Neoplasia lec5

QUICK RECALL

• The balance between tumor suppressor genes and proto-oncogenes in normal cells allows us to renew cells when needed in a controlled, regulated manner.

• Transformed cancer cells lose this balance. They keep proliferating.

• This continuous proliferation is achieved via 1. Gaining the ability to "synthesize" their own growth signals (the first hallmark we already discussed) and 2. Bypassing the negative regulatory mechanisms (the hallmark we will discuss in this lecture)

Second hallmark of cancer: insensitivity to growth inhibitors

• Normally growth inhibition is achieved by tumor suppressor genes. Loss or decreased function of tumor suppressor genes allows cancer cells to proliferate without being affected by inhibitory growth signals.

• Main genes/ pathways mutated to cause insensitivity to growth inhibition:

1. RB gene 2. TP53 gene 3. TGF beta pathway 4. Contact inhibition 5. APC gene

RB gene

• RB gene= retinoblastoma gene = governor of cell cycle

• RB is a key negative regulator of the cell cycle, it is directly or indirectly inactivated in most human cancers

• The function of the RB protein is to regulate the G1/S checkpoint, the portal through which cells must pass before DNA replication starts.

How does RB act? General overview

• Like all our genes RB is translated to a protein, which is the RB protein.

• RB protein acts by binding to DNA. Specifically, RB protein binds to a family of transcription genes called E2F genes.

• E2F genes are responsible for the transcription of cyclin E.

• The binding of RB to E2F inactivates the E2F. So there is no transcription of cyclin E.

• As you know from last lecture, cell cycle progression depends on cyclins and CDKs. So: lack of cyclin E prevents progression of the cell cycle.

How does RB act? The details!

• RB is active at the beginning of G1 phase.

• This activity of RB depends on its phosphorylation state. Strangely RB is active when it's hypo-phosphorylated. Note that most of our proteins are activated by gaining phosphate groups. RB is an exception to this rule.

• So: at the beginning of G1 phase, active, hypo-phosphorylated RB binds to E2F transcription factor family, preventing cyclin E formation.

• Cyclin E/ CDK2 complex is important for initiation of DNA replication (for entering the S phase)

• As long as cyclin E/CDK2 complex is not formed, cells are trapped in the G1 phase and cannot move to the S phase. In other words they cannot cross the G1/S checkpoint.

• Some cells leave cell cycle to G0 or go into senescence at this stage. But if they cross the G1/S they are committed to undergo mitosis without the need of any extra growth signals. That's why this checkpoint is important.

How does RB inactivate E2F?

• This inactivation occurs via 2 mechanisms

• 1. RB sequesters (يحجب) E2F which prevents E2F from interacting with other transcription factors.

• 2. RB recruits proteins that bind to and inhibit E2F promotor. This makes E2F gene insensitive to transcription factors

• The net result is preventing transcription of E2F

Importance of RB function

**RB regulates progression through the G1 phase

** un and hypo- phosphorylated RB protein inhibit the cell from entering a new cell cycle.

• RB inactivation of cell cycle allows the cell to have time to check the cell size, protein content and configuration.

• This pause is important because cells that cross the G1/S are committed to DNA replication. We don't want this to happen unless cells have normal protein content and are really ready to divide.

• However, this inactivation cannot continue forever. We need to deactivate RB at a certain point to allow cells to enter the S phase.

How RB is deactivated/How cells can cross the G1/S checkpoint?

• RB is deactivated by phosphorylation.

• This happens when cells are subject to mitogenic (growth) signals.

• Growth signals cause cyclin D expression. Cyclin D complexes with CDKs, these complexes phosphorylate and inactivate RB.

• Once RB is deactivated, E2F genes can be transcribed, resulting in cyclin E formation.

• Cyclin E/ CDK complexes can start the S phase.

Remember the DEAB sequence we talked about last time. Cyclin D is the first needed, then E.

Notes:

**Note that in the presence of growth inhibitory signals, RB is active and cells cannot divide. With growth stimulatory signals RB is deactivated via cyclin D/ CDKs complexes

**Note that certain viruses can deactivate RB by hyper-phosphorylating it. This is how these viruses cause cancer (become oncogenic)

** Once in S phase cells are committed to division. They don't need additional growth signals

** In M phase phosphate removed from RB, so it goes back to its active state.

Retinoblastoma

• RB is named after a tumor called: Retinoblastoma

• Retinoblastoma is a rare childhood tumor affecting the eye (retino)

• RB gene was first discovered in this tumor and it's named after it. However, RB is mutated in most human cancers, not just retinoblastoma tumor.

Retinoblastoma - 60 % of cases are sporadic, 40% familial

- In familial cases the predisposition to develop the tumor is inherited as an autosomal dominant trait

-However, to develop retinoblastoma: we need both copies of the RB gene to be mutated (remember that tumor suppressor genes' mutations are recessive)

-Loss of the two genes is called the two-hit hypothesis

Two-hit hypothesis

• There are two alleles for RB

• BOTH must be deleted or functionally deactivated before developing a tumor.

• In familial cases there is an inherited, germ line mutation in one of the alleles. The second allele is mutated or deleted later in somatic cells.

• In sporadic cases both alleles need to be mutated and/or deleted to have cancer.

In conclusion:

• Two mutations (hits) required to develop retinoblastoma

• The 2 mutations involve the RB gene on chromosome 13 (13q14) locus

• Both copies of RB gene need to be deactivated to develop retinoblastoma

• In familial cases, one hit is inherited (germ line mutation) the other is acquired - In sporadic cases, both mutations are acquired.

• People with inherited RB have increased risk of other cancers. Mainly osteosarcomas and soft tissue sarcomas

(Back with the genes mutated to cause insensitivity to growth inhibiting signals.)

TP53, the guardian of the genome

• This is also an important regulator of cell cycle.

• Tp53 is one of the most commonly mutated genes in cancer

• It encodes p53 protein, P53 causes growth inhibition by three mechanisms

1. Temporary cell cycle arrest: quiescence

2. Permanent cell cycle arrest: senescence

3. Triggering apoptosis

• P53 monitors internal stress whereas RB senses external signals

• P53 is triggered by several stresses: anoxia, inappropriate oncogene activity (MYC or RAS) or DNA damage.

HOW p53 works?

• In non-stressed healthy cells, p53 is short lived: 20 minutes because it binds MDM2 which is a protein that targets it for destruction via ubiquitin proteasome pathway.

• When cells are stressed, sensors that include protein kinases are activated (ATM is one of these kinases)

• These activated kinases catalyze post translational modifications of p53 and release it from MDM2

• Now p53 has longer life span and can drive transcription of certain genes, hundreds of them. Including:

• 1. Genes mediating cell cycle arrest, like CDKI.

- This occurs late in G1. Caused by p53 dependent transcription of CDKI gene.

- CDKI inhibit cyclin/CDK complexes and prevents phosphorylation of RB

- So cell is arrested in G1 which provides a pause to repair any DNA damage

• 2. DNA repair genes.

-If DNA is repaired successfully, p53 upregulates transcription of MDM2, causing destruction of p53 and removal of the block on cell cycle.

- If DNA not repaired p53 makes cells enter apoptosis or senescence.

• 3. Genes involved in senescence like CDKI

- Senescence needs activation of p53 and or RB and expression of their mediators like CDKI

- Mechanisms of senescence unclear but seem to involve global chromatin change, with permanent change gene expression.

• 4. Pro-apoptotic genes including BAX and PUMA

• More than 70% of human cancers have mutated TP53

• Both copies of the gene need to be lost for cancer to develop

• Mostly somatic

• Rare Li Fraumeni syndrome: inherit defect in one allele. More predisposition to cancer (Sarcoma, breast carcinoma, leukemia and brain tumor)

TGF Beta

• TGF beta is a negative growth regulator.

• It binds to transmembrane receptors

• This binding stimulates second messengers in the cytosol. Of the SMAD family

• The message reaches the nucleus: to inhibit growth through upregulation of CDKI and down regulation of CDK4 and MYC. Resulting in growth inhibition.

• Mutations affecting TGF beta signaling causes cancer

• These mutations involve TGF beta receptor or SMAD molecules that transduce anti-proliferative signals from the receptor to the nucleus

• Mutations affecting TGF beta receptor seen in colon, stomach and endometrial cancer

• SMAD4 is mutated in pancreatic cancer.

• 100% of pancreatic 83% of colon at least one component of is TGF b pathway is mutated

Contact inhibition

• Normally cells proliferate in an organized fashion. Monolayers are formed and contact between adjacent cells inhibits further growth.

• This process is called contact inhibition.

• In cancer cells: contact inhibition is lost so cells pile upon each other

• Contact inhibition is mediated by cadherin molecules.

• If E cadherin (epithelial cadherin) is lost: no contact inhibition, Cells proliferate in an uncontrolled fashion.

• E cadherin is important to “keep cells together”

• Tumors with loss of E cadherin tend to grow in an individual cell fashion: they don’t form glandular or other cohesive structures.

• Example: there are two types of breast carcinoma, invasive ductal and invasive lobular.

The tumor cells in the ductal type form glandular structures, whereas in the lobular type, they grow in individual cell pattern. In this lobular pattern E cadherin is lost.

APC (adenomatous polyposis coli) gene

• APC gene is a tumor suppressor gene

• Suppresses growth by regulating intracellular beta catenin level.

• Beta catenin is a protein that stimulates growth, APC protein acts as a tumor suppressor through inhibiting beta catenin function

Functions of beta catenin

Beta catenin stimulates growth by two ways:

1. Inhibits contact inhibition by stimulating TWIST and SLUG transcription regulators that decrease cadherin expression

2. Stimulates growth by increasing transcription of growth promoting genes like cyclin D1 and MYC.

• APC suppresses growth by being part of a complex that destructs the beta catenin.

• Beta catenin is an important component of WNT signaling. WNT is a protein that induces cell proliferation by binding to a receptor and transmit signals that prevent degradation of beta catenin, Undegraded beta catenin moves to the nucleus where it acts as a transcription activator.

• In quiescent cells not exposed to WNT, cytoplasmic beta catenin is degraded by destruction complex (of which APC is a main component). Loss of APC in means that B catenin is not degraded and WNT pathway activated without the WNT.

• This leads to transcription of growth promoting genes cyclin D1, MYC and transcription regulators: TWIST AND SLUG that repress E cadherin and thus reduce contact inhibition.

** B catenin activates cell proliferation and growth and is usually controlled by APS protein that degrades it to keep balance in proliferation in normal genes

However when APS gene is mutated in cases like cancer, B catenin is not controlled and the cell would proliferate uncontrollably.

Summary

• Insensitivity to inhibitory growth signals is an important cancer hallmark.

• This insensitivity is achieved via inhibiting both copies of tumor suppressor genes.

• Among others, RB and Tp53 are the most important tumor suppressor genes deactivated in human cancer.

• RB was discovered in familial retinoblastoma cases. Patients inherit a defective allele copy in an autosomal dominant fashion, and acquire a second mutation or deletion resulting in tumor formation. These two mutations are called the two hit hypothesis.

• Sporadic cases of retinoblastoma result from two acquired hits.

• RB is mutated in other neoplasms as well.

• RB inhibits cell proliferation by deactivating transcription of F2F genes resulting in decreased cyclin E. Decreased cyclin E prevents cells from entering the S phase.

• RB is deactivated normally by phosphorylation achieved from interaction with cyclin D/ CDK4 complexes formed in response to growth signals.

• Loss of normal cell cycle control is found in all tumors through mutations of RB, cyclin D, CDK4 or CDKN2A (which is a CDKI)

• Tp53 is one of the most commonly mutated genes in cancer

• P53 protein causes growth inhibition by arresting the cell cycle temporarily (quiescence) or permanently (senescence) or triggering apoptosis

• normally p53 has a life span of 20 minutes , it is destructed by ubiquitin proteasome pathway through the MDM2 protein that targets it for destruction.

• When cells are stressed kinases like ATM kinase catalyze post translational modifications of p53 and release it from MDM2. this activates the p53 which stimulates transcription of hundreds of genes including those mediating cell cycle arrest.

• This arrest allows cells to pause to repair any DNA damage.

• If repairing the damage fails, p53 stimulates apoptosis or senescence.

• If mutated, cells will proliferate regardless of DNA damage present and cells will bypass apoptotic and senescence signals.

• TGF beta- SMAD pathway is mutated in several cancers, mainly pancreatic and colorectal. The pathway is the most well understood growth inhibition one and if mutated, loss of growth inhibition occurs.

• Contact inhibition regulates cell growth. It is mainly mediated by E cadherin and merlin.

• Tumors with lost E cadherin result in non-cohesive, usually single cell growth.

• APC gene is a tumor suppressor gene mutated in familial and sporadic colorectal carcinoma.

• APC acts by being part of a destruction complex that destructs a growth stimulator (beta catenin).

• If the destruction complex is lost via deletions or deactivation mutations, Beta catenin will be activated and translocated to the nucleus to stimulate other transcription factors.