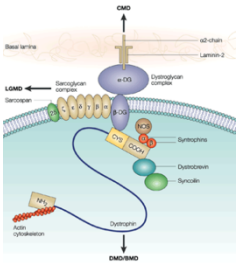
The Dystrophin-Glycoprotein Complex



Dystrophin is tightly associated with a series of transmembrane proteins

- Sarcoglycan complex: mutations here lead to LGMD
- Dystroglycan complex: mutations here lead to congenital muscular dystrophies

Both these complexes link externally to laminin which is a component of the basal lamina
Interaction of dystrophin with the cytoskeleton is mediated via binding to F-actin
The complex is rendered as fragile if part is missing
Appropriate expression of the DGC is needed for both structure and signalling

Physiological Role of Dystrophin

Lack of dystrophin leads to a gradual but remorseless degeneration of skeletal and cardiac muscle; pattern of loss is sporadic
Dystrophin is localised primarily to the cytoplasmic surface of the plasma membrane
Dystrophin is found in much smaller amounts in internal regions of myofibre
Dystrophin (DGC) is postulated to have a structural role in stabilising the sarcolemma during muscle contraction, especially lengthening actions and transmission of force
Although the DGC is important it is not the whole story, there are other important signalling molecules

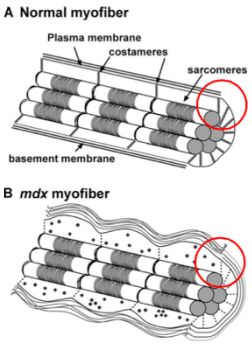
- Many lines of evidence point to a significant contribution by the DGC as a receiver and transducer of signals

Inflammation can be an initiator of protein structure degradation
Normally dystrophin acts as an anchor to confer stability, in DMD this anchor is lots and in BMD the anchor is shorter but still a useable protein

Dystrophin plays a structural role in stabilising the sarcolemma during contraction
DGC forms rib like lattices on the cytoplasmic face of the sarcolemma (costameres)
Mechanical links between sarcomeres within the muscle fibre and laminin located in the extracellular matrix
Anchor sarcolemma to costameres stabilises sarcolemma against forces transduced through costameres during contraction especially when stretched

Effect of dystrophin loss on myofibre function and organisation

- Dystrophin and associated proteins normally found at costameres linking Z-bands with plasma membrane
- Loss of dystrophin results in disorganised costameres and enhanced membrane leak, increased oedema and inappropriate cytosolic Ca^{2+} and ROS generation contribute further to muscle dysfunction
    - Loss of anchoring protein and therefore loss of structural integrity
- Increased extracellular matrix deposition surrounding myofibres also occurs and perturbs membrane integrity
- This interaction is particularly important during muscle contraction



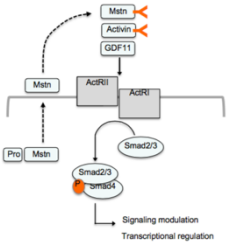
In the early disease stages of pathology there is muscle repair and true hypertrophy but as the protein continues to be susceptible to the same trauma eventually repair becomes exhausted and there is pseudohypertrophy

TGF-β Network as a target for muscle therapeutics

There are several points along the pathway that can be used as a target for therapeutics

- Proteins in the pathway are made in various cells and they are usually made together with their pro domain
- The proteins get secreted from the cell and then enzymes will cut the pro domain
- Ligands will bind receptors and engage the signalling pathways downstream
- Smad proteins are phosphorylated and go into the nucleus to drive gene expression

Engineered antibodies to neutralise specific TGFβ ligands



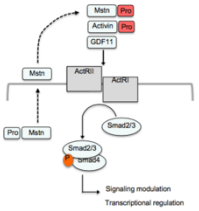
Use of antibodies once the ligand has been released from its prodomain and is in its bioactive state; approach initially started with myostatin

They work to a certain degree in mice but haven't translated well to a human setting; probably due to differences in how the ligand is targeted in humans vs. mice

Given that there are multiple ligands acting on the same types of receptors and driving the same pathway, removing only myostatin will not have a full effect; it is better to target more ligands

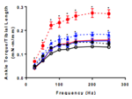
- Blocking both Activins and Myostatins at the same time was superior for increasing mass of primates

Engineered prodomains as inhibitors of specific TGFβ ligands



Putting prodomains onto the ligands once they have been secreted from the cell is able to inhibit their effect

- Blocking both ligands at the same time gives a synergistic effect
- However, Activin doesn't just act on muscle but also other cell types as well so you don't want to block it too vigorously
- There is increased force capacity of a muscle when you remove more than one ligand

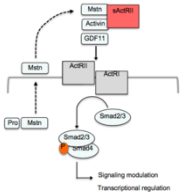


Soluble Activin receptors as ligand traps

Made a protein version of the Activin Type II receptor but modified it slightly so that it was freely circulating; acts as a decoy receptor

- Binds all of the endogenous ligands before they can actually bind to their membrane bound receptors

This is significant for cancer patients where tumours release high levels to Activins to induce cachexia

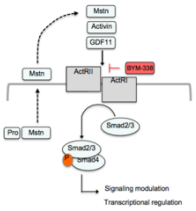


- By giving them these soluble receptors they could wipe up the ligands and not only prevent loss of mass but also increase mass which is associated with increased survival time

However, there were also side effects noted when this treatment was used in DMD

- Included nosebleeds and remodelling of the microvasculature which can be fatal
- These receptors are broad and bind other ligands (e.g. BMP9/10) which are important in regulating microvasculature and cell remodelling

Engineered peptides as receptor antagonists



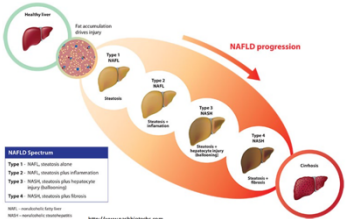
Making a protein antagonist which will bind to the receptor and interfere obscures the receptor binding site so that the ligand won't be able to get to it and bind

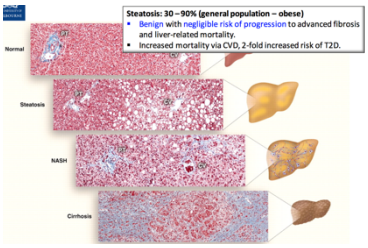
Non-Alcoholic Fatty Liver Disease (NAFLD)

NAFLD can be described as the accumulation of lipid in the liver

Progression of NAFLD

There is a very broad range of disease states associated with NAFLD
Most people have relatively benign accumulation of lipid; as it accumulates we get more inflammation and fibrosis





Normal liver has tightly packed hepatocytes
Steatosis occurs with increased lipid droplets accumulating, especially around blood vessels
NASH occurs with more inflammation and collagen deposition

30-90% of the general obese population have steatosis of the liver; this is usually benign with negligible risk of progression to advanced fibrosis and liver-related mortality

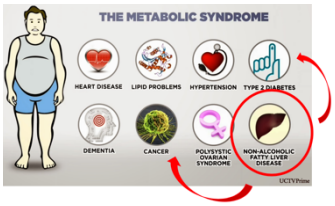
Prevalence of NAFLD

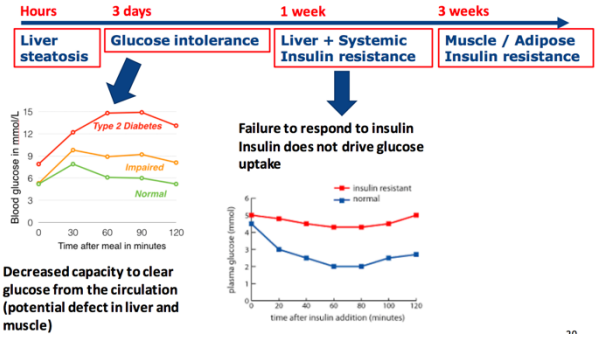
In the general population the prevalence of NAFLD is about 27-34% whilst in the obese population the prevalence is about 75-92%, almost everyone who is obese has fatty liver

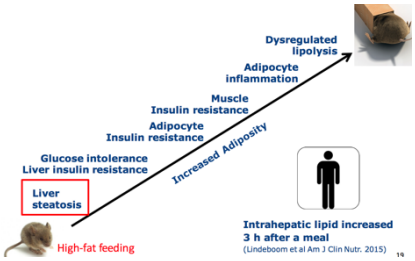
Fatty Liver Disease and Metabolic Syndrome

Fatty liver is thought to be a driver of many metabolic complications and diabetes
Liver Steatosis is a very early event that occurs

- Accumulation of fat in the liver is seen before any issue of glucose control or insulin sensitivity occurs







Liver steatosis occurs very early on (only hours after), glucose intolerance occurs after several days with defective and delayed uptake of glucose from the blood (decreased capacity to clear glucose from circulation)
Insulin resistance first occurs in the liver and then systemically (after 1 week)
Later there is muscle and adipose insulin resistance (after 3 weeks)

Whole body insulin resistance occurs before muscle or adipose tissue insulin resistance; the liver plays a major role in metabolic defects

- Fatty liver occurs very quickly and then drives other factors