Development of nano assisted dual delivery system for delivery of siRNA of P glycoprotein with cancer stem cells targeted anti-cancer drug

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Abstract

Cancer Stem Cells (CSCs) are small sub-population of heterogeneous phenotypes which reside within tumors. They initiate tumor with self-reliant angiogenesis and metastasis. The multidrug resistance (MDR) in cancer stem cells is the current drawback for failure of chemotherapy. ABCB1 gene, members of ATP binding cassette transporters (ABC) is overexpressed in drug resistant tumors. Over expressed ABCB1 may be one reason for drug resistance of CSCs via activating drug efflux pumps in the cell membrane. Gedunin has been identified as a potential drug lead for different type of cancers. Hydrophobicity of Gedunin results poorly soluble in the aqueous environment of cells thus minimizes its bioavailability and pharmacokinetic profile. Therefore nano encapsulated liposomal gedunin has been established by our team with improved anti-cancer activity. However effects of Gedunin or nano encapsulated Gedunin on bCSCs has not yet been studied. Therefore in the present study a nano assisted dual delivery system for gedunin and ABCB1 targeted siRNA (to target ATP binding cassette transporters) was developed with the aim of improving the anti-cancer activity of gedunin on drug resistance CSCs. Unloaded cationic liposomes were synthesized using stearylamine, cholesterol and phosphatidylcholine (egg lecithin) in ratio of 1:4:8 with average particle size of 97 nm and zeta potential of 42mV. Gedunin was successfully loaded into cationic liposome with encapsulation efficiency of 80% with average size of 97.22 ± 7.101 nm and a zeta potential of +35.2 ± 4.57 mV followed by encapsulation of siRNA into liposomal gedunin with average size of 234 ± 44.86 nm with a zeta potential of +41.3 ± 4.48 mV. Anti-proliferative effects of pure gedunin, liposomal gedunin, siRNA coated liposomal gedunin and paclitaxel were investigated by WST-1 assay using WST-1 Cell Proliferation Reagent (ready to use). All these treatments caused dose and time-dependent inhibition of cell proliferation in bCSCs. The siRNA coated liposomal gedunin demonstrated higher anti-proliferative activity in bCSCs with an IC50 of
8.470µg/mL. Liposomal gedunin appeared to be less active (IC50 =19 µg/mL) than siRNA coated liposomal gedunin. Pure gedunin showed less anti proliferative effects (IC50= 40.18µg/mL) than paclitaxel (IC50 = 24.06µg/mL). Immunofluorescence analysis confirmed the inhibition of expression of ABCB1 protein in siRNA coated liposomal gedunin treated bCSCs. The relative mRNA expression of ABCB1 and Cyclin D1 genes investigated in siRNA coated liposomal gedunin treated bCSCs showed a significant (P<0.005) down regulation at both the doses (1 µg/mL -fold change 31 and 2 µg/mL-fold change 3) whereas mRNA expression of Cyclin D1 showed a significant (P<0.05, fold change 3) down regulation only at 2 µg/mL. The siRNA coated liposomal gedunin also modulated the expression of selected apoptotic-related genes (p53, Bax and Survivin). The bCSCs treated with siRNA coated liposomal gedunin demonstrated significant (P<0.05) increase in expression of p53 at both the doses (1 µg/mL-fold change 1 and 2 µg/mL-fold change 7). The expression of Bax is up-regulated at both the doses tested but expression was not significant. Survivin showed a significant [(P< 0.05), fold change 21] down regulation at lower dose (1 µg/mL) and its down regulation at 2µg/mL was not significant. Overall results of the present study suggest that siRNA coated liposomal gedunin exert an antiproliferative effect on bCSCs. Importantly, it was identified for the first time that ABCB1 targeted siRNA coated liposomal gedunin induces antiproliferative effects on bCSCs.