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Reprogramming immunosuppressive tumour-associated dendritic cells with GADD45β inhibitors

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Introduction

The ability of dendritic cells to present tumour antigens efficiently to cytotoxic T-cells has led to a continuous focus on exploiting their unique stimulatory abilities in therapeutic cancer vaccinations.[1] However, existing clinical strategies utilising dendritic cells have failed to induce durable responses.[2] The fact that a dominant immunosuppressive tumour microenvironment (TME) often results in dendritic cells adopting a paralysed or an immunosuppressed phenotype outlines the need to increase the immunogenicity of the TME.[3,4] The role of dysregulated NF-κB signalling has additionally been implicated in various human malignancies, with its effector molecule, GADD45β, shown to suppress pro-inflammatory activation of tumour-associated macrophages.[5] However, as the role of GADD45β in dendritic cells remain unknown, the aim of this study was to investigate whether the immunosuppressive function of GADD45β extends to dendritic cells, and whether inhibiting GADD45β could reprogramme dendritic cells to the pro-inflammatory phenotype.

Materials and methods

Bone marrow-derived dendritic cells (BMDCs) were obtained and pooled together from seven GADD45β$^{-/-}$ mice and seven GADD45β$^{+/+}$ mice. BMDCs were then treated with the inflammatory agents LPS/IFN-γ, followed by the collection of cell lysates and RNA. Western blot techniques were performed to assess the activation of the pro-inflammatory MAPK and STAT1 signalling pathways. The expression of pro-inflammatory genes was additionally measured via quantitative reverse transcription (qRT)-polymerase chain reaction (PCR). The pharmacological relevance of targeting GADD45β in dendritic cells was also performed on the immortal dendritic cell line JAWS II. Western blotting and quantitative PCR techniques were used to assess pro-inflammatory JAWS II activation following a specific GADD45β inhibitor, DTP3, and LPS/IFN-γ co-treatment.

Results and discussion

Western blotting analysis revealed that BMDCs from GADD45β$^{-/-}$ mice showed an augmented p38 signalling phosphorylation compared with their GADD45β$^{+/+}$ counterparts. This indicates the role of GADD45β in suppressing the pro-inflammatory p38–MAPK signalling pathway. GADD45β ablation also corresponded with the upregulation of pro-inflammatory genes, such as *IL-1β*, compared with GADD45β$^{+/+}$ mice (relative messenger ribonucleic acid (mRNA) 375.58 (GADD45β$^{+/+}$; n=1); 1,730.18 (GADD45β$^{-/-}$; n=1)). The activation marker *MHC II* was also upregulated in GADD45β$^{-/-}$ mice compared with GADD45β$^{+/+}$ mice (relative mRNA 369.74 (GADD45β$^{+/+}$; n=1); 640.56 (GADD45β$^{-/-}$; n=1)). This indicates a heightened pro-inflammatory activation state of dendritic cells. Given the established role of GADD45β in macrophages, this potentially recognises GADD45β as an innate-immune checkpoint across cells of the myeloid lineage. Western blot analysis of JAWS II cells treated with DTP3 showed enhanced p38–MAPK signalling compared with untreated control. This corresponded with increased expression of the pro-inflammatory *IL-1β* gene (relative mRNA 1,520.05 (control; n=2); 4,565.27 (treated; n=2)). *MHC II* expression was additionally upregulated relative to untreated control (relative mRNA 29.57 (control; n=2); 39.87 (treated; n=2)). Altogether, this indicates the ability of DTP3 to phenocopy the effects of GADD45β ablation.

Conclusion

These findings highlight the role of the NF-κB-regulated protein GADD45β in suppressing the pro-inflammatory p38 pathway in dendritic cells. Additionally, with the ability of DTP3 to induce pro-inflammatory activation, it indicates the potential capacity to reprogramme dendritic cells from a TME-induced immunosuppressive state to an anti-tumour phenotype. This potentially highlights a new avenue of targeted therapeutics, to increase the likelihood of eliminating even refractory cancers. ■

Conflicts of interest

None declared.

References

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