Agota Apati et al., J Tissue Sci Eng 2016, 7:2(Suppl)
http://dx.doi.org/10.4172/2157-7552.C1.024

Visualization of calcium signals by genetically engineered calcium indicators in human pluripotent stem cells and their differentiated progenies

Agota Apati[1], **Tünde Berecz**[1], **Eszter Szabo**[1], **Tamas I Orban**[1], **Laszlo Homolya**[1], **Janos Rethelyi**[2] and **Balazs Sarkadi**[1]
[1]Research Centre for Natural Sciences, Hungary
[2]Hungarian Academy of Sciences, Hungary

Human pluripotent stem cells (hPSC) provide new approaches to study the development and differentiation of various cell types of the human body and research tools for disease modeling. Meanwhile, the basic signaling events, including $Ca2^{+-}$signals, have not been comprehensively evaluated in these cells. Therefore, we have started to study intracellular calcium responses to various ligands and the calcium signaling pathways in hPSCs and in their differentiated derivatives. First, we developed a protocol using the small molecule fluorescent $Ca2^{+-}$indicator Fluo-4 and confocal microscopy. While this method was informative, several technical problems hindered this approach. To overcome these difficulties (i.e. dye-loading and toxicity), we generated hPSC lines which stably express a genetically encoded $Ca2^{+-}$indicator (GCaMP2) protein by using a transposon-based gene delivery method. In this stable indicator system, we successfully studied the effects of various ligands in undifferentiated hPSCs and in hPSC-derived cardiomyocytes. To extend our knowledge towards $Ca2^{+-}$signals in hPSC-derived neural cell types, we also generated GCaMP6fast expressing neural progenitor cells (NPCs) and then differentiated these to PROX1-positive hippocampal neurons. Next, we investigated the calcium signals in NPCs and PROX1-positive hippocampal neurons expressing GCaMP6fast, or loaded with Fluo-4 in parallel experiments. We found that specific, ligand induced $Ca2^{+-}$signals were similar in the two assays, suggesting the applicability of GCaMP6f for the investigation of fast $Ca2^{+-}$signals in neural cultures. We conclude that characteristics of both the spontaneous and ligand-induced $Ca2^{+-}$signals, as well as their pharmacological modifications can be successfully examined in these model cells by fluorescence imaging.

Biography

Agota Apati has completed her PhD in 2003 from Semmelweis University School of Medicine and Post-doctoral studies from National Blood Service. She is the Leader of Human Pluripotent Stem Cell lab in Research Centre for Natural Sciences of Hungarian Academy of Sciences. She is interested in differentiation, signaling and disease modeling of human embryonic and induced pluripotent stem cells. She has published more than 30 papers in reputed journals.

apati@biomembrane.hu

Notes: