

Introduction

- Good evening everyone, I am Dr. Patricia Berg and I will be talking about multi-step tumorigenesis.
- I thank Dr. Wenge Zhu who has organized this course, Dr. Rong Li, Hafsah Mughal my wonderful grad student whose help was invaluable. My home office technical assistant Anjela Bachman, IT guru Jay Wind for loading the graphics, my husband Bob Weiner, over all graduate student program director Dr. Jack Vanderhoek for very helpful guidance, and his teaching assistant Juntian Wei for tonight's course webex direction.
- In this lecture, I will summarize the topic of multi-step tumorigenesis and how it is related to other parts of our course.
- Multi-step tumorigenesis is a model or hypothesis of how normal cells undergo genetic mutations and epigenetic alterations which will progress into the formation of a tumor.
- The steps of tumorigenesis include initiation, promotion, and progression - which will be discussed in detail.
- Additionally, I will discuss the tumor microenvironment - the environment in which a tumor is able to grow as well as the hallmarks of cancer.

Understanding each of these topics of them can help assess treatments for cancer.

Tumorigenesis and BP1

One reason I am very excited to give today's lecture on multistep tumorigenesis-- the development of tumors—is that a gene we discovered in our laboratory, BP1, is activated more and more the more developed breast and other cancers are. In fact, for the most severe and most developed breast cancer, we found all – 100% have activated BP1.

Announcing BP1's involvement in breast, prostate, and other cancers and its discovery by our lab was a big thing in my life and work. GWU hosted a news conference for the announcement. We had 16 network TV cameras, live interviews on CNN, ABC, NBC, and Fox, and coverage all over the national media—New York Times, Washington Post, Los Angeles Times, Associate Press, Reuters, and lots more. At a follow-on fundraiser, GWU leaders, my department fellow faculty, Department Chairman (so instrumental in helping us) Alan Goldstein, and national leaders came and spoke--- including Hillary Clinton, House Majority Leader Steny Hoyer, Congressional Black Caucus co-founder John Conyers, Maryland State Senate President Mike Miller, and many more. It was bipartisan—Republican House leader Newt Gingrich told me he supported our work. I will never forget this friendship and support by everyone.

So I want to discuss Tumor Oncogenes and Suppressors Regulated by BP1

Breast cancer is the second leading cause of death in women and in 2018 it was estimated that 30% of newly diagnosed cancers in women will be breast cancers. In our laboratory, the BP1 (Beta protein 1) novel homeobox gene is being studied as a potential biomarker for breast cancer prognosis. BP1 acts as a transcription factor which turns other genes on and off, making it critical towards understanding tumor development. It belongs to a homeobox gene family which includes regulatory genes involved in the early development and cell differentiation that is often dysregulated in cancer. Our studies have shown that BP1 expression was significantly higher in estrogen receptor (ER) negative (100%) than in ER positive breast tumors (73%), ($p = 0.03$). Additionally, it was found that BP1 is expressed in 89% of tumors of African American women and 57% of tumors of Caucasian women. BP1 levels increase with the progression of tumor development. The transformation of healthy cells to abnormal cells requires the activation and inhibition of a very tightly regulated system of genes called tumor suppressors and oncogenes. Transcription factors will regulate many of these processes. Knowing that BP1 acts as a transcription factor, it has been shown to have regulatory effects on tumor oncogenes and suppressors. These include C-MYC, BCL-2, VEGF, and BRCA1.

c-Myc

This is a regulator gene that codes for a transcription factor and plays a role in cell cycle progression, apoptosis, and differentiation. BP1 blocks TGF-beta-mediated repression of c-Myc transcription and also induces c-Myc levels independently of TGF-beta/Smad signaling. It inhibits TGF-beta-mediated induction by blocking the TGF-B/Smad signaling pathway in both normal and malignant epithelial cells.²⁸

Bcl-2

This gene is an oncogene and regulates apoptosis by preventing the expression of antiapoptotic genes or by the expression of genes that promote apoptosis. BP1 binds to the regulatory region of the Bcl-2 gene, which results in elevated expression of Bcl-2 and inhibits apoptosis in MCF-7 breast cancer cells challenged with TNF-alpha. Increased BP1 is associated with decreased processing and activation of caspase-7, caspase-8, and caspase-9. High BP1 expression can lead to decreased cell death and increased proliferation.

Fu, S.W., Schwartz, A., Stevenson, H. et al. Correlation of expression of BP1, a homeobox gene, with estrogen receptor status in breast cancer. *Breast Cancer Res* 5, R82 (2003). <https://doi.org/10.1186/bcr602> 32 Lou, Y., Fallah, Y., Yamane, K., & Berg, P. E. (2018). BP1, a potential biomarker for breast cancer prognosis

From an Epidemiological Lens

- Cancer can develop at any age and in any tissue in the body; the clinical presentation of these cells is variable, however all cancers share a common principle - a gradual acquisition of errors in genes.
- In the big picture, the development of cancer is a function of genetic inheritance, environment, diet, and age in both humans and animals.
- Epidemiologic studies have shown that age is a large factor in the incidence of cancer. In fact, the risk of dying from colon cancer is almost 1000 times greater in a 70 year old man than a 10 year boy.
- Steps in multi-step tumor progression proceed faster at later stages because cells in the later growth have acquired multiple oncogenic mutations, have learned to proliferate faster, decrease the timing of clonal expansion, and genome cells are more likely to become mutable.
- Epidemiologists have found that there is a strong age dependence across all cancers.
- Tumorigenesis is a multi-step process in which a sequence of genetic mutation and epigenetic alteration events are required for a tumor to appear.
- The formation of a tumor is a complex process that usually proceeds over a period of decades. Normal cells evolve into cells with increasingly neoplastic phenotypes through a process termed tumor progression. This process takes place at myriad sites throughout the normal human body, advancing further and further as we get older. Rarely does it proceed far enough at any single site to make us aware of its end product, a clinically detectable tumor mass.
- As we grow older, virtually all of us will accumulate cells in many locations throughout our bodies that have completed some, but not all the steps of tumor progression.
- Just by examining the frequencies of mesothelioma in humans, epidemiologists have found that the formation of tumors requires an extended period of repeated exposure to carcinogens.
- The time period of these exposures is what determines the timing of the onset of cancer. In these cases, tumors are created by the actions of exogenous carcinogens rather than occurring spontaneously within the body; these carcinogens increase the rate of tumor progression.
- Tumor progression is driven by a sequence of randomly occurring mutations and epigenetic alterations of DNA that affect the genes controlling cell proliferation, survival, and other traits associated with the malignant cell phenotype. The complexity of this process reflects the work of evolution.
- The cell cycle and other cellular mechanisms create a series of barriers between normal cells and their highly neoplastic derivatives.
- Since most of us will not live long enough for the full schedule of requisite events to be completed we will never realize that any of these tumor progressions had been initiated in our bodies.

Histopathology

- The cells of the human body are very small and not visible to the naked eye - making it difficult to comprehend the total number of cells in the human body. The current accurate estimate is 30 trillion cells in the body.
- As cells age, they are continuously replaced within the average human lifespan.
- All of these cells are derived from preexisting cells which are tightly regulated by the cell cycle and control mechanisms.
- Loss of cell cycle control leads to uninhibited cell division and cancer development.
- The theory that human tumor development is a multi-step process has been histologically documented most clearly in the epithelia of the intestine.
- In fact, the altered histopathology of premalignant and malignant growths is largely a reflection of changes that have occurred in a group of cells forming each of these masses. In the case of carcinomas, the progressive alteration of epithelial cells is accepted to drive the process of tumor progression and the associated changes in histopathology.
- The other cell types in these tumor masses, specifically those in the stroma, are normal cells that have been recruited to the tumor mass and recruited by abnormal epithelial cells to help in the process of tumor formation.¹³

Cancer is a Genetic Disease

- The process of tumorigenesis involves the alterations of four categories of cancer genes where oncogenes are activated, tumor suppressors are inactivated, the evasion of apoptosis genes, and defective DNA repair genes.
- Loss of cellular regulation can give rise to most cases of cancer due to genetic damage.
- During multi-step tumorigenesis, each neoplasm will accumulate at least 80 genetic alterations in its cancer genes, many of which will be ‘driver’ mutations causing uncontrolled growth.
- Mutations in two broad classes of genes have been implicated in the onset of cancer: proto-oncogenes and tumor suppressor genes.
- These proto-oncogenes are activated by mutations to become oncogenes, which will cause the gene to be excessively active in growth promotion, as a result of either increased gene expression or production of a hyperactive product.
- Tumor-suppressor genes normally restrain growth, so damage to them allows uncontrolled growth.
- Many of the genes in both classes encode proteins that help regulate cell birth or cell death by apoptosis; others encode proteins that participate in repairing damaged DNA.⁸
- Cancer commonly results from mutations that arise during a lifetime’s exposure to carcinogens, which include certain chemicals and ultraviolet radiation.
- Cancer-causing mutations occur mostly in somatic cells, not in the germ-line cells.

- These somatic cell mutations are not passed on to the next generation.
- Moreover, certain inherited mutations are able to be carried in the germ line, increasing the probability that cancer will occur at some time.
- Somatic mutations can combine with inherited mutations to cause cancer.
- Carcinogens can activate cellular oncogenes (proto-oncogenes) by a variety of mechanisms including base substitution (point) mutations, chromosomal translocations, and gene amplification.¹

Genetic Mutations and Epigenetic Alterations

- In normal cells, cell growth and proliferation is tightly regulated.
- Normal cells become tumorigenic after multiple genetic and epigenetic alterations.
- Cells accumulate genetic and epigenetic alterations as tumor progression proceeds and this process alters complex signaling networks within cells as well as interactions between cells and the extracellular matrix.
- Cancer develops when a cell acquires specific growth advantages through the stepwise accumulation of heritable changes in gene function.
- This process is directed by changes in two different classes of genes:
 - Tumor suppressor genes which inhibit cell growth and survival
 - Oncogenes which promote cell growth and survival.
- Since several alterations are usually required for a cancer to fully develop, the malignant phenotype is determined by the compound status of tumor suppressor genes and oncogenes.
- Cancer genes may be changed by several mechanisms, which potentially alter the protein encoding nucleotide template, change the copy number of genes, or lead to increased gene transcription.
- In the cell cycle, checkpoints allow for DNA repair before further progression into the cycle.
- The components of checkpoint control act as “brakes” the cycle when overcome by stress or damage.
- Overriding the cell cycle checkpoints with agents such as methylxanthine analogs or pentoxifylline increases the cytotoxicity of DNA-damaging agents.
- The importance of DNA damage in triggering a cell cycle shutdown is obvious.
- Replication of a damaged template would result in irreversible chromosomal aberrations and a high mutation rate.
- Two major checkpoints following DNA damage have been established at the middle to end of G1 (prior to DNA replication) and G2 (prior to chromosome segregation).⁸

Hallmarks of Cancer

- Cancer researchers Robert Weinberg and Douglas Hanahan first published the *Hallmarks of Cancer* in 2000. Which has been the most cited cell article of all time in the journal *Cell* (Cancer Network 2011).
- In 2000, the hallmarks of cancer included six principles: sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, and resisting cell death.
- More than ten years later, in 2011, the *Hallmarks of Cancer* was updated by Hanahan and Weinberg in which they added enabling characteristics and emerging hallmarks. These include deregulating cellular energetics, avoiding immune destruction, tumor-promoting inflammation and genome instability and mutation.
- The hallmarks of carcinogenesis include genetic alterations involved in⁵:
 1. Sustaining proliferative signaling
 2. Evading growth suppressors
 3. Resisting cell death
 4. Enabling replicative immortality
 5. Inducing angiogenesis
 6. Activating invasion and metastasis
 7. Reprogramming energy metabolism
 8. Evading immune destruction

Multi-step Tumorigenesis

- Long term exposure to carcinogens can lead to the development of cancer.
- The later steps in multi-step tumor progression most likely require less time to complete than earlier steps because cells in later growth have acquired multiple oncogenic mutations, have learned to proliferate faster, decreased the time of each clonal expansion, and genomes of cells have become more mutable.
- Tumor growths can arise in any part of our body and the body has a system of checkpoints in each of our organs that keeps it in homeostasis.
- When this equilibrium mechanism is broken, an uncontrolled growth in our body can develop.
- These growths can be benign, but some may become malignant, or cancerous.
- Tumors can grow in solid tissues such as organs, joints, or bones.
- In some cases, a tumor can be felt and other times are only detectable with imaging tests such as an MRI, CT scan, PET scan, endoscopy, or ultrasound.

- A biopsy is often needed so that it can be evaluated under a microscope to determine if it is a benign, precancerous, or malignant tumor.

Initiation

- Carcinogens can induce damage in tumor suppressors or oncogenes in ways which contribute to the transformation of normal cells into tumor cells, known as tumor initiation.
- Additionally, chemical carcinogens are also capable of promoting the outgrowth of those transformed cell clones, causing the generation of visible tumour cell masses known as tumor promotion.⁷
- An initiator is an agent that triggers the first step in multi-step tumorigenesis. Likewise, the promoter is an agent that furthers the progression of multi-step tumorigenesis by non-genetic mechanisms, mainly those involving inflammation and mitogenesis.
- Promoting agents may cause gene repression and derepression in cells.
- These include agents such as drugs, plant products, and hormones, which do not directly interact with host cellular DNA but will influence expression of genetic information.⁷

Promotion

- Promotion is considered that portion of the multistep tumorigenic process where specific agents, known as promoters.
- These will enhance the development of neoplasms from a background of initiated cells.
- Promotion of carcinogenesis in an initiated stem cell will acquire the ability to override all growth-regulatory processes.
- Cancer derived by such a mechanism would be expected to show a well-differentiated, slowly growing phenotype.
- The promotion of multi-step tumorigenesis results when such initiated stem cells develop irreversible autoregulatory control mechanisms that cause abnormal cellular proliferation in differentiation-defective stem cells.⁸

Progression

- The development of a cancer requires a gradual accumulation of mutations in a number of different genes which explains the phenomenon of tumor progression.
- The process of progression involves the initiated cell to expand clonally into a detectable cell mass that can be either benign or preneoplastic.⁸

- An initial mild disorder of cells will evolve gradually into a full-blown cancer. An example of this can be understood from chronic myelogenous leukemia.
- This disorder is characterized by a nonlethal overproduction of white blood cells and continues in this form for several years before changing into a much more rapidly progressing illness that usually ends in death within a few months.
- In the early chronic phase, the leukemic cells are distinguished mainly by the chromosomal translocation.
- In the subsequent acute phase, cells that show not only the translocation but also several other chromosomal abnormalities which overwhelm the hematopoietic system.
- It appears that cells from the initial mutant clone have undergone further mutations that make them proliferate even more vigorously and outnumber both the normal blood cells and their ancestors with the primary chromosomal translocation.⁸

Tumor Progression in Not Linear Path

- The general understanding of clonal succession proposed until now suggests that all of the cells within a tumor mass that participate in a particular clonal expansion are genetically identical to one another and that tumor formation occurs as a consequence of a linear series of these clonal successions.
- Based on this, if we were to examine the cells within a premalignant or malignant cell mass, we would almost always find that a single, genetically homogeneous clone of cells dominates in this mass, since it would have outgrown and largely displaced the preceding cell clone from which it arose.¹
- The actual course of tumor progression is complex. One factor that we must take into account is that as tumor progression advances, tumor genomes often become increasingly unstable.
- The rate at which mutations are acquired during each cell generation becomes exponential.
- As a consequence, rather than looking like a linear series of clonal successions, actual tumor progression in many tumor masses is likely to resemble the highly branched scheme, in which a number of genetically distinct subclones of cells coexist within a single tumor mass.
- Genetic diversification of cells within tumor masses can be obtained by tracking the state of individual genes of interest within various cells of a primary tumor.
- For example, in a human pancreatic carcinoma, detailed genome sequence analysis of different sectors of the tumor revealed genetically distinct subclones, each of which was estimated to comprise at least 100 million cells.

- The localization of individual subclones and associated cells within a tumor is itself unclear.
- Computer-based modeling can give insight on how such subclones arise and are distinguished from one another by heritable differences in DNA sequence or CpG methylation.
- Analysis of individual carcinoma cells within a tumor may reveal this is more complex than imingaine, where the cells of various subclones in fact become intermingled.¹

Tumor Microenvironment

- The cancerous cells in a tumor carry dangerous mutations and have an abnormal structure in comparison to other cells in a tumor.
- The development of a tumor relies on a two-way communication between the tumor cells and the tumor stroma.
- This development is similar to how the normal development of epithelial organs relies on communication between epithelial cells and mesenchymal cells.¹
- The stroma provides a framework for the tumor.
- It is composed of normal connective tissue containing fibroblasts, inflammatory white blood cells, and endothelial cells that form blood and lymphatic vessels with nearby pericytes and smooth muscle cells.
- As a carcinoma progresses, the cancer cells induce changes within the stroma by secreting signal proteins that alter the behavior of the stromal cells, as well as proteolytic enzymes that modify the extracellular matrix.
- The stromal cells in turn act back on the tumor cells, secreting signal proteins that stimulate cancer cell growth and division as well as proteases that further remodel the extracellular matrix.
- As such, the tumor and stroma will evolve together, invade, and the tumor will become dependent on the stromal cells.¹

Angiogenesis

- Angiogenesis has an important role in tumor formation.
- In angiogenesis new blood vessels form which allow the delivery of oxygen and nutrients to the body's tissues.
- In early phases of angiogenesis, the tumor resides in a dormant state, here the rate of cell death counterbalances cell proliferation. Part of this is due to hypoxia and the insufficient available nutrients in the microenvironment.
- Then the tumor activates an angiogenic switch, which is irreversible, and begins to recruit new capillaries which will bring in oxygen and nutrients to both angiogenic cells and surrounding non-angiogenesis cells. This leads to an exponential tumor growth.

- During tumorigenesis, the balance between pro-angiogenic and anti-angiogenic molecules and autocrine and paracrine growth factor stimulation is lost.
- The main mechanism, known as endothelial sprouting, depends on vascular endothelial growth factor upregulation and the development of functional interactions between endothelial cells, pericytes, stromal cells, and the associated extracellular membrane.²
- The "angiogenic switch" is a time-restricted event during tumor progression where the balance between pro- and anti-angiogenic factors tilts towards a pro-angiogenic outcome. The result is a transition from dormant avascularized hyperplasia to an outgrowth of a vascularized tumor and eventually to malignant tumor progression. The molecular mechanism underlying the angiogenic switch has been intensely studied. A large number of pro-angiogenic factors and angiogenic inhibitors activated and repressed in their activities during the angiogenic switch have been identified and characterized.²

Inflammation and Cancer

- The symptoms of inflammation are heat (calor), pain (dolor), redness (rubor), and swelling (tumor).
- Many cancers arise from sites of chronic infection, irritation, and inflammation.
- The tumor microenvironment contains these inflammatory cells and has a large role in tumorigenesis - causing proliferation, survival and migration of cancerous cells.¹⁰
- A link between chronic inflammation and cancer has been suspected for a long time on the basis of epidemiological data such as the observation that chronic inflammation often increases cancer risk in inflamed tissues and long-term use of nonsteroidal anti-inflammatory drugs reduces the risk of several cancers.¹⁰
- Additionally, a variety of cell types are involved in inflammation such as macrophages and lymphocytes as well as cytokines that these cells produce.
- Deletion of certain inflammatory mediators, as seen in mouse studies, reduces cancer susceptibility in these animals.
- Key mediators for the link between inflammation and cancer are NF- κ B and TNF- α . In mouse models, treatment with an anti-TNF- α antibody, or suppression of NF- κ B by induction of the I κ B suppressor of NF- κ B, blocked progression to carcinoma.
- Suppression of NF- κ B function in young mice did not affect carcinoma development - meaning that the promotion–progression phases of malignant transformation are the ones enhanced by inflammation, not the initiation phase.
- In some human and mouse cancers, the malignant cells themselves, in addition to the inflammatory cell types, can produce the offending cytokines.¹⁰

Malignancy

- Carcinogenesis will lead to the formation of malignant tumors that invade and destroy adjacent normal tissue; benign tumors grow by expansion, are usually encapsulated, and do not invade surrounding tissue.
- Benign tumors may push aside normal tissue and may become life threatening if they press on nerves or blood vessels or if they secrete biologically active substances, such as hormones, that alter normal homeostatic mechanisms.¹⁰
- Benign tumors remain localized and do not metastasize and will usually resemble normal tissue more closely than malignant tumors.
- Malignant tumors metastasize through lymphatic channels or blood vessels to lymph nodes and other tissues in the body.
- Malignant tumor cells are less well differentiated (anaplastic) than normal cells of the tissue in which they arise.
- In some cases, malignant neoplastic cells will structurally and functionally resemble the normal tissue in which they arise.
- As the malignant neoplasm progresses it will invade the surrounding tissues, and metastasize.
- The malignant cells will have less resemblance to the normal cell of origin.
- The development of less well-differentiated normal cells in a population of differentiated normal cells is sometimes called dedifferentiation.¹⁰

Conclusion

- While the genetic basis of tumorigenesis may vary between cancer types, the steps, both cellular and molecular, required for metastasis are basically the same.
- In normal tissue, homeostasis is maintained between epithelial cells and their microenvironment, such as vascular endothelial cells, fibroblasts, immune cells, and the extracellular matrix.
- In the cancerous state, these interactions become deregulated.
- The pivotal role of cell proliferation in all phases (e.g., initiation, promotion, progression) of the multistep process of tumorigenesis is completely linked to positive and negative cell cycle control mechanisms as influenced by oncogenes, tumor suppressor genes, growth factors and their cognate receptors, hormones and their receptors, and the action of exogenous agents (e.g., chemicals and viruses) on cell cycle control.

- The **four main points** of emphasis in this lecture are,
 - what factors affect normal cells to become malignant
 - how genetic mutations and epigenetic alterations impact tumor formation

- the differences between initiation, promotion, and progression in tumorigenesis
- how the tumor microenvironment impacts tumor growth
- Now I will take any questions.

References

1. Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell*. New York: Garland Science, 2002. Print.
2. Baeriswyl, Vanessa, and Gerhard Christofori. "The angiogenic switch in carcinogenesis." *Seminars in cancer biology* vol. 19,5 (2009): 329-37. doi:10.1016/j.semcancer.2009.05.003
3. Gerdes, Michael J et al. "Emerging understanding of multiscale tumor heterogeneity." *Frontiers in oncology* vol. 4 366. 18 Dec. 2014, doi:10.3389/fonc.2014.00366
4. Grønbaek, Kirsten et al. "Epigenetic changes in cancer." *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica* vol. 115,10 (2007): 1039-59. doi:10.1111/j.1600-0463.2007.apm_636.xml.x
5. Hanahan, Douglas, and Robert A Weinberg. "Hallmarks of cancer: the next generation." *Cell* vol. 144,5 (2011): 646-74. doi:10.1016/j.cell.2011.02.013
6. Lodish, Harvey F. *Molecular Cell Biology*. 4th ed. New York: W.H. Freeman, 2000.
7. Luch, Andreas. "Nature and nurture - lessons from chemical carcinogenesis." *Nature reviews. Cancer* vol. 5,2 (2005): 113-25. doi:10.1038/nrc1546
8. Malarkey, DE; Hoenerhoff, M; Maronpot, RR. *Carcinogenesis: Mechanisms and Manifestations*. Waltham, MA, Haschek and Rousseaux's Handbook of Toxicologic Pathology Vol 1 (2013) :107-146. doi: 10.1016/B978-0-12-415759-0.00005-4
9. Mierke, Claudia Tanja. *Physics of Cancer*. IOP Publishing Ltd, 2015.
10. Ruddle, Raymond W. *Cancer Biology*. New York: Oxford University Press, 1995.
11. Siddiqui, Imtiaz A et al. "Resveratrol nanoformulation for cancer prevention and therapy." *Annals of the New York Academy of Sciences* vol. 1348,1 (2015): 20-31. doi:10.1111/nyas.12811
12. Scott, R E et al. "Mechanisms for the initiation and promotion of carcinogenesis: a review and a new concept." *Mayo Clinic proceedings* vol. 59,2 (1984): 107-17. doi:10.1016/s0025-6196(12)60244-4
13. Weinberg, Robert A. *The Biology of Cancer*. , 2014. Print.
14. Zhu X; Wetta H. *Genetics and Epigenetics in Tumorigenesis: Separately or Linked?*. Austin Publishing Group (2014)