GFP expression in muscle cells impairs actin-myosin interactions: implications for cell therapy

To the editor: One of the major issues of cell transplantation is the *in vivo* tracking of injected cells to monitor their phenotypic changes and correlate engraftment with function. To this end, GFP and its variants have been most commonly used[1,2]. Although expression of GFP is considered to be innocuous for the cells, deleterious effects have been reported in *in vitro*[3,4] and *in vivo* studies[5,6], and include the development of dilated cardiomyopathy in mice[5]. The therapeutic potential of skeletal myoblasts is increasingly investigated in both cardiology and myology, and GFP is often used to better define the fate of transplanted myoblasts. Because nonspecific sarcomeric labeling of GFP occurs in muscle, we investigated the cellular effects of eGFP expression on muscle cell function.

We demonstrate that eGFP markedly affects myosin function, thereby causing impaired contractile activity of the muscle. EGFP-transduced (using a lentiviral vector, ppt-PGK-eGFP-WPRE) rat primary myoblasts actively proliferated, differentiated and fused to form multinucleated myotubes in culture, which indicates that their myogenic potential is not impaired. To determine the contractile properties of eGFP-transduced and nontransduced myotubes, we performed electromechanical recordings of excitation-contraction coupling in an *in vitro* setting using Fluo-4. Calcium transients were translated into contractions in 39 ± 1% (mean ± s.e.m.) of the nontransduced cells. This percentage was two times lower (21 ± 1%) in the eGFP-transduced subset ($n = 9$ per group, $P < 0.001$; ANOVA). We then postulated that this impairment of excitation-contraction coupling could be due to an inhibition of actin-myosin interactions. To explore this hypothesis, we assessed the mechanical (*in vitro* motility assay) and enzymatic (actin-activated ATPase rate) properties of myosin in the presence of eGFP, purified rabbit heavy meromyosin (HMM) and F-actin. As eGFP concentration increased relative to HMM, the percentage of moving filaments decreased by 27 ± 5% and 90 ± 4% at 1:1 and 1:3 myosin:eGFP ratios, respectively, compared to controls ($P < 0.001$). The maximum ATPase rate decreased by 62 ± 11% and 97 ± 1% at 1:1 and 1:3 myosin:eGFP ratios, respectively ($n = 5$ per group, $P < 0.001$). These results demonstrate that eGFP markedly affected myosin function, as evidenced by severe changes in both mechanical and enzymatic properties of myosin. To provide *in vivo* validation of these findings, we compared the functional effects of cardiac transplantation of eGFP-transduced and nontransduced primary myoblasts in a rat model of myocardial infarction[7]. Echocardiographic data revealed that 1 month after transplantation, left ventricular ejection fraction (a surrogate marker of cardiac contractility) increased from baseline by 9 ± 6% in the nontransduced myoblasts ($n = 12$), whereas it decreased by 14 ± 7% in the eGFP-transduced myoblast group ($n = 9$, $P < 0.05$).

To conclude, our results show that eGFP expression impairs actin-myosin interactions, thereby causing excitation-contraction uncoupling and impaired contractile function of muscle cells. This adverse effect of eGFP should be kept in mind when using this marker to track cells after transplantation because the induced changes in cellular function may confound interpretation of functional data.

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