ON THE COMMONALITY BETWEEN HEMOSTASIS AND ONCOGENESIS

Pathology Grand Rounds
February 12, 2015

David M. Waisman, Ph.D.
Depts. Biochemistry & Molecular Biology
and Pathology

DALHOUSIE UNIVERSITY
Inspiring Minds

Metastatic Cascade

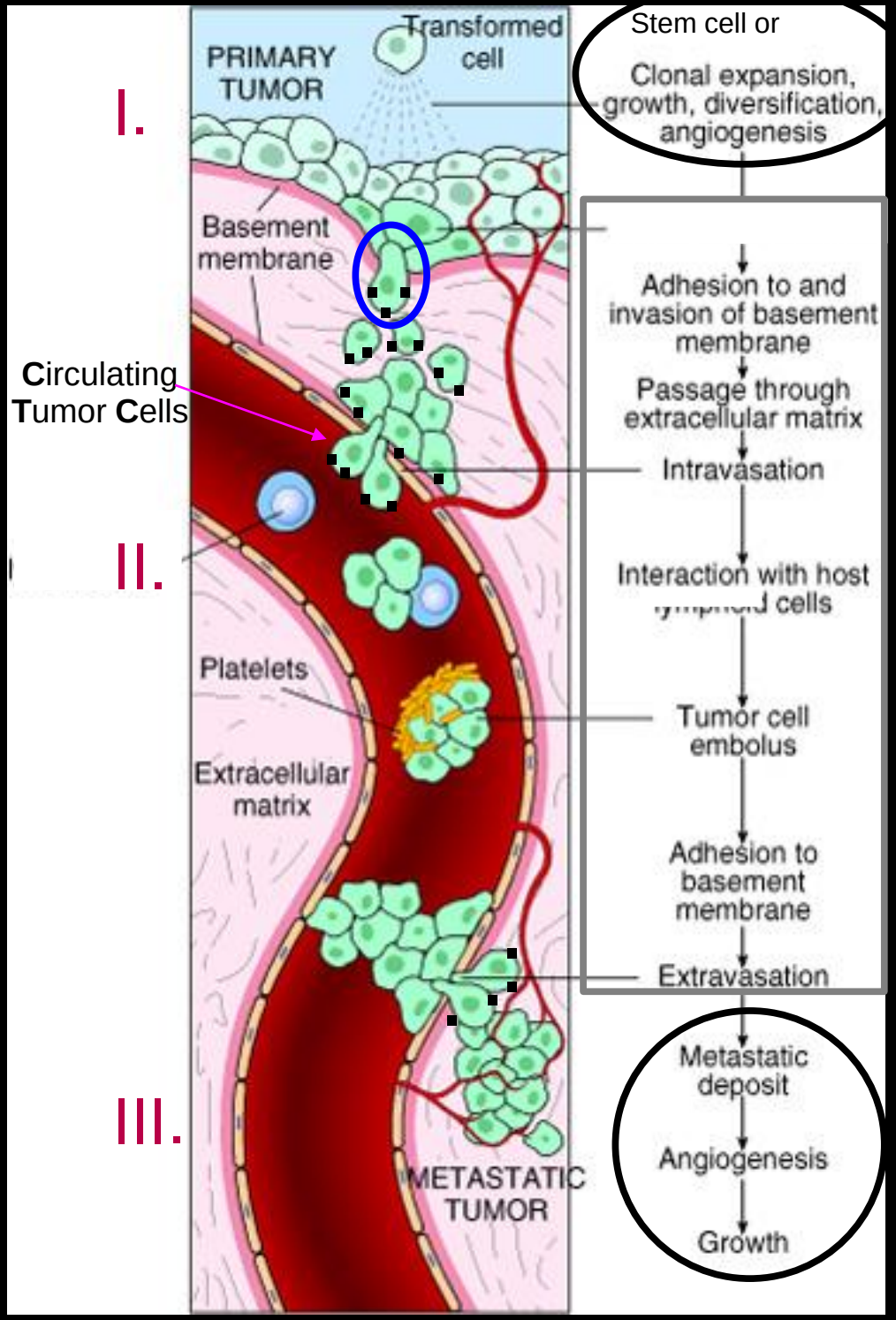
I. Initiation-cellular transformation resulting in tumor growth

II. Progression-acquisition of the metastatic phenotype. Invasion (EMT), intravasation and extravasation.

III. Colonization-MET, micrometastasis (4-50 cells) macrometastases

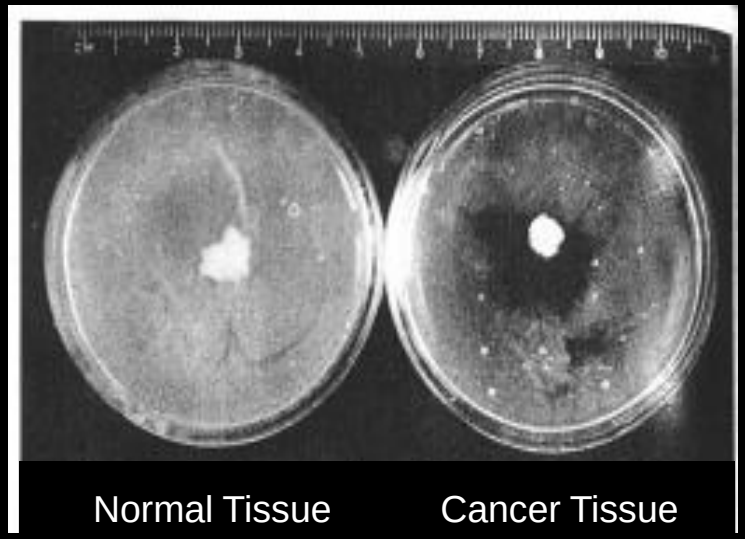
IV. Promoters

ECM (Premetastatic niche)
Inflammation
Angiogenesis



HISTORICAL

Fibrin Plate Assay



Fisher (1925) observed that avian tissue explants transformed to malignancy by viruses generate high levels of fibrinolytic activity under conditions in which cultures of normal cells do not. The enzyme responsible for the fibrinolytic activity was called fibrinolysin and later identified as the enzyme plasmin. Plasmin is produced from the conversion of the blood protein, plasminogen. Fischer, A. 1925. Beitrag zur Biologic der Gewebezellen Eine vergleichendbiologische Stadie der normalen und malignen Gewebezellen in vitro. Arch. Entwicklungsmech. Org. (Wilhelm Roux). 104:210.

Active Fibrinolysis By Transformed Cells

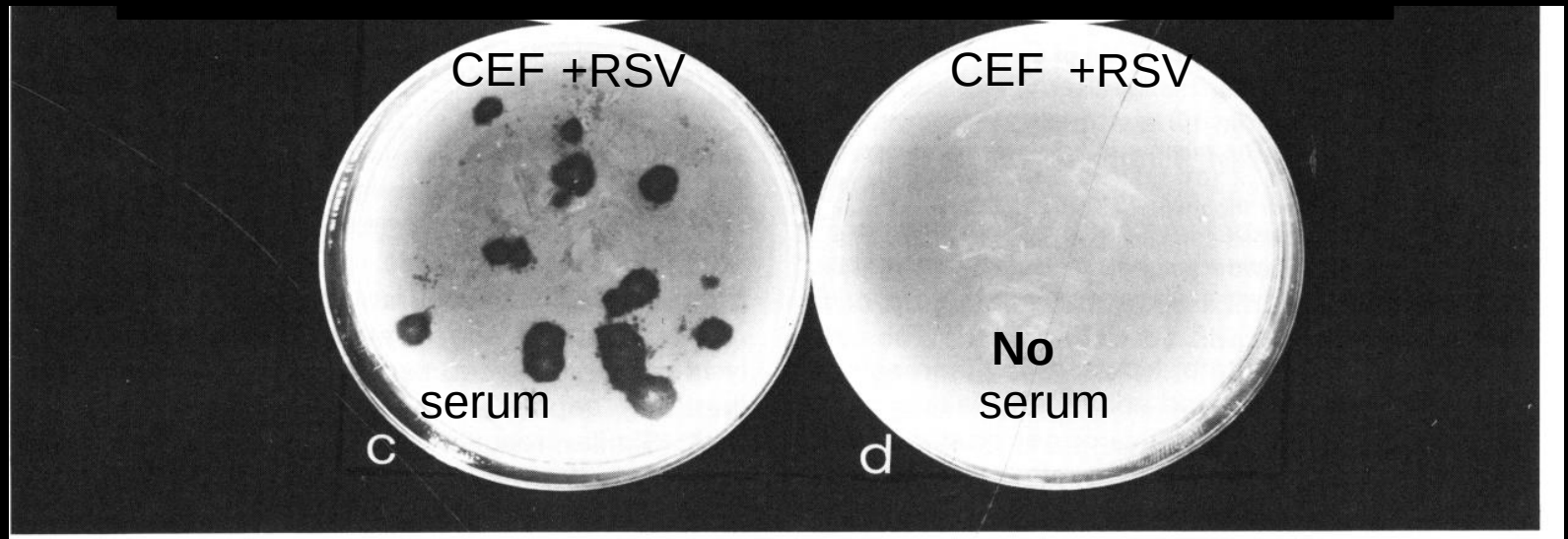
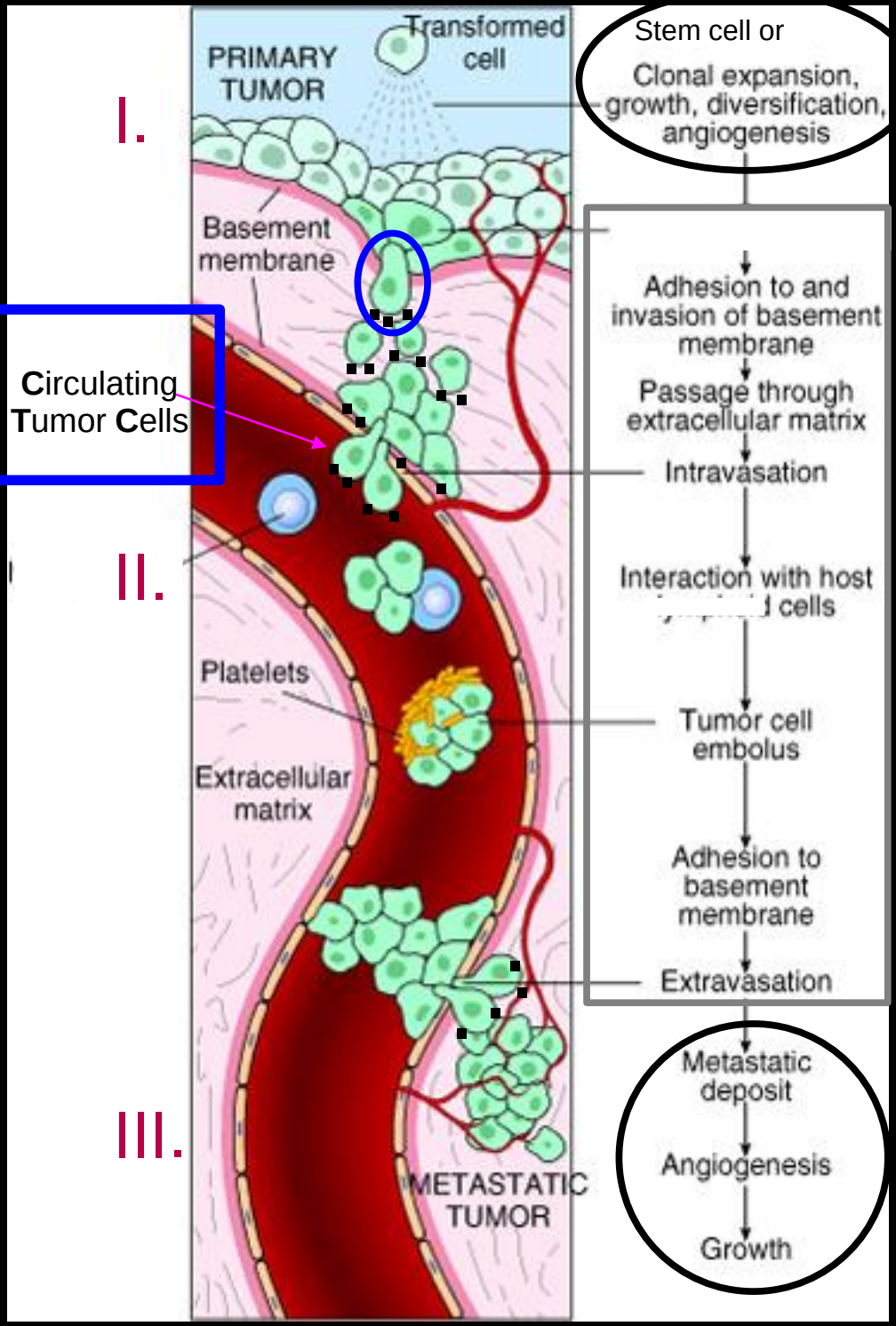


Figure 2. The plasminogen-dependent production of lytic zones by foci of RSV–CEF. CEF (10^6 per 60 mm plate) were infected with Rous sarcoma virus, subgroup A, and overlaid with agar as described by Temin and Rubin (1958). After 5 days, the agar was removed and the fibrin–agar overlay applied, using 5% final concentration of acid-treated fetal bovine serum. The plates were then incubated at 37°C for 18 hr.

The serum contained plasminogen the inactive (zymogen) form of plasmin. The fibrinolytic protease was called *malignant protease* by Reich.

HOW DO WE IDENTIFY THE PROTEINS THAT REGULATE PROTEOLYTIC ACTIVITY OF THE INVADING CANCER CELLS



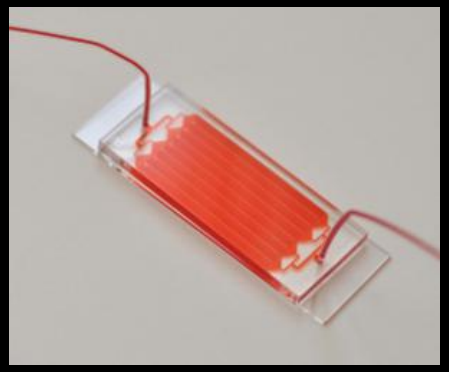
Science

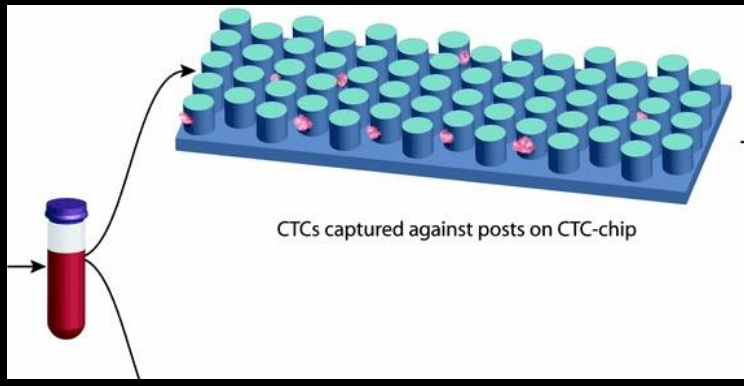
AAAS

Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition

Min Yu *et al.*

Science **339**, 580 (2013);
DOI: 10.1126/science.1228522





→ RNA-seq Analysis

I.D. EMT GENES

- The CTC chip has gone through several iterations, but originally it contained hundreds of thousands of micropillars coated with antibodies, which bind to CTCs in a blood sample without binding normal blood cells. A set of 170 transcripts was enriched in CTCs captured at a mesenchymal predominant time point

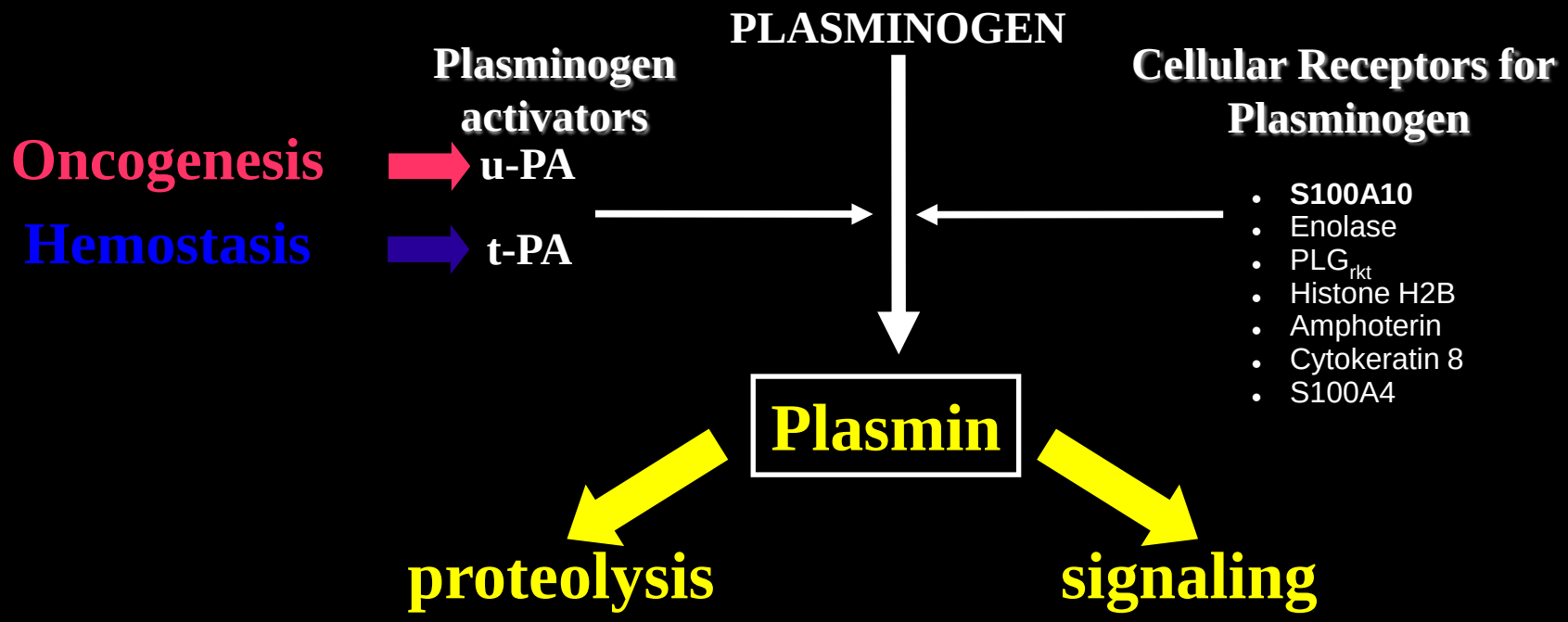
S100A10	S100 calcium binding protein A10	6281	S100A10
TNFRSF21	Tumor necrosis factor receptor superfamily, member 21	27242	TNFRSF21
DOCK6	Dedicator of cytokinesis 6	57572	DOCK6
DSTN	Destrin (actin depolymerizing factor)	11034	DSTN
ITGA6	Integrin, alpha 6	3655	ITGA6
LTBP3	Latent transforming growth factor beta binding protein 3	4054	LTBP3
COL5A2	Collagen, type V, alpha 2	1290	COL5A2
GOLM1	Golgi membrane protein 1	51280	GOLPH2
CRIM1	Cysteine rich transmembrane BMP regulator 1 (chordin-like)	51232	CRIM1
MDK	Midkine (neurite growth-promoting factor 2)	4192	MDK
CD59	CD59 molecule, complement regulatory protein	966	CD59
SLC29A1	Solute carrier family 29 (nucleoside transporters), member 1	2030	SLC29A1
RHOC	Ras homolog gene family, member C	389	RHOC
LIMS2	LIM and senescent cell antigen-like domains 2	55679	LIMS2
AGRN	Agrin	375790	AGRN
FBLN2	Fibulin 2	2199	FBLN2
BCAM	Basal cell adhesion molecule (Lutheran blood group)	4059	BCAM
ANXA2	Annexin A2	302	ANXA2
PTMS	Parathymosin	5763	PTMS

THE LIST OF GSEA GENE SIGNATURES ENRICHED IN THE LIST OF 170 EMT GENES-p11

- Genes upregulated in hepatocellular carcinoma (HCC) compared to normal liver samples.
- Genes upregulated in A4573 cells
- Genes upregulated in pancreatic ductal adenocarcinoma
- Genes upregulated in Wilm's tumor samples compared to fetal kidney
- Genes upregulated in papillary thyroid carcinoma (PTC) compared to normal tissue
- Genes upregulated in RD cells (embryonal rhabdomyosarcoma)
- Genes specifically expressed in samples from patients with pediatric acute myeloid leukemia (AML) bearing inv(16) translocation
- Genes upregulated in melanoma tumours that developed metastatic disease
- Genes upregulated in basal subtype of breast cancer samples
- Genes specifically expressed in FAB subtypes M2, M4, M5 and M7 of pediatric AML (acute myeloid leukemia).
- Genes specifically expressed in samples from patients with pediatric acute myeloid leukemia (AML) bearing 11q23 rearrangements.
- Neural related genes upregulated in melanoma tumors that developed metastases compared to primary melanoma that did not.

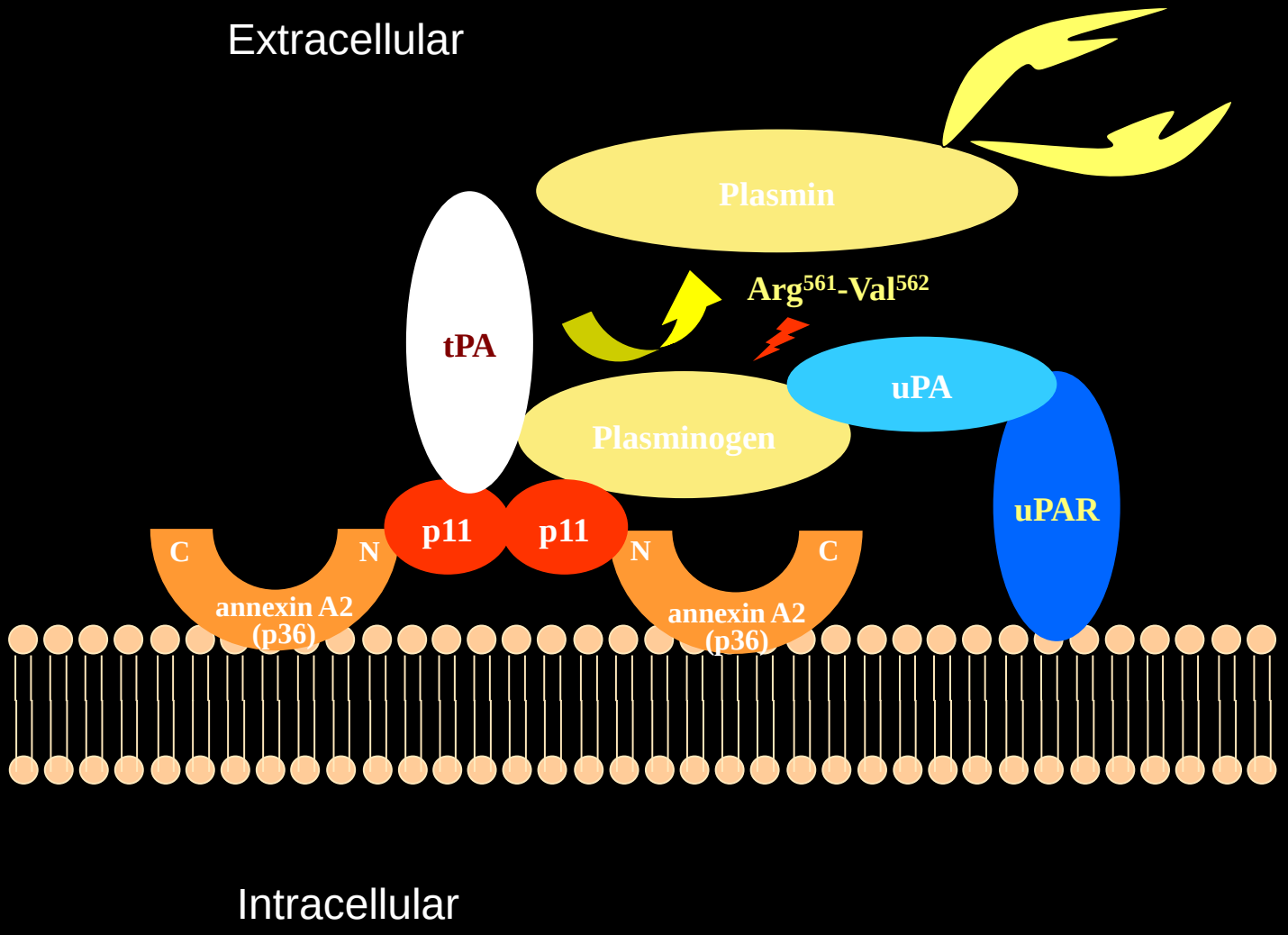
- Genes with promoter regions [---2kb,2kb] around transcription start site containing the motif TGANTCA which matches annotation for JUN: jun oncogene\
- Genes with promoter regions [---2kb,2kb] around transcription start site containing the motif GGGTGGRR which matches annotation for PAX4: paired box gene 4
- Genes with promoter regions [---2kb,2kb] around transcription start site containing the motif RCAGGAAGTGNNTNS which matches annotation for ETS1: v---ets erythroblastosis virus E26 oncogene homolog 1 (avian)
- Genes with promoter regions [---2kb,2kb] around transcription start site containing the motif RGAGGAARY which matches annotation for SPI1: spleen focus forming virus (SFFV) proviral integration oncogene spi1

RELATIONSHIP BETWEEN PLASMIN, HEMOSTASIS AND CANCER



- **Degradation of ECM (laminin, fibronectin/Fibrin)**
- **MMP activation (MMP-3,-9,-12, and -13)**
- **Growth factor activation (TGFb, h-EGF, FGF)**

CDCP1
PAR-1
Indirect (TLR4, growth factors)


Extracellular
Plasmin
Arg561-Val562
tPA
uPA
Plasminogen
uPAR
p11
p11
C
N
N
C
annexin A2
(p36)
annexin A2
(p36)
Intracellular

THE CENTRAL THEME

- S100A10 (p11) plays a key role in plasmin regulation.

- p11 is utilized by cancer cells for growth, invasion and metastasis.

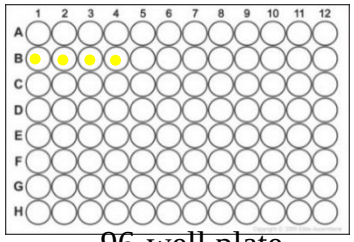
- p11 is utilized by endothelial cells to regulate plasmin-dependent fibrinolysis.

- p11 is utilized by APL (NB4) cells for chemotaxis.

IN VITRO ANALYSIS OF THE REGULATION OF PLASMIN GENERATION BY S100A10

PLASMIN GENERATION ASSAY WITH PURIFIED COMPONENTS AND CHROMOGENIC SUBSTRATE

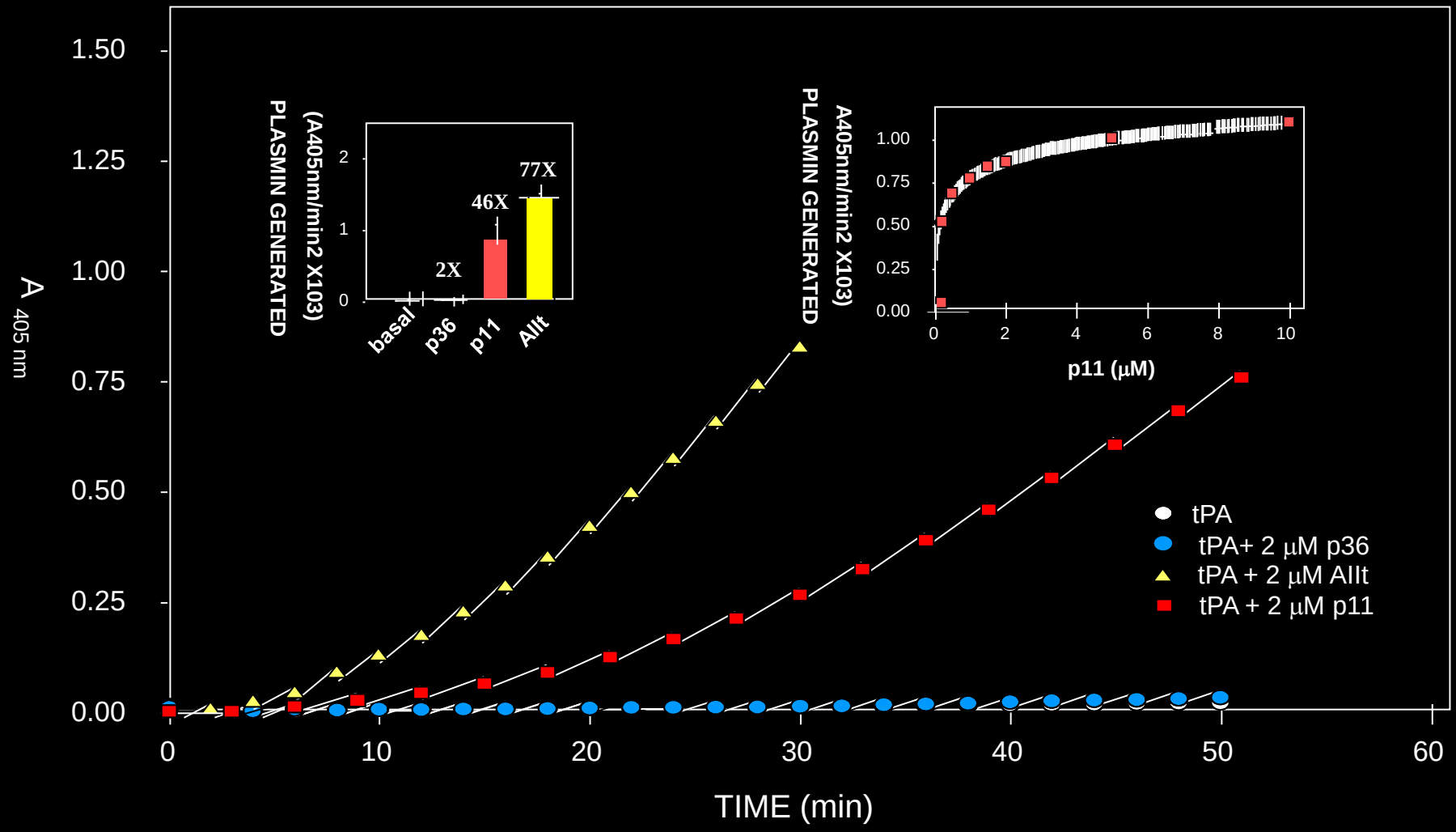
Plasminogen (Pg) + Pro-uPA tPA + Purified proteins: P36, P11, $p36_2p11_2$ + Plasmin substrate chromogenic (A405 nm) →



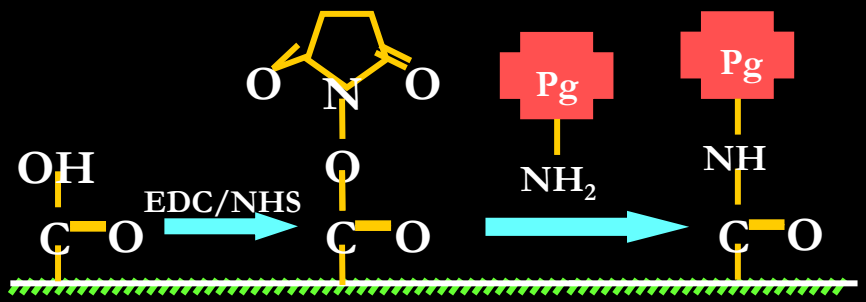
96-well plate

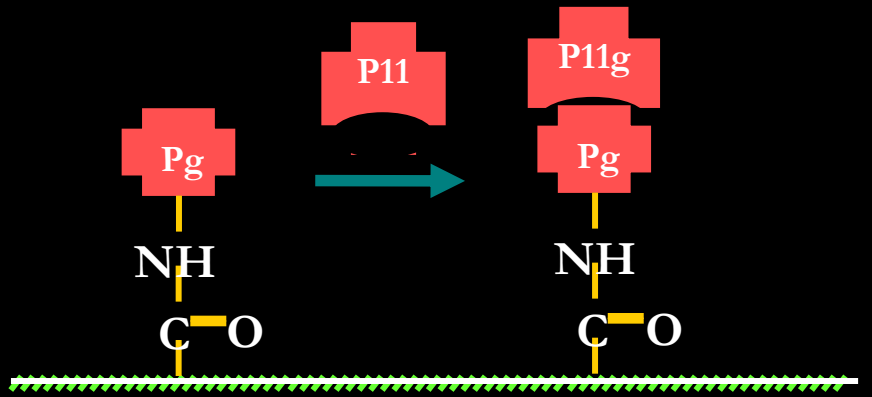
kinetic absorbance assay (plate reader)

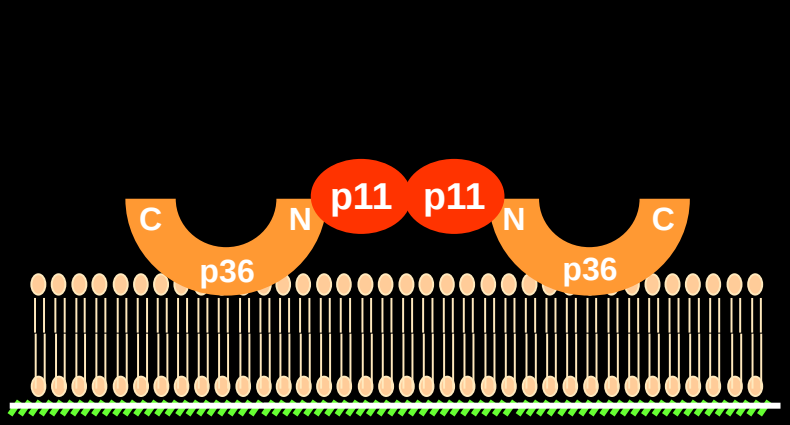
AIIT ACCELERATES tPA-DEPENDENT PLASMINOGEN ACTIVATION

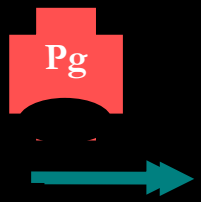


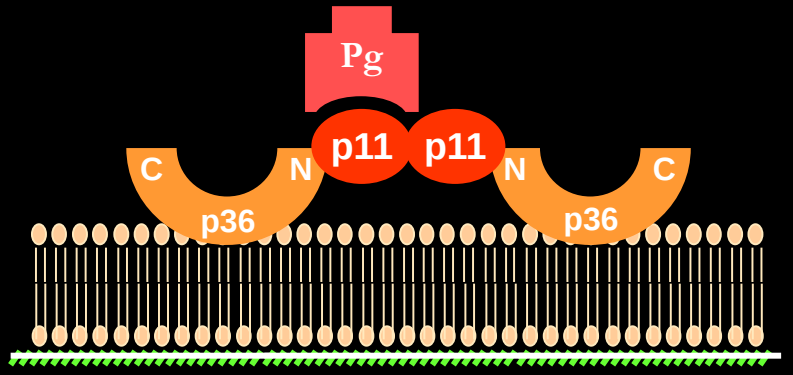
PLASMINOGEN BINDING ASSAYS USING SURFACE PLASMON RESONANCE



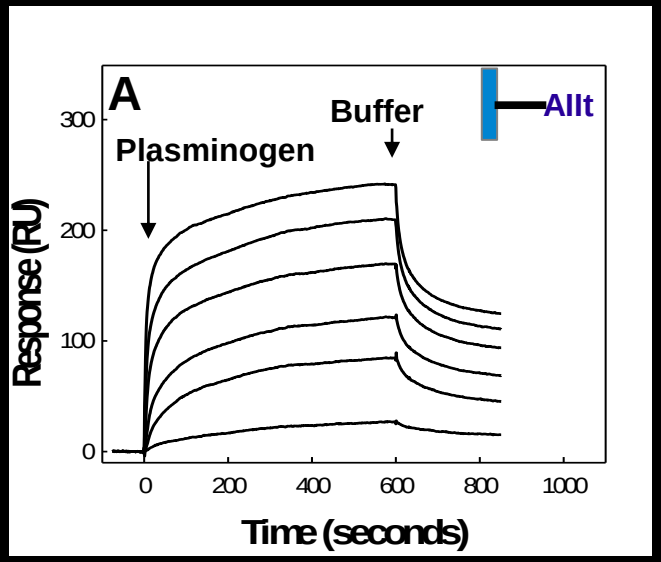


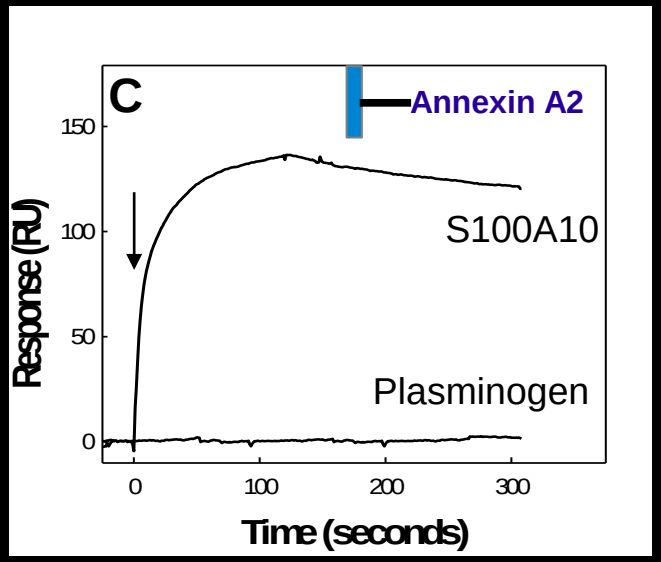


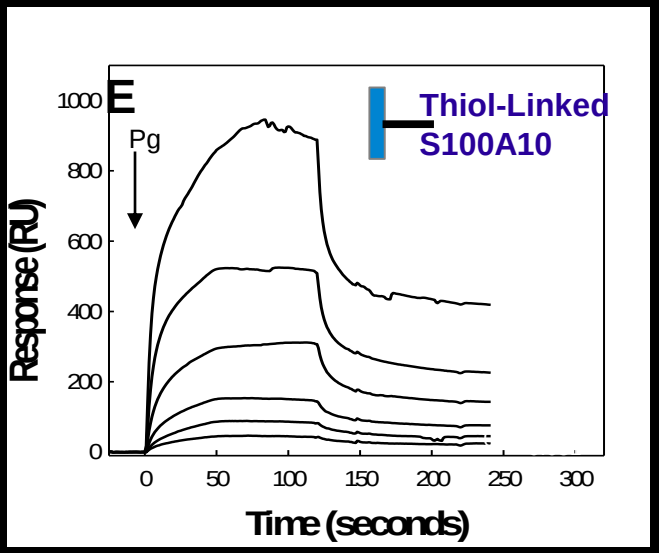


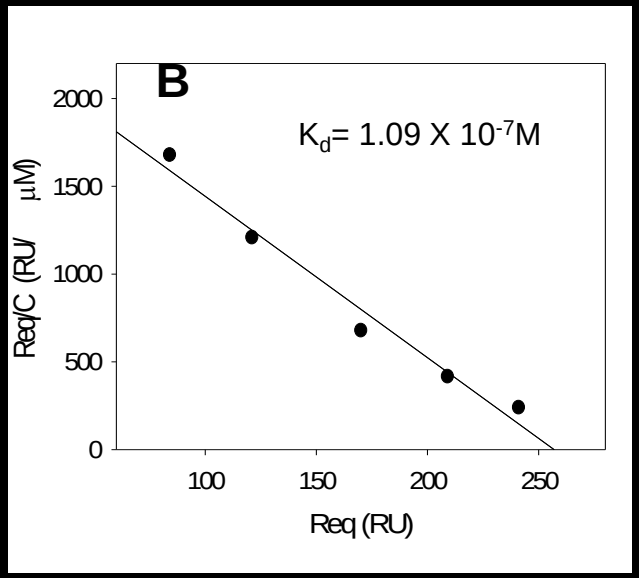


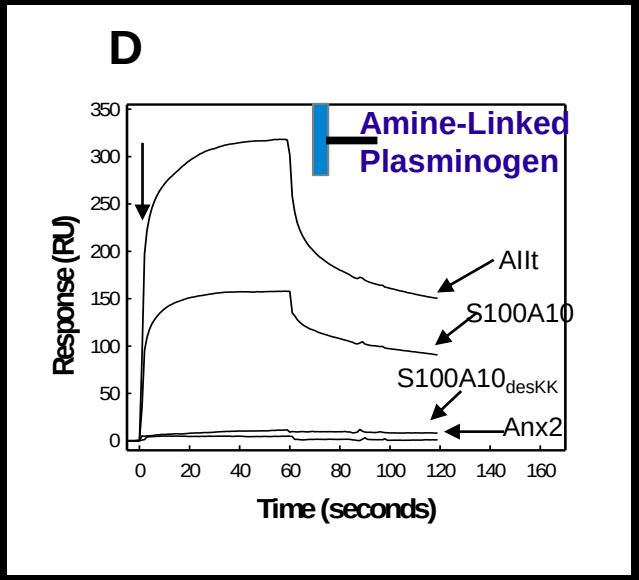
PLASMINOGEN BINDING TO AIIt and SUBUNITS

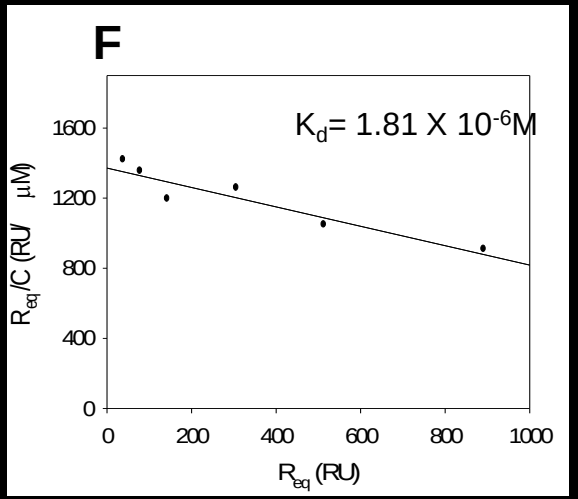












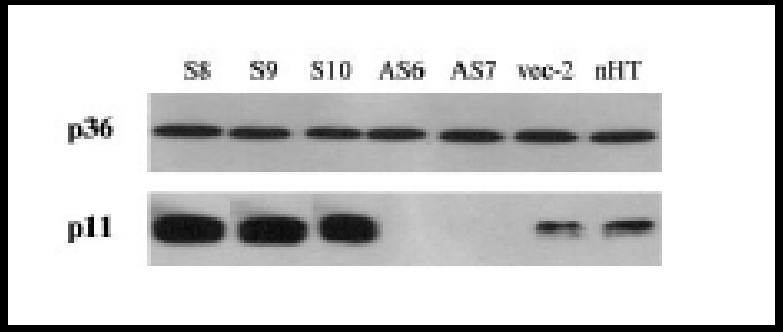
CONCLUSION I

P11 is an EMT gene

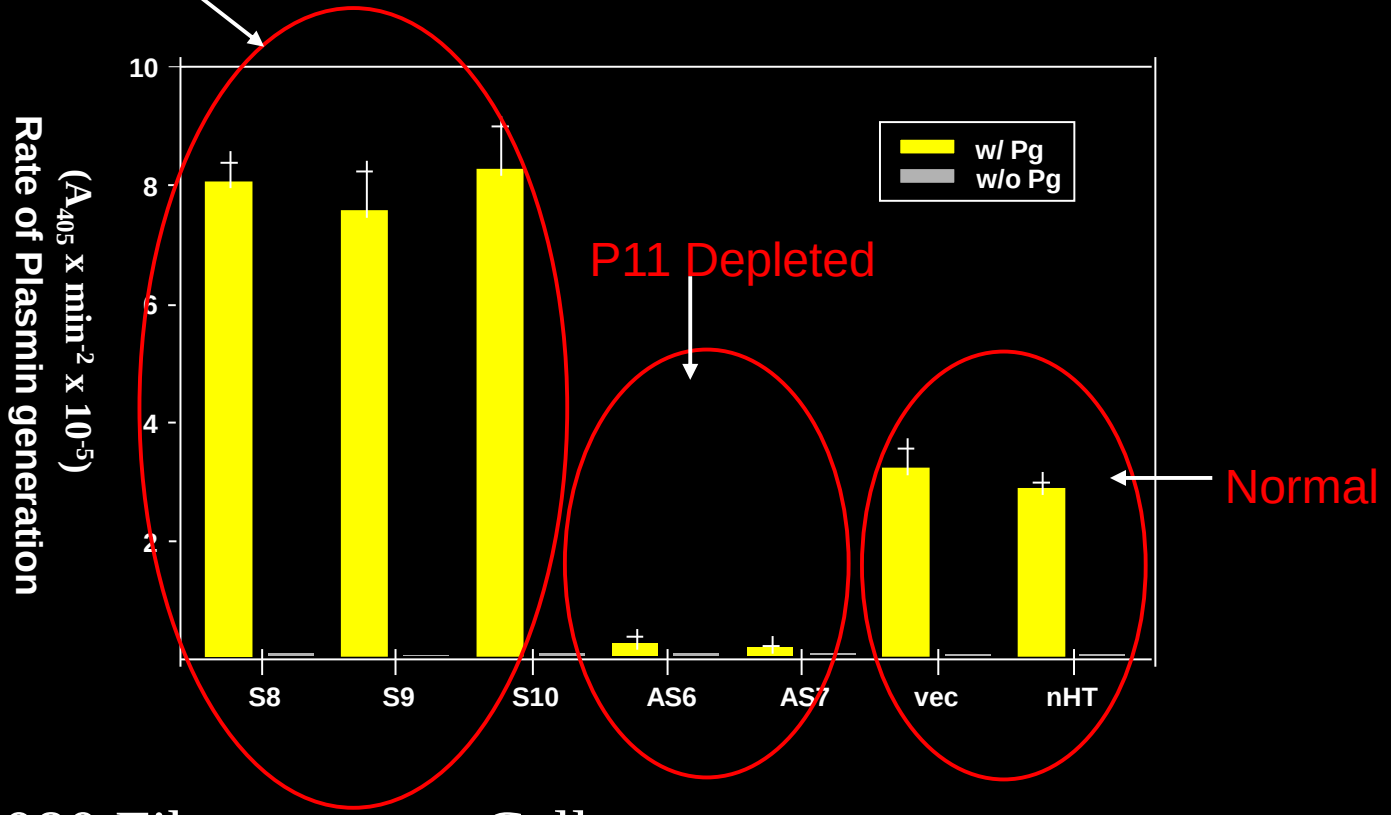
P11 binds tPA and plasminogen and activates tPA-dependent plasmin generation

DOES S100A10 PLAY A ROLE IN TUMOR GROWTH/METASTASIS ?

S100A10 MOIETY OF AIIT REGULATES PLASMIN GENERATION



P11 Overexpressed

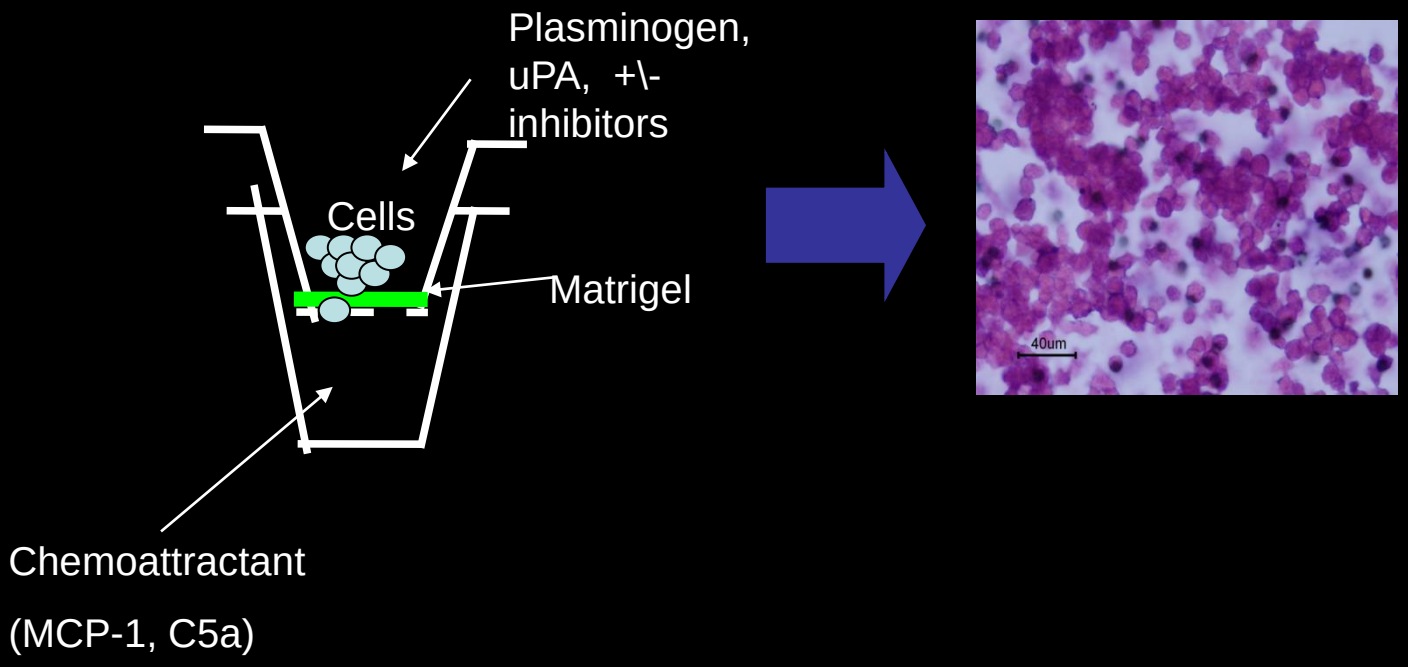


P11 Depleted

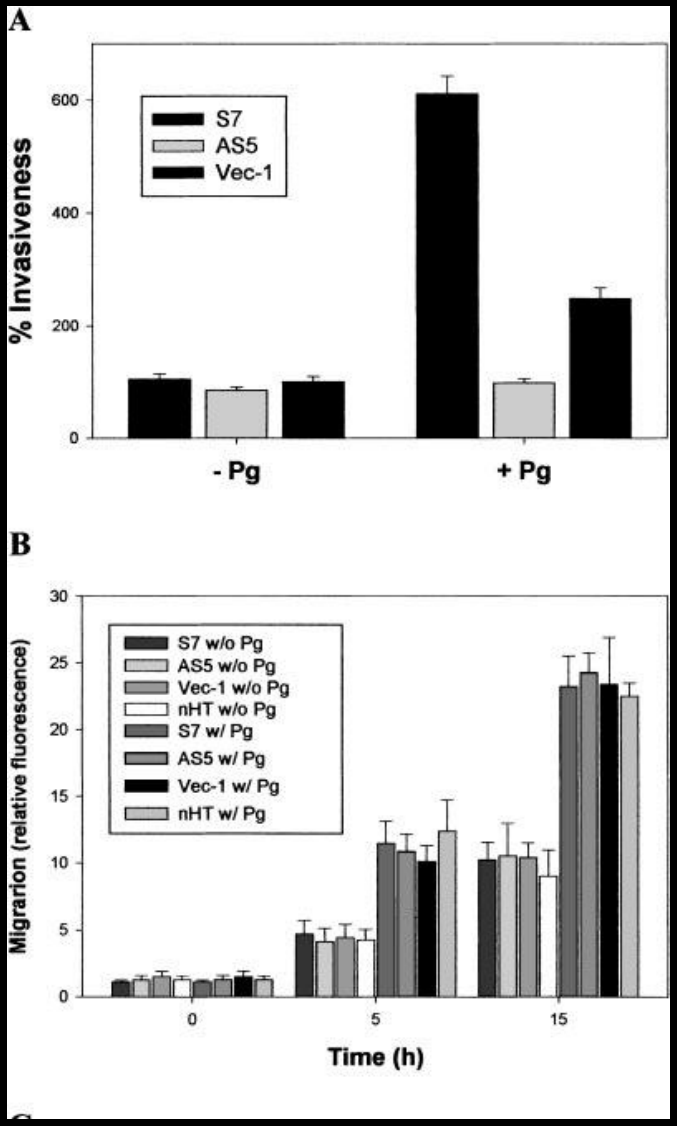
Normal

HT1080 Fibrosarcoma Cells

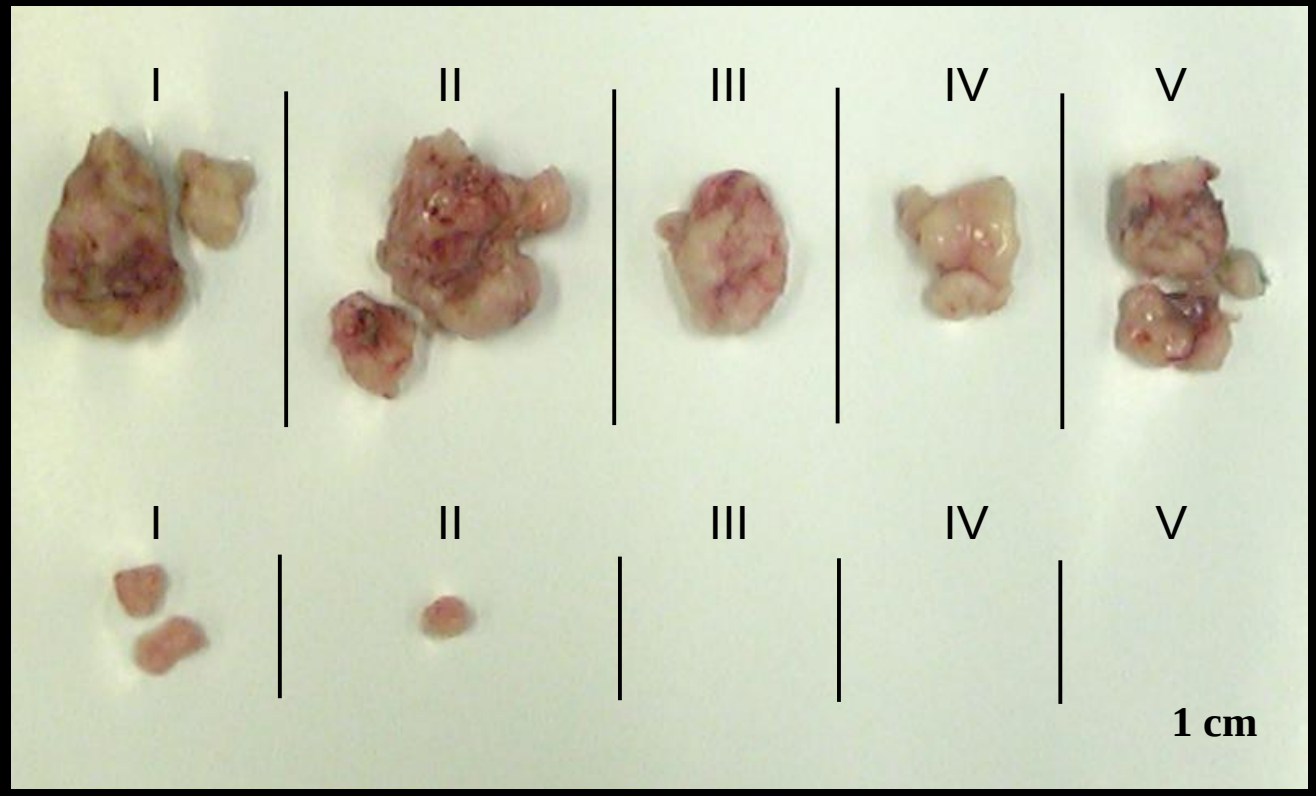
BOYDEN CHAMBER INVASION ASSAY



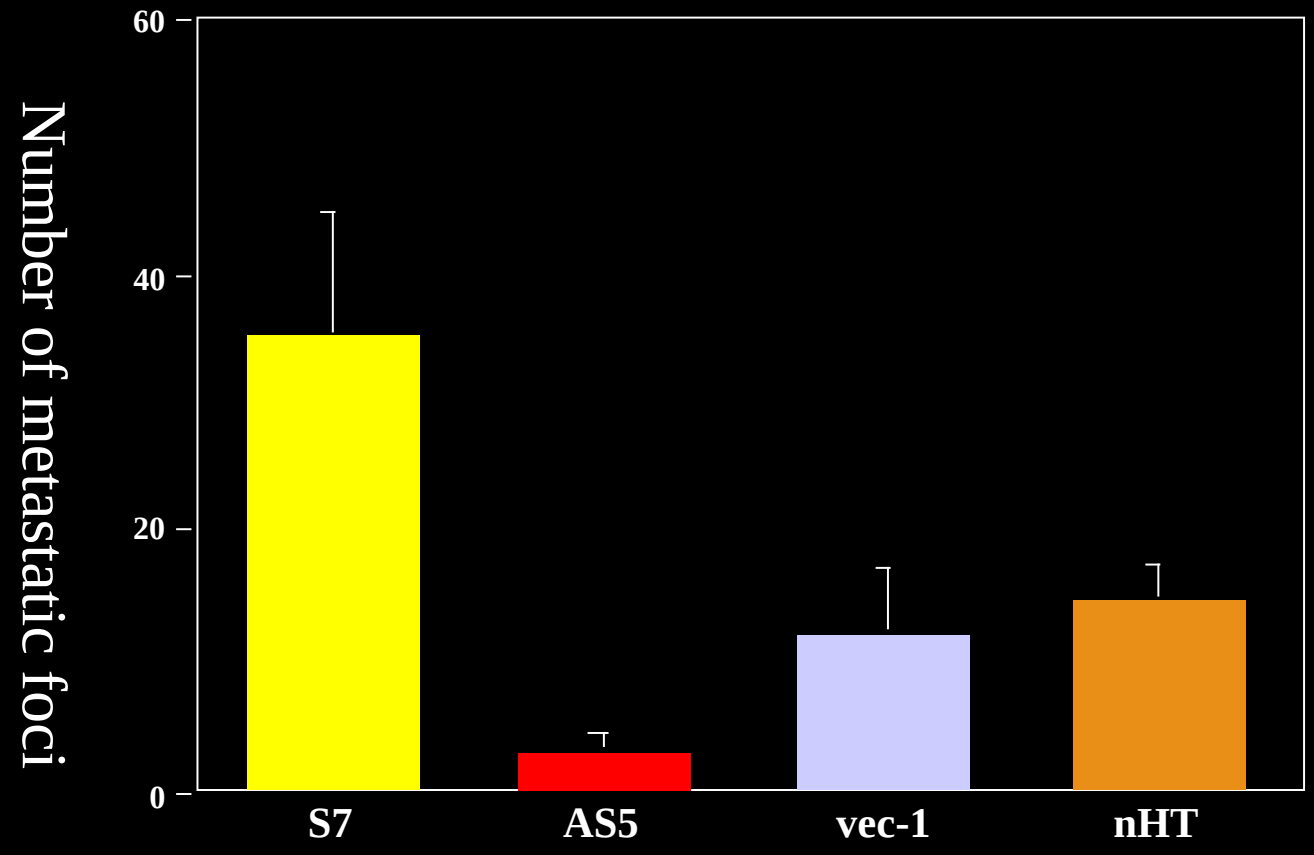
Invasiveness of HT1080 Cells



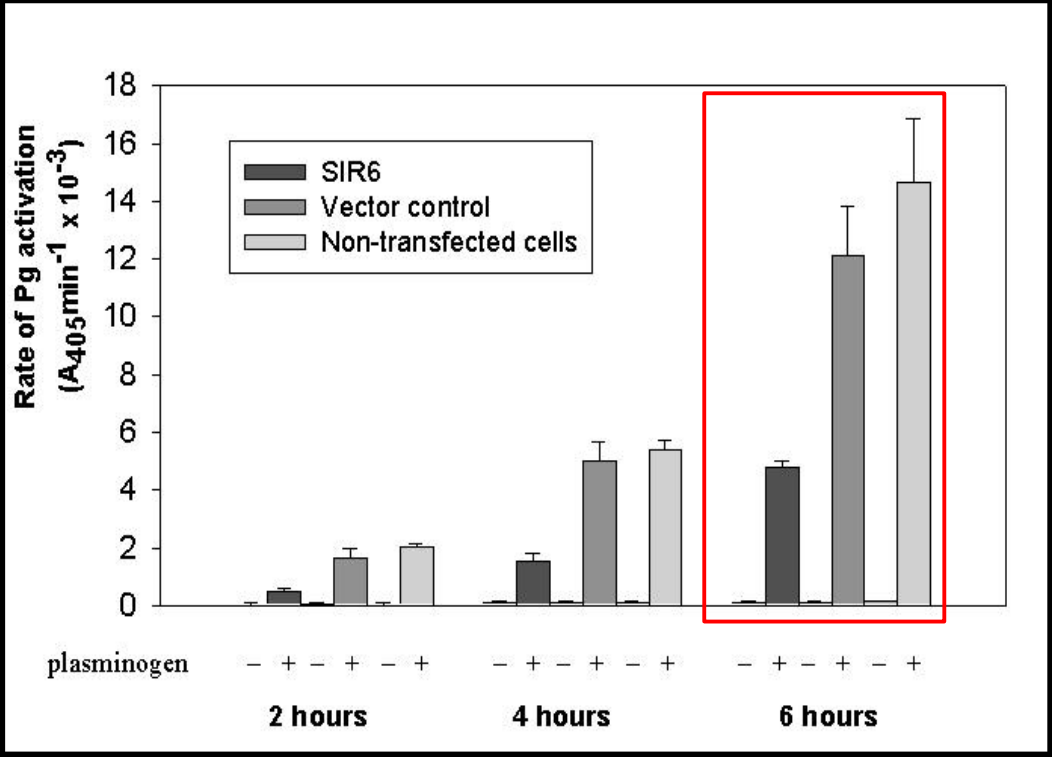
Growth of HT1080 Tumors in SCID Mice



Metastatic Potential of HT1080 Cells in SCID Mice



REDUCTION IN P11 LEVELS AFFECTS PLASMIN FORMATION BY HUMAN COLORECTAL CARCINOMA CELLS



CONCLUSION II

P11 regulates plasmin generation by cancer cells

P11 plays a role in oncogenesis

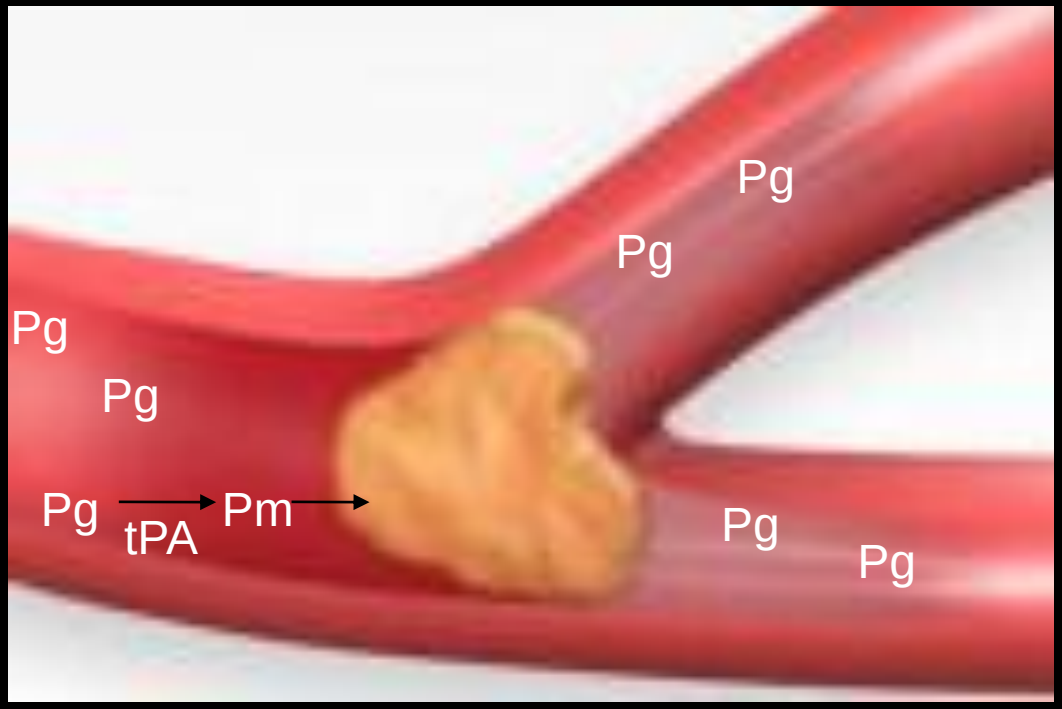
THE S100A10 KNOCKOUT MOUSE

Per Svenningsson, Karolinska Institute, Stockholm, Sweden

- What is the role of S100A10 in normal physiological processes (hemostasis, inflammation)
- What is the role of S100A10 in pathological processes (cancer)

ROLE OF P11 IN HEMOSTASIS

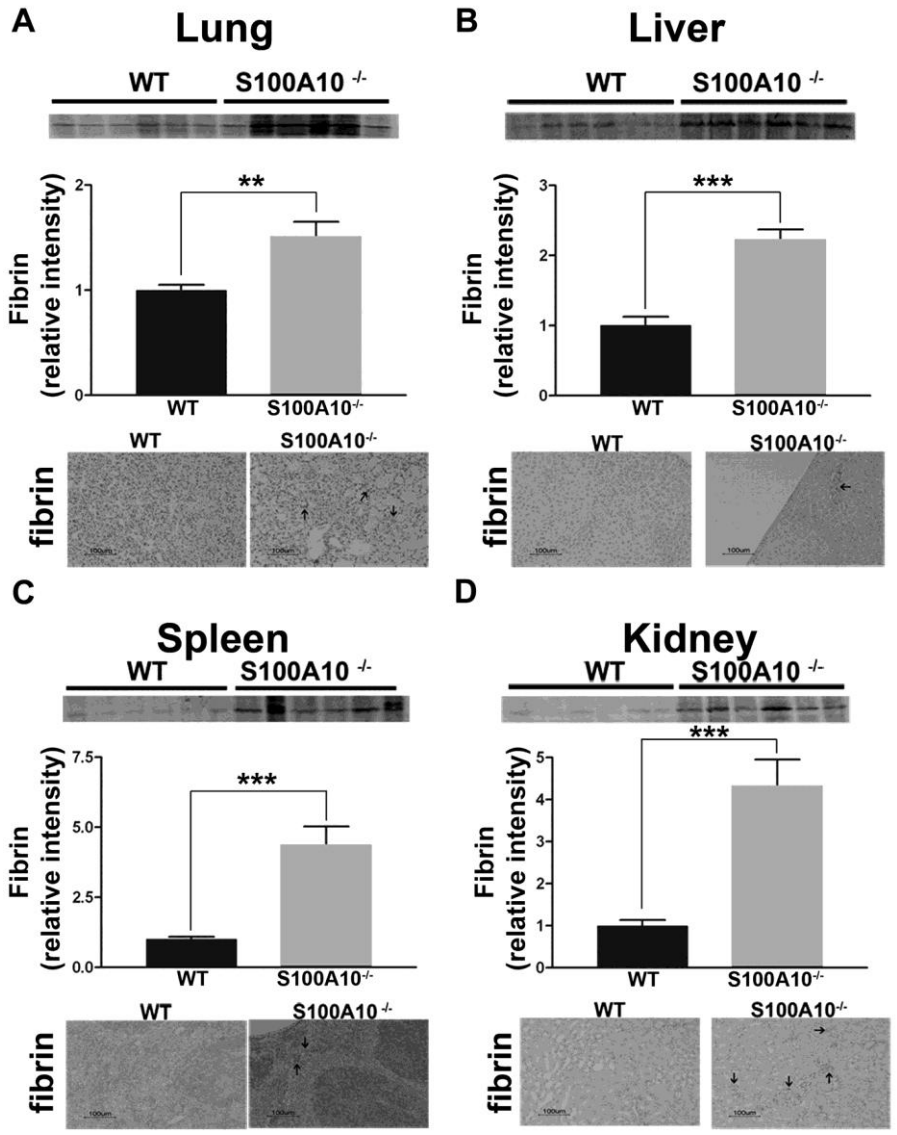
Clot Dissolution is the Responsibility of Plasmin



Plasminogen (Pg) is present in the blood while tissue plasminogen activator (tPA) is released from the endothelial cells. These proteins bind to receptors on the surface of the endothelial cell and as a result plasmin (Pm) is generated.
What is the identity of these cellular receptors?

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

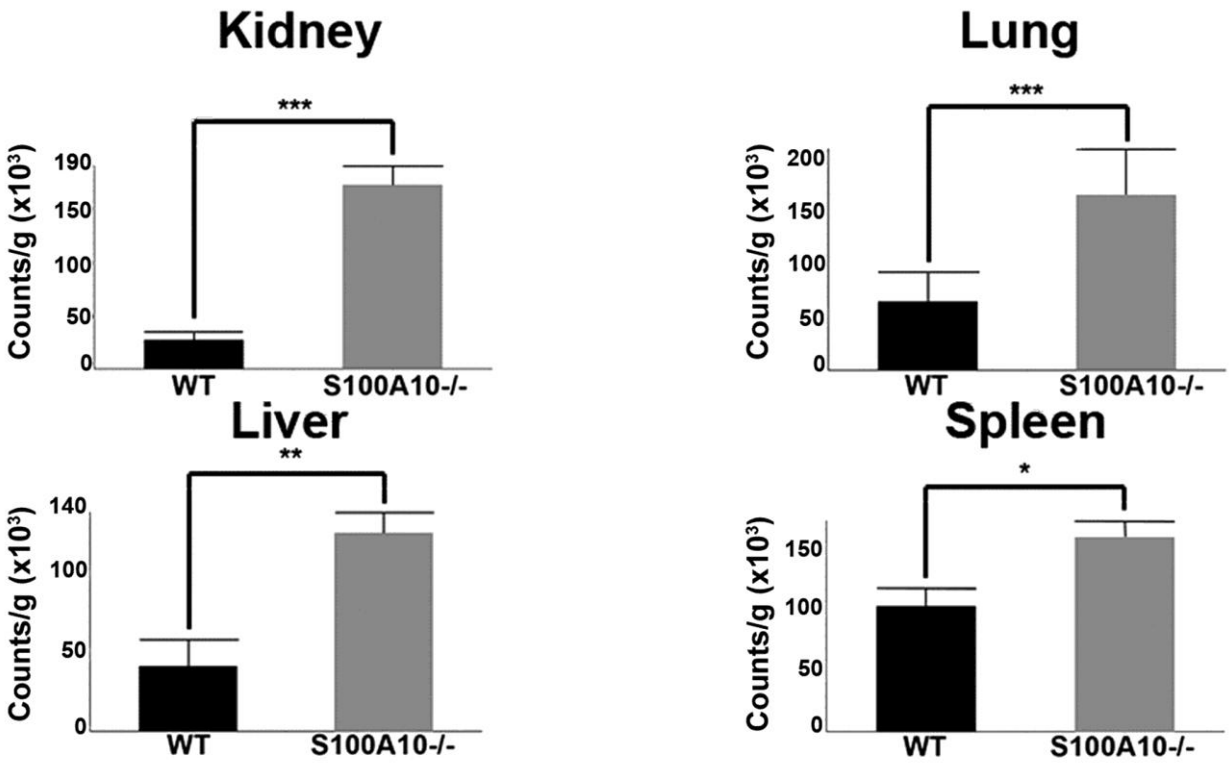
Loss of S100A10 results in increased tissue fibrin deposition.



Surette A P et al. Blood 2011;118:3172-3181

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

S100A10−/− mice have impaired ability to clear induced fibrin clots.

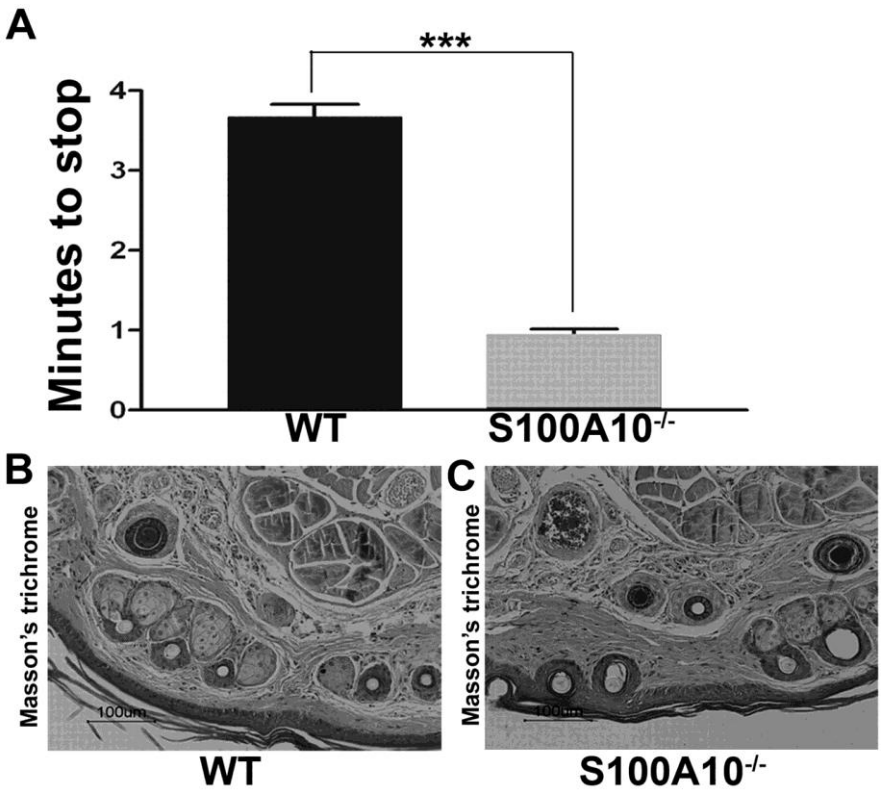


Mouse + ^{125}I-fibrinogen + batroxobin → ^{125}I-fibrin clot formation
(soluble) (insoluble)

Surette A P et al. Blood 2011;118:3172-3181

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

Bleeding time in WT and S100A10−/− mice.



Normal

Stimulus

Clot Formation

Clot Dissolution

No S100A10

Stimulus

Clot Formation

Clot Dissolution

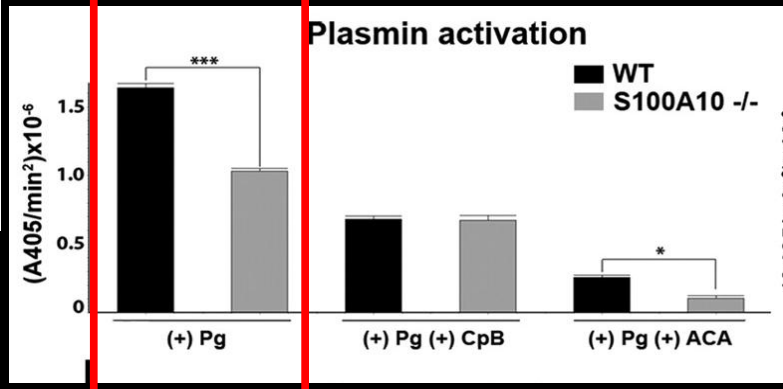
Blood Clot forms faster in S100A10-null mouse because clot dissolution is slower.

Surette A P et al. Blood 2011;118:3172-3181

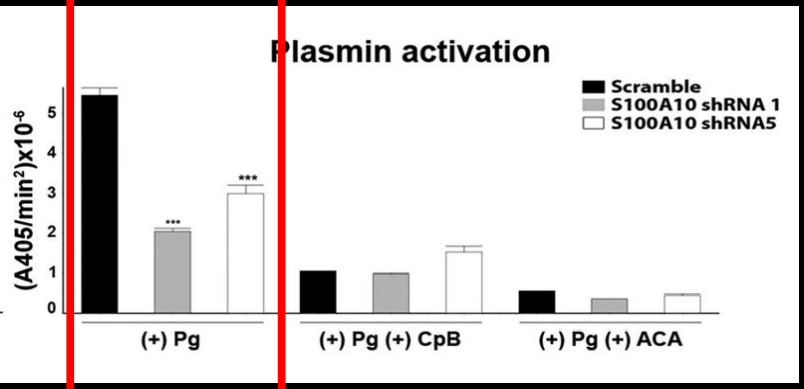
blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

DEPLETION OF S100A10 RESULTS IN DECREASED ENDOTHELIAL CELL PLASMIN GENERATION.

Mouse Endothelial cells



Human Endothelial cells



Surette A P et al. Blood 2011;118:3172-3181

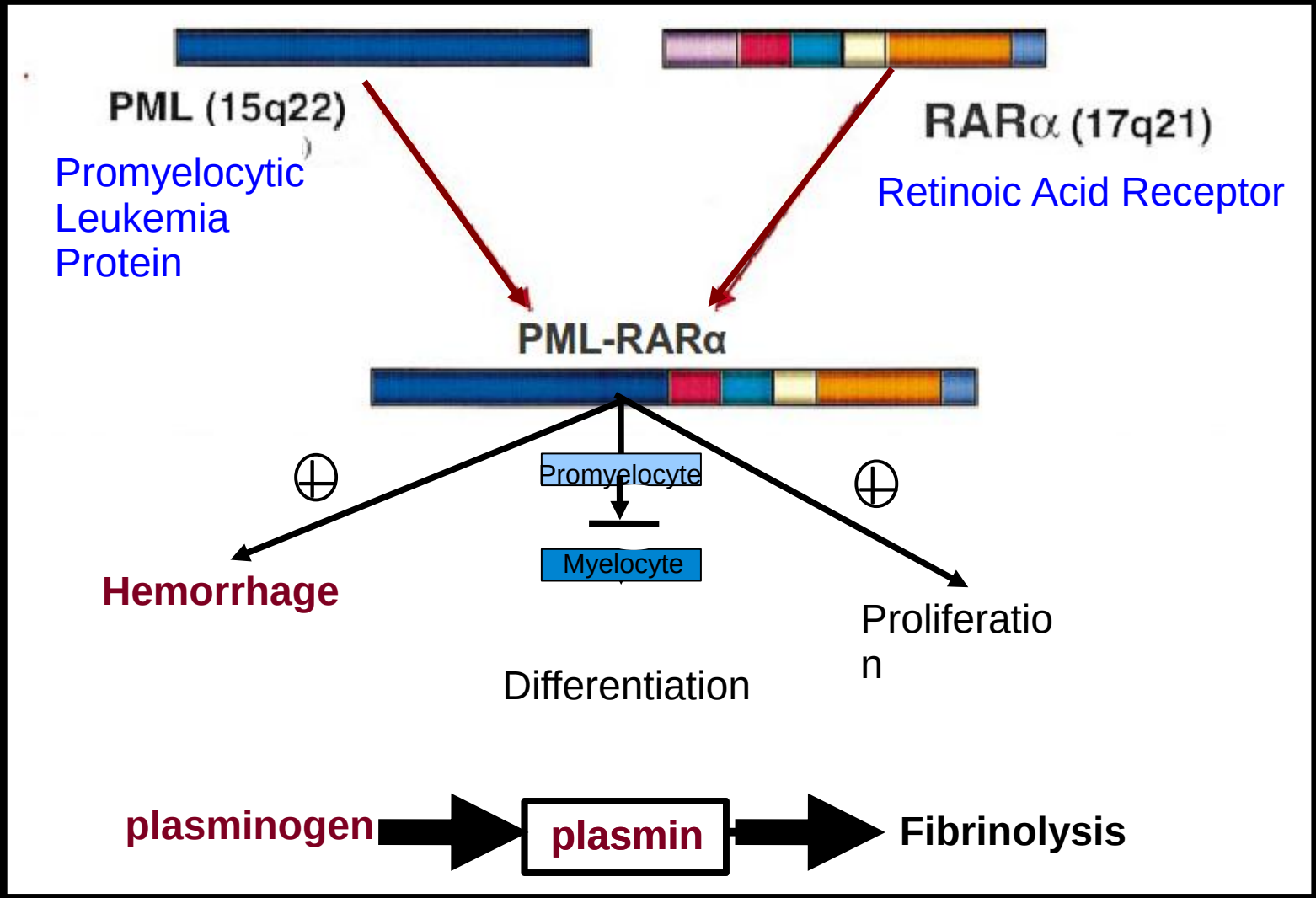
CONCLUSION III

P11 regulates plasmin generation by endothelial cells

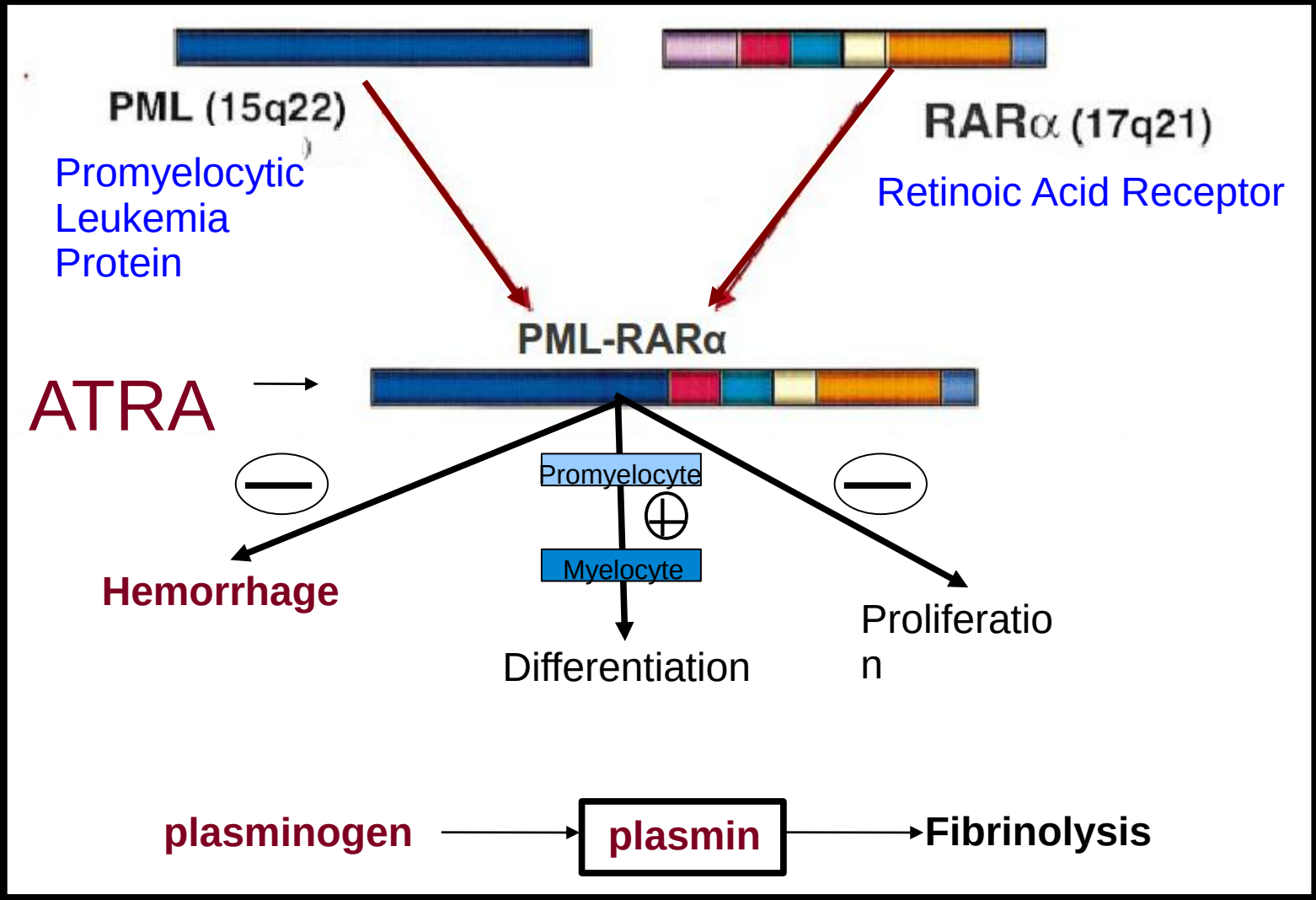
P11 plays a role in hemostasis by regulating fibrinolysis ie. P11 is a key player in the fibrinolytic surveillence system

ROLE OF P11 IN ACUTE PROMYELOCYTIC LEUKEMIA

ACUTE PROMYELOCYTIC LEUKEMIA



ACUTE PROMYELOCYTIC LEUKEMIA



THE CELL LINES

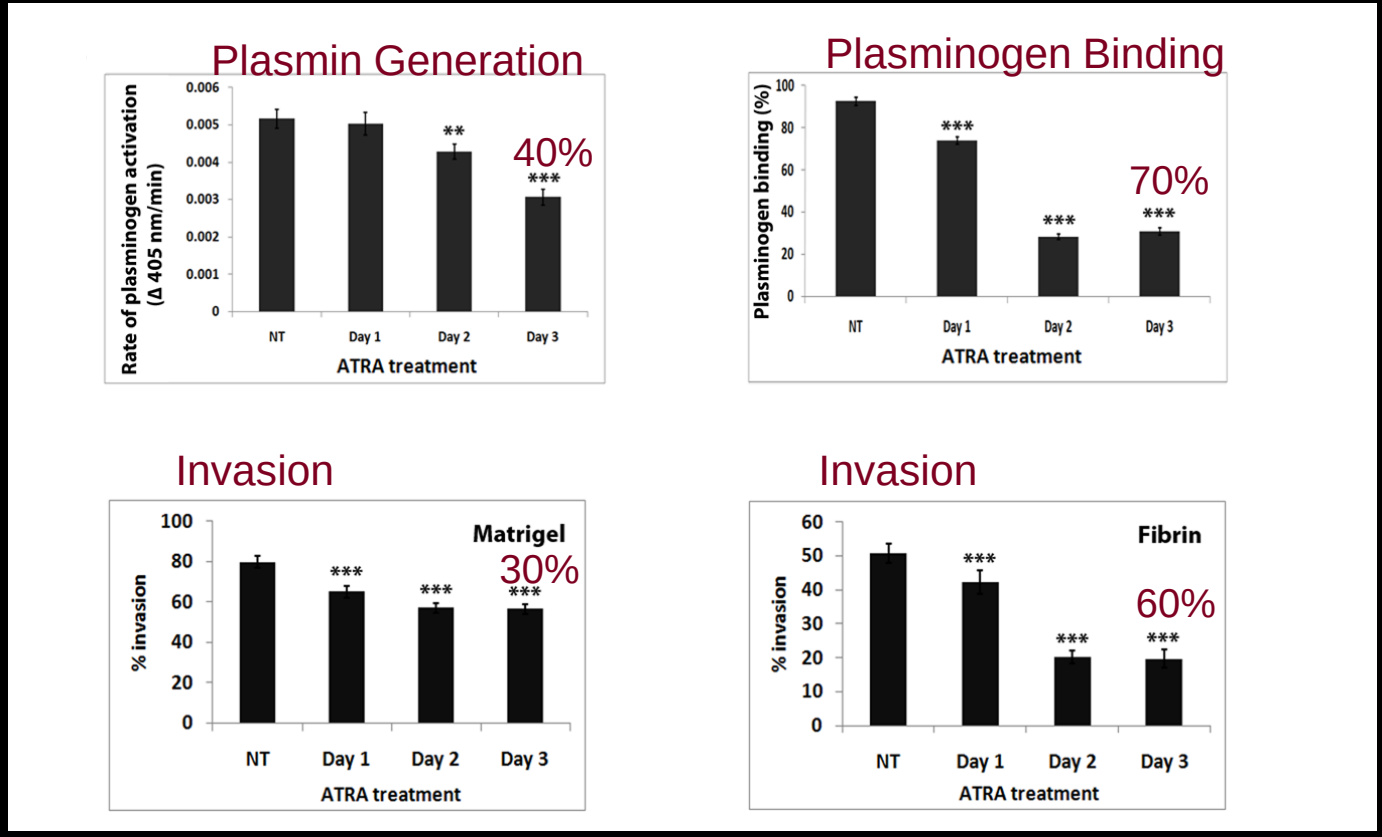
NB4 cells

- derived from a patient with APL
- t(15:17) translocation
- constitutively express PML-RAR fusion protein

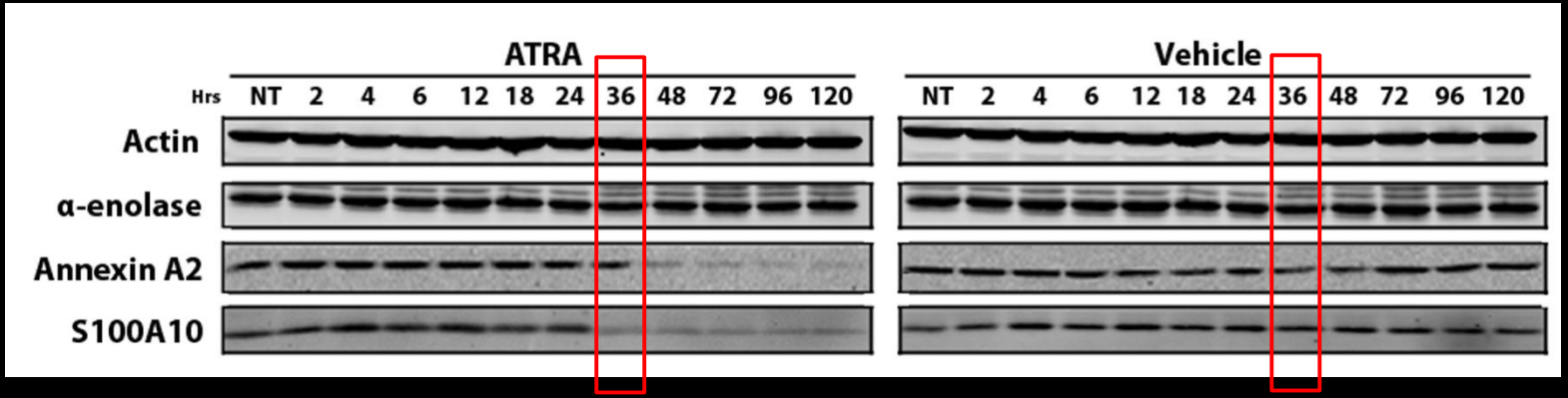
PR9

- derived from U937 (human monocyte lymphoma)
- inducible expression of PML-RAR fusion protein

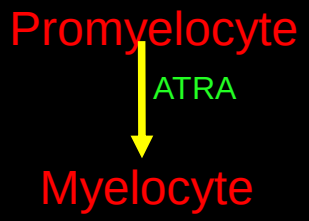
ATRA BLOCKS PLASMINOGEN BINDING AND PLASMIN GENERATION



ATRA BLOCKS S100A10 PROTEIN EXPRESSION IN NB4 CELLS

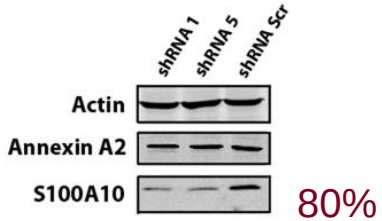


mRNA levels of S100A10 were unchanged



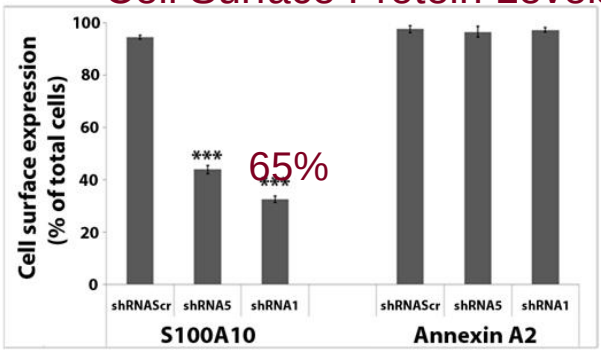
DEPLETION OF S100A10 FROM NB4 CELLS

Total Protein

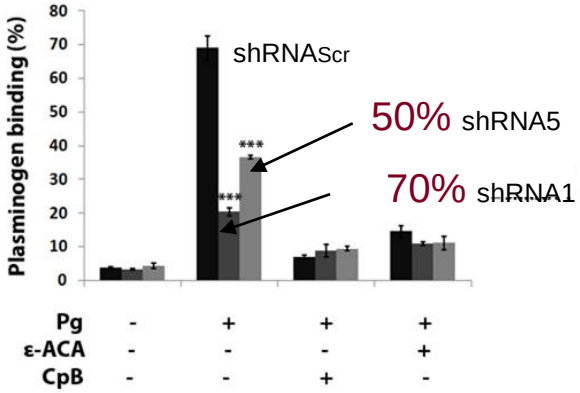


80%

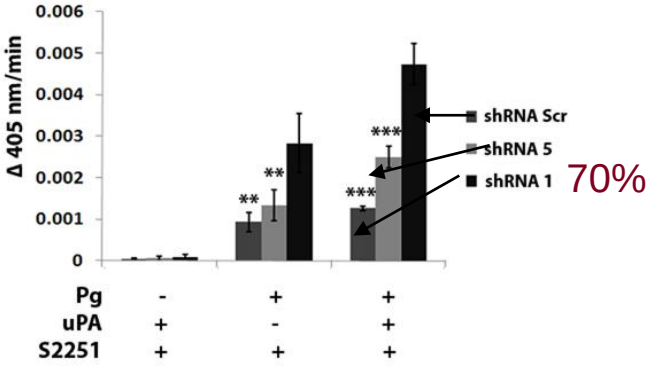
Cell Surface Protein Levels



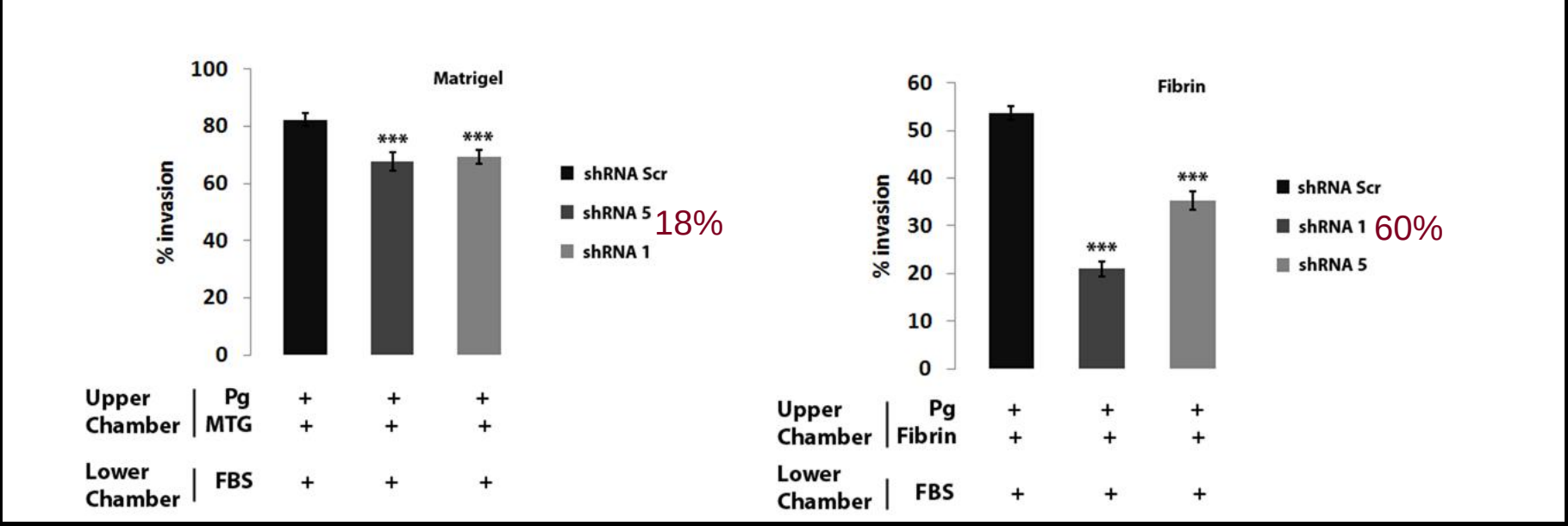
Plasminogen Binding



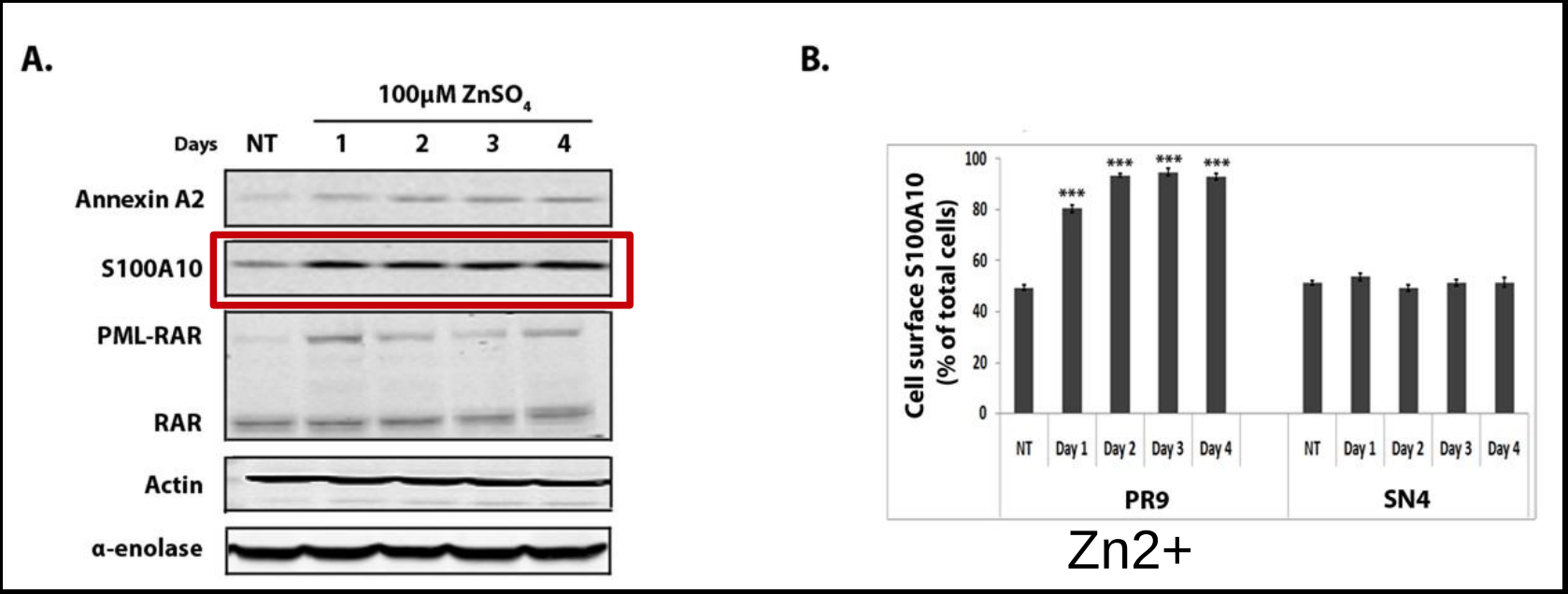
Plasmin Generation



ASSAY OF INVASION BY S100A10 DEPLETED NB4 CELLS

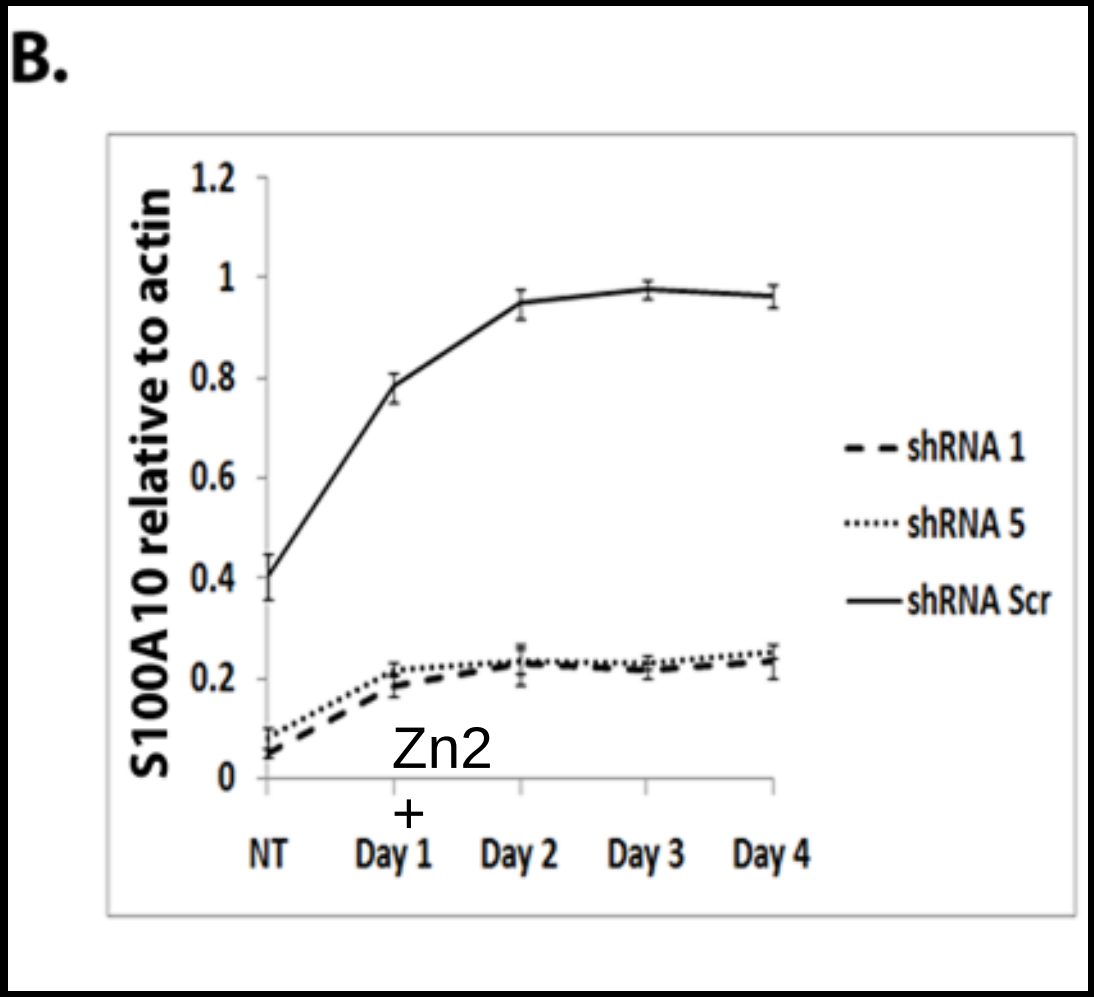


INDUCTION OF PML-RAR ONCOPROTEIN INCREASES P11 AND P36 LEVELS IN PR-9 CELLS

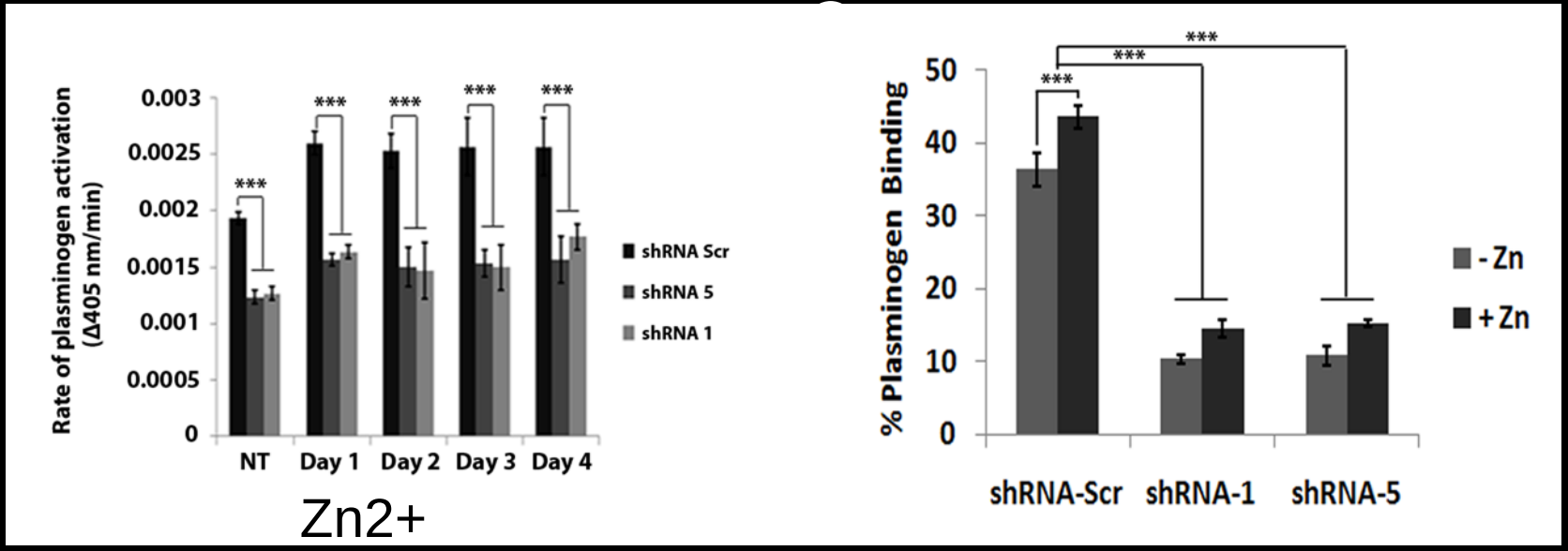


PR9 + Zn^{++} → PML-RAR expression activated

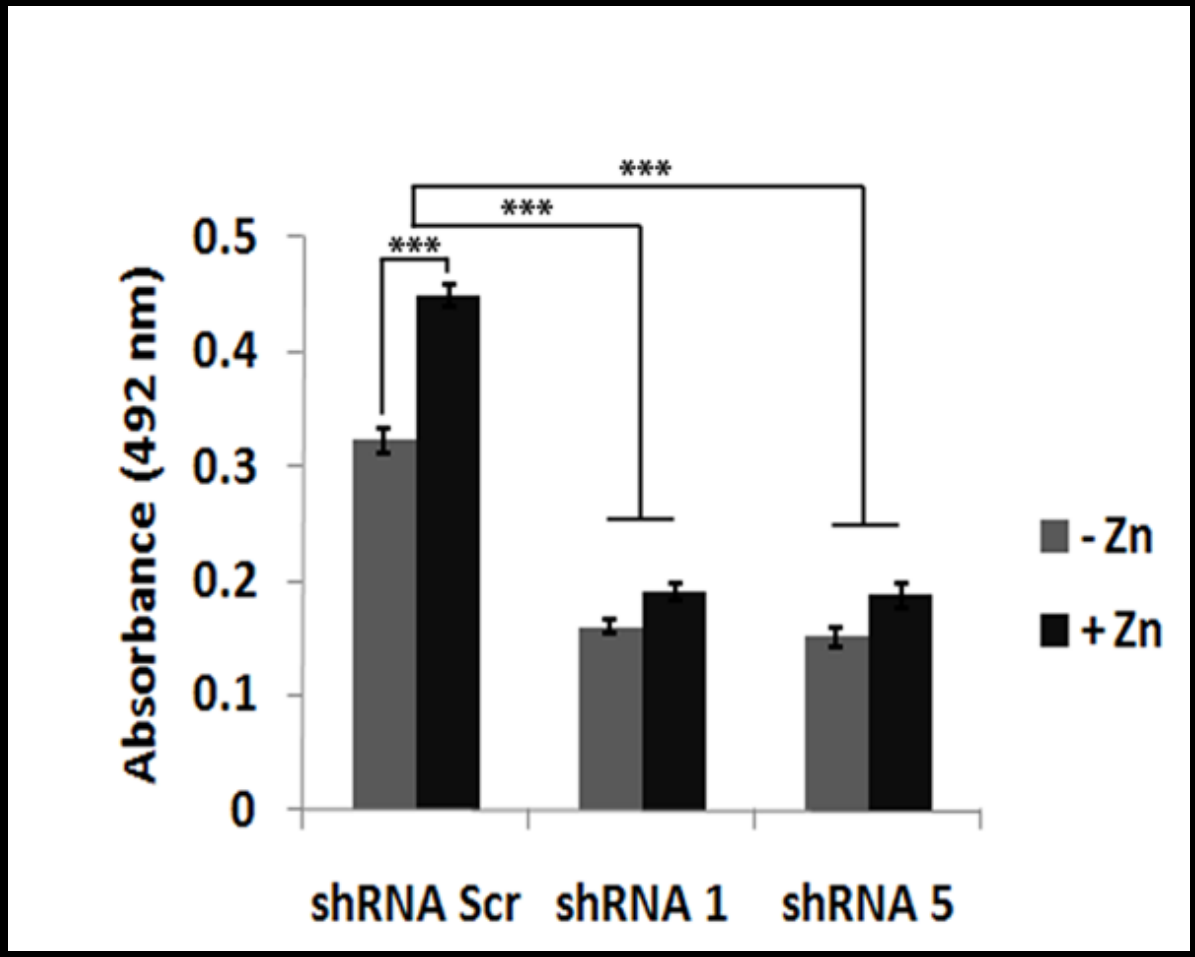
INDUCTION OF PML-RAR ONCOPROTEIN INCREASES P11 LEVELS



PLASMINOGEN BINDING AND PLASMIN GENERATION IS INCREASED BY PML-RAR IN PR-9 CELLS



PML-RAR EXPRESSION ACTIVATES FIBRINOLYSIS



Cells were placed on a fibrin layer for 24 hours in the presence or absence of Zn2+, and the conditioned media was collected and assayed for D-dimer

CONCLUSIONS IV

S100A10 (p11) plays a key role in plasmin regulation.

p11 is a major player in the fibrinolytic pathway of APL cells.

The PML-RAR oncoprotein activates p11 protein expression.

S100A10 is at the cross roads of hemostasis and oncogenesis

WHERE DO WE GO FROM HERE ?

Identify how S100A10 interacts with tPA and plasminogen.

Identify how cell surface levels of p11 are regulated.

Does p11 play a role in the fibrinolytic surveillance system—stroke and MS.

Role of p11 in cancer using genetically engineered mouse models (GEMM) (PYMT and iKras).

Role of p11 in the Epithelial Mesenchymal Transition and invadapodia.

Calgary Lab

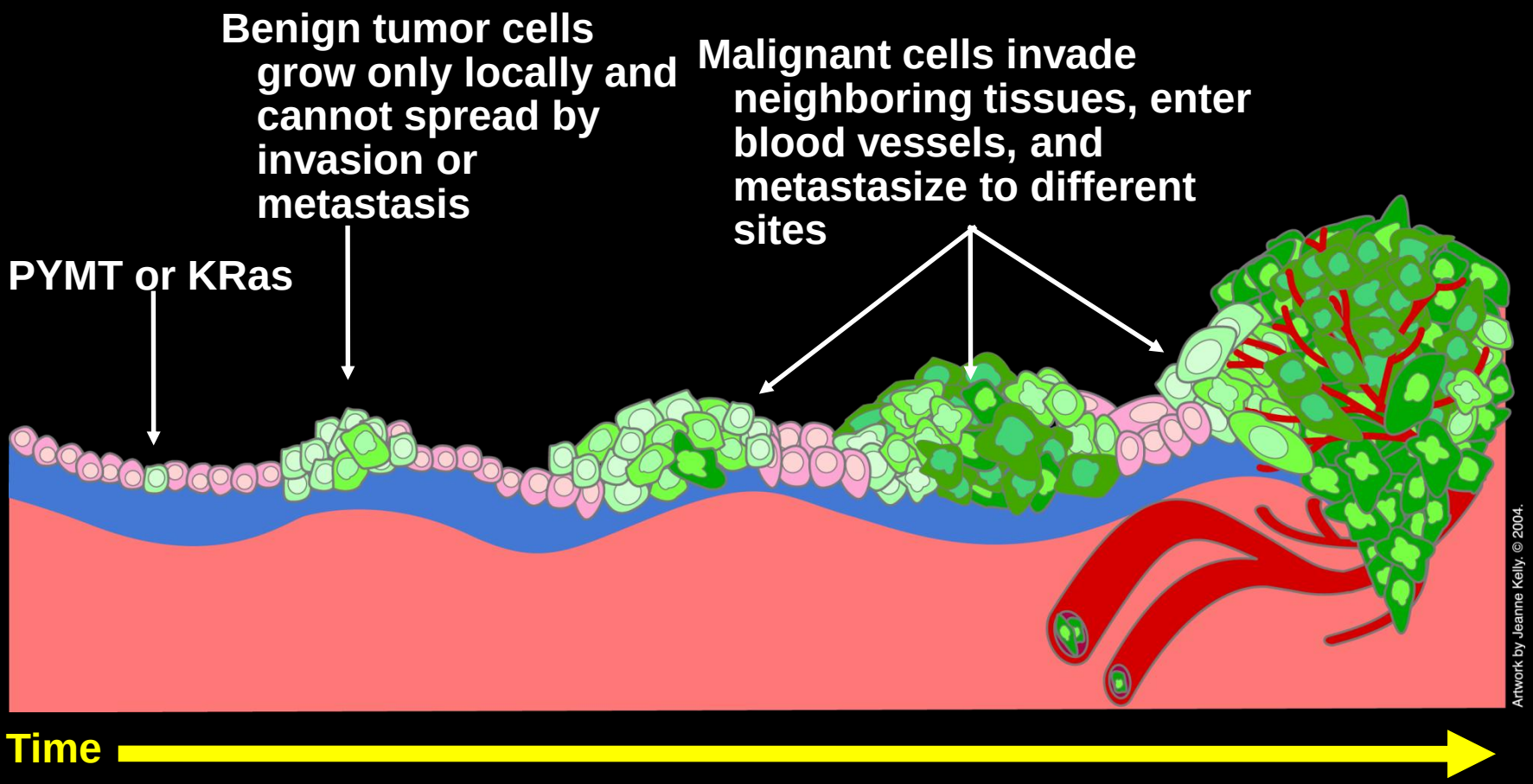
K.-S. Choi
M. Kwon
T. MacLeod
N. Filipenko
C.S. Yoon
L. Zhang
D. Fogg

Dalhousie Lab

Victoria Miller
Patricia Madurieura
Paul O'Connell
Kyle Phipps
Alexi Surette
Yi Zhang
Ryan Holloway
Moamen Bydoun
Alamelu Dharini Bharadwaj
Ludovic Durrieu

Funding from CIHR, HSFNS and CCSRI

GEMM



Tissue expression of CRE recombinase and constitutive expression of TTA. Kras has a LSL stop and is activated by the TO ie. tissue specific and inducible expression of Kras.

Calgary Lab

K.-S. Choi
M. Kwon
T. MacLeod
N. Filipenko
C.S. Yoon
L. Zhang
D. Fogg

Dalhousie Lab

Mike Taboski
Erta Kalaxhi
Victoria Miller
Patricia Madureira
Robert Nuttall
Paul O'Connell
Kyle Phipps
Alexi Surette
Tracy Daley
Yi Zhang
Xin Huang

Funding from Heart and Stroke Foundation

HEART & STROKE FOUNDATION OF NOVA SCOTIA
Finding answers. For life.

GENES ENRICHED IN THE DCIS to IC TRANSITION

Clone ID	*t* statistic	*P* value	Adj. *P* value*	Description
869375	6.86	2.20E-05	0.02683578	IDH2 | isocitrate dehydrogenase 2
504308	6.46	3.60E-05	0.02683578	FLJ10540 | hypothetical protein
825606	5.95	7.10E-05	0.02683578	KNSL1 | kinesin-like 1
951241	5.92	7.40E-05	0.02683578	ANKT | nucleolar protein ANKT
280375	5.58	1.20E-04	0.0304866	PRO2000 | PRO2000 protein
564981	5.58	1.20E-04	0.0304866	Similar to RIKEN cDNA 2810433K01
1476053	5.4	1.50E-04	0.03425014	RAD51
769921	4.97	2.80E-04	0.04935153	**UBE2C | ubiquitin-conjugating enzyme E2C**
128711	4.72	4.10E-04	0.04935153	ANLN | anillin, actin binding protein
814270	4.61	4.80E-04	0.05092141	PMSCL1 | polymyositis/scleroderma autoantigen 1
209066	4.58	5.00E-04	0.05092141	STK15 | serine/threonine kinase 15
2017415	4.52	5.60E-04	0.05338307	CENPA | centromere protein A
823598	4.28	8.10E-04	0.0703179	PSMD12 | proteasome 26S subunit
878330	4.17	9.60E-04	0.07962393	EST
785368	4.05	1.20E-03	0.08461982	TOPK | PDZ-binding kinase
839682	3.96	1.30E-03	0.08461982	UBE2N | ubiquitin-conjugating enzyme E2N
756595	3.89	1.50E-03	0.08461982	S100A10 | S100 calcium-binding protein A10
347373	3.86	1.60E-03	0.08461982	TCEB1 | transcription elongation factor B
624627	3.84	1.60E-03	0.08461982	EST
1517595	3.84	1.60E-03	0.08461982	**RRM2 | ribonucleotide reductase M2**
825470	3.83	1.70E-03	0.08461982	TOP2A | topoisomerase (DNA) II alpha
259950	3.67	2.20E-03	0.08771114	CML66 | CML tumor antigen 66
292936	3.67	2.20E-03	0.08771114	FLJ10468 | hypothetical protein
1416055	3.67	2.20E-03	0.08771114	KIAA0165 | homolog of yeast extra spindle poles
744047	3.55	2.60E-03	0.09967783	PLK | polo-like kinase
705064	3.44	3.20E-03	0.10860726	TACC3
2322367	3.44	3.20E-03	0.10860726	RTN4 | reticulon 4
66406	3.39	3.50E-03	0.116593	EST
462926	3.36	3.60E-03	0.11872875	NEK2 | NIMA-related kinase 2
815501	3.3	4.00E-03	0.12440908	MGC2721 | hypothetical protein
1035796	3.21	4.60E-03	0.13817458	EST
700792	3.15	5.10E-03	0.14848582	CDKN3 | cyclin-dependent kinase inhibitor 3
2018131	3.09	5.80E-03	0.16117884	RACGAP1 | Rac GTPase activating protein 1
743810	3.03	6.30E-03	0.17362215	MGC2577 | hypothetical protein
781047	2.94	7.40E-03	0.18297681	RRM2 | ribonucleotide reductase M2
1422338	2.94	7.40E-03	0.18297681	BUB1
796694	2.92	7.70E-03	0.18720879	BIRC5 | survivin
725454	2.87	8.30E-03	0.18975804	**CKS2 | CDC28 protein kinase 2**

Paired *t* test was performed on 11 patient-matched DCIS–IDC pairs to identify genes with increased expression in IDC. A total of 85 genes were identified at one-sided $P < 0.01$ (Table 7).

*Adjusted *P* value by the Benjamini and Hochberg procedure. The 39 genes shown here are those also identified in the 100-gene grade III signature. Genes in bold are validated by QRT-PCR (Fig. 4*B*).