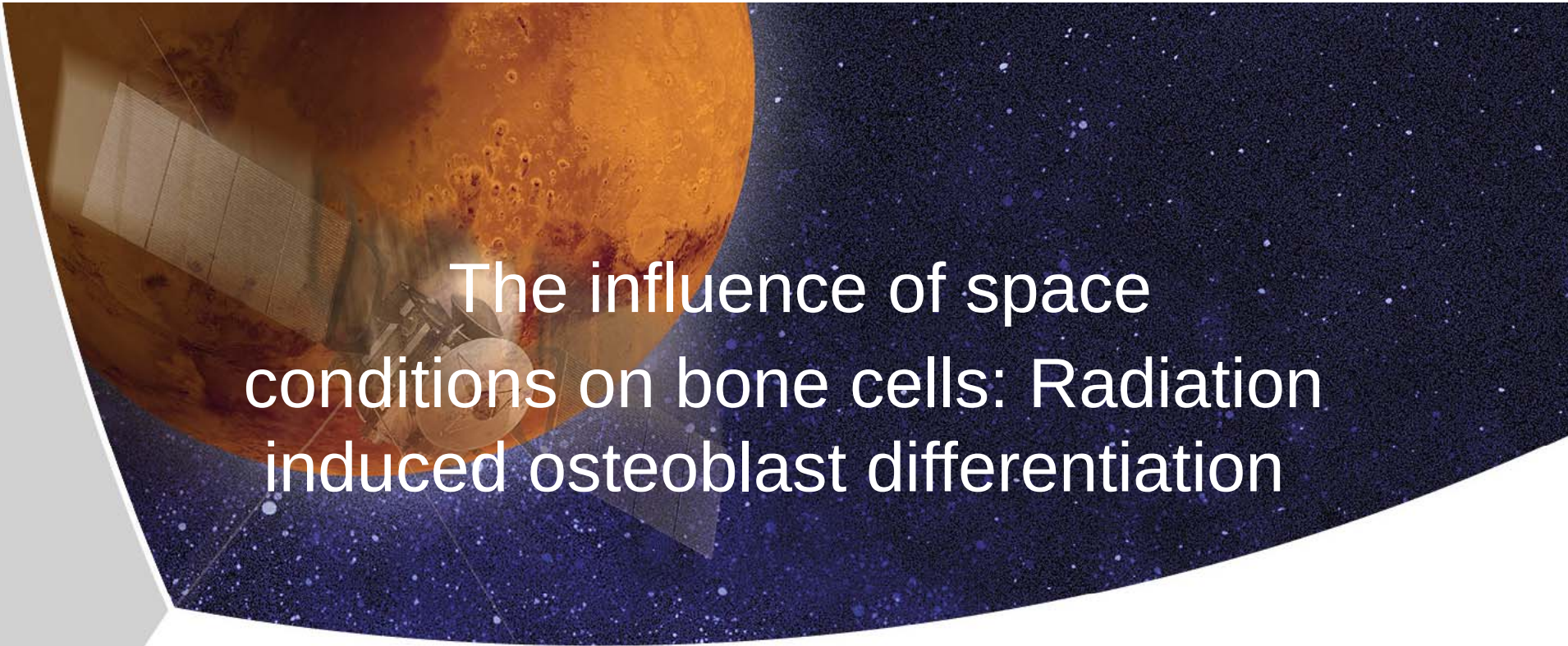




The influence of space conditions on bone cells: Radiation induced osteoblast differentiation

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Introduction:

- Space radiation & microgravity conditions
- Osteoblastic remodeling cycle
- Bone specific marker genes

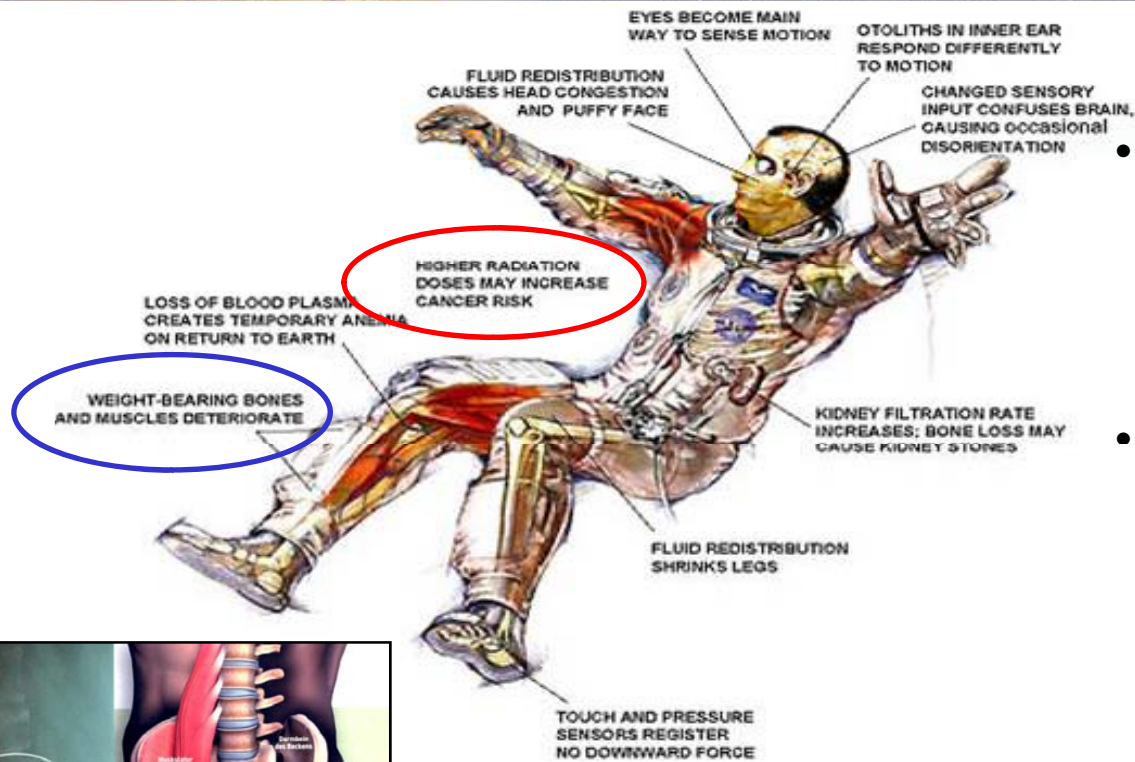
Cell model:

- Mineralization assays
 - Alkaline Phosphatase (ALP)
 - Von Kossa-staining
 - Alizarin Red S

Effects of radiation on:

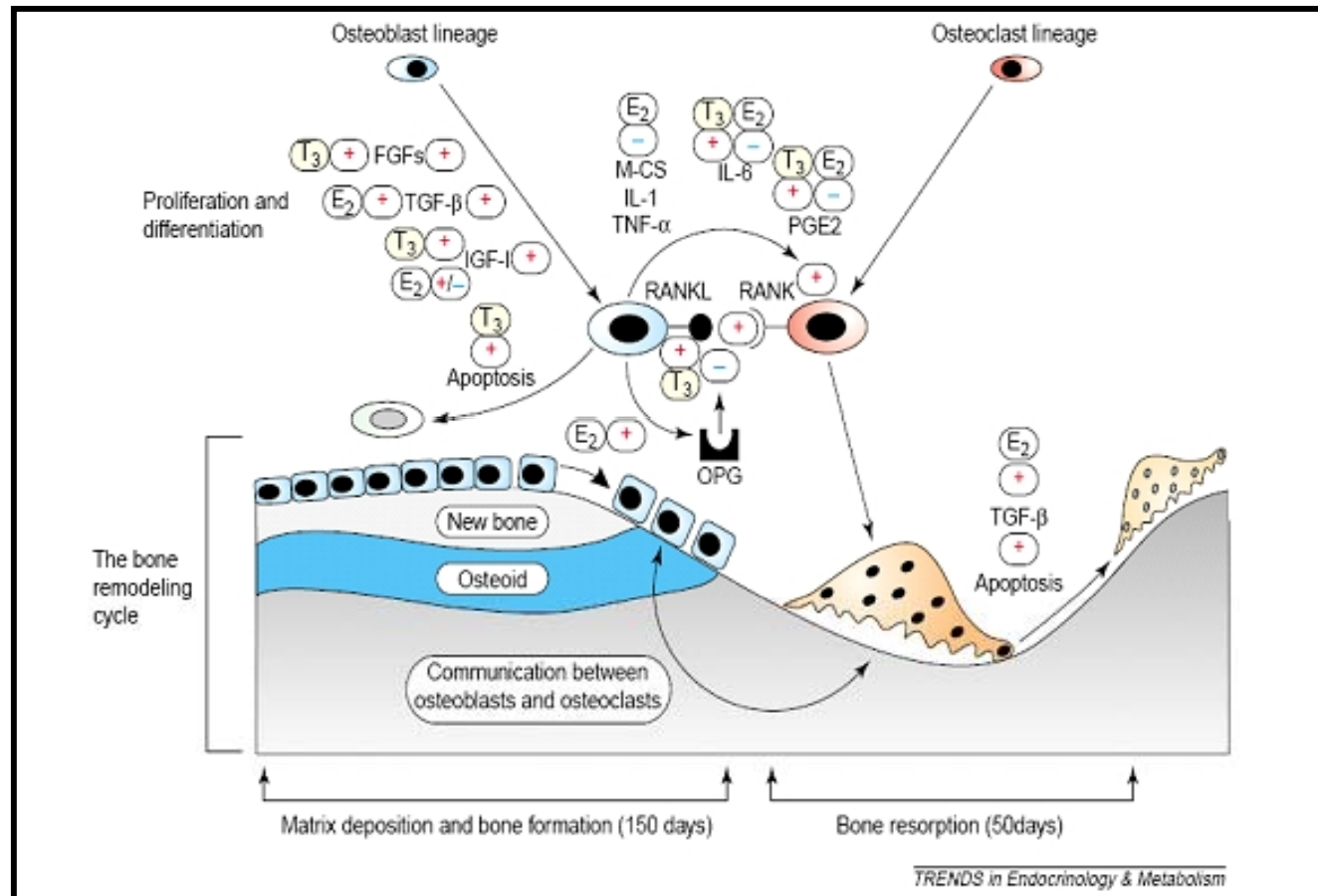
- Cell survival
- Cell cycle progression
- Gene expression analysis



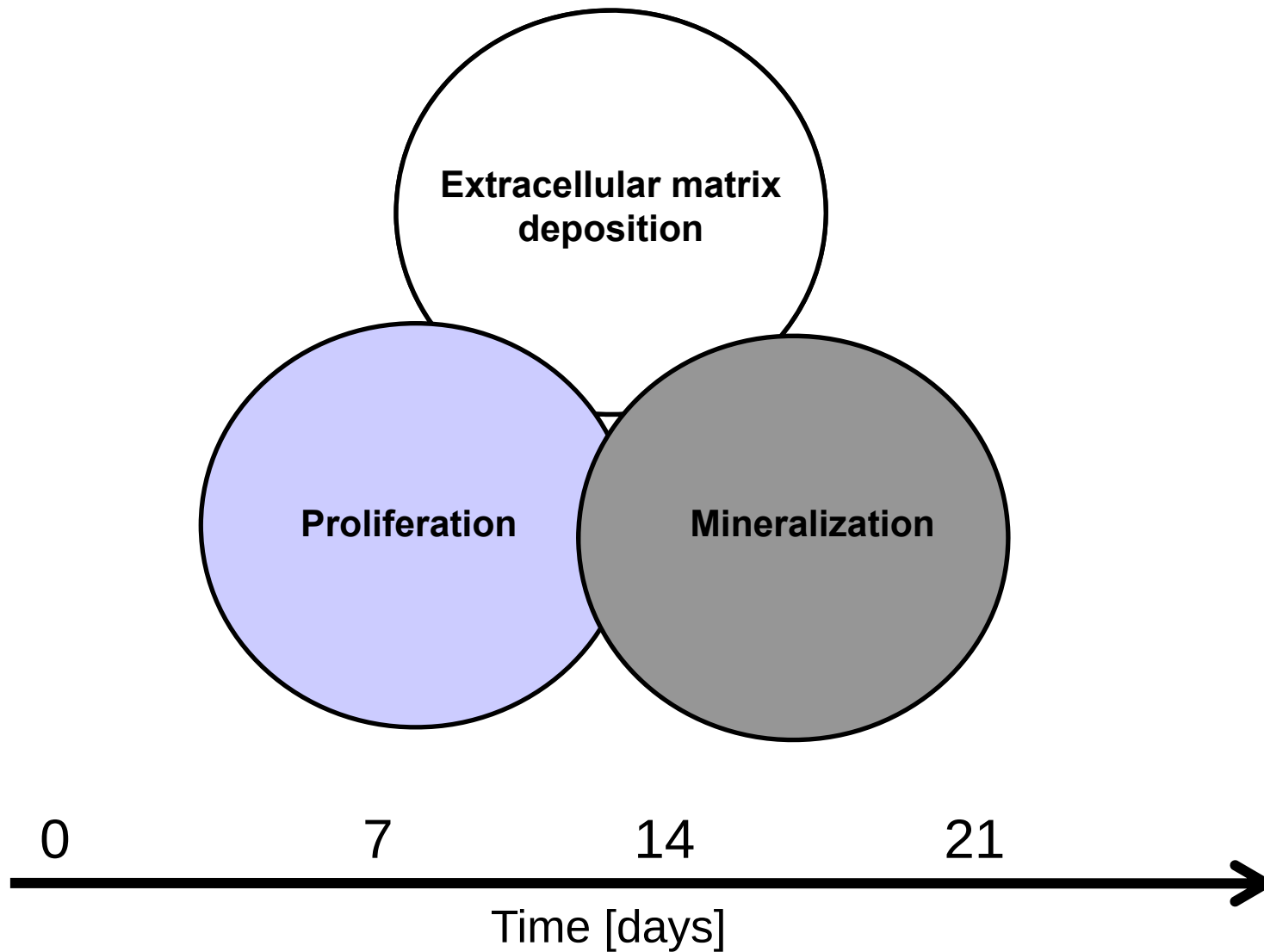


- Prolonged exposure to microgravity has negative effects on the skeletal system (Lang *et al.*, 2004).
- Recent examination of astronauts on the ISS reported 0,8-1,5% bone loss per month in the vertebrae and femoral neck.
- Site- specific losses exceeding 2% per month (Lang *et al.*, 2004).
- Incomplete recovery 5 yr after returning to earth (Tilton *et al.*, 1980).

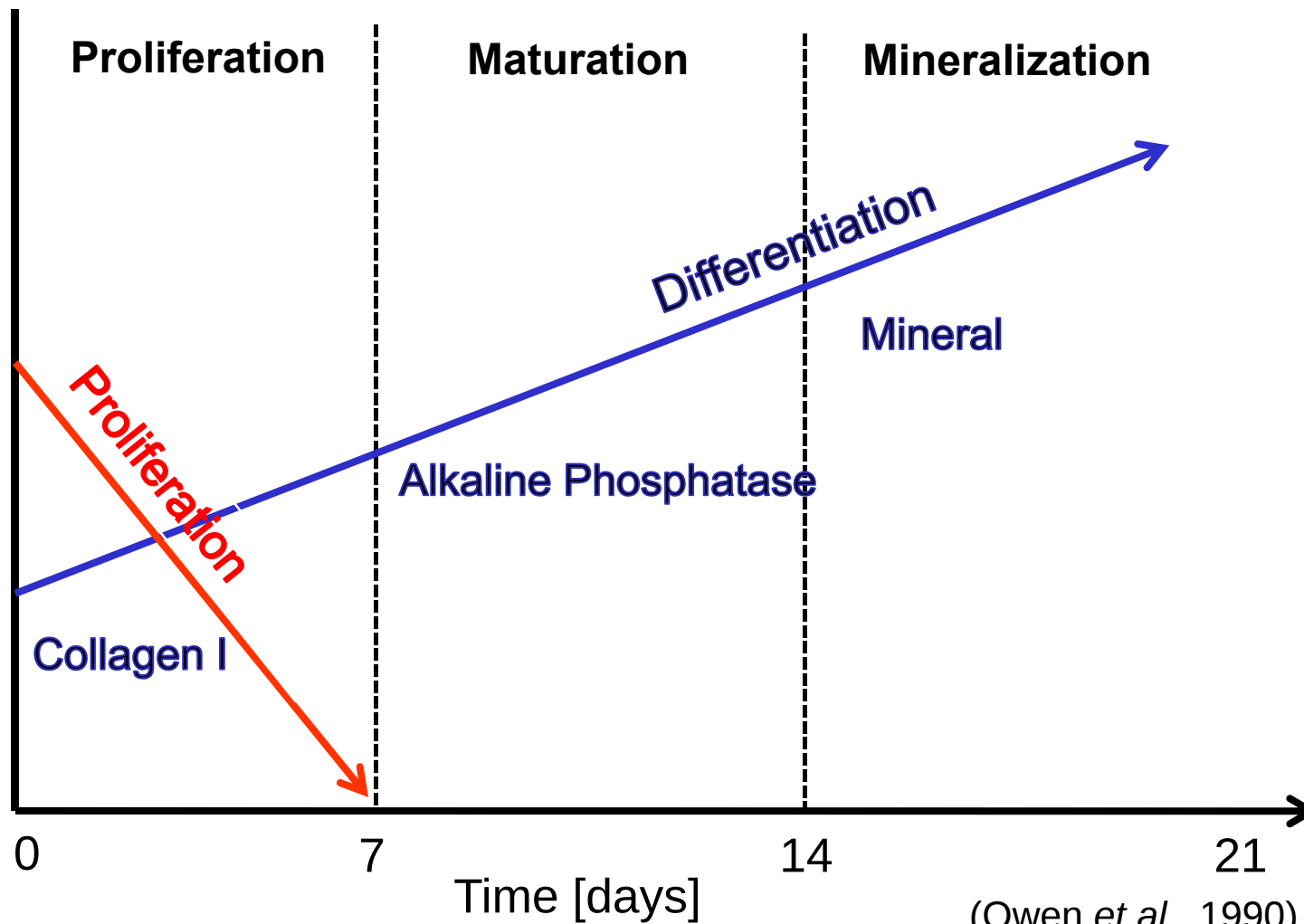
The bone remodeling cycle



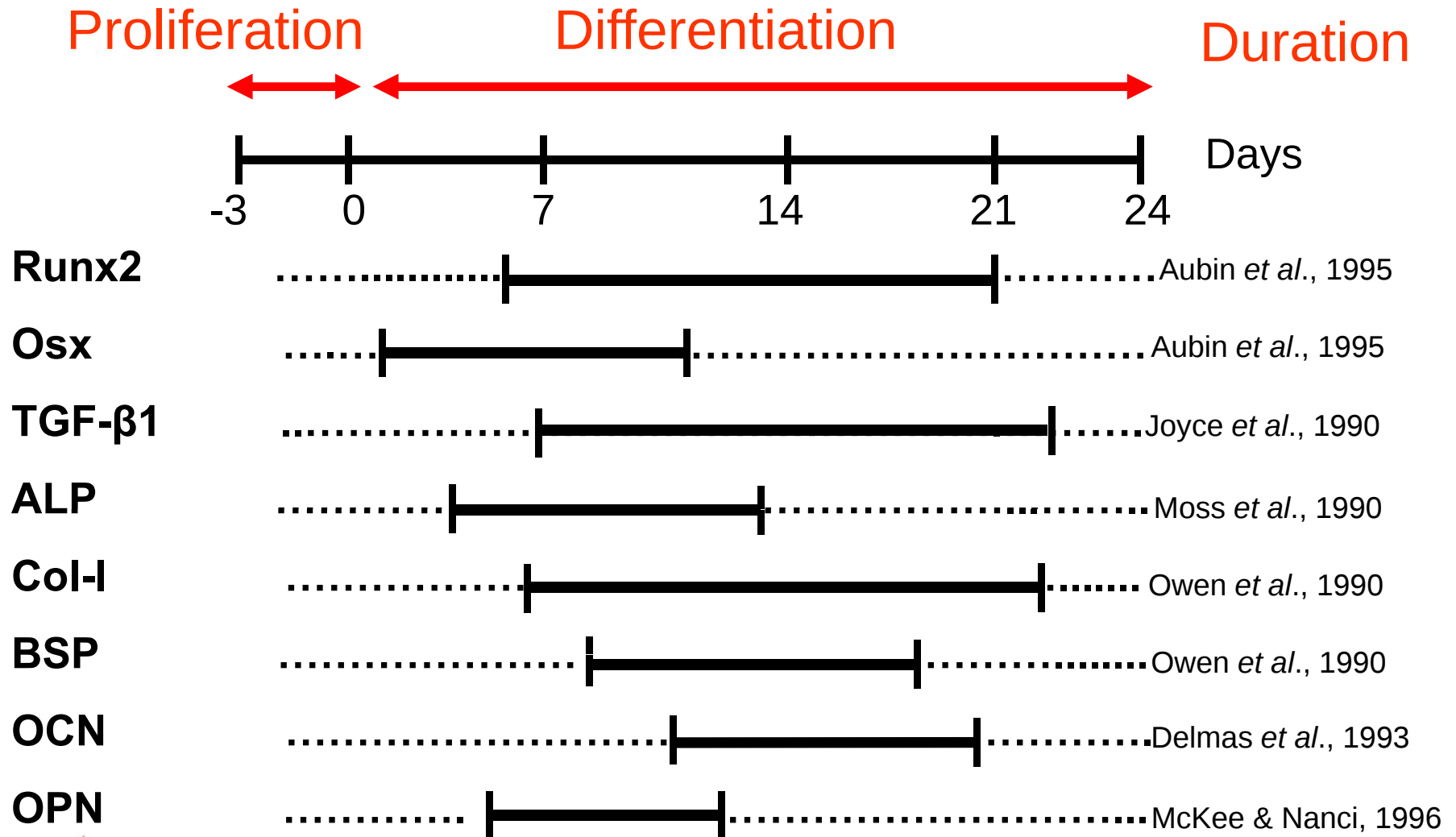
The bone remodeling cycle



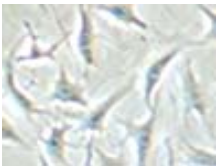
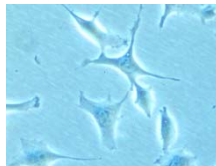
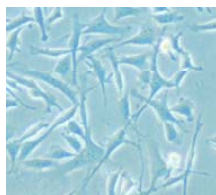
Differentiation of osteoblasts *in vitro*



Bone specific gene expression



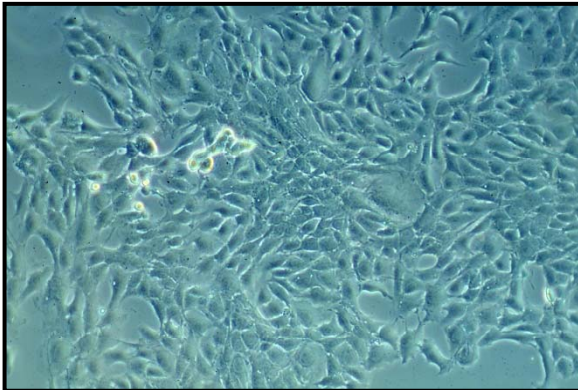
Murine bone cell model

<i>Cell line</i>		<i>Description</i>	<i>Origin</i>	<i>Source</i>
MLO-Y4		Osteocyte	Femur & Tibia	Prof. L. Bonewald Kansas City
OCT-1		Osteoblast	Calvaria	Dr. Di Chen, San Antonio, Texas, USA
MC3T3- E1 S4		Osteoblast like cell	Calvaria	ATCC CRL- 2593
MC3T3- E1 S24		Osteoblast like cell	Calvaria	ATCC CRL-2595

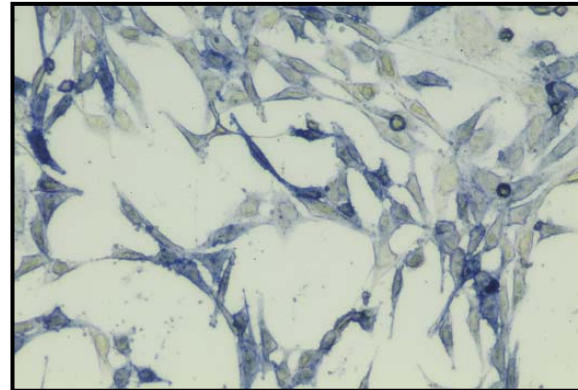
Cultured Osteoblasts



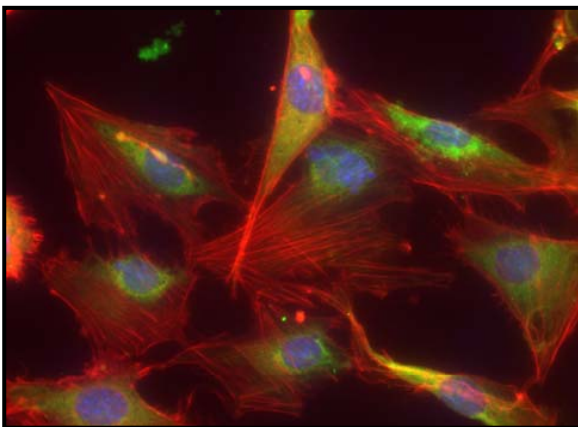
Phase contrast



Alkaline Phosphatase



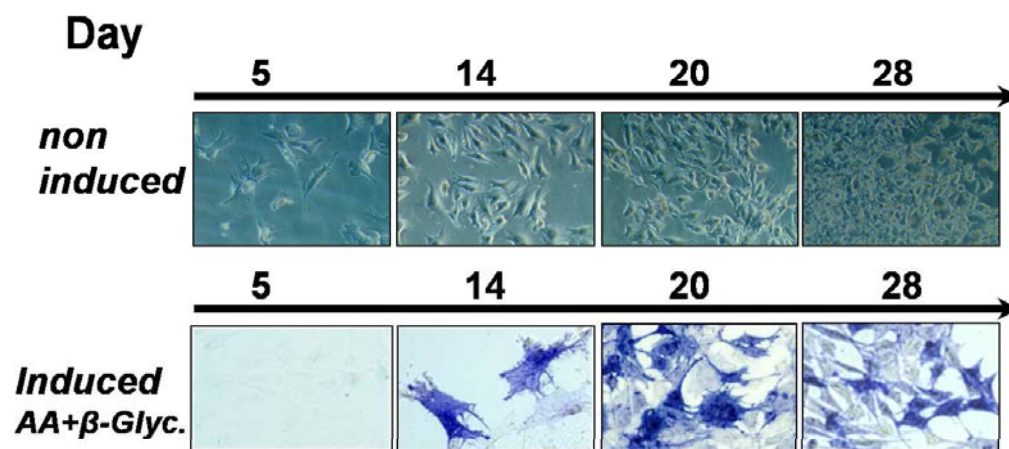
Osteocalcin



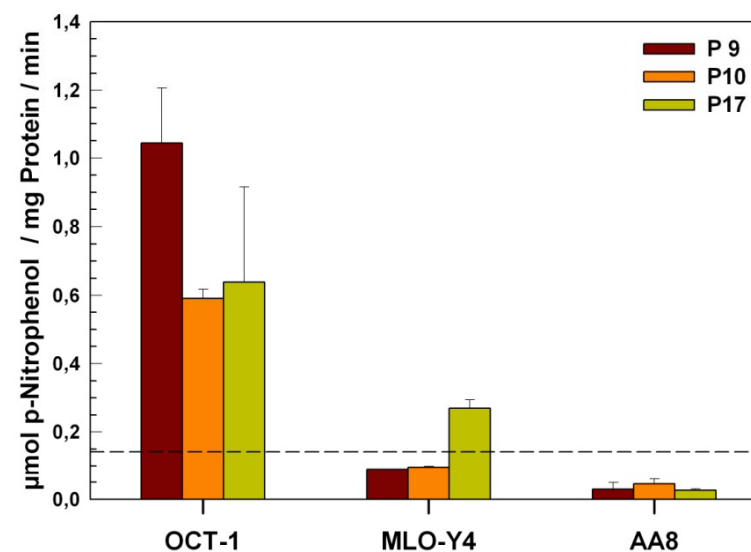
Mineralization



Alkaline Phosphatase activity

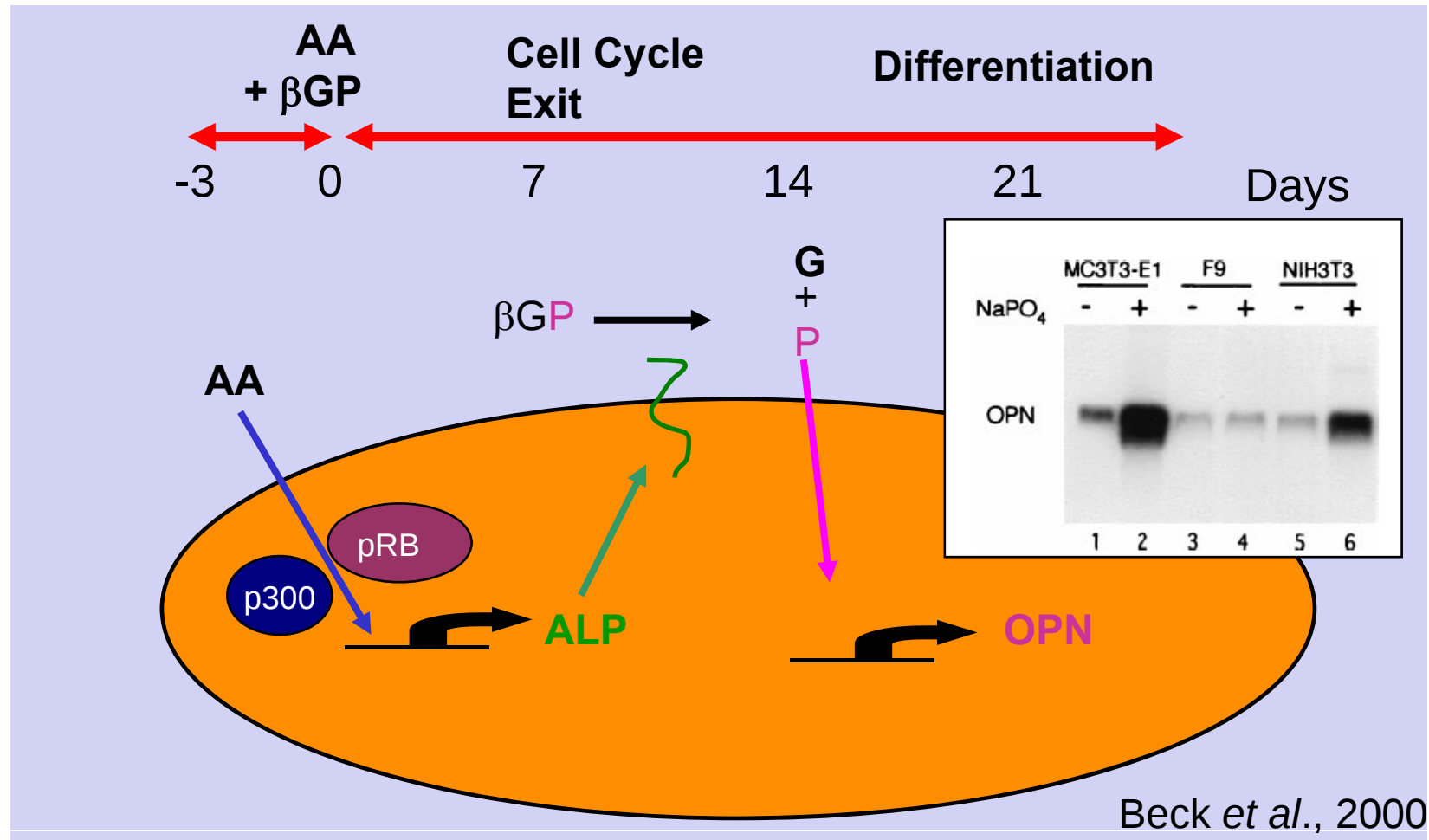


qualitative

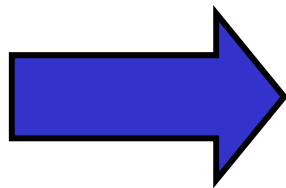
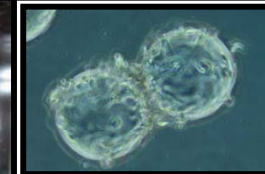
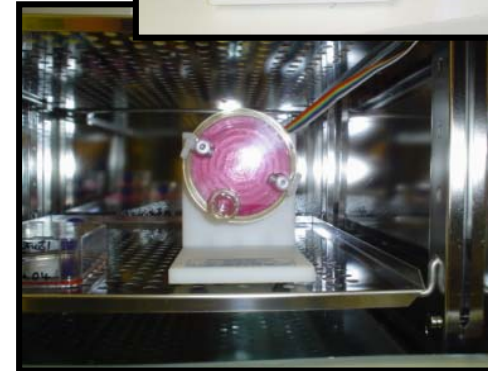
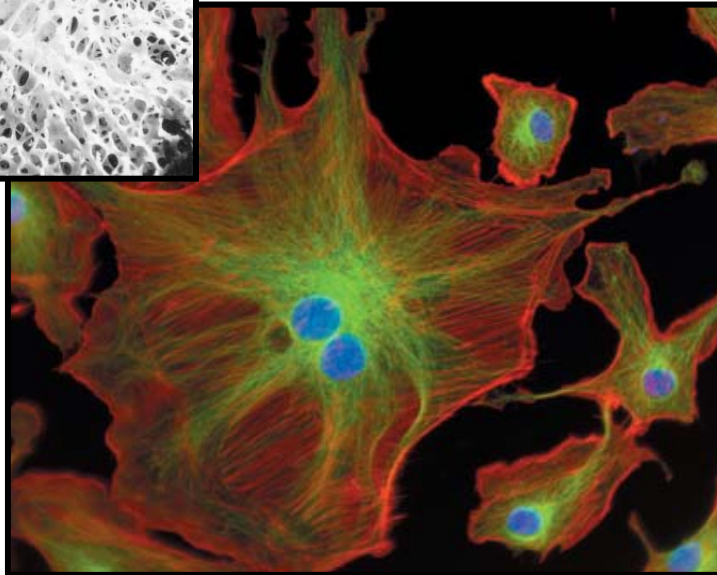
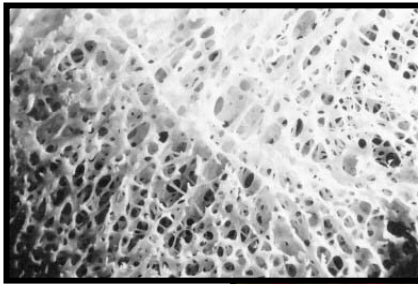


quantitative

Coordination of ALP activity and OPN levels in osteoblasts

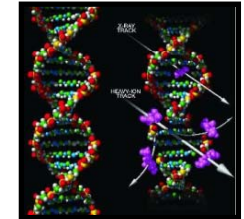


Exposure to modeled microgravity conditions



- Inhibition of the bone specific differentiation process
- Inhibition of ALP activity
- Downregulation of Runx2
- Alteration of local growth factor level (TGF β 1)
- Decreased calcification

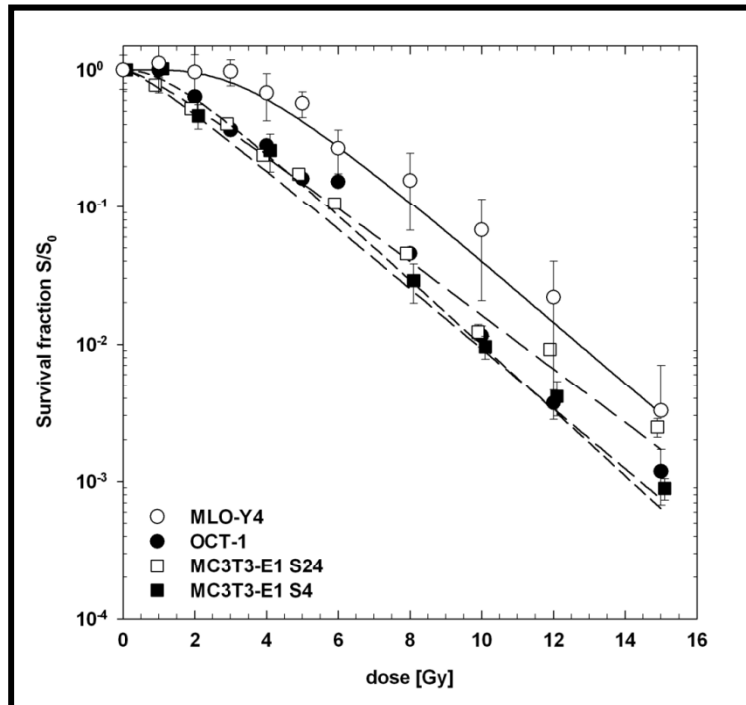
Clinical and Spaceflight application concerning exposure to ionizing radiation



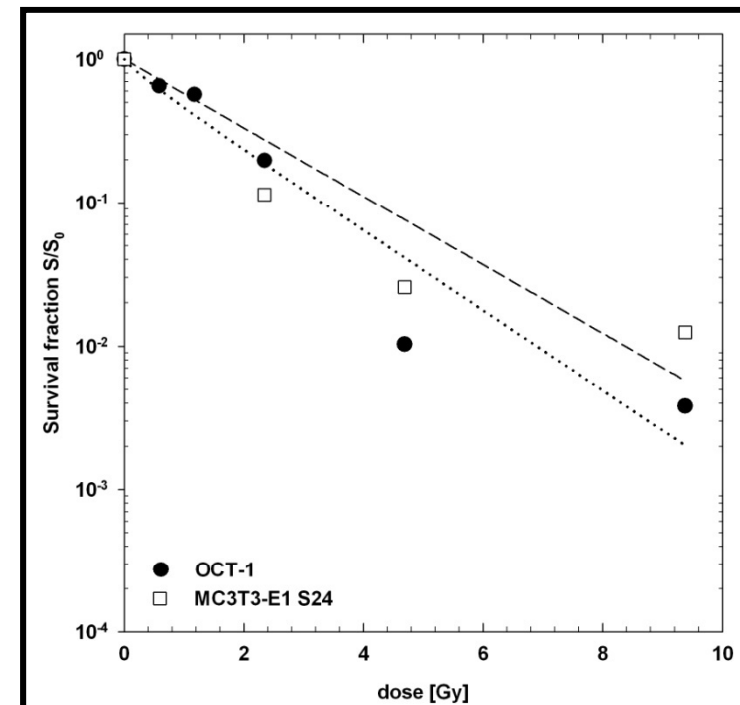
- The effect of ionizing radiation on the skeletal system has not been examined in detail.
- Therapeutic doses of radiation have shown to have deleterious consequences on bone health and maintenance (osteoradionecrosis and spontaneous fractures) (Ergün and Howland 1980)
- Ionizing radiation suppresses normal osteoblast proliferation, modifies gene expression and alters the cell cycle progression (Szymczyk *et al.*, 2004)

It is however unclear how bone already compromised by microgravity will be affected by exposure space radiation.

Survival after exposure to ionizing radiation

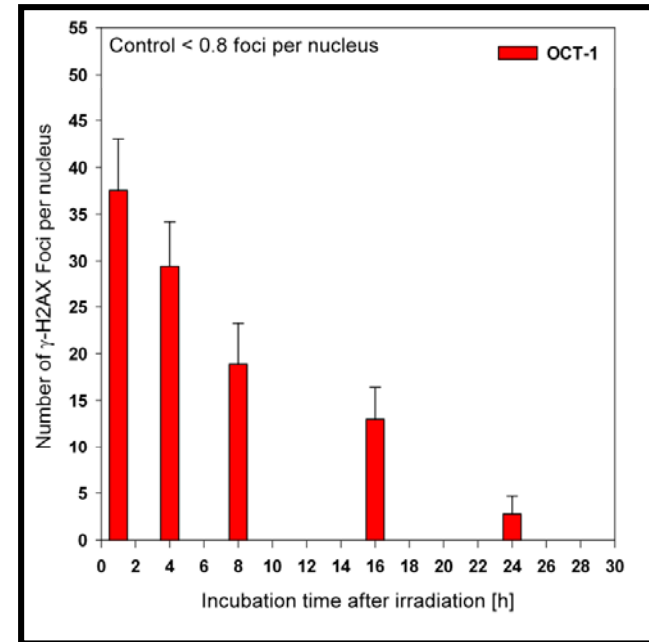
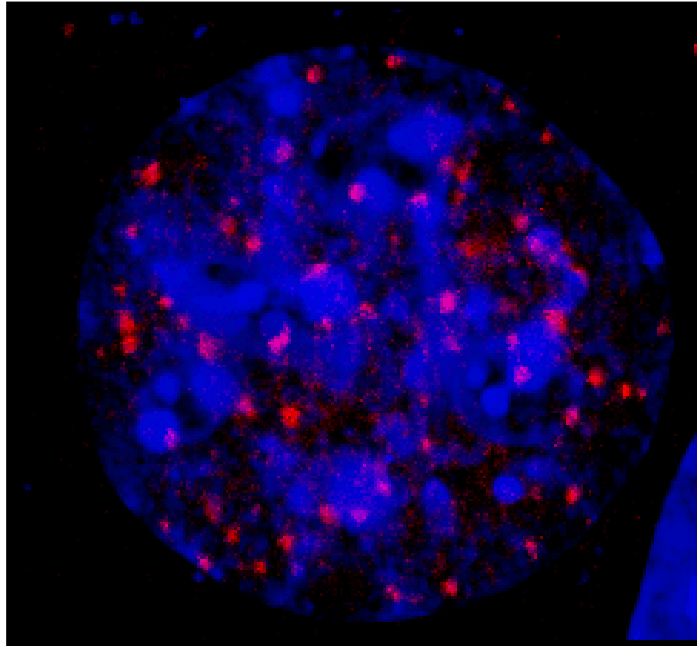


Dose-effect relationship after
X-ray treatment



^{13}C 75MeV/amu
LET 29.28 keV/ μm

DSB Induction and repair (γ -H2AX)



Foci pro nucleus	1 Gy, 1 h	1 Gy, 24 h	Control 24 h	Area nucleus [μm^2]
MLO-Y4	$36,72 \pm 12,26$	$2,79 \pm 1,76$	0,8	$113,5 \pm 29,7$
OCT-1	$37,54 \pm 5,50$	$2,77 \pm 1,91$	0,7	$122,4 \pm 33,8$
S4	$34,26 \pm 6,52$	$2,90 \pm 2,37$	0,8	$112,7 \pm 31,4$
S24	$38,93 \pm 3,54$	$2,87 \pm 1,34$	0,8	$118,1 \pm 32,7$



Cell cycle analysis after X-ray exposure

Control 2 Gy 4 Gy 7 Gy

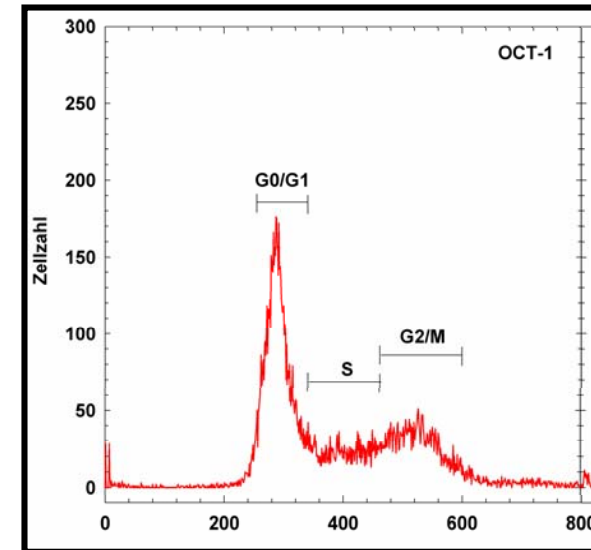
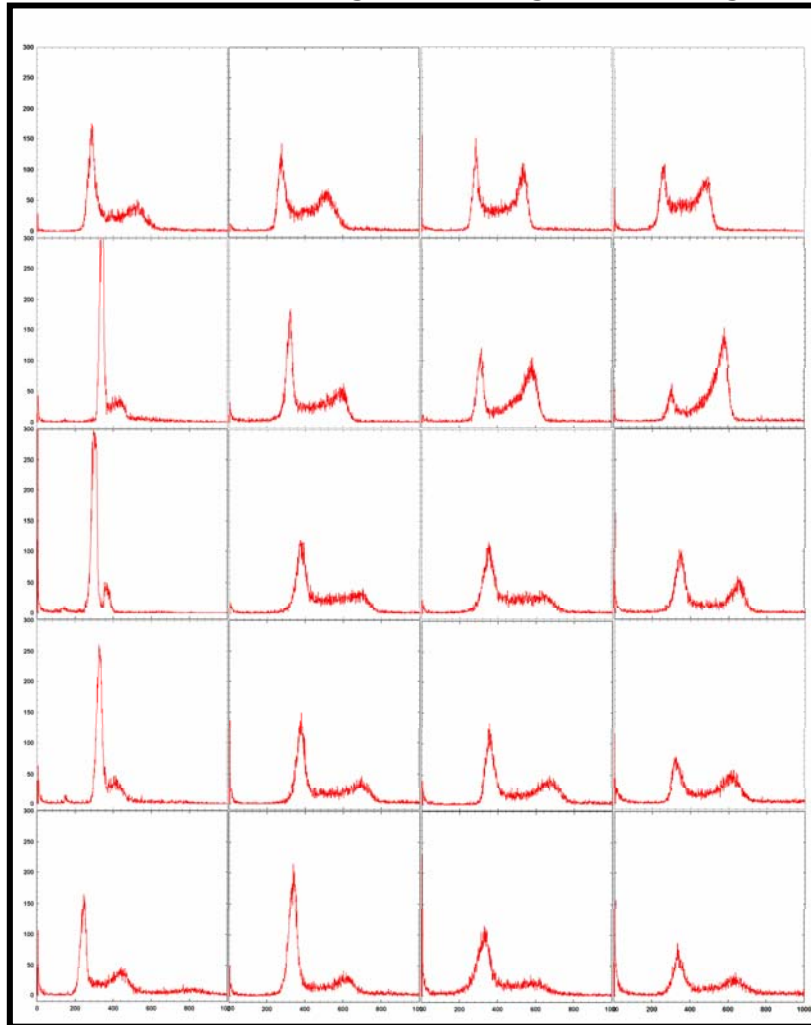
4h

8h

12h

24h

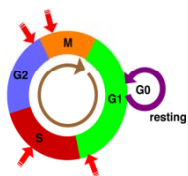
48h



- Cell line : OCT-1
- PI-staining – FACS-Analyse
- Cells: 20000

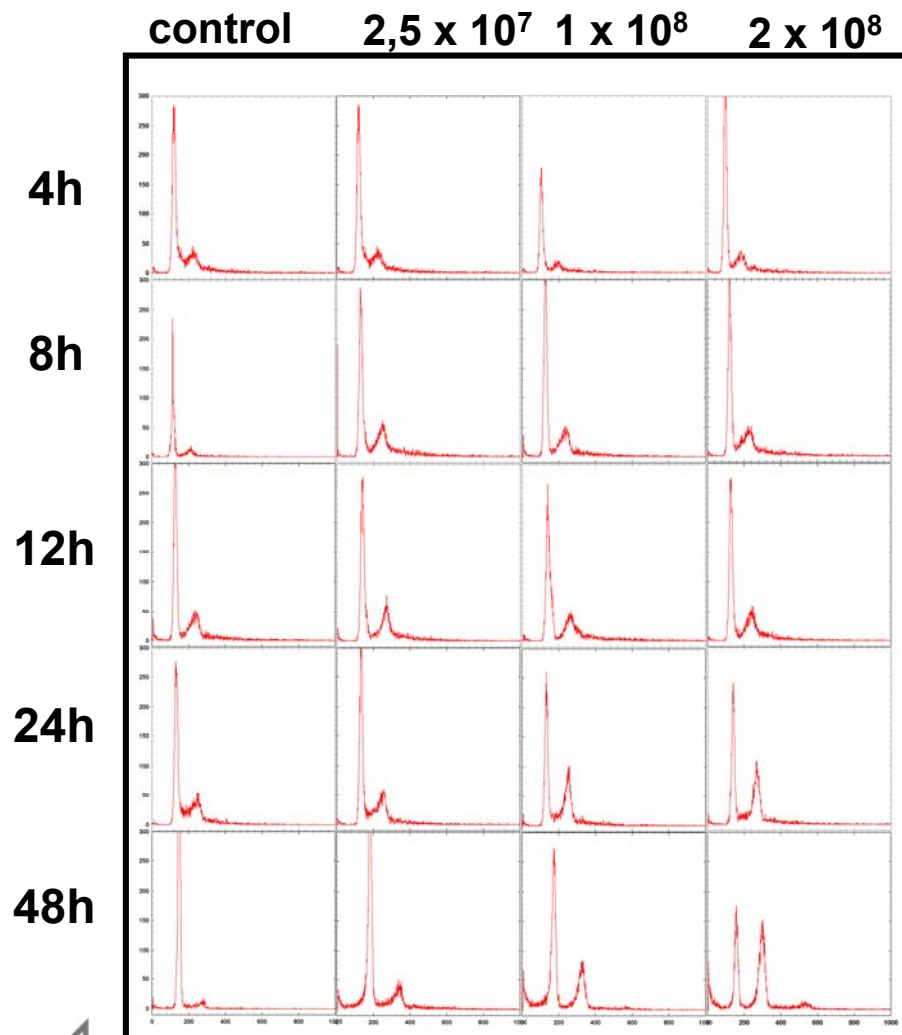


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Cell cycle analysis after exposure to

^{13}C 75 MeV/amu LET 29,28 keV/ μm



P/cm ²	Dose [Gy]	Hits \ Nucleus
2.5×10^7	1.17	25
1×10^8	4.68	100
2×10^8	9.37	200

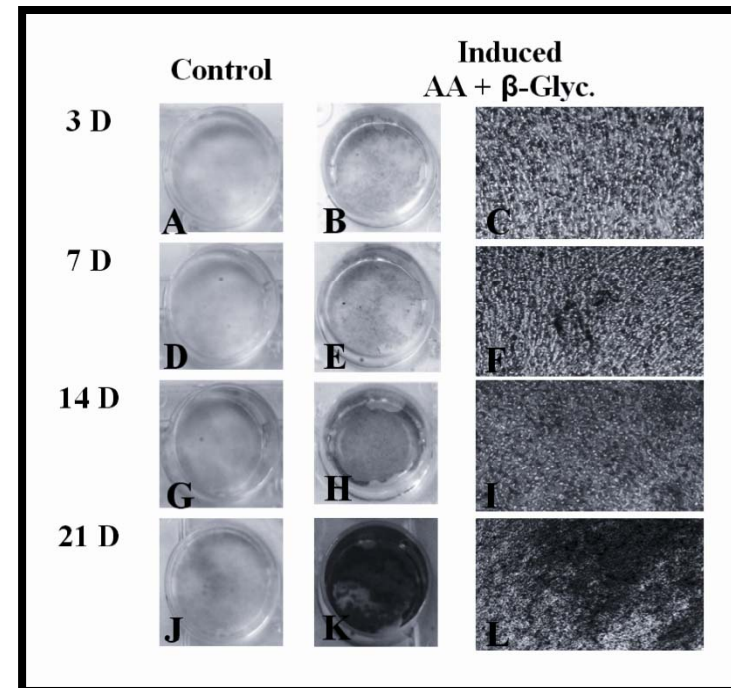
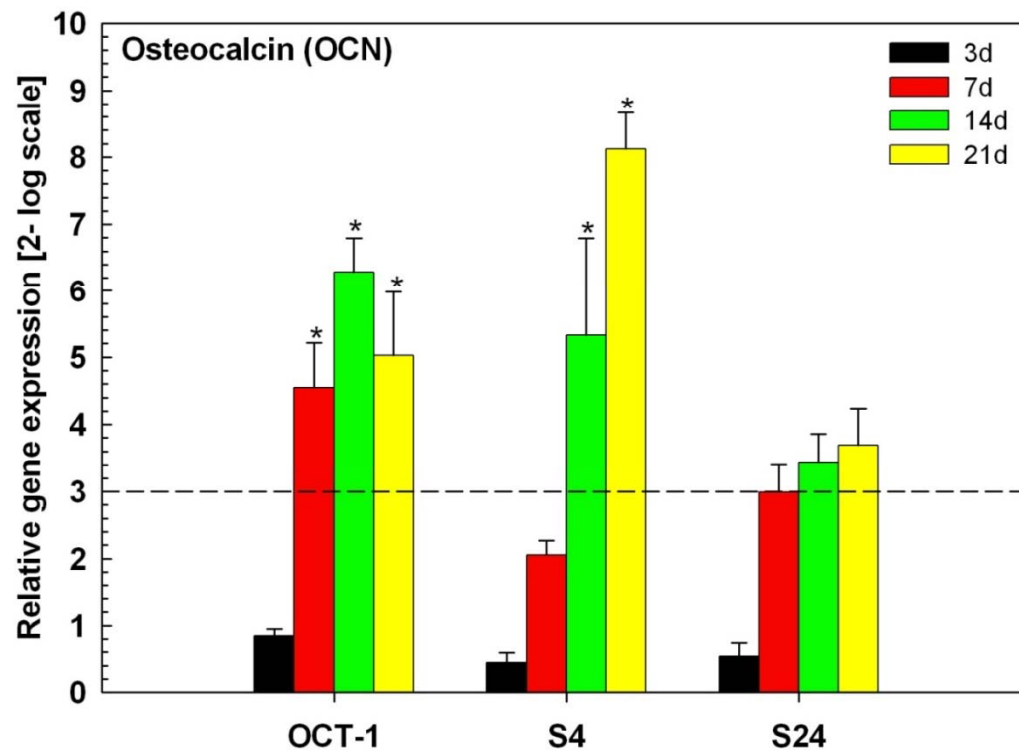
- Cell line : MC3T3-E1 S24
- Staining: PI – FACS-Analyse
- Cells: 20000



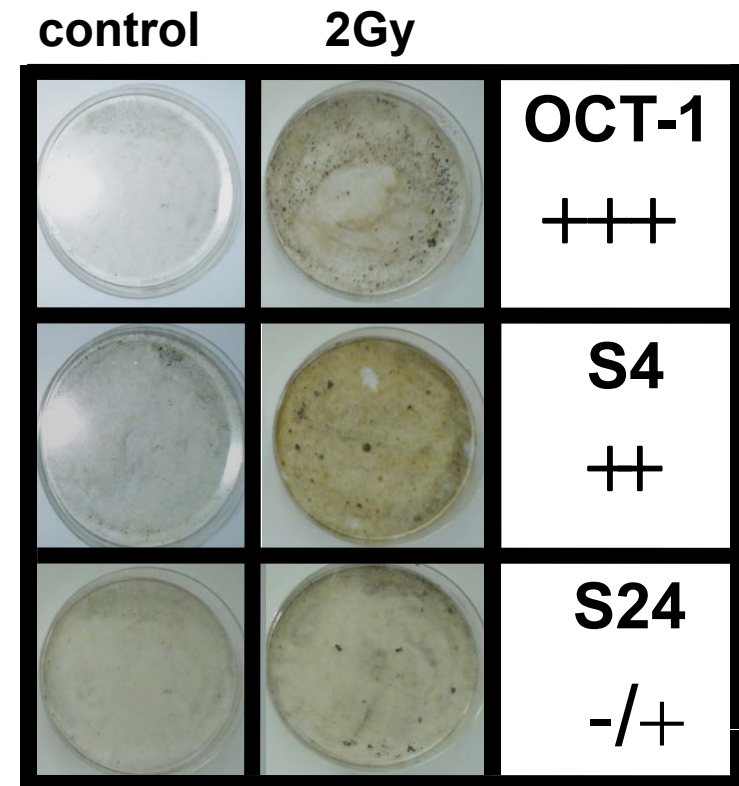
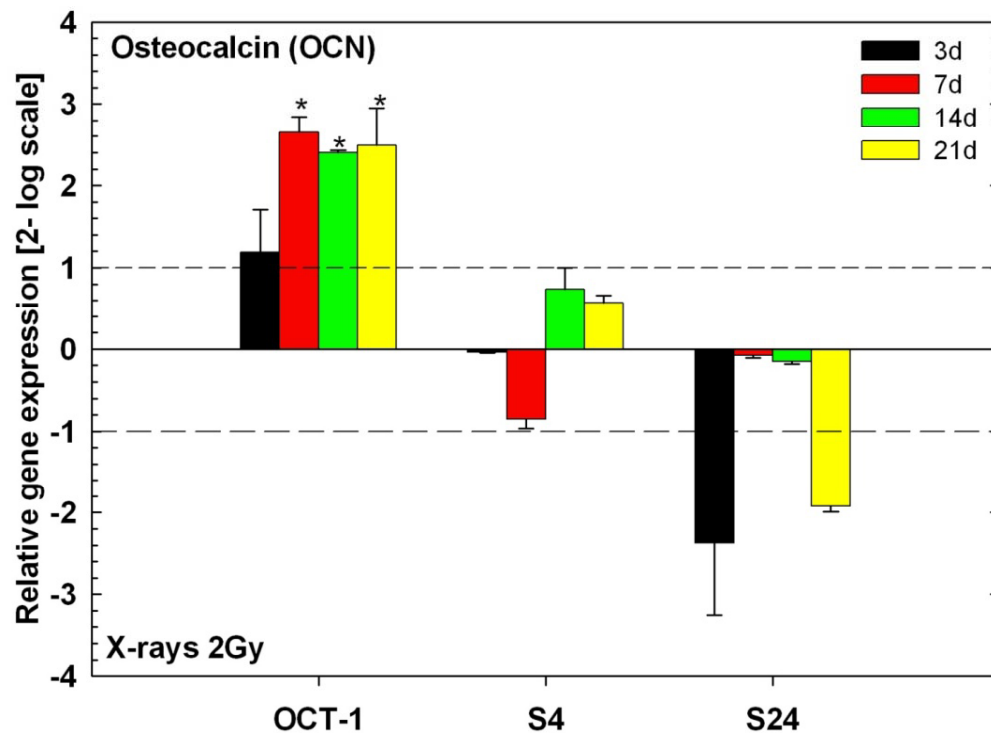
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Gene expression analysis: Osteocalcin (OCN)

- osteogenic induction AA + BG



Gene expression analysis: Osteocalcin (OCN)

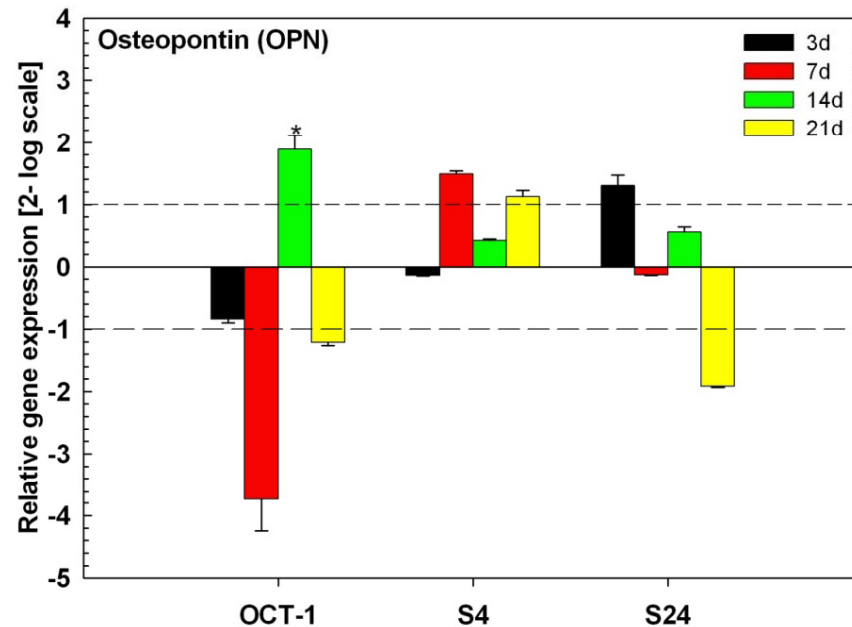


Von-Kossa-staining

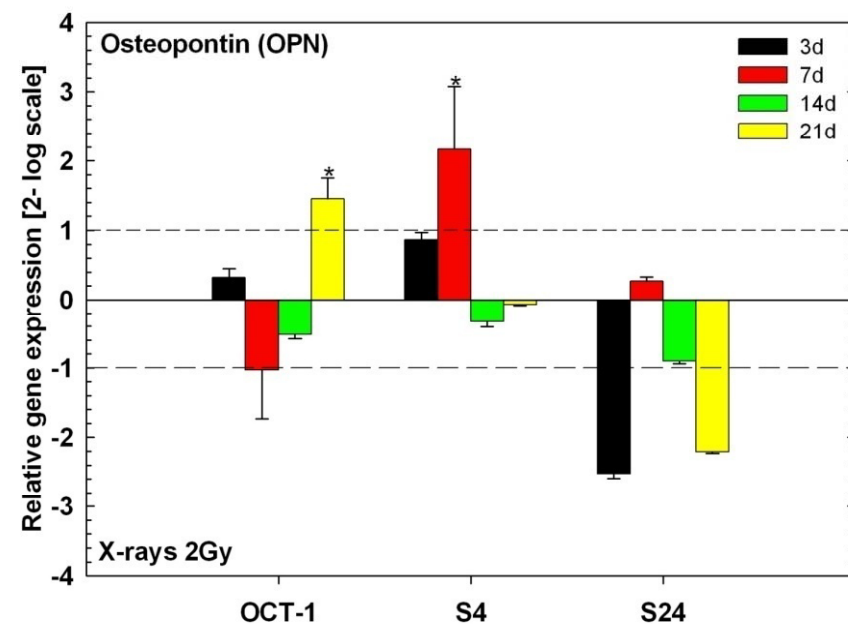


Gene expression analysis: Osteopontin (OPN)

Osteogenic induction



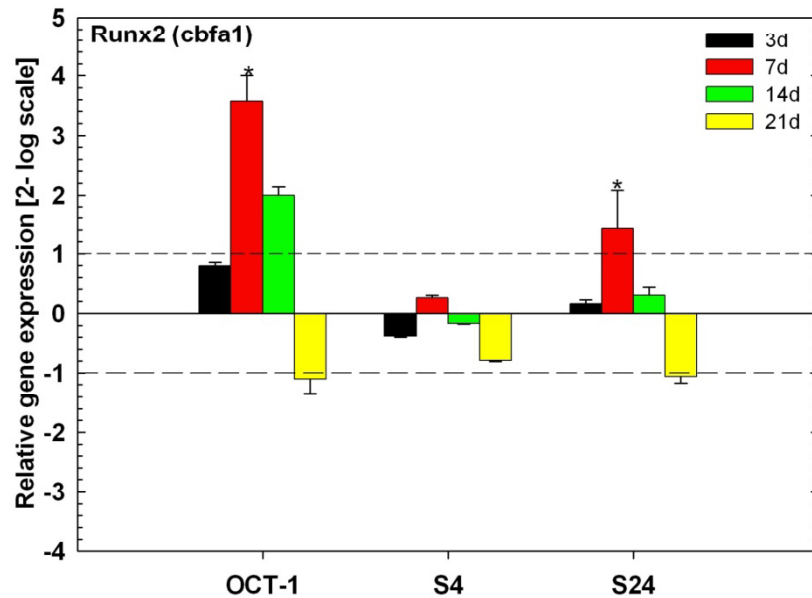
X-rays 2 Gy



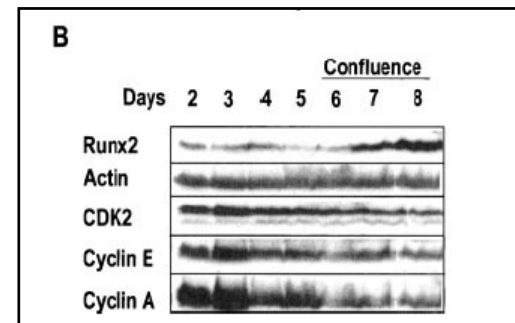
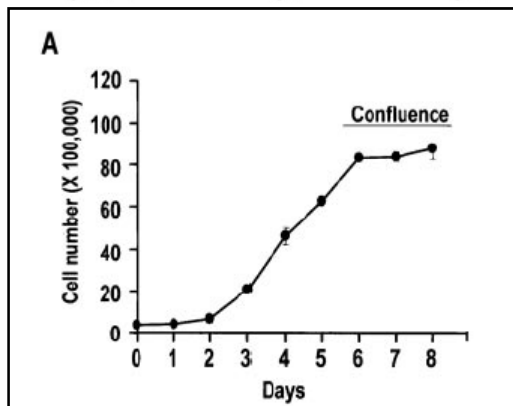
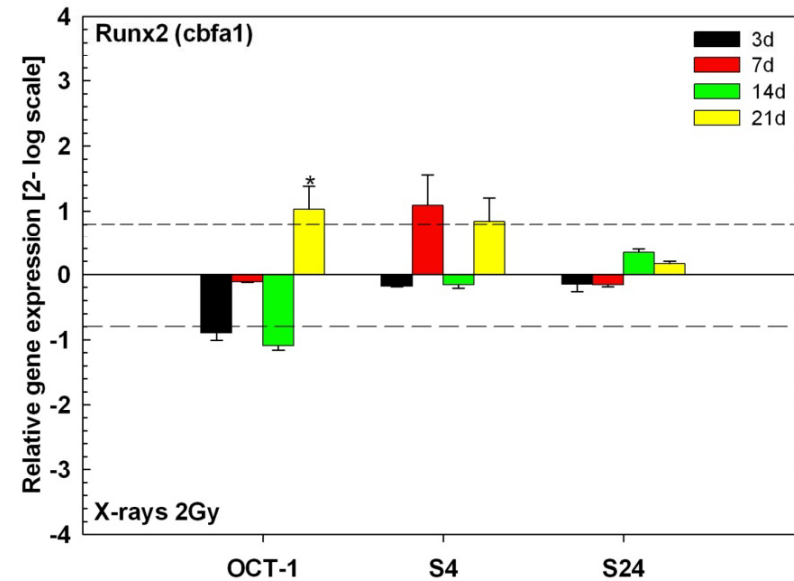
- OPN is a glycoprotein involved in binding calcium ions and hydroxyapatite during the mineralization process.

Gene expression analysis: Runx2 (cbfa1)

Osteogenic induction



X-rays 2 Gy

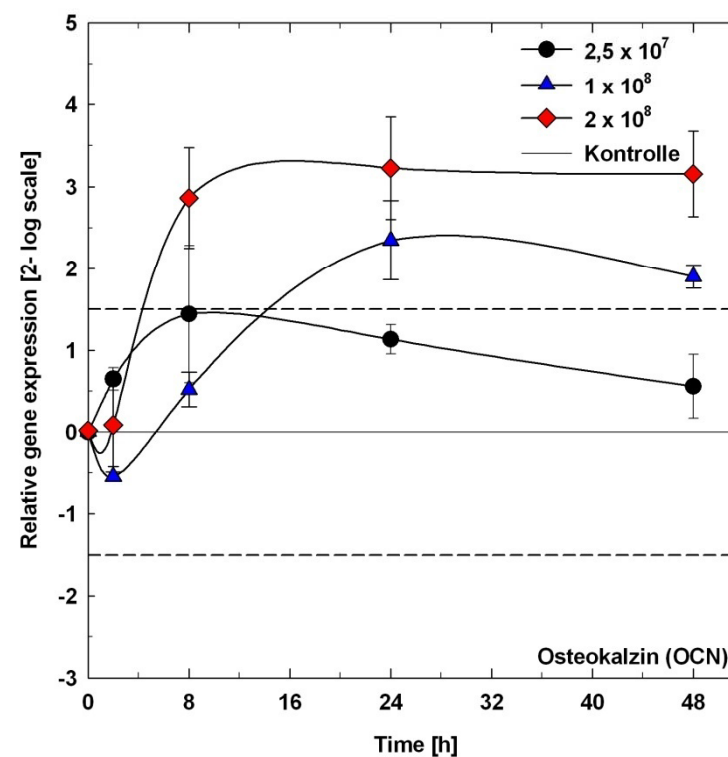
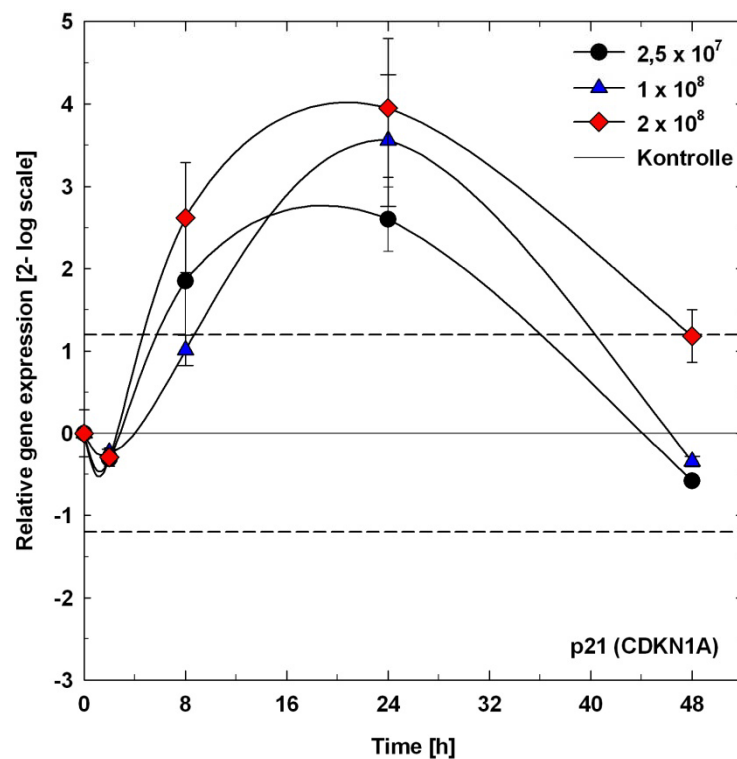


Pratrap *et al.*, 2003



Gene expression analysis after exposure to

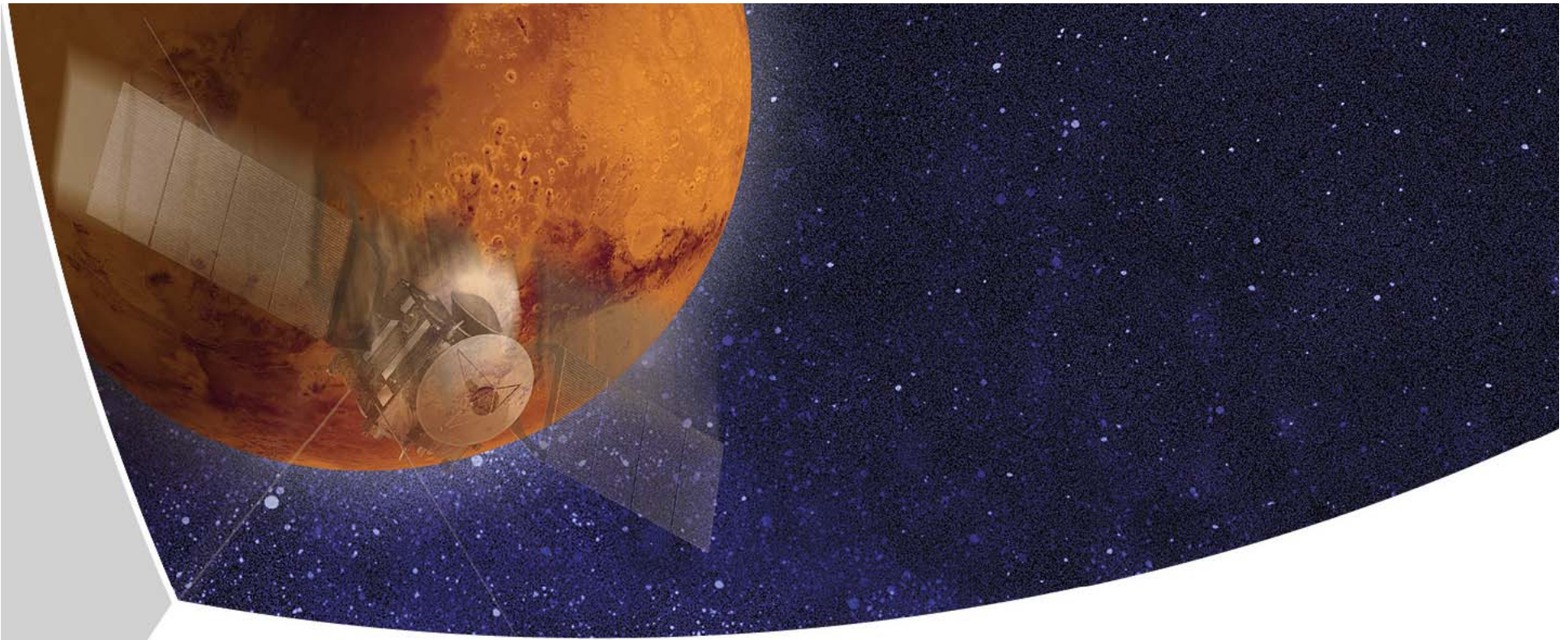
^{13}C -Ions 75MeV/amu LET 29.3 keV/ μm





Relevance of premature osteoblastic differentiation

- Ionizing radiation has negative effects on bone microarchitecture (trabecular bone).
- Impairment of proliferation and osteogenic differentiation potential of bone cells may contribute to post-irradiation osteoporosis.
- Premature differentiation results in a depletion of proliferation competent cells from the osteoblastic precursor pool.
- Decrease of bone mass may be attributed to synergistic effects of exposure to ionizing radiation and weightlessness conditions.
- A cell culture model is necessary, to identify the radiation-specific effects on the skeletal system and to develop countermeasurements.



Thank you
for your attention !



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