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Arsenic-induced cutaneous hyperplastic lesions are associated with the dysregulation of Yap, a Hippo signaling-related protein

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ABSTRACT

Arsenic exposure in humans causes a number of toxic manifestations in the skin including cutaneous neoplasm. However, the mechanism of these alterations remains elusive. Here, we provide novel observations that arsenic induced Hippo signaling pathway in the murine skin. This pathway plays crucial roles in determining organ size during the embryonic development and if aberrantly activated in adults, contributes to the pathogenesis of epithelial neoplasm. Arsenic treatment enhanced phosphorylation-dependent activation of LATS1 kinase and other Hippo signaling regulatory proteins Sav1 and MOB1. Phospho-LATS kinase is known to catalyze the inactivation of a transcriptional co-activator, Yap. However, in arsenic-treated epidermis, we did not observed its inactivation. Thus, as expected, unphosphorylated-Yap was translocated to the nucleus in arsenic-treated epidermis. Yap by binding to the transcription factors TEADs induces transcription of its target genes. Consistently, an up-regulation of Yap-dependent target genes Cyr61, Gli2, Ankrd1 and Ctgf was observed in the skin of arsenic-treated mice. Phosphorylated Yap is important in regulating tight and adherens junctions through its binding to αCatenin. We found disruption of these junctions in the arsenic-treated mouse skin despite an increase in αCatenin. These data provide evidence that arsenic-induced canonical Hippo signaling pathway and Yap-mediated disruption of tight and adherens junctions are independently regulated. These effects together may contribute to the carcinogenic effects of arsenic in the skin.

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1. Introduction

Arsenic exposure through drinking water is a major global public health problem [1]. Approximately 150 million people are exposed to the toxic levels of arsenic worldwide including Bangladesh, Taiwan, Mexico, Mongolia, Argentina, India, Chile, etc. In some parts of the United States of America, high concentrations of arsenic are found in underground water [2]. Exposure to arsenic is associated with the enhanced risk of cancers in various organs including bladder, kidney, lung, liver and skin [3]. In humans, chronic arsenic exposure induces a dry skin phenotype, melanosis, hyperplasia and hyperkeratosis. Some of the precancerous lesions may ultimately progress to basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) [4]. However, these changes in murine models have so far been not described and the mechanism by which arsenic induces these pathological alterations remains elusive.

Hippo signaling pathway is an evolutionarily conserved cascade that controls organ size by regulating cell proliferation, differentiation, apoptosis, and stem cell self renewal [5]. The core Hippo signaling pathway consists of a kinase cascade in which mammalian STE20-like kinase 1/2 (Mst1/2) and Salvador homolog 1 (Sav1) form a complex which phosphorylates and activates a downstream kinase Large tumor suppressor kinase 1/2 (LATS1/2). The regulatory protein MOB kinase activator 1A (MOB1) forms complex with the active LATS1/2 to phosphorylate its downstream transcription co-activators Yes-associated protein (Yap) and transcriptional co-activator with PDZ-binding motif (TAZ). This leads to the inhibition of their activities via their cytoplasmic retention or proteasomal degradation [5]. However, unphosphorylated-Yap and TAZ translocate into the nucleus and interact with transcription factors Tea-domain (TEAD) to induce the expression of their target genes, which were shown to be involved in cell proliferation and apoptosis inhibition [6]. The upstream regulators of core kinase cascade Mst1/2-LATS1/2-Yap/TAZ include proteins such as Merlin, KIBRA, RASSFs, and Ajuba [5]. However, increasing evidence indicates that αCatenin or ZO-2 may independently regulate Yap/TAZ at the adherens junctions and/or tight junctions [7,8]. In addition, the

Abbreviations: Yap, Yes-associated protein; Mst1/2, mammalian STE20-like kinase 1/2; Sav1, Salvador homolog 1; LATS1/2, large tumor suppressor kinase 1/2; MOB1, MOB kinase activator 1A; TAZ, transcriptional co-activator with PDZ-binding motif; TEAD, Tea-domain; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; AMOTL1, Angiomotin-like 1.

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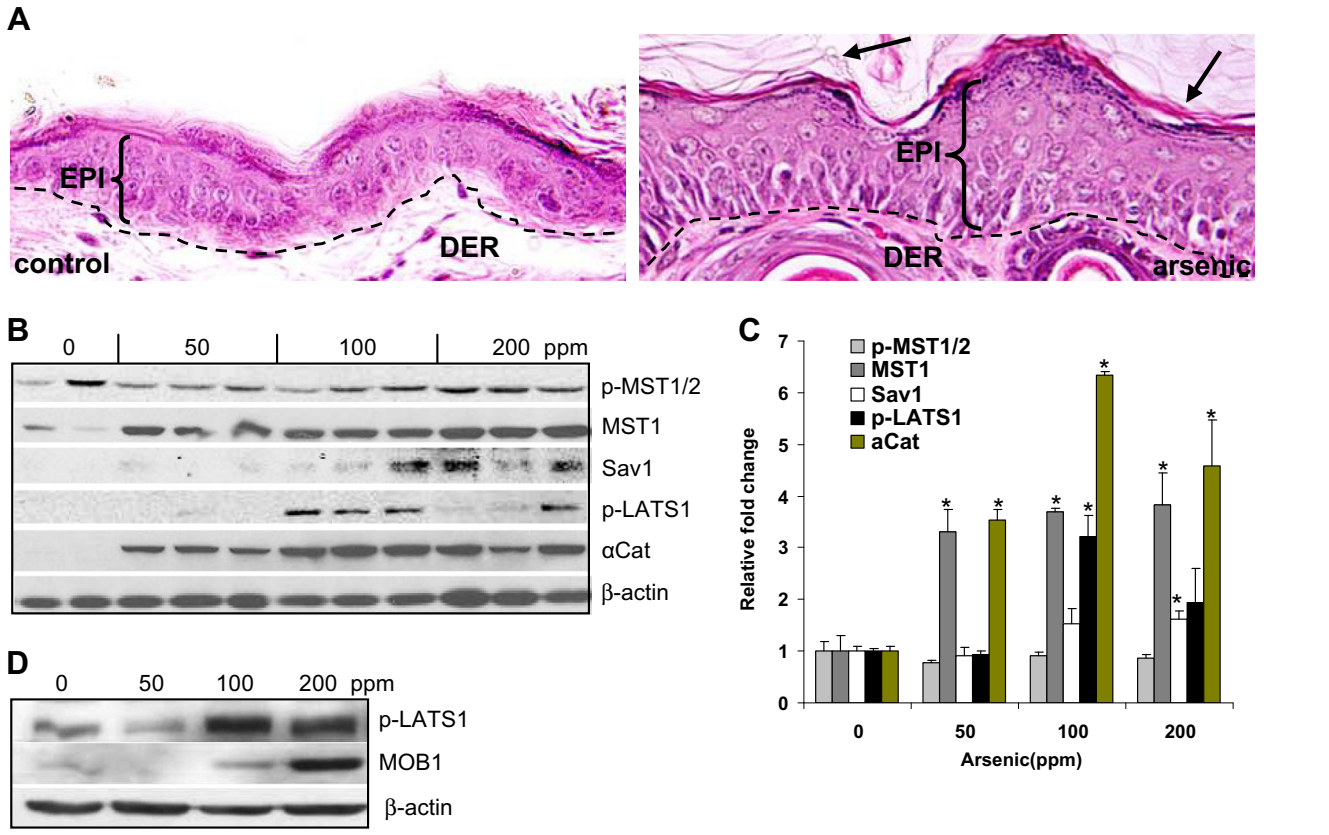

Fig. 1. Arsenic up-regulates Hippo signaling in murine skin. (A) Histological pictures showing arsenic exposure leads to hyperkeratosis, hyperplasia and epidermis disorganization in the skin of SKH-1 mice. Skin samples shown here were from control and 200 ppm sodium arsenite treatment group. Black arrows indicate the stratum corneum. Note that the thickness of stratum corneum was significantly augmented in the focal areas of arsenic-treated skin. The dashed lines delineate the boarder of epidermis and dermis. The braces indicate the thickness of epidermis. Each picture is representative of three independent skin samples. EPI stands for epidermis and DER stands for dermis. (B) & (C) Western blot and statistical analysis showing the expression of p-MST1/2, MST1, Sav1, p-LATS1 and αCatenin in the skin of SKH-1 mice. *indicates $p < 0.05$ when compared to control. Bars represent mean ± SEM ($n = 3$). (D) Western blot analysis showing arsenic up-regulates the expression of p-LATS1 and MOB1 in the skin of SKH-1 mice. For this, three skin samples from one group were pooled together, thus the individual band density represents the average value of protein expression level for each group.

activities of Mst1/2, LATS1/2 and Yap/TAZ may also be regulated by phosphatases, ubiquitination and by the cytoskeleton proteins [8–11]. Dysregulation of the Hippo pathway can lead to cancer development in various organs including skin [5].

In this study, we show for the first time that arsenic activates Hippo signaling pathway in the skin. Treatment of SKH-1 hairless mice with arsenic up-regulates αCatenin without inducing Yap phosphorylation. Instead, arsenic independently activates Yap, leading to its nuclear translocation and transcriptional activation. These data provide a novel mechanism by which some of the cutaneous manifestations of arsenic toxicity and carcinogenicity could be mediated.

2. Materials and methods

2.1. Reagents

Primary antibodies: αCatenin (sc-7894, Santa Cruz, Dallas, TX), MOB1 (3863, Cell Signaling, Danvers, MA), p-MST1/2 (3681, Cell signaling), MST1 (3682, Cell Signaling), p-LATS1 (9157s, Cell Signaling), Yap (4912, Cell Signaling), p-Yap (4911s, Cell Signaling), Sav1 (3507, Cell Signaling), TAZ (4883s, Cell signaling), β-actin (A-5316, Sigma, St. Louis, MO) were purchased.

2.2. Animals

To study the effects of arsenic on Hippo signaling, we utilized the skin samples obtained from the study published earlier [12]. Briefly, 25 age-matched SKH-1 hairless mice (5 mice/group) were fed *ad libitum* respectively drinking water containing arsenic at 0 ppm, 50 ppm, 100 ppm and 200 ppm concentrations for a period of 1 month. Then all of these animals were killed, their skin excised and processed for histology/immunohistochemistry/immunofluorescence studies or Western blot/PCR analysis. All experimental procedures involving animals were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

2.3. Western blot

Western blots were performed as previously described [12]. Briefly, skin tissues were homogenized in an ice-cold lysis buffer (50 mM Tris, pH 7.5, 1% Triton X-100, 0.25% NaF, 10 mM β-glycerolphosphate, 2 mM EDTA, 5 mM sodium pyrophosphate, 1 mM Na_3VO_4, 10 mM DTT and protease inhibitor). Clear lysate was prepared by centrifugation at 10,000*g* for 10 min. Proteins were denatured in 4× loading buffer, subjected to SDS–PAGE and electrophoretically transferred to PVDF membranes. The nonspecific sites were blocked with 5% (W/V) nonfat-dry milk in TBST for 1 h at RT. Blots were probed with primary antibody (4 °C overnight) followed by incubation with HRP-conjugated secondary antibody (1 h at RT). Then the blots were developed with Western blotting luminol reagent (sc-2048, Santa cruz). The integrated density of bands was measured with Image J software (http://rsb.info.nih.gov/ij/). Statistical analysis was conducted using Excel 2003. The same protein lysates were generated for western blot analysis

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