Immunostaining of DDR proteins in senescent cells

Raffaella Di Micco
FIRC Institute of Molecular Oncology
Marzia Fumagalli
FIRC Institute of Molecular Oncology
Fabrizio d'Adda di Fagagna
FIRC Institute of Molecular Oncology

Method Article

Keywords: γH2AX, ATMpS1981, pS/TQ, SMC1pS996, immunofluorescence, DNA damage checkpoints, DNA damage response, senescence, oncogene-induced senescence

Posted Date: December 6th, 2006

DOI: https://doi.org/10.1038/nprot.2006.367

Abstract

Introduction

We present here a brief yet detailed methodology to study the activation of a number of DNA damage response factors in senescent human fibroblasts.

Reagents

poly-D-lysine \(SIGMA P6407 5 mg) Methanol/Aceton 1:1 PBG 10x stock solution: 5% BSA, 2% of gelatin from cold water fish skin \(SIGMA cat. G-7765) in PBS 1x Antibodies: γH2AX mouse Upstate Biotechnology \(cat.05-636) 1:200 ATMpS1981 rabbit Rockland \(cat. 600-401-400) 1:300 pS/TQ rabbit Cell Signaling Technology \(cat. #2851)1:100 SMC1pS996 rabbit Bethyl Laboratories \(cat. A300-050A) 1:500 DAPI \(SIGMA cat.D-9564) Mowiol mounting medium \(CALBIOCHEM cat. 475904)

Procedure

1) Prepare poly-D-lysinated coverslips \(poly D lysine 50µg/ml) 2) Plate 15-20 x 10^3 cells on coverslips 1 day before the staining 3) Wash cells gently 2x5'in PBS1x 4) Fix and permeabilize cells for 2' with methanol/aceton 1:1 at RT 5) Block cells for 30' with PBG1x 6) Stain with primary antibodies diluted in PBG1x at indicated concentration for 1h at RT in a humidified chamber 7) Wash cells gently 3x5'in PBG1x 8) Stain with secondary antibodies diluted in PBG1x for 1h at RT in a dark humidified chamber 9) Wash cells gently 2x5' in PBG1x and 2x5'in PBS1x 10) Stain with Dapi for 5' in the dark 11) Mount coverslips with 3µl of Mowiol mounting medium 12) Let the coverslips dry before microscope analysis

Timing

4 h

Critical Steps

Use always a positive \(X-Rays treated cells 2-5 Gy) and a negative control \(proliferating or even better contact inhibited \(for a short period) quiescient cells) to reduce background

Troubleshooting

Using different type of fixative can allow better staining depending on antibody Lipofuscin accumulation in senescent cells can give an high citosolic autofluorescent background material

References

d'Adda di Fagagna _et al._ A DNA damage checkpoint response in telomere-initiated senescence _Nature_ **426**, 194-198 \(2003)

Figures

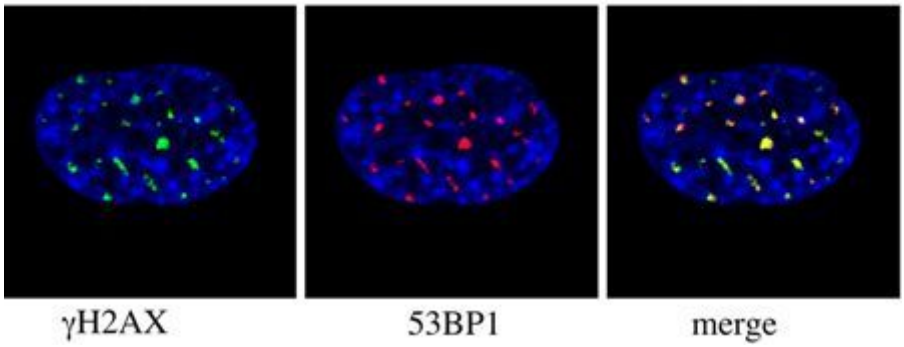

Figure 1

Confocal analysis of DDR proteins (γH2AX=green and 53BP1=red) in oncogene-induced senescent cells. In blue dapi staining.