PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE

Lecture Number: 23

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Carcinogens

Overview:

This lecture aims at introducing you to the various types of carcinogens. We have 3 main types: Chemical, Radiation, and Microbial-of which we have viruses and bacteria.

Of the microbial viruses, we will be talking about: **HTLV-1, HPV, EBV, EBNV-2, HBV and HCV**

Of the microbial bacteria: ***H. pylori***

Chemical

This type of carcinogen has been identified more than 200 years ago. Chimney sweepers, especially in London and northern Europe, where they depended a lot on wood burning to keep warm, you would get a buildup of Creosote and other metabolites in the chimney that could lead to a fire. So they had people come to sweep the chimney and get rid of all these chemical materials. As it turns out, most of these chemicals, ex. Cyclic carbons, turned out to be carcinogenic. And there was a big connection between being a chimney sweep and getting scrotal cancer. So based on that, the Danish chimney sweeper association insisted that all chimney sweeps should bath daily. Just by bathing daily, the rates of scrotal cancer cases decreased. So that's an example of a carcinogen in you work environment that could end up in cancer.

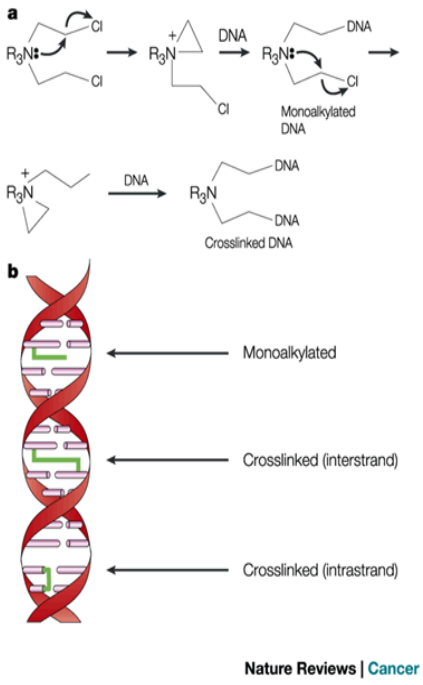
Chemical carcinogens can be either direct acting or indirect (just like the injurious stimuli)

i.e they themselves could cause cancer or their metabolites can cause cancer.

Direct acting chemical agents :-

Those require no metabolic conversion to become carcinogenic ,they are generally weak. For example, alkylating agents(Nitrogen mustard gas , cyclophosphamide

,Chemical warfare) that cause cross linking .Cyclophosphamide which a chemotherapeutic drug that is used in the treatment of Hodgkin Lymphoma but it end up with a second form of cancer which is leukemia which is more tragic .also Cyclophosphamide was used in the treatment of rheumatoid arthritis and Wegener granulomatosis which are non-neoplastic diseases.For that reason Cyclophosphamide prescription should be restricted and those risks should be considered .Typically these Alkylating agents are essentially electrophiles(they love electrons). They attach themselves to DNA to get electrons and cause cross links either between strands or in the same strand which cause DNA damage that needs to be repaired ,they can also attach to RNA, proteins to get electrons and sometimes inactive proteins as well. An example of a protein that can be cross-linked and turned off by this way is the tumor suppressor PETA .



Indirect acting chemical agents :-

Indirect acting they are not carcinogenic but their metabolite is carcinogenic so for these agents to become carcinogenic they require metabolism first. This happens mainly through P450. For example, polycyclic hydrocarbons resulting from fossil fuel burning or cigarette burning. All of these are indirect carcinogens.

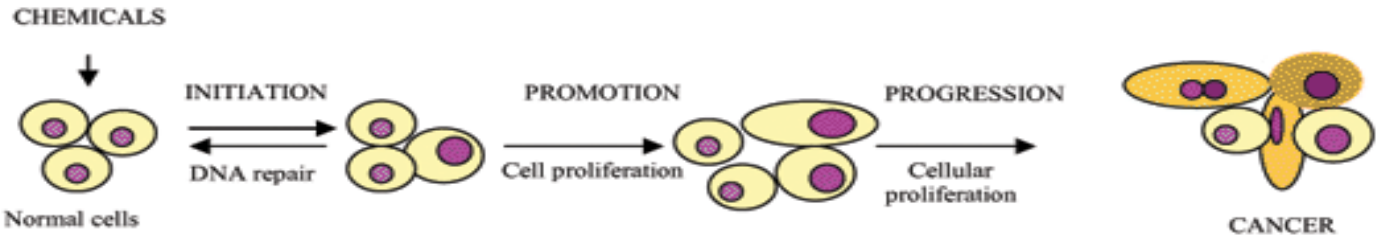
Remember polymorphisms? **Polymorphisms** are variations in genes that are not necessarily mutations because the resulting protein still functions as before but the level of functionality could be higher or lower.

So it means that people exposed to these indirect carcinogens and have polymorphisms in their P450 system will experience different effects. Those that have a polymorphism that increases the P450 function are at a higher risk, whereas those with polymorphisms that have lower P450 function are at a lower risk.

There's one agent we mentioned in the past. We saw that the most common tumors in the world were either lung, colon, prostate in males, or breast tumors in females. But one region specifically stood out in south east Asia. This region had a high incidence of hepatocellular carcinoma. This is an example of environmental effects on that particular cancer. This region has a lot of nuts in their diet. When these nuts are inappropriately stored, they get a type of mold called Aspergillus. This mold produces Aflotoxin. Aflotoxin is a carcinogen that leads to hepatocellular carcinoma. It induces mutations and the signature mutation for Aflotoxin in hepatocellular carcinomas is mutation on the p53 tumor suppressor.

Keep in mind, that that doesn't mean these chemical carcinogens specifically induce mutations in p53 or other tumor suppressor genes, its just that the mutations that are naturally selected to become cancer are the mutations that are important in the pathways leading to cancer, whether its inactivating a tumor suppressor, activating an oncogene, turning off DNA repair genes, etc..

Initiation-Promotion progression



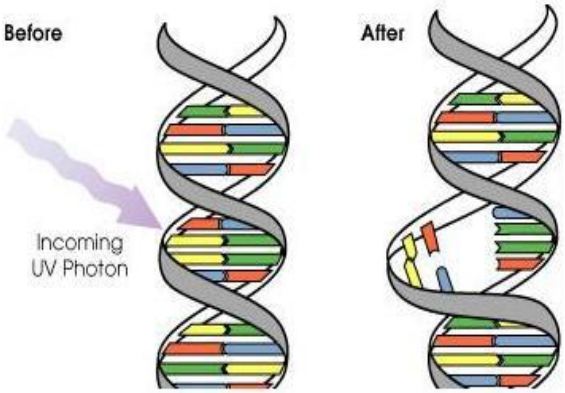
When talking about chemical carcinogens, we're mostly talking about initiators. i.e these chemical initiates the cancer because they can induce damage in the DNA but you need further agents to create a clinically apparent cancer. The chemical carcinogens are the initiating factors ,the DNA repair mechanism is what prevent these mutation from accumulating but at some point DNA repair mechanism cannot keep up with mutagenesis if there is excessive DNA damage , after the DNA repair mechanism is down things that cause cells to proliferate normally in our body will now cause a promotion toward cancer .

For example if you were exposed to a chemical in your endometrial lining and the DNA repair mechanism was down ,estrogen here act as a promoting factor toward carcinogenesis because it normally induces proliferation in the endometrial lining this will lead to progression toward cancer .This example explains the whole

initiation-promotion thing , the chemical is the initiator of DNA damage which is not enough to cause cancer and Estrogen is the promoting agent that induce cellular proliferation and causes cancer .What we've been taking for the last 8 lectures has mostly been about progression, how we move from gaining one hallmark to gaining another.

Radiation.

Radiation DNA damage. Whether its UV, X-ray, or nuclear. The two major radiation events on our planet occurred in Hiroshima and Nagasaki in WW2. Chernobyl was devastated by a nuclear plant leak, and around these areas there was a massive increase in all types of cancer, particularly Myelogenous leukemia. This radiation, whether UV, X-ray, or nuclear, induces chromosomal breakage and translocations. These double stranded breaks are of three cases, where if you have a single stranded break, you can usually copy off the other strand. If it s a double stranded break you can to copy off the other allele. But if its sever damage, then there's no way to fix it. Radiation effects can sometimes (but rarely do) induce point mutations.



Skin cancer and UV.

Which repair pathway fixes UV-induced DNA damage?
The nucleotide excision repair pathway.

So if you get these dimers and you have an inappropriate abnormal nucleotide excision repair which happens in Xeroderma Pigmentosum you can increase your risk of cancer. That doesn't mean that other people who have the normal nucleotide excision repair pathway are not also at increased risk for cancer from UV mediated DNA damage because there's only so much your cells can repair. If you have an accumulative exposure, that means a life time exposure (like being in the sun a lot, or not wearing proper clothing and protection, not applying sun screen) then you're more at risk for non melanoma skin cancer.

Those who get intense intermittent exposure (like tanning at the

beach) are more at risk for melanoma skin cancer. This can also happen at tanning salons.
The risk depends on three factors.
1- The fairness of the skin
2- The geography (and thus the intensity of the sun)
3- Personal Habits, like applying sun screen, wearing long shirts if staying out long

Microbial and viral oncogenes

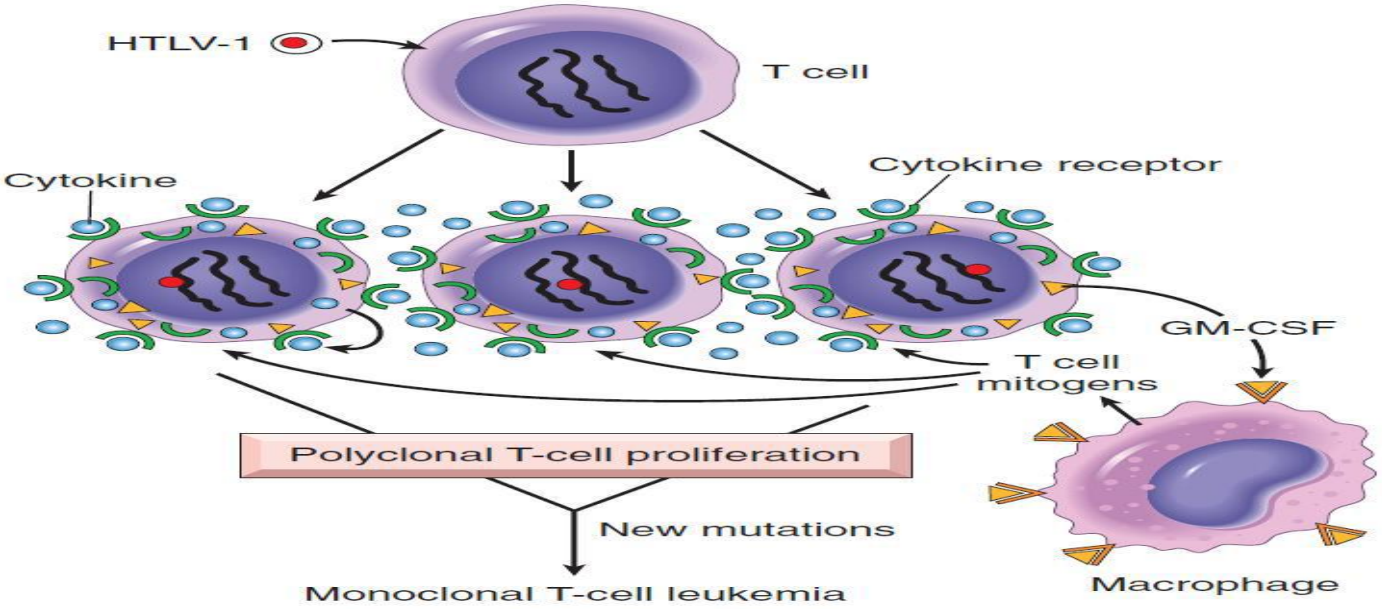
HTLV-1(Human T-lymphotrophic virus)

It is a retrovirus that is very similar to HIV its transmitted through sexual contact, blood or breast feeding in nursing mothers. It is endemic in Japan and the Caribbean. It is also similar to HIV in that it specifically targets CD4 on T-cells. But instead of targeting CD4 t- cells and killing them, it induces cancerous transformation in them. Only 3-5% of all HTLV infections end up in leukemia or lymphoma. There is a very long latency period from 20-50 years like HIV there is latent period where no symptoms occur. So that means the virus alone is not enough to induce transformations (it's a multistep process).
Things need to go wrong, resulting in the accumulation of whatever mutations required to induce leukemia or lymphomas. But that doesn't mean the virus itself doesn't have a role. While the virus itself does not have an oncogene and doesn't integrate into an oncogenic site, it does however produce certain proteins that are important for this process. One such protein is called the TAX protein. TAX has several effects:
1- It inhibits the activation of p53. If we have no p53, then no Senescence
2- It inhibits p16. Inhibition of the cyclin dependant kinase inhibitor leads to the activation of the cell cycle.
3- Activates cyclins and their associated cyclin dependant kinases.
(inhibiting an inhibitor ⟿ > activation) (activating an activator ⟿ > activation)

Because we have a virus which will stimulate and activate the immune system. It'll also have the various Interleukin factors that are important in inflammation, but can also be used by transformed cells to induce their own proliferation. So in

addition to affecting the cell cycle, the virus itself can induce autocrine production of some of these interleukins (IL's) in the affected cells . It can induce IL-2 and it's receptors to reproduce in these cells, as well as IL-15 and IL-15 receptors.

If a cancer cell produces the ligand and the receptor, it is independent from any outside effects. This virus can induce these autocrine loops (IL-2,15 and their receptors). This virus can also induce paracrine effects as well. So the virus can induce cells to produce these Granulocyte Monocyte Colony stimulating factors GM-CSF which will activate macrophages, and the macrophages from your stroma



induce production of other growth factors that are mitogens for T cells, i.e these growth factors induce proliferation of T cells.

So in conclusion HTLV-1 can induce cancer in T-cells by 2 ways :

1-Autocrine mechanism : by synthesizing Interleukine 2 and interleukine 15 and their receptors

2-Paracrine mechanism : by secreting GM-CSF which activates macrophages TAX protein is to send out T cell mitogens that induce T-cell proliferation , TAX necessary and sufficient for tissue lymphomas and leukemia. So it is necessary and sufficient to induce transformation.

HPV: Human Papilloma Virus.

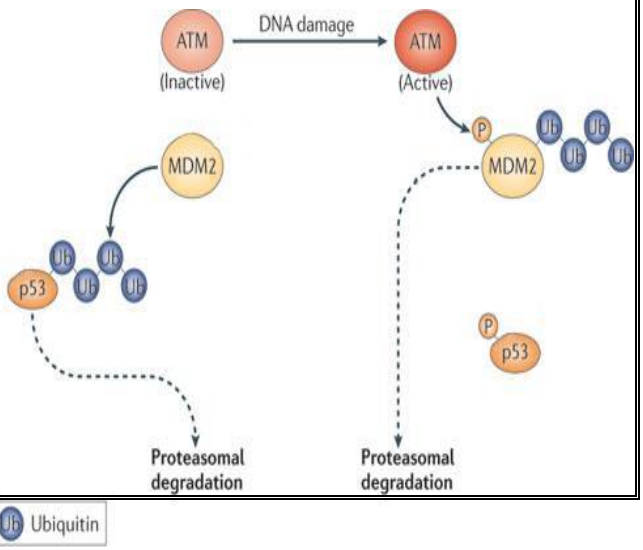
It is related to cervical cancer. There is a vaccine against it for young women to protect them from HPV.
There are many types of HPV. Type **1,2,4 and 7** generally induce warts. Warts are skin lesions, aka neoplastic papillomas. So these are benign squamous papillomas. They have low malignant transformation capabilities, whether they are skin or genital warts.

Genital warts are induced from types 6 and 11.

The high risky types of HPV are of types 16 and 18. These induce squamous cell carcinoma in the cervix and agential regions. The viral proteins responsible for this transformation are known as E7 and E6.
Go back a bit
E7 also has an effect in retinoblastomas.
E7 can inhibit retinoblastoma protein in it's hypophosphorylated form and preventing it from attaching to and sequestering the E2F transcription factors. The E2F transcription factor among other things could increase the production of cyclin E, which is required for G1-S transition. So by inhibiting retinoblastoma it can induce an advance in the cell cycle.
It also inhibits cyclin dependent kinase inhibitors p21 and p27 (and inhibition of an inhibitor = activation)

The difference between these types (the ones that induce benign paplilomas compared to the ones that can induce squamous cell carcinomas of the cervix) is the affinity of these E6 and E7 proteins for their targets. So in types 16 and 18, the E7 protein has a much higher affinity towards retinoblastoma than the types that do not induce cervical squamous cell carcinoma .

The E6 binds to and mediates the ubiquitilation and proteosomal degradation of p53. So this one virus and



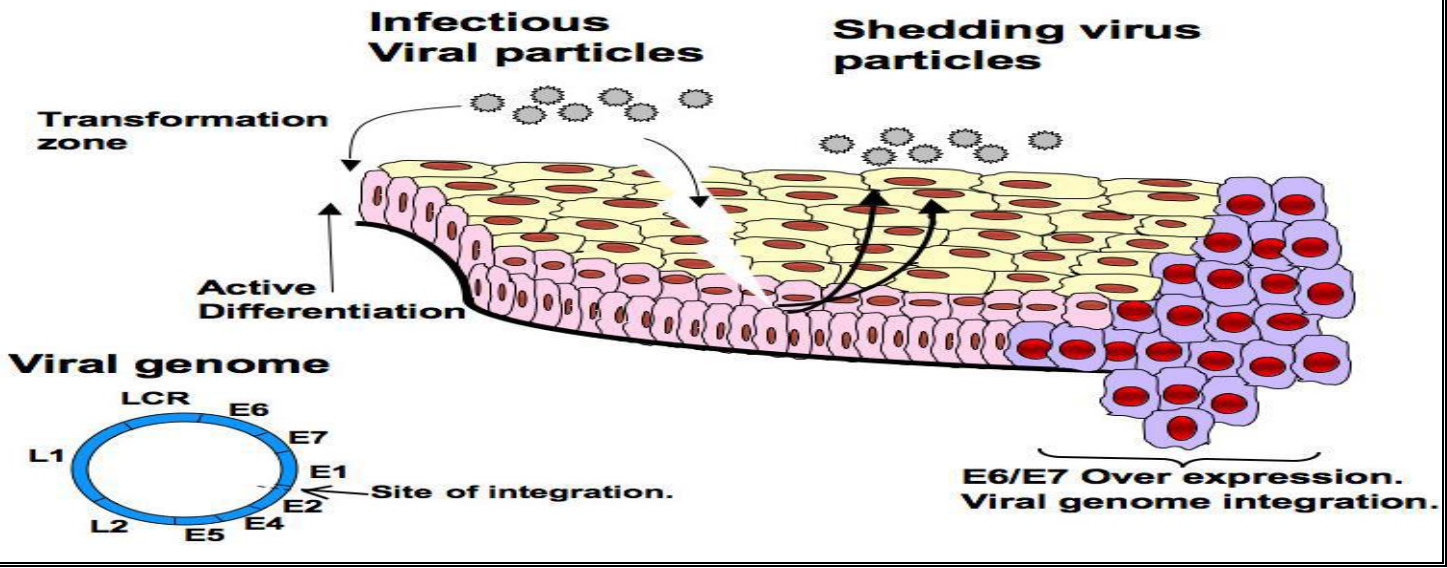
Written by

these 2 protein products turn off RB G1-s check point AND induce p53 destruction. In one blow it has progressed the cell cycle and it has prevented senescence. It has also prevented apoptosis (remember p53 is important in apoptosis), and p53 is also important in cell cycle arrest
p53 is also important for removing mutations so if there is no p53 more mutations will accumulate with no appropriate repair for them and this is called mutational phenotype .

Episomes vs viral integration. HPV typically have a circular genome made into an episome. In this particular case, when the viral genome is an episome, it produces certain proteins that actually inhibit the production of E6 and E7. When the HPV is integrated with the host DNA that particular locus that produces the protein that inhibits E6 and E7 production is split in half. The product of which is no longer able to inhibit the production of E6 and E7. Therefore, the expression of E6 and E7 will increase, ultimately inducing the transformation.

This is important because sometimes the virus just wants to replicate itself (maintain itself), so rather than inducing the transformation and potentially inducing mutations that can kill the cell (and kill the virus with it) all it wants is to produce more episomes and more viral products like proteins, in order to induce infectivity and numbers of the virus so it can transmit elsewhere. Viruses that DO integrate will generally have these effect that we mentioned (E6 and E7 protein production on cells)

E6 and E7 in HPV are not sufficient on their own to induce cancer. There are other environmental factors typically RAS mutations that are required for



cervical cancer to occur in response to HPV infection.

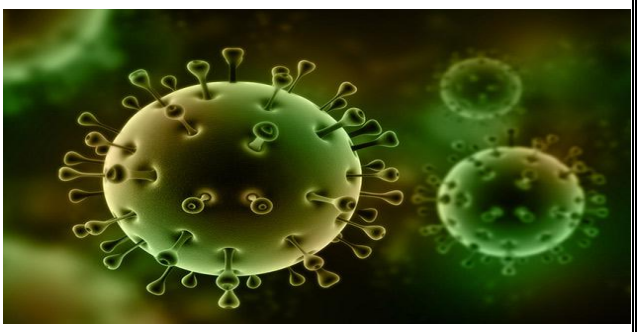
Necessity vs Sufficiency:

Necessity means that it is necessary and without it nothing will happen. For example, Oxygen is necessary for human life, but oxygen alone is not sufficient, i.e you need more than just oxygen to survive like FOOD :D

Sufficiency: If you pour freezing water on your friend's face, that is sufficient to wake him up, but it is not necessary to wake him up, there are other more merciful ways.

So when you say something is necessary AND sufficient it means that without it nothing can occur, but it alone is enough to induce the end result.

Going back, HTVL-1 is necessary AND sufficient for that transformation to occur because TAX induces both autocrine and paracrine mechanisms to induce cancer transformation. HPV however, is not. It is not sufficient, and it is not necessary. Cervical cancers can occur without HPV, and HPV alone is not enough to induce cervical cancer.



EBV: Epstein–Barr virus

EBV cause **Burkett's lymphoma**. In young people, infection with EBV is typically asymptomatic, nothing happens. EBV infections in adults however, especially adolescents, will lead to infectious mononucleosis. This "kissing" disease is commonly called "mono". It is transmitted by salivary exchanges. In areas that are endemic for EBV, there are usually no symptoms, but the virus can stay latent in a person. In countries where EBV isn't endemic, and typically where there's a high concentration of people (like in high schools and colleges), it will cause infectious mononucleosis .

Burkett lymphoma can also occur by a translocation between chromosome 8 and 14 which leads to over-expression of MYC proto-oncogene and cancer will occur as we mentioned before ,here EBV is also associated with Burkett lymphoma

EBV also induces (in addition to BL) a wide range of B and T killer cell lymphomas, carcinomas, and sarcomas. It is endemic in regions like Africa, and

typically, the African infection results in BL.

EBV is also endemic in south east Asia, and these usually result in nasopharyngeal carcinoma.

How?

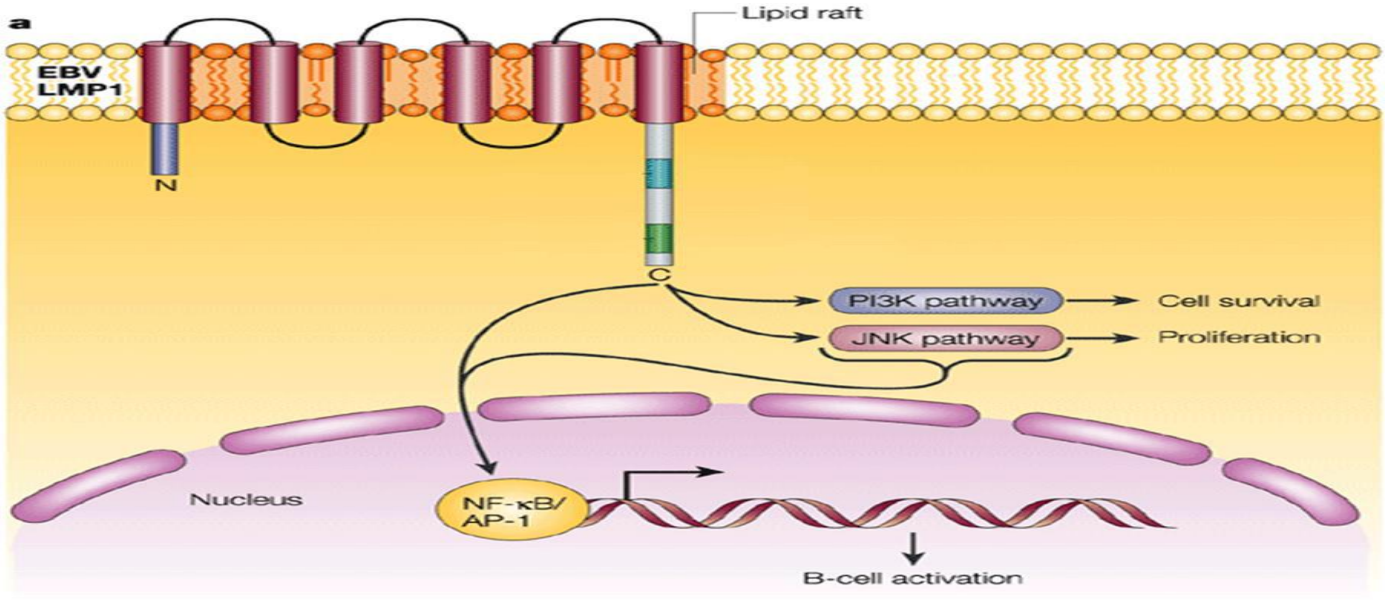
The virus gains access to the cell and either is maintained as an episome and become latent, or it turns certain genes that allow for the replication of the virus inside the cell to increase its number and produce a lytic cycle .

It specifically targets B-cells through CD21 (a chemokine receptor). It fuses with its memebrane and translocates to the nucleus where it can become latent or an active infection.

Epithelial cells can also be infected by EBV and typically through integrins. (The book describes this point as unknown) . It can become latent or lytic by this way. Usually in epithelial cells it is a lytic location. Epithelial cells are used as a stepping stone to get to the B cells (it uses the epithelial cells to replicate inside and increase the number of the viral copies and then move to the B cells)

How does this virus induce cancerous transformation?

It produces an oncogene called LMP-1 (latent membrane protein-1) that mimics CD40 which is TNF receptor this LMP-1 activates signaling pathways such as Nuclear factor Kappa B (Nf-kB) and JAK/STAT which cause B-cell proliferation and cancer .



EBNA-2 is another viral protein that increases the production of cyclin D and protooncogene SRC-proto-oncogene ,but the main oncogene protein is the one that mimics the CD40 receptor which is the LMP-1. The issue with LMP1 is that it is the protein that is recognized by your immune system. So if you look at the primary infection, whether it's directly affecting the B cells or the oralpharaynx if you can recall the epithelial cells, you're lysing the cells. It's a lytic infection. The virus wants to replicate more and get access to your lymph system, and from which to your B cells. One of 2 things could happen: Either it becomes a lytic infection, and this means that it's going to produce the LMP1 protein. This LMP1 protein is recognized by natural cytotoxic killer T cells. This is why, in the primary infection in **IMMUNOCOMPETENT** hosts, the viral infection is kept under control. Then, if cells do not express LMP1, It means that they can escape the immune function. They are not detected by the immune system and they typically remain latent. But LMP1 is required for some of the effects of transformation. So there's a balance between being detected and surviving (natural selection at play) .

In I**MMUNOCOMPROMISED** patients, there is no need for down regulation of LMP1 because these patients cannot recognize LMP1 aslan Because of their weak immunity. If you look at the tumors in immunocompromised patients, you'll see that LMP1 is being expressed in these tumors. No natural selection issue here.

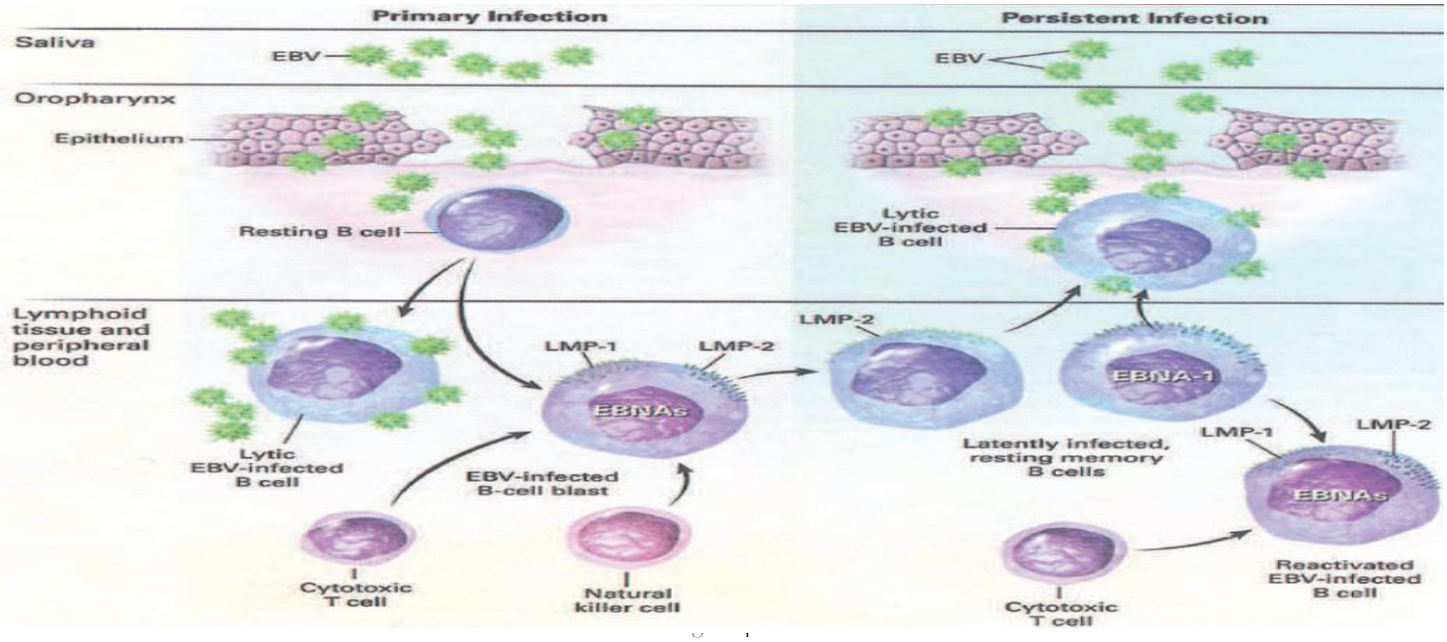
For example, in Africa, where EBV is endemic, there is also a concomitant malarial infection. The same areas that are endemic for EBV are also endemic for malaria. Malarial infections affect your cellular immunity. They affect your immune system's ability to recognize LMP1 and these patients usually end up with BL . Focus here in immunocompromised patients there is an over-expression of LMP-1 and Burkitt lymphoma will arise because of the single transduction pathways that LMP-1 activates and there is no efficient immunity system to detect the LMP-1 expressing B-cell and kill them so they will proliferate easily

But in immunocompetent patients there is an active immunity system that detects the LMP-1 ,because LMP-1 is presented there will be proliferation(not at

high rate) but it will be LIMITED because of the activity of the immunity system that detects LMP-1 and kill some of the B-cells , now because there is some proliferation the B-cell is prone to have mutations by translocation which is 8;14 translocation and Burkitt Lymphoma will arise so you can get infected by HBV virus and no LMP-1 will be found on the B-cell because if there is 8;14 translocation there is no need for LMP-1 to be present , So that's why you will see in patients in Africa even though they got infected by EBV virus they still have 8;14 translocation .

Conclusion : LMP-1 in immune-compromised host is frequently over-expressed because there is no immunity system to down-regulate it but in immune-competent host there is immunity LMP-1 will not be over-expressed but even though in these immune-competent hosts will have Burkitt lymphoma but it's not due to LMP-1 but it's due to increase proliferation which allow 8;14 translocation (we repeated this point a lot because the doctor said it's important)
EBV virus can produce viral Interleukin-10 which inhibits T-cell activation through the Monocyte/Macrophage pathway so in addition to the ability to escape the immunity it can also suppress the immunity as well .

The ability of EBV virus to produce IL-10 is called pirated evolution it means that when the virus became latent it integrated its genetic material with the DNA of the B-cell and we the viral DNA got De-integrated it took along with it the gene that codes for Interleukin-10 production that was found in the DNA of the B-cell so the virus ability to produce IL-10 was actually obtained from YOU .



Quick revision about the viral proteins produced from EBV :

- LMP-1 : Activates B-cell proliferation by activating NF-kB and JACK/STAT signaling pathways
- EBNA2 (Episten Barr nuclear antigen -2) :activates Cycline D and src proto-oncogene family
- Viral Interleukin 10 : its pirated from YOU.

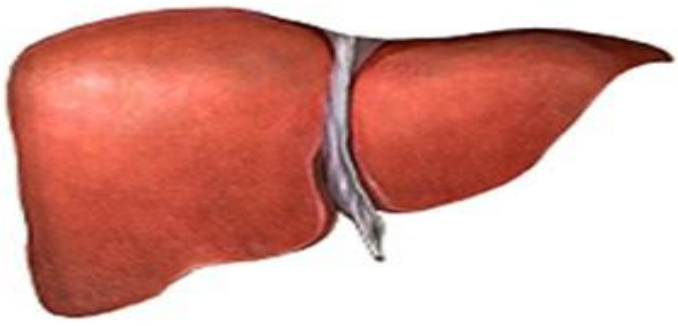
HBV AND HCV: Hepatitis B and C viruses.

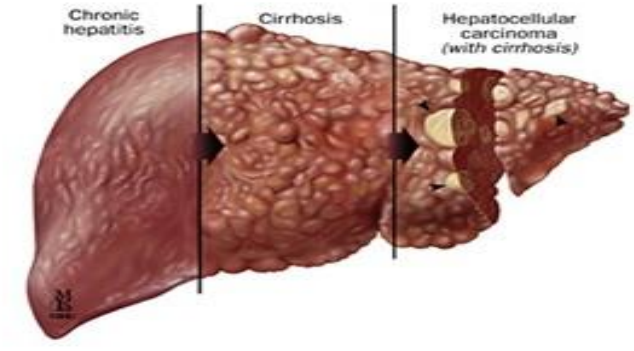
Hepatitis B and C viruses induce injury. They induce inflammation. The liver is a regenerative organ. It is a stable tissue; its cells that are normally quiescent can be induced to go into the cell cycle and replicate. If they are proliferating in the presence of these injurious stimuli (of inflammation, ROS, etc) they are at an increased risk of getting mutations. If they get mutations that select for increased proliferation, inhibition of growth factors, angiogenesis, inhibition of immune system, etc etc this will all lead to cancer.

HBV and HCV can induce production of certain cytokines, IL's, etc. These affect cancer cells or cells that are going to transform. Cancer is an abnormality or an inflammation gone wrong .

In addition, alcohol essentially does the same thing. Necrosis then regenerations, mutagens of oxidative stress are also applied to alcohol and HBV and HCV and afltoxin of nuts in SE Asia all affect p53 and ultimately hepatocellular carcinoma.

Like EBV, some of the viral products like HBX (while not oncogenes themselves) can induce certain pathways like nfkb pathway, which is a transcription factothat can turn on genes responsible for proliferation, etc.





H. pylori.

A bacteria associated with gastric ulcers and duodenal ulcer .

What happens when you get H. pylori infection?

Most commonly, nothing happens, however sometimes ulcers may occurs (along with heartburn and epigastric pain)

In a very small amount of patients, (and this takes decades to happen), H. pylori can induce chronic gastritis followed by atrophy, then intestinal metaplasia, leading to dysplasia, which ends up in gastric carcinoma, or through chronic gastritis because we are inducing inflammation.

We can also produce MALT Lymphoma (mucosal associated lymph tissue lymphoma) . These lymphomas look very much like mucosal associated lymph tissues which is why they're called MALT lymphomas.

How does H. pylori do this?

It induces injury, which induces inflammation. This increases the amount of ROS and inflammatory cells coming in. These induce mutations in gastric mucosa itself (gastric adenocarcinoma) or mutations in lymphoid tissue that comes in because of this inflammation (and it induces MALT lymphoma).

Some strains of H. pylori are more carcinogenic that other. In the strains that are more carcinogenic than other, they have this pathogenicity island that produces a protein called CAGA (cytotoxic associated gene A). This particular protein can turn on the same pathway that was mentioned previously. It can mimic growth factor induction of cells.

So what do growth factors do?

They can induce proliferation and inappropriate continuous growth factor production, thus inducing continuous proliferation.

So now we have inflammation and ROS because of this chronic inflammation which has been induced by this bacteria. Induction of whatever cells that are nearby to inappropriately proliferate occurs, whether they are lymph cells or epithelial cells of the gastric mucosa. So proliferations with a carcinogen =cancer

You're inducing chronic inflammations, which induces mutations, and proliferation of these mutated cells will accelerate the mutations.

Despite all that, it still takes decades to get any cancers. H. Pylori by itself is not an invasive organism, but CAGA can be injected into cells to induce this growth factor, mimicking the signaling pathway.

Review test

1-A pap smear reveals the presence of sever cervical dysplasia in a 35-year old female .Which of the following viruses binds to phosphorylated retinoblastoma protein to increase that risk of lesion :

A- EBV
B- HBV
C- HIV
D- HPV

2-An immunoperoxidase stain for the protease Cathepsin D is performed on the microscopic tissue section from a breast carcinoma in a 61-year old female .There is a noticeable cytoplasmic staining of that stain on the tumor cells The presence of this marker is most likely to predict tumor :

A- Angiogenesis
B- Invasiveness
C- Differentiation
D- Aneuploidy

3-Which of the following principles of carcinogenesis is best illustrated by the study of humans with hereditary nonpolyposis colon cancer (HNPCC) ?

A- Tumor initiators are mutagenic
B- Tumor promoters induce proliferation
C- Carcinogenesis is a multi-step process
D- Inability to repair the DNA predisposes to the development of cancer

4- An 18-year old boy has had multiple basal cell and squamous cell carcinoma of his sun exposed skin on the trunk and extremities . He also has a sister who is similarly affected .This disease is most likely to be the result of defective genes that :

A- Control apoptosis
B- Inhibit the cell cycle
C- Regulate repair of damaged DNA
D- Regulate secretion of growth factors

5-The best example of Viral oncogenesis in human is seen with which of the following neoplasms :

A- Retinoblastoma
B- T-cell leukemia
C- Prostatic Adenocarcinoma
D- Hepatic angiosarcoma

6-pre-malignant conditions include all the following EXCEPT :

A- Endometrial hyperplasia following prolonged estrogen therapy
B- Chronic alcoholism leading to micronodular cirrhosis of the liver
C- Chronic ulcerative colitis
D- Multiple leiomyomas of the uterine endometrium

7-A sequence of epithelial metaplasia followed by dysplasia followed by carcinoma in-situ would be the characteristics for :

A- Chronic gastric ulceration by H-pylori
B- Retroviral infection of T-lymphocyte
C- Influenza virus infection in the lung
D- EBV infection in the B-lymphocyte

1	2	3	4	5	6	7
D	B	D	C	B	D	A

And that concludes this sheet. There's only one sheet left, so shid 7eelak/ik!!!
Im sorry (..) ,for any mistakes you might find within this
But you most definitely won't since Ali Khresat corrected it.

لما تكتب شيت و فاقد الامل فيها
و يرن عليك بنص الليل يتطمن عليك و عليها
بتجيك جرعة امل و صعقة حماس
و بتكمل الشيت مليانة اغلاط من ساسها للراس
مين بساعدك و بنقذك من الدفعة وفضيحتها؟
ما الك غير علي خريسات يصلحها
والله اعطيتياها و حالتها بالويل
و طلعها ابيض من القمر بالليل
و حطلك هل ريفيو تست تتسلوا عليها
و عارف نص الدفعة بتحكي "خلصنا!" بس توصلها
صحيح هاد تسحيج من النوع القوي
بس والله بتستاهل كل خير و شكر يا علي

One last thing, a7la dedications la Abu Zghool (shayef muhannad :P), Rakan Radi, w abu Smadi.
And best of luck, yallah hanat :D
Hasan Hammo.