RECOMBINANT POLYPEPTIDES FOR REGULATABLE CELLULAR LOCALIZATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 62/914,310, filed October 11, 2019, which application is incorporated herein by reference in its entirety.

SUMMARY

Provided are recombinant polypeptides that comprise a protein of interest, a protein localization tag, and a protease cleavage site disposed between the protein of interest and the protein localization tag. In certain embodiments, the recombinant polypeptides further comprise a protease, where the protease cleavage site is a cleavage site for the protease. Also provided are nucleic acids that encode the recombinant polypeptides, cells that comprise such nucleic acids, and compositions (e.g., pharmaceutical compositions) that comprise such cells. Methods of regulating cellular localization of a protein of interest and methods of administering a regulatable cell-based therapy to an individual in need thereof are also provided.

In one aspect, provided herein is a recombinant polypeptide comprising: a protein of interest, wherein the protein of interest is a chimeric antigen receptor (CAR) or a T cell receptor (TCR); a protein localization tag; and a protease cleavage site disposed between the protein of interest and the protein localization tag.

In one aspect, provided herein is a nucleic acid or expression vector encoding the recombinant polypeptide as described herein.

In one aspect, provided herein is a cell comprising the nucleic acid or the expression vector as described herein.

In one aspect, provided herein is a cell comprising a nucleic acid encoding the recombinant polypeptide as described herein or an expression vector encoding the recombinant polypeptide as described herein, wherein the recombinant polypeptide does not comprise a protease, and wherein the cell further comprises a nucleic acid encoding a protease, wherein the protease cleavage site is a cleavage site for the protease.

In one aspect, provided herein is a method of regulating cellular localization of a protein of interest, comprising: contacting the cell as described herein with an inhibitor of the protease when retention of the protein of interest at the cellular compartment determined by the protein localization tag is desired.

In one aspect, provided herein is a pharmaceutical composition comprising: the cell as described herein; and a pharmaceutically acceptable carrier.

In one aspect, provided herein is a method of administering a regulatable cell-based therapy to an individual in need thereof, comprising administering to the individual the pharmaceutical composition as described herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: **Panel A**: A schematic illustration of a recombinant polypeptide according to an embodiment of the present disclosure. The recombinant polypeptide comprises a protein of interest, a protease cleavage site ("cleavage site"), and a protein localization tag (referred to in FIG. 1 as a "retention signal"). In this example, the protein of interest is a chimeric antigen receptor (CAR). Also in this example, the recombinant polypeptide further comprises a protease, where the protease cleavage site is a cleavage site for the protease incorporated in the recombinant polypeptide. The combination of the protease cleavage site and protein localization tag (and if present, a protease that cleaves the protease cleavage site) are sometimes referred to herein as a "STASH tag", because the recombinant construct is designed for intracellular **s**torage by **ta**rgeted **sh**uttling. **Panel B**: Schematic illustration of regulatable cellular localization of the CAR of the recombinant polypeptide shown in panel A. In this example, the protein localization tag is an endoplasmic reticulum (ER) localization tag that directs localization of the recombinant polypeptide to the ER. In the absence of an inhibitor of the protease ("-drug", left), the protease cleaves the protease cleavage site, thereby removing the ER localization tag from the CAR, and enabling expression (and activity) of the CAR on the surface of the cell. In