Targeted Delivery of shRNA to Cancer Cell

Abstract

Novel targeted delivery for shRNA was developed by exploiting bacterial minicells. In this work, shRNA against VEGF A was packaged in minicells. These minicells were linked with folic acid for active targeting of tumor cells via folate receptor *in vitro* and *in vivo*. In order to achieve this, *E.coli* PB114 was selected as a minicell producing strain. Subsequently, *E.coli* PB114 was transformed with pEZ43G-D in order to confirm segregation of plasmid DNA into the minicells. Minicells were purified from *E.coli* PB114 pEZ43G-D by a combination of ceftriaxone lysis and filtration. Ultrapure minicells have been obtained by this new method. Total number of minicells was found to be 3.81×10^{10} minicells/ml from 200 ml of starting culture. Cancer cell lines, LNCaP, HeLa and KB have been selected as positive control whereas A549 was selected as negative control in terms of folate receptor overexpression. shRNA expression vectors, psNIPERDH1A1 and psNIPERDU6A2, were transfected in selected cell lines and gene expression of VEGF A was analyzed by reverse transcriptase PCR in order to validate gene silencing of VEGF A. Both the expression vectors were found to downregulate VEGF A mRNA but psNIPERDU6A2 silenced VEGF A more efficiently, hence psNIPERDU6A2 was selected for *in vitro* and *in vivo* delivery. *In vitro* delivery was studied by delivering 10^9 minicells from each the group, 1) $^{FA}minicells_{pSUPERneo}Scramble$ 2) $minicells_{psNIPERDU6A2}$, 3) $^{FA}minicells_{psNIPERDU6A2}$ in selected cell lines and expression of VEGF A was analysed by RT-PCR. Expression of VEGF A did not changed in any of the group in A549 cell line. In contrast, expression of VEGF A reduced significantly in $^{FA}minicells_{psNIPERDU6A2}$ treated group when compared with other two groups in positive control cell lines. Moreover, uptake of folic acid conjugated minicells was found to be through receptor mediated endocytosis. Tumor xenograft of A549, LNCaP and KB cells were developed in immunosuppressed C57 BL6 mice. Animals were divided in four groups and treated with 1) Saline, and 10^9 of respective minicells 2) $^{FA}minicells_{pSUPERneo}Scramble$ 3) $minicells_{psNIPERDU6A2}$, 4) $^{FA}minicells_{PSNIPERDU6A2}$ intravenously. There was a gradual increase in the tumor volume till the end of treatment in all four groups of A549 xenograft. Whereas in case of LNCaP and KB xenograft, there was a significant decrease in tumor volume in $^{FA}minicells_{psNIPERDU6A2}$ treated group as compared to other groups. Similarly, expression of VEGF A was found to be same in all the groups when compared with the saline treated group

in A549 xenograft. On the contrary, significant downregulation of VEGF A was found in ^{FA}minicells$_{PSNIPERDU6A2}$ treated LNCaP and KB xenograft. Finally, *In vivo* biodistribution study of FITC loaded ^{FA}minicells$_{psNIPERDU6A2}$ revealed majority of fluorescence localize in the tumor followed by liver and heart. These results suggest that majority of ^{FA}minicells$_{psNIPERDU6A2}$ were distributed in tumor tissue which can be attributed to both passive and active targeting of ^{FA}minicells$_{psNIPERDU6A2.}$