

## REVIEW ARTICLE

**The Immunobiology of Cancer: An Update Review**Anna Meiliana<sup>1,2,\*</sup>, Nurrani Mustika Dewi<sup>1,2</sup>, Andi Wijaya<sup>1,2</sup><sup>1</sup>Postgraduate Program in Clinical Pharmacy, Padjadjaran University, Jl. Eijkman No.38, Bandung, Indonesia<sup>2</sup>Prodia Clinical Laboratory, Jl. Cisangkuy No.2, Bandung, Indonesia

\*Corresponding author. E-mail: anna.meiliana@prodia.co.id

Received date: Jun 21, 2017; Revised date: Jul 20, 2017; Accepted date: Jul 28, 2017

**Abstract**

**BACKGROUND:** The introduction of mechanism-based targeted therapies to treat human cancers has been pledge as one of the results of three decades of remarkable progress of research into the mechanisms of cancer pathogenesis. We ponder how the description of hallmark principles is start to inform therapeutic development currently and may increasingly do so in the future.

**CONTENT:** There are 10 biological capabilities involved as the hallmarks of cancer, during the multistep of human tumors development. These hallmarks simplify the complexities of neoplastic disease into a structured rational principles, includes sustaining proliferative signaling,

eluding growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, genome instability, inflammation, reprogramming energy metabolism and evading immune destruction.

**SUMMARY:** The 10 hallmarks of cancer, in other words, the tumor's distinctive and complementary capabilities that enable its growth and metastatic dissemination, continue to provide a solid foundation for understanding the biology of cancer. The acknowledgement of the widespread applicability of these concepts will increasingly influence the development of new manners to treat human cancer.

**KEYWORDS:** hallmark of cancer, cancer genome, inflammation, cancer immunology, metastasis

*Indones Biomed J. 2017; 9(2): 53-72***Introduction**

Hanahan and Weinberg have suggested that 6 hallmarks of cancer together form an organizing principle that offers a logical framework for understanding the remarkable diversity of neoplastic diseases. As normal cells evolve progressively to a neoplastic state, they obtain a succession of these hallmark abilities, and that the multistep process of human tumor pathogenesis could be rationalized by the need of incipient cancer cells to obtain the traits which enable them to become tumorigenic and ultimately malignant.(1) Tumors are not merely insular masses of proliferating cancer cells. Instead, they are complex tissues composed of multiple distinct cell types that engage in heterotypic interactions

with one another. Normal cells recruited can form tumor-associated stroma and shift the cells not again as a passive bystanders but actively participated in tumorigenesis, as such, the stromal cells formed also involved in certain hallmark capabilities development and expression. The biology of tumors can no longer be understood only by enumerating the traits of cancer cells, yet must encompass the contributions of the "tumor microenvironment" to tumorigenesis instead.(1)

Over the last 10 years, the genomic landscapes of common forms of human cancer have been revealed thanks to the comprehensive sequencing attempts. For most cancer types, this landscape consists of a few number of "mountains" (genes changed in a great percentage of tumors) and a much larger number of "hills" (genes changed infrequently). Up

to now, these studies have revealed about 140 genes that, when changed by intragenic mutations, can encourage or “drive” tumorigenesis. A typical tumor contains 2 to 8 of these “driver gene” mutations, the remaining mutations are passengers which give no selective growth benefit. Driver genes can be classified into 12 signaling pathways that regulate three core cellular processes, which are cell fate, cell survival and genome maintenance. A better understanding of these pathways is one of the most importunate needs in basic cancer research. Even at the moment, our knowing of cancer genomes is enough to guide the development of way effective approaches for reducing cancer morbidity and mortality.(2) The results of these attempts and the rigor with which they are implemented will determine whether and how comprehensive tumor genomic information may be incorporated into the routine care of patients with cancer.(3)

Because of the definitive role of inflammatory responses in tumor development at many stages including initiation, promotion, malignant conversion, invasion and metastasis, it affects therapy response and immune surveillance. After infiltrating tumors, immune cells engage in a comprehensive and dynamic crosstalk with cancer cells. Researchers have revealed some of the molecular events that mediate this dialog.(4) The immune system can respond to cancer cells in two ways, either by reacting against tumor-specific antigens (molecules that are unique to cancer cells), immunity to carcinogen-induced tumors in mice is directed into this antigens, or against tumor-associated antigens (molecules that are expressed differently by cancer cells and normal cells).(5,6)

Gotten argumentative idea from immunosurveillance hypothesis, we supposed that the immune system could be harnessed to recognize malignant cells as foreign agents and eliminates them, this was now become available after our understanding about tumor immunity and have been improved with better techniques. In mouse models with gene deletion to eliminate the immune effector mechanisms such as the type 1 interferons, the study showed that immune system clearly reduced the incidence of tumor.(7-11) Three outcomes were possible as the immune system encountered a nascent tumor, initiating a process termed as “immunoediting”(12); elimination of the cancer, cancer equilibrium, when there is immune selection of less immunogenic tumors during an antitumor immune response (13); or tumor escape, which is the growth of tumor variants that resist immune destruction (12).

In the past 25 years, progress in immunology guide to a new understanding and set a bridge of cellular and molecular interplays between the immune system and a

tumor, suggest the cancer research as a myriad phenotypic complexities and manifestations of a small set of underlying organizing principles.(14)

## The Hallmark of Cancer

Cancer is usually viewed as an evolutionary process that results from the accumulation of somatic mutations in the progeny of a normal cell which leads to a selective growth merit in the mutated cells and ultimately to uncontrolled proliferation.(15,16) Human cancer occurred most easily in epithelial tissues such as the skin, colon, breast, prostate or lung.(17) Cancer research has in latest decades characterized the cellular and molecular events that enable the malignant transformation of cells harboring oncogenic alterations. These events include uncontrolled proliferation; evasion of tumor suppression; inhibition of cell death; creation of a particular microenvironment containing blood vessels, stromal and immune cells; and the acquisition of invasive and metastatic potential.(1) Furthermore, our knowledge of oncogenes and tumor suppressor genes has been enriched by the development of next-generation sequencing techniques, which have identified a lot of genes which are mutated in different types of cancer.(18) In other words, the ability to sustain chronic proliferation become the most fundamental character of cancer cells. Carefully, the normal tissues control the production and release of growth-promoting signals which instruct inlet into and progression via the cell growth- and-division cycle, therefrom insuring a homeostasis of cell number and so the maintenance of normal tissue architecture and function. Through these signals, cancer cells can regulate their own destinies. Largely, the enabling signal were transmitted by growth factor which bind cell-surface receptors, typically containing intracellular tyrosine kinase domains.(1)

The proliferative signaling in cancer cells, are capable to sustain in many alternative ways, which are stimulating autocrine proliferative via the expression of cognate receptors, by producing growth factor ligands themselves, or stimulating normal cells within the supporting tumor-associated stroma, which will repay with various growth factors for cancer cells.(19,20) Receptor signaling can also be deregulated by raising the levels of receptor proteins displayed at the cancer cell surface, rendering such cells hyper-responsive to otherwise-limiting numbers of growth factor ligand. The same outcome can result from structural changes in the receptor molecules which facilitate ligand-independent firing. Many tumor suppressors which operate

in various ways to confine cell growth and proliferation have been uncovered through their characteristic inactivation in one or another form of animal or human cancer. Many of these genes have been validated as bonafide tumor suppressors by way of gain- or loss-of-function experiments in mice. The retinoblastoma-associated (RB) and tumor protein (TP)53 proteins are encoded by two prototypical tumor suppressors. Those suppressors are operating as central control nodes within two key complementary cellular regulatory circuits which govern the decisions of cells to proliferate or, alternatively, activate senescence and apoptotic programs.(1)

Cancer cells with blemishes in RB pathway function are missing the services of a critical gatekeeper of cell-cycle progression whose absence approves persistent cell proliferation. Over than last two decades, studies has established a natural barrier to cancer development known as programmed cell death (apoptosis).(20-22) Tumor cells unfold a variety of strategies to confine or circumvent apoptosis. The most common is loss of TP53 tumor suppressor function, which eliminates this critical damage sensor from the apoptosis-inducing circuitry. Other ways to attain this, tumors might downregulating proapoptotic factors (Bax, Bim, Puma), or by short-circuiting the extrinsic ligand-induced death pathway to increase expression of antiapoptotic regulators (C cell lymphoma (Bcl)-2, Bcl-xL) or of survival signals (insulin-like growth factor (IGF)-1/2). The multiplicity of apoptosis-avoiding mechanisms presumably potrays the diversity of apoptosis-inducing signals which cancer cell populations encounter throughtout their evolution to the malignant state.(1,23)

Probably more important, necrotic cell death releases proinflammatory signals into the surrounding tissue microenvironment, in contrast to apoptosis and autophagy, which do not. As consequence, necrotic cells can recruit inflammatory cells of the immune system (4,24) whose dedicated function is to survey the degree of tissue damage and repeal associated necrotic debris. In neoplasia, multiple lines of evidence show that immune inflammatory cells can be actively tumor promoting, knowing that such cells are capable of fostering angiogenesis, cancer cell proliferation and invasiveness. The two barriers to proliferation, senescence and crisis/apoptosis, have been rationalized as crucial anticancer defenses which are hard-wired into our cells, being deployed to impede the outgrowth of clones of preneoplastic and frankly neoplastic cells. Thus, cell senescence is arising conceptually as a protective barrier to neoplastic expansion which can be triggered by various proliferation-associated anomalies, including high levels

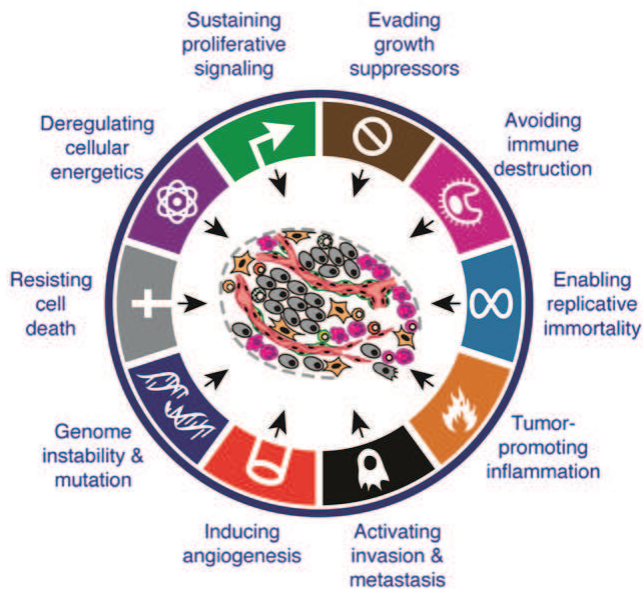
of oncogenic signaling and subcritical shortening of telomeres.(1)

Identical to normal tissues, tumors need sustenance in the form of nutrients and oxygen and also a capability to evacuate metabolic wastes and carbon dioxide. This could be utilized by angiogenesis process to generated tumor-associated neovasculature. The angiogenic switch is ruled by countervailing factors comprised of signaling proteins which is bind to stimulatory or inhibitory cell-surface receptors showed by vascular endothelial cells, then induce or oppose the process.(25,26) The notable prototypes of angiogenesis inducers and inhibitors are vascular endothelial growth factor (VEGF)-A and thrombospondin (TSP)-1, respectively.(1)

VEGF gene expression can be upregulated both by hypoxia and by oncogene signaling.(27-29) A developmental regulatory program, called as the “epithelial-mesenchymal transition” (EMT), has become prominent can obtain the capabilities to invade, to resist apoptosis, and to disseminate.(30-34) By co-opting a process involved in many different steps of embryonic morphogenesis and wound healing, carcinoma cells can concomitantly gain multiple attributes that enable invasion and metastasis. This multifaceted EMT program can be activated transiently or stably, and to differing degrees, by carcinoma cells throughtout the course of invasion and metastasistly implicated as a means by which transformed epithelial cells. We have described the hallmarks of cancer as obtained functional capabilities which allow cancer cells to survive, proliferate and disseminate. These functions are obtained in different tumor types via distinct mechanisms and at various times during the course of multistep tumorigenesis. There are two enabling characteristics facilitating the acquisition. Most prominent is the development of genomic instability in cancer cell that generates random mutations including chromosomal rearrangement. Among these are the rare genetic alterations that can create hallmark capabilities. A second enabling characteristic involves the inflammatory state of premalignant and frankly malignant lesions which is driven by cells of the immune system, some of them are serving to promote tumor progression via various manners.(1)

Another functionally important attribute of cancer cells development might therefore be added to the list of core hallmarks.(35-37) The first involves the major reprogramming of cellular energy metabolism to support continuous cell growth and proliferation, replacing the metabolic program that operates in most normal tissues and fuels the physiological operations of the associated cells.

## The Hallmarks of cancer



**Figure 1. The hallmarks of cancer.**(1) (Adapted with permission from Elsevier).

The second one involves active evasion by cancer cells from attack and elimination by immune cells, this ability highlights the dichotomous roles of an immune system that both antagonizes and enhances tumor development and progression. Both of these capabilities may well prove to facilitate the development and progression of many forms of human cancer and therefore can be considered to be emerging hallmarks of cancer (Figure 1).(1)

### The Cancer Genome Landscapes

Most cancers are driven by genomic changes which dysregulate key oncogenic pathways influencing cell growth and survival. Just now, we can exploit tumor genetic information for its full clinical potential. Over these past years, the convergence of discovery, technology and therapeutic development has established an unparalleled opportunity to examine the hypothesis that systematic knowledge of genomic information from individual tumors can improve clinical outcomes for many cancer patients.(3)

Ten years ago, the idea that all genes changed in cancer could be identified at base-pair resolution would have seemed like science fiction. Now, such genome-wide analysis is routine, done by sequencing of the exome or of the whole genome. Compare to more than \$100,000 cost per case when first prototypical exomic studies of cancer evaluated about 20 tumors, now studies reporting the

sequencing of more than 100 tumors of a given type are the norm with cost that has been reduced 100-fold.(38-40) Although vast amounts of data can now be readily obtained, deciphering this information in meaningful terms is still challenging.(2)

When do mutations occur? Studies in colorectal tumors told a story about a series of mutation over time, evolving the benign into malignant.(41,42) First mutation known as the “gatekeeping”, furnish the normal epithelial cell with selective growth advantage. The gatekeeping capable to outgrow surround cells to become a microscopic clone. Gatekeeping mutations in the colon most often happen in the adenomatous polyposis coli (APC) gene.(43) The small adenoma which results from this mutation grows slowly, but a second mutation in another gene, such as K-Ras, unleashes a second round of clonal growth which allows an expansion of cell number.(42) The cells with only the APC mutation may remain, but their cell amounts are small compared with the cells that have mutations in both genes. The mutation process followed by clonal expansion. Genes mutations such as phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), Smad4 and TP53. In the end will generate a malignant tumor which can invade through the underlying basement membrane, and can metastasize to lymph node to reach a distant organs such as liver.(44)

But, that huge complex information described about cancer genomes sometimes could be misleading. After all, even advanced tumors are not entirely out of control, as evidenced by the dramatic responses to agents that target mutant B-Raf in melanomas (45) or mutant anaplastic lymphoma kinase (ALK) in lung cancers (46). Apparently, even a single mutant gene interfering could stop cancer in its track, though just for a moment. This known as Albeit transient. The driver genes currently known to be classified into one or more of 12 pathways. The discovery of the molecular components of these pathways is one of the biggest achievements of biomedical research, a tribute to investigators working in fields which encompass biochemistry, cell biology and development, and also cancer. Based on core cellular processes, these pathways can be organized into cell fate, cell survival and genome maintenance. Many studies showed the opposing relationship between cell division and differentiation, as the arbiters of cell fate. Cell-autonomous alterations made cancer cells divide abnormally, such as those controlling cell fate, their surrounding stromal cells are absolutely normal and do not keep pace, but with abnormal vasculature of tumors which is asymmetry ramification. As opposed to



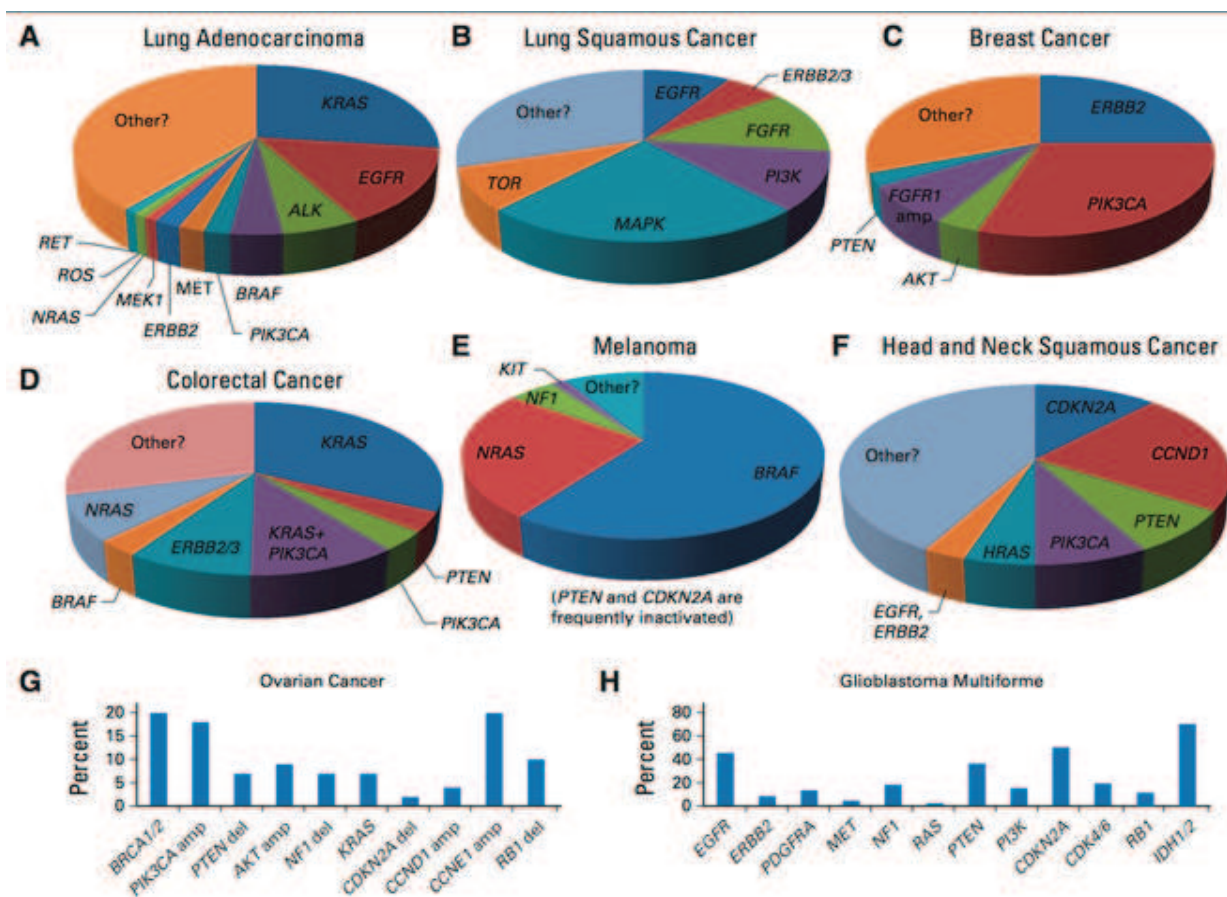
the well-ordered network of arteries, veins, and lymphatics which control nutrient concentrations in normal tissues, the vascular system in cancers is tortuous and lacks uniformity of structure.(47,48) Surrounding with bizarre microenvironment, cancer cells are toxically exposed by many substance, for example reactive oxygen species (ROS). Even without microenvironmental poisons, cells could have mistakes while replicating their DNA or during division (49,50), and checkpoints exist to either slow down such cells or make them commit suicide (apoptosis) under those circumstances (51-53).

In past few years, a consilience of tumor biology, genomics technology, computational innovation and drug discovery has encouraged unprecedented advances in translational cancer research. This revolutionary scientific landscape has been punctuated by a growing amount of examples wherein the knowing of specific tumor genetic underpinnings has announced rational clinical deployment of targeted agents. When a driver genetic alteration (*e.g.*, mutation which dysregulates a protein on which the cancer cell depends critically for viability) is effectively intercepted

by a targeted small molecule or monoclonal antibody, impressive clinical responses may result. Genetic molecular framework raise a promising results and overarching hypothesis for the cancer genome era, especially in non-small-cell lung cancer and melanoma, which previously had been exemplified the limitation of empirical treatment. This hypothesis is undergirded by three main principles gleaned from over past a decade research performed over the past decade, described in detail as follows (3):

### Principle 1

Genetic alteration can enact the molecular pathways involved in tumor survival and progression. Somatic genetic derangement plays an important role in the genesis and maintenance of human cancers in majority. Tumorigenesis can also be promoted by germline mutations in important subsets of patients. The discovery of recurrent genomic alterations dramatically expanded the compendium of oncogenes and tumor suppressor genes while also offering crucial insights into specific cellular processes that become dysregulated in carcinogenesis, such as cell differentiation



**Figure 2. Genomic alterations affecting actionable signaling pathways in common solid tumors.(3)** (Adapted with permission from American Society of Clinical Oncology).

(and arrest thereof), proliferation, apoptosis, tumor metabolism, and chromatin biology.(1) As illustrated in Figure 2, genomic changes that are druggable in principle take place in a substantial proportion of several major tumor types. Most of these tumors also contain additional mutations, which, even though not directly druggable themselves, might dysregulate down-stream effectors targeted by existing or developmental therapeutics. One widely cited example is lung adenocarcinoma, where at least 60% of patients harbor driver oncogenic signaling pathway mutations.(54) Breast cancer is noteworthy in this regard too. Majority of patients harbor driver changes (mutation or gene amplification) affecting genes such as the human epidermal growth factor receptor (HER)2 and fibroblast growth factor receptor (FGFR)1 tyrosine kinases, PIK3CA (which encodes a catalytic subunit of phosphoinositol-3 kinase (PI3K)), or Cyclin D1 gene (CCND1) (which encodes cyclin D, a cell-cycle protein that may render dependency on cyclin-dependent kinases (CDK) and sensitivity to CDK inhibitors).(55-58) Acute myeloid leukemia, brain tumors, and some other malignancies involve repetitive hotspot mutations in the isocitrate dehydrogenases 1 and 2.(39,59), enzymes that initially gained recognition for their roles in basic cellular metabolism (*e.g.*, the tricarboxylic acid cycle). More recently, cancer-associated isocitrate dehydrogenase (IDH)1 and IDH2 mutations were shown to generate an oncometabolite, known as 2-hydroxyglutarate (60,61), which regulates DNA (hyper)methylation (62,63), histone demethylation (64) and oxygen-sensing enzymes (65). Though specific tumorigenic mechanisms remain badly understood, the expanding recognition of epigenetic and metabolic pathways in tumor biology and progression has spawned new drug discovery attempts (66-69) that will likely influence future genomics-driven therapeutic directions.

### Principle 2

Oncogenic pathways targeted anticancer agents, should have entered clinical trials. We have decade-long knowledge about how genomic alteration govern the tumorigenic mechanism, but still it was not enough as a basis to revolutionize the oncology treatment framework. The advances in cancer drug development witnessed unprecedentedly. A vast spectrum of therapeutics directed toward multiple effector proteins spanning most cancer signaling pathways has incorporated clinical trials and, in some cases, clinical practice. Regarding the latter, the National Cancer Institute in 2012 listed 19 targeted therapeutics (including 15 signal transduction inhibitors and four targeted antiangiogenic drugs) which

had received regulatory approval in oncology.(70) This value, which does not include immunotherapy or antibody-drug conjugates, competes the total number of targeted anticancer agents in the whole developmental pipeline just 8 years earlier.(71) Nearly 150 additional compounds are listed as clinical candidates in a public database (72), and dozens more proprietary compounds have entered development.

### Principle 3

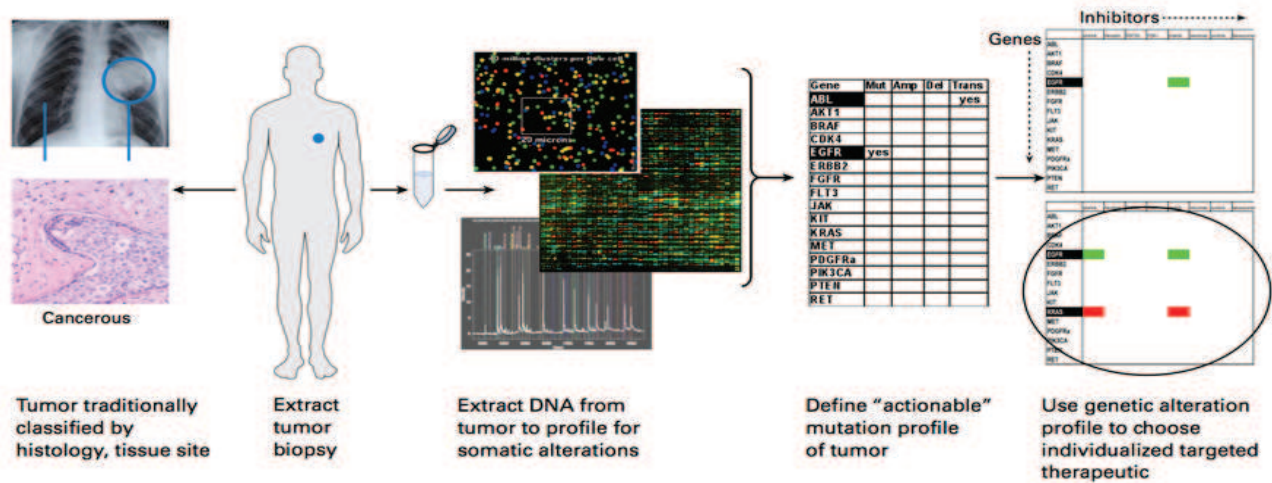
Tumor genomic profiling was now enable in the clinical arena, due to advances genomic technologies. This will support to deliver a targeted precision medicine therapeutic for patients. Subjects have to undergo a stratification based on their individual tumor genetic/molecular make up to find the salient cancer gene mutations especially for patients with advanced disease.

Cancer is a disease of the genome. So it needs a constellation of genomic structure alteration to dictate their clinical behavior as the treatment response. Whereas explaining the nature and importance of these genomic changes has been the objective of cancer biologists for come decades, ongoing global genome characterization efforts are revolutionizing both tumor biology and also the optimal paradigm for cancer treatment at an unprecedented scope. The pace of advance has been empowered, in large part, through disruptive technological innovations which render complete cancer genome characterization feasible on a big scale.(73)

Determining a rational cancer therapeutic choice for better patients outcomes will be enabled with comprehensive genomic information available for oncology field. In the next decade cancer treatment will head to a genomics-driven. The paradigm would be a complementary to other frameworks that shaping the development of oncology therapeutic, i.e immunotherapy, stem cell based therapy or tumor microenvironment targeting therapy (Figure 3).

## Inflammation, Immunity and Cancer

Inflammation is classically regarded as a feature of innate immunity, which is different from adaptive immunity by the receptors mediating its activation and its rapid onset. Innate immunity is also more evolutionarily ancient than adaptive immunity and is triggered by foreign microbial and viral structures, that known as pathogen-associated molecular patterns (PAMP), or normal cellular constituents released upon injury and cell death, called as damage-



**Figure 3. Schema of personalized medicine.** A genome-based vision for personalized cancer medicine will require a paradigm shift in both diagnosis and treatment. Traditionally, tumors are classified by site of origin. In the future, tumor nucleic acid will be profiled for a wide array of genomic alterations with a view to specific, tailored treatment options for each individual patient.(73) (Adapted with permission from American Society of Clinical Oncology).

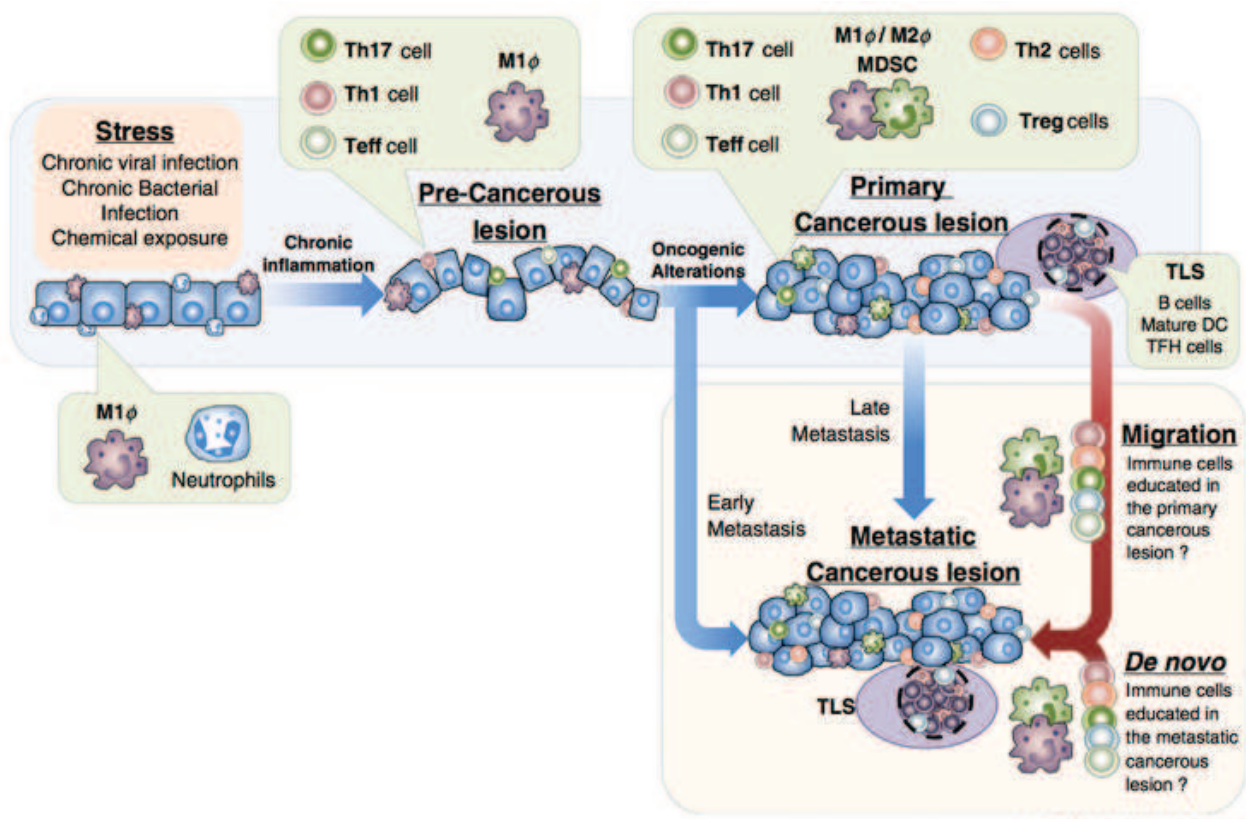
associated molecular patterns (DAMP). PAMPs and DAMPs can be recognized by pattern-recognition receptors (PRRs), whichever belong to the toll-like receptor (TLR) family.(73,74) Once activated, innate immunity results in upregulation of MHC class I and II and costimulatory molecules, a numerous inflammatory chemokines and cytokines which attract and prime T cells for activation through diverse antigen receptors.(75) While in adaptive immunity, T and B lymphocytes will be activated and further amplify the initial inflammatory response. So, type 1 helper T cells (Th1 cells) activate macrophages both through cell-to-cell contact and interferon (IFN)- $\gamma$  secretion, Th2 cells activate eosinophils via cytokine release, and B cells secrete antibodies that activate the complement cascade, as well as phagocytes, NK cells and mast cells, through Fc receptors. (75-80) However, the inflammatory response could be put off by particular immune cells, especially Tregs (81).

The presence of leukocytes within tumors, was observed back in the 1800s by Rudolf Virchow, given the first indication of a possible link between inflammation and cancer. But it is only during the last decade that clear evidence has been obtained that inflammation has an important role in tumorigenesis, and some of the underlying molecular mechanisms have been elucidated.(83) A role for inflammation in tumorigenesis is now generally accepted, and it has become obvious that an inflammatory microenvironment is an essential component of all tumors, including some in which a direct causal relationship with inflammation is not yet proven.(84) The fact is, less than 10% of all cancers are caused by mutations in germline, mostly it was due to somatic mutations coupled with environmental

factors (associated with chronic inflammation). Up to 20% of cancers related to chronic infections, 30% to tobacco smoking and inhaled pollutants (such as silica and asbestos), and 35% was related to dietary factors (20% of cancer burden is linked to obesity).(85)

The link between inflammation and tumor development has been extensively recognized.(84) At precancerous stages, the presence of chronic inflammation in an organ helps promoting cancer outbreak and growth. The inflammatory context may be the result of viral infection such as human papillomavirus (HPV) in cervical (86) and head and neck carcinoma (87) or hepatitis B virus (HBV) and hepatitis C virus (HCV) in hepatocellular carcinoma (88); external bacterial infections, such as *Helicobacter pylori* in gastric cancer (89) and non-Hodgkin gastric lymphoma (90) or the augmented permeability of the epithelial barrier to commensal microbiota promoting the development of colorectal cancer (CRC)(91); or the presence of noxious chemicals such as smoke for lung cancer (92) (Figure 4). The inflammatory milieu can promote tumor growth through the production of cytokines such as interleukin (IL)-6, IL-1 or tumor necrosis factor (TNF)- $\alpha$  (84), in addition to angiogenic molecules such as VEGF-A, transforming growth factor- $\beta$  (TGF- $\beta$ ), adenosine, prostaglandin E2 (93) and suppressor myeloid or T cells attracting chemokines, including c-x-c motif ligand (CXCL)1, CXCL5, c-c motif ligand (CCL)2 and CCL12.(94,95) Indeed, preventive treatments with anti-inflammatory agents, such as aspirin or Cox-2 inhibitors have shown efficacy in decreasing the incidence of CRC.(96) More strikingly, the antibiotics cure of *Helicobacter pylori* infections and the vaccination





**Figure 4. Major events and immune players in cancer natural history.** A cartoon depiction of the development of a cancerous lesion in which the normal tissue is exposed to stress that leads to an influx of inflammatory cells, mainly from the innate immunity. If chronic inflammation continues, there are prominent changes in the infiltration that will then be characterized by M1 macrophages, Th1 and Th17 cells and T effector (Teff) cell presence.(105) (Adapted with permission from Elsevier).

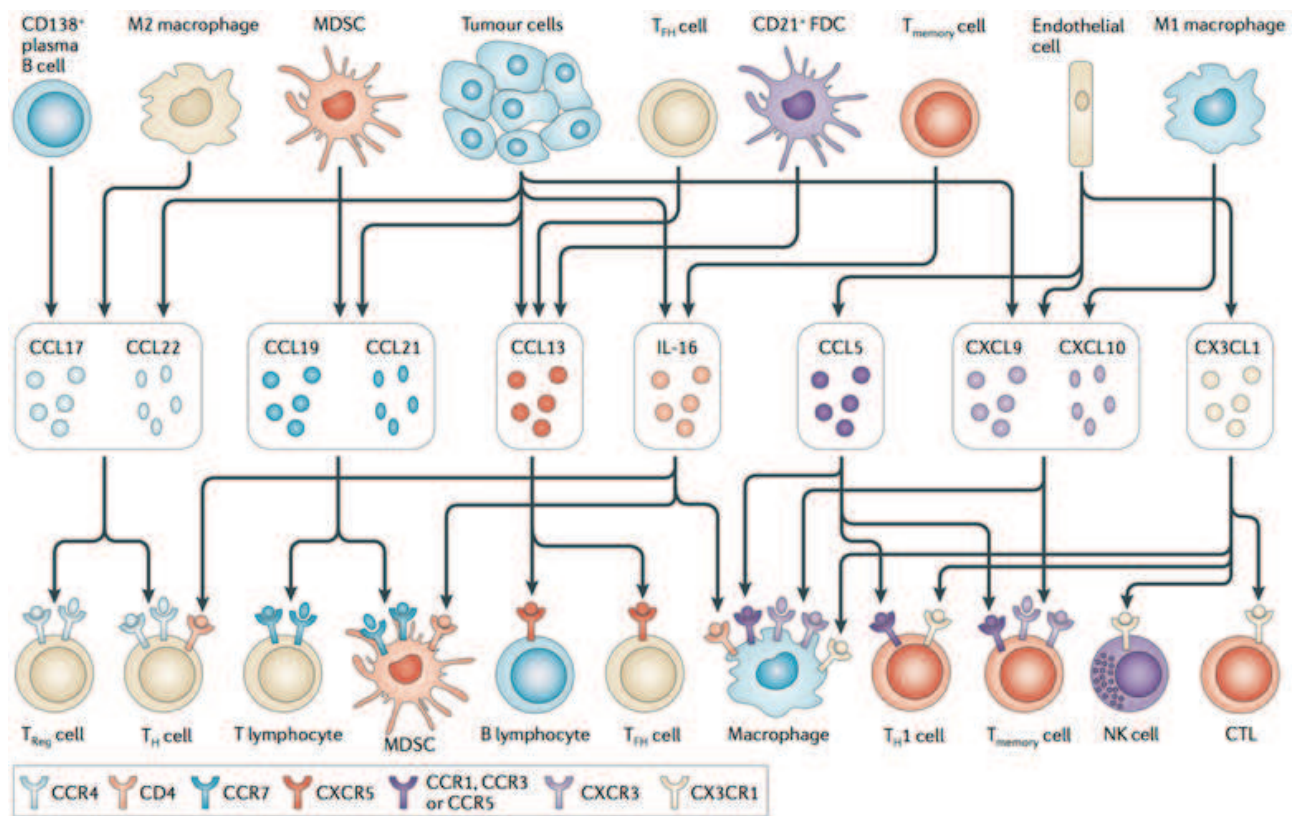
against HPV and HBV have significantly reduced the incidence of gastric cancer (97), cervical carcinoma (98) and hepatocellular cancer (99), respectively. Once a primary cancerous lesion is established, inflammatory cells can also act as tumor promoting elements. Accordingly, the density of tumor infiltrating macrophages or a colony stimulating factor (CSF)-1 response gene signature correlates with poor prognosis and metastasis in breast (100), lung (101), thyroid (102) and liver cancer (103). Moreover, it has been suggested that tumor-associated macrophages are implicated in the metastatic process in human breast cancer.(104)

Even though it is now well known that the induction of inflammation by bacterial and viral infections raises cancer risk (106), new work has shown that in addition to being a tumor initiator by virtue of its high carcinogen content, tobacco smoke is also a tumor promoter because of its ability to trigger chronic inflammation (107). In the same way, obesity, with an alarming rate of growing number in prevalence, promotes tumorigenesis in the liver (108) and pancreas (109). Most solid malignancies show in older individuals, and even old age (110) and cell senescence (111) are postulated to be tumor promoters

which act through inflammatory mechanisms. Along with its pro-tumorigenic effects, inflammation also influences the host immune response to tumors and can be used in cancer immunotherapy (112) and to augment the response to chemotherapy (113). Yet, in some cases, inflammation can diminish the beneficial effects of therapy.(4,114)

Tumors grow within a tangled network of epithelial cells, vascular and lymphatic vessels, cytokines and chemokines, plus infiltrating immune cells. These infiltrated immune cells type will affect in tumor progression which can vary according to cancer type.(115) Indeed, immune infiltrates are heterogeneous between tumor types, and are very diverse from patient to patient. Many types of immune cell that might be found in a tumor, including macrophages, dendritic cells, mast cells, natural killer (NK) cells, naive and memory lymphocytes, B cells and effector T cells (including various subsets of T cell, those are T helper (Th) cells, Th 1 cells, Th2 cells, Th17 cells, regulatory T (T-Reg) cells, T follicular helper (TFH) cells and cytotoxic T cells). These immune cells can be located in the core (the centre) of the tumor, in the invasive margin or in the adjacent tertiary lymphoid structures (TLS). The analysis of the location,





**Figure 5. Cells and chemokines that coordinate the tumor micro- environment.**(115) (Adapted with permission from Macmillan Publishers Limited).

density and functional orientation of the different immune cell populations (which we have termed the ‘immune contexture’ (115) in large annotated collections of human tumors has allowed the identification of components of the immune contexture that are beneficial, as well as those that are deleterious, to patients. In addition, bioinformatics tools (116,117) have permitted the identification of chemokines and cytokines that are involved in shaping the immune contexture (Figure 5) (94).

Tumor growth is assisted by tumor-associated macrophages (TAM), the major leukocyte population infiltrating cancers.(118) Although macrophages have the potential to attack and eliminate tumor cells, TAMs exhibit many protumoral features which are partly shared by macrophages involved in tissue repair, and they interfere with the function and proliferation of immune effectors.(119) In many human tumors, a high number of TAMs associated with poor prognosis.(120)

Another cell used to monitor clinical outcome and response to therapy in human is myeloid-derived suppressor cells (MDSC), whereas the finding of high number of MDSCs shows a bad prognosis (121). MDSCs have a myeloid origin, heterogeneous cell composition, and can negatively regulate adaptive and innate immune responses

to cancer.(122) As an outcome of these different forms of inflammation, the tumor microenvironment conceives innate immune cells (including macrophages, neutrophils, mast cells, MDSCs, dendritic cells and natural killer cells) and adaptive immune cells (T and B lymphocytes) in addition to the cancer cells and their surrounding stroma (that consists of fibroblasts, endothelial cells, pericytes, and mesenchymal cells).(123) To control and shape tumors growth, these cells networking each other in autocrine and paracrine way, by producing cytokine and chemokine, as well as expressing many immune mediators and modulators and abundantly activate different cell types into various state in the tumor microenvironment. This will tip the balance between tumor-promoting inflammation or antitumor immunity.(9,124)

Non-cell-autonomous regulation is important in tumorigenesis of cancer cells. To keep survive, cancer cells need to communicate with stromal cells by humoral factors such as VEGF, FGFs, and Wnt. Many studies recently showed that extracellular vesicles (EV) also have an important role in cell communication and the development of cancer. EVs contain small noncoding RNAs, including microRNAs (miRNAs), which is contribute in cancer cells malignancy.(125)

Tumor-derived exosomes (TEX) are harbingers of tumor-induced immune suppression because they carry immunosuppressive molecules and factors which known to interfere with immune cell functions. By delivering suppressive cargos consisting of proteins similar to those in parent tumor cells to immune cells, TEX directly or indirectly affect the development, maturation, and antitumor activities of immune cells. TEX bring genomic DNA, mRNA, and miRNAs to immune cells, and reprogramming responder cells functions to boost tumor progression. Antitumor immunotherapies can be hampered with TEX that equipped with tumor-associated antigens. In tumor microenvironment, TEX may be associated in antitumor immunity down-regulation signaling pathways. Thus, TEX have a potency to be used as noninvasive biomarkers of tumor progression.(126)

Lately, tumor immunologists and practicing oncologists have seen a dream come true with the clinical implementation and regulatory approval of cancer immunotherapies.(127) It was first proposed by Paul Ehrlich, 50 years after Virchow, that the immune system can fight tumors (128), a suggestion reiterated by the immunosurveillance hypothesis of Burnet and Thomas (129). These hypotheses are based on the idea that cancer-associated genetic changes, along with aberrant quality-control mechanisms and epigenetic reprogramming, result in expression of tumor-specific antigens (neoantigens) and tumor-associated antigens (TAAs), which are non-mutated proteins to which T cell tolerance is probably incomplete due to their restricted tissue expression pattern.(130) These antigens can activate antitumor immunity and under particular circumstances, may also induce rejection of early neoplasms, a concept known as immunosurveillance. (130,131) Yet, the tumor-controlling ability of the immune system was not widely accepted until the successful development of immune checkpoint inhibitors which trigger tumor rejection by activating cytotoxic T lymphocytes (CTL).(132)

The mediators and cellular effectors of inflammation are important constituents of the local environment of tumors. In many cancer types, inflammatory conditions could be detect before a malignancy. Oppositely, an oncogenic change induces inflammatory microenvironment and promotes the tumors progression. Regardless of its origin, 'smouldering' inflammation in the tumor microenvironment has many tumor-promoting effects. It assists in the proliferation and survival of malignant cells, promotes angiogenesis and metastasis, subverts adaptive immune responses, and changes responses to hormones

and chemotherapeutic agents. Now we have untangled the molecular pathways of cancer-inflammation links, we hope in the future new target molecules could be identified to improve diagnosis and treatments.(84)

### Nutrient Competition, Metabolic Reprogramming and Immunoediting of Cancer

The discovery in early 2000s that the immune system controls not only tumor quantity but also tumor quality (immunogenicity) prompted a major revision of the cancer immunosurveillance hypothesis.(7,11) The study showed that tumors in mice with lacked of intact immune system were more immunogenic (will then termed as "unedited"), compare to tumors derived from immunocompetent mice (termed as edited) The idea that the immune system not only protects the host against tumor formation but also shapes tumor immunogenicity as the basis of cancer immunoediting hypothesis, that stresses the dual host-protective and tumor-promoting actions of immunity on developing tumors.(133)

The last 15 years have seen a reemergence of interest in cancer immunosurveillance and a widening of this concept into one termed cancer immunoediting. Strong provocative experimental data in human cancer studies then suggested that immune system not only protects the host against development of primary non-viral cancers but also carves tumor immunogenicity. There are three phases in cancer immunoediting, those are elimination (cancer immunosurveillance), equilibrium and escape. The complete understanding of the immunobiology of cancer immunosurveillance and immunoediting will hopefully stimulate development of more effective immunotherapeutic approaches to control and/or eliminate human cancers (Figure 6).(134)

The understanding of cancer immunoediting principles that involved the dual host-protective and tumor sculpting actions in cancer will set the basis for novel individualized cancer immunotherapies.(131) When oxygen is present, most differentiated cells use mitochondrial oxidative phosphorylation to create energy in the form of adenosine triphosphate (ATP) which can be used to sustain cellular processes. But when oxygen is abstent, such cells revert to much less efficient glycolysis as a means of ATP production. Cancer cells often utilize glycolysis despite the presence of oxygen (aerobic glycolysis or the "Warburg effect").(135) When less efficient at producing energy, it is considered that this form of metabolism supports the macromolecular

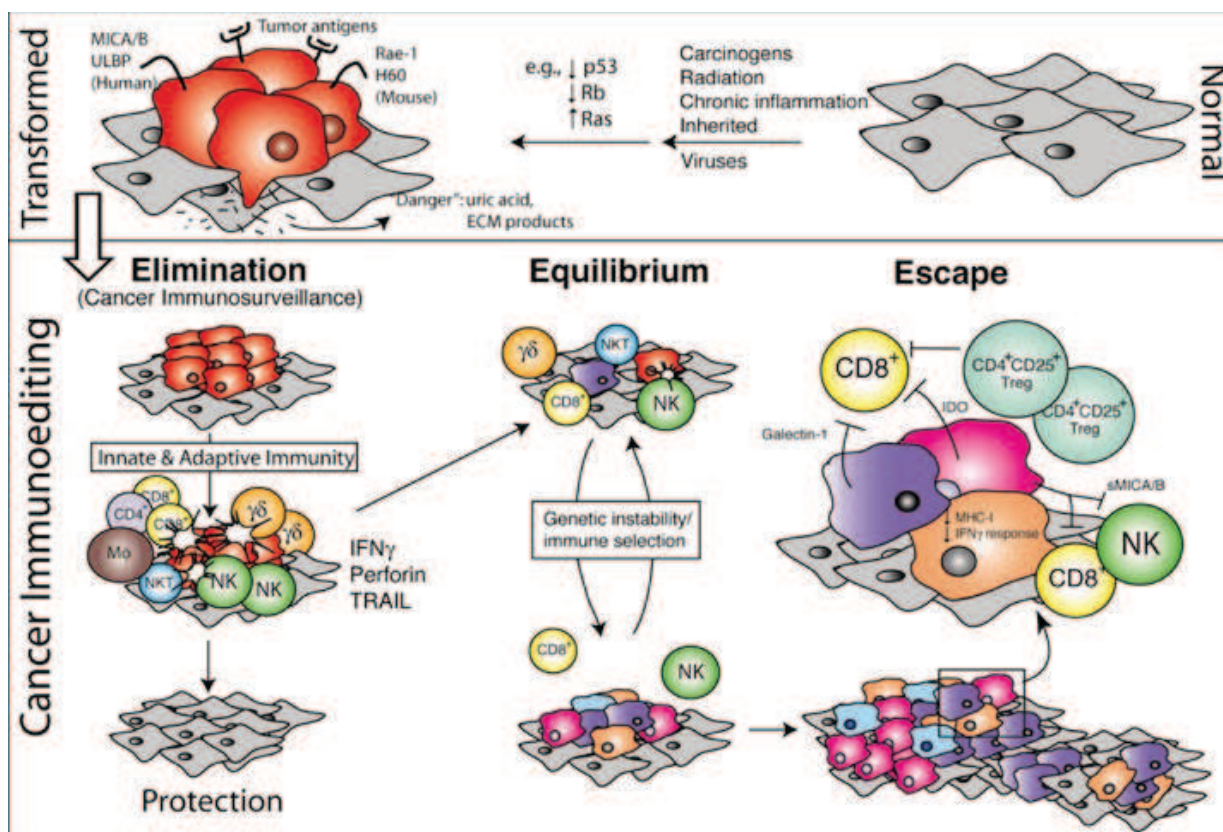


Figure 6. The Three Phases of the Cancer Immunoediting Process.(134) (Adapted with permission from Cell Press)

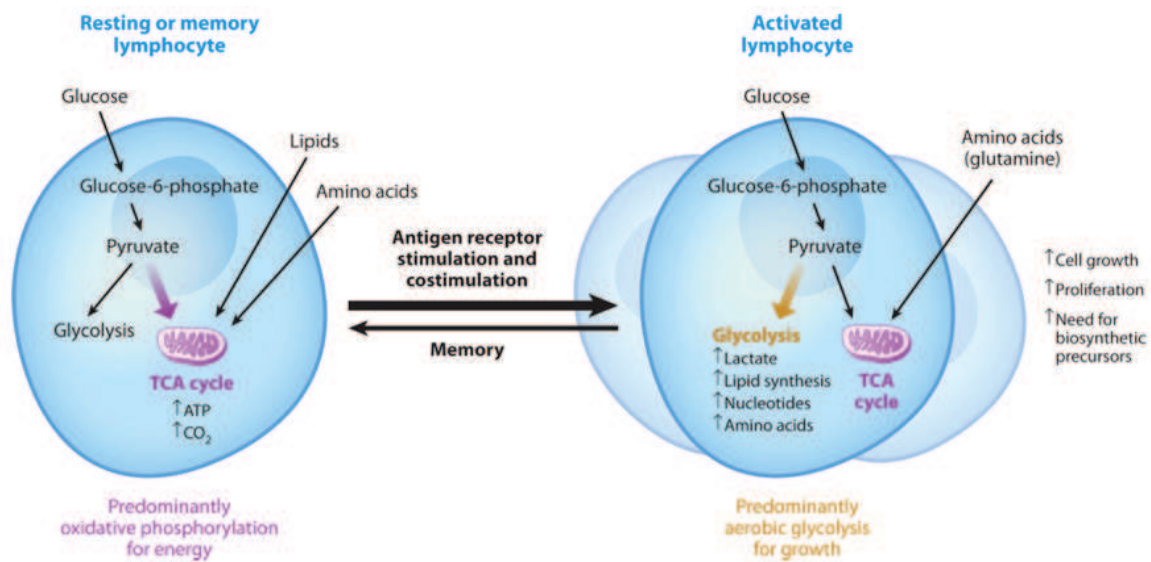
requirements of cell growth and proliferation. Thus, the field has primarily focused on Warburg metabolism as an adaptation that confers intrinsic growth advantages to tumor cells themselves. However, cancer cells may consume nutrients, particularly glucose, in excess of their requirement to sustain proliferation and cell growth.(136) This increases the possibility that nutrient consumption plays additional roles to meeting the intrinsic bioenergetic and biosynthetic requirements of cancer cells.(137,138) Warburg show that metabolism provides tumor cells with a cell-extrinsic advantage, promoting depletion of extracellular glucose that renders tumor-infiltrating T cells dysfunctional.(139)

Instead of manipulating tumor cell metabolism, Ho, *et.al.*, suggests an alternate approach to improve T cell function by imitating nutrient availability within transferred T cells during adoptive cell therapy (ACT). Phosphoenolpyruvate Carboxykinase (PCK1) converts oxaloacetate into phosphoenolpyruvate (PEP). By overexpressing PCK1 in transferred T cells, the PEP levels were able to be artificially increased, restoring T cell antigen receptor (TCR)-induced  $Ca^{2+}$  flux and anti-tumor T cell function despite the presence of low environmental glucose levels within tumors. Blocking glucose metabolism during expansion of T cells for

adoptive immunotherapy withholds effector differentiation and promotes differentiation of memory cells that mediate superior tumor clearance.(140) These findings provide striking examples of how modulating T cell metabolism can improve the outcome of adoptive cell therapy for cancer. Antigenic tumors need glucose to metabolically restrict T cells, and moisten their effector function to allow tumor progression. This resource imbalance can be corrected with checkpoint blockade therapy through a direct effect in the tumor cells (Figure 7).(138,141)

Unfortunately, many tumors can escape not only the recognition by adaptive immune response but also the prime immune cells, makes the role of the immune system in tumor elimination to be ambiguous. The tumors escape through several mechanisms, including direct interference with the cells of the adaptive immune response and indirect immunosuppression by modifying the tumor microenvironment. Tumors can take advantage of a pH control system, via the upregulation of glycolysis and the consequent lowering of pH in the tumor microenvironment, already exploited by specific immune cell subpopulations, to obtain control of the immune system and press both cytotoxic and antigen-presenting cells. This is achieved through the direct competition of tumor cells with





**Figure 7. T cell metabolic programs match functional demands.** Resting T cells oxidize glucose-derived pyruvate, along with lipids and amino acids, to efficiently produce ATP/energy required for immune surveillance.(141) (Adapted with permission from Annual Reviews).

actively proliferating glycolytic immune cells for glucose and indirectly through the creation by the tumor of a microenvironment which interferes with maturation and activation of antigen-presenting cells and naive cytotoxic T cells. Immunosuppressive properties of an acidic microenvironment in the surrounding of the tumor can thus provide additional advantages for upregulation of glycolysis by tumor cells, proposing that the two emerging hallmarks of cancer changed glucose metabolism and immune suppression, are in fact fundamentally linked.(142)

The complex immunologic tumor microenvironment in human cancers, or immune contexture, including the location, density, and functional orientation of infiltrating hematopoietic cells correlate with patients' clinical outcome.(143) In most cases, good prognosis (progression-free survival (PFS) and/or overall survival (OS)) correlated with a strong infiltration of memory CD8<sup>+</sup> T cells and a Th1 orientation. Other parameters as well as immune cells, cytokines, chemokines and other factors also influence the generation of a clinically efficient immune microenvironment. All types of immune cells can be found in many different proportions and locations in tumors. They include myeloid cells, B cells and all subsets of T cells (115,143,144).

The "immunoscore" is a scoring system which came from the immune contexture (114,145,146) and is a clinically useful prognostic marker (147) based on the enumeration of two lymphocyte populations (CD3 and CD8), in both the core of the tumor (CT) and in the invasive margin (IM) of tumors. The immunoscore gives a score ranging from immunoscore 0, in which low densities of both cell types

are found in both regions, to immunoscore 4, in which high densities are found in both regions. Immunoscore classification was known to have a prognostic significance in all stage I, II and III patients with CRC that was superior to that of the American Joint Committee on Cancer/ Union for International Cancer Control (AJCC/UICC) TNM (T stage, N stage, and tumor differentiation) classification system.(148-149) Statistic data reports that tumor invasion was depended on the host's immune reaction. The effector memory T cells ability to recall previously encountered antigens and traffic into tumors may give an advantage in long-term immunity to human cancer.(150,151) These days a clinical validation of the immunoscore with standardized procedures is needed to seize clinical applicability for individual patients with CRC and other cancers.(152,153) The immunoscore also provides a tool and targets for new therapeutic approaches, including immunotherapy (as lately illustrated in clinical trials boosting T cell responses with anti-cytotoxic T-lymphocyte-associated antigen (CTLA)-4, anti-programmed cell death (PD)-1, and anti-programmed cell death ligand (PDL)-1.(153-156)

## Epithelial – Mesenchymal Transition and Metastasis

In spite of the new advances in the treatment of cancer, metastatic disease remains mostly incurable and the main cause of cancer-related deaths. Metastases are the final results of a multistage processes which includes local invasion by the primary tumor cells, intravasation into

the blood or lymphatic system, survival in circulation (hematogenous and/or lymphatic), arrest at a distant organ, extravasation, survival in a novel environment and metastatic colonization. Each of these steps depends on specific phenotypic features of the tumor cell, also interactions with the host microenvironment and the immune system.(157-159) The advance therapy of human cancer, currently still not yet reached the fully treatment for metastatic disease. Metastatic sub-clones can raise either in the early or late stage of primary tumor development. We need a deeper understanding of the genetic evolution of metastatic disease and the biology of metastatic process to reveal different therapies for primary tumors and metastatic diseases.

Tumor metastasis is the most common cause of cancer-associated mortality. To give increase to the outgrowth of metastatic tumors in a new organ microenvironment, cancer cells have to overcome various types of stresses and several rate-limiting steps.(160) During metastasis formation, most dispersed cancer cells were destroyed, but a small subset of those cells can survive and colonize in the new environment. Metastatic niches (specialized tumor microenvironment) are considered to be responsible in nurturing disseminated cancer cells from micrometastases to full macrometastases. Cancer stem cells (CSC) also shown to be linked directly in the metastatic process. Thus, identifying the confining factors which regulate the properties of CSCs and their colonization of metastatic niches is essential for developing strategies to treat patients with metastatic tumors. In a recent issue of Nature, Huelsken and colleagues give a novel insight into how signals from the metastatic niche affect CSC self-renewal and metastatic colonization.(161,162) Disseminated cancer cells need to find a supportive sites to reside their specific microenvironment or “niches” so the stem cells can regulate the self-renewal potential and access to different notions. Various tissues could be in favors for stem cell niches including the intestinal epithelium, hematopoietic bone marrow, epidermis, and brain.(163-166) In primary tumors, cancer cells may interact with native stem cell niches, and the interactions will end as cancer cells leave the tumor. The metastasis-initiating differentiated thyroid cancer (DTC) survival and fitness will depend on specific components of the host environment that play the part of a niche for these cells.(167)

Study performed by Malanchi, *et al.*, demonstrated the crucial role of the stromal periostin for metastatic colonization by regulating the interactions of breast cancer stem cells and their metastatic niche.(162) The transdifferentiation of epithelial cells into motile mesenchymal cells is integral in the development, wound healing and stem cell behavior,

also in contributes pathologically to fibrosis and cancer progression. This switch in cell differentiation and behavior is mediated by key transcription factors, including Snail, zinc-finger E-box-binding (ZEB) and basic helix-loop-helix transcription factors, the functions of which are finely regulated at the transcriptional, translational and post-translational levels. The reprogramming of gene expression throughout EMT, as well as non-transcriptional changes, are initiated and controlled by signaling pathways that respond to extracellular cues. Among these, TGF- $\beta$  family signaling has a predominant role, however, the convergence of signaling pathways is essential for EMT.(168)

Although it is clear that EMT is involved in metastatic events in cancer, its participation in other events may be also highly relevant to tumor progression. TGF- $\beta$  can prevent the progression of incipient tumors and promote tumor invasion and evasion of immune surveillance at advanced stages (169), directing apoptosis or survival plus EMT in many cell contexts. When TGF- $\beta$  acts on activated Ras-expressing mammary epithelial cells, EMT is favored and apoptosis is inhibited, and resistance to TGF- $\beta$ -induced cell death was associated with hepatocytes undergoing EMT.(170) Similarly, several weeks study of breast tumor-derived NMuMG cells exposure to TGF- $\beta$  generates cells that escape apoptosis and performed a sustained EMT (171). This model offers the opportunity to investigate the long-term effect of chronic TGF- $\beta$  exposure during fibrosis in epithelial tissues and in cancer cells.(32) Tumors can escape from immune superintendence by inducing tolerance or utilize immunoediting to modify their phenotype.(172) a study observed tumor relapse in a Neu/HER2-inducible transgenic tumor model after removal of the inducer, indicating that tumors depend on continuous oncogenic signaling is needed for tumor survival. However, all animals had residual foci that finally developed more aggressive new tumors of the EMT type.(173) These two studies suggest that EMT may be involved in the acquisition of resistance to targeted therapies and that cells belonging to the foci of minimal residual disease acquired a mesenchymal phenotype.(32)

Inflammation is known to be correlated with the cancer and fibrosis progression. Interestingly, recent studies point to the inflammation-induced EMT as critical to this connection.(174) In the tumor microenvironment and in course of organ fibrosis (such as occurs with kidney obstruction, diabetes, and glomerulonephritis) different mechanisms converge on the induction of nuclear factor kappa-B (NF- $\kappa$ B), which increase the expression of EMT inducers and Snail in particular both at the transcriptional

and translational levels.(175-177) Together, these data indicate that EMT may not only be necessary for primary carcinoma to invade and disseminate, but also that these pioneer invasive cells with both a mesenchymal and a stem cell-like phenotype can generate a differentiated epithelial-like structure. This reversion to the differentiated phenotype through a process of MET is important for the formation of macrometastasis and thus to form the bulk of the secondary tumor mass. This hypothesis was previously formulated from an analysis of the progression of colon primary tumors and liver metastases, where it was proposed that cancer stem cells could acquire a mesenchymal phenotype, and thereby become migratory cancer stem cells that will form metastasis.(178)

## Cancer Stem Cells

Cancer is a disease of genomic instability, evasion of immune cells, an adaptation of the tumor cells to the changing environment genetic heterogeneity which is caused by tumors and tumor microenvironmental factors forms the basis of aggressive behavior of some cancer cell populations. Several studies have identified a subset of cancer cells, designated as tumor-initiating cell or CSC, with the ability for self-renewal and differentiation into distinct cell lineages. Recently, the hypothesis has emerged, and earned great momentum, that tumors are hierarchically arranged, with CSC being the primary drivers of tumor growth for proliferation, resistance to chemotherapy, and metastasis.(180-182) A combination of flowcytometry and xenotransplantation techniques led to the identification of leukemia-initiating cells (cluster of differentiation (CD)34<sup>+</sup> CD38) and breast cancer-initiating cells (CD44<sup>+</sup> CD24<sup>-lo</sup>) and provided scientific basis for the CSC hypothesis.(182-184)

Tumor heterogeneity and plasticity naturally consequences in many drug resistance development, disease recurrence and metastasis, especially in cells that sensitive to therapy.(185) The hypothesis that tumors rise through sequential mutation and clonal selection has been proposed to be incompatible with a CSC model. Frankly, studies such as that of Jan, *et al.*, have provided compelling evidence that both models are correct.(186) Even though mutations may occur in any cell, those which occur in non-self-renewing cell populations are extinguished through cellular senescence. CSC mutation may lead to these selective population clonal growth, causing further mutation, and keep continue to mutate and evolve even after full

transformation. Thus, cancers can consist of more than one CSC clones.(179,187)

The CSC hypothesis explains that there exists, within a tumor, a minority cell type which has the characteristics of stem cells, these cells can self-renew and can differentiate to mold all of the cell types which constitute the original tumor from a small number of cells.(188) It seems clear that, at least in hematopoietic (189) and some solid tumors including pancreatic (190), prostate (191), colon (163,192,193), breast (194,195), lung (196) and brain (197,198), a small subset of cells can be isolated which can self-renew and form well-differentiated tumors identical to that of the patient's tumor from which they arise. Nevertheless, the CSC hypothesis may not be true for all tumor types or for all of these cells all of the time.(199-202) In reality, it has been offered that stemness may be a dynamic state, which is a function of the cell's interaction with environment.(203).

The CSC model states that tumors are organized hierarchically with a subset of tumor cells at their apex, which possess self-renewal and multilineage differentiation potential.(188,204) This model therefore suggests that tumors are organized in a similar, albeit distorted, mannered as are their tissues of origin, and could potentially explain several phenomena that are currently incompletely understood.(205) According to the CSC model, minimal residual disease and tumor recurrence after treatment would be a result from remaining therapy-resistant CSC fraction, whereas metastatic potential would be a CSC-specific property. This hypothesis could be appealing, but more experimental evidences are needed.(206)

The definition of CSCs as the only self-renewing tumor cells that capable of seeding a new tumor implies that CSCs are also responsible for initiation of metastases, a notion strengthened by the connection between CSCs and EMT (178,206-209) which is associated with metastatic behavior and poor prognosis (210). Transient and long-term quiescence (dormancy), are the constitutional attributes of adult stem cells.(211,212) On the basis of this premise, stem cells are often identified by their propensity to retain DNA labels much longer than their rapidly proliferating offspring. Dormancy might be the crucial mechanism for the CSCs resistance to anti-proliferative chemotherapy. Besides, if indeed CSC occur in a dormant state, this would explain the appearance of local recurrence or distant metastasis after long lag periods.(163)

It is often proposed that CSCs are resistant to therapy in the similar way that normal stem cells are protected against insult. These protections include, for example, mechanisms such as quiescence, expression of ATP-binding



cassette (ABC) drug pumps.(213) Some groups have started to explore if CSCs are indeed more resistant to therapy than their progeny. For example, CD133-expressing glioma cells which were survive from ionizing radiation convicted to have better relative to CD133-tumor cells.(214) CD44<sup>hi</sup> CD24<sup>lo</sup> breast cancer CSCs appear intrinsically resistant to conventional chemotherapy (215) and ionizing radiation (216). Meanwhile chronic myeloid leukemia (CML) is sustained by leukemic stem cells that are relatively resistant to the drug Imatinib.(217,218) Nevertheless, the phenomenon of intrinsically therapy-resistant CSCs cannot be generalized, as for instance, the undifferentiated cells which drive testicular germ cell tumors are more sensitive to radiation or cisplatin therapy than their differentiated cellular progeny.(219) Tumor cells that escape therapy, however, may not be endowed with intrinsic therapy resistance. Rather, they may simply be the stochastic winners of the tumor cell-killing process. On the opposite, when intrinsic differences in the sensitivity of cancer cells to therapy do exist, these may also be determined genetically rather than by epigenetic differences.(220) antiapoptotic proteins and resistance to DNA damage.

In summary, CSC hypothesis, supported by large numbers of evidences data, have a big role in cancer prevention and therapy implications, but we still need more clinical trials to demonstrate that this pathways could really work as a target for reducing cancer incidence and could improve patients outcome.(179)

## Conclusion

Immunity, both innate and adaptive, play an important influence in cancer development, either by promoting or attenuating tumorigenesis, at the same time opposing the cancer therapies effect into resistances, suggesting the mechanisms was correlated with chronic inflammation. Furthermore, cancer development and malignant progression associated with the accumulation of genetic alterations and normal regulatory processes loss, leading to the expression induction of tumor-specific antigens and TAAs, which activate antitumor immune responses. Signals that trigger acute inflammatory reactions usually stimulate dendritic cell maturation and antigen presentation, but chronic inflammation can also be immunosuppressive. This antagonism between inflammation and immunity also influences the outcome of cancer treatment and needs to be considered when designing new therapeutic approaches. Besides cancer cells, tumors exhibit another dimension

of complexities. They contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the “tumor microenvironment.” These concepts was hope to be widespread applicable to promote new means for human cancer successful therapy.

## References

- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144: 646-74.
- Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. *Science*. 2013; 339: 1546-58.
- Garraway LA. Genomics-driven oncology: framework for an emerging paradigm. *J Clin Oncol*. 2013; 31: 1806-14.
- Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010; 140: 883-99.
- Graziano DF, Finn OJ. Tumor antigens and tumor antigen discovery. *Cancer Treat Res*. 2005; 123: 89-111.
- Srivastava PK, Old LJ. Individually distinct transplantation antigens of chemically induced mouse tumors. *Immunol Today*. 1988; 9: 78-83.
- Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoeediting: from immunosurveillance to tumor escape. *Nat Immunol*. 2002; 3: 991-8.
- Dunn GP, Bruce AT, Sheehan KCF, Shankaran V, Uppaluri R, Bui JD, *et al*. A critical function for type I interferons in cancer immunoeediting. *Nat Immunol*. 2005; 6: 722-9. [Erratum in: *Nat Immunol*. 2005; 6: 852.
- Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoeediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol*. 2006; 90: 1-50.
- Simson L, Ellyard JI, Dent LA, Matthaei KI, Rothenberg ME, Foster PS, *et al*. Regulation of carcinogenesis by IL-5 and CCL11: a potential role for eosinophils in tumor immune surveillance. *J Immunol*. 2007; 178: 4222-9.
- Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, *et al*. IFN $\gamma$  and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature*. 2001; 410: 1107-11.
- Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoeediting. *Annu Rev Immunol*. 2004; 22: 329-60.
- Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, *et al*. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature*. 2007; 450: 903-7.
- Finn OJ. Cancer Immunology. *N Engl J Med*. 2008; 358: 2704-15.
- Merlo LMF, Pepper JW, Reid BJ, Maley CC. Cancer as an evolutionary and ecological process. *Nat Rev Cancer*. 2006; 6: 924-35.
- Greaves M, Maley CC. Clonal evolution in cancer. *Nature*. 2012; 481: 306-13.
- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010; 127: 2893-917.
- Stratton MR. Exploring the genomes of cancer cells: progress and promise. *Science*. 2011; 331: 1553-8.
- Cheng N, Chytil A, Shyr Y, Joly A, Moses HL. Transforming growth factor-beta signaling-deficient fibroblasts enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote

- scattering and invasion. *Mol Cancer Res.* 2008; 6: 1521-33.
20. Bhowmik NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature.* 2004; 432: 332-7.
  21. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene.* 2007; 26: 1324-37.
  22. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. *Nature.* 2004; 432: 307-15.
  23. Evan G, Littlewood T. A matter of life and cell death. *Science.* 1998; 281: 1317-22.
  24. Galluzzi L, Kroemer G. Necroptosis: a specialized pathway of programmed necrosis. *Cell.* 2008; 135: 1161-3.
  25. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. *Semin Cancer Biol.* 2009; 19: 329-37.
  26. Bergers G, Benjamin LE. Angiogenesis: tumorigenesis and the angiogenic switch. *Nat Rev Cancer.* 2003; 3: 401-10.
  27. Ferrara N. Vascular endothelial growth factor. *Arterioscler Thromb Vasc Biol.* 2009; 29: 789-91.
  28. Gabhann FM, Popel AS. Systems biology of vascular endothelial growth factors. *Microcirculation.* 2008; 15: 715-38.
  29. Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. *Oncology.* 2005; 69 (Suppl 3): 4-10.
  30. Klymkowsky MW, Savagner P. Epithelial-mesenchymal transition: a cancer researcher's conceptual friend and foe. *Am J Pathol.* 2009; 174: 1588-93.
  31. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer.* 2009; 9: 265-73.
  32. Thiery JP, Acloque H, Huang RYJ, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell.* 2009; 139: 871-90.
  33. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev.* 2009; 28: 15-33.
  34. Barrallo-Gimeno A, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development.* 2005; 132: 3151-61.
  35. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis.* 2009; 30: 1073-81.
  36. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability -- an evolving hallmark of cancer. *Nat Rev Mol Cell Biol.* 2010; 11: 220-8.
  37. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell.* 2009; 136: 823-37.
  38. Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, *et al.* The genomic landscapes of human breast and colorectal cancers. *Science.* 2007; 318: 1108-13.
  39. Parsons DW, Jones S, Zhang X, Lin JCH, Leary RJ, Angenendt P, *et al.* An integrated genomic analysis of human glioblastoma multiforme. *Science.* 2008; 321: 1807-12.
  40. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, *et al.* Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science.* 2008; 321: 1801-6.
  41. Nowell PC. The clonal evolution of tumor cell populations. *Science.* 1976; 194: 23-8.
  42. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell.* 1990; 61: 759-67.
  43. Kinzler KW, Vogelstein B. Gatekeepers and caretakers. *Nature.* 1997; 386: 761-3.
  44. Humphries A, Cereser B, Gay LJ, Miller DSJ, Das B, Gutteridge A, *et al.* Lineage tracing reveals multipotent stem cells maintain human adenomas and the pattern of clonal expansion in tumor evolution. *Proc Natl Acad Sci USA.* 2013; 110: E2490-9.
  45. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, *et al.* Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *Engl J Med.* 2011; 364: 2507-16.
  46. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, *et al.* Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med.* 2010; 363: 1693-703.
  47. Kerbel RS. Tumor angiogenesis. *N Engl J Med.* 2008; 358: 2039-49.
  48. Chung AS, Ferrara N. Developmental and pathological angiogenesis. *Annu Rev Cell Dev Biol.* 2011; 27: 563-84.
  49. Araten DJ, Golde DW, Zhang RH, Thaler HT, Gargiulo L, Notaro R, *et al.* A quantitative measurement of the human somatic mutation rate. *Cancer Res.* 2005; 65: 8111-7.
  50. Kunkel TA. Evolving views of DNA replication (in)fidelity. *Cold Spring Harb Symp Quant Biol.* 2009; 74: 91-101.
  51. Ljungman M, Lane DP. Transcription -- guarding the genome by sensing DNA damage. *Nat Rev Cancer.* 2004; 4: 727-37.
  52. Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. *Nature.* 2000; 408: 433-9.
  53. Medema RH, Macúrek L. Checkpoint control and cancer. *Oncogene.* 2011; 31: 2601-13.
  54. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. *Lancet Oncol.* 2011; 12: 175-80.
  55. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, *et al.* The landscape of cancer genes and mutational processes in breast cancer. *Nature.* 2012; 486: 400-4.
  56. Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, *et al.* Whole-genome analysis informs breast cancer response to aromatase inhibition. *Nature.* 2012; 486: 353-60.
  57. Banerji S, Cibulskis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM, *et al.* Sequence analysis of mutations and translocations across breast cancer subtypes. *Nature.* 2012; 486: 405-9.
  58. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, *et al.* The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature.* 2012; 486: 395-9.
  59. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, *et al.* Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med.* 2009; 361: 1058-66.
  60. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, *et al.* Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature.* 2009; 462: 739-44.
  61. Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Collier HA, *et al.* The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting  $\alpha$ -ketoglutarate to 2-hydroxyglutarate. *Cancer Cell.* 2010; 17: 225-34.
  62. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, *et al.* Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell.* 2010; 18: 553-67.
  63. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, *et al.* IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature.* 2012; 483: 479-83.
  64. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, *et al.* IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature.* 2012; 483: 474-8.
  65. Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, *et al.* Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. *Nature.* 2012; 483: 484-8.
  66. Crea F, Fornaro L, Bocci G, Sun L, Farrar WL, Falcone A, *et al.* EZH2 inhibition: targeting the crossroad of tumor invasion and angiogenesis. *Cancer Metastasis Rev.* 2012; 31: 753-61.

67. Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J, *et al.* Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer Cell.* 2011; 20: 53-65.
68. Cheng LL, Itahana Y, Lei ZD, Chia NY, Wu Y, Yu Y, *et al.* TP53 genomic status regulates sensitivity of gastric cancer cells to the histone methylation inhibitor 3-deazaneplanocin A (DZNep). *Clin Cancer Res.* 2012; 18: 4201-12.
69. Avan A, Crea F, Paolicchi E, Funel N, Galvani E, Marquez VE, *et al.* Molecular mechanisms involved in the synergistic interaction of the EZH2 inhibitor 3-deazaneplanocin A with gemcitabine in pancreatic cancer cells. *Mol Cancer Ther.* 2012; 11: 1735-46.
70. National Cancer Institute [Internet]. Bethesda: National Institute of Health. Targeted cancer therapies [updated 2014 Apr 25; cited 2016 Dec 20]. Available from: <http://www.cancer.gov/cancertopics/factsheet/Therapy/targeted>.
71. Sawyers C. Targeted cancer therapy. *Nature.* 2004; 432: 294-7.
72. Cancer Research UK, Institute of Cancer Research: canSAR Database. <https://cansar.icr.ac.uk>.
73. MacConail LE, Garraway LA. Clinical implications of the cancer genome. *J Clin Oncol.* 2010; 28: 5219-28.
74. Brubaker SW, Bonham KS, Zanoni I, Kagan JC. Innate immune pattern recognition: a cell biological perspective. *Annu Rev Immunol.* 2015; 33: 257-90.
75. Medzhitov R, Horng T. Transcriptional control of the inflammatory response. *Nat Rev Immunol.* 2009; 9: 692-703.
76. Steinman RM. Decisions about dendritic cells: past, present, and future. *Annu Rev Immunol.* 2012; 30: 1-22.
77. Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, *et al.* Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature.* 2002; 415: 536-41.
78. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. *Nat Rev Immunol.* 2014; 14: 478-94.
79. Williams M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN. The function of Fc-gamma receptors in dendritic cells and macrophages. *Nat Rev Immunol.* 2014; 14: 94-108.
80. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat Immunol.* 2015; 16: 343-53.
81. Carroll MC, Isenman DE. Regulation of humoral immunity by complement. *Immunity.* 2012; 37: 199-207.
82. Chaudhry A, Rudensky AY. Control of inflammation by integration of environmental cues by regulatory T cells. *J Clin Invest.* 2013; 123: 939-44.
83. Karin M. Nuclear factor-kappaB in cancer development and progression. *Nature.* 2006; 441: 431-6.
84. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature.* 2008; 454: 436-44.
85. Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clin Cancer Res.* 2009; 15: 425-30.
86. Castle PE, Hillier SL, Rabe LK, Hildesheim A, Herrero R, Bratti MC, *et al.* An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). *Cancer Epidemiol Biomarkers Prev.* 2001; 10: 1021-7.
87. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma. *J Clin Invest.* 2012; 122: 1951-7.
88. Arzumanyan A, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer.* 2013; 13: 123-35.
89. Salama NR, Hartung ML, Muller A. Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. *Nat Rev Microbiol.* 2013; 11: 385-99.
90. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, *et al.* *Helicobacter pylori* infection and gastric lymphoma. *N Engl J Med.* 1994; 330: 1267-71.
91. Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, *et al.* Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature.* 2012; 491: 254-8.
92. Houghton AM. Mechanistic links between COPD and lung cancer. *Nat Rev Cancer.* 2013; 13: 233-45.
93. Motz GT, Coukos G. The parallel lives of angiogenesis and immunosuppression: cancer and other tale. *Nat Rev Immunol.* 2011; 11: 702-11.
94. Mlecnik B, Tosolini M, Charoentong P, Kirilovsky A, Bindea G, Berger A, *et al.* Biomolecular network reconstruction identifies T-cell homing factors associated with survival in colorectal cancer. *Gastroenterology.* 2010; 138: 1429-40.
95. Gabrilovich D, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol.* 2012; 12: 253-68.
96. Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer- reinterpreting paradigms. *Nat Rev Clin Oncol.* 2012; 9: 561-70.
97. Malfertheiner P, Fry LC, Mönkemüller K. Can gastric cancer be prevented by *Helicobacter pylori* eradication? *Best Pract Res Clin Gastroenterol.* 2006; 7: 709-19.
98. Jemal A, Simard EP, Dorell C, Noone AM, Markowitz LE, Kohler B, *et al.* Annual Report to the Nation on the Status of Cancer, 1975-2009, featuring the burden and trends in human papillomavirus (HPV)-associated cancers and HPV vaccination coverage level. *J Natl Cancer Inst.* 2013; 105: 175-201.
99. Cabibbo G, Maida M, Genco C, Antonucci M, Camma C. Causes of and prevention strategies for hepatocellular carcinoma. *Semin Oncol.* 2012; 39: 374-83.
100. Beck AH, Espinosa I, Edris B, Li R, Montgomery K, Zhu S, *et al.* The macrophage colony-stimulating factor 1 response signature in breast carcinoma. *Clin Cancer Res.* 2009; 15: 778-87.
101. Dai F, Liu L, Che G, Yu N, Pu Q, Zhang S, *et al.* The number and microlocalization of tumor-associated immune cells are associated with patient's survival time in non-small cell lung cancer. *BMC Cancer.* 2010; 10. doi: 10.1186/1471-2407-10-220.
102. Qing W, Fang WY, Ye L, Shen LY, Zhang XF, Fei XC, *et al.* Density of tumor-associated macrophages correlates with lymph node metastasis in papillary thyroid carcinoma. *Thyroid.* 2012; 22: 905-10.
103. Kong LQ, Zhu XD, Xu HX, Zhang JB, Lu L, Wang WQ, *et al.* The clinical significance of the CD163+ and CD68+ macrophages in patients with hepatocellular carcinoma. *PLoS ONE.* 2013; 8: e59771. doi: 10.1371/journal.pone.0059771.
104. Robinson BD, Sica GL, Liu YF, Rohan TE, Gertler FB, Condeelis JS, *et al.* Tumor microenvironment of metastasis in human breast carcinoma: a potential prognostic marker linked to hematogenous dissemination. *Clin Cancer Res.* 2009; 15: 2433-41.
105. De Martel C, Franceschi S. Infections and cancer: established associations and new hypotheses. *Crit. Rev. Oncol. Hematol.* 2009; 70: 183-94.
106. Takahashi H, Ogata H, Nishigaki R, Broide DH, Karin M. Tobacco smoke promotes lung tumorigenesis by triggering IKKbeta and JNK1-dependent inflammation. *Cancer Cell.* 2010; 17: 89-97.



107. Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, *et al.* Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell.* 2010; 140: 197-208.
108. Khasawneh J, Schulz MD, Walch A, Rozman J, de Angelis MH, Klingenspor M, *et al.* Inflammation and mitochondrial fatty acid-oxidation link obesity to early tumor promotion. *Proc Natl Acad Sci.* 2009; 106: 3354-9.
109. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med.* 2000; 51: 245-70.
110. Rodier F, Coppé J-P, Patil CK, Hoeijmakers WAM, Muñoz DP, Raza SR, *et al.* Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol.* 2009; 11: 973-9.
111. Dougan M, Dranoff G. Immune therapy for cancer. *Annu Rev Immunol.* 2009; 27: 83-117.
112. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. *Nat Rev Immunol.* 2008; 8: 59-73.
113. Ammirante M, Luo JL, Grivnenikov S, Nedospasov S, Karin M. B cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature.* 2010; 464: 302-5.
114. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer.* 2012; 12: 298-306.
115. Galon J, Fridman WH, Pages F. The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. *Cancer Res.* 2007; 67: 1883-6.
116. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2003; 13: 2498-504.
117. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, *et al.* ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics.* 2009; 25: 1091-3.
118. Balkwill FR, Mantovani A. Cancer-related inflammation: common themes and therapeutic opportunities. *Semin Cancer Biol.* 2012; 22: 33-40.
119. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity.* 2014; 41: 49-61.
120. Zhang Q, Liu L, Gong C, Shi H, Zeng Y, Wang X, *et al.* Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One.* 2012; 7: e50946. doi: 10.1371/journal.pone.0050946.
121. Montero AJ, Diaz-Montero CM, Kyriakopoulos CE, Bronte V, Mandruzzato S. Myeloid-derived suppressor cells in cancer patients: a clinical perspective. *J Immunother.* 2012; 35: 107-15.
122. Ugel S, De Sanctis F, Mandruzzato S, Bronte V. Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages. *J Clin Invest.* 2015; 125: 3365-76.
123. De Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer.* 2006; 6: 24-37.
124. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest.* 2007; 117: 1175-83.
125. Kosaka N, Yoshioka Y, Fujita Y, Ochiya T. Versatile roles of extracellular vesicles in cancer. *J Clin Invest.* 2016; 126: 1163-72.
126. Whiteside TL. Exosomes and tumor-mediated immune suppression. *J Clin Invest.* 2016; 126: 1216-23.
127. Schumacher TN, Kesmir C, van Buuren MM. Biomarkers in cancer immunotherapy. *Cancer Cell.* 2015; 27: 12-4.
128. Ehrlich P. The current state of cancer research (Über den jetzigen stand der karzinomforschung). *Ned Tijdschr Geneesk.* 1909; 5: 273-90.
129. Burnet FM. The concept of immunological surveillance. *Prog Exp Tumor Res.* 1970; 13: 1-27.
130. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science.* 2015; 348: 69-74.
131. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases -- elimination, equilibrium and escape. *Curr Opin Immunol.* 2014; 27: 16-25.
132. Shaloupour S, Karin M. Immunity, inflammation, and cancer: an eternal fight between good and evil. *J Clin Invest.* 2015; 125: 3347-55.
133. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science.* 2011; 331: 1565-70.
134. Dunn GP, Old LJ. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity.* 2004; 21: 137-48.
135. Warburg O. On the origin of cancer cells. *Science.* 1956; 123: 309-14.
136. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009; 324: 1029-33.
137. Ho PC, Bihuniak JD, Macintyre AN, Staron M, Liu X, Amezquita R, *et al.* Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell.* 2015; 162: 1217-28.
138. Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, *et al.* Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell.* 2015; 162: 1229-41.
139. Sukumar M, Roychoudhuri R, Restifo NP. Nutrient competition: a new axis of tumor immunosuppression. *Cell.* 2015; 162: 1206-8.
140. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z, *et al.* Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest.* 2013; 123: 4479-88.
141. MacIver NJ, Michalek RD, Rathmell JC. Metabolic Regulation of T Lymphocytes. *Annu Rev Immunol.* 2013; 31: 259-83.
142. Kereva I, Hahnfeldt P. The emerging "hallmarks" of metabolic reprogramming and immune evasion: distinct or linked? *Cancer Res.* 2013; 73: 2737-42.
143. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, *et al.* Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity.* 2013; 39: 782-95.
144. Teng MWL, Galon J, Fridman WH, Smyth MJ. From mice to humans: developments in cancer immunoediting. *J Clin Invest.* 2015; 125: 3338-46.
145. Angell H, Galon J. From the immune contexture to the immunoscore: the role of prognostic and predictive immune markers in cancer. *Curr Opin Immunol.* 2013; 25: 261-7.
146. Pages F, Kirilovsky A, Mlecnik B, Asslaber M, Tosolini M, Bindea G, *et al.* In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *J Clin Oncol.* 2009; 27: 5944-51.
147. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, *et al.* Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science.* 2006; 313: 1960-4.
148. Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, *et al.* Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clin Oncol.* 2011; 29: 610-8.

149. Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molitor R, *et al.* Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med.* 2005; 353: 2654-66.
150. Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. *Immunity.* 2013; 39: 11-26.
151. Galon J, Pagès F, Marincola FM, Angell HK, Thurin M, Lugli A, *et al.* Cancer classification using the Immunoscore: a worldwide task force. *J Transl Med.* 2012; 10: 205.
152. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, *et al.* Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *J Pathol.* 2014; 232: 199-209.
153. Brahmer JR, Tykodi SS, Chow LQM, Hwu WJ, Topalian SL, Hwu P, *et al.* Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med.* 2012; 366: 2455-65.
154. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010; 363: 711-23.
155. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med.* 2011; 364: 2517-26.
156. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012; 366: 2443-54.
157. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell.* 2011; 147: 275-92.
158. Sethi N, Kang Y. Unravelling the complexity of metastasis -- molecular understanding and targeted therapies. *Nat Rev Cancer.* 2011; 11: 735-48.
159. Turajic S, Swanton C. Metastasis as an evolutionary process. *Science.* 2016; 352: 169-75.
160. Bao S, Ouyang G, Bai X, Huang Z, Ma C, Liu M, *et al.* Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. *Cancer Cell.* 2004; 5: 329-39.
161. Malanchi I, Santamaria-Martínez A, Susanto E, Peng H, Lehr HA, Delaloye JF, *et al.* Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature.* 2011; 481: 85-9.
162. Wang Z, Ouyang G. Periostin: a bridge between cancer stem cells and their metastatic niche. *Cell Stem Cell.* 2012; 10: 111-2.
163. Clevers H. The cancer stem cell: premises, promises and challenges. *Nat Med.* 2011; 17: 313-9.
164. Hsu YC, Fuchs E. A family business: stem cell progeny join the niche to regulate homeostasis. *Nat Rev Mol Cell Biol.* 2012; 13: 103-14.
165. Moore KA, Lemischka IR. Stem cells and their niches. *Science.* 2006; 311: 1880-5.
166. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell.* 2008; 132: 598-611.
167. Oskarson T, Battle E, Massague J. Metastatic stem cells: sources, niches, and vital pathways. *Cell Stem Cell.* 2014; 14: 306-21.
168. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol.* 2014; 15: 179-96.
169. Massagué J. TGF $\beta$  in cancer. *Cell.* 2008; 134: 215-29.
170. Valdés F, Alvarez AM, Locascio A, Vega S, Herrera B, Fernández M, *et al.* The epithelial mesenchymal transition confers resistance to the apoptotic effects of transforming growth factor Beta in fetal rat hepatocytes. *Mol Cancer Res.* 2002; 1: 68-78.
171. Gal A, Sjöblom T, Fedorova L, Imreh S, Beug H, Moustakas A. Sustained TGF beta exposure suppresses Smad and non-Smad signalling in mammary epithelial cells, leading to EMT and inhibition of growth arrest and apoptosis. *Oncogene.* 2008; 27: 1218-30.
172. Knutson KL, Lu H, Stone B, Reiman JM, Behrens MD, Prosperi CM, *et al.* Immunoediting of cancers may lead to epithelial mesenchymal transition. *J Immunol.* 2006; 177: 1526-33.
173. Moody SE, Sarkisian CJ, Hahn KT, Gunther EJ, Pickup S, Dugan KD, *et al.* Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis. *Cancer Cell.* 2002; 2: 451-61.
174. López-Novoa JM, Nieto MA. Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. *EMBO Molecular Medicine.* 2009; 1: 303-14.
175. Julien S, Puig I, Caretti E, Bonaventure J, Nelles L, van Roy F, *et al.* Activation of NF-kappaB by Akt upregulates Snail expression and induces epithelium mesenchyme transition. *Oncogene.* 2007; 26: 7445-56.
176. Strippoli R, Benedicto I, Perez Lozano ML, Cerezo A, Lopez-Cabrera M, del Pozo MA. Epithelial-to-mesenchymal transition of peritoneal mesothelial cells is regulated by an ERK/NF- $\kappa$ B/Snail pathway. *Dis Model Mech.* 2008; 1: 264-74.
177. Wu Y, Deng J, Rychahou PG, Qiu S, Evers BM, Zhou BP. Stabilization of Snail by NF- $\kappa$ B is required for inflammation-induced cell migration and invasion. *Cancer Cell.* 2009; 15: 416-28.
178. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells -- an integrated concept of malignant tumour progression. *Nat Rev Cancer.* 2005; 5: 744-9.
179. Meiliana A, Dewi NM, Wijaya A. Cancer stem cell hypothesis: implication for cancer prevention and treatment. *Indones Biomed J.* 2016; 8: 21-36.
180. Badve S, Nakshatri H. Breast-cancer stem cells: beyond semantics. *Lancet Oncol.* 2012; 13: e43-8.
181. Qi Y, Li RM, Kong FM, Li H, Yu JP, Ren XB. How do tumor stem cells actively escape from host immunosurveillance? *Biochem Biophys Res Commun.* 2012; 420: 699-703.
182. Magee JA, Piskounova E, Morrison SJ. Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell.* 2012; 21: 283-96.
183. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med.* 1997; 3: 730-7.
184. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA.* 2003; 100: 3983-8.
185. Gokmen-Polar Y, Badve SS. Cancer stem cells: nonbeliever's viewpoint. *Clin Chem.* 2013; 59: 208-10.
186. Jan M, Snyder TM, Corces-Zimmerman MR, Vyas P, Weissman IL, Quake SR, *et al.* Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Sci Transl Med.* 2012; 4: 149ra18. doi: 10.1126/scitranslmed.3004315.
187. Azizi E, Wicha MS. Cancer stem cells: the evidence accumulates. *Clin Chem.* 2013; 59: 205-7.
188. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, *et al.* Cancer stem cells: perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res.* 2006; 66: 9339-44.
189. Sarry JE, Murphy K, Perry R, Sanchez PV, Secreto A, Keefer C, *et al.* Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rcc-deficient mice. *J Clin Invest.* 2011; 121: 384-95.
190. Li H, Tang DG. Prostate cancer stem cells and their potential roles in metastasis. *J Surg Oncol.* 2011; 103: 558-62.

191. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, *et al.* Identification of pancreatic cancer stem cells. *Cancer Res.* 2007; 67: 1030-7.
192. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, *et al.* Identification and expansion of human colon-cancer-initiating cells. *Nature.* 2007; 445: 111-5.
193. Vermeulen L, Todaro M, de Sousa Mello F, Sprick MR, Kemper K, Perez Alea M, *et al.* Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. *Proc Natl Acad Sci USA.* 2008; 105: 13427-32.
194. Liu S, Ginestier C, Ou SJ, Clouthier SG, Patel SH, Monville F, *et al.* Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. *Cancer Res.* 2011; 71: 614-24.
195. Charafé-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, *et al.* Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. *Cancer Res.* 2009; 69: 1302-13.
196. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, *et al.* Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ.* 2008; 15: 504-14.
197. Wang J, Sakariassen PØ, Tsinkalovsky O, Immervoll H, Bøe SO, Svendsen A, *et al.* CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. *Int J Cancer.* 2008; 122: 761-8.
198. Pollard SM, Yoshikawa K, Clarke ID, Danovi D, Stricker S, Russell R, *et al.* Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell.* 2009; 4: 568-80.
199. Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS, Johnson TM, *et al.* Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. *Cancer Cell.* 2010; 18: 510-23.
200. Girouard SD, Murphy GF. Melanoma stem cells: Not rare, but well done. *Lab Invest.* 2011; 91: 647-64.
201. Chaffer C, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, *et al.* Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci USA.* 2011; 108: 7950-5.
202. Iliopoulos D, Hirsch H, Wang G, Struhl K. Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc Natl Acad Sci USA.* 2011; 108: 1397-402.
203. Lander AD. The 'stem cell' concept: Is it holding us back? *J Biol.* 2009; 8: 70.
204. Vermeulen L, Sprick MR, Kemper K, Stassi G, Medema JP. Cancer stem cells: old concepts, new insights. *Cell Death Differ.* 2008; 15: 947-58.
205. Vermeulen L, de Sousa e Melo F, Richel DJ, Medema JP. The developing cancer stem-cell model: clinical challenges and opportunities. *Lancet Oncol.* 2012; 13: e83-9.
206. Medema JP. Cancer stem cells: the challenges ahead. *Nat Cell Biol.* 2013; 15: 338-44.
207. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, *et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell.* 2008; 133: 704-15.
208. Morel AP, Lièvre M, Thomas C, Hinkal G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. *PLoS ONE.* 2008; 3: e2888. doi: 10.1371/journal.pone.0002888.
209. Scheel C, Eaton EN, Li SH, Chaffer CL, Reinhardt F, Kah KJ, *et al.* Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. *Cell.* 2011; 145: 926-40.
210. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell.* 2008; 14: 818-29.
211. Fuchs E. The tortoise and the hair: slow-cycling cells in the stem cell race. *Cell.* 2009; 137: 811-9.
212. Essers MA, Trumpp A. Targeting leukemic stem cells by breaking their dormancy. *Mol Oncol.* 2010; 4: 443-50.
213. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. *Nat Rev Drug Discov.* 2009; 8: 806-23.
214. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, *et al.* Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature.* 2006; 444: 756-60.
215. Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, *et al.* Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J Natl Cancer Inst.* 2008; 100: 672-9.
216. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, *et al.* Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature.* 2009; 458: 780-3.
217. O'Hare T, Corbin AS, Druker BJ. Targeted CML therapy: controlling drug resistance, seeking cure. *Curr Opin Genet Dev.* 2006; 16: 92-9.
218. Oravecz-Wilson KI, Philips ST, Yilmaz OH, Ames HM, Li L, Crawford BD, *et al.* Persistence of leukemia-initiating cells in a conditional knockin model of an imatinib-responsive myeloproliferative disorder. *Cancer Cell.* 2009; 16: 137-48.
219. Masters JR, Koberle B. Curing metastatic cancer: lessons from testicular germ-cell tumours. *Nat Rev Cancer.* 2003; 3: 517-25.
220. Sikic BI. Natural and acquired resistance to cancer therapies. In: Mendelsohn J, Howley PM, Israel MA, Gray JW, Thompson CB, editors. *The Molecular Basis of Cancer.* 3rd ed. Philadelphia: Saunders/Elsevier; 2008. p.583-92.