Odegus4: an endogenous parvoviral element that reduces parvoviral replication.

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Introduction

Endogenous viral elements (EVEs) are viral-derived DNA sequences present in the genome of extant species. Some of them possess open reading frames (ORF) that can express proteins with physiological roles in their host. Furthermore, it has been described that some EVEs exhibit a protective role against exogenous viral infection in their host.

Previously our laboratory demonstrated that an EVE derived from *Parvoviridae* family is transcribed in the liver of *Octodon degus*. This EVE, named Odegus4, contains an intact ORF that possess the Rep protein domain of adeno-associated virus, where Rep is an essential protein for viral DNA replication. We also demonstrated that in cells transfected with a plasmid encoding Odegus4, a protein with nuclear localization is expressed. These characteristics lead us to speculate that ORF Odegus4 may function as a cellular coopted protein in degu. The aim of this work was to demonstrate Odegus4 as protein with an antiviral role against exogenous parvovirus.

Methodology

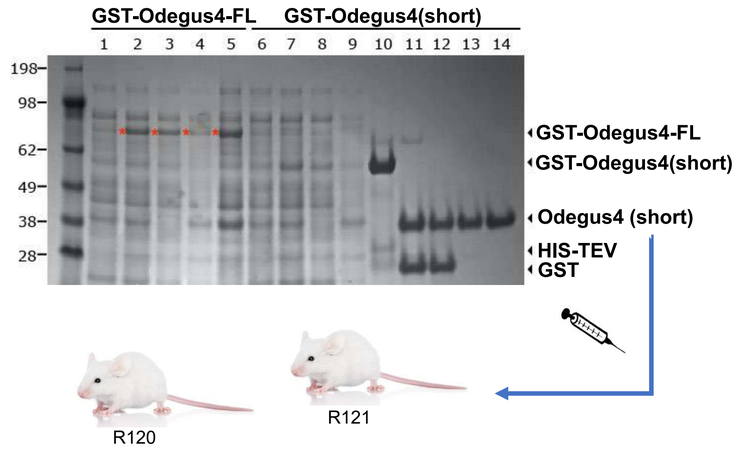

Figure 1. Bacterially produced short segment of Odegus4 (Odegus4 (short)) was purify and 2 mouse were immunized. Serums R119 and R120 were obtained.

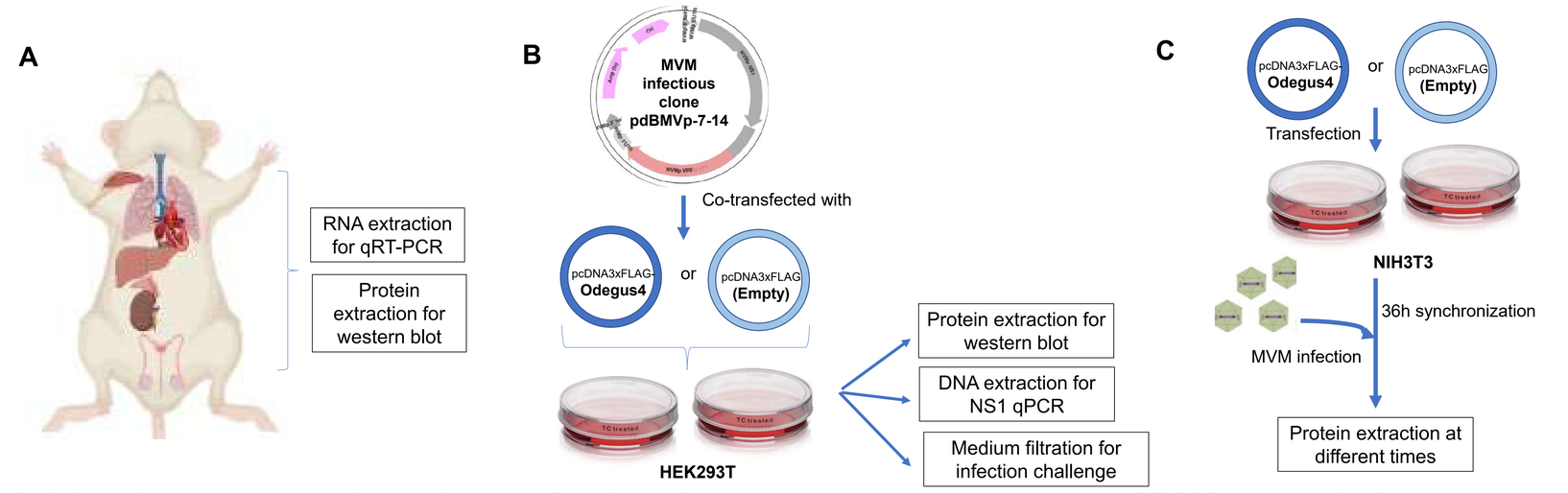

Figure 2. Schematic representation of methodology. A. RNA from different degu's tissues was extracted and a qRT-PCR searching for Odegus4 transcript was performed. In order to find Odegus4 protein in degu, we extracted proteins from tissues and run western blots using R120 and R121 serums against Odegus4. **B.** To analyze if Odegus4 was interfering with parvoviral genome replication, HEK293T cells were co-transfected with MVM infectious clone and pcDNA3xFLAG (empty) or pcDNA3xFLAG-Odegus4 (Odegus4). Cells were lysed and proteins and DNA were extracted. In addition, we tested the viral production using the filtered medium in NIH3T3 cells (a fibroblast mouse cell line susceptible to MVM infection) infected with different viral doses. **C.** NIH3T3 cells were transfected with pcDNA3xFLAG (empty) or pcDNA3xFLAG-Odegus4 (Odegus4). 24hpt cells were subjected to 36 hours of DMEM 0,5%FBS for synchronization. After this time, cells were infected with a viral doses of 1/8 and proteins were extracted at 12, 16, 20 and 24 hours post infection.

Results

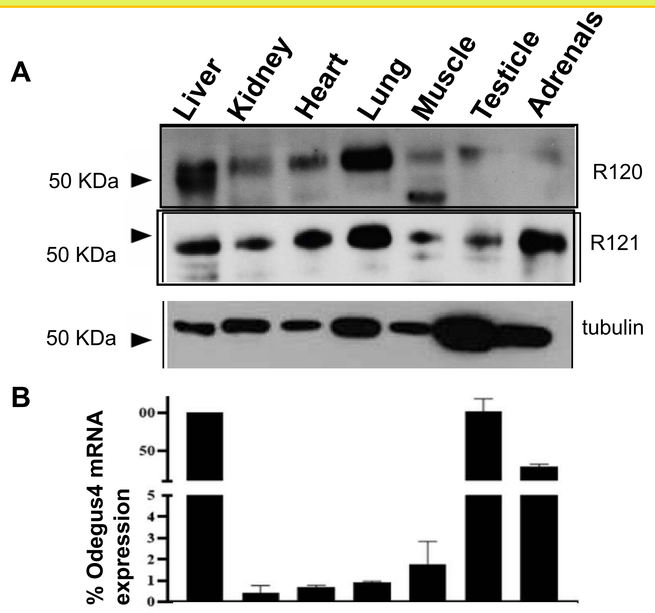

Figure 3. Odegus4 protein is detected in various degu tissues, and shows similar transcript expression level in liver, testicle and adrenals. **A.** Total protein was extracted from liver, kidney, heart, lung, muscle, testicle and adrenals. A western blot using anti Odegus4 serums (R120 or 121) is shown. Tubulin was used as loading control. The antibody used in each blot is shown on the right and the migration of the molecular weight marker is shown on the left-hand side. **B.** RNA was extracted from tissues to make a qRT-PCR using Odegus4 primers and GAPDH as housekeeping. Expression is shown relative to liver. The content of each line and bar is shown on top.

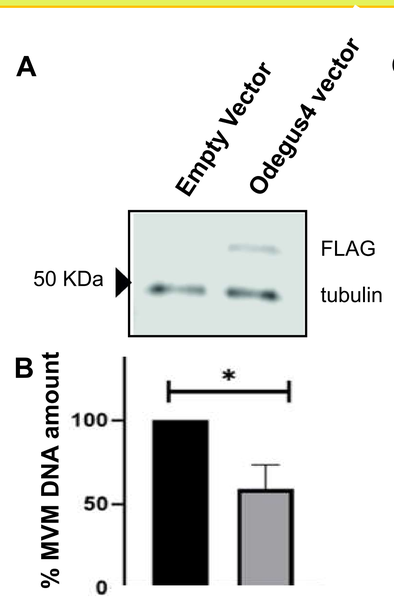

Figure 4. Odegus4 diminishes both parvoviral genome replication and viral production when co-transfected with MVM infective clone. HEK293T cells where co-transfected with MVM infective clone and pcDNA3xFLAG (empty vector) or pcDNA3xFLAG-Odegus4 (Odegus4 vector). 5 days post transfection, cells where lysed and proteins and DNA were extracted. **A.** Western blot anti FLAG was performed to detect Odegus4 expression. Tubulin was used as loading control. **B.** To detect MVM DNA we used NS1 primers to perform a qPCR with GAPDH as housekeeping gene. **C.** Medium of co-transfected cells was filtered and used to infect NIH3T3 cells in different dilutions; 1 is undiluted, from 1/2 - 1/16 dilutions are shown. C- did not received virus. Data is shown as mean of 3 independent replicates ±SD. t student test was used to detect statistical significance ($p<0,5$).

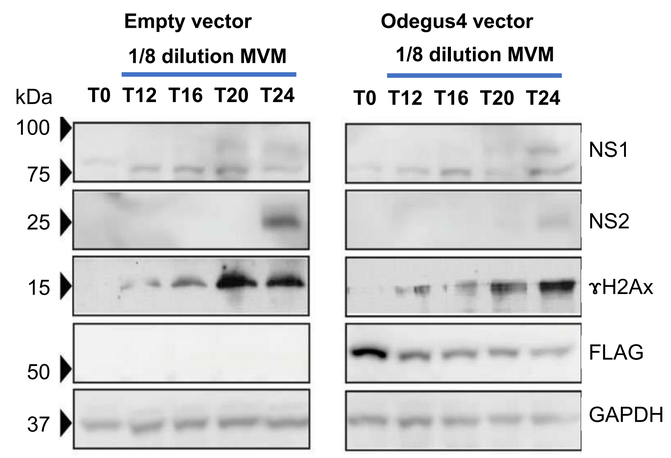

Figure 5. MVM NS1/2 expression is diminished in Odegus4 presence. NIH3T3 cells were transfected with pcDNA3xFLAG (empty vector) or pcDNA3xFLAG-Odegus4 (Odegus4 vector). 24hpt cells were synchronized for 36 hours in DMEM 0,5% FBS. After synchronization, cells were infected with a viral doses of 1/8 and protein extraction was carried out at 12, 16, 20 and 24 hours after infection. T0 is not infected. The content of each line is shown on top, the antibody used in each blot is shown on the right and the migration of the molecular weight marker is shown on the left-hand side. GAPDH was used as loading control.

Conclusions

Our results shows that Odegus4 is being expressed as a protein in degu, and reduced parvovirus replication when expressed *in vitro*.

Acknowledgements

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