PhD Thesis title: Assessing versatility of Tachyplesin in cancer therapeutics and as an antiviral agent

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Abstract

Marine environment is a rich source of various bioactive compounds of which multifunctional biologically active peptides have attracted increasing attention owing to their therapeutic properties. In this study we have employed marine derived antimicrobial cell penetrating peptide, Tachyplesin (Tpl) and assessed its activity in cancer therapeutics and as an antiviral agent. Tpl is cationic, 19 amino acid long peptide stabilized by disulphide bridges, originally isolated from marine horseshoe crab, *Tachypleus tridentatus.* Here, we demonstrated the cell penetrating and cargo delivery ability of Tpl by delivering anti-miR-210 in glioblastoma cell lines. The current treatment regimens for glioblastoma involve surgical resection followed by radiation therapy and chemotherapy and lead to poor prognosis. miRNA based therapeutics are promising candidates, but their activity is limited due to their cellular impermeability. miR-210 is an oncogene and it levels are upregulated in glioblastoma. Therefore, delivery of anti-miR-210 with aid of Tpl could be a promising therapeutic approach. In the present study, Tpl was non-covalently conjugated to anti-miR-210 and cellular uptake of complex was observed in glioblastoma cell lines. After internalization of the complex, significant reduction in miR-210 levels with a concomitant increase in the levels of its target genes was observed. The complex was also found to inhibit cell proliferation, spheroid formation, migration and induced apoptosis in GBM cells. Our findings demonstrate that efficient delivery of anti-miR-210 molecule with Tpl may hold great therapeutic potential for GBM treatment and other solid tumors with high miR-210 levels. Further the action of Tpl as anticancer agent was observed in combination with Doxorubicin. Combination therapy is emerging as an important strategy to achieve synergistic effects. Combining an anticancer peptide with a nonpeptidic cytotoxic drug is an example of this emerging field. This approach potentially reduces drug resistance, as lower therapeutic dosage of each individual agent is required. Cytotoxicity studies were done to determine the sub-cytotoxic concentrations of Tpl and Dox in breast cancer cells. This was followed by determining their anticancer action when used in combination. The combination of Tpl and Dox at their sub-cytotoxic concentrations caused significant cytotoxicity along with induction ROS levels. Keeping in view the results obtained, combination of Tpl and Dox may hold tremendous potential for developing effective breast cancer therapeutic regime. Further, the antiviral activity of Tpl against Hepatitis B Virus was investigated. Although the activity of peptides as antimicrobials is more validated as antibacterial and antifungal but their role as antiviral is less investigated. Uptake of Tpl in human hepatocyte cell lines at non-cytotoxic concentrations has been shown in the present study. Tpl further inhibited early stages of HBV replication by suppressing HBV

precore and HBV pregenomic RNA levels in a widely used cell culture model. Further, a Tpl-mediated reduction in secreted HBV proteins and hepatitis B virion secretion was also observed. In sum, Tpl has demonstrated distinct anti-viral potential against HBV.

In conclusion, the present study signifies potential of Tpl as a promising peptide-based therapeutic in cancer as well as in antiviral research emphasizing its diverse range of actions.