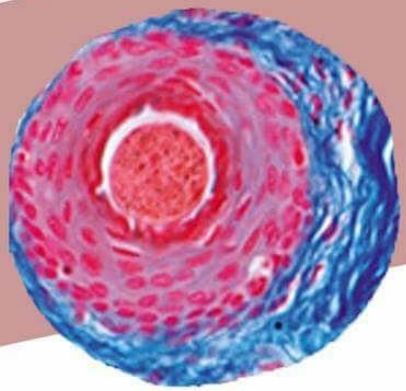
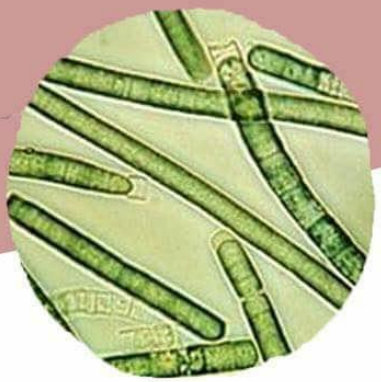
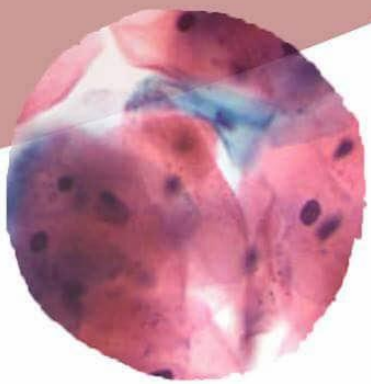
INTRODUCTION TO PATHOLOGY

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Sheet# 20

In this lecture, we're going to continue discussing the hallmarks of cancer.

Immortality

As replication occurs, DNA strands tend to get shorter and shorter. How does replication occur without harming our genetic code then? Telomeres are noncoding regions of DNA located at the end of each chromosome to protect the genetic code every time replication occurs. The unfortunate part is that telomeres also get shorter and shorter with replication and gets closer to the coding genetic part of DNA. After many rounds of replication in somatic cells, senescence (aging) occurs as telomerase's action gets close to DNA's coding region due to telomere shortening. This serves as a problem for the cell. Short telomeres are recognized as a double stranded break during replication. The cell gets stuck between two choices, solving the problem or ending replication and ultimately, apoptosis. As a result, p53 accumulates and signals the end of a cell's lifespan through apoptosis.

An enzyme named Telomerase functions to increase the length of the telomeres after every round of replication so that it doesn't get shorter (it preserves the ends of DNA with noncoding DNA sequences). Guess what? If we had this enzyme in our cells, we'd be immortal. Sadly, though, this enzyme only exists in stem cells-physiologically speaking. Since immortality is a hallmark of Cancer then, you got it, Telomerase also exists in Cancer cells.

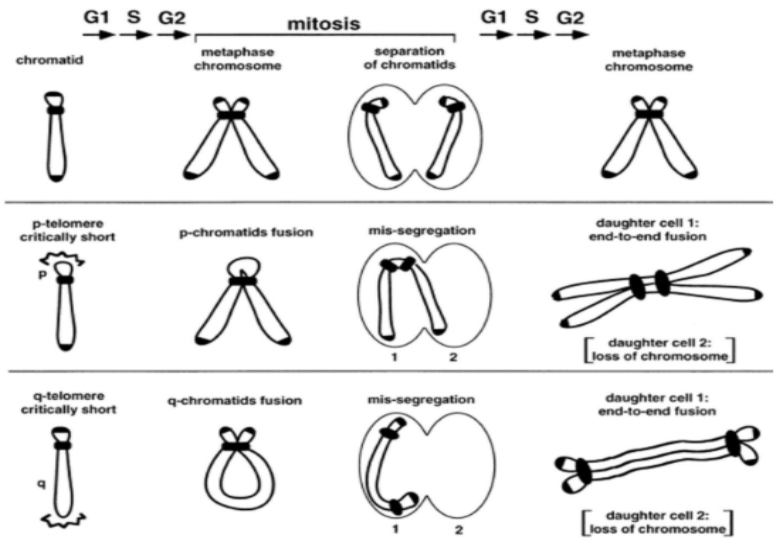
How do cancer cells activate Telomerase activity?

Cancer cells usually overcome p53 and Rb – which are the guardian and the governor of the cell cycle and are what induces cell aging and apoptosis-thus, overcoming them means endless replication. Normally, a somatic cell detects a double stranded break (short telomere) and signals p53 accumulation and cell aging. A cancer cell/mutated cell does that UNTIL it overcomes Rb and p53 activity. In a cell with a mutant copy of Rb/p53 normal signaling and apoptosis will not happen. When this cell faces a double stranded break (a short telomere) it will try to fix it by a last resort called **non-homologous end joining repair.** *Hold on, we still did not explain how telomerase is activated.

Non-homologous End Joining Repair:

An attempt to save the mutated cell with short telomeres. It works by sticking or joining the two ends of chromosomes together. As a result, one of the following situations occur:

(1) Mis-segregation: the cells with joined ends are formed into dicentric chromosomes pulled apart at anaphase, resulting in aneuploidy. New double stranded breaks are introduced into the genome, resulting in catastrophic effects. This is a picture comparing normal cells vs cells with mis-segregtion:



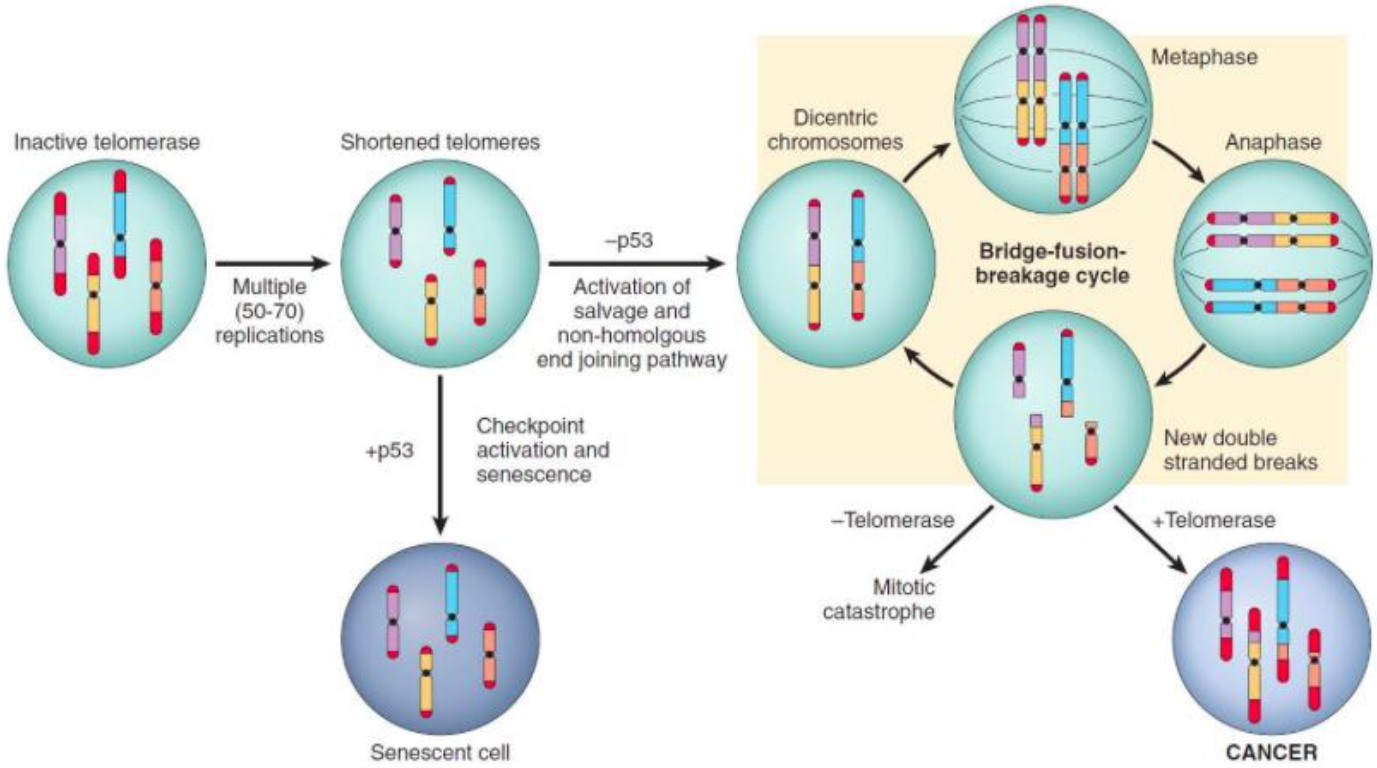
(2) It might break the chromosomes at different regions, also resulting in double stranded breaks and different mutations.

As the cell replicates over and over it will stop by these new double stranded breaks→ no p53→ non-homologous end joining again→ mis-segragation or breaking → new double stranded breaks and mutations

This is what we call the **BRIDGE-FUSION-BREAK CYCLE**. You bridge the problem, fuse the two non-homologous chromosomes and break them apart during mitosis.

This will go one as the tumor grows, creating mutation after mutation, double stranded break after double stranded break. It could break a tumor suppressor gene or fuse an oncogene with an active promoter. With all these mutations, the cell becomes extremely abnormal and reaches a point called **mitotic catastrophe** where it says "enough, I am too abnormal." In order for most cancers not to get into mitotic catastrophe, they reactivate telomerase. In 85%-95% of cancers, telomerase is reactivated. Rather than fixing the ends by non-homologous end joining, they activate telomerase. Thus, with no telomerase, mitotic catastrophe and death results. Now, the mutations already created are stuck in the cancer cells. This is the basis of genomic instability in Cancer cells.

Below is a picture explaining what we discussed:



- ✓ Inactive Telomerase → Short telomeres
- ✓ If p53 active → aging
- ✓ Absence of p53 and/or Rb → dicentric chromosomes → breakage at new points in metaphase/anaphase → Bridge-Fusion-Breakage cycle
- ✓ BFB cycle → if telomerase absent → mitotic catastrophe & death
- ✓ BFB cycle → if telomerase present → CANCER

This pretty much wraps up immortality. Next we're discussing Angiogenesis.

Angiogenesis:

Angiogenesis is the formation of new blood vessels mediated by growth factors (Insulin-like Growth Factor (ILGF), Platelet Derived Growth Factor (PDGF), Granulocyte-Macrophage Colony Stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF).

The difference between angiogenesis in normal cells and angiogenesis in Cancer cells is that new blood vessels in cancer cells do not mature. The resulting vessels are abnormal. They are **leaky**, **dilated** and have **haphazard connections**. Pericytes migrate and everything else occurs but vessels are abnormal. This provides more nutrients, oxygen and growth factors to the tumor and ultimately facilitates metastasis.

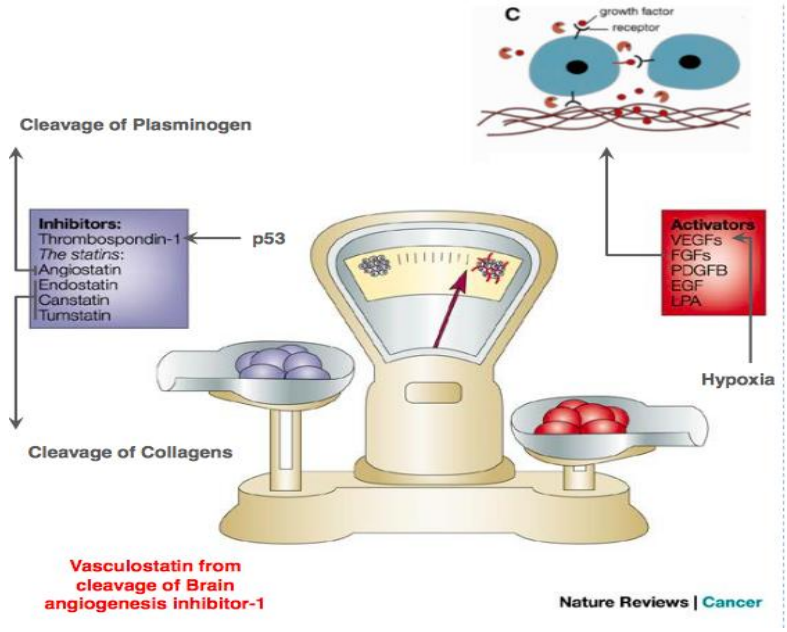
The theoretical limit for diffusion is 1-2mm, which is usual size of the tumor at the beginning. Beyond that limit, it's going to need its own blood supply. Both benign and malignant tumors induce angiogenesis and of course grow beyond 1-2mm. Benign tumors CAN grow beyond 1-2mm.

The Angiogenic switch:

Defined as the switch from no angiogenesis to angiogenesis. There are pro-angiogenic factors as well as anti-angiogenic factors. Like everything else in the body, there is a balance between these two types of factors. Pro-angiogenic factors include VEGF (induced by hypoxia), FGF (released when you destroy ECM). VEGF is also released when you destroy ECM (some is trapped there in an inactive form). Other factors are released such as PDGF, EGF and LPA-activators. Thrombospondin 1 is a major inhibitor of angiogenesis and is a transcriptional target of p53.

Other inhibitors include the statins, they are released by the cleavage of certain compounds:

(1) Angiostatin → cleavage of plasminogen
(2) Vasculostatin → cleavagee of brain angiogenesis inhibitor-1
*Doctor Mazen said the book mentions vasculostatin is released by the cleavage of transthyretin, which is wrong. It's activated by cleavage of brain angiogenesis inhibitor-1.
(3) Endostatin → cleavage of collagen
(4) Canstatin → cleavage of collagen
(5) Tumstatin → cleavage of collagen



Regulation:

- ✓ No p53→ less inhibitor of angiogenesis→ increased angiogenesis
- ✓ Hypoxia→ more VEGF→ increased angiogenesis
- ✓ Increased destruction of ECM by metalloproteases→ increased release of FGF and VEGF → increased angiogenesis

These factors are not only produced by tumor cells, but also by inflammatory cells (macrophages) and tumor associated stromal cells.

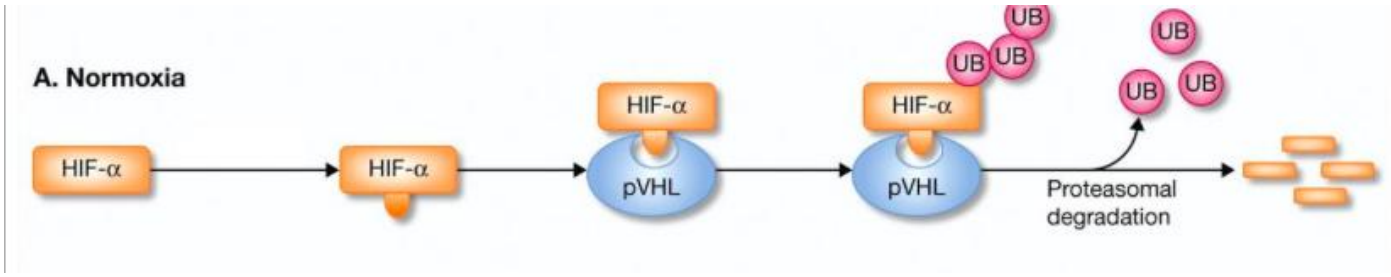
Remember, there's always a two-way conversation between the tumor and stromal cells. If the stroma is not permissive, nothing is going to happen, which explains why cancer is frequent in certain organs and less frequent in others.

<u>VEGF activation in more detail:</u>

VEGF is a transcriptional target of a protein called Hypoxia Inducible Factor alpha (HIF-α)

- Normal Conditions (Normoxia):

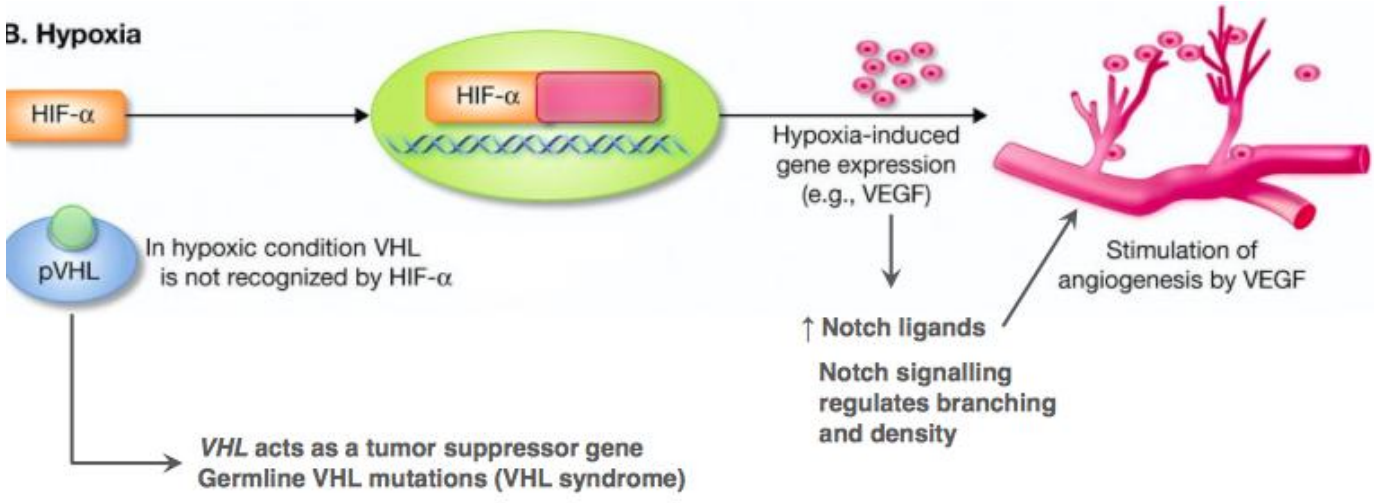
Under normal conditions, HIF-α is constantly produced. It is destroyed by the vin Hippel Lindau protein (VHL) through ubiquitination. VHL is considered a tumor suppressor gene.



- Hypoxic Conditions:

Under hypoxic conditions, which is what happens in the center of a tumor as it grows really big (>1-2mm), VHL cannot detect the presence of HIF-α. Thus,without oxygen, VHL cannot do its function of sensing HIF-α and we still don't know why. HIF-α skips ubiquitinated proteosomal degradation and goes to the nucleus. It induces the expression of VEGF and NOTCH ligands (exists in T-cells and keratinocytes- we just learned the other function). This results in stimulation of angiogenesis through the NOTCH pathway.

*Note: in the slides it says HIF-α cannot detect VHL, but doctor Mazen said VHL cannot detect HIF-α and that's also what's written in the book.



- VEGF is one of the molecules we are targeting as anti-tumor therapy. No oxygen, no nurtients, no tumor. Yes, anti-VEGF antibodies are inhibitors used in various types of cancer. These are beyond the scope of this course.

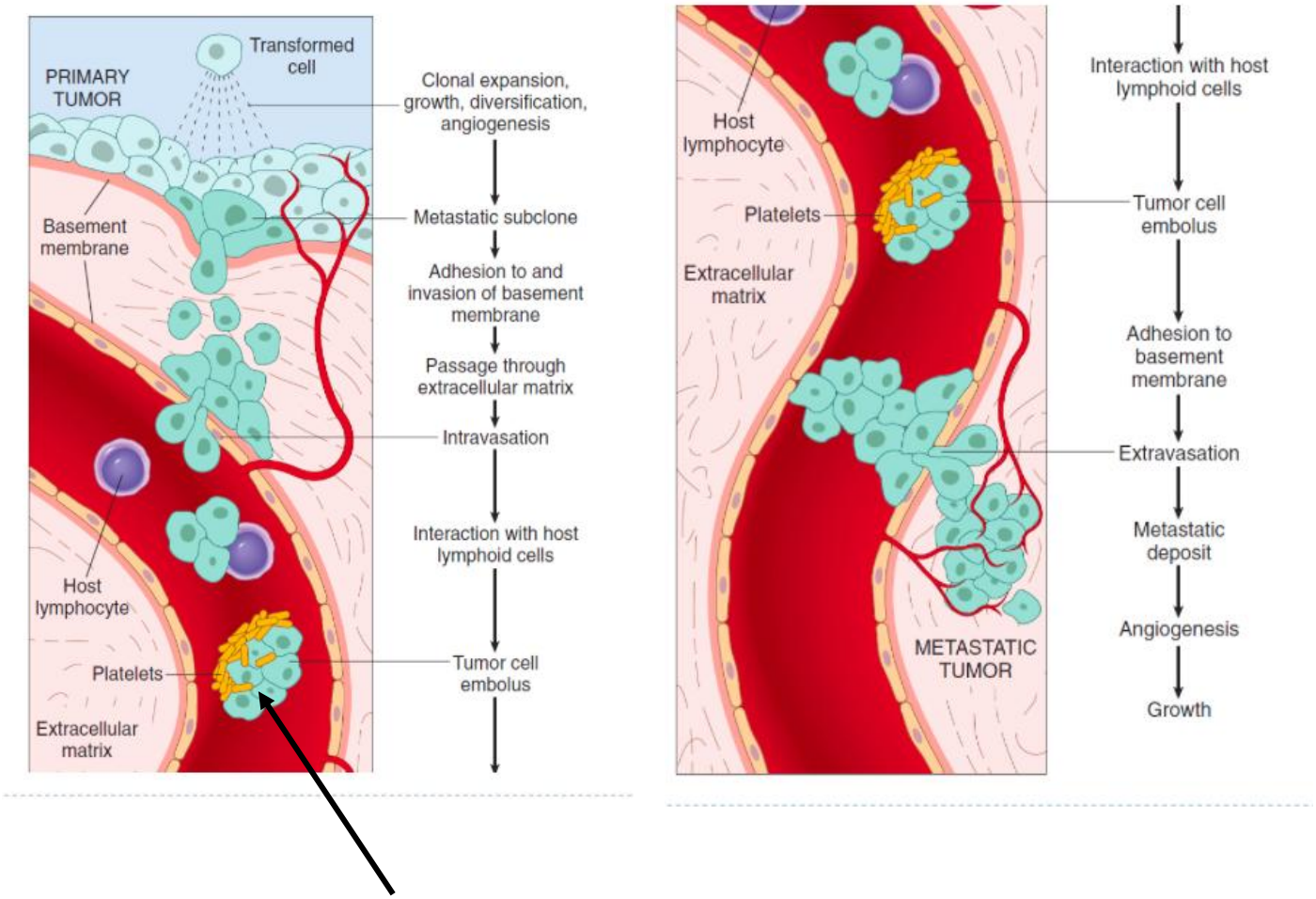
  IF there is a mutation in VHL, there's going to be plenty of HIF-α. This results in vin Hippel Lindau Syndrome. Its symptoms include renal cysts, pheochromocytomas (tumor on the adrenal gland) and various angiomas (an abnormal growth produced by the dilation of new blood vessels).

Next Hallmark is:

<u>Invasion and Metastasis</u>:

One property that the cancer needs for metastasis is **Evasion of the Immune System**. Then it needs to find a new location, permissive stroma, and it needs to induce angiogenesis.

The steps of invasion and metastasis include the following:



For cancers that travel as an **embolus**, the outer shell is a sacrificial (it sacrifices itself) shell. It's killed by WBCs as it travels in the blood stream and the center is left intact until it reaches its destination. However, this is very rare.

Details of the Steps: Which can be divided majorly into (1) ECM Invasion (2) Vascular Dissemination and Homing

(1) ECM invasion

It all starts with epithelial cells. In order for epithelial cells to move, they ought to lose their e-cadherin. There is also an activation of B-catenin, which activates TWIST, SLUG, and SNAIL. This downregulates the expression of e-cadherin, which reduces contact inhibition and induces Epithelial to Mesenchymal Transition.

-It turns out TWIST/SLUG/SNAL upregulation means the tumor is most likely to metastasize.

Our main concern here is EMT:

First step: Losing contact (through downregulation of e-cadherin and loss of contact inhibition).

Second Step: we need to degrade the Basement Membrane. Since there's collagen type IV in the BM, we're going to use Type IV collagenase.

Since we're destroying the ECM, we're releasing all sorts of growth factors, which are chemotactic, angiogenic, and stimulate growth. As you remember, growth factors in repair increase proliferation and migration and inhibit apoptosis. That's a problem.

Some cancers would reduce the tissue inhibitors of metalloprotease(TIMPS) thereby increasing metalloprotease and ECM destruction.

Third Step: Changes in the attachment of tumor cells to ECM proteins. Normally, Integrin is attached to laminin in epithelial cells. When laminin is destroyed or when laminin is located in the wrong places, the cell is going to sense something is wrong and will start migrating. When an epithelial cell isn't attached to the basement membrane, it's going to sense something is wrong- there's no integrin signaling. The predominant signaling now is through cadherin because it's attached to other cells. Cadherin inhibits growth. If it's detached from BOTH (cells and BM) it's going to induce apoptosis. Cancer cells avoid this whole pathway and are resistant to sensing these changes, therefore, detaching doesn't kill them.

Two things stimulate migration:

1. Integrin signaling(resistance of apoptosis)
2. New binding sites on degraded ECM

Fourth Step: Migration. A very complex process that depends on autocrine signaling (cytokines) and paracrine signaling (HGF[Hepatocyte Growth Factor]/SCF[Scatter Factor]).

High concentrations of these factors are found at the leading edges of a Glioblastoma Multiform (highly invasive brain tumor/malignant), contributing to its ability to metastasize. The cancer induces the production of these factors by the stroma at the leading edge. That's why this cancer is considered extremely invasive, HGF and SCF induce lots of cell motility. Thus, we can conclude that the cancer's parenchymal cells are not going to metastasize, they're going to co-opt the stroma to produce the migration and growth factors required.

As you're destroying ECM, bits of it are chemotactic factors that can be used by the normal cells (WBCs and fibroblasts) as well as the cancer cells. Same goes for the chemotactic growth factors (released because ECM is being destroyed), which also stimulate migration, proliferation, inhibition of apoptosis, and alter differentiation. All of this leads to Actin reorganization, which aids the cell in migrating.

(2)Vascular Dissemination and Homing:

At this point, the cancer cell has gotten up from its place and moved through the ECM leaving a trail of destroyed tissue for other cancer cells to follow. It has destroyed the basement membrane of the blood vessel or if it's a very leaky one, it has intravasated into it. Now it needs to avoid WBCs (immune system) as a single cell or embolus. It finally reaches a distant location.

What determines the distant location, though? The distant location is **not** usually located at the first capillary bed the cancer encounters (doesn't occur according to lymph drainage or the expected anatomy). As it turns out, cancer cells have the same receptors that WBCs have that help them bind to the same adhesion molecules, allowing them to roll, stick, and extravasate. Cell adhesion molecules are only produced by certain organs. Lung cancer metastasizes to the adrenals but not skeletal muscles, for example. That's why it doesn't metastasize randomly, but specifically.

Lung cancer metastasizes to the adrenals specifically- due to having SAME adhesion molecules. Breast Cancers produce cytokine/chemokine receptors CXCR4, CCR7. These chemokines are only expressed in certain organs, and thus determine where breast cancer metastasizes-in organs where these ligands for these chemokines exist. Therefore, the cancer metastasizes according to **adhesion molecules, chemokine/cytokine, and permissiveness of the stroma**. The location is **not explained** by anatomy or the first capillary bed it encounters.

For tumors to reach a distant location, there is a very long list of events that has to go right, which is why metastasis is very rare. Remember that according to Murphy's Law, anything that can go wrong, will go wrong. This is why millions of cells are shed before proper metastasis occurs.

Theories concerning how Metastasis occurs:

- **Somatic Mutation Theory**:

This theory states that the genetic alterations required for metastasis are gained through mutation. With every single growth, a new mutation occurs and now the cancer cell can produce collagenase or it might lose e-cadherin. Now this cancer cell can migrate.

*There is contradicting experimental evidence to this theory. Scientists took a biopsy from breast cancer cells that haven't metastasized and from breast cancer cells that have metastasized. They compared to see which mutations had the metastasized tumor gained. Surprisingly, they found there is no difference. This could mean one of two things:

(1) Our current profiling techniques aren't sufficient to detect a small number of cells that have truly gained a new mutation
(2) The stroma is different between the two cancers. Indeed, they had found that the stroma is different. In fact, they took a biopsy of metastasized cancer from one rat and injected it into another rat with a different genetic background. The results showed that the tumor is very unlikely to grow and even metastasize. So the stroma and genetic background matter.

- **Tissue Organization Field Theory**: Stroma is necessary for metastasis to occur. A two-way conversation has to exist between the tumor and the stroma; the stroma has to be permissive.

The truth occurs somewhere between the two mentioned theories. Mutations need to be gained but also there has to be a two-way conversation between the tumor and the stroma for metastasis to occur.

REMEMBER:

"PRECISE LOCALIZATION OF METASTASES CANNOT BE PREDICTED WITH ANY FORM OF CANCER" This is why you need to check everywhere for metastases.

Cancer Dormancy:

Cancers mostly shed millions of cells and mostly in singles. A lot of these cells end up in non-permissive stroma. A lot of these cancer cells could also be found in the bone marrow, again, non-permissive. They're not going to grow, but they're dormant. Is that a problem? Yes. Most of the current treatments are based on active cancer cells. Treatment isn't going to reach them or target them. The treatment is usually specific for an active cancer. This may cause a recurrence of cancer. This is an ongoing theory as to how recurrences occur. The theory is that the dormant cells re-migrate to a permissive stroma. Something changes that makes these cells active again. We don't fully understand the process.

I apologize in advance for anything that you feel isn't clearly explained.
The topics are hard; I tried my best ☺
Please refer to the book for more explanations.

الله يوفقكم جميعا
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