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PERSONAL HISTORY

Mar. 1984. I graduated from School of Medicine, Faculty of Medicine, University of Tokyo.
May. 1984. I passed National Examination of Medical Doctor, Japan.
Jun. 1984. I became a member of medical staff at Tokyo University Hospital.
Jun. 1986. I became a member of The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo.
May. 1989. I became a postdoctoral researcher at Department of Biochemistry, St. Jude Children's Research Hospital, TN, USA.
Aug, 1991. I became an Assistant Professor of The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo.
Aug, 1993. I became the Associate Professor of Department of Molecular Biology, Jichi Medical University.
Apr. 2000. I became the Associate Professor of Division of Functional Genomics, Jichi Medical University.
Jun. 2001. I became the Professor of Division of Functional Genomics, Jichi Medical University.
Sep, 2009. I became the Professor of Department of Medical Genomics, Graduate School of Medicine, The University of Tokyo.

SELECTED AWARDS

Oct. 2008 JCA-Mauvernay Award from The Japanese Cancer Association
Nov. 2008 The Medical Award from The Japan Medical Association
Apr. 2009 The Gold Medal Award from Tokyo Techno-forum 21
Nov. 2009 The Science Award for Special Scientific Research by The Sagawa Foundation for Promotion of Cancer Research
Feb. 2010 The Princess Takamatsu Cancer Research Fund Prize
Oct. 2010 The Academic Award of The Mochida Memorial Foundation
Nov. 2010 The Takeda Prize for Medical Science from The Takeda Science Foundation
Jan. 2011 The Brilliant Scientist Award from The National Institute of Science and Technology Policy, Japan
Mar. 2011 The Uehara Prize from The Uehara Memorial Foundation
Apr. 2011 The Prize for Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, Japan
Jul. 2011 The Takamine Memorial Daiichi Sankyo Prize from The Daiichi-Sankyo Foundation of Life Science

PUBLICATIONS RELATED TO THE *EML4-ALK* ONCOGENE

1) Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, Bando M, Ohno S, Ishikawa Y, Aburatani H, Niki T, Sohara Y, Sugiyama Y & Mano H. "Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer" ***Nature*** 448: 561-566, 2007.
2) Choi YL, Takeuchi K, Soda M, Inamura K, Togashi Y, Hatano S, Enomoto M, Hamada T, Haruta H, Watanabe H, Kurashina K, Hatanaka H, Ueno T, Takada S, Yamashita Y, Sugiyama Y, Ishikawa Y & Mano H. "Identification of novel isoforms of the *EML4-ALK* transforming gene in non-small cell lung cancer" ***Cancer Res*** 68: 4971-4976, 2008.
3) Chen Y, Takita J, Choi YL, Kato M, Ohira M, Sanada M, Wang L, Soda M, Kikuchi A, Igarashi T, Nakagawara A, Hayashi Y, Mano H & Ogawa S. "Oncogenic mutations of ALK kinase in neuroblastoma" ***Nature*** 455: 971-974, 2008.
4) Soda M, Takada S, Takeuchi K, Choi YL, Enomoto M, Ueno T, Haruta H, Hamada T, Yamashita Y, Ishikawa Y, Sugiyama Y & Mano H. "A mouse model for *EML4-ALK*-positive lung cancer" ***Proc Natl Acad Sci U S A*** 105: 19893-19897, 2008.

5) Takeuchi K, Choi YL, Togashi Y, Soda M, Hatano S, Inamura K, Takada S, Ueno T, Yamashita Y, Satoh Y, Okumura S, Nakagawa K, Ishikawa Y & Mano H. "KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer" ***Clin Cancer Res*** 15: 3143-3149, 2009.
6) Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, Yatabe Y, Takeuchi K, Hamada T, Haruta H, Ishikawa Y, Kimura H, Mitsudomi T, Tanio Y & Mano H. "EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors" ***N Engl J Med*** 363: 1734-1739, 2010.

Talk at IAAO2011
Session: Personalized medicine; Targeting ALK-fusion
Title: Discovery of EML4-ALK fusion oncogene

Selected References

1. Soda M, ChoiYL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-smallcell lung cancer. Nature 2007; 448:561-6.

Improvement in the clinical outcome of lung cancer is likely to be achieved by identification of the molecular events that underlie its pathogenesis. Here we show that a small inversion within chromosome 2p results in the formation of a fusion gene comprising portions of the echinoderm microtubule-associated protein-like 4 (EML4) gene and the anaplastic lymphoma kinase (ALK) gene in non-small-cell lung cancer (NSCLC) cells. Mouse 3T3 fibroblasts forced to express this human fusion tyrosine kinase generated transformed foci in culture and subcutaneous tumours in nude mice. The EML4–ALK fusion transcript was detected in 6.7% (5 out of 75) of NSCLC patients examined; these individuals were distinct from those harbouring mutations in the epidermal growth factor receptor gene. Our data demonstrate that a subset of NSCLC patients may express a transforming fusion kinase that is a promising candidate for a therapeutic target as well as for a diagnostic molecular marker in NSCLC. Lung cancer remains the leading cause of cancer deaths in western countries1. Patients with NSCLC, which accounts for ,80% of lung cancer cases, are often diagnosed at advanced stages of the disease. Given that conventional chemotherapeutic regimens only marginally improve the outcome of such individuals, their median survival time is less than one year after diagnosis (ref. 2). A subset of NSCLCs was recently shown to harbour activating mutations in the epidermal growth factor receptor gene (EGFR)3,4; such cancers are responsive to gefitinib, a specific inhibitor of the tyrosine kinase activity of EGFR. The efficacy of targeting key 'growth drivers' in cancer treatment is further exemplified by chronic myeloid leukaemia, for which another tyrosine kinase inhibitor, STI571, is highly effective in reducing the number of cancer cells5. However, EGFR mutations are associated preferentially with NSCLC of non-smokers and Asians4,6. Few oncogenes have thus been identified for NSCLC in individuals with a smoking habit, who constitute most cases of the disease. Retrovirus-mediated complementary DNA expression systems allow expression of the encoded proteins in most of the targeted cells. Through modification of the method used in ref. 7, we have achieved reliable amplification of cDNAs from small quantities of clinical specimens as well as the generation of retroviral libraries for expression of these cDNAs8–10. Application of such a cDNA expression library prepared from an NSCLC specimen to a focus formation assay with mouse 3T3 fibroblasts has now led to the identification of a fusion oncogene.

2. ChenY,TakitaJ, ChoiYL, et al. Oncogenicmutations of ALK kinase in neuroblastoma. Nature 2008;455: 971- 4.

Neuroblastoma in advanced stages is one of the most intractable paediatric cancers, even with recent therapeutic advances1. Neuroblastoma harbours a variety of genetic changes, including a high frequency of MYCN amplification, loss of heterozygosity at 1p36 and 11q, and gain of genetic material from 17q, all of which have been implicated in the pathogenesis of neuroblastoma2–5. However, the scarcity of reliable molecular targets has hampered the development of effective therapeutic agents targeting neuroblastoma. Here we show that the anaplastic lymphoma kinase (ALK), originally identified as a fusion kinase in a subtype of non-Hodgkin's lymphoma (NPM–ALK)6–8 and more recently in adenocarcinoma of lung (EML4–ALK)9,10, is also a frequent target of genetic alteration in advanced neuroblastoma. According to our genome-wide scans of genetic lesions in 215 primary neuroblastoma samples using high-density single-nucleotide polymorphism genotyping microarrays11–14, the ALK locus, centromeric to the MYCN locus, was identified as a recurrent target of copy number gain and gene amplification. Furthermore, DNA sequencing of ALK revealed eight novel missense mutations in 13 out of 215 (6.1%) fresh tumours and 8 out of 24 (33%)neuroblastoma-derived cell lines. All but one mutation in the primary samples (12 out of 13) were found in stages 3–4 of the disease and were harboured in the kinase domain. The mutated kinases were autophosphorylated and displayed increased kinase activity compared with the wild-type kinase. They were able to transform NIH3T3 fibroblasts as shown by their colony formation ability in soft agar and their capacity to form tumours in nude mice. Furthermore, we demonstrate that downregulation of ALK through RNA interference suppresses proliferation of neuroblastoma cells harbouring mutated ALK. We anticipate that our findings will provide new insights into the pathogenesis of advanced neuroblastoma and that ALK-specific kinase inhibitors might improve its clinical outcome.

3. Choi YL,Takeuchi K, Soda M, et al. Identification of novel isoforms of the EML4-ALK transforming gene in

non-small cell lung cancer. Cancer Res 2008;68: 4971- 6.

The genome of a subset of non–small-cell lung cancers (NSCLC) harbors a small inversion within chromosome 2 that gives rise to a transforming fusion gene, EML4-ALK, which encodes an activated protein tyrosine kinase. Although breakpoints within EML4 have been identified in introns 13 and 20, giving rise to variants 1 and 2, respectively, of EML4-ALK, it has remained unclear whether other isoforms of the fusion gene are present in NSCLC cells. We have now screened NSCLC specimens for other in-frame fusion cDNAs that contain both EML4 and ALK sequences. Two slightlydif ferent fusion cDNAs in which exon 6 of EML4 was joined to exon 20 of ALK were each identified in two individuals of the cohort. Whereas one cDNA contained onlyexons 1 to 6 of EML4 (variant 3a), the other also contained an additional 33-bp sequence derived from intron 6 of EML4 (variant 3b). The protein encoded byt he latter cDNA thus contained an insertion of 11 amino acids between the EML4 and ALK sequences of that encoded bythe former. Both variants 3a and 3b of EML4-ALK exhibited marked transforming activity in vitro as well as oncogenic activity in vivo. A lung cancer cell line expressing endogenous variant 3 of EML4-ALK underwent cell death on exposure to a specific inhibitor of ALK catalytic activity. These data increase the frequency of EML4-ALK–positive NSCLC tumors and bolster the clinical relevance of this oncogenic kinase.

4. **Soda M,Takada S,Takeuchi K, et al. A mouse model for EML4-ALK -positive lung cancer. Proc Natl Acad Sci US A 2008;105:19893- 7.**

EML4-ALK is a fusion-type protein tyrosine kinase that is generated in human non-small-cell lung cancer (NSCLC) as a result of a recurrent chromosome inversion, inv (2)(p21p23). Although mouse 3T3 fibroblasts expressing human EML4-ALK form transformed foci in culture and s.c. tumors in nude mice, it has remained unclear whether this fusion protein plays an essential role in the carcinogenesis of NSCLC. To address this issue, we have now established transgenic mouse lines that express EML4-ALK specifically in lung alveolar epithelial cells. All of the transgenic mice examined developed hundreds of adenocarcinoma nodules in both lungs within a few weeks after birth, confirming the potent oncogenic activity of the fusion kinase. Although such tumors underwent progressive enlargement in control animals, oral administration of a smallmolecule inhibitor of the kinase activity of ALK resulted in their rapid disappearance. Similarly, whereas i.v. injection of 3T3 cells expressing EML4-ALK induced lethal respiratory failure in recipient nude mice, administration of the ALK inhibitor effectively cleared the tumor burden and improved the survival of such animals. These data together reinforce the pivotal role of EML4-ALK in the pathogenesis of NSCLC in humans, and they provide experimental support for the treatment of this intractable cancer with ALK inhibitors.

5. **KIF5B-ALK, a Novel Fusion Oncokinase Identified by an Immuno-histochemistry-based Diagnostic Systemfor ALK-positive Lung Cancer (Clin Cancer Res 15, 3143-3149 (2009))**

Abstract Purpose: EML4-ALK is a transforming fusion tyrosine kinase, several isoforms of which have been identified inlung cancer. Immunohistochemical detection of EML4-ALK has proved difficult, however, likely as a result of low transcriptional activity conferred by the promoter-enhancer region of EML4. The sensitivity of EML4-ALK detection by immunohistochemistry should be increased adequately.

Experimental Design: We developed an intercalated antibody-enhanced polymer (iAEP) method that incorporates an intercalating antibody between the primary antibody to ALK and the dextran polymer-based detection reagents.

Results: Our iAEP method discriminated between tumors positive or negative for EML4-ALK in a test set of specimens. Four tumors were also found to be positive forALK in an archive of lung adenocarcinoma (n = 130) and another 4 among fresh cases analyzed in a diagnostic laboratory. These 8 tumorswere found to include1with EML4-ALK variant1,1with variant 2, 3with variant 3, and 2 with previously unidentified variants (designated variants 6 and 7). Inverse reverse transcription-PCR analysis revealed that the remaining tumor harbored a novel fusion in which intron 24 of KIF5B was ligated to intron 19 of ALK. Multiplex reverse transcription-PCR analysis of additional archival tumor specimens identified another case of lung adenocarcinoma positive for KIF5B-ALK.

Conclusions: The iAEP method should prove suitable for immunohistochemical screening of tumors positive for ALK or ALK fusion proteins among pathologic archives. Coupling of PCR-based detection to the iAEP method should further facilitate the rapid identification of novel ALK fusion genes such as KIF5B-ALK.

6. **EML4-ALK Mutations in Lung Cancer That Confer Resistance to ALK Inhibitors (N Engl J Med 2010;363:1734-9 (2010))**

The EML4 (echinoderm microtubule-associated protein-like 4)–ALK (anaplastic lymphoma kinase) fusion-type tyrosine kinase is an oncoprotein found in 4 to 5% of non–small-cell lung cancers, and clinical trials of specific inhibitors of ALK for the treatment of such tumors are currently under way. Here, we report the iscovery of two secondary mutations within the kinase domain of EML4-ALK in tumor cells isolated from a patient during the relapse phase of treatment with an ALK inhibitor. Each mutation developed independently in subclones of the tumor and conferred marked resistance to two different ALK inhibitors.