Genotoxic Stress and Cancer Stem Cells

T.VIROLLE

chemotherapeutic agents

radiotherapy

alcool

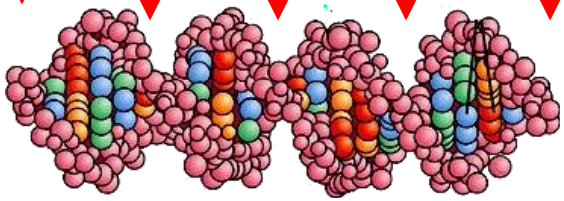
GENOTOXIC STRESS

cigarette smoke

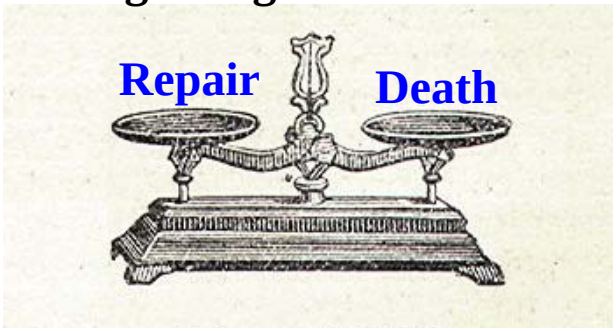
UV

pesticides

heavy metal



DNA damages & genomic Alterations



Anticancer treatment

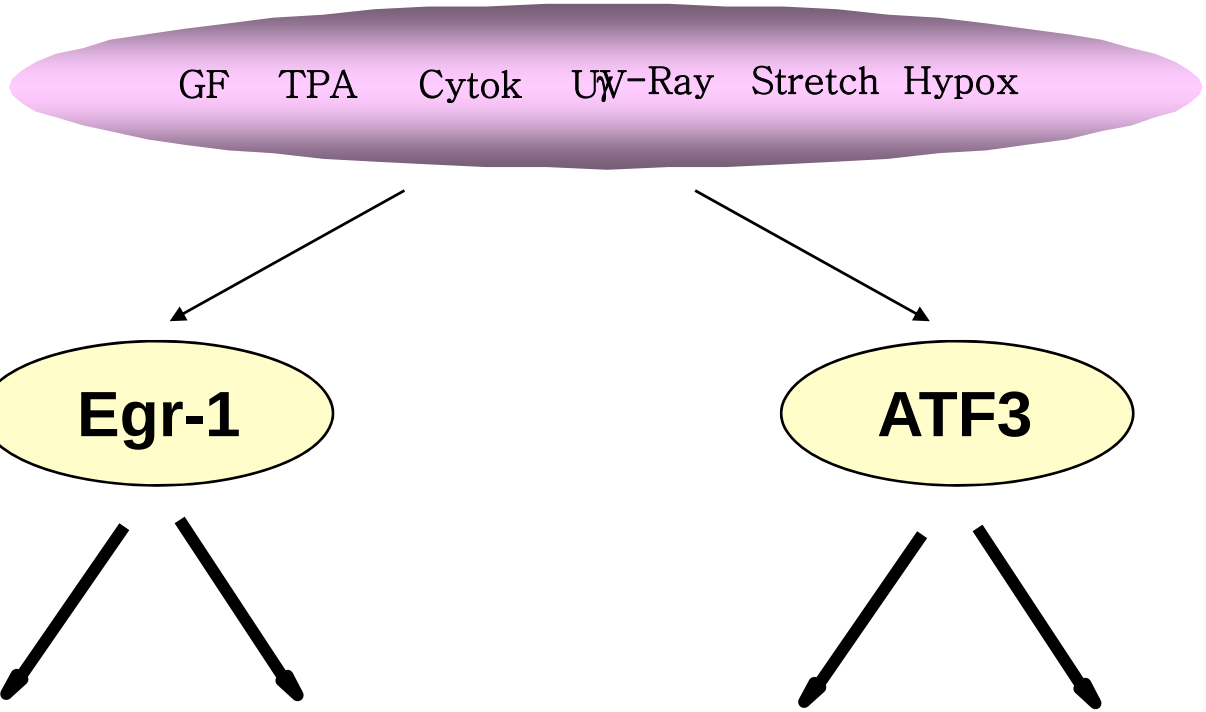
- Cancer Stem cells
  - Self renewal
  - Sensitivity to death

Oncogenesis / Tumor progression

- Egr1 and ATF3 regulation and function

PART 1

Egr1 & ATF3 regulation and function during the genotoxic stress response



Tumor suppressor :
Downregulated
Fibrosarcoma
Breast cancer cells
Huang RP et al. (1995) Cancer Res
Huang RP et al. (1997) Int J Cancer
Virolle et al. (2001) Nat Cell Biol.

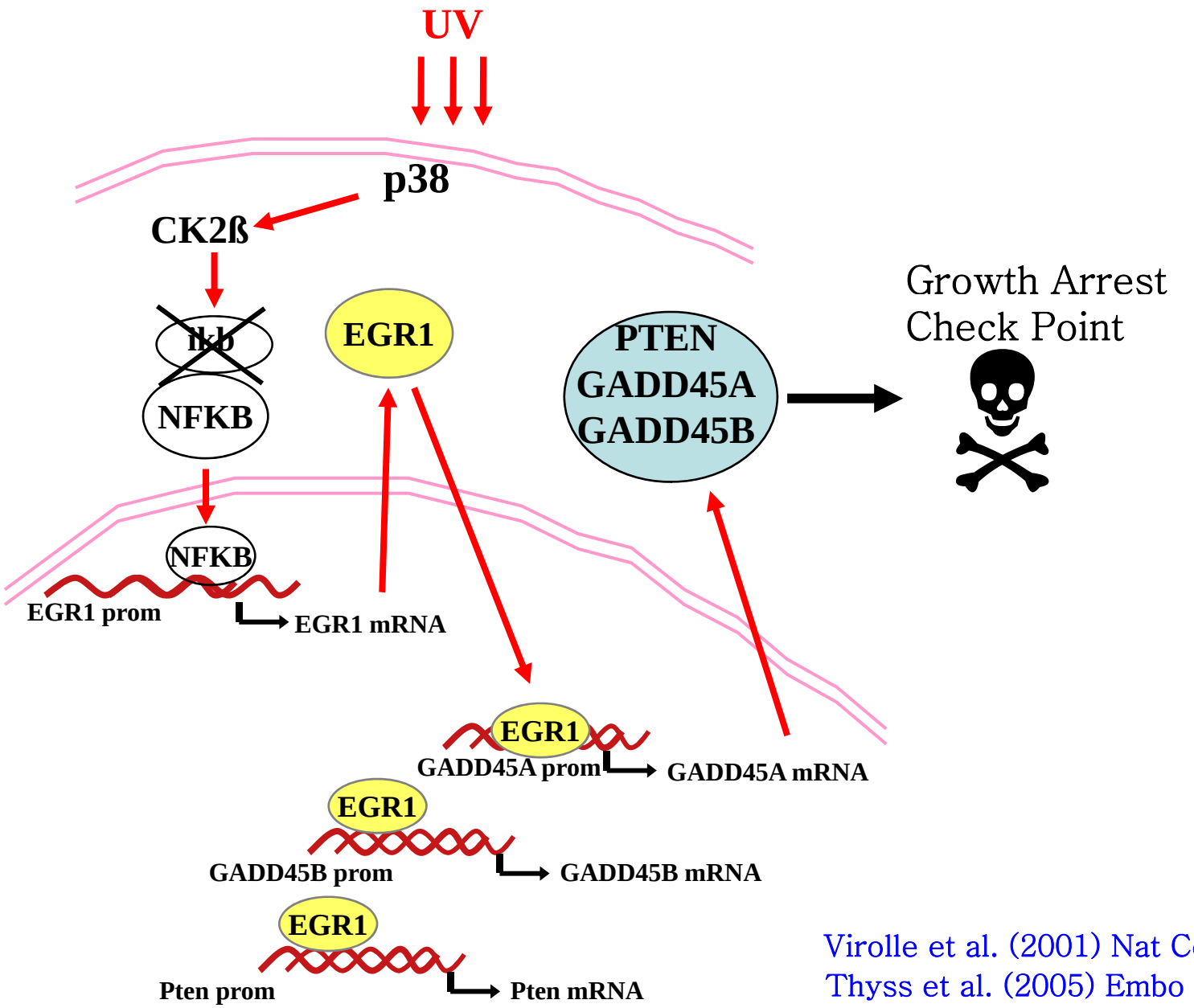
Oncogene :
Overexpressed
Prostate cancer cells
Virolle et al. (2003) JBC
Baron et al. (2003) Oncogene

Tumor suppressor :
Downregulated
Skin, lung, renal
Garber et al.(2001) PNAS
Higgins et al.(2003)
AM J Pathol

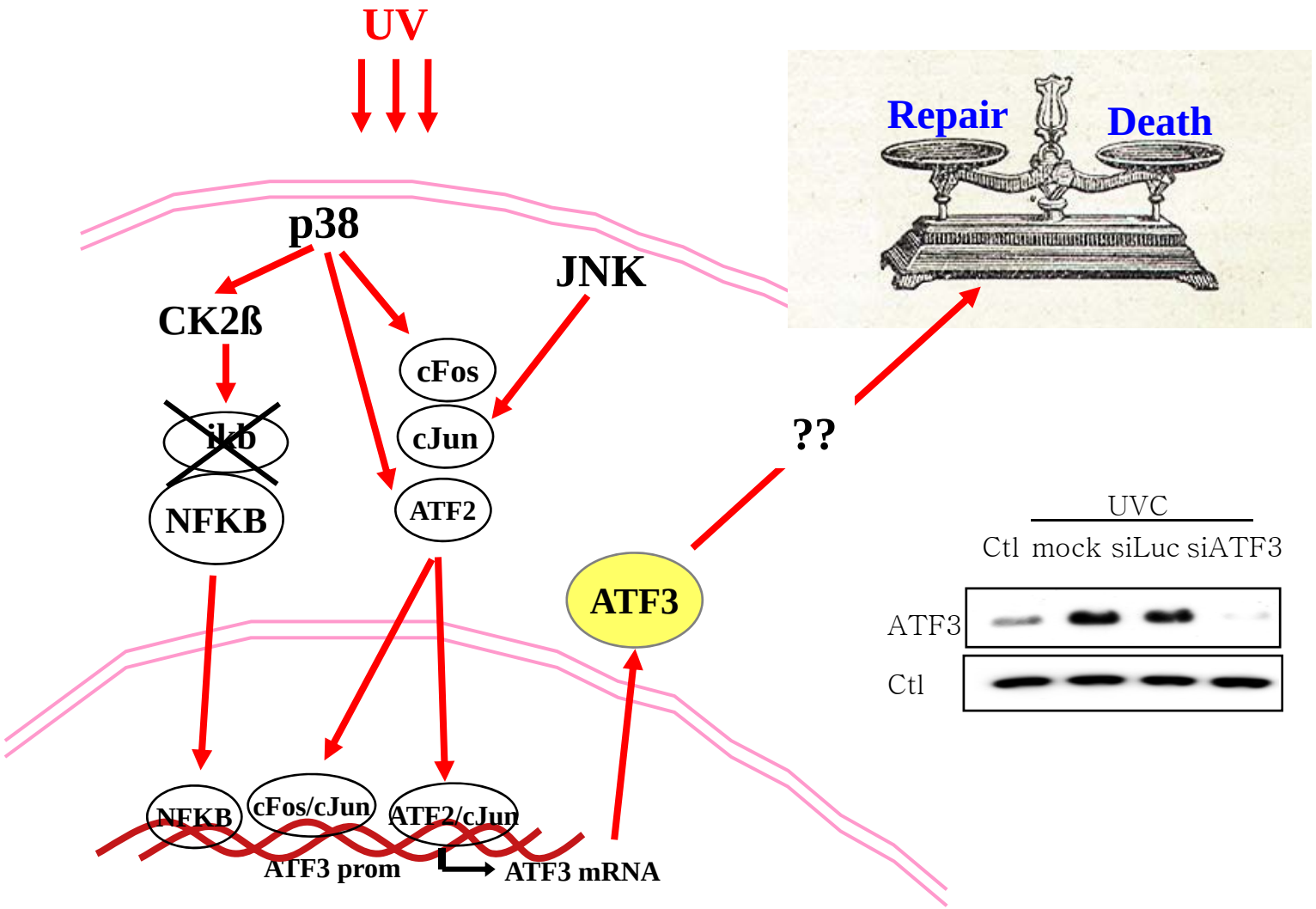
Oncogene :
Overexpressed
Human colon cancer
Ishiguro et al. (2000)
Jpn.J. Cancer Res

Regulation & function
during UV mediated stress

New Egr1 dependent death pathway

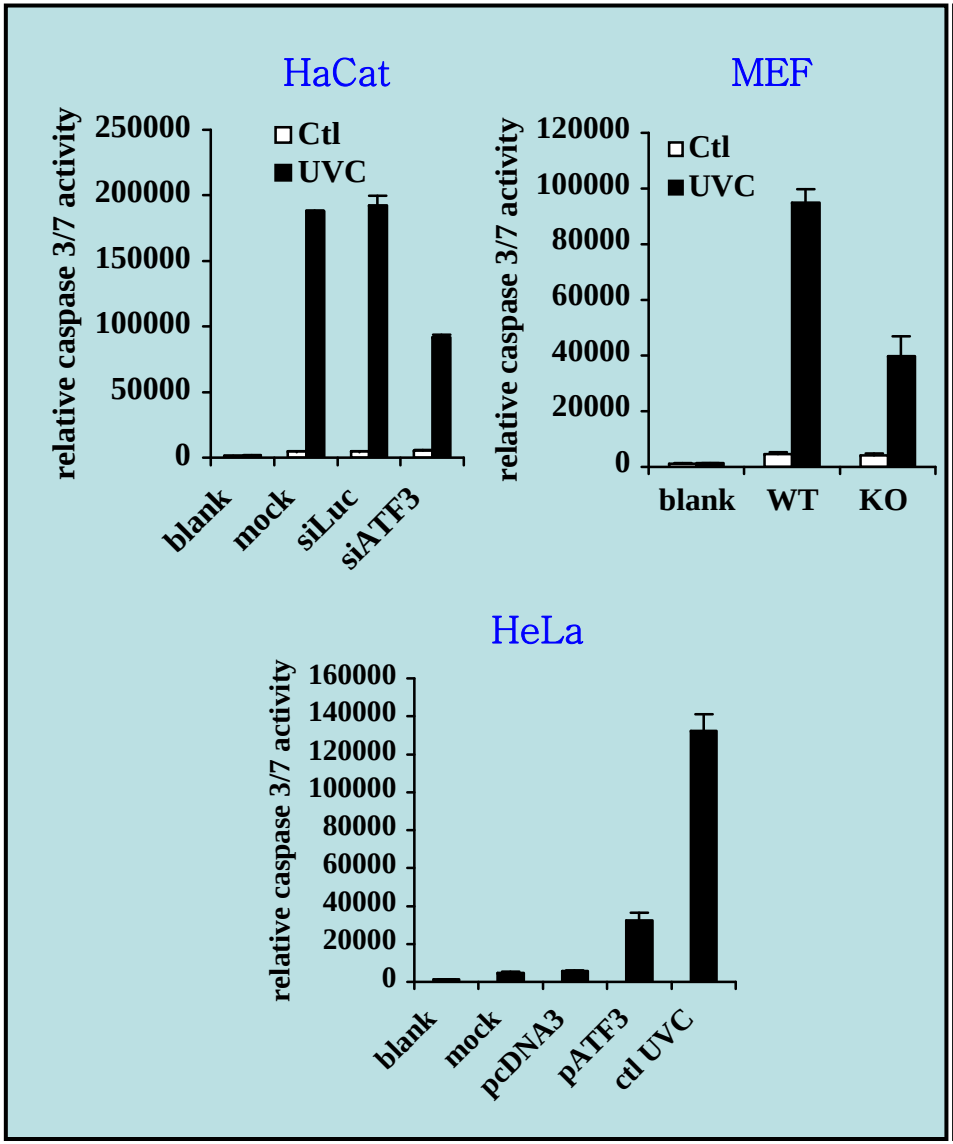


Virolle et al. (2001) Nat Cell Bi
Thyss et al. (2005) Embo J

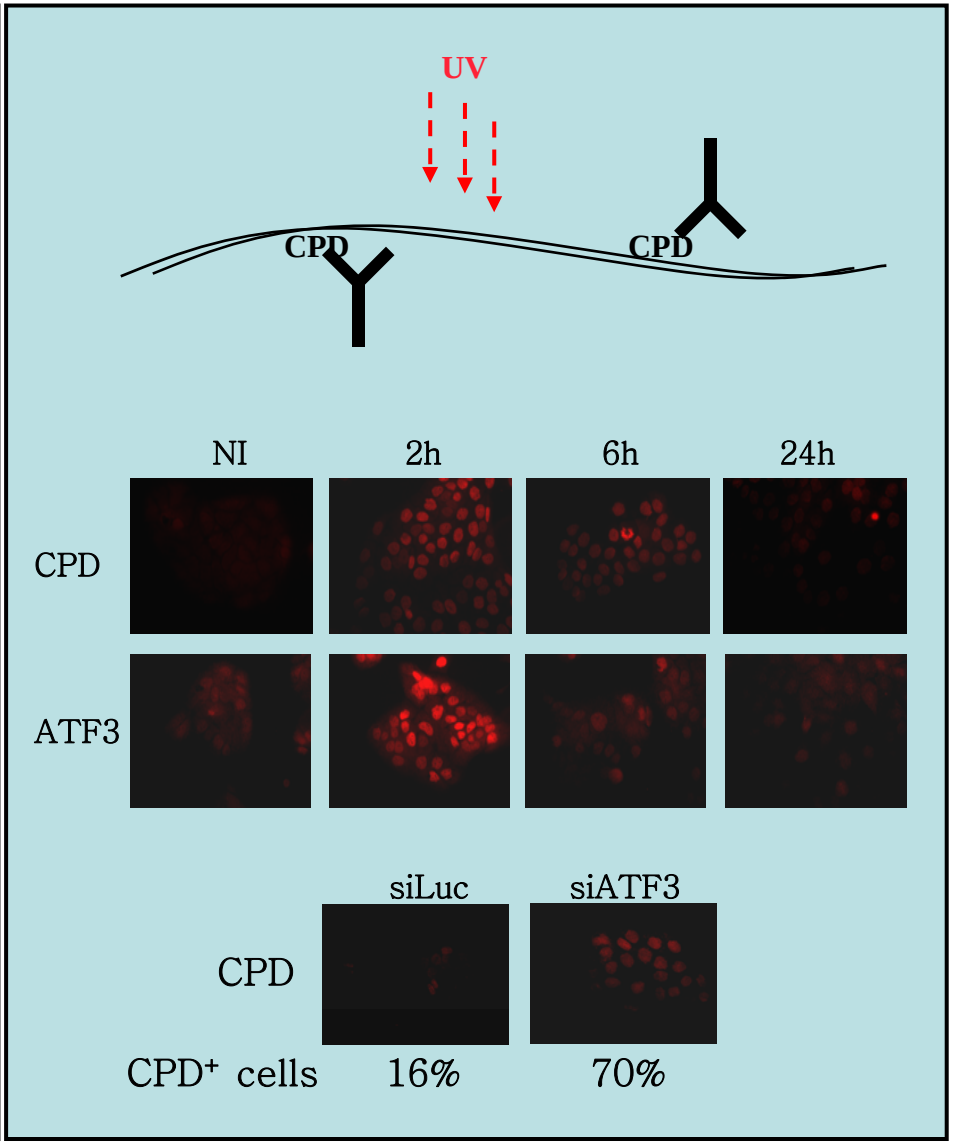

UV
p38
JNK
CK2ß
cFos
cJun
ATF2
IkB
NFKB
??
Repair
Death
ATF3
NFKB
cFos/cJun
ATF2/cJun
ATF3 prom
ATF3 mRNA
UVC
Ctl mock siLuc siATF3
ATF3
Ctl

ATF3 is involved both in death and DNA repair

cell death

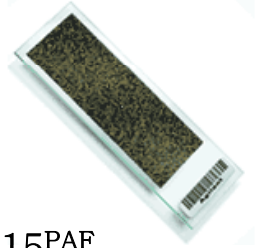


DNA repair

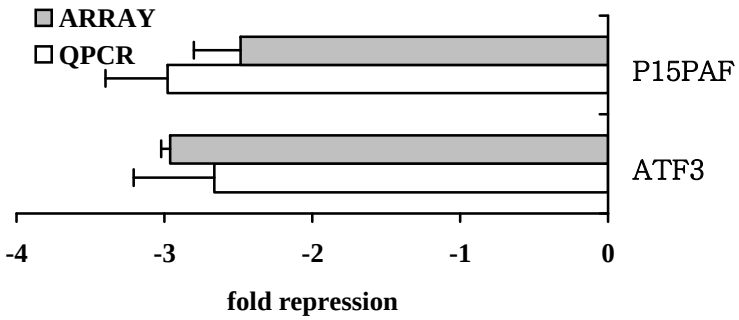


ATF3 target genes ?

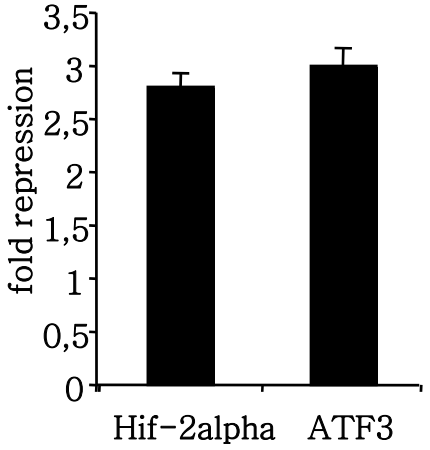
Microarray analysis (IPMC Sophia-Antipolis, FRANCE)

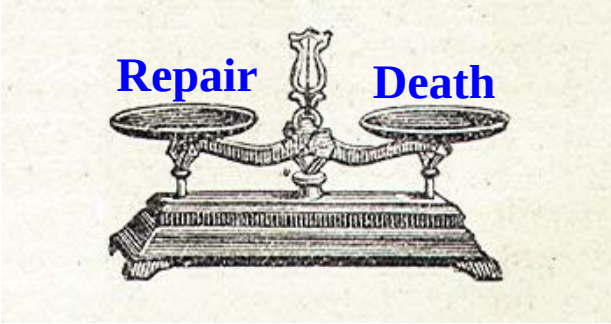


KIAA0101, PCNA associated factor p15^{PAF}

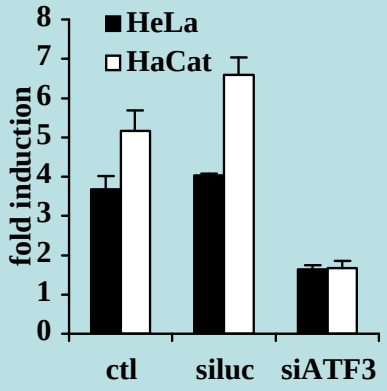


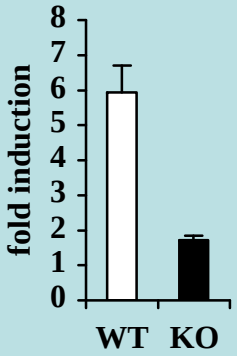
Hif2alpha

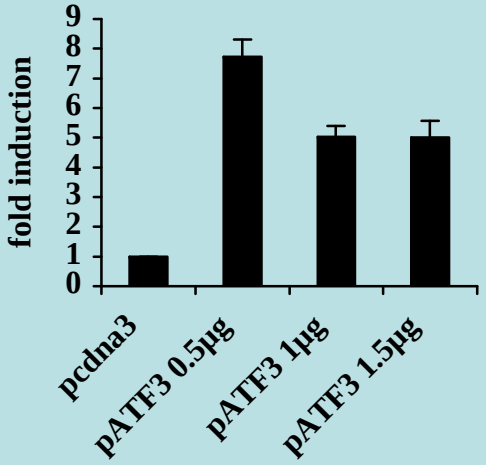


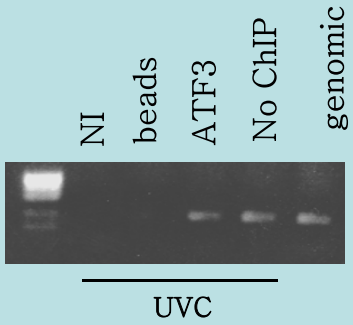


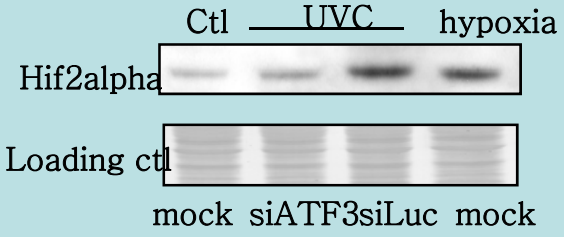
ATF3 regulates Hif2a expression



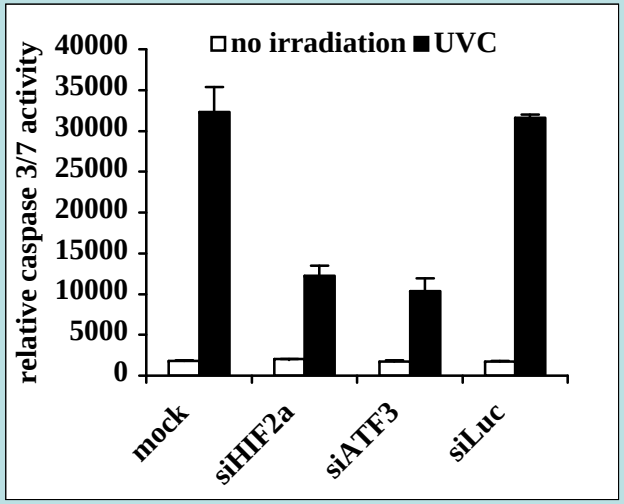


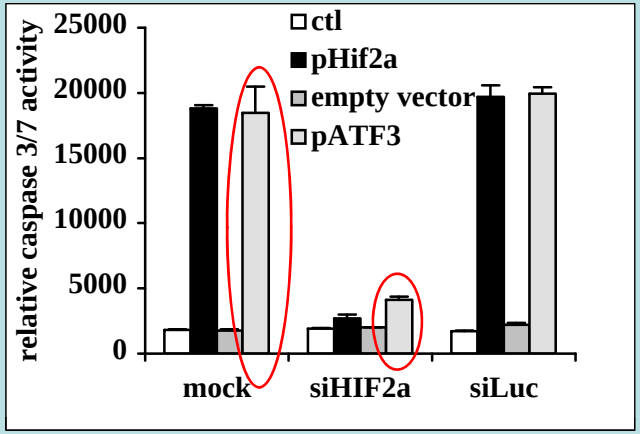






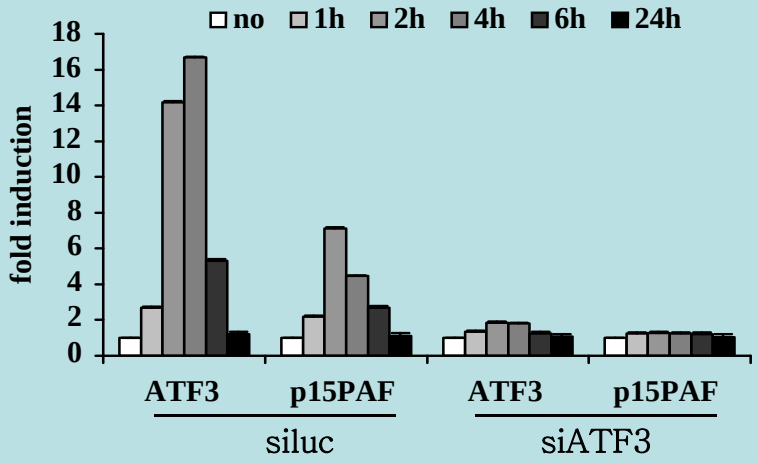
Hif-2a is the death effector of ATF3 upon UV irradiation

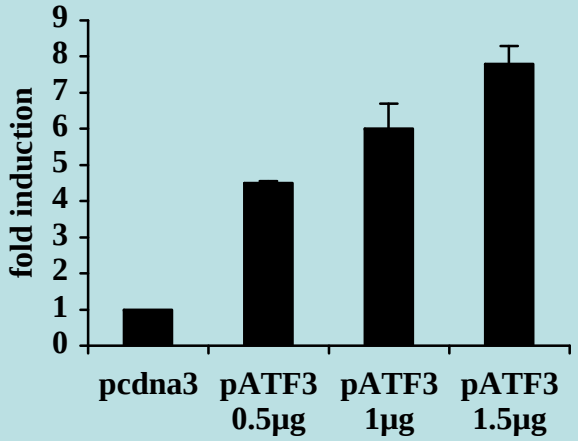


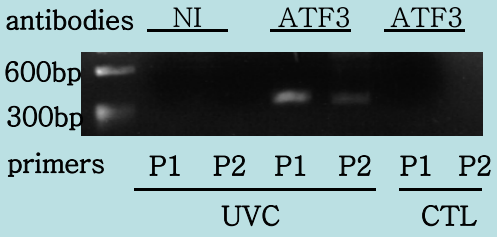


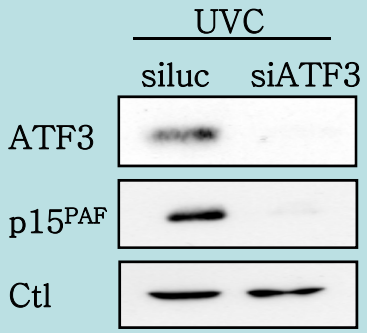
knock down	Hif2-alpha
EGR 1	-1.6
DAPK3	-1.8
GADD45	-1.9
FKHR2	-1.8
EGR 2	-1.9
Casp7	-2.5
TRAIL	**-4.5**
BNIP3	1

ATF3 regulates p15PAF expression

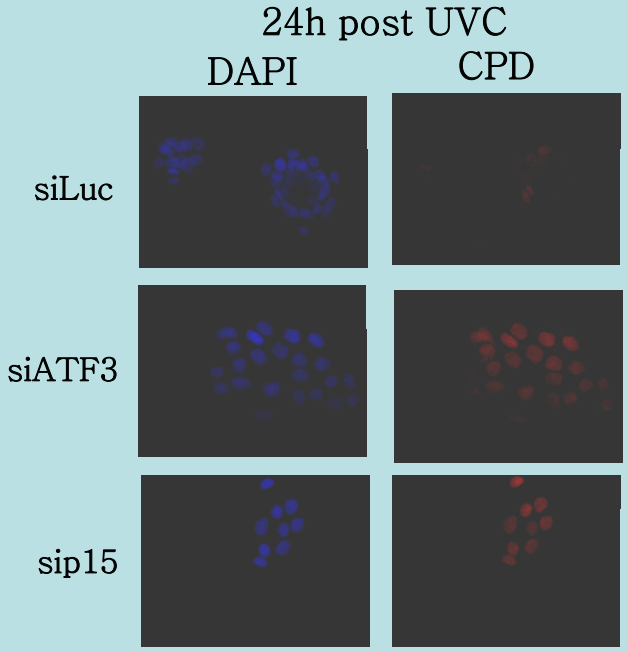




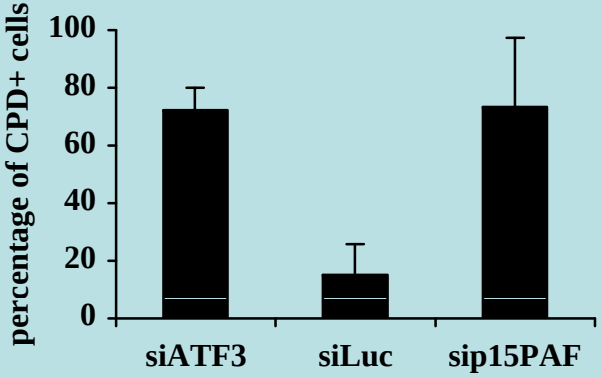




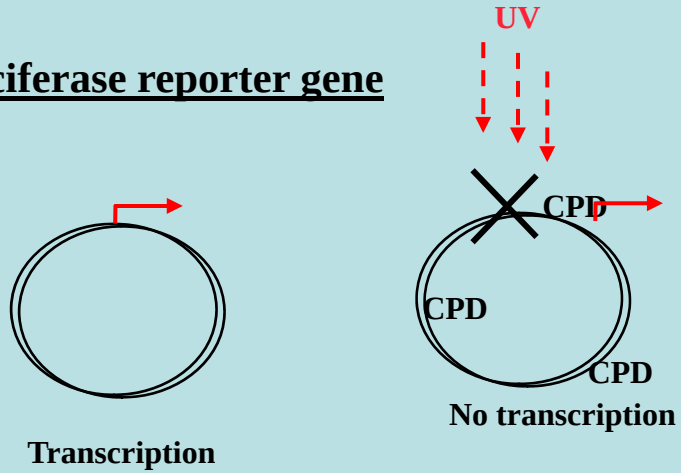
p15^{PAF} is a major effector of ATF3 in DNA repair

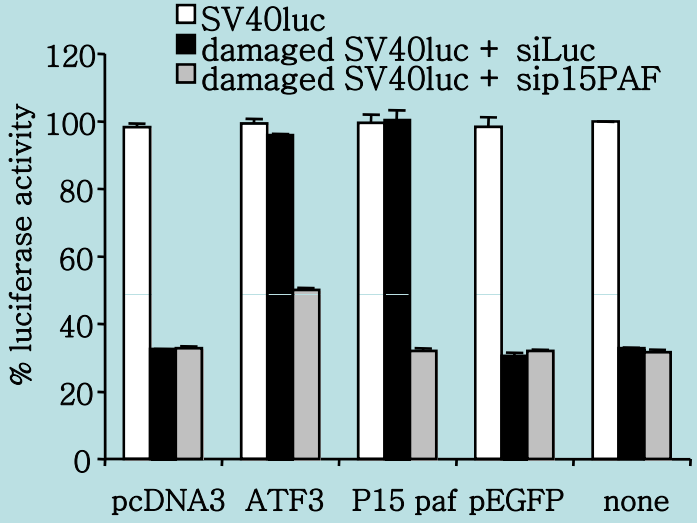


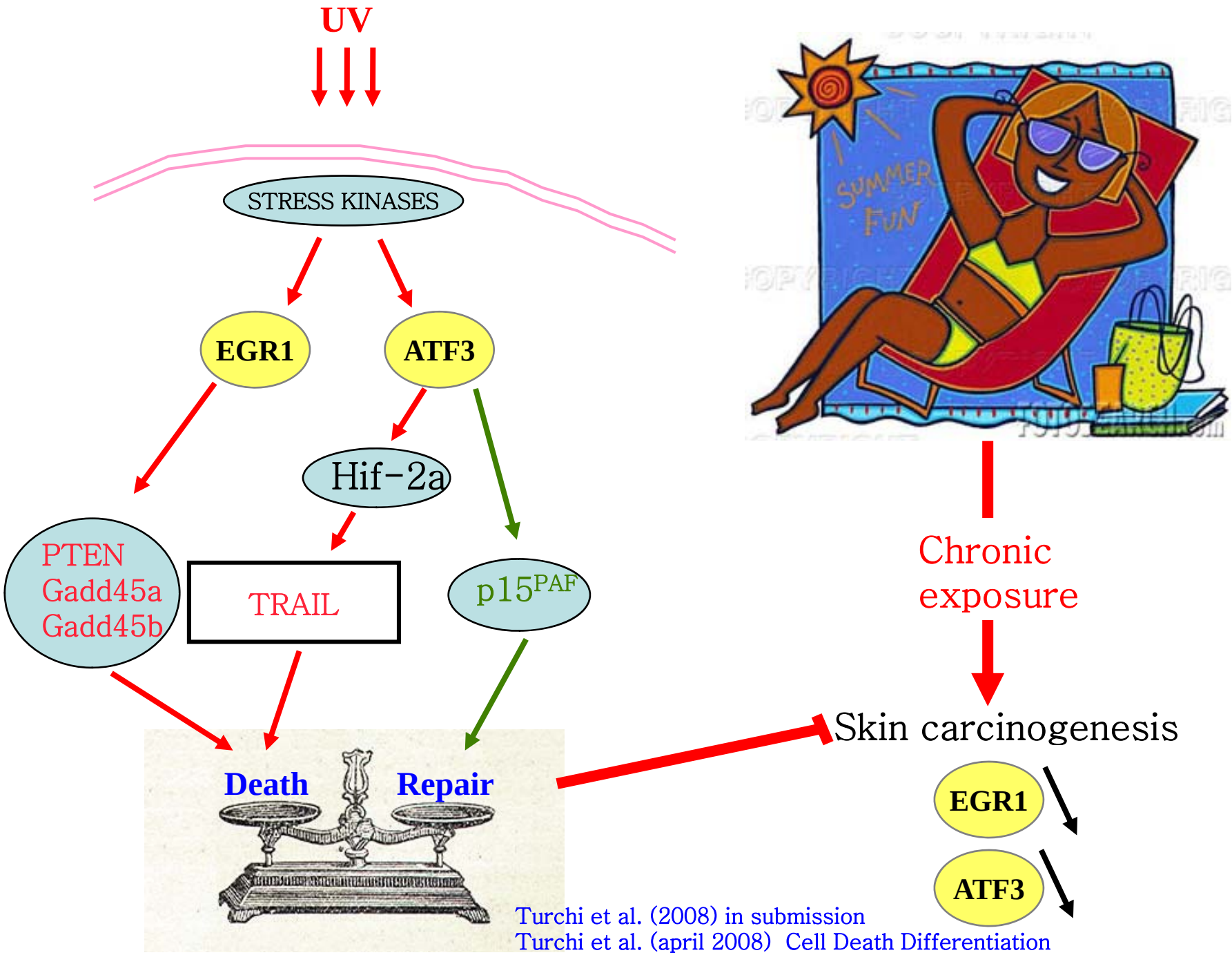
Quantification of CPD positive cells (24h after UVC)



Luciferase reporter gene







Turchi et al. (2008) in submission
Turchi et al. (april 2008) Cell Death Differentiation

Genotoxic Stress : T.Virolle

R. THYSS, Ph.D student

L. TURCHI, Ph.D

E. ABERDAM, Ph.D

Collaborations

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S. KITAJIMA	Kyoto (Japan)
C. WICKING	Melbourne
(Australia)	

Association pour la Recherche sur le Cancer

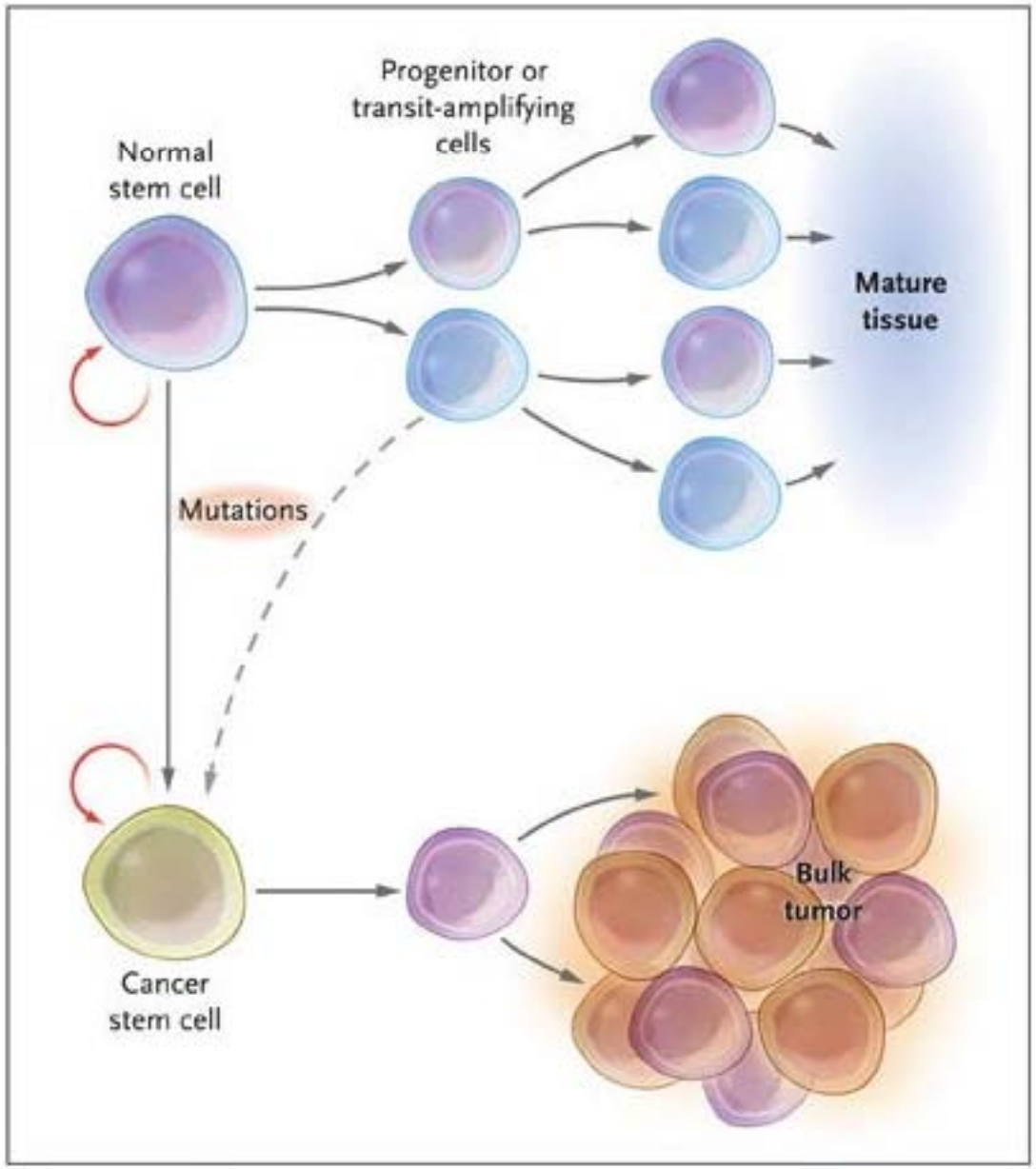
Inserm
Institut national
de la santé et de la recherche médicale

SRD

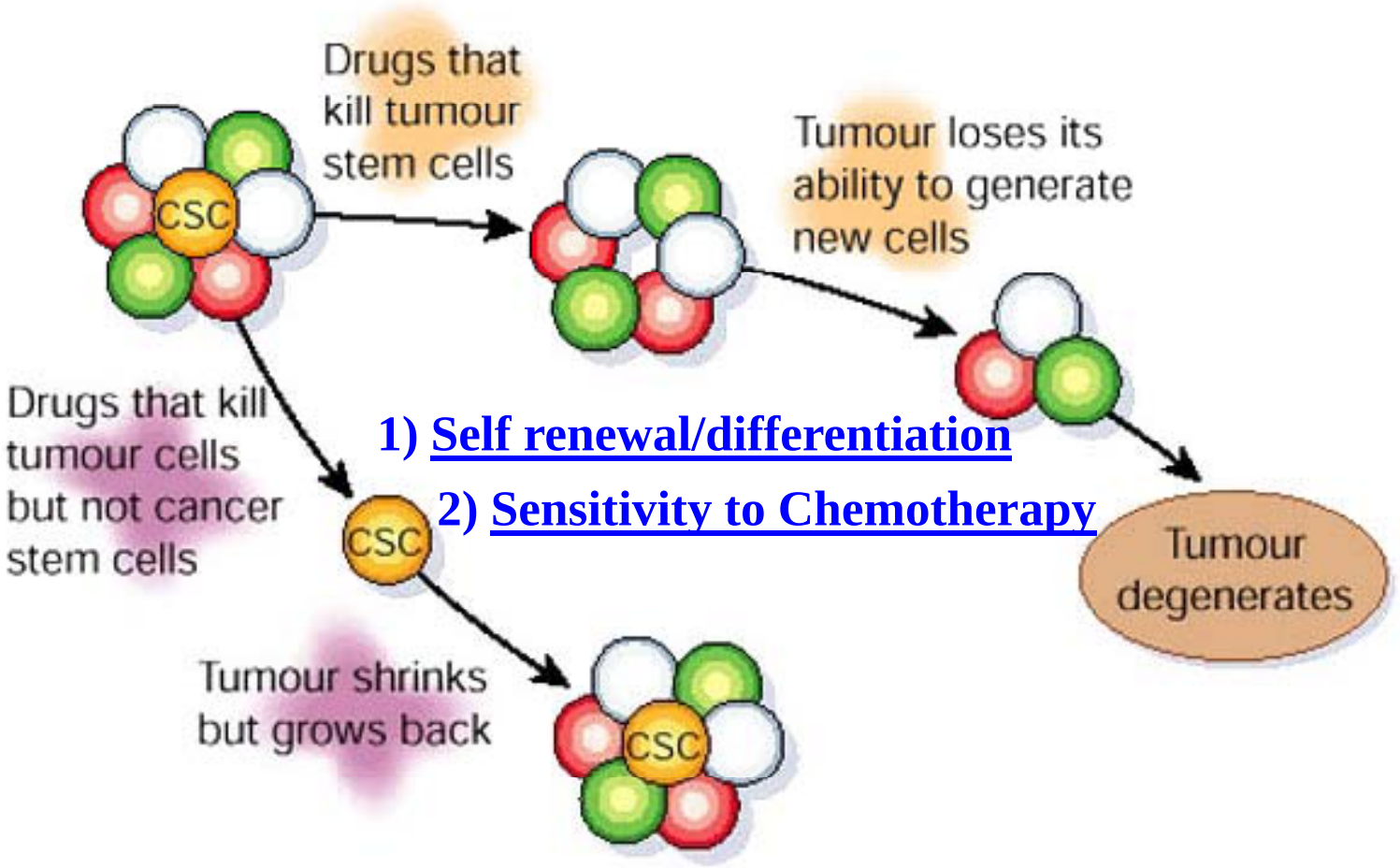
PART 2 :

- **Cancer Stem cells**
  - **Self-renewal/Differentiation**
  - **Sensitivity to Chemotherapy**

Stem cell & Cancer stem cell



Therapeutic approach of cancer



From Reya et al. Nature 2001; 414:105.

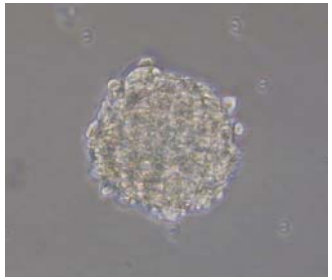
Malignant Glio-Neuronal Tumors (MGNT) & Cancer stem ce

An integrated team in the surgical room

Hervé Chneiweiss, MD PhD
Directeur de l'UMR-S 752 Inserm/Paris 5/CHSA Plasticité Gliale

Dissociated primary cultures

30 to 90 days
Defined medium



TG6

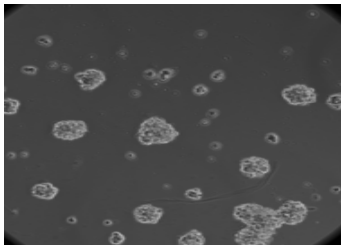
<u>Criteria evaluated</u> :

- Non-adherent sphere formation
- Proliferation (clonogenicity and proliferation rate)
- Self-renewal
- Only few cells (100) are sufficient to make tumor in the brain of nude m

Self renewal and differentiation

TG6 differentiation

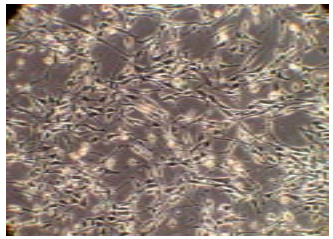
undifferentiated



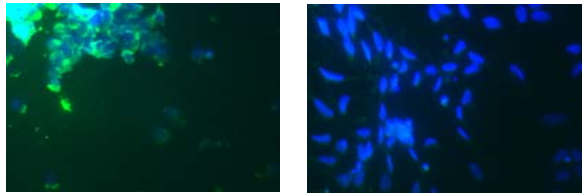
Serum 0.5%

within few hours

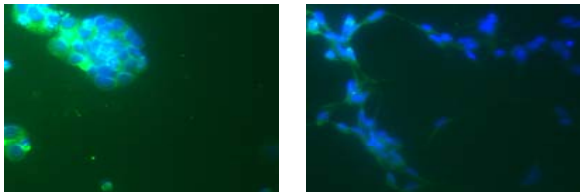
Differentiated
Glioneuronal cells



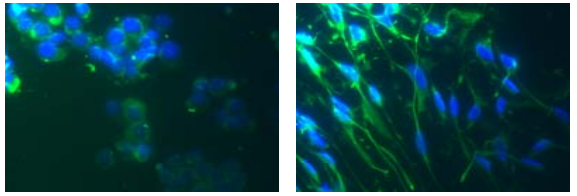
Sox1



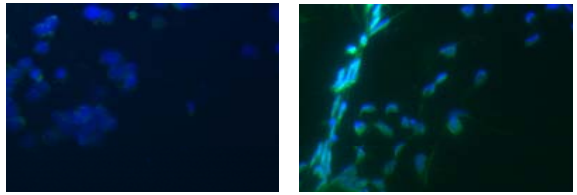
Shh

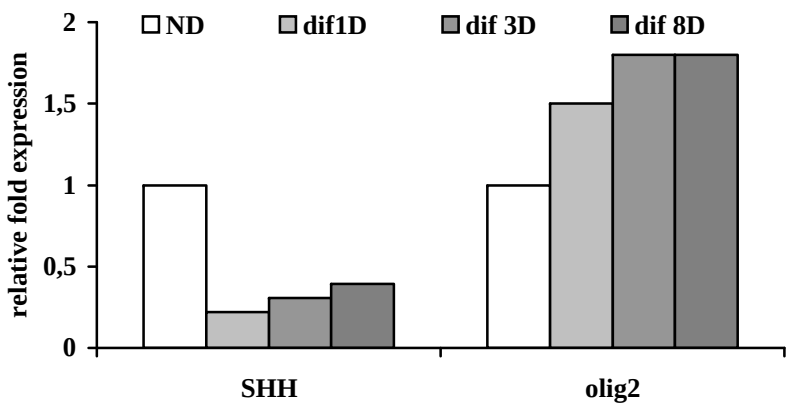


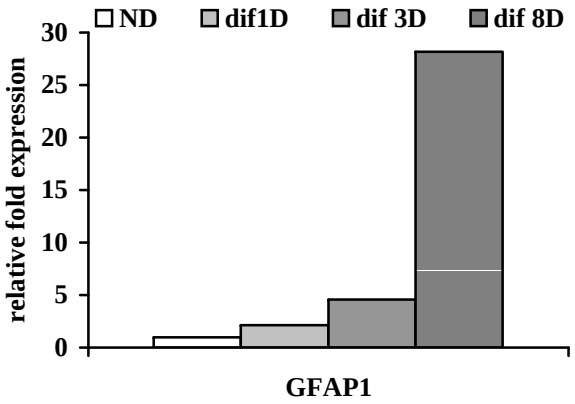
ß3-Tub



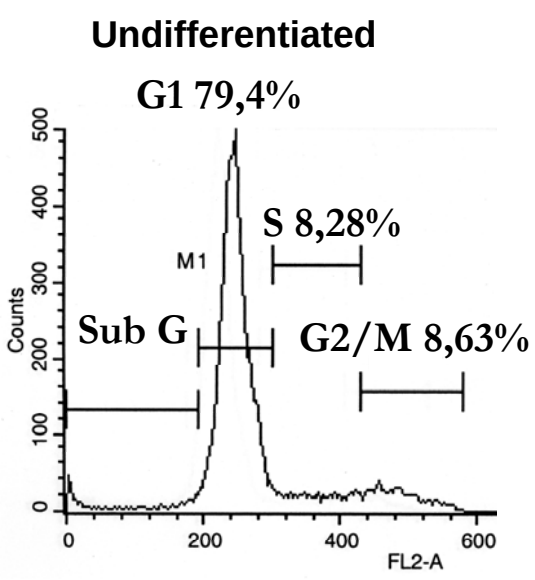
Olig2

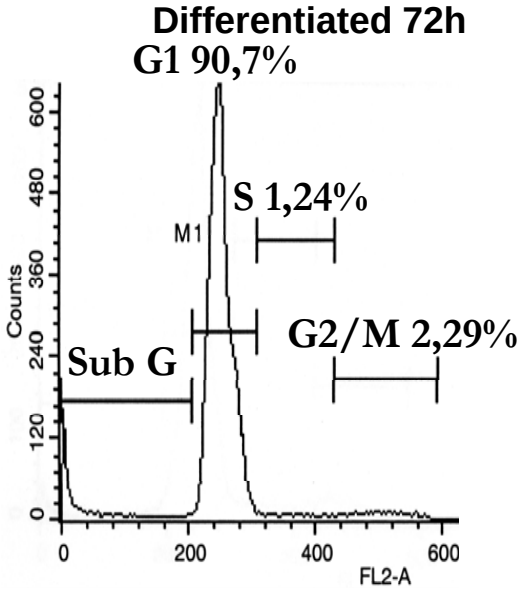


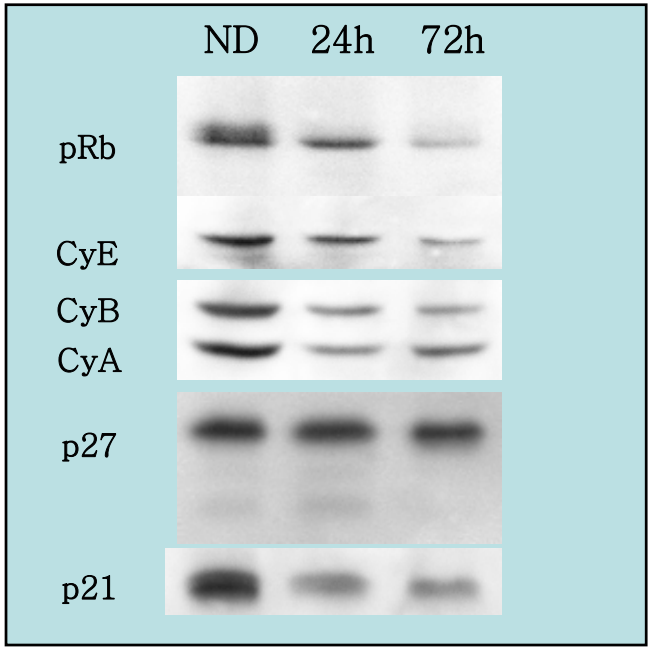




Differentiation & Cell Cycle







TG6 Differentiation

=

1- Adhesion and morphology change

2- Decreased expression of Sox1 and SHH

3- Increased expression of GFAP and Olig2

4- Cell cycle arrest

Molecular Basis of TG6 Differentiation

Transcriptome

microarray analysis

(RNG, IPMC Sophia-Antipolis)

Genes coding for protein, especially transcription factors

Mirnome

micro-RNA Taqman low density array (370miR)

(Applied Biosystems)

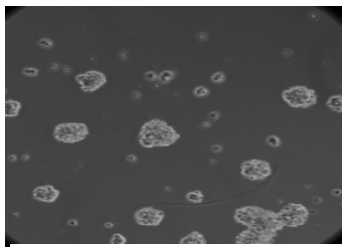
Involvement in stem cell:

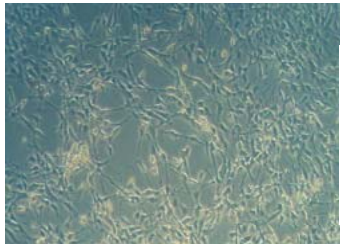
- dicer knock down prevents differentiation of murine ES cells

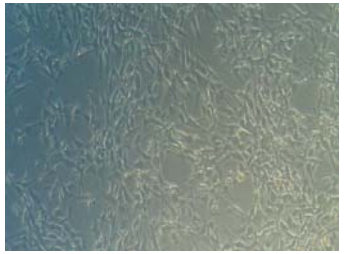
-specifically expressed in undifferentiated human ES cell (miR-371 and miR-302 clusters)

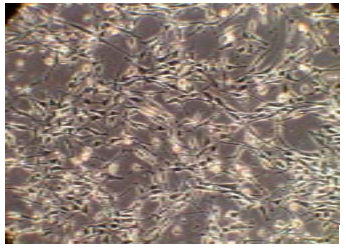
FUNCTIONAL STUDY

Molecular Basis of Differentiation


undifferentiated


24 hours


3 days


Differentiated 8 days

Transcriptome

Ratio < 3

SPOCK1, MGP,
gene not yet characterized

Ratio >3

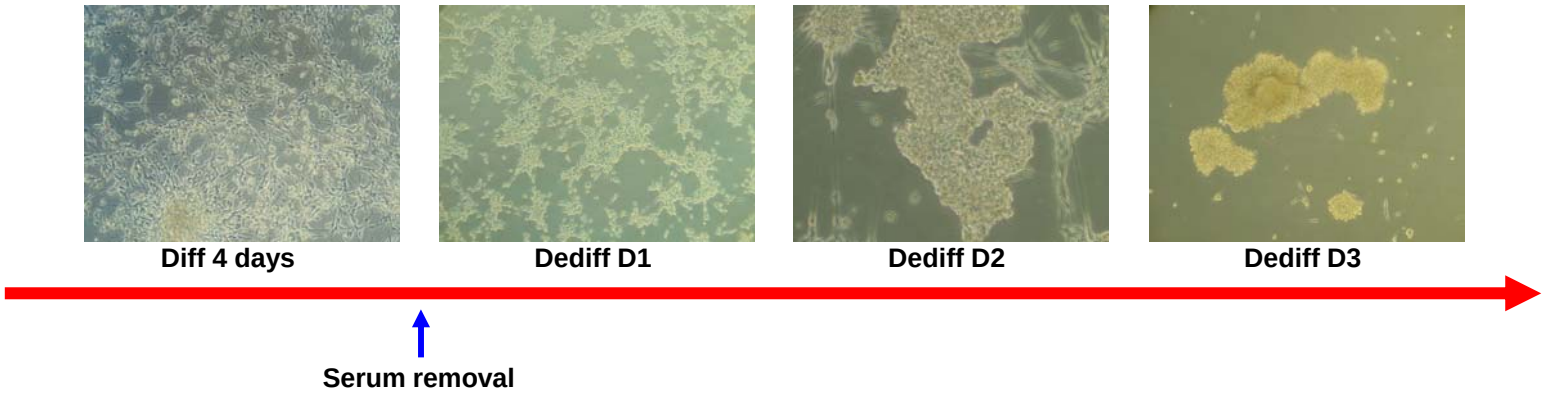
ID2, ID3, Max, PPAR-gamma
GADD45G, GADD45B,
gene not yet characterized

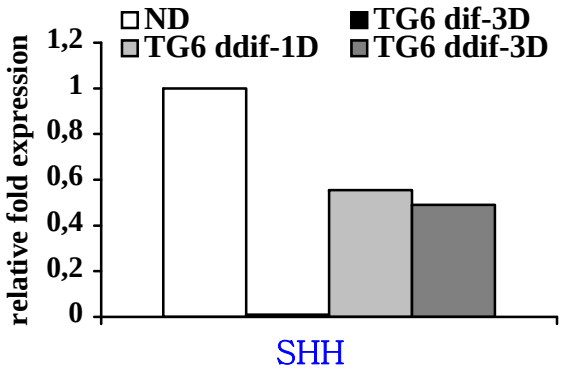
Mirnome

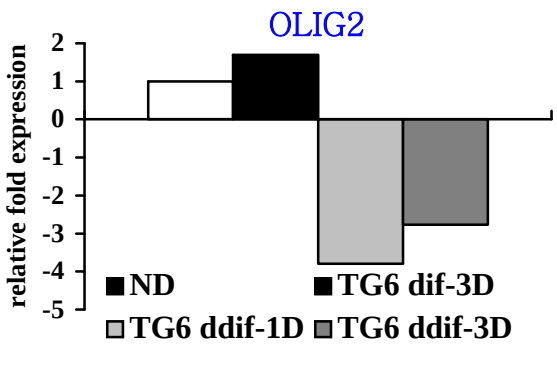
8 miRNA downregulated (ratio <3)

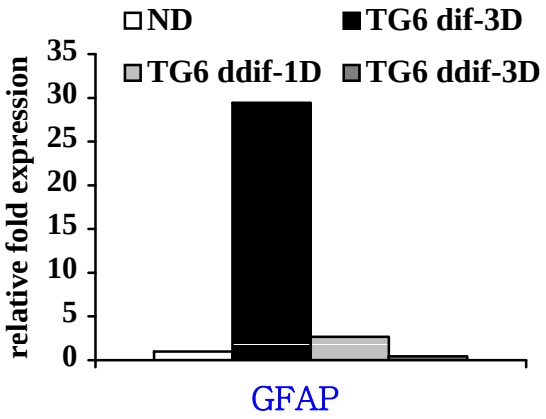
8 miRNA upregulated (ratio >3) :
One cluster (5 miR) only expressed in differentiated cells

Plasticity & TG6

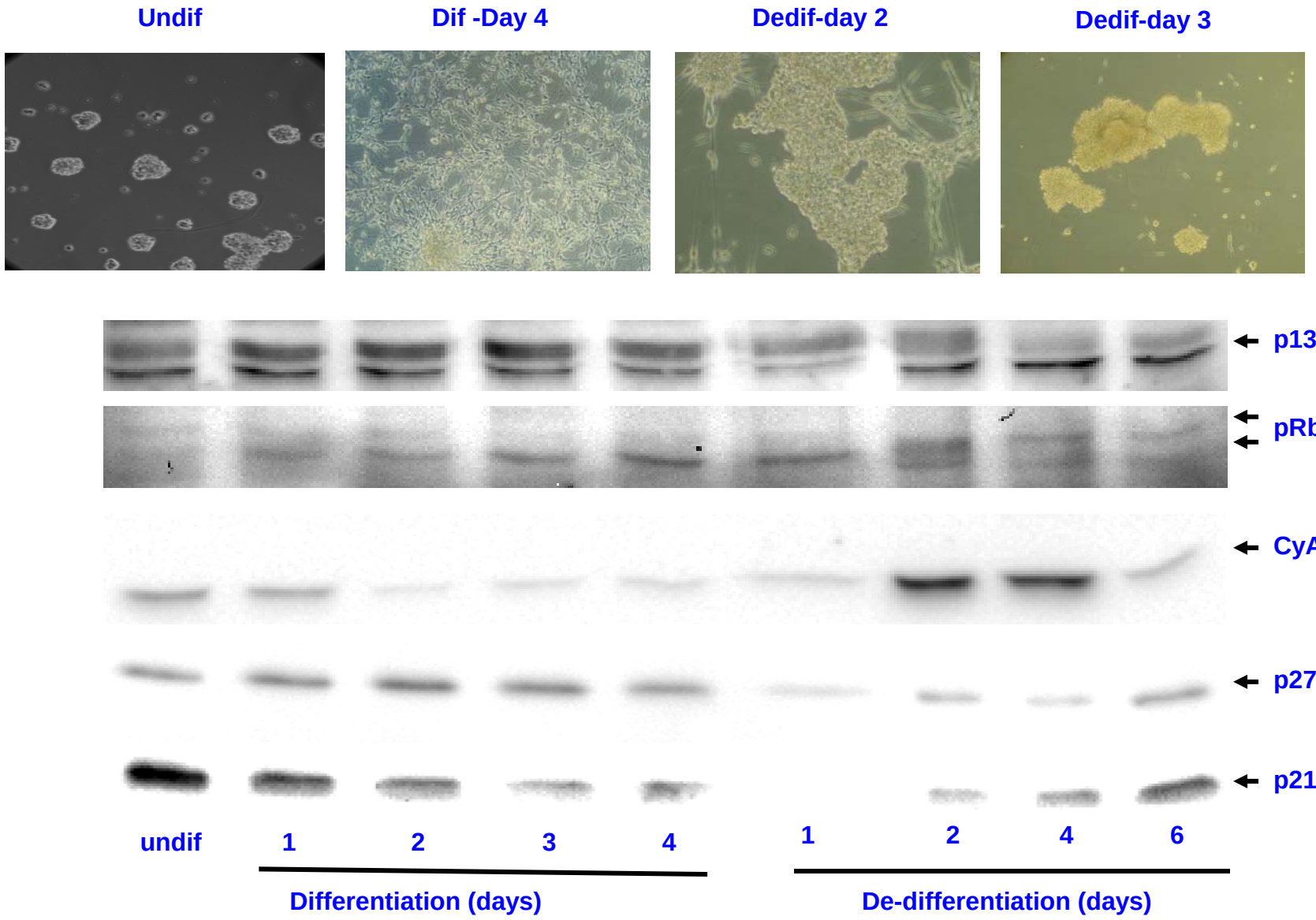




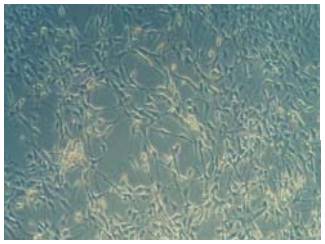




Dedifferentiation and Cell cycle



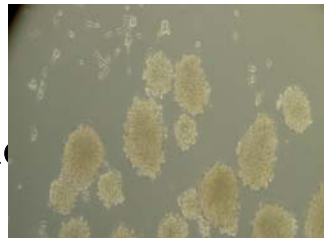
Dedifferentiation process



Loss of markers
of differentiation

Massive inter in cell cycl

1 2 4 6



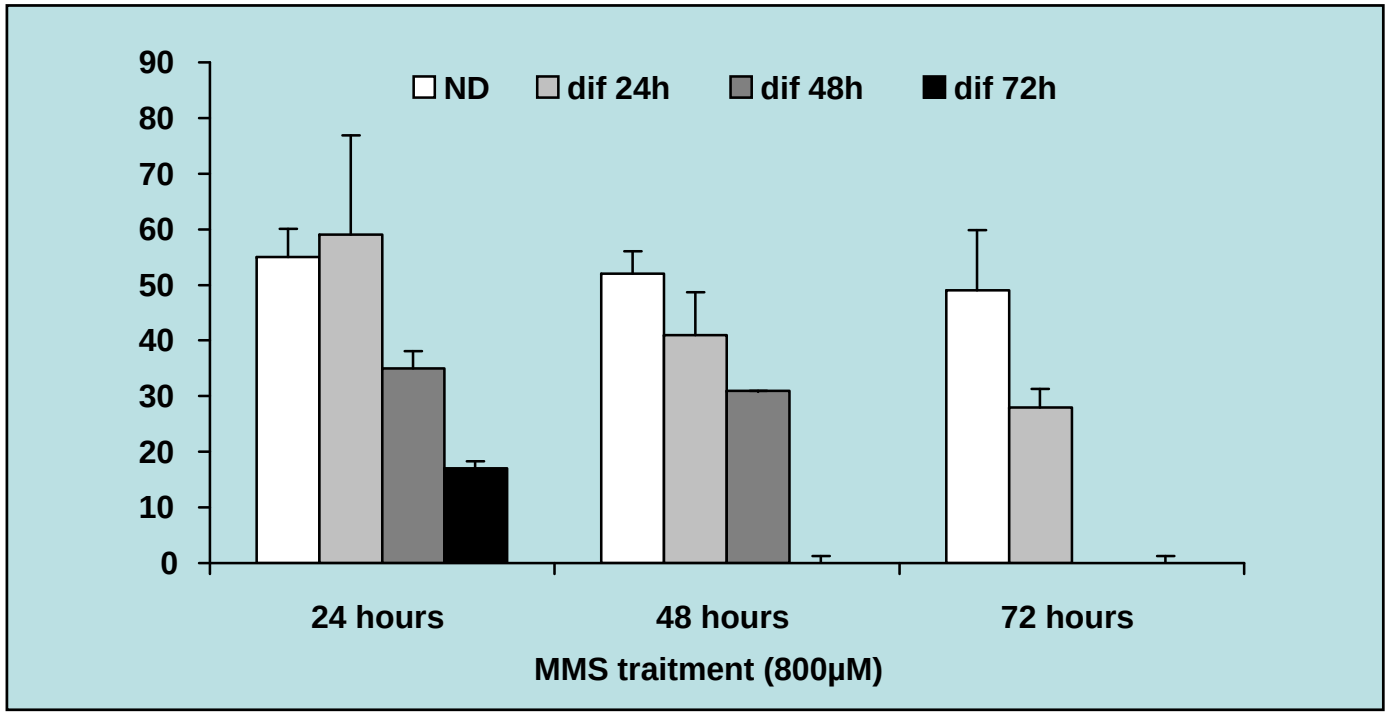
Differentiated TG6

Undifferentiated TG6

Transcriptome and Mirnome analysis

Functional studies

Sensitivity to Genotoxic Stress



Undifferentiated TG6

72h differentiation

Transcriptome & Mirnome

Candidates with Biomedical Interest

gain and loss of function of candidates genes and miRNA (shRNA, cDNA, clear mir etc

Self renewal
Differentiation

Sensitivity to genotoxic stress

Tumor growth

Resistance to anticancer treatment

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