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Review

Precision Oncology-Based Targeting of Signaling Pathways toward a Holistic Approach to Cancer Therapeutics

Manish Kumar

Department of Biotechnology, School of Bio Sciences & Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India; scmanish_16@yahoo.com; kmanish125@yahoo.com

Abstract: The genetic changes appearing in the information system of the cell that program its unregulated growth and proliferation gradually lead to cancer manifestation, and the treatment options need to be guided accordingly. The critical roles played by some of the molecules associated with the signaling pathways and cell microenvironment that often induce tumorigenesis and metastasis have been described precisely in recent years based on findings of the human genome project and other related initiatives undertaken afterward to thoroughly understand the molecular basis of cancer cell behaviors. It is to rely upon the genomic study of cancer cells to fully understand the prognosis and pathways involved in disease progression to selectively target them for a cure. Furthermore, patients with the same cancer types often respond differently to cancer therapies, indicating the need for a patient-specific treatment regimen for cancer. In this direction, precision oncology, defined as the molecular profiling of tumors to identify targetable alterations for custom-tailored personalized treatment, is gaining ground as a potential means of cancer treatment and has started influencing the ways cancer has been treated in clinics. This article intends to comprehensively elucidate the foundations and frontiers of precision oncology in the context of recent advances in cancer genomics and single-cell technologies for assessing its scope and importance in the realization of a proper cure for cancer.

Keywords: mutation; gastric cancer; p53; K-Ras; c-Myc; cancer genomics; targeted therapy; immunotherapy

1. Introduction

Cancer is a devastating disease that causes one in six deaths globally, and a large proportion of these deaths could be prevented by improving the prevention and treatment of the disease. It requires a proper diagnosis of the disease, the development of effective and precise treatment options, and a better understanding of the socioeconomic factors that affect cancer incidence, prevalence, and related deaths worldwide [1,2]. There are more than 100 cancer types with subtypes determined by location, cell of origin, and genetic variations that influence oncogenesis and therapeutic response. Most cancers appear in epithelial cells as carcinomas, such as the lung, skin, breast, liver, colon, prostate, and pancreas. Contrary to carcinoma, sarcomas arise from mesenchymal tissues originating in myocytes, adipocytes, fibroblasts, and osteoblasts. Nonepithelial tumors can develop in hematopoietic tissues such as leukemia and lymphoma in the nervous system, e.g., gliomas and neuroblastomas. These are among the most common cancer types taking a high toll in terms of lives and property across the world [3,4]. Considering the incidences, a formal initiative to address cancer as a leading cause of death was called for on the part of the government system, and it appeared in the United States as the National Cancer Act of 1971 signed by President Richard Nixon aimed at promoting cancer research and application of the outcomes for minimizing cancer incidences and mortality rates related to cancer. The act was euphemistically described as the "War on Cancer", and the passing year of 2021 marked the 50th anniversary of the signing of the act into law [5]. The National Cancer Program that was borne from this initiative resulted at the beginning of a

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concerted effort across the length and breadth of the country to develop the infrastructures required for the treatment, cure and eradication of cancer. A similar approach was adopted by most other developed and developing nations in the following years to combat the menace of cancer, and it has succeeded in satisfying the purpose to a good extent, as the findings reveal that overall morbidity from cancer has decreased and net survival rates, both short-term and long-term, for all cancers combined have increased substantially since then, and as the evidence suggests, the demographic factors as feared do play a role in it [6,7]. The survival rates for cancer types that are responsive to therapy surpass 90% in developed countries, and the prognosis for several other cancer types that were considered the deadliest diseases earlier has improved noticeably in recent decades thanks to rapid advances in clinical oncology [8.9]. However, the fight against cancer is far from over, as estimation by the WHO in 2018 has revealed that cancer incidence would be doubled to approximately 37 million new cases by 2040 with no confirmed remedy for most cancer types in the sight as yet [10,11]. While researchers continue the endeavors to identify the exact causes of cancer types and subtypes and develop strategies for prevention, diagnosis, and treatment, cancer remains the leading cause of death and has a major impact on societies throughout the world. There are many types of treatment available for cancer now, such as chemotherapy, targeted drug therapy, immunotherapy, radiation therapy, surgery, stem cell transplant, and hormonal therapy. Some people may receive a single type of therapy, and some will have a combination of treatments, but whatever the regimen, the result must be a cure. Rigorous cancer research in the last few decades has led scientists to clearly understand that there are specific genetic changes associated with cancer that cause the disease to grow and spread. It has also been observed that every patient responds differently to treatments despite having the same type and stage of cancer. These observations are compelling and have led researchers to look for a treatment option based on the genetic features of vulnerable individuals for effective treatment of the disease [12].

2. Cancer Genomics and the emergence of Precision Oncology

The fundamental abnormality resulting in the development of cancer is the unchecked proliferation of cells due to the loss of cell cycle regulation. Cell cycle regulation involves growth-regulatory signals as well as signals by proteins monitoring the genetic integrity of the cell to ascertain the absence of any genetic damage [13]. Proliferation depends on progression through distinct phases of the cell cycle-regulated by several cyclin-dependent kinases (CDKs) that act in association with their cyclin partners when alterations in the overall expression pattern of the genes responsible for the regulation of cell growth and proliferation could lead the development to go awry, and the factors that cause genetic changes tend to provoke the development of cancer [14]. Every single gene in the body is likely to have undergone mutations on an innumerable number of occasions with a repair mechanism in place to sustain deleterious mutations in genes that regulate cell growth and division. In this way, the generation of cancer has to be conclusively linked to mutagenesis, the introduction of a change in the DNA sequence by the external agents called mutagens and yet a single mutation is unlikely to be enough to change a normal cell into a cancer cell as it will require several changes to accumulate over time for cancerous development to take place. Mitogenic stimulation due to mutations in genes such as Ras or Myc will not lead to unchecked proliferation until the changes in genes that encode essential components of the protective mechanisms, such as Arf or p53, have not occurred. In this way, as multiple genetic changes will be required for cancer manifestation, it can be seen as an evolutionary process involving both genetic change and selection [15]. There can be multiple rate-limiting steps working against the development of cancer along with persistent changes accelerating the process. In this way, most cancers are thought to derive from a single abnormal cell or a small group of cells with certain unwanted gene mutations when additional changes accumulate in some of the descendants

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of the cell, allowing them to outgrow their neighbors and ultimately leading to tumor growth [16].

Cancers can also be driven by epigenetic changes that alter the gene expression pattern of cells caused by physical modifications of chromatin structure often led by DNA methylation or histone modifications inside the cells due to exposure to certain stressful external conditions, such as lifestyle-related factors or environmental factors, without the accompanying alteration in the cell's DNA sequence [17]. Although epigenetic changes may not alter the sequence of DNA, the process can cause point mutations and disable DNA repair mechanisms involved in cancer development. Generally, epigenetic and genetic changes have been seen as two separate mechanisms participating in carcinogenesis, but recent studies from whole-exome sequencing, the technique employed for sequencing all of the protein-coding regions of genes in a genome, for thousands of human cancers have revealed the presence of many inactivating mutations in genes that can potentially disrupt DNA methylation patterns, histone modifications, and nucleosome positioning and hence control the epigenome and contribute to cancer[18]. Thus, considering that both the genome and epigenome can regulate cancer progression through mutations, crosstalk between the two is anticipated and can be exploited to bring new possibilities to cancer research. Furthermore, the majority of the human genome consists of noncoding regions, and studies on alterations in the noncoding regions of the cancer cells are revealing additional mechanisms underlying cancer progression and response to therapies. A systematic approach to understanding the noncoding genome in cancer could help improve cancer diagnosis and therapy [19,20].

Finally, the mutations that alter the DNA sequence of the cells appear to be at the source of all changes in the cell behaviors and remain the most fundamental and universal feature of all cancers, and hence it has to be seen as a genetic disease to be treated accordingly for better results. Biometricians in fact since the nineteenth century have been interested in decoding the relationship between genetics and diseases and have attempted to understand the roles of "constitutional" and environmental factors in the distribution of diseases. Although much less remembered now, Werner Kalow's 1962 textbook 'Pharmacogenetics' had already set the agenda of relating the response of therapeutic drugs to their biochemistry and the role of genetics and evolution in shaping individual-level differences, and the idea seems to be of practical use in the treatment of cancer and has been deliberated for cancer therapy convincingly leading to the emergence of precision oncology as the much needed personalized medicine approach to cancer treatment[21]. The advances in genetic technologies and subsequent understanding of clinically relevant genetic variation over the years are revolutionizing how a range of diseases can be diagnosed and treated in clinics and how they apply to cancer treatment adequately. Precision oncology is the term coined for clinical oncology practice that relies upon genomic profiling of the individual patient's tumor for a complete molecular characterization of the transformed cells and tissues to identify targetable alterations for a cure [22,23]. The precision oncology approach to cancer treatment simply envisages bringing in a custom-tailored treatment plan for personalized treatment by taking into account the unique needs of the person to design a treatment regimen for the best possible results. The use of precision oncology in routine oncogenic practice began approximately 20 years ago but has noticeably enhanced the efficacy of cancer therapy and is on the verge of entering the mainstream of clinical practice results [24,25].

3. Molecular Approach to Cellular Reprogramming and Cancer Evolution

Over the years, technological advances in molecular biology have proven invaluable to the understanding of the pathogenesis of human cancer. The emergence of next-generation sequencing (NGS) in 2005 has proved to be massively important in this direction as the technology is used to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA and has revolutionized biological research, allowing scientists to study biological systems at a level never before possible. It provides new insights into the

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nature of genes and proteins thought to be associated with cancer, and the application of evolving molecular techniques to the study of cancer has not only led to advances in tumor diagnosis but has also provided markers that are proving to be the means for a better assessment of prognosis and disease progression [26]. The important part of tumorigenesis is that cancers of different tissues utilize somewhat different patterns to converge to a relatively common path of cancer development witnessed as tumor growth followed by angiogenesis, invasion, and metastases. Such development is ultimately guided by gene mutations associated with cancer cells and certain epigenetic changes or tissue-specific factors that help the tissue exploit the genetic changes resulting in reprogramming of the molecular events utilized by different cancers, so no gene change is common to all cancers [27,28].

An important genetic feature of cancer is that the population of cells that make up cancer is profoundly heterogeneous at the genetic and epigenetic levels, mainly because the cancer genome is unstable [29,30]. It is based on the fact that mutations are present in the genes that further increase the inherent rate of genetic change, termed mutator mutations, that lead to greater genetic instability resulting in the accumulation of multiple cancer-associated mutations within a cellular lineage at a rate that explains cancer manifestation at different stages in a lifetime. Mutator mutations and genetic instability are generalized concepts in cancer genetics, indicative of only the mutations that lead to an enhanced rate of single nucleotide substitutions but also mutations that lead to microsatellite instability, chromosomal instability, and those responsible for cell activities related to DNA damage [31].

A crucial component of tumor heterogeneity is cancer stem cells (CSCs), which are at the forefront of cancer research owing to their potential to induce cancer development. Recent studies have shown that there can be different subpopulations of CSCs within the tumor mass identified by cancer stem cell surface markers on normal stem cells with similar characteristics as normal stem cells, such as self-renewal and multilineage differentiation capabilities, with a much higher half-life than that of most other cells [32]. The intrinsic properties of self-renewal, multipotency, and longevity could render stem cells more prone to accumulating gene mutations leading to neoplastic transformation, as proposed by the cancer stem cell hypothesis [33,34]. They have been found to be the key driver of tumorigenicity, tumor heterogeneity, recurrence, and drug resistance in many cancer types, and different targeted molecules, including nanoparticles, are being tested for effectively targeting CSC pathways for the cure [35,36,37.38]. Furthermore, the immune cells in the tumor mass could be very different tumors, and the emerging finding of tumor heterogeneity is that tumors from different patients show a different degree of immune cell infiltration and immune cell composition. Immunologically "hot" tumors present high levels of T-cell infiltration, so these tumors are more susceptible to immunotherapy than immunologically "cold" tumors. This immunogenic heterogeneity impacts treatment outcomes and can direct treatment planning [39,40]. Historically, cancer treatments such as chemotherapy and radiation therapy have been targeting actively growing cells of the tissue instead of just attacking diseased cells, so the need for a deeper understanding of the molecular events and related pathways that remain active during cancer progression was realized decades ago for developing treatments that would selectively target the affected cells alleviating the serious side effects of cancer treatment. The functional roles of many critical players involved in tumor growth, tissue invasion, and metastasis have been described precisely in recent decades due to the draft of the human genome and other related developments that took place in the following years [41]. The transcription factor NRF2 is the master regulator of the cellular antioxidant response, and studies have established many new roles for NRF2 in the regulation of metabolism and other essential cellular functions, establishing it as a truly pleiotropic transcription factor. Originally recognized as a target of chemopreventive compounds that help prevent cancer, its protective role is altered in 6-7% of cancer cases, and evidence has established the NRF2 pathway as the driver of cancer progression, metastasis, and resistance to therapy [42]. The c-Myc oncoprotein is a transcription factor that forms a part of a dynamic network whose

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members interact selectively with one another and with various transcriptional coregulators and histone-modifying enzymes. c-Myc is constitutively and aberrantly expressed in over 70% of human cancers, with many of its target genes encoding proteins that initiate and maintain the transformed state [43,44]. Approximately 40-50% of human cancers carry deleterious mutations in the regulatory p53 gene [45]. The overall mutation rate for growth-promoting protein K-Ras is approximately 25% for all tumors and is mutated up to 85-90% in pancreatic ductal adenocarcinoma cases [46]. The treatment of pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer and the leading cause of cancer-related death, has largely been unsuccessful due to the tumor microenvironment, which exhibits an ample number of stromal cells and a complicated extracellular matrix (ECM). Genomic analysis has recently revealed that PDAC harbors frequently mutated genes that include KRAS, TP53, CDKN2A, and SMAD4, which can widely alter cellular processes and change the tumor microenvironment, which in turn affects cancer progression. Mammalian cells express three distinct but closely related Ras proteins (K-RAS, H-RAS, and N-RAS), which may become mutationally activated and promote oncogenesis. The mutation frequency of different Ras isoforms in human cancers varies, and K-Ras is the most frequently mutated isoform leading to uncontrolled cell proliferation, migration, and invasion in many cancers [47]. The enthusiasm created a few years ago with the development of drugs that could block K-Ras was lost sooner, similar to many other targeted cancer drugs, as the affected cells became resistant to the inhibitors, a common problem encountered with drugs designed for targeted cancer therapy [48,49]. The study of K-Ras resistance mechanisms reveals that researchers may have to try several different drug combinations to overcome resistance, and some of these are in the pipeline. Researchers are tirelessly working to determine how to target K-Ras and other signaling proteins behaving abnormally in different cancer cells to develop novel therapeutic options for different cancer types. Some breakthroughs have occurred in certain cancers where understanding the cell signaling underlying the development has led to the development of specific targeted drugs that have revolutionized the treatment of the disease [50,51].

4. Signaling Pathway Dysregulation and Prospective Targets for Cancer Therapeutics

Tumors and cancer are mainly the results of uncontrolled cell division. The root cause of cancer is usually genetic or epigenetic alterations in the affected cells, although the progression of cancer remains dependent on a complex interplay between the tumor cells and surrounding nonneoplastic stromal cells and ECM in the tumor microenvironment [52,53]. As the foremost system of communication, a cell signaling network involves many secreted protein receptors, growth factors, cytoplasmic proteins and kinases, and nuclear transcription factors, enabling individual cells to respond to extracellular signals with physiologically appropriate behavior. In this way, signaling pathways constitute an internal circuitry inside cells guided by external stimuli to help them sense whether their state of attachment to ECM and other cells is appropriate and if different growth factors, hormones, and cytokines guide them to proliferate or differentiate, move or stay put, or commit to cell death by apoptosis or autophagy (Fig. 1) [54]. The signals propagated by growth factors and cytokines can dimply tell individual cells whether to divide or not. Cytokines ordinarily signal the immune s cells to mount coordinated attacks on invading bacteria, and viruses and play important roles in cancer prevention.

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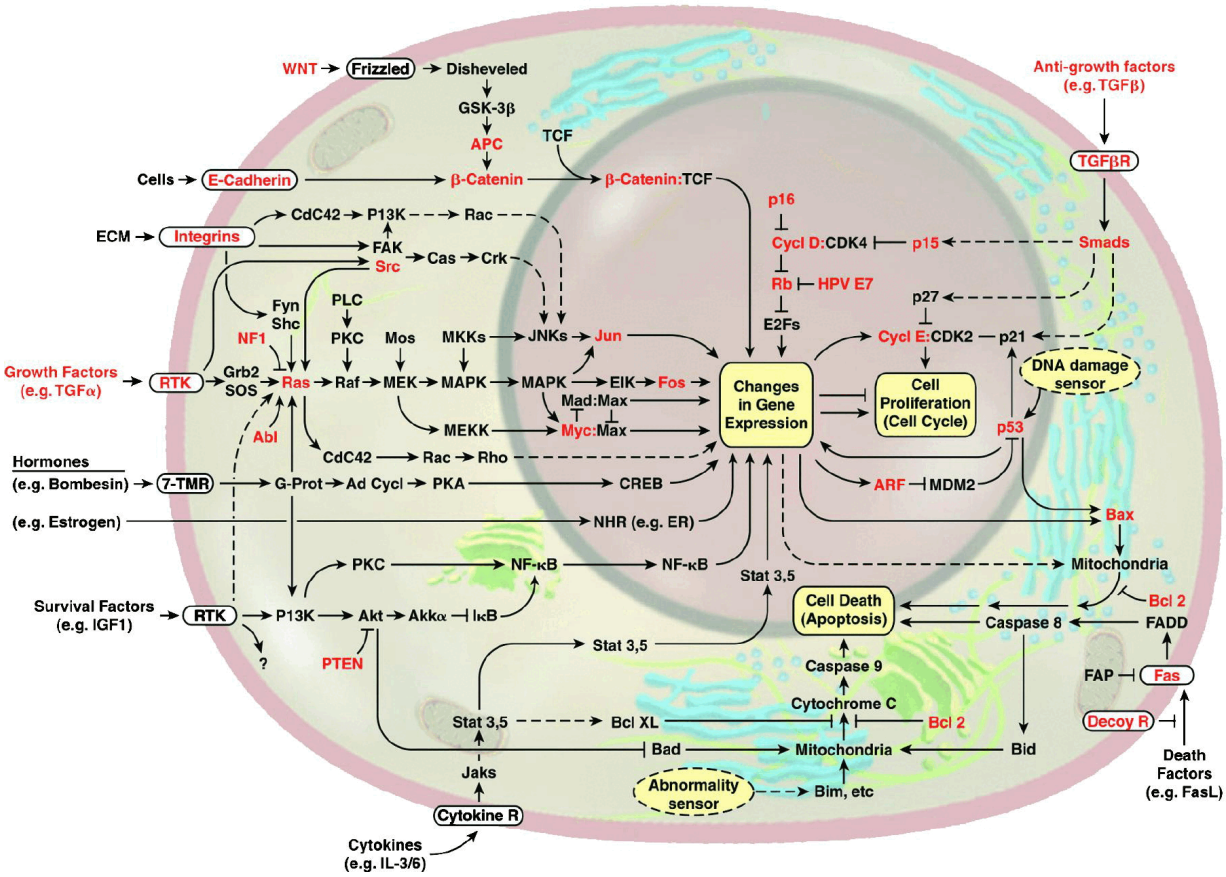

Figure 1. The Emergent Integrated Circuit of the Cell.

Progress in dissecting signaling pathways has begun to layout a circuitry that will likely mimic electronic integrated circuits in complexity and finesse, where transistors are replaced by proteins (e.g., kinases and phosphatases) and the electrons by phosphates and lipids, among others. In addition to the prototypical growth signaling circuit centered around Ras and coupled to a spectrum of extracellular cues, other component circuits transmit antigrowth and differentiation signals or mediate commands to live or die by apoptosis. For the genetic reprogramming of this integrated circuit in cancer cells, some of the genes known to be functionally altered are highlighted in red. (Hanahan and Wienberg [54].)

Normally, cell division is regulated by a group of extracellular growth factors, the proteins that cause resting cells to divide by exploiting the intrinsic signaling process of the cell. The earliest information regarding the relationship between cancer and growth factors came from the observation that normal cells in culture often required serum for proliferation, while cancer cells had a much less requirement for serum. It is known for providing growth factors among other ingredients needed for the overall regulation of cell cycle. The other hints came from gene mutations found in cancer cells observed to cause changes in cell behaviors very similar to those related to the activities of growth factors and their receptors. The oncogenic mutations disrupt the cellular circuits that control cell adhesion and signaling, enabling cells that carry them to over proliferate and invade the other tissues in an uncontrolled fashion. These mutations have been directly linked to the growth factors and their receptor proteins involved with tumor growth, angiogenesis, invasion, and metastases. Oncogenes are the mutated forms of cellular proto-oncogenes that translate into activated versions of signaling proteins that normally participate in the regulation of cell proliferation, growth, and differentiation, as well as control of the cell cycle and cell death. Negatively acting tumor suppressor genes mostly act to repress cell signaling to maintain balance in product formation and are the actual targets for the action of many signaling molecules [55,56]. An important feature of cancer

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cell signaling is that one kind of cell membrane receptor can activate many different downstream intracellular pathways and one pathway can also be activated by several of the upstream surface receptors revealing common signaling components in multiple signaling pathways involved with cancer development. For example, the receptor tyrosine kinases (RTKs), like Epidermal growth factor receptor (EGFR), Fibroblast growth factor receptor (FGFR), Insulin-like growth factor receptor (IGFR), Vascular endothelial growth factor receptor (VEGFR), Platelet-derived growth factor receptor (PDGFR), and G Protein-Coupled Receptors (GPCRs) can all activate the MAPK cascade while the widely studied RTKs such as EGFR/HER family receptor can initiate different signaling pathways including PI3K/Akt, mammalian target of rapamycin (mTOR), and mitogen-activated protein kinase (MAPK) involved in cell proliferation, survival, and differentiation. Because cancer progression frequently involves altered signal transduction pathways due to mutations in the concerned genes, it is satisfying as well as mechanically well-founded that therapeutic interventions taking into account this biology might pave the way for effective treatment of cancer (Fig. 2) [57,58]. Therefore, therapeutic substances that target the signal transduction process are constantly being explored as prospective and efficacious agents for cancer treatments.

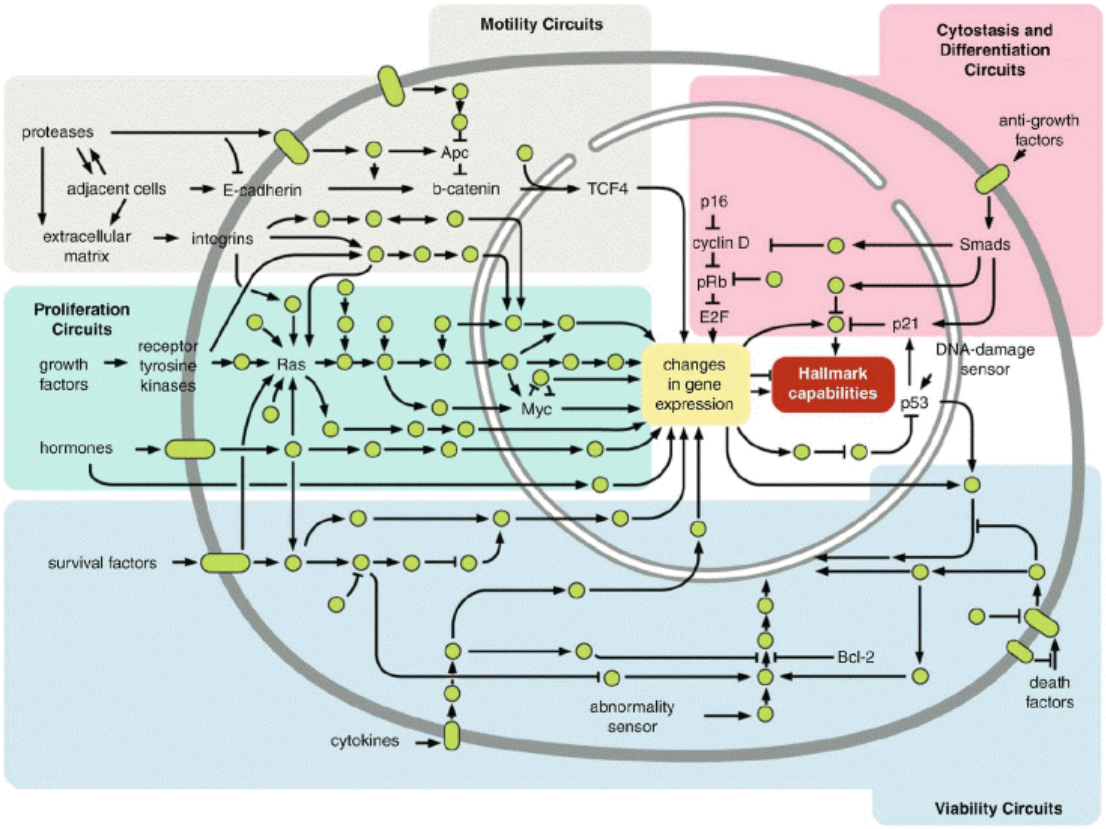

Figure 2. Intracellular Signaling Networks Regulate the Operations of the Cancer Cell.

An elaborate integrated circuit operates within normal cells and is reprogrammed to regulate hallmark capabilities within cancer cells. Separate subcircuits, depicted here in differently colored fields, are specialized to orchestrate the various capabilities. At one level, this depiction is simplistic, as there is considerable crosstalk between such subcircuits. In addition, because each cancer cell is exposed to a complex mixture of signals from its microenvironment, each of these subcircuits is connected with signals originating from other cells in the tumor microenvironment. (Hanahan and Wienberg [57].)

As generally observed, most of the signaling pathways contribute to the development of cancer, and seldom does a cancer type arise from the dysregulation of a single

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pathway. Breast cancer can arise due to elevated expression of estrogen receptor (ER), EGFR/HER, or IGFR, but on many occasions, more than one of these pathways may be dysregulated. Signal transduction leading to tumor growth, cancer cell migration, metastasis, and drug resistance are often complex processes, as cancer cells can develop abnormalities in multiple pathways or rely on the crosstalk between different signaling pathways and also on redundant pathways for growth and survival [59]. It has been observed, although, in clinical practice that targeting a single intermediate or pathway effectively brings considerable results toward recovery possibly because it impedes the synergistic process of disease progression, yet the constitutive activation of a molecular target that is responsible for cancerous developments can be sustained by different mechanisms, and strategies that inhibit multiple targets or redundant pathways simultaneously with molecular-targeted agents may be an even more effective way to treat and overcome resistance in cancer therapy [60]. The major cell signaling pathway involved in cancer reprogramming and the scope for targeting the signaling intermediates for efficient management of the disease are briefly discussed here.It has been observed, although, in clinical practice that targeting a single intermediate or pathway effectively brings considerable results toward recovery possibly because it impedes the synergistic process of disease progression, yet the constitutive activation of a molecular target that is responsible for cancerous developments can be sustained by different mechanisms, and strategies that inhibit multiple targets or redundant pathways simultaneously with molecular-targeted agents may be an even more effective way to treat and overcome resistance in cancer therapy [60]. The major cell signaling pathway involved in cancer reprogramming and the scope for targeting the signaling intermediates for efficient management of the disease are briefly discussed here.

RAS/RAF/MAPK signaling pathway: The MAPK cascade is the key signaling pathway involved in the regulation of normal cells, so this pathway is the main route for extracellular growth factors to transfer signals into the nucleus to stimulate cell proliferation, differentiation, and development, and abnormalities in this pathway are common in many cancer types [61]. The extracellular signal-regulated kinase (ERK) pathway is the MAPK pathway that has been the subject of intense scrutiny in the treatment of cancer. ERK is a downstream component of an evolutionarily conserved signaling system that is activated by rapidly accelerated fibrosarcoma (RAF) kinases. It activates the MAPK/ERK protein kinase MEK, and the mutational activation of Raf in human cancers supports the important role of this pathway in oncogenesis. Importantly, the Raf-MEK-ERK pathway is a key downstream effector of RAS, the small GTPase or G-protein as the product of the most frequently mutated oncogene Ras in human cancers. Growth factor receptors, such as the TGF-β receptors EGFR, VEGFR, PDGFR, FGFR, and IGFR, can all activate RAS and downstream RAF and MEK. Ras proteins act as molecular switches that control the activation and regulation of related pathways that are responsible for numerous cell behaviors [62]. The study with selected inhibitors against the targets in this cascade has shown positive results, such as growth inhibition, anti-angiogenesis, and suppressed metastasis in cancer cell lines and animal models. These results reveal that this strategy is effective at inhibiting cancer cell proliferation and survival, and more clinical validation is ongoing for efficacious treatment of the disease [63].

PI3K/Akt/mTOR signaling pathway: The PI3K/Akt/mTOR pathway is activated by a variety of factors, such as cytokine receptors, GPCRs, RTKs, and integrins, and can stimulate a variety of activities, including protein synthesis, glucose metabolism, cell survival, and proliferation. Persistent activation of the PI3K/Akt pathway in the absence of different stimuli has been frequently observed in many cancers. Adaptive resistance to PI3K/Akt/mTOR pathway inhibitors is common, and combination therapy, if well tolerated, may produce favorable anticancer results [64,65]. mTOR is of particular interest as the master regulator of cellular processes, as it is assembled into a variety of complexes to catalyze the phosphorylation of multiple targets, including protein kinase B (Akt), protein kinase C (PKC), and type-I insulin-like growth factor receptor (IGF-IR), and the components of protein synthesis and activation of mTOR are frequently associated with tumor

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growth and metastasis. Several mTOR inhibitors have been developed to treat cancer, and some are being evaluated in clinical trials for approval [66,67].

Wnt/β-catenin signaling pathway: Wnt signaling is a genetic pathway that functions to promote cell growth in normal cells, and this pathway is carefully controlled by a gene called adenomatous polyposis coli (APC), which functions through the inactivation of β-catenin to prevent excessive cell growth and tumor formation. APC is a negative regulator of canonical Wnt signaling and is capable of binding to a variety of proteins, including β-catenin. Dysregulated Wnt signaling is linked to many cancer types, and mutations that prevent degradation of β-catenin, including certain mutations in β-catenin itself or the destruction complex component APC, hijack the regenerative signaling pathways to contribute to cancer development [68]. The Wnt signaling pathway is important not only in cancer progression but also for many other healthy organs. The Wnt signaling pathway is required to maintain stem cell populations in the gut for tissue repair and wound healing. Its role in immune escape and drug resistance is currently well recognized, and identifying tumor-specific targets is important for developing safe and effective drugs [69]. Numerous inhibitors targeting the molecules associated with this signaling pathway are being explored for a range of different cancers [70].

Hedgehog (Hh) and Notch signaling pathways: These two signaling pathways are involved in cell patterning, cell fate, and differentiation during the developmental stages, and dysregulation in these pathways is implicated in about 25% of cases of oncogenic developments. Hh performs its tasks through a signaling cascade in a context-dependent manner to regulate the change of balance between activator and repressor forms of glioma-associated oncogene (Gli) transcription factors. The activator form of Gli moves to the nucleus to bind to their promoters leading to the transcription of the target genes. Notch signaling is involved in many cellular processes similarly and is activated through cell-to-cell communication. Activation is followed by cleavage of Notch producing Notch intracellular domain (NCID) which translocates to the nucleus where it regulates gene expression involved in the control of cell proliferation, survival, and differentiation [71].

Both Hedgehog (Hh) and Notch signaling are involved in communication between cells and are important for organ development, regeneration, and homeostasis. Constitutive activation of the Hh signaling pathway is associated with an increased risk of developing several malignancies and communication between Hh and major signaling pathways, such as Notch, Wnt, and transforming growth factor β (TGF-β), play critical roles in both embryonic and adult life. The discovery of tumor-initiating cells with self-renewal and differentiation potential, cancer stem cells (CSCs), in cancer progression emphatically supports the role of these signaling pathways in maintaining self-renewal potential for CSCs, causing disease recurrence and chemoresistance [72]. The Hg, Wnt, and Notch pathways are closely related to CSCs, and the components of these three pathways may serve as potential targets in anti-CSC drug discovery for the treatment of cancer [73]. Targeting these pathways with the PI3K/Akt or RAS/RAF/MAPK pathways may be an effective cancer therapy strategy, as they belong to functionally different groups but are of great importance in cancer [74,75].

JAK/STAT signaling pathway: There are seven different signal transducers and activators of transcription (STAT) family proteins in mammals, STAT 1, 2, 3, 4, 5A, 5B, and STAT 6. The Janus kinases (JAK) family comprises four different members, JAK1, 2, 3, and Tyk (tyrosine kinase). This pathway largely involves cytokine signaling which is closely related to the activities of T and B cells and so often linked to the development of hematological malignancies. When a cell is exposed to cytokines such as interleukin-6 (IL-6) or interferon-gamma (IFN-g), JAK kinases associated with the cytokine receptors are activated and phosphorylate a specific tyrosine residue on STATs. STAT family members, especially STAT3 and STAT5, are involved in cancer progression, whereas STAT1 plays the opposite role by suppressing tumor growth. Target genes of STAT5 may regulate processes such as cell cycle progression, survival, and self-renewal, via binding to EGFR and constitutive activation of these transcription factors leads to the high-level expression of genes and proteins, resulting in solid tumors[76,77]. It can be ultimately mediated via

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suppression of p53 activities or crosstalk with NF-kB signaling or expression of RUNX family proteins, leading to inflammation and cancer [78]. The transformed cells depend on STAT3 and STAT5 for growth and survival, whereas non-transformed cells do not, and STAT family proteins act at the intersection of many upstream oncogenic signals, suggesting that STAT-specific inhibition may prevent resistance associated with the activation of parallel signaling pathways. These specificities provide a window for drug development with fewer side effects, and many STAT inhibitors have been tested for the treatment of cancer, yet very few STAT inhibitors have shown clinical efficacy, and STAT inhibition remains an intriguing strategy for cancer treatment [79,80].

NF-κB signaling pathway: NF-κB signaling is initiated by the degradation of IκB proteins via IκB kinase (IKK). IkB binds to the NF-κB dimer in the resting state, preventing it from binding DNA, and its degradation leads to activation of NF-κB and consequent transcriptional activation. The signaling is mediated via both the canonical (NEMO-dependent) pathway and the noncanonical (NEMO-independent) pathway. The canonical pathway is thought to be involved in immune responses and immunosurveillance, while the noncanonical pathway is associated with developmental activities. Thus, canonical and noncanonical pathways have generally been taken to be distinct, but studies have revealed numerous crosstalk mechanisms that connect them, so both pathways may result in a single NF-κB system [81]. Constitutively activated NF-κB signaling may lead to inflammation-related disorders, and its role in pathological inflammation and cancer development is well recognized now [82.83]. Furthermore, NF-κB signaling is associated with the epithelial-mesenchymal transition (EMT), which occurs frequently during tumor progression and metastasis. E-cadherin is a well-known tumor suppressor protein, and the regulation of the adhesive activity of E-cadherin present at the cell surface is important in cancer, and its repression by NF-κB is attributed to EMT induction. NF-kB has been implicated in EMT and metastasis also through the activation of EMT master-switch transcription factors and is highly invasive [84]. Evidence suggests that reversal of EMT is triggered by inhibition of NF-kB signaling, but the activated NF-κB pathway may contribute to antiapoptotic activation, ECM degradation, and E-cadherin-mediated EMT, which results in tumor growth, invasion, and metastasis. NF-κB signaling molecules communicate with many other signaling pathways, and crosstalk can be mediated by other intermediates, such as STAT3 and p53, GSK3-β, p38, or PI3K, which modulate NF-κB transcriptional activity [85,86]. Thus, targeting the NF-κB signaling pathway represents an attractive approach for anti-inflammatory and anticancer therapies, and inhibitors have been developed to block different steps of NF-κB signaling for cancer treatment [87,88].

The cGAS-STING pathway: The cyclic GMP–AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway represents a key signaling process that controls inflammatory responses in the presence of foreign particles based on dsDNA recognition through pattern recognition receptors (PPRs) and thus regulates the overall preparedness for the cell to withstand adversity caused by infection or injury. The binding of cGAS to double-stranded DNA (dsDNA) induces the catalytic activity of the synthase and leads to the production of 2′3′ cyclic GMP–AMP (cGAMP), a second messenger molecule that quickly binds to the stimulator of interferon genes (STING) dimers localized at the endoplasmic reticulum (ER) membrane, which is then released to undergo further processing, finally resulting in the expression of type I interferons, interferon-stimulated genes (ISGs), and several other inflammatory mediators, pro-apoptotic genes and chemokines. STING also binds and stimulates IKK, triggering the transcriptional activation of NF-kB that promotes noncanonical NF-κB responses. This signaling outcome limits type I interferons and the canonical NF-κB pathway as critical negative regulators of STING effector mechanisms, which can have important biological consequences related to immune evasion and metastasis [89,90]. cGAS–STING signaling may also induce autophagy and additionally communicate via p53, MAPK p38, and STAT3 signaling in a context-dependent manner. This finding reveals the complex role of this signaling in the regulation of cell behaviors. Mutations associated with the pathway have been implicated in cancer progression.

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cGAS-STING is an important pathway in cancer immunotherapy, and inhibitors of the pathways are being tried for targeted drug therapy [91,92].

Hippo signaling Pathway: The Hippo pathway is the evolutionarily conserved major signaling pathway involved in cell contact inhibition and control of organ development whose activity can be regulated at multiple levels. Contact inhibition is a process of arresting cell growth when cells come in contact with each other, and it is a powerful anticancer mechanism that is lost in cancer cells. Hippo core activity is controlled by cell density, polarity, stretching, and energy stress as well as ECM stiffness and shear stress, which together can regulate contact inhibition and related development. Cell proliferation and stem cell self-renewal can be directly attributed to contact inhibition governed by this signaling pathway, and dysregulation of the pathway is implicated in oncogenesis and therapeutic resistance [93]. The canonical Hippo pathway is a kinase cascade working as a repressive pathway that phosphorylates and inhibits the transcription coactivators YAP and TAZ, the two major downstream effectors of the pathway, to execute its signal. Phosphatase and protein ubiquitination modulate the activities of the coactivators in the cascade and are also regulated by the cytoskeleton for its performance. When dephosphorylated, YAP/TAZ translocates into the nucleus and interacts with other transcription factors to induce gene expression leading to cell proliferation and inhibition of apoptosis22. The noncanonical Hippo pathway operates in tight and adherens junction complexes to control their localization and activity within the cell. The regulation of YAP1/TAZ may be influenced by many other molecular events, including crosstalk with Wnt/β-catenin signaling, and is oncogenic. The exact nature of extracellular signals and membrane receptors regulating the Hippo pathway remains to be fully understood, but drugs targeting the intermediates of the signaling pathway have been introduced, and some are under investigation for their efficacy in cancer treatment [94.95].

TGF-β/SMAD signaling pathway: SMAD proteins, homologues of the Drosophila protein, mothers against decapentaplegic (MAD) and the Caenorhabditis elegans protein (SMA), comprise a family of structurally similar and well-conserved transcription factors that are the main signal transducers for receptors of transforming growth factor-beta (TGF-B) superfamily proteins, which are critically important for regulating cell development and growth. Transforming growth factor (TGF)-β signaling is known to control various biological processes, including cell proliferation, differentiation, apoptosis, and migration, and plays context-dependent roles in cancer progression. In premalignant cells, TGF-β primarily functions as a tumor suppressor via SMAD-mediated canonical pathways when TGF-β/SMAD-dependent p15/p21 induction or c-MYC suppression works well to maintain growth arrest, apoptosis, and epithelial cell differentiation. However, the situation can be reversed as SMAD- dependent suppression becomes insensitive under the influence of certain aggressive oncogenic mutations mediated by other pathways, and the role of TGF-β becomes antiapoptotic, EMT inducer, and tumorigenic. SMAD inactivation under such a circumstance explains the situation-based role of TGF-β in different cancers. Furthermore, the classical, non-SMAD pathway of TGF-β receptors may involve crosstalk with other signaling pathways, such as the Wnt/β-catenin, Ras/Raf/MAPK, and PI3K/Akt/mTOR pathways, to play a role in cancer development, and a proper understanding of the TGF- β signaling pathway in cancer progression would resolve discrepancies related to the process over time [96,97]. The broad range of functionality associated with TGF-β during oncogenesis has led to the development of multiple therapeutic agents targeting different intermediates of the pathways, and a combination of drugs may achieve more efficient results against metastasizing cancer [98,99].

5. The Cancer Genome Atlas (TCGA) Program

The Cancer Genome Atlas (TCGA) Program, the landmark cancer genomics program initiated by the National Institute of Health (NIH), has contributed immensely to realizing the importance of genomics in cancer research and treatment in the last decade and has begun to change the way the disease has been treated in the clinic. It has been a joint effort

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by the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI), both part of the NIH, that started working in 2006 and brought together researchers from diverse disciplines and multiple institutions to work on the characterization and analysis of cancer at the molecular level for a complete understanding of the genetic basis of human cancer [100,101]. The TCGA Research Network has profiled and analyzed large numbers of human tumors to discover molecular aberrations at the DNA, RNA, protein, and epigenetic levels and has been providing markers for different cancer types since then. As a vast number of mutations generally contribute to cancer and the use of next-generation sequencing-based approaches in clinical diagnostics is leading to a tremendous increase in data and an enormous number of variants of uncertain significance, requiring further analysis and validation by means of accurate techniques will be required to fulfill the purpose satisfactorily [102,103]. Predicting the effects of mutations using in silico tools has become a frequently used approach, but these data may not be analyzed by simply using traditional tools and techniques available to scientists, and even more advanced computational methods will be needed with time to better understand the molecular basis of the origin and evolution of cancer. To meet this end, a cancer hallmark framework through modeling genome sequencing data has been proposed for the systematic identification of representative driver networks to convincingly predict cancer evolution and associated clinical phenotypes [104,105]. It is based on the consideration that possible observable combinations of those mutations must converge to a few hallmark signaling pathways and networks responsible for tumor growth and cancer progression. Thus, it aims to analyze the data to explain how the different gene mutations in different patients have the same downstream effects on the protein machines, ultimately leading to the common path of cancer development and direct treatment options accordingly. In this direction, the cancer cell map initiative (CCMI), developed recently by researchers at the University of California, San Francisco and the University of California San Diego, has been successful in charting how hundreds of genetic mutations involved in breast cancer and cancers of the head and neck affect the activity of certain proteins that ultimately lead to cancer manifestation. As there exists a vast amount of sequence data from many different cancer types, efforts are being made to extract mechanistic insight from the available information, and an integrated computational and experimental strategy will be required to help place these alterations into the context of the higher-order signaling mechanisms in cancer cells [10,107]. This is the defined goal of the CCMI and is likely to create a resource that can be used for cancer genome interpretation, allowing the identification of key complexes and pathways to be studied in greater mechanistic detail to gain insight into the biology underlying different types and stages of cancer [108]. The challenge to identify the relevant genes and signaling molecules for different cancer types using cutting-edge technologies will remain an essential part of cancer research and is most likely to help vulnerable people benefit from it to undergo precise treatment for cancer with time.

Furthermore, researchers funded by the NIH separately completed a detailed genomic analysis based on data available through the TCGA program known as the 'Pan-Cancer Atlas' to provide an independent view of the oncogenic processes that contribute to the development of human cancer [109,110]. By analyzing over 11,000 tumors from 33 of the most prevalent forms of cancer, the Pan-Cancer Atlas provides a uniquely comprehensive, in-depth, and interconnected understanding of how, where, and why tumors arise in humans focusing on how germline genetic variants and somatic mutations collaborate in cancer progression and exploring the influence of mutation on cancer cell signaling [111-112]. The analysis of tumors profiled by TCGA for understanding mutation patterns in 10 major signaling pathways reveals that approximately 90% of tumors had at least one driver alteration in these pathways, and nearly 60% percent of tumors had at least one alteration potentially targetable by the available drugs and 30% of tumors had multiple targetable alterations, providing opportunities for combination therapy. Thus, a comprehensive analysis of tumor signaling pathways reveals patterns of vulnerabilities that will aid in the development of personalized treatments and new combination

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therapies [113]. A synchronizing view on oncogenic processes based on PanCancer Atlas analyses reveals the possible consequences of genome alterations on the different signaling pathways involved with human cancers as well as their influence on tumor microenvironment and immune cell composition, providing new insights for the development of new forms of treatments and immunotherapies. The stemness features extracted from transcriptomic and epigenetic data from TCGA tumors reveal novel biological and clinical insight and potential drug targets for anticancer therapies [114]. Thus, as a singular and unified point of reference, the Pan-Cancer Atlas seems to be an essential resource for the development of new treatments in the pursuit of precision medicine.

6. Integrating Artificial intelligence (AI) with Multi-Omics in Precision Oncology

Artificial intelligence (AI) encompasses multiple technologies with the common aim of computationally simulating human intelligence to solve problems. Referred to as computer programming enabled to perform specific tasks, it is based on the principle that human intelligence can be defined in a way that a machine can easily mimic it and execute tasks from the simplest to even more complex ones successfully [115]. The term may be applied to any machine that shows traits associated with human understanding, such as learning and problem-solving. Artificial intelligence (AI) and related technologies have increasingly been prevalent in finance, security, and society, and are beginning to be applied to healthcare [116]. It has been widely applied in precision medicine in general and in medical oncology practice particularly as many artificial intelligence algorithms have been developed and applied in cancer research in recent years. An exact understanding of the structure of proteins is often the first step to knowing about their roles in cancer progression and therapeutic drugs are often designed using structural information of the target proteins where AI-based techniques can be used for the solutions. The development of next-generation sequencing (NGS), has led multi-omics data on cancer to become available, providing researchers with opportunities to explore the genetic risk and reveal cancer mechanisms to help early diagnosis, exact prognosis, and the discovery, design, and application of specific targeted drugs against cancer. Thus, taking the help of large datasets from multi-omics platforms, imaging techniques, and biomarkers found and mined by artificial intelligence algorithms, oncologists can diagnose cancer early at its onset and help direct treatment options for individualized cancer therapy. The advances in artificial intelligence (AI) now present an opportunity to perfect pathways of diagnosis and prognosis, and to develop personalized strategies for treatment, using large datasets, and future developments in this field are most likely to help many more problems to be resolved swiftly. Artificial intelligence is the future of precision oncology for the prevention, detection, risk assessment, and treatment of cancer [117,118].

Machine learning: Machine learning (ML) is a branch of artificial intelligence that aims to develop computational systems with advanced analytical capabilities. It is concerned with the development of domain-specific programming algorithms with the ability to learn from data to solve a class of problems [119]. Therefore, in healthcare, the most common application of traditional machine learning is precision medicine and is most suited for the data-driven identification of cancer states and treatment options that are crucial to precision oncology (Fig. 3) [120].

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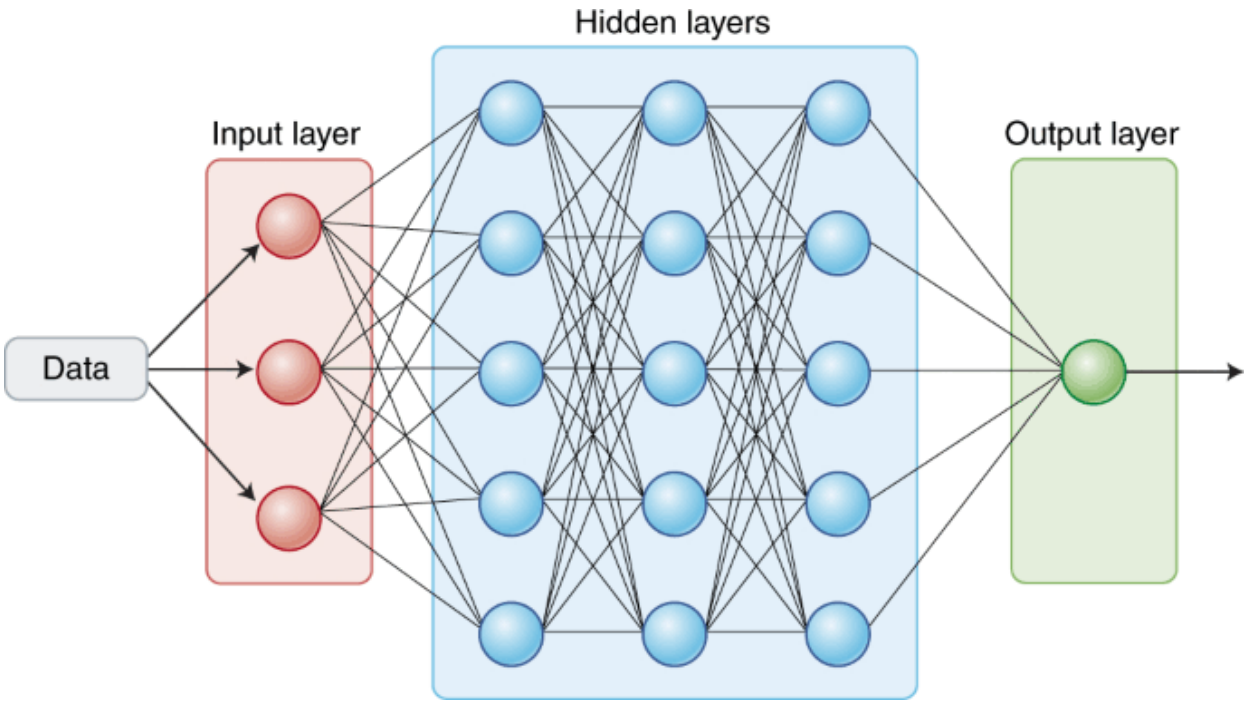

Figure 3. High-performance medicine: the convergence of human and artificial intelligence (Topol, E.J. [120]). .

Deep Learning: Deep learning (DL) is a subbranch of ML that uses statistics and predictive modeling to extract patterns from large data sets to precisely predict a result. A variety of data have been appearing in modern biomedical research, including electronic health records, imaging, multi-omics, sensor data, etc., which are complex, heterogeneous, and poorly defined and need to be mined efficiently to bring correct results. To meet this end, DL uses a machine learning program called artificial neural networks modeled on the human brain that forms a diverse family of computational models consisting of many (deep) data processing layers for automated feature extraction and pattern recognition in large datasets to answer the problems.

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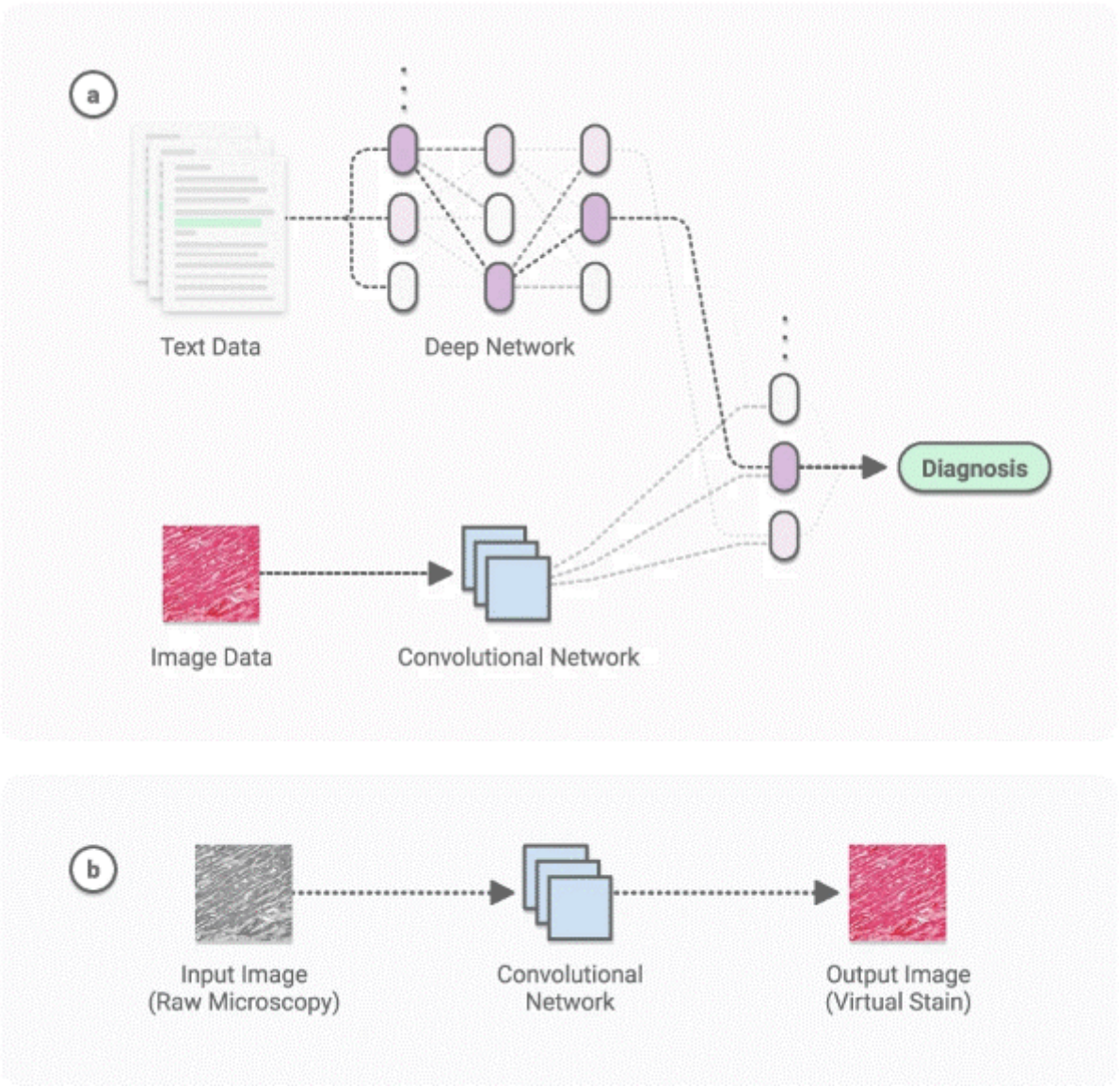

Figure 4. Deep learning-enabled medical computer vision. (Esteva, A., Chou, K., Yeung, S. et al.[121]).

The human brain consists of neurons arranged together as a network of nerves processing a number of pieces of information received from many different senses efficiently to translate into a particular reflex action. In DL, the same concept of the network of neurons can be used on a machine learning platform to emulate human understanding to bring solutions. The neurons are created artificially on a computer as the data processing layers work together to create an artificial neural network where the working of an artificial neuron can be taken as similar to that of a neuron present in the brain. Thus, DL is designed to use a complex set of algorithms, to enable it to process unstructured data such as documents, images, and text to find efficient results [121].

Drug development for cancer therapeutics is the major issue in cancer research and DL is coming of immense help in this direction. Drug combinations targeting multiple pathways or targets are thought to be the answer to the incidences of drug resistance in cancer therapy. Changes in the genetic composition of tumors translate into structural changes in cellular subsystems that require to be integrated into drug design to predict therapy response and concurrently learn about the mechanism underlying a particular drug response. The understanding of the mechanism of drug action can help exploit the importance of heterogeneity of the signaling pathways including some new and uncommon pathways associated with tumors to help develop novel synergistic drugs for

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efficacious therapeutic targeting of diverse types and subtypes of cancer. The new advances in AI are enabling researchers to develop DL-based models to predict tumor cell response to synergistic drug combinations to be employed effectively in precision oncology. Drug combinations targeting multiple pathways or targets are thought to be the answer to the incidences of drug resistance in cancer therapy where computational models can be used to find solutions. Changes in the genetic composition of tumors translate into structural changes in cellular subsystems that require to be integrated into drug design to predict therapy response and concurrently learn about the mechanism underlying a particular drug response. Understanding the mechanism of drug action can help exploit the importance of heterogeneity of the signaling pathways including some new and uncommon pathways associated with tumors to help develop novel synergistic drugs for efficacious therapeutic targeting of diverse types and subtypes of cancer. Recent advances in AI have enabled researchers to develop DL-based models to predict tumor cell response to synergistic drug combinations to be employed effectively in precision oncology [122]. A recent development in the DL system is AlphaFold which is being used to predict the structures of different proteins and the tool has already determined the structures of around 200 million proteins, from every known organism on the planet. Researchers continue to discover proteins that may be the key drivers of cancer and need a fuller understanding of the 3D shape, or structure, of these proteins to decide their exact functions in the cell [123,124]. This new revolutionary development in DL is going to be of great use in understanding the roles of suspected proteins in cancer development and in anticancer drug design. In this way, DL is becoming the main method in precision oncology for the prediction of treatment response, estimation of survival analysis, risk estimation, and treatment planning [125].

Multi-Omics: High-throughput sequencing technologies, also known as next-generation sequencing (NGS), are a comprehensive term used to describe technologies that sequence DNA and RNA rapidly and cost-effectively. It has revolutionized the field of genetics and molecular biology and aided in the study of biological sciences as never before [126]. Technologies using NGS have been developed that measure some characteristics of a whole family of cellular molecules, such as genes, proteins, or metabolites, and have been named by appending the term "-omics. Multiomics refers to the approach where the data sets of different omics groups are combined during sample analysis to allow scientists to read the more complex and transient molecular changes that underpin the course of disease progression and response to treatment, and to select the right drug target for desired results [127]. It forms the basis of precision medicine in general and is at the core of the development of precision oncology, integrating it with artificial intelligence is the need of the hour and is likely to serve the purpose adequately with time [128,129].

7. Single-cell Technology to Unmask Tumor Heterogeneity:

Tumor heterogeneity is a hallmark property of cancer and broadly refers to the differences between tumors of the same type in different patients, the differences between subpopulations of cancer cells within a single tumor, or the differences between a primary tumor and a secondary tumor. The heterogeneous mixture of distinctly differentiated cancer cells includes connective tissue cells, immune cells, cancer stem cells, and vasculature, and these subpopulations of cells can be further distinguished by a variety of features impacting their phenotype, including genetic alterations. Heterogeneity within a single tumor, referred to as genetic intratumoral heterogeneity (ITH), has been documented across most cancers as an outcome of genome instability and clonal evolution [130,131]. Furthermore, researchers from different laboratories in the last decade have established tumor heterogeneity as a phenomenon of critical importance in the history of individual neoplasms [132]. Recent investigations on drug resistance and tumor heterogeneity have converged to focus on the clonal organization of tumors as the underlying basis for drug resistance, thus indicating the need to fully understand the structure and dynamics of ITH to develop treatment strategies for the possible cure [133,134]. Thus, more precisely, the

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cellular composition of a tumor is known, and the mechanisms involved with the diseased cells are understood. More specific therapeutic strategies could be devised to treat the ill. The emergence of single-cell technologies for biological analysis is becoming an important tool in this regard, as they can help carry out single-cell measurements within the tissue to provide a clear picture of the complex biological processes involved and unmask heterogeneity present in the tumor mass [135,136]. The rapid progress in the development of NGS in recent years has provided many valuable insights into cancer genomics, and NGS-based technologies for genomics, transcriptomics, and epigenomics have enabled laboratories to carry out related single-cell measurements efficiently. Single-cell genomics now facilitates the simultaneous measurement of thousands of genes in thousands of 'single' cells from a single specimen, allowing researchers to compare the genomes of individual cells to determine the mutation profile of the cells influencing the changes in the tumor microenvironment. Single-cell sequencing can also be combined with CRISPR screening to enable large-scale studies regarding how genetic modification affects cell behavior or obtain insight into a specific physiological condition that needs to be better understood for designing treatment [137]. Combining both techniques to study gene functions with the concurrent use of single-cell resolution techniques, such as flow cytometry, microfluidics, manual cell picking, or micromanipulation, can have broad applications in cancer treatment, including identifying novel drug targets or studying unknown mechanisms of action of drugs [138]. Furthermore, the importance of epigenetic reprogramming in cancer is well understood, as evidenced by the fact that chromatin regulators are often mutated and that the widespread epigenetic changes throughout cancer genomes can be identified and linked to the activity of known tumor promoters or suppressor genes, such as growth factor-stimulated genes or TP53. The interrelationship between genetics and epigenetics needs to be further examined for the discovery of screening markers to optimize pathways of diagnosis and prognosis and to develop strategies for cancer therapeutics [139]. Epigenetic profiling holds great possibilities for deciphering the cellular states and characterizing phenotypic heterogeneity to help therapeutic options be employed accordingly to pin specific mutations that profoundly affect epigenetic pathways. The inclusion of epigenetics in clinical practice would require the identification of epigenetic signatures that mediate distinct phenotypical changes of clinical relevance, such as mesenchymal transition, stemness, dormancy, and quiescence or therapy resistance.

However, considering that single-cell sequencing technologies have been successful in leading scientists to better understand the cell types and features associated with the tumor, the spatial context of this development is essential to understand how cells organize and communicate across the tissue to fully unlock the repertoire of tumor heterogeneity. It will require a clear understanding of which cells are present, where they are located in tissue, their biomarker expression patterns, and how they organize and interact to influence the tissue microenvironment [140,141]. It is an essential part of spatial biology and adds another dimension to single-cell analysis and the study of tumor heterogeneity. Spatial biology studies ordinarily try to combine whole-slide imaging (WSI), commonly referred to as 'virtual microscopy', at single-cell resolution to visualize and quantitate biomarker expression and reveal how cells interact and organize across the entire tissue landscape. This technique can support research from early biomarker discovery to late-stage translational research and therapy development. The latest development in this direction is spatial transcriptomics to enable researchers to visualize and quantify RNA down to the subcellular level and simultaneously compare gene expression in situ. It is a groundbreaking molecular profiling method that exploits the multi-omics technique, allowing us to measure all the gene activity in a tissue sample and assay the genetic information of single cells within their native tissue environment (Fig. 5) [142,143].

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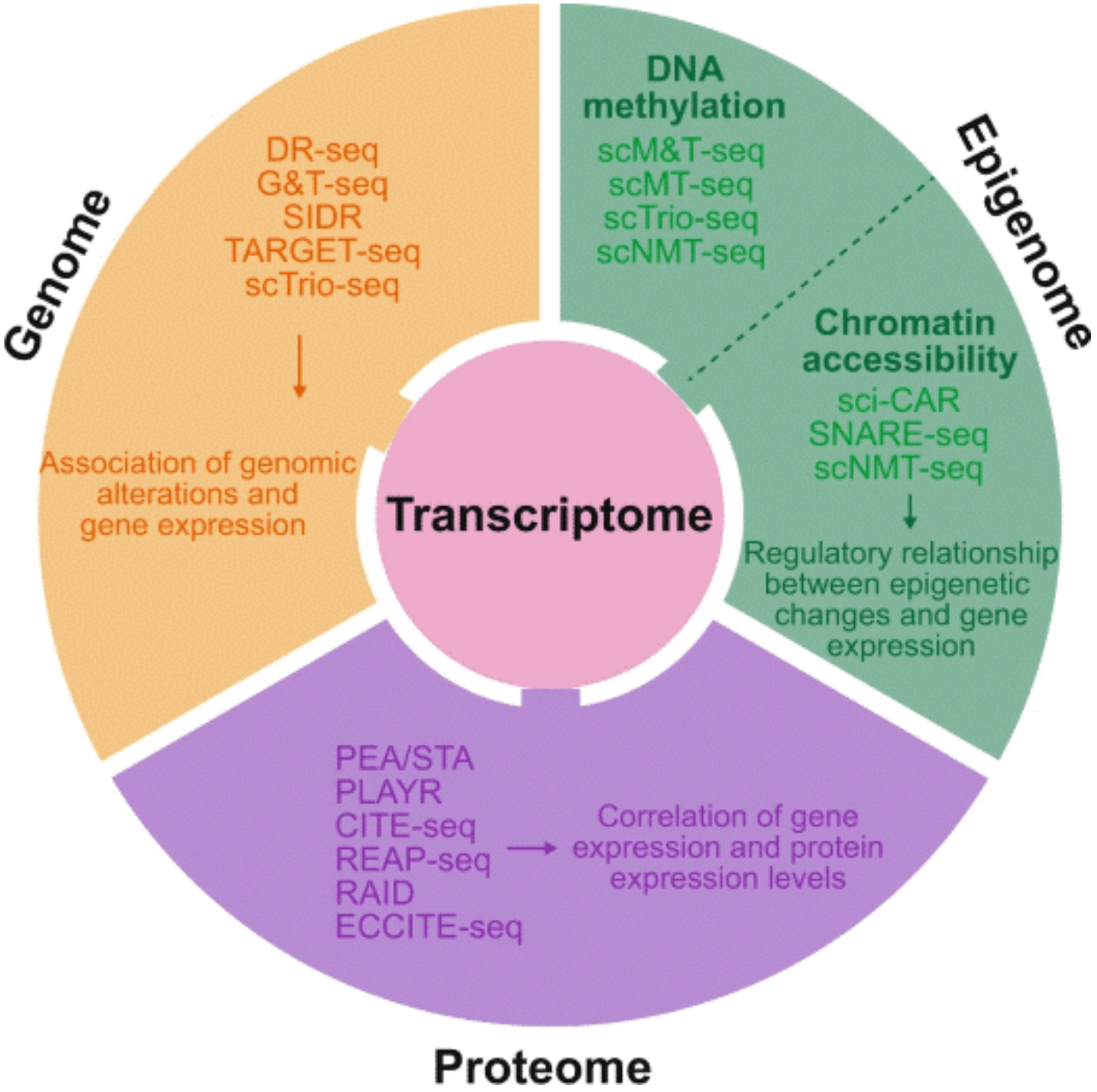

Figure 5. An overview of single-cell multi-omics sequencing technologies.

Technologies that measure more than two types of data are included in multiple categories, e.g., scTrio-seq in transcriptome-genome and transcriptome-DNA methylation categories. (Lee, J. et al. [143].)

The ability to demonstrate the role and function of distinct cell types comprising the tissue is paving the way for a new understanding of the tissue-specific cellular pathways and interactions that lead to cancerous developments. Thus, the precise molecular analysis of cancer cells based on single-cell technologies now aims to present an accurate picture of the most up-to-date development in the tumor microenvironment and detect changes in the genes and proteins responsible for alterations in cellular processes to better understand the prognosis and pathways of cancer progression. New advances in multi-omics techniques powered by AI now enable researchers to integrate genomic, transcriptomic, epigenomic, and other related data to reveal the most accurate information on the activity state of individual genes to help detect novel cancer drivers and genetic vulnerabilities for prevention and cure [144,145]. The advances in single-cell technology thus provide unprecedented insight into the complex genetic and epigenetic heterogeneity within individual tumors for advanced precision oncology-based treatment and are thought to streamline future research direction

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8. Precision Oncology and Targeted Drug Therapy of Cancer

Targeted drug therapy is a new form of cancer treatment that targets specific genes and proteins of cancer-related signaling pathways and molecules in the tumor microenvironment that contribute to cancer development [146.147]. This is in contrast to the single-target approach employed in chemotherapy to primarily target and kill actively dividing cancer cells with serious side effects and can be seen as the natural outcome of decades of studies on cell reprogramming in different cancers. The anticancer drugs employed in targeted therapy are designed to target molecules directly involved with the signaling processes or related molecules in the tumor microenvironment, which are essentially required for tumor growth and cancer progression [148,149]. They are broadly classified as monoclonal antibodies or small molecule drugs. Therapeutic monoclonal antibodies (mAbs) target antigens found on the cell surface, and small molecules can penetrate the cell membrane to interact with targets inside the cell and are usually designed to inhibit the enzymatic activity of target proteins such as the proteasome complex, tyrosine kinases or cyclin-dependent kinases. A type of targeted therapy called tumor agnostic therapy uses drugs and other substances to target certain genetic changes or markers as cancer-specific features to treat the ailment without requiring a focus on the cancer type or where cancer may have started in the body. The use of monoclonal antibodies (mAbs) in targeted therapy is being explored, as they may be exploited successfully for potentiating the natural immune system, addressing the concern related to changes in the immunogenicity of cancer cells. The mAbs may be designed to coat the cancer cells to be recognized and destroyed by the immune cell, block the activity of certain abnormal proteins in the affected cell, or inhibit the immune checkpoints that help cancer cells escape or survive the immune responses [150,151]

Targeted drug therapy and monoclonal antibody-based therapy need to be seen as potent means for cancer treatment and are becoming increasingly important in cancer therapy [152,153]. However, a major concern in cancer chemotherapeutics remains with regard to proper drug delivery to the affected cells and tissue for the desired results. Conventional chemotherapeutics may possess some serious side effects due to nonspecific targeting or inability to enter the core of the tumors, resulting in impaired treatment and a low survival rate. The issue can be addressed with the use of nanotechnology in cancer therapeutics, as it provides the opportunity to obtain direct access to cancerous cells with increased drug localization, solubility, and cellular uptake. Nanoparticles can be programmed for recognizing cancerous cells and allowing selective and accurate drug delivery, avoiding encounters with healthy cells; they will serve the needs better if the treatment is tailored to the requirements of the vulnerable individual or a cohort of patients receiving treatment for the disease [154,155]. The field of precision oncology emerging as the new branch of cancer research is essentially directed at strategizing cancer signaling-based targeted therapy by exploiting the peculiarities of the cancer genomes of the individuals for an efficient treatment. It is dedicated to studying the genetic profile of cancer cells aimed at gaining a thorough understanding of the signaling pathways and related molecular events in the course of tumor growth and metastases and of drug resistance for a proper cure (Fig. 6) [156,157].

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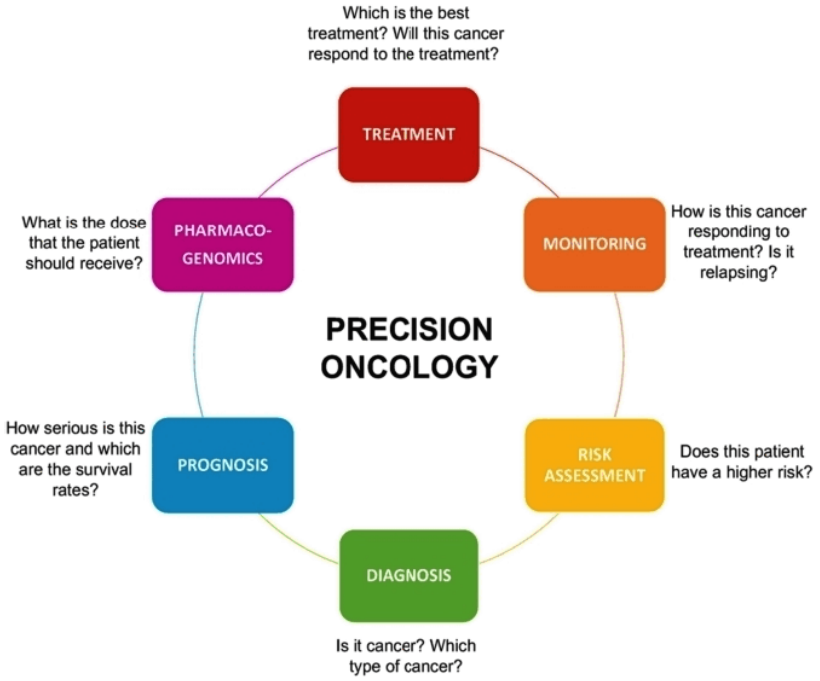

Figure 6. Cancer Genomics in Precision Oncology. Precision Medicine Approach to Diagnosis, Prognosis, and Treatment. (Pereira, M.A. et al. [157]).

Recent advances in cancer genomics and single-cell technologies have certainly made targeted therapy the accepted form of cancer treatment, and yet a huge amount of investment will be needed for future research, drug discovery, and diagnostics to fully unlock its potential and for their application in the management of cancer incidences in the time to come.

Let us not forget that the socioeconomic burden of cancer remains high as the treatment options for most common cancers have been limited thus far and is an indication for a renewed approach to expedited drug development to bring effective anticancer agents from bench to bedside in a cost-effective manner. The lack of understanding of the genetic heterogeneity of individual cancers has traditionally been limiting the search for efficacious agents for cancer treatment and missing a wide range of possibly suitable agents from other disease areas. The use of molecular characterization of different cancer types through cancer genomics can help resolve drug-related issues to a reasonable extent by repurposing the use of certain existing drugs as anticancer agents for a wide range of applications, and it will remain at the forefront of precision oncology [158,159]. Furthermore, the move from tissue- or cancer-specific treatments to genomic or target-based treatments entails the reuse of anticancer drugs prescribed for one type of cancer to treat other cancer types. With the ever-greater understanding of cellular signaling mechanisms and genetic alterations in carcinogenesis in the age of cancer genomics, it is envisaged that considerable progress in cancer treatment will be realized sooner. Thus, considering that academia, industries, and civil society will be working in tandem to cater to the needs of the system, it is hoped that a wide range of people with cancer will benefit from this new development in cancer research in the future to benefit society as a whole [160,161]

9. Conclusion

Precision oncology-based cancer therapeutics propose to develop treatments that target the specific molecular characteristics of an individual's tumor instead of targeting the common features of particular cancer for a cure. A thorough understanding of the genetic composition and heterogeneity of the individual's tumor is now becoming possible through single-cell technologies, and it can help the individuals get the right treatment at the right time rather successfully without requiring more conventional methods of treatment that might not prove most effective at the end. In this way, precision oncology emerging as a new field of cancer researchers working on the identification of specific mutations in the cancer genome to selectively target the pathways involved with disease progression is most likely to bring in proper treatment for the cure. It appears to be the natural outcome of the cancer genome project, and considering the level of support coming from multi-omics platforms, it is destined to satisfy the intended purpose of the initiative satisfactorily. The success of this form of treatment is sure to further strengthen our belief in the possibility of a cure for cancer and is needed to be accessible to a larger number of people with cancer toward the realization of goals with time.

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Supplementary Files:

Table 1. **Cancer Genes: Oncogenes and Tumor Suppressors in Different Cancer Types (https://www.cancerquest.org/cancer-biology/cancer-genes)**

Oncogene Table

Oncogene	Function/Activation	Cancer*	References
ABL1	Promotes cell growth through tyrosine kinase activity	Chronic myelogenous leukemia	[27] [28]
AFF4/MLLT11	Fusion affects the MLLT11 transcription factor/methyltransferase. *MLLT11 is also called HRX, ALL1 and HTRX1*	Acute leukemias	[29] [30]
AKT2	Encodes a protein-serine/threonine kinase	Ovarian cancer	[31] [32]
ALK	Encodes a receptor tyrosine kinase	Lymphomas	[33] [34]
ALK/NPM	Translocation creates fusion protein with nucleophosmin(npm)	Large cell lymphomas	[35] [36]
RUNX1 (*AML1*)	Encodes a transcription factor	Acute myeloid leukemia	[37] [38]
RUNX1/MTG8(ETO)	New fusion protein created by translocation	Acute leukemias	[39] [40]
	Encodes a receptor		
AXL	tyrosine kinase	Hematopoietic cancers	[41] [42]
BCL-2, 3, 6	Block apoptosis (programmed cell death)	B-cell lymphomas and leukemias	[43] [44]
BCR/ABL	New protein created by fusion of bcr and abl triggers unregulated cell growth	Chronic myelogenous and acute lymphotic leukemia	[45] [46]
MYC (*c-MYC*)	Transcription factor that promotes cell proliferation and DNA synthesis	Leukemia; breast, stomach, lung, cervical, and colon carcinomas; neuroblastomas and glioblastomas	[47] [48]

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MCF2 (*DBL*)	Guanine nucleotide exchange factor	Diffuse B-cell lymphoma	[49]
DEK/NUP214	New protein created by fusion	Acute myeloid leukemia	[50] [51]
TCF3/PBX1	New protein created by fusion	Acute pre B-cell leukemia; TCF3 also called E2A	[52] [53]
EGFR	Cell surface receptor that triggers cell growth through tyrosine kinase activity	Squamous cell carcinoma, glioblastomas, lung cancer	[54] [55] [56]
MLLT11	Fusion protein created by a translocation t(11;19).	Acute leukemias	[49] [57]
ERG/FUS	Fusion protein created by t(16:21) translocation. The erg protein is a transcription factor.	Myeloid leukemia	[58] [59]
ERBB2	Cell surface receptor that triggers cell growth through tyrosine kinase activity; also known as HER2 or neu	Breast, salivary gland, and ovarian carcinomas	[60] [61]
*ETS*1	Transcription factor	Lymphoma	[62] [63]
*EWSR1/FLI*1	Fusion protein created by t(11:22) translocation.	Ewing Sarcoma	[64] [65]
CSF1R	Tyrosine kinase	Sarcoma	[66] [67]
FOS	Transcription factor for API	Osteosarcoma	[68] [69]
FES	Tyrosine kinase	Sarcoma	[70] [71]
GLI1	Transcription factor	Glioblastoma	[72] [73]
GNAS (*GSP*)	Membrane associated G protein	Thyroid carcinoma	[74] [75]
HER2/neu	Overexpression of signaling kinase due to gene amplification	Breast and cervical carcinomas	[76] [77]

TLX1	Transcription factor; aka	Acute T-cell leukemia	[78] [79]

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	HOX11		
FGF4	Encodes fibroblast growth factor; aka HST1	Breast and squamous cell carcinomas	[49]
IL3	Cell signaling molecule	Acute pre B-cell leukemia	[49]
FGF3 (*INT-2*)	Encodes a fibroblast growth factor	Breast and squamous cell carcinomas	[49]
JUN	Transcription factor for API	Sarcoma	[80] [17]
KIT	Tyrosine kinase	Sarcoma	[80] [17]
FGF4 (*KS3*)	Herpes virus encoded growth factor	Kaposi's sarcoma	[17]
K-*SAM*	Fibroblast growth factor receptor	Stomach carcinomas	[49]
AKAP13	Guanine nucleotide exchange factor; aka *LBC*	Myeloid leukemias	[17] [81]
LCK	Tyrosine kinase	T-cell lymphoma	[17]
LMO1, LMO2	Transcription factors	T-cell lymphoma	[17]
MYCL	Transcription factor	Lung carcinomas	[49] [80]
*LYL*1	Transcription factor	Acute T-cell leukemia	[49]
NFKB2	Transcription factor. Also called *LYT-10*	B-cell lymphoma	[17]
NFKB2/Cα1	Fusion protein formed by the (10;14) (q24;q32) translocation of NFKB2 next to the C alpha 1 immunoglobulin locus.		[49]
*MAS*1	Angiotensin receptor	Mammary carcinoma	[17]
*MDM*2	Encodes a protein that inhibits and leads to the degradation of p53	Sarcomas	[49] [80]
MLLT11	Transcription factor/methyltransferase (also called HRX and	Acute myeloid leukemia	[82] [17]

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	ALL1)		
MOS	Serine/threonine kinase	Lung cancer	[49] [83]
	Fusion of transcription repressor to factor to a		
RUNX1T1	transcription factor. Also known as MTG8 and AML1-MTG8	Acute leukemias	[49]
MYB	Transcription factor	Colon carcinoma and leukemias	[49]
MYH11/CBFB	New protein created by fusion of transcription factors via an inversion in chromosome 16.	Acute myeloid leukemia	[49]
NEU	Tyrosine kinase. Also called ERBB2 or HER2	Glioblastomas, and squamous cell carcinomas	[84] [49]
MYCN	Cell proliferation and DNA synthesis	Neuroblastomas, retinoblastomas, and lung carcinomas	[84] [49]
MCF2L (*OST*)	Guanine nucleotide exchange factor	Osteosarcomas	[17]
PAX-5	Transcription factor	Lympho-plasmacytoid B-cell lymphoma	[17]
PBX1/E2A	Fusion protein formed via t(1:19) translocation. Transcription factor	Acute pre B-cell leukemia	[49]
*PIM*1	Serine/threonine kinase	T-cell lymphoma	[17]
CCND1	Encodes cyclin D1. Involved in cell cycle regulation. Also called PRAD1	Breast and squamous cell carcinomas	[49]
*RAF*1	Serine/threonine kinase	Many cancer types	[49]
RARA/PML	Fusion protein caused by t(15:17) translocation. Retinoic acid receptor.	Acute premyelocytic leukemia	[84] [49]
HRAS	G-protein. Signal transduction.	Bladder carcinoma	[49]
KRAS	G-protein. Signal transduction	Lung, ovarian, and bladder carcinoma	[49] [80]

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NRAS	G-protein. Signal transduction	Breast carcinoma	[49]
REL/NRG	Fusion protein formed by deletion in chromosome 2. Transcription factor.	B-cell lymphoma	[49] [80]
RET	Cell surface receptor. Tyrosine kinase	Thyroid carcinomas, multiple endocrine neoplasia type 2	[84] [49]
RHOM1, RHOM2	Transcription factors aka LMO1 and LMO2	Acute T-cell leukemia	[49]
ROS1	Tyrosine kinase	Sarcoma	[17]
SKI	Transcription factor	Carcinomas	[17]
SIS (aka PDGFB)	Growth factor	Glioma, fibrosarcoma	[17]
SET/CAN	Fusion protein formed by rearrangement of chromosome 9. Protein localization	Acute myeloid leukemia	[17] [85]
SRC	Tyrosine kinase	Sarcomas	[49] [86]
TAL1, TAL2	Transcription factor.TAL1 is also called SCL	Acute T-cell leukemia	[49] [87]
*NOTCH*1 (*TAN1*)	Altered form of Notch (a cellular receptor) formed	Acute T-cell leukemia	[49] [88]
TIAM1	Guanine nucleotide exchange factor	T-lymphoma	[17] [89]
TSC2	GTPase activator	Renal and brain tumors	[17] [90]
NTRK1	Receptor tyrosine kinase	Colon and thyroid carcinomas	[49] [91]

Tumor Suppressor Table

Tumor Suppressor	Function	Cancer *	References

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APC	Controls the function of specific transcription factors which are involved in tumorigenesis, and development and homeostasis of some cell types including epithelial and lymphoid cells. APC has also been implicated in cell proliferation and other cellular activities such as migration, and adhesion.	Familial adenomatous and non-inherited colorectal carcinomas	[121] [122]
BRCA1, BRCA2	DNA Damage Repair	Inherited breast cancers; ovarian cancers	[123]
CDKN2A	Gene locus that encodes the tumor suppressors p16 and p14ARF.	Brain tumors	1 [124]
DCC	Netrin-1 receptor. Regulation of cell proliferation and apoptosis of intestinal epithelium.	Colorectal carcinomas	[125] [126] [127]
DPC4 (aka SMAD4)	Transciptional factor involved in development; Implicated in metastasis and tumor invasiveness.	Colorectal tumors, pancreatic neoplasia	[128] [129]
MADR2 (aka SMAD2)	Mediates signaling from growth factor receptors. Assists in transport of SMAD4 into nucleus.	Colorectal cancer	[130] [131]
MEN1	Codes for the menin protein that interacts with transcription factors, DNA repair proteins, cytoskeletal proteins and others. Function not	Multiple endocrine neoplasia type 1	[132]

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	clearly defined.		
CDKN2A (aka MTS1)	Inhibitor of cyclin-dependent kinases; regulates cell cycle passage from G1 into S.	Melanomas	[133]
NF1	RAS GTPase activating protein (RAS-GAP)	Neurofibromatosis type 1	[134]
NF2	ERM protein; organize plasma membrane by assembling protein complexes and linking them to actin.	Neurofibromatosis type 2	[135]
	Encodes a transcription	Bladder, breast,	
TP53 (often just p53 in older articles)	factor for p21, a protein that arrests the cell cycle in G1 phase. p53 integrates signals related to cell size, DNA integrity and chrommosome replication.	colorectal, esophageal, liver, lung, prostate, and ovarian carcinomas; brain tumors, sarcomas, lymphomas, and leukemias	[136]
PTEN	Lipid phosphatase. Regulates cell survival	Cowden syndrome; increased risk of breast and thyroid cancer	2 [137]
RB1	Binds to, and inhibits, the E2F transcription factor. Halts cell cycle progression	Retinoblastoma, sarcomas; bladder, breast, esophageal, prostate, and lung carcinomas	[138]
VHL	Cell cycle regulation. May increase stability and activity of p53	Renal cell carcinomas	1 [139]
WRN	DNA helicase and exonuclease. Involved in repair of DNA breaks.	Werner syndrome	2 [140]
WT1	Transcription factor. Essential role in	Wilms tumors (pediatric kidney	1

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	development.	cancer)	

* The cancer types listed in this column are those that are predominantly associated with each tumor suppressor gene but this is not an exhaustive list.

Table 2: Oncology (Cancer) _ Hematologic Malignancies Approval Notifications_FDA (https://www.fda.gov/drugs/resources-information-approved-drugs/oncology-cancer-hematologic- malignancies-approval-notifications)