Improving Central Nervous System Tumor Diagnostics through Methylation Profiling

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Case-Based Questions (please see page 3 for answers)

1.	You are evaluating a lesion that looks like a glioma. Methylation profiling is inconclusive with a classifier score below 0.9. Next generation sequencing detects a TERT promoter mutation (variant allelic fraction=0.15) and genomic copy number array detects EGFR amplification. What is your diagnosis, and how do you explain the methylation results?	
	a.	Metastatic lung adenocarcinoma, EGFR-amplified. Methylation analysis used the wrong classifier library for this lesion.
	b.	IDH mutant oligodendroglioma, grade 3. Insufficient tumor cellularity for reliable methylation analysis.
	c.	IDH wildtype glioblastoma, grade 4. Insufficient tumor cellularity for reliable methylation analysis.

2.	A glioma in a 42 year-old man is immunonegative for IDH1 R132H, yet methylation profiling still classifies it as an IDH mutant astrocytoma. What is the most likely explanation?	
	a.	The tumor contains a non-canonical IDH mutation.
	b.	Insufficient tumor cellularity for reliable methylation classification.
	c.	The tumor is an IDH wildtype glioblastoma mimicking an IDH mutant astrocytoma.

3.	You are reviewing slides from a left temporal mass in a 23 year-old woman with uncontrolled epilepsy. The lesion consists mostly of glial cells with a few neurons. No mitoses, necrosis, or microvascular proliferation is seen. Next generation sequencing, copy number array, and methylation profiling are all negative. What is the most likely diagnosis?	
	a.	IDH mutant oligodendroglioma, WHO grade 2.
	b.	Focal cortical dysplasia.
	c.	Pleomorphic xanthoastrocytoma, WHO grade 2.

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Question 1 Correct Answer and Rationale: **C**

Studies show that if the variant allelic fraction of a heterozygous driver mutation like TERT promoter falls below 0.31, or ~60% tumor cellularity, it is more likely that methylation classification will be confounded by too much admixed nonneoplastic DNA. In those situations, orthogonal approaches like next generation sequencing are essential. Here, the presence of a TERT promoter mutation plus EGFR amplification aligns with an IDH wildtype glioblastoma that was either undersampled at the time of biopsy, or has not yet developed all the histopathologic features of a typical glioblastoma. A different classifier library would indeed be needed to diagnose metastatic lung adenocarcinoma by methylation profiling, but histologically this is a glioma.

Question 2 Correct Answer and Rationale: **A**

Driver mutations like EGFR amplification, H3F3A K27M mutation, and BRAF V600E reshape the tumor epigenome in consistent and predictable ways. Thus, DNA methylation profiling can reliably identify such tumors even if the driver mutation has not been directly tested. All oncogenic IDH1 and IDH2 mutations produce similar methylation patterns in gliomas, which is why such tumors cluster apart from other gliomas. In this case, it is likely that the glioma contains a non-canonical IDH mutation, e.g., IDH1 R132S, that cannot be detected by the widely used IDH1 R132H immunostain. If the tumor cellularity was insufficient, the classifier would probably not misclassify the tumor, it would have failed to classify it at all.

Question 3 Correct Answer and Rationale: **B**

The entire field of molecular diagnostics in CNS tumors has progressed to the point where, if multiple different assays all turn up negative for pathologic alterations, it is possible that the lesion in question is nonneoplastic. In this case, a temporal lobe lesion in a younger patient with intractable epilepsy that is a mixture of glial cells and neurons, and lacks any known pathologic alterations, is probably focal cortical dysplasia. An IDH mutant oligodendroglioma should have been detected by all three molecular assays, and a pleomorphic xanthoastrocytoma would be expected to have some sort of MAPK-activating alteration like BRAF V600E.