The Epigenetic Factors that Drive Cancer Drug Resistance

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1. EPIGENETICS, CANCER, AND CANCER DRUG RESISTANCE

The definition of "epigenetics," initially coined by Waddington in 1942 [1], has evolved over the years [2-6]. However, what is widely acknowledged is that it pertains to processes influencing gene expression that are not based on differences in DNA sequences. If not exclusive to stable, heritable phenotypes [4], epigenetic regulation can cover a broad range of mechanisms involved in gene transcription. These include CpG methylation at the promoter region and modification of histones, both associated with chromatin condensation and the transcription machinery's activation. Various RNA species may also be regulated epigenetically through RNA methylation and the activities of non-coding RNAs (e.g., microRNAs, long non-coding RNAs) [7]. The importance of epigenetics in cancer is evident in the outcome of a PubMed search using the keywords "cancer epigenetics" (> 35,000 publications between 2010 and 2020). Indeed, many of the genes that drive cancer progression are epigenetically regulated through differing mechanisms [8-11]. Hence, epigenetic profiling proved to be a useful tool for the molecular classification of cancer [12]. Both molecular targets and resistance factors can be epigenetically regulated, prompting the interrogation of promoter CpG methylation as surrogate marker in cancer diagnostics. Furthermore, several drugs known to target epigenetic regulators (or epi-drugs) have been approved recently (or currently undergoing clinical trials), usually in combination with other drugs [13]. However, epi-drugs (e.g., inhibitors of histone deacetylases or HDACs, histone acetyltransferases or HATs, DNA methyltransferases, or DNMTs, EZH2, and bromodomain and extra-terminal motif or BET proteins) can modulate transcription at a genome-wide scale. Consequently, epi-drugs can cause unintended down-regulation of tumor-suppressive genes and upregulation of genes that can promote tumor progression and drug resistance (against the other drugs they may be in tandem with).

As often observed in clinics, cancer therapeutics' effectiveness can taper off after a promising initial response from the patient. Simply put, cancer cells begin to rewire and outsmart the drug. Drug-resistant clones could arise from any possible genetic or epigenetic changes in cancer cells, including mutations, alternative splicing, epigenetically-driven expression changes, and even post-translational modification of proteins [14]. The drug resistance that develops can be specific to a family of drugs or may affect a wide array of drugs. Possible mechanisms of drug resistance include over-expression of proteins that metabolically alter the drug itself, reversal of the cytotoxic, cancer-killing damages due to drugs, and simply pumping the drugs out of the cancer cells. Another possibility is dysregulation of apoptosis, such as over-expression of BCL-2 family members and Inhibitor of Apoptosis (IAP) proteins [15]. We can reasonably presume that epigenetics plays essential roles in many of these transcriptional dysregulation-driven drug resistance mechanisms, irrespective of whether the drug-resistant clones arise only after exposure to the drug or were already present (as a minuscule subpopulation) in cancer tissues before treatment [16].

2. STUDIES AT THE INTERFACE OF CANCER DRUG RESISTANCE AND EPIGENETICS

Over the years, our knowledge regarding epigenetics' roles in cancer therapeutics and drug resistance has progressed immensely. This particular field's progress is fueled by the integrated use of modern genomic and epigenomic tools such as microarray (RNA, methylation) and sequencing (bisulfite, RNA Seq, ChIP Seq) technologies, along with the meticulous generation of drug-resistant cancer sublines. The epigenome-wide comparisons between drug-resistant and -sensitive cancer lines (or xenografts) have been employed in recent reports. These include studies on cisplatin resistance in ovarian cancer cell lines (using reduced representation bisulfite sequencing or RRBS technique) [17], trastuzumab resistance in breast cancer lines (using both ChIP-Seq analysis for histones) [18], and carboplatin resistance in Non-Small Cell Lung Cancer (NSCLC) xenografts (using Methylated DNA immunoprecipitation sequencing or MeDIP-Seq) [19]. The most common approach employed to examine if a given gene may be epigenetically regulated is by calculating the correlation coefficient between the gene's expression and the methylation level at its promoter region. The inverse relationship between promoter CpG methylation and transcription has been reported for several genes involved in resistance against classical alkylating chemotherapeutic drugs. These include DNA repair genes [20] coding for proteins that can reverse the DNA-damaging and cancer cell-killing activities of many alkylating drugs. Among the most well-studied epigenetically regulated DNA Repair genes is O6-methylguanine-DNA methyltransferase (MGMT), whose protein product can excise the cytotoxic adducts from O6-methylating/alkylating drugs such as temozolomide and carmustine [21]. Genes involved in metabolic alteration of cancer drugs have also been reported to be controlled epigenetically. Included in this list is glutathione-S-transferases mu 1 (GSTM1) [22], which codes for protein that can catalyze glutathione (GSH) conjugation to (and detoxification of) various chemotherapeutic drugs, including cisplatin, chlorambucil, and melphalan [23]. Also epigenetically regulated are the aldehyde dehydrogenases [24, 25], previously found to be over-expressed in cyclophosphamide (CP)-resistant brain tumor cell line [26]. Aldehyde dehydrogenases (ALDHs) can detoxify oxazaphos-
cyclophosphamide (CP)-resistant brain tumor cell line [26]. Aldehyde dehydrogenases (ALDHs) can detoxify oxazaphosphorines such as cyclophosphamide (CP) by preventing the formation of the reactive phosphoramidemustard [27]. The transcription of the Multidrug Resistance (MDR) genes ABCB1 and ABCG2, which code for ATP-dependent efflux proteins that can pump a wide variety of anticancer drugs (including paclitaxel, olaparib, and etoposide) out of cancer cells, was also found to be dependent on CpG methylation at their respective promoter regions [See [28] for review]. Epigenetic regulation was also observed in the gene GREB1 (growth-regulating estrogen receptor binding 1), which factors in breast cancer tamoxifen resistance bymodulating the activity the drug's target, estrogen receptor 1 (ESR1) [29]. Using TCGA expression and methylation data for select cohorts, it is clear that the expression levels of the genes mentioned are highly dependent (negative R values, see Table 1) on the methylation status of key CpG sites at their respective promoter regions. This analysis was similarly employed by this author in this special issue [30], as well as in earlier publications [31, 32].

When bound to a promoter region, the modified histones H3K4me1, H3K4me3, H3K9ac, H3K36me3, and can promote chromatin decondensation and thereby more active transcription. On the other hand, the markers H3K27me3 and H3K9me3 are associated with promoter silencing [33]. Indeed, the presence of specific histone marks, promoter methylation, chromatin condensation, and transcriptional activation are interrelated events [30-32]. An actively transcribing gene may exhibit a promoter with the following characteristics: the presence of CpG hypomethylation; the histone marks H3K4me3 and H3K9ac; and a decondensed chromatin region that is more accessible to the transcription machinery [30]. In metastatic colorectal cancer cells, the silencing of the pro-apoptotic Fas cell surface death receptor or FAS gene (which factors in resistance against fluorouracil or 5-FU) coincided with enrichment of H3K9me3 at its promoter region [34].

The understanding that epigenetic regulation plays an essential role in cancer progression prompted the exploration of targeting epigenetic regulatory proteins as an option cancer therapeutics. It is surmised that targeting DNMTs (azacitidine, decitabine), HDAC (vorinostat, belinostat, panobinostat), or BRD/BET (JQ1) may cause genome-wide perturbation of transcriptional machinery that is toxic to cancer cells. As demonstrated in several studies, these epi-drugs may also help circumvent drug resistance in cancer cells. For example, the anti-HDAC drugs belinostat and vorinostat were active against cisplatin-resistant NSCLC [35] and bladder cancer [36] lines, respectively. Decitabine (anti-DNMT1) can help reverse 5-FU-resistance in cancer lines [37]. Inhibition of BRD4 through the protein degradation approach was effective against vemurafenib-resistant melanoma cells [38].

Post-transcriptional epigenetic regulation by microRNAs [see [39] for review] has similarly been demonstrated to modulate cancer drug resistance. As an example, mir-381 was capable of suppressing ABCB1/MDR1 mRNA, which would then sensitize breast cancer cells to cisplatin [40]. mir-200c played a role in reversing oxaliplatin-resistance in gastric cancer cells by targeting the DNA repair proteins ERCC3 and ERCC4 [41]. Another epigenetic regulatory mechanism that can influence cancer cells' response to treatment is RNA methylation (such as methylation at the N6 position of adenosine; m6A). The presence of m6A can influence the abundance of RNA transcripts, and therefore the activity of many genes, including those related to drug resistance (e.g., MDR and DNA Repair genes) [42].

Additional analyses of ChIP Seq and DNaseI hypersensitivity data from the ENCODE project [43] would demonstrate that the transcription of a drug resistance gene such as MGMT is likely epigenetically regulated through methylation at its promoter and gene body regions, as well as through modification of bound histone tails [30]. However, it is possible to dig even deeper into other additional TCGA datasets to examine how cancer drug resistance genes' activities may also be influenced by microRNAs, IncRNAs, and mutations in epigenetic regulatory genes. Integrated analyses of publicly available pharmacological, genomic, epigenomic, and proteomic datasets for a broad range of cancer cell lines will further improve our understanding of cancer drug resistance and its associated epigenetic regulatory mechanisms. These datasets include fold-change viability data for >4500 drugs, mutation data, CpG methylation data, expression data (mRNA, miRNA, proteins), and genetic dependency data from genome-wide CRISPR-cas9 screens. The sources of these information include the DepMap (The Cancer Dependency Map) [44], GDSC (Genomics of Drug Sensitivity in Cancer) [45], and CCLE (Cancer Cell Line Encyclopedia) [46] projects.

Knowledge Our knowledge about epigenetic regulation in cancer drug resistance can be consequential in the development of cancer diagnostics and therapeutics. In brain tumors, the methylation at MGMT's promoter region is routinely tested to guide treatment. If the MGMT promoter is highly methylated, a better prognosis is predicted for glioblastoma patients treated with radiotherapy and adjuvant TMZ [47, 48]. Moreover, the interrogation of CpG methylation in plasma-extracted cell-free DNA may become the standard in non-invasive cancer detection and treatment monitoring [49, 50]. Also, as more epigenetic targeting drugs get approved for clinical use (including those that may be used in combination with other drugs), it is crucial to understand if the epi-drugs themselves can help initiate drug resistance.

3. FOR THIS SPECIAL ISSUE

In this special issue of Current Cancer Drug Targets, we are presenting a collection of articles (6 reviews, one original research) contributed by experts working in fields encompassing cancer epigenetics, cancer therapeutics, and cancer drug resistance. The articles will discuss how cancer drug resistance is shaped by a multitude of factors such as mutations in epigenetic regulatory proteins, CpG methylation status at the promoter and gene body regions of cancer drug resistance genes, miRNAs expression, and RNA methylation. Also, we have covered the very timely topic of epigenetic therapy, including the potential of using natural compounds (flavonoids) and protein degradation approach to combat cancer drug resistance.
1. Epigenetic mechanisms of therapy resistance in diffuse large B cell lymphoma (DLBCL), by Yusuke Isshiki and Ari Melnick [51]. In this review, the authors will discuss how recurrent mutations in epigenetic regulator genes (such as CREBBP, KMT2D, EZH2, and TET2) can lead to impairment in immune surveillance, and eventual resistance against immunotherapies (by inducing lymphomagenesis) in Diffuse Large B-cell lymphoma (DLBCL).

2. MGMT epigenetics: the influence of gene body methylation and other insights derived from integrated methylomic, transcriptomic, and chromatin analyses in various types of cancer, by Manny Bacolod and Francis Barany [30]. In this original research article, the authors will describe how an integrated genomic approach (using various publicly available genomic and epigenomic datasets) led to observations that promoter and gene body methylation have a contrasting influence on the expression of MGMT.

3. The role of RNA modifications and RNA-modifying proteins in cancer therapy and drug resistance, by Shaun Wood, Amber Willbanks, and Jason X. Cheng [52]. In this review, the authors will present a comprehensive review on the possible roles that RNA-modifying proteins (RMPs) and post-transcriptional RNA methylations (such as N6 methyladenosine) play in cancer progression and drug resistance.

4. miRNA as regulators of prostate carcinogenesis and endocrine and chemoresistance, by Zoran Culig [53]. In this review, the author will discuss the regulatory microRNAs whose overexpression or down-regulation can modulate drug resistance in prostate cancer.

5. Re-sensitizing tumor cells to cancer drugs with epigenetic regulators, by Stefanie Rauscher, Richard Greil, and Roland Geisberger [54]. In this review, the authors will discuss how some of the widely-studied epigenetic drugs are used to modulate sensitivity towards cancer chemotherapy (including immunotherapy).

6. Flavonoids overcome drug resistance to cancer chemotherapy by epigenetically modulating multiple mechanisms, by Kennet K.W. To and William C.S. Cho [55]. In this review, the authors explore the potential of flavonoids, natural polyphenolic compounds found in plants, fruits, vegetables, and traditional herbs, in reversing cancer drug resistance.

7. PROTACs: Promising approaches for epigenetic strategies to overcome drug resistance by Sarah F. Giardina, Elena Valdambrini, J. David Warren, and Francis Barany [56]. This review describes how a protein target selective degradation approach, known as Proteolysis Targeting Chimera (PROTAC), can be utilized to counter cancer drug resistance.

Table 1. Examples of genes associated with cancer drug resistance. Below are the CpG sites, which exhibit the highest negative Pearson correlation ($R$; CpG methylation v. expression). $Rs$ were calculated from integrated TCGA expression (RNASeq) and methylation (Illumina 450K) data. GBM, glioblastoma multiforme; PAAD, pancreatic adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; BRCA, breast invasive carcinoma.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Gene Description</th>
<th>Associated Resistance</th>
<th>$R$ (CpG meth v. expr)</th>
<th>CpG ID</th>
<th>CpG coord. (bg19)</th>
<th>CpG site Location</th>
<th>TCGA Cohort</th>
<th># Samples Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGMT</td>
<td>O-6-methylguanine-DNA methyltransferase</td>
<td>06-alkylating/methylating drugs (e.g., temozolomide, carmustine)</td>
<td>-0.70</td>
<td>cg12981137</td>
<td>Chr10: 131265575</td>
<td>CpG Island</td>
<td>GBM</td>
<td>65</td>
</tr>
<tr>
<td>ABCB1 (MDR1)</td>
<td>ATP binding cassette subfamily B member 1</td>
<td>various drugs (e.g., etoposide, taxanes)</td>
<td>-0.52</td>
<td>cg16772035</td>
<td>Chr7: 87229880</td>
<td>CpG Island</td>
<td>PAAD</td>
<td>183</td>
</tr>
<tr>
<td>GSTM1</td>
<td>glutathione S-transferase mu 1</td>
<td>various drugs (e.g., chlorambucil, melphalan)</td>
<td>-0.92</td>
<td>cg24506221</td>
<td>Chr1: 110230401</td>
<td>CpG Island</td>
<td>UCEC</td>
<td>197</td>
</tr>
<tr>
<td>GREB1</td>
<td>growth regulating estrogen receptor binding 1</td>
<td>tamoxifen</td>
<td>-0.65</td>
<td>cg25785303</td>
<td>Ch2: 11681908</td>
<td>TSS1500</td>
<td>BRCA</td>
<td>683</td>
</tr>
<tr>
<td>ALDH1A2</td>
<td>aldehyde dehydrogenase 1 family member A2</td>
<td>oxazaphosphorines (e.g. cyclophosphamide)</td>
<td>-0.43</td>
<td>cg02900766</td>
<td>Ch15: 58357319</td>
<td>CpG Island</td>
<td>BRCA</td>
<td>683</td>
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REFERENCES


