

• LIVER CANCER •

# Mechanical properties of hepatocellular carcinoma cells

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## Abstract

**AIM:** To study the viscoelastic properties of human hepatocytes and hepatocellular carcinoma (HCC) cells under cytoskeletal perturbation, and to further to study the viscoelastic properties and the adhesive properties of mouse hepatoma cells (HTC) in different cell cycle.

**METHODS:** Micropipette aspiration technique was adopted to measure viscoelastic coefficients and adhesion force to collagen coated surface of the cells. Three kinds of cytoskeleton perturbing agents, colchicines (Col), cytochalasin D (CD) and vinblastine (VBL), were used to treat HCC cells and hepatocytes and the effects of these treatment on cell viscoelastic coefficients were investigated. The experimental results were analyzed with a three-element standard linear solid. Further, the viscoelastic properties of HTC cells and the adhesion force of different cycle HTC cells were also investigated. The synchronous G<sub>1</sub> and S phase cells were achieved through thymine-2-desoxyriboside and colchicines sequential blockage method and thymine-2-desoxyriboside blockage method respectively.

**RESULTS:** The elastic coefficients, but not viscous coefficient of HCC cells ( $K_1=103.6\pm12.6\text{N}\cdot\text{m}^{-2}$ ,  $K_2=42.5\pm10.4\text{N}\cdot\text{m}^{-2}$ ,  $\mu=4.5\pm1.9\text{Pa}\cdot\text{s}$ ), were significantly higher than the corresponding value for hepatocytes ( $K_1=87.5\pm12.1\text{N}\cdot\text{m}^{-2}$ ,  $K_2=33.3\pm10.3\text{N}\cdot\text{m}^{-2}$ ,  $\mu=5.9\pm3.0\text{Pa}\cdot\text{s}$ ,  $P<0.01$ ). Upon treatment with CD, the viscoelastic coefficients of both hepatocytes and HCC cells decreased consistently, with magnitudes for the decrease in elastic coefficients of HCC cells ( $K_1$ :  $68.7\text{N}\cdot\text{m}^{-2}$  to  $81.7\text{N}\cdot\text{m}^{-2}$ ,  $66.3\%$  to  $78.9\%$ ;  $K_2$ :  $34.5\text{N}\cdot\text{m}^{-2}$  to  $37.1\text{N}\cdot\text{m}^{-2}$ ,  $81.2\%$  to  $87.3\%$ ,  $P<0.001$ ) larger than those for normal hepatocytes ( $K_1$ :  $42.6\text{N}\cdot\text{m}^{-2}$  to  $49.8\text{N}\cdot\text{m}^{-2}$ ,  $48.7\%$  to  $56.9\%$ ;  $K_2$ :  $17.2\text{N}\cdot\text{m}^{-2}$  to  $20.4\text{N}\cdot\text{m}^{-2}$ ,  $51.7\%$  to  $61.3\%$ ,  $P<0.001$ ). There was a little decrease in the viscous coefficient of HCC cells ( $2.0$  to  $3.4\text{Pa}\cdot\text{s}$ ,  $44.4$  to  $75.6\%$ ,  $P<0.001$ ) than that for hepatocytes ( $3.0$  to  $3.9\text{Pa}\cdot\text{s}$ ,  $50.8$  to  $66.1\%$ ,  $P<0.001$ ). Upon treatment with Col and VBL, the elastic coefficients of hepatocytes generally increased or tended to increase while those of HCC cells decreased. HTC cells with  $72.1\%$  of G<sub>1</sub> phase and  $98.9\%$  of S phase were achieved and high  $K_1$ ,  $K_2$  value and low  $\mu$  value were the general characteristics of HTC cells. G<sub>1</sub> phase cells had higher  $K_1$  value and lower  $\mu$  value than S phase cells had, and G<sub>1</sub> phase HTC cells had stronger adhesive forces [ $(275.9\pm232.8)\times10^{-10}\text{N}$ ] than S phase cells [ $(161.2\pm120.4)\times10^{-10}\text{N}$ ,  $P<0.001$ ].

**CONCLUSION:** The difference in both the pattern and the magnitude of the effect of cytoskeletal perturbing agent on the viscoelastic properties between HCC cells and hepatocytes may reflect differences in the state of the cytoskeleton structure and function and in the sensitivity to perturbing agent treatment between these two types of cells. Change in the viscoelastic properties of cancer cells may affect significantly tumor cell invasion and metastasis as well as interactions between tumor cells and their micro-mechanical environments.

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## INTRODUCTION

Current advances in oncology have shown that the continuous growth of malignancy, invasion and metastasis are a multi-step pathophysiological process, which consists of successive steps of tumor cell deformation and locomotion<sup>[1-3]</sup>. For an understanding of the mechanisms involved, advanced methodologies of cellular and molecular biology have been extensively used in the study of the related oncogenes and anti-oncogenes, as exemplified in hepatocellular carcinoma (HCC)<sup>[4-8]</sup>, and in the elucidation of the interaction between tumor cells and vascular endothelial cells<sup>[9,10]</sup>. These studies have already led to considerable knowledge in the event involved in tumor metastasis.

The mechanical properties are very important for biologic behaviors of tumor cells in following reasons. Firstly, tumor cells are destined to experience shear-induced deformation in blood flow if they metastasize through the blood vasculature. The mechanical properties determine whether tumor cells can pass through the microvasculature to form metastases, and probably whether they can survive in the blood shear environment. Secondly, the mechanical properties are related to active pseudopod formation and motility, in which they probably have a similar structural basis and are the main cellular events of tumor cell invasion, and a relationship between active pseudopod formation and cytoskeletal structures has already been demonstrated<sup>[11-13]</sup>. To the aim of this study was to try to understand how the viscoelastic properties of HCC cells are altered compared to those of normal hepatocytes, how the viscoelastic properties of these two types of cells respond to treatment with cytoskeleton perturbing agents [cytochalasin D(CD), colchicines (Col) and vinblastine(VBL)] and what changes occur in viscoelastic properties and the adhesive properties of hepatoma cells in different phases.

## MATERIALS AND METHODS

### Cell sample preparation

HCC cells (SMMC 7721) were obtained from the 2nd Military Medical University (Shanghai, China); HTC cells were kindly given by department of clinical biochemistry of Chongqing Medical University. Normal hepatocytes were prepared from human fetal liver tissue by a combination of  $0.5\text{g}\cdot\text{L}^{-1}$  collagenases IV (Sigma) digestion and density gradient centrifugation<sup>[14-17]</sup>. Cells were maintained in an incubator at  $37^\circ\text{C}$  in an atmosphere of  $950\text{mL}\cdot\text{L}^{-1}$  humidified air and  $50\text{mL}\cdot\text{L}^{-1}$  carbon dioxide. The final concentration of the cells for micropipette experiment was of the order of  $10^6\text{cells}\cdot\text{L}^{-1}$ .

### Preparation of synchronous G<sub>1</sub> and S phase HTC cells

The synchronous G<sub>1</sub> phase cells were achieved through thymine-2-desoxyriboside and colchicines sequential blockage method<sup>[18]</sup>.

### Micropipette system and analysis of the viscoelastic properties of cells

The structure of micropipette system and experimental procedures were described in literatures<sup>[19-21]</sup>. Micropipettes were pulled from capillary glass tubes in a micropipette puller (P87, Sutler Instrument Co, USA). The weighted average values of the internal radius of the pipette used in the present investigation were  $2.47 \pm 0.91 \mu\text{m}$ .

Experimental results were analyzed with a three-element standard linear solid model<sup>[22]</sup>, in which an elastic element,  $K_1$ , was in parallel with a Maxwell element composed of another elastic element,  $K_2$ , in series with a viscous element,  $\mu$ . Viscoelastic coefficients were expressed as  $\bar{x} \pm s$ . Student's *t*-test was used for statistical analysis.

### Analysis of the adhesive properties of HTC cells in different cycle

The adhesive model used was schematically shown in Figure 1,  $F_a$  was adhesive force of cell,  $R_p$  was the inner radius of micropipette,  $\Delta P$  was negative pressure pulled the adhesive cell away from basement membrane coated by collagen IV, and  $\theta$  was the angle of micropipette between basement membrane,  $F_a$  can be calculated from the following equation:  $F_a = R_p^2 \times \Delta P \times \cos \theta$

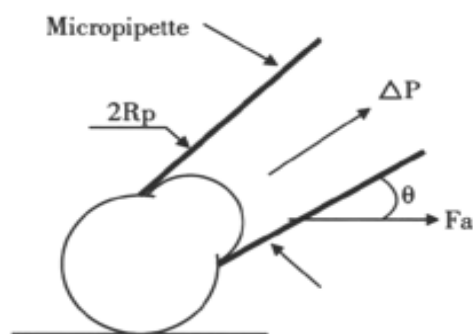


Figure 1 Geometry of adhesive model

## RESULTS

### Viscoelastic properties of HCC cells and hepatocytes and effects of cytoskeleton inhibitions

The values of the viscoelastic coefficients of hepatocytes and HCC and the effects of treatment with CD, Col and VBL were shown in Tables 1-3, respectively. The results were summarized as follows: (1) Compared to those of normal hepatocytes, the values of the elastic coefficients  $K_1$  and  $K_2$  of HCC cells were significantly higher ( $P < 0.01$ ). However, the viscous coefficient,  $\mu$ , of the HCC cells was not significantly different from that of hepatocytes. (2) Treatment with  $1-60 \text{ mg} \cdot \text{L}^{-1}$  of Col resulted in a little but significant

increase in  $K_1$  for hepatocytes with independent of [Col], whereas in the case of  $K_2$  there appeared to be no significant change. In the case of the viscous coefficient, there was a significant decrease with independent of [Col]. In contrast to hepatocytes, the HCC cells resulted in a significant decrease in all 3 coefficients with dependence on [Col] (Table 1). (3) Treatment of the hepatocytes with VBL in the concentration range of  $0.05-2.00 \text{ mg} \cdot \text{L}^{-1}$  resulted in a marked increase in the elastic coefficients at all concentrations, whereas the viscous coefficient only increased significantly at  $0.25 \text{ mg} \cdot \text{L}^{-1}$  and  $0.75 \text{ mg} \cdot \text{L}^{-1}$  of VBL. In the case of the HCC cells,  $K_1$  exhibited a little but significant increase at  $0.05 \text{ mg} \cdot \text{L}^{-1}$  of VBL, but then decreased continuously with increasing [VBL], whereas the values of  $K_2$  and  $\mu$  decreased monotonously with increasing [VBL] (Table 2). (4) Upon treatment with  $0.25$  to  $5.00 \text{ mg} \cdot \text{L}^{-1}$  of CD, the coefficients  $K_1$ ,  $K_2$  and  $\mu$  decreased significantly from the control values, but the decrease exhibited no significant dependence on the perturbing agent concentration. In the case of the  $K_1$  and  $K_2$ , the magnitude of the above decrease was significantly greater for the HCC cells. For  $\mu$ , the magnitude of the decrease for HCC cells was less than that of the hepatocytes (Table 3).

### Viscoelastic and adhesive properties of HTC in different cycle

The synchronization results detected with flow cytometer showed that it could meet the requirements of the experiments nicely. HTC with 72.1% of G<sub>1</sub> phase and 98.9% of S phase were achieved. The values of the adhesive force of HTC on different concentration of artificial basement membrane (collagen IV coated) were shown in Table 4. The adhesive force of G<sub>1</sub> phase HTC on basement membrane coated by collagen IV  $5 \text{ mg} \cdot \text{L}^{-1}$  was  $(275.9 \pm 232.8) \times 10^{-10} \text{ N}$ , and the corresponding value of S phase HTC was  $(161.2 \pm 120.4) \times 10^{-10} \text{ N}$ . Difference between them was considered significant ( $P < 0.001$ ). The viscoelastic coefficients of HTC cells in different cycle were shown in (Table 5).

Table 4 Adhesive forces of HTC on artificial basement membrane ( $\bar{x} \pm s$ )

Concentration of collagen IV ( $\text{mg} \cdot \text{L}^{-1}$ )	$F_a$ ( $10^{-10} \text{ N}$ )
1	$107.8 \pm 65.4$
2	$182.6 \pm 107.9^b$
5	$298.9 \pm 144.1^d$

<sup>b</sup> $P < 0.001$  vs collagen IV  $1 \text{ mg} \cdot \text{L}^{-1}$ ; <sup>d</sup> $P < 0.001$  vs collagen IV  $2 \text{ mg} \cdot \text{L}^{-1}$

Table 5 Viscoelastic coefficients of HTC in different cycle ( $\bar{x} \pm s$ )

Viscoelastic coefficients	General	G <sub>1</sub> phase	S phase
$K_1$ ( $\text{N} \cdot \text{m}^{-2}$ )	$186.5 \pm 35.6$	$215.3 \pm 50.2$	$179.7 \pm 33.0^b$
$K_2$ ( $\text{N} \cdot \text{m}^{-2}$ )	$224.4 \pm 114.5$	$181.9 \pm 102.9$	$188.6 \pm 87.1$
$\mu$ ( $\text{Pa} \cdot \text{s}$ )	$3.1 \pm 2.3$	$2.9 \pm 1.3$	$4.7 \pm 2.4^b$

<sup>b</sup> $P < 0.001$  vs G<sub>1</sub> phase.

Table 1 Viscoelastic properties of hepatocytes and HCC cells under the action of colchicines ( $\bar{x} \pm s$ )

[colchicine] ( $\text{mg} \cdot \text{L}^{-1}$ )	Hepatocytes			HCC cells		
	$K_1$ ( $\text{N} \cdot \text{m}^{-2}$ )	$K_2$ ( $\text{N} \cdot \text{m}^{-2}$ )	$\mu$ ( $\text{Pa} \cdot \text{s}$ )	$K_1$ ( $\text{N} \cdot \text{m}^{-2}$ )	$K_2$ ( $\text{N} \cdot \text{m}^{-2}$ )	$\mu$ ( $\text{Pa} \cdot \text{s}$ )
0.0	$87.5 \pm 12.1$	$33.3 \pm 10.3$	$5.9 \pm 3.0$	$103.6 \pm 12.6$	$42.5 \pm 10.4$	$4.5 \pm 1.9$
1.0	$95.4 \pm 14.1^a$	$33.2 \pm 7.7$	$3.9 \pm 1.7^b$	$86.7 \pm 10.0^b$	$20.6 \pm 2.9^b$	$4.5 \pm 1.5$
15.0	$107.1 \pm 23.0^b$	$39.6 \pm 12.2^a$	$5.3 \pm 1.9$	$31.4 \pm 8.0^b$	$7.0 \pm 1.9^b$	$1.3 \pm 0.6^b$
30.0	$99.5 \pm 11.1^b$	$28.0 \pm 7.3^a$	$4.0 \pm 1.8^b$	$53.5 \pm 12.9^b$	$12.3 \pm 4.8^b$	$2.1 \pm 1.0^b$
60.0	$104.4 \pm 13.0^b$	$30.6 \pm 6.5$	$3.5 \pm 1.1^b$	$61.6 \pm 16.0^b$	$16.5 \pm 6.5^b$	$2.3 \pm 1.2^b$

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs normal control

**Table 2** Viscoelastic properties of hepatocytes and HCC cells under the action of vinblastine ( $\bar{x} \pm s$ )

[vinblastine] (mg.L <sup>-1</sup> )	Hepatocytes			HCC cells		
	K <sub>1</sub> (N.m <sup>-2</sup> )	K <sub>2</sub> (N.m <sup>-2</sup> )	μ (Pa.s)	K <sub>1</sub> (N.m <sup>-2</sup> )	K <sub>2</sub> (N.m <sup>-2</sup> )	μ (Pa.s)
0.00	87.5±12.1	33.3±10.3	5.9±3.0	103.6±12.6	42.5±10.4	4.5±1.9
0.05	115.9±15.9 <sup>b</sup>	42.4±8.8 <sup>b</sup>	6.1±2.3	118.4±19.7 <sup>b</sup>	23.1±6.0 <sup>b</sup>	5.6±2.3
0.25	128.7±2.4 <sup>b</sup>	54.0±6.3 <sup>b</sup>	9.8±1.6 <sup>b</sup>	93.2±11.3 <sup>b</sup>	17.0±3.2 <sup>b</sup>	2.9±1.0 <sup>b</sup>
0.75	138.3±23.2 <sup>b</sup>	51.4±13.4 <sup>b</sup>	8.2±3.3 <sup>a</sup>	84.5±6.2 <sup>b</sup>	15.2±3.1 <sup>b</sup>	2.6±0.8 <sup>b</sup>
2.00	117.0±9.1 <sup>b</sup>	43.9±7.7 <sup>b</sup>	6.2±2.3	53.4±12.0 <sup>b</sup>	8.7±2.8 <sup>b</sup>	1.0±0.5 <sup>b</sup>

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01 vs normal control**Table 3** Viscoelastic properties of hepatocytes and HCC cells under the action of cytochalasin D ( $\bar{x} \pm s$ )

[cytochalasin D] (mg.L <sup>-1</sup> )	Hepatocytes			HCC cells		
	K <sub>1</sub> (N.m <sup>-2</sup> )	K <sub>2</sub> (N.m <sup>-2</sup> )	μ (Pa.s)	K <sub>1</sub> (N.m <sup>-2</sup> )	K <sub>2</sub> (N.m <sup>-2</sup> )	μ (Pa.s)
0.00	87.5±12.1	33.3±10.3	5.9±3.0	103.6±12.6	42.5±10.4	4.5±1.9
0.25	37.7±7.1 <sup>b</sup>	12.9±3.3 <sup>b</sup>	2.0±0.8 <sup>b</sup>	29.5±11.4 <sup>b</sup>	6.5±2.6 <sup>b</sup>	1.6±0.8 <sup>b</sup>
0.50	39.5±6.4 <sup>b</sup>	13.8±3.4 <sup>b</sup>	2.6±1.1 <sup>b</sup>	21.9±5.2 <sup>b</sup>	5.4±1.7 <sup>b</sup>	1.1±0.5 <sup>b</sup>
2.50	42.4±5.9 <sup>b</sup>	16.1±3.3 <sup>b</sup>	2.9±1.4 <sup>b</sup>	31.7±3.8 <sup>b</sup>	7.1±1.8 <sup>b</sup>	2.5±1.0 <sup>b</sup>
5.00	44.9±7.5 <sup>b</sup>	16.1±3.0 <sup>b</sup>	2.6±1.3 <sup>b</sup>	34.9±9.4 <sup>b</sup>	8.0±2.7 <sup>b</sup>	1.9±0.7 <sup>b</sup>

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01 vs normal control

## DISCUSSION

Viscoelasticity is an important mechanical property of a cell that is related to its motility and deformability<sup>[23-25]</sup>. For HCC cells, the viscoelasticity has probably significant bearing on tumor cell invasion and metastasis, in which it determines the flowing behavior of tumor cells in the circulation and whether such cells can be arrested to form metastasis. In addition, mechanical stiffness is closely related to cell adhesion behavior<sup>[26,27]</sup>, which is the first step in tumor cell invasion. With the three-element standard linear solid viscoelastic model, we clearly showed that HCC cells have higher values for the elastic coefficients but not the viscous coefficient than hepatocytes. This result indicated that HCC cells were more rigid than normal hepatocytes under the experimental conditions. One possible explanation of this result is that, in HCC cells, interconnections between microfilaments and microtubules might have changed as compared to those in normal hepatocytes, and thus microfilaments are affected upon disorganization of microtubules.

As the primary force-bearing structure of a cell, the cytoskeleton may be very important in determining cell mechanical behavior<sup>[28-30]</sup>. We used three microfilament- or microtubule- targeting perturbing agents (Col, VBL and CD) to treat hepatocytes and HCC cells and found the effects of agents on viscoelastic properties of HCC cells were different obviously in both pattern and extents from those on hepatocytes. Such differences might reflect differences in the state of the cytoskeleton structure and organization, and in the cell's sensitivity to agents. These results also suggested that cytoskeleton play a role in the maintenance of cell viscoelasticities.

Our results of the viscoelastic properties of HTC in different cycle indicated that high K<sub>1</sub>, K<sub>2</sub> and low μ were the general characteristics of HTC, and these were coincided with the result of HCC cells. G<sub>1</sub> phase cells had higher K<sub>1</sub> value and lower μ value than S phase cells had, but there was no obviously difference in K<sub>2</sub> between two phase cells, which reflected the discrepancies of cytoskeletal protein assemble and synthesis in different cell cycle. Those resultson relevance of cytoskeletal structure to viscoelastic coefficient of HCC cells suggested that microfilaments could play a major rule in the maintenance of cell viscoelasticity, especially in G<sub>1</sub> phase cells. In contrary to these, synthesis of microtubules in S phase cells increased, and more microtubules took part in determination the cells viscoelasticity, which could endow G<sub>1</sub> phase cells with higher elasticity and lower viscosity than S phase of cells. These characteristics evidently contributed to G<sub>1</sub>

phase cells survival from the blood shear environment and arrest to form metastases.

### *The adhesive properties of HTC in different cycle*

Current study has shown that action of tumor cells on basement membrane is a multi-step pathophysiological process<sup>[31]</sup>. Our results showed the adhesive force of HTC cells on basement membrane coated by collagen VI had the obvious correlation with the concentration of collagen. Wang *et al*<sup>[32]</sup> reported that the content of collagen IV and laminin in basement membrane increased along with the growth of tumor, but basement membrane became to decrease, even damaged when tumor transferred. So, the different thickness of basement membrane could reflected the different interaction between tumor cells and the membrane. Increased thickness of basement membrane might play the important rule in tumor cell invasion, which was conducive to the chemotactic motion of tumor cell, active orientation movement, and supplied strong adhesive force and adhesive site for tumor cell. The adhesive force of G<sub>1</sub> phase of HTC was obviously greater than that of S phase cells. In general, expression of fibronectin receptor in transferred tumor cell would decrease and laminin receptor would increase. Fibronectin play a more important rule in improving tumor cell spreading and increasing the synthesis and proliferation of DNA in S/G<sub>2</sub> phase. Laminin has many functions on the tumor cell adhesion and movement<sup>[33]</sup>. So, the difference of adhesive force between G<sub>1</sub> phase cell and S phase cell could reflect the difference of expression of adhesive molecule receptor on the cell surface, especially the difference of periodic distribution of fibronectin receptor and laminin receptor. In addition, a strong affinity existed between laminin and thrombolysin, and both of them binded together to form thrombolysin by activating profibrinolysin and hydrolyzing laminin and fibronectin, and finally activation of procollagen and degradation of basement membrane occurred. The phenomenon of high adhesive force value in G<sub>1</sub> phase cell may be also relevant to these changes, which made G<sub>1</sub> phase cells in active condition in adhesion and movement. In the course of tumor invasion and metastasis, G<sub>1</sub> phase cell were more capable of adhering to and getting through basement membranes than S phase cells.

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