Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (7): 27-32
Published online November 8, 2015 (http://www.cellmolbiol.com)
Received on September 11, 2015, Accepted on November 1, 2015.
doi : 10.14715/cmb/2015.61.7.5

Increasing production of matrix metalloproteinases, tumor necrosis factor-α, vascular endothelial growth factor and prostaglandin E_2 in rheumatoid arthritis synovial fibroblasts by different adiponectin isoforms in a concentration-dependent manner

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Abstract

Adipokines have been known to play a significant role in rheumatic disease via synovial fibroblasts. However, to date, the concentration effects of adiponectin isoforms on the pathophysiology of rheumatoid arthritis (RA) have not been extensively studied. Therefore, the present study examined the different effects of the adiponectin isoforms on rheumatoid arthritis synovial fibroblasts (RASF) and investigated the relations between the concentration of individual adiponectin isoforms and the production of the inflammatory factors of RASF. Articular synovial tissues were obtained from the patients fulfilled with diagnostic criteria of RA, and health people. RASF and human fibroblast-like synoviocytes (HFLS) were isolated and cultured. They were stimulated with increasing concentrations of 25 μg/ml, 50 μg/ml, and 100μg/ml of different human adiponectin isoforms. The levels of matrix metalloproteinase (MMP)-3, MMP-10, tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF), and prostaglandin E_2 (PGE_2) in culture supernatants were measured by immunoassays. The results showed the levels of MMP-3, MMP-10, TNF-α, VEGF and PGE_2 were significantly increased in RASF which were treated with individual adiponectin isoforms compared to untreated RASF ($p<0.01$), and the increases also had significances compared to HFLS which were treated with the same conditions ($p<0.05$). Moreover, the effect of HMW (high molecular weight)/ MMW (middle molecular weight) was the strongest among them. In conclusion, all three adiponectin isoforms may contribute to proinflammatory effect by stimulating the production of MMP-3, MMP-10, TNF-α, VEGF and PGE_2 of RASF in a concentration-dependent manner. HMW/MMW adiponectin could play an important role in matrix destroying and synovial vascular creating of the pathology of RA.

Key words: Adiponectin, isoform, rheumatoid, arthritis, synovial fibroblasts.

Introduction

RA is a chronic systemic autoimmune disease characterised by persistent synovitis and progressive damage to articular cartilage and subchondral bone (1). And synovial fibroblasts are considered to be the lead cells that drive the persistent, destructive characteristics of the disease (2).

Adiponectin, also known as GBP28, apM1, Acrp30, or AdipoQ, was discovered in 1995, and has been characterized in mice as the mRNA transcript most highly expressed in adipocytes. Adiponectin has structural homology with collagens VIII, X and complement factor C1q (3). Interestingly, adiponectin is not a homogenous entity but consists of several isoforms corresponding to different oligomers. Trimeric adiponectin, also called low molecular weight (LMW) adiponectin, is composed of three full-length adiponectin monomers forming a collagen triple helix with a C-terminal globular gC1q domain (head domain). The adiponectin hexamer, the so-called middle molecular weight (MMW) adiponectin, is a combination of two trimeric adiponectin molecules, while an assembly of 12-18 monomers is collectively termed high molecular weight (HMW) adiponectin (4). In addition, individual adiponectin isoforms display distinct biological effect in more than one type of cell (5-6).

Recently, some evidences indicate that adiponectin has a wide range of effects in pathologies with inflammatory components, such as cardiovascular disease (7), endothelial dysfunction (8), type 2 diabetes, metabolic syndrome (9), osteoarthritis (OA) (6) and RA. Articular and synovial adipose tissue can be suspected of playing a role in joint inflammation, but thus far, the local biological function of articular adipose tissue has remained largely unknown. Some experiments found the increasing levels of adponectin in both serum and articular synovial fluid of the patients with RA in comparison to healthy people (10). Even though some studies have investigated the effects of adiponectin isoforms on RASF (11), no study has yet analysed the concentration effects of adiponectin isoforms on the pathophysiology of RA.

Therefore, in this stdudy we investigated whether the adiponectin isoforms differentially affect the expression of relative inflammatory factors of RASF, and the relationship between the concentration of individual adiponectin isoforms and the inflammatory factors production of RASF.

Materials and methods

Cell Culture

Synovial tissue samples were obtained from synovial biopsy specimens from four RA patients who were undergoing joint surgery and four health people who needed traumatic surgery. The patients with RA all fulfilled

with diagnostic criteria of the 1987 American College of Rheumatology (ACR) (12), and the health people were excluded rheumatic disease. Fresh synovial tissues were minced and digested in a solution of collagenase and DNase. Isolated fibroblasts were filtered through 70 μM nylon filters. The cells were grown on the plastic cell culture dishes in Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 15% heat-inactivated fetal bovine serum (FBS; Gibco), 100 units/ml penicillin and streptomycin (Gibco). The dishes were cultured at 37 °C in 95% air-5% CO_2. Fibroblasts from passages four to five were used for the experiments.

Ethics and consent

The study has been approved by the Ethics Committee of China-Japan Union Hospital and has been performed in accordance with the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed consent prior to their inclusion in the study.

Stimulation of RASF and HFLS

RASF and HFLS (4×10^5/well) were distributed in 24-well plates, and cultured overnight. After reaching confluence, cells were stimulated with increasing concentrations of 25 μg/ml, 50 μg/ml, and 100 μg/ml of different human adiponectin isoforms (BioVendor, Heidelberg, Germany) for 15 h : native adiponectin (a mixture of different adiponectin isoforms, recombinantly produced in HEK 293 cells), HMW/MMW-enriched adiponectin (recombinantly produced in HEK 293 cells), and trimeric adiponectin (recombinantly produced in HEK 293 cells; prevented from further oligomerisation by a single amino acid mutation). The total volume of each well was 400 μl. The stimulation time was chosen based on other researches (13). Unstimulated RASF and HFLS served as controls. Supernatants were collected and frozen immediately at -20 °C until further evaluation. Each of the experiments was repeated twice.

Enzyme-linked immunosorbent assays

MMP-3, MMP-10, TNF-α, VEGF and PGE_2 levels in cell culture supernatants were measured using two ELISA kits (MMP-10 and PGE_2) from Abnova and three kits (MMP-3, TNF-α and VEGF) from Boster. Each sample was detected twice.

Statistical analysis

Experimental replicates were used to calculate arithmetic means and standard errors of the mean (SEM). Data are presented as the mean ± SEM. In order to assess the significance of differences, a Student's two-tailed t-test was performed for pairwise comparisons. For multiple comparisons, Tukey's post-hoc test was performed. P values less than 0.05 were considered significant. Statistical calculations were performed using Statistical Product and Service Solutions (SPSS) 19.0.

Results

We found increasing productions of MMP-3, MMP-10, TNF-α, VEGF and PGE_2 in RASF at different levels when stimulated with all three adiponectin isoforms compared to unstimulated RASF. With HMW/MMW adiponectin, basal MMP-3 concentration was 125.39 ng/ml, which was distinctly increased up to 2449.70 ng/ml (19.54-fold increase) when using concentration of 100 μg/ml. Whereas MMP-10 synthesis was increased from 109.81 to 191.21 pg/ml (1.74-fold increase). Similarly, the productions of TNF-α, VEGF and PGE_2 were increased 1.54-fold, 1.70-fold and 1.94-fold respectively (Table 1.). With trimeric adiponectin, basal MMP-

Table 1. Differentially induced inflammatory factors secretion in RASF by increasing concentration of HMW/MMW adiponectin.

Inflammatory factors	Concentration of adponectin (μg/ml)	Mean ± SEM (ng/ml; MMP-10 pg/ml)		p_1 Value	p_2 Value
		RASF	HFLS		
MMP-3	0	125.3872 ± 2.3231	36.4877 ± 0.2355	-	0.027
	25	1753.6785 ± 110.8974	480.9007 ± 16.7045	<0.01	<0.01
	50	2295.2024 ± 43.3746	629.0282 ± 6.9606	<0.01	<0.01
	100	2449.6997 ± 93.2596	690.4022 ± 15.6200	<0.01	<0.01
MMP-10	0	109.8130 ± 0.0566	36.4687 ± 0.0035	-	<0.01
	25	168.2235 ± 0.7943	57.9600 ± 0.1356	<0.01	<0.01
	50	179.2061 ± 0.7190	58.4200 ± 0.5023	<0.01	<0.01
	100	191.2116 ± 1.2061	61.7771 ± 0.2300	<0.01	<0.01
TNF-α	0	31.79095 ± 0.6211	10.5082 ± 0.3143	-	<0.01
	25	43.6004 ± 0.3318	15.4847 ± 0.0858	<0.01	<0.01
	50	43.9705 ± 0.2729	16.0361 ± 0.1559	<0.01	<0.01
	100	48.9083 ± 1.1045	16.6767 ± 0.2970	<0.01	<0.01
VEGF	0	21.5763 ± 0.5379	6.3432 ± 0.0810	-	<0.01
	25	38.0452 ± 0.7330	11.8594 ± 0.1004	<0.01	<0.01
	50	38.7921 ± 1.2377	12.3007 ± 0.2230	<0.01	<0.01
	100	39.6635 ± 0.4433	14.9946 ± 0.1121	<0.01	<0.01
PGE_2	0	27.7920 ± 0.6458	10.0664 ± 0.2566	-	<0.01
	25	61.6466 ± 5.9162	18.0251 ± 0.9466	<0.01	0.01
	50	68.8401 ± 5.8518	18.8092 ± 0.5307	<0.01	0.013
	100	78.0393 ± 9.3568	19.7219 ± 0.8902	<0.01	0.024

p_1 as compared with unstimulated RASF; p_2 as compared with HFLS which were treated with the same conditions; SEM, standard errors of the mean; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; PGE_2, prostaglandin E_2; RASF, rheumatoid arthritis synovial fibroblasts; HFLS, human fibroblast-like synoviocytes; HMM, high molecular weight; MMW, middle molecular weight.

3 concentration was 125.39 ng/ml, which was distinctly increased up to 890.53 ng/ml (7.10-fold increase) when using concentration of 100 μg/ml. Whereas MMP-10 synthesis was increased from 109.81 to 117.61 pg/ml (1.07-fold increase). Similarly, the productions of TNF-α, VEGF and PGE_2 were increased 1.22-fold, 1.37-fold and 1.53-fold respectively (Table 2.). With native adiponectin, basal MMP-3 concentration was 125.39 ng/ml, which was distinctly increased up to 1723.19 ng/ml (13.74-fold increase) when using concentration of 100 μg/ml. Whereas, MMP-10 synthesis was increased from 109.81 to 163.77 pg/ml (1.49-fold increase). Similarly, the productions of TNF-α, VEGF and PGE_2 were increased 1.36-fold, 1.84-fold and 2.8-fold respectively (Table 3.). The levels of MMP-3, MMP-10, TNF-α, VEGF and PGE_2 were significantly increased in RASF which were treated with individual adiponectin isoforms compared to untreated RASF ($p<0.01$), and the increases also had significances compared to HFLS which were treated with the same conditions ($p<0.05$).

In addition, we investigated the effects of increasing concentration of individual adiponectin isoforms on the

Table 2. Differentially induced inflammatory factors secretion in RASF by increasing concentration of trimeric adiponectin.

Inflammatory factors	Concentration of adponectin (μg/ml)	Mean ± SEM (ng/ml; MMP-10 pg/ml)		p_1 Value	p_2 Value
		RASF	HFLS		
MMP-3	0	125.3872 ± 2.3231	36.4877 ± 0.2355	-	0.027
	25	756.7223 ± 21.7850	219.2916 ± 5.2250	<0.01	<0.01
	50	850.1696 ± 60.2834	230.7000 ± 4.2334	<0.01	<0.01
	100	890.5259 ± 20.9807	244.1245 ± 16.6385	<0.01	<0.01
MMP-10	0	109.8130 ± 0.0566	36.4687 ± 0.0035	-	<0.01
	25	113.1960 ± 0.1735	37.6630 ± 0.1579	<0.01	<0.01
	50	115.5218 ± 0.9127	39.8583 ± 0.0251	<0.01	<0.01
	100	117.6078 ± 0.2048	40.8833 ± 0.0484	<0.01	<0.01
TNF-α	0	31.7910 ± 0.6211	10.5082 ± 0.3143	-	<0.01
	25	35.1766 ± 0.1523	11.1334 ± 0.2960	<0.01	<0.01
	50	37.4581 ± 0.2999	12.0178 ± 0.4578	<0.01	<0.01
	100	38.6876 ± 0.3448	12.3955 ± 0.1421	<0.01	<0.01
VEGF	0	21.5763 ± 0.5379	6.3432 ± 0.0810	-	<0.01
	25	25.8587 ± 0.4231	8.1326 ± 0.0654	<0.01	<0.01
	50	28.2370 ± 0.4937	8.9280 ± 0.2428	<0.01	<0.01
	100	29.6485 ± 0.2473	9.7542 ± 0.0887	<0.01	<0.01
PGE_2	0	27.7920 ± 0.6458	10.0664 ± 0.2566	-	<0.01
	25	36.0502 ± 1.8932	12.7629 ± 0.1815	<0.01	<0.01
	50	39.4439 ± 1.7804	13.8960 ± 0.3229	<0.01	<0.01
	100	42.6130 ± 3.9952	14.4224 ± 0.2997	<0.01	<0.01

p_1 as compared with unstimulated RASF; p_2 as compared with HFLS which were treated with the same conditions; SEM, standard errors of the mean; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; PGE_2, prostaglandin E_2; RASF, rheumatoid arthritis synovial fibroblasts; HFLS, human fibroblast-like synoviocytes.

Table 3. Differentially induced inflammatory factors secretion in RASF by increasing concentration of native adiponectin.

Inflammatory factors	Concentration of adponectin (μg/ml)	Mean ± SEM (ng/ml; MMP-10 pg/ml)		p_1 Value	p_2 Value
		RASF	HFLS		
MMP-3	0	125.3872 ± 2.3231	36.4877 ± 0.2355	-	0.027
	25	1084.2904 ± 34.6511	311.8274 ± 10.0091	<0.01	<0.01
	50	1538.2748 ± 28.8827	500.2365 ± 17.2931	<0.01	<0.01
	100	1723.1881 ± 33.2921	572.0954 ± 11.3823	<0.01	<0.01
MMP-10	0	109.8130 ± 0.0566	36.4687 ± 0.0035	-	<0.01
	25	125.5899 ± 0.2018	37.2011 ± 0.0234	<0.01	<0.01
	50	130.8795 ± 1.2053	38.0913 ± 0.0508	<0.01	<0.01
	100	163.7741 ± 0.8738	40.8833 ± 0.0484	<0.01	<0.01
TNF-α	0	31.7910 ± 0.6211	10.5082 ± 0.3143	-	<0.01
	25	41.4470 ± 1.1426	12.9691 ± 0.0699	<0.01	<0.01
	50	41.7149 ± 0.9592	13.8804 ± 0.1848	<0.01	<0.01
	100	43.3624 ± 0.3551	13.9715 ± 0.3413	<0.01	<0.01
VEGF	0	21.5763 ± 0.5379	6.3432 ± 0.0810	-	<0.01
	25	30.0014 ± 0.7808	10.3097 ± 0.1096	<0.01	<0.01
	50	32.8396 ± 0.3333	10.5206 ± 0.0997	<0.01	<0.01
	100	36.6496 ± 0.3965	10.7905 ± 0.4693	<0.01	<0.01
PGE_2	0	27.7920 ± 0.6458	10.0664 ± 0.2566	-	<0.01
	25	43.5123 ± 1.7553	14.5228 ± 1.6084	<0.01	0.01
	50	48.0222 ± 5.5116	16.9067 ± 1.3078	<0.01	<0.01
	100	53.8552 ± 2.7293	17.3971 ± 0.7064	<0.01	<0.01

p_1 as compared with unstimulated RASF; p_2 as compared with HFLS which were treated with the same conditions; SEM, standard errors of the mean; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; PGE_2, prostaglandin E_2; RASF, rheumatoid arthritis synovial fibroblasts; HFLS, human fibroblast-like synoviocytes.

expression of MMP-3, MMP-10, TNF-α, VEGF and PGE_2 in RASF and HFLS (Figure 1.). It is notable that the effects appear not to be restricted to RASF, because up-regulation of these inflammatory factors could also be observed in HFLS. As expected, spontaneous expressions of these inflammation-ralated molecules in HFLS were lower than those in RASF. Generally the treatment of RASF and HFLS with three adiponectin isoforms resulted in a dose-dependent increase in all the inflammatory factors expression.

Among the three adiponectin isoforms, HMW/MMW adiponectin effected the most strongly, and the adiponectin trimeric effected the most weakly. The effect of the native adiponectin was between the two adiponectin isoforms. P values of Tukey's post-hoc test are less than 0.01.

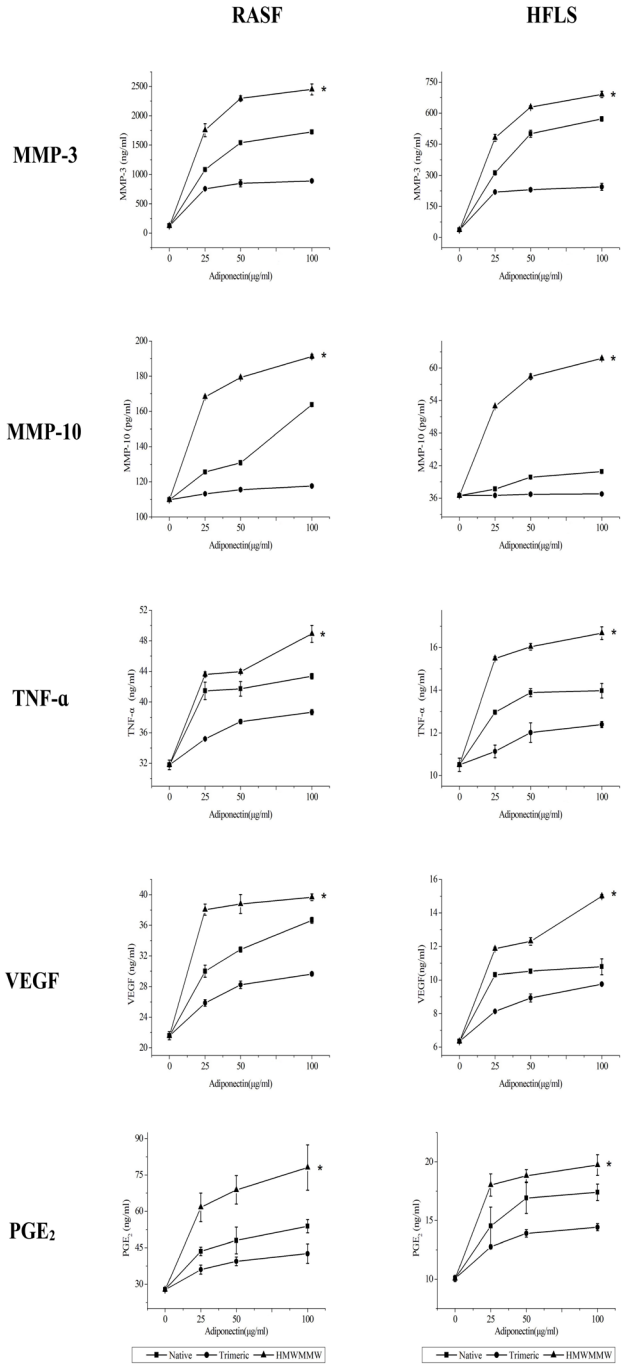

Figure 1. Stimulation of cultured synovial fibroblasts from RA patients and healthy people with increasing concentration of three adiponectin isoforms. * means p value, compared with native and trimeric adiponectin, less than 0.01; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; PGE_2, prostaglandin E_2; HMM, high molecular weight; MMW, middle molecular weight; RASF, rheumatoid arthritis synovial fibroblasts; HFLS, human fibroblast-like synoviocytes.

Discussion

Recently, adipokines have been known to play significant roles in rheumatic disease via target tissues and cells, including cartilage, synovium, bone and various immune cells (14). Activated synovial fibroblasts within the synovial lining, the perivascular area, and the inflamed sublining are primary producers of adiponectin (5). More foundings support that adipokines, especially adiponectin, exert proinflammatory actions in the pathogenesis of RA (15-16). Some reports indicated the concentrations of adiponectin in both serum and synovial fluid of RA patients were higher than OA patients (10, 17). In our previous report, we found the concentrations of different adiponectin isoforms differed in serum of RA patients in comparison to healthy people (18). These researches indicated that adiponectin has a major influence on the pathophysiology of RA. But studies about the effect of the concentrations of different adiponectin isoforms on RASF have not been reported yet. In this study, we explored the relationship between the concentration of individual adiponectin isoforms and the production of the inflammatory factors of RASF, including MMP-3, MMP-10, TNF-α, VEGF and PGE_2.

In RA, MMPs, including MMP-3 and MMP-10, are highly expressed perivascularly and in the synovial lining layer and play pivotal roles in both cartilage and bone destruction (19). In healthy tissue, MMPs are usually barely expressed, whereas tissues undergoing pathologic remodeling are high in MMPs (17). Frommer et al. found all adiponectin isoforms could stimulate the production of MMP-3 and MMP-10 in RASF, and HMW/MMW adiponectin effected the most strongly among the three adiponectin isoforms (11). Our studies are consistent with theirs. Meanwhile, we also found the effect of adponectin depends on the concentration. Accordingly, the higher the concentrations of the individual adiponectin isoforms are, the stronger the effect of matrix destruction is.

There are many researches about the effect of VEGF and TNF-α, which were known as proinflammatory-related mediators, on the pathogenesis of RA (20-21). However, whether adiponectin up-regulate or down-regulate the productions of VEGF and TNF-α is controversial. The study of Ehling et al. showed the expressions of the VEGF and TNF-α were not altered by adiponectin in RASF (13). Whereas, Lee et al. found adiponectin contribute to the production of VEGF, MMP-1, MMP-13, IL-6 and IL-8 in arthritic joints in endothelial cells and osteoblasts (22). And Choi et al. found adiponectin may stimulate VEGF, MMP-1 and MMP-13 expression in RASF more than proinflammatory mediators (23). These researchers all used the native adiopenctin as subject, but didn't evaluate the adiponectin isoforms effect. In our study, we found up-regulation of VEGF and TNF-α in RASF treated with native, trimeric, and HMW/MMW adiponectin. Our results indicated adiponectin isoforms may contribute to synovitis and joint

destruction in RA by stimulating VEGF and TNF-α production. And we also found HMW/MMW adiponectin effected the most strongly among the three adiponectin isoforms.

PGE_2, a very potent lipid mediator produced from arachidonic acid through the action of cyclooxygenase (COX) enzymes, functions significantly in the pathogenesis of RA (24). Kusunoki et al. found that adiponectin induces COX-2 and membrane-associated PGE synthase 1 (mPGES-1) expression, resulting in the enhancement of PGE_2 production by RASF (25). Thus, adiponectin may play a role in the pathogenesis of synovitis in RA patients. But there is no study about the effect of concentration of different adiponectin isoforms on PGE_2. Our results showed that three adiponectin isoforms can stimulate the production of PGE_2 in RASF, and HMW/MMW adiponectin effected the most strongly among the three adiponectin isoforms in a concentration-dependent manner.

Available data have suggested that adiponectin is beneficial for metabolic and cardiovascular health (26), and initial data have indicated that mainly HMW adiponectin is responsible for the vascular-protective effect (27). Our results suggest that three adiponectin isoforms are detrimental in disease progression of RA. Moreover, the HMW adiponectin shows the most strong effect.

All three adiponectin isoforms may contribute to proinflammatory effect by stimulating the production of MMP-3, MMP-10, TNF-α, VEGF and PGE_2 in RASF, and the effect of HMW/MMW is the strongest among them. Thus, HMW/MMW adiponectin could play an important role in matrix destroying and synovial vascular creating of the pathology of RA. Reducing the level of adiponectin in synovial fluid as low as possible, especially the HMW/MMW isoform, may be a new therapy for RA.

Acknowledgments

We acknowledge the expert technical assistance of W.A. Liu.

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