Revisiting the hallmarks of cancer: An interconnected hierarchy as illustrated by cancer-generated lactic acid

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Cancer is a complex disease characterized by diverse biological traits. Hanahan and Weinberg originally proposed six "hallmarks" to simplify and generalize the core features shared by all cancers. Over the past 22 years, this number has expanded to 14, reflecting our growing understanding of cancer biology. This continual expansion highlights a broader challenge: the proliferation of hallmarks without a clear hierarchy or conceptual framework that incorporates them in a coherent manner. As a result, the landscape of cancer traits has become increasingly complex and fragmented. To address this, we propose a functional reorganization of the hallmarks into a ranked hierarchy that clarifies their inter-relationships, rather than treating all traits as equally important. Specifically, we classify the hallmarks into three categories: 1) hallmarks essential for cancer cell survival, 2) hallmarks enhancing aggressiveness, and (3) cancer-enabling characteristics. We further illustrate this hierarchy and interconnectivity using cancer-generated lactic acid as a paradigmatic example. As a product of altered cancer metabolism, lactic acid simultaneously promotes immune evasion, supports cell survival, induces angiogenesis, and enhances metastatic potential. This reclassification of hallmarks provides a clearer conceptual framework to guide the development of foundational therapies targeting multiple cancer hallmarks in an integrated manner.

Cancer is a multifaceted and complex disease, with each subtype exhibiting its own unique characteristics and an array of distinctive molecular and genetic alterations. Nonetheless, common characteristics exist. Hanahan and Weinberg proposed the concept of the "hallmarks of cancer" in 2000 to provide a framework for generalizing these shared characteristics across cancers *(1)*. This concept distilled the essential traits that all cancers must acquire to thrive, regardless of their tissue of origin or their specific mutations. Twenty-two years later, the initial six hallmarks have now increased to 14, reflecting our growing understanding of the complexity of cancer biology. This continued expansion of hallmarks raises important questions: are all such characteristics equally essential to cancer survival and progression? Is the concept now overly complex, making generalizations about cancer biology more difficult?

Complicating the hallmarks landscape is the fact that these characteristics are frequently visualized as equal components around the outside of a circle, expanding as more hallmarks are introduced. While helpful, this representation can unintentionally lead to a misunderstanding of cancer biology. The hallmarks are not equal. Hanahan and Weinberg distinguished "hallmarks" from "enabling characteristics", the latter being phenomena that assist cancers in acquiring the more fundamental hallmark traits *(2)*. This distinction suggests an inherent hierarchy of importance, even if that is not obvious in the now-famous illustration. The side-by-side arrangement also fails to demonstrate the inter-relationship between the characteristics. Biologically, as noted originally by Hanahan and Weinberg, there is an indisputable connection between the various cancer traits, including from a signalling pathway perspective. Certain prominent oncogenes and tumour suppressors (such as c-Myc, Ras, and p53) can regulate multiple hallmark characteristics at the same time *(1)*. The hallmarks are thus interdependent and not isolated from one another.

This commentary revisits the hallmarks of cancer, exploring the nuances and interconnections between these cancer traits. We present a conceptual and visual reorganization into three hierarchical categories based on their functional importance:

- hallmarks essential for cancer cell survival;
- hallmarks enhancing cancer cell aggressiveness; and
- cancer-enabling characteristics.

The traits essential for survival are the most fundamental – without life, there is no opportunity for development or progression. Moreover, these traits often serve as drivers that activate multiple downstream hallmarks and enabling characteristics. Cancer-generated lactic acid is an illustrative example of this interconnectivity, highlighting how one fundamental cancer trait can influence multiple additional hallmarks. Re-examining the hierarchy and interconnectivity of the cancer hallmarks will facilitate the development of new therapeutics that target multiple cancer characteristics simultaneously, potentially improving treatment efficacy.

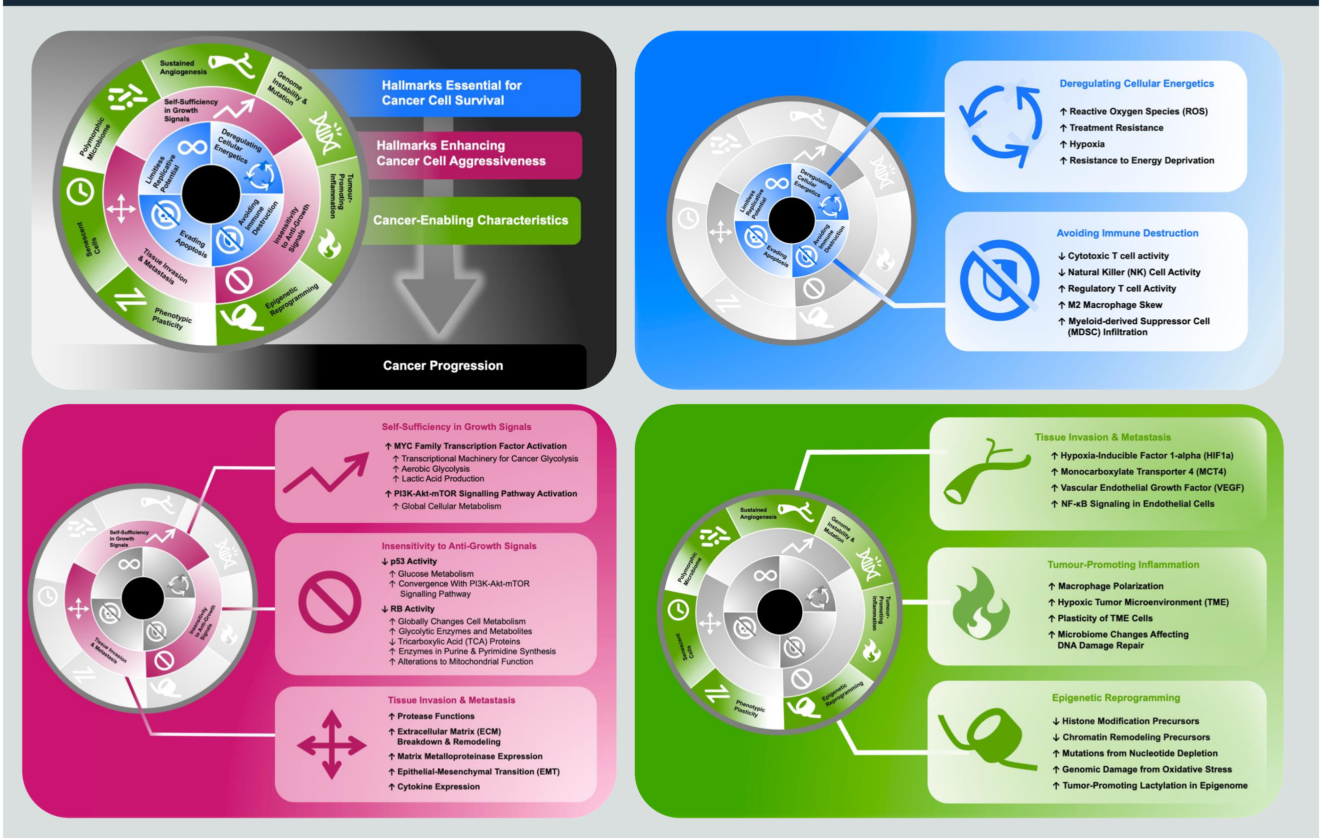
The evolution of cancer hallmarks

The initial hallmarks provided a unifying framework for describing the essential characteristics of cancers. These included self-sufficiency in growth signals, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis *(1)*. Arising from an era of cancer research that emphasized mutational changes to various oncogenes and tumour suppressors, this novel conceptualization of cancer biology was groundbreaking and widely influential.

In 2011, two emerging hallmarks and two enabling characteristics were added: deregulating cellular energetics, avoiding immune destruction, genome instability and mutation, and tumour-promoting inflammation *(2)*. Recent advances were further outlined in the 2022 update, reflecting the continued discovery of new mechanisms involved in cancer development and progression. The addition of unlocking phenotypic plasticity, nonmutational epigenetic reprogramming, polymorphic microbiomes, and senescent cells brings the total number of cancer characteristics to 14*(3)*.

It is highly likely that additional cancer characteristics will be identified in the future. As our understanding of these mechanisms deepens, the framework of hallmarks should also evolve to better incorporate new scientific insights and reflect the biology being described. The current concept is limited by the omission of hierarchy and interconnection, thereby obscuring the true "systems biology" nature of cancer. This risks creating the impression that therapeutic

Figure 1: A hierarchical and interconnected reorganization of the cancer hallmarks. Not every cancer characteristic is equally foundational for cancer growth and progression. Hallmarks essential for cancer survival are placed in the innermost ring and are the most fundamental. Hallmarks enhancing cancer aggressiveness are considered secondary, and cancer-enabling characteristics are tertiary and placed on the outermost ring. Cancer-generated lactic acid, a product of deregulated metabolism, influences multiple cancer characteristics at the same time and is an illustrative example of how the different hallmarks are all interconnected. It has broad influence on multiple hallmark traits across all three hierarchical levels



strategies can be siloed into discrete and independent entities. Instead, significant synergistic effects may be achieved by simultaneously impacting multiple cancer hallmarks.

Reorganizing the cancer hallmarks

To provide clarity and strategic focus in cancer research and treatment, we propose a reorganization of the hallmarks into three hierarchical categories based on functional importance. Hallmarks fundamental to cancer cell survival form the foundation of this hierarchy, while traits that enhance aggressiveness and those that enable and support these processes occupy secondary and tertiary levels, respectively. This tiered structure not only reflects biological dependencies but also suggests a roadmap for prioritizing therapeutic targets.

The three categories (Figure 1) are:

- hallmarks essential for cancer survival;
- hallmarks enhancing cancer aggressiveness; and
- cancer-enabling characteristics.

Category 1: Hallmarks essential for cancer survival

Deregulating cellular energetics

Every living cell requires energy to survive. Cancer cells reprogramme their metabolism to supply energy (i.e., adenosine triphosphate [ATP]) and biomolecular building blocks necessary for rapid proliferation. The most widely observed alteration is a reliance on glycolysis even in the presence of abundant oxygen, a phenomenon known as aerobic glycolysis or the Warburg effect. Beyond glycolysis, cancer cells can also reprogramme additional metabolic pathways involved in glutamine, lipid, nucleotide, and lactic acid metabolism to sustain growth *(4)*. These adaptations not only fuel biosynthesis but also acidify the tumour microenvironment (TME), creating an immunosuppressive niche *(5)*. Understanding these metabolic shifts provides opportunities for metabolism-targeted therapies.

Avoiding immune destruction

Under normal circumstances, mutated cancer cells can be detected and eliminated by the body's immune system in a process known as immunosurveillance. However, as cancer cells continue to form and develop, they acquire the ability to avoid immune detection by downregulating antigen presentation, secreting immunosuppressive factors, and recruiting regulatory immune cells *(5-11)*. In addition, altered lactic acid metabolism leads to the overproduction of lactic acid and the acidification of the TME. This is a major immunosuppressive mechanism that allows cancer cells to evade immune destruction *(10)*. Immunotherapy, which enhances the immune response against cancer cells, has emerged as a promising therapeutic strategy directed at this hallmark.

Evading apoptosis

Cancer cell survival can be enhanced by evading programmed cell death (i.e., apoptosis). This can occur through mutations in apoptosis-related genes, overexpression of anti-apoptotic proteins, and dysregulation of cell death pathways such as autophagy and necroptosis.

Limitless replicative potential

Cancer cells maintain their ability to divide indefinitely by upregulating telomerase, an enzyme that extends telomeres, or by activating alternative lengthening of telomeres (ALT). This offers another direct avenue through which cancer cells can survive and continue to proliferate.

Category 2: Hallmarks enhancing cancer aggressiveness

Self-sufficiency in growth signals

Beyond the basic survival mechanisms that allow cancer to persist in a patient's body, cancer cells also constantly receive proliferation signals due to mutations in signalling pathways such as estimated glomerular filtration rate (EGFR) and Ras. These signals sustain further cancer growth. Ongoing replication also provides cancer cells with the opportunity to develop and accumulate additional aggressive characteristics.

Insensitivity to antigrowth signals

Tumour suppressor genes, such as p53, RB1 and PTEN, typically inhibit cell growth during normal cell division. Cancer cells often harbour mutations that inactivate these genes, allowing uncontrolled proliferation.

Tissue invasion and metastasis

Metastasis is the spread of cancer cells from the primary tumour site to distant organs. This process is facilitated by epithelial-mesenchymal transition (EMT), degradation of the extracellular matrix (ECM), and the establishment of a metastatic niche in the secondary tissue to support continued cancer growth. It is a primary indicator of aggressive disease and is associated with poor prognosis and death.

Category 3: Cancer-enabling characteristics

Sustained angiogenesis

Tumours form new blood vessels (i.e., angiogenesis) to supply oxygen and nutrients to fuel their growth. Anti-angiogenic therapies starve tumours by inhibiting blood vessel growth, although resistance mechanisms can limit their effectiveness.

Genome instability and mutation

Cancer cells exhibit high rates of genetic mutations, driving tumour evolution. This is frequently caused by defects in DNA damage repair pathways, allowing cancer cells to carry a higher mutational burden and propagate these genetic alterations to the next generation of cancer cells.

Tumour-promoting inflammation

Chronic inflammation in the TME supports cancer progression by providing growth factors and survival signals, and by promoting genomic instability. This can be triggered by the death of healthy tissues surrounding tumour cells, and is frequently associated with pro-inflammatory cytokines such as NF-kB, STAT3, and TNF-α.

Unlocking phenotypic plasticity

Cancer cells exhibit remarkable phenotypic plasticity, allowing them to change their behaviours and adapt to diverse environmental or therapeutic pressures. Among the many possible mechanisms, the re-activation of embryonic developmental programmes and the reversion to less differentiated states during treatment may play significant roles in helping cancer cells acquire new traits and develop treatment resistance.

Non-mutational epigenetic reprogramming

Epigenetic modifications, such as DNA methylation and histone modifications, can drive cancer development and progression. They can functionally alter cancer cell behaviour by changing gene expression, without the need for genetic mutations.

Polymorphic microbiome

The microbiome influences cancer development and progression through interactions with the immune system, metabolism, and inflammation. Bacteria, fungi, or viruses may be directly carcinogenic, or they may have microenvironmental effects that modulate cancer growth, the anticancer immune response, or the efficacy of anticancer treatments.

Senescent cells

Senescent or dormant cancer cells, which have stopped dividing but remain metabolically active, contribute to the TME and promote later cancer progression. While they initially represent a barrier to tumourigenesis and a pause in tumour progression, they may eventually secrete cell–cell communication factors that promote the development and aggressiveness of neighbouring cancer cells. Alternatively, dormant cancer cells can also reactivate, becoming proliferating cells that fuel tumour relapse.

Interconnectedness of hallmarks: Cancer-generated lactic acid

It is clear, even from Hanahan and Weinberg's initial conception, that the various hallmarks are not independent entities *(1)*. The overproduction of cancer-generated lactic acid via altered cancer metabolism is an illustrative example of how these various hallmarks are intricately associated with each other *(5)*. There are many other possible examples.

Category 1: Hallmarks essential for cancer survival

Altered cancer metabolism results in excessive generation of lactic acid, which in turn leads to the acidification of the TME. This alteration results in a dramatic increase in glucose consumption, helping cancer cells maintain an adequate supply of precursor molecules for biosynthesis and allowing them to sustain their anabolic needs for rapid proliferation *(12-14)*. Recent research has demonstrated that the acidic TME created by excess lactic acid has multiple downstream cancer-promoting effects, impacting many other cancer hallmarks.

With respect to avoiding immune destruction, it is now well-established that an acidic TME inhibits anticancer effector immune functions while enhancing regulatory immune functions *(5, 10)*. Prior to 2013, few studies treated lactic acid as an immunomodulatory molecule *(11)*. It was considered a metabolic "waste product" instead. We were one of the first to hypothesize that lactic acid acts as a central immune-suppressive molecule in cancer *(5)*. The latest research has demonstrated that cytotoxic T cells and NK cells are less effective in an acidic environment *(15-18)*, while suppressive immune cells such as regulatory T cells are able to maintain their functionality *(19-23)*. An acidic TME can also skew macrophage differentiation towards the tumour-promoting M2 phenotype *(24-26)* and enhance the infiltration and function of immunosuppressive myeloid-derived suppressor cells *(27, 28)*. Inhibition of lactic acid secretion can enhance anticancer immunity, offering a promising therapeutic strategy for anticancer treatments *(29-32)*.

In relation to evading apoptosis, lactic acidosis can prevent apoptosis as induced by a variety of environment stressors, including therapeutic/chemotherapy treatment *(33-35)*, energy deprivation *(36)*, and hypoxia *(37)*. In particular, inhibition of lactate dehydrogenase A (LDHA), the primary enzyme converting pyruvate to lactate in the final step of aerobic glycolysis, can trigger the apoptosis cascade through increased generation of reactive oxygen species (ROS) *(37-40)*.

Category 2: Hallmarks enhancing cancer aggressiveness

Crosstalk occurs between glucose metabolism and growth/antigrowth signals *(41-45)*. Certain oncogenes, especially the MYC family of transcription factors, are primary regulators of glycolysis in cancer development. They can directly upregulate the transcriptional machinery that increases glucose metabolism, facilitates aerobic glycolysis, and increases lactic acid production *(46-48)*. Moreover, multiple oncogenic signals can feed through the PI3K-Akt-mTOR signalling pathway, which also regulates cellular metabolism in a global manner *(49-51)*. Similarly, metabolic deregulation can also occur when tumour suppressors lose their functions. For example, the loss of p53 activity can upregulate glucose metabolism through signalling pathways that also converge on the PI3K-Akt-mTOR axis *(52-54)*. Depletion of the retinoblastoma (RB) gene product can result in global changes to cell metabolism, increasing glycolytic enzymes and metabolites, reducing tricarboxylic acid (TCA) cycle proteins, altering mitochondrial function, and increasing enzymes involved in purine and pyrimidine synthesis *(55-58)*.

Local tissue invasion and distant tumour metastasis are also promoted by an acidic TME through enhanced protease functions, facilitating the breakdown of the ECM and allowing cancer cells to migrate and metastasize *(59)*. Furthermore, reduced oxidative phosphorylation and increased glycolysis can enhance a cancer cell's metastatic potential, especially in terms of facilitating EMT and upregulating matrix metalloproteinase (MMP) and cytokine expression *(59, 60)*. As cancer cells travel in the bloodstream to colonize distant sites, altered metabolism can help circulating cancer cells survive *(61, 62)*.

Category 3: Cancer-enabling characteristics

There is a clear connection between cancer-generated lactic acid and enhanced angiogenesis, particularly in relation to hypoxia. The activation of HIF1a directly increases the expression of the lactic acid transporter MCT4, thereby facilitating increased glycolytic flux *(63)*. HIF1a also regulates multiple pro-angiogenic factors to promote angiogenesis *(64)*. Conversely, the accumulation of lactic acid may act upstream of hypoxia, leading to increased VEGF production and activation of endothelial NF-κB signaling *(65, 66)*.

The correlation between genome instability and cancer-generated lactic acid remains relatively underexplored. However, there are three primary mechanisms by which altered cancer metabolism may negatively impact DNA repair:

- depletion of metabolic precursors necessary for histone modifications and chromatin remodelling *(67-69)*;
- increased oxidative stress from altered cancer metabolism contributing to genomic damage via ROS *(70-73)*; and
- depletion of nucleotides needed for DNA repair increasing the propagation of mutations *(64)*. More studies are needed to fully elucidate how altered cancer metabolism impacts genomic instability.

Tumour-promoting inflammation and a polymorphic microbiome are both intricately linked to lactic acid metabolism through its relationship with anticancer immunity. Lactic acid can alter macrophage polarization, leading to cancer-promoting functions *(25)* Furthermore, both an immunosuppressive tumourigenic microenvironment and chronic inflammation are characterized by hypoxia and elevated levels of lactic acid *(75-77)*. Finally, the presence and depletion of various metabolites, including lactic acid, by bacteria and viruses can affect DNA damage repair *(78, 79)*.

Several mechanisms link metabolic changes to various aspects of phenotypic plasticity. An acidic TME can drive stem-like features in cancer cells *(80, 81)*. Similarly, lactic acid plays a crucial role in driving EMT, a key process in metastasis *(82)*. Altered glucose metabolism also significantly impacts the plasticity of cancer cells, cancer stem cells, and many surrounding non-cancer cell types within the TME *(83)*.

Finally, in addition to traditional methods of epigenetic reprogramming, increased intracellular lactate levels driven by altered cancer metabolism can alter gene expression through a relatively newly discovered epigenetic marker – lactylation. It can have wide-ranging impacts on the transcriptional machinery of cancer cells *(84-86)*, cancer-associated immune cells *(21, 26, 87)*, and other components of the TME *(88)*. Further, lactylation can directly impact various components of basic cellular machinery, broadly altering enzymatic functions as a post-translational modification. This affects PD-L1 *(89)*, p53 *(90)*, DNA repair *(91, 92)*, and glucose metabolism *(93, 94)*. Various lactyltransferases (AARS1/2) and epigenetic modulators (KAT8, HDAC1-3, SIRT1-3) function as important writers and erasers of cellular lactylation *(95-98)*. Lactylation is thus an important and highly relevant mechanism through which cancer-generated lactic acid exerts its cancer-promoting effects.

Conclusion

The hallmarks of cancer provide a framework for understanding the intricacies of cancer biology. The number of hallmarks has increased over the years, reflecting ongoing discoveries and deepening comprehension of cancer's complexity. It is important to reassess how hallmarks are categorized and understood. By reorganizing the hallmarks into a functional hierarchy of survival-critical, aggressiveness-enhancing, and cancer-enabling traits, we provide a framework that captures the intricate interplay driving cancer progression. This structured perspective not only deepens our biological understanding but also offers a strategic lens for developing multi-targeted therapeutic approaches aimed at the most influential drivers of cancer biology. Using the example of altered cancer metabolism, we illustrate how one fundamental trait can significantly influence and activate multiple other hallmark characteristics, highlighting the interconnected nature of these processes. Through this new, holistic perspective, innovative avenues for cancer research and therapeutic development can be identified, ultimately leading to more effective cancer treatments targeting the core drivers of cancer progression.

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Stephen Choi is a Research Scientist at the Vancouver Prostate Centre. In 2013, he pioneered the hypothesis that lactic acid plays a central immunosuppressive role in cancer progression. He now leads translational projects targeting cancer metabolism, angiogenesis, and metastasis using interdisciplinary approaches.

James Killam brings decades of legal experience and strategic leadership to cancer drug development, bridging scientific discovery and commercialization using clear, pragmatic approaches that drive innovation.

Yuzhuo Wang is a cancer biologist known for pioneering patient-derived xenograft models at the Living Tumor Laboratory. His research focuses on tumour heterogeneity, dormancy, and resistance. He has 240+ publications and an H index of 90.

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