Extracellular matrix deterioration

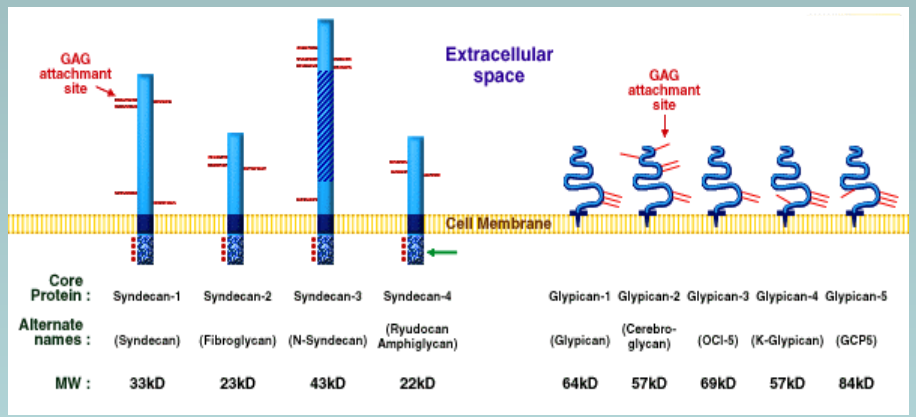
Lessons from laryngeal and colorectal cancer

D. H. Vynios

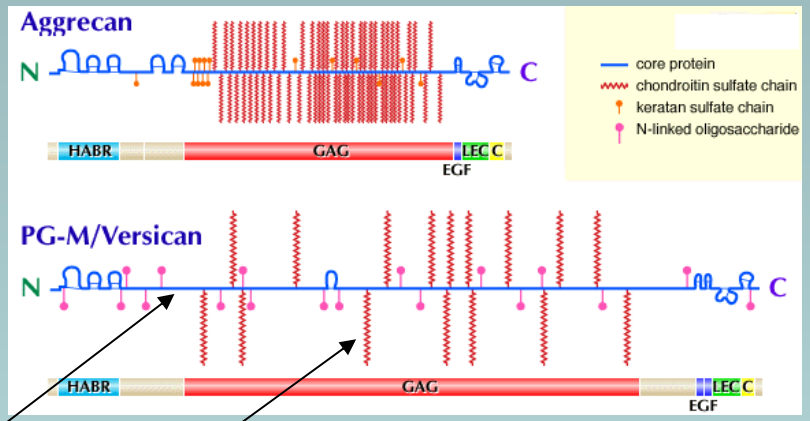
Section of Organic Chemistry, Biochemistry &
Natural Products, Department of Chemistry,
University of Patras, Greece

Proteoglycans: many forms and many functions

Membrane-bound PGs

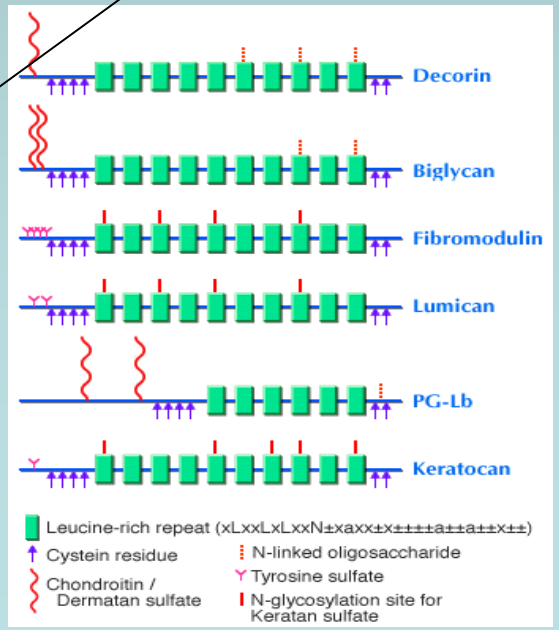


Extracellular PGs



Protein core

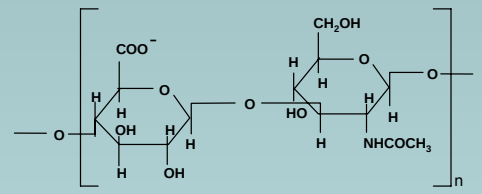
Glycosaminoglycans



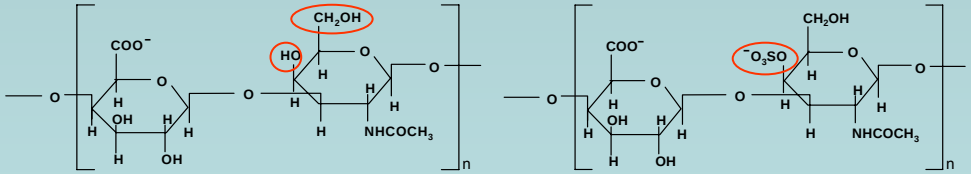
Glycosaminoglycans

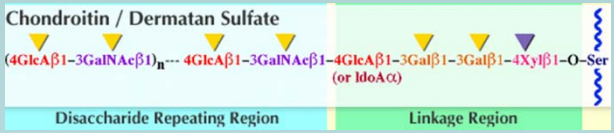
- A specific class of heteropolysaccharides
  - found mainly in the ECM
  - implicated in many physiological and pathological processes

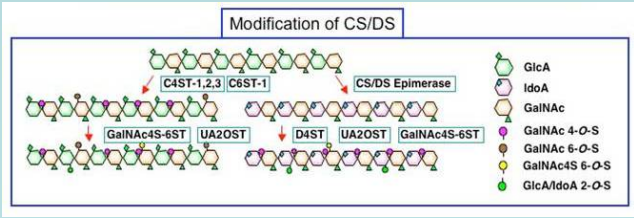
- HA



- CS/DS





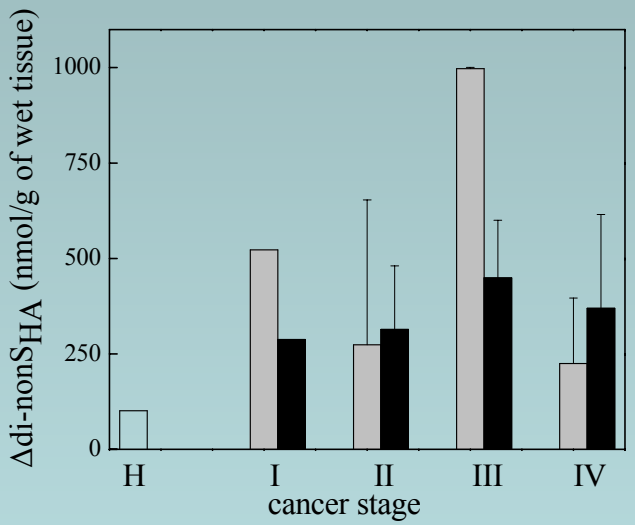


Enzymes in GAGs biosynthesis

- Biosynthesis of HA
  - HAS1, HAS2, HAS3
- Biosynthesis of CS/DS
  - CHSY1, CHSY2 (CHPF), CHSY3
  - C4ST1, CHST3
  - DSE, D4ST1

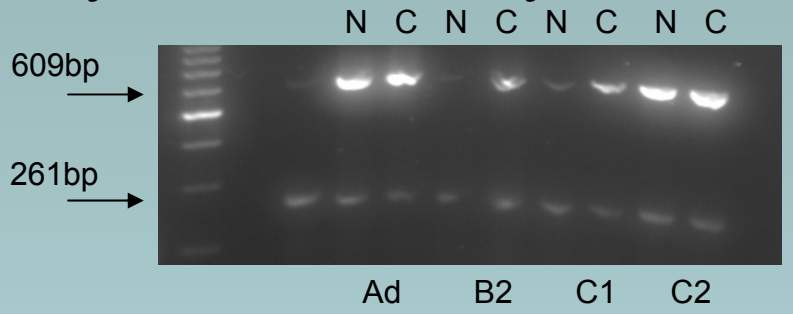
Hyaluronan and colorectal cancer

- **Total HA** ↑

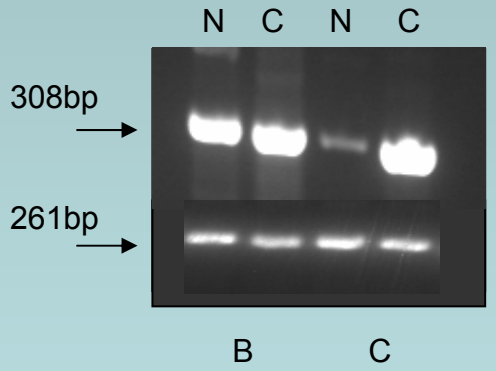


- Increased synthases activity

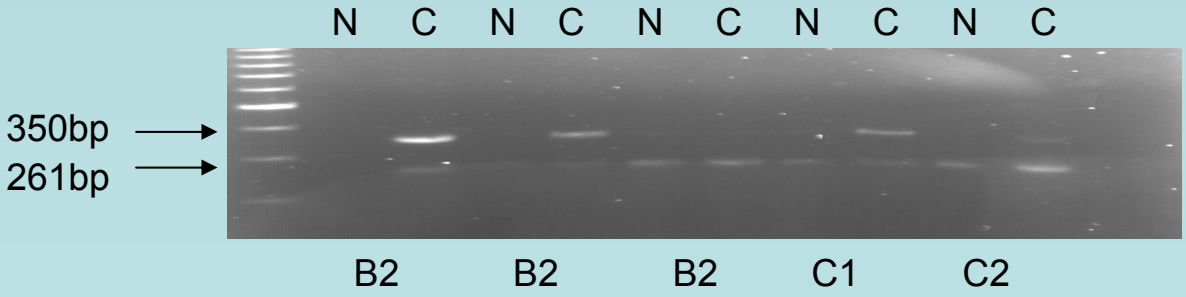
HAS2: 609 bp

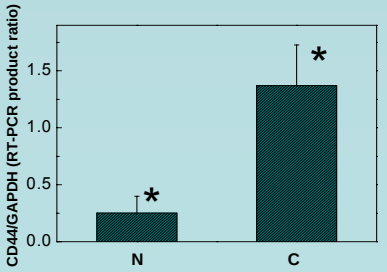


HAS3: 308 bp



- Increased CD44 expression





CS/DS and cancer

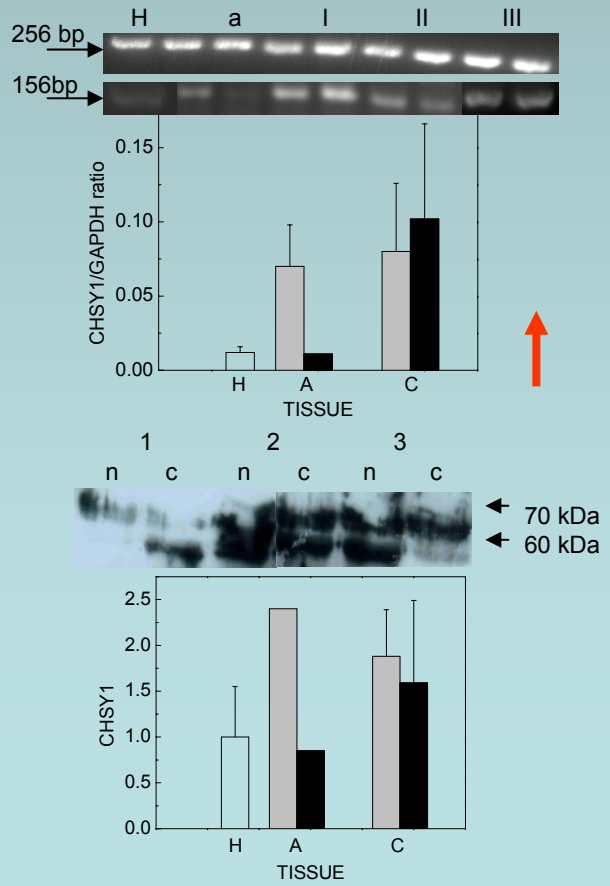
Colorectal cancer

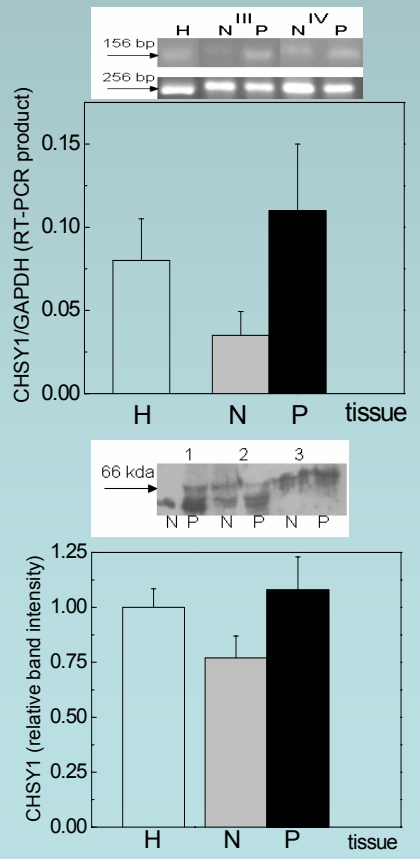
- **Total CS/DS** ↑
- Increased synthases activity

Laryngeal cancer

- **CS** ↓ **DS** ↑
- ? synthases activity

CHSY1





C

CS/DS and cancer

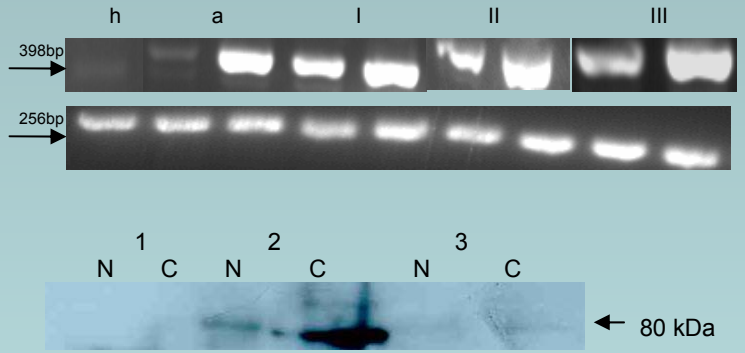
Colorectal cancer

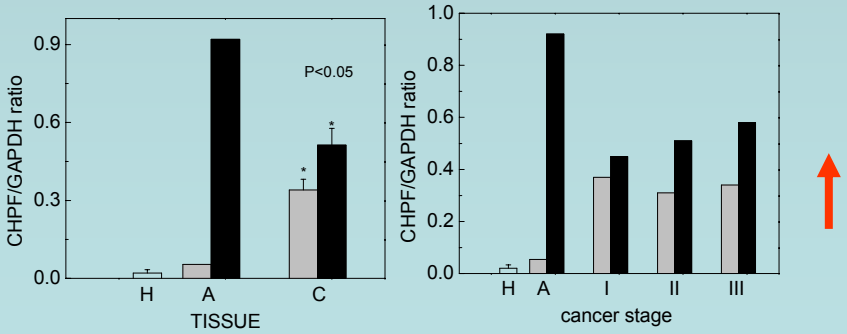
- **Total CS/DS** ↑
- Increased synthases activity

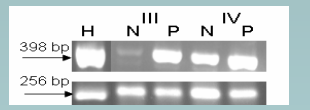
Laryngeal cancer

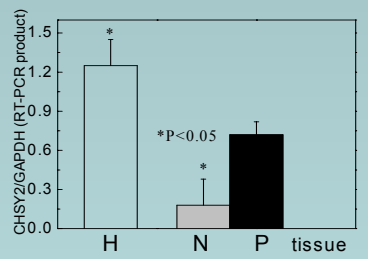
- **CS** ↓ **DS** ↑
- ? synthases activity

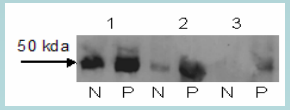
CHSY2 (CHPF)

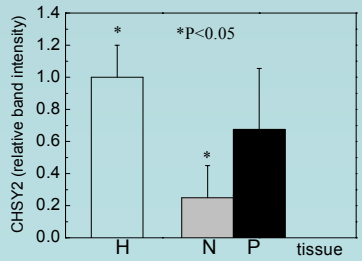












CS/DS and cancer

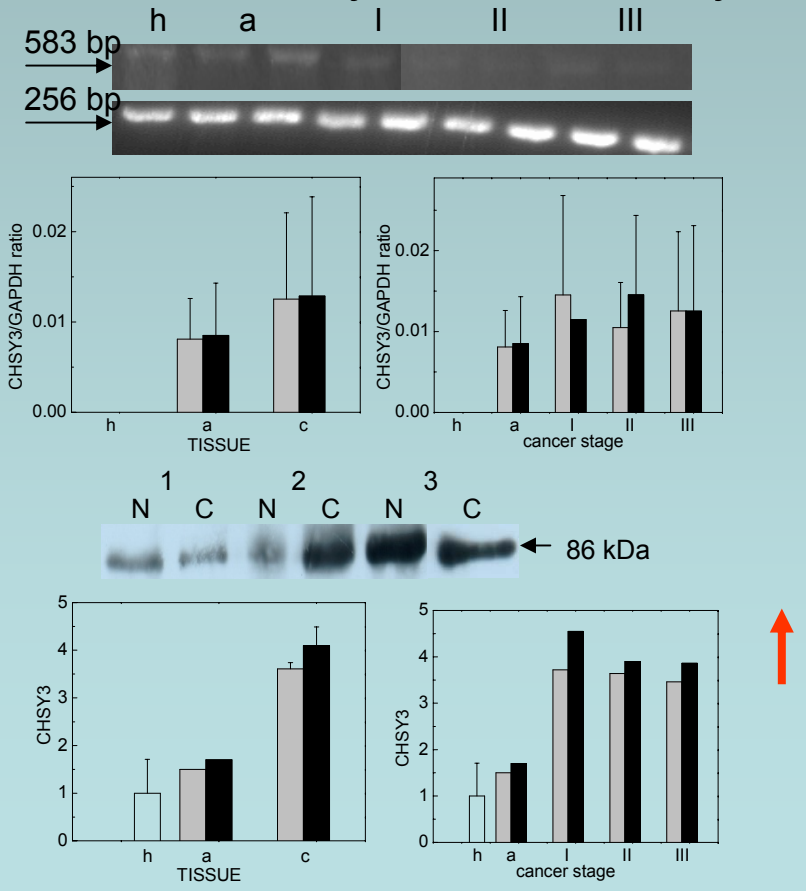
Colorectal cancer

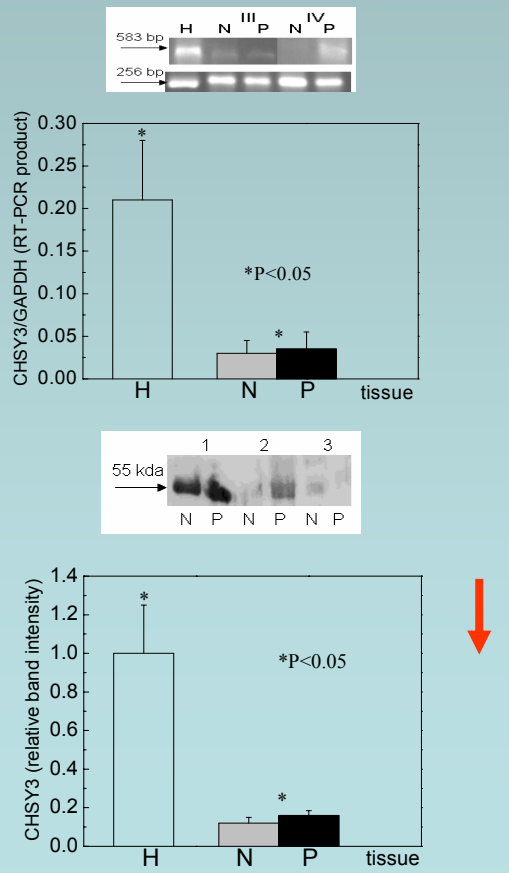
- **Total CS/DS** ↑
- Increased synthases activity

Laryngeal cancer

- **CS** ↓ **DS** ↑
- ? synthases activity

CHSY3

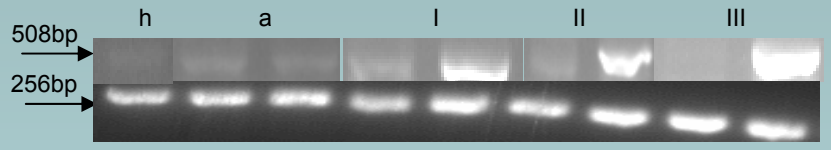




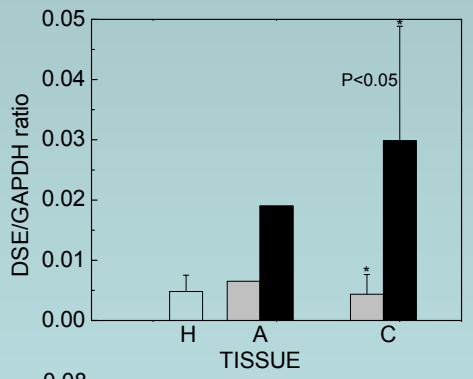
CS/DS and cancer

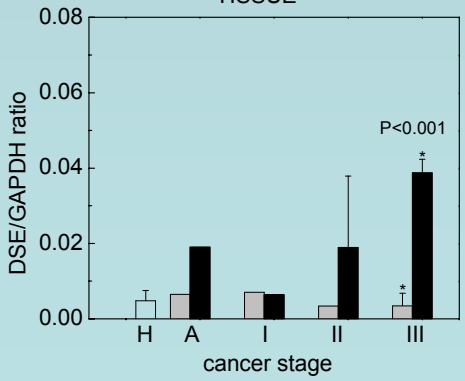
Colorectal cancer

- **CS increases more than DS**



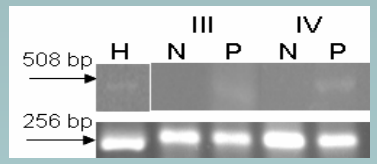
DSE

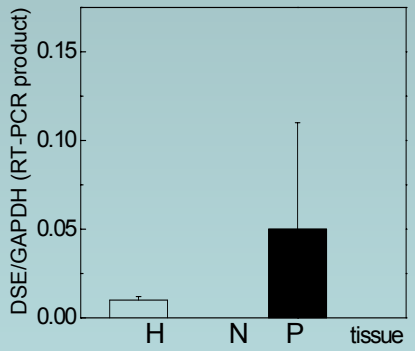


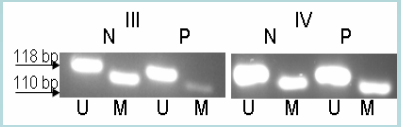


Laryngeal cancer

- **DS increases**



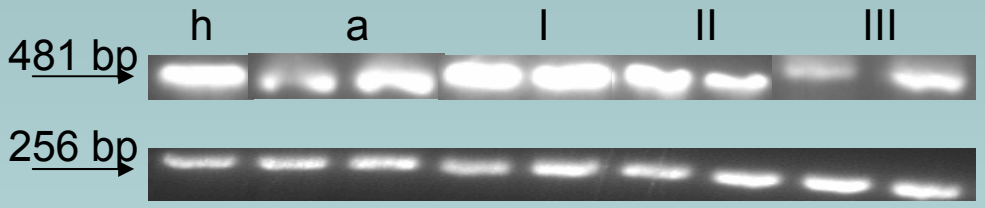


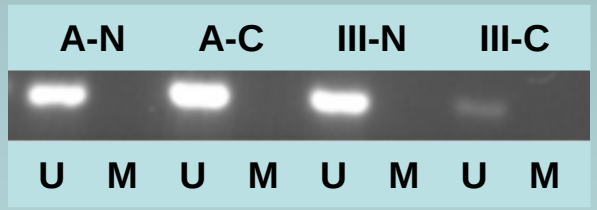


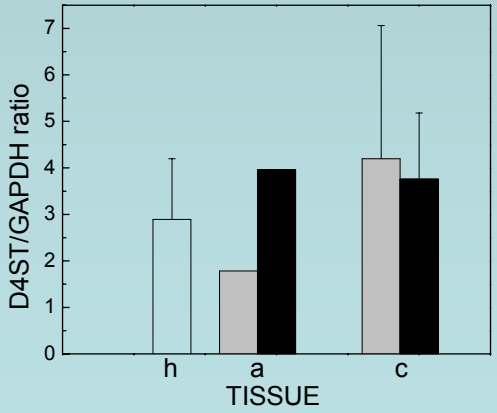
DSE expression is controlled by methylation of the promoter region in certain samples

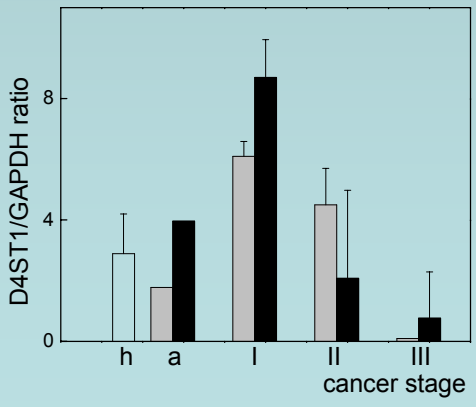
CS/DS and colorectal cancer

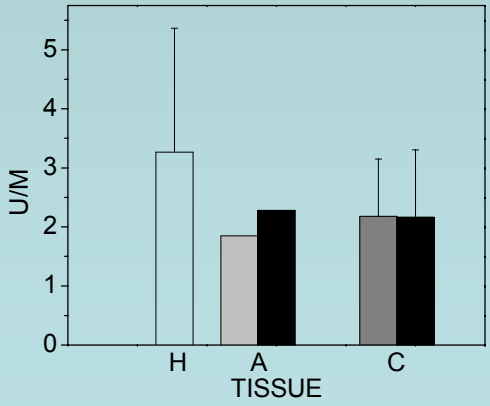
- **CS increases extremely more than DS at late stages**
- D4ST1 activity







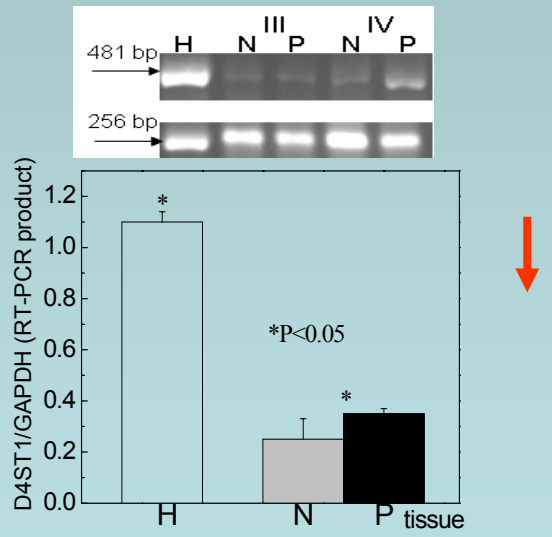




Methylation of the promoter region of the gene occurred at late stages

CS/DS and laryngeal cancer

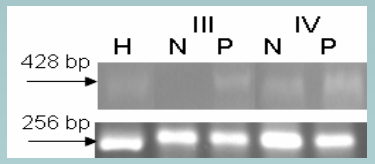
- **CS biosynthesis is favored at late stages**
- D4ST1 activity

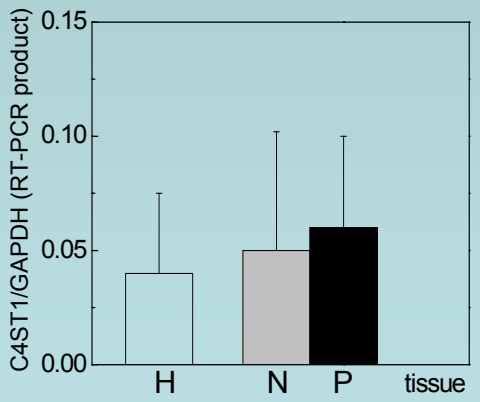


The CpG island near the promoter region was fully unmethylated therefore it did not affect enzyme expression

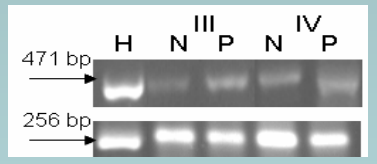
CS/DS and laryngeal cancer

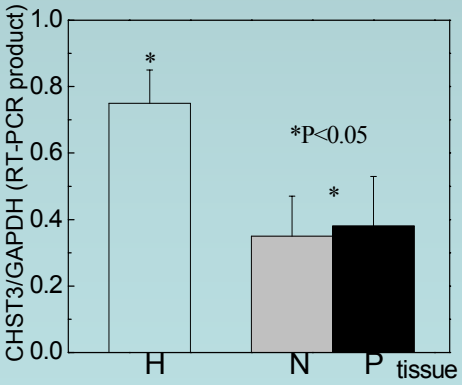
- **C-6S is present in less amounts than C-4S**
  - C4ST1
  - CHST3





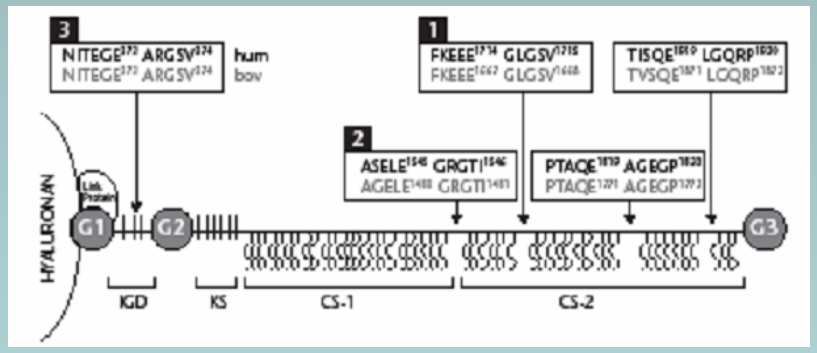
C

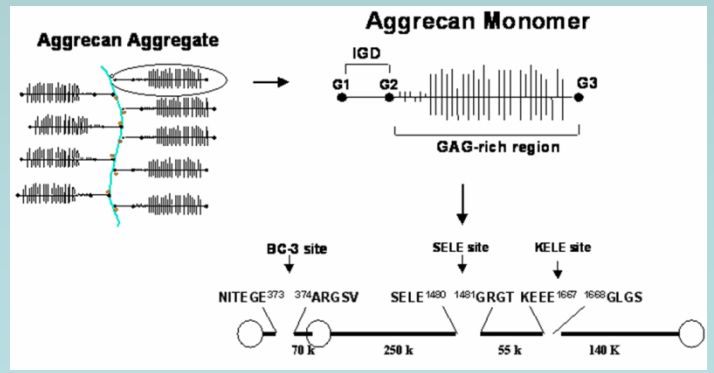




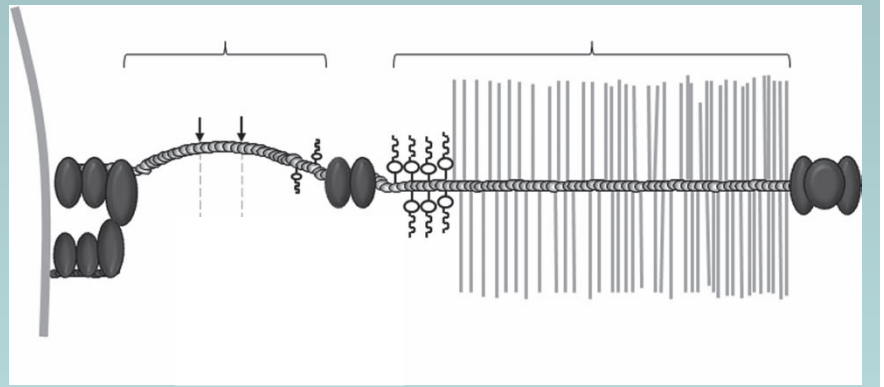
Extracellular degradation of PGs

by ADAMTs



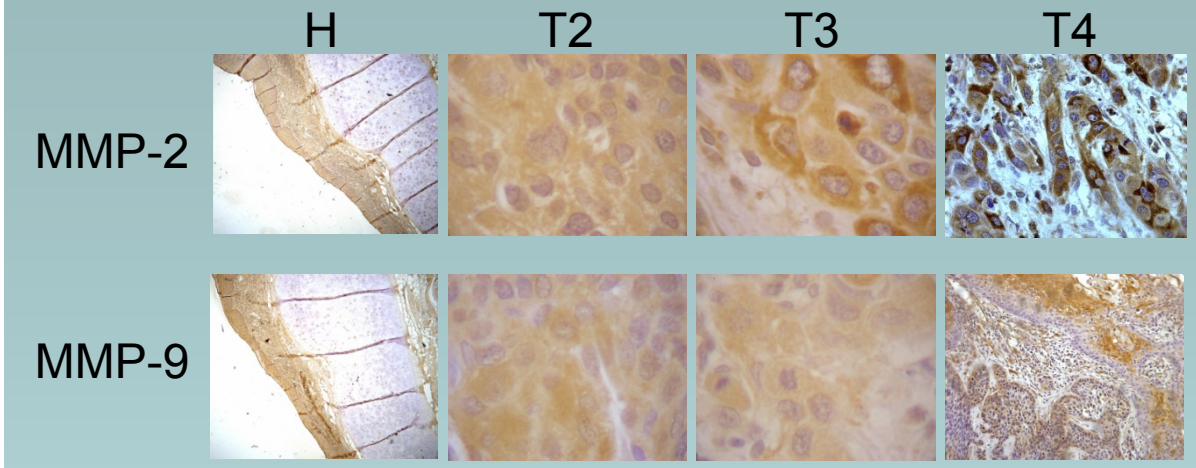


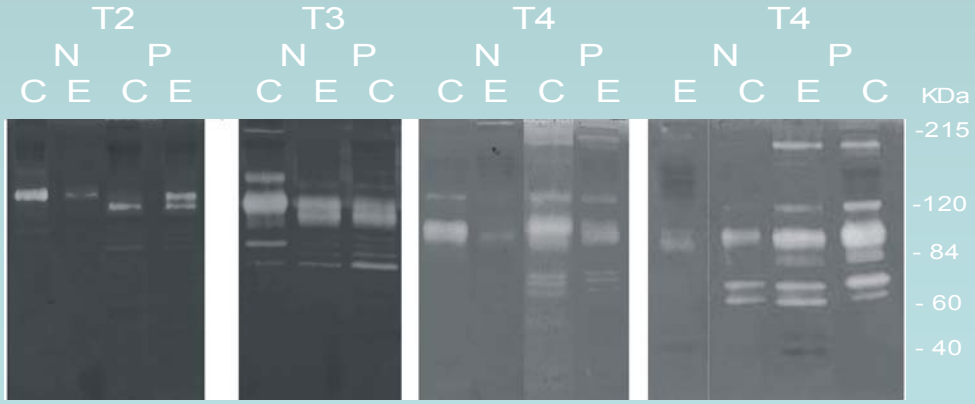
by MMPs



The activity of extracellular proteases is regulated by TIMPs

Gelatinases in laryngeal cancer





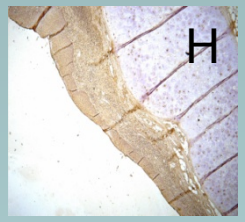
They appeared to be produced from only normal cells

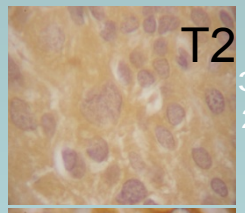
Gelatinases' activity increased with tumor stage, even in macroscopically normal specimens

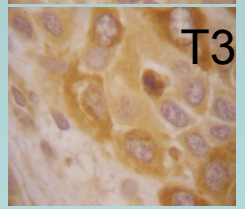
Their active forms appeared in cancer

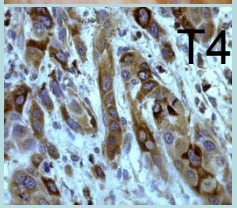
Gelatinases in laryngeal cancer

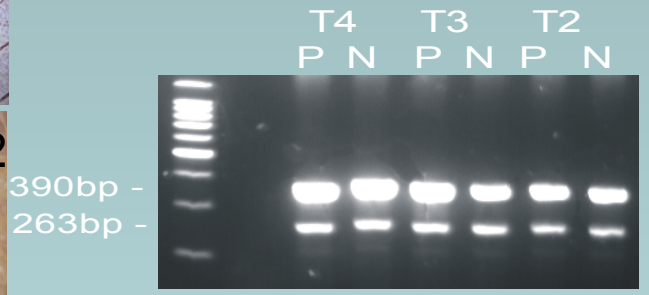
MMP-2

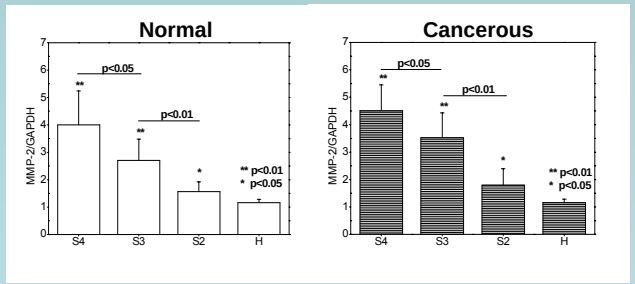












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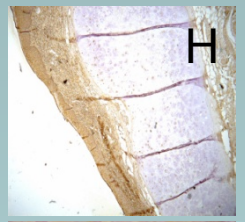
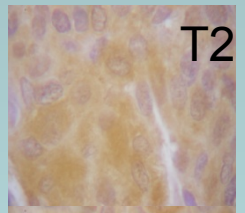
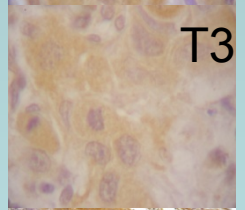
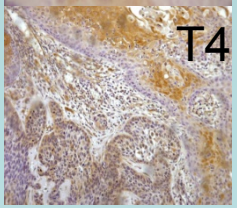
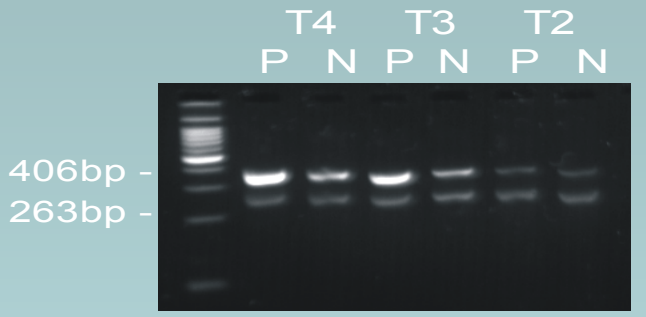
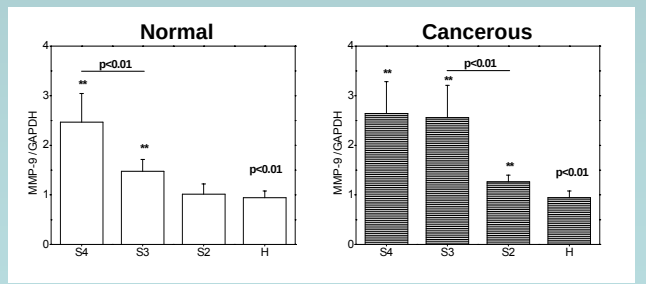








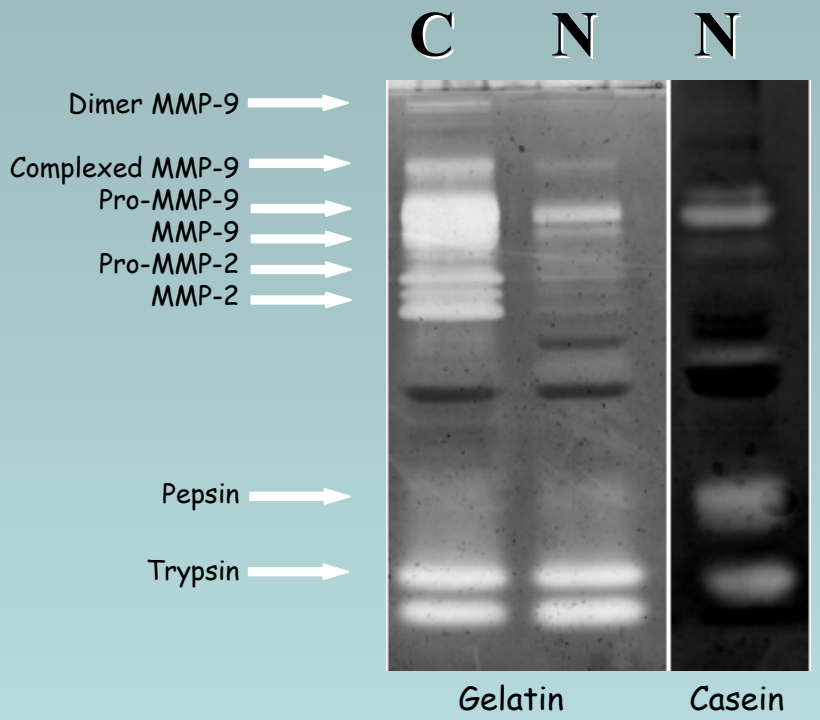


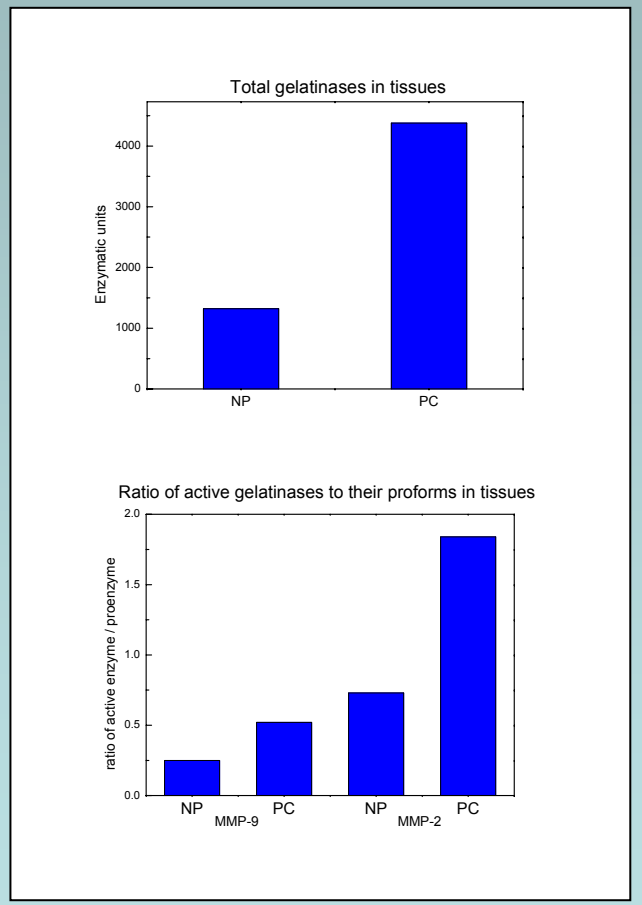


Gelatinases' expression increased

Their translation appeared to be highly regulated

Gelatinases in pancreatic cancer

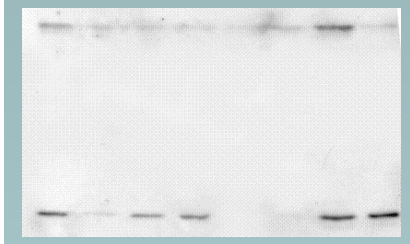




There is a significant increase of both gelatinases, together with substantial increase of the ratio of pro-forms / active forms

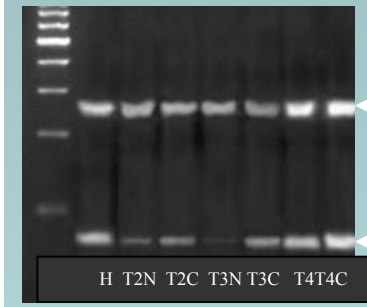
Aggrecanases in laryngeal cancer

ADAMTS-1



Mostly extracted in 4M GndHCl

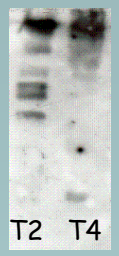
30kDa



263bp GAPDH

95bpADAMTS-1

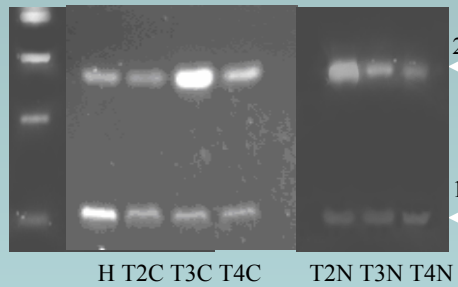
ADAMTS-4



60-75 kDa

Mostly extracted in 0.5M GdnHCl

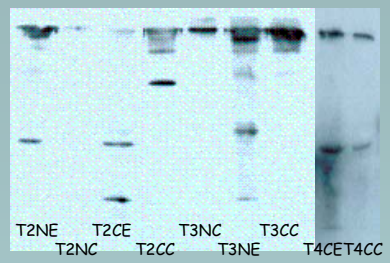
30kDa



263bpGAPDH

100bpADAMTS-4

ADAMTS-5

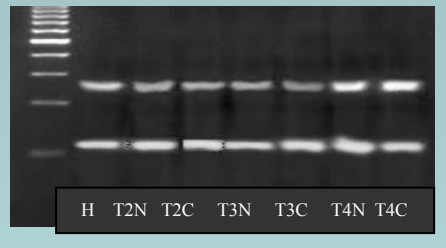


80kDa

50kDa

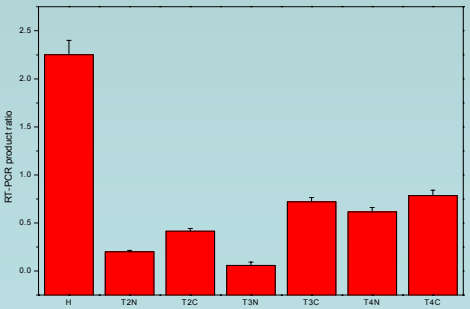
30kDa

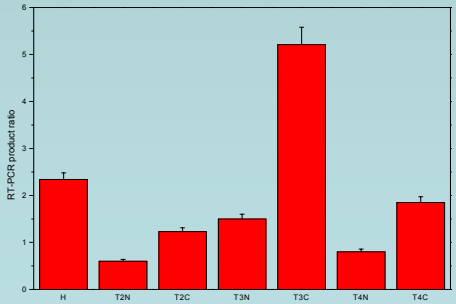
Mostly extracted in PBS

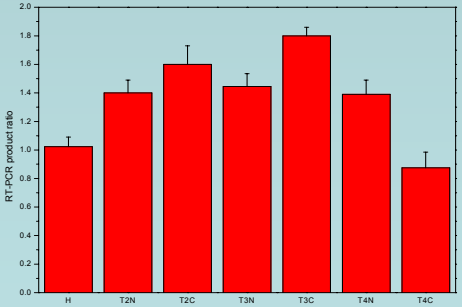


263bp GAPDH

110bp ADAMTS-5

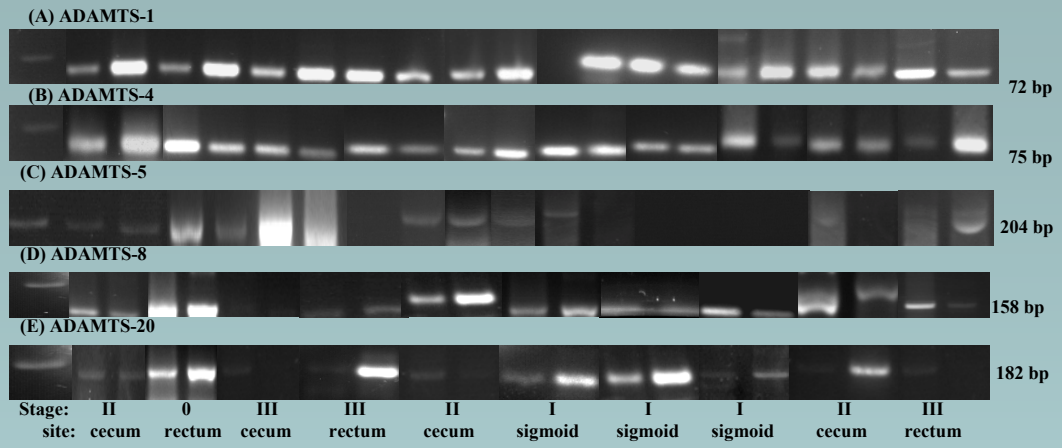




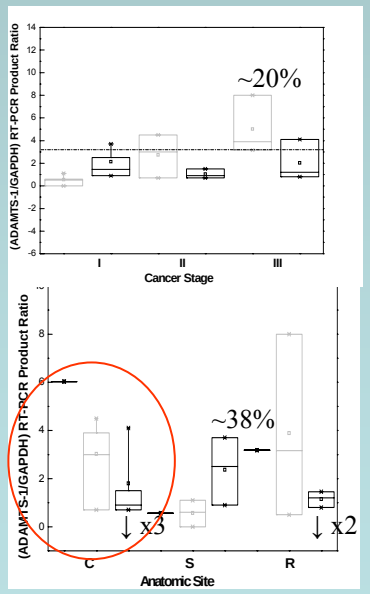


Aggrecanases are variously expressed in laryngeal cancer

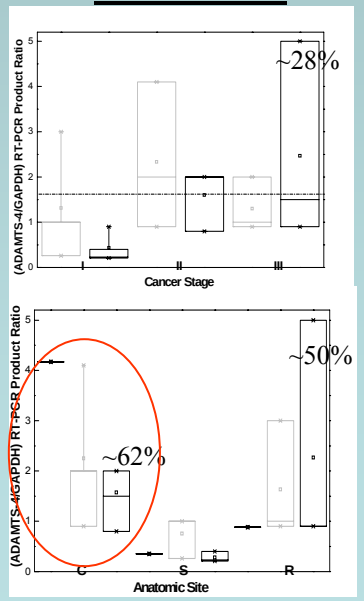
Aggrecanases in colorectal cancer



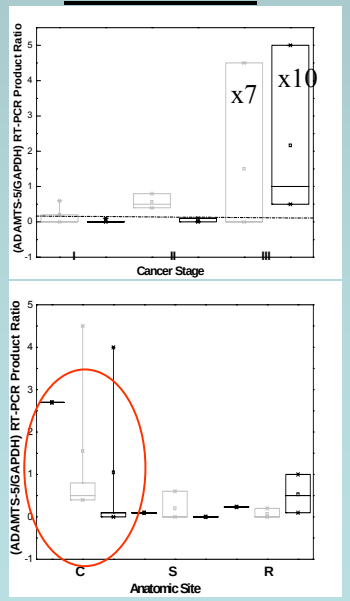
ADAMTS-1



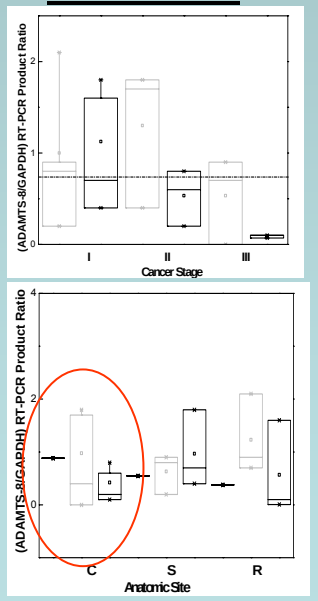
ADAMTS-4



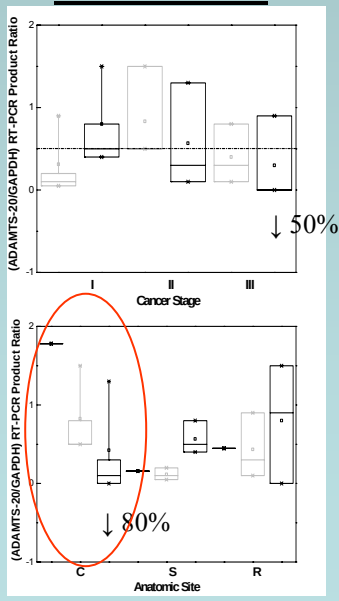
ADAMTS-5



ADAMTS-8



ADAMTS-20



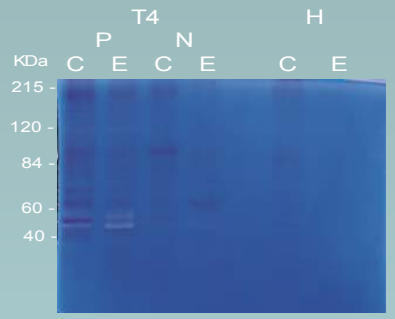
Aggrecanases are variously expressed in colorectal cancer

Extracellular degradation of GAGs

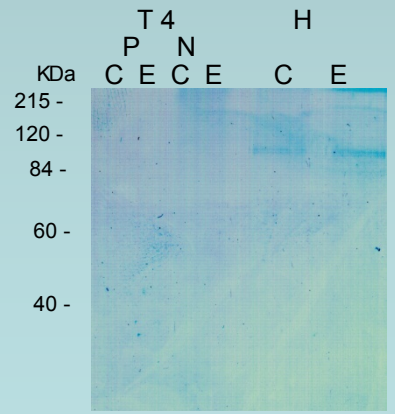
- HYAL1, HYAL2, HYAL3, PH-20
  acting on both HA and CS/DS

Hyaluronidases in laryngeal cancer

Hyaluronidases are key enzymes in cancer
by producing angiogenic sized hyaluronan

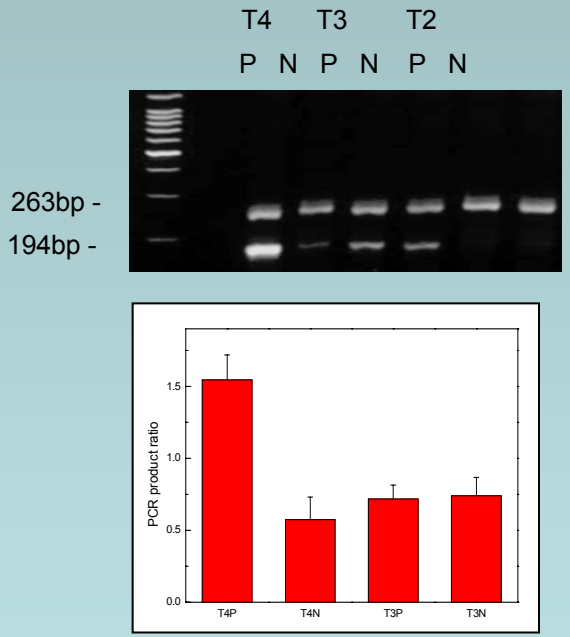


A double band of 45 and 55 kDa with hyaluronolytic activity was present only in cancer

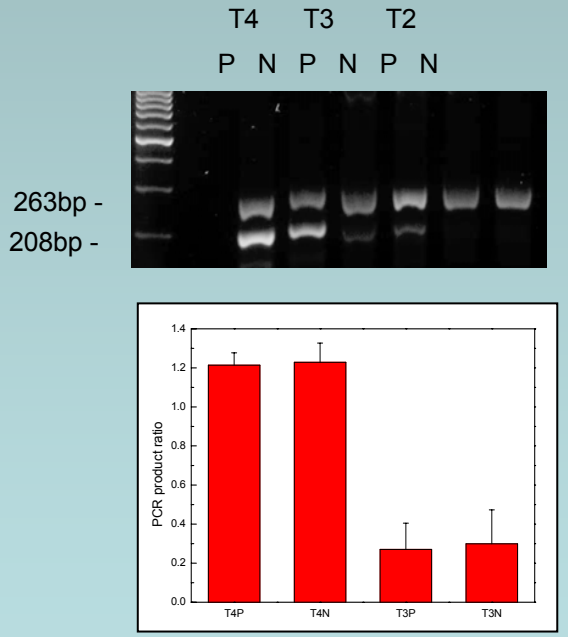


Hyaluronidase's inhibitors were detected only in healthy laryngeal tissues

Hyal-1



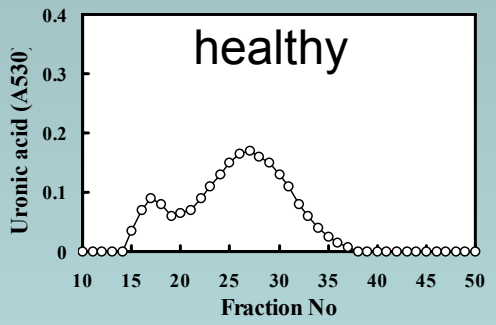
PH-20

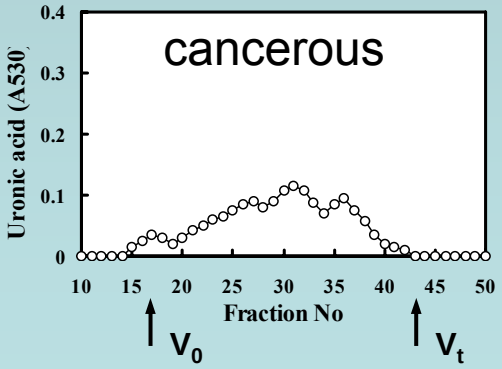


Hyaluronidases increased with tumor stage, more specifically PH-20 than Hyal-1

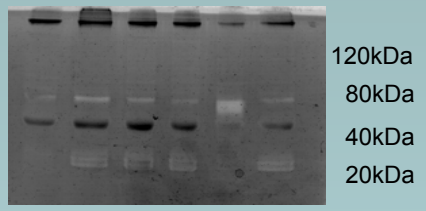
Hyaluronidases in colorectal cancer

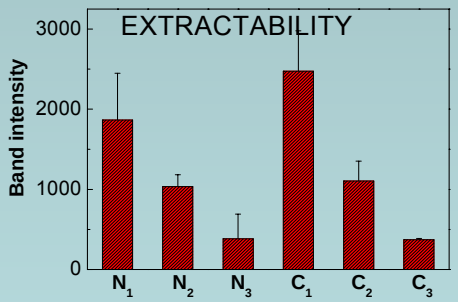
- **HA size on Sepharose CL-2B**

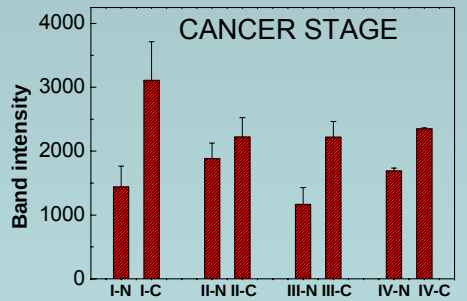


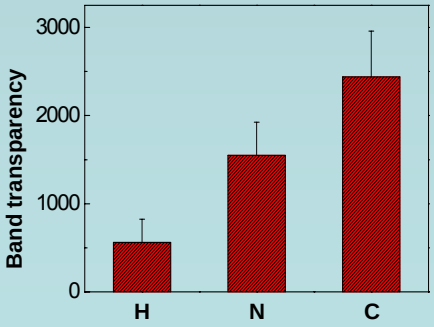


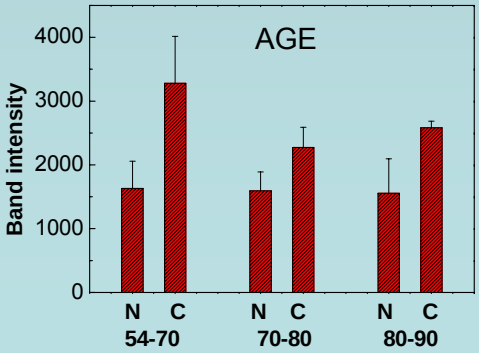
Enzymes were extracted from the tissue sequentially with PBS, 4M GdnHCl and 4M GdnHCl - 1% Triton X-100



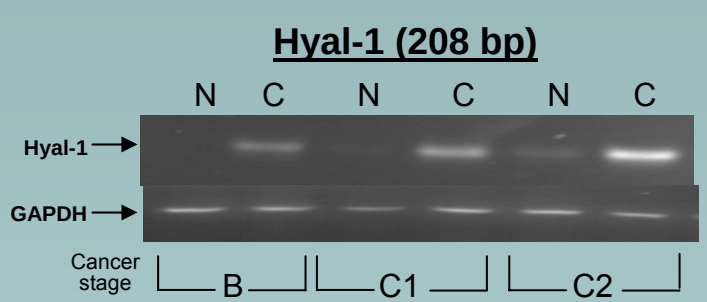


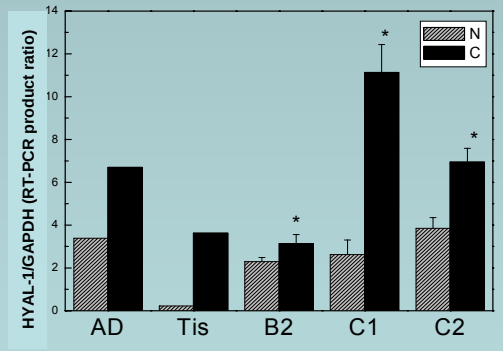


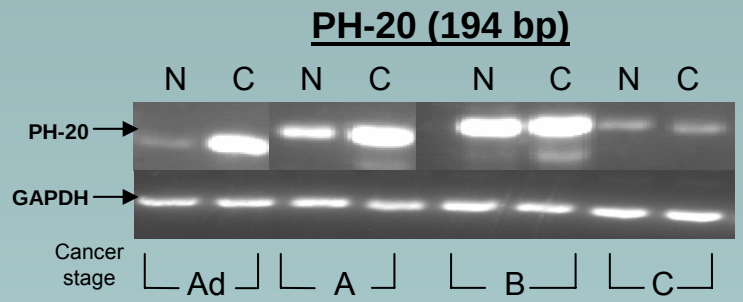


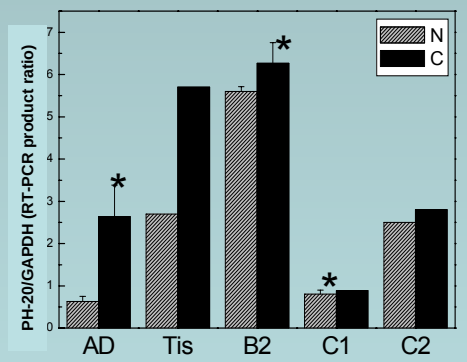


Hyaluronidases in colorectal cancer

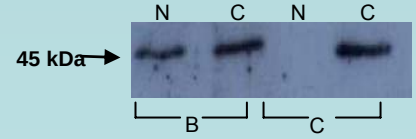




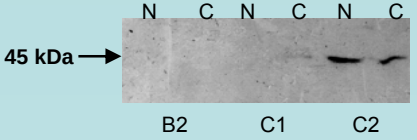




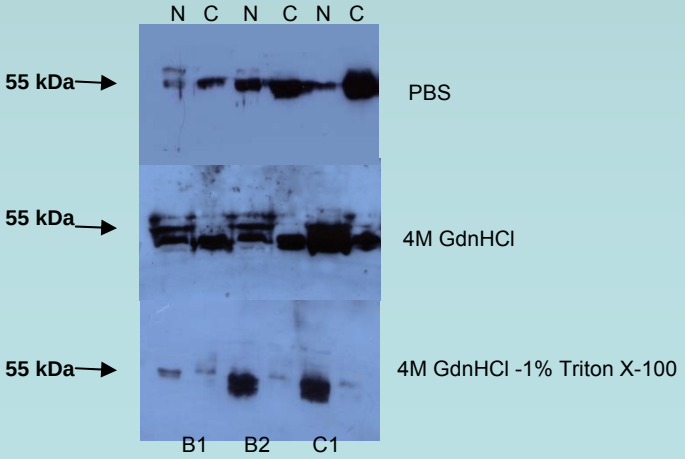
Hyal-1 (72 kDa)
PBS extract



Hyal-2 (45 kDa)
PBS extract



Hyal-3 (45 kDa)
4M GdnHCl -1% Triton extract



CONCLUSIONS

- Expression of enzymes implicated in PGs/GAGs metabolism are highly altered but in a different way in the various types of cancer
- This suggests
  - deep understanding of the mechanisms involved
  - manipulation of enzymes expression
  - synthesis of new and specific inhibitors or regulators

contributors

- Department of Chemistry
  - Prof. N. Papageorgakopoulou
  - Dr. I. E. Triantaphyllidou
  - Dr. S. S. Skandalis
  - Dr. T. Christopoulos
  - Dr. M. Stylianou
  - Dr. E. Bouga
  - Dr. D. Kalathas
  - I. Tsouros
  - E. Filou
  - V. Takouli
  - C. Kolliopoulos
  - E. Tserbini
  - A. Stathakopoulou
  - Prof. A. D. Theocharis
  - Prof. A. J. Aletras
  - Prof. N. K. Karamanos
- Department of Medicine
  - Prof. D. A. Theocharis
- Department of E.N.T., University Hospital
  - Prof. P. Goumas
  - Prof. T. Papadas
  - Prof. N. Mastronikolis
  - G. Tsiropoulos, MD
- Department of Surgery, University Hospital
  - Prof. M. Stavropoulos
  - D. Bounias, MD
  - D. Kyriakopoulou, MD


Thank you!