation. Changes in
213 the expression or function of several cytokines and growth factors have been described to
214 participate in pituitary adenoma development (Perez-Castro et al. 2012), as it is well known
215 that normal pituitary cells are under the auto-/paracrine action of these factors. Altered levels
216 of transforming growth factor alfa and beta protein families, epidermal growth factor, fibroblast
217 growth factor family, bone morphogenetic protein 4 and IL-6/gp130family, have been
218 observed in pituitary tumors (Dworakowska & Grossman 2012; Jiang & Zhang 2013; Jones, et
219 al. 1994; Paez-Pereda, et al. 2003; Perez Castro, et al. 2000; Perez-Castro et al. 2012). It was
220 described that matrix metalloproteinase, secreted by pituitary cells, contribute also to the
221 control of cell proliferation during tumorigenesis (Paez-Pereda, et al. 2005).
222 In particular, the putative oncogenic role of the gp130 protein has been demonstrated in
223 lactosomatotroph GH3 tumor cells, which do not develop into tumors in nude mice after gp130

downregulation, indicating that one or more of the gp130 cytokines might play a role in pituitary tumorigenesis (Castro, et al. 2003). The expression of almost all of the gp130 cytokines and their corresponding receptors was detected either in normal or tumoral pituitary (Hanisch, et al. 2000; Jones et al. 1994; Perez Castro, et al. 2001).

Pituitary tumors do not progress like other solid tumors, which start with hyperplasia, pass a state of benign adenoma and end up with an aggressive carcinoma (Colao, et al. 2010; Farrell & Clayton 1998; Melmed 2008). Pituitary cells are among the few epithelial cell types that rarely undergo malignant transformation. Given that premature senescence occurs in slow growing benign or early stage tumors but not in late stage or malignant tumors and that pituitary adenomas have exhibited stable growth after decades of observation (Levy & Lightman 2003; Melmed 2011), the unique growth of these benign adenomas has been linked with this tumor suppressive mechanism. OIS has been implicated in the arrest of pituitary tumors as in several other types of benign tumors. It has been shown in human and murine melanocytic nevi (Goel, et al. 2009; Michaloglou et al. 2005), human dermal neurofibromas (Courtois-Cox et al. 2006), human schawnnomas (Simonetti, et al. 2014) and human pituitary adenomas (Alexandraki, et al. 2012; Chesnokova, et al. 2007; Chesnokova, et al. 2008; Donangelo, et al. 2006; Lazzerini Denchi & Helin 2005; Sapochnik et al. 2016), but not in malignant adenocarcinomas. Cell senescence has a functional relevance *in vivo*, as a physiological mechanism limiting tumorigenesis in many diseases. Premature pituitary tumor cell senescence appears to bypass pro-proliferative signals, thereby stopping cell proliferation, while preserving vital homeostatic pituitary functions in order to maintain cell viability (Arzt, et al. 2009; Melmed 2011).

IL-6 is produced by tumoral cells themselves but is also delivered to the adenoma cells through IL-6-producing folliculo stellate (FS) cells, which surround or invade the pituitary tumors (Farnoud, et al. 1994; Hofler, et al. 1984; Renner, et al. 1997; Renner et al. 1998; Ueta, et al. 1995; Vajtai, et al. 2007). IL-6 mRNA and protein levels were also detected in cell cultures of all types of pituitary adenomas (Arzt et al. 1999; Borg, et al. 2003; Jones et al.

1994; Sapochnik et al. 2016). Pituitary IL-6 production can be increased by many compounds such as IL-1 (Spangelo, et al. 1991), TNFα, pituitary adenylate cyclase-activating polypeptide (Arzt et al. 1999) and by lipopolysaccharides (Tichomirowa, et al. 2005) and it is inhibited by glucocorticoids (Pereda, et al. 2000). Intrapituitary IL-6 regulated both by neuroendocrine and the immune system, plays a critical role in the pituitary as a neuroendocrine-immune integrator.

Paracrine IL-6 promotes the growth of pituitary cells that could lead to the development of pituitary adenomas. It acts as a stimulatory growth factor (Arzt et al. 1999; Arzt 2001) and also promotes the secretion of vascular endothelial growth factor and matrix metalloproteinases from surrounding FS cells (Gloddek, et al. 1999; Renner et al. 1998), producing not only the expansion of tumoral cells but also vessel formation and extracellular matrix remodeling (Renner et al. 1998). Notably, although this cytokine induced proliferation of GH3 lactosomatotroph cells, it was also shown to inhibit normal pituitary cells (Arzt, et al. 1993). Inhibitory or stimulatory actions of IL-6 were observed in ACTH-, PRL-, GH-secreting and nonfunctioning adenomas, without association to the size or type of the tumor (Pereda, et al. 1996). Activation of different signaling pathways by IL-6/gp130 complex, as discussed above, may explain the differences observed in IL-6 action on the anterior pituitary (Arzt 2001).

AUTOCRINE IL-6 MEDIATES PITUITARY TUMOR SENESCENCE

Different mechanisms and factors involved in the initiation and progression of pituitary adenomas have been described, including cell cycle deregulation, overexpression of growth factors, oncogenes and hormones, defective signaling pathways and an altered intrapituitary microenvironment (Clayton & Farrell 2004; Colao et al. 2010; Dworakowska & Grossman 2009; Farrell 2006; Melmed 2011; Perez-Castro et al. 2012; Vandeva, et al. 2010), as well as inherited or somatic mutations in genes such as AIP (Vierimaa, et al. 2006), GPR101 (Trivellin, et al. 2014) and USP8 (Reincke, et al. 2015). The recent characterization of pituitary stem cells (Fauquier, et al. 2008; Garcia-Lavandeira, et al. 2009; Garcia-Lavandeira, et al.

2015; Vankelecom & Gremeaux 2010) implies the possibility of defining their mechanisms involved not only in pituitary cell renewal but also in pituitary tumorigenesis. The presence of a side population containing cells with high efflux capacity and potentially enriched for tumor stem cells pituitary tumors have been described by several groups (Florio 2011; Gleiberman, et al. 2008; Mertens, et al. 2015). In line with that, it has been reported enhanced self-renewal as a mechanism of tumor initiation in pituitary adenomas (Andoniadou, et al. 2013; Donangelo, et al. 2014; Gaston-Massuet, et al. 2011; Hosoyama, et al. 2010). It has been proposed that the initial mutation that drives tumorigenesis occurs in a cell type (adult pituitary stem cells, SCs) that does not contribute cell autonomously to the tumor. SCs cells, which include FS, secrete factors (such as IL-6) leading to the transformation and proliferation of neighboring cells that generate a tumor (Andoniadou et al. 2013).

FS cells are major agranular cells with a characteristic star-shaped morphology located in the parenchymal tissue of the anterior pituitary gland, representing 5-10% of all pituitary cells. Within the pituitary, FS cells form a three-dimensional anatomical cellular network surrounding hormone-secreting cells, connected to them via gap junctions (Renner et al. 1998). In the normal pituitary IL-6 is produced only by FS cells (Vankelecom, et al. 1989), whereas in pituitary adenomas IL-6 is produced by the pituitary tumor cells themselves (Jones et al. 1994). Intrapituitary IL-6 is assumed to act in a paracrine manner to modulate endocrine cell function and growth in response to external stimuli. IL-6 itself influences hormonal output, i.e. stimulates the secretion of ACTH, GH, PRL, LH, and FSH (Ray & Melmed 1997; Renner, et al. 1996), from the anterior lobe in a paracrine manner. It has been demonstrated a transition zone between normal pituitary tissue and the adenoma that is extremely rich in FS cells (Farnoud et al. 1994). Paracrine IL-6 delivered by FS cells contributes to the development of an adenoma, by promoting tumor cell expansion because of the induction of VEGF release and extracellular matrix-modifying enzymes and tissue inhibitors of metalloproteinases expression (Matsumoto, et al. 1993), which cause extracellular matrix remodeling and vessel formation (Renner et al. 1998). After transformation of a normal pituitary cell to a tumor cell, the further development of the tumor is triggered by the interaction of the FS cells and the

306 tumor cells. *In vitro* studies have shown additional evidence of this. The rat somatotrophic
307 pituitary MtT/S cells overexpressing (sense) or lacking (antisense) gp130 protein were
308 coinoculated with the TtT/GF cell, a mouse FS-*like* cell line, in nude mice (Graciarena et al.
309 2004). At low cell concentration, MtT/S sense and control clones generated tumors of a
310 smaller size than those derived from these same clones plus TtT/GF cells, showing a clear
311 dependence on FS cells. In both cases, MtT/S antisense had an impaired tumor development.
312 Moreover, vessel density was significantly lower in tumors derived from MtT/S antisense plus
313 TtT/GF cells (Graciarena et al. 2004). In these interactive processes, paracrine IL-6 plays a
314 prominent role by stimulating tumor cell proliferation, tumor neovascularization and
315 extracellular matrix remodeling.

316 OIS is linked specifically to the activation of an inflammatory transcriptome, which includes IL-
317 6, in a transduced human melanocytes model (Kuilman et al. 2008). Upon secretion by
318 senescent cells, IL-6 acts promitogenically in a paracrine fashion, but regulates OIS in a cell-
319 autonomous mode, indicating that IL-6 can function as an autocrine or paracrine tumorigenic
320 factor. In line with that, oncogenic stress triggered also the induction of the CDK inhibitor
321 $p15^{INK4B}$, which was dependent on the presence of both IL-6 and C/EBPβ. Taking into account
322 that the stable proliferative arrest in G1 phase of the cell cycle characteristic of senescence is
323 through activation of the p53/$p21^{Cip1}$ and pRb/p16 ^{INK4a} pathways and, consequently,
324 overexpression of cdk inhibitors like $p15^{INK4b}$, this result establishes a link between OIS-
325 activated interleukin signaling and the cell-cycle machinery, suggesting that IL-6 acts in
326 concert with its receptor and $p15^{INK4b}$ to cause cell-cycle arrest in response to oncogenic
327 stress. Thus, IL-6 not only triggers OIS but also maintained it (Kuilman et al. 2008). The
328 protective role of IL-6 in OIS, as discussed below, occurs naturally in pituitary adenomas as a
329 dynamic and slow mechanism, that results in a benign tumor with stable growth arrest.
330 Interestingly, in other endocrine tumors like thyroid nodules, it has been reported IL-6 (and its
331 receptor) expression (Ruggeri, et al. 2002) and also OIS with an associated inflammatory
332 secretome (Vizioli, et al. 2014), suggesting that a senescence process involving IL-6 might

also take place in thyroid tumor progression.

The activation of cell cycle arrest machinery and the involvement of PTTG (Chesnokova et al. 2007; Chesnokova et al. 2008), was also found in pituitary adenomas and more interesting, a differential lineage-specific pathway restricting and controlling pituitary cell cycle progression and triggering senescence was described (Chesnokova, et al. 2011). PTTG exhibits oncogene properties (Pei & Melmed 1997; Zhang, et al. 1999) and its expression results in the activation of DNA-damage signaling pathways, aneuploidy and chromosomal instability *in vitro* and *in vivo* (Kim, et al. 2005; Kim, et al. 2007; Vlotides, et al. 2007), ending in pituitary-specific senescent features (Chesnokova, et al. 2005; Chesnokova et al. 2007). Different to most human GH-producing pituitary adenomas in which PTTG overexpression is associated with p21-dependent senescence (Chesnokova et al. 2008), tumors arising from the gonadotroph lineage also exhibit high PTTG levels, but p21 is not expressed in gonadotroph-derived non-functioning pituitary adenomas, which express $p15^{INK4b}$ and $p16^{INK4a}$. This could be explained by the fact that activation of senescence effector pathways depends on cell and tissue context, the intensity and duration of the signal, and the nature of the damage (d'Adda di Fagagna 2008), which has led to define distinct senescence types (Muñoz-Espín & Serrano 2014).

A recent work (Zhang, et al. 2015) has shown that the expression of IL-6 was significantly increased in aging pituitary tissues, i.e. senescent pituitary, in contrast to normal and tumoral rat pituitaries. Plasma IL-6 concentration was decreased in aging rats compared with normal rats, indicating that the paracrine activity of IL-6 was inhibited in aging rats. As discussed above, IL-6 has opposite dual effects on cell proliferation and growth (Arzt et al. 1993; Arzt et al. 1999; Renner et al. 1996). Taking into account that IL-6 participates in the progression of pituitary tumors, and its role in OIS, this cytokine appears as a candidate for an autocrine/paracrine regulator of pituitary adenoma control. The regulation of OIS by IL-6 has been recently shown using a pituitary tumor senescence cell model (MtT/S cell line) and an *in vivo* senescence model, human pituitary tumor samples (Sapochnik et al. 2016). In both

models, the absence of endogenous IL-6 produces a decrease in senescent biomarkers and, as expected, an increase in cell proliferation and invasion capacity. These findings indicate that the lack of IL-6 allowed tumoral cells to bypass senescence and consequently became tumorigenic. In pituitary tumors IL-6 contributes to maintain the senescent phenotype of these tumoral cells by its autocrine action. Comparing tumors developed by the silencing of IL-6 (i.e. the abolishment of senescence) with tumors resembling the natural situation in which both the paracrine proliferative IL-6 and the autocrine-inducing senescence are on, tumors expressing endogenous IL-6 present a more pronounced senescent phenotype. The dual action of IL-6 in the regulation of two opposite mechanisms occurs in different steps of pituitary tumor development (Figure 2). In the normal pituitary paracrine IL-6 delivered by FS cells do not affect normal cell growth but may act to induce proliferation of tumoral cell and, consequently, the development of an adenoma (Figure 2). However, autocrine IL-6 in the same tumor may induces and maintains senescence and contribute to control aggressive growth and malignant development of these cells (Figure 2).

FUTURE PERSPECTIVES

Senescence is considered an important tumor protection barrier that contributes to stop proliferation and further malignant transformation allowing the pituitary cell to remain viable and perform its homeostatic physiological function. The presence of senescent cells in the tumor and the consequently SASP are important biological factors that favors vital functioning of the pituitary gland for homeostatic control. Thus, pituitary adenomas constitute faithful *in vivo* models of senescence. The presence of senescent cells in the tumor and the relative abundance of different proteins produced by the senescent cells are important biological factors that could have significant prognostic implications for the fate of the disease. The involvement in the senescent process of several oncogenes and mutations recently described in the pituitary (Reincke et al. 2015; Trivellin et al. 2014; Vierimaa et al. 2006) remains an interesting open question.

IL-6 represents an important factor in the regulation of pituitary adenoma development, as promotes tumorigenesis by its paracrine action while restrains further proliferation by inducing and maintaining senescence in the same tumor. Which signaling pathways contribute to each action will certainly enrich to understand this phenomenon. Given its dual and opposite action in the pituitary pathophysiology, IL-6 is an interesting factor for further studies in the outcome of the disease.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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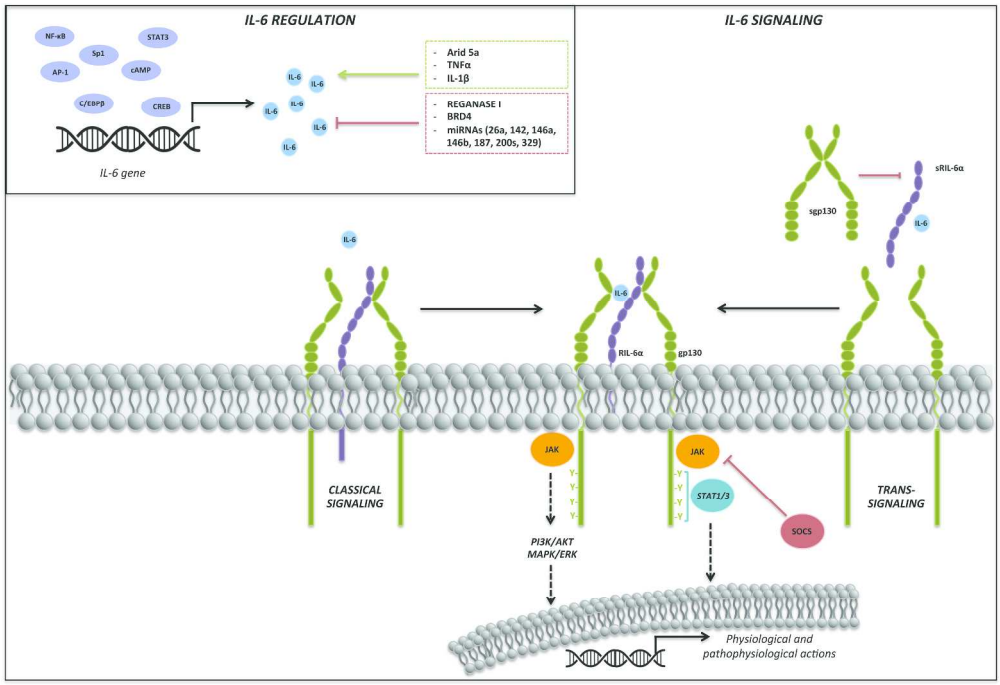
FIGURES

Figure 1. IL-6 regulation and signaling

IL-6 expression and function is highly regulated by many factors that act at a transcriptional and posttranslational level. IL-6 binds to its receptor and then forms a heterotrimer with two gp130 subunits anchored to the plasma membrane. IL-6 signals by to two different pathways: classic IL-6 signaling is mediated via the membrane-bound IL-6R (left), whereas trans-signaling acts via sIL-6R (right). Dimerization of gp130 results in the activation of STAT1/3, MAPK/ERK, and PI3K/AKT signaling pathways, which regulates different physiological and pathophysiological processes.

Figure 2. Pathophysiological role of IL-6 in the pituitary: role of autocrine IL-6 in senescence

IL-6 has a dual role in the anterior pituitary. It is secreted to the normal or adenoma cells by FS cells which, by its paracrine action, induce pituitary cell proliferation at the initial proliferative phase of pituitary adenomas. IL-6 is also secreted by the tumoral cells themselves which, by its autocrine action, stops proliferation and progression of pituitary tumors by inducing and maintaining senescence.



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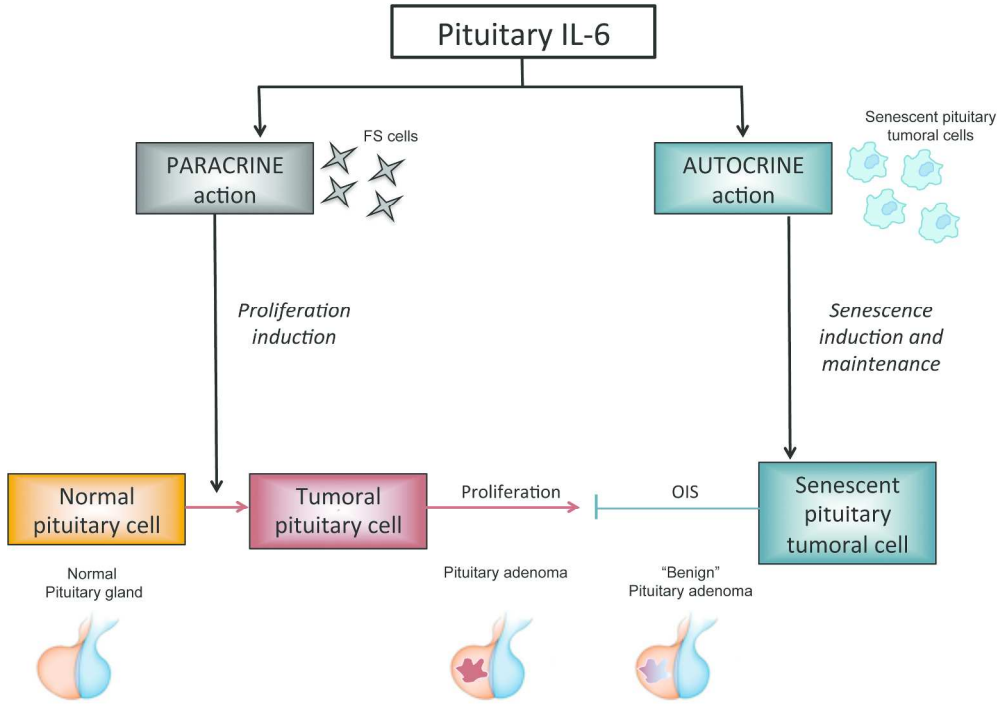

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