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Research Paper

PD-1 (*PDCD1*) Promoter Methylation Is a Prognostic Factor in Patients With Diffuse Lower-Grade Gliomas Harboring Isocitrate Dehydrogenase (*IDH*) Mutations

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ABSTRACT

Immune checkpoints are important targets for immunotherapies. However, knowledge on the epigenetic modification of immune checkpoint genes is sparse. In the present study, we investigated promoter methylation of *CTLA4*, *PD-L1*, *PD-L2*, and *PD-1* in diffuse lower-grade gliomas (LGG) harboring isocitrate dehydrogenase (*IDH*) mutations with regard to mRNA expression levels, clinicopathological parameters, previously established methylation subtypes, immune cell infiltrates, and survival in a cohort of 419 patients with *IDH*-mutated LGG provided by The Cancer Genome Atlas.

PD-L1, PD-L2, and CTLA-4 mRNA expression levels showed a significant inverse correlation with promoter methylation (PD-L1: $p = 0.005$; PD-L2: $p < 0.001$; CTLA-4: $p < 0.001$). Furthermore, immune checkpoint methylation was significantly associated with age (*PD-L2*: $p = 0.003$; *PD-1*: $p = 0.015$), molecular alterations, i.e. *MGMT* methylation (*PD-L1*: $p < 0.001$; *PD-L2*: $p < 0.001$), *ATRX* mutations (*PD-L2*: $p < 0.001$, *PD-1*: $p = 0.001$), and *TERT* mutations (*PD-L1*: $p = 0.035$, *PD-L2*: $p < 0.001$, *PD-1*: $p < 0.001$, *CTLA4*: $p < 0.001$) as well as methylation subgroups and immune cell infiltrates. In multivariate Cox proportional hazard analysis, *PD-1* methylation qualified as strong prognostic factor (HR = 0.51 [0.34–0.76], $p = 0.001$).

Our findings suggest an epigenetic regulation of immune checkpoint genes via DNA methylation in LGG. *PD-1* methylation may assist the identification of patients that might benefit from an alternative treatment, particularly in the context of emerging immunotherapies.

1. Introduction

Gliomas are the most common primary brain tumors accounting for approximately 80% of all brain malignancies in the United States (Ostrom et al., 2016). Diffuse lower-grade gliomas (LGG) often present with very variable clinical appearances and survival rates before fatally progressing to glioblastoma multiforme (Cancer Genome Atlas Research Network et al., 2015). Recent developments in genomic profiling have led to a paradigm shift in the classification of gliomas. As a consequence, the 2016 World Health Organization (WHO) classification includes the molecular characterization of primary brain tumors (e.g. isocitrate dehydrogenase (*IDH*) mutations and codeletions of chromosome arms 1p and 19q (1p/19q co-deletion)) (summarized by Louis et al., 2016). Although the implementation of genetic signatures has led to a better understanding of underlying molecular pathways and more reliable diagnostic criteria, these findings do not fully explain why some LGG patients have far worse courses of disease than others. Recent evidence suggests that DNA methylation profiles might shed light on significantly differing outcomes. Unsupervised cluster analysis of 1122 grade II-III-IV gliomas from The Cancer Genome Atlas (TCGA) identified six methylation groups (LGm1–6) that were in part associated with *IDH* status and further discovered an epigenetic signature that segregated a subgroup of *IDH*-mutant diffuse lower-grade gliomas with unfavorable clinical outcome (Ceccarelli et al., 2016). Mutations in the *IDH1* and *IDH2* genes have previously been identified to lead to a downstream neomorphic enzymatic activity and an accumulation of the onco-metabolite D-2-hydroxyglutarate (D-2HG) in *IDH*-mutant cells (Dang et al., 2009). As D-2HG inhibits key enzymes involved in histone- and DNA-demethylation, excess D-2HG results in DNA hypermethylation. Gliomas harboring *IDH* mutations consequently display a CpG island methylator phenotype (G-CIMP), which is characterized by DNA hypermethylation in CpG-rich domains (Turcan et al., 2012) and has

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been shown to constitute a subset of tumors with a distinct biology and clinical behavior (Noushmehr et al., 2010). These findings emphasize the relevance of epigenetic alterations as an underlying and therapeutically relevant mechanism in glioma.

Gliomas have long been recognized to induce local and systemic immunosuppression, thereby limiting the innate defense against tumor growth (Gousias et al., 2010). Currently emerging immunomodulatory therapies have therefore generated an increasing interest in these novel therapies as potential treatment options for gliomas. Particularly treatments targeting the immune checkpoints programmed cell death 1 receptor (PD-1)/PD-1 ligand 1 (PD-L1) pathway and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) have exhibited dramatic antitumor efficacy in various tumor entities (Chan et al., 2015; Brahmer et al., 2012, 2015; Topalian et al., 2012; Hamid et al., 2013; Wolchok et al., 2013; Larkin et al., 2015; Margolin et al., 2012; Berger et al., 2008; Ribas et al., 2016; Garon et al., 2015). Several clinical trials are currently ongoing to determine the potential of PD-1/PD-L1 and CTLA-4 targeted therapies in high-grade gliomas yielding conflicting results (Omuro et al., 2017; Reardon et al., 2016). Furthermore, several studies have been conducted to determine the prognostic value of PD-L1 in gliomas; however, the results so far have been inconsistent (Xue et al., 2017). The regulation of immune checkpoint genes in glioma, particularly on the epigenetic level, seems to be complex and is only poorly understood. Elucidating the regulatory machinery of immune checkpoints might help to improve patient's treatment, particularly in the view of emerging immunotherapeutic strategies. Recently, inverse correlations between immune checkpoint mRNA levels and promoter methylation indicative of an epigenetic regulation as well as significant associations of immune checkpoint methylation levels with survival have been reported for several hematopoietic and solid neoplasms including acute myeloid leukemia (AML), prostate cancer, colorectal adenocarcinomas, and head and neck squamous cell carcinomas (HNSCC) (Franzen et al., 2018; Gevensleben et al., 2016; Goltz et al., 2016a, 2016b, 2017a, 2017b). However, epigenetic association studies regarding tumors of the central nervous system are lacking so far.

In the present study, we investigated DNA promoter methylation of the immune checkpoints genes *PD-1* (Human Genome Organisation (HUGO) gene symbol: *PDCD1*), *PD-L1* (*CD274*), *PD-L2* (*PDCD1LG2*), and *CTLA-4* (*CTLA4*) in patients with LGG harboring *IDH* mutations with regard to mRNA expression, clinicopathological parameters, previously established methylation subtypes, immune cell infiltrates, and survival.

2. Materials and Methods

2.1. Patients and Clinical Endpoints

The results shown are entirely based on gene methylation data created by the TCGA Research Network (http://cancergenome.nih.gov/). The cohort comprised fresh-frozen tissues from 419 patients with histologically confirmed LGG from several international centres involved in the TCGA project. Clinical, cytological, and mutational data were obtained from the TCGA Research Network. Additional information on methylation subtypes was taken from Ceccarelli et al. (2016). Patients' characteristics are described in detail in Table 1. Overall survival (OS) was defined as time to death or last follow-up. The mean OS was 24.81 months. The TCGA Research Network acquired written informed consent from all participants. All experiments were carried out according to the World Medical Association Declaration of Helsinki.

Table 1
Association of clinicopathological parameters with *PD-L1*, *PD-L2*, *PD-1*, and *CTLA4* promoter methylation in diffuse lower-grade glioma patients ($n = 419$).

Variable	All patients	[%]	Mean *PD-L1* methylation [%]	*p*-Value	Mean *PD-L2* methylation [%]	*p*-Value	Mean *PD-1* methylation [%]	*p*-Value	Mean *CTLA4* methylation [%]	*p*-Value
All patients	419	100.0	36.11		64.49		47.61		91.97	
Gender										
Male	231	55.1	36.56	0.31[c]	64.91	0.57[c]	48.8	0.081[c]	92.31	0.24[c]
Female	187	44.6	35.57		63.88		46.53		91.55	
Unknown	1	0.2								
Age [years]										
Mean	40.87									
Median	39									
≤41 years	245	58.5	35.95	0.94[c]	62.75	0.003[c]	49.71	0.015[c]	91.87	0.43[c]
>41 years	173	41.3	36.36		66.87		45.05		92.12	
WHO classification (2016)										
IDH-mut, 1p/19q-codel	169	40.3	36.43	0.44[c]	74.89	<0.001[c]	44.76	0.002[c]	92.52	0.028[c]
IDH-mut, 1p/19q-non-codel	250	59.7	35.9		57.46		49.96		91.61	
Methylation subgroups[a]										
LGm1	45	10.7	30.59	<0.001[b]	50.57	<0.001[b]	40.48	0.001[b]	88.18	<0.001[b]
LGm2	251	59.9	36.72		60.82		50.06		92.32	
LGm3	123	29.4	36.9		77.06		46.07		92.66	
MGMT promoter status[a]										
Methylated	389	92.84	36.43	<0.001[c]	65.33	<0.001[c]	47.44	0.068[c]	92.12	0.11[c]
Unmethylated	30	7.2	31.9		53.58		53.29		90.06	
ATRX status										
Mutant	181	43.2	36.19	0.79	57.86	<0.001[c]	51.15	0.001[c]	91.71	0.63[c]
Wildtype	238	56.8	36.05		69.53		45.36		92.17	
TERT promoter status										
Mutant	93	22.2	36.94	0.035[c]	73.53	<0.001[c]	45.47	<0.001[c]	93.22	<0.001[c]
Wildtype	143	34.1	36.69		58.19		52.6		92.45	
Unknown	183	43.7								

[a] Data taken from Ceccarelli et al. (2016).
[b] Data taken from Kruskal-Wallis test.
[c] Data taken from Wilcoxon-Mann-Whitney test.

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