Editorial

Editorial on "The genomic landscape of *TERT* promoter wildtype-*IDH* wildtype glioblastoma"

Rui Batista[1,2,3], Tiago Bordeira Gaspar[1,2,3,4], Paula Soares[1,2,3], João Vinagre[1,2,3]

[1]Cancer Signaling & Metabolism Group, Institute for Research and Innovation in Health Sciences (i3S), University of Porto, Porto, Portugal; [2]Cancer Signalling & Metabolism Group, Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal; [3]Medical Faculty of University of Porto (FMUP), Porto, Portugal; [4]Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto, Porto, Portugal

Correspondence to: João Vinagre. Institute for Research and Innovation in Health Sciences (i3S), Rua Alfredo Allen 208, 4200-135 Porto, Portugal. Email: jvinagre@ipatimup.pt.

Comment on: Diplas BH, He X, Brosnan-Cashman JA, *et al.* The genomic landscape of TERT promoter wildtype-IDH wildtype glioblastoma. Nat Commun 2018;9:2087.

Submitted Jul 30, 2018. Accepted for publication Aug 02, 2018.
doi: 10.21037/tcr.2018.08.06
View this article at: http://dx.doi.org/10.21037/tcr.2018.08.06

In 2016, with the publication of the novel guidelines for the classification of the central nervous system (CNS) tumours by the World Health Organization (WHO) (1), there was a paradigm shift in the classical diagnosis, mainly based in microscopy, by the integration of molecular markers in the novel stratification. Due to the intrinsic limitations of the collected CNS study material and the majority of the conservative procedures that ends in scarce material, and sometimes of difficult histology, this added value of the molecular data introduced a significant advance in tumour homogenous classification. The genetic classification is based on canonical events that have been collected throughout the recent years (2-5). Regarding the pivotal events that are used to stratify glioblastoma (GBM), we have the mutations in the isocitrate dehydrogenase 1 and 2 (IDH) genes (2,5) and in the promoter of the telomerase gene (*TERTp*) (5-10). These alterations are used to aid in the diagnosis of up to 80% of GBM and can cluster patients with distinct prognostic features (5). With the molecular subgroups there is also an association for a particular mechanism used for telomere maintenance. Telomere maintenance can rely in mechanisms that are dependent in telomerase re-activation or re-expression, or in a telomerase non-dependent mechanism, the so-called alternative mechanism for telomere maintenance (ALT) (11). In a telomerase re-activation setting, *TERTp* mutations are so far recognized as the most frequent event and have been reported in several human cancers (6,8,12-15). In an ALT mechanism setting, the Alpha Thalassemia/Mental Retardation Syndrome X-Linked (*ATRX*) and Death Domain Associated Protein (*DAXX*) gene mutations were documented as the most frequently altered genes and were, until the study by Diplas *et al.* (16), the only known genes to be directly involved in ALT mechanism promotion. *TERTp* mutations are present in about 70% of *IDH*-wildtype GBM, associated with older patients that present a poorer prognosis with a shorter survival; this subgroup represents up to 90% of GBM (1,5). The remaining are *IDH*-mutant GBM, and present *ATRX* mutations in 70% of the cases (with ALT phenotype); this subgroup is composed of younger patients and presents a better outcome with increase overall survival (OS) when compared with the previous (1,4,5,8). In the study by Diplas *et al.* (16), the authors set to determine the genetic landscape of $TERTp^{WT}$-IDH^{WT} GBM. For this purpose, they identified a cohort of 16.9% of $TERTp^{WT}$-IDH^{WT} GBM from a series of 260 GBM that had been previously studied (5); of these, when 1p/19q co-deletion status was available it was negative, in this way this subset lack the three main glioma markers ($TERTp^{WT}$-IDH^{WT}-$1p/19q^{WT}$)—triple negative tumours (17). The whole exome sequencing of this subgroup presented recurrent mutated genes in classical pathways as the RTK/RAS/PI3K (88%), P53 (40%), and RB (24%) pathways, as well as, copy number variations in *PDGFRA* (8%), *MDM2* and *MDM4* (12%) and *CDKN2B* (12%) genes. Analysis for glioma-associated driver alterations identified mutations in classical associated genes as *PTEN* (32%), *NF1* (24%), *EGFR* (28%), *TP53* (24%), *ATRX* (20%), and *BRAF* (20%), and in two novel candidate drivers that were not previously associated with *GBM*, *SMARCAL1* (16%) and *PPM1D* (8%).

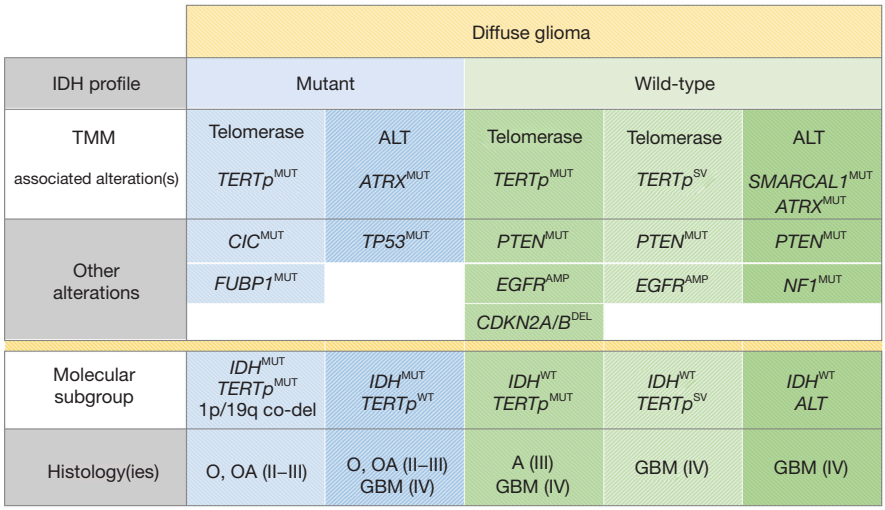

Figure 1 Novel genetic subtypes of gliomas defined in the study by Diplas *et al.* (16). TMM, telomere maintenance mechanism; WT, wild-type; MUT, mutations; AMP, amplification; SV, structural variation; DEL, deletion; GBM, glioblastoma.

The results obtained revealed several interesting findings. There was an enrichment of *BRAF* (V600E) mutations (20%) in the *TERTp*^{WT}-*IDH*^{WT} GBM, whereas *BRAF* alterations that were previously associated to low-grade pediatric gliomas, being rare in adult gliomas (18,19), had a high representation in this subgroup; with 80% of the patients being less or equal than 30 years old. The second novelty with this study was the detection of mutations in *SMARCAL1* and *PPM1D* as novel gliomagenesis genes. The *SMARCAL1* gene, that stands for the SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A Like 1 protein was the main focus of the study since it encodes an adenosine triphosphate (ATP)-dependent annealing helicase responsible for rewinding of Replication Protein A (RPA)-bound DNA at stalled replication forks for resolving telomere-associated replication stress (5); such similarity with *ATRX* function, also a member of the SWI/SNF family of chromatin remodelers, made it immediately an attractive candidate (16,20,21). All these facts led the authors to expand the cohort of *TERTp*^{WT}-*IDH*^{WT} GBM, and in 21% of these cases, *SMARCAL1* mutations were detected. Given the similarities with *ATRX*, it was determined if *SMARCAL1* inactivation was comparable to *ATRX* loss-of-function, and ALT initiation. The authors performed a battery of assays demonstrating classical ALT phenotypic features (16,20,21), such as the presence of ultrabright telomeric foci and C-circles and establishing a novel link of a gene to ALT development. Additionally, *SMARCAL1*-mutant GBM were mutually exclusive of GBM with ATRX loss of expression, reinforcing the independent contribution of each gene for the ALT mechanism. However, the authors noted that still other mechanisms should be involved because 61.5% of *TERTp*^{WT}-*IDH*^{WT} GBM remained without a telomere maintenance mechanism. To decipher further mechanisms, it was taken a whole genome sequencing approach in 8 *TERTp*^{WT}-*IDH*^{WT} GBM and structural variants (SV) of *TERT* were identified in 75% of the cases. This structural alteration consisted in half of the cases in translocations to other chromosomes and in the remaining cases in inversions within the same chromosome. In the expanded analysis of the *TERTp*^{WT}-*IDH*^{WT} GBM for *TERT* SV identification, the authors used break-apart FISH probes and found that half of the cases presented *TERT* structural rearrangements. Functionally, *TERT*-rearranged GBM expressed significantly higher levels of telomerase in comparison with the ALT-positive (*ATRX*- or *SMARCAL1*-mutated GBM) but with no striking differences in comparison with *TERTp* mutated GBM.

All the previous data together allowed the creation of new genetic subgroups of *TERTp*^{WT}-*IDH*^{WT} GBM (*Figure 1*). Within the previous *IDH*^{WT}-ALT subgroup we are now aware that besides *ATRX* mutations (with absence of *IDH* and *TP53* mutations), there was a fraction of cases that presented *SMARCAL1* mutations (38.5%), a novel mechanism with phenotypic markers compatible with ALT. The *SMARCAL1*-mutant GBM contrary to the *ATRX*-mutated GBM often presented mutations in *TP53* as well as in *PTEN* and *NF1*; such co-occurrences may be necessary for gliomagenesis. In addition to the subgroups with ALT, we have the *IDH*^{WT} GBM subgroup that rely on telomerase re-expression due to *TERT* SVs. Altogether, this novel genetic events account for more than 80% of the *TERTp*^{WT}-*IDH*^{WT} GBM. In the study by Diplas *et al.* (16),

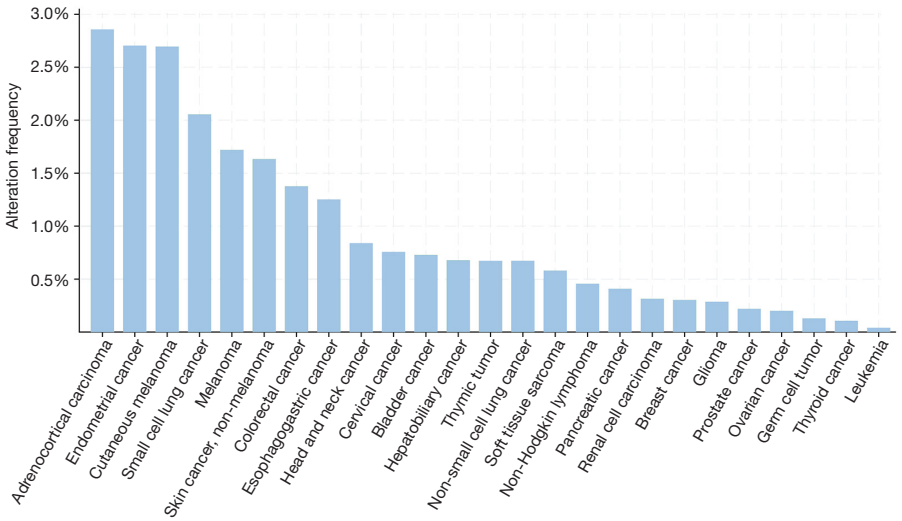

Figure 2 *SMARCAL1* mutations within several cancer types present in cBioPortal (23,24).

it was also evaluated how this subgroup genetic integration impacts patients' survival. Both IDH^{WT} GBM, either with ALT or with *TERT* SVs exhibited the poorest OS (14.9 and 19.7 months, respectively), similarly to the IDH^{WT} GBM with *TERTp* mutations (OS: 14.7 months); contrasting with the better outcome of GBM patients with *IDH* mutations and absence of *TERTp* alterations (OS: 37.1 months).

Apart from the definition of these novel subgroups of $TERTp^{WT}$-IDH^{WT} GBM, there was also a novel finding, the discovery of a new gene associated to ALT. The authors created a set of experiments to elucidate *SMARCAL1* contribution for this process. The first approach was to start with cancer cell lines that presented mutations in this gene and to evaluate if these cell lines recapitulated the findings detected in the human GBM samples. Both *SMARCAL1*-mutant cell lines had abolished expression of the SMARCAL1 protein and maintained intact expression of ATRX and DAXX. Still, ALT phenotypical markers were present and included ALT-associated promyelocytic leukemia (PML) bodies (APBs), DNA C-circles, and the classical ultrabright telomere DNA foci by FISH (22); exogenous expression of *SMARCAL1* was able to restore, or partially restore, the previous changes. The second approach went the opposite way and was the evaluation of GBM cell lines after *CRISPR/Cas9* gene removal of the *SMARCAL1* gene. Under this approach, isogenic $SMARCAL1^{-/-}$ GBM cell lines were assessed for the ALT markers, and it was observed that there was a significant increase of C-circles, ultrabright telomere foci formation and APBs presence; this increase was more evident when the alterations that targeted SMARCAL1 helicase domains, pointing its domain important role.

Overall, in the study by Diplas *et al.* (16), two novel subgroups of $TERTp^{WT}$-IDH^{WT} GBM were identified. They represent two genetically defined GBM subgroups, IDH^{WT}-ALT and IDH^{WT}-$TERT^{SV}$ that present similarities with the established IDH^{MUT} and $TERTp^{MUT}$, both subgroups relying in novel genetic alterations result in ALT-mediated or telomerase-mediated mechanisms for telomere maintenance with novel alterations (*Figure 1*). Within the gliomas, *SMARCAL1* mutations seem to be rare in lower-grade gliomas (WHO grade II–III) and only present in high grade gliomas (WHO grade 4); also, *TERT* SV were only detected in GBM (WHO grade 4). The detection of a high frequency of *BRAF* mutations was a novelty that opens a new therapeutic option for this subgroup of patients younger than 30 years old, since this is an alteration that is drug-targetable. It remains now to be understood what is the true expression of this novel gene associated to ALT, the *SMARCAL1* and its prevalence within cancer. In a short overview of the cancer genomic data accessed in cBioPortal (23,24) for cancer genomics (*Figure 2*) we detected that, besides GBM, several cancers present mutations in *SMARCAL1* and despite the prevalence of these alterations to be inferior to 3% it may be useful to select patients with particular clinicopathological features.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Duo Liu (Harbin Medical University Cancer Hospital, Harbin Medical

University, Harbin, China).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.08.06). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Louis DN, Ohgaki H, Wiestler OD, et al. WHO classification of tumours of the central nervous system. World Health Organization classification of tumours. Lyon: International Agency For Research On Cancer (IARC), 2016.
2. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765-73.
3. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807-12.
4. Jiao Y, Killela PJ, Reitman ZJ, et al. Frequent ATRX, CIC, FUBP1 and IDH1 mutations refine the classification of malignant gliomas. Oncotarget 2012;3:709-22.
5. Killela PJ, Pirozzi CJ, Healy P, et al. Mutations in IDH1, IDH2, and in the TERT promoter define clinically distinct subgroups of adult malignant gliomas. Oncotarget 2014;5:1515-25.
6. Vinagre J, Almeida A, Populo H, et al. Frequency of TERT promoter mutations in human cancers. Nat Commun 2013;4:2185.
7. Batista R, Cruvinel-Carloni A, Vinagre J, et al. The prognostic impact of TERT promoter mutations in glioblastomas is modified by the rs2853669 single nucleotide polymorphism. Int J Cancer 2016;139:414-23.
8. Killela PJ, Reitman ZJ, Jiao Y, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci U S A 2013;110:6021-6.
9. Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. Cell 2013;155:462-77.
10. Labussiere M, Boisselier B, Mokhtari K, et al. Combined analysis of TERT, EGFR, and IDH status defines distinct prognostic glioblastoma classes. Neurology 2014;83:1200-6.
11. Gaspar TB, Sa A, Lopes JM, et al. Telomere Maintenance Mechanisms in Cancer. Genes (Basel) 2018;9. doi: 10.3390/genes9050241.
12. Huang FW, Hodis E, Xu MJ, et al. Highly recurrent TERT promoter mutations in human melanoma. Science 2013;339:957-9.
13. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. Science 2013;339:959-61.
14. Vinagre J, Pinto V, Celestino R, et al. Telomerase promoter mutations in cancer: an emerging molecular biomarker? Virchows Arch 2014;465:119-33.
15. Vinagre J, Nabais J, Pinheiro J, et al. TERT promoter mutations in pancreatic endocrine tumours are rare and mainly found in tumours from patients with hereditary syndromes. Sci Rep 2016;6:29714.
16. Diplas BH, He X, Brosnan-Cashman JA, et al. The genomic landscape of TERT promoter wildtype-IDH wildtype glioblastoma. Nat Commun 2018;9:2087.
17. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. N Engl J Med 2015;372:2499-508.
18. Basto D, Trovisco V, Lopes JM, et al. Mutation analysis of B-RAF gene in human gliomas. Acta Neuropathol 2005;109:207-10.
19. Horbinski C. To BRAF or not to BRAF: is that even a question anymore? J Neuropathol Exp Neurol 2013;72:2-7.
20. Heaphy CM, de Wilde RF, Jiao Y, et al. Altered telomeres in tumors with ATRX and DAXX mutations. Science 2011;333:425.
21. Jiao Y, Shi C, Edil BH, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science 2011;331:1199-203.
22. Amorim JP, Santos G, Vinagre J, et al. The Role of ATRX in the Alternative Lengthening of Telomeres (ALT) Phenotype. Genes (Basel) 2016;7. doi: 10.3390/genes7090066.
23. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
24. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401-4.

Cite this article as: Batista R, Gaspar TB, Soares P, Vinagre J. Editorial on "The genomic landscape of *TERT* promoter wildtype-*IDH* wildtype glioblastoma". Transl Cancer Res 2018;7(Suppl 7):S762-S765. doi: 10.21037/tcr.2018.08.06