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Perspectives

Use of omics analysis for low-dose radiotoxicology and health risk assessment: the case of uranium

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Abstract

Exposure to environmental pollution and the increase in the incidence of multifactorial diseases in the population have become health problems for industrialized countries. In this context, the question of the health impact of exposure to these pollutants is not clearly identified in the low-dose range. This article looks at this problem using the example of preclinical studies of the effects of chronic low-dose exposure to uranium in rats. These studies demonstrate the value of molecular screening analyses (omics) and multimodal integrative approaches, of which the extreme sensitivity and breadth of observation spectrum make it possible to observe all the biological processes affected and the mechanisms of action triggered at the molecular level by exposure to low doses. They also show the value of these analytical approaches for finding diagnostic biomarkers or indicators of prognosis, which can be necessary to evaluate a risk. Finally, the results of these studies raise the question of the health risk caused by epigenomic deregulations occurring during critical developmental phases and their potential contribution to the development of chronic diseases that are metabolic in origin or to the development of certain cancer liable in the long term to affect the exposed adult and possibly its progeny.

Key words: uranium; low doses; omics; metabolomics; transcriptomics; epigenetics

Introduction

Uranium is naturally present throughout the Earth's crust at varying concentrations. It is a heavy metal that is chemically toxic and radiologically toxic since all its isotopes (234, 235 and 238) are radioactive with a very long half-life (several hundred million years to several billion years). Uranium is an emitter of high-energy alpha particles which, depending on its level of enrichment with the isotope 235, makes it a particularly radiologically toxic element [1–3]. The natural form (ore) and the form depleted (after enrichment) of ^{235}U are mainly chemically toxic. Uranium can spread in the environment and accumulate in the soil and living organisms by forming complexes with organic matter or by binding to certain organic molecules such as proteins. In plants, uranium content is closely linked to the nature of the plant and to the levels of phosphates, sulphates, carbonates and organic matter in the soil. In humans, average daily absorption associated with the ingestion of food products and drinking water is estimated at between 1 and 4 $\mu g \cdot d^{-1}$ [4]. The human body naturally contains ~90 μg of uranium [5]. Its civil and military use makes it a source of anthropic pollution [6] and potentially of chronic exposure for populations living near mining sites [7], areas where ore refining, spent fuel processing and waste storage activities are carried out, but also military sites and battlefields contaminated by depleted uranium (DU) munitions [8]. Other industrial activities like those requiring the use of phosphate fertilizers (with which uranium forms strong complexes) and the use of coal-fired power plants (where uranium concentrates in the ash) are also likely to cause pollution and environmental dispersion.

The health risk associated with small quantities of uranium liable to be incorporated into the body is a subject that raises questions for society because, whereas uranium toxicity is relatively well documented in situations of acute exposure at high doses, it is much less so in situations of chronic exposure at low doses. Uranium is a radioactive element that emits high-energy alpha radiation and can create breaks in deoxyribonucleic acid molecules (DNA) [9] and form reactive oxygen species that are highly oxidative and a source of genetic instability and oxidative stress for cells [10]. Uranium is also a heavy metal, and its chemical toxicity is also a source of metabolic effects and oxidative stress. It accumulates mainly in the kidneys and bones and can cause kidney disease and cancer.

By contrast, at low doses, the effects of the exposure levels measured in the environment are much less well documented, and the results of epidemiological studies conducted on the subject are too contradictory [11–14] to be able to estimate an exposure threshold that can be linked to a health risk. Moreover, at this range of doses, the exposome [15] is a confounding factor that

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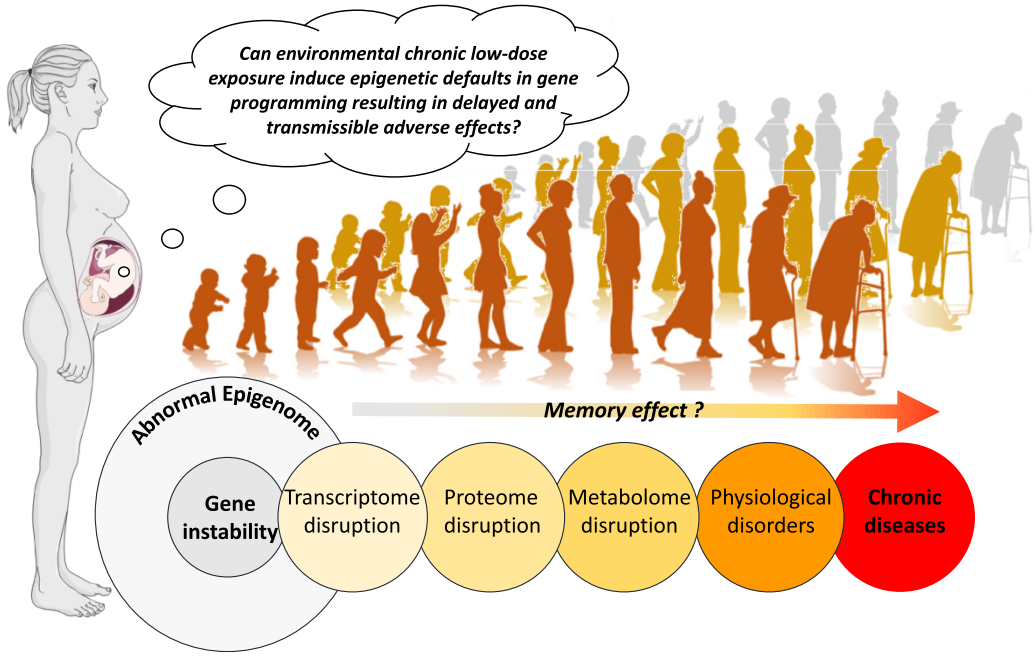

Figure 1: scientific concept of the epigenetic origin of multifactorial chronic diseases and multi/transgenerational effects

can modulate the nature and amplitude of the biological effects of uranium and cause variations in the value of the biological thresholds observed [16]. Among the endogenous and exogenous factors that can modulate uranium toxicity, the age of the individuals is known to influence the rate of intestinal absorption of uranium (20–100 times higher in newborns than in adults) [17] and the epigenetic sensitivity of the individuals during the embryonic and foetal periods of development, the delayed effects of which can affect the individual in adulthood or even its progeny [18] (Fig. 1). Sex can also modulate the renal excretion rate of uranium and thus its toxicokinetics (preclinically, this rate is higher in males than in females) [19] as can species, so genetic dimorphisms also have an influence on uranium toxicity [20].

So at these low exposure levels, interpretation of the biological effects of uranium is muddied by the effects of numerous physical and biological parameters that interfere in the organism's allostatic response [21, 22] and consequently reduce the statistical power of the results of any epidemiological studies that may be carried out [23–25] to (i) find a possible causal link between environmental exposure to uranium and an increase in the frequency of occurrence of certain diseases [26] and (ii) draw any conclusions about health risk [13, 21, 27, 28].

Experimentally, most of the preclinical studies conducted *in vivo* by exposure to non-nephrotoxic doses of uranium have not been able to observe significant physiopathological effects on rodent models either [29]. However, these studies have revealed the existence of many deregulations at the molecular level that affect the metabolism in different physiological systems [29–31]. These deregulations, which could be a direct consequence of exposure to uranium, could be used as biomarkers of exposure but also as predictors of delayed effects or even as bioindicators of the risk of pathological effects. Now, estimating the health risk of exposure requires the ability to identify physical or biological indicators that signify a specific metabolic or physiological deregulation of these. These indicators can be used as diagnostic tools for a condition linked to exposure, as predictive sensors of more delayed adverse effects [32] but also as tools necessary for the identification of factors of vulnerability or predisposition to certain diseases such as multifactorial diseases [33].

In the low-dose range, the observation of biological effects proves complex. The biological effects produced are generally small in amplitude [34] and heavily affected by environmental factors of confusion. In addition, the temporal dynamics of the biochemical reactions that take place at different molecular levels mean that it is not always possible to identify a significant effect at the time of observation [28, 35]. So to be able to identify indicators that predict effects and factors of vulnerability, predictive toxicology must be able to take account of and adapt to this analytical difficulty [36, 37]. In view of this problem, it is essential to implement innovative, sensitive and specific analytical strategies for observing the dynamics of biological systems as a whole in order to identify the beginnings of a biological effect that could lead to a physiological dysfunction or even to a disease at a later stage. In this field, the use of analytical techniques like the 'omics', which can simultaneously measure a large number of biological variables with very high precision [38], proves to be a major advantage. These techniques, which provide large datasets, make it possible to refine the identification and understanding of the allostatic mechanisms that can produce major effects or cause delayed physiopathological states [39, 40]. At the same time, although they are certainly not specific to an environmental pollutant, the biomarkers identified in the metabolome and transcriptome could be used as complementary exposure diagnostic tools. They will make it possible to increase the sensitivity and statistical power that epidemiological studies generally lack [41] and thus improve risk assessment and radiation protection standards.

Based on the results of a couple of studies, we would like to present in this article the interest of omics to identify the effects of chronic low-dose uranium exposure.

Methods, Results and Discussion

Metabolomics

An individual's metabolome is defined by all the metabolites measurable in a biological compartment at a given instant and considered as the molecular fingerprint of their own phenotype [42]. Also, a metabolomic analysis does not technically allow a complete analysis of all the chemical forms represented by all the metabolites, and the techniques currently available only allow the simultaneous observation of certain chemical families (hydrophilic, lipophilic and volatile). The level of sensitivity and the extent of the analysis spectrum will also be strongly dependent on the detection tools used (Nuclear magnetic resonance or mass spectrometry). The following studies focused on the analysis by liquid chromatography–mass spectrometry. Indeed, to supplement knowledge about the effects of chronic contamination at non-toxic doses of uranium, the metabolomic analysis was carried out on a preclinical model of rats. The results of this study showed that contamination by ingestion of 2 μg of uranium per day for a period of 9 months significantly altered the animals' urinary metabolome [43]. To refine our understanding of the mechanisms of action of uranium, a monitoring study of the dose–response relationship was also carried out during the uranium contamination to (i) find the limits of rats' metabolic sensitivity to uranium, (ii) find predictive or early markers of any adverse effects and (iii) identify the metabolic pathways liable to be significantly impacted by chronic uranium exposure at a non-toxic dose [44]. The uranium concentration contained in the aqueous solution used was between double the concentration measured in a natural water source in Finland (40 $mg \cdot l^{-1}$) [27] and half the maximum concentration recommended by the World Health Organization for drinking water (0.015 $mg \cdot l^{-1}$) [45]. The rats were contaminated from birth to enhance the effect of the uranium [46]. In this study, the results confirmed those of a previous study [43] and also showed that at a very low concentration (0.15 $mg \cdot l^{-1}$), uranium had an effect on the urinary metabolome of the contaminated rats. This study also showed that the effects observed depend on both the dose and the length of exposure to the uranium in a non-linear relationship. This type of non-linear relationship has already been observed in other fields of low-dose exposure [47, 48]. In terms of impact on the metabolism, these studies also showed that the nicotinate–nicotinamide pathway (metabolism of vitamin B3) was among the pathways most affected by uranium, confirming in passing the results of old studies carried out in the 1970s and the 1980s on renal insufficiency caused by exposure to toxic doses of uranium [49, 50]. Another more recent study also observed similar results in the saliva of Kuwaiti children who were potentially exposed to uranium during childhood [51]. In another preclinical study, a comparative analysis of urinary and renal metabolomic profiles shows the existence of significant differences in the molecular profiles measured in these biological compartments but also between those of animals of different sexes. However, despite these dimorphisms, some of which are directly linked to the animal's sex, the results of these analyses still confirm a strong mechanistic link between uranium and nicotinate–nicotinamide metabolism but also with that of the biosynthesis of unsaturated fatty acids. These two pathways could be metabolic targets of the effects of uranium in renal tissue and also indicators of a biological effect on renal function [52]. In the future, these studies could be enriched by performing focused analyses on lipid metabolism (lipidomics), which is closely associated with inflammatory processes.

Transcriptomics

In order to study the transcriptional (messenger RNA (mRNA)) and post-transcriptional (microRNA) activities of genes in the presence of uranium, transcriptomic analyses were carried out from renal biopsies of rats [36]. For this purpose, different analytical methods are available depending on the objective. For the simplest analyses, aimed at the relative measurement of expression levels of genes of interest known and well described to be involved in certain biological processes, the performance of tests carried out by qPCR may be more than sufficient. On the other hand, for exploratory analyses with less precise research objectives or, on the contrary, for mechanistic studies that seek to precisely describe reaction cascades, analysis methods that allow the measurement of many identities simultaneously are very useful. In this field, a distinction is made between microarray and RNA sequencing methods. In the following study, microarrays were used to analyse the levels of mRNA produced and therefore to compare the activation status of genes between uranium-treated and uranium-untreated animals. MicroRNAs were measured using TaqMan low-density arrays which are based on the single complex qPCR assay technique.

The results of these analyses revealed significantly different levels of gene expression in the contaminated animals compared to the control animals, and these deregulations mainly concerned genes associated with gene regulation mechanisms (24%), cell signalling processes (24%), cell structuring processes (16%), developmental processes and processes of cell proliferation (8%) and apoptosis (8%). A post-transcriptional regulation activity was also revealed through the identification of 70 differentially expressed microRNAs in the kidneys of the contaminated animals [52]. All these results prove that uranium has a genetic effect on the renal cells, even at dose levels considered to be non-toxic. Finally, to understand the mechanistic complexity of the molecular regulation systems at the cell level, a multimodal integration was carried out. This multiomic analysis carried out using data from omics analyses obtained at different molecular levels (transcriptomic and metabolomic) makes it possible to qualify more finely the cell's mechanisms of action in response to the presence of uranium. In this study, this methodological approach showed that uranium acted on the kidney at different molecular levels and that different metabolisms, such as the metabolisms of fatty acids, unsaturated fatty acid biosynthesis and nicotinate–nicotinamide but also of tryptophan, glycosphingolipids, arachidonic acid, aspartate and glutamate, and also vitamin D, were significantly affected. The metabolisms of nicotinate–nicotinamide and of unsaturated fatty acid biosynthesis still seemed to be the ones most affected by uranium.

Epigenomics

From a mechanistic point of view, the different molecular signatures observed at metabolome and transcriptome levels could be mediated by epigenetic regulations that are known to be involved in the mechanisms of adaptation to environmental exposure [53, 54] but also in the evolution of species and even, in some cases, in causing adverse health effects where these mechanistic effects prove inadequate [38, 55]. The involvement of effects of epigenetic origin in the delayed development of certain diseases raises more and more questions for society regarding the health impact of environmental exposure [56]. In this field, recent studies conducted in humans have identified a heightened risk for the

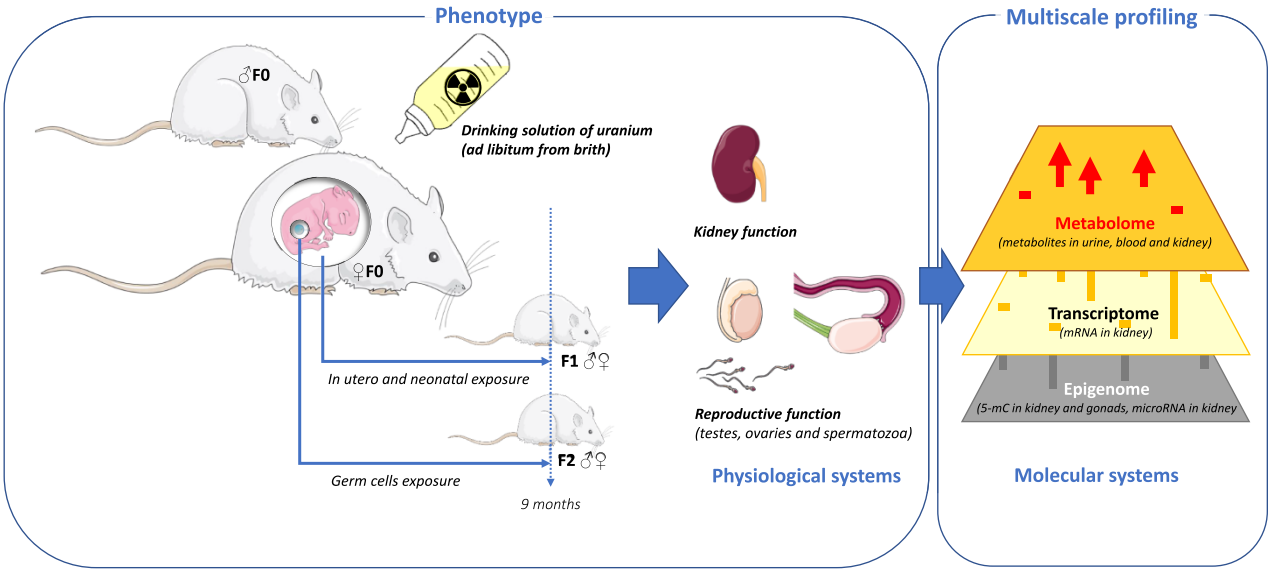

Figure 2: experimental design of the study on the multigenerational effects of chronic low-dose contamination to uranium in rats

progeny of individuals exposed to certain chemical compounds, particularly if exposure occurs during the epigenetic programming phases of the (embryonic, somatic and germ) cells of the exposed individuals or their progeny [57]. These delayed effects of epigenetic origin could be due to changes in DNA methylation profiles [58]. Also, despite the fact that too few studies have yet been carried out on the subject, the worrying results from these few studies raise questions for the scientific community about the level of risk to progeny attributable to environmental exposure.

To address this question, a protocol to study the multigenerational effects of chronic exposure to low doses of uranium was developed in rats. In this study, the overall DNA methylation levels in the rats' kidneys (males and females) were analysed over three generations of rats: (F0) contaminated for 9 months from birth, (F1) contaminated *in utero* and then until weaning and potentially affected by the paternal (F0) germ cells and, lastly, (F2) only potentially affected via the germ cells of their parents (F1) [59] (Fig. 2).

The results revealed a slight increase in the body mass of the males in the first generation (F1) after exposure to uranium (the body mass of the females did not vary compared to the control group) and then a slight drop accompanied by a reduction in the mass of the kidneys, observed only in the males in the second generation (F2). A significant increase in the overall DNA methylation level of the kidneys was also observed in the first-generation (F1) males and in the second-generation (F2) males, bred from generation F0 of parents contaminated with uranium [59]. Concerning the reproduction system, the results of analysis of the methylation levels of the male and female gonads provided proof of principle of an epigenetic effect, revealing an overall DNA hypomethylation effect in the ovaries and an overall DNA hypermethylation effect in the testicles after exposure to uranium. This effect was observable for all three generations of exposed animals [60]. Although very preliminary, these results raise questions about the biological significance of these epigenetic fingerprints and about the possibility of transmission of effects to subsequent generations via the gametes. To partially answer these questions, a recent study carried out using the same experimental model showed that the DNA of the spermatozoids of generation F2 was hypomethylated overall in the animals in the group previously exposed to uranium. As a reminder, this last generation is still considered to have been directly exposed to uranium because it originates from parental (F1) germ cells that had been exposed [61]. This result is very interesting because it raises questions about the possibility of a direct effect on the fertility of the males in this last generation (F2) but also about the possible transmission of genetic effects to the next generation (F3), which could be associated with transgenerational effects as described and observed experimentally using other sources of exposure [18, 62, 63]. Although these analyses show that chronic exposure to uranium has a significant effect on the epigenome of the kidneys and gonads by modifying the global level of DNA methylation, these results do not make it possible to identify precisely the genes involved in this effect nor to know their level of methylation. In the future, an in-depth study could be carried out using enhanced reduced representation bisulphite sequencing or whole-genome bisulphite sequencing to measure DNA methylation throughout the genome. Other studies could also be carried out at the chromatin level, analysing the interactions between proteins and DNA by chip sequencing or estimating the level of accessibility of chromatin by assay for transposase-accessible chromatin using sequencing.

Conclusion and Perspective

Omics methods focusing on other molecular levels have also been used to conduct studies on the biological and toxicological effects of uranium. In addition to monitoring DNA damages (breaks, mutations), genomics can also be used to evaluate other parameters such as gene of instability or kidney cells adaptive response to uranium [64]. These measurements can be used to identify and understand carcinogenesis [65] and a risk, such as lung cancer in uranium miners exposed to radon [66]. Genomic instability in F1 progeny from DU-exposed fathers was also observed [67]. Downstream to gene expression, the study of the proteome provides a more deterministic view of the functional state of metabolisms and stress areas. Proteomics, another high-throughput analysis method, can be rich in information by providing complementary

data located between the level of gene expression (transcriptomics) and the product of various metabolisms (metabolomics). Although few epidemiological, *in vivo* and *in vitro* studies in radiotoxicology have been interested in the differential analysis of proteomes after exposure to uranium, the main objectives of these protein screenings help to decipher fine molecular mechanisms of cell effects [68, 69] and biological retention of uranium and toxicokinetics [70] and to look for biomarkers of exposure [71] and even early indicators of kidney toxicity and carcinogenicity [72–74]. In this respect, specific bonds of uranium with certain protein cites have been identified in the plasma, and the study of interference with metalloproteins or the effectiveness of the renal and pulmonary antioxidant system has also been studied to better describe the toxicity of uranium [75]. These different preclinical studies confirm the pertinence of omics analyses in radiotoxicology.

In the low-dose field, metabolomics is a powerful diagnostic tool that offers the most deterministic vision of an individual's phenotype and makes it possible to describe the molecular mechanisms involved in an organism's allostatic response and to identify diagnostic markers or even predictive indicators of a delayed physiopathological risk. Multiomic analysis also seems very pertinent because, although it comes up against problems like temporal delays in reactions and biological threshold effects, it can be used to carry out more in-depth analyses of the molecular mechanisms and interactions associated with the effects of uranium. It can also reveal molecular targets of uranium that can be modulated by environmental factors of co-exposure and that make them factors of intrinsic vulnerability. Beyond the absence of observable physiopathological effects in a population of rats chronically exposed to low doses of uranium, the observation of epigenetic effects over several generations provides a new picture of uranium toxicity risk in terms of radiation protection and scientific knowledge about the multigenerational effects of chronic low-dose exposure [43, 44, 52, 60, 61]. Nevertheless, although these experimental data help to enrich the current state of scientific knowledge, they still do not enable any conclusions to be drawn in terms of risk because the molecular effects observed in these studies can also be influenced by other factors associated with the exposome, such as the age, sex and genetics of the populations, which are also risk factors for vulnerability that can modulate the amplitude of the effects of uranium through additive or synergic effects [19, 46, 76]. However, these results raise questions because they provide elements of scientific proof and attest to the need for more in-depth research on the risk for the progeny of individuals exposed to environmental pollution.

With this objective in mind, conducting studies involving different exposure or population models, based on rigorous study protocols including accurate analytical methods focused on other molecular levels such as proteomics to make a mechanistic link between genomics and metabolomics, would be relevant to offer any hope of arriving at conclusions about this complex subject. To do this, the use of molecular screening analyses (omics) seems essential to give greater depth to these studies and to make sense in health terms of the molecular mechanisms that can be triggered by environmental exposure [37].

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Data Availability

Data are available from the corresponding authors upon request.

Conflict of interest statement. The authors declare that they have no conflicts of interest.

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