A systems pharmacology approach to improve drug therapy in NSCLC: Establishing a CESAR network

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Introduction

The epidermal growth factor receptor (EGFR) plays an important role in activation of signaling pathways responsible for proliferation of non-small cell lung cancer (NSCLC) cells. However, EGFR targeting therapy is only effective in a subset of EGFR-positive tumors that harbour activating mutations in the receptor kinase domain occurring in a frequency of 5 – 20% of EGFR-positive tumors in Caucasian lung tumor populations. As several studies showed that EGFR mutation-negative tumors responded better to classical chemotherapy, regimens based on platinum complexes or taxanes are still the treatment of choice for those patients [1]. A major drawback in both regimes, targeted as well as chemotherapy treatment, is the development of drug resistance. Mechanisms of drug resistance include alterations in target structure and function, compensating signaling activation as well as changes in drug delivery and accumulation within the tumor cell. Typically, drug resistance is not restricted to a single resistance mechanism but is the result of an accumulation of several mechanisms. Therefore, strategies of combination therapy are increasingly investigated to overcome drug resistance [2]. In order to tailor the most beneficial combination therapy to an individual patient, knowledge on potential drug resistance mechanisms and respective biological markers in a global scale are required. Systems pharmacology presents an innovative discipline which combines systematic collections of experimental data from whole cells, tissues or organisms with the mathematical models of drug pharmacokinetics and pharmacodynamics and natural disease progression models to understand drug action at a global scale [3]. Within CESAR a research network was established to set up systems pharmacology approaches to unravel EGFR-associated drug resistance in NSCLC.

Models and methods

Cell lines of non-small-cell lung cancer origin were selected that express either wild-type EGFR or EGFR harboring activating mutations. The NSCLC cell lines HCC4006 and A549 were obtained from ATCC (Manassas, VA, USA). Sub-lines with acquired resistance to cisplatin, gefitinib, or erlotinib were established as described previously [4]. The cell lines were designated as A549(CDDP)2000 (cisplatin-resistant), HCC4006(EGFI) (gefitinib-resistant), and...
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HCC4006\textsuperscript{ERLO1} (erlotinib-resistant) and are part of the Resistant Cancer Cell Line (RCCL) collection. The A549 cell lines were cultivated in MEM, the HCC4006 in DMEM/HAM’s F12 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified 5% carbon dioxide atmosphere.

Data collection will consist of investigating the EGFR interactome (affinity purification and high-resolution mass spectrometry), characterizing exosomes (microRNA content, protein content, EGFR status), analyzing the cellular transcriptomes (microarray technologies) and metabolomes (mass spectrometry), and determining uptake and distribution of cisplatin, erlotinib, and gefitinib (intracellular accumulation and transwell experiments) in the investigated cell lines. The experimental data will be used to develop predictive, coherent in silico models of the resistance-associated signaling networks.

**Resulting concept**

The structure of the research consortium is shown in Figure 1. The role of EGFR in NSCLC will be studied at three levels of complexity:

- 1) The EGFR function will be studied at the molecular level. The EGFR-mediated signaling, the EGFR interactome, and the intracellular localization of EGFR in time and space will be analyzed in dependence on the receptor genetics, the activation state, and the response to anticancer drugs. These data will be used to develop computer-based models of the EGFR subcellular kinetics and the dynamic EGFR signaling and interaction at a systems level.

- 2) Cellular aspects of drug response and drug resistance that are not directly related to EGFR function are studied. This includes the studying of the intracellular accumulation and distribution of anticancer drugs, since cancer cell drug resistance mechanisms include decreased cellular drug uptake, increased drug efflux, and changes in sub-cellular drug location. In addition, transcriptomics analyses will reveal regulatory changes in response to drug resistance.

- 3) A third aspect addresses molecular changes outside of the cells, i.e., secreted species such as exosomes as well as the metabolome. These secreted species play a pivotal role as markers to be extrapolated to the patient situation. Differential secretion of metabolites and exosome content, particularly microRNAs will be investigated based on EGFR receptor genetics, activation state, and drug response phenotype, characteristics of exosomes will be correlated with whole tumor cells, and specifically identified metabolites and microRNA species will be correlated to transcriptome and drug response phenotype.

Finally, the data will be integrated into a multi-level network modeling approach to develop predictive models for drug resistance depending on EGFR status and drug treatment by the core bioinformatics and pharmacometrics units.

**Conclusions**

Here, we will develop a systems pharmacology approach in order to understand the
role of EGFR in NSCLC 1) as a drug target and 2) in the response to anti-cancer therapy in general which will provide the basis for the development of improved therapies.

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References


