Proximity ligation assay evaluates IDH1R132H presentation in gliomas.

For a targeted cancer vaccine to be effective, the antigen of interest needs to be naturally processed and presented on MHC by the target cell or an antigen-presenting cell (APC) in the tumor stroma. The presence of these characteristics is often assumed based on animal models, evaluation of antigen-overexpressing APCs in vitro, or assays of material-consuming immune precipitation from fresh solid tissue. Here, we evaluated the use of an alternative approach that uses the proximity ligation assay (PLA) to identify the presentation of an MHC class II-restricted antigen in paraffin-embedded tissue sections from patients with brain tumors. This approach required a specific antibody directed against the epitope that was presented. We used an antibody that specifically binds an epitope of mutated isocitrate dehydrogenase type 1 (IDH1R132H), which is frequently expressed in gliomas and other types of tumors. In situ PLA showed that the IDH1R132H epitope colocalizes with MHC class II in IDH1R132H-mutated glioma tissue. Moreover, PLA demonstrated colocalization between the class II epitope-containing melanoma antigen New York esophageal 1 and MHC class II. Collectively, our data suggest that PLA may be a useful tool to
acquire information on whether an antigen is presented in situ, and this technique has potential to
guide clinical studies that use antigen-specific cancer immunotherapy.