Targeted peptide strategy against anaplastic thyroid carcinoma: EGFR-mediated delivery and PIP3-driven inhibition of the PAM pathway.

Zehra-Cagla Kahvecioglu[1], Rachel Baillif[1], Samuel Vandecasteele[1], Marine Bougard[1], Alison Daubry[1], Olivia Rasson[1], Céline Martens[1], Maurine Appels[1], Sophie Laurent[1,2], Sven Saussez [3], Carmen Burtea[1]

[1]*NMR and Molecular Imaging Laboratory, General, Organic and Biomedical Chemistry Unit, Faculty of Medicine, Pharmacy and Biomedical Sciences, University of Mons, B-7000 Mons, Belgium*
[2]*Center for Microscopy and Molecular Imaging (CMMI), Charleroi, Belgium*
[3]*Human Anatomy and Experimental Oncology Unit, Faculty of Medicine, Pharmacy and Biomedical Sciences, University of Mons, B-7000 Mons, Belgium*

zehracagla.kahvecioglu@umons.ac.be

Background:
Anaplastic thyroid carcinoma (ATC) is a rare but highly aggressive malignant neoplasm with a median survival of only four months post-diagnosis. Despite its low incidence (1–2% of all the thyroid cancers), it accounts for up to 39% of thyroid cancer-related deaths. Current treatments offer limited survival benefits, highlighting the urgent need for novel targeted therapies. One of the hallmarks of ATC is the dysregulation of key signaling cascades, particularly the PI3K/AKT/mTOR (PAM) pathway, which is critically involved in tumor cell survival, proliferation, and therapy resistance. EGFR overexpression and aberrant PAM signaling are major contributors to ATC aggressiveness.
Methods:
We developed a therapeutic peptide (TP) that disrupts PIP3-dependent protein interactions, thereby inhibiting AKT activation. To enhance specificity and reduce systemic toxicity, a vectoring peptide (VP) was designed to bind EGFR, which is overexpressed in ATC cells. Both peptides, identified via phage display technology, were chemically linked to form a peptide complex (PC). EGFR and AKT expression were assessed by western blot, immunofluorescence (IF), and immunohistochemistry (IHC) in ATC cell lines (8505c, Cal-62) compared to normal thyroid cells (Nthy-ori 3-1). Apoptosis was evaluated via Annexin V flow cytometry and activated caspase-3 detection. Subcellular trafficking was analyzed by IF co-localization of EGFR–endoplasmic reticulum (ER), VP–ER, and VP–caveolae. *In vivo* safety of VP was assessed in NMRI mice, while therapeutic efficacy of PC was tested in athymic nude mice bearing Cal-62 or 8505c tumors. Biodistribution was evaluated by optical imaging using IRDye800CW-conjugated VP.
Results:
ATC cells showed elevated EGFR and AKT activation, with nuclear localization of p-AKT (S473). PC induced significantly greater apoptosis than TP alone, with an EC_{50} of 5 µM (vs. IC_{50} of 24 µM for TP). IF co-localization studies revealed retrograde intracellular trafficking of the VP and nuclear accumulation of p-AKT. *In vivo*, PC treatment reduced tumor size and enhanced apoptosis, confirmed by activated caspase-3 (IF). Masson's Trichrome staining corroborated apoptotic features, including pyknotic nuclei. Toxicological analyses showed no morphological damage in major organs, suggesting minimal systemic toxicity. Liver alterations revealed by caspase-3 activation and elevated AST/ALT ratio were likely anesthesia-related. Elevated BUN levels indicated possible proteolysis and metabolic imbalance. Overall, histology and plasma biomarkers confirmed low systemic toxicity. CYP450 screening showed CYP3A4 inhibition by both PC and TP, suggesting potential interaction with hepatic metabolism. *In vitro*, KRAS and BRAF proteins exhibited increased nuclear localization especially in Cal-62 cells, reflecting their aggressive phenotype. *In vivo* fluorescence imaging confirmed tumor-specific accumulation of VP, validating its targeting capacity.
Conclusion:
This EGFR/PIP3-targeted peptide complex shows strong potential for the intracellular delivery of therapeutic agent in ATC cancer cells and demonstrates potent antitumor activity and limited toxicity in ATC models by selectively inhibiting the PI3K/AKT/mTOR pathway.