

Review

The Ten Hallmarks of Cancer in Cutaneous Malignant Melanoma

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ABSTRACT / Malignant tumors are complex and therapeutic intervention often ineffective. To bring more structure in the complex nature of tumors, the hallmarks of cancer framework was developed. This conceptual framework encompasses ten cellular functions that make normal cells malignant cancer cells. These hallmarks are: genomic instability and mutation, tumor promoting inflammation, sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, deregulating cellular energetics and finally avoiding immune destruction. These hallmarks are functional within the context of the tumor microenvironment. The hallmarks of cancer form a general framework which can be applied to interpret alterations that occur in different types of cancer. Therefore it should predict that any specific type of cancer shows oncogenic alterations that match the hallmarks described by Hanahan and Weinberg. The type of cancer selected to test this hypothesis is melanoma. This form of skin cancer represents only 4% of human skin cancers but is responsible for 75-80% of skin cancer deaths.

Introduction

The hallmarks of cancer framework was developed to structure the complex nature of cancer. Malignant tumors are complex and often not responding to therapeutic intervention. In order to structure this complexity the hallmarks of cancer framework were developed^{1,2} (Fig. 1). The hallmarks are: genomic instability and mutation, tumor promoting inflammation, sustaining proliferative signaling, evading growth suppressors, resisting cell death, replicative immortality, inducing angiogenesis, invasion and metastasis, deregulating cellular energetics and finally avoiding immune destruction. These hallmarks are functional within the context of the tumor microenvironment. Early stage tumor cells can acquire adaptations that improve one of the hallmark capabilities, giving them a competitive advantage over its neighboring cells. With each newly acquired advantage, a subclone outgrows the other cancer cells, enabling the hallmark capabilities to be acquired via a series of clonal expansions

The hallmarks of cancer form a framework that can be applied to interpret adaptations found in different types of cancer. Therefore, it predicts oncogenic adaptations found in a specific type of cancer matching the hallmarks as described by Hanahan and Weinberg. The type of cancer that is selected to test this hypothesis is melanoma. The hypothesis of this article is therefore: *oncogenic alterations, which are predicted by the hallmarks of cancer conceptual framework, can be found in melanoma*. The aim of this article is not to give a complete overview of all oncogenic alterations reported for melanoma. The

aim of the article is testing the predictive value of the hallmarks of cancer conceptual framework. For this purpose one oncogenic alteration, reported for melanoma, is discussed for each hallmark.

Figure 1 / The Ten Hallmarks of Cancer. This conceptual framework encompasses ten cellular functions that make normal cells malignant cancer cells, these hallmarks are functional within the context of the tumor microenvironment. This framework was developed to structure the complex nature of tumors and to rationalize the development of anti-cancer therapies.



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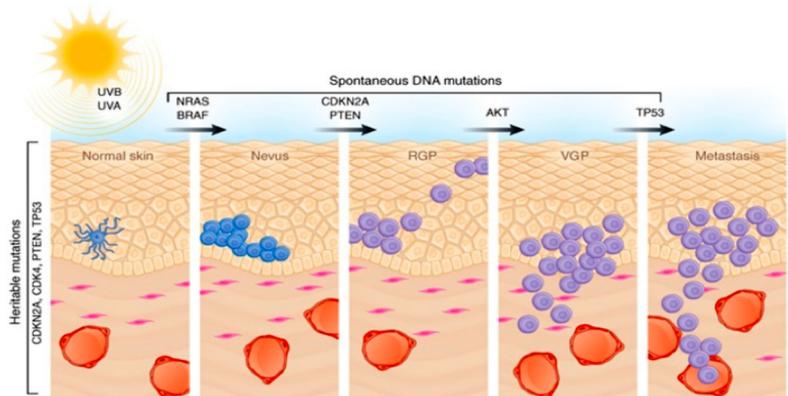
Cutaneous malignant melanomas represent only 4% of human skin cancers but are responsible for 75-80% of skin cancer deaths^{3, 4}. Melanoma originates from melanocytes, the skin cells producing the pigment melanin. These melanocytes are found in the bottom layer of the epidermis, the pigmented parts of the eye and mucosal surfaces lining the intestines and the reproductive tract⁵. Melanocytes found in all these tissues can give rise to melanomas. The most common type is cutaneous malignant melanomas (CMM) and this article will focus on this type of melanoma. The development of CMM is illustrated in Fig. 2. A single mutated melanocyte develops, via a multi-step process, into a metastatic tumor. This process is initiated by ultra violet irradiation, heritable mutation, or a combination thereof.

1 Genomic instability and mutation

An important hallmark to the development of malignant tumors is genomic instability, which enables an increased rate at which mutations are acquired. Genomic instability enables other hallmarks to develop and drives tumor progression. For cells it is essential to keep the genome stable, therefore they have evolved an arsenal of proteins preventing and repairing mutations. When DNA damage is recognized, the cell cycle can be halted until the damage is repaired. If the DNA damage is beyond repair tumor, suppressor proteins such as p53 activate senescence or apoptosis². Despite all these safety mechanisms cells can form malignant tumors.

Genomic instability in CMM is caused or aggravated by mutagenic properties of ultraviolet (UV) radiation. The risk of developing CMM is positively correlated with UV exposure⁶⁻¹⁰. Melanoma shows many mutations with an "UV signature", such as a cytosine to thymine transition^{11, 12}. UV radiation is non-visible light with a wavelength between 100 and 400 nanometer (nm). UV radiation is categorized as UV-c (100-280 nm), UV-b (280-315 nm) and UV-a (315-400 nm). Light of shorter wavelengths contains more energy, which makes UV-c potentially the strongest mutagenic factor. Fortunately, all UV-c in sun light and most of UV-b is absorbed by ozone in the stratosphere as shown in Fig. 3. However, damage to the ozone layer causes the amount of UV-b reaching the surface of earth to increase. It is also important to note that none of the UV-a is filtered out by the ozone layer¹³.

Figure 2 / The multi-step progression of CMM, normal melanocytes develop into cancer cells. This process is initiated by ultra violet irradiation, heritable mutation or a combination thereof. Spontaneous mutations lead to the development of hallmark functions finally resulting in a metastatic tumor³⁵.

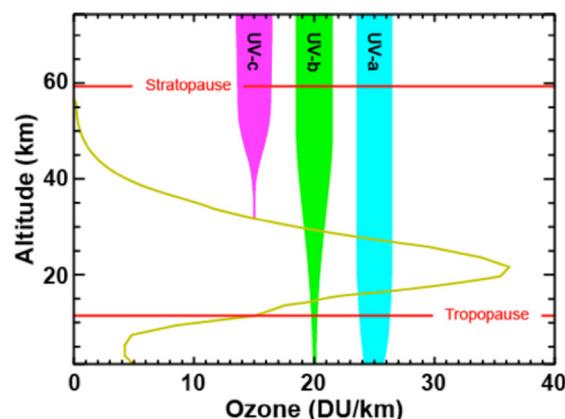


2 Tumor-promoting inflammation

The immune system has evolved over millions of years to protect the human body against invading pathogens. Additionally, the immune system eliminates rogue somatic cells, which are sacrificed to ensure the survival of the organism as a whole. The immune system plays an important role in controlling cancer growth and is a promising therapeutic tool to enhance anti-tumor activity. However, inflammation has been shown to be a double-edged sword.

Chronic inflammation enables tumor progression in several ways. Immune cells secrete a multitude of signaling molecules, enzymes and reactive oxygen species (ROS) into the tumor microenvironment. These signaling molecules can activate proliferating cells.

Figure 3 / UV radiation, has a shorter wavelength than visible light. These shorter wavelengths contain more energy; this gives UV its mutagenic properties. UV is categorized in UV-c, UV-b and UV-a. UV-c is the strongest mutagenic factor, followed by UV-b and then UV-a. The main sources of skin cancer are UV-b and UV-a since UV-c and most of UV-b are blocked by the ozone layer³⁶.

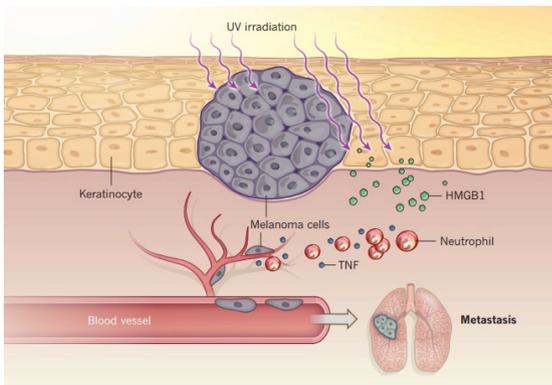


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erative signaling, resistance to cell death, contribute to genomic instability, stimulate angiogenesis and metastasis².

Chronic inflammation in CMM is aggravated by UV irradiation. Not only melanocytes but also keratinocytes can be damaged by UV irradiation. This damage causes keratinocytes to release the cytokine HMGB1 (high mobility group box 1) as is shown in Fig. 4. The release of this protein recruits neutrophils to the tumor microenvironment where they secrete the pro-inflammatory proteins like TNF (tumor necrosis factor). TNF in turn facilitates angiogenesis and metastasis^{7,14}.

Figure 4 / UV induced inflammation contributes to tumor progression. UV irradiation has been identified a new source of an inflammation response. Keratinocytes damaged by UV irradiation secrete the cytokine HMGB1 which attracts neutrophils to the tumor site. These immune cells secrete TNF contributing to the tumor-promoting inflammation^{7,14}.



3 Sustaining proliferative signaling

Sustained proliferative signaling and evading growth suppressors are prominent hallmarks of cancer. A common metaphor for these two hallmarks is a car with a stuck gas paddle and malfunctioning brakes, respectively. Sustained proliferative signaling enlarges the effects of genomic instability since there are more cells available for evolutionary selection. Moreover there is less time for DNA damage repairs.

Sustaining proliferative signaling in CMM in 40-60% of melanomas is caused by an activating BRAF mutation affecting the RAS/RAF/MEK/ERK pathway⁶. The most common mutation is the BRAF V600E, changing the amino acid valine into a glutamic acid making the protein constitutively active^{4,10,15}. This

activated BRAF protein sustains activation of the downstream MEK and ERK kinases resulting in excessive proliferation.

Treatment of melanoma was improved when the BRAF inhibitor Vemurafenib was approved for patients of advanced malignant melanoma⁴. A phase 3 clinical trial was done comparing Vemurafenib with Dacarbazine (DTIC) in patients with BRAF V600E positive advanced melanoma. Compared to DTIC, Vemurafenib slowed initial tumor progression. However, they both result in a similar overall survival at 18 months of 30%¹⁶.

4 Evading growth suppressors

The uncontrolled growth of cancer cells is caused by both evading growth suppressors and sustained proliferative signaling. Two major tumor suppressor proteins are p53 (described as the “guardian of the genome”) and retinoblastoma protein (pRB). Both these proteins can arrest the cell cycle, and induce senescence or apoptosis. Growth suppressor resistance is therefore closely linked to senescence and apoptosis resistance².

Growth suppressor resistance in CMM is often caused by deactivating mutations in the tumor suppressor gene CDKN2A^{10,17-19}. CDKN2A encodes the protein p16, which can arrest the cell cycle in the G1 phase by inhibiting phosphorylation of pRB. p16 inhibits cyclin-dependent kinases CDK4 and CDK6 from phosphorylating pRB. When the CDKN2A gene is mutated p16 can lose functionality. This means the p16 no longer inhibits CDK4 and CDK6. When p16 inhibition is lost CDK4 and CDK6 phosphorylate pRB allowing the continuation of the cell cycle from G1 phase to S phase and continuing proliferation.

5 Resisting cell death

Programmed cell death or apoptosis is essential for a multicellular organism. In embryonic development of humans, programmed cell death is vital. For example, the separation of fingers and toes is achieved by induction of apoptosis of the connecting skin cells.

Resisting cell death in CMM can be the result of defects in apoptotic pathways. DNA damage results in the release of cytochrome-C from mitochondria. Cytochrome-C together with Apaf-1 protein and caspase 9 form a structure called the apoptosome. This apoptosome further activates the pathway finally resulting in apoptosis. In metastatic melanomas the loss of Apaf-1 has been reported²⁰.

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6 Enabling replicative immortality

Enabling replicative immortality is an essential hallmark of tumor progression. Replicative immortality is caused by upregulation of telomerase, a telomere-lengthening enzyme. Telomeres have two important functions. First, telomeres protect the ends of chromosomes from degradation. Second, telomeres indicate that the end of a chromosome is not a double stranded break in need of repair²¹. With each replication the telomeres become shorter, limiting the reproductive potential of chromosomes. Once cells can sustain proliferative signaling, they divide faster and deplete their telomeres. Once telomeres are depleted cells enter a state called replicative senescence.

Replicative immortality in CMM can be caused by upregulation of telomerase reverse transcriptase (TERT) gene. The telomerase complex is formed by TERT and the telomerase RNA component (TERC). In familial and sporadic CMM, mutations are found in the promoter region of the TERT gene. This mutation results in a twofold increase of transcription, which results in replicative immortality²²⁻²⁵.

7 Inducing angiogenesis

In tumors the tissue pressure can be very high due to rapidly dividing cancer cells which press on each other. This high pressure leads to reduced blood perfusion. Without adequate blood flow, the tumor microenvironment becomes starved of nutrients and oxygen and filled with carbon dioxide and metabolic waste products.

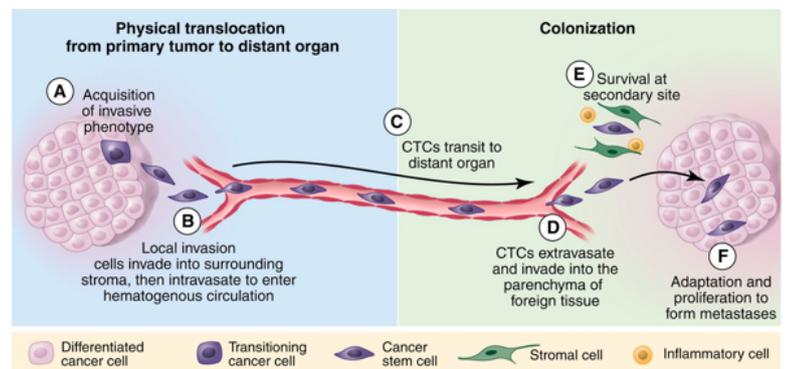
In response to this reduced perfusion and hypoxia tumors can activate the process of angiogenesis. Existing blood vessels are stimulated to sprout new vessels and to develop into mature vessels.

Angiogenesis in CMM is stimulated by melanoma cells secreting glycoproteins which increase the affinity of endothelial cells for FGF (fibroblast growth factor)²⁶. The secreted linear polysaccharides called heparin sulfates are thought to assist in the formation of active FGF/FGF-receptor signaling complexes. This way the endothelial cells lining the interior surface of blood vessels can be stimulated to participate in angiogenesis²⁷.

8 Activating invasion and metastasis

90% of cancer deaths are due to metastatic dissemination to other organs²⁸. Fig. 5 shows the process of metastatic dissemination, starting by “invading” the

Figure 5 / The process metastatic dissemination. The first step in this process is to acquire an invasive phenotype (A). The next step is “intravasation” as either blood or lymphatic vessels (B). The cells spread throughout the body via these vessels (C). During “extravasation” the cancer cells escape the vessels and invade the distant organ (D). At the metastatic site the first challenge for the cancer cells is to survive at the new microenvironment (E). The cells that survive can proliferate and form metastases (F)²⁸.



surrounding tissue. The next step is “intravasation”, as the cancer cells enter blood or lymphatic vessels. During “extravasation” the cancer cells escape the vessels and invade the distant organ. At the metastatic site the first challenge for the cancer cells is to survive at the new microenvironment. The cells that survive can proliferate and form metastases²⁸.

Invasion and metastasis in CMM is accomplished by degradation of the basal membrane and the extracellular matrix (ECM) by invadopodia. Many different proteins are involved in the organization of invadopodia and ECM degradation. The protein N-Wasp is involved in the reorganization of the actin cytoskeleton. The protein cortactin regulates the trafficking of vesicles. These vesicles contain matrix metalloproteinases (MMPs) from the Golgi and are exocytosed by the cell. MMPs are released from the tip of the invadopodium through small holes in the plasma membrane and degrade the ECM. After degradation of the ECM the melanoma cells can spread to other parts of the body²⁹.

9 Deregulating cellular energetics

Under normal physiological conditions the energy metabolism of the cell is carefully regulated. Tissues are perfused with blood via the capillary bed, supplying oxygen, nutrients and removing metabolic waste products. In tumors the sustained proliferative signaling of cancer cell results in an increase

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of the number of cells. These cells press on each other resulting in a high tissue pressure. This high pressure leads to hypoperfusion which results in a lack of nutrients, hypoxic conditions and metabolic waste build-up. As a response to the hypoxic conditions cells switch from aerobic oxidative phosphorylation to aerobic glycolysis. This metabolic pathway is much less efficient in converting glucose to energy carriers such as ATP, therefore the cells upregulate glucose importers GLUT1.

Energy metabolism reprogramming in CMM can be observed using positron emission tomography (PET). The increased glucose uptake by cancer cells can be made visible by injecting the patient with the glucose analog fludeoxyglucose-F18. In this radiopharmaceutical marker a glucose hydroxyl group is replaced by a positron-emitting radioactive isotope fluorine-18.

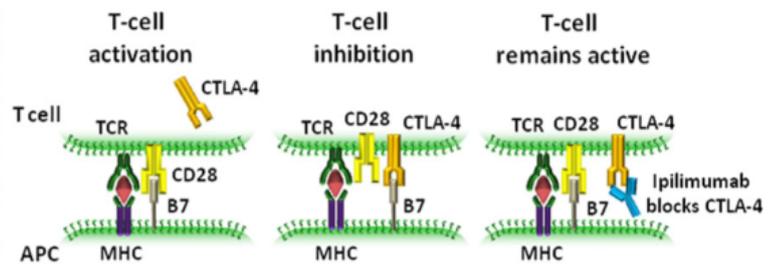
10 Avoiding immune destruction

The tumor microenvironment has been described as an ecological system including somatic evolutionary selection. In this environment there is surveillance and predation by immune cells³⁰. The immune system can detect and eliminate both invading microorganisms and somatic cells showing abnormal behavior. Cancer cells which can escape detection or downregulate immune destruction have an important selective advantage.

Avoiding immune destruction in CMM is being therapeutically targeted by enhancing the activation of immune cells. This approach uses the Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) antibodies Ipilimumab (Fig. 6). The APC displays an antigen bound to a major histocompatibility complex (MHC) which is recognized by the T-cell receptor (TCR). The membrane protein B7 binds to cluster of differentiation 28 (CD28) which assists in activating the T-cell. When CTLA-4 binds to B7 the T-cell activation is inhibited. This is where the drug Ipilimumab intervenes: by binding to CTLA-4 it prevents B7 binding and the T-cell is activated. This cytotoxic T-lymphocyte is now able to recognize and destroy melanoma cells⁴.

In 2011 Ipilimumab was approved to be used in patients with advanced melanoma⁴. A phase 3 clinical trial was done testing Ipilimumab in patients with metastatic melanoma. A significant improvement in overall survival was reported. However, there can be severe side effects which can even be life threatening³¹.

Figure 6 / CTLA-4 antibody Ipilimumab. APC can activate T-lymphocytes by displaying a MHC bound antigen which is recognized by the T-lymphocyte receptor. This activation can be inhibited if CTLA-4 binds B7. Ipilimumab binds CTLA-4 and prevents this inhibition. The activated cytotoxic T-lymphocyte is now able to recognize and destroy melanoma cells⁴.



Tumor microenvironment

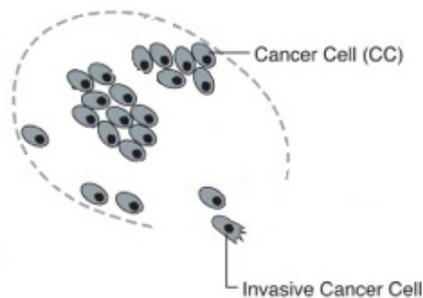
The hallmarks discussed above all function within context of the tumor microenvironment (Fig. 7). This microenvironment consists of cancer cells, seemingly normal cells and is filled with a plethora of messenger molecules. There are immune cells which cause tumor promoting inflammation. In the tumor microenvironment there are growth factors which stimulate sustained proliferative signaling, evading growth suppressors and resisting cell death. The release of ROS present contributes to genomic instability and mutation. The released proangiogenic factors and matrix-modifying enzymes stimulate angiogenesis, activate invasion and metastasis. Finally the hypoxic conditions contribute to deregulating cellular energetics. This highly complex microenvironment can be seen as an ecological system, where by a process of somatic evolution the most fit cancer cells are selected³⁰.

Tumor microenvironment in CMM has been shown to be exported via exosomes to sentinel lymph nodes. The primary tumor microenvironment allowing cancer cells to thrive was thought to be absent at a potential metastatic site such as a sentinel lymph node. However, studies have shown that exosomes released by melanoma cells prepare sentinel lymph nodes for metastasis^{32,33}. These lipid bilayer enclosed vesicles carry growth factors and matrix remodeling enzymes which are thought to prepare the metastatic site for colonization.

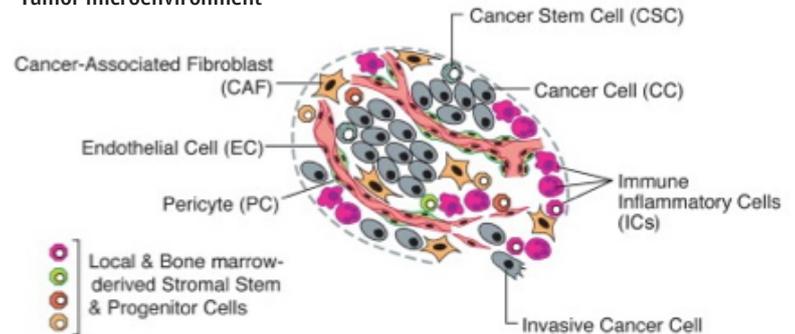
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Figure 7 / Tumor microenvironment complexity. The hallmarks of cancer conceptual framework can be seen as reductionist thinking. By reducing this complex subject into usable parts it is a great tool. However, the individual hallmarks are often interdependent and function within the tumor microenvironment. It is important to note that Hanahan and Weinberg are aware of the limitations of a reductionist view (adapted from <http://science-blogs.com/insolence/2011/08/23/the-complexity-of-cancer/> and Hanahan and Weinberg, 2011)

Reductionist view



Tumor microenvironment



Discussion

The oncogenic alterations described above support the main statement of this article. *The oncogenic adaptations predicted by the hallmarks of cancer can indeed be observed in CMM.* For each of the hallmarks of cancer at least one oncogenic alteration is reported for CMM.

2011 was an important year in the treatment of melanoma with the approval of the immune stimulating antibody Ipilimumab and the BRAF inhibitor Vemurafenib. This offers new prospects for patients of advanced malignant melanoma⁴. With these drugs two of the ten hallmarks (Fig. 1), sustaining proliferative signaling and avoiding immune destruction, are being targeted in CMM. However, despite these developments cancer in general and CMM in particular remains a challenge to treat.

The hallmarks of cancer conceptual framework was developed to structure the complex nature of tumors and to rationalize the development of anti-cancer therapies. The hallmarks of cancer conceptual framework can be seen as reductionist thinking with some limitations (Fig. 7). First, the ten hallmarks described are often interdependent and function within the tumor microenvironment. It can be difficult to discriminate between the different hallmarks. Second, there are many factors contributing to each individual hallmark. Third, the acquisition of a hallmark should be seen as a gradual process depending on many factors rather than flicking an On-Off switch. It is important to note that Hanahan and Weinberg are clearly aware of the limitations of a reductionist view. Despite these limitations, reducing this complex subject into usable parts makes the hallmarks of cancer framework a great tool.

An integral approach of targeted therapy is need-

ed to successfully treat cancer. Despite recent advances, cancer has not been reduced to a disease of the past like smallpox (*Variola major*). The smallpox virus is nowadays only known to exist in highly secured labs of Russia and the United States³⁴. There are many challenges in treating cancer. First, unlike smallpox, there is no external pathogen invading the body. This endogenous nature of cancer makes it hard to discriminate between normal and malignant tissues making it difficult to eradicate cancer cells and at the same time prevent toxicity for healthy cells. Second, the hallmark genomic instability and mutation causes cancer to be a very dynamic and adaptive disease. Third, effectively targeting a specific signaling pathway is hindered by “pathway redundancy”. This means that alternative signaling routes can be activated when a route is impaired by a targeted drug. Fourth, on the level of the tumor as a tissue there is a big heterogeneity in cancer cells. When a specific drug is able to destroy many cells, a resistant subclone can survive and regrow. This subclone will have little competition in the beginning since many other cancer cells have been eradicated.

In order to overcome tumor resistance and relapse an integral approach is needed. Such an integral approach would encompass targeting all of the ten hallmarks shown in Fig. 1. It is important to continue the funding of cancer research to explore the complexities of different types of cancer. This growing body of knowledge on the molecular mechanisms of cancer enables the development of more targeted therapies. The hallmark of cancer framework formulated by Hanahan and Weinberg is of great value in understanding the complex nature of cancer. This framework can be used to guide to rational design towards integrated targeted therapies.

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