

3rd International Conference and Exhibition on **Immunology Summit-2014**
Clinical & Cellular Immunology

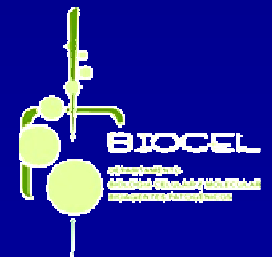
THE ROLE OF MAST CELL PROTEASES IN TUMOR ANGIOGENESIS

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MAST CELLS

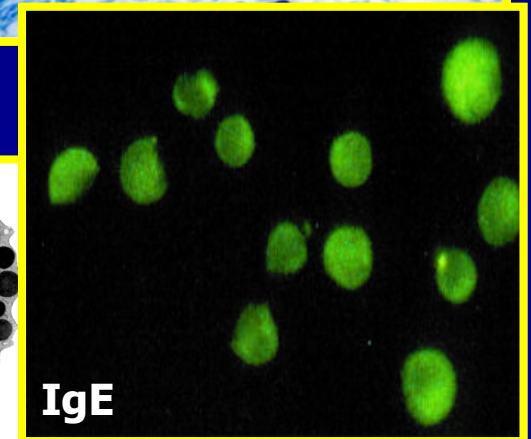
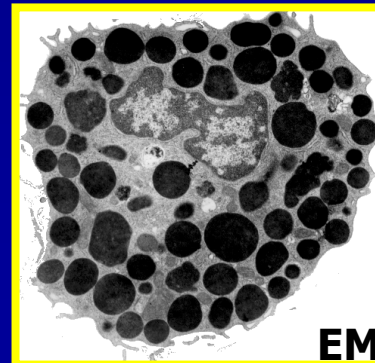
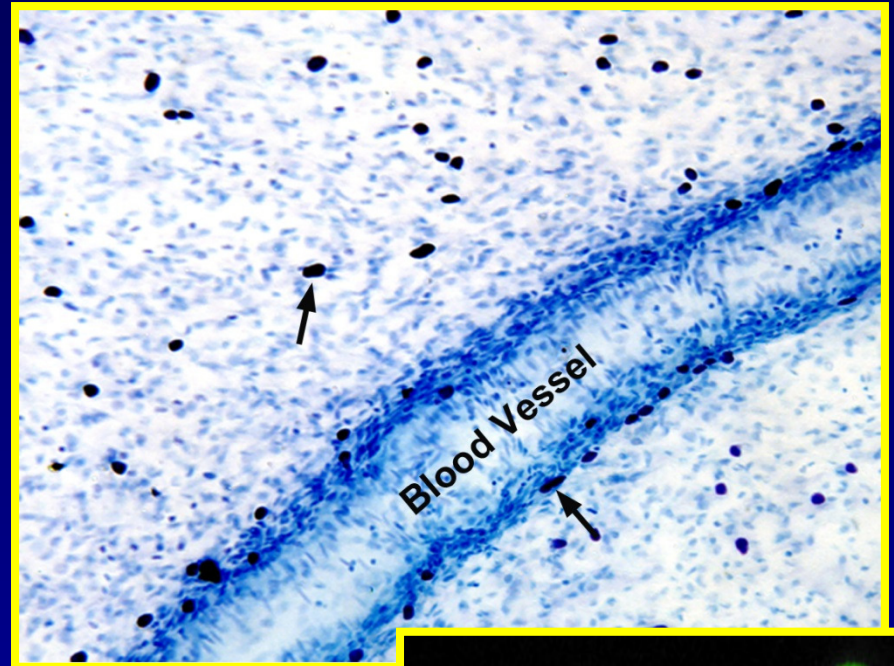
Local effector cells of the immune system that participate in :

Defense of the host organism

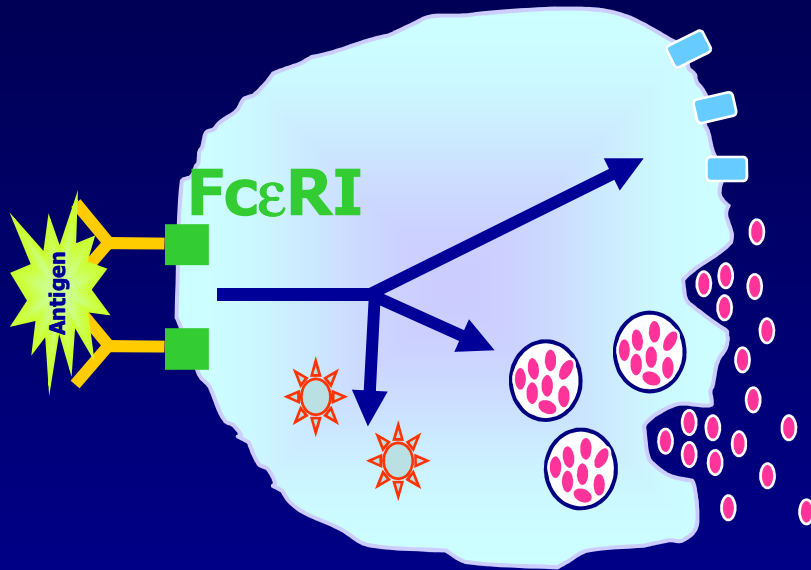
- Inflammatory reactions
- Elimination of parasites
- Innate immunity against bacterial and viral infections
- Allergic reactions

Diseases

- Asthma
- Diseases of the central nervous system: Alzheimer's disease
- Diseases of the cardiovascular system: Atherosclerosis
- Tumor Progression



MAST CELL FUNCTION IS RELATED TO THE RELEASE OF MAST CELL MEDIATORS

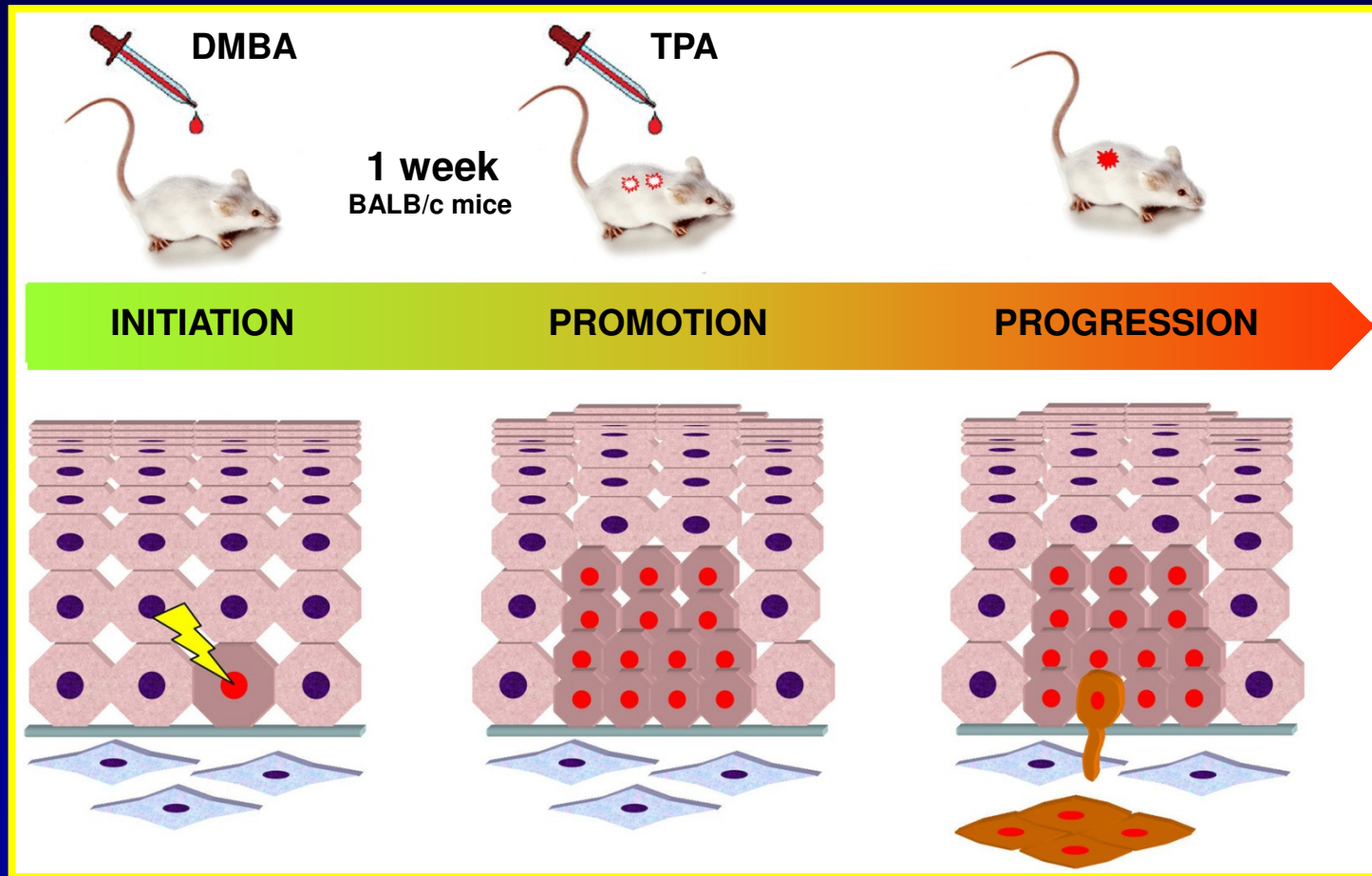


Newly Synthesized Mediators
Transcription Factor Activation
Growth Factors
Cytokines

Newly Formed Mediators
Phospholipase A₂ Activation
Chemotactic leukotrienes
Prostaglandins
Thromboxanes
Activating Factors, PAF

Preformed Mediators
Granule Release
Histamine
Heparin
Enzymes
Chemotactic factors
Mast cell specific proteases

TUMOR INDUCTION

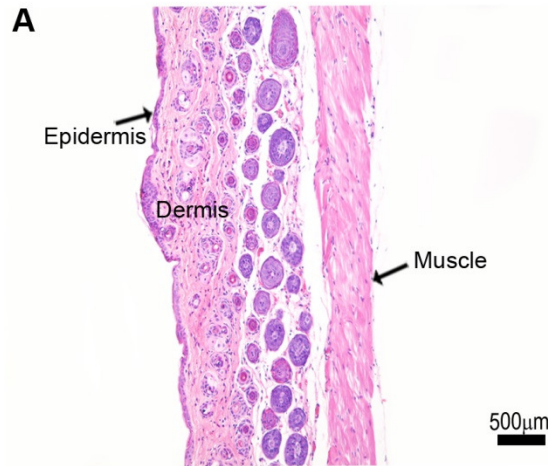


DMBA (7,12-Dimethylbenz(a)anthracene)
TPA (12-O-Tetradecanoylphorbol-13-acetate)

Diagram Modified from the German Cancer Research Center

TUMOR MORPHOLOGY CHANGES WITH TUMOR PROGRESSION

Control



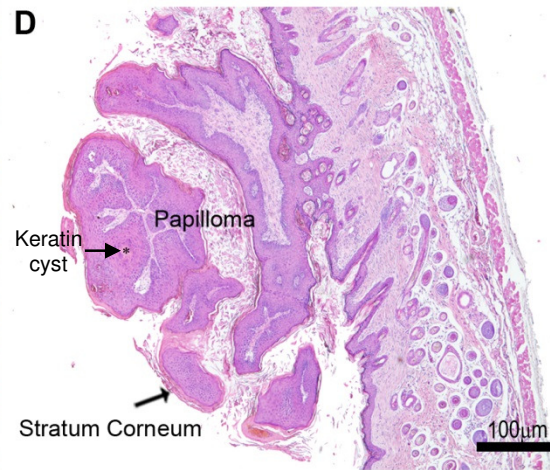
Phase I



Phase II

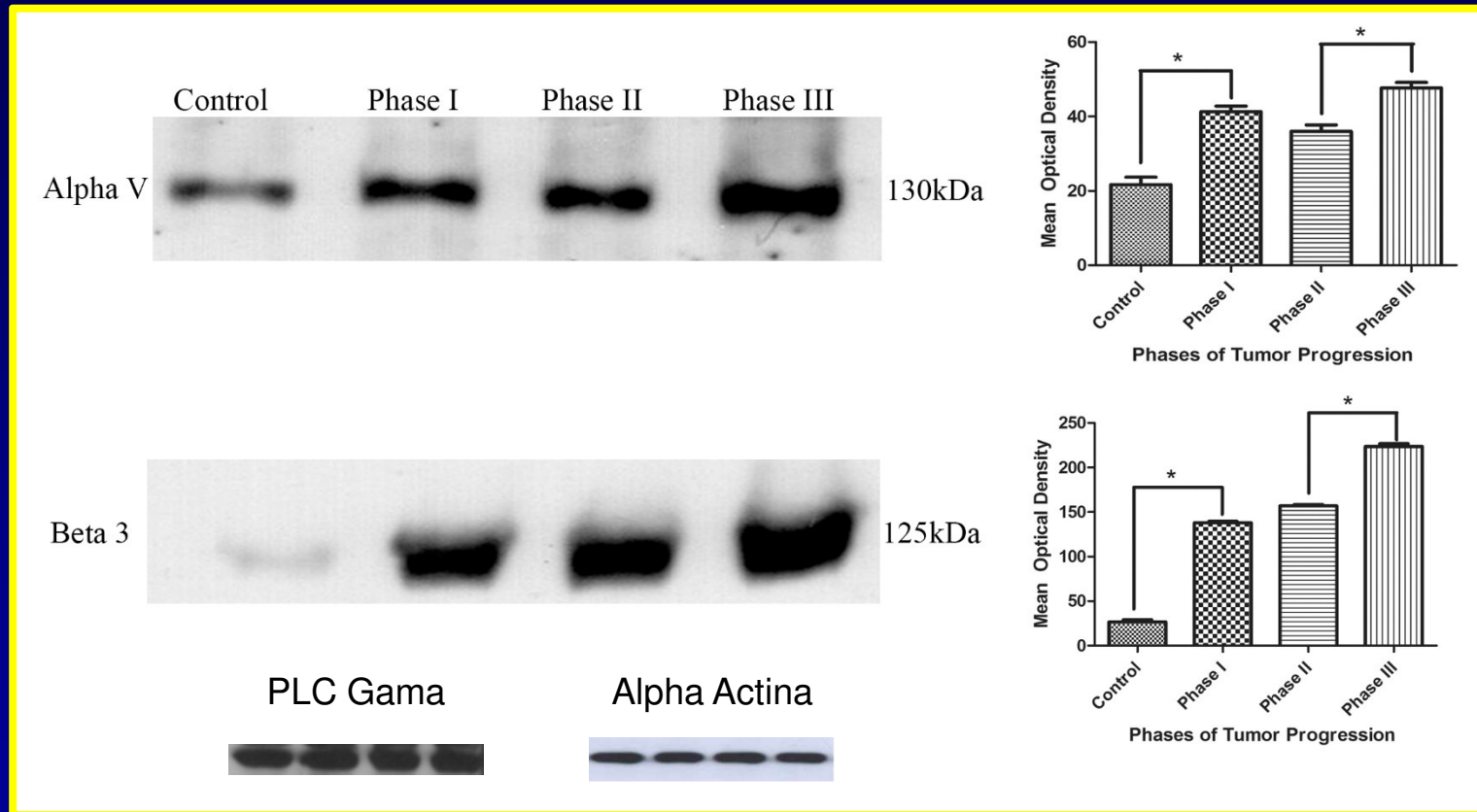


Phase III



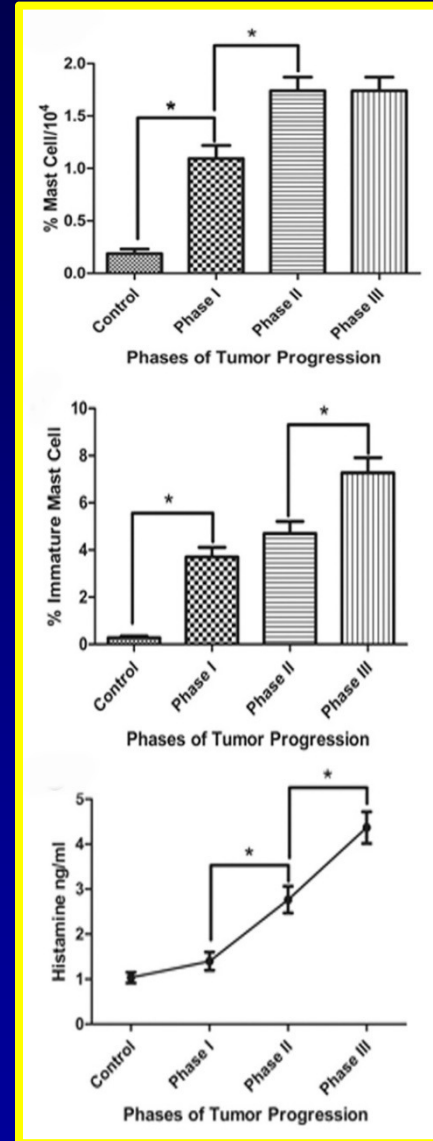
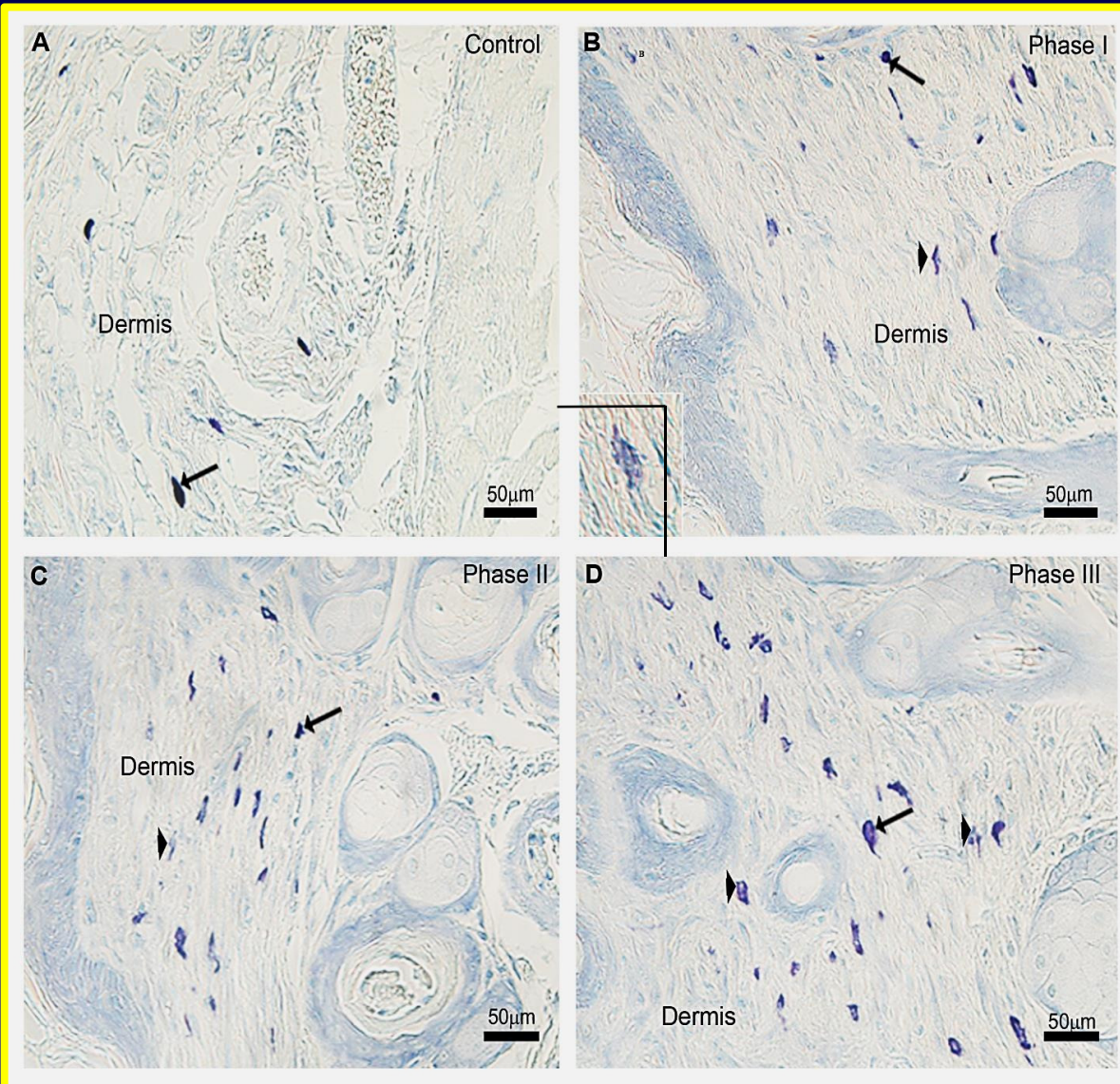
The division into morphological phases facilitates the analysis of mast cells during tumor progression.

MARKERS OF TUMOR PROGRESSION INCREASE WITH TIME



Expression of αV and $\beta 3$ integrin subunits

MAST CELL NUMBERS INCREASE IN THE DERMIS DURING TUMOR PROGRESSION



MAST CELL SPECIFIC PROTEASES

- Exclusively expressed by mast cells and include chymases, tryptases and carboxypeptidase A
- These proteases constitute ~25% of the granule content
- Stored as active enzymes
- Implicated in several pathological conditions:
 - Arthritis
 - Allergic airway inflammation
 - Glomerulonephritis
 - Aortic aneurism
 - Tumor angiogenesis

Caughey, Immunol. Reviews, 2007
Pejiler et al., Blood 2010
da Silva et al., J.Histochem. Cytochem, 2014

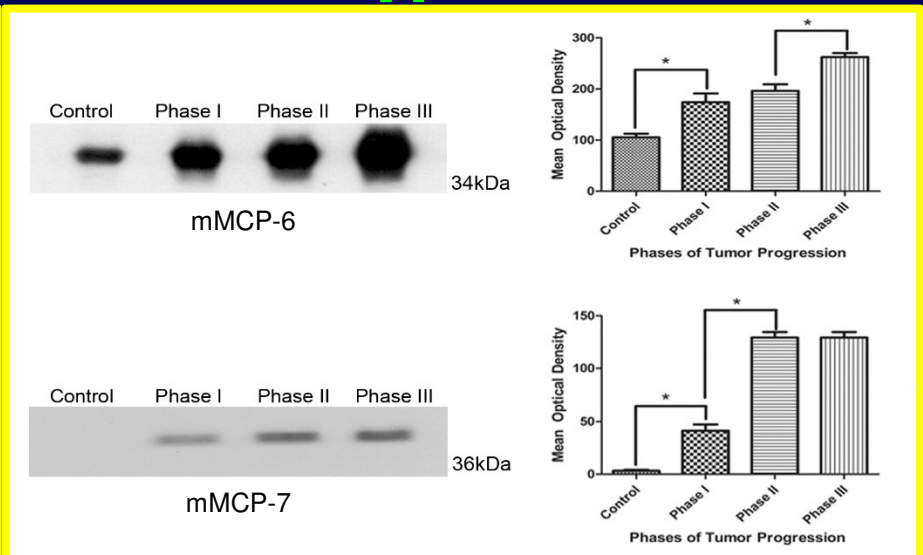
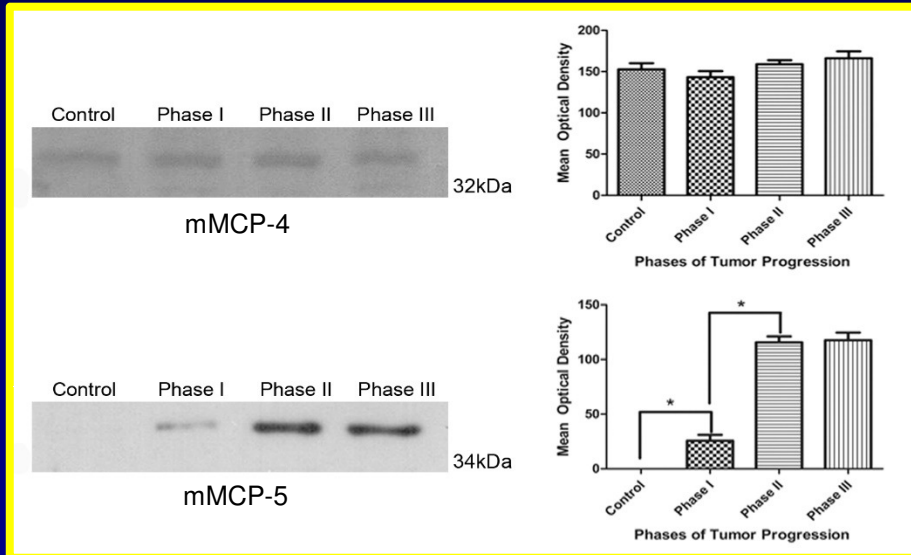
MOUSE MAST CELL SPECIFIC PROTEASES

PROTEASE	MW	CLASS	TYPE	ACTIVITY
Chymase	~36 kDa	Serine protease	mMCP-1, 2, 4 mMCP-5	Chymotrypsin-like Elastase
Tryptase	~36 kDa	Serine protease	mMCP-6, 7	Trypsin-like
Carboxypeptidase A	~50 kDa	Metalloprotease	mMCP-CPA	Carboxypeptidase

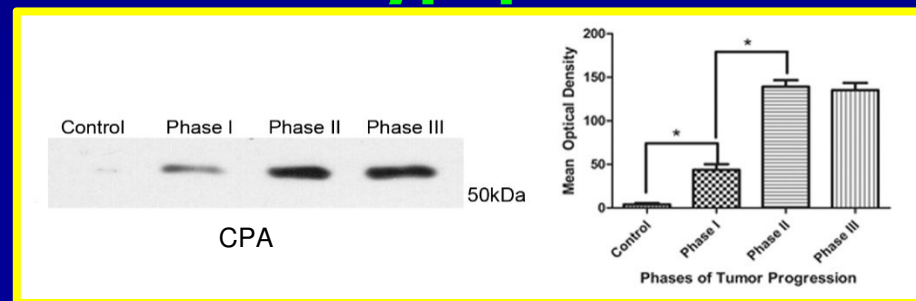
EXPRESSION OF MAST CELL SPECIFIC PROTEASES IS ALTERED DURING TUMOR PROGRESSION

Chymases

Tryptases

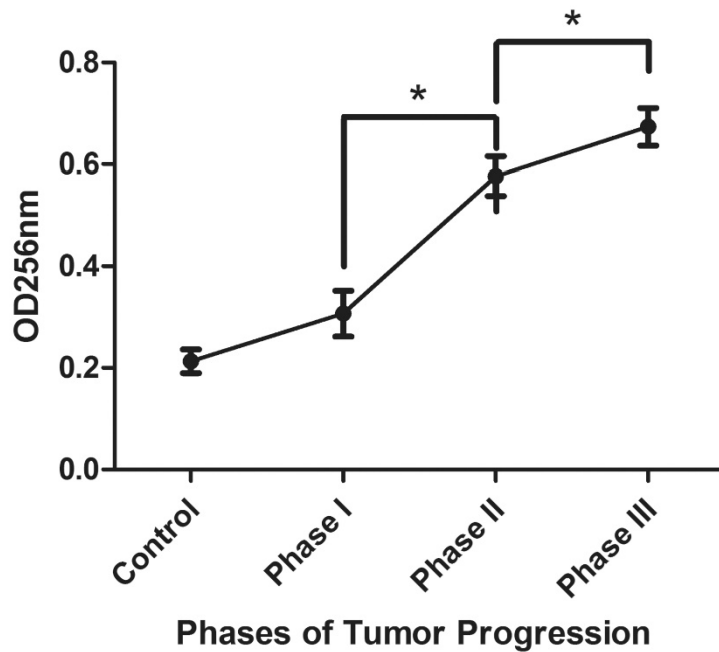


Carboxypeptidase A

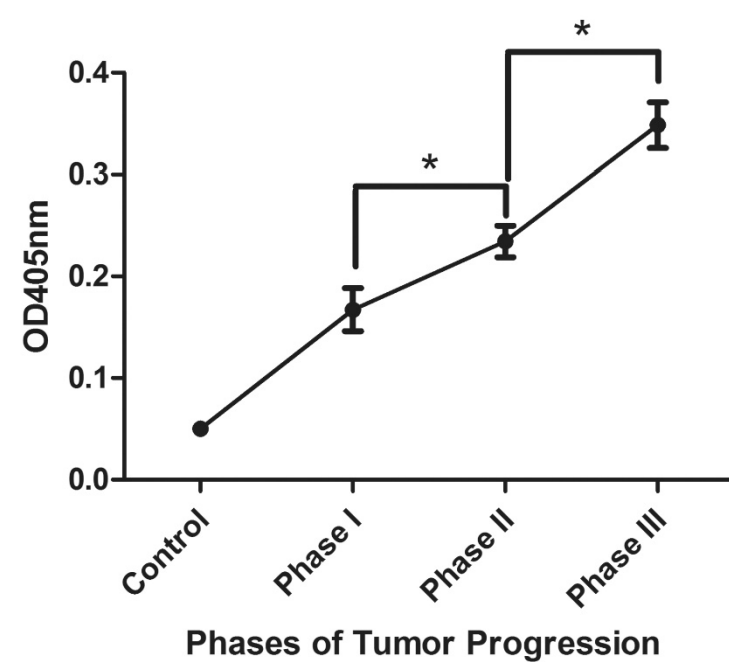


ENZYMATIC ACTIVITY OF CHYMASE AND TRYPTASE INCREASE WITH TUMOR PROGRESSION

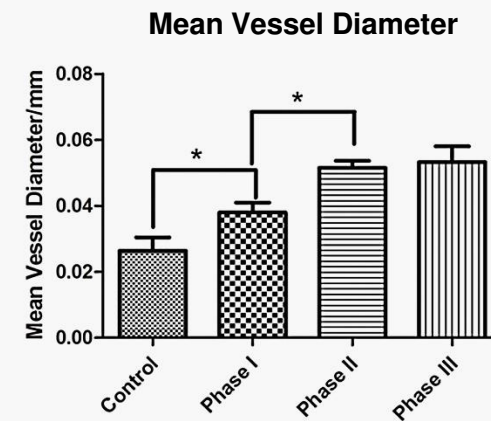
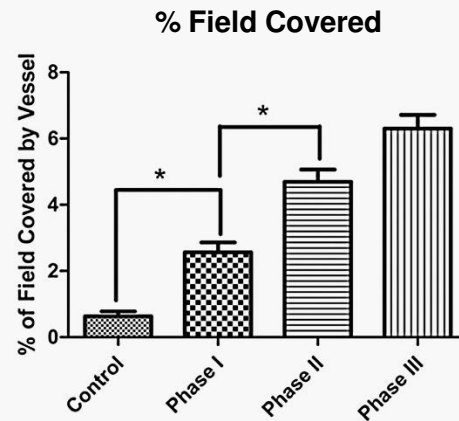
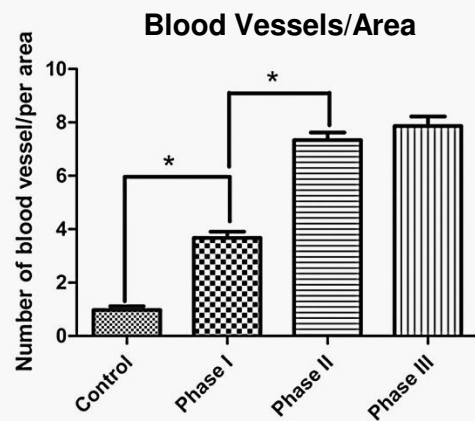
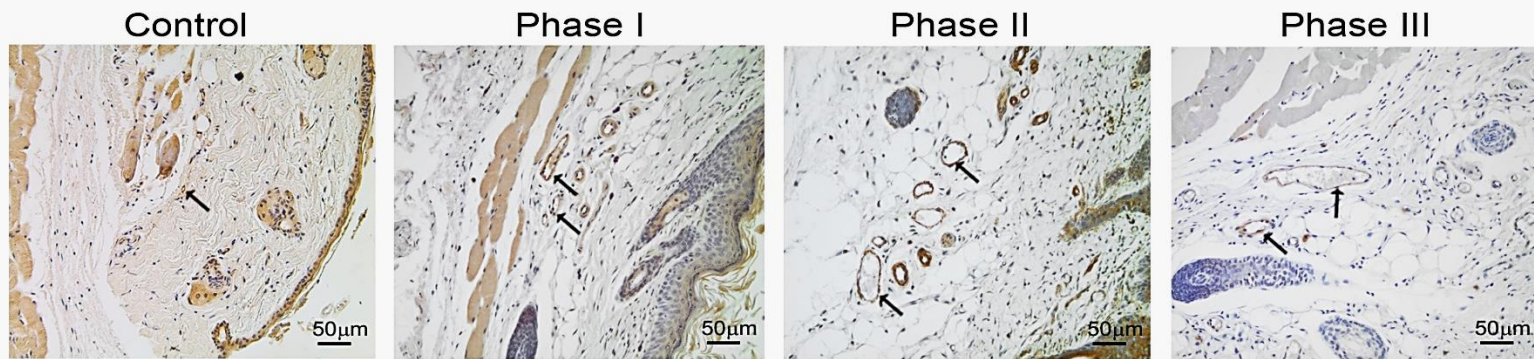
Chymase



Tryptase



BLOOD VESSEL NUMBER AND DIAMETER INCREASE DURING TUMOR PROGRESSION



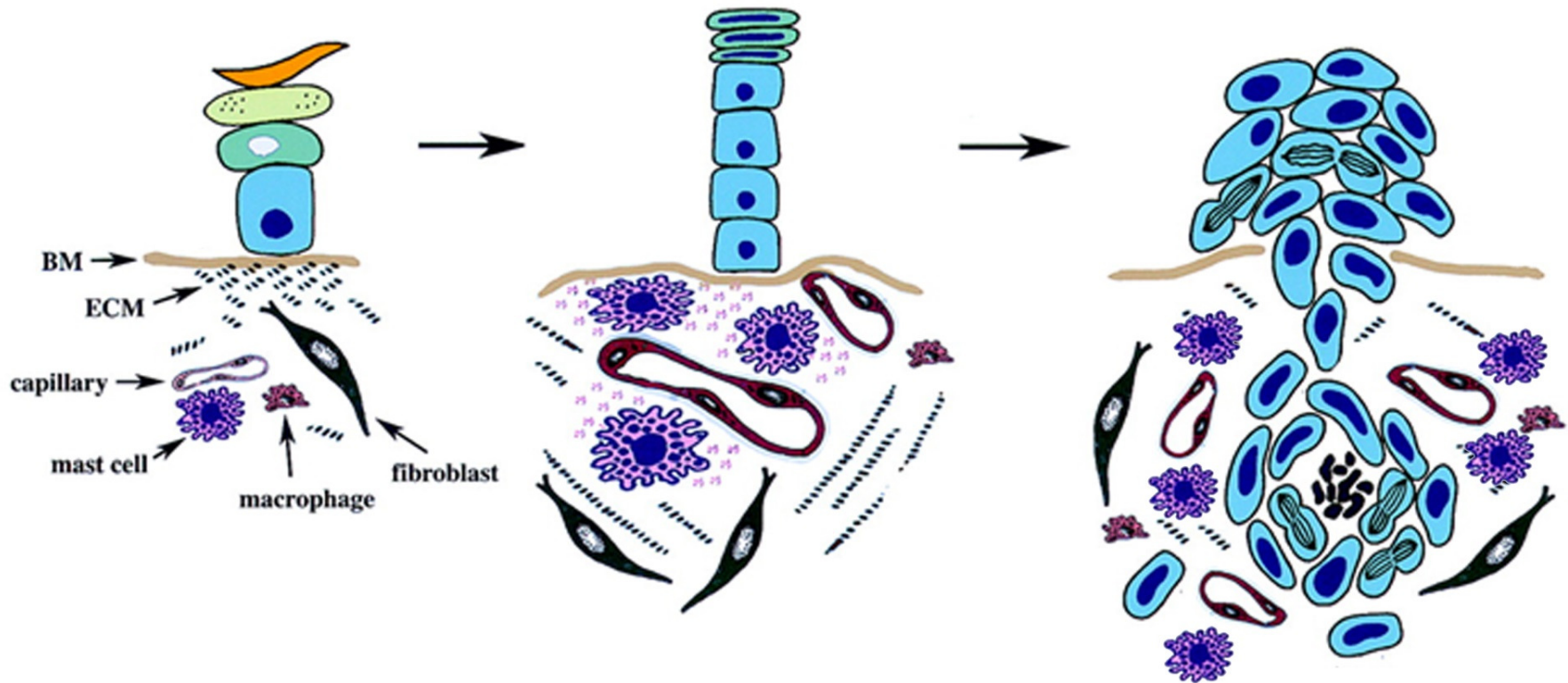
Area = 1.9 mm²

THERE IS A POSITIVE CORRELATION BETWEEN BLOOD VESSELS AND MAST CELLS NUMBER AND PROTEASE EXPRESSION

	Number of Vessels/Field*		% of Field* Covered by Vessels	
	Correlation coefficient (r)	Significance (p)	Correlation coefficient (r)	Significance (p)
Number of Mast Cells	0,98	0,018	0,94	0,05
Protease Expression	(r)	(P)	(r)	(P)
mMCP-5	0,98	0,016	0,95	0,04
mMCP-6	0,92	0,07	0,97	0,02
mMCP-7	0,99	0,007	0,96	0,03
mMC-CPA	0,99	0,009	0,95	0,04

*Field = 1.9mm²

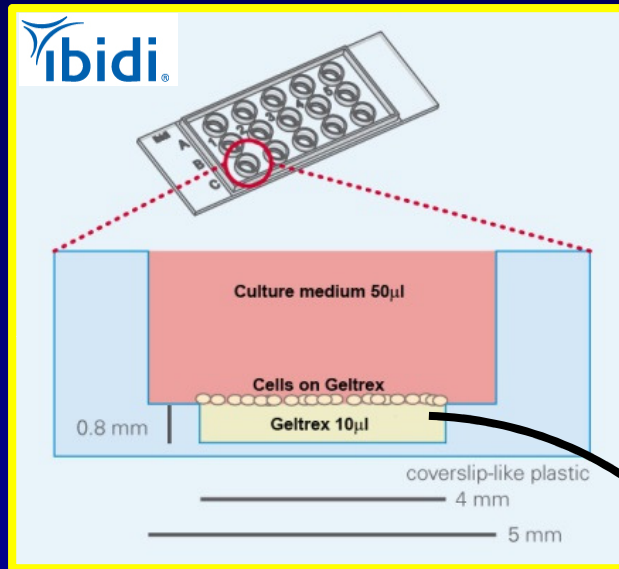
Tumor Progression



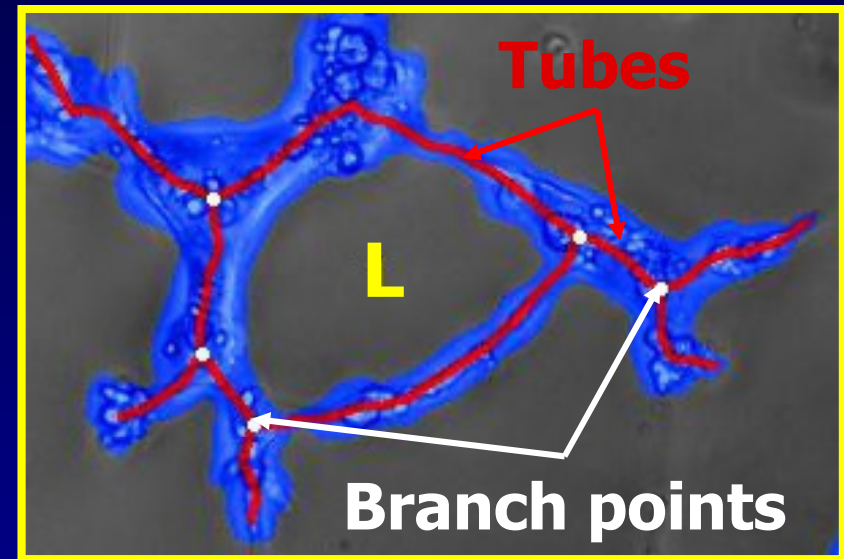
Mast Cell Proteases

Blood Vessels

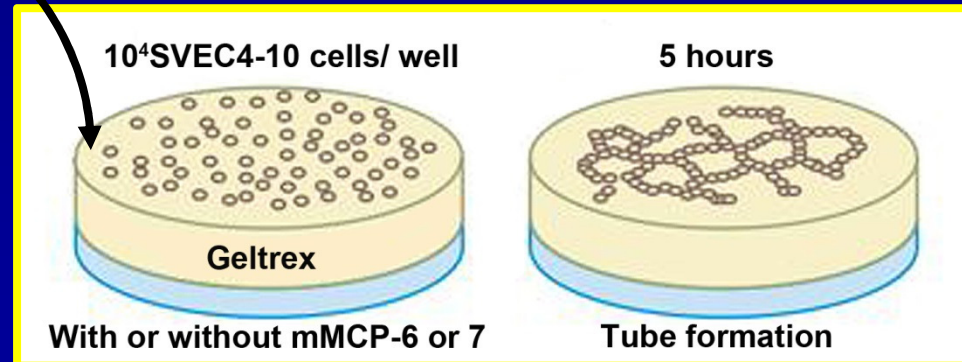
IN VITRO ANGIOGENESIS TUBE FORMATION ASSAY



**Ibidi μ-Slide
Angiogenesis®**

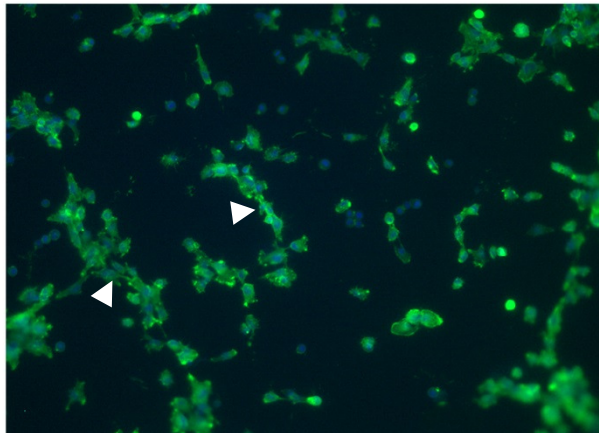


Wimasis WimTube

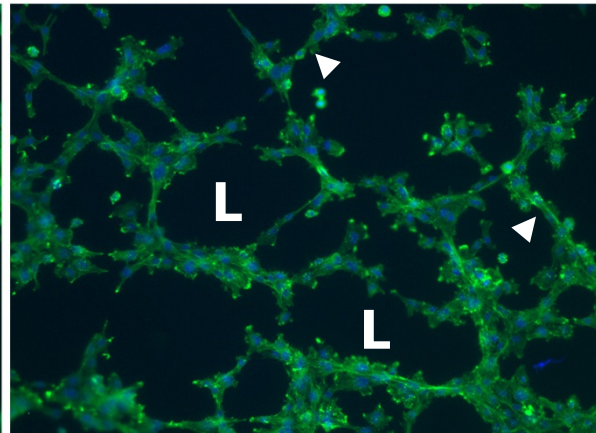


rMMP-6 AND -7 ACCELERATED TUBE FORMATION BY ENDOTHELIAL CELLS

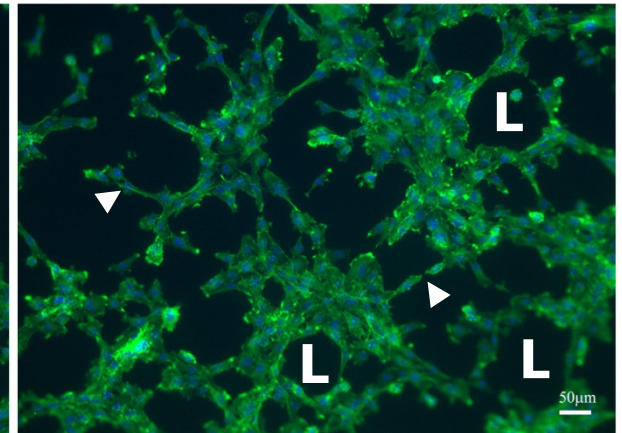
SVEC4-10 CELLS
without proteases



SVEC4-10 CELLS
rmMCP-6



SVEC4-10 CELLS
rmMCP-7



A few cells are spread and occasional tubes are seen

Cells are spread and tubes and loops are present

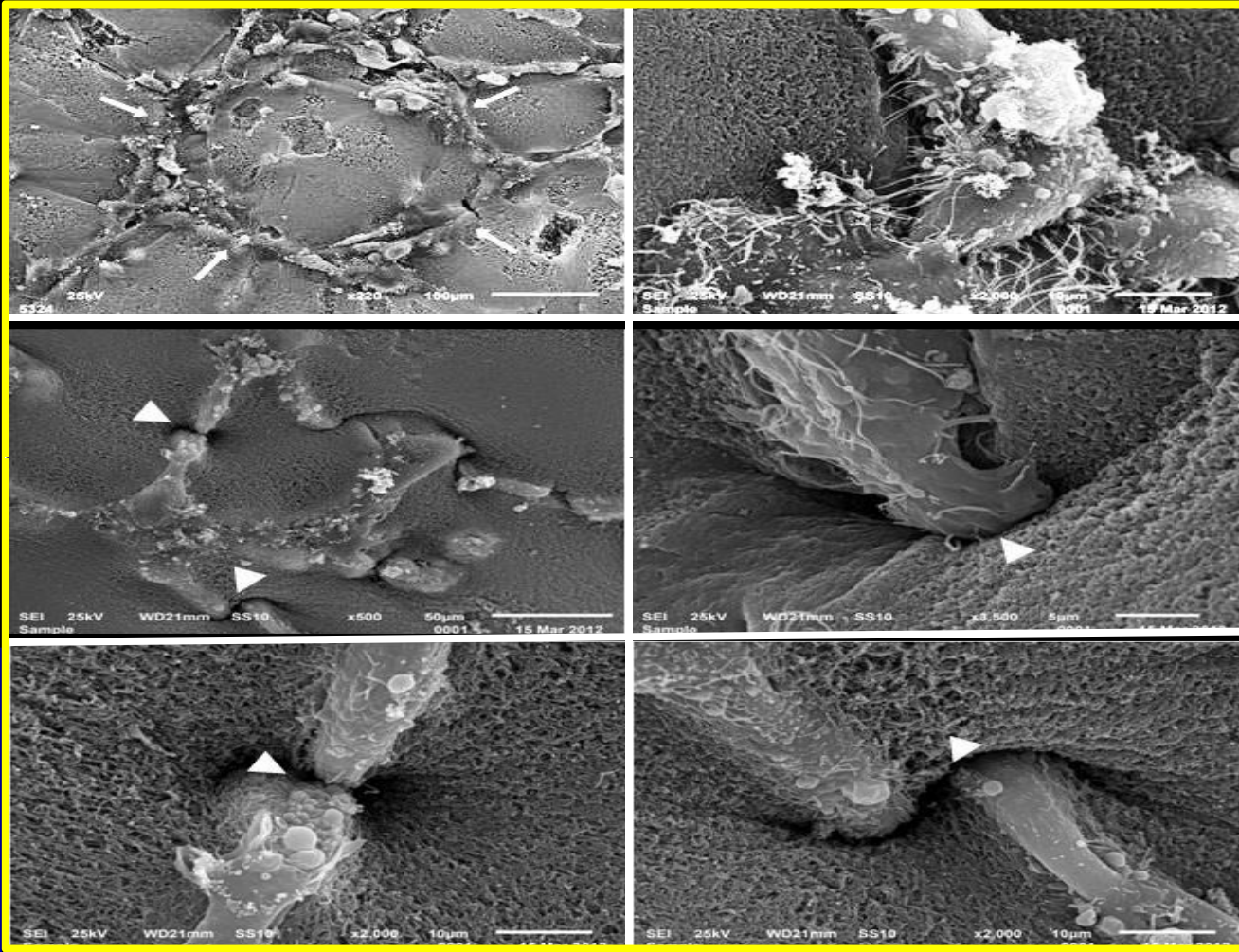
Tubes and loops are more prevalent

▶ Tube

L = Loop

Cells were cultured for 5 hours at 37°C on Geltrex, fixed, stained with phalloidin conjugated to Alexa 488 and counterstained with DAPI .

rMMCP-6 AND -7 ACCELERATE TUBE FORMATION AND MATRIX PENETRATION



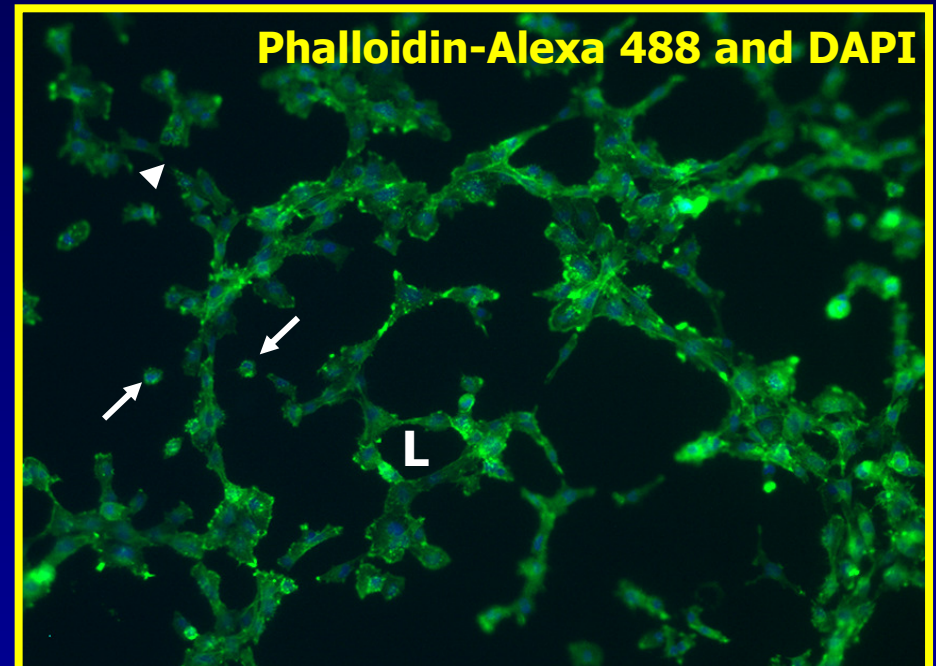
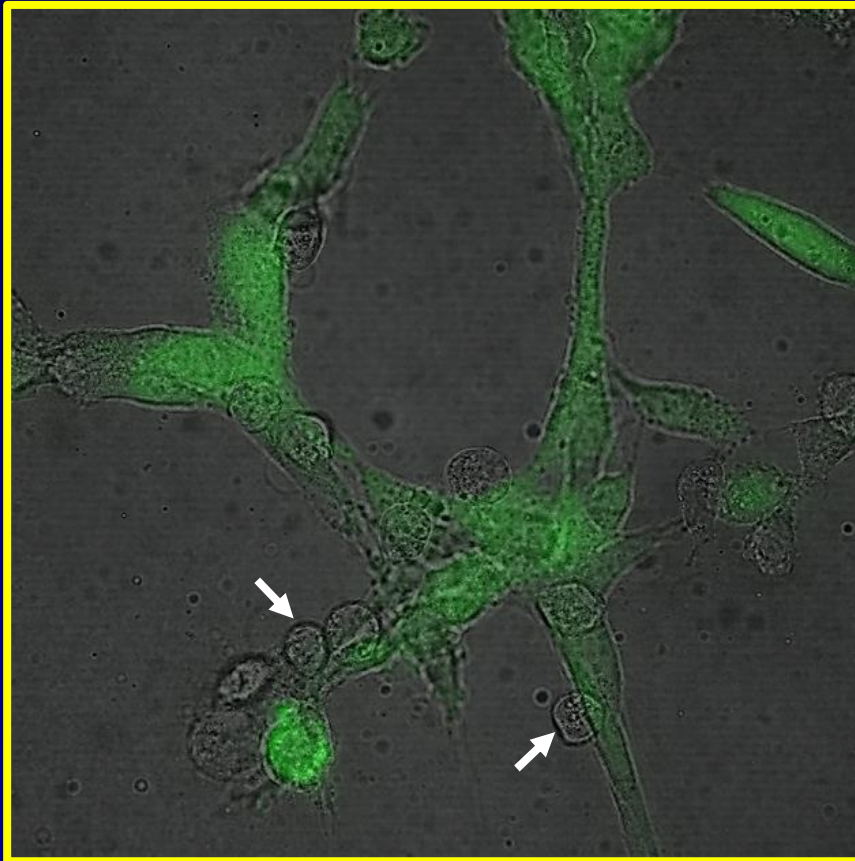
Control

rmMCP-6

rmMCP-7

**Cells were cultured for 5 hours at 37°C on Geltrex
Scanning Electron Microscopy**

CO-CULTURE OF SVEC4-10 ENDOTHELIAL CELLS WITH P815 MAST CELLS INDUCES FORMATION OF TUBES AND LOOPS

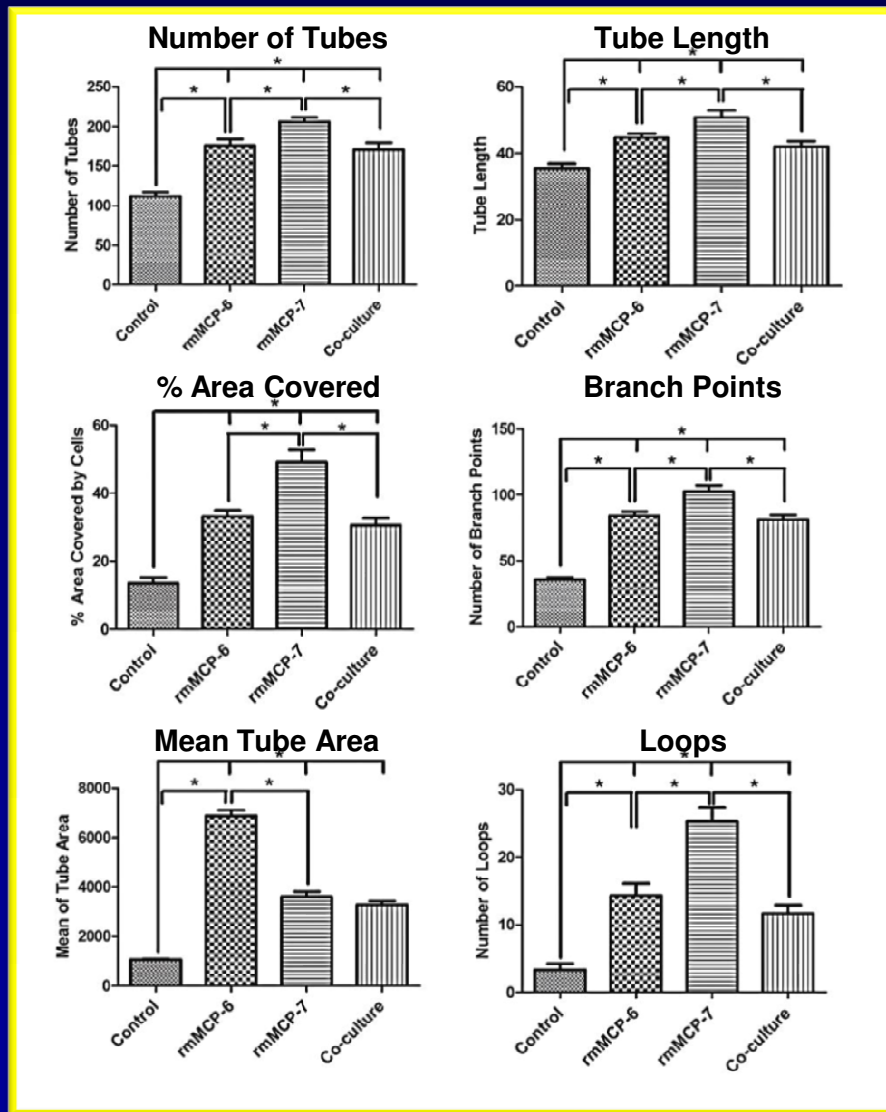


There are fewer tubes and loops than when the SEVEC4-10 cells are cultured with proteases

Cells were cultured for 5 hours at 37°C on Geltrex

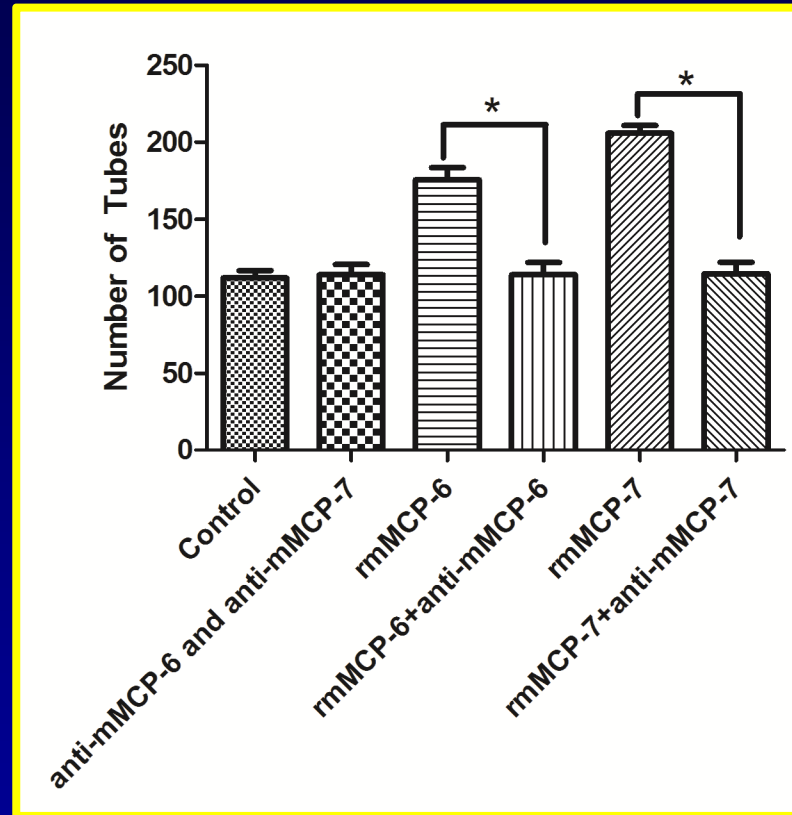
GFP-SVEC4-10 cells → **P815 Mast Cells** ▶ **Tube** **L = Loop**

rmMCP-7 IS MORE EFFECTIVE IN INDUCING TUBE FORMATION



- Number of tubes, area covered by tubes, tube length, branching points and loops are higher when the SVEC4-10 cells are incubated with rmMCP-7
- The average area occupied by the tubes was higher when cells were cultured with rmMCP-6

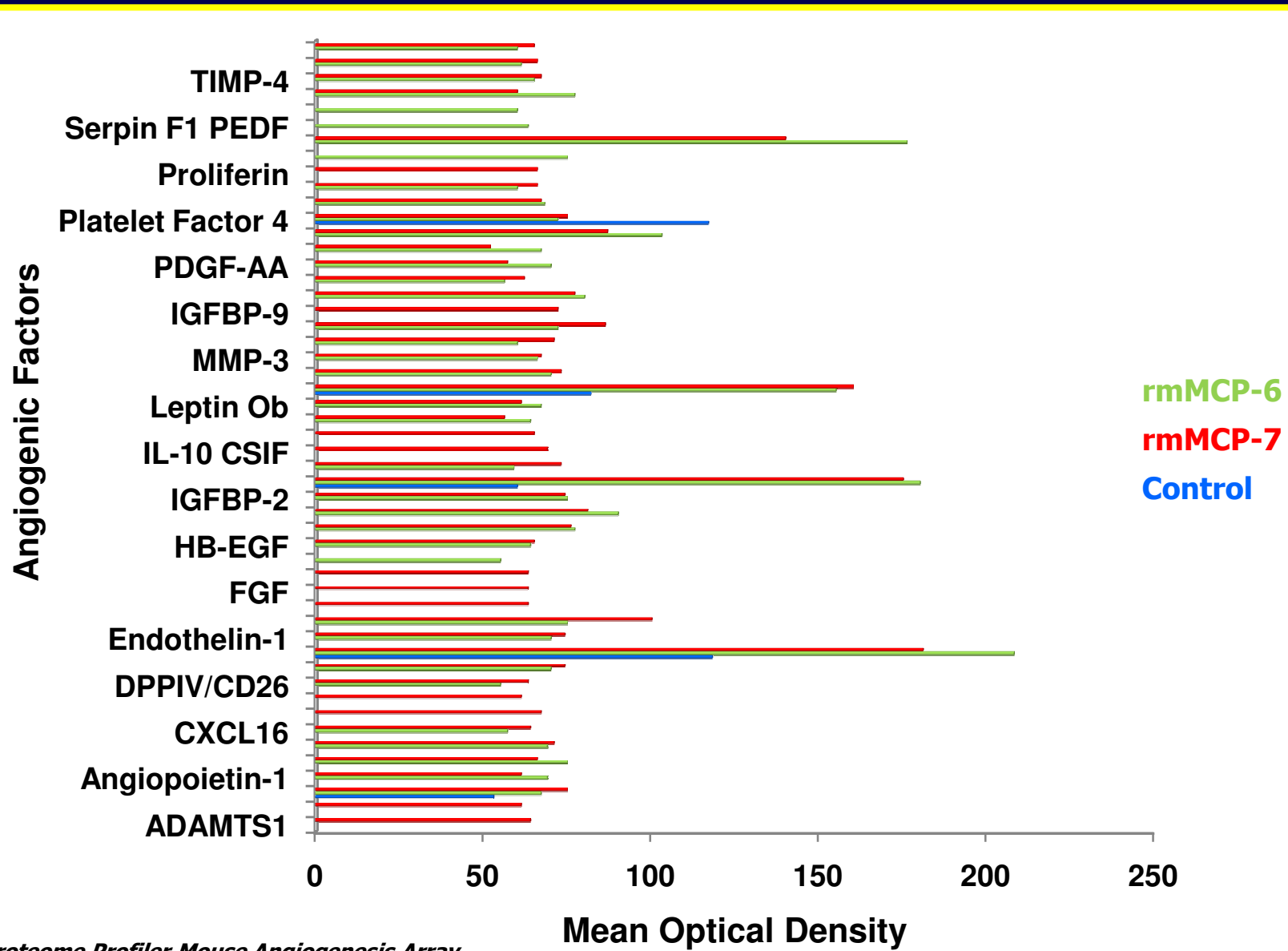
ANTI-mMCP-6 AND ANTI-mMCP-7 REDUCE TUBE FORMATION



Control cells were cultured in absence of antibodies
or proteases

Antibodies were a gift from Dr. Michael Gurish, Brigham and Women's Hospital, Harvard University

rmMC TRYPTASES INDUCE THE RELEASE OF ANGIOGENIC FACTORS



Proteome Profiler Mouse Angiogenesis Array

CONCLUSIONS

- **Mast cells are involved in the induction of angiogenesis in the early stages of tumor development**
- **Mast cells modulate blood vessel growth in the later stages of tumor progression**
- **Mast cell specific proteases play a critical role in angiogenesis by inducing release of angiogenic factors**



Collaborators:

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Antonio Carlos Borges
Constance Oliver

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Thank You!

