Tissue tumor marker expression in normal cervical tissue and in cervical intraepithelial neoplasia, for women who are at high risk of HPV (Human papilloma virus infection).

Raghad Samir
MD PhD
Verksamhet chef IVF
Falun
Introduction

• Etiology, co-factors and risk factors
• Cervical intraepithelial neoplasia, grade
• Tumor markers definition, mode of action

The aims

Material and methods

Results

Conclusions

• Strengths and weaknesses
• Future research directions
ETIOLOGY AND CO-FACTORS

- Infection with HR-HPV.

- Co-factors:
  - Smoking
  - Contraceptive use
  - Sex steroids
  - Genetic predilection
RISK FACTORS

- Age
- HIV/Aids, immunodeficiency
- Poor socioeconomic status
- Poor access to health services
- Multiple sex partners
- High parity
HUMAN PAPILLOMA VIRUS
(HPV)
HPV AND CERVICAL CANCER

The epidemiological and clinical studies have clearly demonstrated that HR-HPV are the major etiologic agents of neoplasia of the cutaneous and mucosal epithelia.

HR-HPV positivity in cervical cancer is estimated to be between 90% and 95% (zur Hausen, 1991; Munoz, 2003).
Infection of metaplastic epithelium in TZ* with HR-HPV

Persistent infection with oncogenic HPV

Progression of the persistent infection to precancerous changes

Invasion through the basement membrane

* Transformation zone
CERVICAL INTRAEPITHELIAL NEOPLASIA

- CIN is used to describe the histological changes detected with biopsy and it is divided into three degrees of severity:
  - CIN 1
  - CIN 2
  - CIN 3
- Borderline lesions are called ASCUS (atypical squamous cells of undetermined significance).
Another terminology which is used to describe these changes at the cytological and histopathological level uses the terms LSIL and HSIL.

- LSIL (Low squamous intraepithelial lesion) = CIN1
- HSIL (High squamous intraepithelial lesion) = CIN2 +
TUMOR MARKERS
DEFINITION

The National Cancer Institute (NCI), defines biomarker as:

“A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease.”
Tumor markers are biochemical substances elaborated by tumor cells either due to the cause or effect of malignant process.

These markers can be normal endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells.
Tumor markers

- Etiology
- Pathogenesis
- Detection
- Disease

- HR-HPV infection
- Latency
- Precancerous lesion
- HSIL
- Cervical cancer
- Cofactors
- Risk factors
- Diagnosis and screening
### Tumor Markers and Mode of Action

<table>
<thead>
<tr>
<th>Category</th>
<th>Marker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation</td>
<td>Ki-67, EGFR</td>
</tr>
<tr>
<td>Tumor suppressor</td>
<td>P53, P16, Rb, FHIT</td>
</tr>
<tr>
<td>Cell-cell adhesion</td>
<td>E-Cadherin</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Cox-2</td>
</tr>
<tr>
<td>Immunological marker</td>
<td>IL-10</td>
</tr>
<tr>
<td>Onco-protein</td>
<td>C-myc</td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>CK-10</td>
</tr>
<tr>
<td>Immune defense</td>
<td>CD4+</td>
</tr>
</tbody>
</table>
TUMOR SUPPRESSORS

The main tumor suppressors that regulate the cell cycle are:
- P53
- Rb
TUMOR SUPPRESSOR PROTEINS AND HR-HPV ONCO-PROTEINS

E6 → P53 → abnormal cell division

E7 → Rb → Immortal cells
The aims of the Thesis
To study whether the epidemiological findings of a correlation between smoking, sex steroids and contraceptive, and CIN are reflected in the expression of tissue tumor markers, thus supporting a biological role of these co-factors.
Materials and methods
MATERIALS AND METHODS:

The study population comprised 228 women of whom 188 were recruited at our colposcopy clinic for laser cervical conisation or a directed punch biopsy on indication of:

- Vaginal smear that showed CIN
- Vaginal smear suggesting CIN
- Smears repeatedly showing ASCUS.

In addition 40 healthy volunteers in fertile ages and with normal Pap smears were recruited.
MATERIALS AND METHODS:

- The mean and median age of the entire study population were 36.6 years and 34.0 years, respectively.

- These samples were collected between 2005 - 2007.
MATERIALS AND METHODS:

A structured questionnaire included:

- Date of birth, age, last menstruation, cycle day, menopausal status, history of abnormal Pap smear, contraceptive use if any, and present or past smoking (cigarettes per day and duration).

- Blood samples were collected (serum cotinine, serum progesterone, serum estradiol).
The results
High-risk HPV infection and CIN grade correlates to expression of c-myc, CD4+, FHIT, E-cadherin, Ki-67 and p16\textsuperscript{INK4a}

Article I
Sufficient and representative material was available from 116 women for analysis of HR-HPV.

64 (55.2%) were positive for one or more of the 12 HR-HPV subtypes.
- 94 (82.5%) were in pre-menopausal status, HR-HPV positive biopsies were found in 59.6% of them.

- 20 women (17.5%) were in post-menopausal status, HR-HPV positive biopsies were found in 35.0% of them.
CONCLUSIONS, PAPER I

- Ki-67 (tumor proliferative) was the only marker that independently predicted both the presence of HR-HPV and the severity of cervical lesions.
Tissue tumor marker expression in smokers, including serum cotinine concentrations, in women with cervical intraepithelial lesion.
83 (36.9%) of the women were smokers, defined as daily smoking and serum cotinine levels above 12 ng/mL, and 142 (63.1%) women were non-smokers.

There were no findings of measurable serum cotinine in the latter group.
Smoking seems to be associated with a negative molecular expression pattern.

This was true for a lower tumor suppressor expression, p53 and FHIT, and a higher Cox-2 and Ki-67 expression, associated with tumor proliferation, among smokers than nonsmokers. This was particularly true in women in fertile age.
Oral contraceptive and progestin-only use correlates to tissue tumor marker expression in women with cervical intraepithelial neoplasia

Article III
195 women were included in this study:
- 57 COC (combined oral contraception) users.
- 15 MID (medicated intrauterine device) users.
- 24 systemic progestin-only contraceptive users.
- 99 nonusers.
THE HISTOPATHOLOGICAL DIAGNOSIS IN THE WHOLE STUDY.

- CIN including borderline cases was diagnosed in 121 (62.1%) women, while in 74 (37.9%) women the cervical epithelium was considered normal.
The biological reason for the strong association between COC use and increased \textbf{Cox-2} expression is unclear, but might be an unfavourable finding in the light of the reports on invasive cancer.

Increased \textbf{p53 expression} in the progestin-IUD (MID) users compared to nonusers was an unexpected finding which may indicate a protective effect.
Increased levels of serum progesterone and estradiol correlate to increased Cox-2 tissue expression in cervical intraepithelial neoplasia
- The study population comprised 80 women.

- Sixty women were consecutively recruited from the out-patient surgery, Department of Obstetrics and Gynecology, Falun County Hospital.

- 20 healthy volunteers.
Inclusion criteria were premenopausal status, regular menstruation, and no oral contraceptive or progestin-only contraceptive use.

Menstrual cycle phase did not influence the recruitment.
CONCLUSION PAPER IV

- Serum levels of progesterone and estradiol appear to correlate with increased COX-2 expression in CIN.
Conclusions
Ki 67 (proliferation factor) expression could be used as a surrogate marker for HPV infection.

A high Cox 2 expression (proliferation, angiogenesis, Inflammation) was found in fertile smokers, oral contraceptive users, and in group with elevated serum progesterone and estradiol levels.
The finding of increased expression of p53 (tumor suppressor) in the progestin IUD users compared to non-user is interesting and required further studies.
Final conclusions
This study shows the possibility of using certain tumor markers as diagnostic or prognostic indicators in the development of cervical intraepithelial neoplasia.

The negative influence of HPV infection, smoking, serum progesterone as well as oral contraceptive use on the development of cervical intraepithelial neoplasia was confirmed.
FUTURE RESEARCH DIRECTIONS

HPV-Vaccine.

HPV test as primary screening test.

Scoring system:
Age (HRT and post-menopausal status).
Genetic/family history,
Smoking.
Contraception.
Future studies should involve additional biological markers, of possible importance in CIN and invasive cervical cancer.
Thank you!