RD114 envelope proteins provide an effective and versatile approach to pseudotype lentiviral vectors

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Therapeutic lentiviral vectors are emerging as vital tools for molecular medicine as evidenced by the growing number of clinical trials using these vector systems. From a basic research standpoint, lentiviral vectors are very intriguing substrates. On the one hand, the HIV-1 genome offers expanded cloning capacity and the capability to transduce nondividing cells such as hematopoietic stem cells (HSCs) and neurons. However, concerns associated with the potential risk of generating replication competent lentiviral particles require the removal of significant portions of the HIV genome, most notably the envelope protein (env).

In their work published as the feature article in the October issue of Experimental Biology and Medicine, Bell et. al show that envelope proteins derived from the endogenous feline virus RD114 provide a versatile and effective method to pseudotype lentiviral vectors. The work was carried out by Anthony Bell; working together with David Fegen, Maureen Ward and Arthur Bank at Columbia University Medical School.

Dr. Bell stated, "The majority of lentiviral vectors are pseudotyped or packaged with the envelope from the vesicular stomatitis virus (VSV-G). VSV-G equips lentiviral vectors with a broad host cell tropism and increased particle stability. Increased particle stability enables viral supernatants to be concentrated via high-speed centrifugation to enhance their infectivity. VSV-G is cytotoxic, however, a feature that essentially

prohibits the constitutive expression of lentiviral vectors pseudotyped with this envelope. Therefore, nontoxic viral envelopes such as RD114 are being investigated. RD114 is a particularly attractive alternative because this envelope also provides increased particle stability and its receptor is widely expressed on HSCs. Hence, RD114 could prove to be an essential component of therapeutic lentiviral vector systems.

Together, the research team compared the packaging efficiency of three envelopes: VSV-G, RD114 and RDpro with two lentiviral vectors. RDpro is an RD114-HIV chimera designed to pseudotype lentiviral vectors with higher efficiency. In the transient expression format, as expected VSV-G, provided the higher relative titer values. The nontoxic nature of the RD114, however, enabled typical transfection reactions to be extended from 48 to 96 hours. VSV-G transfections are generally limited to 48 hours. Hence, RD114 envelopes offer an effective means to increase the total viral yield of lentiviral supernatants vs. VSV-G. The versatility of RD114 proteins was exploited further to generate a "mixed" lentiviral expression system. This expression system is composed of stably expressed RD114 proteins that pseudotype lentiviral vectors generated in trans.

Dr. Bell said, "We are very excited about the data received in the mixed expression format. We were also pleasantly surprised that the native RD114 envelope outperformed the chimeric envelope, RDpro. In regard to gene therapy, we show that RDpro supernatants effectively transduce peripheral blood HSCs. We believe that this level of transduction can be enhanced further upon optimization of the concentration protocol."

Dr. Steven R. Goodman, Editor-in-Chief of *Experimental Biology and Medicine* said "This exciting study by Anthony Bell and colleagues demonstrates the value of using nontoxic envelope proteins as a viable alternative to pseudotype lentiviral vectors. This has great potential for future gene therapeutic approaches".