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Epigenetics Advancing Personalized Nanomedicine in Cancer Therapy

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Abstract

Personalized medicine aims to deliver the right drug to a right patient at the right time. It offers unique opportunities to integrate new technologies and concepts to disease prognosis, diagnosis and therapeutics. While selective personalized therapies are conceptually impressive, the majority of cancer therapies have dismal outcome. Such therapeutic failure could result from no response, drug resistance, disease relapse or severe side effect from improper drug delivery. Nanomedicine, the application of nanotechnology in medicine, has a potential to advance the identification of diagnostic and prognostic biomarkers and the delivery of right drug to disease sites. Epigenetic aberrations dynamically contribute to cancer pathogenesis. Given the individualized traits of epigenetic biomarkers, epigenetic considerations would significantly refine personalized nanomedicine. This review aims to dissect the interface of personalized medicine with nanomedicine and epigenetics. I will outline the progress and highlight challenges and areas that can be further explored perfecting the personalized health care.

Keywords

Epigenetics; Biomarkers; Targeted therapy; Nanotechnology; Nanoparticles; Drug delivery; Personalized medicine; Nanomedicine; Personalized nanomedicine

1. Introduction

Epigenetic changes (e.g. DNA methylations and histone modifications) are the heritable pattern of alteration in chromatin and hence genes [1]. While there is no impact on gene sequences, epigenetic modifications can specify gene expression patterns throughout differentiation and development. Thanks to the cooperation of epigenetic and genetic codes, a single fertilized egg with one set of genetic material can accurately develop into complicated biological systems, like tissues and organs with distinct morphology and functions. The crucial role of genetic-epigenetic crosstalk in physiology and pathology is also evident by the following key observations. The monozygotic (MZ) twins display huge differences in developing diseases when they grow up separately, and such disease potential is dramatically attenuated when they live under similar environment [2]. Furthermore,

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Conflict of Interest

The author states no conflict of interest.

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99.9% of the human genome is identical among the individual persons with only 0.1% uniqueness (http://www.ornl.gov/sci/techresources/Human_Genome). The interaction of this 0.1% unique genetic material together with epigenetic modifications generates vast diversity in the human population. In some cases, when the physiological balance is broken by genetic and/or epigenetic aberrations, it results in human illnesses (e.g. systemic cancerous lesions).

Traditionally, the patients are treated by a “one-size-fits-all” approach within a standard protocol. The treatment decisions are mainly decided basing on the clinical stage of the disease and the patient’s medical and family history, but ignoring the individual’s biology driving such diseases [3]. While these approaches did achieve remarkable therapeutic efficacy in curing certain cancers (e.g., testicular cancers, and pediatric leukemias) [4], the overall survival rate of cancer patients has improved little in the last decades, arguing for the need of novel ideas and strategies in disease management. The new treatment decision should integrate comprehensive disease biology, right therapeutic reagents and drug delivery vehicle into other personal attributes (age, sex, nutrition, psychological conditions and gut microbiome [5–7]). Such integration has brought the concept of personalized medicine that addresses the right drug to the right person at the right time.

Personalized medicine emphasizes the uniqueness of a person, a disease and a drug at specific time points whereby delivering individualized health care to patients with the maximum therapeutic efficacy at the cost of the minimal adverse effects. Personalized medicine has been widely and successfully applied to the management of numerous human diseases, particularly cancers. The best example of personalized cancer therapy comes from the identification and clinical testing of the estrogen receptor (ER) in breast cancer and BCR/ABL in CML. Despite the impressive clinical benefits from the individualized health care, the current chemotherapies still have dismal outcomes. In such cases, further disease characterization using suitable biomarkers (e.g. genetic and epigenetic alterations) may aid the clinicians to decide the best means to deliver the drug in order to provide the maximal efficacy. Among many biomarkers, epigenetic modifiers are the most essential players in the design of personalized medicine, because these markers are tightly correlated to individualized attributes. Additionally, barriers contributing to current chemotherapeutic failure could be secondary to suboptimal drug delivery. As such, the nanomedicine could overcome these pitfalls by specifically sending the drugs to the right place with likely reduced systemic toxicity. New nanotechnology (e.g. nano-imaging reagents and devices) can also assist the discovery of novel biomarkers from the limited materials in non-invasive ways thereby promoting the individualized health care. To that end, the true sense of personalized medicine should be the seamless integrations of medicine, nanotechnology and personal attributes, with epigenetic consideration placed at the central location. Certainly personalized nanomedicine will be theoretically beneficial to all types of patients, but this article mainly emphasizes its influences on the cancer management, given that cancer is the representatively systemic lesions which are the most lethal diseases worldwide.

2. Personalized medicine in cancer management

2.1. Concept of personalized medicine

Every single person is truly unique, and so are the cancerous lesions. Each person’s uniqueness includes clinical, genomic and environmental information, as well as the nature of diseases, including their onset, course of progression and response to treatment. Such uniqueness should be used to guide the disease management. Personalized medicine is individualized or individual-based-therapy, which takes into account the unique profiles of a single patient and a single disease. Through the discovery of genetic, genomic and clinical markers, personalized medicine allows us to accurately predict a person’s susceptibility of

developing disease, the progression of disease, the patient's response to therapy, and maximize the therapeutic index for treating or curing illness with the least toxicity for a specific patient [8, 9].

Personalized medicine also requires the integration of other individual physiological factors, such as age, race, sex, living environment, nutrition, genetic background, drug metabolism and health condition into therapeutic decisions. Utilization of such specific marker-based therapy can avoid unnecessary medication-associated toxicity and costs. For example, higher drug metabolism rate indicates the rapid clearance of drug from systemic circulation and requires continuous infusion for maintenance of therapeutic plasma concentration in these patients. Even with the similarity of intrinsic drug metabolism, the drug dose may need to be changed accordingly to the "personal" gut microbiome, which recently has been shown to significantly affect therapeutic efficacy [5–7]. Other examples come from cancer patients, where individual biomarkers become important to allow physicians to make decisions and deliver more personalized care to a specific patient. As an example, the classifications of lung cancer patients into non-small-cell lung and small-cell lung carcinoma are not sufficient to determine the best treatment. Physicians are taking into account these disease characteristics: tumor size, histology, stage, presence or absence of distant metastases, plus the patients' age and smoking history, and gene mutation status (e.g. KRAS, EGFR). Such individualized approaches with a focus on novel targeted therapies can take over the "one-size-fits-all" standard protocol for treating many other cancers [10]. The promising effects of personalized cancer therapy are also evident by the identification and clinical testing of the estrogen receptor (ER), PR and HER2 in breast cancer. About 31% patients increased 5-yr overall survival in ER-positive patients, however, no significant favorable outcomes were achieved from tamoxifen in ER-negative breast cancer. Moreover, the benefits of personalized medicine have been evidenced in other human diseases, including neuropsychiatric disorders, cardiovascular disease, neurodegenerative diseases, diabetes, and obesity. Overall, the performance of personalized medicine will help to make more informed and suitable medical decisions, execute possible early intervention by prompt disease detection, minimize the toxicity and healthcare costs by providing accurate treatment plan and avoiding unnecessary medication, foretell and monitor the disease progression thereby achieving higher probability of desirable outcomes.

2.2. Personalized oncology

The application of "personalized medicine" in cancer therapy is termed "personalized oncology". Taking into consideration the diversity of single tumor and cancer cells is one of the most important components in the development of personalized oncology. Cancer surpasses cardiovascular disease as the number one causes of death throughout the world. More than 1500 people died from all forms of cancer each day in USA in 2010 [11]. The tumor heterogeneity is the major hamper to accomplish effective cancer diagnosis and treatment, although lots of factors contribute to the chemotherapy failure. The massive spectrum of tumor types within a given cancer and the cell-to-cell discrepancy have been discovered within tumors [12]. Cancers even clinically displaying as the same type or subtype could potentially be very disparate at the molecular level and thus act distinctly in response to therapeutic interventions [13, 14]. Further, some individual primary tumors contain small cancer cell subpopulations (e.g. cancer stem cells) that vary in metastatic ability and treatment response. Cancer cells in the tumor tissue and circulating system can display different responsiveness to therapies. Specifically, breast cancer, lung cancer and colon cancer stand for multiple diverse disease entities with genetic and epigenetic variations. Hansen *et al* identified cancer specific DNA-methylated regions (cDMRs) distinguishing cancer from normal tissues in colon cancer, and recently they showed stochastic methylation variation of the same cDMRs in colon, lung, breast, Wilms' tumors

and adenomas [15]. Thomas *et al* applied high-throughput genotyping to interrogate 238 known oncogene mutations across 1,000 human tumor samples, and observed an unexpectedly high number of co-occurring mutations [16]. Stephens *et al* evidenced several types of somatic rearrangements in human breast cancer genomes, highlighting their potential contribution to cancer pathogenesis [17]. Such cancer heterogeneity nicely explains why it is unrealistic to develop “block-buster” cancer drugs that cure all patients with a given disease, and also requires that the effective chemotherapies should be delivered not only at the level of the individual patient and disease, but also a specific tumor or tumor cell, the central points of personalized medicine.

2.3. Biomarkers provide the fundamental guidance for personalized medicine

Molecular signatures or biomarkers of tumor or tumor cell are indicators of tumorigenesis, which is mostly determined by the interaction or coordination of diverse signaling pathways and a result of the combined tumoral, stromal and inflammatory factors of the original heterogeneous tumor. The molecular biomarkers include mutant genes, RNAs, proteins, lipids, carbohydrates and small metabolite molecules, whose expression levels are positively or negatively correlated to biological behaviors or clinical outcomes. Clinical biomarkers encompass diagnostic biomarkers indicating the presence or absence of a disease, and prognostic and therapy-optimization biomarkers indicating patient and disease characteristics that influence treatment plans. All these biomarkers are the “flags” to deliver the true sense of individual health care. They can be utilized separately, but the best practice is to apply comprehensive and unbiased biomarkers simultaneously to make the most suitable therapeutic regimens for a given patient.

2.3.1. Chromosomal abnormalities—Chromosomal abnormalities can appear when chromosomes break and the fragments relocate to other chromosomes. These breakpoints cause the loss of genetic materials or the alteration of gene microenvironment or the generation of novel fusion genes. The characterization of patient-specific translocations has revolutionized the personalized cancer therapy. For example, many structurally different types of chromosomal abnormalities have been defined in leukemia, including t(15;17)(q21;q12)--PML/RAR α , t(11;17)(q23;q12)--PZLF/RAR α , t(8;21)(q22;q22)--AML1/ETO, t(8;16)(p11;p13)--CBP/MOZ, t(11;23)(q23;q13)--MLL/p300, and inv(16)--CBF β /SMMHC. These genetic fusions change the biological function of the original genes, such as DNA binding affinity or the physical interaction with other transcriptional partners, like AML1/ETO interacts with DNMT1/HDAC1, instead of HAT, to suppress the expression of target gene *IL-3* [18]. Another famous example is BCR/ABL fusion gene, which is derived from the rearrangement of the breakpoint cluster region (BCR) on chromosome 22 with the c-ABL proto-oncogene on chromosome 9. BCR/ABL fusion gene is present in virtually all CML (>95% of cases) [19] and some ALL [20] patients and produces hyperactivated ABL-driven kinase signaling. Its inhibitors (e.g. Imatinib) have accomplished striking therapeutic efficacy in CML patients, representing one of the most fruitful performances of targeting translocation biomarkers in cancer therapy.

2.3.2. Gene mutations—Two main types of genes involved in cancer pathogenesis are oncogenes and tumor suppressor genes (TSGs). While most of the gene mutations don't implicate disease phenotypes, cancerous lesions start when cells accumulate genetic errors and multiply unlimitedly. TSGs with acquired mutations have been evaluated in many cancers, like lung, colorectal, breast cancer and leukemia. For example, the abnormalities of TP53 gene have been seen in more than 50% of human malignancies. Although very crucial to normal cell growth, numerous proto-oncogenes, when mutated, are hyper-activated leading to cancer pathogenesis. The discovery of disease-causing mutations in a family can foretell “at-risk” individuals whether they are susceptible for cancer or other diseases [21]

and how they will response to certain medications, thereby prompting individualized therapy. Mutations of some well-known oncogenes include DNA methyltransferases (DNMT3a [22], DNMT1 [21, 23]), protein kinases (e.g. EGFR, KIT and FLT3, ErbB2) and GTPase (e.g. KRAS). The identifications of KIT and FLT3 mutations as well as the application of their specific inhibitors to the relevant patients have advanced the personalized cancer management. In the clinics, specific KIT mutation in codon 822 can predict the response to imatinib, whereas mutations in codon 816 are not and can be targeted successfully with midostaurin or dasatinib [24]. Specific mutations in the EGFR gene as predictors of response to erlotinib or gefitinib is the first example of using biomarkers to predict response to targeted agents [25]. Further, increasing evidence demonstrates the non-specific induction of gene mutations by chemotherapy. Discovery of chemotherapy-induced gene mutations can help develop special inhibitors and also predict how the given patients will response to the selected drugs [25], an important concept of personalized medicine. To this end, all the treatment and therapies are tailored to target the unique spectrum of mutations that define individual tumors and tumor cell subpopulations [12, 26–28].

2.3.3. Single nucleotide polymorphisms (SNPs)—Single nucleotide variations (SNPs) occur at a specific site in coding and noncoding regions of the genomic DNA. SNPs are taking place every 100 to 300 base pairs along the human genome and making up about 90% of all human genetic variation. In the past two decades over 10 million SNPs have been discovered [29]. While most SNPs have no obvious impacts on critical biological processes, some of them did implicate disease phenotypes or affect patients' response to a drug thereby representing favorable biomarkers for diagnosis and prognosis. For example, Irinotecan is a form of chemotherapy for colon carcinoma patients. A single SNP governing irinotecan metabolism can predict whether this patient subgroup responses to irinotecan therapy. SNPs in BRCA1 and TP53 genes associate with increased risk for breast cancer, whereas other SNPs, like rs2352028 at chromosome 13q31.3, indicate higher susceptibility for lung cancer in never-smokers [30]. Another example is the polymorphic nature of the cytochrome P450 (CYP) genes, including copy number variants (CNV), mutations, insertions and deletions, which significantly affect individual drug response. Thus employment of CYP polymorphism biomarkers in clinics can improve drug response and diminish treatment costs [31].

Recently SNPs have been identified in microRNA (miR) genes, their processing machinery and target binding sites, which function as another indicator for cancer risk, patient prognosis and treatment efficacy. miRs are regulatory non-coding RNA of ~22 nucleotides that regulate gene expression by hybridization to the mRNA 5'UTR or 3'UTR or even the coding region [32]. Abnormal miR expression causes various human diseases and miR expression signatures can uniquely classify cancer types [33]. SNPs of miRs could significantly modulate their biological activities thereby representing attractive biomarkers for making clinical decisions. Overall, it is increasingly clear that SNPs will be an important enabler to design and develop personalized medicine. Because of SNPs' clinical potential, several groups, like U.S. Human Genome Project (HGP) and SNP Consortium, are creating human genome SNP maps, in order to identify useful prognostic and diagnostic biomarkers. Knome is providing complete genome sequencing to consumers who look for a personal genetic profile [34]. Other companies (23andMe, deCODE Genetics and Navigenics) are utilizing high-density SNP array to dissect the genetic information of an individual person. Currently "23andMe" can determine a person's SNPs for less than a tenth of the cost, when compared to genome-sequencing. However, the largest barrier for SNP clinical application in individualized medicine is the "Genetic Discrimination" issue.

2.3.4. Circulating DNA, RNA and tumor cells—In our blood system circulates various types of RNA and DNA in the form of free or wrapped in red blood cells and platelets or

nucleosomes. These oligos are released by apoptotic and dead cells or cancer and regular cells as messengers, which is evident by the transformation function of these oligos from cancer patients. Circulating DNA was first demonstrated in human blood from healthy donors, pregnant women and clinical patients in 1940s, but no interest was developed until high DNA levels were found in the blood of patients with systemic lupus erythematosus and also cancer. These DNAs could be used (a) as an early marker for cancer detection due to its elevated level and (b) for monitoring therapeutic efficacy when their levels back to normal.

miRs can activate [35] or suppress target gene expression and are the largest class of gene regulators. In circulating blood, differential expression of miRs between normal people and patients has been evidenced. For example, the level of *miR-155*, *miR-210* and *miR-21* in B-cell lymphoma patients' serum is elevated [36]. Mitchell *et al* reported that serum levels of *miR-141* can distinguish patients with prostate cancer from healthy controls [37]. These circulating miRs are emerging as important non-invasive biomarkers (reviewed in [38]), which can benefit the diagnosis and prognosis, even function as a flag for therapeutic efficacy. Additionally, in the bloodstream circulates tumor cells, which are derived from a primary tumor with the potential to grow in another part of the body. Such circulating cancer cells (CTCs) can provide clinicians with a more advanced non-invasive way to find out the stage of cancer metastasis, the individualized approaches for patients, and the way to monitor disease progress. In general, the aforementioned biomarkers (DNA, RNA, miR and CTC) are distinct between cancer and normal cells, even the cancer cells at different stage of disease progression. Such diversified biomarkers offer a crucial platform to understand the etiology and pathology of cancers with a strong support to personalized medicine.

2.3.5. “omics” data offer the most comprehensive information for the design of personalized medicine—Although the identifications of protein kinase mutations led to the fruitful targeted therapy, the survival rate of cancer patients has increased little over decades. These traditional *in vivo* and *in vitro* assays are capable of measuring only a few parameters at best thereby providing a mere snapshot of the possible effects of a given drug. To overcome such high rate of drug failure, what we need is the customized therapeutic care based on the comprehensive genetic and epigenetic biomarkers, which can only be successfully uncovered by “-omics”, including genomics, transcriptomics, proteomics, metabolomics and epigenomics, through the utilization of high throughput technologies (e.g. high-throughput sequencing in a genome-wide scale [39]). “omics” technologies can discover and characterize tumor cells, tumors and person-specific biomarkers that will advance the individualized therapy [40]. Recently, Feinberg *et al* run the unbiased genome-scale analysis of ~4 million CpG sites among 74 individuals with comprehensive array-based relative methylation assay, and found 227 regions showing huge inter-individual variability (variably methylated regions, VMRs) across the genome. Half of these VMRs were stable among individuals over an average of 11 years and these VMRs defined a personalized epigenomic profile [41], which can be used as diagnostic biomarker in individualized health care. Moreover, drug-specific modulation of oncogenic pathways in patient subpopulations can predict response to targeted therapy. For example, Andersen *et al* used a pathway-based phosphor-profiling approach to identify and quantify clinically relevant, drug-specific biomarkers for phosphatidylinositol 3-kinase (PI3K) pathway inhibitors. They found that 71 out of 375 non-redundant PI3K pathway-relevant phosphopeptides were drug-related and 11 of them by all selected-inhibitors [42]. These drug-specific markers could be used for the development of patient-tailored therapies. Further, analysis of four colorectal and two breast cancers with massively parallel sequencing revealed an average of nine rearranged sequences (range 4 to 15) per tumor [43], indicating that consideration of the uniqueness of a single tumor is crucial for the successful personalized medicine.

However, the application of omics data in personalized cancer therapy is very challenging. While numerous “omics” data have been generated from patient samples and cell lines, high-quality “omics” data, which can produce meaningful results, are lacking [3]. The physiological conditions in tumors and persons are dynamics and may change over time. Even the most advanced techniques can only yields a snap shot of the disease, thus decreasing the clinical value of “omics” data. The capability and the procedures to interpret “omics” data are also disputable. Therefore, very few have been translated from bench to bedside, although plenty of “omics” data have been generated. This is why, instead of using “omics” data in making treatment decision, currently physicians prefer to adopt “simple” biomarkers, such as kinase mutation, ICH staining, hormone receptor expression or/and chromosomal aberrations.

2.4. The challenge of personalized medicine

Personalized therapy offers great opportunities to maximize therapeutic benefits by targeting the genomic or epigenomic abnormalities while minimizing the toxicity at the same time by avoiding the treatment in non-responders. Despite the impressive achievement in cancer management, personalized medicine faces numerous challenges including the unexplained high failure rate of targeted therapeutics and the difficulties in discovering and validating biomarkers [44, 45]. Personalized medicine is also challenged by the difficulties in quantifying some personal factors (e.g. living environment and psychological conditions), “the \$1000 genomes, the \$100,000 analysis” problem [46], the ways to accurately deliver the drugs to the action sites, the capacity to dissect two morphologically identical tumors, as well as the heterogeneity and various biologic processes in solid cancers, including angiogenesis, tumor microenvironment and dormancy. The solutions to these problems will definitely help clinicians to design patient-specific treatments basing on the unique signatures of patient’s tumor at given stage of disease progression.

3. Nanotechnology in personalized medicine

3.1. Concept of nanomedicine

While mortality has reduced recently due to the advanced understanding of cancer etiology and pathology as well as the improved diagnostic devices and treatments, cancer remains the world’s most lethal diseases. Current diagnostic and prognostic classifications do not precisely reflect the clinical heterogeneity of tumors and are insufficient to make predictions for patient outcomes [47–49]. The current available regimens for cancer management, including surgery, radiotherapy and chemotherapeutic drugs, are non-selective with unacceptable toxicity. There is no available therapeutic window to come out further benefits to the patients who have high risk for early relapse or advanced multidrug resistance and refractoriness. Addressing these challenges requires identification of novel anti-cancer agents, and importantly selection of right drug delivery carriers (e.g. nanoparticles) conjugated with correct individualized ligands or biomarkers from patients.

Nanomedicine, referred by the National Institutes of Health, is the application of nanotechnology in medicine. These applications include the drug delivery, disease detection, diagnosis, and treatment. The disease areas include cancerous lesions [50], cardiovascular system, lungs, blood, neurological diseases, diabetes, inflammatory/infectious diseases, Parkinson’s or Alzheimer’s disease, orthopaedic problems and so on. The types of nanoparticulates utilized are 1) biological mimetics and functionalized carbon tubes, 2) nanofibers and polymeric nanoconstructs, 3) nanoscale microfabrication-based devices and silicon microchips for drug release, 4) nanomachines made from DNA parts and DNA scaffolds, and 5) nanoscale particle technologies for delivering drug, genetic materials and diagnostic agents [51].

3.2. Therapeutic application of nanoparticles

Nanocarriers are classified into passively and actively targeting particles. Passively targeting nanocarriers accumulate in the tumor by enhanced permeability and retention effect. These particles were approved for clinical practice in 1980s and marketed in 1990s. In contrast, actively targeting nanocarriers are conjugated with molecules binding to antigens or receptors overexpressed in tumor cells. The individualized biomarkers could enhance the specificity of targeted nanocarriers in a given patients.

3.2.1. Role of nanoparticles in drug delivery—In cancer chemotherapy, most drugs do not greatly differentiate cancerous cells from normal ones, leading to systemic toxicity and adverse effects in other tissues, including bone marrow suppression, cardiomyopathy and neurotoxicity. No selection of drug targets significantly constrains the maximal allowable doses *in vivo*. Nanoparticles can specifically transport complex cargoes to the metastasis sites, such as lungs, liver and lymph nodes, and target specific cell populations within these organs when conjugated with disease or patient-relevant biomarkers [52]. To this end, nanoparticles have been used to override several pitfalls of conventional drug delivery systems, such as nonspecific biodistribution and targeting, lack of water solubility, poor oral bioavailability, and low therapeutic index which influence both cancer and normal cells, limitation of the achievable dose in tumor, strong serum binding affinity (e.g. about 80% of bortezomib binds to plasma protein, <http://www.drugs.com/ppa/bortezomib.html>) and faster clearance in the circulating system. As chemo-drug delivery vehicles, nanocarriers can 1) improve the therapeutic efficacy of anti-cancer drugs, since the optimized size and surface characteristics can increase their circulation time in bloodstream, 2) selectively deliver drugs to cancer cells in passive accumulation through their enhanced permeability and retention effect, 3) in active targeting, selectivity can be enhanced by the conjugation of tumor specific ligands or antibodies, and 4) overcome drug resistance through escaping from P-glycoprotein, one member of adenosine 5'-triphosphate-binding cassette (ABC) transporter family. As an example, monoclonal antibody nanoparticles can enhance diagnosis and cancer therapy by targeting drug to tumor specifically and sparing the surrounding normal tissues. Nanochannel electroporation is able to deliver well-defined amounts of materials (e.g. miRs and siRNA) into a single living cell [53]. While the application of this nanochannel electroporation is still *in vitro*, it has great potential to target the small population of cancer stem cells in a given tumor, the most difficulties in personalized cancer therapy.

3.2.2. Application of nanoparticles assists biomarker discovery—Nowadays various nano-therapeutics have reached patients, including anticancer drugs and imaging agents, but there are only few clinically approved nanocarriers that incorporate molecules to selectively bind and target cancer cells [54]. Individual biomarkers are the keys to successfully develop and employ personalized therapies, because biomarkers are crucial to distinguish individual patient or tumor tissue or even a single tumor cell at a given stage of disease development and predict the clinical outcomes. It's highly difficult to identify clinically meaningful biomarkers from a given patient, specifically with barely available or detectable materials. In such cases, nano-particulate systems may be very useful in uncovering prognostic and diagnostic markers, detecting circulating tumor cells, monitoring therapeutic efficacy in non-invasive way. For example, nano-materials (e.g. biocompatible and stable fluorescent silver/dendrimer nanocomposites) are suitable for labeling cells *in vitro*. Quantum dots as molecular labels have potential to discover the earliest signs of cancers. Careful selection and design of nanoparticle surface can specifically condense a subset of biomarkers [55, 56] and sequester them for further investigations by high-sensitivity "omics" tests. Nanobiosensors can improve cancer biomarker discovery by sensitively detecting multiproteins in various clinical samples [57]. Magnetic nanoparticles

can capture circulating tumor cells in bloodstream [58] thereby promoting tumor detection. Overall, we expect that, by means of novel nanotechnology, the detection of sparse biomarker will become true *in vivo* even when the tumor growth is still undetectable by typical techniques.

3.2.3. The challenge of nanomedicine—Although nanomedicine has brought considerable advances in fighting cancer regarding to the diagnosis, prognosis and therapeutics, it is still at the early stage of development and meets various challenges. For example, there are no efficient ways to determine the body distribution of nanoparticulate carriers after system administration, and no suitable imaging modalities to vision the biodistributions over time [59], although radiological imaging can track and quantify particle distribution [60]. Nanoparticles are toxic to several organs [61–66], particularly those particles with little solubility or no biodegradation at the accumulation location. Regarding to the FDA-approved drug-containing nanoparticles, while liposome-encapsulated doxorubicin has brought striking clinical benefits with reduced toxicity (e.g. cardiotoxicity), its therapeutic efficacy against the tested tumors was unsatisfactory. PXL remarkably increases the efficient of hydrophobic cancer drug delivery, but there is no obvious enhancement in survival rate. There are multiple hurdles contributing to such nanomedicine failure, but non-integration of individualized factors (drug metabolism rate, epigenetic biomarkers, and tumor heterogeneity etc.) may speak really loudly here.

3.3. Application of nanotechnology in personalized therapy

Personalization of cancer therapies relies on a better understanding of the disease at the molecular level, including the mechanisms of cancer initiation and invasion, the identifications of prognostic and diagnostic biomarkers as well as the development of novel therapeutic agents. Nanotechnology can create multi-functional platforms, which advance the anti-cancer drug discovery and delivery, the identification of individual “omics” information [67], and the monitoring of disease course and treatment outcomes. However, without combining nanotechnology with personalized attributes – “personalized nanomedicine”, we would expect the chemotherapeutic failure to be continuously arisen from such independent practice. The uniqueness of nanoparticulates is their multi-functionality. Biotargeting ligands (e.g. antibodies, peptides or small molecules), imaging labels, therapeutic drugs and others can be integrated into a single nanoplatform, which can maximize the therapeutics by specifically delivering drugs (even drug cocktails) to the action sites, identifying diagnosis/prognosis biomarkers and monitoring disease progress after chemotherapy. Such multi-functional nanoplatform nicely fits the features of personalized therapy: early detection, combination of diagnostics with therapeutics.

To date, nanotechnologies have already brought benefits to the personalized treatment. Nanotechnology can efficiently and specifically deliver biological therapies, such as cells and genes that come from a given patient. Some nanoparticles and nanodevices (e.g. nanobiosensors and nanobiochips) have been conjugated with patient-specific ligands targeting special tumor cells to improve clinical efficacy [68], and such nanoparticle-based drug delivery plus the right dose and time is required by successful individualized medicine [69]. Molecular imaging is an important basic technology for personalizing health care. Novel molecular imaging tools (e.g. superparamagnetic contrast agent and perfluorocarbon nanoparticles) will enable non-invasive characterization of patients for delivering personalized therapy [70]. For example, $\alpha v\beta 3$ -targeted paramagnetic nanoparticles have been used to noninvasively detect very small regions of angiogenesis associated with nascent melanoma tumors [71] and deliver higher doses of drugs directly to the tumor site without systemic toxicity. The aforementioned achievements offer opportunities for the progression of personalized oncology in which cancer detection, diagnosis and therapy are

tailored to each individual's molecular signature thereby maximizing efficacy, but sparing patients unnecessary toxicity.

4. Potential role of epigenetics in the design of personalized nanomedicine

4.1. Epigenetic deregulations cause human cancer

4.1.1. Concept of epigenetics—Genetic alterations (e.g. deletions and mutations) could lead to the abnormal signaling pathways and sometimes drive illnesses. This concept provides a sound rationale for pathway-based drug discovery, in which the targeted therapy is built to against both mutant oncoproteins (KIT, BCR/ABL) [72–74] and wild-type effectors [75] residing upstream or downstream of the dysfunctional pathways. In contrast, epigenetics is dealing with the modifications of DNA and proteins (e.g. core histones). The term “Epigenetics” was first used by British biologist, Conrad Waddington, in 1950s to explain why some “genetic” changes do not develop new phenotypes, and how genes and their environments cooperatively determine the phenotypes. Today epigenetics is referred to the heritable patterns of changes in chromatin and gene expression, but no impact on DNA bases [1, 76]. Although no change in genomic codes, epigenetic modifications (e.g. DNA methylation) are inheritable throughout successive cell divisions and can even be passed onto the next generation [77]. Aberrant epigenetics causes numerous human diseases, but these modifications are reversible representing promising targets for therapeutic interventions. As such, epigenetics has become one of the most attractive fields in the current biomedical research landscape since the inception of epigenetics in the past century.

4.1.2. Environmental and intrinsic signals critically influence epigenetics—

What factors determine epigenetic balance? It is reported that environmental and lifestyle-related influences, such as nutrition, toxins and drugs as well as exposure to stress, can induce epigenetic changes, indicative of epigenome as a biochemical record of relevant life events [76, 78–80]. Special environment, like maternal epigenetics and maternal behavior, also impacts epigenetic variations [81, 82], which is strongly supported by the observed aging-related epigenetic changes [83, 84] and the discoveries showing that monozygotic twins have greater differences in global DNA methylation in older than in younger. Such divergence of epigenome in monozygotic twins is decreased when they are living in similar environment [2]. Specifically, the enzymatic activities, which catalyze histone modifications, are governed by environmental and intrinsic stimuli [85]. Such stimuli-induced histone modifications can remodel the chromatin and yield short-term or/and long-term outcomes [86].

Noticeably the change of environmental and intrinsic stimuli for a given person is frequent and dynamic. The dynamic environments create the dynamic epigenetic modifications changing over time, which are demonstrated by the observation showing that an 11-year span of global DNA methylation alteration in participants of an Icelandic cohort, and age- and tissue-related changes in some CpG islands from an array of 1413 arbitrarily chosen CpG sites near gene promoters [84, 87]. The dynamic cooperation of all epigenetic modifications remodels the chromatin structure in a specific region to turn on/off gene expression [88] at a given time and environment. Such personal environmental/epigenetic influence should be incorporated into the treatment decisions in personalized medical practice.

4.1.3. Epigenetic codes—Eukaryotic nucleosomes, consisting of a histone octamer (two H2A, H2B, H3 and H4) wrapped by 147 base pairs of DNA fragment, are the fundamental repeating units of chromatin and carry epigenetically inherited codes through covalently modified histones and DNAs. Epigenetic codes mainly have histone modifications and DNA methylation. Some studies [89–91] also include microRNA, other non-coding RNA and

even nucleosome positioning, but I view these as upstream regulators or downstream factors of epigenetics. Histone modifications occur mainly on their N-terminal tails, including acetylation, methylation, phosphorylation, sumoylation and ubiquitination. The enzymes involved are HATs, HDACs, HMTs, HDMTs, kinase, phosphatase, ubiquitin ligases, deubiquitinase, SUMO ligase, protease and so on [92, 93]. Integration of all histone modifications constitutes the “histone code”, which modulates diverse biological processes including gene regulation, DNA repair and chromosome remodeling. In contrast, DNA methylation represents a covalent chemical modification of DNA without disruption of DNA sequence. In the presence of S-adenosyl-methionine (SAM) that serves as a methyl donor, a methyl group is added to C-5 position of cytosine residues generating 5-methylcytosine. DNA methylation critically influences X chromosome inactivation, genome imprinting, transposons silencing and gene transcriptional regulation [94]. However, the expression profile of a given gene is the consequences of the dynamic cooperation among DNA methylation and histone modifications.

4.1.3.1. Aberrant HDAC activities drive cancer: Among the numerous posttranscriptional modifications, acetylation and deacetylation are the most extensively studied histone changes, and typically catalyzed by HAT and HDACs, respectively. In general, histone deacetylation results in chromatin condensation with the consequence of gene silencing, in contrast, histone acetylation leads to chromatin relaxation and gene reactivation. In line with these, abnormal HDAC expression or activity has been shown to be the driving forces in the development of human malignances [95], because aberrant HDAC activities could condense the chromatin and silence TSGs, resulting in unlimited cancer cell proliferation.

On the basis of sequence identity and domain organization, HDACs are classified into four classes [96], class I (HDAC1, 2, 3 and 8), class II (HDAC4, 5, 6, 7, 9, 10), HDAC III (sirtuins in mammals, SIRT6) and HDAC IV (HDAC 11). Classes I, II and IV are referred to as “classical” HDACs representing promising anti-cancer drug targets [95]. Nowadays there are numerous HDAC inhibitors (e.g. SAHA, Valproic acid, TSA etc.) being studied *in vitro* and in the clinics (reviewed in [95]). These inhibitors block HDAC activities, remodel the chromatin and turn on the TSG expression, thereby leading to a broad spectrum of anti-cancer activity. However, the HDAC inhibitor trials as monotherapy have showed severe toxicities and failed to induce a long-term response. These dismal outcomes could result from the dynamic coordination of distinct histone modifications with DNA methylation as well as the cooperation of different histone modifications themselves, which is a sound rationale for the combination of HDAC and DNMT inhibitors. Moreover, other contributors to the therapeutic failure of HDAC inhibitors could be the tumor heterogeneity, in which the elevated expression of class I or II HDAC isoforms associates with improved prognosis and predicts better clinical outcome in specific-tumor entities [97]. As such, the effective strategies involving in HDAC inhibitor trials should be to pinpoint the most relevant HDAC molecules in a given tumor from a specific patient and select the appropriate compound for mono- or combinational therapy, a concrete application of personalized medicine.

4.1.3.2. Abnormal DNA methylation in cancer: DNA methylation is under control of DNMTs, including DNMT1, DNMT1b, DNMT2, and DNMT3a and DNMT3b [98]. Among those, DNMT1 is the maintenance enzyme in dividing cells and preferentially replicates already existing methylation patterns, but DNMT3a and 3b are *de novo* enzymes responsible for establishing *de novo* methylation. Although the mechanisms leading to DNA hypermethylation remain to be fully explored, accumulative evidence demonstrate that all DNMT isoforms cooperatively determine DNA methylation status [99–101], since knockdown of one isoform only did not cause the complete loss of methylation in mouse stem cell [102] and colon cancer [103]. Further, mutations of DNMT1 and DNMT3a have been found to reduce DNMT activities [21, 22]. Interestingly, ten-eleven-translocation

(TET) family, a fusion partner of the MLL gene in AML, has been found to play critical roles in determining DNA methylation status [104, 105]. This gene family has three members: TET1, TET2 and TET3, which catalyze the conversion of methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), and knockdown of TET1 by siRNA led to the decreased 5hmC [106]. It is also reported that isocitrate dehydrogenase (IDH) 1/2 mutations in AMLs are associated with global DNA hypermethylation [107], arguing the potential regulatory role of IDH in DNA methylation machinery.

So far strong correlation between epigenetic aberrations and cancers has been established [108–110]. On the one hand, increased levels of DNMT1 and DNMT3a/3b have been observed in human malignancies (e.g. lung cancer [91], leukemia [111], breast cancer [112]), indicative of DNMT overexpression in driving tumorigenesis. On the other hand, tumors often exhibit global DNA hypomethylation, in conjunction with regional hypermethylation at CpG islands [113]. Hypermethylation of CpG islands on the promoter region silences gene expression [114] and usually occurs at the early stage of tumorigenesis, indicative of early cancer detection, especially in patients with inherited genetic jeopardy [115, 116]. Almost 50% of the genes have been shown to go through methylation-mediated silencing in various cancer [117]. The type of genes regulated by CpG hypermethylation include TSGs, cell cycle related genes, DNA mismatch repair genes, hormone receptors and tissue or cell adhesion molecules. As an example, the TSGs, like FHIT [118], WWOX [118, 119] and RASSF1A [120] in lung cancer [91, 111], p15 and estrogen receptor [121] in leukemia, are hypermethylated. TSG suppression offers proliferative benefits to tumor cells. It is also clear that DNA methylation plays an important role in the generation of mutations in human tumors, which is evident by the observation that the high occurrence of C-to-T transitions found in p53 TSG is attributed to the spontaneous deamination of 5-methylcytosine residues. Since epigenetic modifications could mark individualized environmental effects and mediate genetic alterations [122], epigenetic consideration would significantly advance personalized cancer therapy.

4.1.3.3. Deregulated epigenetics in non-cancer diseases: Epigenetic modifications regulate phenotypic variations in both health and disease, providing an important window to understand how environment-genome crosstalk controls health and initiates diseases. Environmental factors (intrinsic and outside stimuli) dynamically change DNA and histones leading to new phenotypes as a result of the altered gene and miR expression [123]. In turn, the dynamic epigenetics ensure a correct response of individual to a frequently changing environment [123]. When this dynamic balance is lost, illness occurs. Such epigenetic-driven disease will cause further alteration of other genetic and epigenetic signaling, resulting in numerous human disorders, including autoimmunity-related diseases, heart failure [124], rheumatoid arthritis and systemic lupus erythematosus [125], diabetes [126], mental disorders such as schizophrenia [127], osteoarthritis (OA) [128] and others [41]. Similar to cancer, the molecular mechanisms under these epigenetic-driven “common” diseases are the abnormal expression of disease-specific genes or miRs [128, 129] resulting from aberrant DNA methylation and histone modifications. These deregulated genes or miRs impact not only the disease development, but also the response to chemotherapy. For example, through regulating the expression of drug metabolism and transport genes (e.g. P450), DNA methylation status creates inter-individual variation in drug responsiveness [130]. Therefore, successful overcome of these diseases relies on the discovery of epigenetic biomarkers and the application of appropriate inhibitory compounds in a disease and patient-dependent manner.

4.2. Epigenetic biomarkers in individualized cancer therapy

Cancers usually display global DNA hypomethylation, specific CpG hypermethylation and global aberrant histone modifications. While the human illnesses are driven by the dynamic crosstalk between DNA methylation and core histone modifications, currently the development of epigenetic biomarkers mainly focuses on DNA methylation (reviewed in [80]), because of the well-established role in cancer pathogenesis and the ease to be quantified by means of current techniques. DNA methylation is the orchestrated consequence of many individual attributes and an important biological mediator of environmental effects on human diseases [131]. Very sensitive techniques have been developed for the measurement of DNA methylation [132, 133], including PCR or HPLC-based quantifications. Although it is dynamic over time, some scientists support the concept of inherited DNA methylation pattern and believe its variation across individuals [134]. Regardless, on the one hand, the regions (e.g. VMRs [41]) that are not changed over time could be used as an epigenetic signature for an individual, similar to genotype, and can be considered as candidates for assessment of methylation associated disease [41] and developing personalized epigenomics in medicine. On the other hand, the regions that do not change over the stage of cancer pathogenesis could be the right biomarkers for specific tumor type in a given person. Moreover, radiation- and chemo- therapies, to a given patient, are very important epigenetic modulators and will result in the altered gene expression, gene mutations, protein modifications, chromosome instability, and noticeably therapy-related leukemia [135–137]. To this end, pharmacoeigenomics, although a new field, is emerging to hold promise for personalized medicine, not only in cancer [138], but also other common diseases (Type 2 diabetes mellitus [139], psychoses [76]). It is defined as the “epigenomic manipulation of the genome to enable the development of more effective medications tailored for an individual’s genome” [140]. Pharmacoeigenomics covers all fields of clinical medicine [140] and is creating a platform to address the inter-individual variations in drug response, dissect drug-induced gene expression signature, predict drug side effects in preclinical trials and discover novel drugs ([126] and reviewed in [169]).

Regional CpG hypermethylation has been utilized as tool to diagnose cancer in patients using biological fluids and tissue biopsies [141, 142]. Cancer specific hypermethylation can be evidenced in the serum of cancer patient [143], and such markers have been clinically utilized in different types of human malignancies [144–146]. For example, the best DNA methylation marker for cancer detection and the one most likely to succeed as an epigenetic biomarker is the hypermethylation of glutathione S-transferase gene (*GSTP1*) in prostate cancer, which was used to help distinguish between prostate cancer and benign lesions [116, 147]. The detection of *GSTP1* hypermethylation in urine [148] and serum [149] strongly support the clinical application. However, both hypo- and hyper-methylation have been observed at various loci and stages in cancer diseases. These observations argue for the difficulties and complexities to figure out whether these epigenetic changes are driving forces for pathogenesis or the consequence from the altered physiology in cancer cells. Thus, the application of epigenetic biomarkers in curing cancers (e.g. colorectal cancer) has to be cautious due to the inconsistent and inconclusive results from methylation-based trials [138]. Under some circumstances, global hypomethylation and hypomethylating agents can promote tumorigenesis [126], due to the reactivated oncogenes, supporting the urgent need of all-inclusive epigenetic biomarkers with equally counting on both TSGs and oncogenes.

4.3. Hypermethylation-targeted therapy

The promoter regions of approximately 50% of human genes contain one or more CpG islands [150], once methylated, gene silencing occurs. Each tumor type has a unique profile of hypermethylated CpG islands [151, 152]. Given the reversible nature of epigenetic modifications [153], aberrant DNMT activities are the most attractive targets for both

biomarker development and anti-cancer drug generation. The aim of targeting DNMT activities or “epigenetic therapy” is to reverse epigenetic silencing and reactive various TSGs *in vitro* and *in vivo* to induce a therapeutic effect, like cell differentiation, growth arrest or apoptosis [154]. Azacitidine (Vidaza) and decitabine (Dacogen), the DNMT inhibitors, were the first successfully developed and FDA-approved epigenetic drugs in myelodysplastic syndromes (MDS) and being investigated in AML [155–157]. These were followed by FDA-approved HDAC inhibitors, vorinostat (Zolinza) and romidepsin (Istodax). There are other “epigenetic drugs” currently being used in clinical trials or under various stages of preclinical development. To date, epigenetic therapy has achieved impressive therapeutic efficacy and numerous patients have complete remission, particularly in leukemias [158].

However, epigenetic-targeted therapies face many challenges, including inefficient drug delivery, lack of predictive biomarkers, unexplained mechanisms of sensitivity and resistance, and few responses in solid cancers [159–161]. Because there are no suitable biomarkers available to detect cancer cell metastasis and invasion, over 60% of cancer patients have hidden or over metastatic colonies when they show up at the clinics. At this stage the tumors display the highest heterogeneity, thereby no suitable therapeutic regimes are available. Further, the common chemotherapy, like decitabine, suffers many pitfalls, such as low stability in current delivery vehicle [162], cell cycle-dependent activity [163], low efficiency of DNA incorporation [164], lack of obvious inhibitory effect on DNMT3a and DNMT3b [164, 165], and the development of drug resistance [166, 167]. Among those, I believe that drug delivery efficiency is the most critical barrier to effectively translate the leukemia accomplishment to solid cancer [161].

5. Conclusion and future perspective

The main emphasis of this article is rendered to the interface or the coordination of personalized- and nano- medicine taking into account epigenetics in cancer management. Such selection is originated from the unique feature and requirement of personalized health care, the only strategy that can be used to cure cancer. Today, individualized medicine, nanomedicine and epigenetic targeted therapy, mainly from independent practice point of view, have been broadly employed in clinics. While remarkably therapeutic efficacy has been accomplished, overall survival of cancer patients has changed little. Certainly multiple factors contribute to such discouraging outcomes, but the chemotherapeutic failure could be mainly as result of the influences of “one-size-fits-all” standard protocol. As such, the ideal therapeutics relies on the maximal consolidation of personalized therapy into nanomedicine and epigenetics, applying the right drug and dose with appropriate delivery vehicles conjugated with specific biomarker to a given patient at the right time (refer to the diagram figure A).

Epigenetic aberrations and genetic alterations cooperatively and dynamically drive almost all human illnesses, thereby representing attractive targets for therapeutic interventions. The identifications and employment of genetic and epigenetic biomarkers have revolutionized disease management. Among them, kinase-targeted therapies represent several cases of successful personalized medicine, because these approaches emphasize patient’s personal attributes at the molecular or chromosomal level. However, the information used here represents only tiny part of patient’s molecular signatures, and most clinical and biological characterization still remains unexplored. This can explain why such targeted therapy eventually failed, while successful initially. To overcome the aforementioned therapeutic pitfalls, comprehensive characterization of the disease biology for specific patient (responder versus non-responder or complete remission versus relapse) is a must, which shall be assisted through systemic approach using “omics” technologies. The “omics” results

are useful for early detection of disease, predicting patient response to therapy and developing biomarkers to enable effective targeting of drug delivery modalities to the disease site. Among the “omics” technologies, genomics have achieved the most detailed and comprehensive studies, but most gene expression signatures from cell lines and patient samples, which reflect only small time window of disease development, are lacking therapeutic meaning thereby not into clinic yet [168]. Proteomic profiling has huge potential to yield more direct answers to functional and pharmacologic questions than transcriptional profiling [141], because physiological functions are executed by proteins. However, the inconsistency of protein modifications dynamically limits the application of proteomics marker to individualized medicine.

Of all “omics” information, epigenomics would be the most essential leader in developing personalized- and nano- medicine, as epigenetics is the record of personal internal and external stimuli (e.g. maternal, nutrition and living), not only determining other omics (e.g. transcriptomics and metabolomics), but also inducing gene mutations thereby affecting the genomics. Furthermore, all chemotherapies themselves are one of the most important drivers to epigenetic changes. Actually there exists a feedback loop between epigenetics and chemotherapies, in which aberrant epigenetic biomarkers implicate and determine the selection of chemotherapies, whereas the chemo-treatment non-specifically modulates epigenetic codes thereby generating new epigenetic aberrations, eventually even chemotherapy-induced cancer (e.g. leukemia). Different patients would have distinct response to even the same drug delivered by similar vehicles thereby generating patient-specific drug-induced epigenetic biomarkers. Thus epigenetic changes fully represent personalized traits and explain why cooperation of epigenetics would enhance personalized nanomedicine.

The last point to be considered for the development of epigenetic-mediated personalized nanomedicine is the dynamics of cancer pathogenesis as well as pre- and post-drug treatment. The communication and interaction among tumor cells, stromal cells and host immune system are undergoing changes all the time. As such, the biomarkers for cancer diagnostics and therapeutics, the choice of drugs, delivery vehicles and the vehicle-conjugated tumor specific ligands should be changed judiciously, not only from patient to patient, tumor to tumor, but also from cell to cell and pre- to post- drug treatment. Novel nanotechnologies will be indispensable to discover these dynamic epigenetic biomarkers, specifically the sparse biomarkers hidden in barely available materials. Although being largely confined to cancer in this article, epigenetics-driven personalized nanomedicine will become greatly attractive options for clinicians working on other common diseases (e.g. heart failure, osteoarthritis). Overall, we are increasingly hopeful that knowledge about a patient’s proteomic, genetic, epigenetic and metabolic figures could be integrated to tailor medical care to that individual’s needs. It is also hopeful that the alliance of personalized nanomedicine with epigenetics would bring to us the unsurpassed health care.

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Abbreviations

5mC	5-methylcytosine
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5hmC	5-hydroxymethylcytosine
ABC	adenosine 5'-triphosphate-binding cassette
AML	acute myeloid leukemia
ATP	adenosine 5'-triphosphate
BCR	breakpoint cluster region
BRCA1	breast cancer type 1
CBFβ	core binding factor β
CBP	CREB binding protein
CML	chronic myelogenous leukemia
CREB	cAMP response element-binding
DNMT	DNA methyltransferase
DOX	doxorubicin
EGFR	epidermal growth factor receptor
ER	estrogen receptor
ErbB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2
ETO	eight-twenty-one
FHIT	fragile histidine triad
Flt3	fms-like tyrosine kinase receptor-3
GSTP1	glutathione S-transferase gene
HAT	histone acetylase
HDAC	histone deacetylase
HDMT	histone demethyltransferase
HER2	human epidermal growth factor receptor 2
HGP	Human Genome Project
HMT	histone methyltransferase
IDH	isocitrate dehydrogenase
IL-3	interleukin 3
MDS	myelodysplastic syndrome
miRs	microRNAs
MOZ	monocytic leukemia zinc finger
MRI	magnetic resonance imaging
MZ	monozygotic
PI3K	phosphatidylinositol 3-kinase
PZLF	promyelocytic leukemia zinc-finger protein
RAR-α	retinoic acid receptor alpha
RASSF1A	Ras-association domain family 1A gene

SAM	S-adenosyl-methionine
SMMHC	smooth muscle myosin heavy chain
SNPs	single-nucleotide polymorphisms
TET	ten-eleven-translocation
TSGs	tumor suppressor genes
UTRs	untranslated regions
VMRs	inter-individual variability
WWOX	WW-domain containing oxidoreductase

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