MINI-REVIEW

Update of Research on Drug Resistance in Small Cell Lung Cancer Chemotherapy

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Abstract

Small cell lung cancer (SCLC) is characterized by a short cell doubling time, rapid progression and early occurrence of blood-borne and lymph metastasis. The malignancy is the highest of all lung cancer types. Although SCLC has a relatively good initial response to chemotherapy as well as radiotherapy, relapse or disease progression may occur quickly after the initial treatment. Drug resistance, especially multi-drug resistance, is the most important cause of failure of SCLC chemotherapy. This article provides a brief update of research on mechanisms of drug resistance in SCLC and reversal strategies.

Keywords: Small cell lung cancer (SCLC) - chemoresistance - targeted therapy

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Introduction

Small cell lung cancer (SCLC) originates from neuroendocrine bronchial cells. It accounts for approximately 15%-20% of all cases of lung cancer throughout the world (Rodriguez et al., 2010). Compared with non-small cell lung cancer (NSCLC), SCLC is considered distinct from other lung cancers because of their clinical and biologic characteristics. SCLC exhibits aggressive behavior, with rapid growth, early spread to distant sites, and therefore its malignancy is the highest of all lung cancer types.

Although SCLC has a relatively good initial response to chemotherapy as well as radiotherapy, relapse or disease progression may occur quickly, and the 5-year survival is in less than 2% (Jackman et al., 2005). Drug resistance, especially multi-drug resistance (MDR), is the most important reason for failure of SCLC chemotherapy. Acumulating evidences show that either a single factor alone or multiple factors together could lead to MDR, although none of these mechanisms could fully explain MDR, indicating that there are other unknown mechanisms of MDR. MDR is therefore a crux that needs to be solved urgently in chemotherapy. The mechanism of MDR is complex, because tumor cells can produce MDR through various pathways. This article is a review of the update of research on mechanisms of drug resistance in SCLC and reversal strategies in recent years.

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SCLC Drug Resistance Mechanisms

The mechanism of MDR in SCLC is complex, involving multiple factors. The known drug resistance mechanisms include over-expression of outer membrane proteins such as P-glycoprotein (P-gp) as a member of the ATP-binding cassette (ABC) family and lung resistance-related proteins; abnormality of the intracellular enzyme system such as topoisomerase (Top) and gamma-glutamyl transpeptidase (γ-GGT); enhancement of the cell repair system; and dysregulation of cell apoptosis, such as over-expression of anti-apoptotic gene Bcl-2 and c-myc. Changes of the key genes involved in these processes at either genetic or epigenetic level could trigger the formation of drug resistance phenotypes of tumor cells (Roberti et al,2006).

SCLC gene expression profiles are relatively specific, including activation of proto-oncogenes and inactivation or loss of tumor suppressor genes. Fong et al. (1999) classified SCLC as classic and variant types. The former has relatively high differentiation and sensitivity to chemotherapy, while the latter has relatively low differentiation, a quick growth rate and poor chemotherapy

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response. In the variant type, proliferation and up-regulation of c-myc expression are often found in SCLC metastatic foci with variant cells, while in the classic type the c-myc expression level is relatively low.

Abnormal Expression of Membrane Protein Drug Pumps

MDR refers to drug resistance of tumor cells to a particular chemical, as well as cross resistance to other chemicals of unrelated structures and different action mechanisms. MDR is related to over-expression of outer membrane proteins such as P-gp of the ABC family and MDR-related proteins such as MDR1, MRP1 and MRP2. It was found that P-gp and MRP-1 expression levels were increased in drug resistant SCLC tissues and cell lines cultured in vitro (Yeh et al., 2005). MDR is also related to high expression of lung resistance-associated protein (LRP), which mediates DNA-targeted chemotherapeutic agents by preventing cisplatin, carboplatin and other alkylating agents from entering the nucleus. It plays a role of an intermediate pass by preventing drugs from entering the nucleus, or transporting the drugs that have entered the nucleus out of the nucleus via transporters, or transporting the drugs in the cytoplasm to transport vesicles and distributing them in an atrioventricular form, and then excreting the drugs out of cells via the molecular mechanism of exocytosis. Recently, it was reported that low expression of microRNA (miRNA) miR-134 was closely correlated with up-regulation of drug-resistant MRP1 in SCLC cells (Guo et al., 2010), suggesting the regulation of MDR-related proteins by epigenetic mechanisms.

Abnormality of intracellular enzyme systems

DNA topoisomerase (Top) is a ribozyme for DNA replication and transcription. Top inhibitors are the most commonly used chemotherapeutic agents in SCLC treatment. Down-regulation of Top expression and change in the type of expression are important reasons for SCLC resistance to Top inhibitors (GUAN et al., 2011). A recent study (Song et al., 2005) found that transient transfection with liposome-mediated artificial siRNA could effectively induce the inhibition of Top I and sensitivity to Etoposide of human SCLC cell H446, suggesting that the down regulation of Top I expression was accompanied with an upregulation of Top II expression. The inhibitory effect of Top I on Top I and II inhibitors may be related to compensation of the two enzymes. Both in vitro and in vivo studies have demonstrated that combined use of Top I and II inhibitors could yield an obvious synergistic effect (Knez et al., 2011).

Lawson et al. (2011) used cDNA chips to screen and analyze drug resistance-related genes in SCLC, and artificially changed the expression level of the candidate target genes in order to observe their effect on SCLC chemosensitivity. The result showed that the high expression of DNA polymerase β and neuroendocrine transcription factor NKX2.2 may be related with SCLC resistance to etoposide. This conclusion was also confirmed in SCLC tissue chip analysis.

Abnormality of Cell Apoptosis

Apoptosis escape is a common feature of tumor cells, and also an important reason for SCLC chemoresistance. Mechanisms are regarded as follows: activation of anti-apoptotic pathways via extracellular signals; insensitization of the endogenous cell death machinery via resisting apoptotic protein "addiction"; and loss of apoptosis-promoting tumor suppressor genes via accumulated mutations.

Integrin plays an important role in inducing cell apoptosis and blocking metastasis. In cell adhesion-mediated drug resistance, extracelluar matrix (ECM) protein can resist apoptotic signals induced by cytotoxic drugs. ECM increases the expression of PKB and GSK3β in the downstream target area and blocks cell apoptosis through mediation of β1 integrin (Hodkinson et al., 2007) CD9 is a member of the tetraspanin family, and is over-expressed in metastatic SCLC tissues and cisplatin or carboplatin-resistant SCLC cell lines. Mediated by β1 integrin, drug resistant SCLC cells positive for CD9 bind with fibronectin more closely, making them less sensitive to chemotherapy-induced apoptosis by activating the PI3K/AKT/mTOR pathway. Use of CD9-sepcific monoclonal antibody ALB6 or small interfering RNA (siRNA) could trigger apoptosis of the above drug-resistant cells (Kohmo et al., 2010).

Interestingly, activation of the PI3K/AKT/mTOR pathway can promote glycolysis, and on the other hand the PI3K/AKT/mTOR pathway depends on sufficient glucose to obtain energy (Mason et al., 2010). Only when there is sufficient glucose can activated AKT prevent degradation of anti-apoptotic protein Mcl-1 and inhibit expression of pro-apoptotic protein Bim, thus promoting apoptosis escape of tumors cells and increasing their survival. Insufficient glucose could trigger expression of pro-apoptotic protein Bim and Puma, promoting apoptosis of tumor cells (Coloff et al., 2011).

In more than 80% SCLC cases, there is a loss of wild-type p53 activity. Inhibition of the expression of cyclin-dependent kinase inhibitors p21 (WAF1) may cause abnormality of cell cycle checkpoints, and eventually lead to uncontrolled cell proliferation and apoptosis blockage, which are known to be associated with disease progression, poor prognosis and chemoresistance (Gemba et al., 2000).

The B-cell lymphoma-2 (Bcl-2) family proteins are crucial factors in the regulation of apoptosis, vital for proper tissue development and cellular homeostasis. Overexpression of Bcl-2 is often detected in SCLC tissues and in drug resistant SCLC cell lines induced in vitro (Sartorius et al., 2002).

Enhancement of Cell Repair Systems

Studies in recent years show that DNA mismatch repair (MMR) plays a very important role in the progression of SCLC chemoresistance. Some researchers found that down-regulation of MMR gene MLH1 and MSH2 may

be associated with the occurrence and MDR of SCLC, but the exact mechanism still remains unclear. It may be linked to silencing of MMR genes induced by acetylation and phosphorylation of histones and hypermethylation of promoters (Morimoto et al., 2005).

New Strategies to Overcome SCLC Drug Resistance

Drug resistance-reversing agents

Targeted mitochondrial apoptosis pathway is an SCLC drug resistance-reversing strategy that has been studied most extensively at present. Over-expression of anti-apoptotic gene Bcl-2 was commonly found in SCLC cell lines and tumor tissues (Hann et al., 2008), which participated in apoptosis escape of tumor cells and was closely associated with chemoresistance (Rudin et al., 2004). Bcl-2-tragetd inhibitors include two categories: antisense oligonucleotides and small molecular weight inhibitors. It was found in a phase I clinical trial that Bcl-2 targeted inhibitor Oblimersen relieved 86% of untreated ES-SCLC cases (Oltersdorf et al., 2005). A clinical trial (NCT00445198) on small molecular weight stimulant ABT-263 specific to apoptosis-promoting protein Bad is underway in SCLC patients (Tahir et al., 2010). It was found in a pre-clinical study that drug resistance to ABT-263 in a SCLC cell line and a transplanted tumor model was accompanied with the increased concentration of apoptosis-promoting protein Bax, Bim and NOXA (Harley et al., 2010).

It is noteworthy that spindle toxic drugs such as taxol-like drugs may cause mitotic arrest by activating spindle assembly checkpoints, which may activate two independent but mutually competing pathways: 1) inducing degradation of anti-apoptotic protein Mcl-1 and causing mitochondrial outer membrane permeability (MOMP), thus promoting apoptosis of tumor cells (Tan et al., 2011; Wertz et al., 2011) and 2) escaping from mitotic arrest through slow hydrolysis of cyclin-B and entering the next cycle of G1 or death, or remaining in this cycle of abnormal G1 in the tetraploid form, or continuing with proliferation. These findings suggest that combined use of spindle toxic drugs and apoptosis-targeted inhibitors may produce a synergistic pro-apoptotic effect through double inhibition of anti-apoptotic protein Mcl-1 and Bcl-xl. It has been demonstrated in SCLC cell line in vitro and NSCLC transplanted tumor models that combined use of Bcl-xl-targeted inhibitor Navitoclax (ABT-263) and spindle toxic drugs could induce apoptosis of tumors cells in the phase of mitotic arrest (Inoue et al., 2010).

New chemotherapeutic agents

New chemotherapeutic agents for SCLC include amrubicin, SABA, picoplatin, belotecan and vinflunine. Amrubicin is a synthetic anthracycline that blocks NDA repair by inhibiting Top I. It has been approved as the first line drug for ES-SCLC in Japan. Clinical trials have shown that the response rate of combined use of amrubicin and picoplatin is as high as 88%, and the median survival is 13.6 months. It could expectedfully replace irinotecan (Tang et al., 2011). Picoplatin is a platinum analogue that can overcome platinum drug resistance. Phase II clinical trials have shown its therapeutic activity in patients with relapsed SCLC due to sensitivity and drug resistance (Jeong et al., 2010). The ototoxicity and nephrotoxicity of picoplatin are lower than those of other platinum drugs. Belotecan is a new-type hydroxy camptothecin analogue, and phase II clinical trials have shown its good activity (Demedts et al., 2010).

Molecularly targeted therapy

Advances in molecular biology have provided SCLC treatment with many new targets. Some molecularly targeted drugs (combined use or single use) have entered phase II clinical trials, including matrix metalloproteinase (MMP) inhibitors, thalidomide, biological vaccines, and small molecular weight inhibitors directed at receptor protein tyrosine kinases such as EGFR, c-Kit and VEGFR. Unfortunately, the therapeutic effects are unsatisfactory. At present, no single molecularly targeted drug has been approved for routine clinical treatment of SCLC (Rigas et al., 2003).

MMPs regulate extracellular matrix modeling, and play an important role in maintaining cell growth and morphology. Up-regulation of MMP is considered a marker for poor prognosis of SCLC. Synthetic MMP inhibitors can inhibit SCLC infiltration, metastasis and angiogenesis. However, some large-scale randomized phase III clinical trials suggest that use of MMP inhibitor marimastat and tanomastat as maintenance therapy after failing induction therapy did not seem to prolong the survival of NSCLC patients (Dy et al., 2002).

KIT gene can encode transmembrane tyrosine kinase growth factor receptor of the platelet-derived growth factor receptor (PDGFR) family. 80% of SCLC tumors express c-Kit protein, stimulating cell growth signaling pathways in an autocrine or paracrine manner. Imatinib can inhibit activities of c-Kit receptor tyrosine kinase, bcr/abl fusion protein and PDGFR tyrosine kinase (Spigel et al., 2007). However, phase II clinical trials have shown that imatinib did not prolong progression-free survival (PFS) of sensitivity and drug resistance-induced relapse of SCLC patients whose c-Kit was highly expressed, and no potentiating effect was observed when it was used on the basis of the platinum+irinotecan protocol (Lee et al., 2008).

p53 protein can respond to DNA injury signals, activate the growth arrest pathway (inhibiting DNA repair) and the apoptosis pathway. The prognosis of SCLC patients with p53 gene loss or mutation is usually poor, often accompanied with resistance to radiochemotherapy. Antonia et al (Antonia et al., 2006) used a dendritic cell (DC) vaccine that was transfected with full-length wild-type p53 gene for pretreatment of SCLC patients before initiation of systemic chemotherapy, and achieved an overall response rate (ORR) of 61.9% in patients with relapsed and progressive SCLC.

Thalidomide is a multi-target angiogenesis inhibitor, and can inhibit vascular endothelial growth factor (VEGF), fibroblast growth factor β (FGFβ) and tumor necrosis factor α (TNFα), and modify intracellular matrix. Phase II clinical trials using thalidomide on the

basis of carboplatin+etoposide and using thalidomide for maintenance therapy showed satisfactory tolerance and response (Khanzada et al., 2006), and this protocol has entered phase III clinical trail.

It was found in pre-clinical studies that simvastatin could inhibit SCLC growth, induce tumor cell apoptosis, and increase the sensitivity of SCLC to etoposide. Pravastatin also exhibited its inhibitory effect on SCLC growth and sensitizing effect on chemotherapeutic agents (Weiss et al., 1999). Phase III clinical trials on pravastatin in combination with the first-line chemotherapy protocols are under way in the United Kingdom (NCT00433498).

Summary

Using gene and tissue chip techniques to compare differentially expressed gene/protein profiles in sensitive and drug resistant SCLC tissues/cells may provide new research methods for exploring the mechanism of SCLC drug resistance and potential therapeutic targets, which is of significance especially in the treatment of relapsed SCLC cases with drug resistance. It is noteworthy that as in vitro cultured cell lines are outside the tumor microenvironment in vivo, their phenotypes and biologic behaviors are somewhat different from those in vivo. Therefore, SCLC cells from biopsy or surgical specimens may have a more practical research value. How to converse the research results in vitro to actual clinical situations remains to be a problem in SCLC drug resistance research.

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