Analyzing cytokine-induced growth arrest

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Method Article

Keywords: growth arrest, senescence markers, cytokines

Posted Date: February 11th, 2013

DOI: https://doi.org/10.1038/protex.2013.020

Abstract

Here, we present the details of the experimental procedure used to analyze cytokine-induced growth arrest of different cancer cells in vitro.

Introduction

The most stringent criterion defining cellular senescence is stable and permanent growth arrest in the absence of the inducing agent. Thus, we developed a method to first treat the cells with defined combinations of cytokines, then remove the cytokines and further cultivate the cells in the absence of cytokines.

Reagents

Human cancer cell lines from the NCI 60 panel. Human IFN-gamma \(R&D Systems, Wiesbaden, Germany). Human TNF \(R&D Systems). Trypan Blue Stain 0.4% from Gibco \(Life Technologies; Darmstadt, Germany). MultiscreenTM HTS 96 well Filtration Plates \(Millipore, Billerica, MA, USA). BrdU-based Cell Proliferation ELISA \(Roche Diagnostics, Mannheim, Germany). Vector®SG Substrate Kit for Peroxidase from Vector Laboratories \(Burlingame, CA, USA).

Equipment

Zeiss Axiovert 25 microscope \(Zeiss, Oberkochen, Germany). Neubauer counting-chamber \(Karl Hecht GmbH, Sondheim, Germany). ELISPOT reader \(Bioreader®-3000; BIO-SYS, Karben, Germany).

Procedure

1. Seed the different cancer cells at a density of 10 000 cells/cm2. 2. Incubate the cells overnight at 37°C; 5% CO2 to ensure adherence. 3. Treat the cells with either control medium or the cytokines mentioned above \(standard concentration 100 ng/ml IFN-gamma plus 10 ng/ml TNF) for 4 - 5 d. 4. After treatment, remove the medium and trypsinize the cells. 5. Count viable cells \(use trypan blue exclusion) under a Zeiss Axiovert 25 microscope using a Neubauer counting-chamber. 6. Reseed the cells at 20000 cells/cm2 in the absence of cytokines. 7. Grow the cells in complete RPMI 1640 medium until the control cultures reach confluence. 8. Repeat steps 4 - 6. 9. After passage 1 - 2 \(p1 - p2), seed 1000 - 3000 viable cells on MultiscreenTM HTS 96 well Filtration Plates. 10. Incubate the cells overnight at 37°C; 5% CO2 to ensure adherence. 11. Measure proliferation by the BrdU-based Cell Proliferation ELISA in combination with the Vector®SG Substrate Kit for Peroxidase to visualize BrdU-incorporating cell clusters. 12. Count BrdU-positive spots with an ELISPOT reader.

Timing

The overall procedure takes about 2 - 3 weeks.

Troubleshooting

1. Ensure that at the beginning of cytokine treatment, the cells are 5 - 25% confluent. 2. The duration of the treatment depends on the growth kinetics of the cancer cells \(incubate for approx. two duplication times). 3. The cytokine sensitivity of the different cancer cell lines may differ over a broad concentration range. 4. Toxic side effects of the cytokines have to be evaluated beforehand.

Anticipated Results

A cell line that is driven into senescence should show complete growth arrest after removal of the cytokines, i.e. there should not be any increase of the cell number of the cytokine-treated cells.

References

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