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Adeno-associated virus finds its disease

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Abstract

Adeno-associated virus (AAV) vectors have been widely adopted for use in gene therapy. A new study raises concerns regarding this approach, reporting that chromosomal insertions of AAV serotype 2 seem to activate proto-oncogenes in human hepatocellular carcinoma.

AAV and cancer

Hepatocellular carcinoma (HCC) is one of the few cancers showing an increasing frequency of incidence in the developed world, with most cases occurring in the context of liver cirrhosis and chronic inflammation and frequently in association with hepatitis B virus (HBV) infection¹. Several proto-oncogenes have been found to be overexpressed in human HCC and are thought to be causally related to cancer development. A subset of these genes, including *TERT* (telomerase reverse transcriptase), *CCNE1* (cyclin E1) and *KMT2B* (lysine-specific methyltransferase 2B), are associated with HBV insertions², with *TERT* activation being the most frequent genetic event in all HCCs and presumably an early event in the ontogeny³. In this issue of *Nature Genetics*, Jessica Zucman-Rossi and colleagues add to this story by sequencing human HCC samples and finding clonal integrations of sequences derived from wild-type AAV2 at several known HCC driver genes⁴. *In vitro* modeling demonstrated that these insertions increased the expression of the proto-oncogenes and that the partial AAV sequences could act as both promoters and enhancers in hepatocytes (Fig. 1). These data strongly suggest that the AAV integrations actually caused the tumors, similarly to the scenario with HBV integrations.

The results are surprising in several ways, especially because AAV has long been considered a nonpathogenic virus that even has anti-oncogenic properties⁵. Most of the insertions included a 3' portion of the AAV2 capsid gene and the 3' inverted terminal repeat (ITR), which was previously shown to have promoter properties⁶ and presumably also contains an enhancer element, as implied by the reverse orientation of some of the insertions. Approximately 6% of the HCCs studied contained clonal AAV insertions at

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proto-oncogene loci, with 21% of matched non-tumor liver specimens containing nonclonal AAV sequences. The HCCs with AAV insertions were enriched in patients without underlying cirrhosis, suggesting that AAV-induced inflammation was not a major contributor to oncogenicity (unlike in HBV-associated HCC). More than 50% of the population of the United States is thought to be infected with AAV, but no chronic hepatitis seems to have developed as a result. The absence of chronic liver injury in AAV infections may explain why the relative risk of developing HCC is lower than with HBV. Nonetheless, these results clearly indicate that insertional mutagenesis by AAV can cause malignant transformation in the liver, apparently without additional insults. This is remarkable given that AAV is generally considered a respiratory virus and requires a helper virus, such as adenovirus, for productive infection. The frequent presence of viral sequences in liver specimens suggests that the virus can also enter the bloodstream and infect internal organs at high levels. It remains to be seen whether similar oncogenic insertions occur in other types of human tumors.

Gene therapy and hepatocellular carcinoma

In mice, HCC can be induced by chromosomal integration of transposons, lentiviral vectors or AAV gene therapy vectors^{7–9}. However, most of these findings were obtained in mice with tumor-prone or disease-associated genotypes, and they stand in contrast to the excellent safety record of AAV vectors in large animal models as well as in numerous preclinical studies in mice that have supported moving them forward in human clinical trials. A notable exception is the observation that AAV vector integration into the *Rian* locus in normal newborn mice causes HCC, with increased expression of a set of surrounding microRNAs and small nucleolar RNAs (snoRNAs) whose counterparts are also overexpressed in some human HCCs¹⁰. Interestingly, these integrations are centered around one specific microRNA gene in the *Rian* locus (*Mir341*) that is not conserved in humans¹¹, which could explain the lack of wild-type AAV insertions found in the syntenic human *MEG8* locus by Nault *et al.*⁴ (Fig. 1).

Does the finding that wild-type AAV integration can lead to human HCC shed light on the risks of gene therapy? In one sense, this study is reassuring because the critical 3' capsid gene fragment present in wild-type AAV insertions is absent from AAV vectors. However, a larger concern is the novel observation that an enhancer-promoter element packaged in an AAV virion can integrate and activate a proto-oncogene in human hepatocytes, as most AAV vectors by necessity include a strong enhancer-promoter that is active in the target tissue. In liver-directed gene therapy, integration in as few as 0.1% of hepatocytes would still result in tens of millions of integration events. Consequently, it is likely that some patients will have vector sequences inserted at proto-oncogene loci. The tumorigenic impact of these insertions is difficult to predict because multiple oncogenic hits may be required for transformation. Even so, it is hard to imagine an exposure to wild-type AAV that is equivalent to the intravenous delivery of $>10^{13}$ vector particles that a patient undergoing gene therapy typically might receive, and we now know that, in some cases, exposure to wild-type AAV might be enough to cause a tumor.

Moving forward, there are several steps that gene therapists can take to improve the safety of AAV vectors. Careful design of enhancer and promoter elements may minimize the risks of insertional mutagenesis, as demonstrated for integration in the mouse *Rian* locus¹¹. Eventually, the field may adopt promoterless vectors that integrate site specifically, as suggested by the recent demonstration of clotting Factor IX expression from an albumin locus knock-in vector¹². When targeting tissues other than liver, one should choose vector serotypes with reduced liver tropism and enhancer and promoter elements that are not active in hepatocytes. Chronic hepatic inflammation and cirrhosis are clear contributors to HCC evolution, and patients with such conditions might not be suitable candidates for liver-directed gene therapy. Notably, obesity is a frequent cause of chronic hepatitis¹³ and thus could also be a risk factor for AAV-mediated oncogenesis. Similarly, AAV integrations occur more frequently in dividing cells¹⁴, so the risk of tumor formation could be higher in any setting with hepatocyte proliferation, especially in young children with growing livers. Finally, there should be renewed efforts to eliminate even low levels of contaminating replication-competent AAV from clinical-grade vector stocks, as these particles could deliver the oncogenic capsid gene element. Close follow-ups of patients treated with AAV vectors will shed light on some of these issues, and renewed research into the potential oncogenicity of AAV vectors is now more important than ever.

References

1. Njei B, Rotman Y, Ditah I, Lim JK. *Hepatology*. 2015; 61:191–199. [PubMed: 25142309]
2. Sung WK, et al. *Nat Genet*. 2012; 44:765–769. [PubMed: 22634754]
3. Paterlini-Brchot P, et al. *Oncogene*. 2003; 22:3911–3916. [PubMed: 12813464]
4. Nault JC, et al. *Nat Genet*. 2015; 47:1187–1193. [PubMed: 26301494]
5. Weitzman MD, Linden RM. *Methods Mol Biol*. 2011; 807:1–23. [PubMed: 22034024]
6. Haberman RP, McCown TJ, Samulski RJ. *J Virol*. 2000; 74:8732–8739. [PubMed: 10954575]
7. Riordan JD, et al. *PLoS Genet*. 2013; 9:e1003441. [PubMed: 23593033]
8. Donsante A, et al. *Science*. 2007; 317:477. [PubMed: 17656716]
9. Ranzani M, et al. *Nat Methods*. 2013; 10:155–161. [PubMed: 23314173]
10. Wang PR, et al. *Proc Natl Acad Sci USA*. 2012; 109:11264–11269. [PubMed: 22733778]
11. Chandler RJ, et al. *J Clin Invest*. 2015; 125:870–880. [PubMed: 25607839]
12. Barzel A, et al. *Nature*. 2015; 517:360–364. [PubMed: 25363772]
13. Jiang CM, et al. *World J Gastroenterol*. 2014; 20:16464–16473. [PubMed: 25469014]
14. Russell DW, Miller AD, Alexander IE. *Proc Natl Acad Sci USA*. 1994; 91:8915–8919. [PubMed: 8090744]

Figure 1.

AAV and hepatocellular carcinoma. Genomic integration of wild-type (WT) or engineered AAV sequences in human or mouse hepatocytes can lead to HCC through activation of proto-oncogene expression. Pro/enh, promoter-enhancer.