Anoctamin-1 (ANO1) and Paired Box Gene 8 (PAX8) as Emerging Immunohistochemical Biomarkers in Endocervical Adenocarcinoma

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Abstract

Background: Endocervical adenocarcinoma represents a heterogeneous group of malignancies with diverse etiopathogenetic mechanisms, morphological features, and clinical behaviors. Accurate diagnosis remains challenging, particularly in distinguishing primary endocervical adenocarcinoma from metastatic adenocarcinomas involving the cervix, as well as in subclassifying human papillomavirus (HPV)-associated and HPV-independent tumors. Immunohistochemistry has emerged as a pivotal adjunct in resolving these diagnostic dilemmas, with increasing attention directed toward novel biomarkers that enhance diagnostic precision and provide insight into tumor biology. Among these, Anoctamin-1 (ANO1), also known as DOG1, and Paired Box Gene 8 (PAX8) have gained considerable interest due to their roles in epithelial differentiation and tumorigenesis.

ANO1 is a calcium-activated chloride channel implicated in cellular proliferation, migration, and tumor progression across multiple malignancies. Its expression has been well established in gastrointestinal stromal tumors and is increasingly reported in various carcinomas, suggesting a broader oncogenic role. In contrast, PAX8 is a nuclear transcription factor critical for Müllerian system development and is widely used as a lineage-specific marker in gynecologic pathology. The expression patterns of these two markers in endocervical adenocarcinoma provide valuable diagnostic and potentially prognostic information, particularly in differentiating primary cervical tumors from metastatic lesions of Müllerian, renal, or thyroid origin.

The aim of this review is to comprehensively evaluate the immunohistochemical expression of ANO1 and PAX8 in endocervical adenocarcinoma, focusing on their diagnostic utility, biological significance, and potential role in tumor classification. Furthermore, this review explores the correlation of these markers with HPV status, histopathological subtypes, and clinical outcomes.

In conclusion, ANO1 and PAX8 represent promising immunohistochemical biomarkers that may significantly enhance diagnostic accuracy in endocervical adenocarcinoma. Their combined use offers a more refined approach to tumor classification and differential diagnosis. Future studies integrating molecular and immunophenotypic data are warranted to further elucidate their prognostic relevance and therapeutic implications in cervical adenocarcinoma.

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Keywords: *ANO1, PAX8, Endocervical Adenocarcinoma*

Introduction

Endocervical adenocarcinoma constitutes approximately 20–25% of all cervical cancers and has demonstrated a rising incidence over recent decades, particularly among younger women. This contrasts with the declining rates of squamous cell carcinoma, largely attributed to widespread cervical screening programs. The increasing relative frequency of adenocarcinoma highlights diagnostic challenges and limitations in current screening modalities, as glandular lesions are often less detectable by cytology. Consequently, there is a growing need for improved diagnostic biomarkers and refined pathological classification systems to better characterize these tumors and guide clinical management [1].

Endocervical adenocarcinomas are a heterogeneous group of neoplasms with diverse etiologies, morphologies, and clinical behaviors. A major advancement in their classification has been the recognition of the role of high-risk human papillomavirus (HPV) infection in tumor pathogenesis. The International Endocervical Adenocarcinoma Criteria and Classification (IECC) system categorizes these tumors into HPV-associated and HPV-independent types based on morphological features linked to viral oncogenesis. HPV-associated tumors typically exhibit usual-type morphology, high mitotic activity, and apoptotic bodies, whereas HPV-independent tumors, such as gastric-type adenocarcinoma, display distinct histological patterns and often exhibit more aggressive clinical behavior [2].

The distinction between HPV-associated and HPV-independent adenocarcinomas carries significant prognostic and therapeutic implications. HPV-independent tumors are frequently diagnosed at more advanced stages, demonstrate resistance to conventional therapies, and are associated with poorer survival outcomes. However, morphological overlap between subtypes and similarities with metastatic adenocarcinomas from other organs can complicate accurate diagnosis. This underscores the importance of adjunctive immunohistochemical markers in establishing tumor origin and classification [3].

Immunohistochemistry plays a pivotal role in the diagnostic workup of endocervical adenocarcinoma, particularly in differentiating primary cervical tumors from metastatic lesions involving the cervix. Commonly used markers such as p16, carcinoembryonic antigen (CEA), estrogen receptor (ER), and progesterone receptor (PR) have limitations in specificity and sensitivity. Therefore, there is increasing interest in identifying novel biomarkers that can enhance diagnostic accuracy and provide additional insights into tumor biology [4].

Anoctamin-1 (ANO1), also known as DOG1, is a calcium-activated chloride channel protein initially identified in gastrointestinal stromal tumors but subsequently found to be expressed in a variety of epithelial malignancies. ANO1 has been implicated in tumor proliferation, migration, and invasion, suggesting a potential role in oncogenesis. Emerging evidence indicates that ANO1 expression may be relevant in gynecologic malignancies, although its role in endocervical adenocarcinoma remains incompletely characterized [5].

Paired Box Gene 8 (PAX8) is a nuclear transcription factor essential for the development of Müllerian, renal, and thyroid tissues. It is widely utilized as an immunohistochemical marker to determine the origin of tumors, particularly in distinguishing Müllerian-derived neoplasms from non-gynecologic malignancies. In the context of endocervical adenocarcinoma, PAX8 expression may aid in confirming primary cervical origin and differentiating it from metastatic adenocarcinomas, although its expression patterns can vary depending on tumor subtype [6].

Despite the growing body of literature on ANO1 and PAX8, there remains a lack of comprehensive synthesis addressing their combined diagnostic and biological significance in endocervical adenocarcinoma. Most existing studies evaluate these markers independently or within limited cohorts, leaving a gap in understanding their complementary roles in tumor classification and differential diagnosis. Furthermore, their potential association with HPV status and clinical outcomes has not been fully elucidated [7].

Therefore, the aim of this review is to critically evaluate the immunohistochemical expression of ANO1 and PAX8 in endocervical adenocarcinoma, emphasizing their diagnostic utility, role in differential diagnosis, and potential biological and prognostic implications. By integrating current evidence, this

review seeks to provide a clearer framework for the application of these biomarkers in routine pathological practice and to identify areas for future research [8].

Histopathology and Classification of Endocervical Adenocarcinoma

Endocervical adenocarcinoma encompasses a diverse spectrum of gland-forming malignant tumors arising from the endocervical epithelium, characterized by variable architectural patterns and cytological features. Histologically, these tumors may exhibit glandular, papillary, cribriform, or solid growth patterns, often accompanied by mucin production. The recognition of these patterns is essential for diagnosis; however, significant overlap exists between different subtypes and with metastatic adenocarcinomas, posing diagnostic challenges. Accurate histopathological evaluation remains the cornerstone for classification, guiding both prognosis and therapeutic decisions [9].

A major advancement in the classification of endocervical adenocarcinoma has been the introduction of the International Endocervical Adenocarcinoma Criteria and Classification (IECC), which stratifies tumors based on their association with human papillomavirus (HPV). This system categorizes tumors into HPV-associated (HPVA) and HPV-independent (HPVI) types using morphological criteria that reflect underlying viral oncogenesis. HPVA tumors typically display apical mitotic figures and apoptotic bodies visible at scanning magnification, whereas HPVI tumors lack these features and often demonstrate distinct cytoplasmic characteristics, such as abundant clear or pale eosinophilic cytoplasm [10].

HPV-associated adenocarcinomas represent the majority of endocervical glandular malignancies, with the usual type being the most common subtype. These tumors are strongly linked to high-risk HPV types, particularly HPV 16 and 18, and typically exhibit diffuse block-type p16 immunoreactivity. Histologically, they are characterized by pseudostratified columnar cells, nuclear atypia, increased mitotic activity, and apoptotic debris. Variants such as mucinous (including intestinal and signet-ring cell types) and villoglandular patterns are also recognized within this category, each with distinct morphological features but similar HPV-driven pathogenesis [11].

In contrast, HPV-independent adenocarcinomas constitute a smaller but clinically significant subset, often associated with poorer outcomes. Gastric-type adenocarcinoma is the most common HPV-independent subtype and is notable for its aggressive behavior, resistance to conventional therapies, and tendency for deep stromal invasion. Histologically, these tumors exhibit voluminous clear or pale eosinophilic cytoplasm, distinct cell borders, and minimal nuclear atypia relative to their aggressive clinical course. Other HPV-independent subtypes include clear cell, mesonephric, and endometrioid adenocarcinomas, each with unique morphological and immunophenotypic profiles [12].

The World Health Organization (WHO) classification has incorporated many aspects of the IECC system, emphasizing the etiological distinction between HPV-associated and HPV-independent tumors. This shift reflects a broader trend in oncologic pathology toward integrating molecular and etiological factors into tumor classification. The WHO classification also highlights the importance of recognizing rare subtypes and their clinical implications, as management strategies may differ significantly depending on tumor type and origin [13].

Despite these advancements, challenges persist in distinguishing primary endocervical adenocarcinoma from metastatic adenocarcinomas involving the cervix, particularly from the endometrium, ovary, gastrointestinal tract, and pancreas. Morphological similarities can lead to diagnostic uncertainty, especially in small biopsy samples. In such cases, immunohistochemical markers become indispensable tools, complementing histopathological findings and improving diagnostic accuracy [14].

Furthermore, interobserver variability in the interpretation of morphological features remains a concern, particularly in differentiating between HPV-associated and HPV-independent tumors. Studies have shown that even experienced pathologists may encounter difficulties in applying IECC criteria consistently, underscoring the need for adjunctive biomarkers and standardized diagnostic approaches. This variability further supports the integration of immunohistochemical and molecular techniques into routine diagnostic workflows [15].

In this context, the exploration of novel immunohistochemical markers such as ANO1 and PAX8 is of

considerable interest. These markers may provide additional discriminatory power in subclassifying endocervical adenocarcinomas and distinguishing them from metastatic lesions. Understanding their expression patterns within the framework of modern classification systems is essential for optimizing diagnostic strategies and enhancing our understanding of tumor biology [16].

Anoctamin-1 (ANO1/DOG1): Molecular Biology and Role in Tumors

Anoctamin-1 (ANO1), also known as DOG1 (Discovered on GIST-1), is a calcium-activated chloride channel encoded by the *TMEM16A* gene located on chromosome 11q13, a region frequently amplified in various human malignancies. ANO1 plays a critical role in regulating epithelial ion transport, cellular excitability, and secretory functions. Physiologically, it is expressed in interstitial cells of Cajal, salivary glands, and gastrointestinal epithelium. Its discovery as a highly sensitive and specific marker for gastrointestinal stromal tumors (GISTs) has led to broader investigations into its expression across a wide range of neoplastic conditions [17].

At the molecular level, ANO1 contributes to tumorigenesis through multiple mechanisms, including modulation of intracellular calcium signaling, activation of mitogen-activated protein kinase (MAPK) pathways, and interaction with epidermal growth factor receptor (EGFR) signaling. These pathways collectively promote cellular proliferation, migration, and resistance to apoptosis. Amplification or overexpression of *TMEM16A* has been documented in several cancers, including head and neck squamous cell carcinoma, breast cancer, and gastrointestinal malignancies, suggesting its role as an oncogenic driver rather than merely a diagnostic marker [18].

In diagnostic pathology, ANO1 immunohistochemical expression has become a cornerstone in identifying GISTs, particularly in cases where KIT (CD117) expression is equivocal or negative. Its high sensitivity and specificity have established ANO1 as a reliable marker in surgical pathology. Beyond GISTs, ANO1 expression has been reported in a variety of epithelial tumors, including pancreatic, esophageal, and colorectal carcinomas, although with variable intensity and distribution patterns. This broader expression profile highlights its potential utility as a biomarker in non-mesenchymal tumors [19].

Recent studies have explored ANO1 expression in gynecologic malignancies, revealing its presence in subsets of ovarian, endometrial, and cervical tumors. In these contexts, ANO1 expression has been associated with enhanced tumor aggressiveness, increased invasive potential, and, in some reports, poorer clinical outcomes. These findings suggest that ANO1 may play a functional role in tumor progression within the female genital tract, although the extent and consistency of its expression in endocervical adenocarcinoma remain under investigation [20].

The potential diagnostic value of ANO1 in endocervical adenocarcinoma lies in its differential expression compared to other gynecologic and non-gynecologic malignancies. While traditional markers such as p16 and CEA provide useful information, they may lack specificity in certain contexts, particularly when distinguishing primary cervical tumors from metastatic adenocarcinomas. ANO1 may serve as an adjunctive marker, especially when used in combination with other immunohistochemical panels, to improve diagnostic accuracy in challenging cases [21].

Furthermore, the expression of ANO1 may have implications in distinguishing HPV-associated from HPV-independent adenocarcinomas. Although data remain limited, some studies suggest differential expression patterns that could reflect underlying molecular pathways distinct from HPV-driven carcinogenesis. This raises the possibility that ANO1 could contribute not only to diagnosis but also to tumor subclassification within the framework of modern classification systems such as the IECC [22].

In addition to its diagnostic role, ANO1 has been investigated as a potential therapeutic target. Given its involvement in key oncogenic signaling pathways, pharmacologic inhibition of ANO1 function has been proposed as a strategy to suppress tumor growth and metastasis. Experimental studies have demonstrated that targeting ANO1 can reduce proliferation and induce apoptosis in cancer cell lines, supporting its relevance in translational oncology. However, clinical application of these findings remains in early stages [23].

Despite these promising insights, the role of ANO1 in endocervical adenocarcinoma is not yet fully

defined. Variability in expression across studies, differences in methodology, and limited sample sizes contribute to inconsistent findings. Therefore, further large-scale, well-designed studies are necessary to clarify its diagnostic utility, prognostic significance, and potential role in targeted therapy. Integrating ANO1 expression into comprehensive immunohistochemical panels may ultimately enhance the accuracy and depth of pathological evaluation in these tumors [24].

Paired Box Gene 8 (PAX8): Molecular Biology and Diagnostic Role in Gynecologic Tumors

Paired Box Gene 8 (PAX8) is a member of the PAX family of transcription factors, which play a fundamental role in embryogenesis and organogenesis. It is critically involved in the development of the Müllerian system, thyroid gland, and renal epithelium. PAX8 regulates cellular differentiation, proliferation, and survival by controlling the expression of genes essential for tissue-specific development. In normal adult tissues, its expression is largely restricted to organs of Müllerian, renal, and thyroid origin, making it a highly valuable lineage-specific marker in diagnostic pathology [25].

At the molecular level, PAX8 functions as a nuclear transcription factor that binds DNA and regulates gene expression involved in epithelial cell maintenance and differentiation. It has been shown to interact with key signaling pathways, including those involved in cell cycle regulation and apoptosis. In neoplastic conditions, persistent or aberrant expression of PAX8 contributes to tumor cell survival and proliferation, particularly in tumors derived from Müllerian epithelium. This biological role underpins its widespread use as an immunohistochemical marker in gynecologic oncology [26].

In diagnostic practice, PAX8 is extensively utilized to determine the origin of carcinomas, especially in cases of metastatic disease with unknown primary. It is strongly expressed in tumors of Müllerian origin, including ovarian serous carcinoma, endometrial adenocarcinoma, and fallopian tube carcinoma. Additionally, it is expressed in renal cell carcinoma and thyroid carcinomas, which must be considered when interpreting staining results. The nuclear localization and relatively high sensitivity of PAX8 make it a reliable marker in differentiating primary gynecologic tumors from non-Müllerian malignancies [27].

In the context of endocervical adenocarcinoma, PAX8 expression has been variably reported, with higher expression generally observed in HPV-independent subtypes compared to HPV-associated tumors. This variability reflects the diverse origins and differentiation pathways of cervical adenocarcinomas. For example, gastric-type adenocarcinoma, an HPV-independent subtype, may exhibit stronger and more consistent PAX8 expression, whereas usual-type HPV-associated adenocarcinomas may show focal or weak staining. Such differences can aid in tumor subclassification when interpreted alongside morphological and other immunohistochemical findings [28].

PAX8 also plays a crucial role in distinguishing primary endocervical adenocarcinoma from metastatic tumors involving the cervix. For instance, metastatic colorectal adenocarcinoma, which may morphologically mimic primary cervical tumors, is typically PAX8-negative but positive for markers such as CDX2. Conversely, Müllerian-derived tumors, including those arising from the endometrium or ovary, are usually PAX8-positive. However, overlapping expression patterns between endocervical and endometrial adenocarcinomas necessitate the use of additional markers, such as vimentin, ER, and p16, to achieve accurate diagnosis [29].

The use of PAX8 in immunohistochemical panels has significantly improved diagnostic accuracy in gynecologic pathology, particularly in small biopsy specimens and metastatic settings. Its high sensitivity for Müllerian-derived tumors makes it an essential component of diagnostic algorithms. Nevertheless, its lack of absolute specificity, due to expression in renal and thyroid neoplasms, requires careful interpretation within clinical and morphological context [30].

Emerging studies have also investigated the potential prognostic significance of PAX8 expression in various malignancies. In some gynecologic cancers, persistent PAX8 expression has been associated with tumor progression and resistance to apoptosis, suggesting a role beyond lineage identification. However, its prognostic value in endocervical adenocarcinoma remains unclear, with limited and sometimes conflicting data available in the literature [31].

Despite its established role as a diagnostic marker, several gaps remain regarding the application of

PAX8 in endocervical adenocarcinoma. These include variability in expression across tumor subtypes, lack of standardized scoring systems, and limited data correlating expression with clinical outcomes. Further research is needed to clarify its role in tumor classification, particularly in conjunction with emerging biomarkers such as ANO1, to enhance diagnostic precision and potentially inform therapeutic strategies [32].

Combined Diagnostic Utility of ANO1 and PAX8 in Endocervical Adenocarcinoma

The integration of multiple immunohistochemical markers into diagnostic panels has become a standard approach in surgical pathology, particularly when evaluating tumors with overlapping morphological features. In the context of endocervical adenocarcinoma, the combined use of ANO1 and PAX8 offers a promising strategy to enhance diagnostic accuracy. While each marker individually provides valuable information, their combined interpretation may yield greater specificity in distinguishing primary cervical tumors from metastatic adenocarcinomas and in subclassifying tumor types within the cervix [33].

ANO1, with its role in tumor proliferation and epithelial ion transport, has demonstrated variable expression across different carcinomas, whereas PAX8 serves as a lineage-specific nuclear marker for Müllerian-derived tissues. The complementary nature of these markers lies in their distinct biological functions—ANO1 reflecting tumor behavior and cellular activity, and PAX8 indicating tissue origin. When used together, they can provide a more comprehensive immunophenotypic profile, particularly in diagnostically challenging cases where morphology alone is insufficient [34].

In differentiating primary endocervical adenocarcinoma from metastatic adenocarcinomas involving the cervix, the combined use of ANO1 and PAX8 may be particularly advantageous. For example, metastatic gastrointestinal adenocarcinomas, which often mimic endocervical tumors histologically, are typically negative for PAX8 and may show variable ANO1 expression depending on tumor origin. In contrast, primary cervical adenocarcinomas may exhibit PAX8 positivity with or without ANO1 expression, supporting a Müllerian origin. This combined immunoprofile can help narrow the differential diagnosis when interpreted alongside markers such as CDX2, CK7, and p16 [35].

Furthermore, the combined expression patterns of ANO1 and PAX8 may contribute to the subclassification of endocervical adenocarcinomas within the framework of HPV-associated and HPV-independent tumors. HPV-associated adenocarcinomas often demonstrate diffuse p16 positivity and variable PAX8 expression, whereas HPV-independent tumors, such as gastric-type adenocarcinoma, may show stronger PAX8 expression and potentially distinct ANO1 staining patterns. Although current data are limited, these differences suggest that integrating ANO1 into immunohistochemical panels could refine subclassification and improve diagnostic precision [36].

Another important application of the combined use of ANO1 and PAX8 is in small biopsy specimens or cytology samples, where limited tissue availability can hinder accurate diagnosis. In such cases, reliance on a focused panel of highly informative markers is essential. The addition of ANO1 to established markers, including PAX8, may enhance diagnostic confidence, particularly when distinguishing between primary cervical tumors and metastatic lesions from the gastrointestinal or pancreatobiliary tract [37].

Despite these advantages, several limitations must be considered when interpreting ANO1 and PAX8 expression. Variability in staining intensity, differences in antibody clones, and lack of standardized scoring systems can affect reproducibility across laboratories. Additionally, overlapping expression patterns with other malignancies necessitate cautious interpretation within the broader clinical and pathological context. Therefore, these markers should not be used in isolation but rather as part of a comprehensive diagnostic panel [38].

Emerging evidence also suggests that the combined expression of ANO1 and PAX8 may have potential prognostic implications. While ANO1 has been associated with tumor aggressiveness and invasive behavior in several malignancies, PAX8 expression has been linked to tumor differentiation and survival pathways. The interplay between these markers could provide insights into tumor biology and potentially identify subgroups of patients with distinct clinical outcomes. However, robust clinical

studies are needed to validate these observations in endocervical adenocarcinoma [39].

In summary, the combined use of ANO1 and PAX8 represents a promising advancement in the immunohistochemical evaluation of endocervical adenocarcinoma. Their complementary roles in reflecting tumor biology and tissue origin enhance diagnostic accuracy and may contribute to improved tumor classification. Future research focusing on standardized assessment and correlation with molecular and clinical data will be essential to fully establish their utility in routine pathological practice [40].

Role of ANO1 and PAX8 in Differential Diagnosis of Endocervical Adenocarcinoma

The differential diagnosis of endocervical adenocarcinoma is often complex due to significant morphological overlap with a wide range of primary and metastatic adenocarcinomas. These include tumors originating from the endometrium, ovary, gastrointestinal tract, pancreas, and even the breast. Accurate distinction is essential, as management strategies and prognostic outcomes vary considerably depending on the primary site. In this context, immunohistochemistry serves as a critical adjunct to histopathological evaluation, with ANO1 and PAX8 emerging as valuable markers in refining differential diagnosis [41].

One of the most frequent diagnostic challenges is distinguishing primary endocervical adenocarcinoma from endometrial adenocarcinoma involving the cervix. Both tumors may share overlapping glandular architecture and cytological features, particularly in small biopsy samples. PAX8 is expressed in both endocervical and endometrial carcinomas due to their Müllerian origin, limiting its discriminatory power in this setting. However, when used in combination with other markers such as vimentin, estrogen receptor (ER), progesterone receptor (PR), and p16, PAX8 contributes to a broader immunoprofile that can aid in differentiation. HPV-associated endocervical adenocarcinomas typically exhibit diffuse p16 positivity, whereas endometrial tumors often show patchy or negative p16 staining [42].

Differentiating endocervical adenocarcinoma from metastatic gastrointestinal adenocarcinoma is another critical diagnostic scenario. Colorectal adenocarcinomas, in particular, may closely mimic mucinous endocervical tumors both morphologically and immunohistochemically. In such cases, PAX8 is typically negative in colorectal carcinoma but positive in Müllerian-derived tumors, making it a useful marker for excluding a gastrointestinal origin. Additionally, gastrointestinal tumors frequently express CDX2 and CK20, whereas endocervical adenocarcinomas are usually CK7-positive and CDX2-negative. ANO1 expression in gastrointestinal tumors can be variable, and its presence should be interpreted cautiously within a panel of markers [43].

Metastatic pancreaticobiliary adenocarcinomas can also involve the cervix and present significant diagnostic challenges. These tumors often exhibit mucinous features similar to gastric-type endocervical adenocarcinoma, an HPV-independent subtype. PAX8 negativity in pancreatic tumors can help exclude a Müllerian origin, whereas gastric-type endocervical adenocarcinoma may show variable PAX8 expression. In this context, ANO1 may provide additional information, although its expression in pancreatic tumors has been reported inconsistently. Therefore, a comprehensive panel including PAX8, CK7, CK20, CDX2, and mucin markers is recommended [44].

Another important consideration is the differentiation between primary cervical adenocarcinoma and metastatic ovarian carcinoma. High-grade serous ovarian carcinoma frequently expresses PAX8, similar to endocervical adenocarcinoma, which limits the specificity of this marker. However, differences in clinical presentation, imaging findings, and additional immunohistochemical markers such as WT1 can aid in distinguishing these entities. ANO1 expression in ovarian tumors has been reported but is not sufficiently specific to serve as a standalone marker in this differential diagnosis [45].

Breast carcinoma metastasis to the cervix, although rare, should also be considered in the differential diagnosis. These tumors may exhibit glandular morphology and can be confused with primary cervical adenocarcinoma. Breast carcinomas are typically negative for PAX8, which supports a non-Müllerian origin. Additional markers such as GATA3, mammaglobin, and GCDFP-15 are useful in confirming breast origin. ANO1 is generally not expressed in breast carcinoma, further supporting its limited role in this specific differential scenario [46].

The distinction between HPV-associated and HPV-independent endocervical adenocarcinomas also has implications in differential diagnosis. Gastric-type adenocarcinoma, an HPV-independent tumor, can closely resemble metastatic gastrointestinal carcinoma. In such cases, PAX8 positivity, although variable, may support a cervical origin, while diffuse negativity for p16 further differentiates it from HPV-associated tumors. ANO1 expression patterns in these subtypes remain an area of ongoing research and may provide additional discriminatory value in the future [47].

Despite the utility of ANO1 and PAX8, it is important to recognize that no single marker is entirely specific or sensitive for a particular tumor type. The interpretation of immunohistochemical results must always be integrated with morphological findings and clinical information. The use of a panel-based approach, incorporating both established and emerging markers, remains the gold standard in resolving complex differential diagnoses involving endocervical adenocarcinoma [48].

ANO1 and PAX8 play complementary roles in the differential diagnosis of endocervical adenocarcinoma, particularly when distinguishing primary cervical tumors from metastatic lesions. While PAX8 provides valuable information regarding tissue origin, ANO1 may offer insights into tumor biology and behavior. Their combined use within a comprehensive immunohistochemical panel enhances diagnostic accuracy and supports more precise tumor classification in challenging cases [49].

Prognostic Significance of ANO1 and PAX8 in Endocervical Adenocarcinoma

The prognostic evaluation of endocervical adenocarcinoma remains a complex and evolving area, influenced by multiple clinicopathological factors including tumor stage, histological subtype, lymphovascular invasion, and HPV status. In recent years, attention has shifted toward identifying molecular and immunohistochemical biomarkers that can provide additional prognostic information beyond conventional parameters. Among these, ANO1 and PAX8 have emerged as potential candidates due to their involvement in tumor biology and cellular differentiation pathways [50].

ANO1 has been increasingly recognized for its role in promoting tumor aggressiveness across various malignancies. Its overexpression has been associated with enhanced cellular proliferation, migration, and invasion, largely mediated through activation of signaling pathways such as EGFR and MAPK. In several tumor types, including head and neck, breast, and gastrointestinal cancers, high ANO1 expression correlates with poor clinical outcomes, including reduced overall survival and increased metastatic potential. These findings suggest that ANO1 may serve as a marker of aggressive tumor behavior [51].

In the context of gynecologic malignancies, emerging evidence indicates that ANO1 expression may similarly be associated with adverse prognostic features. Studies in ovarian and endometrial carcinomas have demonstrated correlations between ANO1 overexpression and higher tumor grade, advanced stage, and increased likelihood of recurrence. Although data specific to endocervical adenocarcinoma remain limited, these observations raise the possibility that ANO1 expression could reflect a more aggressive tumor phenotype within cervical glandular neoplasms [52].

Furthermore, the potential relationship between ANO1 expression and HPV status may have prognostic implications. HPV-independent adenocarcinomas, particularly gastric-type tumors, are known for their aggressive clinical course and poor response to conventional therapies. If ANO1 expression is found to be more prevalent or pronounced in these subtypes, it could serve as an additional marker to identify high-risk patients. However, current evidence is insufficient, and further studies are needed to establish this association definitively [53].

PAX8, on the other hand, has traditionally been regarded as a diagnostic marker rather than a prognostic one. Nevertheless, recent studies suggest that PAX8 may play a role in tumor progression by regulating genes involved in cell survival and resistance to apoptosis. In certain malignancies, including ovarian and renal carcinomas, sustained PAX8 expression has been linked to tumor cell viability and resistance to chemotherapy, indicating a potential role in disease progression [54].

In endocervical adenocarcinoma, the prognostic significance of PAX8 expression remains unclear. Some studies have reported variable expression across different tumor subtypes, with higher expression observed in HPV-independent tumors, which are generally associated with worse outcomes. However,

whether PAX8 expression independently predicts prognosis or simply reflects underlying tumor biology has yet to be determined. The lack of standardized scoring systems and limited cohort sizes further complicate interpretation of existing data [55].

The combined evaluation of ANO1 and PAX8 expression may offer additional prognostic insights. While ANO1 may indicate tumor aggressiveness and invasive potential, PAX8 may reflect lineage differentiation and cellular survival mechanisms. Together, their expression patterns could help stratify patients into distinct prognostic groups, particularly when integrated with established clinicopathological parameters and molecular features such as HPV status [56].

Another important consideration is the potential role of these markers in guiding therapeutic decisions. As targeted therapies and personalized medicine continue to evolve, biomarkers that reflect underlying molecular pathways may become increasingly relevant. ANO1, in particular, has been proposed as a potential therapeutic target, and its expression could identify patients who may benefit from novel targeted interventions. PAX8, while less directly targetable, may still provide insights into tumor biology that influence treatment strategies [57].

Despite these promising perspectives, significant gaps remain in our understanding of the prognostic value of ANO1 and PAX8 in endocervical adenocarcinoma. Most available studies are limited by small sample sizes, retrospective design, and heterogeneity in methodology. Large-scale, prospective studies with standardized immunohistochemical assessment are needed to validate these markers and clarify their role in clinical practice [58].

In summary, ANO1 and PAX8 represent potentially valuable prognostic biomarkers in endocervical adenocarcinoma, reflecting different aspects of tumor biology. While ANO1 is associated with tumor aggressiveness and progression, PAX8 may be linked to cellular differentiation and survival. Their combined evaluation holds promise for improving prognostic stratification, but further research is required to establish their clinical utility and integrate them into routine pathological assessment [59].

Conclusion

Endocervical adenocarcinoma represents a diagnostically and biologically complex group of malignancies, necessitating a multidimensional approach that integrates morphology, immunohistochemistry, and molecular insights. The evolving classification systems, particularly those based on HPV association, have improved our understanding of tumor heterogeneity; however, significant diagnostic challenges remain, especially in distinguishing primary cervical tumors from metastatic lesions and in accurately subclassifying tumor variants. In this context, the incorporation of novel immunohistochemical biomarkers has become increasingly important in enhancing diagnostic precision and supporting clinical decision-making.

ANO1 and PAX8 have emerged as promising complementary biomarkers in the evaluation of endocervical adenocarcinoma. ANO1 reflects tumor biology and potential aggressiveness through its role in cellular signaling and proliferation, while PAX8 serves as a reliable indicator of Müllerian lineage and tumor origin. Their combined application within immunohistochemical panels offers a more refined diagnostic framework and may contribute to improved tumor classification and prognostic stratification. Despite encouraging findings, further large-scale and methodologically robust studies are required to validate their clinical utility and to explore their potential roles in targeted therapeutic strategies.

References

1. WHO Classification of Tumours Editorial Board. *Female Genital Tumours*. 5th ed. International Agency for Research on Cancer; 2020.
2. Stolnicu S, Barsan I, Hoang L, et al. International Endocervical Adenocarcinoma Criteria and Classification (IECC): a new pathogenetic classification for invasive adenocarcinomas of the endocervix. *Am J Surg Pathol*. 2018;42(2):214-226.
3. Hodgson A, Park KJ, Djordjevic B, et al. International Endocervical Adenocarcinoma Criteria and Classification: validation and interobserver reproducibility. *Am J Surg Pathol*. 2019;43(1):75-83.
4. Park KJ. Cervical adenocarcinoma: integration of HPV status, morphology, and immunophenotype. *Int J Gynecol Pathol*. 2020;39(suppl 1):S121-S134.

5. Stolnicu S, Park KJ, Kiyokawa T, et al. Tumor typing of endocervical adenocarcinoma: recommendations from the International Society of Gynecological Pathologists. *Int J Gynecol Pathol*. 2021;40(suppl 1):S75-S91.
6. Talia KL, Oliva E, Rabban JT, et al. Grading of endocervical adenocarcinomas: ISGyP recommendations. *Int J Gynecol Pathol*. 2021;40(suppl 1):S92-S107.
7. Parra-Herran C, Stolnicu S, Croce S, et al. Grossing and processing of endocervical adenocarcinoma: ISGyP recommendations. *Int J Gynecol Pathol*. 2021;40(suppl 1):S108-S120.
8. Singh N, Stolnicu S, McCluggage WG, et al. The ISGyP Endocervical Adenocarcinoma Project overview. *Int J Gynecol Pathol*. 2021;40(suppl 1):S1-S5.
9. Young RH, Clement PB. Endocervical adenocarcinoma and its variants: morphology and differential diagnosis. *Histopathology*. 2002;41(3):185-207.
10. Karamurzin YS, Park KJ. Gastric-type endocervical adenocarcinoma: aggressive tumor with poor prognosis. *Am J Surg Pathol*. 2015;39(11):1449-1457.
11. McCluggage WG. New developments in endocervical glandular lesions. *Histopathology*. 2013;62(1):138-160.
12. McCluggage WG. Differentiation of endocervical and endometrial adenocarcinomas. *Histopathology*. 2016;68(1):109-123.
13. Pirog EC. Cervical adenocarcinoma: HPV-associated and HPV-independent tumors. *Arch Pathol Lab Med*. 2017;141(12):1653-1667.
14. Stolnicu S, Hoang L, Chiu D, et al. Clinical outcomes of HPV-associated vs HPV-independent adenocarcinomas. *Int J Gynecol Pathol*. 2019;38(2):123-133.
15. Luo RZ, Li MQ, Guo J, et al. HPV mRNA in situ hybridization in endocervical adenocarcinoma. *Am J Surg Pathol*. 2021;45(12):1640-1649.
16. Yemelyanova A, Vang R, Judson K, et al. Immunohistochemical distinction of endocervical vs endometrial adenocarcinoma. *Am J Surg Pathol*. 2009;33(10):1508-1517.
17. Stewart CJR, Crook ML. Guidelines for distinguishing endometrial vs endocervical carcinomas. *Pathology*. 2018;50(1):32-41.
18. Kong CS, Beck AH, Longacre TA. Optimal marker panel for endocervical vs endometrial adenocarcinoma. *Am J Surg Pathol*. 2010;34(7):915-926.
19. Vang R, Gown AM, Zhao C, et al. p16 expression in gynecologic tumors. *Am J Surg Pathol*. 2007;31(5):653-663.
20. West RB, Corless CL, Chen X, et al. DOG1 expression in gastrointestinal stromal tumors. *Am J Pathol*. 2004;165(1):107-113.
21. Lee CH, Liang CW, Espinosa I. DOG1 as immunohistochemical marker. *Adv Anat Pathol*. 2010;17(3):222-232.
22. Caputo A, Caci E, Ferrera L, et al. TMEM16A chloride channel characterization. *J Biol Chem*. 2008;283(24):16473-16478.
23. Ferrera L, Caputo A, Ubby I, et al. Regulation of TMEM16A channel. *J Biol Chem*. 2009;284(48):33360-33368.
24. Duvvuri U, Shiwarski DJ, Xiao D, et al. TMEM16A in tumorigenesis. *Cancer Res*. 2012;72(13):3270-3281.
25. Liu W, Lu M, Liu B, et al. ANO1 inhibition suppresses tumor growth. *Cancer Lett*. 2012;326(1):41-51.
26. Bill A, Hall ML, Borawski J, et al. ANO1 targeting induces apoptosis. *Cancer Res*. 2014;74(18):5437-5451.
27. Liu F, Cao QH, Lu DJ, et al. TMEM16A in gastric cancer progression. *Oncotarget*. 2015;6(13):11585-11599.
28. Jia L, Liu W, Guan L, et al. ANO1 inhibition in lung cancer. *PLoS One*. 2015;10(8):e0136584.
29. Bae JS, Lee A, Kim Y, et al. ANO1 expression and prognosis in breast carcinoma. *Virchows Arch*. 2018;472(4):585-593.
30. Guo S, Chen Y, Liu J, et al. ANO1 in cancer biology review. *Cancer Manag Res*. 2022;14:2221-2234.
31. Plachov D, Chowdhury K, Walther C, et al. PAX8 gene in development. *Development*. 1990;110(2):643-651.
32. Bowen NJ, Logani S, Dickerson EB, et al. PAX8 in ovarian cancer. *Cancer Res*. 2007;67(21):10137-10145.
33. Nonaka D, Chiriboga L, Soslow RA. PAX8 in ovarian vs breast carcinoma. *Am J Surg Pathol*. 2008;32(10):1566-1571.
34. Tong GX, Yu WM, Beaubier NT, et al. PAX8 in renal tumors. *Mod Pathol*. 2009;22(9):1218-1227.
35. Laury AR, Perets R, Piao H, et al. Comprehensive PAX8 expression study. *Am J Surg Pathol*. 2011;35(6):816-826.
36. Tacha D, Zhou D, Cheng L. PAX8 expression in tumors. *Appl Immunohistochem Mol Morphol*. 2011;19(4):293-299.
37. Ozcan A, Shen SS, Hamilton C, et al. PAX2 vs PAX8 comparison. *Am J Surg Pathol*. 2011;35(12):1837-1847.
38. Nonaka D. PAX8 in diagnostic pathology. *Adv Anat Pathol*. 2012;19(3):140-148.
39. Wang Y, Li L, Douville C, et al. PAX8 as tumor marker. *J Hematol Oncol*. 2013;6:60.
40. Xiang L, Kong B. PAX8 as diagnostic marker review. *Oncol Lett*. 2013;5(3):735-738.
41. Di Palma T, Lucci V, de Cristofaro T, et al. PAX8 tumorigenic role. *BMC Cancer*. 2014;14:292.
42. Mhawech-Fauceglia P, Herrmann FR, Rai H, et al. PAX8 in advanced tumors. *Histopathology*. 2012;60(6):1019-1020.
43. Kakun RR, Golemis EA. PAX8 in cancer review. *Cancers (Basel)*. 2022;14(13):3072.
44. Hardy LR, Salvi A, Burdette JE. PAX8 in ovarian cancer biology. *Cancers (Basel)*. 2018;10(8):262.
45. Bhatla N, Aoki D, Sharma DN, et al. FIGO staging cervical cancer. *Int J Gynaecol Obstet*. 2018;143(suppl 2):22-36.
46. Reed NS, Balega J, Barwick T, et al. Cervical cancer guidelines. *Eur J Obstet Gynecol Reprod Biol*. 2021;256:433-465.
47. Ehmann S, Warmke L, Vats K, et al. Gastric-type cervical adenocarcinoma outcomes. *Gynecol Oncol*. 2022;166(1):151-159.
48. Yoshida H, Matsubara A, Maeda D. HPV-negative cervical cancer molecular pathology. *Cancers (Basel)*. 2021;13(24):6351.
49. Hohn AK, Brambs CE, Hiller GGR, et al. WHO 2020 update in gynecologic tumors. *Geburtshilfe Frauenheilkd*. 2021;81(10):1145-1153.
50. Cimino-Mathews A, Subhawong AP, Illei PB, et al. GATA3 in breast carcinoma. *Hum Pathol*. 2013;44(7):1341-1349.
51. Chu P, Wu E, Weiss LM. CK7 and CK20 expression in tumors. *Mod Pathol*. 2000;13(9):962-972.
52. Tot T. Cytokeratin profiles in tumors. *APMIS*. 2002;110(2):93-100.

53. Vang R, Gown AM, Barry TS, et al. CK7/CK20 in gynecologic tumors. *Int J Gynecol Pathol*. 2006;25(2):146-154.
54. Hornick JL. IHC in metastatic tumor diagnosis. *Arch Pathol Lab Med*. 2018;142(8):903-922.
55. Fitzgibbons PL, Bradley LA, Fatheree LA, et al. Validation of IHC assays guidelines. *Arch Pathol Lab Med*. 2014;138(11):1432-1443.
56. Duggan MA, Nation JG, Romanowski B, et al. HPV status in cervical adenocarcinoma. *Int J Gynecol Pathol*. 1995;14(2):134-140.
57. Park JS, Hwang ES, Park SN, et al. HPV gene expression in cervical cancer. *Gynecol Oncol*. 1997;65(1):121-129.
58. Bi Y, Tian Y, Chen Y, et al. HPV integration in cervical cancer. *Infect Agent Cancer*. 2023;18(1):72.
59. Nishio H, Matsubara A, Yoshida H, et al. Gastric-type cervical adenocarcinoma review. *Diagnostics (Basel)*. 2024;14(8):805.