抄読会(2023/12/15)

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p16 overexpression and Rb loss correlate with high-risk HPV infection in oropharyngeal squamous cell carcinoma

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【要旨】

背景：

HPV の感染は中咽頭扁平上皮癌（OPSCC）の発症における重要な要因の 1 つであり、p16 免疫組織化学によって HPV を検査することが推奨されている。また p16 陽性の OPSCC は p16 陰性の OPSCC と比べ予後良好であり、UICC 第 8 版において TMN 分類のダウンステージングが記載された。p16 免疫組織化学 は HPV 感染に対して非常に感受性が高いが、完全な特異的代替マーカーではなく、HPV 感染率が高い欧米と比較し HPV 感染率が低い地域や国では一致率が低くなる傾向がある。

また HPV 感染をより正確に検出するために、PCR や DNA in-situ hybridization、RT-PCR、RNA in-situ hybridization などの HPV 検査がある。しかし p16 免疫組織化学よりも技術的に高度かつ高価であるため広く利用できない。

本研究では、OPSCC 症例における HPV 感染と p16 および Rb の発現状態を調べ、HPV 感染および患者予後との関係性について調べた。

対象と方法：

177 例の OPSCC 症例における p16 および Rb の発現状態を免疫組織化学で確認し、

HPV 感染の有無は mRNA in-situ hybridization を使用した

結果：

177 例中、p16 + /HPV + ( n = 105, 59.3%)、p16 + /HPV − ( n = 8, 4.5%)、p16 − /HPV − ( n = 64, 36.2%) 群に分けられた (Table 2)。p16 + /HPV - ( n = 8) および p16 - /HPV - ( n = 64) の群は、p16 + /HPV + の群 ( n = 105)よりも全生存期間 (OS) が悪化する、もしくは OS が有意に悪化する傾向があった (それぞれ P = 0.0610、P = 0.0004)。(Figure 4)

Rb の染色状態を preserved expression (> 90 %、n = 68)、partial loss ( 10- 90 %、n = 97)、および complete loss (< 10%、n = 12) に分類した (Figure 1, 補足)。HPV 陽性症例 (n = 105) の Rb の染色パターンは partial loss (n = 97 /105、92.4%) と complete loss (n = 8 /105、7.6%) で、preserved expression の症例は認めなかった (n=0 /105, 0%)。一方、HPV 陰性の症例 (n = 72) では、Rb の染色パターンは preserved expression (n = 68 /72、94.4%)、と complete loss (n = 4 /72、5.6%) があり、partial loss は認めなかった (n=0/72, 0 %)。p16 単独染色例と比較し、p16 と Rb 染色の partial loss / complete loss の併用は、優れた感度(それぞれ 100%)と特異度(88.9% 対 97.2%)および陽性的中率(92.9 % 対 98.1 %)を示した。(Table 3)

結論：

p16 単独例と比較し、p16 と Rb の併用例は信頼性が高く費用対効果の優れた方法であることが示唆された (Figure 3)。しかし p16 や Rb 免疫組織化学の併用で HPV 陽性・陰性が確定しない場合は、mRNA in-situ hybridization などの HPV 検査を追加する必要がある。

【take home message】

- p16 陽性が必ずしも HPV 陽性とは限らず、ダウンステージングによって患者に不利益になる可能性がある。

**Table 1.** Clinicopathological findings of 177 cases of OPSCC

| | | All ($n$ = 177) |
|---|---|---|
| Age (y) | Mean (range) | 62.8 (37–84) |
| Sex (%) | Male | 147 (83.1) |
| | Female | 30 (16.9) |
| History of smoking (%) | Yes (current/former) | 138 (78) |
| | No (never) | 30 (16.9) |
| | Unknown | 9 (5.1) |
| Tumor site (%) | Anterior wall | 33 (18.7) |
| | Lateral wall | 132 (74.6) |
| | Posterior wall | 2 (1.1) |
| | Superior wall | 10 (5.6) |
| T-stage (%) | T1/T2 | 103 (58.2) |
| | T3/T4 | 74 (41.8) |
| N-stage (%) | N0 (N-) | 37 (20.9) |
| | N1,2,3 (N+) | 140 (79.1) |
| Clinical stage, 7$^{th}$ UICC (%) | I/II | 24 (13.6) |
| | III/IV | 153 (86.4) |
| Clinical stage, 8$^{th}$ UICC (%) | I/II | 97 (54.8) |
| | III/IV | 80 (45.2) |
| Clinical stage, modified 8$^{th}$ UICC (%) | I/II | 95 (53.7) |
| | III/IV | 82 (46.3) |
| Tumor differentiation (%) | KSCC | 64 (36.2) |
| | NKSCC | 113 (63.8) |
| Event after initial therapy (%) | Residual tumor (PD, SD, PR) | 14 (7.9) |
| | CR followed by recurrence/metastasis | 35 (19.8) |
| | Durable CR | 125 (70.6) |
| | Unknown | 3 (1.7) |

**Table 1.** (*Continued*)

| | | All ($n$ = 177) |
|---|---|---|
| Last contact (%) | No evidence of disease | 118 (66.6) |
| | Alive with disease | 7 (4) |
| | Died of disease | 45 (25.4) |
| | Died of other cause | 7 (4) |

UICC, Union for International Cancer Control; KSCC, keratinising squamous cell carcinoma; NKSCC, non-keratinising squamous cell carcinoma; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response; OPSCC, oropharyngeal squamous cell carcinoma.

**Table 2.** Association between clinicopathological variables and HR-HPV infection in 177 cases of OPSCC

| Variable | *n* = 177 | HR-HPV ISH Positive 105 (59.3%) | HR-HPV ISH Negative 72 (40.7%) | *P*-value |
|---|---|---|---|---|
| Age, years | | | | |
| ≤ 60 | 69 | 47 (31.9%) | 22 (68.1%) | 0.0397* |
| > 60 | 108 | 58 (59.3%) | 50(40.7%) | |
| Sex | | | | |
| Male | 147 | 85 (57.8%) | 62 (42.2%) | 0.4195 |
| Female | 30 | 20 (66.7%) | 10 (33.3%) | |
| History of smoking | | | | |
| Yes | 138 | 73 (52.9%) | 65 (47.1%) | 0.0005* |
| No | 30 | 26 (86.7%) | 4 (13.3%) | |
| Unknown | 9 | 6 (66.7%) | 3 (33.3%) | |
| Tumour site | | | | |
| Anterior | 33 | 12 (36.4%) | 21 (63.6%) | < 0.0001* |
| Lateral | 132 | 93 (70.5%) | 39 (29.5%) | |
| Posterior | 2 | 0 (0%) | 2 (100%) | |
| Superior | 10 | 0 (0%) | 10 (100%) | |
| T-stage | | | | |
| T1/T2 | 101 | 69 (68.3%) | 32 (31.7%) | 0.0056* |
| T3/T4 | 76 | 36 (47.4%) | 40 (52.6%) | |
| N-stage | | | | |
| N0 | 37 | 12 (32.4%) | 25 (67.6%) | 0.0003* |
| N1, 2, 3 | 140 | 93 (66.4%) | 47 (33.6%) | |
| Clinical stage, 7th UICC | | | | |
| I/II | 24 | 7 (30.4%) | 17 (69.6%) | 0.0016* |
| III/IV | 153 | 98 (63.6%) | 55 (36.4%) | |
| Clinical stage, 8th UICC | | | | |
| I/II | 97 | 78 (80.4%) | 19 (19.6%) | <0.0001* |
| III/IV | 80 | 27 (33.8%) | 53 (66.2%) | |
| Clinical stage, modified 8th UICC UICC UICC UICC | | | | |
| I/II | 95 | 78 (82.1%) | 17 (17.9%) | <0.0001* |
| III/IV | 82 | 27 (32.9%) | 55 (67.1%) | |
| Tumour differentiation | | | | |
| KSCC | 64 | 18 (28.1%) | 46 (71.9%) | <0.0001* |
| NKSCC | 113 | 87 (77.0%) | 26 (23.0%) | |
| p16-IHC | | | | |
| Positive | 113 | 105 (92.9%) | 8 (7.1%) | < 0.0001* |
| Negative | 64 | 0 (0%) | 64 (100%) | |
| Rb-IHC | | | | |
| Preserved | 68 | 0 (0%) | 68 (100%) | < 0.0001* |
| Partial loss | 97 | 97 (100%) | 0 (0%) | |
| Complete loss | 12 | 8 (66.7%) | 4 (33.3%) | |

HR-HPV, high-risk human papillomavirus; ISH, *in-situ* hybridisation; UICC, Union for International Cancer Control; KSCC, keratinising squamous cell carcinoma; NKSCC, non-keratinising squamous cell carcinoma; IHC, immunohistochemical staining.
*Statistically significant.

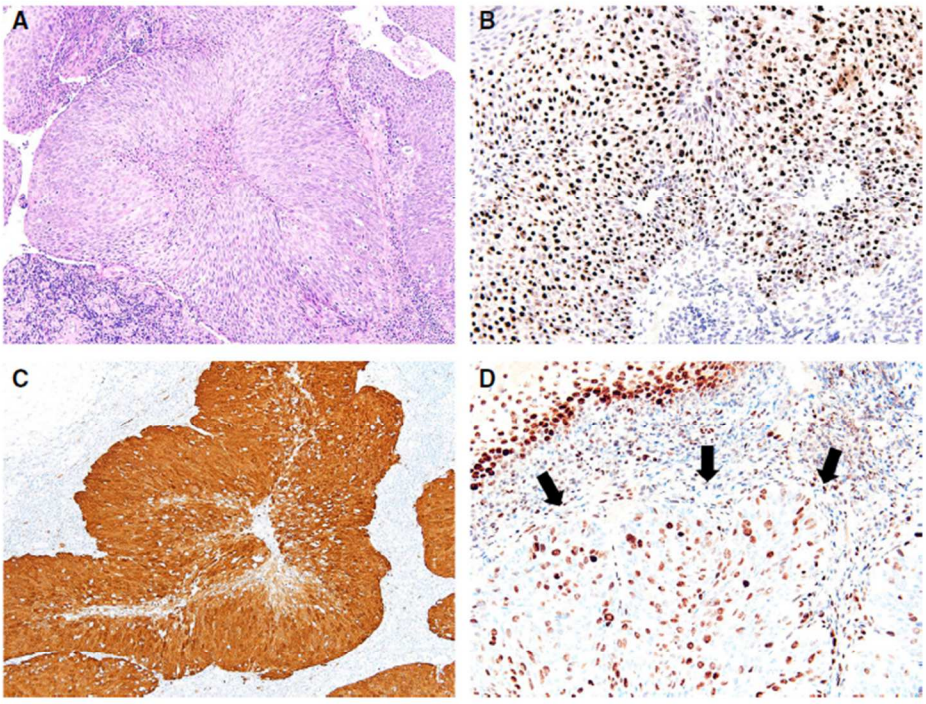



Figure 1. Representative histology, p16 overexpression and Rb loss in high-risk human papillomavirus (HR-HPV)$^+$ oropharyngeal squamous cell carcinomas (OPSCCs). Non-keratinising squamous cell carcinoma (A, haematoxylin and eosin). Nuclear and cytoplasmic signals are diffusely observed by HR-HPV mRNA *in-situ* hybridisation (ISH) (B). Diffuse nuclear and cytoplastic expression of p16 is revealed by IHC (C). Cancer cells show a partial loss of Rb nuclear expression by IHC (indicated by arrows), while non-neoplastic squamous epithelium (left upper) and internal lymphocytes are positive (D).

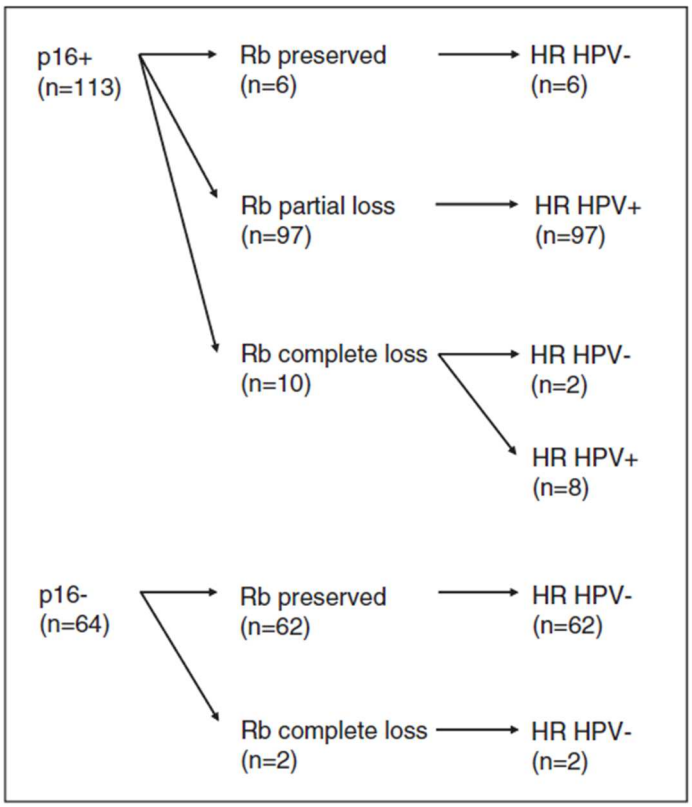



Figure 2. The correlations among p16 expression, Rb status and human papillomavirus (HPV) infection. p16$^+$/Rb-partial loss oropharyngeal squamous cell carcinomas (OPSCCs) are exclusively positive for high-risk (HR)-HPV. In contrast, p16$^-$/Rb-preserved OPSCCs are exclusively negative for HR-HPV. p16$^+$/Rb-complete loss OPSCCs are either positive or negative for HR-HPV. [Colour figure can be viewed at wileyonlinelibrary.com]

**Table 3.** Sensitivity, specificity, PPV and NPV of p16 and Rb to predict HR-HPV infection in OPSCC

| | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| p16$^+$ alone | 100 | 88.9 | 92.9 | 100 |
| p16$^+$/Rb partial loss | 92.4 | 100 | 100 | 90 |
| p16$^+$/Rb partial or complete loss | 100 | 97.2 | 98.1 | 100 |

PPV, positive predictive value; NPV, negative predictive value; HR, high-risk; HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma.

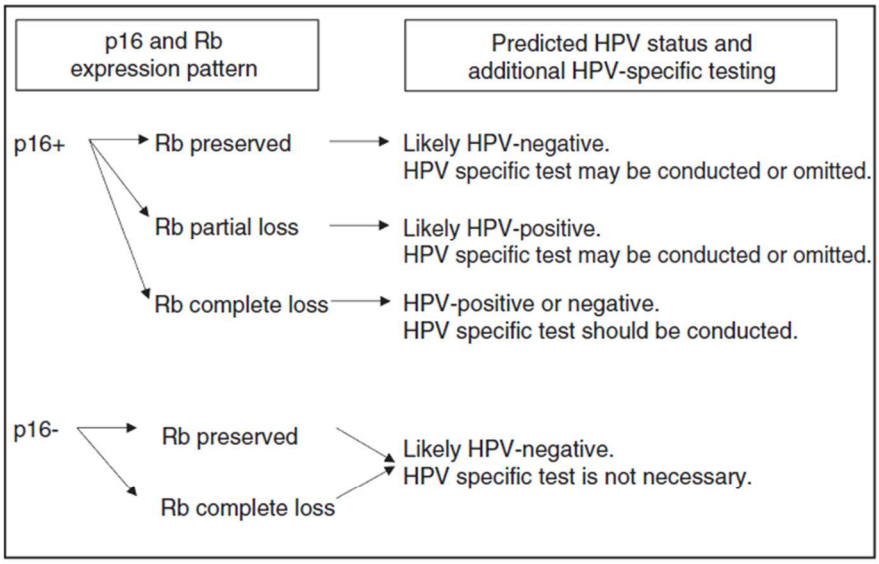



Figure 3. A proposed algorithm for the practical use of p16-immunohistochemistry (IHC), Rb-IHC and human papillomavirus (HPV)-specific testing in oropharyngeal squamous cell carcinomas (OPSCC). In all likelihood, $p16^+$/Rb-partial loss cases are $HPV^+$ and $p16^+$/Rb-preserved cases are $HPV^-$; thus, an additional HPV-specific test could be omitted for these cases. In contrast, $p16^+$/Rb-complete loss cases need an additional HPV-specific test. All $p16^-$ OPSCCs can be considered $HPV^-$ and an additional HPV-specific test is not necessary. [Colour figure can be viewed at wileyonlinelibrary.com]

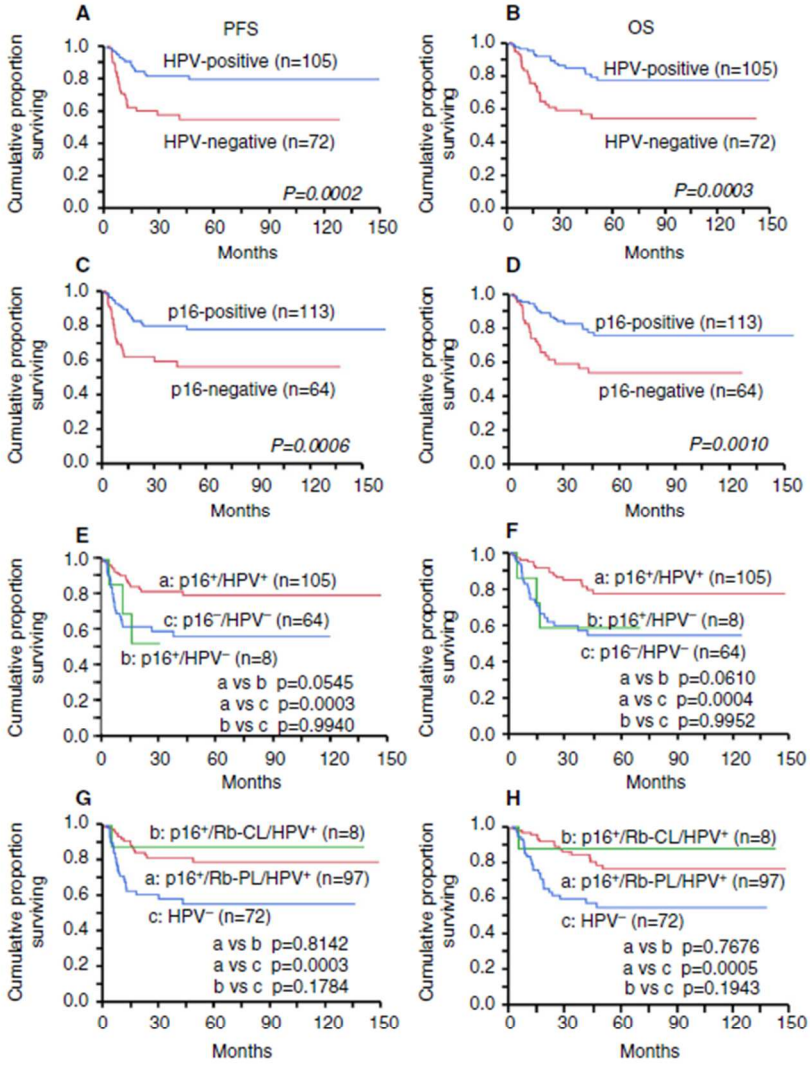
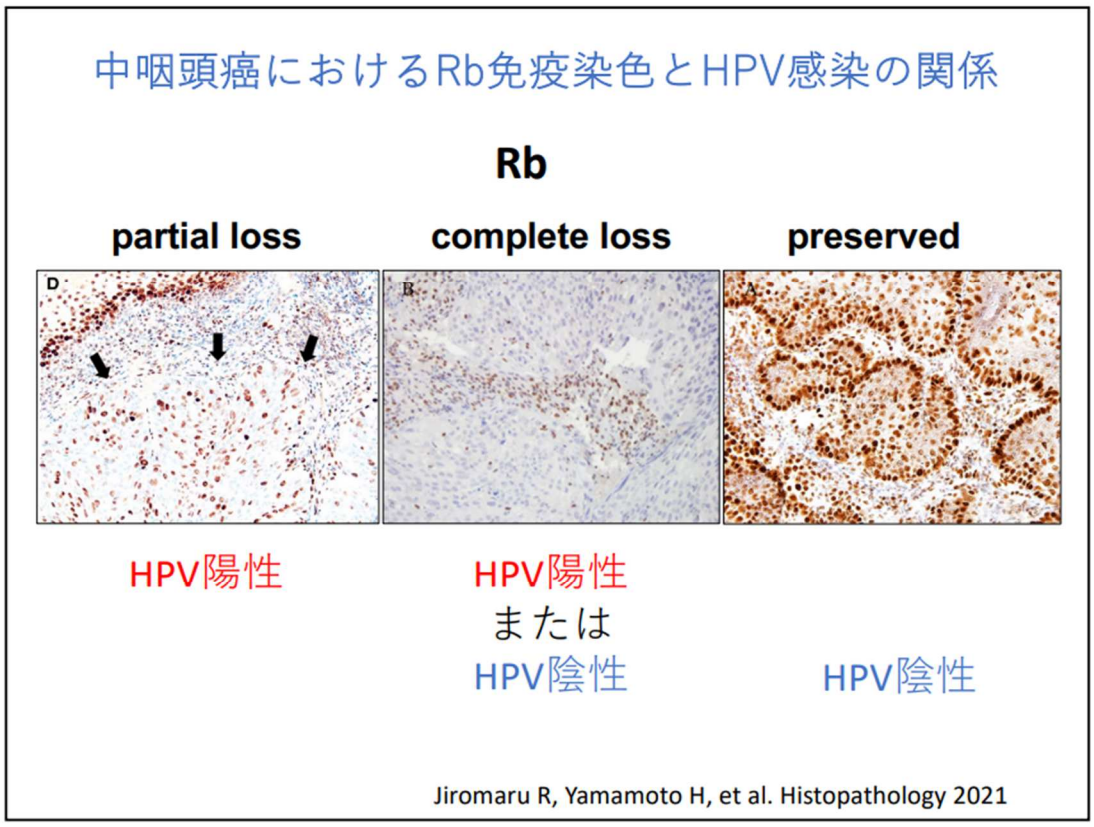



**Figure 4.** The Kaplan–Meier analyses for progression-free survival (PFS) (A,C,E,G) and overall survival (OS) (B,D,F,H) in oropharyngeal squamous cell carcinomas (OPSCC). A,B, Human papillomavirus (HPV) infection is significantly correlated with a better prognosis (PFS, $P = 0.0002$; OS, $P = 0.0003$). C,D, The p16-positive OPSCCs show significantly better prognoses than the p16-negative OPSCCs (PFS, $P = 0.0006$; OS, $P = 0.0010$). E,F, $p16^+/HPV^+$ OPSCCs show significantly better prognoses than the $p16^-/HPV^-$ OPSCCs (PFS, $P = 0.0003$; OS, $P = 0.0004$). The $p16^+/HPV^-$ OPSCCs show prognoses as poor as those of $p16^-/HPV^-$ OPSCCs. G,H, The $HPV^+$/Rb-partial loss (PL) cases and $HPV^+$/Rb-complete loss cases show similar prognostic trends (PFS, $P = 0.8142$; OS, $P = 0.7676$) and the $HPV^+$/Rb-PL cases have better prognoses compared to the $HPV^-$ cases (PFS, $P = 0.0003$; OS, $P = 0.0005$).

補足

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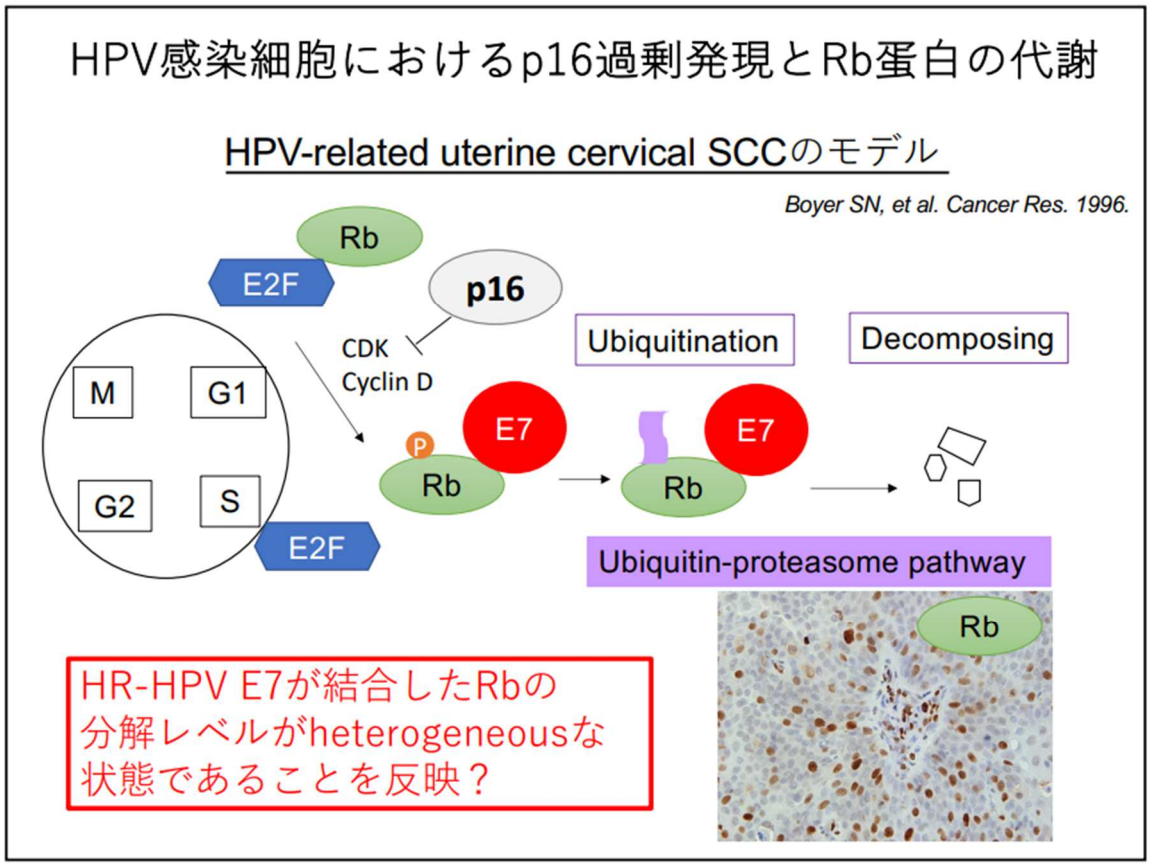


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参考文献: IAP 日本支部病理学教育セミナー(2023/11/11) 教育講演 2 山元英崇先生ハンドアウトより