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Tissue Factor Promotes the Glioma Stem Cell Phenotype, and Is Suppressed by Mutant IDH1

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Abstract: Isocitrate dehydrogenase 1 mutant (IDH1^{mut}) gliomas have global genomic hypermethylation, are less aggressive than IDH1 wild-type (IDH1^{wt}) gliomas, and generally grow poorly *in vitro* and *in vivo*. Yet little data exist that connect specific hypermethylation targets to this unique phenotype. We previously reported that the gene encoding Tissue Factor (TF), *F3*, is among the most hypermethylated and downregulated genes in IDH1^{mut} gliomas relative to IDH1^{wt} gliomas. In addition to promoting normal blood clotting and abnormal cancer-induced thrombosis, TF directly increases the malignancy of many cancers through protease-activated receptor 2 (PAR2), though its activity in gliomas is not well understood. In multiple IDH1^{wt} and IDH1^{mut} patient-derived glioma cell lines, *F3* was hypermethylated in IDH1^{mut} cells compared to IDH1^{wt} cells, with consistently reduced TF protein expression. Treatment of IDH1^{mut} glioma cells with a demethylating agent, decitabine, increased *F3* transcription 5-fold, but had no effect in IDH1^{wt} cells. TF knockdown greatly reduced the *in vitro* proliferation and colony formation of IDH1^{wt}/EGFRvIII^{amp} GBM6 cells and IDH1^{wt}/EGFR^{amp} GBM12 cells, but not of GBM43 cells, which contain an *NF1* mutation downstream of EGFR. TF knockdown specifically reduced the *in vitro* stemlike behavior of GBM6 and GBM12 cells, as indicated by limiting dilution assays and expression of glioma stem cell (GSC) markers. The stemlike behavior of GBM43, however, was unaffected by TF knockdown. *In vivo*, TF knockdown doubled the median survival of mice intracranially engrafted with GBM6, and caused complete regression of GBM12 ($P = 0.001$), whereas GBM43 xenograft growth was unimpeded. Conversely, TF induction enhanced the proliferation and colony formation of IDH1^{mut} GBM164 and TB09 cells, though the effect was more pronounced in GBM164. TF induction also increased the *in vivo* "take rate" of intracranial GBM164 xenografts from 0% to 100% ($P = 0.0001$), but did not enable TB09 xenograft growth. Further investigation to explain the stronger effects of TF manipulation in GBM6, GBM12, and GBM164 cells revealed that, in those cells, TF-PAR2 activated receptor tyrosine kinases (RTKs), including EGFR in GBM6 and GBM12, and PDGFRβ in GBM164. This occurred through a Src-dependent intracellular pathway, even when extracellular RTK stimulation was blocked. In contrast, baseline expression of RTKs was much lower in GBM43 and TB09 cells. RNA-Seq analysis showed that, out of the entire transcriptome, only two genes showed downregulation after TF knockdown in GBM6 and GBM12, as well as upregulation after TF induction in GBM164: *PROM1*, encoding CD133, and *CTNND2*, encoding δ-catenin. In contrast, TF manipulation did not alter expression of either gene in GBM43 or TB09 cells. Analysis of Cancer Genome Atlas gliomas confirmed that high *F3* mRNA correlated with enrichment of multiple GSC markers, and that, among GBMs, high *F3* mRNA was a significant adverse prognostic marker, independent of patient age and IDH1^{mut} status. Together, these data suggest that: (i) TF-PAR2 acts through RTKs to promote a GSC phenotype; (ii) CD133 and δ-catenin may be critical downstream effectors of TF-induced GSC behavior; (iii) TF suppression is one reason why IDH1^{mut} gliomas are less aggressive; (iv) TF-PAR2 is an attractive, novel therapeutic target in IDH1^{wt} gliomas.

Keywords: Tissue Factor, Glioma, Stem cell, IDH1.

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