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Authors

Navarro, Mariana
Tedeschi, Giulia
Scipioni, Lorenzo
et al.

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Abstract 2708

Imaging Macrophage Polarization with Bioluminescent Reporters and Spectral Phasor Analysis

Mariana Navarro, *University of California-Irvine*

Giulia Tedeschi, Lorenzo Scipioni, Tanvi Sondhi, Michelle Digman, Jennifer Prescher

Bioluminescence spectral phasor analysis macrophage polarization gene expression reporters BRET.

Macrophages are intricately involved in tissue development and repair. These cells exhibit a spectrum of behaviors upon activation, and are generally classified as one of two types: inflammatory (M1) or anti-inflammatory (M2). M1 macrophages are typically associated with pathogen killing, while M2 macrophages play central roles in tissue healing and growth. A complete understanding of these phenotypes, along with intermediate behaviors along the M1-M2 axis, requires methods to track dynamic macrophage function. Imaging tools have much to contribute in this regard, and several macrophage reporter cells have been developed. However, many existing cell lines are limited to either *in vitro* model systems or tracking a single marker over time. We are developing bioluminescent macrophage reporters to enable multiplexed imaging *in vivo* over time. Bioluminescence involves light production from luciferase enzymes and luciferin small molecules and is advantageous for serial studies because no excitation light is required. Bioluminescence has historically been limited, though, to tracking single biological features over time due to a lack of distinguishable reporters. Bioluminescent probes generally have large overlapping spectra, preventing facile identification. To address this limitation, we developed bioluminescence resonance energy transfer (BRET) based reporters for imaging macrophage polarization. BRET reporters provide a multicolor readout via fusion of luciferase enzymes to various fluorescent protein acceptors. M1- and M2- specific promoters were used to drive the expression of various BRET reporters in macrophage cell lines. Further resolution was obtained via spectral phasor analysis. This approach transforms the emission data collected in each pixel into spectral phasors. With BRET-based reporters and spectral phasor analysis, we monitored the expression of multiple reporters over time in RAW 264.7 and THP-1 macrophages. Changes in reporter expression were analyzed upon macrophage stimulation with various cytokines. Spectral phasor analysis could readily assign the distinct signals, enabling single-cell imaging of macrophage polarization over time. Ongoing work focuses on expanding the number of M1- and M2- specific reporters in the cell lines and applying the reporter cells in various *in vivo* models. Overall, this work broadens the scope of tools for monitoring macrophage polarization and efforts to dial in desired immune cell behaviors.

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