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Development of a Novel Anti-SSTR Bispecific T-Cell Engager (BiTE)-like Molecule for the Treatment of Neuroendocrine Tumors

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BACKGROUND

Well-differentiated neuroendocrine tumors (NETs) are characterized by the overexpression of somatostatin receptors (SSTRs). To efficiently engage and activate tumor infiltrating lymphocytes against NET cells, we designed a novel bispecific T-cells engager (BiTE) composed of 2 molecules of somatostatin-14 (SST14), the hormone that physiologically binds SSTRs, linked with a single chain variable fragment (scFV)-based anti-CD3.

METHODS

The optimized sequence of the BiTE was subcloned into a vector (pAcGP67a) designed for protein expression in insect cells using Baculovirus. Trichoplusia-ni (High Five) cells were used to express the recombinant protein, which was isolated from the supernatant using nickel affinity chromatography. Flow cytometry and confocal microscopy were used to determine the binding potential of the BiTE towards CD3 and SSTR2. CD3+ T cells isolated from the peripheral blood of healthy donors were co-incubated with 293T cells stably transduced to concurrently express SSTR2 and green fluorescent protein (GFP) in the absence or presence of the BiTE. The SSTR2- parental 293T cell line was used as negative control, while anti-CD3/CD28 beads were added as a positive control. The BiTE-induced T cell activation was evaluated measuring the secretion of IFNγ and granzyme B by ELISA and OX40, 41BB and CD69 by flow cytometry.

RESULTS

At a concentration of 100 nM, the BiTE bound the CD3 receptor of approximately 85% of T cells. By confocal microscopy, the BiTE was found to coat SSTR2+ 293T cells. IFN-γ secretion was significantly higher when the T cells were co-cultured with SSTR+ 293T cells in the presence of the BiTE as compared with parallel preparations with SSTR- 293T cells or without the BiTE, suggesting that the BiTE-induced T cell activation is specific. At high concentration of BiTE, OX40, CD69 and 41BB on T cells were upregulated regardless of the presence of target cells.

However, at the same concentration, the granzyme B concentration increased only in presence of SSTR+ target cells.

CONCLUSIONS

To our knowledge, this is the first BiTE to incorporate a hormone in one binding site. Its non-antibody-like structure efficiently engaged SSTR2 and T cells enabling the formation of immune synapsis.

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