NF-κB-mediated regulation of astrocyte-secreted signals modulating the differentiation potential of adult hippocampal neural progenitor cells

S. Cvijetic[1], V. Bortolotto[1], E. Marengo[2], M. Manfredi[2,3], E. Ranzato[2], P.L. Canonico[1], M. Grilli[1]

[1]Dept. Scienze del Farmaco, Università del Piemonte Orientale, Via Bovio 6, 28100, Novara, Italy
[2]Dept. di Scienze ed Innovazione Tecnologica, Università del Piemonte Orientale, v. T. Michel 11, 15121, Alessandria, Italy
[3]ISALIT srl - Dept. di Scienze ed Innovazione Tecnologica, Università del Piemonte Orientale, v. T. Michel 11, 15121, Alessandria, Italy

The hippocampal SubGranular Zone (SGZ) is characterized by the presence of the neurogenic niche, a highly specialized microenvironment which is both instructive and permissive for adult neural progenitor cells (NPC) and their progeny. In previous work we proved that members of the NF-κB family of transcription factors are important contributors of signalling pathways in the SGZ neurogenic niche and in the response of adult NPC to several clinically relevant drugs. Within the family, the p50 subunit appears to play a crucial role since p50KO mice display dramatically reduced adult hippocampal neurogenesis in association with short-term memory defects. However, when adult NPC derived from wt and p50KO mice are cultured *in vitro*, no significant differences can be observed in their neurogenic potential, suggesting a potential contribution of other cell subpopulations within the niche to defective neurogenesis in mutant mice. To this purpose we have set up enriched astrocyte cultures from hippocampi of p50KO and wt neonatal mice and studied their influence on wt or p50KO NPC by using astrocyte-conditioned medium (ACM). When wt NPC where exposed to wt ACM, an increased rate of differentiation towards both neuronal and astroglial lineages was observed, in comparison with standard medium. Conversely, p50KO ACM significantly increased the percentage of newly generated astrocytes, but lacked proneurogenic effect on wt NPC. Moreover, wt and p50KO ACM promoted neither neurogenesis nor gliogenesis in p50KO NPC. We then decided to actively pursue the identification of proneurogenic and/or antineurogenic signals that, under control of NF-kB p50, may be differentially regulated in astrocytes. To this purpose we have analyzed wt and p50KO astrocyte secretome through a high throughput label-free protein quantitation method called SWATH-MS which allows the relative determination of secreted proteins and identified several novel molecules which appeared differentially modulated in absence or presence of p50.
Altogether our data suggest that neurogenic defects observed *in vivo* in p50KO mice are both cell autonomous and non-cell autonoumous, and that they may also involve phenotypic changes in the secretome of astroglial cells. In addition, they add another level of complexity to the role of NF-κB p50 in the regulation of adult hippocampal neurogenesis.