The bitter side of epigenetics: variability and resistance to chemotherapy

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Abstract

One of the major obstacles to the development of effective new cancer treatments, and the main factor for the increasing number of clinical trial failures, appears to be the paucity of accurate, reproducible, and robust drug resistance testing methods. Most research assessing the resistance of cancers to chemotherapy has concentrated on genetic based molecular mechanisms, while the role of epigenetics in drug resistance has been generally overlooked. This is rather surprising given that an increasing body of evidence pointing to the fact that epigenetic mechanism alterations appear to play a pivotal role in cancer initiation, progression, and development of chemoresistance. This results in a series of clinical trials involving epi-drug as single treatment or combined with cancer conventional drugs.

Histone deacetylases (HDAC) expression contributes to cancer development and drug resistance.

Histone lysine modifications directly influence activation and repression of transcription, and are also essential for higher chromatin order structure; depending on which residue is modified and at what position in the gene such a residue is located. HDAC and histone acetyltransferases (HAT) control the acetylation state of lysine residues, including those situated in the N-terminal “tails” of the histones. HATs fulfil simultaneously the function of transcription coactivators, while HDACs are co-repressors. Overall, posttranslational modifications (PTMs) of histones create an epigenetic mechanism for the regulation of a variety of normal and disease-related processes. Histone modification patterns based on acetylated H3 Lysine K18 can predict the risk of tumour recurrence for cancer, and global hypoacetylation of H4K12 is considered to be informative of tumour stage. Interestingly, we and others found that the loss of H4K16 acetylation can be used as a hallmark of multidrug resistance cancer cells.

Aberrant expression patterns of HDACs are implicated in a number of cancers, for example, SIRT1(HDAC class III), and it is consistently up-regulated in malignant cells or tissues from patients with leukemia, glioblastoma, prostate, colorectal or pancreatic cancer and is the only HDAC that is significantly overexpressed in leukemia lymphoblasts as compared with normal lymphoblasts. We recently showed that reduction in HDAC2 expression level plays an essential role in vitro and in vivo in cancer response to DNA damaging agents alone or combined with HDAC inhibitors. Furthermore, sustained-suppression of HDAC2 in lung cancer results in regression of tumour cell growth and activation of cellular apoptosis via p53 and Bax activation and Bcl2 suppression.

Increasing number of studies that have reported that specific inhibition of certain HDACs (e.g. Sirt1) leads to down-regulation of multidrug resistance protein1 (MDR1) and promotes cell death by acetylating the DNA-damage-protecting protein NBS1. In the light of this, we showed that hMOF (HAT) and SIRT1(HDAC) expression levels are critical parameters in HDAC inhibitor-mediated sensitization of multidrug-resistant cancer cells to topoisomerase II inhibitor and increased chromatin relaxation through H4K16 acetylation.

The overexpression of certain HDACs in cancer cells is implicated in genotoxic insult protection, silencing of tumour suppressor genes, alteration of DNA repair pathways, and increased resistance to DNA damaging agents by the activation of non-histone proteins that are required for DNA stability.
These outcomes are to a large extent cell-type specific and have raised the potential that HDAC inhibitors may represent a promising new class of antineoplastic agents which may reverse chemo-resistance and stratify patients according to potential for chemoresponse in cancer.

HDAC inhibitors have been shown to be effective therapeutic anticancer agents via multiple mechanisms, inducing cell-cycle arrest, intrinsic and extrinsic apoptotic mechanisms, mitotic cell death, autophagic cell death, reactive oxygen species, inhibiting angiogenesis and improving NK cell-mediated tumour immunity. These diverse effects on cancer cells make HDAC inhibitors attractive agents not only for monotherapy but also for combination therapy with other anticancer modalities. HDACs can modulate cellular responses to cancer conventional treatment. Although many combination strategies have been shown to be both effective and synergistic, the exact mechanism(s) for this synergy are poorly understood and likely different according to the combination regimen utilised.

**DNA methylation and drug resistance**

Another epigenetic mechanism that has been shown to be associated with drug resistance is the covalent modification that occurs by the addition of methyl group at the 5-carbon of cytosine in a DNA CpG dinucleotide and catalyzed by DNA methyltransferases (DNMTs) enzyme. Genomic DNA sequencing analysis has shown elevated rates of abnormal CpG promoter methylation (5% to 10%) in several types of cancer. There are three fundamental associations between drug resistance and DNA methylation status: i) drug resistance associated with hypo or hypermethylated genes; ii) cellular heterogeneity and iii) induction of tumor cells sensitivity through adjuvant treatments.

**i) Resistance induction:** The main reorganisation of DNA methylation related to drug-resistant is the hypermethylation of the CpG islands on gene promoters of certain genes. This contributes to carcinogenesis through silencing of tumor suppressor genes (e.g. E-cadherin, pRB, P53, and CDKN2A).

The hypermethylated androgen receptor (AR) gene promoter causes resistance to anti-androgens in prostate cancer (CaP). Hypermethylated promoters in MLH1, WTH3 and BMP6 genes are also involved in breast adenocarcinoma drug resistance. Furthermore, the hypermethylation of C22orf2 and BCL2-like11 promoters by DNMT1 induces resistance to tyrosine-kinase inhibitors in chronic myeloid leukemia.

On the other hand, a global DNA hypomethylation in cancer targets diverse genomic sequences, including repetitive elements, transposons, intronic CpG dinucleotides, and gene deserts, increasing genomic instability and activating proto-oncogenes. DNA hypomethylation may also be involved in anticancer drug resistance, which leads to an accumulation of the multi-drug resistance genes as MDR1 in breast cancer or in oral squamous cell carcinoma (cisplatin resistance inductor). Glioma tumor cells resistant to conventional drugs showed a significant DNA hypomethylation compared with their counterpart nonresistant tumor cells in vitro. Furthermore, drug resistance could be driven by the combination of hyper- and hypomethylation alterations depending where the alteration of DNA methylation occurs. For instance, the sulfatase2 precursor gene hypomethylation and the hypermethylation of estrogen receptor α gene induced loss of estrogen responsiveness by estrogen metabolism deregulation in MCF-7 drug-resistant cells.
ii) Cellular heterogeneity: DNA methylation heterogeneity defines a disease spectrum in many tumors. It has been demonstrated that epigenetic abnormalities have an important role in the plasticity of cell states during tumorigenesis, and this could lead to acquired drug resistance. Reversible states of cells that survive to chemotherapeutic drugs exposure may drive multistep epigenetic fixation of gene expression changes during the acquisition of drug resistance\textsuperscript{18}. Heterogeneity in a tumor cell population, based on dynamic variation in epigenome configurations, is thought to provide a non-genetic variance source for selection of drug-resistant cells\textsuperscript{19}.

iii) Induction of tumor sensitivity: DNA methylation modulators have shown to sensitize the multi-drug-resistant tumor cells to conventional treatment. For instance, increased global methylation level was reduced in recurrent cases of colorectal cancer (CRC) by the use of 5-aza-2'-deoxycytidine that restore CRC sensitivity to 5-FU\textsuperscript{20}. On the other hand, demethylating agents have been used in ovarian cancer to overcome acquired resistance to carboplatin\textsuperscript{21}. In addition, demethylation agent also restored the sensitivity to cisplatin, taxol, and oxaliplatin in cervical cancer\textsuperscript{22}. However, the use of DNA methylation regulation agents could activate unwanted genes, including new drug resistance genes and others that induce tumor progression. Still more to know about hypermethylation resistance nodules whether emerged earlier or induced by chemotherapy.

MicroRNA and Drug resistance in cancer

MicroRNAs (miRNAs) are small non-coding RNAs (19-25 nucleotides in length) implicated in most physiological processes and are tightly related with several diseases, including cancer\textsuperscript{23}. The first evidence of a correlation between microRNAs and cancer was reported in 2002. Calin et al., describe deletions and down-regulation of miR-15a and miR-16-1 in approximatively 68% of chronic lymphocytic leukemia patients\textsuperscript{24}. After these initial observations, miRNAs have been shown to play an important role during tumorogenesis, act as either tumor suppressors or oncogenes depending on the cellular context and the expression of the miRNA targets in the concrete malignant tissues\textsuperscript{25}. The involvement of miRNAs in resistance to anticancer drugs is emerging field. Several studies indicate that a significant changes in miRNA expression profiles occur in drug-resistant cancer cells in comparison with parental drug-sensitive cancer cells\textsuperscript{26} and other studies shown that the alteration of specific miRNAs expression, such as miR-21, miR-221/222, miRNA let-7 family, are responsible for drug resistance in tumor cells\textsuperscript{27}. One of the most important links between miRNA function and cancer drug resistance is represented by the effect on the expression of tumor suppressor genes. Those miRNAs are considered as oncogenes and usually promote tumor development by negatively inhibiting tumor suppressor genes and/or genes that control cell differentiation or apoptosis\textsuperscript{28}.

Many reported research findings showed that certain miRNA influences cancer chemoresistance and the difference in their expression occurs simultaneously rather than by an individual chemoresistance miRNA mechanism\textsuperscript{29}. In recent years, researchers linked miRNA dysregulated expression with different cellular pathways that are directly influencing tumour chemoresistance. MiRNAs activity affects apoptotic pathway, proliferation response to DNA damage, and regulation of multidrug resistance genes\textsuperscript{30-32}. The complexity of
understanding miRNA regulation in cancer is due to the high number of target genes that can be regulated by one single miRNA as predicted by bioinformatics analysis. Additionally, by the fact that each cell type of cancer may be defined by a unique set of miRNAs that controls drug resistance.

Epigenetic mechanisms cross-talk in cancer drug resistance

The mainstream of epigenetic and drug resistance in cancer research field stresses the importance of individual epigenetic mechanisms. However, the interaction between different regulators of epigenetic mechanisms in cancer drug resistance is overlooked despite of strong evidence of these interactions. For instance, hypermethylation has been shown to affect the expression of miR-129-5p that modulates the level of the multi-drug resistance ABC transporters (ABCB1, ABCC5 and ABCG1) genes in gastric cancer.\textsuperscript{33} Interestingly, miRNAs can directly target HATs/HDACs and subsequently influence the level of histone acetylation and transcription factor activation. Furthermore, MiRNAs can affect the level of $\text{DNMT}$ expression. Recently, Masoumeh et al (2017) found in tissues and cells of pancreatic cancer (PC) that miR-377 expression was inversely correlated with $\text{DNMT1}$ expression. Downregulating $\text{DNMT1}$ expression by miR-377 led to reactivation of tumor suppressor genes BNIP3 and SPARC via promoter DNA hypomethylation and subsequently reduction of proliferation and apoptosis induction in PC cells.\textsuperscript{34}

The emerging strategies to regulate epigenetic regulators in cancers include the activation or inactivation of their expression to increase drug effectiveness. This indicates that these regulators can be considered as sensitive biomarkers for drug resistance and as a potential therapeutic target to break drug resistance. However, a deeper understanding of epigenetic cross-talk could increase the efficiency and the use of selective and combined epigenetic drugs for therapeutic use.
References:


