AN OPTIMIZED PROTOCOL OF ELECTROPORATION OF *HEPATOCYTE NUCLEAR FACTOR 1 ALPHA (HNF-1α)* IN MESENCHYMAL STEM CELLS

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*The objective of this study was to investigate the optimization of electroporation of hepatocyte nuclear factor 1 alpha (Hnf1-α) in murine mesenchymal stem cells (mBM-MSCs). mBM-MSCs were phenotypically observed and confirmed by positive expression of stemness markers with differentiation capacity into osteocytes. Hnf1-α plasmid DNA was transfecDVted via Neon electroporation system into the mBM-MSCs. The cells were maintained in a complete DMEM medium. Following single 0.5 μg Hnf1-α electroporation the differences in viability of mBM-MSCs were statistically insignificant at 24, 72, and post-21 days. Fluorescence imaging of turbo green fluorescence protein (tGFP) was detected for the efficiency of transfection. The transfection efficiency was detected at parameters of 1000 pulse voltage (v), 10 pulse width (ms), and at 3 pulse number at 24 hours (***p-value < 0.001, 66.5 ± 12.2) in mBM-MSCs. The efficiency of transfected 0.5 μg Hnf1-α was decreased at 72 hours (40.2 ± 10.9) and 21 days (31.7 ± 5). 250 μg/ml G418 Sulfate was used for the selection of Hnf1-α transfected positive cells. TaqMan-qRT-PCR results of independent experiments revealed significant fold differences in Hnf1-α expression with above mentioned defined parameters. Therefore, 0.5 μg Hnf1-α plasmid into 2.5 × 10^5 mBM-MSCs with a pulse voltage of 1000 v, pulse width of 10 ms, and pulse number of 3, was optimized, which was not reported before. These parameters can be considered for transfection with cell viability of 65–96 % from 24 hours to 21 days and 60 –70 % transfection efficiency after 24 hours. Hence, this optimized procedure with efficient transfection rates can be applied for further gene functions and differentiation studies in the liver, pancreas, kidney, intestine, and for other tissues in specialized niches.*

Key words: *Alamar blue, G418, Pulse, Stem Cells, Transfection Efficiency, Viability, Voltage.*

ОПТИМІЗОВАНИЙ ПРОТОКОЛ ЕЛЕКТРОПОРАЦІЇ ЯДЕРНОГО ФАКТОРА 1 АЛЬФА ГЕПАТОЦИТІВ *(HNF-1α)* У МЕЗЕНХИМАЛЬНИХ СТОВБУРОВИХ КЛІТИНАХ

Мета цього дослідження полягала у вивченні оптимізації електропорації ядерного фактора 1 альфа гепатоцитів (Hnf1-α) у мезенхимальних стовбурових клітинах мишей (мКМ-МСК). мКМ-МСК були виявлені фенотипічно і підтверджені за позитивною експресією маркерів стовбуровості зі здатністю до диференціації в остеоцити. ДНК плазміди *Hnf1*-α трансфікували за допомогою системи електропорації Neon в мКМ-МСК. Клітини тримали в повному середовищі DMEM. Після одноразової електропорації 0,5 мкг *Hnf1*-α відмінності в життєздатності мКМ-МСК були статистично незначними через 24, 72 год та 21 день. Ефективність трансфекції визначали за допомогою флуоресцентної візуалізації турбо-зеленого флуоресцентного білка (tGFP). Ефективність трансфекції визначали за параметрів імпульсної напруги 1000 (v), тривалості імпульсу 10 (мс) та 3 імпульсів на 24 години (***p-значення <0,001, 66,5 ± 12,2) в мКМ-МСК. Ефективність трансфекції 0,5 мкг *Hnf1*-α знижувалася через 72 год (40,2 ± 10,9) та 21 день (31,7 ± 5). Для відбору позитивних клітин, трансфікованих Hnf1-α, використовували 250 мкг/мл сульфату G418. Результати TaqMan-кЗТ-ПЛР у незалежних експериментах виявили значні відмінності в експресії Hnf1-α при вищезазначених параметрах. Таким чином, було оптимізовано 0,5 мкг плазміди *Hnf1*-α в 2,5 × 105 мКМ-МСК з імпульсною напругою 1000 В, тривалістю імпульсу 10 мс і кількістю імпульсів – 3, про що раніше не повідомлялося. Ці параметри можна розглядати для трансфекції з життєздатністю клітин 65 % – 96 % від 24 годин до 21 дня та ефективністю трансфекції 60 % – 70 % через 24 год. Таким чином, ця оптимізована процедура з ефективними показниками трансфекції може бути застосована для подальших досліджень функцій генів та диференціювання в печінці, підшлунковій залозі, нирках, кишківнику, а також для інших тканин у спеціалізованих нішах.

Ключові слова: аламаровий синій, G418, пульс, стовбурові клітини, ефективність трансфекції, життєздатність, напруга.

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Received April 15, 2023
Received July 21, 2023
Accepted May 18, 2024