

## REVIEW

## A systematic review of the possible carcinogenic role of the aristolochic acid

TIVADAR BARA JR.<sup>1)</sup>, SIMONA GURZU<sup>2,3)</sup>, HARUHIKO SUGIMURA<sup>4)</sup>, TIVADAR BARA<sup>1)</sup>,  
 MARIUS ALEXANDRU BELEAUA<sup>2)</sup>, IOAN JUNG<sup>2)</sup>

<sup>1)</sup>Department of Surgery, University of Medicine and Pharmacy of Tîrgu Mureş, Romania

<sup>2)</sup>Department of Pathology, University of Medicine and Pharmacy of Tîrgu Mureş, Romania

<sup>3)</sup>Department of Pathology, Clinical County Hospital, Tîrgu Mureş, Romania

<sup>4)</sup>Department of Tumor Pathology, Hamamatsu University, Hamamatsu, Japan

### Abstract

Aristolochic acid (AA) is a bioactive component of Chinese herbs, dietary supplements, slimming pills and contaminated flour, which is known to induce chronic tubulointerstitial disease. AA is also shown to be involved in the genesis of the upper urinary tract urothelial carcinoma (UTUC) and some other cancers, but its tumorigenic role is far to be understood. We performed a systematic literature review regarding the involvement of AA in malignant processes and molecular pathways of carcinogenesis. Twenty representative papers were selected for this review. These papers reveal that AA exposure increases the risk for UTUC, renal cell carcinoma, hepatocellular carcinoma, gastric and small intestine cancer. The role of AA in lymphomagenesis is also proposed. The A:T to T:A transversions occurring in the 5'-CpApG-3' trinucleotide context of the *TP53* gene is considered to be the signature mutation of AA. Genes including *H-ras*, *FGFR3*, *N-ras* and *BRCA2* are also involved. For further understanding of AA's role in tumorigenesis, the exploration of the AA's molecular signature is necessary.

**Keywords:** aristolochic acid, carcinoma, lymphoma, testis, gastric, intestine.

### Introduction

*Aristolochia* species (such as *Aristolochia* and *Asarum*) are used in the Chinese traditional medicine as bioactive components of the herbal medicines (such as *fangchi* and *mutong*) and ethnobotanicals. The isolation of aristolochic acid (AA) from various *Aristolochiaceae* and its possible role in cell division were first reported by Ganshirt & Deufel in 1953 [1–4].

First examinations performed in 1961 showed that the nitrophenanthrene derivative AA could exert an anti-inflammatory and anti-neoplastic role and herbs were extensively used as analgesics [5]. Although products containing AA were withdrawn from the market in the early 1980s due to their role in carcinogenesis [6], in Asian countries the plant drugs derived from *Aristolochia* are still used for the treatments of snakebites, arthritis, gout, and coronary artery diseases [7]. In Europe, the report about the presence of AA in the dietary supplements and slimming pills containing *Aristolochia fangchi* and its related nephrotoxicity was firstly presented in 1991, referring to females from Belgium [7, 8]. Up to 5% of them presented chronic tubulointerstitial disease and many developed upper urinary tract urothelial carcinoma (UTUC) [8].

Nowadays, AA I and II is well-known to be geno- and nephrotoxic inducing the Balkan endemic nephropathy in people living in the alongside rural communities of the Danube's River tributaries in the Balkan Region [8–10]. The Balkan endemic nephropathy is a chronic tubulointerstitial disease with progressive renal failure that is especially common in people from Romania, Serbia,

Bulgaria, Bosnia and Herzegovina and Croatia [9, 10]. In these regions, it is supposed that the AA is contained in the homemade flour based bread whose grains were contaminated with *Aristolochia clematitis* [2, 8, 10]. One-third of Taiwan's population is also exposed to AA-containing herbs [2, 8].

The role of AA in carcinogenesis, except for the UTUC, is still unknown. In this paper, we intended to present a data review from literature regarding the possible role of AA in carcinogenesis of the urinary tract or other organs. The underlying molecular mechanisms are also presented.

### Methodology

Systematic search of literature on the *PubMed* database using keywords such as “aristolochic acid”, “aristolochic acid and carcinoma” and “aristolochic acid and cancer” has been conducted for this review. The possible role of AA in carcinogenesis was the main focus. Both experimental studies and publications referring to human tissues were taken into account, while review-type articles and papers referring to the non-tumor lesions were excluded. Based on these criteria, from about 1021 publications identified on the *PubMed* database using these terms, we have further selected a total of 20 representative studies that were considered to be eligible (Figure 1).

### Aristolochic acid and urothelial carcinoma

The UTUCs especially involves the renal pelvis and the upper ureter, representing 5–10% of all urothelial cell carcinomas and 10% of renal tumors [1, 11]. The main

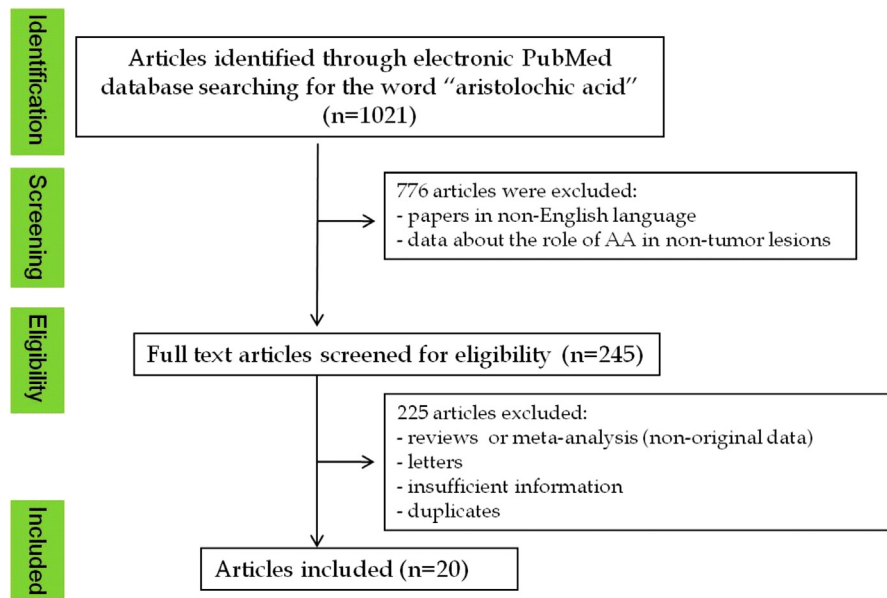
risk factors are represented by smoking and AA exposure [1, 8, 11–14]. Some studies revealed that 50% of patients with previous AA exposure have a higher risk of developing an UTUC or a bladder carcinoma [7].

The world highest incidence of UTUC is certified to be in Taiwan [8]. The AA-associated UTUC tumors are more frequent in females. They are highly aggressive, usually diagnosed as high grade and high stage carcinomas and the relapse can also occur in the contralateral upper urinary tract [15, 16].

The UTUC developed on the background of the Balkan

endemic nephropathy is considered to be a multifactorial disease. The environmental nephrotoxic agents (lead, metal and metalloids, *Aristolochia clematitis*, ochratoxin A and Pliocene lignite, and viruses), genetic predisposition and epigenetic mechanisms (DNA methylation, histone acetylation and miRNA interference) are all probably involved in tumorigenesis [2, 9].

After kidney transplantation, the AA exposure is a risk factor of developing a UTUC, which occurs in 14% of the recipients compared with 1–2% of the non-exposed patients [13].



**Figure 1** – Preferred reported items for systematic reviews and meta-analyses (PRISMA) flow diagram adapted for the data involving the role of aristolochic acid (AA) in tumorigenesis published on the PubMed database between 1953 and 2016 (September 1).

### ☞ Aristolochic acid and other cancers

The most recent studies revealed a link between AA and genesis of renal cell carcinoma, the clear cell variant [1]. The mutagenic dA-AL-I adducts were proved to be present in the renal DNA of 76% of the Taiwanese patients with renal cell carcinomas who have priorly ingested a cumulative quantity of more than 250 mg of AA [1].

There are few studies that proved a link between AA exposure and non-urothelial cancer. Based on the fact that the hsa-miR-30a-5p and miR 4795 5p is downregulated not only in UTUC (Table 1) but also in patients with colorectal cancer and in lung cancer the hsa-miR-30a-5p is down-regulated [2, 9], it might be supposed a possible role of this acid in carcinogenesis. Similarly to AA-associated UTUC, miR-200c is lost in pancreatic cancer [2].

**Table 1** – The molecular pathways involved in the genesis of aristolochic acid (AA)-associated tumors

Type of disorder	Gene mutations		Modified miRNA	
	Name of the gene	Type of mutation	Downregulated	Upregulated
Upper urinary tract urothelial carcinoma	<i>TP53</i>	A:T to T:A transversion in the 5'-CpApG-3' trinucleotide	Common: hsa-miR-30a-5p, hsa-miR-127-3p, hsa-miR-154-5p	Common: hsa-miR-1290, hsa-miR-205-5p, hsa-miR-3620-3p, hsa-miR-3656, hsa-miR-373-5p, hsa-miR-4322, hsa-miR-99b-3p
	<i>H-ras, N-ras</i>	NS	Rare: hsa-miR-4795, hsa-miR-4784, hsa-miR-330-3p, hsa-miR-3916, hsa-miR-181c, hsa-miR-15a-5p, hsa-miR-10a-5p, hsa miR-200c-3p	Rare: hsa-miR-488-3p, hsa-miR-4274, hsa-miR-4434, rno-miR-21-5p, rno-miR-32a-5p, hsa-miR-224-3p
	Other genes: <i>FGFR3, BRCA2, mTOR, MAPK, Akt, STAG2</i>	NS		

Type of disorder	Gene mutations		Modified miRNA	
	Name of the gene	Type of mutation	Downregulated	Upregulated
Renal cell carcinoma	TP53	A:T to T:A transversion		
	H-ras – codon 61	CAA → CTA		
	N-ras – codon 61			
	K-ras – codon 12/13	GGT → GAT		
	C-myc	NS		
	TGF- $\beta$ 1	NS		
Hepatocellular carcinoma	C-myc	NS		
	H-ras	NS		
Colorectal and gastric cancer	TP53	A:T to T:A transversion	hsa-miR-30a-5p, miR-479-5p	
	H-ras	NS		
Ovarian or testicular toxicity	Bcl-2	NS		
	Akt	NS		

A high risk for carcinomas of the forestomach, urinary tract and fibrohistiocytic sarcomas at the AA injection site was proved in animal models [10]. Within 56 weeks after the start of a three-week AA I exposure, multiple mice's tumors were reported [7]. In rats, the dose-dependent gastrotoxic effect of ingested AA I consists of necrosis, ulcer, hyperkeratosis and hyperplasia of epithelial cells in the forestomach [11, 12]. This effect occurs prior to renal injuries [11].

After 15 weeks of oral exposure of rats to AA (10 mg/kg/day, five times a week), the papillomatosis or the squamous cell carcinoma of the forestomach occurred in 38% of rats, 18% of them developed ear duct squamous cell carcinomas and 58% of animals developed an adenocarcinoma or a sarcoma of the small intestine [17]. Adenocarcinoma of the kidney and hyperplasia of the pancreas were also described in these animals that presented the AT→TA transversion mutation at codon 12/13 and 61 of H-ras and K-ras genes and at codon 61 of the N-ras gene [17].

Regarding lymphoid neoplasms, lymphomas were rarely described in rats after 15 weeks of AA exposure [17] and only one case of human splenic large B-cell lymphoma was reported in the literature occurring at 17 years after AA exposure [18].

In the East Asian countries, the AA dose-dependent risk for hepatocellular carcinoma was supposed but not confirmed [7, 9, 12]. After AA I oral administration for at least one week, changes in the TP53 knock-in mice and canines livers were proved to be associated with c-Myc oncoprotein and oncofetal RNA-binding protein Lin28B overexpressions [7]. The miRNAs that codify the signal transduction of interleukin (IL)-6 and NF- $\kappa$ B also seems to be involved in hepatocarcinogenesis [7].

The AA exposure was recently proved to cause toxicity during ovarian or testicular maturation through an apoptosis-induced cell death mechanism [19]. The AA-related impending apoptosis is realized through the inhibition of the anti-apoptotic markers bcl-2 and ERK1/2, the suppression of Akt activation and the stimulation of the pro-apoptotic agents such bcl-2-associated X protein, poly (ADP-ribose) polymerase, caspase-3 and caspase-9 [19]. The cytotoxicity is exerted upon both germ cells and somatic cells [19] but the relation between AA and the development of an ovarian or testicular cancer was not yet proved.

### ▣ Molecular pathways of AA-associated tumors

The exact molecular mechanisms of the AA-associated carcinomas are still unknown. The AA is classified as a Group 1 carcinogen by the *World Health Organization – International Agency for Research on Cancer* (WHO – IARC) [10]. The whole-exome sequencing revealed that more than 524 genes are mutated in each of the AA-associated UTUC [8]. The metabolomics studies showed that the AA binds covalently to genomic DNA and induce the formation of the aristolactam (AL)-DNA adducts [1, 2].

The molecular pathways seem to be mainly related on AA-associated TP53 gene mutations, especially for the UTUC and the renal cell carcinoma (RCC) [2, 10, 15, 16]. The A:T to T:A transversions occurring in the 5'-CpApG-3' trinucleotide context of the TP53 gene is the signature mutation of AA that occur in more than 70% of AA-related tumors and less than 10% of smoking-induced UTUC [2, 8, 10, 15, 16]. In patients with UTUCs, the dA- and dG-AL-DNA adducts are also present in the renal cortex which is used as a biomarker for AA exposure [2, 10, 15, 16].

Other genes proved to be mutated in human UTUCs are FGFR3 (8% of the case), H-ras (4%), N-ras (15%), STAG2 (27%), BRCA2 (19%) and the driver genes involved in the chromatin modification pathway (MLL2 – 62%, CREBBP – 38% and KDM6A/UTX – 15%) [8].

Short-term exposure of animals to AA I upregulates c-Myc oncoprotein expression in the kidney, this gene being probably involved in the AA-mediated molecular pathways [7]. Induction of the H-ras or K-ras mutations are also supposed to mediate the renal carcinogenic effect of AA (Table 1) [12]. The hedgehog signaling is triggered by the AA-mediated TGF- $\beta$ 1 activation that induces type II or fibrosis-related epithelial-to-mesenchymal transition (EMT) of tubular epithelial cells and kidney fibrosis [20]. There are no known data about the role of AA in the type III or tumor-related EMT.

A dose-dependent induction of heterozygous H-ras mutations (exons 2, 3, 5, 7, 8) was observed in rodents' forestomach, liver and kidney as a potential mechanism of AA-associated cancer [12, 13].

In the most recently published papers, the authors tried to outline the miRNA profiling of the AA-associated human

UTUC. They pointed out that the hsa-miR-205-5p is expressed in almost all UTUCs, independently from the presence or absence of AA exposure. The specific AA signature seems to include 10 commonly downregulated or upregulated miRNAs (Table 1) [2, 9]. These miRNAs target the genes such as FGFR3, mTOR or MAPK and modulate the Akt signaling pathways, cell–cell focal adhesion, angiogenesis (*VEGF-A* gene), apoptosis, invasion, metastasis (*RAS* gene), etc. [2]. The miR21 target the *TP53* gene [2].

### ☒ Summary and future perspectives

Only scattered data were published in literature about the role of AA in genesis of non-renal cancers. Talking about a dose-dependent lesion and occurrence of tumors in animal models after short-term exposure to AA, it is suggested that the future researches should be focused on developing anti-AA antibodies. Their possible role in predicting tumor progression and invasion capacity of the AA-containing tumor cells of the gastrointestinal tract, liver and pancreas should be also explored.

### Conflict of interests

None of the authors has any competing interests in the manuscript.

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### Corresponding author

Simona Gurzu, Professor, MD, PhD, Head of Department of Pathology, University of Medicine and Pharmacy of Tîrgu Mureş, 38 Gheorghe Marinescu Street, 540139 Tîrgu Mureş, Romania; Phone +40745–673 550, Fax +40372–653 183, e-mail: simonagurzu@yahoo.com