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Estrogen receptor alpha and ADAM9 expression in CIN lesions and cervical cancer

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Cervical cancer is one of the most frequent cancers in women and develops from premalignant lesions called cervical intraepithelial neoplasia (CIN1, CIN2, CIN3). It is now generally accepted that HPV plays a central role in the development of premalignant and malignant lesions of the uterine cervix. However, HPV infection alone is not sufficient for the development of cervical cancer and other cofactors are necessary for the malignant transformation. The cervix is a steroid-hormone-dependent tissue, and malignant tumors arising from the cervical epithelium may therefore be estrogen dependent. The estrogen receptor alpha (ERα) content of breast carcinomas is an important prognostic and predictive biomarker but little is known about the influence of estrogens on the malignant transformation of the cervix. Data on the role of ER in cervical carcinoma are controversial. ADAM9 is a member of the ADAM family which is involved in cellular processes such as cell adhesion, migration, and signaling. ADAM9 overexpression has been described in many solid tumors. There is some evidence that matrix metalloproteinases (MMPs) could be involved in cervical carcinogenesis. Since MMPs and the ADAMs are members of the same metzincin superfamily, it may therefore be speculated that ADAM9 is also involved in cervical carcinogenesis, but its role in cervical neoplasia has not been yet investigated. The aim of the present study was to evaluate the expression of ERα and ADAM9 in samples from the normal cervical epithelium, CIN3 and carcinoma of the cervix. For ADAM9, immunohistochemical (IHC) expression was studied in archived paraffin-embedded tissue blocks from biopsy or surgery specimens obtained from 50 subjects (36 with CIN3 lesions and 14 with squamous cell cancer (SCC) of the cervix); 44 cases of normal squamous epithelium and 49 cases of glandular cervix epithelium were analyzed as normal controls. IHC staining for ERα was

performed in 123 paraffin-embedded specimens of normal stratified squamous epithelium and 149 specimens of pathological epithelium of the uterine cervix (94 of CIN3, 46 of SCC and 9 cases of adenocarcinoma). A total of 38 samples (23 of the CIN3 and 15 of invasive cervical cancer (13 SCC and 2 adenocarcinoma) were analyzed by real-time quantitative PCR (RT Q-PCR) for ERα gene (*ESR1*) amplification.

Strong or moderate intranuclear staining for ERα was detected in the basal, parabasal, and intermediate layer in almost all specimens of normal stratified squamous cervical epithelium. A positive nuclear reaction for ERα was observed in 42.6% of CIN3 lesions, in 10.9% of SCC, and in 33.3% (3 cases) of adenocarcinoma. A statistically significant trend of decreased of ERα positivity was noted with advancing stage of malignancy.

Amplification was found in 28.9% (11/38) specimens, 5 amplifications were observed in the CIN3 group and 6 in the SCC group. In the CIN3 group, amplification was detected in 21.7% (5/23) specimens; in the SCC group amplification was found in 40.0% (6/15) specimens. Of the 11 amplified samples, 7 lesions (63.6%) were negative for ERα IHC staining and 4 (36.4%) were positive.

Weak cytoplasmic and membrane staining for ADAM9 was found in the normal cervical epithelium. Evident staining for ADAM9 was detected in 86.1% (31/36) of CIN3 lesions and in 92.9% (13/14) SCC of the uterine cervix. Staining was stronger in SCC compared to CIN3 lesions. Moderate staining was detected in 64.3% (9/14) of SCC and in 36.1% (13/36) of CIN3 lesions. Weak staining was observed in 50.0% (18/36) of CIN3 lesions and in 28.6% (4/14) of SCC. The difference in the ADAM9 protein expression between cervical squamous carcinomas and normal epithelium was highly significant. Statistical significance was also found for the increased expression observed in CIN3 lesions versus normal squamous epithelium.

In conclusion, results from this study show for the first time that ADAM9 expression is low in the squamous epithelium of the cervix, but is increased in CIN3 lesions as well as SCCs, the increase being statistically significant in both cases. Expression of ERα was observed in the normal stratified squamous cervical epithelium. A statistically significant trend of decreased ERα expression was noted with advancing stage of malignancy. *ESR1* amplification was found in cervical cancer and precancerous lesions and, surprisingly, was more frequent in samples with negative IHC staining for ERα expression than in samples with positive staining. The possible diagnostic and prognostic value of ADAM9 and ERα expression in cervical cancer needs to be investigated further.