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Background Protein Kinase A (PKA) is a heterotrameric holoenzyme that consists of two regulatory and catalytic subunits. During T cell activation, one of the regulatory subunits (PRKAR1A) localizes to the immune synapse, inhibiting several central proteins in the T-cell signalling cascade and leading to T cell inactivation. Previously, the disruption of localisation of PKA type I R1α (PRKAR1A) to the immune synapse using disruptor peptides has been shown to improve chimeric antigen receptor (CAR) T cell function.1 2 However, the effect of PRKAR1A knockdown in T cells (including CAR T cells) has not been studied yet. In this study, we have utilized microRNAs (miR); miR96/183 or miR155 to knockdown PRKAR1A and explored the advantages of PRKAR1A knockdown on T cell activation and function.

Materials and Methods MicroRNAs (miR); miR96/183 or miR155 were cloned from human genomic DNA into a sleeping beauty vector under a doxycycline inducible promoter (TCE). Overexpression of miRNA and target knockdown was assessed at both transcript level (by real time RT-PCR) and/or protein level (by western blot) respectively while target validation was done by luciferase assay. The fate of PRKAR1A knockdown on Jurkat T cells activated with anti-CD3 and anti-CD28 antibodies were determined by measuring IL-2 production (ELISA) and CD69 surface expression (flow cytometry). The effect of miR96/183 or miR155 overexpression in primary T cells expressing HER2-CAR were also compared.

Results We efficiently overexpressed both miRNAs and downregulated PRKAR1A expression in HEK293 cells at both mRNA and protein level. Luciferase assay confirmed miRNA mediated specific knockdown of PRKAR1A; mutated 3’UTR of PRKAR1A was used as negative control. Overexpression of miRNAs also downregulated PRKAR1A expression in Jurkat cells which resulted in enhanced expression (CD69 expression) and IL-2 production following anti-CD3/CD28 stimulation compared to untransfected controls (with normal PRKAR1A expression). Additionally, miRNA 96/183 and miRNA155 were found to target inhibitory proteins of TCR signalling such as CTLA4, Foxo3 and ptpn2 and resulted in superior T cell function. A third-generation lentiviral system has been optimised to express either miR96/183 or miR155 and HER2-CAR in the same vector and currently we are assessing the effect of PRKAR1A knockdown on primary CAR T cells.

Conclusions Overexpressing miRNA for knockdown of inhibitory proteins could be an efficient way of enhancing T cell function against solid tumours. Additionally, co-expressing CAR and miRNAs using lentiviral system would benefit such approaches for cancer immunotherapy.

REFERENCES

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