# Papillary Immature Metaplasia and Thin High-Grade Squamous Intraepithelial Lesion Originate in Early Metaplastic Epithelium of the Cervix

*To the Editor.*—We read with interest the article by Hong et al[1] describing 26 cases of papillary immature metaplasia (PIM) of the cervix. The authors conclude that this variant of low-grade squamous intraepithelial lesion (LSIL) originates from human papillomavirus (HPV)-infected cells at or proximal to the squamocolumnar junction (SCJ) via "top-down and bottom-up differentiation," whereas LSILs reported as condylomata originate from HPV-infected cells more distal to the SCJ. We agree that PIMs and condylomata originate from epithelia of different differentiation and maturation, but we find the terms of "top-down and bottom-up differentiation" and the postulated origin of these 2 lesions from cells "more distal or proximal" of the SCJ problematic.

Anatomically, the SCJ is the border between the squamous epithelium and the mucin-producing columnar epithelium of the cervix.[2] Most gynecologists consider the transformation zone (TZ) as the area where squamous metaplasia has occurred. But the TZ can also be considered the area where squamous metaplasia potentially *may* occur; this area reaches from the original squamous epithelium across the cervix and along the endocervical canal, including the crypts, to the epithelium of the uterine isthmus.[2] As a rule, squamous metaplasia starts at or near the SCJ. Transformation is preceded by the appearance of subcolumnar reserve cells, initially as a single layer. With progressive proliferation, immature and mature squamous metaplasia become manifest. Squamous metaplasia may be a focal process and be bordered by mucin-producing columnar epithelium, both on the surface and in glands.[2] It is unclear to us why the biologic processes of reserve cell proliferation/hyperplasia and subsequent squamous metaplasia/dysplasia should be complicated by the terms "top-down and bottom-up differentiation."[1,3]

Condylomata usually arise after low-risk (LR) HPV infection of a well-developed stratified squamous epithelium; thus, these lesions show thick epithelium with koilocytes in the superior cell layers. The mature epithelium allows the LR HPVs to complete the productive life cycle with assembly of the viral particles. This process is accompanied by expression of the late HPV genes L1/L2. High-risk (HR) HPV infection of a stratified squamous epithelium can result in thick high-grade squamous intraepithelial lesion (HSIL; Figure 1). By contrast, PIMs develop from LR HPV infection of reserve cells or early metaplastic squamous epithelium only a few cell layers thick. Infection of the same early immature metaplastic epithelium or proliferated reserve cells with HR HPVs will lead to a thin HSIL.[4] Thin HSILs develop de novo directly as HSILs and do not contain koilocytes (Figure 2).

Current thinking suggests that the epithelial site and the differentiation of the infected epithelium along with the HPV subtypes predicts outcome.[5] The key is that both PIMs and thin HSILs develop from early immature metaplastic epithelium or reserve cell proliferation of the TZ on the surface and/or in the crypts. In conclusion, PIMs of the cervix represent the low-grade equivalent of thin HSILs. We suggest a simple distinction between (1) HPV infection of proliferated reserve cells/early immature metaplasia, and (2) HPV infection of mature squamous metaplasia (Figures 1 and 2).

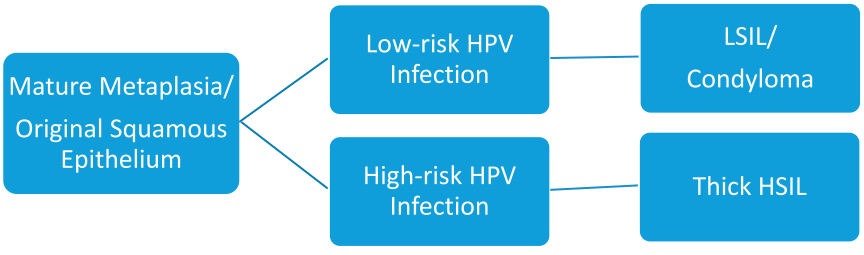


**Figure 1.** *Outcome after human papillomavirus (HPV) infection of stratified squamous epithelium of the cervix. Abbreviations: HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.*

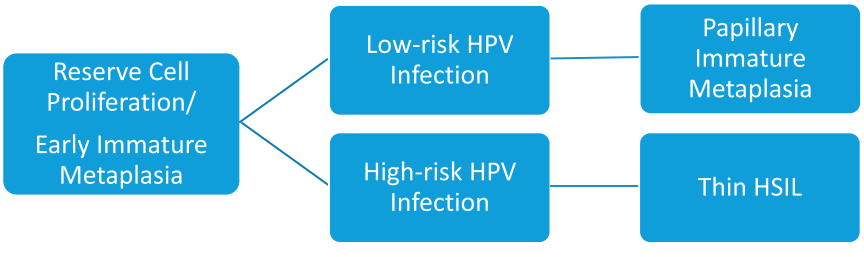


**Figure 2.** *Outcome after human papillomavirus (HPV) infection of nonstratified squamous epithelium of the cervix. Abbreviation: HSIL, high-grade squamous intraepithelial lesion.*

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*In Reply.*—We appreciate the submitted comments.

Reich and Regauer's hypothesis is not so different from ours.[1] We also believe

that low-grade squamous intraepithelial lesion (LSIL)/condyloma arises from original squamous epithelium of the exocervix or mature squamous metaplasia that arises from the columnar cells in the transformation zone and goes through the process of reserve cell proliferation, immature metaplasia, and then maturation (mature metaplasia). We also believe that papillary immature metaplasia (PIM) arises from immature squamous metaplasia infected with human papillomavirus (HPV). However, as shown in our figures (Figure 1, A and C through F), PIM can mature and disclose mature squamous epithelium and koilocytosis in many cases, as the presence of mucin-secreting cells in the middle layers of mature squamous epithelium and on top of the papillary lesions signifies.

Our additional thought was why and how PIMs have papillary configuration in most cases whereas the LSIL/condyloma are flat. The normal endocervical mucosa has a papillary configuration (see Figure 4, A). Additionally, PIM is more frequently identified in the endocervix and the transformation zone than elsewhere. Thus, we interpret this appearance of PIM as a variant of LSIL that had originated from HPV-infected immature squamous metaplastic cells at or proximal to the squamocolumnar junction.

"Top-down differentiation" in our paper denotes the process whereby the single-layered reserve cell formation is induced from the columnar epithelium lying above. "Bottom-up differentiation" refers to the reserve cell proliferation and immature and then mature squamous differentiation from the single-layered reserve cells. It explains not only the mechanism of PIM formation, but also for general process by which immature and mature squamous metaplasia forms.

However, we cannot fully agree that only the mature squamous epithelium allows low-risk HPV to complete the productive life cycle with assembly of the viral particles. Immature squamous epithelium infected with HPV likely also goes through the maturation process, then permitting the assembly process of the viral particles producing koilocytosis to complete, as our cases show.

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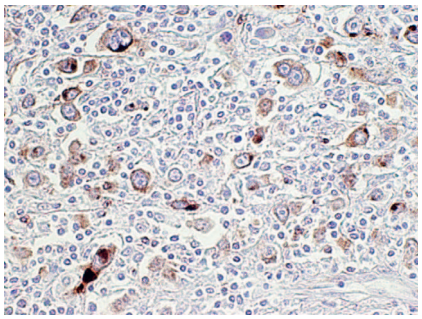
## Validation of CD137 Immunohistochemical Stain on Paraffin-Embedded Tissue as a Marker to Facilitate Distinction Between Classic Hodgkin Lymphoma, Nodular Lymphocyte–Predominant Hodgkin Lymphoma, T-Cell/Histiocyte-Rich Large B-Cell Lymphoma, and Anaplastic Large Cell Lymphoma

*To the Editor*.—Remarkable efforts are underway to exploit classic Hodgkin lymphoma's (cHL) complex interplay of molecular alterations, loss of B-cell programming, and dependency of tumor microenvironment.[1] One potential novel immunotherapeutic target, anti-CD137, was recently addressed by Makkouk et al.[2] CD137 is a member of the tumor necrosis factor receptor superfamily and has been shown to induce proliferation and enhance the survival and function of T cells, natural killer cells, and dendritic cells. Conceptually, agonistic anti-CD137 is categorized as a costimulatory immunotherapy with the potential to manipulate the tumor microenvironment and accentuate antitumor response. Ectopic expression of CD137 has been demonstrated in Hodgkin and Reed--Sternberg (HRS) cells and is thought to play a role in the reduction of the antitumor response.[3,4]



*Immunohistochemical stain for CD137 performed on paraffin-embedded tissue sections (cytoplasmic and membranous). Thermo Scientific, monoclonal BBK-2 clone (original magnification ×400).*

Anderson et al[5] reported significant sensitivity of immunohistochemical (IHC) staining for CD137 on HRS cells (179 of 208; 86%), comparable to that of CD15 staining. Their data also demonstrated CD137 positivity in several cHL cases where HRS cells were negative for CD15. Additionally, CD137 IHC was negative in all lymphocyte-predominant cells of nodular lymphocyte–predominant Hodgkin lymphoma (NLPHL) cases evaluated (0 of 17). They also observed that CD137 IHC is a relatively clean stain with little nonspecific background staining.[5]

We were primarily interested in the usefulness of CD137 IHC to facilitate the diagnosis of cHL. We constructed tissue microarrays from formalin-fixed, paraffin-embedded tissue including 42 cases of cHL, 33 cases of NLPHL, 16 cases of anaplastic large cell lymphoma, and 7 cases of T-cell/histiocyte–rich large B-cell lymphoma. We validated anti-CD137 (Thermo Scientific, monoclonal BBK-2 clone) using protease retrieval and a dilution of 1:100 (Figure). Tissue microarrays were then stained with CD137 as well as CD15, CD20, and CD30 and reviewed by 2 pathologists. Positive staining was assigned if 20% or more of the malignant cells were positive regardless of strength of staining. Of the cHL cases, 76% were positive for CD137, 85% were positive for CD15, 100% were positive for CD30, and 13% were positive for CD20. Interestingly, CD137 was positive in all cHL cases where CD15 was negative.