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Imaging and measuring the biophysical properties of Fc gamma receptors on single macrophages using atomic force microscopy

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ABSTRACT

Fc gamma receptors (FcγR), widely expressed on effector cells (e.g., NK cells, macrophages), play an important role in clinical cancer immunotherapy. The binding of FcγRs to the Fc portions of antibodies that are attached to the target cells can activate the antibody-dependent cell-mediated cytotoxicity (ADCC) killing mechanism which leads to the lysis of target cells. In this work, we used atomic force microscopy (AFM) to observe the cellular ultra-structures and measure the biophysical properties (affinity and distribution) of FcγRs on single macrophages in aqueous environments. AFM imaging was used to obtain the topographies of macrophages, revealing the nanoscale cellular fine structures. For molecular interaction recognition, antibody molecules were attached onto AFM tips via a heterobifunctional polyethylene glycol (PEG) crosslinker. With AFM single-molecule force spectroscopy, the binding affinities of FcγRs were quantitatively measured on single macrophages. Adhesion force mapping method was used to localize the FcγRs, revealing the nanoscale distribution of FcγRs on local areas of macrophages. The experimental results can improve our understanding of FcγRs on macrophages; the established approach will facilitate further research on physiological activities involved in antibody-based immunotherapy.
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1. Introduction

The use of monoclonal antibodies (mAbs) in cancer therapy has achieved considerable success in recent years, and antibody-based immunotherapy is now one of the most important strategies for treating patients with hematological malignancies and solid tumors [1,2]. Rituximab is the first mAb for treating CD20 + non-Hodgkin's lymphoma (NHL) [3]. The unprecedented success of rituximab in clinical practice promotes the development of new anti-CD20 mAbs [4], but to date none of the newer anti-CD20 mAbs have been shown to be clinically more effective than rituximab [5]. In the three killing mechanisms of rituximab, it is widely accepted that antibody-dependent cell-mediated cytotoxicity (ADCC) is critical, while the role of complement-dependent cytotoxicity and programed cell death is still disputed [6]. In ADCC, the mAbs Fab portions bind to the antigens (CD20) on tumor cells, and then the Fc portions of mAbs bind to the Fc gamma receptors (FcγR) on effector cells (e.g., NK cells, macrophages) [7]. The effector cells then cause the lysis of tumor cells by the release of cytotoxic granules containing granzymes and perforin [8] or phagocytosis [9]. In vitro experiments indicate that the NK cell is the prominent effector cell in ADCC [6], but this suggestion stems largely from the use of peripheral blood mononuclear cell in ADCC assays where NK cells are the main effector cells [10]. In vivo, perhaps macrophages are much more important [10]. Enhancing the binding affinity of Fc portion to FcγR is emerging as one of the most promising ways to further increase the clinical potential of mAbs in patients [11], particularly in those patients who express a low affinity version of FcγR on their effector cells [9]. Many new anti-CD20 mAbs with enhanced FcγR binding affinity have been developed, but the efficacy of these new mAbs in rituximab-refractory disease is yet to be determined in clinical practice [12].

Despite the undeniable value for treating B-cell NHL, rituximab is not effective in all patients, and many patients develop resistance to the rituximab therapy [13]. In current practice, the contribution of rituximab to the resistance phenomenon is difficult to evaluate, because rituximab is typically combined with cytotoxic chemotherapy [14]. Recent evidence suggests that antigenic modulation appears to play a key role in the development of resistance to rituximab therapy [15]. In addition to the resistance, the rituximab treatment costs are expensive and serious reactions can occur (including hypotension, rigors, bronchospasm, and angioedema) in as many as 10% of patients [5]. Hence the urgent need in mAb therapy is to predict the drug efficacy [16] to identify the patients who can benefit from the therapy [17], helping the insensitive patients to avoid non-effective treatments and unnecessary economic costs. If we can exactly predict the treatment efficacy, then we make a

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major step in the era of personalized medicine. To obtain this goal, we need to fully understand the physiological activities involved in the mAb mechanisms. Much of our understanding of molecular reactions in cells has come from traditional ensemble experiments done in test tubes with purified biomolecules [18] which are unable to reveal the behaviors of single molecules. In recent years, single-molecule experiments [19] have attracted the attention of many researchers. Probing single molecules can reveal the fundamental chemical, biological and physical properties that are hidden in ensemble experiments [20], complementing the information obtained by traditional methods [21]. Atomic force microscopy (AFM) [22] is a commonly used single-molecule technique. With AFM single-molecule force spectroscopy (SMFS), we can directly measure the binding force between molecules [23] and localize the receptors on the cell surface [24]. Currently, to our knowledge, the single molecule behaviors in Fc–FcγR interactions are largely unknown. In this work, the binding affinities and nanoscale distribution of FcγRs were directly measured on single macrophages by AFM force spectroscopy.

2. Materials and methods

2.1. Sample preparation

Macrophage cell line RAW264.7 was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and maintained in DMEM containing 10% fetal bovine serum at 37 °C (5% CO_2). Cells were cultured in Petri dishes for 24 h before experiments. After removing the culture medium in the Petri dishes, 4% paraformaldehyde was added for chemical fixation. After washing the Petri dishes with phosphate-buffered saline (PBS), fresh PBS was added and then the Petri dishes were placed on AFM stage. For FcγR fluorescence labeling, rituximab solution was added into the Petri dishes and incubated for 3 h at room temperature. After washing the Petri dishes with PBS three times (each time 10 min), goat-anti-human IgG (conjugated with red-fluorescent dye RBITC) was added and incubated for 30 min. After washing the Petri dishes with PBS three times (each time 10 min), the Petri dishes were placed on the AFM stage for fluorescence observation.

2.2. Tip functionalization

The prerequisite of measuring the molecular forces with AFM force spectroscopy is attaching the antibodies onto the AFM tip. Tip functionalization was according to the procedure described in the reference [25]. The heterobifunctional PEG molecule NHS-PEG3500-MAL (JenKem Technology, Beijing, China) was used to link rituximabs (Chinese Affiliated Hospital of Military Medical Academy of Sciences) onto the AFM tip. The nominal spring constant of the probe was 0.01 N/m (Bruker, Santa Barbara, CA, USA). Fluorescence experiments were performed to verify whether rituximab molecules were linked onto the AFM tip. The functionalized probe was placed in a petri dish and then RBITC-conjugated goat-anti-human IgG solution was added. After washing the functionalized probe with PBS three times, the functionalized probe was placed on the stage of fluorescence microscope for fluorescence observation.

2.3. AFM imaging and SMFS measurements

AFM imaging and SMFS measurements were performed in PBS at room temperature using a Bioscope Catalyst AFM (Bruker, Santa Barbara, CA, USA) which was set on an inverted fluorescence microscope (Ti, Nikon, Japan). For cell imaging, AFM probe (unfunctionalized) was moved onto the macrophages under the guidance of an optical microscope, and the imaging mode was the contact mode. For binding affinity measurement, AFM probes (functionalized) were used, and force curves were obtained on different areas of the cells at five different loading rates (60,000–120,000 pN/s). The spring constant of the probe was calibrated by thermal noise method [26]. To demonstrate the specific rituximab–FcγR binding, force curves were obtained on macrophages after blocking experiments (adding free rituximab molecules to mask the FcγRs on macrophages). Also the force curves on the bare area of Petri dishes were obtained. In order to localize the FcγRs on macrophages, two Petri dish macrophages were prepared (one Petri dish was blocked by adding rituximab molecules). Arrays of force curves were first obtained on macrophages (without blocking), and then on blocked macrophages. To verify the activity of functionalized tips, arrays of force curves were obtained on macrophages (without blocking) again. The force curves were analyzed by Matlab 7.6.0. After converting the binding force values into 0–255, gray maps were constructed by image processing software.

3. Results and discussion

FcγRs, widely expressed throughout the hematopoietic system, belong to the large immunoglobulin superfamily and are type I transmembrane glycoproteins [27]. FcγRs are key players in both the afferent and efferent phases of the immune responses [28]. Binding of FcγRs to the Fc portion of the antibody triggers effector functions that are important in the antibody-based immunotherapy of cancer [2]. RAW264.7 cell is a mouse macrophage cell line that has been used extensively for studying FcγR-mediated phagocytosis [29]. Here we used RAW 264.7 cells for the SMFS experiments. Before SMFS experiments, we first examine the expression of FcγR on macrophages by immunofluorescence labeling. Rituximab can bind to the FcγRs on macrophages and fluorescent-conjugated goat-anti-human IgG can bind to rituximab. Fig. 1(A, B) was optical image and fluorescence image of macrophages. From the fluorescence image, we can clearly see that macrophages exhibited bright red fluorescence, and this indicated that there were FcγR on the macrophages. Then we examined the rituximab molecules on functionalized probes. Fig. 1(C) was the fluorescence image of the functionalized probe. We can see that the probe exhibited bright red fluorescence and this indicated that rituximab molecules had been linked onto the AFM tip after functionalization.

Fig. 2 shows the AFM images of macrophages. Macrophages can attach and spread on the substrate. Hence we can image them by directly culturing them in Petri dishes. Under the guidance of optical image [Fig. 1(D)], AFM tip was moved onto the macrophages. Fig. 2(A and B) shows the AFM height image and deflection image of two adjacent macrophages. The scan size was 80 μm. We can see that many lamellipodium and filopodium were discernible in AFM images. The line profile of the height image [Fig. 2(C)] indicated that the height of the two cells was about 4 μm and the diameter was about 25 μm. To observe the fine structures of the macrophages, AFM imaging on a local area of a macrophage was performed, as shown in Fig. 2(D and E). The scan size was 26 μm. From the deflection image [Fig. 2(E)], we can clearly see that the surface of macrophage was rough and ruffles [30] were observed on the surface (denoted by the arrows). The line profile (red curve in Fig. 2(F)) indicates that the diameter of ruffles was about 1 μm and the height was about 200 nm. Additionally, the lamellipodium were clearly discernible in the image [Fig. 2(E)] and the line profile of a lamellipodia (black curve in Fig. 2F) indicated that its width was about 4 m. Cells come in different shapes and sizes, and cell geometry affects cell fate in many processes [31]. Cell shape emerges from the interaction of many elements (such as

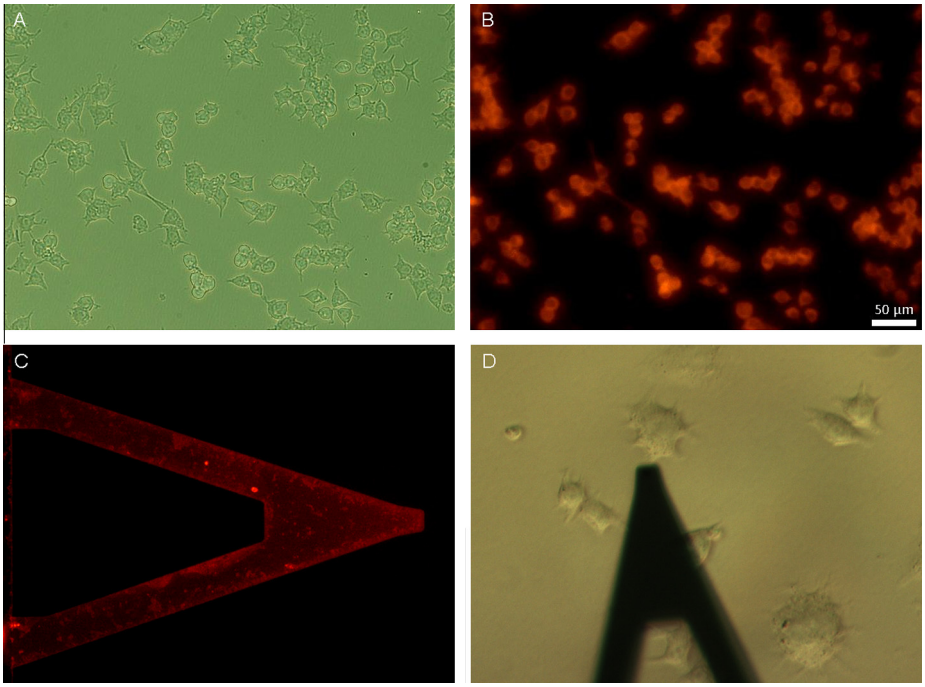

Fig. 1. Observing the express of FcγR on the macrophages and the functionalized probes by immunofluorescence labeling. Optical image (A) and the corresponding fluorescence images (B) of macrophages. Fluorescence image (C) of the functionalized probe. Under the guidance of optical image (D), the AFM tip was moved to the macrophages.

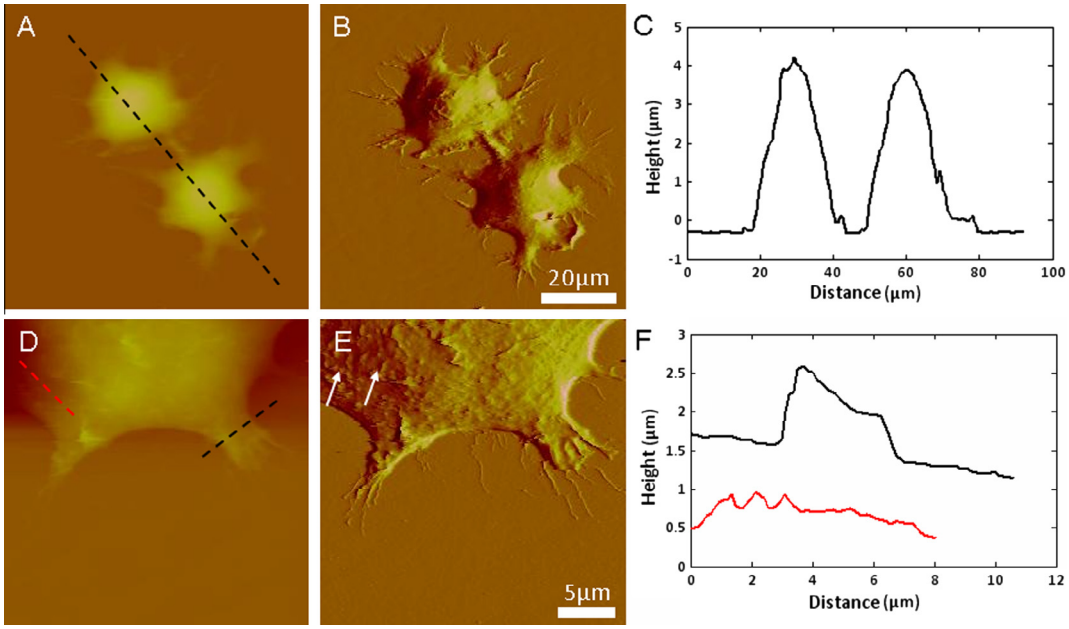

Fig. 2. Nanoscale morphologies of the macrophages obtained by AFM imaging in PBS. AFM height image (A) and deflection image (B) of two macrophages. Line profile (C) taken in the height image (A) along the dashed line. AFM height image (D) and deflection image (E) of the local area of a macrophage. (F) Line profiles taken in the height image (D) along the dashed lines.

cytoskeleton, cell membrane, and cell adhesions), and the mechanism by which global morphology is generated and maintained at the cellular scale is yet unknown [32]. The observed ultra structures of macrophages are related to their biological functions [30]. Recent evidence indicates that macrophages play an important role in the development of metabolic disease [33]. The imaging results indicate that AFM can reveal the cellular ultra-structures which is useful for understanding cellular physiological activities.

Fig. 3 shows the results of measuring the rituximab–FcγR binding forces on macrophages. AFM-based SMFS has been widely used to quantify the molecular interactions, but these studies are mainly performed on purified biomolecules (in most cases, the transmembrane receptors are purified in truncated forms consisting only of their extracellular domains [23]) in vitro which are quite different from the native environments around the receptors [34]. In order to better reflect the rituximab–FcγR interactions, we measured

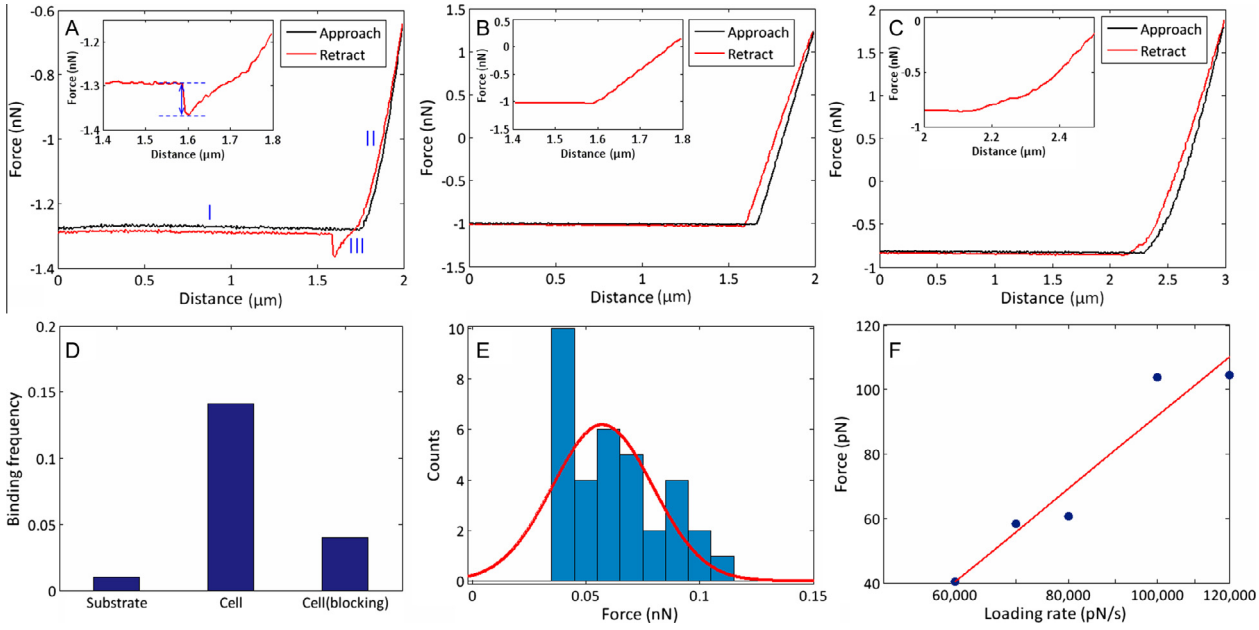

Fig. 3. Measuring the rituximab–FcγR interactions by obtaining force curves on macrophages using rituximab-conjugated tips. Typical force curves obtained on macrophages (A), on substrate (B), and on macrophages after blocking (C). The insets in (A–C) were the enlarged views of retract curves. (D) Binding frequency on macrophages, on substrate and on blocked macrophages. (E) Force histogram at a loading rate. (F) Forces are linearly related to the logarithm of the loading rates.

the rituximab–FcγR binding forces directly on cells. Fig. 3(A) shows a typical force curve obtained on macrophages with a specific molecular unbinding peak. Each force curve contains two portions, approach curve and retract curve. When the tip did not touch the cell surface, the deformation of the probe cantilever was kept unchanged, and the force curve was flat (denoted by the I in Fig. 3(A)). After the tip touched the cell surface, the deformation of the probe cantilever changed, and the force curve became bent (denoted by the II in Fig. 3(A)). During the approach-retract cycle, if rituximab molecules on the AFM tip bound to the FcγR on macrophages, then the rituximab–FcγR pair was stretched (denoted by the III in Fig. 3(A)) when the tip retracted from the cell surface to its original position. The molecular binding strength is far smaller than the covalent bonds between PEG linker and AFM tip, and thus the molecular pair will first rupture during the stretching process [25,35]. When the molecular pair ruptured, there was a specific molecular peak in the retract curve, as shown in the inset of Fig. 3(A). The magnitude of the peak indicated the binding force (denoted by the double-headed blue arrow). When obtaining force curves on the substrate [Fig. 3(B)], the force curves were straight, and there was no specific peak in the retract curve. This is because the substrate is stiff, and there were no FcγR molecules on the substrate. After the blocking experiments (adding free rituximab to bind the FcγR on macrophages), no specific molecular peak appeared in the retract curve [Fig. 3(C)]. This is because the added rituximab molecules had blocked the FcγR molecules on the cell surface. For each situation (on macrophages, substrate and blocked macrophages), after obtaining 500 force curves, the binding frequency was computed, as is shown in Fig. 3(D). We can see that the binding frequency on macrophages was significantly larger than on the substrate and blocked macrophages, indicating the specific rituximab–FcγR interactions.

Fig. 3(E and F) shows the rituximab–FcγR binding forces measured at five different loading rates. Fig. 3(E) was the histogram of rituximab–FcγR binding force measured at a certain loading rate (70,000 pN/s). The Gaussian fit indicates that the mean force was 58 pN. For biomolecules, ligand-receptor affinity is mainly determined by the rate of ligand-receptor complex dissociation [36]. According to the Bell–Evans model [37,38], the ligand-receptor unbinding force was linearly related to the logarithm of the loading rate of the external pulling force. Recent researches indicate that not only the molecular bonds but also the mechanical properties of proteins and membranes are loading-rate dependent [39]. Fig. 3(F) shows the relationship between rituximab–FcγR binding force and the logarithm of loading rates. We can clearly see that the rituximab–FcγR binding force is linearly related to the logarithm of the loading rate. In single-molecule dynamic force spectroscopy experiments, measuring the unbinding force at different loading rates is useful for assessing kinetic parameters of the unbinding process, including dissociation rate constant, association rate constant, and relative heights of energy barriers [35,40,41]. During the ADCC mechanism, the Fab region of rituximab can bind to the antigen (CD20) on the lymphoma cell surface and the Fc region of the rituximab can bind to the FcγR on the macrophage surface. This means that there are two types of molecular binding during the ADCC mechanism. We havepreviously measured the binding force between rituximab and CD20 on the lymphoma cell surface [25,42]. Here the binding forces between rituximab and FcγR on the macrophage surface were measured, improving our understanding of Fc–FcγR interactions.

Fig. 4 shows the distribution of FcγRs on macrophages. Besides measuring the molecular binding force, SMFS can also localize the receptors on the cell surface by obtaining arrays of force curves on the local area of the cell surface [43]. We first obtained arrays (16×16) of force curves on different local areas (500×500 nm) of three macrophages. Fig. 4(A) is a typical force curve with molecular specific unbinding peak. Fig. 4(B–D) are the force maps. From the maps, the gray pixels indicated the FcγR. We can see that the FcγRs were dispersedly located on the cell surface, and some FcγRs aggregate. In order to verify that the maps reflect the distribution of FcγR on the macrophage surface, we used the functionalized tips to obtain arrays of force curves on macrophages that had been blocked, and the results are shown in Fig. 4(E–H). The majority of the force curves obtained on blocked macrophages did not have specific unbinding peaks, as shown in Fig. 4(E). From the maps [Fig. 4(F–H)], we can see that there were very few gray pixels. This

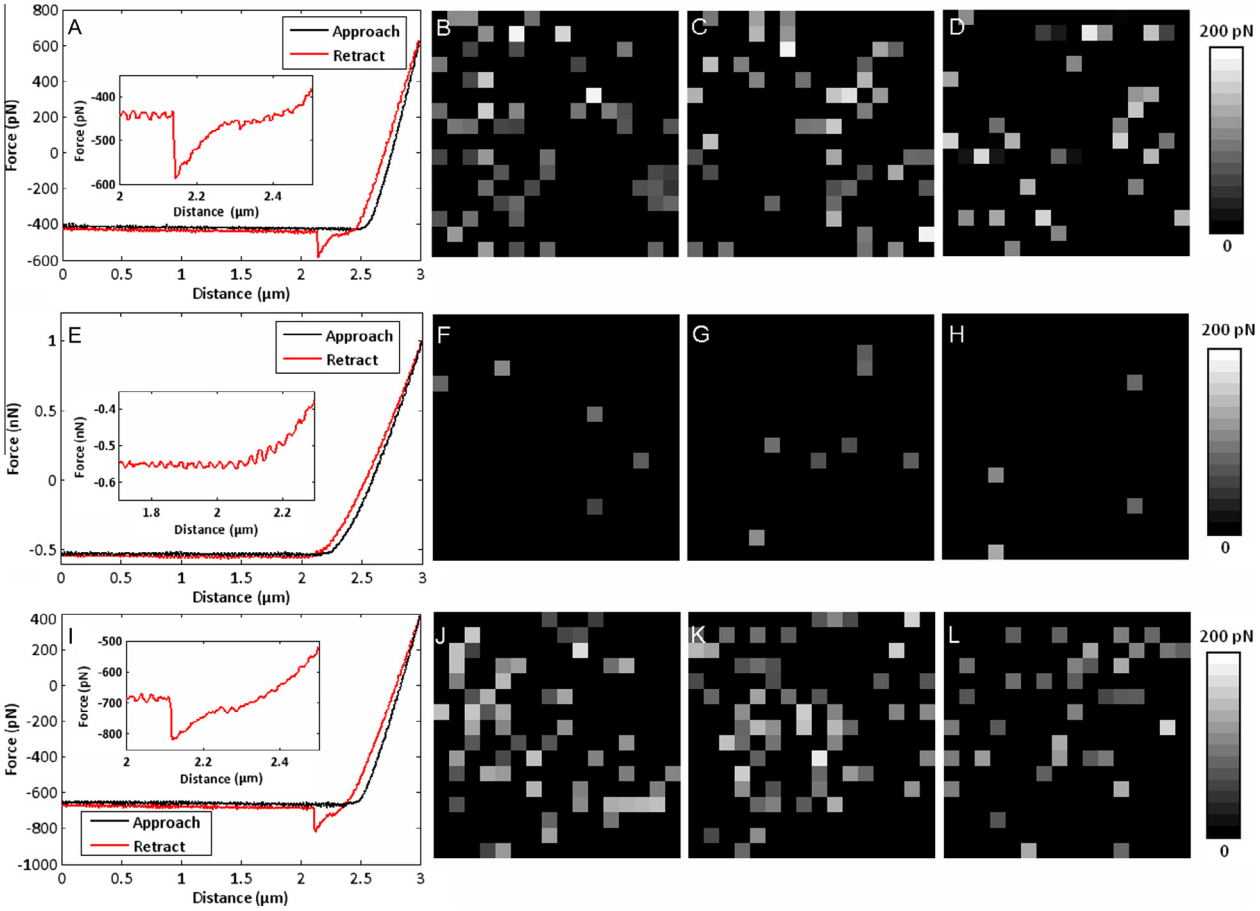

Fig. 4. Localizing the FcγR on macrophages by obtaining arrays of force curves on a local area of the cell surface. (A–D) were from macrophages. (A) A typical force curve. (B–D) were force maps constructed by obtaining 16 × 16 force curves on 500 × 500 nm area of the cell surface. (E–H) were from rituximab-blocked macrophages. (E) A typical force curve. (F–H) Force maps. (I–L) were from macrophages (without blocking). (I) A typical force curve. (J–L)Force maps. The insets in (A, E and I) were enlarged views of retract curves.

is because that the FcγRs on the cell surface had been blocked by the added rituximab molecules. This may also be because the rituximab molecules on the AFM tip surface had lost activity. Hence in order to verify whether the rituximab molecules on the functionalized tips were still active, we used the same functionalized tips to obtain arrays of force curves on macrophages (without blocking) and the results are shown in Fig. 4(I–L). Many force curves had specific unbinding peaks [Fig. 4(I)], and there were many gray pixels in the maps [Fig. 4(J–L)]. This demonstrates that the rituximab molecules on the functionalized tips were still active and the maps correspond to the distribution of FcγRs. AFM has nanometer spatial resolution and can work in liquids. These advantages offer unprecedented opportunities for detecting the life activities at single-cell and single-molecule levels [21]. After attaching specific ligands onto the AFM tip, we can nanoscopically detect the receptors on the cell surface. Researchers have used an AFM adhesion force map to investigate the distribution of various membrane proteins on the cell surface, such as sensors [24], adhesions [43], receptors [44], and fibronectin attachment proteins [45]. Traditional methods for characterizing the distribution of proteins on cell surfaces are based on optical microscopy which has a 200 nm resolution limit. AFM can detect the proteins at nanometer scale and thus provides novel insights for understanding the distribution of proteins on the cell surface.

In summary, the cellular ultra-structures and FcγR's biophysical properties (affinity and distribution) were investigated on single macrophages in this work. Due to the fact that ADCC mechanism involves two types of molecular binding, CD20-rituximab and rituximab–FcγR, we should investigate both these two types of molecular binding to better understand ADCC. In previous studies, we investigated the CD20–rituximab interactions on patient cancer cells [42], and here rituximab–FcγR interactions were investigated. In the future, we want to use SMFS to simultaneously measure the CD20–rituximab interactions and rituximab–FcγR interactions on patient cancer cells. Nowadays, to our knowledge, almost all SMFS studies measured the binding force of one type of receptor–ligand interaction. But the fact is that in many activities, such as the immunotherapy mechanism [2], there is more than one type of receptor–ligand interaction. If we can simultaneously measure multiple types of receptor–ligand interactions, then we can obtain novel information of the cellular activities that may be meaningful for identifying the biomarkers of cancer that predict the targeted therapy [16].

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References

[1] A.M. Scott, J.D. Wolchok, L.J. Old, Antibody therapy of cancer, Nat. Rev. Cancer 12 (2012) 278–287.

[2] L.M. Weiner, J.C. Murray, C.W. Shuptrine, Antibody-based immunotherapy of cancer, Cell 148 (2012) 1081–1084.

[3] B.D. Cheson, J.P. Leonard, Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma, N. Engl. J. Med. 359 (2008) 613–626.
[4] S. Cang, N. Mukhi, K. Wang, D. Liu, Novel CD20 monoclonal antibodies for lymphoma therapy, J. Hematol. Oncol. 5 (2012) 64.
[5] D.G. Maloney, Anti-CD20 antibody therapy for B-cell lymphomas, N. Engl. J. Med. 366 (2012) 2008–2016.
[6] S.H. Lim, S.A. Beers, R.R. French, P.W.M. Johnson, M.J. Glennie, K.S. Cragg, Anti-CD20 monoclonal antibodies: historical and future perspectives, Haematologica 95 (2010) 135–143.
[7] G. Cartron, H. Watier, J. Golay, P. Solal-Celigny, From the bench to the bedside: ways to improve rituximab efficacy, Blood 104 (2004) 2635–2642.
[8] E. Reefman, J.G. Kay, S.M. Wood, C. Offenhauser, D.L. Brown, S. Roy, A.C. Stanley, P.C. Low, A.P. Manderson, J.L. Stow, Cytokine secretion is distinct from secretion of cytotoxic granules in NK cells, J. Immunol. 184 (2010) 4852–4862.
[9] E. Oflazoglu, L.P. Audoly, Evolution of anti-CD20 monoclonal antibody therapeutics in oncology, MAbs 2 (2010) 14–19.
[10] M.J. Glennie, R.R. French, M.S. Cragg, R.P. Taylor, Mechanisms of killing by anti-CD20 monoclonal antibodies, Mol. Immunol. 44 (2007) 3823–3837.
[11] A.C. Chan, P.J. Carter, Therapeutic antibodies for autoimmunity and inflammation, Nat. Rev. Immunol. 10 (2010) 301–316.
[12] W. Alduaij, T.M. Illidge, The future of anti-CD20 monoclonal antibodies: are we making progress, Blood 117 (2011) 2993–3001.
[13] G.J. Weiner, Rituximab: mechanism of action, Semin. Hematol. 47 (2010) 115–123.
[14] A.R. Rezvani, D.G. Maloney, Rituximab resistance, Best Prac. Res. Clin. Haematol. 24 (2011) 203–216.
[15] R.P. Taylor, M.A. Lindorfer, Antigenic modulation and rituximab resistance, Semin. Hematol. 47 (2010) 124–132.
[16] C.L. Sawyers, The cancer biomarker problem, Nature 452 (2008) 548–552.
[17] M.A. Hamburg, F.S. Collins, The path to personalized medicine, N. Engl. J. Med. 363 (2010) 301–304.
[18] X.S. Xie, J. Yu, W.Y. Yang, Living cells as test tubes, Science 312 (2006) 228–230.
[19] K.C. Neuman, A. Nagy, Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy, Nat. Methods 5 (2008) 491–505.
[20] L.B. Oddershede, Force probing of individual molecules inside the living cell is now a reality, Nat. Chem. Biol. 8 (2012) 879–886.
[21] D.J. Muller, Y.F. Dufrene, Force nanoscopy of living cells, Curr. Biol. 21 (2011) 212–216.
[22] G. Binnig, C.F. Quate, C. Gerber, Atomic force microscope, Phys. Rev. Lett. 56 (1986) 930–933.
[23] D.J. Muller, J. Helenius, D. Alsteens, Y.F. Dufrene, Force probing surfaces of living cells to molecular resolution, Nat. Chem. Biol. 5 (2009) 383–390.
[24] J.J. Heinisch, V. Dupres, S. Wilk, A. Jendretzki, Y.F. Dufrene, Single-molecule atomic force microscopy reveals clustering of the yeast plasma-membrane sensor Wsc1, PLoS One 5 (2010) e11104.
[25] M. Li, L. Liu, N. Xi, Y. Wang, Z. Dong, G. Li, X. Xiao, W. Zhang, Detecting CD20–rituximab interaction forces using AFM single-molecule force spectroscopy, Chin. Sci. Bull. 56 (2011) 3829–3835.
[26] J.L. Hutter, J. Bechhoefer, Calibration of atomic-force microscope tips, Rev. Sci. Instrum. 64 (1993) 1868–1873.
[27] F. Nimmerjahn, J.V. Ravetch, Fc receptors as regulators of immune responses, Nat. Rev. Immunol. 8 (2008) 34–47.
[28] F. Nimmerjahn, J.V. Ravetch, Fc receptors: old friends and new family members, Immunity 24 (2006) 19–28.
[29] T. Pham, P. Mero, J.W. Booth, Dynamics of macrophage trogocytosis of rituximab-coated B cells, PLoS One 6 (2011) e14498.
[30] S. El-Kirat-Chatel, Y.F. Dufrene, Nanoscale imaging of the candida–macrophage interaction using correlated fluorescence-atomic force microscopy, ACS Nano 6 (2012) 10792–10799.
[31] A. Mogilner, K. Keren, The shape of motile cells, Curr. Biol. 19 (2009) 762–771.
[32] K. Keren, Z. Pincus, G.M. Allen, E.L. Barnhart, G. Marriott, A. Mogilner, J.A. Theriot, Mechanism of shape determination in motile cells, Nature 453 (2008) 475–480.
[33] S. Nishimura, I. Manabe, M. Nagasaki, K. Eto, H. Yamashita, M. Ohsugi, M. Otsu, K. Hara, K. Ueki, S. Sugiura, K. Yoshimura, T. Kadowaki, R. Nagai, CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity, Nat. Med. 15 (2009) 914–920.
[34] Y.F. Dufrene, E. Evans, A. Engel, J. Helenius, H.E. Gaub, D.J. Muller, Five challenges to brining single-molecule force spectroscopy into living cells, Nat. Methods 8 (2011) 123–127.
[35] P. Hinterdorfer, Y.F. Dufrene, Detection and localization of single molecular recognition events using atomic force microscopy, Nat. Methods 3 (2006) 347–355.
[36] X. Shi, L. Xu, J. Yu, X. Fang, Study of inhibition effect of herceptin on interaction between heregulin and erbB receptors HER3/HER2 by single-molecule force spectroscopy, Exp. Cell Res. 315 (2009) 2847–2855.
[37] G.I. Bell, Models for the specific adhesion of cells to cells, Science 200 (1978) 618–627.
[38] E. Evans, K. Ritchie, Dynamic strength of molecular adhesion bonds, Biophys. J. 72 (1997) 1541–1555.
[39] I.D. Medalsy, D.J. Muller, Nanomechanical properties of proteins and membranes depend on loading rate and electrostatic interactions, ACS Nano 7 (2013) 2642–2650.
[40] Y. Li, H. Qiao, W. Yan, J. Zhang, C. Xing, H. Wang, B. Zhang, J. Tang, Molecular recognition force spectroscopy study of the dynamic interaction between aptamer GBI-10 and extracellular matrix protein tenascin-C on human glioblastoma cell, J. Mol. Recognit. 26 (2013) 46–50.
[41] X. Zhang, X. Shi, L. Xu, J. Yuan, X. Fang, Atomic force microscopy study of the effect of HER 2 antibody on EGF mediated ErbB ligand–receptor interaction, Nanomedicine: NBM 9 (2013) 627–635.
[42] M. Li, X. Xiao, L. Liu, N. Xi, Y. Wang, Z. Dong, W. Zhang, Atomic force microscopy study of the antigen-antibody binding force on patient cancer cells based on ROR1 fluorescence recognition, J. Mol. Recognit. 26 (2013) 432–438.
[43] V. Dupres, F.D. Menozzi, C. Locht, B.H. Clare, N.L. Abbott, S. Cuenot, C. Bompard, D. Raze, Y.F. Dufrene, Nanoscale mapping and functional analysis of individual adhesions on living bacteria, Nat. Methods 2 (2005) 515–520.
[44] M. Hu, J. Chen, J. Wang, X. Wang, S. Ma, J. Cai, C.Y. Chen, Z.W. Chen, AFM- and NSOM-based force spectroscopy and distribution analysis of CD69 molecules on human CD4+ T cell membrane, J. Mol. Recognit. 22 (2009) 516–520.
[45] C. Verbelen, Y.F. Dufrene, Direct measurement of mycobacterium–fibronectin interactions, Integr. Biol. 1 (2009) 296–300.