the preceding 6 months and the first 6 months of life.

Marini is concerned that there was no measurable increase in lumbar bone density. However, bone mineralization is not uniform throughout the skeleton. Furthermore, bone density measurements may not be the most appropriate means to express bone mineralization in pediatrics[7]. For this reason, we chose to measure changes in total bone mineral content with dual energy X-ray absorptiometry. This is a more sensitive assay of overall bone mineralization than measurements of the spine, which is only a small portion of the skeleton. Marini also confirms the presence of mesenchymal engraftment, but questions how so low a level of engraftment can produce the profound changes seen. We agree that this is puzzling, and in our article and the accompanying *News & Views* article[8], several possible mechanisms were proposed. Marini discounts many of these based on the fact that they are not consistent with predictions made after studying mosaic carriers of OI. It is an equally valid argument, however, that mosaic carriers of OI simply fail to accurately model the effects of normal osteoblasts in children with severe disease.

Finally, Bishop correctly states that other children with HLA-compatible siblings have been enrolled subsequently in our protocol. There are two such additional children and both demonstrated histologic and clinical findings similar to the reported patients, corroborating our initial results. We emphasize that our work represents only the 'proof of principle' of mesenchymal cell engraftment, and encourages future investigations of both cell and gene therapy of mesenchymal disorders. Only extensive study of carefully selected patients will demonstrate whether or not such therapies are the optimal treatment for children with severe osteogenesis imperfecta.

EDWIN M. HORWITZ[1], DARWIN J. PROCKOP[2], LORRAINE A. FITZPATRICK[3], WINSTON W.K. KOO[4], JEFFREY C. MARX[1], MALCOLM K. BRENNER[1]

[1]Cell & Gene Therapy Program
St. Jude Children's Research Hospital
332 N. Lauderdale, Room D-4026
Memphis, Tennessee 38101, USA
[2]Allegheny University of the Health Sciences, 15th and Vine Streets,
Philadelphia, Pennsylvania 19102, USA
[3]Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, USA
[4]Wayne State University,
4707 St. Antoine Boulevard
Detroit, Michigan 48201, USA

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Adeno-associated virus 2 co-receptors?

To the editor—We were surprised to read two papers in the January issue of *Nature Medicine* claiming both human fibroblast growth factor receptor[1] (FGFR) and αVβ5 integrin[2] as co-receptors for adeno-associated virus 2 (AAV2) infection.

FGFR is the high-affinity receptor for basic fibroblast growth factor[3] (bFGF), although it has been shown that low-affinity binding to heparan sulfate molecules is prerequisite for the binding of bFGF with FGFR (ref. 4). This interaction between FGFR and heparan sulfate led to the proposal that herpes simplex virus, which uses heparan sulfate as an attachment molecule[5], may use FGFR as its cellular receptor[6]. Qing *et al.* made an analogous suggestion[1] about the binding of AAV2 to FGFR. The data on the relationship between HSV/FGFR and AAV2/FGFR were almost identical: bFGF was able to compete the viral binding and infection, and transfection of FGFR cDNA was capable of increasing viral binding and infection in 'nonpermissive' cells. However, subsequent research has shown that FGFR is not required (e.g., ref. 7) for HSV infection. Part of the viral-inhibiting properties of the bFGF is due to competition with binding to cell surface heparan sulfate, and cell lines such as Hep-2 and A431 that do not have FGFR on their cell surface are fully susceptible to HSV. We have done similar experiments for AAV2 (Table). Flow cytometry confirmed the absence of FGFR on Hep-2 or A431 cells[7], yet they were readily transduced by rAAV2, showing that FGFR is also not required for AAV2 infection.

In the paper by Summerford *et al.*[2], αVβ5 integrin was identified as a co-receptor for AAV, mainly on the basis of a viral overlay reported to show binding to denatured β5. Using the same viral overlay technique, we found a strongly binding, 150-kDa AAV2 binding protein[8]. We have also identified a weaker band at about 110 kDa, and have identified the protein as human nucleolin, based on peptide sequencing and antibody detection[9]. However, using viral overlay, we were unable to detect any binding of AAV2 to purified or recombinant β5 integrin. In addition, despite the title of the Summerford paper ("αVβ5 integrin..."), the gels seem to have been truncated so that binding to αV (150 kDa) could not be seen. This is surprising, because for adenovirus, most of the viral interaction is through the αV motif, and any β subunit can substitute (although typically αVβ3 is used for attachment and internalization) (ref. 10). In addition, adenovirus binding seems to depend on conformation and RGD motif and seems to be not detected by viral overlay. Finally, the transfection of β5 cDNA only increased by 260% the transduction of AAV2 infection, with significant transduction in CS cells (which lack β5). As in the Qing paper[1], the ability to significantly infect or transduce cells that do not have the putative receptor suggests that these molecules are not essential in initiation of AAV2 infection.

Table FGFR and AAV2

Cell line	FGFR (mAb VBS1)	% rAAV2 transduction
HeLa S3	++	60
Hep-2	-	40
A431	-	10
U937	-	0
Mo7e	+	0
CHO-K1	++	70

Analysis of FGFR on cell surfaces, using flow cytometry with monoclonal VBS1 (Chemicon, Temecula, California). AAV2 transduction was done by infection with rAAV-LacZ (6.5×10^3 genome copies/cell); β-galactosidase positive cells were counted 2 days later.

JIANMING QIU[1], HIRO MIZUKAMI[2] & KEVIN E. BROWN[1]

[1]Hematology Branch
NHLBI,NIH, 9000 Rockville Pike
Bethesda, Maryland 20892, USA
[2]Division of Genetic Therapeutics
Center for Molecular Medicine
Jichi Medical School, 3311-1 Yakushiji,
Minamikawachi-machi, Tochigi 329-0498, Japan

Qing et al. reply—The conclusion by Qiu *et al.* that HEp-2 and A431 cells do not express FGFR is wrong. The Muggeridge *et al.* report[7] they quote clearly shows that the FGFR number per cell is approximately 300.

There are few details of how Qiu *et al.* generated the data presented in their table. The remarkably high multiplicity of infection used in these experiments is not standard, and it is difficult to reconcile their 60% transduction rate for HeLa cells when others have reported that AAV vectors do not transduce these cells well because of the rate-limiting viral second-strand DNA synthesis[11,12]. Transduction efficiencies of 40% for HEp-2 and 10% for A431, respectively, are cited as proof that these cells can be transduced in the absence of FGFR expression. Yet, as stated above, these cells do indeed express FGFR (ref. 7). Thus, it seems that the analysis of FGFR by Qiu *et al.* using flow cytometry with a monoclonal antibody is inadequate to draw such a conclusion.

We have compared the transduction efficiency of a recombinant AAV-*lacZ* vector (4×10^3 particles/cell) and found transduction efficiencies in HeLa and 293 cells of approximately 4% and 20%, respectively, and <1% in A431 cells which are known to efficiently bind AAV (ref. 13). The lack of trangene expression in A431 cells has previously been reported to be due to very high levels of expression of the epidermal growth factor receptor (EGFR) protein tyrosine kinase known to limit the viral second-strand DNA synthesis[13]. The observed lack of transduction of M07e cells, which we showed do express FGFR (ref. 1), has previously been shown to be due to lack of expression of heparan sulfate proteoglycan[14] (HSPG), a co-receptor of AAV. The absolute requirement for the deliberate expression of both HSPG and FGFR1 in Raji cells, which are known to lack expression of both of these genes[15], to render these cells permissive for AAV infection, strongly supports our contention that both HSPG and FGFR1 serve as co-receptors for AAV. Of course, other co-receptors may be used in other cells.

KEYUN QING, JONATHAN HANSEN, AND ARUN SRIVASTAVA
Department of Microbiology and Immunology
Medical Science Building 247
Indiana University School of Medicine
635 Barnhill Drive
Indianapolis, Indiana 46202-5120, USA

Samulski et al. replies—Using the procedure that Mizukami *et al.* used[8], we also observed a 150-kDa protein (Fig. 1, lane 1); however,

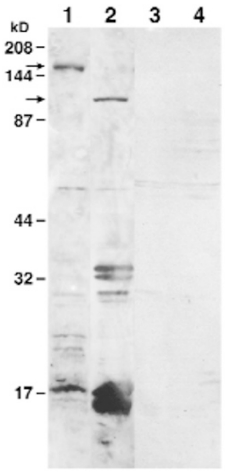

Fig. 1 Viral overlay analysis of two different membrane preparations. Lanes 1 & 2 represent an AAV-2 overlay on equivalent amounts of membrane purified by the Chong & Rose method and the Hennache & Boulanger method respectively. Arrows point to the 150 kDa (lane 1) and 100 kDa (lane 2) proteins that interact with AAV.

this method (described by Chong and Rose[16]) does not stringently purify plasma membrane proteins. In our paper[2], we used a method that specifically enriched cell surface proteins by 30-fold (ref. 17), as assessed by 5'-nucleotidase activity. In these more stringent conditions, binding to the 150-kDa protein was not detected. (Fig. 1, lane 2), thus our submitted gel[2] was truncated to save space. This protein may be a non-plasma membrane protein (for example, nucleolin as identified by Qiu and Brown), or a cell surface protein that migrates in a different fraction with our procedure. As the 'fold' enrichment of plasma membrane proteins was not monitored in Mizukami's study[8], all interpretations are plausible.

As for β5 integrin, we also did not see interaction with the purified form, possibly because of the absence of essential post-translational modification. It should be noted that we observed AAV binding to immunoprecipated β5 integrin, supporting the specificity of this interaction. Furthermore, we established that there is a role for integrin in AAV-2 infection (ref. 2, Figs. 2 and 3). The presence of integrin influences viral infection, but is not essential, as is the case with adenovirus[10,18]. Figure 3 of our study[2] clearly demonstrates that expression of β5 substantially increases AAV-2 internalization in a time-dependent manner, indicating a role in AAV entry, which may have important consequences *in vivo*[2,10,18].

As for the transduction data, the 260% enhancement we observed is very similar to that seen for adenovirus (320%), whose use of αVβ5 integrin as a co-receptor is well established. In addition, it is not surprising that AAV may interact with integrin in a non-RGD manner. A ligand does not have to use an RGD or RGD-like motif in order to interact with integrin.

Integrin αVβ3 and αVβ5 facilitate adenovirus infection; however, it is αVβ5 integrin that has been shown to have a dual role in facilitating both membrane permeabilization and internalization[17]. In addition, compared with αVβ3 integrin, αVβ5 internalizes adenovirus at a faster rate and renders cells significantly more susceptible to infection[18]. These studies and our data strongly suggest that both Ad and AAV use αVβ5 as a co-receptor to mediate viral entry.

RICHARD JUDE SAMULSKI, JEFFREY S. BARTLETT & CANDANCE SUMMERFORD
Gene Therapy Center
University of North Carolina at Chapel Hill
7119 Thurston-Bowles CB7352
Chapel Hill, NC 27599

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