

Hepatocyte apoptosis/anoikis is suppressed by Fas down-regulation on PVLA, a galactose-carrying polymer

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INTRODUCTION: Extracellular matrices (ECMs) have been known to decide cell fate such as differentiation, proliferation, migration, and survival [1]. Especially, it was reported that survival of epithelial cells requires integrin-mediated adhesion to ECM molecules. Integrin interacts with ECM components to activate PI3K/Akt and MEK/ERK signaling pathways and maintains cell viability. When the interaction between integrin and ECM is disrupted, cell is led to apoptosis, named anoikis. In our previous study, a galactose-carrying polystyrene (PSt), poly (*N*-*p*-vinylbenzyl-4-*O*- β -D-galactopyranosyl-D-gluconamide) (PVLA), was developed as a hepatocyte-specific adhesive matrix, and hepatocytes attached to PVLA through asialoglycoprotein receptor (ASGP-R), that could survive. Kim et al. also reported that hepatocyte adhered on PVLA strongly suppressed integrin signaling, because ECM deposition beneath the hepatocytes is inhibited by coated-PVLA molecules [2]. Interestingly, inspite of integrin signaling suppression, hepatocyte can attach to PVLA and survive. It is, thus, suggested that the mechanism of anoikis suppression without integrin signaling might exist.

In this study, we compared the mechanism of anoikis suppression by integrin-mediated adhesion and non-integrin-mediated one.

METHODS: Mouse hepatocytes were cultured on adhesive matrices (PVLA, poly-L-lysine (PLL), collagen-1, and fibronectin) and non-adhesive matrices (agarose gel). Hepatocyte death was measured as leaked LDH activity. Akt and ERK phosphorylation levels were determined by Western blot analysis. Fas expression level was examined by RT-PCR method and western blot analysis.

RESULTS and DISCUSSION: FAK autophosphorylation in hepatocytes on PVLA, PLL, and agarose gel was suppressed although that on collagen-1 and fibronectin was strongly detected. This indicates that integrin signal is suppressed in hepatocytes on PVLA and PLL. Hepatocyte anoikis was suppressed on PVLA and PLL without integrin signal although hepatocytes on agarose gel underwent apoptosis. To analyze the suppression mechanism of anoikis, we focused

on two survival signalling molecules, ERK and Akt. ERK was phosphorylated in both adherent and non-adherent conditions. In the case of Akt, phosphorylated Akt was detected only on integrin-mediated adhesive matrices (collagen-1 and fibronectin). This result indicates hepatocytes survive on non-integrin-mediated adhesive matrices without involvement of Akt and ERK activation. In the next step, we focused on Fas/Fas ligand system to clarify the mechanism of hepatocyte anoikis. Fas up-regulation was observed in hepatocyte only on agarose gel. To confirm whether Fas/Fas ligand system is involved in hepatocyte anoikis, we used *gld/gld* mice whose Fas ligand has point mutation and loses the ability to induce apoptosis. Anoikis of hepatocytes from *gld/gld* mice was suppressed compared with those from wild type (WT) mice (Figure 1). These results indicate Fas down-regulation without integrin signal rescues hepatocytes from anoikis. Our studies using the artificial matrices that can regulate cell adhesion mechanism will provide us the new insights to regulate anoikis and other cellular behaviour by cell-ECM interaction. Furthermore, these results give us not only information to understand the regulation mechanism of anoikis but also strategical guideline to avoid decline of cell viability in cell transplantation for regenerative medicine and carcinoma metastasis.

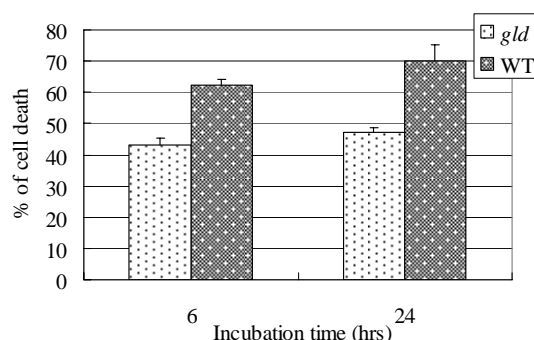


Figure 1: Hepatocytes from *gld/gld* mice were suppressed anoikis compared with those from wild type mice

REFERENCES: [1] F.G. Giancotti, and E. Ruoslahti, (1999) *Science* **285**: 1028-1032. [2] S.-H. Kim, and T. Akaike, et al (2003) *FEBS Lett.* **553**: 433-439.