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ORIGINAL ARTICLE

Prognostic Role of Aryl Hydrocarbon Receptor Interacting Protein (AIP) Immunohistochemical Expression in Patients with Resected Gastric Carcinomas

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Abstract

Aryl hydrocarbon receptor (AHR) interacting protein (AIP) is a chaperone which binds to inactive AHR in the cell cytoplasm. AHR is best known for mediating the toxicity of halogenated aromatics, but it has also been linked to carcinogenesis and tumor progression in several tumor types. Our aims are to assess the features of AIP immunohistochemical (IHC) staining and to evaluate its possible role as a prognostic marker in gastric cancer (GC). Retrospective study of 147 cases of resected GC. Clinicopathological features were collected, tissue microarrays were constructed for AIP IHC and statistical analysis were performed. AIP staining was observed in 50.3% of tumors. All AIP-positive cases exhibited cytoplasmic or membranous staining, variably associated with nuclear co-staining. 93.2% of AIP-positive tumors showed AIP immunoreactivity in 100% of cells. Staining intensity was mild, moderate and intense in 33.8%, 13.5% and 52.7% of cases. Tumors were stratified according to AIP staining intensity into low expression (no or mild AIP immunoreactivity) and high expression (moderate or intense AIP immunoreactivity). 36.6% of our cases showed high AIP expression. High AIP expression was significantly and independently correlated to tumor progression and cancer death. Tumors with high AIP expression showed lower survival and higher progression rates. AIP expression might be useful for determining GC prognosis. More studies are needed to clarify the role of AHR pathway in GC, AIP expression and its potential use as a surrogate marker for selecting patients for AHR modulation therapy.

Keywords Gastric cancer · Prognosis · Aryl hydrocarbon receptor interacting protein · AIP · Immunohistochemistry

Introduction

Gastric cancer (GC) is the fifth most common malignant tumor in the world, and the third cause of cancer-related deaths [1]. In western countries, most patients are diagnosed at advanced stages, and the 5-year-survival rate is estimated to range between 10 and 30% [2, 3]. GC is a heterogeneous disease, both at the histological and molecular level, which occurs as a combination of environmental and genetic factors [4]. Several classification systems have been proposed, but the TNM classification is still the main prognostic tool for patient stratification and management [5]. Recent advances in molecular medicine have had a little impact on the current practice of GC. Most GCs are treated by surgery, radiation therapy and chemotherapy [6]. Trastuzumab and Ramucirumab are the only targeted therapies approved for GC treatment [7]. Since the tissue-agnostic approval of Pembrolizumab by the U.S. Food and Drug Administration (FDA) in 2017, immunotherapy can also be administered to patients with immunosensitive tumors [8, 9]. However, GC prognosis

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remains poor and there is an urgent need to identify novel biomarkers to improve GC classification, outcomes and therapy [6].

The aryl hydrocarbon receptor (AHR) is a member of the bHLH/PAS (basic helix-loop-helix / period [Per]-aryl hydrocarbon receptor nuclear translocator [ARNT]-single minded [SIM]) family of transcription factors [10]. ARH is a multidomain cytosolic protein which can be activated by ligands [11]. It was first linked to toxicity of several environmental contaminants, such as TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin) and related halogenated aromatics (HAs) [12]. It was first purified by Poland et al. in the 70s, and subsequent studies confirmed its function as a toxicity mediator [12, 13]. Microarray studies have found that AHR regulates genes involved in energy metabolism, lipid and cholesterol synthesis, xenobiotic metabolism and cell transporters [11]. Several reports have shown that AHR is also involved in physiological processes: it seems to be highly conserved among species, it is expressed during development and in mature tissues, and studies in mice lacking AHR expression have revealed phenotypic alterations [14]. AHR seems to be implicated in neurogenesis, hematopoietic stem regulation, cellular stress response or immune response [10]. Moreover, epidemiological and experimental data have supported a role for AHR in cancer [15]. Exposure to HAs have been associated with leukemia, lung, liver and oral cancers. Even without exogenous ligands, AHR has been found to be overexpressed and operative in breast, lung, gastrointestinal, central nervous system or gastrointestinal tumors [16]. AHR also mediates functions related to cell proliferation, differentiation and apoptosis [10]. Furthermore, AHR could be a therapeutic target and several compounds have been found to modulate its activity [11].

Inactive AHR is located in the cell cytoplasm, combined with molecular chaperones such as heat shock protein 90 (HSP90), p23 and AHR-interacting protein (AIP) [10]. When a ligand binds to AHR, the receptor translocates to the nucleus, where it heterodimerizes with the aryl hydrocarbon nuclear translocator (ARNT) or the aryl hydrocarbon nuclear repressor (AHRR) [14]. The resulting complex regulates transcription of genes, and AHR then returns to the cytoplasm and is degraded by the proteasome [14].

AHR has been only recently studied beyond its role as a toxic mediator. Early cancer research was focused on tumors caused by exogenous ligands, such as some types of lung or liver cancer [11]. Studies on GC are scarce, but published data have shown that AHR is overexpressed in GC as compared with normal tissues, and that it could be a therapeutic target [17, 18]. AHR has been found to be involved in GC carcinogenesis and progression in functional studies [19, 20]. No previous study has analyzed AIP protein expression in malignant tumors. Our aims are to assess the characteristics of AIP immunohistochemical (IHC) staining and to evaluate its possible role as a prognostic or predictive biomarker in GC.

Material and Methods

This is a retrospective study of 147 patients who underwent curative resection for GC at a tertiary referral hospital in Madrid (Spain), from 2001 to 2009. Clinical records were reviewed and demographic data were collected, including patient age, sex, symptoms, tumor location, progression and cause of death. Gross findings were retrieved from the database of the Surgical Pathology Department (PatWin).

Specimens were formalin-fixed and paraffin-embedded. All slides were reviewed by two independent pathologists. Main microscopical features were assessed, including tumor type (Laurén classification), histologic grade, perineural infiltration, lymphovascular invasion, growth pattern, tumor extension, margins, number of lymph nodes dissected and number of positive lymph nodes. Lymph node ratio was calculated by dividing the number of metastatic lymph nodes by the total number of lymph nodes removed. Tumors were classified according to the 8th edition of the AJCC-TNM classification of tumors.

IHC Study

Four tissue microarrays were constructed for immunohistochemical analysis and contained two cores per case, corresponding to the tumor center and the leading edge of the tumor. We used the MTA-1 tissue arrayer (Beecher Instruments, Sun Prairie, USA). Each core (diameter: 1 mm) was punched from pre-selected tumor areas in paraffin embedded tissues. IHC staining was performed in 2 μm sections. Slides were deparaffinised by incubation at 60 °C for 10 min and incubated with PT-Link (Dako, Denmark) for 20 min at 95 °C in a high pH buffered solution. Holders were incubated with peroxidase blocking reagent (Dako, Denmark) to block endogenous peroxidase. Biopsies were incubated overnight with the primary antibody at room temperature, followed by incubation with the appropriate anti-Ig horseradish peroxidase-conjugated polymer (EnVision, Dako, Denmark) to detect antigen- antibody reaction. Sections were visualized with 3,3′-diaminobenzidine as a chromogen for 5 min and counterstained with haematoxylin. Sections of the TMA block were immunostained for AIP (Rabbit Anti-AIP Polyclonal Antibody, Elabscience, reference number: E-AB-16169-120) with negative and positive controls of the technique. The anti-AIP antibody was used at 1/100 dilution.

IHC stain was evaluated by two independent pathologists, and discrepant cases were re-reviewed in order to reach a consensus. Staining pattern, intensity and percentage of cells stained were assessed. Intensity ranged from 0 (none) to 3

(intense). Staining patterns included cytoplasmic staining, cytoplasmic with membranous accentuation, membranous staining, and any of the above associated with nuclear immunoreactivity. Nuclear staining alone was not detected.

Statistical Analysis

All the information was stored in an anonymized Excel file and analyzed using IBM SPSS statistical package for Mac, version 24. Qualitative variables are described using percentages and frequencies. Quantitative variables are expressed as means and standard deviation (SD) or median and range. For the analysis of the association between variables, we have performed either $\chi 2$ (chi)-squared test (qualitative data) or Student's t test (to compare means between dichotomic quantitative variables). Statistical significance was settled at a p value <0.05. Multivariate logistic regression models were adjusted for potential confounders. Survival curves according to the Kaplan Meier method were plotted, and significance was tested by log-rank test.

A literature review on AIP expression in solid tumors was conducted, and our results were compared with those previously published.

Results

147 cases were included in our study. Clinicopathological features of our patients are summarized in Table 1. 52.7% of patients were male, and mean age at diagnosis was 70 years (SD: 12.4). Most patients were symptomatic (90.6%). 66.4% and 57.8% of patients presented local and systemic symptoms, respectively. Tumors were located in the gastric antrum (53%), body (35.6%), fundus (8.3%) and cardia (2.3%). Tumor size ranged from 4 to 120 mm (mean: 49, SD: 25). Macroscopically, GCs were ulcerative (32.6%), fungoid (32.6%), polypoid (20,6%) or flat (14.2%). Neoadjuvant therapy was not administered to any of our patients. Microscopically, tumors were intestinal (56.2%), diffuse (33.6%) or mixed (10.3%), according to Laurén classification. Perineural and lymphovascular invasion were detected in 53.1% and 41.4% of tumors, respectively. Growth pattern was infiltrative in 61.1% of cases. Most GCs were diagnosed at T3 stage (62.2%). T2, T4 and T1b lesions were detected in 12.8%, 11.9% and 2.1% of patients, respectively. Lymph node metastases were identified in 65.7% of cases (N1: 19%, N2: 25.5%, N3: 21.1%). Mean lymph node ratio was 0.24 (SD: 0.29). 20.8% of patients received adjuvant therapy (postoperative chemoradiation). Immunotherapy was not used in any case. During follow-up, 43.1% of patients experienced disease progression, with distant metastasis in 67.7% of them. Mean disease-free survival was 47 months (SD: 54). 25.9% of patients died of disease, with a mean overall survival of 51 months (SD: 53).

AIP IHC Expression

AIP immunoreactivity was detected in 50.3% of cases. Immunohistochemical features of AIP-positive cases are presented in Table 2. Among AIP-positive tumors, staining intensity was mostly moderate (52.7%). 33.8% and 13.5% of AIP-positive cases showed mild and intense AIP expression (Fig. 1). Mean percentage of AIP-stained cells in AIP-positive cases was 97.7%. All AIP-positive cases expressed AIP in ≥50% of cells: 93.2% of AIP positive cases expressed AIP in all cells. Only 6.8% of positive cases ($n = 5$) showed AIP expression in less than 100% of cells, but in all of these cases AIP expression was detected in ≥50% of cells (50%, 50%, 70%, 80% and 80%).

In most tumors, AIP immunoreactivity was cytoplasmic with varying degrees of membranous accentuation (56.7%) or cytoplasmic only (28.4%) (Fig. 2a and b). 2.8% of cases showed mainly membranous staining (Fig. 2d). 12.2% of AIP-positive cases showed both cytoplasmic and nuclear staining (membranous accentuation in 12.5% of them). All of these cases except one showed nuclear coexpression in scattered cells (5–8%), and nuclear staining intensity was equal or less intense than cytoplasmic staining (Fig. 2c). Only one of our cases showed moderate nuclear coexpression in most cells (Fig. 3). Isolated nuclear expression was not detected in any case.

In summary, all AIP-positive cases expressed AIP in ≥50% of cells (93.2% of AIP-positive cases expressed AIP in all cells), all positive cases showed cytoplasmic positivity, and nuclear coexpression was infrequent and scarce. No significant relationship was observed between AIP expression pattern or percentage of AIP-positive cells and clinicopathological features, including tumor recurrence and death.

Due to these findings, we stratified our cases according to AIP intensity. Samples were divided into two groups: low expression (no AIP immunoreactivity or mild AIP immunoreactivity) and high expression (moderate or intense AIP expression). 36.6% of our cases showed high AIP expression.

In univariate analysis (Table 3), high expression of AIP was significantly associated with tumor progression and death ($p = 0.026$ and $p = 0.023$, respectively). 55.3% of GC with high AIP expression showed recurrences, as compared with 35.3% of GC with low expression. Tumor death occurred in 40.5% of cases with high AIP expression and 20% of cases with low AIP expression.

To test the relationship between AIP expression and tumor progression and death, Kaplan-Meier curves were plotted and significance was tested by log-rank test. AIP expression was

Table 1 Clinicopathological features of our patients

Features		Patients, *n* (valid %) *N* = 147
Demographic data		
Age, years [mean (SD)]		70 (12.4)
Male gender		77 (52.7%)
Clinical and imaging data		
Symptoms	Symptomatic	106 (90.6%)
	Local symptoms	77 (66.4%)
	Systemic symptoms	67 (57.8%)
Location	Cardias	3 (2.3%)
	Fundus	11 (8.3%)
	Body	47 (35.6%)
	Antrum	70 (53%)
Pathology		
Size, mm [mean (SD)]		49.4 (25.1)
Macroscopic type	Fungoid	46 (32.6%)
	Flat	20 (14.2%)
	Ulcerative	46 (32.6%)
	Polypoid	29 (20.6%)
Laurén	Intestinal	82 (56.2%)
	Diffuse	49 (33.6%)
	Mixed	15 (10.3%)
Lymphovascular invasion		60 (41.4%)
Perineural infiltration		77 (53.1%)
Treatment		
Gastrectomy	Subtotal	101 (68.7%)
	Total	46 (31.3%)
Lymphadenectomy	D1	6 (4.1%)
	D2	23 (15.6%)
	Not specified	118 (80.3%)
Adjuvant therapy		25 (20.8%)
Survival		
Recurrence		62 (43.1%)
Disease-free survival, months [mean (SD)]		47.07 (54.4)
Tumor death		30 (25.9%)
Overall survival, months [mean (SD)]		51.4 (53.7)
Tumor staging		
T stage*	T1	3 (2.1%)
	T2	34 (12.8%)
	T3	89 (62.2%)
	T4	17 (11.9%)
N stage*	N0	47 (34.3%)
	N1	26 (19%)
	N2	35 (25.5%)
	N3	29 (21.1%)
pTNM stage*	I	20 (14.8%)
	II	54 (40%)
	III	61 (45.2%)
LN ratio [mean (SD)]		0.24 (0.29)

* According to the AJCC Cancer Staging Manual, 8th edition

Table 2 Immunohistochemical features of AIP-positive cases

Features		Patients, *n* (valid %) *N* = 74
Expression pattern	Cytoplasmic	21 (28.4%)
	Cytoplasmic and membranous	42 (56.7%)
	Membranous	2 (2.8%)
	Cytoplasmic and nuclear	8 (10.8%)
	Cytoplasmic, membranous and nuclear	1 (1.4%)
Nuclear positivity	0%	0 (0%)
	5–8%	8 (10.8%)
	>50%	1 (1.4%)
Staining intensity	Mild	25 (33.8%)
	Moderate	39 (52.7%)
	Intense	10 (13.5%)
Percentage of positive cells	50%	2 (2.7%)
	70%	1 (1.35%)
	80%	2 (2.7%)
	100%	69 (93.2%)
Mean percentage of positive cells [mean (SD)]		97.7% (9.3)

significantly associated with disease-free survival (Fig. 4, $p = 0.004$) and overall survival (Fig. 5, $p = 0.011$). Patients with high AIP expression showed significantly lower survival and higher progression rates compared to those with low expression.

Multivariate analysis including patient sex, tumor size, Laurén subtype, vascular and perineural invasion, growth pattern, AIP IHC expression and TNM classification (T stage and lymph node ratio) were performed (Table 4). AIP expression was independently associated with tumor progression [$p = 0.002$, Exp (B) = 4.38, 95% CI: 1.74–11.03], along with perineural invasion, Laurén subtype and T stage. AIP expression was also independently associated with tumor death [$p = 0.014$, Exp (B) = 4, 95% CI: 1.318–12.28], along with Laurén subtype and lymph node status (lymph node ratio).

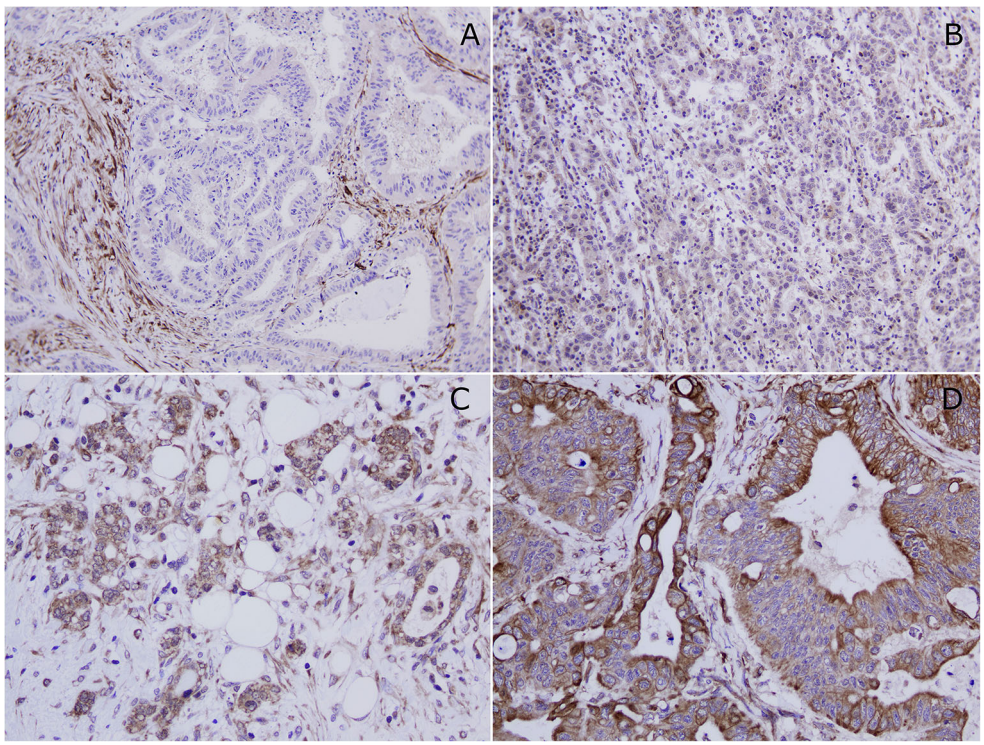

Fig. 1 AIP expression by IHC: staining score. **a** Negative staining. AIP, ×200. **b** Mild staining. AIP, ×200. **c** Moderate staining. AIP, ×400. **d** Intense staining. AIP, ×400

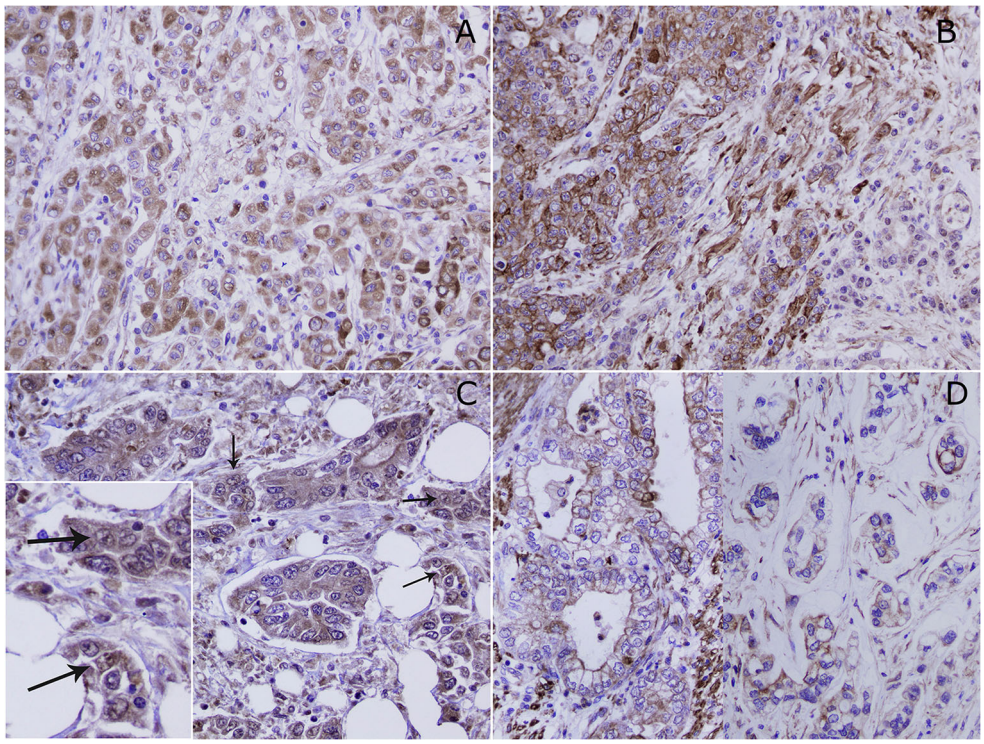

Fig. 2 AIP expression by IHC: expression pattern. **a** Cytoplasmic staining. AIP, ×400. **b** Cytoplasmic and membranous staining. AIP, ×400. **c** Cytoplasmic and nuclear staining. Inset: positive nuclei were scarce (arrows). **d** Membranous staining. AIP, ×400

Discussion

Epidemiological and experimental data have revealed an association between AHR and cancer [15]. AHR is involved in cell proliferation, apoptosis and differentiation, processes intimately related to tumor initiation and progression [21]. Moreover, AHR plays a role in immune response, which can also modulate cancer aggresivity [11].

Exposure to toxic substances has been connected to several tumor types [14, 22]. Toxic HAs and alkylating agents such as 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) and benzo(a)pyrene (BaP) were the first exogenous ligands of AHR detected [12]. Other industrial compounds, endogenous biochemical and chemoprotective phytochemicals also bind to AHR to mediate toxic or carcinogenetic effects [12]. Recent research has shown that AHR is overexpressed and active in several tumors even without environmental ligands [23].

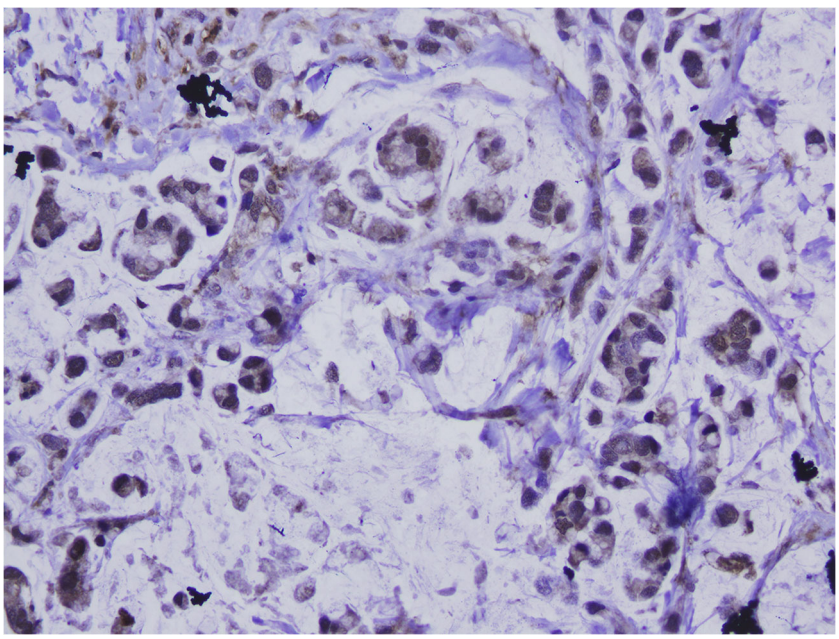

Fig. 3 Area of mucinous carcinoma showing extensive nuclear and cytoplasmic positivity. AIP, ×400

Table 3 Univariate analysis, variables associated with tumor death and/or progression

Feature	Tumor death			Recurrence		
	p	OR (95% CI)		*p*	OR (95% CI)	
Size	0.634			0.037		
Laurén	0.004	Intestinal	1	0.014	Intestinal	1
		Diffuse	1.63 (0.68–3.93)		Diffuse	2.83 (1.35–5.93)
		Mixed	2.94 (0.62–13.95)		Mixed	0.93 (0.29–2.99)
LV inv[a]	0.037	2.47 (1.04–5.84)		0.146		
PN inf[b]	0.228			0.019	2.45 (1.23–4.88)	
T stage	0.034	T1–2	1	0.009	T1–2	1
		T3–4	3.74 (1.035–13.5)		T3–4	3 (1.29–7)
N stage	0.002	N0	1	0.067	N0	1
		N1	1.93 (0.57–6.54)		N1	2.87 (1.05–7.85)
		N2	0.32 (0.06–1.67)		N2	1.85 (0.73–4.68)
		N3	5.06 (1.51–17)		N3	3.28 (1.22–8.81)
LNR[c]	0.023			0.056		
AIP IHC[d]	0.023	2.73 (1.13–6.57)		0.026	2.27 (1.09–4.7)	

[a] LV inv.: lymphovascular invasion

[b] PN inf: perineural infiltration

[c] LNR: lymph-node ratio

[d] IHC: immunohistochemistry

Thus, ARH can also be activated by endogenous ligands and/or be constitutively active. AHR activity has been linked to genitourinary, neurological, lung, head and neck, skin, gastrointestinal, liver, breast and hematologic tumors [16].

Regarding GC, in 2002 Andersson et al. showed that transgenic mice with a constitutively active AHR mutant developed gastric glandular tumors [19]. Lesions were well differentiated and cystic. Despite this low-grade histology, tumors penetrated all stomach layers and even adhered to surrounding organs. The authors concluded that AHR is involved in cell proliferation and gastric tumorigenesis. Ma et al. studied 60 and 57 cases of GC and normal tissue, respectively, and found that AHR was overexpressed in GC, probably through up-regulation of CYP1A1 expression [24]. Peng et al. also observed nuclear translocation of AHR and AHR overexpression in GC tissues and GC cell lines, with no differences in ARH expression between Laurén subtypes. Moreover, the toxic substance TCDD functioned as a tumor inhibitor by arresting cell growth at the G1-S phase [17]. Later, this group found that AHR was also involved in GC progression: after

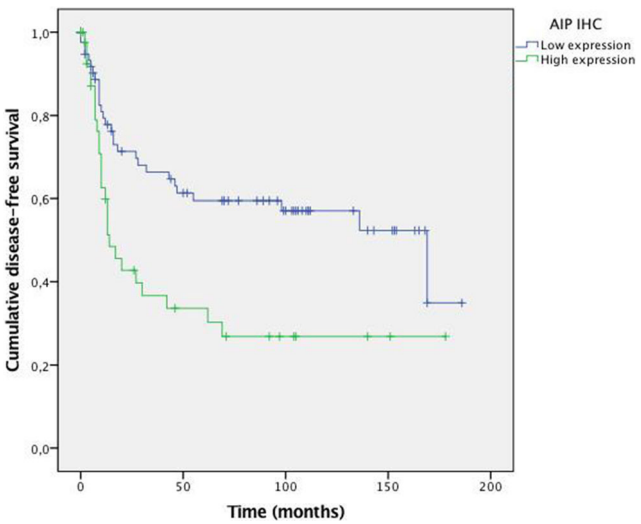

Fig. 4 Progression-free survival curves according to AIP expression by IHC

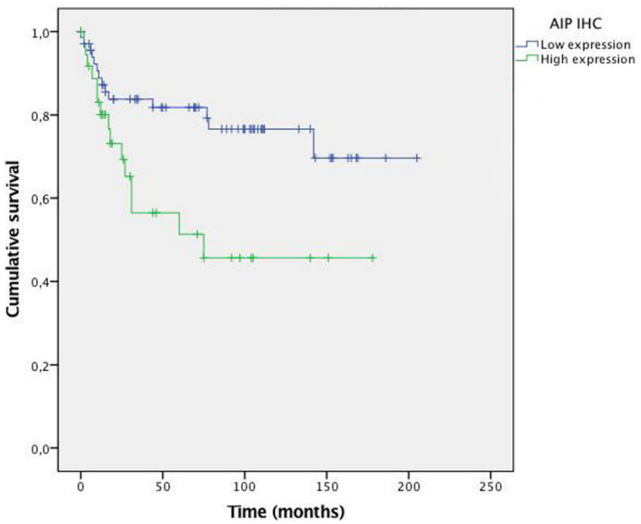

Fig. 5 Overall survival curves according to AIP expression by IHC

Table 4 Multivariate analysis

Dependent variable	Factor		*p*	Exp (B)	95% CI for Exp (B)	
					Lower	Upper
Tumor death	AIP IHC		0.014	4.022	1.318	12.28
	Lymph node ratio		0.03	6.794	1.198	38.52
	Laurén	Intestinal	0.031			
		Diffuse	0.008	4.718	1.487	14.97
		Mixed	0.303	2.457	0.444	13.603
Recurrence	AIP IHC		0.002	4.38	1.74	11.03
	Perineural infiltration		0.053	2.522	0.989	6.432
	Laurén	Intestinal	0.058			
		Diffuse	0.017	3.324	1.248	8.856
		Mixed	0.699	1.306	0.329	5.177
	T stage	T3-T4	0.075	2.939	0.898	9.621

TCDD treatment and AHR pathway activation, AGS cells increased migration distance and invasion abilities, with a dose-dependent manner. This was associated with an increase in matrix metalloproteinase (MMP)-9 expression [20]. Yin et al. observed that downregulation or AHR expression by RNA interference in GC cells decreased cellular growth, delayed cell cycle progression and increased apoptosis. It also decreased cell invasive ability and expression of MMP-2 and MMP-9 [25]. Wei et al. exposed human GC cells to BAP and it induced cell proliferation, migration and invasion, probably through upregulation of MMP-9 and c-myc [26].

Li et al. analyzed the expression levels of the AHR repressor (AHRR) by PCR, western blotting and IHC in GC and non-tumoral tissues, and found that GC showed lower levels of AHRR expression. Reduced expression of AHRR was significantly and independently related to overall survival [6].

Besides that, Zhu et al. investigated AHR and AHRR expression in gastritis and GC tissues, and showed that AHR and AHRR expression was decreased in gastritis and GC tissues with *H. pylori* infection. The authors suggested a role for AHR in *H. pylori*-related gastric pathogenesis and mucosal resistance against inflammation [18].

Finally, Yin et al. and Lai et al. found AHR modulators that could have therapeutic impact in GC: 3,3′-Diindolylmethane (DIM) and biseugenol [27, 28]. DIM is a relatively non-toxic indole derivative, product of indole-3-carbinol, which suppress cell proliferation in breast, colon or pancreatic tumors [27]. Biseugenol (4-allyl-2-methoxyphenol) is a phenolic constituent of *Syzigium aromaticum* with anticancer, antioxidant and anti-inflammatory activity. Lai et al. observed that AHR inhibition by biseugenol in GC mouse models decreased tumor growth and peritoneal dissemination. In addition, tumors exposed to biseugenol gained epithelial features and increased endoplasmic reticulum stress [28].

Some of the previously discussed studies have assessed AHR expression by IHC. AHR overexpression and nuclear translocation by IHC have been associated with gastric carcinogenesis and tumor progression [17]. As for AHRR, Zhu et al. analyzed its expression by IHC, and decreased IHC expression was correlated with poor prognosis [18]. Interestingly, tumors showed cytoplasmic and no nuclear staining.

As mentioned above, AIP is one of the chaperone proteins which bind to inactive AHR in the cell cytoplasm [29]. Previous reports have examined AIP expression in pituitary adenomas, because mutations or the AIP gene lead to predisposition to pituitary adenomas, particularly in the context of familial isolated pituitary adenoma [30]. However, we have not found any study assessing AIP expression in malignant tumors. According to The Human Protein Atlas (http://www.proteinatlas.org), AIP expression is mainly cytoplasmic, and can be associated with membranous and nuclear co-staining [31]. In our study, most tumors showed cytoplasmic staining with or without membranous accentuation (86.7% of our cases). A minority of tumors showed nuclear coexpression (12.2%) or mainly membranous staining (2.8%). AIP stained all tumor cells in most cases, and positive cases showed mostly moderate expression. We divided our cases into two groups: low AIP expression (negative or mild staining) and high AIP expression (moderate or intense staining). AIP IHC evaluation and stratification of cases into two groups was relatively straightforward, because mild staining was faintly visible and cases with moderate or intense staining were by comparison clearly positive.

In this study, we have performed univariate and multivariate analysis and we have found that AIP overexpression by IHC (moderate or intense staining) is significantly and independently associated with tumor progression and cancer death. As illustrated by Kaplan-Meier curves shown in

Figs. 4 and 5, patients with high AIP expression showed lower survival and higher progression rates than patients with low AIP expression. As stated previously, we have not found any other study assessing the prognostic role of AIP expression in cancer tissues. However, data analyses from the Human Protein Atlas database showed that AIP overexpression might be a prognostic factor in colorectal cancer. Tumors with high AIP expression showed lower survival rates than tumors with low AIP IHC expression, as seen in our results (http://www.proteinatlas.org) [31]. Thus, accumulation of AIP could be an indirect indicator of AHR activity or its deregulation, which has shown to be related to gastric carcinogenesis and increased cell migration [20].

Previous studies have revealed that the effect of ligands or AHR overexpression is tissue-specific [25]. Future research on AHR modulators should consider the complexity of modulating AHR activity: both AHR agonists and antagonists could be useful as cancer therapy under different circumstances [32]. AIP expression, as a possible surrogate marker of AHR activity, could be helpful for selecting patients for AHR modulation therapy. On the other hand, AHR activity could influence response to targeted or conventional treatment. In 2017, Ye et al. found that AHR activation caused resistance to EGFR tyrosine kinase inhibitors in non-small cell lung cancer [33]. The role of AHR pathway in targeted therapy for GC remains to be elucidated.

Conclusions

AHR is best known for mediating the toxicity and carcinogenic effects of DCPP, but it is also involved in physiological processes, activated constitutively or by endogenous ligands. Recent studies have linked AHR to carcinogenesis and tumor progression in several types of tumors, including GC. AIP is a chaperon protein which binds to inactive AHR in the cell cytoplasm. No previous study has assessed AIP protein expression by IHC in cancer. In this study, we have evaluated AIP expression by IHC in surgical resection specimens from GC patients. All AIP-positive GC cases exhibited cytoplasmic or membranous staining (variably associated with nuclear co-staining), and in most of these cases AIP stained 100% of tumor cells. High AIP expression by IHC was significantly and independently related to tumor progression and cancer death. Tumors with high AIP expression showed lower survival and higher progression rates. More studies are needed to clarify the role of AHR pathway in GC, AIP expression and its potential use as a surrogate marker for selecting patients for AHR modulation therapy.

Author Contribution Díaz del Arco C. data acquisition, analysis, interpretation, manuscript draft, approval and agreement.

Estrada Muñoz L. data acquisition, analysis, interpretation, manuscript revision, approval and agreement.

Barderas Manchado R. data acquisition, manuscript revision, approval and agreement.

Peláez García A. data acquisition, manuscript revision approval and agreement.

Ortega Medina L. study design, data interpretation, manuscript revision, approval and agreement.

Molina Roldán E. data acquisition, manuscript revision, approval and agreement.

Solís Fernández G. data acquisition, manuscript revision, approval and agreement.

García Gómez de las Heras S. data interpretation, manuscript revision, approval and agreement.

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Compliance with Ethical Standards

Conflict of Interest No conflict of interest regarding the publication of this paper.

Research Involving Human Participants and/or Animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Retrospective study; for this type of study formal consent is not required.

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