Novel Germline Mutation in the Transmembrane Domain of HER2 in Familial Lung Adenocarcinomas

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We encountered a family of Japanese descent in which multiple members developed lung cancer. Using whole-exome sequencing, we identified a novel germline mutation in the transmembrane domain of the human epidermal growth factor receptor 2 (HER2) gene (G660D). A novel somatic mutation (V659E) was also detected in one of 253 sporadic lung adenocarcinomas. Because the transmembrane domain of HER2 is considered to be responsible for the dimerization and subsequent activation of the HER family and downstream signaling pathways, we performed functional analyses of these HER2 mutants. Mutant HER2 G660D and V659E proteins were more stable than wild-type protein. Both the G660D and V659E mutants activated Akt. In addition, they activated p38, which is thought to promote cell proliferation in lung adenocarcinoma. Our findings strongly suggest that mutations in the transmembrane domain of HER2 may be oncogenic, causing hereditary and sporadic lung adenocarcinomas.

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Familial lung cancers are rare among human malignancies. Recent studies have reported that germline mutations in the epidermal growth factor receptor (EGFR) gene predispose the development of lung cancer. Reported familial lung adenocarcinomas with a germline EGFR mutation, such as T790M, carry secondary somatic EGFR mutations, including exon 19 deletion and exon 21 L858R mutation (1–4). We encountered a family of Japanese descent in which multiple members developed lung cancer (Figure 1). The proband (III-4) was a 53-year-old woman with multiple lung adenocarcinomas in bilateral lungs. She was a light smoker with a 1.2-pack-year history of smoking. She had undergone a left lower lobectomy for multiple lung adenocarcinomas at the age of 44 years. Her mother (II-4), a never smoker, also had multiple lung adenocarcinomas. Partial pulmonary resections of two tumors were performed for II-4 for the purpose of diagnosis after pleural dissemination was found during surgery, and multiple lesions were removed in a lobectomy or partial resections in III-4. A histological examination of the resected tumors in II-4 revealed nonmucinous adenocarcinoma in situ and nonmucinous minimally invasive adenocarcinoma, whereas

Figure 1. Pedigree chart of a Japanese family in which multiple members developed lung cancer. The boxes and circles indicate men and women, respectively. The numbers at the bottom of each member indicate the age at the time of death or the time of the analysis. An oblique line shows deceased family members. The proband (III-4) had multiple lung adenocarcinomas (arrow). Tumor tissue, nonmalignant lung tissue, and peripheral blood samples were obtained from III-4. The proband’s mother (II-4) also had multiple lung adenocarcinomas, and tumor and nonmalignant lung tissue samples were available. The proband’s father (II-5) and sister (III-5) were both unaffected, and peripheral blood samples were obtained from these individuals. Some family members who were not considered as critical for this study were excluded from the pedigree chart to preserve confidentiality. Whole-exome sequencing was performed for individuals II-4, II-5, III-4, and III-5.
the histological findings of pleural dissemination indicated mucus-containing adenocarcinoma. Those of III-4 contained various subtypes of adenocarcinoma, including non-mucinous and mucinous adenocarcinoma in situ and invasive mucinous adenocarcinoma. In addition, normal-appearing lung parenchyma obtained from a lobectomy in III-4 revealed innumerable small pre-invasive lesions, implying the presence of precancerous changes throughout the lung (Supplementary Figure 1, available online). Sequencing analyses of EGFR exons 18 to 21 and KRAS as well as an immunohistochemical staining for ALK protein in the resected tumors indicated no genetic alterations in these genes. The pedigree chart suggested that lung cancer was inherited in an autosomal dominant manner.

After obtaining permission from the Institutional Review Board at Okayama University Hospital and informed consent from the patients and other family members, we performed a whole-exome sequencing study. Tumor DNA samples from II-4, tumor and peripheral blood DNA samples from III-4, and peripheral blood DNA samples from two unaffected family members (II-5 and III-5) were used for the analysis. The candidate germline alterations were restricted to 29 variants by comparing the whole-exome sequencing results between the patients and the unaffected family members. Among them, we focused on a point mutation in the human epidermal growth factor receptor 2 (HER2/neu) gene (NM_004448, G660D, GGC to GAC), which was located in exon 17 encoding the transmembrane domain of HER2 (Supplementary Tables 1–3). This alteration was confirmed by direct sequencing (Figure 2A). We also confirmed that there was no copy number gain of HER2 in the examined tumors based on the degree of read-depth in the whole-exome sequencing results. Of note, no mutations in genes known to cause lung cancers were detected for tumors from III-4 and II-4.

We considered that somatic mutations in the HER2 transmembrane domain might act as driver mutations in lung cancer. Hence, we sequenced exon 17 of the HER2 gene in the tumor and peripheral blood DNA samples from III-4. Sequencing performed on tumor DNA from III-4 indicated G660D. Sequencing of the peripheral blood DNA from III-4 failed to detect the G660D mutation, indicating that this alteration was of germline origin.

Figure 2. DNA and amino acid sequences in the transmembrane domain of HER2. A) Direct Sanger sequencing of the proband (III-4), her affected mother (II-4), and her unaffected sister (III-5). The results indicated that G660D was a germline mutation. B) Direct sequencing of a sporadic lung adenocarcinoma with a HER2 V659E mutation. V659E was found to be of somatic origin based on the sequencing results of the peritumoral lung tissue from the same specimen. All the sequence variants were confirmed by independent polymerase chain reaction amplifications and were sequenced in both directions. C) Interspecies conservation of the transmembrane domain of HER2 (UCSC Genome Browser, http://genome.ucsc.edu, accessed September 12, 2013). The yellow highlight indicates the N-terminal glycine zipper motif Thr652-Xe-Ser655-Xe-Gly660, a tandem variant of a GG4-like motif of human HER2. Codons 659 and 660 in human HER2 are highly conserved among the listed vertebrate species (shown in red). X. tropicalis = Xenopus tropicalis.
to be activated by HER2 by phosphati-
dylinositol 3-kinase and leads to increased
cell growth and survival (12,13). Also, the
activation of p38 was shown to contribute to
the viability of lung adenocarcinoma
cells derived from never or light smokers
(14,15). A western blot analysis for Akt and
p38 successfully confirmed the upregu-
lation of both phospho-Akt and phospho-p38
expression in the mutant HER2 transfect-
ants (Supplementary Figure 2B).

Because the G660D alteration in HER2
might have been the cause of the lung can-
cer in the pedigree studied, we investigated
whether familial aggregation of cancer in
other organs could be seen in this pedi-
gree. We found that II-1 and II-6 de-
veloped renal and gastric cancers, respectively;
however, both of them also had lung cancer.
The reason why other types of clinically
apparent malignances were rarely found in
this pedigree is unclear. The G660D ger-
mline mutation may be tolerated in organs
other than the lung.

This study had some limitations. First,
the carcinogenic potential of the HER2
mutation at the transmembrane domain
should be confirmed in other models such as
gastric cancer in mice. Accordingly,
hereditary ERBB2 kinase mutations are
rarely found in human tumors, oncogenic
transformation (8). In addition, in vivo
experiments showed that the HER2 V659E
mutation contributed to the stability of HER2
dimers, resulting in the dysregu-
lated receptor activation and subsequent
cell transformation (9,10). Furthermore,
the novel mutations were located within the
glycine zipper motif Thr612-Xaa-Ser616,
Xaa-Gly620, a tandem variant of the
GGG-like motif, at the N-terminal portion
of the transmembrane domain, which was
critically related to the dimerization
of HER2 (Figure 2C) (9,11). Accordingly,
we performed a functional analysis of the
mutant HER2 proteins. We found that
the degradation of HER2 protein after
the administration of cycloheximide was
slower in G660D and V659E mutants as
compared with wild-type (Supplementary
Figure 2A), indicating the higher stabil-
y of the mutant proteins than wild-type
protein. In addition, results of a phospha-
mitogen–activated protein kinase array
indicated the activation of Akt and p38α
(data not shown). Indeed, Akt is known

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Note
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