SMALL MOLECULES TO TREAT DIABETIC RETINOPATHY: IN-SILICO AND IN-VITRO APPROACHES FOR DISCOVERY OF HUR INHIBITORS

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Purpose: Diabetic retinopathy (DR) is an inflammatory and microvascular disease. The ELAVL-1(HuR) RNA binding protein is able to stabilize the mRNA of TNF-alpha and VEGFA, whose corresponding proteins play a key role in DR-associated inflammation and angiogenesis, respectively. Inhibition of HuR-mRNA binding can represent a novel approach towards DR treatment. To this aim, we searched for novel compounds able to interfere with the HuR-mRNA complex.

Material and methods: Crystal structure of HuR (PDB:4EGL) and HuR-mRNA (PDB:4ED5) have been used for molecular docking of HuR inhibitors and inactive compounds. Compounds to be docked were selected from a proprietary database by means of Tanimoto similarity search on the basis of indomethacin structure. Therefore, twenty indole derivatives have been docked at 4EGL and 4ED5. Docking was carried out with the Schrodinger® package. Docking results have been re-scored with MM-GBSA calculations. Structural interaction fingerprints (SIFts) have been calculated for further analysis of docking results. Human retinal endothelial cells (HREC) were cultured in medium containing physiological glucose concentration (5mM) or high glucose concentrations (25mM). Lead compounds identified from virtual screening were tested at different concentrations, in order to evaluate by western blotting and Elisa their capability to modulate TNF-α and VEGFA expression via interaction with HuR protein.

Results: Two indole compounds VP12_14and VP12_110 were identified as best lead compounds and inhibitor of HuR-mRNA interaction. These two compounds, together with dyhidrotanshinone (positive control), protected HRECs from high glucose damage preventing the increase of TNF-α and VEGFA expression.

Discussion and conclusions: VP12_14and VP12_110, on the basis of pharmacological assays carried out, can be further developed as novel innovative anti-angiogenic and anti-inflammatory drugs for treatment of diabetic retinopathy.