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Editorial

Koilocytes in the era of molecular viral detection: Still a useful cytopathic effect of human papillomavirus?

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Dear Editor,

Historically, koilocytes have been recognized as essential cytopathic changes to identify the presence and pathogenic effects of human papillomavirus (HPV) in cervical smears.[1] As cervical smears became an established worldwide method for the early detection of HPV-related cervical lesions, cytopathologists were trained to recognize characteristic koilocytic and dyskeratotic alterations to support the diagnosis of viral infection.[2]

The mechanisms underlying the formation of koilocytes, with a characteristically hyperchromatic nucleus and perinuclear vacuolation, are not entirely clear.[3,4] However, HPV oncoproteins are proposed to play a role in producing this cytopathic effect. In particular, the small HPV E5 oncoprotein is proposed to drive koilocytosis through its C-terminal region, while E6 potentiates this effect.[5] More recently, the HPV E4 protein was also proposed to play a role in inducing koilocytosis.[6] While some animal papillomavirus also cause similar cytological changes, others are preferentially associated with cytoplasmic basophilia, large keratohyalin granules, and dyskeratosis.[7] These findings indicate that different viral genotypes induce different cytopathic effects, reinforcing the role of viral oncogenes and their protein products.

Besides cervical cancer, infection with low-risk and high-risk HPV is associated with a number of benign and malignant lesions in multiple anatomic locations, including laryngeal papillomatosis, oropharyngeal squamous cell carcinoma, as well as intraepithelial neoplasia, condylomas, and squamous cell carcinomas of non-cervical genital regions such as the vulva, vagina, penis, and anus.[8-10] Beta-HPV genotypes have also been implicated in cutaneous carcinogenesis.[11] In addition, there are limited data suggesting the involvement of HPV in some specific subsets of breast cancer. However, additional research is needed to demonstrate the putative etiological role of HPV in breast cancer.[12]

The diagnosis of HPV lesions includes morphological and molecular analyses to demonstrate the presence of the virus as well as its etiologic role, including cytopathological and histopathological techniques, polymerase chain reaction (PCR)-based methods for detecting viral DNA,

real-time PCR and *in situ* hybridization methods for confirming transcription of viral ribonucleic acid and the immunohistochemical detection of $p16^{Ink4a}$ as a surrogate marker of HPV activity.[13] In recent years, PCR-based methods for the detection of HPV have been adopted in multiple world regions as a cost-efficient alternative for cervical cancer screening, either replacing traditional cytological methods or in co-testing approaches with cervical smears.[14-16]

Considering these developments, one should ask what the relevance of cytopathic lesions such as koilocytes is now that HPV is detected through molecular techniques? Are these cytological alterations still useful, and what are their current applications? Perhaps unsurprisingly, koilocytes remain relevant in multiple settings, both for diagnostic and research purposes.

Cervical smears remain in use for confirming the occurrence of viral lesions in PCR-positive cases[16], and koilocytes remain a central cytopathological finding in this context. In addition, the presence of koilocytes is also helpful to classify HPV-associated lesions in some, but not all, non-cervical anatomic locations.

In penile squamous cell carcinomas, koilocytes are useful cytological features that help distinguish histological subtypes associated with HPV (such as warty and warty-basaloid tumors) from others that are HPV-independent, such as verrucous and papillary tumors.[17] However, immunohistochemical detection of $p16^{Ink4a}$ is considered the preferred method for assigning penile lesions into HPV-related and HPV-independent groups.[18]

Among head-and-neck cancers, HPV plays a growing role in oropharyngeal squamous cell carcinomas[10] while in non-oropharyngeal head-and-neck sites, the incidence of HPV-related cancer is much lower. In oral and oropharyngeal sites, koilocytes are not considered a reliable predictor of $p16^{Ink4a}$ positivity.[19,20] The clinical significance of oral koilocytic dysplasia remains a matter of discussion.[21] Importantly, there is limited evidence to suggest that, in non-oropharyngeal head-and-neck sites, koilocytosis may define squamous cell carcinoma subsets with a particularly favorable prognosis.[22] In anal cytology, koilocytes are not a frequent finding,[23] and histological data suggest that they are more often found in high-grade intraepithelial lesions rather than in low-grade or invasive carcinoma lesions.[24] In Bowen's disease, koilocytosis is preferentially associated with HPV-positive rather than HPV-negative lesions, but koilocytes are not considered reliable morphological markers for HPV-related Bowen's disease.[25]

Interestingly, koilocytes have also been suggested as markers of breast cancer subgroups potentially linked to HPV.[12] However, the role of HPV in breast cancer remains highly controversial, and further research is required before the potential use of this morphological marker in breast pathology is defined.

To this date, koilocytes remain an essential feature of HPV-induced lesions in multiple settings. Nonetheless, its applicability in diagnostic procedures is variable, and in some anatomic locations, koilocytes are not considered reliable markers of HPV-induced pathology. In fact, the biopathological changes leading to koilocyte formation are only partially understood. It will be interesting to elucidate the mechanisms underlying those cytopathic changes, as they may help understand how HPV differentially affects different non-cervical anatomic locations. Recent genome editing technologies should be useful to support these research efforts. Overall, the biology and the clinical relevance of koilocytes require further refinement through additional research.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

ABBREVIATIONS

DNA: Deoxyribonucleic acid
HPV: Human papillomavirus
PCR: Polymerase chain reaction
RNA: Ribonucleic acid

AUTHOR CONTRIBUTIONS

HOB and RMGC: Concept or design of the study; LM: Acquisition of data; HOB, LM, and RMGC: Drafting the article or revising it critically for important intellectual content; HOB, LM, and RMGC: Final approval of the version to be published; HOB, LM, and RMGC: Aptitude to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript. All authors meet ICMJE authorship requirements.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

EDITORIAL/PEER REVIEW

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