Editorial

Emerging role for USP1 in glioblastoma stem cell maintenance and radioresistance: a potential target for glioblastoma therapy

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Glioblastoma (GBM) is the most common primary brain malignancy with limited treatment options. The standard of care for GBM, including maximal safe resection, radiotherapy, and concomitant and adjuvant temozolomide is currently recommended for all patients (1) but only a small proportion of patients survive 2 years or longer and, eventually, virtually every tumor recurs (2). GBM is characterized by a high degree of intra-tumoral cellular heterogeneity. Recently, genome-wide expression profiling in the context of The Cancer Genome Atlas (TCGA) project has led to the identification of initially three, and then four main subtypes of GBM (3,4), namely proneural (P), mesenchymal (M), neural (N) and proliferative (or classical, C), thus revealing the existence of a profound degree of inter-tumoral cellular heterogeneity. An alternative scheme has also been reported in which GBMs clusterized in six subtypes that correlated better with survival than histology (5). The exact number of subtypes, their validity and inter-observer reproducibility, and their clinical and prognostic correlate, all are still a matter of debate. However, the opposing picture of the P and M GBM subgroups is widely accepted. The P subtype is strongly associated with secondary GBM, hypermethylator phenotype, younger age, lack of enhancement on MRI and, in selected reports, better prognosis. Conversely, M subtype GBMs tend to be more invasive, displaying angiogenesis, necrosis and contrast enhancement (3,6-8).

Cancer cells with stem cell-like features (CSCs) seem to influence tumor growth and recurrence in many malignancies. Although there is no generally accepted definition of these so-called CSCs in GBM (GSCs), they have been characterized as slow-cycling tumor cells with enhanced self-renewal potential and high tumor-forming potential (9). Analogous to normal stem cells, GSCs seem to be endowed with increased resistance to chemotherapy and radiotherapy, due to the over-expression of DNA damage repair enzymes (10) as well as to metabolic traits which allow GSCs to grow under hypoxic conditions (11,12) thus, shielding them from the oxygen-dependent effects of radiotherapy. Increased expression of membrane proteins associated with drug-resistance has been reported in GSCs, too (13,14). The role of GSCs in determining the molecular subtype of GBM according to TCGA classification is not well understood. However, expression profiling of GSC cultured *in vitro* from primary GBM appears to divide them into two groups: one is characterized by proneural-like gene expression signature, in which GSCs resemble normal neural stem cells, are CD133-positive, grow as floating spheres and display high invasiveness *in vivo* ("full" GSCs), whereas the other one shows mesenchymal-like expression signature, with cells more similar to adult normal neural stem cell lines, displaying no CD133 expression, *in vitro* adherent growth, and low invasive behavior *in vivo* ("restricted" GSCs) (15).

However, irrespective of the subtype, the discovery of GSCs as the cell population responsible for tumor development and propagation has led to the search of novel targeted therapies aimed at eradicating this cellular core, rather than the differentiated, non-stem tumor bulk. This has led to a push to identify the specific signaling pathways

which stem cells depend on.

Carcinogenesis is a complex process finely regulated at multiple levels by post-translational modifications (PTMs). Ubiquitination is one of the most important PTMs responsible for regulating the stability and activity of modified proteins. Deubiquitination has a pivotal role in the ubiquitin system through specific deconjugation of ubiquitin from target proteins. The human genome encodes at least 98 deubiquitinases (DUBs), grouped into six families based on sequence and structural similarity, reflecting the need for specificity in their function (16). The wide functional diversity of DUBs has a profound impact on the regulation of multiple biological processes as cell-cycle control, DNA repair, chromatin remodeling and several signaling pathways that are frequently altered in cancer (17). Consistent with this, altered DUB function has been related to several diseases, including cancer and thus, DUBs are increasingly regarded as a potential target in cancer therapy. The link between genetic damage repair and cancer development is widely documented by the increased tumor rates reported for those disorders associated with deficient DNA repair mechanisms such as Fanconi's anemia. A critical role in the regulation of several important steps in the Fanconi's anemia pathway is played by USP1, a member of DUBs, through the deubiquitination of Fanconi's anemia protein FANCD2 and the subsequent stabilization of CHEK1 (18). USP1, upon forming a molecular complex with UAF1 (WDR48), deubiquitinates substrates including PCNA, FANCI, FANCD2, and the short-lived Inhibitor of DNA Binding (ID) proteins ID1, ID2, and ID3 (19). Members of the Id gene family, including Id2, have been proposed as mediators of GBM and are known to be highly expressed in a number of different tumor types (20).

Starting from this background, in a recent work Lee and co-authors have further defined a role for USP1 in maintaining the stem-cell properties of GSCs and thus in the regulation of GBM aggressiveness and resistance to therapies (21). In the paper, the authors focus on USP1 after an analysis of public genomic and proteomic databases and demonstrated that this protein is correlated with glioma malignancy. Consistently, analysis of GSC cultures showed that USP1 expression paralleled expression of Sox2, which is a stemness marker. Then, the author inhibited USP1 expression in GSCs cultures, using both lentivirus-mediated short-interference RNAs and pharmacological inhibition through pimozide. As expected, USP1 blockade induced apoptosis and strongly reduced clonogenic potential of GSCs. Noticeably, such an effect was not obtained in normal NSCs, confirming that the stemness-maintaining activity of DUBs is unique of neoplastic cells. In a further, elegant set of experiments, the authors confirmed the causal link between USP1 expression and stabilization of ID1 and CHK1 in GSCs. ID proteins are key regulators in gliomagenesis and in the maintenance of the stem cell phenotype of GSCs (22); consistently, USP1 down-regulation decreased the expression of stemness markers while increasing that of differentiation markers, whereas the opposite occurred when USP1 was up-regulated. Moreover, USP1 blockade, through inhibition of DNA checkpoint kinases like CHK1, resulted in a reduced radio-resistance of GSCs.

Interestingly, in a previous work by our group (23), we showed that the multikinase-inhibitor staurosporine-derivative UCN-01 inhibited the growth of GSCs, both *in vitro* and *in vivo*, through the blockade of CHK1 and of the receptor tyrosine kinase pathway. Taken together, these evidences support the assumption that, to achieve a therapeutic response against GBM, it is fundamental to target not only the stemness and/or growth factors pathways (like RTKs or ID), but also the DNA-repair machinery. Consistent with this, in an *in vivo* GSC-derived GBM model in mice, USP1 inhibition leads to reduced tumor growth and prolonged survival of animals. Interestingly, GBM-bearing mice treated with irradiation and the USP1 inhibitor pimozide survive longer than controls and single-treatment arm, with tumor cells showing increased DNA damage and reduced expression of stemness markers.

Conclusions

Lee *et al.* have provided early results describing a role of USP1 in regulating critical regulators of DNA damage repair and stem cell maintenance in GBM. Moreover, they showed that genetic or pharmacologic inhibition of USP1 significantly impairs *in vitro* clonogenic ability and *in vivo* tumorigenic potential of GSCs.

While the cellular and molecular complexity of GBM is gradually unraveled, it is crucial to notice that the therapeutic strategies that are more likely to carry a therapeutic success necessarily hit more targets at the same time. DUBs have the advantage to act not only on molecular heterogeneity, targeting several pathways at a time, but also on cellular heterogeneity, reducing the stem cell properties of GSCs and therefore hitting the cellular core of GBM. Moreover, their key role in blocking DNA damage repair enzymes increases the susceptibility of GSCs to chemo-radiation.

The results showed in this work, together with the recent success of clinically targeting the ubiquitin proteasome system in cancer, reinforce the concept of DUBs as appealing targets for the development of new specific therapies against human malignancies, including GBM. So far, no DUB-targeted strategies have reached clinical trials and many challenges remain before translating this information into clinical benefits for cancer patients. Thus, more work is required to achieve a better understanding of the basic mechanism of USP1 function and regulation for the development of USP1 inhibitors useful as anticancer drugs for GBM treatment. Hopefully, the knowledge derived from this and similar studies will provide new insights into the multiple questions still open in relation to DUBs and may lead to the introduction of DUB-targeting strategies as an essential component of molecular therapies against cancer.

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References

1. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
2. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10:459-66.
3. Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006;9:157-73.
4. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010;17:98-110.
5. Gravendeel LA, Kouwenhoven MC, Gevaert O, et al. Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. Cancer Res 2009;69:9065-72.
6. Pelloski CE, Ballman KV, Furth AF, et al. Epidermal growth factor receptor variant III status defines clinically distinct subtypes of glioblastoma. J Clin Oncol 2007;25:2288-94.
7. Gutman DA, Cooper LA, Hwang SN, et al. MR imaging predictors of molecular profile and survival: multi-institutional study of the TCGA glioblastoma data set. Radiology 2013;267:560-9.
8. Colman H, Zhang L, Sulman EP, et al. A multigene predictor of outcome in glioblastoma. Neuro Oncol 2010;12:49-57.
9. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. Nature 2004;432:396-401.
10. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756-60.
11. Pistollato F, Abbadi S, Rampazzo E, et al. Intratumoral hypoxic gradient drives stem cells distribution and MGMT expression in glioblastoma. Stem Cells 2010;28:851-62.
12. Li Z, Bao S, Wu Q, et al. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell 2009;15:501-13.

13. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 2006;5:67.
14. Liu Q, Nguyen DH, Dong Q, et al. Molecular properties of CD133+ glioblastoma stem cells derived from treatment-refractory recurrent brain tumors. J Neurooncol 2009;94:1-19.
15. Marziali G, Signore M, Buccarelli M, et al. Metabolic/Proteomic Signature Defines Two Glioblastoma Subtypes With Different Clinical Outcome. Sci Rep 2016;6:21557.
16. Komander D, Clague MJ, Urbé S. Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol 2009;10:550-63.
17. Sacco JJ, Coulson JM, Clague MJ, et al. Emerging roles of deubiquitinases in cancer-associated pathways. IUBMB Life 2010;62:140-57.
18. García-Santisteban I, Peters GJ, Giovannetti E, et al. USP1 deubiquitinase: cellular functions, regulatory mechanisms and emerging potential as target in cancer therapy. Mol Cancer 2013;12:91.
19. Williams SA, Maecker HL, French DM, et al. USP1 deubiquitinates ID proteins to preserve a mesenchymal stem cell program in osteosarcoma. Cell 2011;146:918-30.
20. Lasorella A, Benezra R, Iavarone A. The ID proteins: master regulators of cancer stem cells and tumour aggressiveness. Nat Rev Cancer 2014;14:77-91.
21. Lee JK, Chang N, Yoon Y, et al. USP1 targeting impedes GBM growth by inhibiting stem cell maintenance and radioresistance. Neuro Oncol 2016;18:37-47.
22. Martini M, Cenci T, D'Alessandris GQ, et al. Epigenetic silencing of Id4 identifies a glioblastoma subgroup with a better prognosis as a consequence of an inhibition of angiogenesis. Cancer 2013;119:1004-12.
23. Signore M, Pelacchi F, di Martino S, et al. Combined PDK1 and CHK1 inhibition is required to kill glioblastoma stem-like cells in vitro and in vivo. Cell Death Dis 2014;5:e1223.

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