IS THERE AN INFLUENCE OF ENVIRONMENTAL POLLUTANTS IN THE DEVELOPMENT OF PITUITARY TUMORS?
A TRANSLATIONAL PERSPECTIVE RELAVANT TO ACROMEGALY

[1, 2, 3, 7, 8] Roberto Toni, [1]Marco Alfieri, [1]Fulvio Barbaro, [1]Elia Consolini, [1]Davide Dallatana, [4]Andrea Faccini, [5, 6]Lisa Elviri

[1] RE.MO.BIO.S Lab (Regenerative Morphology and Bioartificial Structures) - DIMEC, [2] Museum and Historical Library of Biomedicine, [3] Center for Sport and Exercise Medicine (SEM); [4] Centro Interdipartimentale Misure; [5] Dept. of Food and Drug Sciences, [6] M3DATEK, [1-6] University of Parma School of Medicine; [7] Academy of Sciences, Bologna; [8] Division of Endocrinology, Diabetes, and Metabolism, Tufts Medical Center - Tufts University School of Medicine, Boston, MA, USA

Germ-line inactivating mutations in the aryl hydrocarbon receptor (AhR)-interacting protein (AIP) are likely causative to the development of familial isolated pituitary adenomas (2% of pituitary tumors), predisposing to young-onset aggressive GH-secreting macroadenomas, acromegaly and gigantism (around 20% of families and up to 40% of those with isolated familial GHoma, including autosomal dominant conditions at incomplete penetrance) as well as to 20% of apparently sporadic, pediatric pituitary adenomas. In addition, in sporadic non-AIP mutant cases low levels of tumoral AIP associates with increased tumoral aggressiveness of GH-omas. Collectively, these data suggest that AIP restrains adenomatous proliferation of human somatotrophs (1). Molecular evidence in the rat adenomatous somato-lactotroph cell line, GH3 supports this assumption showing interference of AIP with the tumorigenic activity of estrogens (Fig.1): A) wild type AIP downregulates estrogen receptor alpha (ERα)-dependent transcription at its response element (ERE) and B) stabilizes AhR with its nuclear transport protein (ARNT). Such a complex C) promotes ubiquitination and proteasomal destruction of ERα, D) competes for specific cofactors (AP1, NF-1, SP1) required for ERα-dependent transcription, and E) blocks ERα-dependent transcription by binding to the inhibitory xenobiotic response element (iXRE). In addition, wild type AIP maintains F) normal levels of phosphodiesterase 4A5 (PDE4A5, homologous of human PDE4A4) thus avoiding intracellular increase of the well known somatotroph growth factor, cAMP and unpregulates the zinc finger protein PLAG1 favouring cell cycle arrest and somatotroph apoptosis. As a result, AIP acts as a tumor suppressor. Remarkably, the C-terminal region of AIP consists of a tetratricopeptide repeat (TRP) domain that allows for binding of chaperones like Hsp70 through the common C-terminal motiv EEVD, and around 75% of AIP mutations affects the TRP site indicating that it is critical to AIP tumor suppressor activity (2). Since Hsp70 is a protein-folding chaperone whose binding to TRP induces stabilization of the target protein through a number of co-chaperones (3), it is presumed that excess availability of Hsp70 molecules might unbalance the normal folding activity of a target molecule like AIP, leading to its structural hyperstabilization and inhibition of tumor suppressor function. Indeed, endocrine disruptors (ED) like organochlorines (pesticides), heavy metals (zinc, cadmium, mercury), and polychlorinated biphenyls (PCB, released in water, soil, and air from electric material and inceneration of municipal waste, stored in human adipocytes) all have the ability to increase intracellular levels of Hsp70 (4), making them potential candidates for xenobiotic-dependent misfolding of AIP (Fig. 2). Previously, in rat models we have shown that pre-, peri-, and post-natal exposure to PCB may induce an ED-dependent, non thyroidal illness-like syndrome that favours hypogonadotropic hypogonadism acting at the level of the hypohysiotrophic tuberoinfundibular axis, and possibly interferes with the immune homeostasis and immune-dependent anti-tumoral surveillance (Fig. 3). Therefore, in a rat model of GH-secreting pituitary adenoma like GH4C1 cells we have hypothesized that increased levels of Hsp70 and related co-chaperons might be indicative of a deranged protein-folding machinery, potentially affecting a number of functional proteins including AIP. Rat GH4C1 cells offers the advantage of reportedly having an *in vitro* GH secretion largely comparable to that observed in *in vitro* human GHomas, and being pathogenetically related to the estrogenic milieu as a consequence of their original development in an adult female rat (Fig. 4). In GH4C1 cells, qualitative proteomic analysis of cell membrane proteins (including intracellular organelles) preliminarily showed presence of substantial amounts of Hsp70 and a related molecule (Hyou1), suggesting involvement of this chaperone folding machinery in the survival

and growth of rat adenomatous GH-secreting cells (Fig. 5). As a result, we have put forth a working hypothesis relevant to the role of xenobiotics to induce pituitary tumors, primarily GHomas. Increased availability of Hsp70 by prolonged exposure to xenobiotics would favour AIP misfolding acting at its TRP domain and, similar to the effect of the nonsense mutation at Arginine 304 (AIP-R304) the chaperone might continue to bind the AIP-TRP domain but not its client proteins PDE4A5 and Ahr. This would lead to inability of AIP to restrain the ERα-dependent transcriptional activity, that would be upregulated potentiating estrogen-dependent tumorigenic effecs. In addition, altered AIP would not be able to bind AhR leading to decreased levels of PLAGL1 and PDE4A5, reduced apopotosis of somatotrophs, and increased cAMP that provides a cell trophic stimulus and ligand-independent, AhR nuclear translocation. Since nuclear shuttling of Ahr stimulates transcription of mitogenic growth factors and activation of the D cyclin machinery (5), this would promote somatotroph proliferation. Such a mitogenic cascade is supported by the evidence that increased Hsp70 in pancreatic cancer is associated to reduced apoptosis (Fig. 6). In conclusion, overexpression of chaperone Hsp70 by exposure to EDs is in a position to conformationally alter AIP and repress the signaling checkpoints restraining activation of the estrogen tumorigenic machinery (Fig. 7). An hypothesis on conformational changes in the AIP-Ahr molecular interactions may offer an additional explanation to the increased prevalence of acromegaly in highly polluted areas where mutations in either AIP, Ahr or other proteins / genes of their transduction pathway as well as modifications in their epigenetic imprinting involve only a minority of cases. This possibility is also consistent with recent theoretical assumptions on the conformational effects of polymorphisms in the Ahr gene (6), and the potential positive selection of the transcriptional expression downstream to the AIP/Ahr complex during ancient vertebrate evolution to gigantism, eventually providing exquisite susceptibility of pituitary somatotrophs to grow in humans chronically exposed to polycyclic aromatic hydrocarbons and dioxins from smokes of fossil fuels (7).

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4. Ait-Aissa S, Porcher JM, Arrigo AP, Lambre´ C. Activation of the *hsp*70 promoter by environmental inorganic and organic chemicals: relationships with cytotoxicity and lipophilicity. Toxicology 145: 147–157, 2000

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Unit of Biomedical, Biotechnological, and Translational Sciences

Center for Sport and Exercise Medicine, Unit of Anthropometry and Constitutional Medicine

RE.MO.BIO.S. Lab
Regenerative Morphology and Bioartificial Structures

DIMEC, University of Parma School of Medicine, Parma, Italy

TMC -TUSM
Boston, MA, USA

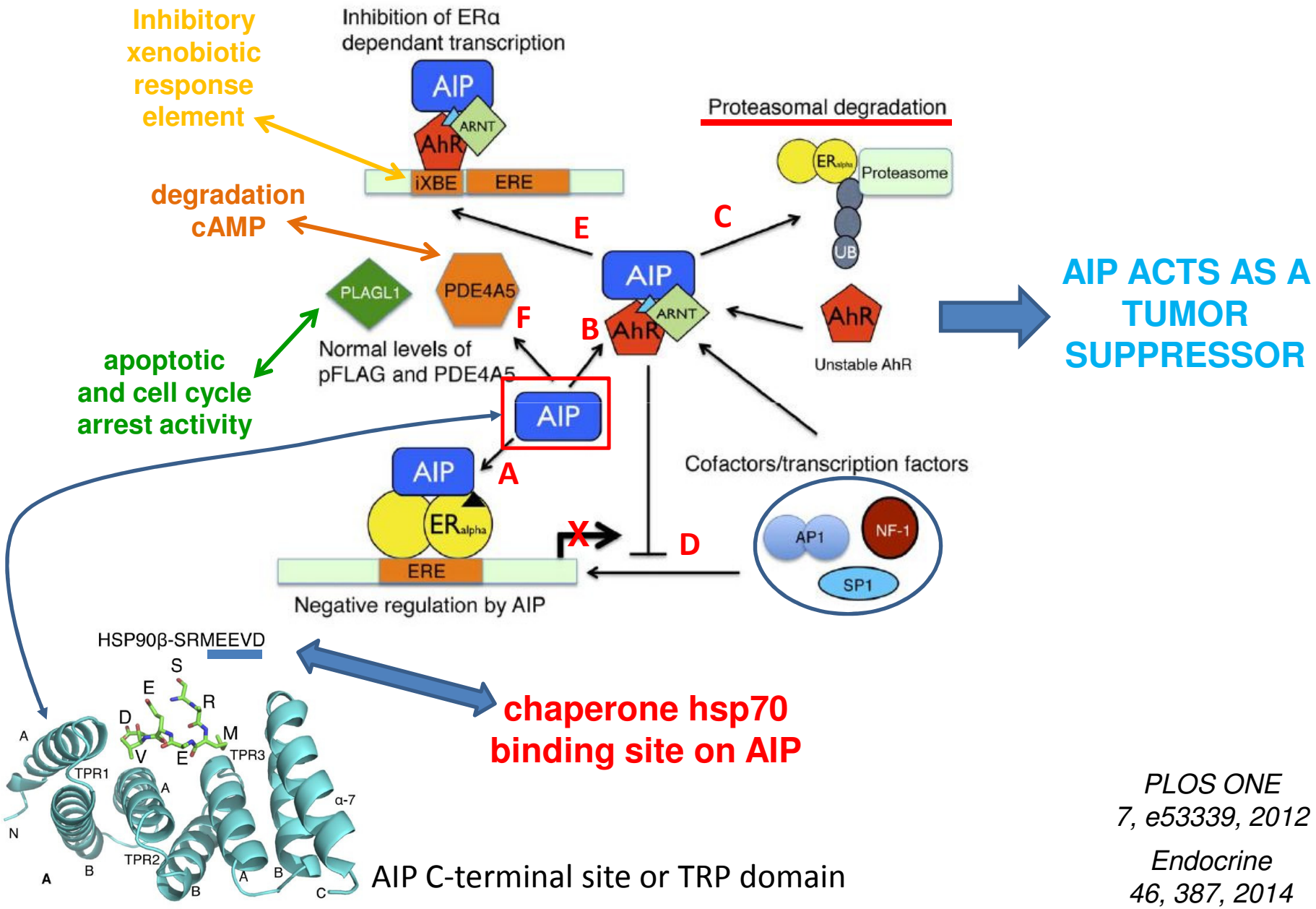
Division of Endocrinology, Diabetes, and Metabolism

IS THERE AN INFLUENCE OF ENVIRONMENTAL POLLUTANTS IN THE DEVELOPMENT OF PITUITARY TUMORS? A TRANSLATIONAL PERSPECTIVE RELEVANT TO ACROMEGALY

Roberto Toni

I° meeting
Club SIE Endocrinologia Ambientale
Roma, 19 giugno 2017

SIGNALLING CROSS-TALK BETWEEN THE AHR-AIP COMPLEX AND THE ER α INVOLVED IN SOMATOTROPH TUMORIGENESIS BY XENOBIOTICS (rat GH3 cell line)



PLOS ONE
7, e53339, 2012

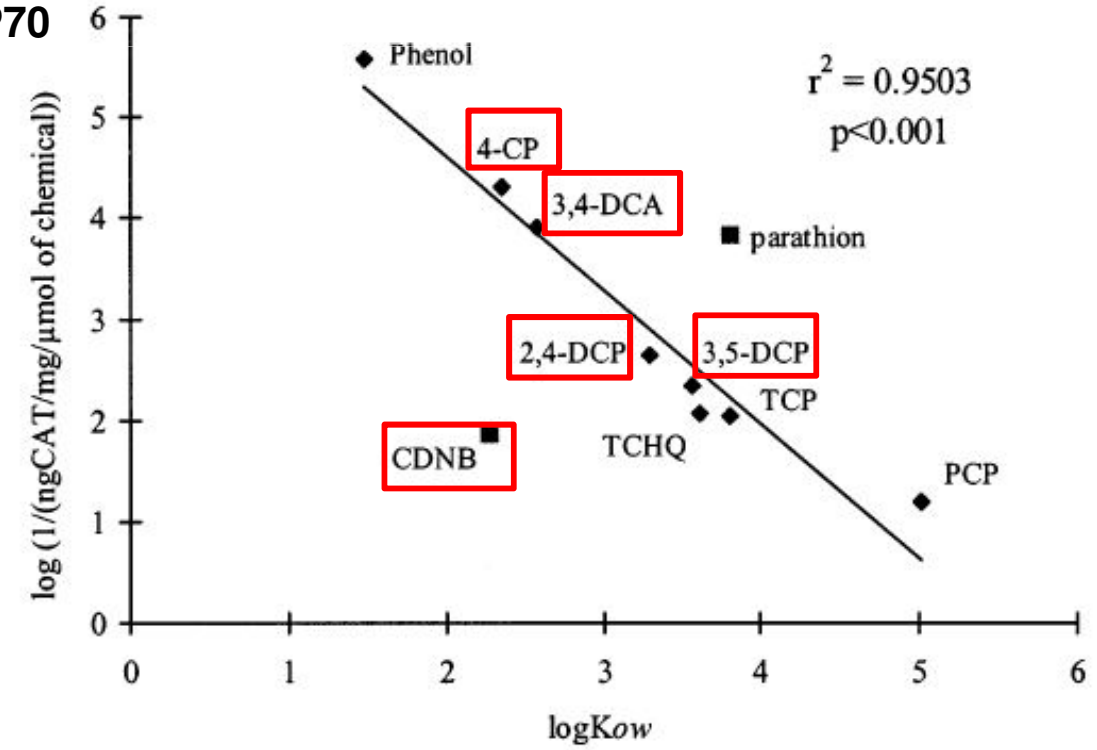
Endocrine
46, 387, 2014

Lipophylic organochlorine compounds that increase espression of HSP70 in HeLa human uterine cancer cells:

chlorophenol derivatives - 4,CP; DCP
tetrachlorohydroquinone,
3,4-dichloroaniline – 3,4-DCA
ethyl parathion,
1-chloro-2,4-dinitrobenzene - CDNB

AND HEAVY METALS: ZINC, CADMIUM, MERCURY

HSP70



What about lipophilic PCB?

Toxicology 145, 147, 2000

Effects of hypothyroidism and endocrine disruptor-dependent non-thyroidal illness syndrome on the GnRH-gonadotroph axis of the adult male rate

R.Toni[1, 3, 6], C. Della Casa[1], S. Castorina[2, 3], D. Cocchi[4], and F. Celotti[5]

J Endocrinol Investig, 28, suppl to 11, 1, 2005

Neuroendocrine regulation and tumor immunity

R. Toni,[1,2] P. Mirandola,[1] G. Gobbi,[1] M. Vitale[1]

Eu J Histochem 51, suppl to 1, 133, 2007

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FRI 126

Perinatal Exposure to Aroclor 1254 Exerts a Stimulatory Effect on Parvocellular PVN TRH mRNA Levels And Has an Inhibitory Action in Response to Salt-Loading Stress

Edith Sánchez-Jaramillo[1], Eduardo Sánchez-Islas[1], Gabriela Berenice Gómez-González[1], Fidelia Romero[2], Victor Rivelino Juarez-Gonzalez[2], Nashiely Yáñez-Recendis[1], Fulvio Barbaro[3], Roberto Toni[3, 4] and Martha León-Olea[1]

[1]Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Ciudad de México, México, [2]Instituto de Biotecnología UNAM, Cuernavaca, Morelos, México, [3]SBIBIT Dept. Univ. Parma, Italy and [4]Endocrinology Division, Tufts Medical Center- Tufts University, Boston, MA, USA

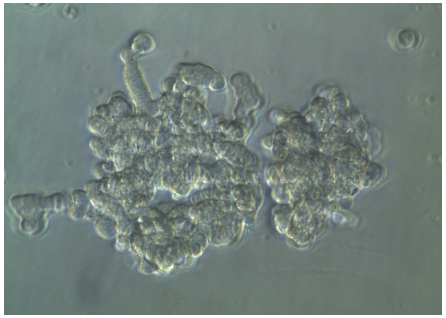
SALUD
SECRETARÍA DE SALUD

ENDO 2016

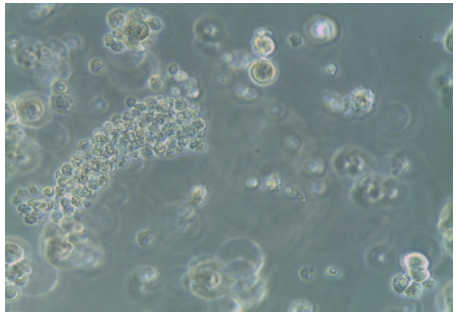
Endocrine Society Abstract, 2016

Fulvio Barbaro

Adenomatous, rat somatomammotroph GH4C1 cell line



GH4C1 2D culture (40x)



GH4C1 3D matrigel culture (20x)

Marco Alfieri

Organism: Rattus norvegicus, rat (female , 7 months) **PITUITARY ADENOMA**
Karyotype: hypertriploid rat cell line modal number = 65; range = 60 to 68

GH4C1 cells: growth hormone 60 ng or less hormone/mg cell protein

versus

HUMAN GHomas

	6 (ng/μg protein)	7 (pg/μg protein)	8 (pg/μg protein)	9 (pg/μg protein)	10 (pg/mg tissue)
GH-secreting adenomas *In vitro* (I) C	39 ± 17	34 ± 24	10 ± 3	56 ± 35	764 ± 191
	39 μg/mg	**34 ng/mg**	**10 ng /mg**	**56 ng /mg**	**10 ng/mg**

JCEM 49, 737, 1979

GH4C1 MEMBRANE PROTEINS

Proteomic HPLC - MS analysis -ORBITRAP

Lisa Elviri

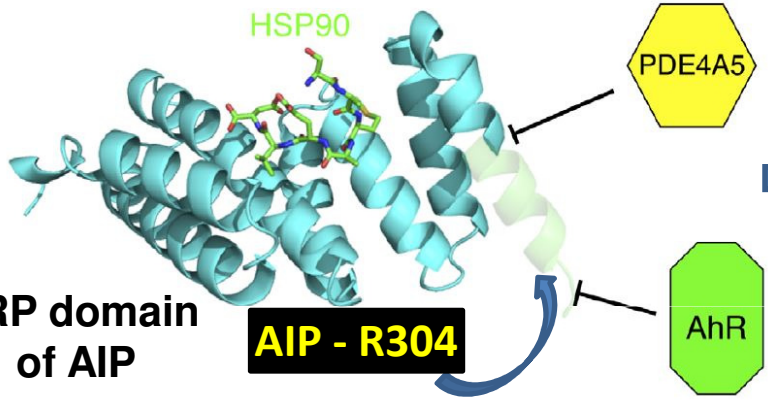
Marco Alfieri

Alfieri M, Barbaro F, Consolini E, Dallatana D, Faccini A, Elviri L and Toni R, 2017, unpublished

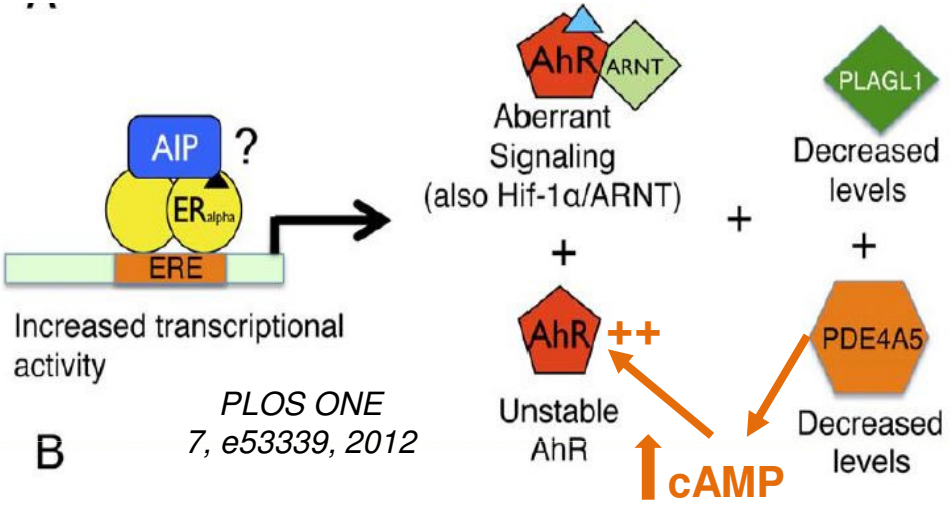
A WORKING HYPOTHESIS OF XENOBIOTIC - AIP - ERα INDUCTION AND PROMOTION OF ADENOMA IN PITUITARY SOMATOTROPHS

organochlorines

HSP70

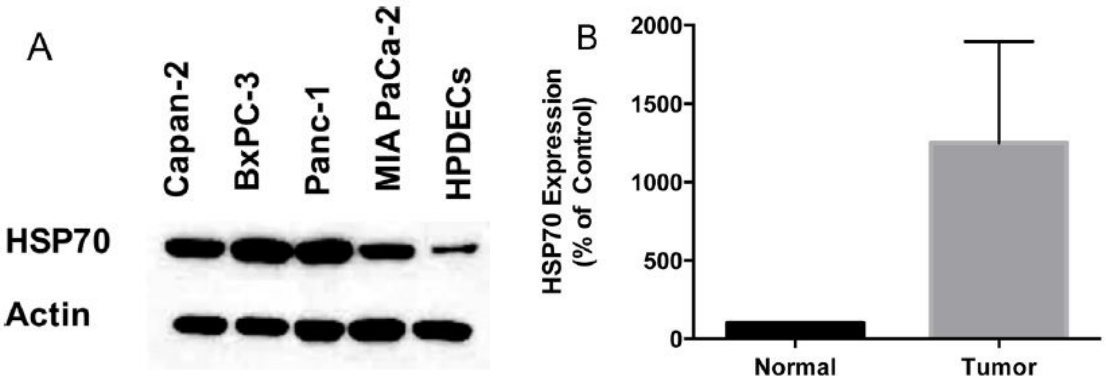


Chaperone hsp70-dependent AIP misfoldig ?



PLOS ONE 7, e53339, 2012

HSP70 overexpression in pancreatic cancer inhibits apoptosis



J Surg Oncol. 9999,1, 2017

TAKE-HOME MESSAGES

1. **Wild-type AIP act as a tumor suppressor in rodent pituitary cells of the somatomammotroph lineage (e.g. GH3) and disruption of this action results in human pituitary adenoma, primarily somatotropinomas (FIPA and sporadic types). Its activity is consistently related to restraint of the estrogen tumorigenic signalling (ERα);**

2. **The C-terminal, TRP domain of AIP is under 3D folding control by chaperones like HSP90 and HSP70;**

3. **Organochlorines (possibly including PCB) may induce overexpression of chaperone HSP70 in human cancer cells. This overexpression is detectable also in rat, adenomatous GH4C1 cells, supporting a role for HSP70 to induce AIP misfolding;**

4. **Xenobiotic-dependent misfolding of AIP is in a position to inhibit the signalling checkpoints restraining activation of the estrogen tumorigenic machinery (ERa) and favour cAMP, ligand-independent activation of unbound AHR enhancing cell cycle progression and tumor-promoting growth factors also in pituitary somatrotrophs.**

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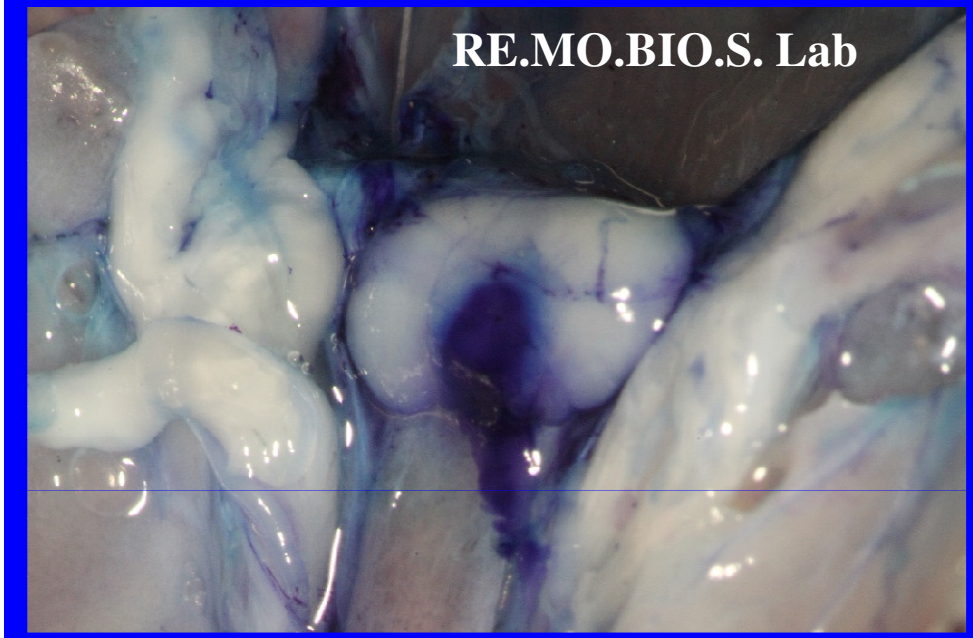
di Biomedicina



Grazie dell'attenzione

Roberto Toni

roberto.toni@unipr.it
roberto.toni@unibo.it
roberto.toni@tufts.edu