Targeting CD38 in the tumor microenvironment: a novel approach to treat glioma

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Glioblastoma multiforme (GBM) is one of the most lethal human cancers, accounting for about 15% of all primary brain tumors in adults. Tumor-associated microglia/macrophages (TMMs) are a major constituent of the tumor mass and the tumor microenvironment where they support tumor progression. We previously demonstrated that the NAD⁺ utilizing ectoenzyme CD38 regulates microglia activation and that loss of CD38 inhibits glioma progression and extends the survival of glioma-bearing mice. These results indicated that targeting CD38 in the tumor microenvironment may serve as a novel therapeutic approach to treat glioma. To test this hypothesis, we identified small molecules that inhibit CD38 enzymatic activity (NAD⁺ glycohydrolase): the natural anthranoid rhein, its water-soluble tri-potassium salt (K-rhein), and the polyphenol tannic acid (TA). Microglial properties regulated by CD38 (e.g., NO secretion and LPS/IFN-γ activation induced cell death) were inhibited in primary microglia treated with rhein in a CD38-dependent manner. Furthermore, wild-type mice intracranially injected with GL261 mouse glioma cells and intranasally treated with K-rhein or TA, exhibited significant reduction in tumor volume and prolonged life-span compared to vehicle treated mice. On the other hand, these inhibitors had only a modest effect on tumor-bearing Cd38−/− mice. Taken together, our results demonstrate that small molecule CD38 inhibitors such as K-rhein and TA can target CD38 in the tumor microenvironment and offer a novel and useful strategy for glioma treatment.

Keywords: CD38; microglia; tumor microenvironment; tannic acid; K-rhein; brain tumors


Glioma is the most common primary brain tumor in adults and its aggressive form, Glioblastoma multiforme (GBM), is one of the most lethal human cancers [1]. Worldwide, in developed countries, an estimated 3.5 GBM cases per 100,000 people are diagnosed per year [2]. GBM is not a surgically curable disease; tumor cells invade the surrounding brain, rendering complete surgical resection virtually impossible. Furthermore, GBM tumors are among the most resistant to radiation and cytotoxic chemotherapy [1, 3]. Thus, GBM patients survive, on average, 12-15 months despite aggressive surgical resection and conventional therapy [4]. New treatment approaches are desperately needed.

It is now well established that the tumor microenvironment supports glioma progression [6-11]. Thus the ineffectiveness of the current treatments may result, at least in part, from their inability to suppress the tumor microenvironment’s supportive effect. Notably, the microenvironmental cells are genetically stable in contrast to
the tumor cells, which are very heterogeneous. Therefore, targeting glioma microenvironment is a promising useful approach for glioma treatment, which is expected to attenuate glioma progression in a broad spectrum of gliomas regardless of the heterogeneity of the tumor cells. Moreover, such treatment can be used in combination with other approaches targeting the tumor cells. This, in turn, is expected to cause a synergistic beneficial effect since each treatment acts through a different pathway.

The cells that mainly contribute to the supportive effect of the glioma microenvironment on glioma progression are tumor-associated microglia/macrophages (TMMs) [5-8]. Tumor microglia originate from resident brain microglia, while tumor-associated macrophages arise from infiltrating monocytes. TMMs invade the tumor mass and comprise the most common cells in the tumor’s microenvironment. The amount of TMMs in the tumor positively correlates with glioma grade and invasiveness [9-11]. Therefore, targeting the glioma supportive effect of TMM in the tumor microenvironment can serve as a novel methodology to treat glioma. This can be done by targeting CD38, an ectoenzyme that regulates microglia/macrophage activation [12].

In this Research Highlight we summarize our results showing that CD38 is indeed a good target for glioma treatment, emphasizing on the effect of newly discovered CD38 inhibitors on glioma progression using a mouse model for glioma progression.
CD38 is an ectoenzyme which utilizes nicotinamide adenine dinucleotide (NAD$^+$) and nicotinamide adenine dinucleotide phosphate (NADP$^+$) for the production of the calcium mobilizing metabolites: adenosine diphosphate ribose (ADPR), cyclic ADPR, and nicotinic acid adenine dinucleotide phosphate (NAADP) [13]. Among the cell types

Figure 2. TA decreases tumor volume and prolongs lifespan of glioma-bearing mice. WT (n = 24) and Cd38$^{-/-}$ (n = 14) male mice were injected with GL261 cells and treated with vehicle (H$_2$O) (n = 13 and 7 for WT and Cd38$^{-/-}$, respectively) or TA (n = 11 and 7 for WT and Cd38$^{-/-}$ respectively). At 17 and 22 days post-tumor cells injection, the brains of these mice were scanned by CT. CT images of representative WT (A) and Cd38$^{-/-}$ (B) mice treated with vehicle (upper panel) or TA (lower panel) taken at 22 days post-injection are shown. (C-D) Quantification of the tumor volumes of vehicle- or TA-treated WT (C) and Cd38$^{-/-}$ (D) mice. Results are presented as mean ± S.E.M (bars). (E-F) Kaplan-Meier survival curves of the same WT (E) or Cd38$^{-/-}$ (F) mice treated with vehicle or TA.
which express CD38 are myeloid-derived cells such as microglia, in which we showed that their activation is regulated both in vitro and in vivo by CD38 [12, 14]. To examine the effect of CD38 targeting on glioma progression we first used genetic targeting of CD38 [CD38 deficient mice (Cd38−/−)] and a mouse model for glioma progression. In these experiments wild-type (WT) or Cd38−/− C57BL/6 mice were intracranially injected with the syngeneic GL261 glioma cells. The results showed that loss of CD38 in the tumor microenvironment reduced tumor size and extended the life span of glioma-bearing mice [15].

CD38 targeting inhibits glioma progression at least partially by inhibiting the tumor-supporting features of TMMs. Accordingly, CD38 is expressed in TMMs and its expression increases in these cells in the context of a tumor. Moreover, CD38 deficiency does not affect the amount of TMMs in the tumor, but rather alters their properties, as indicated by the reduced expression of the microglia/macrophages activation marker F4/80 as well as of matrix metalloproteinases (e.g., MMP-12 and MMP-13) [15]. Taken together these findings suggest that the inhibition of CD38 enzymatic activity can mediate the effect of loss of CD38 and thus that small molecule inhibitors of CD38 enzymatic activity could represent a useful therapeutic approach to fight glioma.

Previously, a potent CD38 inhibitor, AraF-NAD+, was described [16]. However, its complex synthesis and sensitivity to degradation under physiological conditions limit its use in vivo. Flavonoids such as luteolin, luteolinidin, and apigenin were reported recently to inhibit CD38 [17, 18]. However, the poor water solubility of these compounds limits their use as effective drugs. Recently, we reported that 4, 5-dihydroxyanthraquinone-2-carboxylic acid (rhein) and its water-soluble derivate, K-rhein, are low micromolar noncompetitive CD38 inhibitors. Rhein is a natural anthranoid produced by herbs such as *Rheum officinale* and is commonly used in traditional Chinese medicine [19]. Notably, rhein and K-rhein inhibited microglial activation features [activation induced cell death (AICD) and NO production] previously shown to be regulated by CD38 [12] and microglia migration [20].

Moreover, treatment of WT mice with K-rhein suppressed GL261 glioma progression and prolonged the lifespan of the glioma-bearing mice [20]. Notably, rhein and K-rhein have also CD38-independent activities, some of which can target the tumor cells. Accordingly, these compounds inhibited GL261 cell viability and migration in a CD38-independent manner. In addition, the effect of K-rhein treatment on glioma progression was stronger than CD38 deficiency. However, although the effect of K-rhein on tumor progression can be mediated also by CD38-independent pathway(s), the major action of K-rhein on glioma progression is CD38 dependent since the effect of this compound in Cd38−/− was substantially lower than its effect in WT mice.

An additional compound that can act as a small molecule CD38 inhibitor in glioma treatment is tannic acid (TA) a polyphenolic compound (Fig. 1A) produced by food plants and extensively used as industrial food additives [21]. TA administration was shown to suppress several cancer types in different murine models [22–24]. TA was identified as a novel CD38 inhibitor by screening of water-soluble hetero-aromatic compounds using the Ba/F3 cell-based system in which CD38 enzymatic activity is measured by accumulation of the ε-NAD+ substrate, ε-ADPR. Ba/F3[CD38], but not the parental CD38neg control cells, hydrolyze ε-NAD+ and generate the fluorescent product ε-ADPR [25]. Incubation of Ba/F3[CD38] cells with 5, 10, and 50 µM of TA inhibited ε-ADPR formation in a dose-dependent manner, in which 50 µM TA almost completely eliminated production of ε-ADPR (Fig. 1B). In order to characterize the inhibition mechanism of TA we used recombinant mouse CD38. The enzyme was incubated with ε-NAD+ in the presence of increasing concentrations of TA and ε-ADPR generation was monitored. TA inhibited ε-ADPR production with an IC50 value of 33.8 ± 8.9 nM and a Ki value of 16.0 ± 3.0 nM (Fig. 1C, Table 1). Notably, this inhibitory effect of TA was substantially stronger than that of K-rhein [IC50 value of 840 ± 110 nM and Ki value of 420 ± 55 nM [20] (Table 1)]. Kinetic analysis revealed that in contrast to rhein and K-rhein, which act as noncompetitive inhibitors, TA inhibits CD38 in a competitive manner (Fig. 1D). We next examined the effect of TA on glioma progression in vivo using the syngeneic GL261 model of glioma progression. WT mice were nasally treated with TA (0.25 mg/10 µL, equivalent to 10 mg/kg) or with vehicle. The mice were treated with TA or vehicle 24 h prior to GL261 cells injection followed by two days interval of the treatments. Similar experiments were conducted on Cd38−/− mice to evaluate the dependence of the effect of TA on glioma progression on CD38. CT scanning was used to monitor tumor volume at 17 and 22 days after tumor cells injection. Fig. 2A and B (CT images) and Fig. 2C

<p>| Table 1: IC50 values and modes of inhibition for CD38 inhibitors |
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<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC50 (nM)</th>
<th>Ki (nM)</th>
<th>Mode of Inhibition</th>
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<tbody>
<tr>
<td>Rhein</td>
<td>1.24 ± 0.10</td>
<td>620 ± 50</td>
<td>Uncompetitive</td>
</tr>
<tr>
<td>K-rhein</td>
<td>840 ± 110</td>
<td>420 ± 55</td>
<td>Uncompetitive</td>
</tr>
<tr>
<td>Luteolin</td>
<td>97.6 ± 6.4</td>
<td>48.8 ± 3.2</td>
<td>Uncompetitive</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>33.8 ± 5.9</td>
<td>16.0 ± 3.0</td>
<td>Competitive</td>
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and D (quantification) show the tumor volume in the TA-untreated and TA-treated WT and Cd38<sup>-/-</sup> mice, respectively. The results show that at days 17 and 22, TA inhibited tumor volume by 68% and 72%, respectively in WT mice (significant effect for treatment, repeated measures ANOVA, p = 0.0002) whereas in Cd38<sup>-/-</sup> mice no significant effect was found for the treatment. Kaplan-Meier survival analysis (Fig. 3E) indicated that TA also significantly improved the survival of the tumor-bearing WT mice (median survival of TA-treated WT mice was 28 vs 23 days of vehicle-treated WT mice, p = 0.0004). On the other hand, in glioma-bearing Cd38<sup>-/-</sup> mice, TA did not have a significant effect on the lifespan of the tumor-bearing mice (Fig. 2F). Collectively these results demonstrate that TA attenuates the progression of glioma and that the effect results mainly from the inhibition of CD38 activity.

Previous studies showed that TA exerts various biological effects including anti-inflammatory [26], antiviral [27], antibacterial [28] and anticancer [29]. The latter can be mediated by inducing apoptosis [30]. TA also has anti-oxidant activity and acts as a scavenger of reactive free radicals [31]. Moreover, a TA-based medical food supplement is currently in clinical trials for the treatment of diarrhea, in which it was demonstrated to be safe and effective [32].

Our findings demonstrated that both TA and K-rhein act as low micromolar inhibitors of CD38. Furthermore, like genetic targeting of CD38 they inhibited glioma progression and prolong the lifespan of the glioma-bearing mice. This study therefore provides experimental evidence supporting our hypothesis that the inhibition of CD38 in the tumor microenvironment can be used as a new therapeutic approach for glioma treatment.

Conflicting interests

The authors have declared that no competing interests exist.

References


