DNA transfection of the quail cell line QT6 using the BIONTEX K2® transfection system

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Materials & Methods

The quail cell line QT6 which is chemically transformed by methylcholanthrene [1] was cultivated on 100-mm dishes in a medium containing 1x HAM's F10 with glutamine, 0.295% (w/v) tryptose phosphate broth, 5% (v/v) calf serum, 2% (v/v) chicken serum, 1.875% (w/v) $NaHCO_3$, 0.5% (v/v) DMSO, and 1x antibiotics/antimycotics at 37°C, 5% CO_2 in a humidified atmosphere. Prior to transfection, cells were seeded onto MP-12 dishes (3 x 10^5 cells/well). The next day, the medium was replaced by culture medium (1 ml/well) containing 1% (v/v) K2® Multiplier (10 µl), and then cells were incubated for 2 h. Each 6 µg DNA (1 µg of luciferase reporter plasmid, 5 µg of expression plasmids) diluted to 0.25 µg/µl (24 µl) were mixed with 156 µl culture medium without serum and 20 µl of K2® Transfection Reagent (final concentration 10%), and incubated at room temperature for 15 min. Then, the transfection solution was dropwise added in triplicate to the cells (each 66 µl, corresponding to 2 µg DNA/well), and cells were incubated for 24 h. Preparation of cell extracts, fluorescence microscopy, and luciferase assays were done as described previously [2].

Results

Ectopic expression of the v-Myc oncoprotein leads to efficient transcriptional activation of the *WS5* promoter (Fig. 1) which contains canonical Myc binding sites. The *WS5* gene is highly expressed in cells transformed by the *myc* oncogene and represents a direct transcriptional target of Myc [3]. The *WS5* promoter activation is inhibited by the Myc suppressor BASP1 as reported previously [2]. The amino-terminal 11-amino acid effector domain of BASP1 fused to the green fluorescent protein (GFP), which is sufficient in inhibit Myc-mediated cell transformation [2], also interferes with transcriptional Myc activity as shown here (Fig. 1).

Comment

The estimated transfection efficiency for QT6 cells using the BIONTEX system is > 60%. Using the applied concentrations, no toxic effect was observed. Besides QT6, also normal quail embryo fibroblasts (QEF), and the chicken cell line DF-1 [4] were transfected with high efficiencies. Compared to calcium phosphate-mediated gene transfer, or to other commercially available lipofection-based systems, the BIONTEX system yielded the highest transfection efficiencies. Only the electroporation-

based protocol termed nucleofection (LONZA/AMAXA) resulted into a higher transfection rate of avian fibroblasts but in this case the cytotoxicity was considerably higher. Therefore, considering cell viability and efficient transfection, the BIONTEX system currently represents the most efficient tool for gene transfer into chicken or quail embryo fibroblasts (CEF, QEF), or into cell lines derived from these primary cells. Further advantages are high reproducibility, easy execution, and the low amount of time spent to carry out this transfection protocol.

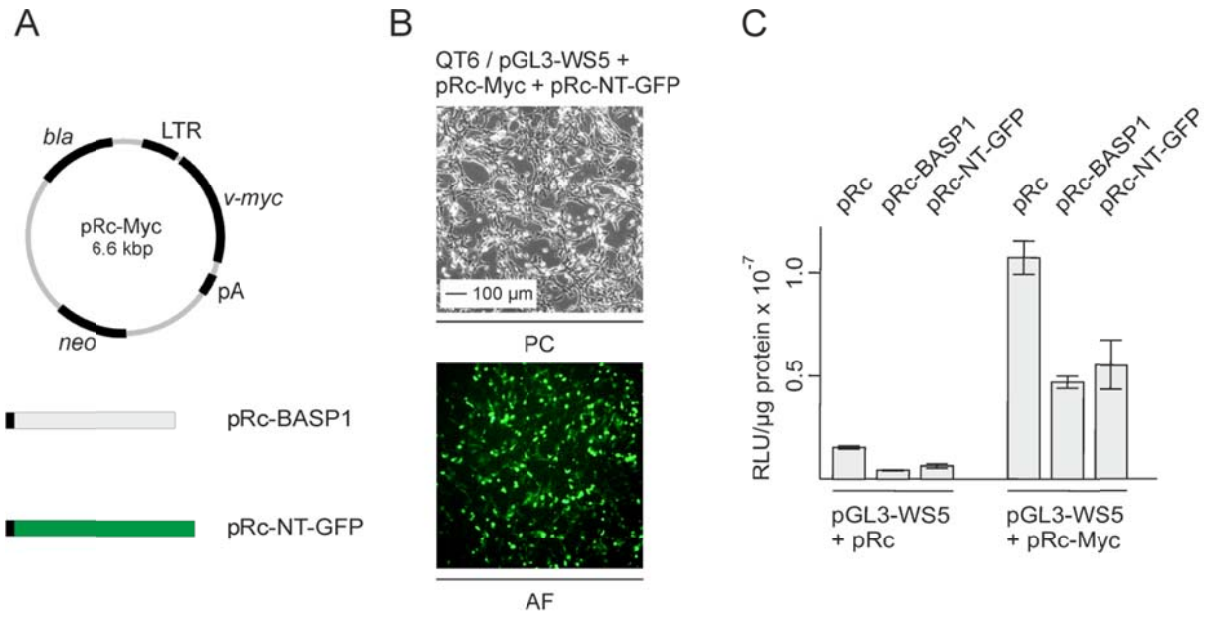

Fig. 1. Inhibition of Myc-mediated transcriptional *WS5* activation by BASP1. (A) Structure of the expression vectors pRc-Myc, pRc-BASP1, and pRc-NT-GFP encoding the v-Myc oncoprotein, the brain acid-soluble protein 1 (BASP1), and a fusion protein consisting the the 11-amino acid BASP1 effector domain (NT) and the enhanced green fluorescent protein (GFP). (B) Micrographs of the chemically transformed quail cell line QT6 co-transfected with the constructs depicted under *A* and a luciferase reporter plasmid containing the *WS5* promoter (pGL3-WS5), using the K2 Biontex system (PC, phase contrast; AF, autofluorescence). (C) Luciferase activities measured 24 h after transfection from cell extracts (RLU, relative light units).

References

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