A novel isoform of human Glucocorticoid-induced TNFR-related (GITR) protein that modulate activation and proliferation in effectors and regulatory T-cells

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Glucocorticoid-induced TNFR-related (GITR, also know as TNFRSF18) protein is a gene coding for a member of the TNF receptor superfamily. GITR activation influences the activity of effector and regulatory T cells (Treg), thus participating in the modulation of immune response against tumours and infectious agents, as well as in autoimmune and inflammatory diseases1,2. In mouse, four GITR splice variants have been identified3 (GITR, GITR-B, GITR-C and GITR-D), which are characterized by different downstream pathways thus acting in different manner on T-cells differentiation and proliferation. GITR is activated by its ligand (GITR-L)4 thus resulting in a co-stimulatory signal in effector T-cells5, both in mouse and humans. Recently Secreted and transmembrane (SECTM) 1A protein was identified as a novel GITR ligand in mouse6. In humans, the GITR system exerts diverse effect depending on the type of T-cells sub-populations; signaling of human GITR (hGITR) may be different from that of murine GITR (mGITR) due to its structural differences7,8. The aim of our study was to verify if novel hGITR isoforms exist and modulate the activation and differentiation of human effector T-cells and Treg. We found some hGITR splice variants, one of which, hGITR-4, maintains the fourth intron of GITR gene. This isoform entails a shift in the frame of the cytoplasmic region thus leading to a different translation from the main hGITR splice variant. GITR-4 is the ortholog of murine GITR-C and it appears conserved at protein level. Using qPCR we demonstrated that GITR-4 is expressed at a lower level in effector T-cells than in Tregs of healthy donors and after their activation (by aCD3/28 beads or PMA/ionomincine treatment) increases quickly at the mRNA level; moreover, the ratio hGITR/GITR-4 is equal to one in Treg cells isolated from SLE patients and much lower in healthy donors cells. We plan to study the role of GITR-4 in effector T-cells and Treg. To this aim we demonstrated by duolink experiments that SECTM1 is able to bind to the extracellular domain of hGITR; we also demonstrated, by qPCR, that SECTM1 is more expressed than GITR-L in T-lymphocytes at basal levels and under activation stimuli. Interestingly the binding of SECTM1 by an anti-SECTM1 antibody inhibits the effector cells and Treg proliferation while the binding of GITR-L by an anti-GITR-L antibody inhibits only effector T-cells proliferation. The possibility that SECTM1 binds hGITR and hGITR-4 at different level is still under investigation. In conclusion we identified a novel hGITR isoform that is involved in T-Cell differentiation and proliferation.