Mapping and analysis of cathepsin cleavage site distribution on the 3-D model of human thyroglobulin for potential inhibitor design.

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Hyperthyroidism is one of the abnormalities seen with the thyroid gland often resulting in Graves' disease with highly increased metabolism. The auto-antibodies generated by these patients often result in constitutively active downstream signalling by the thyroid stimulating hormone receptor leading to excess production of thyroxine (T4). High levels of T4 in the blood causes the symptoms of elevated metabolism in Graves' disease. In this study we hypothesized that by inhibiting the cathepsin cleavage sites on human thyroglobulin (hTg), one can reduce the hTg degradation by the lysosomal cathepsins and thereby reducing the formation of T4 in the thyroid gland. In order to achieve this goal, a homology model of hTg was built using SWISS MODEL server and all the possible cathepsin cleavage sites were mapped to understand their accessibility for inhibitor design. A total of 7 sites were identified and successfully mapped.

Key words: Human thyroglobulin, cathepsins, cleavage sites, molecular modeling, inhibitor design.

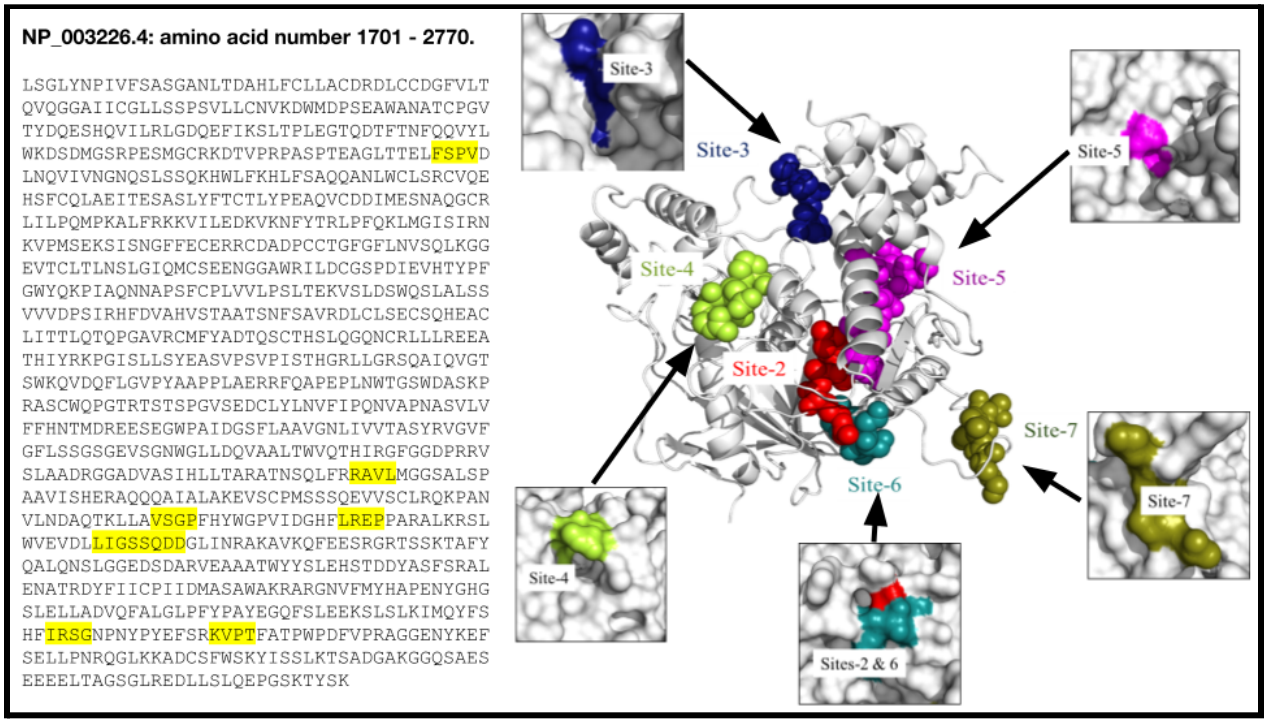

Figure 1. Human thyroglobulin amino acid reference sequence taken from the NCBI (NP_003226.4) with cathepsin cleavage sites highlighted in yellow (left) and corresponding homology model built using SWISS MODEL server with cathepsin sites highlighted in different colors.

Graves' disease (also known as hyper -thyroidism) is one of the thyroid disorders in which the patients make auto-antibodies against the thyroid stimulating hormone receptor (TSHR). Due to constitutive stimulation of the TSHR, the thyroid follicular cells produce more triiodo -thyronine (T3) and thyroxine (T4) that are released into the blood. The elevated levels of T3 and/or T4 in blood cause hypermetabolism and related symptoms of hyperthyroidism. Normally the negative feedback controls the stimulation of TSHR but in this case, due to the constitutive stimulation of TSHR by the auto-antibodies, the negative feedback control fails. Both T3 and T4 are produced from the tyrosine side chains of human thyroglobulin (hTg) and the lysosomal proteases release T3 and T4 by degrading the hTg. A panel of lysosomal proteases such as Cathepsins B, D, K, L, etc. are involved in the degradation of hTg at various stages releasing the T3 and T4. In other words, lysosomal protease-mediated degradation of hTg is required for the release of T3 and T4.

The auto-antibodies induce constitutive production of the hTg which, inturn, produces more T3 and T4 in the case of hyperthyroidism. Since it is challenging to control such auto-antibodies, one can always target the downstream pathway that involves hTg in order to control the excess production of T3 and T4. However, targeting the lysosomal proteases may result in non-selective complications and toxicity hence, hTg becomes an interesting therapeutic target. Not much has been explored about the hTg in terms of targeting its degradation to control hyperthyroidism. Hence, hTg should be further explored from a therapeutic point of view not only to design possible therapeutics but also to understand the mechanisms further in detail.

In this study we hypothesized that by using small molecules one can selectively target the lysosomal cleavage sites on hTg such that the protease-mediated degradation of hTg is reduced which in turn, decreases the release of T3 and T4 as a possible therapeutic approach for hyperthyroidism. Alternately, one can use inhibitors to inhibit the lysosomal proteases but this results in loss of selectivity because these inhibitors will inhibit lysosomal proteases in all cells, not just within thyroid follicular cells.

In the absence of structural data for human thyroglobulin (hTg), a homology model was built using the SWISS-MODEL server (2, 3). In order to understand the overall organization of the hTg, 3-dimensional analysis of its homology model was performed using Computational Biology tools. The homology model of hTg was prepared by uploading the amino acid sequence to the SWISS-MODEL server. The analysis includes evaluation of the secondary structure (alpha-helices and beta-strands) followed by hydrogen bonding analysis using PyMOL software. As shown in Figure 1, seven lysosomal protease cleavage sites were identified and mapped on the primary amino acid sequence of hTg. Out of the 7 sites, six sites are mapped onto the homology model as shown in Figure 1. Sites 2, 5 and 6 were found to be within the same vicinity while the rest of them were found well separated. Most of these sites matched for Cathepsins B, D and L. Small molecule inhibitor design to target these sites is currently in progress. Once designed, the small molecules have to be synthesized using synthetic organic chemistry tools. The synthesized small molecules have to be characterized for quality analysis followed by their evaluation *in vitro* using biochemical, biophysical and cell culture techniques for binding affinity potency analysis. The ongoing and upcoming research on the small molecule inhibitor design and their evaluation will be published in the future issues of TCABSE-J.

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