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TERT promoter mutation as a diagnostic marker for diffuse gliomas

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See the article by Diplas et al, in this issue, pp. 440-450

TERT, the telomerase reverse transcriptase gene, is a catalytic subunit of telomerase, which maintains the telomere length and plays a central role in conferring immortality to cancer cells.[1] Although telomerase activation has been observed in most cancers, the activation mechanism was not known until recurrent mutations in the promoter region of *TERT* were discovered in melanomas and many other cancers, in particular, gliomas.[2]

TERT promoter mutations are the most common single gene mutations in adult diffuse gliomas. The patterns of *TERT* promoter mutations are highly specific to glioma subtypes. They are present in virtually all oligodendrogliomas, which harbor isocitrate dehydrogenase (*IDH*) mutations and 1p/19q codeletion as defined in the revised fourth edition of the World Health Organization (WHO) classification of tumors of the central nervous system.[3] *TERT* promoter mutations may also be found in up to 80% of *IDH* wild-type (wt) glioblastomas. However, they are rarely found in other types of glial tumors, with only very few exceptions.[4] The highly recurrent and subtype-specific pattern of *TERT* promoter mutation makes it an attractive candidate as a diagnostic marker for diffuse gliomas.[5,6]

The majority of astrocytomas are *IDH*-mutated, and *TERT* promoter mutations are entirely absent in these tumors. Nevertheless, a considerable proportion of astrocytomas are *IDH*-wt, although such entities are provisional in the current classification. A subset of *IDH*-wt astrocytomas show aggressive clinical behavior and have short survival. Therefore, it is necessary to develop additional markers to define a clinically relevant subgroup among *IDH*-wt diffuse gliomas.

Considering these concerns, cIMPACT-NOW (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) recently recommended diagnostic criteria to define "diffuse astrocytic glioma, *IDH*-wildtype, with molecular features of glioblastoma, WHO grade IV." *TERT* promoter mutation was one of the 3 molecular criteria (others are amplification of epidermal growth factor receptor [*EGFR*] and the combination of whole chromosome 7 gain and whole chromosome 10 loss) to define *IDH*-wt diffuse astrocytic gliomas that are biologically equivalent to glioblastoma, and hence designated as WHO grade IV.[4] *TERT* promoter mutation has thus been acknowledged as a promising diagnostic marker in adult diffuse gliomas.

However, *TERT* promoter mutations are notoriously difficult to detect. The mutations occur at one of the 2 hotspots as C to T transition in the coding strand (designated as C228T and C250T, which are located at -124 and -146 bp, respectively, upstream of the start codon) in a mutually exclusive manner. Either mutation creates an identical 11 bp sequence, which renders it difficult to discriminate by high-resolution melting point analysis. Furthermore, because they are in the middle of a large cytosine-phosphate-guanine island, high guanine-cytosine content of the region makes it difficult to amplify/sequence by conventional methods, including next-generation sequencing. Additionally, diffuse gliomas are highly invasive, and their tissues are frequently mixed with nonneoplastic cells, particularly at their periphery, as well as massive necrosis. These may mask mutations in the tumor cells and cause diagnostic challenges.

In this issue of *Neuro-Oncology*, Diplas et al present GliomaDx, a method for sensitive and rapid detection of *TERT* promoter and *IDH* mutations in diffuse gliomas.[7] In their assay, a locked nucleic acid base, a nucleic acid analog that has higher melting temperature when hybridized, was incorporated at the 3′ end of the mutation-specific primer to greatly increase specificity during quantitative PCR. The method was validated by serially diluted mutated DNA and cross-validated using other sensitive methods, including a droplet digital PCR and next-generation sequencing. The sensitivity was as low as 0.1% mutant allele fraction, which corresponds to 0.2% tumor cell contents of the tissue. They also developed GliomaDx Nest, a nested PCR system that allows the amplification of low-input samples such as formalin-fixed paraffin embedded ones. Upon successful validation of the method, they revisited a cohort of tumors that had previously been determined to be *TERT*-wt and *IDH*-wt. Surprisingly, they found that 26.3% of them harbored mutations at a low allele frequency by using GliomaDx. Those cases showed significantly shorter overall survival than that of the remaining *TERT*-wt cases. Estimation

of tumor cell contents by whole exome sequencing showed that those tumors with low mutation percentage had indeed tumor purity of less than 30%.

Their study has several implications. First, the technology allows a rapid and sensitive detection of 2 of the most important biomarkers in diffuse gliomas, which can facilitate molecular diagnostic application. In fact, the concurrent *TERT* and *IDH* mutations are almost invariably associated with the presence of 1p/19q codeletion. Detection of 1p/19q codeletion for the entire chromosomal arm, as recommended by the WHO classification, is troublesome. Practically, this test may be circumvented by a combination of IDH and alpha thalassemia/mental retardation syndrome X-linked (ATRX) immunohistochemistry; however, this may often be inconclusive. A combined *IDH*/*TERT* test as presented by Diplas et al may serve as an accurate and easy surrogate for 1p/19q testing.

Second, we need to be aware that molecular diagnosis based on samples that are not validated for tumor contents may sometimes be misleading, when mutations are present in a minor cell population. Immunohistochemical detection of IDH1 R132H has an advantage in this respect; however, *TERT* promoter mutations cannot be detected by immunohistochemistry. Careful cross-validation of histopathological and molecular findings is key for accurate diagnosis.

Third, a highly sensitive assay for mutation detection opens up a possibility of liquid biopsy, at least in CSF, for non-invasive diagnosis of gliomas.[8] This may be useful for rapid detection of early recurrence or monitoring disease control under treatment, particularly when a specific inhibitor of mutant IDH is used.

IDH and *TERT* mutations are likely to be the founder mutations of oligodendrogliomas.[9] Whether *TERT* mutation is a founder mutation for glioblastoma remains to be seen, although Lee et al recently provided interesting findings that may support this idea.[10] Regardless, it is certain that *IDH* and *TERT* play a key role in the development of diffuse gliomas, although *TERT* seems to contribute to the development of oligodendrogliomas and glioblastomas in different ways. An accurate detection of *IDH* and *TERT* mutations is essential for developing proper molecular diagnosis and understanding the biology of diffuse glioma.

Disclaimer

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