

New Insights into the Discovery of Novel Drugs to Treat Human Pulmonary Adenocarcinoma

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How to cite this paper: Wang, Y., Zhang, H.S., Chen, Y.X. and Wang, L.N. (2019) New Insights into the Discovery of Novel Drugs to Treat Human Pulmonary Adenocarcinoma. *Journal of Cancer Therapy*, 10, 178-187.

<https://doi.org/10.4236/jct.2019.102014>

Received: February 1, 2019

Accepted: February 18, 2019

Published: February 21, 2019

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Abstract

Lung cancer is one of the most prevalent cancers in the world. A cisplatin-based chemotherapy regimen is commonly used to major patients who have adenocarcinoma. Because of the limited remission rate, many patients need receive second-line treatment after receiving first-line chemotherapy. Drug screenings carried out currently are mainly from single phase. In consideration of systemic treatment, evaluation of a novel drug candidate from the point of view of first-line and second-line treatment is supposed to be important in the process of drug screening. In this review, we propose a flow of searching for new drugs, which is tailored to accommodate to first-line and second-line treatment in clinic.

Keywords

Cisplatin, Docetaxel, Pemetrexed, Adenocarcinoma, *In Vitro*, *In Vivo*, Clinical Trial

1. Introduction

Non-small cell lung cancer (NSCLC) is about 75% - 80% of the primary lung cancers. Many patients need secondary chemotherapy after receiving first-line treatment due to the limited remission rate [1] [2] [3] [4]. Researchers are making great efforts to search for more effective drugs. Traditionally, the first-line chemotherapy is cisplatin based regimen, including another third generation drug, such as docetaxel, paclitaxel and gemcitabine [5] [6] [7]. For the second-line treatment, pemetrexed and docetaxel are constantly used [1] [2] [3] [8] [9]. The relationship between histology and first-line regimen has been realized in recent years; there are reports indicating that pemetrexed is suitable to be used for

nonsquamous histology which can give a better OS, while not for squamous histology [3] [10] [11]. Based on these reports, previously we designed an experiment to see the effect when human adenocarcinoma A549 cells received first-line and second-line treatment *in vitro* [12]. We analyzed the inhibiting effect of cell proliferation from the group by adding docetaxel following treatment with cisplatin and pemetrexed (Pem-Doc group) and compared with that from another group in which pemetrexed was added subsequent to treatment with cisplatin and docetaxel (Doc-Pem group). Pem-Doc group showed an increased inhibiting effect of cell proliferation. Concerning cisplatin resistance gene excision repair cross-completion gene1 (ERCC1) [13] [14], the gene expression and protein levels of ERCC1 in the Pem-Doc group were decreased. Interestingly, results from this treatment model are consistent with clinical findings. This makes it possible to evaluate new drug candidate taking first-line and second-line effect into account. Therefore, the first-line and second-line treatment model could be thought as an appropriate step for screening of drugs for first-line and second-line treatment in clinic. Here, we propose a flow of searching for new drugs that is suitable for first-line and second-line treatment in clinic (Table 1).

2. Sources

Sources for screening include chemical compounds, natural products (plants, marine organisms), peptides, antibodies *etc.* [15] [16] [17] [18]. Compounds used as chemotherapy drugs mainly come from natural plants or from synthesized compounds and their derivatives. Chemical synthesis remains an important method providing compounds. Traditionally, compounds were synthesized by trial-and-error method, with the advent of combinatorial chemistry, available compounds increased dramatically. For example, 44,000 compounds were screened with the result that a novel small molecule compound (iMDK) could be a potential therapeutic approach for the treatment of lung cancers that are driven by MDK [19]. Similarly, plant-derived anticancer drugs have a long history of use in cancer treatment. Paclitaxel, the most excellent anticancer drug discovered in recent years was isolated from the leaves of various taxus species. Paclitaxel promotes microtubule polymerization and inhibits depolymerization, which results in the death of cancer cells. Docetaxel, an analogue of paclitaxel, is widely used to treat many kinds of cancers including NSCLC [5] [6] [7]. Marine organisms constitute a rich source with the feature of structural diversity and extremely potent biological activeness. For instance, tunicate-derived ET-743 is a marine natural product which was approved in Europe as an antitumor agent [20]. Peptide library is also a good source of targeted ligands [21]. By screening a random cyclic peptide phage display library, a cyclic peptide CIGB-300 was found to exhibit anticancer properties and seems to have synergistic effect when used with chemotherapeutic agents together for lung cancer [22]. Due to small sizes, peptides can be synthesized in large quantities, with high affinities for protein receptors which make them more attractive. Antibody library is another

Table 1. Recommended flow of searching for new drugs.

Stage	Sources: chemical compounds; natural products (plants, marine organisms); peptides; antibodies	
<i>In vitro</i>	Methods: high throughput screening (HTS), high content screening (HCS), surface plasmon resonance (SPR) biosensor, yeast two hybrid	Minimizing candidates
<i>In vivo/Ex vivo</i>	Animal models: wild type transgenic/knockout xenograft models: <i>in vivo</i> study (cultured cancer cells): subcutaneously, orthotopically, systemically <i>ex vivo</i> study (cancer tissues from patients surgically): subcutaneously, orthotopically	Analyzing pharmacokinetics, effect and toxic side effect
<i>Super vitro</i>	First-line second-line treatment model with pemetrexed-docetaxel as standard control	Accommodating to first-line and second-line treatment in clinic
<i>In vivo</i>	Clinical trials Phase I: safety and dosage Phase II: efficacy and side effects Phase III: efficacy and monitoring of adverse reactions Phase IV: safety and efficacy	

good choice for screening because of high biological activities. Epidermal growth factor receptor (EGFR), a member of the ErbB family, is commonly over expressed in several cancers including NSCLC. Due to inverse correlation with prognosis, EGFR has been studied and proved to be a molecular target for NSCLC treatment. Necitumumab, which is a monoclonal antibody screened by a monoclonal antibody library, can bind the extracellular domain of EGFR and block EGF signal pathway [23]. Necitumumab was proved to be effective and approved to be used for treating squamous lung cancer by FDA [24].

3. *In Vitro* Stage

Several commonly used screening techniques include high throughput screening (HTS), high content screening (HCS), surface plasmon resonance (SPR), yeast two hybrid *et al.* HTS is the process to study the effects of large numbers of samples on the biological activity of targets such as proteins, enzymes, ion channels, receptors [25] [26]. According to the status of targets, HTS assays could be divided into cell-free assay or cell-based assay in which target resides in the cell. Also HTS could be divided into radiometric assay or fluorescence-based assay. For example, 2802 compounds were tested by a cell-based fluorescence assay with the result that aclacinomycin can reduce EGFR levels and radiosensitize EGFR-mutant non-small cell lung cancer [27]. Comparing with the simplicity of

HTS which only observes one parameter, HCS can detect multiple parameters including cellular and subcellular phenotypes, cellular image readout, morphology analysis, cell counting *etc.* [28]. In an image-based HCS, 1280 pharmacologically active compounds were screened with a consequent that identifying twelve potent high drug-efflux cancer cells (HDECC) inhibitors, which may potentially be used as potential adjuvant to improve the efficacy of chemotherapeutic drugs [29]. Furthermore some researchers developed modified screening by adding new methods. For instance, a high content clonogenic survival drug screen identifies MEK inhibitors as potent radiation sensitizers for KRAS mutant non-small cell lung cancer [30]. Surface plasmon resonance (SPR) biosensor is the method to detect the interaction between low molecular weight fragment of compounds and membrane protein drug targets, such as G-protein coupled receptors (GPCRs) [31] [32]. In contrast to conventional high-throughput screening, SPR is suitable to screen compounds with low molecular weight because low affinities can be detected. Yeast two hybrid is a method to detect the interaction between two proteins in living cells. As the protein-protein interactions are promising drug targets, are being studied by many researches in the field of drug screening [33].

4. *In Vivo/Ex Vivo* Stage

Animals are used to study the pharmacokinetics, effect and toxic side effect of candidate compound since long time ago. In recent years, transgenic/knockout mice are used to analyze gene function, protein function, chemotherapy effect *et al.* For instance, transgenic mice expressing activated EGFR invariably developed multifocal lung adenocarcinomas [34]. Similarly, IL-6 deficient mice developed much more lung tumors after an activating mutant of K-Ras was induced in the lungs comparing with wild type mice [35]. Xenograft models are created by injecting human cancer cells into immunocompromised mice, subcutaneously, orthotopically, or systemically [36] [37] [38]. Xenograft models are primarily used to examine chemotherapy effect *in vivo*. *Ex vivo* models are created by grafting cancer tissues into the immunocompromised mice subcutaneously or orthotopically immediately after surgeries [39] [40] [41]. With these murine models, more information about therapeutic effect and molecular mechanism of novel drug can be got and lead to further research.

5. *Super Vitro* Stage

Drug screenings carried out currently are mainly by studying effect during single phase. However, second-line even third-line treatment is common in clinic. To get better therapeutic effect for an individual patient, possible second-line treatment following first-line should be considered. Estimating new drug candidate taking first-line second-line effect into account becomes necessary. Previously we designed an experiment to simulate first-line and second-line treatment in clinic [12], which compared inhibiting effect of cancer cell proliferation from the group by adding docetaxel following treatment with cisplatin and pemetrexed

(Pem-Doc group) with that from another group in which pemetrexed was added subsequent to treatment with cisplatin and docetaxel (Doc-Pem group). Pem-Doc group showed an increased inhibiting effect of cell proliferation which is consistent with results from clinical trials. Thus Pem-Doc group can be used as a control in case of cisplatin-based chemotherapy regimen. Therefore, we recommend to use this first-line and second-line treatment model as a good step to screen drugs for first-line and second-line treatment in clinic as it reflects better than traditional single phase. In the *super vitro* stage, as shown in **Table 2**, new candidate can be combined with cisplatin in first-line treatment followed by docetaxel in second-line treatment or be added in second-line treatment following treatment with cisplatin and pemetrexed in first-line treatment. This experiment will help us to determine if candidate is suitable for first-line treatment or second-line treatment, respectively. Also we can test effects of two candidates (A, B) simultaneously as shown in **Table 2**. For those with better cell proliferation assay (CPA) results, candidates could be studied further to see the gene and protein expression of ERCC1 by using Real-Time PCR and western blots.

6. In Vivo Stage

Clinical trials are divided into 4 phases (Phases I, II, III, and IV). In Phase I, 20 to 100 healthy volunteers or people with pulmonary adenocarcinoma participate with a period of several months. In Phase II, up to several hundred people with pulmonary adenocarcinoma participate with a period of several months to 2 years. In Phase III, 300 to 3000 volunteers who have pulmonary adenocarcinoma participate with a period of 1 to 4 years. In Phase IV, several thousand volunteers who have pulmonary adenocarcinoma participate.

7. Conclusion

Pulmonary adenocarcinoma is about 50% of the NSCLC. How to increase chemotherapy effect remains to be a problem both in clinic treatment and in the field of research. A cisplatin-based chemotherapy regimen has been the main solution for treating advanced NSCLC. Docetaxel and pemetrexed are two commonly used drugs, which have shown stable effects on many treatments. With further study, some mutations in case of adenocarcinoma were discovered including KRAS, EGFR, BRAF, HER2 *etc.* [42]. This led to the discovery of novel target EGFR-TKI drugs, such as erlotinib and gefitinib, which could inhibit proliferation of cancer cells through impairing EGFR kinase activating [43]. These EGFR-TKI drugs execute effects on condition that there are EGFR mutations. Reports indicated that EGFR mutations were found in about 16.6% of total Caucasian lung adenocarcinoma patients. It is thought that about 15% of patients with adenocarcinoma of the lung are ideal candidates for these targeted therapies [43] [44]. Recently, a report indicated that EGFR mutations were found in about 50.2% of patients of mainland China [45]. Samples can be used to detect EGFR mutations which include tumor tissues from surgery or needle biopsy, peripheral blood and pleural fluid. A report indicated that concordance

Table 2. Recommended flow of *super vitro* study.

	Standard control	Candidate A	Candidate A	Candidate A, B
First-line	Pem + DDP	A + DDP	Pem + DDP	A + DDP
Second-line	Doc	Doc	A	B
	CPA	CPA	CPA	CPA

Doc: docetaxel; Pem: pemetrexed; DDP: cisplatin; CPA: Cell proliferation assay.

rate in EGFR mutation detection between peripheral blood and tumor tissue was about 85.1% [46]. Detection from tissue is thought to be the most accurate, but it fits patients with surgeries or biopsies. Nevertheless, there are primary resistance and acquired resistance to EGFR-TKI therapy [47]. There is another kind of target drug monoclonal antibodies, which can block the signal transmission by combining with relevant receptors. Currently, there are monoclonal antibodies against EGFR (necitumumab for squamous cancer) or against VEGFR (ramucirumab) [48] [49]. Ramucirumab can be used to treat non-squamous NSCLC in combination with standard chemotherapy [49] [50]. Therefore, cisplatin-based chemotherapy regimen still is the main solution to major patients who have adenocarcinoma. In this review, we proposed that a flow probably could be used for screening for novel drugs fitting first-line and second-line treatment in clinic.

Funding

The present study was supported by a grant from Shenyang Medical College for the Returned Overseas Chinese Scholars of Lining Wang (No. 20144062).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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