

ENDEMIC (BALKAN) NEPHROPATHY IS ARISTOLOCHIC ACID NEPHROPATHY

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Abstract

Endemic nephropathy is a syndrome that comprises two entities: chronic interstitial nephropathy and urothelial cell cancers predominantly of the upper urinary tract. The etiological agent for the disease is aristolochic acid, a compound found in the plants of *Aristolochia* spp. The development of urothelial cancers is characterized by the formation of aristolactam DNA adducts leading to mutations, predominantly A: T- > T: A transversions. In order to comprehensively understand the gene regulation programs in upper urothelial cancers we performed integrated miRNA and mRNA expression profiling of paired tumours and unaffected urothelium samples. The obtained data will help us to understand the carcinogenesis caused by aristolochic acid and might be the source for the design of a diagnostic biomarker.

Key words: endemic nephropathy, aristolochic acid nephropathy, aristolochic acid, upper urothelial cancers, microRNA.

Endemic (Balkan) Nephropathy (EN), nowadays known as Aristolochic acid nephropathy, is a syndrome that comprises two entities: chronic interstitial nephropathy and urothelial cell cancers predominantly of the upper urinary tract. Based on our results, the etiological agent for the disease was recently discovered: the presence of the disease in the endemic regions is due to the plant called *Aristolochia clematitis*, a weed that grows in the farm fields and whose seeds, comingled with wheat seeds, eventually lead to chronic intoxication by poisoned flour/bread [1, 2]. The culprit lies in aristolochic acid, a nephrotoxic, mutagenic and carcinogenic compound that all *Aristolochia* species plants contain [3].

As mentioned, aristolochic acid in endemic regions was usually ingested via contaminated bread. Once ingested it metabolizes and in genetically predisposed individuals covalently binds to the DNA, predominantly to the purine bases, forming DNA-adducts. Deoxyadenosine-aristolactam is the most frequent one. Aristolactam DNA adducts persist in the renal cortex for many years (even lifelong), and therefore present a biomarker of previous exposure to aristolochic acid. There are two target tissues affected by aristolochic acid: the renal cortex, especially the proximal renal tubule and urothelial cells of the upper urinary tract. Recent studies show that aristolochic acid may play a role in liver disease and possibly other exposed organs [4].

The aforementioned DNA adducts lead to mutations, predominantly A: T → T: A transversion, which are now considered to be the fingerprint mutations of aristolochic acid. These mutations were firstly described on the p53 tumour suppressor gene [2, 5]. However, our recent results showed that this mutation is widely present throughout the genome (Karanović et al., unpublished data). The mutated adenosines are predominantly present on the nontranscribed strand of DNA and thus resistant to the global genomic nucleotide excision repair, eventually leading to the development of cancer. It is important to stress that genetic factors play a significant role in the development of the nephropathy/tumour. There is a certain genetic predisposition/susceptibility since not all exposed subjects do exhibit the disease [6, 7].

Urothelial cancers in en

Urothelial carcinomas of the upper urinary tract (pyelocaliceal cavities and ureter) are extremely rare in the general population with an incidence of 2 new cases per 100.000 inhabitants [8]. Interestingly, as many as 50% of patients with endemic nephropathy develop upper urothelial cancers [9]. The specific mortality from this type of cancer in the Croatian endemic focus is 55 times higher than in other parts of Croatia [10]. These tumours can occur simultaneously with renal disease, can precede it, but also appear independently of it [11]. Upper urothelial cancers in endemic regions equally affect both genders, with a peak incidence in the 7th decade of life. The tumours are often bilateral with frequent relapses but are usually of low grade and rarely metastasize.

MICRORNAs in EN

MiRNAs (miRs) are small, non-coding, endogenous RNAs that have been involved in the regulation of gene expression both in normal development but also in disease. They are approximately 21–25 nucleotides in length, single-stranded RNAs that are widely expressed in eukaryotes. The first microRNA was discovered in *Caenorhabditis elegans* in 1993, and since then the roles of miRNAs in various diseases have been investigated. Approximately 1,872 precursors and 2,578 mature miRs

have been identified in humans. In tumours microRNAs are usually dysregulated, either over- or underexpressed. In the last few years a number of studies have been published showing miRs' roles in cancer pathogenesis, chemo/radiotherapy resistance, tumour relapse, and metastasis. Its importance lies in the possibility of the development of new targeted therapeutics [12].

In this part of our research, our aim is to determine specific patterns of gene expression and their posttranscriptional regulation in patients with upper urothelial cancers from endemic regions using methods of functional genomics. In order to comprehensively understand the gene regulation programs we performed integrated miRNA and mRNA expression profiling of paired tumours and the adjacent unaffected urothelium of a dozen of patients from endemic areas who underwent surgery due to transitional cell cancers of the upper urinary tract. Patients were enrolled consecutively between 2009 and 2010 and were residing in two endemic regions, one in Croatia and the other in Bosnia and Herzegovina. Detailed medical history, residency information, dietary practices and habits were acquired prior to the surgery. During surgery fresh tumour, renal cortex and normal urothelial tissue were taken and used for mutational, DNA adduct and mRNA and microRNA profiling analyses. Pathohistological diagnosis was confirmed by two independent pathologists. The level of AL-DNA adducts in the renal cortex DNA was determined using ³²P-postlabelling polyacrylamide gel electrophoresis [13]. TP53 mutations in DNA isolated from tumour samples were identified with the AmpliChip p53 detection algorithm (Roche Molecular Diagnostics, Pleasanton, CA) that detects all single basepair substitutions and single-base deletions in the TP53 gene. MiRNA profiling was performed by high-capacity qPCR using Applied Biosystems RT primers and TaqMan Low Density Arrays, while mRNA profiling of the same RNAs was performed using Affymetrix arrays. At the moment data are being analysed using bioinformatical and biostatistical methods. The final step in the study will be data validations, i.e. results obtained by molecular analysis will be validated/confirmed by means of immunohistochemistry.

Scientific contribution

We are hoping the obtained data will help us better understand the carcinogenesis caused by aristolochic acid. The data might be the source for the design of diagnostic biomarkers, or even more could be used as a basis for new therapeutic strategies. Since we now know that endemic nephropathy is an environmental form of aristolochic acid nephropathy, a disease present around the world, these achievements might be applicable worldwide.

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Резиме

ЕНДЕМСКАТА (БАЛКАНСКА) НЕФРОПАТИЈА Е НЕФРОПАТИЈА ОД АРИСТОЛОХИЧНАТА КИСЕЛИНА

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Ендемската нефропатија е синдром кој се состои од два ентитета: хронична интерстицијална нефропатија и канцери на уротелијалните клетки, претежно на горниот уринарен тракт. Етиолошкиот агенс за болеста е аристорохичната киселина, соединение кое се наоѓа во растенијата на *Aristolochia* spp. Развојот на уротелијалниот канцер се карактеризира со формирањето на aristolactam ДНК-адукти доведуваат до мутации, претежно трансверзиите на А: Т -> Т : А. Со цел сеопфатно да се разберат програмите за регулација на гените на канцерот кај горниот уротелиум, изведовме интегрирана

miRNA и mRNA-експресија со профилирање на парирани тумори и незасегнатите примероци на уротелиум. Добиените податоци ќе ни помогнат да ја разбереме карциногенезата предизвикана од аристорохичната киселина и може да

биде извор за дизајнирање дијагностички биомаркер.

Клучни зборови: ендемска нефропатија, нефропатија од аристорохичната киселина, аристорохична киселина, канцери на горен уротелијалеум, microRNA.