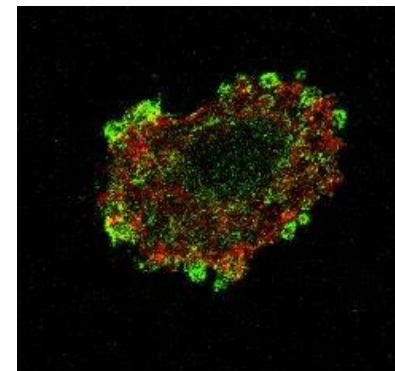


# ***Structural characterization and in vivo pro-tumor properties of a highly conserved matrikine***

**Bertrand BRASSART**

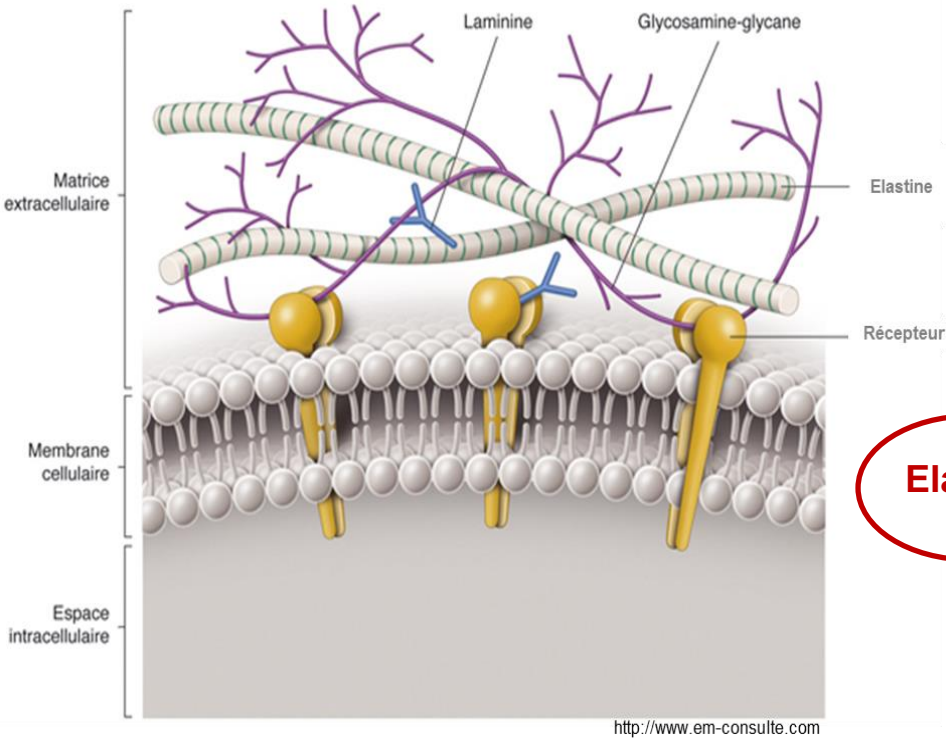
UMR CNRS 7369,  
Matrice Extracellulaire et Dynamique Cellulaire – MEDyC  
Reims, France.

11<sup>ème</sup> forum Cancéropôle Est  
Reims  
16 novembre 2018

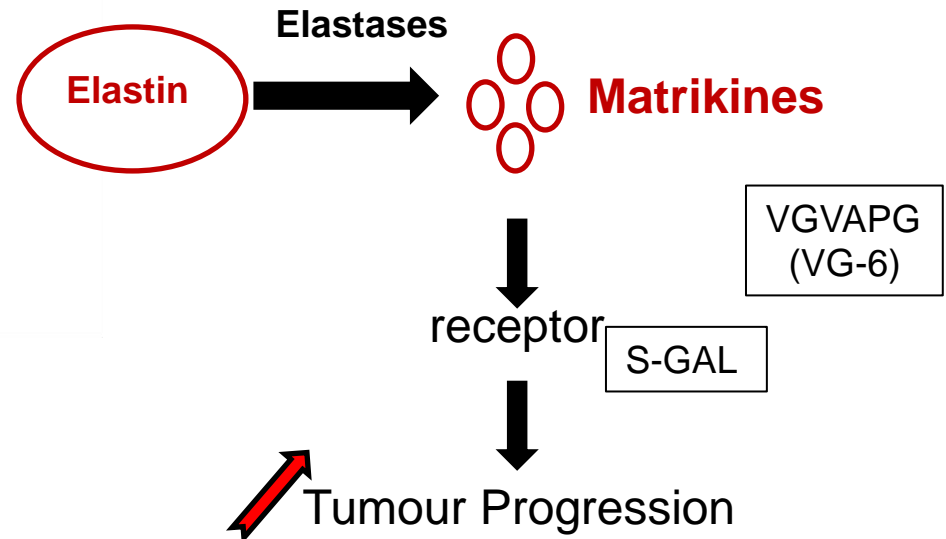


# Elastin

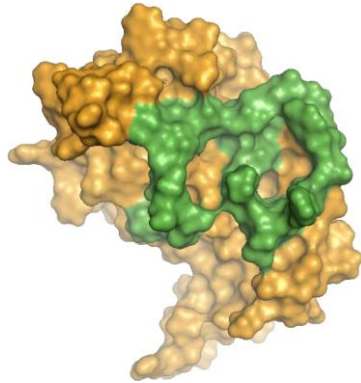
## Extracellular Matrix



- Hydrophobic protein
- Lung, skin, blood vessels...

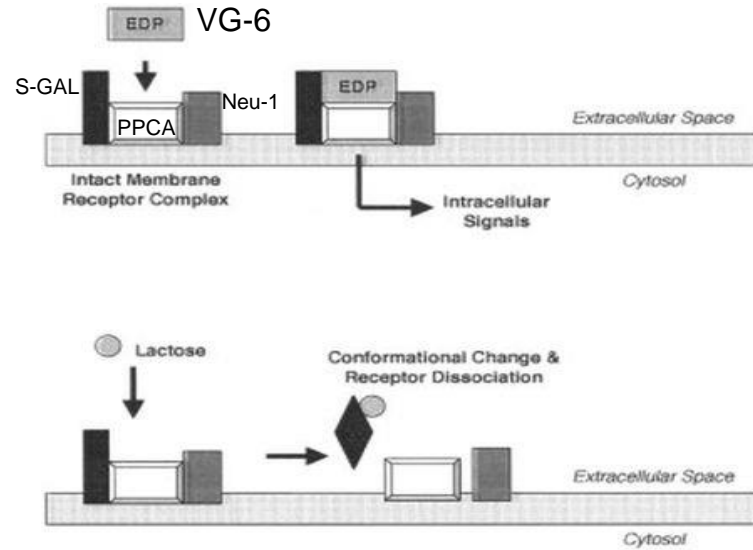


# Receptor : S-Gal / EBP (Elastin Binding Protein) ?

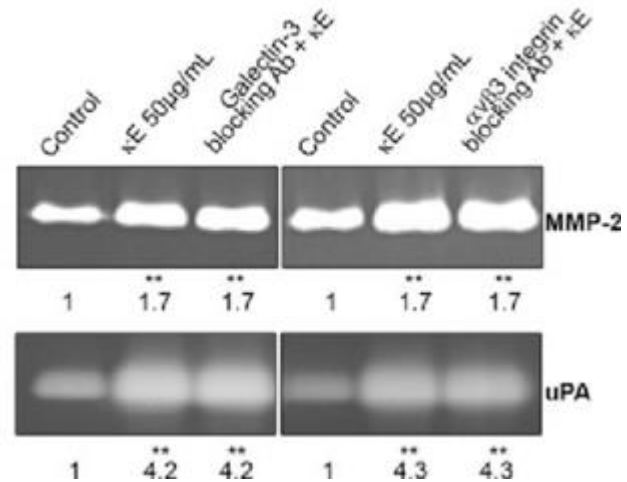
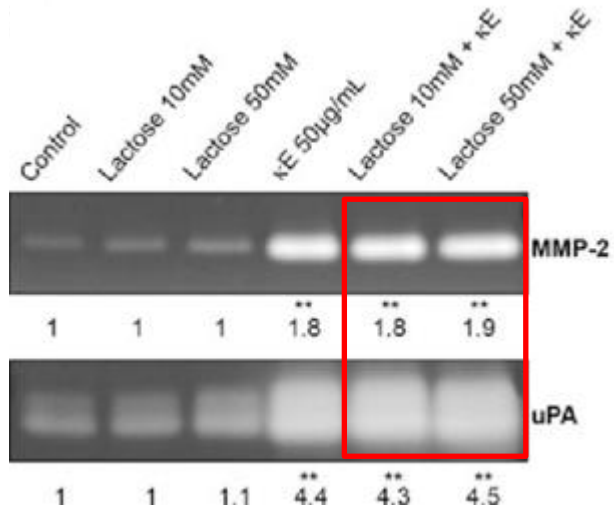


**β-Gal** : Excision : exons 3, 4 and 6  
 Reading frame change: exon 5  
 Reading frame restoration : exon 7

MW: 67kDa



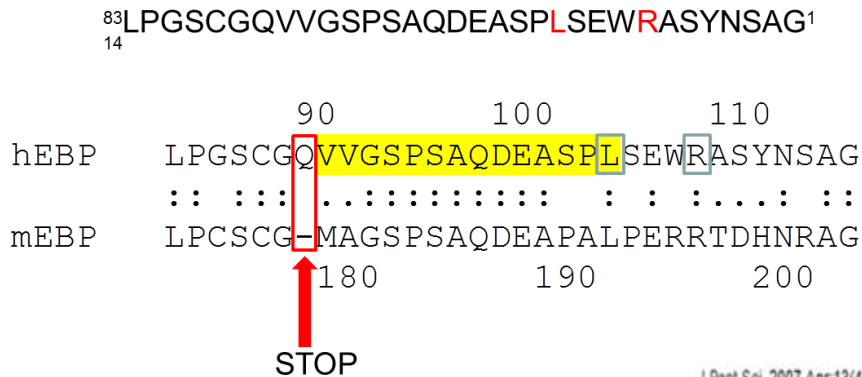
Hance *et al*, 2002



Toupance *et al*, 2012

# Receptor : S-Gal / EBP ?

## Human / Mouse



[J Pept Sci. 2007 Apr;13\(4\):263-8.](#)

### Induction of macrophage migration through lactose-insensitive receptor by elastin-derived nonapeptides and their analog.

[Maeda I<sup>1</sup>, Mizoiri N, Briones MP, Okamoto K.](#)

[Author information](#)

AG-9

#### Abstract

Elastin, one of the extracellular matrix components, is present in tissues requiring extensibility and resilience such as the aorta, lungs, ligaments and skin. Degradation of elastin is observed in diseases such as atherosclerosis, emphysema and metastasis. It has been suggested that degraded elastin-derived peptides interact with a variety of cell types and are involved in development of diseases. Two nonapeptides, Ala-Gly-Val-Pro-Gly-Leu-Gly-Val-Gly (AGVPGFGVG) and Ala-Gly-Val-Pro-Gly-Phe-Gly-Val-Gly (AGVPGFGVG), exist in hydrophobic regions of elastin. In this paper, we characterized these elastin-derived nonapeptides by macrophage migration assay. Both nonapeptides induced a maximal migration at 10(-8) M and elicited the same degree of responsiveness. To investigate the role of the sixth residue of the nonapeptides, seven analog peptides in which Leu or Phe is substituted by Ile, Val, Ala, Gly, Pro, Lys or Glu were synthesized and their macrophage migration activity tested. Among the nonapeptide analogs, only Ala-Gly-Val-Pro-Gly-Ile-Gly-Val-Gly induced the migration of macrophages at the optimal concentration of 10(-9) M and its responsiveness was the same as that of parent nonapeptide AGVPGFGVG. Results of the deactivation tests and the effect of lactose on macrophage migration showed that a lactose-insensitive receptor which mainly recognizes Ala-Gly-Val-Pro-Gly-Ile-Gly-Val-Gly is presumably present on the membrane of macrophages in addition to the elastin-binding protein (EBP) sensitive to lactose. These results suggest that Leu, Phe and Ile residues at the sixth position of elastin-derived nonapeptides are crucial for inducing macrophage migration and in particular, Ile residue is important for the recognition by receptor insensitive to lactose.

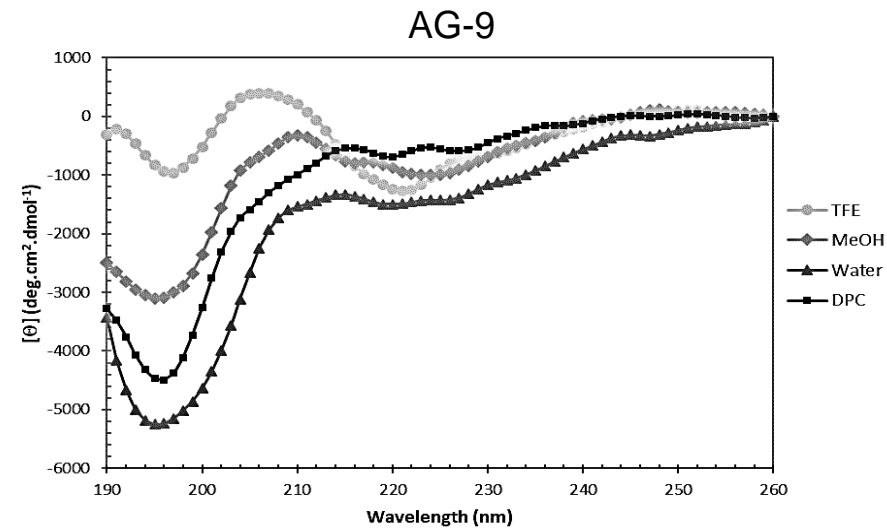
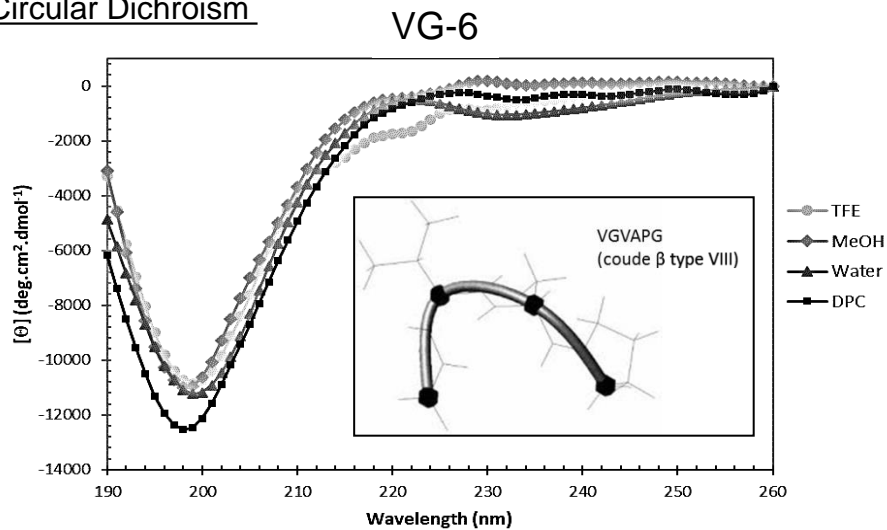
# Aims

- Determine the structural characteristics of the AGVPGLGVG (AG-9) elastin peptide.
- Define the *in vitro* and *in vivo* pro-tumor biological activities of the AG-9 peptide.
- Identify the AG-9 elastin peptide receptor insensitive to lactose.

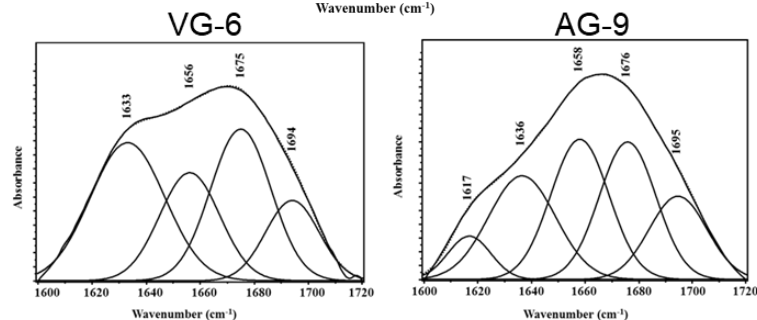
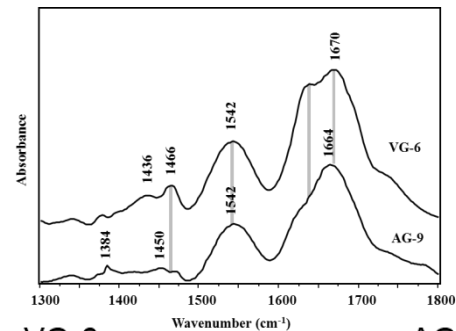


# Structural Characterization

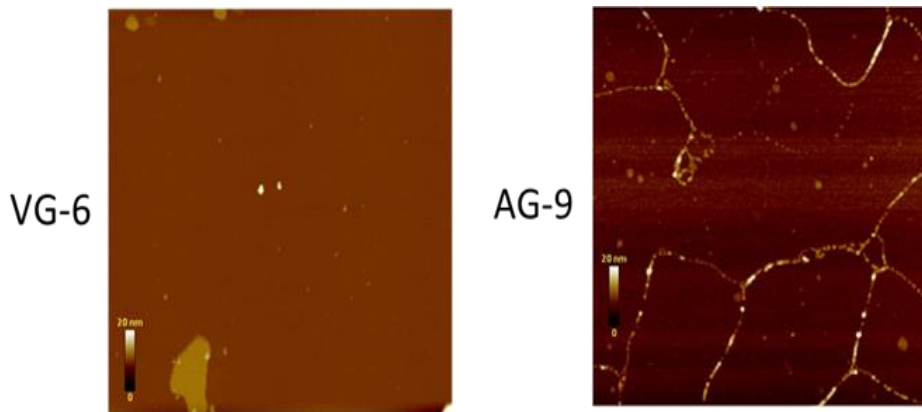
## Circular Dichroism



## Fourier-transform infrared spectroscopy



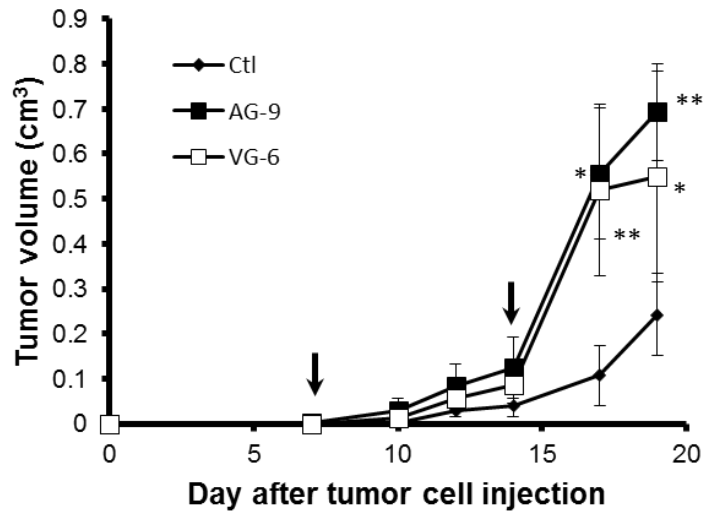
## Atomic Force Microscopy



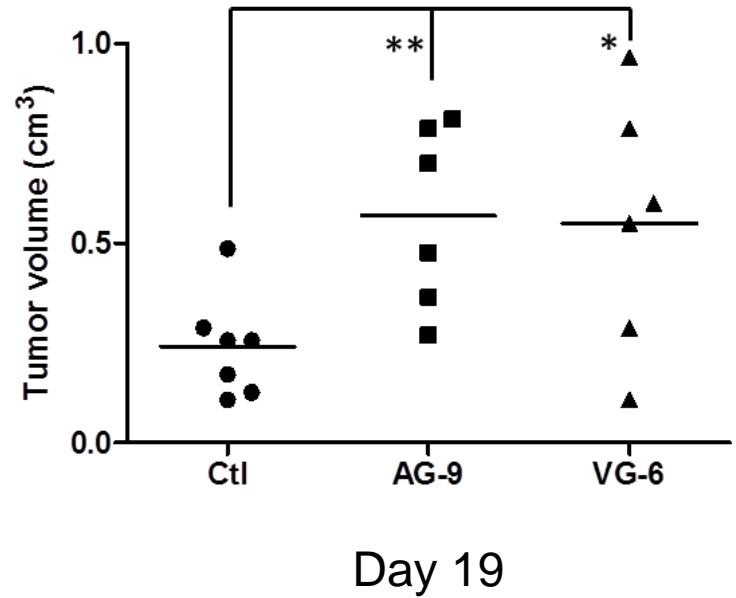
➔ NMR → β-turn



## In vivo Pro-tumour Properties



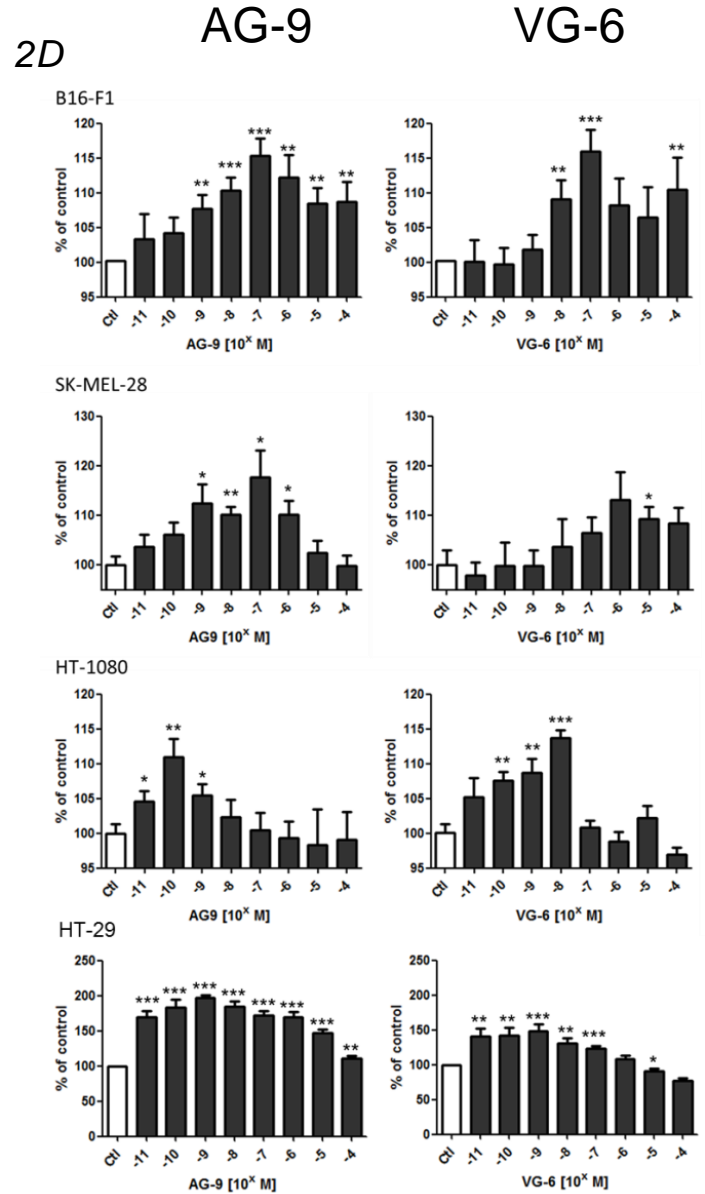
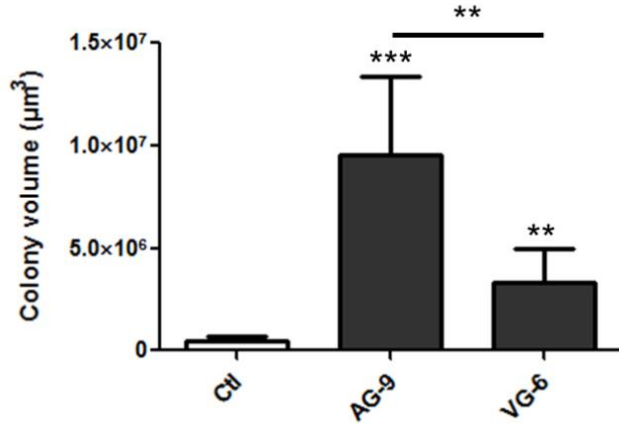
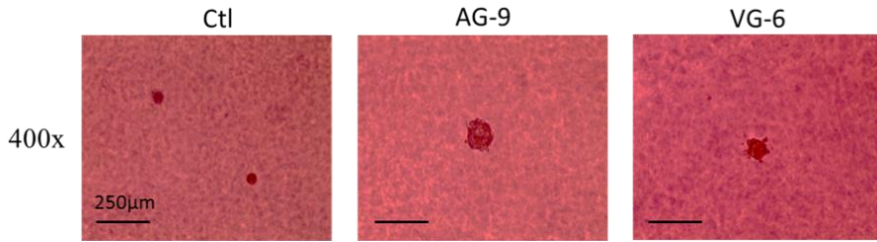
Injection: J7 and J14  
AG-9 and VG-6 : 10mg/kg



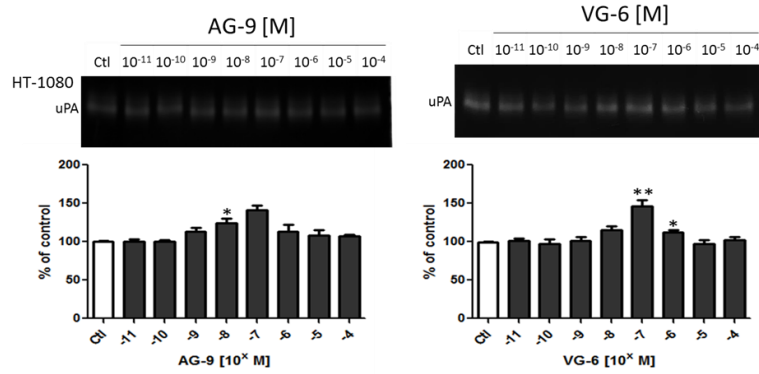
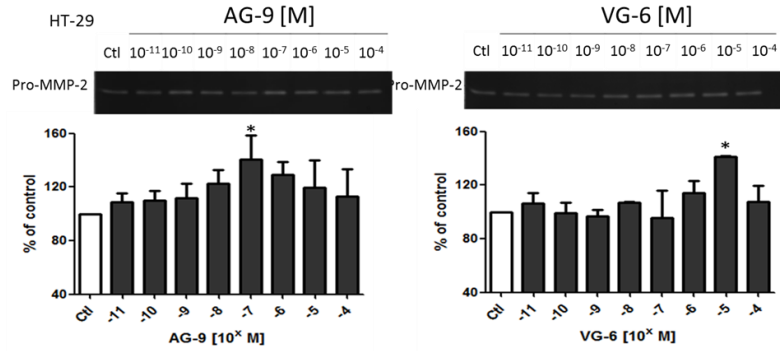


*In vitro* Proliferation

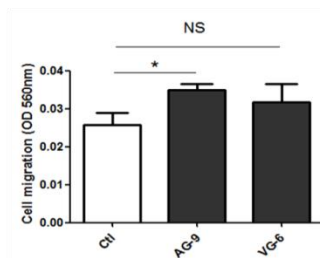
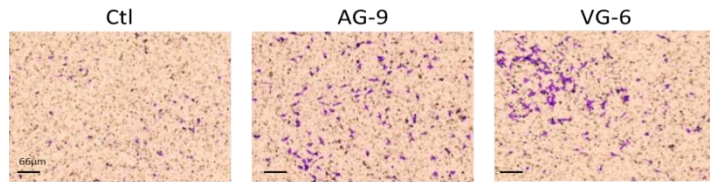
Soft-Agar colony Formation Assay (3D)



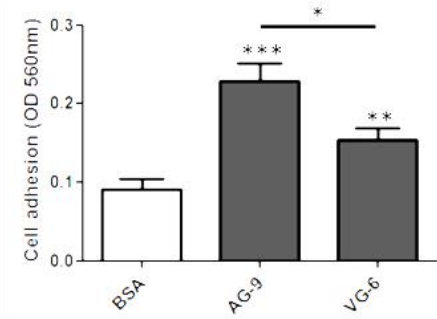
# Proteinase Secretion



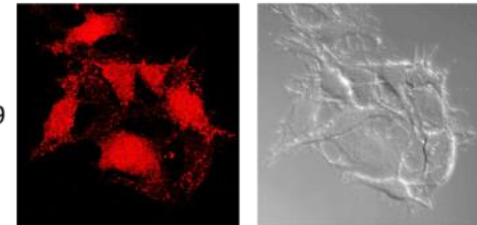
# Cell Migration



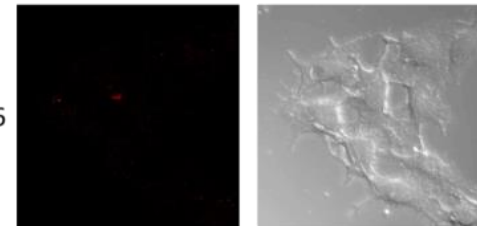
# Adhesion



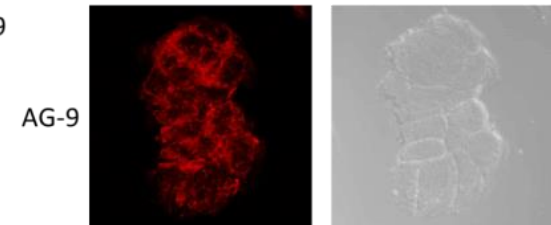
B16-F1



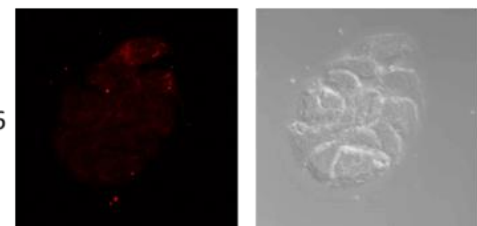
VG-6



HT-29



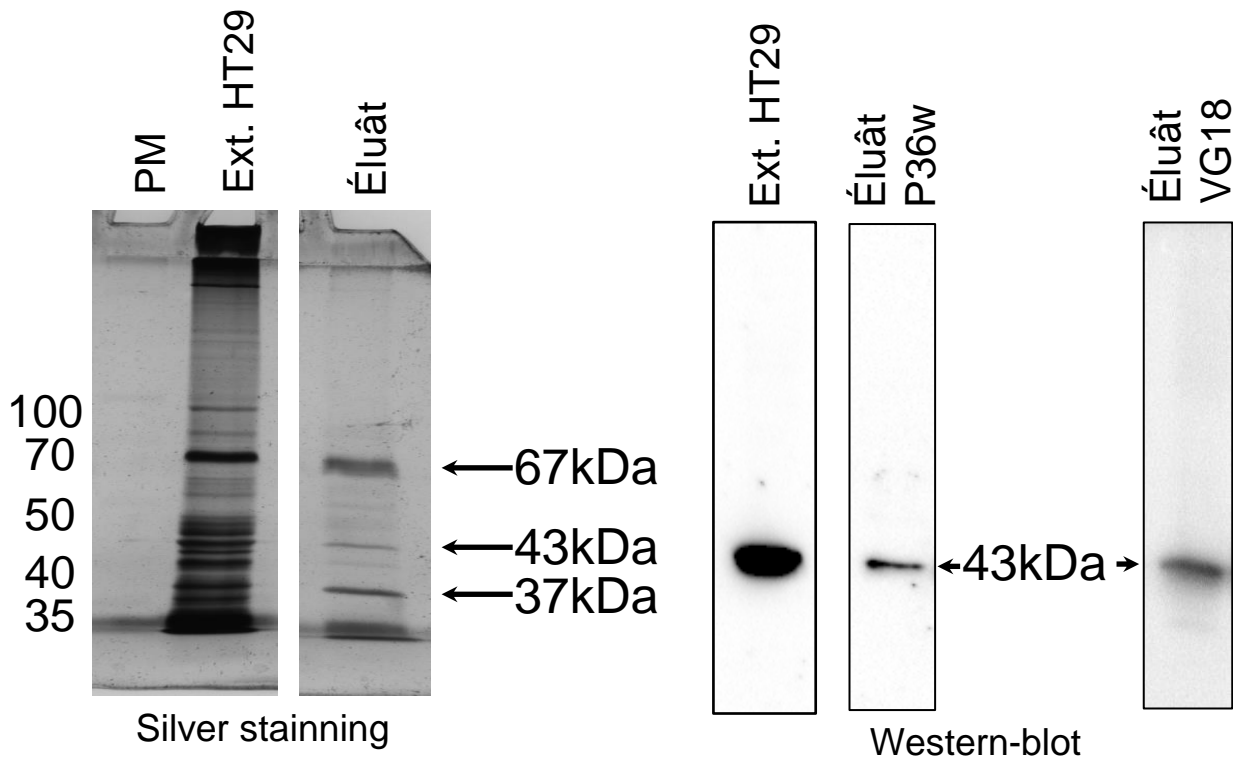
VG-6



Peptide-TAMRA / +4°C

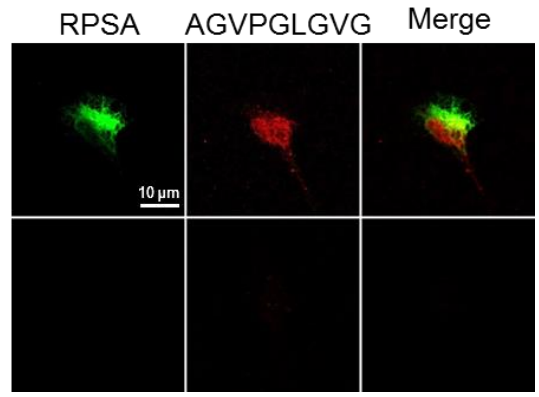
# Identification of the AG-9 elastin peptide receptor insensitive to lactose: **Ribosomal Protein SA (RPSA)**

➔ Affinity Chromatography



P36W : AGIPGLGVG-VGVPGLGVG-**AGVPGLGVG**-AGVPGWGAG

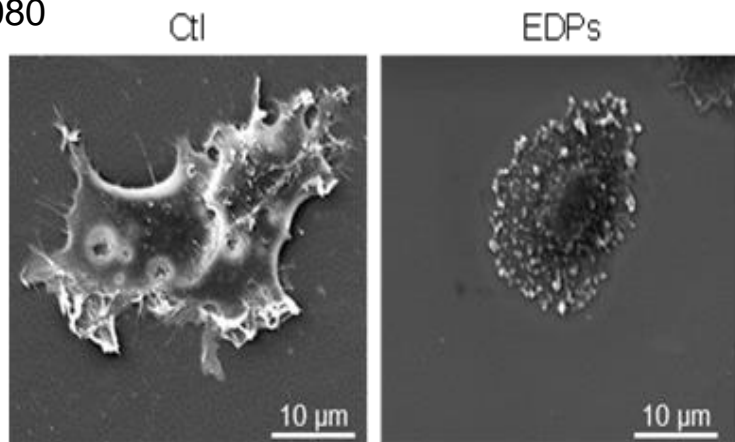
VG18 : VGVAPG-VGVAPG-VGVAPG



➔ **RPSA : receptor for AG-9 and VG-6 peptides**

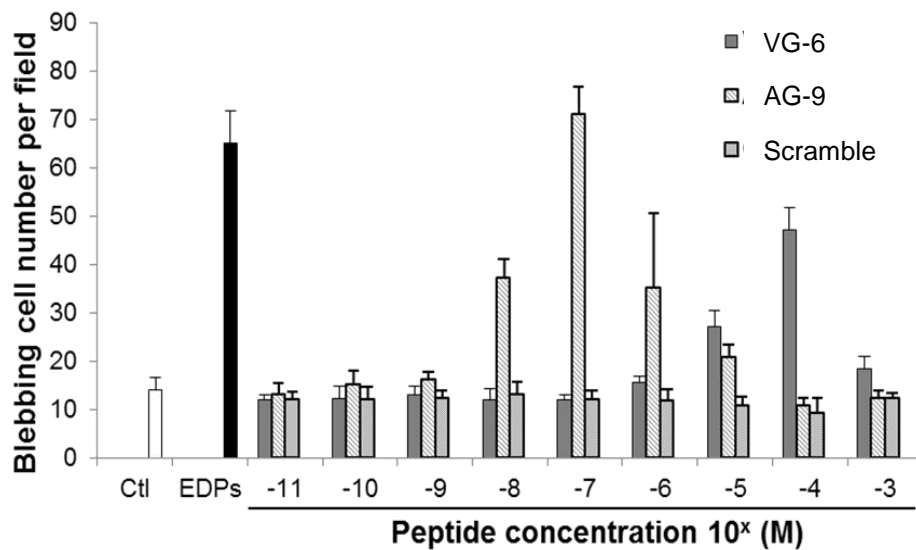
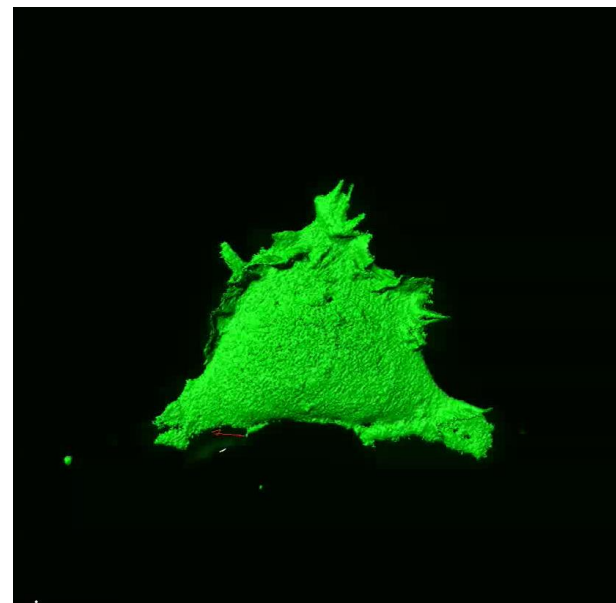
# Blebbing and Extracellular Vesicle Shedding

HT-1080

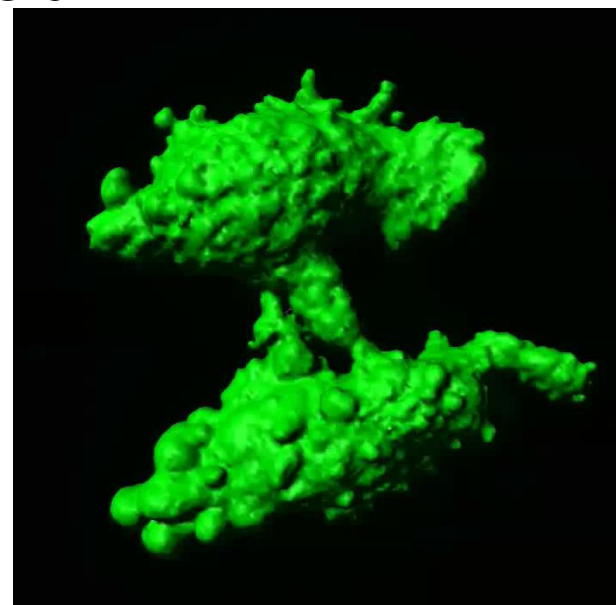


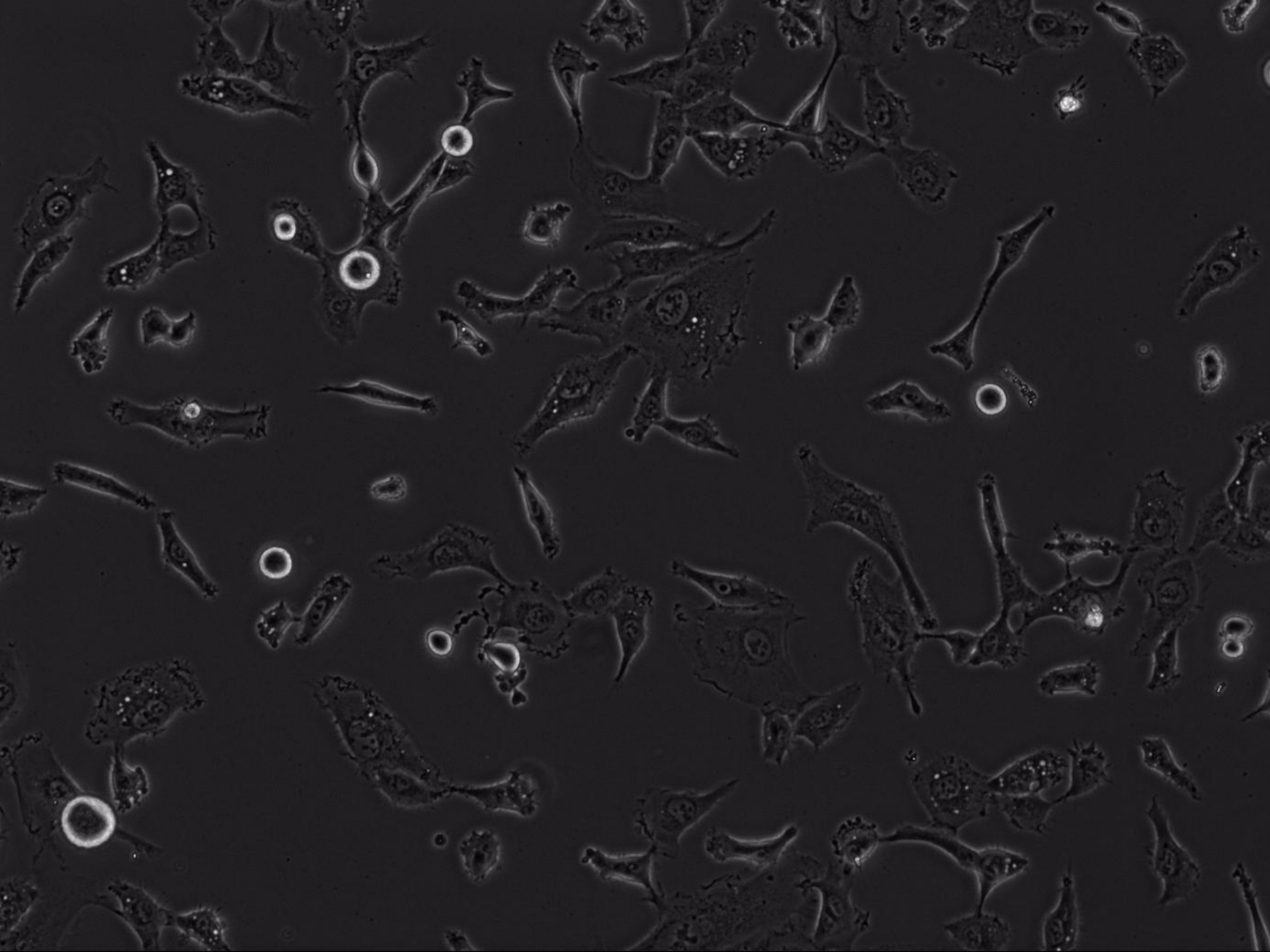
Control

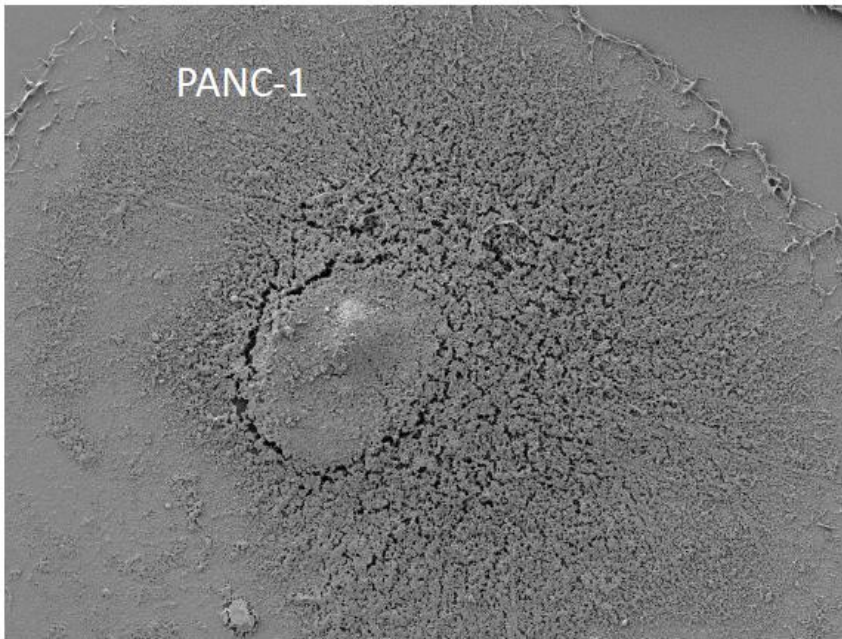
GFP-Hsp90



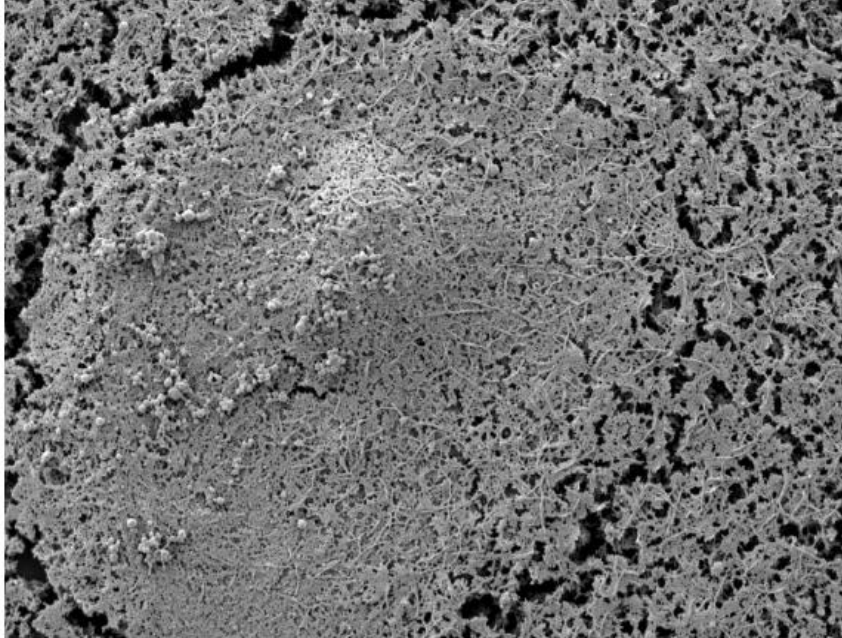
AG-9



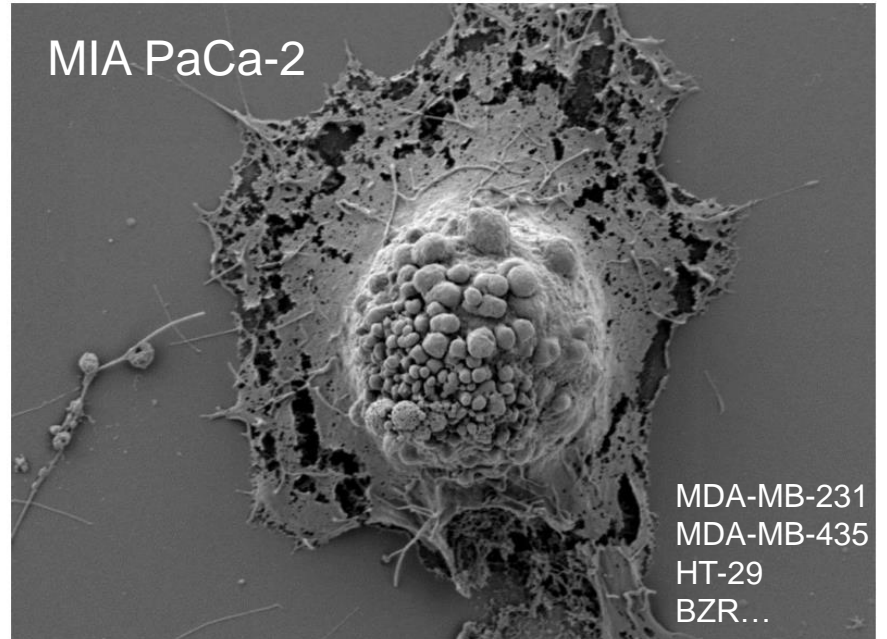




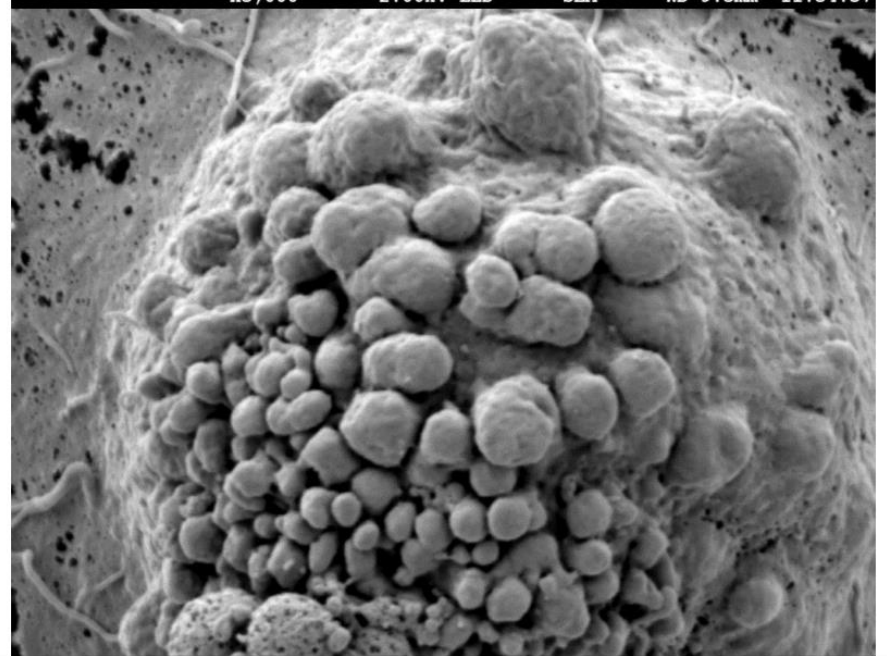
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x1,500 2.00kV LED SEM WD 9.3mm 11:35:03



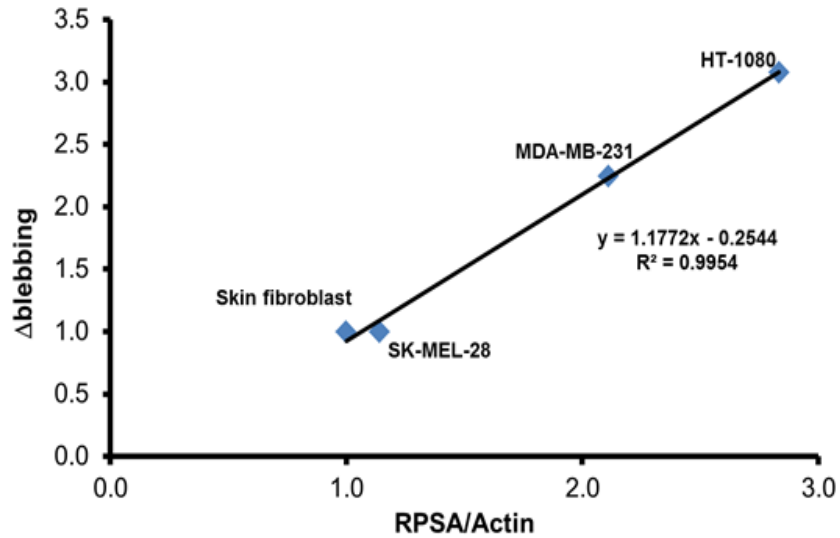
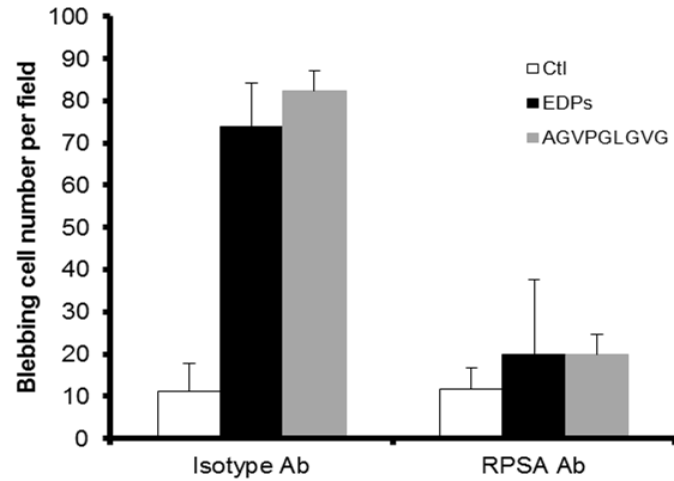
1µm JEOL 26/10/2018  
x5,000 2.00kV LED SEM WD 9.3mm 11:36:06



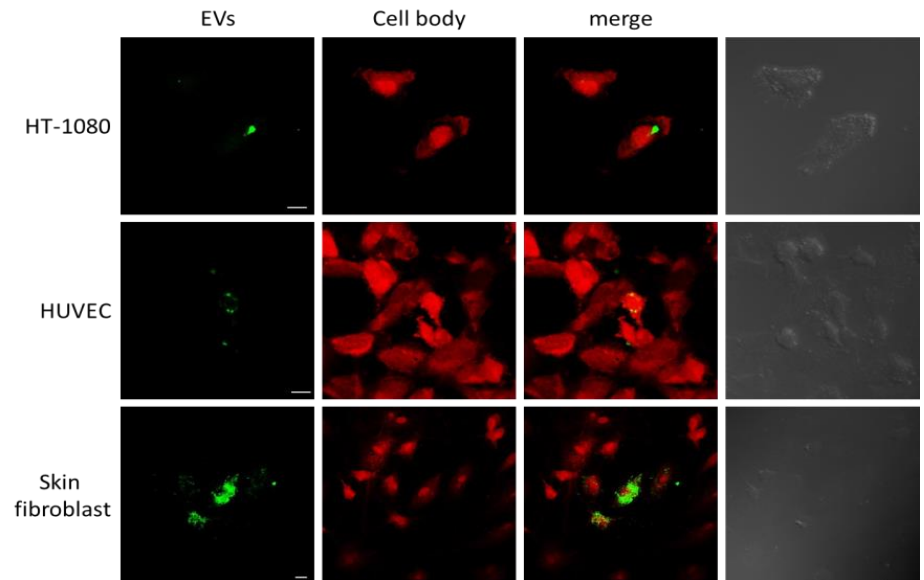
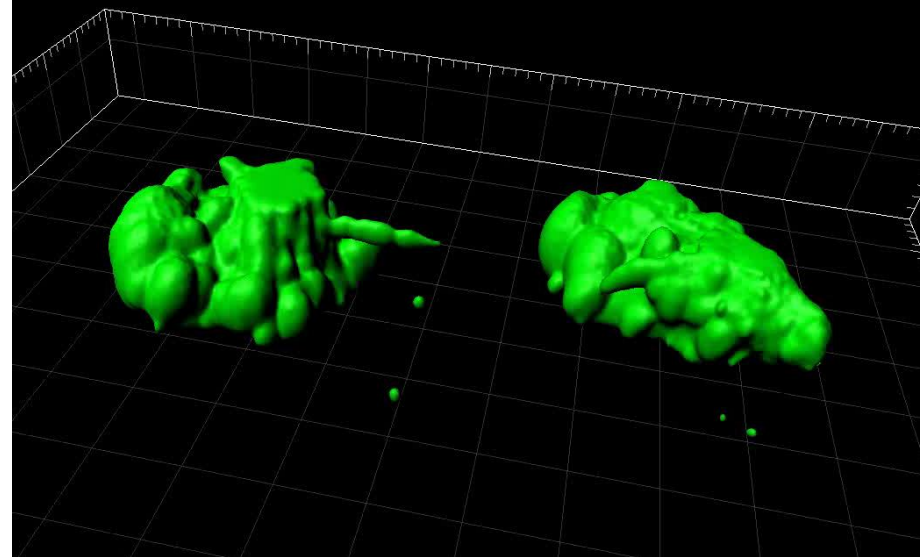
1µm JEOL 26/10/2018  
x5,000 2.00kV LED SEM WD 9.5mm 11:54:57



## Role of RPSA?



## Blebbing and Extracellular Vesicle Shedding



## Conclusion

- AG-9 is highly more conserved than VG-6 peptide
- AG-9 and VG-6 structural characteristics are similar (DC, NMR, FTIR)
  - same conformation ( $\beta$ -turn).
- Different supraorganization (AFM)
  - VG-6 : dots
  - AG-9 : fibers (amyloid-like)
- *In vivo* : AG-9 > VG-6
- *In vitro*
  - 2D and 3D proliferation,
  - migration,
  - adhesion,
  - proteinase secretions
  - tubulogenesis (angiogenesis)

Optimal Concentration  
AG-9 :  $10^{-7}$ M / VG-6:  $10^{-4}$ M
- Cancer : RPSA (AG-9 and VG-6 receptor)
- Blebbing and Extracellular Vesicle shedding
  - Invasion and cell/cell communication

AG-9 and its RPSA receptor influence tumor progression

→ New antitumor therapies?



### UMR CNRS 7369 MEDyC

#### Equipe « Matrikines »

Jordan Da silva

Mathieu Villemin

Aurélie Dupont-Deshorgue

Pr Laurent Ramont

Dr Abdelilah Beljebbar

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Dr Sylvie Brassart-Pasco

Pr François-Xavier Maquart

### EA4676 – UPJV - Amiens

Pr Mathieu Gautier

### EA4682 – URCA

Dr Alexandre Berquand

Pr Michael Molinari

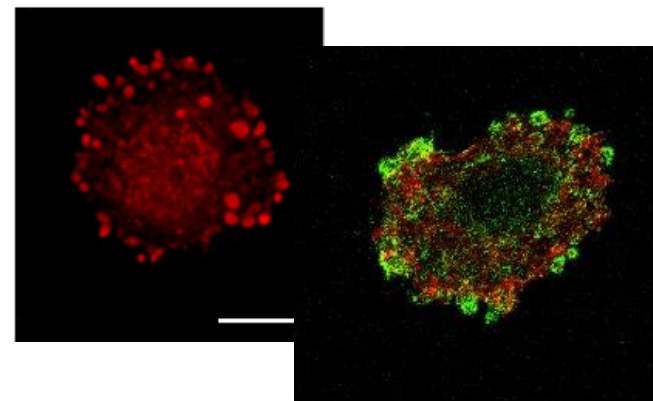
### ICMR, CNRS UMR 7312 – URCA

Dr Pedro Lameiras

Dr Jean-Marc Nuzillard

### Plateau Imagerie - URCA

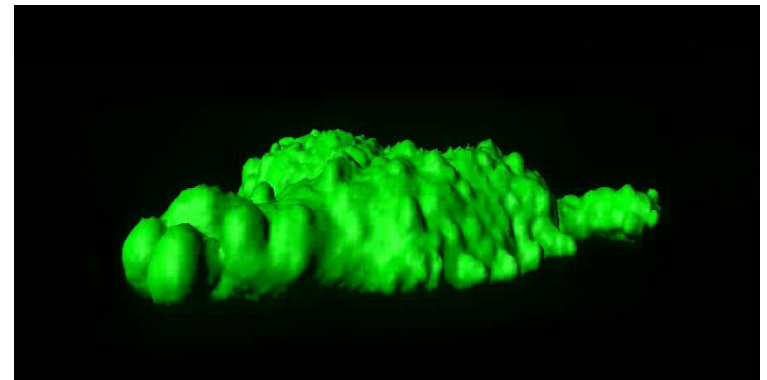
Dr Christine Terryn



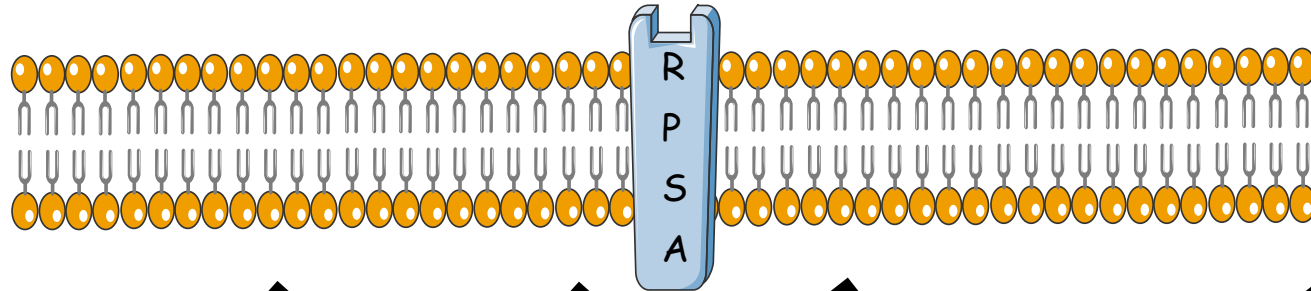


# récepteur Ribosomal Protein SA (RPSA)

- Récepteur transmembranaire
- Récepteur de surface pour la laminine (Rea *et al*)
- Précurseur 37 kDa, forme mature 67 kDa
- Nécessaire pour la formation de la sous-unité ribosomique 40S
- Voie d'entrée de nombreux pathogènes tels que les virus ou les bactéries
- Permet l'adhésion des cellules à la membrane basale
- Récepteur aux peptides d'élastine
- Régulation de l'activité protumorale des peptides d'élastine (*Blebbing* et vésicules extracellulaire)



# Ribosomal Protein SA (RPSA)



Assemblage  
des  
ribosomes

génération  
des ARNr

Viabilité  
cellulaire

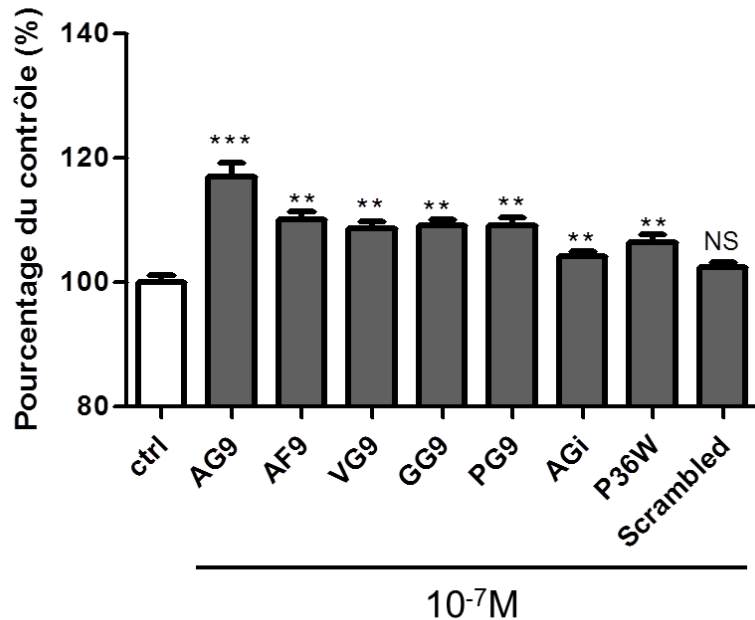
- Croissance
- Migration
- Adhésion
- Invasion
- Remodelage de la MEC
- Survie cellulaire

Surexprimé  
dans le cancer

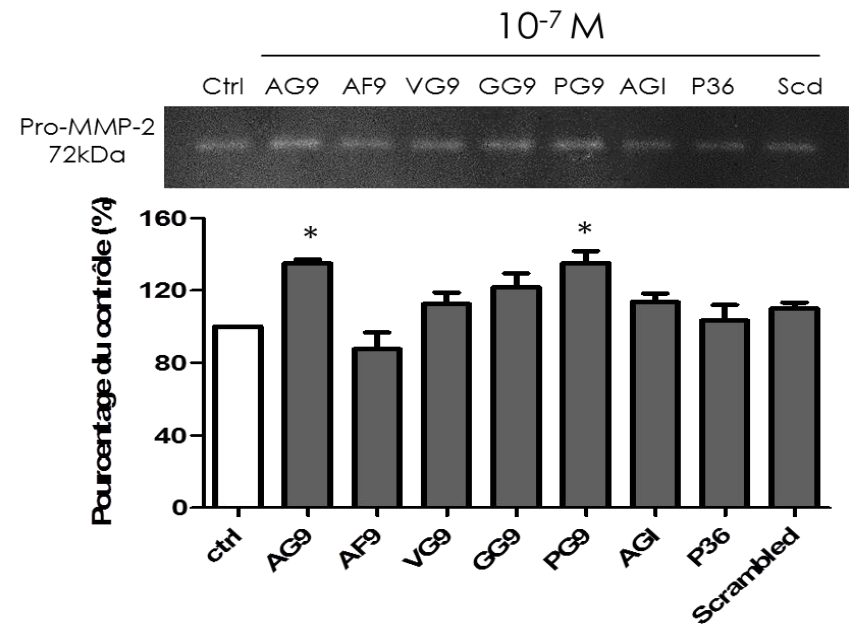
Cible thérapeutique

## Prolifération

Peptide	Séquence (aa)
AG9	AGVPGLGVG
AF9	AGVPGFAG
VG9	VGVPGLGVG
GG9	GGFPGFVG
PG9	PGGPGFGPG
AGI	AGIPGLGVG
P36	AGIPGLGVGVGPGLGVGAGVPGLGVGAGVPGFAG
Scrambled	AVGGGGPLV

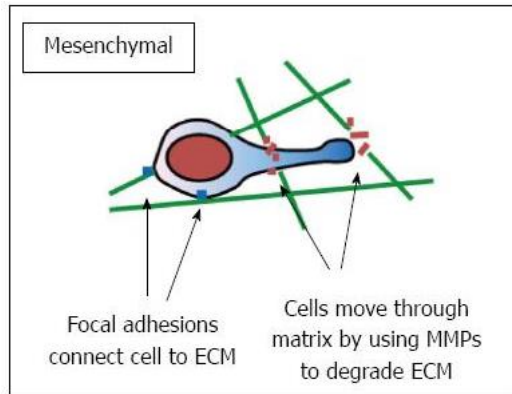


## Sécrétion de protéases



# Invasion et formation de Métastases

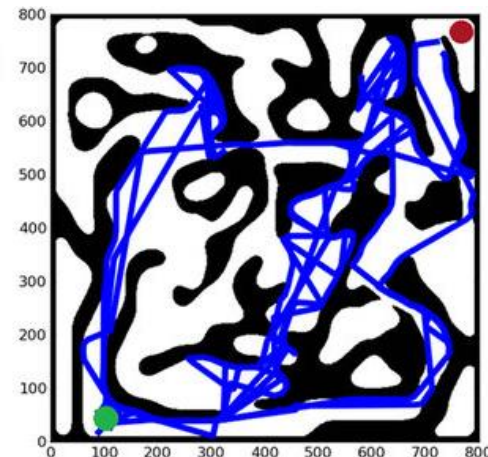
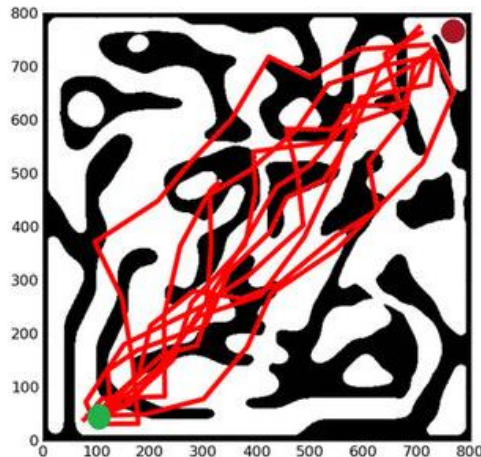
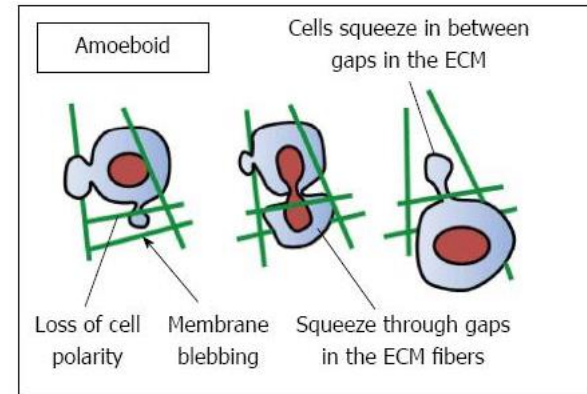
## Mesenchymal



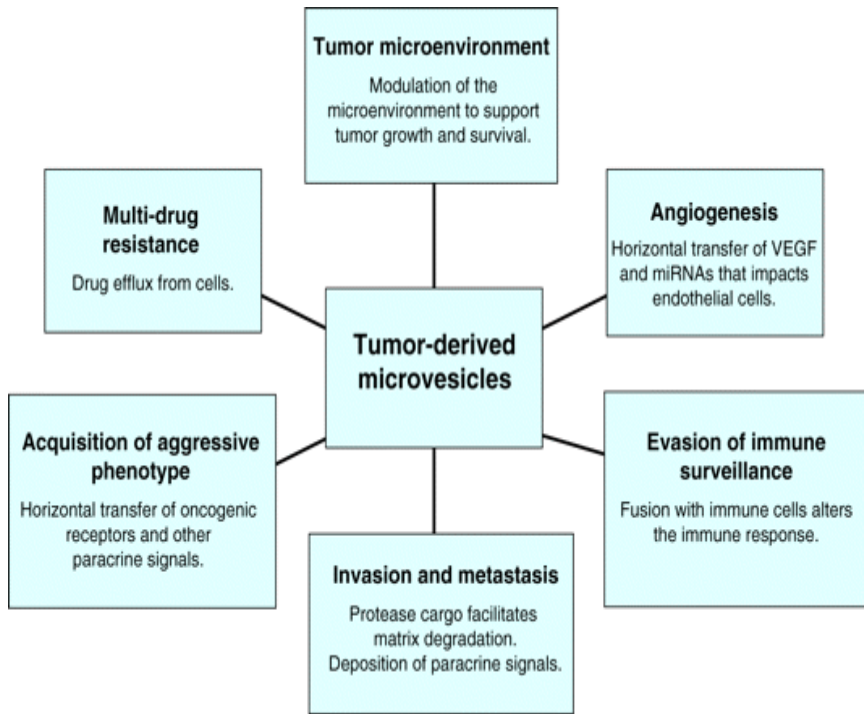
ECM degradation ↓  
Cell-substrate adhesion ↓



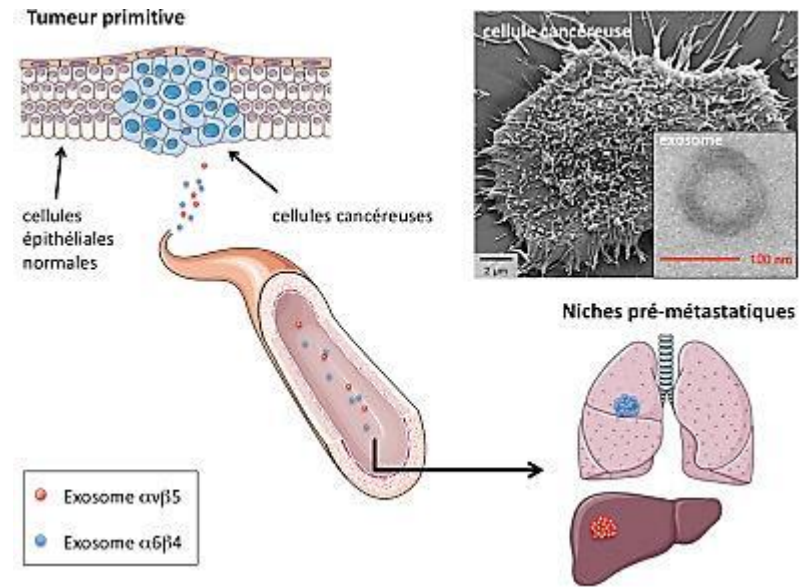
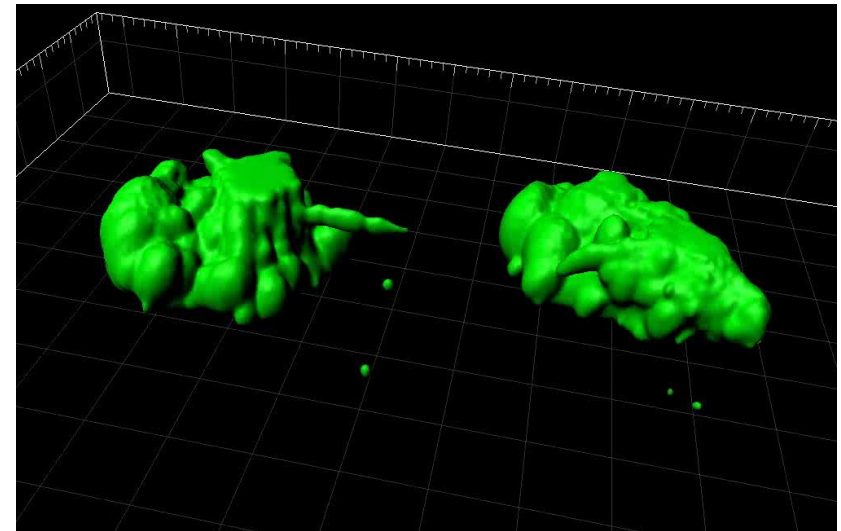
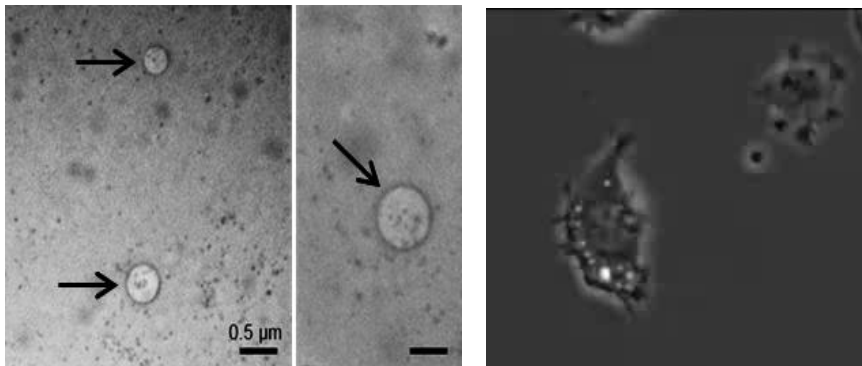
## Amiboïde



# Microvésicules tumorales



D'après Muralidharan-Chari V *et al*, 2010



# Cell motility through plasma membrane blebbing

Fackler OT, Grosse R - J. Cell Biol. (2008)

