ImmunoTools IT-Box-Cy55M-Award 2013

Giulia Magni

PhD Supervisor: Prof. Dr. Maria Pia Abbracchio

Italian Institute of Technology, Genova, and Department of Pharmacological and Biomolecular Sciences,
University of Milan, via Balzaretti 9, 20133 Milan, Italy

THE PURINERGIC SYSTEM AS TARGET FOR INNOVATIVE ANTI-MIGRAINE DRUGS

Migraine is a chronic neurovascular disorder affecting 15% of adults in the Western World, typically characterized by recurrent attacks of disabling headaches and associated autonomic symptoms. Despite recent advances in the comprehension of migraine pathophysiology, its pharmacological treatment still remains unsuccessful in a significant number of patients. For this reason, new efforts in clarifying the mechanisms at the basis of its onset, in discovering innovative targets, and in developing new drugs are strongly needed. While the search for new pharmacological approaches to the management of migraine pain has mainly focused on neurons, growing evidence suggests an important role for glial cells as well. In fact, several data clearly indicate that satellite glial cells (SGCs) modulate excitability of sensory neurons in the trigeminal ganglion (TG). Among the possible signaling pathways involved in this cell-to-cell communication, the purinergic system is one of the most intriguing and yet not fully explored, although evidence is accumulating on its role in pain transmission. Data obtained in the laboratory where I currently work in Milan suggest that extracellular nucleotides operate as key signaling molecules in neuron-to-non-neuronal cell communication within the TG, in close interconnection with other already known pain mediators like the calcitonin gene-related peptide (CGRP) signaling pathway. In fact, our data show that, by activating its specific neuronal receptors, the pro-algogenic mediator BK induces the release of CGRP, which in turn stimulates the ERK1/2 MAP kinase signaling pathway in surrounding SGCs, and increases P2Y receptor-mediated intracellular calcium responses.

My PhD project is therefore aimed at dissecting out the molecular basis of the purinergic system hypersensitivity, and its crosstalk with other pro-algogenic mediators, with the final goal of opening new perspectives for the discovery of effective pharmacological approaches. To reach our purpose, I am utilizing an in vitro model of mixed neuron-SGCs TG cultures from C57BL6 mice previously set up in our laboratory, and to better investigate the molecular pathways linking glial CGRP receptor activation to the purinergic system on glial cells, I have also generated an *in vitro* model of purified SGCs cultures, completely depleted of neurons.

Based on literature data showing that a basal pro-inflammatory state is crucially involved in the development of trigeminal sensitization, I have investigated changes

in the extracellular level of several immunomodulatory molecules after CGRP treatment. I could observe that overnight exposure to 1 µM CGRP significantly modulated the release of 12 cytokines by at least 1.5-fold. The vast majority of both pro- and anti-inflammatory mediators was up-regulated, with only pro-inflammatory IL17 and IFNγ levels being reduced. These results show that exposure to CGRP produced an extracellular immunomodulatory milieu, which could possibly participate to the development of migraine pain and trigeminal sensitization. My current hypothesis is that the observed inflammatory milieu could be important in the P2Y receptors potentiation observed in our experimental model.

For this reason, I am planning to investigate in detail the role of cytokines/chemokines and other inflammatory mediators in P2Y receptors activation on SGCs. In particular, we are interested in evaluating if cytokine release is a cause or a consequence of P2Y receptors potentiation after CGRP exposure. Therefore, I will treat our SGCs purified cultures with cytokines included in the ImmunoTools *IT-Box-Cy55M,* and I will verify by means of single cell calcium imaging if they can either increase or decrease the activation of glial P2Y receptors. Moreover, I will also check for the expression levels of the P2Y receptor subtypes of interest in the presence of the selected inflammatory mediators. I will start with the mediators which, based on my previous analysis, are released by SGCs upon CGRP exposure, namely IL-3 and IL-2, but I will also test combinations of different pro- or anti-inflammatory mediators included in the *IT-Box-Cy55M*. These experiments will allow me to elucidate the exact role of pro- and anti-inflammatory molecules in TG sensitization, and in the modulation of the purinergic system, to be subsequently validated as a pharmacological target to develop new anti-migraine drugs.

ImmunoTools *IT-Box-Cy55M* for **Giulia Magni**
includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9, rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1α / CXCL12a, rm SDF-1β / CXCL12b, rm TNFα, rm TPO, rm VEGF DETAILS