Journal of **Kim Il Sung** University (Natural Science)
Vol. 3 No. 3
Juche103(2014)

The Differentiation of Adipose-Derived Stem Cells into Hepatocyte-Like Cells

Yom Tong Yun, Ri Ryol and *O Hak Chol*

Abstract We separated the rats' adipose tissue, prepared the cell suspension by collagenase Ⅱ, cultured the cells in vitro and isolated the adipose-derived stem cells (ADSCs). The ADSCs was proliferated to 80% confluence at 7^{th} day of original culture. It was identified that the cultured cells express CD29, CD44 and unexpressed CD34, CD106 by the immunohistochemical method. On the other hand, we removed the 70% liver of normal rat, detached and minced the remained liver tissue of the rat with injured liver 2 days later. After the minced liver tissue fragments were cultured for 12 hours, supernatant was recovered from the culture flask and added to the culture flask contained ADSCs. As the result, the ratio of the oval or polygonal cells got higher from 3^{rd} day and more than 80% ADSCs were differentiated into hepatocyte-like cells at 14^{th} day. The abilities of urea-composition and glucose-conversion appeared at 5^{th} day and were maintained at fixed level to 21^{st} day. The glycogen-granules appeared at 7^{th} day and (75.1±3.7)% cells had glycogen-granules at 21^{st} day.
Key words ADSCs, differentiation

Introduction

The great leader Comrade **Kim Jong Il** said as follows.

"**In addition, in-depth studies must be conducted to explore new fields of medical science and introduce the latest advances of science and technology into curative and preventive services**."("ON THE FURTHER IMPROVEMENT OF THE HEALTH SERVICE" P. 18)

Until now there are no drugs and treatment methods to treat hepatic diseases, hepatoma, hepatic cirrhosis and so on [5, 6, 8].

How to treat hepatic diseases using functional hepatocytes is the focus of research and many researchers think it is very important to use the differentiated hepatocyte-like cells from MSCs [2, 3, 7, 9].

There are several reports which described the differentiation of MSCs into hepatocyte-like cells using cytokines such as HGF, but the differentiation of ADSCs into hepatocyte-like cells is rarely reported [6, 10].

We studied to differentiate ADSCs into hepatocyte-like cells using the culture supernatant of the hepatocytes separated from the rat with injured liver and found the optimal condition of ADSCs differentiation.

1. Research Materials and Method

1.1. Materials

We detached the ADSCs from the rat's adipose tissue cultivated the tissue fragments in vitro [1, 4] and used the rats. (Body weight; 120～180g)

1.2. Method

We made the model rats with injured liver by the method of resetting the 70% liver and separated hepatocytes from the remained liver tissue after 1st day. When ADSCs were cultured to 80% confluence, they observed CD29, CD44, CD34, CD106 marker with the immunohistochemical method.

And ADSCs were inoculated in the supernatant obtained from hepatocytes culture medium after 1 week. While ADSCs were differentiated in the supernatant, their ability of urea-composition or glucose-conversion and the ratio of glycogen-granules were studied.

2. Results and Discussion

2.1. The culture characters of ADSCs

We separated the rats' adipose tissue, prepared the cell suspension by collagenase II, cultured the cells in vitro and isolated the adipose-derived stem cells (ADSCs). As seen in Fig. 1, mononuclear cells were cultured to 80% confluence at 7th day. It is said that ADSCs has high multiplication capacity as MSCs.

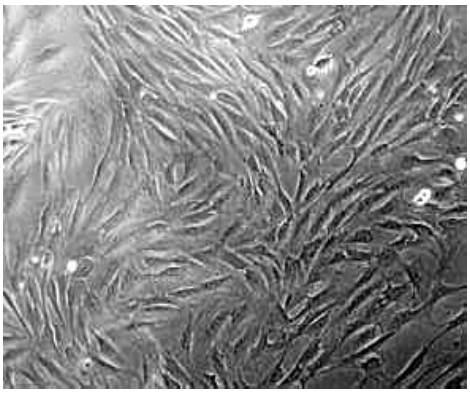

Fig. 1. 7d after ADSCs inoculation

And then the cellular markers expressed from cell-surface were observed by the immunohistochemical method. As seen in Fig 2 and 3, CD29, CD44 were positive and CD34, CD106 were negative. The results of the immunohistochemical method showed that the cultured cells are exactly ADSCs.

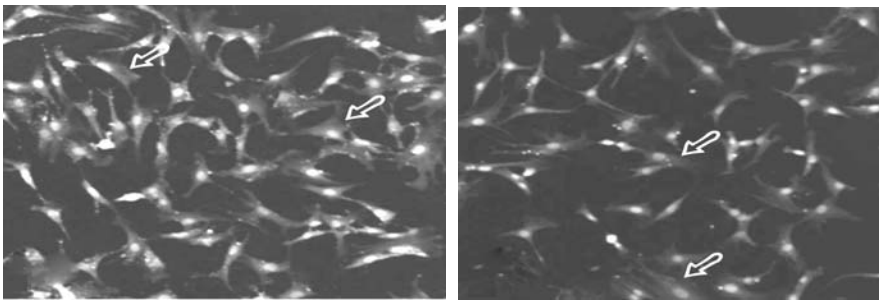

Fig. 2. CD29(left) and CD44(right) positive of the cultured cells

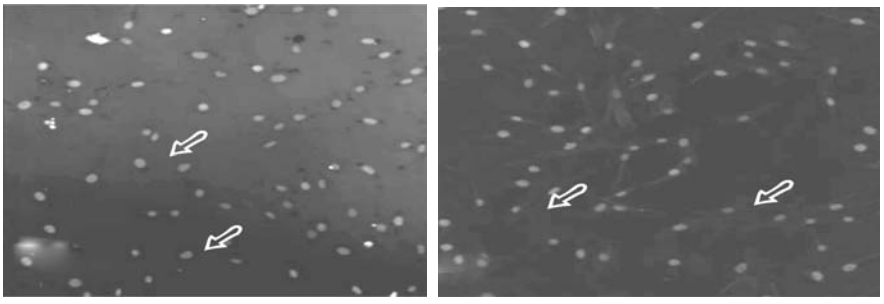

Fig. 3. CD34(left) and CD106(right) positive of the cultured cells

2.2. Differentiation of ADSCs into hepatocyte-like cells

While we added supernatant of the mice hepatocyte medium to the ADSCs and culture, observed the variation of the cellular shape and the abilities of urea-composition, glucose-conversion and glycogen-granules production.

The cytomorphosis of the differentiated hepatocyte-like cells is same as table 1.

Table 1. The cytomorphosis of the differentiated hepatocyte-like cells/%

Distribution	Spindle cells/oval or polygonal cells according to the days				
	3d	5d	7d	14d	21d
ADSCs	100/0	100/0	100/0	100/0	100/0
Hepatocytes of the rat	0/100	0/100	0/100	0/100	0/100
Hepatocyte-like cells	85/15	60/40	35/65	20/80	15/85

As seen in table 1, the oval or polygonal cells were more and more from 3rd day. At 14th day, more than 80% ADSCs were differentiated into hepatocyte-like cells. The cultured ADSCs without supernatant still had spindle and were very different from the differentiated cells.

The urea-composition ability of the differentiated hepatocyte-like cells is same as table 2.

Table 2. The urea-composition ability of the differentiated hepatocyte-like cells

Distribution	Urea-composition ability according to the days /($\mu g \cdot 10^{-6} cell \cdot d^{-1}$)				
	3d	5d	7d	14d	21d
ADSCs	—	—	—	—	—
Hepatocytes of the rat	67.46±3.45	68.64±3.48	68.16±3.62	67.20±3.43	65.04±3.41
Hepatocyte-like cells	—	25.32±1.44	55.78±2.75	56.54±2.78	55.67±2.68

$n=7$

As seen in table 2, the urea-composition ability of the differentiated hepatocyte-like cells is different from the rat's hepatocytes, but it appeared at 5th day after co-culture with hepatocytes and sustained at fixed level to 21st day. However, ADSCs had no urea-composition ability in the whole culture period.

The glucose-conversion ability of the differentiated hepatocyte-like cells is same as table 3.

Table 3. The glucose-conversion ability of the differentiated hepatocyte-like cells

Distribution	Glucose-conversion ability according to the days /($\mu g \cdot 10^{-6} cell \cdot d^{-1}$)				
	3d	5d	7d	14d	21d
ADSCs	—	—	—	—	—
Hepatocytes of the rat	49.37±2.09	48.25±2.08	49.58±2.10	48.64±1.93	48.07±1.91
Hepatocyte-like cells	—	15.20±0.64	35.34±1.82	36.01±1.92	36.88±1.96

$n=7$

As seen in table 3, the glucose-conversion ability of the differentiated hepatocyte-like cells was different from hepatocytes of the normal rats, but appeared from 5th day after supernatant was added and sustained at fixed level up to 21st day. However, ADSCs had no glucose-conversion ability in the whole culture period.

The positive glycogen-granules of the differentiated hepatocyte-like cells are same as table 4.

Table 4. The positive glycogen-granules of the hepatocyte-like cells (%)

Distribution	Period of coculture/d				
	3	5	7	14	21
ADSCs	0	0	0	0	0
Hepatocytes of the rat	100	100	100	100	100
Hepatocyte-like cells	0	0	25.3±1.7	65.8±2.9	75.1±3.7

n=3

As seen in table 4, after the ADSCs were inoculated in the supernatant, the glycogen-granules appeared at 7th day and (75.1±3.7)% cells had glycogen-granules at 21st day. But the glycogen-granule of the ADSCs without supernatant was negative in the whole culture period.

From these results, it is proved that the differentiated hepatocyte-like cells have not only the abilities of urea-composition and glucose-conversion sustains at fixed levels, but also have the figure of the hepatocytes and glycogen-granules.

Conclusion

1ADSCs are proliferated to 80% confluence at 7th day of culture and express CD29 or CD44, unexpressed CD34 or CD106.

ADSCs are differentiated into hepatocyte-like cells, using the supernatant of the hepatocytes culture medium of the rat with 70% removed liver.

References

[1] H. Mizuno et al.; Plastic and Reconstructive Surgery, 109, 1, 199, 2002.

[2] J. R. Mauney et al.; Biomaterials, 26, 31, 6167, 2005.

[3] K. Yoshimura et al.; Cell Physiol., 208, 1, 64, 2006.

[4] L. Aust et al.; Cytotherapy, 6, 1, 7, 2004.

[5] L. Hong et al.; Ann. Biomed. Eng., 33, 4, 511, 2005.

[6] L. J. Dai et al.; Stem Cell Res., 2, 1, 16, 2009.

[7] T. P. Wolter et al.; Ann Plast Surg., 55, 4, 365, 2005.

[8] 李宾公 等; 中华器官移植杂技, 27, 3, 143, 2006.

[9] 牟洪善 等; 生命科学仪器, 4, 6, 22, 2006.

[10] 韩明子 等; 中华器官移植杂志, 24, 4, 251, 2003.