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Original Article

Linc-POU3F3 promotes cell proliferation in gastric cancer via increasing T-reg distribution

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Abstract: Long noncoding RNA (lncRNA) have been proved to participate in the oncogenesis or development of gastrointestinal tumors. In this study, we aimed to identify the function of lncRNAs in the differentiation of peripheral blood T cells especially the distribution of regulatory T cells (T-reg) in gastric cancer. The distribution of T-reg was detected by flow cytometry. Peripheral blood T-reg cells were significantly up-regulated in plasma samples of gastric cancer patients. LncRNA microarray detection indicated an aberrant expression profiling of lncRNAs in T-reg cells between gastric cancer patients and controls in which linc-POU3F3 was selected as a potential biomarker with the highest fold change value as well as the most stable expression level in each group. In addition, over-expression of linc-POU3F3 elevated Treg distribution *in vitro* and promoted tumor cell proliferation in the co-culture system. We further found that linc-POU3F3 could recruit TGF-beta which increased the phosphorylation of SMAD2/3. In conclusion, we found that linc-POU3F3 could promote the distribution of Tregs in peripheral blood T cell which caused an enhanced cell proliferation of gastric cancer cells by recruiting TGF-beta as well as activating TGF-beta signal pathway. This finding may provide a theoretical basis for the further exploration of lncRNAs function in immune cell cells of gastric cancer.

Keywords: Tregs, tranwell, TGF-beta, lincRNA, phosphorylation

Introduction

Tumor cell-mediated immunity is generally held to be the main mechanism of anti-tumor immunity [1]. Recent studies have revealed that a subset of $CD4^+$ T cells, referred to as $CD4^+CD25^+Foxp3^+$ Treg cells, may accumulate in the tumor microenvironment and suppress tumor-specific T-cell responses, thereby hindering tumor rejection [2-4]. $CD4^+CD25^+$ Treg cells have been shown to suppress the activity of nearby T cells in a contact-dependent manner and seem to have quite a dual role in host immune responses [5, 6]. On the one hand, Treg cell activity will have a positive effect on autoimmune and graft-versus-host responses in that they induce tolerance towards self and foreign antigens [7, 8]. On the other hand, the actions of Treg cells might lead to suppression of tumor-specific T cell responses at the tumor site [9].

Long non-coding RNAs (lncRNAs) are non-protein coding transcripts which are longer than 200nt in length [10, 11]. In functional genomics, they belong to a new type of regulatory genes. The detailed function of majority of lncRNA still remains unclear. More and more evidences showed that lncRNAs play critical roles in human disease, including stem cell development, pluripotency, cell growth and apoptosis in malignant tumors [12-14]. LncRNAs was identified to regulate protein coding gene through chromatin remodeling, transcriptional control and post-transcriptional processing [15, 16]. Many researchers showed altered lncRNA levels could affect the expression of gene products [17, 18]; however, majority was focused on the function of lncRNAs on tumor cells, the further exploring of lncRNAs involved in the immune cells of cancer is rare.

In this study, we firstly determined the distribution of T-reg cells in gastric cancer patients and

Table 1. Correlation between expression level of linc-POU3F3 and clinical characteristic of patients

	Linc-POU3F3		
Feather	Low	High	*P* value
All cases	20	20	
Age			
<60	15	11	0.185
≥60	5	9	
Gender			0.677
Male	16	17	
Female	4	3	
Tumor size (cm)			0.006
≤5 cm	17	4	
>5 cm	3	16	
Tumor location			0.633
Middle	3	2	
Lower	17	18	
Tumor capsular			0.151
Incomplete	4	1	
Complete	16	19	
TNM stage (I:II:III)	9:6:5	10:5:5	0.102

controls. We next focused on the aberrant expressed lncRNAs in T-reg cells in gastric cancers. LncRNAs were screened by Arraystar Human LncRNA Microarray v3.0. Finally, we selected the linc-POU3F3 as the candidate lncRNA to further analyze the function in regulating the distribution of T-reg.

Materials and methods

Clinical samples

40 cases of gastric cancer (GC) patients with gastric cancer during February 2013 to December 2014 were recruited from the Second Affiliated Hospital of Nanjing Medical University (Nanjing, China). All patients had negative histories of exposure to either chemotherapy or radiotherapy before, and there was no co-occurrence of diagnosed cancers. 40 healthy controls were enrolled in this study. Healthy blood donors were healthy physicians and post-graduate students from the Second Affiliated Hospital of Nanjing Medical University. Whole blood samples of donors were collected in EDTA anti-coagulated evacuated tubes. This study was approved by the Ethical Committee of Nanjing Medical University, and every donor had written informed consent.

Cell isolation and culture

Whole blood from both patients and healthy controls was collected. PBMCs were separated by Ficoll, and Ficoll-separated cells were used as the reference samples. Isolated PBMC were then resuspended in M200 medium with IL-2 (300 U/mL, PeproTech, USA) was added to enable survival of the lymphocytes in vitro. and cultured in TAKARA lymphocyte medium (Takara, Dalian, China). Gastric cancer cells were cultured in RPMI 1640 medium (Gibco, Grand Island, USA) containing 10% fetal calf serum (Gibco, Grand Island, USA). The detailed correlation of linc-POU3F3 with clinical characteristic of gastric patients was presented in **Table 1**.

Flow cytometric setup and analysis

All samples were sorted using a BD FACS Aria flow cytometer (BD, USA). Flow cytometric analysis was performed by using FACS Diva software (BD Bioscience). Fluorochrome-labeled mouse anti-human monoclonal antibodies targeted against CD4-PerCP, CD25-APC, Foxp3-PE (BD Phamingen) were used together with appropriate isotype controls to allow identification of positive and negative cell populations.

Microarray analysis of lncRNA

Total RNA from each sample was quantified by the NanoDrop ND-1000 (Thermo, CA, USA). For microarray analysis, Agilent Array platform was employed. The sample preparation and microarray hybridization were performed based on the manufacturer's standard protocols with minor modifications. Then, each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcripts without 3' bias utilizing a random priming method. The labeled cRNAs were hybridized onto the Human LncRNA Array v3.0 (8 x 60K, Arraystar). After having washed the slides, the arrays were scanned by the Agilent Scanner G2505C (Agilent, CA, USA).

Quantitative PCR and western blot

Total RNA from $CD4^+$ and $CD4^+CD25^+Foxp3^+$ cells was extracted by QianGen (QianGen, Beijing, China) according to the manufacturer's protocol. cDNAs from all sample were synthesized from 1 mg of total RNA by PrimeScript

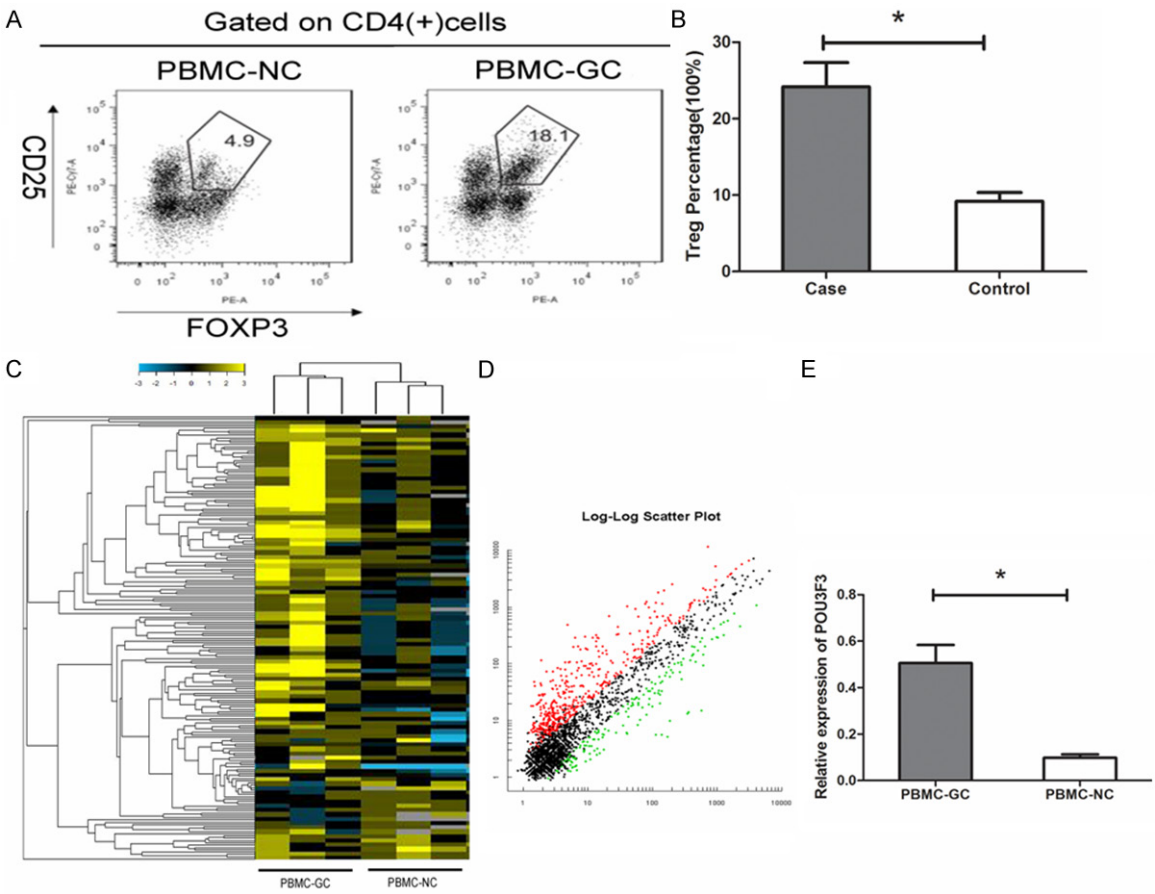

Figure 1. Elevated T-reg in blood of gastric cancer patients harbored an ectopic expression of lncRNAs comparing with control patients. A, B. The frequencies of $CD4^+CD25^+Foxp3^+$ cells detected by flow cytometry are significantly higher in patients (n = 40) than in the healty (n = 40). C, D. Different expression level of lncRNAs detected by lncRNA microarray presented as heatmap and scatter diagram (Fold change>2). E. Up-regulated linc-POU3F3 was confirmed by qRT-PCR in 40 GC patients. Data was presented as the mean ± SD. *P<0.05.

RT Master Mix kit (Takara, Dalian, China). Expression levels were calculated relative to GAPDH. Data analyses were performed using the $2^{-\Delta\Delta Ct}$ method.

Total protein was extracted using cell lysis buffer for western and IP (Beyotime, Shanghai, China) in accordance with the manufacturer's instruction. Typically, 20 mg of the protein was loaded per lane. he membranes were blocked with 5% non-fat dry milk for one hour at room temperature and incubated at 4°C overnight with primary antibody (Abcam, London, UK). GAPDH (Abcam, London, UK) was used to normalize the quantity of the protein.

RNA immunoprecipitation assay

RNA immunoprecipitation (RIP) experiments were performed using a Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA), according to the manufacturer's instructions. Antibody for RIP assays of TGF-beta (Cell Signaling Technology, Beverly, MA) was used as described before. Co-precipitated RNAs were detected by RT-PCR.

Transwell co-culture system and EDU assay

To investigate the interaction of T cell and cancer cell, the Transwell chambers were used to co-culture T cell and cancer cell. According to the experimental groups, T cells were seeded onto the upper insert of a 6-well Transwell (with 0.4 μm pores; Millipore) at a density of 5×10^5/ml placed above the cancer cells. After 5-day culture, cell proliferation was measured by EDU.

Cell proliferation was assayed using CCK8 and EDU (5-ethynyl-2'-deoxyuridine). (Roche, Basel, Switzerland) as described previously [19].

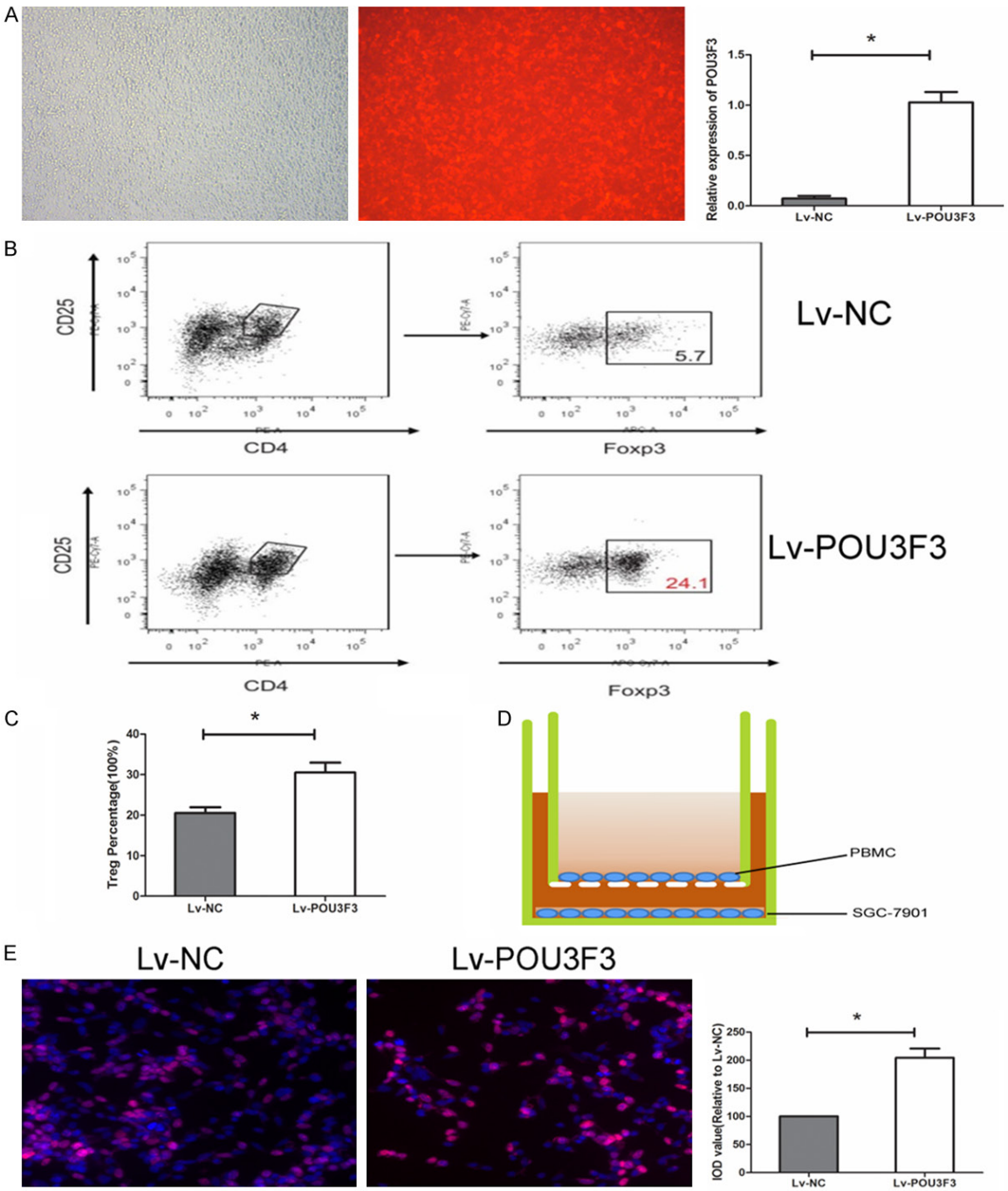

Figure 2. Up-regulated linc-POU3F3 promoted T-reg differentiation resulting in increasing cancer cell proliferation *in vitro.* A. The efficiency of lentivirus to up-regulate linc-POU3F3 was confirmed by both fluorescence intensity and qRT-PCR. B, C. The ratio of T-reg cells was detected by FACS in cell treated with linc-POU3F3 lentivirus and empty control. D. Schematic diagram of co-culture system. E. Cell proliferation ability was measured by EDU assay. All tests were performed in triplicate and presented as the mean ± SD. **P*<0.05.

Statistical analysis

The results of qRT-PCR and other variables were expressed as the mean (S.D.). The Student's t-test and Mann-Whitney unpaired test analysis of variance were used to evaluate statistical differences in patients and controls. Statistical analysis was performed using STATA

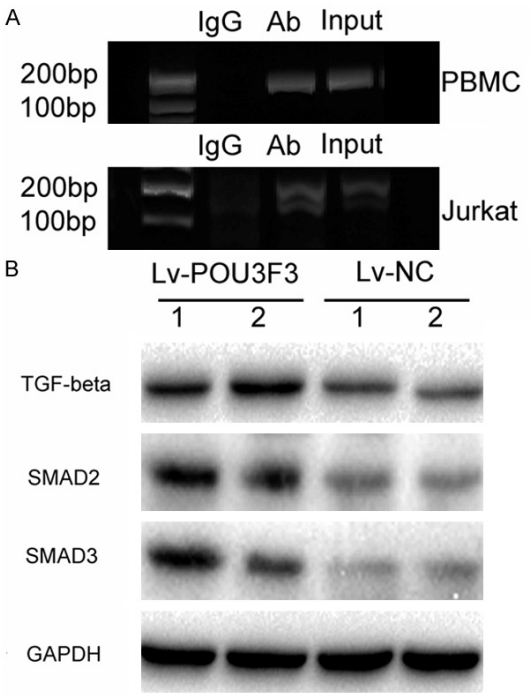

Figure 3. Linc-POU3F3 activated TGF-beta signal pathway by recruiting TGF-beta. A. RIP assay was performed to detected the binding ability of linc-POU3F3 to TGF-beta in both T cells isolated from patients and Jurkat cell line. Input was used as positive control while IgA as negative control. B. Expression level of TGF-beta and the phosphorylation of SMAD2/3 were detected by western blot. All tests were performed in triplicate.

9.2 and presented with the GraphPad prism software. In all cases, $P<0.05$ was considered significant.

Results

Elevated $CD4^+CD25^+Foxp3^+$ T-reg in blood of gastric cancer patients

We first examined the distribution of T-reg cells in peripheral blood of 40 gastric cancer patients and 40 controls. We characterized $CD4^+CD25^+Foxp3^+$ cells as T-reg and determined the ratio of T-regs in total $CD4^+$ cells as the frequencies. $CD4^+CD25^+Foxp3^+$ T-reg are abundantly present in blood of gastric cancer patients but nearly absent in healthy volunteers (**Figure 1A**, **1B**).

Aberrant expression of lncRNAs in T-reg isolated from GC patients and controls

To determine whether lncRNA was involved in the elevated distribution of T-reg in GC patients, we applied a lncRNA microarray to detect the different expression level of lncRNA in these T-reg cells. As presented in **Figure 1C** and **1D**, we found a significant aberrant expression profiling between the two groups which indicated that lncRNA might participated in the aberrant up-regulating ratio of T-reg in GC patients. We further ranked the different expressed lncRNA according to the fold change as well as the detection density which indicated the detect ability of the lncRNAs. We found that linc-POU3F3 presented the highest fold change value and the most stable detection density. Therefore, we selected linc-POU3F3 as candidate lncRNA to explore whether the different distribution of T-reg was associated with linc-POU3F3. We first detected the expression level of linc-POU3F3 in a larger samples mentioned above. QRT-PCR confirmed the up-regulation of linc-POU3F3 in peripheral blood T-reg cells of GC patients comparing with the healthy controls (**Figure 1E**), further analysis showed that increased linc-POU3F3 was highly associated with tumor size as presented in **Table 1** indicating that linc-POU3F3 might involve in the ectopic distribution of T-reg in GC patients.

Up-regulated linc-POU3F3 promoted T-reg differentiation resulting in increasing cancer cell proliferation in vitro

To explore the affection of linc-POU3F3 on T cell differenation especially on the distribution of T-reg, we initially over-expressed linc-POU3F3 in Naive T cells isolated from GC patients. The efficiency of over-expression assay by lentivirus was confirmed (**Figure 2A**). T-reg subpopulation detection indicated that up-regulated linc-POU3F3 could increase the ratio of T-reg *in vitro* (**Figure 2B**, **2C**). To further understand the interaction of T-reg cell and cancer cell induced by linc-POU3F3, we applied the transwell co-culture system. As presented in **Figure 2D**, the upper chamber was seeded with T cell treated with linc-POU3F3 lentivirus and empty controls while the bottom was supplied with GC cell line (SGC-7901). After co-cultured for 5 days, the proliferation of SGC-7901 cells was detected by EDU assay. We obtained that co-cultured the T cells with up-regulation of linc-POU3F3 could promote the proliferation of cancer cells (**Figure 2E**).

Linc-POU3F3 activated TGF-beta signal pathway by recruiting TGF-beta

The differentiation of T-reg was known highly associated with the TGF-beta signal pathway.

Although linc-POU3F3 has been proved interacting with POU3F3 which could also increasing cell proliferation in cancer cells [20, 21], in this study, we mainly focused on the function role of linc-POU3F3 in immune cells instead of cancer cells. Primary RIP assay has confirmed that linc-POU3F3 could not bind with EZH2 in human peripheral blood lymphocyte (data not shown). Besides, The Human Protein ATLAS database (http://www.proteinatlas.org) indicated that POU3F3 hardly expressed in human lymphoid cells.

Accordingly, due to the essential role of TGF-beta, we next detected the interaction of linc-POU3F3 with TGF-beta. RIP assay was performed by using TGF-beta antibody in both human peripheral blood lymphocyte and Jurkat (known as T cell lines origin from acute lymphocytic leukemia). We found that linc-POU3F3 could directly binding with TGF-beta (**Figure 3A**) and caused an up-regulation of TGF-beta was confirmed by western blot as presented in **Figure 3B**. We further analyzed whether the TGF-beta was activated by determinded the phosphorylation of SMAD2/3. We obtained that both the phosphorylation of SMAD2 (Ser467) and SMAD3 (Ser423) was up-regulated following by the up-regulation of TGF-beta (**Figure 3B**).

Discussion

T-reg is an important subpopulation of T lymphocytes. They were involved in many different functions in immune processes and play crucial roles in human disease, such as cancer, allergy [22, 23]. It has been reported that the population of Treg cells in PBLs is significantly higher than that in normal tissue in several malignancies [24]. T-reg cells could decrease the functions of innate and adaptive immune cells [6, 25]. Due to the important roles of T-reg in the development of human malignant tumors, the exploration of mechanism involved in the aberrant distribution of T-reg in human cancer was very necessary.

In our research, we mainly focused on the lncRNAs participating in T-reg cells of GC patients. We showed that $CD4^+CD25^+Foxp3^+$ Treg cells were increased in peripheral blood of gastric cancer patients, as compared to healthy donors. These results correlated well with previous findings of increased frequencies of $CD4^+CD25^+Foxp3^+$ Treg cells in tumor tissue, detected by immunohistochemical analysis [26]. Highthrought screening was applied to explore the potential lncRNAs involved in GC patients and healthy controls. Linc-POU3F3 was identified as the target lncRNA for further investigation. We found that linc-POU3F3 could promote the distribution of T-reg and further increase the ability of cell proliferation. Linc-POU3F3 was recently reported as an important factor in the tumorigenesis of esophagus cancer by interacting with EZH2 and further influenced the expression of POU3F3 [20]. Thus, we first determined to examine whether linc-POU3F3 could regulated POU3F3 as reported, RIP assay indicated that linc-POU3F3 could not directly bind with EZH2 which was not consistence with previous research. Further detection obtained that POU3F3 hardly expressed in human T cells as well as Jurkat cell line.

LncRNA is confirmed that could regulate gene expression at different levels, including chromatin modification, transcription and post-transcriptional processing; however, according to the function of linc-POU3F3, we first detected the function of linc-POU3F3 in post-transcription level. Thus, we decided to examine whether linc-POU3F3 could interact with crucial factors involved in the differentiation of T-reg. Antibody targeting TGF-beta was selected in the next RIP assay, we found that linc-POU3F3 could recruit TGF-beta protein which caused a up-regulation of TGF-beta and activation of TGF-beta signal pathway.

In conclusion, the results demonstrated the role of linc-POU3F3 in T-reg from peripheral blood of gastric cancer patients. And furthermore, we preliminarily found that up-regulation of linc-POU3F3 could promote the distribution of T-reg resulting in increasing cell proliferation by recruit TGF-beta. Our research might provide a new foundation for the mechanism exploration of gastric cancer.

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Disclosure of conflict of interest

None.

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References

[1] Wang C, Zhou Q, Wang X, Wu X, Chen X, Li J, Zhu Z, Liu B and Su L. The TLR7 agonist induces tumor regression both by promoting CD4+ T cells proliferation and by reversing T regulatory cell-mediated suppression via dendritic cells. Oncotarget 2015; 6: 1779-1789.

[2] Mizukami Y, Kono K, Kawaguchi Y, Akaike H, Kamimura K, Sugai H and Fujii H. CCL17 and CCL22 chemokines within tumor microenvironment are related to accumulation of Foxp3+ regulatory T cells in gastric cancer. Int J Cancer 2008; 122: 2286-2293.

[3] Wolf AM, Wolf D, Steurer M, Gastl G, Gunsilius E and Grubeck-Loebenstein B. Increase of regulatory T cells in the peripheral blood of cancer patients. Clin Cancer Res 2003; 9: 606-612.

[4] Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, Maxeiner J, Hansson M, Taube C, Quiding-Jarbrink M and Muller A. DC-derived IL-18 drives Treg differentiation, murine Helicobacter pylori-specific immune tolerance, and asthma protection. J Clin Invest 2012; 122: 1082-1096.

[5] Victor CT, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, Benci JL, Xu B, Dada H, Odorizzi PM, Herati RS, Mansfield KD, Patsch D, Amaravadi RK, Schuchter LM, Ishwaran H, Mick R, Pryma DA, Xu X, Feldman MD, Gangadhar TC, Hahn SM, Wherry EJ, Vonderheide RH and Minn AJ. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 2015; 520: 373-7.

[6] Liu G, Bi Y, Xue L, Zhang Y, Yang H, Chen X, Lu Y, Zhang Z, Liu H, Wang X, Wang R, Chu Y and Yang R. Dendritic cell SIRT1-HIF1alpha axis programs the differentiation of CD4+ T cells through IL-12 and TGF-beta1. Proc Natl Acad Sci U S A 2015; 112: E957-965.

[7] Bin Dhuban K, d'Hennezel E, Nashi E, Bar-Or A, Rieder S, Shevach EM, Nagata S and Piccirillo CA. Coexpression of TIGIT and FCRL3 Identifies Helios+ Human Memory Regulatory T Cells. J Immunol 2015; 194: 3687-96.

[8] Koenecke C, Lee CW, Thamm K, Fohse L, Schafferus M, Mittrucker HW, Floess S, Huehn J, Ganser A, Forster R and Prinz I. IFN-gamma production by allogeneic Foxp3+ regulatory T cells is essential for preventing experimental graft-versus-host disease. J Immunol 2012; 189: 2890-2896.

[9] Kurose K, Ohue Y, Sato E, Yamauchi A, Eikawa S, Isobe M, Nishio Y, Uenaka A, Oka M and Nakayama E. Increase in activated Treg in TIL in lung cancer and in vitro depletion of Treg by ADCC using an antihuman CCR4 mAb (KM2760). J Thorac Oncol 2015; 10: 74-83.

[10] Tang J, Jiang R, Deng L, Zhang X, Wang K and Sun B. Circulation long non-coding RNAs act as biomarkers for predicting tumorigenesis and metastasis in hepatocellular carcinoma. Oncotarget 2015; 6: 4505-4515.

[11] Tang J, Zhuo H, Zhang X, Jiang R, Ji J, Deng L, Qian X, Zhang F and Sun B. A novel biomarker Linc00974 interacting with KRT19 promotes proliferation and metastasis in hepatocellular carcinoma. Cell Death Dis 2014; 5: e1549.

[12] Cao C, Sun J, Zhang D, Guo X, Xie L, Li X, Wu D and Liu L. The long intergenic noncoding RNA UFC1, a target of MicroRNA 34a, interacts with the mRNA stabilizing protein HuR to increase levels of beta-catenin in HCC cells. Gastroenterology 2015; 148: 415-426, e18.

[13] Thum T and Condorelli G. Long Noncoding RNAs and MicroRNAs in Cardiovascular Pathophysiology. Circ Res 2015; 116: 751-762.

[14] Fritah S, Niclou SP and Azuaje F. Databases for lncRNAs: a comparative evaluation of emerging tools. RNA 2014; 20: 1655-1665.

[15] Cech TR and Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. Cell 2014; 157: 77-94.

[16] Marchese FP and Huarte M. Long non-coding RNAs and chromatin modifiers: their place in the epigenetic code. Epigenetics 2014; 9: 21-26.

[17] Lopez-Pajares V, Qu K, Zhang J, Webster DE, Barajas BC, Siprashvili Z, Zarnegar BJ, Boxer LD, Rios EJ, Tao S, Kretz M and Khavari PA. A LncRNA-MAF:MAFB transcription factor network regulates epidermal differentiation. Dev Cell 2015; 32: 693-706.

[18] Ramos AD, Andersen RE, Liu SJ, Nowakowski TJ, Hong SJ, Gertz CC, Salinas RD, Zarabi H, Kriegstein AR and Lim DA. The long noncoding RNA Pnky regulates neuronal differentiation of embryonic and postnatal neural stem cells. Cell Stem Cell 2015; 16: 439-47.

[19] Zhuo H, Tang J, Lin Z, Jiang R, Zhang X, Ji J, Wang P and Sun B. The aberrant expression of MEG3 regulated by UHRF1 predicts the prognosis of hepatocellular carcinoma. Mol Carcinog 2015; [Epub ahead of print].

[20] Li W, Zheng J, Deng J, You Y, Wu H, Li N, Lu J and Zhou Y. Increased levels of the long intergenic non-protein coding RNA POU3F3 promote DNA methylation in esophageal squamous cell carcinoma cells. Gastroenterology 2014; 146: 1714-26, e5.

[21] Tong YS, Wang XW, Zhou XL, Liu ZH, Yang TX, Shi WH, Xie HW, Lv J, Wu QQ and Cao XF. Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. Mol Cancer 2015; 14: 3.

[22] Jayaraman P, Alfarano MG, Svider PF, Parikh F, Lu G, Kidwai S, Xiong H and Sikora AG. iNOS expression in CD4+ T cells limits Treg induction by repressing TGFbeta1: combined iNOS inhibition and Treg depletion unmask endogenous antitumor immunity. Clin Cancer Res 2014; 20: 6439-6451.

[23] Yin Y, Cai X, Chen X, Liang H, Zhang Y, Li J, Wang Z, Zhang W, Yokoyama S, Wang C, Li L, Hou D, Dong L, Xu T, Hiroi T, Yang F, Ji H, Zhang J, Zen K and Zhang CY. Tumor-secreted miR-214 induces regulatory T cells: a major link between immune evasion and tumor growth. Cell Res 2014; 24: 1164-1180.

[24] Lin ZW, Wu LX, Xie Y, Ou X, Tian PK, Liu XP, Min J, Wang J, Chen RF, Chen YJ, Liu C, Ye H and Ou QJ. The expression levels of transcription factors T-bet, GATA-3, RORgammat and FOXP3 in peripheral blood lymphocyte (PBL) of patients with liver cancer and their significance. Int J Med Sci 2015; 12: 7-16.

[25] Alizadeh D and Larmonier N. Chemotherapeutic targeting of cancer-induced immunosuppressive cells. Cancer Res 2014; 74: 2663-2668.

[26] Gu Y, Wang C, Han D and Zhang L. Increased frequencies of CD4+ CD25+ FOXP3+ regulatory T cells in human nasal inverted papilloma. Head Neck 2011; 33: 1005-1012.