Review

Cancer Epigenomics and Beyond: Advancing the Precision Oncology Paradigm

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ABSTRACT

How cancers are characterized and treated has evolved over the past few decades. Major advances in genomics tools and techniques have revealed interlinked regulatory pathways of cancers with unprecedented detail. Early discoveries led to success with rationally targeted small molecules and more recently with immunomodulatory agents, setting the stage for precision oncology. However, drug resistance to every agent has thus far proven intractable, sending us back to fill the gaps in our rudimentary knowledge of tumor biology. Epigenetics is emerging as a fundamental process in every hallmark of cancer. Large-scale interrogation of the cancer epigenome continues to reveal new mechanisms of astounding complexity. In this review, I present selected experimental and clinical examples that have shaped our understanding of cancer at the molecular level. Translation of our collective erudition into revolutionary diagnostic and treatment strategies will advance the precision oncology paradigm.

Keywords: cancer, genomics, epigenomics, epitranscriptomics, noncoding RNA, targeted therapy, molecular therapy, immuno-oncology, immune checkpoint inhibitors

EVOLVING ONTOLOGY OF CANCER AND THERAPY

Canonical tumor classification is based on the primary organ or tissue of origin. In combination with staging information, microscopic histopathologic diagnosis remains the gold standard for guiding treatment decisions. Although this approach has well-served oncology for the past half century, new and increasingly granular details of cancers have led to major advances in diagnostics and therapy. Since the discovery of oncogenes in the 1970s, DNA mutations, chimeric fusion proteins, and dysregulated biochemical pathways have been identified across all cancers. Specific genes and their protein products emerged as actionable biomarkers for characterizing cancer subtypes. A seminal example is the Philadelphia chromosome in chronic myeloid leukemia (CML), a consequence of chromosomal translocation resulting in the oncogene *BCR-ABL*. Successful treatment with the selective inhibitor, imatinib (Gleevec) sparked the explosive growth of targeted molecular therapy.[1,2] These historic *BCR-ABL* (fusion of *BCR* and *ABL* genes resulting from translocation of chromosomes 9 and 22) studies unequivocally linked a cancer with a specific mutant gene product that could be precisely targeted with a specific molecule, a defining paradigm for molecular medicine.

The Promise and Reality of Molecular Medicine

Molecular classification of cancer types are now commonly defined by the expression status of druggable targets such as human epidermal growth factor receptor 2 (HER2) erythroblastic oncogene B (ERBB2), epidermal growth factor receptor (EGFR), and *BRAF*.[3–6] However, mounting clinical experience with single-target therapies demonstrated small to modest improvement in survival benefit, underscoring our incomplete knowledge of cancer biology.[7] Moreover, drug resistance, universally observed for all drugs to date, reveals the limitations of imperfect oversimplified pharmacologic models. Strategies to overcome resistance centered on combination therapy, rationalized by the notion that many targets are better than one. This sentiment grew from the experience with combination cytotoxic chemotherapy in the 1960s.[8] And indeed, drug combinations were found to be more effective as exemplified by the PRIME phase 3 study comparing panitumumab (anti-EGFR) combined with folinic acid (leucovorin), fluorouracil, and oxaliplatin (FOLFOX4) vs FOLFOX4 alone.[9] More recently, the BEACON colorectal cancer (CRC) study reported the best

response with triplet therapy using encorafenib (inhibitor of *BRAF*), binimetinib (MAPK/ERK kinase inhibitor), and cetuximab (anti-EGFR antibody) compared to doublet or single agent therapy in metastatic *BRAF* (V600E mutant) CRC.[10] Despite incremental improvements in response, tumors invariably exhibit greater resiliency with concomitant phenotypic aggressiveness.

Shortcomings of Targeted Therapies

Although a number of specific gene mutations were identified in a variety of tumors, targeted therapeutic attempts have resulted in mixed responses. However, newer molecular techniques that interrogated gene function revealed that mutations in two distinct but dependent genes could elicit tumor cell death, a processed called synthetic lethality.[11] Early examples of synthetic lethal strategies targeting poly(ADP-ribose) polymerases (PARPs) exploited defective DNA repair mechanisms frequently observed in tumors. Clinical response to PARP inhibitors (PARPis) depends on the presence of germline *BRCA1* or *BRCA2* mutations or other gene mutations that result in a *BRCA*-like ("BRCAness") phenotype in the setting of wild-type *BCRA1/2*.[12–14] Consistent with this mechanism, recent results from three studies evaluating different PARPis in ovarian cancer demonstrated improved progression-free survival (PFS) in patients with germline *BCRA1/2* mutations and for homologous recombination (HR) deficiency cohorts, but not in the HR-competent cohort.[15–17] Although data to assess overall survival have not yet matured in the referenced trials, the interim findings suggest nondurable responses.

Our nascent understanding of drug resistance is facilitated by advances in research tools. It is now experimentally and economically feasible to assess the effects of perturbing one or more specific pathways on a genome-wide scale. Dynamic networks of interacting genes, their products, and metabolic pathways, collectively referred to the "interactome," link genomic variations and phenotype.[18,19] Kennedy et al[20] have recently shown that CRC cell lines with the oncogenic $KRAS^{G13D}$ mutation display extensive "rewiring" within the EGFR protein-protein interaction network. Such changes are expected to predict targeted drug resistance, particularly for genes interconnected to multiple compensatory and/or functionally redundant pathways. At present, how tumors achieve gene reprogramming to evade targeted therapy remains largely unknown, but growing evidence points to epigenetics.

Beyond the Gene: Implications for Drug Selection

Mechanistic studies of PARPi and cisplatin resistance revealed that initial germline *BRCA2* mutations were functionally restored by secondary mutations.[21,22] Experimental evidence suggests that PARPs are directly involved in regulating chromatin structure through extensive cross talk with epigenetic pathways.[23–26] A role for DNA methylation in DNA double-strand break repair was established in preclinical studies that showed synergistic antitumor effects with a PARPi and a DNA methyltransferase inhibitor (DNMTi), regardless of *BRCA* status.[27,28] Moreover, defects in the DNA damage repair pathway have been shown to correlate with increasing tumor mutational burden (TMB) and production of neoantigens.[29–31] Collectively, these findings provide compelling justification for clinical evaluation of combining PARPis with immune checkpoint inhibitors (ICIs) or epigenetic modifying drugs (epi-drugs). Effectiveness of this strategy will be assessed as data accrue from a number of ongoing trials.[29]

Although synthetic lethal gene pairs are not always druggable, knowledge of the intimate cross talk between molecular pathways can uncover new therapeutic opportunities.[12] This concept was demonstrated by Kumar et al[32] using *KRAS* mutant non–small cell lung cancer (NSCLC) cell lines in genome-wide chromatin screening studies. The investigators found that cell survival was dependent on GATA2, a transcription factor with regulatory roles in several pathways, including those of proteasome and Rho signaling (G proteins). Using a *KRAS* mouse model and clinically available drugs that targeted the proteasome and Rho pathways, tumor growth was markedly inhibited.[32] These findings demonstrate that new synthetic lethal targets can be intuitively deduced from regulatory networks empirically determined by interrogating globally activated chromatin. Furthermore, their preclinical antitumor effect was achieved with approved compounds, illustrating the potential for drug repositioning to accelerate clinical testing.

Addressing Drug Resistance With Epigenetic Priming

Chromosomal instability in cancers has been known for quite some time. Karyotypic abnormalities in myeloid malignancies were known to correlate with response to intensive therapy (allogeneic stem cell transplantation).[33] Epigenetic abnormalities, particularly hypermethylation of regulatory genes, were also frequently observed in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).[34] This prompted the evaluation of nucleoside analogs that were originally intended as anti-DNA synthesis agents but were found to inhibit DNMT at lower doses.[35] Early clinical studies evaluating azacitidine and decitabine—first-generation DNMTis also called hypomethylating agents (HMAs)—showed modest overall efficacy in MDS and AML but were notable for favorable toxicity profiles.[36–41] These studies demonstrated that targeting a presumably global epigenetic process, DNA methylation, can control disease, at least for hematologic malignancies. However, innate and acquired drug resistance has remained a formidable problem for the first-generation HMAs, motivating the development of a second-generation DNMTi, guadecitabine, with improved pharmacokinetics. A phase 2 study

in patients with intermediate or high-risk MDS who were treatment naïve or relapsed on prior HMA therapy showed comparable response in both cohorts, indicating that acquired resistance could be circumvented, albeit transiently.[42] Despite the improved pharmacokinetic profile, a large phase 3 study of guadecitabine as monotherapy in AML failed to achieve primary endpoints vs treatment of choice (ASTRAL-1).[43]

Testing of HMAs turned to solid tumors, justified by preclinical studies that showed improved effectiveness when combined with cytotoxic chemotherapy. Epigenetic modifications, particularly DNA methylation, were known to be associated with drug resistance in ovarian cancer, and early clinical studies with a first-generation HMA, decitabine, established the feasibility of epigenetic "priming" to resensitize tumors to platinum.[44] Disappointingly, guadecitabine combined with carboplatin in a recent phase 2 study failed to meet its endpoint over treatment of choice in recurrent platinum-resistant ovarian cancer.[45] A noteworthy observation was that the median PFS at 6 months was higher in the experimental arm (37% vs 13%), suggesting dose schedule was suboptimal or epigenetic status was rapidly reestablished. Conducted in parallel, genomic and epigenomic analyses of tumor biopsies taken pre- and post-guadecitabine administration revealed a number of genes involved in DNA repair, metabolism, and immune activation.[46] Interestingly, a subset of the total number of genes that were silenced by promoter methylation pretherapy were demethylated and reactivated following two cycles of guadecitabine + carboplatin. Among the therapy-induced reactivated genes, those involved in metabolic pathways and regulatory networks of embryonic stem cells were enriched. These findings illustrate the remarkable plasticity of the tumor epigenome that can be directly monitored.

Chromatin Modifications and Immune Response

Of particular interest is the reactivation of immune response pathways observed in the preceding HMA study. Consistent with an earlier trial in ovarian cancer, DNA methylation and a repressive histone mark (H3K27me3) correlated with poor tumor infiltration of T cells and resistance to immune checkpoint blockade. Peng et al[47] found that selective inhibitors of DNMT1 and enhancer of zeste homolog 2 (EZH2)—proteins that catalyze their respective epigenetic marks—reversed transcriptional repression and improved response to programmed death-ligand 1 (PD-L1) blockade as well as to adoptive T-cell therapy. Two other preclinical studies demonstrated that HMAs produce their antitumor effects by activating viral defense genes through induction of double-stranded RNAs derived from endogenous retroviral elements.[48,49] There are several key points taken from these hypomethylating drug studies. First, epigenetics mediate reversible chromatin states that are repressive or permissive to the expression of multiple pathways, including immune response genes. Switching between states is catalyzed by specific enzymes that are druggable. It is noteworthy that high expression of viral defense pathways in melanoma patients correlated with durable ICI response. Thus, the epigenetics-activated antiviral mechanism may offer a novel approach to improving durability in response to immune checkpoint blockade.

The failure of HMAs in recurrent ovarian cancer suggests the need for drugs with greater target specificity. Illustrating this point is the recent accelerated Food and Drug Administration (FDA) approval of tazemetostat, a potent selective inhibitor of EZH2, a histone methyltransferase, for adults and pediatric patients (16 years and older) with advanced epithelioid sarcoma.[50] This first-in-class drug is also undergoing clinical testing in mesothelioma, diffuse large B-cell lymphoma (DLBCL), and follicular lymphoma with encouraging early findings.[51,52]

Histone modifications constitute major processes of the epigenetic machinery. Histone acetylation is an essential evolutionarily conserved mechanism catalyzed by enzymes that add or remove acetyl groups, the histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Substantial preclinical work on HDAC inhibitors (HDACis) and promising early clinical studies led to the FDA approval of vorinostat (SAHA), romidepsin (FK228), panobinostat (LBH589), and belinostat (PXD101) for cutaneous T-cell lymphoma, peripheral T-cell lymphoma, and multiple myeloma. Combination with conventional and targeted therapies with HDACis is also being evaluated with promising early findings.[53] However, similar to DNMTis, resistance to HDACis is frequently observed with limited efficacy against solid tumors as monotherapy.[54] Combining a DNMTi and HDACi was correlated with a reversal of cancer-associated epigenetic marks, albeit transiently, suggesting the complementary action of their respective pathways.[36,55–57] Many other HDACis are undergoing clinical studies, and upcoming data will be informative. Other epi-drugs such as inhibitors of histone demethylases LSD1/KDM1A, BET, and EZH2 as monotherapy or in combination with ICIs, await maturation of clinical trials. Despite encouraging preliminary data, intractable drug resistance remains universally challenging, and multipathway targeting with combination therapy will likely be the rule in clinical trials.[58]

Immuno-oncology drugs, in particular ICIs targeting cytotoxic T-lymphocyte–associated protein-4 (CTLA-4), programmed death-1 (PD-1), or PD-L1, have set a new standard in cancer therapy. Despite their success in a number of tumor types, the majority of patients do not respond. Moreover, a high percentage of initial responders eventually acquire drug resistance. Substantial experimental evidence support a role for epigenetics in tumor immune evasion, resistance to checkpoint inhibition, and T-cell exhaustion.[59–65] Numerous clinical trials evaluating epi-drugs and ICIs are currently under way

and comprehensively reviewed elsewhere.[65–67] Although too soon to fully assess the therapeutic benefit of ICI–epi-drug combination therapy, early preliminary observations are encouraging. In a recent report of a single-arm phase 2 study, Daver et al[68] showed impressive response to nivolumab + azacitidine in relapsed/refractory AML with an overall response rate (ORR) of 33%, which included 22% complete remission (CR) compared to the historical 20% ORR (10%–16% CR) with hypomethylation-based salvage therapy.[68]

In contrast, a phase 2 study evaluating pembrolizumab + azacitidine vs pembrolizumab + placebo Levy et al[69] observed no significant difference in PFS or overall survival (OS) in previously treated advanced NSCLC patients, with the caveat of lower dosing in the test arm due to adverse events. Pre- and on-therapy global DNA methylation changes in the pembrolizumab + azacitidine arm also showed no significant correlation in response, albeit changes were difficult to interpret without a control group comparison. Whether the observed outcomes reflect pharmacologic differences in solid and hematologic cancers such as tumor drug penetration or dosing is unknown but warrants further consideration.[70] Nonetheless, this preliminary negative result suggests that testing of epi-drugs will likely benefit from predictive biomarkers for specific epigenetic signatures. This notion is supported by a recent in silico study that revealed distinct methylation levels across several costimulatory and coinhibitory ligand genes of the immunologic synapse between certain tumors and counterpart normal tissues.[71] Interestingly, costimulatory ligand genes such as *CD40* were generally found to be hypermethylated in immunogenic tumors, whereas coinhibitory genes such as *PD-L1* were hypomethylated. Although such findings await clinical validation, prognostic epigenetic signatures in a tissue-specific context are expected to greatly facilitate future biomarker-driven drug trials.

CANCER EPIGENOMICS AND BEYOND

Our nascent exploration of epigenomics, transcriptomics, proteomics, and other "omics" have revealed an astounding array of complex, interlinked, and baffling mechanisms encoded within our genetic script. We are only now beginning to understand the basic structure and rules of the epigenome and its role in tumorigenesis. Early genomics research focused on nucleotide sequence changes based on the tenet that cancer is a direct consequence of gene mutations. It still remains perplexing that genome-wide studies found that only approximately 1% of all protein encoding genes are causally implicated in tumorigenesis, the so-called cancer "driver" genes or mutations. Among these, protein kinases were most frequently identified, followed by transcription regulatory proteins.[72] Although driver gene mutations are conceptually attractive as targets of intervention, other noncausative mutations or "passenger" mutations present challenges in identifying druggable targets.[73] Recent high throughput sequencing has uncovered other driver genes, and nearly half of them were involved in epigenetic mechanisms such as DNA methylation and histone modifications.[74] Epigenetics-mediated alterations in the expression of nonmutated genes may enhance or reduce tumorigenicity through both direct and indirect means, adding another layer of complexity in differentiating drivers and passengers.

The OncoArray Consortium performed extensive genome-wide association studies incorporating data of enhancer–promoter interactions and epigenetic marks, among other annotations.[75] They reported numerous single nucleotide polymorphisms associated with breast cancer risk, and many were located in regulatory elements, particularly for transcription factor binding sites.[76,77] Recent interrogation of the genome identified a total of 20,352 (potentially) protein-coding genes with approximately the same number of noncoding genes.[78] Furthermore, alternative promoters and alternative splicing, both pervasive throughout the genome,[79,80] significantly increase the number of distinct entities and isoform diversity. Epigenetic mechanisms have been shown to regulate alternative splicing[81,82] and, in certain cases, influence production of entirely different proteins. This remarkable phenomenon is illustrated by the *CDKN2A* gene locus from which two distinct tumor suppressor proteins, p16(INK4A) and p14(ARF), are produced from the same overlapping gene sequence by translating from an alternative open reading frame.[83]

Long-range interactions of gene control elements such as enhancers and promoters are mediated by higher order chromatin structure characterized by chromosomal territories, chromatin loops, and topologically associating domains arranged into hierarchical layers.[84–87] Comprehensive analysis of chromatin folding across a wide variety of tumors revealed DNA territories organized into epigenetically distinct domains; the folding patterns were found to be markedly disrupted in complex chromatin rearrangements in cancer cells.[88,89] Such structural changes are predicted to alter gene expression programs. Indeed, a large-scale genome-wide analysis identified over 2000 new gene fusions. Surprisingly, some of the fusions, termed "bridge" and "composite" fusions, were assembled from three or more genomic segments.[90] These studies provide insight into how the physical architecture of chromatin can impact the regulation of a large number of genes, hitherto invisible by DNA-only analytical techniques.

Small chromosomal fragments called "double minutes" have been observed in a large number of cancers, a result of catastrophic rearrangement events called chromothripsis.[91] Distinct from mitochondrial DNA, a variety of extrachromosomal DNAs have been characterized. Similar to prokaryotic plasmids, these DNA fragments vary in size and gene content, locate in the nucleus and cytoplasm, and are present in both normal and cancer cells.[92]

Larger chromatin fragments called extrachromosomal DNA (ecDNA) are circular, replicate with cell division, amplify encoded oncogenes, and contribute to tumor evolution.[93] Interestingly, the chromatin structure of ecDNAs was found to be more accessible than of chromosomal DNA, suggesting higher transcriptional potential.[94] Importantly, ecDNAs lack centromeres, resulting in random distribution of copies in daughter cells during mitosis.[95] These findings raise important issues for characterizing oncogene amplification since ecDNAs essentially function as autonomous entities uncoupled from the regulatory constraints imposed on chromosomes. Stochastic distribution of ecDNAs can generate intratumoral heterogeneity and present a major challenge for therapy. This is illustrated by a study of ecDNA containing an *EGFR* mutant gene in glioblastoma multiforme. Mutant *EGFR* gene was found almost entirely amplified on ecDNA, and EGFR-specific tyrosine kinase inhibitors (erlotinib and lapatinib) dramatically reduced copy number; however, copy number increased to a high level within 1–2 weeks after drug withdrawal.[96] Hence, biological response to therapeutic pressure can be markedly different for oncogenes that are ecDNA encoded. Clinical implications are profound, and continued work in this field is essential to better understand this phenomenon.

Noncoding RNAs and RNA Epigenetics

The cancer transcriptome reflects the underlying genomic and epigenomic changes characterized by changes in copy number of RNAs, alternative splicing, gene fusions, and/or combinations thereof. However, it has been well-known that the majority of cellular RNAs have functions other than encoding proteins. Well-known noncoding RNAs (ncRNAs) are essential components of ribosomes, spliceosomes, telomerases, and ribonucleases such as RNase P. Notably, the CRISPR-Cas9 system (clustered regularly interspaced short palindromic repeats-Cas9 gene editing system) also has an essential RNA component. Aside from these functional RNA entities, a growing number of ncRNAs ranging in size from approximately 20 to >200 nucleotides are being characterized with fascinating experimentally determined and proposed functions.[97,98]

Long noncoding RNAs (lncRNAs) constitute a significant portion of the total pool of RNA generated from the genome, approximated four times that of protein-coding RNAs.[99] Yet, their function remains largely unknown. A lncRNA called *HOTAIR* has been shown to increase breast cancer invasiveness and metastasis through epigenetic silencing of suppressor genes by recruiting of polycomb repressive complex 2 (PRC2), which has histone methyltransferase activity.[100] Clinically important, high overexpression of HOTAIR lncRNA (>125 fold) in primary breast tumors was a strong independent predictor of subsequent metastasis and OS.[100]

LncRNAs appear to function as gene expression regulators through the formation of ribonucleoprotein complexes.[97] A large-scale screen for lncRNAs using a modified CRISPR system revealed chromosomal features that suggest higher order chromatin structures are important for functional specificity.[101] Abundant smaller ncRNAs, called microRNAs (miRNAs), are known to affect protein expression and are themselves modified, reflective of extensive crosstalk with epigenetic regulatory pathways.[102]

Methylation of ncRNA on adenine and cytosine bases are increasingly recognized to be important in cancers.[103] Methylation and other covalent chemical modifications of noncoding and other classes of RNAs, termed "epitranscriptomics," is emerging as an active area of cancer research made feasible by new chemical probing and sequencing techniques.[104,105] The RNA-specific enzymes that catalyze the chemical modification, the so-called epigenetic writers, readers, and erasers, are similar to those for DNA.[106] This nascent field can add a new dimension to our knowledge of cancer biology and is poised to be a productive area of discovery.

Epigenetic Signatures as Cancer Biomarkers

Although PD-L1 overexpression in tumors is a key mechanism for immune evasion, its use as a biomarker is suboptimal as a predictor of ICI response.[107,108] ICIs represent a distinct class of therapeutics with evolving paradigms that are turning to genomics-based biomarkers such as TMB in an effort to improve patient selection.[109–113] Clinical studies with other immune checkpoint targets such as LAG-3,[114] TIM-3,[115] and TIGIT,[116] as well as the macrophage checkpoint CD47,[117,118] are currently under way. Whether their respective or related biomarkers accurately correlates with therapy response remains to be determined.

Recent work surveying the epigenetic landscape of certain tumors uncovered an intriguing mechanism across a wide range of gene promoters. Qamra et al[119] and Sundar et al[120] demonstrated that tumors expressed genes from different start sites relative to paired normal tissues, thereby producing RNA transcripts differing in length and base sequence. Alternative promoter usage is known to occur in about 50% of expressed genes in normal tissues.[121] Remarkably, the level of alternative promoter activity was found to correlate with ICI resistance in gastric adenocarcinoma. Subsequently, an extensive in silico interrogation of large transcriptomic datasets of >18,000 samples covering 42 cancer types showed that alternative promoter switching is pervasive, tissue specific, and correlative with survival.[122] These studies illustrate how tumor epigenetic states can significantly increase RNA isoform diversity from the same DNA template without obligate sequence mutations. In turn, the greater complexity in RNAs can create or expand the repertoire of tumor survival pathways. Hence, alternative promoter signatures may serve the role of a transcriptomic biomarker for epigenetic regulatory networks in cancers.

A few cancer-specific epigenetic tests have entered the clinic.[123] Epi proColon (Epigenomics AG, Berlin, Germany) is a blood-based test that detects methylated *SEPTIN9* DNA for CRC screening. This single epigenetic biomarker is observed in >90% of colorectal tumors, with minimal levels in normal tissues.[124] Although the assay was independently validated,[125] results from a clinical study, PRESEPT (NCT00855348) demonstrated suboptimal sensitivity particularly for early cancers and advanced adenomas.[126] An improved version of the assay was developed and approved by the FDA in April 2016, the first and only approved blood-based CRC screening test. The company has also developed a blood-based lung cancer screening test that detects methylated *SHOX2* and *PTGER4* genes and has received the Conformitè Europëenne (CE)-IVD mark in 2017; however, the FDA approval status is undisclosed. Another notable FDA-approved epigenetic biomarker assay is Cologuard, a stool-based test detecting the methylated genes, *NDRG4* and *BMP3* (Exact Sciences Corp., Madison, WI, USA) with fourth quarter 2019 revenue of $229 million.[127]

In a retrospective study, epigenetic profiling was shown to significantly improve the classification of tumors that were initially of unknown primary through a machine learning–based algorithm using DNA methylation patterns.[128] This technology was developed through a public-private partnership in Barcelona, Spain, and received CE mark for its microarray DNA methylation assay called EPICUP, based on a commercial microarray platform that interrogates >450,000 methylation sites in the human genome (Infinium Human Methylation 450 BeadChip; Illumina, Inc., San Diego, CA, USA). This example is noteworthy because it leverages two powerful technologies positioned to revolutionize precision oncology—high throughput next-generation sequencing (NGS) and machine learning/artificial intelligence—to accelerate the identification of disease patterns in large genome-scale datasets.

Expanding interest in targeted and immuno-oncology drugs will continue to drive biomarker discovery for disease detection, predicting response, and monitoring. Cost-effective next-generation screening tests for early cancer detection also remain in high demand. Companion diagnostics for targeted drugs is now commonplace with regulatory agencies. These trends point in the direction of precision medicine, as the one-size-fits-all model is gradually replaced with bespoke cancer treatments.

MAKING SENSE OF IT ALL

NGS, new gene interrogation techniques, and increased computational power have produced massive datasets that are beginning to pay dividends. The collective body of experimental and clinical evidence reveals a complex interplay between the molecular casts of a cancer cell interlaced with misreads and precarious improvisations of the original genetic script. Duly, the basic tenet of DNA mutations as the primary inciting event in tumorigenesis continues to be expanded and revised, together with evolving frameworks to conceptualize the molecular basis of cancers.

Base sequence changes are now characterized in the context of dynamic chromatin states orchestrated by precise chemical modifications of the epigenetic code. Global epigenomic and transcriptomic profiles of tumors are dominated by gene programs that reflect the normal tissues from which they originate.[122,129] Maintenance of cell identify—of both normal and malignant phenotypes—during and immediately after mitosis has been shown to be mediated by epigenetic mechanisms that act on specific chromatin loci, a process called mitotic gene bookmarking.[130] Hence, neoplastic transformation and tumor dedifferentiation are likely sequelae of epigenetic aberrations coupled to genomic instability in a vicious cycle. In contrast to sequence mutations, epigenetic signatures are in flux, provoked by external signals or environmental pressures. Although such plasticity is essential for normal development, tumors can also usurp these same mechanisms for survival in hostile environments created by the immune system or drug attack.[131]

RNA has recently enjoyed the spotlight as its role in virtually every cancer-related biochemical process is painstakingly annotated. Protein-coding and noncoding RNAs of widely varying sizes are no longer considered "intermediate" inert molecules but in fact represent essential components of the cellular regulatory machinery. Classically, the workhorse RNAs, such as rRNAs and tRNAs, were relegated to roles in protein synthesis, but ncRNAs are now recognized as integral chromatin regulators through direct interaction with histone-modifying nucleoprotein complexes. Indeed, these emerging roles of RNAs may partially explain the function of 99% of the human genome that does not encode proteins. Covalent modifications of RNAs greatly expand their biochemical capabilities, analogous to chromatin mechanisms, raising the notion of DNA-epigenetics as a molecular palimpsest of a prebiotic past.[132] This emerging field of epitranscriptomics, although nascent in scope, has already suggested a treasure trove of potential druggable targets for cancers.

The mechanisms described herein, albeit rudimentary, can be visualized as pieces of a jigsaw puzzle that assemble into a practical framework to conceptualize tumorigenesis (Figure 1). How cancer epigenomics will ultimately impact patients will depend on the effective translation of discoveries and insights. Momentum in this field is accelerating as more drugs are being developed and tested in concert with epigenetic modifying agents. Although the first-generation DNA-hypomethylating agents and HDACis had limited success, the clinical experience will guide future drug development programs for later-generation therapies with greater target specificity. Successful clinical translation will also

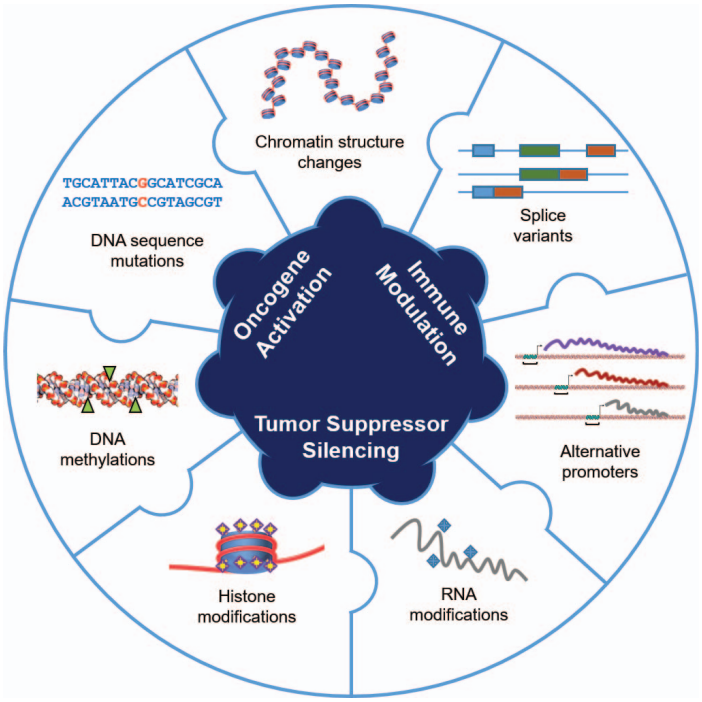

Figure 1.—Tumorigenesis model. Multiple genomic/epigenomic mechanisms that can independently or in combination lead to oncogene activation, tumor suppressor silencing, and/or immune modulation.

require judicious use of appropriate biomarkers for patient selection, carefully designed basket or umbrella trials, and monitoring approaches to not only assess response and toxicities but also to capture essential data for subsequent iterations. When NGS techniques can perform whole genomic/epigenomic sequencing routinely and cost effectively, comprehensive molecular profiling may provide a customized compendium of genetic pathways to target for each and every cancer patient. Together with the expanding armamentarium of new molecular drugs, precision oncology can be realized.

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