# DICHIARAZIONE

## Relatore: NADIA PASINETTI

Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

- Posizione di dipendente in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Consulenza ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Partecipazione ad Advisory Board **(NIENTE DA DICHIARARE)**
- Titolarietà di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Altro

XXIV CONGRESSO NAZIONALE
AIRO2014
Associazione Italiana Radioterapia Oncologica
Padova, 8-11 novembre

*SIMPOSIO AIRO-AIRB*
*Microambiente e*
*modulazione della risposta alla radioterapia*

# STUDIO *in vitro* DELL'EFFETTO FIBRINOLITICO INDOTTO DALL'INIBIZIONE DELLA VIA Rho/ROCK ATTRAVERSO L'ATTIVAZIONE DELLA METALLOPROTEASI 2 (MMP2)

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UNIVERSITA' DI BRESCIA

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The microenvironment of malignant solid tumors is totally different from that of normal tissues, being characterized by marked diversities in pH, the distribution of nutrients, and oxygen concentrations, and so forth.

To understand this heterogeneity is important in cancer radiation therapy because it influences the effect of ionizing radiation through various mechanisms.

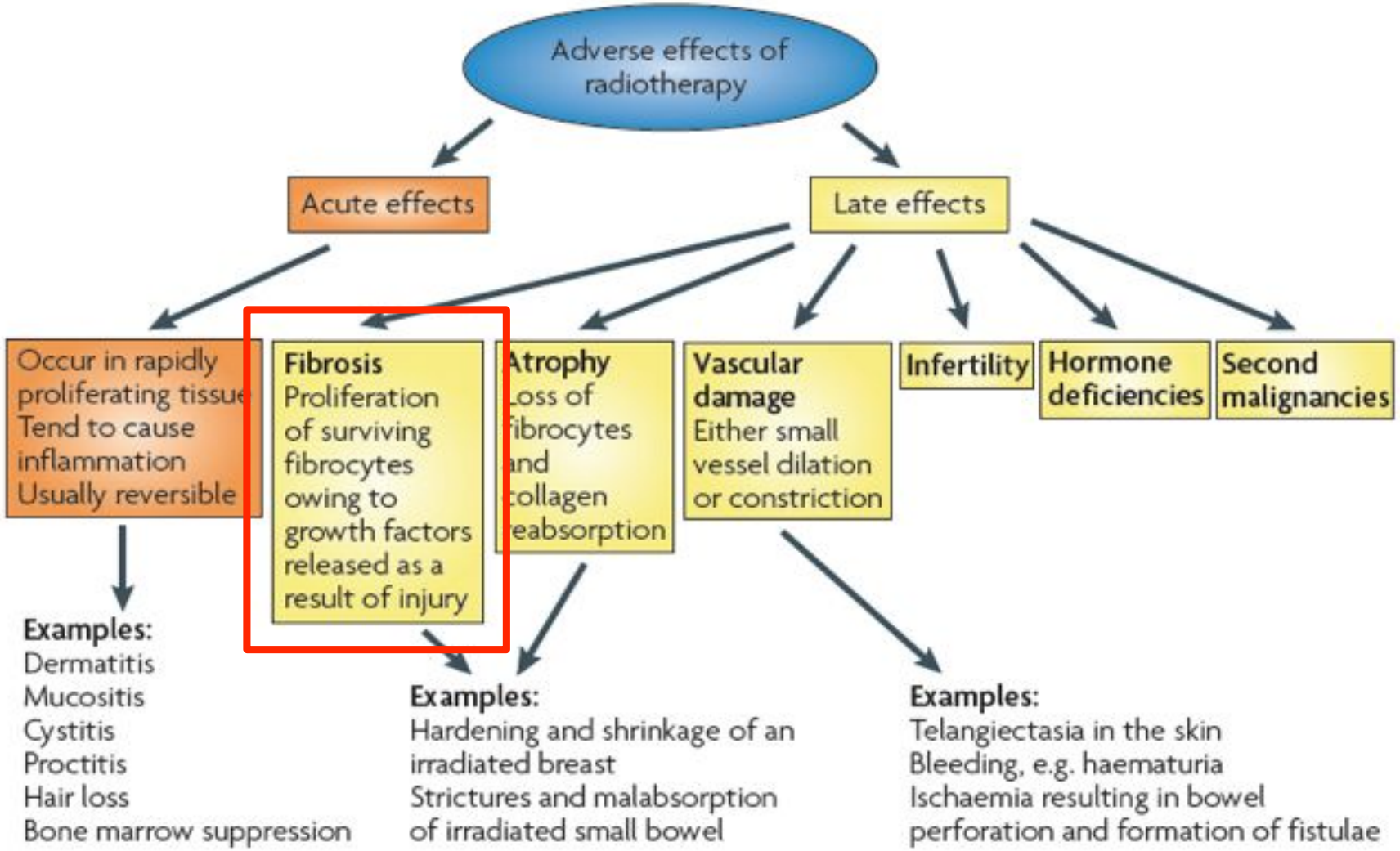
# RADIATION EFFECTS ON NORMAL TISSUES

Radiation therapy remains a cornerstone of modern cancer management.

In Italy, > 150000 new patients every year need a treatment.

One great challenge of moderne radiation protection and radiation therapy is the development of **individualized treatment regimes** by:

- early prediction of individual radiosensitivity and outcome
- protecting normal tissues from radiation injury by increasing its tolerance or treating the cellular and molecular defects that disturbe tissue homeostasis and interfere with proper wound healing.




Barnett GC. Et al., **Nature Rew Cancer 2009**

# BACKGROUND

*Clin Cancer Res* 2007;13:5331-5340.

**Pravastatin Inhibits the Rho/CCN2/Extracellular Matrix Cascade in Human Fibrosis Explants and Improves Radiation-Induced Intestinal Fibrosis in Rats**

Valérie Haydont,[1,4] Céline Bourgier,[1,4] Marc Pocard,[2,6] Antoine Lusinchi,[3] Jocelyne Aigueperse,[5] Denis Mathé,[1] Jean Bourhis,[1,3] and Marie-Catherine Vozenin-Brotons[1,4]

ELSEVIER

Int. J. Radiation Oncology Biol. Phys., Vol. 68, No. 5, pp. 1471–1482, 2007

0360-3016/07/$–see front matter

doi:10.1016/j.ijrobp.2007.03.044

**BIOLOGY CONTRIBUTION**

**SUCCESSFUL MITIGATION OF DELAYED INTESTINAL RADIATION INJURY USING PRAVASTATIN IS NOT ASSOCIATED WITH ACUTE INJURY IMPROVEMENT OR TUMOR PROTECTION**

VALÉRIE HAYDONT, PH.D.,*† OLIVIER GILLIOT, M.D.,* SOFIA RIVERA, M.D.,* CÉLINE BOURGIER, M.D.,*† AGNÈS FRANÇOIS, PH.D.,*† JOCELYNE AIGUEPERSE, PH.D.,‡ JEAN BOURHIS, M.D., PH.D.,* AND MARIE-CATHERINE VOZENIN-BROTONS, PH.D.*†

*Current Drug Targets*, 2010, *11*, 1395-1404 1395

**Modulation of the Rho/ROCK Pathway in Heart and Lung after Thorax Irradiation Reveals Targets to Improve Normal Tissue Toxicity**

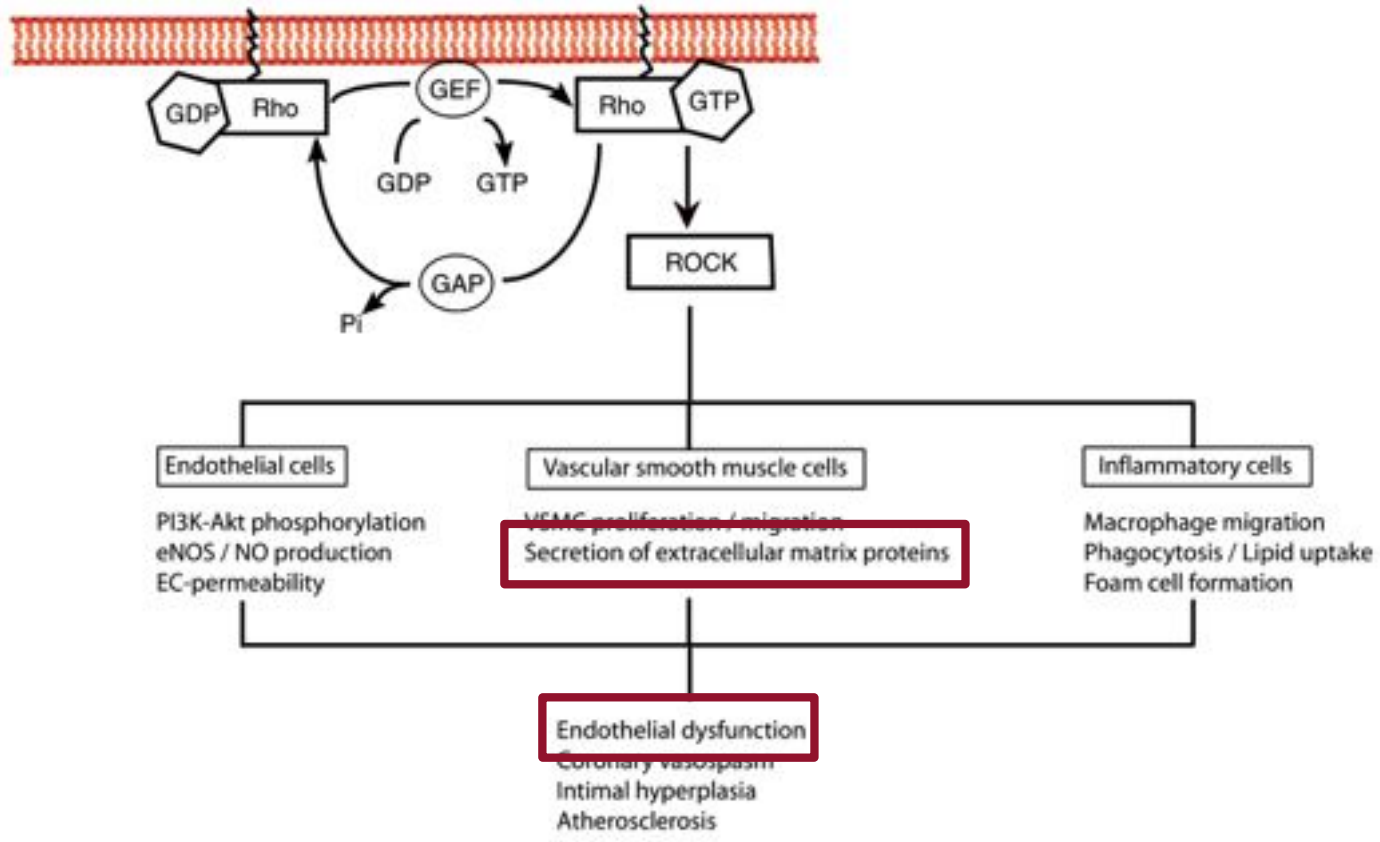
Virginie Monceau[1,2], Nadia Pasinetti[1,6], Charlotte Schupp[1], Fred Pouzoulet[1,5], Paule Opolon[3] and Marie-Catherine Vozenin[*,1,2,4]

Radiation-Induced Pulmonary Fibrosis is a chronic, progressive, fibrosing interstitial pneumonia.

The characteristics of the histopathology include fibroblast foci, interstitial fibrosis and honeycomb changes, which are generated due to fibroblast proliferation and excessive ECM deposition.

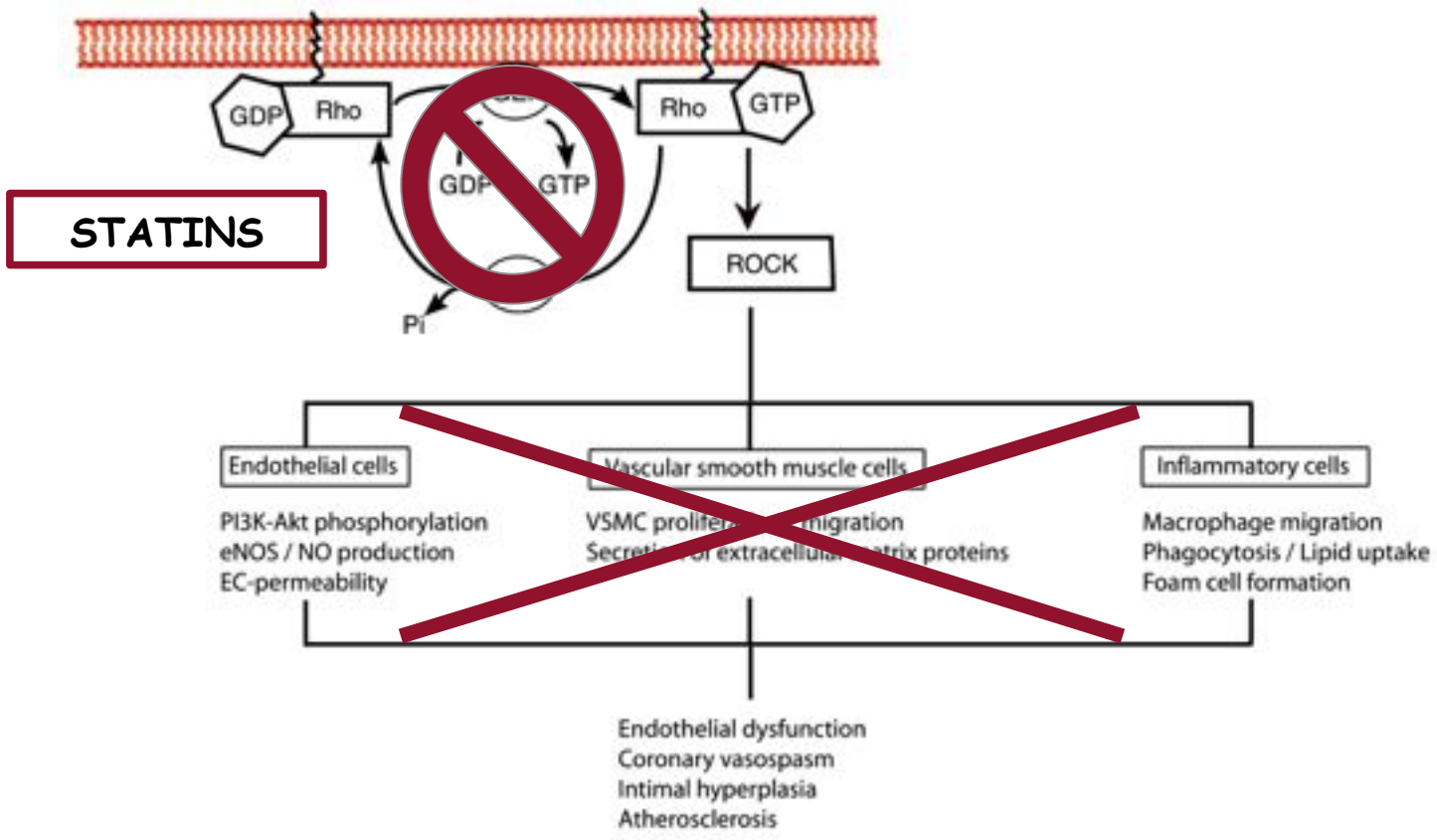
J IH et al., **Biomed Rep. 2014**

# Regulation of the Rho GTPase cycle




Zhou Q, Liao JK **Circulation Journal 2010**

# Regulation of the Rho GTPase cycle




Zhou Q, Liao JK **Circulation Journal 2010**

**Statins** are HMG-CoA reductase inhibitors that were originally developed as lipid-lowering agents. However, they have also demonstrated potent anti-inflammatory and antithrombotic properties.

Jain MK, Ridker PM. **Nature Rev. Drug Discov. 2005**

**Statins** have shown potential as mitigators of late radiation damage by decreasing lung fibrosis and increasing survival when started 8 weeks post-irradiation.

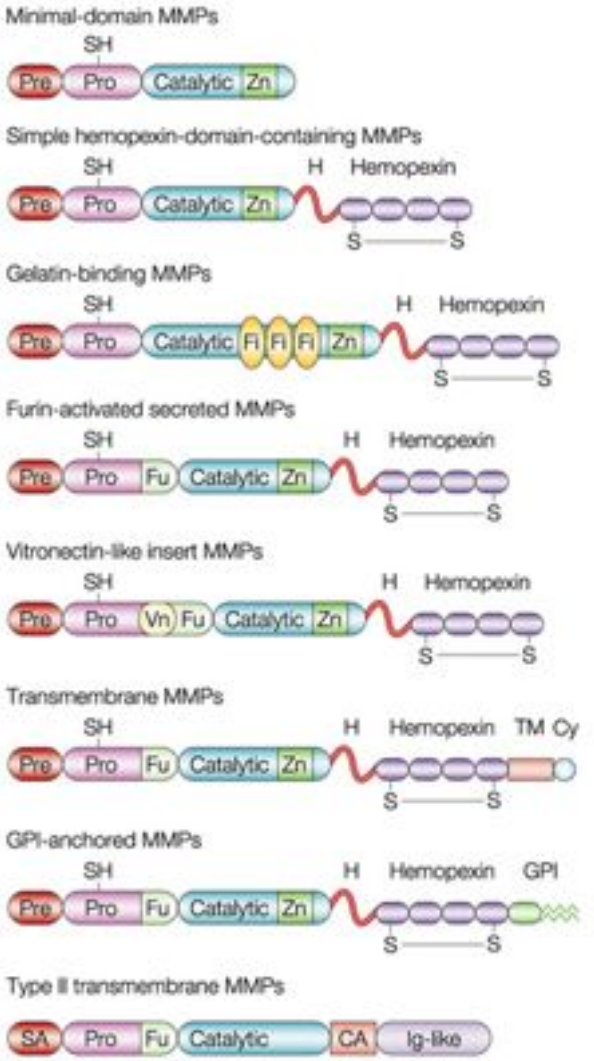
Williams JP, et al. **Radiat. Res. 2004**

**Statins** can ameliorate delayed radiation-induced damage in the intestine when started 2 weeks before radiation.

Wang J, et al. **Int. J. Radiat. Oncol. Biol. Phys. 2007**

**Statins** have potential in radiation oncology, as they are relatively safe and can mitigate radiation-induced enteropathy when given 14 days alter radiation without allecting the efficacy of radiation on tumours.

Haydont V, et al. **Int. J. Radiat. Oncol. Biol. Phys. 2007**

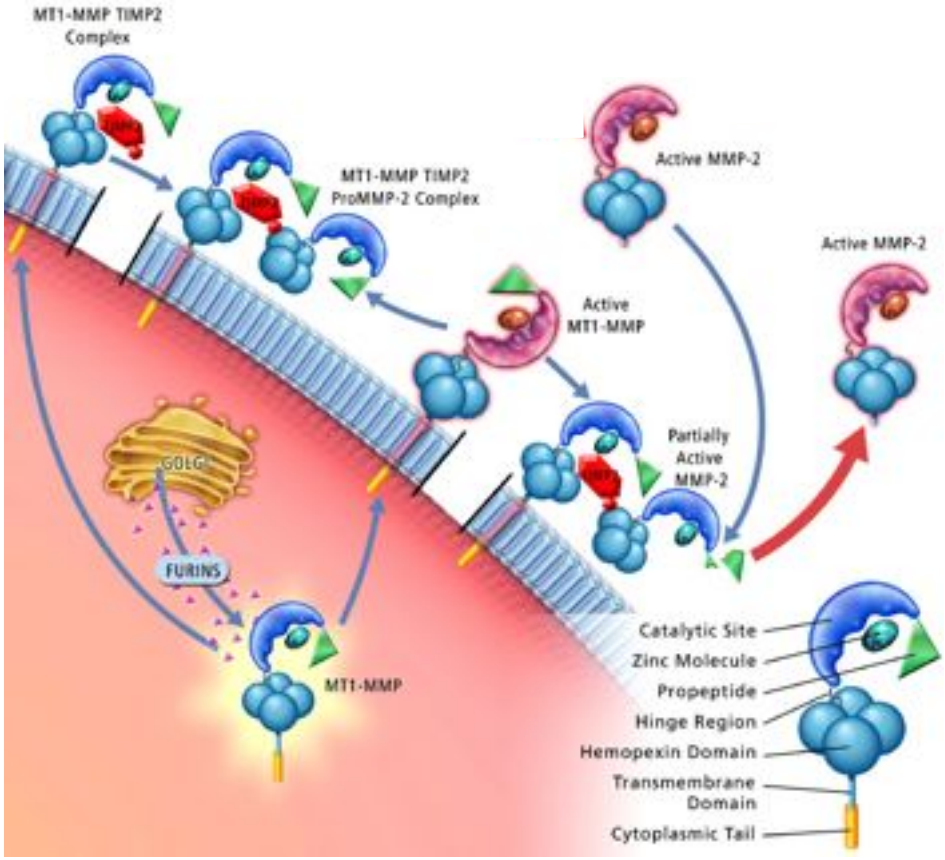



## Metalloproteinases (MMP)

Family of zinc-dependent proteins, that are capable of degrading the major components of the extracellular matrix (ECM). The MMPs include the collagenases, gelatinases and many others. They are inhibited by proteins known as tissue inhibitors of matrix metalloproteinases (TIMP).

Metalloproteinases play an important role in the degradation of the collagen fibers present in the fibrotic tissue.

Egeblad M and Werb Z, **Nature Reviews Cancer 2002**



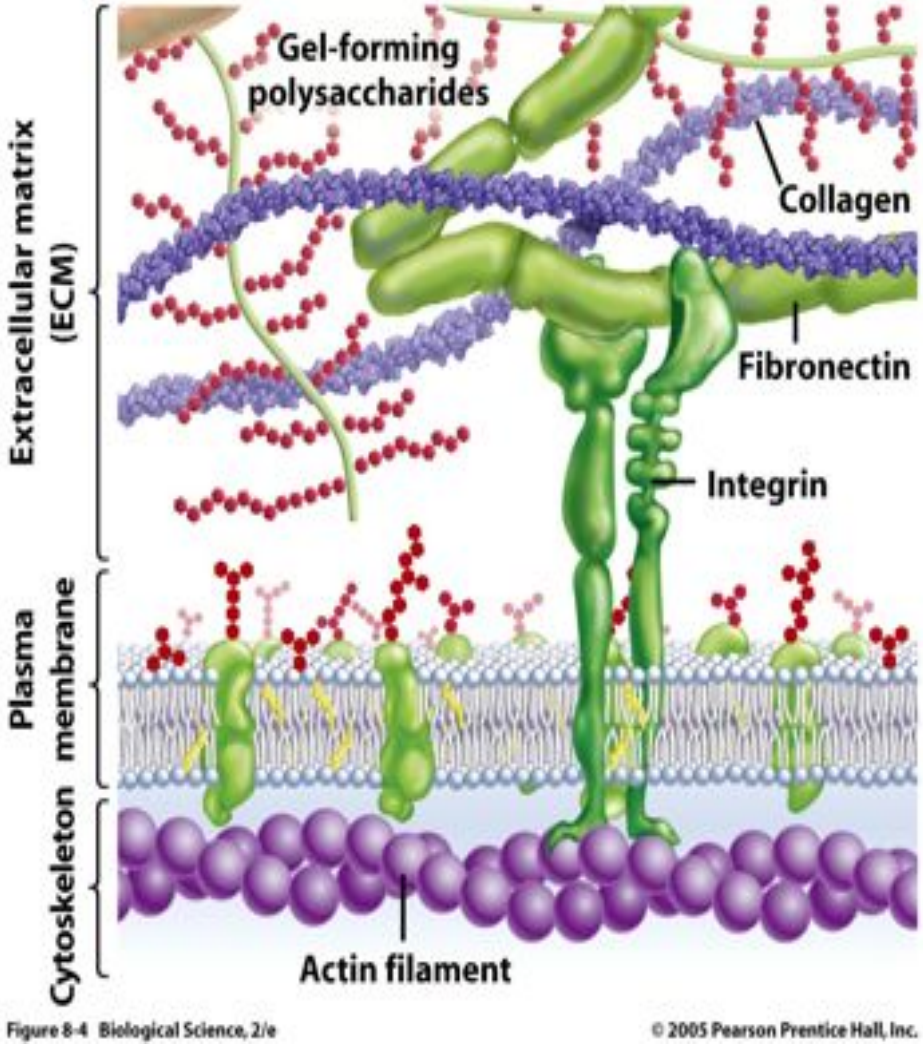

## Metalloproteinase 2

Primary function is degradation of proteins in the extracellular matrix. It proteolytically digests gelatin (denatured collagen), and types IV, V, VII, IX and X collagen. Physiologically, MMP2 in coordination with other MMPs, play a role in normal tissue remodeling events and wound healing.

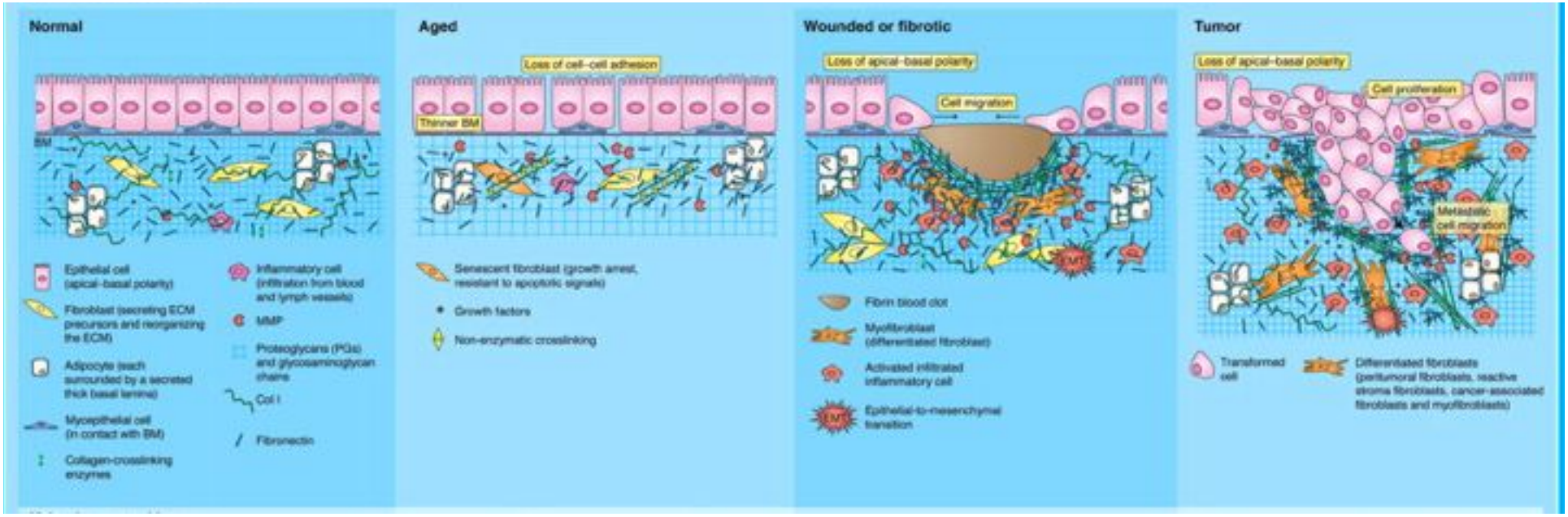
Dollery C.M., Libby P., **Cardiovascular Research 2006**

## EXTRACELLULAR MATRIX (ECM)

The extracellular matrix is composed of structure proteins : collagen and elastin, and of a fluid gel made of sulphated proteoglycans, such as hyaluronic acid, glucosaminoglycans (*GAG*), chondroïtine sulphate, keratin sulphate and glucosamine sulphate.

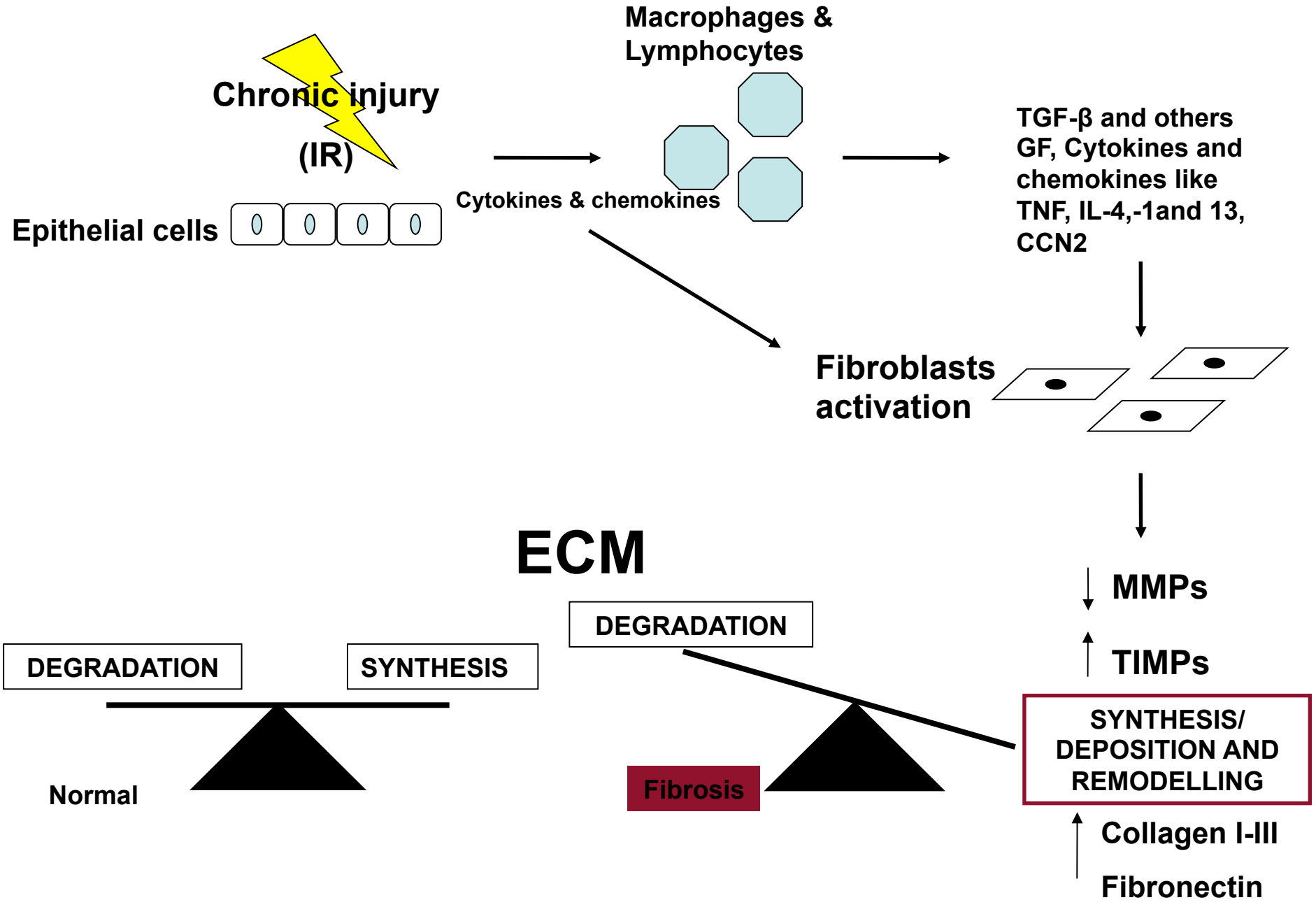


# ECM STRUCTURE




Frantz G. et al., **J Cell Sci 2010**

# Extracellular matrix (ECM) and MMPs in fibrosis

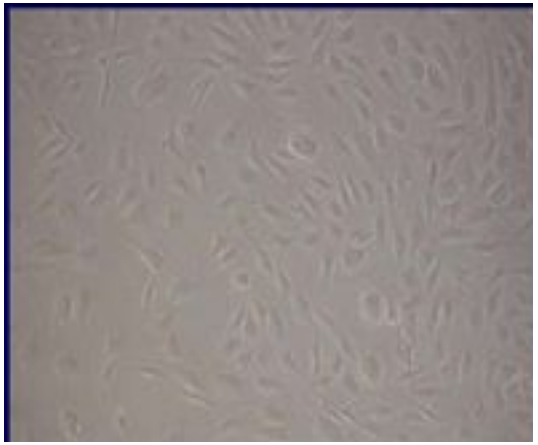


## QUESTION 1:

Is there an *in vitro* effect on MMP2 fibroblast secretion after radiation ?

# MATERIALS & METODS

IR 2Gy – 8Gy – 16Gy



C57BL6 Lung fibroblast cultures

4 - 8 - 24 and 48h after IR

Cells

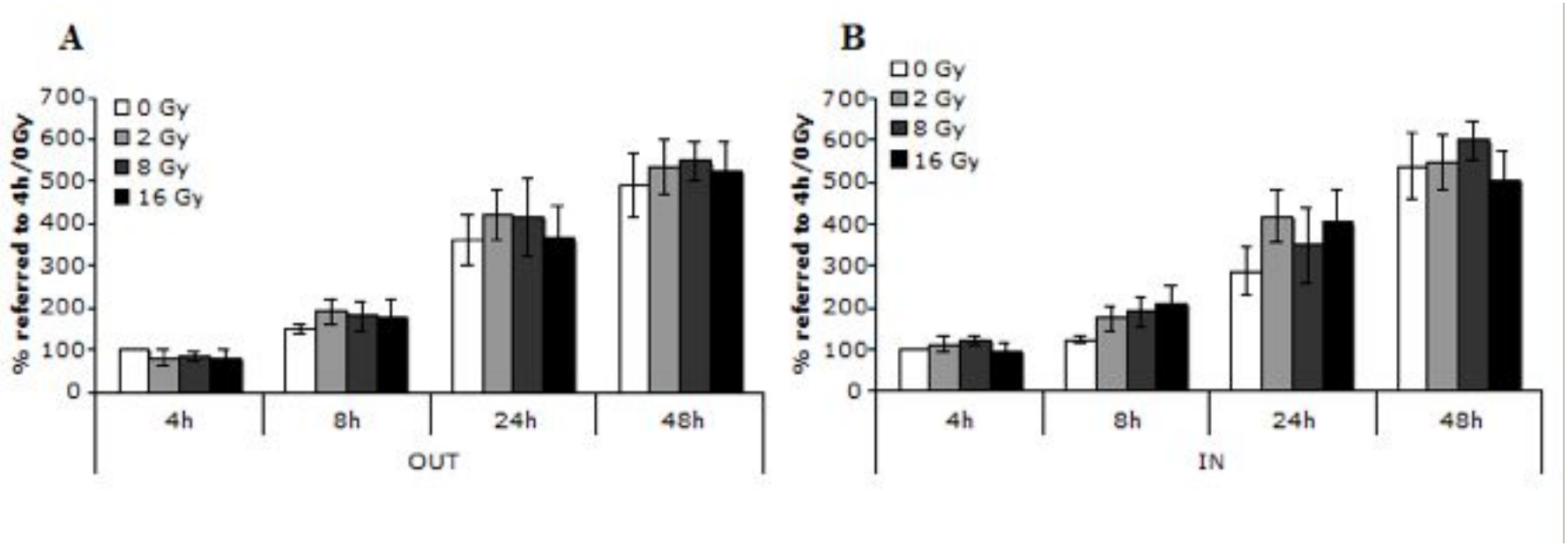
Conditioned medium

STUDY of MMP 2 ACTIVITY by ZYMOGRAPHY

# RESULTS

## Extracellular MMP2

## Intracellular MMP2




Radiations does not influence the viability of the cells

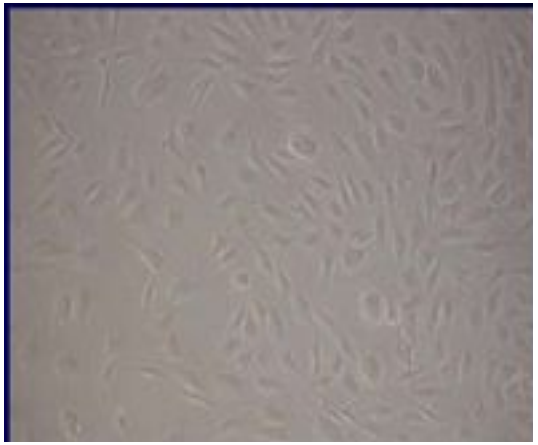
No significant differences between the relative amount of extracellular and intracellular MMP2 deriving from radiated samples compared to non-irradiated samples

## QUESTION 2:

Is there an *in vitro* effect on MMP2 fibroblasts secretion after radiation and Pravastatin exposure ?

# MATERIALS & METODS

PRAVASTATIN 500$\mu$M or 1000$\mu$M



C57BL6 Lung fibroblast cultures

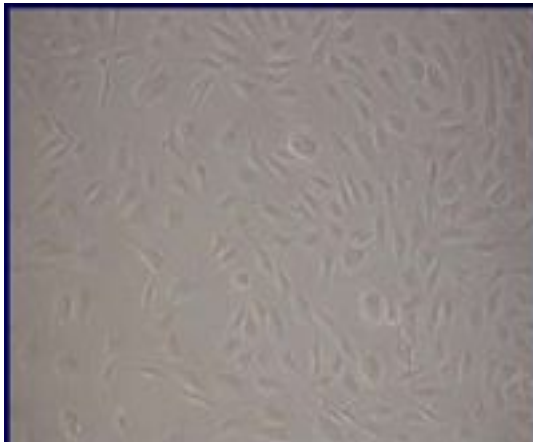
4 - 8 - 24 and 48h after IR

Cells

Conditioned medium

STUDY of MMP 2 ACTIVITY by ZYMOGRAPHY

IR 2Gy – 8Gy – 16Gy
PRAVASTATIN 500μM or 1000μM



C57BL6 Lung fibroblast cultures

4 - 8 - 24 and 48h after IR

Cells

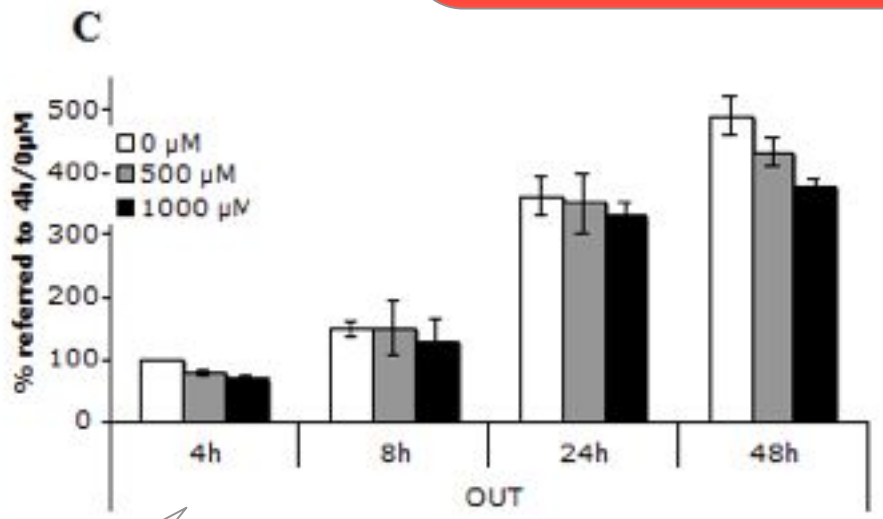
Conditioned medium

STUDY of MMP 2 ACTIVITY by ZYMOGRAPHY

# RESULTS

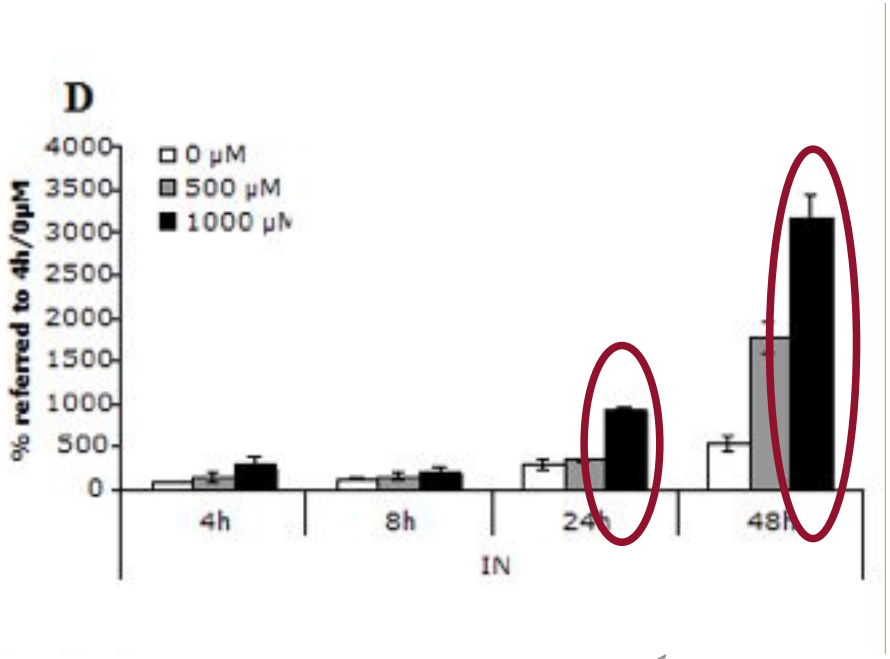
## Extracellular MMP2

By prolonging the time-exposure to the drug, we observed fluctuations in the relative amount of MMP2 in the culture media while after 48h treatment, significant differences were observed compared to control media, with the highest significance at the higher dose.




After 4h we observed a reduced relative amount of MMP2 in the culture media of Pravastatin-treated cells compared to untreated controls.

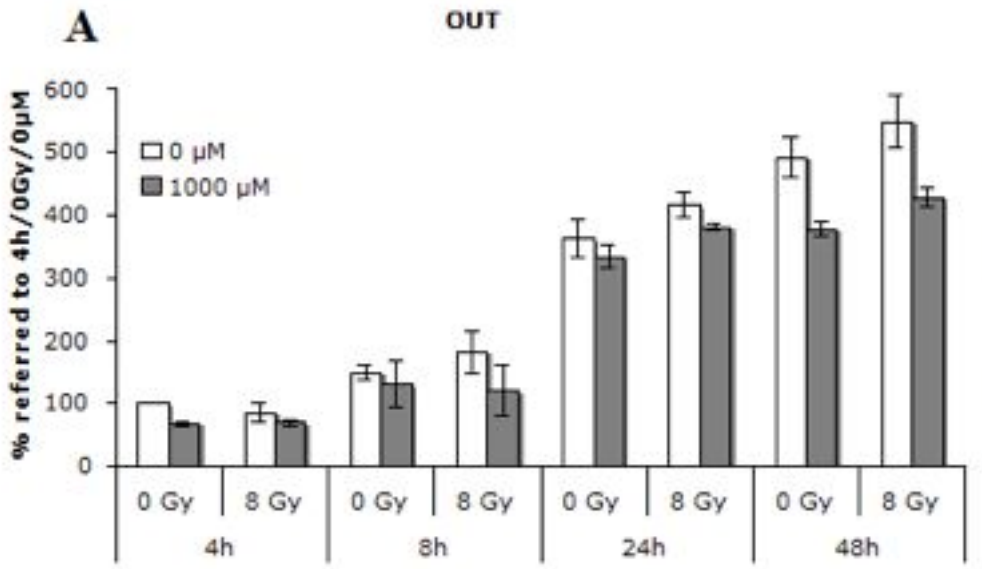
# Intracellular MMP2




Significant changes were observed at short times (4h) and in particular the treatment with the drug induced an increase of the amount of intracellular collagenase, especially evident for the maximum dose and long times (24h/48h).

These results suggest that cell irradiation has little, if any, effect on the secretion of MMP2 while Pravastatin seems to play an important role in the mechanisms that control the secretion of MMP2.

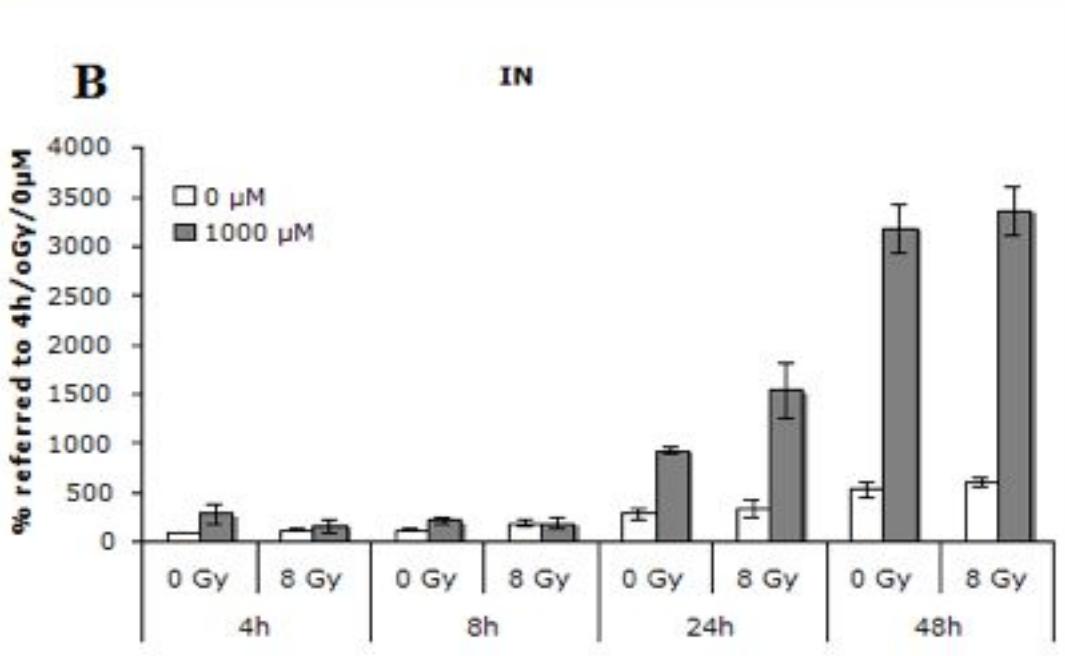
# Extracellular MMP2




The relative amount of MMP2 secreted into the culture medium increases with time and in every experimental condition ---> cells do not lose viability.

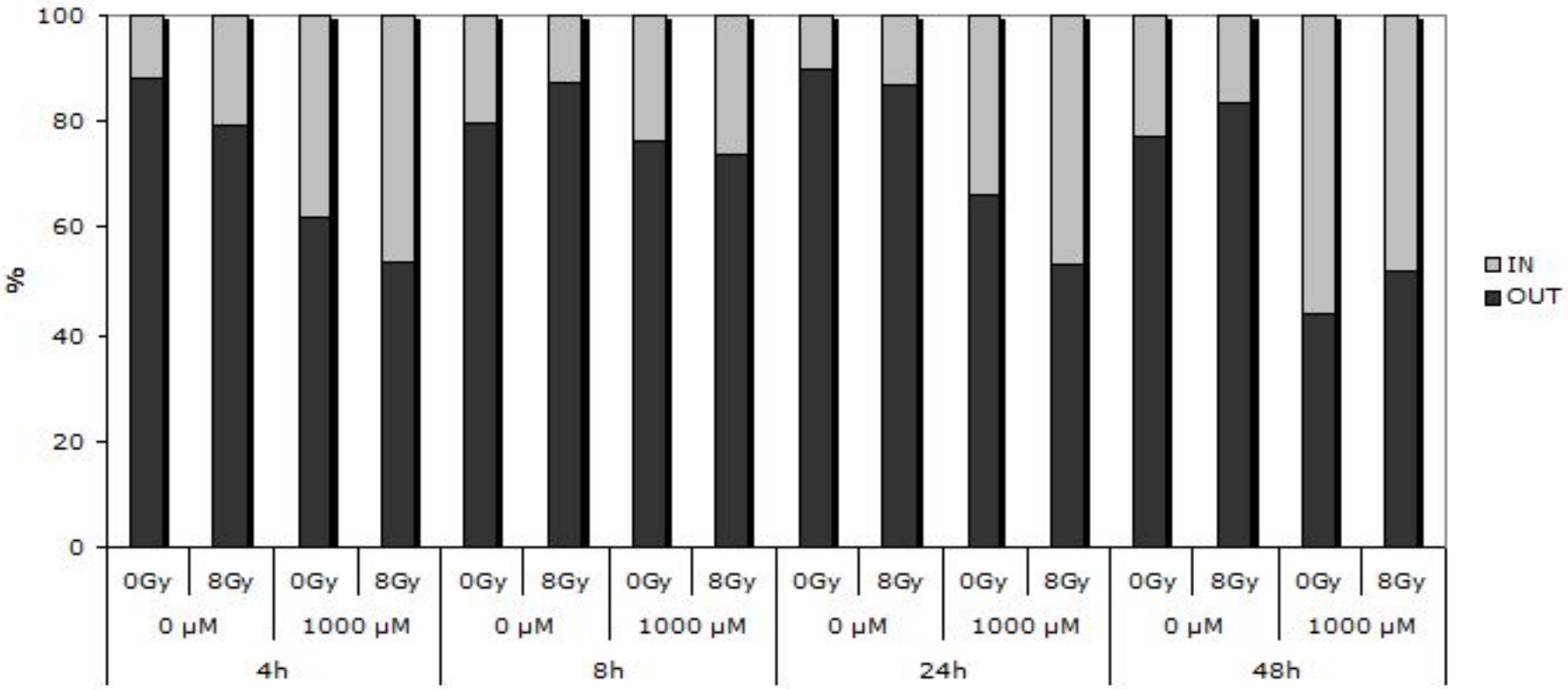
The combined treatment (radiation & Pravastatin) leads to a significant **reduction** of the secretion of MMP2 already after 4h treatment and this is particularly evident at 48h.

# Intracellular MMP2




Independently from radiations treatment, Pravastatin significantly **increases** the amount of intracellular MMP2.

This supports the hypothesis of a possible and specific role played by Pravastatin in the secretion of MMP2.



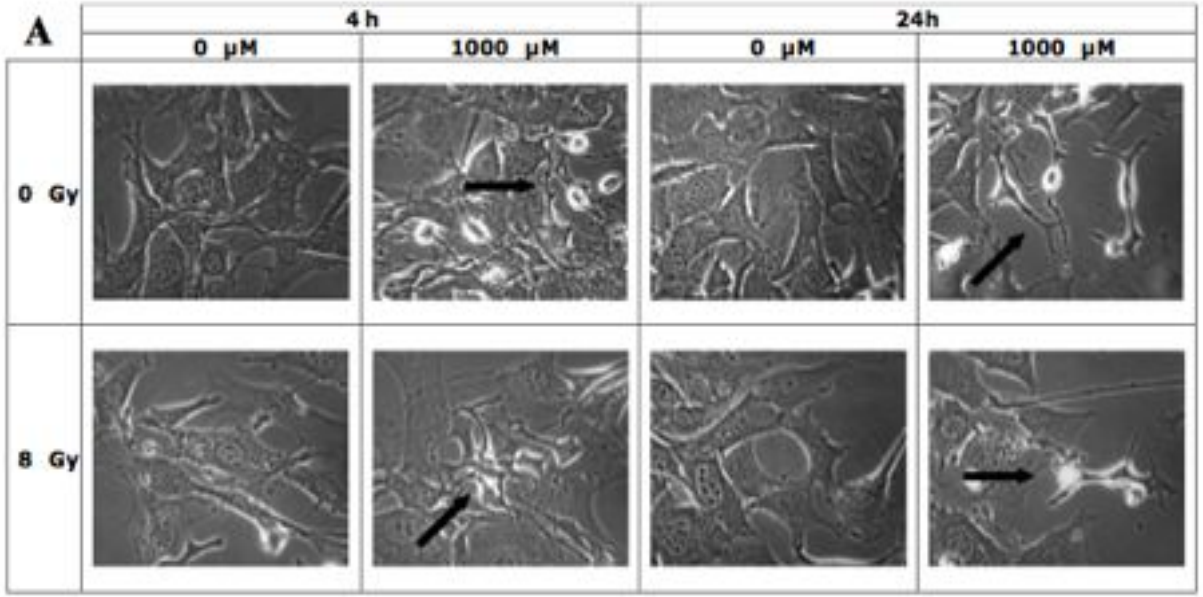

In control samples the % of total MMP2 secreted into the culture medium remains constant over time and is around 85% of total MMP2.

Treatment with radiation (8Gy) does not significantly influence this distribution.

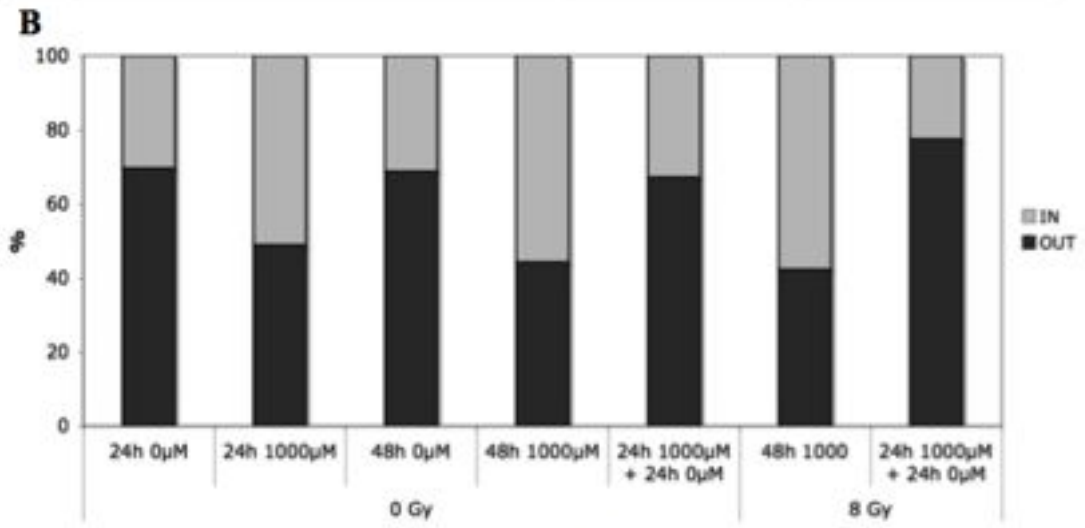
Treatment with Pravastatin higher dose, in association with radiation, decreases to 50% the amount of MMP2 in the medium in favor of the intracellular fraction.

**Pravastatin plays an effect on the MMP2 secretory mechanisms**

# Morphological analysis




Radiation brings to an increase in the cell volume, with the appearance of numerous intracellular vacuoles and an increase in the number of multinucleated cells




After further 24 hours the percentage of MMP2 in the culture media of treted cells shifts from 50% to 80%, a value comparable to non-treated cells.

Cells recovered the typical morphology of control fibroblasts (data not shown).

# IN CONCLUSION

These results show that **radiation** do not lead to significant changes in the mechanisms of secretion of MMP2.

Conversely, treatment with **Pravastatin**, in association with radiation, induces a reduction of the relative amount of MMP2 in the culture medium, probably exerting **a specific effect on the secretion of this collagenase.**

↓

**ANTI-FIBROTIC EFFECT ??**

# PERSPECTIVES

Cumulative experimental evidence indicates that the irradiated tumor microenvironment actively contributes to such aggressive behavior.
Also in normal tissues secondary effects, microenvironment modulated by ionizing radiation and drugs (such as statins) plays an important role.

We now need to move on and conduct innovative translational studies and combination trials to validate or invalidate in patients the molecular pathways uncovered in preclinical models.

**In addition, we need to determine predictive biomarkers (such as gene expression signatures or circulating molecules), to identify patients who are more prone than others to develop delayed healthy tissue toxicity after radiation therapy**