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Toxicogenomic responses of human alveolar epithelial cells to tungsten boride nanoparticles

Hasan Türkez [a], Mehmet Enes Arslan [a,*], Erdal Sönmez [b,c], Abdulgani Tatar [d], Metin Açikyildiz [e], Fatime Geyikoğlu [f]

[a] Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, Turkey
[b] Department of Physics, Kazım Karabekir Education Faculty, Atatürk University, Erzurum, Turkey
[c] Advanced Materials Research Laboratory, Department of Nanoscience & Nanoengineering, Graduate School of Natural and Applied Sciences, Ataturk University, Erzurum, Turkey
[d] Department of Medical Genetics, Medical Faculty, Atatürk University, Erzurum, Turkey
[e] Department of Chemistry, Faculty of Science and Art, Kilis 7 Aralık University, Kilis, Turkey
[f] Department of Biology, Faculty of Arts and Sciences, Atatürk University, Erzurum, Turkey

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A B S T R A C T

During the recent years, microarray analysis of gene expression has become an inevitable tool for exploring toxicity of drugs and other chemicals on biological systems. Therefore, toxicogenomics is considered as a fruitful area for searching cellular pathways and mechanisms including cancer, immunological diseases, environmental responses, gene-gene interactions and chemical toxicity. In this work, we examined toxic effects of Tungsten Borides NPs on gene expression profiling of the human lung alveolar epithelial cells (HPAEpiC). In line with this purpose, a single crystal of tungsten boride (mixture of WB and W_2B) nanoparticles was synthesized by means of zone melting method, and characterized via using X-ray crystallography (XRD), transmission electron microscope (TEM), scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDX) techniques. Cell viability and cytotoxicity were determined by 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT), neutral red (NR) and lactate dehydrogenase (LDH) release tests. The whole genome microarray expression analysis was performed to find out the effects of WB and W_2B NPs mixture on gene expression of the HPAEpiC cell culture. 123 of 40,000 gene probes were assigned to characterize expression profile for WB/W2B NPs exposure. According to results; 70 genes were up-regulated and 53 genes were down-regulated ($\geq$2 fold change). For further investigations, these genes were functionally classified by using DAVID (The Database for Annotation, Visualization and Integrated Discovery) with gene ontology (GO) analysis. In the light of the data gained from this study, it could be concluded that the mixture of WB/W2B NPs can affect cytokine/chemokine metabolism, angiogenesis and prevent migration/invasion by activating various genes.

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1. Introduction

Recent studies have shown that nanoparticles (NPs) are important chemicals for transcription profiling analysis because they have high impact on various biological processes with their gene activating or silencing effects [1]. Furthermore, metal borides are commonly preferred component for industrial applications because of their high level of temperature, corrosion, and wear resistance applications and their strong compositions. Tungsten borides (W_2B and W_2B_5) have electronic conductivity, high hardness values and chemical dormancy properties which are suitable for manufacturing [2–4].

Tungsten borides are one of the biggest rivals of traditional super hard materials with their transition-metal/light-element compound characteristics. Also W_2B has distinct features to make itself good candidate for industry with its such features as high resistance to oxidation and acids, high melting point and extreme hardness [5]. However, toxicological properties of WB and W_2B are not investigated yet and there is no performed toxicogenomic

* Corresponding author.
E-mail address: enes.aslan@erzurum.edu.tr (M.E. Arslan).

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analysis on this NPs. Additionally, toxic genomics is a rapidly developing area for genomic collection analysis that compares toxicity mechanisms with gene expressions. This technology uses microarray studies where high-density hybridization arrays monitor the whole genome gene expression simultaneously. Researches have shown that using compounds with specific toxic effects are resulted in resembling gene expression profiles and this investigation give insight about building a database including toxic molecules effects on expression profiles can be used as a template for unknown compound toxicity [6]. Furthermore, DNA microarrays can analyze expression level of thousands of genes at the same time by using a specific mRNA sample. To compare transcription levels in clinical conditions, this high-throughput profiling of gene expression can be used in order to monitor disease classification, therapy responses, prognostic/diagnostic biomarker and the genes involved in disease occurrence [7–9]. Hence, possible applications of array technology in toxicology research have a huge potential since microarray based assays are more reliable than cell death assays when high throughput assay markers with comprehensive transcriptional libraries are developed for specific compounds [10–12].

In this work, synthesized WB/W2B NPs NPs mixture was characterized by using XRD, TEM, SEM and EDX techniques. WB/W2B NPs were applied on the human lung alveolar epithelial cell line (HPAEpiC). After 72 h of incubation, MTT, LDH and NR assays were performed to find out the cytotoxicity of WB/W2B NPs on the cell line. IC_{20} value for the mixture of WB/W2B NPs was determined, and then total RNAs were isolated from the cultures and investigated in microarray analysis. In the light of these findings, 50 most affected genes from WB/W2B NPs mixture treatment were appointed and functional categories for these genes revealed to understand effects of WB/W2B NPs on biological pathways. The main purpose of this article was to fill the missing parts in the literature by revealing any hazardous effect of WB/W2B NPs mixture toxicity.

2. Materials and methods

2.1. Synthesis and characterization of tungsten boride (WB/W2B) NPs

Mechanical activation-assisted self-propagating system was used to synthesize WB/W2B NPs mixture. For a stimulating appropriate condition for this system, a stoichiometric compound of tungsten, tungsten trioxide, boron oxide and magnesium elements were mixed in a high-energy planetary mill. To get combustion process W, WB and MgO molecules were added to the mixture and grinded for 60 min. After grinding WB particles partially disappeared,W2B, W and MgO triple nano composites were produced. Unwanted MgO was discarded via washing step and W2B/W phase composites were constituted. Nanoparticles characterization was carried out by using XRD, TEM, SEM and EDX techniques.

2.2. Nanoparticles characterizations

The micro-structural investigations of WB/W2B NPs were made by X-ray diffraction (XRD) measurement at room temperature via using a Rigaku/Smart Lab diffractometer with CuKα radiation ($\lambda = 0.154059$ nm) operated at 40 kV and 30 mA. The measurement was taken in the geometry of coupled θ-2θ varied between 100 and 850 with the step of 0.020. The surface morphology and particle size of tungsten boride nanoparticles were investigated with a scanning electron microscope (FEI inspect S50 SEM) and transmission electron microscopy (JEOL JEM-ARM200CFEG UHR-TEM). Finally, chemical characterization of mixture of WB/W2B NPs was conducted with energy-dispersive x-ray spectroscopy (EDS, EDX).

2.3. Cell culture

The HPAEpiC (California, USA, ScienceCell®) cells were seeded in 48-well plates and incubated at 37 °C in a humidified 5% CO_2 with Alveolar Epithelial Cell Medium (AEpiCM, California, USA, ScienceCell®). Negative control (only cell culture), hydrogen peroxide (H_2O_2; 25 μM Sigma-Aldrich®) as positive control and 12 experimental groups with different WB/W2B NPs concentrations were created to test cytotoxicity of the compound. First, W2B NPs were dissolved in DMSO (Sigma-Aldrich) and medium (<1% DMSO final concentration) and then incubated at different concentrations (0.625, 1.25, 2.5, 5, 10, 20, 80, 160, 320, 640 and 1280 mg/L) for 72 h.

2.4. MTT assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was used according to the manufacturer's instructions (Cayman Chemical Company®, Ann Arbor, MI, USA). Shortly, MTT was added to the cell cultures for 3 h and after incubation formazan crystals were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich®), and the plates were analyzed using a plate reader at 570 nm wavelength [13].

2.5. LDH release assay

LDH cytotoxicity assay kit (Cayman) was used to carry out LDH assay according to the manufacturer's protocol. The cells were seeded in 96-well plates and exposed to different concentrations of W2B for 72 h. After exposure, 96-well plate was centrifuged at 400*g* for 5 min to get rid of WB/W2B NPs in the wells. Then, 100 μL supernatant was transferred to a fresh well of 96-well plate with 100 μL of the reaction mixture from Cayman kit and incubated for 30 min at room temperature. Finally, the absorbance of the solution was measured at 490 nm using a microplate reader [14].

2.6. Neutral red assay

Neutral red stock solution was prepared according to the manufacturer's instructions in phosphate-buffered saline (Sigma-Aldrich®, USA). The HPAEpiC cells were incubated with the NR solution for 2 h at 37 °C for the lysosomal dye uptake of viable cells. Then, the mixture of formaldehyde (0.125%) and CaCl2 (0.25%) was used to wash cell culture and the NR solution was removed. The cells were incubated with a mixture of acetic acid (1%) and ethanol (50%) at room temperature for 30 min to extract the NR from the HPAEpiC cells. The optical density of each sample was then measured at 540 nm with a microplate reader (Bio-Tek Instruments, USA) [14].

2.7. Microarray analysis

ND-1000 Spectrophotometer (NanoDrop, Wilmington, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA) were used to evaluate RNA purity and integrity. TargetAmp-Nano Labeling was used to amplify and purify total RNA for Illumina Expression BeadChip (EPICENTRE, Madison, USA) using to get biotinylated cRNA according to the manufacturer's instructions. By using a T7 oligo (dT) primer 500 ng of total RNA was reverse-transcribed to cDNA, second-strand cDNA was synthesized, in vitro transcribed, and labeled with biotin-NTP and cRNA was quantified using the ND-1000 Spectrophotometer. Human HT-12 v4.0 Expression Beadchips were hybridized with 750 ng of

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