Insights to the Pathophysiology of Non-Alcoholic Fatty Liver Disease

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Objectives

- Overview of the complex pathophysiology of NAFLD
- to learn modulators which can affect the progression of NAFLD
- Understand the potential targets of current and future therapeutic options
NAFLD

• Defined as presence of fat in hepatocytes of > 5%.
• Spectrum of disease from simple steatosis to NASH and cirrhosis
• Worldwide, the prevalence rates range from 5% to 30%
• In Singapore, recent survey showed up to 40% of population have NAFLD

George Goh *Gastroenterol Rep* 2016
Natural history of NAFLD

NAFLD
9-46% PREVALENCE

NASH
6-13% PREVALENCE

NASH
9-20%

NASH/Cryptogenic Cirrhosis
40-60% over 5-7 years

Progression of Fibrosis
26-37%

Stable or Regression of Fibrosis
52-79%

Cirrhosis related complications including HCC

Starley *Hepatology* 2010
Pathogenesis of NAFLD

- Multiple hit model

William Peverill *Int J Mol Sci* 2014
Pathogenesis of NAFLD

William Peverill *Int J Mol Sci* 2014
Insulin resistance

• Obesity leads to insulin resistance
• The prevalence of NAFLD is high in persons with obesity (30–76%), even higher in morbid obesity (up to 98%)
• adipocyte dysfunction is believed to occur in the setting of increased calorie intake and adipocyte hypertrophy

Liu CJ J Gastro and Hepato 2012
Adams LA CMAJ 2005
Adipose tissue and insulin resistance

Sun K JCI 2011
Insulin resistance

- Associated with an increase in lipolysis and circulating FFA
- Dysregulated adipokines production
  - Adiponectin
    - Enhancing lipid clearance and beta-oxidation of FA, suppressing TNF-alpha in the liver
    - Reduced circulating levels correlate with the severity of liver histology in NASH
  - Resistin
    - Worsens insulin resistance
Lipid metabolism in liver

- FFA in liver
  - undergo beta oxidation
  - esterified to TGs and incorporated into VLDL, to be secreted into the systemic circulation
  - Store in hepatocyte as TG which is a stable form of lipid

Michele VaccaSem Liv dis 2015
Lipotoxicity

• In insulin resistance, the hepatocytes are unable to accommodate the increased influx of FFA
• The resultant excess FA causes oxidative stress with generation toxic metabolites such as ceramides, diacylglycerols, lysophosphatidyl choline, and oxidised cholesterol metabolites, which act as reactive oxygen species (ROS)

Michele Vacca *Sem Liv disease* 2015
Lipotoxicity

• Excess FFAs promote mitochondrial dysfunction via the production of ROS and induces hepatocyte apoptosis

• Hepatocyte apoptosis and mitochondrial dysfunction activate inflammatory cascade by cytokines
Kupffer cells

- Kupffer cells act to stimulate fibrogenic responses via the release of TGFβ1, MMPs, platelet-derived growth factor, and ROS
- Kupffer cells also express TLR 2, 3, 4, and 9 and these receptors are responsive to induce signalling pathways that regulate pro-inflammatory cytokines and chemokines
Hepatic stellate cell and fibrosis

• The hepatic stellate cell (HSC) is responsible for collagen production and scar formation
• It is stimulated by tissue growth factor-beta (TGFβ), lipid peroxidation products and connective tissue growth factor (CTGF)
• HSCs also express cytokines and chemokines that perpetuate inflammation and fibrogenesis
Modulators of NAFLD
PPAR

- Peroxisome proliferator-activated receptors (PPAR) are nuclear receptors
- Control metabolic programs and to regulate energy homeostasis
PPARα/δ

- Present in hepatocyte
- modulates fatty acid uptake and lipoprotein metabolism
  - Promotion of LPL and apoA proteins
  - inhibition of the LPL inhibitor apoCIII
  - promotes β-oxidation
  - inhibits De novo lipogenesis (DNL)
PPAR γ

- Present in adipose tissue
- Regulate adipocyte differentiation and function
- Can expand subcutaneous adipose tissue due to an enhanced differentiation of preadipocytes in mature adipocytes, this promotes lipid repartitioning from visceral fat, liver, and skeletal muscle to the subcutaneous adipose tissue and improve insulin resistance
- Also promote adiponectin in adipose tissue
Gut microbiome

- complex immunological, neurohumoral, and metabolic interactions between the microbiota and the host
- In animal study, gut microbiota caused obesity
  - higher efficiency in extracting calories from the diet
  - increased production of short chain fatty acids (SCFAs) that in turn induce a slowing of intestinal transit, thereby enhancing nutrient absorption

Turnbaugh PJ *Nature* 2006
Machado MV *Ann Hepatol* 2012;
Gut microbiome

- Overgrowth of Gram-negatives organisms and impaired gut barrier function in liver disease facilitate translocation of whole organisms and/or LPS
- These activate the inflammasome complex via binding of LPS to TLR4 receptors located on the cell surface of Kupffer cells, resulting in a cascade of proinflammatory cytokine production that ultimately leads to liver injury and can progress to fibrosis.

Eamonn M Sem Liv disease 2015
Gut microbiome

Eamonn M Sem Liv disease 2015
Genetics in NAFLD

• Not sufficient to determine disease outcome at an individual level, usually combine with other factors eg environmental, diet for its effect

• Recent genome-wide association studies identified a variant of PNPLA3 and TM6SF2 as important genetic factors associated with NAFLD
Patatin-like phospholipase 3 (PNPLA3)

- carriage of the PNPLA3 variant sensitizes the liver to metabolic stress
- Possible affect lipid transport
- Presence of G allele is associated with a greater degree of steatosis for any given degree of insulin resistance or adiposity
- more severe hepatic necroinflammation and fibrosis across different ethnic groups

Pirazzi C J Hepatol 2012
Petta S J Hepatol 2012
Transmembrane 6 superfamily member 2 (TM6SF2)

• Individuals carrying the minor (T) allele appear prone to developing NAFLD with advanced fibrosis but less likely to develop cardiovascular morbidity and mortality.

• Conversely, carriage of the C allele is associated with dyslipidemia and cardiovascular disease

Dongiovanni P Hepatology 2015
Dongiovanni P Hepatology 2015
Lean NAFLD

• a high percentage of Asia-Pacific NAFLD subjects were found to be non-obese even using the ethnic-specific criteria. (BMI Normal: 17.5–22.4 kg/m², Overweight: 22.5–24.9 kg/m²)

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Proportion of non-obese subjects in reported series of non-alcoholic fatty liver disease (NAFLD) from Asia</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Taiwan</td>
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<tr>
<td>BMI &lt; 25 kg/m²</td>
<td>17%</td>
</tr>
</tbody>
</table>

• In Singapore, mean BMI for subjects with NAFLD was <25

Liu CJ *J Gastro and Hepato* 2012
George Goh *Gastroenterol Rep* 2016
Lean NAFLD

- Metabolic syndrome was present in approximately 70% of Chinese patients with NAFLD in one study.
- Obesity at baseline and any increase in weight gain are important predictors for the development of NAFLD.

Wong VW. Aliment. Pharmacol. Ther. 2006
Fan JG. Zhonghua Gan Zang Bing Za Zhi 2010
Predictors of NAFLD in non obese patient

<table>
<thead>
<tr>
<th>Authors</th>
<th>Population</th>
<th>Risk factors</th>
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<tbody>
<tr>
<td>Chen et al. (Taiwan)(^{16})</td>
<td>Adult (health check-up)</td>
<td>Aged 40 to 60 years, Elevated serum ALT, Triglyceride $\geq$ 150 mg/dL</td>
</tr>
<tr>
<td>Fu et al. (Taiwan)(^{23})</td>
<td>Adolescents (students)</td>
<td>Increased serum triglyceride, Elevated HOMA index, Elevated serum ALT</td>
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<tr>
<td>Kim et al. (Korea)(^{29})</td>
<td>Adults (health check-up)</td>
<td>Increased waist circumference, Increased serum triglyceride, Elevated HOMA index</td>
</tr>
<tr>
<td>Das et al. (India)(^{14})</td>
<td>Adults (community-based)</td>
<td>Abdominal obesity, Dysglycemia (fasting plasma glucose $&gt;100$ mg/dL or elevated HOMA index), Higher body fat content</td>
</tr>
<tr>
<td>Fan et al. (China)(^{30})</td>
<td>Adults (health check-up, prospective cohort)</td>
<td>At baseline: advanced age, elevated BMI, elevated serum triglyceride and total cholesterol, obesity, and hypertension During FU: weight gain and increase of serum triglyceride</td>
</tr>
</tbody>
</table>

Liu CJ *JGH* 2012
PNPLA3 variant in patients with or without metabolic syndrome

- Greater impact of genetic variant on hepatic steatosis in patients without metabolic syndrome

J Shen *AP&T* 2014
Current and future therapeutic options

- Insulin resistance
  - FFA + cytokines
    - Steatosis + metabolic dysregulation
      - Oxidative stress + ER stress + mitochondrial injury
        - Inflammatory signalling + apoptosis cell death
          - Stellate cell activation
            - fibrosis

- Weight loss
  - Insulin sensitizers
    - Omega 3 fibrates
    - PPARα/δ compound
    - FXR ligand
    - antioxidant
    - pentoxifylline
    - Anti TNF
    - ACEI/ARB
    - Bovine colostrum
Summary

• Although lipotoxicity, oxidative stress, exogenous factors (e.g., drugs, microbiota, alcohol, and fructose consumption), and genetic predisposition have been proposed as modulators in this progression, more detailed insights are needed to elucidate the mechanisms responsible for the evolution of NAFLD.

• Due to the complex mechanism of the disease, it is difficult to find a universal effective pharmacological treatment at the moment.