7 Summary

In vitro characterization of Aplysia punctata ink toxin (APIT) for its anti-cancer efficacy against human cancer cell lines and establishment of xenograft models for *in vivo* analyzing of APIT.

The increasing number of patients suffering from different forms of cancer remains a major health problem in industrialized countries. Although commonly used as standard treatment options, surgery, radiation and classical chemotherapy often result in severe adverse reactions, or are even unsuccessful due to the emerging problem of resistance linked to various drugs. For this reason, oncology research is focused on the search for novel drugs which are highly specific and efficacious. In the last decade in particular, substances isolated from marine organisms have been considered for their anti-tumor activity. The purple ink of the sea hare *Aplysia punctata* acts as an effective defense mechanism against natural predators. An active component of this ink, the 60 kDa *A. punctata* ink toxin (APIT) protein was isolated and subsequently analyzed for its cytotoxic activity. The aim of this work was to develop appropriate *in vivo* and *in vitro* model systems to evaluate the pharmacokinetics and cytotoxic/anti tumor activity of APIT.

First the efficacy of this novel drug was assessed *in vitro* against various human cancer cell lines representing not only the most prevalent forms of cancer including lung, breast, prostate and colon carcinoma, but also different forms of leukemia and various treatment resistant cancer types. In cell culture experiments all tested cell lines were shown to be highly sensitive to APIT. Both cancer cells of the hematopoietic system (Jurkat neo, CEM neo and K562) and cell lines representing solid tumors (GLC4, MCF7, SK-BR-3, PC3, DU145, HT29, RD-Es and A673) were efficiently killed by the cytotoxic activity of APIT. Very encouraging results were also obtained for the resistant cell lines GLC4/ADR and MCF7Bcl-X_L. The concentration of APIT required to kill 50% of cells (inhibitory concentration-IC_{50}) correlated well with the size of the cells and ranged between 3 and 12 ng/ml. Cells treated with APIT exhibit characteristic morphologies: adherent cell types lose their appendices and subsequently detach from neighboring cells, cells in suspension no form longer colonies, but exist individually instead. Both cell types were found to form vacuoles in their cytoplasm and exhibit an arrest in intracellular plasma and organelle movement. The additional/subsequent loss of metabolic activity and permeablization of the cell membrane ultimately lead to cell death.

In order to examine the efficacy of APIT *in vivo* murine xenograft models were established. For this purpose, various cancer cell lines shown to be sensitive for APIT's cytotoxic activity *in vitro* were injected subcutaneously into irradiated immuno deficient mice. After defining suitable experimental parameters, a cell concentration of 1 x 10^7 cancer cells was found to be ideal to successfully establish subcutaneous tumors in the xenograft models. Xenograft models were implemented for the leukemia cell line CEM neo, the lung carcinoma cell line GLC4 and its multidrug resistant phenotype GLC4/ADR, for the colon carcinoma cell line HT29 and the sarcoma cell line A673, all of them exhibited a growth rate of at least 80%. The xenograft models established in this work has laid the foundations for future systematic investigations on the anti-tumor activity of APIT.