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Lasting glycolytic stress governs susceptibility to urethane-induced lung carcinogenesis in vivo and in vitro

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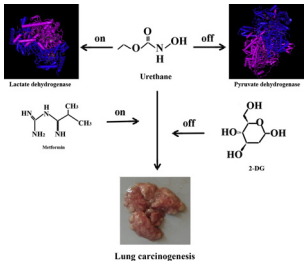
HIGHLIGHTS

- The mouse strains have different susceptibility to lung carcinogenesis.
- Urethane-induced lung carcinogenesis is associated with glycolytic stress.
- Lasting glycolysis removes difference of susceptibility to lung carcinogenesis.
- Glycolytic metabolism is a direct initiator of tumorigenesis.
- 10× injection of urethane is a valuable model for studying lung carcinogenesis.

GRAPHICAL ABSTRACT



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ABSTRACT

Urethane is a recognized genotoxic carcinogen in fermented foods and beverages. This study is to compare susceptibility of ICR mice, BALB/c mice and C57BL/6 mice to urethane-induced lung carcinogenesis. The mice were injected intraperitoneally with 600 mg/kg of urethane for three times or ten times at 7-day intervals. At week 26, lung carcinogenic incidence was found in 40% ICR mice, 20% BALB/c mice and 10% C57BL/6 mice of the 3× injection group, respectively, whereas 100% lung tumor incidence took place in three mouse strains of the 10× injection group. In the 10× injection group, urethane induced lasting glycolytic stress of lung with an increase in lactate, monocarboxylate transporter 1 (MCT-1), reactive oxygen species(ROS) and 7,8-dihydro-8-oxo-29-deoxyguanosine (8-OHdG) and a decrease in pyruvate dehydrogenase (PDH) and cytochrome C oxidase (COX). In the 3× injection group, urethane also promoted lung glycolytic stress at the end of urethane injection but it lasted no more than 7 days besides in lung tumor-bearing mice. Metformin as a glycolytic enhancer promoted urethane carcinogenic efficacy in the 3× injection group, whereas 2-deoxy-glucose (2-DG) as a glycolytic inhibitor decreased urethane carcinogenic efficacy in the 10× injection group. Further, urethane promoted tumor survival in A549 cells by inducing cancer stem-like cellular state. These data suggest that lasting glycolytic stress is sufficient for urethane-induced lung tumorigenesis, and that urethane 10× injection-induced lung cancer can serve as a valuable model for lung tumor biology and tumor prevention.

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1. Introduction

Lung cancer is one of the most common and lethal forms of cancer (Liang et al., 2015). Although there are advances in therapeutic strategies, the prognosis of lung cancer remains poor with a 5-year survival rate of only 15% (Yentz and Wang, 2011). It is recognized that genetic mutations underlie malignant transformation (Milyavsky et al., 2005). Based on this idea, next-generation sequencing of human tumors has refined our understanding of the mutational processes operative in cancer initiation and progression, yet major questions remain regarding the factors that induce driver mutations and the processes that shape mutation selection during tumorigenesis (Westcott et al., 2015). Obviously, the presence of mutations by themselves is not sufficient for tumor formation (Watson et al., 1991), understanding the necessary alterations of the cancer development is critical for identifying novel complementary agents.

Notable characteristics of growing tumor cells are their increased glycolytic rate and their decreased oxidative respiration, even in the presence of oxygen (Zhou et al., 2015). This quintessential hallmark of cancers is known as the "Warburg effect". Despite the fact that reversing the Warburg effect may offer a generalized anticancer strategy (Esteves et al., 2014), there is much debate about the causal role of the Warburg effect (Krisher and Prather, 2012). Urethane is a recognized genotoxic carcinogen, with widespread occurrence in fermented foods and beverages (Lachenmeier, 2005). Compared to human lung adenocarcinoma, the mouse-urethane model exhibits similar histological appearance and molecular changes, and therefore serve as a valuable tool not only for understanding basic lung tumor biology but also for the development and validation of new tumor intervention strategies (Meuwissen and Berns, 2005). We demonstrated previously that the mitochondrial dysfunction played an important role in urethane-induced lung carcinogenesis (Du et al., 2013). Herein, we used this mouse model to establish a clearly link between glycolytic metabolism stress and lung carcinogenesis. Our results showed that the mouse strains had differential susceptibility to urethane-induced lung carcinogenesis with a differential glycolytic stress. Alternatively, lasting glycolytic stress caused the same carcinogenic incidence in different mouse strains, whereas metformin as a glycolytic enhancer promoted urethane carcinogenic efficacy although it was reported that metformin had antitumor activity in a variety of cancers (Ben Sahra et al., 2008) and delayed tobacco carcinogen-induced lung cancer (Memmott et al., 2010). Our results suggest that glycolytic metabolism is not only a secondary side effect of cancer transformation but also a direct initiator of tumorigenesis.

2. Materials and methods

2.1. Reagents

Ethyl carbamate (urethane), basic fibroblast growth factor (bFGF) and heparin were purchased from Sigma Chemical Co. The antibodies used including E-cadherin, N-cadherin, Vimentin, Cytokeratin 18, Nanog, Octamer-binding transcription factor-4 (Oct4) and proliferating cell nuclear antigen (PCNA) were purchased from BD Pharmingen. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG polyclonal antibody and Peroxidase substrate 3-amino-9-ethylcarbazole (AEC) were purhased from Nichirei Bioscience (Tokyo, Japan). Lactate kit and pyruvate kit were purchased from BioVision (Mountain View, CA, K609-100). Tumor Necrosis Factor α (TNF-α), high-sensitivity C-reactive protein (hs-CRP), ROS, 8-OHdG, nicotinamide adenine dinuleotide reduced (NADH)-oxidase, succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) ELISA kit were purchased from BioVision Research Products. 2′,7′-dichlorofluorescein diacetate (DCFH-DA) was purchased from Wako Ltd. (Osaka, Japan). Adenosine triphosphate (ATP) bioluminescent somatic cell assay kit was purchased from Sigma (St. Louis, MO, USA).

2.2. Animals

The female ICR mice, BALB/c mice and C57BL/6 mice, 5–7 weeks of age, and experimental diets were obtained from Beijing Weitong Lihua Animal Co. All mice were housed in individual ventilated cages under a 12 h light–dark cycle (lights on 7:00 AM–7:00 PM). The animals were fed standard rodent chow and water and were maintained under pathogen-free conditions within the institutional animal facility. Food and tap water were given ad libitum.

All animal procedures in this study were approved by the animal experimentation ethics committee of Henan University, all procedures were performed in strict accordance with the guide for the care and use of laboratory animals and the regulation of animal protection committee to minimize the suffering and injury.

2.3. Urethane-induced lung adenocarcinoma model

The mice were administered freshly prepared urethane to induce lung adenocarcinoma according to our previously published protocol (Du et al., 2013). In the first experiment, the mice were given an intraperitoneal injection of urethane (600 mg/kg body weight) dissolved in sterile 0.9% NaCl once weekly for 3 weeks. Following the last injection of urethane, the mice were treated with metformin 250 mg/kg by oral gavage once daily for 23 weeks. In the second experiment, the mice were administered an intraperitoneal injection of urethane (600 mg/kg body weight) dissolved in sterile 0.9% NaCl once weekly for 10 weeks. Following the first injection of urethane, the mice were treated with 2-DG 500 mg/kg by oral gavage once daily for 25 weeks. The control mice were treated with vehicle (0.5% carboxymethylcellulose in PBS) by oral gavage. During the studies, the health of the mice was monitored daily, and body weights were measured weekly. At week 26, the mice were euthanized by CO_2 asphyxiation, and the lung tumor incidence, multiplicity, and tumor load (sum of tumor per lung in average) were determined according to previously established criteria (Stearman et al., 2005). The lungs were fixed in 4% paraformaldehyde solution overnight for histopathologic and immunohistochemical evaluations.

2.4. Histopathology analysis

The animals were euthanized by CO_2 asphyxiation, and the lungs were immediately excised. Subsequently, the lungs were washed with PBS, and the number of external nodules in each pulmonary lobe was counted. After the gross examination, a part of each lung lobe was preserved in 4% paraformaldehyde solution. Randomly selected, the paraformaldehyde-fixed lung tissues were routinely processed and embedded in paraffin. Histological sections (5 μm) were stained with hematoxylin and eosin and analyzed. Proliferative lesions in the lungs were classified as hyperplasia, adenoma or adenocarcinoma based on the recommendations published by the Mouse Models of Human Cancers Consortium (Nikitin et al., 2004).

2.5. Immunohistochemical study

Lung histological sections (5 μm) from the paraffin blocks were obtained in silanized slides. The sections were deparaffinized, rehydrated and washed with PBS. After blocking with 3% hydrogen peroxide for 10 min, 0.1% Triton X-100 was used for 20 min at room temperature and the sections were rinsed with PBS. Antigen