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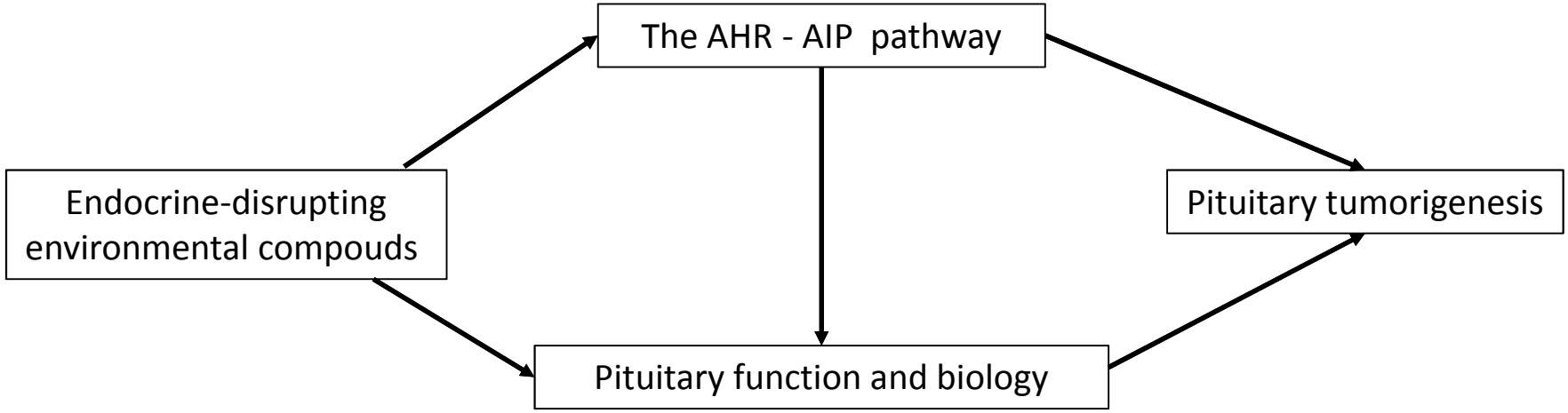

AHR: Aryl Hydrocarbon Receptor
AIP: AHR-Interacting Protein

ACROMEGALY, GENETIC VARIANTS OF THE ARYL HYDROCARBON RECEPTOR PATHWAY AND ENVIRONMENTAL BURDEN

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ABSTRACT

Increasing evidence suggests that environmental contaminants can exert endocrine disruptors activities and that pollution exposition can have a role in tumorigenic processes. Several environmental pollutants have been shown to affect pituitary cells biology and function. The aryl hydrocarbon receptor (AHR) pathway is involved in xenobiotics' metabolism and in tumorigenesis. A deregulation of the AHR pathway could have a role in pituitary tumours' pathophysiology, especially in the GH secreting ones. AHR-interacting protein (AIP) is one of the key partners of AHR and is implicated in pituitary tumours' pathogenesis. Moreover, an increased prevalence of acromegaly has been reported in a highly polluted area of the province of Messina (Sicily, Italy). Nevertheless, at present, few data are available about the potential role of environmental factors in the pathogenesis and clinical expression of GH secreting pituitary tumours. This review is aimed at discussing the evidences on the potential links among environmental pollutants, the AHR pathway and the pathophysiology of GH-secreting pituitary adenomas.

***Key terms*:** acromegaly, AHR, AIP, pollution, endocrine disruptors

Introduction

Acromegaly is a rare disease characterised by an abnormal growth of bone, soft tissues and organs, as a consequence of a growth hormone (GH) excess due – in most of the cases – to a pituitary adenoma (Broder, Chang, Cherepanov et al., 2016; Capatina and Wass, 2015; Dal, Feldt-Rasmussen, Andersen et al., 2016; Daly, Rixhon, Adam et al., 2006; Melmed, 2009; Mestron, Webb, Astorga et al., 2004). The estimated prevalence of acromegaly has been variably reported between 34-125 cases per million of persons (cpm), although it has been shown to be even higher in some studies (Cannavo, Ferrau, Ragonese et al., 2010). Acromegaly is generally diagnosed after several years from disease onset with considerable clinical, economical and social consequences (Capatina and Wass, 2015). To date, the pathogenic mechanisms underlying the development, progression and clinical impact of GH-secreting pituitary tumours are only partially disclosed.

Some studies have shown that environmental pollutants with endocrine disruptors activities can impact on pituitary function and biology (Gore, 2010). It's known that the aryl hydrocarbon receptor (AHR) is the main actor of the intracellular mechanisms of xenobiotics' metabolism and detoxification, and can influence the major stages of tumorigenesis (Figure 1) (Dietrich and Kaina, 2010; Feng, Cao and Wang, 2013; Hao and Whitelaw, 2013). However, data on the role of environmental pollutants and of the alterations of the AHR pathway in pituitary tumorigenesis are scanty. On the other hand, the AHR cytosolic stabilization, function and signalling pathway are strictly dependent on the AHR-interacting protein (AIP) that in turn has a key role in pituitary tumour development (Beckers, Aaltonen, Daly et al., 2013; Beischlag, Luis Morales, Hollingshead et al., 2008; Hao and Whitelaw, 2013; Petrulis and Perdew, 2002; Trivellin and Korbonits, 2011). Indeed, AIP gene mutations have been found in about 20% of patients with familial isolated pituitary adenoma syndrome (FIPA) and in 40% of patients with isolated familial somatotropinomas, as well as in up to 4% of patients with apparently sporadic acromegaly (Beckers et al., 2013; Ferrau, Romeo, Puglisi et al., 2016; Hernandez-Ramirez, Gabrovska, Denes et al., 2015; Lloyd and Grossman, 2014). Pituitary tumours associated with AIP gene mutations are

mainly GH and/or PRL secreting, show an aggressive clinical and biochemical phenotype, occur more frequently in young patients and are more resistant to conventional treatments (Alband and Korbonits, 2014; Beckers et al., 2013; Martucci, Trivellin and Korbonits, 2012). In this review, the evidence on the potential links among environmental pollutants, the AHR pathway and the pathophysiology of GH-secreting pituitary adenomas will be discussed.

The AHR-AIP pathway

The AHR is a transcription factor belonging to the basic helix-loop-helix/Per/ARNT/Sim (PAS) family (Dietrich and Kaina, 2010). It is stimulated by several natural compounds which are present in food, as indoles and flavonoids, or by tryptophan derivatives, arachidonic acid metabolites and other endogenous products (Denison and Nagy, 2003; Denison, Pandini, Nagy et al., 2002; Dietrich and Kaina, 2010). The most potent AHR ligand known so far is the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) but more than 400 exogenous compounds act as AHR ligands, most of which are environmental endocrine disruptors, including a variety of polycyclic aromatic hydrocarbons and planar polychlorinated biphenyls (PCBs) (Denison and Nagy, 2003; Denison et al., 2002; Dietrich and Kaina, 2010).

It is generally accepted that the metabolic responses to environmental pollutants can be the direct consequence of AHR activation. In the cytosol, the unbound receptor forms a complex with AIP, the co-chaperone protein p23 and two heat shock protein 90 (Hsp90) molecules. Ligand binding results in nuclear translocation of AHR, dissociation from the chaperone proteins, heterodimerization with a nuclear translocator (ARNT) and subsequent binding to xenobiotic-responsive elements (XREs) (Figure 1). This leads to transactivation of several genes encoding phase I and II xenobiotic metabolizing enzymes, such as cytochrome P450s (CYP1A1, CYP1A2 and CYP1B1), as well as other genes coding for non-enzymatic molecules or proteins like the cyclin-dipendent kinases inhibitor p27Kip1 (Denison and Nagy, 2003; Dietrich and Kaina, 2010; Feng et al., 2013; Hao and Whitelaw, 2013). The functional relevance of the AHR pathway is not

restricted to the cellular response to the toxic insult, since others 'non-canonical' effects (on the cell-cycle, contact inhibition, cell adhesion, function and metabolism of the oestrogen receptors, and DNA repairing processes) can be the direct consequence of the activation of this transcriptional factor, further contributing to the disruption of cell homeostasis (Denison and Nagy, 2003; Dietrich and Kaina, 2010; Feng et al., 2013).

The AHR plays a relevant role in tissue-specific embryonic development, hematopoietic stem cell self-renewal, pluripotent stem cell and neural stem cell differentiation, and erythroid stem cell growth, as well as in development of cells with cancer stem cell-like qualities (Gasiewicz, Singh and Bennett, 2014; Mulero-Navarro and Fernandez-Salguero, 2016; Stanford, Wang, Novikov et al., 2016). The AHR interacts directly and affects the activity of Sox2, a master regulator of self-renewal that is also required for pituitary progenitor proliferation (Goldsmith, Lovell-Badge and Rizzoti, 2016; Stanford et al., 2016).

The AHR is thought to be involved in tumorigenic processes, although the molecular mechanism underlying the AHR-related carcinogenesis is largely unknown and probably species and tissue specific (Feng et al., 2013; Murray, Patterson and Perdew, 2014). Abnormal AHR expression and/or activity have been shown in a variety of sporadic human cancers (Dietrich and Kaina, 2010; Feng et al., 2013; Harper, Riddick and Okey, 2006). Some studies suggested that AHR is also implicated in the transition from a benign to a malignant tumour, and that the AHR pathway activation associates with increased tumour invasiveness (Villano, Murphy, Akintobi et al., 2006; Yang, Liu, Murray et al., 2005). Moreover, the pattern of regulation of the AHR gene has been identified as one of the most altered in a recent study aimed to identify the driver genes in colorectal cancer (Aziz, Periyasamy, Al Yousef et al., 2014). Among the endocrine tumours, AHR has been found overexpressed in papillary thyroid carcinomas of patients with and without acromegaly, especially in tumour samples carrying the BRAF V600E mutation (Mian, Ceccato, Barollo et al., 2014).

AIP is one of the main partners of AHR, being crucial for its cytoplasmic stabilization and function, and both of them are expressed in the pituitary (Denison et al., 2002; Trivellin and Korbonits, 2011). AIP is a co-chaperone protein that interacts with several other proteins such as nuclear receptors (peroxisome proliferator activated receptor alpha, estrogen receptor alpha, thyroid receptor beta1 and glucocorticoid receptor), oncogenes and components of the cAMP-signalling pathway (Trivellin and Korbonits, 2011). AIP gene mutations have been described in families with cases of isolated pituitary adenomas, predominantly presenting as GH and/or PRL secreting tumours (Beckers et al., 2013; Vierimaa, Georgitsi, Lehtonen et al., 2006). AIP gene mutations have been also found in young subjects with sporadic pituitary adenomas presenting with aggressive disease and less responsive to conventional medical treatment (Cazabat, Bouligand, Salenave et al., 2012; Daly, Tichomirowa, Petrossians et al., 2010; Lloyd and Grossman, 2014). AIP has been suggested to act as a tumour suppressor in pituitary cells (Leontiou, Gueorguiev, van der Spuy et al., 2008). The precise tumorigenic mechanism is not well understood but some recent data point to a deregulation of the cAMP pathway. Indeed, it has been demonstrated that AIP deficiency leads to elevated cAMP concentrations through defective inhibitory G protein α subunits (Gα)i-2 and Gαi-3 that normally inhibit cAMP synthesis (Tuominen, Heliovaara, Raitila et al., 2015). The same authors demonstrated, by immunostaining, that AIP deficiency is associated with a reduction in Gαi-2 protein expression in human and mouse GH-secreting pituitary adenomas, thus indicating defective Gαi signalling in these tumours (Tuominen et al., 2015). Moreover, a recent study showed a reduced half-life of AIP mutants, due to enhanced proteosomal degradation, that would correlate with the clinical phenotype (Hernandez-Ramirez, Martucci, Morgan et al., 2016).

Some studies suggested that AIP gene mutations could impact on AHR signalling pathway (Nukaya, Lin, Glover et al., 2010; Trivellin and Korbonits, 2011). Indeed, AIP gene mutations, known to be pathogenic and affecting the C-terminal region of the protein, have the potential to disrupt the AHR and phosphodiesterase (PDE)4A5 client-protein interaction (Morgan, Hernandez-Ramirez, Trivellin et al., 2012). Among more than 20 interactions of AIP, that one with the AHR-

cAMP-PDE signalling pathway seems to be the most promising in terms of a potential involvement in pituitary tumorigenesis (Dietrich and Kaina, 2010). On the other hand, the cAMP pathway has been shown to be relevant in GH-secreting pituitary cells' biology and is a target of the action of somatostatin analogues (SSA) that are widely used to control acromegaly (Melmed, 2003). Moreover, cAMP is a non-ligand activator of AHR and nucleocytoplasmic shuttling of AHR induced by cAMP is inhibited by PDE2, which in turn is stabilised by AIP. Furthermore, AIP has been demonstrated to reduce forskolin-induced cAMP signalling in GH3 cells (GH and PRL secreting rat pituitary tumour cells) (de Oliveira, Hoffmeister, Gambaryan et al., 2007; Formosa, Xuereb-Anastasi and Vassallo, 2013; Oesch-Bartlomowicz, Huelster, Wiss et al., 2005).

However, the AIP effects on AHR are still a matter of debate. Some authors showed that AIP could increase the transcriptional activity and expression of AHR, while others suggested the opposite (Kazlauskas, Poellinger and Pongratz, 2000). Some evidence suggests that AIP prevent AHR from ubiquitinous-dipendent proteosomal degradation (Kazlauskas et al., 2000; Morales and Perdew, 2007; Trivellin and Korbonits, 2011). Recently, Lecoq A. et al. showed that AIP protein level in fibroblasts from AIP mutated patients was reduced as compared to healthy subjects, without any significant change in terms of AHR expression (Lecoq, Viengchareun, Hage et al., 2016). However, in the same study, gene expression analysis showed significant alterations in the expression of the AHR target genes CYP1B1 and AHRR in AIP-mutated fibroblasts, both before and after stimulation with the endogenous AHR ligand kynurenine. AHR activation increased CYP1B1 expression to a greater extent in GH3 cells overexpressing wild type AIP than in cells expressing mutant AIP. Furthermore, AHR related CYP1B1 induction was reduced in AIP-knockdown GH3 cells as well as the reduced expression of AIP affected the kynurenine-dependent GH secretion of GH3 cells. This data would suggest an impairment of AHR transcriptional activity in AIP-mutated cells (Lecoq et al., 2016).

Moreover, there are several functional cross talks between the AHR-AIP pathway and other intracellular signalling pathways that in turn could mediate the effects of environmental pollutants

and could be also implicated in the tumorigenic process. For instance, AHR is involved in androgen and oestrogen receptors (ER) function, promoting the ubiquitination and proteosomal degradation and modulating the actions of sex steroids. Moreover, the AHR/ARNT complex antagonizes the estrogenic action by inhibiting competitively the binding of ERalfa with the oestrogen responsive elements when close to or overlapping the XRE (Klinge, Bowers, Kulakosky et al., 1999; Shanle and Xu, 2011). The interaction among AHR and ER and AR would modulate in a positive or negative fashion the oestrogen/androgen signalling pathways according to the cellular context. In this regard, it's noteworthy that several endocrine disruptors can interfere with the oestrogen receptor signalling in GH3 cells (Kim, Jung, Choi et al., 2012; Vo, An, Yang et al., 2012; Wang, Knosp, Tai et al., 2014).

The AHR pathway in GH-secreting pituitary tumours

In GH-secreting pituitary adenomas, lower AIP expression, as detected by immunohistochemistry, has been shown to associate with a more aggressive disease, since it correlated with higher pre-operative GH and IGF-1 levels, more invasive pituitary tumours and higher Ki67 index (Jaffrain-Rea, Angelini, Gargano et al., 2009; Kasuki, Colli, Elias et al., 2012; Kasuki, Vieira Neto, Wildemberg et al., 2012). Nevertheless, the expression of AHR and its partners in pituitary tumours is more controversial. It's worth of noting, however, that AHR expression and signalling could be differently affected according to pituitary tumour phenotype, in line with the postulated cell and tissue specific actions of AHR. In 2009, a study from Jaffrain-Rea et al. showed a lower expression of AIP and AHR in invasive somatotropinomas compared to non-invasive ones, suggesting that the down-regulation of both could be associated to disease aggressiveness (Jaffrain-Rea et al., 2009). The same group in a following study found some AHR cytoplasmic (AHRc) immunostaining in 94% of cases and AHR nuclear (AHRn) staining in 49% of samples, with a positive correlation between the AHRc and AHRn and between AIP and AHRc, which would suggest a role for AIP in stabilising AHR. The GH-secreting adenomas with higher AHR expression (32% of the analysed

cases) were smaller than the other ones and included a higher percentage of pure GH-secreting tumours as compared to those expressing AHR at low levels. Moreover, the AHR content or localization was not statistically influenced by preoperative SSA treatment, excluding a relevant role for AHR in the pharmacological response to SSA. However, SSA treatment was able to reduce the correlation between AHRc and AIP content, suggesting different effects of these drugs on the expression of the two proteins or a destabilization of their complex (Jaffrain-Rea, Rotondi, Turchi et al., 2013). The same authors found that Gsp status could have some effects on AHR expression or localization. Indeed, AHR nuclear content was reduced in Gsp^{+ve} tumours, probably due to a reduced cAMP concentration with consequent inhibited AHR shuttling, while pre-operative SSA treatment was able to increase AHR expression in Gsp^{-ve} cells. However, octreotide treatment of primary cell cultures of human somatotropinomas did not increase AHR expression, regardless of Gsp status (Jaffrain-Rea et al., 2013).

On the other hand, Heliovaara et al. found ARNT to be underexpressed and AHR nuclear content to be increased in AIP mutated pituitary adenomas compared to AIP wild type tumours. The authors suggested that the down-regulation of ARNT could be connected to an imbalance in AHR/ARNT complex formation arising from aberrant cAMP signalling (Heliovaara, Raitila, Launonen et al., 2009). It's worth to be mentioned, however, that in this study 3 out of 14 AIP mutated samples were pure PRL-secreting adenomas. However, AIP silencing in HeLa, HEK293, or Aip-null mouse embryonic fibroblast cells did not show reduced expression of ARNT, but in GH3 cells caused a partial reduction of ARNT and a clear increase in cell proliferation (Heliovaara et al., 2009).

Xenobiotics, the AHR and pituitary tumours: *in vitro* studies.

Some studies showed that in pituitary cell lines, the activation of AHR could result in endocrine effects. Indeed, in GH3 cells, the reversible AHR agonist β-naphthoflavone induced the expression of CYP1A1 and impaired PRL but not the GH expression, without any clear effect on cell proliferation, although the levels of the anti-proliferative signalling cytokine TGFbeta1 were

suppressed (Moran, Brannick and Raetzman, 2012). Similarly, in the rat pituitary gland cells, TCDD was demonstrated to upregulate known AHR target genes, to upregulate ER1 expression and to abrogate estradiol-induced PRL expression (Cao, Patisaul and Petersen, 2011).

On the other hand, several environmental contaminants have been shown to act as endocrine disruptors in pituitary cells.

Perfluoroalkyl acids, perfluorinated compounds with endocrine disruption potential in humans and animals, have been shown to affect GH3 cells growth and the T3-induced cell proliferation, and to interfere with the thyroid hormone receptor (TR) and AHR function (Long, Ghisari and Bonefeld-Jorgensen, 2013).

PCBs, chemicals that can disrupt the endocrine function and promote the incidence of tumours, could bind to AHR if they have a dioxin like structure (Giesy and Kannan, 1998; Ludewig and Robertson, 2013). Some authors showed that non-dioxin like PCBs could be involved in the regulation of rat pituitary cells apoptosis with a pro-apoptotic or an anti-apoptotic effect, depending on their chemical structure. They also showed that the regulation of pituitary apoptosis by PCBs involves multiple pathways, occurring through an AHR or a TR-dependent mechanism, and is associated with changes in the expression level and activity of caspases (Raggi, Russo, Urbani et al., 2016).

A very recent *in vitro* study showed that incubation with some endocrine disruptors, e.g. phenol and bis-(2-ethylhexyl)-phthalate, increases cell viability, energy content and proliferation, as well as Ccnd1, PTTG, AhR and AIP expression in normal rat pituitary cells (Tapella, Sesta, Cassarino et al., 2016). In addition, the long-term benzene exposition increased GH synthesis in GH3 cells, and this effect was associated with decreased AIP and increased AHR expression (Zunino, Catalano, Guaraldi et al., 2014). In the same study, exposition to benzene also increased the expression of the somatostatin receptor subtype 2 but decreased ZAC1 expression, effect in accordance with a potential impairment of the sensitivity to SSA (Zunino et al., 2014).

Phytoestrogens are natural plant components that can interfere with the hormones' actions or secretion. It has been shown that single isoflavonoid metabolites and their mixture and coumestrol induced GH3 cell growth and AHR transactivity dose-dependently (Long, Kruger, Ghisari et al., 2012).

Pesticides have been also proved to interfere with TR signalling and AHR function in vitro and might have the potential to cause endocrine disruption in GH3 cells (Ghisari, Long, Tabbo et al., 2015).

Xenobiotics effects on GH , PRL and POMC: studies in animal models.

Several data suggest the pituitary gland to be a direct target for TCDD or other toxins and the AHR system to play a physiological role in controlling neuroendocrine functions (Huang, Ceccatelli and Rannug, 2002). In mice, maternal exposure to TCDD attenuates gonadotropin-regulated steroidogenesis and GH expression leading to the impairment of pup development and sexual immaturity via mechanisms that involve AHR activation (Takeda, Taura, Hattori et al., 2014). In mice, loss of AHR leads to a reduction in PRL mRNA, while GH is unaffected (Moran et al., 2012). In 129/SV/C57BL/6 mice, TCDD or beta-naphthoflavone treatment increased the levels of CYP1A1 mRNA and protein, as well as the AHR repressor (AHRR) mRNA levels, in the pituitary gland. Furthermore, a three-fold increase in POMC mRNA was observed in the pituitary of TCDD treated mice. POMC mRNA level was also increased in the pituitary cell line AtT-20 after TCDD treatment (Huang, Ceccatelli, Hoegberg et al., 2003). Exposure to PCBs can affect growth and pituitary GH content in chicken embryos as well (Gould, Cooper and Scanes, 1997). In the rainbow trout's pituitary gland, the treatment with o,p'-DDT (o,p'-dichlorodiphenyltrichloroethane), a xenoestrogen, resulted in a significant induction of GH and PRL mRNA. Co-incubation of pituitaries with TCDD and alpha-napthoflavone (ANF), which is an AHR inhibitor, caused an inhibition of TCDD-induced PRL mRNA increase at the higher and lower concentrations, but these

effects were less consistent on GH mRNA levels. However, the responses of PRL and GH mRNA to co-incubation with TCDD and ANF were bi-phasic, since stimulation was seen at the low concentrations and inhibition at the high concentrations. The o,p'-DDT and TCDD effects on the expression of GH and PRL genes in the rainbow trout pituitary were mediated by mechanisms involving the ER and AHR (Elango, Shepherd and Chen, 2006).

The relationship among environmental pollutants, the AHR pathway and acromegaly: clues from *in vivo* studies.

Several evidences suggest that environmental contaminants with a carcinogenic potential, can disrupt the hypothalamic/pituitary function with neuroendocrine consequences. However, few studies have investigated in humans or animals the in vivo effects of environmental pollution exposition, or of the AHR pathway alterations on epidemiology, pathophysiology and clinical features of pituitary tumours.

In 2008, Pesatori et al. investigated the incidence of pituitary tumours in the Seveso population exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin following an industrial accident occurred in 1976. They found a higher risk, despite non statistically significant, of developing pituitary adenomas in subjects exposed to high-intermediate dioxin concentrations as compared with the non-exposed population, suggesting the need for an extended follow-up (Pesatori, Baccarelli, Consonni et al., 2008).

In 2010, we investigated the epidemiology of acromegaly in the province of Messina, one out of nine provinces in Sicily, focusing on the relationship between the geographical pattern of disease distribution and environmental context (Cannavo et al., 2010). On the basis of the degree of exposition to environmental pollutants mostly due to industrial emissions, we identified four distinct zones in the province (Figure 2, Table 1): i) area A (the Ionian area, 31 towns, 76338 inhabitants), low industrial density; ii) area B (the Tyrrhenian area, 71 towns, 287328 inhabitants),

middle-low industrial density; iii) area C (the city of Messina, 1 town, 243381 inhabitants), middle industrial density; and iv) area D (5 towns, 47554 inhabitants), high industrial density. The area D is officially identified as a high-risk zone for environmental crisis by the Department of the Environment of the Italian Government, because of the presence of an oil refinery, a steel plant, a thermoelectric power station, a lead recovery plant, and several small factories (Cannavo et al., 2010). In the air of area D, elevated atmospheric concentrations of non-methanic hydrocarbons (NMHC) and volatile organic compounds (VOC) have been detected. In this highly polluted area the prevalence of acromegaly was 210 cpm, dramatically higher than that one reported in literature or found in the other less polluted areas of the province of Messina (Table 1). The Relative Risk (RR) calculation in our province showed a significant increased risk to develop acromegaly in the population residing in the highly polluted area D, assuming the population of low polluted area A as a reference (Table 1). RR estimation demonstrated a sort of gradient of increasing prevalence of acromegaly related to an increasing degree of pollution in the different zones (D>C>B>A), as shown by the finding of an increased relative risk also in area C, at middle industrial density (Cannavo et al., 2010). Recent unpublished investigations in the polluted area suggest that the current prevalence is 330 cpm.

On the basis of the studies suggesting an involvement of the AHR pathway in pituitary tumours' pathophysiology, we evaluated the role of the pollution exposition and of the genetic variants of AHR and AIP in acromegaly patients.

Several AHR gene polymorphisms have been described, mostly in the exon 10. Some of these genetic variants have been associated with an altered induction of the activity of CYP1A1 and CYP1A2 in response to specific ligands (Harper, Wong, Lam et al., 2002). In one of our study published in 2014, we screened a population of acromegalic patients searching for AHR gene variants. In this cohort of patients, we found only two polymorphisms in the exon 10, the rs2066853 and rs4986836. The AHR rs2066853 variant, which consists in a G>A substitution causing the replacement of a arginine residue with a lisin within the transactivating domain, has been found in a

quarter of the patients and associated with increased IGF-1 levels at diagnosis, more invasive pituitary tumours and increased likelihood of developing other neoplasms such as those of thyroid, bladder and emolimphatic organs (Cannavo, Ferrau, Ragonese et al., 2014). This polymorphism has been associated with an increased risk of developing cancer in some studies but not in others (Chen, Tian, Wang et al., 2009; Luo, Zou, Ji et al., 2013; Perez-Morales, Mendez-Ramirez, Moreno-Macias et al., 2014). In our cohort of acromegalics the AHR rs4986826 variant was rare and always associated with the rs2066853 (Cannavo et al., 2014). Interestingly, a recent study suggested that the effect of the rs2066853 SNP could interfere with the AHR activity by affecting its secondary structure and stability and, potentially, with ligands' interaction (Aftabi, Colagar and Mehrnejad, 2016).

Furthermore, we recently evaluated the impact of AHR and AIP gene variants on clinical phenotype of acromegaly in patients living in highly polluted areas (Cannavo, Ragonese, Puglisi et al., 2016). Two hundred and ten patients with acromegaly, from 5 Italian regions (Sicily, Calabria Region, Apulia, Veneto Region and Marche Region), have been searched for AIP and AHR genetic variants. We compared their clinical, biochemical and radiological data after stratification on the basis of the area in which they lived for at least 20 years before diagnosis. The areas have been considered as highly polluted or not polluted on the basis of the official classification of the Department of Environment of the Italian Government that identified 57 districts of national interest (SIN) because of environmental pollution. One of the SIN is located in Veneto Region, 1 in Calabria Region, 2 in Marche Region, 4 in Apulia and 4 in Sicily. Twelve of our patients were from 2 SIN in Sicily, 7 from the Sin in Veneto Region, 2 from 2 SIN in Marche Region, 1 from a SIN in Apulia and 1 from the SIN in Calabria Region. In these areas the Regional Agencies for Environmental Protection have detected an increased concentration of non-methanic hydrocarbons, volatile organic compounds (especially benzene), and heavy metals (especially cadmium, chromo, and lead). The presence of asbestos has been reported only in the SIN in Apulia. We found that acromegaly is more severe in patients living in polluted areas if they also carry AHR and/or AIP genetic variants.

Indeed, these patients with AHR/AIP gene variants and coming from polluted areas (HR/VAR^{+ve}) showed higher IGF-1 levels and larger pituitary tumours as compared to the other cases. Moreover, these patients were more resistant to the treatment with SSA administered as first line for six months. Indeed, SSA normalized IGF-1 levels only in 14% of HR/VAR^{+ve} patients, while in the 54-56% of the other patients. Similarly, SSA halved GH values in 14% of the HR/VAR^{+ve} patients while in 54-80% of the acromegalics of the other groups (Figure 3), and significantly reduced GH and IGF-1 levels in all the patients but in the HR/VAR^{+ve} ones (Cannavo et al., 2016).

Interestingly, some authors found an increased plasma concentration of organ-alogenated compounds, like PCBs and other substances with oestrogen like activity, in acromegalic domestic cats, thus suggesting an impairment of the xenobiotic metabolizing enzymatic system (Dirtu, Niessen, Jorens et al., 2013).

Conclusions

The number of environmental contaminants and toxic agents activating the AHR system and with endocrine effects is remarkable. On the other hand, increasing evidence suggests that environmental pollution exposition is associated with human and animal tumorigenesis, but at present few data are available about the potential role of environmental factors in GH secreting pituitary tumours. In vitro and in vivo studies carried out so far showed that: i) cancer-related environmental contaminants, acting also via AHR, can alter pituitary cells biology and function, affecting proliferation and hormone secretion; ii) the AHR signalling pathway has significant functional cross-talk with molecular pathways known to be involved in pituitary tumour pathogenesis; iii) a deregulation of the AHR pathway occurs in somatotropinomas; iv) there is an increased prevalence of acromegaly in a highly polluted area of the province of Messina (Sicily, Italy); v) the AHR gene SNP rs2066853 is more frequent among acromegalics then in the general population and among patients associate to a worse clinical, biochemical profile and more invasive pituitary tumour; vi) patients with AHR or AIP gene variants and living in polluted areas have larger pituitary tumours,

higher IGF-1 levels at diagnosis and are more resistant to SSA treatment as compared to acromegalics living in polluted areas but not carrying AHR/AIP gene variants or living in non polluted areas; vii) acromegalic cats have been found with increased levels of environmental contaminants. Nevertheless, the role of specific environmental pollutants and of the AHR pathway in the development, biological features and therapeutic outcome of human GH-secreting pituitary tumours is still far from being definitively cleared. Further efforts are needed to gain knowledge in this complex and intriguing field.

FIGURE LEGENDS

Figure 1. In the cytosol, the unbound aryl hydrocarbon receptor (AHR) forms a complex with AHR-interacting protein (AIP), the co-chaperone protein p23 and two heat shock protein 90 (Hsp90) molecules. AIP is an oncosuppressor involved in the pathogenesis of pituitary tumours. AHR recognises several exogenous and endogenous ligands, whose binding results in nuclear translocation of AHR, dissociation from the chaperone proteins, heterodimerization with a nuclear translocator (ARNT) and subsequent binding to xenobiotic-responsive elements (XREs) modulated by other coregulators. This leads to transactivation of several genes encoding phase I and II xenobiotic metabolizing enzymes, such as cytochrome P450s (CYP1A1, CYP1A2 and CYP1B1), as well as other genes coding for non-enzymatic proteins. Other genomic and non-genomic 'non-canonical' effects (on the cell-cycle, contact inhibition, cell adhesion, function and metabolism of the oestrogen receptors, and DNA repairing processes) can be the direct consequence of the activation of this transcriptional factor, contributing to the disruption of cell homeostasis and tumorigenic processes. AHRR: AHR repressor.

Figure 2. Map of the province of Messina, 108 towns distributed in4 zones characterized by different environmental context: i) area A (the Ionian area), low industrial density; ii) area B (theTyrrhenian area), middle-low industrial density; iii) area C (the city of Messina), middle industrial density; and iv) area D, high industrial density.

Figure 3. Percentage of acromegaly patients who normalized IGF-1 levels or reduced GH concentrations >50%. HR/VAR^{+ve}: patients with AIP/AHR variants and living in polluted areas; Other cases: patients living in polluted areas but without AIP/AHR variants and patients living in non polluted areas, regardless of AIP/AHR gene status.

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Table 1: Demography, environmental characteristics and epidemiology of acromegaly in the Province of Messina

Area	Characteristics*	Inhabitants	Prevalence of acromegaly (c.p.m.)	RR
A (Ionian)	Low industrial density	76338	26	1
B (Tyrrhenian)	Middle-low industrial density	287328	84	3.19 (CI 0.75-13.49)
C (City of Messina)	Middle industrial density	243381	115	4.39 (CI 1.05-18.43)
D (High-risk for health zone)	High industrial density	47554	210	8.03 (CI 1.76-36.63)
Province of Messina (overall)		654601	97	-

RR: relative risk; CI: 95% confidence intervals; c.p.m.: cases per million of inhabithants
*: on the basis of a qualitative (different degree of industrialization and urbanization) and quantitative (different concentrations of atmospheric contaminants) evaluation.

Figure 1

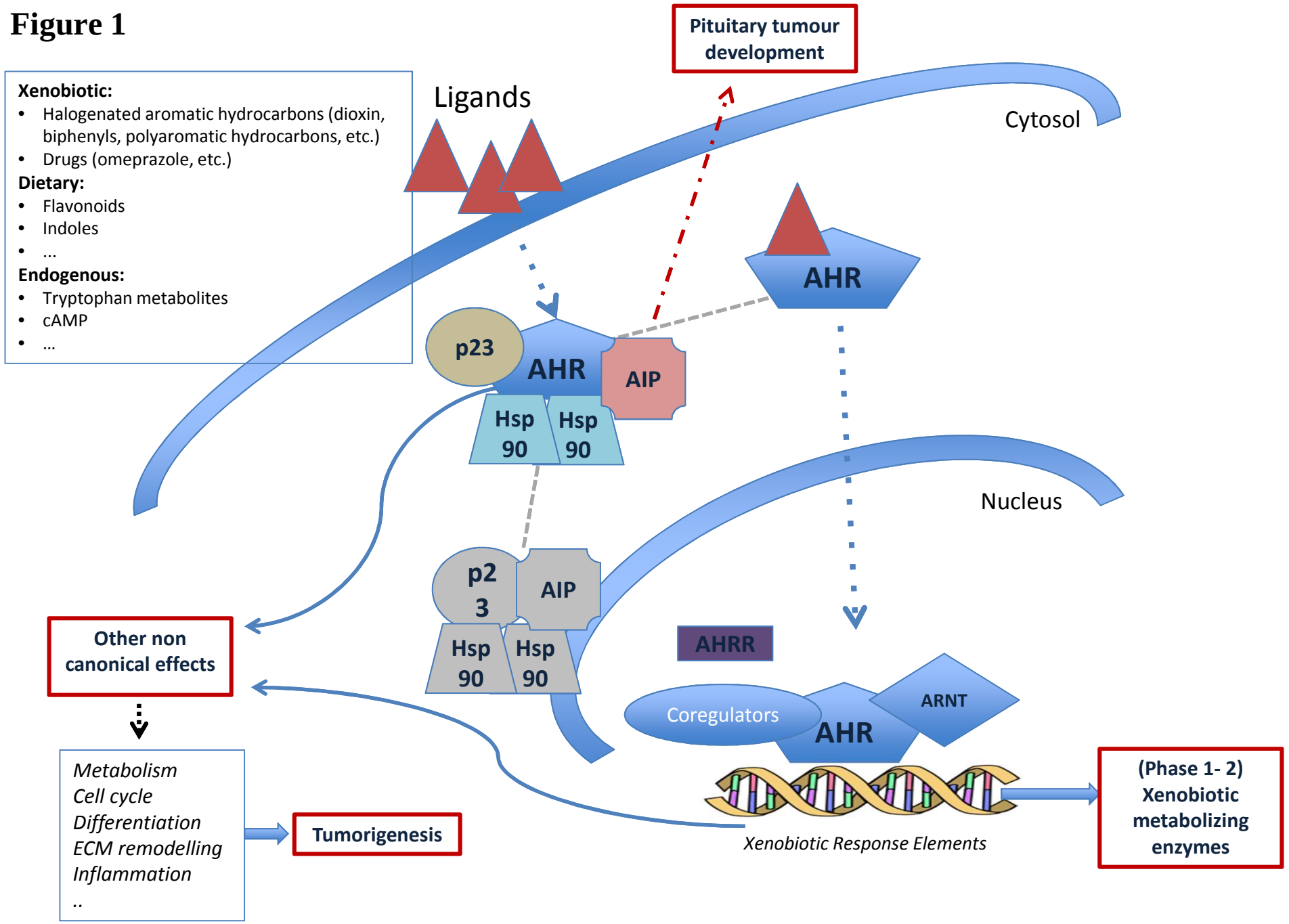

Pituitary tumour development
Ligands
Cytosol
Xenobiotic:
• Halogenated aromatic hydrocarbons (dioxin, biphenyls, polyaromatic hydrocarbons, etc.)
• Drugs (omeprazole, etc.)
Dietary:
• Flavonoids
• Indoles
• …
Endogenous:
• Tryptophan metabolites
• cAMP
• …
AHR
p23
AHR
AIP
Hsp 90
Hsp 90
Nucleus
p2 3
AIP
Hsp 90
Hsp 90
AHRR
Coregulators
ARNT
AHR
Other non canonical effects
Metabolism
Cell cycle
Differentiation
ECM remodelling
Inflammation
..
Tumorigenesis
Xenobiotic Response Elements
(Phase 1- 2) Xenobiotic metabolizing enzymes

Figure 2

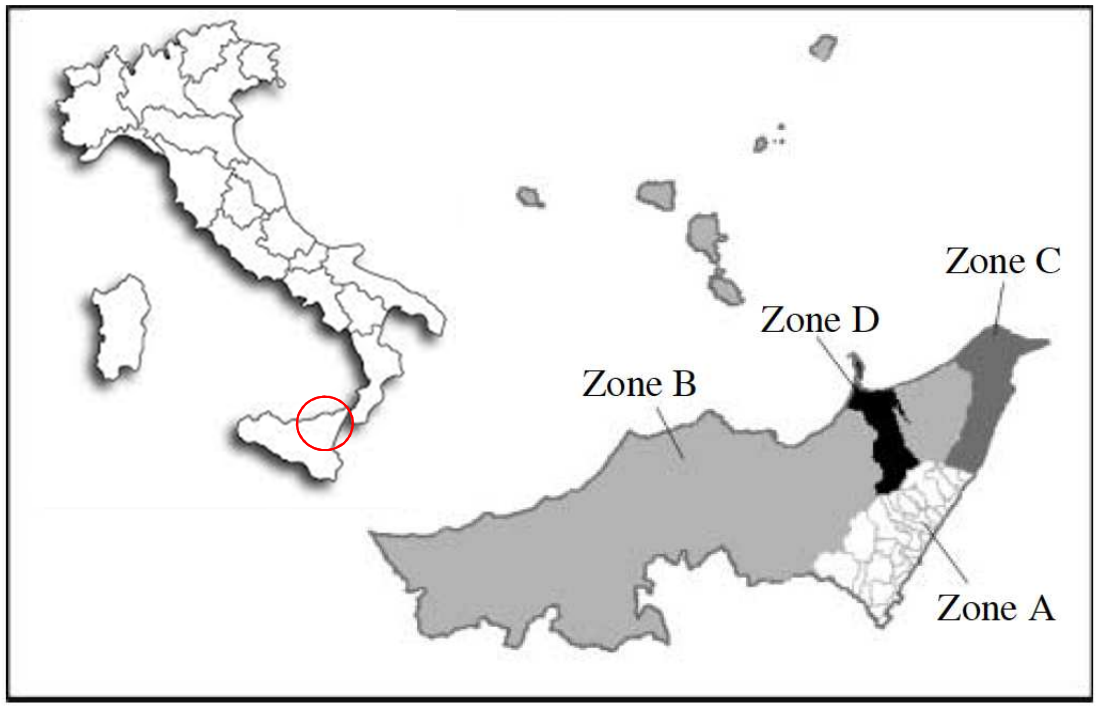
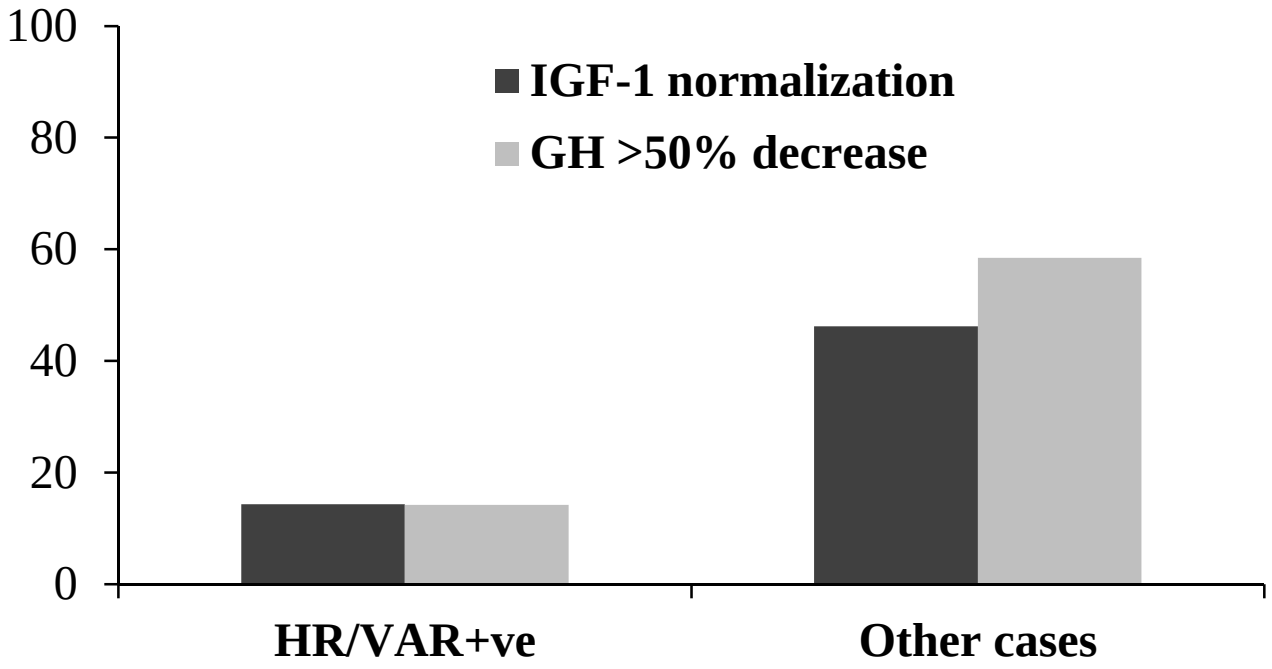


Figure 3



HR/VAR^{+ve}: patients with AIP/AHR variants and living in polluted areas;
Other cases: patients living in polluted areas but without AIP/AHR variants and patients living in non polluted areas, regardless of AIP/AHR gene status.

Highlights

- AHR is involved in cells' detoxification mechanisms and in tumorigenic processes.
- Endocrine-disrupting environmental compounds can affect pituitary function via AHR.
- The AHR key partner is AIP, which is involved in pituitary tumorigenesis.
- Pollutants exposition, along with AHR/AIP variants, impacts on acromegaly features.