Evaluation of MCM7 and activin βA as potential biomarkers for cervical cancer prognosis.

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INTRODUCTION: Cervical cancer is the fourth most frequently diagnosed cancer, and the fourth leading cause of cancer death in women worldwide. There is epidemiological evidence that persistent infection of the cervix with high-risk Human Papillomavirus (HR-HPV) types of (HPV), plays a preponderate role in the development of uterine cervical carcinoma. However, one of the most important challenges for oncology medicine is the prediction of cancer case outcome in variable types of tumors. In this context, the development of cell markers that could evaluate the prognosis of cancer patients is of extreme relevance, for the planning of effective follow-up and management actions. In this

study we evaluated the immunolabeling of Minichromosome maintenance protein 7 (MCM7) and activin ßa subunit (from the activin/inhibin complex) as prognostic biomarkers of cervical cancer by using immunohistochemistry (IHC). MCM7 is part of a complex responsible for licensing DNA replication, and previous data from our study group, demonstrated a strong association between MCM7 immuno-labeling and the presence of HPV-DNA. activin ßa is a member of the TGF-beta family involved in cellular signaling, differentiation, apoptosis and cell-migration. Proteins from the activin-inibin axis can exhibit a suppressor or a tumor-promoting function that may differ among different tumor types. Presence of HPV and HR-HPV DNA was also analyzed in Squamous cell carcinoma (SCC) paraffin tissue-samples. **METHODS**: In this retrospective-study 29 cervical-tissue samples were obtained by total-sterectomy from women with invasive SCC. Based on the FIGO criteria, tumor-samples were histopathologically classified as Moderately-differentiated (MD) and Poorly-differentiated (PD)-SCC, which are associated with a better and a worse prognosis, respectively. Evaluation of HPV-DNA presence and the genotyping for HR-HPV-DNA was performed using *Nested*-PCR and *Hemi-nested* PCR techniques. MCM7 and activin ßa immunostaining was scored by using the follow parameters: percentage of labeled-cells (1) undetectable; (2) 0-25% of labeled-cells, (3) 26-50%, (4) 51-75%, (5) more than 75%; intensity of labeling (1) undetectable, (2) weak, (3) moderate, (4) strong, (5) very-strong. **RESULTS**: HPV-DNA was detected by *Nested*-PCR in 42% of SCC samples, with 35% of MD-SCC and 50% of PD-SCC tissue-samples exhibiting positive-results. HPV35 was detected in 24% of samples, followed by HPV16, present in 21% of SCC cases. The MCM7 immuno-labeling was only observed in nuclei of atypical epithelial-cancer cells, while activin ßa presented nuclear and cytoplasmic labeling patterns. Additionally, two distinct cancer-cell populations were characterized: the type-I, with variable nuclei-shape and size, and abundant cytoplasm, and type-II, exhibiting morphological aspects characteristic of immature cells. According to the FIGO criteria established to describe the histopathology classification of cervical cancer, cells with characteristics similar to type-II are commonly present in poorly-differentiated tumors, and exhibit a more aggressive phenotype. In moderately-differentiated cancer tissue-samples, a significant increase in intensity of MCM7 and activin ßa immuno-labeling was observed in the nucleus and the cytoplasm of samples from the mature type-I subpopulation group, respectively ($p<0.0001$). **CONCLUSION**: In SCC tissue-samples, MCM7 demonstrated to be a potential proliferation marker, able to detect cell-populations under proliferative-events, while activin ßa was able to identify cancer-cells subpopulations possibly more committed with cell-differentiation pathways.