Introduction to Molecular biology of Head and Neck cancer

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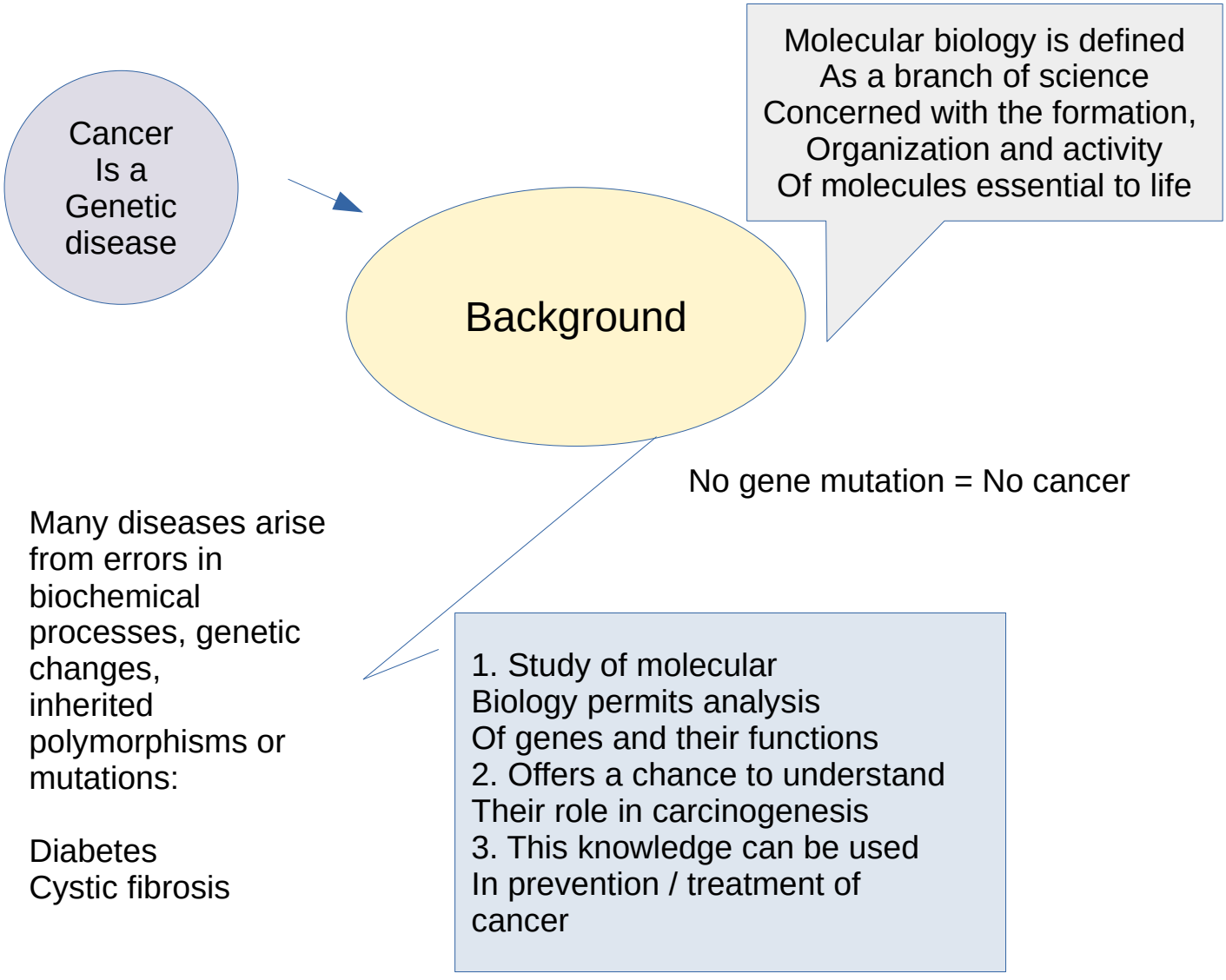


What has changed?

Current technology in molecular biology can analyze tumors and is capable of identifying all the mutated genes in a biopsy specimen taken from an individual tumor site.

Rapid tests are also available:

One step nucleic acid amplification (OSNA)
This test provides perioperative information such as nodal involvement to the surgeon

Understanding the function of mutated genes will help:

1. In learning how it would impact the cellular process
2. Tumor behavior in terms of growth and progression can be predicted
3. Prediction of how the tumor cells would behave to a specific therapy

Role of molecular biology in modern therapy

Monoclonal antibody drugs like:

Cetuximab, Novolumab, and Adalimumab are used to treat cancers and rheumatoid arthritis

These drugs are manufactured using molecular biological techniques like DNA cloning and sequencing.

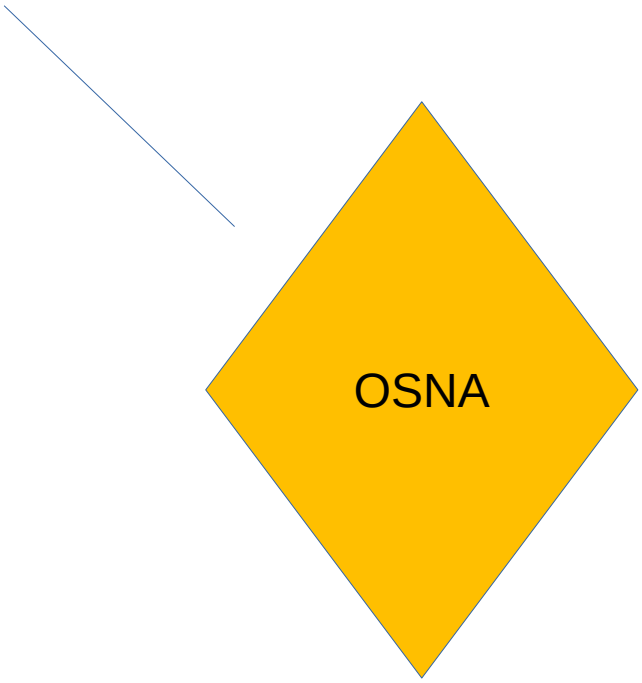
cetuximab

1. Epidermal growth factor receptor inhibitor
2. Used in treatment of colorectal cancer, and head and neck cancer
3. Can be used in combination with radiotherapy
4. KRAS gene encodes G protein on the epidermal growth factor (EGFR) pathway. EGFR inhibitors will work only on tumors in which KRAS gene is not mutated. KRAS mutational analysis is a must before prescribing these drugs

No Gene mutations = No cancer Even when virus (carcinogen) is present

Identification of mutated genes in biopsy tissue

One Step Nucleic acid amplification



Functions of mutated genes can be studied. It would also reveal that how they would impact the cellular Process. It could also predict the tumor behavior also.

Can help in identification of biomarkers. Identification of p16 in patients with oropharyngeal squamous cell carcinoma is one good example

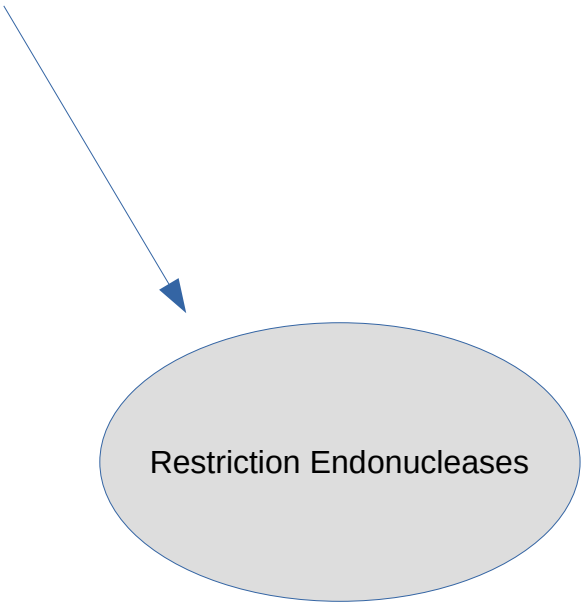
1. This test identifies all mutated genes from biopsied tissues
2. This test can be performed in hours
3. Provides excellent perioperative information like nodal involvement

Understanding the functions of mutated genes will help in:

1. How they impact cellular process
2. Prediction of behavior of tumor cells in terms of its growth and progression
3. Understanding how tumor cells would respond to therapy
4. Understanding how the body is going to respond to the tumor

The Technology

Identification of these enzymes has revolutionized the field of molecular Biology



These enzymes have the ability to cleave double stranded DNA at predefined sites as it can clearly recognize specific sequences of DNA.

Di-deoxy sequencing method: Invented by Fred Sanger

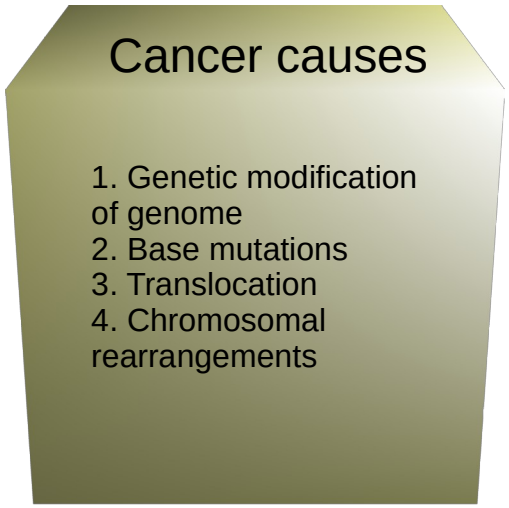
This involves amplification of specific sequences of DNA using PCR.

Illumina / Solexa / SOLiD:

These technologies are also involved in gene amplification and splicing. They have contributed enormous amount of data that is proving to be useful in better understanding of head and neck cancers. They also help to examine the transcriptome (the messenger RNA products of genes that are being actively transcribed).

TNAscope:
This helps in the analysis of fixed tissue biopsy sections. This test is highly sensitive in detecting human papilloma virus gene expression.

Molecular biology of Head & Neck Cancer

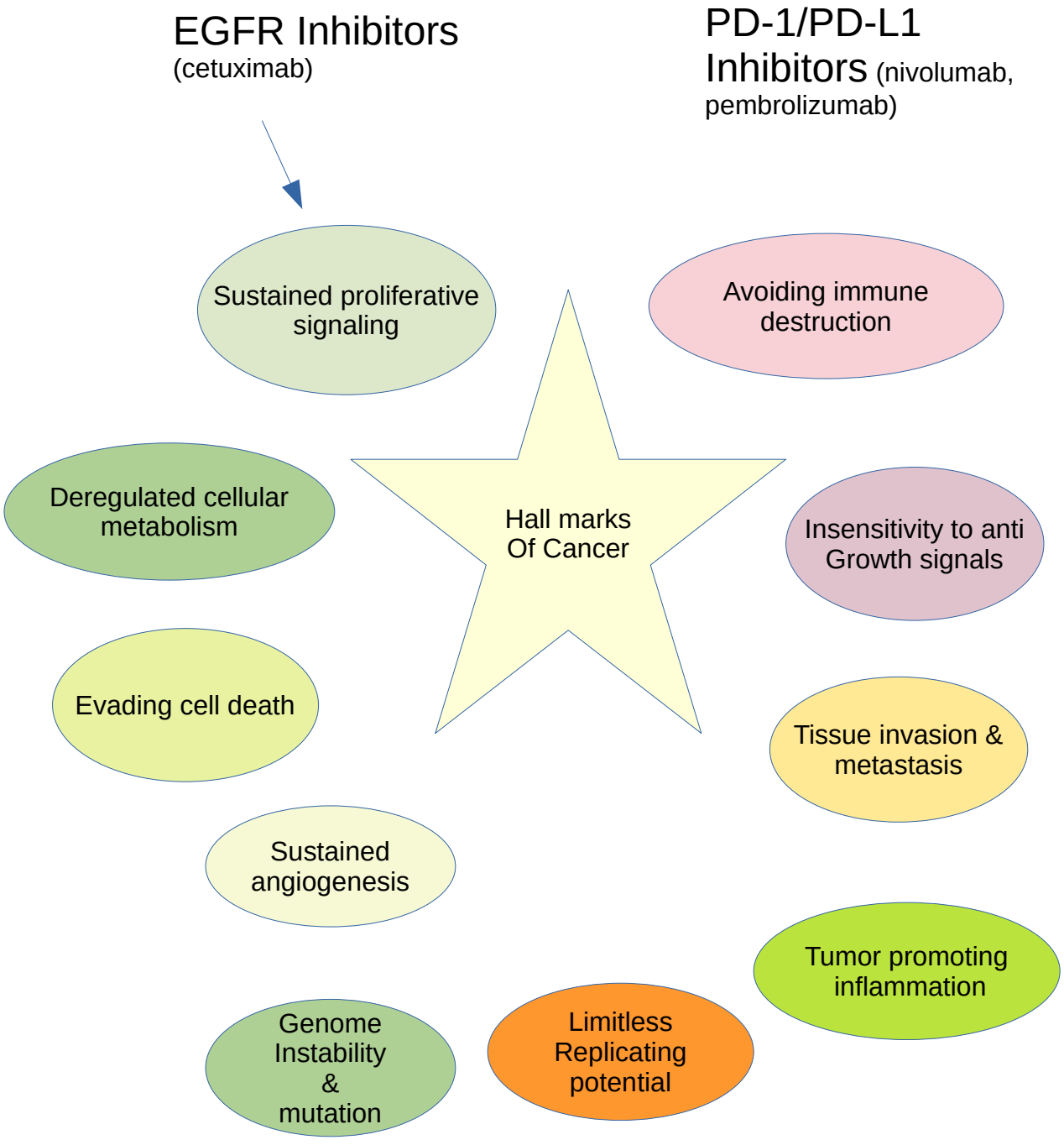


Gene Mutations:
Could be inherited/acquired.
No identified common inherited cancer syndrome has been identified in Head and neck like that of colon / breast

Indirect effects:
Altered gene regulation
Via epigenetic alterations
Affecting chromatin structure

Carcinogenic events:
1. Affect the function of critical subset
Of regulatory genes(survival genes, proliferation, Invasiveness, and interaction with host).

Hallmarks of Cancer



Fanconi Anemia

1. Rare autosomal recessive disease

2. Affects approximately 3 in 10000000 individuals

3. These patients have increased risk of developing cancers of Head & neck

4. None of the genes implicated in the different complementation groups of this disease have been found to be mutated in sporadic head and neck cancers

This term indicates a situation in which mutations in different genes can cause the same phenotype I.e disease.

If matings between two affected individuals produce healthy offspring, then the mutation cannot be in the same gene. This is only applicable for a recessive trait.

Cancer associated syndromes

1. Fanconi syndrome: Anaemia with increased incidence of Head and neck cancers
2. Lynch II syndrome: Commonly implicated genes include MSH2, 6 or MLH1. There is associated increased risk of Colo rectal and endometrial cancer
3. Bloom syndrome: inherited mutations in the BLM gene. Associated with short stature and increased risk of malignancy
4. Xeroderma pigmentosum: Associated with defects involving DNA repair genes
5. Ataxia telangiectasia: Associated with mutations in ATM gene
6. Li-Fraumeni syndrome: Involving mutations in the TP 53 and CHEK2 genes

Only TP53 and CHEK2 gene mutations have shown increased Risk of head and neck cancers. The concept of field cancerization in oral cancers can be explained by the process of sporadic mutations and clonal selection. As mutations arise and the populations of cells harboring the mutations expand they ultimately become cancers.

Populations of cells that arise early on don't disappear. They may continue to survive, proliferate and occupy areas outside the cancer area.

Mutagenic events

1. DNA damage occurs every day in our body cells

2. Replication errors also occur

3. Much of DNA damage gets repaired and hence don't lead to Stable alterations / mutations

4. If DNA damage is not repaired then it would lead to established mutations

5. Common source of DNA damage is the normal biochemical process that generates reactive oxygen species (ROS). This is done by oxidative phosphorylation through the electron transport chain. ROS are known to cause oxidation of one of the DNA bases, guanine to 8-oxo-guanine. These can be repaired by base excision pair (BER) mechanisms initiated by an enzyme that recognizes this condition

Processes that promote head and neck cancers

1. Mutations arising from exposure to chemical carcinogens (tobacco, And alcohol).

2. Infection by oncogenic viruses (specifically oncogenic strains of HPV) Typically leading to loss of tumor suppressor gene function

3. Epigenetic modifications, leading to loss of tumor suppressor gene expression

Environmental carcinogens Cause significant mutagenic Events and they result in Cancers with poor outcomes. Their effects on DNA could Not be repaired that easily

Tobacco smoke has About 60 substances That could affect DNA

Alcohol as a carcinogen

1. Alcohol consumption is a major risk factor for developing head and neck cancer

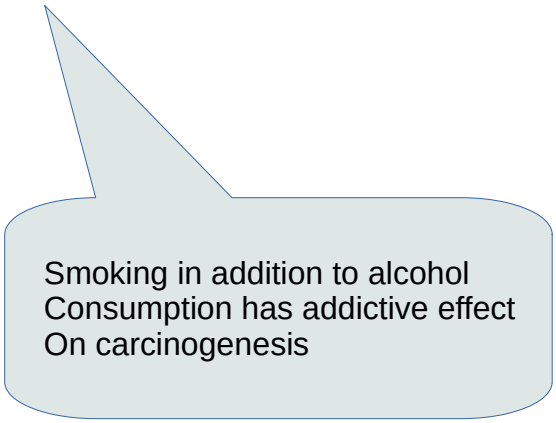
2. Alcohol provokes inflammatory response

3. Alcohol can cause mutations

4. Alcohol can cause epigenetic effects

5. Acetaldehyde a metabolite of alcohol is a genotoxin

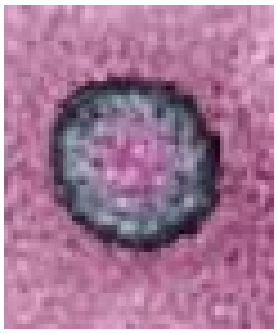
6. Majority of alcohol gets metabolized in the liver while oral cavity mucosa and oesophagus can also metabolize alcohol thereby releasing Acetaldehyde locally



Virus as a carcinogen

1. Incidence of oropharyngeal squamous Cell carcinoma has shown an increase Despite a decrease in the incidence of Smoking

2. Presence of viral DNA derived from human Papilloma virus 16 inside the malignant cells Has really opened our eyes towards this fact



HPV is a double Stranded DNA virus

These viruses ensure That the host cell Provides the DNA Replication machinery For their proliferation

These viruses push The host cell into S phase

Epigenetic modifications

1. Epigenetic modification of gene expression and function is a mechanism That promotes cancer in the head and neck.
2. Methylation of cytosine residues at the so called CpG islands in the promoter Regions of TSGs.
3. In most genes the concentration of CpGs is higher in the non-coding promoter Regions known as the “islands”.
4. In head and neck cancers CDKN2A hypermethylation is associated With reduced overall survival rate

1. DNA methylation
2. Histone modification
3. Non coding RNA

Genetics is the study of Heritable changes in gene Expression that do not Involve changes to the underlying DNA sequence. It is also known as The change in phenotype without a change in geneotype

The Cancer Genome Atlas

Loss of chromosomal
Regions in 3p and 8p
And gains of 3q, 5p and 8q
Are the most common

Majority of tumor cells
Harbored copy number alterations
In this phenomenon sections of the genome
Are repeated and the number of
Repeats varies between
individuals

1. 30 genes have been described in TCGA that are critical For carcinogenesis
2. These genes have a mutation frequency ranging from 80-0%
3. Depending on the presence / absence of HPV DNA tumors Can be classified into HPV negative and HPV positive
4. HPV status is decided from whether viral oncogenes E6 And E7 are expressed
5. Expression of cellular protein p16 is a surrogate marker For HPV infection

These changes in the genome causes:
1. Loss of self sufficiency in growth signals
2. Insensitivity to antigrowth signals
3. Evading apoptosis
4. Limitless replicative potential
5. Sustained angiogenesis
6. Tissue invasion & metastasis

Enabling characteristics:
Genome instability & mutation
Tumor promoting inflammation

Emerging hallmarks:
Avoiding immune destruction
Deregulating cellular energetics

Gene mutations in Head & Neck Cancers

TP 53 – encodes p53 proteins. Guardian of the genome

CDKN2A – Critical gene for cell cycle regulation
It encodes protein p16 which is a regulator of cell cycle progression

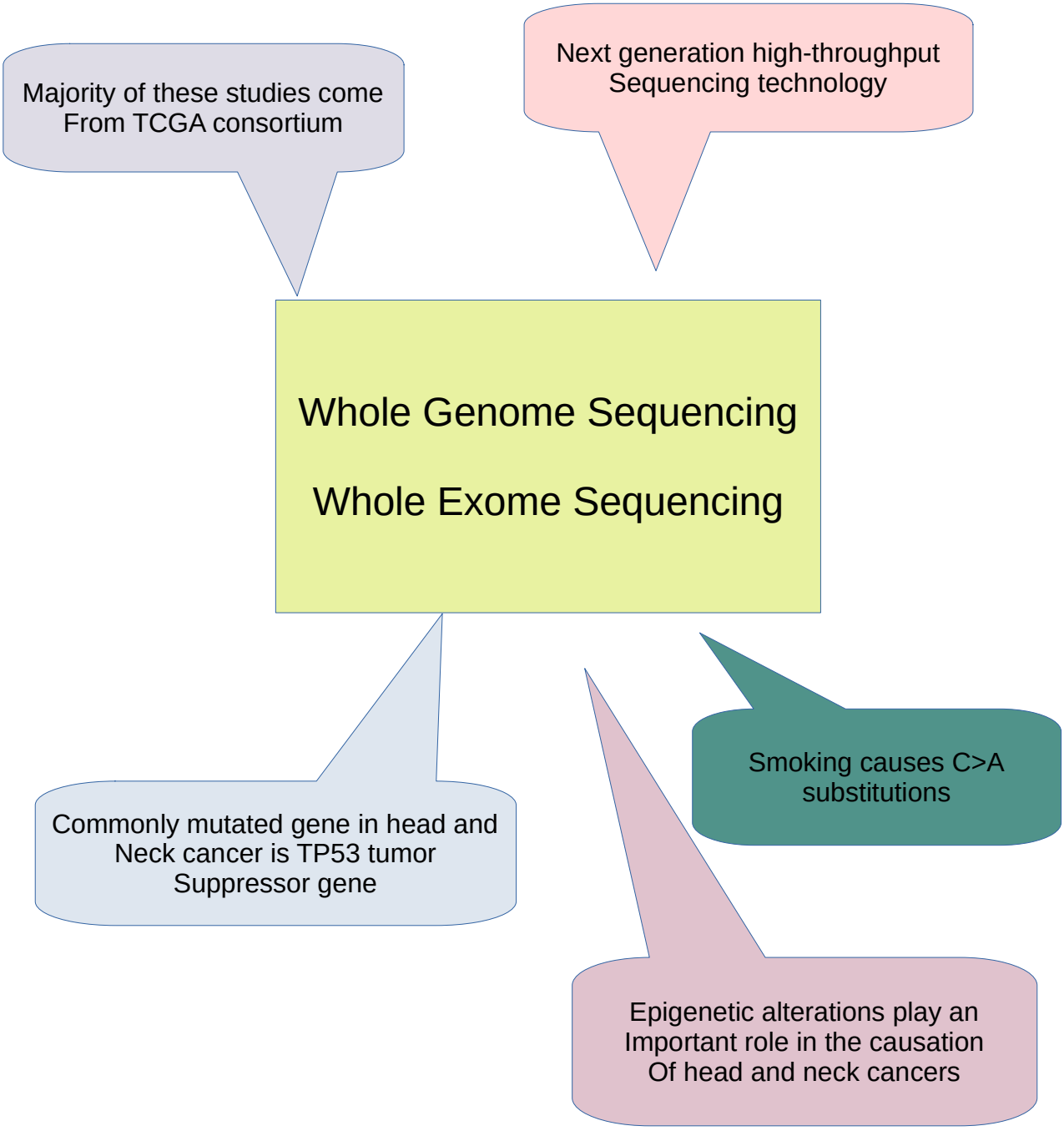
CCND1

PIK3CA

MYC

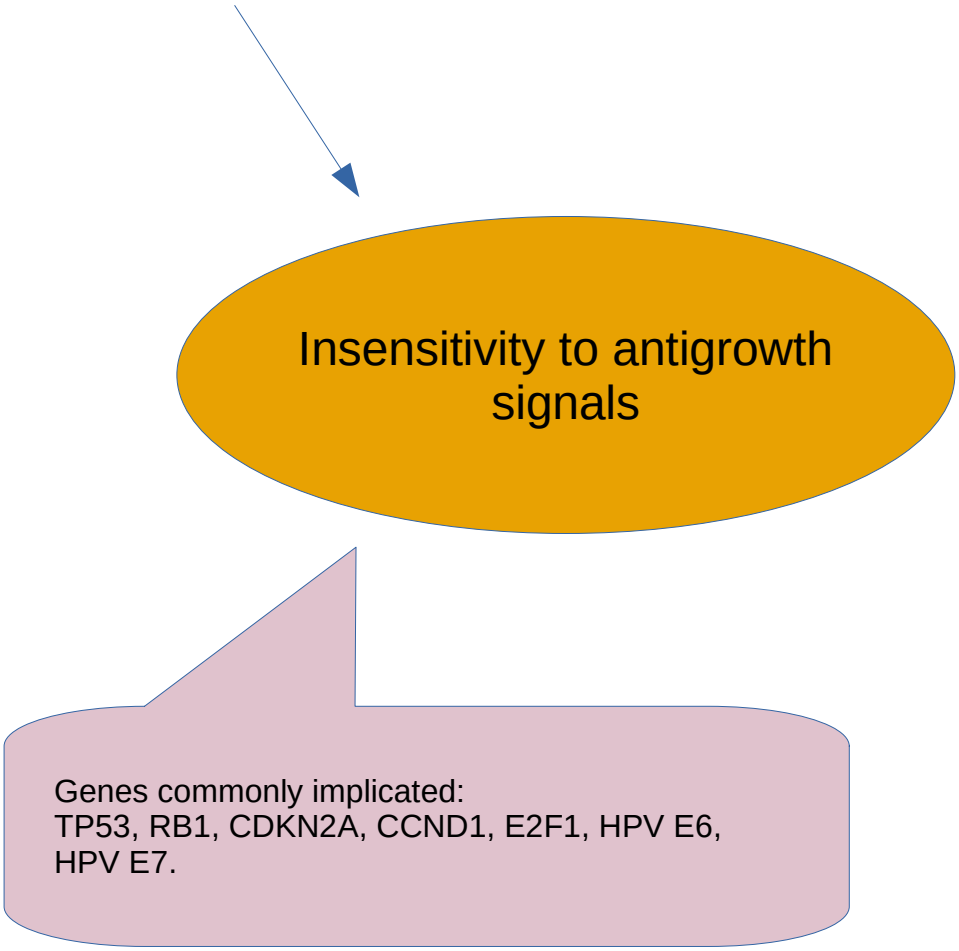
1. Insensitivity to antigrowth signals
2. Evading apoptosis
3. Increased cell survival
4. Sustained angiogenesis

WGS / WES


Majority of these studies come From TCGA consortium
Next generation high-throughput Sequencing technology
Whole Genome Sequencing
Whole Exome Sequencing
Commonly mutated gene in head and Neck cancer is TP53 tumor Suppressor gene
Smoking causes C>A substitutions
Epigenetic alterations play an Important role in the causation Of head and neck cancers

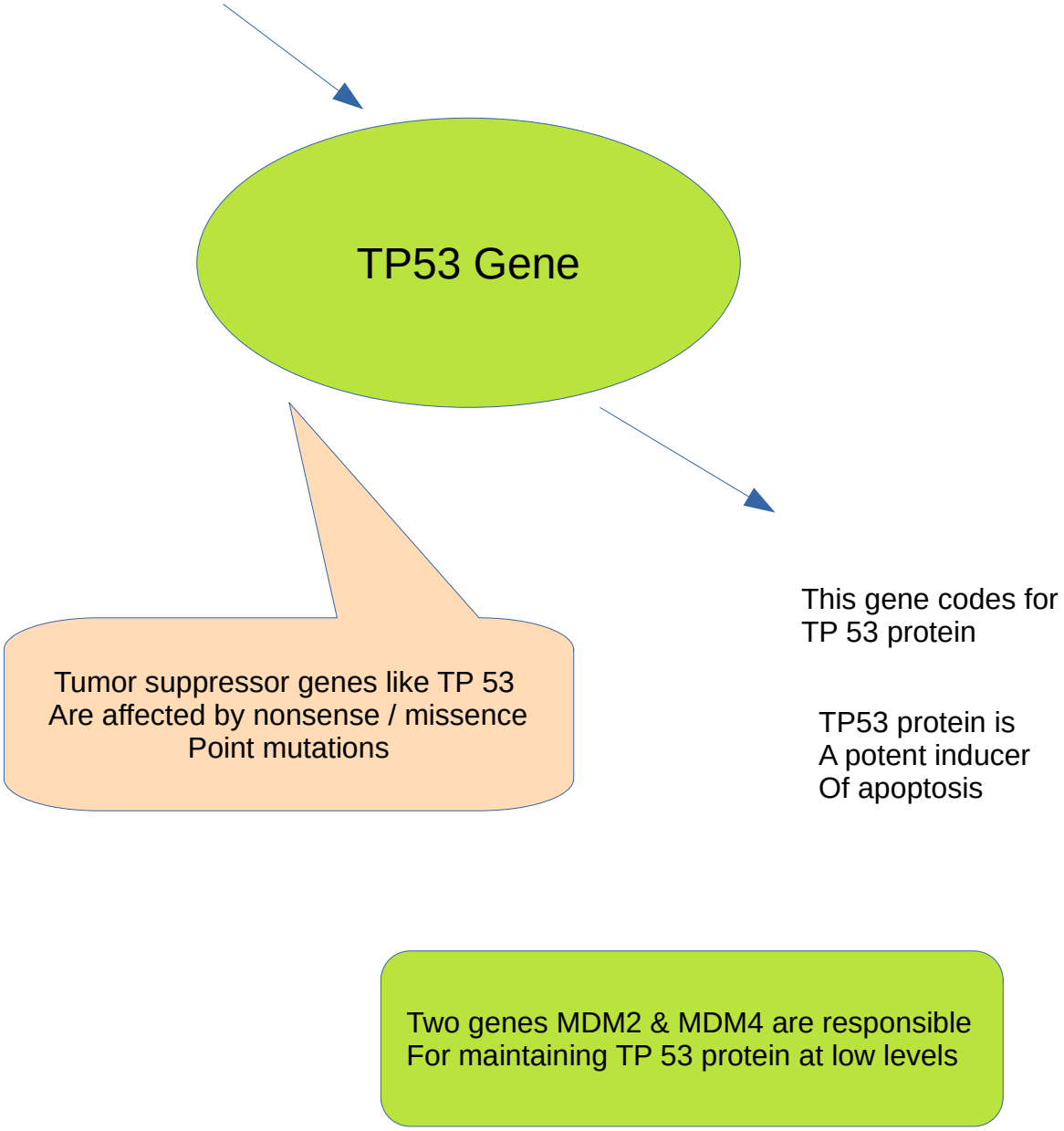
Loss of cell cycle control

This is the most common pathway affected in both HPV+ and HPV- tumors



TP53

1. Most common mutations in head & neck cancers Occurs in this gene
2. Mutation of this gene occurs in 60% of all head & neck Cancers
3. Missence point mutations change in codon in such a Way that a different amino acid is encoded



Tumor suppressor Gene

CDKN2A gene encodes two Proteins p14, p16 which has Tumor suppressor activity Hypermethylation inactivates This gene

These genes regulate G1-S phase transition

RB, E2F1 and CDKN2A

RB gene is also known as Retinoblastoma gene

These genes regulate mitogens Which facilitate mitosis. Mitogens Include:
Epidermal growth factor
Cyclin dependent kinases
Phosphorylation of RB genes inactivates it

CCND1

let-7c is a micro RNA
That regulates many
Target genes

CCND1 gene codes for Cyclin D1 protein.
This gene is found amplified in head and neck
Cancers.

Cyclins are critical regulators of cell cycle
These proteins are short lived

Cyclin D1 is regulated
By MAPK pathway

MYC

1. This gene plays a role in cell cycle regulation

2. MYC gene has effects on the following genes:
CCND1, TP53, TERT (Telomerase a gene implicated in immortalizing Cancer cells)

3. Modulates stability of recently synthesized RNA molecules

4. Amplification of MYC locus occurs in nearly 20% of HPV positive Head and neck cancers

HPV E6, E7 and E2f1

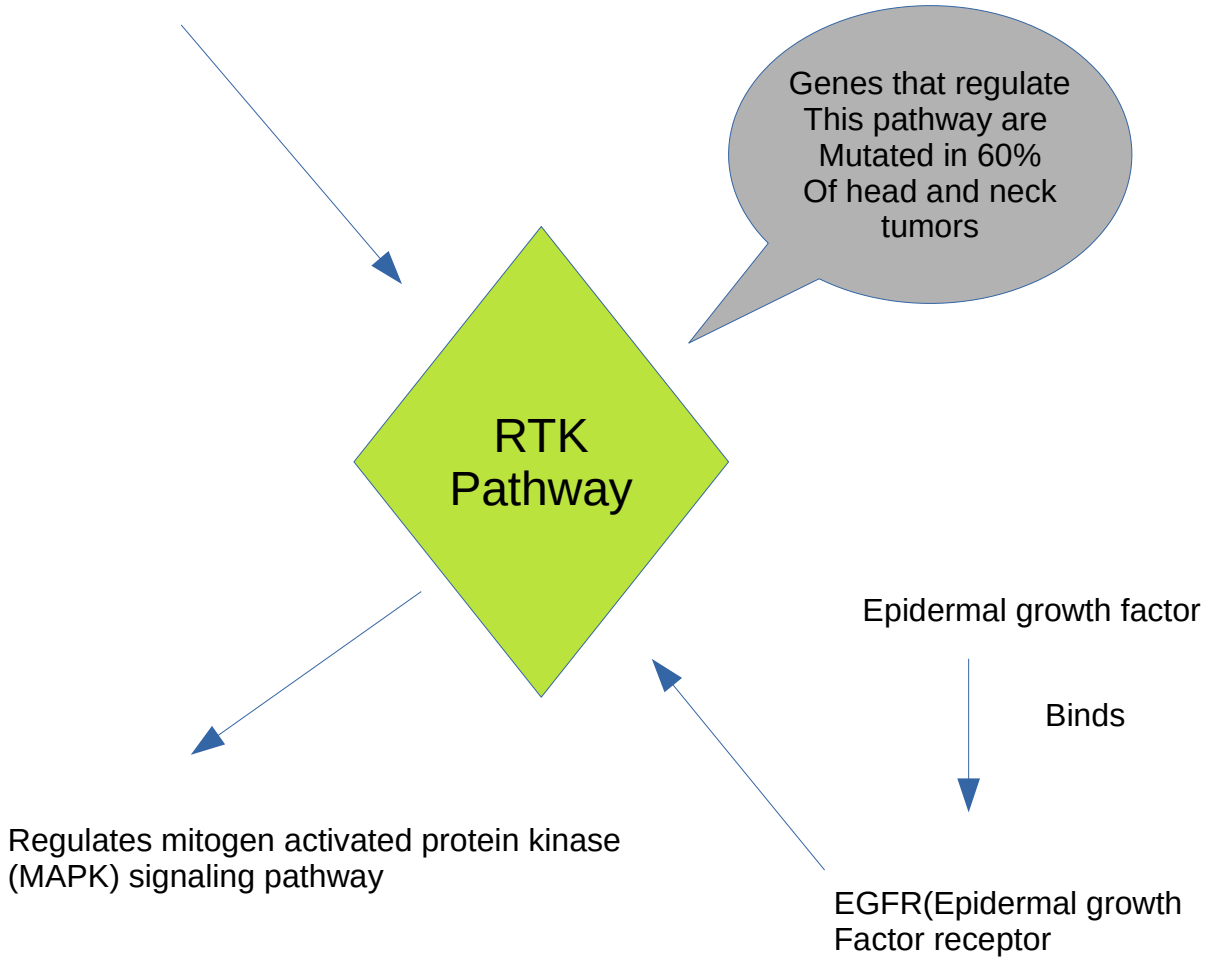
E6 & E7 inactivates TSG, TP53 and RB. This pushes the affected cell into S Phase progression

E6 and E7 are expressed as Episomal genes. These genes Replicate independent of Chromosomal DNA

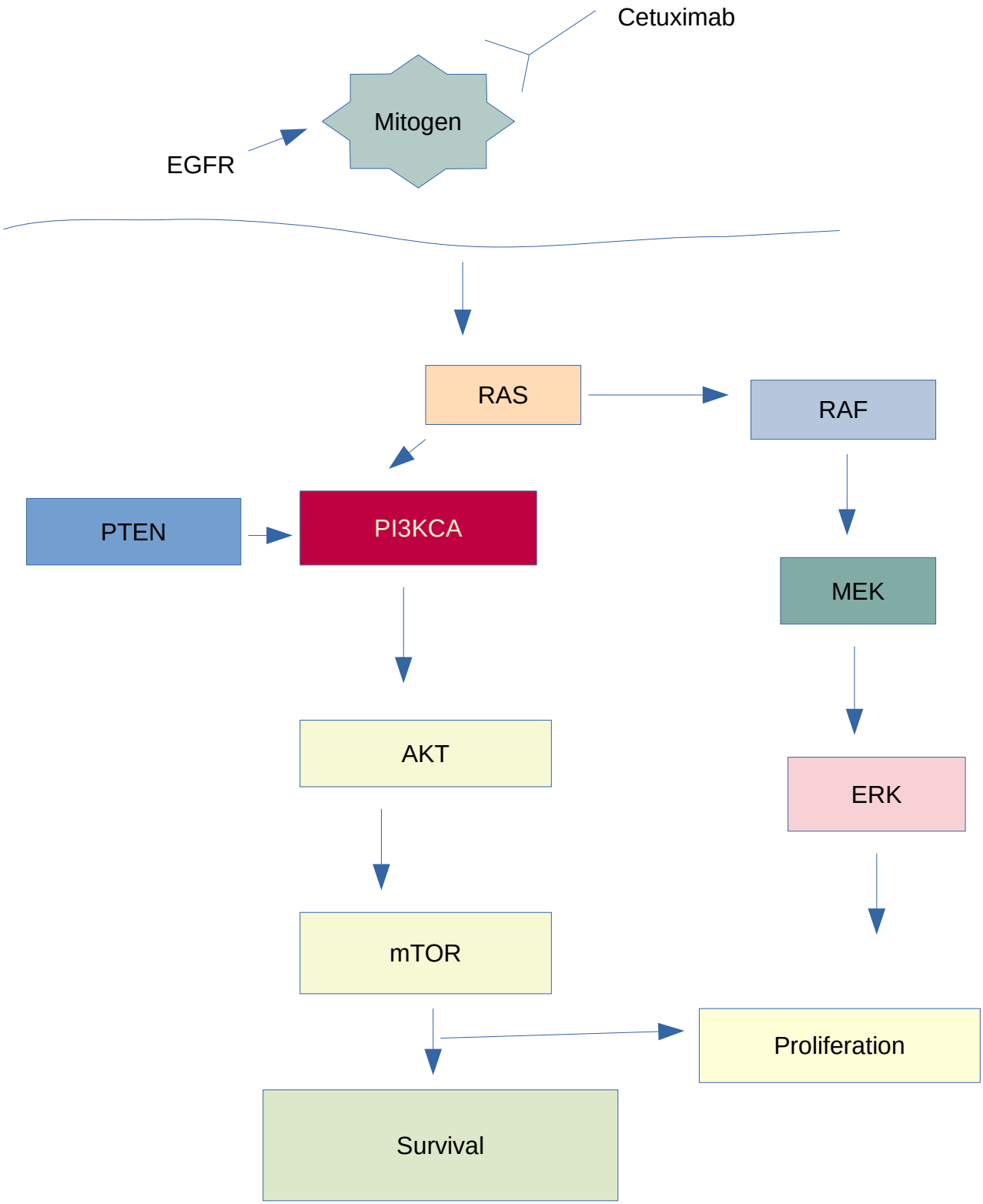
Human papilloma viruses are known to have double stranded DNA.
They require host cell DNA mechanism for replication.
HPV encodes two critical onco proteins E6 and E7 which promotes S-phase progression in infected cells.
Sometimes DNA material from HPV gets integrated into the genome so the infected cell retains the oncogenes E6 and E7. These genes promote deregulated cell division.

Growth & Proliferation phase Signaling

Receptor Tyrosine Kinase signaling pathway



EGFR pathway



NOTCH

1. This gene was the first to be discovered as cause of cancers in head and neck in current human genome studies.

2. It has three alleles (Notch 1, Notch 2, and Notch 3)

3. This gene prevents differentiation of stem cells

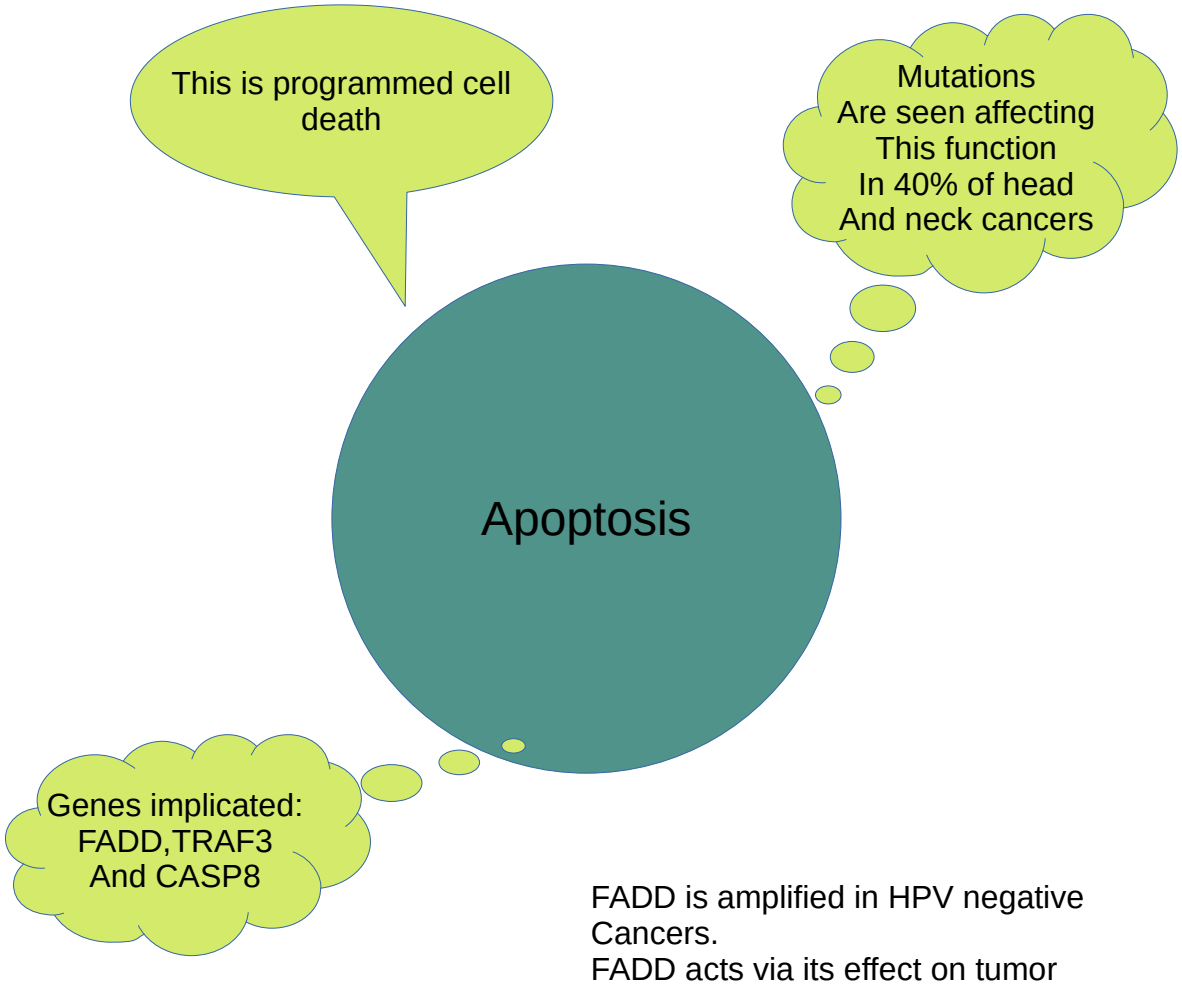
4. Mutations that inactivate this gene has antiproliferative effect

5. Mutations that cause activation of this gene causes cancer

6. NOTCH – p53 interplay maintains the balance between cellular proliferation and differentiation

Inflammation is a major risk factor in promoting carcinogenesis. One of the key regulators of inflammation is NF-κB. Genes like FADD, TRAF 3 has an impact on the regulation of its activity.

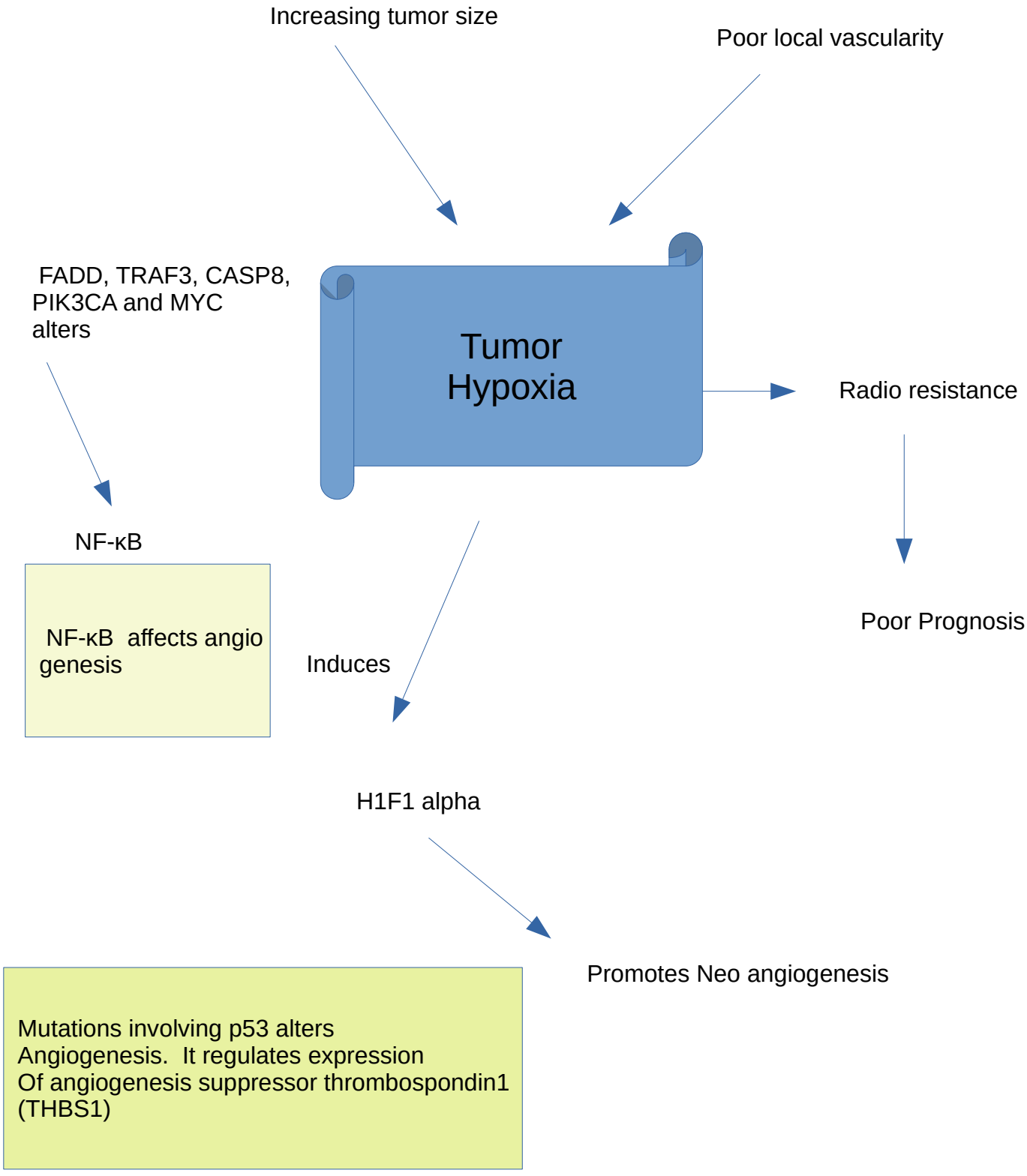
Cell death regulation (Evasion of Apoptosis)



FADD is amplified in HPV negative Cancers.
FADD acts via its effect on tumor necrosis factor

Two pathways for apoptosis:
Extrinsic: Regulated by cell surface receptor signaling
Intrinsic: like DNA damage, Hypoxia and metabolic stress

Sustained Angiogenesis

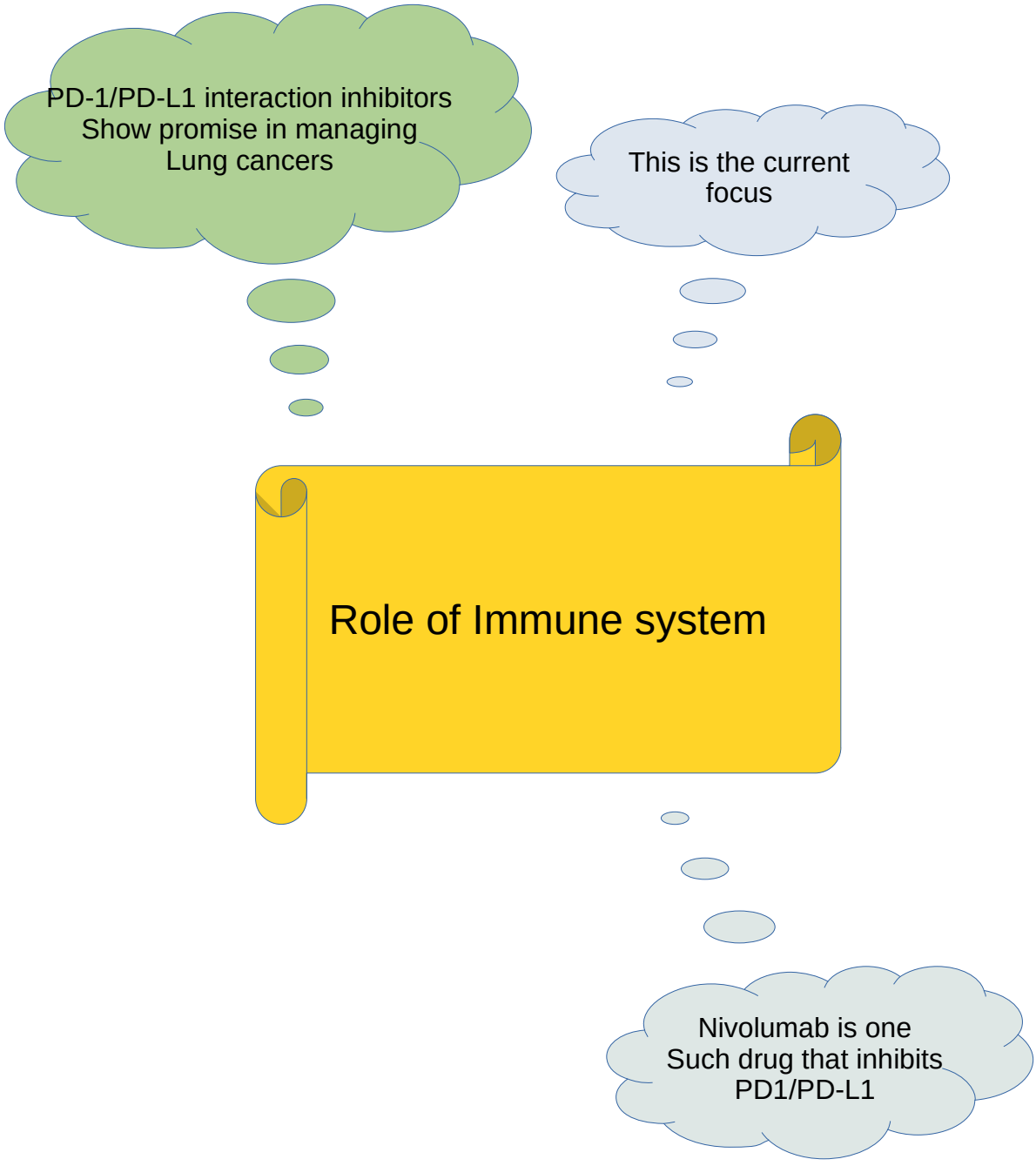


Tissue invasion & Metastasis

1. The ability of cancer cells to invade surrounding tissue and metastasize to distant Sites is a hall mark of cancer
2. Metastasis is a complex multi-step process
3. During metastasis the cancer cells leave the primary site migrates to adjacent areas
4. Distant metastasis occurs via transmission through angiolymphatics

EMT (Epithelial mesenchymal Transition) endows enhanced Migratory capacity to tumor cells

Avoiding immune destruction



Tumor cellular energetics