ZVex® lentiviral vector strongly activates pro-inflammatory, antigen processing, and anti-viral defense response pathways in monocyte-derived dendritic cells

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#5092

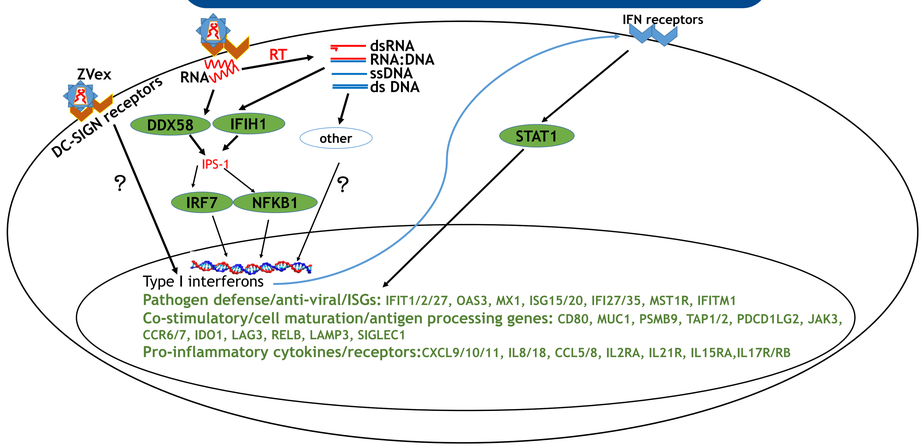
Introduction

Dendritic cells (DCs) are professional antigen presenting cells that effectively bridge the innate and adaptive immune responses and require activation for successful priming of naïve T-cells. Immune Design is developing therapeutic agents from ZVex, an integration deficient, DC-targeted, non-replicating, lentiviral vector-based platform. Unlike wild type lentiviruses that normally don't efficiently infect DCs, ZVex vectors are engineered to target DC-SIGN, which is expressed on immature myeloid DCs, for genetic immunization against tumor antigens. Little is known about the functional effect of normal LV transduction of conventional DCs. Here, the effect of DC transduction with ZVex vectors was studied by gene expression profiling.

Results

- DCs transduced with ZVex vectors displayed statistically significant up-regulation of genes involved in pro-inflammatory, antigen processing, and anti-viral defense pathways (Fig. 1 and 2).
- An RT-deficient vector also induced these genes, suggesting that incoming LV-RNA itself can activate these pathways in DCs (Fig. 1 and 2).
- Noteworthy among the up-regulated genes were anti-viral response genes like DDX58, OAS3, MX1, CXCL10, IFIT1, IFIT27 and interferon stimulated genes like ISG15 and ISG20. Upregulation of MX1, CXCL10, IFIT1,and IFIT27 was further confirmed using qPCR (Fig. 3)
- ZVex-mediated activation resulted in enhanced surface expression of B7-1 (CD80, Fig. 4) consistent with observed antigen presentation and T-cell priming as previously shown

Proposed MOA of ZVex in DCs



ZVex binds to DC-SIGN receptors expressed on the dendritic cell surface. Engagement of the DC-SIGN receptor, entry of the viral RNA, and reverse-transcription all contribute to the induction of an anti-viral, pathogen defense, pro-inflammatory response that results in dendritic cells activation, antigen presentation, and T cell activation. Shown in green boxes and listed in green text are a subset of genes that were found up-regulated by ZVex lentiviral vectors in the current study. Through this study it was confirmed that ZVex lentiviral vectors, though unique (non-integrating, DC targeting/transducing) amongst other third generation LVs, are capable of mounting a potent anti-viral, antigen-presentation, DC activation response.

Conclusions

- ZVex induces potent innate immune activation in DCs and results in the induction of an anti-viral, pathogen defense, DC activation response.
- Viral RNA is sufficient for induction of the DC activating innate response, though reverse transcription seems to augment it on a case-by-case basis.
- DCs transduced with ZVex vectors displayed statistically significant up-regulation of genes involved in pro-inflammatory, antigen processing, and anti-viral defense pathways.
- Up-regulation of some of the genes was further confirmed for a subset of donors via independent technologies like flow cytometry (CD80), and qPCR (MX1, CXCL10, IFIT1, IFIT27).
- Other engineered vector particle components such as viral structural proteins also seem to contribute to low level of DC activation.
- CMB305, a product of the ZVex platform, is currently in ongoing Phase 1 and 2 studies.

IMMUNE DESIGN

ZVex induces potent innate immune activation in DCs

Fig. 1. Gene expression changes in ZVex transduced moDCs

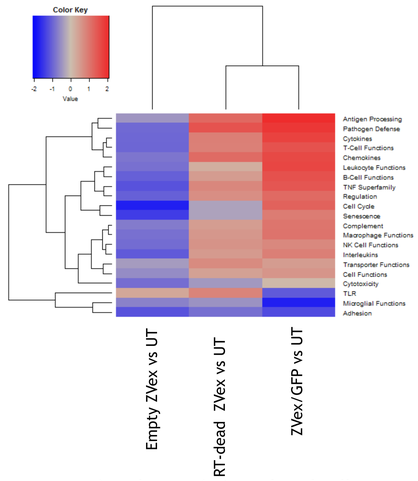
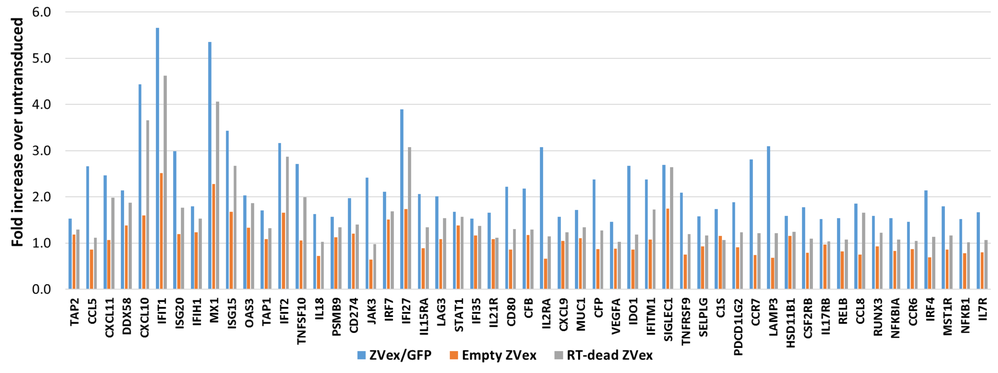


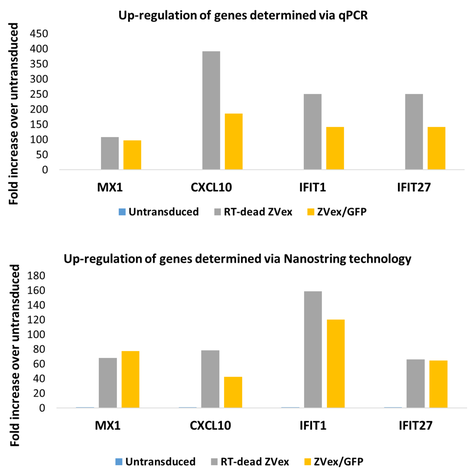
Fig. 2. Up-regulation of pro-Inflammatory, antigen presenting, anti-viral defense genes



Gene expression changes in transduced cells compared to untransduced cells

Methods: CD14+ monocytes from 4 donors were treated with 100 ng/ml GM-CSF and 50 ng/ml IL-4 for 5 days. The treated cells were transduced by co-incubation with ZVex/GFP, ZVex with nonfunctional reverse transcriptase (RT-dead ZVex), ZVex particles generated to contain no vector genome (empty ZVex), or left untransduced (UT). Total cellular RNA was isolated at 18, 42 and 66 hours post transduction and used for gene expression analysis using the Nanostring human pan cancer immune kit consisting of 770 genes.
Results: The heatmap in Fig. 1 shows global gene expression changes in transduced cells compared to untransduced (UT) control cells for all the 4 donors at the 3 different time points combined. Red and blue colors denote gene over-expression and under-expression, respectively. Fig. 2 shows a subset of 53 genes that are significantly up-regulated > =1.5 fold in ZVex/GFP over untransduced (UT) control cells ($p < 0.05$, N=4 donors). Cells treated with empty ZVex and especially RT-dead ZVex also show up-regulation of a subset of the genes. Incubation of DCs from one donor with heat-inactivated vector did not result in gene up-regulation (not shown).

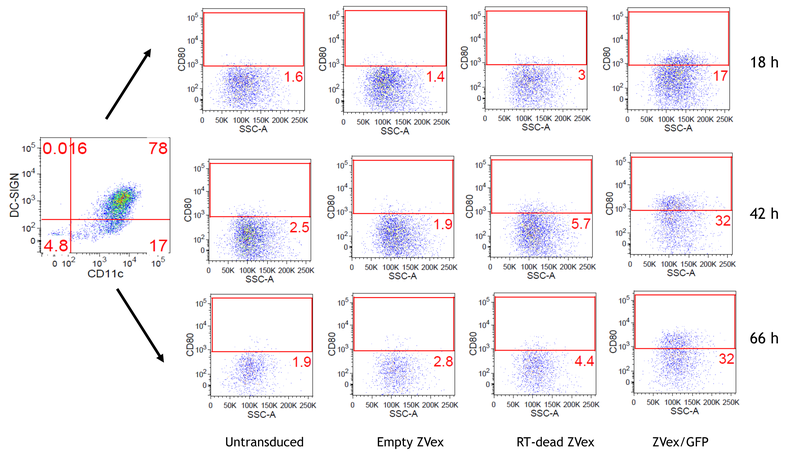
Fig. 3. Up-regulation of viral response genes confirmed by qPCR



Expression of MX1, CXCL10, IFIT1, IFIT27 by qPCR (upper panel) and Nanostring technology (lower panel) in total RNA extracted out of ZVex transduced moDCs from one donor

Methods: RNA used for the Nanostring analysis (Fig. 2) was also analyzed via qPCR using quantitative Taqman assays for the MX1, CXCL10, IFIT1, and IFIT27 genes. The upper panel shows fold gene up-regulation in transduced cells compared to untransduced cells. The lower panel shows gene up-regulation in the same sample as determined by Nanostring technology (human pan cancer panel).
Results: MX1, CXCL10, IFIT1,and IFIT27 genes were up-regulated to similar extents as determined via Nanostring technology and independent qPCRs. There is no significant difference in fold up-regulation obtained via ZVex/GFP or RT-dead ZVex transduction ($p < 0.05$).

Fig. 4. ZVex-mediated surface up-regulation of CD80



Surface expression of activation marker CD80 on ZVex transduced moDCs

Methods: moDCs cultures were transduced with ZVex vectors, as indicated. At 18, 42, and 66 hours post transduction, cells were fixed and analyzed for CD80 expression on DCs (CD11c+/DC-SIGN+ cells), see left panel). The flow cytometry dot plots above are from one experiment and are representative for 3 out of 4 donors whereas DCs from a 4th donor did not up-regulate CD80.
Results: DCs transduced with ZVex/GFP show increased CD80 expression compared to untransduced DCs in 3 out of 4 donor cell samples. Interestingly, cells co-incubated with the RT-dead ZVex also induced CD80 albeit at a lower level than RT-competent ZVex (ZVex/GFP).