Viral infections and metabolism: a clue to antiviral therapy?

Patrice André
CIRI, INSERM U1111, CNRS UMR 5308, Université Claude Bernard Lyon1, Ecole Normale supérieure Lyon, Hospices Civils de Lyon
What are the general rules which define the Topology of neuronal connections?

How is regulated the flux of metabolites in metabolic pathways? How stable is a steady state equilibrium?
Help!

Differentiated tissue:
- Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate $\rightarrow$ CO$_2$
- Oxidative phosphorylation: $\sim 36$ mol ATP/mol glucose

Proliferative tissue:
- Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate $\rightarrow$ CO$_2$
- Aerobic glycolysis (Warburg effect): $\sim 4$ mol ATP/mol glucose

Tumor:
- Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate $\rightarrow$ CO$_2$
- $+/-$O$_2$

Biomass
Questions

• Do viruses replicate only in cells which have the appropriate metabolism or do they modify the cell metabolism?
Metabolo- and fluxomic of fibroblasts infected by human cytomegalovirus

- Infection of primary human fibroblast by human cytomegalovirus

- Increased glycolysis with increased lipid, aminoacid and nucleotide synthesis

- Increased TCA feed by Glu

Fatty acids synthesis inhibitors (ACC and FAS inhibitors) repress viral replication
All-omic studies of hepatitis C virus infection

Studies in primary human hepatocytes, at 10 days post-infection
All-omic studies of hepatitis C virus infection

- Transcriptional regulatory analysis of glycolysis showed enrichment of HNF4α targets
- HNF4α inhibitor Medica 16 represses HCV RNA replication

Infection-induced metabolism changes favor viral replication
All-omic studies of hepatitis C virus infection

- Increased lipid oxidation and decreased bile salts synthesis
- Transcriptional regulatory analysis of fatty acid oxidation showed significant enrichment of FXR targets
- FXR antagonists favor HCV RNA replication

Infection-induced metabolism changes limit viral replication

Answers and new questions

- Viral infections alter cell metabolism
- Some metabolic modifications favor and others limit virus replication (defining a new equilibrium supporting both viral replication and cell survival?)
- Limiting the virus-induced metabolic modifications by chemicals targeting specific metabolic pathways alter viral replication

- Do viruses directly modify metabolic factor?
- Does metabolites consumption by viral replication oblige cell metabolism to respond and reach a new equilibrium?
Investigating the mechanisms underlying the metabolism changes

Pairwise screen of interaction between HCV proteins and glycolysis enzymes by protein complementation assay in mammalian cells

Glucose → Glc6-P → Glyceraldehyde-3-P → Pyruvate → Lactate

Glucose → Nucleic acids synthesis (UTP, CTP)

Acetyl-CoA → Citrate → Triglycerides and phospholipids synthesis

NS5A (D2) → Hexokinase → Glc6-P → PFK → Glyceraldehyde-3-P → PK → ATP → Lactate

Glutamine → NADH → amino acids synthesis
Functional impact of NS5A and HK interaction on HK activity

Purified recombinant HK2 protein, specific activity=0.1UI/mg
Purified NS5A full length and Core proteins produced with wheat germ cell-free expression system (Cell-Free Science, Japan) provided by F. Penin.

Michaelis and Menten curves

- NS5A = allosteric activator of hexokinases
- Cell expression of NS5A sufficient to increase the uptake of glucose and the excretion of lactate

Lineweaver and Burk projection

$V_{max} +50\%$

$K_m -27\%$
On-going work and perspectives

Can virus-alteration of HK activity sufficient to induce remote metabolome modifications (e.g. fatty acids synthesis...)?

Does NS5A modifies other regulatory loops within the metabolome (e.g. NR expression, enzyme activity modulation...)?
On-going work and perspectives

- Comparative metabolomic and fluxomic analysis of Huh-7 cells infected or non-infected with HCV; expressing or not NS5A; expressing HK4 or 2 that have different catalytic activities
- Could modeling predict the role of HK and other factors in the observed metabolic changes
- What algorithms can be used, Recon2.2, topological, differential...
Acknowledgements

CIRI U1111 “Cell Biology of Viral Infections”

Vincent Lotteau
Laure Perin Coccon
Christophe Ramière
Karim Mouzzanar
Camille Ménard
Caroline Charre
Marine Porcherot

Olivier Diaz
Clémence Jacquemin
Anne Aublin
Claire Curtil
Pauline Radreau
Baptiste Planthu