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Smart Cell-Specific Protein Therapeutics for Head and Neck Cancer

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Direct protein delivery is a safer alternative to gene therapy vectors as rapid, robust, and short-term protein expression can be achieved without the accompanying risk of insertional mutagenesis. Cell-penetrating peptides (CPPs) are a diverse class of peptides that have been derived from viral proteins or developed synthetically. These peptides can readily translocate into live cells in vitro and in vivo, and when fused to proteins, they are an invaluable tool for protein internalization and manipulation of intracellular protein levels. The arginine and lysine residue-rich HIV TAT transduction peptide (47–57 aa) has been widely used because of its superior ability to deliver a variety of payloads across the cell membranes. Close proximity of the cationic peptide to the negatively-charged cell membrane induces membrane deformation and cytoskeletal remodeling, which facilitates intracellular uptake of attached biological macromolecules by macropinocytosis (Mishra et al., 2010). Cellular entry of CPP-fusion proteins however, is not cell-specific (Figure 1A), and targeted delivery remains a major challenge. Engineering cell-specificity in protein delivery can greatly minimize the unwanted effects in non-targeted cells.

Human Torsionless-like kinase 1 (TLK1) functions in chromatin remodeling, regulation of cell cycle and repair of genomic breaks (Sunavala-Dossabhoy et al., 2018). Preemptive delivery of TAT-TLK1 fusion protein to salivary cells in culture or salivary glands in vivo was shown to prevent radiation-associated loss of cell viability and function (Sunavala-Dossabhoy et al., 2012). In an attempt to improve therapeutic safety especially, in context of cancer care, Nair et al. (2019) tailored the protein to sense biological signals and achieve cell-selective protein transduction. A majority of head and neck cancers express matrix-metalloproteinases (MMP) in abundance (Rosenthal and Matrisian 2006) The biochemical rationale was, therefore, to introduce a MMP-sensitive peptide linker (MS) between TAT peptide and TLK1 protein such that extracellular cleavage in response to pathological levels of MMP in the cancer environment curtails TLK1 entry in cancer cells. The molecular targeting strategy achieves protein delivery specifically to healthy cells (Figure 1B).

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TLK1 and TLK2 share 84% sequence identity at the amino acid level, and they homo- or heterodimerize through coiled-coiled domains in the N-terminal regulatory regions to achieve full activation through autophosphorylation. By exploiting the structural and functional characteristics of TLK1, manipulation of a MMP-response linker between the regulatory and kinase domains results in protease-dependent release of the regulatory region in cancer cells and their sensitization to radiation (Figure 1b). Using protein engineering, a smart dual-purpose protein therapeutic was developed to protect healthy cells and in addition, sensitize cancer cells to radiation.

Protein engineering provides greater control of protein delivery and dynamics. Balancing therapeutic safety and efficacy has always been a challenge in medicine. Antibody-drug conjugate systems and controlled-release drug systems have been developed to increase the therapeutic safety index. With the development of sensor-based smart protein therapeutics that integrate biological inputs for targeted release or activation/inactivation, cell-selectivity with fewer off-target effects can be realized along with a multifold increase in therapeutic ratio. The unique protein expression system has immense therapeutic potential as the biological signal-sensing concept can be readily applied to other protein therapeutics in the treatment of head and neck diseases and disorders.

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Figure 1.

A. Transduction of TAT- β -galactosidase reporter protein in human OKF6 oral keratinocytes (Opitz et al. 2001), NS-SV-AC salivary acinar cells (Azuma et al. 1993), and FaDu HNSCC (ATCC). Fusion protein (10 μ g) was layered on cells and after 4 h, cells were washed, fixed, and reacted with X-gal. **B.** A schematic display of MMP-sensitive TAT-TLK1 transduction in tumor and healthy cells. Cleavage of the protein in a MMP-rich cancer milieu results in cellular entry of TAT or a kinase-devoid TLK1. The latter dimerizes and sequesters endogenous TLK1 in tumor cells resulting in sensitization to genotoxic stress. On the other hand, entry of full-length, functional TLK1 radioprotects healthy cells. *Color scheme: TAT (black); MMP-sensitive peptide (yellow); N-terminal TLK1 (solid blue); C-terminal catalytic domain of TLK1 (patterned blue).*