INVESTIGATION OF A PUTATIVE NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) RECEPTOR

1)Torretta S. 2)Colombo G. 3)Manfredi M. 4)Travelli C. 5)Ambra AA. 6)Genazzani AA.

Università del Piemonte orientale

Nicotinamide phospho ribosyltransferase (NAMPT) is a pleiotropic protein that exists in two forms: an intracellular form (iNAMPT), which acts as an important enzyme in NAD biosynthesis and an extracellular form (eNAMPT), a cytokine released by various cell types with autocrine and paracrine effects. However, it has been reported that eNAMPT has a physiological role on a wide range of cells: (i) controls angiogenesis, migration and invasion of endothelia and cancer cells; (ii) it has a cardio-protective and neuro-protective effects on cardiomyocytes and neurons; (iii) it induces proliferation and it increased insulin secretion in adipocytes and □-cells; (iv) last, eNAMPT controls differentiation and polarization of macrophages (Grolla et al., 2016). Although, eNAMPT might potentially remain enzymatically active upon release, it has been postulated that might act through binding to a putative receptor. The nature of this receptor is still unknown, however to date two putative receptors have been proposed: CCR5 (C-C chemokine receptor type 5) and TRL4 (Toll-like receptor 4). Indeed, it has been reported that eNAMPT-induced NFκB activation might occur via TLR4 binding (Camp et al. 2015). Furthermore, a physical interaction between eNAMPT and CCR5 has been also proven (Van der Bergh et al., 2012).

We first investigated the ability of eNAMPT to bind CCR5. We demonstrated that, despite CCR5 did not seem to be the main eNAMPT receptor, a link exists between them. Indeed, eNAMPT did not activate CCR5 signalling, but it reduced RANTES (the natural ligand of CCR5)-dependent calcium signalling, suggesting that eNAMPT could bind CCR5, but mainly acting as a partial antagonist. The involvement of TLR4 in eNAMPT signallig was also investigated and no significant results were obtained.

Starting from these evidences, we changed our strategy and we decided to investigate the existance of another possible eNAMPT receptor. We set up ligand-binding assay using fluorescently-labelled eNAMPT (eNAMPT-488 conjugated) on purified plasma membrane obtained from brain, heart, kidney, liver, lung and bone marrow of adult mice (C57Bl/6). A dose response curve of fluorescent 488-eNAMPT (1-5 μg) was made and un-labeled eNAMPT was used to set up a competitive binding assay. Our preliminary data demonstrated a binding of 488-eNAMPT to the membrane of all tissues tested, with high intensity of fluorescent in the brain and bone marrow, moderate binding in heart, kidney and liver, and lower in the lung. Importantly, the binding of 488-eNAMPT is reverted by the pre-treatment with high amount of un-labeled eNAMPT (500μg). Next, we immuno-precipitated eNAMPT (NI-NTA resin) with the plasma membrane, then the samples were cross-linked and solubilized membrane proteins were analysed through Western-blot and silver staining. We identified 6 putative bands conserved between organs, and we are analysing them by LC-MS technique to identify a novel putative receptor.

References

Grolla AA et al., (2016) Br J Pharmacol. 173(14):2182-94

Camp et al., (2015). Sci Rep. 14;5:13135

Van den Bergh et al., (2012) PLoS One. 7(4):e35074