Rodent Parvoviruses as Anti-cancer Agents

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Normal cell spared

Schematic representation of tumor-selective viral replication and oncolysis (Kirn et al., 2001)
(Autonomous) Parvoviruses: Structure

Icosahedral capsid
non enveloped
diameter ~25 nm

ssDNA
~ 5100 bases

Modified from Sgro and Spencer, Encyclopedia of Virology
Autonomous Parvoviruses: Lytic Cycle

- Virus Production
- Viral SS DNA Displacement Synthesis
- Production of Viral Capsid Proteins
- Production of Viral Regulatory Proteins
- Viral DNA Amplification
- Viral SS → DS DNA Conversion
- Nuclear Translocation Decapsidation
- S Phase
- Uptake
(Autonomous) Parvoviruses: Biology

**SUSCEPTIBLE SPECIES:**
- Mammals (rodents, human, felines, canines, porcines, bovines, ...)
- Birds (?)

**REQUIREMENTS TOWARDS HOST CELLS:**
- **Common:** cell proliferation (S factor)
- **Specific:** animal species
  - cell differentiation

**IN VIVO BIOLOGY**
- Cytopathic effect restricted to proliferating tissues
- Pathogenesis in adult < new-born < foetus
- Antineoplastic effect
Oncotropism of H-1PV in i.t. Injected RG-2 Glioma-bearing Immunocompetent Rats

**Brain**
- NS1 protein (Western Blotting)
- Viral DNA (PCR)

**Periphery**

**Brain**
- NS1 protein (IF)
- Viral RNA (RT-PCR)
- Virus multiplication

**Periphery**
- tumor area
- adjacent tissue

Inoculum 2 d p.i.
- Infectious Units (10^6)
Regulation of Parvovirus DNA Replication and Gene Expression by Proliferation/Transformation-dependent Cellular Factors

Examples

**Cyclin A activates the conversion of parvovirus ss DNA into ds replicative form**

**The early promoter P4 is activated by E2F/ATF families of transcription factors**

- Cyclin A activates the conversion of parvovirus ss DNA into ds replicative form
- The early promoter P4 is activated by E2F/ATF families of transcription factors

Diagram showing the interaction between cyclin A, cdk2, Pol δ, PCNA, RFC, RPA, and the activation of the P4 promoter by E2F/ATF transcription factors.
NORMAL FIBROBLAST

EXTRACELLULAR SPACE

MVMp

CYTOPLASM

PRRs

NUCLEUS

type-I IFN genes

ANTIVIRAL STATE

PV multiplication

αIFN Ab

type-I IFN genes

antiviral genes
Searching for Viral Oncotoxins
The Parvoviral NS1 Product Is Sufficient to Kill Oncogene-transformed Cells
NS1 Interaction with CKIIα:
a Determinant of Parvovirus Cytotoxicity

• NS1 cytotoxicity mutant S473A is defective in CKIIα binding

• CKIIα is involved in the induction of cytopathic effects

Mock | MVM 24 h p.i. | MVM 48 h p.i.

A9 cells

A9-E81A (P38:dnCKIIα)
Adaptor Model of NS1 Cytotoxic Functions

Colony Formation Inhibition Assay
Improvement of the Antitumor Immunomodulating Effect of H-1PV through Arming with CpG Elements

TARGETS
MH3924A hepatoma cells

AUTOLOGOUS TUMOR CELL VACCINE

Immunostimulation (mediastinal lymph nodes)

Suppression of lung metastases

Vaccine treatment
Mock H-1 wt H-1GC H-1CG

IFNγ CD80 CD86 β-actin

lung metastases
Oncolytic Parvovirotherapy of Non-Hodgkin B-cell Lymphomas
Why Burkitt’s Lymphoma?

• Form of non-Hodgkin lymphoma, divided in endemic, sporadic and immunodeficiency-associated types

• Initiating role for Epstein-Barr Virus in tumorigenesis (in partic. endemic type)

• An extremely aggressive human cancer: among the fastest growing tumors

• The 1-year survival rate for adult patients with advanced disease is 15%

• Current treatment: standard chemotherapy in combination with rituximab
  ➢ short- and long-term side effects
  ➢ drug resistance
  ➢ early or late relapse: durable remission may not be achieved
Burkitt’s Lymphoma-derived Cell Lines Are Sensitive to H-1PV-induced Killing

Irrespective of EBV presence

Irrespective of rituximab resistance

Through necrotic cell death

EBV - EBV +

Mock

H-1PV (10 pfu/cell)

CD20 expression (rituximab sensitivity)

H-1PV-induced cell killing

RAMOS Namalwa

* 10 pfu/cell, 72 hpi

Annexin-V

PI

R1: apoptotic cell fraction
R2: necrotic cell fraction

24 hpi

48 hpi

2.3

0.5

0.3

1.1

27.7

0.9

1.3

80.8

R1

R2

R1

R2
H-1PV Suppresses Burkitt’s Lymphomas in a SCID Mouse Model

Early treatment (14 d post tumor initiation)

Late treatment (28 d post tumor initiation)

Days post i.t. injection of H-1PV

Days post tumor initiation

* Sacrificed
H-1PV-induced Regression of BLs Correlates with i.t. Expression of the Viral Cytotoxic Protein NS1 (SCID mouse model)

IHC detection of NS1 protein in necrotic areas of treated tumors

RT-PCR detection of NS1 transcripts in treated and (contralateral) untreated tumors

<table>
<thead>
<tr>
<th>Single tumors</th>
<th>Double tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV.H-1PV</td>
<td>H-1PV</td>
</tr>
<tr>
<td>H-1PV</td>
<td>H-1PV</td>
</tr>
<tr>
<td>H-1PV</td>
<td>H-1PV</td>
</tr>
</tbody>
</table>

Animal: 1 2 3 4 5 6 7 8
Oncolytic Parvovirotherapy of Pancreatic Ductal Adenocarcinoma
Why Pancreatic Ductal Adenocarcinoma (PDAC)?

- A highly malignant disease (almost 100% lethal)
- The 4th (Western Europe) and 5th (North America) leading cause of cancer-related deaths
- The worst 5-year survival rate of all human cancers: <5% of all PDAC patients survive 5 years
- Surgery is the only curative treatment currently available
- Most patients present with advanced PDAC: > 80% have unresectable disease at the time of diagnosis
- Highly resistant to conventional cytotoxic agents (e.g. Gemcitabine)
Therapeutic Effect of H-1PV in a Rat Orthotopic PDAC Model

Features

- Tumor progression

1. Local tumor development (T)
2. Lymph node metastases (N)
3. Liver metastases (M)

- Immunocompetent model

- Stromal reaction present

Animals survival

![Graph showing survival index over 120 days with treatment comparisons.](image)

- At treatment
- 8 weeks after mock treatment
- 8 weeks after H-1PV treatment
Orthotopic PDAC Suppression through Adoptive Immune Cell Transfer (Rat Model)

Survival of Recipients of splenocytes from H-1PV vs mock-treated Donors

Neutralizing Ab response in H-1PV-treated Donors but not in Recipients therefrom

Survival index

days post tumor initiation

- Recipients from mock-treated Donors
- Recipients from H-1PV-treated Donors

Serum dilutions

Mock-treated Donors
H-1PV-treated Donors
Recipients from H-1PV-treated Donors
H-1PV Kills Human PDAC Cells Irrespective of their Resistance to Gemcitabine
Wild-type SMAD4 Expression: a Predictive Marker of PDAC Cell Permissiveness for H-1PV Infection

Changes in SMAD4 status during PDAC progression

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>SMAD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panc-1</td>
<td>Primary PDAC</td>
<td>wt</td>
</tr>
<tr>
<td>MiaPaCa-2</td>
<td>Primary PDAC</td>
<td>wt</td>
</tr>
<tr>
<td>AsPC-1</td>
<td>Metastasis</td>
<td>mt</td>
</tr>
<tr>
<td>CFPac-1</td>
<td>Metastasis</td>
<td>loss</td>
</tr>
</tbody>
</table>

SMAD4-mediated signalling

Correlation of wt SMAD4 status with enhanced PDAC cell sensitivity to H-1PV

Stimulation of H-1PV production by SMAD4

Panc-1

0 0.8 1.6 2.4 3.2
0 1 4

Total progeny viruses [RFU x 10^6]

Days post-infection

Input Virus

Functional knock-down

pΔ

pΔnSmad4

cell line

short-term culture
Conclusion

Arming with regulatory elements
Mutagenesis  Transgene addition

(1) Survival and death pathways
(2) Anticancer immune responses
(3) Cell cycle checkpoints
(4) Cytoskeleton dynamics

Neoplastic Cell

PROSPECTS OF CANCER PARVOVIROTHERAPY
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SMAD4: a Positive Regulator of H-1PV Gene expression

**Presence of putative SMAD-binding elements (SBE) in the H-1PV P4 promoter**

**Correlation of NS1 accumulation with wt SMAD4 expression**

**Activation of H-1PV P4 promoter by SMAD4**
Oncolytic Parvovirotherapy of Glioblastoma Multiforme
Why Glioma?

Glioblastoma multiforme is the most common of primary brain tumors. 2–3% of all human malignancies.

Malignant gliomas have an extremely bad prognosis: 50% of patients die within 12–16 months after diagnosis (grade IV-gliomas). < 5% long-time survivors.

Human Glioma Cells Are Targets for H-1PV Oncolytic Activity

**H-1PV kills human glioma cells resisting DNA-damaging agents and/or soluble death-ligands**

- Survival (%)
- NCH-89, NCH-82, U-138, normal astrocytes

**Cathepsins are involved in H-1PV-induced glioma cell death**

- Rescue of NCH82 cells from H-1PV infection (5 pfu/cell) (cell lysis)
  - NCH82, NCH82 gfp, NCH82 StefinB

**Acidic vesicles accumulate in the cytosol**
- Acridine orange staining
  - NCH82 cells
  - Mock
  - H-1PV 5 pfu/cell 24h p.i.

**Cathepsins relocate in the cytosol**
- Cathepsin B staining

**Cytosolic cathepsin-inhibitors are down-regulated**

- NS1
- ß-tubulin
- CystatinC
- StefinB
Early infection

- H-1PV

Late infection

- H-1PV

**Days post-implantation**

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Early Infection</th>
<th>Late Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.0</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>0.6</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**U87 human glioma:**
- 10⁵ cells/rat
- H-1PV (i.c./i.v. combination): 6x10⁹ pfu/rat

**NS1 expression in tumor**
Population

- up to 25 patients
- histologically confirmed glioblastoma, WHO grade IV
- single center (University of Heidelberg)

Further main inclusion criteria

- recurrent and resectable GBM progressing (MRI-Scan) in spite of radio- and/or chemotherapy
- chemotherapy stop for at least 4 weeks prior to treatment with H1PV

Main exclusion criteria

- multifocal disease
- poor Karnofsky-Score (<70)
- severe systemic infection
- pregnant or breast feeding women

Administration of virus

- escalated dosage; i.t. (pre-/intra-OP); i.v. (pre-OP)

Study design developed with the advice of NCT and KKS, with provisional approval of the National Authority (Paul-Ehrlich Institute)
Enhancing Potency of Oncolytic Parvoviruses
Selection of H-1PV Variants Adapted for Multiplication in Human Glioma Cells

Virus selection

RG2 (rat glioma)
- rat H-1PV
- large production

NCH (hum glioma)
- rat H-1PV
- little/no production

NCH149 (hum glioma)
- rat H-1PV
- > 25 passages
- large production

Enhanced fitness of hgH-1PV to propagate in human glioma cell cultures

rat RG-2
- H-1PV
- hgH-1PV

hum NCH 89
- H-1PV
- hgH-1PV

hum NCH 82
- H-1PV
- hgH-1PV

hum NCH 37
- H-1PV
- hgH-1PV

Input fold input

m.o.i: 5 1.5 0.5 0.1 0.05

H-1PV
hgH-1PV
H-1PV
hgH-1PV
H-1PV
hgH-1PV
H-1PV
hgH-1PV
H-1PV
hgH-1PV
Production of recombinant parvoviral vectors

- recombinant parvoviral DNA plasmid
- packaging helper plasmid

Co-transfection

Excision and amplification

Packaging

Capsid formation

Amplification VP-expression

Release of virus

293T cells
IP-10 (human) /CRG-2 (mouse)
Interferon-inducible protein of 10 kD

• Cellular sources: monocytes, T cells, fibroblasts, osteoblasts, keratinocytes and endothelial cells
• Member of the CXC family of chemokines
• Chemoattractant for activated monocytes, T cells and NK cells
• Inducer of lymphocyte adhesion to endothelium
• Activator of NK cells
• (In)direct antiangiogenic effects
• Potent suppressor of vascular(ized) tumours
Tumor Necrosis Factor- α (TNF- α)

- Cytokine, 2 receptors (TNF-R1, -R2)
- Pro-apoptotic factor
- Anti-angiogenic properties
- Immunostimulant (DC maturation, macrophage activation)

→ use of TNF-α in combination with chemokines which can recruit but not always activate immune cells
Tumour growth inhibition following infection of glioma cells with recombinant parvoviruses

GL 261 cells, C57/Bl6 mice