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Arsenic carcinogenesis in the skin

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Summary

Chronic arsenic poisoning is a world public health issue. Long-term exposure to inorganic arsenic (As) from drinking water has been documented to induce cancers in lung, urinary bladder, kidney, liver and skin in a dose–response relationship. Oxidative stress, chromosomal abnormality and altered growth factors are possible modes of action in arsenic carcinogenesis. Arsenic tends to accumulate in the skin. Skin hyperpigmentation and hyperkeratosis have long been known to be the hallmark signs of chronic As exposure. There are significant associations between these dermatological lesions and risk of skin cancer. The most common arsenic-induced skin cancers are Bowen's disease (carcinoma in situ), basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Arsenic-induced Bowen's disease (As-BD) is able to transform into invasive BCC and SCC. Individuals with As-BD are considered for more aggressive cancer screening in the lung and urinary bladder. As-BD provides an excellent model for studying the early stages of chemical carcinogenesis in human beings. Arsenic exposure is associated with G2/M cell cycle arrest and DNA aneuploidy in both cultured keratinocytes and As-BD lesions. These cellular abnormalities relate to the p53 dysfunction induced by arsenic. The characteristic clinical figures of arsenic-induced skin cancer are: (i) occurrence on sun-protected areas of the body; (ii) multiple and recrudescent lesions. Both As and UVB are able to induce skin cancer. Arsenic treatment enhances the cytotoxicity, mutagenicity and clastogenicity of UV in mammalian cells. Both As and UVB induce apoptosis in keratinocytes by caspase-9 and caspase-8 signaling, respectively. Combined UVB and As treatments resulted in the antiproliferative and proapoptotic effects by stimulating both caspase pathways in the keratinocytes. UVB irradiation inhibited mutant p53 and ki-67 expression, as well as increased in the number of apoptotic cells in As-BD lesions which resulted in an inhibitory effect on proliferation. As-UVB interaction provides a reasonable explanation for the rare occurrences of arsenical cancer in the sun-exposed skin. The multiple and recurrent skin lesions are associated with cellular immune dysfunction in chronic arsenism. A decrease in peripheral CD4+ cells was noticed in the inhabitants of arsenic exposure areas. There was a decrease in the number of Langerhans cells in As-BD lesion which results in an impaired immune function on the lesional sites. Since CD4+ cells are the target cell affected by As, the interaction between CD4+ cells and epidermal keratinocytes under As affection might be closely linked to the pathogenesis of multiple occurrence of arsenic-induced skin cancer. In this

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review, we provide and discuss the pathomechanisms of arsenic skin cancer and the relationship to its characteristic figures. Such information is critical for understanding the molecular mechanism for arsenic carcinogenesis in other internal organs.

Introduction

Arsenic (As) is one of the most toxic metals (metalloids) derived from the natural environment. Arsenic occurs in two oxidative states: a trivalent form, arsenite (As III), and a pentavalent form, arsenate (As V). As III is 2–10 times more toxic than As V [1]. Organic As is non-toxic whereas inorganic As is toxic. Over the centuries, As has been used for a variety of purposes [2]. Arsenic has been used as a drug or poison for nearly 4,000 years. Inorganic As has been used for the treatment of syphilis, psoriasis and leukemia. Arsenic trioxide is now widely used to induce remission in patients with acute promyelocytic leukemia. In industry, As is used to manufacture paints, fungicides, pesticides, insecticides, herbicides etc. Gallium arsenide and aluminum gallium arsenide crystals are components of semiconductors, high emitting diodes, lasers and variety transistors. Because of the natural distribution of As in the rust of the earth, drinking water is the most common resource of As exposure for the general population [3, 4]. The World Health Organization suggests that maximum permissible limit of groundwater As concentration is 50 μg/l [5, 6]. Currently, the drinking water As standard in Taiwan, Japan, and U.S. is 10 μg/l. In human beings, the adult's acute As poisoning dosage is 0.17–0.87 mg/kg. This acute As exposure dosage causes conditions ranging from diarrhea, vomiting, liver and kidney toxicity [5–7]. Long-term exposure to low dosage of As may induce serious adverse health effects in multiple organs. Chronic health effects of As exposure via drinking water include skin and internal cancers, peripheral vascular disease, ischemic heart disease, cerebral infarction, diabetes mellitus and hypertension. Skin, lung, bladder, kidney, liver and uterus are considered as the sites related to arsenic-induced malignancies [2, 8]. The skin is thought to be perhaps the most sensitive site. Arsenic-induced skin cancers are usually occur on the sun-protected areas with multiple and recrudescent lesions [9–11]. This review discusses the pathomechanisms of arsenic skin cancer and the relationship to its characteristic figures.

Mechanisms of arsenic carcinogenesis

The International Agency for Research on Cancer (IARC) has classified As as a human carcinogen [12]. Exposure to As in drinking water is almost exclusive to inorganic As. Various hypotheses have been proposed to explain the carcinogenicity of inorganic arsenic. Oxidative stress, chromosomal abnormality and altered growth factors are possible modes of action in arsenic carcinogenesis [13, 14]. The mode-of-action studies suggest that the arsenic might be acting as a cocarcinogen, a promoter or a progressor of carcinogensis [15].

Reactive oxygen species

In human beings, As can be metabolized by s-adenosylmethionine dependent methylation. Arsenic methylation has been generally considered a detoxification process, because the methylated compounds are less genotoxic [16] and are excreted more rapidly in urine than inorganic forms [17]. After ingestion, inorganic As is taken up by red blood cells and then distributed primarily to the liver, kidney, spleen, lung, intestine and skin [18, 19]. As V is reduced to As III in blood. Arsenic is metabolized in the liver to various methylated forms. Enzymatic transfer to arsenite produces monomethylarsenic acid (MMA V), which is reduced to monomethyl arsonous acid (MMA III). A second methylation reaction methylates MMA III to dimethylarsinic acid (DMA V). Some DMA V can then be reduced to DMA III [20]. In this methylation process, reactive oxygen species (ROS) including peroxyl radical, superoxide radical, and hydroxyl radical could be generated [21, 22]. Arsenic is a strong ligand to the thio-group (-SH) of proteins [23]. Arsenic can react with -SH of the reduced glutathione (GSH). Since GSH is one of the most important free radical scavengers, the effects of As on GSH activity will affect cellular abilities of oxidative stress elimination. Directly or indirectly, arsenic-induced oxidative stress can induce further damages in cells, and these oxidative injuries are reported to associate

with arsenical carcinogenesis. Both in mouse and human skins, arsenic can induce oxidative damage in cellular DNA and generate 8-hydroxyl-2′-deoxylguanosine (8-OHdG) oxidative DNA adducts [24–26]. Clinical studies in arsenic-induced Bowen's disease (As-BD) indicate that the increased 8-OHdG levels are positively correlated to the lesional arsenic concentration [26], suggesting the involvement of oxidative stress in arsenical skin carcinogenesis. In vitro studies indicated that ROS induced by low concentrations of arsenic ($< 5\ \mu$M) can increase the transcription of the activator protein-1 (AP-1) and the nuclear factor kappa B (NF-κB) [27–30], which results in subsequent stimulation of cell proliferation [31, 32].

Genotoxicity

Arsenic is known to induce genetic toxicity in mammalian cells. Arsenic is reported to increase the rate of chromosome aberration and sister chromatid exchange that associated with arsenical carcinogenesis [33, 34]. Chromosome aberrations and endoreduplication were induced by arsenite in human fibroblasts and Chinese hamster ovary cells at higher concentrations, in contrast, arsenic induced sister chromatid exchanges at lower concentrations [35, 36]. These chromosomal abnormalities are reported closely relate to arsenic-induced oxidative DNA damage [26, 37]. Arsenite exposure induces micronuclei (MN) formation in human fibroblasts [38]. Low dose exposure to arsenite results mainly in kinetochore-positive (K^+) MN (MN contain centromere), whereas high dose treatment causes K-negative MN (MN without centromere). K^+ MN are usually derived from whole chromosome and are induced by agents that cause aneuploidy, whereas X-rays and other clastogens induce (K^-) MN [39, 40]. Therefore at low dose, arsenite acts as an aneugen, but at high dose it acts as a clastogen [15]. An increased frequency of MN has been detected in exfoliated bladder cells, buccal cells, sputum cells and lymphocytes from arsenic-exposed population [41–43]. Chien et al. reported that arsenite induced an increased frequency of MN in HaCaT cells which was associated with tumorigenicity in nude mice [44].

Altered DNA repair

Arsenic is able to inhibit DNA repair systems [45, 46]. The incision step and the ligation step of nucleotide excision repair were inhibited by arsenite [47]. Arsenite has been reported to decrease the DNA ligase III activity which results in DNA base excision repair [48, 49] and DNA strand break rejoining [50]. Arsenic is also reported to inhibit other DNA repair regulatory proteins including DNA ligase I, DNA ligase II, DNA ligase III, DNA polymerase β, O^6-methyl-guanine-DNA methyltransferase and poly (ADP-ribose) polymerase (PARP) [13, 49, 51]. Interfering of these DNA repair proteins by arsenic is shown to affect genome stabilities of the cells. Arsenite enhances the mutagenicity of carcinogeneic stresses (such as UV, X-rays, and chemical agent) in mammalian cells [52–55]. It is proposed that spontaneous or induced mutations in key genetic sites can then lead to subsequent mutation via inhibited DNA repair by arsenic.

Altered transcription factors

Arsenic is a strong ligand to the -SH, an important active residue for some regulatory proteins. It is known that about 200 proteins could be affected by arsenic-thio interaction [56]. Among these proteins, the proto-oncogene c-Jun is well-studied. Arsenic can block Jun N-terminal kinase (JNK) phosphatase activity via binding with its -SH. Since JNK phosphatase functions as a negative regulator of JNK, arsenic-induced JNK phosphatase dysfunction will cause irreversible activation of JNK. This JNK activation can further activate proto-oncogene c-Jun and the subsequent gene expression regulated by c-Jun/c-Fos complex (AP-1) [30, 57]. The effects of arsenic on transcription factor AP-1, as well as NF-κB, can induce series of abnormalities in cell functions. In which, the abnormalities in growth factor expression, cell cycle regulation, and apoptotic signaling are most closely associated with arsenic carcinogenesis. It is reported that long-term low dose arsenic exposure can enhance cellular sensitivity and response to epithelial growth factor (EGF) [31, 58] which can further inhibit cell cycle inhibitory protein p27 expression and cause cell hyperproliferation via c-myc

and E2F-1 regulatory pathway [59]. Arsenic can also enhance keratinocytes to express TGF-α, GM-CSF, IL-6 and IL-8 [31, 58, 60]. These arsenic-induced growth factors and cytokines expression are reported to associate with arsenic-induced cutaneous tumorigenesis via AP-1 and NF-κB regulation [61]. High concentrations of arsenic can induce significant cellular and DNA injuries. Arsenic-induced DNA damages are reported to activate p53-associated cell cycle checking and result in G2/M cell cycle arrest. Since arsenic exposure can inhibit DNA repair system, this p53-associated cell cycle checking will possibly fail and p53-regulated apoptotic cell death will be activated [62–65].

Arsenic and skin cancer

Arsenic tends to concentrate in ectodermal tissue including the skin, hair and nail. Thus, skin lesions (both malignant and non-malignant lesions) are considered to be the most common adverse health effects associated with chronic arsenic exposure in humans [66–68]. Skin hyperpigmentation and hyperkeratosis have long been known to be the hallmark signs of chronic arsenic exposure. They were the most common health effects found in populations exposed to arsenic-contaminated drinking water in many countries including Taiwan [9], Chile [69], Argentina [70], India [71, 72] and Bangladesh [73]. Hyperpigmentation occurs as diffuse brownish black pigmentation with a characteristic ''rain drop'' hypopigmentation. The hyperkeratosis may appear as a uniform thickening or as discrete nodules. It is emphasized that both palmar and plantar keratosis are a significant diagnostic criterion [71, 74]. There was a significant association between the concentration of arsenic in well water and the prevalence of hyperpigmentation and hyperkeratosis among the residents living in the arsenic-exposed areas [9, 71]. Both arsenic-induced skin lesions may be considered as a long-term biomarker of arsenic exposure [8]. There were significant associations between these dermatological lesions and risk of skin cancers. Tseng et al revealed a dose–response relationship between arsenic levels in drinking water and skin cancers. The most common arsenic-induced skin cancers are Bowen's disease, basal cell carcinoma and squamous cell carcinoma [9].

Bowen's disease is a carcinoma *in situ* of the skin, precancerous in nature, and has been well documented as a consequence of arsenical exposure [9–11, 66]. Clinically, Arsenic-induced Bowen's disease can be distinguished from non-arsenical Bowen's disease by its occurrence loci on sun-protected areas of the body and its multiple and recrudescent lesions [9, 11, 75]. Abnormal cellular proliferation and dysplasia are observed in the epidermal lesion of BD with significant apoptotic and dyskeratotic keratinocytes[11, 66]. Most of non-arsenical BD showed complete remission after surgical operation, however, many of As-BD may recur after surgery. Furthermore, As-BD lesion is able to transform into invasive SCC, BCC and combined forms of the skin cancer [9, 11, 76, 77]. Arsenic-induced cancers of other internal organs are reported to associate with As-BD lesions [10, 11, 76]. Individuals with documented As-BD are considered for more aggressive screening for long-term complications, especially the development of malignancies in the lung and urinary bladder [77–80]. It was indicated that As-BD started within 10 years, invasive skin cancer after 20–30 years [81], and pulmonary cancer after 30 years following the suspected arsenic exposure [76]. Therefore, the characteristic pathological and clinical features of As-BD may provide evidences of arsenic-induced cellular responses in the early stages of chemical carcinogenesis.

Pathomechanisms of arsenic-induced Bowen's disease

In vitro investigations had identified that arsenic could induce p53 accumulation through an ATM-dependent pathway [65, 82]. Histopathological studies indicated that p53 protein was highly expressed in As-BD as compared with non-arsenical BD [83, 84]. The over-expressed p53 in As-BD lesions was a mutant form [85, 86]. Most of the p53 mutation sites are located on exon 5 and exon 8. Furthermore, the mutation types of p53 gene mutation in arsenic-associated skin cancers are different from those in UV-induced skin cancers [87]. Chromosomal instability and aneuploidy were also commonly observed in As-BD lesions [88]. These findings suggest that dysplasia in As-BD is associated with p53 mutation. However, other study did not find significant association between p53 mutation and As-BD [86]. Although the linkage between p53 mutation and arsenic exposure is not

clear, the affect of arsenic on p53 regulation is well documented. Both in vitro and As-BD lesion studies indicated that arsenic exposure was associated with G2/M cell cycle arrest and DNA aneuploidy [88–90]. These cellular abnormalities may associate with p53 dysfunction induced by arsenic.

Hyperproliferative and dyskeratotic (apoptotic) keratinocytes co-existed in As-BD lesions. In vitro study indicated that the co-existence of hyperpoliferative and dyskeratotic keratinocytes might relate to the biological effects of arsenic on human keratinocytes [91]. The effects of As on keratinocytes depend on the concentrations of arsenic. At lower concentrations ($\leq 1\ \mu M$), arsenic induced keratinocyte proliferation and enhanced both NF-κB and AP-1 activity [91]. Keratinocyte apoptosis was not induced at low arsenic concentration (1 μM), which may relate to the anti-apoptotis signals of NF-κB [92] or the apoptosis resistant nature of keratinocytes. At higher concentrations ($\geq 5\ \mu M$), arsenic induced keratinocyte apoptosis by Fas/Fas ligand (FasL) pathway. At apoptosis inducing concentrations, NF-κB activity was not enhanced, however, AP-1 activity was further enhanced [91]. Since promoter regions of FasL contain binding sites for AP-1, arsenic-activated Fas/FasL signaling may associate with arsenic-induced AP-1 activation [93–95].

Effects of UVB on arsenic-induced skin cancer

Clinically, As-induced skin cancer lesions are usually on sun-protected skin. UVB has been used to treating many hyperproliferative dermatoses including psoriasis and cutaneous T-cell lymphoma. UVB may play a modulatory role in the skin arsenic carcinogenesis. Chai et al revealed that UVB irradiation reduces mutant p53 and ki-67 expression, as well as decreases in the number of apoptotic cells in As-BD lesions [85] which results in an inhibitory effect on cell proliferation (Figure 1).

Arsenic is not mutagenic in bacterial or mammalian cells, but it reinforces mutations induced by various mutagens including UVB. Reports investigating the interaction of UVB and arsenic have focused on the DNA excision repair and replication. Inhibition of pyrimidine dimers excision [96] and postreplication repair [45, 48] by arsenic is responsible for the cytotoxicity and mutagenesis of UV in Chinese hamster ovary cells.

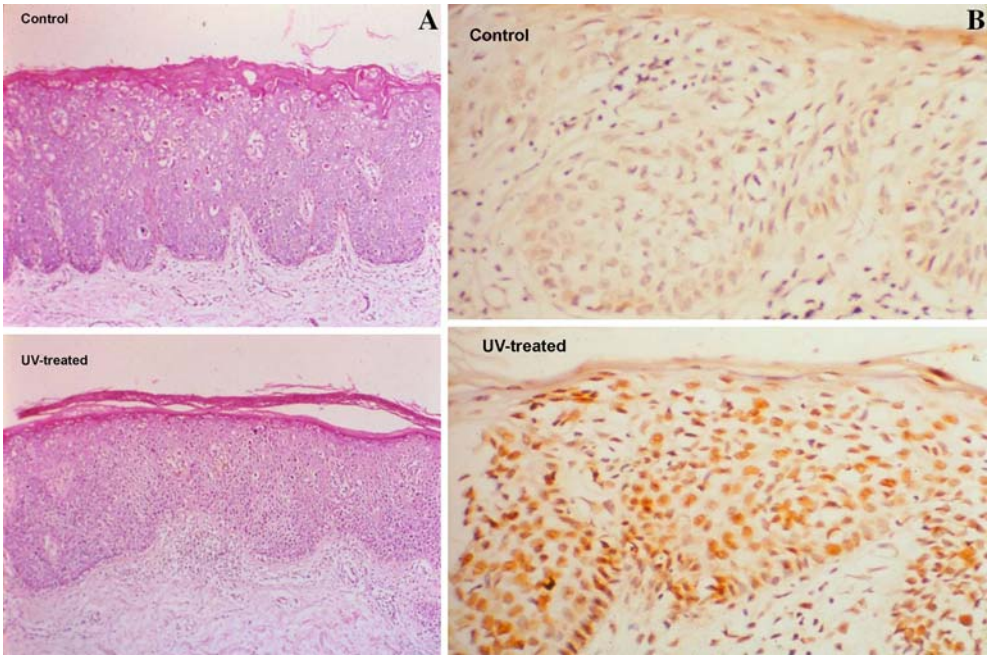

Figure 1. UVB irradiation on As-BD lesions. (a) HE staining of As-BD lesion from a patient before and after UVB irradation (75 mJ/cm^2 × 5 times/wk × 2 = 750 mJ/cm^2 UVB total). UVB irradiation showed significant effects on decreasing dysplasia and dyskeratotic cells of the lesion. (b) TUNEL staining of apoptotic cells after UVB irradiation. UVB irradiation induced apoptosis in lesional kerationcytes.

Arsenic treatment enhances the cytotoxicity, mutagenicity, and clastogenicity of UV light in Chinese hamster ovary cells [97]. UV-induced DNA damage leads to p53-mediated apoptosis [98, 99]. Upon severe DNA damage, p53 upregulates Bax that binds to the mitochondria membrane and activates caspase-9 and caspase-3, leading to downstream apoptotic responses [100, 101]. Arsenic causes apoptosis of human keratinocytes through the Fas/FasL pathway with enhanced AP-1 activity. Downstream signals of Fas/FasL pathway, including FADD, caspase-8, caspase-3 and PARP cleavage, are activated [91]. Therefore, activation of a different primary caspase is involved in apoptosis induced by As as compared to UVB-induced apoptosis. In vitro study revealed that arsenic enhances UVB-induced keratinocyte apoptosis via suppression of Bcl-2 expression and stimulation of caspase-8 activity (Figure 2). Combined UVB and arsenic treatment resulted in the antiproliferative and proapoptotic effects in the keratinocytes [75]. As-UVB interaction provides a reasonable explanation for the rare occurrences of arsenical cancer in the sun-exposed skin.

Immunological dysfunction in arsenic-induced skin cancer

Previous reports indicated that the multiple and recurrent skin lesions are associated with cellular immune dysfunction in patients with chronic arsenism. It was reported that arsenic exposure was associated with the decreased number in CD4+ cells (T helper) both in adults and in children [102, 103]. Furthermore, increased arsenic exposure is associated with decreased proliferative response to mitogen (phytohemagutinin) stimulation in CD4+ cells. Patients with arsenic-induced skin cancer showed increased gene expression of inflammatory molecules, such as IL-1β, IL-6, CD14, C-C and C-X-C chemokine motif ligand [104]. Impaired delayed-type hypersensitivity response to 2,4-dinitrochlorobenzene was observed in patients with As-BD. The association of impaired cellular immunity may be attributed to the effects of arsenic on human lymphocytes. The defective cell-mediated immune function in As-BD was related to an impairment of IL-2 receptor expression and a decrease in CD4+ cells after chronic arsenic exposure [102]. The decreased CD4+ cell number was related to arsenic induced CD4+ cell apoptosis via the TNF-R1 pathway [105]. In addition to these systemic effects in immune cells by arsenic, immune cell alternations in As-BD lesions were also observed in As-BD. There was a progressive decrease in the number of Langerhans cells in the order of normal skin, normal appearing skin in As-BD, and As-BD lesion. The Langerhans cell density in As-BD was not correlated with the perilesional infiltrates.

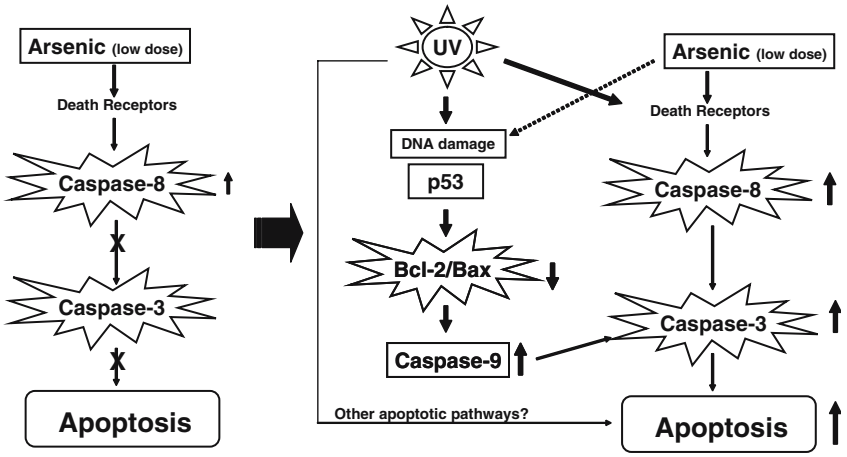

Figure 2. A scheme of arsenic and UVB interaction in keratinocyte apoptosis. Low doses (≤ 1 μM) exposure of arsenic enhanced human keratinocytes to express death receptors (including Fas) and its downstream caspase-8 expression. However, low dosage of arsenic did not activate further caspase cascade and apoptosis in keratinocytes (left). UVB irradiation induces keratinocyte apoptosis majorly via caspase-9 pathway. Combined UVB irradiation, low doses of arsenic further enhanced UVB-induced caspase-9 activation, as well as caspase-8 activation. In this condition, the downstream effector caspase (caspase-3) was significantly activated and keratinocyte apoptosis was increased. Nontoxic concentrations of arsenic can significantly increase UVB-induced keratinocyte apoptosis via further activating UVB-associated caspase signals (right).

Most of the infiltrating cells in the peritumoral area of arsenic-induced skin cancer are T cells [106]. Langerhans cells are known to be one of the antigen presenting cells for T lymphocytes. They play a pivotal role in the presentation of tumor-associated antigens in neoplastic tissue, thereby facilitating T cell-mediated antitumoral immune responses [107–109]. The decreased Langerhans cells in As-BD lesions implied an impaired immune function on the lesional epidermis itself. CD4+ cells carrying acquired dendritic cell antigen-presenting machinery can efficiently stimulate cytotoxic T lymphocyte response [110]. Since CD4+ cell is the target cell affected by arsenic, the interaction between CD4+ cells and epidermal keratinocytes under arsenic affection might be closely linked to the pathogenesis of the multiple and recrudescent arsenic-induced skin cancer.

Epidemiological studies revealed that long-term exposure to arsenic induces cancers in lung, urinary bladder, kidney, liver, uterus and skin. Arsenic skin cancer is usually located on sun-protected areas with multiple and recrudescent lesions. In this review, we provide and discuss the pathomechanisms of arsenic skin cancer and the relationship to its characteristic clinical figures. Such information is critical for clarifying the molecular mechanism for arsenic carcinogenesis in other internal organs.

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