MTR-07. GLIOBLASTOMA ADAPTATION TO SUSTAINED cdk4/6 INHIBITION INVOLVES SUPPRESSION OF Rb EXPRESSION THROUGH HISTONE H3K4 MODIFICATION

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We have previously shown that pharmacologic inhibition of cdk4/6, in preclinical, Rb proficient models of glioblastoma (GBM), slows tumor growth, and extends the survival of animal subjects with intracranial GBM xenografts. In the current study we examined molecular characteristics of two distinct GBM xenograft models that developed resistance to cdk4/6 targeted therapy when subjected to sustained inhibitor treatment. For each model, mice with intracranial GBM xenograft, established from G55 and U87 cells, received daily administration of inhibitor, which slowed tumor growth, as indicated by bioluminescence imaging, and significantly extended animal subject survival (p < 0.001). However, tumor bioluminescence slowly increased during the course of treatment, and all treatment group mice eventually succumbed to tumor burden. Intracranial tumor, from mice subjected to continuous daily inhibitor treatment, was dissected from mouse brain, then minced and injected subcutaneously into new athymic host mice, with host mice continuing to receive daily administration of cdk4/6 inhibitor until engrafted subcutaneous tumors showed growth rates comparable to corresponding subcutaneous tumors that were left untreated. Subcutaneous tumors exposed to continuous daily inhibitor treatment were used as cell sources for intracranial therapy response experiments, with results showing that tumors subjected to continuous extended daily administration of inhibitor no longer responded to inhibitor treatment, as indicated by bioluminescence imaging and survival analysis. Immunoblot analysis revealed that resistant tumors showed greatly reduced or a complete absence of Rb protein expression. RB sequence analysis of DNA from resistant tumors failed to reveal any sequence alterations, and there was no indication of altered cytosine methylation in RB regulatory sequences. In contrast, chromatin immunoprecipitation revealed 11x (U87) and 7x (G55) reductions in RB promoter sequence association with histone H3K4 trimethyl (H3K4me3) antibody, indicating suppression of Rb expression, in GBM subjected to sustained cdk4/6 inhibition, through a histone modification associated with gene silencing.