Molecular Disease Mechanisms

Lecture 4: Viral carcinogenesis and radiation-induced cancer

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Topics for lecture 4

1. Viral carcinogenesis

   viruses associated with cancer

   pathways disrupted

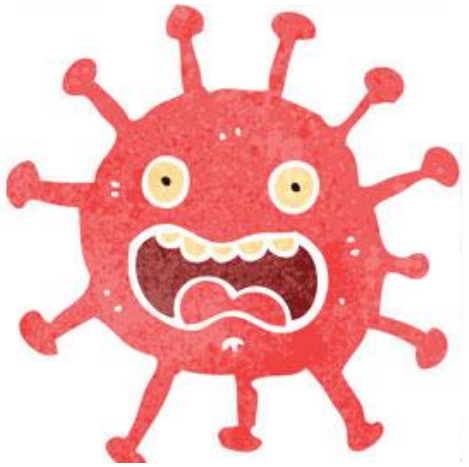
2. Radiation and cancer

   sources of radiation

   mechanism of genotoxic damage

Lecture 4, Part 1

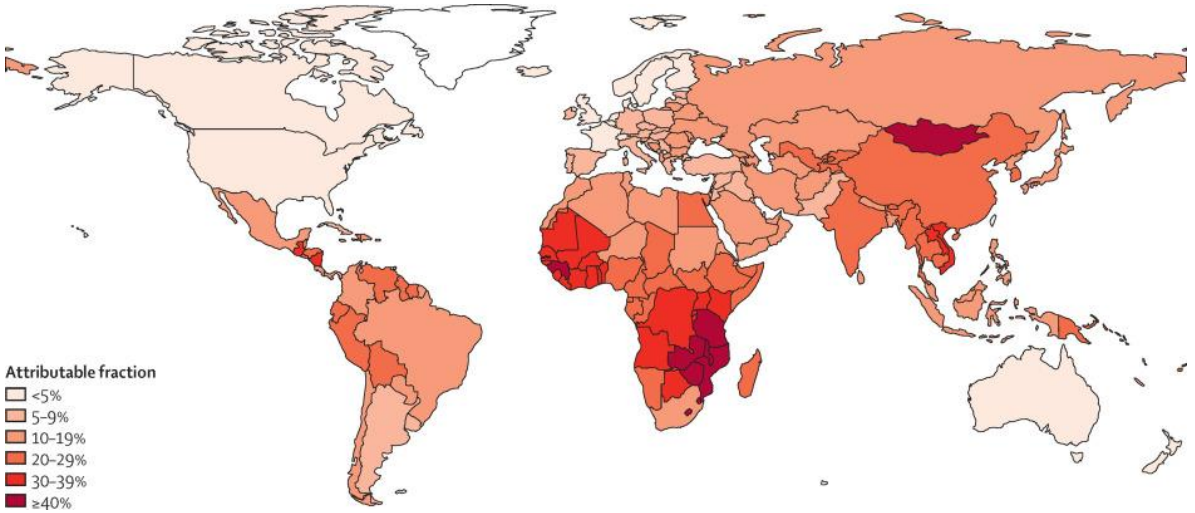
VIRAL CARCINOGENESIS



Cancer caused by infectious agents

- 14 million new cancer cases in 2012 – 2.2 million (15·4%) were attributable to carcinogenic infections.
- The most important infectious agents worldwide were **Helicobacter pylori** (770 000 cases), **human papillomavirus** (640 000), **hepatitis B virus** (420 000), **hepatitis C virus** (170 000), and **Epstein-Barr virus** (120 000).

Attributable fraction of cancer related to infection, 2012



Virus basics

- Infectious particles consisting of RNA or DNA molecules packaged in a protein capsid
- Can multiply only inside a host cell
- Outcome of viral infection:
  - lysis of infected cells with release of viral particles
  - Integration of nucleic acid sequence into host chromosome

How did we discover the link between viruses and cancer??

Let’s talk about cancer in chickens…

Let’s talk about cancer in chickens…

Rous Peyton (American virologist)

-seminal studies in chickens to show the role of a virus in cancer transmission

-Many scientists did not believe him at first

-Won the Nobel Prize in 1966 (40 years post discovery)

What did Peyton do?

Rous (1910) became interested in the transplantability of tumors when a woman came to the Rockefeller Institute with a Plymouth Barred Rock hen with a large tumor (Fig. 2)

Figure 2. The original Plymouth Barred Rock fowl bearing the tumor presented to Rous and held by somewhat arthritic hands. Reproduced from Rous, 1910.

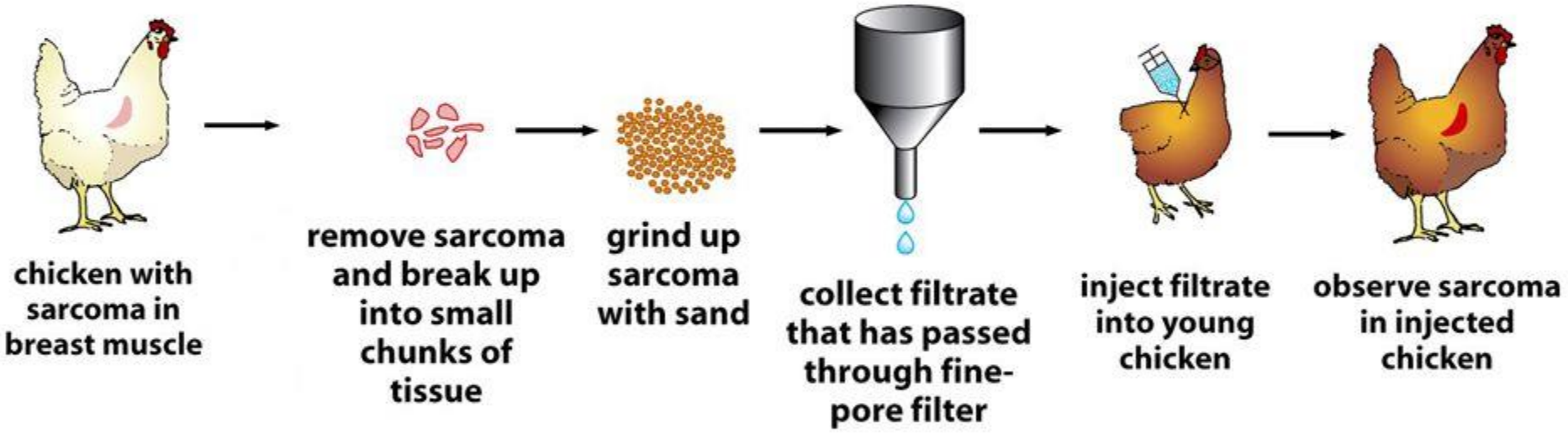
What: he found that a sarcoma in chickens was transmissible to other chickens.

Rous P (1911) A sarcoma of the fowl transmissible by an agent separable from the tumor cells. *J Exp Med* 13:397–411.

…nobody believed him, although it was the foundation of tumor virology!

The Rous Sarcoma Virus (RSV)

A virus can transform a normal cell into a tumor

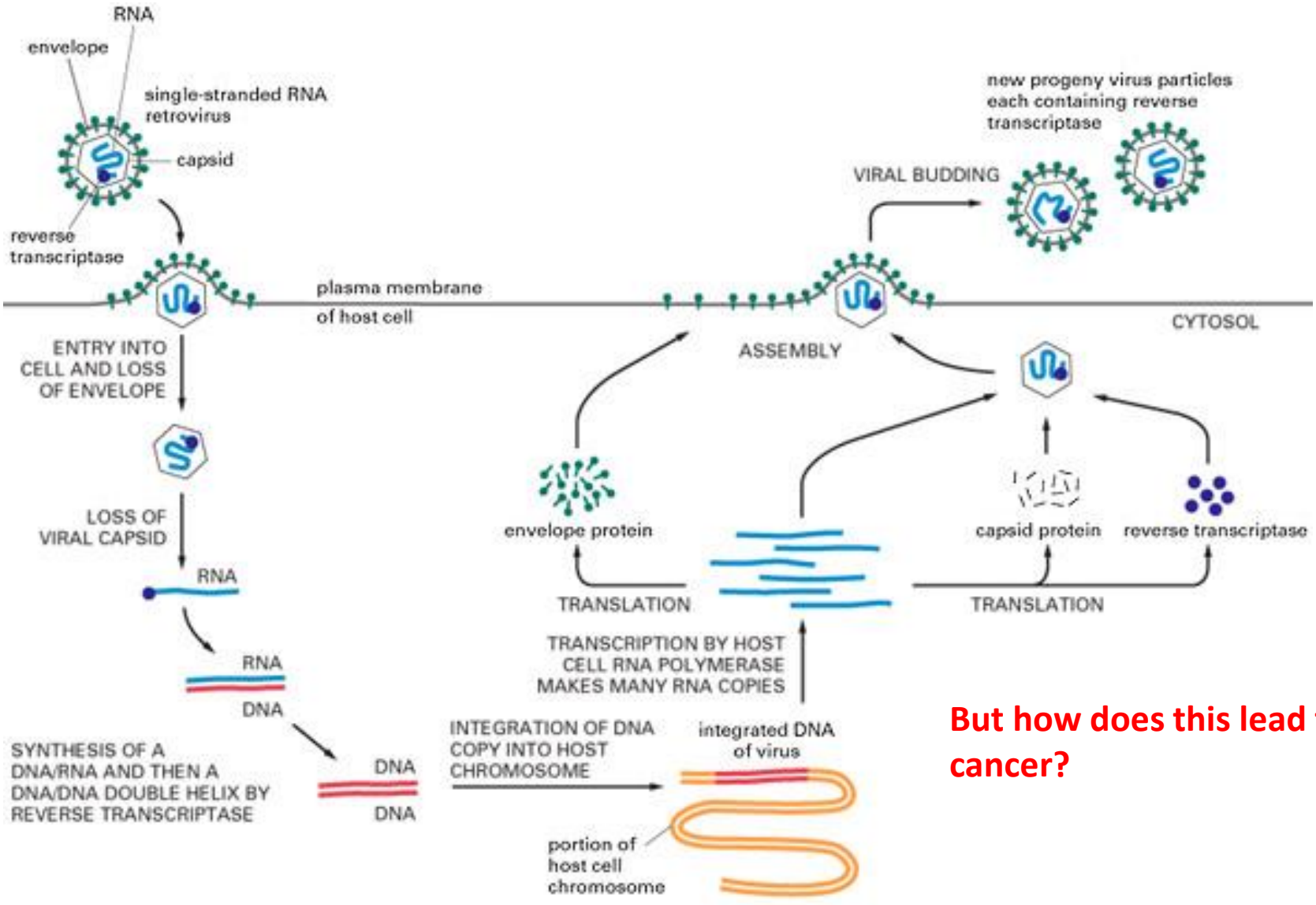


How: took a tumor extract that was passed through a filter (too fine to contain chicken cells or bacteria) and it caused cancer in a healthy chicken.

-could transmit this to further progeny

- the tumorogenic agent was a virus, later became known as RSV

RSV host infection



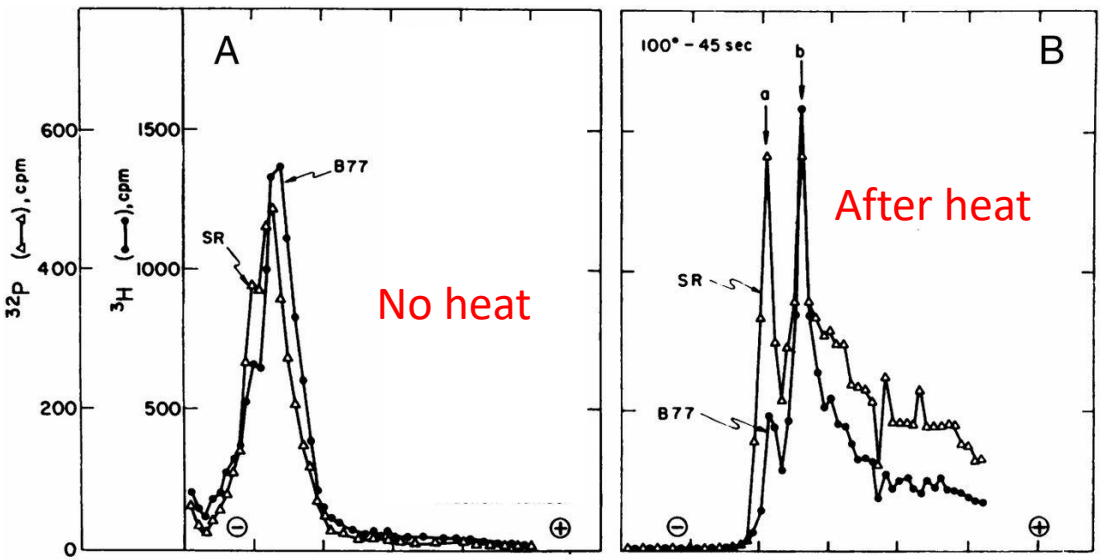
But how does this lead to cancer?

RSV: Rous Sarcoma Virus

- RSV is a virus with a RNA genome
- RSV has four genes:
  - gag – encodes the capsid proteins
  - pol – encodes for reverse transcriptase
  - env – encodes for the envelope gene
  - src – encodes a tyrosine receptor kinase that attaches phosphate groups to the amino acid tyrosine in the host cells proteins

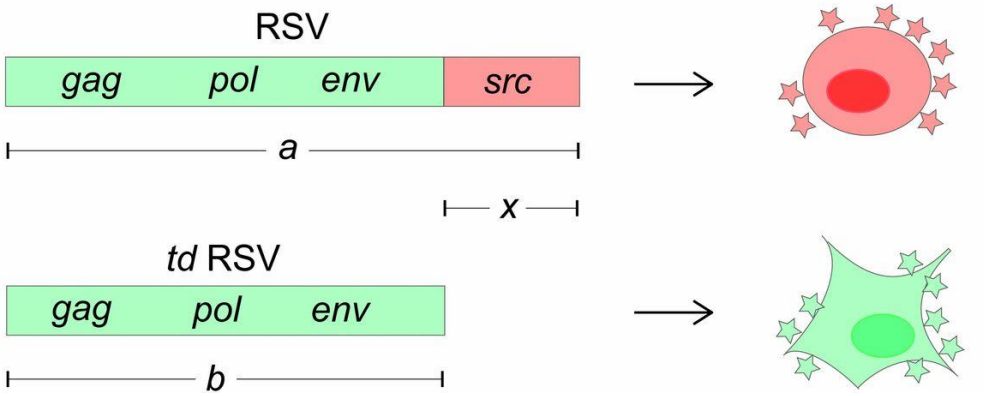
What was the cause of the transforming ability in the virus?

- v-src is involved in the transformation to cancer
  - THE DISCOVERY OF ONCOGENES!
- How was this determined?



2 types of RSV mutants:
RSV transforming
(a and b fractions)

RSV non-transforming, but still replication competent (only b fraction)



PNAS paper by Duesberg and Vogt

a-b = x….(x = src - for sarcoma)

Viral Oncogenes

Retrovirus oncogenes derived from normal cellular genes

Retrovirus	Viral oncogene	Cellular proto-oncogene
Rous sarcoma virus	v-src	c-src (src)
Simian sarcoma	v-sis	c-sis (sis)
Harvey murine sarcoma	v-H-ras	c-H-ras (H-ras)
Kirsten murine sarcoma	v-K-ras	c-K-ras (K-ras)
FBJ murine osteosarcoma	v-fos	c-fos (fos)
Avian myelocytomatosis	v-myc	c-myc (myc)
Abelson leukemia virus	v-abl	c-abl (abl)
Avian erythroblastosis	v-erbB	c-erbB (erbB)

First oncogene identified →

- viral oncogenes are ~80-99% homologous to cellular proto-oncogenes
- viral oncogenes in general are copies of cellular mRNA and lack introns

v-*src*/c-*src* relationship

- Seminal finding: *src* gene of RSV (v-*src*) is a transduced allele of a cellular gene (c-*src*) that the virus picked up by recombination during the retroviral life cycle
- For this discovery '*the cellular origin of retroviral oncogenes*'

Bishop and Varmus were awarded the Nobel Prize in Physiology or Medicine in 1989

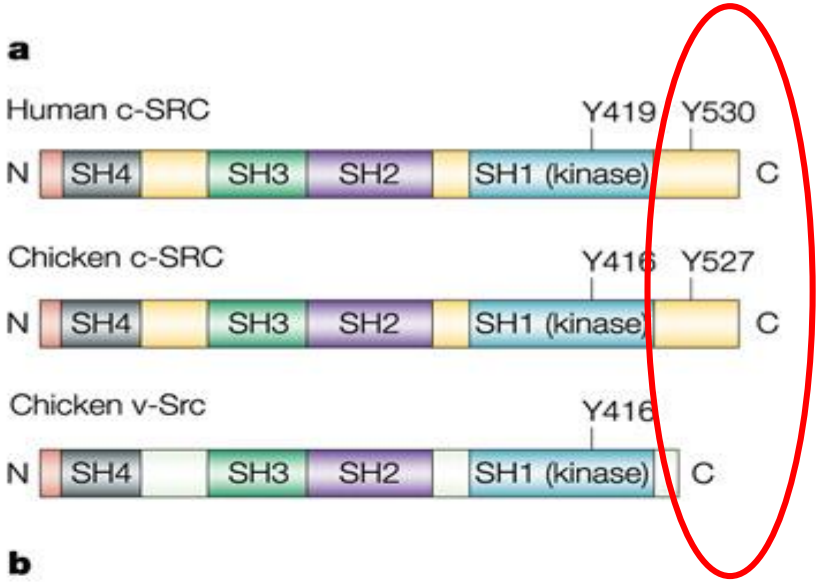
J. Michael Bishop
Prize share: 1/2

Harold E. Varmus
Prize share: 1/2

c-SRC

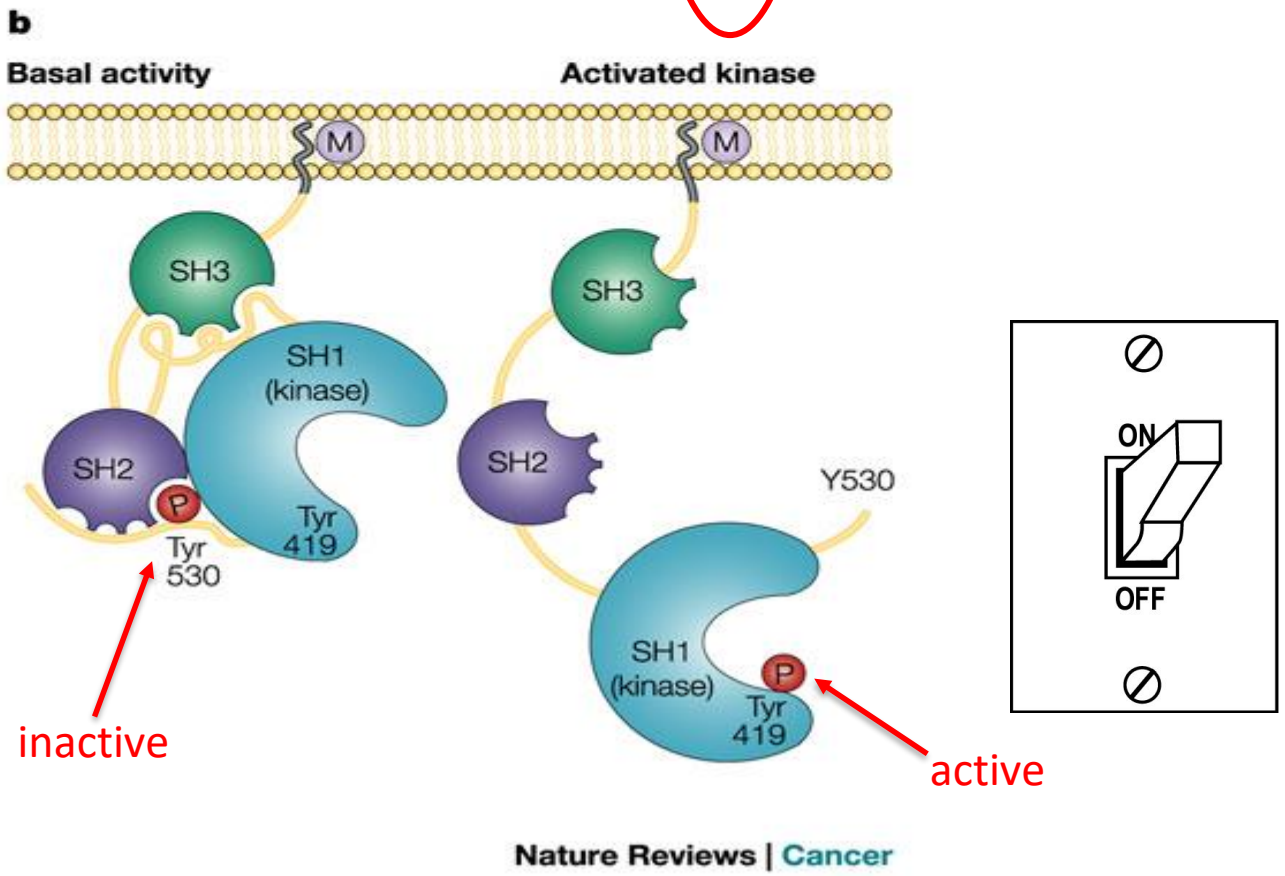
- Non-receptor tyrosine kinase overexpressed in many cancers
- Most tyrosine kinases phosphorylate serine and threonine, Src phosphorylates tyrosine residues
- SRC structure:
  - Four Src homology domains (SH)
  - SH1: autophosphorylation site
  - SH2: interacts SH1 (negative regulator)
  - SH3: interacts SH1 (kinase domain)
  - SH4: lipid motif for membrane localization
  - C terminus: site of phosphorylation (Tyr530 in humans) for negative regulation

Src Protein



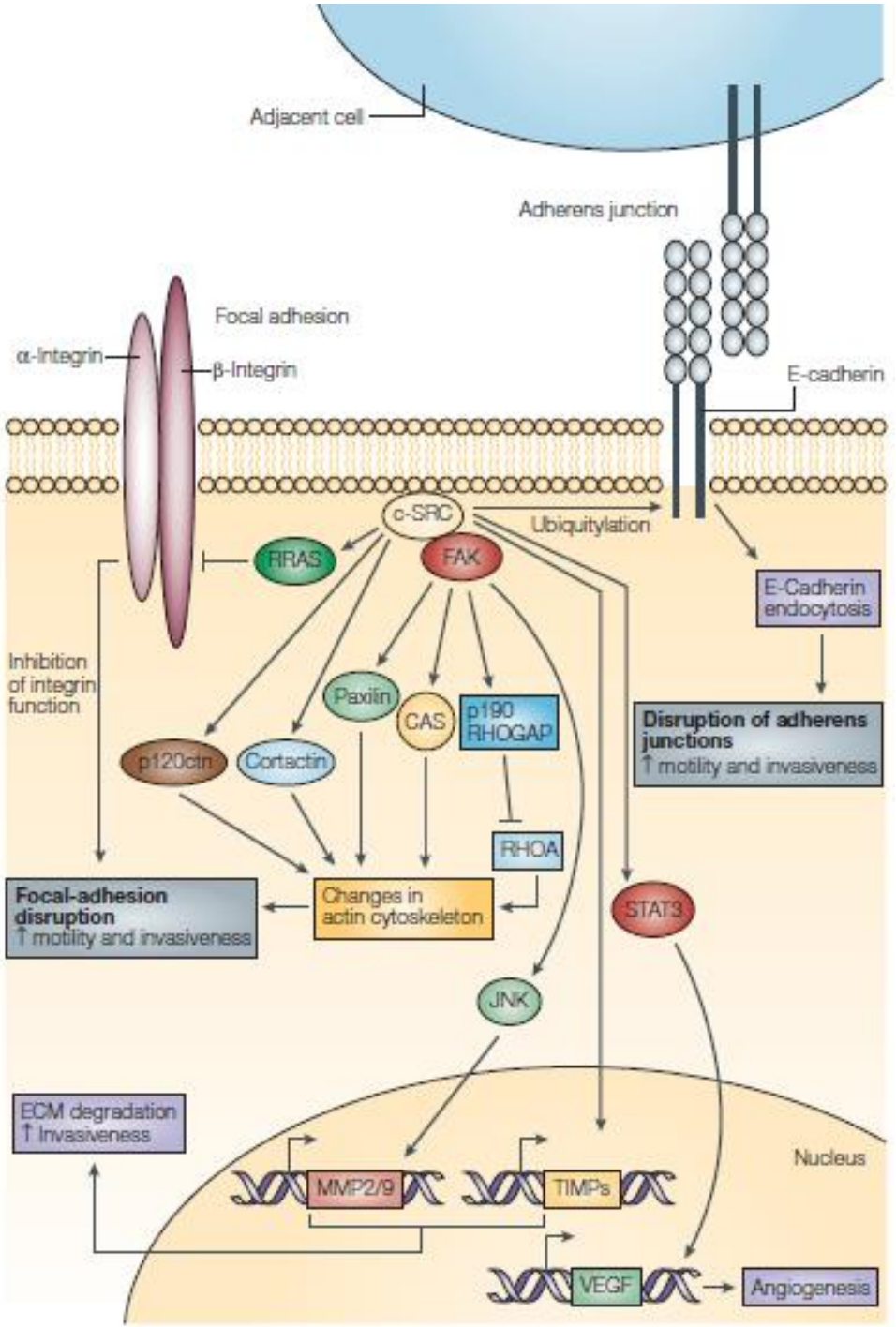
*Differs in carboxy terminal deletions

*v-src is a <u>constitutively active</u> receptor tyrosine kinases due to no regulatory c-domain



Effect of src on tumor behaviour:

-increased cell motility
-ECM degradation
-adheren junction degradation
-stimulated angiongenesis



Viral carcinogenesis in mammals

Many other examples of tumor-inducing viruses in **rabbits, mice, cats, and nonhuman primates** eventually followed (Shope and Hurst, 1933; Bittner, 1942; Gross, 1951; Sweet and Hilleman, 1960),

The **first oncogenic human virus**, Epstein Barr virus, was observed in 1964 (Epstein et al., 1964)

VIRUSES CARCINOGENIC TO HUMAN

- Epstein-Barr virus
- Hepatitis B virus
- Hepatitis C virus
- Kaposi's sarcoma herpes virus
- Human immunodeficiency virus type 1 (HIV-1)
- Human T cell lymphotropic virus type 1 (HTLV-1)
- Human papilloma virus

Viral Carcinogenesis

Causal relationship between putative cancer-causing virus and human cancer:

1. Epidemiological evidence
2. Serological evidence (presence of certain antibodies)
3. Insertion of viral genome into host genome
4. Consistent chromosomal translocation
5. Experimental evidence of viral-induced transformation

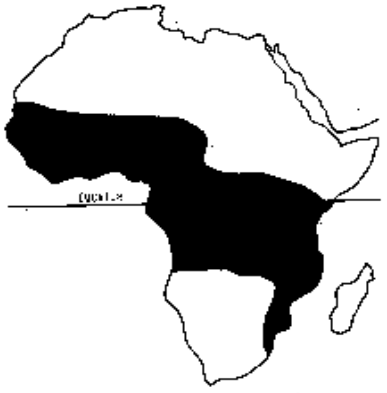
Recognition of Burkitt's Lymphoma

Cancer that starts in immune B-cells
Recognized as fastest growing human tumor

1934-57
Descriptions of Jaw Tumors and High Frequency of Lymphomas in African Children

1958
Denis Burkitt describes a Clinical Syndrome

Endemic in equatorial Africa



Epstein Barr Virus (EBV)

1964

Epstein, Achong and Barr discover EBV in cultured tumor cells derived from African Burkitt's lymphoma tissue by electron microscopy

mature particles

immature particles

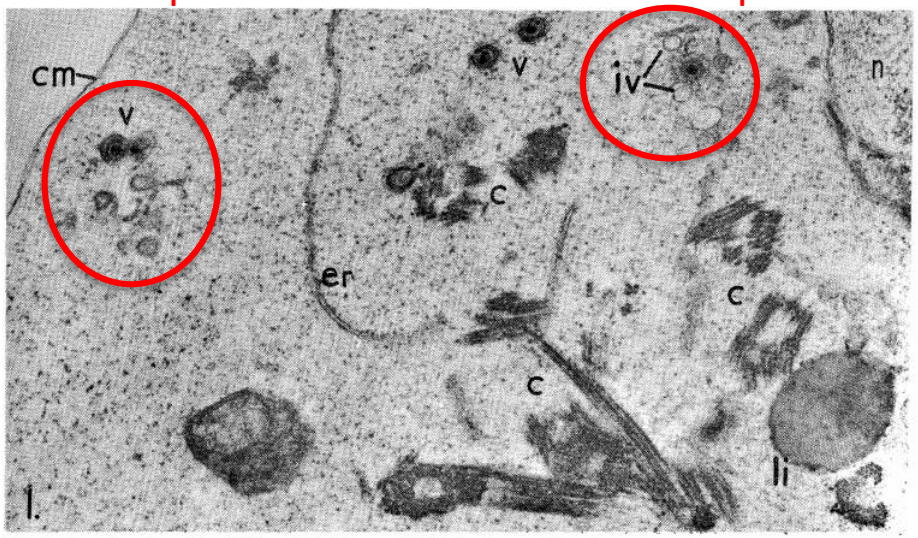


Fig. 1—Part of a cultured lymphoblast derived from a Burkitt lymphoma. The cell membrane (cm) crosses the top left corner and the nucleus (n), bounded by its double membrane, lies in the upper right portion of the field. The intervening cytoplasm contains several mature virus particles (v) within spaces enclosed by fine membranes, some immature particles (iv), and crystals (c) cut in various planes; a large lipid body (li) and endoplasmic reticulum (er) can also be seen. In addition profuse free ribosomes lie scattered throughout the cytoplasmic matrix. Electronmicrograph × 42,500.

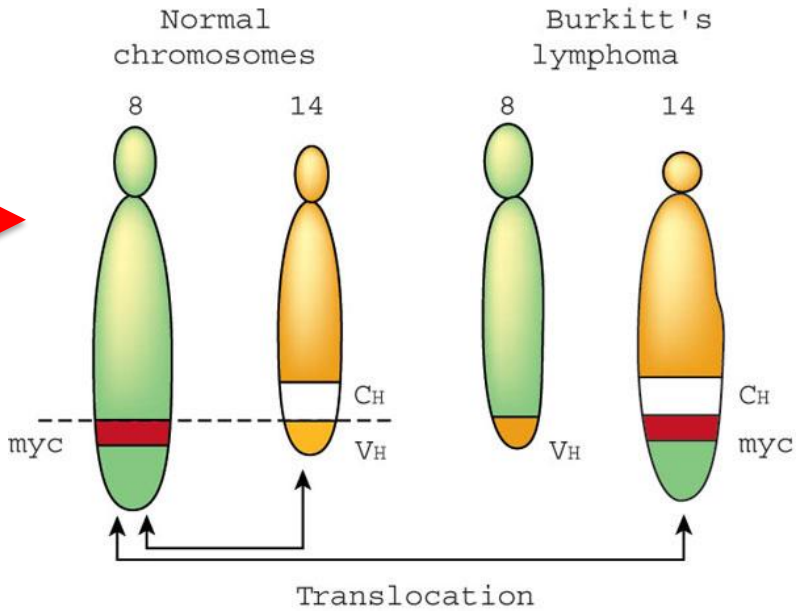
1967-68

Henle's, Diehl and Pope show EBV transformation of B cells

EBV and cancer

- First human tumor shown to be associated with a virus
- Inspired search for others; prevention by vaccine
  - (Hepatitis B virus, Human Papilloma virus)

Chromosomal translocation identified; shown to juxtapose Ig sequences to *myc*

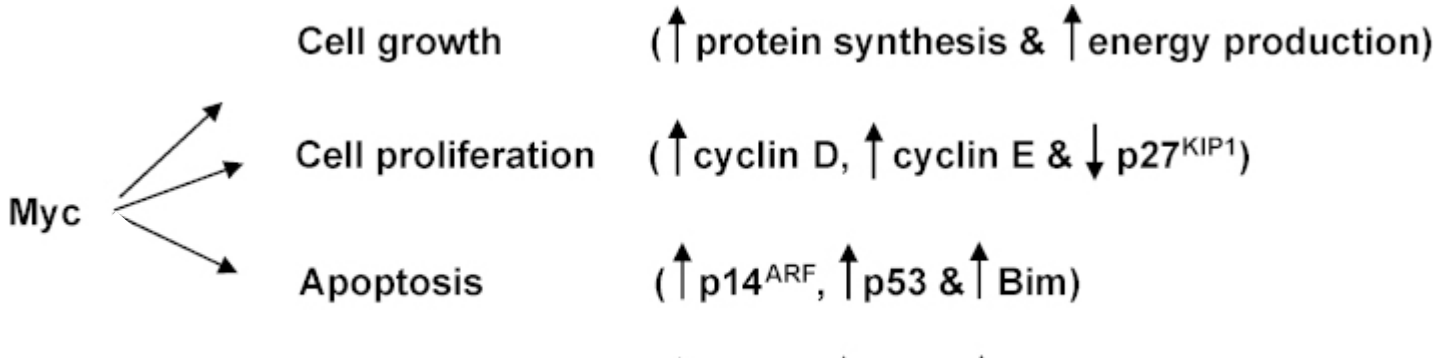


-translocation of myc puts it under the control of IgH (highly active) promoter

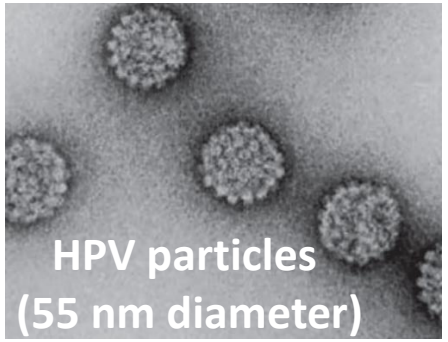
-leading to understanding molecular pathogenesis and providing a model for other lymphomas and leukemias

Myc translocation in the pathogenesis of Burkitt's lymphoma

- a reciprocal chromosomal translocation activates the *Myc* oncogene by *juxtaposing it to an immunoglobulin gene loci*
- This juxtaposing brings the proto-oncogene under the control of a transcriptionally active *Ig* locus
  - (thus a deregulated constitutive expression of the translocated *Myc* gene)
- Myc protein accumulates to higher levels than in normal B cells



Human papillomavirus (HPV)



- DNA virus from the papillomavirus family
- Group of more than 200 related viruses (~40 spread sexually)

High-risk HPVs cause several types of cancer (~5% of worldwide cancers).

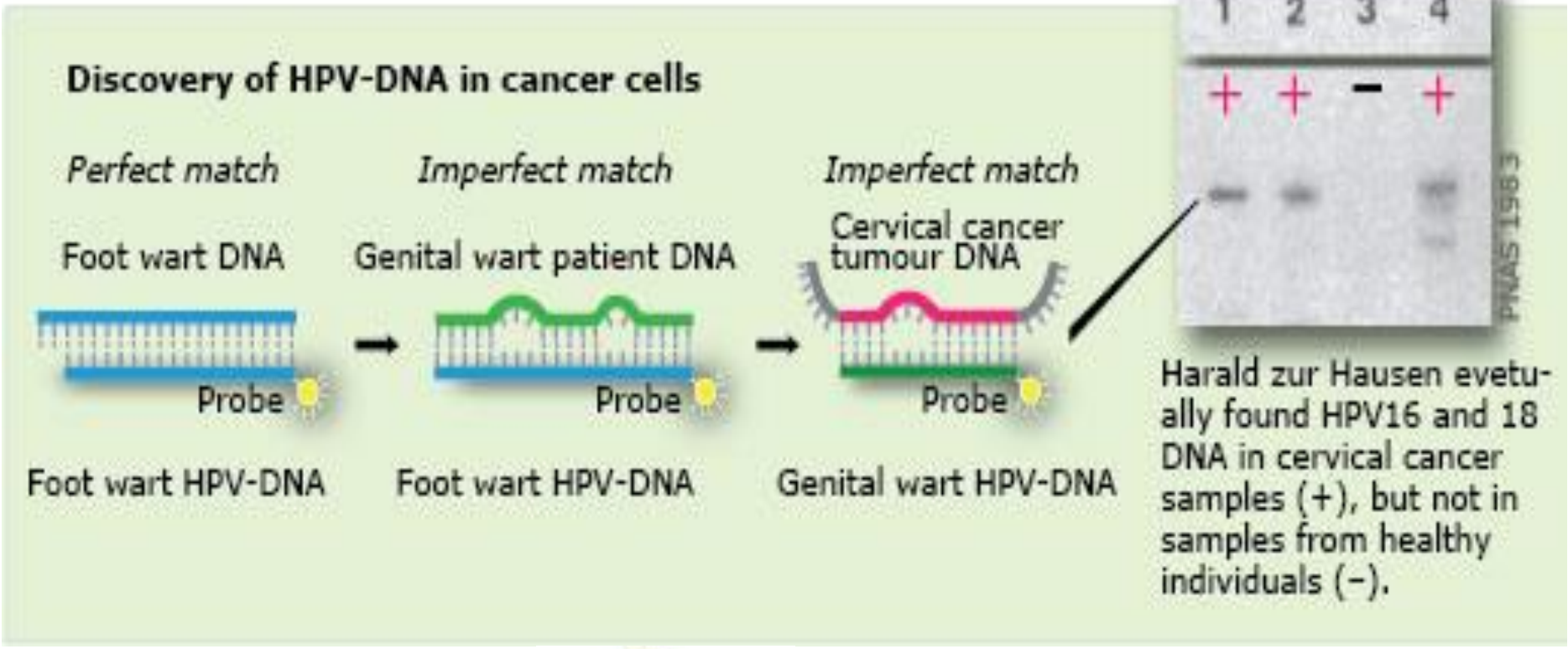
Cervical cancer: Virtually all cases of cervical cancer are caused by HPV, and just two HPV types, 16 and 18, are responsible for about 70% of all cases.

Anal cancer: About 95% of anal cancers are caused by HPV. Most of these are caused by HPV type 16.

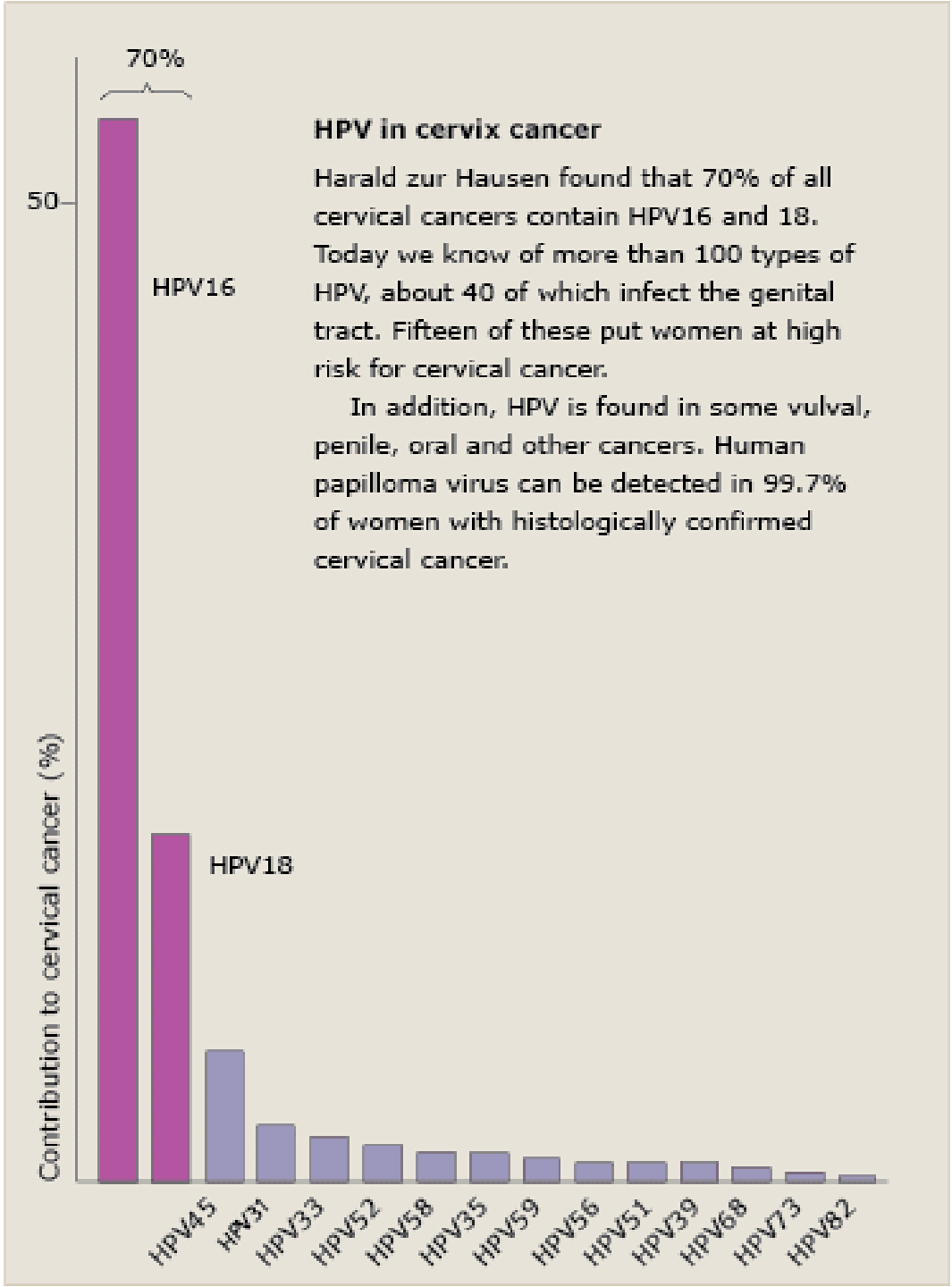
Oropharyngeal cancers (cancers of the middle part of the throat, including the soft palate, the base of the tongue, and the tonsils): About 70% of oropharyngeal cancers are caused by HPV. In the United States, more than half of cancers diagnosed in the oropharynx are linked to HPV type 16.

Rarer cancers: HPV causes about 65% of vaginal cancers, 50% of vulvar cancers, and 35% of penile cancers. Most of these are caused by HPV type 16.

HPV and cancer



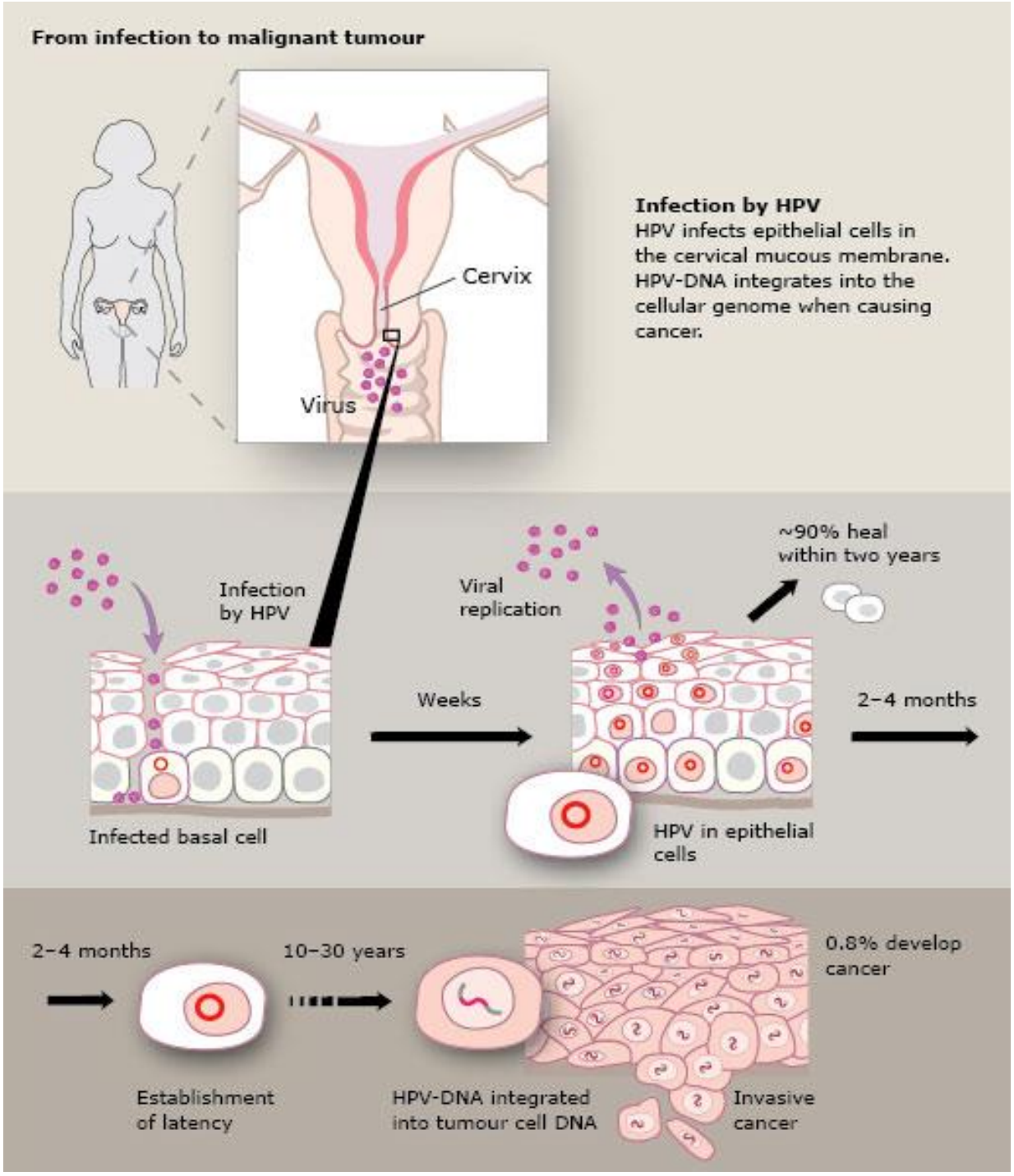
Harald zur Hausen (German virologist), Nobel Prize 2008

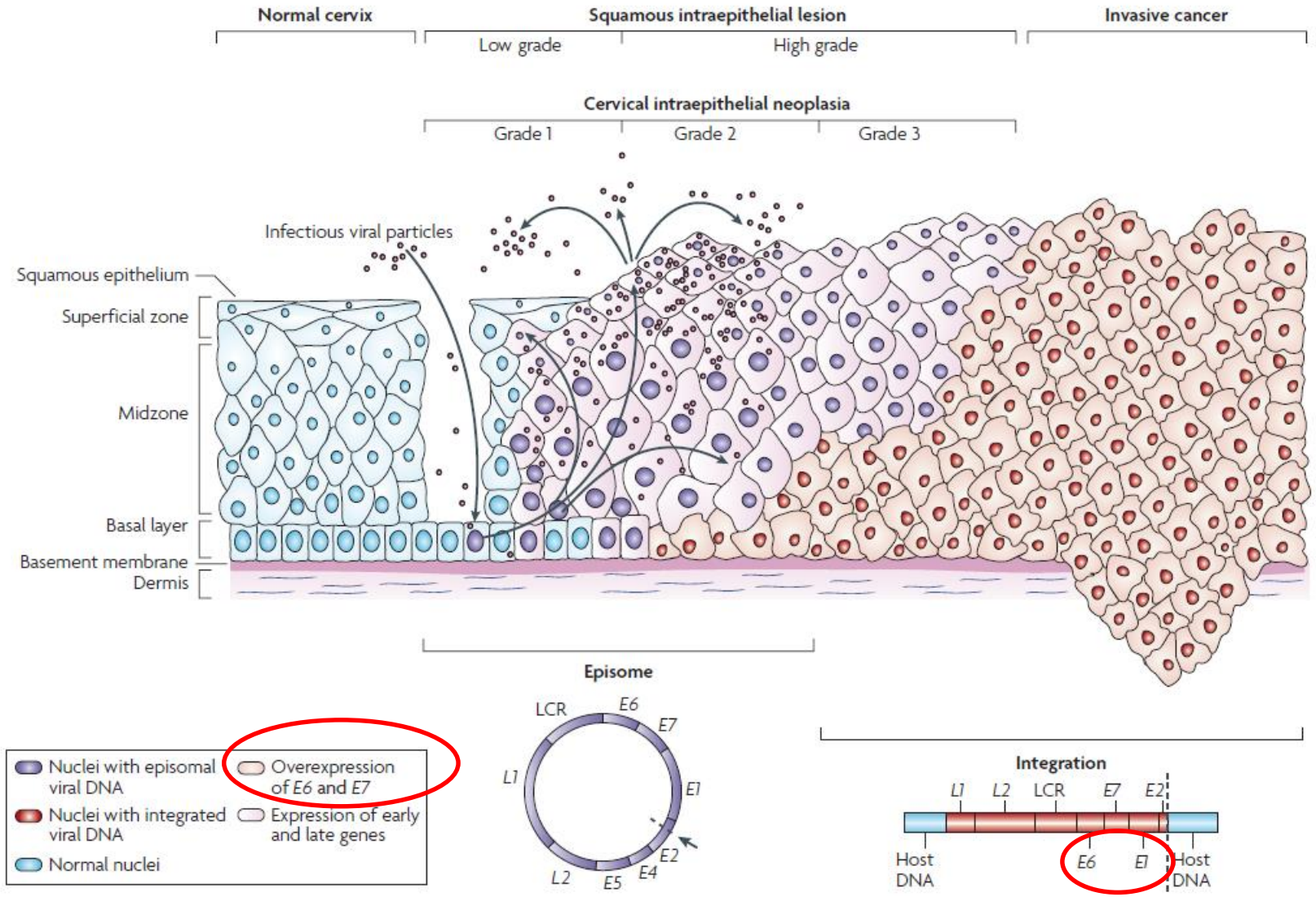


HPV in cervix cancer

Harald zur Hausen found that 70% of all cervical cancers contain HPV16 and 18. Today we know of more than 100 types of HPV, about 40 of which infect the genital tract. Fifteen of these put women at high risk for cervical cancer.

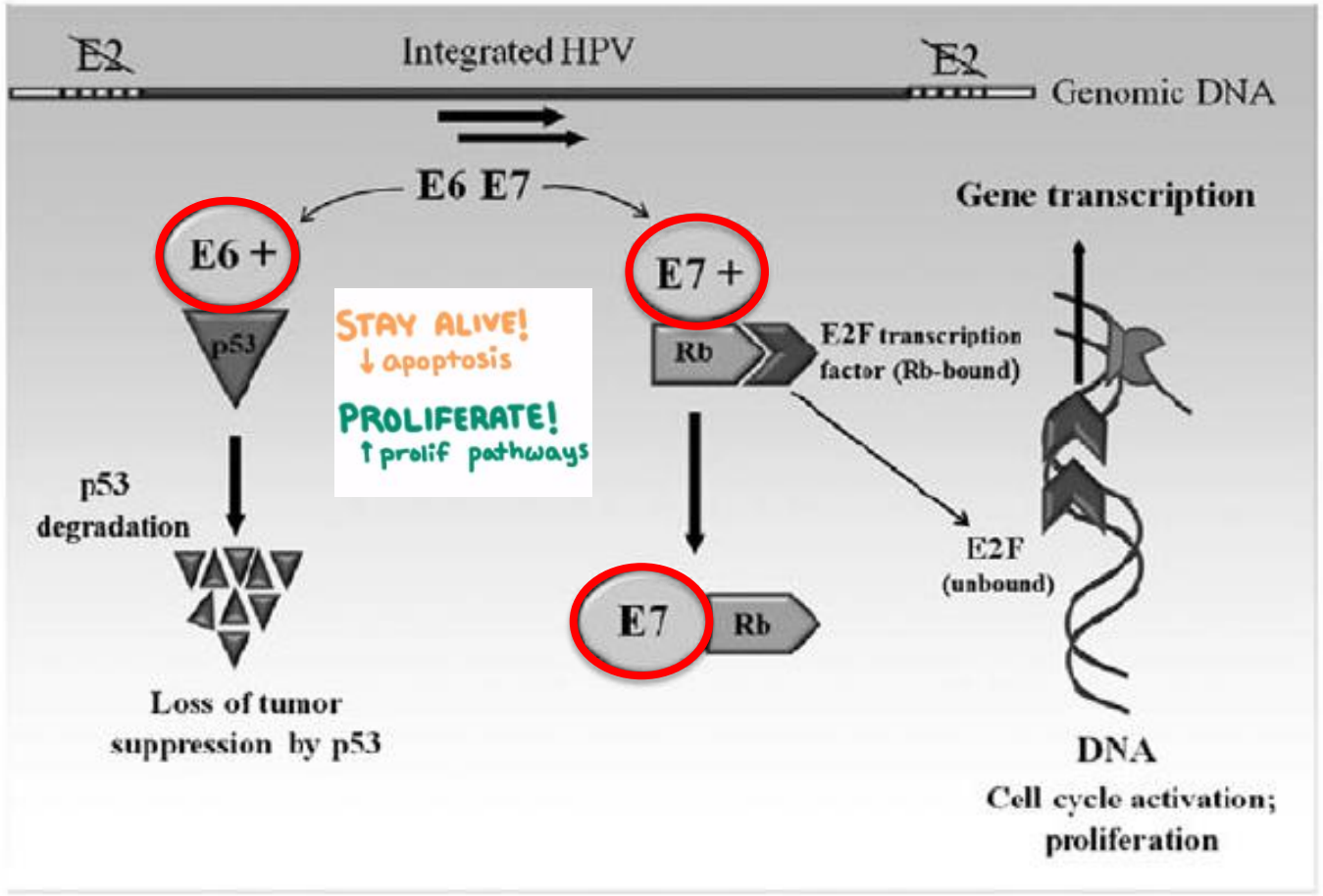
In addition, HPV is found in some vulval, penile, oral and other cancers. Human papilloma virus can be detected in 99.7% of women with histologically confirmed cervical cancer.


From infection to malignant tumour
Cervix
Virus
Infection by HPV
HPV infects epithelial cells in the cervical mucous membrane. HPV-DNA integrates into the cellular genome when causing cancer.
Infection by HPV
Infected basal cell
Weeks
Viral replication
~90% heal within two years
2–4 months
HPV in epithelial cells
2–4 months
Establishment of latency
10–30 years
HPV-DNA integrated into tumour cell DNA
Invasive cancer
0.8% develop cancer

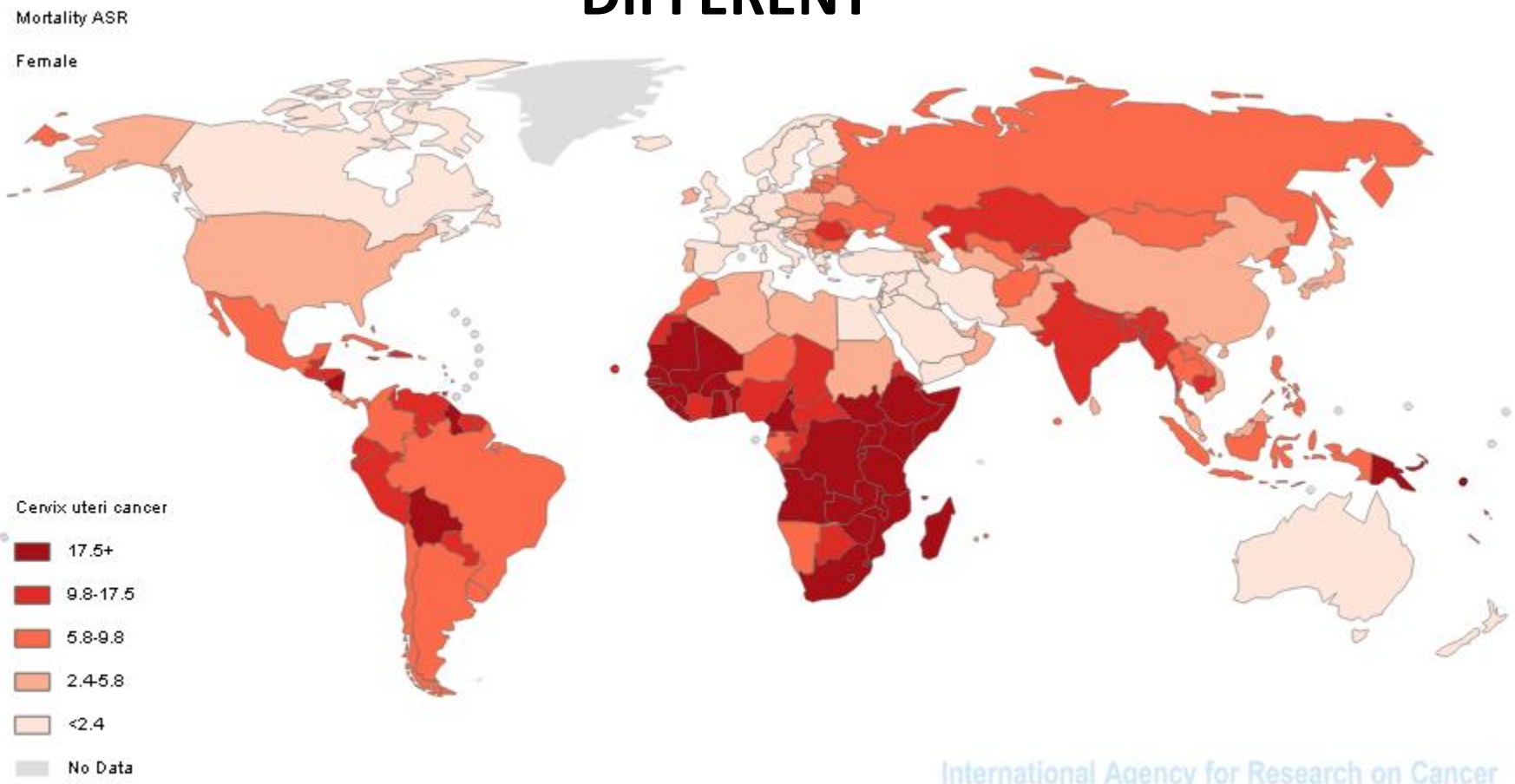

Normal cervix
Squamous intraepithelial lesion
Low grade
High grade
Invasive cancer
Cervical intraepithelial neoplasia
Grade 1
Grade 2
Grade 3
Infectious viral particles
Squamous epithelium
Superficial zone
Midzone
Basal layer
Basement membrane
Dermis
Episome
LCR
E6
E7
L1
E1
E2
L2
E5
E4
Integration
L1
L2
LCR
E7
E2
Host DNA
E6
E1
Host DNA
Nuclei with episomal viral DNA
Nuclei with integrated viral DNA
Normal nuclei
Overexpression of E6 and E7
Expression of early and late genes

Viral oncogenes E6 and E7

- These oncogenes inhibit Rb and p53 tumor suppressors



CERVICAL CANCER MORTALITY IS GEOGRAPHICALLY DIFFERENT

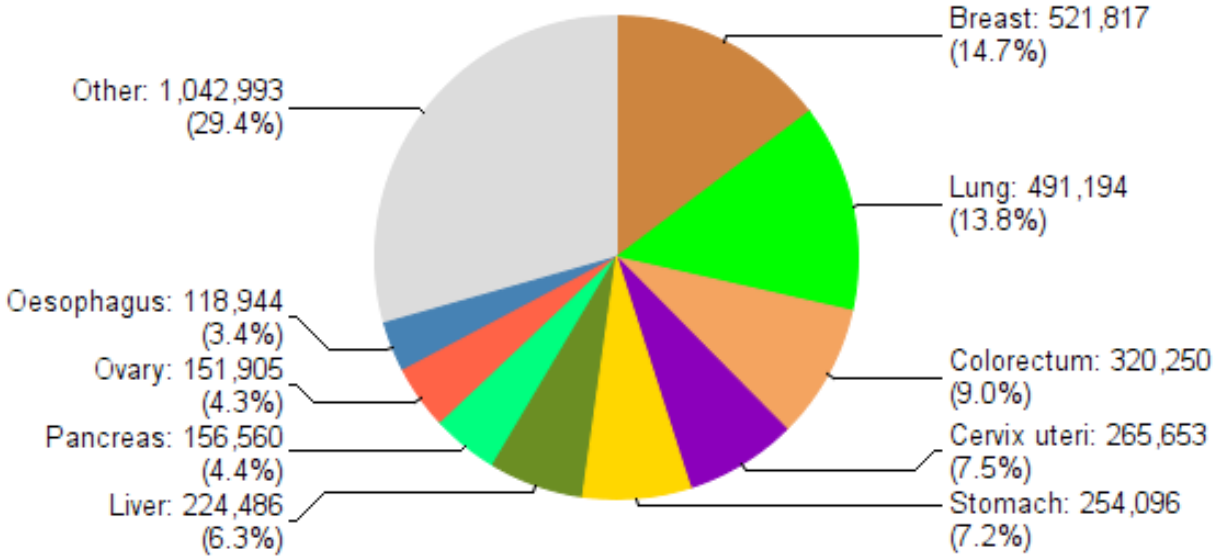


Source: GLOBOCAN 2012 (IARC)

World: Female

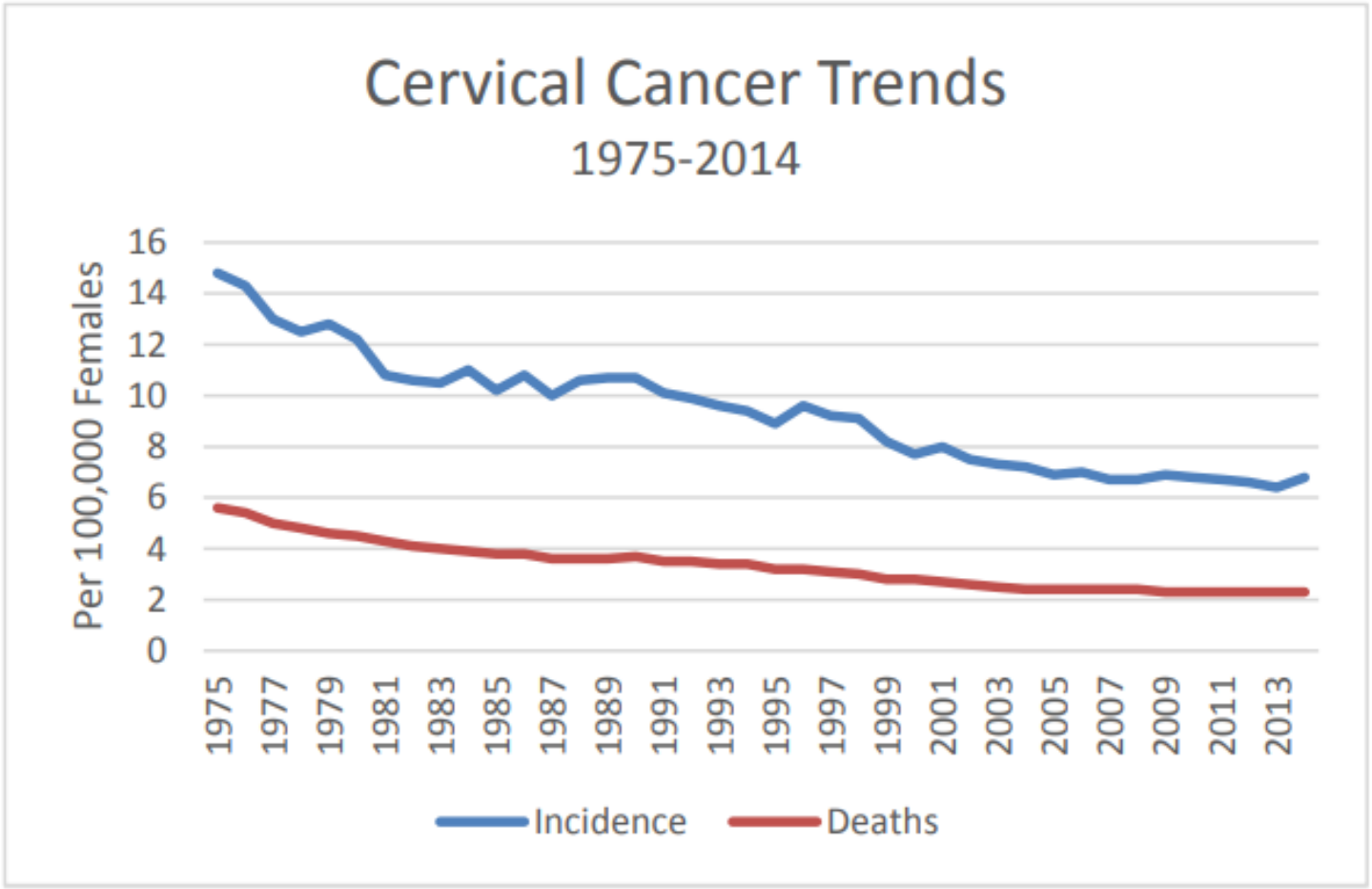
Estimated number of cancer deaths, all ages (total: 3547,898)

CANCER MORTALITY: CERVICAL CANCER IS THE FOURTH CAUSE IN WOMEN



GLOBOCAN 2012 (IARC) - 29.4.2014

CERVICAL CANCER NEW CASES ARE DECREASING

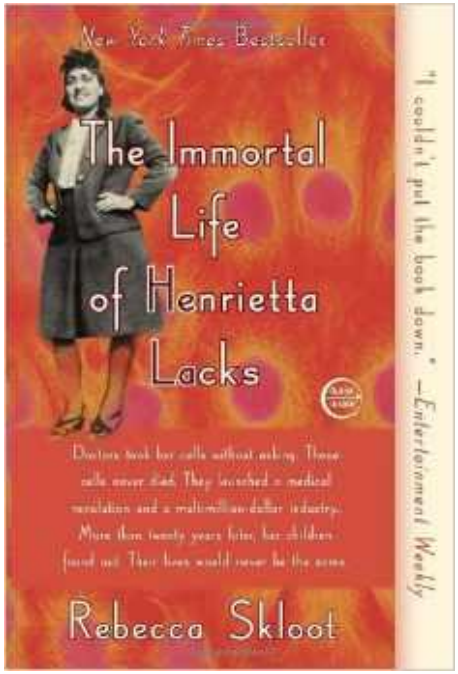


Sources: Incidence: Surveillance, Epidemiology, and End Results (SEER) Program, SEER 9 registries, National Cancer Institute, 2017. Mortality: National Center for Health Statistics, Centers for Disease Control and Prevention, 2017. Age adjusted to the 2000 US standard population; incidence rates adjusted for reporting delays.

www.seer.cancer.gov

Preventing HPV cancers with vaccination

- 2006 first vaccination for HPV available to the public
- Gardasil, Gardasil 9 and Cervarix
  - (prevention, not treatment)



Henrietta Lacks (scientists know her as HeLa). She was a poor black tobacco farmer whose cells—taken without her knowledge in 1951—became one of the most important tools in medicine

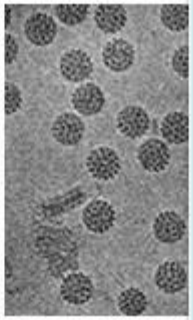
Hela cells are HPV18 positive

Viral transforming genes

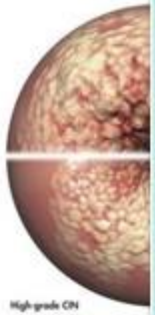
- Two general strategies:
  - Permanent activation of cellular signal transduction cascades
  - Disruption of cell cycle regulation

Important discoveries and events in tumor virology

1933
Richard Shope discovers a papillomavirus in the horns of cottontail rabbits (CRPV)



1937
Dr Shope observes that rabbits that overcome CRPV are immune to re-infection



1977
Dr Harald zur Hansen links HPV to human cervical cancer

2006
A vaccine against HPV-16 and 18 is made available to the public

1935
Dr Rous describes the progression of papilloma warts into cancer

1966
Dr Rous is awarded the Nobel Prize for his work on the causes and treatment of tumours in the chicken

1995
WHO declares HPV-16 and HPV-18 cancerous

2008
Harald zur Hansen receives a Nobel Prize for his work on HPV