Molecular biology :- Cancer genetics – lecture 11

-We have talked about 2 group of genes that is involved in cellular transformation : proto-oncogenes and tumour suppressor genes , and it isn't enough to activate a number of oncogenes so the cell must also inactivate a number of tumor suppressor genes

-oncogenes can be Growth factors (PDGF), Growth factor receptors(PDGFR) ,Signal transduction proteins (Ras),Transcription factors (myc) ,Cell cycle regulators(cyclin and cyclin dependent kinases), Regulators of apoptosis

*** cell cycle regulators** :- **<u>cyclin and cyclin dependent kinases (CDKs)</u>**

-cyclin the bind to CDKs and form active complexes (cyclins--- they cause the cell to cycle / cyclin dependent kinases --- they work by phosphorylating other proteins but their activity depends on the presence of cyclins

- CDKs are always present but what's going up and down are the cyclins

For example, to pass from G1 to S, cyclin D must form complex with CDK4 or CDK6 (<u>cyclin D is induced by the transcription factor Myc</u>

- It's not enough to stop producing cyclin D, the already produced cyclin D will be inhibited by

<u>Cyclin dependent kinase inhibitors CDKI</u>

<u>If these inhibitors get mutated, cells will proliferate Uncontrollably</u>

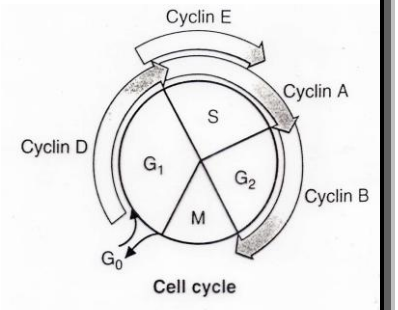


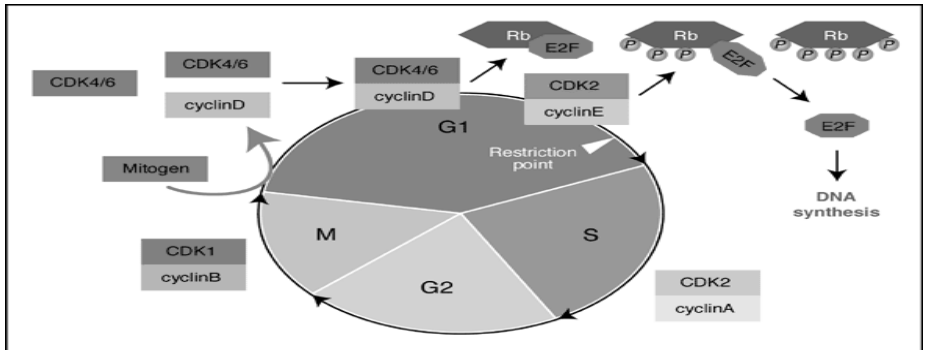
Fig. 18.6. Cyclin synthesis during different phases of the cell cycle.

- Two families:

1). Cip/Kip family (p21, p27, p57): Broad specificity,
inhibit all cyclin/CDK complexes

2) INK4 family (p15, p16, p18 and p19): Specific for cdk4 & cdk6

*note :- p stands for protein while the numbers represent their molecular weight



Mitogens drive cell cycle progression by induction of cyclinD and inactivation of the retinoblastoma (Rb) protein

- Growth factor binds to its receptor
-activation of ras/raf signal pathway
-induction of cyclin D (cyclin D's transcription factor is myc)
-cyclin D form complexes with CDK4 , CDK6
-the complex phosphorylate target proteins one of them is the RB protein (retinoblastoma protein which is product of RB gene , first discovered in tumor called retinoblastoma but it's present in every cell regulating the G1/S checkpoint)
*when RB is not phosphorylated it binds a transcription factor called E2F and keeps it into the cytoplasm and when it's phosphorylated it dissociate from E2F . now E2F is free --- go to nucleus helps in transcription of genes needed for the S phase (DNA replication). When its job is done, inhibitors will work to inhibit it.

Tumor suppressor genes:-
- Proteins that inhibit cell proliferation in response to DNA damage "Brakes on cell growth" (whether directly or indirectly)
- BOTH alleles of a tumor suppressor gene are mutated in cancer cells (Oncogenes: one mutated copy is enough)

A. Tumor suppressors directly regulating cell cycle:

1. <u>Retinoblastoma (*rb*) gene</u>:
- Works on transition from G1 to S phase
Controls the E2F family of TFs
<u>- Familial retinoblastoma</u>: (rare type of familial cancers while the most common are breast and colon cancers
* first allele is mutated: high probability to gain second allele mutation
* happen in many families at an early age
- <u>Sporadic retinoblastoma</u>:
*two alleles mutated during life time
* happen in less number , at a late age (because the probability of 2 mutations to happen at the same cell sporadically is much less than if the person was born with one mutant copy

2. <u>p53, The guardian of the genome:</u>
-the most important tumor suppressor gene (actually it's the most important cancer related gene i.e mutation of p53 is involved in almost every cancer)
- A transcription factor that responds to DNA damage
- Loss of both alleles in > 50 % of all tumors (by deletion)

(Loss of function: > 90 %) (mutations in the promoter or in the coding region , for example it works as a tetramer – mutation in the region where the subunits bind so that it becomes monomer
Or it might activate a protein called mdm2 which destroys p53 ,so you find that many p53 are produced but they are inhibited by this protein
* one of the greatest mistakes happened in molecular biology was that someone thought that p53 is an oncogene because he found a large number of it in a cancer but when he studied other cancers he didn't get the same results as the first one to discover later that in that case of cancer the cell was trying to get rid of cancer by overexpression of p53 but they were inactive because of mdm2

- Main mechanisms of action:
1) . recognition of DNA damage by UV or chemicals
(it's always present in low amount scanning DNA for damage , if it finds damage ---- level rises)
2) induce transcription of p21 inhibits cyclin/CDK complexes
3) p53 also stimulates GADD45 transcription(Growth Arrest and DNA Damage): a DNA repair enzyme
a- if repair was successful: p53 down regulates it self
b- If damage is beyond repair:
4) p53 activates *bax* which induce apoptosis

B. Tumor suppressors affecting receptors and signal transduction:

1- <u>Regulators of ras oncogene</u>: every signal transduction protein, has an inhibitor

- e.g. GAPs (GTPase-activating proteins) They cause hydrolysis of GTP into GDP:

Remember that Ras when bound to GTP is active and inactive when bound to GDP, and has a GTPase domain which inactivates it

NF-1 is a Tumor suppressor that bind to GTPase domain and activates it

* Ras is a signal transduction proto-oncogene while NF-1 is a signal transduction tumor suppressor

We have many signal transduction pathways which are controlled by negative regulators (tumor suppresors) , some of them are active in different places , others are active in different stages

2- <u>Patched and Smoothened</u>:

- Encode receptor for hedgehog class of signaling peptides

- Normally control growth during embryogenesis

Patched receptor bind & inhibits Smoothened

- Smoothened: Proto-oncogene/Patched: Tumor suppressor

3) tumor suppressors involved in cell adhesion (E-cadherin)

-in the last stages of transformation , cancer cells are going to form tumors in secondary places (metastasis) through blood vessels or lymphatics but the cells are attached together by adhesion molecules (ex:- E-cadherin) so mutation in E-cadherin gene – cells separate – metastasis (so in this case E-cadherin is a tumor suppressor)

- E-cadherin holds the cells from outside, but from inside by actin filaments attached to E-cadherin , the E-cadherin is attaches to actin filaments by cateinins alpha and beta the more important is beta-catenin and since it is involved in cell adhesion it's a tumor suppressor

-but if beta catenin detaches and goes to nucleus it works as a transcription factor for myc (i.e an oncogene)

Remember that myc is a transcription factor for cyclin D --- cyclin d binds to CDK4,6 --- phosphorylate RB --- E2f is free --- go to nucleus – synthesize proteins for S phase

So it's very dangerous , so it's regulated by APC (adenomatous polyposis coli) which is a tumor suppressor , APC bind to beta catenin --- destroy it , if APC is mutated (not functional) – beta catenin is free to go to nucleus causing cell proliferation

Familial colon cancer caused by 2 types :- the most common is HNPCC (mutation in mismatch repair genes – MMR) the second is by APC less common but more dangerous

- Important note :- familial colon cancer. Breast cancer involving BRCA1,2 they are dominant condition but that's different from other conditions like thalassemia ,… here if the person is born with one mutated copy there is a high probability for the second copy to be mutated and affected with cancer (example in breast cancer if person born with one mutated copy 90% will have the cancer 10-40 years , in case of colon involving MMR 80% between 20-50depending on the environment

APOPTOSIS :- programmed cell death

-active process unlike necrosis which is passive
-can be caused by reduction in telomeres length to a certain limit but the main pathways involved in cancer are the death receptor and mitochondrial integrity
-last resort to any transforming cells to prevent tumor formation

A. Normal Apoptotic Pathways:

- Apoptosis is divided into three phases:

1) Initiation Phase: The occurrence of an event that leads to apoptosis (not the normal shortening of the telomere)

- By External signals (virus can initiate such a signal via lymphocytes)
  - Death Receptors: e.g. Tumor Necrosis Factor (TNF), the death ligand might come from a cytotoxic T cell.
  - Deprivation of growth hormones
- By Intracellular events (high oxidative stress, or a high amount of damage). The amount of damage is measured by the integrity of the mitochondria. If the mitochondria loses integrity, then the cell will die. When the mitochondria leaks cytochromes (especially cyt c) then the cell will die.
  - affecting mitochondrial integrity (O_2 deprivation, radiation,...)
  - Extensive DNA damage

2). Signal integration phase:

Balancing the above pro-apoptotic signals against the anti-apoptotic signals by different pathways including BCL-2 protein family. The BCL-2 family contains both pro and anti-apoptotic proteins

3) Execution phase:

Carried out by proteolytic enzymes called Caspases

Caspases:

- Cysteine proteases that cleave peptide bonds next to an aspartate residue.

- Found as Pro- caspases: zymogens activated by proteolysis of inhibitory peptide sequence

- Two groups:

i. Initiator Caspases: specifically cleave other procaspases

activated by:
- Death Receptor Pathway – caspase 8
- Mitochondrial Integrity Pathway – caspase 9

ii. Execution Caspases:

pathways to apoptosis :-

1) **mitochondrial integrity pathway (internal pathway**) :- something wrong happens inside the cells (oxidative stress, DNA damage)

if the damage reached the mitochondria causing destruction of its membranes then the damage is severe , proteins will get out of the mitochondria one of the cytochrome C which will bind to Apaf forming apoptosome which will activate initiator caspase 9

2) **death receptor pathway (external)** :- signal inside the cell for example a virus but not severe , will be recognized by immune cell (CTCs) from outside so it sends a death ligand which will bind to death receptor on surface –bind adaptor protein(FAD) on intracellular domain – 2 procaspases 8 – autocatalysis – active caspase 8 (initiator caspases) which will cause Bid--- tBid (truncated protein) which will activate the mitochondrial pathway by causing destruction in the mitochondria (release of cytochrome c)

*BCL2 family :- proapoptotic proteins which activates apoptosis and antiapoptotic proteins which inhibits apoptosis

-antiapoptotic (Bcl-2 , Bcl-x ,bcl-w)

-goes to mitochondria and close the channels to prevent cytochrome c from going outside

-binds Apaf to prevent binding of cytochrome c forming the apoptosome

Channel forming proapoptotic (Bax)

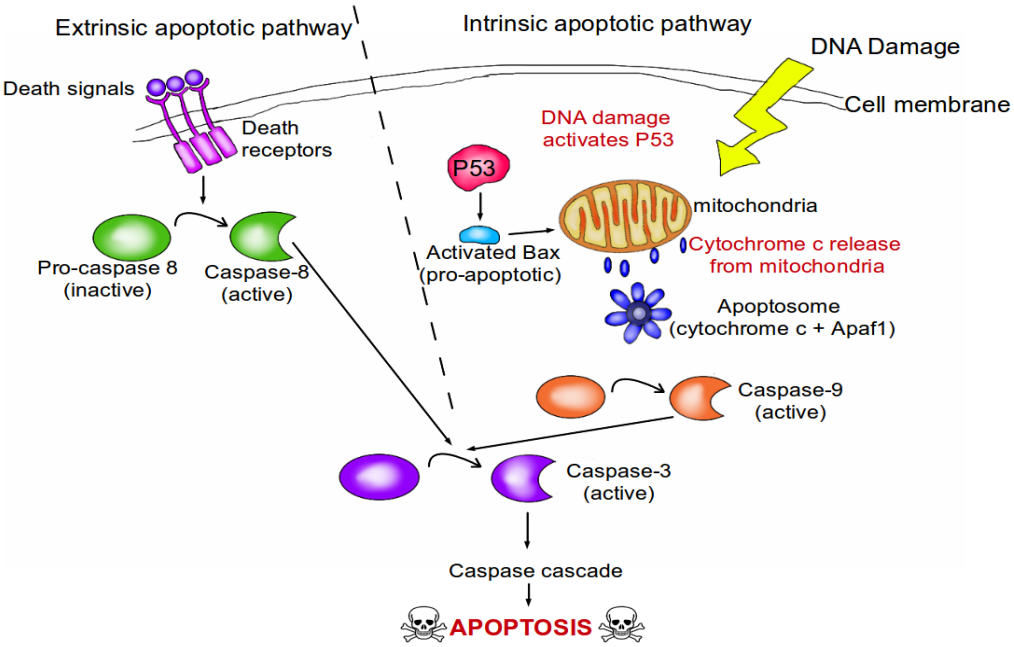
-goes to mitochondrial forming more channels

BH3-only:- (Bid, Bad) similar to the others in only one domain ,the BH3 domain

-prevent Bcl-2 binding and closing channels

-helps Bax in its function to cause more release of cytochrome c to cytosol

Whoever is faster and more efficient, will determine the fate of the cell.



Done by Ramiz alyacoub

Good luck in the finals ☺