# Characterization of visfatin as a novel natural ligand of CCR5

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Visfatin/NAMPT/PBEF is a pro-inflammatory cytokine with several functions in physiology and pathology. Its circulating levels have been reported increased in several inflammatory and metabolic disorders, including cancer, in which it promotes cancer cells proliferation and migration. It has been reported that visfatin exerts its effect mainly though activation of different pathways such as MAPK, NF-kβ, Akt and STAT3. The activation of these pathways suggests that visfatin binds to a putative receptor, however the nature of this receptor is still unknown, it has been postulated to be the insulin receptor by Fukuhara et al., however this paper has been retracted. In 2012, Van den Bergh et al. found an interaction of visfatin with CCR5 using the *Surface Plasmon Resonance (SPR)*. However, no additional data have been documented so far on the real interaction in biological condition. Therefore, starting from this proof of principle, the aim of our study was to shed light on the possible interaction of visfatin with CCR5.

CCR5 (CD195) is the C-C chemokine receptor type 5 and it is involved in inflammatory response, thought migration and the polarization of immune cells, HIV infection, and in cancer, in which it regulates cancer progression and metastasis. To date several natural ligands of CCR5 are known; the agonists are RANTES, MIP-1α and 1-β and CCL2-8-11-14, while MCP-3 is known to act as natural antagonist. When an agonist binds to the receptor induces PLC-γ activation and calcium influx. In order to study the ability of visfatin to interfere with CCR5, we analysed the ability to modulated calcium signalling.

We took advantage to microglia cells, which are well known to over-express a large amount of CCR5. Pure microglia cultures and microglia/astrocytes cultures were obtained from brain of P2 mice pups and calcium signalling was analysed loading cells with FURA-2AM. Despite literature data, we failed to obtain any statistical results using RANTES (the most characterized agonist) in these cells. We therefore created a stable HeLa cell line over-expressing murine CCR5 (HeLa/CCR5) and compared the activity of RANTES and visfatin in HeLa/CCR5 vs HeLa/WT. In HeLa/CCR5 cells, RANTES induces a sustained calcium influx compared to HeLa/wt cells, demonstrating that our tool was working. In these cells, RANTES induces a concentration (>50 ng/ml) dependent calcium signalling. Surprisingly, visfatin, also at high concentration (500 ng/ml), failed to induce similar calcium effects in HeLa/CCR5. These raw data suggest that visfatin is not a natural CCR5 agonist, however we cannot exclude that visfatin is still able to bind to CCR5 and exerts antagonistic effect. Indeed, the pre-treatment of HeLa/CCR5 cells with visfatin (500 ng/ml) blocks RANTES-dependent calcium signalling in a dose-dependent manner. Moreover, the inhibitory effect of RANTES-dependent calcium signalling was similar to that of maraviroc, a negative allosteric modulator of CCR5 and retroviral drug used in the treatment of HIV infection.

This preliminary data suggest a possible interaction between visfatin and CCR5, demonstrating an antagonistic affect of visfatin preventing RANTES calcium signalling. To validate and to characterize this interaction, we will investigate the ability of visfatin to modulate migration of HeLa/WT and HeLa/CCR5 and to activate CCR5 downstream pathways. Finally a competition binding study is needed to demonstrate the real nature of visfatin as a new antagonist of CCR5 and this new finding will open new direction in understanding the natural visfatin receptor and its function.