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**Title: ADAMDEC1 and FGF2/FGFR1 signaling constitute a positive feedback loop to maintain GBM cancer stem cells**

**Authors: Ana Jimenez-Pascual<sup>1</sup>, Justin D. Lathia<sup>2,3</sup>, Florian A. Siebzehnrubl<sup>1</sup>**

**Affiliations:** <sup>1</sup>European Cancer Stem Cell Research Institute, Cardiff University School of Biosciences

<sup>2</sup>Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA

<sup>3</sup>Case Comprehensive Cancer Center, Cleveland, OH, USA

**Abstract:** Identification of targetable mechanisms that maintain glioblastoma cancer stem cells (CSCs) remain a priority. Our study reveals a new mechanism by which a disintegrin and metalloproteinase domain-like protein decysin 1 promotes CSC maintenance through the activation of a fibroblast growth factor autocrine signaling loop, which can be blocked pharmacologically.

**Summary:** Glioblastoma (GBM) is a highly infiltrative and heterogenous tumor, formed by cancerous cells harboring diverse molecular signatures which contribute to therapeutic resistance. Tumor recurrence and poor prognosis are the consequence of resident GBM cancer stem cells (CSCs), which can be quiescent and capable of self-renewal, invasion and initiation of new tumors<sup>1</sup>. Tumor progression and local invasion of GBM cells into the tissue surrounding the tumor is facilitated by the secretion of metalloproteinases which enable the degradation of extracellular matrix (ECM) causing the release of growth factors and cytokines in the tumor microenvironment<sup>2</sup>.

Our work identifies a disintegrin and metalloproteinase domain-like protein decysin 1 (ADAMDEC1) as the most highly expressed protease of the A disintegrin and metalloproteinase (ADAM) family in GBM<sup>3</sup>. ADAMDEC1 lacks a transmembrane domain, contains an altered catalytic domain and is the only soluble protease of the ADAM family<sup>4</sup>. ADAM family proteases have been associated with tumor growth and invasion, but the functions of ADAMDEC1 in CSC maintenance remained unknown. Using *in vitro* stem cell assays, we found ADAMDEC1 to be highly expressed and secreted by CSCs as compared to non-stem tumor cells (NSTCs). As this protease cleaves growth factors from immobilized precursors<sup>4</sup>, we performed an ELISA assay to identify candidate cytokines released from CSCs and NSTCs after recombinant ADAMDEC1 treatment. Fibroblast growth factor (FGF) 2 emerged as the top candidate and was released only from CSCs.

FGF2 has been linked to malignancy and progression in GBM as it promotes angiogenesis, proliferation and CSC self-renewal<sup>5</sup>. However, the specific pathways of how this growth factor acts to maintain stemness in GBM CSCs remain incompletely understood. Using the cancer genome atlas (TCGA) gene expression data, we observed a significant correlation between FGF2 and the stemness-associated transcription factors ZEB1, SOX2 and OLIG2. These transcription factors are known to mediate self-renewal, tumor formation capacity and therapy resistance in GBM patients<sup>6,7</sup>. Protein analysis of patient-derived cell lysates treated with FGF2 demonstrated that this growth factor induces the expression of ZEB1, SOX2 and OLIG2. To further assess stemness, we tested a pharmacological inhibitor that blocks the interaction between FGF2 and its receptors and found it reduced clonogenicity and sphere formation of patient-derived GBM cells. From this, we concluded that FGF2 signaling contributes to CSC maintenance and that this can be targeted.

We next sought to identify which FGF receptor (FGFR) was activated by FGF2 on CSCs. FGF2 binds to four different receptors FGFR1-4 whose functions have not been fully elucidated in GBM. *FGFR1* expression is associated with higher glioma grade, radioresistance and malignancy, as well as increased expression of ZEB1<sup>8</sup>. In contrast, *FGFR2* has been

described as a tumor suppressor in GBM and FGFR3-transforming acidic coiled-coil (TACC) 3 fusion proteins have been identified as oncogenic<sup>9</sup>. Less information is currently available for FGFR4 in brain tumors and our TCGA data analysis showed a lack of expression of this receptor in GBM patient samples. Using short hairpin (sh) RNA-mediated knockdown of *FGFR1-3* we found that only FGFR1 was necessary for CSC self-renewal. As FGF2 treatment induced the expression of stem cell-associated transcription factors, we examined whether FGFR1 was transducing FGF2 signals. Interestingly, we found that only FGFR1 loss decreased ZEB1, SOX2 and OLIG2 expression while its overexpression had the opposite effect. This indicated that FGFR1 was upstream of these stemness-associated transcription factors.

To further substantiate the pivotal role of FGFR1 in CSC maintenance, we demonstrated that limiting-dilution orthotopic transplantation of FGFR1 knockdown cells decreased tumor initiation capacity. Kaplan-Meier analysis of the TCGA GBM data revealed that *FGFR1* was associated with poorer patient survival, consistent with previous studies that identified *FGFR1* as a prognostic factor for overall and progression-free survival in GBM patients<sup>10</sup>. As our results linked FGFR1 with self-renewal, we speculated that FGFR1 may be a surface marker of CSCs. FACS purification yielded a FGFR1+ population of highly tumorigenic CSCs, while the FGFR1- population was less capable of initiating tumor growth. These results identify FGFR1 as a potential cell surface marker of CSCs.

Since ADAMDEC1 releases FGF2 from the ECM, we examined a possible link between this metalloproteinase and FGFR1. Combinational Kaplan-Meier analysis of the TCGA GBM dataset revealed that *FGFR1* and *ADAMDEC1* correlated with poorer patient survival. Analyzing how ADAMDEC1 expression was regulated in GBM CSCs, we found that FGF2 increased ADAMDEC1 expression and that this was mediated through FGFR1. Interestingly, ZEB1 could also regulate ADAMDEC1 levels indirectly through the repression of miR-203.

In order to identify how FGFR1 signals to ADAMDEC1 and ZEB1, we assessed the expression of different signaling cascades known to be hyperactivated in GBM. Our data shows an increase in ERK1/2 phosphorylation after FGF2 treatment and a decrease upon FGFR1 loss. Importantly, ERK1/2 inhibition reduced the expression of both ADAMDEC1 and ZEB1.

In conclusion, our study identifies a novel and targetable feedback loop where ADAMDEC1 promotes FGF2/FGFR1-mediated CSC self-renewal by the activation of stem cell transcription factors, which in turn regulate expression of ADAMDEC1 (Fig. 1). This contributes to CSC maintenance in GBM and provides a mechanism through which CSCs can access key growth factors embedded in their surrounding microenvironment to drive self-renewal.

**Figure 1. Diagram depicting ADAMDEC1-FGFR1-ZEB1 feedback loop.** ADAMDEC1 releases FGF2 to the tumor microenvironment which binds to FGFR1 and mediates the activation of ERK1/2 signaling. The latter increases the expression of ADAMDEC1 and the stem cell-associated transcription factor ZEB1. Simultaneously, ZEB1 regulates ADAMDEC1 expression, completing a positive feedback loop that contributes to GBM CSC self-renewal and maintenance. Image created with biorender.com. ADAMDEC1: a disintegrin and metalloproteinase domain-like protein decysin 1; FGF2: fibroblast growth factor (FGF) 2; FGFR1: FGF receptor 1; GBM: glioblastoma; CSC: cancer stem cell.

## References

1. Lathia JD, Mack SC, Mulkearns-Hubert E, Valentim CL, Rich JN. Cancer stem cells in glioblastoma. *Genes Dev.* 2015;29(12):1203-1217. doi:10.1101/gad.261982.115.tumors
2. Le DM, Besson A, Fogg DK, Choi K-S, Waisman DM, Goodyer CG, Rewcastle B, Yong VW. Exploitation of astrocytes by glioma cells to facilitate invasiveness: a mechanism involving matrix metalloproteinase-2 and the urokinase-type plasminogen activator-plasmin cascade. *J Neurosci.* 2003;15(23):4034-4043.
3. Jimenez-Pascual A, Hale JS, Kordowski A, Pugh J, Silver DJ, Bayik D, Roversi G, Alban TJ, Rao S, Chen R, et al. ADAMDEC1 maintains a growth factor signaling loop in cancer stem cells. *Cancer Discov.* 2019. doi:10.1158/2159-8290.CD-13-0172
4. Chen R, Jin G, McIntyre TM. The soluble protease ADAMDEC1 released from activated platelets hydrolyzes platelet membrane pro-epidermal growth factor (EGF) to active high-molecular-weight EGF. *J Biol Chem.* 2017;292(24):10112-10122. doi:10.1074/jbc.M116.771642
5. Pollard SM, Yoshikawa K, Clarke ID, Danovi D, Stricker S, Russell R, Bayani J, Head R, Lee M, Bernstein M, et al. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell.* 2009;4(6):568-580. doi:10.1016/j.stem.2009.03.014
6. Siebzehnrubl FA, Silver DJ, Tugertimur B, Deleyrolle LP, Siebzehnrubl D, Sarkisian MR, Devers KG, Yachnis AT, Kupper MD, Neal D, et al. The ZEB1 pathway links glioblastoma initiation, invasion and chemoresistance. *EMBO Mol Med.* 2013;5(8):1196-1212. doi:10.1002/emmm.201302827
7. Singh DK, Kollipara RK, Vemireddy V, Yang XL, Sun Y, Regmi N, Klingler S, Hatanpaa KJ, Raisanen J, Cho SK, et al. Oncogenes Activate an Autonomous Transcriptional Regulatory Circuit That Drives Glioblastoma. *Cell Rep.* 2017;18(4):961-976. doi:10.1016/j.celrep.2016.12.064
8. Gouazé-Andersson V, Ghérardi M, Lemarié A, Gilhodes J, Lubrano V, Arnauduc F, Moyal Cohen-Jonathan E, Toulas C. FGFR1/FOXM1 pathway: a key regulator of glioblastoma stem cells radioresistance and a prognosis biomarker. *Oncotarget.* 2018;9(60):31637-31649. doi:10.18632/oncotarget.25827
9. Singh D, Chan JM, Zoppoli P, Niola F, Sullivan R, Castano A, Liu EM, Reichel J, Porrati P, Pellegatta S, et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science.* 2012;337(6099):1231-1235. doi:10.1126/science.1220834
10. Ducassou A, Uro-Coste E, Verrelle P, Filleron T, Benouaich-Amiel A, Lubrano V, Sol JC, Delisle MB, Favre G, Ken S, et al.  $\alpha\beta 3$  Integrin and Fibroblast growth factor receptor 1 (FGFR1): Prognostic factors in a phase I-II clinical trial associating continuous administration of Tipifarnib with radiotherapy for patients with newly diagnosed glioblastoma. *Eur J Cancer.* 2013;49(9):2161-2169. doi:10.1016/j.ejca.2013.02.033