Lysosomes and lysosomal disorders
Eukaryotic cell
Lysosomes

- Degradation of macromolecules
- Calcium store
- Cholesterol homeostasis
- Lysosomal exocytosis - plasma membrane repair
- Cell death
Lysosomes
Lysosomes are the principal sites of intracellular degradation of macromolecules

about 40 types of acid hydrolases - proteases, nuclease, 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 endsosome

Endolumenal vesicle

Phagosome (autophagosome)

Endolysosome

Hydrolase

Lysosome

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“
“Unusual” lysosomes
Secretory lysosomes /Lysosome-related organelles

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

$H^+$
Transport of proteins and material for degradation to lysosomes
Multiple pathways deliver material to lysosomes

- Golgi
- LE (late endosome)
- phagocytosis
- autophagy
- endocytosis
- macropinocytosis
- LY (lysosome)
- EE (early endosome)
- M6PR (mannosa-6-phosphate receptor)
- NC (nucleus)
- exocytosis
- secretory vesicle

M6PR - “scavenger pathway”

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Image M.H.
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
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
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
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
Sorting of proteins containing MP6 signal

protein-M6P-M6PR → lysosome

protein-M6P protein

protein → Secretion pathway

cis-Golgi

trans-Golgi
MP6 receptors capture lysosomal enzymes by receptor-mediated endocytosis at plasma membrane.

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M6PR - „scavenger pathway“
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
General features of lysosomal disorders
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.
LSD: Common phenotypical features and affected organs

Central nervous system: neurodegeneration, ...
Spleen, liver: hepato and splenomegaly, hepatopathy ...
Skeleton: Facial dysmorphy, dysostosis multiplex, ...
Peripheral nervous system: peripheral neuropathy,..
Heart – cardiomyopathy, valve disease, ...
Kidney: renal failure, nefrolithiasis ...
Skin: agiokeratomas, ...
Eye: cataracts, corneal clouding, cherry-red spot, retinal degeneration, ...
Ear: Sensorineural deafness, ...
Bone marrow: anemia
Lungs: 

MPS VI, http://www.ojrd.com/content/figures/1750-1172-5-5-4-l.jpg
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
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 autofagy
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
Overview of lysosomal disorders
Lysosomal enzymes

30 enzymes – hereditary deficiencies of which cause human diseases

**lipids** – lipidoses, including sphingolipidoses

**glykosaminoglycans** – mucopolysaccharidoses

**N-glycans, oligosaccharides** – glycoproteinioses

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

**proteins** – proteinioses
Lipidoses – 9 types

Gaucher disease – glucocerebrosidase deficiency

Fabry disease – alpha-galactosidase A deficiency

Niemann-Pick disease type A/B – acid sphingomyelininase deficiency

Niemann-Pick disease type C - deficit of proteins involved in intracellular transport of unesterified cholesterol

Krabbe disease - beta-galactosylceramidase deficiency

Metachromatic leukodystrophy – arylsulfatase A deficiency
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 – symptoms

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
Females are mosaics

The size of X-inactivation patches differs between tissues

Skewing of X-inactivation may influence phenotype

Patch size may confound testing of clonality, enzyme activity etc. in tissues

Patch size may influence cross-correction of the defect by endocytosis of enzyme from cells expressing wild-type allele (in heterozygotes)

Marco Novelli et al. PNAS 2003;100:3311-3314
G6PD staining in the intestine in G6PD carriers
Example of arrhythmia in Fabry disease: Atrial fibrillation with slow ventricular response and a heart rate of 56 bpm. Criteria for LVH with diffuse abnormal repolarization.

http://www.lysosomalstorageresearch.ca/Fabry_eClinic/electrocardiography-ecg.html
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 (GBA) is a susceptibility factor for Parkinson’s disease (PD).

Molecular mechanism is not clear, ? tau protein or α-synuclein transport disorder?

Strong epidemiologic evidence for the association, 5%-10% of PD patients carry GBA mutations, Odds-ratio 16-28

Mutant glucocerebrosidase is present in Lewy bodies in Gaucher patients with Parkinson disease

Association with GBA mutations also shown in dementia with Lewy bodies
Niemann-Pick disease type C

- 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 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)
**Systemic involvement**

(hepato) Splenomegaly
- Absent in ~15% of cases
- Age of onset is variable
  - always before neurological signs
- May regress with age

*Neonatal Splenomegaly*
- Neonatal fatal
- Liver
- Foetal ascites/hydrops

*Age, years*
- Birth
- 1
- 2
- 3
- 6
- 10
- 20
- 30
- 40
- 50
- 60

**Neurological involvement**

*Early* Infantile
- Delay in motor milestones
- Hypotonia

*Late* Infantile
- Gait problems
- Speech delay
- Cataplexy

*Juvenile*
- School problems
- Ataxia
- (Seizures)
- (Cataplexy)

*Adult*
- Psychiatric problems
- Ataxia, Dystonia
- (Dementia)

*Vertical supranuclear gaze palsy*

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*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

Vanier 2010
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 glycocalix at the lumenal face of the lysosome
- Treatment in trials: propyl beta cyclodextrin intrathecally
Mucopolysaccharides

Polysaccharides

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

G1
- HA binding region

G2
- IgG-like
- KS chain binding region
- Leucine-rich repeats

G3
- EGF-like
- Lectin-like
- CRP-like

BIGLYCAN
- CS/DS binding region

AGGREGAN
- Transmembrane Domain
- Constant domain 1
- Variable domain
- C2 PDZ-binding domain

SYNDENCAN-3
- HS chain binding region

GLYPICAN-3
- Cysteine-rich region
- HS chain binding region
- GPI anchor

PERLECAN
- SEA homology
- LDH receptor module
- HS/CS binding region
- Laminin EGF-like
- Laminin homology 2
- Ig-like repeats
- EGF-like
- Laminin homology 1
Glycosaminoglycans are degraded by sequential action of glycosidases.
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
Mukopolysaccharidosis III, MPS III
Sanfilippo disease

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
Glycoproteinoses: Hereditary deficits of enzymes degrading sugar moieties of glycoproteins
Clinically similar to mucopolysaccharidoses

Fig. 140-4 Probable steps in degradation of complex oligosaccharide structure.
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

don't work in the endoplasmic reticulum.
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 (Mucolipidosis II)

Deficiency of GLCNac-phosphotransferase
Coarse facies, thickening of gums, small hepatomegally and splenomegally, 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).

Dysostosis multiplex in I-Cell disease

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: myopathy sudden death

X-linked disease - females have usually milder phenotype

Accumulation of autophagic vacuoles predominantly in cardiac and skeletal muscle
Danon disease

- **Cardiomyopathy**: hypertrophic, dilated, Wolf-Parkinson-White syndrome
- **Skeletal myopathy**: proximal muscle weakness
- **Intellectual disability**

Earlier onset in males: typically after the first decade of life

Females: diagnosis typically in third decade of life

X-linked disorder

Treatment: no causal therapy, heart transplantation, defibrilators

Differential diagnosis: Pompe disease, vacuolar myopathies
Deficiencies of lysosomal permeases lead to lysosomal accumulation of small molecules

- **Cystinosis:** Cystinosin deficiency
  - renal disease with Fanconi syndrome
  - corneal crystals, photophobia, growth retardation
  - hypothyroidism
  - normal intelligence
  - lysosomal accumulation of cystine

- Isolated ocular form

- Mixed disulfide with cysteamine is transported by permease for lysine

Lysosomal transporters deficiencies

**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**
Cystinosis

cystin
cysteamin
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
Enzyme supplementation therapy

Supplementation of deficient enzyme in regular infusions

Gaucher disease (glucocerebrosidase)
Fabry disease (alpha galactosidase A)
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Production of recombinant enzymes
Genzyme, TKT, Biomarin, Shire, Inotech, ...
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