

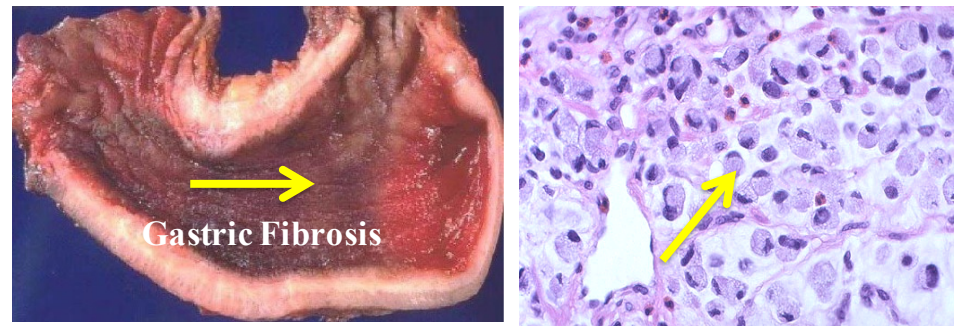
Absence of thrombotic events in the Gastric signet-ring cell adenocarcinoma patients with high expression of heparanase

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Gastric Cancer

- Fifth most common cancer (significant global health problem) ¹.
- Gastric cancer in **Europe** and **USA** is decreased ².
- BUT some specific form of gastric cancer, named **Signet ring cell adenocarcinoma (SRCA) : INCREASE**



- 29% of patients with gastric cancer had a **SIGNET RING CELL** type tumor histology ⁴.
- Radical multimodality treatments: Titanium silicate (TS)-1, 5 Fluorouracil, Cisplatin
- Survival rate of patient undergoing only curative surgery is low ⁵.

1- Torre, Lindsey A., et al. "Global cancer statistics, 2012." *CA: a cancer journal for clinicians* 65.2 (2015): 87-108.

2-Amiri, M., Janssen, F., & Kunst, A. E. (2011). The decline in stomach cancer mortality: exploration of future trends in seven European countries. *European journal of epidemiology*, 26(1), 23-28.

4-Antonioli, D.A. and H. Goldman, *Changes in the location and type of gastric adenocarcinoma*. *Cancer*, 1982. **50**(4): p. 775-781

5-Macdonald, John S., et al. "Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction." *New England Journal of Medicine* 345.10 (2001): 725-730.

Objectives

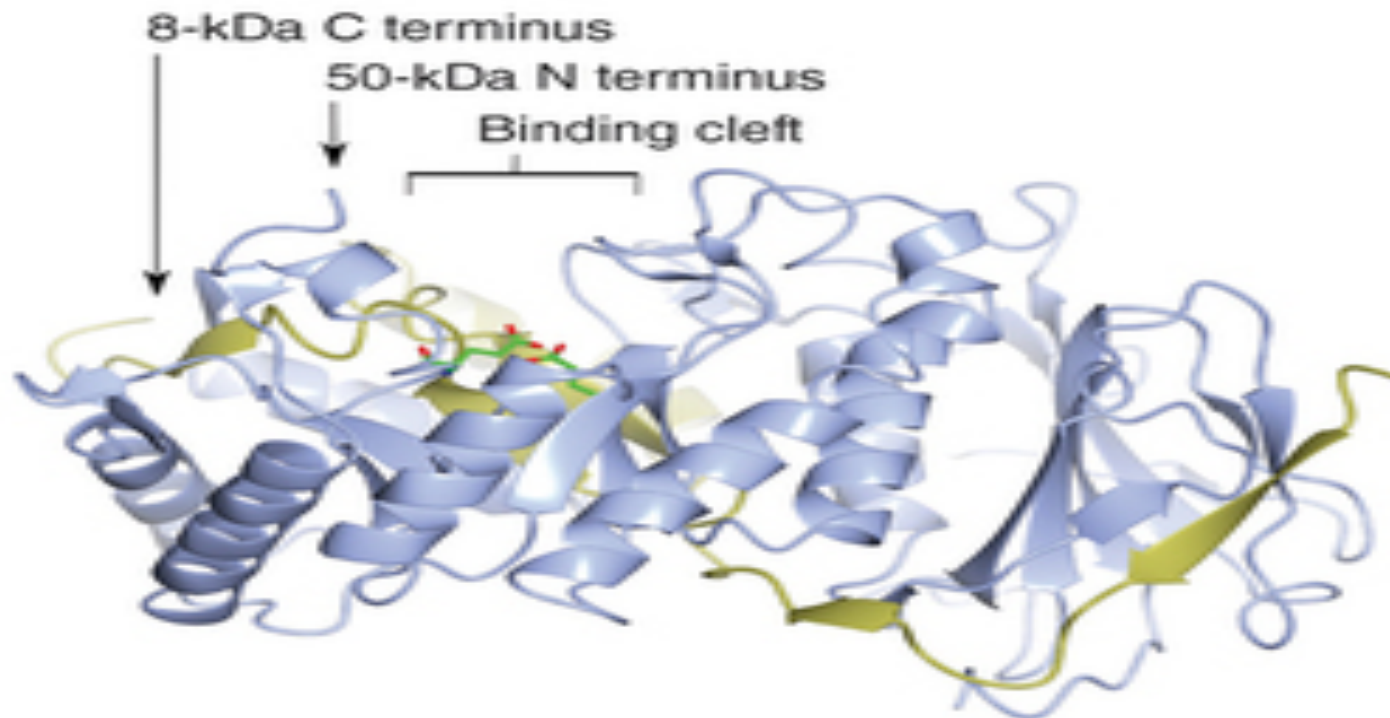
- **Co-relation between level of heparanase expression and incidence of venous thromboembolism (VTE) in SRCA patients**

What is Heparanase ?

- **Enzyme:** Endo- β -D-glucuronidase

Cleavage heparin sulfate (HS) chain of HSPGs of extracellular matrix and cell surface at pH 5.5

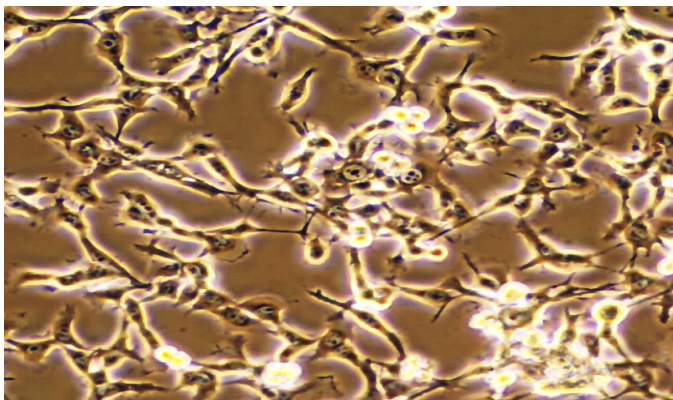
- Synthesis as an **Inactive form** 65 kDa precursor.
- **Active form:** 2 in units of 8 and 50 kDa



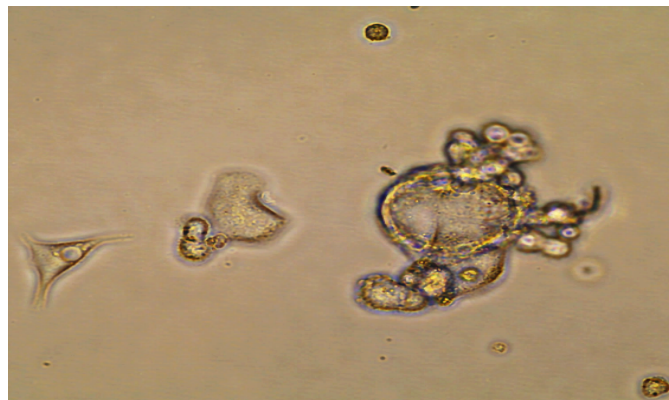
Materials

- Human cancer cell lines used were: Ovarian (**OVCAR-3** and **SKOV-3**), breast (**MDA-MB231** and **MCF7**), gastric (**AGS**, **KATO-III**), intestinal (**LS174T**), lung (**A549**), leukemia (**K562**), cervical (**HELA**) and human microvascular endothelial (**HMEC-1**) were obtained from American Type Culture Collection (ATCC, Manassas, VA).
- Tumor and corresponding normal gastric tissue specimens (**SRCA tumoral**, **SRCA peri-tumoral**, **Non-SRCA tumoral** and **Non-SRCA peri-tumoral**) were obtained from 21 patients and **Ascites fluids** from 14 cancer patients from General and Digestive Tract Surgery Department at Lariboisière Hospital in Paris (France).
- Drugs used in this study were: **Suramin** (Sigma Chemical Co, St. Louis, MO, USA)
- **Human Phospho-Kinase Array** (R&D Systems, Minneapolis, MN 55413 USA)
- **HepAnalyze™ Heparanase ELISA Kit** (InSight Biopharmaceuticals Ltd. Rehovot, Israel)
- **Rabbit Anti-Heparanase Polyclonal Antibody, FITC Conjugated** (Bioss 03103 Montlucon Cedex - France)

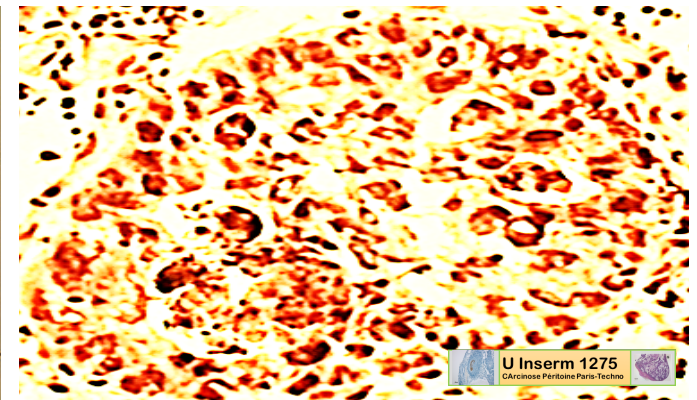
Primary Cells (Operative room)



(KATO-III Cell line)



Tissue (RNA and frozen samples)



Methods used in this Project

Ficol Protocol → to isolate mononuclear cells from ascitic fluid of cancer patient

Cell Culture → to maintain live cell lines (KATO-III)

Tissues / (Primary Cells) → Gastric linitis

PCR → to investigate Gene Expression

Cells/Tissue → RNA isolation → Reverse Transcriptase (cDNA) → PCR

- RT-PCR (Determination of length)
- q-PCR (Determination of quantity)

ELISA → for the quantitative determination of proteins

Immunofluorescence → to visualize the localization of heparanase proteins

Phosphokinase array → to observe changes in phosphorylation profiles of various kinases

Ascitic fluid

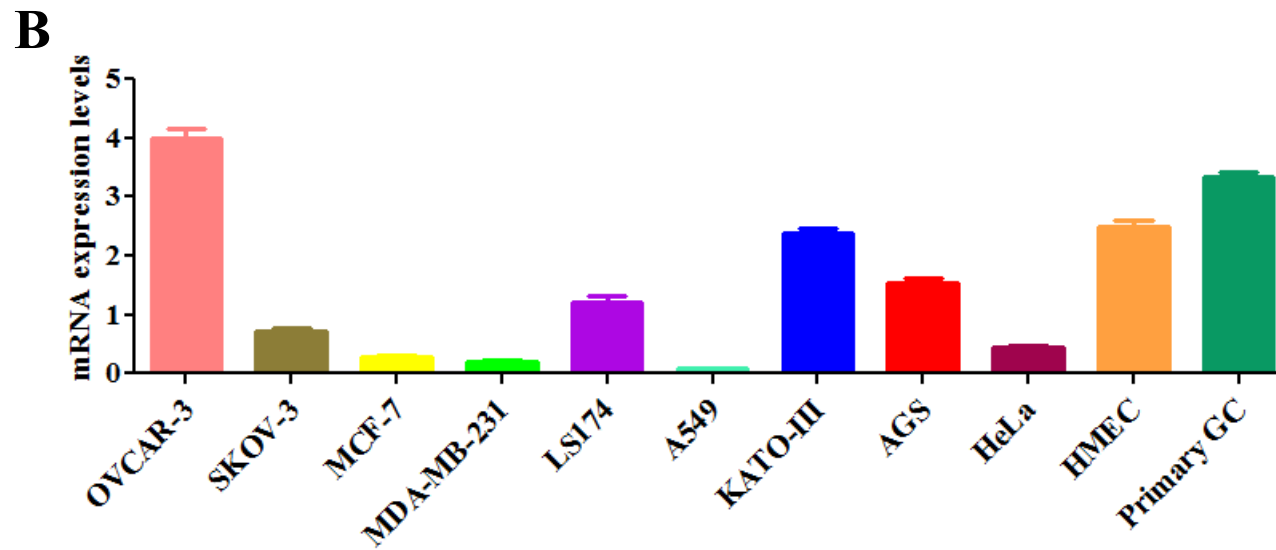
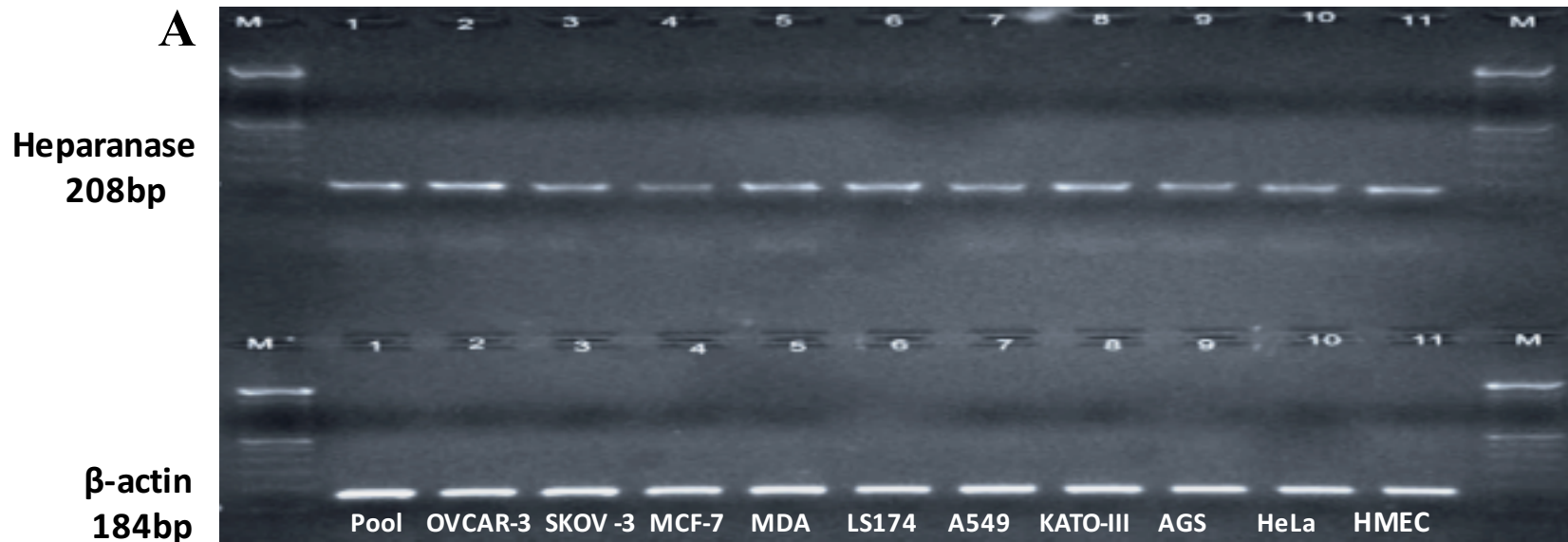
Mononuclear cells

Red Blood Cells

Ficol Protocol



Tumor cell lines express Heparanase in vitro by RT-PCR and qPCR



- ❑ A-Demonstration of the presence of heparanase in the cell lines by RT-qPCR
- ❑ B-Quantitative analysis of heparanase RNA in tumor cell lines

Immunofluorescence of KATO-III cell line

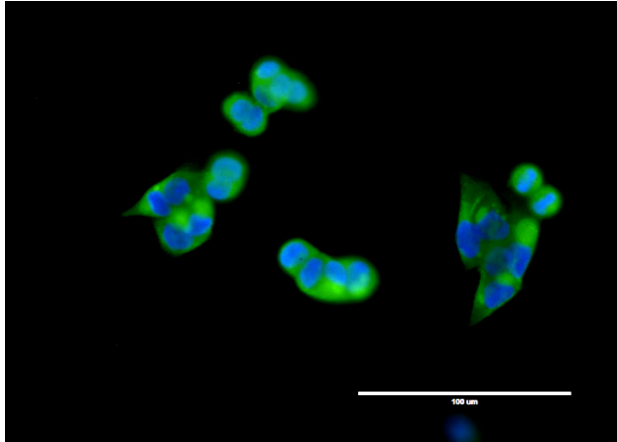
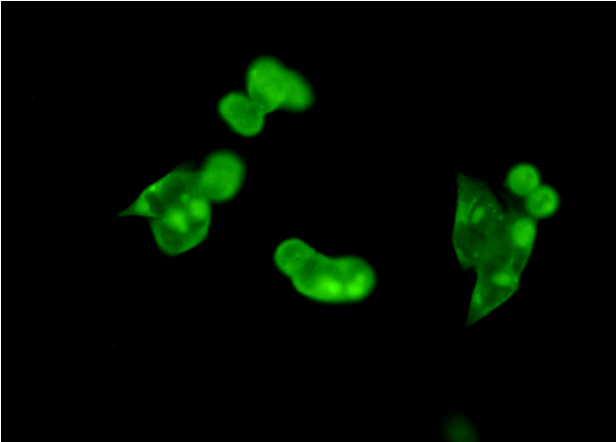
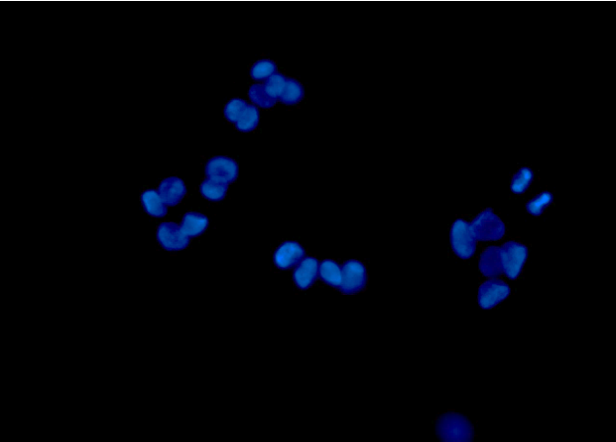
Result-2

DAPI

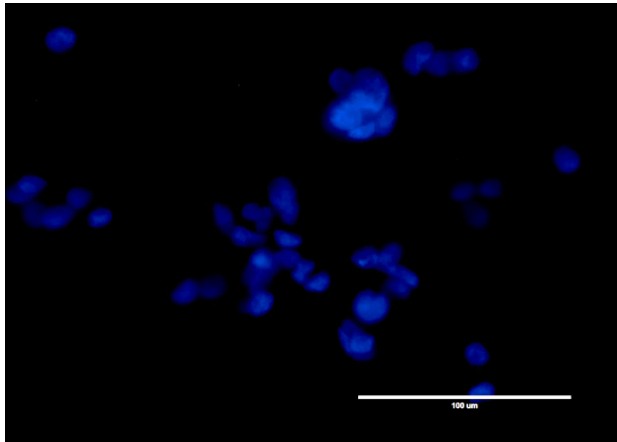
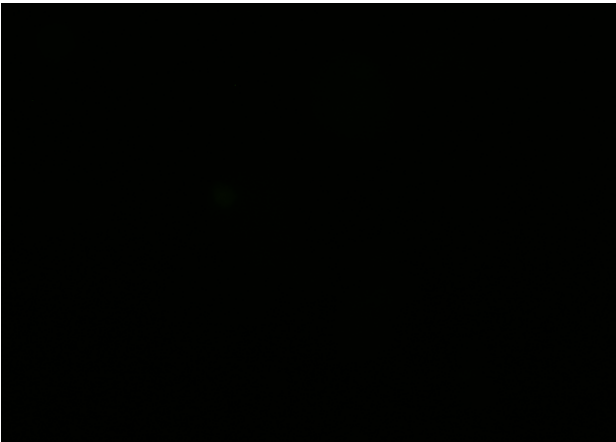
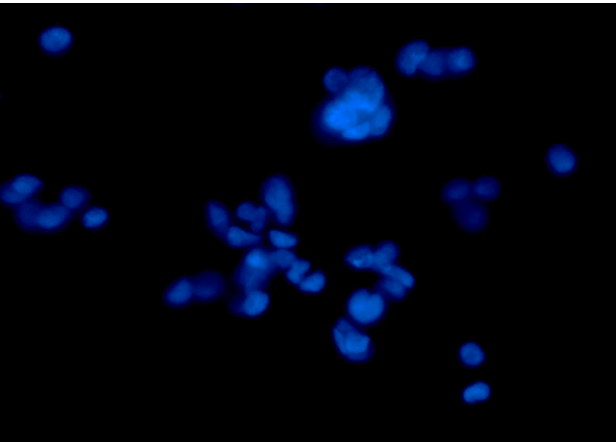
Staining of Heparanase

MERGE

40x
With primary antibody



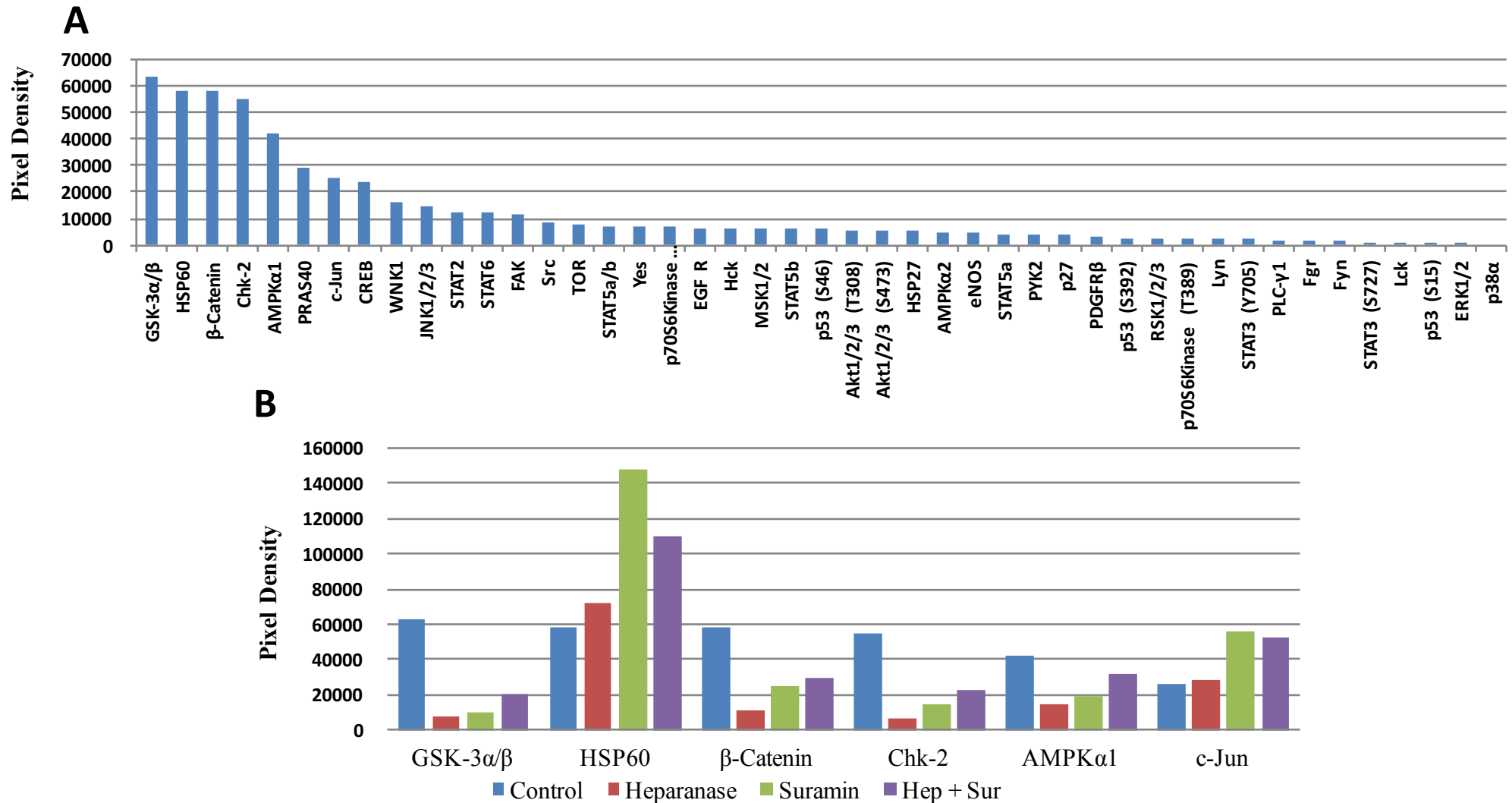
40x (Control)
No primary antibody



High expression of heparanase protein found in KATO-III cell line

Phospho-Kinase Array of KATO-III treated with Heparanase/Suramin

Result-3

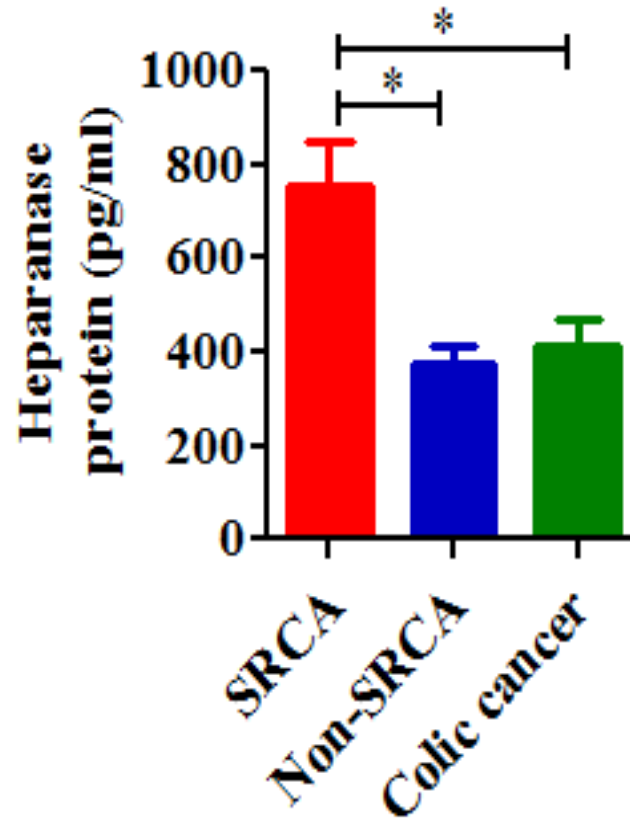


A- 45 different kinases in normal KATO-III Cell line

B- 6 different kinases of KATO-III treated with heparanase (0.2 μ g/ml) or suramin (200 μ M) or both for 5 hours in comparison to control

❑ Suramin has not any antagonist effect in phosphorylation pathway induced by heparanase.

Heparanase Expression in ascitic fluid of different cancer patients by ELISA



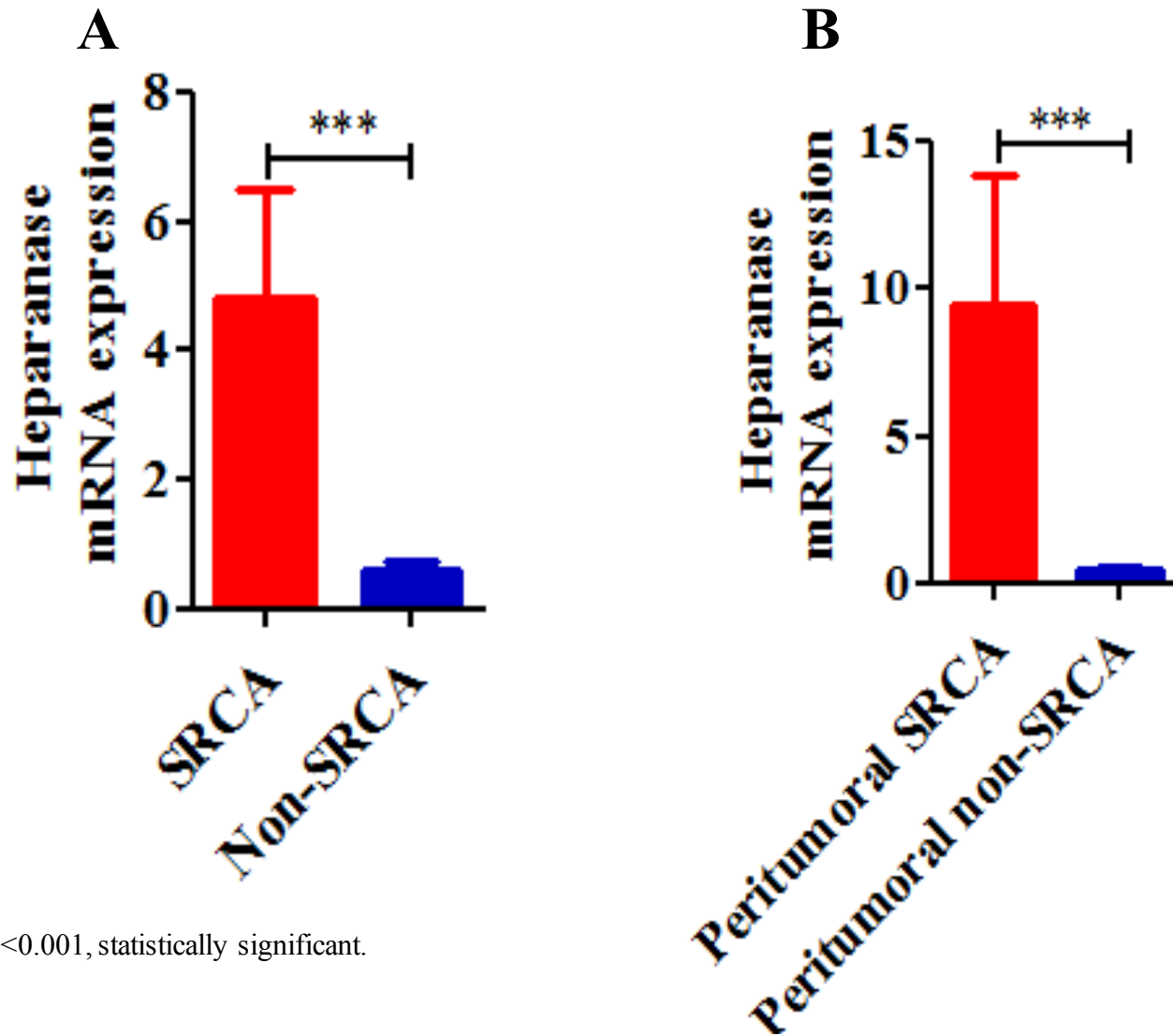
*P<0.05, statistically significant.

❑ High heparanase level found in the ascitic samples of primary SRCA of stomach as compared to Non-SRCA of stomach and colic cancer.

(SRCA n=5, Non-SRCA n=3 and colic cancer n=6)

Heparanase expression in the tumoral tissue of SRCA and Non-SRCA and their peri-tumoral areas via qPCR

Result-5



□ High heparanase mRNA level found in the tumoral and peri-tumoral tissue samples of SRCA as compared to non-SRCA of stomach.

(Tumoral SRCA n=11, Peri-tumoral-SRCA n=7, Tumoral non-SRCA n=10, Peri-tumoral non-SRCA n=8)

Querying clinical databases for Thrombotic events

Variable	Total N =302 (%)	Non SRCA N=205 (67,9%)	SRCA N=97 (32,1%)	p
Pulmonary embolism				1.000
No	271(89.7)	177 (86.3)	94 (96.9)	
Yes	9 (3.0)	6 (2.9)	3 (3.1)	
Unspecified	22 (7.3)	22 (10.7)	0 (0.0)	
DVT (Deep vein thrombosis)				0.660
No	272 (90.1)	176 (85.9)	96 (99.0)	
Yes	5 (1.7)	4 (2.0)	1 (1.0)	
Unspecified	25 (8.3)	25 (12.2)	0 (0.0)	
PE and/or DVT				1.000
No	266 (88.1)	173 (84.4)	93 (95.9)	
Yes	12 (4.0)	8 (3.9)	4 (4.1)	
Unspecified	24 (7.9)	24 (11.7)	0 (0.0)	

□ No significant difference in thrombotic events was observed between SRCA (8.2%) and non-SRCA (8.7%) of stomach

Conclusions

- For the first time, we found **Heparanase** expression in SRCA of stomach.
- A significant difference in heparanase level expression between SRCA and non SRCA of stomach was observed.
- But no such a difference was found in thrombotic events between SRCA and non SRCA of stomach (8-9% events was observed in both).

Therefore,

- No correlation  between heparanase level and increase of thrombotic events.