Transfection of murine melanoma cell lines (B16F1 and B16F10) with Biontex K2® Transfection System

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Materials and Methods

Cell lines and plasmid

Two subtypes of murine melanoma cell line; B16F1 and B16F10 (American Type Culture Collection, Manassas, VA) were cultured in advanced minimum essential medium (AMEM, Gibco, Life Technologies, Grand Island, NY) supplemented with 5 % FBS (Life Technologies), 10 mM/l L-glutamine (Life Technologies), 100 U/ml penicillin (Grünenthal, Aachen, DE) and 50 mg/ml gentamicin (Krka, Novo mesto, Slovenia) in a 5 % CO_2 humidified incubator at 37°C.

Plasmid CMV-EGFP-N1 (pEGFP, BD Biosciences Clontech, Palo Alto, CA), encoding enhanced green fluorescent protein under the control of the CMV promoter, was used in the study. The concentration of the plasmid was 1 mg/ml.

Transfection with K2 transfection system

One day before transfection 8 x 10^4 B16F1 and B16F10 cells were plated on 24-well plates (Corning Incorporated) in 1 ml AMEM media with 5% FBS. Next day 9.5 µL of K2® Multiplier were added into each well and incubated for 2 h at 37 °C in a 5 % CO_2. In the meantime, solution A, containing 28.5 µL serum free medium and 0.5 µg DNA, and solution B, containing 28.5 µL serum free medium and 2.28 µL K2® Transfection Reagent, were prepared. Solution A was then added to solution B, mixed gently by pipetting and incubated at room temperature for 15 – 20 min. Immediately after 15 – 20 min incubation the mixture was added to appropriate wells and cells were incubated overnight.

Transfection efficiency

Transfection efficiency was determined 24 h after transfection by flow cytometry. Samples were trypsinized and resuspended in 400 µl of phosphate buffered for flow cytometry analysis. The measurements were performed with FACSCanto II flow cytometer (BD Biosciences, San Jose, CA), where a 488-nm laser (air-cooled, 20 mW solid state) and 530/30-nm band-pass filter were used for the excitation and detection of GFP fluorescence, respectively. Cells were gated and histogram of gated cells against their fluorescence intensity was recorded. The number of fluorescent cells and their median fluorescence intensity were determined (software: BD FACSDiva V6.1.2).

Bright-field and fluorescent (exposure 400 ms) images of the cells were taken before preparation of the samples for flow cytometry for better visualization of the cells. Three different observation fields at x 100 objective magnification with Olympus IX-70 (Hamburg, Germany) and appropriate filters (excitation: 460-490 nm, emission: 505 nm).

Results

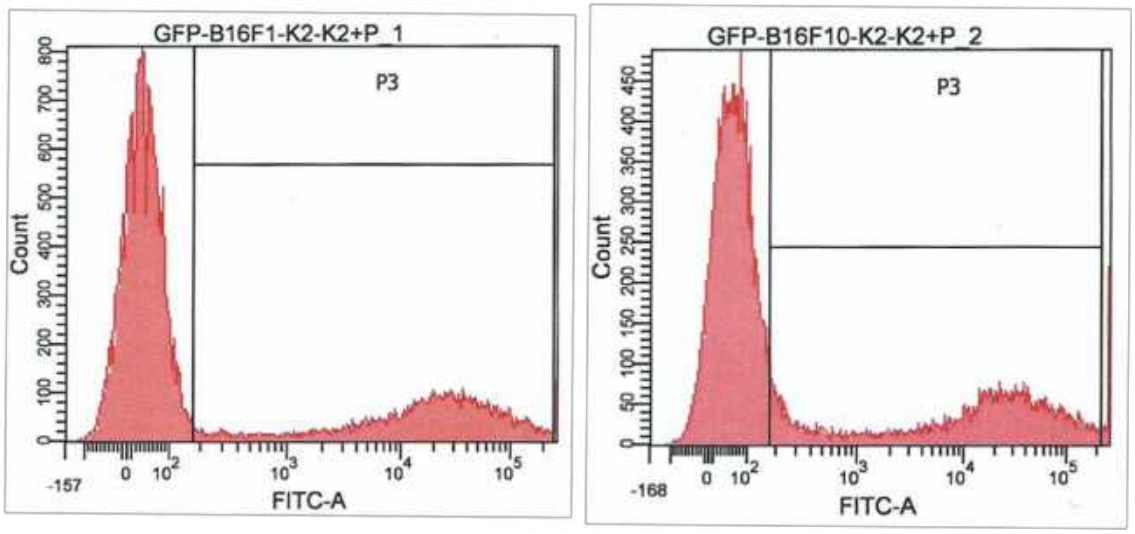

Figure 1 Representative flow cytometry graphs of B16F1 (left) and B16F10 (right) cells 24 h after transfection with Biontex K2® Transfection System.

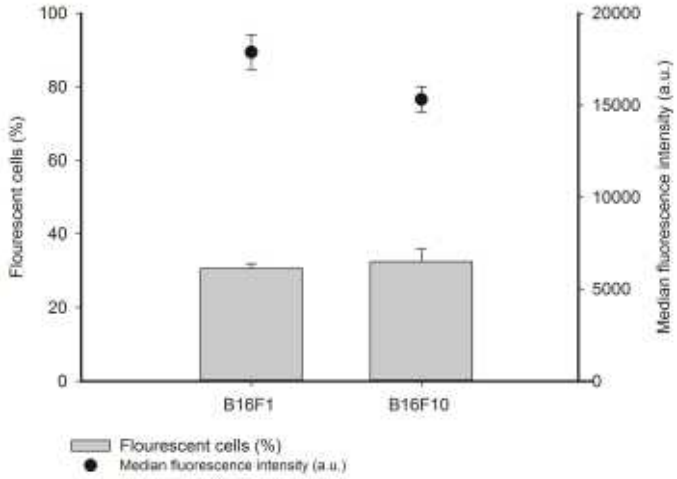

Figure 2 Percentage of fluorescent cells and their median fluorescent intensity 24 h after transfection with Biontex K2® Transfection System.

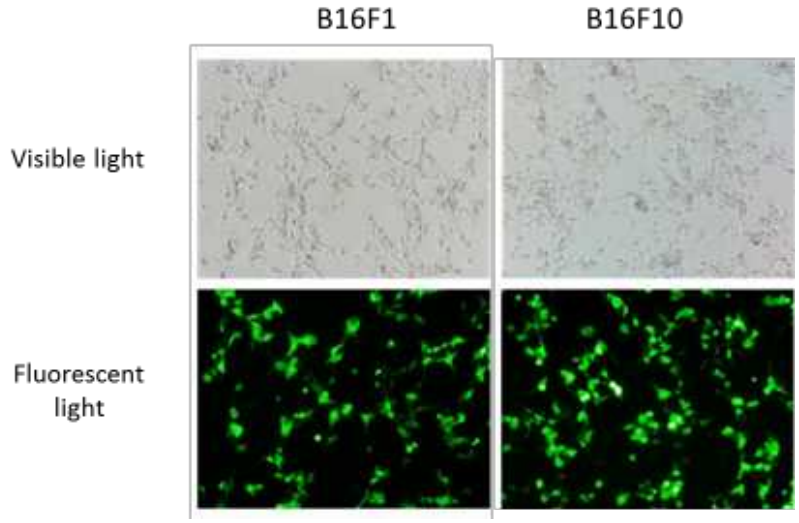

Figure 3 Bright-field and fluorescent images of melanoma cell lines.

Conclusion

Biontex K2® Transfection System is efficient transfection system for DNA transfer into murine melanoma cell lines. Transfection efficiency is similar in both subtypes of B16 melanoma cell line; B16F1 with low metastatic potential and B16F10 with high metastatic potential. Already very low amount of DNA results in high transfection efficiency 24 h after transfection.