Artificial internalizing receptors for mammalian cells

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Receptor-mediated endocytosis is one of the core cellular functions and is often exploited for targeted drug delivery. The natural presence of cell surface receptors enables the action of antibody-drug conjugates (ADCs), a highly successful modality of drug delivery. However, targeting natural receptors is often associated with a high risk of off-targets and side effects. We therefore try to develop new dedicated artificial communication routes, which could be essential in the improvement of current cell-based therapies e.g. CAR T cell therapy. Inspired by Nature, we have designed chemical, artificial internalizing cell receptors that mimic the natural process of receptor-mediated endocytosis. The chemical receptor design includes a cholesterol amine anchor that enables association with the phospholipid membrane of mammalian cells, and a spacer moiety, which separates the anchor from the crucial recognition motif, fluorescein (Figure 1A). This xenobiotic, fluorescent moiety supports the visualization of the artificial receptor and enables extracellular targeting with a cognate anti-fluorescein antibody (Figure 1C). Targeting of the artificial receptor in primary human T-cells using a Monomethyl auristatin E (MMAE)-based ADC was proved to be of nanomolar potency (Figure 1B, D). Furthermore, the eradication of a receptor-equipped 3D cell spheroid was achieved. This emphasizes the ability to use our artificial receptor in tumour-infiltrating engineered cells, where on-demand deactivation would not only lead to the killing of the equipped cell but also of the surrounding cancerous tissue, by the inherent bystander effect of the released drug (MMAE) [1]. In new unpublished data, we made significant improvements in the receptor-mediated ADC delivery by demonstrating enhanced potency relative to the free drug (70-fold), increased selectivity by elimination of the bystander effect, significantly faster action, and long sustainability of the receptor. These improvements increase the applicability of using artificial receptors in the design of a selective communication route only to the pre-engineered cells, such as a functional “suicide switch” installed in CAR T cells in case unwanted immunological responses or side effects arise. Additionally, we work on investigating the scope and potential limits in terms of cargo that can be internalized using artificial receptors. To do so, we inverted the design and equipped the anti-fluorescein antibody itself with an anchor moiety. This allowed easy incorporation into mammalian cells, with both fluorescein-labelled antibodies and serum albumin being successfully internalized. This highlights that the internalization of cargo is potentially only limited by the ability to be labelled with the commonly used fluorescein moiety. Our artificial fluorescein-based recognition system illustrates how the use of xenobiotics can overcome the problems faced when targeted drug delivery is based on naturally occurring antigens. Furthermore, our designs demonstrate how artificial receptors can be very useful tools with many future applications within important areas of biomedicine and biotechnology.

References

[1] Monge, P., et al., Advanced Science, 7 (2020).

[2] Monge, P., et al., Adv. Drug Deliv. Rev, 170 (2021), 281-293.

Figures

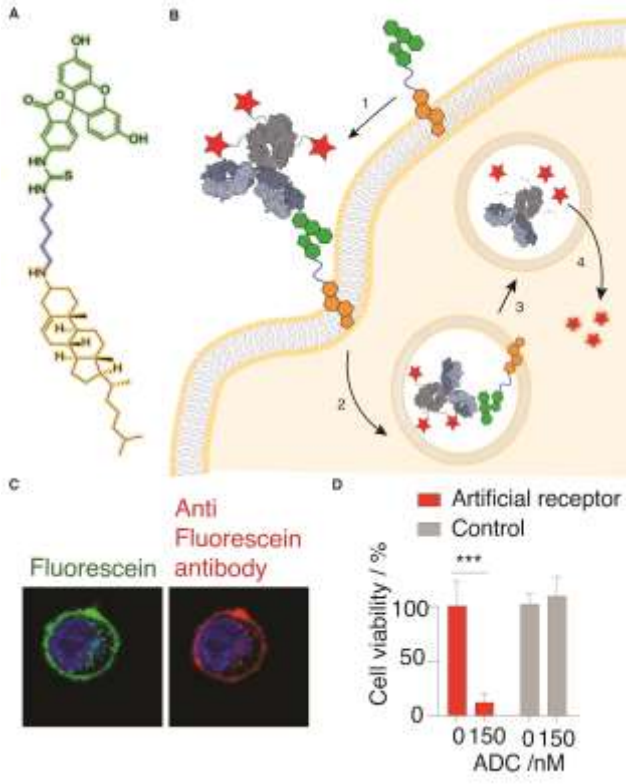

Figure 1. Artificial internalizing receptors [1].
A. Structure of the artificial receptor including the anchor (orange), spacer (purple), and recognition motif (green). **B.** Illustration of the artificial receptor-mediated internalization mechanism. 1) Binding of the antibody-drug conjugate to the artificial receptor 2) Internalization and pH-dependent cargo dissociation 3) lysosomal degradation 4) drug release. **C.** Nanomolar potency of the antibody-MMAE conjugate in artificial receptor-equipped primary human T cells.