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C-reactive protein induces G2/M phase cell cycle arrest and apoptosis in monocytes through the upregulation of B-cell translocation gene 2 expression

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ABSTRACT

We hypothesized that C-reactive protein (CRP) may affect the cell cycle and induce apoptotic changes of monocytes. CRP (~25 μg/ml) significantly increased expressions of B-cell translocation gene 2 (BTG2) mRNA and protein in human monocytes through pathways involving CD32/NADPH oxidase 2/p53, which eventually induced G2/M phase arrest and apoptotic cell death. Such pro-apoptotic effect of CRP was not found in thioglycollate-elicited intraperitoneal monocytes/macrophages harvested from BTG2-knockout male C57BL/6 mice ($n = 5$). Within atheromatous plaques obtained from CRP-transgenic male LDLR$^{-/-}$ C57BL/6 mice ($n = 5$) and human coronary arteries, BTG2 co-localized with CRP, p53 and monocytes/macrophages. Therefore the pro-apoptotic pathway of CRP-CD32-Nox2-p53-BTG2 may contribute to the retardation of the atherogenic process.

1. Introduction

Monocytes egress from bone marrow, circulate in the blood, and have a short life-span (17–72 h) [1]. The milieu of cytokines and growth factors present in inflammatory lesions, such as interleukin-1 beta (IL-1β), IL-8, IL-10, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), macrophage and granulocyte/macrophage-colony stimulating factors (GM-CSF), enable recruited monocytes to escape from spontaneous apoptosis and undergo differentiation and proliferation [1]. Conversely, any factors that facilitate apoptotic changes in monocytes may reduce the inflammatory response and eventually retard the process of inflammation in the arterial wall, e.g. atherosclerosis.

C-reactive protein (CRP), a prototype acute phase reactant [2], directly induces a number of innate immune responses including the production of potentially pro-apoptotic cytokines and inflammatory mediators [3] through the activation of Fc-gamma receptors (FcγRs) [4,5]. CRP is detected in atherosclerotic plaques [6] and stimulates monocytes/macrophages and vascular smooth muscle cells (VSMCs) to produce pro-apoptotic cytokines such as IL-1β and TNF-α, and reactive oxygen species (ROS) [7,8]. We previously reported CRP-induced apoptotic changes in VSMCs via activation of NADPH oxidase (Nox) and subsequent ROS generation [5]. However, it is not clear whether CRP is pro-apoptotic for monocytes.

We therefore investigated if cell kinetics and life span of monocytes can be changed by CRP. The present study demonstrates *in vitro* and *ex vivo* evidences that CRP upregulates B-cell translocation gene 2 (BTG2), a member of the antiproliferative (APRO) gene family, through elaborate signaling pathways involving a CD32-Nox2-ROS-p53 cascade. As a consequence, monocytes exposed to CRP developed G2/M phase arrest and eventually underwent apoptotic death.

2. Materials and methods

2.1. Preparation of monocytes and CRP

Circulating human monocytes were freshly isolated from healthy donors using 1077 Histopaque (Sigma–Aldrich, St. Louis,

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MO) and a negative isolation technique using magnetic beads (Miltenyi Biotech, Auburn, CA) as described previously [9], and maintained in serum-free complete media (Gibco BRL, Grand Island, NY). Flow cytometry showed that the monocytes were >80% pure as determined by staining with an anti-CD14 antibody. Informed consent was obtained from the blood donors and the protocol conformed to the principles outlined in the 'Declaration of Helsinki'. Human monocytes were cultured in RPMI1640 medium containing 10% FBS as described previously [9]. In a subset of experiment, cell cycle of monocytes was synchronized at G1/S phase (G1 phase; 43%, S phase; 52%, G2/M phase 5%) by treating with 100 ng/ml nocodazole for 18 h as described [10] and was then removed to progress cell division cycle. Murine monocytes/macrophages were collected from 6-week-old C57BL/6 male mice [wild-type, p53 knockout ($p53^{-/-}$), and BTG2 knockout ($BTG2^{-/-}$), $n = 5$ per group] at 5 days after intraperitoneal injection of 3% thioglycollate (1.0 ml/mouse, Sigma–Aldrich, St. Louis, MO). The harvested cells seeded into 6-well plates in RPMI-1640 supplemented with 20% FBS were allowed to adhere for 2 h before non-adherent cells were washed out. C57BL/6 male mice containing $LDLR^{-/-}$/hCRPtg ($n = 5$) were fed a high-cholesterol/low-fat diet comprising cholesterol-enriched regular mouse chow (0.5% cholesterol and 4.4% fat; TD97234, Harlan–Teklad, Madison, WI) for 24 weeks before being sacrificed for immunohistochemical analysis. The animal study was approved by the University of Ulsan Animal Subjects Committee and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Human recombinant CRP stock (Calbiochem Millipore, Billerica, MA) was confirmed as "IgG-free" (<1.0 ng/μg CRP), conformationally intact (by electrophoresis on non-denaturing gels) [9], and endotoxin-free (<1.0 ng/ml; determined by a timed-gel endotoxin kit assay (Sigma–Aldrich). The complete cDNA sequence of human BTG2 (GenBank accession no. NM_006763) was amplified by PCR and the PCR product cloned into pCRII-TOPO (Invitrogen, Carlsbad, CA). The nucleotide sequence of the inserted BTG2 cDNA was confirmed by direct sequencing followed by subcloning into the expression vector, pcDNA3.1 (Invitrogen).

Human isolated monocytes were transfected using the Lipofectamine 2000 reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Briefly, 5×10^5 monocytes in a 6-well plate in RPMI 1640 medium were treated with either 4 μg p53 dominant negative DNA (pCMV-p53mt135; Clontech, Mountain View, CA) plus 10 μL Lipofectamine 2000, or 100 pmole siRNAs against Nox2, DGKζ, PKCδ, and BTG2 (Santa Cruz Biotechnology, Dallas, TX) plus 5 μl Lipofectamine 2000 for 4–6 h. The medium was then changed to RPMI 1640 containing 10% FBS and the cells were further incubated at 37 °C in a CO_2 incubator for 24 h. The transfection efficiency, determined using a pcDNA3.1 vector encoding green fluorescent protein, was estimated to be >70%. In a subset of experiments, monocytes were incubated with N-acetyl-L-cysteine (NAC, Sigma–Aldrich, St. Louis, MO), anti-human CD64 or anti-CD32 antibodies (R&D System, Minneapolis, MN).

2.2. Measurement of mRNA expression levels

The expression of mRNA encoding p53, BTG2, Nox2, β-actin, and GAPDH was determined by real-time PCR with SYBR Green I Faststart Master Mix (Roche Molecular Diagnostics, Pleasanton, CA) and specific primers. PCR amplification conditions were 45 cycles at 95 °C for 15 s, 60 °C for 5 s, and 72 °C for 25 s with the following specific primers;

Human p53: 5′-CAGTCTACCTCCCGCCATAA-3′ (forward)
5′-CCACAACAAAACACCAGTGC-3′ (reverse)
Human BTG2: 5′-CAGAGCACTACAAACACCAC-3′ (forward)
5′-AGACTGCCATCACGTAGTTC-3′ (reverse)
Mouse p53: 5′-GTTCCGGGAGCTGAATGAGG-3′ (forward)
5′-CAGGCCCCACTTTCTTGACC-3′ (reverse)
Mouse $BTG2^{/TIS21}$: 5′-TGGACCCCATCATCAGCAAG-3′ (forward)
5′-TGCCCAGCATCATCTGGTTC-3′ (reverse)
GAPDH: 5′-GACCCCTTCATTGACCTC-3′ (forward)
5′-GCTAAGCAGTTGGTGGTG-3′ (reverse)

β-Actin or GAPDH mRNAs were employed as the internal standards and analyzed under the identical conditions using a pair of specific primers. The Ct value (the cycle number at which emitted fluorescence exceeds an automatically determined threshold) for the target cDNA was corrected using the Ct value for GAPDH and expressed as ΔCt. Data were expressed as fold changes in the amount of mRNA, which was calculated using the following formula: -Fold change = $2^{(\Delta Ct \text{ for untreated cells} - \Delta Ct \text{ for treated cells})}$.

2.3. Immunohistochemistry

To detect proteins of p53, BTG2, CRP and monocytes/macrophages in the plaque, thoracic aorta of $LDLR^{-/-}$/CRPtg mice and human atherosclerotic plaques in coronary artery were analysed. Cross sections of mouse thoracic aorta specimen were blocked with 5% goat serum, incubated overnight at 4 °C with a specific antibodies detecting mouse p53 (1:250, PAb240, Abcam, Cambridge, UK), mouse BTG2 (1:100, ab58219, Abcam, Cambridge, UK), mouse F4/80 (1:50; Abcam, Cambridge, UK), or CRP (1:500; Sigma–Aldrich). Mouse thoracic aorta specimen were incubated with appropriate biotin-labelled secondary antibodies and counterstained with hematoxylin QS (Dako, Glostrup, Denmark). The reactions were visualized using the LSAB2 system/streptavidin-HRP kit with DAB staining. Human atherosclerotic coronary artery was incubated with an anti-human antibodies for CD68 (Abcam), or BTG2 (Abcam) and cell-associated antibodies were detected by secondary antibodies labeled with Alexa Flour 488 or Rhodamine TRITC (Abcam). Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) and fluorescent images were obtained using an Olympus DP71 microscope and Olympus DPController software.

2.4. Immunoblotting assay

Immunoblotting was performed using mouse anti-human monoclonal IgG antibodies (1:1000) against p53 (Cell Signaling Technology, Boston, MA), BTG2, or DGKζ (Santa Cruz Biotechnology, Dallas, TX) and developed using goat anti-mouse IgG-HRP (1:10,000, Jackson ImmunoResearch Laboratories, Inc, West Grove, PA) and a chemiluminescence detection kit (ECL-kit, PIERCE from Thermo Fisher Scientific Inc., Waltham, MA). The amount of protein was quantified using the MULTI-IMAGE analysis system and Quantitation software (Bio-Rad Laboratories Inc., Hercules, CA).

2.5. Proliferation and apoptosis of monocytes

Human monocytes or mouse peritoneal macrophages were labelled with 30 μM BrdU for 1 h at 37 °C, washed with PBS containing 0.5% BSA (wash buffer), and fixed with 70% ethanol for 30 min at room temperature. DNA was denatured in 2 N HCl for 20 min at RT and neutralized with 0.1 M sodium borate (pH 8.5) for 2 min. Cell-associated BrdU was labelled with 20 μl FITC-conjugated anti-BrdU IgG (R&D System, Abingdon, UK). Nonspecific binding of anti-BrdU IgG was blocked by preincubation with 10 μg/ml of Fc Fragments (ChromPure Human IgG; Jackson ImmunoResearch Laboratories, West Grove, PA) for 30 min. To detect apoptosis in monocytes, cells were fixed with 90% ethanol for 20 min at room temperature and stained with 5 μl propidium iodide (PI, 25 μg/ml, R&D System, Abingdon, UK) for 15 min at room temperature in

the dark. Cell-associated fluorescence was detected by flow cytometry on a FACScan flow cytometer and data analysis was performed with CELLQUEST software (BD Biosciences, San Jose, CA).

2.6. Statistical analysis

Values are expressed as mean ± S.D., and the differences between two groups were evaluated by the unpaired *t* test and the differences among multiple groups were by two-way ANOVA. Statistical differences $P < 0.05$ by SPSS (version 10.0, SSPS Inc., Chicago, IL) were regarded as significant.

3. Results

3.1. CRP upregulates p53 and BTG2 expressions in monocytes

Freshly-isolated human monocytes were incubated with recombinant human CRP (0–25 μg/ml), and then subjected to immunoblot and real-time RT-PCR analyses to evaluate protein and mRNA expressions of p53 and BTG2. CRP increased both total and phosphorylated forms of p53 proteins, and BTG2 protein in a dose-dependent manner (Fig. 1A) ($P < 0.01$). Moreover, mRNA expressions of both p53 and BTG2 were increased by up to 3–6 folds within 4 h of CRP treatment (Fig. 1A). We also confirmed CRP (~25 μg/ml for 24 h) increased expressions of p53 and BTG2 proteins in THP-1 human monocytic cultured cell line, too (Supplementary Fig. 1).

We harvested and cultured murine thioglycollate-elicited peritoneal monocytes/macrophages and confirmed CRP-stimulated expression of p53 and BTG2 proteins (25 μg/ml for up to 72 h) in *ex vivo* conditions (Fig. 1B). Interestingly, parallel experiments using $p53^{-/-}$ littermates failed to show BTG2 upregulation by CRP treatment whereas p53 upregulation was observed in $BTG2^{-/-}$ mice in 48 hours after CRP treatment, suggesting p53 is required for the effect of CRP to complete BTG2 upregulation. Then we localized BTG2, p53, and CRP proteins in atherosclerotic lesions in $LDLR^{-/-}$/hCRPtg mice, whose serum CRP levels were maintained over 10.0 mg/l. Immunohistochemistry analysis showed that

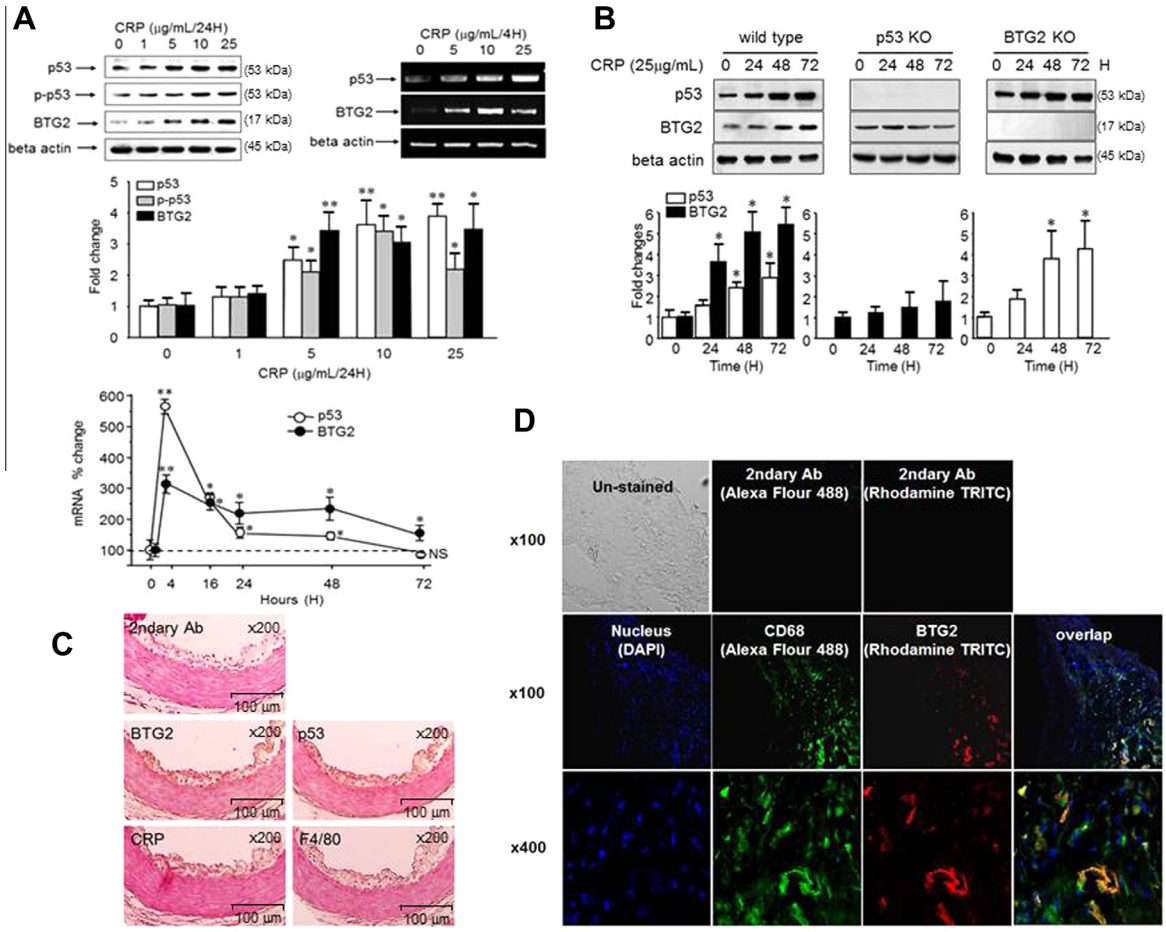

Fig. 1. CRP-induced upregulations of p53 and BTG2 expressions in monocytes, and co-localization of CRP, p53 and BTG2 with monocytes/macrophages in the atherosclerotic plaque. (A) Freshly-isolated human monocytes were incubated with 0–25 μg/ml CRP, and the changes of total and phosphorylated forms ('p-p53') of p53 and BTG2 proteins in cell lysates were examined by immunoblot analysis and the fold changes were presented as a bar graph. Detection of p53 and BTG2 mRNA expressions by both semi-quantitative and real time RT-PCR and the percent changes were expressed as a dot-line graph. Data represent the mean ± S.D. of triplicates and were analyzed by unpaired *t*-test (*$P < 0.05$, **$P < 0.01$ *vs.* control; NS, not statistically significant). (B) Thioglycollate-induced peritoneal macrophages derived from the 6-week-old C57/BL6 wild-type ('wild type') male mice, $p53^{-/-}$ ('p53 KO') or $BTG2^{-/-}$ ('BTG2 KO') littermates ($n = 5$ each) were treated with CRP (25 μg/ml for up to 72 h), and the protein expressions of p53 and BTG2 in the cell lysates were detected by immunoblot analyses. Data represent the mean ± S.D. of triplicates and were analyzed by unpaired *t*-test (*$P < 0.05$). (C) The thoracic aorta derived from hCRPtg $LDLR^{-/-}$ mice was stained with specific antibodies against CRP, p53, BTG2, and F4/80. Hematoxylin and eosin staining was applied as a counter-stain. Scale bar, 100 μm. (D) Human atherosclerotic plaque in coronary artery was stained with specific antibodies against BTG2 and CD68, a specific marker for monocytes/macrophages and cell-bound antibodies were detected by either Alexa Flour 488 (green color) or Rhodamine TRITC (red color). Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) as a background (blue color).

neointimal areas of the thoracic aorta enriched with F4/80(+) monocytes/macrophages were positively stained with antibodies specific for p53, BTG2, and CRP (Fig. 1C). We also performed the immunofluorescence staining in order to detect BTG2 expression by human monocytes/macrophages resident in atherosclerotic plaques. Human atherosclerotic coronary artery was stained with specific antibodies recognizing either CD68(+) monocytes/macrophages or BTG2 protein and cell-bound antibodies were detected by either Alexa Flour 488 (green color) or Rhodamine TRITC (red color). The nucleus was stained by DAPI (blue color) as a background. The results clearly showed the co-localization of BTG2 with CD68(+) monocytes/macrophages. (Fig. 1D)

3.2. CRP-stimulated BTG2 expression facilitates apoptosis of monocytes through the induction of G2/M cell cycle arrest

In order to quantify cells undergoing apoptotic changes, murine thioglycollate-elicited peritoneal monocytes/macrophages were prepared and treated with CRP (25 μg/ml/up to 48 h) and stained with PI and annexin V, and flowcytometry was performed. The proportion of cells from wild type in sub-G0/G1 phase exceeded 60% after 48 h of CRP treatment while the number of sub-G0/G1 cells from BTG2$^{-/-}$ mice was significantly lower than those from the wild type (Fig. 2A). Moreover, the proportion of annexin V-high/PI-high BTG2$^{-/-}$ monocytes/macrophages were also significantly less than wild type cells (Fig. 2B), suggesting BTG2 may facilitate the apoptotic change of CRP-treated monocytes. The effect of CRP to induced apoptosis and the role of BTG2 were further confirmed using freshly-isolated human monocytes, in which CRP treatment for 48 h or longer clearly increased numbers of cells in subG0/G1 phase (Fig. 2C). Same treatment also increased the number of monocytes in annexin V-high/PI-high fraction and the transfection with either p53DN or siRNA-BTG2 significantly blocked the effect of CRP to induce apoptotic cell death (Fig. 2D). In order to confirm the role of BTG2 on the development of apoptotic changes in monocytes, BTG2 expression was induced by transfecting a pcDNA3.1 vector encoding complete cDNA sequence of human BTG2 to human monocytes, which enhanced the amount of BTG2 protein by more than 3 folds in 24 h (Supplementary Fig. 3A). Flow cytometry clearly showed that the enhanced BTG2 expression significantly increased the number of monocytes in annexin V-high/PI-high fraction (Supplementary Fig. 3B).

To investigate if CRP may affect cell cycle kinetics, the human monocytes were synchronized at G1/S phase (G1 phase; 43%, S

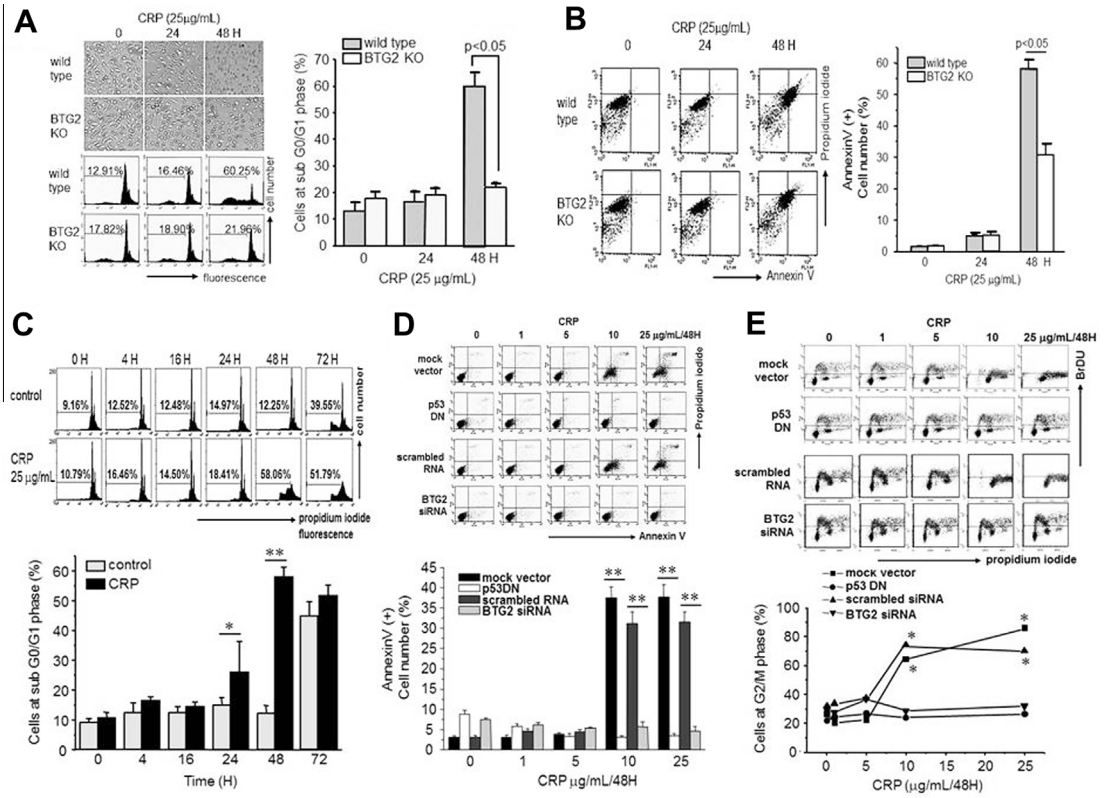

Fig. 2. Regulation of CRP-induced apoptotic changes by BTG2. (A and B) Thioglycollate-induced peritoneal macrophages derived from the 6-week-old C57/BL6 wild-type ('wild type') male mice or BTG2$^{-/-}$ ('BTG2 KO') littermates (n = 5 each) were cultured with CRP (25 μg/ml for 24 or 48 h) and stained with 25 μg/ml propidium iodide (PI) and the fraction of apoptotic cells in sub G0/G1 phase were assessed on the basis of DNA content and quantified as% of the total cells (A). The cells were also co-stained with PI and annexin V-FITC, and the fraction of annexin-V-high early apoptotic cells were quantified as% of the total cells, too (B). (C and D) Human monocytes were cultured with CRP (25 μg/ml for up to 72 h), and then stained with 25 μg/ml PI. The fraction of apoptotic cells distributed in sub G0/G1 phase was obtained as described in A (C). In a subset of experiments, human freshly-isolated monocytes were transfected with pCMV ('mock vector') or pCMV-p53mt135 ('p53DN'). Alternatively, human monocytes were transfected with scrambled RNA or siRNA specific for BTG2 ('BTG2 siRNA'), and were subsequently cultured for 48 hours with CRP (up to 25 μg/ml). The monocytes were stained with PI and annexin V-FITC, and the fraction of annexin-V-high early apoptotic cells were quantified as% of the total cells (D). The data represent the mean ± S.D. of triplicates and were analyzed by unpaired t-test ($^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control). (E) Human monocytes were cultured with CRP (up to 25 μg/ml/48 h), and stained with 25 μg/ml PI and labelled with 30 μM BrdU. The cell-associated BrdU was labeled with FITC-conjugated anti-BrdU antibody. The cell populations in G0/G1-, S-, and G2/M-phase were analyzed by flow cytometry. Prior to the treatment with CRP, monocytes were transfected with pCMV ('mock vector') or pCMV-p53mt135 ('p53DN'), or transfected with scrambled RNA or siRNA specific for BTG2('BTG2 siRNA'). Data represent mean ± S.D. of triplicates and were analyzed by unpaired t-test [$^{*}P < 0.05$ *vs.* control (p53DN or scrambled RNA)].

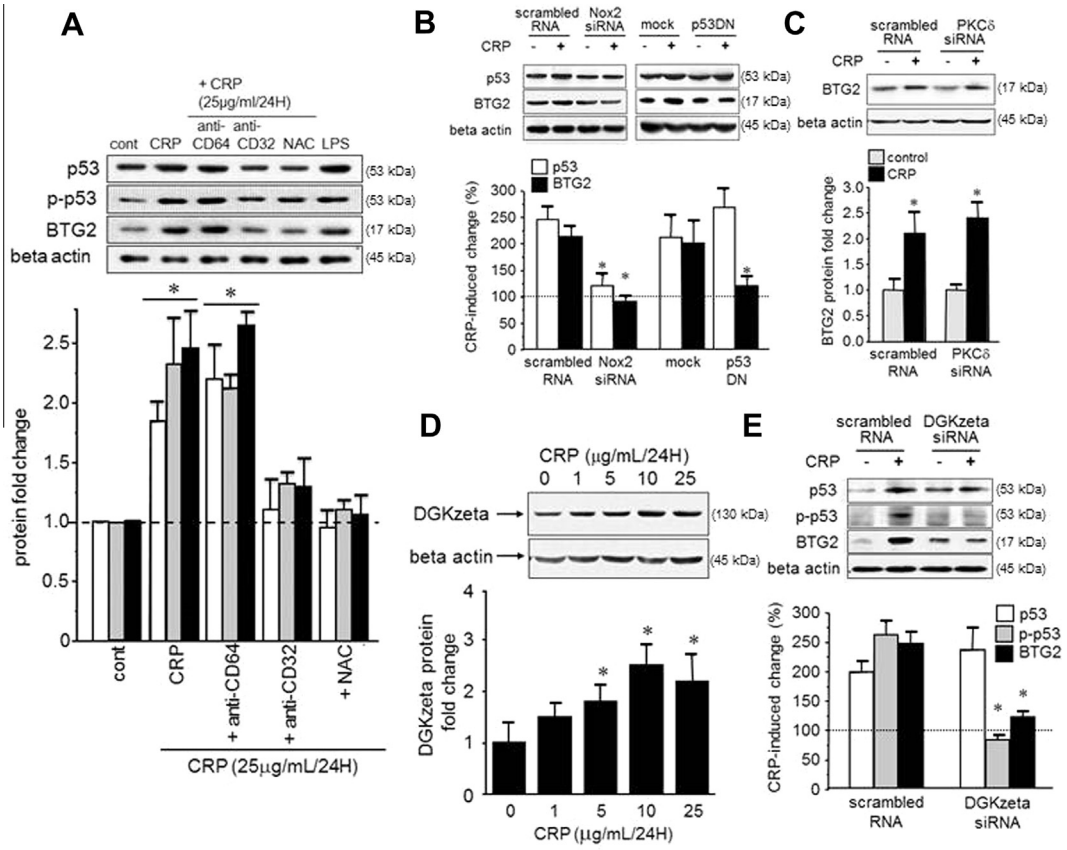

Fig. 3. Regulations of CRP-induced p53 and BTG2 expression by the signals through CD32, Nox2, and DGKζ. (A) Human monocytes were incubated with or without CRP (25 μg/ml). In a subset of experiments, specific antibodies against CD64 or CD32 (10 μg/ml each), or NAC (an oxygen radical scavenger; 10 mM) were pre-treated 30 min prior to CRP treatment, and then maintained for 24 h. Stimulation with lipopolysaccharide (100 ng/ml/24 h) was performed as a positive control. Protein levels of p53, phosphorylated form of p53 ('p-p53') and BTG2 were quantified by immunoblot analysis (*$P < 0.05$ *vs.* untreated control). (B) Human monocytes were transfected with scrambled RNA or siRNA specific for Nox2 ('Nox2 siRNA'). Alternatively, human monocytes were transfected with pCMV ('mock') or pCMV-p53mt135 ('p53DN'). After incubation with CRP (25 μg/ml) for 24 h, p53 and BTG2 proteins in the cell lysates were measured by immunoblot analysis. (C) Human monocytes were transfected with either scrambled RNA or siRNA specific for PKCδ ('PKCδ siRNA'), treated with CRP (25 μg/ml/24 h) and BTG2 protein was detected in cell lysates was detected by immunoblotting. (D and E) Human monocytes were transfected with either scrambled RNA or siRNA specific for DGKζ ('DGKzeta siRNA'). After incubation with CRP (25 μg/ml/24 h), DGKζ (D) and BTG2 proteins (E) in the cell lysates were measured by immunoblot analyses. Data represent the mean ± S.D. of triplicates and were analyzed by unpaired *t*-test (*$P < 0.05$ *vs.* control; NS, not statistically significant).

phase; 52%, G2/M phase 5%) and treated with CRP (25 μg/ml) for up to 72 h and the amount of BrdU incorporation into the cells was determined by flowcytometry. Human monocytes was accumulated at G2/M phase without further progression after the CRP treatment and the transfection with either p53DN or siRNA-BTG2 prevented abolished CRP-induced cell cycle arrest (Fig. 2E).

3.3. CRP positively regulates BTG2 expression through a CD32/Nox2/ROS/p53 pathway in monocytes

Our previous report described that CRP-bound CD32(FcγRII) generates ROS through activation of NADPH oxidase 4 (Nox4) [5]. Similarly, results of our immunoblot analysis also showed that either treatment with anti-CD32 neutralizing antibody or NAC, the scavenger of ROS, blocked the upregulatory effects of CRP on p53 and BTG2 (Fig. 3A). Since our preliminary experiments confirmed the dominant Nox2 expression in human monocytes, we therefore transfected siRNA-Nox2 to the freshly isolated human monocytes, which suppressed Nox2 mRNA expression by more than 55% (Supplementary Fig. 2). The transfection of siRNA-Nox2 significantly inhibited CRP-stimulated expressions of both p53 and BTG2 (Fig. 3B). Moreover, the effect of CRP on BTG2 expression was blocked by transfection with the dominant-negative form of p53 (p53DN), suggesting the p53 is required for CRP-stimulated BTG2 expression in human monocytes (Fig. 3B).

Although our study results highly suggest that CRP-CD32-Nox2-ROS-p53 pathway may exclusively upregulate BTG2 expression, a previous report showed that BTG2 expression in monocyte-like U937 cultured cells may be regulated through protein kinase C delta (PKCδ) without involvement of p53 [10]. Our immunoblotting clearly proved that transfection of siRNA-PKCδ, which knockdown its expression by 75% (Supplementary Fig. 2), did not affect CRP-stimulated BTG2 expression in human monocytes (Fig. 3C). Activation of CD32 can trigger the production of diacylglycerol (DAG), a known negative regulator of BTG2 [11], whereas DAG is catabolized to phosphatidic acid by diacylglycerol kinase-zeta (DGKζ). Interestingly, the treatment of human monocytes with CRP (up to 10 μg/ml/24 h) enhanced expression of DGKζ (Fig. 3D) and CRP-induced BTG2 upregulation and p53 phosphorylation was disappeared in human monocytes transfected with a specific siRNA for DGKζ (Fig. 3E), which suppressed mRNA expression of DGKζ over 60% (Supplementary Fig. 2).

4. Discussion

The present study provides a number of evidence that CRP may affect cell cycle kinetics of monocytes via CD32(FcγRII)/Nox2/ROS/p53/BTG2 cascade, which induced G2/M phase arrest and eventually facilitated apoptotic death. CD32 had been described to trigger apoptotic signals in granulocytes and eosinophils [12], and is

highly expressed in a subset of $CD14^{high}$ monocytes that may polarize to M1 pro-inflammatory macrophages [13] Therefore, our results suggest that one of the functional activities of CRP may alleviate macrophage-driven pro-inflammatory responses. Teupser et al. recently generated CRP-deficient female mice with $LDLR^{-/-}$ C57BL/6 backgrounds, which had a relatively higher burden of macrophages in the atherosclerotic lesion when compared with control littermates [14]. However, the majority of other previous murine intervention studies did not show the compositional change in the plaque including the number of apoptotic monocytes/macrophages in the presence of CRP [15]. As a possible explanation, CRP-bound apoptotic monocytes/macrophages may be immediately removed via FcγR-mediated phagocytosis [16]. Indeed CRP can trigger a wide variety of cell signaling pathways other than BTG2 upregulation, which may show complex contributions to the process of atherosclerosis and apoptotic changes may not be an exclusive consequence of CRP stimulation in a subset of monocytes/macrophages. As we described previously, monocytes in the lesion might be replenished by CRP stimulation itself through activating PLD/CCR2/MCP-1-dependent recruitment process [9]. Moreover, other previous studies show that CRP also activates NFκB [17] and increases the production and secretion of GM-CSF [18], which can trigger macrophages to undergo proliferation and polarize to the M1 type.

The present study clearly identified BTG2 as a downstream effector of CRP and BTG2 could be more specific target for the retardation of inflammatory process mediated by monocytes. We proved that the enhanced expression of BTG2 was potent enough to orchestrate G2/M arrest and subsequently induce apoptotic changes in CRP-treated monocytes. Our results show that p53 activation is required for the positive regulatory effect of CRP on BTG2 expression. The amount of total and phosphorylated form of p53 protein is increased in the advanced human plaques [19] and our study showed that BTG2 protein is colocalized with p53 and CRP in the early stage of atherosclerotic plaques enriched with monocytes/macrophages. Therefore, BTG2 may conceivably contribute to anti-atherosclerotic effects of p53 as described in earlier animal intervention studies [20–22]. Our previous study [23] and others [24] have examined in vivo tissue-specific expression of BTG2, in which lymphoid organs (such as thymic cortex, spleen white pulp and lymph node) lung and stomach express high levels of BTG2, while myocardium showed little BTG2 expression. The functional expression of BTG2 has been described as negatively correlated with the severity of various cancers and the inflammatory conditions in pancreas [25]. Taken together, our results raise the possibility that targeting the BTG2-specific pathway rather than CRP itself can be more selective and effective strategy for the suppression of various inflammatory and cancerous conditions including the growth of atherosclerotic lesions.

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Conflict of interest

The authors do not have any conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.01.008.

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