Effect of adenoviral transduction of hepatic stellate cells with Adv5-optHGF-RFP on their phenotype

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Abstract Adenovirus is a promising gene delivery vector that has a high efficiency and relative ease of construction. These advantages make this system attractive for diverse research applications. In this project we performed transduction of hepatics stellate cells (HSC) with adenoviral vector containing hepatocytes growth factor (HGF) and red fluorescent protein (RFP) as a reporter (Adv5-optHGF-RFP), which let us to visualize the transduced cells. Further changes of phenotype were studied by real-time PCR and immunocytochemical staining. According to our results most of the cells were transduced and demonstrated stable expression of RFP. Effectiveness of the transduction was also confirmed by high expression of HGF (1200 times higher than in control HSC culture). Adenoviral transduction of HSC with HGF gene didn`t change their morphology, but stimulated expression of HSC marker desmin, lead to HSC activation (α-SMA) and appearance of α-FP, one of the hepatoblasts markers. During the entire experiment there were Ki-67+ cells, meaning that proliferation of transduced HSC was not affected. Thus, adenoviral transduction of HSC with Adv5-optHGF-RFP is a good gene delivery system with stimulating effect on HSC.

Keywords hepatic stellate cells, adenovirus, transduction, differentiation

1 Introduction

Hepatic stellate cells (HSC) are being studied for a long time and in numerous researches it was demonstrated, that activated HSC transdifferentiate into myofibroblasts (expressing myofibroblasts marker α-SMA) and produce extracellular matrix components, among which is collagen [1]. On that ground this hepatic cell population was thought to be the "causer" of liver fibrosis. However during the last decade there were published series of contributions, describing HSC as hepatic stem cells, able to grow and proliferate *in vitro*, express stem cells markers, differentiate into hepatocytes and thus, restore damaged liver cells *in vivo* [1, 2]. By some researchers, including our group, it was shown that these cells population plays an important role in liver development and differentiation of progenitor cells into hepatocytes [3,4]. This becomes possible due to creation of specific microenvironment, including extracellular matrix components and an amount of HSC growth factors, among which are fibroblast growth factor 4 (FGF4), stem cell growth factor (SCF) [1,5] and the key factor - hepatocytes growth factor (HGF), responsible for cells migration, proliferation and differentiation into hepatocytes [6]. It should be noted, that the same growth factors are used for differentiation of mesenchymal stem cells and HSC into hepatocytes

in vitro [6,7]. There are various ways of these factors delivery into the cells: simple addition of commercial factors to the culture media of the growing cells [7], plasmid transfection or transduction with adenoviruses carrying gene of the growth factor [8]. The last technique is of particular interest, while it provides reliable delivery of the genes into the cells [9]. HSC transduction with adenoviral vector (Adv) containing HGF and its effect on the cells phenotype became an aim of this project.

2 Material and Methods

This work has been carried out in accordance with Kazan Federal University on the use of laboratory animals (ethical approval by the Institutional Animal Care and Use Committee of Kazan State Medical University N6-24.06.2014). Hepatic stellate cells were isolated from Wistar rats (6 month old) liver by consequent collagenase-pronase liver perfusion with further separation of the cells in Histodenz gradient [10]. The cells were cultivated in DMEM medium supplemented with 10 % of Fetal Bovine Serum, 200 mM L-Glutamine and penicillin-streptomycin (PanEco). HSC of the 3rd passage (3 replicates) were transduced in serum-free medium with Adv containing genes of HGF and reporter gene of red fluorescent protein (RFP) Adv5-optHGF-RFP, MOI 10. Next day the medium was changed to normal one. As control sample were taken HSC grown in normal medium and standard conditions. To study the effect of transduction on phenotype of the cells were performed immunocytochemical staining and real-time PCR with samples collected on 3, 7, 10, 14 and 21 days of cultivation. Staining was performed with following antibodies against: desmin **(**marker of HSC) – Dako (M076001), 1:15; α-SMA (marker of myofibroblasts and smooth muscle cells) – Dako (M0851), 1:50; α-Fetoprotein (α-FP, one of the hepatoblasts proteins) – Abcam (ab3980), 1:100; Ki-67 (nuclear protein, necessary for cellular proliferation and ribosomal RNA transcription) – Abcam (ab66155), 1:200. For real-time PCR RNA was isolated by Yellow Solve Kit (Sileks). Aliquots of total RNA were reverse-transcribed into cDNA (were used reagents of Fermentas) and reverse transcriptional products were subjected to PCR amplification using SYBR Green as the fluorescence indicator on a Bio-Rad iCycler System (Bio-Rad, Hercules, CA). The primers used in this study were synthesized by Sileks, the sequences were: for rHGF-SG sense CATTGGTAAAGGAGGCAGCTATAAA, antisense GGATTTCGACAGTAGTTTTCCTGTAGG; for α-SMA-SG sense ACTACTGCCGAGCGTGAGAT, antisense AAGGTAGACAGCGAAGCCAA; for rDesm-SG sense CTTCAGGAA CAGCAGGTC, antisense ATCTCGCAGGTGTAGGAC; for α-FP-SG sense GGAAGATGGTGAGCATTG, antisense AGCCTCCTGTTGGAATAC.

3 Results and Discussion

During the entire experiment HSC kept their size and shape, so the transduction didn`t affect their morphology. The effectiveness of the adenoviral transduction was proved by stable red fluorescence of almost all the cells (around 90%) during the experiment (Fig.1A). Another confirmation of the transduction was expression by the HSC gene of HGF. Analyzing the staining and quantitative real-time PCR results (Fig.1) we could follow the dynamics of HGF production: gradual increase till the 7th day (when around 70% of the cells expressed HGF), than strong decline on the 10th day and achievement of maximal level on the 14th day (more than 1200 times higher expression than in control group) and gradual return to normal meanings on the 21st day. Similar tendency was visualized for α-SMA gene (marker of activated HSC, myofibroblasts) expression (Fig.1B). Perhaps, expression of HGF and α-SMA are interrelated, because HGF leads to activation and migration of the cells, that require contractile activity and expression of the α-SMA. However, the level of α-SMA expression was very low, so adenoviral transduction is relative safety and do not lead to transdifferentiation of all HSC into myofibroblasts.

Maximal level of another two genes expression, desmin and α-FP [11], was detected also on the 14th day of the experiment, but the dynamics was different (Fig.1C). In compare to two previous two genes expression, the tendency of desmin and α-FP expression gradually increased till the 14th day (more than 750 times of desmin and more than 350 times of α-FP respectively). Probably, expression of desmin and α-FP are also interrelated. As far as α-FP is one of the hepatoblast markers we can propose that adenoviral transduction with gene of HGF stimulated HSC to differentiate in hepatogenic direction.

Interestingly, at day 10 the expression for all markers dropped, that could be related to the reduced activity of the adenoviral vector (they are active over the period of around 10-14 days). Further increase of genes expression could be caused by switch of adenoviral genes expression to expression of HSC original HGF with consequent increase of desmin and α-FP. For now it is only our speculation and it needs to be studied and proved.

The Ki-67 nuclear staining in control and transduced cultures were without significant changes, so the proliferation of the cells was not affected by viral transduction.

Thus increased level of desmin, α-SMA, α-FP expression were caused by transduction of the HSC with adenovirus, containing HGF. At the same time the effect of adenoviral transduction is not longstanding and is followed by gradual return of all expression levels to normal meanings on the 21st day of the experiment.

4 Conclusions

Transduction of HSC with adenovirus is the simple and effective method of growth factor gene delivery, that was visualized by high RFP fluorescence. Transduction of HSC with Adv5-optHGF-RFP lead to their activation and increase of desmin, α-SMA expression, didn`t change the morphology and proliferation of the cells (Ki-67 staining) and induced hepatogenic differentiation (appearance of α-FP). Adenoviral transduction of HSC is applicable for short time stimulation of genes expression and probably can be used for the study of liver regeneration by transplantation of gene-modified cells.

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Conflicts of interest

The authors declare no conflicts of interest.

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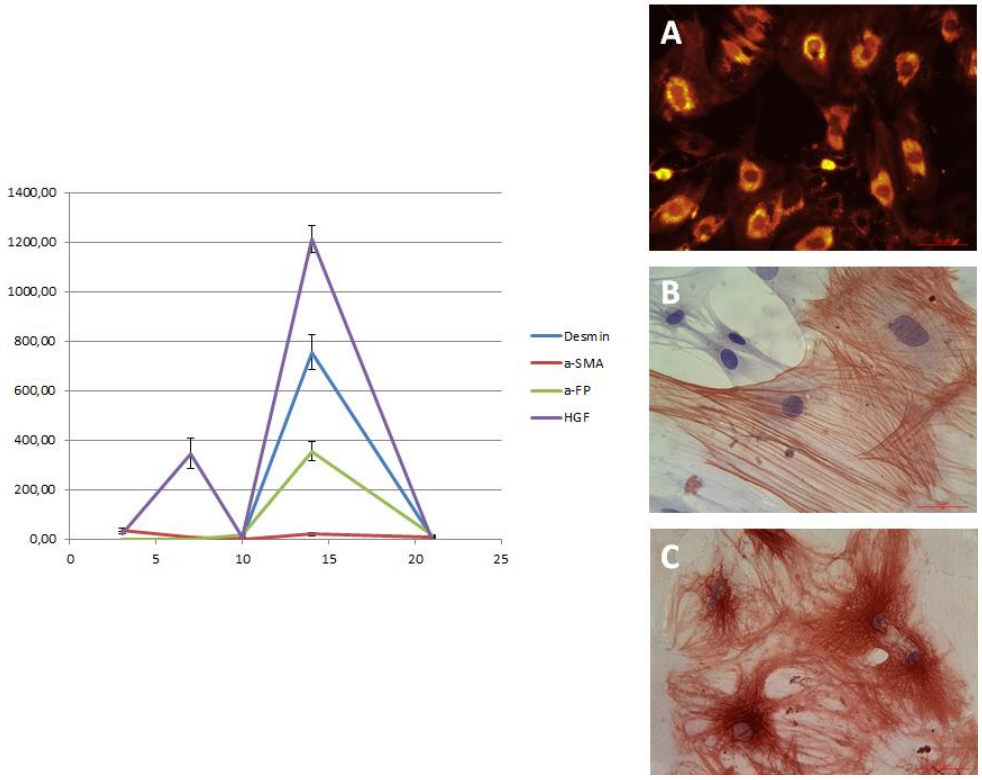

Figure legends

Figure 1. Changes of HSC phenotype after transduction with Adv5-optHGF-RFP. Left – levels of HGF, desmin, α-SMA and α-FP expression, quantified by the real-time PCR. Right: A – RFP fluorescence, 14th day, 20x magnification. Results of HSC staining: B – α-SMA, C – desmin, 14th day after transduction, 40x magnification.