Rapid IDH1 Gene Mutation Analysis for Intraoperative Pathological Diagnosis

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Abstract

Isocitrate dehydrogenase 1 (IDH1) R132 mutations occur frequently in low-grade glioma and secondary glioblastoma. We established a high-resolution melting analysis method that uses single nucleotide polymorphism analysis based on real-time PCR and that reliably and quickly detect IDH1 mutations. Further, we attempted to apply it in rapid diagnosis during glioma surgery. DNA extracted in 15 min from glioma tissue collected during surgery was used to test for R132 point mutations of the IDH1 gene by using real-time PCR/high resolution melting analysis method. Normally, detecting IDH1 mutations requires about 80 min from the start of the PCR cycle. For our rapid diagnosis, however, DNA extension and annealing times in the PCR cycle were reduced by half. Regular analysis and rapid diagnosis analysis were used to detect IDH1 mutations in 6 glioma cases. Both methods produced the same results in all cases. Our method can determine all IDH1 R132 mutation-positive gliomas during surgery in 50–60 min after the tissue is collected. Further, this method could aid in intraoperative pathological diagnoses to differentiate IDH1 mutation-positive low-malignancy gliomas from gliosis and other non-neoplastic tissues.

INTRODUCTION

Isocitrate dehydrogenase 1 (IDH1) R132 mutations occur as point mutations at a frequency of 70% or more in gliomas, including astrocytoma and oligodendroglioma, of WHO grades 2 and 3, as well as secondary glioblastoma [1-3]. Because this gene alteration is not observed in normal cells or non-glioma brain tumors [1], the diagnosis of glioma is nearly certain if an IDH1 mutation is detected. Moreover, the occurrence of IDH1 mutation was associated with the early stage of gliomatogenesis [1,4]. We established a high-resolution melting analysis method that uses single nucleotide polymorphism analysis based on real-time PCR and that sensitively and quickly detect IDH1 mutations. Further, we attempted to apply it in rapid diagnosis during glioma surgery.

CASES AND METHODS

Six glioma cases (diffuse astrocytoma, 2 cases; oligodendroglioma, 2 cases; anaplastic astrocytoma, 1 case; glioblastoma, 1 case) were studied. Genomic DNA was extracted from fresh frozen biopsy specimens using DN easy Tissue kit (Qiagen, Hilden, Germany). DNA extraction was completed in 15 min. Next, the Light Cycler 480 Real-Time PCR System (Roche Diagnostics) and its high-resolution melting (HRM) analysis was used [5] to screen the presence or not of IDH1 R132 mutations. The primer sequences used to amplify IDH1 R132 (exon 4) were: 5’-CGGTTCTCAGAAGAACCCATT-3’ (sense), and 5’-CACATTATTGCAACATGAC-3’ (anti-sense) as previously described [6]. Briefly, each PCR run contained 50 ng of genomic DNA in a total volume of 20 μl: 2 X master mix containing high-resolution melting dye (Roche Diagnostics, Mannheim, Germany), 3 mM Mg²⁺, and 500 nM of each primer. Cycling conditions were 3 min at 95, followed by 30 cycles of denaturation at 94 for 30 s, annealing at 57 for 30 s, and extension at 72 for 40 s. The melting step was 95 for 1 min, 50 for 1 min, 72 for 5 s, and continuous acquisition to 95 at 30 acquisitions per 1. Melting data collected using the LightCycler480 Instrument can be analyzed by the “Gene Scanning” algorithm that converts the melting profiles into derivative plots, which allows wild-type and mutated samples to be distinguished (Figure 1). These were the method for rapid diagnosis. For regular diagnosis, DNA extension and annealing times in the PCR cycle were extended to double. Our results were compared with those obtained using the regular method.

RESULTS AND CONCLUSION

Both regular and rapid diagnosis methods produced the same results in all cases. Representative case is shown in Figure 2. Direct DNA sequencing and immunostaining can be used to identify IDH1 mutations. However, because analyses by using the former method require several hours, it cannot be used in rapid diagnosis. The latter method could not identify all R132 mutations, because the current commercially available antibodies can only detect R132H mutant proteins. This method can detect...
IDH1 mutation in even 1% of tumor purity [7] and determine all IDH1 R132 mutation-positive gliomas during surgery in 50–60 min after the tissue is collected.

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REFERENCES