Rapid Detection of Epidermal Growth Factor Receptor Mutations in Lung Cancer by the SMart-Amplification Process

Kanako Hoshi,1,4 Hideki Takakura,1,4 Yasumasa Mitani,1,2 Kenji Tatsumi,1,4 Nobuyoshi Momiyama,4 Yasushi Ichikawa,4 Shinji Togo,4 Toru Miyagi,6 Yuki Kawai,1,2 Yasushi Kogo,1 Takeshi Kikuchi,1,2 Chiaki Kato,1,2 Takahiro Arakawa,1 Syuji Uno,1,3 Paul E. Cizdziel,1 Alexander Lezhava,1 Noboru Ogawa,5 Yoshihide Hayashizaki,1,3 and Hiroshi Shimada4

Abstract Purpose: A positive response to gefitinib in non–small cell lung cancer (NSCLC) has been correlated to mutations in epidermal growth factor receptor (EGFR) gene. Previous reports have been based mainly on diagnostic screening by sequencing. However, sequencing is a time-consuming and complicated procedure, not suitable for routine clinical use.

Experimental Design: We have developed rapid, simple, and sensitive mutation detection assays based on the SMart Amplification Process (SMAP) and applied it for analyzing EGFR gene mutations in clinical samples. By using SMAP, we can detect mutations within 30 min including sample preparation. To validate the assay system for potential use in clinical diagnostics, we examined 45 NSCLC patients for EGFR mutations using sequencing and SMAP.

Results: The outcomes of the SMAP assay perfectly matched the sequencing results, except in one case where SMAP was able to identify a mutation that was not detected by sequencing. We also evaluated the sensitivity and specificity of SMAP in mutation detection for EGFR. In a serial dilution study, SMAP was able to find a mutation in a sample containing only 0.1% of the mutant allele in a mixture of wild-type genomic DNA. We also could show amplification of mutated DNA with only 30 copies per reaction.

Conclusions: The SMAP method offers higher sensitivity and specificity than alternative technologies, while eliminating the need for sequencing to identify mutations in the EGFR gene of NSCLC. It provides a robust and point-of-care accessible approach for a rapid identification of most patients likely to respond to gefitinib.

Non–small cell lung cancer (NSCLC) is the most common cause of death by cancer worldwide (1). As the global burden of NSCLC continues to increase, new agents are being developed for more effective treatment within a wide range of modalities, including surgery, radiotherapy, and chemotherapy, as the first and second lines of treatment. However, many patients still experience a relapse in cancer growth after cytotoxic chemotherapy.

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, gefitinib (Iressa, ZD1839), has emerged as an effective therapeutic agent for some patients with advanced NSCLC (2). Clinical trials revealed that there is a significant variability in the response to gefitinib. Recently, two groups reported an association between somatic mutations in the EGFR gene and dramatic positive clinical responses to gefitinib in patients with NSCLC (3, 4). Among 848 cases of EGFR mutations in database,7 739 occurred in three “hotspots” associated with the response to gefitinib. Of these, 53.2% (393 of 739) were multinucleotide in-frame deletions encoded by exon 19, 44.0% (325) cases were point mutations in exon 21 that resulted in a specific amino acid substitution at position

7 http://www.cityofhope.org/cmdl/egfr%5Fdb/

Human Cancer Biology

Authors’ Affiliations: 1Genome Exploration Research Group (Genome Network Project Core Group), RIKEN Genomic Sciences Center, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho, 1-7-29 Suehiro-cho, Tsurumi-ku; 2Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, 3-9 Fukaura, and 3Graduate School of Integrated Science, 6-16-1 Tomiokahigashi, Kanazawa-ku, Yokohama, Kanagawa, Japan; and 4Department of Respiratory Surgery, Kanagawa Cardiovascular and Respiratory Center, 6-16-1 Tomiokahigashi, Kanazawa-ku, Yokohama, Kanagawa, Japan; and 5Wakunaga Pharmaceutical Co. Ltd., 1624 Shimokotachi, Koda-cho, Akitakata-shi, Hiroshima, Japan

Received 3/1/07; accepted 5/9/07.

Grant support: RIKEN Genome Exploration Research Project from the Ministry of Education, Culture, Sports, Science, and Technology of the Japan (MEXT) to Y. Hayashizaki, and RIKEN “Research Collaborations with Industry” Program to K. Shibata. S. Kuramitsu is supported by the Research Grant for National Project on Protein Structure and Functional Analysis from MEXT.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Requests for reprints: Kanako Hoshi, Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, 3-9 Fukaura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan. Phone: 81-45-787-2650; E-mail: Canaty@aol.com.

©2007 American Association for Cancer Research. doi:10.1158/1078-0432.CCR-07-0509

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).
858 (L858R), and 2.8% (21) were point mutations in exon 18 (G719S). The remaining mutations were nonsynonymous and scattered throughout exons 18 to 21 or in-frame duplication/insertions in exon 20.

Information about the EGFR mutation status in clinical samples is becoming incorporated into clinical decisions and designs for future clinical trials. The previous standard for experimental detection of genomic mutations is the sequencing of DNA amplified from tumor tissues. However, as long as EGFR mutation testing is based on sequencing, it is unlikely to be a widely adopted approach. Diagnostic sequencing techniques are too complex, time-consuming, and expensive for routine pretherapeutic screening programs. Adding to the complexity is that clinical samples often contain a subpopulation of mutant cells mixed within an excess of normal tissue, sometimes causing the mutations to be missed by sequencing due to technical limitations of the technology. For conclusive determination of a mutant subpopulation, the sequence needs to be present at a minimum of ~25% of the total genetic content. At levels below this threshold, the signal may not be present or confused with background noise and not recognized. With the rapid development of mutation-detection techniques, many methods have been reported for detection of EGFR mutations (5–18). These techniques are based on PCR-related (5–14), sequence-related (15), and other methodologies (16–18).

Some are more accurate and sensitive than sequencing, but all are still time consuming, complicated, and unsuitable for routine clinical examination. We therefore were motivated to develop a fast, easy, inexpensive, and accurate EGFR-mutation detection method.

It was previously shown that the Smart Amplification Process (SMAP) method can detect single nucleotide polymorphisms with a high sensitivity, specificity, and within 30 min under isothermal conditions (19). We adapted the SMAP method to target the three EGFR hotspot mutations and showed the detection of these sequence changes in tumor samples within 30 min. The main goal of this study was to develop a reliable method for rapid screening of EGFR mutations that had potential for point-of-care testing (POCT).

Materials and Methods

The principle of SMAP used for EGFR mutation detection. The SMAP method is a unique genotyping technology that can detect a mutation within 30 min under isothermal conditions and in a single step (19). The basic principle of SMAP is that “DNA amplification equals detection,” no further analysis is required. The critical milestone in establishing the SMAP method was to suppress exponential background amplification and ensure single nucleotide precision for amplification of mutant-specific sequences.

The basic primer design of SMAP is presented in Fig. 1. A set of five specially designed primers that recognize a total of six distinct sequences on the target DNA enable the precise amplification of only mutant-specific sequences. The five primers are the folding primer (FP), turn-back primer (TP), boost primer (BP), and two outer primers (OP1 and OP2). One key advantage of SMAP is the flexibility of primer design; mutation detection primers can be designed on various positions at the 3' end of FP, TP, or BP and at the 5' end of TP. For suppression of background (nonspecific) amplification, SMAP employs two strategies; use of an asymmetrical primer design, which minimizes potential misamplification pathways, and inclusion of Taq MutS in the assay mix. Taq MutS is a mismatch binding protein that recognizes mismatched primer-template pairs and prevents the complex from being an active template for DNA synthesis (20, 21). Through use of a strand-displacing DNA polymerase, amplification occurs to generate amplicons of two short intermediate products <200 bp. These two intermediates then are capable of self-priming through three possible pathways to synthesize concatenated products containing multiples of the amplicons up to several hundred units in length. Exponential amplification occurs continuously until the reaction components are exhausted and copious amounts of DNA are generated. The DNA can then be detected or its generation monitored in real time by intercalating SYBR Green I (Molecular Probes) dye during the reaction. The SMAP method for detecting the L858R mutation was carried out in a total of 25 μL reaction mixture containing 1.6 μmol/L of each FP...
and TP, 0.8 μmol/L BP, 0.1 μmol/L each OP1 and OP2 (primers), 0.5 mmol/L deoxynucleotide triphosphates (dNTP), 0.6 mol/L betaine, 10 mmol/L (NH₄)₂SO₄, 2.5 mmol/L MgCl₂, 10 mmol/L KCl, 2 mmol/L MgSO₄, 1:100,000 diluted SYBR Green I, 20 mmol/L Tris-HCl (pH 8.8), 0.1% TritonX-100, 8 units Bst DNA polymerase (New England Biolabs), 1 μg Taq MutS (Nippon Gene), and 1 μL of prepared template. After amplification, the products of the SMAP reaction were cleaved by MscI and run on 3% of Nusieve GTG agarose gel for sizing and comparison.

For amplification of the G719S mutation, the components of reaction mix were identical to that of the L858R assay, except for changes in the concentration of the following components: 0.2 μmol/L each of OP1 and OP2, 0.4 mol/L betaine, and 5 mmol/L MgCl₂.

The SMAP method for deletion detection in the EGFR gene on exon 19. We designed EGFR mutation detection primer sets that were specific for SMAP amplification of the seven highest frequency deletions observed in NSCLC. The location and sequences of primers for SMAP-based deletion typing of EGFR are shown in Fig. 3. In this case, we chose to use the BP as the mutation detection primer. The two outside primers (OP1, OP2), the FP, and the TP are all commonly used among the exon 19 deletion detection assays. Specificity for detecting each deletion mutant resides exclusively in the design of the BPs. Each BP was made to anneal perfectly to a different deletion mutant with a primer sequence that spans across the actual deletion site and has perfect homology on either side. The wild-type BP [BP(Wt)] sequence is shown in Fig. 3D, and the sequence of all Boost deletion primers [BP(DE-A) to BP(DE-G)] are also shown. The precise locations of the seven high-frequency deletions are shown as underlined nucleotides in Fig. 3C. They are also described in more detail in Supplementary Data S1.

The SMAP reactions for detecting the exon 19 deletion alleles were done with the same reaction mixture used for detecting the exon 19 wild-type allele, except that BP(DE-X) was used instead of BP(Wt). Reactions were done in 25 μL of a SMAP reaction mixture containing 10 mmol/L (NH₄)₂SO₄, 2.5 mmol/L MgCl₂, 10 mmol/L KCl, 2 mmol/L MgSO₄, 1:100,000 diluted SYBR Green I, 20 mmol/L Tris-HCl (pH 8.8), 0.1% Triton X-100, 8 units Bst DNA polymerase (New England Biolabs), 1 μg Taq MutS (Nippon Gene), 0.5 mol/L betaine, 0.45 mmol/L each dNTPs, 0.5 μg Taq MutS, 2.0 μmol/L each of FP and TP, 0.25 μmol/L each of OP1 and OP2, 0.8 μmol/L BP, and 1 μL of prepared template. The reactions were incubated at 60 °C for 30 min and monitored by real-time PCR as described in the previous section for substitution mutation detection.
Patients and tissues. Tumor samples were obtained from 45 consecutive NSCLC patients surgically treated at the Kanagawa Cardiovascular and Respiratory Center between November 1995 and December 2005. Institutional approval and informed consent from all patients were obtained in writing. Of these 45 patients, 15 cases were subsequently treated with gefitinib. The entire group consisted of 32 males and 13 females with an age at diagnosis ranging from 32 to 83 years (median, 65.6 years). Among the 45 lung cancer patients, 28 (62.2%) were diagnosed as having adenocarcinomas, 10 (22.2%) as having squamous cell carcinomas, 3 (6.7%) as having adenosquamous carcinomas, and 4 (8.9%) as having other types of cancer. After surgical removal, all tumor samples were immediately frozen and stored at -80°C until assayed.

Sample preparation and EGFR gene analysis. The EGFR mutations were analyzed by DNA sequencing of the relevant regions of exons 18 and 21. For the PCR-RFLP analysis and sequencing, four sets of previously reported primers (5) were used to amplify the EGFR gene. PCR products were digested with MscI overnight at 37°C. The digested products were analyzed on a 3% NuSieve GTG agarose gel, which was stained with ethidium bromide. Genotype assessments were based on the gel pattern of the restricted PCR fragments.

Results

Identification of EGFR exon 18 and exon 21 point mutations by SMAP. Before testing clinical samples with our mutant primer sets and SMAP assay, controlled tests on known genomic templates were done. The SMAP reaction was done in the presence of an intercalating dye (SYBR Green I) and was mixed with 1 mL of 30 mmol/L NaOH, stirred, and incubated at 95°C for 5 min. After chilling on ice, the tumor lysate was diluted 1:10 in water, and 1 µL of the diluted solution was used as the template for SMAP assays carried out in the same way as described in the previous sections.
monitored with the Mx3000P System. Genomic DNA isolated from the NC1-H1975 cell line known to be carrying the L858R substitution mutation (exon 21) was used as a template to test the reliability of the SMAP mutation detection assay. SMAP amplification primer sets with a FP specific for detecting the L858R point mutation (2573T > G) as described in Fig. 2 were shown to rapidly amplify (in 20 min) the NC1-H1975 cell line DNA, whereas the same primer set was incapable of amplifying wild-type genomic control DNA even after 60 min (Fig. 4A). A “no-template” control reaction was also negative after 60 min. The graphs in Fig. 4A are composite graphs, each line representing a different SMAP assay using a wild-type primer set (left) and a mutant primer set (right) and a different genomic template. Each assay was done in duplicate. Note that NC1-H1975 shows amplification with both primer sets, confirming that the cell line is heterozygous for the L858R mutation.

Electrophoretic analysis of the amplified DNA showed ladder patterns expected and typical of SMAP amplification (Fig. 4B). The DNA bands of ~50 bp in size (indicated by asterisk) are likely to correspond to the monomeric self-primed amplification product bounded by the 5’ ends of the FP and TP primers. Larger sized bands are alternative forms (derived from intermediate forms, IM1 and IM2), self-hybridized species, and multiple-unit length amplicons of each species. To confirm the accuracy of genotype-specific products by SMAP, restriction analysis followed by electrophoresis was done. The MscI enzyme recognition sequence exists in the wild-type sequence of EGFR exon 21, but not the L858R mutant. As expected, the amplification products derived from the H1975 cell line were digested by MscI. In similar experiments on genomic DNA templates, we could also detect the G719S mutation by SMAP within 30 min (data not shown). The G719S mutation assay also showed similar high specificity, allowing reliable discrimination of mutants from wild-type specimens.

Identification of EGFR exon 19 deletions by SMAP. Again, before testing clinical samples with our mutant primer sets and SMAP assay, controlled tests on known templates were done. For deletions detection, engineered plasmid templates were used because no cell lines were available that have these specific EGFR mutations. The SMAP reaction was done in the presence of an intercalating dye (SYBR Green I) and was monitored with the Mx3000P real-time PCR instrument. Seven different allele-specific primer sets for deletion detection were developed and examined for specificity in the SMAP assay. The primer sets described previously and illustrated in Fig. 3 were each used in SMAP assays with exact match plasmid templates and against all other deletion mutants. In all cases, only the template completely identical with each allele-specific primer set was amplified within 30 min. No amplification was observed from
Fig. 4. Analysis of allele-specific SMAP reaction for EGFR mutation. A, amplification curves of L858R mutation detection. Left, wild-type–specific primer amplification on ●, 20 ng of NCI-H1975 cell line DNA as a template. ×, no template. Right, L858R mutant-specific primer amplification on ▲, 20 ng of NCI-H1975 cell line DNA; □, 20 ng of wild-type human genomic DNA as a control; and ×, no template. B, SMAP-amplified NCI-H1975 DNA cut by restriction endonuclease MscI. MscI is capable of digesting wild-type amplified DNA at a single site; amplified L858R mutant DNA will not cut. SMAP reaction products were run on a 3% of NuSieve GTG agarose gel and stained by ethidium bromide. Lane M, 20-bp ladder used as size marker (TAKARA). Lane 1, uncut DNA of NCI-H1975 amplified by SMAP wild-type specific primers. Lane 2, MscI digestion of DNA used in lane 1. Lane 3, uncut DNA of NCI-H1975 amplified by SMAP L858R mutant-specific primers. Lane (4): MscI digestion of DNA used in lane 3. A single band in lane 2 is the only visible digestion product, consisting of unit lengths of the EGFR amplicon cut once by MscI. C, verification of deletion primer specificity on cloned deletion targets. Both graphs are composite amplification profiles when using all deletion primer sets to amplify 20 ng of wild-type human genomic DNA (left) and 3,000 copies of DE-A plasmid (right). Assays were done in duplicate with primer set for wild-type (●), primer set for DE-A (■), primer set for DE-B (▲), primer set for DE-C (◇), primer set for DE-D (●), primer set for DE-E (○), primer set for DE-F (△), primer set for DE-G (★). Only the primer set matching the template DNA displayed amplification in 60 min. D, sensitivity of SMAP. Left, SMAP reaction using L858R point mutation-specific primer set with serial dilutions of H1975 cell line genomic DNA. Right, SMAP reaction using DE-A deletion mutation-specific primer set with serial dilutions of H1650 cell line genomic DNA. E, specificity of mutant DNA detection in the presence of wild-type DNA. Left, composite graph of SMAP reactions using L858R primer set with a mixture of wild-type genomic DNA and 10%, 5%, 1%, 0.5%, and 0% of target H1975 cell line DNA (in duplicate). Right, composite graph of SMAP reactions using DE-A primer set with a mixture of wild-type genomic DNA and 10%, 1%, 0.1%, and 0% of H1650 cell line DNA (in duplicate).
any mismatched combination of primers and templates even when monitored for 60 min (Fig. 4C, Supplementary Fig. S2). Each experiment was done in duplicate; hence, each graph shows two positive amplification profiles for each of the deletion primer sets.

**Sensitivity of SMAP-based mutation detection in mixed-cell populations.** Tumor samples frequently consist of numerous subpopulations of cancer cells. A useful diagnostic for mutation detection must be able to detect mutations in heterogenous genomic DNA samples. To test SMAP for this capability, we conducted serial dilution experiments to examine detection sensitivity and genomic DNA mixing experiments to examine the sensitivity of detecting mutants as a subpopulation in a background of wild-type DNA. In the serial dilution experiments, using the NC1-H1975 and NSC-H1650 cell line genomic DNAs, the allele-specific primers for amplification of the exon 21 L858R point mutation, and the exon 19 E746-A750 deletion could each detect 30 copies in SMAP amplification (25 μL reaction) in 30 min when using the respective full-match cell line DNA (Fig. 4D).

To determine the minimal detection limits for mutant sequences in a background of wild-type DNA, mutant cell line DNA was mixed with increasing amounts of wild-type DNA, and SMAP assays were done with full-match mutant primer sets. Using the NC1-H1975 cell line genomic DNA and the allele-specific primers for amplification of the exon 21 L858R point mutation, the mutant sequences could be detected even when present at only 0.5% in 60 min. Likewise, using the NC1-H1650 cell line genomic DNA and the allele-specific primers for amplification of the exon 19 E746-A750 deletion, the mutant sequences could be detected even when present at only 0.1% in 60 min (Fig. 4E).

**Genotyping of clinical samples.** We purposefully set out to design a mutation detection system that was both accurate and fast. We therefore minimized the genomic DNA sample preparation to a simple lysis in NaOH as described in Materials and Methods and usually within several minutes had material ready for the SMAP assays. With this crude extraction procedure and SMAP analysis, we were able to diagnose specific mutations from clinical samples in about 30 min.

The genomic status of the EGFR gene was evaluated in a series of 45 primary NSCLC specimens by both SMAP and conventional sequencing. Nine mutations were found by sequencing, of which four cases were substitution in exon 21, and five cases were a deletion in exon 19. All of these mutations were believed to be heterozygous, having also one wild-type allele. The nine cases that were proven to have mutations by sequencing, of which four cases were substitution in exon 21, and five cases were a deletion in exon 19. All of these mutations were believed to be heterozygous, having also one wild-type allele. The nine cases that were proven to have mutations by sequencing, of which four cases were substitution in exon 21, and five cases were a deletion in exon 19. All of these mutations were believed to be heterozygous, having also one wild-type allele.

**Discussion**

The SMAP method has several advantages for EGFR mutation detection over genotyping technologies besides conventional sequencing. More newly developed methods including PCR-related technologies (PNA-LNA PCR clamp, TaqMan PCR assay, SURVEYOR analysis, mutant-enriched PCR, PCR-SSCP, ARMS, TaqMan-MGB probes, and others), other sequencing platforms (microreector-based pyrosequencing), and other methods such as high-resolution melting amplicon analysis and nanoscale engineered biomagnetite, all have serious limitations on comparison to SMAP. These new methods are more accurate, sensitive, and convenient than sequencing, but they are still time consuming (shortest time, 3.5 h), require careful DNA extraction, and involve several steps (DNA extraction, PCR, electrophoresis, etc.). They are therefore unsuitable for routine pretherapeutic examination and difficult to implement at most medical institution. The SMAP method is based on strand-displacing DNA polymerase activity and can amplify and detect mutations directly from blood samples requiring only a simple
heat lysis and denaturation step. We have also shown that the simple sample preparation is effective on lysed tissue samples from human lung cancer. It is not known why some strand-displacing polymerases can effectively amplify DNA from such crude samples, but this feature is one crucial merit of the SMAP method.

The application of SMAP to molecular diagnostics has significant potential as evident from this study. Sample preparation was extremely minimal, setup was very simple, detection time was <30 min, and the specificity displayed single-nucleotide precision with no mist calling. The unique primer design, background suppression technology, and isothermal nature of the assay provide a powerful combination of attributes for mutation detection that stands alone, making SMAP perhaps the first practical tool for bedside usage or POCT in molecular diagnostics.

In this study, we employed the SMAP method for EGFR mutation detection to explore its utility as a tool to rapidly diagnose NSCLC tumors that are potentially gefitinib sensitive.

We have shown that the SMAP method is capable of detecting as little as 30 copies per reaction of the EGFR mutation. This high sensitivity of SMAP is an advantage, particularly when analyzing small biopsies, or in cases where cytodiagnosis of the sputum and bronchoalveolar lavage are used such as for inoperable patients from which it is not possible to obtain enough clinical material for analysis using existing mutation detection methods. In addition, SMAP allows the detection of as little as 0.1% to 0.5% of targeted mutant alleles in DNA samples without any background amplification, which is similar or better than other newly developed methods. In our study, a gefitinib-sensitive case with a two bases-substitution mutation could be detected by SMAP, where sequencing was unable to identify it at all. This observation suggests that some clinical cases may be inappropriately treated if the diagnosis was dependent exclusively on DNA sequencing for EGFR mutation detection.

One limitation of SMAP technology is that it requires pre-engineered primer sets and, therefore, is effective only on
already known mutations. Among cases which have mutations in the EGFR gene, 387 (45.6%) mutations consisted of small in-frame deletion between codons E746 and P753, and 325 (38.3%) were at codon 858 (L858R), and 21 (2.5%) were at G719S. Taken together, these three alterations account for 86.4% of the total number of mutations thus far detected in the EGFR gene. We decided to evaluate these three hotspots because they were mainly related to gefitinib sensitivity. It is possible that similar, but nonidentical mutations, might exist in these same codons and also render the cancer sensitive to gefitinib. These new mutations would be missed by the SMAP method because of its precision and, thus, present a risk of misdiagnosis. Sequencing, which has sensitivity limits and its own risks (already discussed), may be able to detect these new mutations. However, at the pace of current research in this area, it is likely that most occurring EGFR mutations will be identified and mapped for sensitivity to gefitinib. The SMAP genotyping method for cancer metastases in lymph nodes, peritoneal disseminations, resection margins, etc. The SMAP genotyping method for cancer detection (SMAP), pioneered here first for EGFR mutation in NSCLC, is likely to become widely employed in the near future as the age of tailor-made medicine emerges in the operating room and clinic.

The SMAP method can be applied to known point mutations, in-frame deletions, insertions, and duplications at fixed positions. To facilitate the design of primer sets for SMAP applications, a software specific for SMAP primer design can be freely accessed online. The software generates primer sequences and suggested arrangements suitable for any target sequence of interest, and optimization and assay design recommendations are supported as well. Cancer-related gene polymorphisms are said to participate in every cancer phase such as carcinogenesis, cell proliferation, metastasis, and drug sensitivity. Ongoing global research have already found several mutations, such as those in p-53 (22), k-ras (23), c-kit (24, 25), BRCA (26), etc. As more and more clinically important cancer-related mutations and genes are described, a quick and simple diagnostic method will become indispensable for prescribing effective cancer therapies. Rapid and sensitive quantification methods for detecting mutated DNA has great potential for providing new intraoperating (real-time) diagnosis of micro-metastases in lymph nodes, peritoneal disseminations, resection margins, etc. The SMAP genotyping method for cancer mutation detection (SMAP), pioneered here first for EGFR mutation in NSCLC, is likely to become widely employed in the near future as the age of tailor-made medicine emerges in the operating room and clinic.

**Table 1. Clinical and molecular features of the 10 lung cancer patients with EGFR mutations**

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age</th>
<th>Gender</th>
<th>Histotype</th>
<th>Stage*</th>
<th>Sequence</th>
<th>SMAP</th>
<th>PCR-RFLP</th>
<th>Deletion type</th>
<th>Gefitinib therapy</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>68</td>
<td>M</td>
<td>ADC</td>
<td>III A</td>
<td>Wild type</td>
<td>L858R</td>
<td>L858R</td>
<td></td>
<td>+</td>
<td>CR</td>
</tr>
<tr>
<td>14</td>
<td>83</td>
<td>F</td>
<td>ADC</td>
<td>I B</td>
<td>L858R</td>
<td>L858R</td>
<td>L858R</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>74</td>
<td>F</td>
<td>ADC</td>
<td>I A</td>
<td>L858R</td>
<td>L858R</td>
<td>L858R</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>52</td>
<td>F</td>
<td>ADC</td>
<td>III A</td>
<td>L858R</td>
<td>L858R</td>
<td>L858R</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>60</td>
<td>M</td>
<td>ADC</td>
<td>III B</td>
<td>L858R</td>
<td>L858R</td>
<td>L858R</td>
<td>+</td>
<td>-</td>
<td>PD</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>F</td>
<td>ADC</td>
<td>I A</td>
<td>2235-2249</td>
<td>2235-2249</td>
<td>-</td>
<td>DE-A</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>61</td>
<td>M</td>
<td>ADC</td>
<td>I B</td>
<td>2239-2247</td>
<td>2239-2247</td>
<td>-</td>
<td>DE-D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>54</td>
<td>F</td>
<td>ADC</td>
<td>III B</td>
<td>2239-2248</td>
<td>2239-2248</td>
<td>-</td>
<td>DE-G</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>72</td>
<td>M</td>
<td>ADC</td>
<td>III A</td>
<td>2236-2250</td>
<td>2236-2250</td>
<td>-</td>
<td>DE-B +</td>
<td>PD</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>64</td>
<td>F</td>
<td>ADC</td>
<td>III A</td>
<td>2235-2249</td>
<td>2235-2249</td>
<td>-</td>
<td>DE-A +</td>
<td>CR</td>
<td>-</td>
</tr>
</tbody>
</table>

NOTE: L858R mutations are encoded on exon 21. Deletion mutations (DE-A, DE-B, DE-D, DE-G) are encoded on exon 19. Abbreviations: F, female; M, male, ADC, adenocarcinoma; BAC, bronchioalveolar carcinoma; CR, complete response; PD, progressive disease. *According to the American Joint Committee on Cancer staging system.

References


http://www.smapdna.com


Rapid Detection of Epidermal Growth Factor Receptor Mutations in Lung Cancer by the SMart-Amplification Process

Kanako Hoshi, Hideki Takakura, Yasumasa Mitani, et al.


Updated version Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/13/17/4974

Supplementary Material Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2007/09/11/13.17.4974.DC1

Cited articles This article cites 24 articles, 11 of which you can access for free at: http://clincancerres.aacrjournals.org/content/13/17/4974.full.html#ref-list-1

Citing articles This article has been cited by 10 HighWire-hosted articles. Access the articles at: /content/13/17/4974.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.