SV40 Carcinogenesis

Species and cell types susceptible to SV40 infection and transformation
Why human mesothelial cells and malignant mesothelioma (MM)?

SV40-infected fibroblast  SV40-infected mesothelial cell
Why human mesothelial cells?
Why human mesothelial cells?
SV40 and asbestos are co-carcinogens in vitro

**TRANSFECTIONS (ori-)**

- $T^+t^+$: mesothelial cells $4.6 \times 10^{-4}$
  fibroblasts $6.0 \times 10^{-6}$
- $T^+t^-; T^-t^+; T^-t^-$: 0.0 (mes. cells and fibr.)
- Asbestos: 0.0 (mes. cells and fibr.)
- $T^+t^+$ and asbestos: mesothelial cells $6.0 \times 10^{-4}$
  fibroblasts $9.3 \times 10^{-6}$
- $T^+t^-$ and asbestos: mesothelial cells $4.0 \times 10^{-4}$
  fibroblasts $3.6 \times 10^{-6}$
- Asbestos and $T^-t^+$ (or $T^-t^-$): 0.0 (mes. and fib.)
Summary

• Human fibroblasts infected with SV40 are lysed, thus are not transformed.
• Human mesothelial cells are very susceptible to SV40 infection. SV40 persists in these cells without causing lysis.
• The prolonged expression of Tag in 100% of mesothelial cells causes a very high rate of malignant transformation.
Summary

• Asbestos promotes SV40-mediated mesothelial cell transformation
• Our data provide a rationale for the observation that SV40 has been identified in mesothelial cells and mesothelioma
• Our data suggest that individuals infected with SV40 may be more susceptible to asbestos carcinogenicity
SV40 carcinogenesis

SV40 and several other DNA tumor viruses are seldom oncogenic in their natural host, but become oncogenic when they cross species.
DNA Viruses and Cancer

• Human adenoviruses cause tumors in hamsters
• Human polyomavirus JC cause brain tumors in owl monkeys and rodents
• Monkey polyomavirus SV40 cause tumors in hamsters, mastomys and some mice
SV40 oncogenicity

- Species in which oncogenicity is studied
- Susceptible cell types and target organs
- Individual susceptibility and immune status
Cell types susceptible to SV40 carcinogenesis

<table>
<thead>
<tr>
<th>Tumors Caused by SV40 in Hamsters</th>
<th>Tumors Associated with SV40 in Humans</th>
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</thead>
<tbody>
<tr>
<td>Mesotheliomas*</td>
<td>Mesotheliomas</td>
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<tr>
<td>Lymphomas*</td>
<td>Lymphomas</td>
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<tr>
<td>Bone Tumors*</td>
<td>Bone Tumors</td>
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<tr>
<td>Brain Tumors*</td>
<td>Brain Tumors</td>
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<tr>
<td>Sarcomas*</td>
<td></td>
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<tr>
<td>• Systemic intracardiac administration</td>
<td></td>
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<tr>
<td>* Local intracerebral injection</td>
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<td>+ Local subcutaneous injection</td>
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</tbody>
</table>

- Mesothelioma 33 papers from 22 labs
- Lymphoma 4 papers from 4 labs
- Bone tumors 7 papers from 7 labs
- Brain tumors 17 papers from 16 labs
SV40 carcinogenesis

Detection of SV40 in human tumors
Specificity

• Carbone et al., 1994, 1997. Staining and in situ hybridization shows that Tag and Tag mRNA are present only in the tumor cells.
• Shivapurkar et al., 1999. SV40 in 57/118 MM and in 1/75 nearby normal tissue microdissected from the same slide.
• Ramael et al., 1999. PRINS SV40 only in Mesothelioma cells, not in nearby stroma.
Carbone et al., Oncogene 1994

- 29/48 mesothelioma
- 1/28 matching lung biopsies
- 2/24 lung ca (all types)
- 1/6 gastric ca
- 0/3 additional ca
- 0/14 lung biopsies
SV40: Pathogen vs Passenger

• The presence of SV40 in a given tumor type does not establish causation because only specific cell types are susceptible to SV40 oncogenicity
SV40 detection

T antigen domains

- Pol-α
- RP-A
- Host range
- DNA binding
- ATPase
- Helicase
- TEF-1
- p53
- p300?
- p130
- p107
- Hsc70
- Rb
- 1, 82, 164, 246, 260, 517, 708
SV40-negative reports

- 3 reports from 2 different labs
- Techniques: PCR and presence of neutralizing antibodies
Mesothelioma biopsies contain mostly non-malignant cells
Short Communication

Simian Virus 40 and Pleural Mesothelioma in Humans


Viral Epidemiology Branch, National Cancer Institute, NIH, Bethesda, Maryland 20892 [H. D. S., J. J. G., C. S. R.]; Armed Forces Institute of Pathology, Washington, D. C. 20306 [M. F., W. D. T.]; Jerome H. Holland Laboratory, American Red Cross, Rockville, Maryland 20855 [A. E. W.]; Department of Molecular Microbiology and Immunology, School of Public Health, The Johns Hopkins University, Baltimore, Maryland 21205 [R. W. D., K. V. S.]

percentage of cases. In addition, a number of the tumor specimens tested were also shown by immunochemistry to contain SV40 large T antigen (11, 12). In a subsequent PCR study, SV40 DNA was reported to be present in osteosarcomas, osteoblastomas, chondrosarcomas, giant cell tumors, Ewing’s sarcomas, liposarcomas, and Li-Fraumeni cell lines (13).

The public health implications of a relationship between SV40 and human cancers could be substantial. More than one
Mulatero et al. 1999

- 12 British mesotheliomas SV40- using PCR and ethidium bromide staining. Sensitivity 1 copy/cell as per HPV
SV40-positive reports

- 61 reports from 49 labs
- Techniques: Virus rescue, EM, Southern blot genomic DNA, PCR, PRINS, Microdissection/PCR, mRNA in situ, Antisense Tag, Co-immunoprecipitation Tag-p53/Rb, Western blot, Antibodies anti-Tag, Specific induction of oncogenes in SV40+ tumors.
Testa et al., Cancer Res. 1998

- Laboratories: Testa, Kalili, Carbone, Linnainmaa.
- PCR and immunostaining
- Results: 10/12 specimens were SV40 positive.
Strickler et al., Cancer Epi, Bio, Prev. 2001

- 9 laboratories including Dr. Shah (negative) and Drs. Butel, Miller, Jasani (positive)
- Shah’s laboratory was the most sensitive in detecting SV40 in the positive controls
- Butel’s and other labs were not contaminated and reliably detected SV40 in controls
- Mesothelioma were negative
- Half of the negative controls were positive
Geographical differences

• Hirvonen et al., 1999; SV40 present in 3/5 USA mesotheliomas but not in 49 Finnish mesotheliomas.
• Emri et al., 2000; SV40 in 2/2 Italian and 0/29 Turkish mesothelioma.
• De Rienzo et al., 2002. SV40 in 4/11 USA and 0/9 Turkish mesothelioma.
Geographical differences

• Leithner et al., 2002 SV40 present in 3 tumors from Italy and USA, absent in all 24 bone tumors and 8 MM Austrian samples

• Waggen et al., 2000, 2/27 ependymomas contained SV40. Both positives were USA patients, 25 negatives were from Germany

• Heinsohn et al., 2000, 2/42 osteosarcomas, 1/4 sarcomas contained SV40, Germany
Geographical differences

• Finland, Turkey and Austria did not receive contaminated poliovaccines, Germany it is unclear
• Geographical differences are found also with other DNA tumor viruses
Technical differences

• Gordon et al. Surgical Forum 2001 and Oncology Report 2002, found SV40 in 2/35 mesothelioma biopsies and 3/7 mesothelioma cell lines

• The same authors state that initially they had failed to detect SV40 in the same specimens. When they changed the technical approach they detected SV40
Is SV40 present in human cancer?

• “The presence of SV40 in some human tumors has been convincingly demonstrated in the past 4 years”
SV40 carcinogenesis

Sources of human infection
Human exposure to SV40

- Vaccines
- Direct contact with monkeys
- Laboratory personnel
Documented vaccine exposure in the USA

- Parenteral poliovaccines 1955-1963
- Oral poliovaccines 1960
- Parenteral adenovaccines 1957-1960
- Adeno 3 and 7 vaccines 1961-1965
- Recombinant Adeno-SV40 viruses
- Hepatitis A vaccine 1980 (only 150 newborns).
Amount of infectious SV40 in vaccines

- Parenteral poliovaccines $10^{2.3} - 10^{3.3}$
  Batches tested 2
- Oral poliovaccines $10^4 - 10^6$
- Parenteral Adenovaccines ?
- Adeno 3 and 7 vaccines ?
- Hepatitis A vaccine ?
Significance of infectious dose

- The significance is unclear because human cells are permissive to SV40 infection. Thus, millions of viral particles are produced following infection.
- Rodent cells on the other hand are non-permissive. SV40 does not replicate and the effects are dependent on the initial dose.
Poliovaccination in the US until September 1961

- < 1 year 2.4 millions (55% of age group)
- 1-4 years 14.6 millions (87%)
- 5-9 years 18.4 millions (93%)
- 10-14 years 16.9 millions (94%)
- 15-19 years 11.4 millions (84%)
- 20-39 years 26.3 millions (60%)
- 40-59 years 8.4 millions (19%)
SV40 and mesothelioma

• More than 34.7 million people aged 20-59 were vaccinated. The enormous increase in the incidence of mesothelioma in the second half of the 20th century was in this cohort.

• Therefore, the statement that “the increased incidence of mesothelioma occurred in a cohort unlikely to have been vaccinated” is inaccurate.
SV40 and mesothelioma

• Comparing the incidence of mesothelioma in infants/children vaccinated or not with contaminated vaccines, Strickler et al., stated that there was “no significant cohort effect” in a total of 71 MM.

• Of those only 2 MM were in the unexposed cohort, while 45 and 23 were in the childhood and infancy exposed cohort. One we do not know
Non-documented exposure

• It has been stated that some poliovaccine manufacturers did not take the required steps to document the absence of SV40 in their products after 1961.
SV40 in poliovials after 1963

• Rizzo et al., Cancer Res. 1999, No SV40 in 6 oral and 6 parenteral poliovials prepared in 1996. Verified by 45 PCR cycles and Southern blot hybridization with primers that amplify less < 400 bp of SV40 DNA

• Two more studies tested poliovials prepared after 1963. However, technical problems make the results unreliable
<table>
<thead>
<tr>
<th>nt no.</th>
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<th>Vaccine isolate</th>
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<td>36</td>
<td>G</td>
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<td>732</td>
<td>T</td>
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<td>747</td>
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<td>2239</td>
<td>T</td>
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<td>2716–2721</td>
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<tr>
<td>2795</td>
<td>—</td>
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<td>4879</td>
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Some SV40 found in humans comes from the poliovaccines

- In Rizzo et al. 1999 we demonstrated the presence of 2 unique strains of SV40 in poliovials prepared in 1954. Strain MC028846B and MC028863B.
- Butel’s team found strain MC028846B in 3 lymphoma patients: 42, 60 and 30 year old (HIV+).
Hypothesis: SV40 and asbestos co-carcinogenesis in vivo
SV40 carcinogenesis

Mechanisms
Can SV40 cause human cancer?

- Disruption of the intracellular pathways regulated by large Tag and small tag, oncogenic ras, and telomerase activity suffices to create a human tumor cell.
SV40 induces telomerase activity

• SV40 induces telomerase activity early after infection of human mesothelial cells
• Telomerase induction creates a background of immortal cells capable of continuous growth when malignantly transformed
  – Foddis et al., Oncogene 2002
SV40 genetic damage in humans.
Cell with 85 chromosomes
Can SV40 cause human cancer?

- SV40-transformed human cells grew as subcutaneous tumors when injected into human volunteers.
Antisense to SV40 causes apoptosis of SV40+ tumor cells

• SV40+ Mesothelioma cell cultures have low levels of Tag expression
• Antisense Tag treatment causes growth arrest and apoptosis of these cells, but not of SV40 negative mesothelioma cell cultures
• Antisense Tag induced expression of p21 indicating restoration of the p53 pathway
  – Waheed et al., Cancer Res. 1999.
Can SV40 cause human cancer?

- Yes, because SV40 is a potent human carcinogen
- However, viruses are seldom complete carcinogens and cancer development is not an inevitable outcome of virus infection in any viral system
- SV40 carcinogenesis must be studied together with the environmental and genetic factors associated with SV40+ tumors
SV40 transformation

- HIT AND RUN transformation can occur
- Marolokau et al., PNAS 1994
- Moorwod et al., Exp Cell Res 1996
- Ewald et al., Science 1996
- Tzeng et al., Oncogene 1998
- Salewski et al., Cancer Research 1999
SV40 Tag inhibits p53 and Rb

- Carbone et al., Nature Medicine 1997
- Co-expression of Tag and wt-p53 in mesothelioma biopsies
- Co-precipitation of Tag and p53
- Lack of p21 induction in p53 + biopsies

- De Luca et al., Nature Medicine 1997
- SV40 DNA and proteins in 30/36 mesothelioma biopsies
- Co-precipitation of Tag and pRb, p107 and p130
Co-expression of Tag and p53 in mesothelioma
Co-precipitation of Tag and p53 in mesothelioma
Inactivation of RASSF1A in SV40+ mesotheliomas

- RASSF1A is a tumor suppressor gene
- The frequency of RASSF1A methylation is significantly higher in SV40+ mesotheliomas compared to SV40 negative tumors
- SV40 induces RASSF1A methylation in human mesothelial cells
  - Toyooka et al., Cancer Res. 2001; and Oncogene 2002.
SV40 induces HGF secretion and met activation in mesotheliomas

• Some mesotheliomas secrete HGF
• SV40 transfection induces HGF secretion, met activation and proliferation of mesothelial cells
• Met was activated only in SV40+ mesothelioma cell lines and this was caused by Tag inhibition of RB.
SV40 causes VEGF secretion in SV40+ mesotheliomas

- VEGF is released by some mesotheliomas and it is associated with shorter survival
- VEGF is released by mesothelial cells transfected with SV40 and stimulates vein cell proliferation
- VEGF is released specifically by tumor cells from SV40+ mesotheliomas
  – Cacciotti et al., Am J Resp Cell Mol Biol 2002
SV40-induces Notch 1

- Notch 1 is a pivotal gene in regulating cell fate, growth, and differentiation
- SV40 induces Notch-1 in mesothelial cells. Downregulation of Notch-1 blocks SV40+ mesothelial cell growth
- Notch-1 is overexpressed in SV40 positive mesotheliomas
Hill criteria for causality: SV40/mesothelioma

- Strenght of association
- Consistency
- Specificity
- Temporal relationship

- ~ 50% in the USA
- 32 reports found SV40 using multiple techniques, 3 did not.
- The virus is present in the tumor cells
- 10% HM vs 50%MM
- MM increase followed poliovaccination 55-63
Hill criteria for causality: SV40/mesothelioma

- Biologic gradient
- Biologic plausability
- Coherence
- Unknown significance
- SV40 is the only complete carcinogen for HM and cause MM in hamsters
- Helps understanding mesothelioma in non-asbestos exposed
Hill criteria for causality: SV40/mesothelioma

- Experimental evidence
- In vitro and in vivo evidence supports a causal role for SV40
- Analogy
- Other DNA tumor viruses cause human tumors by inhibiting RB and p53. SV40 cause mesothelioma in hamsters.
Conclusions

• SV40 is present in some human tumors
• Multiple lines of evidence indicate that SV40 is a pathogen, not a passenger, when present in certain human cell types and that it contributes to tumor development
• It is likely that some SV40 comes from the contaminated poliovaccines