Targeting TRIBBLES1 (TRIB1) Pseudokinase in GBM: A New Therapeutic Strategy

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Purpose/Objective(s): GBM (WHO grade IV) is the most aggressive form of glioma with a 5-year survival rate of 5%. The current therapeutic regimen involves radiation therapy and concurrent and adjuvant TMZ (Stupp protocol). Due to dismal survival outcomes, there is an urgent need to identify druggable therapeutic targets in GBM for drug development. In this study we identified TRIB1, a Ser/Thr pseudokinase that acts as a scaffold protein to initiate Ubiquitin Proteasome System-mediated degradation of target proteins in the cell. TRIB1 mRNA and protein have been found upregulated in several cancers (e.g. NSCLC, prostate etc.). It has also been shown to contribute towards chemotheraphy resistance in NSCLC and CRC. It has also been observed that COP1, a TRIB1 associated E3-ligase is also upregulated in glioma cells.

Materials/Methods: We utilized a patient-centered reverse translational approach to identify novel therapeutic targets. TRIB1 was identified by statistical association (Cox regression analysis) and logic-based network analyses of the patient-derived methylation data generated using EPIC methylation array. TRIB1 was functionally validated in vitro by overexpression and knockdown approaches. For knockdown of TRIB1, a doxycline inducible system was used. Stable cell lines were generated by puromycin selection and cell sorting. Protein levels were detected by western blotting.

Results: The global methylation analysis on a Utrecht GBM cohort revealed that TRIB1 promoter methylation was associated with better OS of GBM patients (HR = 0.81 (0.62-1.05); p = 0.11). We also observed that overexpression of TRIB1 caused a decrease in apoptosis of PDX GBM cell lines after radiation exposure and TMZ treatment. Unoverexpression of TRIB1 also increased the phosphorylation of ERK and Akt in PDX cell lines. However, in TRIB1 knockdown cell lines only phosphorylation of Akt was decreased. It was also observed that TRIB1 levels were upregulated in p53-mutant cell lines suggesting a correlation between TRIB1 and p53.

Conclusion: TRIB1 is a potential therapeutic target for GBM therapy as targeting TRIB1 could promote radio-sensitivity of p53-mutant glioma cells by enhancing TMZ action. Targeting TRIB1 would also reduce oncogenic signaling in these cells; overall providing an additional treatment strategy for GBM.


Gene Expression Profiles of Fibroblasts Irradiated with Two Protocols in vitro

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Purpose/Objective(s): Irradiation of exponentially growing fibroblasts induces premature differentiation to postmitotic functional cells depositing increased amounts of extracellular matrix (ECM) proteins. On the other hand, a predictive test for patients’ risk of developing fibrosis was based on a gene expression signature after fractionated irradiation of confluent cultures (Alsner et al., Radiother. Oncol. 83:261-6, 2007). The purpose was to characterize the radiation-induced gene expression profiles for the two protocols.

Materials/Methods: Established skin fibroblast cultures were irradiated with 6 MV X-rays in vitro. Fibroblast differentiation was determined by cytomorphological scoring in clonal culture. Gene expression was determined using Affymetrix HG-U133+ microarrays. After batch normalization, differentially expressed genes were identified by ANOVA using JMP Genomics software. The expression of selected genes was validated by real-time PCR. Pathway analysis was performed by Gene Set Enrichment Analysis using the R-package ReactomePA, filtering pathways with normalized enrichment scores (NES) >1.5 or < -1.5 and adjusted p-values <0.05.

Results: On day 5 after irradiation with 4 Gy, exponentially growing cultures showed the terminally differentiated phenotype. Microarray analysis showed >4-fold upregulation of 157 genes, including several collagen genes and >4-fold downregulation of 244 genes, including many cell cycle-related genes. Irradiation of confluent cultures with 3×4 Gy yielded 91 up- and 195 down-regulated genes (4–fold). Differential gene expression for the two protocols correlated better for down- than for up-regulated genes but GDF15 was strongly upregulated in both. Pathway analysis showed 37 common pathways, 51% of which were related to ECM or glycosaminoglycans (GAG), 14% to inflammation, and 11% to cholesterol and bile acid/salts. 37 pathways were more specific for 1×4 Gy (exponential cultures) with 41% related to GAG or cell-cell/cell-matrix interactions, and 16% to inflammation. 27 pathways were more specific to 3×4 Gy (confluent cultures) with 22% related to cholesterol and bile acid/salts, 15% to each of translation and metabolism, and 11% to inflammation.

Conclusion: The expression profile of exponentially growing cells reflected premature terminal differentiation to a postmitotic phenotype. However, both protocols upregulated ECM and, notably, inflammatory pathways. Additional upregulated pathways represented GAG and cell-cell/cell-matrix interactions relating to differentiation after 1×4 Gy (exponential cultures). By contrast, upregulated cholesterol and bile acid/salts, translation, and metabolism pathways after 3×4 Gy (confluent cultures) might indicate a role of lipid metabolism. Thus, different aspects of the fibrogenic process appear to be detected with the two protocols which may complement each other as model systems for mechanistic studies of radiation-induced fibrosis.

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Effect of Carbogen On Tumor Oxygenation Status By Probe pO2 Measurement And Hypoxia Imaging Study

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Purpose/Objective(s): Tumor hypoxia, a common phenomenon in solid neoplasms has been associated with aggressive malignant phenotypes and resistance to chemotheraphy and radiotherapy. The purpose of the study was to investigate the effect of carbogen on oxygenation status in tumoral and muscular tissues by direct probe pO2 measurement, to compare the difference of hypoxia biodistribution by imaging exogenous and endogenous hypoxia markers and to explore potential hypoxia-modifying modality to enhance the effect of radiotherapy.

Materials/Methods: BALB/c-nu/nu nude mice were used as tumor-bearing host. pO2 measurements in tumoral and muscular tissues were