Cell Biology – FS 2017

(c.f. Molecular Biology of the Cell – Alberts et al. 6th edition; papers referred to in the lecture is on moodle) – (Suter's part)

Exam: The lecture is the basis of the exam.
Good repition: https://www.youtube.com/watch?v=OKr-9WJTHME

Questions to be discussed: How do cells form a tissue (and tissues an organ). Also, it will be looked at their development, regeneration, diseases and homeostasis.

Make a list with factors responsible for hypermyelination and hypomyelination.

Make an overview of all growth factors.

We learned many different factors involved in tumors. Make some overview (mind-map) what is involved in tumorigenesis.

The chosen model: The Central Nervous system

Myelination of the nervous system (=: NS)
Main purpose: increase of speed and efficiency at which neural impulses travel along myelinated fibers. Improvement of the regulation of information transfer.
For example, myelination increases the speed of the action impulses to travel by a tenfold (30 ms if myelinated, 300 ms if not myelinated.)

In the CNS, they are called oligodendrocytes and in the PNS, Schwann cells.

(I always need to know how a system is built and optimized for its function, which is equivalent to its anatomy and structure as the basis of physiology.)

Significance of myelination
Major diseases: Leukodystrophies (genetic myelin deficiencies), MS, Neonatal white matter disorder, amyotrophic lateral sclerosis (metabolic support of neurons and axons), psychiatric disorders (neural circuit abnormalities), memory decline in aging (correlated with reduced amounts of myelin), relation or repair (spinal cord injury, trauma, stroke).

Vertebrate myelination is an evolutionary advancement that is essential for motor, sensory and cognitive function.

Basics of functional anatomy and physiology – a cell biology point of view

The cell body has an axon initial segment which initiates an action potential. This action potential propagates along the myelinated fiber to the next cell.

The brain needs so much ATP, because it needs to pump out the Na+ out of the neuron.

Structure determines function:
What is the mechanism by which an oligodendrocyte extends its plasma membrane to wrap axons and to generate the multilayered compact myelin sheath around axons?

Reorganization of the cytoskeleton (one of the reasons).
Process: glial-axonal contact and retraction, axon segment selection, contact stabilization and polarization, lateral and radial expansion also called wrapping, compaction, maturation and incisure closure (cytoplasmic channels spiralling through developing myelin).
Myelin outgrowth occurs at the innermost tongue and the lateral edges.

Conceptual steps leading to CNS myelination:
Proliferation and migration of oligodendrocyte precursor cells in white matter tracts
Recognition of target axons and axon-glia signalling
Differentiation of OPCs into myelinating oligodendrocytes
Trafficking of membrane components
Myelin compaction (extrusion of cytoplasm)
Nodes of Ranvier formation

Achievement of proper myelin compaction as a requirement for correct function:
(PIC OF ANATOMY OF NEUROMUSCULAR SYSTEM)

Myelin proteins in CNS and PNS:

In CNS: PLP, MOG, MAG, MBP, CNP

In PNS: P0, CNP, MAG, FASN, no PLP

PMP22 (peripheral myelin protein 22) is a gene that is expressed into a 160 amino acids long protein in Schwann cells (in the PNS). PMP22 does not seem to appear in the CNS.
Malfunctions of PMP22 have been linked to Marie-Charcot-tooth type 1A disease (=: CMT1A) when it is underproduced and to HNPP when it is overproduced.

Proteolipid protein. Myelin PLP, also known as the Folch-Lees protein [11], has the unusual physical property of solubility in organic solvents. The molecular weight of PLP from sequence analysis is about 30,000, although it migrates anomalously fast on SDS gels. The amino acid sequence, strongly conserved during evolution, contains several membrane-spanning domains.

(In addition to PLP, myelin of the CNS has lesser quantities of a related protein, DM-20, named for its Mr of 20,000. This protein is coded by an alternative splicing of the RNA, which gives rise to the major PLP. Both DNA and protein-sequencing data indicate that the structure of DM-20 is related to that of PLP by a deletion of 35 amino acids [13,14]. DM-20-related message appears earlier than PLP during development, even before myelin formation in some cases; and it might have a role in oligodendrocyte differentiation in addition to a structural role in myelin. The PLP and DM-20 proteins may be evolved from an ancestral gene encoding a pore-forming polypeptide [15], lending support to the hypothesis that myelin may be involved in ion movement. Although PLP and DM-20 serve important functions, they are not essential. Contrary to the general expectation that PLP would be needed for formation of compact, multilamellar myelin, a knockout mouse for PLP/DM-20 [16] is relatively normal with respect to myelin formation, although there is a difference at the level of the intraperiod line. In this knockout mouse, life span and sophisticated motor performance also are affected. In contrast, a variety of naturally occurring

mutations in PLP (see Chap. 39) or overexpression of normal PLP [17] have severe functional consequences, apparently due to cellular toxicity of mutated forms of the protein or even just excess amounts of normal PLP. A curiosity is that, although significant amounts of PLP and DM-20 are restricted to the CNS, mRNA for PLP is expressed in the PNS and small amounts of protein are synthesized but not incorporated into myelin in appreciable amounts.)

Myelin basic protein has long been of interest because it is the antigen that, when injected into an animal, elicits a cellular immune response that produces the CNS autoimmune disease experimental allergic encephalomyelitis (EAE) (see Chap. 39). **MBP** can be extracted from myelin as well as from white matter with either dilute acid or salt solutions; once extracted, it is very soluble in water. The amino acid sequence of the major basic protein is similar in a number of species [11]. These proteins have molecular weights of around 18,500; they are highly unfolded, with essentially no tertiary structure in solution.

MBP is located on the cytoplasmic face of the myelin membranes corresponding to the major dense line. The rapid turnover of the phosphate groups present on many of the MBP molecules [18,19] suggests this post-translational modification might influence the close apposition of the cytoplasmic faces of the membrane. It also has been speculated that phosphorylation may modify this process in a dynamic manner. Of interest is that mRNA coding for MBP is preferentially localized far from the cell perikaryon, in the region where myelin compaction is taking place [20].

P0 is the major PNS myelin protein. Gel-electrophoretic analysis (Fig. 4-11A, C) shows that a single protein, of 30 kDa, P0 accounts for more than half of the PNS myelin protein. The cloning and sequencing of the message for this protein [30, 31] led to derivation of amino acid sequences from several species. From this, it has been deduced that the protein has about 220 amino acids with an intracellular domain, a hydrophobic transmembrane domain and a single extracellular immunoglobulin-like domain. The amino-terminal extracellular domain includes a signal sequence for insertion of protein into the membrane and a glycosylation site. In addition to the well-characterized carbohydrate chain, other post-translational modifications include sulfation, phosphorylation and acylation.

It is interesting to note that PLP and P0 protein, although different in sequence, post-translational modifications and structure, may have similar roles in the formation of structures as closely related as myelins of the CNS and PNS. These proteins are not mutually exclusive; they are coexpressed in certain fish and amphibians [32]. Transfection of non-neural cells with the P0 gene results in cell—cell interaction, which can be demonstrated to be due to homophilic interactions of the extracellular domains of P0 [33,34]. Elucidation of the crystal structure of the extracellular domain of P0 shows tetrameric packing of P0 molecules, suggesting that the extracellular domains of P0 project from the myelin membrane surface as tetramers [35]. The complete knockout of P0 has profound consequences on myelin structure and function [36], in contradistinction to the previously noted, relatively benign consequences for CNS in animals with a deletion of the PLP gene.

Myelin basic protein content in the PNS varies from approximately 5 to 18% of total protein, in contrast to the CNS, where it is on the order of 30%.

Myelin has a high lipid-protein ration (around 70-80% lipids and 20-30% proteins). This is rather unusual for a cell membrane. Cholesterol needed.

Myelin increases the resistance of the axons and decreases its capacity, such that the action potentials can travel faster.

Note that diseases affecting PLP and MBP are not as severe in the PNS as they are in the CNS, since the PNS has P0 (unlike the CNS) to compensate for eventual losses in proper myelination with MBP or PLP.

Summary of the functions of interactions between axons and myelinating cells at the node of ranvier:

The node is defined as an area free of the compact glial sheath. They localize the Na+ channels in the axonal membrane, since a demyelination causes usually a redistribution of the sodium channels and reduce nerve conductivity. The potassium channel is localized on either side (critical). The ends of the myelin sheath attach to the axon on either side of the node.

MS and nodes of Ranvier: node elongation; node and paranode elongation and redistribution of K-channels in paranode; myelin retraction and mixing of Na- and K- channels and CASPR.

Why and when does myelination make sense:

Fiber diameter is directly proportional to conduction velocity in myelinated axons (linear). It is a strategy to increase speed of nerve impulses without increasing the axonal calibres tremendously.
Non-myelinated axons are inverse quadratic proportional regarding diameter and velocity. There are no non-myelinated fibers below 1 micrometer (diameter becomes too thick if it wants to reach the same speed as myelinated fibers).
For vertebrates, it is an evolutionary advantage (predators, escaping, survival etc.). It is energetically favourable.

Sometimes, an axon might not be myelinated along its whole length. In pyramidal neurons (in the CNS), there are parts that are non-myelinated and then myelinated and so on.

Large unmyelinated gaps along the axons will increase their capacitance and thereby modify the speed of conduction. But speed is not everything, precision is also important. A neuron integrates the input it gets, so it makes a different if there are two subsequent inputs or two inputs at the same time (inputs are action potential of course).

In the PNS, axons with a calibre more than 1 micrometre are myelinated since they profit from this myelination. It optimizes conductivity. Myelin thickness is roughly proportional to axon calibre (thicker myelin sheaths for bigger axonal calibre).

In the PNS, the myelin thickness is always optimized. In the CNS, often just below the theoretical optimum, so that it has room for improvement before the critical (optimal) thickness is reached.

For thinly myelinated axons, they have reduced conduction. For de-myelinated axons, they have no conduction (blocked).
Along an axon, it might have different levels of myelination for the internodes.

Due to de-myelination, muscles and so on do not receive sufficient (or no) stimuli, which leads to motor and sensory neuropathies.

Myelin thickness is proportional to axon diameter.

Common pathway in Charcot-Marie-Tooth disease
CMT disease genes are PMP22, MPZ, GJB1 etc. They lead to CMT pathology, that is when Schwann cells myelinate poorly. Schwann cells then fail to support axons. Diagnostic criteria is then the reduced NCV defines CMT1 and CMT4. That is demyelinating CMT followed by axonal damage and degeneration.

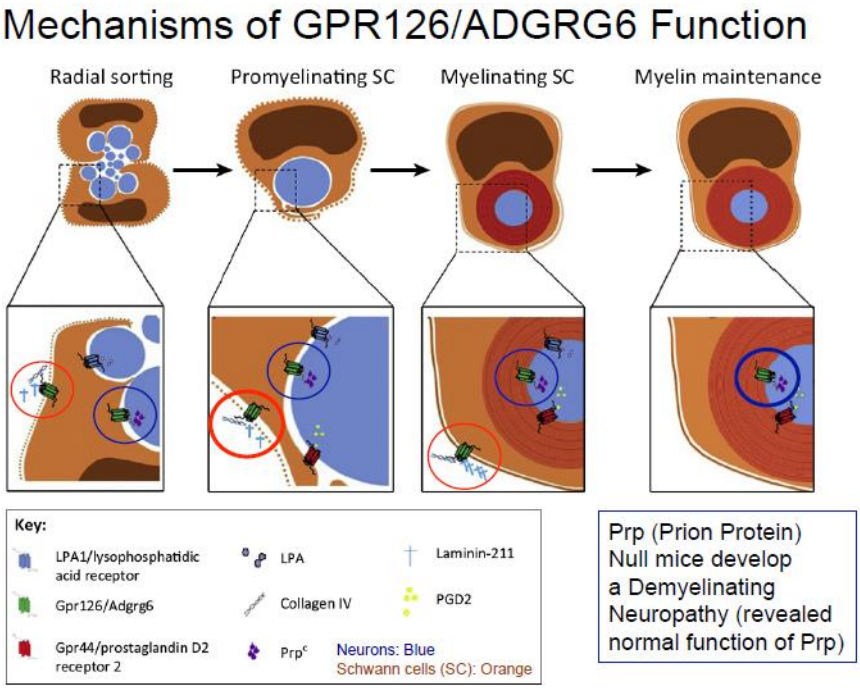
Moreover, Schwann cells failing to support the axons lead to axonal transport defects and to progressive axonal loss. Finally, muscle denervation and sensory loss.

One always needs to choose a suitable disease model. When and why is a model suited?

Often, the zebra fish model is used. In zebra fish, the oligodendrocytes look for axons to engage with. Imaging can be done fast and easily and so can genetic screenings.
In PNS myelination of the zebra fish, gpr126 is essential for myelination as it activates Adcy6 and cAMP after it.

What are the cellular and molecular mechanisms behind these findings? What activates the pathway?

Schwann cells development: Neural crest cell, Schwann cells precursors, immature Schwann cells. Now, there are two possible ways: immature Schwann cells develop into non-myelinating Schwann cells OR birth occurs; immature Schwann cells develop into pro-myelinating Schwann cells, then into myelinating Schwann cells.



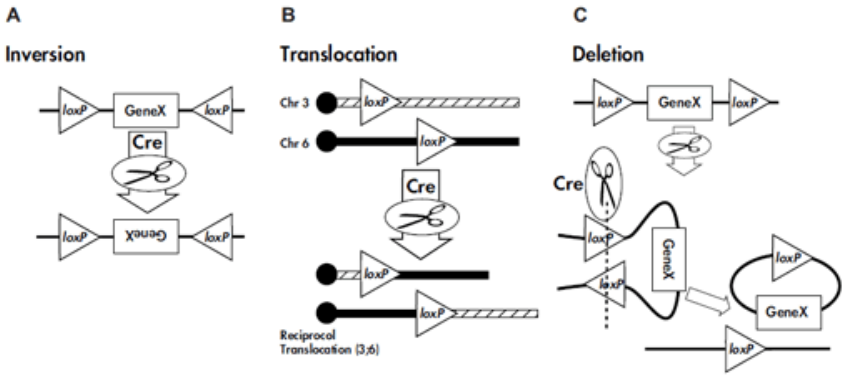
Def. the prion theory (prion := proteinaceous infectious particle): A theory that proposes that some nervous diseases are not caused by bacteria or viruses, but by abnormally folded proteins. Those abnormal proteins (prions) touch normally folded proteins of the same kind, changing their folding and making them into prions. Prions are not destroyed by cooking and they are not recognized by the immune system as dangerous. Often, this leads to an accumulation of prions in the (nervous) tissue, eventually killing the cell and the tissue. Ex.: the prion protein Prp.

Why the mouse as a model?

Mammalian experimental system, highly relevant for disease:
Inflammation, immune system, metabolism;
Standardized behavioural analysis;

Myelination is a vertebrate invention, often affected by inflammation;
Mammalian specificity, for example complexity of neural progenitor;
Features and regulation of myelination (regional differences);
Small, they can be housed easily as colonies;
Genome completely sequenced and known, easy to change genes for KO mice;

Cre-loxP system: Origin in bacteriophage P1. This system is used to produce genotype mutations in specific tissues in animals. Mutating the embryo might be lethal, so cre-loxP is used instead:
First animal has the Cre-gene (Cre := creating recombinase), which is an enzyme that recognizes loxP sites and produces deletions, inversions and translocations depending on the orientations of the loxP sites. Preceding the Cre-gene, there will be a suitable promoter that can either be unspecific and express Cre in all cells of the animal or it can be specific only expressing Cre in specific tissues (useful to study KO in tissues only without affecting every cell in the organism).
Second animals have 2 loxP sites. They flank the targeted gene [(**loxP)-TARGET_GENE-(loxP)].**
After mating, the offsprings will have the loxP configuration and Cre-gene with its promoter.
The Cre protein recognizes the 13 bp palindorminc sequence of loxP and forms a dimer. It recoginzes a second loxP site and its palindromic sequence and forms a tetramer. Now, it splices the loxP sites.
It is: 1) deletion ⇔ loxP sites have the same orientation (direction).
2) inversion ⇔ loxP sites have opposite directions.
3) translocation ⇔ loxP sites are on different chromosomes.



Ex.: A typical LoxP site: ATAACTTCGTATA -NNNTANNN-TATACGAAGTTAT (34 bp long in total).

Def. Transgene: A gene or genetic material which is either mechanically or naturally introduced into the genome of an organism. By definition, a transgene is a foreign gene that has been added to a foreign genome often by engineering means.

28.02.2017

Hypothesis: Understanding the basic molecular control mechanisms of myelination (development, maintenance, regeneration) will enable us to develop strategies to cure such diseases.

Development of Schwann cells:
They come from the neural crest then develop into Schwann cell precursors around week 12 in mice. A few weeks later, they differentiate into immature Schwann cells, then to promyelin Schwann cells. They become the final Schwann cell.

Sympathetic neurons have small axons. Not even in control cells you do have myelin, because the axon calibre is too small. It is not the calibre itself that control myelination, but the neuronal ectopic expression (NRG1 type III). The level of NRG1 typed III on the axon determines myelin thickness, the more NRG1 type III leads to thicker myelin.

Sox10 is an important transcription factor in Schwann cells to be myelinating. Bacteria might enter the Schwann cells and remove Sox10, therefore, the Schwann cell loses its Schwann cell characteristic and becomes a stem cell like cell. Schwann cells can persist in the peripheral nerve and remain there, but they do not become Schwann cells again and do not start myelination.
These reprogrammed stem cells can cross the blood nerve barrier and directly differentiate into infected skeletal or infected smooth muscles. Alternatively, via macrophages they infection can spread. (not sure)

What might be involved in the control of active NRG1 levels?

For example, NRG1 transcription, intracellular transport to the surface, processing, stability on the surface etc.

BACE1 is involved in correct myelination. In KO mice, there was hypomyelination. BACE1 cleavage generates a positive myelination signal.

TACE lacking mice were hypermyelinated in their motor neurons. TACE cleavage generates a negative myelination signal.

A balance between BACE1 and TACE and other protease activities acting on NRG1 type III is required for correct myelination. Other parallel mechanisms are likely to be part of fine tuning myelination.

On PI3K/AKT pathway: Very complex pathway that is not yet fully understand. It is involved in many different mechanisms. Downstream effects of AKT: it binds to BAX protein in order to inhibit apoptosis. It can activate the mTOR pathway. PI3K activates AKT after a cascade of in between processes.
(READ IT UP IN THE MOLECULAR BIOLOGY OF THE CELL.)

In biological systems, there are always drivers and stoppers. In the myelination case, PTEN and Dlg1 interact together and this stabilises PTEN. Result is less AKT activation and less myelin. PTEN is in that case (see slide, part 2 number 38) the main stopper.

Therefore, ErbB2/ErbB3 is a driver and PTEN/DLG1 are stoppers acting as a counter force and eventually taking over and stopping myelination in axons altogether.
Too thin myelin leads to bad conductivity (hypomyelination) while too thick myelin leads to instability (hypermyelination).

In the PNS, the main protein complex regulating myelination in the PNS is mTOR. It is is part of mTORC1 and mTORC2 (it is the effective part of these protein complexes – see later in the lecture series on mTOR).

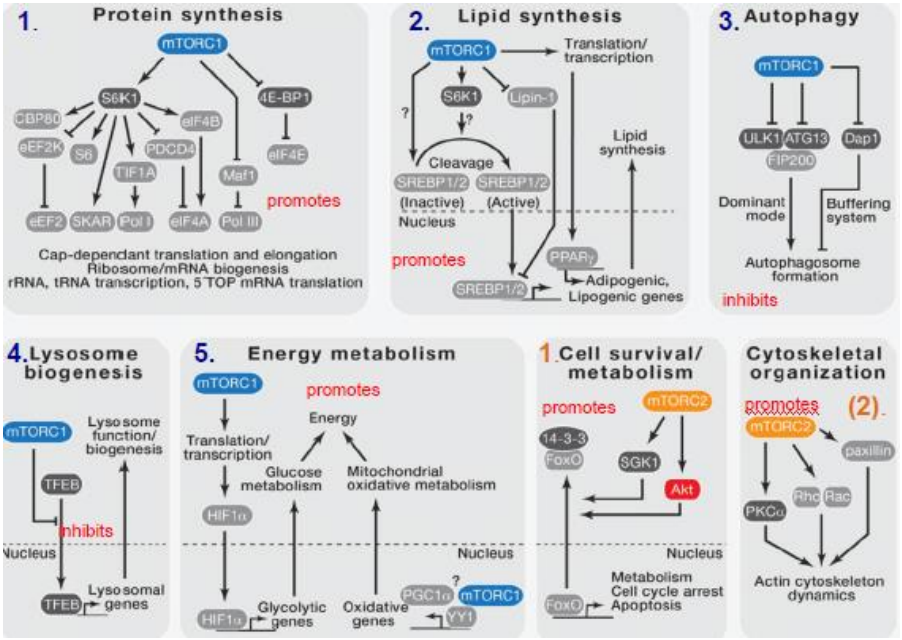
Key outputs of mTORC1 and mTORC2 pathways

mTOR (Serine / Threonine kinase): mechanistic Target of Rapamycin

mTORC1: negatively regulates lysosome biogenesis; positively regulates energy metabolism; regulates protein synthesis; regulates autophagy; regulates lipid synthesis

mTORC2: regulates cell survival/metabolism; regulates cytoskeletal

Def. Rapamycin: A compound that has immunosuppressive functions; especially useful to prevent kidney transplant rejection. It inhibits the activation of T and B cells by reducing the production of interleukin-2 (IL-2). It also seems to have an antiproliferative effect in cancer cells, thus making it suitable for cancer treatment in combination with other drugs.



Progression of PNS myelination in raptor mutants

Control group has normal myelination and after 12 months, the myelin is significantly thicker than at the beginning. The raptor mutants that are hypomyelinated, also myelinate but very, very slowly before they die. **PNS myelination is dependent on mTORC1.**

6.3.2017

FASN (fatty acid synthase) is also an important factor for myelination and its regulation. FASN is the only source of fatty acids (apart from diet). Is it required from PNS myelination? It is important for proper myelination initiation and myelin growth, apparently, mutants can cancel the defect out after some months, although mTORC1 deficiency problems can occur, so it is healthier to have FASN at disposal.

Def. Tomacula: Sausage-like myelination structure due to genetic mutations in the PMP22 gene. Such axons are often hypermyelinated which later leads to severe demyelination of axons, loss of function and death.

Def. HNPP (hereditary neuropathy with liability to pressure palsy): A peripheral neuropathy associated with malfunctioning myelination. It is caused by a mutation in PMP22 gene (dominant autosomal disease, at least one parent must be affected). It leads to hypermyelination (tomaculi), patients experience mild to severe symptoms, such as numbness in limbs, decline in life-quality etc.
Not all of the axon has to be hypermyelinated, most of the time, only some parts along an axon form tomaculi.

Erk1/2 also regulations myelination and myelin thickness.

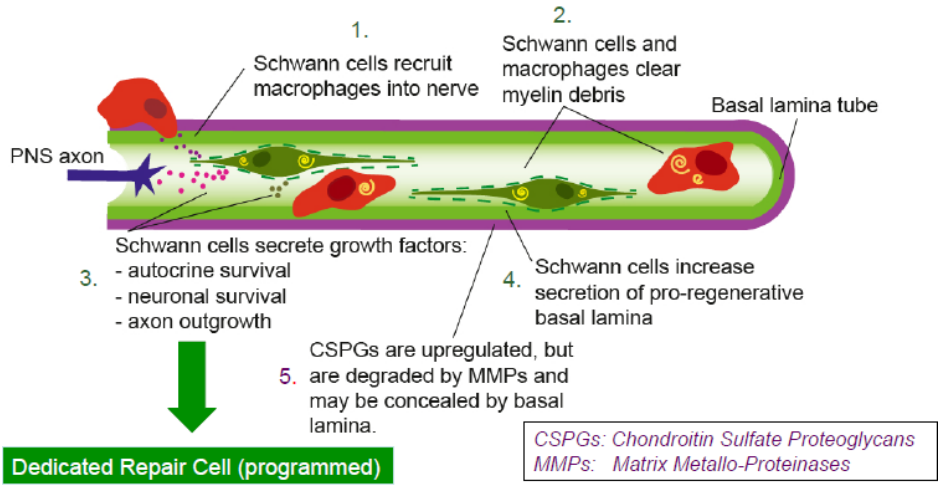
The ROSA26 is produced in every cell in the mouse. ROSA26 is always expressed and it can be controlled by another regulator such as CRE.

Is ERK signalling per se required for myelination?

Not going much into it: Take double Erk1/2 KO in mouse. Result is, myelination defect in mutants. Therefore Erk1/2 is required for proper myelination.

Summary of major results of basic science: In the PNS: myelination initiation, myelin growth, restraining and control of myelin thickness, demyelination after injury.
These processes are controlled by NRG1 signalling mediated by mTORC, AKT, PI3K and MEK-ERK.

Schematic axon after PNS injury: Why does it regenerate?



Major difference between the injured PNS and CNS (in addition to some intrinsic qualities of the neurons themselves): Pro-regenerative response of Schwann cells in changing positively the neural environment

CNS after axon injury: What impedes regeneration?

Myelin debris persist distal to the site of injury (inhibitory substances including NOGO). Reactive astrocytes secrete CSPGs that wall of lesion site. Oligodendrocytes undergo apoptosis or become quiescent. Microglia regulate the inflammatory milieu (microglia are CNS tissue-resident macrophages).

From here on, we will discuss the CNS in more detail. Especially how do cells from a tissue in diseases.

On multiple sclerosis (=: MS)

In multiple sclerosis (MS), the immune system attacks CNS myelin leading to impaired sensory and motor nerve function, also cognitive function, and in most cases some degree of disability, depending on the brain and/or spinal cord area that is affected (autoimmune disease). The myelin sheath is protective and supportive for axons and allows neurons to transmit impulses quickly and effectively. In MS, the myelin sheath is damaged, causing varying symptoms that include increased difficulty of moving and progressive weakness, including often vision problems (optic nerve affected).

Facts summary on MS

• Most common neurodegenerative disorder among young people.

• Inflammatory reaction in the CNS causing demyelination (myelin-autoreactive T cells).

• Loss of myelin causes reduced or blocked nerve conduction resulting in attacks of numbness, loss of vision, weakness, bladder problems, ataxia (tremor).

• Initially efficient functional recovery in 85% of patients. In about 15%, the illness is progressive from the onset, with or without preceding inflammatory phase.

Natalizumab / Tysabri are is an effective drugs against MS. Why is that so? Write summary of how it works schematically.

Natalizumab/Tysabri: The drug is believed to work by reducing the ability of inflammatory immune cells to attach to and pass through the cell layers lining the intestines and blood–brain barrier. Natalizumab has proven effective in treating the symptoms of both diseases, preventing relapse, vision loss, cognitive decline.

Def. Haploinsufficiency: In diploid organisms, one copy of a gene has been lost, often due to deletion leading to a loss of function. The wild type phenotype can no longer be sufficiently expressed, since only one copy of the wild type allele is present.

13.3.2017

Diphtheria toxin: An exogenic toxin secreted by the bacterium Corynebacterium diphteriae. It causes the disease diphtheria in humans etc.

Conclusions of induced oligodendrocyte death in adult mice:

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Current hypothesis for symptoms progression in RRMS:

First phase dominated by inflammation, associated with damaged blood-CNS barrier. Variable in temporal progression: Remission involves resolving local inflammation, sodium channels redistribution along denuded axolemma and remyelination; axonal damage starts. Neurodegeneration is the major cause of permanent neurological disability in MS Second (chronic) phase dominated by neurodegeneration. Progresses in most patients at the same rate. Blood-brain barrier repaired, but some inflammation remains locally in parenchyma. Axonal degeneration continues in chronic MS lesions without major inflammation. Anti-inflammatory drugs: No effects in chronic progressive phase and primary progressive MS. Uncertain influence on timing of entry in chronic disease progression.

APP is transported in axons and if axons are damaged, they start to accumulate which leads to transporttation problems.

A few (auto) antigen-specific T cells migrate through the blood–brain (CNS) barrier into the perivascular space between the capillary endothelium and the glia limitans, the beginning of the CNS parenchyma

There, T cells are confronted with dendritic cells that have been collecting (auto) antigens and present them to the T cells

Auto-antigen-specific T cells start to proliferate and migrate to the sites of primary inflammation

T cells mediate damage via direct cytotoxicity and secretion of cytokines

Surrounding tissue cells such as *neurotoxins-secreting microglial cells and astrocytes are activated, producing further damage

Cautious conclusions:

The axonal damage that is associated with genetic ablation of myelinating oligodendrocytes argues in

favor of a primary function of oligodendrocytes in supporting axonal integrity. There is very likely some protective role of living oligodendrocytes on axons, although there might still be indirectly contributing mechanisms in the oligodendrocyte death-induced phenotype, e.g. mediated by the observed activation of the innate immune system, that may contribute to the damage (or neurotoxicity of the myelin debris?)

Immunology definitions

The Immune System
Cells in our bone marrow, thymus, and the lymphatic system of ducts and nodes, spleen, and blood that function to protect us.
Antigen
Anything causing an immune response, usually foreign material but may be our own tissues.
Pathogen
Any disease causing micro-organism.
Tolerance
Non-reactivity of the immune system, usually refers to "self" but may include foreign tissue in organ transplants.
Autoimmunity
A failure of tolerance, the immune system reacts to self.
Innate immunity
Protection that is always present. Includes phagocytic (cells that eat other cells) macrophages and dendritic cells.
Adaptive immunity
Protection that arises by an immune response, including humoral immunity producing antibodies and cellular immunity.

Technical analysis methods for axonal transport in the living mouse:

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It seems the second main function of oligodendrocytes in the CNS is to preserve the health of the axons next to increasing conductivity speed. But what are oligodendrocytes then doing?

Experimental strategies for such a question:
Candidate approach: Study known single molecules, singalong pathways, metabolic pathways etc.
Screening approach: Study and identify novel single molecules, signalling pathways, metabolic pathways etc.
Global (Systems) approach: Use genomics, proteomics, lipidomics, metabolomics etc. We need a bioinformatics analyse to identify the candidates again (single muscles, singling pathways, metabolic pathways etc.)

Methods: Fluorescence-activated cell sorting (FACS): (NVM)

Laser capture microdissection: (NVM)

Known the following table conceptually

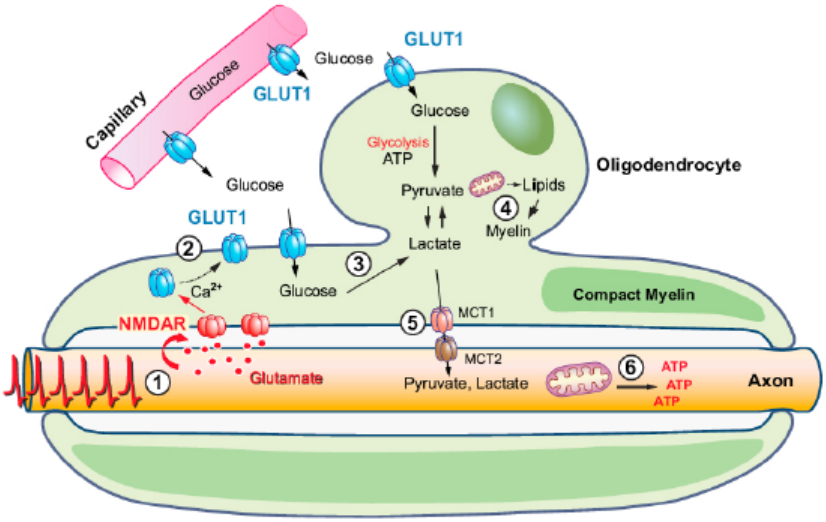
Models of Adult Demyelination / Remyelination

Model	Type	Mechanism/Description
Cuprizone	Toxin	- Orally administered copper chealator; inhibits copper-dependent mitochondrial enzymes monoamine oxidase and cytochrome oxidase - Causes selective cell death of oligodendrocytes resulting in widespread CNS white matter demyelination
Lysolecithin	Toxin	- Focally injected membrane solubilizing agent - Induces demyelination in focal lesions that cause local cell death of predominantly oligodendrocyte in the surrounding tissue
Ethidium Bromide	Toxin	- Focally injected DNA-intercalating agent - Similar to lysolecithin: Focal lesions are caused by the non-specific cell death of all cell types in the surrounding tissue
Experimental Autoimmune Encephalomyelitis (EAE)	Immune Mediated	- Injectable antigen induced encephalomyelitis (purified myelin, myelin proteins - MBP, PLP, MOG and spinal cord homogenate) - Causes an autoimmune inflammatory response resulting in more pronounced white matter vs. gray matter deymyelination
Theiler's Murine Encephalomyelitis (TME)	Immune Mediated	- Injectable virus (single-stranded RNA cardiovirus) induced encephalomyelitis - Causes an autoimmune inflammatory response affecting all cells of the CNS and results in demyelination, predominantly in the spinal cord
Murine Hepatitis	Immune Mediated	- Injectable virus (positive-strand RNA coronavirus) induced encephalomyelitis - Similar to the TME virus: causes an autoimmune inflammatory response affecting all cells of the CNS and results in widespread demyelination
Diphtheria Toxin (DT) Receptor Targeting	Genetic	- Two inducible Cre-mediated strategies for the selective ablation of oligodendrocytes in adult animals: 1) DT injections administered to transgenic mice that express DT receptor in oligodendrocytes of the CNS, 2) Transgenic mice that express DT receptor in oligodendrocytes of the CNS where no DT administration is necessary. Upon Cre recombination DT-A mediated cell death of oligodendrocytes occurs. - Selective elimination of oligodendrocytes results in widespread CNS demyelination

Environmental risk factors for MS: Sun light (the further away from the equator the higher the risk), smoking, Epstein-Barr virus.

Perhaps, oligodendrocytes deliver energy transport to the axon: they lactate when the myelinate. Myelinating Oligodendrocytes take up blood-derived glucose and deliver glycolysis products (lactate / pyruvate) via Monocarboxylate Transporters (MCT1 and MCT2) to myelinated axons.

But how do oligodendrocytes "sense" the energy needs of an axon?



(Working Model)

(1) During myelination, NMDA receptors respond to axonal glutamate indicating increased axonal electrical activity and energy needs, causing **(2)** more glucose uptake via more glucose transporters. Glycolysis products **(3)** are initially used for ATP and lipid synthesis **(4)**. Afterwards, myelinating Oligodendrocytes release lactate (or pyruvate) to fuel the axonal compartment **(5)** for mitochondrial ATP production **(6)**.

Why is the additional energy from the oligodendrocytes so critically important to the axons?
Loss of this energy means that the axon itself will need to produce more energy in some way, which can lead to self-intoxication. It has to get rid of the Na+ influx by pumping it out (requires energy). (see slide 48 part 4 for a nice picture/overview.)

14.3.2017

In a Rotarod study, mice without myelination could not stay on the rotating rod and they fell down. Those who survived were able to recover their demyelination and became almost as good as the control group indicating that myelination is indeed protective.

Some of the adult OPCs had differentiated into myelinating oligodendrocytes in the adult indicating that myelination is going on in the adult.

Adaptive myelination:

It is likely that new neuronal connections are formed, or existing connections strengthened, in response to repetitive firing of neural circuits that elicit a particular sequence of movements. The increased activity in these circuits might then stimulate myelination of their axons, or myelin remodeling, making the circuit more efficient. There might be a reserve of preformed, parallel circuits in the brain, and motor training selects the best of these by stimulating myelination in the most active circuits (and may fix the outcome).

Kor.: Implication of the Concept:
Neuronal electrical excitability modifies myelin plasticity. In turn, myelin plasticity feeds back to modulate neural activity and behaviour.

(ADAPTIVE MYLEINATION SEE SLIDE 35ff PART 5)

Activation of neurons in the mouse premotor cortex increases the proliferation of oligodendrocyte precursors, which differentiate into oligodendrocytes. The increased myelin thickness observed could have several possible explanations (not mutually exclusive and possibly cumulative):

(A) Myelination of unmyelinated axons by the new oligodendrocytes (as commonly occurring in the adult corpus callosum).

(B) Intercalation of new sheaths on myelinated axons (as observed in the adult optic nerve, with shorter intercalated internodes).

(C) Thickening of existing sheaths.

20.3.2017

As of today, we begin with Wutz's part (cell plasticity and developmental biology). Lecture slides are the basis for the exam.

Systematic language in mouse genetics: In italics, we denote the gene with 3 letters and numbers. E.g. *Sox2*. In normal font, we denote the protein, which is often just the letter sequence of the gene. E.g. Sox2.

Def. epiblast: In embryology, the epiblast is one of two distinct layers arising from the inner cell mass in the mammalian blastocyst. During gastrulation, the three primary layers endoderm, mesoderm and ectoderm are derived from the epiblast. The extraembryonic mesoderm also originates from the epiblast.

Def. hypoblast: A tissue type that forms the inner cell mass and it lies beneath the epiblast. The extraembryonic endoderm is derived from the hypoblast cells.

Def. Oct4 (marker gene): A protein that is critically involved in the self-renewal of undifferentiated embryonical stem cells. (It is active during the preimplantation period of the embryo.)

21.3.2017

Def. EpiSCs: From the epiblast-derived stem cells. The inner cell mass of the blastocysts segregates into two layers one of which is called epiblast (the other is called hypoblast).
EpiSCs are more similar to human embryo stem cells than are blastocyst derived stem cells (also sometimes called naïve stem cells). The segregation does not occur ex vivo, the blastocyst must be added into a uterus.

27.3.2017

Mesoderm development

(suggested book: principles of development, 4th edition, Lewis Wolpert (and others) – book is rather short and easy to read. Actually, it is quite long.)

Subdivisions of mesoderm: axial (notochord, precordal plate), paraxial (somites and presomitic plate), intermediate (kidney and gonad), lateral plate mesoderm (splanchnic, somatic and extraembryonic); heart and circulatory system, formation and differentiation of the somites.

Lecture summary:

Development of the ectoderm:

neural tube development gives rise to CNS; surface ectoderm forms the outer layer of the skin – epidermis - including hair follicles; neural and surface ectoderm contribute to the eye development

Loss of a specific cell type or the structure leads to functional impairment that manifests itself as a disease. Parkinson's disease and AMD are caused by loss of cells that are not regenerated

Impairment of the function of a particular cell type can disrupt normal tissue function: epidermolysis bullosa severely impairs the function of skin not due to cell loss but deficits in the extracellular matrix that connects the layers of the skin

Embryonic stem cells from blastocysts: Which one do human ES cells resemble more closely: Mouse ES cells or mouse EpiSCs? Explain the characteristic growth properties and differences of these three cell types. ES cells can differentiate into all cell types of the organism (pluripotent developmental potential)

How can this be shown? Are ES cells unrestricted in their developmental potential? Which cell types of the extraembryonic tissues would you expect cannot be formed from ES cells? The embryonic axis can only be minimally approximated when ES cells differentiate in culture. Which prominent gene group would you expect could be affected in culture derived somatic cell types?

(LEARN A.WUTZ SLIDES, SINCE MY SUMMARY IS SOMEHWAT SCARCE.)

28.3.2017

(from now on, Sabine Werner's part begins – read the signalling chapter of "molecular biology of the cell" since it is the basis for the lecture.)

For a cell to grow in a medium, there needs to be some sort of serum that contains growth factors. Almost all cells produce growth factors. They are usually small and secreted peptised. Each cell requires a certain set of growth factors present and they are already active in very small concentrations (in the range of nano- to picomolars). The growth factors act on cells that possess the right receptors.

There are signalling cells that produce growth factors for other cells. This is important for tumours, because if they can produce the growth factors themselves they can act independently of their neighbours.

Def. PDGF: PDGF := platelet derived growth factors. 100 microrgams have been derived from 200 litres of human blood.

Mechanisms of growth factor activation:

Juxtacrine: signalling cell is basically next to the target cell and secretes the growth factors via membrane.

Paracrine: Signalling cell produces for several target cells locally.

Autocrine: Signalling cell is its own target cell.

Endocrine: Signalling cell produces growth factors that enter the blood stream in order to travel to the target cell.

Functions of growth factors: They regulate proliferation, differentiation, survival and migration of the cell. They are always required for tissue repair, during development (e.g. during embryo development), and for tumorigenesis.

Def. Cytokines: Molecules that regulate proliferation, differentiation, survival and other cellular functions at extremely low concentrations (includes classical growth factors).

A growth factor can bind to different sites that must not be exactly identical. The signalling will then be different.

Signalling proteins bind to domains, since the receptor needs to be sure to get the exactly right signalling protein. That is realized by recognizing not only the desired signalling protein that can be phosphorylated for example, but also 2-3 amino acids up and down of the protein.

2 domains: SH2 and PTB (**slide 18 part 1.1**)

Signalling proteins that are activated by receptor tyrosine kinase: PLC-gamma, Src, c-cbl, Pl3-kinase, structural proteins, adaptor proteins such as Grb-2, Shc, Eps15, Epsin.

On PLC-gamma: An enzyme that hydrolizes PIP2, DAG, IP3. There are different types of PLC, they differ in regulation and localization of the organism. These enzymes are involved in the transduction of signals from out of the cell to the inner cell.

On Pl3-kinase: An enzyme that occurs in every eukaryotic cell. It is a key factor in processes such as cell growth, cell differentiation, cell proliferation, cell migration, cell adhesion and in the survival of the cell. It catalyses phosphorylation.

Kor.: Its antagonist is PTEN which is involved in the suppression of tumors.

On adaptor proteins: These proteins contain several different domains that allow protein-protein interaction with a variety of other proteins for cell signal transduction. They themselves do not possess any intrinsic enzymatic activity.

Adaption/Desensitation: Prolonged exposure of GF to a cell reduces the cellular response. Cells can respond to changing GF concentrations. (**Principle: delayed negative feedback**)

Methods on how to analyse growth factors in the lab:

Analysis of cell proliferation:

Seed equal number of cells and count cells at different time points.

Incorporation of 3H-thymidine: measure incorporated radioactivity or identify labelled cells by autoradiography

Incorporation of 5-bromo-2`-deoxyuridine (BrdU; nucleotide analogon): Identify labelled cells with an antibody directed against BrdU

3.4.2017

On PDGF

Embryonic development: kidneys (mesangial cells), blood vessels (smooth muscle cells, pericytes), lungs (alveolar smooth muscle cells), CNS (oligodendrocytes) and it is involved in the stimulation of wound healing.

PDGF is also involved in some diseases such as cancer (autocrine stimulation leading to brain cancer and sarcoma; paracrine stimulation leading to carcinomas and the stimulation of stromal cells), atherosclerosis and in fibrotic conditions such as lung fibrosis or liver cirrhosis.

H2O2 can also be used as a signalling molecule and it is produced by the Fenton reaction via superoxide. H2O2 is also produced at a wound site to attract immune cells (as seen in zebrafish and flies).

On HERCEPTIN: A monoclonal antibody used to treat breast cancer that is HER2 receptor positive.

10.4.2017

On Angiogenesis

Excessive and insufficient angiogenesis

Different steps in angiogenesis: Angiogenic signal: inflammation, hypoxia; Degradation of extracellular matrix; Migration of endothelial cells; Proliferation of endothelial cells; Contact to the extracellular matrix; Lumen formation; Stabilization by association with pericytes and smooth muscle cells.

Def. VEGF: Vascular endothelial growth factors expressed for vasculogenesis, lymphangiogenesis and angiogenesis. There are VEGF-A,B,C,D,E,F.

Def. PLGF: placenta growth factors

Biological functions of VEGF types:

VEGF-A: *In vitro*: induces migration and proliferation of endothelial cells, prevents apoptosis of endothelial cells.
In vivo: involved in various steps of vasculogenesis and angiogenesis, induces sprouting of capillaries, increases vascular permeability, induces survival of blood vessels, can also stimulate lymphangiogenesis via VEGFR2.
Regulation of VEGF-A production: Most important stimulus: hypoxia: activates hypoxia-inducible transcription factor, which in turn activates VEGF gene expression. Other inducers: pro-inflammatory cytokines, growth factors, reactive oxygen species, UV, oncogenes

Tumors stimulate angiogenesis: The role of VEGF in tumors: Strongly upregulated in almost all tumors by hypoxia, inflammatory cytokines, oncogenes, ROS.
Induces survival, migration and proliferation of endothelial cells and sprouting of new vessels.
Recruits endothelial progenitor cells from the bone marrow that contribute to the formation ofnew vessels.
Recruits inflammatory cells and also stimulates some tumor cells directly.

Ephrins and Eph receptors: Involved in the development of the nervous system, angiogenesis, vasculogenesis etc. Ephrins are remarkable, since they are transmembrane proteins. They use a juxtacrine mechanism of action and require cell to cell contact (bidirectional signalling).

Ephrins do not stimulate cell proliferation, but they regulate cell migration, cell attachment and cell-matrix contacts.

Inhibitory factors of angiogenesis:

Synthetic inhibitors: Metalloproteinase inhibitors, TNF-70 - a homologue of the antibiotic fumagillin, thalidomide

Endogenous inhibitors: Angiopoietin-2, angiostatin, endostatin, IL-12, interferon-α

Biological antagonists: VEGF antibodies (Avastin), VEGF antisense RNAs or ribozymes, VEGF receptor tyrosine kinase inhibitors Soluble VEGF receptors, soluble TIE-2

Endostatin: Advantage: Broad-spectrum inhibitor that blocks different angiogenic factors. Disadvantage: Expensive production, dosing is critical and thus difficult – low concentrations are efficient, but high concentrations are not.

Avastatin: Humanized monoclonal antibody against VEGF. Approved in combination with chemotherapy (5-fluorouracil) for the treatment of metastatic colon and rectal cancer, non-small cell lung cancer and breast cancer. Side effects: Bleeding, hypertension, holes in the colon, impaired wound healing, kidney damage. Additional problem: Endothelial cells become resistant - they use other factors, e.g. FGF2 for angiogenesis. VEGF antagonism may increase even tumour growth.

SUTENT (Sunitinib): Inhibits VEGFR1-3, PDGF receptors, KIT. Inhibits angiogenesis, reduces vessel stabilization by pericytes and inhibits (some) tumor cells directly.

Nexavar (Sorafenib): Inhibits VEGFR2, PDGFRβ and Raf kinase. Inhibits angiogenesis and (some) tumor cells directly.

MACUGEN: Pegylated anti-VEGF aptamer. Prevents excessive angiogenesis and vascular leakage in the eye.

LUCENTIS: A humanized antibody FAB fragment, affinity maturated (six amino acid changes compared to wild-type protein): Used for the treatment of macular degeneration.

Lymphangiogenesis in cancer: Inhibited by VEGF-C, VEGF-D, VEGFR3 (suppress tumour formation and metastasis). Overexpression of VEGF-C leads to increased rate of metastasis.

Def. ischemia: Restriction of blood supply (oxygen and glucose) to tissues in order to keep it alive.

On Fibroblast growth factors (FGFs)

There are 23 different FGFs (FGF1-23, note that FGF15 is absent in humans but present in mice, while FGF19 is present in humans and absent in mice). They play a key role in cell differentiation and growth and are critical to embryonal development.

In adults, FGF plays an important role in tissue regeneration, angiogenesis, regeneration of nerves and creation of cartilage (dt. Knorpel) and in wound healing.

KO situations in mice:

FGF5: negative regulator of hair growth: KO leads to longer hair in mice, mice are fully viable.
FGF10: mice have no limbs and die of lung failure
FGF8: it has a similar phenotype like FGF10, but most often, FGF4 is in the same area and it can compensate for the FGF8 KO to some extent.
FGF7: it mostly has protective effects. FGF7 KO means less protection, leading to a number of health problems.
FGF23: premature aging. Also, bones will be fragile sooner and they will look messed up after some time. FGF23 regulates Ca2+ and phosphate (high levels of both molecules in KO mice). It also regulates vitamin D metabolism and phosphate homeostasis in the liver. FGF23 requires the transmembrane protein Klotho. Klotho KO mice show very similar phenotypes like FGF23 mice.
FGF11: mutations lead to an early on set in ataxia, epilepsy and sensory deficits. FGF11 acts in the nervous system.
FGF14: FGF14 SNPs are associated with major depression, schizophrenia, alzheimer's disease and substance abuse.

FGFR1: die during gastrulation
FGFR2: die after implantation
FGFR3: bone deformation and extension, inner ear defect. FGFR3 is a negative regulator for bone growth. Too much FGFR3 will lead to shorter bones. Mutation is solely passed on from the father.
FGFR4: liver regenerates slowlier

Double KO mice: FGFR1 and 2 KO in transgenic floxed mice (in keratinocytes): they lose hair and are naked (progressive epidermal thickening). There is also an enhanced transepidermal water loss. ➔ it resembles human atopic dermatitis.

FGF7 is extremely upregulated in wound healing and FGF10 and FGF22 are also expressed during wound healing.

More on FGF7: FGF7 mostly has a protective function in organisms. A FGF7 KO results into a number of different health problems for the organism:
on cells of the bladder: prevents ulcerative hemorrhagic cystitis after cyclophosphamide injection; on alveolar cells: FGF7 can prevent lung injury in various model systems (e.g. hyperoxia, acid); on cells of the gastrointestinal tract: pre-treatment of mice with recombinant FGF7 reduces injury induced by radiation and/or chemotherapy (increased mucosal thickness, increased crypt cell survival)

Diseases caused by FGF7 KO: oral mucositis: severe oral pain, gastrointestinal bleeding, difficulties in eating and swallowing, difficulties in speaking

FGF7 also protects keratinocytes (such as skin cells), protecting it from UV light and reactive oxygen agents and reducing cell damage and apoptosis. In response to reactive oxygen species (=: ROS), FGF7 reduces protein oxidation, thus protecting their structure. Also, FGF7 is cytoprotective for human hair follicle keratinocytes in organ culture.

Endocrine FGFs

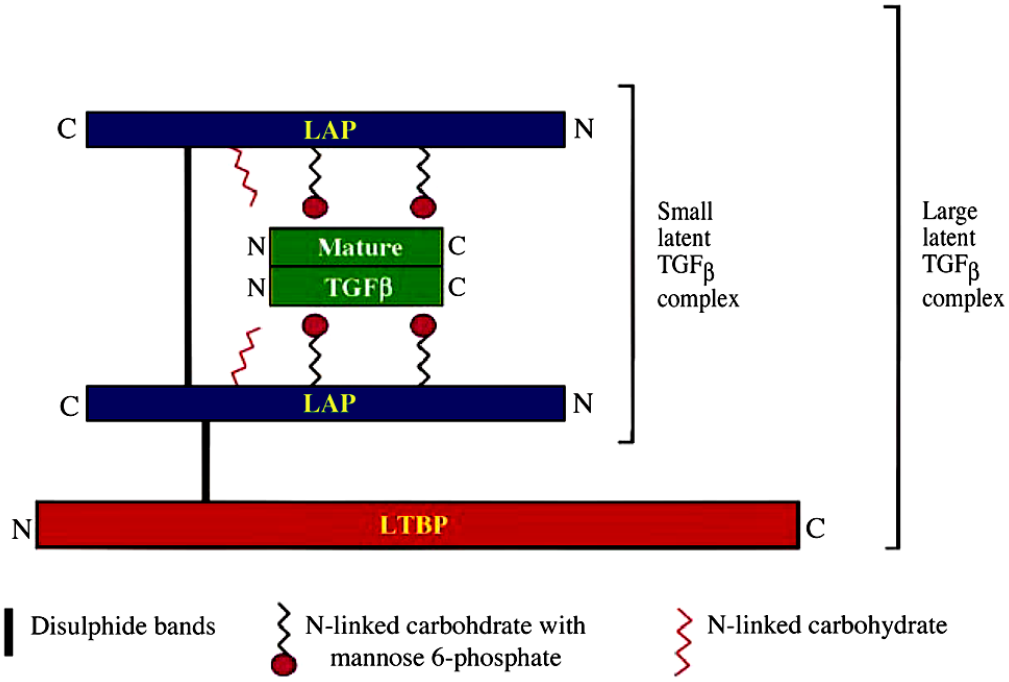
FGF19 (FGF15 in mice), FGF21 and FGF23 show reduced heparin binding and therefore act as endocrine hormones. Receptor activation requires a co-receptor protein (Klotho or Klotho β). FGF19 regulates bile acid synthesis and gall bladder filling. It is produced in the intestine and stimulates hepatocytes of the liver. It is also involved in energy and lipid homeostasis. FGF21 is produced in the liver. It regulates response to fasting by signalling to adipose tissue and brain. Involved in energy, lipid and glucose homeostasis. FGF21 is regulating eating behaviour, telling the brain to eat more or less.

Def. Klotho and Klotho beta: Transmembrane protein that are involved with some FGFs. KO of Klotho protein leads to similar phenotypes that are observed in FGF KO mice.

On transforming growth factor-beta (TGF-beta):

TGF-beta is the prototype for many different growth factors and it has a huge super family. It is typically 112 amino acids long, it has 9 cysteine residues, one of them is used for dimerization while the other 8 cysteine residues are used for intramolecular disulphide bridges. It is commonly produced as a large and inactive precursor. It needs to be activated first, before it can have any effect in an organism.

The longer form has a longer half time and is normally thought to be the storage form of the TGF-beta complex.



Ways to activate TGF-beta:

In vitro: lower pH value, apply mechanical tension on the complex.
In vivo: Proteinases, thrombospondin (induces conformational change), locally low pH, reactive oxygen species, binding of LAP to mannose-6-phosphate receptors or integrin αvβ6 (induces conformational change), mechanical tension.

TGF-beta receptors (Ex. Betaglycan, type-III receptor) are transmembrane proteins and they do not have an enzymatic activity. They promote the binding of the ligand to the signaling receptors. The type-I and type-II receptors are also transmembrane proteins, type-I transmits the signal and type-II phosphorylates type-I, so it can transduce the signal at all. Type-I and type-II form a receptor dimerization.

Biological functions of TGF-beta: Inhibit proliferation of most cell types, including epithelial cells: mutations in the TGF-β signaling pathway are frequently found in epithelial cancers, e.g. Smad4 mutations in pancreatic carcinoma (DPC), Smad2 mutations in colon carcinomas. Exception: Proliferation of fibroblasts is stimulated by TGF-β.

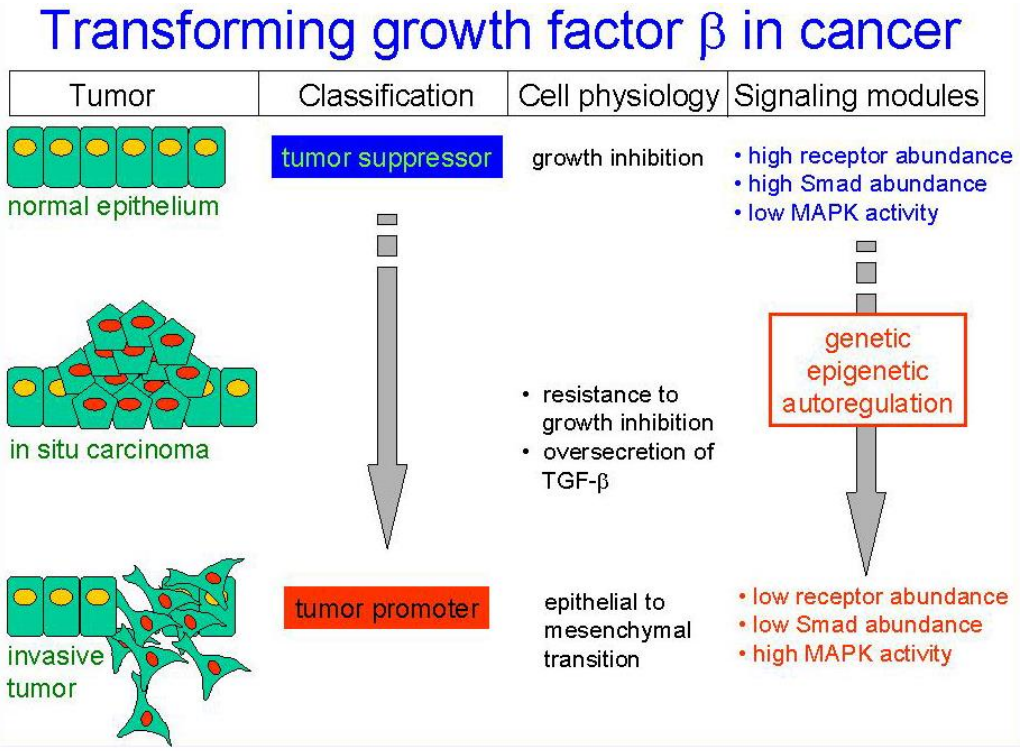
Furthermore, TGF-beta has anti-inflammatory properties: KO mice suffer from severe inflammation. TGF-beta also stimulates the production of regulatory T cells, thus inhibiting autoimmune responses (might be used to treat MS via viral vectors).

Also, TGF-beta induces cell migration, proliferation of fibroblasts and cell matrix production by these cells. TGF-beta KO could also bring about phenotypes typical to FGF KO, since the fibroblasts do not proliferate, I think. It promotes differentiation of fibroblasts into myofibroblasts (in between form of muscle cells and fibroblasts, containing contractile actin- and myosin filaments and they also have a high endogenic collagen production), in particular in combination with mechanical tension: Cells acquire contractile properties.

TGF-beta mutation: overexpression

Often over-expressed in fibrotic disease where functional epithelial tissue is replaced by non-functional connective tissue, e.g. lung fibrosis, liver cirrhosis, hypertrophic scars and keloids etc.

TGF-beta's role in cancer:



Def. SMAD protein: SMADs are intracellular proteins that transduce extracellular signals from TGF-beta ligands to the nucleus where they activate downstream gene transcription.

Kor.: 1) Receptor-Smads: Smad2 and Smad3 bind to TGF-β und Activin receptors.
2) Smad1, Smad5, Smad8 bind to BMP (:= bone morphogenetic protein) receptors.
3) Smad4 binds to receptor Smads.
4) Smad6 and Smad7 are inhibitory Smads.

24.4.2017

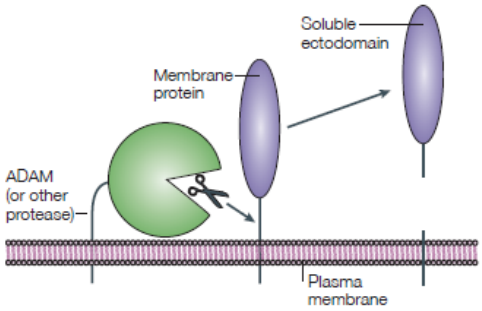
Proteases as modulators for growth factors

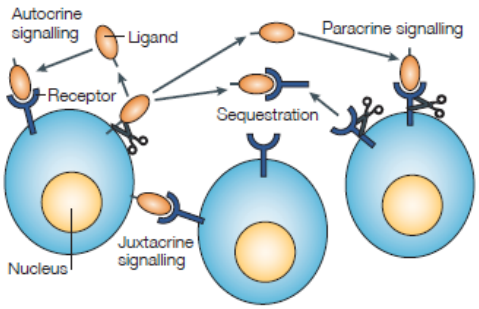
Proteolysis and its biological consequences:

During proteolytic processing, the initiator methionine is removed. The protein matures, increases stability and it can be localized more easily on a receptor or so. These proteins are sometimes zymogens, proenzymes, which are in itself inactive and they require a suitable protease to active these enzymes (these enzymes can be other inactive proteases themselves).
Also, processes for domain shedding are involved such as ligand release, receptor processing, and transsignaling and lastly, altered receptor binding upon ligand cleavage, control of growth factor and cytokine bioavailability, release from precursors.

Moreover, proteases are involved in biodegradation (food to energy source) or as quality control and protein degradation.

Domain shedding: Active growth factors are shed from membrane bound proteins (left picture). Released ligands can be sequestered (dt. absondern) by shed receptors (right picture).





Def. Tumor necrosis factor: It is a proinflammatory, transmembrane cytokine that arranges in stable homotrimers. It mediates apoptosis, inflammation, proliferation and differentiation.

Def. metallo matrixproteins (MMPs): … .These enzymes are capable of degrading all components of the extracellular matrix.

Def. Zymography: An electrophoretic technique for the detection of hydrolytic enzymes, based on the substrate repertoire of the enzyme. In the lecture, we looked at a SDS-PAGE gel example and at a in situ zymography.

Kor.: SDS-PAGE gel: (NVM)
In situ zymography: (NVM)

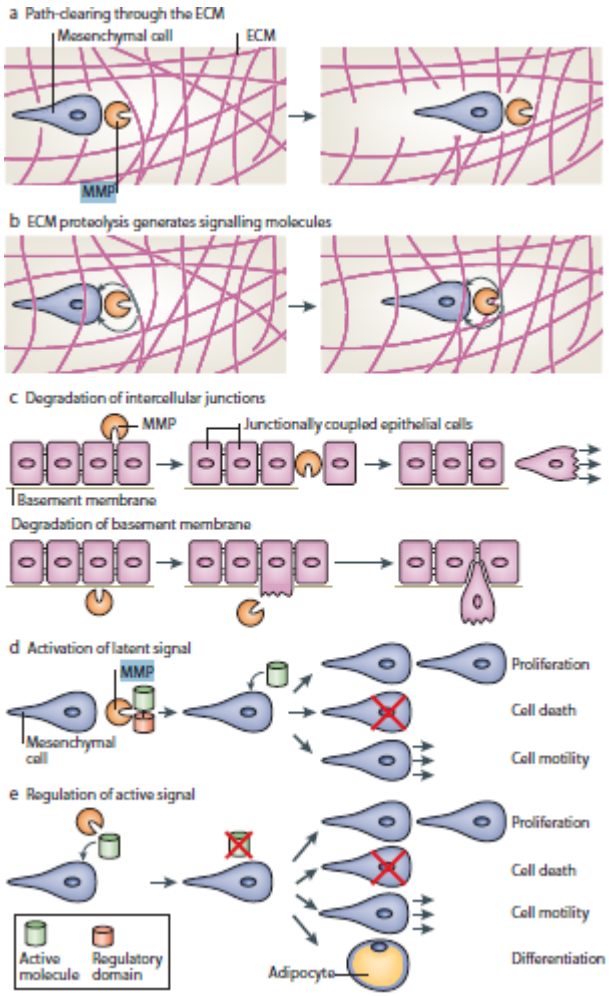
Functions of metallo matrixproteins (MMPs) in response to injury:

MMPs are responsible for the ECM degradation. MMPs are highly expressed at an injury site (probably to degrade damages cells). They are tissue inhibitory MMPs, called TIMPs. Probably present all the time at healthy (undamaged), or else, MMPs would degrade healthy ECMs.

MMPs are highly expressed in tumors. Probably to degrade the healthy cells, so the tumor cells have building material for themselves.

25.04.2017

Modes of MMP action: MMP's aim is to degrade the extracellular matrix (=: ECM). They release cryptic growth factors from ECM molecules. Then, degradation of extracellular junctions and basement membrane follows. Latent signals are activated and active signals are regulated.



Def. HARP: heparin affin regulatory peptide.
Def. CTGF: connective tissue growth factor.

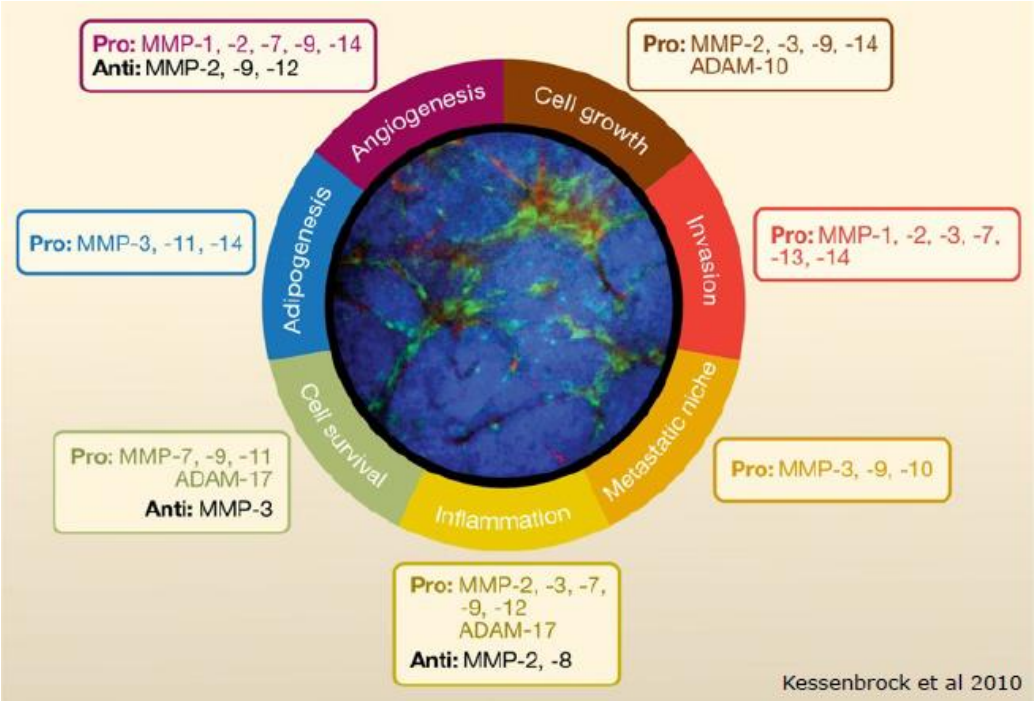
Def. chemokine: Small enzymes, specifically zytokines (signal proteins), that control/induce the movement of the cell. The cells move along a concentration gradient of some sort towards the highest concentration

MMPs control influx and clearance of neutrophils and macrophages at sites of injury by chemokine processing.

Functions of MMPs in response to injury: Not only does it degrade the ECM, it is also involved in other processes during injury: MMP7 sheds FASL that leads to apoptosis, which is an innate defense. It also sheds syndecan-I which leads to a release of CXCL8 that is needed for immune cell recruitment. MMP9 cleaves inhibitor of neutrophil elastase that induces an antimicrobial activity. ADAM17, MMP7 and MMP12 all have pro-inflammatory activities. Lastly, MMPs also modulate chemokine activity.

Some MMPs such as MMP8 (also known as collagenase-2) have protective properties in carcinogenesis. KO mice got terrible skin cancer.

One does not always get a phenotype when a MMP is knocked out. In any case, MMPs are used as targets and anti-targets in cancer therapy:



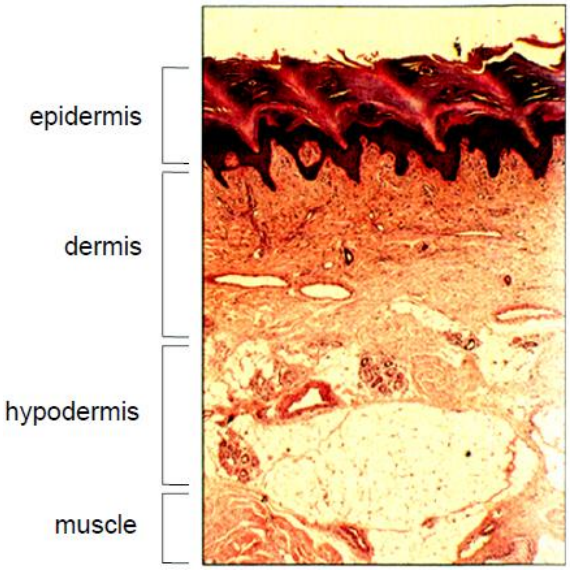
Take inflammation for example: The pro MMPs lead to more inflammation, while the anti MMPs lead to less inflammation. In that case, inhibiting pro MMPs would lead to less inflammation from a conceptual point of view. From a biochemical point of view, one would need to observe the signalling pathways and understand the network in order to know, where activity is inhibited. If anti-MMPs are inhibited, we would simply have more inflammations occuring probably.

In the case of cell survival, the pro-MMPs enhance cell survival while anti-MMPs lead to apoptosis or decrease cell survival in general. Knocking out MMP-3 would favour tumorigenesis, since such KO cells would grow indefinitely (or simply more than they normally would do, there are also other processes regulating cell survival and cell death).

In the case of angiogenesis, the formation of new blood vessels from pre-existing blood vessels, the pro-MMPs would favour angiogenesis, while the anti-MMPs would act inhibitory on angiogenesis. Knocking out anti-MMPs could lead to tumors or to excessive angiogenesis, since there is no downregulator.

SDS-PAGE and DIGE (difference gel electropherosis):

(know the conceptual idea behind it, why it is used and how it is used)



Wound healing – Matthias Schäfer's part (paying good attention is sufficient for the exam)

Functions of the skin: protection from UV, toxins, chemicals, irradiation, pathogens, physical insults (since the skin has multiple layers it can really efficiently absorb physical insults) etc.;

regulation of the body temperature;
barrier against water loss;
sensory organ

The ECM gives the skin its elasticity/flexibility and softness.

The skin has different layers:
1) epidermis = **suprabasal** (stratum corneum, stratum granulosum, stratum spinosum) + **basal** (stratum basale, basal lamina).
The epidermis is made not only of keratinocytes (90%), but also of merkel cells, immune cells (T-cells, langerhans cells), melanocytes (produce melanin, therefore protect cells form UV light). Especially, the DNA is protected within the cells.

2) dermis = fibroblasts (synthesize ECM and collagen), immune cells (T-cells, neutrophils, macrophages), endothelial cells (form the interior surface of blood vessels and lymphatic vessels), cutaneous nerves (sensory innervation) and smooth muscle cells (responsible for the erection of the hair e.g.).

Method: H&E staining: … **(maybe write about it, it was used in observing keratinocyte differentiation. The markers used were K10 and K14.)**

3 phases of wound healing: inflammation (blood clot formation, invasion of immune cells), proliferation, remodeling. The processes are overlapping.

Inflammation: 1) blood clot formation:
Damage of blood vessels => platelets bind to interstitial connective tissue;
Aggregation of platelets => degranulation and release of growth factors (such as TGF-beta, VEGF, PDGF etc.) and chemotactic factors for neutrophils and macrophages;
Blood coagulation => formation of fibrin clot, plugs the wound and serves as a provisional matrix.

Function of blood clot formation: protection of wound tissue (e.g. against invading microorganisms, water loss etc.), matrix for migrating cells, reservoir of cytokines and growth factors.

Cytokines and growth factors: They recruit inflammatory cells initiate re-epithelialization and formation of the granulates tissue and … .

2) invasion of immune cells:
Neutrophils and monocytes migrate concurrently to the wound site. Neutrophils arrive first at the wound site due to their large abundance in the circulation.

Activities of neutrophils:

Destroy bacteria: Via phagocytosis and via enzymatic pathways and release of reactive oxygen species (ROS).

Secrete pro-inflammatory cytokines: IL1α, IL1β, TNFα leads to activation of macrophages, fibroblasts, keratinocytes.

Neutrophil infiltration ceases within a few days: Entrapped in blood clot, extruded with eschar or become senescent, phagocytosed by macrophages.

Activities of macrophages:

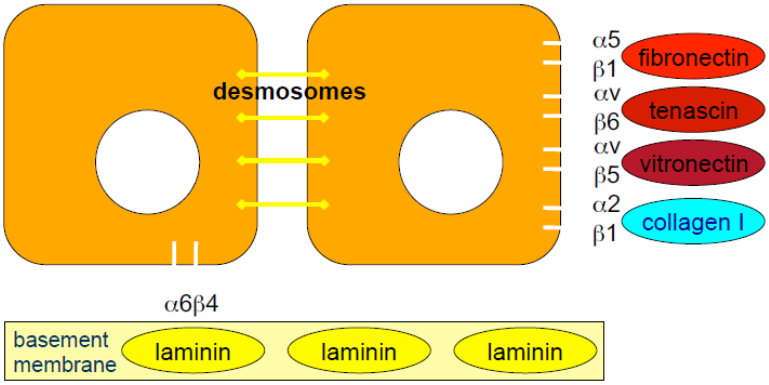
Angiogenesis through growth factors (bFGF, VEGF) and cytokines (TNF-alpha).
Antimicrobial function phagocytosis through oxygen radicals and nitrous oxide.
Cell recruitment and activation through growth factors (PDGF, TGF-beta, EGF, IGF) and cytokines (TNF-alpha- IL1, IL6).
Wound debridement phagocytosis enzymes such as collagenase and elastase.
Matrix synthesis through growth factors (TGF-beta, EGF, PDGF) and cytokines (TNF-alpha, IL1, IFN-gamma) and enzymes (collagenase, elastase).

Second phase: Proliferation phase is divided into re-epithelialization and formation of granulation tissue (fibroblast migration and proliferation, matrix deposition and wound contraction, neovascularization).

Kor.: re-epithelialization = keratinocyte migration and proliferation.

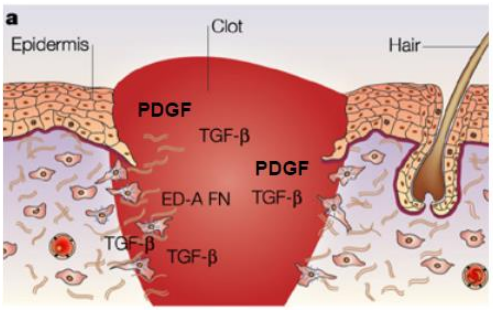
Stimulations leading to re-epithelialization:
1) The free-edge stimulus: absence neighboring cells.
2) A high concentration of growth factors such as the EGF family (EGF, HB-EGF, TGF-alpha) and HGF and KGF.

Def. desmosome: A cell structure that is specialized for cell-to-cell adhesion.

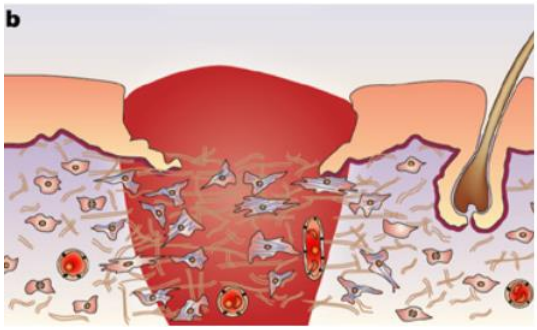


The basement membrane is right below the epidermis.

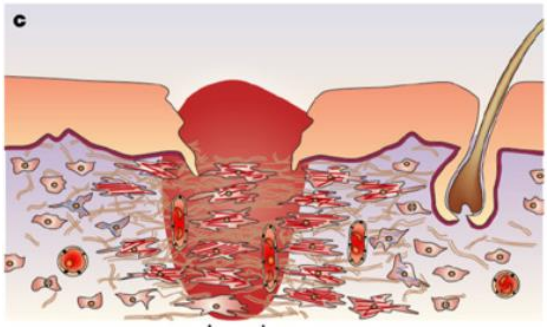
Granulation tissue formation – fibroblast migration and proliferation



PDGF, TGF-β
↓
activation of fibroblasts
↓
proliferation and migration
↓
production of ECM
(fibronectin, laminins, tenascin-C,
onset of collagen production

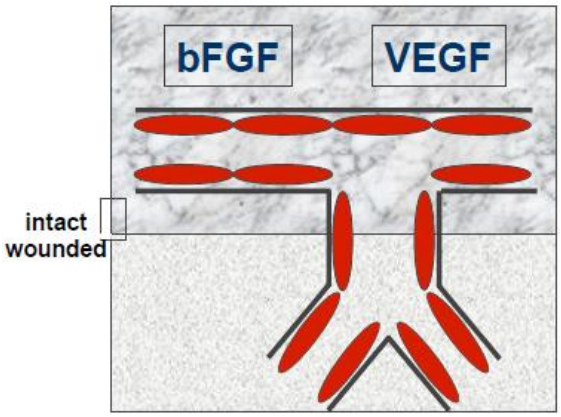


fibroblasts produce large amounts of collagen
↓
Gradual replacement of provisional matrix by collagenous matrix
↓
matrix remodeling begins



proto-myofibroblast
↓
myofibroblast: synthesis of smooth muscle actin
↓
increased contractile force

Neovascularization: proliferation and migration of endothelial cells (tube formation); deposition of own ECM; formation of new basement membrane around vessels.



Third phase: Remodelling: Epithelium becomes the epidermis and the granulation tissue becomes the dermis.

Remodelling is the transition from the granulation tissue to a mature scar. It then induces: Apoptosis of fibroblasts/myofibroblasts, the regression of capillaries, collagen remodeling.

Collagen remodeling also implies: collagen synthesis by fibroblasts, collagen catabolism: tightly regulated process involving metalloproteinases and their inhibitors, formation of larger collagen bundles, and alterations of intermolecular cross-links.

Outcome of the wound healing process: Skin is neither aesthetically nor functionally perfect, it losses its skin appendages such as hair follicles, sweat glands and sebaceous glands and it has reduced tensile

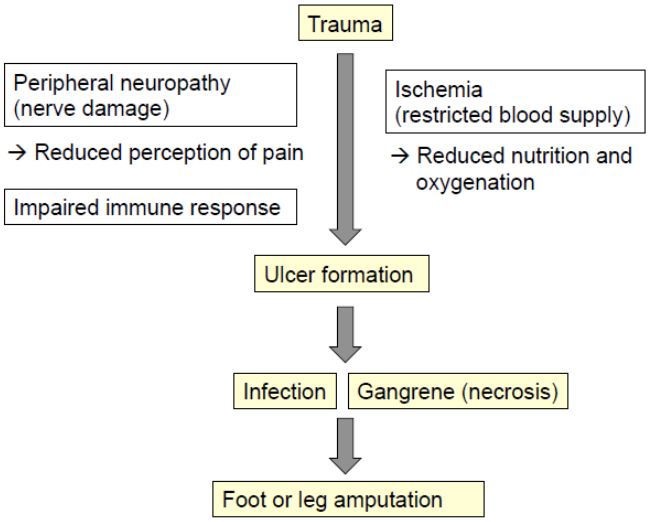
strength (the mature scar only has 70% tensile strength of the unwounded skin and only 20% of the final strength after 3 weeks of healing).

Fetal wounding: A fetus will not have a scar until the third trimester when it is wounded.

Cellular level in fetal wound healing: fast re-epithelialization,fetal fibroblasts, no myofibroblasts but acctin cable, low inflammation, nerve regeneration.

Molecular levvel in fetal wound healing: low levels of TGF1-beta, high levels of hyaluronic acid, high levels of MMPs.

Diabetic foot ulcer



Histopathological features:

• Reduced migration and proliferation of keratinocytes and fibroblasts

• Delay in mature granulation tissue formation

• Reduced Angiogenesis

• Disturbed extracellular matrix formation and remodeling by MMPs

• Reduced epidermal nerve formation

Molecular changes:

• Fibroblasts: senescent and reduced response to growth factors

• Macrophages: reduced secretion of cytokines (Il1β, VEGF)

• Growth factors: trapped and reduced expression

• MMPs: excessive activation implies impaired cell migration, degradation of matrix proteins and growth factors

• Nitric oxidase: reduced levels lead to reduced fibroblast proliferation and collagen production and to reduced angiogenesis

Standard therapy for diabetic foot ulcer:

1. Removal of excessive cells and fluid: Wound debridement (surgical removal of tissue); Negative pressure

2. Treatment of infection

3. Correction of perfusion/ oxygenation: Hyperbaric oxygen therapy; Negative pressure

4. Enhancement of wound closure: Wound dressing

Parallels between wound healing and cancer

	Wound	Cancer
Fibrin matrix	**Blood clot formation** (damaged blood vessels)	**Chronic fibrin deposition** (hyperpermeability of vessels)
Inflammation	**transient**	**persistent** → protumorigenic → stimulates angiogenesis + ECM breakdown → enhances cancer cell motility + invasion → promotes malignancy (ROS, NOS)
Epithelial Proliferation + Migration	**transient**	**persistent**
Epithelial-mesenchymal transition (EMT)	**partial** → Remaining intercellular junctions + keratin expression	**complete** (metastasis) → Loss of cell-cell contacts → Fibroblast like morphology → Expression mesenchymal markers
	Stimulation: HGF, TGFβ, TNFα, MMPs, *only tumor:* Ras mutations	

	Wound	Cancer
Fibrous tissue	**Granulation tissue** → fibrous tissue	**Persistent Stroma formation** Microenvironment: → tumor progression → cancer cell invasion
	Fibroblast activation: PDGF, TGFβ and others	
Angiogenesis	**transient**	**Persistent + imperfect** → essential for tumor growth
	Stimulation: VEGFA, PLGF, FGF2 and others Inhibition: TSP1, IP10	

Require:	Appropriate animal facilities
	Training (in house and official courses)
	Application and permission
	Regular reports
Control mechanisms:	Written application
	Control of reports
	Unannounced controls in the lab
Experiment:	Anaesthesia, including pain control
	Shaving, surgery
	Daily controls

8.5.2017

From here on, Kovacs' part begins. Exams: those things we spend a lot of time on, such as mechanisms, pathways etc. Not really something we just rushed through. (Focus will be on mammalian metabolism.)

Oxygen sensing and signaling

Oxic reactions evolved from anoxic reactions. the core is very similar, it's not easy to see because it is so dense. Oxygen made completely new reactions/pathways that more or less directly depend on O2 presence.

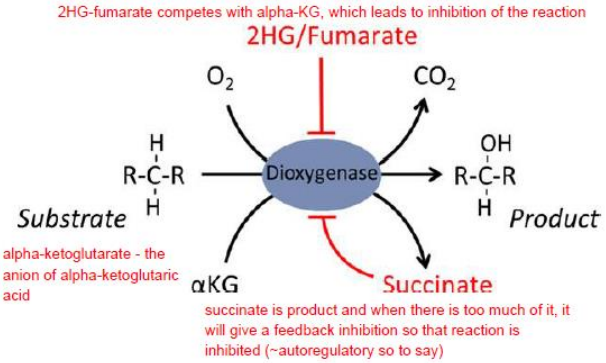
Def. Oxygenase: A molecule that transfers one or more oxygens to its substrate. Often, NADPH/NADH is used in these reactions. Dioxygenase would transfer two oxygens to its substrate.

Def. oncometabolite: Any metabolite that is associated with cancer. Ex.: **Succinate**, **fumarate**/fumarate hydratase (=: FH), **2-hydroxyglutarate** (oncogenic metabolite in leukaemia that disregulates epigenetics and cell differentiation), … .

On the pseudohypoxia part: Pseudohypoxia is the stabilization of HIF-alpha subunits under non-hypoxic (under normal) conditions.

Def. fumarate hydratase (FH): An enzyme for fumarate that hydrates fumarate to malate.

Def. hypoxia: Regions of the body or an organ that is suppressed of adequate oxygen supply (too little oxygen in these cells, mostly there is no oxygen supply, since there are also no vessels supplying it).



Def. Warburg effect: Cancer cells consume high amounts of glucose and produce lactic acid; provides cancer cells growth advantages in the tumor microenvironment. **(CHECK THAT DEF. AGAIN)**

Def. Pasteur effect: D-glucose is used up more quickly under anaerobic conditions in order to increase energy production when oxygen is not available to the cell (anaerobic glycolysis). Oxygen is an inhibitor for the fermentation process.

Def. Lymphoma: A group of blood cell tumors that develop from lymphocytes.

Def. Mitophagy: Autophagy of mitochondria.

Target genes of HIF1/2-alpha: HIF1-alpha: BNIP3/BNIP3L (cell death and mitophagy), VEGF (angiogenesis), glycolytic enzymes (metabolism and energy).
HIF2-alpha: EPO (erythropoiesis; also HIF1-alpha is involved but mostly HIF2-alpha).

PHDs regulate the HIF-alphas by degrading them:

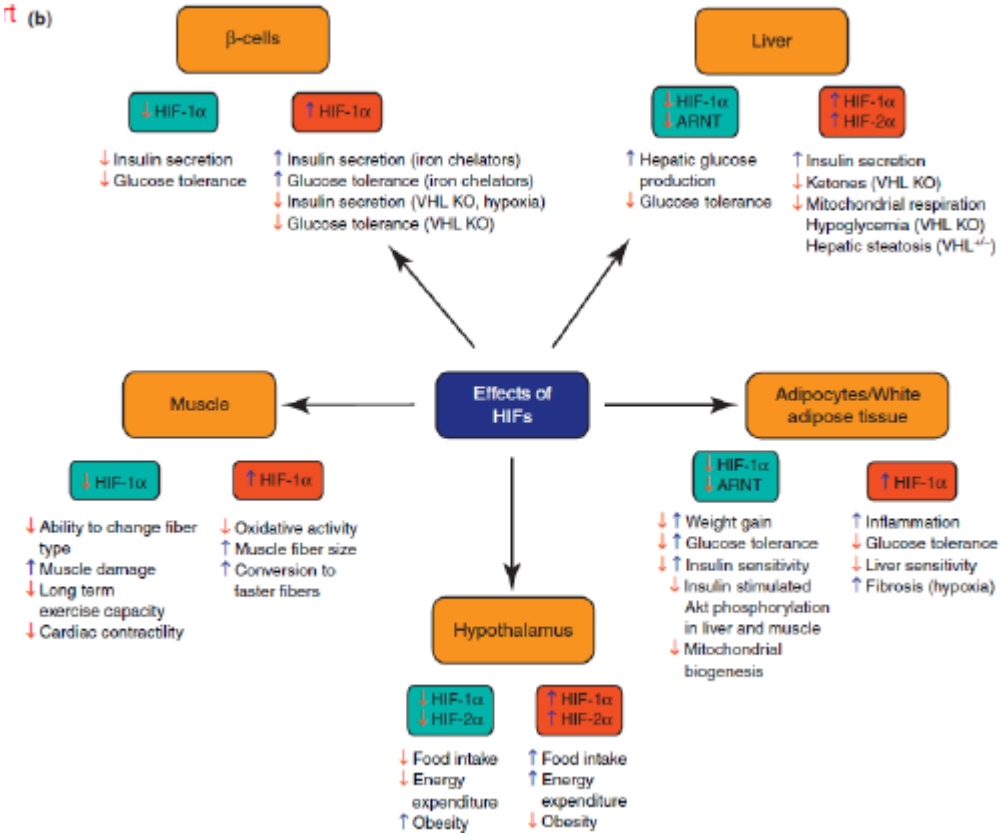
	HIF-α isoform target	Tissue distribution in normoxia	Induction by hypoxia
PHD1	HIF-2α>HIF-1α (normoxia) HIF-1α=HIF-2α (hypoxia)	Testis>liver=heart=brain=kidney	No
PHD2	HIF-1α≫HIF-2α (normoxia) HIF-1α>HIF-2α (hypoxia)	Heart>liver=testis=kidney>brain	Yes
PHD3	HIF-1α=HIF-2α (normoxia) HIF-2α>HIF-1α (hypoxia)	Heart>testis=kidney=liver=brain	Yes
FIH	Unknown	Breast=testis=ovary>pancreas>liver>kidney	No

enzyme names gene names

PHD1 = EGLN2
PHD2 = EGLN1
PHD3 = EGLN3

Use the gene names as the correct names.

Links between HIFs, type 2 diabetes and metabolic syndrome



You must be able to answer the following questions:

What is the sense of sensing oxygen?

What are the mechanisms?

What is the nature of adaptive response?

How do oxygen levels affect metabolism?

What does the oxygen pathway play for a role in disease and which diseases?

15.5.2017

Def. sphingomyelin: A type of sphingolipid that is found in animal cell membranes. It is also found in the membranous myelin sheath that surrounds some nerve cell axons.

Def. glycosphingolipids: Glycosphingolipids are a subtype of glycolipids containing the amino alcohol sphingosine. (Alternatively, they may be considered as sphingolipids with a carbohydrate attached.)

Kor.: Sphingomyelins is involved in signal transduction and also in the apoptotic signaling pathway.

Cholesterol homeostasis in the liver:

Uptake of cholesterol through blood or synthesis from acetate Ac to cholesterol Chol.
A cell will normally prefer the uptake to synthesis, since cholesterol synthesis involves many different

reactions and also requires the cell to regulate other processes too (keep in mind that a cell is a network and the reactions within a cell are parts of a network – they are not independent processes).

Mechanisms to get rid of cholesterol: Catabolic reactions: Chol to bile acids. Bile acids are secreted into the bile.
The liver can package cholesterol into packages and remove it from the liver. Those packages are used for lipid supply to other cells (ULDL).
Another way would be to make LD (lipid droplets), but this process does not normally occur within a cell.

Def. low-density lipoprotein (=: LDL): A transport vehicle in the blood plasma for water insoluble molecules such as cholesterol, phospholipids, fatty acids triglycerides etc. LDLs are sometimes implanted into arteries, leading to arteriosclerosis.

Kor. Ultra low-density lipoprotein (=: ULDL): Analogous to LDL, but density of proteins is even lower (ULDLs are very huge and can be seen through a light microscope at maximum magnitude).

LDL receptor:

- Binding, internalization, disassembly
- Regulated by cellular cholesterol
- Determines LDL level in blood
- Defective in familial hypercholesterolemia (FH)
- Induced by “Statin” drugs

Uptake pathways:

Def. proprotein convertase subtilisin/kexin type 9 (=: PCSK9): An enzyme that is ubiquitously expressed in many tissues and cell types. It binds to LDLR and destroys them. Note that the brain is dependent of endogenous synthesis of cholesterol and LDL/LDLR. The question arises: How low should one go?

Possible ways to inhibit PCSK9: Gene silencing: PCSK9 anti-sense oligonucleotide (=: ASO), locked nucleic acid ASO, siRNA in lipidoid nanoparticles, induction of loss-of-function mutations in mice using CRISPR-CAS9. All these possibilities have shown to reduce PCSK9 levels.

(Pcsk9 stuff …)

16.5.2017

On the regulation of cholesterol in eukaryotes

…

Def. HMG CoA reductase: An enzyme in the mevalonate pathway, which is necessary for cholesterol production. Mevalonate pathway occurs in the cytoplasm. (**formally**: HMG CoA with HMG CoA reductase gives mevalonate).

Inhibitors of HMG CoA: Atorvastatin, Simvastatin, Pravastatin.

Those statins also increase the expression of LDLR and therefore increase the uptake of LDL.

Def. sterol regulatory element-binding protein (:= SREBP): Transcription factors that are involved in the cholesterol metabolism. They bind to the sterol coding regulatory element DNA sequence. They are indirectly required for cholesterol biosynthesis and uptake and for fatty acid biosynthesis.

SREBPs as activators of the complete cholesterol and fatty acid synthesis:

• Lipid homeostasis in vertebrate cells is regulated by a family of membrane-bound transcription factors designated sterol regulatory element-binding proteins (SREBPs).

• SREBPs directly activate the expression of genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids, as well as the NADPH cofactor required to synthesize these molecules.

• The mammalian genome encodes three SREBP isoforms, designated SREBP-1a, SREBP-1c, and SREBP-2.

• SREBP-1a and -1c are derived from a single gene through the use of alternative transcription start sites that produce alternate forms of exon 1, designated 1a and 1c.

• At normal levels of expression, SREBP-1c favors the fatty acid biosynthetic pathway and SREBP-2 favors cholesterologenesis.

• SREBPs comprise a subfamily of bHLH leucine zipper (bHLH-LZ) proteins. SREBPs bind both the canonical inverted-repeat E-box site, characteristic of most bHLH proteins, and the SREBP-specific direct-repeat-binding element or SRE.

Def. Insulin induced gene protein 1 (=: insig-1): Located in the ER and apparently expressed everywhere, especially in the liver. It is involved in the cholesterol biosynthesis; it binds to SCAP in order to make the SREBP/SCAP complex stay longer in the ER. It inhibits SCAP to carry over SREBP to the golgi complex. Effectively, it blocks SREBP from acting as a transcription factor for the SRE in the promoter region of HMG CoA reductase gene and results in decreased expression of the HMG CoA reductase enzyme.
Also, it binds to the sterol sensing domain of HMG CoA reductase, resulting in the enzyme's increased degradation.

Def. Insulin induced gene protein 2 (=: insig-2): Insulin activates the human INSIG2 promoter. Insig2 is upregulated under hypoxic conditions and is sometimes associated with pancreatic cancer.
It works pretty much like insig1.

22.5.2017

Niemann-Pick disease type C: Lysosomal storage disease with mutations in NPC1 and/or NPC2. Also in this disease, free unesterified cholesterol accumulates in the lysosomes. This leads to a deficiency where cholesterol should be present (membranes and in steroid synthesis).

Cholesterol in the central nervous system:

•Plasma lipoproteins do not cross the blood-brain barrier

•Brain depends on intracerebral de novo synthesis of cholesterol

•Brain is the most cholesterol-rich organ in the body (25% of unesterified cholesterol in the whole body)

•Cholesterol concentration in the brain is 15-20 mg/g tissue, average cholesterol concentration in other tissues is ~2 mg/g tissue

•2 major cholesterol pools in the CNS: myelin sheaths (i.e., oligodendroglia) and plasma membranes of astrocytes and neurons

•Cholesterol is found predominantly in white matter

•Up to 70% of the brain cholesterol is associated with myelin

•Myelin consists of 70% lipids and 30% proteins

•Cholesterol is an essential signal for synaptogenesis and the formation, function and stability of synapses are sensitive to disturbances in cholesterol metabolism

•Cholesterol has a half-life of 4-6 months in rat brain

CNS disorders and its connection to cholesterol:

•Increased cholesterol turnover in neurodegenerative disorders such as Alzheimer‘s disease (AD) and Niemann-Pick type C disease

•Patients with elevated plasma cholesterol levels have increased susceptibility to Alzheimer‘s disease

•Hypercholesterolemia is associated with increased brain amyloid b (Ab) immunoreactivity

•Apolipoprotein E (ApoE) is the major cholesterol transporter in the brain; increased expression of the ApoE4 allele is associated with increased risk of AD development at a younger age

•Determinants of cardiovascular health, especially midlife dyslipidemia, are associated with an increased risk of dementia based on molecular and epidemiological data.

•Statins might have beneficial effects in several neurologic diseases (e.g., multiple sclerosis, AD, ischaemic stroke)

Cholesterol metabolism and in embryogenesis

Role of cholesterol in the embryo and fetus

•Membrane formation

•Maintains membrane integrity and consequently the structure and function of membrane-bound proteins

•Cholesterol is part of lipid rafts and caveolae, which are critical for directing the location and thereby activity of proteins into lipid-rich or-poor membrane microdomains. Numerous signaling processes originate in lipid microdomains, including those related to growth (i.e., insulin signaling).

•Hedgehog processing

•Precursor for bile acids, steroid hormones, and oxysterols. Bile acids are key integrators of metabolism in addition to being involved in lipid absorption. Some steroid hormones are essential for normal development of the fetus (i.e., lack of estrogen affects morphology of the gonads).

The role of maternal cholesterol (during pregnancy)

•Maternal transfer of cholesterol to the fetus does occur, but the significance of the maternal contribution of cholesterol and other sterols is still in debate.

•It is accepted that early development depends on maternal cholesterol, while later on, endogenous cholesterol synthesis capacity is indispensable.

•What are the consequences of less exogenous cholesterol? Less cholesterol could be presented to the fetus due to lower maternal cholesterol concentrations, less uptake of lipoproteins, lower sterol synthesis rates in the placenta or yolk sac, less transport of sterol to the basolateral side, and/or less efflux or secretion to the fetal-facing side of trophoblasts or placental epithelial cells.

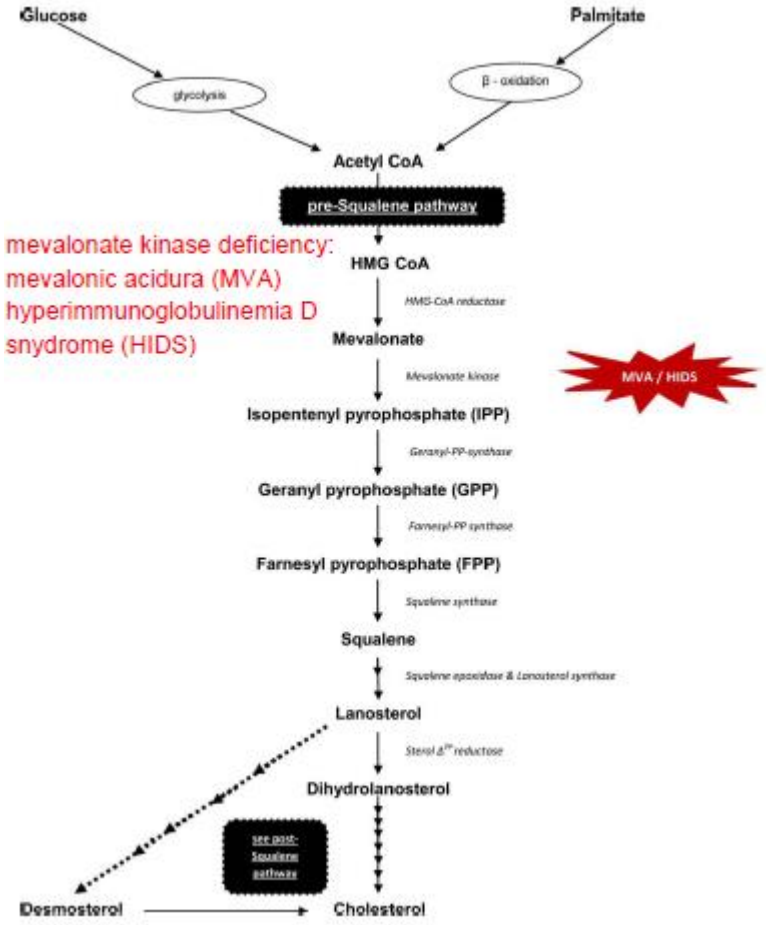
•Recently, the effect of lower maternal cholesterol concentrations on a more subtle outcome of gestation, i.e., birth weight, has been evaluated. Women with lower plasma cholesterol concentrations had smaller newborns. There is also a correlation between low plasma cholesterol and microcephaly. Newborns with abnormal in utero growth rates, which lead to intrauterine growth-restricted infants and macrosomic infants (newborn with excessive birth weight), have an increased risk of developing age-related diseases.

Genetic disorders in cholesterol synthesis

There are several disorders which we can categorize in the pre-squalene pathway and in the post-squalene pathway:

Def. squalene: A 30-C organic compound that is vital for the synthesis of all plant and animal sterols. It is central for the synthesis of cholesterol, vitamin D and steroid hormones in the human body.

Pre-squalene pathway

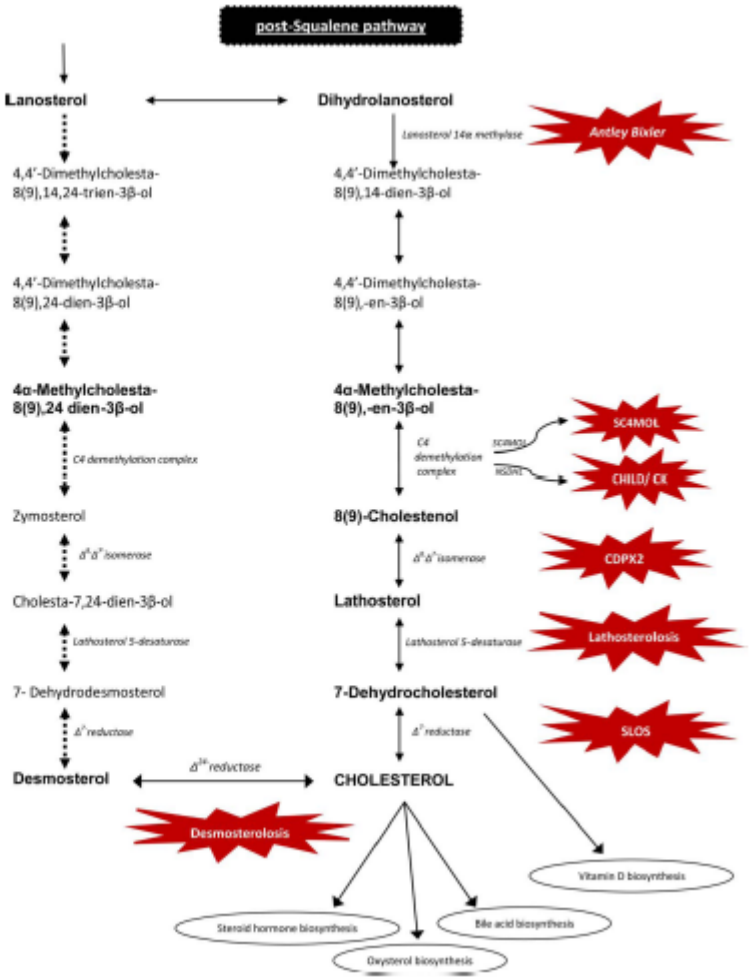


Post squalene pathway

why are earlier steps embryonically lethal, but latter steps are viable?

the sooner a gene acts in cholesterol synthesis the earlier the phenotype occurs (see nexgt slide)

with latter occurrence, some earlier functions at least can be rescued



This leads to several inborn errors in cholesterol synthesis:

•The sooner a gene acts in cholesterol synthesis, the earlier the phenotype occurs.

•Dependence of fetal development on endogenous cholesterol synthesis

•Inhibitors of cholesterol biosynthetic enzymes are potentially teratogenic. Drugs inhibiting human cholesterol synthesis are better avoided in early pregnancy.

•Consequences of cholesterol deficiency and the potential consequence of accumulation of bioactive or toxic precursor sterols

•Disorders of sterol metabolism frequently present with structural abnormalities of the brain, skeleton, and skin.

•Neuro-developmental/ behavioral abnormalities are frequent and variable

•Defects in cholesterol synthesis are generally lethal in mice, while humans with impaired later steps of the pathway can survive with severe malformations.

The biological basis for cholesterol phenotypes are deficiency in the final product cholesterol, excess or deficiency of sterol intermediates and modifications in the hedgehog signaling pathway.

On Smith-Lemly-Opitz syndrome (=: SLOS): Inborn error in the synthesis of cholesterol. Specifically, a mutation in the DHCR7, dehydrocholesterol reductase enzyme 7.
Phenotype: mild intellectual disability (cholesterol is essential in the brain for myelination and since cholesterol cannot pass the blood brain barrier, the brain cannot get exogenous cholesterol and it has to produce it itself through biosynthesis) up to lethal malformations.
Biochemical phenotype: Hypocholesterolemia (lowered cholesterol levels in the blood plasma), but in 10% of all patients, there is no hypocholesterolemia. Also, elevated 7-dehydrocholesterol levels (obviously, since the reducing enzyme is not expressed).

On the hedgehog signaling pathway: A signaling pathway central to a proper development in many animals. This pathway transmits information to embryonic cells for proper cell differentiation. Malfunctions in this pathway often lead to basal cell carcinoma. (**LOOK IN MOLECULAR BIOLOGY OF THE CELL FOR MORE INFO IF THERE IS INFO ON IT**)

Mammals have three hedgehog homologues: Sonic hedgehog (SHH), Indian hedgehog (IHH) and Desert hedgehog (DHH). (SHH is the best studied one.)

Perturbations in the hedgehog signaling pathway

•Hedgehog family is comprised of three different proteins: Sonic hedgehog (SHH), Indian hedgehog (IHH), and Desert hedgehog

•Cholesterol is a covalent ligand of the hedgehog family of developmental patterning proteins

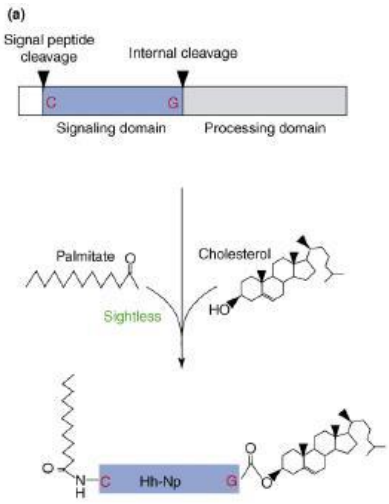
•Shh-deficient mice: cyclopic, defects in the ventral neural tube, somite, and foregut patterning. Later defects include distal limb malformation, absence of vertebrae and ribs, and failure of lung branching.

•These phenotypes are consequences of dysfunction in patterning during early embryogenesis.

•Possibilty that congenital abnormalities associated with defects in cholesterol synthesis arise because the autocatalytic processing of hedgehog proteins is disrupted.

•Hypothesis that cholesterol biosynthesis phenotypes are phenocopies of hedgehog defects, in which the properties of hedgehog are altered when processed with sterols other than cholesterol.

Cholesterol is vital for the proper maturation of hedgehog proteins.



Sterol intermediates: toxicity or gain of function:

•Inhibition of enzymes early in the sterol biosynthetic pathway leads to early embryonic lethality (e.g., HMGCR, MVK, SREBP-2, squalene synthase).

•Most fetuses with defects in sterol biosynthesis late in the pathway are viable until late in gestation or until just after birth.

•Even though the endpoint of all reactions is cholesterol, early inhibition of the process results in the lack of isoprenoids as well as a lack of cholesterol. Likewise, late inhibition results in a lack of cholesterol and a buildup of different intermediates.

•Lack of isoprenoids occurs in reactions prior to farnesol synthesis. Isoprenoids, including geranylgeraniol and farnesol, are essential for basic cellular processes (e.g., cell proliferation). The proteins modified by isoprenoids include proteins of the the ras, rab, and rho families; GTP-binding proteins; and G proteins. Farnesyl pyrophosphate is a precursor for dolichol, which is essential for survival of blastocysts past implantation.

On bile acids

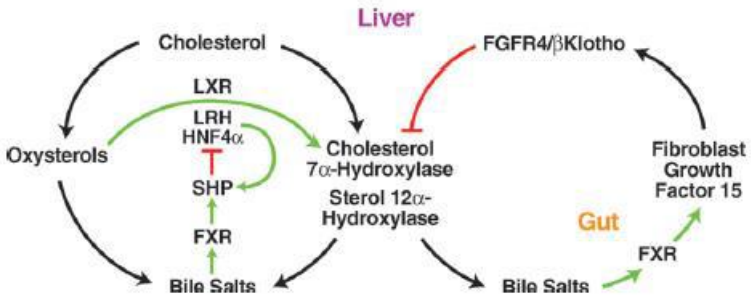
•Dietary lipid absorption

•Cholesterol homeostasis

•Inherited mutations that impair bile acid synthesis cause a spectrum of human disease; this ranges from liver failure in early childhood to progressive neuropathy in adults.

•Bile acids (BAs) as signaling molecules: BAs activate mitogen-activated protein kinase (MAPK) pathways; BAs are ligands for the G-protein-coupled receptor (GPCR) TGR5; BAs activate nuclear hormone receptors such as FXR.

Regulation of bile acid synthesis



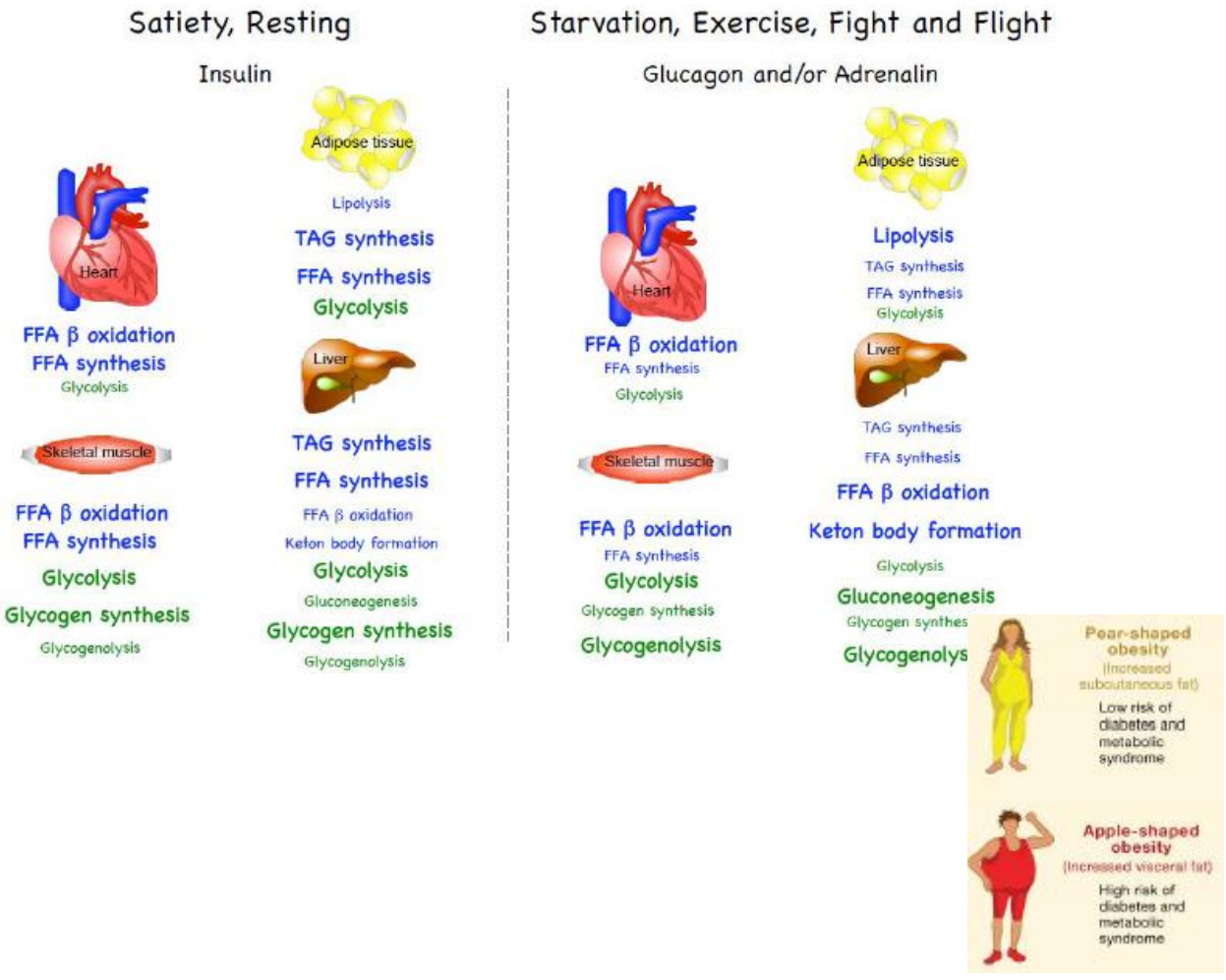
More on lipid metabolism:

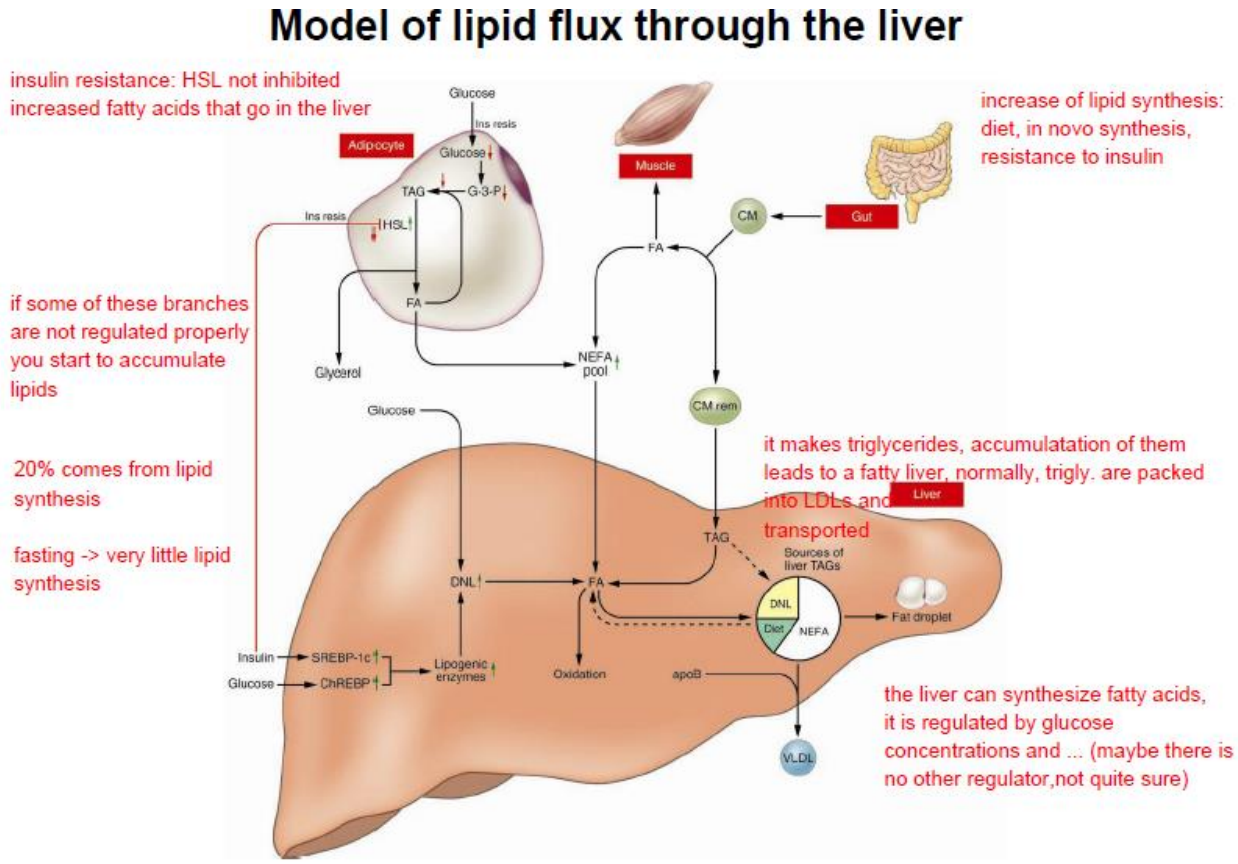
On obesity

Def. BMI: BMI := 1[kg]/[cm**2] * [normalizing_factor]. Obesity ⇔ BMI > 30

One also uses the hip-to-waist index as a second reference measure.

Lipid and glucose homeostasis in different tissues under different physiological tissues





Functions of glucose and insulin in liver and cholesterol homeostasis

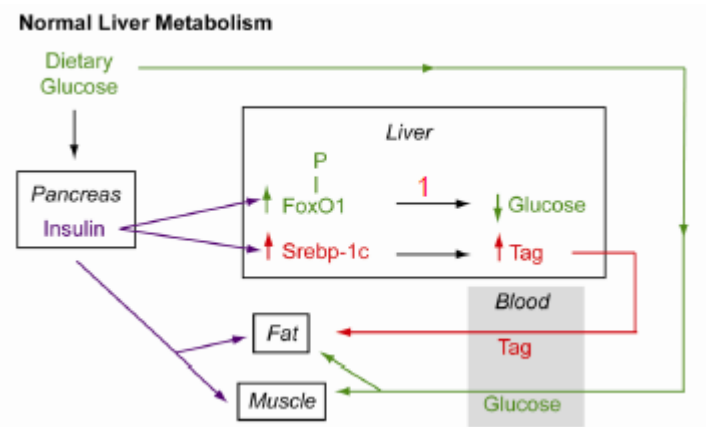
Glucose: Influences pathway, since increased glucose levels mean an increase in glucolysis.

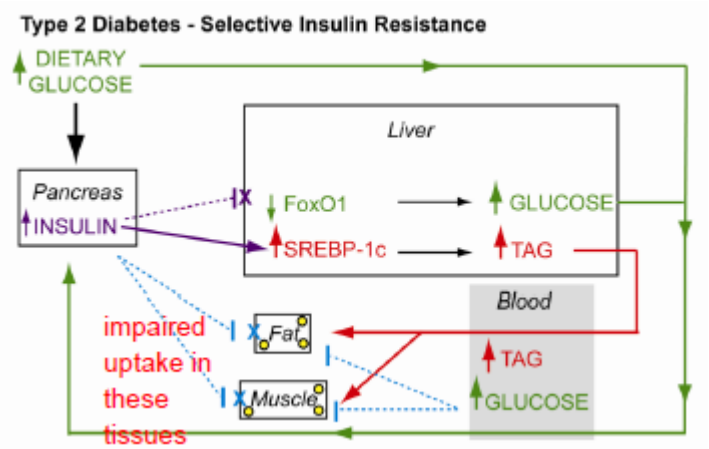
…

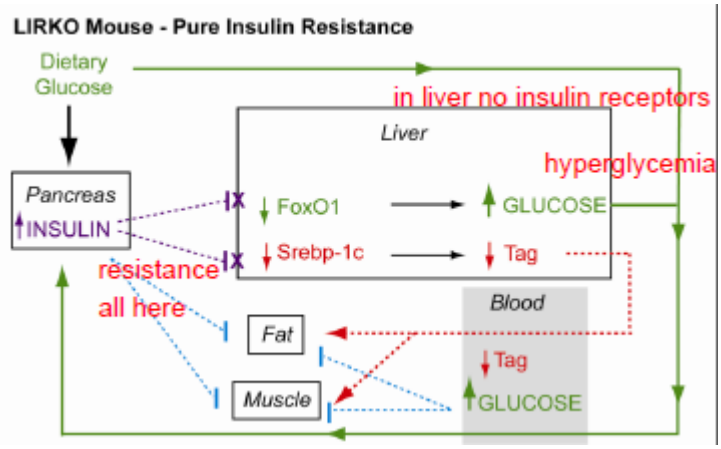
Insulin: Essential for the glucose uptake out of blood.

…

3 cases where insulin plays an important role (with diseases):





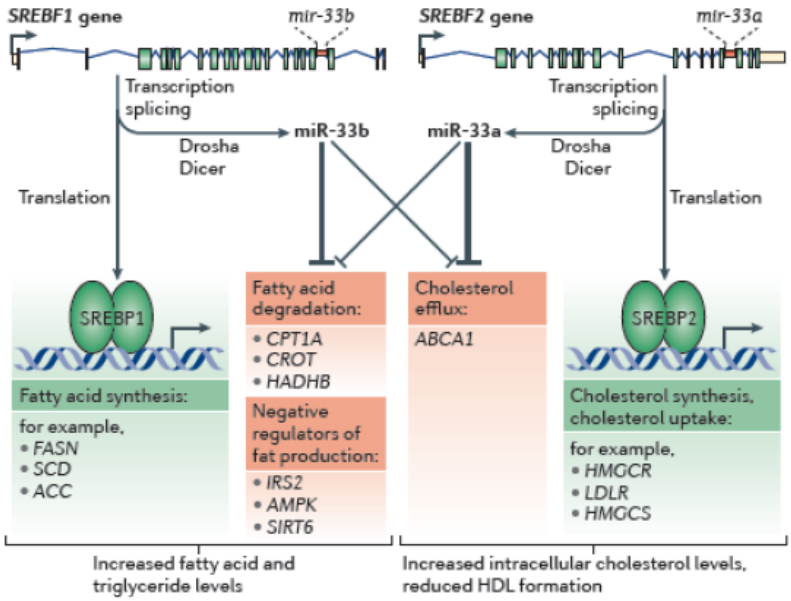


1: Shuts down a pathway and gluceogenesis is inhibited.

Pure insulin resistant mice do not develop coronary heart disease unlike the type 2 diabetes mice.

Def. miR-33b: Precursor of a mature micro RNA. miR-33b is involved in the regulation of genes such as SREBP-1. In lipid metabolism, it downregulates ABC transporters that are responsible for the regulation of cholserol and HDL generation. Also, it is suggested that miR-33b is involved in the regulation of fatty acid metabolism and insuline signaling.
Location: in intronic regions beween two protein coding genes (SREBP-2 and SREBP-1).

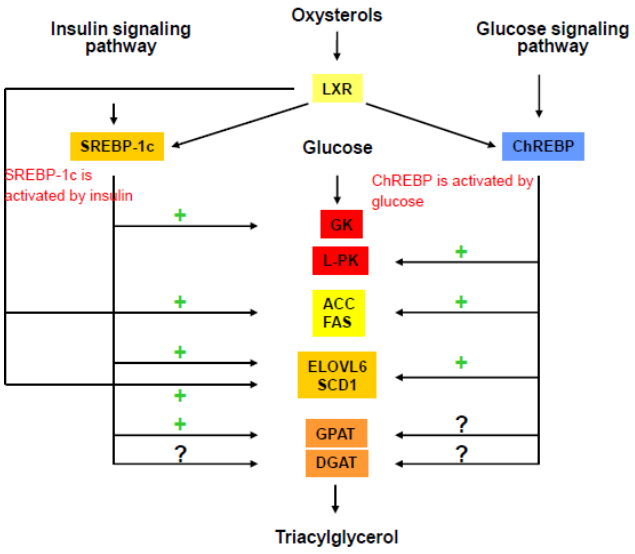
Model of the SREBP and miR-33b circuit

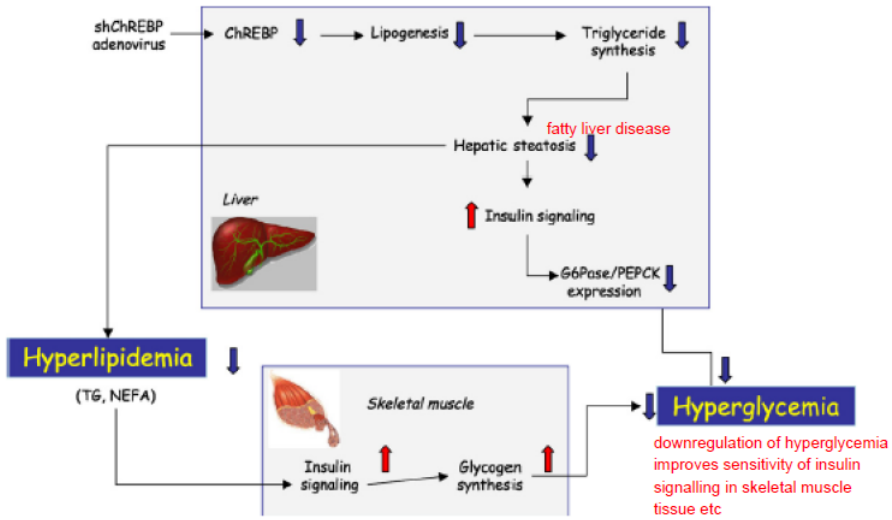


(WHAT IS THIS GOOD FOR AND WHAT DOES IT DO)

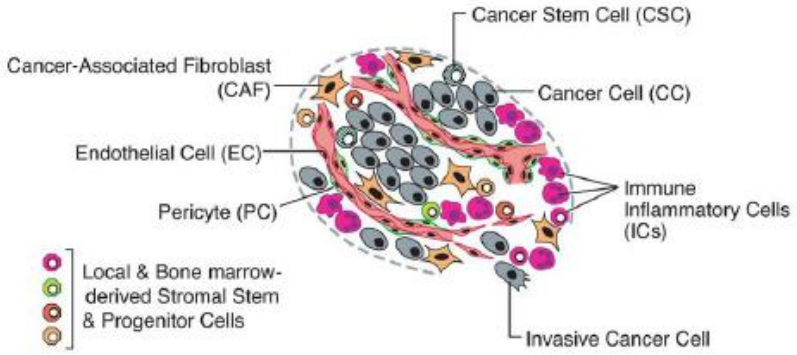
Def. Carbohydrate responsive element binding protein (=: ChREBP) (naive): In the liver, ChREBP mediates several different regulatory enzymes of glycolysis and lipogenesis. It is a protein that interacts with other glucose related proteins and carbohydrates by forming heterodimers and thus binding and activating them. Knockouts in the MLXIPL gene (it codes for ChREBP) leads to various malfunctions in the pathways involved.

Def. Adenovirus: A virus that uses humans as its host. Depending where the virus is active it can cause different diseases. Genetically engineered, it can invade a human liver and introduce the synthesis of exogenous proteins.





Lipid metabolism in cancer



Def. Adipose tissue: Loose connective tissue mainly composed of adipocytes – body fat and fat cells. An adipocyte stores energy as fat.

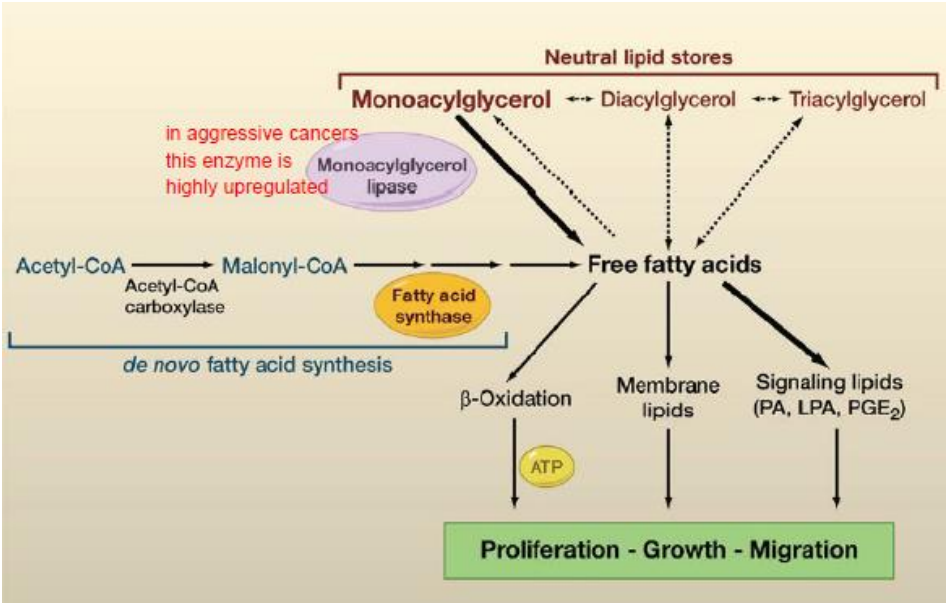
Def. cachexia: Decreased body mass and less fatty tissue accumulation. Also, it involves muscle atrophy, fatigue and loss of apetite.

Common phenotypes in virtually all aggressive cancers:

Metabolic reprogramming: mutations in cancer genes and alterations to cell signaling.
Aerobic glycolysis (Warburg effect): Consumption of high amounts of glucose and production of lactic acid leads to an advantage of growth for tumor cells in their microenvironment.
Increased glutamine metabolism: glutamine derived alpha-ketoglutarate contributes to the production of citrate.
Increased DNA synthesis and protein synthesis (e.g. mTOR pathway).
Increased de novo fatty acid synthesis.

Def. clear cell renal cell carcinoma: most common type of kidney cancer; malignant epithelial cells with clear cytoplasm and a compact-alveolar (nested); very small tubes that transport waste from liver to urine.

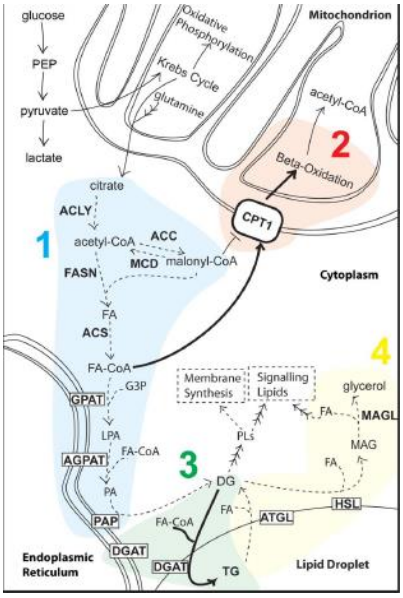
An overview: free fatty acids and tumorigenesis



Lipids can promote different aspects of cancer development: de novo fatty acid synthesis and diet mobilisation from adipose tissue leads to fatty acids. Those fatty acids are used by cancer cells for: membrane synthesis (cell growth and proliferation), membrane saturation (oxidative stress resistance), lipid droplet formation (survival under energy stress), NADPH oxidation (redox balance), cholesterol lipid hormones (proliferation and invasion).

An example of an exam question (commentary from slides copied):

How can we interefere? Inibit/acitvate pathways etc.?



Com.: “inhibit uptake of fatty acids. prob: there are many uptake enzymes that can compensate (to some extent)”

Com.: “ can we influence the synthesis pathway?yes. important is:we need citrate (1).cancer cells need to interefere with citrateshuttle serivce and pre-cursor.

Also, need to interfere with CPT1 to inhibit fatty acid synth (transport of FA to mitoch.)”

Com.: “Also, inhibit glycolysis => prevent FA release (inhibit lactase or mobilization of FA => see 4)”

Com.: “Also, we can influence storage (3): promote storage so FA stay in lipid droplets. But prevent mobilization, so it's never really used. What about oxidation? (see 2): induce oxidation to break down FA.

prob: when FA very energy rich, we get ATP, which will be used for other processes by cancer cells. Tricky situation. => in some cancers, inhibiting of oxid, might be better: it's context dependent."

Com.: "Can we also affect a normal cells? normal cell will be satisifed of uptake of fatty acids (costs energy for de novo snythesis)"

Analysis: There are several smaller networks interacting with one another. Those networks have their own pathways that in one way or the other will modulate the other pathways. For the exam, you can analyze each network and think about inhibition or activation in cancer cells, but also in normals.

De novo fatty acid synthesis (summary slide):

• Two sources: exogenously-derived (dietary) FAs and endogenously-synthesized FAs

• Biosynthesis is catalysed by the multifunctional, homodimeric fatty acid synthase (FASN)

• Predominant product of FASN is palmitate (C16:0)

• In well-nourished individuals the role of FASN is of minor importance owing to sufficient levels of dietary fat.

• Most normal cells and tissues, even those with high cellular turnover, seem to preferentially use circulating lipids for the synthesis of new structural lipids.

• In normal conditions FASN converts excess carbohydrate into FAs that are then esterified to storage TAGs.

• De novo FA synthesis is very active during embryogenesis and in fetal lungs (production of lung surfactant).

• A wide variety of tumors and their precursor lesions undergo exacerbated de novo biogenesis of FAs irrespective of the levels of circulating lipids.

• Neoplastic lipogenesis is reflected by significantly increased activity and coordinate expression of several lipogenic enzymes in tumor cells [e.g., FASN, ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACACA)].

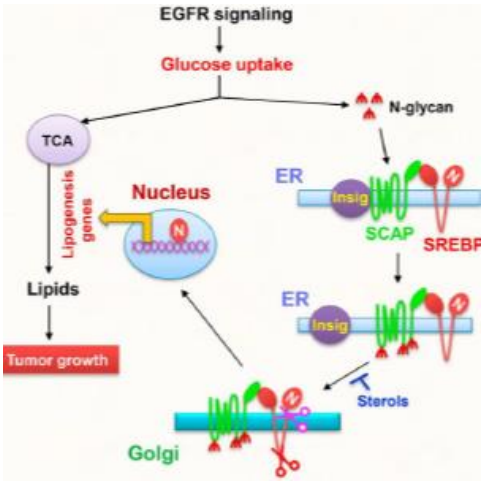
• Upregulation of FASN represents a nearly-universal phenotypic alteration in most human malignancies.

• FAs synthesized in cancer cells are esterified predominantly to phospholipids and incorporated into membrane lipids by proliferating cells.

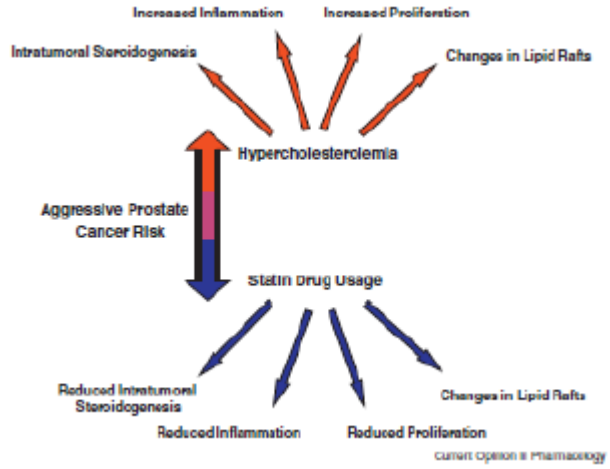
• Many of the genes that encode the enzymes of the FA biosynthetic pathway, including ACLY, ACACA, FASN, reside on human chromosome 17q. This is a common site for gene rearrangement and is the location of many oncogene amplifications. However, only one study evaluating the correlation of the expression levels of lipogenic enzymes with gene copy number alterations has detected a significant increase in FASN copy number in prostate cancer.

• Increased FA synthesis in tumor cells seems to involve the modulation of multiple lipogenic enzymes at various levels (e.g., increased transcription, enhanced protein stabilization).

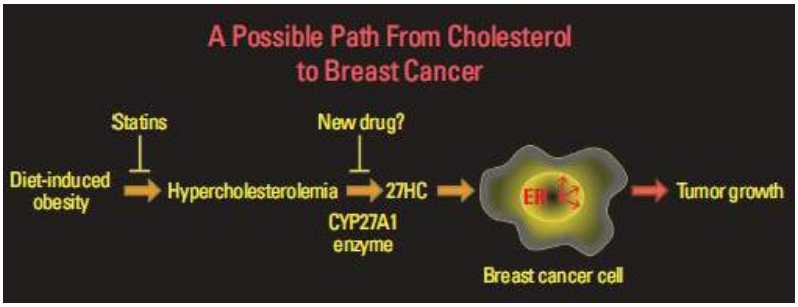
SCAP links glucose to lipid metabolism in cancer cells:



Lipid metabolism genes are important for transformation and are upregulated in cancer tissue. Also, it seems that **melavonate** plays an important role in cancer, since it is a precursor molecule to many other molecules regulating the cell cycle (GGPP, FPP, dolichol – GGPP and FPP regulate Rho and Ras; those are regulatory proteins for signal transduction of several membrane receptors related to cell proliferation, differentiation and apoptosis). It is probably even more important than cholesterol.



Cholestorol and breast cancer:



27HC mimics estrogen in certain tissues. Estrogen driven breast tumors may rely on 27HC when estrogen is unavailable.

Com. (generalized form): "in cancer (e.g.): when A is not available, cancer cells might makeuse of B, which mimics the function of A in certain areas (either a subset of all areas or all areas)"

ADDENDUM: Why mice are not a good model system for research in lipids and what we can do to make the suitable according to our needs: … (don't have miR-33b and SREBP-1, different concentration of HDL/VLDL, …)

29.5.2017

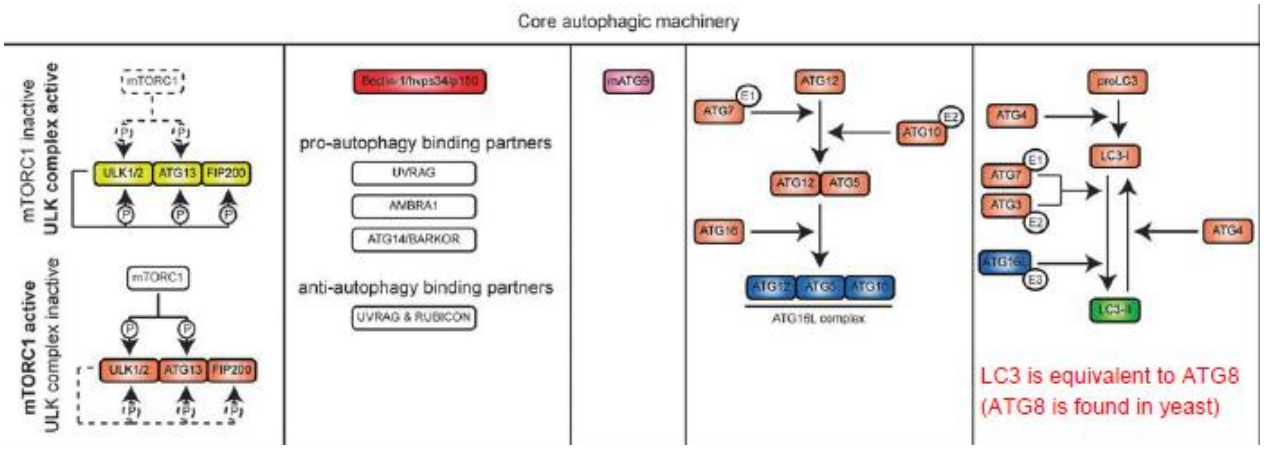
Autophagy

Def. phagophore: A double membrane that encloses and isolates the cytoplasmic components during macroautophagy.

Def. macroautophagy: A phagophore forms around the material to be digested before it fuses with the lysosome.

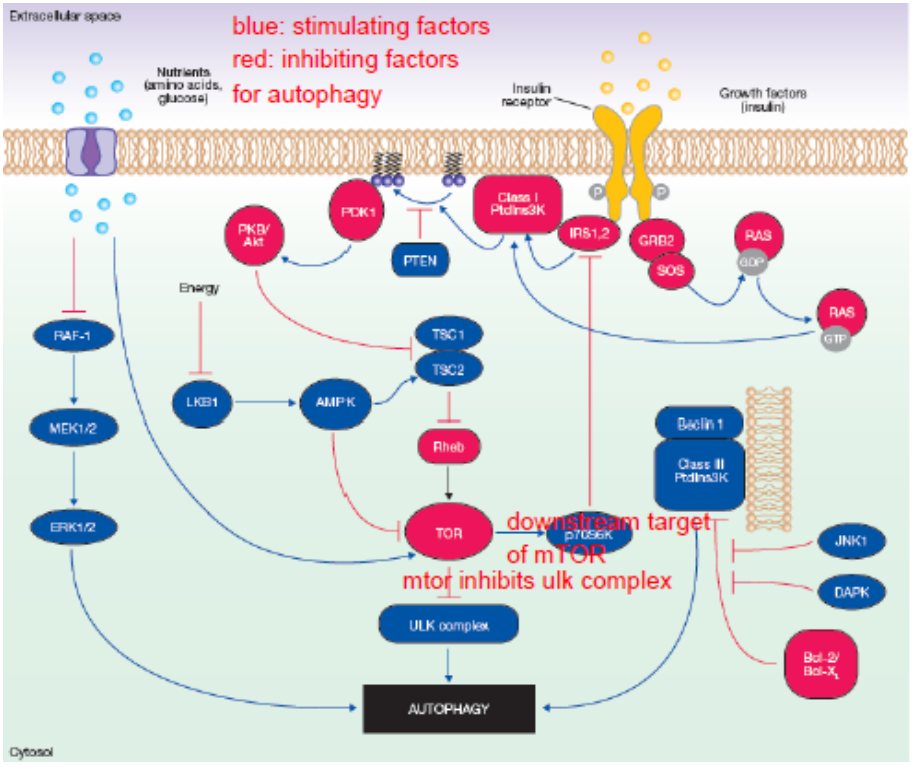
Def. microautophagy: The material to be digested fuses directly with the lysosome without forming a phagophore first.

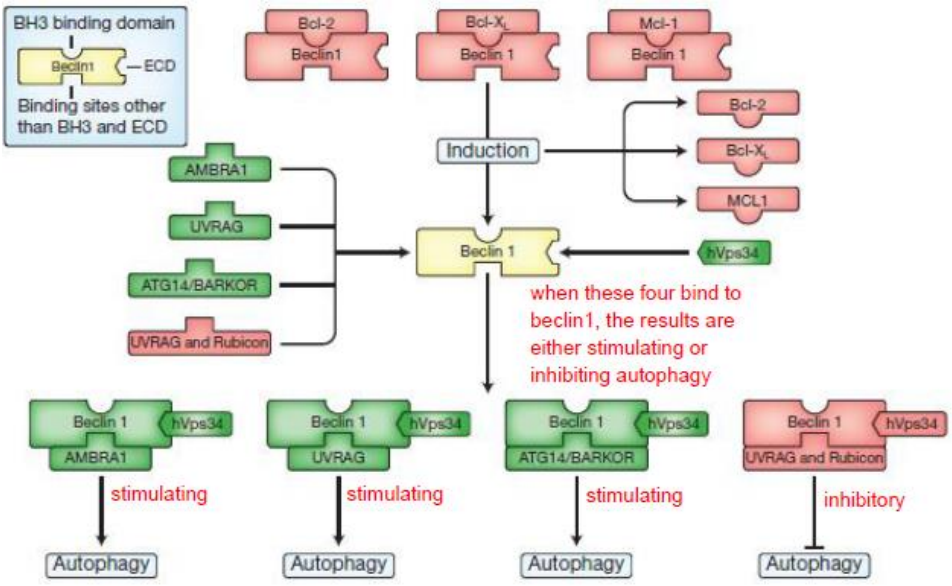
ULK1/2: orthologues of yeast Atg1
BARKOR: Beclin-1-associated autophagy-related key regulator
UVRAG: protein product of the ultraviolet radiation resistance gene
AMBRA1: activating molecule in
Beclin-1-regulated autophagy
RUBICON: RUN domain and cysteine rich domain



mTORC1: mTOR+ RAPTOR
mTORC2: mTOR + RICTOR
RAPTOR: reuglatory associated protein of mTOR
RICTOR: rapamycin insensitive companion of mTOR

Becyclin-1 was initially identified as a tumorsuppressor gene.

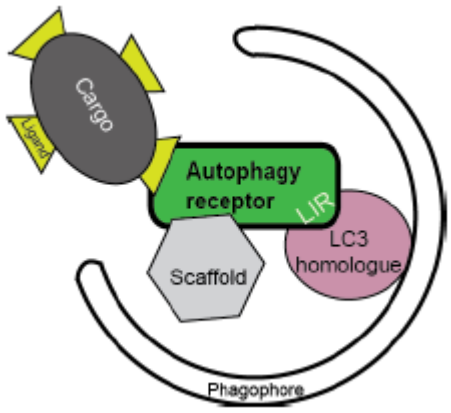




Com.: “one can increase ph value to inhibit degradation of proteins, I think”

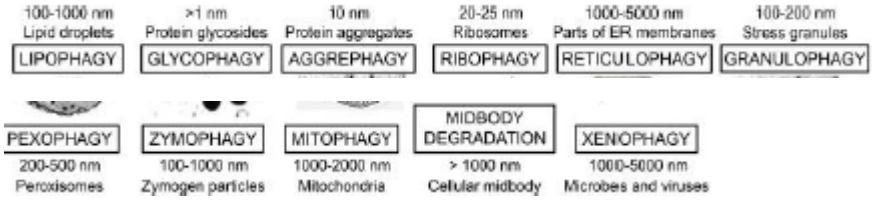
Autophagy in diseases

4 key steps of selective autophagy: induction; cargo tagging; sequestration; degradation of cargo and receptor



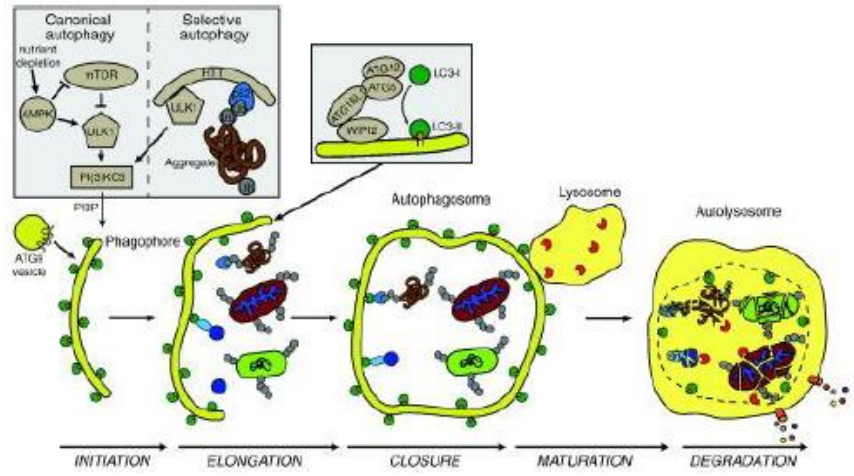
LC3-interacting region (=: LIR) motif ensures the targeting of autophagy receptor LC3 (or other ATG8 family proteins) anchored in the phagophore membrane.

Types of selective autophagy mammalian cells



There are two common mechanisms in organellophagy: one is a receptor mediated process the oter is a ubiquting mediated process.

Summary of the porcess and reuglation of selective autophagy



Exam question: how does HIF downregulate mitochondrial metabolism?

HIF induces glycolysis for energy production and for biosynthetic purposes. (see HIF-signaling for mitochondrial metabolism downregulation).

Another posibility is to decrease abundance of mitochondria.
Other possibility: Inhibit mitochondrial biogenesis.
(This would be enough to answer the question, one would not need to go into detail, I think.)

Ubiquitin mediated autophagy: Cargo recognition:

There is a cooperative function of the autophagy-lysosome system with the ubiquitin-proteasome system to manage the turnover of damaged proteins to maintain the proteome.

The ubiquitin-proteasome system requires unfolding of substrates for degradation via the proteasome core.

The autophagy-lysosome system is capable of handling much larger protein aggregates or tightly folded proteins without a requisite unfolding step.

There is some overlap in specificity for ubiquitylated cargo among selective autophagy receptors. In some cases this overlap is cooperative to mediate delivery to autophagosomes (e.g., mitophagy). In other cases multiple different autophagy receptors appear capable of mediating the process individually (e.g., xenophagy).

Post-translational modifications of both the selective autophagy receptors as well as the cargo (and in some cases ubiquitin itself on the cargo) are integral to regulating autophagy receptor function.

Additional complexity given that many of the selective autophagy receptors have non-autophagy functions.

Mitochondrial stress

Various insults can cause damage: environmental (radiation, toxic chemicals); genetic factors such as mutations in genes for metabolix processes or repair pathways; spontaneous facotrs such as ROS being generated as a byproduct of electron transport.

Types of damage: DNA, proteins, lipids.

Problems: loss of metabolic functions (ATP synthesis); more ROS made by defective mitochondria, etc.

Cellular response to damage: DNA repair, proteases, lipases, mitophagy, apoptosis, mitochondrial unfolded protein response.

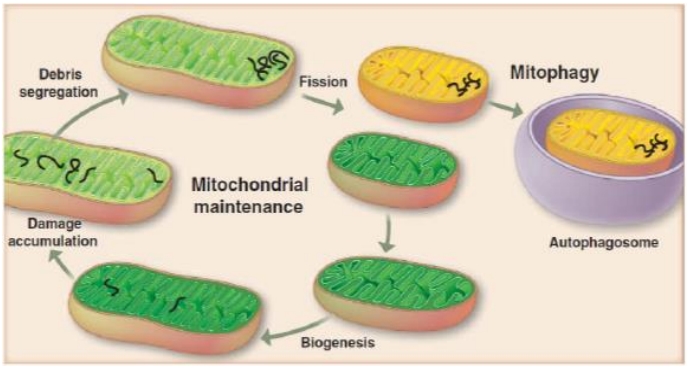
Fission and fusion in mitochondira:

A damages mitochondira can fuse with a healthy mitochondria in order to reduce total damage. Alternatively, a fissure can occur in the mitochondria to remove the malfunctioning site.

Fission proteins: dynamine related GPTase (Drp1/Dlp1), mitochondrial fission factor (Mff), Fission 1 (Fis1), GDAP1

Fusion proteins: optic atrophy 1 (Opa1), Mitofusin 1 (Mfn1), Mitofusin 2 (Mfn2)

Fusion is stimulated by energy demand and stress. Fission generates new organelles and faciliates quality control.



Example: Parkinson's disease (=: PD)

… (maybe it's not so important after all, an understanding might be enough without going into too deep detail.)

Parkin is an E3 ubiquitin ligase. **PINK1** is PTEN-induced kinase 1. PINK1 accumulates on depolarized mitochondria, since Parkin recruitment requires PINK1.
(MAYBE WRITE MORE ON PARKIN, AS IT SEEMS TO BE INVOLVED IN A COUPLE OF IMPORTANT PROCESSES.)

Short summary:

1. Mitochondria are depolarized (with CCCP or due to damage)
2. Parkin translocates to damaged mitochondria.
3. Parkin ubiquitylates mitochondrial surface proteins (e.g., MFN1, MFN2, VDAC).
4. Proteasome translocates to damaged mitochondria.
5. Surface proteins are degraded.

How does Parkin detect damaged mitochondria?

How does the autophagic machinery detect these mitochondria?

(HAVE A LOOK AT THE END OF THE AUTOPHAGY PDF.)

Peroxisomes

Metabolic functions of peroxisomes

• beta-oxidation of fatty acids (e.g., very long-chain, branched-chain & dicarboxylic fatty acids)

• aalpha-oxidation of fatty acids (e.g., phytanic acid, 2-hydroxylated fatty acids)

• Ether phospholipid (plasmalogen) synthesis

• Cholesterol and isoprenoid synthesis

• Bile acid synthesis (i.e., b-oxidation of the cholesterol side chain)

• Synthesis [e.g., docosahexaenoic acid (DHA)] and degradation of polyunsaturated fatty acids

• H2O2 degradation by catalase

• Degradation of eicosanoids

• Degradation of amino acids, polyamine, and purine

• Metabolism of reactive oxygen species (ROS)

• Synthesis of pyrimidines and purines

Novel peroxisomal functions

• Viral innate immune defense

• GPI-anchor biosynthesis

• H2O2 signaling in hypothalamic neurons

• Platform for cytomegalovirus evasion from cellular immune response

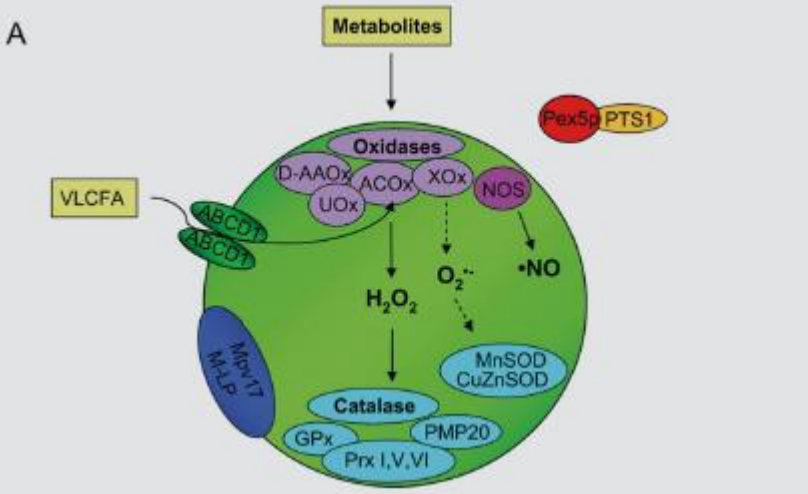
• Peroxisome inheritance is coupled with mitosis to balance growth and differentiation

• Peroxisomal ether lipid synthesis regulates inflammation by sustaining neutrophil membrane composition and viability

• Peroxisome proliferation contributes to the physiological response to sound exposure; impaired in patients with pejvakin mutations

• PEX13 required for selective autophagy of Sindbis virus (virophagy) and of damaged mitochondria (mitophagy)

Schematic overview of peroxisomal ROS homeostasis



B

Type of ROS/RNS produced	Generating reaction	Produced in PO by	Scavenged in PO by
Hydrogen peroxide (H_2O_2)	$O_2^{\bullet-} + H^+ \rightarrow HO_2^{\bullet}$, $2HO_2^{\bullet} \rightarrow H_2O_2 + O_2$	Acyl-CoA oxidase (several types) Urate oxidase Xanthine oxidase D-amino acid oxidase D-aspartate oxidase Pipecolic acid oxidase Sarcosine oxidase L-alpha-hydroxy acid oxidase Polyamine oxidase	Catalase Glutathione Peroxidase Peroxiredoxin 1 PMP20
Superoxide anion ($O_2^{\bullet-}$)	$O_2 + e^- \rightarrow O_2^{\bullet-}$	Xanthine oxidase	MnSOD CuZnSOD
Nitric oxide ($^{\bullet}NO$)	L-Arg + NADPH + H^+ + O_2 → NOHLA + $NADP^+$ + H_2O, NOHLA + ½ NADPH + ½ H^+ + O_2 → L-citrulline + ½ $NADP^+$ + $^{\bullet}NO$ + H_2O	Nitric oxide synthase	

Peroxisomes play an important role in the synthesis of bile acids. Cholesterol is biosynthesized to C27 bile acid in the liver. C27 bile acids are transformed to C24 bile acids in peroxisomes then to conjugated bile acids with BAAC and they are secreted into the bile in the end.

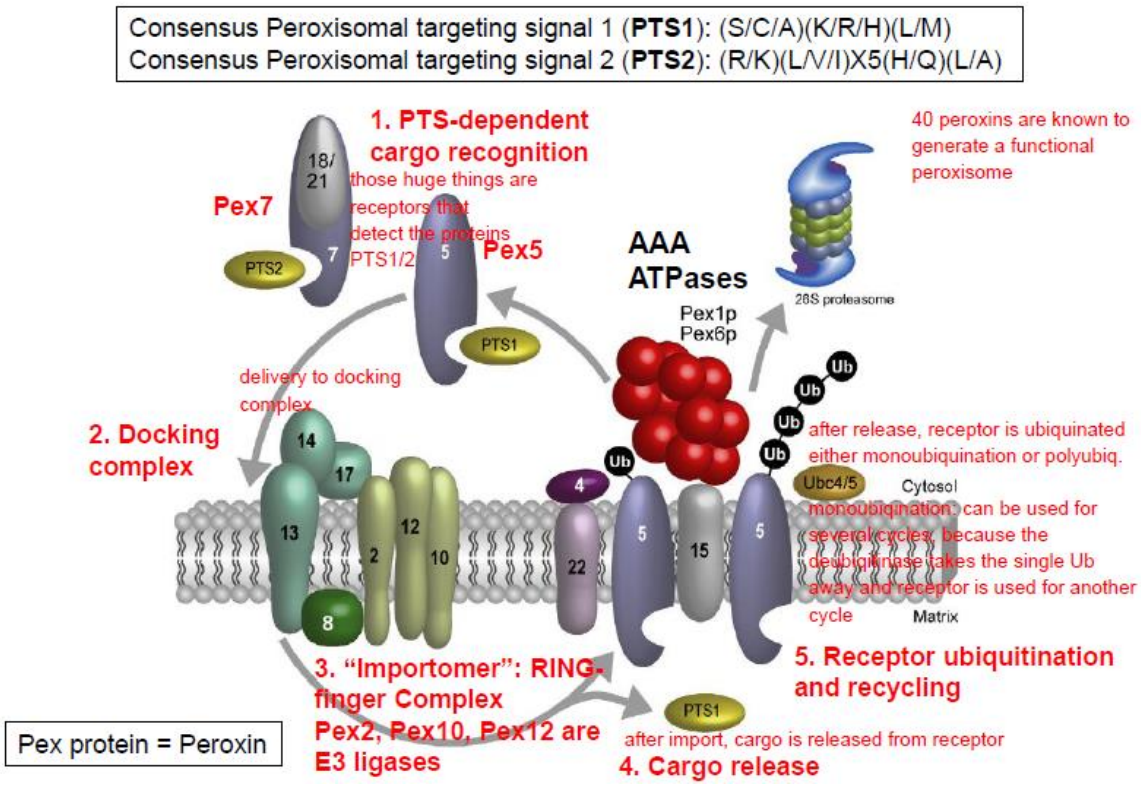
Peroxisomes play a key role in the formation of DHA (subsequent beta-oxidations occur in peroxisomes).

Def. plasmalogene: Similar to glycerin, but instead of a fatty acid ester, there is an enolether and some other differences. This phospholipid ether occurs in many tissues, such as the brain, but also in the immune and circulatory system.

Plasmalogenes can protect animals from reactive oxygen species (ROS) and they act as signaling molecules and modulators of membrane dynamics.

Plasmalogene biosynthesis starts in peroxisomes and thus, peroxisomes are indispensable.

How are proteins directed to their subcellular location in the cell?



Explanation of the picture above:

1. Proteins harboring a peroxisomal targeting signal are recognized and bound by the import receptors Pex5 and Pex7 in the cytosol.

2. The cargo-loaded receptor is directed to the peroxisomal membrane and binds to the docking complex (Pex13/Pex14/Pex17).

3. The import receptor assembles with Pex14 to form a transient pore and cargo proteins are transported into the peroxisomal matrix in an unknown manner. Cargo release might involve the function of Pex8 or Pex14.

4. The import receptor is monoubiquitinated at a conserved cysteine by the E2-enzyme complex Pex4/Pex22 in tandem with E3-ligases of the RING-complex (Pex2/Pex10/Pex12).

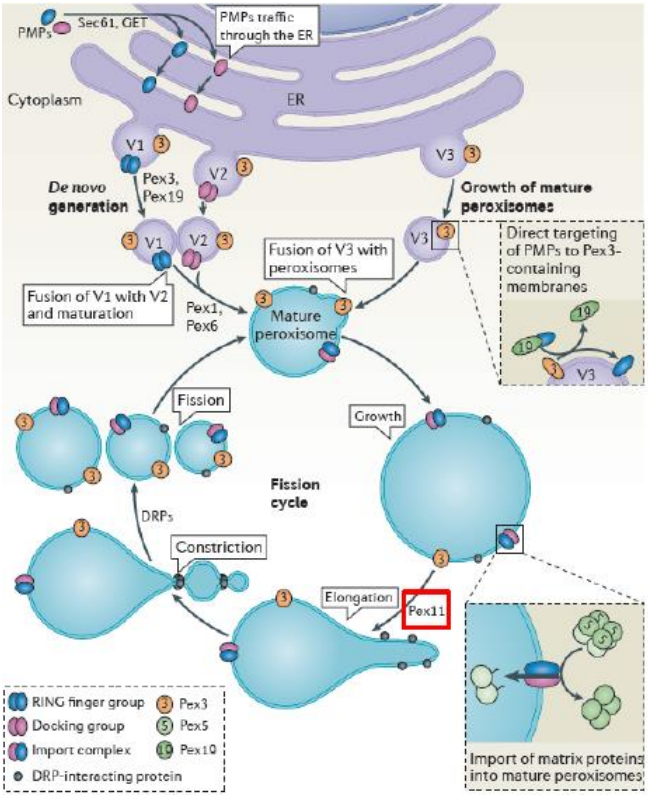
5. The ubiquitinated receptor is released from the peroxisomal membrane in an ATP-dependent manner by the AAA-peroxins Pex1 and Pex6, which are anchored to the peroxisomal membrane via Pex15. As the last step of the cycle, the ubiquitin moiety is removed and the receptor enters a new round of import.

Models for peroxisome multiplication:

Vesicle fusion model: Class 2 peroxisomal membrane proteins are produced in the ER. PEX 13 and 14 and RING-finger PMPs fuse to membranes + fission matrix protein import realizes the mature peroxisome.

Growth and division model: A peroxisome grows until it is large enough to be elongated. The elongation is constricted and smaller peroxisomes are removed from the initial peroxisome.

Reintroduction of peroxisomes: PEX13/14 are probably combined with membrane import proteins and then with matrix protein imports in order to produce de novo peroxisomes.



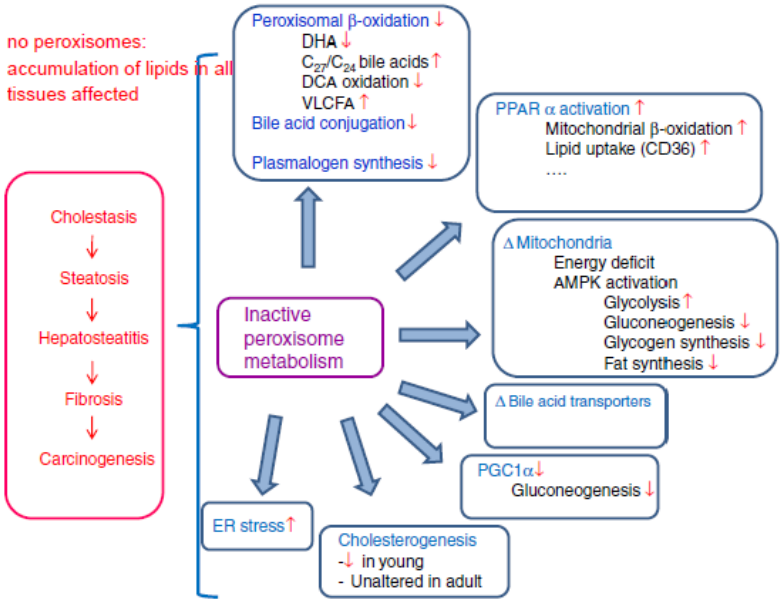
Peroxisomal disorders (disorders in the biogenesis of peroxisomes)

KO in peroxisome genes lead to a multitude of disorders. Example: Zellweger spectrum disorder (Zellweger syndrome, neonatal andrenoleukodystrophy, infantile resum disease), Rhizomelic chondrodysplasia puncata etc. These disorders all affect the nervous system (keep in mind that for example plasmalogenes are used abundantly in the central nervous system. These molecules rely on metabolic processes occurring within the peroxisomes (probably beta-oxidation etc.)).

In Zellweger syndrome specifically, there is no cell membrane formed. Such individuals only live for 1-6 weeks normally.

Def. peroxisomal membrane ghost: A structure (organelle) that peroxisomal integral membrane proteins, but it lacks most of the matrix proteins. It is assumed that there are genetic defects in the import of newly-synthesized peroxisomal proteins in the organelle.

In Zellweger syndrome, for example, actual peroxisome organelles are not present, but some building blocks of peroxisomes still occur. Therefore, it was assumed that there are mutations in the import of matrix proteins.



Degradation pathways of peroxisomes and pexophagy:

3 main pathways: Macroautophagy, then peroxisomal Lon protease (Lonp2), then 15-lipoxygenase (Alox15; “this enzyme ruptures the membrane and other proteases take over then. this enzyme is not selective on peroxisomes, it affects all organelles like mitochondria etc.”).

General themes for selective autophagy pathways

The key decision point in any selective autophagy pathway is the mechanism by which the core autophagy machinery is redirected to degrade primarily selective cargo.

1. Every selective autophagy pathway studied to date requires a specific cargo receptor.

2. These cargo receptors typically have a tripartite role in (a) cargo binding, (b) interaction with Atg11, and (c) interaction with Atg8 (LC3 in mammals) via an Atg8-interaction motif (LIR motif).

3. The selective autophagy receptors are often synthesized even under conditions wherein the cargoes are not degraded, but receptor activation often relies on protein modifications, such as phosphorylation or ubiquitination.

4. Specialized membrane structures, such as the MIPA, are needed for micropexophagy, not for macropexophagy.

5. Generally the receptors are degraded in the vacuole (lysosome) along with the cargo.

Peroxisomes and HIF-alpha signaling

Peroxisomes are highly dependent on O2. They consume around 20% of all O2 in the liver (there are ten times more mitochondria in the liver than there are peroxisomes). On the other hand, O2 availibility regulates HIF-alpha signaling.
What to do when hypoxic conditions arise?

To minimize O2 consumption under hypoxic conditions, Hif-a signaling either inhibits O2-dependent peroxisomal metabolic pathways or decreases peroxisome abundance. Reduced peroxisomal activity would be part of metabolic reprogramming causing changes in lipid metabolism.

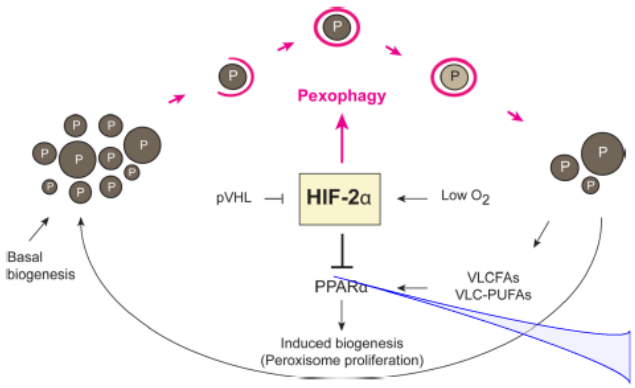
In the liver, it was observed that liver specific loss of VHL caused severe accumulation of lipids (there are about ten times more peroxisomes in the liver than there are mitochondria).

Liver specific loss of VHL causes hepatic steatosis (fatty liver). Peroxisome abundance is reduced or there are intact peroxisomes.

HIF-1alpha signaling has no effect on peroxisomes whatsoever, but HIF-2alpha signaling influences peroxisome abundance in Vhl livers. Amongst other various changes in the metabolism of Vhl -/- livers, there is an accumulation of C27 bile acids, since they are not transformed to C24 bile acids (requires peroxisomes to do that).

In Vhl livers, the whole biogenesis machinery for peroxisomes is still intact. But the peroxisomes colocalize in the autophagosomes (a key structure for macroautophagy which degrades intracellular contents).
If autophagy in a Vhl -/- liver can be inhibited, there will be an increase of abundance of peroxisomes.

Peroxisome numbers are reduced in ccRCC with high HIF-2alpha levels.



Loss of tumor suppressor *Vhl* in hepatocytes decreases peroxisome number
Hif-2α promotes mammalian pexophagy
Hif-2α mediates changes in lipid composition reminiscent of peroxisomal disorders
Human ccRCCs with high HIF-2α levels have decreased peroxisome abundance

Peroxisome abandunce in tumors

Human ccRCCs with high HIF-2a levels have decreased peroxisome abundance.

HIF-2a stabilization is observed in the vast majority of solid tumors and might lead to reduced peroxisome abundance in other cancer types.

Excessive peroxisome proliferation leads to hepatocellular carcinomas in rodents.

Peroxisomal branched-chain fatty acid b-oxidation enzymes are induced in prostate cancer. Is peroxisome abundance also increased?

Peroxisomes are essential for ether lipid synthesis – aggressive cancers have high levels of ether lipids, and inhibition of ether lipid synthesis reduces tumor growth.

Decrease in peroxisome abundance has also been observed in other tumors such as hepatocellular carcinoma, colon carcinoma, breast cancer.