Innate immune response activation in long-term AAV transduction

Xintao Zhang1, Zheng Chai 1, Wenwei Shao 1, R.Jude Samulski 1,2, Chengwen Li 1,3,4*

1 Gene Therapy Center, University of North Carolina at Chapel Hill (UNC-CH), Chapel Hill, NC 27599, 2 Department of Pharmacology,, UNC-CH, Chapel Hill, NC 27599, USA

3 Department of Pediatrics, UNC-CH, Chapel Hill, NC 27599, USA. 4 Carolina Institute for Developmental Disabilities, UNC-CH, Chapel Hill, NC, 27510, USA

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

ABSTRACT

Liver damage has been consistently seen in a subset of patients after AAV systemic gene delivery. These side effects usually occur at a relatively late time, are typically related to high doses and independent of AAV serotypes. All these clinical studies have led to hypothesize that there could be a common biological mechanism at play. It has been shown that AAV ITR has promoter function, the 3' ITR induces minus strand RNA that can anneal with the positive strand RNA from vector promoter in the cytosol, and form dsRNA. The dsRNA can be recognized by cytosolic RNA sensors and then activate innate immune response. Our studies have documented that an innate immune response can be triggered by minus strand RNA generation after long term AAV transduction in human hepatocyte cell lines and primary human hepatocytes. This observation was further established *in vivo* in chimeric mouse xenograft model using human hepatocytes after systemic AAV administration. Mechanistic analysis demonstrated that 1) dsRNA sensor MDA5 was involved, 2) transient knockdown of MDA5 inhibited IFN-β expression while increasing transgene production, 3) transgene expression was enhanced with dramatic decrease of IFN-β mRNA levels in AAV transduced MAVS knockout human hepatocyte cells. Further, AAV vector specifically expressing shRNA to silence MAVS induced a higher transgene expression and lower innate immune activation. These results highlight for the first time the importance of late innate immune response activation following AAV transduction. Most importantly, this study provides valuable insights to design novel AAV vectors to overcome innate immunity.

RESULTS

Fig.1

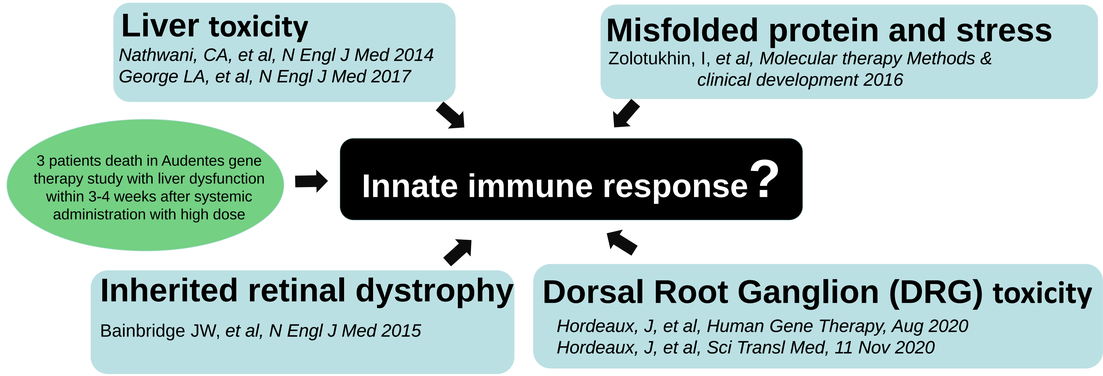
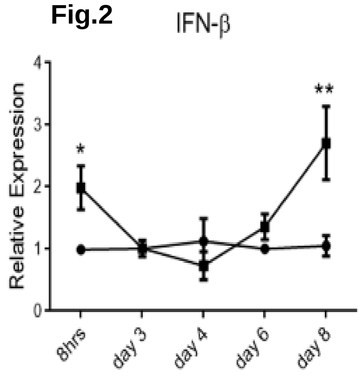
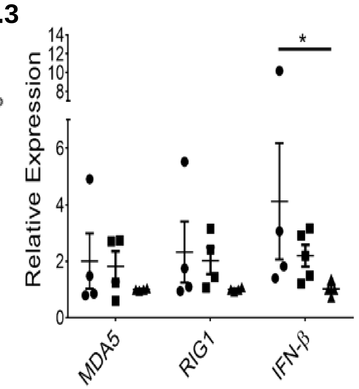


Fig.2



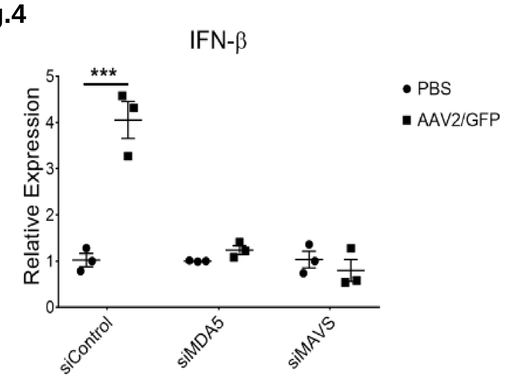
In vitro: Hela cells

Fig.3



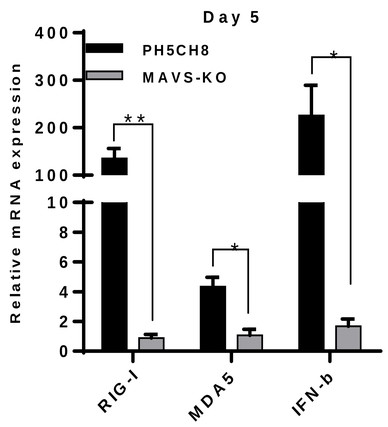
In vivo: human hepatocytes xenograft mice at 4 weeks

Fig.4



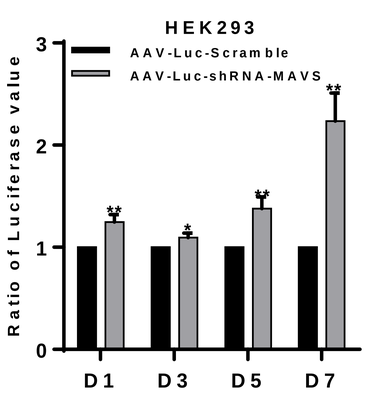
siRNA knockdown dsRNA sensors down-regulates IFN-β expression activated by AAV transduction

Fig.5



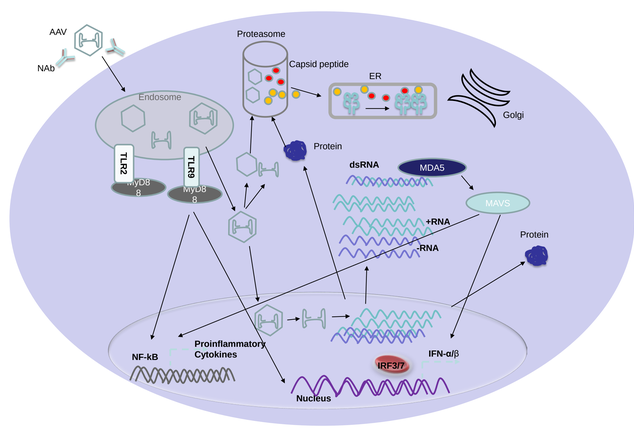
knockout of MAVS sensor decreases dsRNA pathway activation

Fig.6



shRNA knockdown MAVS enhances long-term AAV transduction

Fig.7



Immune response to AAV vectors after AAV transduction