metabolic reprogramming -
a hallmark of oncogenic viruses

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France
### 30 to 40% of all cancer have a viral etiology

<table>
<thead>
<tr>
<th>Oncovirus</th>
<th>Cancer types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Papillomavirus</td>
<td>All cervical cancers</td>
</tr>
<tr>
<td></td>
<td>Majority of anal and vaginal carcinomas</td>
</tr>
<tr>
<td></td>
<td>Cancers of the oral cavity and pharynx</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Oncogenic in rodents but not humans</td>
</tr>
<tr>
<td></td>
<td>Transforming capacity in vitro</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td></td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>Burkitt, Hodgkin lymphoma</td>
</tr>
<tr>
<td></td>
<td>T-cell / NK-cell lymphomas</td>
</tr>
<tr>
<td></td>
<td>Nasopharyngeal and gastric carcinomas</td>
</tr>
<tr>
<td>Kaposi’s Sarcoma-associated Herpes Virus</td>
<td>Kaposi sarcoma</td>
</tr>
<tr>
<td></td>
<td>Primary effusion lymphoma</td>
</tr>
<tr>
<td>Human Cytomegalovirus</td>
<td>‘Oncomodulator’</td>
</tr>
<tr>
<td>Human T-cell lymphotrophic virus type 1</td>
<td>Adult T-cell leukemia and lymphoma</td>
</tr>
</tbody>
</table>
Oncoviruses are necessary but not sufficient for cancer development:

Prevalence of virus in the population $>>$ Cancer incidence
How do Oncoviruses contribute to cancer?

Integrations that activate/inactivate oncogenes or tumor suppressors

Expression, stabilisation, degradation of tumor suppressors/oncogenes or cellular factors that drive key signal transduction pathways

Chronic cellular stress and activation of inflammatory responses

Multi hit model of carcinogenesis
Most virally-induced cancers emerge in the context of persistent infections

- Avoid apoptosis (p53, Bcl2)
- Modulate and evade immune responses (Pd1)
- Stimulate growth and proliferation (pRB, E2F1, etc)
- Induction of vascularization (HIF1a)
- Induction of cell migration

Viruses and cancer cells share similar needs
Most virally-induced cancers emerge in the context of persistent infections

- Avoid apoptosis \((p53, Bcl2)\)
- Modulate and evade immune responses \((Pd1)\)
- **Stimulate growth and proliferation** \((pRB, E2F1, etc)\)
- Induction of vascularization \((HIF1a)\)
- Induction of cell migration

Viruses and cancer cells share similar needs
Viruses need biomass for virion production

Tumor cells need biomass for cell division
Key metabolic enzymes and pathways that are targeted by oncogenic viruses
Key metabolic enzymes and pathways that are targeted by oncogenic viruses
HCV upregulates glucose and glutamine utilization

Glucose uptake

<table>
<thead>
<tr>
<th></th>
<th>glucose utilization (A.U.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mock</td>
<td>90</td>
</tr>
<tr>
<td>HCV</td>
<td>120</td>
</tr>
</tbody>
</table>

Glutamine uptake

<table>
<thead>
<tr>
<th></th>
<th>glutamine utilization (A.U.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mock</td>
<td>100</td>
</tr>
<tr>
<td>HCV</td>
<td>180</td>
</tr>
</tbody>
</table>

Standardized to cell numbers
HCV upregulates key factors of glutamine metabolism

Glutaminolysis

- Glutamine
- SLC1A5, SLC7A5
- GLS2, GLS
- Glutamate
- α-Ketoglutarate
- TCA cycle

Transcript levels

Protein levels

HCV: cyto. nuclear
cell lysates

- Myc
- PARP
- Actin
- Core
HCV decreases glutamine and induces glutamate levels in supernatant

- Glutamine/glutamate quantif. in supernatant

Medium with:
- Glucose/Glutamine
- Glucose/-
- -/ Glutamine

Glutamine → GLS → Glutamate ↔ AA

α Ketoglutarate

TCA cycle

Standardized to cell numbers
Glycolysis versus glutaminolysis?

Glucose → Pyruvate → Lactate

Acetyl-CoA → α-Ketoglutarate → TCA cycle → Citrate → Malate → OxPhos?

NH₃ → Glutamate → Glutamine → Glutamine

Lactate production (A.U.)

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Pyruvate</th>
<th>Lactate</th>
<th>Acetyl-CoA</th>
<th>TCA cycle</th>
<th>Citrate</th>
<th>Malate</th>
<th>OxPhos?</th>
<th>Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>Glutamate</td>
<td>Lactate</td>
<td>Lipids</td>
<td>OAA</td>
<td>Citrate</td>
<td>Malate</td>
<td>α-Ketoglutarate</td>
<td>NH₃</td>
</tr>
</tbody>
</table>

Lactate production (day 3 post inf)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucose</th>
<th>Glutamine</th>
<th>Lactate Production (A.U.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock</td>
<td>+</td>
<td>+</td>
<td>1,22 fold</td>
</tr>
<tr>
<td>HCV</td>
<td>-</td>
<td>-</td>
<td>1,36 fold</td>
</tr>
<tr>
<td>Mock</td>
<td>+</td>
<td>-</td>
<td>2,8 fold</td>
</tr>
</tbody>
</table>

Amino Acids

Day 3    Day 4      Day 5

OxPhos?  ATP?
Increased respiratory activity in HCV-infected cells
HCV augments superoxide anion production

BRAULT et al, GUT 2016
Proliferation of HCV-infected cells depends on glutamine

![Graph showing cell growth from day 1 to 5 with and without HCV infection, under different conditions (control medium, no glucose, no glutamine, no glutamine + αKG).]
HCV depends on glutaminolysis

Glutaminolysis

- Glutamine
- SLC1A5
- SLC745
- GLS
- Glutamate
- α Ketoglutarate
- TCA cycle

HCV RNA (% control)

- Control
- No Glu
- No Glu + NEAA
- No Glu + Glu
- No Glu + αKGl

HCV RNA

- ***
HCV replication is sensitive to MYC and Glutaminase silencing

Huh7.5 +/- shRNA

Day 0

Day 3

RTqPCR

HCV replication

MYC/GLS silencing efficiency

HCV RNA (% control)
Inhibition of biosynthesis or induction of energy stress

Anti-cancer

Anti-virus
HCV replication is sensitive to MYC and Glutaminase inhibitors

Preventive effect of inhibitors on neo-infection

Therapeutic effect of inhibitors on established infection

HCV replication is sensitive to MYC and Glutaminase inhibitors

anti-MYC

anti-Glutaminase
CB-839 blocks HCV-induced superoxide production
Conclusion

Glutaminolysis
- is induced by HCV
- is required for HCV infection
- stimulates cell growth of infected cells in vitro

Importance of glutamine in HCV infection:
→ Redox balance
→ ATP / electron acceptor generation
→ Carbon/nitrogen donor for anabolic processes

What role does glutaminolysis play in fibrosis progression and incidence of hepatocellular carcinoma?

Is induced glutaminolysis reversible, and can we prevent hepatocarcinogenesis by targeting glutaminolysis?
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