Dean&Francis

ISSN 2959-409X

Experimental research to verify the effect of targeted drugs in epigenetic cancers

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Abstract:

Epigenetic regulation plays a key role in the development of cancer, and drugs targeting epigenetic modifications (such as DNA methyltransferase inhibitors, histone deacetylase inhibitors, etc.) [2] have become new strategies for cancer treatment. This study aims to verify the anti-tumor effect and mechanism of action of a novel epigenetically targeted drug LSD1 in vitro and in vivo models. Through cell proliferation assays, epigenetic modification detection, transcriptome analysis, and xenograft tumor models, we have confirmed that LSD1 can significantly inhibit cancer cell growth and reverse abnormal epigenetic modifications. This study provides an experimental basis for the development of epigenetic anticancer drugs. Epigenetics is a theory proposed by Waddington in 1942, which refers to heritable changes caused by the unchanged DNA sequence. Many phenomena that cannot be explained by traditional genetics can be well explained by epigenetics. For example, identical twins have exactly the same DNA sequence, but sometimes they can show very different phenotypes; inactivation of female X chromosome and gene silencing. Epigenetic research mainly includes DNA modification, histone modification, chromatin remodeling, and regulation of non-coding RNAs. Among them, DNA methylation and histone modification are the most thoroughly studied, and many inhibitors have been produced for protein factors involved in these modifications.

Keywords: epigenetics, cancer treatment, targeted drugs, DNA methylation, histone modifications

1. Introduction

Aberrant alterations in epigenetic modifications (e.g., DNA methylation, histone modifications) are important features of cancer and can lead to silencing of tumor suppressor genes or activation of proto-oncogenes [1]. In recent years, epigenetically targeted drugs (e.g., 5-azacytidine, vorinostat) have shown anti-cancer potential in clinical practice [4]. However, drug resistance and toxic side effects limit their application, so it is crucial to develop new epigenetic

drugs with high efficiency and low toxicity.

In this study, the drug LSD1, a novel histone deacetylase inhibitor, was used to verify its antitumor effect and explore its molecular mechanism through in vitro and in vivo experiments.

2. Existing research results

2.1 DNA methyltransferase inhibitors

DNA methyltransferase inhibitors such as azacitidine and decitabine are currently widely studied and used epigenetic therapeutics. They can inhibit the activity of DNMT, demethylating DNA, thereby restoring the expression of some tumor suppressor genes that are silenced by hypermethylation [3]. Among hematologic malignancies, azacitidine and decitabine have been approved for the treatment of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), among others. Clinical studies have shown that azacitidine therapy can achieve complete response in some patients with AML who are not candidates for intensive chemotherapy or hematopoietic stem cell transplantation [6]. In the treatment of MDS, these drugs are also effective in improving the patient's condition, improving the quality of life, and prolonging survival.

2.2 Histone deacetylase inhibitors

Histone deacetylase inhibitors (HDACi) can inhibit the activity of HDAC, increase the level of acetylation of histones, loosen the chromatin structure, and promote gene expression [2]. At present, a variety of HDACi have entered the clinical trial stage, such as vorinostat, romidepsin and others. In the treatment of lymphoma, HDACi combined with chemotherapy or immunotherapy has achieved certain efficacy. For example, in the treatment of diffuse large B-cell lymphoma, HDACi combined with drugs such as rituximab can achieve complete remission in some patients [6]. In addition, HDACi has shown some potential in the treatment of other solid tumors such as breast cancer and lung cancer, but its efficacy is limited when used alone, and it often needs to be used in combination with other treatments [4].

2.3 Dual-target or multi-target epigenetic modulators

With the in-depth understanding of epigenetic mechanisms, the development of dual-target or multi-target epigenetic modulators has become a new research direction. For example, the new generation of "epigenetic modulators" JBI-802 has an inhibitory effect on two epigenetic targets, histone demethylase 1 (LSD1) and histone deacetylase 6 (HDAC6) [5]. Preclinical studies have shown that JBI-802 has better synergistic anti-cancer effects than the previous generation of single-target drugs, and the safety profile is good. In a phase 1 clinical trial, JBI-802 showed good antitumor activity in 2 patients with small cell lung cancer that was resistant to immunotherapy, resulting in significant tumor shrinkage and symptom relief [7].

3. Materials and methods

3.1 Cell culture and drug treatment

Cell line: Human cancer cell lines (such as MCF-7 breast cancer, A549 lung cancer, HCT116 colon cancer) and normal control cells (such as MCF-10A) are selected.

Drug treatment: The experimental group was treated with LSD1-DMSO solution gradient concentration (0.1-10μM) for 48-72 hours, and the control group was treated with DMSO for the same time.

3.2 Cell proliferation and apoptosis detection

During the drug treatment process, the following indicators of the experimental group and the control group were tested and compared every 4 hours

· CCK-8 method: Detect cell viability.

Flow cytometry: AnneLSD1in V/PI double staining to detect apoptosis rate.

Clone formation experiments: Evaluate the effects of long-term drug effects.

3.3 Epigenetic modification analysis

During drug treatment, the experimental group and the control group were tested for genetic modifications every 4 hours and compared

· DNA methylation detection: Methylation-specific PCR (MSP) or whole genome methylation sequencing (WGBS) analyzes the methylation status of tumor suppressor genes (e.g., p16, BRCA1).

Histone modification detection: Western blot detects the modification levels of histone H3K27ac, H3K9me3 and other modifications.

ISSN 2959-409X

· ChIP-qPCR: Validation of histone modification changes in promoter regions of specific genes.

3.4 Transcriptome sequencing (RNA-seq)

During drug treatment, transcriptome sequencing was performed on the experimental group and the control group every 4 hours, and differentially expressed genes (DEGs) were analyzed before and after drug LSD1 treatment, and GO/KEGG enrichment analysis was performed.

3.5 In vivo experiments (xenograft tumor model)

Animal model: BALB/c nude mice with the same physical condition were subcutaneously inoculated with HCT116 cells and randomly divided into an equal number of control group (normal saline) and drug LSD1 group (10mg/kg, intraperitoneal injection, three times a week).
Tumor volume monitoring: Measure the tumor size of the two groups of nude mice every three days, and take the average value multiple times and compare them.
Immunohistochemistry (IHC): The expression indexes of Ki-67 (proliferation marker) and Cleaved Caspase-3 (apoptosis marker) were detected and compared between the two groups of nude mice.

3.6 Statistical analysis

The data were expressed as mean ±SD, t-test or ANOVA analysis, and *P<0.05 was considered statistically significant.

4. Results

4.1 The drug LSD1 inhibits the proliferation of cancer cells and induces apoptosis

· CCK-8 experiments showed that the drug LSD1 inhibited cancer cell viability in a concentration-dependent manner (IC50≈2μM).
Flow cytometry showed that the apoptosis rate increased significantly after 48 hours of treatment with 10 μM LSD1 (P<0.01).

4.2 Drug LSD1 reverses abnormal epigenetic modifications

· MSP analysis showed that the drug LSD1 reduced the level of methylation of the promoter of the p16 gene.
· Western blot showed a significant increase in histone H3 acetylation level (P<0.05).

4.3 RNA-seq reveals the key signaling pathway regulated by drug LSD1

· DEGs are enriched in cell cycle, apoptosis, and epigenetic regulatory pathways (e.g., p53, Wnt signaling pathways).

4.4 In vivo experiments confirm the anti-tumor effect of the drug LSD1

The tumor volume of the LSD1 group was reduced by 50% compared with the control group (P<0.01).
· IHC displays that Ki-67 expression is reduced, Cleaved Caspase-3 expression is elevated.

5. Conclusion

This study confirmed that the drug LSD1 inhibits cancer cell proliferation and induces apoptosis by regulating DNA methylation and histone modification. The application of LSD1 can inhibit the occurrence of abnormal methylation, thereby activating silent tumor suppressor genes to achieve the purpose of treating tumors. Due to the inherent cytotoxic effect of LSD1, the non-covalently bound non-nucleoside DNA methylase inhibitors have high specificity, which can directly block the active site of DNA methylases, thus avoiding the toxic side effects caused by the covalent binding of nucleoside DNA methylase inhibitors to enzymes. RNA-seq analysis suggests that it may play a role through the p53 pathway and promote histone modification. In vivo experiments further verified its anti-tumor effect, and no significant toxicity was observed.
Compared with existing epigenetic drugs such as vorinostat, the drug LSD1 has higher selectivity and lower toxicity side effects, but further clinical research is still needed to optimize the dosing regimen. ’
As a new epigenetically targeted drug, LSD1 has shown significant anti-cancer activity in vitro and in vivo, and greatly reduced the toxicity and side effects of traditional epigenetically targeted drugs. The mechanism involves epigenetic reprogramming and apoptosis induction. This study lays the foundation for subsequent clinical trials.

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