Recent advances in adenosquamous lesions of the cervix

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OUTLINE

• To describe morphology and diagnostic criteria of cervical adenosquamous carcinoma and mimickers
• To discuss useful tools for differential diagnosis
• To provide information on etiology, clinical features and outcome of cervical adenosquamous lesions in comparison with other cervical malignant tumors
Introduction

WHO 2014

• Squamous cell carcinoma invasive/HSIL
• Glandular carcinoma invasive/In situ adenocarcinoma
• Adenosquamous carcinoma/In situ adenosquamous carcinoma
• Others
Adenosquamous carcinoma (AS)

• First described as mixed carcinoma by Glucksmann and Cherry in 1956

• The term „adenosquamous” was introduced by Greene in 1963

• WHO 2014: malignant epithelial tumor composed of a mixture of adenocarcinoma and squamous cell carcinoma (both invasive)

• However the cutoff is missing
Adenosquamous carcinoma

• Historically, glassy cell carcinoma and mucoepidermoid carcinoma—considered malignancies lying within the spectrum of AS

• WHO 2014: glassy cell carcinoma as an AS subtype

• WHO 2014: mucoepidermoid carcinoma only for rare lesions identical to those occurring in the salivary glands, containing squamous, intermediate and mucin-producing cells
Adenosquamous carcinoma

• In practice: tumors historically diagnosed as AS appear to represent a spectrum of lesions, some of which do not exhibit definitive malignant squamous and/or glandular differentiation

• This degree of architectural and cytologic heterogeneity has led to variable reported AS prevalence

• AS prevalence: 2 to 50% of all invasive cervical carcinomas
Adenosquamous carcinoma

• Different morphology
• Different etiology (associated rate with HPV infection)
• Different clinical presentation at diagnosis
• Different clinical outcome
• Different management
IECC study: 462 cases of ECA and adenosquamous carcinoma

53 cases excluded

Missing blocks, only biopsies, few slides, reclassified (in situ, pure squamous, endometrial and uterine adnexal adenocarcinomas involving cervix, carcinosarcoma)

409 cases included for morphology (65 cone and 344 hysterectomies)

354 cases excluded being ECAs (IECC)

59 cases excluded being adenosquamous carcinoma, glassy cell carcinoma and mimickers
Definitions
Pure AS: unequivocal invasive malignant glandular and squamous differentiation, each component representing at least 10% of the tumor
Glassy cell carcinoma: >90% of tumor cells with sharp cytoplasmic margins, “ground glass” eosinophilic cytoplasm, large round/ovoid nuclei, prominent nucleoli
Mucoepidermoid carcinoma: nests of three cell types (squamoid, intermediate and mucin-producing)
HPV-associated ECA with benign squamous metaplasia: not considered as AS
Pure stratified mucin-producing carcinomas (iSMILE):
>90% iSMILE morphology; not considered as AS
iSMILE with component: >10% but <90% iSMILE morphology, in a background of usual, mucinous, AS type; not considered as AS
Stolnicu et al., Mod Pathol, 2018: Study results

Total cohort: 409 cases
354 cases excluded: endocervical adenocarcinomas

59 cases included: adenosquamous carcinomas, glassy cell carcinomas, and mimickers

- Pure adenosquamous carcinoma: 34 cases
- Glassy cell carcinoma: 2 cases
  - Immunohistochemical stains: reclassified as adenocarcinoma not otherwise specified
- Pure invasive stratified mucin-producing carcinoma: 9 cases
- Invasive stratified mucin-producing carcinoma with components: 10 cases
- Usual or mucinous endocervical adenocarcinoma with squamous metaplasia: 4 cases
Pure AS

• None of the patients with AS were pregnant (or recent history of pregnancy)
• Mean age: 46y; median age: 44y
• Clinically: patients presented with painless vaginal bleeding and discharge
• Macroscopically: similar to ECAs (ulcerated, nodular or polypoid firm mass)
• FIGO stage I: 65%, stage II: 32%, stage III: 3%
Pure AS

- 50% of AS were high-grade microscopically
- LVI in 76%
- 29% of cases with LNM
- Pelvic metastasis occurred in one patient (to the ovary)
- 65% had a precursor lesion: AIS, HSIL, a combination or SMILE
Associated with HSIL and AIS
AS immuno profile

Of interest:

• Expression of squamous (p63, p40, CK5) and glandular (mucins) markers may vary, particularly in poorly differentiated lesions

• Markers can highlight benign elements admixed with the tumor (areas of entrapped benign glands or squamous metaplasia)

• Caution in their interpretation is advised
Mimics
Glassy Cell Carcinoma of the Uterine Cervix: Histochemical, Immunohistochemical, and Molecular Genetic Observations

Noriko Kato, M.D., Yousei Katayama, M.D., Mitsuomi Kaimori, M.D., and Teiichi Motoyama, M.D.

- Described by Glucksmann and Cherry in 1956
- Poorly differentiated AS
- Electronic microscopy: evidence of glandular differentiation and squamous (less obvious)
- Aggressive tumor, in association with pregnancy; poor response to radiation/surgery
- Another theory: multipotential stem or reserve cell

### TABLE 2. Expression of mucin

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mucin subtype</th>
<th>Mucin core protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC (n = 3)</td>
<td>+ (Sialo)</td>
<td>++</td>
</tr>
<tr>
<td>ASC (n = 4)</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Glandular component</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Nonkeratinizing SCC (n = 5)</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Mucinous AC, endocervical type</td>
<td></td>
<td>++ (Sulfo &gt;&gt; Sialo)</td>
</tr>
<tr>
<td>Nonneoplastic cervix (n = 10)</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Endocervical glands</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Reserve cells</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Metaplastic squamous cells</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Mature squamous epithelium</td>
<td></td>
<td>++</td>
</tr>
</tbody>
</table>

GCC, glassy cell carcinoma; ASC, adenosquamous carcinoma; SCC, squamous cell carcinoma; AC, adeno-carcinoma.

++, diffusely positive; +, focally positive; −, negative.
Sialo, sialomucin; Sulfo, sulfomucin; Neut, neutral mucin.
Glassy cell carcinoma

- Glassy type: 2 cases reinterpreted as poorly differentiated ECAs: p63 and p40 were both negative
- Both cases were positive for p16 and HPV
Poorly differentiated ECAs: p16 and HPV+

p16 positive

HPV positive
Glassy cell carcinoma

• No squamous differentiation
• Poorly differentiated ECAs
• All are HPV +
• Should be managed as HPVAs
• Further studies are needed to elucidate this rare entity
• Glassy cell carcinoma should be deleted from the future WHO classification
Stratified Mucin-Producing Intraepithelial Lesions of the Cervix: Adenosquamous or Columnar Cell Neoplasia?

Park, Jeong-Ja; Sun, Deqin; Quade, Bradley; Flynn, Cythia; Sheets, Ellen; Yang, Annie; McKeon, Frank; Crum, Christopher

AJSP 24(10):1414-1419, October 2000

• Intraepithelial lesion with stratified cells similar to HSIL
• Varying proportions of intracytoplasmatic mucin vacuoles
• Mucicarmine highlights mucin throughout
• Presence of mucin produces spaces between nuclei
• Mitotic figures, apoptotic bodies
• Absence of classic gland formation (AIS)
• In situ adenosquamous lesion or AIS variant?
• Hybrid morphology
• 9/18 (50%) associated with invasive carcinoma
  • Adenocarcinoma (n=4)
  • Adenosquamous carcinoma (n=5)
  • SCC (n=1)

Marker of phenotypic instability
Coexistence with other findings:
- 7 cases of SMILE associated with invasive ca
- 6 SCC, 1 Adeno
- AIS present in 42%
- HSIL present in 93%

IHC
- p16 diffuse strong, ki67 index high
- p63 and p40 diminished (distinct from HSIL)
- IMP3 negative (closer to SIL than AIS immunophenotypically)

Fluidity of differentiation in SMILE
- “Reserve cell dysplasia” preferred, management like AIS
Invasive Stratified Mucin-producing Carcinoma and Stratified Mucin-producing Intraepithelial Lesion (SMILE) 
15 Cases Presenting a Spectrum of Cervical Neoplasia With Description of a Distinctive Variant of Invasive Adenocarcinoma

Ricardo R. Lastra, MD,** Kay J. Park, MD,‡ and J. Kenneth Schoolmeester, MD§§

iSMILE is characterized by

- Nests of stratified columnar cells
- Mucin/clear vacuoles throughout thickness (variable in amount)
- Peripheral palisading
- Bland round/ovoid nuclei without prominent nucleoli
- Apoptotic bodies and mitoses
- Neutrophilic infiltrate

8 invasive SMILE (mean and median: 44y): 7 pure and 1 associated with usual ECA (Lastra, 2016)
2 pure iSMILE
1 case of iSMILE associated with SCC
SMILE is a precursor of iSMILE
iSMILEs and SMILEs were + for HPV (16 and 18)
• All iSMILEs were + for p16, CAM 5.2 and focally + for IMP3
• iSMILEs were – or focally + for p63 and CK5/6
• Electron microscopy: a stratified structure with mucin vacuoles, abundant mitochondria, no tonofilaments (suggesting glandular differentiation)
• 5 additional cases of iSMILE
• Large tumor size with polypoid exophytic appearance
• All developed pelvic recurrences during a mean time of 8 months
• 2 patients with distant metastases (one with lung and one with inghinal lymph nodes, the liver and skin)
• 4 out of 5 died of disease while one patient is alive with disease
Survival in iSMILE; Horn, 2019

- Lastra, 2016: 3/8 cases developed distant tumor recurrence
- Onishi, 2016: all 3 patients with iSMILE were alive NED (59.3 months)
- Horn, 2019: iSMILEs associated with pelvic LNM; may represent an aggressive tumor with early recurrent disease and risk of distant metastatic disease (lungs)
- Hodgson, 2019: 50% of iSMILEs developed recurrence (2 local and 2 distant recurrence), 25% died of disease; iSMILEs had significantly worse DFS and DSS compared with other HPVA types
iSMILE

• Architectural diversity
• Various cytologic features
• Extravasated mucin
• Hyaline-like globules
• Pure or mixed
Pure iSMILE: insular architecture
Pure iSMILE: glandular architecture
Pure iSMILE: solid architecture
Pure iSMILE: papillary architecture
Pure iSMILE: trabecular architecture
iSMILE with micropapillary features
Pure iSMILE: isolated tumor cells
iSMILE with eosinophilic cytoplasm
iSMILE with cytoplasmic clearing
iSMILE with “glassy cell” like features
iSMILE with bizarre nuclei
iSMILE with signet ring-like cells
iSMILE with squamoid differentiation
iSMILE with extravasated mucin
iSMILE with hyaline-like globules
iSMILE with components (usual type ECA)
iSMILE with components (neuroendocrine carcinoma)
Stolnicu, 2019, under review

- 52 cases of iSMILE reviewed by experienced pathologists
- 29 pure invasive SMILEs (56%) and 23 iSMILE mixed with other components (44%) were identified
- 13 cases were associated with UEA
- 6 cases with ASC
- 3 were associated with MUC
- 1 was associated with NEC
iSMILE differential diagnosis

• poorly differentiated usual or mucinous type adenocarcinoma (both of HPVA subtypes)
• serous adenocarcinoma or clear cell adenocarcinoma (both of NHPVA subtypes)
• adenosquamous carcinoma
• squamous cell carcinoma
iSMILE

p16: 62.5%

HPV: 100%
<table>
<thead>
<tr>
<th>Marker</th>
<th>Pure invasive stratified mucin-producing carcinomas n (%)</th>
<th>Adenosquamous carcinomas n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV*</td>
<td>8/8 (100)</td>
<td>19/23 (82.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P16</td>
<td>5/8 (62.5)</td>
<td>18/25 (72)</td>
<td>0.1757</td>
</tr>
<tr>
<td>PAX8*</td>
<td>2/7 (28.5)</td>
<td>10/23 (43.4)</td>
<td>0.0382</td>
</tr>
<tr>
<td>p40*</td>
<td>2/7 (28.5)</td>
<td>15/23 (65.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p63*</td>
<td>3/8 (37.5)</td>
<td>15/25 (60)</td>
<td>0.0018</td>
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<tr>
<td>PR</td>
<td>2/8 (25)</td>
<td>6/25 (24)</td>
<td>1.0000</td>
</tr>
<tr>
<td>AR</td>
<td>0/8 (0)</td>
<td>0/25 (0)</td>
<td>1.0000</td>
</tr>
<tr>
<td>CAIX</td>
<td>4/7 (57.1)</td>
<td>16/23 (69.5)</td>
<td>0.4022</td>
</tr>
<tr>
<td>MUC6*</td>
<td>4/7 (57.1)</td>
<td>5/23 (21.7)</td>
<td>&lt;0.0001</td>
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<tr>
<td>HIK1086</td>
<td>0/8 (0)</td>
<td>1/23 (4.3)</td>
<td>0.1212</td>
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<tr>
<td>HNF-1beta*</td>
<td>1/7 (14.2)</td>
<td>7/23 (30.4)</td>
<td>0.0023</td>
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<tr>
<td>GATA3</td>
<td>1/7 (14.2)</td>
<td>2/23 (8.7)</td>
<td>0.2747</td>
</tr>
<tr>
<td>Vimentin*</td>
<td>1/8 (12.5)</td>
<td>0/25 (0)</td>
<td>0.0003</td>
</tr>
<tr>
<td>HER2</td>
<td>1/8 (12.5)</td>
<td>1/25 (4)</td>
<td>0.0652</td>
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<tr>
<td>SATB2</td>
<td>0/8 (0)</td>
<td>0/23 (0)</td>
<td>1.0000</td>
</tr>
<tr>
<td>CDX2</td>
<td>0/8 (0)</td>
<td>0/23 (0)</td>
<td>1.0000</td>
</tr>
<tr>
<td>p53*</td>
<td>2/7 (28.5)</td>
<td>2/25 (8)</td>
<td>0.0004</td>
</tr>
<tr>
<td>CK7*</td>
<td>8/8 (100)</td>
<td>20/23 (86.9)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

*Statistically significant differences
**Immunohistochemical profile in iSMILE**

- p40 and p63 less often positive, patchy pattern, typically in peripheral cells
- PAX8 less frequently positive
- The expression of p63/p40 and PAX8 in iSMILE suggests that it is likely to arise from HPV-infected reserve cells, retaining pluripotential ability
- More frequent aberrant p53 staining (28.5% vs 8%) (although p53 mutation not demonstrated by recent study- USCAP 2019)
Molecular genetic pathways in iSMILE

Are currently being investigated

Parra-Herran (USCAP, 2018):
- higher mutational burden in the tumors with adverse outcome compared to indolent tumors
- 2 of 5 cases of iSMILE (40%) presenting with STK11 alterations

IMPACT study (MSKCC): multiple genes amplified, deleted, mutated and gene rearrangement (STK11 and KMT2D) in iSMILEs cases (unpublished data)

May be biologically different from other HPV-associated adenocarcinomas
Mucoepidermoid carcinoma

• No cases with a morphology suggesting mucoepidermoid carcinoma in our study
• Mucoepidermoid carcinoma of the major and minor salivary glands harbours a characteristic t(11;19)(q21;p13) chromosomal translocation
• Similar molecular abnormalities have been demonstrated in a small number of cervical tumours which are morphologically similar
• These molecular abnormalities were not found in cervical adenosquamous carcinomas
• Cervical mucoepidermoid carcinoma is an entity distinct from conventional adenosquamous carcinoma

McCluggage, 2012, Histopathology
Other mimics of AS

- HPVA usual-type (with benign squamous metaplasia)
- Endometrioid ECA with squamous differentiation (very rare in the cervix!)
- Clear cell carcinoma (if glycogen rich)
- Adenoid-basal cell carcinoma- if glycogen rich (no atypia, bland morphology)
- SCC- if intracellular mucin/glycogen-rich clear cells (no glandular component)
- A collision tumor with SCC and ECA (both components, not admixed, very rare!)
- Secondary involvement by endometrioid endometrial adenocarcinoma with squamous differentiation

Loureiro, Hirschowitz, Russel
Older literature suggested that AS has a worse OS and DFS than ECA and SCC.


Advanced stage AS also has worse prognosis than ECA and SCC

**Adenosquamous Histology Predicts a Poor Outcome for Patients with Advanced-Stage, but Not Early-Stage, Cervical Carcinoma**

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Jay W. Carlson, M.D.²
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Edward R. Kost, M.D.⁴
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**BACKGROUND.** The objective of this study was to compare survival between patients with adenosquamous carcinoma and patients with adenosquamous carcinomas of the cervix.

**METHODS.** Patients who were diagnosed with invasive cervical carcinoma from 1988 to 1999 were identified from the Automated Central Tumor Registry for the United States Military Health Care System. Clinical data, including age at diagnosis, histology, tumor grade, disease stage, lymph node status, treatment modality, and survival, were collected. Survival analysis was performed with Kaplan-Meier survival curves and compared using the log-rank test.

**RESULTS.** A total of 273 women were identified, 185 women with a histologic diagnosis of adenosquamous carcinoma (AC) and 88 women with a diagnosis of adenosquamous carcinoma (ASC). Among the women with ASC, only 5% had Grade 1 tumors, and 66% had Grade 3 tumors. By comparison, among the women with AC, 27% had Grade 1 tumors, and 26% had Grade 3 tumors (P < 0.001). There was no difference in the incidence of positive lymph nodes or in the number of patients who underwent radical hysterectomy as primary treatment between patients with ASC and patients with AC. More patients with ASC received radiation therapy (51% vs. 28%) or chemotherapy (23% vs. 12%) as treatment (P < 0.001). Patients who had tumors with ASC histology had a significantly decreased 5-year survival rate compared with patients who had tumors with AC histology (65% vs. 83%; P < 0.002).

When patients with early-stage cervical carcinoma (International Federation of Gynecology and Obstetrics Stage I) were examined separately, there was statistically significant difference in the 5-year survival rate (AC, 89%; ASC, 86%; P = 0.044). However, when patients with advanced-stage disease (FIGO Stages II–IV) were analyzed, ASC was associated with a significant decrease in median and overall survival (P = 0.01). When the results were analyzed by grade, patients who had tumors with AC histology had a shorter survival compared with patients who had AC histology of any grade; however, this was a significant difference only for patients with Grade 1 tumors: The 5-year survival rate for patients with Grade 1 AC was 93%, compared with 50% for patients with Grade 1 ASC (P < 0.01).

**CONCLUSIONS.** ASC histology appears to be an independent predictor of poor outcome in women with cervical carcinoma compared with their counterparts who have pure AC. The significant decrease in survival was observed only in patients with advanced-stage cervical carcinoma. This decreased survival may be related mainly to the grade of ASC. Cancer 2003;97:1996–202.

Published 2003 by the American Cancer Society.

DOI: 10.1002/jcc.11371


Address for reprints: John H. Farley, M.D., Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Tripler Army Medical Center, 1 Jarrett White Road, TMC, Honolulu, HI 96859-5000. Fax: (808) 433-1552; E-mail: john.farley@amedd.army.mil.

The views expressed herein are those of the authors and do not reflect the official policy or opinion of the Department of Defense, the United States Army, or the United States Navy.
More recent literature suggests that there is no difference between survival in SCC, ECAs and AS carcinomas especially in early stage treated with radical surgery.

Cervical cancer- does the morphological subtype affect survival rates? Emmett M et.al; J Obstet Gynaecol, 2018

Stolnicu, 2018: Analysis of OS between AS, iSMILE without/with components and usual type ECAs
Stolnicu, 2018: Analysis of DFS between AS, iSMILE without/with components and usual type ECAs
Conclusions

• AS carcinoma of the cervix is rare
• All linked with HPV infection
• Glassy cell carcinoma should be removed from WHO classification
• Mucoepidermoid is not part of the spectrum of AS
Conclusions

• The great mimickers (HPVA with benign squamous metaplasia, pure iSMILE and iSMILE with components) should not be regarded as AS (no malignant squamous component)

• IECC 2019 (including iSMILE) diagnostic criteria should be used

• No differences in outcome between all these entities but differences in origin, biology, morphology and immunohistochemical profile
Thank you