Evidence for a Tetraploid Intermediate in the Development of Cervical Cancer

David A. Eastmond, Andrew Olaharski, Maria Gonsebatt

University of California, Riverside and Universidad Nacional Autonoma de Mexico (UNAM)
Cervical cancer

- Second most common female malignancy worldwide:
  - ~ 500,000 new cases diagnosed annually
- Is the leading cause of death from cancer for women in many developing countries
- Is a relatively slow developing cancer with several well-defined, pre-neoplastic stages that can lead to invasive carcinoma
Mechanisms to develop aneuploidy: Definitions

- **Diploid (2n)**: 46 chromosomes
- **Tetraploid (4n)**: 92 chromosomes
- **Near diploid aneuploid cell (2n±1)**: 45 or 47 chromosomes
- **Near tetraploid aneuploid cell (4n±1)**: 91 or 93 chromosomes
Hypothesized relationship between cellular ploidy level and Pap smear classification

<table>
<thead>
<tr>
<th>Normal</th>
<th>Inflam.</th>
<th>ASCUS</th>
<th>LSIL</th>
<th>HSIL</th>
<th>Invasive carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Diploidy
- Aneuploidy
- Tetraploidy
Cervical Carcinogenesis Study

- Methods
  - 143 Mexican women with Pap smears representative of all diagnostic categories were recruited
  - Multiple probe fluorescence *in situ* hybridization (FISH) was used to simultaneously analyze 1000 cervical cells for numerical alterations in chromosomes 3 & 17
  - Questionnaires were also administered to obtain basic background information for the participating women
Patient recruitment and sample preparation

Instituto Nacional de Cancer, Mexico (MNCI)

A cytobrush is used to exfoliate cervical cells

Cytobrush is vortexed in Carnoy’s fixative in order to obtain residual cervical cells

Residual cervical cells are shipped in suspension to UCR where “secondary” Pap smears are made for cytogenetic (FISH) analysis

Diagnostic Pap smear to be analyzed by several pathologists at MNCI

Cytogenetic and diagnostic results are compared
Methodology: Interphase FISH

1. Denaturation
2. Application of labeled probe
3. Reanneal

Images showing fluorescently stained cells.
Relationship between Tetraploidy and Pap Smear Classification

Inflammation: p-value = 0.001
ASCUS: = 0.0006
LSIL: = 0.0011
HSIL: < 0.0001

N=39
Relationship between Aneuploidy and Pap Smear Classification

Inflammation: p-value < 0.0001
ASCUS: < 0.0001
LSIL: = 0.0013
HSIL: < 0.0001

# Hyperdiploid aneuploid cells/1000

Inflammation: p-value < 0.0001
ASCUS: < 0.0001
LSIL: = 0.0013
HSIL: < 0.0001
Origin of Micronuclei

Pancentromeric probe: used to characterize the origin of micronuclei
- Cen + chromosome loss
- Cen - chromosome breakage

human all-centromere probe
Frequencies of Micronucleated Cervical Cells by Diagnostic Category

![Bar chart showing frequencies of micronucleated cervical cells by diagnostic category.](chart.png)

- **Normal (n=24)**
- **Inflammation (20)**
- **ASCUS (1)**
- **LSIL (6)**
- **HSIL (23)**

Legend:
- **MN+ (chromosome loss)**
- **MN- (chromosome breakage)**
Correlation Between Micronuclei and Tetraploid Cervical Cells

P < 0.0001; R² = .67
Preferential Loss of Chromosome 17 in Near-tetraploid Aneuploid Cervical Cells

Near-tetraploid cervical cell exhibiting loss of cs 17

<table>
<thead>
<tr>
<th>Normal N=39</th>
<th></th>
<th>HSIL N=41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal or no loss 35/39</td>
<td></td>
<td>Normal or no loss 13/41</td>
</tr>
<tr>
<td>Cs 17 3/39</td>
<td></td>
<td>Cs 17 28/41</td>
</tr>
<tr>
<td>Cs 3 1/39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-value < 0.0001
HPV, chromosomal instability and cervical cancer

• Objective
  – Identify if infection with human papillomavirus is associated with the development of numerical chromosomal aberrations and the formation of micronuclei

• Methods
  – Nested PCR using a combination of the My09/11 and Gp5/6 primers to amplify HPV DNA
Tetraploidy is significantly associated with the presence of HPV

P-value < 0.0001
Chromosomal instability is significantly associated with presence of HPV

![Bar chart showing MN chromosomal breakage and loss, with P-values 0.001 and 0.016 for HPV Negative and Positive respectively.](chart.png)
Proposed Model for the Development of Aneuploidy in Cervical Cancer

Cervical cell infected with high-risk HPV type

HPV infection persists

Abrogation of key tumor suppressor proteins by the HPV E6 and E7 oncoproteins

Tetraploid cervical cell

Chromosomal loss (Cs 17) and breakage

Aneuploid cervical cell near-tetraploid

Development of micronuclei (formed through chromosomal loss and breakage)

Gain/losses of chromosomes (e.g. trisomy 3)

Cervical cancer near-tetraploid

Continued chromosomal loss and breakage

Cervical cancer near-diploid

Apoptosis

Regression
Summary

• The induction of tetraploidy and then aneuploidy appear to be intermediate steps in the development of cervical cancer. Two separate pathways appear to be involved.

• In ~55% of women diagnosed with a LSIL or a HSIL lesion, aneuploidy results from the loss of chromosomes from a tetraploid intermediate.

• Because tetraploidy is a transient and genomically unstable phenotype often observed to precede the development of aneuploidy, elevated levels of tetraploid cervical cells may be useful in identifying women that have an elevated risk of developing cervical cancer.
Summary

• Tetraploidy and aneuploidy are strongly associated with the presence of HPV in women suggesting that they occur as a consequence of HPV infection.

• This association suggests that environmental agents that interfere with immune function can increase the risks of developing cervical cancer. For example, increases in cervical cancer have been seen in women chronically using immunosuppressive drugs. Cigarette smoking is also associated with an increased risk of developing cervical cancer.
Acknowledgments

University of California, Riverside
• Drew Olaharski
• Leslie Hasegawa

UNAM/MNCSI
• Maria Gonsebatt, Gilberto Solorza-Luna, Rita Sotelo, Patricia Guzman, Alejandro Mohar

University of California Mexus Program