

## CBER research related to neoplastic cell substrates

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### Goal of presentation

- At 11/98 VRBPAC meeting, the need for additional research to answer fundamental questions was discussed
- Show how CBER research program is addressing issues associated with using neoplastic cell substrates

## Areas of research

- Risk of residual DNA
  - Study of potential tumorigenicity of injected oncogene DNA
  - Infectious risk
- PrP stability
- Adventitious agent detection

## DNA-related issues

- 1998 WHO requirements for the use of animal cells as in vitro substrates for the production of biologicals: 10 ng limit per dose
- Does not apply to: products given orally, products derived from microbial, diploid, or primary cell culture systems
- Tumor-derived cells were not considered

**ID<sub>50</sub>/TPD<sub>50</sub> of injected viral genomes**

<b>Virus</b>	<b>Animal model</b>	<b>Estimated ID<sub>50</sub> of DNA</b>	<b>Estimated TPD<sub>50</sub> of DNA</b>	<b>Estimated Risk per μg of DNA @ 1 genome /cell*</b>
SIV	monkey	~38 μg		2.5 x 10 <sup>-8</sup>
murine retrovirus	mouse	2.5 μg		3.8 x 10 <sup>-7</sup>
polyoma virus	mouse	4 ng	0.5 μg	1.2 x 10 <sup>-4</sup> 1 x 10 <sup>-6</sup>
herpes virus saimiri	marmoset		1-10 μg	10 <sup>-5</sup> - 10 <sup>-6</sup>
SV40	Syrian hamster		~2 μg	~ 10 <sup>-6</sup>

\*Assuming integrated genomic DNA is as infectious as linearized genomic DNA

## Calculation of “safe” residual DNA level based on worst case assumptions

- Assume cells contain viral genomes that are as infectious as polyomavirus DNA
- Assume each cell has 50 copies of infectious genomes
- Assume no degradation of cell DNA
- Assume viral genomic DNA is proportionally as infectious when incorporated in cellular DNA as when injected directly
- Then: 160 pg of residual DNA could be associated with one infection per million vaccinees

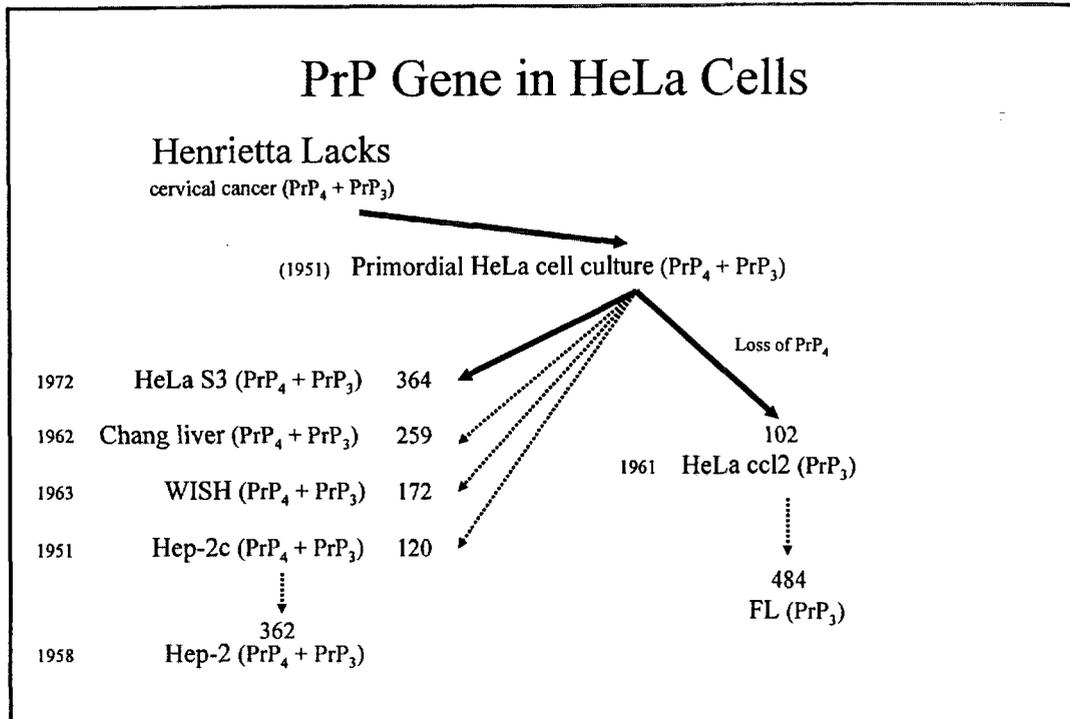
## Experiments to address safety of residual DNA

- Determine relative infectivity of integrated vs. non-integrated virus genomes
- Determine effect of DNA fractionation on infectivity of integrated vs. non-integrated virus genomes

## PrP-related issues

- According to prion hypothesis, mutations in PrP genes can lead to generation of infectious prions
- Greater tendency toward mutations among some neoplastic cells might increase likelihood of such mutations occurring
- To study PrP gene stability, PrP of different HeLa cell lineages were sequenced

## PrP Gene in HeLa Cells



## PrP study: conclusions

- HeLa progenitor cells were heterozygous in PrP C-terminal repeat region
- Different HeLa lines have different PrP sequences
- Sequence differences may have resulted from chromosome loss with repeated passage of HeLa cells
- No point mutations were observed
- Study implies potential value to sequencing PrP gene

## Adventitious Agent Issues

- Immortalized cells are presumed to have higher risk of containing oncogenic viruses
- This risk of oncogenic viruses may include viruses that have not yet been discovered
- There is a need to develop new methods to non-specifically detect viruses

## Non-molecular non-specific methods to detect viruses

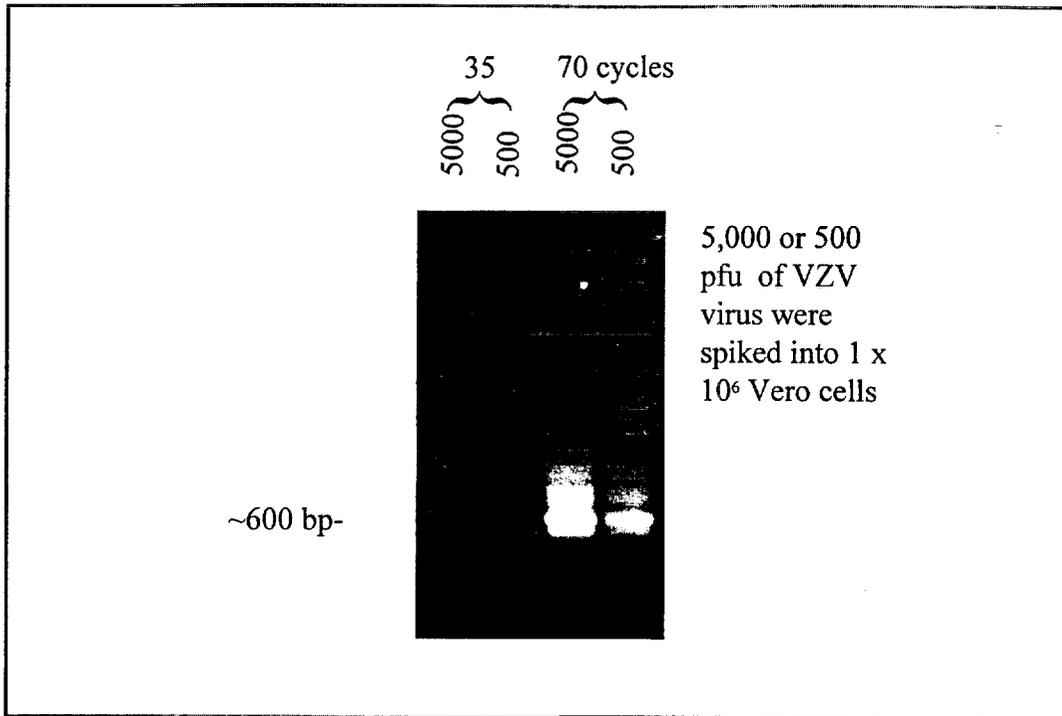
- Tissue culture
- Electron Microscopy
- Animal models
  - antibody production tests
  - adult & suckling mice
  - general safety tests
  - tumorigenicity endpoint

## Non-specific molecular methods for virus detection

- PCR-based RT assays
  - use in induction studies
- Use DNA chips to identify cellular response to infection
- Use consensus PCR primers to detect related viruses
- Non-specifically amplify viral nucleic acids

## Non-specific amplification of viral nucleic acids

- Use physical methods (nuclease, ultracentrifugation) to separate viral nucleic acids from cellular nucleic acids
- Use non-specific PCR methods to amplify resulting nucleic acids
- Clone & sequence PCR products



## Sensitivity of non-specific virus detection

Virus	Spiked into	Sensitivity
VZV	10 <sup>6</sup> cells	100 pfu
SCMV	10 <sup>6</sup> cells	100 TCID50
SV40	10 <sup>6</sup> cells	100 pfu
AAV	10 <sup>6</sup> cells	1000 pfu
Polio	Vaccine	~10 <sup>6</sup> IU*
Influenza	10 <sup>6</sup> cells	1:2048*

\*Lowest dilution tested

## Conclusion

- Ongoing research at CBER will help to further define risks associated with neoplastic cell substrates
- Investigators
  - Krause
  - Chumakov
  - Asher
  - Peden
  - Lewis
  - Khan