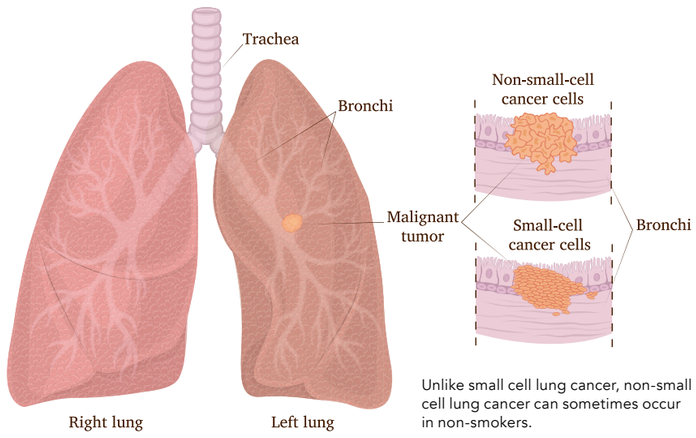
Understanding the *EGFR* mutation aids the fight against lung cancer

Genetic mutations are known to be responsible for several different types of cancer. One type, known as non-small cell lung cancer, is sometimes caused by mutations in a gene called EGFR. *A group of drugs called tyrosine kinase inhibitors are often used to treat patients with this type of cancer. Unfortunately, patients can develop genetic alterations, namely mutation, amplification (an increase in the number of copies of a gene) or methylation (methyl groups are added to the DNA molecule), that trigger resistance to these drugs. In-depth research by Dr Toshimitsu Yamaoka and colleagues at Showa University, Tokyo, Japan, aims to uncover the mechanisms of drug resistance in lung cancer patients so that new treatment options can be explored.*

Genetic alterations have been associated with many different forms of cancer. In the *EGFR* gene, several different mutations have been found to be linked with lung cancer. Normally, *EGFR*, which is a transmembrane protein, transduces their signals from the environments, then carries the instructions a cell need in order to adapt to the environment to the nucleus. The role of the receptor protein is to bind to other proteins outside the cell, known as ligands, and to help the cell communicate with its environment.

Occasionally, the *EGFR* gene develops mutations that disrupt the function of the receptor protein. When this occurs, the receptor protein is effectively always 'on'. This means that the cell continuously receives signals that tell it to survive and, crucially, grow – leading to the formation of a tumour. Mutations in *EGFR* are somatic; that is, they develop during a person's lifetime, rather than being inherited from their parents.



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Unlike small cell lung cancer, non-small cell lung cancer can sometimes occur in non-smokers.

EGFR MUTATIONS AND LUNG CANCER

Most mutations in *EGFR* trigger a type of cancer called non-small cell lung cancer. Non-small cell lung cancer (which, unlike other types of lung cancer, is weakly associated with smoking) accounts for about 80–85% of lung cancer cases in the UK. Mutations in *EGFR* are particularly common in people of Asian ethnicity. Research has revealed that, among non-small cell lung cancer patients, 32% of Asians have mutations in the *EGFR* gene, compared to just 7% of patients of other ethnicities.

Lung cancer caused by *EGFR* mutations is often treated with a group of chemotherapy drugs called *EGFR* tyrosine kinase inhibitors (TKIs). These drugs work by binding to the malfunctioning receptor proteins in the cell membrane, blocking their activity and therefore stopping the unchecked growth of the cell. This group of drugs includes the medications gefitinib, erlotinib, afatinib, and osimertinib. When given to patients as a first-line treatment for non-small cell lung cancer, the response rate to these drugs is 70–80% (i.e. in 70–80% of patients, the tumour stops growing, shrinks or disappears).

Unfortunately, most patients treated with *EGFR* TKIs develop resistance to the drugs after one to two years. This resistance is at least partly due to the appearance of secondary mutations in the *EGFR* gene. When this happens, the drugs are no longer effective and the patient's chances of survival are significantly lowered. These patients often then face treatment with traditional, cytotoxic (i.e. cell-destroying) chemotherapy, which is less effective and frequently causes severe side-effects.

UNDERSTANDING THE GENETICS OF DRUG RESISTANCE

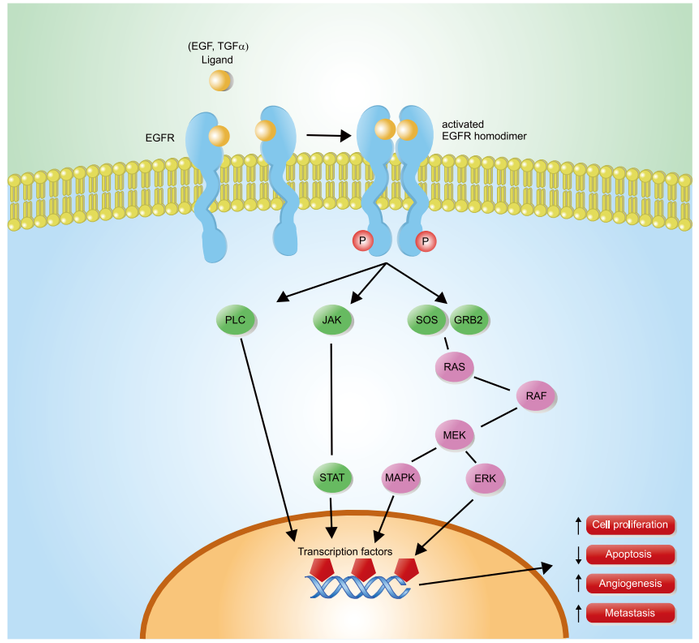
Dr Toshimitsu Yamaoka of Showa University, Japan, investigates the complex relationships between mutations in the *EGFR* gene, *EGFR* TKI drugs, and drug resistance in non-small cell lung cancer. The goal of Dr Yamaoka and his colleagues is to overcome the problems generated by *EGFR*-mediated drug resistance, which should ultimately allow the development of new, improved cancer therapies.

In recent research, Dr Yamaoka and a team at Showa University investigated the particular mechanisms that cause resistance to the third-generation *EGFR* TKI drugs rociletinib and osimertinib. Previously, the team had identified a specific genetic mutation in lung cancer cells that causes resistance to afatinib. In the subsequent study, they used cancer cells known to carry the afatinib-resistance mutation to find out why resistance to rociletinib and osimertinib develops.

In this study, Dr Yamaoka used cells that were originally obtained from a previously untreated non-small cell lung cancer patient in the 1980s. To generate resistance to afatinib, the cells were grown for several months in a medium that contained increasing concentrations of the drug. These cells were then subject to a repeat of the same process, but this time with either rociletinib or osimertinib in the growth medium, in order to trigger resistance to these medications. The team subsequently used various techniques, including genomic sequencing, to ascertain the genetic changes that had occurred in the two groups of drug-resistant cancer cells.

RESISTANCE REVEALED

When they began this research, the team expected that resistance to rociletinib and osimertinib would be triggered by novel genetic changes. This idea was based on previous work, in which the team discovered three distinct mechanisms that lead to afatinib resistance in lung cancer cells. One of these mechanisms is the emergence of a particular mutation, termed *EGFR*-T790M, that is linked to non-small cell lung cancer. When this mutation arises in cancerous cells, resistance to afatinib follows.



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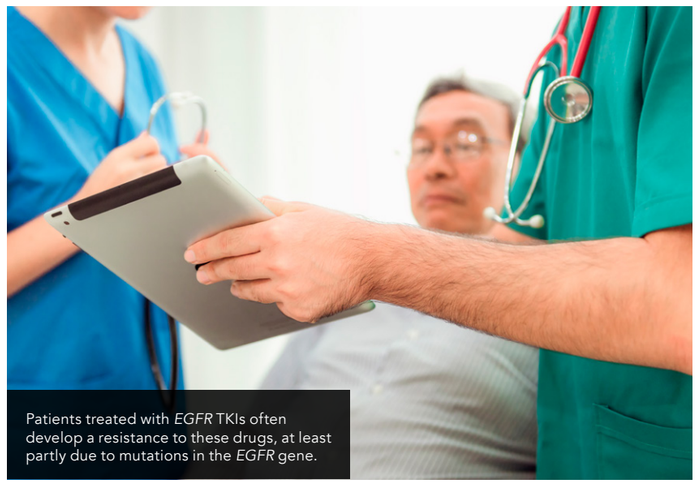
When EGFR mutations occur, it disrupts the function of the receptor protein, meaning it continuously signals the cell to grow, resulting in a tumour.

Their results revealed that resistance to rociletinib and osimertinib is indeed a result of unique genetic mutations.

As Dr Yamaoka and his colleagues had hoped, their results revealed that



Patients treated with *EGFR* TKIs often develop a resistance to these drugs, at least partly due to mutations in the *EGFR* gene.

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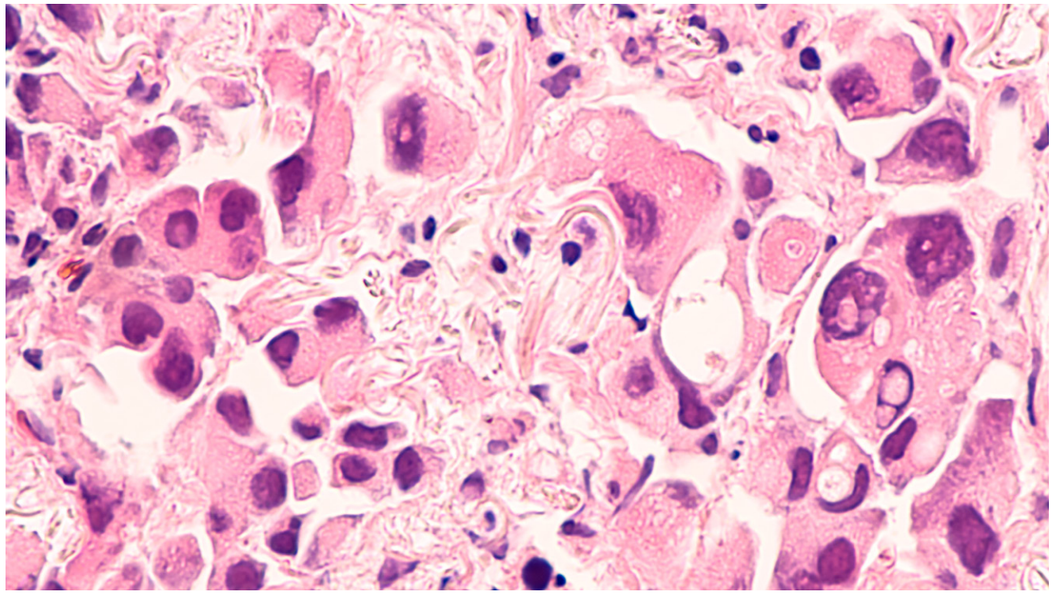

A microscopic image of lung cancer.

resistance to rociletinib and osimertinib is indeed a result of unique genetic mutations. Gene amplification – an increase in the number of copies of a gene – was observed in both *EGFR* and *KRAS*, another gene involved in cell signalling, in the rociletinib-resistant cells. Amplification and subsequent overexpression (where genes are activated inappropriately, as when *EGFR* mutations cause the receptor protein to be always 'on') has been seen in different types of cancer and could be responsible for a tumour becoming malignant.

Dr Yamaoka also spotted *KRAS* amplification in the osimertinib-resistant cells. The degree of resistance to osimertinib appeared to grow as the concentration of the drug was increased. Importantly, in one group of osimertinib-resistant cells, resistance was revealed to be reversible; when the drug was removed, KRAS activity was dulled, and the cells eventually regained sensitivity to osimertinib. In these cells, osimertinib resistance was found to be caused by a particular complex of proteins. Two months after osimertinib was withdrawn, this protein complex broke down. This suggests that when patients develop resistance to osimertinib, the treatment could be withdrawn for two months; on being reinstated, the cancer should respond to the drug once again. Dr Yamaoka believes that this would be a better treatment option than continuous administration of *EGFR* TKIs.

Importantly, in one group of osimertinib-resistant cells, resistance was revealed to be reversible.

Interpreting the results of this study has enabled Dr Yamaoka to suggest that an effective treatment for non-small cell lung cancer could be a combination of third-generation *EGFR* TKIs, like rociletinib and osimertinib, with another group of drugs called MEK inhibitors. These drugs target a particular cell-signalling pathway that is known to be overactive in some cancers. MEK inhibitors block out-of-control cell proliferation and 'kill' cancer cells. At present, preliminary results already suggest that osimertinib following afatinib treatment greatly slows the progression of lung cancer.

FROM RESEARCH TO REAL PATIENTS

How to treat patients who have become resistant to *EGFR* TKI drugs represents a significant medical problem, particularly for patients in the Asia-Pacific region, including Japan. By successfully describing how resistance to two third-generation *EGFR* TKIs, rociletinib and osimertinib, is acquired, Dr Yamaoka and his colleagues have taken the first steps along potential new treatment routes.

Ideally, the team would like to test their ideas in tissue samples taken from non-small cell lung cancer patients. However, such samples are difficult to take from the lungs without causing excessive damage. Another possibility would be to explore *EGFR*-TKI resistance using circulating tumour DNA. These are DNA fragments that, having been shed from the primary and/or metastatic tumour, travel around the body in the blood – they are therefore much easier to sample than tissues deep within a core organ such as the lungs. This is the direction that Dr Yamaoka and his colleagues will take, as they pursue new treatment avenues for patients suffering from non-small cell lung cancer.

Behind the Research

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Research Objectives

The clinical trial "The continuous evaluation of *EGFR* mutation in *EGFR*-mutation positive lung cancer patients during *EGFR* TKI treatment" identifies novel resistance mechanisms in *EGFR*-mutation positive lung cancer patients to help develop more effective anti-cancer treatments.

Detail

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Bio

Dr Yamaoka's medical career began at the Division of Allergology & Respiratory Medicine at Showa University Hospital (Tokyo, Japan), providing medical services for patients with lung cancer, asthma, COPD, pneumonia and chronic bronchial infection. His research career started at the Institute of Molecular Oncology (now called the Advanced Cancer Translational Research Institute) from 1998. Shortly after, Yamaoka's mentor, Dr Ohmori, discovered an *EGFR*-activating mutation in a non-small cell lung cancer cell line, but not in human tissue, motivating them to discover the resistance mechanisms to *EGFR* TKIs.

Funding

Showa University Research Funds

Collaborators

- The members of the Division of Allergology and Respiratory Medicine
- Advanced Cancer Translational Research Institute at Showa University

SHOWA University

References

Nakatani, K. *et al.* (2019). *KRAS* and *EGFR* amplifications mediate resistance to rociletinib and osimertinib in acquired afatinib-resistant NSCLC harbouring exon 19 deletion/T790M in *EGFR*. *Mol Cancer Ther*, 18(1), 112–126.

Personal Response

How do you envision future treatment of EGFR TKI drug-resistance?

"The therapies for patients with *EGFR*-activating mutations can improve as first-line therapy and after second-line therapy. For the first-line therapy, we should find a more effective combination therapy with *EGFR* TKI to pursue longer survival and develop another concept to target *EGFR*-activating mutation. For the after second-line therapy, it is necessary to clarify the various resistance mechanisms and to obtain therapeutic tools which can overcome the corresponding resistance mechanisms, continuously."