Smoking and cervical cancer health disparity

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Sanford Research/USD

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Over-view:

- Sanford Heath and Sanford Research/USD
Sanford Health and Sanford Research/USD

- Sanford Health is an integrated health system headquartered in the Dakotas and is now the largest rural, not-for-profit health care system in the nation with locations in 126 communities in seven states.
- Sanford Health includes 35 hospitals, 140 clinic locations and nearly 1,200 physicians in 70 specialty areas of medicine.
- With more than 25,000 employees, Sanford Health is the largest employer in North and South Dakota.
- The system is experiencing dynamic growth and development in conjunction with Denny Sanford's nearly $700 million in gifts, the largest ever to a health care organization in America.
- These gifts are making possible the implementation of the several initiatives including global children's clinics, multiple research centers and finding a cure for type 1 diabetes and breast cancer.

http://www.sanfordhealth.org/About
• South Dakota: Population of 824,000
• Native American: 8.9% (73,300 people)
Cervical Cancer

• Globally: Cervical Cancer is the 2\textsuperscript{nd} most common cancer among women
  – Per year, approximately 470,000 new cases, 233,000 deaths
  – Majority of cases occur in the developing world
  – In many developing countries, cervical cancer is the leading cause of cancer mortality in women
  – Screening programs have not been successfully established

• In the United States, during 2011
  – ~ 12,710 cases of invasive cervical cancer were diagnosed
  – ~ 4,290 women in the US died

• Strong correlation between infection with a high risk genotype of Human Papillomavirus and Cervical Cancer
• Development of the GARDASIL HPV vaccine (targeting HPV 16/18 and 11/6) prevents HPV infection and therefore reduces cervical cancer and genital warts caused by these genotypes of HPV
  – Promising, however, cervical cancer screening will still be important…

http://www.cancer.org
Cervical Cancer 1999–2008

Incidence Rates* by Race and Ethnicity, U.S.,

Mortality Rates* by Race and Ethnicity, U.S.,


Data from SD Department of Health (2007 to 2009).

Percentage of U.S. Women Aged 18 Years and Older Who Have Had a Pap Test in the Last 3 Years by Race and Ethnicity

Available data suggests a similar or higher rate of cervical cancer screening is obtained for American Indian women living in South Dakota.

HPV is more commonly detected in AI women and HPV Positive AI women have higher rates of abnormal PAP tests.
Smoking Rates

Prevalence of Smoking

Percent

Caucasian  AI

12%  49%
Prevalence of HPV Genotypes (% Based on Positives)

% of HPV infections

Caucasian  AI

% of HPV infections
Natural Progression of HPV Infection and a Role for Chemoprevention...

Maher et al, Advances in Gynecological Oncology; 2010

Control of Human Papillomavirus gene expression by transcription factors and the upstream regulatory region
• HPV E6/E7 from high risk HPV genotypes are required for the development of cervical cancer
  
  – **Most famous pathways**
  • E6 – degrades p53 (tumor suppressor)
  • E7 – interferes with retinoblastoma protein (tumor suppressor)

  – **Other important pathways**
  • E6 – activation of telomerase, degradation of proteins with PDZ domains (roles in cell signaling and adhesion)
  • E7 - up-regulation of AKT pathway, and interactions with various cell signaling molecules (cyclin A and E, p27, p21 etc.)

• Reducing the amount of HPV E6/E7 should be beneficial in interrupting the development of invasive cervical cancer.
Curcumin (diferuloylmethane)

Fig. 2. Schematic showing multiple biological activities of turmeric/curcumin. Maheshwari, et al. 2006. Life Sciences.
Curcumin treatment suppresses cervical cancer cell growth in monolayer and organotypic raft culture systems

A. % Change with varying concentrations of curcumin for different cell lines.

B. Clonogenic potential of Caski and SiHa cells with different concentrations of curcumin.

C. DMSO Control, 20 µM Curcumin, 40 µM Curcumin.

D. Collagen images showing effect of curcumin treatment.

A PLGA nano-formulation of Curcumin effectively suppresses cervical cancer cell growth

Micromolar concentrations

MTS assay 24 hours after treatment
Curcumin treatment induces apoptosis in cervical cancer cells via caspase-mediated signaling.

![Image A: 24 hr DMSO, 10 µM, 20 µM, 40 µM Curcumin](image1)

![Image B: 24 hr 7AAD Staining](image2)

![Image C: 24 hr PARP, Caspase 3, Caspase 9, β-actin](image3)

* p<0.05


D = DMSO
Curcumin treatment represses the expression of HPV oncogenes E6 and E7

RNA: 6 hr after addition of curcumin

* \( p < 0.005 \)


D = DMSO
Curcumin treatment represses the activity of the HPV16 URR (upstream regulatory region)

**HPV 16 URR**

Plasmid Transfection (48 hr)

- Curcumin Treatment

- Collect supernatant and analyze for Luciferase activity

- Normalize data to control plasmid (Tk-CLuc) and DMSO

**HPV URR activity in cervical cancer cells**

(25 µM Curcumin for 6 hr)
Curcumin restores the expression of tumor suppressor proteins: p53, Rb, PTPN13 (each known to be degraded when HPV E6 or E7 is expressed)


D = DMSO
REL: relative expression level
Curcumin treatment inhibits the motility of cervical cancer cells

Curcumin treatment inhibits the motility of cervical cancer cells. Curcumin treatment inhibits the motility of cervical cancer cells. **p<0.005**

Smoke Carcinogen: Benzo[a]pyrene (BaP)

- Cigarette smoking is known to be a risk factor for cervical cancer
  - However, the molecular link between smoking and HPV is unknown
- BaP is a polycyclic hydrocarbon. These compounds are generated by burning carbon containing materials, such as:
  - Tobacco, Charbroiled Meat, Fried Food (especially when the same oil is used repeatedly, Wood
- BaP is detected in cervical mucus of women who smoke

![Prevalence of Smoking Graph]

- 12% Caucasian
- 49% AI
Tobacco smoke compound BaP up-regulates the expression of HPV oncogenes, NFκB and AP1 but is suppressed by curcumin treatment

Schematic model of increased oncogenic signals via HPV E6/E7 oncoproteins and molecular effect of curcumin on HPV associated cellular events.

**Top:** HPV oncoproteins degrade tumor suppressor proteins, increasing the risk of developing cancer. BaP increases the expression of HPV oncoproteins, potentially increasing oncogenic signals and even in the presence of only a few copies of HPV genome.

**Bottom:** Curcumin specifically inhibits the expression of HPV oncoproteins, even in the presence of BaP, thereby reducing the oncogenic signals and potentially inhibiting cancer development.

BaP exposure activates the AhR pathway increasing CYP1A1 expression

Representative data from 3 independent experiments
AhR is aberrantly expressed and localized in pre-neoplastic and invasive cervical cancer tissues.

Normal (n=10), cervical intraepithelial neoplasia I (CIN I, n=10), CIN II (n=10), CIN III (n=6), and invasive cervical cancer (n=15).
Development of an orthotopic cervical cancer mouse model

A) Stable tdTomato expression in cervical cancer cells

Red Fluorescence

B) *In vivo* detection of tdTomato expressing cervical cancer cells

<table>
<thead>
<tr>
<th>WT</th>
<th>10 x 10^6 cells</th>
<th>2.5 x 10^6 cells</th>
<th>1.25 x 10^6 cells</th>
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Phase Contrast

14767. 38133. 61499. 84665. 108231.
Growth of orthotopic cervical tumors

Mouse injected with 2.5x10^6 TdTomato expressing cells

Control Mouse

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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<td><img src="week3.png" alt="Image" /></td>
<td><img src="week4.png" alt="Image" /></td>
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Mean Fluorescent Intensity

- Week 1: 27426.10
- Week 2: 45530.38
- Week 3: 69534.85
- Week 4: 81738.82
- Week 5: 99843.20

Mouse injected with 2.5x10^6 TdTomato expressing cells
Cervical Cancer Orthotopic Mouse Model
Treatment of orthotopic cervical tumors

Mice were treated by intratumoral injection 2x’s per week
BaP increases oncogenic signals from HPV infection
Curcumin may inhibit this signaling in vivo
Smoke exposure may modulate the local immune response, favoring persistent HPV infection and development of cervical cancer
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