

Métabolisme Lipidique et Mort Cellulaire



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EA7270 - Equipe 'Biochimie du Peroxysome, inflammation et Métabolisme Lipidique'

Faculté des Sciences Gabriel

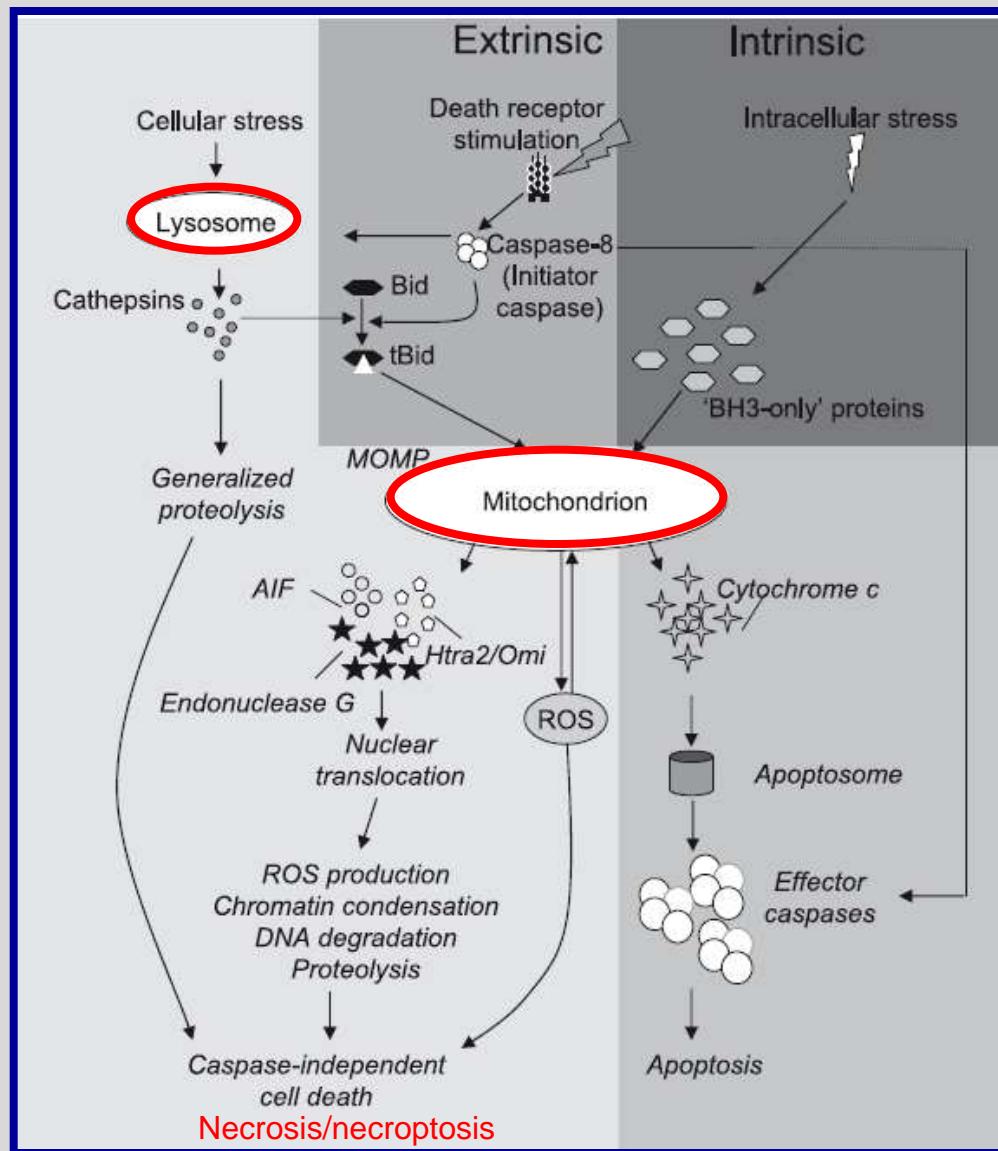
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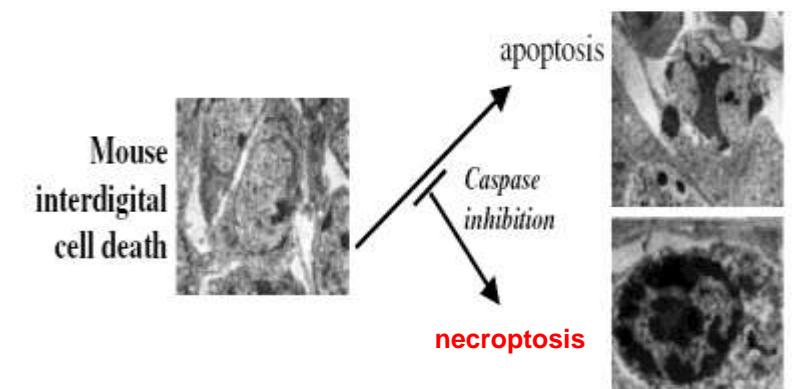
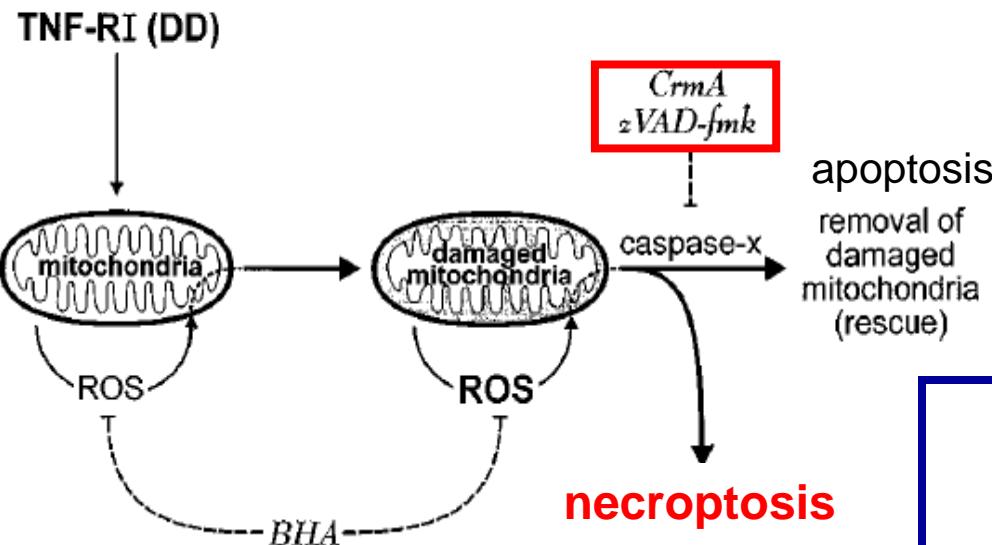
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ORGANELLES INVOLVED IN CELL DEATH

Organelles	<i>Apoptosis</i>	<i>Autophagy</i>	<i>Necrosis / Necroptosis</i>
- Mitochondria	+	+ (mitophagy)	+
- Lysosomes	+ / -	+	+ / -
- Endoplasmic- reticulum (ER)	+ / -	+	+ / -
- peroxisome	?	+ (pexophagy)	?

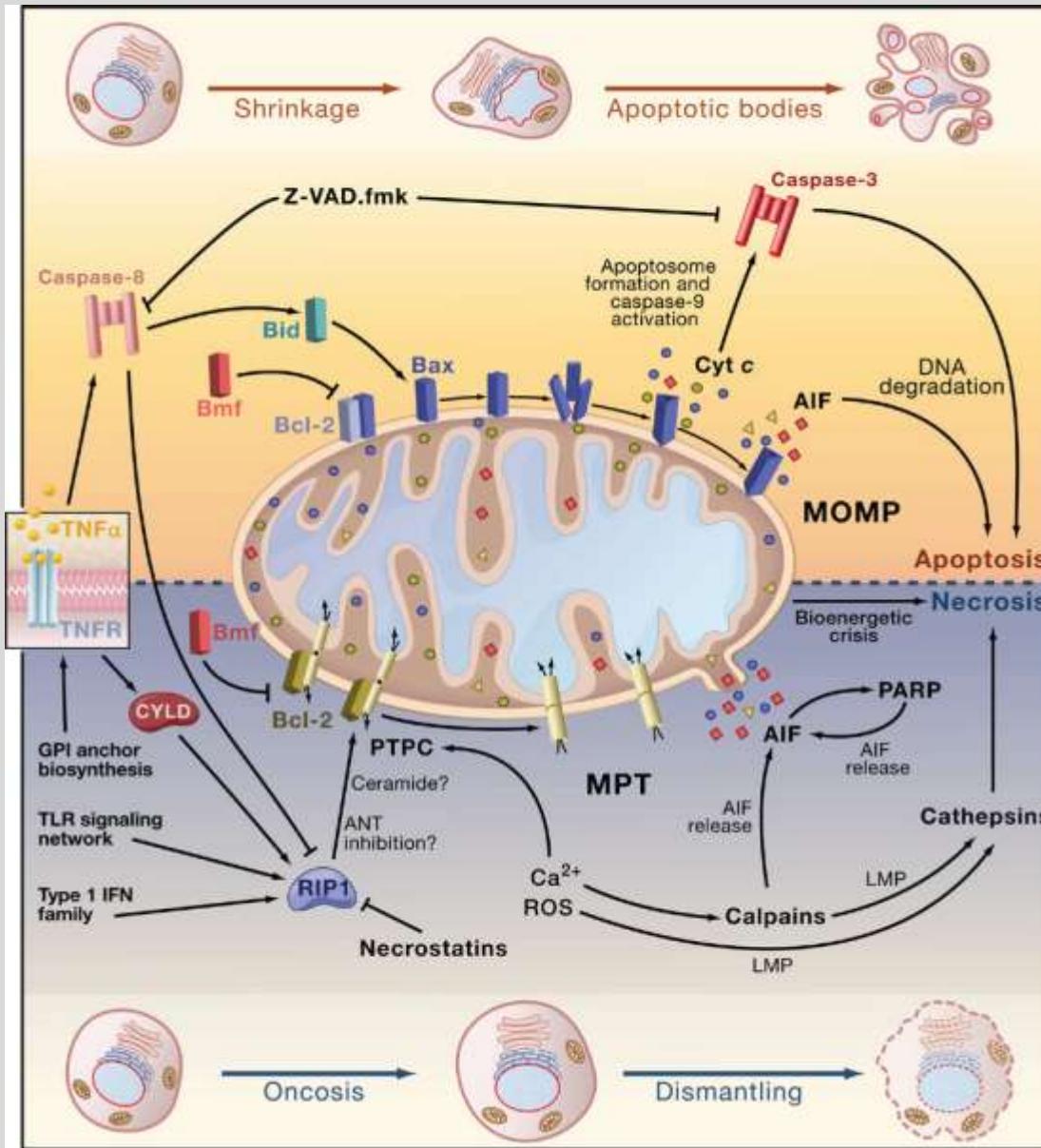
CONNECTION BETWEEN LYSOSOME AND MITOCHONDRIA





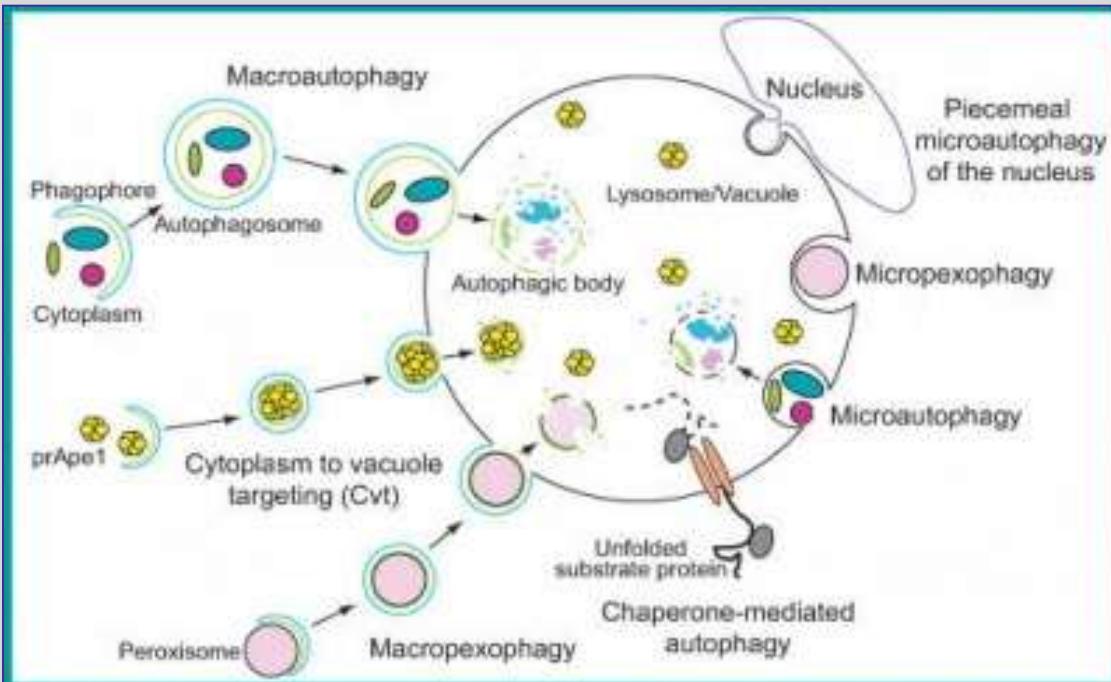
**Different pathways can co-exist in the same cell
and can be switched on by specific stimuli**

SWITCHING FROM APOPTOSIS TO NECROPTOSIS

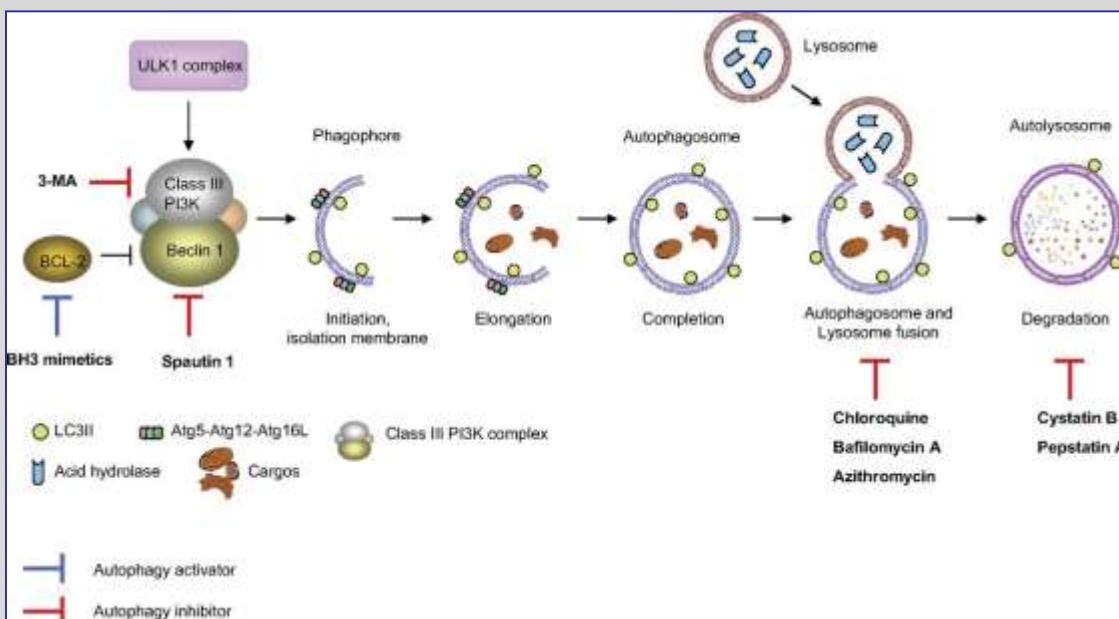


Three types of autophagy:

- Macroautophagy
- Microautophagy
- Chaperone-mediated autophagy

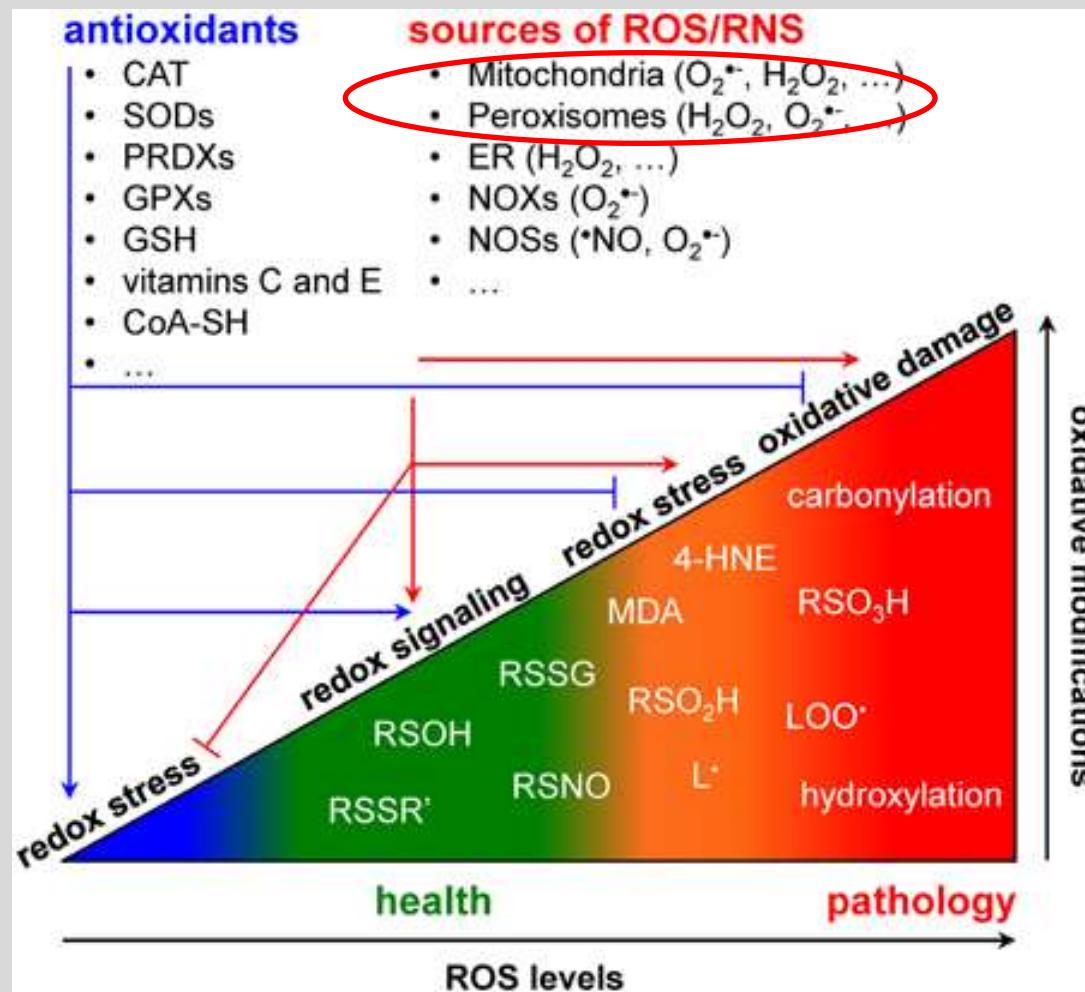


Sequences of autophagy



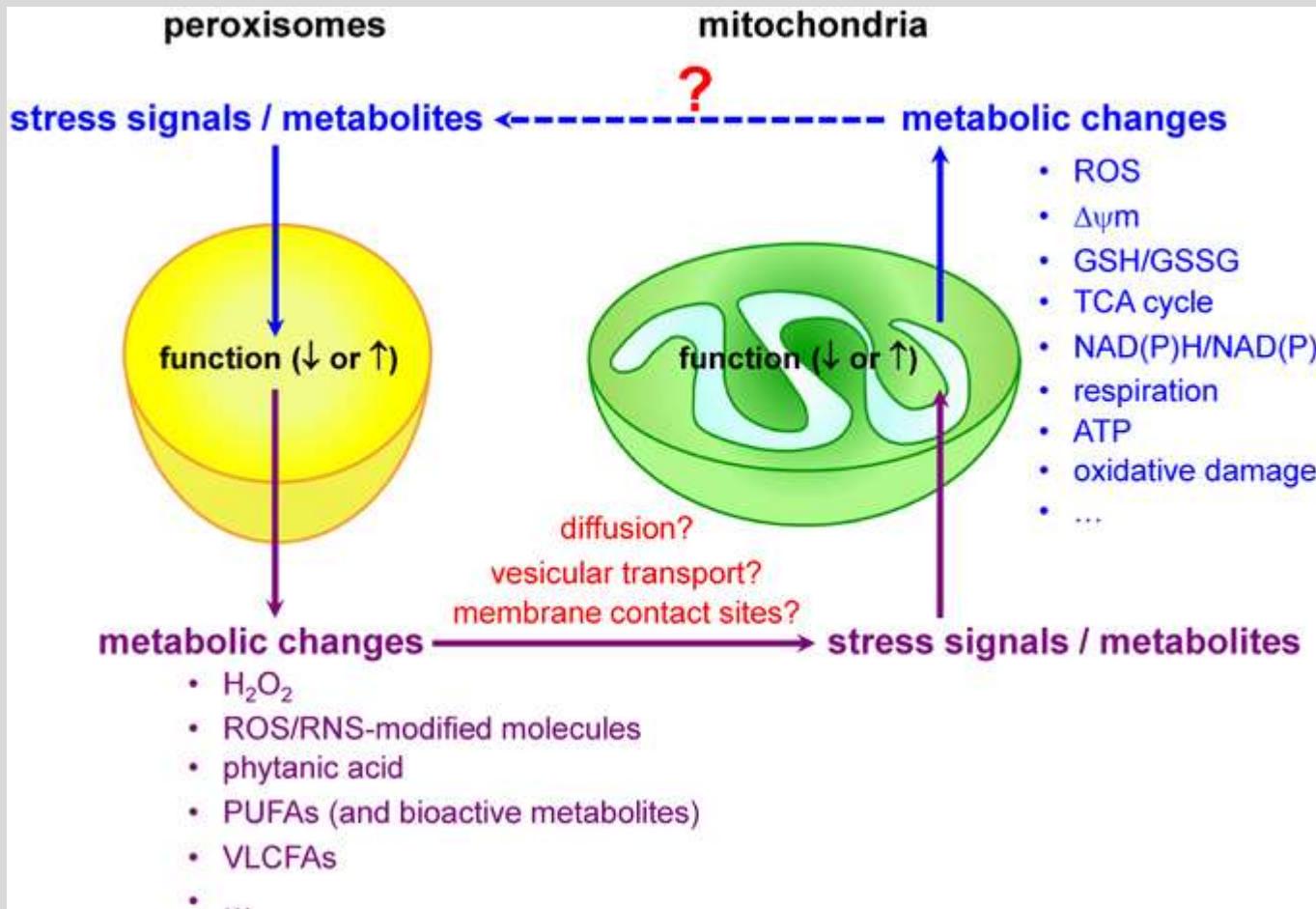
Rupture de l'équilibre RedOx et induction de mort cellulaire

Implication du peroxysome et de la mitochondrie



Relations peroxysome / mitochondrie

Implication dans le contrôle de l'équilibre RedOx et l'activation de la mort cellulaire



Lipids and Cell Death

Les lipides : structures et classification

Basé sur la structure chimique, on distingue

✓ **Acides gras**

✓ **Lipides simples**

- glycérides
- stérols et stéroïdes
- cérides (esters d'alcools gras + acides gras)

✓ **Lipides complexes**

- phospholipides
- sphingolipides
- plasmalogènes

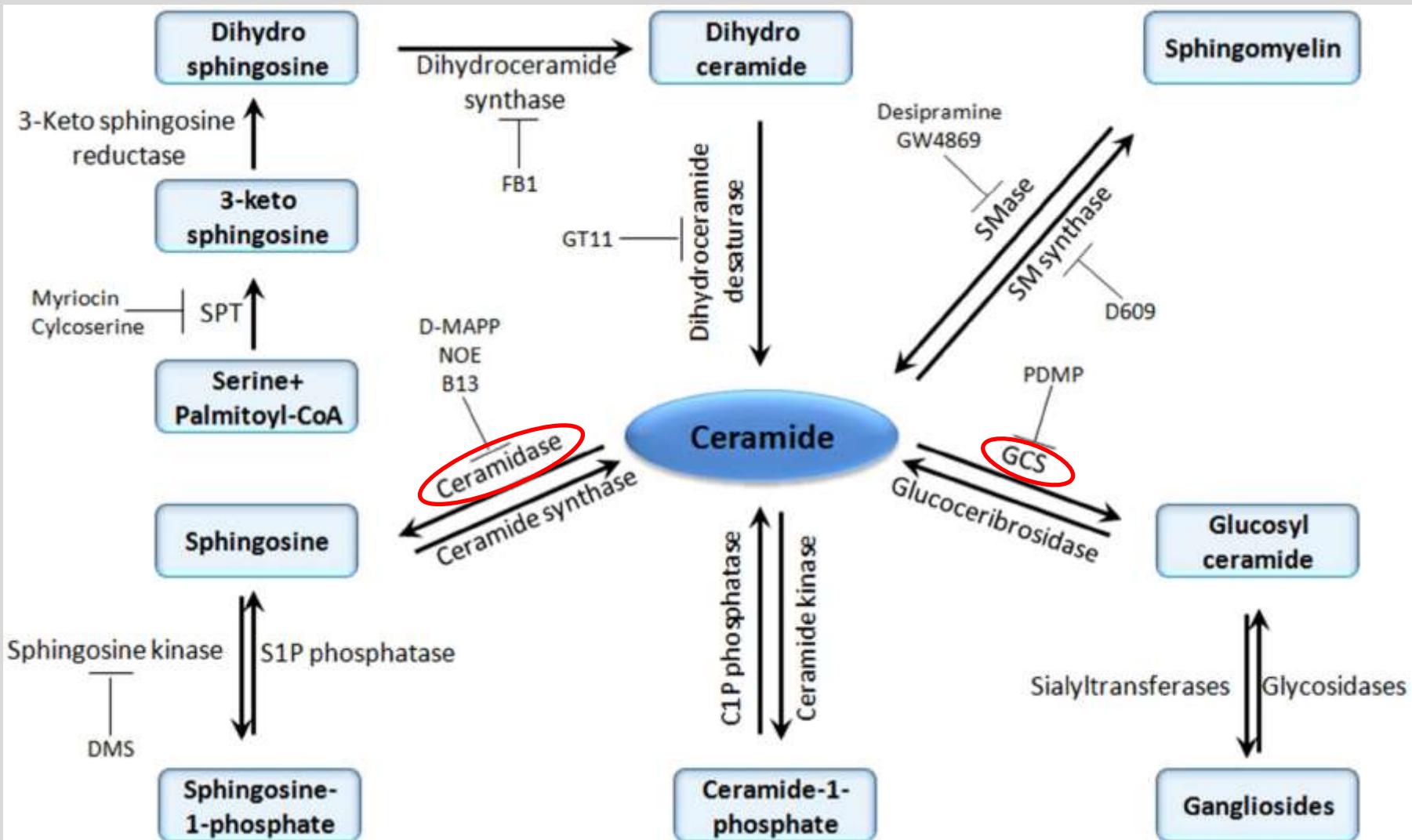
✓ **Lipides isopréniques**

- carbures isopréniques (polymères d'isoprènes: terpènes)
- quinones et vitamines liposolubles

Insolubles dans l'eau, solubles dans les solvants organiques apolaires

(groupements chimiques communs: -CH=CH-; -CH₂-; -CH₃-)

Biosynthèse des céramides



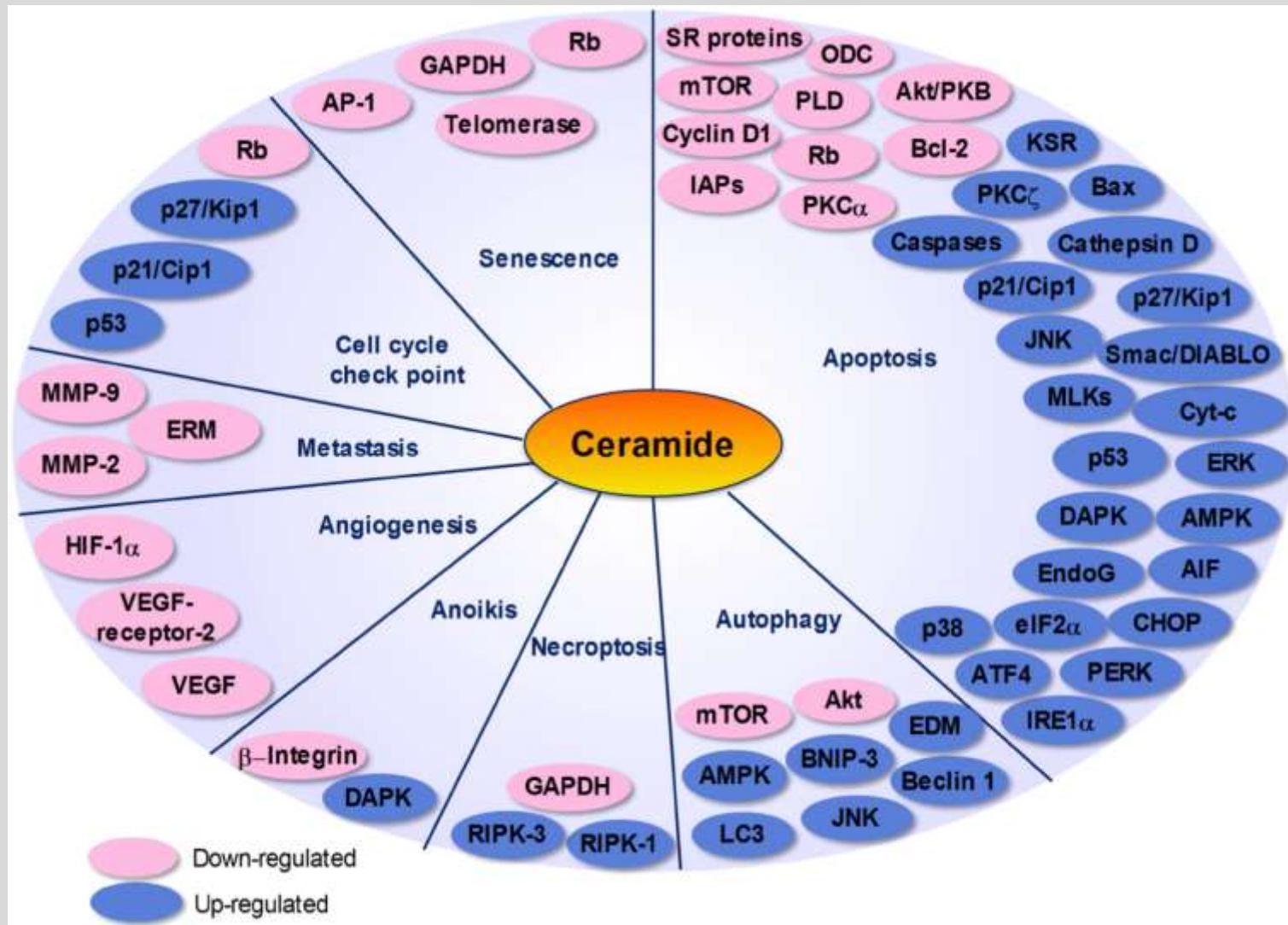
GCS: glucosylceramide synthase

Galadari S, Rahman A, Pallichankandy S, Thayyullathil F. Tumor suppressive functions of ceramide: evidence and mechanisms. *Apoptosis*. 2015;20(5):689-711.

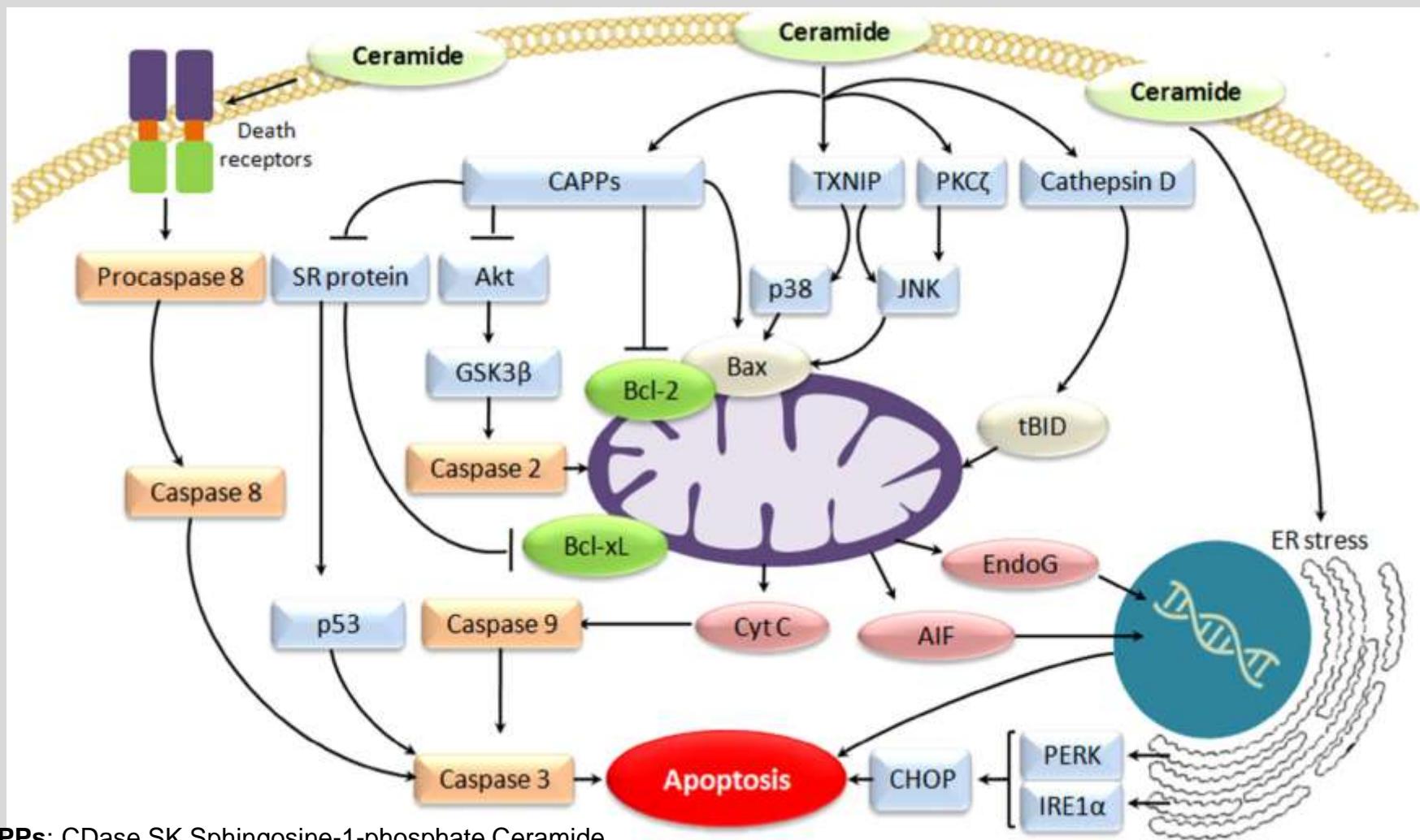
Céramides : cibles thérapeutiques

Enzymes	Cancer species (cell line)	Drug	Resistance
GCS ↑	Breast cancer (MCF-7)	Adriamycin	↑
	Colon cancer (SW620)	Adriamycin	↑
	Epidermoid carcinoma (KB-3-1)	Adriamycin	↑
		Vinblastine	
	Leukemia (HL-60)	Vincristine	↑
	Melanoma (MeWo)	Etoposide	↑
GCS ↓	Leukemia (HL-60)	Doxorubicin	↑
	Adriamycin-resistant MCF-7	Adriamycin	↓
	Adriamycin-resistant MCF-7	Vinblastine	↓
		Paclitaxel	
	Adriamycin-resistant MCF-7 and murine breast cancer (EMT6)	Doxorubicin	↓
	Adriamycin-resistant SW620	Doxorubicin	↓
Acid Ceramidase ↑	Doxorubicin-resistant ovarian carcinoma (A2780)	Doxorubicin	↓
	Doxorubicin-resistant cervical cancer (KB-A1)	Doxorubicin	↓
	Hepatoma (HepG2)	Doxorubicin	↓
	Multidrug-resistant leukemia (K562/A02)	Adriamycin	↓
Acid Ceramidase ↓		Doxorubicin	
	Prostate cancer (DU145)	Cisplatin	↑
		Etoposide	
		Gemcitabine	
Acid Ceramidase ↓	Hepatoma (HepG2, Hep-3B, SK-Hep and Hep1c1c7)	Daunorubicin	↓

Céramides : effecteurs et cibles moléculaires



Céramides : inducteurs d'apoptose / activité anti-tumorale

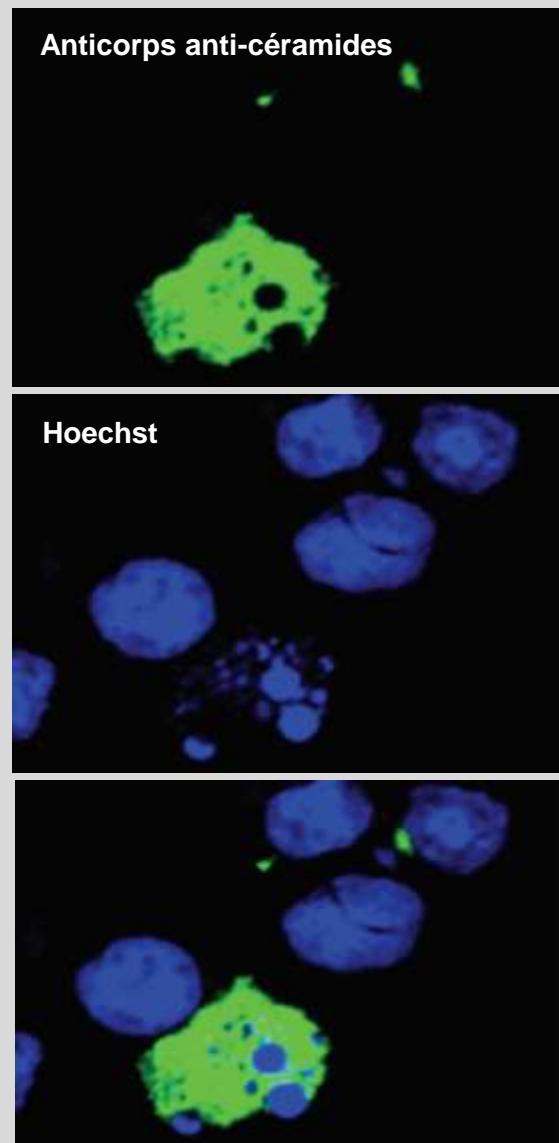
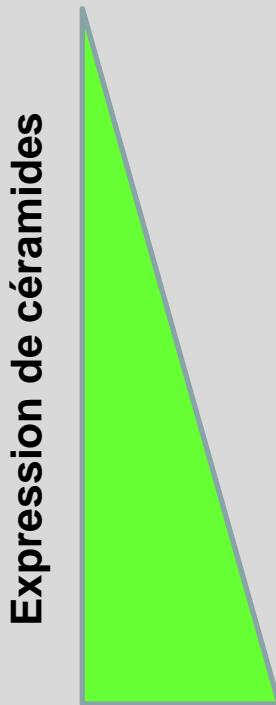
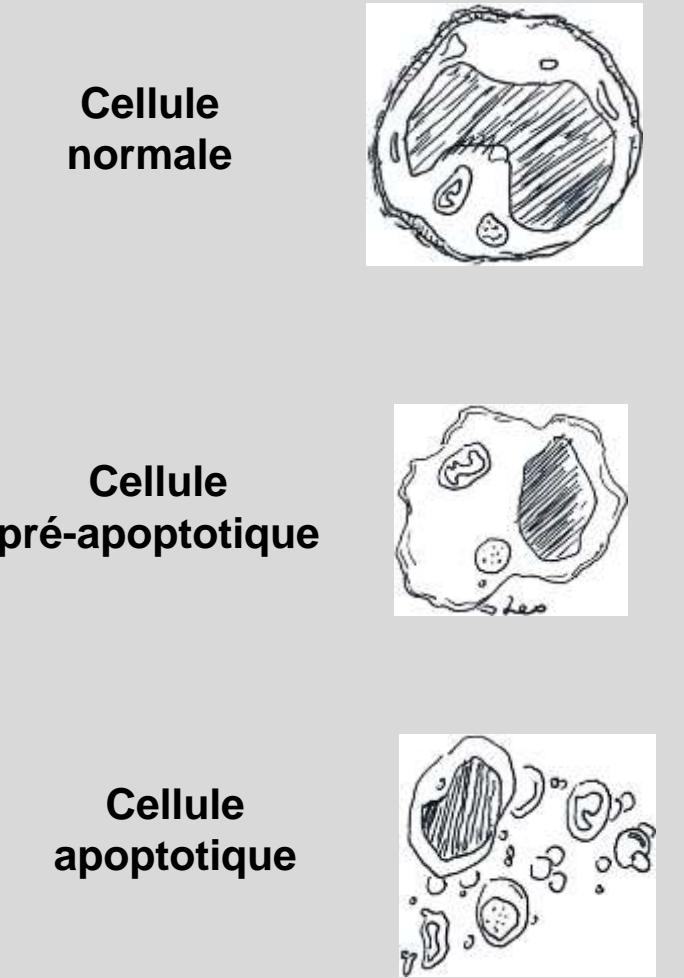


CAPPs: CDase SK Sphingosine-1-phosphate Ceramide

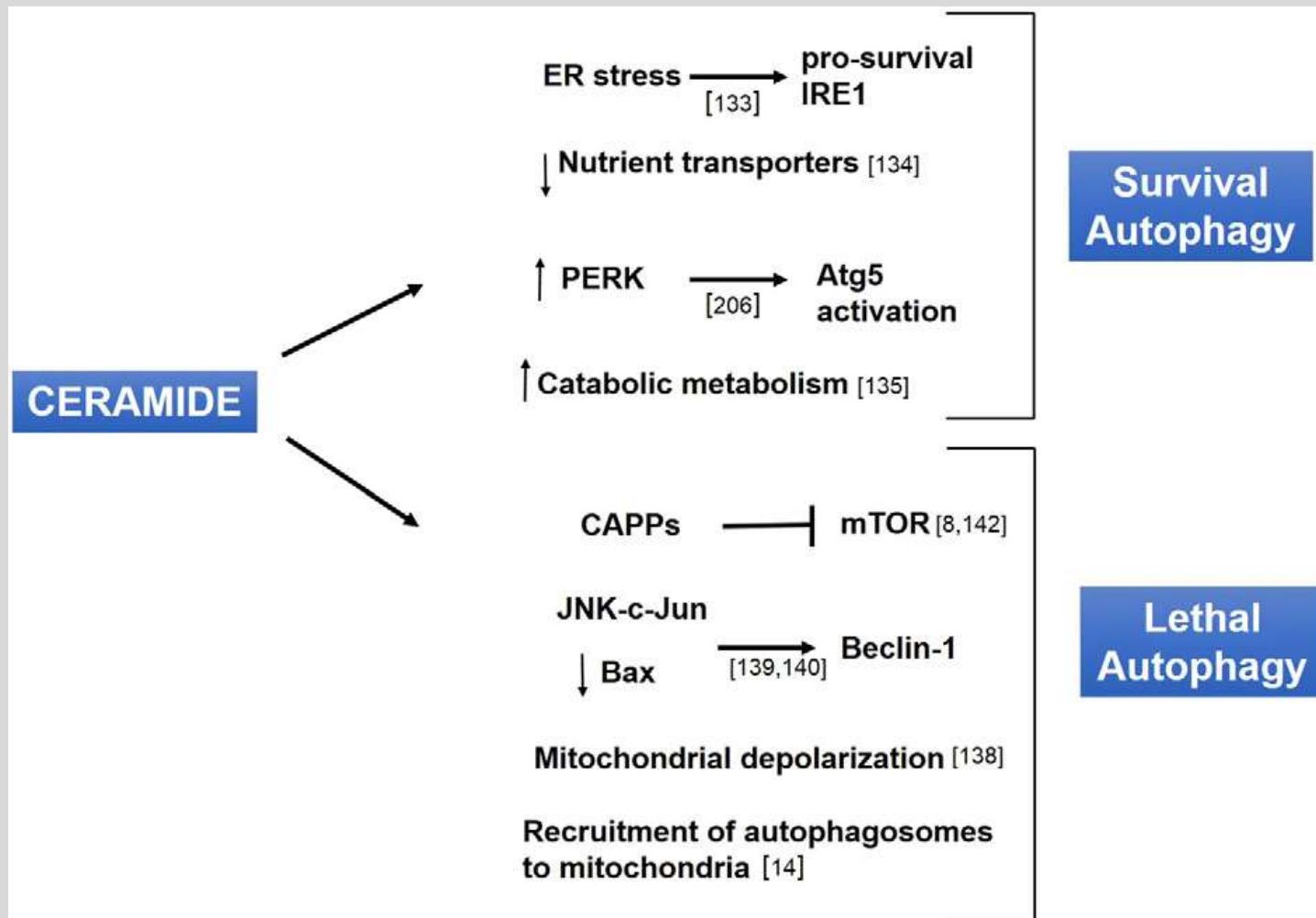
TXNIP: Thioredoxin-interacting protein

Galadari S, Rahman A, Pallichankandy S, Thayyullathil F. Tumor suppressive functions of ceramide: evidence and mechanisms. *Apoptosis*. 2015;20(5):689-711.

Stimuli apoptotique : génération de céramides (déttection par immunofluorescence)

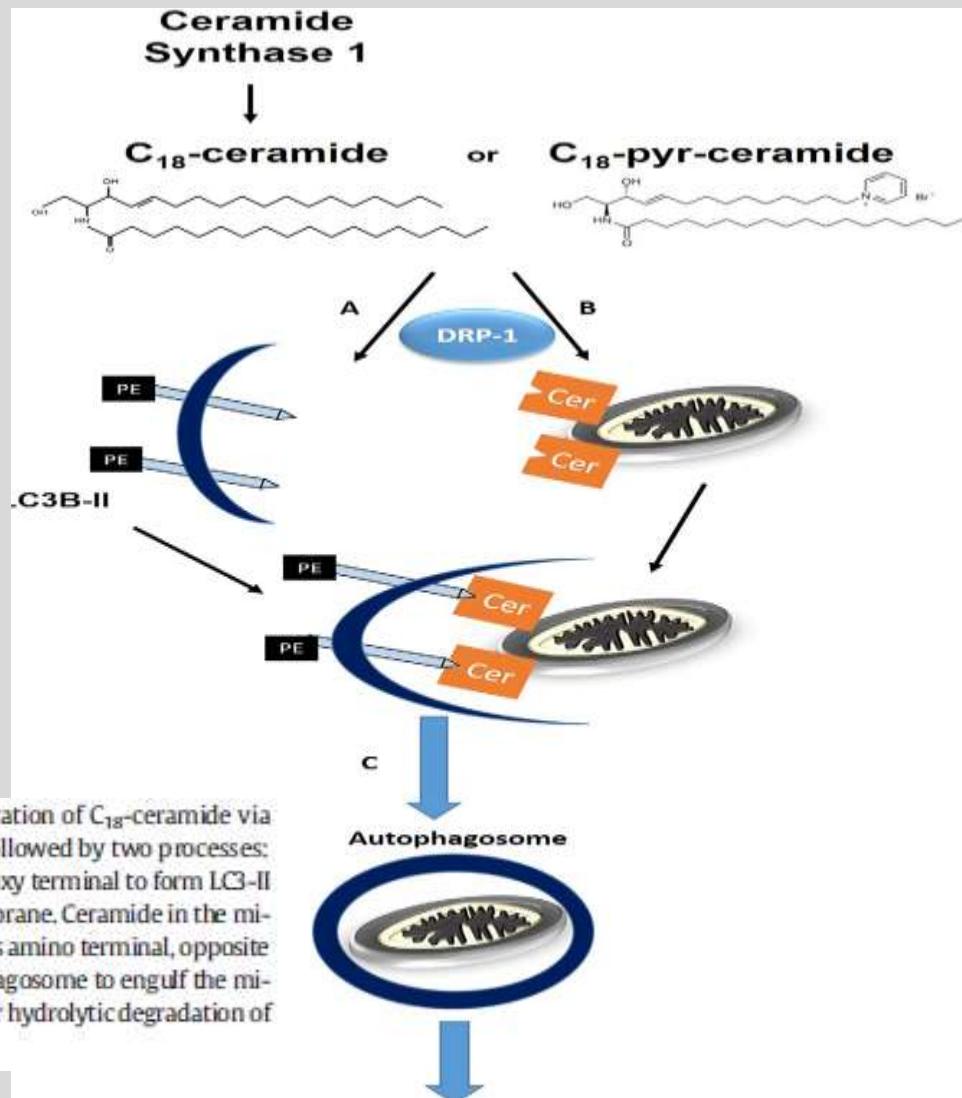


Céramides : autophagie cytoprotectrice et léthale



Dany M, Ogretmen B. Ceramide induced mitophagy and tumor suppression. *Biochim Biophys Acta*. 2015;1853(10 Pt B):2834-45.

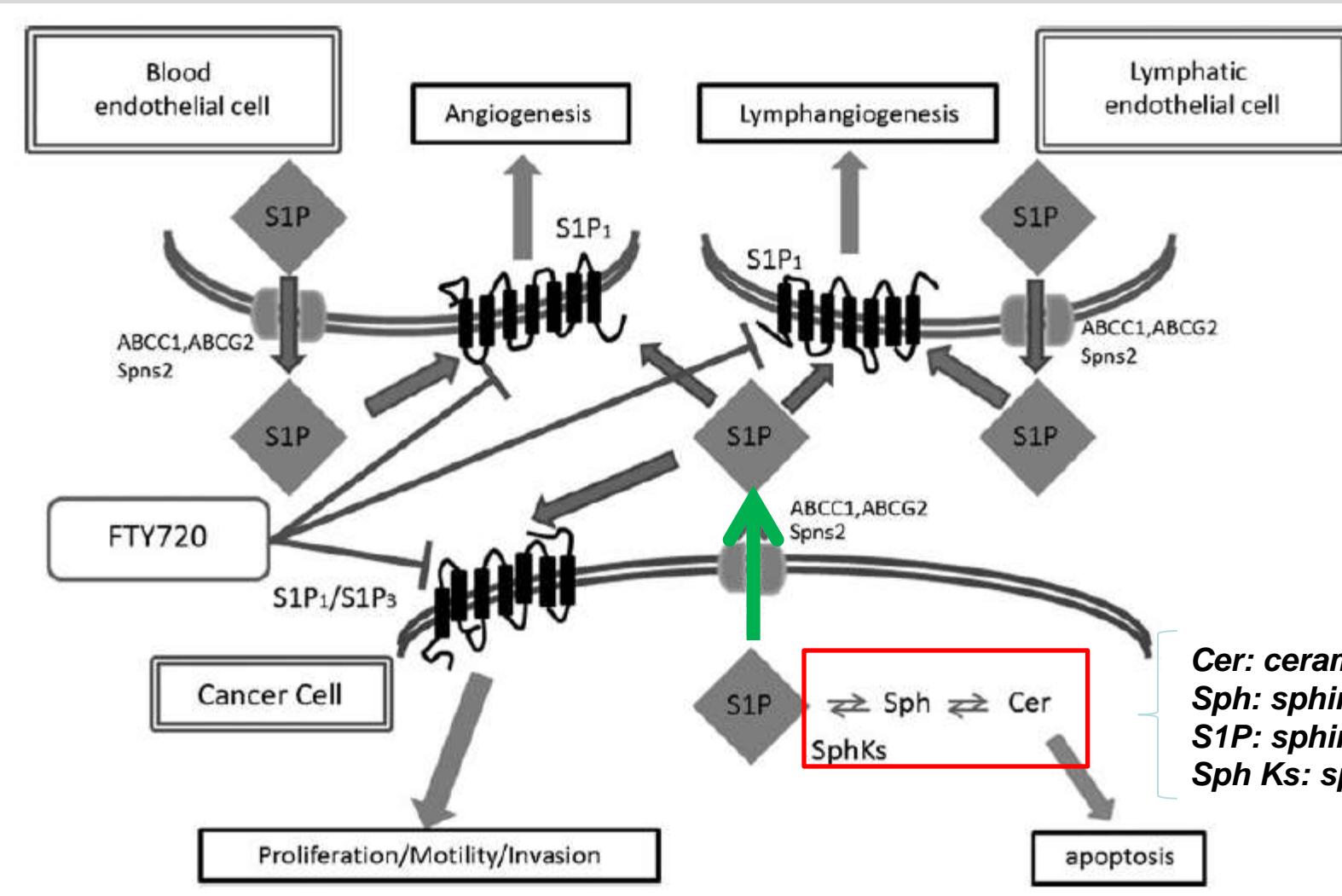
Céramides : régulation de la mitophagie



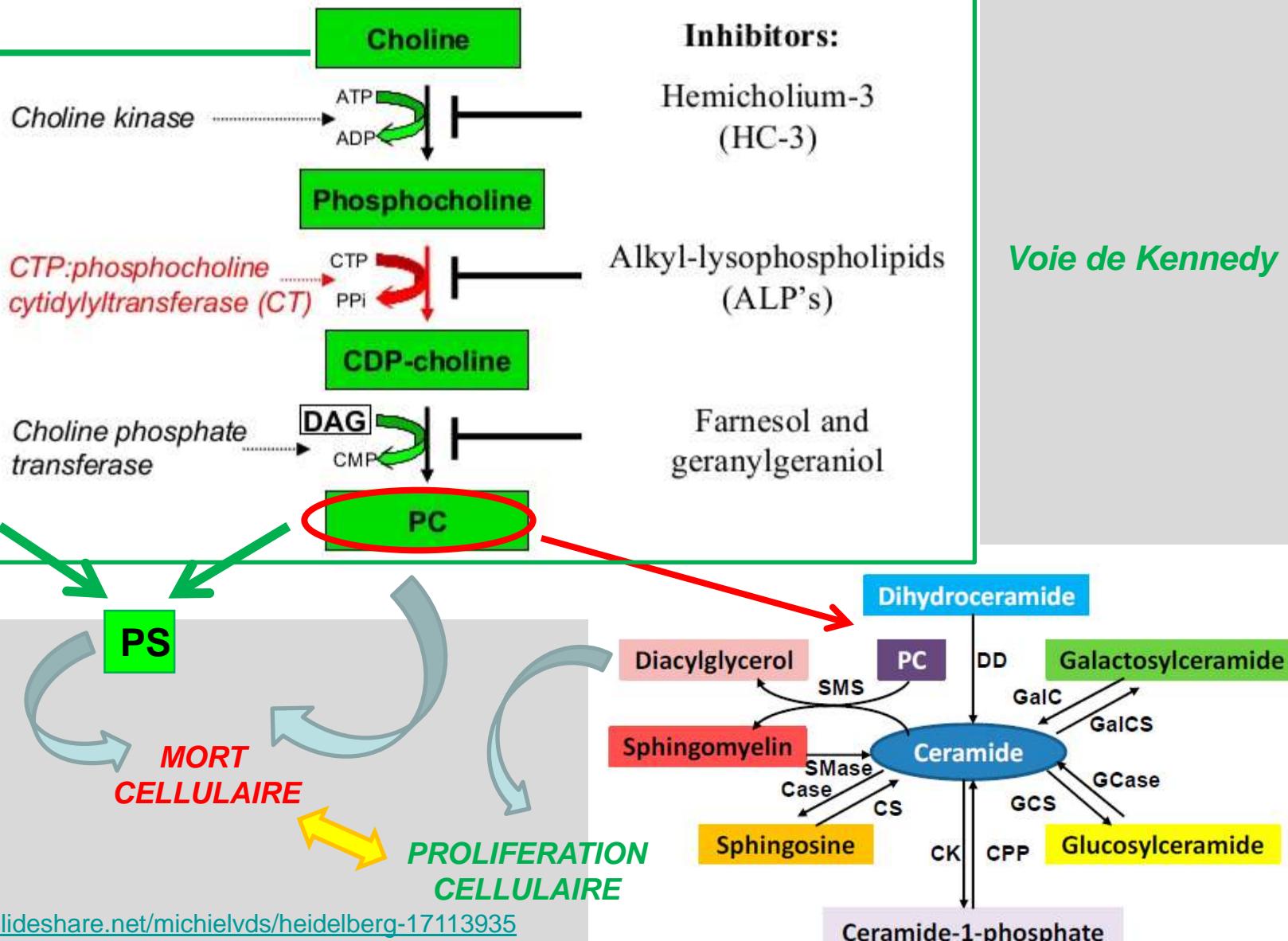
Regulation of mitophagy by ceramide. Endogenous generation of C₁₈-ceramide via CerS1 or exogenous treatment by C₁₈-pyridinium-ceramide is followed by two processes: A. conjugation of LC3-I to phosphatidylethanolamine on the carboxy terminal to form LC3-II and B. accumulation of ceramide in the mitochondrial outer membrane. Ceramide in the mitochondrial membrane acts as a receptor to LC3-II by binding to its amino terminal, opposite to where PE is conjugated. This results in C, recruiting the autophagosome to engulf the mitochondria. Lysosomes then fuse with the autophagosomes (D) for hydrolytic degradation of the contents.

Tumor suppression

Sphingosine 1 kinase / Sphingosine 1 phosphate (S1P) : facteur de risque associé à plusieurs cancers



Synthèse de novo de phosphatidylcholine : voie de Kennedy

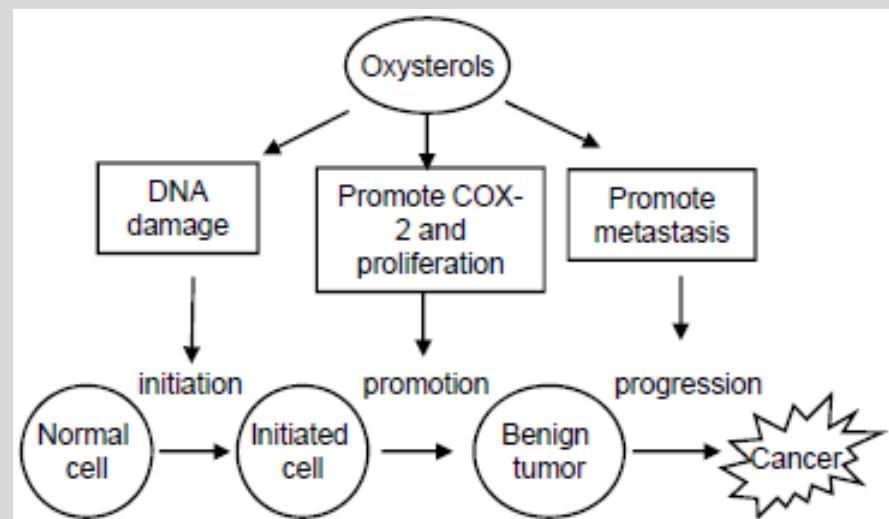
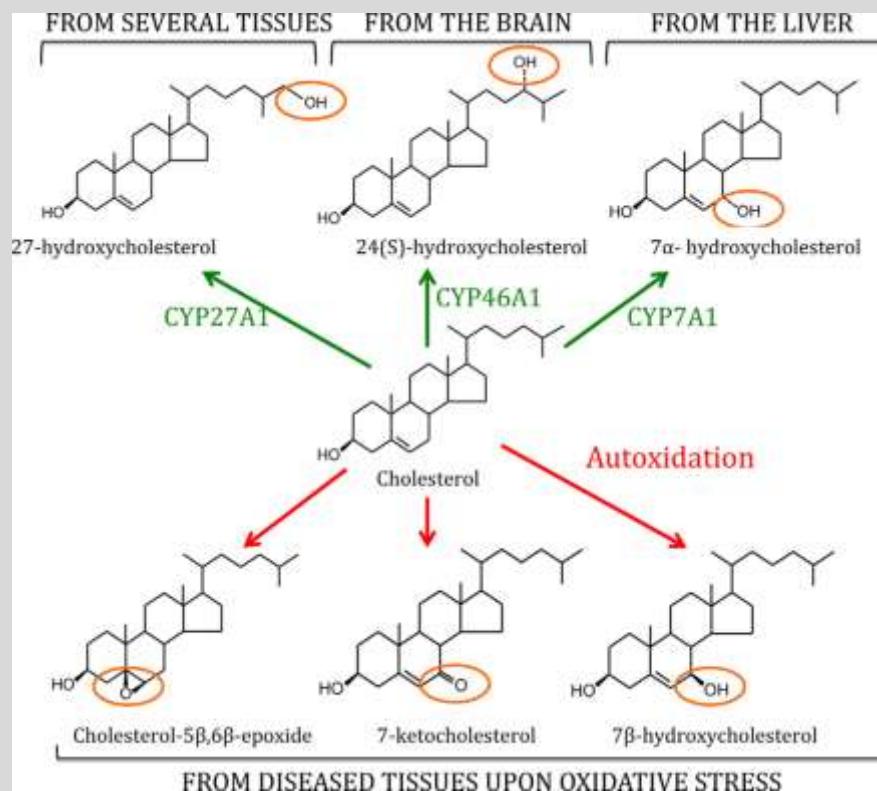


<http://fr.slideshare.net/michielvds/heidelberg-17113935>

Gibellini F & Smith TK Life 2010, 62(6): 414-428.

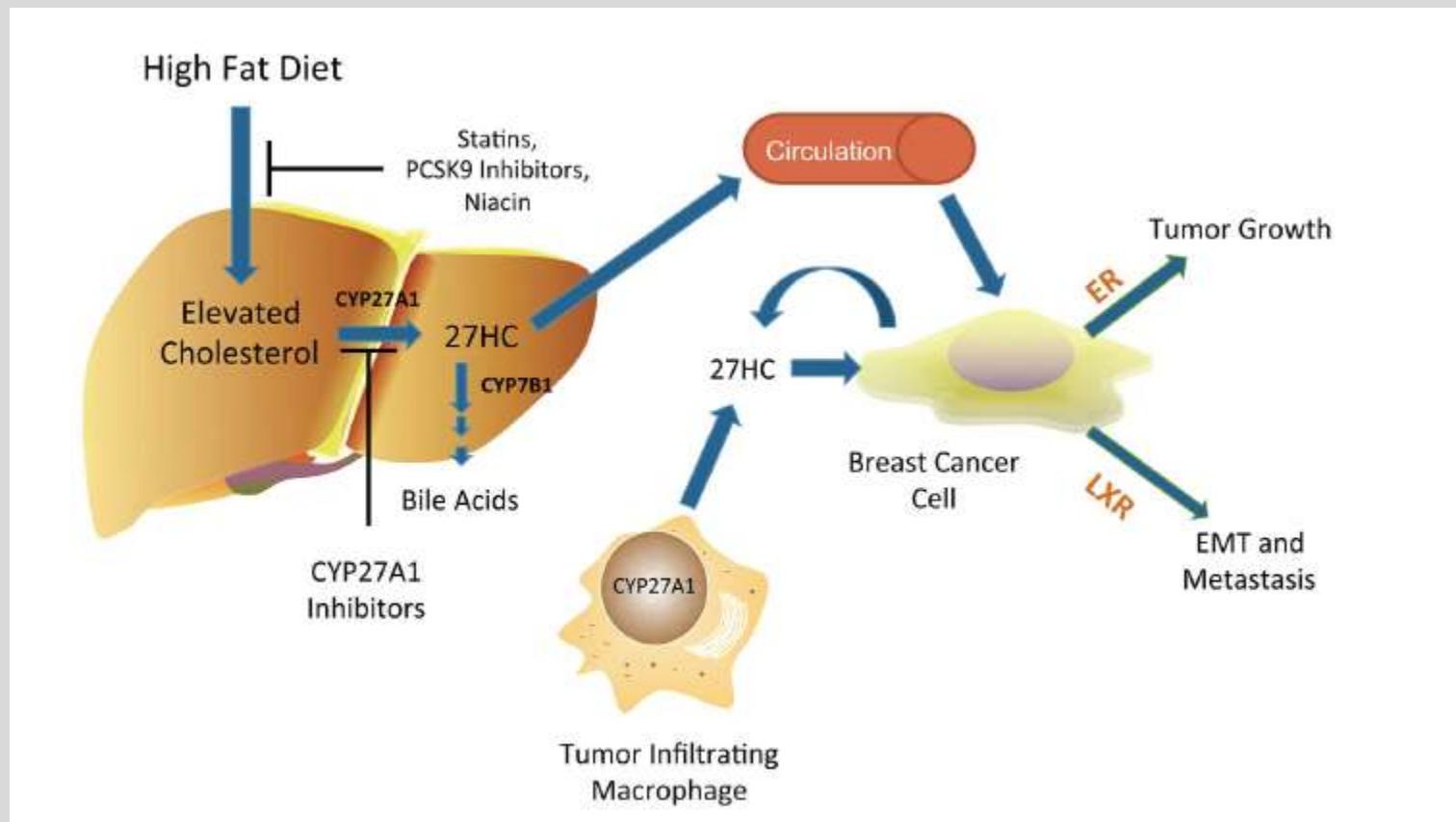
Huang C & Freter C Int. J. Mol. Sci. 2015, 16, 924-949

Oxystérols et cancer



Activités pro-tumorales des oxystérols ??????

Cancer du sein : implication du cholestérol et du 27-hydroxycholestérol

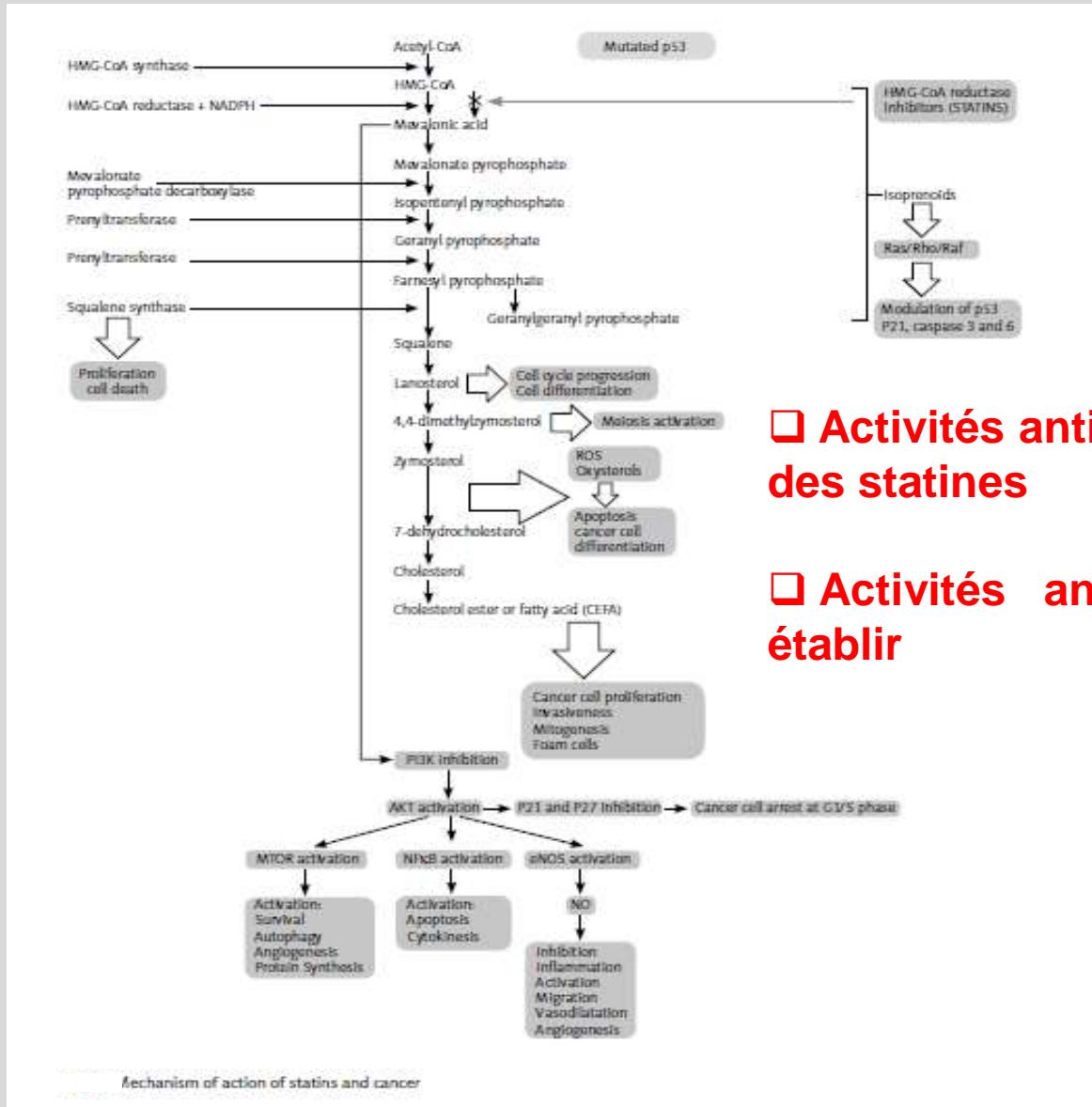


CYP27A1
Obésité → cholestérol → 27-hydroxycholestérol (27HC) → Récepteurs aux oestrogènes (ER)

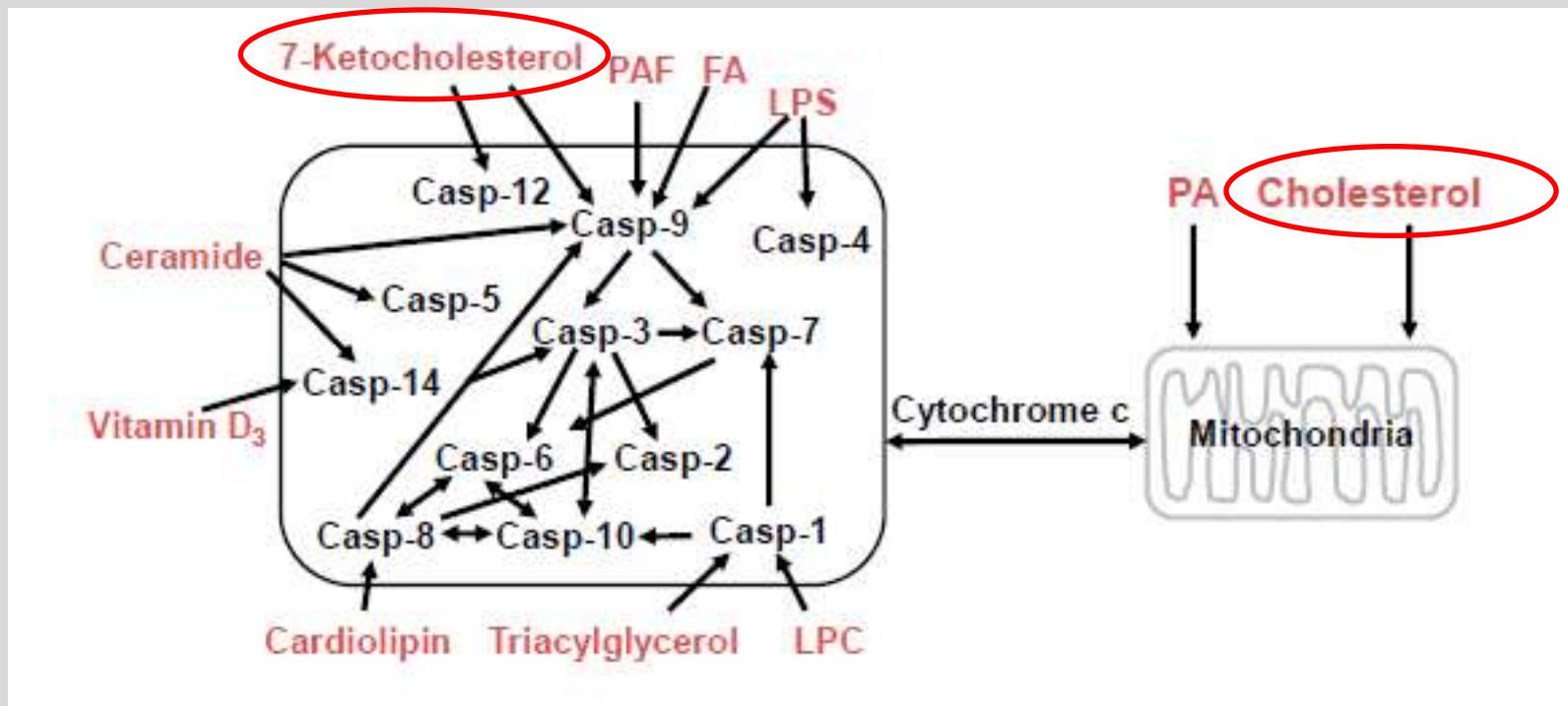


Prolifération tumorale / métastases
(activité pro-tumorelle)

Statines et cancer

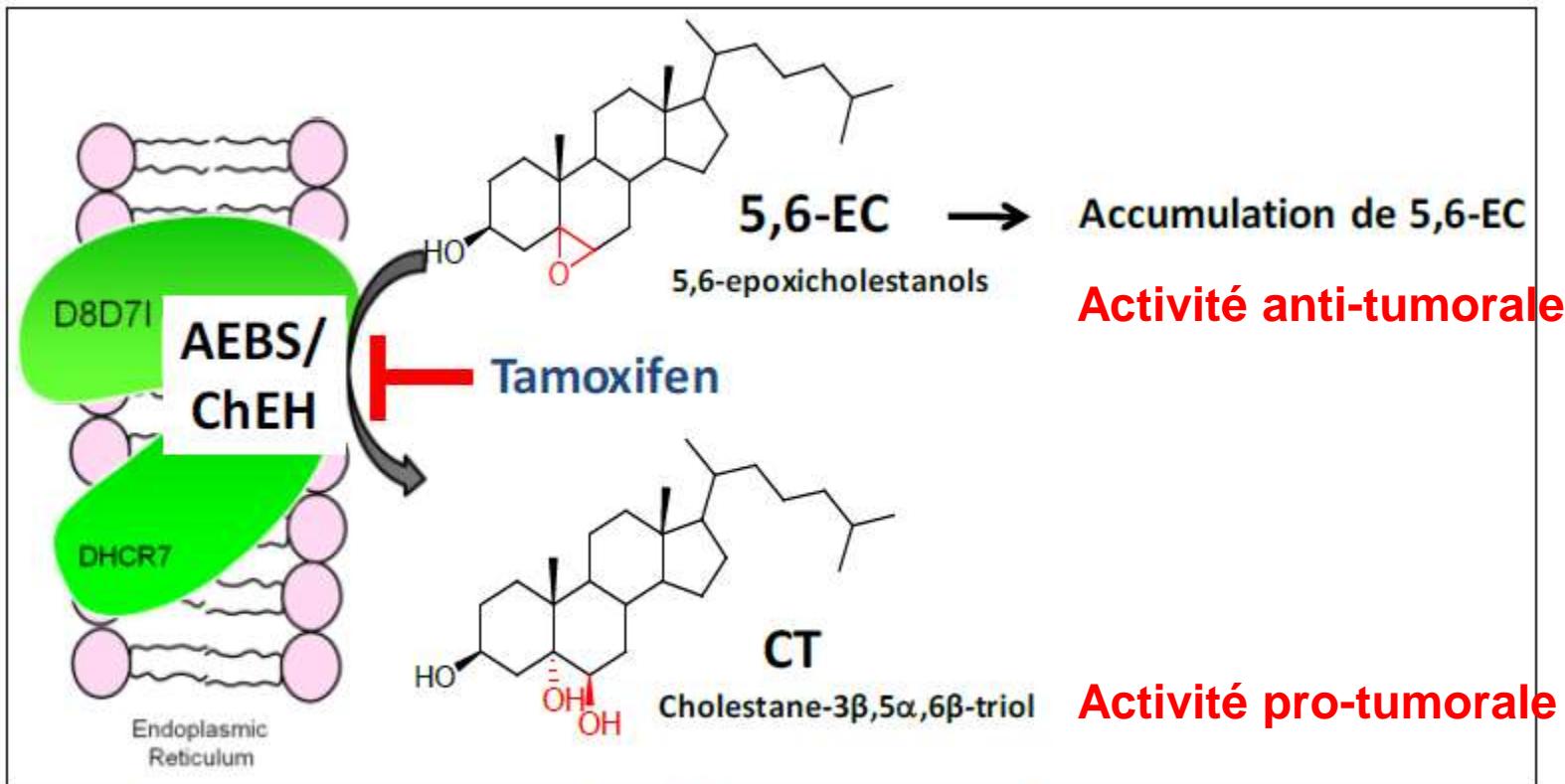


Implication du cholestérol et de ses dérivés oxydés (7-cétocholestérol) dans la cancérogénèse



Le cholestérol et les oxystérols sont impliqués dans la régulation de la signalisation apoptotique (**activités anti-tumorales**)

Tamoxifen et cancer du sein



Le site AEBS porte l'activité ChEH . En absence de ligands, les 5,6-EC sont hydrolysés en CT. Lorsqu'un ligand, comme le Tam vient se fixer, il provoque l'inhibition de la ChEH et l'accumulation des 5,6-EC.

D8D71: 3 β -hydroxystérol- Δ 8- Δ 7-isomérase

DHCR7: 3 β -hydroxystérol- Δ 7-réductase

AEBS: anti-estrogen binding site

ChEH: cholesterol ester hydrolase

Oxydation des acides gras et carbonylation protéique

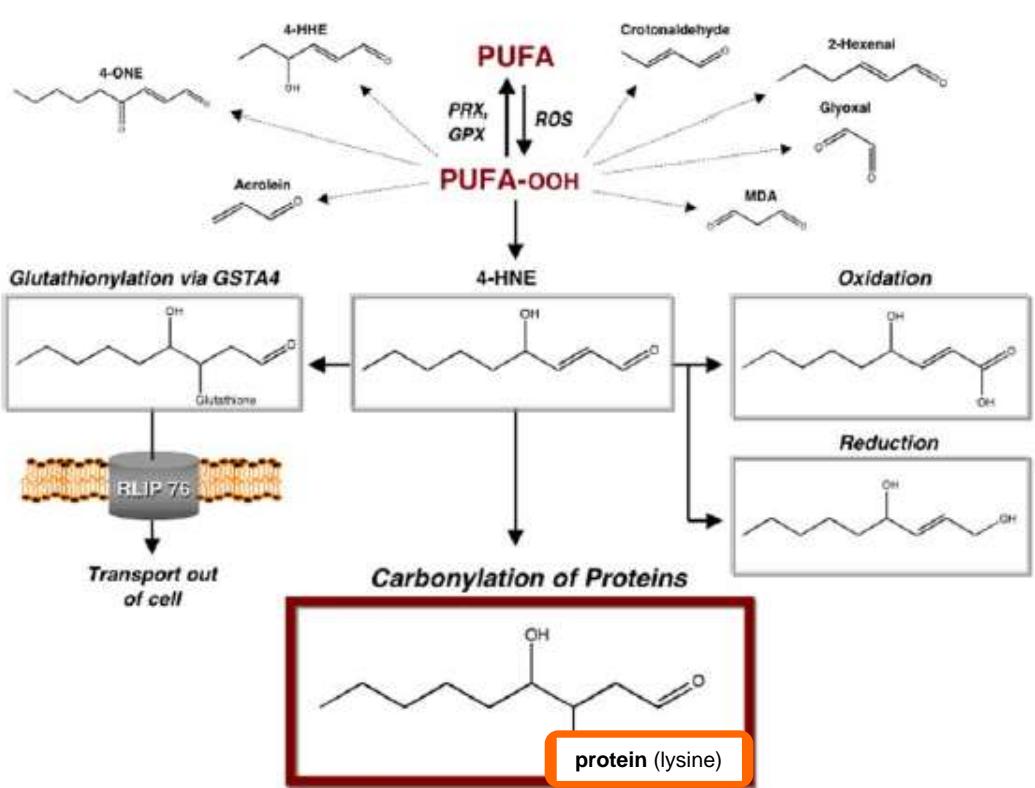
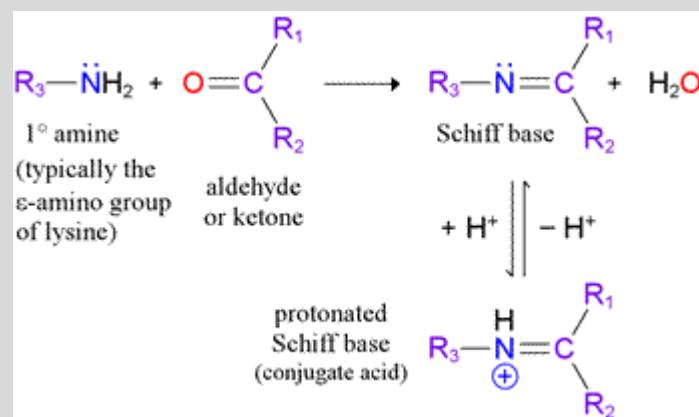
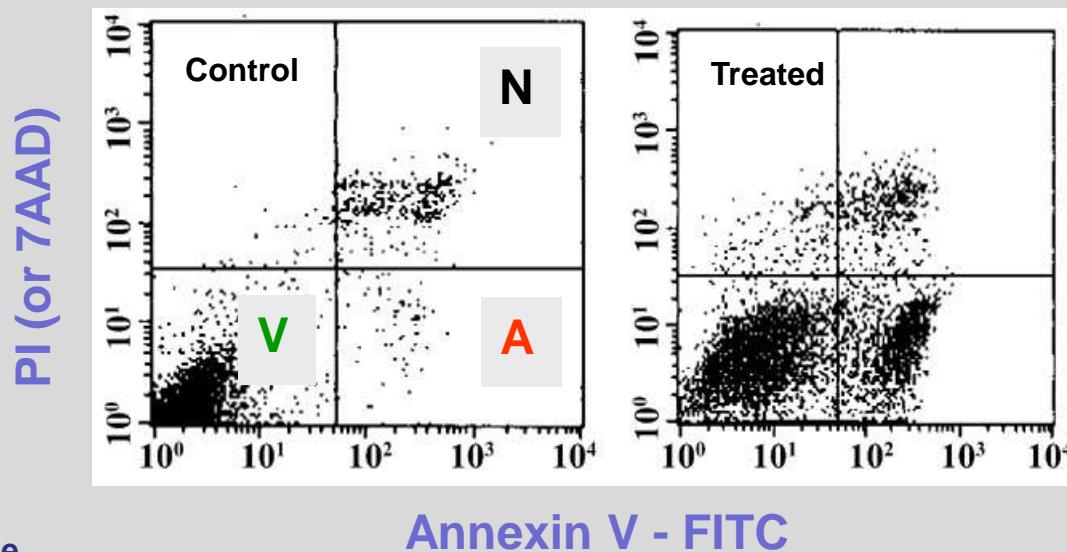
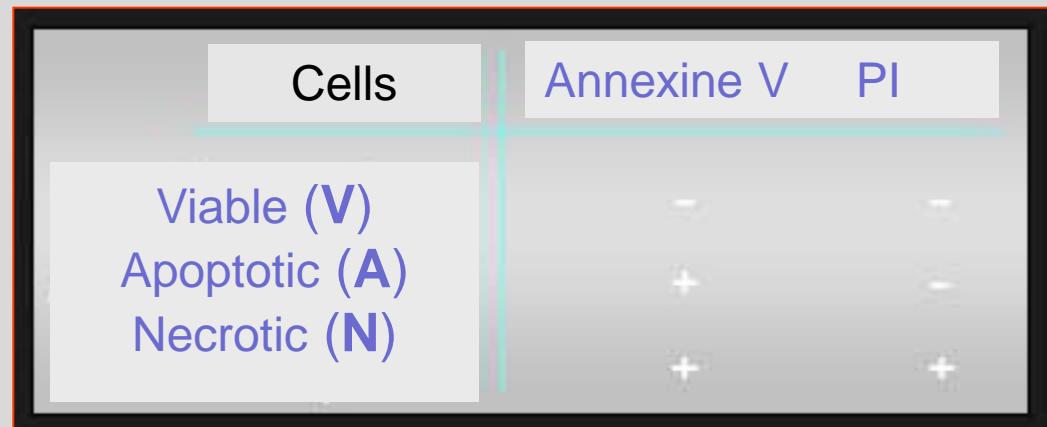
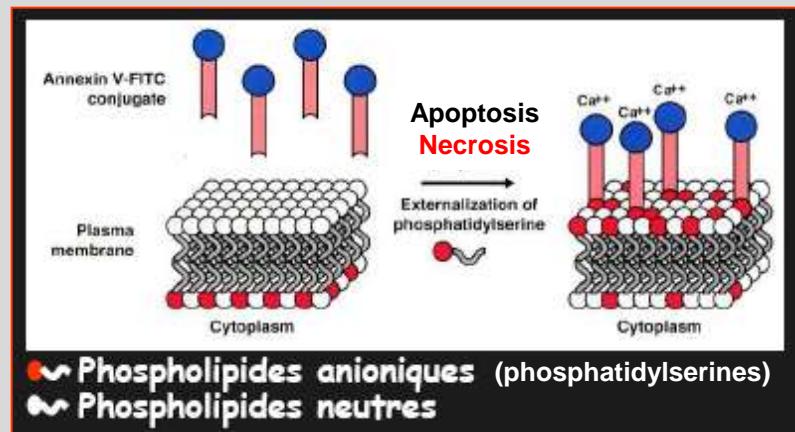


FIG 1 Molecular fates of α,β -unsaturated aldehydes and carbonylated proteins. ROS stimulate peroxidation of polyunsaturated fatty acids (PUFA), an oxidative event that is reversible through reduction by peroxiredoxin (PRX) and glutathione peroxidase (GPX) enzymes. The lipid hydroperoxides (PUFA-OOH) generated are unstable and lead to a variety of reactive aldehydes. The lipid peroxidation products generated include the α,β -unsaturated aldehydes 4-HNE, 4-ONE, 4-hydroxy-(2E)-hexanal (4-HHE), (2E)-hexenal, crotonaldehyde, and acrolein as well as the dialdehydes glyoxal and malondialdehyde (MDA). GSTA4 catalyzes the conjugation of the highly reactive α,β -unsaturated aldehydes to glutathione, leading to their efflux from the cell by the glutathione conjugate transporter RLIP76. In addition, oxidation by aldehyde dehydrogenase or reduction by alcohol dehydrogenase, aldehyde reductase, or aldose reductase converts free aldehydes into less toxic molecules. The α,β -unsaturated aldehydes that escape cellular metabolism serve as electrophiles in the covalent modification of proteins via non-enzymatic Michael addition. The resulting aliphatic carbonyl adducts on cysteine, histidine, or lysine residues may alter the activity of protein targets or cause them to become degraded by the proteasome.



AVAILABLE METHODS ALLOWING THE CHARACTERIZATION OF APOPTOSIS, NECROSIS / NECROPOTOSIS, AND AUTOPHAGY

Phosphatidylserine externalization : Annexin V-FITC / PI test



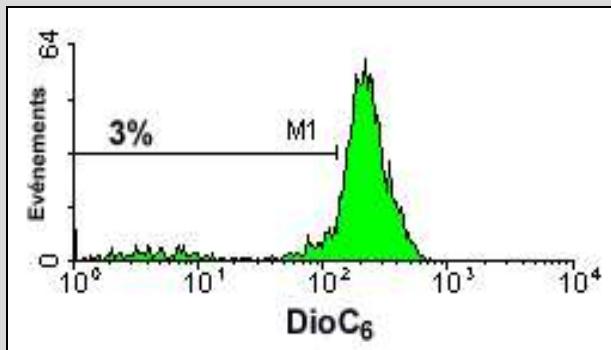
PI: propidium iodide

7 AAD: 7 amino actinomycin D

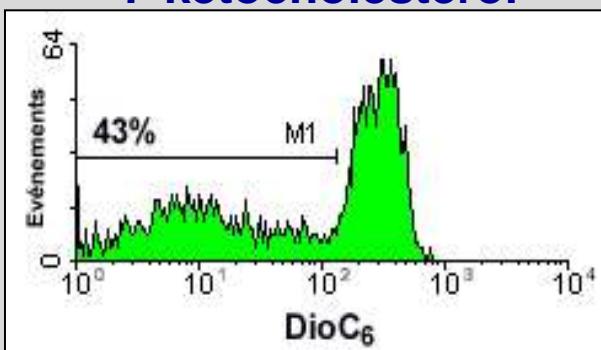
Gérard Lizard,

Mitochondrial potential (FCM)

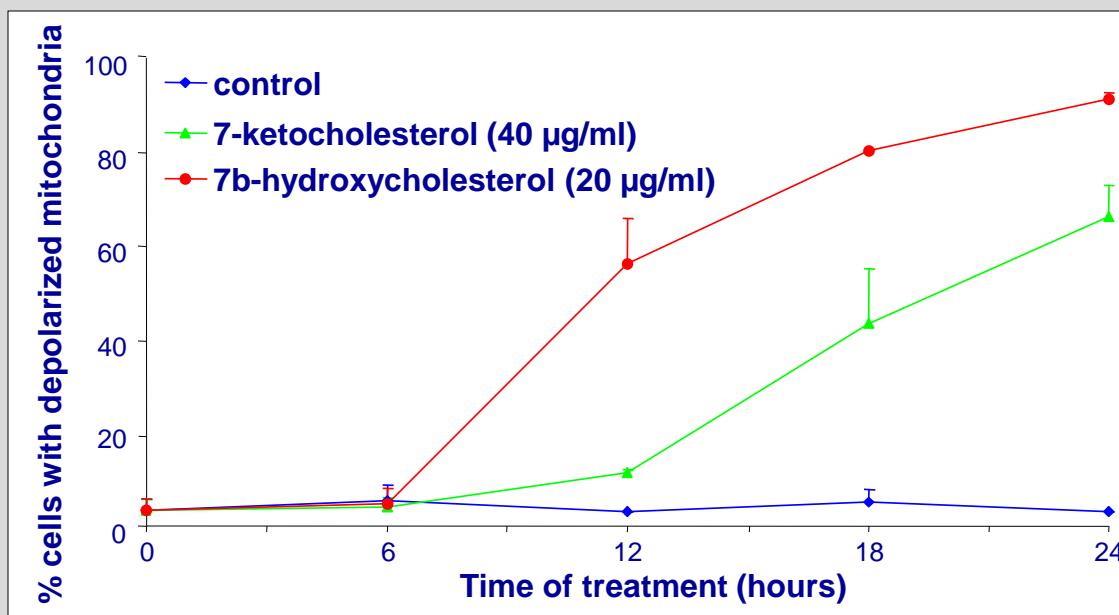
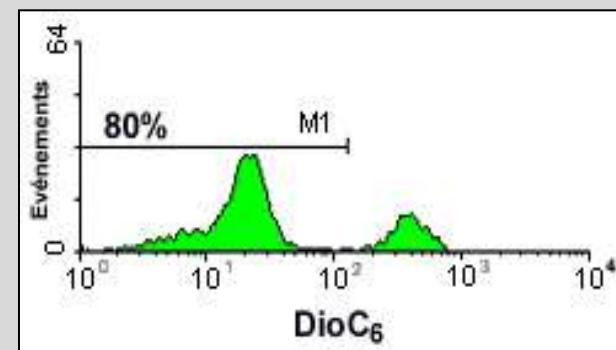
Control



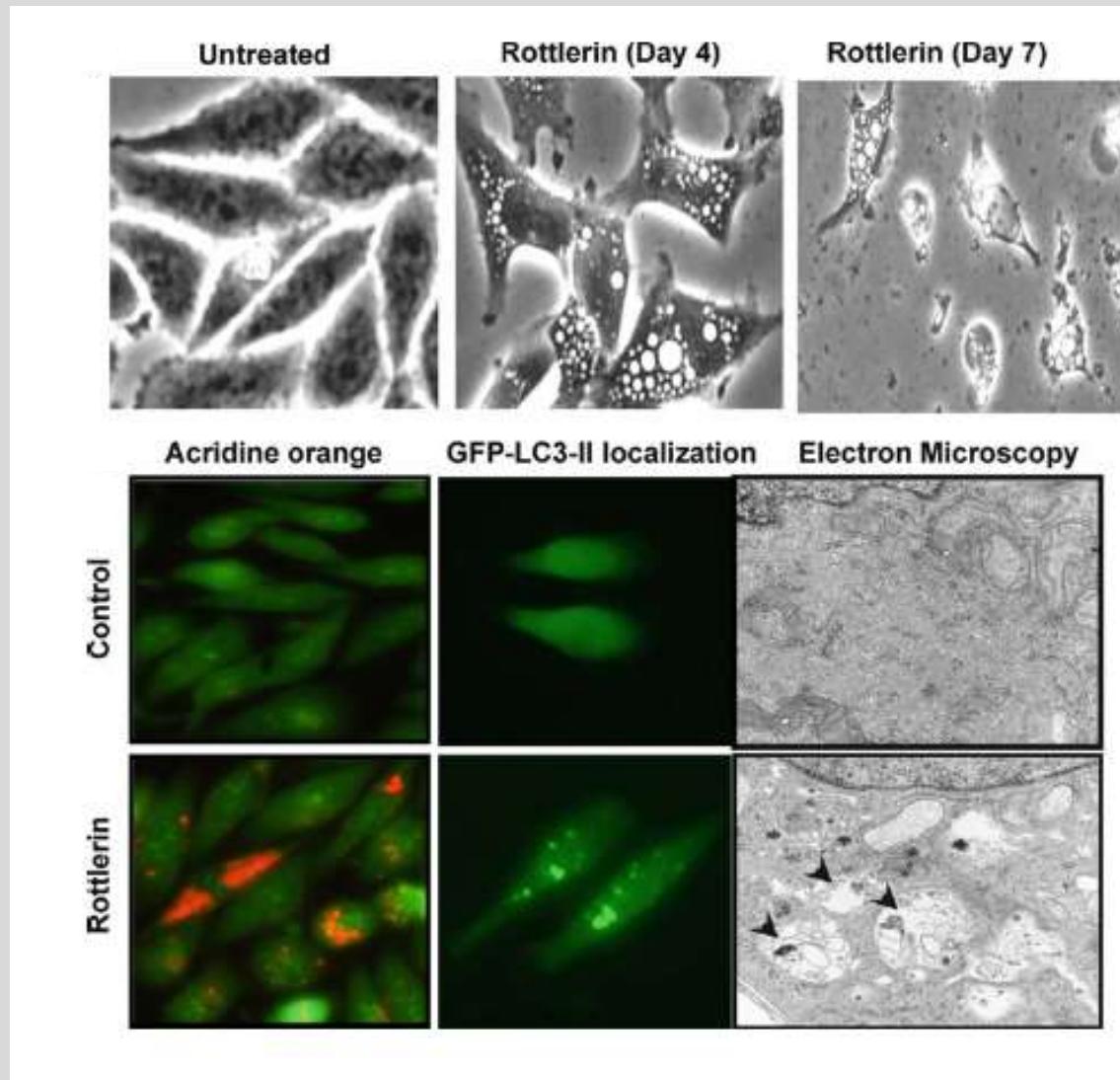
7-ketocholesterol



7 β -hydroxycholesterol

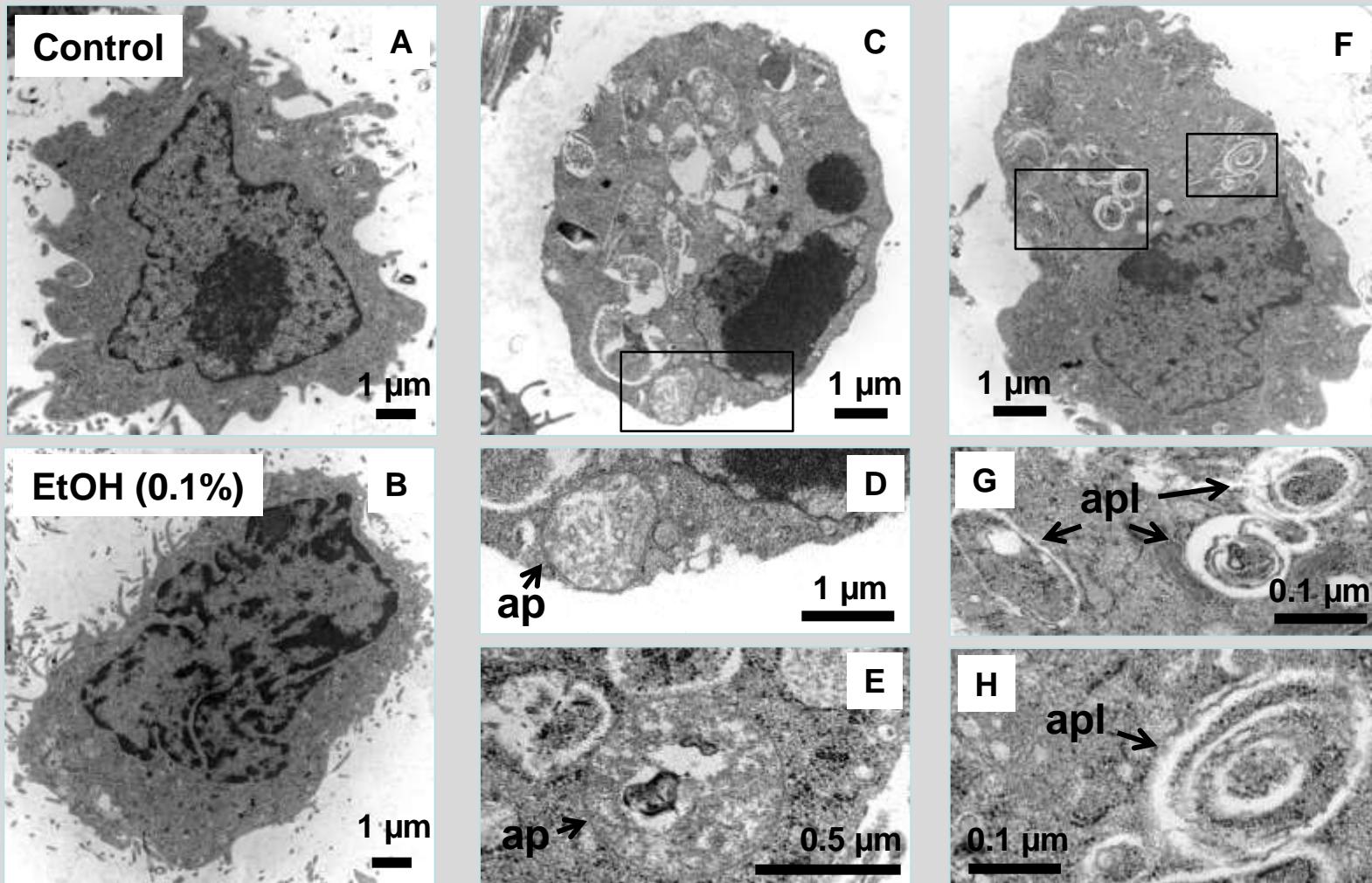


CHARACTERIZATION OF AUTOPHAGY: ‘VISUAL’ CRITERIA - LIPOPHAGY



CHARACTERIZATION OF AUTOPHAGY: ULTRASTRUCTURAL CRITERIA – Transmission Electron Microscopy

7KC (50 μ M, 24 h)

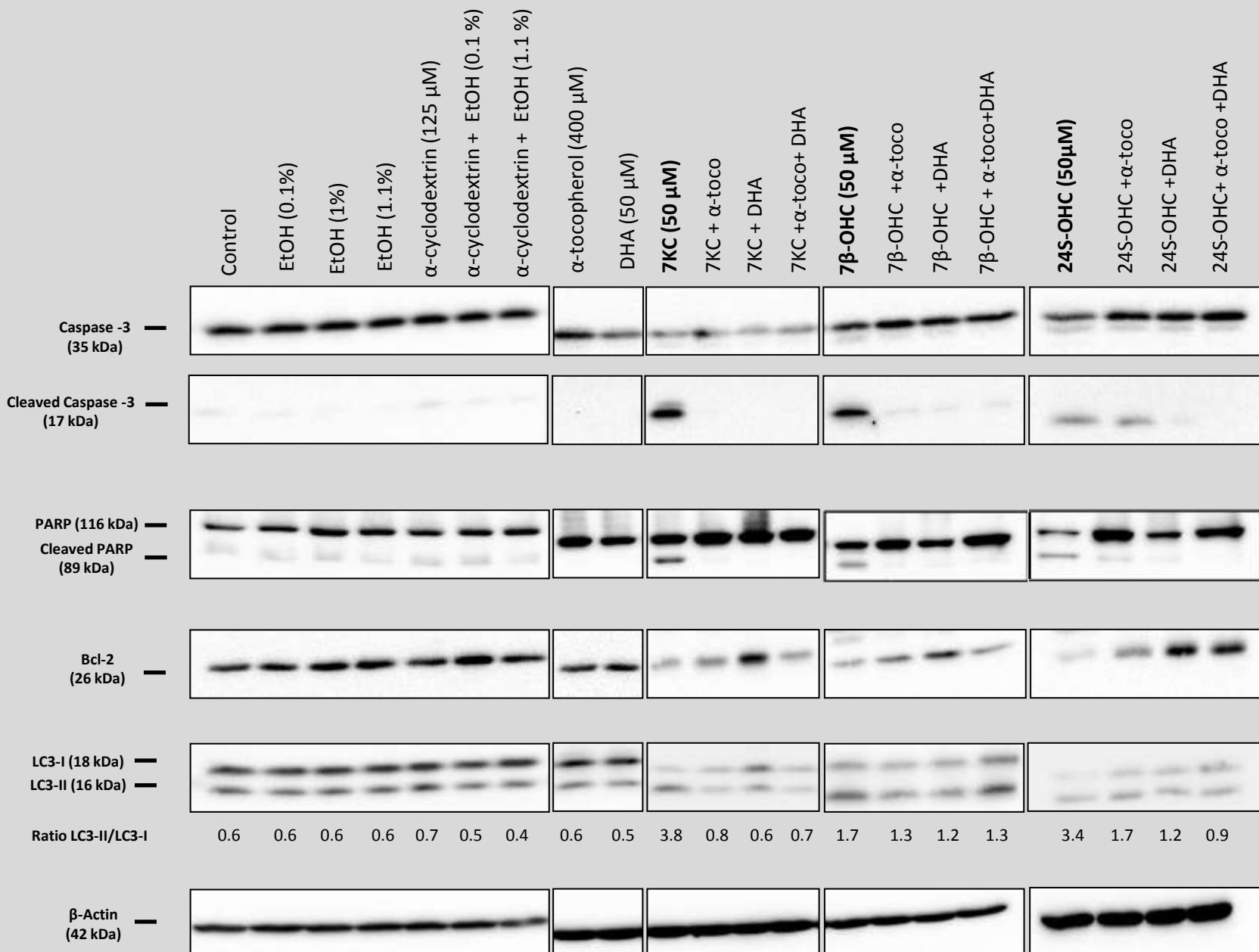


ap: autophagosome; **apl:** autophagolysosome

Gérard Lizard

CHARACTERIZATION OF AUTOPHAGY: BIOCHEMICAL CRITERIA

Murine oligodendrocytes (158N) - autophagy and apoptosis (oxiaapoptophagy)



Gérard Lizard,

Etude du Métabolisme Lipidique : Utilisation du Nile Red

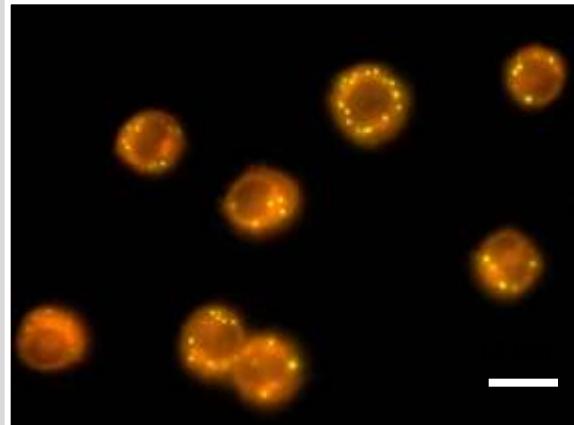
Nile Red



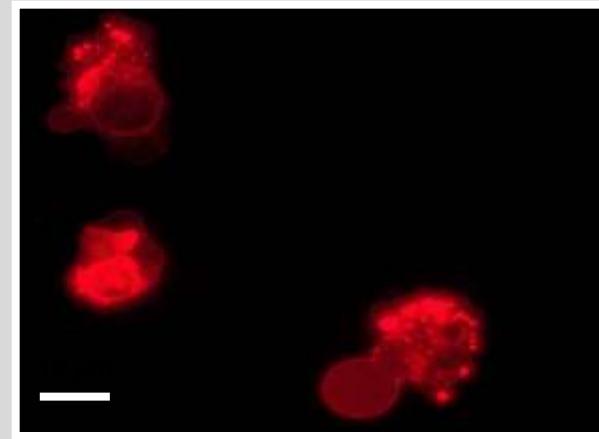
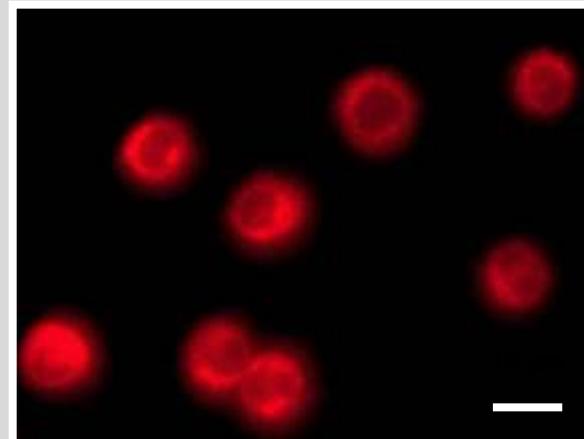
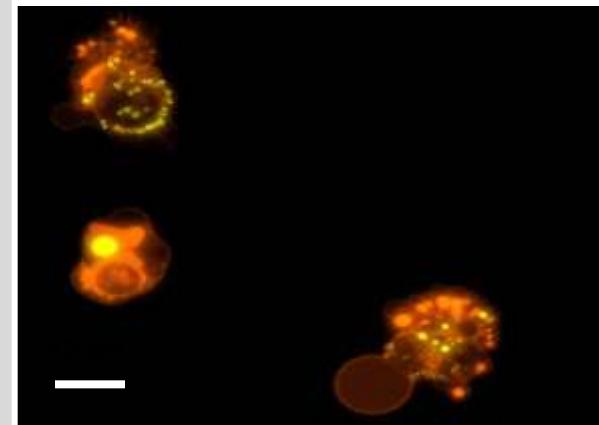
- ° Excitation 488 nm
- ° Emission > 515 nm

- ° Excitation 534–558 nm
- ° Emission > 590 nm

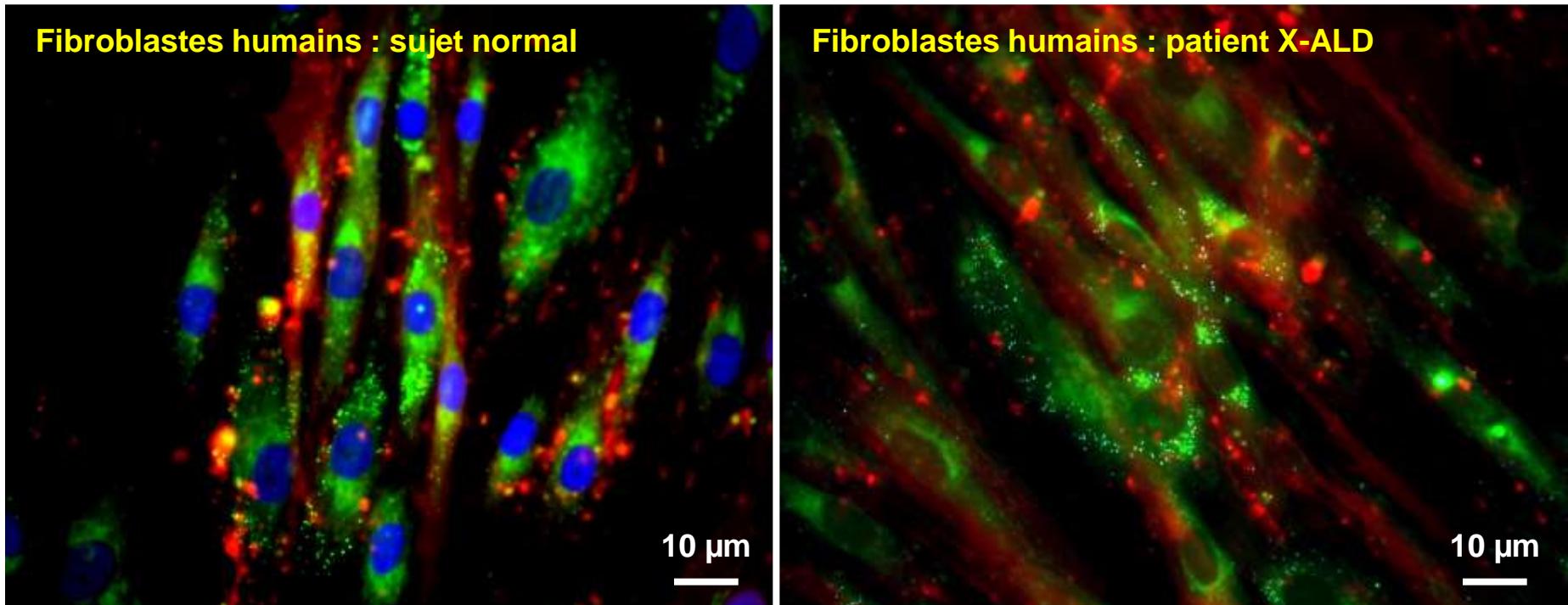
Cellules U937 non traitées



Cellules U937 traitées 7-cétocholestérol



Distinction des Lipides Neutres et polaires avec le LipidTOX



LipidTOX (Réf H34158; Invitrogen)

* Lipides neutres : fluorescence verte * Lipides polaires : fluorescence rouge
Contrairement au Nile Red, l'extinction de fluorescence est faible
Microscopie confocale possible dans de bonnes conditions

Fluorochromes dédiés à l'analyse des lipides

Sondes	Cibles	Utilisation	Applications
Nile Red	Lipides polaires et neutres	Microscopie, cytométrie en flux, analyse spectrale	Phospholipidose, stéatose
HCS LipidTOX	Lipides neutres et phospholipides	Microscopie, analyse spectrale	Phospholipidose, stéatose
Filipine	Cholestérol non estérifié	Microscopie, cytométrie en flux	Hypercholestérolémie
BODIPY	Lipides neutres	Microscopie, cytométrie en flux	Métabolisme des lipides, radeaux lipidiques
Laurdan	Phospholipides	Polarisation de fluorescence, microscopie confocale multiphotonique	Fluidité membranaire, radeaux lipidiques
Mérocyanine 540	Phospholipides	Microscopie, cytométrie en flux	Externalisation des phosphatidylsérines
Cis-parinaric acid	Acides gras insaturé	Microscopie, cytométrie en flux	Peroxydation lipidique
DPH	Lipides de la membrane plasmique	Polarisation de fluorescence	Fluidité membranaire