

Genetic Mutations and Humanized Monoclonal Antibody Treatment in Small-Cell Lung Cancer: A Review

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Abstract

Small-cell lung cancer (SCLC) is one of the deadliest type of cancer with fast tumor growth and rapid metastatic dissemination. The most common genetic modifications associated with SCLC is connected to tumor suppressor and oncogenic genes, genes that control the cycle arrest of the cells. Standard treatment for SCLC is a combination of drugs such as cisplatin and etoposide and sometimes radiotherapy of the chest can be used. But these treatments have not been sufficient to decrease death rate in SCLC patients and new drugs have been studied, specially humanized monoclonal antibodies (mAb). Therefore, the aim of this work is to review the main genetic alterations in SCLC and the mAb that has been tested to improve life expectancy in these patients.

Keywords: SCLC; Lung cancer; Humanized monoclonal antibody; Tumor suppressor genes

Introduction

Small-cell lung cancer (SCLC) is one of the most malignant neoplasia and it is characterized by fast tumor growth and early metastatic dissemination [1,2]. The majority of SCLC cases are already metastatic at the time of diagnosis, and the patients need effective systemic therapies [3]. The standard therapy for SCLC is a combination of cytotoxic drugs, such as cisplatin or carboplatin with etoposide [4]. The survival rate for this cancer is 2% and it has not changed over the past 30 years [5,6]. There are no FDA-approved specific drugs for SCLC and there is a profound need to find novel forms of diagnosis and therapies to elevate the survival rate of this type of cancer [7].

The most common genetic alterations in SCLC are related to tumor suppressor and oncogenic genes. Examples of genes are TP53, RB1, MYC, PI3K/AKT pathway, DLL3, RTK, FGFR1, EGFR and KRAS [8]. MicroRNAs (miRNAs) are non-coding RNA that regulate translational processes. MiRNAs have also been related to SCLC although their role is not completely understood [9].

The first line treatment of SCLC consists in isolated chemotherapy and chemoradiotherapy [10]. Patients normally respond very well in the beginning of the treatment but the survival rates are still very low. New types of therapeutic approaches have been studied and humanized monoclonal antibodies (mAb) have been tested [8].

Therefore, the aim of this study is to revise the main genetic alterations associated to SCLC and the mAb that has been tested to improve drug therapy in this type of cancer.

Clinical Aspects

Small-cell lung cancer (SCLC) is a neuroendocrine most aggressive form of lung cancer [1,2]. It differs from non-small-cell lung cancer

(NSCLC) for its rapid growth, early metastatic dissemination and good initial response to chemotherapy and ionizing radiotherapy [2].

SCLC represents about 15% of all cases of lung cancer, behind the adenocarcinoma and epidermoid carcinoma histological types that belongs to NSCLC [12]. SCLC is divided according to its staging, being classified as limited stage when restricted to ipsilateral hemitorax, or extensive stage when it is confirmed metastatic sites by physical examination or image diagnosis [8]. Approximately 2/3 of the patients are diagnosed in the extensive stage of the disease and due to its highly metastatic potential a large part is symptomatic and presents multiple metastasis in organs such as brain, liver, bones and lymph nodes that causes symptoms as pain, paresis and dyspnea [9,12,13].

At long term, survival is really rare for these patients. 12 months is the medium deadline of survival after the diagnosis [10,11,14]. The main risk factor for SCLC is smoking, a characteristic that is present in 90% of all SCLC patients. The risk of developing this cancer increases the larger the exposure time to tobacco carcinogenic agents, since they induce mutations that end in malignant transformation in lung cells changing its adhesion, migration and resistance abilities [11,15]. Although smoking is the main factor to initiate SCLC, passive smoking and radon, asbestos and arsenic exposure are also described as important factors [2,16].

Genetic Mechanisms

Genetic alterations most frequently found in resected tumor tissues of patients with SCLC are tumor suppressor genes inactivation, specially TP53 and RB1 genes, MYC amplifications, histone-modifying mutations (CREBBP and EP300), changes in PI3K/AKT/mTOR pathway (PIK3CA, PTEN, AKT2, AKT3, RICTOR and mTOR), DLL3 expression modifications and changes in activation of receptor tyrosine kinase (RTK) genes, specially FGFR1. There is rare oncogenic involvement but the most common oncogenes involved are epidermal growth factor receptor (EGFR) gene and KRAS gene [17].

Loss of function of the tumor suppressor genes TP53 and RB1 are present in about 75% to 90% of the SCLC patients and it plays an

important role in the cancer pathogeny [12,18]. This genetic modification is correlated to mutations in 3p chromosome that codifies several tumor suppressor genes [19]. These genes when concomitantly mutated can act in type 2 alveolar cells to present SCLC phenotype [20].

TP53 gene suppresses directly or indirectly the expression of numerous target genes as a master regulatory suppressor gene, blocking cell proliferation or initiating cell death programs, specially apoptosis, to suppress tumor development and growth [21]. TP53 gene also down-regulates IDO-1 gene, a gene that can exert a potent immunosuppressive effect by inhibition of T-lymphocytes and other immune cells [22]. IDO-1 has been shown to induce immunosuppression to favor tumor growth and progression in animal models of lung cancer [23,24]. When TP53 is suppressed, IDO-1 expression is increased and it is highly associated with lymph node-metastasis [22].

RB1 gene participates of the cell cycle regulation suppressing cell transition from G1 phase to S phase and so regulating cell differentiation. TP53 in hypoxia or cell damage situation can play a role in genomic integrity maintenance, stopping the cell cycle or inducing cell death. Thus, it is possible to relate genetic abnormalities of one of these genes with genomic instability [12]. Another gene related to SCLC are the MYC genes that codify transcription factors that regulate other genes that act in the cell cycle progression and in development regulation. Amplifications or pronounced expression of these genes are found in approximately 20% to 30% of the SCLC patients [12,18].

Another important tumor suppressor gene involved in SCLC is the phosphatase and tensin homolog (PTEN) gene localized in 10q23.31 chromosome that inhibits the oncogenic pathway of phosphatidylinositol 3-kinase (PI3K). Some studies have demonstrated that homozygous deletions in PTEN gene are present in 10% of the SCLC patients. These deletions cause a greater activation of the PI3K pathway which leads to uncontrolled cell growth and proliferation [12,25].

Angiogenesis is known to be important in the development and maintenance of tumors, and it is responsible for the recruitment of new blood vessels from pre-existing vessels, occurring in response of the high demand of oxygen and nutrients by tumor cells [8,26]. Vascular endothelial growth factor (VEGF) genes are frequently very expressed in SCLC patients [8], and fibroblast growth factor (FGF) and angiopoietin-2 genes also sustain the continuous activation of angiogenesis [27].

The insulin-like growth factor type 1 receptor (IGF-1R) gene is also highly expressed in SCLC patients which is associated to a bad prognosis since IGF-1R pathway is activated in mitogenic, anti-apoptotic and metastatic events [28,29]. The human epidermal growth factor type 2 receptor (HER2) gene is also correlated to a bad prognosis in SCLC patients since when the tumor cells are positively regulated to HER2 expression the cells often become chemo resistant [13,30].

Delta-like protein 3 (DLL3) gene codifies a highly tumor-selective cell surface protein expressed in high-grade neuroendocrine lung tumors including SCLC [32]. The DLL3 protein is an inhibitory ligand of the Notch signaling pathway that is normally expressed exclusively on intracellular membranes [32]. In SCLC patients, DLL3 is associated with clonogenic capacity, early metastatic spread and rapid tumor

repopulation after exposure to chemotherapy [33]. This gene and its protein have been studied for tumor-cell specific therapy [7].

Micro RNAs

MicroRNAs (MiRNAs) are small single stranded non-coding RNA (RNA) with 19 to 22 nucleotides in length. MiRNAs are highly conserved among different organisms and play an important regulatory role in animals by targeting messenger RNA (mRNA) for translational repression or degradation [34]. MiRNAs regulate many protein-coding genes and serve as biomarkers and therapeutic tools for several types of cancer [35].

Aberrant MiRNAs expression have been associated to lung cancer in many studies [36-39]. The first miRNA identified in lung cancer is Let-7, a tumor suppressor miRNA that is associated with poor prognosis [40]. Let-7 suppresses some oncogenes as MYC, RAS and HMGA2 [41,42]. Another tumor suppressor miRNA associated with lung cancer is MiR-34, an important component of TP53 function [43]. Down-regulation of MiR-34 upregulates MET and BCL-2 leading to cell proliferation [44].

MicroRNA	Expression	Biology and Target Genes
Tumor suppressor microRNAs		
Let-7	Decreases	i) Regulates cell proliferation (MYC, RAS, HMGA2); ii) Regulates miRNA biogenesis (DICER1); iii) Regulates cell cycle (CDK6).
miR-34	Decreases	Regulates TRAIL-induced cell death and cell proliferation (BCL-2, MET, PDGFRA, PDGFRB).
miR-200	Decreases	Promotion of EMT and metastasis (CDH1, VIM, ZEB1, ZEB2).
miR-126	Decreases	Cell proliferation, migration, and invasion through PTEN/PI3K/AKT pathway.
miR-195	Decreases	Cell proliferation, migration and invasion (CHEK1).
Oncogenic miRNAs		
miR-21	Increases	Cell proliferation, migration, and apoptosis (PDCD4, PTEN, SOCS1, SOCS6, TPM1).
miR-155	Increases	i) Resistance to chemotherapy (TP53); ii) Cell proliferation and apoptosis (PTEN, SOCS1, SOCS6).
miR-17-92	Increases	Carcinogenesis and cell proliferation (HIF1A, E2F1, PTEN).
miR 221/222	Increases	Cell migration and apoptosis (PTEN, TIMP3).
miR-31	Increases	i) Cell proliferation and apoptosis (BAP1); ii) Promotion of KRAS/MAPK signaling (SPRE1, SPREAD 2, SPRY1, SPRY3, SPRY4, RASA1).
EMT: Epithelial-Mesenchymal Transition; TRAIL: TNF-Related Apoptosis-Inducing Ligand.		

Table 1: Tumor suppressor and oncogenic microRNAs in lung cancer (adapted from Inamura, 2017 [34]).

Some of the miRNAs involved with lung cancer play a role as oncogenic miRNA. MiR-21 is one of the most studied since it is over-expressed in many cancers and its high expression is associated with poor prognosis [45]. This miRNA promotes carcinogenesis inhibiting the negative regulators of RAS/MEK/ERK pathways and by apoptosis suppression [34]. MiR-155 is also an oncogenic miRNA and it directly targets TP53. Studies have shown that this miRNA when increased is associated to chemotherapy resistance [46].

Further studies are required to determine the roles of miRNAs in lung cancer development and treatment. Table 1 resumes the roles of tumor suppressor and oncogenic miRNAs in lung cancer.

Tumor microenvironment and immune cells in SCLC

The environment in which the tumor exists includes surrounding blood and lymph vessels, fibroblasts, extracellular matrix (ECM) and many types of immune and inflammatory cells [47]. Abnormal ECM contributes to cancer progression, since it promotes cellular transformation and metastasis and it can contribute to angiogenesis and inflammation [48]. For SCLC, it has been demonstrated that proteins from ECM support SCLC cell growth [49] and it can enhance tumorigenicity and apoptosis resistance [50].

ECM is deposited around the cells by fibroblasts which regulate epithelial differentiation, inflammation and wound healing [51]. SCLC cells can secrete proteins, such as gastrin-releasing peptide (GRP) that promotes fibroblasts proliferation, and transforming growth factor- β (TGF- β) that increases ECM production, epithelial-mesenchymal transition in malignant cells and immunosuppression [52-56].

Besides fibroblasts, the tumor microenvironment also contains various immune cells. In SCLC, the local immune cells are not very well described. In other type of cancers, the tumor infiltrate includes tumor-infiltration lymphocytes: T cells and B cells, natural killer (NK) cells, natural killer T (NKT) cells, dendritic cells (DCs) and macrophages. The presence of these cells can initiate a tumor-tolerant microenvironment by immunomodulatory mechanisms [47]. This effect can enhance tumor growth and metastasis [57].

Immunological modulation and treatment in SCLC

Patients with SCLC present important local and systemic immune defects that can be correlated to increased mortality [58]. In peripheral blood, the ratio of CD4/CD8 lymphocytes is decreased, which is associated to impaired secretion of IL-2 that is strongly associated to poor survival in SCLC patients [59]. Histological analysis showed that in SCLC the amount of inflammatory cells infiltration is lower than in other lung cancers [60]. Lymphocytes isolated from SCLC biopsies are ineffective in lysing tumor cells [61], and alveolar macrophages of SCLC patients have been showed to possess impaired phagocytic function and the production of inflammatory cytokines (TNF-, IL-1 and IL-6) is very low [62]. Furthermore, the expression of major histocompatibility complex class II (MHC-II) antigen, intercellular adhesion molecule 1 (ICAM-1) and CD83 are reduced in these patients [62].

In patients with SCLC, the expression of MHC-I is also very decreased [63], although MHC-I is involved with the development of SCLC in the early stage. The programmed cell death-1 (PD-1) receptor is an immunoinhibitory checkpoint receptor that is expressed in NK, T and B cells [64]. The ligand of this receptor (PDL-1) inhibits T lymphocyte proliferation and induces apoptosis of the tumor-specific

T cells [65]. That way, cancer cells down-regulate immune response by expressing PDL-1, and in SCLC, 70% of the tumor cells express this ligand. PDL-1 is positively correlated to patient survival [66].

Another immunoinhibitory checkpoint receptor is the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) that works to downregulate T-cell function through a variety of mechanisms [67]. CTLA-4 is important to maintain normal immunologic homeostasis, and it was evidenced by an experiment that showed that CTLA-4 deficient mice died from fatal lymphoproliferation [68]. CTLA-4 has been the first immune checkpoint receptor to be targeted by therapeutic agent, and it has been extensively studied as SCLC treatment [69,70].

SCLC therapy depends on the tumor staging. In the limited stage, first line treatment consists on the combination of chemotherapy and radiotherapy of the chest, and it can be followed by prophylactic cranial irradiation [11,71,72]. The most used chemotherapy drugs are cisplatin associated to etoposide, and the combination induces a first response of 80% with a medium survival of 17 months and a healing rate of 12% to 25% [11,73]. In the extensive stage the only treatment is the chemotherapy with the combination of cisplatin and etoposide with a first response of 50% to 80% and an overall survival (OS) of 7 to 9 months [8,11].

In rare cases, SCLC patients are diagnosed in an early stage with a localized and resectable tumor. For these patients, tumor resection is indicated followed by adjuvant chemotherapy. Patients in extensive stage do not have the indication for the surgery, since it has no benefits for them [74].

Although most of the patients present a good initial response to chemotherapy and radiotherapy, almost all of the patients present treatment relapse and they manifest resistant disease about 6 to 12 months after the beginning of the therapy [12]. Since patient survival remains low with first line therapy, new studies and research are being conducted to assess new line of treatments. And since genetics and molecular biology of SCLC have exponentially grew, new promising therapies have emerged, such as the humanized monoclonal antibodies [75].

Humanized monoclonal antibodies treatment

Humanized monoclonal antibodies (mAb) production was initiated in the 1980 decade, being the Orthoclone (OKT3) the first one to be approved for use in 1986 [75] and the first mAb approved to be used in cancer treatment was Rituximab in 1997 [76]. The purpose of use of these mAb is that they possess the ability to connect specifically to an antigen and destroy only the cancerous cells, thus saving the normal ones [77]. There are many mechanisms involved in the action of the mAb, such as modification of the patient's response to tumor cells, cytotoxicity, direct connection to antigens in the surface of the cancerous cells and immunity modulation [77].

Bevacizumab

Bevacizumab is a mAb used against VEGF proteins, specifically VEGF-A, resulting in an inhibitory mechanism of the angiogenesis [2,78]. A randomized phase II-III clinical trial with Bevacizumab administered associated to chemotherapy did not show an improvement of the results in SCLC patients in advanced stage. The progression-free survival (PFS) was of 5,5 months in the association of chemotherapy to Bevacizumab against 5,3 months of the isolated

chemotherapy. The OS was also not better in the association of chemotherapy to Bevacizumab with a medium of 11,1 months against 13,3 months in the isolated chemotherapy [79].

A randomized phase II clinical trial using Irinotecan, Carboplatin and Bevacizumab in the treatment of SCLC patients in advanced stage showed that this combination is effective but also more toxic. Although the addition of Bevacizumab in the first line treatment can be positive, more studies should be accomplished. Bevacizumab treatment was associated with thrombocytopenia, dehydration and fatigue in 26% of the patients [10].

In another randomized phase II clinical trial Bevacizumab was used in combination of line one chemotherapy treatment (cisplatin or carboplatin + etoposide) to SCLC patients that were not treated previously. The PFS was better in the group that combined Bevacizumab with chemotherapy with a medium of 5,5 months against 4,4 months of the group that used placebo combined with chemotherapy. The toxicity in this study was very tolerable. However, the OS of the patients was not improved by the combination, but was statistically better in the group that used Bevacizumab with a medium of 10,9 months against 9,4 months of the placebo group [80].

Bevacizumab was associated to cisplatin and etoposide in a randomized phase III clinical trial in advanced stage SCLC patients. The PFS of the group that used Bevacizumab was statistically better than the group that did not use the mAb, with a medium of 6,7 and 5,7 months, respectively. The OS, although, was not statistically different between treated and not treated group, with a medium of 9,8 and 8,9 months, respectively. The survival rates in the group treated with Bevacizumab was better than the not treated group being 37% to the treated group and 25% of the not treated group [81].

Trastuzumab

Trastuzumab was the first mAb against HER2, very effective in breast and gastric cancer [82]. SCLC patients can also be HER2 positive and some clinical trials have analyzed its therapeutic efficiency in this type of cancer. One of these studies showed that Bevacizumab can be associated to Trastuzumab in patients that express resistance to the second one being HER2 positive. Antiangiogenic effects of Bevacizumab can be complementary to the immunomodulation of Trastuzumab [13].

The main antitumor activity of Trastuzumab in HER2 positive patients occurs by the antibody-dependent cell-mediated cytotoxicity (ADCC) and not by direct inhibition of HER2 [13].

Ipilimumab

Ipilimumab is a mAb anti-CTLA-4 totally human. It works inhibiting the interaction of CTLA-4 with its binders CD80/CD86 resulting in more T cells activation and proliferation that can infiltrate the tumor. The benefit of associating Ipilimumab with chemotherapy is that the chemotherapeutics induce the release of tumor antigens that initiate the activation of T cells [82-85].

A randomized double-blind and multicenter phase II trial was conducted with the association of Ipilimumab with paclitaxel and carboplatin in 130 extensive stage SCLC patients. This study showed no exacerbated adverse effects in the use of Ipilimumab associated with chemotherapy. In fact, it showed a satisfactory clinical response [82].

Anti-CTLA-4 antibodies enhance immune responses in some cancer models [86]. The blockade of CTLA-4 may prolong T-cell activation and also intensify T-cell-mediated antitumor responses, potentially restricting tumor cell evasion of the immune system [87].

Cixutumumab

Cixutumumab is a mAb anti-IGF1R which presents as mechanisms of action the inhibition of the anti-insulin growth factor [2]. A randomized phase II clinical trial evaluated the combination of cisplatin and etoposide with Cixutumumab in extensive stage SCLC patients presenting disappointing results with no improvement in PFS compared with isolated chemotherapy [73]. Other study also showed that Cixutumumab associated to chemotherapy did not raise PFS, OS and response rate [14].

Ganitumumab

Ganitumumab is a mAb totally human anti-IGF1R. The mechanism of action of Ganitumumab is based on the blocking IGF-1 binding to its receptor and on the induction of the receptor degradation in the cell. IGF-1 inhibition results in the activation of PI3K-AKT and consequently in the inhibition of cellular growth [85, 88].

This mAb was also evaluated in combination with cisplatin in a randomized phase II clinical trial in extensive stage SCLC patients and the results did not show significant improvements in OS and PFS when compared to cisplatin alone [11,83].

Figitumumab

Figitumumab is a mAb totally human anti-IGF1R that presents antiproliferative and antitumor effects in cancerous cells [28,89]. It has been shown that patients treated with Figitumumab presented an elevation of the activation of MEK/ERK pathway after the treatment. This same study showed that the use of Metformin associated to Figitumumab significantly raised the efficacy of the treatment. Metformin also decreases hyperglycemia rate in the blood, one of the collateral effects of Figitumumab [28]. Figitumumab has been discontinued [19].

Rovalpituzumab Terisine

Rovalpituzumab Terisine (RT) is an antibody-drug conjugate (ADC) that acts against DLL3 [20,90]. A randomized phase I clinical trial evaluated the security and effectiveness of RT in 74 SCLC patients. There were very severe collateral effects related to the treatment in 38% of the patients and 18% presented a relevant response [90].

Conclusion

In conclusion, although some of the most important genetic mutations was revealed and it can be used as new therapeutic targets against small-cell lung cancer along with immunotherapy, it has been shown that there is an urgent need for new treatments since the survival rate for this type of cancer is very low. Current treatment options for SCLC are limited and prognosis remains poor. And although current standard treatment approaches are successful at inducing initial response, the final outcomes are not good.

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