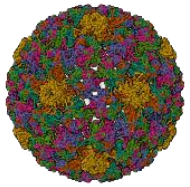

HPV Molecular Diagnostics

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دانشگاه علوم پزشکی اصفهان
مرکز تحقیقات پیشگیری از سرطان

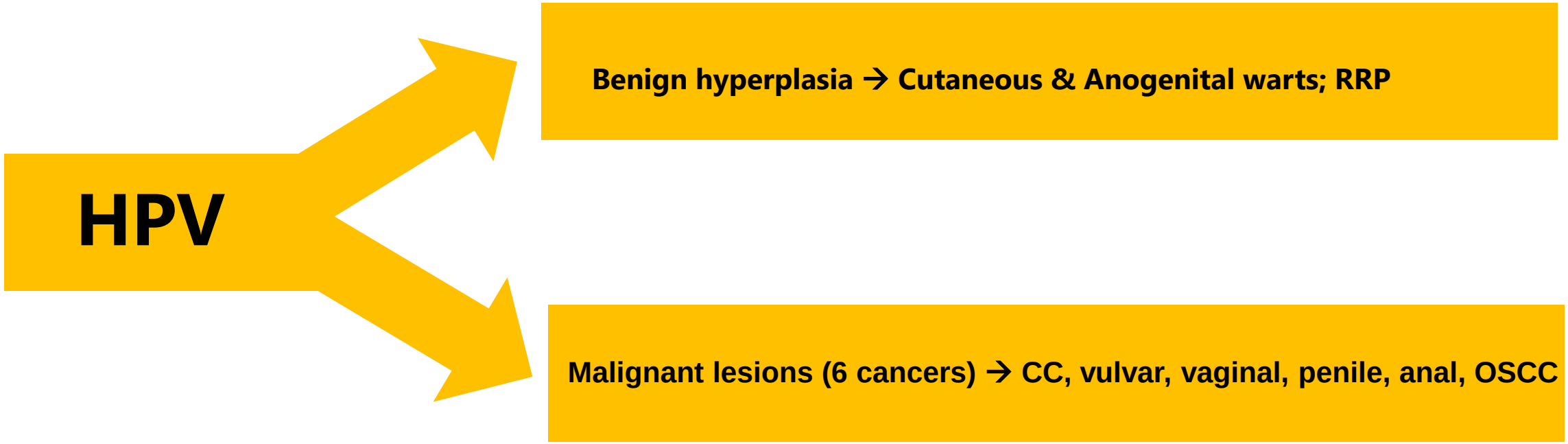
HISTORY OF PAPILLOMAVIRUSES (PV)

- Name of papillomavirus: 1. Latin Papilla: protuberance & 2. Greek Oma: mass/tumor
- Warts; known to ancient Greeks and Romans (infectious nature)
- Considered as a form of syphilis or gonorrhea until 19th century
- 1907: Viral nature was demonstrated in early (cell-free filtrates)

- 1935: Richard Shope showed PV → tumors in rabbits (Shope papillomavirus → CRPV)
- 1970s–1980s: Harald zur Hausen linked specific HPV types to cervical cancer
- 2008: Harald zur Hausen awarded Nobel Prize for discovering the role of HPV in cervical cancer

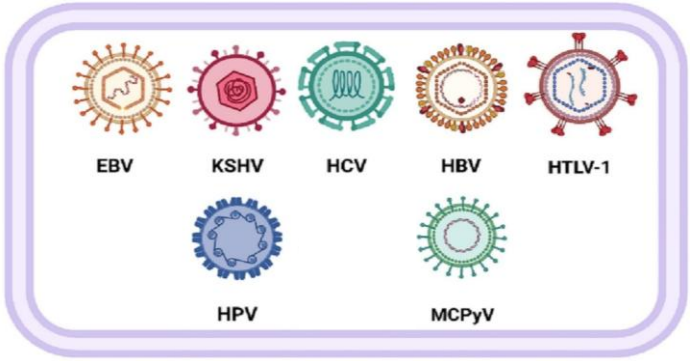
CLINICAL IMPORTANCE of HPV

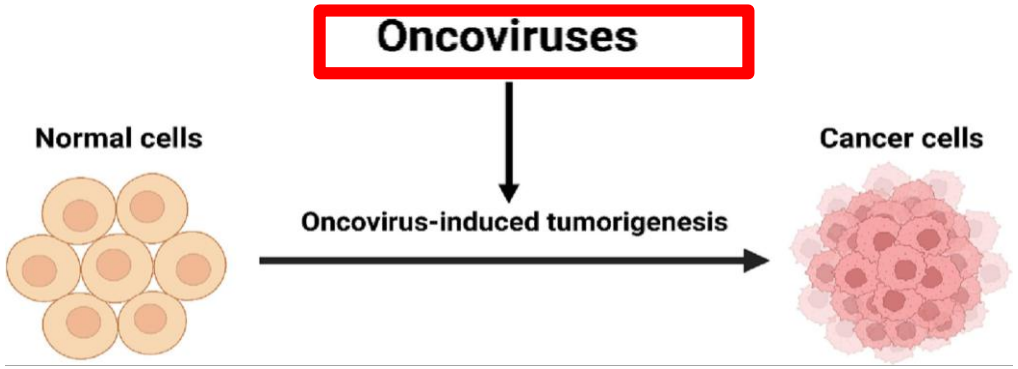
PVs infect vertebrates—including birds, fish, reptiles, and mammals— not invertebrates



CLINICAL IMPORTANCE of HPV

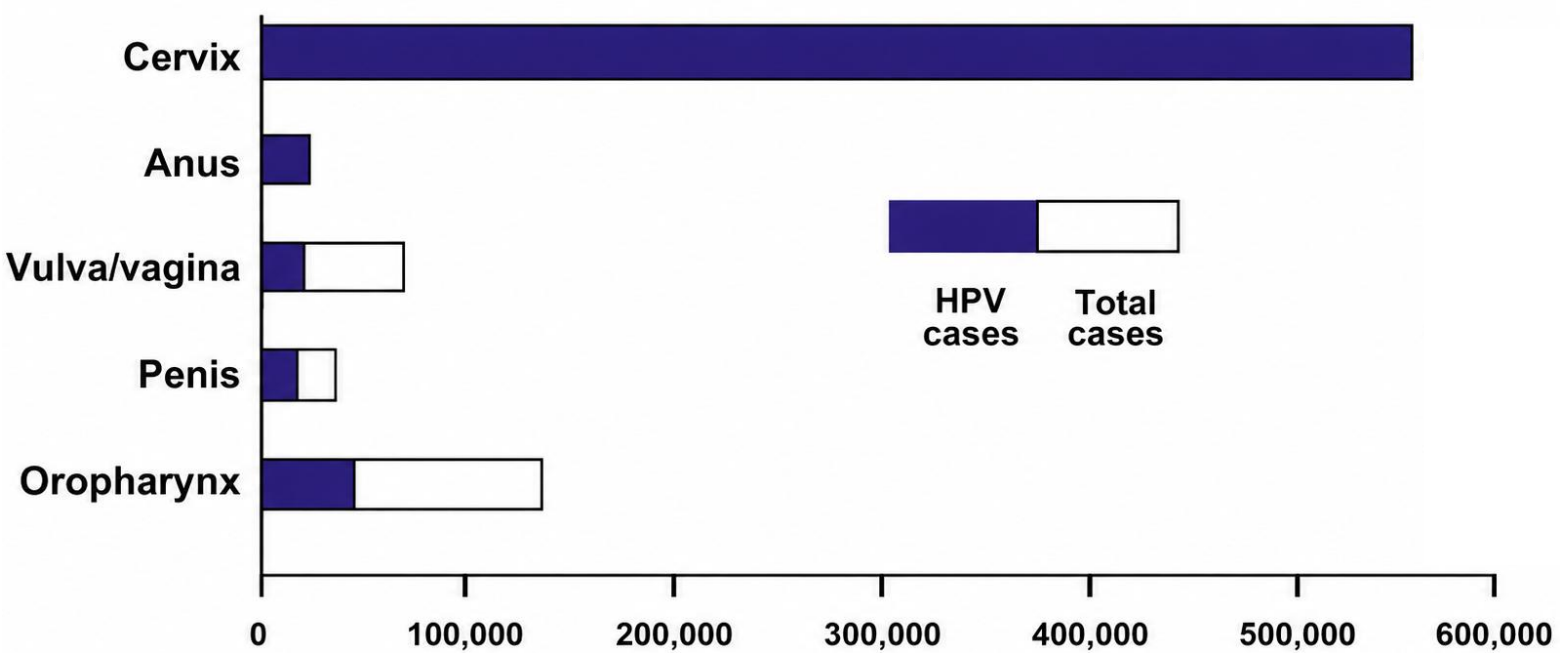
- **Infections → at least a sixth (~10 - 16%) of all cancer cases worldwide → ~ 30% in LMICs**
- **Oncogenic viruses → ~10–12% of all cancer cases worldwide**
- **HPV → 5% of all new cancer cases world**
- **HPV → 30% of all infection-related cancers**





CLINICAL IMPORTANCE of HPV

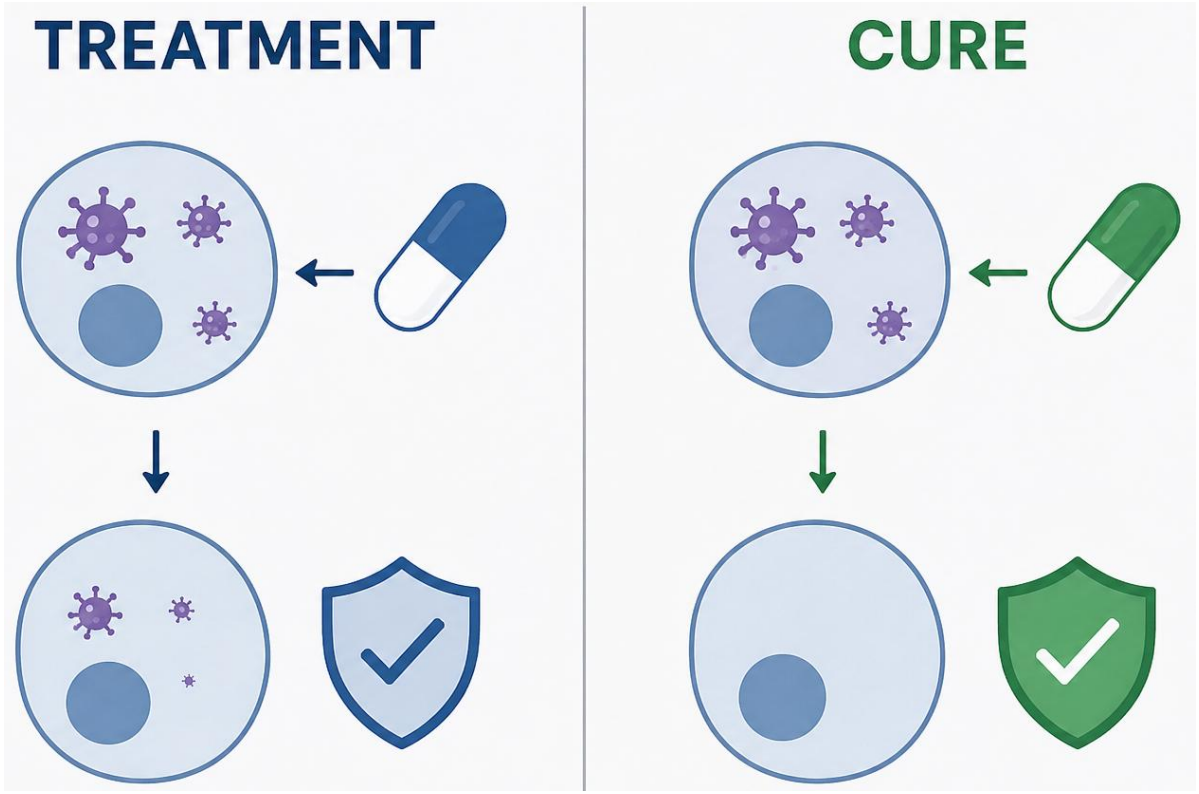
- **HPV → 6 types of cancer**



Annual number of HPV-related cancer worldwide

CLINICAL IMPORTANCE of HPV

- **HPV; most common STI**
- **≥1 lifetime HPV infection in sexually active**
- **STIs → Treatable, Generally Not Curable**
- **Bacterial, Fungal, Parasitic STIs → Curable**



CLINICAL IMPORTANCE of HPV

- Worldwide, at least 290 million women have been infected with HPV
- HPV: 2nd leading infectious cause of cancer worldwide (after H. Pylori)
- CC: 4th common cancer in women globally → 600,000 new case annually → 300,000 death ~~(CFR 50%)~~
- CC: 14th most common cancer in women in Iran → 1000 new case annually → 644 death in Iran
- Mostly (95-97%) asymptomatic & transient infection (After 6m – 2y cleared by Immune system)
- Persistent high-risk infection (3-5%) → Precancerous lesion development → HPV-associated cancer

International Agency for Research on Cancer

World Health Organization

CLINICAL IMPORTANCE of HPV

Globally, cervical cancer is the fourth most common type of cancer among women. In 2020, an estimated:

604 000 women were diagnosed with cervical cancer worldwide*

About 342 000 women died from the disease.*

International Agency for Research on Cancer

World Health Organization

CLINICAL IMPORTANCE of HPV

About 90% of new cervical cancer cases and deaths worldwide in 2020 occurred in low-and middle-income countries.*

International Agency for Research on Cancer

World Health Organization

CLINICAL IMPORTANCE of HPV

International Agency for Research on Cancer

World Health Organization

90-70-90 targets, to be met by 2030

90%

of girls fully vaccinated with HPV vaccine by age 15 years.

70%

of women are screened with a high-performance test by 35 years of age and again by 45 years of age.

90%

of women identified with cervical disease receive treatment (90% of women with precancer treated, and 90% of women with invasive cancer managed).

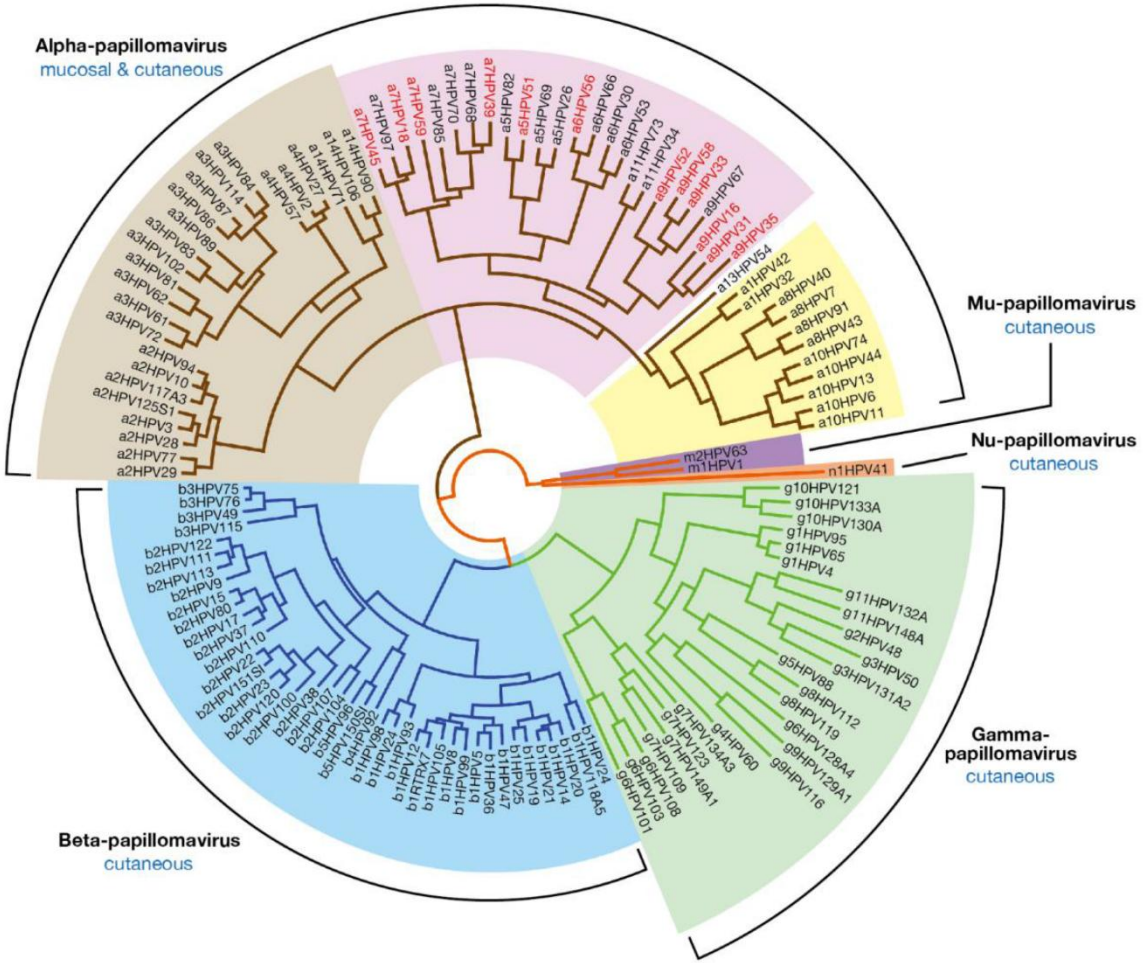
HPV Classification & Nomenclature

- **HPV classification → L1 ORF (most conserved) nucleotide sequence similarity**
- **Different genera → <60% L1 sequence similarity**
- **Different species within a genus → 60–70% L1 sequence similarity**
- **Species designation → genus + species number (e.g., Alphapapillomavirus-9)**
- **Different HPV types → <90% L1 sequence similarity**
- **Variant → <2% L1 sequence difference**

CLASSIFICATION

- 655 PV types isolated from human, mammals, birds, reptiles, fish, etc.
- 440 HPV types identified, 231 of which are officially established
- No PV identified in non-vertebrate species so far
- PV are species-specific
- More HPV types expected; ongoing discoveries continue

CLASSIFICATION

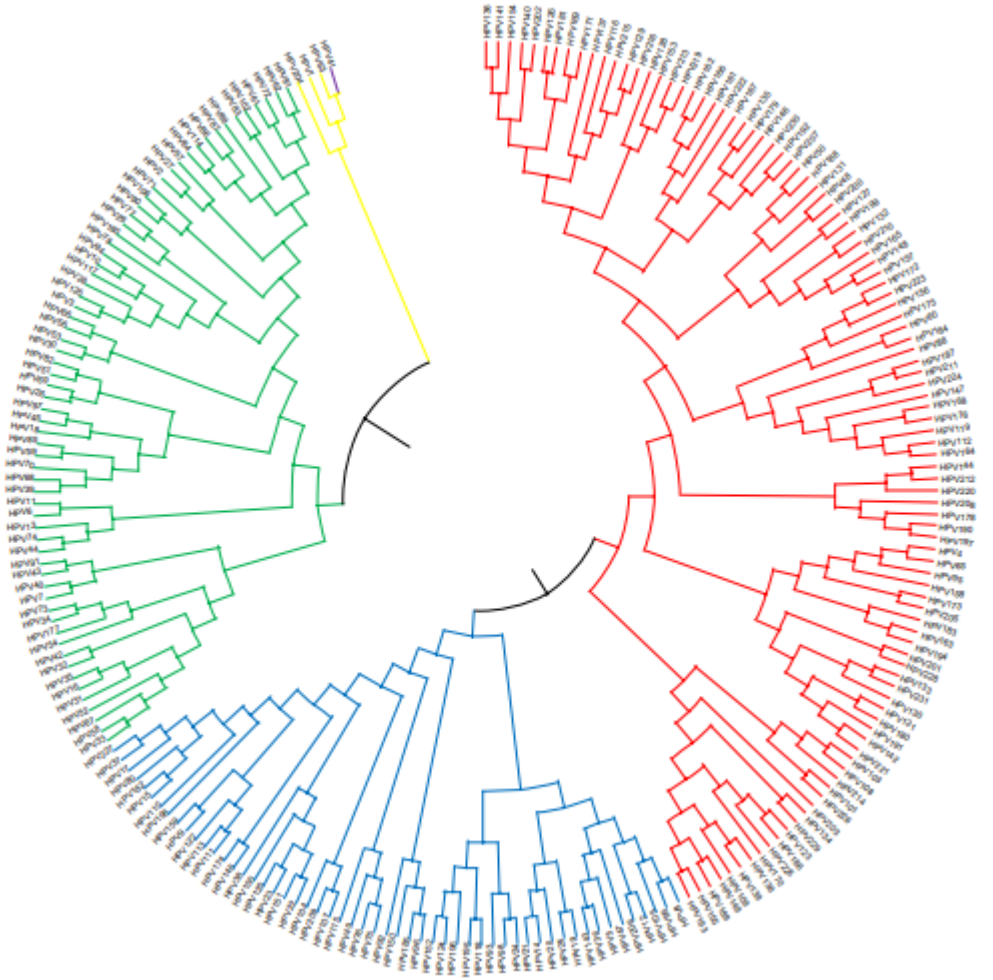


- **Pvs comprise >50 genera**

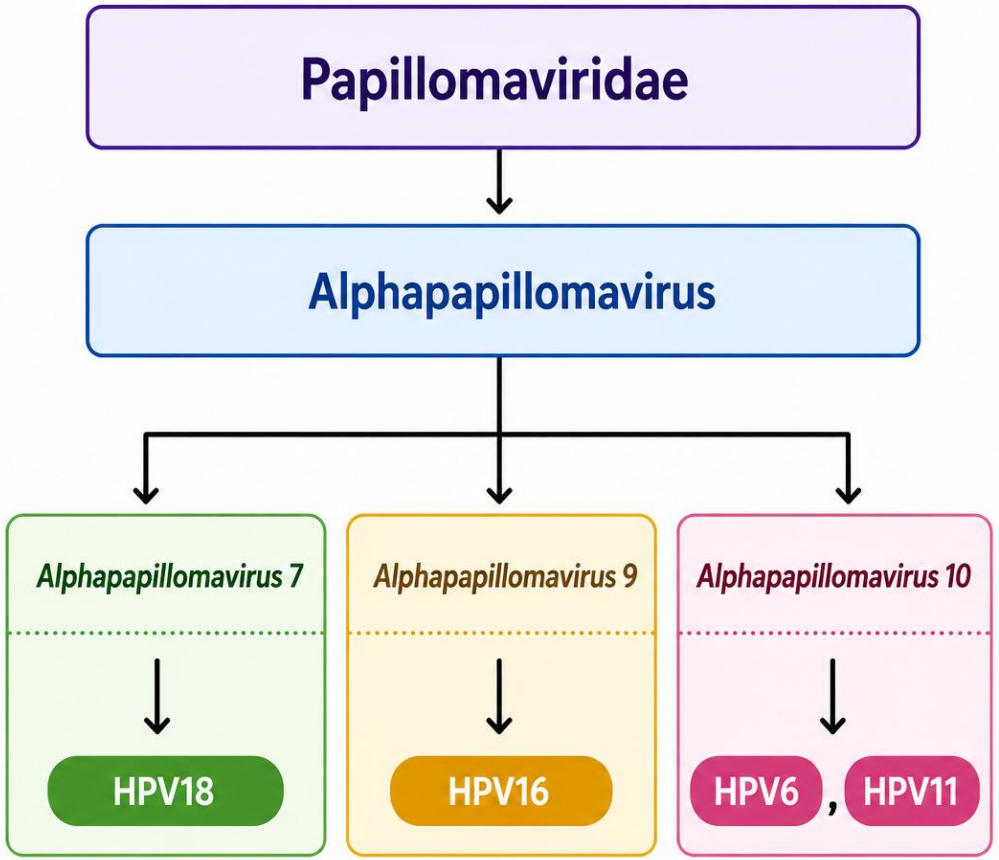
- **HPVs grouped in 5 genera**
  - **α: Major clinical importance (Mucosal & cutaneous)**
  - **β: Cutaneous; often commensal or asymptomatic**
  - **γ: Cutaneous; often commensal or asymptomatic**
  - **Mu: Cutaneous; common warts on hands and feet**
  - **Nu: cutaneous; common warts on hands and feet**

Phylogenetic tree

- based on L1 sequences
- Current official HPV types → 225 established types
- α → Red
- β → Green
- γ → Blue
- δ → Yellow



CLASSIFICATION



GENOTYPES

International Agency for Research on Cancer

World Health Organization

- Approximately 40 HPV types preferentially infect genital mucosa
- HPVs grouped according to epidemiologic link with cancer: HR & LR

1: agent is carcinogenic to humans

2: at one extreme, human evidence is sufficient, other extreme, no human data, animal data is sufficient

2A: probably carcinogenic to humans (limited evidence in humans, sufficient in animals)

2B: possibly carcinogenic to humans (limited evidence in humans, less than sufficient in animals)

3: not classifiable as to its carcinogenicity to humans (inadequate evidence in humans and animals)

GENOTYPES

International Agency for Research on Cancer
World Health Organization

- HR / carcinogenic / Group 1: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
  - 16, 18 → most frequently in SCC of cervix → 70% of CC
  - 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 → most frequently in AC of cervix
  - 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 → virtually cause all CCs
  - All belong to α genus
- Probably carcinogenic / Group 2A: type 68
  - 68 → in patients with epidermodysplasia
- Possibly carcinogenic / Group 2B: types 5, 8, 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97

not classifiable as to its carcinogenicity to humans / Group 3: types 6, 11, genus β (except types 5, 8), genus γ

Stratification of Group 1 (cHPV) into 4 groups

Based on prevalence of each HPV type in CC

Group 1a:	HPV16 is singularly carcinogenic and causes about 60% of cases of squamous cell carcinoma (SCC).
Group 1b:	HPV18 and HPV45 cause 15% and 5% of SCC cases, respectively.
Group 1c:	Other closely related HPV types – HPV31, HPV33, HPV35, HPV52 and HPV58 – together account for 15% of SCC cases.
Group 1d:	The remaining carcinogenic types – HPV39, HPV51, HPV56 and HPV59 – are much less carcinogenic and together cause about 5% of SCC cases.

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World Health Organization

GENOTYPES

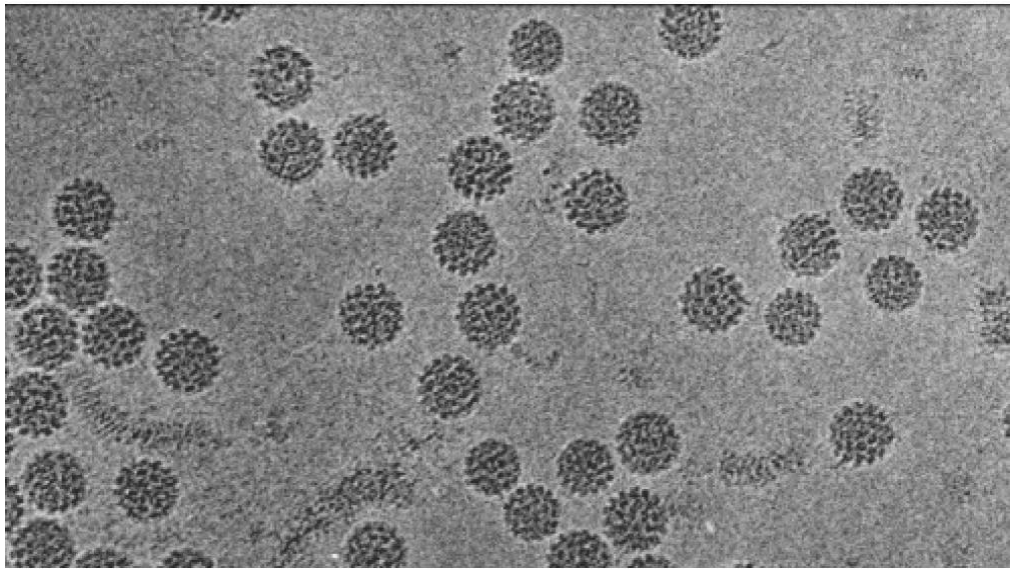
- FDA: 14 HR HPV types for broad screening (66 & 68 associated with some pre-cancer lesions)
  - types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- IARC: 13 HR HPV types with proven epidemiologic carcinogenic evidence in humans
  - types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66

International Agency for Research on Cancer

World Health Organization

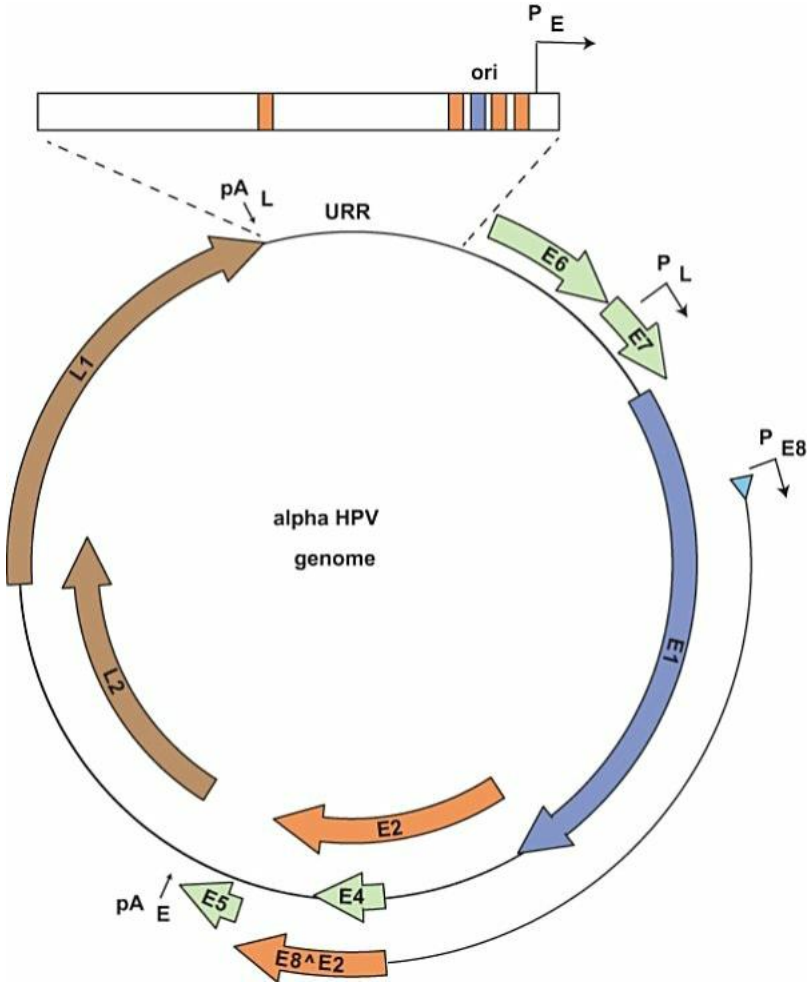
VIRION STRUCTURE

- Virions
  - non-enveloped
  - Capsid (protein) + Nucleic acid
  - Icosahedral capsids (with 72 capsomer)
  - ≃ 60 nanometers in diameter



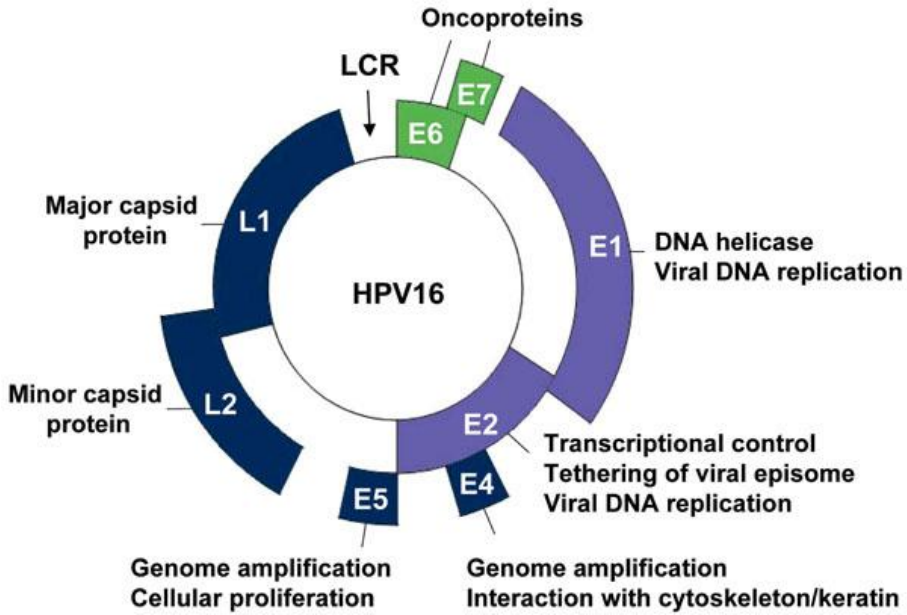
HPV GENOME STRUCTURE

- dsDNA
- Circular
- histone-associated
- **8 kb in length**



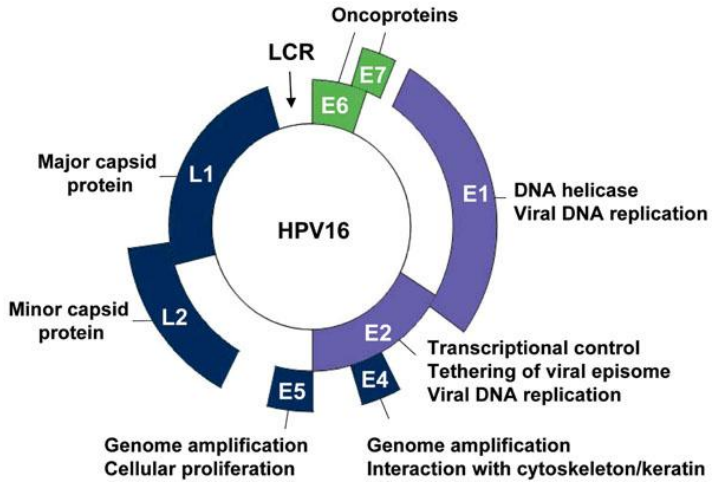
HPV GENES

- 8 open reading frames (ORFs)
- ORFs divided into
  - 1. early
  - 2. late
- Early ORFs encode E1, E2, E4, E5, E6, E7, E8
- Late ORFs encode L1, L2



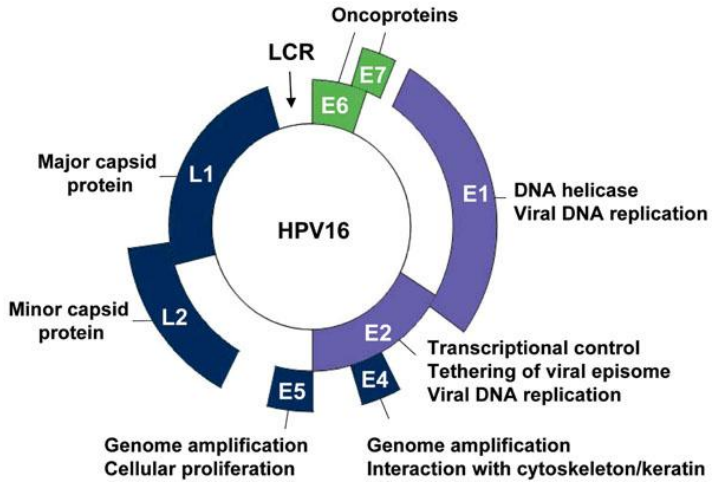
HPV GENES

- **E1, E2: regulate viral replication**
- **E4: disrupting intermediate filaments in cytoskeleton → virion release**
- **E5, E6, E7: transform and stimulate cell growth (oncogenes)**
- **L1: major capsid protein (360 per capsid)**
- **L2: minor capsid protein (12 – 72 per capsid)**



HPV GENES

- **HPV Long Control Region (LCR)**
- **Contains: viral promoter, enhancer, origin of replication**
- **Functions → transcriptional regulation & viral DNA replication**



Transmission

- **Sexual contact → Vaginal, anal, oral sex; genital-to-genital contact; major route (~85–90%)**
- **Condoms → ~70% reduction in infection risk; not complete protection**
- **Direct skin-to-skin contact → Genital touching; heteroinoculation**
- **Autoinoculation → Self-transmission between body sites**
- **Fomites / indirect contact → Clothing, towels, razors/blades, waxing tools**
- **Caregiver-to-child → Contact with genital/hand warts**
- **Mother-to-child → Perinatal transmission; associated with RRP**
- **Intact skin/mucosa → No efficient penetration; microtrauma required**
- **Swimming pools/toilets → HPV detection reported; no evidence of transmission**

RISK FACTORS/DETERMINANTS OF HPV ACQUISITION

- number of lifetime sexual partners (one of main factors)
- Lack of circumcision
- Lack of condom use
- Smoking & Alcohol use
- use of OCPs
- Parity
- hormonal changes
- Nutritional deficits
- other STIs
- age at first sexual intercourse
- host susceptibility
- immunosuppression
- irradiation

INCUBATION PERIOD

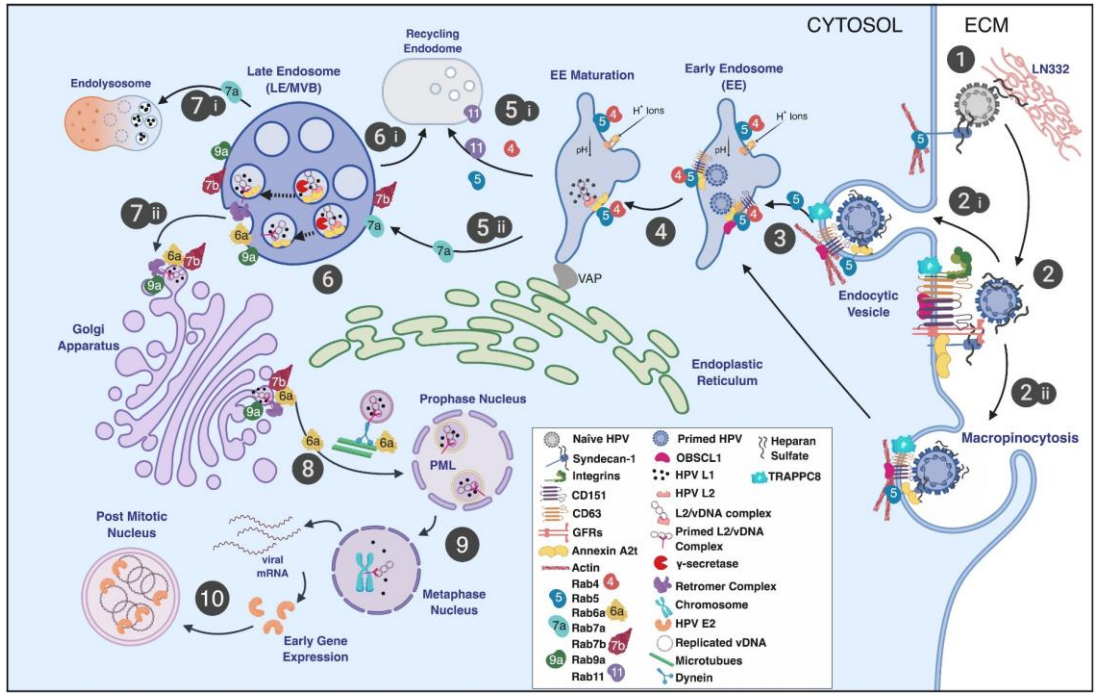
- Warts grew as early as
  - 6 weeks or as long as 2 years after inoculation
  - Most often within 3 to 4 months

PATHOGENESIS; VIRUS REPLICATION

- HPV is highly species specific
- HPV has specific tropism for squamous Eps
- stages of viral replication are linked to differentiation state of Eps
- basal cell is only cell in the squamous epithelium capable of sustained division
- Thus, virus must infect basal cell to establish a long-term, persistent infection
- In basal cells: viral mRNAs, some E PROs, very low levels of viral DNA
- in differentiated Eps: DNA synthesis, production of capsid PROs, assembly

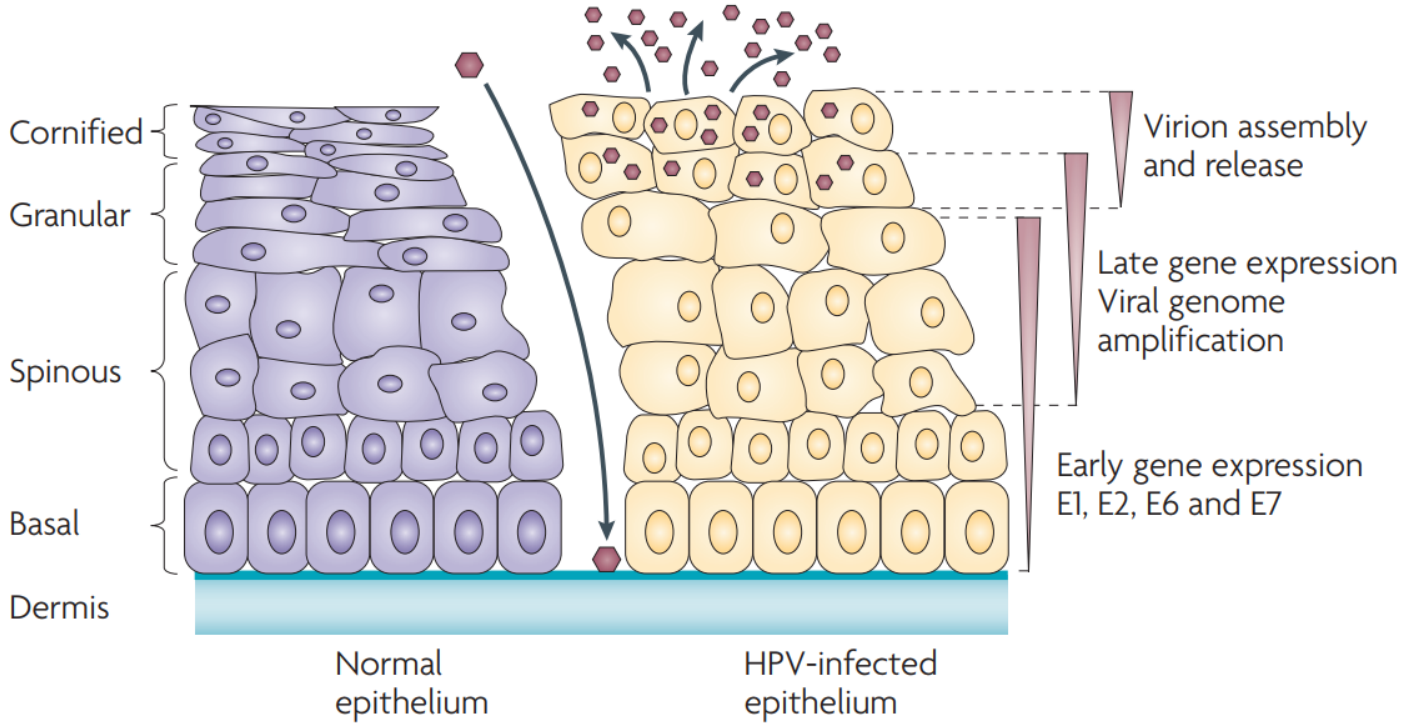
VIRUS REPLICATION

- Virions are internalized by endocytosis
- Viral genome enter to cell nucleus
- Viral transcription
- Protein synthesis
- Genome replication
- Viral assembly

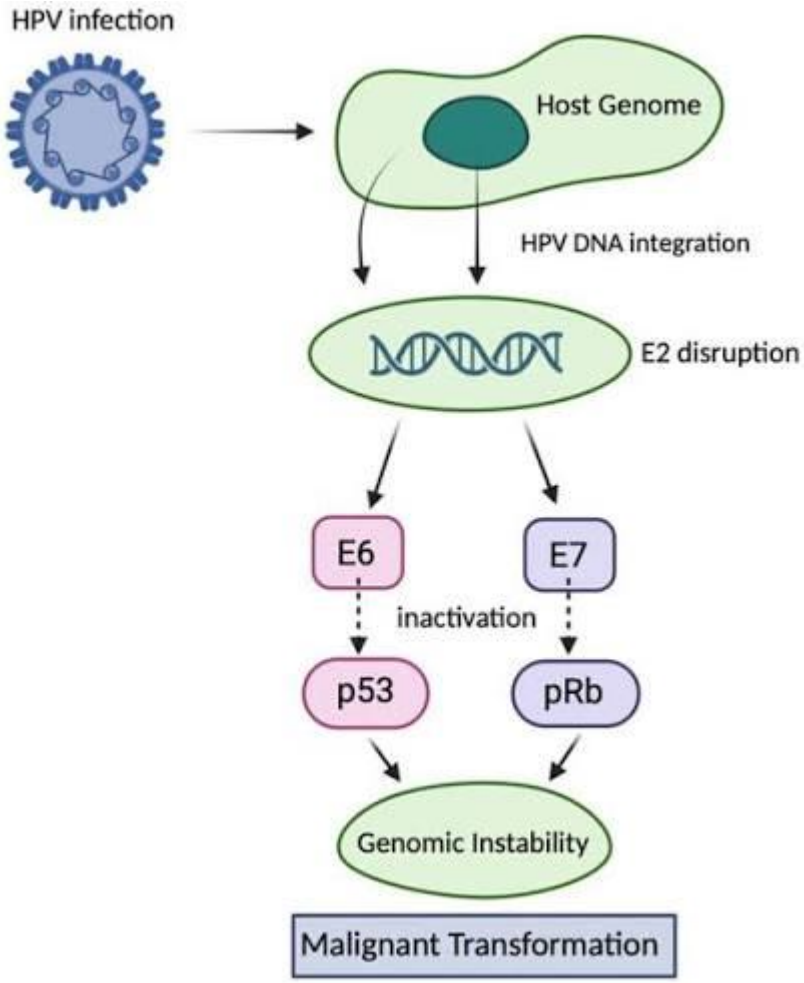


LIFE CYCLE

- **Life cycle is dependent on cell differentiation**
- **Initial infection occur by microtrauma of Ep**
- **→ entry of virion into cells of basal layer**



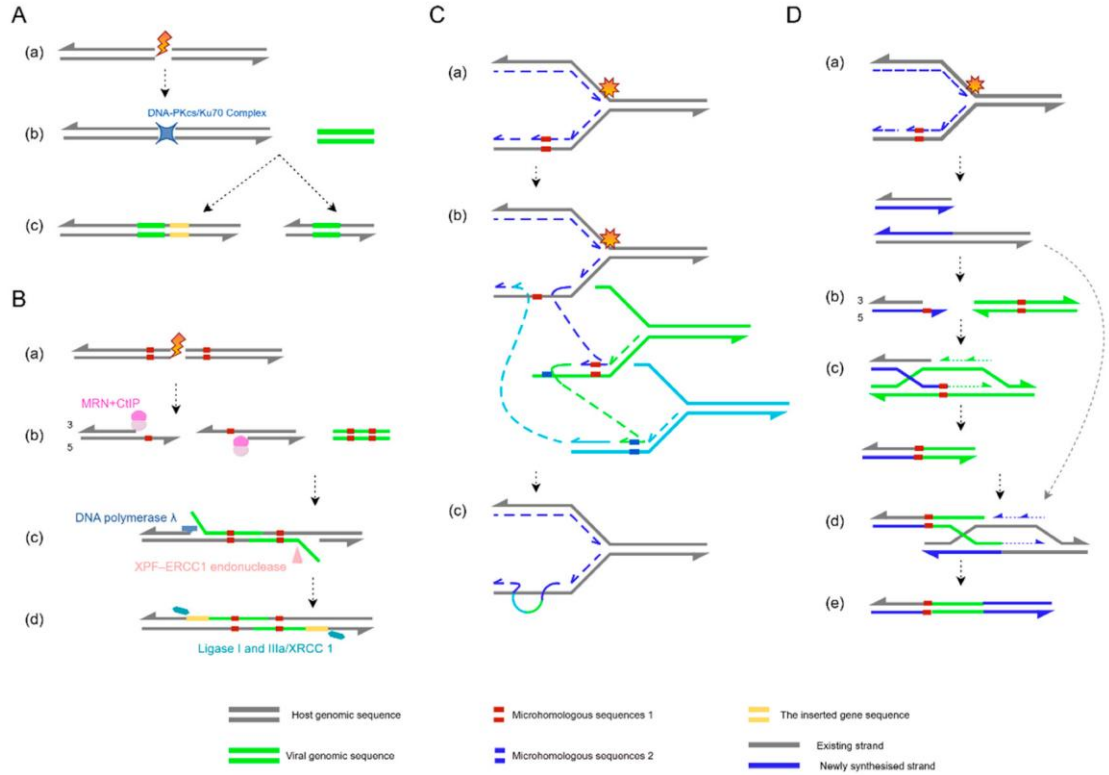
MOLECULAR MECHANISMS HPV CARCINOGENESIS



MECHANISMS OF HPV INTEGRATION IN HOST GENOME

Only in cancer cells

1. **Non-homologous end joining (NHEJ)**
2. **Microhomology-mediated end joining (MMEJ)**



NATURAL HISTORY OF HPV INFECTION

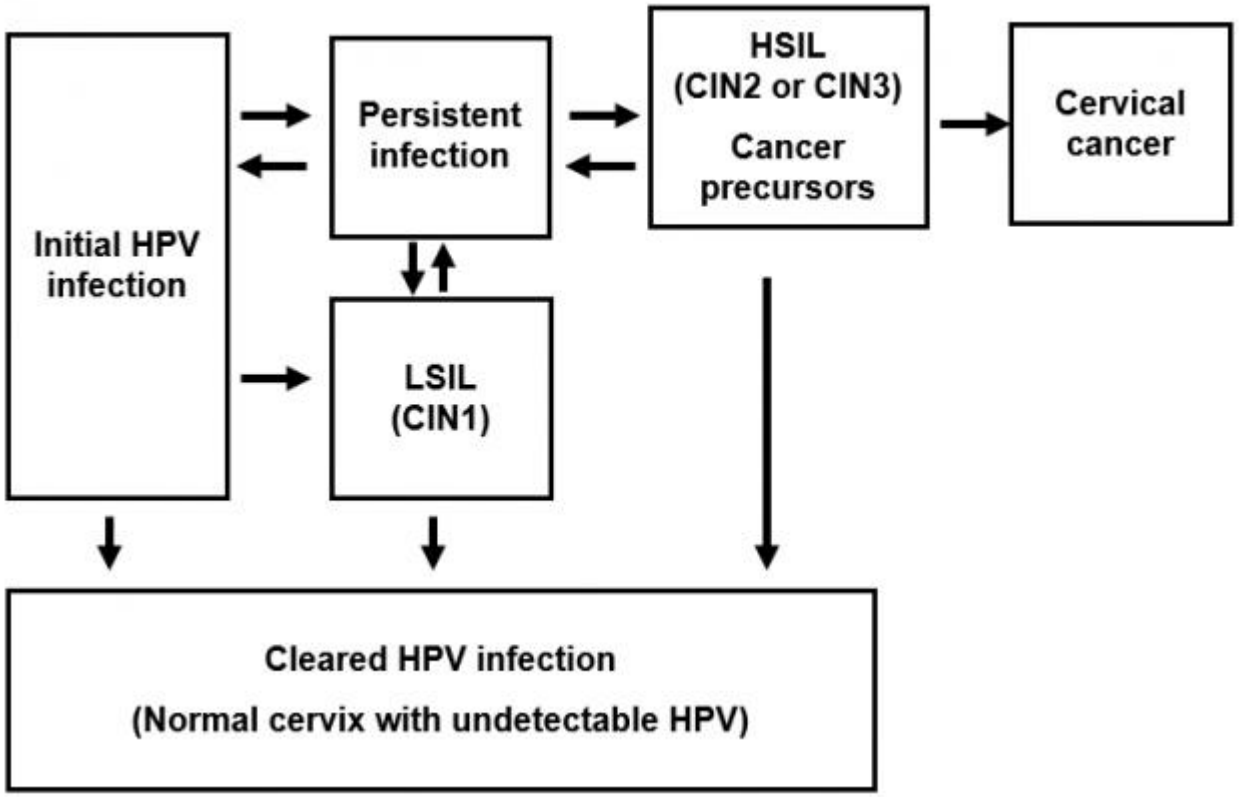
Transient Infection

- ✓ Common in sexually active individuals
- ✓ Usually controlled by immune system
- ✓ Viral clearance within 1–2 years
- ✓ No progression to significant cervical lesions
- ✓ Low risk for long-term complications

Persistent Infection

- ✓ Long-term persistence of HR-HPV
- ✓ Failure of effective clearance
- ✓ Major risk factor for cervical precancer
- ✓ development of cervical intraepithelial lesions
- ✓ Potential progression from CIN1 to CIN2/CIN3
- ✓ Increased risk of invasive CC

Pathogenesis of HPV Infection and Cervical Cancer



Clinical application & Limitations of HPV Testing

- Clinical application
- **Detection of oncogenic HPV types**
- **Risk stratification by HPV genotype**
- **CC screening**
- **management/follow-up of abnormal pap smear result**
- **management/follow-up of HPV-positive women**
- **Guidance for colposcopy and clinical management**
- **Longitudinal assessment of HPV positivity → ?clearance / persistence**
- Limitations of HPV Testing
- **Not indicated → Male partners of HPV-positive women**
- **Not indicated → Women aged <25 years**
- **Not indicated → Diagnosis of genital warts**
- **Not indicated → General STI testing**
- **Not recommended → routine screening of Men**

Types of sample

- HPV test result quality ↔ quality of the sample taken
- Cervical samples → taken during GYN exam by HCW
- Vaginal self-collection → important, equally valid option → ↑ acceptability of screening

- **by Clinical Purpose**
- **Cervical cancer screening** → Brush / Swab / Tampon / Urine / …
- **Wart-like lesion assessment** → Biopsy / brush / …

Patient Preparation

- Women & Men → No sexual intercourse ≥2–3 days before sampling
- Women → No vaginal douching or local medications ≥2 days before sampling
- Women → No specimen collection during menstruation
- Women & Men → Sampling day → No genital washing, bathing/showering, or underwear change

HPV Sampling

- **Swabbing → Firm rubbing across all designated sites**
- **Transport → transport media**
- **Urethral sampling → Thin swab**

HPV Sampling

- **Preferred timing** → First-morning, first-void urine; **≥8 h bladder retention**
- **Preparation** → Hand washing/drying; **no genital washing/cleaning**
- **Collection** → Direct collection without interrupting urine flow; **sterile container**
- **Volume** → ~10 mL urine
- **Handling** → Secure capping → prompt laboratory delivery
- **Females** → First-void urine → maximized cervical–vaginal component
- **Men** → Urine testing → **not routinely recommended; selected circumstances only**

HPV Sampling

- **Wart sampling → Firm circular swabbing of the lesion surface for ~10 seconds**
- **Transport → VTM tube; vigorous agitation ~10–15 times**
- **Multiple warts → Sampling of larger, clustered, or adjacent lesions**
- **No need → Sampling every lesion**
- **Pre-collection → Compliance with recommended preparation instructions**
- **Typical wart + negative HPV genotype test → Consider clinical history and lesion duration**
- **Warts >12 months → Possible viral clearance with persistent virus-free lesion**
- **Virion-free lesion → Persistent wart despite absence of detectable HPV DNA**
- A negative genotype result in a typical wart is more consistent with a virion-free persistent lesion when the wart has been present for more than 12 months.

HPV Sampling

- **Cervical Exfoliated Cell Specimens — HCW-Collected**
- **Goal** → Cellular specimen with preserved DNA from infection site
- **Target** → Cervical transformation zone
- **Cervical mucus** → Remove excess mucus with swab → biohazard waste
- **Sampling** → 360° rotation of swab over exocervix → transport medium
- **Alternative** → Vaginal sampling when speculum insertion is not feasible
- **Biopsy specimens** → Protection against drying (gauze/paper moistened with normal saline)

HPV Sampling

Anal Specimen Collection

- **Swab insertion** → ~3–5 cm beyond anal opening
- **Swabbing** → Gentle rotation for 5 seconds
- **Specimen transfer** → Preservative/transport medium in liquid collection tube

HPV Sampling

Male External Genital Specimen Collection

Routine HPV testing → Not routinely recommended for male genital specimens

Preparation → Uncircumcised men → retract foreskin before sampling

Sampling → 3 saline-prewetted Dacron swabs; firm rubbing of each site for ~10 s

Swab 1 → 360° coronal sulcus + glans penis

Swab 2 → Penile shaft

Swab 3 → Scrotum

Transport → All 3 swabs combined in a single vial with transport medium

Pre-extraction → 3 specimens combined → single DNA extract

Handling → Tight tube closure → leakage prevention during shipment

Safety

- **Specimens → Biohazardous material**
- **Routine handling → BSL-2**
- **Specimen processing → Biological safety cabinet (BSC)**
- **PPE → Gloves, laboratory coat**

Laboratory Layout & Biosafety

- **Facility** → **BSL-2 laboratory standards**
- **Laboratory access** → **Restricted**
- **Separate areas** → extraction, Master mix, adding, Amplification, Electrophoresis, hybridization
- **Dedicated equipment, materials, consumables & PPE** for each area
- **Unidirectional workflow:** Clean → dirty
- **Positive / negative pressure** → According to laboratory design & risk assessment
- **Air-lock / ante-room** → Where contamination risk exists
- **Pipette tips** → Aerosol-filter tips
- Workstation-labeled; no transfer between workstations
- **BSC** → Performance monitoring, maintenance & upkeep
- **Waste segregation** → Infectious vs. non-infectious waste
- **Infectious waste** → Autoclave decontamination
- **Sharps** → Safety box; maximum **⅔ full** → decontamination
- **Decontamination** → Workbenches, equipment, under workstations, under hoods
- **Floors** → disinfection

STABILITY OF THE VIRUS ON SURFACES

- HPV non-enveloped virion → Highly stable; heat resistant
- Drying resistance → ~50% infectivity retained after 3 days at room temperature
- Drying → Up to 7 days; ~30% infectivity retained
- Fomite surfaces → Stable and infectious PV in a mouse model
- Fomite survival → ≥8 weeks without squames; up to 1 year with squames
- Fomite-to-new-site transfer → ~10-fold reduction in virus titre
- Free virions vs. infected keratinocytes → Relative contribution to transmission uncertain
- Terminally differentiated keratinocytes → Keratin-rich cellular structure
- Keratin network → Secondary protective structure → Environmental stress protection
- HPV within keratinocytes → Greater stability than free virions

HPV Self-Sampling

- **Self-sampling** → ↑ Screening access, uptake & equity; reduced cultural barriers
- **Acceptability** → Private, discreet, painless, quick, convenient & preferred over clinician collection
- **Clinical performance** → Comparable HPV detection; cervical–vaginal results correlated not perfectly concordant
- **Specimen** → Self-collected cervicovaginal/vaginal-pool swab; greater vaginal contribution than HCW
- **Overall impact** → ↑ Screening participation and access; **limited improvement in clinical follow-up**

Sample Rejection Criteria

- **Inadequate patient/test identification** → Missing label or incomplete patient name
- **Insufficient specimen volume**
- **Leakage / damaged specimen container**
- **Inappropriate specimen collection tube**
- **Improper specimen storage or transport temperature**
- **Specimens submitted in fixatives** → e.g., formalin
- **Mismatch** → Test request form vs. specimen type/specifications
- **Potential test-result interference** → Blood, excessive secretions, non-sterile materials/equipment
- **Cotton swabs → × for urethral or endocervical sampling** → Cotton-fiber adherence → poor cell release
- **Formalin → ×** → PCR inhibition / PCR reaction interference

transport

room temperature up to 14 days.

4°C for three weeks

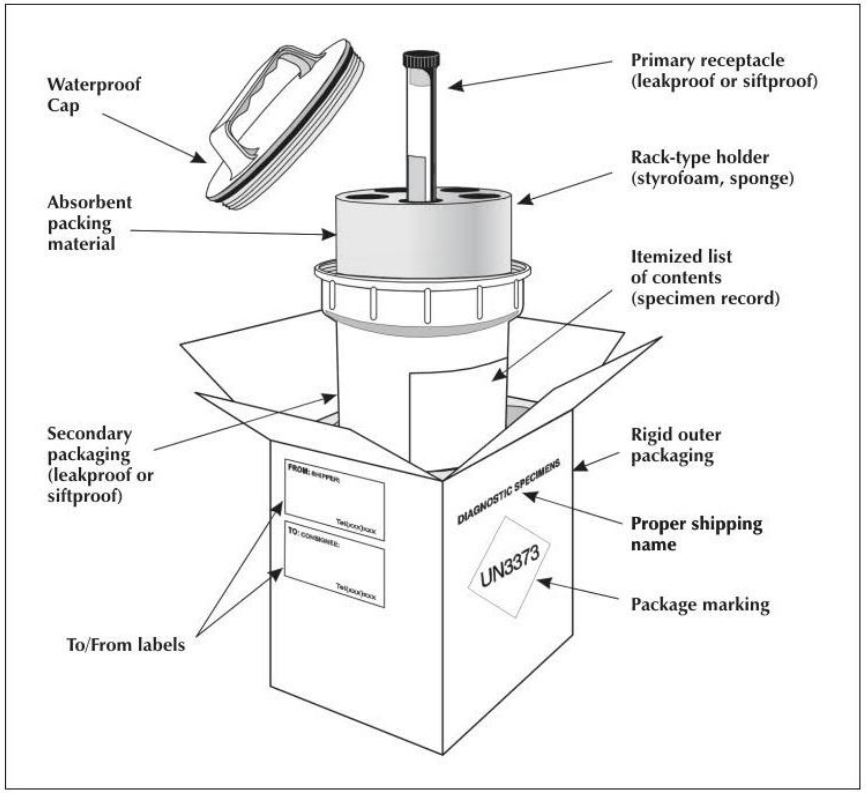
longer storage, freeze at -20°C.

•HPV specimens → Category B

- Category B → No permanent disability, life-threatening, fatal in humans/animals

•Packaging → Appropriate containers + triple packaging

•Labeling → UN 3373



Surface Disinfection & HPV Inactivation

- **Chemical disinfection** → Glutaraldehyde, hydrogen peroxide, sodium hypochlorite/chlorine compounds, peracetic acid, iodophors, phenolics, quaternary ammonium compounds

Spill Management

- Allow aerosols to settle
- Wear appropriate protective clothing
- Cover spill with absorbent paper towel
- Apply appropriate disinfectant
- Clean from periphery toward center
- Allow adequate disinfectant contact time
- Dispose of contaminated waste → Autoclave before disposal

THANKS FOR YOUR ATTENTION