XVI Congreso Chileno de Hematología
La Serena, Septiembre, 2008

A NOVEL FAMILY OF MITOCHONDRIAL RNAs: TARGET FOR DIAGNOSTIC AND THERAPY OF CANCER.

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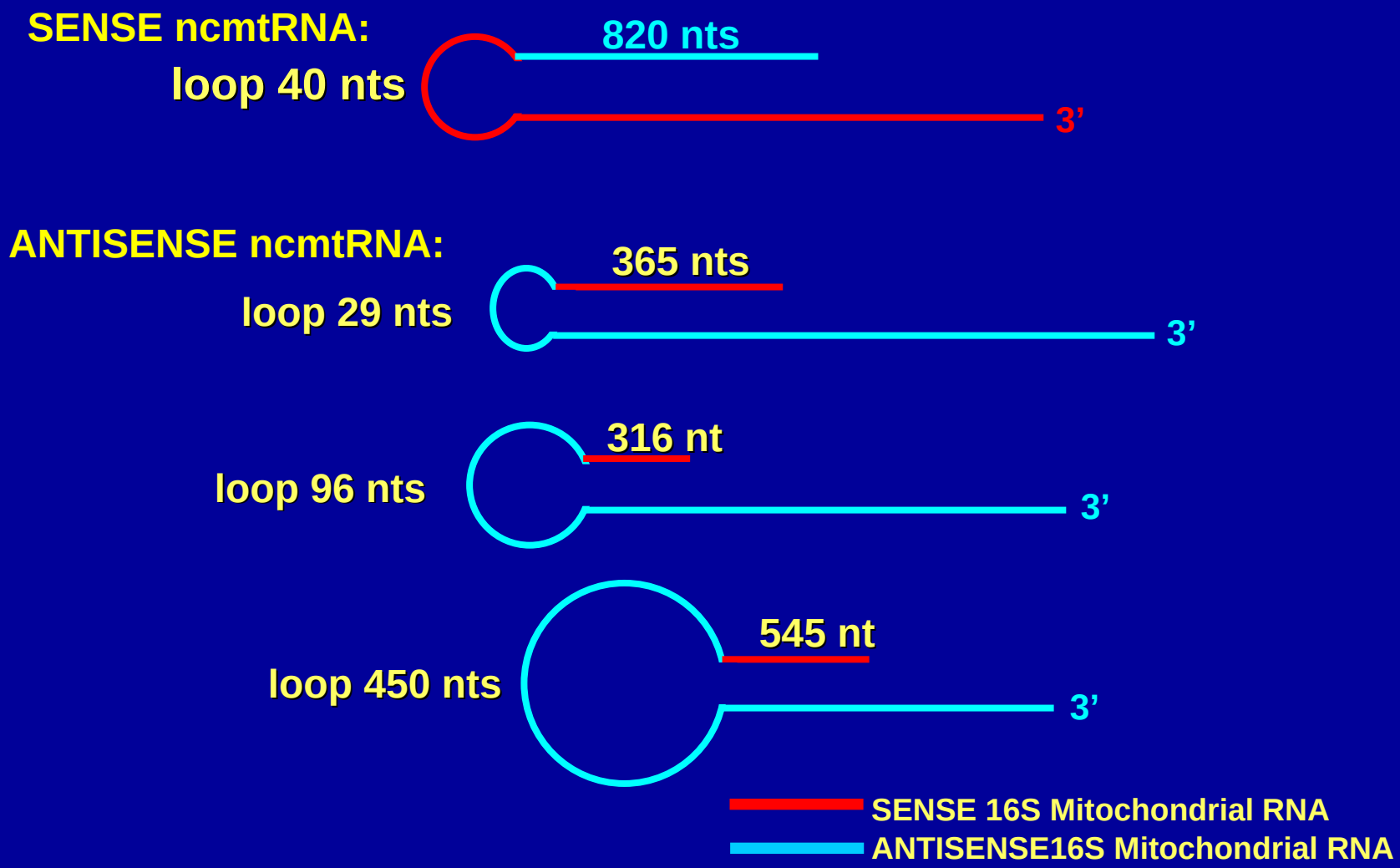
ANDES BioSciences SA

FCPV
FUNDACION
CIENCIA PARA LA
VIDA

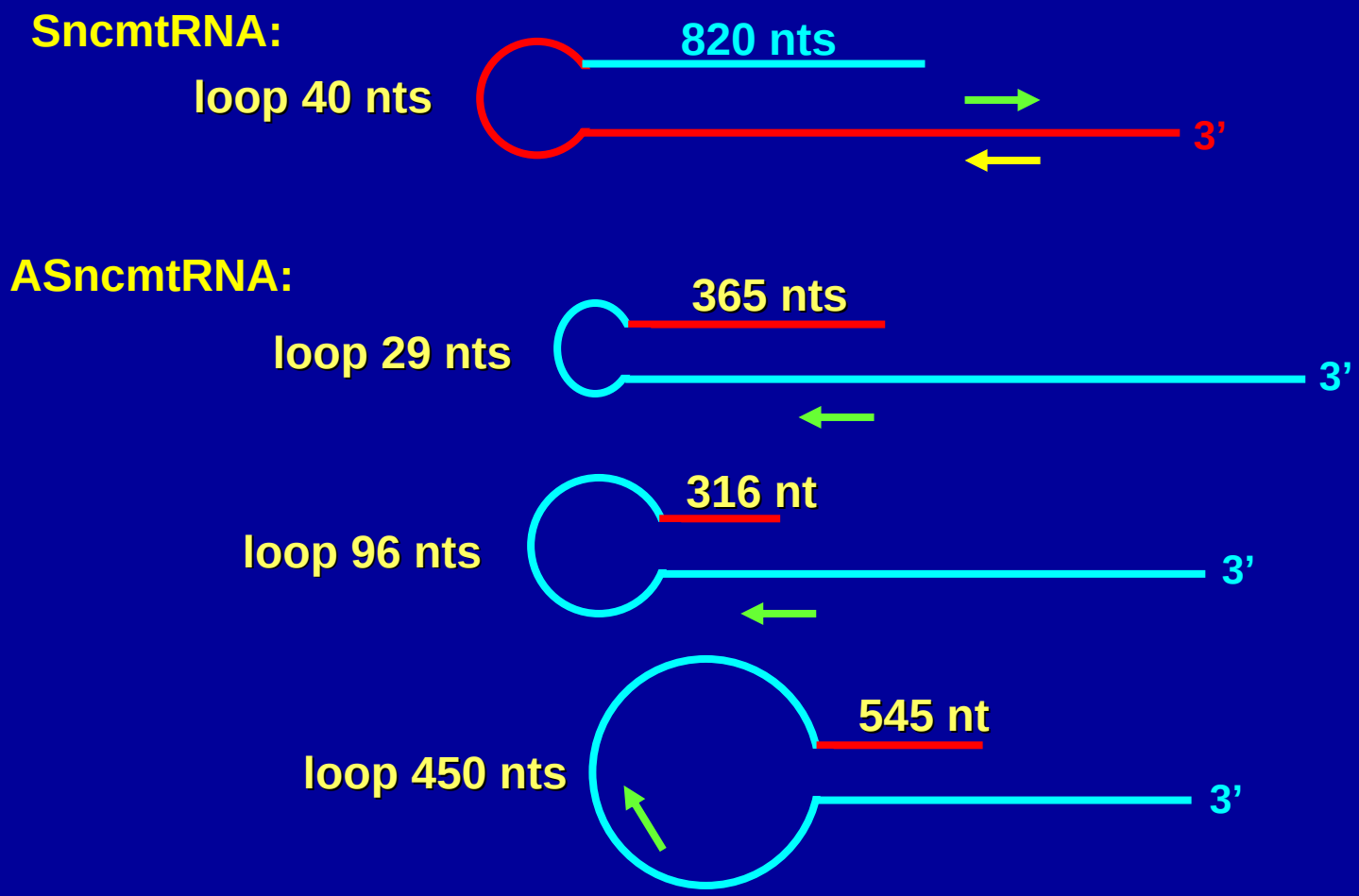
UNIVERSIDAD ANDRES BELLO

Grupo Bios SA

STRUCTURES OF THE HUMAN NON-CODING MITOCHONDRIAL RNAs (ncmtRNAs).

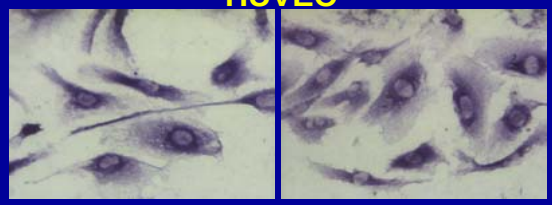


EXPRESSION OF THE SENSE AND ANTISENSE ncmtRNAs



EXPRESSION OF THE SncmtRNA AND THE ASncmtRNAs IN NORMAL PROLIFERATING CELLS

HUVEC

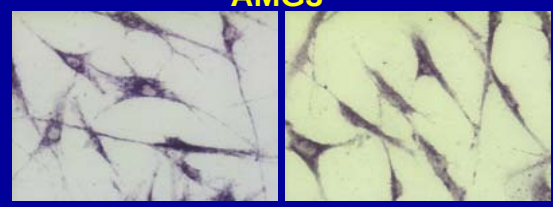


SncmtRNA ASncmtRNA

HFK cells

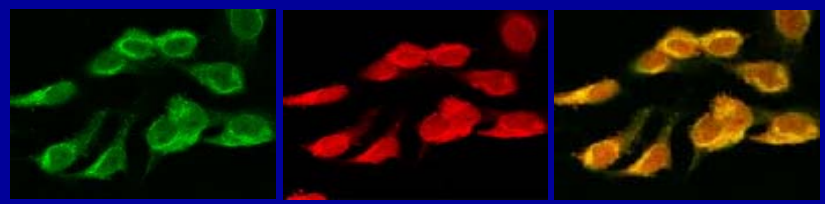
SncmtRNA ASncmtRNA

AMG3

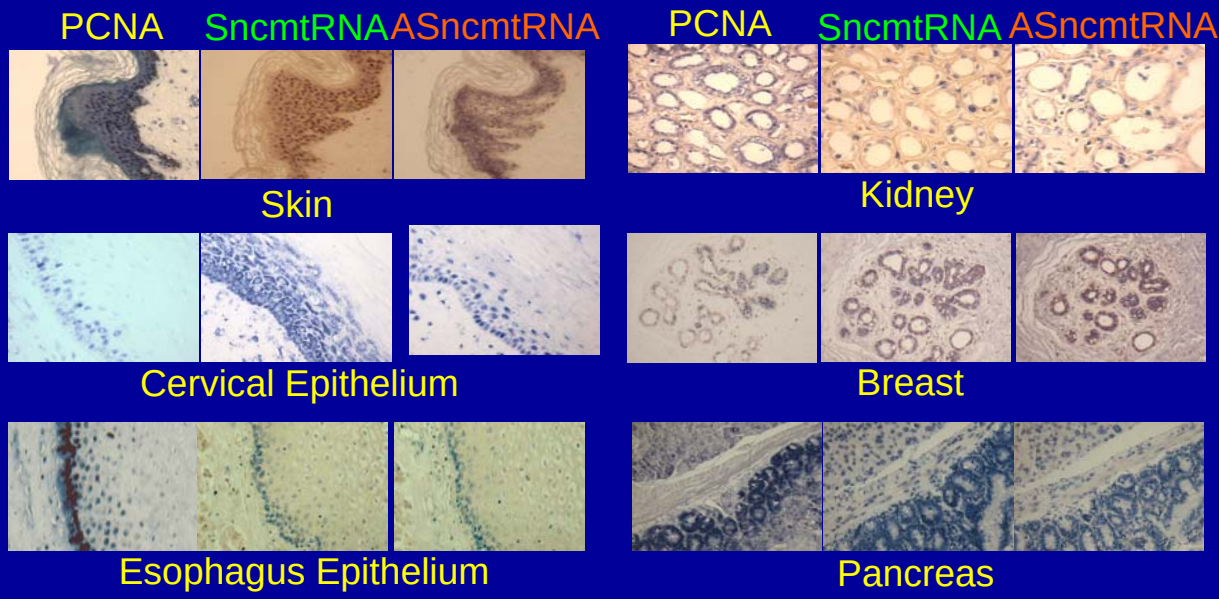


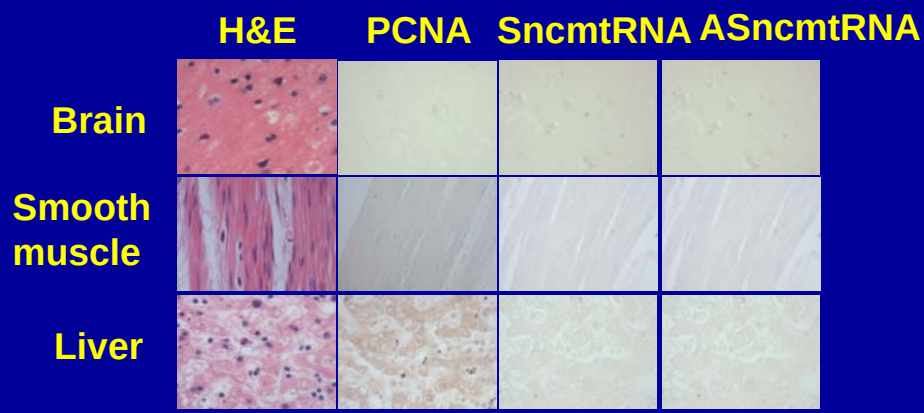
SncmtRNA ASncmtRNA

SncmtRNA ASncmtRNA Merger



EXPRESSION OF THE SncmtRNA AND THE ASncmtRNAs IN NORMAL PROLIFERATING TISSUES

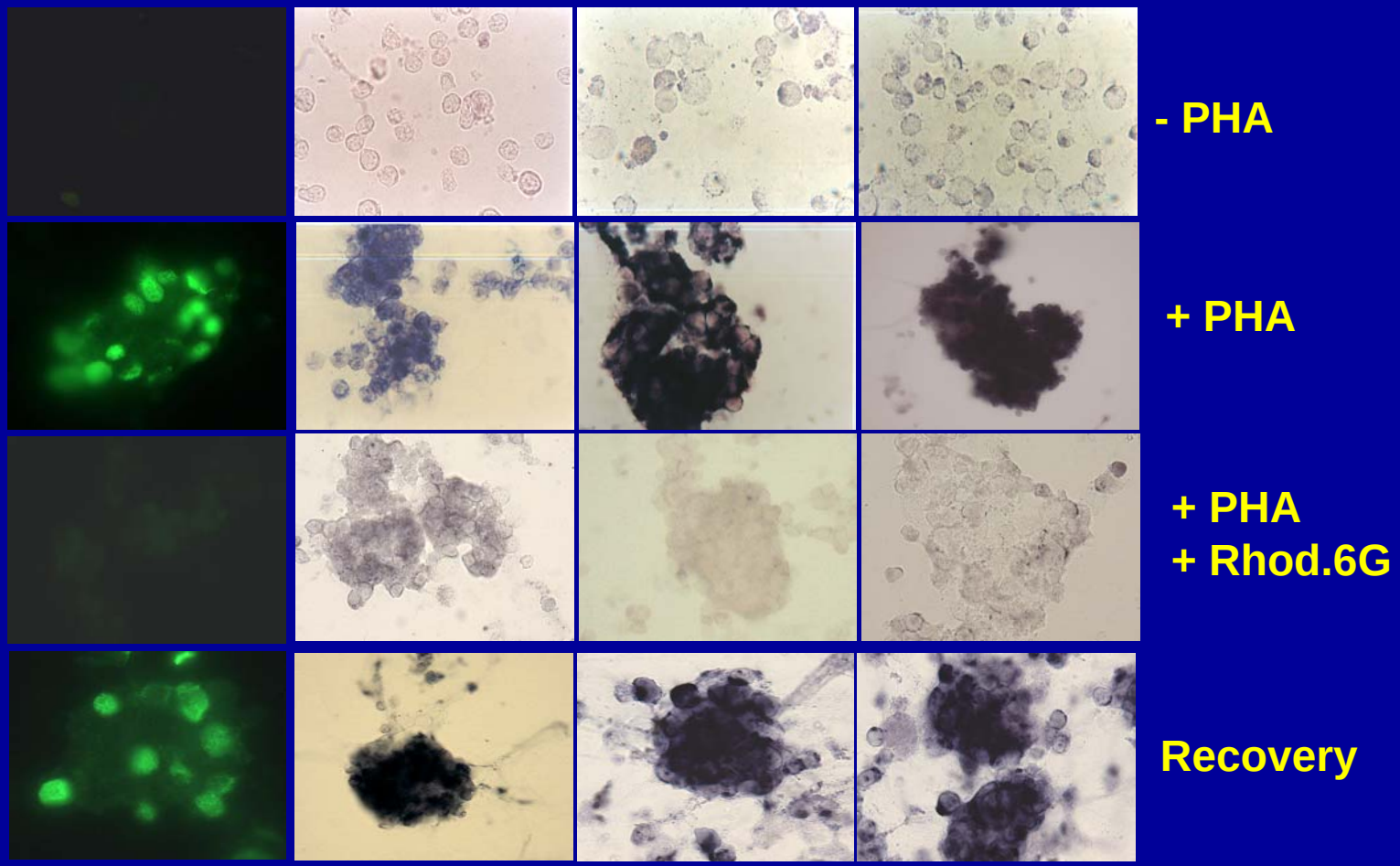




ESPRESSION OF THE SncmtRNA AND THE ASncmtRNA IN DIFFERENT NORMAL PROLIFERATING TISSUES.

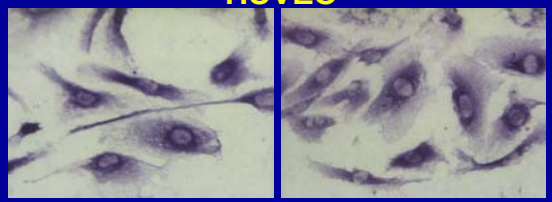
Tissue	No Cases	SncmtRNA	ASncmtRNAs
Normal skin	14	Positive	Positive
Cervical epithelium	22	Positive	Positive
Sebaceous gland	9	Positive	Positive
Esophagus epithelim	4	Positive	Positive
Kidney	6	Positive	Positive
Breast	8	Positive	Positive
Pancreas	5	Positive	Positive
Spleen	6	Positive	Positive
TOTAL	74		

THE EXPRESSION OF THE SncmtRNA AND ASncmtRNAs CAN BE INDUCED IN NON-DIVIDING HUMAN LYMPHOCYTES.

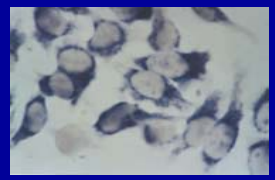
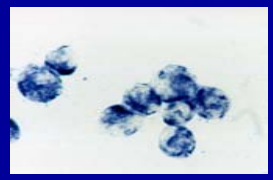
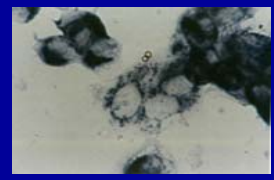
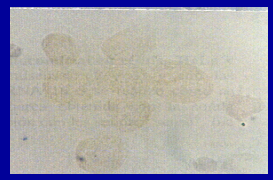
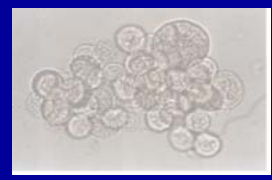


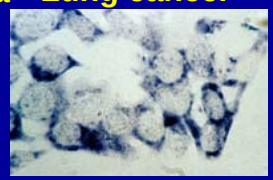
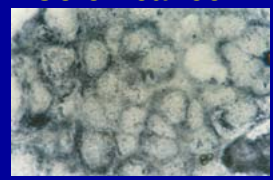
EXPRESSION OF THE SncmtRNA AND THE ASncmtRNAs IN TUMOR CELL LINES

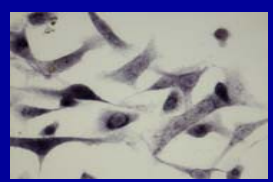
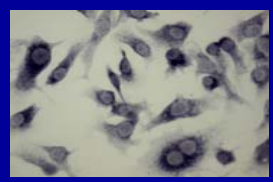
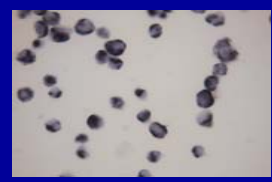
HUVEC



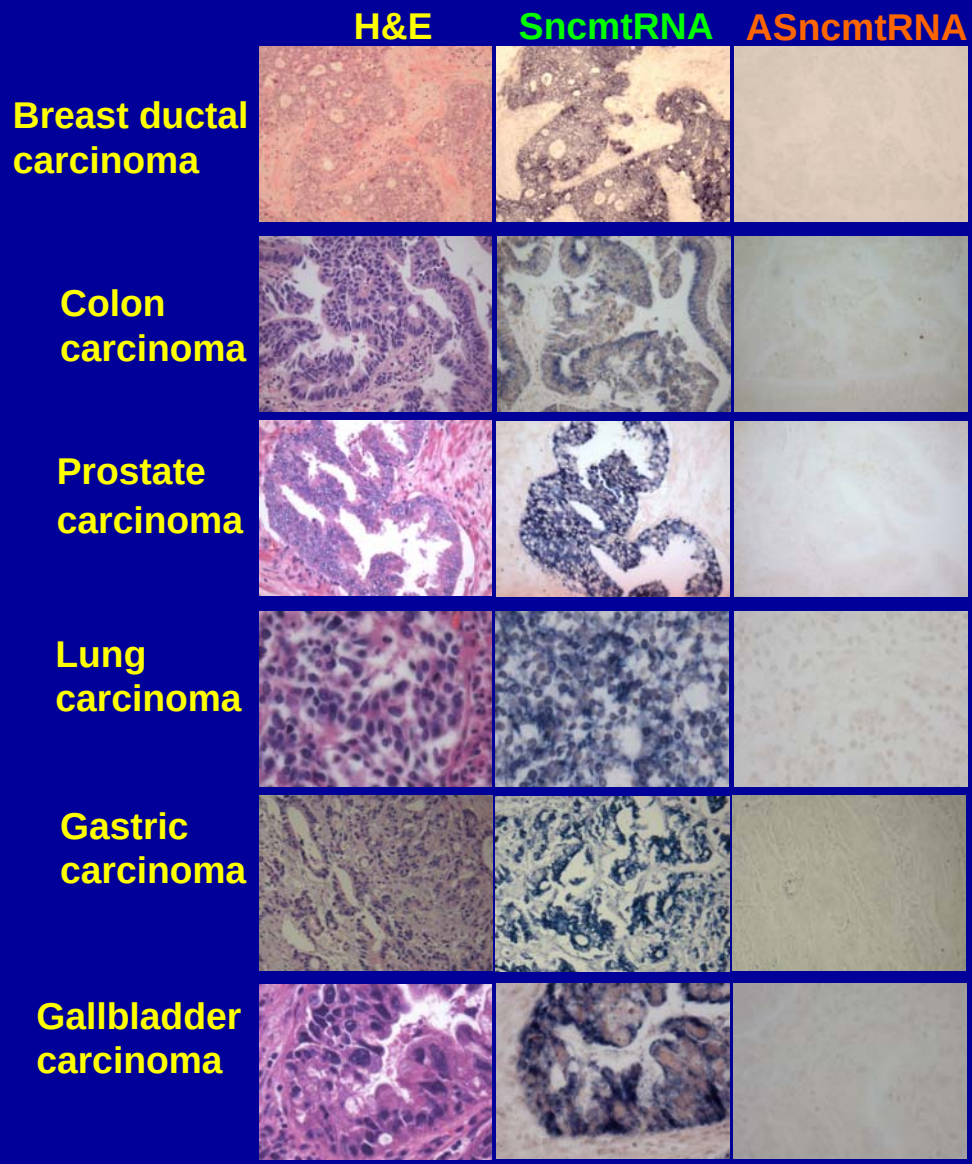
SncmtRNA ASncmtRNA

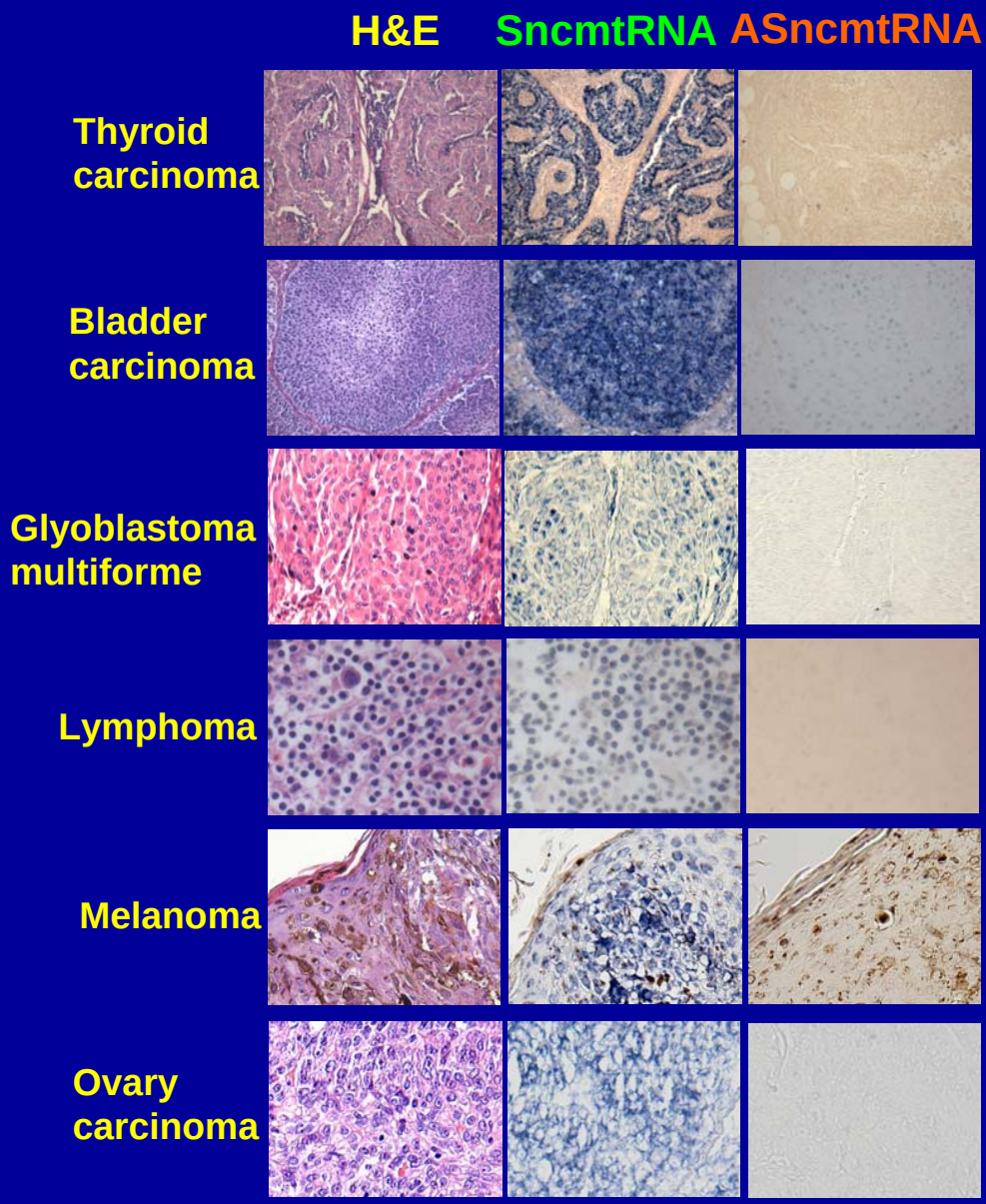
	HeLa Cervical carcinoma	HL-60 Leukemia	MCF/7 Breast Carcinoma
SncmtRNA			
ASncmtRNA			

	DU145 Prostate carcinoma	H441 Lung cancer	Caco-2 Colon cancer
SncmtRNA			
ASncmtRNA			

	MDA-MB-231	OVCAR3	Jurkat
SncmtRNA			
ASncmtRNA			

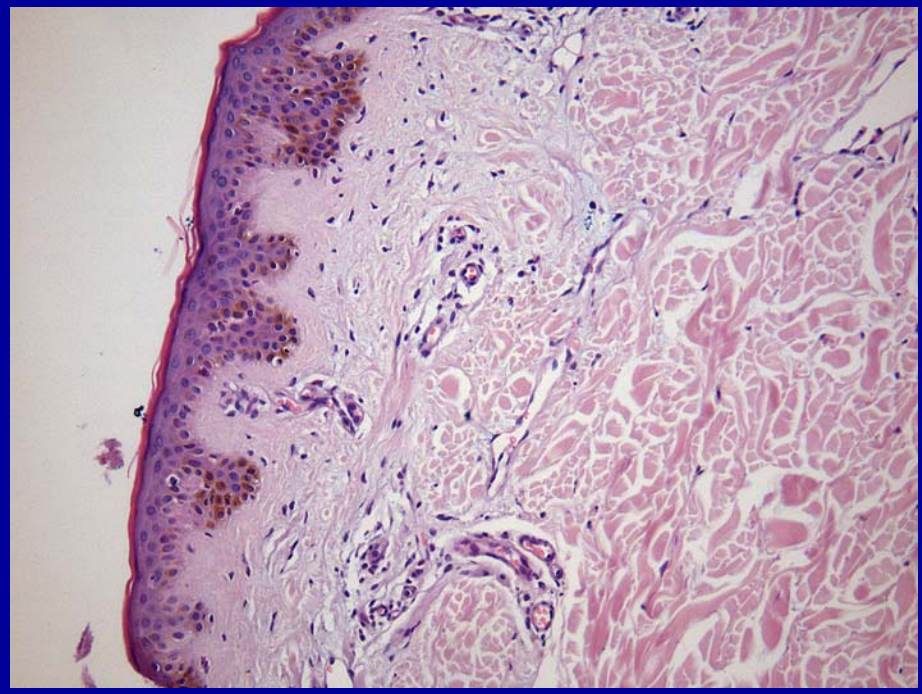
EXPRESSION OF THE SncmtRNA AND THE ASncmtRNAs IN TUMORS



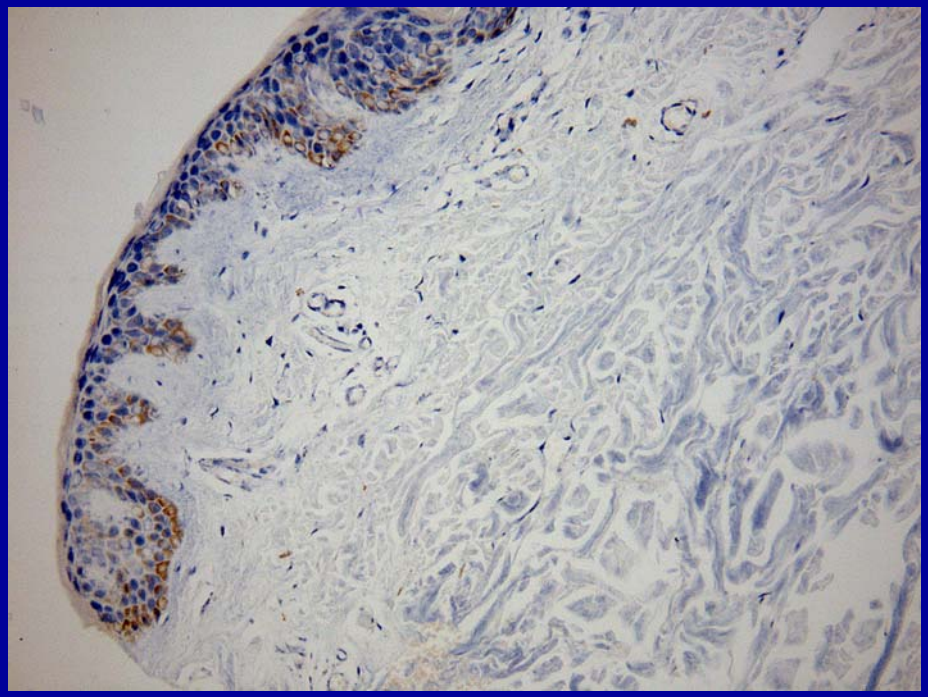

H&E
SncmtRNA
ASncmtRNA
Thyroid carcinoma
Bladder carcinoma
Glyoblastoma multiforme
Lymphoma
Melanoma
Ovary carcinoma

NORMAL SKIN

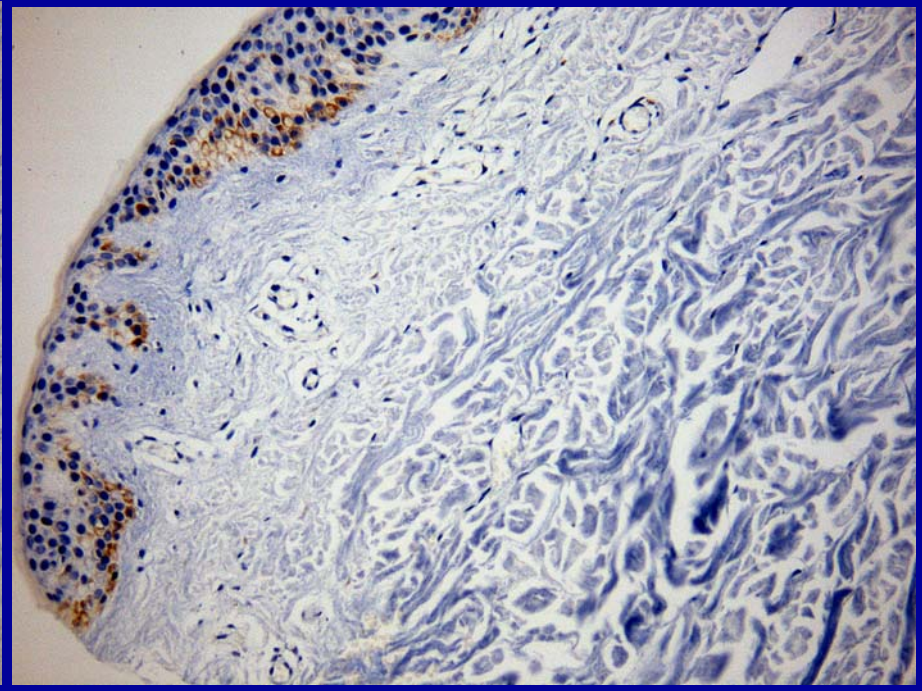
H & E



SncmtRNA

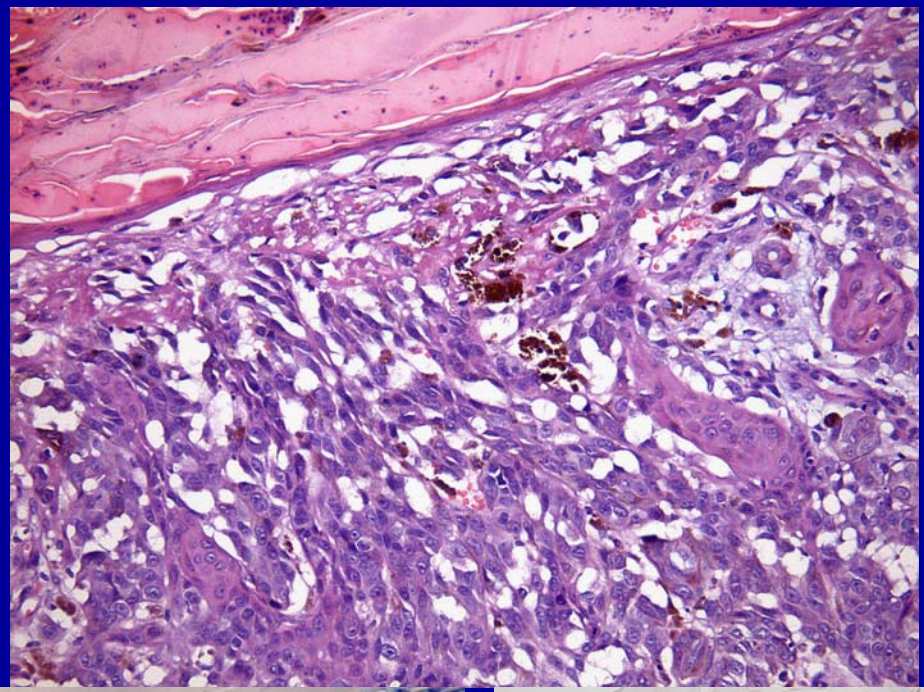


ASncmtRNAs

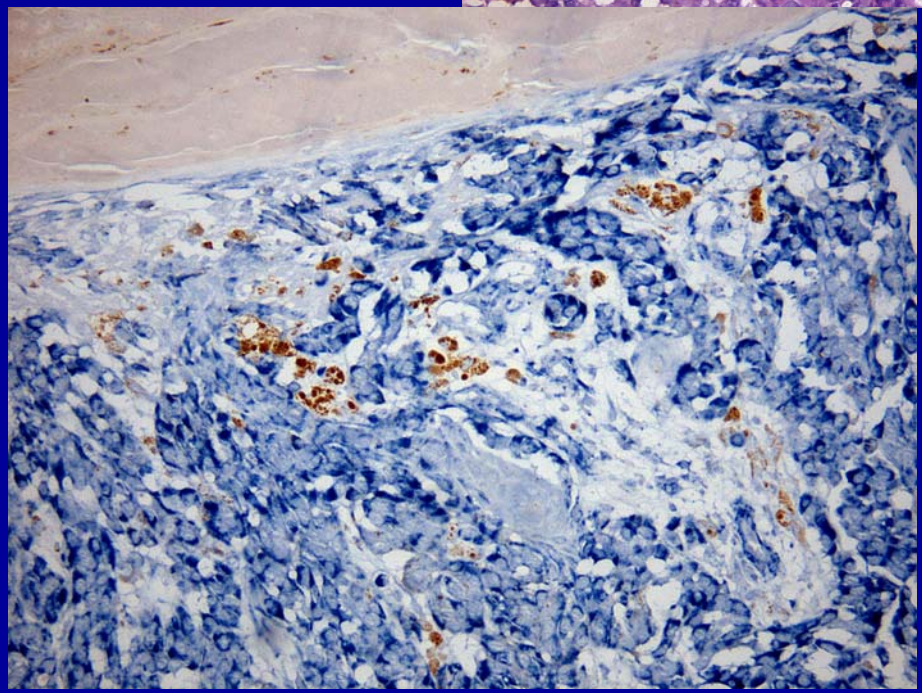


MELANOMA

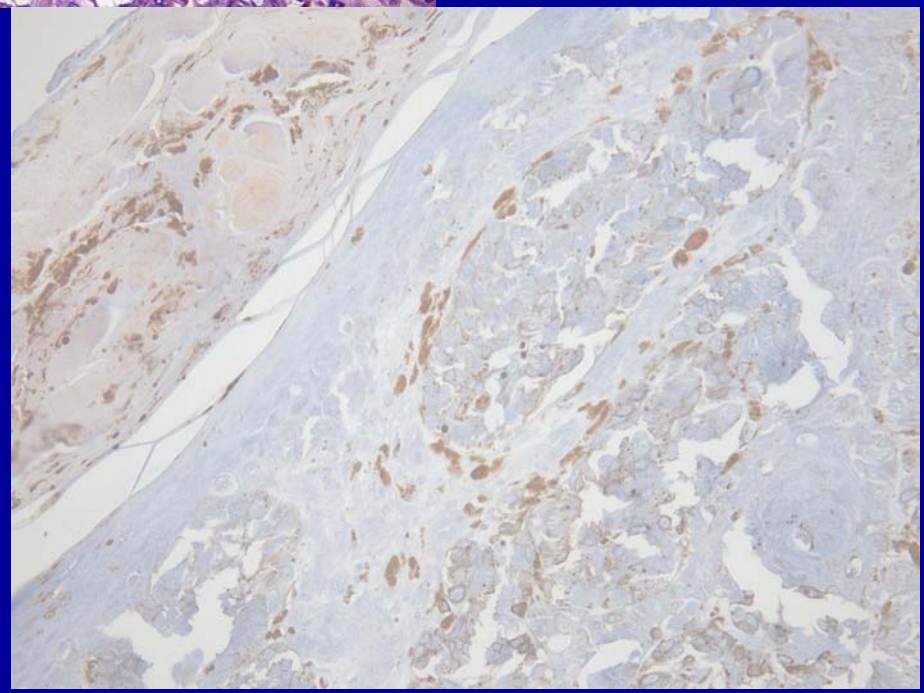
H & E



SncmtRNA



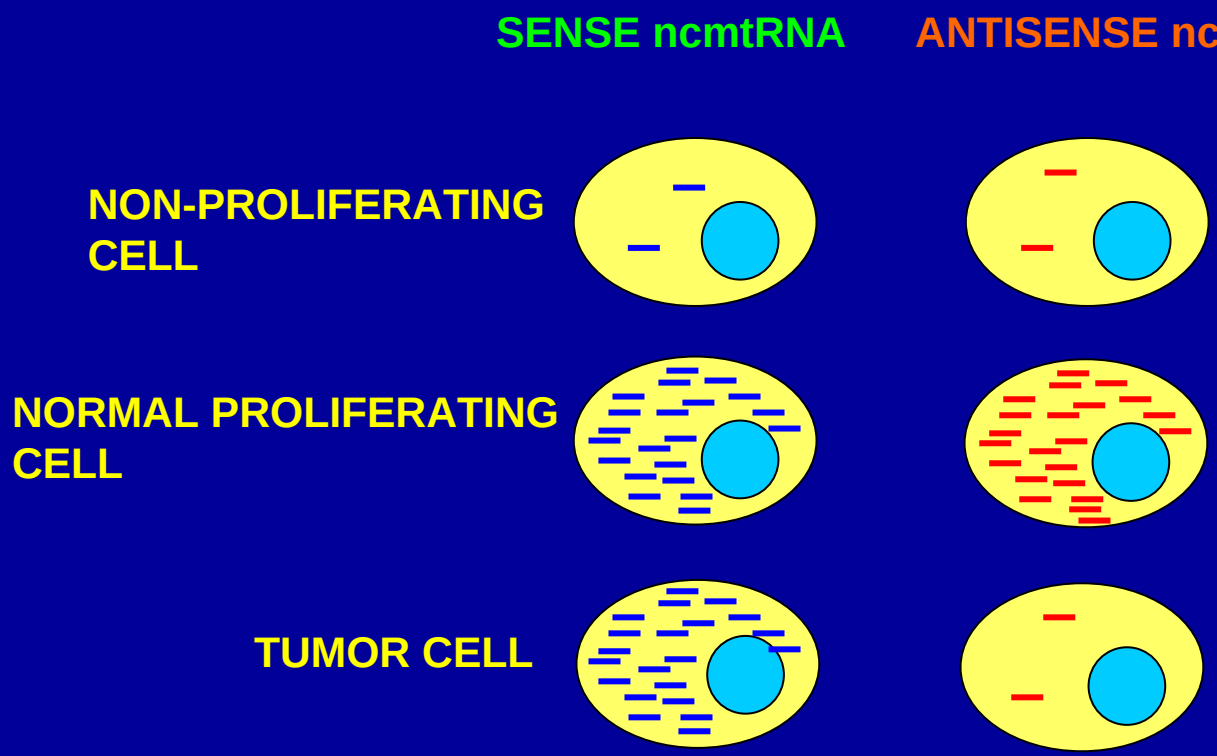
ASncmtRNAs



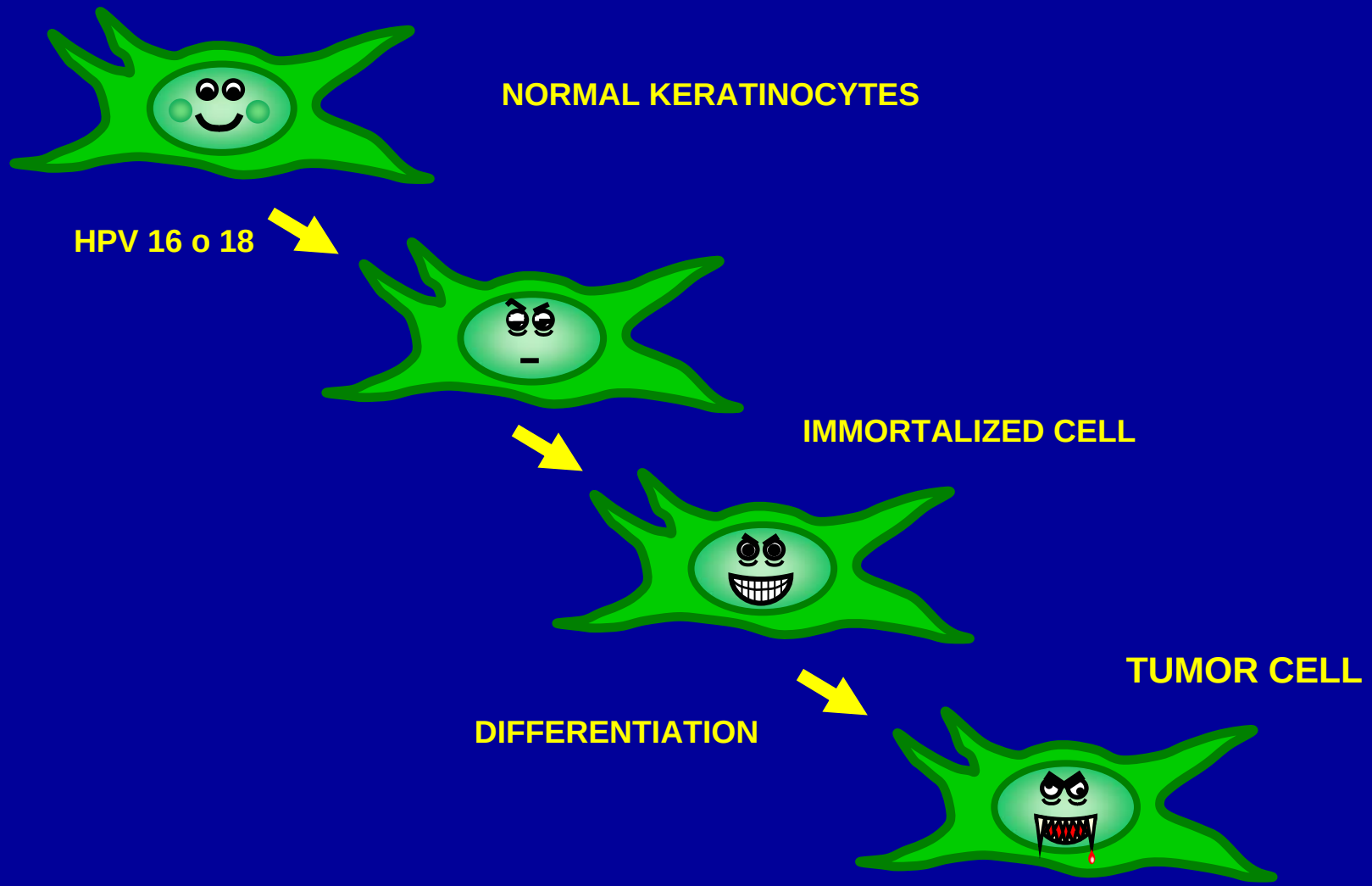
ESPRESSION OF THE SncmtRNA AND THE ASncmtRNA IN DIFFERENT TYPES OF CANCER.

Tissue	No Cases	SncmtRNA	ASncmtRNAs
Breast carcinoma	64	Positive	Negative
Prostate carcinoma	27	Positive	Negative
Lung Carcinoma	14	Positive	Negative
Colon	17	Positive	Negative
Galldbladder	11	Positive	Negative
Stomach carcinoma	22	Positive	Negative
Uterine Cervix*	27	Positive	Negative
Brain tumors**	12	Positive	Negative
Kidney carcinoma	9	Positive	Negative
Hepatocarcinoma	5	Positive	Negative
Thyroid carcinoma	12	Positive	Negative
Ovary	8	Positive	Negative
Bladder	12	Positive	Negative
Melanomas	12	Positive	Negative
Lymphoma	6	Positive	Negative
Small intestine Carcinoma	3	Positive	Negative
TOTAL	261		

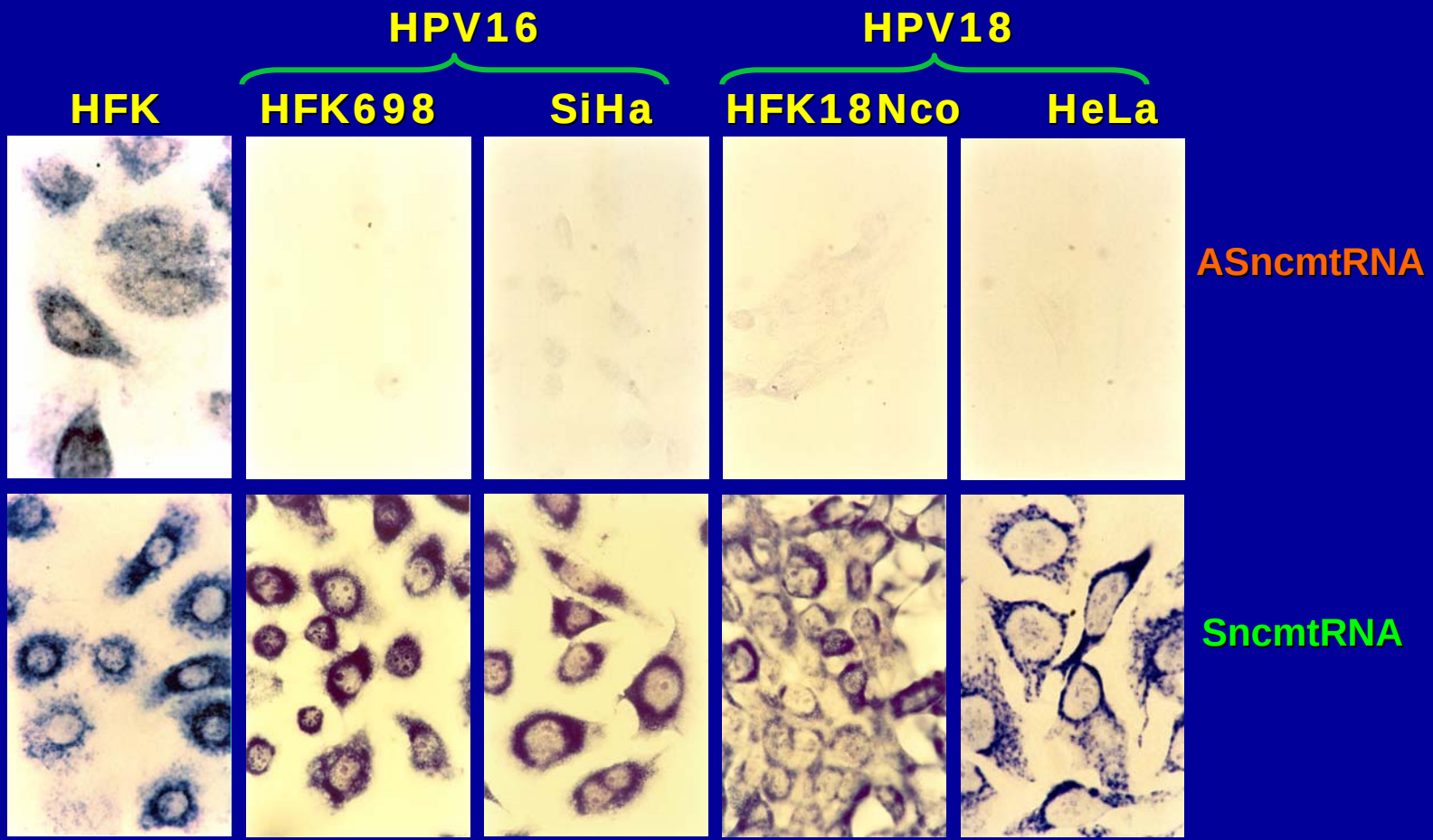
SUMMARY ON THE EXPRESSION OF THE ncmtRNAS IN HUMAN CELLS.



IMMORTALIZATION OF HUMAN KERATINOCYTES WITH PAPPILOMA VIRUS 16 OR 18



KERATINOCYTES IMMORTALIZED WITH HPV 16 or 18 INDUCE DOWN REGULATE THE ASncmtRNAs.



CONCLUSIONS:

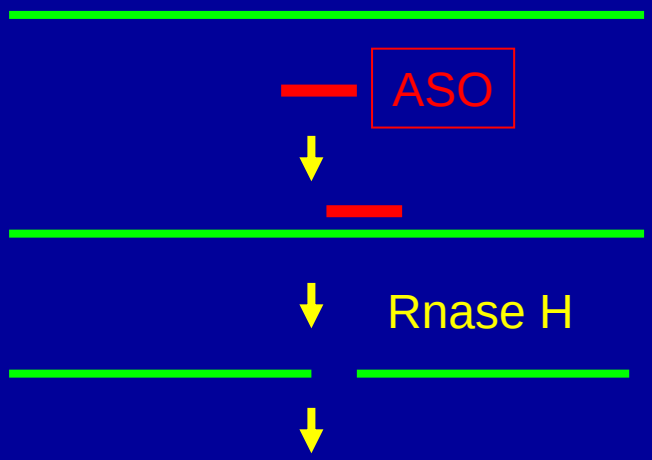
1. **THE SncmtRNA IS AN IMPORTANT FACTOR FOR CELL PROLIFERATION**

2. **THE ASncmtRNA IS DOWN-REGULATES IN CANCER CELLS**

3. **HYPOTHETICALY, THE ASncmtRNAs ARE NOVEL TUMOR SUPRESSORS MITOCHON-DRIALLY-ENCODED**

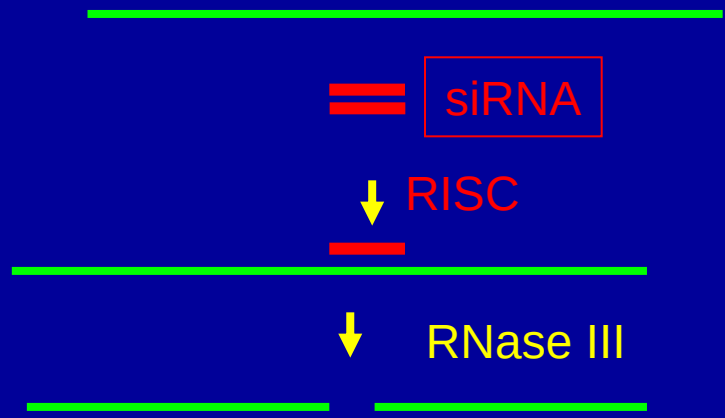
HOW TO STUDY THE FUNCTION OF THESE TRANSCRIPTS?
KNOCK-DOWN OF THE RNAs

ASO or ANTISENSE OLIGONUCLEOTIDES



NO SYNTHESIS OF PROTEIN

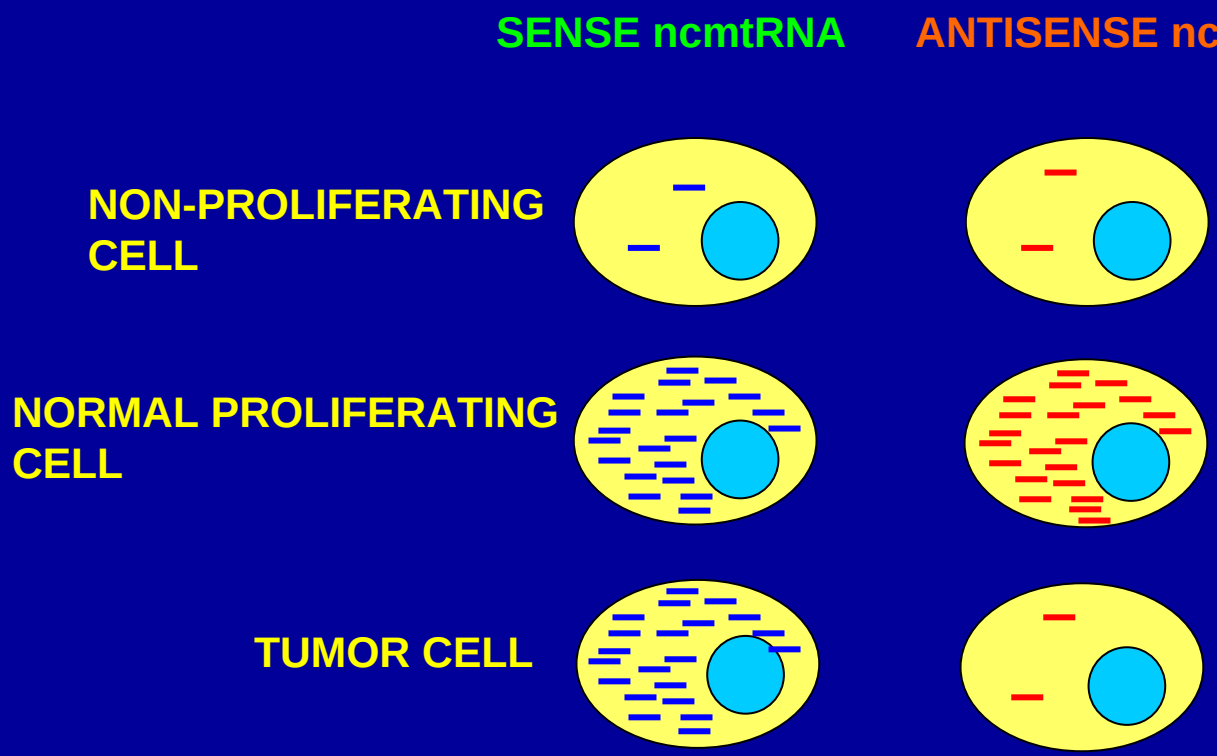
siRNAs or SMALL INTERFERING RNAs



NO SYNTHESIS OF PROTEIN

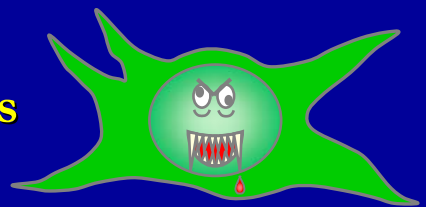
PHYSIOLOGICAL CHANGES OF THE CELL

SUMMARY ON THE EXPRESSION OF THE ncmtRNAS IN HUMAN CELLS.



THE ASncmtRNAs AS TARGET FOR CANCER THERAPY

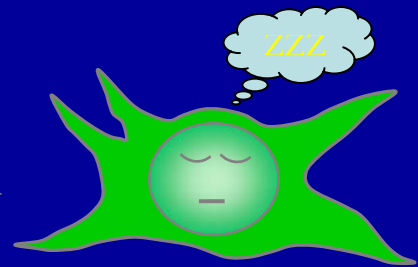
Hypothesis



Tumor Cells

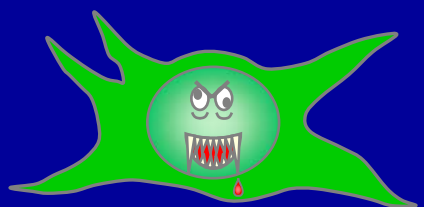
ASO against

ncmtRNAs



Quiescence

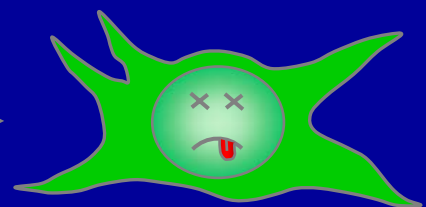
Results



Tumor Cells

ASO against

ncmtRNAs



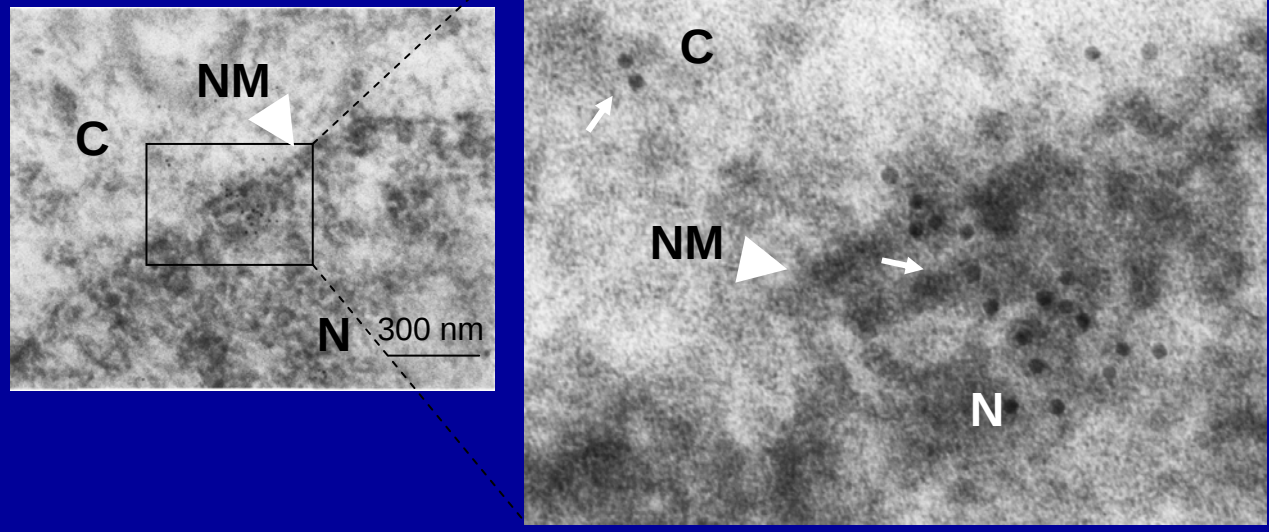
CELL DEATH

TWO MAIN QUESTIONS

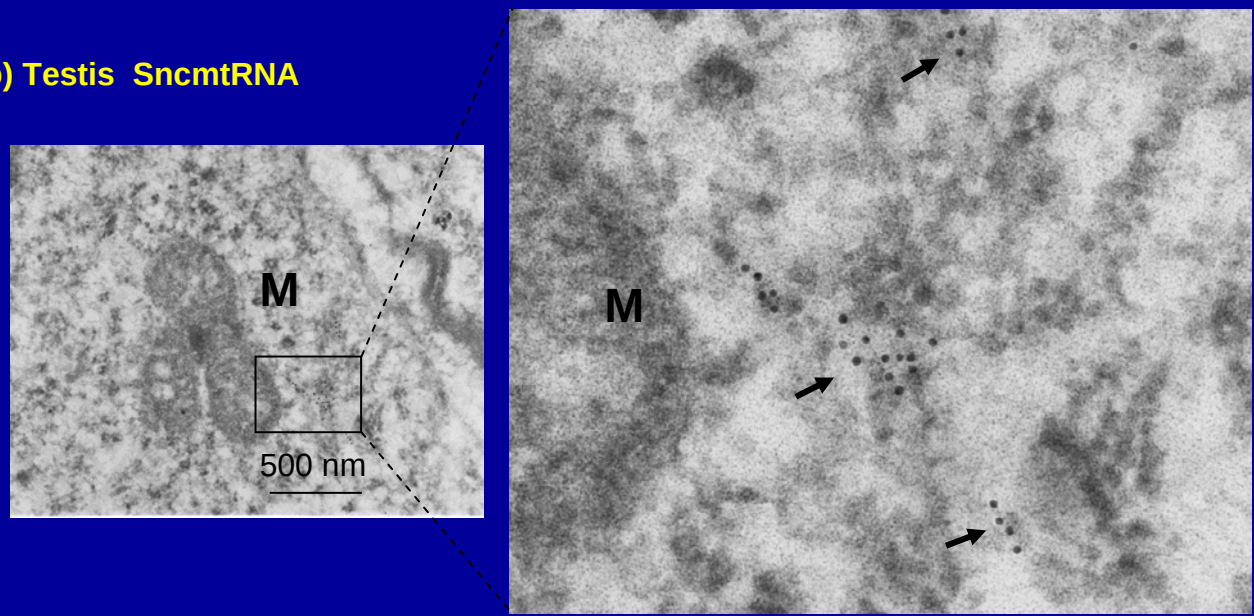
1. WHICH TYPE OF CELL DEATH?

2. KNOCK-DOWN OF MITOCHONDRIAL TRANSCRIPTS???

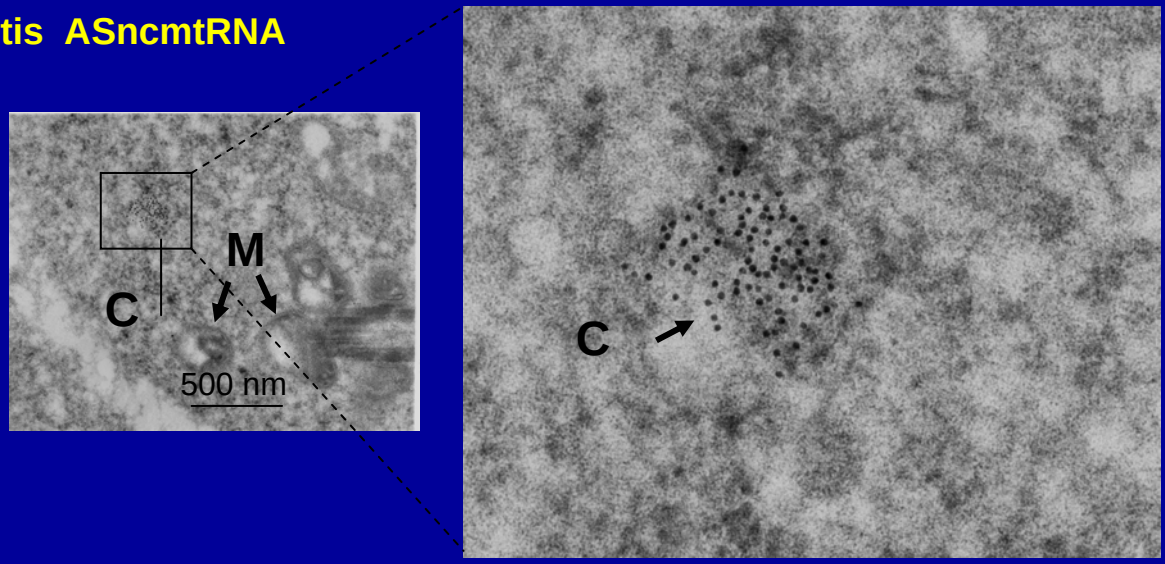
a) Testis SncmtRNA



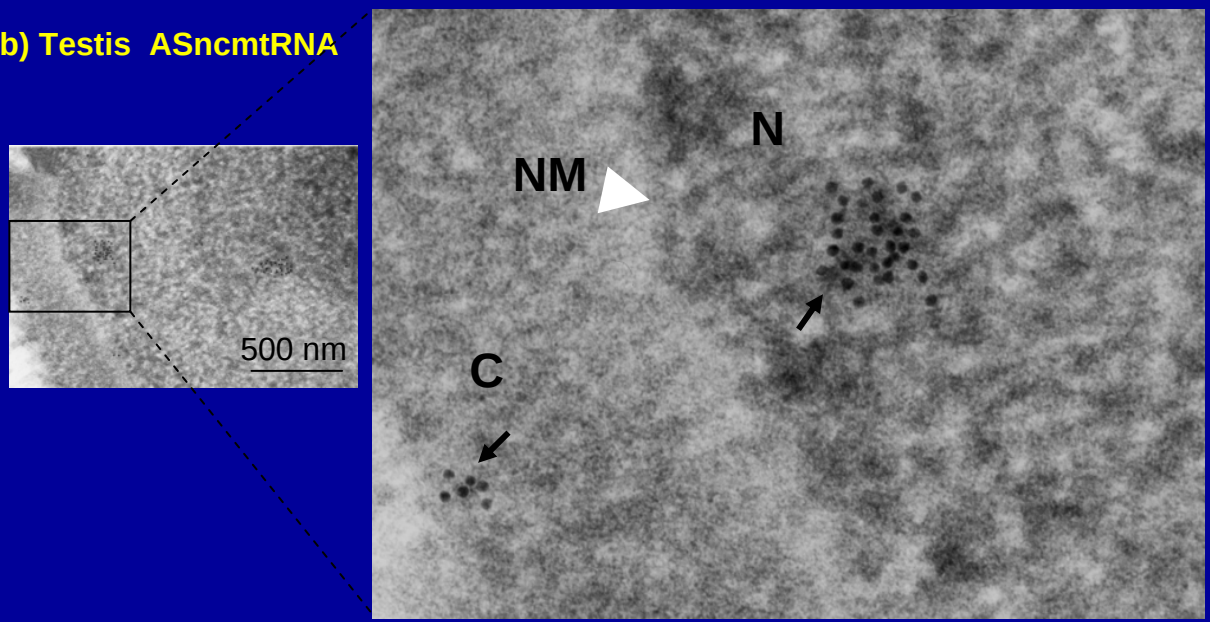
b) Testis SncmtRNA



a) Testis ASncmtRNA



b) Testis ASncmtRNA



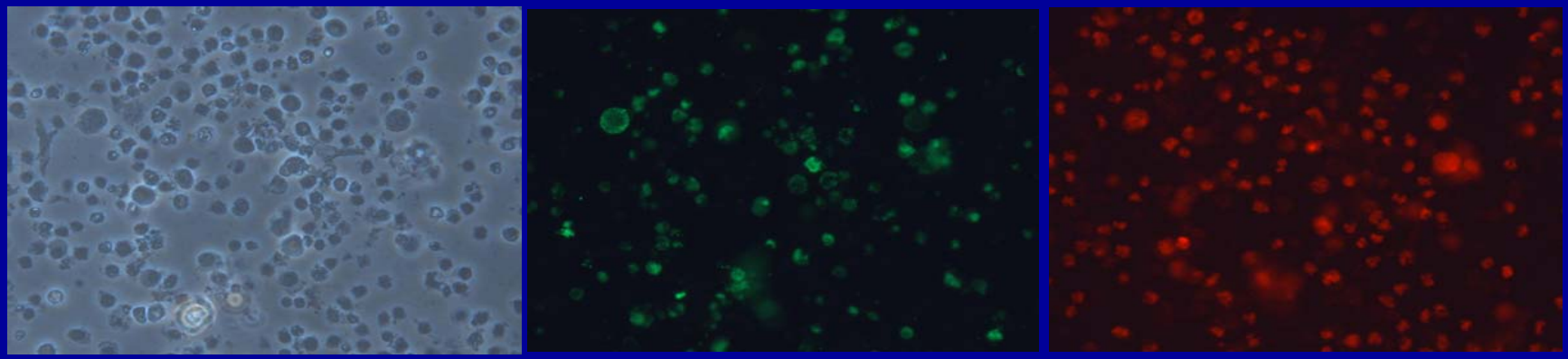
PROGRAMED CELL DEATH OR PCD

PCD TYPE 1 OR APOPTOSIS
- Translocation of phosphatidylserine
- Caspase activation
- DNA fragmentation
- Nuclear Fragmentation

PCD TYPE 2 OR AUTOPHAGY
- Autophasgic vacuoles (AV) formation
- Fusion of AV with lysosomes (Autophagolysomes)
- Processing of LC3

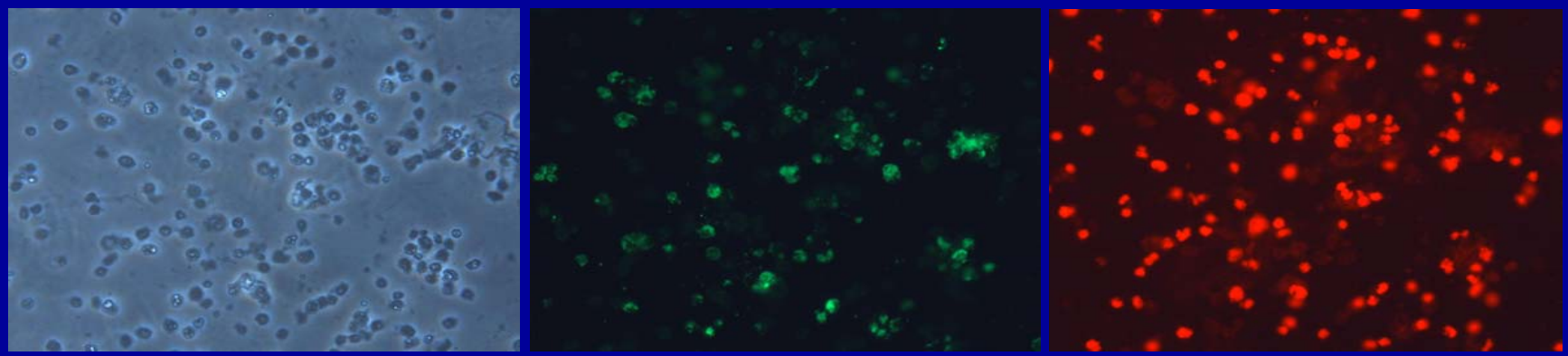
TRANSLOCATION OF PHOSPHATIDYLSERINE

PHASE | ANEXIN V | Propidium I



ASO FOR THE ASncmtRNAs 0.1 uM for 18 H

PHASE | ANEXIN V | Propidium I

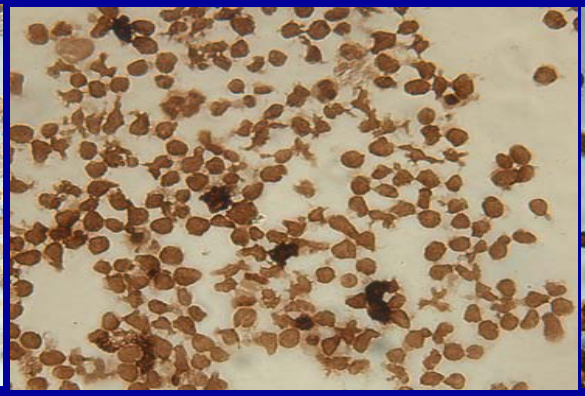


Staurosporin 4uM FOR 18 H

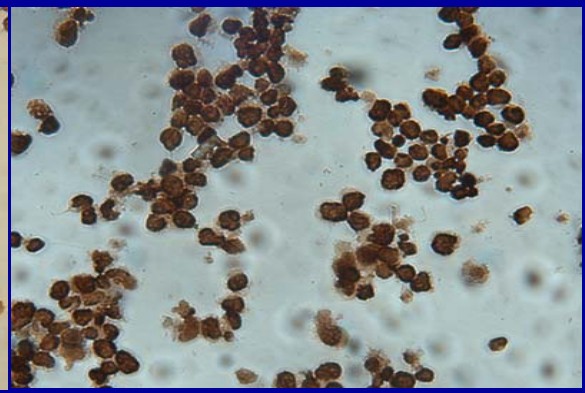
DNA FRAGMENTATION = TUNEL ASSAY

Control ASO

ASO x SncmtRNA

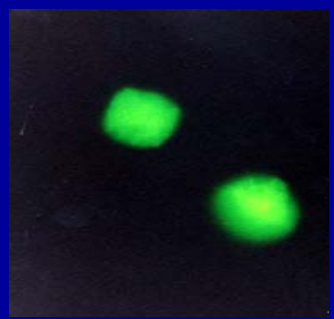


ASO x ASncmtRNA



CASPASE ACTIVATION = BINDING OF z-VADfmK-FITC

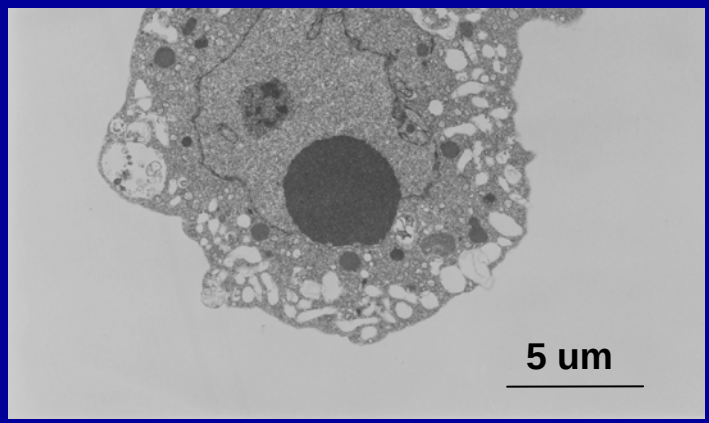
Control ASO

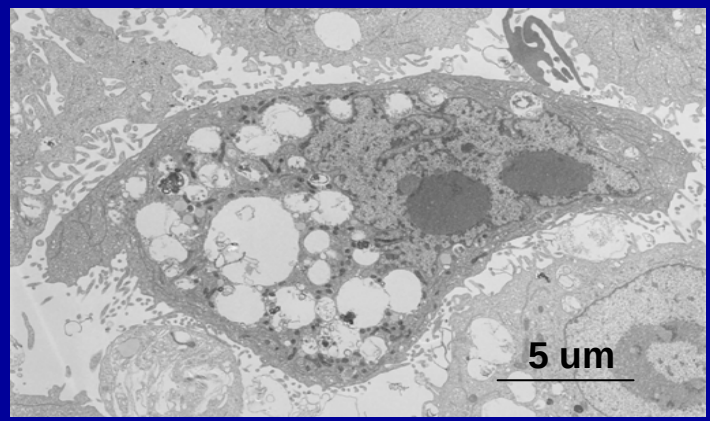


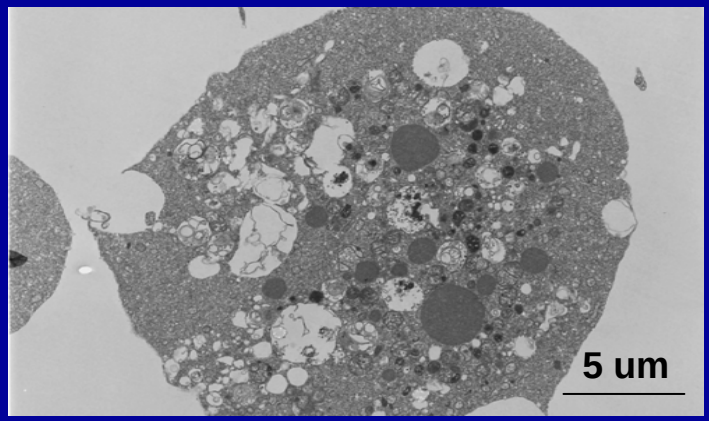
ASO x ASncmtRNA

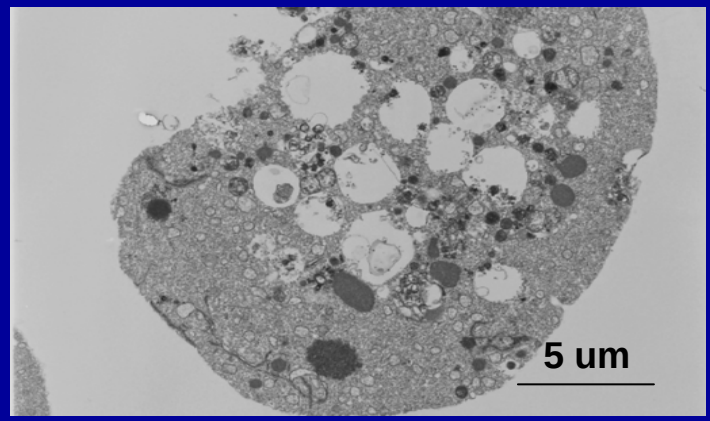
STAUROSPORIN

NUCLEAR FRAGMENTATION & CHROMATIN CONDENZATION



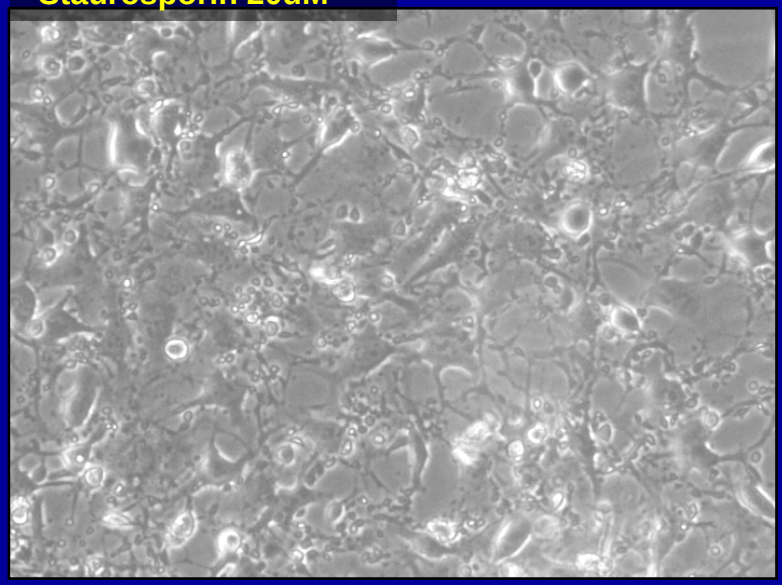




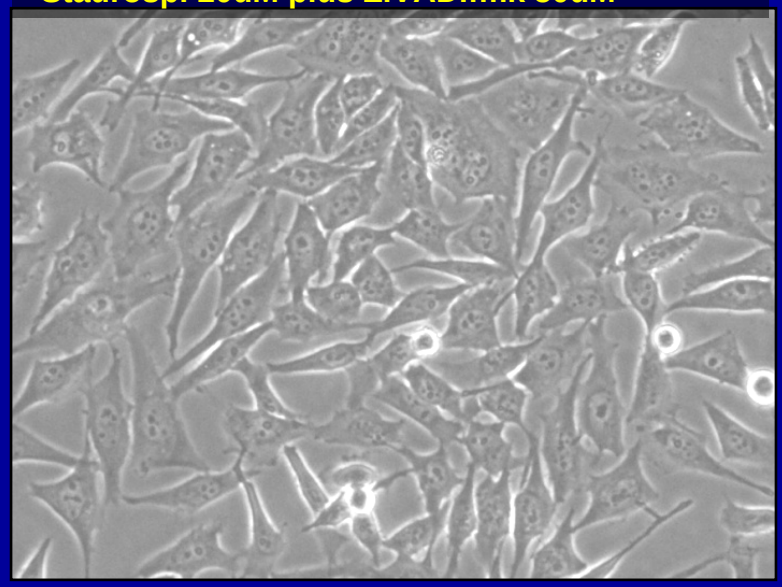


INHIBITION OF CASPASES & CELL DEATH

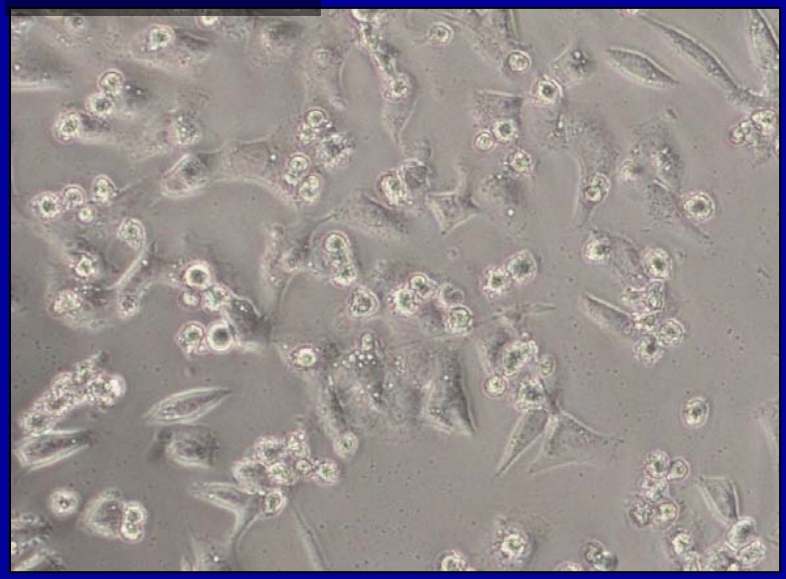
Staurosporin 20uM



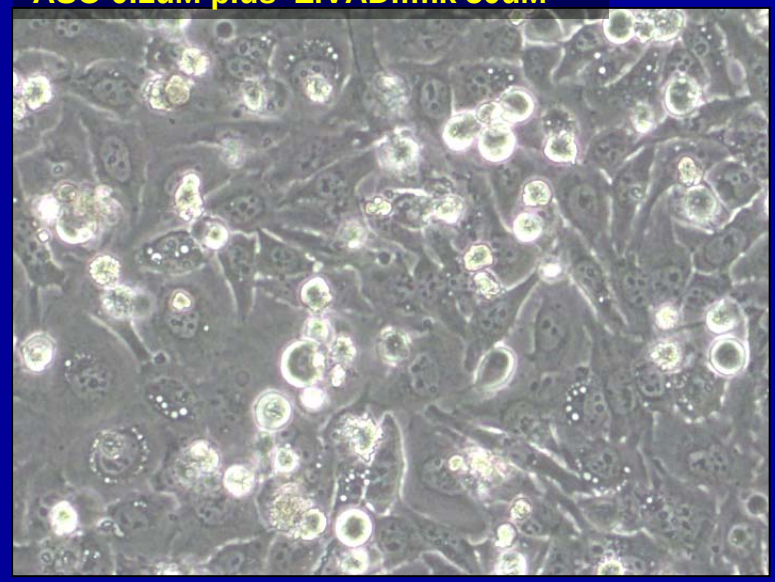
Staurosp. 20uM plus Z.VAD.fmk 50uM



ASO 0.1uM 18h

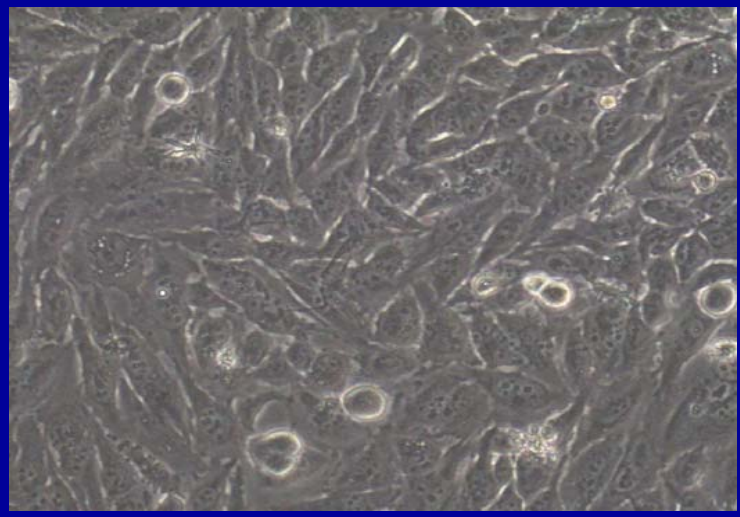


ASO 0.1uM plus Z.VAD.fmk 50uM

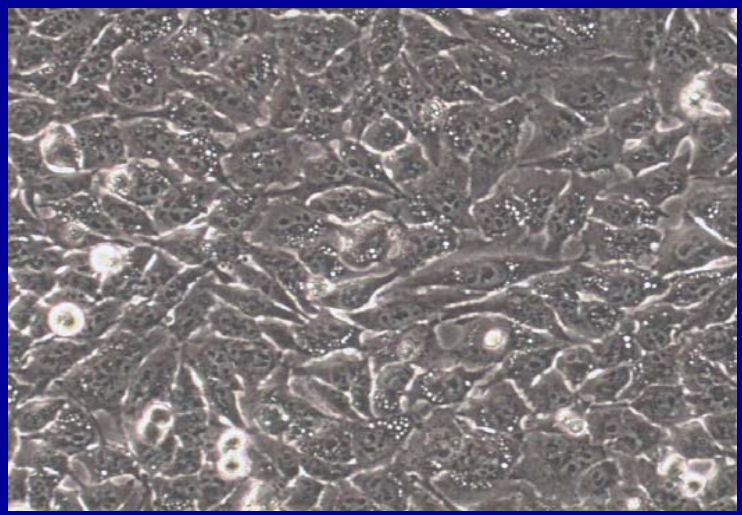


DOES KNOCK-DOWN OF THE ASncmtRNAs ALSO INDUCE PCD TYPE 2 OR AUTOPHAGY?

CONTROL ASO



ASO 1537



ASO 1537