Lysosomes and lysosomal disorders
Eukaryotic cell
Lysosomes

- Degradation of macromolecules
- Calcium store
- Cholesterol homeostasis
- Lysosomal exocytosis - plasma membrane repair
- Cell death

Ca++/Na+

pH ~ 4-5

ATP
ADP + Pi

Image M.H.
Late-endosomal Intralumenal vesicles are formed from domains on the endosome membrane.

Ubiquitylated membrane proteins are sorted into endosomal membrane domains, which sequestrate to form intralumenal vesicles.

Multivesicular bodies = maturing endosomes with intralumenal vesicles.
Lysosomal („storage“) diseases

Deficiencies of proteins from the lysosomal system lead to storage of material in lysosomes
Disorders of transport of enzymes into lysosome or disorders of substrate transport (e.g. due to a disruption of vesicular transport inside the cell) can also lead to lysosomal storage.
Lysosomal disorders

Hereditary disorders associated with storage of material within the lysosomes

1. Disorders of glycan degradation - mucopolysaccharidoses and glycoproteinoses
2. Lipidoses
3. Proteinoses
4. Disorders of lysosomal transport of metabolites
5. Disorders of transport of proteins into lysosomes
Lysosomes
Lysosomes are the principal sites of intracellular degradation of macromolecules.

About 40 types of acid hydrolases - proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases.

Acidic pH optimum – protection of cytosol (neutral pH)

Acidic environment – (pH 4.5 -5) – maintained by vacuolar H+ ATPase

H+ gradient drives transport of small molecules across the membrane

Lysosomal membrane proteins are highly glycosylated – protection from proteolytic attack

Provide interface for various lysosomal functions
Maturation of lysosomes

Late endosome

Endolysosome

Endoluminal vesicle

Phagosome (autophagosome)

Lysosome

Hydrolase

adapted from Alberts et al. Molecular cell biology
Lysosomes and vacuolar transport

- EE – early endosome
- LE – late endosome
- M6PR – mannosa-6-phosphate receptor
- LY – lysosome
- NC – nucleus

M6PR - „scavenger pathway“

endocytosis → phagocytic vacuole → chaperone mediated autophagy → LE → LY → exocytosis

secretory vesicle → Golgi → M6PR
LYSOSOMES

can KILL !!!!

and their relatives
In some cells (often of haematopoietic origin) there are organelles that have properties of both lysosomes and secretory granules:
- acidic pH
- lysosomal membrane and lumenal proteins
- exocytosis in response to a stimulus

Lysosome-related organelles (LRO)
- lytic granules (NK cells and cytotoxic T-lymphocytes)
- azurophilic granules
- melanosomes
- “external“ lysosomes of osteoclasts
- delta-granules in platelets
Lysosome-related organelles - osteoclast

- Sealing zone
- Ruffled border
- Bone

H^+ H^+ H^+
Multiple pathways deliver material to lysosomes

EE – early endosome
LE – late endosome
M6PR – mannose-6-phosphate receptor
LY – lysosome
NC - nucleus

M6PR - „scavenger pathway”

endocytosis
macropinocytosis

phagocytosis

chaperone mediated autophagy

exocytosis
secretory vesicle

autophagy
Autophagy

Macroautophagy

Microautophagy

Chaperone-mediated autophagy

- proteins containing specific signal sequence
- translocation of proteins driven by binding of chaperones
- internalization via lamp2a receptor in the lysosomal membrane

Lysosomal membrane protein **LAMP2** is a receptor involved in fusion of autophagic vacuoles with lysosomes
Autophagy is a process of self-degradation of cellular components.

Double-membrane autophagosomes sequester organelles or portions of cytosol and fuse with lysosomes.

Autophagy is upregulated in response to signals such as:
- starvation
- growth factor deprivation
- ER stress
- pathogen infection.

Mizushima, Genes and Development, 2007
Morphology of autophagosome and autolysosome

- **Arrows**: autophagosomes
- **Double arrows**: autolysosomes/amphisomes.
- **Arrowheads**: fragments of endoplasmic reticulum inside the autophagosome

Mizushima, Genes and Development, 2007
Import of lysosomal proteins into lysosome

**Soluble lysosomal proteins:**
– mannose-6 phosphate receptor

**Lysosomal membrane proteins:**
- signals in short C-terminal “tail”)
- signals are recognised by adaptor proteins (AP3..)

**Other**
- glucocerebrosidase, lysosomal acid phosphatase
- prosaposin
- sortilin, LIMPII
Alteration of metabolic, signalling, and transport pathways in lysosomal disorders
Alteration of metabolic, signalling, and transport pathways in lysosomal disorders

- Accumulation of secondary metabolites
- Alterations of calcium homeostasis
- Free radicals and oxidative stress
- Neuroinflammation
- Abnormal autophagy
Alteration of metabolic, signalling, and transport pathways in lysosomal disorders

- **Accumulation of secondary metabolites**

- In many lysosomal disorders are stored also metabolites unrelated to the primary defect, very often lipids or hydrophobic proteins

- Frequently gangliosides GM3, GM2 or cholesterol ... although the protein machinery for their degradation or transport is intact

- Example: in some mucopolysaccharidoses (storage of polysaccharides) is in the brain present storage of glycolipids - gangliosides GM2 a GM3

Heparan sulfate/heparin (HS)
Alteration of metabolic, signalling, and transport pathways in lysosomal disorders

- **Alteration of calcium homeostasis**

- Disorders of calcium homeostasis can contribute to the pathogenesis of the disease

- **Example:**
  - Glucosylceramide: the glycolipid stored in Gaucher disease modulates the function of ryanodine receptors in neurons and leads to more prominent release of calcium from ER to cytosol

- In other lysosomal disorders were described different alterations of calcium homeostasis - different mechanisms

Structure of predominant species
Alteration of metabolic, signalling, and transport pathways in lysosomal disorders

- **Free radicals and oxidative stress**

- signs of increased production of free oxygen radicals and oxidative stress

- there is no obvious mechanism - secondary elevation of free radical production due to e.g. endoplasmic reticulum stress

- Oxidative stress can contribute to pathogenesis of lysosomal disorders, especially in the brain
Alteration of metabolic, signalling, and transport pathways in lysosomal disorders

• **Neuroinflammation**

• Signs of neuroinflammation is present essentially in all lysosomal disorders with CNS involvement

• Activation of immune system – microglia and astrocytes

• Similar findings are present in „classic“ neurodegenerative disorders

• **Chronic glial activation** in lysosomal disorders apparently contributes to neuronal damage
Alteration of metabolic, signalling, and transport pathways in lysosomal disorders

• **Abnormal autophagy**

• vacuolar mechanism for degradation of damaged organelles and long-life proteins
• signs of increased autophagy is present in many lysosomal disorders, can lead to cell damage and cell death
• the mechanism of activation of autophagy is not clear, but may contribute to cell damage

• (Danon disease – deficiency of LAMP2 – accumulation of autophagic vacuoles)
Transport of soluble lysosomal proteins by mannose-6-phosphate receptors
The majority of soluble (luminal) lysosomal proteins is transported into lysosome via mannose-6-phosphate receptor.
M6P signal is built on N-linked oligosaccharides of hydrolases by Glc Nac phosphotransferase in cis-Golgi

N-acetylglucosamine phosphotransferase (GlcNac phosphotransferase) recognises a 3-D pattern on lysosomal enzymes

Protective GlcNac group is enzymatically removed in trans-Golgi, leaving M6P exposed

GlcNac phosphotransferase

GlcNac di-phospho uridine

Hydrolase with N-linked oligosaccharide

GlcNac

Hydrolase carrying M6P moiety

UMP

Image M.H.
MP6 receptors capture lysosomal enzymes by receptor-mediated endocytosis at plasma membrane.
Sorting of proteins containing MP6 signal

protein-M6P-M6PR → lysosome

protein-M6P protein → protein

protein → Secretion pathway

cis-Golgi

mannose-6-phosphate
Lysosomal membrane proteins
Lysosomal membrane contains more than 100 proteins, majority of which have unknown function. Proteins with known function include receptors, molecules participating in vesicular transport, transporters of small molecules, vacuolar ATPase etc.

Oligosaccharide chains at the inner face of lysosomal membrane for a glycocalix protecting the membrane from the attack of hydrolases

LAMP 2 (lysosomal associated membrane protein 2) is a receptor for autophagic vacuoles
Activators of lysosomal hydrolases
Exoglycosidases participating in degradation of oligosaccharide moieties of glycolipids require protein activators for glycolipids with less than 3 residues.
Activators of lysosomal hydrolases

Saposins A,B,C,D

deficits of saposins lead to variant forms of disorders caused by deficiencies of enzymes they activate

GM2 activator activates hexosaminidase A
Overview of lysosomal disorders
Lysosomal enzymes

30 enzymes – hereditary deficiencies of which cause human diseases

**lipids** – lipidoses, including sphingolipidoses

**glykosaminoglycans** – mucopolysaccharidoses

**N-glycans, oligosacharides** – glycoproteinoses

**glycogen** – glycogenosis type II (Pompe)

**proteins** – proteinoses
N-acetyltransferase activity

- Patients: n=5
- Heterozygotes: n=22
- Controls: n=103

R412X/wt
Fabry disease – alpha-galactosidase A deficiency

X-linked disease

lysosomal storage of glycolipids with terminal alpha-galactose, predominantly globotriaosylceramide

storage in vessel endothel, smooth muscle of the vessels, cardiomyocytes, glomerules and tubules and other cell types
Fabry disease – clinical picture

hypertrophic cardiomyopathy, arythmias

chronic progressive renal disease leading to renal failure

TIA, parestesias

angiokeratomas, cornea verticilata

X-linked disease

In females the severity of phenotype depends on X-inactivation
Gaucher disease

Lysosomal storage disorder

Deficiency of glucocerebrosidase (acid beta glucosidase)

Accumulation of glucosylceramide preferentially in cells of macrophage origin (Gaucher cells)

Multisystem disorder

Hepatomegaly, splenomegaly, bone disease, trombocytopenia, anemia, lung infiltration

In type 2 and 3 Gaucher disease: CNS disease

Clinical variability, chronic progression
Type 1: chronic non-neuronopathic
Type 2: acute neuronopathic
Type 3: chronic neuronopathic
Heterozygosity or homozygosity for a mutation in the glucocerebrosidase gene is a susceptibility factor for Parkinson's disease.

Molecular mechanism is not clear, ? tau protein transport disorder ?

Strong epidemiologic evidence for the association

Mutant glucocerebrosidase is present in Lewy bodies in Gaucher patients with Parkinson disorder.
Niemann-Pick type C disease

- Disorder of intracellular lipid trafficking, especially of cholesterol
- Accumulation of unesterified cholesterol and glycolipids in late endosomes/lysosomes
- Disorder of LDL-derived cholesterol
- Abnormal fusion of late endosomes and lysosomes, abnormal filling of lysosomes with Ca$^{++}$

Mutations in two cholesterol-transporting proteins: NPC1 and NPC2

NPC1 is more frequent (about 95% of NPC)

- (Note: Niemann-Pick type A and B are caused by the deficiency of acid sphingomyelinase)
Figure 2 Niemann-Pick disease type C as a neurovisceral disease. Schematic representation of the main forms of the disease, with particular emphasis on type and age of onset of first neurological symptoms.
• Disorder of **intracellular lipid trafficking**

• Neurovisceral disorder: highly variable clinical picture
• Prolonged neonatal jaundice of cholestasis, hepatosplenomegaly or isolated splenomegaly
• Later **progressive neurological disease** – ataxia, clumsiness, falls, spasticity, seizures, dysarthria or dysphagia
• Typical signs: vertical gaze palsy, gelastic cataplexy
• **Psychiatric signs:** presenile cognitive decline, dementia, paranoia (hallucinations, ...)

Niemann-Pick disease type C
Intracellular transport of LDL cholesterol
Function of NPC1 and NPC2

- Soluble NPC2 binds LDL-derived cholesterol and transfers it to NPC1
- NPC1 transfers cholesterol molecules across glycocalyx at the lumenal face of the lysosome
Mucopolysaccharides

Polysaccharides

Heparan sulfate
Dermatan sulfate
Keratan sulfate
Chondroitin sulfate
Families of proteoglycans expressed in cartilage: representative members

- **BIGLYCAN**
  - CS/DS binding region
  - Leucine-rich repeats
  - HS/CS binding region
  - SEA homology
  - LDH receptor module

- **GLYPICAN-3**
  - Cysteine-rich region
  - HS chain binding region
  - Ig-like repeats
  - EGF-like
  - Laminin homology 1

- **SYNDIECAN-3**
  - Transmembrane Domain
  - Constant domain 1
  - Variable domain
  - C2 PDZ-binding domain

- **AGGREGAN**
  - EGF-like
  - Lectin-like
  - CRP-like

- **PERLECAN**
  - Laminin EGF-like
  - Laminin homology 2

Legend:
- HA binding region
- KS chain binding region
- CS chain binding region
- HS chain binding region
- GPI anchor
Mucopolysaccharidoses

11 disorders

**Most common:**
MPS I Hurler disease - deficiency of alpha-iduronidase, AR-inheritance
MPS II - Hunter disease - deficiency of iduronate sulfatase, X-linked

**Common symptoms**
Progressive dementia, hepatosplenomegaly, coarse features (gargoylism), bone disease (dysostosis multiplex), corneal opacities, cardiac disease

Alberts *et al* : Molecular biology of the cell 6 edition
Mukopolysaccharidosa III, MPS III
Sanfilippova choroba

In the first years of life normal development
At 2 – 6 years of age prominent hyperactivity, sleep disorders, slowly progressive dementia

Coarse facies, coarse hair
drsné vlasy, small
hepatosplenomegaly

Spasticity, dementia,
death usually
between 15 - 25 years of age
Fig. 140-4 Probable steps in degradation of complex oligosaccharide structure.
Activators of lysosomal hydrolases

Saposins A, B, C, D

deficits of saposins lead to variant forms of disorders caused by deficiencies of enzymes they activate

GM2 activator
activates
hexosaminidase A
I-cell disease (mucolipidosis II)

Disorder of transport M6P-tagged lysosomal proteins due to mutations in GlcNAC phosphotransferase

**Increased activities of lysosomal proteins in extracellular fluid**

**Decreased activities of multiple lysosomal enzymes in lysosomes**

**Enlarged lysosomes**
Mutations in GlcNAc transferase gene
Mutations in GlcNAc transferase gene

Proteins transported normally by M6PR are not targeted to lysosomes... instead, they are secreted out of the cell.
I-cell disease

Coarse facies
thickening of gums
small hepatomegally and
splenomegally
bone disease - dysostosis
multiplex
psychomotor delay, mental deficit
elevated activities of lysosomal hydrolases in
plasma, low activities in tissues

Vacuolization of lymphocytes („Inclusion cell“)
= storage lysosomes
Figure 1 A lymphocyte with many vacuole-like inclusions (original magnification, x900).

Figure 3 Electron microscopic image of lymphocytic vacuoles containing round osmiophilic structures (original magnification, x15 000).

Figure 2a: X-ray of hand showing shortening of tubular bones and proximal tapering of 2nd to 5th metacarpals.

Figure 2b: Lateral X-ray of the spine showing ovoid vertebral bodies and "hammer shaped" vertebrae. The ribs are widened and "ear shaped".
Danon disease – LAMP2 deficiency

Lamp 2 participates in fusion of lysosomes with autophagic vacuoles

Cardiomyopathy - usually hypertrophic
Arrhythmia - typically preexcitation syndrome - WPW

Intelectual disability in some patients

Other symptoms

X-linked disease - females have usually milder phenotype

Accumulation of autophagic vacuoles predominantly in cardiac and skeletal muscle
Cystinosis – cystinosin deficiency
renal disease with Fanconi syndrome
renal failure – renal transplantation
corneal crystals, photophobia
growth retardation
hypothyroidism
normal intelligence

ocular form

Sialuria – sialin deficiency
B  Untreated cystinotic lysosome

C  Cysteamine-treated cystinotic lysosome

Cystine

Cysteamine

Mixed disulfide of cysteine and cysteamine

Cysteine

Cystine

Cysteamine
Cystinosis
Cystinosis

Figure 4. Renal Function in Patients with Cystinosis Treated with Cysteamine and in Untreated Patients, According to Age.
Disorders of lysosome-related organelle biogenesis and function

A group of hereditary disorders often associated with
- albinism (melanosome dysfunction)
- visual impairment
- bleeding tendency (platelet dysfunction)
- inflammatory bowel disease
- lung fibrosis
- immunodeficiency
- “huge lysosomes” in tissues

Heřmanský-Pudlák, Griscelli, Chediak-Higashi syndromes
Diagnostics and treatment of lysosomal disorders
Treatment

Supplementation of deficient protein

Bone marrow transplantation

Enzyme replacement therapy

Reduction of stored substrate

Substrate inhibition therapy
Bone marrow transplantation

Haematopoietic stem cell transfer

Pro:
In contrast to enzyme replacement therapy can influence CNS disease

Con:
High morbidity and mortality

Lysosomal disorders
Mucopolysacharidosis I
  Modifies natural course of the disease
  Early treatment can prevent neurological disease
  Residual disease
Other MPS disorders
MPS III – no improvement of neurological progression
Other lysosomal disorders

Peroxisomal disorders
X-ALD

Enzyme supplementation therapy

Supplementation of deficient enzyme in regular infusions

Gaucher disease (glucocerebrosidase)
Fabry disease (alpha galactosidase A)
Pompe disease (acid alpha glucosidase)
MPS I (alpha iduronidase)
MPS II (alpha iduronate sulfatase)
MPS VI, Maroteaux-Lamy (arylsulfatase B)
Niemann-Picko disease B (acid sphingomyelinase)
MPS IVA, Morquio A, ...

Production of recombinant enzymes
Genzyme, TKT, Biomarin, Shire, Inotech, ...
Enzyme supplementation therapy in Gaucher disease

Receptor-mediated endocytosis

Macrophage targeted glucocerebrosidase - treatment with exoglycosidases

Mannose receptor (macrophages, endothelia, liver)

Regular infusions

Originally glucocerebrosidase isolated from human placentas (Ceredase, Genzyme)

Recombinant enzyme

Cerezyme (Genzyme) – Cho cells

Does not cross haematoencephalic barrier

High costs
b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

„Substrate inhibition (reduction) therapy“
Substrate inhibition therapy

Mutant enzymes have residual activities

N-butyldeoxyjirinomycin (Zavesca)

Inhibitor of glucosylceramide synthase

Gaucher disease, GM1 gangliosidosis
Diagnostics

Measurement of metabolites

Enzyme activity measurement

Mutation analysis

Morphological diagnostics