

Review Article

PAX1/JAM3 methylation-a novel biomarker for early detection and accurate management of cervical adenocarcinoma

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Abstract: Cervical adenocarcinoma is a relatively special pathological type of cervical cancer. Due to its hidden location, low sensitivity of traditional cytology, and insufficient specificity of high-risk human papillomavirus (HR-HPV) detection, it is used in early diagnosis and accurate management. It presents huge challenges. Epigenetic markers represented by DNA methylation provide new breakthroughs in the diagnosis and treatment of cervical cancer. Among them, the dual-gene methylation detection of pairing box gene 1 (PAX1) and junction adhesion molecule 3 (JAM3) has become a new molecular marker with broad prospects because of its significant hypermethylation status in cervical adenocarcinoma tissues and excellent diagnostic sensitivity and specificity. This review summarizes the biological characteristics and carcinogenic mechanism of PAX1/JAM3 double gene methylation and the latest research progress in cervical adenocarcinoma screening and diagnosis, and discusses its application value, existing challenges and future development directions in order to provide new insights and directions for the pathogenesis research and clinical diagnosis and treatment strategies of cervical adenocarcinoma.

Keywords: Cervical adenocarcinoma, PAX1 gene, JAM3 gene, DNA methylation, precision diagnosis, biomarker

Introduction

Cervical adenocarcinoma (CA) is a special pathological type of cervical cancer, accounting for about 10%-25% of all cervical cancer cases. It originates from columnar epithelial cells in the endocervical membrane, and the focus location is hidden, resulting in the detection rate of early lesions by traditional liquid based cytology (TCT) based on morphology is significantly lower than that of cervical squamous cell carcinoma [1]. Despite the high sensitivity of high-risk human papillomavirus (HR-HPV) detection, the positive predictive value is low in adenocarcinoma and HPV infection is not detected in approximately 15% of CA cases, increasing the risk of missed diagnosis or misdiagnosis [2]. In addition, CA has a high risk of recurrence and metastasis after treatment, and the overall prognosis is poor. Therefore, objective molecular markers with high sensitivity and high specificity are urgently needed to make up for the

shortcomings of existing screening methods and realize early identification and accurate management of CA. DNA methylation is a key epigenetic modification mechanism, playing a central role in regulating gene expression, mediating transcriptional silencing and driving tumor development [3]. In recent years, several studies have confirmed [4], hypermethylation of PAX1 and JAM3 gene promoter regions is a key early molecular event in cervical carcinogenesis, especially in CA, showing high methylation levels and significant clinical relevance. Specifically, when the promoter region of PAX1 gene is hypermethylated, its transcriptional activity is inhibited, resulting in decreased protein expression, which in turn affects the normal expression of downstream target genes, and ultimately disrupts the balance between cell proliferation and differentiation, promoting malignant transformation of cervical epithelial cells [5]. At the same time, the abnormal methylation silencing of JAM3 gene not only weak-

ens the adhesion function between cells, but also induces epithelial-mesenchymal transition (EMT) by regulating multiple signaling pathways including HIF-1 α /VEGFA, thus promoting the migration and invasion ability of cervical cancer cells [6]. Therefore, it is very important to investigate the methylation status of PAX1 and JAM3 genes in CA for revealing the molecular mechanism behind tumor growth and evolution. This article aims to summarize the biological characteristics and carcinogenic mechanisms of PAX1 and JAM3 double methylation and the latest research progress in CA screening and diagnosis, focusing on its potential application in improving screening efficiency, accurately predicting lesion severity and dynamically evaluating treatment response. At the same time, the article will also sort out the key problems and challenges existing in the current research, and look forward to the future development direction, in order to provide a solid scientific basis for promoting the precise prevention and control strategy of CA.

Biological characteristics of methylation of PAX1 and JAM3 genes and its carcinogenic mechanism

Molecular characteristics of PAX1 gene and its carcinogenic mechanism

PAX1 (Pairedbox1) gene is an important member of Pairedbox gene family, which has nine members in mammals and encodes a series of highly conserved transcription factors, which are widely found in vertebrates and invertebrates [7]. PAX1 gene is located in human chromosome 20 short arm 1 region 1 band 2 subband (20p11.22) [8]. PAX proteins generally contain two conserved DNA binding domains, a paired domain (PD) and a homologous domain (HD), and a conserved octapeptide sequence (OP). PD consists of about 128 amino acid residues and is the core module that specifically recognizes and binds to target DNA sequences, HD consists of 61 amino acids and presents three alpha helices, and OP presents three alpha helices linked to HD. PAX genes can be further divided into four subgroups according to whether they have HD region at N-terminal and OP sequence, including PAX gene subgroups II (PAX2, PAX5 and PAX8) and III (PAX3 and PAX7), which are mainly involved in important processes such as cell survival, motility

and tumor progression, while subgroups I (PAX1 and PAX9) and IV (PAX4 and PAX6) are less important in cancer progression [9]. It is noteworthy that Rychel et al. found that PAX1 belongs to a subgroup lacking homology domains, and its DNA binding and transcriptional regulation functions mainly depend on pairing-domain mediated interactions [10, 11]. PAX1 plays an important role in tissue homeostasis, especially in the development of tissues derived from pharyngeal sac (such as thymus), parathyroid gland and skeletal system [12].

At the molecular level, recent studies have revealed that PAX1 can inhibit the classical Wnt signaling pathway. Specifically, PAX1 competes with SUMO E3 ligase PIASy for binding to transcription factor TCF7L2 (TCF4), thereby interfering with SUMOylation of TCF7L2, reducing its transcriptional activity and protein stability, and achieving negative regulation of Wnt signaling [13], as shown in **Figure 1**. This mechanism plays a dual role in definitive endoderm and foregut/pharyngeal endoderm cells differentiated from human embryonic stem cells (hESCs), which eventually develop into thymic epithelium, and is essential for normal development. Biallelic loss-of-function mutations in PAX1 have been identified as the cause of severe combined immunodeficiency (SCID) with orofacial neck syndrome type 2 (OTFCS2), the molecular basis of which is the impaired ability of PAX1 to inhibit Wnt signaling [14]. In addition to its central role in developmental biology, abnormal expression of PAX1 is closely related to the occurrence and development of various human tumors, especially confirmed as an important tumor suppressor gene [15]. Hypermethylation of its promoter region is a key mechanism leading to epigenetic silencing of this tumor suppressor gene in cervical cancer [13]. Numerous clinical studies confirm [16, 17], the methylation frequency of PAX1 gene promoter in cervical cancer tissues was significantly higher than that in normal cervical tissues, and the methylation level was positively correlated with the severity of cervical lesions, that is, from normal tissues, low-grade squamous intraepithelial lesions (LSIL) to high-grade squamous intraepithelial lesions (HSIL) and cervical cancer (CC), the methylation level increased in turn [18]. This hypermethylation leads to the silencing of PAX1 expression, which prevents

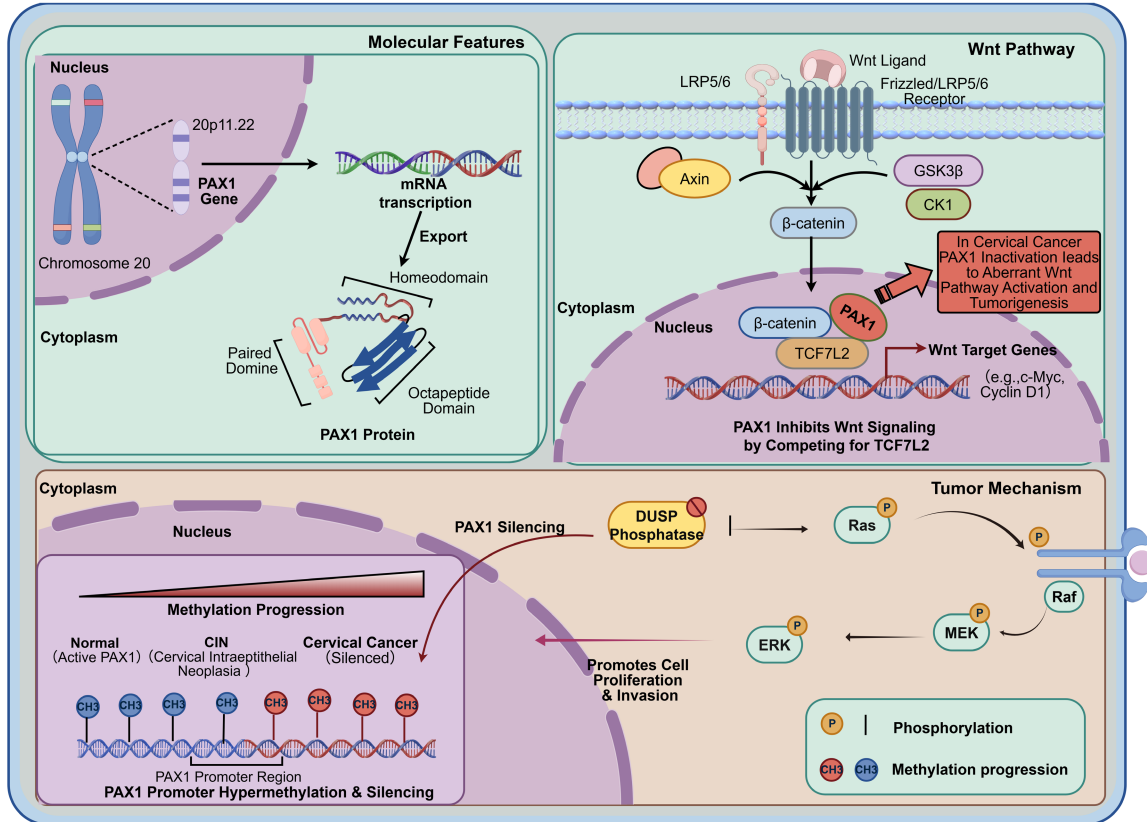


Figure 1. Subcellular localization and pathway molecular characteristics of PAX1 gene and cervical carcinogenesis Wnt pathway (by figdraw). PAX1 gene is located on chromosome 20 and encodes a protein containing multiple domains. In Wnt pathway, PAX1 gene is activated by competitive binding to TCF7L2 inhibitory pathway. In cervical cancer, PAX1 promoter hypermethylation silences PAX1 gene, which releases the inhibition of Wnt pathway and promotes cell proliferation and invasion.

PAX1 from inhibiting cell proliferation, regulating differentiation and inducing apoptosis by activating phosphatases such as DUSP1/5/6 and inhibiting tumor-promoting signaling pathways such as EGF/MAPK [19]. Therefore, PAX1, as a key transcription factor, plays an essential role in embryonic development and tissue homeostasis through its paired domain, which plays a role in DNA binding and transcriptional regulation. Loss of Wnt function (either through gene mutation or promoter hypermethylation) disrupts normal cell differentiation and proliferation regulation, leading to severe developmental diseases (such as SCID) on the one hand, and to the release of inhibition of oncogenic signaling pathways on the other hand, thus driving tumor initiation and development, especially inhibition of Wnt pathway, providing important theoretical basis for developing new therapeutic strategies.

Molecular characteristics and carcinogenesis of JAM3 gene

JAM3 (Junctional Adhesion Molecule3) is an important member of immunoglobulin superfamily (IgSF), located at chromosome 11 long arm region 2 band 5 (11q25). It plays a key role in cell adhesion, signal transduction and various physiological and pathological processes [20, 21]. The encoded product is a type I transmembrane glycoprotein containing two extracellular IG-like domains and an intracellular PDZ binding motif [22], they are integral to cell-cell contact and migration and are a key process in early tumor metastasis [23]. This structural feature determines its core biological function: mediating cell-to-cell adhesion by binding to homotypic or heterotypic receptors on the surface of adjacent cells, such as other JAM family members (the latter integrins), and thus acting as a “molecular glue” in maintaining

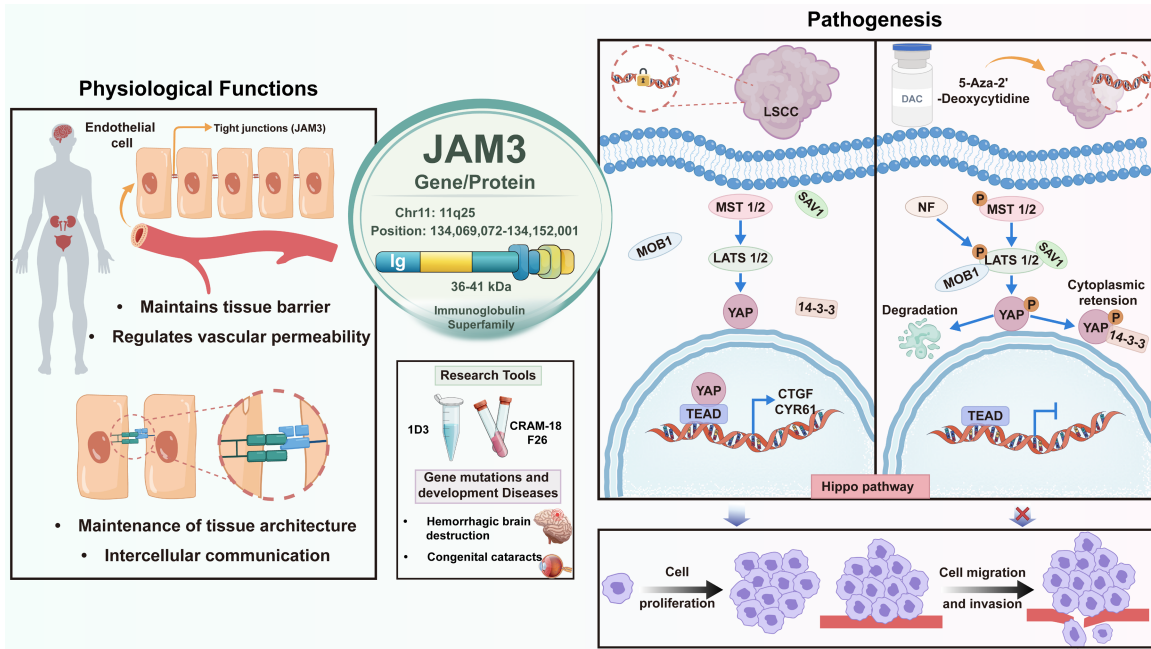


Figure 2. JAM3 gene function and regulatory mechanism diagram (by Adobe Illustrator). The left side shows JAM3 physiological functions, including maintaining tissue barriers and regulating vascular permeability; the middle introduces JAM3 gene localization and protein characteristics; the right side explains pathogenesis mechanism, revealing the influence of JAM3 abnormality on Hippo pathway and cell behavior.

the integrity of epithelial and endothelial tissue barriers [20, 24]. In addition, JAM3 gene expression is precisely controlled by upstream regulatory sequences, and its promoter/enhancer region contains multiple transcription factor binding sites, such as AP-1, COUP-TF, HNF-4 α , HOXA5, POU2F1, etc. These transcription factors may respond to different developmental signals, hormonal stimuli or stress signals, thus regulating JAM3 transcription level under specific spatial and temporal conditions.

However, recent studies have revealed that JAM3 functions far beyond physical adhesion. It is an active signal transduction molecule deeply involved in regulating cell fate. JAM3 exhibits clear tumor suppression function in a variety of solid tumors, as shown in **Figure 2**. For example, in esophageal cancer, numerous studies have demonstrated that JAM3 expression is epigenetically regulated by CpG island methylation in its promoter region. When JAM3 expression is normal, it can effectively inhibit the classical Wnt/ β -catenin signaling pathway, which directly translates into suppression of malignant phenotype of esophageal cancer cells: JAM3 overexpression significantly inhibits proliferation, clone formation, migration

and invasion of esophageal cancer cells, and induces cell cycle arrest in G1/S phase and apoptosis. These findings confirm that JAM3 acts as a tumor suppressor gene in esophageal cancer by inhibiting Wnt pathway [20]. In addition, recent studies indicate that JAM3 exerts tumor suppression in laryngeal squamous cell carcinoma (LSCC) mainly through activation of Hippo pathway, which is a key tumor suppressor pathway regulating organ size and cell proliferation [25]. When JAM3 is expressed normally, it helps to maintain the activity of Hippo pathway, thereby inhibiting the nuclear translocation of downstream oncogenic transcription coactivator YAP/TAZ and inhibiting abnormal proliferation and migration of cancer cells. In conclusion, JAM3 may be epigenetically downregulated and may function as a novel tumor suppressor gene in LSCC via the Hippo pathway, providing insights for the development of targeted therapies and diagnostics [26].

In addition to the above pathways, recent studies have also found [6] that JAM3 promotes the migration and invasion of cervical cancer cells by activating HIF-1 α /VEGFA pathway, mainly because JAM3 overexpression signifi-

cantly upregulates the mRNA and protein expression of HIF-1 α and VEGFA, thus activating HIF-1 α /VEGFA pathway. JAM3 promotes migration and invasion of cervical cancer cells by activating HIF-1 α /VEGFA pathway, mainly because overexpression of JAM3 significantly upregulates mRNA and protein expression of HIF-1 α and VEGFA, thus activating HIF-1 α /VEGFA pathway. JAM3 has also been reported to negatively regulate invasion, apoptosis and brain metastasis of breast cancer cells through TGF/Smad signaling pathway [27]. Interestingly, JAM3's role in tumors is not static, but rather is significantly "environment-dependent" or "cancer-specific". In contrast to its role as a tumor suppressor in solid tumors, JAM3 acts as a tumor promoter in hematological malignancies such as leukemia. The study found that JAM3 is highly expressed in leukemia-initiating cells (LICs), and maintains the self-renewal ability and dryness of LICs by activating the LRP5/AKT/ β -catenin/CCND1 signaling axis, thus driving the occurrence and development of leukemia [28]. In conclusion, JAM3 is a key molecule with multiple functions. Under normal physiological conditions, it is an adhesion molecule that maintains tissue barriers. In most solid tumors, it is an epigenetically silenced tumor suppressor gene, mainly through inhibition of Wnt pathway and activation of Hippo pathway. However, in cervical cancer, HIF-1 α /VEGFA pathway is activated to promote tumor cell migration and invasion, and in leukemia, it can be transformed into tumor promoting factors that maintain cancer dryness. This complex and important role transition makes JAM3 a valuable molecular target for understanding tumorigenesis and developing novel diagnostic markers and specific targeted therapeutic strategies.

Molecular mechanism of DNA methylation in cervical carcinogenesis

DNA methylation refers to the covalent modification of CpG dinucleotide sites of cytosine bases in DNA molecules, which is an epigenetic mechanism that can stably regulate gene expression without changing DNA sequence [29]. During tumorigenesis, CpG islands in the promoter region of tumor suppressor genes are hypermethylated abnormally, which will recruit inhibitory protein complexes and change chromatin structure, resulting in gene transcription

being silenced [30]. In cervical cancer, hypermethylation of PAX1 promoter region results in its expression being turned off. Studies have shown that PAX1 methylation level is significantly positively correlated with the severity of cervical lesions, and the incidence or level of methylation increases from normal cervix, low-grade lesions (LSIL) to high-grade lesions (HSIL) and cervical cancer (CC). For example, one study showed [31] that methylation of PAX1 gene diagnosed CIN1+, CIN2+ and CIN3+ at 0.52 (95% CI: 0.43-0.62), 0.88 (95% CI: 0.80-0.97), and 0.88 (95% CI: 0.75-1.00), and in HPV 16/18 negative patients, the methylation assay had a sensitivity of 32.4% and specificity of 83.7% for CIN1+, and a sensitivity of 100% for both CIN2+ and CIN3+. PAX1 may form a complex with WDR5 and SET1B to enhance methylation of histone H3K4, thereby activating the expression of phosphatases such as DUSP5 and PTPRR and regulating the phosphatase-kinase balance of cervical epithelium [19]. When PAX1 is silenced by methylation, this balance is disrupted and kinase signaling pathways are overactivated, driving abnormal cell proliferation, resistance to apoptosis, and transformation to malignant phenotypes. JAM3 is an important protein that maintains cell-cell junction and barrier function, and also participates in signal transduction. Hypermethylation of its promoter also leads to gene silencing. As a member of the junction adhesion molecule family, epigenetic silencing of JAM3 directly weakens the physical connection and polarity of epithelial cells, creating preconditions for tumor cell exfoliation and invasion. One investigator confirmed [32] that methylation testing successfully identified 2 hrHPV and liquid-based cytology (LBC)-negative adenocarcinoma cases and 7 non-16/18 hrHPV infected patients missed by LBC. PAX1 and JAM3 methylation levels were higher in older CIN2+ women than in younger women, and were independent of HPV type. Mechanically speaking, the silencing of JAM3 promotes tumor progression from another dimension. The loss of JAM3 function directly destroys the tight junction and barrier integrity of epithelial cells, which not only facilitates local invasion of tumor cells, but also affects leukocyte migration and angiogenesis, changes tumor microenvironment and promotes metastasis. In addition, JAM3 may exert its anti-tumor effect by affecting Hippo, PI3K/Akt and other pathways closely related to cell

proliferation and survival, and its silencing will release the inhibition of these pathways. Methylation of PAX1 and JAM3 often occurs simultaneously, resulting in synergistic effects. Studies have shown that [32] PAX1/JAM3 methylation detected CIN2+ lesions with sensitivity of 93.2% [95% CI: 85.7%-100%], CIN3+ lesions with sensitivity of 97.2% [95% CI: 91.9%-100%], the specificity of CIN1+ lesions was 93.6% [95% CI: 89.9%-97.3%], Better than LBC (CIN2+/CIN3+ lesions with sensitivity: 75% [95% CI: 60.9%-89.1%]; the specificity of: 52.3% [95% CI: 44.9%-59.8%]). This double gene silencing may disrupt the normal homeostasis of cells more comprehensively from two aspects of “intracellular signal regulation” and “intercellular interaction and microenvironment regulation”, and greatly accelerate the carcinogenesis process. Therefore, the dual-gene methylation test of PAX1 combined with JAM3 has been proved to have higher diagnostic sensitivity and specificity than single-gene testing, and has become the focus of clinical attention.

The role of PAX1/JAM3 double methylation in the diagnosis and treatment of cervical adenocarcinoma

Improve screening sensitivity and specificity, optimize screening and early diagnosis

Traditional cervical cancer screening relies mainly on cytology (TCT) and high-risk human papillomavirus (hrHPV) detection, but there are limitations in sensitivity, specificity and ability to detect adenocarcinoma. PAX1/JAM3 double gene methylation test, as a molecular diagnostic technology based on DNA epigenetic changes, provides an innovative solution for improving the accuracy of screening by quantifying stable molecular markers in diseased tissues. Its core value lies in that it can more objectively and directly reflect the malignant transformation status of cervical epithelial cells, thus significantly optimizing the sensitivity and specificity of screening.

As hrHPV positive shunt tool: achieving precise risk stratification

For the large hrHPV-positive population, accurately distinguishing transient infections from patients at risk for high-grade cervical lesions (CIN2+) is also a central challenge for clinical management. PAX1/JAM3 double gene methylation

assay showed excellent shunt efficiency in this segment, especially in high specificity and high positive predictive value. A recent study [33] evaluating cytology, histopathology, and DNA methylation at multiple sites in 360 HR-HPV patients showed that in CIN2+, combined methylation testing of candidate genes such as PAX1 and JAM3 showed 97.87% (95% CI: 0.954-0.990) specificity, higher than any single gene methylation test. Additionally, several studies have shown that PAX1 methylation testing has higher sensitivity and specificity than conventional HPV testing and TCT, Wang et al. [34] demonstrated that the sensitivity, specificity and accuracy of PAX1 in detecting HSIL were 0.77, 0.88 and 0.90, respectively. Studies have also confirmed [35] that when this technology is used to diagnose high-grade cervical intraepithelial lesions (CIN2+), the sensitivity is 89.0% and the specificity is 95.3%, which is significantly better than traditional cytology and can make unnecessary referrals for colposcopy. The rate is reduced by approximately 50%-60%. This means that individuals with positive test results are highly likely to have high-grade lesions, thus providing a strong molecular basis for clinical decisions to immediately perform colposcopy and intervention, effectively avoiding over-diagnosis.

In addition, PAX1/JAM3 methylation detection outperformed traditional joint strategies in diagnostic accuracy. Studies have further confirmed [33] that the accuracy of detecting CIN2+ by combining hrHPV typing (such as HPV 16/18) with polygenic methylation test is significantly higher than that of hrHPV typing and cytological test. Li et al. Found [36] that the specificity of HPV 6/E7 mRNA detection (37.32%) and PAX1 methylation test (97.18%) were significantly higher than HR-HPV test. By calculating the area under the curve (AUC) of cervical cancer, PAX1 methylation test had the largest AUC (0.919) for HSIL+ diagnosis, which was significantly higher than HPV 6/E7 mRNA detection and HR-HPV test. This further indicates that PAX1 has high detection efficiency and can be used as an important tool for future screening. When combined with high-risk human papillomavirus (HR-HPV) testing or thin-panel cytology (TCT), it significantly improves the specificity of screening and facilitates accurate triage and management of high-risk patients [37]. This establishes the molecular

basis for methylation testing as a better triage tool after hrHPV positivity, increasing the robustness of the overall screening pathway.

Accurate identification of cytologically abnormal populations: compensating for morphological diagnostic uncertainty

There is uncertainty in the management of patients with cytological findings of atypical squamous cells of equivocal significance (ASC-US) or low-grade squamous intraepithelial lesions (LSIL). PAX1/JAM3 methylation detection does provide objective molecular supplementation that not only significantly improves sensitivity and specificity, but also identifies occult adenocarcinoma lesions. In ASC-US/LSIL populations [38], methylation assays for single genes (e.g. PAX1 or JAM3) already have some sensitivity (approximately 64.8%-70.3%) to CIN2+, whereas dual gene combinations increase sensitivity to 83.8% while maintaining high specificity of 95.8%. This combination of high sensitivity and specificity makes it superior to HPV-DNA testing alone in distinguishing high-grade lesions in women with mild cytological abnormalities, providing more reliable decision support for clinical practice. The cytological characteristics of cervical adenocarcinoma are atypical, and the association with HPV is more complex than squamous cell carcinoma. The missed diagnosis rate of traditional screening methods is high, which becomes the difficulty of clinical diagnosis. Some studies have shown [39], that double gene methylation testing can effectively identify occult adenocarcinoma lesions that are negative for hrHPV or missed cytologically. This finding is decisive for compensating for the current screening system's relative insensitivity to adenocarcinoma and is expected to reduce the missed diagnosis rate of adenocarcinoma. In a recent multicenter study [40], PAX1/JAM3 methylation was found to be significantly more positive than high-risk HPV in cytologically negative patients with histologically confirmed adenocarcinoma. Therefore, dual-gene combination testing provides a powerful molecular solution to complement the current status of screening for adenocarcinoma insensitivity.

Achieving dynamic risk stratification and prognostic prediction: accurate management from qualitative to quantitative

It is worth noting that PAX1/JAM3 methylation levels are not only diagnostic, but also closely

related to disease severity and clinical outcome. Studies have shown [41], that methylation levels (ΔCt values) are significantly negatively correlated with cervical pathology results, i.e., the lower the ΔCt value (representing higher methylation levels), the more severe the lesion. This quantitative relationship allows the test results to reflect the degree of molecular grade malignancy of the lesion. Based on quantitative results, more refined management strategies can be implemented in the clinic. Based on quantitative results, more refined management strategies can be implemented clinically. For example, high levels of methylation strongly suggest a risk of high-grade disease or invasive cancer, suggesting immediate referral to colposcopy [42], while mild methylation may reflect a lower risk state or early precancerous disease, and conservative strategies such as shorter follow-up intervals and enhanced surveillance can be adopted [43]. This risk stratification based on molecular indicators is the key to achieving precision medicine. Some studies have also shown [32] that the methylation level of PAX1 and JAM3 genes is significantly correlated with prognostic indicators such as recurrence risk and survival outcome of patients. Hypermethylation status before treatment often indicates poor prognostic characteristics, such as lymph node metastasis and deep stromal invasion. Among them, PAX1 hypermethylation was closely associated with shorter disease-free survival and higher recurrence rate, consistent with its silencing as a tumor suppressor gene [44], while JAM3 hypermethylation was significantly associated with lower overall survival rate [45]. Combined detection of methylation status of the two drugs can evaluate prognosis more accurately and provide evidence for clinical treatment decision. Studies have confirmed [46] that its dynamic changes can be used as an important biomarker for monitoring efficacy. If methylation continues to be positive or does not decrease significantly after cervical conization, it often indicates an increased risk of residual lesions or recurrence. Dynamic monitoring of methylation levels during chemoradiation and chemotherapy can also help to assess treatment response and predict recurrence risk, thus providing molecular basis for individualized follow-up management [47]. In addition, methylation status was an independent predictor of treatment outcome [48], and the sensitivity and specificity of JAM3 gene methylation

status in cervical exfoliated cells for predicting recurrence after treatment were as high as 94.8% and 93.7%, respectively, in patients with CIN2/3 after treatment. This means that the test can effectively identify patients at high risk of recurrence, guiding closer follow-up and early intervention where necessary.

Personalized diagnosis and treatment strategy: precise navigation based on epigenetic markers

Cervical adenocarcinoma presents a significant challenge to existing treatments because of its unique biological behavior, showing a higher tendency to relapse and metastasis than squamous cell carcinoma. Patients with early CA usually have a good prognosis after radical surgery (such as total hysterectomy), with a 5-year survival rate of 70% to 90%. However, patients with advanced or recurrent CA have a significantly reduced 5-year survival rate, and their efficacy is affected by multiple factors such as treatment options and individual response differences [49]. Tumor microenvironment (TME) composed of immune cells and stromal cells plays a key role in the development of CA [50], and its complex cellular interaction network further increases the difficulty of treatment. Multidimensional comprehensive intervention strategies are urgently needed. In this context, personalized diagnosis and treatment strategies based on the methylation status of PAX1 and JAM3 genes are moving from theoretical exploration to clinical practice, showing great potential for transforming epigenetic information into precise treatment decisions. Studies have shown that gene methylation status is closely related to the efficacy of different treatment modalities [51]. For patients with early CA, surgery is the primary treatment option, but its effectiveness may be affected by methylation levels. The study suggests [52], that hypermethylation of the PAX1 gene may reduce susceptibility to surgical resection, and surgery alone may not be sufficient to remove all high-risk cells in such patients. Therefore, the results of preoperative methylation detection can be used as an important reference to prompt doctors to consider the combination of drugs targeting DNA methylation (such as demethylation drugs) in the perioperative period, in order to restore the function of tumor suppressor genes before or

after surgery, and eliminate potential micrometastases to consolidate the surgical effect. At the same time, for early patients with strong fertility intentions, methylation levels can also be used as a key indicator to assess the risk of recurrence after fertility preservation surgery (such as cervical conization), helping to make a more precise trade-off between organ preservation and risk reduction. In the treatment of advanced CA, chemoradiotherapy effects are also associated with double gene methylation status, e.g. JAM3 gene methylation may reduce chemotherapy response by weakening cell adhesion and enhancing invasion and drug resistance [53]. Therefore, when patients experience elevated levels of JAM3 methylation during treatment, this is not only a signal of poor response, but may also point to a specific resistance mechanism. Based on this epigenetic signature, doctors can select targeted treatment options that can overcome this mechanism, such as targeted drugs targeting cell invasion or specific signaling pathways, thereby improving the accuracy and success of subsequent treatments.

In addition, PAX1/JAM3 methylation detection has the advantage of being quantifiable and reproducible, making it an ideal dynamic monitoring tool during treatment. Regular detection of changes in methylation levels during radiotherapy, chemotherapy or targeted therapy allows real-time assessment of treatment response. For example, a significant decrease in methylation levels after treatment often indicates a good response to treatment, whereas persistent or elevated levels strongly indicate possible drug resistance or disease progression [4]. This allows clinical management to shift from a traditional “treatment-wait-image assessment” model to an active closed-loop model of “treatment-real-time molecular monitoring-timely protocol adjustment”, providing the possibility to intervene before imaging or clinical symptoms of disease appear. Long-term management after treatment is also critical to improving survival in CA patients.

Multivariate analysis confirmed [39, 54], that PAX1/JAM3 methylation levels were independent prognostic factors affecting progression-free survival (PFS) and overall survival (OS). Survival curves clearly showed that patients with high methylation levels had significantly

lower survival rates than those with low methylation levels. Therefore, double gene methylation detection plays an irreplaceable role in prognosis assessment and recurrence monitoring. In the future, further integration of other molecular markers and clinical parameters to build a multidimensional prognostic model is expected to significantly improve the accuracy and overall efficacy of CA diagnosis and treatment [55]. Through the above epigenetic marker-based individualized strategy, CA patients are expected to achieve substantial improvement in long-term survival and quality of life.

Challenges to clinical translation and future research directions

Challenges of clinical transformation

The methylation of PAX1/JAM3 double gene involves complex interactions between multiple signaling pathways and transcription factors, which significantly increases the difficulty of related research. PAX1, as a key tumor suppressor gene, is regulated by multiple epigenetic modification enzymes, mainly relying on DNA methyltransferase (DNMTs) as the core complex, DNMTs catalyze CpG island cytosine methylation, directly shut down gene transcription [13]. At the same time, histone deacetylase (HDACs) activity is closely related to DNA methylation, histone deacetylation usually forms dense chromatin structure, which cooperates with DNA hypermethylation to construct an inhibitory epigenetic environment, so that PAX1 and other tumor suppressor genes are stably silenced [56]. The methylation of JAM3, an important member of cell adhesion molecule family, may be involved in the initiation and development of CA by interfering with Wnt/ β -catenin [20], PI3K/Akt [57], and other key signaling pathways. Loss of signal transduction may affect cell survival and migration by interfering with multiple pathways including PI3K/Akt. In particular, recent studies have revealed a previously underappreciated mechanism of JAM3 tumor suppression [25], in squamous cell carcinoma of the larynx, JAM3 exerts tumor suppression by activating Hippo signaling, and its epigenetic silencing leads to inhibition of Hippo signaling, which drives tumor progression. This suggests that JAM3 may also play a role in cervical cancer by regulating Hippo, a core pathway that controls organ size and cell

proliferation. Therefore, PAX1/JAM3 silencing may not result in a single pathway abnormality, but may cause the imbalance of Wnt/ β -catenin, PI3K/Akt, Hippo and other key oncogenic networks jointly or separately, and there are complex cross-dialogues among these pathways, forming a second layer of regulatory complexity [26]. Furthermore, interactions between environmental factors and genetic background may have profound effects on methylation status [58]. This multi-layered and multidimensional regulatory system requires further understanding of the biological function of PAX1/JAM3 double methylation in CA. By further analyzing this complex network, we are expected to further develop PAX1/JAM3 methylation from a powerful biomarker to a key target for understanding disease mechanisms and developing novel therapies.

Although PAX1/JAM3 double gene methylation detection has shown potential clinical value in CA screening and diagnosis, it still lacks uniform standards in sample collection, detection technology and result interpretation standards [59], which seriously restricts the reliability and popularization of this technology in clinical practice. Therefore, it is urgent to establish standardized test procedures and consistent result determination thresholds (Δ Ct values) to ensure comparability and consistency of test results between different laboratories [43]. Previous studies have focused on validation of diagnostic accuracy (sensitivity, specificity), but large-scale confirmatory studies in a wide range of populations, especially geographically and ethnically diverse populations, are still limited. The long-term effect of reducing the incidence and mortality of cervical adenocarcinoma needs to be confirmed by prospective, large-scale, multicenter cohort studies. In addition, the high cost of methylation detection based on high throughput sequencing has become a key obstacle to its widespread application. Clinical efficacy data on PAX1/JAM3 double gene methylation detection are still limited, especially the lack of confirmatory studies in a wide range of populations, resulting in its guiding role in clinical decision making. In the future, it is necessary to systematically evaluate whether the combined benefits of this technology as a screening or shunt tool in reducing unnecessary colposcopy referrals, avoiding overtreatment, and early detection of occult

lesions (especially adenocarcinoma) are sufficient to offset its increased detection costs. Therefore, promoting technological innovation to reduce detection costs and actively carrying out high-quality clinical validation studies are the core paths to realize the transformation of this detection technology from scientific research to clinical practice.

Future research direction

PAX1/JAM3 double gene methylation detection is an emerging tool for accurate screening and diagnosis of CA, and its clinical translation has achieved initial results. However, in order to ultimately reduce the incidence and mortality of cervical cancer (especially adenocarcinoma), future research needs to be deepened and expanded in multiple dimensions. First, future research should focus on exploring other epigenetic markers associated with PAX1/JAM3 methylation, and construct more refined molecular typing systems by conducting multigene methylation joint analysis. For example, PAX1 and JAM3 can be compared with other genes that have been shown to be abnormally methylated in cervical cancer. (such as SOX1, DAPK1, etc.). These genes may jointly regulate biological behaviors such as proliferation, invasion and immune escape of tumor cells through different signaling pathways (such as Wnt, PI3K-AKT, etc.) [60]. Through bioinformatics analysis and large-scale clinical cohort data, mapping multigene methylation regulatory networks including PAX1/JAM3 can not only improve the detection sensitivity of high-grade lesions (CIN2+), but also distinguish different pathological subtypes (such as squamous cell carcinoma and adenocarcinoma) and predict the risk of disease progression, providing a basis for realizing truly individualized risk assessment. In addition, methylation data alone are still scarce, and the frontier in the future lies in the fusion of multiomic data, i.e., the integration of DNA methylation with genomic mutations, transcriptome expression, proteome and immune microenvironment characteristics. For example, abnormal expression of key hub genes such as CDKN2A and BIRC5 has been found in cervical adenocarcinoma, and is associated with PI3K-AKT signaling pathway and immune cell invasion depth [61]. Combining such expression profile data with methylation status can more fully reveal the driving

mechanism of tumor, so as to identify more powerful prognostic models and new therapeutic targets. At the same time, on the basis of existing HPV screening, further clarify the best strategy of methylation detection as a shunt tool. Research should focus on determining a more accurate methylation threshold (Cut-off value) to balance sensitivity and specificity, and explore its unique diagnostic value in special populations (such as postmenopausal women, type 3 transformation zone, HPV negative adenocarcinoma) to make up for the blind spots of traditional screening methods. Future studies may explore targeted therapeutic strategies for PAX1/JAM3 aberrant methylation, such as demethylation drugs (e.g., decitabine) to attempt to restore the function of these tumor suppressor genes. It is also possible to study upstream regulatory enzymes (such as DNMTs) that specifically interfere with methylation based on gene editing technology or small molecule inhibitors [62] providing a new potential pathway for preventive intervention and treatment of cervical cancer.

Summary and prospect

As an innovative molecular diagnostic strategy based on epigenetic changes, PAX1/JAM3 double gene methylation detection makes up for the lack of sensitivity of traditional screening methods in identifying cervical adenocarcinoma and provides key support for risk identification of core pathological stages. Its complete clinical application closed-loop value has been preliminarily verified from early diagnosis, risk stratification to efficacy monitoring and prognosis evaluation. The future of PAX1/JAM3 double gene methylation research is a systematic deepening process from double genes to multi-omics networks, from technology to application. With the continuous standardization of detection technology, systematization of operation process and continuous enrichment of clinical evidence-based basis, this marker is expected to be formally incorporated into the comprehensive prevention and control system of cervical cancer, especially playing an irreplaceable role in accurate screening and individualized management of cervical adenocarcinoma, providing solid technical support for further improving and accelerating the strategic goal of eliminating cervical cancer.

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Disclosure of conflict of interest

None.

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