Construction of a polycistronic regulatable vector expressing the yamanaka genes for reprogramming fibroblasts to cardiomyocytes

Micaela Lopez-Leon, Paula C. Reggiani, Claudia B. Herenu, Rodolfo G. Goya

Institute for Biochemical Research at La Plata (INIBIOLP), Histology B & Pathology B, School of Medicine, National University of La Plata. La Plata city, Argentina.

Heart failure remains as one of the major causes of morbidity and mortality throughout the world, worsening as the population ages. Since the adult myocardium has a weak endogenous regenerative capacity which decreases further with age, the generation of cardiomyocytes (CM) by reprogramming somatic cells from the patient is a goal of keen interest for the treatment of myocardial infarction. It has been reported that CM can be produced by direct reprogramming of mouse fibroblasts. In this procedure, the four pluripotency genes Oct4, Sox2, cMyc and Klf4 (the Yamanaka genes) are briefly overexpressed, taking the fibroblasts to a stage of “epigenetic instability” which under appropriate culture conditions revert towards cardiomyogenic precursors (CP) or terminal CM, that may constitute up to 40% of the transdifferentiated cell population. Unfortunately, clinical use of CM thus generated is severely compromised by the fact that current reprogramming strategies to produce them involve integrative gene transfer with the well-known risk of insertional mutagenesis or gene silencing. Adenoviral vectors offer a safer reprogramming alternative due to the fact that they do not integrate their genes into the target cell’s genome, thus avoiding the risk of insertional mutagenesis. In particular, advanced helper-dependent (HD) regulatable adenovector systems constitute a promising tool for pluripotency gene transfer and direct cell reprogramming. We report here the construction of a regulatable expression vector simultaneously expressing the pluripotency genes Sox2, c-Myc, Klf4 y Oct4 and the reporter gene for humanized green fluorescent protein (hGFP). These genes were cloned in a regulatable bidirectional system constructed in our laboratory. The system consists of a regulatable bidirectional cassette which expresses hGFP and has an empty multiple cloning site (MCS) available for cloning genes of interest. The MCS was used for cloning a tandem (termed STEMCCA) harboring the four pluripotency genes mentioned above. Both, the hGFP gene and the pluripotency gene tandem are under the control of a single regulatable (Tet-Off) bidirectional promoter which can be reversibly inhibited by the antibiotic doxycycline (DOX). We characterized the DOXdependent regulatability of the system in HEK293 cell culture and demonstrated, by PCR, RT-PCR, immunocytochemistry, Western-Blot, FACs and fluorescence microscopy, the expression of the four pluripotency genes and the hGFP reporter as well as the dose-dependent ability of DOX to inhibit the expression of the 5 transgenes. This expression vector will be used to construct a regulatable HD adenovector expressing hGFP and the STEMCCA tandem. With this gene delivery tool we plan to implement a non-integrative direct reprogramming protocol in mouse fibroblasts in which the Yamanaka genes will be expressed for 4 days in order to obtain epigenetically unstable (EU) fibroblasts which are responsive to a variety of differentiation factors. In order to overcome the lower transduction efficiency of adenoviral as compared to retroviral vectors, we plan to use the magnetofection technique during cell reprogramming. The EU intermediaries so generated will be exposed for about 10 days to suitable cardiomyogenic factors in order to induce CP and terminally differentiated CM. Advanced adenovectormediated non-integrative pluripotency gene delivery, should allow a safe generation of clinical grade CP and CM by direct reprogramming.

Keywords: myocardial infarction - regenerative medicine – transdifferentiation