The role of regulatory B cells on hepatocellular carcinoma progression

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Background

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide with a poor prognosis of limited survival. Human regulatory Breg cells(Bregs), a new subset of B cells, play an important role in autoimmune disease. However, the role of Bregs in the HCC progression and the underlying mechanisms is still unknown.

Objective

- To study the roles of Bregs in liver tumor growth and invasion
- To investigate the underneath mechanisms of Bregs regulating HCC progression

Materials and methods

- Clinic study: abundance of circulating Bregs, the distribution of B cells in tumor tissues of HCC patients and their clinical correlation.
- *In vitro* study: the role of Bregs on HCC growth and migration in coculture system
- *In vivo* study: the role of Bregs on HCC growth further using SCID mice liver cancer model

Results

1. Human intrahepatic B cells and peripheral B cell subsets participated in HCC progression.

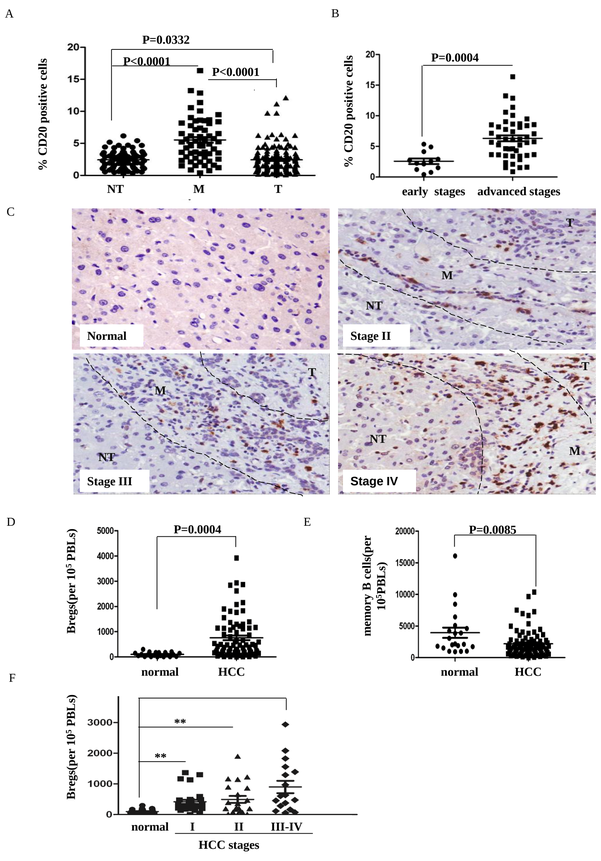

Fig1. (A and B) higher number of CD20 positive B cells were found at tumor margin of advanced HCC tumors. NT: non-tumor region, M: tumor margin region, T: tumor region. (C) B cells were increased with the advance of TNM stages of HCC. (D and E) A significantly higher percentage of Bregs and lower percentage of memory B cells from bloods of HCC patients than that from normal bloods was founded in HCC patients.(F) The number of Bregs per 105 PBLs was increased with progressive stages of HCC patients.

Table. Association between intrahepatic B cells or circulating Bregs and the clinicopathological parameters of HCC patients.

Clinicopathological parameters	B cells in tumor margin, %		p-value
Tumor size (cm)	<5 (n=7)	≥5 (n=52)	
	3.81 (0.41-12.88)	5.22 (0.75-16.38)	**0.004***
Tumor multiplicity	simple (n=48)	multiply (n=11)	
	5.07 (0.41-16.38)	6.18 (1.61-11.4)	0.263
Encapsulation	absent (n=53)	present (n=6)	
	5.26 (0.41-16.38)	4.38 (1.22-5.68)	**0.029***
Venous infiltration	absent (n=20)	present (n=39)	
	2.63 (0.41-16.38)	5.68 (1.53-13.26)	**0.006***
UICC stages	early (n=27)	late (n=32)	
	4.92 (0.41-12.88)	5.42 (1.53-16.38)	**0.025***

Clinicopathological parameters	Circulating Bregs per (10^5 PBLs)		p-value
Tumor size	<5 cm (n=40)	≥5 cm (n=34)	
	289.6 (12.96-2868.08)	419.5 (56.83-2938.22)	0.15
Tumor multiplicity	simple (n=66)	multiply (n=8)	
	333.8 (12.96-2938.22)	1072 (165.87-2080.87)	**0.023***
Encapsulation	absent (n=66)	present (n=8)	
	347.7 (12.96-2938.22)	1027 (25.67-1829.73)	0.102
Venous infiltration	absent (n=36)	present (n=38)	
	319.5 (18.2-1900.1)	433.6 (12.96-2938.22)	**0.029***
UICC stages	early (n=57)	late (n=17)	
	305 (12.96-2860.08)	606.4 (56.83-2938.22)	**0.019***

Numbers of patients, median (range) and p-values were presented as shown in table. * p<0.05 ; ** p<0.01

2. Human Bregs engrafted in SCID mice and promoted tumor growth.

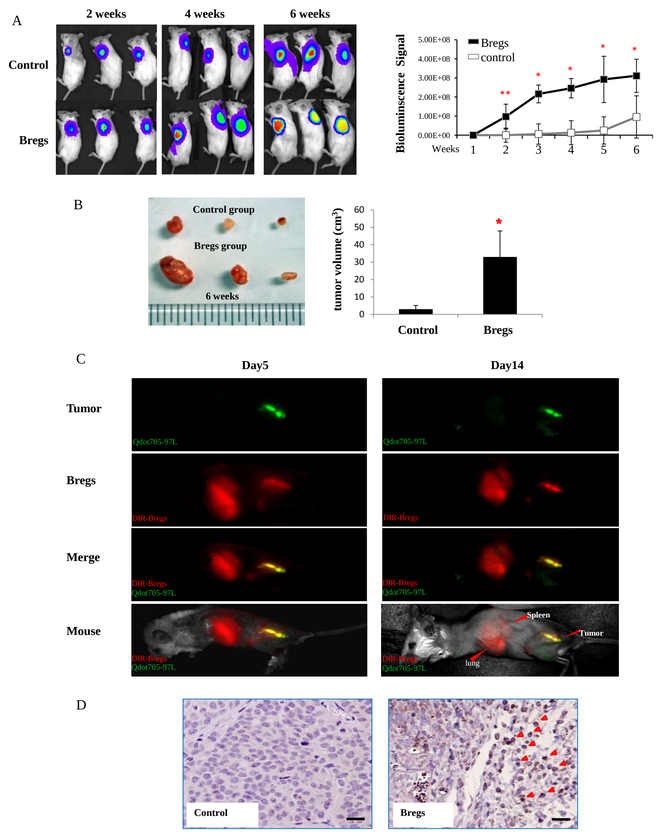

Fig2. (A and B) In vivo, Bregs in SCID mice increased the size of HCC tumor time-dependently. (C)In vivo imagining showed that Bregs could migrate into tumor site. * p<0.05 ; ** p<0.01 Tumors were stained by Qdot705 (green); Bregs were stained by DiR (red). (D) Bregs were detected in the tumor region.

3. Bregs promoted proliferation and invasion of HCC cells.

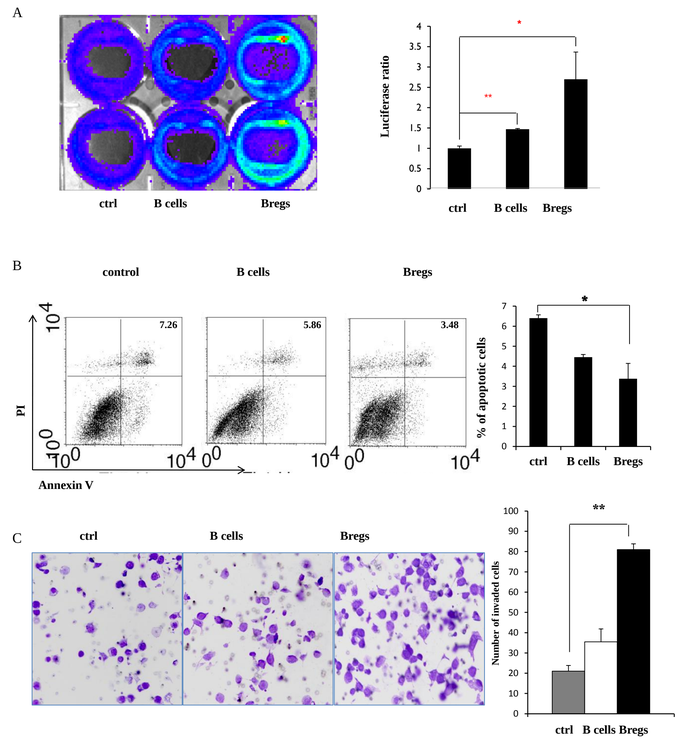

Fig3. (A) Luciferase-labeled human HCC cell line MHCC-97L cells were cocultured with B cells or Bregs. Bregs could promote more MHCC-97L cells proliferation than B cells. (B) Apoptotic assay demonstrated that the percentages of late apoptotic MHCC-97L cells were decreased after coculture with Bregs. (C) The number of invaded MHCC-97L cells was increased after coculturing with Bregs compare to coculturing with B cells.

4. CD154 neutralization abolished Bregs induced tumor growth.

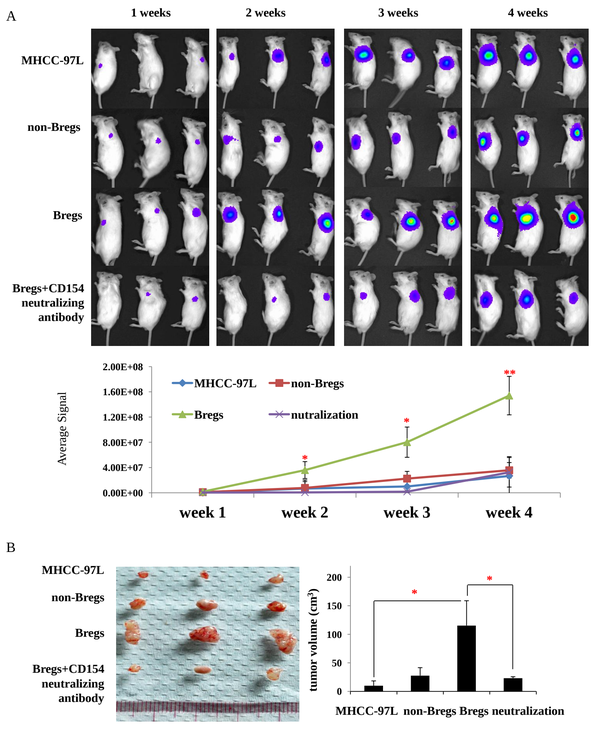

Fig4. HCC tumor growth was faster in Bregs group than non-Bregs injection or HCC control group. In addition, the Bregs induced tumor growth was inhibited by anti-CD154 neutralizing antibody treatment, compared with Bregs group.

5.Bregs interacted with HCC cells through CD40-CD154 signaling.

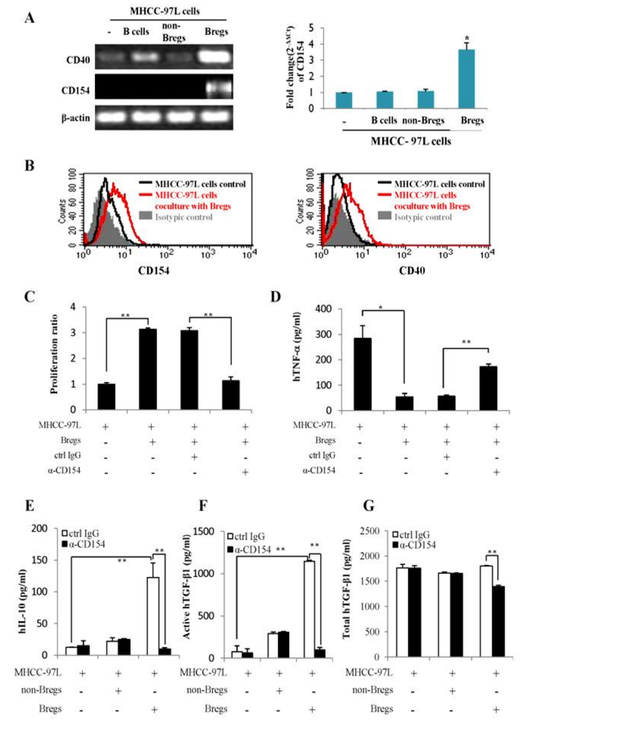

Fig5. (A and B) Bregs induced MHCC-97L cells to express high level of CD40 and CD154 protein. (C) Bregs could promote MHCC-97L cells proliferation. Anti-CD154 neutralizing antibody reversed the induction of HCC proliferation by Bregs. (D) Human TNF-α secretion was decreased concurrently with the induction of HCC cell proliferation. (E) IL10 secretion was highly induced in Bregs-HCC cells coculture. (F and G)The expression of human active and total TGF-β1 was measured.

Conclusion

- Abundance of B cells at HCC tumor margin was associated with cancer progression.
- Circulating regulatory B cells (Bregs) were associated with HCC progression.
- Bregs promoted HCC progression through CD40-CD154 interaction *in vivo* and *in vitro*.
- Suppression of Bregs may be an appealing therapeutic strategy in the treatment of HCC.

(Shao Y, et al, Cancer Letters, 2014. 264-272)

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