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ORIGINAL ARTICLE

Rapid sensitive analysis of *IDH1* mutation in lower-grade gliomas by automated genetic typing involving a quenching probe

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ABSTRACT

The authors recently found that 80% of lower-grade gliomas (LGGs) harbored a mutation in *IDH1*. Intraoperative detection of the mutated *IDH1* helps not only differentiate LGGs from other type of brain tumors, but determine the resection border. In the current study, the authors have applied an automated genetic typing involving a quenching probe to detect the mutated *IDH1*. If tumor cells with the mutated *IDH1* contained 10% or more in the mixture of normal and tumor cells, the device could detect it sensitively. The intraoperative assessment of *IDH1* mutation is useful in brain tumor surgeries.

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Introduction

Gliomas are the most common primary malignancies of the central nervous system and account for 28% of brain tumors (1). These tumors are stratified into grades I–IV by the World Health Organization (WHO) on the basis of histopathological and clinical criteria (2). Grade II–IV tumors show diffuse infiltration. Grade II and III gliomas (lower-grade gliomas [LGGs]) are usually less aggressive tumors but can progress further and are generally incurable (3). In particular, the appearance of LGGs is similar to that of normal brain tissue; thus, intraoperative decision of the extent of resection possible has depended on the surgeon's subjective assessment for many years. Although recently developed modalities such as neuronavigation and fluorescence staining involving 5-aminolevulinic acid help guide tumor resection (4–8), they are often inaccurate because of brain shift or false-positive/negative staining. As previously reported, we analyzed cohorts of LGGs from Japan and The Cancer Genome Atlas consortium by whole-exome and targeted deep sequencing, as well as by single nucleotide polymorphism (SNP) array analysis (9). We found that 80% of LGGs harbored a mutation in *IDH1*, which was ubiquitous and was the earliest mutational event in the majority of LGGs (9). In the current study, we investigated whether mutation in *IDH1* could be detected by an automated rapid system using a quenching probe and have discussed real-time intraoperative application (10).

Materials and methods

We analyzed the mutation frequency of *IDH1* with a new automated genetic typing device (i-densy; Arkray, Kyoto, Japan). This device automatically performs pretreatment of reagents, polymerase chain reaction (PCR), and SNP typing in approximately 80 min (11). The SNP typing involves a quenching probe (QProbe) that has a fluorescently labeled cytosine base terminal; QProbe fluorescent emission is quenched by DNA hybridization (12, 13). The PCR primers were; forward, tgtgagtggatgggtaaaacctatc, and reverse, cacattattgccaacatgac. QProbe was TAMRA-cataagcatgacgacctat-Phosphate residue. The fluorescence increases once the probe dissociates (Figure 1). We used tumor DNAs from four LGGs (two anaplastic oligodendrogliomas, an oligodendroglioma,

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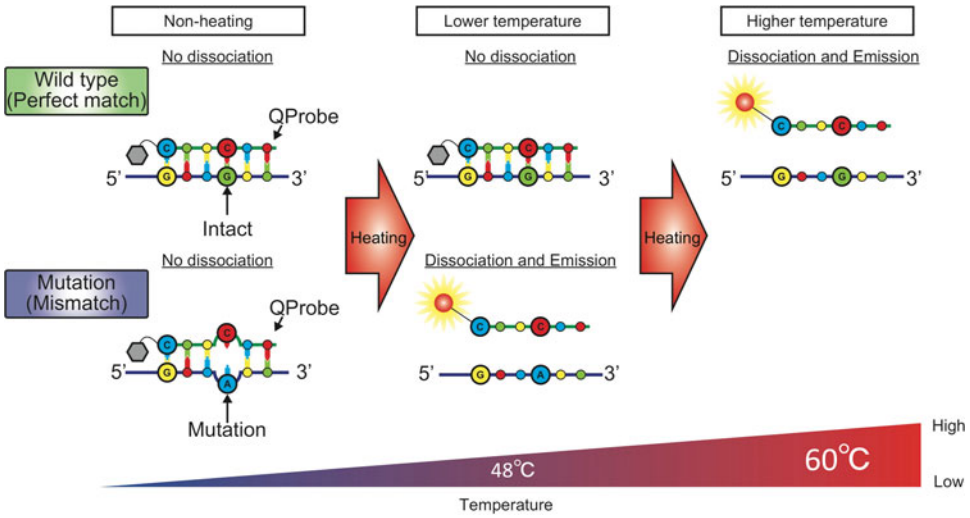

Figure 1. System of I-densy. The SNP typing involves a quenching probe (QProbe) that has a fluorescently labeled cytosine base terminal; QProbe fluorescent emission is quenched by DNA hybridization. The fluorescence increases once the probe dissociates.

and an anaplastic astrocytoma) in which variant allele frequency (VAF) of the mutated *IDH1 (IDH1-R132H)* was known based on deep sequencing analysis of the PCR amplicons performed in our earlier study. We adjusted the concentration of tumor DNA with mutant *IDH1* and DNA from the blood of a normal healthy volunteer to 6 ng/μL, and mixed them to obtain various wild type/mutation ratios (0, 20, 50, 80, and 100). The

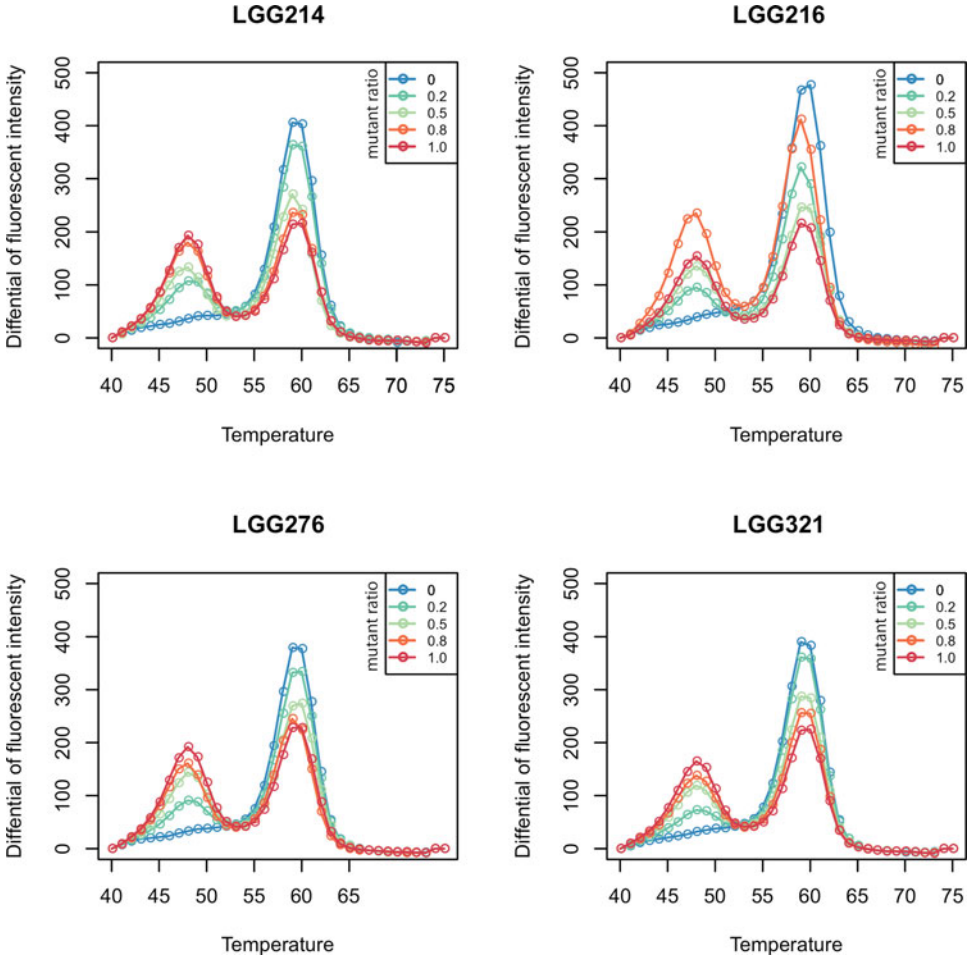

Figure 2. Fluorescence curve over temperature. There are two clear peaks at 48°C and 60°C, derived from a mismatch (point mutation) and a perfect match (wild type), respectively. LGG (lower-grade glioma) 276 and LGG321, anaplastic oligodendrogliomas; LGG214, oligodendroglioma; LGG216, anaplastic astrocytoma. Mutant ratio = mutated DNA/(wild-type DNA + mutated DNA).

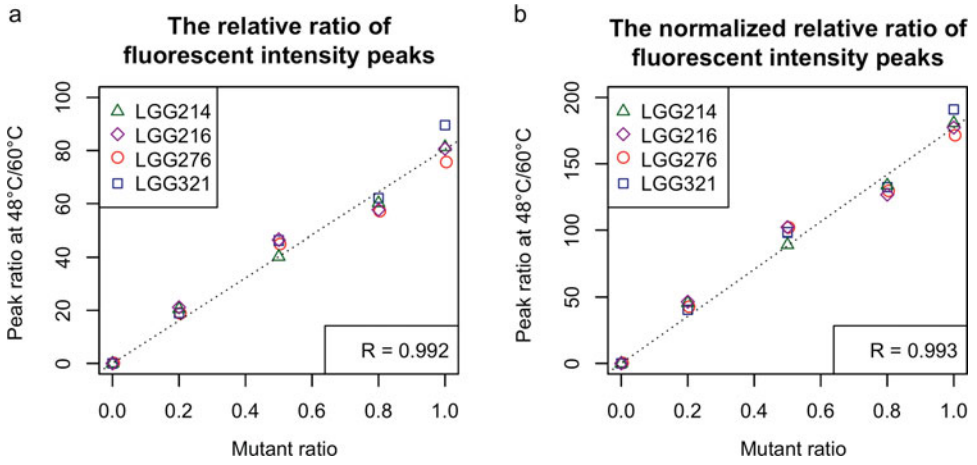

Figure 3. (a) The relative ratio of fluorescent intensity peaks ["fluorescence at 48°C" − "fluorescence of the ratio = 0 sample at 48°C"/fluorescence at 60°C] ×100]. (b) Further, the relative ratio of fluorescent intensity peaks is normalized by dividing it by the IDH1-R132H variant allele frequency of each sample. The relative ratio of fluorescent intensity peaks is reproducibly proportional to the mutant ratio. Mutant ratio = mutated DNA/ (wild-type DNA + mutated DNA).

mutant ratios are calculated as follows, mutation ratio = mutated DNA/(wild-type DNA + mutated DNA).

Results

We could obtain two clear peak curves at 48°C and 60°C, derived from a mismatch (point mutation) and a perfect match (wild type), respectively in all samples (Figure 2a–d). The relative ratio of fluorescent intensity peaks (fluorescence at 48°C/fluorescence at 60°C × 100) was reproducibly proportional to the mutant ratio ($R = 0.992$) (Figure 3a). Further, the relative ratio of fluorescent intensity peaks was normalized by dividing it by the *IDH1-R132H* VAF of each sample ($R = 0.993$) (Figure 3b). The fact that *IDH1-R132H* VAF ranged from 0.4 to 0.5 of each sample suggested that actual tumor cells contained by 40% to 50% in the bulk tumor tissues. Our data indicated that if the proportion of tumor cells with mutant *IDH1* was 10% and more within bulk tumor tissue, the device could detect the *IDH1* mutation sensitively.

Discussion

IDH1/2 mutations are seen in 80% of LGGs, and in most cases, the *IDH1/2* mutation is *IDH1R132H* (14). Because *IDH1/2* mutations are the earliest mutational event, they are thought to be related to the tumorigenicity of LGGs. In addition to the possible biological/functional implications of *IDH1* mutation, the presence of this mutation may aid in differential diagnosis between LGGs and other tumors (15,16) and between glioma and reactive gliosis (17). The extent of tumor resection is one of the factors that affects prognosis in patients with LGGs (18–20). The similarity of the appearance of the tumor to that of the surrounding tissue often limits gross total resection. Therefore, intraoperative real-time molecular diagnosis is required to maximize tumor removal. To our knowledge, the current study is the first to demonstrate that automated PCR with a quenching probe can be used for sensitive detection of the *IDH1* mutation. Although extracted DNA samples were utilized in this study, bulk tumor tissue can be used with this device. Further study is required to determine whether the *IDH1* mutation can be detected even in fresh LGG samples.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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