Personalized Targeted Lung Cancer Therapy Identified From Fine Needle Aspirate Samples

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Abstract

- The American Cancer Society estimates in 2017 that approximately 222,500 new cases of lung cancer will emerge in the U.S., causing about 155,870 deaths [1].
- Epidermal growth factor receptor (EGFR) is overexpressed in lung cancer, and is thus currently targeted by drugs.
- Certain mutations in EGFR make cells sensitive or resistant to these drugs.
- We screened H2228 and H1975 cell lines and 20 fine needle aspirate (FNA) non-small cell lung patient samples for mutations in EGFR.
- We treated the lung cancer cell lines with Tarceva (Erlotinib) and quantified cell death with flow cytometry.
- Cells with no mutations in EGFR are sensitive to Tarceva while cells with T790M mutations are resistant.
- From the DNA sequencing results, we identified which patient samples had mutations. By analyzing which mutations the patients have, our findings will allow us to predict whether or not Tarceva will be an effective treatment.

Introduction

- Every cancer patient has unique cancer cells, so no two cancers are alike and different drugs may work for different patients.
- As an alternative to trial and error, which is common but time-consuming and inefficient, scientists and doctors are beginning to use personalized medicine to determine which drugs will be effective for patients' specific mutations.
- Lung cancer results from abnormal lung cells that grow out of control. One type, non-small cell lung cancer (NSCLC), accounts for 80 to 85 percent of all lung cancer cases in America [1].
- A significant number of lung cancer cases are associated with mutations in the epidermal growth factor receptor (EGFR) gene [2]. EGFR is a transmembrane receptor that binds to the epidermal growth factor, leading to a multitude of effects in the cell, including cell proliferation.
- Since certain mutations in EGFR render cells sensitive or resistant to different drugs, many different drugs are designed to target specific EGFR mutations [3].
- Erlotinib hydrochloride (Tarceva) is used to treat NSCLC, and is a reversible tyrosine kinase inhibitor, acting on the intracellular domain of the EGFR which bears the Tyrosine Kinase domain.
- Our goal was, after screening NSCLC cell lines for EGFR mutations and correlating their mutational status with resistance to Tarceva, to use this data to predict whether patient samples would be resistant to Tarceva.
- It is known that some mutations are sensitive to the drug, while some are resistant. By analyzing DNA sequencing data from the patient samples, we could determine if the patients had mutations in EGFR and thus if Tarceva would be ineffective.
- Our research question was as follows: For which patients would Tarceva be an effective treatment for NSCLC?
- We hypothesized that we will be able to identify EGFR mutations in patient FNA smears and thereby predict the efficacy of Tarceva

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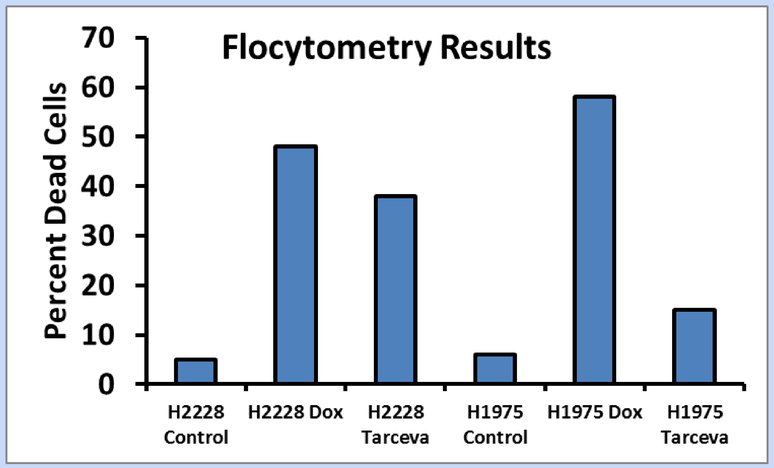
Materials & Methods

- We tested the cell lines H2228 (no EGFR mutation) and H1975 (contains EGFR mutation), which were provided by Dr. Goswami.
- After extracting the DNA from the cell lines, we performed polymerase chain reactions (PCR) to amplify it. In the PCR tube, we combined 10 ng of DNA, 10 µL of ImmoMix, and 1 µL of primer mix, and then added nuclease free water to reach a total volume of 20 µL. The initial denaturation was at 95ºC for 10 minutes, followed by 35 cycles of 95ºC for 15 seconds, 55ºC for 15 seconds, and 72ºC for 30 seconds, with a terminal extension of 8 minutes at 72ºC.
- We stored the samples overnight at 4ºC, then ran a 2% agarose gel electrophoresis, and sent the samples out for sequencing to identify which cell line had a mutation in EGFR.
- The flowcytometry results indicated that H2228 experienced 38 percent cell death when treated with Tarceva, while H1975 experienced 15 percent cell death. This confirmed our expectation that H2228 is sensitive to Tarceva and H1975 is resistant to it.
- Twenty different patient FNA samples, provided by Dr. Goswami, were also tested for mutations in EGFR. We repeated the PCR and gel electrophoresis procedures, and sent the samples out to be sequenced.
- After analyzing the DNA sequencing results to identify mutations in EGFR, we predicted which patient samples Tarceva would not be effective for.



EGFR mutation detection results for cell lines:

1. H2228 Not Mutant
2. H1975 Mutant : T790M (C→T mutation in exon 20)

Patient Sample Analysis

Case #	EGFR Status	Mutations	Predicted Sensitivity
1	Mutant	T790M	Resistant
2	Mutant	L858R	Increased sensitivity
3	Not Mutant		Sensitive
4	Not Mutant		Sensitive
5	Mutant	T790M	Resistant
6	Not Mutant		Sensitive
7	Not Mutant		Sensitive
8	Not Mutant		Sensitive
9	Not Mutant		Sensitive
10	Mutant	L858R	Increased sensitivity

Case #	EGFR Status	Mutations	Predicted Sensitivity
10	Not Mutant		Sensitive
12	Not Mutant		Sensitive
13	Not Mutant		Sensitive
14	Mutant	L858R	Increased Sensitivity
15	Mutant	T790M	Resistant
16	Not Mutant		Sensitive
17	Not Mutant		Sensitive
18	Not Mutant		Sensitive
19	Mutant	L858R	Increased Sensitivity
20	Not Mutant		Sensitive

The mutation T790M has a substitution of a T for a C in exon 20 and the mutation L858R has a substitution of a G for a T in exon 21.

Discussion

- Our results supported our hypothesis, confirming that some patient samples should be resistant to Tarceva and some should be sensitive.
- Cancerous cells containing a T790M mutation in the EGFR gene are resistant to Tarceva, while cells without this mutation are sensitive, and cells with a L858R mutation experience increased sensitivity.
- Based on our patient sample sequences, we can predict that Tarceva will work for 17 out of the 20 patients in our cohort.
- DNA sequencing to identify EGFR gene mutations can have a significant impact on medicine, for patients who do not have the T790M mutation can hopefully be successfully treated with Tarceva. Doctors will also know that Tarceva is not a good treatment option for patients who have the mutation, and will thus look to other drugs for treatment, saving time that would otherwise be spent in trial and error.
- Further studies should test more drugs and more mutations. Ideally, it should be known exactly which drugs will work for a patient based off of their cell's DNA sequence, giving them the most effective treatment as soon as possible.

References

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