Characterization of a possibile interaction between CCR5 and eNAMPT

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Visfatin/eNAMPT/PBEF is a cytokine released by various cell types[1]. Increased circulating levels of eNAMPT have been reported in several inflammatory and metabolic disorders, including cancer. It has been postulated that eNAMPT acts through a putative receptor and activates pathways such as MAPK, NF-kB, Akt and STAT3. However, the nature of this receptor is still unknown. The insulin receptor was first postulated to act as an eNAMPT receptor[2], but the manuscript was retracted and the finding was never confirmed. More recently, Camp et al. have reported that eNAMPT-induced NFκB activation might occur via TLR4 (toll like receptor-4) ligation[3].
Van den Bergh et al.[4] have also reported a specific interaction between eNAMPT and CCR5 although their manuscript did not investigate the physiological role of this interaction. CCR5 is the C-C chemokine receptor type 5 and its natural agonists are RANTES (CCL5), MIP-1Î±, MIP-1β (CCL3 and CCL4 respectively) and CCL2-8-11-14, while MCP-3 (CCL7) is known to act as the natural antagonist. The CCR5-RANTES interaction activates different pathways such as transient calcium influx via PLC-γ, or proliferative pathways such as MAPK, NF-kB, Akt and AMPK.
The aim of the present work was to evaluate whether eNAMPT could bind the CCR5 receptor to explicate its effects on the cells. This could be translated in a role of CCR5 as a possible receptor for eNAMPT.
We have generated a stable cell line that markedly over-expressed murine CCR5 (HeLa-CCR5) and investigated phosphorylation of ERK and STAT3 as well as cAMP and calcium signalling.
RANTES induced a strong activation of MAPK in Hela-CCR5. On the contrary, eNAMPT did not induce MAPK activation and it was not able to revert the effect of the natural ligand. Similarly, cAMP production mediated by RANTES seemed was unmodified by eNAMPT.
On the contrary, pre-treatment of HeLa-CCR5 with eNAMPT resulted in the blockage of RANTES-dependent calcium signalling. In addition, eNAMPT did not modify other calcium signalling pathways triggered by ATP and carbachol, suggesting that the effect of eNAMPT may be specific for CCR5.
Last, we evaluated whether eNAMPT could be an allosteric modulator of CCR5 by investigating receptor internalization. Yet, eNAMPT pre-treatment was not able to modify CCR5 internalization.
Our work, which follows reports on a physical interaction between eNAMPT and CCR5 by plasmon resonance[4], suggests that a link exists between these two pathways (in particular regarding calcium signalling) but is unable to confirm that this is directly linked to eNAMPT binding to the CCR5 receptor.

References

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