PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE

Lecture Number: 21

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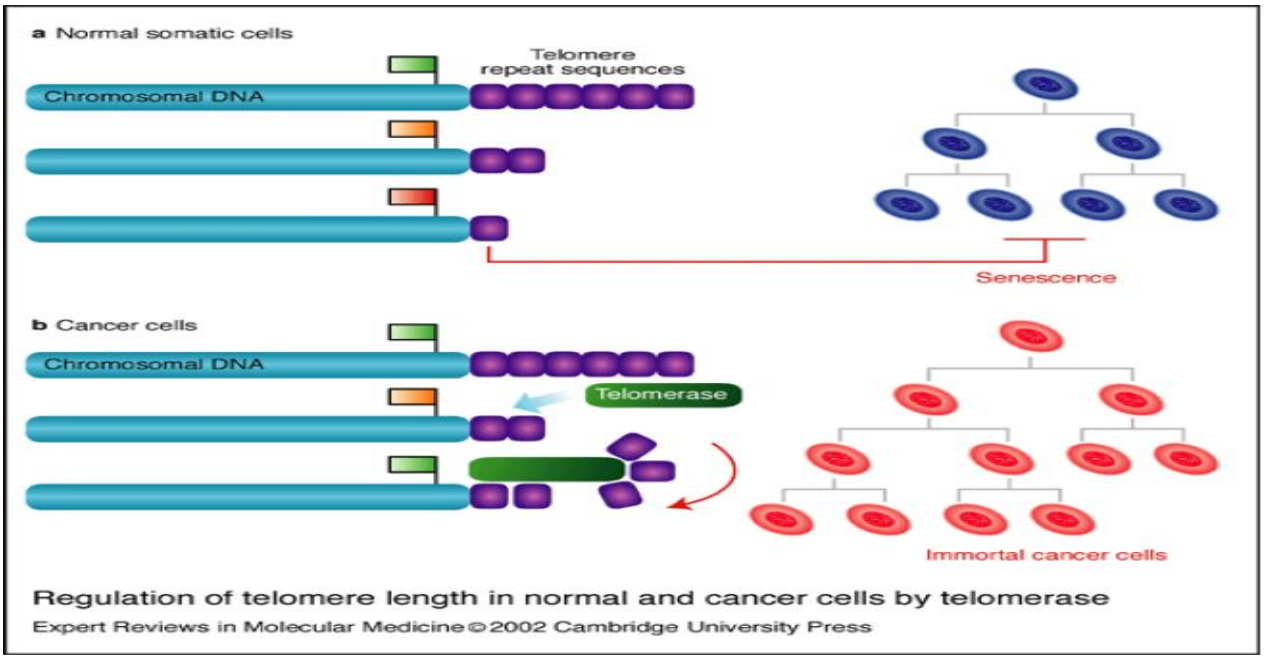
Hallmarks of cancer-3

as you remember from the previous lectures : One hallmark is not enough to create a clinically apparent cancer cell for example if you over-express certain oncogene it will cause cells to proliferate (one hallmark) but if you didn't inhibit the pathways which inhibit cell growth like P53 , TGF-B , RB the cancer cell will stop proliferating so always remember ONE HALLMARK IS NOT ENOUGH .

- as you know ,Somatic cells don't have telomerase activity so normally after certain number of replications , the somatic cell can no longer replicate and you have to activate other pathways that allows it to continue replication and gain replicative immortality .



Regulation of telomere length in normal and cancer cells by telomerase
Expert Reviews in Molecular Medicine © 2002 Cambridge University Press

NOW as you can see from the figure above , in somatic cells the telomeres function is to protect the ends of chromosomes from damage and these telomeres will shorten after each replication and when we reach the point of replication at which this telomere is very short the genetic material will become exposed to endonucleases which causes DNA damage so the telomere will be recognized as a " double-stranded break " .

Written by Omar Alqeisi

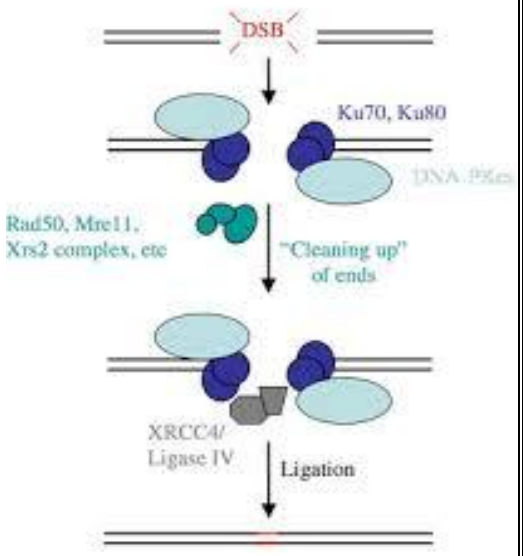
now the cell defense mechanism for that is **cell senescence** .

and if you remember , cell senescence is **permanent** exit of the cell cycle in response to DNA damage , and it requires P53 and / or RB pathway activation .

however , cancer cells will abnormally continue to replicate by increasing the expression of certain enzymes , such as telomerase which increases the length of the telomere , thus allowing the cell to continue replicating so they become immortal .

originally (in a normal cell cycle) : the chromosomes will replicate into 2 sister chromatids , and during the cell cycle , one part is detached to one end of the cell and other part is detached to the other end of the cell (the sister chromatids are separated) , so you will one full copy of that chromosome in each cell .

NOW, if the telomere is critically short , and the cell does not sense that damage and could not stop the cell cycle , as in the presence of an abnormal P53 , or the RB is not doing its function which is stopping the G1-S check-point , inappropriately , some DNA repair pathways will be activated , one of these pathways is the " **nonhomologous end-joining pathway** " which is a pathway that repairs double-strand breaks in DNA by ligating the ends of nonhomologous chromosomes .



- off note : the telomere prevents the joining of the ends of the chromosomes together .

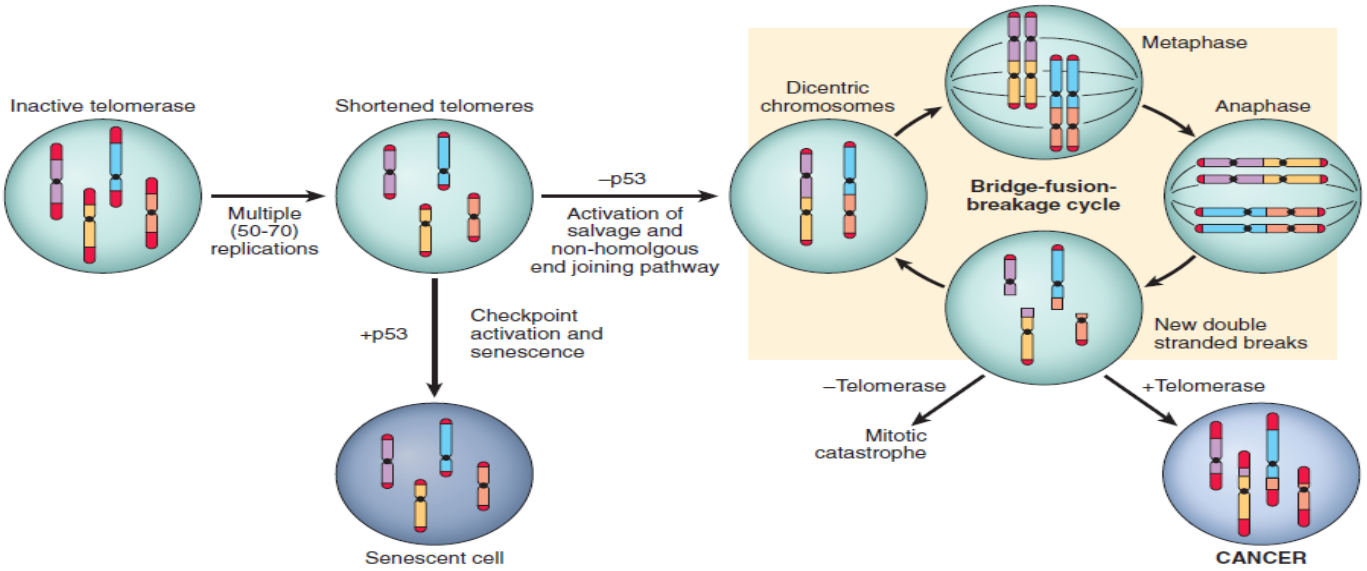
this ends up in a chromosome that is **dicentric** which is a chromosome that has 2 centromeres , NOW one of two things can happen :

1- the whole chromosome goes to one cell which means that cell gets double of the amount of the genetic material , and the other cell does not get anything , so both cells end up in **aneuploidy**

2- despite having a dicentric chromosome ,sometimes you also pull on those 2 centrosomes on that chromosome , here you are going to create a new

double stranded break ,that double stranded break will be detected and nonhomologous end-joining is repeated again ,this is called the bridge-fusion-breakage cycle .

look at the picture below and you will understand what has been said above



when you have a mutated P53 or mutated RB you are going to inappropriately activate that bridge-fusion-breakage pathway , where normally you would induce the cell to stop replicating .

SO , this cell continue to replicate and it's accumulating more double stranded breaks which means it accumulate more mutations , as we said before some of these mutations are beneficial and some are not .

if the cell continue to replicate and accumulates mutations to a point where it induces mitotic catastrophe (the chromosomes are so abnormally arranged) , the whole set of genes is not enough for the cell to continue its life cycle , or you have deleted genes , or you have mutated certain important genes , thats going to induce **apoptosis** . UNLESS you activated telomerase and stopped that cycle (the bridge-fusion-breakage cycle)

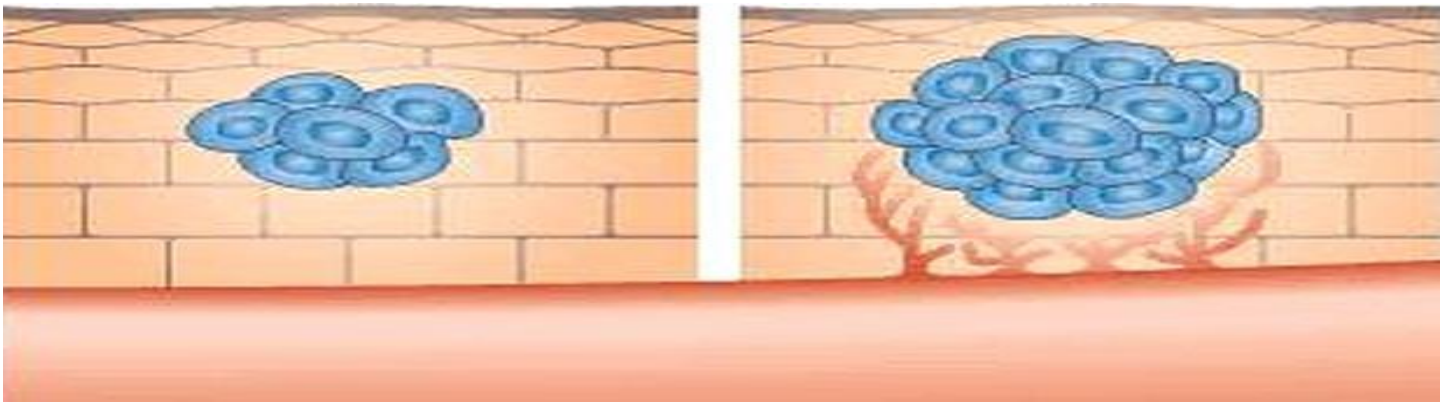
so early on , in a tumor if you have a mutated P53 or RB you are going to get this cycle , but if they are normal (P53 and RB) you are going to get cell senescence

remember one of the characteristics of cancer is **hypomethylation** i.e we are activating a lot of genes , and one of these genes could be telomerase and we stop that bridge-fusion-breakage cycle , here the beneficial mutations for the cancer cell that have accumulated are now FIXED .As we said the cell have averted the mitotic catastrophy .

telomerase while it's expressed in stem cells , it's not normally expressed in somatic cells , however 85% - 95% of all tumors have activated their telomerase , the rest tumors that have not activated telomerase have activated other pathways inappropriate DNA recombination repair to make sure that these double stranded breaks that resulted from the bridge-fusion-breakage cycle do not lead to mitotic catastrophy .

so this is an example of the multistep nature of carcinogenesis .

angiogenesis



we said that cancer needs oxygen , nutrients , so it needs to get access to the human vasculature .

at any case , tumors initially grow very slowly and they may take years to accumulate these mutations .

so many cancers will remain at the 1 - 2 millimeter size for a very long time , and they rarely grow beyond that until they induce angiogenesis , apparently this is the theoretical limit for diffusion , diffusion of nutrients , oxygen and getting rid of waste products that is the worth of being 1-2 millimeter size .

after that , for cancer to grow (or ever large benign tumors) they need to induce angiogenesis , you remember how we induce angiogenesis : normal vessel > sprouting > growing of new vessels (migration and proliferation) ,but the difference here in cancer is that the vessels DO NOT mature (they remain abnormal) they are leaky and dilated and they don't have normal connections i.e they are haphazard and random , so the last step which is maturation does not occur in cancer .

additionally to the functions of angiogenesis which are bringing oxygen , metastasis etc , some newly formed endothelial cells stimulate the growth of adjacent cancer cell by secreting growth factors like insulin-like growth factor , PDGF .

so not all functions are gained from mutations , some of the functions are gained because of the interaction with the stromal cells as we just mentioned .

remember we mentioned that in the bone marrow of the adults you can produce precursors for the endothelial cells , and this is typically what happens in vasculogenesis in the embryo , but we don't know if it has a role in repair in angiogenesis , apparently it may have a role in angiogenesis in cancer .

so there are 2 ways for angiogenesis in cancers :

1-new vessels sprout from previously existing capillaries

2-Vasculogenisis,in which endothelial cells are recruited from the bone marrow

Mechanism of angiogenesis in cancer

typically you have a balance between pro-angiogenic and anti-angiogenic factors , in cancer you increase the production of the pro-angiogenic factors ex: VEGF, FGF or decrease the production of anti-angiogenic factors ,

as you remember FGF can be released from the ECM when it's being remodeled (degraded) , so in cancer there are a lot of proteases that degrade the ECM and allow release of these growth factors that have been sequestered in the ECM so the balance tip toward angiogenesis in cancer .

normal P53 induces the production of an inhibitor called thrombospondin-1 (TSP- from the fibroblast , so if you don't have normal P53 so you cannot induce the production of TPS-1 .

the book also mentions angostatin and endostatin (they are angiogenic inhibitors) they are cleavage products of plasminogen and collagen , and it mentions another one which is vasculostatin which is a cleavage product of tranthyretin WHICH IS WRONG IN THE BOOK the correct statement is that vasculostatin is a product of the cleavage of brain angiogenesis inhibitor 1 .

so essentially if have you cleaving plasminogen and collagen which occur normally in repair , you are increasing these inhibitors .

there may also be collagen degradation in cancer but the overall balance has been tipped because you are no longer producing the most potent inhibitor which is TSP-1 and you are over expressing VEGF and allowing the release of FGF at the same time , so again this is the angiogenic switch , it's a balance , if you are increasing the production of pro-angiogenic factors more than reducing the anti-angiogenic factors you are going to get angiogenesis .

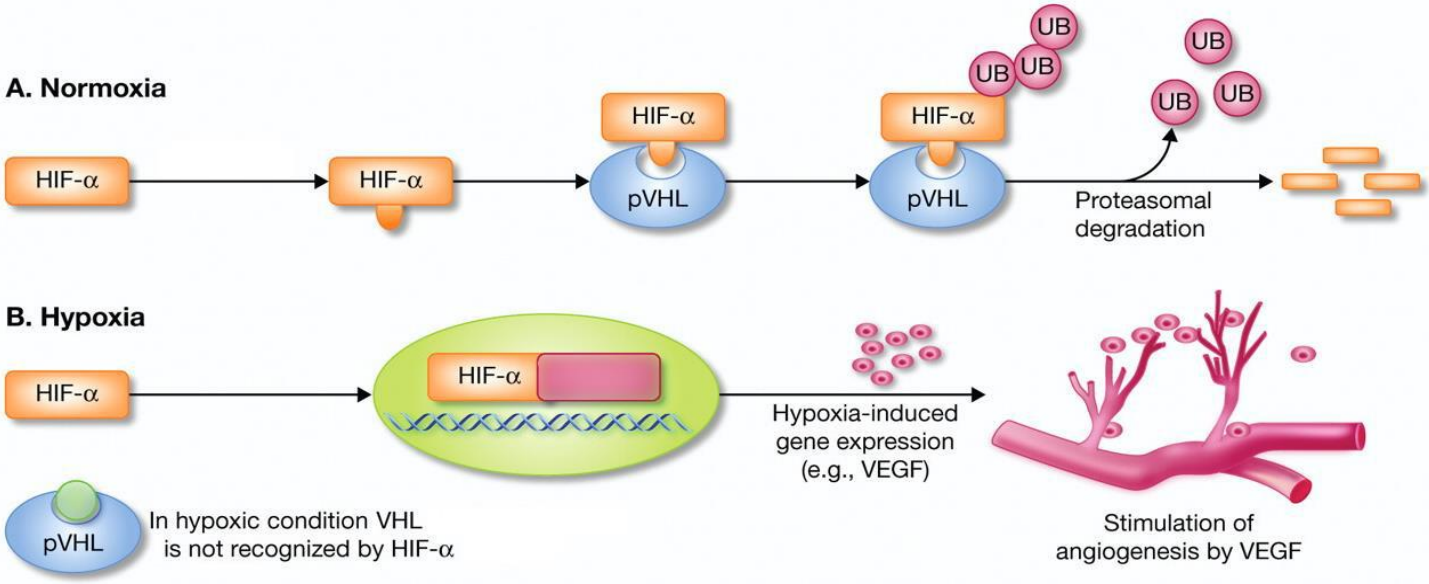
we just discussed how FGF is released , now we will talk about VEGF :

normally VEGF is not induced , under normoxia (normal oxygen) there is a transcription factor called HIF-1 alpha (hypoxia inducible factor alpha) , that's identified by the VHL protein , when it identifies HIF-1 alpha > HIF-1 aplha is ubiquitilated and sent off to proteosomal degradation .

if you have hypoxia for example in tissue damage or in cancers that are growing so fast , they are outpacing their blood supply and you have cells that are hypoxic , here in this case HIF-1 alpha is no longer recognized by VHL protein , so it escaped degradation , then it goes to the nucleus and induce the transcription of VEGF .

VEGF can affect transcription of other pathways , in this instance it can increase the transcription of NOTCH ligands , and NOTCH ligands are responsible for the branching and organization of those new blood vessels .

now if you have abnormal VHL protein , the angiogenesis will be easier because you are no longer destroying the transcription factors for VEGF .



it turned out that mutations in the VHL gene results in VHL syndrome , these patients have renal cell carcinomas , pheochromocytoma , CNS angiomas , retinoangiomas , renal cyst .

now if tumors cannot grow beyond a certain size , this means that these cancers will not be able to replicate more frequently and it will not be able to accumulate more mutations and it will not gain more hallmarks because they haven't gone beyond that small size , so if you inhibit angiogenesis , you could potentially inhibit metastasis , mutation accumulations etc .This is another example of one hallmark is not enough if the cells replicate indefinitely without activation of angiogenesis they will die by hypoxia .

so it turned out that inhibitors of VEGF have been very effective in a wide variety of cancers and they are currently used in the treatment of cancer very effectively .

invasion and metastasis

metastasis means that the cancer cells after they have grown , they degrade the basement membrane , get into the interstitial matrix to reach a blood vessel and go through that blood vessel or lymph vessel , where it reaches the first capillary bed or first lymphatic area , and then it needs to extravasate from that vessel , again degrading the basement membrane , degrading the interstitial matrix , co-opt the new environment (remember that the stromal cells are not a passive barrier , they are sometimes cooperating with the cancer because the cancer sends signals , the stromal cells produce for example growth factors in response to these signals) .

so that means , in order for the cancer to reach that area , it needs to find stroma that is willing to cooperate (not all stroma wants to cooperate) that is why cancer sheds a lot of metastatic cells (it's inefficient process) these cells could be in single form (most cancers shed them in a single form) or they can be emboli were they can be aggregate by the help of some platelets through the blood stream .

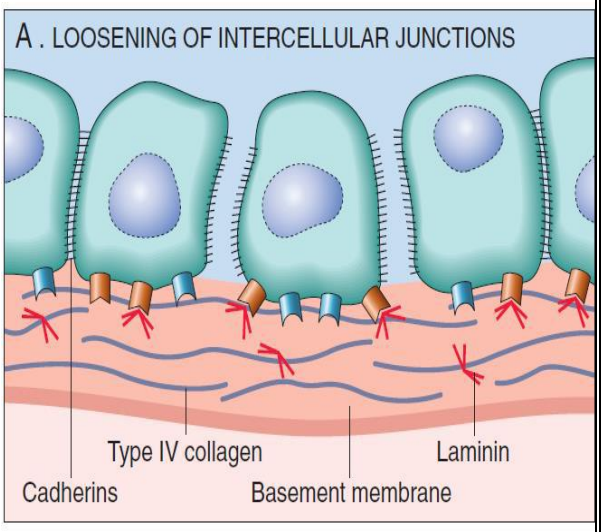
now if you think about it , a single cell is more likely to be caught and identified by the immune system where as in embolus , the surface cells may be attacked , the cells in the middle are less likely to be attacked because it's hidden .

so the cells also have to escape the immune system then go to the basement membrane , find stoma that's willing to cooperate and continue their growth as they were with the original stroma , so that is why it is an inefficient process .

so tumor produce a lot of these tumors , and not all of these cells will end up with metastasis , some small tumors produce millions and millions of these cells throughout its life and they never end in metastasis .

now we will discuss the steps by which the above happens :

- first : we need to get through the basement membrane



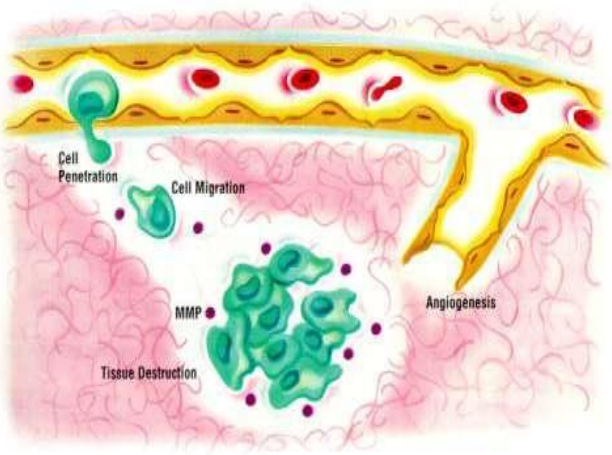
1- loosening of the intracellular junctions remember when we talked about epithelial to mesenchymal transition , we lose the E-cadherin junction between these cells (it turns out that E-cadherin function is lost in almost all epithelial cancers) either the E-cadherin gene is mutated , or we activated the B-catenin pathway , as you remember B-catenin is attached to E-cadherin , and if you lose E-cadherin , B-catenin can be released either because of signals or an abnormality in the destruction complex , and B-catenin can turn on certain genes for proliferation like : MYC , cyclin D , but more importantly it can also enhance the transcription of the master regulators we mentioned before : TWIST , SNAIL , SLUG , and those inhibit the transcription of E-cadherin .

sometimes these master regulators can be inappropriately over-expressed independent of B-catenin .

also you remember when we talked about Merlin or NF2 (the mutated gene in neurofibromatosis type 2) , that if there is E-cadherin signaling it inhibits oncogenic gene expression , but if you lose that E-cadherin signaling , you allow oncogenic gene expression by inhibiting Merlin entry to the nucleus .

so by losing that E-cadherin , there will an increase in the proliferation , there will be more deregulation of E-cadherin because you are increasing the B-catenin > further inhibiting the production of E-cadherin .

2- degrading the basement membrane and the interstitial matrix .



we said that one of the enzymes that are responsible for this degradation are metalloproteinases MMP , and we said that they are inhibited by tissue inhibitors of metalloproteinasesTIMP , now in cancer cells there's an increase production of these metalloproteinases along with cathepsin D and urokinase plasminogen activator , these are all proteases that degrade the basement membrane , specifically MMP 2 and MMP 9 those are really over-expressed , for example if you look at breast cancer tumors , you will find that their benign counterpart do not over express these proteases , were if you looked at their malignant counterpart they over-express these proteases .

also as we mentioned when you are degrading the ECM you are releasing growth factors ex: FGF inducing angiogenesis , other growth factors are also released .

so the ECM itself when it's degraded , these fragments could act as chemotactic , angiogenic and they can also stimulate growth .

now in repair you increase the production of theses proteases and you want to enhance proliferation , migration of fibroblasts , you want to bring in some blood cells to help in the repair , so that same mechanism that is beneficial to you during repair , tumors express the same proteases that are needed in the repair but extensively , leading to this chemotaxis i.e enhancing migration , increased growth and angiogenesis .

you know that the basement membrane has type 4 collagen , and some of these metalloproteinases have a degrading activity on collagen type 4 , and that is how they get through the basement membrane .

typically malignant tumors have an increased expression of type 4 collagenase activity whereas benign tumors typically do not .

3-changes of attachment of tumor cells to the ECM

remember when you are degrading the basement membrane that the cell has integrins , those integrins are attached to actin and they are attached to collagen type 4 and laminin , normally if a cell loses attachment with the basement membrane it will die off by apoptosis , however , cancer cells do not because they have changed their internal signals for example through mutations .

in cancer cells internal signaling has changed to a point that it inhibits apoptosis , we will not discuss the mechanism because it is not fully understood

in addition because you are degrading ECM , some parts of the ECM may still bind to those integrins , but binding is altered , that means the signaling through the integrins will also be altered .

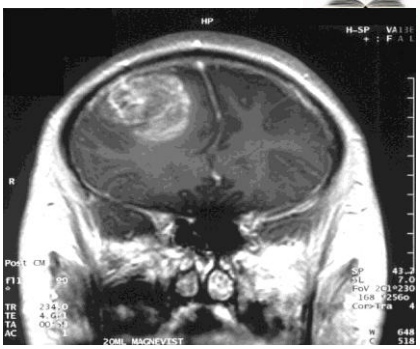
remember when we said that signaling through integrins could affect proliferation , migration , and differentiation , so if you are playing with that signaling , you could potentially : increase proliferation , increase migration and affecting differentiation of these cells , all of which are important in carcinogenesis .

4- now we have degraded the basement membrane and the interstitial matrix now the cells want to move this is the **migration** point .

it's a very complex signaling , it's a combination of autocrine (like autocrine motility factor)

so the cell has a receptor , the cell produces a ligand for that receptor , that leads to actin organization , which leads to cell moving .

paracrine : i.e the stromal cells are co-opted , they are being permissive , where the tumor signals to the stromal cells , the stromal cells produce certain growth factors like : hepatocyte growth factor and scatter growth factor , to allow for this movement (migration) .



clinical case on this point : glioblastoma multiforme which is a very invasive brain tumor that has hyperplastic blood vessels and an over-expression of HGF and HCF at the leading front of the tumor which cause the tumor to move .

ECM degradation products :

remember when we said that the degradation product can signal through the integrins can affect proliferation , migration and differentiation it also act as chemotaxis .

these degradation products tells the cell that has produced the MMP to follow that devastation pathway (the degradation pathway) ,because chopping off these ECMs actually signals to the cell to move to this way (the degradation way) .

as you are chopping the ECM , you are releasing growth factors like : FGF , VEGF (3aref eljomle hay en3adat 5mseen mrra bs t7ammalo b3een allah :P) other growth factors like insulin growth factor 1 and 2 work as chemotactic growth factors and they are released from their pockets , from their local high concentration in the ECM , allowing these cells to move ,all of this leads to actin reorganization and we come back to what we just mentioned : down-regulation of E-cadherin , affecting the cell-cell junction , you are changing polarity because you have changed the signaling through the integrins , you have reorganized actin , up-regulation of MMP , and you are remodeling the extracellular proteins , and because you are damaging the ECM you are releasing growth factors that could enhance migration , angiogenesis , proliferation and tell the cell to move this way .

now once the cell has reached the blood vessel we want to avoid host immunity , either by being an embolus or by certain mechanisms where we mask the antigen or we don't present the antigen etc , to be able to get to a distant site .

remember when talked about metastasis as a mechanism of the malignant tumor , we said that vascular and lymphatic anatomy explains most but not all of where the metastasis shows up , lung cancers frequently involve in the adrenals but it doesn't

involve in the skeletal muscles , even though the skeletal muscles have plenty of capillary beds < this in not explained by anatomy .

so the explain by tumor biology : where certain tumors may express an adhesion molecules (remember that these adhesion molecules are useful for WBC for migration , they are expressed on the endothelium) , these tumor cells use the same mechanism to migrate to a specific tissue , where in that particular region of the endothelium of a certain tissue express the ligand of the adhesion molecule of the tumor cell , and that is where that tumor goes to .

chemokine homing :

cancers produce chemokine receptors ex: breast cancer produces CXCR4 an CCR7 , and their ligands CXCL12 and CCL21 , they are highly expressed where breast cancer ends up .

so if you inhibit that chemokine receptor you can inhibit metastasis , once we get metastasis it's very hard to treat the patient .

now remember when we said that cancer cells produce a lot of cells in a very inefficient process , this is related to cancer dormancy , some of these micro-metastasis i.e they have not really inhabited the new space ,because they have not found a new stroma that is permissive , may become quiescent , they don't act and replicate , and they potentially could escape from treatment , and they may not be detectable by the different techniques .

so you treat the patient and you think he has fully recovered and he is free from cancer , but after 3 years the cancer comes back and may be worst than ever .

these dormant cells have escaped treatment because it's not acting like the rest of the tumor cells and they can be potentially found in the bone marrow , in the blood stream , so it can escape treatment .

according to the somatic stem cell theory , as the cancer cells grow they accumulate more mutations , and some of these cells may gain the functions we just talked about (ECM invasion , MMP , chemokine receptors activation) and those who gained these functions are the cells that will metastasize where the rest of the tumor doesn't .

now if you look at both metastatic cancer and the original cancer and you do a chain profiling of the two i.e which genes are activated , it turns out that the genes that are activated in the metastatic cancer and the original cancer are the same .

now there are 2 hypothesis here : the first one claims that when researchers / scientists perform chain profiling of a cancer , they're profiling over a million cells , so it would be virtually impossible to detect a single additional mutation in a single cell. the other one claims that perhaps whenever an additional mutation / gene expression is elicited in one cell . certain pathways allow all other cells of the cancer to express this change , making it impossible to detect .

now these two hypothesis non of either has proven , you need to be aware of both .

tissue organization field theory : maybe it's not a matter of up-regulating a gene or down-regulating a gene , maybe it's an interaction with the stroma , unless you get a stroma that is permissive that respond to the cancer , and allows that cancer to migrate and invade you cannot get metastasis , so potentially it's not a matter of gaining a mutation , it's a matter of having that particularly in a particular tissue in a particular stromal space that allows its migration to happen , given that precise localization of metastasis cannot be predicted with any form of cancer , now there are common trails : this cancer commonly goes here and that cancer commonly goes there BUT you do not evaluate the patient about what is common , you assume it can go anywhere

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