DOI 10.1007/s10517-015-3082-7
Bulletin of Experimental Biology and Medicine, Vol. 159, No. 6, October, 2015

ERRATUM

To the article «Suppression of *ITGB4* Gene Expression in PC-3 Cells with Short Interfering RNA Induces Changes in the Expression of β-Integrins Associated with RGD-Receptors»

by E. N. Knyazev, K. M. Nyushko, B. Ya. Alekseev, T. R. Samatov, M. Yu. Shkurnikov, Vol. 159, No. 4, pp. 541-545, August, 2015

The section "Materials and Methods" should be as follows:

MATERIALS AND METHODS

The shRNA-mediated depletion of ITGB4 in PC3 cells and the corresponding xenograft experiment as well as the isolation of RNA used for the described microarray analyses were performed at the Institute of Anatomy and Experimental Morphology, University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf (Prof. Dr. T.Lange, Dr. D.Wicklein, Prof. Dr. U.Schumacher).

For obtaining *ITGB4* knockdown PC cells, cells of the PC-3 line were transfected with pLVX vector (Clontech) that carried a sequence encoding short hairpin RNA (shRNA), a precursor of small interfering RNA (siRNA), complimentary to a locus in *ITGB4* gene (CGAGAAGCTTCACACCTAT).

DNA sequence encoding anti-ITGB4 shRNA (http://bioinfo.clontech.com/rnaidesigner/oligoDesigner.do) is presented in Figure 1. Cloning was performed as described previously [14]. The obtained plasmid clones were sequenced.

The knockdown efficiency was assessed by flow cytofluorometry. PC-3 cells with integrin β4 protein (ITGB4) expression not exceeding the baseline 3% were used.

Immunodeficient male $Pfp^{-}/Rag2^{-}$ mice [15] aging 8-12 weeks and weighing 20-25 g were used as xenograft recipients. *ITGB4*-knockdown and control PC-3 cells resuspended in nutrient medium (~10^6 cells in 200 μl) were injected subcutaneously between the scapulae to mice narcotized with O_2/CO_2 (10 animals for each variant of cells). After the tumor attained 2 cm^3 or skin lesion appeared, the mice were anesthetized and sacrificed by cervical dislocation. Primary tumors were obtained for isolation of total RNA.

Xenograft specimens were frozen in liquid nitrogen and homogenized in a porcelain mortar with a porcelain pistil [10]. Then, 500 μl QIAzol Lysis Reagent (Qiagen) was added and the samples were repeatedly homogenized using QIAshredder columns (Qiagen). Cell line samples were lysed in 500 μl QIAzol Lysis Reagent. RNA was isolated using miRNeasy Mini Kit [8] and its concentration was measured on a NanoDrop 1000 spectrophotometer (Thermo Scientific). The quality of isolated RNA was evaluated using Experion RNA StdSens Analysis Kit on Experion station (Bio-Rad). Only samples with RQI (RNA Quality Indicator) ≥7 were used for further analysis.

Gene expression was analyzed using GeneChip Human Transcriptome Array 2.0 (Affymetrix) according to manufacturer's instructions. The analyzed microarray data were preprocessed and then expression of samples included in microarray was measured. Preprocessing and expression evaluation were performed using Affymetrix Expression Console.

The data were analyzed using limma package in Bioconductor project. The analysis was applied to logarithms of expression, modified Student's test (moderated *t* statistics) was used for statistical processing of the results.

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