NEWS AND COMMENTARY

Allele-Specific RNA Interference in FOP

Silencing the FOP gene

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Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant disease in which bone is formed in soft tissues through heterotopic ossification (HO). HO is often a problem for patients who have received a traumatic insult, either to musculoskeletal tissues (traumatic HO) or to the skin or spinal cord (neurologic HO). Although trauma-induced HO can be painful, it is often transitory and easily treated with conventional therapies such as bisphosphonates, radiation and surgical removal of the heterotopic bone. In sharp contrast, the unpredictable and progressive nature of FOP leads to severe impairment of mobility and eventual paralysis due to ossification of joints. In addition, as this form of HO characteristically occurs on the chest, back and spine, damage to internal organs, particularly compression of the lungs, contributes to the lethality of FOP. There is currently no treatment for FOP, due in part to the rarity of the disease and the difficulty in obtaining samples from FOP patients for study – even minimal trauma, such as a tissue biopsy, can trigger HO to occur in these patients. Thus, the discovery of the genetic mechanism of FOP in 2006 was a tremendous accomplishment and renewed the hope of finding a cure.¹

FOP is caused by heterozygous mutations in the *Activin Receptor Type 1A* (*ACVR1*, also known as *ALK2*).² All cases of classic FOP examined to date carry a single recurrent mutation in *ACVR1* (R206H), though other mutations in *ACVR1* have been reported in variant FOP. Both protein modeling and *in vitro* studies indicate that these mutations in *ACVR1* result in constitutive activation of the receptor.² This is of importance, as *ACVR1* is a type 1 receptor for bone morphogenetic proteins (BMPs), whose exogenous administration in soft tissue leads to ectopic bone formation. Thus, inhibition of mutant *ACVR1* (*caACVR1*) signaling in FOP patients represents an attractive therapeutic approach. However, pharmacological inhibitors that are currently available to block BMP signaling are unable to distinguish between *caACVR1* and normal BMP type 1 receptors, and could, therefore, cause unintended inhibition of global BMP signaling.^{3–5} This would be of considerable consequence as BMP signaling is required for the normal functioning of most organs and tissues.⁶

In two separate reports published in *Gene Therapy*, Kaplan *et al.*⁷ and Takahashi *et al.*⁸ provide preliminary evidence of the ability to selectively suppress *caACVR1*. Both studies utilize the use of allele-specific RNA interference (ASP-RNAi) to target *caACVR1* mRNA for degradation (Figure 1). Importantly, careful design of the siRNAs allows these authors to discriminate between mutant and normal mRNAs, thus preserving expression of normal *ACVR1*. By doing so, the level of BMP signaling in cells obtained from FOP patients, which is basally higher than control cells, can be reduced to normal levels. These studies are the most recent examples of the growing applicability of the use of ASP-RNAi for treating human disease. ASP-RNAi has been preliminarily applied to the study of several diseases,^{9–13} most often of a neuronal or neurodegenerative nature including Alzheimer's disease,^{14,15} Parkinson's disease,¹⁶ Huntington's disease^{17–22} ALS,²³ Machado-Joseph disease,^{18,24,25} dystonia^{26–28} and others,^{29–31} and a phase I

clinical trial determining the safety and toxicity of ASP-RNAi for treatment of pachyonychia congenita was recently completed (NCT00716014). Classic FOP is an ideal candidate disease for ASP-RNAi because of the strikingly recurrent single nucleotide mutation (c.617G>A). This important distinction is of clear translational advantage, even over many other monogenic autosomal dominant diseases associated with heterogeneous mutations, as it would allow for careful validation and clinical trial of a single set of siRNAs that could potentially treat all classic FOP patients.

Although the results of Kaplan *et al.*⁷ and Takahashi *et al.*⁸ provide promising proof-of-principle for the allele-specific silencing of *caACVR1A* in the treatment of FOP, there are outstanding questions that must be answered before this technology can be translated to humans. For instance, it is presently unclear how mild constitutive activation of *ACVR1* leads to severe HO. It is of note that *caACVR1* is likely expressed in all cells that also express normal *ACVR1*; however, HO only occurs in a considerably smaller domain in FOP patients. Furthermore, although FOP seems to be caused by constitutive activation of *ACVR1*,² surprisingly, conditional loss of *ACVR1* in osteoblasts results in higher bone mass,³²

Figure 1. (a) FOP patients express both normal *ACVR1* and mutant *ACVR1* (*caACVR1*). (b) Administration of allele-specific siRNA leads to selective targeting of *caACVR1* mRNA without affecting normal *ACVR1* expression.

leading these authors to conclude that ACVR1 negatively regulates endogenous bone formation. Collectively, these findings are highly suggestive that specific responses downstream of ACVR1 might be regulated in a cell type- and/or context-dependent manner. Moreover, they underscore the importance of identifying the cell type(s) responsible for HO in FOP. In addition, they also raise the intriguing possibility that mutant ACVR1 might not only be constitutively active but might also possess novel gain-of-function properties not associated with normal ACVR1 signaling.³³ Recent evidence has pointed to vascular endothelial cells as a major contributor to HO through endothelial-to-mesenchymal transition.^{34,35} However, not all cells in FOP lesions are of endothelial origin; other sources posited include circulating osteogenic precursors,³⁶ skeletal myoblasts³⁵ and vascular smooth muscle cells.³⁷ Identification of the cell type(s) involved in HO will also aid in solving a general concern for use of ASP-RNAi *in vivo*, which is how to preferentially target RNA-mediated silencing to sites of disease. Previous approaches have included the use of viruses with significant tropism for cells/tissues of interest,¹⁴ and one could envision utilizing cell type-specific promoters to drive expression of shRNAs *in vivo*. With these considerations in mind, FOP therapy has come a step closer to actuation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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