Efficient Generation of Functionally Mature Left Ventricular Cardiomyocytes from Human iPSCs for Cardiac Research and Drug Testing

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Processo FAPESP 20/14635-1; Processo FAPESP 24/09281-7

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INTRODUCTION: Cardiomyocytes derived from induced pluripotent stem cells (CM-iPSCs) are essential tools for pathophysiological studies and the development of novel cardiovascular therapies[1,2].However, maintaining the pluripotent characteristics and ensuring efficient differentiation requires standardized culture conditions to preserve cell viability[3,4]. Typically, CM-iPSCs exhibit immature properties reminiscent of fetal cardiomyocytes, including low sarcomeric organization, limited mitochondrial oxidative capacity, and altered calcium handling. These features constrain their utility in pharmacological and toxicological applications[5,6]. To overcome these limitations, metabolic modulation has been explored as a strategy to promote cardiomyocyte maturation, enhancing their functional characteristics and advancing them toward a more adult-like phenotype[7,8]. Additionally, region-specific differentiation protocols such as those targeting the generation of left ventricular cardiomyocytes have yielded more homogeneous and functionally mature populations[1]. The generation and propagation of action potentials (APs) in cardiomyocytes depend critically on ionic currents, particularly the sodium current (INa). Both fetal and adult cardiomyocytes can exhibit modulation of INa in response to pyrethroid insecticides and other drugs, providing insights into the electrophysiological properties of these cells[9,10]. **Aim:** To establish a rapid and effective protocol for the differentiation and maturation of induced pluripotent stem cells into a homogeneous and functional population of mature left ventricular cardiomyocytes (LV-iPSCs).**METHODS:** Human iPSCs were cultured and subjected to two differentiation protocols: a standard method for generating CM-iPSCs and a region-specific protocol for generating LV-iPSCs. The effects of penicillin-streptomycin (PS), varying concentrations of CHIR and BMP4, and metabolic maturation (MM) were systematically evaluated. Following differentiation, cardiomyocytes were characterized through histological, molecular, functional, and pharmacological analyses, including Patch-Clamp technique.**RESULTS:** Optimization of the iPSC culture revealed that eliminating PS from the medium was critical, as its presence adversely affected AP generation. Electrophysiological recordings showed that CM-iPSCs did not generate APs 30 days after the initiation of differentiation. At 90 days, these cells displayed immature APs with low INa amplitude (~1nA). However, when subjected to MM, CM-iPSCs exhibited this immature AP and increased INa amplitude (~3nA) after just 30 days. Among the conditions tested for LV-iPSC differentiation, the combination of 5 μM CHIR and 3 ng/mL BMP4 proved most effective. After 30 days, LV-iPSCs displayed a phenotype comparable to CM-iPSCs treated with MM, showing robust APs and similar INa amplitude (~3nA). Notably, when LV-iPSCs were also subjected to MM, INa amplitude increased markedly (~12nA), and AP morphology closely resembled that of mature cardiomyocytes. These findings indicate that this protocol effectively yields a functionally mature and homogeneous population of ventricular cardiomyocytes. Furthermore, exposure to the pyrethroid β-cyfluthrin selectively increased the late component of INa and prolonged AP duration in LV-iPSCs, but not in standard CM-iPSCs, suggesting that LV-iPSCs possess a more mature and responsive electrophysiological profile.**CONCLUSIONS:** This study highlights the importance of optimizing both iPSC culture conditions and differentiation protocols. The combination of region-specific cardiac differentiation and metabolic maturation significantly accelerated the generation of mature left ventricular cardiomyocytes. These cells exhibited enhanced electrophysiological properties and responded predictably to pharmacological agents, underscoring their suitability for use in toxicology, pharmacology, and regenerative therapies for cardiovascular diseases. The proposed protocol is a cost-effective and time-efficient method for producing functionally mature cardiomyocytes.

Keywords: Human cardiomyocytes derived from iPSCs; Cell differentiation; Metabolic maturation;

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