Immunostaining of DDR proteins in senescent cells

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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

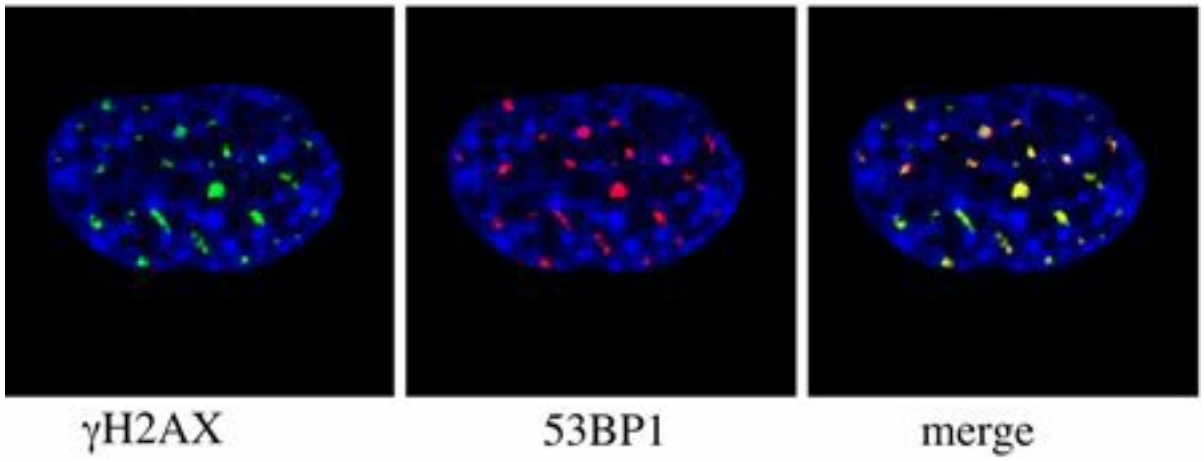

Figure 1

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

Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication

by Di Micco, R. et al.

Nature (03 October, 2006)