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CANADIAN Feature Article:

"Cancer: No Longer a Black Box"

By: Shairaz Baksh, Ph. D.

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Cancer:
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INTRODUCTION

Cancer is a disease affecting 1 in 3 adults and 1 in 330 children worldwide. The identification of defined cancer biomarkers and linkages to specific genetic changes has been intensely researched in the last two decades. It is now widely believed that cancer is purely a genetic disease. Our understanding of cancer initiation and progression since the first proto-oncogene, Ras, was first identified in 1993 has been exponential.[1] We have made incredible strides in identifying specific cancer biomarkers, characterizing cancer stem cells and uncovering global gene expression changes in cancer cells versus normal cells. In addition, we can now non-invasively monitor tumour appearance and progression, sequence the human and "cancer" genomes to reveal undiscovered oncogenes and tumour suppressor genes and, lastly, comprehensively understand the six hallmarks of cancer documented in the seminal paper by Hanahan and Weinberg in 2000.[2] It is widely accepted that cancer is a complex disease and its complexity has stimulated a worldwide inderdisciplinary approach to understand what drives the survival of a cancer cell. In this article, I will summarize the current status of cancer research and present a commentary on what lies ahead for the next two decades for deciphering cancer signaling networks.

A SHORT HISTORY OF CANCER

Cancer is a disease that arises when cells have uncontrolled growth. It is a disease that was initially thought to be one of old age. However, this ideology has now been demonstrated to be not entirely accurate as cancer can strike at any age. It has been estimated that > 10% of the human population will die of cancer annually worldwide. Canada will see about 173 800 new cases of cancer in 2011 while the United States will see 1 529 560 new cases over the next year.[3,4] It is considered to be the second leading cause of death in both Canada and the United States behind heart disease. Cancer can occur in the majority of our tissues and was first documented by the Greek physician, Hippocrates (460 – 370 BC) by using the words "karkinos" and "carcinoma" to describe non-ulcer and ulcer forming tumours, respectively. It was later translated into Latin by the Roman physician, Celsus (28 – 50 BC) to "cancer", the Latin word for "crab". Hippocrates described cancer as similar in appearance to the finger-like projections of a crab. The Roman physician, Galen (130 – 300 AD) used the word "oncos" to describe the observed tumours. Oncos is the Greek word for swelling and thus was appropriately described by Galen.[5] During the Renaissance period leading into the 19th century, human diseases were studied in a more systematic way by scientists like Galileo and Newton that generated a more detailed account of the appearance and progression of cancer. We now have a better understanding of this devastating disease that does not discriminate by age, sex or ethnicity.

It is now widely accepted that all cancers arise due to the accumulation of mutations in genes that will allow the tumour cell to achieve several characteristic "hallmarks" or phenotypic characteristics as described by Hanahan and Weinberg in 2000.[2] These include a limitless potential to replicate, evade apoptosis, survive by promoting neo-vascularization, invade and spread, escape growth suppressor pathways and sustain proliferative signaling. Defining the hallmarks of cancer in 2000 has provided the framework for cancer research over the past decade and will continue to drive new discoveries over the next several decades. Unlike diseases such as muscular dystrophy and cystic fibrosis whereby changes in a single gene results in a disease state, cancer arises due to multiple genetic changes to growth regulatory pathways. The appearance of cancer follows Knudson's two hit hypothesis[6] whereby at least two "hits" or genetic changes are required in order to achieve the cancer state. However, there is evidence that cancer most likely arises as a result of 2 - 3 genetic alterations in humans and up to 5 alterations in mice.[7] These changes alter the replicative ability of the cell and promote the growth and survival of the resulting cancer cell. Understanding how to detect these changes before additional changes occur is a monumental challenge that will only be achieved through intense interdisciplinary research, the creation of innovative technologies and increased funding support for cancer research. We have already seen the benefits of cancer research in the survival rates of several cancers. Examples include the > 70% survival rate in childhood leukemias, early diagnostics for prostate cancer (PSA – prostate specific antigen detection) and breast cancer (BRCA1 expression). Additionally, research has identified epigenetic silencing of genes in cancer as a major mechanism of modulating the growth rates of cancer cells. This has been demonstrated for the tumour suppressor

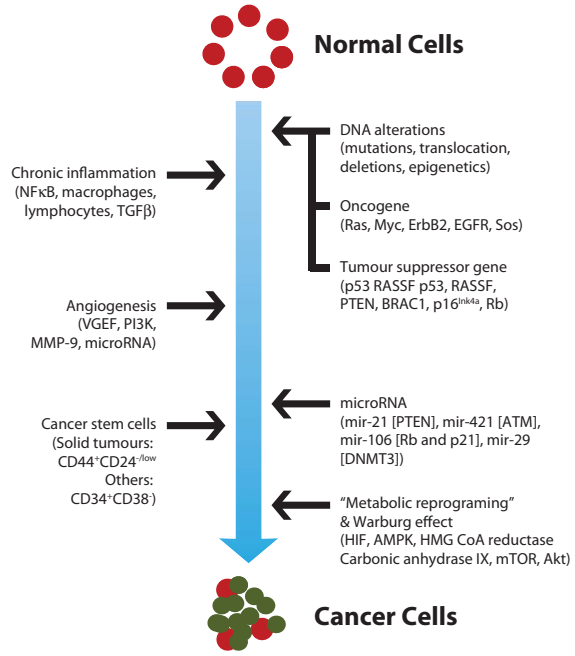

Figure 1. ***Selective pressures responsible to drive a normal cell to a cancer cell phenotype.*** Several elements are responsible for driving and maintaining the cancer phenotype as indicated. Names in brackets are examples of genes that are involved in each element listed. The genes listed for the micro RNA (mir) are the ones that the microRNA may regulate. The cluster determinant (CD) numbers for the cancer stem cell denotes the surface biomarker that identifies a solid tumour cancer stem cell or a hematopoietic cancer stem cell. The Warburg effect denotes the reprogramming of energy production by the cancer cell to rely on glycolysis versus mitochondrial metabolism to produce ATP (a state of "aerobic" glycolysis). Please see text for more details.

gene family, Ras similarity family (or RASSF) and the cyclin dependent kinase inhibitor gene, $p16^{Ink4a}$. Epigenetic silencing of RASSF1A has been established as an early diagnostic test for ovarian cancer in the U. S. and can also be utilized as a diagnostic test for other carcinomas as it is commonly silenced in cancer cells.[8] The epigenetic silencing of RASSF1A is considered to be an early event in tumour formation and thus very suitable as an early diagnostic test. The hope is that with an interdisciplinary approach to understand cancer, we can continue to identify other cancer biomarkers to aid in better diagnosis and prognosis.

THE HALLMARKS OF CANCER

Hanahan and Weinberg have eloquently summarized years of cancer research information into six defining characteristics of cancer.[2] As mentioned earlier, this seminal paper in Cell has been the framework for the cancer research conducted in many groups over the past decade. I will only highlight what was documented in the article in 2000 and describe what challenges we face with respect to cancer research for over the next 1 - 2 decades.

The hallmarks of cancer focused on the ability of cancer cells to have several unique properties that include limitless replicative potential, evasion of apoptosis, ability to stimulate neo-vascularization, invasion and metastasis, and to inhibit suppressor pathways and sustain proliferation. These principles are found in all neoplastic diseases. These defining characteristics are never acquired at once but are acquired through a "multi-step process of human pathogenesis that allows the cancer cells to acquire the traits to enable them to become "tumorigenic" or in a state of "chronic proliferation".[9] Since these characteristics were documented in 2000, it has been quickly realized that two more "emerging" hallmarks need to be recognized – the ability of cancer cells to evade the immune system and their ability to carry out metabolic reprogramming to ensure the availability of nutrients for their survival **(Fig. 1)**. Cancer cells thus have a number of selective pressures to drive their phenotype as shown in Fig. 1.

The ability to have limitless replicative potential is a result of genetic alterations (such as mutations, translocation, deletions and epigenetic changes) to numerous genes. These genes can be categorized into two groups of "cancer causing" genes - the driver genes (changes that promote a growth advantage to the cancer phenotype) and the passenger genes (changes to genes that support/maintain the cancer phenotype).[10] Through the efforts of Bert Volgelstein and his colleagues in sequencing the "cancer" genomes, they came to the conclusion that about 10% of driver genes code for oncogenes that function to promote accelerated growth. However, about 90% of the driver genes code for tumor suppressor genes that inhibit growth **(Fig. 2)**. The remaining genes are passenger genes whose genetic change occurred coincidently or, most likely, subsequently to the presence of the driver mutation. Therefore, tumour appearance is heavily determined by the function of tumour suppressor genes. It was further determined that tumour suppressor genes have a much higher mutation rate than oncogenes and thus would be more of a driving force to promote tumourigenesis if inactivated.[10,11]

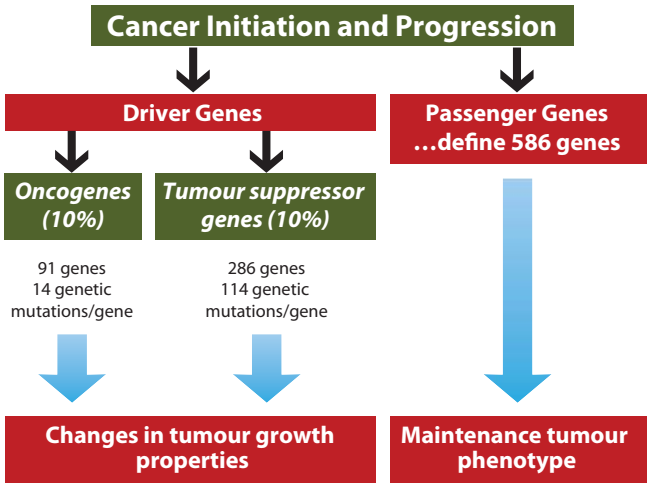

Figure 2. ***Both oncogenes and tumour suppressor genes drive the cancer phenotype.*** Data from this figure was largely gathered from the work of Bert Vogelstein and colleagues that suggest that in any one particular cancer genome, the indicated number of driver and passenger genes exist with the indicated number of mutations/gene. As indicated, passenger genes support or maintain the cancer phenotype and account for the rest of the genes that are involved in the appearance and progression of cancer.

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Vogelstein and colleagues demonstrated that effort should be focused on understanding how driver genes function and contribute to tumourigenesis. Ras was the first oncogene identified in 1993[1] and was found to be a critical element in epidermal growth factor signaling in many other growth factors signaling pathways.[12] Oncogenes typically arise from point mutations, translocations and deletions such that the modified form of the original gene (now termed an oncogene) has the ability to promote enhanced growth factor signaling. Several oncogenes are listed in Fig. 1 and all have been well characterized to be involved directly or indirectly in the Ras-Raf-MAPK (the Ras oncogene),[12] PI3K-Akt/PKB/mTOR (the ErbB2 oncogene)[13] or the MKK/p38 or MKK/JNK (the Myc oncogene)[14] growth factor signaling pathways. Similarly, the loss of tumour suppressor genes can also contribute to enhanced growth factor signaling and proliferation. A lot of effort has gone into understanding how tumour suppressor genes function, as their role in biology is quite varied when compared to oncogenes. However, the end stage result of the loss of normal function of oncogenes or tumour suppressor genes is the same – enhanced proliferation. Numerous tumour suppressor genes have been identified over the last 20 years with p53, pRb, RASSF1A, PTEN and $p16^{Ink4a}$ commonly mutated or epigenetically silenced in cancer cells. Generally, functional inactivation of tumour suppressor genes in cancer cells occurs by point mutations that interfere with their role(s) in cell cycle control or apoptosis.[9,15] In other cases, such as for $p16^{Ink4a}$ and RASSF1A, functional inactivation results from epigenetic methylation of their promoters to result in loss of expression and failed ability to modulate cell cycle control and proliferation. In the case of RASSF1A, the ability of this tumour suppressor gene to promote cell death (apoptosis) and cell cycle arrest is lost, resulting in a selective advantage to promote the survival of the cancer cell.[4,16] In fact, the RASSF gene family is one of the most commonly silenced tumour suppressor genes with RASSF1A, RASSF2, RASSF5A and RASSF8 all considered as tumour suppressor genes.[16] A complete understanding of the roles for this gene family and other tumour suppressor genes will be needed as 90% of driver genes in cancer are tumour suppressor genes that will promote the cancer phenotype.

The other phenotypic characteristics of cancer are detailed in an updated version of the classic article published in Cell in 2011.[9] In that article, Hanahan and Weinberg continue to emphasize the validity of the six hallmarks of cancer. We have already discussed oncogenes and tumor suppressor genes and how they are involved in apoptotic and growth signaling pathways. Over the past decade, we are beginning to understand the complex process of other characteristics of cancer such as neo-vascularization and the invasive abilities of tumour cells. Both are essential to promote tumour progression and metastasis and are essential for the survival of tumour cells. Hanahan and Weinberg describe at length the players and outcomes of new blood vessel formation and tumour invasiveness.[9] In the next decade, our understanding of neo-vascularization and tumour invasiveness will substantially aid in designing treatment schemes for patients to better manage their cancer and prevent the spread to secondary sites such as those of the brain and bone.

NEW ADVANCES IN UNDERSTANDING TUMOUR SURVIVAL

The original six defining characteristics of cancer focused on a fundamental understanding of how cancer cells affect proliferation, apoptosis, cell cycle regulation and new blood vessel formation. While understanding these is important, Hanahan and Weinberg in their 2011 updated publication realized that there are more fundamental differences between normal and cancer cells.[9] Two prominent and new emerging phenotypes of cancer cells are their ability to evade the immune system and their ability to metabolically re-program to utilize glycolysis instead of mitochondrial respiration as source of energy (the Warburg effect). Both of these issues have been areas of intense research over the past decade with surprising results.

> ***// Patients die not from the cancer but from the potent autoimmunity reaction that can occur before or shortly after malignancy ensues. //***

The immune system is composed of both the innate and adaptive immunity that function as our defense against foreign pathogens for protection against excessive tissue damage.[17] We have a great deal of knowledge of how both systems work and how the immune system can distinguish between 'self' and 'non-self'. A tumour cell was originally a natural part of the body and would be recognized as 'self' by the immune system. We know this to be true as the majority of tumours are well tolerated in the body within multiple organs. Hanahan and Weinberg do point out that there are documented cases of, "increases in certain cancers in immunocompromised individuals"[9,18] suggesting that tumour "surveillance" by the immune system may actually occur but not to the extent that the body would recognize and attack an invading pathogen. Therefore, the immune system may have a role in the occurrence of cancers. This idea of immune attack on tumours is supported in the case of paraneoplastic antigen syndromes whereby the immune system does attack the tumour in a robust anti-tumour response. This is the only documented case of a biologically active anti-tumour response. Paraneoplastic antigen syndromes manifest themselves in many forms and anti-tumour responses are mounted to systemic tumours of the breast, lung and ovaries.[19] What usually occurs in paraneoplastic antigen syndromes is the upregulation of proteins in the tumour that are normally not expressed or are expressed at a low level. Once this occurs, it triggers a $CD4^+$ driven humoral response to generate antibodies against these additional components on the surface of these tumours. This is advantageous as it can effectively kill the tumour. However, the antibodies produced will migrate to areas where the upregulated protein(s) are normally highly expressed and an

autoimmune response proceeds. Patients die not from the cancer but from the potent autoimmunity reaction that can occur before or shortly after malignancy ensues.

The paraneoplastic neurological disorders (PNDs) are a subset of the paraneoplastic antigen syndromes that are particularly devastating as the proteins upregulated in systemic tumours formed in patients with PNDs are usually present in the brain – the paraneoplastic antigen markers (of which PNMA1-4 are an example of such a family of markers). Following antibody production, the anti-PNMA antibodies will cross the blood/brain barrier and induce an autoimmune reaction and/or promote cell death at various positions in the central nervous system resulting in neuronal destruction.[20] This is of particular importance to our research group as PNMA4 (also known as modulator of apoptosis 1 [MOAP-1]) is an interacting partner to the tumour suppressor protein, RASSF1A – the focus of our research. Understanding why these PNMAs are upregulated in some cancer cells, how the anti-tumour immunity is driven and the role of PNMAs in neuronal function is important as we might be able to utilize this information to generate an effective anti-tumour response without the autoimmunity effect. The RASSF1A/PNMA4 pathway is involved in numerous aspects of biology including cell cycle control, apoptosis, cell migration, microtubule stability and inflammation. It will be interesting to explore the role for both RASSF1A and PNMA4/MOAP-1 in the appearance of PNDs and the molecular partners involved.

The above response is mainly driven by the production of anti-tumour antibodies by the adaptive immune system. Equally as important is the innate immune system that will respond to invading pathogens and to the presence of damaged tissues.[17] As mentioned earlier, inflammation is a complex defense mechanism against biological and chemical insults. It must be activated rapidly and shut down in a timely manner in order to prevent damage to surrounding healthy cells. Although inflammation is generally beneficial, it is well documented that persistent or excessive inflammation can cause cellular damage if not properly down-regulated and can predispose individuals to other diseases later in life, including numerous cancers. In fact, it has been documented that about 1/3 of all cancer cases are preceded by chronic inflammation that is most likely initially driven by the innate immune system. Examples include *Helicobacter pylori* and gastric cancer,[21] inflammatory bowel disease (IBD) leading to colorectal cancer,[22] chronic bronchitis leading to lung cancer[23] and chronic pancreatitis resulting in pancreatic cancer.[24] Therefore, we need to understand how to manage chronic inflammatory states in order to reduce the risk of developing cancer later in life. Again, our research into RASSF1A has begun to allow us to understand the link between inflammation and cancer. RASSF1A is a tumour suppressor gene that functions to activate cell death and induce cell cycle arrest when needed.[16] We have begun to explore how it may be involved in innate immunity and in the modulation of NFκB activity. What we can conclude from our experiments is that RASSF1A appears to be an important modulator of inflammation. Mice missing RASSF1A have elevated production of cytokines and NFκB activity as well as severe colonic damage following innate immunity insults (unpublished observations). These data suggest that the loss of RASSF1A can promote a state of chronic inflammation. In support of this data, it has been documented in cell lines that elevated levels of IL-6 can drive expression loss of RASSF1A by upregulating DNA methyltransferases (DNMTs) function to methylate the promoter region of exon 1A of RASSF1A[25,26] (an epigenetic inactivation). We are currently confirming this result in wild type mice by inducing a state of chronic inflammation followed by expression testing for RASSF1A. It will be interesting to see if we can observe loss of RASSF1A expression that correlates with the time frame of increased IL-6 production and formation of colonic tumours. Chronic inflammation may also result in the expression/functional loss of oncogenes and/or other tumour suppressor genes that would also predispose inflammatory disease patients to getting cancer later in life. Focusing on this aspect of the origins of cancer may be an important way to reduce the incidence of cancer worldwide.

"Although inflammation is generally beneficial, it is well documented that persistent or excessive inflammation can cause cellular damage if not properly down-regulated and can predispose individuals to other diseases later in life, including numerous cancers."

Hanahan and Weinberg also discussed the importance of metabolic re-programming in cancer cells.[9] This is an emerging characteristic phenotype of cancer that has gained importance over the past several years. Most cells rely on classical mitochondrial respiration in order to produce the necessary energy requirements needed to survive. However, cancer cells eventually switch to "aerobic" glycolysis by utilizing the glycolytic pathway to produce energy (ATP) in order to drive their survival. Hanahan and Weinberg suggest that cancer cells might upregulate key components to ensure that aerobic glycolysis is utilized for energy production, such as the upregulation of glucose transporter GLUT1.[9] Evidence exists for altered function of other components of metabolism such as AMPK/LKB1,[27] p53,[28] and others.[29] It will be interesting to see if the information gathered from a metabolic understanding of cancer cells will lead to new diagnostics or therapies for some cancers.

EPIGENETICS AND microRNA

The concept of heritable changes in gene expression without alterations in primary DNA sequence has been around for a while. This is, in essence, epigenetics and is commonly used to inactivate fetal genes shortly after birth. Research over the past two decades has revealed the occurrence of epigenetic control of diseases that range from schizophrenia to diabetes. Cancer is a major disease type whereby epigenetic regulation is very prominent and is

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the subject of intense research.[30] Epigenetics involves the addition of a methyl group to the 5' position of cytosine in the context of "CpG" dinucloetides in mammals. Several genes have multiple CpG residues grouped in "CpG islands" within their promoter area that are susceptible to DNA methylation by the three DNA methyltransferase (DNMT), DNMT1, DNMT3a and DNMT3b.[31] The presence of several CpG islands in their promoter region confers susceptibility to be methylated by DNMTs, leading to loss of expression and functional loss of biological activity. Therefore, what is heritable in epigenetics is not the methylated DNA but the presence of CpG islands that will confer susceptibility to the next generation. What is increasingly becoming evident is that it is imperative to explore the temporal and spatial roles for DNMTs in inflammation and cancer. More importantly, can we devise methods to selectively inhibit their activity in cancer cells that have epigenetically inactivated important tumour suppressor and pro-apoptotic genes? RASSF1A, $p16^{Ink4a}$, death associated protein kinase (DAPK) and caspase-8 are frequently methylated in human cancers and quite often in the same tumour subsets.[32] Since these genes are important components of the apoptotic and proliferation machinery, cancer cells are quite intelligent in epigenetically silencing them in order to promote their survival. Several platforms are now available for genome wide epigenetic studies from Roche NimbleGen, Affymetrix, Illumina and Agilent as well as several methodologies for identifying specific genes with methylated DNA such as pyrosequencing, methylation specific PCR and combined bisulfate restriction enzyme analysis (COBRA).[33] Over the past decade, several genome wide methylation analyses have been carried out in cancer patients that have helped in our understanding of epigenetic control of cancer.[34] Over the next decade we will see an increased amount of genome wide methylation analysis as the platforms become more cost effective and provide increased sensitivity.

Rapidly following the rejuvenated importance in epigenetics is the discovery of microRNA (miRNA). These are usually 21 - 23 nucleotides long that function post-translationally to target specific messenger RNA (mRNA) by the association of its complimentary ends to the target mRNA.[35] They are thought to be evolutionarily conserved and function to destabilize mRNAs, resulting in the loss of protein expression. It is currently estimated that about 1000 microRNAs are present to regulate the expression of >30% of protein-coding genes with some microRNA regulating several genes.[36] microRNA are distinct from small interfering RNA (siRNA) and are thought to be the more abundant forms of small RNAs. Currently, about 70 reported diseases are associated with microRNA dysregulation such as DiGeorge Syndrome and fragile-X syndrome to mention a few.[37] A partial list of microRNA to cancer specific genes is shown in Fig. 1. Once again, RASSF1A does not escape being regulated by microRNA and it is thought that miR-181,[38] miR-373,[39] mir-602, miR-148a, miR-152[25] can modulate RASSF1A expression in numerous cancers. It is plausible that the RASSF1A inhibitory microRNAs have tissue specific localization that would allow them to modulate RASSF1A expression in different tissues. Further detailed analysis is warranted to answer these and other questions. Understanding the complexities of microRNA function will add another dimension to the complex regulation of elements targeted in cancer.

CANCER STEM CELLS

Cancer cells are quite a homogenous population of cells that seem to escape all the multitude of checks that an invading pathogen would normally have to encounter. At some point in the progression from localized malignancy to metastatic malignancy cancer cells find a way to "evolve" and differentiate

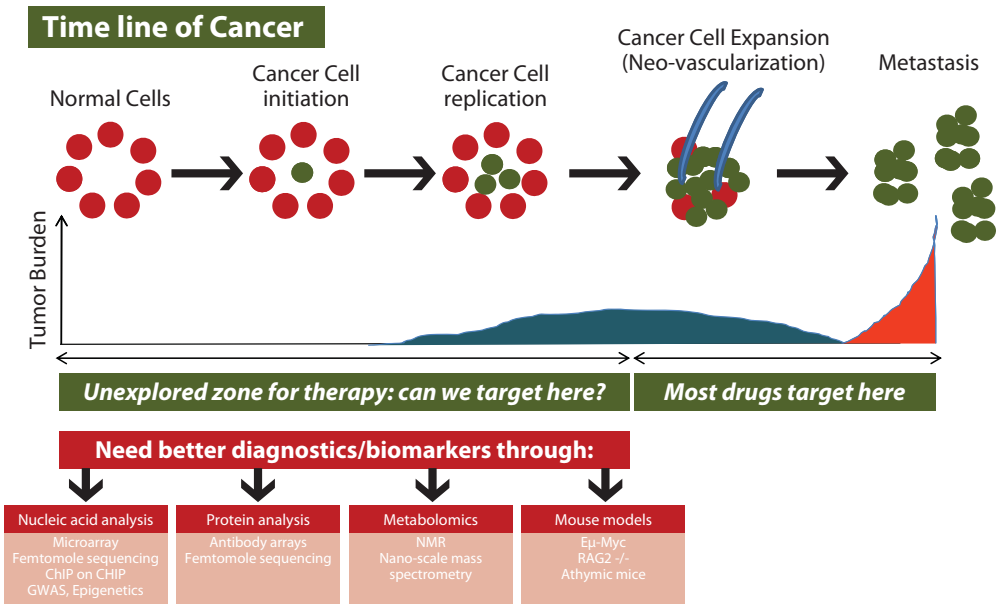

Figure 3. ***The time line of cancer progression.*** This figure illustrates the progression of a normal cell to a cancer cell and, eventually, to a metastatic cell. The majority of cancer therapy schemes will target an individual whereby the tumour can be clearly detectable. However, by this stage many cancer cells may have already acquired the ability to grow uncontrollably and even begin the switch to a metastatic state. The challenge facing cancer therapeutics is to design methods to detect the tumour cell much earlier before they have acquired all the alterations to become a cancer cell. Several platforms are available for early detection of cancer cells as well as for cancer research as indicated. Tumour burden can be tumour size or tumour numbers. The mouse models indicated represent a small group of what is available. Please see text for more details. GWAS, genome wide association studies; ChIP, chromatin immunoprecipiation; CHIP, microarray chip; NMR, nuclear magnetic resonance.

into a form that continues to elude the body and become heterogeneous. What is increasingly becoming evident is the presence of tissue specific stem cells that have the imprinting necessary to differentiate into diverse specialized cell types or can self renew to produce more stem cells.[40] A large effort has been funneled into understanding the surface topology of a stem cell with respect to surface markers, contacts with the tissue microenvironment and genetic and epigenetic changes. The concept of a cancer stem cell spawned from the increasing need to understand the origin and function of tissue stem cells. Cancer stem cells are, by definition, cells that have all the necessary regulatory factors to produce new tumour cells. The devastating possibility of the presence of a cancer stem cell has fueled efforts into creating stem cell research departments/divisions/institutes, separate funding streams for stem cell research, focused meetings on stem cells and an effort to define, understand, manipulate and destroy a cancer stem cell.

As one can appreciate, stem cells are a low abundant population of cells that have built in mechanisms to survive in order to repopulate themselves. The concept of a cancer stem cell is slowly being accepted and if true, it will be imperative to eradicate not only the cancer cells but also the cancer stem cells in order to find a cure for cancer. Cancer stem cells were first identified in hematologic tumours followed by their identification in solid tumours of the breast. Specific surface antigens were subsequently characterized as shown in Fig. 1. These and other markers have routinely been used to isolate cancer stem cells and culture them *ex vivo*. We know that these isolated populations of cancer stem cells can form tumours as xenograft assays have been carried out as proof of principle. However, it is currently uncertain as to the exact origin of these cells, but we do know that they are capable of self-renewal to form new tumour cells. What is also being realized is that epithelial to mesenchymal transition (EMT) may support the cancer stem cell phenotype and actually aid in cancer metastasis and in the repopulation of cancer cells at the desired destination reached following metastasis.[9,41] Several signaling molecules and pathways are altered in cancer stem cells such as the loss of PTEN and upregulation of β-catenin as well as abnormal Wnt, Hedgehog and Notch signaling pathways.[42] Interestingly, these pathways are important during the development and differentiation of normal cells suggesting that they can support the survival of cancer stem cells that can renew themselves. The mere presence of a cancer stem cell adds a complexity to cancer research that is only now beginning to be realized. However, it does give us an alternative target for therapy and the hope that if cancer stem cells can be eradicated then the cancer can be as well. A detailed understanding of the origins, propagation, survival and specificity of cancer stem cells may be brought to light over the next decade that will certainly aid in our understanding of how cancer originates and spreads.

> ***// The mere presence of a cancer stem cell adds a complexity to cancer research that is only now beginning to be realized. //***

THE FUTURE OF CANCER RESEARCH

I have tried to highlight some of the important advances in the conceptual evolution of cancer from a black box to an understanding of it as a cell having complex genetic and protein regulatory networks successfully escaping our defense mechanisms. As we learn more about the complex molecular circuitry within a cancer cell we might stand a better chance at finding a way to eradicate these cells. Current therapies that target cancer cells usually do so only after tumours have formed and have acquired several alterations to define them as a cancer cell. What will be truly groundbreaking is our ability to treat patients at a stage before a cancer cell has acquired some of the hallmarks defined by Hanahan and Weinberg[9] **(see Fig. 3)**.

The development of early diagnostic tests for cancers is a monumental task as tumour appearance varies from patient to patient and also from tumour to tumour. However, as we know more about the origins of cancer we can attempt to organize temporal changes with phenotypic changes to allow for rational intervention strategies. Understanding these phenotypic changes will require several platforms and methodologies as described at the bottom of Fig. 3. These include nucleic acid analysis for gene expression, femtomole sequencing by nano-electrospray mass spectrometry, genome wide association studies (GWAS) for both mRNA and epigenetic variations in gene expression, and the use of NMR to determine the differences in the metabolites produced in normal versus tumour cells (the growing field of metabolomics). These and other technologies will greatly aid in the search for distinct signatures of cancer cells.

Bert Volgelstein and colleagues have begun the task to decipher the molecular signatures of cancer cells. They have now completely sequenced > 100 patients with cancer in order to define the composition of a cancer genome. This work culminated in a theory that abnormal proliferation of cancer cells can be traced to alterations in 12 "core" signaling pathways that include TGFβ, Wnt, HIFα, Jak/STAT, Notch, PI3K/PTEN, Ras/Raf, Hedgehog signaling pathways together with general pathways for cell adhesion, apoptosis, DNA damage control and control of G1/S transition.[43,44] Cancer can be understood by the analysis of these core signaling pathways and as we know more about the differences between normal and cancer cells, i.e., identification of cancer specific biomarkers, one can then envision the beginning of personalized medicine. For cancer therapy, personalized medicine may be needed due to the genetic heterogeneity of cancer. Therapies that are being investigated include RNAi and antisense approaches, cancer stem cells, tumour immunotherapy, gene delivery systems (via viral and non-viral mechanisms), apoptosis and DNA synthesis/repair activators (mainly via cisplatin, etoposide and doxirubicin treatment) and radiation therapy to mention a few.

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Mouse models have emerged as excellent mediums to explore the origin and progression of cancer due to the extensive physiological and molecular similarities to humans, their small size and short life span of 3 years, and the benefit of having their genome completely sequenced. With respect to cancer therapeutics, they have emerged as a useful model to test emerging new drugs for short term and long term effects. Several models have been extensively used in cancer research such as the Eμ-Myc mouse for understanding B cell lymphomas,[45] the RAG2$^{-/-}$ mice for investigating cell autonomous effects and blood cancers, the K-Ras transgenic mouse for investigating lung cancer and numerous other carcinomas,[46] and the athymic (nude) mouse for carrying out xenograft assays in order to explore tumour growth potential. Mouse models offer a novel method to investigate several aspects of cancer research, especially the interplay between tumour cells and their endogenous microenvironments[9,47] and the environmental hazards that can drive tumourigenesis. Protocols to manipulate the mouse genome are well established and sophisticated such that genes can be overexpressed and targeted to specific tissues, deleted in specific tissues and turned on and off in specific tissues using tetracycline based promoters and Cre-LoxP recombination systems.[47] For most of these manipulations the ultimate goal is to recapiculate the human disease with respect to genetic and phenotypic characteristics. Although the latter is not always possible, mouse models are an important methodology utilized in cancer research that allows us to explore the complexity of a very hetereogenous disease.

Emerging new therapeutic approaches are continually being tested in mice and other animal models. One of the more exciting therapeutic approaches is the use of nanoparticles for drug delivery. These particles are usually between 1 to 100 nm in diameter and can be modified with lipid moieties in order to cross the lipid bilayer.[48] They can also be coated with surface markers to reach defined destinations, filled with chemotherapeutic or siRNA particles for drug delivery. They are small enough to cross the blood vessel wall and enter into target tissues and have the ability to cross the lipid bilayer.[49] The large surface area of a nanoparticle can allow for a number of target molecules to be added to specifically recognize cancer cells or cancer stem cells. Once they reach their destination, they are internalized by receptor mediated endocytosis followed by breakdown in the cytoplasm and release of the drug or reagent. The limitation to this methodology is prior knowledge of the surface of a cancer cell and the efficiency of target acquisition, i.e. the cancer cell. Once the chemotherapeutic drug is released in the cancer cell, this will trigger an apoptotic response to cause its demise. Researchers have already documented that some receptors, such as the TNFα related apoptosis inducing ligand receptor (TRAIL-R), are actually upregulated in cancer cells while expressed at very low levels in normal cells.[50] TRAIL-R is a death-inducing receptor so it is unusual for it to be upregulated in cancer cells. It is plausible that TRAIL-R is involved in non-apoptotic signaling and confers a selective advantage to cancer cells. Can this be coupled to a nanoparticle for delivery to cancer cells? Further research is needed to utilize TRAIL-R or other surface molecules for nanoparticle delivery for cancer therapy.

// Over the next decade, the combination of "medicinal" nanoparticle design and delivery together with a complete understanding of the signaling pathways and genetic alterations in cancer cells will allow for early detection, selective treatment, inhibition of metastasis and personalized medicine. //

Once a topic for science fiction, the "magic bullet" cure for cancer may be in the form of a nanoparticle. Again, accurate and precise delivery is a limitation of the use of nanoparticles and is currently a subject of intense research. Once we really understand the genetic composition of a cancer cell[44] we can engineer methods to selectively eradicate them amongst a milieu of normal cells. Over the next decade, the combination of "medicinal" nanoparticle design and delivery together with a complete understanding of the signaling pathways and genetic alterations in cancer cells will allow for early detection, selective treatment, inhibition of metastasis and personalized medicine. I am optimistic that the next decade of cancer research will open up unexplored avenues of research that will allow us to understand the complex framework of what drives a normal cell to become a cancer cell.

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Abbreviations used:

AMPK, 5'-AMP activated protein kinase; **DNMT**, DNA methyltransferase; **EMT**, epithelial to mesenchymal transition; **MAP**, mitogen activated kinase; mir, microRNA; **MOAP-1**, modulator of apoptosis; **NFκB**, nuclear factor of kappa light polypeptide gene enhancer in B-cells; **PI3K**, phosphatidylinositol 3-kinase; **PKB**, protein kinase B; **RAS**, Rat Sarcoma; **RASSF**, Ras association domain family; **RBD**, Ras binding domain.

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