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Immunohistochemistry in Cervical Cancer

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ABSTRACT

Uterine cervical cancer is caused, mostly, by persistent infection with some types of Human Papilloma Virus **(HPV)**, with types 16 and 18 showing higher risk for developing the disease. Because there is no effective drug treatment, early diagnosis of cervical cancer is very important for prognosis. The objective of this paper is to review the current immunohistochemical markers used in diagnosis, prognosis and treatment response in uterine cervical cancer, according to each type of lesion.

Keywords: Immunohistochemistry; Cervical cancer; intraepithelial lesion; Cervical squamous cell carcinoma; HPV

INTRODUCTION

Cervical cancer is the second most common malignancy affecting women's health. Each year worldwide, about 500,000 women are diagnosed and 200,000 die from this disease [1,2]. Most cases of cervical cancer is due to human papillomavirus infection caused by sexual contact, with types 16 and 18 high risk, representing 59.8% and 15%, respectively [2]. It has a long latency and has no obvious symptoms in the early stages. Currently, there are effective drugs for the treatment of cervical cancer, with early diagnosis of great importance for treatment and prognosis.

According to the World Health Organization (**WHO**, 2008), precursor lesions of cervical squamous cell carcinoma are classified as CIN I, II and III, but may also be referred to as high and low grade due to the difficulty in the diagnosis of such precursors [2]. While screening for cervical cancer depends on cervical cytology and detection of high-risk HPV, the histological diagnosis and, specifically, the extent of the injury, is the main parameter that drives the clinical management of positive evaluation of women [3]. Several studies have evaluated various immunehisto chemical markers in an attempt to find ways to assist the diagnosis and prognosis of precursor and malignant lesions of the cervix.

PRECURSOR LESIONS OF SQUAMOUS CELL CARCINOMA

An intraepithelial lesion (CIN) morphologically diagnosed may regress spontaneously in over half of all cases but sometimes persist and progress to carcinoma. However, this is not based only on morphology [3]. It is suggested that the lack of major protein L1 capsid may be a feature of progressive lesions [4], while the expression of minor protein L2 has not been extensively evaluated [3]. Yemelyanova et al showed capsid protein expression in 40% of cases of intraepithelial lesion low grade (CIN I), 6% of high-grade / CIN II and in any case high-grade / CIN III. On the other hand, among the cases with varying degrees of injury in the same sample, none with HSIL / CIN III expressed capsid proteins anywhere / degree of injury [3].

Cyclooxygenase 2 **(COX-2)** regulates the production of prostaglandins and appears to have a role in the onset and progression of various malignant tumors and premalignant conditions [5]. Balan et al showed cytoplasmic immunohistochemical expression of COX-2 weaker in samples with low grade (LSIL) and stronger in people diagnosed with high-grade lesions (HSIL), being more intense in CIN III. With these findings, they concluded that increased COX-2 expression in cervical cancer precursors certifies that it may play a role in the development and progression of squamous intraepithelial lesions of the cervix [5].

Several studies have highlighted the role of p16 as a cervical carcinoma marker and the disease progression is directly related to the presence of HPV [2,6,7]. However, positive immunohistochemical expression of p16 has also been tested as an alternative to improve diagnostic accuracy squamous intraepithelial high-grade lesion (HSIL) [7-9]. Cardoso et al showed that it can be considered as a biomarker of cervical intraepithelial neoplasm grade 3 in cases with high risk of recurrence or progression to invasive carcinoma [6]. Kim et al noted that the combined use of p16 and Proliferating Cell Nuclear Antigen **(PCNA)** has greater diagnostic accuracy for HSIL. Moreover, positive expression of p16 is also strongly associated with risk of progression to high grade CIN in women with low-grade lesions [10].

In the PALMS study held recently in five European countries, there was a high positive predictive value of cytology diagnosed with ASCU-S or doubly stained LSIL by immunohistochemistry for p16 and Ki-67 for the presence of high-grade CIN. They concluded that these markers may help to reduce the number of unnecessary referrals to colposcopy [11].

By comparing the accuracy of double staining for p16 / Ki-67 and molecular testing for high-risk HPV in identifying high grade intraepithelial neoplasm (CIN2 / CIN3) in women with cervical cytology ASC-US and LSIL, Posati-Resende et al showed that both present similar performance in predicting HSIL among women with ASC-US and women over 30 years with LSIL [12]. However, in patients less than 30 years diagnosed with LSIL, double staining p16 / Ki-67 had greater accuracy in identifying high-grade precursor lesions [12].

There is strong evidence that the host immune system plays a crucial role in the development of malignant and pre-malignant cervical lesions related to papillomaviruses [13]. When analyzing patients with High Grade Squamous Intraepithelial Lesion (HGSIL), Origoni et al correlated a high index of CD4 + cells, dendritic cells (CD11c +) and transcription factor T-bet + with more favorable clinical results, reinforcing the importance of host's immune status in the natural history of cervical disease [13].

The precursor lesions of the squamous cell carcinoma may also have differential diagnosis with non-malignant lesions such as atypical immature metaplasia, reactive immature squamous metaplasia, atypia, atrophy, and hyperplasia of basal cells. Selvi et al noted that immunohistochemical panel comprising p16, p63 and CK17 can be a precious help to distinguish these lesions from intraepithelial precursors (NIC) [14]. Aslani et al suggests that Ki67 and p16 markers are recommended for the differentiation between non-dysplastic and dysplastic lesions, however, does not discriminate between CK17 Immature metaplasia with and without dysplasia [15].

CERVICAL SQUAMOUS CELL CARCINOMA (SCC)

Besides being useful in differentiating between precursor neoplastic lesions types, immunohistochemistry can also be used to distinguish neoplastic lesions *in situ*, and superficial invasive tumors [16]. Most superficially invasive squamous cervical carcinomas show immune phenotypic changes consistent with Epithelial-Mesenchymal Transition **(EMT),** and particularly the expression of cyclin D1 may be useful for diagnosis. Similar changes in CIN III can indicate increased invasive potential [16].

As the p16 protein [17], positive expression of NF-κB (nuclear factor-κB) and c-IAP2 (inhibitor of protein-2 apoptosis) and low expression of caspase-3 in cervical cancer may be important in tumorigenesis and development of cervical cancer, and they can be useful as markers to estimate the progression of cervical cancer [18].

Cury et al suggested the use of p16, E-cadherin and Ki-67 in cervical biopsies with difficult diagnostic could assist in the early diagnosis of malignant lesions. However there was no association between the diagnosis of biopsy and persistence of the lesion, as these markers do not distinguish between persistent and non-persistent lesions [2].

Different markers such as Lgr5 (Leucine-rich repeat-containing G protein-coupled receptor 5) [19], Sphingosine Kinase 1 **(SphK1)** an oncogenic kinase [20], Stomatin-Like Protein 2 **(STOML2)**

[21] and Th17 / Foxp3 [22] can play an important role in the development and progression of cervical carcinoma. Galectin-7 and S100A9, in turn, play important roles in protecting from cervical squamous cell carcinoma [23].

Several immunohistochemical markers have been studied as prognostic factors of cervical cancer. Zhu et al showed that protein expression levels of related autophagy beclin-1 and LC3 was significantly lower in squamous cancer cells cervical than in normal squamous epithelial cells, concluding that expression of beclin 1 and LC3 may have significance prognostic in squamous cell carcinoma of the cervix at an early stage [24]. High expression of Interacting Receptor Protein Kinase 4 **(RIPK4)**, a regulatory protein β-catenin signaling, can act as a potential biomarker prognostic and independent diagnosis for cervical squamous cell cancer [25]. Positive expression of HMGB1 both nuclear and cytoplasmic is an independent factor of poor prognosis in early stage cervical carcinoma [26]. Liu et al showed that positivity for p16 and survivin may also be correlated with prognosis [27].

MARKERS LINKED TO TUMOR METASTASIS

Galectin 1 is a binding lectin laminin involved in important biological tumor mechanisms including neoplastic transformation, cell survival, angiogenesis, proliferation and metastasis [28]. Kim et al revealed that high expression of galectin in peritumoral stroma was significantly associated lymph node metastasis and galectin 1 can be functionally involved in cell proliferation and invasion [28].

Levels of protein kinase inhibitor Raf (RKIP) may be elevated in normal tissue, lower in primary cancer and minimal or absent in metastatic cancer [29]. Moreover, positive expression of p16 is possibly associated with tumorigenesis, but not to metastasis or prognosis of cervical carcinoma [30].

THE POSSIBLE RESPONSE EVALUATION TO CHEMOTHERAPY

Neoadjuvant Chemotherapy **(NAC)** is an important therapeutic strategy for cervical cancer and some markers may predict response to this treatment [31-33]. The first Aldehyde Dehydrogenase **(ALDH1)**, a stem cell marker of cancer chemoresistance is linked in a variety of cancers. Xie et al suggested that positive tumor expression of pre-NAC ALDH1 could be a predictive marker of poor response to CAN and positive expression of post-NAC ALDH1 could be a marker of poor prognosis for cervical cancer [31].

Abnormal expression of Annexin A2 and S100A proteins has been reported to induce sensitivity / resistance to chemotherapy in a variety of cancers. Jin et al found that high expression of S100A8 and S100A9 in tumor cells, S100A4 in stromal cells and low expression of Annexin A2 in tumor cells suggest significant increase in sensitivity to cisplatin-based chemotherapy of cervical cancer [32]. Therefore, the high expression of annexin A2 cells in the stroma and low S100A8 expression in tumor cells may indicate poor prognosis [32].

Nuclear Factor Kappa B **(NF-kB)** is an active transcription factor in cervical cancer and is part of an important pathway which leads to treatment resistance in many tumor types [33]. Garg et al suggested that nuclear expression NF-kB pre-treatment may be associated with a poor prognosis for cervical cancer patients treated with chemoradiation [33].

ADENOCARCINOMA

Endocervical adenocarcinoma account for about 10-30% of cases of cervical cancer and displays a variety of different morphologies, including, mucinous, hairy, serous and endometrioid, presenting challenging clinical-pathological diagnosis [34].

Positive immunohistochemical staining for p53 and Ki67 (> 50%) and negative for CA125 can aid the discrimination of adenocarcinoma diagnosis at an early stage [35]. In the differential diagnosis of glandular endocervical benign and malignant lesions, immunohistochemical analysis PAX8 and IMP3 can be helpful. However, there is some degree of overlap staining, both benign and malignant group. Thus, IMP3 and PAX8 should always be interpreted with caution and in combination with histomorphology [36].

P16 and Epidermal Growth Factor Receptor **(EGFR)** are frequently expressed in adenocarcinoma of the cervix. Bodner et al observed a tendency for the increase of lymphatic vessel invasion in p16 positive tumors, however, the investigation of p16 and EGFR is of limited use to assess prognosis and to guide tumors clinically [37].

Barbu et al showed immunohistochemical typical adenocarcinoma profile (ER-/RP-/VIM-/CEA+) in only 62.5% of the cases and that the endometrioid variant is the poor prognosis, while the hairy and have serous more favorable prognosis to be diagnosed in less advanced stages of the disease [34].

The protein 2 binding of Latent Transforming Growth Factor Beta-1 **(LTBP-2)** is a member of fibrillin / family of extracellular matrix proteins, which has been found to be over expressed in certain malignancies [38]. Ren et al noted that the immunohistochemical expression of LTBP2 was higher in the cervical adenocarcinoma than in epithelial tissue of normal cervix and that its expression is related to clinical stage, cervical tumor size, invasion depth and cervical lymph node metastasis stroma, suggesting that LTBP2 can serve as a prognostic factor in the clinical evaluation of patients with cervical adenocarcinoma [38].

CONCLUSION

There are many studies with immunohistochemical markers for cervical cancer, not only for diagnosis and prognosis of squamous cell carcinoma but also in cervical Adenocarcinoma (Table 1). In addition, new markers related to tumor metastasis and to predict response to chemotherapy are arising, but more researches in these fields are required.

Table 1: Immunohistochemical markers in diagnosis and prognosis of squamous cell carcinoma and cervical adenocarcinoma.

	Diagnosis	Poor Prognosis	Protection
Cervical squamous cell carcinoma (SCC)	Ciclina D1(+) p16(+) E-caderina(+) Ki-67(+) NF-κB(+) c-IAP2(+) Caspase-3(-) Lgr5(+) SphK1(+) STOML2(+) Th17/Foxp3(+)	Beclin-1(-) LC3(-) RIPK4(+) HMGB1(+) p16(+) Survivina(+)	Galectina-7(+) S100A9(+)
Adenocarcinoma	p53(+) Ki67 (> 50%) CA125(-) p16(+) EGFR(+) ER(-) RP(-) Vimentina(-) CEA(+)	LTBP2(+)	

(+): Positive expression; (-): Negative or weakly positive expression; **NF-kB**: Nuclear Factor-κB; **c-IAP2**: Inhibitor Of Apoptosis Protein-2; Lgr5: Leucine-rich repeat-containing G protein-coupled receptor 5; SphK1: esfingosina quinase 1; **STOML2**: Stomatin-Like Protein 2; **RIPK4**: Receptor Interacting Protein Kinase 4; **EGFR**: Epidermal Growth Factor Receptor; **ER**: Estrogen Receptor; **PR**: Progesterone Receptor; **LTBP2**: Latent Transforming Growth Factor Beta-1.

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