Transport and Targets

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THE PLEIOTROPIC FUNCTIONALITY OF ENDOGENOUS 3-IODOTHYRONAMINE (T1AM) AND SYNTHETIC THYRONAMINE-LIKE ANALOGS: A POWERFUL TOOL TO TARGET INTERLINKED DISEASES SUCH AS OBESITY AND NEURODEGENARATION

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Accumulating evidence has suggested the presence of a strong correlation between obesity and neurodegeneration. Neurodegenerative diseases (NDDs) are characterized by a progressive loss of memory and cognition, which ultimately can lead to death. This deterioration is mostly due to inflammation triggered by aberrant protein deposition, oxidative stress and modification in lipid pathways. Because of these multifactorial aspects, the design of multi-target directed ligand (MTDL) could represent a potential strategy for the treatment of NDDs. In this context, the polypharmacology described in good detail for naturally occurring 3-iodothyronamine (T1AM), and rapidly emerging also for thyronamine-like analogs SG-1 and SG-2, may provide a novel pleiotropic therapeutic approach for the treatment of NDDs.

With the aim to provide a detailed characterization of the pharmacological profile of these new drug candidates, in the present work we evaluated their ability to promote lipolysis in HepG2 cells, as well as, to activate clearing pathways, such as autophagy (ATG) and ubiquitine proteasome (UP) in human glioblastoma cells (U87-MG).

Methods: Cultured HepG2 cells were incubated for 24 h with 10 μM T1AM or SG-2 and Oil-red O staining was used to monitor intracellular lipid accumulation. Cell culture supernatants were also collected and analyzed for free glycerol release.

In another set of experiments, cultured U87-MG cells were treated with 1 μM T1AM, SG-1, SG-2 or vehicle for 30 min, 4, 8 and 24 h and the induction of ATG was monitored morphologically by using transmission electron microscopy (TEM) and immunofluorescence (IF) microscopy. Ultrastructural morphometry, based on the stoichiometric binding of immunogold particles, allowed the quantitative evaluation of ATG and UP component (i.e. LC3 and P20S, respectively) within autophagosomes and autophagoproteasomes. RT-qPCR and Western blot assays were applied to detect the expression of ATG and UP indicators.

Results: A significant decrease in lipid accumulation was observed in HepG2 cells treated with T1AM or SG-2, possibly due to increased lipolytic activity, further confirmed by accumulation of glycerol (an end product of triglyceride lipolysis) in the culture media.

Treatment with T1AM, SG-1 or SG-2 induced autophagy in U87-MG cells, by promoting autophagosome formation and up-regulating LC3-II expression and p62 degradation.

Notably, increased 20S proteasome recruitment to autophagosome was also observed, suggesting that these compounds might modulate both ATG and UP protein clearing pathways within the autophagoproteasomes.

Conclusions: Our studies highlight the potential of T1AM and its synthetic analogs, SG-1 and SG-2, as novel drugs for the treatment of obesity and NDDs.

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IDENTIFICATION OF ZINC TRANSPORTER ZNT8 IN THYROID TISSUES FROM CHILDREN AND ADOLESCENTS WITH THYROID DISEASES

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Introduction: Recent studies have revealed the presence of zinc and the expression of zinc transporter (ZnT) family members in most endocrine cell types. It has been demonstrated that the ZnT family plays an important role in the synthesis and secretion of different hormones. Furthermore, ZnT8Ab (zinc transporter-8 autoantibodies) together with GADAb (glutamic acid decarboxylase antibodies), IAA (insulin autoantibodies) and IA-2Ab (islet antigen-2 antibodies) are markers of autoimmunity in patients with type 1 diabetes mellitus (T1DM). We studied the expression of ZnT8 transporter in thyroid tissues from patients with thyroid nodular goiter (TNG).

Material and Methods: The study was performed in the group consisting of 17 patients with thyroid nodular hyperplasia (mean age, 17.8 years ± 4 years) and patients with pancreatic tumor as a positive controls. Patients were recruited from Polish endocrine centers. The ZnT8 expression protein was evaluated using immunohistochemistry. The specimens were paraffin embedded tissues, derived from the pediatric patients, who had thyroid nodular hyperplasia. The antibody against ZnT8 was goat polyclonal antibody (Santa Cruz Biotechnology USA; sc-98243). The antigen was retrieval was done using high pH (PTLink DAKO) and antibody was incubated in 4°C overnight in 1:50 dilution.

Results: In all of the examined cases we observed the ZnT8 expression in the thyroid follicular cells. He staining was strong and diffuse and observed in almost all thyroid follicular cells. The staining was observed in the cytoplasm. However in 2 out of 17 cases we observed C cells hyperplasia and ZnT8 expression was identified in those cells, also in the cytoplasm and the perinuclear area of the hyperplastic C cells.

Conclusion: According to our knowledge this is the first investigation which identified ZnT8 transporter in pediatric thyroid tissues. Further studies in thyrocytes covered by an autoimmune process are scheduled to confirm ZnT8 as a new thyroid autoantigen.

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REGIONAL HYPERTHERMIA ENHANCES SELECTIVE MESENCHYMAL STEM CELL MIGRATION TOWARDS THE TUMOR STROMA

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The strong tropism of mesenchymal stem cells (MSCs) for tumors provides the basis for a "Trojan Horse"-like therapy approach, in which genetically modified MSCs deliver a therapeutic gene into the critical microenvironment of growing tumors. Due to its dual role as reporter and therapy gene, the sodium iodide symporter (NIS) allows detailed noninvasive imaging of transgene expression and, subsequently a highly effective application of therapeutic radionuclides. To enhance the selective migratory properties of MSCs to the tumor stroma and thereby trigger targeted delivery of the NIS gene to the tumor, we are examining the pre-treatment of tumors with regional hyperthermia, as heat induces the secretion of immunomodulatory chemokines, cytokines and growth factors, well-known attractants of MSCs.

Human hepatocellular carcinoma cells (HuH7) were heat-treated in a water bath at 41° C for 1 h, followed by incubation at 37° C for 0–48 h. Chemokine mRNA analysis by quantitative real-time PCR indicated a substantial increase in expression levels of chemokines and growth factors after heat exposure,