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INTRINSIC ACTIVITY

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MEETING ABSTRACT

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TRPC6 photopharmacology enables therapeutic modulation of the mast-cell signaling signature

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Background: Mast cells release a wide range of potent inflammatory mediators that give rise to these immune cells' complex role within the tumor microenvironment. Release of pre-stored mediators is likely to promote tumor progression, while nuclear factor of activated T cells (NFAT) transcriptional activation in mast cells leads to the *de novo* synthesis of potentially beneficial, antitumor mediators. Mast-cell degranulation involves a heterogenous set of cellular mechanisms, and to date specific interference, especially interventions that dissect Ca^{2+}-mediated exocytosis from Ca^{2+} transcription coupling in mast cells are lacking. Here, we tested a novel optochemogenetic approach, which targets TRPC6 Ca^{2+} channels, for suitability to specifically tailor the immunological signature of mast cells.

Methods: All experiments were performed using rat basophilic leukaemia cells (RBL-2H3) cells, genetically modified to overexpress TRPC6 channels along with a reporter of NFAT translocation and CD63 as a standard marker for immune (mast)-cell degranulation. Intracellular Ca^{2+} changes were monitored by fluorescence imaging using Fluo-4 AM.

Results: Genetic manipulation of RBL cells to overexpress TRPC6 conferred susceptibility to photopharmacological control of temporal features of Ca^{2+} signaling. Exposure of cells to the inactive *trans* isomer of the photochromic benzimidazole OptoBI-1, failed to induce degranulation and/or NFAT transcriptional activity. Repetitive photoactivation of OptoBI-1 generated oscillatory Ca^{2+} signals associated with rapid and efficient NFAT nuclear translocation. Importantly, OptoBI-1-mediated oscillatory Ca^{2+} signals introduced by a sequence of short (15 s) light pulses generated robust NFAT activation without detectable degranulation of the mast cells.

Discussion: Our results demonstrate that TRPC6/OptoBI-1-based optical modulation of mast-cell Ca^{2+} signals allows for unprecedented specificity in targeting mast-cell functions. The described optochemogenetic approach was found suitable to initiate specific transcriptional activation of the NFAT pathway coupled with the generation of a potentially antitumor immunological signature. Our results provide evidence for a potential therapeutic value of (photo)pharmacological strategies for high-precision immunomodulation in cancer therapy.

Keywords: TRPC6 – photopharmacology – mast-cell degranulation – NFAT

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