



**Trip Report:
Keystone Symposia: Inflammation and Cancer
Breckenridge, Colorado
February 27 – March 3, 2005**

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***Abstract:** This was a Keystone Meeting focusing on the current developments in the understanding of the role of inflammation in the initiation and progression of cancer. Although an old view of cancer is that it is a wound that does not heal, it has only been in the last year or so that evidence of a molecular link between inflammation and cancer has been reported. So far, 15-18% of tumors have been demonstrated to have a bacterial cause and this number is going to increase as more tumors are looked at from this angle. Tumor formation is basically the end result of a breakdown of the immune system, in some cases it is even the root cause.*

The initial set of changes within a cell that lead to sustained growth and immortality can be brought about by a number of factors including exposure to carcinogens, genetic problems and also by the reactive oxygen species produced by the immune system. Cells that have undergone these changes are normally recognized by the innate immune response and targeted for destruction by the adaptive immune response. This process involves the release of immunoglobulins by B-cells, subsequent release of cytokines and chemokines, which attract killer cells like macrophages, and finally activation of T-cells. In tumors, this link between the innate and adaptive responses has been corrupted – the adaptive immune response is no longer active. What is left is very beneficial to the tumor. Recruitment of red blood cells with accompanying angiogenesis creates a new blood supply that brings in more nutrients and provides a pathway for tumor metastasis. The release of cytokines and chemokines sets up a feedback loop, as the cells that are attracted are those that release more cytokines and chemokines.

This conference highlighted many new approaches to the treatment of cancer: restoration of the adaptive immune system could result in the body's own immune system destroying the tumor, elimination of inflammation has the potential to halt the progression and metastasis of the tumor, and treatment with anti-infectives could remove the source of many tumors.

KEYNOTE ADDRESS: "Integrating Inflammation and Cancer Induce Neovascular Stroma by Common Mechanisms"

Harold F. Dvorak.

Dvorak was responsible for the discovery of vascular permeability factor, now known as vascular endothelial growth factor - VEGF

Normal tissue can be divided into two compartments: parenchyma (epithelial cells) and stroma (benign vasculature connective tissue). Stroma is needed for cell growth. Tumors are made up of a lot of stroma – breast, 80%, and colon 50%. All tumors have stroma. Stroma is not linked to malignancy, but it is not always benign (in vitro, carcinoma associated fibroblasts render SU-40 cells tumorigenic). Tumor stroma has increased levels of interstitial fluid and structural proteins (like tenascin and fibrin) and proteoglycans and glycosaminoglycans (increased and abnormal). It also contains abnormal blood vessels, new lymphatics, inflammatory cells, and mesenchymal cells. Tumor cells contain lots of fibrin but this maybe related to the selection of tumors for in vivo work that grow quickly. Fibrin is normally produced more slowly. The blood vessels in tumors lack plasminogen; they are hyperpermeable (leaky). The clotting system in the stroma is activated – F-VII, F-X etc. The clotting of fibrin forms a gel, which is an excellent surface for cell migration. If fibrin gel is injected into an animal, lots of angiogenesis occurs. VEGF-A is produced which increases microvessel permeability, causes endothelial cell migration and proliferation and protects endothelial cells from senescence and apoptosis. VEGF-A expression is regulated by cytokines, TGF- α , TGF- β , EGF, PDGF, b-FGF, NF- κ B etc.

When VEGF-A is delivered by virus (into immune compromised mice) very large, balloon like blood vessels called mother vessels form. The irregular shapes of these vessels causes turbulence and thrombosis. This is followed by basement membrane degradation, pericyte detachment, and vessel enlargement. These mother vessels sometimes become divided by membranes of endothelial cells, or joined together. They can be stabilized by smooth muscle cells and form arteries. They can also form bulbous vascular structures resembling glomeruloid bodies. The presence of these glomeruloid bodies correlates with poor prognosis for the patient. VEGF-A also induces the formation of abnormal lymphatics. The formation of mother vessels and all subsequent effects are also seen in wound healing, psoriasis, sinusitis and rheumatoid arthritis.

“Innate Immunity and Cancer,”

Alberto Mantovani.

Until recently, a common view of tumors was that, until they become malignant, they are simply tissue masses with a clear, defining line between cancerous tissue and normal tissue. As far as the local environment is concerned, they were viewed as silent. This changed recently and it is now recognized that tumors are not silent, they are screaming. The tissue surrounding the tumor is highly inflamed with the innate immunity response in full swing but the adaptive immunity not responding; a weakening of the adaptive immune response with age explains why cancer becomes

more frequent with advancing age. Tumors are very dynamic about remodeling the skin around them.

Examination of late stage tumors showed that the activation state of circulating monocytes (white blood cells that are precursors to macrophages) was responsible for determining tumor outcome. Monocytes can be activated either classically (M1) or alternatively (M2) and it is the balance between them that is important. In mouse models, M1 activation leads to tumor regression, M2 to tumor growth. STAT6 determines the M1 or M2 phenotype – remove STAT6 and tumors do not grow. M1 macrophages have high levels of IL-6, IL-12, and TNF- α ; they cause the TH-1 response that kills pathogens. M2 macrophages fall into three subclasses, M2a, M2b, and M2c, they all promote cancer. There are also tumor-associated macrophages (TAM) - these have much higher levels of IL-10 and CXCL10 than normal macrophages.

There is a frequent, but not universal correlation between high macrophage infiltration and poor prognosis. The expression of B-cell genes, e.g. TLR5, in infiltrating normal cells is an indicator of poor prognosis in follicular lymphoma; follicular lymphoma is a malignant lymphoma arising from follicular B-cells.

MCP-1 is a cytokine that attracts macrophages, they accumulate in hypoxic areas. Hypoxia upregulates CXCR4 in human monocytes via HIF-1 α . Activated macrophages lead to tissue remodeling, tumor survival and angiogenesis.

“The Role of Inflammatory Cytokines and Chemokines in Cancer Progression,”

Frances R. Balkwill.

TNF α and tumor promotion.

Autocrine TNF α is made by epithelial cells during the earliest stage of tumor production. In later stages, TNF α can be either produced autocrine or paracrine. High TNF α causes massive necrosis of tumor cells yet at low doses is important for tumor growth.

TNF α -/- mice are resistant to chemically induced skin carcinomas. TNF α -/- mice do produce chemokines but production is delayed. TNF α -/- mice do not release MMP9 chemokines at the right time for migration of activated epithelial cells. TNF α induces TNF α but only transiently in normal cells. In cancer cells, TNF α production is much stronger and prolonged. CXCL12 induces TNF α , as does IL2.

CXCR4 is the receptor for CXCL12. The ligand is found on the surface of epithelial cells in organs that the tumor cells metastasize to. Other tumor cell types produce different CXC-receptors and these home to CXC-ligands on other organs. The TNF α antibody Infliximab lowers CXCR4 – there is an autocrine loop between TNF α and CXCR4 increasing malignancy. Stromal TNF α has been implicated in tumor promotion in an inflammation-induced liver cancer model. It is detected in many human cancers associated with a poor prognosis.

Anti- TNF α antibody is useful in 70-80% of rheumatoid arthritis patients. Thalidomide has been shown to inhibit cancer, it lowers TNF α .

A clinical study of Infliximab in ovarian cancer has demonstrated a dramatic lowering of the percentage of AUA-1 tumor cells in ascites drains. 4/6 Patients showed down regulation of chemokines, cytokines, and their receptors, e.g. MMP2, 9, 14 and VEGFR. VEGF was unchanged. The results from a study on renal cancer showed that it did work but that results were slow. – 120-250 days. The antibody induces static state not regression so needs to be used in conjunction with other chemotherapeutic agents.

“Innate and Adaptive Immune Interactions Promote Cancer Development,”

Lisa M. Coussens.

Leukocyte infiltration correlates with enhanced angiogenesis and poor clinical outcome in mammary, renal and lung cancer.

Chronic inflammation by *H. pylori* is linked to gastric cancer and inflammatory bowel disease to colon cancer.

Aspirin and NSAIDS decrease cancer by blocking recruitment of mast cells. This works in very early cancers – hyperplasia and dysplasia but not in carcinoma. **If recruitment of mast cells is blocked, angiogenesis is not turned on and malignancy is prevented.** The presence of the protein - complement -is very important. Chronic inflammation is complement-independent, acute inflammation is complement-dependent. Animals that have a B-cell deficiency do not have an innate immune response and do not have mast cell migration. B-cells are lymphocytes; they produce antibodies and are transformed into plasma cells by T-helper cells. Plasma cells produce lots of antibodies. [Macrophages](#) attract T helper cells and [present](#) an [antigen](#) onto which the T helper cell binds. The T helper cell is then activated and produces and [secretes potent lymphokine hormones](#) that [stimulate B-cell production](#) of [antibodies](#), signals [natural killer](#) or [cytotoxic T-cells](#), and attracts more macrophages - i.e. a loop in which B-cells activate macrophages which recruit T-helper cells, which produce more B-cells.

RAG1 $-/-$ mice do not make B-cells. They essentially do not make MMP9 or proMMP9. They show no induction of VEGF. Tumors in these animals become 100% hyperplastic but 0% dysplastic. B-cells act indirectly through immunoglobulin (Ig) deposition. If adoptively transfer in B-cells, see rescue of tumor progression - get Ig deposition and infiltration of neutrocytes and mast cells, and angiogenesis returns. If serum from normal mice is injected into RAG $-/-$ mice, lots of rescue is observed. Antibodies in serum home appropriately but the recovery is partial, not total. B-cells and Igs lead to mast cell activation that leads to tissue remodeling, angiogenesis, epithelial cell proliferation, which in turn leads to neoplastic progression and cancer development.

I.e. innate immune response is critical to cancer malignancy, chronic inflammation critical for cancer development.

“Tumor-Educated Macrophages Promote Mammary Tumor Progression and Metastasis,”

Jeffery W. Pollard.

Tumors comprise of fibroblasts, adipocytes, macrophages, mast cells, blood vessels etc. Over expression of the macrophage growth factor CSF-1 (colony stimulating factor) correlates with a poor prognosis. It is found in association with CSF1R in approx 50% of tumors cell and 90% of

infiltrated macrophages. Macrophage density also correlates with a poor prognosis in >80% of human adenocarcinomas, especially in breast. Macrophage depletion slows tumor progress. CSF-1 $-/-$ cells doesn't metastasize to the lung (wt 100%). If a promoter is used to turn on CSF-1, all adenocarcinomas progress to carcinomas (become malignant). CSF-1 is a chemokine; it causes the release of MMP, VEGF, and TNF α . Macrophages recruit other cells like neutrophils and mast cells, re-model vasculature, and allow tumor cells to get into vasculature and so metastasize. If CSF-1 is injected into a tumor, within 5 minutes the macrophages are moving. EGF works too but PDGF does not. If an EGF inhibitor is injected with CSF-1, no recruitment of carcinoma cells or macrophages occurs. If CSF-1 is injected along with an EGF-R, the same result ensues. Tumor cells and macrophages have a paracrine loop. CSF-1 and EGFR are expressed by carcinoma cells, CSF-1R and EGF are expressed by macrophages. Both are required for movement of both cell types. **Block either CSF-1 or EGF signaling and** the paracrine loop is shut down. Without this gradient of chemokines, the maximum migration of cells would only be a couple of millimeters. In human breast cancer, EGF and CSF-1 are the most important indicators of a poor prognosis.

Macrophage clusters are required for intravasation. Macrophages stick to blood vessel walls and don't migrate – not yet known why. Pollard showed a video in which single macrophages were moving in response to chemokines but clusters of them remained stationary.

“Radical Causes of Human Cancer,”

Curtis C. Harris.

18% of human cancers are related to infection. Over 30 years, 15% of ulcerative colitis patients develop colon cancer. Inflammation generates radicals – OH \cdot , O $_2^{\cdot-}$, NO \cdot , ONOO \cdot , N $_2$ O $_3$ which cause protein damage and lipid peroxidation. iNOS is always active. It leads to the formation of aldehydes and the oxidation of L-arginine to citrulline and NO \cdot . It protects against pathogens. NO \cdot release leads to activation of p53 after 1-2 hours and also p21. In cells that have intact p53 and p21, get G $_2$ M accumulation. If either is null, there is no G $_2$ M halt. p53 also trans represses iNOS, therefore NO \cdot can be anti-carcinogenic. iNOS 2 can be induced by HIF-1 α and cytokines like IL1 β and TNF α . NO \cdot can also induce p53 mutations prior to cancer. Many chronic inflammatory diseases show an increase cancer risk via NO \cdot and p53.

“p16 Silencing and Premalignant Progression Involve COX-2,”

Thea D. Tlsty.

One of the problems with breast cancer is that it is detected too late. It takes about 10 years for a tumor to reach a size large enough to be detected by radiography (1 cm) and 11 years by physical exam (2 cm). Biomarkers are needed to allow much earlier detection.

One possible indicator is breast tissue density. Breast density can be sub-divided into four types with increasing density correlating with increasing risk for breast cancer. A 1% increase in density correlates with a 2% increase in risk. The risk associated with density may account for >30% of all breast cancers. Density is easy to measure non-invasively and can be changed through diet and hormone use. Higher density tissue contains less fat, more collagen, and increase in collagen remodeling. The collagen remodeling is caused by activated stroma (e.g. wound healing). The presence of TGF- β and IGF-1 both lead to high levels of collagen. Breast tissue can be separated

into fibroblasts and epithelial cells. Carcinogen associated fibroblasts (CAFs) are found in the cancer tissue, their presence provides an indicator of tumor progression. The fibroblasts from high and low-density tissue are different. When fibroblasts are co-injected with tumor cells into nude mice, low density associated fibroblasts (LDAF) lead to a delayed onset of tumors, high density associated fibroblasts (HDAF) to an accelerated onset of tumors.

Fibroblasts from high-density tissue show increases in TGF β , PAI-1, tenascin, and IGF-1. An increase in IGFBP-5 leads to an increase in IGF-1 as does a decrease in IGFBP-3. IGFBP-3 sequesters IGF. Serum from high-density tissue shows an increase in IGF-1 and a decrease in IGFBP-3 and this correlates with an increased cancer risk. In wounds, TGF- β activates IGF-1 and the same is true here: too much IGF-1 is produced for IGFBP-3 to handle and the excess leads to proliferation

Tlsty previously identified a rare subpopulation of variant human mammary epithelial cells (vHMEC) that have the capacity to propagate beyond an in vitro barrier and accumulate multiple chromosomal changes. As these chromosomal changes were similar to those seen in pre-malignant lesions of breast cancer, she postulated that these cells might be precursors to this disease. One of the hallmarks of vHMECs is hypermethylation of the p16 gene. A histological examination of normal tissue showed that there are patches of methylated p16 cells within the normal, disease free breast tissue of most women and that these cells will lead to lesions. The methylation of p16 leads to over expression of COX-2. COX-2 stimulates aromatase activity, angiogenesis, proliferation, invasion, and prostaglandin synthesis. The increase in prostaglandins leads to an inhibition of apoptosis. In 15% of the cases examined, the p16 cells don't have over expressed COX-2 - but the surrounding tissue does. vHMEC cells expressing Ha-Ras become immortal.

HDAF and CAF, but not LDAF, induce EMT (epithelial to mesenchymal transition) in vHMECs and stimulate invasion. p16 methylated cells have a proliferative advantage over normal cells under normal conditions. Add centrosomal abnormalities, leading to the loss of telomere shortening (immortality), and, in the presence of HDAF or CAF in the stroma, get cancer. vHMECs are a lot like stem cells. Stem cells have to silence p16 to transform and they do this using BMI. BMI is also over expressed in vHMECs.

One problem here is that there are many normal changes in breast tissue that occur with monthly cycle, pregnancy, lactation etc. This provides a link between early breast cancer and early and late menopause. The effects of multiple pregnancies are not yet known.

Short Talk: "Interleukin 6 (IL-6) Regulates and Maintains Epigenetic Silencing of Tumor Suppressor and DNA Repair Genes in Human Multiple Myeloma Cells,"

William L. Farrar.

Many cancer types are associated with IL-6 secretion – many make it, others respond to it. IL-6 is involved in the re-methylation of genes but it is not know if it is involved in the initial methylation of them. Methylation is one of the mechanisms in which genes are silenced. IL-6 leads to activation of PI3K and Akt. Akt phosphorylates DNMT, which silences BERs (Base Excision Repair), NERs (Nucleotide Excision Repair), p53, p16, MnSOD etc.

“C-Reactive Protein Levels and Subsequent Cancer Outcomes: Results from a Prospective Cohort Study and Implications for Prevention,”

Kathy Helzlsouer.

C-Reactive protein (CRP) has strong links to cardiovascular disease as well as cancer. It is a non-specific marker of inflammation and higher levels have been observed in people who go on to develop cardiovascular disease.

Helzlsouer talked about two studies of a large group of people from Washington County, somewhere near Baltimore. In 1974, a large number of people provided answers on a questionnaire about family history of diseases, work etc and donated serum which was stored so that it could be used to study the impact of family and environment etc on disease. This study was called CLUE. In 1989, a second study was done in the same area in which plasma and red and white blood cells were also collected. A large group of people participated in both –this group called Odyssey.

The Odyssey set was very useful because it allowed for research into early biomarkers on patients who subsequently developed cancer or suffered from a stroke in the time between the two studies. C-Reactive protein (CRP) was found to have strong links to cardiovascular disease as well as cancer. It is a non-specific marker of inflammation and higher levels have been observed in people who go on to develop cardiovascular disease. CRP is produced in response to inflammatory molecules like IL1, IL6 and TNF α .

In the patient population it was found that colon cancer was slightly increased in people who smoked, decreased by NSAID use, increased by hormone use, and increased in people with a high body mass index (this was really important). CRP level was important in colon cancer but not rectal cancer. The higher the level of CRP, the higher the risk, even more true in non-smokers. CRP was also found to be a marker for insulin resistance and obesity (metabolic/dysmetabolic function).

There was no association between CRP and prostate cancer. Breast, ovarian and endometrial cancer is currently being examined.

A study was done looking into the use of compounds to prevent diseases. Two sets of data were generated: NNT – number needed to treat (number of people than need to be treated to result in one prevention) and NNH - number needed to harm (number of people treated to cause one problem).

		Tamoxifen	Aspirin	COX-2
NNT	Invasive breast cancer	78		
	In situ breast cancer	198		
	Hip Fracture	674		
	Chronic heart disease		107	
	Polyps			NA
NNH	endometrial cancer	317		
	stroke	474		
	Deep vein thrombosis	511		

Hemorrhage	1004	
Cataract	97	
GI ulcers	500	
Chronic heart disease		Vioxx 64
		Celoxocib 200 mg 77
		400 mg 42

One concern with this study is that the patient populations for inclusion were selected to include similar profile people in both the disease and non-disease groups. The people who participated in this study were from a fairly small area and included many related people. The relationships of the people were not included when selecting people for each group so it is possible that the results are biased by genetic factors.

“Premalignant Lesions as Targets for Cancer Vaccines,”

Olivera J. Finn.

Inflammation – the recognition by the innate immune response that groups of abnormal cells have become dangerous and should be eliminated. Elimination of the abnormal cells is the job of the adaptive immune response. When it fails, the inflammation becomes chronic and a tumor forms. Tumors are normally destroyed by neutrophils, macrophages, and NK cells. Cancer vaccines can change the context and balance of the immune response but one problem with them is that cancer patients already have a failed immune system. The aim of this program is to create and test antibodies against MUC-1 and cyclin B1.

MUC-1 is an antigen that is expressed on all human carcinomas (>83% of all human tumors). In normal cells, MUC-1 is present at very low levels and is extremely densely glycosylated. In tumor cells, it is very over expressed and has few attached sugars. A vaccine against MUC-1 has been developed. It was safe, presenting no sign of any autoimmunity – i.e. no long-term problems. It worked in chimpanzees but, for the most part, not in humans. Every so often, there would be a positive response seen in humans.

Why doesn't a patient who has had a primary tumor respond to vaccines?

Removal of tumor doesn't return immune system to normal

Cancer patient has a different cell balance – lots of active granulocytes

High levels of isoprostane – a biomarker for oxidative stress

T-cells in cancer patients make very little IFN γ , and upon activation, die

Systemic chronic inflammation suppresses adaptive immune response and promotes tumor growth

Polyps – early form of colon cancer – express high levels of tumor-type MUC-1. Antibodies to this type of MUC-1 are generated by bone fracture, mastitis, current smoking, + others. The risk of ovarian cancer is lower in patients who have five or more of these risk factors.

Currently, the use of this antibody is being investigated in patients who do not have tumors. Can induction of a strong anti-MUC-1 immunity prevent colon cancer? This is expected to be more effective than immunizing people who already have a tumor because cancer patients will likely already have an immune response against MUC-1 and it is being overridden.

Similarly, many heavy smokers already have an antibody to cyclin B1. Looking at immunizing these patients to strengthen their immune response.

Cyclin B1 is a cell cycle regulatory protein that is aberrantly expressed in the cytoplasm of tumor cells where it is found in large amounts never encountered in normal cells. All cells that over express cyclin B1 have inactive p53.

During questions, an oncologist noted that during chemotherapy, the more neutropenia seen, and the more likely the chemotherapy would be successful.

“Molecular Immunology and Tumor Immunotherapy,”

Drew M. Pardoll.

STAT3 is constitutively active in over 50% of tumors but is not mutated. It is activated as a result of being downstream of oncogenic pathways like IL6, IL10, EGF, HGF, and Src. Activation of STAT3 induces cyclin D1, Bcl-X_L, and VEGF. STAT3 β is a dominant negative form of STAT3. Increased levels of STAT3 in tumor cells leads, via an increase in VEGF, to increased levels of STAT3 in dendritic cells. Upregulation of STAT3 in dendritic cells blocks their maturation and this blocks the adaptive immune response. Dendritic cells (DCs) are the bridge between the innate and adaptive immune responses. They carry antigens from the tumor to the T-cells in the lymph nodes. DCs can be activated by LPS. This activation is blocked by IL10 or tumor supernatant. STAT3 -/- DCs do not respond to LPS.

A historical view of cancer is that tumors are wounds that do not heal. This view is becoming very relevant.

	Tumor	Wound	via STAT3
Turn on cell proliferation	✓	✓	✓
Inhibit apoptosis	✓	✓	✓
Enhance angiogenesis	✓	✓	✓
Inhibit immunological danger signals (inflamed wounds don't heal)	✓	✓	✓

If STAT3 is knocked out in hematopoietic cells tumors either don't grow or grow very slowly. STAT3 restrains tumor surveillance. If STAT3 were blocked, tumor would die.

CPA is a first generation STAT3 inhibitor. It has no effect upon MB49 cells in vitro but in vivo it dramatically decreases tumor growth rate.

“Sequential Cancer Immunotherapy,”

Glenn Dranoff.

Host responses to bacterial challenge are shaped by the mixture of cytokines GM-CSF and IFN γ . Knockout of these cytokines leads to mice that are very susceptible to pneumonia. Mice that survive to mid age are very susceptible to tumors, especially ovarian, and this is always in the setting of chronic inflammation. If enrofloxacin is put into the drinking water, these mice do not get pneumonia or tumors. NSAIDs work similarly but not as efficiently as antibiotics. Cytokine

deficiency links chronic inflammation and cancer. GM-CSF was the most potent molecule of the 30 tested for enhancing tumor immunity following gene transfer into tumor cells.

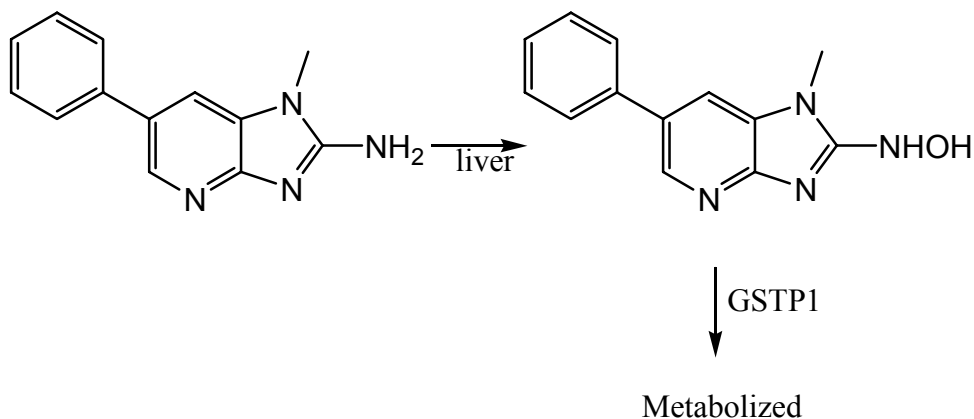
“Inflammation and Prostate Carcinogenesis,”

William G. Nelson.

30% of 30 year olds and 50% of 50 year olds have prostate cancer, many of which are undiagnosed. It is largely asymptomatic and has a high inflammation involvement. There is a high level of heredity (~40%) but a shared gene has proven difficult to find mainly due to the difficulty of getting DNA samples from several generations. So far, no common organism has been found to infect the prostate.

Two genes have been found to be linked to prostate cancer, RNASEL and MSRA (macrophage scavenger factor), both are typically used to fight off infection. MSRA is needed for ingestion of *Neisseria* bacteria.

GSTP1 (P1a glutathione S-transferase) has been found to be inactive in >90% of prostate cancers. It is inactivated by hypermethylation of a CpG island. In its active form, it is not methylated at all. Loss of GSTP1 leaves cells vulnerable to oxidative damage via reactive oxygen species (ROS). It increases cell survival in spite of the DNA damage caused by ROS. Lycopene (from tomatoes) has been found to lower prostate cancer risk. Eating meat increases it. Nelson showed a table showing the effect of different grilling methods on the cancer risk from grilling steak. A single flip on a very hot grill was the worst as this is the conditions under which most Phip (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) is formed. Phip is the most abundant of the heterocyclic amines formed during the cooking of fish and meat, it is carcinogenic via a DNA intercalation mechanism. Multiple flips on a lower temperature grill are a much healthier way to cook meat.



MDB2 is responsible for silencing GSTP1. A HTS screen has been run against MDB2.

As a side note, it was noted that estrogen is most responsible for prostate cancer. Estrogen is produced from testosterone.

“Helicobacter pylori Diversity and Interaction with Gastric Epithelial Cells,”

Martin J. Blaser.

Helicobacter pylori are ancient organisms that have populated the human stomach for millennia. They are spiral, gram –ve bacteria that live offshore in the mucus layer. As a result of antibiotics and diet changes, the percentage of the human population that has *h. pylori* has dropped from all to around 50%. Apparently, there are 10^{14} microbial cells and 10^{13} eukaryotic cells in an average human! There are several outcomes of an *h. pylori* infection: 1) peptic ulcer, 2) gastric neoplasms, 3) no clinical consequences, 4) protection from esophageal diseases (e.g. adenocarcinoma of the esophagus).

There is lots of diversity within *h. pylori*. CagA + strains have increased bacterial density, more acid, increased frequency of peptic ulcer and gastric carcinoma and increased expression of IL-8. CagA is translated into endothelial cells lining the stomach, which affects cell cycle and secretion of pro-inflammatory cytokines and metalloproteases, all of which are relevant to the development of neoplasia. I.e. *h. pylori* is signaling through CagA. AGS cells change their phenotype upon exposure to CagA. CagA transfection turns on IL-8. All of this suggests that *h. pylori* signals its host and the host signals back.

H. pylori is generally present in several strains, which are in equilibrium. The return signal from the host affects the equilibrium. CagA- strains make only a little MMP1. The formation of stomach cancer is linked to *h. pylori* but often by the time that the cancer is detected, it is gone, the gastric environment has been damaged and much less friendly bacteria have moved in.

“Chemokine Receptors in Tumor Progression and Metastasis,”

Anja D. Müller.

Do tumor cells adopt chemokine-mediated functions to metastasize? Yes. CXCR4 is dramatically upregulated in mammary tumors. The ligand for CXCR4, CXCL12, is found in lymph, lung, liver, and bone marrow and this is where mammary tumor cells metastasize to. Similarly, malignant melanoma express the chemokine receptor CXCR10. The receptor for this is CXCL27 and this is found on skin, again the major site for metastasis. CXCR4 is induced by IL-1 β , TNF α , VEGF, and TGF β , the same growth factors responsible for tumor growth etc. CXCR4 is also stimulated by sub-lethal chemotherapy. For example, it is dose dependently induced by cisplatin. Exogenous CXCL12 rescues cells from apoptosis caused by cisplatin. Block CXCR4 with antibodies and there is no change in adhesion but extravasation is blocked. Block CXCL12 and extravasation is promoted.

Short Talk: “Inflammation Associated Angiogenesis: A New Potential Target for Tumor Therapy,”

Douglas M. Noonan.

Induction of angiogenesis by CXCL1 involves neutrophils. CXCL1 binding to its receptor leads to VEGF release, which in turn induces angiogenesis through MIP2. Under normal inflammatory conditions e.g. wound healing, the role of the neutrophils is complete within 24 hours and they die and are replaced by macrophages. The process by which neutrophils and macrophages kill other

cells is known as necrosis. This is better for a developing tumor than apoptosis because it leads to the release of a lot of factors that suppress the immune response.

Noonan is studying the effects of green tea polyphenols; he has shown that they inhibit angiogenesis and tumor growth through inhibition of neutrophil migration (both in vitro and in vivo).

“Hypoxia, Angiogenesis, and Tumor Progression,”

M. Celeste Simon.

Under low oxygen conditions, cells switch from oxidative phosphorylation to glycolysis. Angiogenesis is triggered and the kidney begins producing more erythropoietin which increases red cell mass. Hypoxia Inducible Factors (HIFs) are released which directly activate at least 100 genes. HIF1 is a heterodimer with an α and β subunit, it is regulated by the α subunit. HIF1 β is also called ARNT. In normoxia, HIF α is hydroxylated at Pro564, ubiquitinated by the von Hippel-Lindau E3 ligase complex and destroyed by the proteasome. In low oxygen conditions this doesn't happen, the HIF1 α builds up and forms a dimer with HIF1 β leads to formation of an active transcriptional complex.

HIF2 α is found in a wide range of tumors. It is exclusively expressed in the endothelium during embryonic development. Replacement of HIF1 α with HIF2 α leads to over expression of VEGF and TNF α . HIF1 α promotes tumor growth more than HIF2 α .

Short Talk: “Mechanisms Linking Innate Hemostatic Factors and Innate Immunity to Metastatic Potential,”

Joseph S. Palumbo.

Palumbo made the interesting comment that you need to inject 100,000 tumor cells to get 100 tumors to form in a mouse. I.e. metastasis is remarkably inefficient. This is a good thing because biopsy releases lots of tumor cells into the bloodstream.

Hemostatic derangements are common in cancer. Studies of gene-targeted mice lacking clotting function (i.e. fibrinogen-null mice) or platelet activation function (e.g. G α q-null mice) show a decrease in the rate of metastasis. The G α q null mice clear tumor cells from their lungs 10x more efficiently than wt mice. Fibrin(ogen)/platelets support metastatic success by preventing the elimination of tumor cells by natural killer cells (tumor surveillance). Once tumors are established, inhibition of fibrin/platelets has no effect upon tumor growth. Thrombin catalyses the polymerization of fibrinogen to fibrin. Thrombin inhibition can block metastasis but has no effect upon tumor growth. Anti-platelet treatment during biopsy could therefore be beneficial but the major side effect, bleeding, could be a problem. Yet another role for aspirin.

“TGF β , Inflammation, and Colon Cancer Progression,”*Thomas Doetschman.*

Inactivation of TGF β is found in 15-30% of colon cancer cases. It is at the apex of a signaling pathway that may be second only to the most commonly disrupted pathway in human colon cancer, the Wnt pathway.

Doetschman described the development of a mouse model for colon cancer created by introducing a TGF β null mutation into an immunodeficient (Rag2 $-/-$) strain of mouse. The Rag2 $-/-$ mutation was necessary as TGF β $-/-$ alone leads to death from an autoimmune disease. The double mutant animals survive to 6 months. Rag2 $-/-$ survival suggests that T-cells are responsible for the mouse deaths. TGF β $-/-$ Rag2 $-/-$ animals go rapidly through hyperplasia – dysplasia – adenoma – mucinous carcinoma.

In hyperplasia (increase in number of normal cells in a normal arrangement in tissue), TGF β $-/-$ does not affect growth or genetic stability. It has no effect upon apoptosis, proliferation, APC, or β -catenin and p53 is not mutated – i.e. no other signatures of colon cancer, and no increase in innate immune response.

In dysplasia (alteration in size, shape, organization of normal cells of adult cells), there is an increase in phosphorylated-Akt and a decrease in PTEN. There is an enhanced immune response to both gram + and – bacteria, a decrease in MUC-2, an increase in NF- κ B, a decrease in the mucosal system, a decrease in goblet cell differentiation and an increase in TNF- α . There is loss of growth control but no dysregulation of the APC pathway or genetic instability.

TGF β $-/-$ scid mice have colitis and hyperplasia but not cancer. DSS does induce cancer in these animals. If TGF β $-/-$ Rag2 $-/-$ are maintained in a germ free environment they do not get cancer. When these animals were returned into the normal lab setting, they developed colon cancer over the course of a year but when put into the knockout lab they did not. Doetschman is currently examining the microflora to look for differences between the two rooms. It is possible that there are protective bacteria as well as harmful ones. So far they have found *helicobacter hepaticus*, *b. thetoiotomicron* (IBD antagonist), *b. frugalis* (progression factor), and *b. distasonis* (associated with ulcerative colitis) in the cancer room. Overall, they see 6-30x more bacteria in the double knockout mice and there are lots of other roles played by bacteria that do not necessarily involve inflammation, for example binding to fibrinogen. It is proving challenging to grow up all of the species of bacteria.

TGF β is a potent chemoattractant responsible for getting inflammation going. It is also a very potent inhibitor of inflammation, more potent than cyclosporine, though it depends upon the amount present. One problem here is that the mice have no adaptive immune system at all.

“The IKK Complex: Providing a Link between Inflammation and Cancer,”
Michael Karin.

The effect of IKK β on tumors in mice is reported to be the first evidence of the molecular link between inflammation and cancer.

Inhibition of NF- κ B activation in cancer cells converts inflammation-induced tumor growth to inflammation-induced tumor regression. NF- κ B transcription factor regulates apoptosis.

Inflammation	Innate Immunity	Anti-apoptosis
iNOS, COX-2	Defensins	cIAP 1/2
PLA2, TNF- α	Chemokines	A1
IL-1 etc	Cytokines	Bcl-X _L
	Adhesion Molecules	Cflip

The complex (?) is activated by TNF- α , IL-1, LPS, LTA, dsRNA, ISS-DNA, IR etc. There are two pathways through which it is activated. One involves the dimerization of IKK α - this is not important in cancer. The other involves dimers of IKK α , β , and γ . Of these, IKK β is the most important. There are 5 isoforms of NF- κ B. Individual isoforms are redundant and single knockouts give partial phenotypes. NF- κ B activation blocks caspases and JNK - which blocks apoptosis and Bcl-X_L - which stops activation of macrophages. It also blocks formation of reactive oxygen species (ROS). Elevated levels of NF- κ B can provide resistance to anti-cancer drugs and especially ‘death’ cytokines like TRAIL. Early on in cancer, NF- κ B suppresses apoptosis of cells with DNA damage, chromosomal aberrations, or activated oncogenes.

IKK -/- mice have fewer tumors. These tumors look the same but have more inflammation. The decreased incidence of tumors is probably due to increased epithelial cell apoptosis.

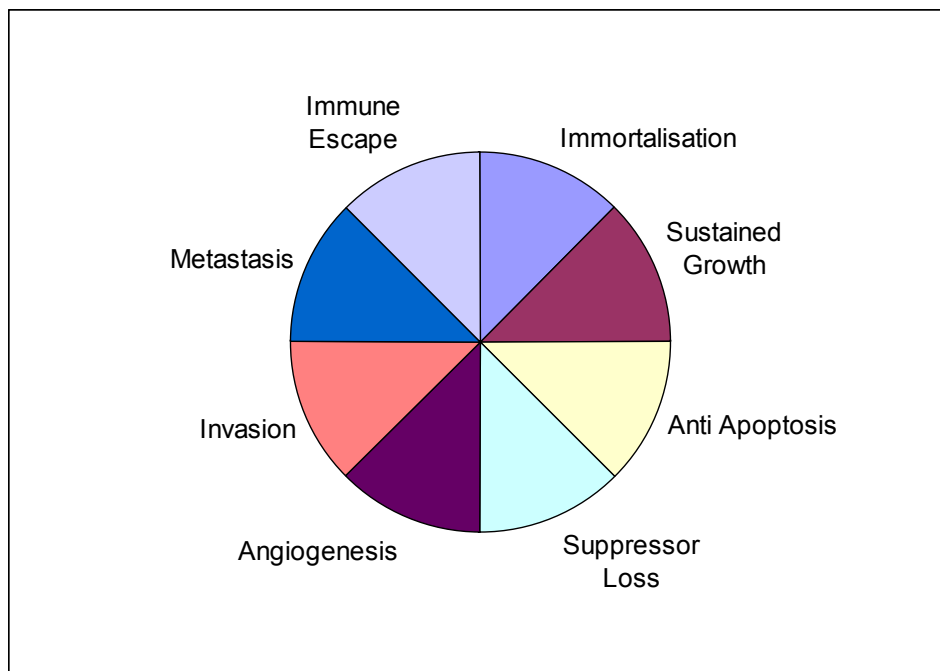
Karin described work on the effect of IKK β -/- on a model of hepatocellular carcinoma. Injection of diethylnitrosamine gives 10% cancer induction. The IKK β -/- mice get more, and larger, tumors. However, these tumors are killed by radiation. IKK β +/- tumors do not respond to radiation. This effect is linked to BCL-2 and is important in the liver because the liver is capable of regeneration much more than other organs.

NF- κ B inhibition leads to sustained activation of JNK. JNK activates ROS. If give the antioxidant BHA for 4 days around the time of injection of diethylnitrosamine, the mice tumor burden is returned to normal.

Diethylnitrosamine causes death by apoptosis and necrosis. Necrosis leads to cytokine release and activation of macrophages.

Short Talk: “IDO in Immune Suppression, Cancer, and Cancer Therapy,”
George C. Prendergast.

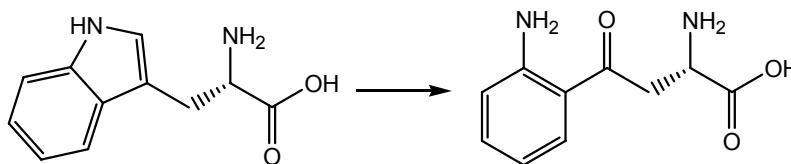
In clinical cancer, mutations are of lesser importance than modifiers and microenvironment. Cancer can be viewed as being made up of the following elements:



Prendergast discussed two proteins: Bin1 and IDO.

Bin1 doesn't initiate cancer but drives it. It integrates actin/membrane interactions and suppresses cancer. It is found downregulated or mis-spliced/located in approximately half of tumors and seems to affect NF-κB and STAT trafficking.

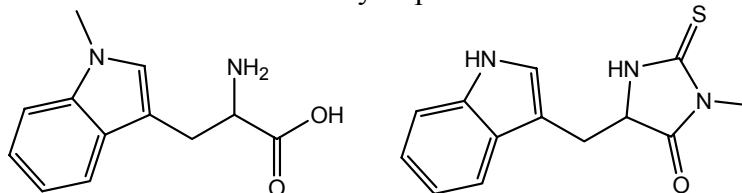
IDO (indoleamine-2,3-dioxygenase) is over expressed in almost all tumors. It catalyzes the catabolism of tryptophan to produce kynurenine. Kynurenine blocks the activation of T-cells (the adaptive immune response) and thus allows immune escape. IDO is produced in activated macrophages upon stimulation by IFN γ .



Several inhibitors of IDO have been found. MTH-tryp has an IC₅₀ of 10 μ M. 1-methyltryptophan (1MT) works quite well in mice due to good PK. Taxol + 1MT gives regression. MTH-tryp + taxol works very well – gives a few cures and no overt toxicities seen during trial or at necropsy.

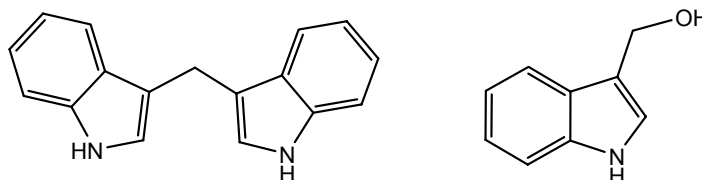
It is not just boosting taxol as taxol was used close to its MTD. It doesn't work alone or in conjunction with vinblastine or rapamycin. It does work with cisplatin. Brassinin is one of several natural products that have been found to inhibit IDO.

IDO -/- are viable. The human genome has no IDO-related enzymes and there is a facile pre-clinical and clinical PK/PD assay (serum analysis). IDO inhibitors do not work in xenografts – nude mice don't have T-cells. IDO becomes very important in mice that lack Bin1.



1MT

MTH-trypt



Brassinin

Indole-3-carbinol

“COX-2, Inflammatory Mediators and Cancer Prevention,”

Raymond N. DuBois.

Use of NSAIDs leads to a significant decrease in the risk of cancer (40-50% for colon cancer). Colorectal cancers have very large amounts of COX-2 but little COX-1. Very large adenomas have lots of COX-2 too. NSAIDs block prostaglandin synthesis (arachidonic acid to PGE₂). PGE₂ is produced in most tumors. PGE₂ binds to EP1, 2, 3 and 4. Binding to EP4 leads to activation of the PI3K, Akt pathway, which leads to proliferation, cell spreading, motility, and invasion. COX-2 is turned on by TNF α . In normal cells, the increase in COX-2 is transient. In colon cancer cells it stays at elevated levels. PGE₂ accelerates adenoma growth – presence of high levels of PGE₂ leads to more and larger tumors in the mouse. Small polyps have COX-2 in the stroma, large polyps have COX-2 in the stroma and endothelial cells. Mice lacking the EP1, 2 and 4 receptors show a markedly reduced growth and progression of tumors.

Dosing of Celecoxib at the levels normally used to treat rheumatoid arthritis leads to the prevention and halt of tumor growth. The results of a phase II trial of Celecoxib against familial adenomatous polyposis (FAP) showed a 41% decrease in polyps at 400 mg bid and a 15% decrease at 100 mg bid. A subset of patients showed complete regression. Serum profiling showed a peak that, if present, no response to treatment was observed and, if absent, there was a strong response. VIOXX showed heart problems but only after a long time. At 18 months, there was no apparent difference, at 24 months the increase of heart problems was statistically insignificant. VIOXX did however, give a 20-30% decrease in polyps. A diet low in unsaturated fats lowers polyps. Increasing the proportion of ω -fatty acids should help too.

PGE₂ affects cellular organization and the migration and proliferation of endothelial cells. It causes the release of GRO- α (CXCR2 is the receptor for GRO- α). To overcome the problems associated with COX-2, the Japanese company ONO is looking at inhibitors of PGE₂ and EP receptor antagonists. GRO- α is, apparently, a very similar enzyme to COX-2.