Mastomys coucha: A natural animal model for papillomavirus-induced skin carcinogenesis

Kai Schaefer, Julia Nafz, Myriam Ibberson and Frank Rösl

Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, Heidelberg
Risk Factors of Non Melanoma Skin Cancer (NMSC)

- **Cumulative UV exposure**
  - Outdoor workers (farmers, sailors)
  - Outdoor sport (tennis, golf etc.)
  - Age

- **Individual susceptibility**
  - Skin type
  - Hair type
  - Immunosuppression
  - HPV infection
Human Papillomaviruses (HPV)

- more than 100 types known to date

- **Mucosal types**
  - low risk: anogenital warts
  - high risk: HPV16/18 ⇒ cervical cancer

- **Skin types**
  - warts, papillomas, keratoacanthomas
  - Non Melanoma Skin Cancer (NMSC) ?
Association between HPV and NMSC first described in patients with *Epidermodysplasia verruciformis* (EV)

EV-HPV types (HPV 5, 8, 9, 12, 14, 15, 17, 19-25, 38)

30-60% develop multiple cutaneous squamous cell carcinoma (SCC) on UV-exposed sites after 10 - 30 years

These SCC mostly harbour the oncogenic types HPV 5 and HPV 8
HPV and Non-melanoma Skin Cancer

- Patients under **systemic immunosuppression** (e.g. organ transplant recipients)
  - multiple warts and NMSC at UV-exposed sites
  - HPV-DNA in up to 80% of tumors, but here **not necessarily** every cell is HPV positive

kindly provided by Prof. E. Stockfleth, Charité Berlin
Causal Role of Viruses in Carcinogenesis as indirect carcinogen

1. Regular presence of the genome or parts of it in every cell is not given.

2. Transfection of the nucleic acid into tissue culture cells should lead to immortalization or tumor induction. Animal models.

3. Prevention of DNA repair and apoptosis

4. Epidemiological case/control studies should identify this virus as an essential co-factor for the respective tumor

5. Vaccination against this agent should provide protection
UV-exposure of the skin

Non-infected cells

- p53 induction
  - Growth arrest & DNA repair
    - Normal phenotype
  - Bak induction & Apoptosis
    - Elimination of cells with abnormal phenotype

HPV-positive cells

- p53 induction
  - p53 mediated induction of ΔNp73
    - E6-mediated Bak degradation & XRCC1 inactivation
    - E7-mediated MMP induction
      - Accumulation of cells with an abnormal phenotype

Elimination of cells with abnormal phenotype

Normal phenotype
Non-melanom skin cancer (NMSC)

Vaccination prior to transplantation

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Requirement of a Model
Mastomys coucha

- African multimammate mouse
- Natural occurring animal model
- Immune competent
- Mastomys population latently infected with

*Mastomys natalensis*
Papillomavirus (MnPv)

⇒ Spontaneous development of benign epithelial tumors of the skin and the anogenital tract

- Age-dependent appearance in 40% of the animals (> 8 months)
- Treatment with a tumor promoter: skin cancer
Spontaneous appearance of lesions

papillomas, keratoacanthomas (skin, eyes, snout, ears and anus)
Time course of lesion development

![Graph showing the time course of lesion development for different types of lesions: Skin Papilloma, Papilloma eyelid, Condyloma, and Papilloma ear. The x-axis represents time in months, ranging from 0 to 20, and the y-axis represents tumor incidence in number of cases, ranging from 0 to 25. The graph illustrates the progression of lesion development over time for each type of lesion.]
MnPV genomic organisation

- 7 ORFs
- E5 is missing like in other EV-HPVs and cutaneous HPV types
- one coding DNA strand
Histological detection of viral DNA in infected tissues

DNA-\textit{In Situ}-Hybridisation

Skin

Skin tumor
Is there any oncogenic potential of MnPV?
Expression of MnPV E6 in transgenic mice

A

B

C

D

E

F

- 269 bp

- 599 bp

MnPV E6

\[ \text{K14} \rightarrow [\beta-glo] \rightarrow E6 \rightarrow RES \rightarrow \text{LacZ} \rightarrow pA \]
Time course of tumor formation

A. Papillomas per Animal

B. Papilloma Incidence (%)

C. Carcinoma-Bearing Animals (%)

D. Keratoakanthoma-Bearing Animals (%)

Weeks after DMBA Treatment
Detection of H-ras mutations in MnPV E6 transgenic mice by RT-PCR RFLP analysis

DMBA induces a A → T transversion at codon 61
Inverse correlation between Ha-ras activation and E6 expression in SCC of MnPV E6 transgenic mice
### H-ras mutations in MnPV E6 transgenic animals

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>pos. 12</th>
<th>pos. 13</th>
<th>pos. 61</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillomas</td>
<td>0/34</td>
<td>0/34</td>
<td>13/34</td>
</tr>
<tr>
<td>Keratoacanthomas</td>
<td>0/14</td>
<td>0/14</td>
<td>4/14</td>
</tr>
<tr>
<td>SCC</td>
<td>0/23</td>
<td>0/23</td>
<td>0/23</td>
</tr>
</tbody>
</table>
Distribution and propagation of Mastomys Papillomaviruses in different tissues
Absence of MnPV in anogenital and tongue lesions

MnPV L1

MnPV E6

actin

1 = normal skin
2 = papilloma eye
3 = condyloma anus

1 = normal skin
2 = papilloma eye
3 = condyloma vulva

1 = papilloma eye
2 = condyloma anus
3 = papilloma tongue
Condylomas

- benign tumors of the anogenital tract
- conjunctional epithelia
Phylogenetic tree: difference between MnPV and McPV2
Distribution of MnPV and McPV2 DNA: PCR analysis
MnPV and McPV2 infection of the tongue

EM localization of tumor

PCR

MnPV-L1
McPV2-L1
Actin

localization of tumor

MnPV-L1
McPV2-L1
Actin
MnPV can be predominantly detected in skin tumors but also in apparently healthy skin.

In transgenic animals, MnPV E6 can substitute or circumvent DMBA induced Ha-ras mutations; 100% formation of SCCs

There is no indication for a trans-placental/germ-line transmission route of the virus.

MnPV DNA is also present in other organs (cell specificity, viral spread?)

McPV2 is responsible for the less frequently occurring tumors of the anogenital tract and other conjunctional epithelia like mouth and eye.
Immunological events controlling the virus infection
Case-control study: 120 animals analyzed

collecting tissue samples and serum

PCR

Detection of viral DNA

GST-Capture-ELISA

Detection of antibodies
Seroreactivity against MnPV-L1

- High antibody responses to L1 correlate with the presence of papillomas.
- Low antibody level in young animals ⇒ delayed immune response or latency?
Seroreactivity against early proteins

MnPV

A450

Tumor

L1 L1 E6 E6 E7 E7 E2 E2

+ - + - + - + -
Seroreactivity against early proteins

McPV2

*only low E2 responses compared to MnPV*
MnPV: early E2-responses

- E2 seroreactivity: marker for early infection and latency, measurable by ELISA earliest 4 weeks after birth, preceding L1

- Maternal transmission of E2 antibodies, but no L1 antibodies

- L1 seroreactivity: productive infection and the onset of tumors
How we want to use this animal model?
Prophylactic vaccination against cutaneous HPV for OTR before transplantation

Mastomys as model for a vaccine against skin PV

VLP-vaccination against MnPV-induced skin tumors

- immunization of young animals
- induction of neutralizing antibodies at early stages

⇒ Prevention of primary infections or viral dissemination in older animals
Summary

- MnPV and McPV2 are the etiological agents for a variety of epithelial tumors in *M. coucha*.

- The antibody reactivity against L1 is significantly increased in tumor-bearing animals. High IgG-titers produced as response to PV-reactivation and the massive viral multiplication in older animals are not sufficient to prevent viral spread and tumor development.

- MnPV-E2 is highly immunogenic and seroreactivity precedes the L1 response, marking early and latent stages of infection.

- Unique read-out model for vaccination studies: VLPs will be applied as a prophylactic vaccine to prevent skin tumor development; Testing the efficacy of VLPs vaccines under immunosuppressed conditions.
Thanks for your interest
Maternal transmission of antibodies
MnPv: early E2-responses

Follow-up study: 84 animals

⇒ 4 weeks after birth: only very weak L1 responses

⇒ E2 responses coincide and precede L1 responses

⇒ E2 seroreactivity: early infection and latency
⇒ L1 seroreactivity: productive infection and the onset of tumors
Stomach cancer (gastric carcinoids and adenocarcinoma):

H2 receptor blocker Loxtidine or by Helicobacter pylori – co-infection with PV?
(Model of chronic inflammation caused by bacteria and virus?)

Transfer of MnPV and McPV2 to nude mice: effect of UV

Genetic susceptibility? (CGH, gene profiling)
Detection of virus particles in skin sections

mature viruses are released in stratum corneum

Stratum corneum

Stratum granulosum

Stratum spinosum

Stratum basale

basal membrane
Prophylactic PV vaccine consists of a single protein.

Production in insect cells or yeast:

- L1 protein
- Spontaneous assembly
- 360 copies

Virus-like particle (VLP)
ISH of McPV2 in lesions of the vulva
MnPV-DNA in hair follicle cells: a possible virus reservoir?

E = Epidermis  hf = hair follicle