Molecular Biology

By: Dr. Fatin Al-Sayes
MSC-FRCPath
Consultant Hematologist
Associate Professor
King Abdulaziz University Hospital

- Normal somatic cell has 46 chromosome = diploid
- Ova and sperm has 23 chromosome = haploid
- Aneuploid: A somatic cell with more or less than 46 chromosome.

Hyperdiploid: More than 46 chromosomes.

Hypodiploid: Less than 46 chromosomes.

Pseudodiploid: 46 chromosomes but with rearrangements

Each chromosome has two arms: short arm = p and long arm =q.

Centromere: Short and long arms meet at the center.

Telemores: Ends of the chromosomes.

Each arm is divided into *regions* numbered outwards from the centromere.

Each region is divided into *bands.*

-or + shows loss or gain of the chromosome.

Del: part of the chromosome is lost, e.g. del (16q).

Add: additional material has replaced part of chromosome.

t: Translocation e.g. t(9;22)

Inv (inversion); part of the chromosome runs in the opposite direction.

An isochromosome (i) is a chromosome with identical chromosome arms at each end, e.g. I (17q) has two copies of 17q joined at the centromere.

(●) Karyotype shows the chromosomes from a mitotic cell in numerical order.

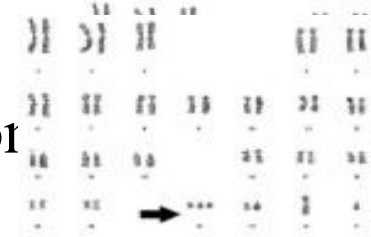
(●) Clonality ~ at least two cells having the same extra chromosome or structural rearrangement and acquire a proliferative advantage.

CYTOGENETICS

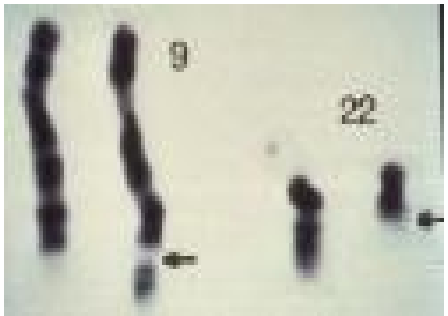
GOOD/COMMON

- Tel-aml
  - ETV6-CBFA2;t(12;21)
- Hyperdiploidy
  - >50

BAD/UNCOMMON



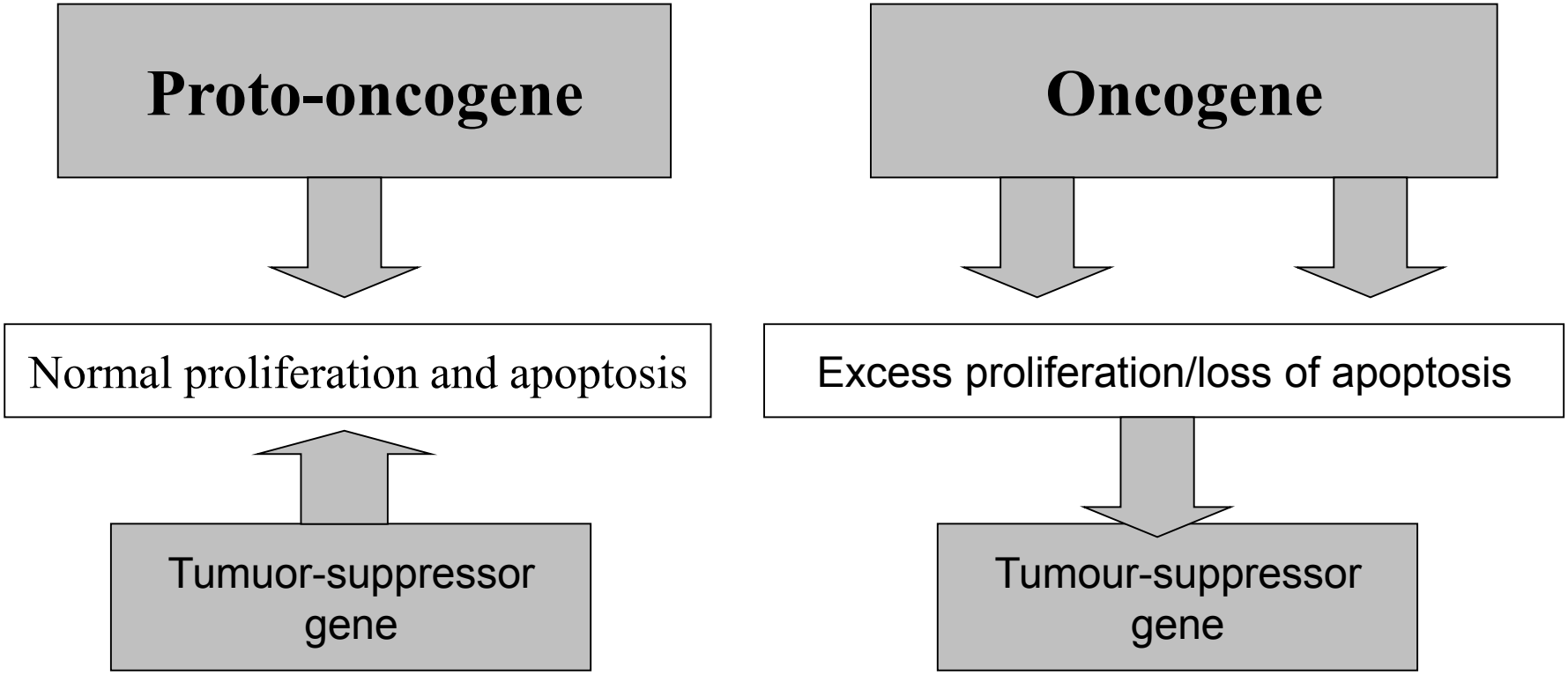
- Philadelphia chromosor
  - BCR-ABL;t)9;22)
- MLL rearrangement



Genetics of Haematological Malignancies

Haematological malignancies are mostly clonal disorders resulting from a genetic alteration.

Genes involoved: oncogenes and tumour-suppressor genes.



Oncogenes

Oncogenes result from gain-of-function mutations of proto-Oncogenes that would normally control the activation of genes.

Translocation may lead to:

(a) over-expression of an oncogene under the control of the promoter of another gene, e.g. an immunoglobulin or T cell receptor gene as seen in lymphoid malignancies.

(b) Fusion of segments of two genes creating a novel fusion gene and thus a fusion protein, e.g. in CML.

Tumour-Suppressor Genes

Tumour-suppressor genes are subject to loss-of-function Mutations (point mutation or deletion) and thus malignant transformation.

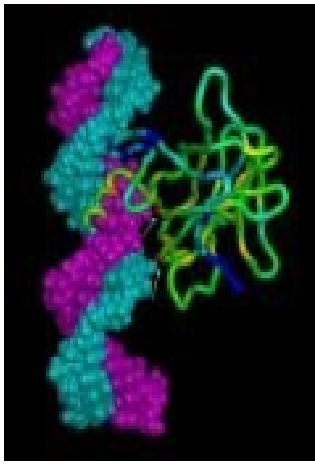
Tumour-suppressor genes help regulate cells to pass through different phases of the cell cycle, e.g. G_1 to S, S to G_2 and mitosis.

Clonal Progression

Malignant cells may acquire new characteristics resulting from new chromosamal changes causing acceleration.

p53 Protein

- One gene one monomer
- Its consists of 4 different monomers
- If one of the monomers is dysfunctional the whole protein becomes defective.
- Thus all it takes its one mutant gene for the protein to become defective.
- Cytosol levels rise rapidly in response to DNA damaging agents
- If damage is found in the template or complementary strand then duplication stops
- The amount of p53 will stop Synthesis in the cell cycle
- If it reaches a threshold level then it induces the cell to undergo apoptosis
- Evolutionary homology with murines, reptiles, even yeast.



Causes of leukemia???

- Clonal expansion → a cell that has the ability to self-replicate but unable to differentiate
- **Genetics**
  - Higher incidence in siblings and twins
- **Virus**
  - Clusters of leukemia
- **Ionizing radiation**
  - Survivors of Hiroshima and Nagasaki

Syndromes with higher incidence

- Down's
- Bloom's
- Fanconi's
- Klinefelter's
- Ataxia telangiectasia

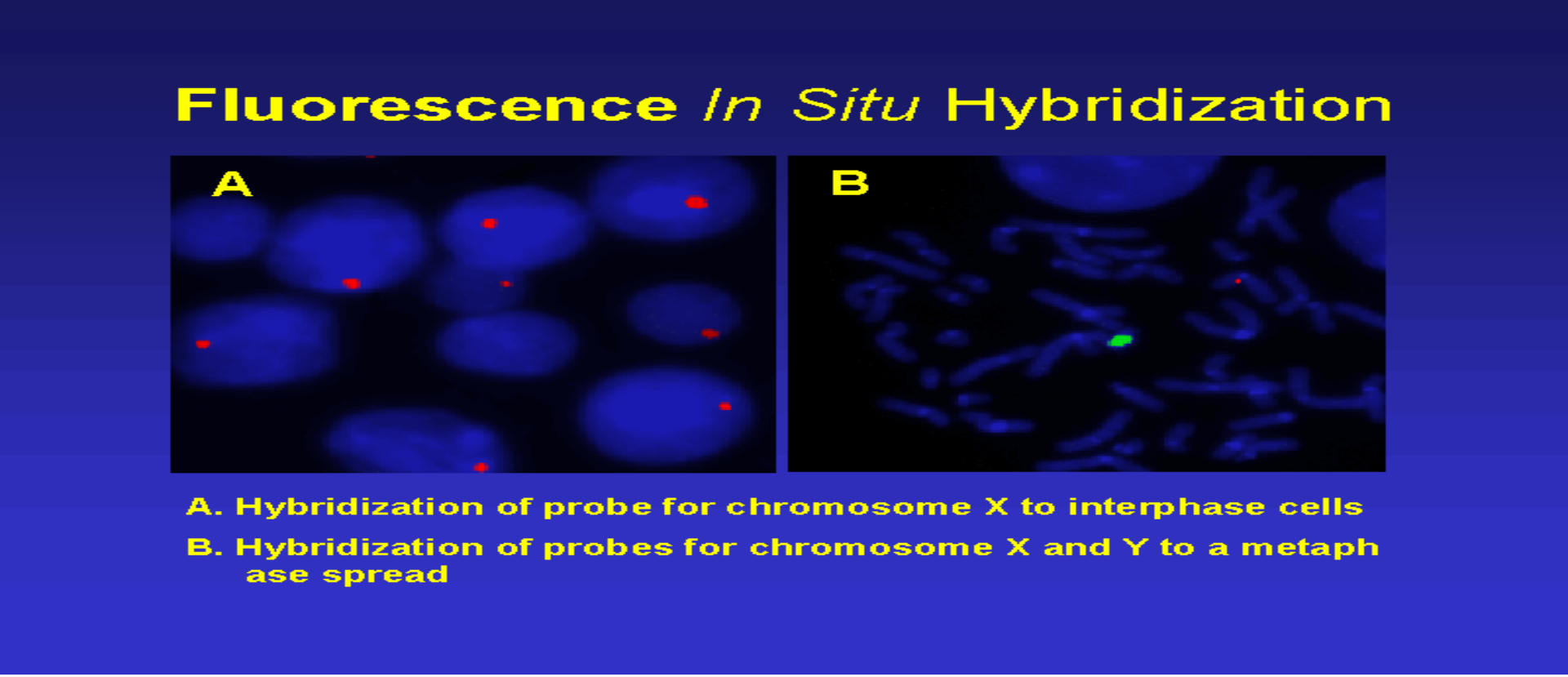
Methods used to Study the Genetic of Malignant Cells

Karyotype Analysis (Cytogenetic studies)

Images of chromosomes are captured when cell is in metaphase

Immunofluorescence Staining

Can be useful for a few chromosomal abnormalities, e.g. Promyelocytic leukaemia protein which normally has a punctate Distribution but is diffusely scattered in acute promyelocytic Leukaemia with the t(15;17) translocation. Abnormal fusion Proteins may also be detected by specific monoclonal antibodies.



Fluorescent in situ Hybridisation (FISH)

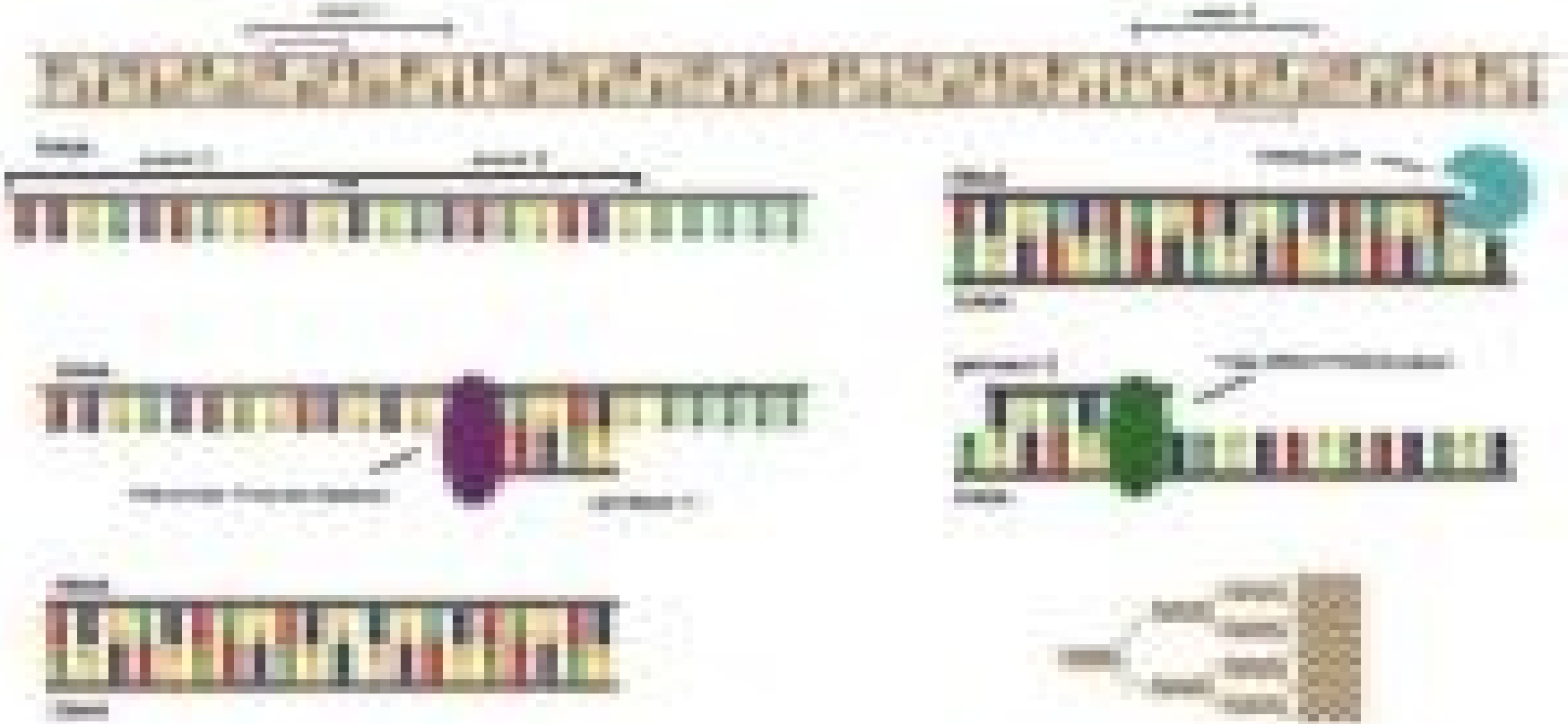
Fluorescent-labelled genetic probes hybridise to specific parts of the genome. Can pick up extra copies of genetic material in both metaphase and interphase, e.g. trisomy 12 in CLL. Translocations can be seen by using two different probes.

Southern Blot Analysis

Restriction enzyme of DNA, gel electrophoresis and “blotting” To a suitable membrane. DNA fragments are hybridised to a probe complementary to the gene of interest. If the probe recognises a segment within the boundaries of a single fragment One band is identified. If the gene has been translocated to a new area in the genome a novel band of different electrophoretic mobility is seen.

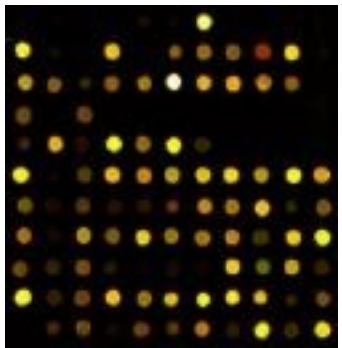
Polymerase Chain Reaction (PCR)

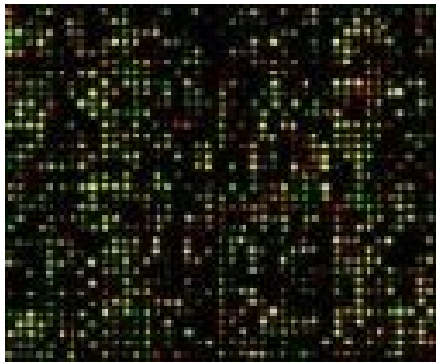
Can identify specific translocations, e.g. t(9;22). Can also detect clonal cells of B- or T-cell lineage by immunoglobulin or T-cell receptor (TCR) gene rearrangement analysis. Sensitivity (can detect one abnormal cell in $10^{5-}10^{6}$ normal cells) makes this of value in monitoring patients with minimal residual disease (MRD).



DNA Microarry Platforms

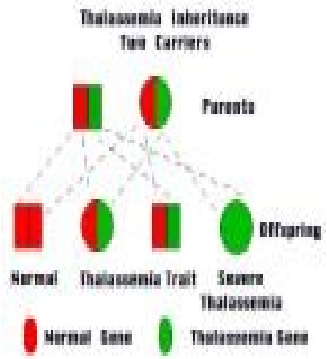
Rapid and comprehensive analysis of cellular transcription by hybridising labelled cellular mRNA to DNA probes immobilised on a solid support. Oligonucleotides or complementary DNA (cDNA) arrays are immobilised on array and fluorescent labelled RNA from the cell sample is annealed to the DNA matrix. Can determine the mRNA expression pattern of different leukemia subtypes.





Thalassemias

- Thalassemias are a heterogenous group of genetic disorders
  - Heterozygous individuals exhibit varying levels of severity
  - the disorders are due to mutations that decrease the rate of synthesis of one of the two globin chains (α or β).The genetic defect may be the result of:



Thalassemias

- Beta (β) thalassemia

- The disease manifests itself when the switch from γ to β chain synthesis occurs several months after birth
- There may be a compensatory increase in γ and δ chain synthesis resulting in increase levels of hgb F and A_2. The genetic background of β thalassemia is heterogenous and may be roughly divided into two types:
  - β^0 in which there is complete absence of β chain production.
    This is common in the Mediterranean.

Cont:

- β^+ in which there is a partial block in β chain synthesis. At least three different mutant genes are involved:
  - » $\boldsymbol{\beta}^{+1}$ – 10% of normal **β**chain synthesis occurs
  - » $\boldsymbol{\beta}^{+2}$ **-** 50% of normal **β** chain synthesis occurs
  - » $\boldsymbol{\beta}^{+3}$ - >50% of normal β chain synthesis occurs

Thalassemias

- The clinical expression of the different gene combinations (1 from mom and 1 from dad) are as follows:
  - β^0/β^0,β^{+1}/β^{+1}, or β^0 /$\beta^{+1,+2,\ 0r\ +3}$=thalassemia major, the most severe form of the disease.
    - » Imbalanced synthesis leads to decreased total RBC hemoglobin production and a hypochromic, microcytic anemia.
    - » Excess α chains precipitate causing hemolysis of RBC precursors in the bone marrow leading to ineffective erythropoiesis
    - » In circulating RBCs, α chains may also precipitate leading to pitting in the spleen and decreased RBC survival via a chronic hemolytic process.
    - » The major cause of the severe anemia is the **ineffective erythropoiesis**

Thalassemias

- A mutation in the noncoding introns of the gene resulting in ineffecient RNA splicing to produce mRNA, and therefore, decreased mRNA production
- The partial or total deletion of a globin gene
- A mutation in the promoter leading to decreased expression
- A mutation at the termination site leading to production of longer, unstable mRNA
- A nonsense mutation

- Any of these defects lead to:
  - An excess of the other normal globin chain
  - A decrease in the normal amount of physiologic hemoglobin made
  - Development of a hypochromic, microcytic anemia

The clinical applications of sequenced susceptibility genes.

Susceptibility Gene	Clinical Application
● Prothrombin Mutation:G20210A.	Hereditary thrombophilia
● Factor V Leiden Mutation: R506Q	
● Platelet GP Ia Mutation:	
●C807T and 648A (HPA-5).	Bleeding tendency due to Platelets dysfunction
* Platelet GP IIIa: Mutation:T393C(HPA-la/b=P1A1/P1A2)	
* Factor IX propeptide Mutations at ALA-10	Coumarin hypersensitivity