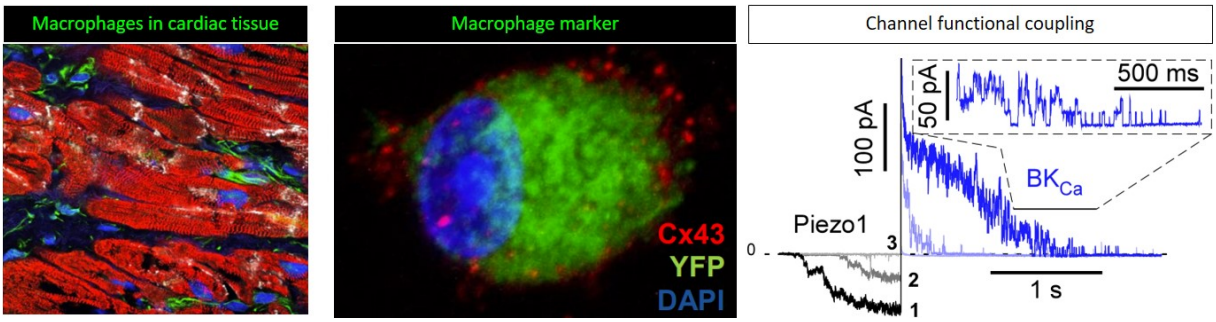
Master / MD Thesis Project

Fields involved: cell biology, mechano-biology, signalling, biophysics, electrophysiology
Duration: 6 to 12 months (M.Sc. or Dr. med. project)

Title: Mechano-sensitive channels in cardiac macrophages: Interaction between Piezo1 and BK_{Ca} channels

Throughout a lifetime, the heart pumps blood to all parts of the body by rhythmic contraction and relaxation. This mechanical activity exposes all cells within the heart to cyclic changes of stretch and compression. We, and others, have identified stretch-activated channels in various cells types that can sense mechanical forces and transduce them into cellular responses[1-5]. It is well established that cardiomyocytes are sensitive to their mechanical environment via stretch-activated ion channels that may provide mechano-electric feedback[6]. Cardiac fibroblasts (FB) play a crucial role for the mechanical stability of the heart by controlling production of the extracellular matrix[7, 8]. In cardiac FB we have recently detected the stretch-activated channel Piezo1, which is capable of changing cell stiffness (Emig et al. 2021)[3]. In addition, we characterized a functional interaction between Piezo1 channels and the Ca^{2+} activated K^+ channel of large (big) conductance (BK_{Ca} channel) (Jakob et al. under revision).
Besides FB and cardiomyocytes, cardiac immune cells represent an abundant cardiac cell population, both in healthy myocardium and following cardiac injury[9, 10]. These cells do not only play an important role for tissue maintenance by clearing cell debris, and during inflammation, but may also be involved in electrophysiological integrity as they were shown to couple directly to cardiomyocytes via connexins. Cardiac resident macrophages express multiple functional ion channels[11], but it is not known whether these are required for electrophysiological or immunological function. Recently it was shown that macrophages in the lungs sense cyclic hydrostatic pressure changes via stretch-activated Piezo1 channels and transform them into a coordinated inflammatory defence response (Solis et al. 2019)[12].

In search for stretch-activated ion channels in primary cultures of mouse and human macrophages, the candidate will do cell-attached patch clamp studies and simultaneously apply stretch to the membrane via controlled suction device. Electrophysiological, molecular and pharmacological tools will be applied to identify SAC. In particular, he/she will look for the cation nonselective SAC Piezo1 and the potassium-selective channel BK_{Ca}.



Macrophages staining in tissue, confocal image of a macrophage in culture, channel functional coupling present in FB and to be investigated in macrophages

Project 1: Interaction of Piezo1 and BK_{Ca} channels in mouse and human macrophages

Background: The heart consists of distinct cell types including resident macrophages, which were recently shown to functionally express different ion channels. It was suggested that macrophages

exert indirect electrophysiological effects via electrotonic coupling to cardiomyocytes. Since macrophages are exposed to mechanical cues during the contraction-relaxation cycle of the heart, we hypothesize that they can sense their mechanical environment via stretch-activated channels.

Central aim: This project aims at understanding mechano-sensing and mechano-transduction in cardiac resident macrophages.

Preliminary data: Preliminary experiments suggest that macrophages express two functional stretch-activated channels, Piezo1 and BK_{Ca}. The aim of this project is to characterize the electrophysiological properties of these SAC channels and to study their putative interactions.

Specific aims:

- Electrophysiological and pharmacological characterization of Piezo1 channels using cell attached patch clamp technique
- Similar characterization of BK_{Ca} channels, including Ca^{2+} sensitivity
- Investigation of the functional interactions between Piezo1 and BK_{Ca} in patch-clamp experiments.

Material: Murine and human cardiac resident macrophages in primary culture
Techniques: Cell culture, patch clamp, imaging

Project 2 : Piezo1 and BK_{Ca} channel remodelling in atrial fibrillation

Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia, affecting 33.5 million people worldwide. With an ageing population, prevalence and number of individuals with atrial fibrillation will continue to grow in the coming years. Tissue remodelling in general and fibrosis more specifically, combined with atrial mechanical overload are major substrates of AF, favouring its progression. Mechano-sensitive channels have been implicated in tissue remodelling in different organs and diseases. We show that Piezo1 and the Big Potassium channel (BK_{Ca}), two mechano-sensitive channels, are both active in human atrial fibroblasts and see their activity remodelled in cells isolated from AF patients.

Central aim: this project aims at characterising the interactions between the two mechano-sensitive channels and assessing their contribution to AF-induced fibrosis.

Preliminary data: Piezo1 and BK_{Ca} channel activities have been recorded in the lab and our data show a remodelling of their activity in cells isolated from AF patients compared to cells isolated from patients in sinus rhythm (SR). The activity of Piezo1 is increased in AF cells while the one of BK_{Ca} is decreased. From the literature, we know that Piezo1 can conduct calcium and BK_{Ca} are calcium-activated channels.

Specific aims:

- By using the patch clamp technique, assess if BK_{Ca} stretch activation could be due to/influenced by Piezo1.
- By using imaging techniques, identify where Piezo1 and BK_{Ca} are located
- By using immunocytochemistry, characterise the organisation and the composition of the extracellular matrix produced by cells expressing or not Piezo1 and BK_{Ca}

Material: Human macrophages in primary culture
Techniques: Patch clamp, imaging, cell culture, immunocytochemistry

References

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