Galectin-1, a potential molecular imaging biomarker of well-differentiated thyroid cancers

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The worldwide incidence of the thyroid cancer, the most common endocrine malignancy, is still increasing nowadays. The main challenge is to diagnose the patients who really need a surgery. Indeed, despite the frequency of thyroid nodules, 90% of surgeries are made for benign lesions. Current diagnosis approaches imply painful and often useless thyroid surgeries. Thereby, this project aims to develop a new and non-invasive diagnosis approach of papillary carcinoma, which is the most frequent thyroid malignancy.

This work is based on the targeting of galectin-1 (gal-1) as a diagnostic tool for well-differentiated thyroid cancers. Gal-1 is a small adhesion protein expressed in muscles, neurons and some embryonic tissues in non-pathologic conditions. Mostly secreted in the extracellular compartment, this protein can also be found in the cytoplasm and the nucleus. It is involved in cellular adhesion, aggregation, migration, cytoskeleton reorganisation and cell cycle regulation phenomena. Moreover, gal-1 is overexpressed in a large variety of cancers (head and neck, skin, lungs, bladder, prostate, ovaries, colorectal region) and also in thyroid cancers. In fact, gal-1 is implied in tumour-induced immunosuppression, angiogenesis, hypoxia and metastasis.

During this project, the phage display technique has been used to identify peptides targeting gal-1. Six successive rounds have been performed by exposing the immobilized target to a phage library. Each phage clone wears on its capsid a different random peptide sequence. The pre-selection has been carried out on a control protein and after washing and elution steps, the phages bound to gal-1 have been collected and amplified to start a new round. The affinity towards gal-1 of these 6 outputs has been evaluated by ELISA and showed an increase along the rounds. From the sixth output, 50 clones have been isolated and their affinity evaluated. Two clones over fifty showed a good specific affinity towards gal-1. The peptide sequence has been revealed after DNA extraction and the two peptides were synthesized.

The affinity of these two molecules has been evaluated by ELISA ($K_d^{gal\text{-}1}$ = 2,38 x 10^{-6} and 4,1 x 10^{-8} for Peptide 1 and Peptide 8 respectively) and their cellular localization has been demonstrated by immunohistochemistry on human well-differentiated thyroid cancer slices. Clone 8 and its respective peptide appeared to be the best, according to ELISA. However, Peptide 1 has shown a better specific affinity on histological sections. Moreover, peptide 1 has perfectly co-localized with gal-1 on TPC-1 cells (derived from papillary thyroid cancer) as demonstrated by immunofluorescence. Interest will be henceforth focused on this peptide, which has been thereafter synthesized and conjugated to a contrast agent (USPIO) in order to visualize tumours *in vivo* by MRI.

Peptide 1 seems to be a promising targeting agent against gal-1 for the thyroid cancer imaging, once used to functionalize a contrast agent for MRI. After in vitro assays, vectorized contrast agents will be assessed on murine models of papillary thyroid cancer.

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