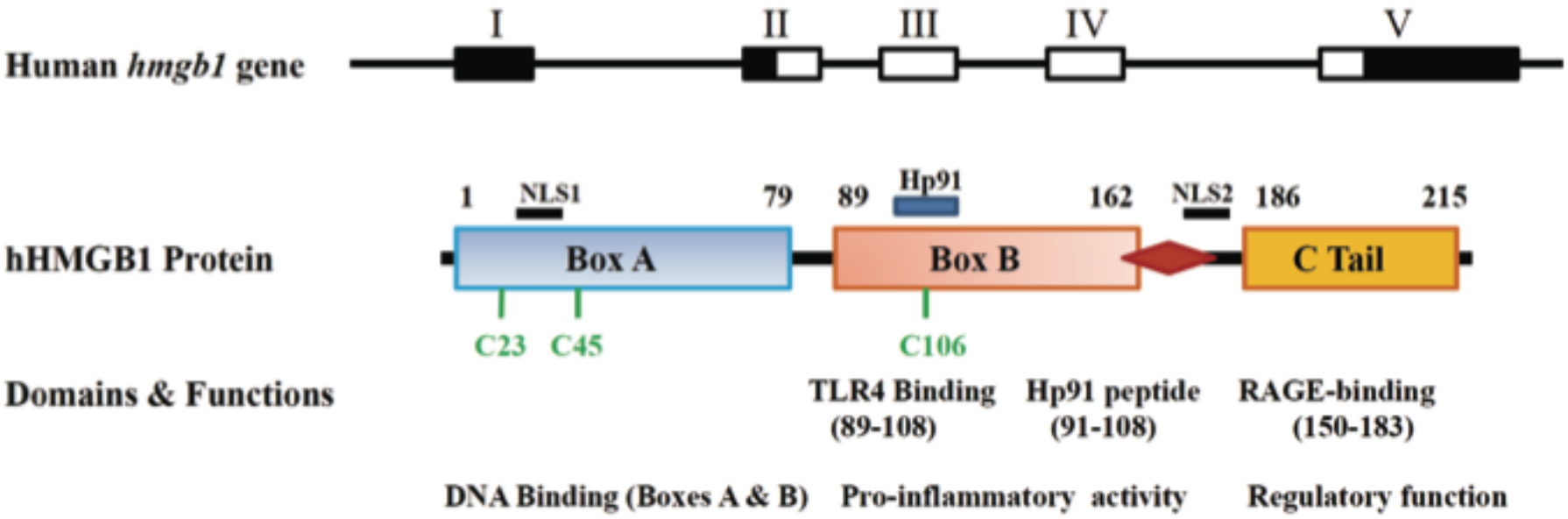
HMGB1 in tumor microenvironment

Prof. Stefania Mardente

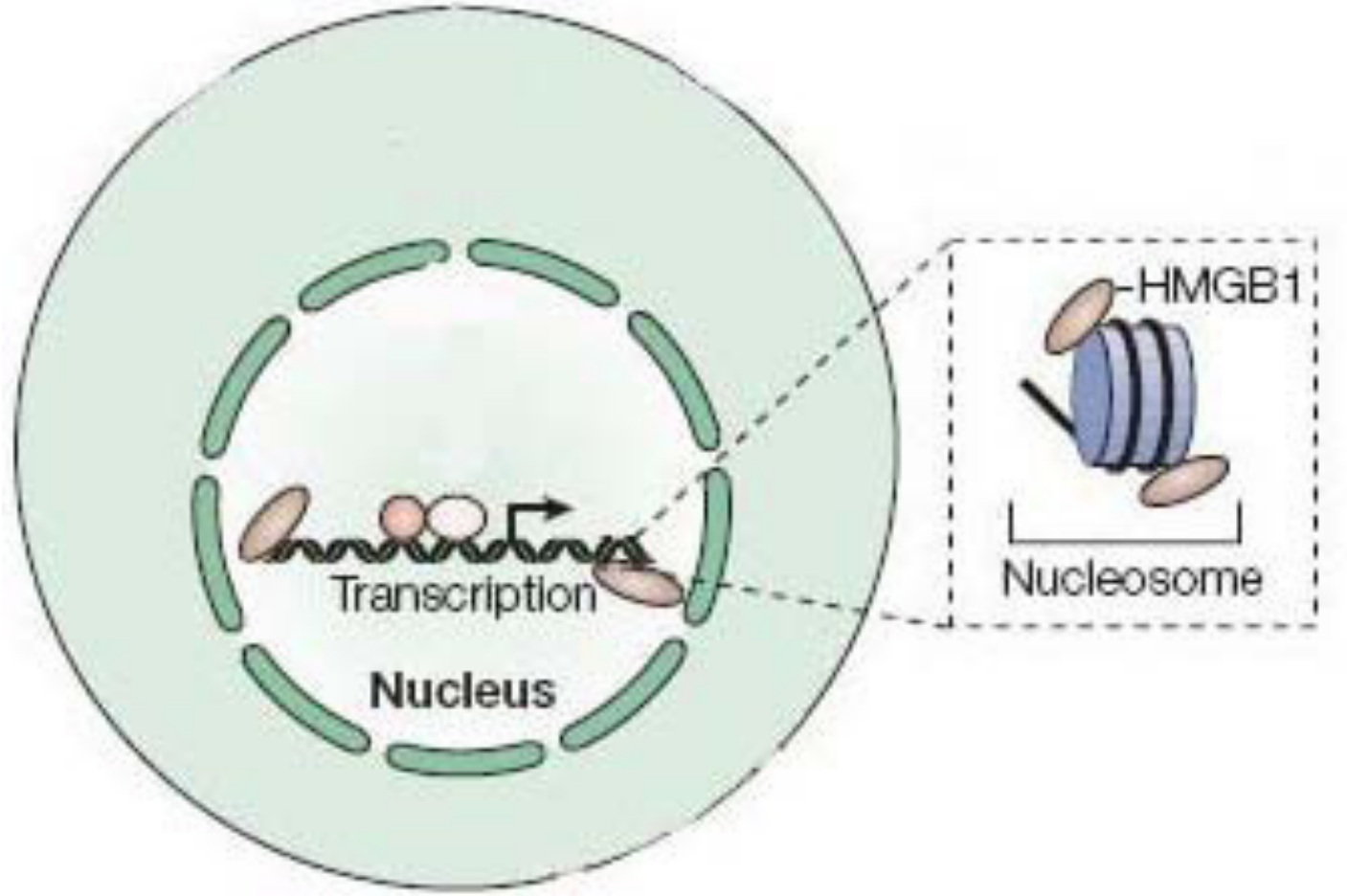
Department of Experimental Medicine

HMGB1: gene, proteins and functions

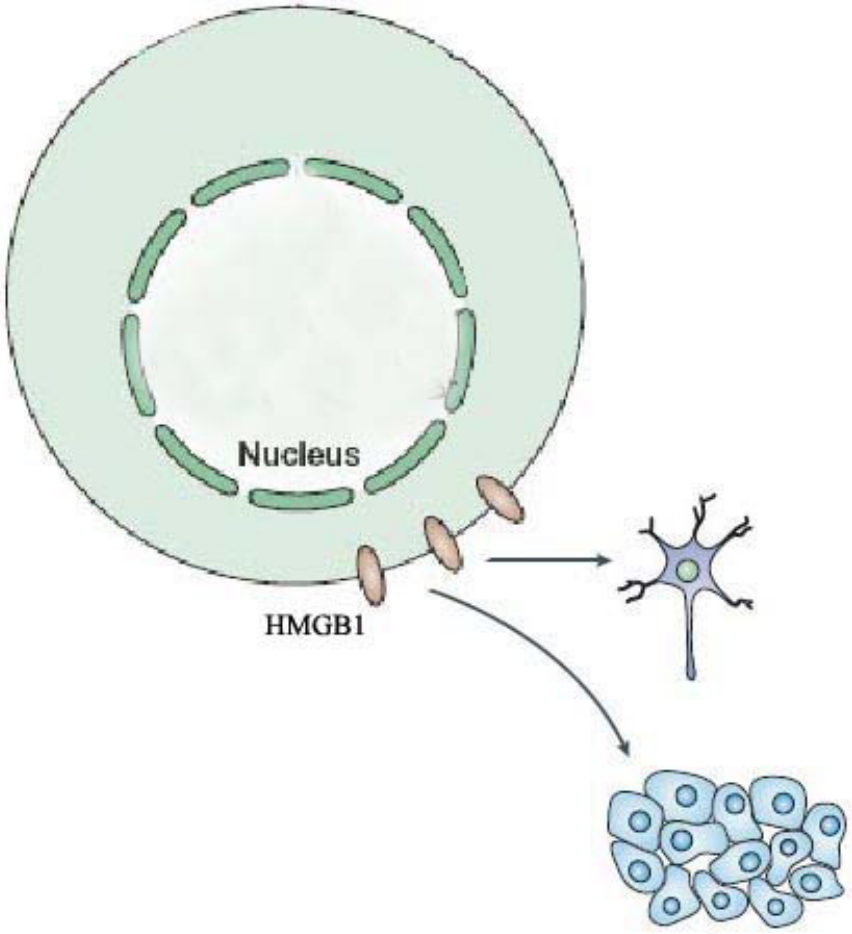


Am J Cancer Res 2013;3(1):1-20

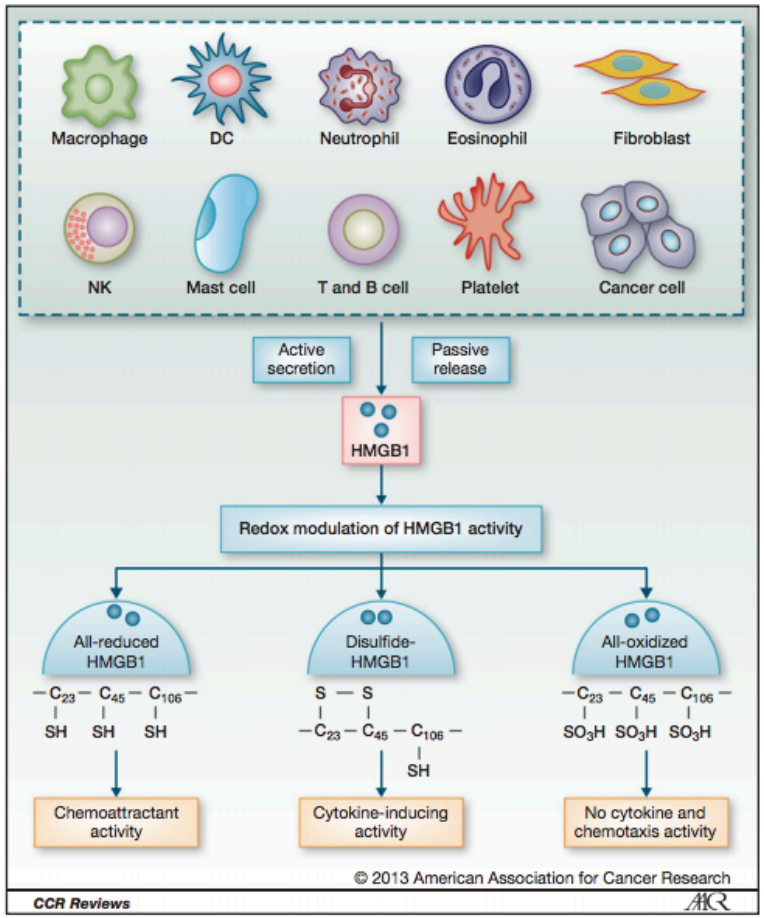
High mobility group box 1- HMG-1
Amphoterin



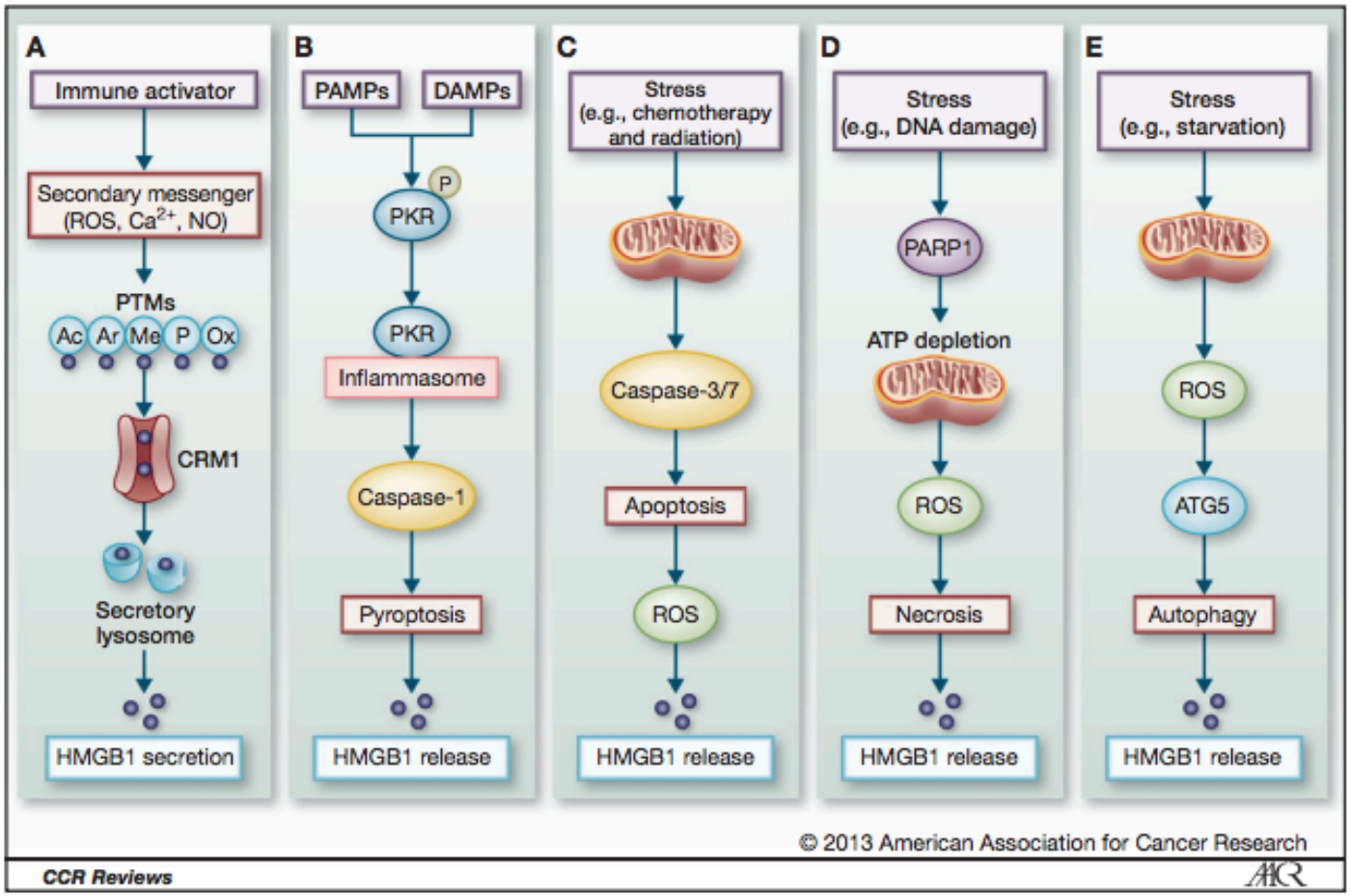
HMGB1 at the cell surface and in extracellular fluid

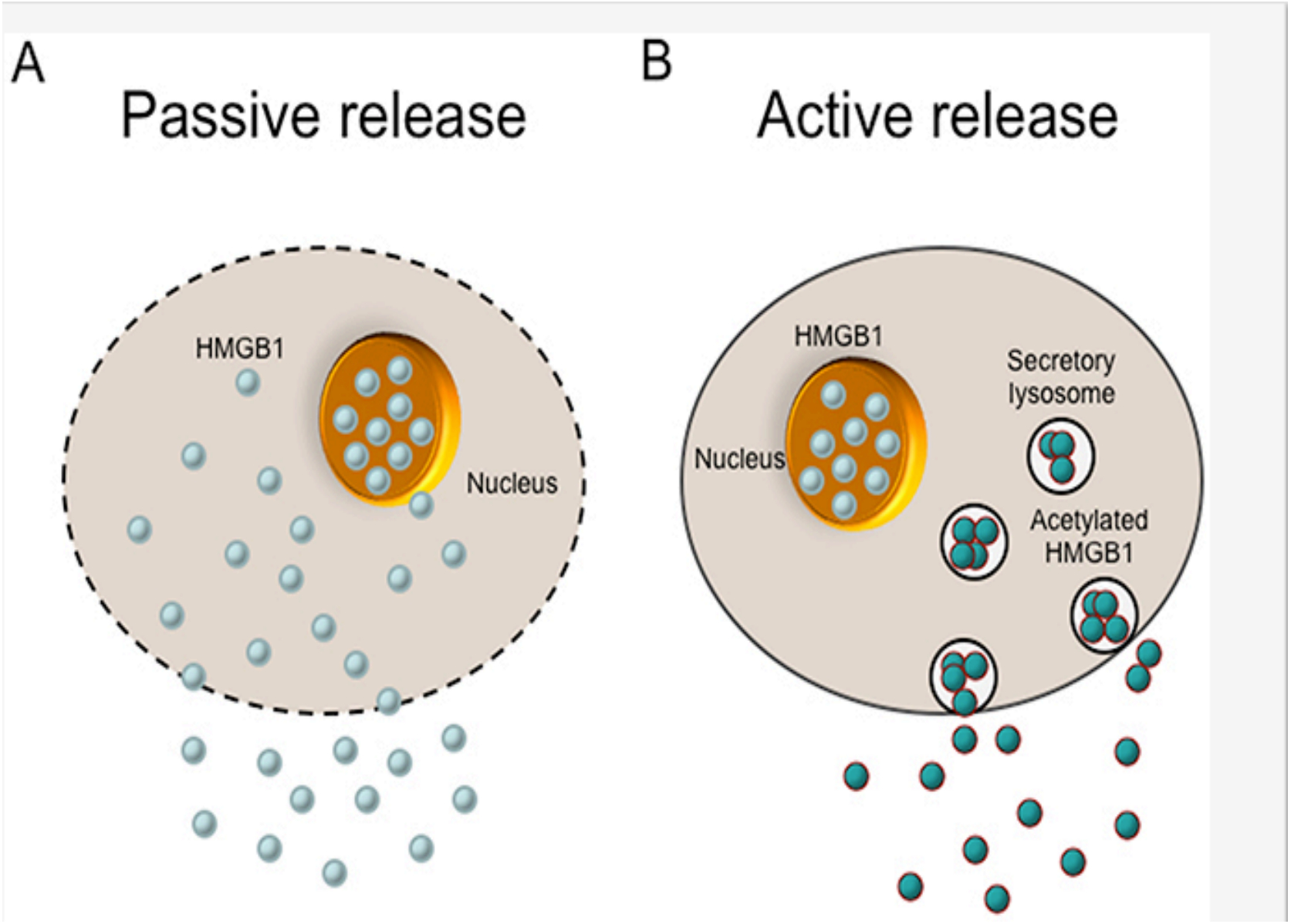


DAMP


Macrophage
DC
Neutrophil
Eosinophil
Fibroblast
NK
Mast cell
T and B cell
Platelet
Cancer cell
Active secretion
Passive release
HMGB1
Redox modulation of HMGB1 activity
All-reduced HMGB1
Disulfide-HMGB1
All-oxidized HMGB1
Chemoattractant activity
Cytokine-inducing activity
No cytokine and chemotaxis activity
© 2013 American Association for Cancer Research
CCR Reviews

HMGB1 is secreted or released



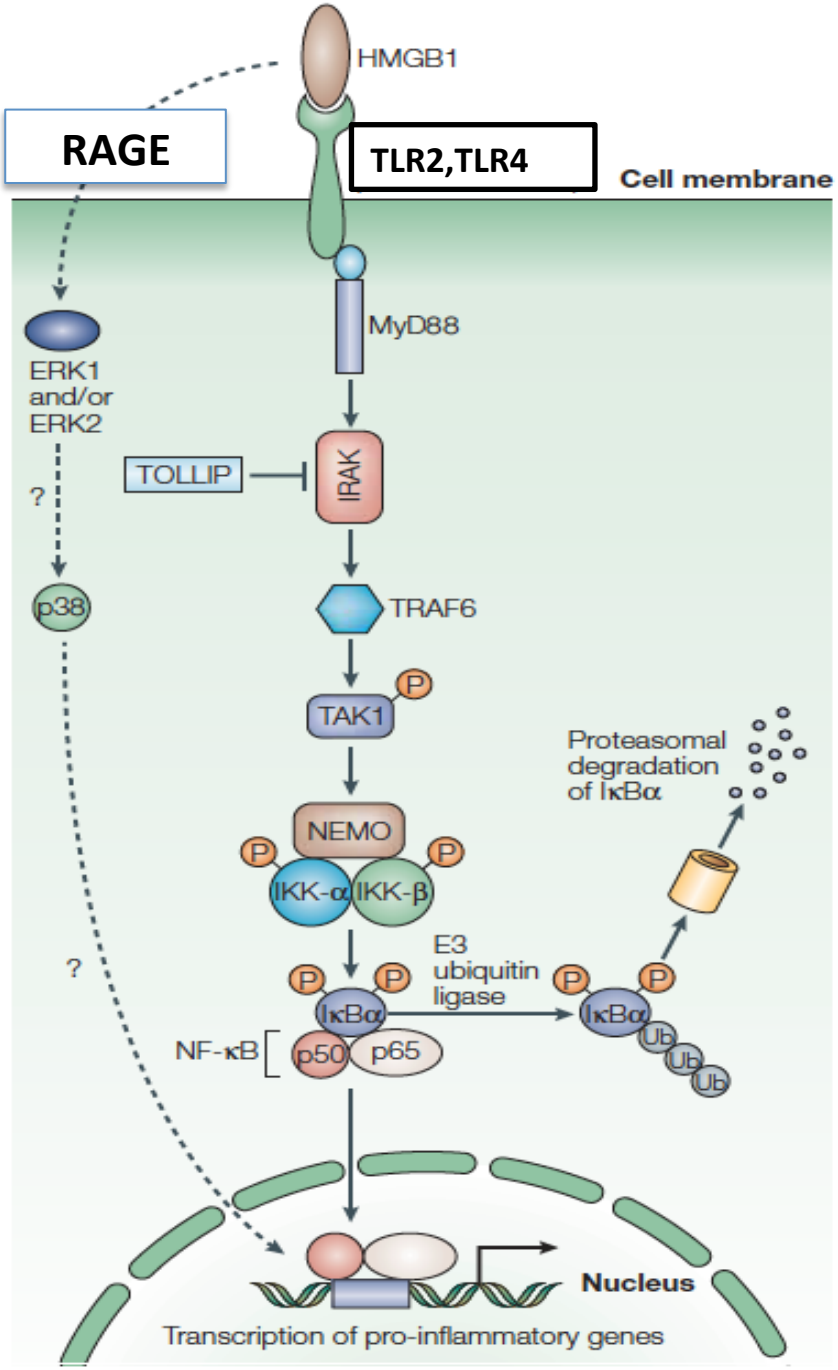


Si Jia Hee et al. Oncotarget 2017

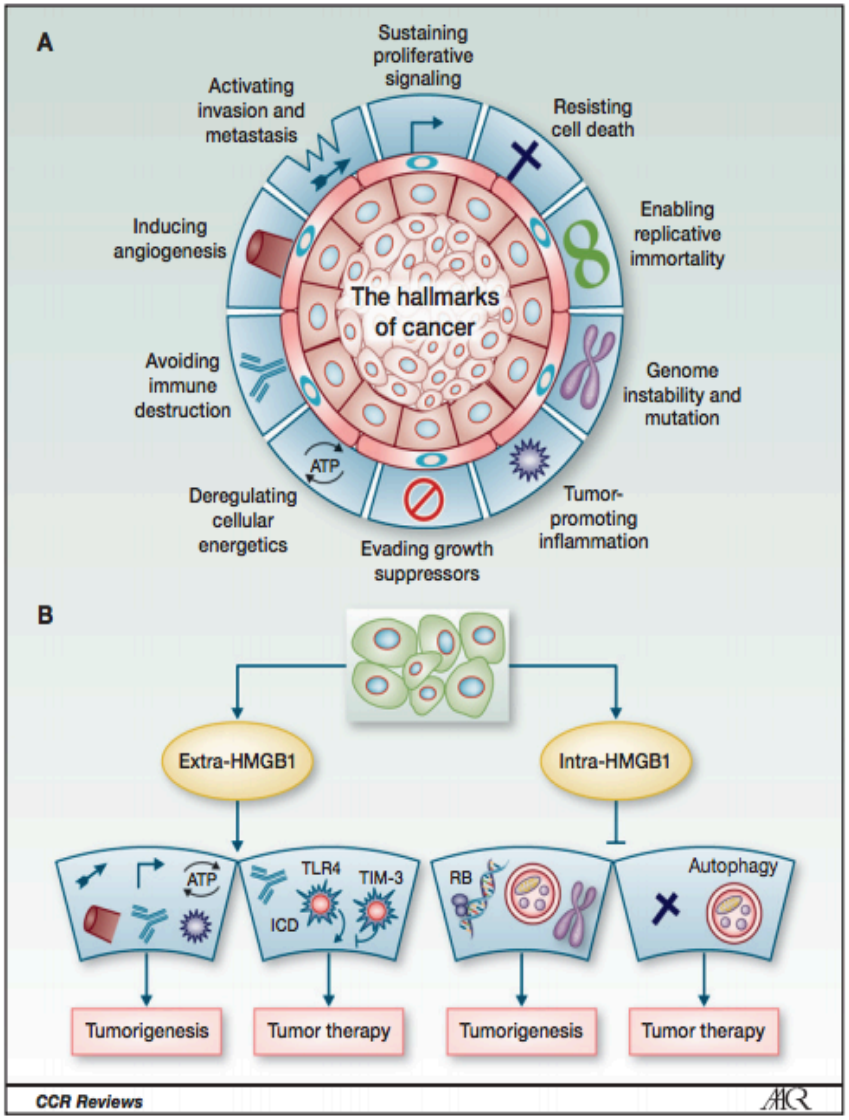
Signal Transduction through RAGE uses two different pathways that converge on NfKB

Signal transduction from TLR2-TLR4 activates transcription protein MyD88

The final activatory transcription factor is NfKB

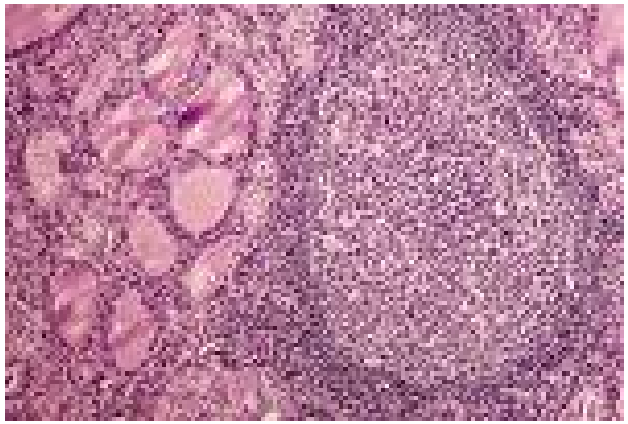


Hallmarks of cancer



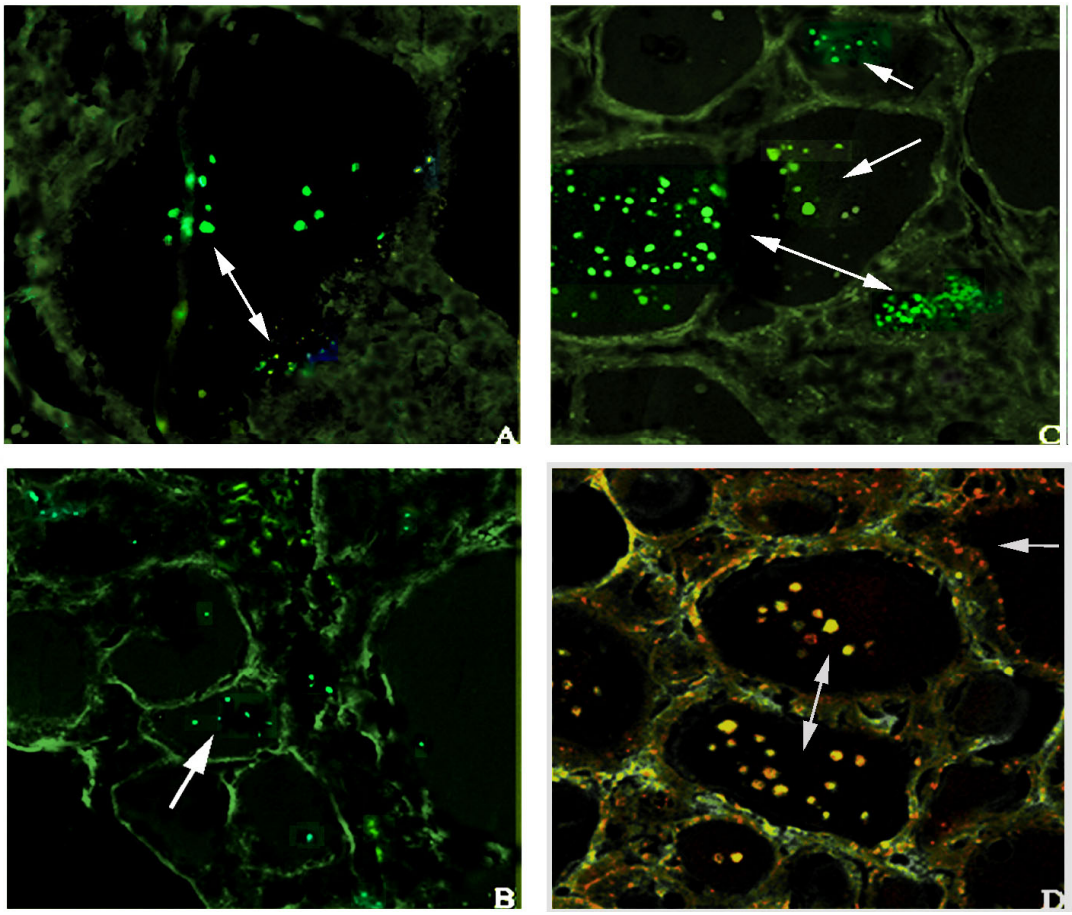
Clin Cancer Res; 19(15)Aug 1,2013

Chronic inflammation : a risk factor for cell transformation

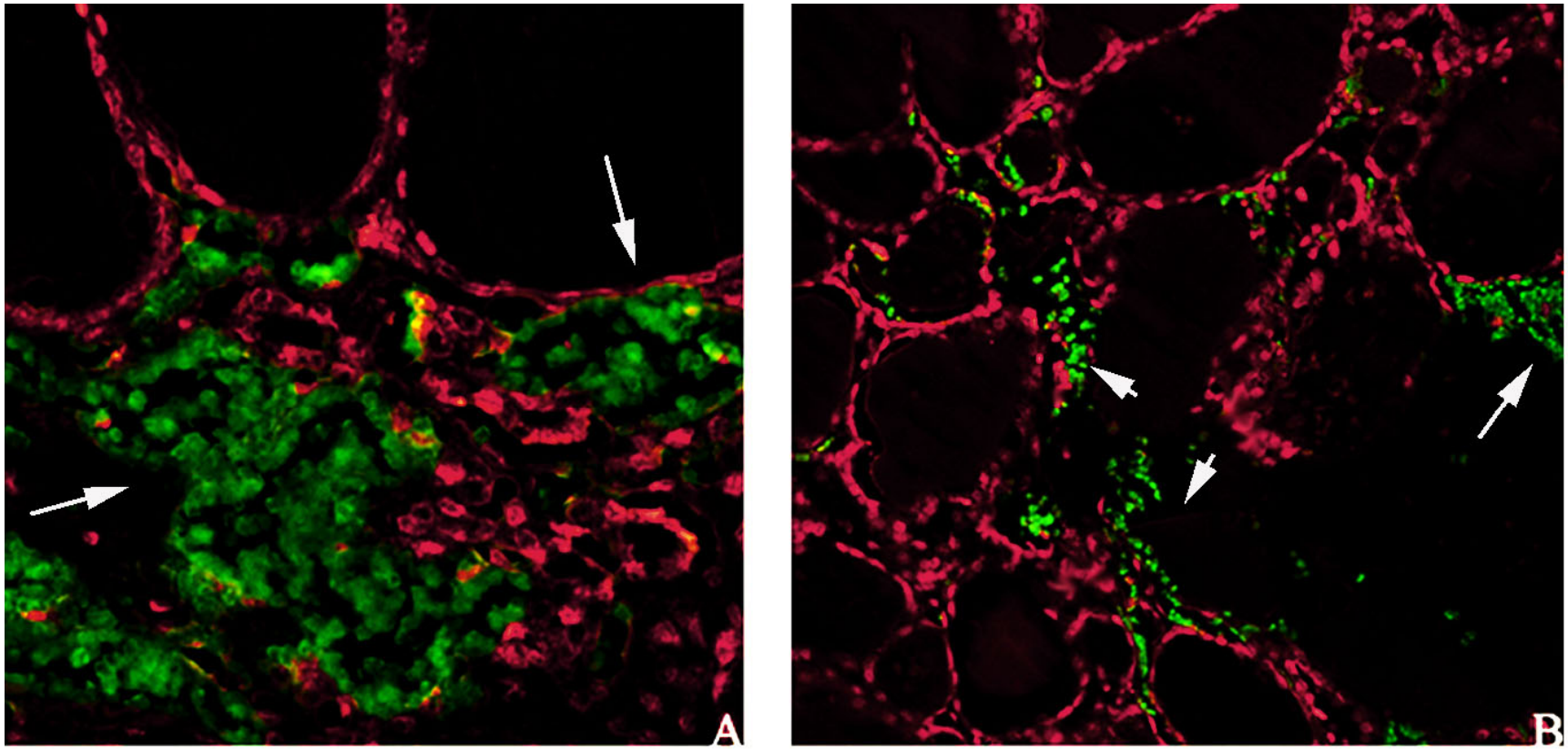


Hashimoto Thyroiditis

Green: IFNγ− CD4+ and CD8+
Red: IL 4- CD4+ and CD8+



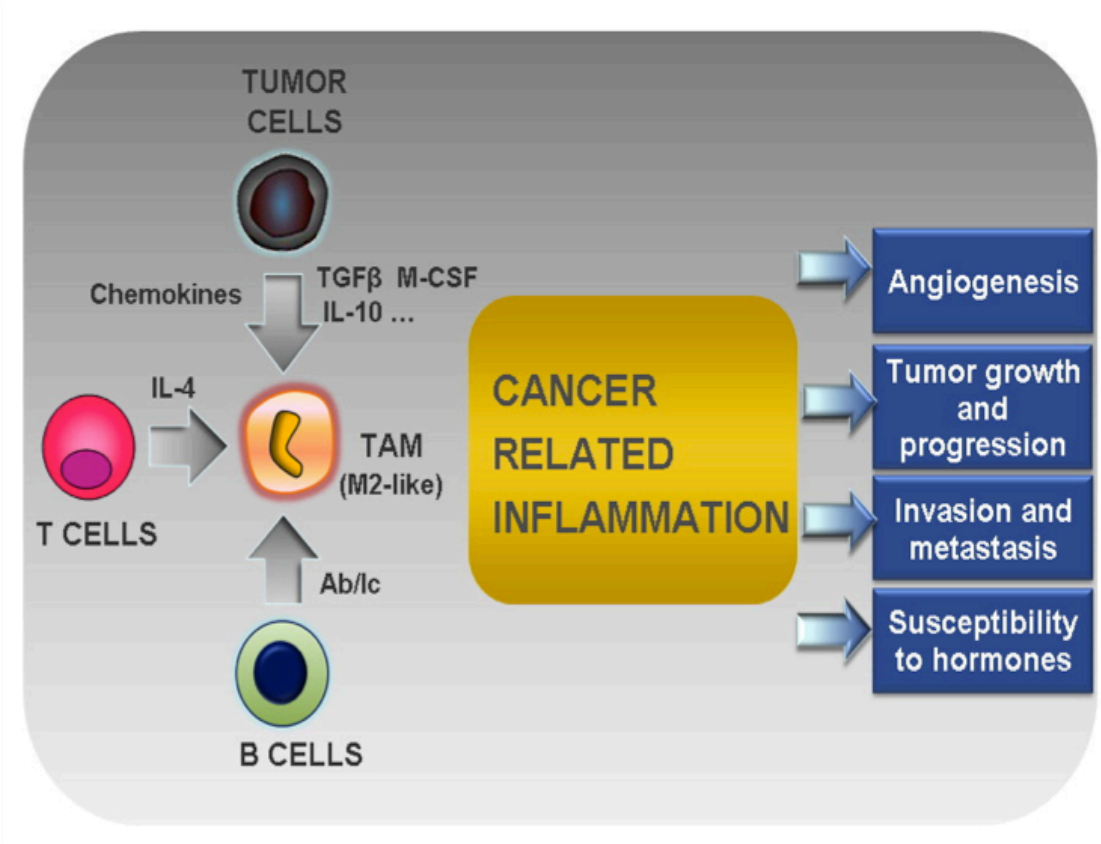
Papillary cancer of thyroid



Green: IL4- CD4+ and CD8 +

Mardente at al. Anticancer Research 25:2483-2488 (2005)

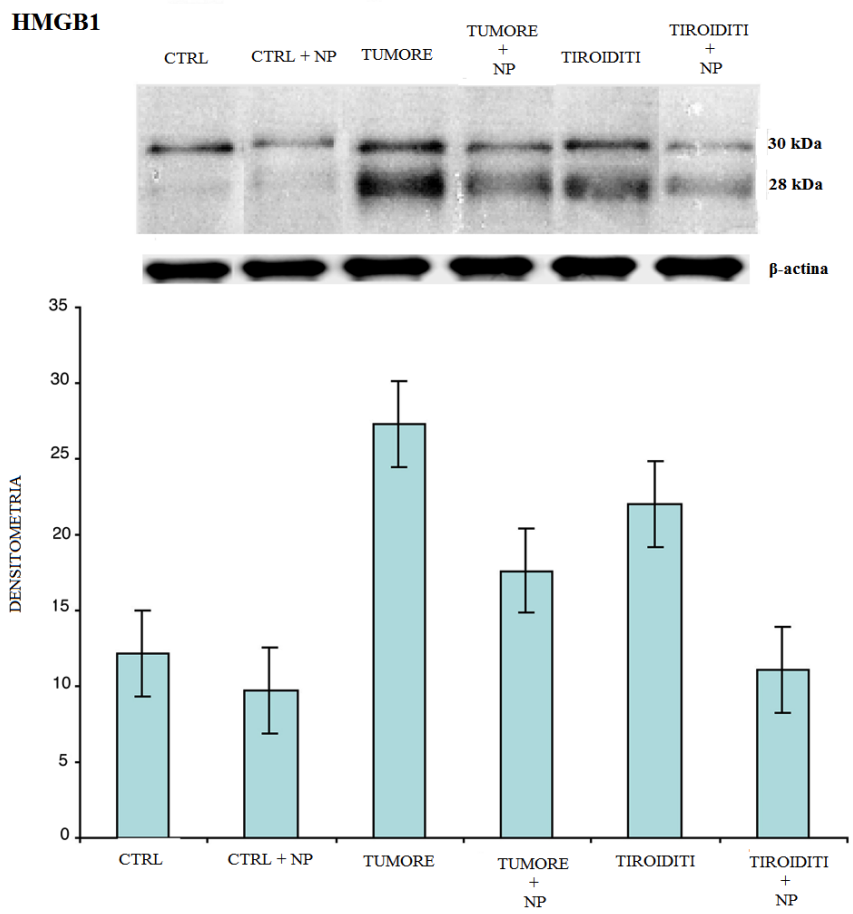
Orchestration of TAM in cancer-promoting inflammation



Mantovani A and Sica A Curr Opin Immunol 2010

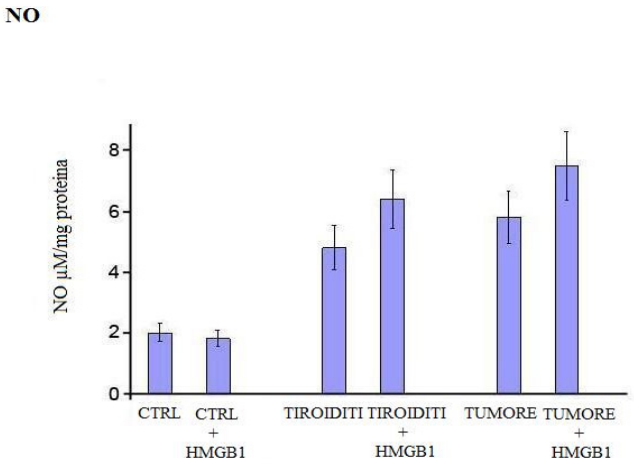
Expression of HMGB1 in primary cultures of thyreocytes

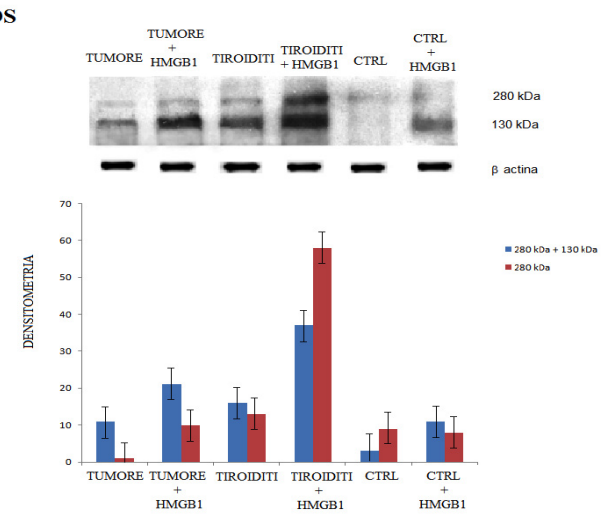
- HMGB1 expression increases in thyreocytes from thryroiditis and papillary cancer
- NO is able to reduce its expression.
- This means that the two mediators interact
- Acetylated HMGB1 (30 Kda) is the most active isoform.



ONCOLOGY REPORTS 24:
1455-1461, 2010

Thyreocytes release NO and expression of iNOS increases

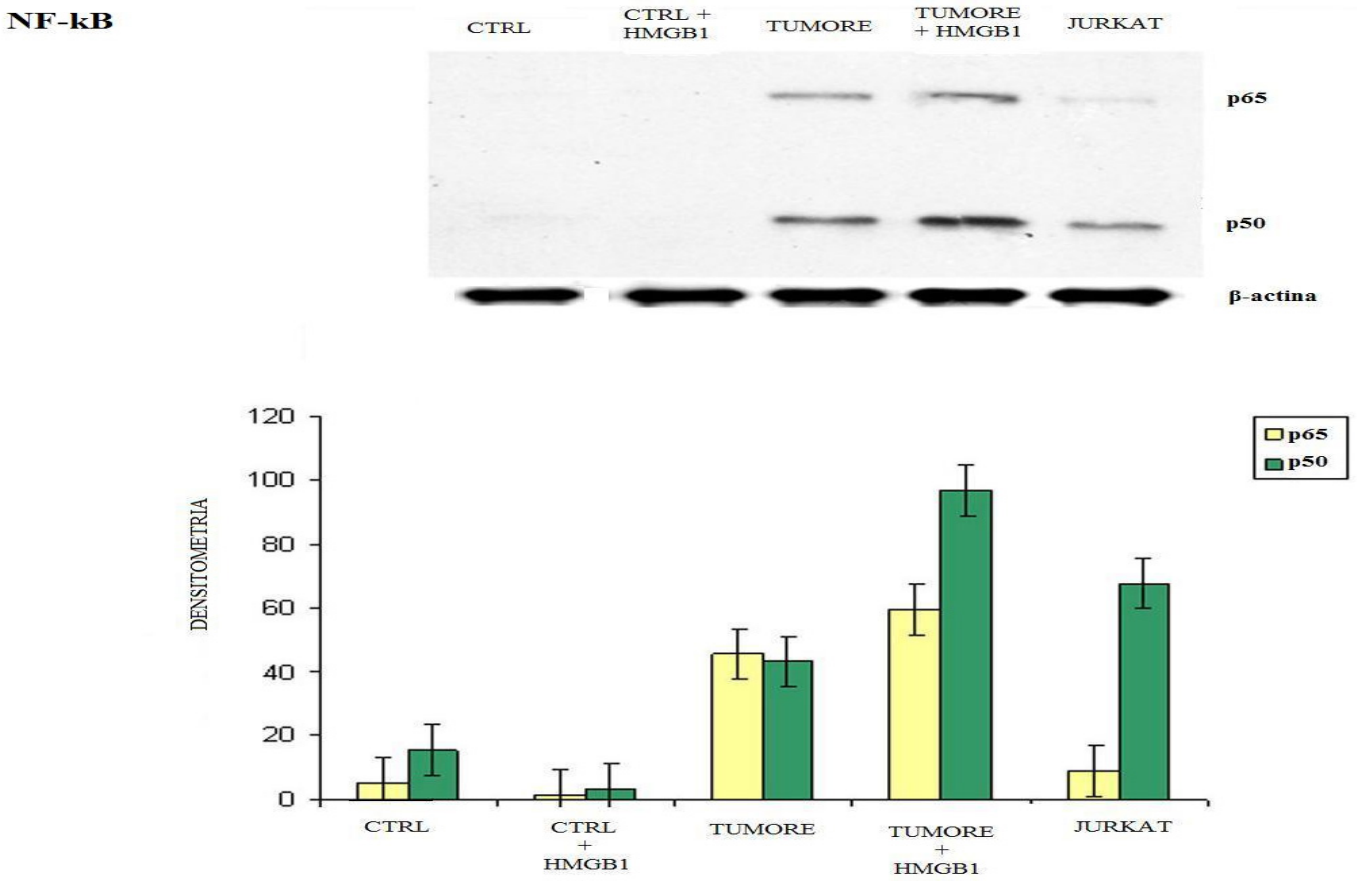




- NO is released in supernatants of 24 h primary cultures. Addition of HMGB1 in culture medium increases the effect

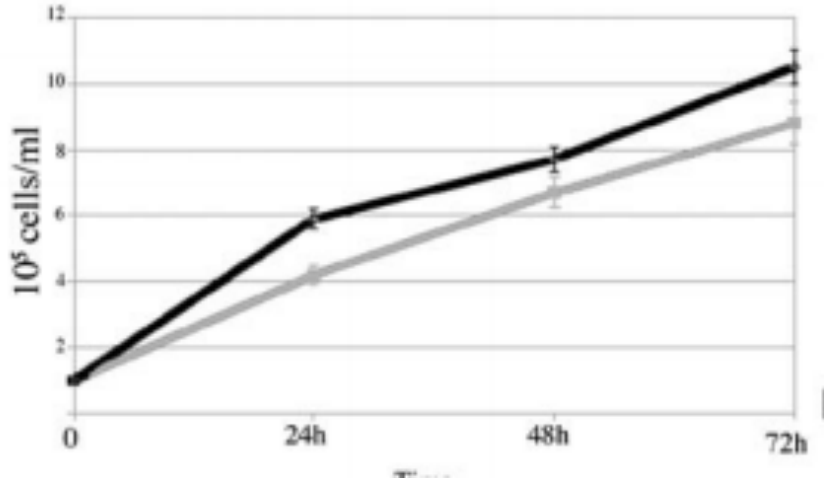
- iNOS 260KDa increases in thyroiditis and cancer before and after addition of HMGB1. There is no increase of the 260 Kda active isoform in control cells.

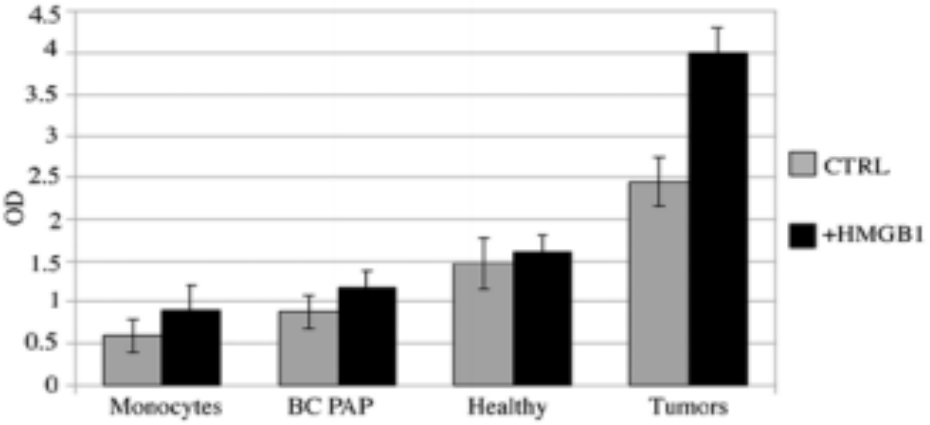
HMGB1 uses NFkB for signal transduction



Mardente et al. Oncol Rep 2010

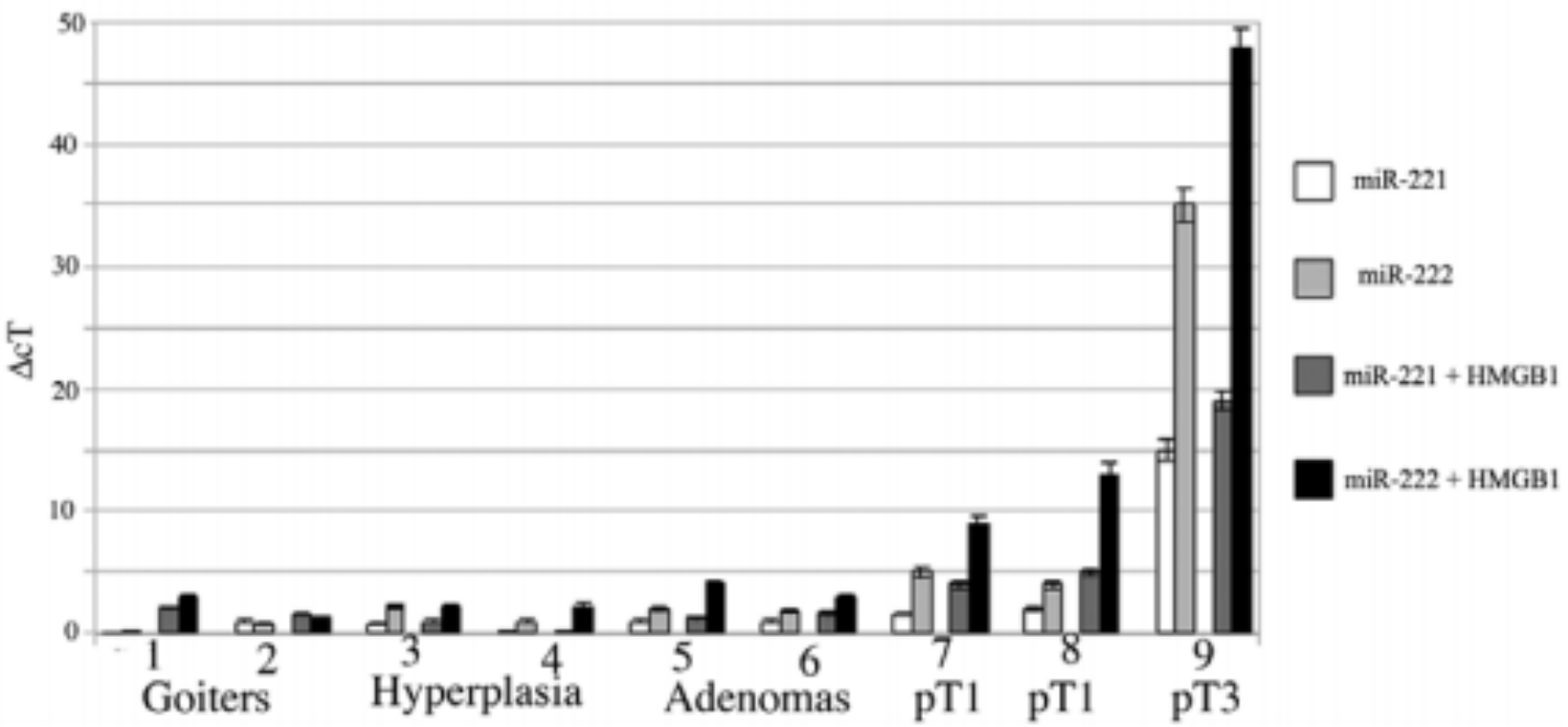
BC PAP growth and migration



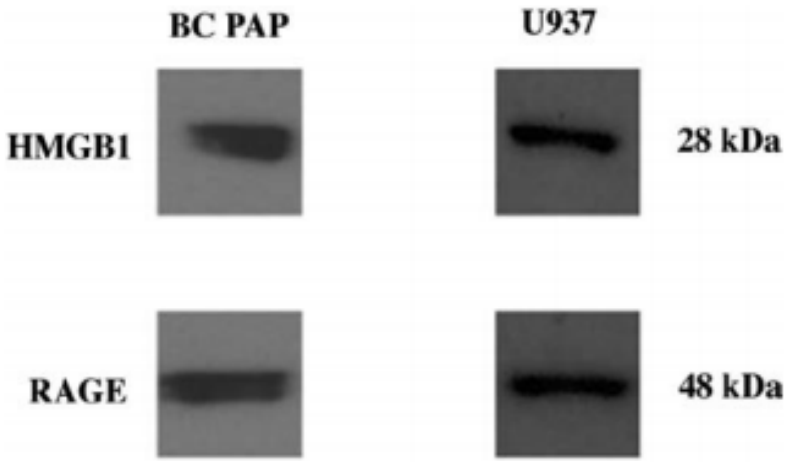


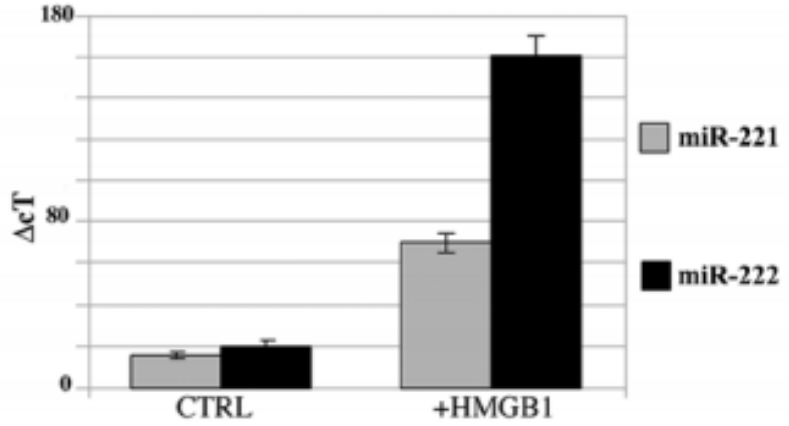
ONCOLOGY REPORTS 28:
2285-2289, 2012

Expression of miR 221 and 222

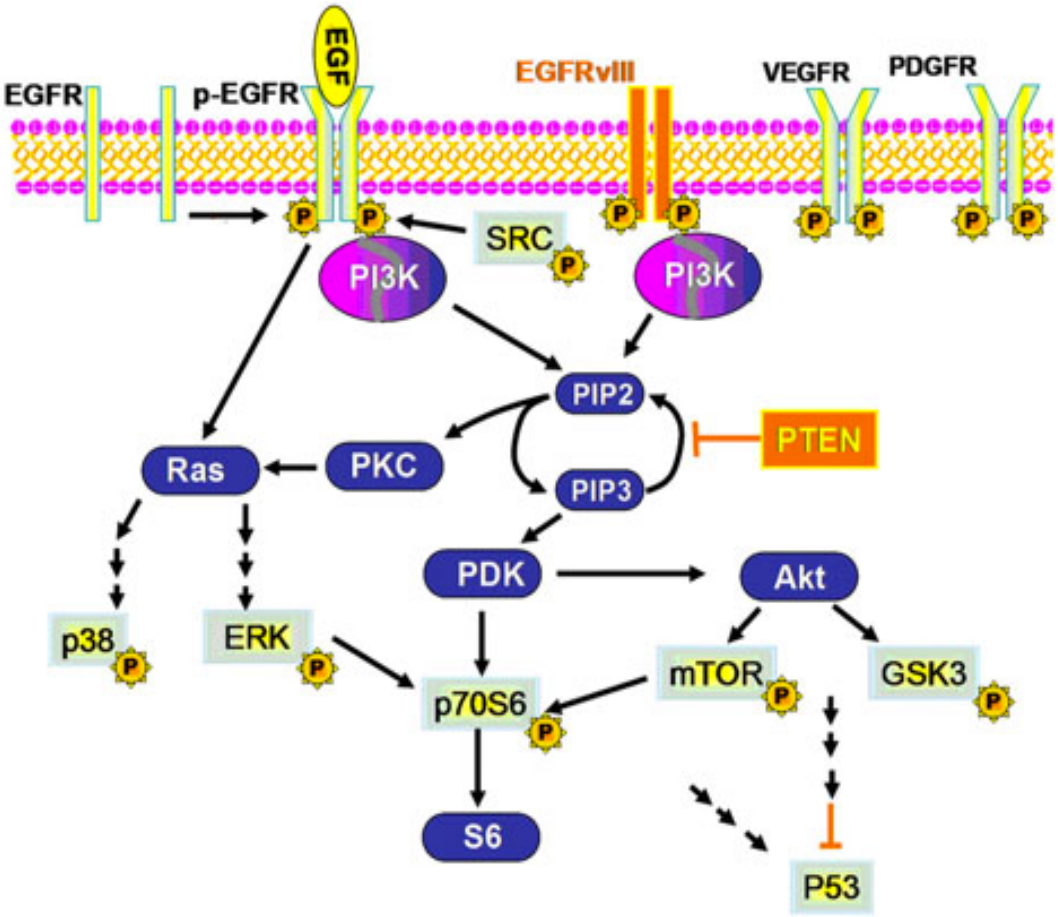


Expression of miR 221 and 222 in BCPAP cells is increased by treatment with exogenous HMGB1





Background

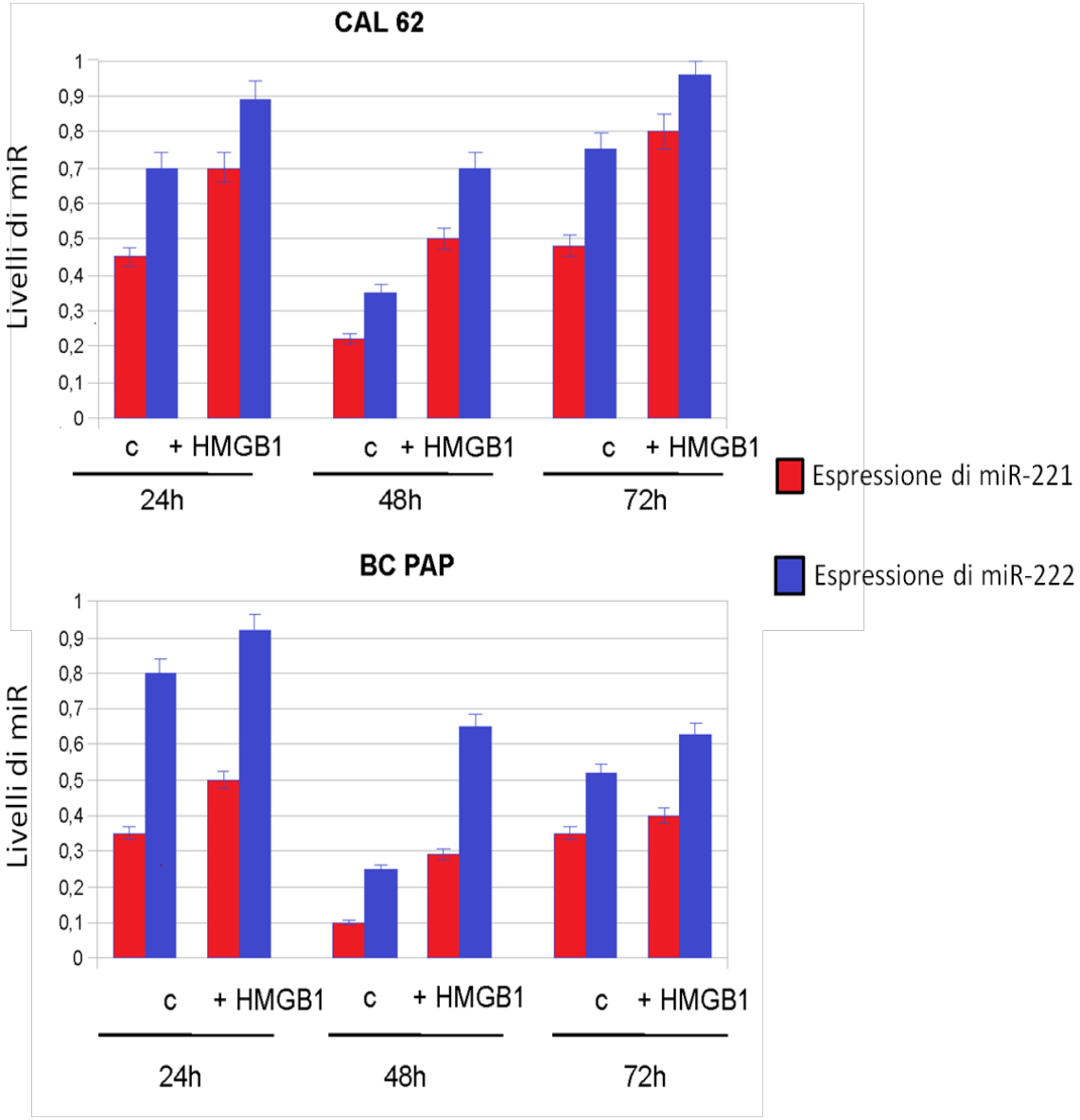


- PTEN is oncosuppressor phosphates that negatively regulates the PDk-AkT signalling pathways.
- Silencing of PTEN is implicated in thryroid cancerogenesis.
- PTEN is a target of the oncogenic cluster miR221/222

HMGB1 increases

Expression of

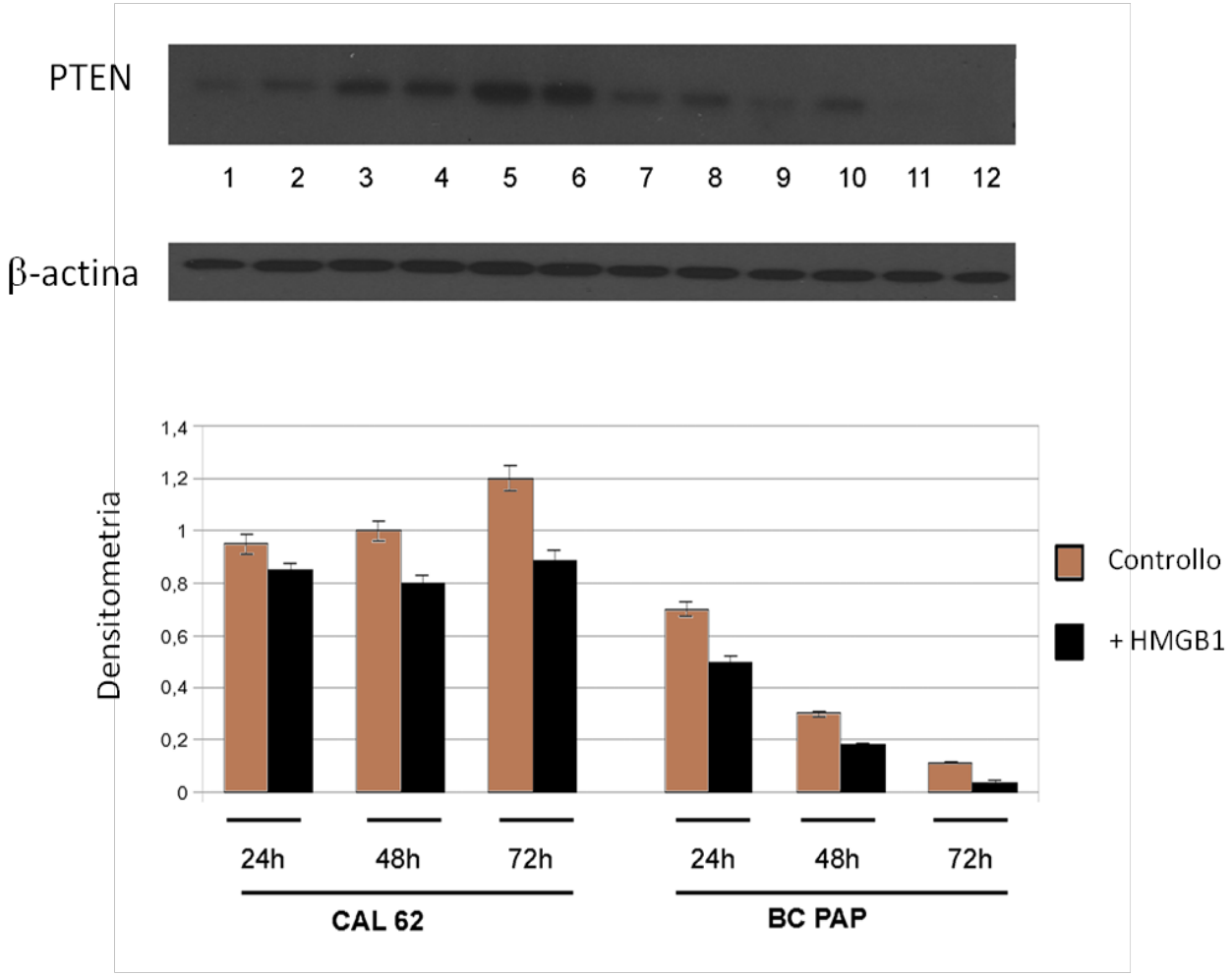
miR-221 e miR-222



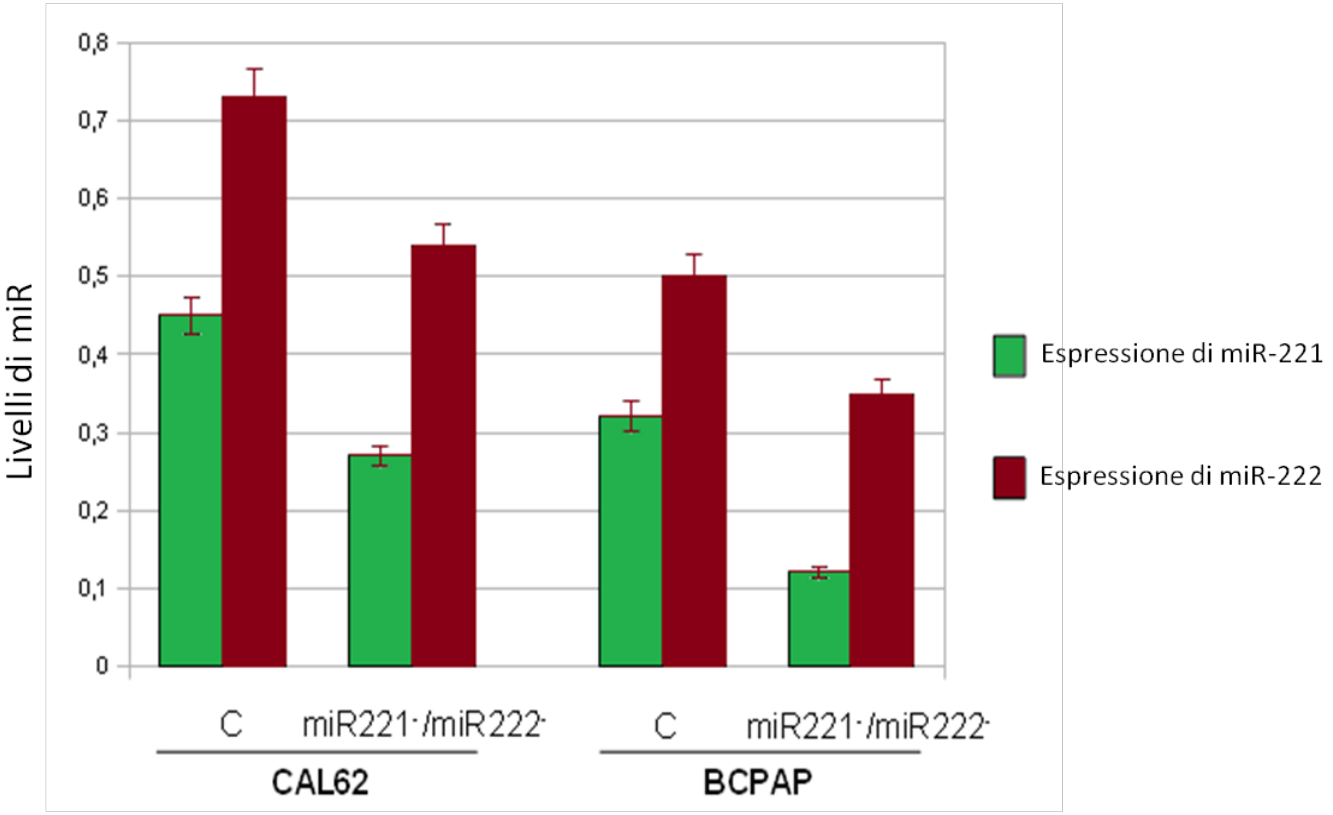
Oncol Rep 2012; Biomed res Int 2015; Oncol lett 2016

HMGB1 reduces expression of PTEN

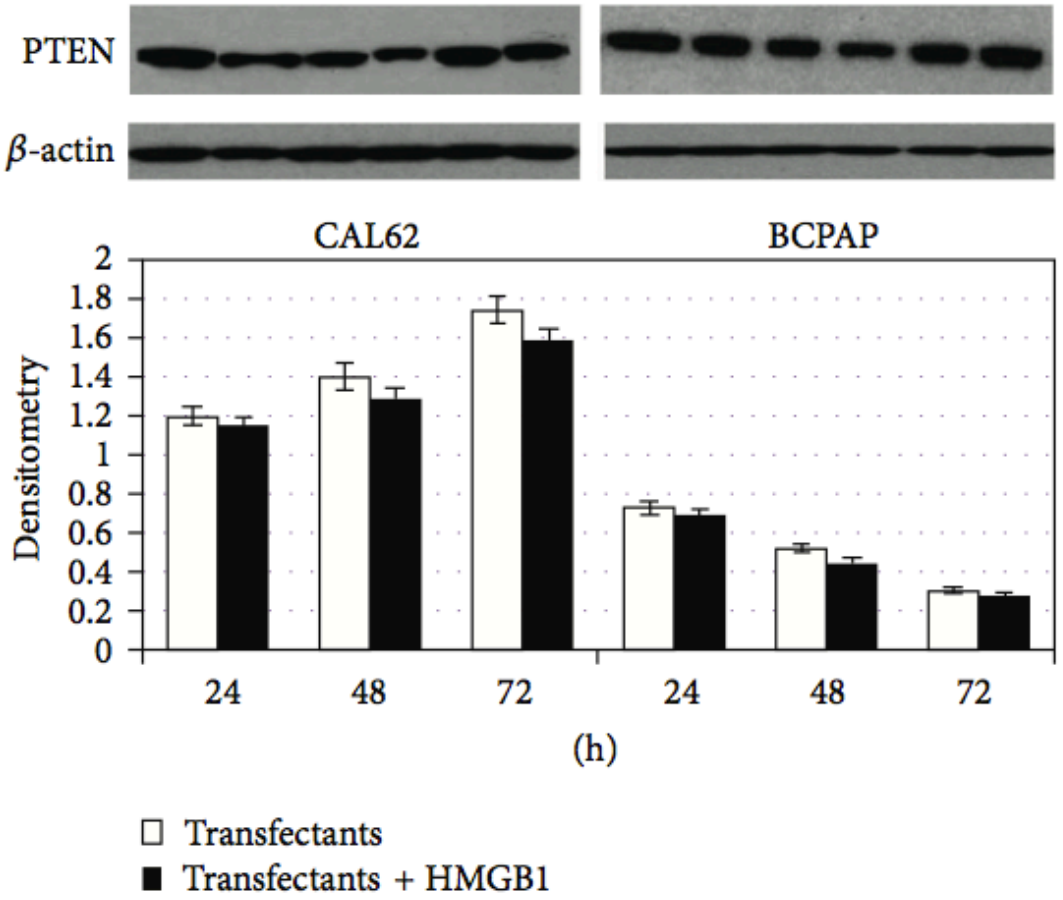
In CAL62 and in BC PAP cells



miR 221 and 222 expression in CAL and in BC PAP cells before and after silencing with anti-sense oligonucleotides



PTEN expression is not reset by HMGB1 in miRs silenced cells



Extracellular HMGB1 released in inflammatory states and in tumor microenvironment

- ✓induces iNOS
- ✓Interacts with NO
- ✓Induces NfKB signalling
- ✓Induces miR 221 and miR 222
- ✓Suppresses PTEN

PLATELET- HMGB1

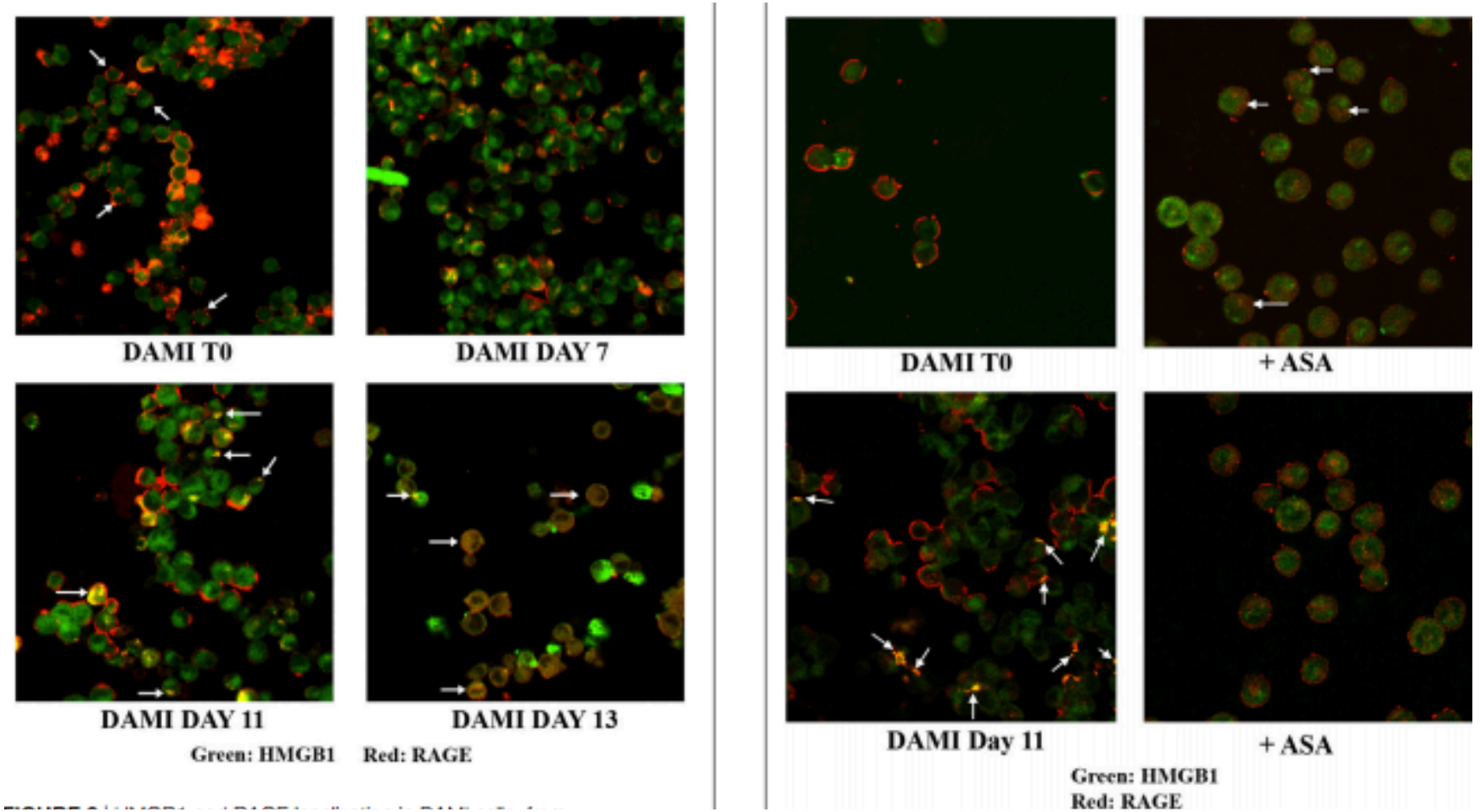
- HMGB1 and RAGE are overexepressed in thrombi.
- Direct interaction between ASA and HMGB1 (Faseb J 2016; Pharmacol res 2016;)
- ASA delays mesothelioma growth by inhibiting HMGB1-mediated tumor progression

Experimental setting : in vitro
Dami cells

- DAMI cells: a human megakaryocitic cell line deriving from human megakaryoblastic leukemia .

- DAMI cells were induced to differentiate in vitro into platelets for 7 days with TPO and PMA.
- ASA (50μg) was added to cultures before platelet formation (day 5 or 6)

Cellular localization of HMGB1 and RAGE in DAMI cells

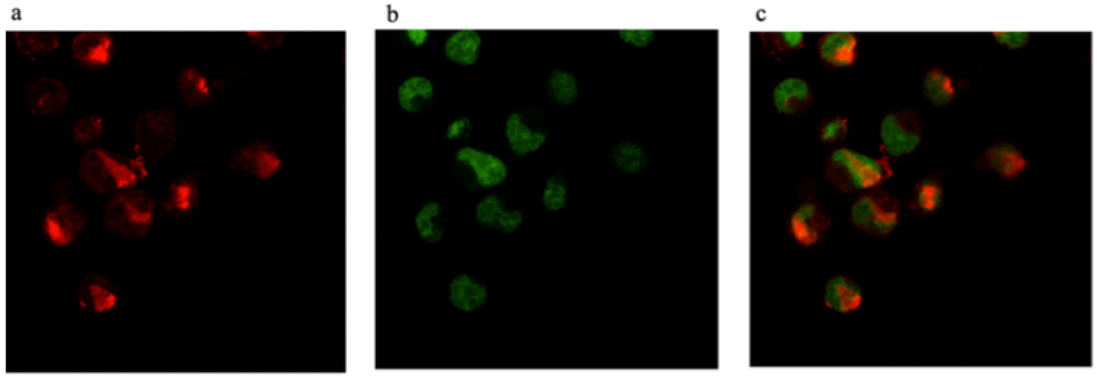


Human megakaryocytes

- Human Hematopoietic Progenitor Cells obtained from peripheral blood of healthy donors isolated by Ficoll gradient.

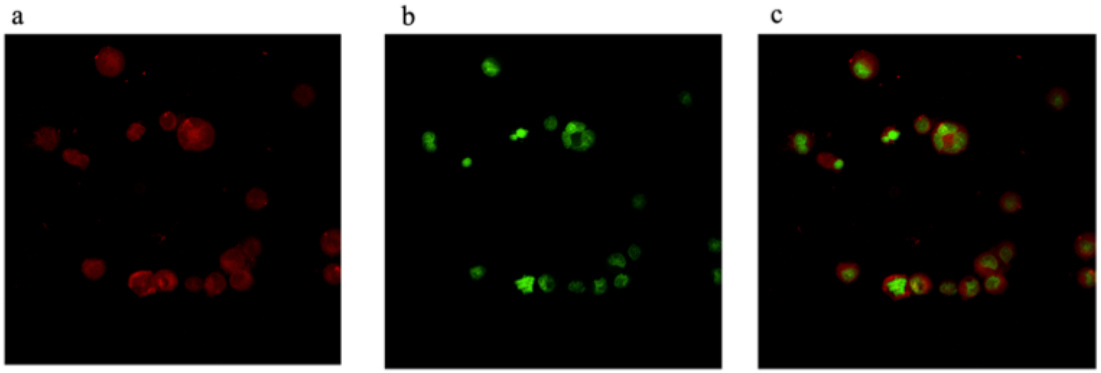
- CD34+ cells (90%) were purified and allowed to differentiate into MKs in vitro while they were treated with ASA. Platelets were obtained at day 14.

HMGB1 in MKs at different stages of development



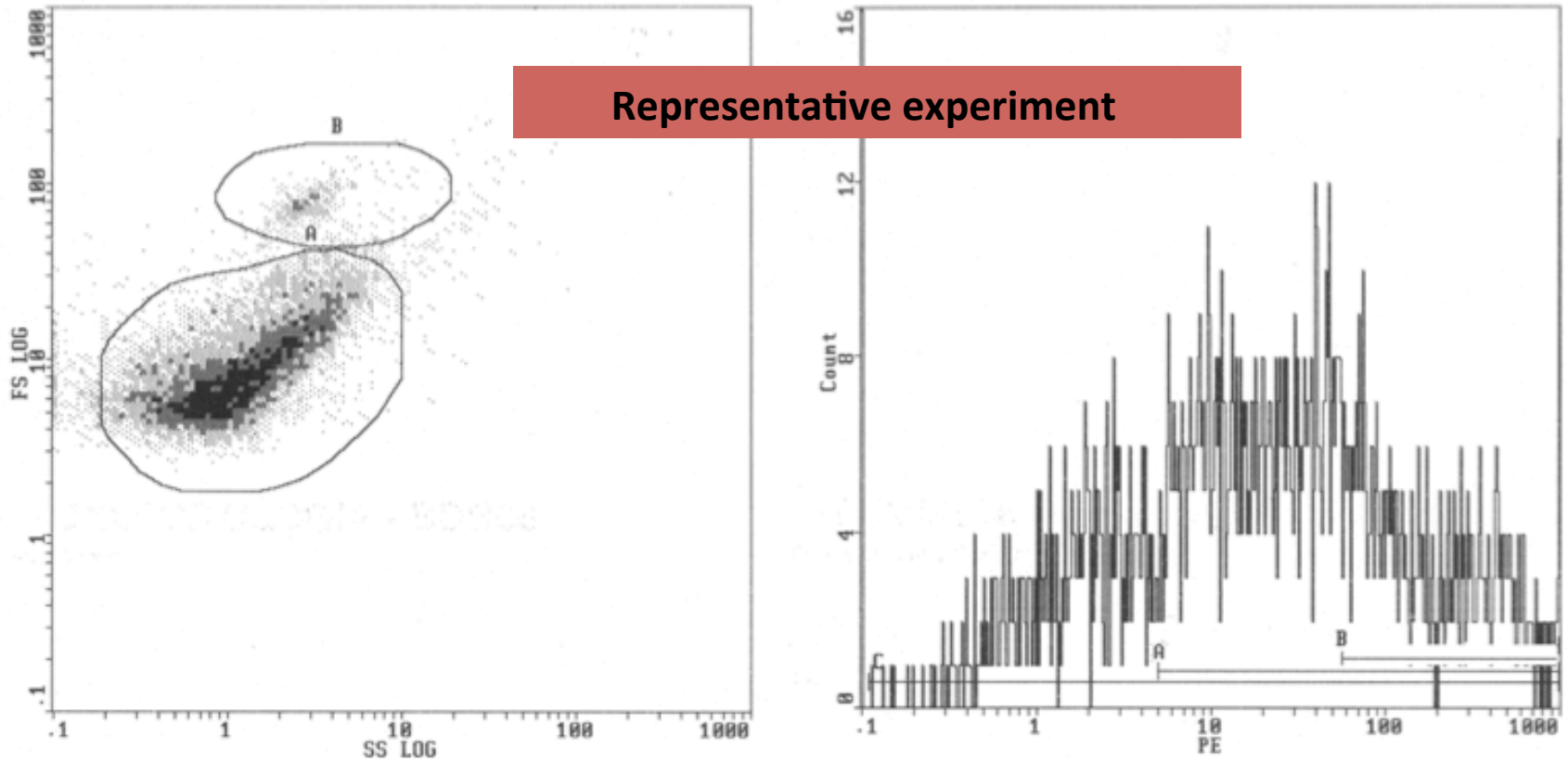
MKs day 7: a. HMGB1 red; b. Sytox green nuclei; c. merge (magnification 60X)

B



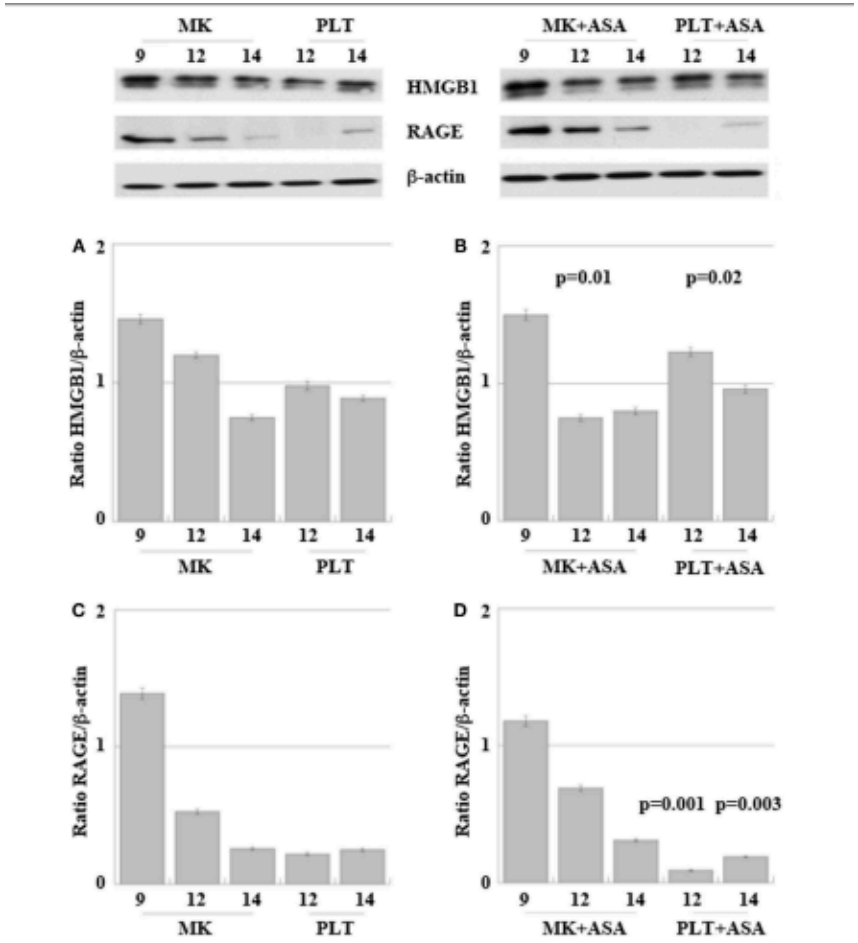
MKs day 14: a. HMGB1 red; b. Sytox green nuclei; c. merge (magnification 60X)

Platelets and pro-platelets obtained in vitro from human MK at day 14



Immunofluorescence with CD61 monoclonal antibody of platelets in Gate A (66,9% positive events) and pro platelets in Gate B (80.9% positive events)

Aspirin decreases HMGB1 expression in human MK



Day of maturation and treatment	Supernatants
MK day 9	18.25 ± 0.3
MK day 9 + ASA	17.45 ± 0.2
MK day 12	19.45 ± 0.2
MK day 12 + ASA	18.02 ± 0.3
MK day 14	22.19 ± 0.2
MK day 14 + ASA	20.13 ± 0.3

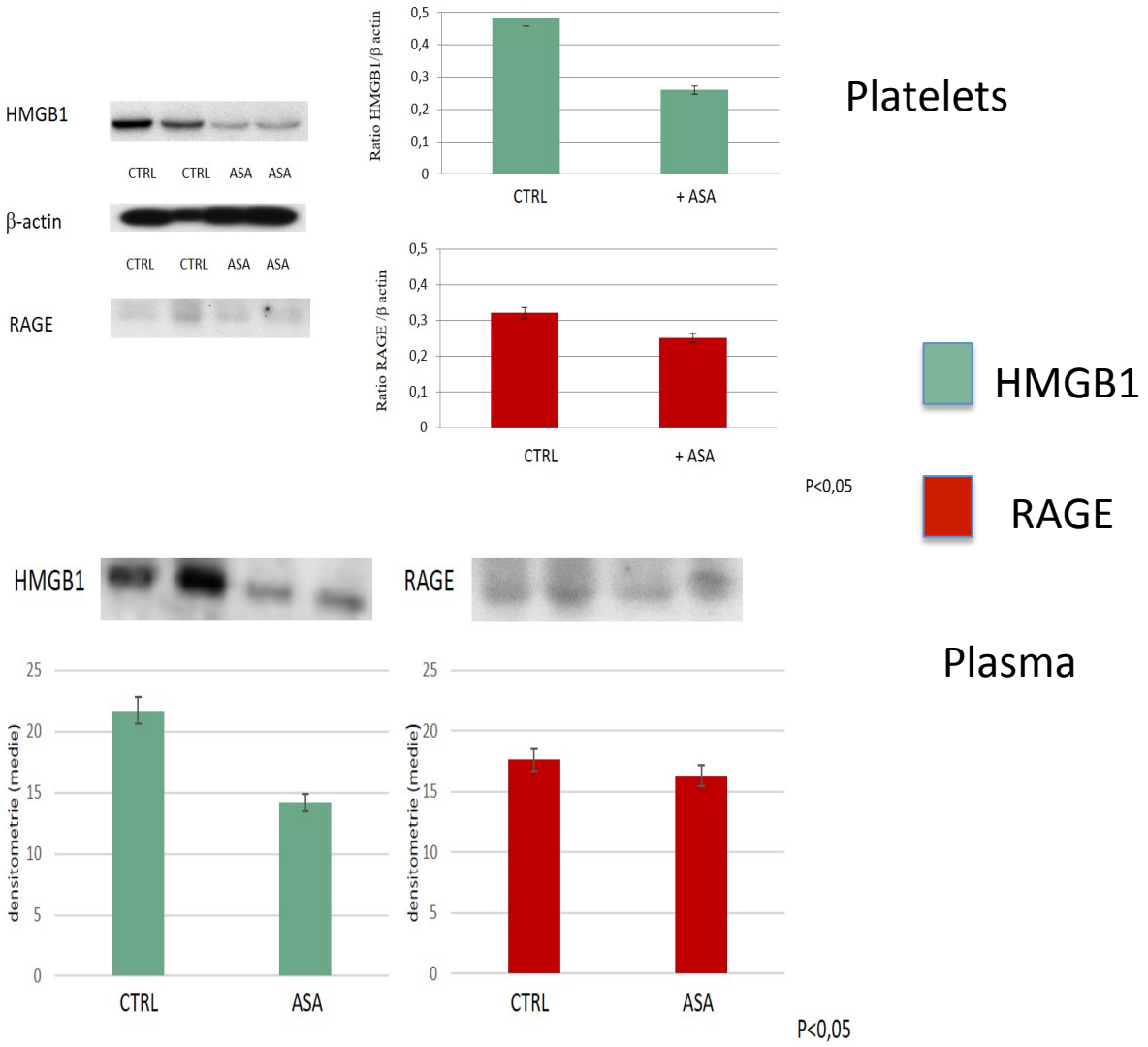
Experimental model 2

Table 1

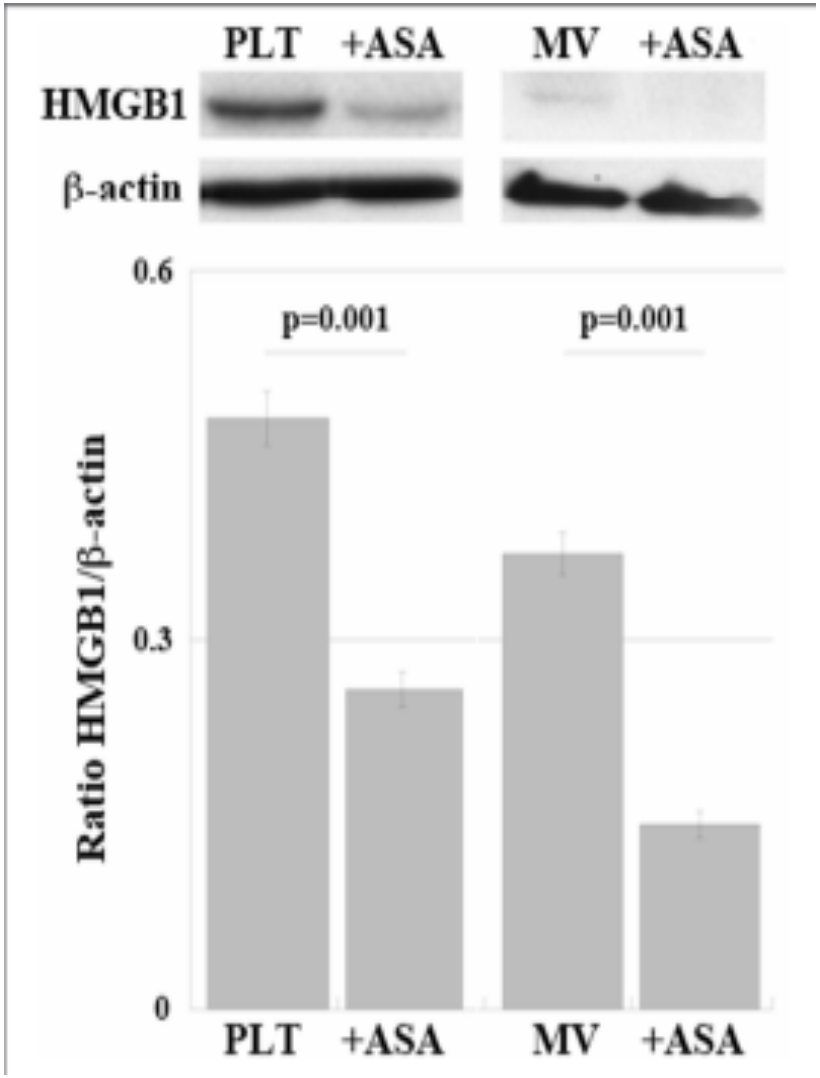
Description of donors used in the study.

Subjects	Group 1, healthy volunteers (HVs)	Group 2, HVs (ASA 300 mg/day *per os*)	Group 3, high-risk thrombosis patients	Group 4, high-risk thrombosis patients (ASA 100 mg/day/*per os*)
Total number	10	10	10	10
Male/female	6/4	6/4	7/3	7/3
Age range	25–55	25–55	58–75	58–75

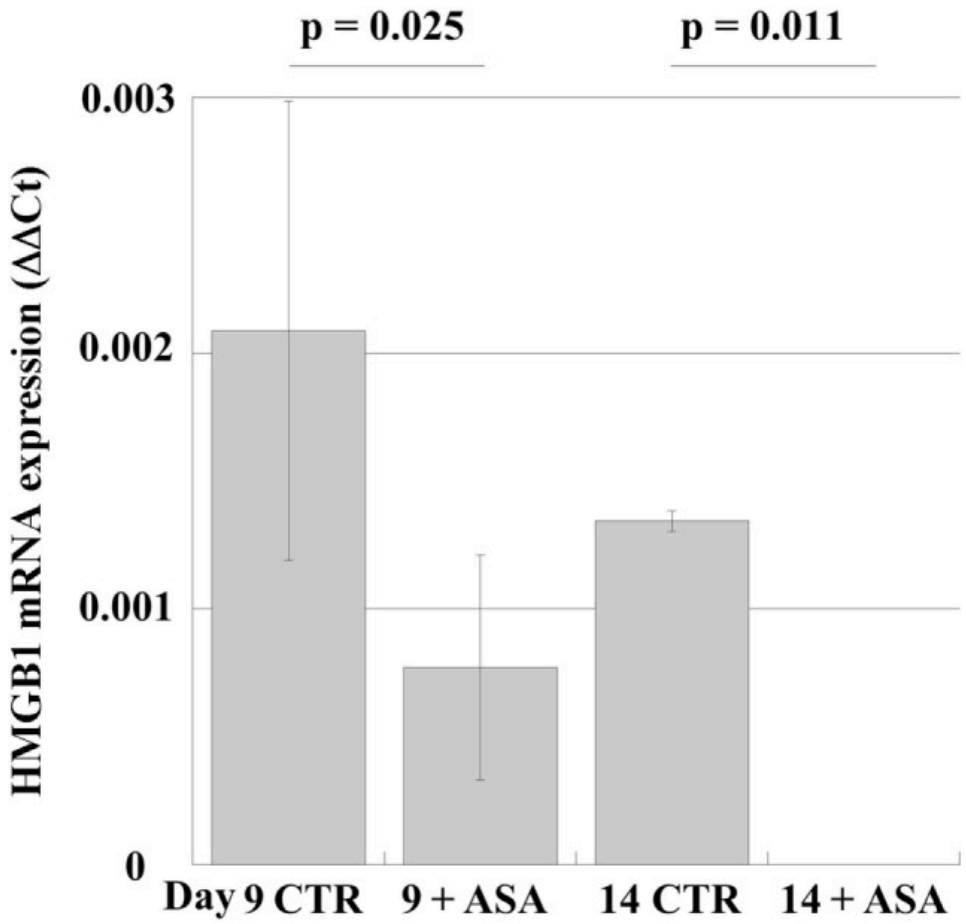
Administration of ASA (100mg/die) decreases HMGB1 and RAGE expression in platelets and in plasma of patients



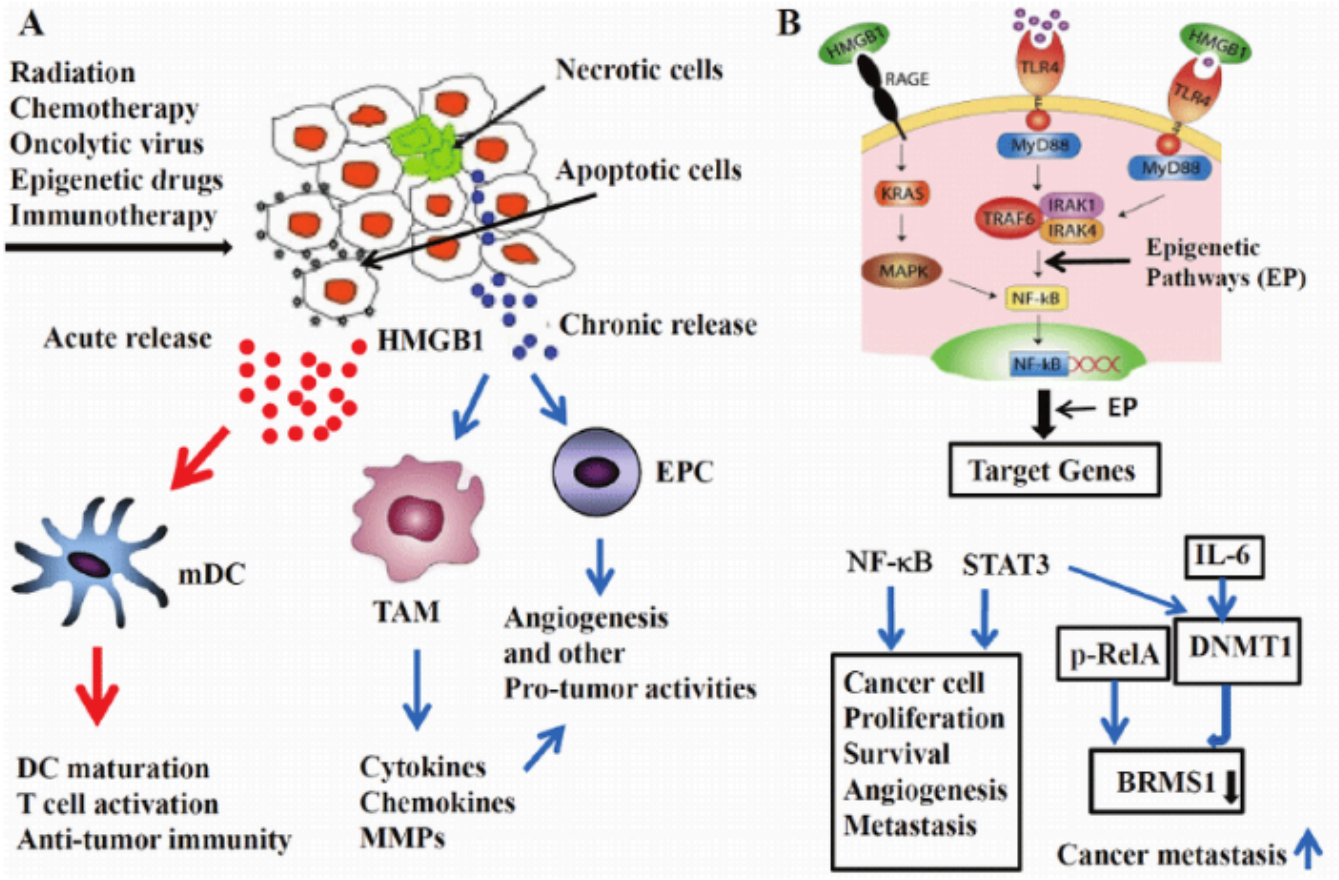
HMGB1 expression in platelets and platelet derived MV in patients treated with ASA



Aspirin decreases HMGB1 mRNA in human MK



Mardente et al. Frontiers in Immunology (2018)


A
Radiation
Chemotherapy
Oncolytic virus
Epigenetic drugs
Immunotherapy
Necrotic cells
Apoptotic cells
Acute release
HMGB1
Chronic release
mDC
TAM
EPC
DC maturation
T cell activation
Anti-tumor immunity
Cytokines
Chemokines
MMPs
Angiogenesis
and other
Pro-tumor activities
B
HMGB1
RAGE
TLR4
HMGB1
TLR4
MyD88
MyD88
KRAS
TRAF6
IRAK1
IRAK4
Epigenetic
Pathways (EP)
MAPK
NF-kB
NF-kB
EP
Target Genes
NF-κB
STAT3
IL-6
p-RelA
DNMT1
Cancer cell
Proliferation
Survival
Angiogenesis
Metastasis
BRMS1
Cancer metastasis


Work in progress