VIRO3001 Virology Lecture notes

Contents (2 lectures per week)

Preview: Lecture 10.1
10.1 Hepatitis C Virus and control of viral infections

Learning outcomes:

- Describe the replication of HCV including virus entry, the function of IRES and expression of polyprotein
- Describe the targets of HCV antivirals and their modes of action

Hepatitis:
Inflammation of the liver, for which there are a variety of causes including excess alcohol, medications, auto-immune disorders and viral infection.
Hepatitis can lead to impairment of liver function (cirrhosis), liver failure, tiredness, yellowing of the skin and whites of the eyes, abdominal pain and diarrhoea.

Viral causes of hepatitis:
These virus cause inflammation of the liver, they are not closely related genetically.

- Hep A:

+ssRNA, picornaviridae
faecal-oral transmission
vaccine preventable using inactivated virus

- Hep B:

retro DNA, hepnaviridae
contaminated body fluids: sex, childbirth, blood transfusion, needle sharing
vaccine preventable using yeast expressed envelope protein (subunit)

- Hep C:

+ssRNA, flaviridae
- Zika virus is also a flaviviridae
contaminated body fluids: blood transfusion, tattoo, piercing, childbirth, needles
don't have a good vaccine yet

- Hep D:

-ssRNA, viriod, requires Hep B coinfection
exacerbates symptoms of Hep B

- Hep E:

+ssRNA, hepeviridae
faecal-oral transmission, risk to immunocompromised otherwise acute, self-limiting

Hep C is a major cause of mortality

- major cause of liver disease and liver cancer (Hepatocellular carcinoma: 25% caused by HCV)
- chronic infection around the world, many asymptomatic cases which is a challenge as people unknowingly spread the virus

Acute infection →
Most cases progress to chronic infection → some patients are fine
Decades pass, 5-20% experience cirrhosis of the liver →
Progressive cirrhosis leading to liver failure or Hepatocellular Carcinoma

HCV types (1-6) are substantially divergent genetically, despite presenting similar clinical symptoms. These variations are a challenge for antivirals and vaccine design. For example a treatment that targets a specific viral protein may only be effective on one type of HCV. In the developed world, strain 1 is the most prevalent, however other strains are common around the world.

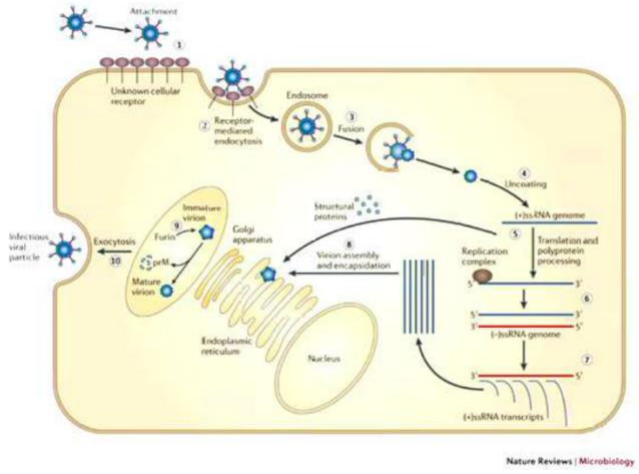
HCV was discovered in 1989, challenging to culture and productively infect animals. Mice with xenografts of human hepatocytes and chimps were used- recent development of infectious cell culture.

About Hep C:

- +ssRNA, flaviridae
- enveloped, 50nm diameter with a 10kb genome
- nucleocapsid, envelope proteins E1 and E2
- T3 icosahedral shape
- Single ORF for polyprotein, during viral replication the polyprotein is cleaved into constituent protein by viral and cellular proteases.
- RNA dependent RNA polymerase (this gene is encoded and ready to be made into protein, enzyme itself not present in capsid as required for –ssRNA)

Replication:

- Affinity with host cell
- Receptor-mediated endocytosis
- intracellular endosome
- Nucleocapsid released into cytoplasm, release +ssRNA genome ready to be translated (functional mRNA)
- Translation and polyprotein processing- Make proteins involved in viral assembly, boost in gene expression, polyprotein cleaved into constituent proteins by host and viral proteases
- RNA dep RNA Pol makes -ssRNA template for genomes
- Gene expression
- Replication, virion assembly at ER, maturation and encapsidation
- Cell lysis, release of progeny

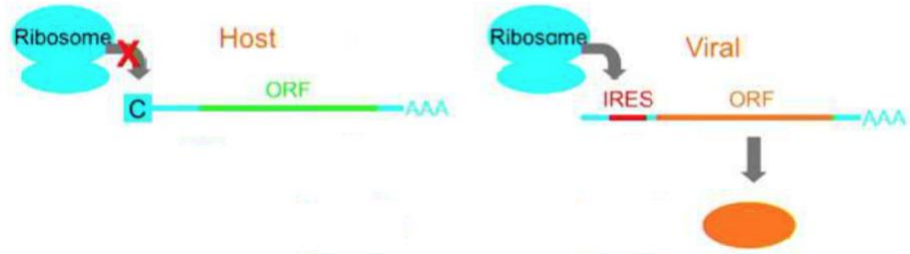


Entry of HCV

- Number of cellular proteins involved in mediating entry, initial attachment to cell surface, transport to the tight junction and internalisation, leading to an endosome with a virus particle connected to lipid droplet
  Movie:
- LVP: Lipoviral protein, protect from antibody neutralisation, reaches hepatocyte. Low affinity bonds activate co-receptors, expose binding site to CD-81, signal transduction initated, interacts with claudin-1 clathrin-mediated endocytosis, low endosomal pH causes a conformational change in viral E proteins resulting in HCV endosome fusion, permitting virus to escape, uncoat and release its genome into the cytosol for translation and replication.

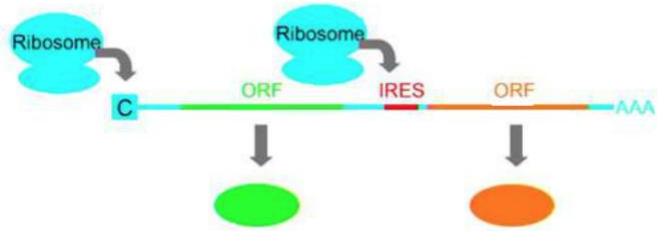
- initial attachment promoted by GAGs and low-density liprotein receptor. E2 envelope protein directly interacts with tetraspanin CD81, scavenger receptor B1 and tight junction (between 2 cells) proteins claudin-1 and occludin. Binding followed by clathrin-mediated internalisation and pH dependent release of genome to cytoplasm.
- Drop in pH triggers membrane fusion between the viral envelope and endosomal membrane, release of icosahedral nucleocapsid into cytoplasm (similar process to influenza virus and segmented nucleocapsid)
- Hepatocytes (liver cells) express high levels of LDL-R and SR-B1 proteins as well as liver-specific miRNA (micro RNA) miR122, leading to sensitivity to infection by HCV. Humanised mice that express SR-B1 and OCLN can mediate HCV entry- expressing correct entry receptors.

Genomic polyprotein:
Translation of polyprotein in ER, trans golgi network, released.
IRES: Internal Ribosome Entry Site
The HCV genome lacks a 5' cap so it uses IRES to mediate ribosome binding to translate the ORF.
Hairpin loops, regions of base complementarity.
This allows the virus to control gene expression- modification of host ribosome by viral proteases inhibits expression of host genes, so cell makes only viral protein products.

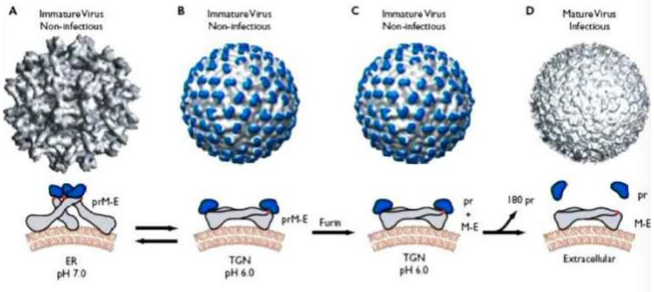


Express proteases, which cleave ribosome that block its ability to bind to the 5' cap, inhibiting host gene expression which can limit antiviral responses.
IRES can function internally in an RNA molecules, having applications in molecular biology to drive expression of two ORFs from a single transcript. For example 5' cap and then IRES.

Molecular biology example: maybe see which cells have been infected



Maturation of virus:
Drop of pH, ER to trans golgi network, furin cleavage



Flavivirus particle maturation. Nascent, noninfectious, immature particles undergo a pH-dependent conformational rearrangement of the viral glycoproteins E (gray) and precursor M (prM) (blue). Mature, infectious particles form upon furin cleavage of prM and release of the pr fragment.

Treatment of HCV- Interferon

- Early treatment with Interferon 1980s
- Interferons protect cells from viral infection, activating immune cells and increase host defences
- Pegylated interferon is a more chemically stable form, increasing efficacy and fewer administrations needed. Intravenous delivery
- Interferon treatment is a successful treatment in 5-40% of cases.
- Interferon has many side effects (depressed, fatigue, rash, myalgia etc), long treatment- many patients decide to quit their course of treatment
- Challenge for patient compliance: additionally many patients are difficult to access such as drug users, homeless. These patients are likely to be reinfected/spread infection.

Interferon plus ribavirin

- Combine Peg-IFN with ribavirin
- Ribavirin is a guanosine nucleoside analogue
- Interferes with RNA synthesis during viral replication- activity against HSV, Ebola etc
- Much more effective! But depends on genotype – 1, 2, 3 etc.

Ideally we want a sustained virological response (SVR): no virus relapse after completing treatment.

Selective antivirals based on viral proteins as drug targets:
inhibit

- entry
- translation
- IRES
- **Serine Protease**
- **RNA dep RNA Pol**
- Helicase

Current treatment options:

- 95-98% success rate!! Sustained virological response.
- Patient friendly: pills for 12 weeks
- Fewer side effects
- Ledipasvir and sofosbuvir
- Insanely expensive $100, 000, manufacture cost $100
- Australia has unlimited access to HCV treatments, $1 billion deal
- Govt should invest more $$ in pharma research
- Resistance mutations NS5A
- Deliver multiple drugs with different targets

Drug targets: