Cancer Microenvironment and Therapeutic Implications
Cancer Microenvironment and Therapeutic Implications

Tumor Pathophysiology Mechanisms and Therapeutic Strategies
The book is dedicated to Pietro M Gullino,
an uncommon friend and teacher
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<tr>
<td>BDMCs</td>
<td>Bone marrow derived myeloid cells</td>
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<tr>
<td>CCL22</td>
<td>Chemokine ligand 22</td>
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<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
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<tr>
<td>DCs</td>
<td>Dendritic cells</td>
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<tr>
<td>G-CSF</td>
<td>Granulocyte colony stimulating factor</td>
</tr>
<tr>
<td>GF-β</td>
<td>Transforming growth factor beta</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage colony stimulating factor</td>
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<tr>
<td>GAL-1</td>
<td>Galectin 1</td>
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<tr>
<td>HIF-1</td>
<td>Hypoxia inducible factor 1</td>
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<tr>
<td>HSP</td>
<td>Heat shock protein</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
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<tr>
<td>IDO</td>
<td>Indoleamine 2,3-dioxygenase immunoregulatory cells</td>
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<tr>
<td>IL-1</td>
<td>Interleukin 1</td>
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<td>IL-2</td>
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<td>Interleukin 12</td>
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<tr>
<td>IF</td>
<td>Interstitial fluid</td>
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<tr>
<td>IFP</td>
<td>Interstitial fluid pressure</td>
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<tr>
<td>LAK</td>
<td>Lymphokine-activated killer cell</td>
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<tr>
<td>MDSC</td>
<td>Immature myeloid-derived suppressor cells</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
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<tr>
<td>NF-kB</td>
<td>Nuclear factor kappa B</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>PDGF</td>
<td>Platelet derived growth factor</td>
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<tr>
<td>pH_e</td>
<td>Low extracellular pH</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SDF-1</td>
<td>Stromal cell derived growth factor 1</td>
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<tr>
<td>TAA</td>
<td>Tumor associated antigens</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>TLR</td>
<td>Toll like receptors</td>
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<td>Treg</td>
<td>Regulatory T cells</td>
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<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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Introduction

Revolutionary changes have swept through cancer diagnosis and treatment over the past century. In the early 1900s the struggle was to diagnose. Then came the struggle to cut it out. Then the struggle to keep it away.

In the late 1900s scientists and physicians turned inward towards cancer cell mechanics and that is when the explosion of oncologic sciences erupted. A fountain of genes, transcription factors, signaling pathways, degradation fates, apoptosis chains, receptor interactions and cell-cell contact points provide a wellspring of opportunity to interfere with runaway cell growth and metastasis.

In the post-genomic era, cancer is a genetic disease.

But somewhere in the rush of genetic reductionism, the cancer research community has increasingly neglected the importance of the tumor microenvironment and the interplay between cancer cells, dysplastic cells and normal cells.

It is in this realm that this book is written.

The genesis of cancer is not as simple as a gene abnormality or even a set of gene abnormalities. A permissive environment must allow unchecked cell division. For cancer to initiate, the immune system must look the other way, nutrients and oxygen must be served at the right time, temperature and concentration, and a hospitable bed of stroma and extracellular matrices must support burgeoning growth and spread. Cancer is a chance co-op with a panoply of factors traded between normal and malignant.

Chief interactions in this co-op are exposed herein and implications for anti-cancer therapies are discussed. This book is not intended to serve as a simple review; rather, to spark new ideas and provocative questions so that better anti-neoplastic therapies are conceived and promulgated.

In this book we assembled a team of contributors who share amongst them serious experiences at the laboratory bench and in the clinic. These physician-scientists have dedicated themselves to the tension between the urgency for breakthroughs and the technical challenges of discovery. Their thoughts and perspectives on the state of cancer biology, ecology and implications for treatment are gathered herein. We intend for this book to roadmap outstanding questions and potential answers for the eradication of cancers.
Part I
Tumor Physiopathology
and Microenvironment Genesis
Chapter 1
Inflammation and Carcinogenesis: A Change in the Metabolic Process

L. Schwartz, M. Israël and Icard Philippe

Abstract Since the seminal work it is known that inflammation is a major risk factor for cancer. Inflammation can be caused by agents as diverse as heat, cold, foreign body or chemicals. In every case, there is a protein leak from the damaged capillaries. This results in increased oncotic pressure which is in turn responsible for the methylation of the PP2A phosphatase. The activation of PP2A results in the translocation of NFκB and the activation of several metabolic pathways.

Keywords Inflammation · Chronic inflammation · Carcinogenesis · Methylation · PP2A phosphatase · NF-κB

Cancer is frequently associated with pre-existing inflammation and fibrosis. Between 60% and 90% of hepatocellular carcinoma occurs in patients with hepatic macronodular cirrhosis (De Vita et al. 1993, Podolsky and Isselbacher 1994). Chronic liver disease of any type is a risk factor for liver cancer. The cancer may be caused by hepatitis B or C, alcoholic liver disease, antitrypsin deficiency, hemochromatosis, and tyrosinemia. Its features result from hepatocyte necrosis, extensive fibrosis, connective tissue deposition, vascular distortion and nodular regeneration of the remaining tissue parenchyma (Podolsky and Isselbacher 1994). Evidence for a cause-effect link between cirrhosis and hepatocarcinoma is lacking. The relation may often be one of chance alone, since not all cirrhotics develop cancer. Nonetheless, diseases that cause cirrhosis also increase the risk of hepatocarcinoma (Podolsky and Isselbacher 1994). Furthermore, the more disorganized the liver becomes, the higher the risk of hepatocarcinoma (Podolsky and Isselbacher 1994, Baffis et al. 1999).

Similarly, lung cancer is most common among patients suffering from any form of chronic lung disease (Ernster 1996, Maitre et al. 1996). History of chronic bronchitis, emphysema, primary lung fibrosis, chronic lung infection and even lung
irradiation are associated with increased risk (Ernster 1996, Maitre et al. 1996). There is no evidence for a relation of bronchitis or emphysema with lung cancer that could not be explained by independent links to exposure to tobacco smoke or other noxious agents. Nevertheless, the risk of lung cancer increases with the extent of disruption of normal lung architecture. For example, the risk of lung cancer is higher among patients suffering from chronic bronchitis and severe impairment of the lungs’ carbon monoxide. Breast cancer genesis also seems to be linked to architectural changes. A woman’s reproductive history is one of the most important determinants of breast cancer risk. This is not a new notion. Ramaziani in 1700 first showed that breast cancer risk was higher among nuns (Haagensen 1986). Early in the 1900s, investigations noted that nulliparity and a history of having never breast-fed an infant were risk factors. Modern epidemiological cohorts have confirmed the increased risk for breast cancer after early puberty, late menarche, hormonal stimulation (Haagensen 1986).

In 1977, Ing reported a disproportionate increase of post menopausal breast cancer in the left breast of Tanka women of Hong-Kong thought to have nursed only with the right breast (Ing et al. 1997). All these risk factors and others, like radiation to the developing breast, are related (causally or not) to change in architecture of the breast.

Breast cancer is rare among young women. It is before the menopause, when the architecture of the mammary gland starts to undergo fatty tissue involution, that the incidence of cancer rises (Haagensen 1986). With the completion of menopause, the breast changes, it becomes somewhat smaller and less dense. There is a decrease in the number and size of the ducts. These atrophic lobules are seen lying in a dense fibrous matrix. Increase in the connective tissue is a prominent feature of this aging process.

The rare cases of breast cancer in young women are often due to hereditary anomalies. The most studied gene is BRCA-1. BRCA-1 is a nuclear phosphoprotein expressed in a broad spectrum of tissues during cell division. The inheritance of a mutant BRCA1 allele dramatically increases a woman’s lifetime risk for developing both breast and ovarian cancers. This increased risk may be secondary to architectural changes. Analysis of a prophylactic subcutaneous mastectomy after genetic counseling for either carrying the BRCA-1 gene or belonging to a pedigree with familial breast cancer shows a different architectural pattern. The BRCA-1 or related genes may have a functional role in the branching pattern of the breast during lobular development, mainly in epithelial-stroma interaction (Russo et al. 2001). BRCA-1 deficient mice display multiple malformations (Cressman et al. 1999).

Cancers occurring during childhood (nephroblastoma, medulloblastoma, retinoblastoma or Li-Fraumeni syndrome) are also associated with tissue disorganization from embryonic remnants (De Vita et al. 1993, Pivnick et al. 1998, Boyle et al. 2000). Patients suffering from genetically-encoded hereditary tumors like Li-Fraumeni syndrome and retinoblastoma have both mutated epithelial cells and fibroblasts with impaired growth, resulting in concomitant malformations (Boyle et al. 2000, Le Couter et al. 1998).
Experimental Evidence that Chronic Inflammation Induces Fibrosis and Cancer

**Chemical Carcinogenesis**

Exposure to chemical carcinogens is considered to cause most human cancer (De Vita et al. 1993). In animal carcinogenesis experiments, a first chemical (initiator) is responsible of an intense inflammatory reaction. A second chemical (promotor) is genotoxic. The word “genotoxic” has been created to replace the older previous term: toxic. The effect of genotoxic compounds is not confined to the epithelial cell; they kill epithelial and stromal cells. Genotoxicity causes tissue disruption through cell killing and replacement. For example, hepatocyte necrosis induced by genotoxic compounds, which precedes hepatic carcinoma, is associated with substantial damage to surviving hepatocytes, as well as extensive mesenchymal changes and loss of normal liver architecture (Ames and Gold 1990).

In vitro, however, carcinogens have not always successfully transformed normal human cells in culture (McCormick et al. 1990). For these normal cells to be transformed, they often need to be immortalized by transfection with a cancer-associated virus prior to exposure to a carcinogen (Dip ao et al. 1986).

**Radiation-Induced Cancer**

Ionizing radiation induces cancer in humans and animals (Rhim et al. 1993, Barcellos-Hoff and Ravani 2000). In vitro, the vast majority of attempts to achieve transformation of normal human cells into cancer cells have been unsuccessful (Barcellos-Hoff and Ravani 2000). In fact radiation induced carcinogenesis appears to be a consequence of inflammation and fibrosis.

The female mammary gland is unique among all glands in that the epithelium develops after the birth from a rudiment that can be easily removed at about three weeks of age. Barcellos-Hoff irradiated the whole mammary gland of nurturing mice. After the irradiation, the epithelial cells are surgically removed and replaced with transplanted normal mammary cells. The cancer arises from these normal non-irradiated epithelial cells. Tumor growth appears as a consequence of the changes in the irradiated stroma (Barcellos-Hoff and Ravani2000).

**Physical Carcinogenesis**

Chemicals and radiation induce inflammation and fibrosis. The question is whether these architectural changes and the fact that tissue disruption is a risk factor for neoplasia may be explained by chronically increased epithelial cell proliferation resulting in an increased rate of mutation (Moore and Tsuda 1998). The answer lies in the old literature on physical carcinogenesis. It has been documented that some
foreign bodies induce cancer (Sonnenschein and Soto 1999, Brand 1982, Stanton and Wrench 1972, IARC 1999, Lipkin 1980). The carcinogenicity of foreign bodies is linked to their shape. Cellulose membrane filters of specific shape, texture or size generate sarcoma. Intense inflammation and proliferative fibrosis precede tumor formation. The combination of shape and size (about the width of a human cell) may also be critical (Lipkin 1980). The carcinogenicity of this chemically inert molecule is also linked to particle shape and size.

The International Agency for Research on Cancer evaluated the carcinogenic effect of surgical implants and other foreign bodies in humans (IARC 1999). The evaluation resulted in a group 2B classification (possibly carcinogenic for humans) for polymeric implants prepared as thin smooth films, and implanted foreign bodies consisting of metallic cobalt, or nickel, and a particular alloy powder consisting of 66–67% nickel, 13–16% chromium and 7% iron. The evaluation also resulted in a group 3 classification (not classifiable as to their carcinogenicity to humans) for organic polymeric materials as a group, orthopaedic implants of complex composition, cardiac pacemakers, silicone breast implants, dental materials and ceramic implants.

Physical carcinogenesis may also be a “transforming” factor. In nude mice, the implantation of both colon adenoma cells and of a plastic plate are necessary for tumorigenic growth. Again, locally, there is intense inflammation (Okada et al. 2000).

Inflammation Is a Metabolic Disease

Of the cardinal symptoms of inflammation: rubor, calor, dolor and tumor, we shall particularly consider a cellular aspect covered by the word tumor. Indeed, inflammation, like cancer, triggers the mitosis of a variety of cells, and common mechanisms may have to be controlled in order to remain within physiological limits. We shall first recall a few basic facts.

Inflammation is a natural response of the organism to an aggression, which can be traumatic, bacterial or viral. The defense involves first non-specific (innate) mechanisms, and second specific immunological mechanisms. The innate mechanisms cover the complement cascade, which leads to the activation of proteases able to destroy cells, the complement found in the serum, responds to an antigen-antibody complex, or might be directly activated by bacterial antigens. The innate defense is also associated to the release by the liver, of a variety of proteins the Hageman factor for example. The latter, induces a variety of events, first the coagulation cascade (thrombine-fibrinogen-fibrine); then the induction of the fibrinolytic-plasmine cascade; finally, the activation of the so called kallikrein-kinin system, which will have major actions on vascular permeability, leading to infiltration of tissues and pain, and to the release of lipid mediators of inflammation, eicosanoids-leucotrienes. The liver releases many other proteins (C reactive protein) the latter, binds phospholipids from bacteria, controlling the recruitment of macrophages, it also promotes the activation of the complement. The increase of proteins in the serum
increases the sedimentation velocity of red blood cells, measuring the intensity of inflammation.

An essential player of the innate line of defense is the mast cell; it will release histamine, causing vasodilatation, and TNF α (tumor necrosis factor); it will produce and release Interleukins (IL1) and generate lipid mediators from arachidonic acid: leukotrienes, prostaglandins. The mast cell also releases a platelet activation factor (PAF), which promotes the release of serotonin from platelets. The activation of the mast cell may result from an injury, or from an allergen-IgE recognition. Endothelial cells neutrophils and macrophages, which are attracted on the site by chemotactic factors (e.g., RANTES), form this first line of defense. There is a vasodilatation, the site is red and hot (histamine-serotonin), the vascular permeability increases (prostaglandin), swelling gives pain since nerves are compressed, and bradykinine is involved. As for IL1 and TNF α, they act on the hypothalamic center controlling fever. They cytokines also trigger the release of prostaglandin (E2), which activates cAMP dependent processes and catabolism, providing substrates to the site of inflammation. The patient experiences cachexia, anorexia, fatigue, and fever. This innate line of defense already explains three of the major symptoms of inflammation: rubor, calor, and dolor. But what about cell multiplication and “tumor”? We shall see that both lines of defense trigger the increased mitosis leading to the accumulation of neutrophils and macrophages.

The mitogenic effect involves signaling processes mediated by tyrosine phosphorylations, that have common features with tyrosine kinase receptors such as the insulin receptor. If we consider for example the Toll/IL1-1 receptors, or the TLR4 receptors that respond respectively to interleukin 1 (IL1) or to bacterial lipopolysaccharides, they both have a tyrosine kinase, intracellular domain. Downstream of the tyrosine kinase signal, and with the help of an adapter protein (MyD88), the IL-1R kinase (IRAK) gets activated, which leads after several steps, to a phosphorylation and to proteolysis of Iκb. This dissociates Iκb from its partener NF-κb. The latter can then move to the nucleus and transcribes proteins controlling cell mitosis. Nitric oxide released by macrophages may nitrosylate Iκb and dissociate it from NF-κb.

We shall now discuss mitogenic effects associated to the specific defense line. When a virus infects a cell for example, it presents on its surface the antigen, viral proteins for example, associated to the major histocompatibility complex of class 1 (MHC1). The complex will be recognized by a T cell lymphocyte of the cytotoxic type. The T cell receptor recognizes not only MHC1 as a self-identification device, but also motifs of the antigen. The cytotoxic T cell receptor will signal via its CD3 components, and via an essential protein (CD8) that a correct recognition of both the antigen and MHC1 took place, which activates again a tyrosine kinase (P56lck). In the case of cytotoxic T cells, the tyrosine kinase signal induces synthesis and secretion of enzymes perforating the contaminated cell, which is eliminated. The situation is different for helper T cells, they recognize antigens presented by the major histocompatibility complex of class 2 (MHC2) that appear on the surface of macrophages or B lymphocytes. The helper T cell receptor recognizes not only self-MHC2, but also the presented antigen. The helper T cell receptor will signal via
its CD3 component, but also via an essential protein of helper T cells (CD4), that the recognition of the antigen-MHC2 complex took place, which activates again the P56lck kinase. A variable menu of lymphokines are consequently produced, triggering a massive multiplication of immunologically competent cells. In all cases interleukin 2 (IL-2) autoactivates the multiplication of the helper T cell. In the case of an antigen presented by a macrophage, the helper T cell and the macrophage secrete a cocktail of lymphokines: II-6, TNFα, INFα, and colony stimulating factors for granulocyte and macrophages (G-CSF and GM-CSF). In the case of an antigen presented to helpers T cells by a B lymphocyte, which binds circulating antigens, the helper T cell secretes IL-4 and IL-6, which induce proliferation of the antigen presenting B lymphocyte. The selected population will proliferate and convert into a plasmocyte secreting antibodies in the plasma, which neutralize circulating antigens. For macrophage presentation, a cocktail of the same lymphokines induces their proliferation.

After this brief survey of immunological defense mechanisms, the point we want to make in relation to cancer is that these lymphokines will act on receptors that activate cellular proliferation via similar MAP kinase signaling pathways. In spite of many common features, there are different modalities to consider. In response to growth factors, the receptor is autophosphorylated on tyrosines, which bind the SH2 domains of adapter proteins. Then, RAS-GTP steps activate the Raf serine-threonine kinase, follows the mixed MEK kinase, which finally activates ERK. The nuclear translocation of ERK will allow the phosphorylation of transcription factors, regulating the expression of genes involved in mitosis. Two other MAP kinase pathways have been identified, they are particularly activated in response to inflammatory cytokines; in these pathways, JNK or P38 replaces ERK. At the receptor level, there are also differences, particularly when the receptor itself has no kinase activity. In this case it recruits a tyrosine kinase JAK, or src. In the case of JAK, there is a short cut, which activates STAT, the latter being translocated to the nucleus.

An interesting modality is related to the CD45 phosphatase that serves as receptor in lymphocytes, it will then modulate a downstream tyrosine kinase step, promoting mitosis. It is probable that scaffold proteins are involved for selecting among the different MAP kinase pathways.

After having recalled these basic features of inflammation, we shall discuss some possible links with cancer.

The proliferation of immunologically competent cells, the multiplication of embryonic tissues, or the proliferation of tumor cells seems to require the activation of a signaling mechanism, which indeed, depend on MAP kinases. Their activation first depends on a tyrosine kinase reaction, mediated by specific enzymes, or result from auto-phosphorylations, which can be induced by the binding of a ligand, or an antigen, to the tyrosine kinase receptor.

In cancer formation, oncogenes often results in a perturbation of normal signaling pathways. Similar perturbations can also occur in inflammation, when proliferating cells involved in immunologic defense escape from homeostatic controls. Indeed, if factors that trigger the proliferation of cells related to inflammation,
interleukins for example, are continually secreted because inflammation persists, the enhanced tyrosine kinase signals may result in uncontrolled proliferation of tissues and then oncogenic transformation. We know that tyrosine kinase receptor signals are not only triggers for the MAP kinase cascade, but that they also activate PI3 kinase, this is the case for the insulin receptor for example. Receptors coupled to G proteins, activated by transmitters released by inflammatory cells, may also activate PI3 kinase, leading in both cases to the conversion of phosphatidyl inositol 4,5, bisphosphate (PIP2) into phosphatidyl inositol 3,4,5 (PIP3). We know from the work of Hokin and Hokin (Hokin and Hokin 1953) that transmitters such as acetylcholine (ACh) acting on muscarinic receptors, elicit hydrolysis of phosphoinositides. Other transmitters released during inflammation (serotonin or histamine) may also activate such “Gq coupled” receptors, increasing phosphoinositides via the stimulation of a phospholipase C. If PI3 kinase is activated as well, inositol 1,3,4,5 (IP4) is formed, if not, inositol 1,4,5 (IP3) is released. In general, Gq coupled receptors activate phospholipase Cβ while tyrosine kinase receptors activate phospholipase Cγ. The released inositides mobilize calcium, thereby helping the exocytotic incorporation of the glucose transporter. Another action of phospholipids, like diacyl glycerol (DAG), activates protein kinase C, which mimics the tumoral action of phorbol esters, probably via a direct activation of ERK. The inflammatory transmitters also activate a phospholipase A2, releasing arachidonic acid. The latter, lead via cyclooxygenases (COX1, COX2) to prostaglandins and thromboxanes or to leukotrienes via lipoxygenases. The preventive effect of non-steroidal anti-inflammatory drugs in colon cancers likely depend on the inhibition of COX2, decreasing inflammation. But above all, we should recall that PTEN phosphatase hydrolyses phosphoinositides, decreasing their effects. Hence, if this phosphatase is down regulated, as it is observed in cancer, the PI3 kinase pathway and related lipidic mediators of inflammation will exaggerate their effects. If PI3 kinase signaling is upregulated, PTEN control is lost, resulting in increased frequency of mitosis. This may not necessarily lead to cancer, unless some other perturbation takes place at the level of the MAP kinase pathway, which controls mitosis. The synthesis of cell cycle proteins or tumor suppressor proteins is partly controlled by this pathway through transcription factors activated by ERK, JUNE, or P38, after their translocation in the nucleus.

So what might perturb the MAP kinase pathway in inflammation, and why should this favor the transformation of cells? Fig. 1.1 answers in part the questions raised. The tyrosine phosphorylation step is essential. In order to limit the mitogenic effects of the MAP kinase pathway, we have a natural inhibitory system performing tyrosine nitrosylation of proteins. This inhibitory system competes with phosphorylation of these tyrosines when the pathway is activated. Normally, nitrosylation is operated by peroxynitrite, formed when NO meets superoxides, which are generated by NADH oxidase, or if respiration does not generate enough electrons to fully reduce oxygen. If an aggression takes place, hypoxia induces via HIF1/Von Hippel-Lindau factor: NO synthase, glycolytic enzymes, carbonic anhydrase, VEGF, COX2, leading to inflammation; except that cell mitosis is still limited by the tyrosine nitrosylation mechanism. Then, purine catabolism gets activated,
Fig. 1.1 Inflammation. Metabolic stressors of tissues, such as hypoxia, induce via HIF1α and Von Hippel-Lindau factors: NO synthase, VEGF, COX2-prostaglandins, which explains three of the cardinal signs of inflammation (rubor, calor, dolor). Chemokines secreted at the site attract leukocytes and macrophages. Glycolytic enzymes are also induced in the inflamed tissue. The other sign of inflammation (tumor), involves mitosis of cells ensuring immunological defense.

xanthine oxidase becomes not only a source of superoxide, but also a source of an essential product: uric acid. The latter, is the natural inhibitor for tyrosine nitrosylations, which increases tyrosine phosphorylation and triggers the MAP kinase cascade. Mitosis occurs resulting in tumor formation, a cardinal sign of inflammation. In primates, who lack urate oxidase (Oda et al. 2002, Spitsin et al. 2002), uric acid becomes the natural activator of the MAP kinase pathway since it removes the nitrosylation brake on the MAP kinase pathway (Teng 2002). But uric acid also
inhibits formation of nitrocatechols and nitroindols, which normally inhibit methylases (Perez and Avila 1999, Huotari et al. 2001). Hence, uric acid helps methylation of PP2A and its activation, thereby counteracting the MAP kinase trigger of mitosis and limits its tumorigenic effects.

In other works we have discussed the role of nitrocatechols and nitroindols in relation to neurologic diseases, and compared them to endogenous neuroleptics (Israël 2004). The levels of these molecules decrease when uric acid increases. In normal conditions, uric acid controls this defense mechanism in primates; it is still operational when an excess of peroxynitrite or peroxycarbonate is formed, such as after inhaling NO or CO2 with cigarette smoke. But in this case, peroxynitrite converts urate into triurate, which inhibits tyrosine nitration, and thus pushes the MAP kinase pathway. Triurate has deleterious actions on lipid peroxidation (Robinson et al. 2004) and cannot inhibit formation of nitrocatechols as well as urate. Nitrocatechols increase, and the methylase is more inhibited, leading to PP2A becoming poorly methylated and inactive towards given substrates. We have seen that this situation favors mitosis. In sum, toxin exposure generates triurates which lead to poorly activated PP2A, which then fails to counteract the MAP kinase activation of mitosis.

A protection against the effects of triurate on lipid peroxidation and on PP2A could come from ascorbate (Frei 1991) or vitamine E, but primates do not synthesize these vitamins. Other mammals, such as rodents, can synthesize ascorbate. Rodents use ascorbate, as a single protection mechanism against lipid peroxidation and tyrosine nitrosylation. Probably, this explains why cigarette smoke interferes with urate defenses of primates, resulting in lung cancer, while rodents with different defense mechanisms, based on ascorbate, rather than urate, are more resistant to developing lung cancer after toxin exposure.

In this discussion we highlight major players in inflammation such as PP2A and PTEN. Specifically, PTEN is down regulated in cancer because the gene is hypermethylated, while PP2A is inactivated because of a cytosolic hypomethylation.

Cancer as a Metabolic Disease: Back to Otto Warburg

To understand regulation of human cell division, a detour to the world of microbiology is necessary, beginning with the work of Louis Pasteur himself. Pasteur (1822–1895) showed that the conversion from sugar to ethanol required living organisms, rather than a chemical catalyst, demonstrating that by decreasing the oxygen content in a yeast broth, the yeast cells could be made to divide, multiply, and ferment vigorously (the “Pasteur Effect”). In modern terms, this effect could be described as an activation of anaerobic glycolysis to meet cellular ATP needs.

Otto Warburg developed the work of Pasteur on fermentation. During his lifetime Warburg was generally regarded as the greatest biochemist of the 20th century. Warburg, Krebs and Meyerhoff showed that cancer cells were anaerobic in nature, more akin to fungus or bacteria than to normal mammalian cells.
A vicious circle leading to cancer. 1. There are conditions related to food: methyl sources /methyl baits, vitamins, or genetic susceptibilities, that lead to a methylation deficit in the cytosol.

2. This affects the activity of PP2A phosphatase, this enzyme has hundreds of variable isoforms which may be affected by many different external agents: viral, physical or chemicals that after the different isoforms. The resulting effect is a hypomethylation in affected cells. The PP2A subunits do not assemble.

3. The phosphatase loses its specificity for given protein targets, leading to effects on proteins controlling mitosis, and also to a glycolytic metabolic "bottle neck", because Pyruvate kinase remains in its inactive M2 phosphorylated form, rather than switching to the active M4 tetramer.

4. There is a compensatory insulin signaling process, entering glucose, saturating mitochondria shuttles above the neck, Lactate dehydrogenase forms NAD+ and lactate (Warburg effect). Below the neck, Alanine transaminase (ALAT), the Malic enzyme, form pyruvate, but the lactate sink is deep. Hence, fatty acid catabolism has to form the necessary acetyl-coA, not provided by pyruvate dehydrogenase, oxaloacetate comes via an abnormal carboxylation of phosphoenolpyruvate by mitochondrial PEP carboxy kinase.

5. The activation of insulin–tyrosine kinase–MAP kinase signaling, activates PP1 phosphatase; 6. PP1 acts on the cell cycle
As early as 1920, Warburg knew how to inject tumoral suspensions into the peritoneum of mice, and how to measure their gas concentration. He understood that cancer is a disease of cellular breathing and that cancers are often hypoxic.

He also understood that all cancer-producing substances (arsenic, tars, and cyanide) decrease cellular respiration. Cancer cells ferment even in presence of oxygen. Either oxygen cannot reach the cell, or it cannot be utilized. In the 1920’s, Warburg had identified these two phases: first, hypoxia alters cell metabolism; second, if the cell survives these anomalies, the latter will produce cancer. Despite the Nobel prizes discerned to Otto Warburg, Krebs and Meyerhoff their work has largely been forgotten. The main reason for this ignorance of the work done at the beginning of the past century lies in the multiple dramas of that period.

And what could be easier, when you understand cellular respiration than to choke it? From the start of the First World War, German scientists working on cellular respiration lent their knowledge to the German war effort, with the resulting devastation caused by yperite and other combat gases. Yet another poison was synthesized: Zyklon B, used so “successfully” in the death camps of the Second World War. Needless to say, this research on cellular respiration mechanisms – whatever it’s original merits – suffered greatly from the ensuing madness. Furthermore, Warburg was an eccentric genius who had many enemies. He proposed a theory of cancer dependent on glycolysis that was initially greeted enthusiastically but was later widely ridiculed in academic circles (Nachmansohn 1979, Guillemim and Krasnow 1997). Although Warburg’s data were impeccable, other scientists later claimed to find exceptions to Warburg’s rule. In time, Warburg’s theory became not just old-fashioned but anathema to a scientific establishment that was increasingly focused on viruses and aberrant genes as the source of cancer. Since then, the Positron Emitting Tomography (PET) scan has revived Warburg’s work.

For long, the prodigious expense and ponderous size of the cyclotron (housed in its own building and managed via a network of intricate controls) and the cumbersome requirements of staff prohibited its clinical use. Over the years, refinements made it more practical, more manageable, and less complicated. The cyclotron was downsized. It could fit into a hospital.

Fig. 1.2 (continued) synergistically with the PP2A deficit, leading to the permanent activation of mitosis. 7-MAP kinases affect lamine phosphorylation, the nuclear membrane becomes permeable to methyl donors such as S-adenosyl methionine (SAM), it is retained in the nucleus, cytosolic methylase become less active, while the nuclear methylases are boosted. 8-There is an increase of nuclear methylations, the hypermethylation of promoters will silence genes (PTEN), but also demethylase inhibitors; thus, other parts of the genome get hypomethylated, activating other genes (hexokinase gene). 9-Consequently PTEN and HPM1 phosphatases are silenced, increasing insulin – PI3 kinase – MAP kinase effects, while hexokinase and glucose influx increase, aggravating the effect of the PP2A failure, on the cell cycle. In sum there is a cytosolic hypomethylation and a nuclear hypermethylationhypomethylation process, which upsets the phosphatases and leads to catastrophic changes of metabolism and mitosis, generating cancer.
Using a radioactive analog of glucose, PET scan examination has revived Warburg’s work. Modern imaging confirms the increased uptake by cancer of large quantities of glucose.

Fermentation provides energy, though less efficiently than respiration (Guillemin and Krasnow 1997, Schwartz 2004) for, in the presence of sufficient oxygen, glucose is completely degraded into water and carbonic gas, hence the efficiency of respiratory metabolism. At lower concentrations of oxygen, by contrast, the glucose is incompletely degraded and waste is produced, a portion of which is released into the extracellular component. Some wastes such as ethanol produced by glycolysis are highly valuable.

Other “waste products” of anaerobic glycolysis remain within cells, causing cell mass to increase, and reacting to form amino acids, lipids, glucids and nucleic acids (Schwartz 2004, Palmer 1985, Wang et al. 1995, Gerasimovskaya et al. 2002, Wada et al. 2002). These amino acids, in turn, form proteins, the lipids are transformed into hormones; and the nucleic acid provides DNA and RNA. In this manner, anaerobic glycolysis provides cells with all the requirements for mitosis, both physical (sufficient cell mass) and biochemical (suitable molecular species).

Modern biology has been hindered by the discoveries of hundreds of what have been identified and named or, rather, misnamed, “growth factors”. Originally, the concept of “growth factors” was limited to polypeptides, but it now extends to include sugars and fats such as triglycerides. All such molecules have one thing in common: they deliver energy to the cell. There are, for example, no polypeptidic “growth factors” which cannot be transformed into energy. These so-called growth factors can be utilized on the spot or exported as secretions to be used by distant cells, as in the case of steroid hormones (derivatives of cholesterol, that is, of fat). Some growth factors like insulin or insulin like growth factor (IGF1 and 2) further increase the glucose uptake and metabolism.

Pharmacologists modify “growth factors” and synthesize false nutrients that cannot be transformed into available energy for the cell. As a result, cell metabolism is reduced and the cell may ultimately starve to death.

Once cancerous, the metabolism of a cancer cell remains glycolytic even in the presence of oxygen (the Warburg Effect), with concurrent hypoxia responsible for an elevated incidence of mutations whose pattern is similar to that observed in tumors (Reynolds et al. 2002). For example, an oncogene originally activated by hypoxia will result in the accumulation of p53 and an increased concentration of the Hypoxia Inducible Factor (HIF), thus mimicking a situation of reduced oxygen availability (Chan et al. 2002). Hypoxia also results in the secretion of proteases. These may destroy the basement membrane and enable the cancer cells to invade the surrounding normal tissue and spread to distant organs.

Most if not all of the properties of cancer can be explained by hypoxia and the resulting anaerobic glycolysis: carcinogenesis, cancer fractal growth, cell proliferation, loss of cell differentiation, loss of cell polarity, metastasis, and resistance to conventional cancer.
Inflammation and Cancer Are Dysmethylation Syndroms

Recent work from Abdolhassani (Abolhassani et al. 2008) and his colleagues demonstrates that inflammation is a consequence of a dysmethylation syndrome. In short, they show that in every model of inflammation studied, the inflammatory cascade is a direct consequence of the methylation of the catalytic subunit of PP2A.

Similarly work by Israel (Israel and Schwartz 2006) and then Guenin (Guenin et al. 2008) demonstrates that tumor response is controlled by methylation of PP2A adding further insight into the epigenetic regulation of cancer.

The cholinergic deficit and the increase of homocysteine are often found in Alzheimer’s disease. Another essential methylation is also deficient in this disease: the methylation of PP2A phosphatase. As previously stated, this phosphatase controls key enzymes of glycolysis, pyruvate kinase, but also the phosphorylation of other proteins, Tau protein, controlling in this way, tubulin polymerization. The PP2A deficit leads to hyperphosphorylated Tau and tangles. Homocysteine also reacts with serine, giving cysteine, a step requiring vitamin B6. Cysteines like glutathion control the folding of proteins, their proteolysis and are in this way involved in the formation of plaques.

The same effect could take place in inflammation and tumors, and the PP2A block towards given proteins, presumably shuts down pyruvate kinase, leading to a “bottle neck” in glycolysis, and to the Warburg effect (Israel and Schwartz 2006). The PP2A methylation deficit also alters tubulin polymerization and perturbs the spindle. But in cancer, the possible SAM decrease, which impaired the cytosolic methylation of PP2A, seems to be associated to an excess of nuclear DNA methylations, leading to the silencing of genes such as PTEN, and to changes activating demethylases, which favor the expression of other genes such as hexokinase. The metabolic result, is a facilitation of PI3kinase signals linked to the insulin-tyrosine kinase pathway, which also gets enhanced. Consequently MAP kinases are activated, and mitosis is triggered.

The activation of nuclear methylases, could be a consequence of inflammation or hypoxia acting via arachidonic inflammatory derivatives on these methylases, the nuclear membrane becomes permeable to SAM, while the cytosolic methylases are blocked in a non-specific configuration. We have abundantly discussed the different external triggers leading to this metabolic situation in which cytosolic hypomethylations are associated to nuclear hypermethylations-hypomethylation shifts. In comparison and schematically, Alzheimer’s disease displays only the cytosolic methylation deficit, while tumors alter as well their nuclear methylation programs.

Tumors also secrete proteases that disrupt the controls of differentiation, which are also linked to the proteolysis of contact proteins. Mitosis and the development of the cancer mass is certainly most impressive, but the catastrophic metabolic situation in which the organism burns proteins and lipids for burning glucose is really terrible. Clinically, this can present as cancer cachexia.