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Journal of Cancer Biology & Research

Research Article

Review: Pulmonary Carcinoma Associating with EML4-ALK

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Abstract

Conventionally, it has been believed that oncogenesis accompanying chromosomal translocations such as BCR-Abl in CML is limited to particular tumors such as hematologic diseases, etc. However, the presence of the EML4-ALK fusion gene in lung cancer induced by chromosomal translocations in chromosome 2q was reported in 2007. This is present in approximately 5% of pulmonary adenocarcinomas in which ALK inhibitor, crizotinib, is greatly responsive with respect to EML4-ALK lung cancer. Crizotinib was approved by the FDA in 2012 at an unprecedented speed. A summary of EML4-ALK lung cancer is provided in this report.

Special Issue on

Lung Cancer

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Submitted: 27 January 2014

Accepted: 26 February 2014

Published: 15 March 2014

Keywords

- EML4-ALK
- Lung cancer
- Adenocarcinoma
- Crizotinib

ABBREVIATIONS

EGFR: Epidermal Growth Factor Receptor; EML-4: Echinoderm Microtubule-associated protein-Like 4; ALK: Anaplastic Lymphoma Kinase

INTRODUCTION

Regarding hematologic diseases, the oncogenesis mechanism accompanying chromosomal translocation represented by BCL-Abl in Chronic Myelocytic Leukemia (CML) has been previously well-known [1]. At the same time, it was believed that such oncogenesis caused by a genetic abnormality due to chromosomal translocation was relatively rare in solid tumors including lung cancer. The presence of a genetic mutation in lung cancer was reported for the first time in 2004 [2]. That is, point mutation and 15-base pair deletion in the intracellular tyrosine kinase domain of Epidermal Growth Factor Receptor (EGFR) gene. This was discovered when EGFR inhibitor, such as gefitinib, started being used in medical practice in 2002 as the first molecularly-targeted drug against lung cancer, and a clinical characteristic was found in which gefitinib was well-responsive against particular patient groups; namely, groups including those with adenocarcinoma, never smokers, females, and Asians.

Subsequently, Soda et al. reported in 2008 the presence of echinoderm microtubule-associated protein-like 4 (EML-4) - Anaplastic Lymphoma Kinase (ALK) fusion gene in lung cancer caused by chromosomal translocation [3]. This was the first report on the driver oncogene in lung cancer caused by chromosomal translocation.

It is said that the incidence of EML4-ALK lung cancer accounts for approximately 5% of all lung cancers. Further, crizotinib, which is a molecularly-targeted drug having an effect of being completely responsive against ALK lung cancer, was approved by the FDA in 2012, 4 years following the report by Soda, thus leading to the adoption thereof in medical practice.

A summary of EML4-ALK lung cancer is provided in this report.

Discovery of EML4-ALK lung cancer

Soda and Mano et al. extracted the genes of tumor cells of lung cancer to make a cDNA library, introduced the cDNA to mouse 3T3 fibroblasts using a retroviral vector, and conducted screening of their proliferation. EML4-ALK fusion genes were confirmed from cDNA collected from one transformed clone, that was from the surgery specimen of pulmonary adenocarcinoma of a 62-year-old smoking male patient. The 5' side was approximately half the EML4, which is a type of microtubule assembly protein, while the 3' side was coding the intracellular tyrosine kinase domain of ALK, which is a type of receptor type tyrosine kinase. Both such genes were present inside the short arm 2q of chromosome 2, in which small reverse chromosomal translocation generated in this domain, causing the fusion gene of EML4 and ALK [3].

As a result of creating transgenic mice specifically expressing the EML4-ALK fusion gene in the alveolar epithelium, multiple adenocarcinomas generated in both lungs several weeks following birth. Further, these tumors shrunk due to the oral administration of ALK inhibitor, therefore EML4-ALK was proven to be a strong oncogenic drivergene [4].

Regarding ALK, a fusion-protein with NPM1 due to chromosomal translocation in the anaplastic large cell lymphoma

had been originally confirmed [5]. However, this is the first report on the discovery of an oncogenic driver gene due to gene translocation in a solid tumor.

Generally, receptor type tyrosine kinase such as EGFR forms a dimer due to the binding of a ligand, thereby activating the kinase site. ALK is also a similar receptor type tyrosine kinase. ALK is a cell membrane protein comprising a transmembrane domain configured from 1,620 amino acids in humans, and has a tyrosine kinase domain in the intracellular domain. It is believed to activate due to the extracellular binding of a ligand; however, the specific ligand of ALK in higher eukaryotes has not been clarified as of yet. Meanwhile, EML4 comprises a dimer-forming site referred to as the coiled-coil domain; therefore, in EML4-ALK fusion protein, it is believed that the dimer is formed by the coiled-coil domain of EML4, thus causing the ALK to be constantly activated, and thereby promoting cell proliferation.

The exon 13 of EML4 jointed to exon 20 of ALK was first found regarding the fusion part of EML4 and ALK, and subsequently, it was found that there are more than 10 types of variants thereof [6]. While ALK are all cut on the 5' side of the exon 20, the cleavage site of EML4 varies. The difference in biological activity and the difference in reactivity to the ALK inhibitor due to such a difference in the fusion part is not clear. Moreover, due to the presence of multiple fusion sites, diagnosis with RT-PCR is becoming difficult.

Furthermore, regarding the partner of the ALK fusion gene, several fusion genes such as KIF5B, KLC1, etc. have been reported in addition to the initially reported EML4 [7,8].

Clinical background of ALK lung cancer

From the report on EML4-ALK lung cancer onwards, there have been several reports on the clinical background thereof [9-15]. Once comprehended, it was determined to account for approximately 2-7% of all non-small cell lung cancers [11,13,14], and in the same manner as EGFR mutation lung cancer, the incidence of adenocarcinoma is high, often being observed in women and non-smokers. Moreover, another characteristic is the high incidence in younger people. Inamura reported that EML4-ALK lung cancer accounts for 36% when limited to patients aged

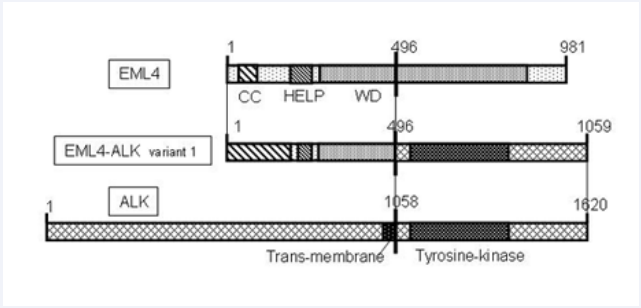

Figure 1 Formation of EML4-ALK fusion gene in lung cancer.
EML4 gene and ALK gene are both present in the short arm of chromosome 2. Reverse translocation generates in a narrow domain, thereby causing fusion of the site comprising the coiled-coil domain of the EML4 gene and the site comprising the intracellular thyrosine kinase domain of the ALK gene, thereby forming the EML4-ALK fusion gene.

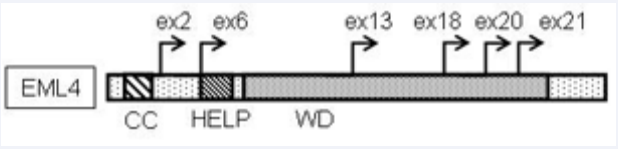

Figure 2 Various variants of EML4-ALK.
Coiled-coil (CC) domain, HELP domain, WD repeat Domain (WD) are present in the EML4 protein, and fuse with exon 20 of the ALK gene in-frame from the site indicated with arrows.

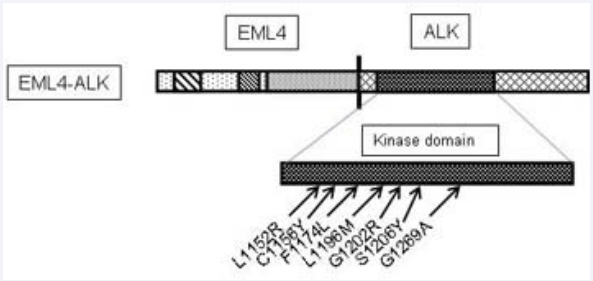

Figure 3 Crizotinib resistance mutations.
This shows the amino acid substitutions causing crizotinib resistance that have been clarified to date. They are all present in the tyrosine-kinase domain of ALK; however, among all of these, the L1196M mutation is the most frequent.

50 years old or younger [12]. It is relatively rare in heavy smokers and squamous cell carcinoma.

It is relatively exclusive with other known oncogenic driver genes such as EGFR gene mutation and KRAS gene mutation, and it is rare for these to be simultaneously present from the initial diagnosis [11,12,16].

The tumor rarely exhibits ground-glass opacity (GGO) in Chest CT images, and it has been determined that a solid pattern is exhibited in most cases [15].

Furthermore, pathomorophological characteristic features of ALK lung cancer by previous reports indicated acinar growth pattern, cribiform pattern, and/or mucinous pattern with signet ring cells [10-12,17,18]. Especially, patients with signet ring cell type are frequently seen in Western [13].

Diagnosing EML4-ALK lung cancer

A diagnosis regarding whether or not EML4-ALK fusion gene is present in the lung cancer cells provides essential information with regard to determining whether or not to administer crizotinib, as mentioned later. Currently, a diagnosis for EML4-ALK lung cancer is made via a combination of the brake-apart FISH method, RT-PCR, and immunohistostaining. However, there are many factors that act as barriers against diagnosis, including: both genes of EML4 and ALK being present in normal cells; the expression of ALK protein itself not being strong even when EML4-ALK fusion gene is present; translocation at the short arm of chromosome 2 being a translocation within a very narrow range; there being several fusing patterns; the possibility that EML4 is not the partner regarding ALK lung cancer, etc., and in the current state, it cannot be said that a definite test has been

sufficiently established. A review is provided regarding each method.

First, the brake-apart FISH method is a method in which FISH is carried out using a probe set on the 5' side and 3' side adjacent to the fusion part of the ALK gene. If there is no translocation of the ALK gene, both probes will appear overlapped, and when the sites at the point in which the two types of probes binding are distant from each other due to translocation, the color of each probe may be confirmed. It is capable of confirming all types of ALK chromosomal translocation and may be carried out in the paraffin-embedded tissue. However, it is problematic in that there are many cases in which making a determination regarding overlapping and separation of signals is difficult, in addition to the fact that the partner gene of ALK gene fusion may not necessarily be EML4.

The RT-PCR method is capable of detecting the EML4-ALK fusion gene at high sensitivity by designing a primer in a manner sandwiching the fusion part of EML4 and ALK and subsequently carrying out RT-PCR. A multiplex RT-PCR method capable of detecting various fusion variants has also been developed [19,20]. Although the RT-PCR method is capable of detecting a variety of translocations, a frozen specimen must be ensured for RT-PCR when sampling specimens from patients, and so there is a major problem in that enforcement with paraffin embedded specimens is impossible.

An anti-ALK antibody is used for immunohistostaining. Although ALK protein is barely expressed in normal pulmonary tissues, the expression of ALK protein is promoted under the presence of ALK gene translocation, so this is detected by immunohistostaining. However, detection of the ALK protein is not stable by normal methods, so Takeuchi et al. developed an intercalated Antibody-enhanced Polymer (iAEP) method for improving the detection sensitivity [7]. This allows occasional enforcement using paraffin embedded specimens, making it a useful method in terms of screening. Meanwhile, the promoted expression of ALK protein does not directly prove the presence of ALK gene translocation; therefore, a re-test by FISH becomes necessary.

In any event, determination by a single method is difficult, and so diagnosis for ALK lung cancer is conducted by combining a plurality of methods; however, improvement of the detection rate will be a future problem.

Treating EML4-ALK lung cancer

Following the indication of the efficacy of ALK inhibitor against pulmonary adenocarcinoma caused by the EML4-ALK fusion gene in animal models [4], various drug industries started developing ALK inhibitors. Among these, the first clinical trial was conducted using crizotinib (PF-02341066) manufactured by Pfizer, Inc. The development of crizotinib has already been commenced as an inhibitor of c-MET, which is a Hepatocyte Growth Factor (HGF) receptor; however, its effect of inhibiting ALK was confirmed, and so the test was switched to the clinical trial of EML4-ALK lung cancer. Crizotinib specifically binds to the ATP-binding pocket to decrease the activity of ALK. In the Profile 1001 trial, which is the phase I study of crizotinib, the oral administration of crizotinib 500mg/day was conducted in 82 cases of EML4-ALK lung cancer diagnosed as ALK translocation-positive by the FISH method; as a result, a good response was acquired with the ORR (the overall response rate) at 57% and 1-year progression-free survival at 74%, and complete remission was observed in 1 case [14,21]. Regarding adverse events, grade 1 vomiting/diarrhea was common, with grade 3 pneumonitis and grade 4 hepatic dysfunction observed in 1 patient, respectively. Moreover, visual disturbance has been reported as a side effect peculiar to ALK inhibitor. This causes prominent afterimages following the transition between dark and bright fields; however, no ophthalmologic abnormalities have been indicated.

Based on the evident efficacy in the Phase I study, the U.S. FDA approved crizotinib as a drug in August 2011, and it was subsequently approved in Japan in March 2012.

Currently, Profile 1007 trial involving a randomized comparison with Docetaxel or Pemetrexed is being carried out in the 2nd line, and Profile 1014 trial involving a randomized comparison with platinum doublet is being carried out in the 1st line as phase III studies. Moreover, multiple ALK inhibitors other than crizotinib are currently under development [22].

In the NCCN guideline, regarding inoperable non-small cell lung cancers not suitable for radical irradiation, in non-squamous cell carcinoma, a gene mutation of EGFR and ALK is being searched for, and if it is positive for ALK gene translocation, crizotinib is to be used as the first line [23]. On the other hand, ALK rearrangement testing is not routinely recommended for those with squamous cell carcinoma.

RESISTANCE OF CRIZOTINIB

Crizotinib has been observed with dramatic effects against ALK lung cancer upon commencement of administration; however, in many cases, resistance to the drug is acquired within a year, causing re-enlargement of the tumor. An investigation is being carried out into the mechanism thereof, with a secondary mutation of the ALK gene itself having already been reported. In the first report, point genetic mutations of C1153Y and L1196M were found from different clones of the same patient [24]. It has been proven that resistance against crizotinib *in vitro* is generated when this point genetic mutation is adopted [25]. Among these, L1196M was a site homologous to T790M, which is a secondary mutation known as the resistance mechanism of lung cancer comprising the EGFR mutation in terms of the structure of kinase. It was inducing the mutation of the innermost amino acid of the ATP-binding pocket referred to as the gate keeper site. Subsequently, many secondary mutations of ALK have been reported in addition to this. Moreover, in addition to the secondary mutation of ALK gene, secondary mutations of the KRAS gene and EGFR gene have also been reported; however, the details thereof have not been clarified as of yet.

Furthermore, there are reports mentioning that ALK inhibitors other than crizotinib were effective *in vitro* against the secondary mutation of the ALK gene [26,27], and further investigation in the future is required regarding the treatment following resistance to crizotinib.

Discovery of a new fusion-type tyrosine kinase

In succession to the discovery of the EML4-ALK fusion gene,

KIF5B was found as a partner of the ALK fusion gene [7]. Upon further investigation, Takeuchi et al. reported a case in which RET, which is another receptor type tyrosine kinase, was fused with KIF5B in pulmonary adenocarcinoma [16]. In the same manner as EML4-ALK, KIF5B was fused with the intracellular domain of RET due to chromosomal translocation. This KIF5B-RET fusion gene also proved to have prominent oncogenesis capacity. It has been indicated to be present in 1.2% of pulmonary adenocarcinoma. In addition, CCDC6-RET has also been reported [4].

Moreover, it has been reported that ROS1, which is another receptor type tyrosine kinase, also forms a fusion gene, and six types of fusion genes have been reported, including SLC34A2-ROS1, etc [28] .

A clinical trial of a molecular target drug, which is hoped to have an effect against RET lung cancer and ROS1 lung cancer [28,29], has already been initiated.

CONCLUSION

The clarification of driver oncogene in lung cancer has been greatly developed since the report on the EML4-ALK fusion gene following EGFR mutation. Also, it is innovative in that it directly connects to the early development of a specific molecularly-targeted drug. Further progress of research is hoped for in the future.

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Cite this article

Mori M, Yamaguchi T, Hirano H, Yokota S (2014) Review: Pulmonary Carcinoma Associating with EML4-ALK. J Cancer Biol Res 2(1): 1027.