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.

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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,

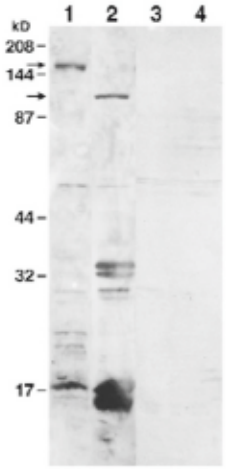

Fig. 1 Viral overlay analysis of two different membrane preparations. Lanes 1 & 2 represent an AAV-2 overlay on equivalent amounts of membrane purified by the Chong & Rose method and the Hennache & Boulanger method respectively. Arrows point to the 150 kDa (lane 1) and 100 kDa (lane 2) proteins that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

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