Title: Use of Lentiviral vector for improved system of protein expression in mammalian cells

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Lentiviral vectors can efficiently deliver genetic payloads, making it possible to deliver expression cassettes that direct long term expression of recombinant transgene products, in broad range of cell types. The present dissertation reports development and efficacy validation of HIV-2 derived multiple differently configured transfer vectors with expanded utility for \textit{in vitro} and \textit{in vivo} transgenesis. Among the features imparted, the new ones include a blue/white colony screening platform, a reduced vector backbone and availability of default dual tags for functional proteomics studies. Simultaneously, panels with different utilities were also made these include neomycin or puromycin selection markers, with options of default promoter and availability of dual multiple cloning site (MCS). Each transfer vector format was tested by appropriate transgene expression function by transduction of target cells. During lentiviral transgene delivery, only the cells that are transduced by the vector receive the effect of the transgene coded recombinant protein. To bypass this limitation, we report a novel strategy to amplify the effect of the lentivirally delivered gene product in bystander cells. In this vector system the transgene coded protein is secreted with a cell penetrating peptide (CPP) allowing entry of the same to nearby untransduced bystander cells resulting effectively in an increased biodistribution of the delivered gene product. The efficacy of the enhanced biodistribution system was tested \textit{in vitro} and \textit{in vivo} using GFP as a transgene product and protein transfer to neighboring untransduced cells was observed when green fluorescent protein (GFP) was secreted with a CPP tag. This novel lentiviral vector platform can thus be used to effectively deliver recombinant proteins with enhanced bioavailability into the target organ for desired effect. Furthermore, LV platform was also effectively used for the development of novel reporter cell based antiviral screening assay for rapid evaluation of Tat-TAR interaction inhibitors. The system was validated by establishing a stable cell line and treating with \textit{tat} targeted shRNA and a small molecule inhibitor K37 \textit{in vitro}. This is the simplest assay developed so far requiring test material addition as the only manipulation for screening of putative Tat-TAR antagonists for adjunct AIDS therapy. Utility of this vector system was further expanded to device a mammalian expression system for the production of therapeutic recombinant human erythropoietin (rhEPO) in serum free medium. The expanded configurations of this indigenously developed LV will significantly aid in preferential applications and thus increase its utility as a versatile system for gene transfer technology. Appropriate vector formatting with methods of cell type specific gene delivery will further assist in better gene therapy strategy evaluation using Lentiviral vectors.