The Study of Adhesive Properties of Hepatocellular Carcinoma Cells to Collagen Type I

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1. INTRODUCTION

Malignant tumors are a serious disease about human health and life. The clinical treatment about malignant tumors is one of difficult medical problems all over the world. The present work focuses on the hepatic tumor invasion and metastasis in clinical treatment, and select the hepatocytes(HTCs) or hepatocellular carcinoma cells (HCCs) as study objects firstly. The studies about adhesive properties of HCCs to one of main components of liver—collagen type I are little[1,2]. The main aim is at finding out a satisfactory experimental method, so as quantitatively to discuss the adhesive property of hepatocytes or HCCs to collagen type I. A micropipette aspiration technique that is a very mature biomechnical experimental technique[3] was employed to investigate the adhesive properties of hepatocellular carcinoma cells (HCCs) to collagen type I, based on the isolation and primary culture of hepatocytes(HTCs) and the culture of HCCs. This investigation suggested a quantitative analysis methodology for the metastasis of hepatocellular carcinoma.

2. EXPERIMENTAL METHOD

The paper developed a new, a simple, low cost, and rapid method for the isolation and purification and cultivation of primary human liver sinusoidal endothelial cells (LECs) and hepatic parenchymal cells. The two cells were isolated by collagenase IV perfusion of the liver, and separated by speed gradient centrifugal method. After getting liver, it was possible to insert the plastic tube further in the portal vein without rupture of the vessel. The inferior vena cava beneath the liver was immediately cut and preperfusion started with 37°C balanced salt solution. About 50ml of balanced salt solution was flushed repeatedly through the liver at a flow rate of 10ml/minute, during which the inferior vena cava above the liver was ligated with a clamp. Directly after the preperfusion, 40ml of 37°C collagenase solution was pumped repeatedly through the liver at a flow rate of 5ml/minute. After 20 minutes, the cell suspension was then filtered through nylon gauze to remove undigested tissue. The cell suspension was centrifuged at 50g for 3 minutes to isolate most of parenchymal cells in the bottom of pipette. After washing and purification of parenchymal cells at 50g, its can be inoculated in culture dish[4]. The supernatant enriched in sinusoidal cells was then centrifuged for 10 minutes at 500g. The pellet was resuspended and centrifuged alternately for 5 minutes at 50g or 500g, in order to purify the liver sinusoidal cells. After inoculation of LECs suspension for 2 hours, inoculated supernatant again to remove Kupffer’s cells. In the meantime, the problem of the anchorage of LECs in a chamber using PDL and collagen type I was solved.

The collagen type I-coated technology is described firstly in this paper. The matrix was coated with PDL(Poly-D-lysine), collagen type I and BSA(Bovine Serum albumin). Firstly, 200 μl of PDL(2μg/ml) was perfused in the marked circle area of chamber to help collagen type I adhesion to substrate. After incubation for 30 minutes, remove PDL solution from chamber. PBS solution wash chamber twice. Secondly, 200 μl of collagen type I solution was perfused in same area of chamber. After incubation for 40 minutes, remove collagen type I solution from chamber. The 200 μl of 0.5% BSA solution was perfused in same area for filling a vacant position after collagen type I-coated. After incubation for 15 minutes, it was washed by PBS solution and put on the stage for experiment. The coated method was tested and verified about methodology at same time and presented that collagen type I was the most important role. The paper researched the adhesive properties of hepatocytes or HCCs to PDL, collagen type I and BSA. The result denoted that PDL and BSA would benefit collagen type I-coated process. We developed one quantitative method to measure the adhesive property of HCCs or hepatocytes to collagen type I-coated surface based on cell culture technology and micropipette aspiration technology. The cells were inocate in chamber with collagen type I. The chamber was incubated for 30 minutes in 37°C incubator. After 30 minutes, it was put on the 37°C stage. Regulate the microscope so as to focus on the cells. Remove manipulator in order that the micropipette appeared in field of microscope vision. The pressure system produced negative pressure to grasp a cell. We increase the negative pressure to remove a cell critically from bottom of chamber and record the pressure value.
3. RESULTS AND DISCUSSION

Cell-substrate adhesive model is showed in Fig.1. Different diameter of micropipette and cells will produce different relative adhesive stress. We defined the relative adhesive force(RAF) $F_a$ and relative adhesive stress(RAS) $S_a$ in Fig.1 in order to compare the changes of RAF or RAS for different experimental groups.

$$F_a = \Delta P \cos \theta \pi R_p^2$$  \hspace{1cm} (1)

$$S_a = \Delta P \cos \theta (R_p/R_a)^2$$  \hspace{1cm} (2)

Fig. 1 Geometry of adhesive model

The experimental results that were dealt with mathematics method presented that the normal hepatocellular carcinoma cells showed stronger relative adhesive force and relative adhesive stress in contrast to hepatocytes under every time duration or concentration. The time and density dependence of HCCs showed that the relative stress or force would increase to the saturation value with increasing of time and density. The relative force and stress of HCCs decreased after increase with time increase, and showed different adhesive properties during invasion and metastasis process. Therefore we obtained a new view about adhesive properties of HCCs to collagen type I using quantitative mechanics method. The results will benefit to explain some questions on biomechanical views about how the HCCs peeled off primary position and how the HCCs invaded to secondary position.

4. CONCLUSION

The adhesive experimental results presented show that relative adhesive stress of HCCs is so stronger than HTCs that HCCs could adhere to tissues or cells around it as well as possible. It will provide better condition for HCCs crawling, spreading and infiltrative growth during cancer invasion or metastasis. The conclusion could give a better theory basement for clinical medicine treatment.

REFERENCES


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