

Detailed description of high-risk HPV RNA in situ hybridization patterns in endocervical adenocarcinoma and correlation with p16 immunohistochemistry

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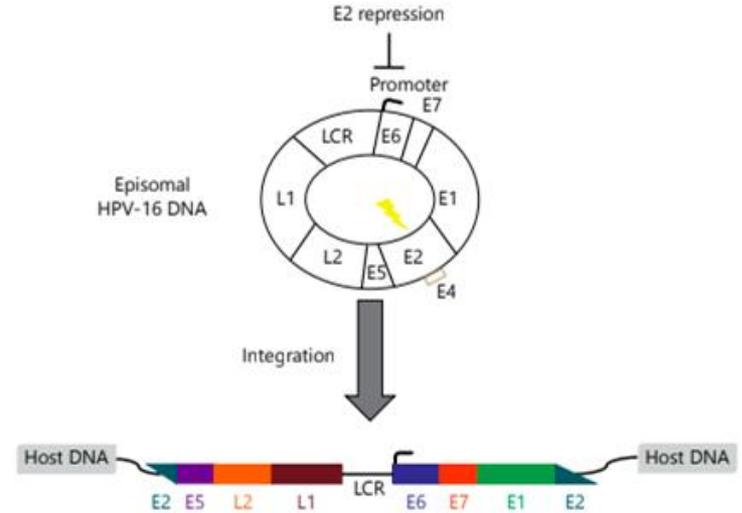
Conflict of interest

No conflict of interest to declare.

HPV functions in two forms: Episomal and Integrated.

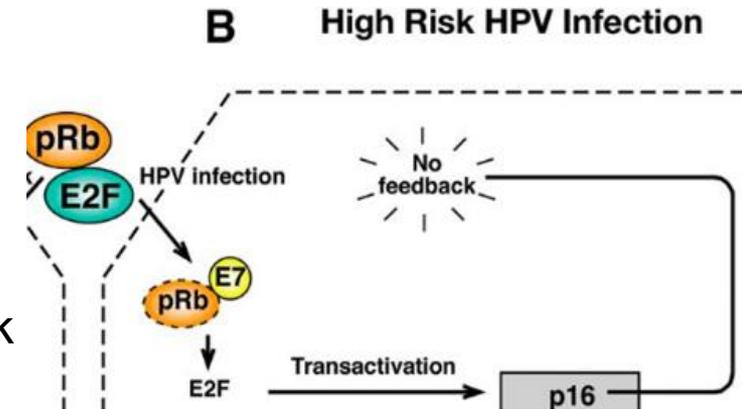
HPV infection (in nutshell)

- ❖ Access to basal epidermal layer
- ❖ Endocytosis and viral capsid disassembly (L2 mediated, episomal form)
- ❖ Viral genome replicates in synchrony with host cell DNA (E1,E2, E4,E5)
- ❖ E2 open reading frame disruption > viral genome integrates into the host DNA
- ❖ E2 no longer inhibits E6 and E7.



HPV E7 protein Hijacks normal function of pRB, leading to overexpression of p16 gene

- ❖ P16 is a tumor suppressor protein > can be overexpressed in different tumors
- ❖ HPV E7 protein binds to pRB, preventing inactivation of E2F > cell cycle activation > overexpression of p16 with no negative feedback



P16 IHC is being used as a surrogate marker for HPV-associated lesions

In squamous lesions (cervix, vulva/vagina, anal) (Darragh et al, The CAP-ASCCP LAST Project Arch Pathol Lab Med—Vol 136, October 2012)

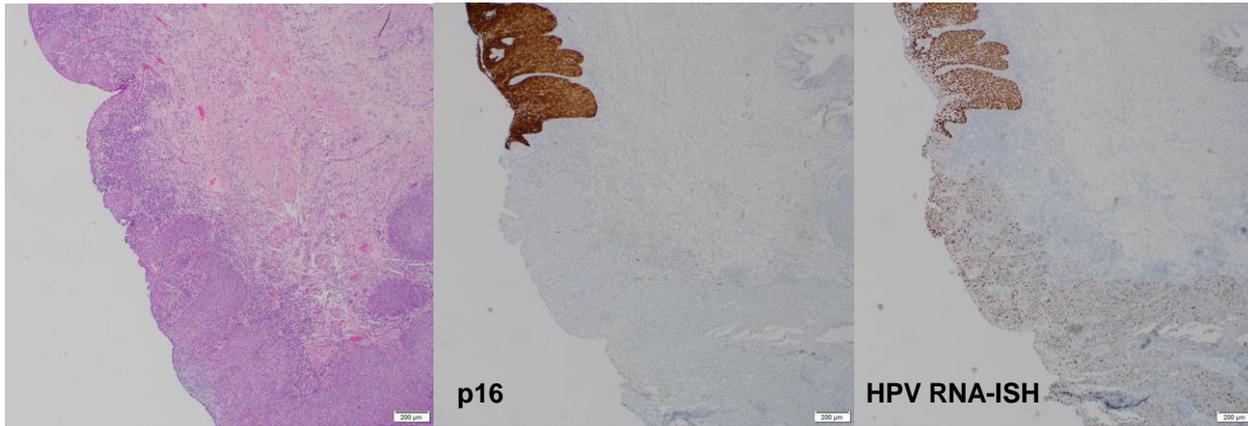
- ❖ HSIL vs mimickers
- ❖ CIN1 vs CIN2 (with the caveat that 40-50% LSILs are p16 positive)
- ❖ How much is positive? 6 contiguous cells horizontally above lower $\frac{1}{3}$ (Am J Surg Pathol Volume 41, Number 5, May 2017).

In glandular lesions:

- ❖ HPV-related glandular lesions vs benign
- ❖ HPV-related glandular vs non-HPV related lesions

P16 IHC is not always cooperating!

- ❖ Rare cases of “null” p16 in squamous intraepithelial lesions, possibly due to CDKN2A gene methylation or allelic loss (Proc Natl Acad Sci U S A. 1999;96:1254–1259, Virchows Arch 2011 Feb;458(2):221-9)



- ❖ False positive cases > tubal metaplasia, endometrial carcinomas

High-risk HPV ribonucleic acid in situ hybridization (HR HPV RNA-ISH): a very sensitive tool for detection of HPV infected epithelia.

- ❖ mRNA E6/E7 transcripts > integration of the viral genome in host's > a decisive indicator of biological activity
- ❖ More robust than HR DNA ISH > 84% of HPV DNA ISH (-) HNSCC were RNA ISH (+) with 94% concordance with p16 (Bishop et al. Am J Surg Pathol. 2012 Dec; 36(12): 1874–1882.)
- ❖ More robust than HPV PCR in squamous lesions (Milles et al. Am J Surg Pathol Volume 41, Number 5, May 2017)
 - ❖ HPV PCR is not necessarily an indicator of viral genome integration
 - ❖ 40% of PCR invalid and 20% of PCR (-) had evidence of HPV infection

HR HPV RNA-ISH signal patterns has been studied in squamous intraepithelial lesions (PLoS One. 2014 Mar 13;9(3):e91142.)

❖ Two distinct patterns:

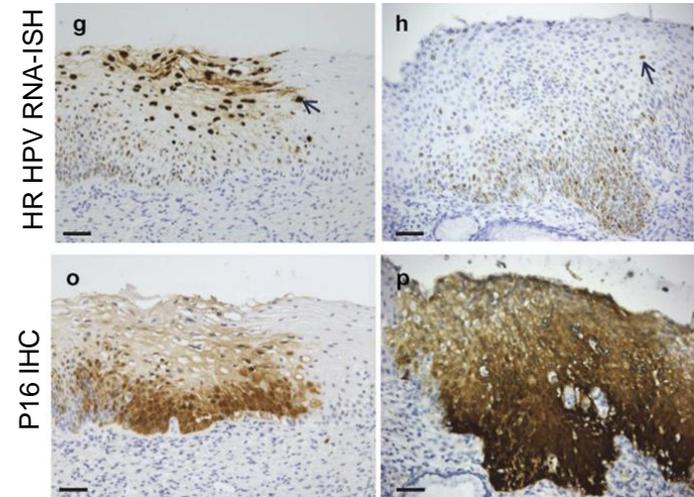
- Diffuse nuclear: 81% of CIN1 and 63% of CIN2 (productive phase)

- Punctuate (nuclear and cytoplasmic): (transformative phase)

❖ Diffuse nuclear pattern resistant to RNase treatment > suggests some DNA hybridization when DNA load is high

❖ CIN 1 with diffuse p16 showed punctate integrated signal pattern

CIN 1 cases with different p16 and RNA-ISH patterns



Does HR HPV RNA-ISH signal pattern in cervical adenocarcinoma correlate with p16 IHC?

Objectives:

- ❖ To study different pattern of p16 staining in cervical adenocarcinoma
- ❖ To evaluate any correlation of p16 and HR HPV RNA ISH pattern of staining

Material and Methods:

- ❖ Two TMAs composed of 200 cases of invasive endocervical adenocarcinoma (1994 -2015) including 6 cores for each case
- ❖ P16, Ki-67 IHC and HR HPV RNA-ISH (ACD RNAScope).

HPV RNA chromogenic in situ hybridization scoring:

- ❖ Overall viral load - apparent on high magnification (40x) ;apparent on medium magnification (10x); apparent on low power (4x)
- ❖ Nuclear signal coarseness - signal <10% of nuclear diameter; 10-20%; >20%
- ❖ Percentage of nuclei with coarse signal
- ❖ Pattern of nuclear signal - single, dual, multiple
- ❖ Amount of cytoplasmic signals - not present; rare or difficult to find; abundant or easy to find; 2- in between
- ❖ Presence of “reservoir-like cells” (cells with very dense load of cytoplasmic signals which covers the nucleus)

P16 IHC scoring:

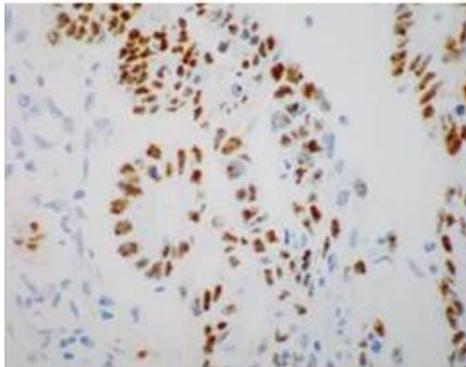
- ❖ Positive: diffuse, strong, block-like
- ❖ weak/variable positive: diffuse but weak; strong but patchy (variable in different cores)
- ❖ Negative

Ki-67 scoring:

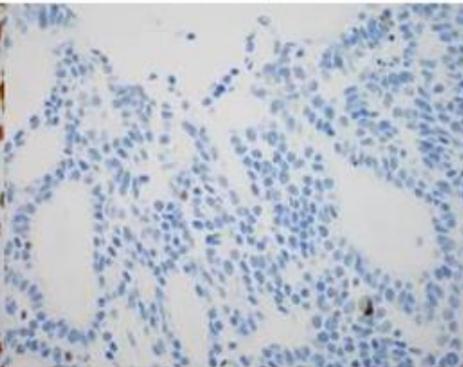
- ❖ Quantifying the positive malignant nuclear staining (percentage)

Various patterns of HPV-ISH staining

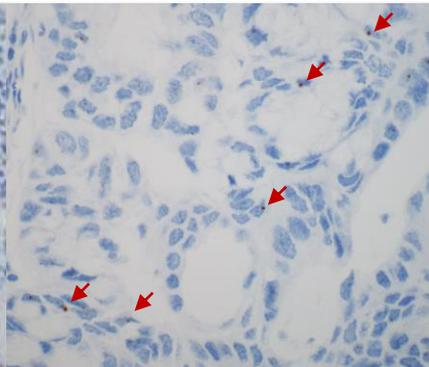
Viral load score 3 with multiple fine and coarse nuclear signals



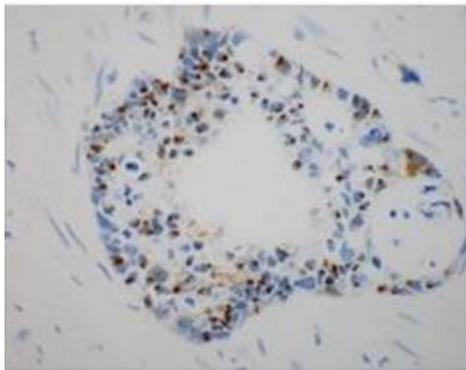
Viral load score 2 with single coarse nuclear signals



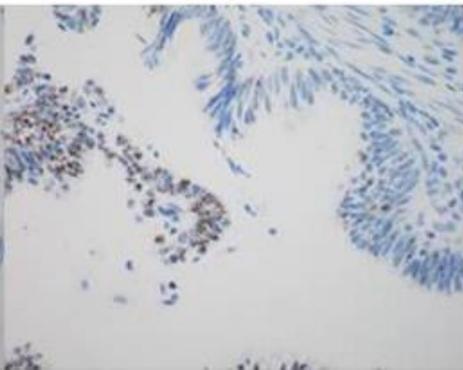
Viral load score 1 with fine nuclear signals



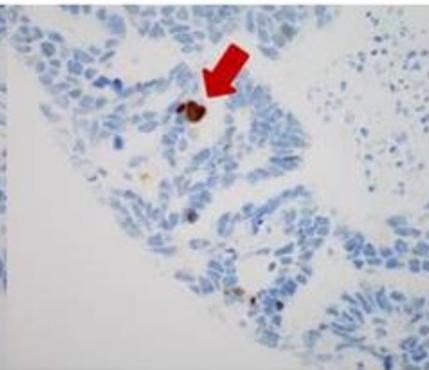
Single /dual coarse nuclear signals with abundant cytoplasmic signals



Multiple coarse nuclear signals with scattered cytoplasmic signals



Reservoir-like signals



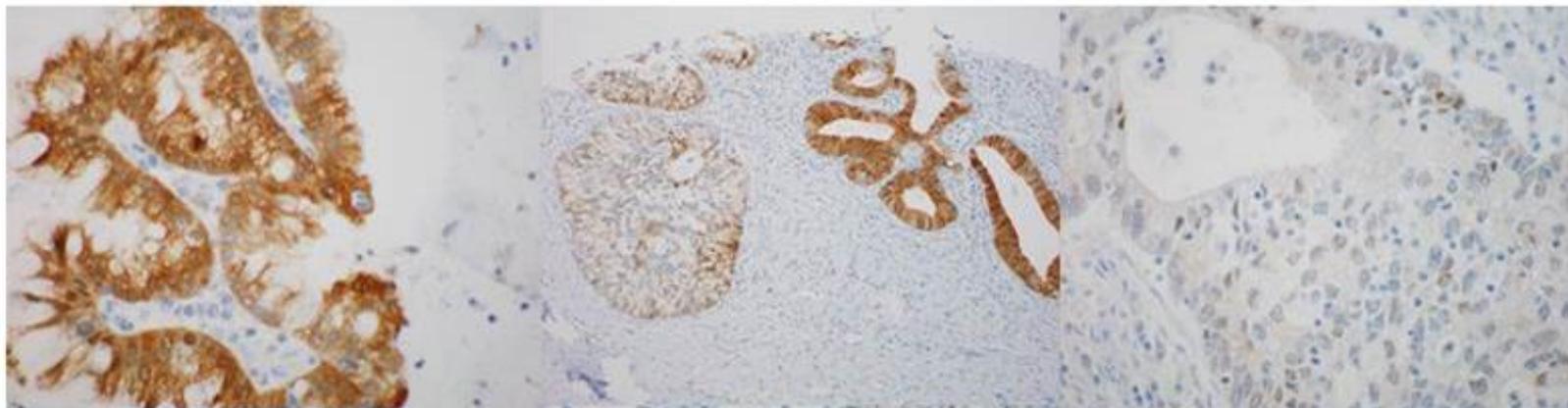
Patterns of p16 staining in HPV-ISH positive groups

Strong and diffuse

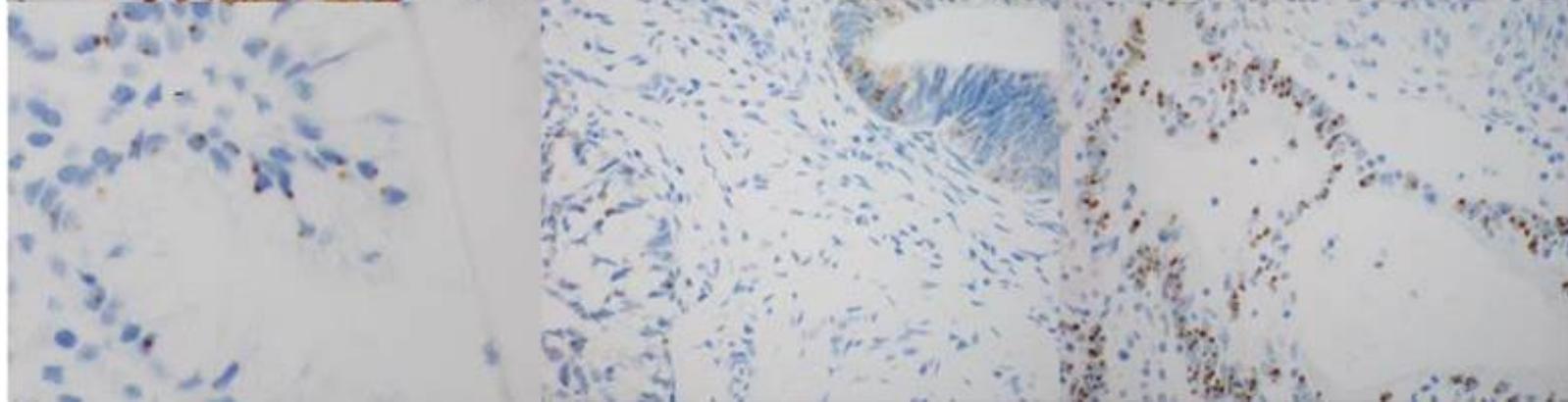
Weak and variable

Negative

P16
IHC



HPV
ISH



P16 IHC and HR HPV RNA ISH concordance rate in cervical adenocarcinoma: 79.4%

	HR HPV RNA-ISH	P16	# (%)
G1	Negative	Negative	32 (18.4%)
G2	Positive	Positive	106 (61%)
		Block-like	69 (39.7%)
		Variable/weak	37 (21.3%)
G3	Negative	Positive	8 (4.5%)
		Block-like	2 (1.1%)
		Variable/weak	6 (3.4%)
G4	Positive	Negative	28 (16.1%)

174 cases had conclusive p16 staining and HR HPV ISH results

Ki-67 proliferation index is higher in p16 (+) group regardless of HPV status * and in HPV (+) group regardless of p16 status **

Ki-67 proliferation index median		P16	
		negative	positive
HR HPV RNA ISH	negative	G1: 35% (n=32)	G3: 33% (n=8)
	positive	G4: 43% (n=28)	G2: 55% (n=106)

Mann-Whitney U test: * p-value: 0.013, ** p-value: ;0.016

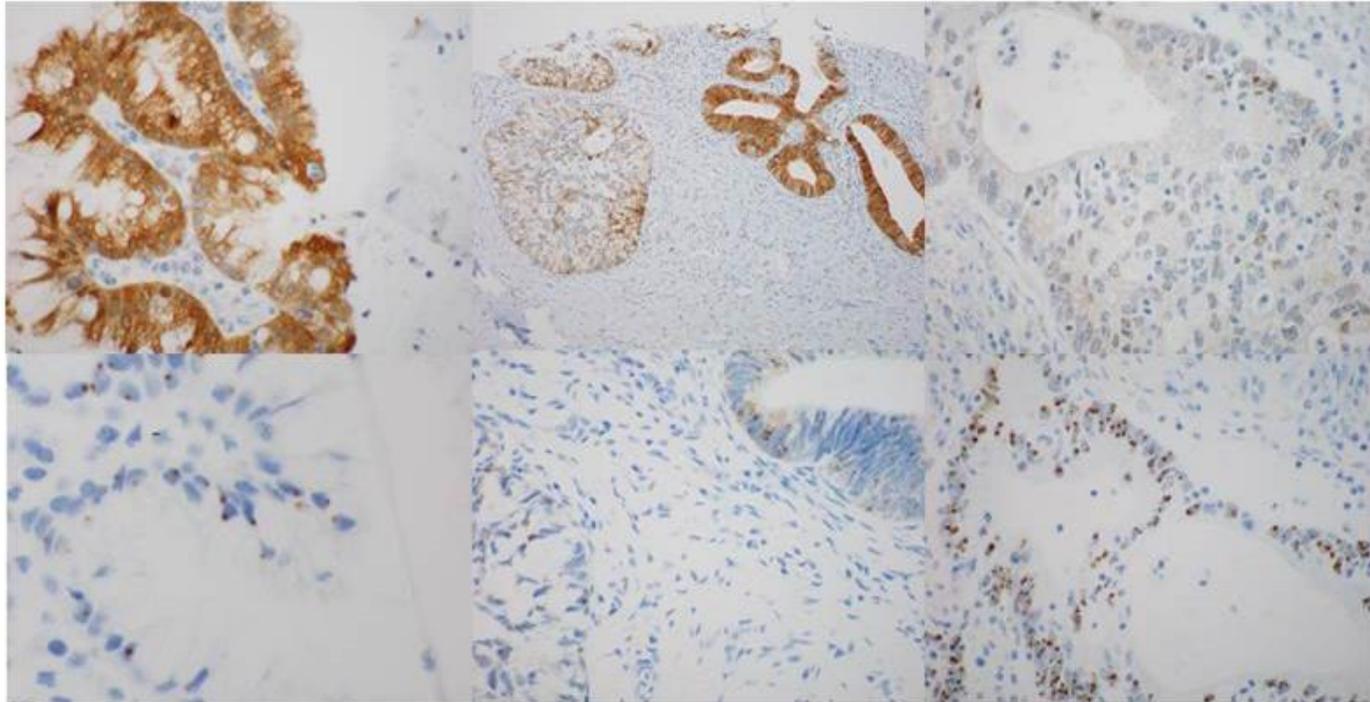
P16 status did not correlate with viral load (number of signals in the tumor cells)

Strong and diffuse

Weak and variable

Negative

P16
IHC



HPV
ISH

Summary

- ❖ P16 is not entirely sensitive or specific for detecting HPV in cervical adenocarcinomas
 - ❖ In a large scale study (n=174), more than a third of HPV-related endocervical adenocarcinoma were either totally negative (16%) or focally/weakly positive (21.3%) for p16.
 - ❖ Rare cases of NHPVA showed diffuse p16 staining.
- ❖ HPV RNA-ISH patterns in endocervical adenocarcinoma are varied.
 - ❖ Coarse, fine, single, multiple, nuclear, cytoplasmic
- ❖ P16 status does not necessarily correlate to the quantity and intensity of HR HPV RNA-ISH positivity.
- ❖ Diffuse strong p16 is associated with higher proliferation index.
- ❖ HR HPV RNA-ISH is recommended to distinguish HPV versus non-HPV related cervical adenocarcinoma.

Thank you
for your attention.

Any questions?



	G1: HPV -/p16-	G2: HPV+/p16+ (wk/var)	G3: HPV-/p16+ (wk/var)	G4: HPV-/p16-
Usual	14	56 (19)	3 (1)	20
Adenosquamous	4	12 (2)	2 (2)	1
Intestinal	3	4	0	1
Gastric-type	1	2 (1)	0	0
Endometrioid	2	2 (2)	0	0
iSMILE	0	2	0	0
Clear cell	1	3 (2)	0	0
Serous	1	0	0	1
NOS	2	1 (1)	0	0
Unknown	4	24 (11)	3 (3)	5
	32	106	8	28

HR HPV RNA-ISH signal patterns has been studied in squamous intraepithelial lesions

- ❖ Episomal versus integrated pattern (J Clin Pathol. 1991 May; 44(5): 406–409.)
- ❖ Higher percentage of basal layer cells with punctate (integrated pattern) signals (versus diffuse “episomal” signal patterns) by HR HPV DNA ISH may predict progression to CIN2/3 (Gynecologic Oncology 112 (2009) 114–118).
- ❖ Higher number of punctate signals predicts progression of CIN2 to CIN3 (Am J Clin Pathol 2007;128:208-217).
- ❖ A small cohort of CIN 1 cases with diffuse p16 showed diffuse integrated signal pattern (PLoS One. 2014 Mar 13;9(3):e91142.).

CIN 1 cases with different p16 and RNA-ISH patterns

