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BRIEF COMMUNICATION

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 the transmembrane domain of *HER2* 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

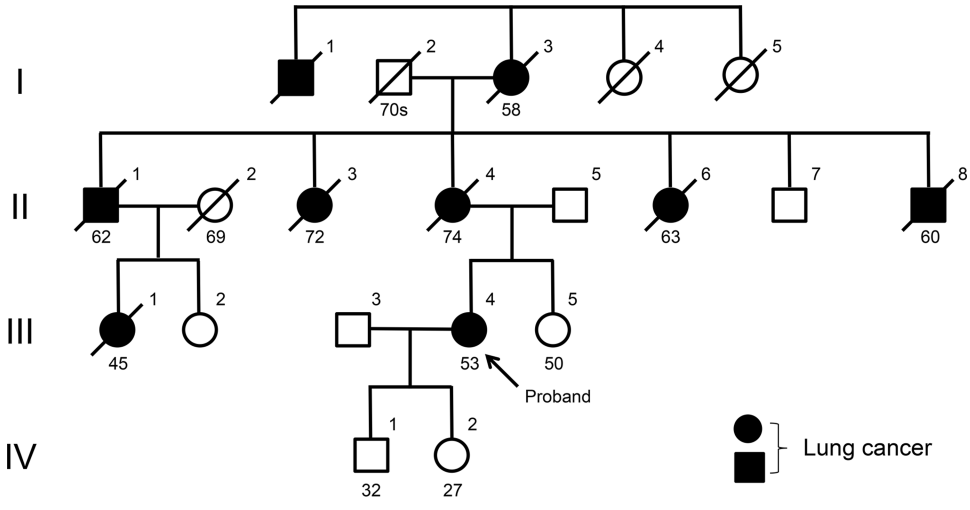

Figure 1. Pedigree chart of a Japanese family in which multiple members developed lung cancer. The **boxes** and **circles** indicate men and women, respectively. The **numbers** at the bottom of each member indicate the age at the time of death or the time of the analysis. An **oblique line** shows 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 nonmucinous 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 preinvasive 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*

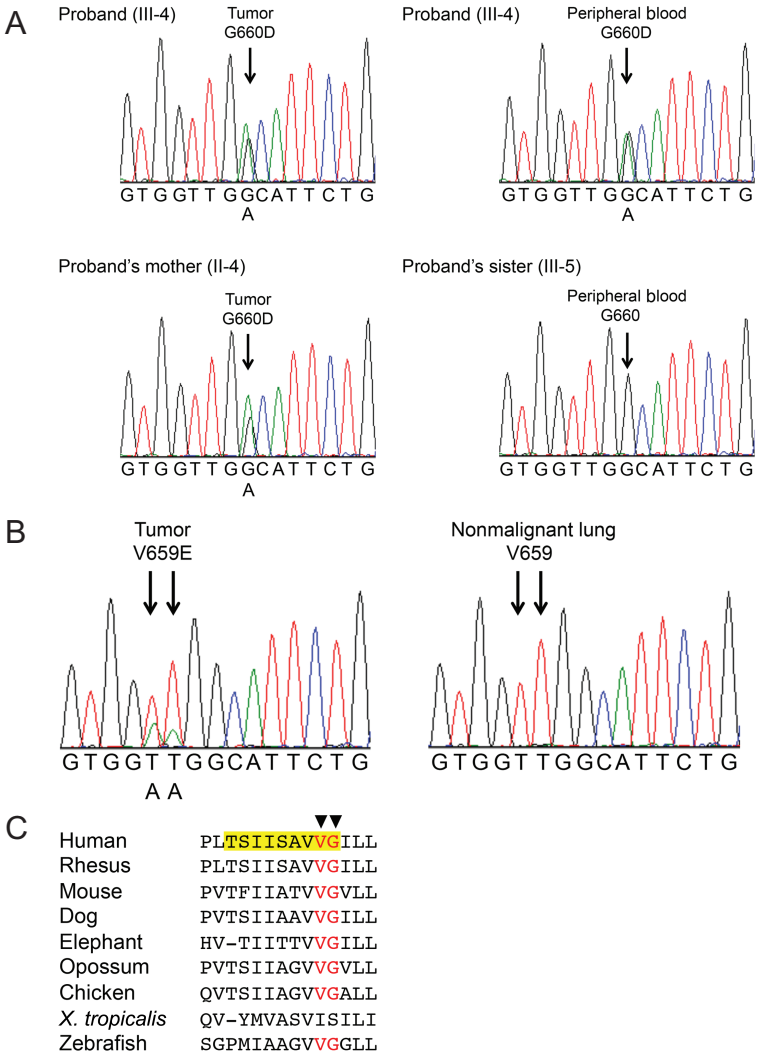

Figure 2. DNA and amino acid sequences in the transmembrane domain of *HER2*. **A**) Direct Sanger sequencing of the proband (III-4), her affected mother (II-4), and her unaffected sister (III-5). The results indicated that G660D was a germline mutation. **B**) Direct sequencing of a sporadic lung adenocarcinoma 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 Thr^{652}-X_3-Ser^{656}-X_3-Gly^{660}, 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*.

in the tumor samples of 315 sporadic non–small cell lung cancer patients, of which 253 were adenocarcinomas. Although the *HER2* G660D mutation was not detected, a novel nonsynonymous mutation, V659E (GTT to GAA), next to codon 660 was identified in one of these patients. This patient was histologically diagnosed as nonmucinous adenocarcinoma in situ, and the patient had neither smoking history nor apparent family history of lung cancer. This V659E mutation was certainly a somatic mutation because it was not identified in the peritumoral lung tissue of the same patient (Figure 2B). The alignment of *HER2* amino acid sequences showed high conservation of valine 659 and glycine 660 among vertebrates (Figure 2C).

HER2 somatic mutations have been reported in 2% to 4% of lung adenocarcinomas (5–7). However, all reported mutations were restricted to its tyrosine kinase domain (6,7). According to the cBioPortal for Cancer Genomics (http://www.cbioportal.org/public-portal/, accessed September 12, 2013), the same genetic mutation in the *HER2* has not been reported in any type of cancer. Interestingly, a previous study reported that a mutation in the transmembrane domain (V664E) of the rat *neu* gene, which corresponds to V659E in its human homolog *HER2*, induced oncogenic transformation (8). In addition, in vivo experiments showed that the *HER2* V659E mutation contributed to the stability of HER2 dimers, resulting in the dysregulated receptor activation and subsequent cell transformation (9,10). Furthermore, the novel mutations were located within the glycine zipper motif Thr^{652}-X_3-Ser^{656}-X_3-Gly^{660}, a tandem variant of the GG4-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 stability of the mutant proteins than wild-type protein. In addition, results of a phospho-mitogen–activated protein kinase array indicated the activation of Akt and p38α (data not shown). Indeed, Akt is known to be activated by HER2 by phosphatidylinositol 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 upregulation of both phospho-Akt and phospho-p38 expression in the mutant *HER2* transfectants (Supplementary Figure 2B).

Because the G660D alteration in *HER2* might have been the cause of the lung cancer in the pedigree studied, we investigated whether familial aggregation of cancer in other organs could be seen in this pedigree. We found that II-1 and II-6 developed 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 germline 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 transgenic mice. Second, the rarity of these mutations in sporadic lung cancers may be the limitation for generalizability to other cases even if targeting therapies for similar types of *HER2* mutation were developed.

In conclusion, we identified a novel germline mutation in the transmembrane domain of the *HER2* in familial lung adenocarcinomas. Somatic mutation in the *HER2* transmembrane domain may be a possible cause of sporadic lung adenocarcinomas.

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Note

H. Yamamoto, J. Soh, S. Miyoshi, and S. Toyooka conceived the project. K. Higasa, M. Sakaguchi, K. Shien, and K. Ichimura performed the experiments. H. Yamamoto, J. Soh, M. Furukawa, S. Hashida, N. Takigawa, K. Kiura, K. Tsukuda, and S. Toyooka collected the samples and assisted with the experiments. H. Yamamoto, K. Higasa, K. Shien, and K. Matsuo analyzed the data. H. Yamamoto, K. Higasa, M. Sakaguchi, F. Matsuda, and S. Toyooka prepared the manuscript with input from the other authors. S. Miyoshi, F. Matsuda, and S. Toyooka supervised the project. The authors declared no conflicts of interest.

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