DIVISION OF BIOCHEMICAL TECHNOLOGY OF THE AMERICAN CHEMICAL SOCIETY

BIOT

The Best of BIOT Awards: September 25, 2018

Area	Date	Time	Presenter	Institution
Emerging Technology	Tuesday, January 15th	12:00-12:30 PM	**Lieser, Rachel**	University of Delaware
		Controlled EGFR ligand display on cancer suicide enzymes for targeted intracellular delivery		
		12.30 -1:00 PM	**Madsen, Sean**	Tulane University
		Decoy TRAIL receptor CD264: A predictor of in vitro regenerative potential for mesenchymal stem cells		

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Controlled EGFR Ligand Display on Cancer Suicide Enzymes for Targeted Intracellular Delivery

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Proteins have emerged as a new class of therapeutics in the past several decades due to breakthroughs in molecular engineering that allow researchers to customize proteins for a multitude of clinical applications. However, despite great interest and investment in advanced protein therapeutics, most FDA-approved proteins have extracellular targets, despite the existence of multiple diseases that could be treated through intracellular protein therapies. Engineering efforts to address delivery limitations often rely on modifying proteins through direct conjugation of polymers and peptides using reactive residues on naturally occurring amino acids. The key shortcoming of this method is the inability to modify a specific site within a protein, which can significantly reduce pharmacological action. Additionally, such approaches do not offer control over variables such as ligand clustering, which is an important determinant of targeting efficacy.

Unnatural amino acid (UAA) incorporation provides a method to modify proteins with site-specific, biorthogonal reactive moieties through nonsense codon replacement. This method of modification not only allows site-specific functionalization of therapeutics for improved bioactivity, but also allows exploration of the arrangement of delivery molecules on proteins for optimal intracellular delivery. Previous work has demonstrated the ability to incorporate biorthogonal chemistries into model proteins through UAA incorporation, enabling modification with simple ‘click’ chemistry techniques. In our work, we demonstrate application of this approach for conjugation of epidermal growth factor receptor (EGFR) targeting peptides in fluorescent proteins, using varying EGFR peptide arrangements to control cellular internalization in inflammatory breast cancer (IBC) cells. Furthermore, we demonstrate the ability to adapt this system for delivery of a suicide enzyme to enable IBC-targeted cell death through prodrug application and conversion. Through this approach, we have identified the importance of ligand display for targeted protein delivery and applied these finding to enhance enzyme delivery to IBC cells. Future work will refine the efficacy of the approach via incorporation of endosomal escaping peptides and hydrophilic polymers, enabling tailorable intracellular protein delivery *in vivo*.

Decoy TRAIL Receptor CD264: A Predictor of in Vitro Regenerative Potential for Mesenchymal Stem Cells

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The regenerative potential of marrow-derived mesenchymal stem cells (MSCs) exhibits significant variation, particularly among older patients. The objective of this study is to identify a cell-surface marker whose expression is predictive of the in vitro proliferation and differentiation potential of MSCs. This study evaluates surface expression of decoy TRAIL receptor CD264, in vitro regenerative potential and metrics of cellular aging for marrow MSCs from 12 donors, 20-60 years old. Male and female donors were age-matched. When CD264(+) cell content was 20% to 35%, MSC cultures from young (20 < age < 40) and older (45 < age < 60) donors proliferated rapidly and differentiated extensively. Older donor MSCs containing < 35% CD264(+) cells had a small size and negligible senescence despite the donor's advanced chronological age. Above the 35% threshold, CD264 expression inversely correlated with proliferation and differentiation potential. When CD264(+) cell content was 75%, MSCs were enlarged and mostly senescent with severely compromised regenerative potential. There was no correlation of the older donors' chronological age to either CD264(+) cell content or the regenerative potential of the donor MSCs. CD264 was upregulated after p53 and had a similar expression profile to that of p21 during serial passage of MSCs. No sex-linked differences were detected in this study. The strong inverse correlation of CD264(+) cell content to the in vitro regenerative potential of MSCs has possible application to predict the therapeutic potential of patient MSCs, and to standardize the composition and efficacy of MSC therapies.