Drug resistance in cancer therapy and the possible role of epigenetics

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Abstract
Effective leukemia treatment is seriously hampered by drug resistance. The possible roles of epigenetic mechanisms have recently been considered in cancer drug resistance. With conventional anti-cancer drugs, including alkylating drugs, anti-metabolite drugs, topoisomerase inhibitors, and microtubule inhibitors, which have been available for half a century, the drug resistance often occurs due to decreased expression of target enzymes, with increased expression of drug export pumps. The alterations of target gene expression and increased export pump function might be caused by epigenetic changes, such as changes of methylation status, as well as changes of histone acetylation status. In addition, newly developed anti-cancer drugs, including small molecule drugs such as kinase inhibitors, antibody drugs, and immune modulatory drugs, also showed development of drug resistance within a year, although these drugs show significant efficacy in conventional anti-cancer drug-resistant patients. The resistant cells showed increased expression of bypass pathways, activation of downstream cascades, decreased expression of antigens of tumor cells, increased DNA repair activity, and increased expression of drug export pumps, also suggesting epigenetic changes. In this paper, drug resistance to cancer therapy and the possible roles of epigenetic mechanisms are reviewed.
Introduction
Drug resistance remains a serious problem in cancer therapy, and children with cancer cells exhibiting *in vitro* resistance to anti-cancer agents have substantially worse prognoses than children whose cancer cells are drug-sensitive. The pattern of gene expression in cancer cells that eventually acquire resistance prior to the initiation of treatment is important because drug-resistant subpopulations grow selectively as treatment progresses. Nevertheless, how cells acquire drug resistance is unclear. Many signaling pathways and genes that may affect the response of cancer cells to therapy have been identified. Because many factors are involved in the mechanism of acquisition of drug resistance, the “one gene: one outcome” hypothesis cannot adequately explain acquired resistance in cancer. Thus, multiple mechanisms and multiple genes, rather than a single pathway or gene, likely mediate acquired resistance.

Aberrant methylation has been shown to play a potent role in tumorigenesis, where genome-wide hypomethylation and regional hypermethylation of tumor suppressor gene promoters are characteristic hallmarks in many cancers. DNA methylation occurs in eukaryote DNA at CpG sites, usually enriched in the promoters of genes. Increasing evidence has shown that epigenetic changes can be a crucial driving force behind the acquisition of drug resistance, with changes in gene expression occurring after chemotherapy without gene mutations. Wei et al. used drug-resistant cell lines and differential methylation hybridization and showed many differences in CpG island methylation and epigenetic regulation after drug treatment.

Histones also control gene expression by modulating the structure of chromatin and the accessibility of regulatory DNA sequences to transcriptional activators and repressors. Acetylation of histone increases gene expression by relaxing chromatin structure, allowing access of transcription factors to DNA.

In this paper, drug resistance to cancer therapy and the possible roles of epigenetic mechanisms are reviewed.

**Conventional anti-cancer drugs**
Conventional anti-cancer drugs include alkylating drugs, anti-metabolite drugs, topoisomerase inhibitors, and microtubule inhibitors, which have been available for half a century. In the 1990s, there were many studies of drug resistance, especially multiple drug resistance. The following mechanisms were generally accepted for anti-cancer drug resistance: (1) increased drug metabolism; (2) decreased uptake of drugs; (3) decreased expression of target molecules; and (4) mutation of target molecules.
I. Alkylating drugs

1) Mechanisms of drug action

Alkylating agents bind alkyl bases of DNA in tumor cells and make a covalent conjunction between drugs (dacarbazine, temozolomide) and one alkyl base or between drugs (cyclophosphamide, melphalan) and two alkyl bases. These modified alkylated bases are not able to repair because of a lack of a DNA repair mechanism in leukemic cells, and this leads to apoptosis in leukemic cells.

2) Mechanisms of resistance to alkylating drugs

As mechanisms of resistance to alkylating drugs, (1) decreased drug transfer into cells, (2) inactivation of glutathione as a detoxifying enzyme, (3) O6-alkylguanine methyl converting enzyme (MGMT) enhancement, (4) enhancement of nucleotide excision repair as a DNA repair function, and (5) a deficit of mismatch repair have been reported.

(1) Decreased uptake into tumor cells

Decreased drug uptake into cancer cells occurs by increased expression and function of export pumps, which include ATP Binding Cassette (ABC) transporters. These transporters export the anti-cancer drugs to outside of the cancer cells using ATP. There are 49 proteins (transporters) identified as ABC transporters (Tsuruo). We could not find any mutations in the promoter area of ABC transporter genes in drug-resistant cancer cell lines with alkylating agents. Now, the increased expression of ABC transporter genes is considered to occur through epigenetic regulation.

(2) Increased detoxification effect in tumor cells by increased expression of glutathione transferases

Increased detoxification generally occurs by increased expression and function of glutathione transferases. Increased expression of these genes might be related to epigenetic mechanisms.

(3) Increased expression of O6-alkylguanine methyl converting enzyme (MGMT)

MGMT is a DNA repair enzyme, and low expression of MGMT leads to higher drug sensitivity in cancer cells. Because lower expression is related to methylation of the promoter region of MGMT gene, hypomethylation might occur in leukemia cells with resistance to alkylating drugs.
(4) Enhancement of nucleotide excision repair as a DNA repair function
Alkylating agents damage tumor cells by covalently linking the alkyl group to DNA. Enhancement of the repair mechanism leads to resistance to alkylating agents. These alterations of expression of DNA repair enzymes might be caused by epigenetic modulations.

(5) Lack of mismatch repair
The alkylating agent forms a base pair with thymine by alkylation at the O6 position and causes mismatch formation. In resistant cells, apoptosis is not induced by eliminating the mismatch repair, and resistance against alkylating agents occurs.

II. Anti-metabolite drugs
This category of drugs includes pyrimidine analogs (cytosine arabinoside (AraC), enocitarabine (BHAC), gemcitabine), purine analogs (fludarabine, 6-mercaptopurine, nelarabine, clofarabine), and anti-folic acid drugs (methotrexate).

1) Drug actions of pyrimidine nucleoside analogs
The processes involved in the drug actions of pyrimidine nucleoside analogs (especially Ara C) are (1) uptake to cancer cells by nucleoside transporter (human equilibrative nucleoside transporter 1 (hENT1); (2) phosphorylated by deoxycytidine kinase; and (3) binding DNA and blocking elongation of DNA chains and induction of apoptosis.

2) Mechanisms of resistance to pyrimidine analogs (Ara C)
(1) Decreased expression of nucleoside transporters
Decreases in expression and function of nucleoside transporters are related to resistance to pyrimidine analogs. The decreased expression might be caused by increased methylation of the promoter region of hENT1 gene or decreased acetylation of hENT1 gene.

(2) Increased expression of cytidine deaminase and decreased activity of pyrimidine analogs
AraC is inactivated by the action of cytidine deaminase. When cytidine deaminase activity increases, drug resistance is acquired because AraC activity is reduced.

(3) Decreased expression or mutation of deoxyctydine kinase and decreased phosphorylation of pyrimidine analogs
Intracellular AraC is phosphorylated to Ara-CMP by the action of deoxycytidine kinase, and Ara-CMP is further phosphorylated to become active Ara-CTP. Active Ara-CTP becomes a substrate for DNA polymerase and is translocated into the nucleus, and it causes inhibition of the elongation of the DNA chain and finally induces apoptosis. Reduced activity of deoxycytidine deaminase causes drug resistance\textsuperscript{19}.

(4) Decreased expression of deoxycytidine monophosphate (dCMP) deaminase and increased production of dCTP
AraCMP is phosphorylated and becomes an active form, AraCTP, which causes cell damage. However, a decrease in dCMP deaminase activity increases the conversion of AraCMP to Ara-UMP, resulting in resistance. Furthermore, dCTP competitively inhibits DNA polymerase with AraC, so that increasing dCTP results in resistance to AraC\textsuperscript{20}.

(5) Increased expression of anti-apoptosis protein
Increases in Bcl-2 expression and function lead to an anti-apoptotic action in resistant cells\textsuperscript{21}.

(6) Increased expression of 3'-\textgreater 5' endonuclease
Ara-C is translocated to the nucleus, and 3' \textgreater 5' endonuclease leads to removal of DNA that is integrated with pyrimidine analogs, such as AraC. Thus, the increased endonuclease activity causes AraC resistance.

3) Mechanisms of drug action of purine nucleotide analogs
The drug actions of purine nucleotide analogs (fludarabine, 6-mercaptopurine, nelarabine, clofarabine) are as follows.

(1) Uptake into tumor cells by nucleotide transporter
The purine nucleotide analog is transferred to the cell via a nucleotide transporter, such as AraC.

(2) Phosphorylated by deoxycytidine kinase (dCK)
In the cell, purine nucleotide analogs are phosphorylated by deoxycytidine kinase (dCK) and become triphosphates, and the purine nucleotide analogs become their active forms.

(3) Integrated into DNA and inhibition of DNA synthesis
The triphosphorylated purine nucleotide analogs translocate into the nucleus and are
incorporated into DNA, causing DNA synthesis inhibition.

4) Mechanisms of resistance to purine analogs
(1) Decreased transport (hENT1) into tumor cells
hENT1 is considered a pump necessary for transporting a drug into a cell. Decreased uptake into cells causes drug resistance.

(2) Decreased phosphorylation by decreased expression of deoxycytidine kinase (dCK) gene
Purine nucleotide analogs taken into cells are phosphorylated by dCK. If the function of dCK is reduced, the purine nucleotide analogs are phosphorylated and do not become their active forms, thereby causing drug resistance. Yamanishi et al. reported that resistance to clofarabine was caused by decreased expression of dCK gene due to decreased deacetylation of histone in the promoter region of dCK and a concomitant decline in function, resulting in drug resistance, as summarized in Table 1.

5) Drug action of 6-mercaptopurine (6-MP)
6-MP is an especially key drug for childhood acute lymphoblastic leukemia. 6-MP is transferred into the cell and converted to thioinosine monophosphate (TIMP), and it inhibits biosynthesis of adenyllic acid and guanylic acid and exhibits antitumor activity.

6) Mechanisms of resistance to 6-MP
As mechanisms of resistance to 6-MP, (1) oxidation to the inactivated form by xanthine oxidase and (2) methylation by thiopurinemethyltransferase (TPMT) have been reported. TPMT also has a genetic polymorphism with reduced activity. A correlation between gene polymorphism and 6-MP metabolic activity has also been reported, and the mutant type of TPMT shows severe bone marrow suppression compared to wild-type.

7) Drug actions of anti-folic acid drugs (methotrexate)
Folic acid is essential for cell survival. Because de novo folic acid biosynthesis is not possible in mammals, it is necessary to incorporate folate from outside of the cells and use it for DNA synthesis. Methotrexate (MTX) is an enzyme that inhibits the action of dihydrofolate reductase (DHFR), which reduces folic acid to the active folic acid required for nucleic acid synthesis, and then methotrexate inhibits thymidylate and purine synthesis and suppresses cell growth.
8) Mechanisms of resistance to MTX
As mechanisms of resistance to MTX, (1) decreased cellular uptake of folic acid, (2) increased expression of thymidylate synthase (TS), (3) increased copy number of dihydrofolate reductase (DHFR) gene or mutation, and (4) decreased polyglutamine synthesis by decreased folypoly-glutamate synthase (FPGS) activity have been reported\textsuperscript{23, 24}.

(1) Decreased cellular uptake of folic acid
MTX has a similar structure to folic acid and is actively transported into cells by reduced folate carrier (RFC). The decreased RFC activity causes decreased uptake of MTX into cells.

(2) Increased expression of thymidylate synthase (TS)
DNA is synthesized using the thymidine kinase pathway. MTX is converted to the polyglutamine type and accumulates in the cells, and it binds dihydrofolate reductase (DHFR). This binding inhibits nucleic acid synthesis and causes apoptosis.

(3) Increased copy number or gene mutation of dihydrofolate reductase (DHFR)
Increased copy number of dihydrofolate reductase (DHFR) or mutation of dihydrofolate reductase (DHFR) prevents depletion of reduced folic acid and prevents leukemic cell death.

(4) Decreased polyglutamine synthesis by decreased folypoly-glutamate synthase (FPGS) activity
Decrease folypoly-glutamate synthase (FPGS) activity causes decreased polyglutamine synthesis and inhibits accumulation of the polyglutamine type of methotrexate (MTX).

III. Topoisomerase inhibitors
1) Mechanisms of drug action
Topoisomerase is a key enzyme for duplication and transcription of single and double-stranded DNA. Topoisomerase I cuts and ligates single-strand DNA. An inhibitor of topoisomerase I is camptothecin. Topoisomerase II cuts and ligates double-stranded DNA, and the inhibitors of topoisomerase II include etoposide and anthracycline\textsuperscript{4}.

2) Mechanisms of resistance to topoisomerase inhibitors
(1) Increased expression of export pumps: ATP-binding cassette (ABC) transporter, ABCB1 (p-glycoprotein), BCPR (breast cancer resistance protein)

Increased expression of ABC transporters in drug-resistant cancer cells was not associated with mutations in the promoter area of these genes, suggesting that the mechanism of increased expression of ABC transporters might be related to epigenetics. However, MX2, which is a morpholino anthracycline derivative with highly lipophilic properties, did not increase expression of ABC transporters.

(2) Mutation of binding sites of topoisomerase

Mutations of the binding sites of topoisomerase result in failure of the drug to interact with the enzyme in established cell lines. Although mutations have been reported in *in vitro* cell lines, mutations from clinical specimens from patients with drug resistance are rarely reported.

(3) Increased expression of DNA repair enzymes

DNA repair enzyme activity is increased in damaged cancer cells, which improves their survival.

(4) Decreased expression of topoisomerase II or mutation of topoisomerase II

Decreased expression of topoisomerase II gene is frequently observed in topoisomerase II-resistant cells, and we found that the decreased expression was caused by increased methylation of promoter regions of topoisomerase IIα gene (Asano 2005). We could not find mutations in topoisomerase IIα gene and the promoter in leukemia cells resistant to topoisomerase IIα inhibitors.

(5) Increased expression of glutathione reductase

The metabolism of camptothecin, an inhibitor of topoisomerase I, requires glucuronate conjugation. Increased expression of glutathione S transferase causes increased glucuronate conjugation and increased detoxification.

IV. Anthracycline

1) Mechanisms of drug action

The antitumor effect occurs by inhibiting DNA and RNA synthesis, DNA polymerase, and RNA polymerase, and by topoisomerase II inserted between DNA base pairs of tumor cells.
2) Mechanisms of resistance to anthracyclines

(1) Emerging multiple drug-resistant proteins: P-glycoprotein, multiple drug resistance protein (MRP), lung resistant related protein (LRP)

Increased expression of multidrug-resistant proteins by enhanced expression of P-glycoprotein, multiple drug resistance protein (MRP), lung resistant related protein (LRP) etc. enhances the function of excreting anticancer drugs from leukemia cells and induces anthracycline drug resistance.

(2) Decreased activity of topoisomerase

Decreased expression and decreased activity of topoisomerase gene are frequently observed in anthracycline-resistant tumor cells and with decreased expression related to epigenetic mechanisms. The mutations of topoisomerase gene are observed in resistant cell lines, but they are rare in human samples.

(3) Increased detoxification

Increased expression of glutathione S-transferase and increased activity of thymidylate synthase are observed in anthracycline-resistant cells. The alteration of gene expression might be related to epigenetic mechanisms.

5. Microtubule inhibitors (vincristine)

1) Mechanisms of drug action

Microtubules are essential proteins for cell mitosis. Although the details of the mechanism of action of microtubule inhibitors (especially vincristine sulfate) have not yet been clarified, vinca alkaloids bind directly to tubulin in microtubules and inhibit tubulin polymerization, thereby stopping cell division and resulting in cytotoxicity. On the other hand, taxanes are depolymerization inhibitors that stabilize microtubules and induce cell death by causing hyperplastic microtubules.

2) Mechanisms of resistance to microtubule inhibitors

Resistance to vinca alkaloids occurs relatively quickly. The mechanisms are (1) mutation of the binding site of tubulin and (2) enhancement of extracellular excretion by the drug excretion pump.

(1) Changes of binding sites with tubulin

Changes of binding sites of drugs with tubulin are usually caused by point mutations.
(2) Increased activity of DNA repair
DNA repair enzyme activity is increased in damaged cancer cells, which improves their survival.

(3) Increased expression of export transporter
Increased expression of export transporter might be caused by epigenetic mechanisms. In particular, it is known that P-glycoprotein also excretes vinca alkaloids as a drug excretion pump, and increased expression of ABCC1, a drug excretion transporter, also promotes intracellular drug excretion.
Newly developed drugs

Recently, several newly developed drugs, including 1) small molecule drugs, such as kinase inhibitors, 2) antibody drugs, and 3) immune modulatory drugs, have become available in the clinic. These drugs show significant efficacy in conventional anti-cancer drug-resistant patients. However, these newly developed drugs have also shown drug resistance within a year, and problems have emerged.

I. Molecular targeted drugs

1) Mechanisms of resistance to molecular targeted drugs

Although some targeted hematopoietic tumors can be cured by targeted drugs, many cancer types have a problem in that, even if the tumors respond to the targeted drug once, they relapse due to resistance. The main mechanisms of targeted drug resistance include changes in target genes (gatekeeper mutations or secondary mutations, gene amplification), activation of collateral pathways, downstream activation of targets, and small cell carcinoma transformation, epithelial mesenchymal transition (EMT), cancer stem cell traits, and other mechanisms.

(1) Mutations of gatekeeper genes

Bcr-Abl resistance is related to T315I mutation. Epithelial growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) resistance is related to T790M mutation. Anaplastic lymphoma kinase (ALK)-TKI resistance is related to L1196M mutation, and C-kit resistance is related to T670I substitution.

As solvent-front mutations, ALK resistance is related to G1202R, and c-ros oncogene 1 (ROS1) is related to G2032R substitution.

(2) Increased expression of bypass pathways

When ligands attach to EGFR, activation of the PI3K/Akt, MEK/MAPK, and JAK/STAT pathways occurs. EGF-TKI-resistance shows amplification of hepatocyte growth factor (c-Met) and activation of insulin-like growth factor 1 receptor (IGF-1R), and it affects the MEK/MAPK pathway. ALK resistance increases expression of receptor of stem cell factor (c-kit).

(3) Activation of downstream signal pathways

EGFR-TKI-resistant cells show further activation of the downstream signal pathways such as PI3K/Akt, MEK/MAPK, and JAK/STAT, and they finally overcome suppression of downstream signals by TKI. Cells resistant to BRAF inhibitors also show increased
expression of ARAF and CRAF and then lead to activation of MAPK, overcoming suppression of downstream signals by BRAF inhibitors.

(4) Epithelial to mesenchymal transition (EMT)
A reversible change from epithelial to mesenchymal cells that possess migration properties occurs in embryogenesis, as well as wound healing and metastasis of tumor cells. The EMT leads to drug resistance. This phenomenon is caused by epigenetics. The following process occurs in the EMT.

(i) Disassembly of cell-cell contacts
Loss of epithelial markers (E-cadherin, claudin, occludin), decreased number of structures of inter-cell attachment (tight junction, adherence junction, desmosome), and consequently, loss of polarity of cells, lead to loss of cell-cell contacts.

(ii) Changes of the cytoskeleton (cytoskeletal modification)
In the EMT, changes of the cytoskeleton occur, and tumor cells gain cellular motility. Actin forms stress fibers, and intermediate filaments change from cytokeratin to vimentin.

(iii) Changes in gene expressions
Gene expressions that are controlled by SNAIL, ZEB, and the bHLH family, which are transcription factors, change in the EMT process.

(iv) Gain of motility
The motility is acquired by the switch from E-cadherin to N-cadherin, rearrangement of the cytoskeleton, and expression of matrix metalloprotease.

II. Immune antibodies
1) Mechanism of resistance to immune antibodies (antibody-based biopharmaceuticals)
Mechanisms of resistance to immune antibodies are 1) decreased expression of antigens of tumor cells, 2) increased DNA repair activity, and 3) increased expression of drug export pumps.

2) Drug actions of rituximab
Rituximab is an antibody drug that selectively binds the CD20 surface antigen that appears on the surface of B lymphocytes, and it is one of the molecular target drugs. Rituximab binds to CD20 and activates the human immune response to attack tumorous
B lymphocytes and is effective against B cell lymphomas\cite{35-37}.
It is considered that rituximab’s B-lymphocyte-damaging action is mediated through pre-complementary cytotoxicity (complement-dependent cytotoxicity) and antibody-dependent cell-mediated cytotoxicity.
Rituximab was found to lyse human-derived CD20-positive cells in the presence of human complement, but it did not lyse human-derived CD20-negative cells, confirming that it has complement-dependent cytotoxicity for cells having the CD20 antigen. In addition, rituximab was found to lyse human CD20-positive cells in the presence of human effector cells, but it did not lyse human CD20-negative cells, confirming that it has antibody-dependent cell-mediated cytotoxicity with respect to cells having the CD20 antigen.

3) Mechanisms of resistance to rituximab\cite{38-42}
(1) Inhibition of conjunction between rituximab and CD20
Decreased expression of CD20, increased internalization of CD20, and abnormalities of CD20 gene cause inhibition of conjunction between rituximab and CD20.

(2) Abnormality of reactivity after conjunction between rituximab and CD20
Abnormal reactivity includes abnormal signal transduction, apoptosis, clustering to lipid rafts, and complement conjunction.

(3) Abnormality of reactivity of effector cells (NK cells, monocytes, macrophages)
Abnormality of effector cells causes destruction of CD20-positive cells with rituximab.

4) Drug actions of trastuzumab
Drug actions of trastuzumab, which is a humanized monoclonal antibody against human epidermal growth factor receptor 2 (HER2) (15-20% of breast cancers express HER2), including disturbing the Ras-MAPK pathway, inhibiting proliferation of cancer cells, and inhibiting the PI3K-Akt-mTOR pathway, causing increased apoptosis of cancer cells and induction of antibody-dependent cellular cytotoxicity (ADCC) by NK cells, monocytes, and macrophages. However, in some HER2-positive patients, cases refractory to trastuzumab are observed, and in half of the patients who were responsive to trastuzumab, resistance to trastuzumab occurred within one year.

5) Mechanisms of resistance to trastuzumab\cite{43}
(1) Hetero-dimer formation with HER2 and HER family
Trastuzumab inhibits homodimer formation of HER2/HER2. In resistant cancer cells, more heterodimer formation with HER2 and HER family (HER1, HER3, HER4) occurs than in sensitive cells. Heterodimer of HER shows survival and proliferation activity in tumor cells.

(2) Activation of downstream signaling pathways
Increased expression of the PI3K/Akt signaling pathway is observed in resistant cells.

6) Mechanism of drug action of immune checkpoint inhibitors
Cancer cells express neoantigen, which is different from antigens expressed in the host. The neoantigen is recognized by dendritic cells, and dendritic cells express the neoantigen with major histocompatibility complex (MHC) class I or II on their surface. CD4-positive T cells recognize the neoantigen combined with MHC class II on dendritic cells, and they are activated by co-stimuli with the conjunction of CD28 on the T cells and CD28 ligands (CD80 or CD86) on the dendritic cells. CD8-positive T cells become cytotoxic T cells (CTL) by recognizing neoantigen combined with MHC class I through T cell receptors. To avoid excess immune reactions, cytotoxic T lymphocyte-associated protein-4 (CTLA-4) is expressed on the CD4-positive T cells. By binding with CTLA-4 and CD28 on T cells, CD4 proliferation and inflammatory cytokine expressions are inhibited. CTL expresses programed cell death 1 (PD-1). When PD-1 on CTL binds programmed cell death ligand-1 (PD-L1) expressed on the dendritic cells and/or tumor cells, cytokine production and cytotoxic activity by CTL are suppressed. Immune checkpoint inhibitors (anti-CTLA antibody, anti-PD-1 antibody) block inhibitory actions caused by CTLA-4 and PD-1/PD-L1. Recently, Toffalori et al reported that in relapsed patients after allogenic hematopoietic cell transplantation, the increase in the expression of PD-1 on donor-derived T cells paralleled the rise of minimal residual disease markers and anticipated clinical relapse.

7) Drug action of anti-CTLA-4 antibody
Anti-CTLA-4 antibody stimulates production of interferon-γ. Then, interferon-γ activates interferon-γ receptor on the cancer cells and activates the JAK/STAT pathway. Activating the STAT pathway leads to upregulation of MCH class I, and consequently, increases targeting against activated T cells.

8) Drug actions of anti-PD-L1 antibody
Anti-PD-L1 antibody blocks the interaction between PD-1 on T cells and PD-L1 on
dendritic cells and tumor cells and leads to restoration of suppression of the immune mechanism.

9) Resistance mechanisms to anti-CTLA-4 antibody\textsuperscript{45}
In anti-CTLA-4 antibody-resistant cells, inactivation of the JAK/STAT pathway by a decreased copy number of activation genes and an increased copy number of inhibitory genes occurs.

10) Resistance mechanism to anti-PD-L1 antibody\textsuperscript{46}
Mutations of JAK1 or JAK2 cause lack of function of JAK1 or 2, or inactivation of the STAT pathway leads to decreased expression of MCH class I. So far, JAK mutation is a major mechanism against anti-PD-L1 antibody.

**Conclusion**
Drug resistance is a serious problem in cancer therapy. Among the mechanism of drug resistance, I consider that epigenetic mechanisms may play potent roles in drug resistance. To overcome resistance to drugs against cancers in children, we need to explore the epigenetic mechanisms of drug resistance.

Conflict of Interest: None declared
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Table 1

1) Drug actions of 6-mercaptopurine
(1) Transported into the tumor cells and becomes thioinosine monophosphate (TIMP)
(2) Inhibition of synthesis of adenyl acid and guanyl acid.

2) Mechanisms of resistance to 6-mercaptopurine
(1) Inactivation by xanthine kinase
(2) Decreased expression of thiopurine methyltransferase#
(3) Decreased expression of thiopurine methyltransferase (TPMT) by polymorphism

3) Drug actions of anti-folic acid drugs (methotrexate)
(1) Mammals cannot synthesize folic acid.
(2) Folic acid needs to be imported actively into cells by reduced folate carrier (RFC) and is used for DNA synthesis.
(3) Methotrexate is actively transported into tumor cells by the same reduced folate carrier (RFC).
(4) Methotrexate binds to dihydrofolate reductase by folypoly-glutamate synthetase.
(5) Methotrexate inhibits deoxidation of the oxidative type of folic acid and leads to decreased deoxidation of folic acid.
(6) Methotrexate inhibits DNA synthesis along with thymidylate synthase and causes cell death.

4) Mechanisms of resistance to methotrexate
(1) Decreased expression of reduced folate carrier (RFC), which causes decreased uptake of folic acid.
(2) Increased expression of thymidylate synthase
(3) Increased copy number of dihydrofolate reductase (DHFR) gene and/or mutation of dihydrofolate reductase (DHFR) gene
(4) Inhibit polyglutamine oxidation by decreased expression of folypoly-glutamate synthetase##

#The decreased expression of thiopurine methyltransferase (TPMT) might be caused by increased methylation of the promoter region of the TPMT gene or decreased acetylation of the TPMT gene.
##Decreased expression of reduced folate carrier, increased expression of thymidylate synthase, and decreased expression of folypoly-glutamate synthetase might be caused by epigenetic mechanisms.