ISSN 2573-5985

Trends in Immunotherapy

Volume 3, Issue 2, 2019 https://systems.enpress-publisher.com/index.php/ti/index

ISSN 2573-5985 02
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2019 | Volume 3 | Issue 2

Trends in Immunotherapy

https://systems.enpress-publisher.com/index.php/ti/

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Trends in Immunotherapy (2019) Volume 3 Issue 2, pp.62–68.
doi: 10.24294/ti.v3.i2.42

ORIGINAL RESEARCH ARTICLE

Clinical experience of combination therapy of infliximab and total glucosides of paeony for severe psoriasis with liver disorder history

Yu Hu, Lin Lin, Pangen Cui, Xu Yao, Chao Luan, Zhimin Hao, Min Chen*

Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing, Jiangsu, People's Republic of China

ABSTRACT

Severe psoriasis patients are reported to have a higher risk of liver abnormalities. Treatment option for severe psoriasis patients with liver disorder history remains a great challenge. Hepatic toxicity and long-term safety are the major concerns. Hence, it is necessary to look for safer and more effective treatment for those patients. This retrospective review evaluated the safety and efficacy of combination therapy of infliximab and total glucosides of paeony (TGP) in treating 13 severe psoriasis patients with liver disorder history. Patients with severe psoriasis, comprising eight men and five women with a mean age of 37.3 ± 12.3, were observed. The patients experienced a mean course of psoriasis of 11.2 ± 7.1 years. The mean psoriasis area and severity index (PASI) score was 29.3 ± 12.9. All patients have the history of liver disorder. In our study, these patients were treated with infliximab at a dose of 5 mg/kg and TGP at a dose of 1.8 g/day. No liver test abnormalities were seen during combination therapy. After treatment, 61.5% patients showed PASI 50 response at week 2, and 81.8% patients have PASI 75 response at week 6. The mean time for achieving PASI 75 and PASI 90 improvement was 4.2 weeks and 9.6 weeks, respectively. Our observation demonstrates that combined therapy of infliximab and TGP is effective and safe in the treatment of severe psoriasis, especially for patients with liver disorder history.

Keywords*: psoriasis; infliximab; total glucosides of paeony; liver disorder; treatment*

ARTICLE INFO
Received: September 11, 2019
Accepted: October 5, 2019
Available online: October 19, 2019

***CORRESPONDING AUTHOR**
Min Chen, Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing, Jiangsu, People's Republic of China; drchenmin@126.com

CITATION
Hu Y, Lin L, Cui P, *et al*. Clinical experience of combination therapy of infliximab and total glucosides of paeony for severe psoriasis with liver disorder history. Trends Immunother 2019; 3(2): 62–68. doi: 10.24294/ti.v3.i2.42.

Introduction

Psoriasis is a chronic inflammatory skin disease characterized by proliferation of keratinocytes and infiltration of inflammatory cells into both dermis and epidermis. The prevalence of psoriasis is about 2% of the world's population[1]. Psoriasis is proved to be associated with a series of comorbidities especially in severe forms, including the metabolic syndrome, cardiovascular disease and liver abnormalities[2]. Recent studies indicate that drug-induced hepatitis and non-alcoholic fatty liver disease (NAFLD) may account for liver test abnormalities in severe psoriasis patients[3,4]. Considering the risk of abnormal liver function, treatment options need to be chosen carefully in severe psoriasis patients with liver disorder history.

Conventional systemic agents such as methotrexate (MTX) and acitretin are not recommended in treating those patients, for potential liver toxicities. While biological agent such as infliximab may be an appropriate option for patients with liver disorder history, it is still important to be cautious about the risk of HBV reactivation and other adverse effects[5,6]. Though the incidence of liver injury is relatively low, the existing several cases suggest that liver injury has a strong correlation with anti-TNF-α medications[7,8]. In addition, it is suggested that some patients lack a complete response to infliximab treatment. Hence, the application of combined medication is necessary to improve safety and efficacy.

Total glucosides of paeony (TGP), extracted from roots of *Paeonia lactiflora* Pall, has been approved by State Food and Drug Administration as an anti-inflammatory and disease-modifying drug in China. TGP has been widely used as disease-modifying antirheumatic drugs (DMARDs) in the

treatment of rheumatoid arthritis and psoriasis with good efficacy and fewer side effects[9]. In addition, it is well known that TGP has protective effects on liver function[10]. A double-blind, randomized, placebo-controlled trial reflected that TGP combined with acitretin is effective and safe in treating moderate-to-severe plaque psoriasis[11]. However, up to now, there have been no studies involved in the combination therapy of infliximab and TGP in treating psoriasis.

Thus, in this paper, we aim to share our experience about the efficacy and safety of combination therapy of infliximab and TGP in severe psoriasis patients with liver disorder history.

Methods

We retrospectively reviewed the medical records of severe psoriatic patients with liver disorder history who were treated with infliximab from 2013 to 2015 at the Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College (Nanjing, Jiangsu Province, China). All patients were diagnosed as severe psoriasis, as their psoriasis area and severity index (PASI) scores were all >10. The patients all had history of liver function disorder because of the use of conventional systemic therapies. The clinical data collected included age, gender, weight, history of smoking and drinking, course of disease, comorbidities and prior medication use. All patients were provided written informed consent before the treatment of infliximab.

All patients were treated with infliximab at a dose of 5 mg/kg in a scheduled or episodic therapy. Scheduled therapy was defined as patients receive infliximab infused at 0, 2 and 6 weeks, followed by scheduled infusions every 8 weeks. Episodic therapy was aimed for the induction of clinical remission, thus receiving infusions if necessary. All patients were also treated with TGP at a dose of 1.8 g/day. The numbers of infliximab infusions and adverse events were collected from the medical records. For the evaluation of the severity of psoriasis and the response to infliximab therapy, PASI was calculated before the first treatment of infliximab and at 2, 4 and 6 weeks, as well as at each follow-up visit. PASI 50, PASI 75 and PASI 90 refer to 50%, 75% and 90% reduction in the PASI scores compared to the baseline, respectively, and have been recognized as the significant endpoint in the assessment of psoriasis[12]. Clinical laboratory tests such as blood and urine routine tests, hepatic and renal function and other biological tests were also performed and data were collected. Chest X-ray and tuberculosis examination were included.

Statistical analyses

Statistical analyses were performed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). All continuous variables were expressed as mean ± standard deviation (SD), and discrete variables were described as sample number and percentage.

Results

Thirteen patients were enrolled in the study (**Table 1**). All patients were negative for HIV or active infections such as hepatitis, pneumonia or tuberculosis; the patients also had no history of malignant tumors, and were not pregnant or lactating. They all had the history of liver function damage because of the use of conventional systemic therapy, and the hepatic enzymes returned to normal by liver-protecting treatment before using infliximab. The backgrounds of their liver diseases are reported in **Table 2**. Eight men and five women with a mean age of 37.3 ± 12.3 years (range 18–60 years) were enrolled. The mean weight was 68.8 ± 9.0 kg. The patients experienced a mean course of psoriasis of 11.2 ± 7.1 years. The mean PASI score before therapy was 29.3 ± 12.9 (range 14.4–52.0). Of all the patients in this study, one (7.7%) had psoriatic arthritis and nine (69.2%) patients had nail involvement. Two (15.4%) active smokers were also included in these patients. Last but not least, all the patients' previous treatments before infliximab therapy are reported in **Table 1**.

Of all 13 patients, eight received scheduled therapy and the remaining five received episodic the-

Table 1. Clinical characteristics of patients in the study

Characteristics	Psoriasis (*n* = 13)
Male/Female, *n* (%)	8(61.5%)/5(38.5%)
Age, years (mean ± SD)	37.3 ± 12.3
Weight, kg (mean ± SD)	68.8 ± 9.0
Psoriasis duration, years (mean ± SD)	11.2 ± 7.1
Psoriatic arthritis, *n* (%)	1 (7.7%)
Nail involvement, *n* (%)	9 (69.2%)
Active smoker, *n* (%)	2 (15.4%)
Chronic hepatitis, *n* (%)	0
Psoriasis medication history, *n* (%):	
Acitretin	10 (77.0%)
Tripterygium wilfordii	9 (69.2%)
Methotrexate	2 (15.4%)
Cyclosporine	1 (7.7%)
Etanercept	1 (7.7%)

Table 2. Clinical characteristics of liver disorder history

Characteristics	Psoriasis (n = 13)
Liver injury-related drugs, n (%):	
Acitretin	8
Tripterygium wilfordii	3
Methotrexate	2
Liver injury type, n (%):	
Hepatocellular	7
Cholestatic	4
Mixed	2
Treatment of liver disorder:	
Withdraw	10
Stronger Neo-Minophagen C	3
Hepatitis:	
HBV	0
HCV	0

rapy. All patients received at least one infusion of infliximab, and the detailed information is included in the flowchart (**Figure 1**). As for TGP therapy, 11 patients received combination therapy of infliximab and TGP from the first infusion of infliximab, while the remaining two patients started the treatment of TGP when they experienced adverse events.

As for liver function monitoring, one patient had positive biological detection of liver abnormalities during monotherapy of infliximab. No liver test abnormalities were seen during the combination therapy of infliximab and TGP.

The PASI 50, PASI 75 and PASI 90 responses of all these patients after each infliximab therapy are summarized in **Table 3**. At week two, eight had at least 50% (PASI 50) improvement, five had a 75% (PASI 75) improvement and one had at least 90% (PASI 90) improvement compared with baseline. At week six, the PASI 50, PASI 75 and PASI 90 responses were for 10, 9 and 7 of the remaining 11 patients, respectively. Except for the only patient with no response to the therapy, all the other 12 patients maintained at least 75% improvement compared with baseline after the last infusion. Moreover, we also calculated the mean time to achieve PASI 75 and PASI 90 improvements among all patients.

Of all 13 patients, we have observed two patients with different circumstances during the combination therapy. These two interesting cases are described as follows:

Case 1: A 31-year-old man had three years of history of plaque-type psoriasis. When the patient presented at our department, physical examinations

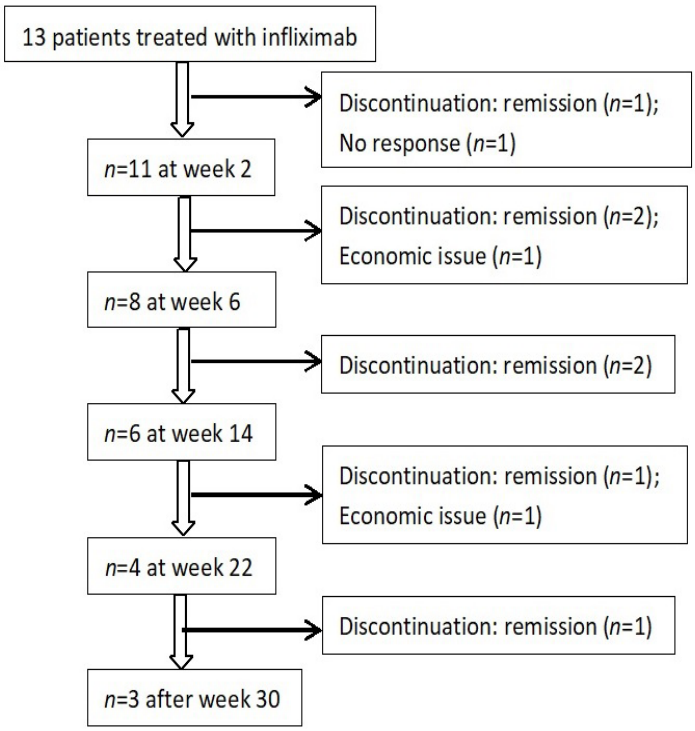

Figure 1. Flowchart of psoriasis patients treated with infliximab in our study

Table 3. Clinical response after infliximab treatments

Week	Patients (*n*)	PASI 50 (*n*)	PASI 75 (*n*)	PASI 90 (*n*)
2	13	8	5	1
4	11	9	8	6
6	11	10	9	7
10	8	8	7	7
14	8	8	7	7
22	6	6	6	6
Mean time of achieving PASI 75 improvement: 4.2 weeks				
Mean time of achieving PASI 90 improvement: 9.6 weeks				

revealed generalized symmetric distribution of erythematous scaly plaques involving more than 70% of the body surface, and the PASI score was nearly 52 (**Figure 2**). There was nothing abnormal from the blood and urine routine tests and biochemical test. Although the HBeAb, HBcAb and HBsAb were all positive, the level of HBV DNA in serum and hepatic enzymes were all normal. Then, he was treated with infliximab (5 mg/kg) combined with topical moisturizer. Within one week after the first treatment, the plaques and papules were resolved, the effusion lesions were significantly reduced and the PASI score dropped to 30. He missed the second treatment two weeks later for the slight elevation of the level of hepatic enzymes, so he was treated with TGP. Two weeks later, hepatic enzymes became normal, but the lesions relapsed. Then, he continued the treatment with infliximab at the week 4, 8 and 16 combined with TGP, and the improvement continued. All lesions cleared up after his eight-week treatment (**Figure 2**). Before each treatment, we performed blood and urine routine tests, biochemical test, the level of HBV DNA and T-Spot, and no adverse event occurred.

Case 2: A 48-year-old man had a 10-year history of plaque-type psoriasis. He had been treated with *Tripterygium wilfordii*, a traditional Chinese medication which has immunodepressive and anti-inflammatory effects but with only limited efficacy. Oral administration of acitretin (20 mg/day) had been considered as an anti-psoriatic therapy for him, but it also produced an adverse reaction (hepatotoxicity), which made him discontinue acitretin therapy. When this patient first presented at our outpatient department, his lesions covered about 35% of the body surface, and the PASI score was 19 (**Figure 3**). The results of his blood and urine routine tests, biochemical test and other laboratory data were normal. We treated this patient with infliximab (5 mg/kg). Two weeks after the first infliximab infusion, the lesions did not regress. Cutaneous examination revealed that scaly plaques on the trunk and thighs became thinner, but unfortunately the scaly erythematous plaques were confluent and the range of lesions was broader. At that time, the lesions covered about 52% of the body surface, and the PASI score was 23.2 (**Figure 3**). The treatment with infliximab was maintained and we also combined it with TGP. At the follow-up visit after the second infliximab therapy, his cutaneous symptoms gradually ameliorated, and laboratory data were all normal.

Discussion

Treatment options are limited for the management of psoriasis patients with liver disorder history. Liver function is the major concern when selecting the optimal treatment in those patients. Systemic drugs such as MTX, acitretin and even biological agents are reported to cause liver test abnormalities[13–15]. In our clinical study, patients were treated with combination therapy of infliximab and TGP. No liver test abnormalities were seen during the combination therapy. What is more, in Case 1, the elevated hepatic enzyme returned to normal after the combination therapy with TGP. This phenomenon may be attributed to the anti-inflammatory and disease-modifying function of TGP. A clinical trial demonstrates that TGP can significantly reduce the incidence and severity of liver damage caused by MTX and leflunomide in treating active rheumatoid arthritis (RA) patients[10]. Moreover, several animal experiments reflect that TGP plays a role on liver histopathology. Wang *et al*. showed that TGP could retard the progression of hepatic fibrosis in rats by the inhibition of collagen synthesis and by decreasing oxidative stress[16]. Qin *et al*. also found that TGP protects hepatocytes from carbon tetrachloride (CCl_4)-induced oxidative stress by inhibiting the expression of proinflammatory mediators[17]. Considering that all patients in our study did not have severe adverse effects, infliximab combined with TGF is thought to be a promising combination therapy for patients with liver disorder history.

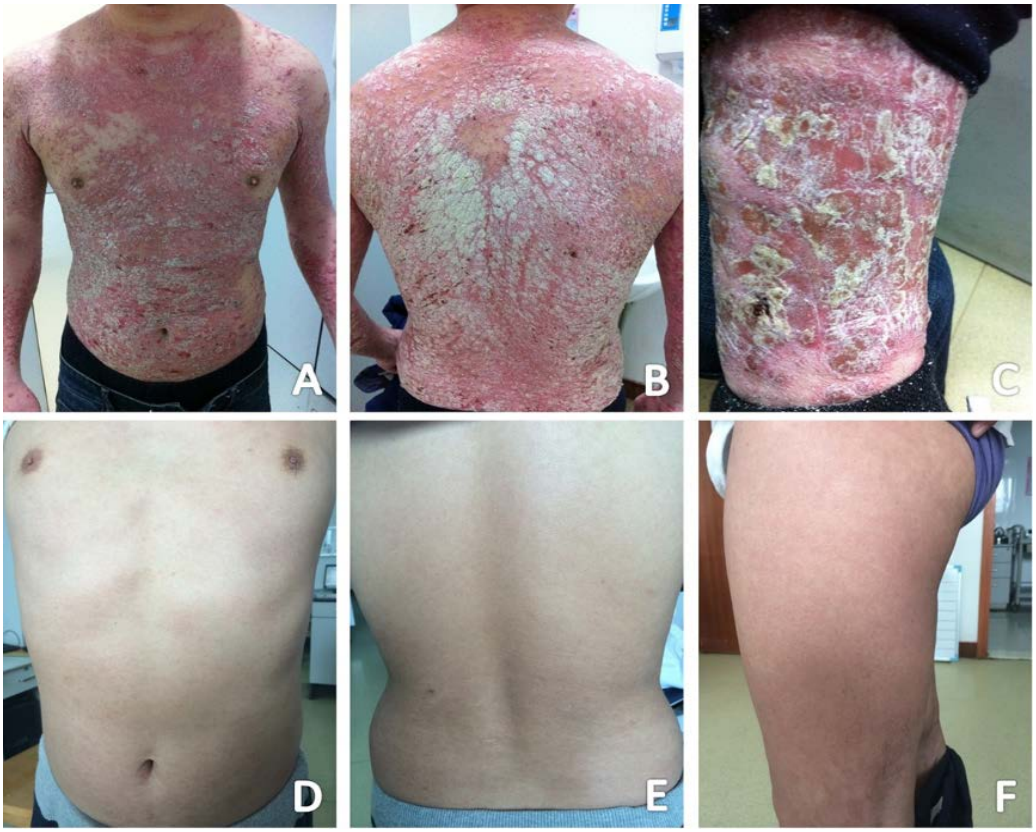

Figure 2. Generalized plaques with active border, red papules and effusion lesions of plaque psoriasis images before (**A**, **B** and **C**) and after (**D**, **E** and **F**) treatment with infliximab

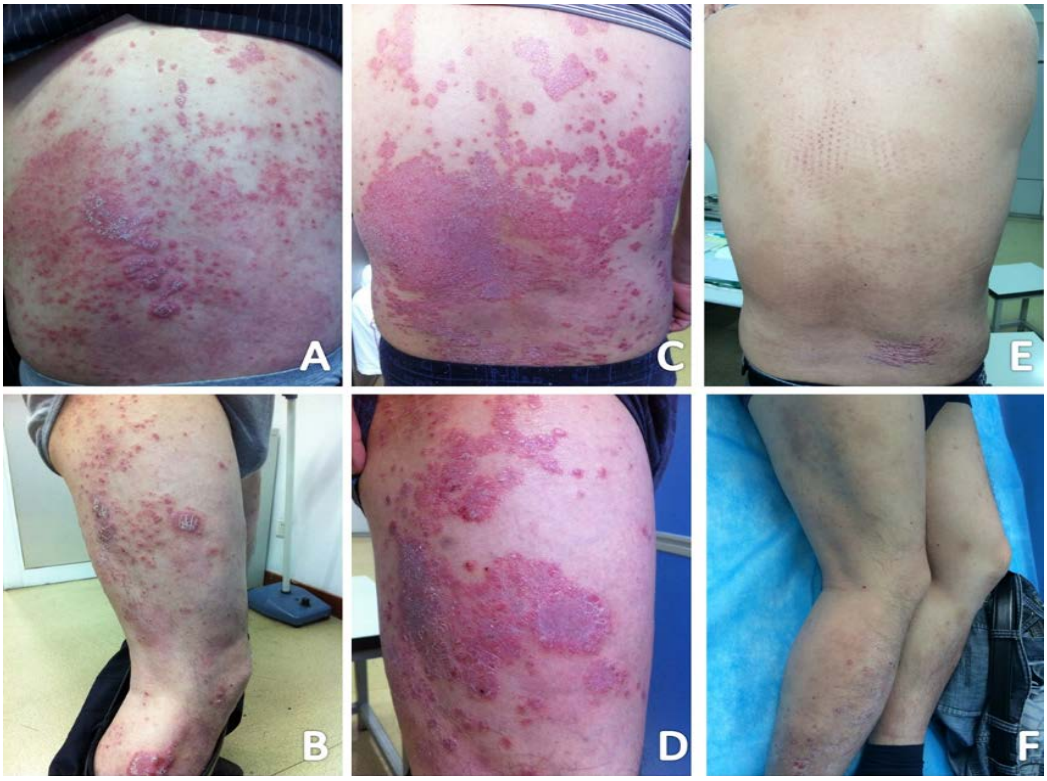

Figure 3. Erythematous plaques on the scalp, trunk and extremities, with scales covering the plaques before treatment (**A** and **B**). Two weeks after the first infusion of infliximab, the scaly erythematous plaques were confluent and the range of lesions was broader (**C** and **D**). Most scaly plaques were relieved after the second treatment of infliximab (**E** and **F**).

Compared with infliximab monotherapy, combination therapy of infliximab and TGP provides higher clinical remission rates. Previous studies have proved the safety and efficacy of infliximab monotherapy in treating psoriasis[18,19]. Three randomized clinical studies from Western countries showed that the PASI 75 response at week 14 were 75.5% (EXPRESS2), 87.9% (SPIRIT) and 72.4% (RESTORE1)[20–22]. Another double-blind trial published in Lancet reported that 80% of patients treated with infliximab achieved PASI 75 and 57% achieved PSAI 90[23]. As for the week 2 response, the PASI 50 rates ranged from 35.4% to 40%[21–23]. In our study, 61.5% (8/13) had at least 50% improvement in PASI score at week 2. Moreover, 81.9% (9/11) achieved a PASI 75 response at week 6. The mean time to achieve PASI 75 improvement was 4.2 weeks and the mean time to achieve PASI 90 improvement was 9.6 weeks. These results above suggested that our patients had higher clinical remission rates than those reported previously, which reflected that the combination therapy of infliximab and TGP might be more effective in treating psoriasis patients by comparing with the monotherapy of infliximab.

In Case 2 discussed above, we found that the patient developed an erythematous rash after the first infusion of infliximab. To our surprise, anti-TNF medications may also induce psoriasiform skin lesions[24]. Grinblat and Scheinberg reviewed the literature of this phenomenon between 2005 and 2007, and reported that more than 25 cases of all 50 cases were associated with the application of infliximab[25]. Several mechanisms such as infections and cytokine imbalance may be associated with the phenomenon. Interferon (IFN)-α produced by dermal plasmacytoid dendritic cells has been identified as a key element in psoriatic skin lesion formation. As TNF-α regulates IFN-α production and the inhibition of TNF-α has been shown to induce the overexpression of IFN-α–regulated genes, therefore it is proposed that TNF-α inhibition might induce locally sustained IFN-α production in patients developing psoriasis while undergoing anti-TNF therapy[26]. In another research, anti-TNF drug-induced psoriasiform skin lesions are attributed to the infiltrates of interleukin (IL)-17A/IL-22-expressing Th17 cells and IFN-expressing Th1 cells, and the severity of skin disease were positively correlated with the number of IL-17A-expressing T cells[27]. At the same time, TGP can inhibit the maturation and function of dendritic cells (DCS) by selectively blocking the activation of TLR4/5 activation *in vivo*, which in turn reduces T cell proliferation and leads to impaired Th1 and Th17 differentiation[28,29]. This might help to explain why the infliximab-induced exacerbation in Case 2 got resolved after combination with TGP.

One limitation of our study is the small sample size. Furthermore, because of the retrospective study, the treatment duration was varied among our patients, which might probably induce potential bias.

Conclusion

We present the experience of combination therapy of infliximab and TGP in psoriasis patients with liver disorder history. Considering that all patients in our study achieved remarkable improvements and did not have liver test abnormalities, infliximab combined with TGP is thought to be a promising combination therapy, especially for patients with liver disorder history. Further randomized controlled studies in large populations are needed in the future for a better understanding of the combination treatment.

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of their article.

References

1. Gladman DD. Clinical features and diagnostic considerations in psoriatic arthritis. Rheum Dis Clin North Am 2015; 41(4): 569–579. doi: 10.1016/j.rdc.2015.07.003.
2. Gottlieb AB, Dann F. Comorbidities in patients with psoriasis. Am J Med 2009; 122(12): 1–9. doi: 10.1016/j.amjmed.2009.06.021.
3. Finet A, Viguier M, Chazouillères O, *et al*. Liver test abnormalities in patients admitted for severe psoriasis: Prevalence and associated risk factors. J Eur Acad Dermatol Venereol 2016; 30(10): 1742–1748. doi: 10.1111/jdv.13674.
4. Tula E, Ergun T, Seckin D, *et al*. Psoriasis and the liver: Problems, causes and course. Australas J Dermatol 2016. doi: 10.1111/ajd.12460.
5. Viganò M, Degasperi E, Aghemo A, *et al*. Anti-TNF drugs in patients with hepatitis B or C virus infection: Safety and clinical management. Expert Opin Biol Ther 2012; 12(2): 193–207. doi: 10.1517/14712598.2012.646986.
6. Moustou AE, Matekovits A, Dessinioti C, *et al*. Cutaneous side effects of anti-tumor necrosis factor biologic therapy: A clinical review. J Am Acad Dermatol 2009; 61(3): 486–504. doi: 10.1016/j.jaad.2008.10.060.
7. French JB, Bonacini M, Ghabril M, *et al*. Hepatotoxicity associated with the use of anti-TNF-alpha agents. Drug Saf 2016; 39(3): 199–208. doi: 10.1007/s40264-015-0366-9.

8. Ghabril M, Bonkovsky HL, Kum C, *et al*. Liver injury from tumor necrosis factor-alpha antagonists: Analysis of thirty-four cases. Clin Gastroenterol Hepatol 2013; 11(5): 558–564.e3. doi: 10.1016/j.cgh.2012.12.025.
9. Min WQ, Wei Q, Li H, *et al*. (Mandarin) [A clinical study of total glucosides paeony in the treatment of rheumatoid arthritis: A multi-center trial]. Chin J Rheumatology 2005; 9(8): 487–491. doi: 10.3760/j:issn:1007-7480.2005.08.011.
10. Xiang N, Li XM, Zhang MJ, *et al*. Total glucosides of paeony can reduce the hepatotoxicity caused by Methotrexate and Leflunomide combination treatment of active rheumatoid arthritis. Int Immunopharmacol 2015; 28(1): 802–807. doi: 10.1016/j.intimp.2015.08.008.
11. Yu C, Fan X, Li Z, *et al*. Efficacy and safety of total glucosides of paeony combined with acitretin in the treatment of moderate-to-severe plaque psoriasis: A double-blind, randomised, placebo-controlled trial. Eur J Dermatol 2017; 27(2): 150–154. doi: 10.1684/ejd.2016.2946.
12. Carlin CS, Feldman SR, Krueger JG, *et al*. A 50% reduction in the Psoriasis Area and Severity Index (PASI 50) is a clinically significant endpoint in the assessment of psoriasis. J Am Acad Dermatol 2004; 50(6): 859–866. doi: 10.1016/j.jaad.2003.09.014.
13. Kalb R, Strober B, Weinstein GM, *et al*. Methotrexate and psoriasis: 2009 National Psoriasis Foundation Consensus Conference. J Am Acad Dermatol 2009; 60(5): 824–837. doi: 10.1016/j.jaad.2008.11.906.
14. Zachariae H. Dangers of methotrexate/etretinate combination therapy. Lancet 1987; 1(8582): 422.
15. Saurat JH, Stingl G, Dubertret L, *et al*. Efficacy and safety results from the randomized controlled comparative study of adalimumab *vs*. methotrexate *vs*. placebo in patients with psoriasis (CHAMPION). Br J Dermatol 2008; 158(3): 558–566. doi: 10.1111/j.1365-2133.2007.08315.x.
16. Wang H, Wei W, Wang NP, *et al*. Effects of total glucosides of peony on immunological hepatic fibrosis in rats. World J Gastroenterol 2005; 11(14): 2124–2129. doi: 10.3748/wjg.v11.i14.2124.
17. Qin Y, Tian YP. Protective effects of total glucosides of paeony and the underlying mechanisms in carbon tetrachloride-induced experimental liver injury. Arch Med Sci 2011; 7(4): 604–612. doi: 10.5114/aoms.2011.24129.
18. Chaudhari U, Romano P, Mulcahy LD, *et al*. Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: A randomised trial. Lancet 2001; 357(9271): 1842–1847. doi: 10.1016/S0140-6736(00)04954-0.
19. Shear NH, Hartmann M, Toledo-Bahena M, *et al*. Long-term efficacy and safety of infliximab maintenance therapy in patients with plaque-type psoriasis in real-world practice. Br J Dermatol 2014; 171(3): 631–641. doi: 10.1111/bjd.13004.
20. Barker J, Hoffmann M, Wozel G, *et al*. Efficacy and safety of infliximab *vs*. methotrexate in patients with moderate-to-severe plaque psoriasis: Results of an open-label, active-controlled, randomized trial (RESTORE1). Br J Dermatol 2011; 165(5): 1109–1117. doi: 10.1111/j.1365-2133.2011.10615.x.
21. Menter A, Feldman SR, Weinstein GD, *et al*. A randomized comparison of continuous *vs*. intermittent infliximab maintenance regimens over 1 year in the treatment of moderate-to-severe plaque psoriasis. J Am Acad Dermatol 2007; 56(1): 31.e1–15. doi: 10.1016/j.jaad.2006.07.017.
22. Gottlieb AB, Evans R, Li S, *et al*. Infliximab induction therapy for patients with severe plaque-type psoriasis: A randomized, double-blind, placebo-controlled trial. J Am Acad Dermatol 2004; 51(4): 534–542. doi: 10.1016/j.jaad.2004.02.021.
23. Reich K, Nestle FO, Papp K, *et al*. Infliximab induction and maintenance therapy for moderate-to-severe psoriasis: A phase III, multicentre, double-blind trial. Lancet 2005; 366(9494): 1367–1374. doi: 10.1016/S0140-6736(05)67566-6.
24. Sfikakis PP, Iliopoulos A, Elezoglou A, *et al*. Psoriasis induced by anti-tumor necrosis factor therapy: A paradoxical adverse reaction. Arthritis Rheum 2005; 52(8): 2513–2518. doi: 10.1002/art.21233.
25. Grinblat B, Scheinberg M. The enigmatic development of psoriasis and psoriasiform lesions during anti-TNF therapy: A review. Semin Arthritis Rheum 2008; 37(4): 251–255. doi: 10.1016/j.semarthrit.2007.05.004.
26. de Gannes GC, Ghoreishi M, Pope J, *et al*. Psoriasis and pustular dermatitis triggered by TNF-{alpha} inhibitors in patients with rheumatologic conditions. Arch Dermatol 2007; 143(2): 223–231. doi: 0.1001/archderm.143.2.223.
27. Afzali A, Wheat CL, Hu JK, *et al*. The association of psoriasiform rash with anti-tumor necrosis factor (anti-TNF) therapy in inflammatory bowel disease: A single academic center case series. J Crohns Colitis 2014; 8(6): 480–488. doi: 10.1016/j.crohns.2013.10.013.
28. Zhou Z, Lin J, Huo R, *et al*. Total glucosides of paeony attenuated functional maturation of dendritic cells via blocking TLR4/5 signaling *in vivo*. Int Immunopharmacol 2012; 14(3): 275–282. doi: 10.1016/j.intimp.2012.07.012.
29. Lin J, Xiao L, Ouyang G, *et al*. Total glucosides of paeony inhibits Th1/Th17 cells via decreasing dendritic cells activation in rheumatoid arthritis. Cell Immunol 2012; 280(2): 156–163. doi: 10.1016/j.cellimm.2012.12.005.

Trends in Immunotherapy (2019) Volume 3 Issue 2, pp.69–75.
doi: 10.24294/ti.v3.i2.96

MINI-REVIEW

Mast cells in collagen diseases

Naoya Mikita*, Yutaka Inaba, Takashi Yoshimasu, Nobuo Kanazawa, Fukumi Furukawa

Department of Dermatology, Wakayama Medical University, Wakayama-shi, Wakayama, Japan

ABSTRACT

Mast cells are involved in many immune reactions and diseases through 1) the expressions of several receptors, 2) productions of various mediators such as histamine, cytokines, and chemokines, 3) direct interactions with immune cells. Besides allergic diseases, the involvement of mast cells has been also investigated in autoimmune diseases such as bullous pemphigoid, rheumatoid arthritis, and multiple sclerosis. Moreover, several studies reported the involvement of mast cells in collagen diseases. In this article, we review recent findings about the role of mast cells especially in systemic lupus erythematosus and systemic sclerosis. In these diseases, mast cells seem to be involved in local inflammation and tissue damage in the targeted organ or local immunosuppression rather than the development of autoimmunity including production of autoantibodies.

***Keywords**: mast cells; systemic lupus erythematosus; systemic sclerosis*

ARTICLE INFO
Received: July 12, 2019
Accepted: August 8, 2019
Available online: August 20, 2019

***CORRESPONDING AUTHOR**
Naoya Mikita. Department of Dermatology, Wakayama Medical University, Wakayama-shi, Wakayama, 641-0012, Japan;
naoya@wakayama-med.ac.jp

CITATION
Mikita N, Inaba Y, Yoshimasu T, *et al*. Mast cells in collagen diseases. Trends Immunother 2019; 3(2): 69–75. doi: 10.24294/ti.v3.i2.96.

Introduction

Mast cells are hematopoietic cells which have characteristic secretary granules in their cytoplasm[1]. Mast cells generally distribute in host-environment interface sites such as skin and mucosal tissues of digestive and respiratory tracts. In these tissues, mast cells response to various pathogens[2]. Mast cells play important roles in the defense against some parasitic and bacterial infections. In response to various allergens, mast cells degranulate their secretary granules including histamine, and induce immediate type hypersensitivity and Th2 immune response. Furthermore, mast cells also play important roles in tissue remodeling and fibrosis[1]. Due to these diverse functions, the involvement of mast cells has been reported in various diseases including allergic diseases, malignant tumors[3] and autoimmune diseases[4–6]. In this review, we summarize current knowledge about the role of mast cells in collagen diseases.

Mast cells in autoimmune diseases

Mast cells express several receptors on their cell surface. High-affinity IgE receptor (FcεRI) is a well-known receptor that binds specific IgE for various allergens. Allergen stimuli cause crosslinking of FcεRI-bound IgE, and induce allergic reactions by degranulation of their cytoplasmic granules. KIT with tyrosine kinase activity is important for mast cell maturation and proliferation. Furthermore, mast cells also express toll-like receptors which recognize pathogen-associated molecular patterns, FcγRI and FcγRIII for IgG, and complement receptors for C3a and C5a[7,8]. The production of various autoantibodies and activation of complement system are key events in many autoimmune diseases. Therefore, expressions of receptors for these components implicate the involvement of mast cells in collagen diseases.

Additionally, mast cells release many types of mediator with or without degranulation. In addition to chemical mediators such as histamine, protease, and lipid mediators, mast cells also produce and release various cytokines (*e.g.* TNF-α, IL-4, IL-5, IL-10, and TGF-β), and chemokines such as CCL2 and CXCL8[9,10].

Moreover, mast cells directly interact with various immunocompetent cells through some ligands and costimulatory molecules[5]. For example, following antigen stimulation, OX40L on mast cells contributes to T cell activation through interaction with OX40 on T cells[11,12]. Inversely, OX40 on regulatory T cells (Tregs) has been reported to inhibit degranulation of mast cells and allergic reaction through interaction with OX40L on mast cells[13]. Moreover, mast cells with PD-1 expression directly contact with immature dendritic cells (DCs) with PD-L1 expression, and induce tolerogenic indoleamne-2,3-deoxoygenase expressing DC[14].

Besides these diverse functions of mast cells, recent studies have shown their anti-inflammatory and tolerogenic effects through the production of anti-inflammatory cytokine such as IL-10 and TGF-β and direct interaction with Tregs and DCs.

On the basis of these diverse immunological effects, the involvement of mast cells has been reported in autoimmune diseases and malignancies. Direct data clarifying their involvement have been reported in several autoimmune diseases such as bullous pemphigoid, rheumatoid arthritis, and multiple sclerosis in human and experimental model[4–6]. In rheumatoid arthritis patients, an increased number of mast cells and degranulated mast cells were observed in synovial tissue, and mast cell-derived mediators were shown in synovial fluid[15]. In a rheumatoid arthritis model mice, K/B×N mice, administration of their serum-induced arthritis in control mice, but did not induce arthritis in mast cell-deficient *Kit*$^{W/W\text{-}V}$ mice[16]. Some reports proposed the importance of FcγRIII on mast cells[17], IL-1 production by mast cells[18], and synovial membrane-derived IL-33 with the IL-33 receptor (ST2) on mast cells[19] in rheumatoid arthritis model. Though in most of these earlier studies, KIT-dependent mast cell-deficient mice were studied, these mice have immunological abnormalities other than mast cell deficiency. Recently, KIT-independent mice with specific mast cell-deficiency and fewer other immunologic abnormalities have been frequently studied by applying the gene recombination technique targeting a gene specifically expressed by mast cells[20]. In the experiment using autoimmune disease models prepared with these genetically modified mice, mast cell deficiency did not influence the disease in antibody-mediated rheumatoid arthritis or T cell-mediated experimental autoimmune encephalomyelitis model[21], but it is necessary to investigate whether similar findings are obtained in humans and other model mice.

Compared with the diseases described above, knowledge of the role of mast cells in the other collagen diseases is limited. The roles of mast cells in systemic lupus erythematosus (SLE), systemic sclerosis (SSc), dermatomyositis[22,23], and Sjögren's syndrome[24] have been reported. The roles of mast cells in SLE and SSc are described below.

Mast cells in systemic lupus erythematosus

SLE is the major collagen disease, which affects diverse organs including kidney, skin and central nervous system. The individual susceptibilities and environmental factors are involved in the development of SLE. Multiple organ damages are closely associated with loss of tolerance and characteristic autoantibody production. This abnormality in immune system results from defective clearance of immune complexes and biological waste, nucleic acid sensing, and interferon production pathway. Various immune cells such as T cells, B cells, dendritic cells and neutrophils are the major cell types involved in SLE. The involvement of mast cells in SLE has been investigated mainly for renal lesions (**Table 1**). In normal human kidney, only a few mast cells are present preferentially in the interstitial space[25]. In lupus nephritis, the number of mast cells increases also in the renal tubule-interstitial area[26]. The number of renal mast cells is correlated with renal fibrosis development[27] and differed between classes of lupus nephritis[26]. In pristine-induced experimental lupus nephritis model with mast cell-deficient *Kit*$^{W/W\text{-}V}$ mice, the development of humoral autoimmunity, such as hypergammaglobulinemia and autoantibodies, was comparable to their wild-type counterparts[28]. However, in the absence of mast cell, diffuse proliferative glomerulonephritis was more frequently observed compared to wild-type mice. These findings indicated that mast cells were dispensable in the development of humoral autoimmunity, but had limited inhibitory effects for lupus nephritis in this study.

Further investigations are still required for this issue. Clear mechanism and signaling by which mast cells caused lupus nephritis have not been elucidated. Firstly, the involvement of mast cells may not be specific for autoimmune renal disease. Because besides lupus nephritis, the involvement of mast cells in renal inflammation and fibrosis has been reported in various kidney diseases including diabetic nephropathy[29]. Secondarily, some studies in human and mice showed no correlation between mast cell and lupus nephritis. In human, no clear correlation was observed between the number of mast cells and

Table 1. The involvement of mast cells in SLE.

Species	Lupus model	MC depletion	Role of mast cell	Organ	Findings	References
Human	–	–	Not defined	Kidney	The number of MCs increased in tubule-interstitial area of LN.	[26]
					The number of MCs correlated with the classes of LN.	
					The number of MCs correlated with renal fibrosis in LN.	[27]
					The number of MCs did not correlate with the severity of LN.	[30]
				Skin	MCs preferentially infiltrated in reticular dermis and peri-pilosebaceous in CLE	[32]
Mouse	Pristine-induced	$Kit^{W/W\text{-}V}$	Suppressive	Kidney	MCs deficiency increases the severity of lupus nephritis partially.	[28]
			No effect	Humoral immunity	MCs deficiency has no effect on hypergammaglobulinemia and autoantibodies.	
	$Lyn^{-/-}$ spontaneous SLE model	$Kit^{W\text{-}sh/W\text{-}sh}$	No effect	Kidney	MCs deficiency has no effect on lupus nephritis.	[31]
				Humoral immunity	MCs deficiency has no effect on autoantibodies and ANA.	
	MRL/lpr spontaneous SLE model	$Kit^{W\text{-}sh/W\text{-}sh}$	Suppressive	Skin	MCs suppress the development of skin lesions.	[35]
			Promotive	Kidney	MCs exacebrated the degree of proteinuria.	
			Various	Others	MCs improved survival rate, but exacebrated serum ds-DNA antibody level.	
	B6/lpr spontaneous SLE model	$Kit^{W\text{-}sh/W\text{-}sh}$	No effect	Kidney	MCs exacebrated the degree of proteinuria	[36]
				Humoral immunity	MCs improve survival rate, but exacebrated serum ds-DNA antibody level.	

Abbreviation: MC: mast cell; LN: lupus nephritis; SLE: systemic lupus erythematosus; ANA: antinuclear antibodies

lupus nephritis severity[30]. Src family protein kinase Lyn-deficient mice develop a strong and constitutive Th2 skewing in early life and an autoimmune disease similar to SLE such as production of autoantibodies and lupus-like nephritis in late life. $Lyn^{-/-}$ mice deficient in mast cells, $Kit^{W\text{-}sh/W\text{-}sh}$; $Lyn^{-/-}$ mice, exhibited comparable severity of lupus nephritis to those in $Lyn^{-/-}$ mice, so lupus-like nephritis was independent of mast cells in $Lyn^{-/-}$ mice[31]. In this study, basophils activated by IgE autoantibodies promoted autoantibody production and resulted in lupus-like nephritis.

Skin is exposed to several external agents including pathogens and allergens. Skin is the resident tissue of mast cells, which involved in the cutaneous immunity and diseases. Most of the collagen diseases including SLE also affects skin, but the role of mast cells in skin lesions of lupus patients remains unclear. Thus, we have investigated the role of mast cells in LE, especially focused on the skin lesions. In skin lesions of lupus patients, mast cells abundantly infiltrated mainly the reticular layer of the dermis and around pilosebaceous[32]. Moreover, we used MRL/lpr mice as SLE model to investigate lupus skin lesions. MRL/lpr mice are characterized by dysregulated apoptosis due to homozygous *lpr* mutation in the *Fas* gene. MRL/lpr mice produce autoantibodies including anti-nuclear antibodies and anti-ds DNA antibodies. These mice develop lupus nephritis and lupus dermatitis-like skin lesions with immune complexes deposition. Lupus dermatitis-like skin lesions in MRL/lpr mice exhibit immunoglobulin deposition in the epidermis-dermis boundary similar to human cutaneous lupus erythematosus, infiltration of many mast cells in the dermis[33] and abnormal histamine metabolism[34]. These findings implicate

the involvement of mast cell in the skin lesions of MRL/lpr mice. Hence, we generated MRL/lpr mice deficient in mast cells, MRL/lpr-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, and analyzed autoimmune features and symptoms[35]. MRL/lpr-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice also developed lupus dermatitis-like skin lesions similar to those in control MRL/lpr mice with mast cells. Histologically, the degree of infiltrating inflammatory cells such as T cell, macrophage, and neutrophils were comparable among MRL/lpr-*Kit*$^{W\text{-}sh/\ W\text{-}sh}$ and control MRL/lpr mice. However, skin lesions in MRL/lpr-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice occurred earlier, and their age-matched severity scores were higher than those in control MRL/lpr mice. Moreover, MRL/lpr-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice showed higher mRNA expression of various inflammatory cytokines such as IL-1α, IL-2, IL-4, IL-10, IL-33 and TGF-β in the dorsal skin. Systemically, survival rate, serum anti-ds-DNA antibodies levels and proteinuria in MRL/lpr-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice were lower than those in control MRL/lpr mice. These results indicated that mast cells played some protective roles in LE-like autoimmune symptoms, especially skin lesions and except for survival rate, of MRL/lpr mice.

Nevertheless, van Nieuwenhuijze *et al*. reported the inconsistent results concerning the role of mast cells in SLE[36]. They used lupus C57Bl6(B6)$^{lpr/lpr}$ mice as lupus model, which exhibit lymphoproliferation, production of autoantibodies, and lupus-like nephritis, but their phonotype is somewhat milder than MRL/lpr. B6$^{lpr/lpr}$-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice did not change autoantibody production, proteinuria, the composition of T and B cell or autoimmune pathology, except for minor findings such as enhanced splenomegaly and reduced IL-4 production by CD4^{+} T cell. They concluded that mast cell deficiency did not affect lpr-induced systemic autoimmunity similar to SLE. As SLE patients in human are relatively heterogeneous group, the different results by mice strains may result from genetic background. Anyway, most studies could not indicate the effect on the level of autoantibody. Mast cells involve in local inflammation and tissue damage in the targeted organ rather than the development of autoimmunity including production of autoantibodies.

Mast cells in systemic sclerosis

SSc is another autoimmune connective tissue disease characterized by tissue fibrosis and microangiopathy. SSc affects many internal organs such as the gastrointestinal tract, lungs, kidneys, and heart. Skin symptoms include scleroderma by skin fibrosis, digital ulcer and Raynaud's phenomenon by microanigopathy. These skin symptoms generally appear early in clinical course and are important as diagnostic criteria. Mast cells are closely associated with fibroblasts involved in tissue fibrosis. Moreover, mast cells produce and release mediators such as TGF-β and tryptase, which promote tissue fibrosis. The involvement of mast cells has been investigated in several lung, kidney, and heart diseases with tissue fibrosis. Some studies also investigated the role of mast cells in SSc characterized by tissue fibrosis. In SSc patients, mast cells significantly increase in skin lesions of the forearm and fingers[37], and have also been reported to be one of the main sources of TGF-β[38] (**Table 2**). Tyrosine kinase inhibitor, imatinib mesylate, which inhibits the downstream of TGF-β and PDGF signaling, candidate for the treatment of SSc[39,40]. Imatinib mesylate also inhibits the growth of cells with KIT receptor. Mast cells also express KIT receptor, so imatinib may have partially effect on mast cells in the treatment for SSc. Moreover, some reports described SSc cases associated mastocytosis, which is the proliferative disease in the mast cell compartment[41,42].

In SSc model mice, bleomycin-induced scleroderma model[43] and tight skin mice[44], mast cells increase in the skin, similarly to those in humans. Moreover, chymase inhibitor alleviated skin fibrosis in tight skin mice[45]. However, in the bleomycin-induced scleroderma experiments with mast cell-deficient *Kit*$^{W/W\text{-}V}$ mice[46] and gene-modified Mcpt5Cre/iDTR mice[47] scleroderma was induced regardless of the presence or absence of mast cells. These findings suggest that the involvement of mast cells is limited, and further investigation using different models is necessary.

Conclusion

The involvement of mast cells in collagen diseases, especially SLE and SSc, has been investigated, but evidence is still insufficient and controversial. Taken together, mast cells involve in local inflammation and tissue damage in the targeted organ or local immunosuppression rather than the development of autoimmunity including production of autoantibodies.

Though corticosteroids and immune-suppressive agents are still therapeutic mainstay for various autoimmune diseases, side effects of these agents are occasionally problematic. New insights into pathogenesis of collagen diseases have contributed to new targeted therapy, such as B-cell targeted therapy for SLE or SSc[48,49]. There are no drugs solely targeted mast cells, but some therapeutic agents suppress the function of mast cells. As mentioned above, some pharmacological agents associated mast cells are effective in human and mouse collagen diseases.

Table 2. The involvement of mast cells in SSc.

Species	SSc model	MC depletion	Role of mast cell	Organ	Findings	References
Human	–	–	Not defined	Skin	Dermal MC density in fingers correlated with severity of skin sclerosis.	[37]
			Promotive		Skin MCs are a source of TGF-β.	[38]
Mouse	Bleomycin-induced	–	Not defined	Skin	The number of dermal MCs gradulally increased as the skin sclerotic changes developed.	[46]
		$Kit^{W/W-V}$	No effect	Skin	The MC deficiency did not affect the development of skin fibrosis but the onset of skin fibrosis was delayed in mice without MCs.	[43]
	Tight skin mouse	$Kit^{W/W-V}$	Promotive	Skin	The number of cutaneous MC was correlated with development of fibrosis in old mice.	[44]
		–			Chymase inhibitor attenuated fibrous proliferation.	[45]
	Bleomycin-induced	*Mcpt5Cre/iDtR*	No effect	Skin	The MC deficiency did not affect the development of skin fibrosis.	[47]

Abbreviation: MC: mast cell; SSc: systemic sclerosis; TGF-β: transforming growth factor-β

Through KIT receptors, imatinib may exert some beneficial effects on mast cells for SSc treatment. The effectiveness of chymase inhibitor for SSc model mice is prospective data for human SSc treatment. Moreover, further knowledge of mast cell involvement in collagen disease may lead to new treatment targeting mast cells[50].

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

References

1. Wernersson S, Pejler G. Mast cell secretory granules: Armed for battle. Nat Rev Immunol 2014; 14(7): 478–494. doi: 10.1038/nri3690.
2. Abraham SN, St John AL. Mast cell-orchestrated immunity to pathogens. Nat Rev Immunol 2010; 10(6): 440–452. doi: 10.1038/nri2782.
3. Varricchi G, Galdiero MR, Loffredo S, *et al*. Are mast cells MASTers in cancer? Front Immunol 2017; 8: 424. doi: 10.3389/fimmu.2017.00424.
4. Benoist C, Mathis D. Mast cells in autoimmune disease. Nature 2002; 420(6917): 875–878. doi: 10.1038/nature01324.
5. Sayed BA, Christy A, Quirion MR, *et al*. The master switch: The role of mast cells in autoimmunity and tolerance. Annu Rev Immunol 2008; 26: 705–739. doi: 10.1146/annurev.immunol.26.021607.090320.
6. Yu X, Kasprick A, Petersen F. Revisiting the role of mast cells in autoimmunity. Autoimmun Rev 2015; 14(9): 751–759. doi: 10.1016/j.autrev.2015.04.008.
7. Yu Y, Blokhuis BR, Garssen J, *et al*. Non-IgE mediated mast cell activation. Eur J Pharmacol 2016; 778: 33–43. doi: 10.1016/j.ejphar.2015.07.017.
8. Ali H. Regulation of human mast cell and basophil function by anaphylatoxins C3a and C5a. Immunol Lett 2010; 128(1): 36–45. doi: 10.1016/j.imlet.2009.10.007.
9. Cardamone C, Parente R, Feo GD, *et al*. Mast cells as effector cells of innate immunity and regulators of adaptive immunity. Immunol Lett 2016; 178: 10–14. doi: 10.1016/j.imlet.2016.07.003.
10. Metz M, Maurer M. Mast cells – key effector cells in immune responses. Trends Immunol 2007; 28(5): 234–241. doi:10.1016/j.it.2007.03.003.
11. Bulfone-Paus S, Bahri R. Mast cells as regulators of T cell responses. Front Immunol 2015; 6: 394. doi: 10.3389/fimmu.2015.00394.
12. Nakae S, Suto H, Iikura M, *et al*. Mast cells enhance T cell activation: Importance of mast cell costimulatory molecules and secreted TNF. J Immunol 2006; 176(4): 2238–2248. doi:10.4049/jimmunol.176.4.2238.
13. Gri G, Piconese S, Frossi B, *et al*. $CD4^{+}CD25^{+}$ regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction. Immunity 2008; 29(5): 771–781. doi: 10.1016/j.immuni.2008.08.018.
14. Rodrigues CP, Ferreira AC, Pinho MP, *et al*. Tolerogenic IDO^{+} dendritic cells are induced by PD-1-expressing mast cells. Front Immunol 2016; 7: 9. doi: 10.3389/fimmu.2016.00009.

15. Nigrovic PA, Lee DM. Mast cells in autoantibody responses and arthritis. In: Chadwick DJ, Goode J (editors). Mast cells and basophils: Development, activation and roles in allergic/autoimmune disease: Novartis Foundation Symposium 271. Chichester, UK: John Wiley & Sons, Ltd; 2005. p. 200–209. doi: 10.1002/9780470033449.ch17.
16. Lee DM, Friend DS, Gurish MF, *et al*. Mast cells: A cellular link between autoantibodies and inflammatory arthritis. Science 2002; 297(5587): 1689–1692. doi: 10.1126/science.1073176.
17. Corr M, Crain B. The role of FcγR signaling in the K/B×N serum transfer model of arthritis. J Immunol 2002; 169(11): 6604–6609. doi: 10.4049/jimmunol.169.11.6604.
18. Nigrovic PA, Binstadt BA, Monach PA, *et al*. Mast cells contribute to initiation of autoantibody-mediated arthritis via IL-1. Proc Natl Acad Sci USA 2007; 104(7): 2325–2330. doi: 10.1073/pnas.0610852103.
19. Xu D, Jiang HR, Li Y, *et al*. IL-33 exacerbates autoantibody-induced arthritis. J Immunol 2010; 184(5): 2620–2626. doi: 10.4049/jimmunol.0902685.
20. Rodewald HR, Feyerabend TB. Widespread immunological functions of mast cells: Fact or fiction? Immunity 2012; 37(1): 13–24. doi: 10.1016/j.immuni.2012.07.007.
21. Feyerabend TB, Weiser A, Tietz A, *et al*. Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmunity. Immunity 2011; 35(5): 832–844. doi: 10.1016/j.immuni.2011.09.015.
22. Yokota M, Suzuki K, Tokoyoda K, *et al*. Roles of mast cells in the pathogenesis of inflammatory myopathy. Arthritis Res Ther 2014; 16(2): R72. doi: 10.1186/ar4512.
23. Shrestha S, Wershil B, Sarwark JF, *et al*. Lesional and nonlesional skin from patients with untreated juvenile dermatomyositis displays increased numbers of mast cells and mature plasmacytoid dendritic cells. Arthritis Rheum 2010; 62(9): 2813–2822. doi: 10.1002/art.27529.
24. Konttinen YT, Hietanen J, Virtanen I, *et al*. Mast cell derangement in salivary glands in patients with Sjögren's syndrome. Rheumatol Int 2000; 19(4): 141–147. doi: 10.1007/s002960050118.
25. Blank U, Essig M, Scandiuzzi L, *et al*. Mast cells and inflammatory kidney disease. Immunol Rev 2007; 217: 79–95. doi: 10.1111/j.1600-065X.2007.00503.x.
26. Kaczmarczyk K, Kosalka J, Soja J, *et al*. Renal interstitial mast cell counts differ across classes of proliferative lupus nephritis. Folia Histochem Cytobiol 2014; 52(3): 218–224. doi: 10.5603/FHC.2014.0025.
27. Danilewicz M, Wagrowska-Danilewicz M. Quantitative analysis of interstitial mast cells in lupus and non-lupus membranous glomerulopathy. Pol J Pathol 2001; 52(4): 211–217.
28. Lin L, Gerth AJ, Peng SL. Susceptibility of mast cell-deficient W/Wv mice to pristane-induced experimental lupus nephritis. Immunol Lett 2004; 91(2–3): 93–97. doi: 10.1016/j.imlet.2003.11.014.
29. Madjene LC, Pons M, Danelli L, *et al*. Mast cells in renal inflammation and fibrosis: Lessons learnt from animal studies. Mol Immunol 2015; 63(1): 86–93. doi: 10.1016/j.molimm.2014.03.002.
30. Ravinal RC, Costa RS, Coimbra TM, *et al*. Mast cells, TGF-β1 and myofibroblasts expression in lupus nephritis outcome. Lupus 2005; 14(10): 814–821. doi: 10.1191/0961203305lu2188oa.
31. Charles N, Hardwick D, Daugas E, *et al*. Basophils and the T helper 2 environment can promote the development of lupus nephritis. Nat Med 2010; 16(6): 701–707. doi: 10.1038/nm.2159.
32. Uede M, Nakamura Y, Yamamoto Y, *et al*. No differences in number and distribution of dermal mast cells between acute and chronic cutaneous lupus erythematosus. Autoimmune Dis Ther Approach 2014; 2: 109. doi: 10.14437/2378-6337-1-109.
33. Furukawa F, Tanaka H, Sekita K, *et al*. Dermatopathological studies on skin lesions of MRL mice. Arch Dermatol Res 1984; 276(3): 186–194. doi: 10.1007/BF00414018.
34. Tachibana T, Furukawa F, Taniguchi S, *et al*. Histamine metabolism in skin of MRL/l mice. Arch Dermatol Res 1985; 278(1): 57–60. doi: 10.1007/BF00412497.
35. Inaba Y, Kanazawa N, Yoshimasu T, *et al*. Severer lupus erythematosus-like skin lesions in MRL/lpr mice with homozygous *Kit*$^{wsh/wsh}$ mutation. Mod Rheumatol 2017; 11: 1–8. doi: 10.1080/14397595.2017.1341591.
36. van Nieuwenhuijze AE, Cauwe B, Klatt D, *et al*. Lpr-induced systemic autoimmunity is unaffected by mast cell deficiency. Immunol Cell Biol 2015; 93(10): 841–848. doi: 10.1038/icb.2015.49.
37. Yukawa S, Yamaoka K, Sawamukai N, *et al*. Dermal mast cell density in fingers reflects severity of skin sclerosis in systemic sclerosis. Mod Rheumatol 2013; 23(6): 1151–1157. doi: 10.1007/s10165-012-0813-8.
38. Hügle T, Hogan V, White KE, *et al*. Mast cells are a source of transforming growth factor β in systemic sclerosis. Arthritis Rheum 2011; 63(3): 795–799. doi: 10.1002/art.30190.
39. Fraticelli P, Gabrielli B, Pomponio G, *et al*. Low-dose oral imatinib in the treatment of systemic sclerosis interstitial lung disease unresponsive to cyclophosphamide: A phase II pilot study. Arthritis Res Ther 2014; 16(4): R144. doi: 10.1186/ar4606.
40. Gordon J, Udeh U, Doobay K, *et al*. Imatinib mesylate (Gleevec™) in the treatment of diffuse cutaneous systemic sclerosis: Results of a 24-month open label, extension phase, single-centre trial. Clin Exp Rheumatol 2014; 32(6 Suppl 86): S189–S193.
41. Bagnato G, Roberts WN, Sciortino D, *et al*. Mastocytosis and systemic sclerosis: A clinical

association. Clin Mol Allergy 2016; 14: 13. doi: 10.1186/s12948-016-0050-3.
42. Frigui M, Dupin N, Carlotti A, *et al*. Telangiectasic mastocytosis with systemic sclerosis. Presse Med 2013; 42(5): 902–904. doi: 10.1016/j.lpm.2012.10.014.
43. Yamamoto T, Takagawa S, Katayama I, *et al*. Animal model of sclerotic skin. I: Local injections of bleomycin induce sclerotic skin mimicking scleroderma. J Invest Dermatol 1999; 112(4): 456–462. doi: 10.1046/j.1523-1747.1999.00528.x.
44. Everett ET, Pablos JL, Harley RA, *et al*. The role of mast cells in the development of skin fibrosis in tight-skin mutant mice. Comp Biochem Physiol A Physiol 1995; 110(2): 159–165. doi: 10.1016/0300-9629(94)00127-F.
45. Shiota N, Kakizoe E, Shimoura K, *et al*. Effect of mast cell chymase inhibitor on the development of scleroderma in tight-skin mice. Br J Pharmacol 2005; 145(4): 424–431. doi: 10.1038/sj.bjp.0706209.
46. Yamamoto T, Takahashi Y, Takagawa S, *et al*. Animal model of sclerotic skin. II. Bleomycin induced scleroderma in genetically mast cell deficient WBB6F1-W/W(V) mice. J Rheumatol 1999; 26(12): 2628–2634.
47. Willenborg S, Eckes B, Brinckmann J, *et al*. Genetic ablation of mast cells redefines the role of mast cells in skin wound healing and bleomycin-induced fibrosis. J Invest Dermatol 2014; 134(7): 2005–2015. doi: 10.1038/jid.2014.12.
48. Dörner T, Lipsky PE. Beyond pan-B-cell-directed therapy – New avenues and insights into the pathogenesis of SLE. Nat Rev Rheumatol 2016; 12(11): 645–657. doi: 10.1038/nrrheum.2016.158.
49. Yanaba K. Strategy for treatment of fibrosis in systemic sclerosis: Present and future. J Dermatol 2016; 43(1): 46–55. doi: 10.1111/1346-8138.13026.
50. Singh J, Shah R, Singh D. Targeting mast cells: Uncovering prolific therapeutic role in myriad diseases. Int Immunopharmacol. 2016; 40: 362–384. doi: 10.1016/j.intimp.2016.09.019.

Trends in Immunotherapy (2019) Volume 3 Issue 2, pp.76–78.
doi: 10.24294/ti.v3.i2.121

MINI-REVIEW

Potentials of interleukin-27 (IL-27) as an immunotherapeutic cytokine in cancer therapy

Wei Boon Yap[1*], Shaktypreya Nadarajah[1], Nadiah Shidik[1], Noorjahan Banu Mohammed Alitheen[2]

[1] *Program of Biomedical Sciences, School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia*

[2] *Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM Serdang, Selangor, Darul Ehsan, Malaysia*

ABSTRACT

Cancer immunotherapy using cytokines has been sought as an alternative therapeutic approach for treating cancers. Besides remarkable immunoregulatory properties, interleukin (IL)-27 has recently been shown to possess promising anticancer functions; hence, its potential roles in cancer immunotherapy. Although proven to be effective against cancer cell growth and angiogenesis, given its dual immune-regulating functions (pro-inflammatory and anti-inflammatory), the use of IL-27 as a cancer immunotherapeutic cytokine could possibly be a two-edged sword without meticulous and thorough research. This mini-review mainly discusses the functions and future prospects of IL-27 as an effective anticancer cytokine. Hopefully, it imparts useful insights into the potential applications of IL-27 in cancer immunotherapy

Keywords*: interleukins; IL-27; cytokines; immunotherapy; cancers*

ARTICLE INFO
Received: July 2, 2019
Accepted: August 2, 2019
Available online: August 15, 2019

***CORRESPONDING AUTHOR**
Wei Boon Yap, Program of Biomedical Sciences, School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia; yapweiboon@ukm.edu.my

CITATION
Yap WB, Nadarajah S, Shidik N, *et al*. Potentials of interleukin-27 (IL-27) as an immunotherapeutic cytokine in cancer therapy. Trends Immunother 2019; 3(2): 76–78. doi: 10.24294/ti.v3.i2.121.

Introduction

Given various detrimental side effects of modern anticancer treatments, cytokine immunotherapy has been sought as an alternative therapeutic approach. A combinatorial immunotherapy of interleukin (IL)-12 with a breast cancer-specific monoclonal antibody, trastuzumab and an anticancer drug namely paclitaxel was found to inhibit breast cancer cell growth as high as 52% in the first-phase clinical trial[1]. However, repeated administration of IL-12 did somehow render toxicity to metastatic kidney cancer and breast cancer patients[2]. As a result, to optimize the use of cytokines in cancer immunotherapy, scrupulous research on therapeutic cytokines especially those capable of triggering both pro-inflammatory and anti-inflammatory reactions is undoubtedly entailed. In this mini-review, the anticancer functions of IL-27, with pro- and anti-inflammatory effects, and its future prospects in cancer immunotherapy are highlighted.

Interleukins are more than just immune modulatory cytokines

Interleukins (ILs) have long been recognized for their immune modulatory functions such as regulating local and systemic immunity by controlling immune cell proliferation, differentiation and motility. By doing so, immune homeostasis is achieved between pro- and anti-inflammatory reactions[3]. In the recent decades, the potentials of ILs have been further explored and found effective as immunotherapeutics against various cancers. ILs such as IL-2 has been approved by US Food and Drug Administration (FDA) for use in cancer immunotherapy, for instance in treating metastatic melanoma[4,5]. Besides IL-2, IL-12 was also found to exhibit direct inhibition on cancer cell growth while promoting activation of immune cells with cancer killing functions[2]. The anticancer properties of IL-12 were well observed in various

maglinant models such as B16 melanoma, breast ductal adenocarcinoma, C26 colon carcinoma and metastatic lung carcinoma[6].

Although proven with prominent anticancer functions, IL-12 immunotherapy was, however, reported to render adverse side effects such as hypersplenism in melanoma patients[7] which in turn results in extravascular hemolysis and enlargement of spleen. To overcome the adverse effects, IL-27, which is also a member of IL-12 family, has been merited for its antitumor benefits without causing much adverse toxic effects in cancer models[4]. Of note, IL-27 could be harnessed as an alternative anticancer cytokine in cancer immunotherapy[8].

IL-27 is composed of Epstein-Barr virus-induced gene-3 (EBI-3) and p28 subunits that interact with cell receptors that are made up of WSX-1 and gp130 subunits[9]. IL-27 is often produced by antigen presenting cells and particularly important in T-cell proliferation. IL-27 functions as both pro- and anti-inflammatory cytokine, for instance, it promotes Th1 responses but inhibits proliferation of Th17 that is responsible for many inflammatory reactions[10]. As a result, the use of IL-27 as a therapeutic agent entails scrupulous and rigorous investigation so it does not produce undesired outcomes in recipients. This is particularly crucial in cancer therapy as any agents that generate excessive inflammation would cause drug resistance and metastasis in cancers[10].

Inhibitory functions of IL-27 on cancer cells

To better employ IL-27 as an immunotherapeutic cytokine for cancers, the mechanisms of actions on how IL-27 exerts its anticancer functions have to be unraveled. As an immunoregulatory cytokine, IL-27 signals activation of natural killer (NK) and cytotoxic T cells which possess cancer killing properties[11]. Besides regulating antitumor immune responses, IL-27 has also been proven to execute direct inhibitory functions on cancers. For instance, inhibiting pediatric B-acute lymphoblastic leukemia cell proliferation[12], prohibiting angiogenesis in melanoma B16F10 cell line and blocking metastasis of lung cancer in mouse model[13]. Inhibition of metastasis of lung cancer by IL-27 is mostly attributed to suppression of cyclooxygenase-2 (COX-2)[14] and downregulation of epithelium-mesenchymal transition (EMT) in lung cancer cells[15].

Apart from the aforementioned antitumour activities, cancer cell death *via* apoptosis is often targetted by many antitumour agents. Antitumour agents with apoptotic properties trigger mitochondrial oxidative stress which then results in the release of cytochrome-C, activation of caspase cascade and eventually cell death[16]. Ruiz-Ruiz *et al*.[17] showed that IL-27 was able to trigger apoptosis in breast cancer cells by stimulating interferon (IFN)-γ expression. Activation of IFN-γ subsequently increases the expression of pro-apoptotic protein, caspase-8 in MCF-7 and MDA-MB-231 breast cancer cell lines. Through caspase-8 activity, the intrinsic pathway is activated and cytochrome-c is released and interacts with protease activating factor-1 (Apaf-1) which in turn promotes caspase-9 and caspase-3 activation and apoptosis (**Figure 1**). Collectively, the immunoregulatory, direct inhibitory and apoptosis-promoting functions of IL-27 add up to its potentials as an immunotherapeutic agent.

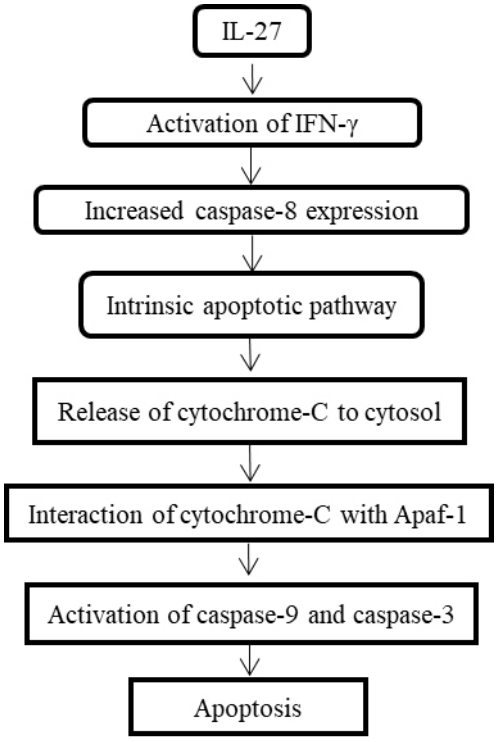

Figure 1. Activation of apoptotic protein cascade by IL-27. Through activation of IFN-γ, IL-27 is able to induce apoptosis in breast cancer cell lines.

Future prospects of IL-27 in cancer immunotherapy

IL-27 is unequivocally a prominent immunoregulatory cytokine that promotes both pro-inflammatory and anti-inflammatory responses. In order to avoid treatment outcomes that could

encourage metastasis and drug-resistance in cancers which in turn undermine an anticancer therapy regime[10], any claims on the potential use of IL-27 as an immunotherapeutic cytokine should be preceded by meticulous and rigorous experimentations. Anticancer functions of IL-27 can be further explored by (a) detailed investigation on the ability of IL-27 in promoting NK cells and tumor-specific T cell proliferation *ex vivo* and how these activated cells target tumors in adoptive cell therapy; (b) expanding the IL-27-induced apoptosis networks in cancer models. With great breadth of understanding on how IL-27 effects anticancer functions in cancer cells, this ensures more precise use of IL-27 as an immunotherapeutic agent without causing much damaging side effects to cancer patients. As a conclusion, IL-27 does hold prominent potentials in cancer immunotherapy. Rigorous empirical evidence would be able to help further unveil its importance in cancer immunotherapy.

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgments

The authors thank the Ministry of Higher Education, Malaysia for funding the work under the Fundamental Research Grant Scheme (FRGS/1/2014/SG05/UKM/02/4).

References

1. Bekaii-Saab TS, Roda JM, Guenterberg KD, *et al*. A phase I trial of paclitaxel and trastuzumab in combination with interleukin-12 in patients with HER2/neu-expressing malignancies. Mol Cancer Ther 2009; 8(11): 2983–2991. doi: 10.1158/1535-7163.MCT-09-0820.
2. Tugues S, Burkhard SH, Ohs I, *et al*. New insights into IL-12-mediated tumor suppression. Cell Death Differ 2015; 22: 237–246. doi: 10.1038/cdd.2014.134.
3. Zhang JM, An JX. Cytokines, inflammation and pain. Int Anesthesiol Clin 2007; 45(2): 27–37. doi: 10.1097/AIA.0b013e318034194e.
4. Sorensen EW, Gerber SA, Frelinger JG, *et al*. IL-12 suppresses vascular endothelial growth factor receptor 3 expression on tumor vessels by two distinct IFN-γ-dependent mechanisms. J Immunol 2010; 184(4): 1858–1866. doi: 10.4049/jimmunol.0903210.
5. Lee S, Margolin K. Cytokines in cancer immunotherapy. Cancers 2011; 3: 3856–3893. doi: 10.3390/cancers3043856.
6. Shi X, Liu J, Xiang Z, *et al*. Gene expression analysis in interleukin-12-induced suppression of mouse mammary carcinoma. Int J Cancer 2004; 110(4): 570–578. doi: 10.1002/ijc.20145.
7. Gollob JA, Mier J, Veenstra K, *et al*. Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: Ability to maintain IFN-γ induction is associated with clinical response. Clinic Cancer Res 2000; 6: 1678–1692.
8. Yoshimoto T, Chiba Y, Furusawa JI, *et al*. Potential clinical application of interleukin-27 as an antitumor agent. Cancer Sci 2015; 106(9): 1103–1110. doi: 10.1111/cas.12731.
9. Pflanz S, Timans JC, Cheung J, *et al*. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive $CD4^+$ T cells. Immunity 2002; 16(6):779–790.
10. Li MS, Liu ZZ, Liu JQ, *et al*. The Yin and Yang aspects of IL-27 in induction of cancer-specific T cell responses and immunotherapy. Immunotherapy 2015; 7(2): 191–200. doi: 10.2217/imt.14.95.
11. Hisada M, Kamiya S, Fujita K, *et al*. Potent antitumor activity of interleukin-27. Cancer Res 2004; 64: 1152–1156. doi: 10.1158/0008-5472.CAN-03-2084.
12. Canale S, Cocco C, Frasson C, *et al*. Interleukin-27 inhibits pediatric B-acute lymphoblastic leukemia cell spreading in a preclinical model. Leukemia 2011; 25(12): 1815–1824. doi: 10.1038/leu.2011.158.
13. Shimizu M, Shimamura M, Owaki T, *et al*. Antiangiogenic and antitumor activities of IL-27. J Immunol 2006; 176(12): 7317–7324. doi: 10.4049/jimmunol.176.12.7317.
14. Ho MY, Leu SJ, Sun GH, *et al*. IL-27 directly restrains lung tumorigenicity by suppressing cyclooxygenase-2-mediated activities. J Immunol 2009; 183(10): 6217–6226. doi: 10.4049/jimmunol.0901272.
15. Airoldi I, Tupone MG, Esposito S, *et al*. Interleukin-27 re-educates intratumoral myeloid cells and down-regulates stemness genes in non-small cell lung cancer. Oncotarget 2015; 6(6): 3694–3708. doi: 10.18632/oncotarget.2797.
16. Liou GY, Storz P. Reactive oxygen species in cancer. Free Radic Res 2010; 44(5): 479–496. doi: 10.3109/10715761003667554.
17. Ruiz-Ruiz C, Munõz-Pinedo C, López-Rivas A. Interferon-γ treatment elevates caspase-8 expression and sensitizes human breast tumor cells to a death receptor-induced mitochondria-operated apoptotic program. Cancer Res 2000; 60(20): 5673–5680.

Trends in Immunotherapy (2019) Volume 3 Issue 2, pp.79–88.
doi: 10.24294/ti.v3.i2.95

REVIEW ARTICLE

Role of targeting nanoparticles for cancer immunotherapy and imaging

***Ru Wen*[1*], *Afoma C. Umeano*[2*]**

[1] *Department of Chemistry, University of Georgia, Athens, GA, United States*
[2] *Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL, United States*

ABSTRACT

Cancer immunotherapy involves the delivery of immunogenic compounds and/or the priming, or induction, of the body's natural immune system to target cancer. The use of cancer immunotherapy has led to various means of cancer prevention and treatment that have produced prolonged life expectancy and stabilized disease. Nanoparticles are promising vehicles or adjuvants for effective delivery of therapeutics, antigens, stimulatory effectors, or antibodies for therapeutic invention. Targeting nanoparticles are especially useful due to their capability of accumulating in specific sites of interest like tumors and, thereby, decreasing risks of damage to normal tissue. Targeting can be achieved by incorporation of cell-surface related binding molecules or antibodies. This review explores the role of targeting nanoparticles as delivery or adjuvant systems to modulate immune response, and as imaging tracking systems for cancer immunotherapy.

***Keywords**: nanoparticles; cancer immunotherapy; imaging; targeting*

ARTICLE INFO
Received: July 11, 2019
Accepted: August 7, 2019
Available online: August 18, 2019

***CORRESPONDING AUTHORS**
Ru Wen, Department of Chemistry, University of Georgia, Athens, GA 30602, United States; soso2012@uga.edu

Afoma C. Umeano, Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL 33136; acu14@med.miami.edu

CITATION
Wen R, Umeano AC. Role of targeting nanoparticles for cancer immunotherapy and imaging. Trends Immunother 2019; 3(2): 79–88. doi: 10.24294/ti.v3.i2.95.

Introduction

Surgery, radiation, chemotherapy and their combinations are well established standards for treating cancer, one of the most life-threating diseases worldwide[1]. Metastatic spreading of cancer cells, however, inspires the development of new strategies towards eradicating cancer cells from normal tissue. Cancer immunotherapy uses the body's natural immune systems as a defense for fighting existing cancer or preventing potential cancer(s). Various uses of cancer immunotherapy have provided promising strategies towards achieving long-lasting and effective cancer therapy.

The body is equipped with a natural defense against cancers, viruses, and bacterial pathogens. The primary immune system responsible for cancer immunotherapy is the adaptive immune system. The adaptive immune system is comprised of two major responses: (1) humoral-mediated response and (2) cell-mediated response. Both processes occur when an antigen of interest is phagocytosed by an antigen presenting cell (APC). APCs process phagocytosed antigens and present them on their cellular surface using major histocompatibility complex (MHC) class II. Once a presented antigen is recognized through binding a specific T-cell population, one of the two processes occurs. The process of humoral-mediated or antibody-mediated response, results in the mass production of antibodies by B-cells, activated by T-cells, which bind and target pathogenic bacteria for phagocytosis by macrophages and neutrophils and is often not the employed or desired response for cancer immunotherapy. Cell-mediated immune response occurs through cytokine activation of T-cells, which target and destroy affected/infected cells that present dysregulated cellular or viral proteins through MHC class I receptors. The overview of both processes is outlined in **Figure 1**. Cancer cells mask themselves from the body's natural immune defense in various ways. However, recent technologies have been developed to re-prime the body's existing cell-mediated immune response to destroy these cancer cells.

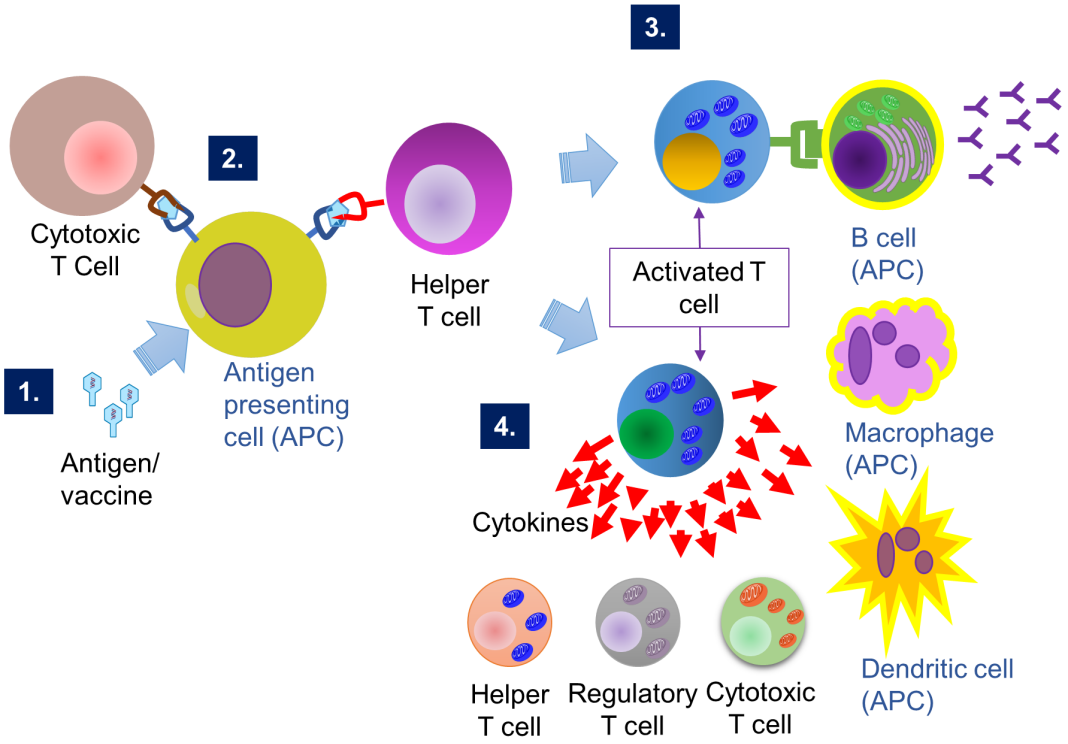

Figure 1. Humoral- and cell-mediated immune response to antigen stimulation. (1) Antigen is phagocytosed by antigen presenting cells (APCs), such as dendritic cells, macrophages/monocytes and B cells. (2) APCs present processed antigen via major histocompatibility complex (MHC) class II receptors to T cells. (3) Helper T-cells stimulate B-cell mediated antibody production in humoral immune response. (4) Cytotoxic and helper T-cells promote cytokine activation of effector cell populations for cell-mediated immune response, or MHC class I-mediated destruction of affected cells.

Several clinical trials have demonstrated the success of cancer immunotherapy, such as immune checkpoint therapy using blocking antibodies to cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1), and chimeric antigen receptor (CAR) therapy, for priming the immune system[2]. Human papillomavirus (HPV) and hepatitis B vaccine have been available for routine vaccination and have improved overall cancer prevention[3–5]. Initial clinical trials on the combination of immunotherapy and radiation in various tumor sites show better localization control, decreased metastatic spreading, and improved overall survival rate[6].

Through the incorporation of nanotechnology, nanoparticles are used as vehicles or adjuvants to effectively deliver imaging agents, therapeutics, antigens, stimulatory effectors, or antibodies to sites of tumor for immunotherapy. Various nanoparticles have been constructed as delivery systems, such as liposomes, quantum dots (QDs), nano-dendrimers, nano-micelles, nano-hydrogels, polymer-based, carbon-based, and inorganic metal or metal oxide based nanoparticles. Introducing immunotherapies with targeted treatments at the molecular level is of particular interest and can be a promising strategy for effective cancer therapy. This review discusses the targeted nanoparticle-based intervention for immune modulation as carrier or adjuvant system as well as its application in immune system tracking as imaging agents or delivery systems.

Targeting nanoparticles for immunotherapy

Rapid progress has been made in the application of nanotechnology to various diseases, either as drug or imaging agent delivery system, or as adjuvant therapeutic agents. Targeting control is essential to reduce side effects associated with off-target distribution and enhance therapeutic intervention when vaccine is administrated in clinical settings. The exclusive delivery to specific cell types or tissues by nanotechnology is of great interest and promising for effective therapeutic intervention. The properties of nanoparticles can be modified to control their distribution behavior by various ways, wherein a specific targeting ligand can be attached *via* surface modification, conjugating or as part of the nanoparticle components. Nanoparticle delivery system can be constructed by various formulations,

such as liposome[7], polymers[8], micelle[9], or lipid-polymer hybrid[10]. The targeting of the adaptive immune system plays a significant role in effective vaccine or immunotherapy, in which antigen is largely or exclusively enriched in the specific sites inducing immunomodulatory effectors in lymphoid environment[11]. Possible cellular targets for nanoparticles are illustrated in **Figure 2**.

APCs consist of professional APCs such as dendritic cells (DCs) expressing antigens and MHC class II molecules, and non-professional APCs expressing MHC class I molecules such as fibroblast and endothelial cells[12–14]. APCs can present antigens to T cells for immune response through binding with MHC class I molecules for T cells expressing CD8 cells, and MHC class II molecules for CD4 cells[15]. The APCs mainly include DCs, macrophages and B cells. DCs are mostly studied as targets for immunotherapy[16], other types of APCs such as astrocytes may be used as targets for brain tumor therapy. Therefore, APCs are possible targets to nanoparticle-based delivery system for stimulating efficient immune response for cancer therapy.

Dendritic cells

DC is one of the most potent APCs for initiating and modulating immune response. DCs have been used as targets for antigen-loaded nanoparticle system in most studies. Size control is a key regulator in targeting nanoparticle system for DCs. An *in vivo* mice model indicates that large (1000–2000 nm) polystyrene particles were predominantly detected

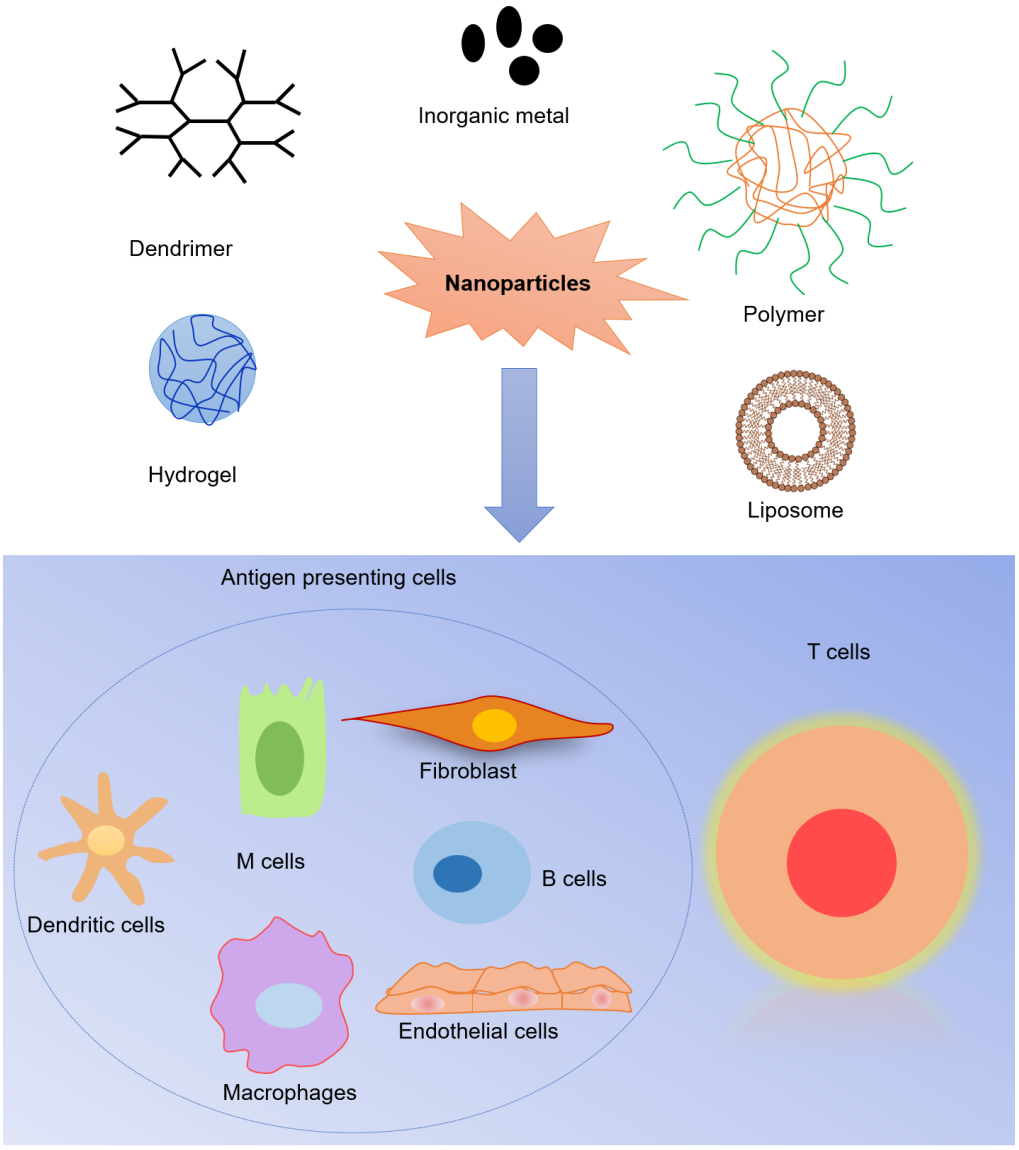

Figure 2. Potential cell level targets for nanoparticle-based immunotherapy

in DC and small (20–200 nm) nanoparticles were mainly located in lymph-nodes resident DCs[17]. Poly(ethylene glycol)-stabilized poly(propylene sulfide) (PPS) nanoparticles are reported to reside in ~50% of DCs in the lymph nodes without a targeting ligand. The small size range of 20–45 nm PPS nanoparticles was suitable for both uptake and retention in lymph node, and 100-nm PPS nanoparticles were not found in lymphatic site[18]. Poly(γ-glutamic acid) (PGA) nanoparticles demonstrate effective internalization in DCs when injected in mice and induced the generation of interleukin (IL)-12p40 and IL-6. The ovalbumin (OVA)-loaded PGA nanoparticles showed significant levels of interferon (IFN)-γ, antigen specific immunoglobulin G (IgG), IgG1, and IgG2a antibodies and more potent cytotoxic T lymphocyte (CTL) response than free OVA in a 7-day immunization study. The incorporation of listeriolysin O (LLO) antigen in PGA nanoparticles significantly enhanced the survival rate of mice in a fourteen-day immunization for lethal *Listeria monocytogenes* prevention[19].

It is possible to effectively target antigen-nanoparticles to DC *via* cell-surface molecules to enhance strong immune potency for cancer therapy. A targeting ligand such as an antibody is commonly used to achieve the goal of nanoparticles targeting. A humanized targeting antibody hD1 is reported to target DCs *via* interaction with human C-type lectin receptor DC-SIGN. Poly(lactic-co-glycolic acid)-based nanoparticles (PLGA-NPs) could be effectively delivered to DCs when incorporating hD1 as targeting moiety[20]. The DEC-205 antibody was used as DC targeting ligand as well for nanoparticles 200–250 nm in diameter. DEC-205-OVA PLGA-NPs showed similar level of IFN-γ and IL-2 production, but significantly more of IL-5 secretion than non-targeted nanoparticles, and a production of IL-10 cytokine *in vitro*. *In vivo* model confirmed that DEC-205-OVA CFA nanoparticles could induce T cell production of IL-10 and IL-5, with more cytokine generated upon greater density of anti-DEC-205[21]. The PLGA-NPs could also be selectively internalized to DCs when using α-CD40-mAb targeting moiety. The multi-compound loaded PLGA-NPs composed of a protein antigen (*e.g.* OVA), Pam3CSK4 and Poly(I:C) and α-CD40-mAb showed highly efficient delivery to DC in the *in vivo* model when injected in mice, stimulated significantly boosted $CD8^+$ T cell responses, and prolonged the survival rate in vaccinated mice compared to non-treated and non-targeted nanoparticle control[22]. A comparative study of targeting various DC cell-surface molecules indicated that CD40, DEC-205 or CD11c targeted nanoparticle showed similar capability to induce $CD8^+$ T cell immune response[23].

Active targeting of antigens in DCs by nanoparticles can also be achieved by other targeting ligands, such as mannose or lactose residues, *via* endocytic receptor interaction to elicit robust vaccines[24]. Mannan (MN)-decorated PLGA-NPs showed an increased cellular uptake in DCs by both flow cytometry and confocal microscope[25]. Fc fragment of human IgG is used as an efficient targeting system *via* Fc receptor interaction for gold nanoparticles (AuNPs) and liposomes for superior intracellular uptake and antigen presentation in DCs to that of free peptide control[26]. Surface-functionalized mannose polyanhydride nanoparticles are reported to effectively activate DCs through mannose receptor. The dimannose-modified nanoparticles demonstrated the improved MHC II, CD86 and CD40 antigen presentation, increased CD206 and *CIRE* expression, higher level of pro-inflammatory IL-6 and TNF-α compared to non-modified nanoparticles[24]. Asparagine-glycine-arginine (NGR) cell-specific peptide is reported as a targeting ligand to target PEGylated polyplex to DC *via* enhanced phagocytosis. The NGR peptide-PLGA-PEG-PLGA nanoparticles showed non-toxicity to DCs at an optimal concentration of 0.25% of copolymer[27].

Subcellular compartments are potential targets for nanoparticles to improve antigen presentation and potentially induce robust immune responses[28]. The pH-sensitive nanoparticles have been developed to directly disrupt endosomes and/or lysosomes in response to acid environment to release antigens into cytosol. Nanoparticles composed of hydrophobic pH-responsive tertiary amines core and hydrophilic shell 2-aminoethyl methacrylate (AEMA) cross-linked by poly(ethylene glycol) methacrylate (PEGDMA) showed effective delivery of OVA antigen to cytosol of DCs, and 4-fold increasing of IFN-γ production from T cells than pH-insensitive and free OVA controls[29]. Cationic acid-degradable nanoparticles are reported to direct improved OVA antigens presentation *via* MHC class I pathway in response to acidic lysosomes. The cationic nature elicited nanoparticles to be endocytosed or phagocytosed into bone marrow-derived DCs (BMDCs) at the initial stage of cellular uptake. The CpG-oligodeoxynucleotides (ODN)-coated acid-degradable nanoparticles produced 10 more fold of IL-12 than free CpG ODN, or nanoparticles without CpG-ODN in BMDCs[30].

Endoplasmic reticulum (ER) is an alternative subcellular target for immunotherapy. ER-targeting

can be achieved by a peptide or sequence containing ER targeting moieties like KKXX signal. ER-targeting peptide is reported to intracellular traffic PLGA-NPs to ER. This targeting peptide-conjugated nanoparticles induced low efficiency of SIINFEKL cross-presentation at initial time (2 h) and prolonged cross-presentation of SIINFEKL up to 48 h *in vitro*[31]. Intensive *in vitro* and *in vivo* studies of this nanoparticle system are not yet reported. Gelatin nanoparticles are reported to target tetramethylrhodamine-dextran (TMR-dextran) to lysosomes of DCs[32]. Cationic gelation nanoparticles loaded with CpG ODN strongly inducted stimulatory effectors for immune response within DCs both *in vitro* and *in vivo*[33]. Efficient gene targeting can be realized from endosomal escape by using a nanoparticle system for cancer immunotherapy. Octa-arginine (R8)-modified lipid nanoparticles are reported to deliver short interfering RNA (siRNA) to DCs for gene knockdown. R8 modification facilitates cellular uptake of nanoparticles *via* macropinocytosis to avoid lysosomal degradation. The siRNA-loaded R8-modified nanoparticles efficiently reduced the endogenous *SOCS1* gene expression and showed enhanced *in vivo* vaccine potential with suppressed tumor growth after immunization compared to the control BMDCs[34].

Macrophages

Macrophages are possible targets in stimulating immune response and/or tolerance as well for nanoparticle-based cancer vaccine and/or therapy. Macrophages express various surface molecules such as Fc receptors, Toll-like receptors (TLRs), integrin, glycosylphosphatidylinositol (GPI)-anchored, mannosyl, and galactosyl receptors[35,36]. One approach is to incorporate targeting ligands to nanoparticles for efficient cellular uptake *via* binding with macrophage surface receptors. Similar to DCs, polyanhydride nanoparticles were also used for macrophage targeting when conjugated with dimannose and galactose *via* C-type lectin receptors. The dimannose functionalized nanoparticles induced significantly higher levels of macrophage mannose receptor, while galactose-modified nanoparticles expressed more macrophage galactose lectin (MGL). The functionalized nanoparticles significantly enhanced the expression of CD40 and cytokine production of cytokines IL-1β, IL-6 and TNF-α by alveolar macrophages[37]. Liposomes are mostly used for various drug delivery to macrophage, ligands such as antibody L-Ab is able to modify liposomes for delivery of various materials such as dideoxycytidine triphosphate (ddCTP) to human macrophages *via* Fc receptor mediated pathway[38]. These systems may be used as antigen carriers or adjuvants for macrophage-related tumor vaccine.

Microfold cells

Microfold cells (M cells) are a type of epithelial non-APCs lacking rigid cytoskeleton and presenting broad membrane microdomains on apical surfaces. The antigen-loaded system firstly undergoes endocytic or phagocytic uptake by M cells and antigens are rapidly directed to the intraepithelial pocket containing immune cells like B and T lymphocytes *via* transcytosis for immune responses[39]. The density of M cells is usually very low in the gastrointestinal tract, hence, specific targeting of M cells by nanoparticle delivery system is attractive for robust immune responses. Fievez *et al*. reported arginine-glycine-aspartate (RGD)-grafted PLGA-PEG nanoparticles (RGD-NPs) as oral vaccine system for M-cell targeting through selectively interacting with integrin. OVA-loaded RGD-NPs elicited immune response with significantly higher level of IgG antibodies and IFN-γ *in vivo* intragastric administration[40]. Surface receptors related to M cell endocytosis or phagocytosis can be used as targets for antigen-nanoparticle system. Claudin 4 is such a receptor that highly expressed in M cells. C-terminal targeting peptide, CPE30 is reported as targeting ligand to bind Claudin 4 in PLGA-NPs system. HA-HT-CPE30 nanoparticles showed effective uptake by M cells both *in vitro* and *in vivo* as targeted mucosal vaccines[41].

T lymphocytes

T lymphocytes possess various antigen-specific receptors, such as Ti and T3[42], which can be served as potential targets for antigen-nanoparticles to elicit T-cell immune responses. The binding of CTLA-4 with CD80 or CD86 to inhibits the T-cell activation and blockage of CTLA-4 can be used to enhance T-cell activation and memory[43]. The combination of CTLA-4 with other vaccines such as GM-CSF and PD-1 can significantly suppress the tumor progress *via* modulating autoimmune[44,45]. Tumor associated antigens (TAAs) can be directly carried to T cells for promoting immune secretory activation. PLGA-NPs are reported to load tyrosinase-related protein 2 (TRP2) as TAA and 7-acyl lipid A as TLR4 to induce robust $CD8^+$ T cell-activated anti-tumor immunity *in vivo*[46]. Cui *et al*. reported human immunodeficiency virus type 1 (HIV-1) vaccine by delivering Tat (1–72) antigen in a nanoparticle system to promote Tat-specific T cell proliferation for robust immune response *via* generating antibodies and cytokines[47].

Imaging role of targeted nanoparticles

Imaging tracking of immune cells is important in understanding the latent mechanisms of immune cell-based therapy in clinical practice. Nanoparticle system is a possible strategy for noninvasively monitoring immune cell trafficking in a longitudinal fashion. Nanoparticles can be used as delivery systems incorporating imaging agents or as agents themselves for cell/tissue monitoring.

Fluorescent-based labeling

The most commonly used agent in cell tracking is fluorescent or bioluminescent-based probe, in which reporter genes expressing fluorescent protein, fluorescent or bioluminescent probe are used for labeling. Fluorescent nanoparticles for immune cells imaging can be made with fluorochromes or gene expressing fluorescent protein loading in polymeric system, inorganic metal oxide such as silica nanoparticle and liposomes. The reporter gene is superior to fluorescent-based probe as it lasts as long as a cell lives by continuously expressing optimal-based protein for imaging. However, the fluorescent- or bioluminescent-based methods have low tissue penetration. Yu *et al*. reported TopFluor-labeled cholesterol as lipid component for constructing virus-like nanoparticles which were surface modified by CD169, which was used as nanoparticles tracking for uptake behavior in DCs[48]. Near-infrared (NIR) fluorochromes (700–1000 nm) are widely used in imaging system as it penetrates deep tissues[49].

In addition, the nanoparticles alone can also be used as fluorescent probes, such as QDs, carbon dots (CDs) and upconversion nanoparticles (UCNPs), which are good alternatives to fluorescent dyes due to high resistance to photobleaching, deep penetration, high physicochemical and photochemical stability[50–52]. OVA-loaded UCNPs (OVA-UCNPs) are used to stimulate DCs for immune secretory as well as *in vitro* and *in vivo* precise nanoparticle tracking[53]. UCNPs can conduct photodynamic therapy (PDT) as well as be used as imaging agents in cancer immunotherapy. The unique NIR-to-visible upconversion process can be used to activate photosensitive therapeutic agents such as ZnPc[54] for TAAs production to stimulate immune secretory in cancer vaccine. A recent study incorporated a photosensitizer Ce6 and TLR-7 agonist R837 into UCNPs (Ce6-R837-UCNPs). The constructed Ce6-R837-UCNPs were able to penetrate to deep tumor tissue under NIR photoirradiation and caused TAAs generation promoting strong immune response *in vivo*. The UCNPs showed good NPs labeling efficiency *in vitro* and this may also be detected *in vivo* before conducing PDT treatment[55].

Magnetic resonance imaging contrast agent-based labeling

Magnetic resonance imaging (MRI) technique utilizes magnetic field to visualize organs and structures of body system[56]. Inorganic paramagnetic metal or metal oxide is used for MRI, such as iron oxide and gadolinium-based nanoparticles[57,58]. The contrast agents-loaded nanoparticle system effectively enhances the cellular uptake by modulating the size and surface modification with targeting ligand in cancer immunotherapy. Fe_3O_4-ZnO core-shell nanoparticles (Fe_3O_4-ZnO-NPs) were reported to play dual roles in cancer immunotherapy by delivering carcinoembryonic antigen into DCs and simultaneously being used as an MRI agent. The Fe_3O_4-ZnO-NPs were effectively internalized into DCs, which were used for immunization *in vivo* and showed better tumor growth inhibition and prolonged survival than controls[59]. Besides, molecule probe that contains ^{19}F such as perfluorocarbon or induces chemical exchange saturation transfer can be possible MRI contrast agent in biomedical imaging[56]. PLGA-perfluorocarbon nanoparticle is reported to distinct plasmacytoid and myeloid DCs by ^{19}F MRI in clinical settings[60].

Computed tomography contrast agent-based labeling

Computed tomography (CT) is X-ray based imaging technique widely used in biomedical field[61]. CT contrast agents are used for tracking behavior to evaluate the recruitment of immune cells and monitor the cellular immunotherapies. Nanoparticle is a possible system to deliver CT contrast agents for imaging since attributed to long retention time and fine-tuned size control. Many nanoparticle carriers are used in medical imaging, including liposomes, micelles, lipoproteins, polymeric nanoparticles, inorganic metal nanoparticles, silica and carbon nanotubes[62], these nanoparticles can be applied as imaging vesicles for immune cells as well. A cell-specific targeting ligand would facilitate the localization of nanoparticles in immune cells of interested. Inorganic metal nanoparticles are potential CT contrast agents, which includes AuNPs, iodine-based and bismuth-based nanoparticles[63]. AuNPs of 20 nm in diameter are reported as CT agents to *in vivo* image cancer-specific T cells. The Au labeling was not toxic and did not affect T cell function at a concentration less than 0.75 mg/mL *in vitro*. The targeted T-cells labeled with AuNPs for CT showed increased accumulation of T-cells in tumor

sites than non-targeted control when intravenously administrated *in vivo*, and biodistribution investigation indicated that targeted AuNPs labeled T-cells were mainly migrated to lung and spleen, and these AuNPs were secreted out of body system after two weeks. In a comparison study of CT imaging to fluorescence imaging, T cells dual-labeled with AuNPs and reporter gene overexpressing green fluorescent protein (GFP) demonstrated similar trends of T cells distribution and movement *in vivo.* The incorporated AuNPs also acted as anti-tumor agents in this system, in that targeted T-cells with AuNPs-labeling significantly inhibited tumor progression compared to nontargeted or non-treated controls or AuNPs alone[64].

Other types of labeling

A bimodal-labeled nanoparticle system provides more accurate and quantitative information for cellular tracking compared to single-labeled controls. A dual mode imaging system composed of gadolinium as MRI and aza-BODIPY as NIR was reported to label DCs for *in vivo* tracking[65]. A nanoparticle delivery system can be used to incorporate MRI and NIR agents to enhance the labeling efficiency at low concentrations and to reduce cytotoxicity in DCs. Pittet *et al*. reported the construction of magnetofluorescent-nanoparticle for T-cell labeling. The nanoparticles were composed of MRI contrast agent iron oxide, NIR fluorochromes such as VT680, AF680 or Cy5.5, and HIV-Tat peptide or protamine as clinically viable transfecting agent for effective cellular uptake and displayed membrane-translocating properties[66]. The FePt nanoparticles are reported to play dual roles as contrast agents for both MRI and CT scan[67]. Radionuclide (*e.g.* iodine) labeled AuNPs were quantitatively tracked in dendritic cells by positron emission tomography and Cerenkov luminescence[68,69].

Conclusion

Combining targeted nanoparticles with other conventional approaches such as radiation and surgery is a promising strategy to enhance the immune-based therapeutic or preventing intervention of cancer. Targeting nanoparticle is emerging as a promising tool for immune cell-mediated cancer therapy. The targeting function of nanoparticles can be achieved by tagging a targeting ligand such as receptor-related molecules. Various targeting cells in the immune system have been identified for nanoparticle systems to directly stimulate immune response or suppress the tumor-related microenvironment. The immune responses of targeted nanoparticles for inhibiting tumor growth are well studied both *in vitro* and *in vivo*, but pathways of their cellular uptake, factors influenced the uptake and antigen release, and degradation and secretion pathways of nanoparticles are still in need of further investigation.

Non-invasive imaging by targeted nanoparticles is a potential strategy for understanding these pathways/mechanisms involved in the development of immune therapies. Nanoparticle can be used as carrier systems for fluorescent/bioluminescent probes and contrast agents for MRI and CT, and/or as imaging agents themselves, for imaging immune cells during cancer treatment. However, real-time *in situ* tracking of immune system is still in infancy and much more intensive studies, preclinical and clinical trials are necessary before translating targeted nanoparticles into commercial markets. Outside of cancer treatment, nanoparticles may be used for targeted immunotherapy and imaging of other diseases as well and further studies should be dedicated to exploring this field.

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of their article.

References

1. Schuster M, Nechansky A, Kircheis R. Cancer immunotherapy. Biotechnol J 2006; 1(2): 138–147. doi: 10.1002/biot.200500044.
2. Couzin-Frankel J. Cancer immunotherapy. Science 2013; 342(6165): 1432–1433. doi: 10.1126/science.342.6165.1432.
3. Stokley S, Jeyarajah J, Yankey D, *et al.* Human papillomavirus vaccination coverage among adolescents, 2007–2013, and postlicensure vaccine safety monitoring, 2006–2014 – United States. MMWR Morb Mortal Wkly Rep 2014; 63(29): 620–624.
4. Liang X, Bi S, Yang W, *et al.* Reprint of: Epidemiological serosurvey of Hepatitis B in China—Declining HBV prevalence due to hepatitis B vaccination. Vaccine 2013; 31(Supplement 9): J21–J28. doi: 10.1016/j.vaccine.2013.08.012.
5. Williams WW, Lu P-J, O'Halloran A, *et al.* Vaccination coverage among adults, excluding influenza vaccination – United States, 2013. MMWR Morb Mortal Wkly Rep 2015; 64(4): 95–102.
6. Crittenden M, Kohrt H, Levy R, *et al.* Current clinical trials testing combinations of immunotherapy and radiation. Semin Radiat Oncol 2015; 25(1): 54–64. doi: 10.1016/j.semradonc.2014.07.003.

7. Schwendener RA. Liposomes as vaccine delivery systems: A review of the recent advances. Ther Adv Vaccines 2014; 2(6): 159–182. doi: 10.1177/2051013614541440.
8. Chen H, Ruckenstein E. Micellar structures in nanoparticle-multiblock copolymer complexes. Langmuir 2014; 30(13): 3723–3728. doi: 10.1021/la500450b.
9. Chen H, Ruckenstein E. Formation and degradation of multicomponent multicore micelles: Insights from dissipative particle dynamics simulations. Langmuir 2013; 29(18): 5428–5434. doi: 10.1021/la400033s.
10. Fan Y, Sahdev P, Ochyl LJ, *et al*. Cationic liposome–hyaluronic acid hybrid nanoparticles for intranasal vaccination with subunit antigens. J Control Release 2015; 208: 121–129. doi: 10.1016/j.jconrel.2015.04.010.
11. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 2012; 12(4): 237–251. doi: 10.1038/nrc3237.
12. Pichler WJ, Wyss-Coray T. T cells as antigen-presenting cells. Immunol Today 1994; 15(7): 312–315. doi: 10.1016/0167-5699(94)90078-7.
13. Cruse JM, Lewis RE, Wang H. Antigen presentation. In: Immunology guidebook. San Diego: Academic Press; 2004. p. 267–276.
14. Flaherty DK. Antigen-presenting cells. In: Immunology for pharmacy. Saint Louis: Mosby; 2012. p. 37–44. doi: 10.1016/B978-0-323-06947-2.10005-7.
15. Sprent J. Antigen-presenting cells: Professionals and amateurs. Curr Biol 1995; 5(10): 1095–1097. doi: 10.1016/S0960-9822(95)00219-3.
16. Figdor CG, de Vries IJM, Lesterhuis WJ, *et al.* Dendritic cell immunotherapy: Mapping the way. Nat Med 2004; 10(5): 475–480. doi: 10.1038/nm1039.
17. Manolova V, Flace A, Bauer M, *et al.* Nanoparticles target distinct dendritic cell populations according to their size. Eur J Immunol 2008; 38(5): 1404–1413. doi: 10.1002/eji.200737984.
18. Reddy ST, Rehor A, Schmoekel HG, *et al. In vivo* targeting of dendritic cells in lymph nodes with poly(propylene sulfide) nanoparticles. J Control Release 2006; 112(1): 26–34. doi: 10.1016/j.jconrel.2006.01.006.
19. Uto T, Wang X, Sato K, *et al.* Targeting of antigen to dendritic cells with poly(γ-glutamic acid) nanoparticles induces antigen-specific humoral and cellular immunity. J Immunol 2007; 178(5): 2979–2986. doi: 10.4049/jimmunol.178.5.2979.
20. Cruz LJ, Tacken PJ, Fokkink R, *et al.* Targeted PLGA nano- but not microparticles specifically deliver antigen to human dendritic cells *via* DC-SIGN *in vitro*. J Control Release 2010; 144(2): 118–126. doi: 10.1016/j.jconrel.2010.02.013.
21. Bandyopadhyay A, Fine RL, Demento S, *et al.* The impact of nanoparticle ligand density on dendritic-cell targeted vaccines. Biomaterials 2011; 32(11): 3094–3105. doi: 10.1016/j.biomaterials.2010.12.054.
22. Rosalia RA, Cruz LJ, van Duikeren S, *et al.* CD40-targeted dendritic cell delivery of PLGA-nanoparticle vaccines induce potent anti-tumor responses. Biomaterials 2015; 40: 88–97. doi: 10.1016/j.biomaterials.2014.10.053.
23. Cruz LJ, Rosalia RA, Kleinovink JW, *et al.* Targeting nanoparticles to CD40, DEC-205 or CD11c molecules on dendritic cells for efficient $CD8^+$ T cell response: A comparative study. J Control Release 2014; 192: 209–218. doi: 10.1016/j.jconrel.2014.07.040.
24. Carrillo-Conde B, Song E-H, Chavez-Santoscoy A, *et al.* Mannose-functionalized "pathogen-like" polyanhydride nanoparticles target C-type lectin receptors on dendritic cells. Mol Pharm 2011; 8(5): 1877–1886. doi: 10.1021/mp200213r.
25. Ghotbi Z, Haddadi A, Hamdy S, *et al.* Active targeting of dendritic cells with mannan-decorated PLGA nanoparticles. J Drug Target 2011; 19(4): 281–292. doi: 10.3109/1061186X.2010.499463.
26. Cruz LJ, Rueda F, Cordobilla B, *et al.* Targeting nanosystems to human DCs *via* Fc receptor as an effective strategy to deliver antigen for immunotherapy. Mol Pharm 2010; 8(1): 104–116. doi: 10.1021/mp100178k.
27. Moffatt S, Cristiano RJ. Uptake characteristics of NGR-coupled stealth PEI/pDNA nanoparticles loaded with PLGA-PEG-PLGA tri-block copolymer for targeted delivery to human monocyte-derived dendritic cells. Int J Pharm 2006; 321(1): 143–154. doi: 10.1016/j.ijpharm.2006.05.007.
28. Li WA, Mooney DJ. Materials based tumor immunotherapy vaccines. Curr Opin Immunol 2013; 25(2): 238–245. doi: 10.1016/j.coi.2012.12.008.
29. Hu Y, Litwin T, Nagaraja AR, *et al.* Cytosolic delivery of membrane-impermeable molecules in dendritic cells using pH-responsive core-shell nanoparticles. Nano Lett 2007; 7(10): 3056–3064. doi: 10.1021/nl071542i.
30. Kwon YJ, Standley SM, Goh SL, *et al.* Enhanced antigen presentation and immunostimulation of dendritic cells using acid-degradable cationic nanoparticles. J Control Release 2005; 105(3): 199–212. doi: 10.1016/j.jconrel.2005.02.027.
31. Sneh-Edri H, Likhtenshtein D, Stepensky D. Intracellular targeting of PLGA nanoparticles encapsulating antigenic peptide to the endoplasmic reticulum of dendritic cells and its effect on antigen cross-presentation *in vitro*. Mol Pharm 2011; 8(4): 1266–1275. doi: 10.1021/mp200198c.
32. Coester C, Nayyar P, Samuel J. *In vitro* uptake of gelatin nanoparticles by murine dendritic cells and their intracellular localisation. Eur J Pharm Biopharm 2006; 62(3): 306–314. doi: 10.1016/j.ejpb.2005.09.009.
33. Zwiorek K, Bourquin C, Battiany J, *et al.* Delivery by cationic gelatin nanoparticles strongly

increases the immunostimulatory effects of CpG oligonucleotides. Pharm Res 2008; 25(3): 551–562. doi: 10.1007/s11095-007-9410-5.
34. Akita H, Kogure K, Moriguchi R, *et al.* Nanoparticles for *ex vivo* siRNA delivery to dendritic cells for cancer vaccines: Programmed endosomal escape and dissociation. J Control Release 2010; 143(3): 311–317. doi: 10.1016/j.jconrel.2010.01.012.
35. Taylor PR, Martinez-Pomares L, Stacey M, *et al.* Macrophage receptors and immune recognition. Annu Rev Immunol 2005; 23: 901–944. doi: 10.1146/annurev.immunol.23.021704.115816.
36. Ahsan F, Rivas IP, Khan MA, *et al.* Targeting to macrophages: Role of physicochemical properties of particulate carriers–liposomes and microspheres–on the phagocytosis by macrophages. J Control Release 2002; 79(1): 29–40. doi: 10.1016/S0168-3659(01)00549-1.
37. Chavez-Santoscoy AV, Roychoudhury R, Pohl NL, *et al.* Tailoring the immune response by targeting C-type lectin receptors on alveolar macrophages using "pathogen-like" amphiphilic polyanhydride nanoparticles. Biomaterials 2012; 33(18): 4762–4772. doi: 10.1016/j.biomaterials.2012.03.027.
38. Betageri G, Black C, Szebeni J, *et al.* Fc-receptor-mediated targeting of antibody-bearing liposomes containing dideoxycytidine triphosphate to human monocyte/macrophages. J Pharm Pharmacol 1993; 45(1): 48–53. doi: 10.1111/j.2042-7158.1993.tb03678.x.
39. Neutra MR, Frey A, Kraehenbuhl J-P. Epithelial M cells: Gateways for mucosal infection and immunization. Cell 1996; 86(3): 345–348. doi: 10.1016/S0092-8674(00)80106-3.
40. Fievez V, Plapied L, des Rieux A, *et al.* Targeting nanoparticles to M cells with non-peptidic ligands for oral vaccination. Eur J Pharm Biopharm 2009; 73(1): 16–24. doi: 10.1016/j.ejpb.2009.04.009.
41. Rajapaksa TE, Stover-Hamer M, Fernandez X, *et al.* Claudin 4-targeted protein incorporated into PLGA nanoparticles can mediate M cell targeted delivery. J Control Release 2010; 142(2): 196–205. doi: 10.1016/j.jconrel.2009.10.033.
42. Perez P, Hoffman RW, Shaw S, *et al.* Specific targeting of cytotoxic T cells by anti-T3 linked to anti-target cell antibody. Nature 1985; 316(6026): 354–356. doi: 10.1038/316354a0.
43. O'day SJ, Hamid O, Urba WJ. Targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4). Cancer 2007; 110(12): 2614–2627. doi: 10.1002/cncr.23086.
44. Van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med 1999; 190(3): 355–366. doi: 10.1084/jem.190.3.355.
45. Duraiswamy J, Kaluza KM, Freeman GJ, *et al.* Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. Cancer Res 2013; 73(12): 3591–3603. doi: 10.1158/0008-5472.CAN-12-4100.
46. Hamdy S, Molavi O, Ma Z, *et al.* Co-delivery of cancer-associated antigen and Toll-like receptor 4 ligand in PLGA nanoparticles induces potent $CD8^+$ T cell-mediated anti-tumor immunity. Vaccine 2008; 26(39): 5046–5057. doi: 10.1016/j.vaccine.2008.07.035.
47. Cui Z, Patel J, Tuzova M, *et al.* Strong T cell type-1 immune responses to HIV-1 Tat (1–72) protein-coated nanoparticles. Vaccine 2004; 22(20): 2631–2640. doi: 10.1016/j.vaccine.2003.12.013.
48. Yu X, Feizpour A, Ramirez N-GP, *et al.* Glycosphingolipid-functionalized nanoparticles recapitulate CD169-dependent HIV-1 uptake and trafficking in dendritic cells. Nat Commun 2014; 5: 4136. doi: 10.1038/ncomms5136.
49. Frangioni JV. *In vivo* near-infrared fluorescence imaging. Curr Opin Chem Biol 2003; 7(5): 626–634. doi: 10.1016/j.cbpa.2003.08.007.
50. Medintz IL, Uyeda HT, Goldman ER, *et al.* Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater 2005; 4(6): 435. doi: 10.1038/nmat1390.
51. Yang S-T, Cao L, Luo PG, *et al.* Carbon dots for optical imaging *in vivo*. J Am Chem Soc 2009; 131(32): 11308–11309. doi: 10.1021/ja904843x.
52. Wang F, Banerjee D, Liu Y, *et al.* Upconversion nanoparticles in biological labeling, imaging, and therapy. Analyst 2010; 135(8): 1839–1854. doi: 10.1039/C0AN00144A.
53. Xiang J, Xu L, Gong H, *et al.* Antigen-loaded upconversion nanoparticles for dendritic cell stimulation, tracking, and vaccination in dendritic cell-based immunotherapy. ACS Nano 2015; 9(6): 6401–6411. doi: 10.1021/acsnano.5b02014.
54. Idris NM, Gnanasammandhan MK, Zhang J, *et al. In vivo* photodynamic therapy using upconversion nanoparticles as remote-controlled nanotransducers. Nat Med 2012; 18(10): 1580–1585. doi: 10.1038/nm.2933.
55. Xu J, Xu L, Wang C, *et al.* Near-infrared-triggered photodynamic therapy with multitasking upconversion nanoparticles in combination with checkpoint blockade for immunotherapy of colorectal cancer. ACS Nano 2017; 11(5): 4463–4474. doi: 10.1021/acsnano.7b00715.
56. Ahrens ET, Bulte JW. Tracking immune cells *in vivo* using magnetic resonance imaging. Nat Rev Immunol 2013; 13(10). doi: 10.1038/nri3531.
57. Na HB, Song IC, Hyeon T. Inorganic nanoparticles for MRI contrast agents. Adv Mater 2009; 21(21): 2133–2148. doi: 10.1002/adma.200802366.
58. Caravan P. Strategies for increasing the sensitivity of gadolinium based MRI contrast agents. Chem Soc Rev 2006; 35(6): 512–523. doi: 10.1039/b510982p.

59. Cho N-H, Cheong T-C, Min JH*, et al.* A multifunctional core-shell nanoparticle for dendritic cell-based cancer immunotherapy. Nat Nanotechnol 2011; 6(10): 675–682. doi: 10.1038/nnano.2011.149.
60. Srinivas M, Tel J, Schreibelt G*, et al.* PLGA-encapsulated perfluorocarbon nanoparticles for simultaneous visualization of distinct cell populations by ^{19}F MRI. Nanomedicine 2015; 10(15): 2339–2348. doi: 10.2217/NNM.15.76.
61. Lusic H, Grinstaff MW. X-ray-computed tomography contrast agents. Chem Rev 2012; 113(3): 1641–1666. doi: 10.1021/cr200358s.
62. Toy R, Bauer L, Hoimes C*, et al.* Targeted nanotechnology for cancer imaging. Adv Drug Deliver Rev 2014; 76: 79–97. doi: 10.1016/j.addr.2014.08.002.
63. Cormode DP, Naha PC, Fayad ZA. Nanoparticle contrast agents for computed tomography: A focus on micelles. Contrast Media Mol 2014; 9(1): 37–52. doi: 10.1002/cmmi.1551.
64. Meir R, Shamalov K, Betzer O*, et al.* Nanomedicine for cancer immunotherapy: Tracking cancer-specific T-cells *in vivo* with gold nanoparticles and CT imaging. ACS Nano 2015; 9(6): 6363–6372. doi: 10.1021/acsnano.5b01939.
65. Kim E-J, Bhuniya S, Lee H*, et al. In vivo* tracking of phagocytic immune cells using a dual imaging probe with gadolinium-enhanced MRI and near-infrared fluorescence. ACS Appl Mater Inter 2016; 8(16): 10266–10273. doi: 10.1021/acsami.6b03344.
66. Pittet MJ, Swirski FK, Reynolds F*, et al.* Labeling of immune cells for *in vivo* imaging using magnetofluorescent nanoparticles. Nat Protoc 2006; 1(1): 73–79. doi: 10.1038/nprot.2006.11.
67. Chou S-W, Shau Y-H, Wu P-C*, et al. In vitro* and *in vivo* studies of FePt nanoparticles for dual modal CT/MRI molecular imaging. J Am Chem Soc 2010; 132(38): 13270–13278. doi: 10.1021/ja1035013.
68. Lee SB, Ahn SB, Lee S-W*, et al.* Radionuclide-embedded gold nanoparticles for enhanced dendritic cell-based cancer immunotherapy, sensitive and quantitative tracking of dendritic cells with PET and Cerenkov luminescence. NPG Asia Mater 2016; 8(6): e281. doi: 10.1038/am.2016.80.
69. Lee SB, Lee S-W, Jeong SY*, et al.* Engineering of radioiodine-labeled gold core-shell nanoparticles as efficient nuclear medicine imaging agents for trafficking of dendritic cells. ACS Appl Mater Inter 2017; 9(10): 8480–8489. doi: 10.1021/acsami.6b14800.

Trends in Immunotherapy (2019) Volume 3 Issue 2, pp.89–95.
doi: 10.24294/ti.v3.i2.122

REVIEW

Sirtuins in wound healing

Akihiro Aioi[1,2]

[1] *Septem-Soken, Osaka, Japan*
[2] *Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland, Australia*

ABSTRACT

Sirtuins (SIRTs) are initially recognized as NAD^+-dependent histone deacetylase. SIRTs attract attention for their role as calorie restriction-induced "longevity proteins" to be expected to extend human life span and to promote health. As advancing studies, SIRTs have been recognized as cell signaling regulators which contribute to anti-inflammation, cell differentiation and so on. Therefore, SIRTs are supposed to affect wound healing which is comprised highly orchestrated complex four phases: hemostasis, inflammation, tissue formation and tissue remodeling. This review highlights the roles of SIRTs in wound healing process and provides a foundation and impetus for future basic and clinical research.

Keywords*: sirtuin; wound healing; anti-inflammation; re-epithelialization*

ARTICLE INFO
Received: September 5, 2019
Accepted: October 11, 2019
Available online: October 22, 2019

***CORRESPONDING AUTHOR**
Akihiro Aioi, Septem-Soken, Osaka, Osaka 530-0003, Japan; a-aioi@septem-so.com

CITATION
Aioi A. Sirtuins in wound healing. Trends Immunother 2019; 3(2): 89–95. doi: 10.24294/ti.v3.i2.122.

Introduction

Since the discovery of *silent information regulator 2* (*SIR2*) gene in 1997, *SIRT*, the *SIR2*-like genes, have been found in bacteria, plants and animals, and their function has been investigated the key roles. Sir2 protein was initially identified as a member of NAD^+-dependent deacetylases and ADP-ribosyltransferases in *Saccharomyces cerevisiae*[1] and subsequent studies showed that SIR2 extends replicate life span of yeast by suppressing rDNA recombination and decreasing extrachromosomal rDNA circle[2,3]. Following the studies on longevity of life span in *Caenorhabditis elegans* and *Drosophila*[4–6], the studies on mammalian sirtuins embarked. Human sirtuins comprise seven members of protein (SIRT1–7) localized in cytosol, nucleus and mitochondria, which are involved in pleiotropic cellular functions by deacetylation of histone and/or non-histone proteins (**Table 1**). The SIRTs attract attention for their role as calorie restriction-induced "longevity proteins" to be expected to extend human life span and to promote health[7–9]. The primary function of skin, dominantly comprised by fibroblasts and keratinocytes, is to serve as a protective barrier against environment. Wound, which disrupts the primary function of skin, may lead to major disability or even death. Chronic skin ulcers such as bedsore and diabetic foot ulcer emerged as the issue to be addressed in "aged society", as well as acute wounds caused by injury and burns in all generations. On the other hand, previous studies suggested that SIRTs expressed in fibroblast and keratinocyte may concern cutaneous physiology. However, their role is gaining interest in the field of dermatology. Herein this review highlights the role of SIRTs in wound healing.

Acute Wounds

Wound healing is a physiological response to restore skin integrity and is comprised highly orchestrated complex four phases: hemostasis, inflammation, tissue formation and tissue remodeling[10]. In inflammation phase to begin with the formation of a hemostatic plug by aggregated platelets, many kinds of mediators are involved through the wound healing processes. Previously, a great deal of studies focused on growth factors, cytokines and chemokines in the process (**Table 2**)[11]. Platelets aggregated around the wound site not only

Table 1. Sirtuins

Sirtuin	HDAC Class	Localization	Function
Sirtuin1	Class I	Nucleus Cytosol	Cell survival Life span regulation Metabolism regulation Inflammation Oxidative stress response
Sirtuin2	Class I	Nucleus Cytosol	Cell cycle regulation Nerve system development
Sirtuin3	Class I	Nucleus Cytosol Mitochondria	Mitochondrial metabolism
Sirtuin4	Class II	Mitochondria	Mitochondrial metabolism
Sirtuin5	Class III	Mitochondria	Apoptosis
Sirtuin6	Class IV	Nucleus	Genome stability DNA repair
Sirtuin7	Class IV	Nucleus	rRNA transcription regulation Cell cycle regulation

facilitate the formation of a hemostatic plug, but also secrete kinds of mediators such as platelet-derived growth factor (PDGF), that attracts and activates macrophages and fibroblasts. The activation of infiltrated cells and residing cells in wound site leads to up-regulation of proinflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α in inflammation phase. IL-1 released from macrophages and keratinocytes induces keratinocyte migration for re-epithelialization and the secretion of FGF-7 from activated fibroblasts for extracellular matrix formation[28,29]. IL-6 expression is induced in neutrophils and macrophages immediately after wounding and the expression is sustained during healing process[18,19]. IL-6 attracts neutrophils which cleanse the wound site of foreign particles and bacteria. Additionally, IL-6 has a mitogenic and proliferative effect on keratinocytes for re-epithelialization[22,23]. A previous study suggested that TNF-α accelerates re-epithelialization *via* induction of FGF-7 in fibroblasts like IL-1[31]. However, TNF-α has also been reported to suppress the wound healing *via* the induction of type V collagenase at the high concentration[32].

Nuclear factor kappa B (NF-κB), a transcription factor, is well-known as a major regulator of proinflammatory cytokine expression. In unstimulated cells, NF-κB is bound to inhibitory protein, IκB, localizing in cytosol. Extracellular stimuli recognized by receptors initiates a signaling cascade leading to the activation of IκB kinase (IKK). The phosphorylation of IκB by IKK induces degradation of IκB by proteosome and releases NF-κB from the complex. The freed NF-κB translocates into the nucleus where it binds to the target gene promoter region and activates gene transcription[33]. Yeung *et al*. demonstrated that SIRT1inhibits the transcriptional activity of NF-κB by deacetylation of RelA/p65 subunit of NF-κB[34]. Indeed, resveratrol (RSV), a well-known SIRT activator, inhibited IL-6 production from normal human dermal fibroblast by lipopolysaccharide, which binds to TLR4 and activates NF-κB, in our laboratory (**Figure 1**). TNF-α, which is a pivotal cytokine in inflammation, up-regulates the expression of matrix metalloproteinase-9 (MMP-9), IL-1β and IL-6 in 3T3 fibroblasts. SIRT1 activation by RSV suppressed TNF-α expression by the inactivation of NF-κB, followed by down-regulation of MMP-9, IL1β and IL-6[35].

Other than peptide mediators, nitric oxide (NO), a short-lived free radical, has been reported as a regulator in wound healing. NO is formed from arginine by NO synthase (NOS) which exist in three distinct isoforms, two constitutive (endothelial and neuronal) isoforms and one inducible isoform. The highest NOS activity was detected in the early phase in wound healing[36] followed by sustained NO synthesis[37] and the highest expression of inducible NOS was confirmed in the early phase as well[38,39]. During the healing process, NO is involved in re-epithelialization, neovascularization and collagen synthesis[40]. SIRT1 activation suppressed inducible NOS (iNOS) expression through the inhibition of

Table 2. Growth factors and cytokines in wound healing

Factors	Source	Function
Platele-derived growth factor[11]	Platelet Macrophage Keratinocyte	Fibroblast proliferation Chemoattraction
Vascular endothelial growth factor[11]	Epidermal cell Macrophage	Angiogenesis Increase vascular permeability
Epidermal cell growth factor[11,12]	Platelet Macrophage Fibroblast	Cell migration Cell proliferation
Fibroblast growth factor[11,13,14]	Macrophage Mast cell Endothelial cells Keratinocyte Fibroblast	Angiogenesis Fibroblast proliferation Keratinocyte migration
Transforming growth factor b1[11,15,16]	Platelet Macrophage Lymphocyte Keratinocyte Fibroblast	Cell migration Chemoattraction Granulation tissue formation Re-epithelialization Extracellular matrix synthesis
Tumor necrosis factor-α[11,17–18]	Neutrophil Macrophage	Growth factor expression Re-epithelialization
Interleukin-1[18–20]	Neutrophil Macrophage Fibroblast	Initiation of inflammation Re-epithelialization
Interleukin-6[11,18,19,21–23]	Neutrophil Macrophage Fibroblast	Re-epithelialization Formation of granulation tissue Angiogenesis
Interleukin-8[24–27]	Neutrophil Macrophage	Re-epithelialization

TNF-α expression[35], SIRT1/2 has been described to enhance endothelial NOS (eNOS) activity eliciting significant increase in NO production by deacetylation at lysines 496 and 508 in the calmodulin-binding domain of eNOS[41,42]. On the other hand, class I histone deacetylase (HDAC2), which is the only member of this class known to be S-nitrosylated directly by NO inhibits the expression of growth factors such as epidermal growth factor (EGF) by attaching the promoter regions. These suggest that post-translational S-nitrosylation of HDAC2 leads to enhance the production of growth factors by detachment of HDAC2 from the promoter regions[43]. Consequently, SIRT-dependent NO production enhances wound closure by evocation of keratinocyte proliferation. Taken together, SIRT1 engages profoundly with the expression of proinflammatory mediators, suggesting SIRT1 could be one of the therapeutic targets for wound healing.

Chronic Wounds

Chronic wounds are defined as those which do not follow the normal healing process and show no signs of effective healing within 3 months after the injury. The cause of failure to complete wound healing is mainly to stagnate at the early inflammation phase[44]. The features characteristic for the chronic wounds are shown in **Table 3**. Avishai *et al*. demonstrated risk factors such as autoimmune diseases, aging, obese and diabetes mellitus[45]. In "aged-society", the strategy of therapy for chronic wounds is the issue to be addressed, because older adults suffering from vascular disease, venous insufficiency, unrelieved pressure and diabetes mellitus, are more likely to have chronic wounds than younger people[50]. As the morphology of resident cells is similar to that seen in senescent cells in chronic wounds, it is supposed that the treatment against cell senescence as well as against prolonged inflammation. Expression of aging

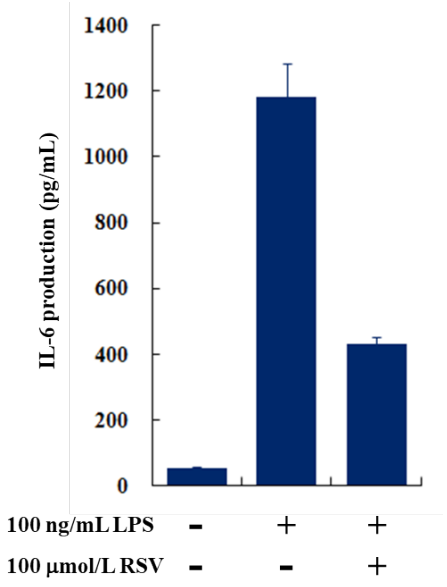

Figure 1. RSV suppressed LPS-induced IL-6 production. 100 ng/mL LPS induced abundant IL-6 production from normal human dermal fibroblasts. Addition of 100 mmol/L RSV reduced significantly the production to 38.4%.

biomarkers including procollagen I and VII, SIRT1 and SIRT6 were down-regulated in passaged human dermal fibroblasts[51]. Growth of chronic wound fibroblasts was significantly decreased compared with fibroblasts isolated from acute wound and normal dermis[52].

Table 3. Features in chronic wounds

Features	References
Prolonged inflammation	[44]
Excessive inflammation	[44]
Excessive neutrophil infiltration	[46]
Infection	[47]
Atypical biofilms	[48, 49]

Moreover, the abnormalities in mitogen activating protein kinase (MAPK) and Smad pathway were observed in venous ulcer fibroblasts suppressed TGF-β type II receptor expression[53]. These suggest that TGF-β-induced collagen synthesis is suppressed in fibroblasts isolated from chronic wounds as well as senescent fibroblasts. Collagen remodeling during the transition from granulation tissue to scar is dependent on synthesis and catabolism. Collagen degradation is controlled by MMPs. Cigarette smoke exposure reduced SIRT1 expression and activity in lung tissue of A/J mice, accompanied with elevation of MMP-9. The elevation was blocked by SRT2127, a small molecular SIRT1 activator[54]. In skin, RSV and metformin significantly inhibited up-regulation of MMP-9 expression and prevented collagen degradation after ultra-violet irradiation[55]. Similarly, SIRT6 has been reported to regulate negatively the expression of MMP-9[56,57]. It is easily presumed that this negative regulation of MMP-9 expression is due to inhibition of NF-κB pathway, because MMP-9 expression is regulated by NF-κB which is a deacetylation target of SIRTs. Like so, suppressed MMP-9 expression contributes to collagen re-modeling in dermis. However, MMP-9 deficiency leads to impaired wound healing, because MMP-9 also contributes keratinocyte migration in re-epithelialization phase. On the other hand, high glucose impaired keratinocyte migration by inducing levels of MMP-9 expression in diabetic mouse model. This glucose-sensitive elevation of MMP-9 expression was blocked by deletion of FOXO1, which is also a deacetylation target of SIRT1, concomitant with improved wound healing[58]. Therefore, the appropriate expression of MMP-9 is required for orchestrated wound healing process.

Conclusion

SIRTs attract attention for their role as calorie restriction-induced "longevity proteins" with expectation to extend human life span by promoting health and wellness. As studies advance on SIRTs, it has been emerged that SIRTs are involved in pleiotropic functions *via* deacetylation of histone and/or non-histone proteins (**Figure 2**)[59]. Of them, inflammation, cell proliferation and cell migration are vital events in wound healing process. SIRTs have not only anti-inflammatory effects but also promotive effect on cell proliferation and cell migration to enhance wound healing, suggesting SIRTs activation could be one of the therapeutic strategies for wound healing. SIRTs activators have been found in nature and synthesized such as RSV and its derivatives[60]. The effect of RSV on wound healing and chronic diseases has been evaluated not only in animal model and human EpiDerm full thickness model, but also in clinical applications[61–63]. Taken together, SIRTs are involved in orchestrated wound healing processes and its activation provides an approach for acceleration of wound therapy. RSV could be a major candidate compound for wound healing because it is a polyphenol found in nature such as within plants, or the consumed such as grapes and wine.

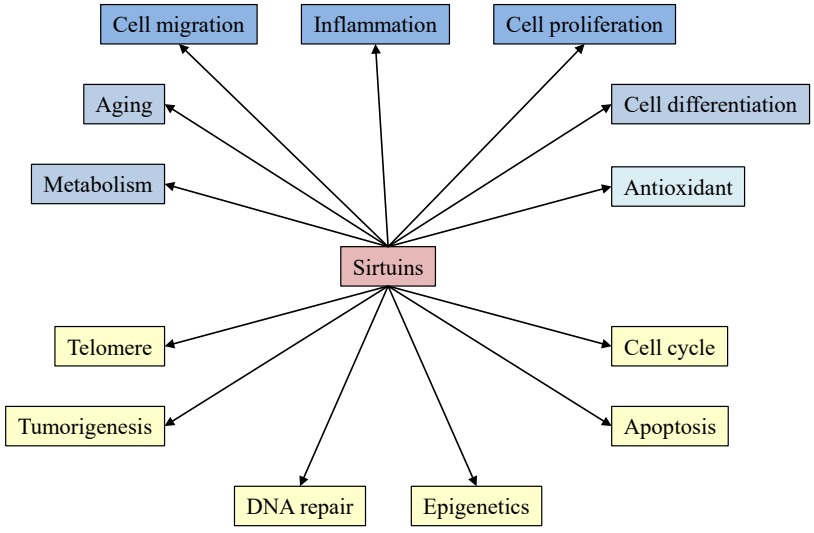

Figure 2. Multi-functions of SIRT. SIRTs possess multi-functions other than originally recognized function of life longevity. Blue-shaded functions are vastly involved in wound healing.

Conflict of interest

The author declares no potential conflict of interest with respect to the research, authorship, and/or publication of his article.

References

1. Imai S, Armstrong CM, Kaeberlein M, *et al*. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 2000; 403(6771): 795–800. doi: 10.1038/35001622.
2. Sinclair DA, Guarente L. Extrachromosomal rDNA circles—A cause of aging in yeast. Cell 1997; 91(7): 1033–1042. doi: 10.1016/S0092-8674(00)80493-6.
3. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev 1999; 13(19): 2570–2580. doi: 10.1101/gad.13.19.2570.
4. Tissenbaum HA, Guarente L. Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*. Nature 2001; 410(6825): 227–30. doi: 10.1038/35065638.
5. Wang Y, Tissenbaum HA. Overlapping and distinct functions for a *Caenorhabditis elegans* SIR2 and DAF-16/FOXO. Mech Ageing Dev 2006; 127(1): 48–56. doi: 10.1016/j.mad.2005.09.005.
6. Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci USA 2004; 101(45): 15998–16003. doi: 10.1073/pnas.0404184101.
7. Colman R, Anderson R, Johnson S, *et al*. Caloric restriction delays disease onset and mortality in rhesus monkey. Science 2009; 325(5937): 201–204. doi: 10.1126/science.1173635.
8. Guarente L, Picard F. Calorie restriction—The SIR2 connection. Cell 2005; 120(4): 473–482. doi: 10.1016/j.cell.2005.01.029.
9. Haigis M, Mostoslavsky R, Haigis K, *et al*. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell 2006; 126(5): 941–954. doi: 10.1016/j.cell.2006.06.057.
10. Singer AJ, Clark RAF. Cutaneous wound healing. N Eng J Med 1999; 341(10): 738–746. doi: 10.1056/NEJM199909023411006.
11. Barrientos S, Stojadinovic O, Golinko MS, *et al*. Growth factors and cytokines in wound. Wound Rep Reg 2008; 16(5): 585–601. doi: 10.1111/j.1524-475X.2008.00410.x.
12. Shen C, Sun L, Zhu N, *et al*. Lindlin-1 contributes to EGF-induced re-epithelialization in skin wound healing. Int J Mol Med 2017; 39(4): 949–959. doi: 10.3892/ijmm.2017.2911.
13. Meyer M, Muller AK, Yang J, *et al*. FGF receptors 1and 2 are key regulators of keratinocyte migration *in vitro* and in wounded skin. J Cell Sci 2012; 125(Pt 23): 5690–5701. doi: 10.1242/jcs.108167.
14. Takamiya M, Saigusa K, Nakayashiki N, *et al*. Studies on mRNA expression of basic fibroblast growth factor in wound healing for wound age determination. Int J Legal Med 2003; 117(1): 46–50. doi: 10.1007/s00414-002-0354-3.
15. Zhang C. Tan CK. McFarlane C. *et al*. Myostatin-null mice exhibit delay skin wound healing through the blockade of transforming growth factor-β singnaling by decorin. Am J Cell Physiol 2012; 302(8): C1213–C1225. doi: 10.1152/ajpcell.00179.2011.

16. Ishida Y, Kondo T, Takayasu T, *et al*. The essential involvement of cross-talk between IFN-γ and TGF-β in the skin wound-healing precess. J Immunol 2004; 172(3): 1848–1855. doi: 10.4049/jimmunol.172.3.1848.
17. Ashcroft GS, Jeoung MJ, Ashworth JJ, *et al*. Tumor necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. Wound Rep Reg 2012; 20(1): 38–49. doi: 10.1111/j.1524-475X.2011.00748.x.
18. Grellner W. Time-dependent immunohistochemical detection of proinflammaory cytokines (IL-1β, IL-6, TNF-α) in human skin wounds. Forensic Sci Int 2002; 130(2–3): 90–96. doi: 10.1016/S0379-0738(02)00342-0.
19. Grellner W, George T, Wilske J. Quantitative analysis of proinflammatory cytokines (IL-1β, IL-6, TNF-α) in human skin wounds. Forensic Sci Int 2000; 113(1–3): 251–264. doi: 10.1016/S0379-0738(00)00218-8.
20. Ishida Y, Kondo T, Kimura A, *et al*. Absence of IL-1receptor antagonist impaired wound healing along with aberrant NF-κB activation and a reciprocal suppression of TGF-β signal pathway. J Immunol 2006; 176(9): 5598–5606. doi: 10.4049/jimmunol.176.9.5598.
21. Gallucci RM, Sugawara T, Yucesoy B, *et al*. Interleukin-6 treatment augments cutaneous wound healing in immunosuppressed mice. J Interferon Cytokine Res 2001; 21(8): 603–609. doi: 10.1089/107999001525478 67.
22. Gallucci RM, Sloan DK, Heck JM, *et al*. Interleukin 6 indirectly induces keratinocyte migration. J Invest Dermatol 2004; 122(3): 764–772. doi: 10.1111/j.0022-202X.2004.22323.x.
23. McFarland-Mancini MM, Funk HM, Pluch AM, *et al*. Differences in wound healing in mice with deficiency of IL-6 versus IL-6 receptor. J Immunol 2010; 184(12): 7219–7228. doi: 10.4049/jimmunol.0901929.
24. Kondo T, Oshima T, Mori R, *et al*. Immunohistochemical detection of chemokines in human skin wounds and its application to wound age determination. Int J Legal Med 2002; 116(2): 87–91. doi: 10.1007/s004140100260.
25. Engelhardt E, Toksoy A, Goebeler M, *et al*. Chemokines IL-8, GROα, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound-healing. Am J Pathol 1998; 153(6): 1849–1860. doi: 10.1016/S0002-9440(10)65699-4.
26. Ishida Y, Gao JL, Murphy PM. Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function. J Immunol 2008; 180(1): 569–579. doi: 10.4049/jimmunol.180.1.569.
27. Aibina JE, Mills CD, Henry WL, *et al*. Temporal expression of different pathways of l-arginine metabolism in healing wounds. J Immunol 1990; 144(10): 3877–3880.
28. Komine M, Rao LS, Kaneko T, *et al*. Inflammatory *versus* proliferative process in epidermis. Tumor necrosis factor-α induces K6b keratin synthesis through a transcriptional complex containing NFκB and C/EBPβ. J Biol Chem 2000; 275(41): 32077–32088. doi: 10.1074/jbc.M001253200.
29. Tang A, Gilchrest BA. Regulation of keratinocyte growth factor gene expression in human skin fibroblasts. J Dermatol Sci 1996; 11(1): 41–50. doi: 10.1016/0923-1811(95)00418-1.
30. Sato M, Sawamura D, Ina S, *et al*. *In vivo* introduction of the *inteleukin-6* gene into human keratinocyte: Induction of epidermal proliferation by the fully spliced form of interleukin-6, but not by the alternatively spliced form. Arch Dermatol Res 1999; 291(7–8): 400–404. doi: 10.1007/s004030050429.
31. Brauchle M, Angermeyer K, Hubner G, *et al*. Large induction of keratinocyte growth factor expression by serum growth factors and proinflammatory cytokines in cultured fibroblasts. Oncogene 1994; 9(11): 3199–3204.
32. Unemori EN, Hibbs MS, Amento EP. Constitutive expression of 92-kD gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. J Clin Invest 1991; 88(5): 1656–1662. doi: 10.1172/JCI115480.
33. Napetschnig J, Wu H. Molecular basis of NF-κB signaling. Annu Rev Biophys 2013; 42: 443–468. doi: 10.1146/annurev-biophys-083012-130338.
34. Yeung F, Hoberg JE, Ramsey CS, *et al*. Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 2004; 23(12): 2369–2380. doi: 10.1038/sj.emboj.7600244.
35. Zhu X, Liu Q, Wang M, *et al*. Activation of Sirt1 by resveratrol inhibit TNF-α induced inflammation in fibroblasts. PloS One 2011; 6(11): e27081. doi: 10.1371/journal.pone.0027081.
36. Schaffer MR, Tantry U, Barbul A, *et al*. Nitric oxide metabolism in wounds. J Surg Res 1997; 71(1): 25–31. doi: 10.1006/jsre.1997.5137.
37. Frank S, Madlener M, Pfeilschiter J, *et al*. Induction of inducible nitric oxide synthase and its corresponding tetrahydrobiopterin-cofactor-synthesizing enzyme GTP-cyclohydrolase I during cutaneous wound healing. J Invest Dermatol 1998; 111(6): 1058–1064. doi: 10.1046/j.1523-1747.1998.00434.x.
38. Reichner JS, Meszaros AJ, Louis CA, *et al*. Molecular and metabolic evidence for the restricted expression of inducible nitric oxide synthase in healing wounds. Am J Pathol 1999; 154(4): 1097–1104. doi: 10.1016/S0002-9440(10)65362-X.
39. Paulsen SM, Wurster SH, Nanney LB. Expression of inducible nitric oxide synthase in human burn wounds. Wound Rep Reg 1998; 6(2): 142–148. doi: 10.1046/j.1524-475X.1998.60208.x.
40. Rizk M, Witte MB, Barbul A. Nitric oxide and wound healing. World J Surg 2004; 28(3): 301–306. doi: 10.1007/s00268-003-7396-7.

41. Mattagajasingh I, Kim CK, Naqvi A, *et al*. SIRT promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci 2007; 104(37): 14855–14860. doi: 10.1073/pnas.0704329104.
42. Valente S, Mellini P, Spallotta F, *et al*. 1,4-Dihydropyridines active on the SIRT1/AMPK pathway ameliorate skin repair and mitochondrial function and exhibit inhibition of proliferation in cancer cells. J Med Chem 2016; 59(4): 1471–1491. doi: 10.1021/acs.jmedchem.5b01117.
43. Spallota F, Cencioni C, Straino S, *et al*. A nitric oxide-dependent crosstalk between class I and II histone deacetylases accelerates skin repair. J Biol Chem 2013; 288(16): 11004–11012. doi: 10.1074/jbc.M112.441816.
44. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: Molecular and cellular mechanisms. J Invest Dermatol 2007; 127(3): 514–525. doi: 10.1038/sj.jid.5700701.
45. Avishai E, Yeghiazaryan K, Golubnitschaja O. Impaired wound healing: Facts and hypotheses for multi-professional considerations in predictive, preventive and personalized medicine. EPMA J 2017; 8(1): 23–33. doi: 10.1007/s13167-017-0081-y.
46. Martin P, Nunan R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. Br J Dermatol 2015; 173(2): 370–378. doi: 10.1111/bjd.13954.
47. Edwards R, Harding KG. Bacteria and wound healing. Curr Opin Infect Dis 2004; 17(2): 91–96. doi: 10.1097/00001432-200404000-00004.
48. Wolcott RD, Rhoads DD, Dowd SE. Biofilm and chronic wound inflammation. J Wound Care 2008; 17(8): 333–341. doi: 10.12968/jowc.2008.17.8.30796.
49. Thomson CH. Biofilms: Do they affect wound healing? Int Wound J 2011; 8(1): 63–67. doi: 10.1111/j.1742-481X.2010.00749.x.
50. Gould L, Abadir P, Brem H, *et al*. Chronic wound repair and healing in older adults: Current status and future research. J Am Geriartr Soc 2015; 63(3): 427–438. doi: 10.1111/jgs.13332.
51. Kim KS, Park HK, Lee JW, *et al*. Investigate correlation between mechanical property and aging biomarker in passaged human dermal fibroblasts. Microsc Rec Tech 2015; 78(4): 277–282. doi: 10.1002/jemt.22472.
52. Agren MS, Steenfos HH, Dabelsteen S, *et al*. Proliferation and mitogenic response to PDGF-BB of fibroblasts isolated from chronic venous leg ulcers is ulcer-age dependent. J Invest Dermato 1999; 112(4): 463–469. doi: 10.1046/j.1523-1747.1999.00549.x.
53. Kim BC, Kim HT, Park SH, *et al*. Fibroblasts from chronic wounds show altered TGF-β-signaling and decreased TGF-β type II receptor expression. J Cell Physiol 2003; 195(3): 331–336. doi: 10.1002/jcp.10301.
54. Nakamura Y, Vuppusetty C, Wada H, *et al*. A protein deacetylase is a negative regulator of metalloproteinase-9. FASEB J 2009; 23(9): 2810–2819. doi: 10.1096/fj.08-125468.
55. Lee JS, Park KY, Min HG, *et al*. Negative regulation of stress-induced matrix metalloproteinase-9 by Sirt1 in skin tissue. Exp Dermatol 2010; 19(12): 1060–1066. doi: 10.1111/j.1600-0625.2010.01129.x.
56. Kang L, Hu J, Weng Y, *et al*. Sirtuin 6 prevents matrix degradation through inhibition of the NF-κB pathway intervertebral disc degeneration. Exp Cell Res 2017; 352(2): 322–332. doi: 10.1016/j.yexcr.2017.02.023.
57. Thandavaryan RA, Garikipati VNS, Joladarashi D, *et al*. Sirtuin-6 deficiency exacerbates diabetes-induced impairment of wound healing. Exp Dermatol 2015; 24(10): 773–778. doi: 10.1111/exd.12762.
58. Zhang C, Lim J, Jeon HH, *et al*. FOXO1 deletion in keratinocytes improves diabetic wound healing through MMP9 regulation. Sci Rep 2017; 7(1): 10565. doi: 10.1038/s41598-017-10999-3.
59. Serravallo M, Jagdeo J, Glick SA, *et al*. Sirtuins in dermatology: Applications for future research and therapeutics. Arch Dermatol Res 2013; 305(4): 269–282. doi: 10.1007/s00403-013-1320-2.
60. Villalba JM, Alcaın FJ. Sirtuin activators and inhibitors. Biofactors 2012; 38(5): 349–359. doi: 10.1002/biof.1032.
61. Zhao P, Sui BD, Liu N, *et al*. Anti-aging pharmacology in cutaneous wound healing: Effects of metformin, resveratrol, and rapamycin by local application. Aging Cell 2017; 16(5): 1083–1093. doi: 10.1111/acel.12635.
62. Lephart E, Sommerfeldt JM, Andrus MB. Resveratrol: Influences on gene expression in human skin. J Func Foods 2014; 10: 377–384. doi: 10.1016/j.jff.2014.07.017.
63. Wahab A, Gao K, Jia C, *et al*. Significance of resveratrol in clinical management of chronic diseases. Molecules 2017; 22(8): 1329. doi: 10.3390/molecules22081329.

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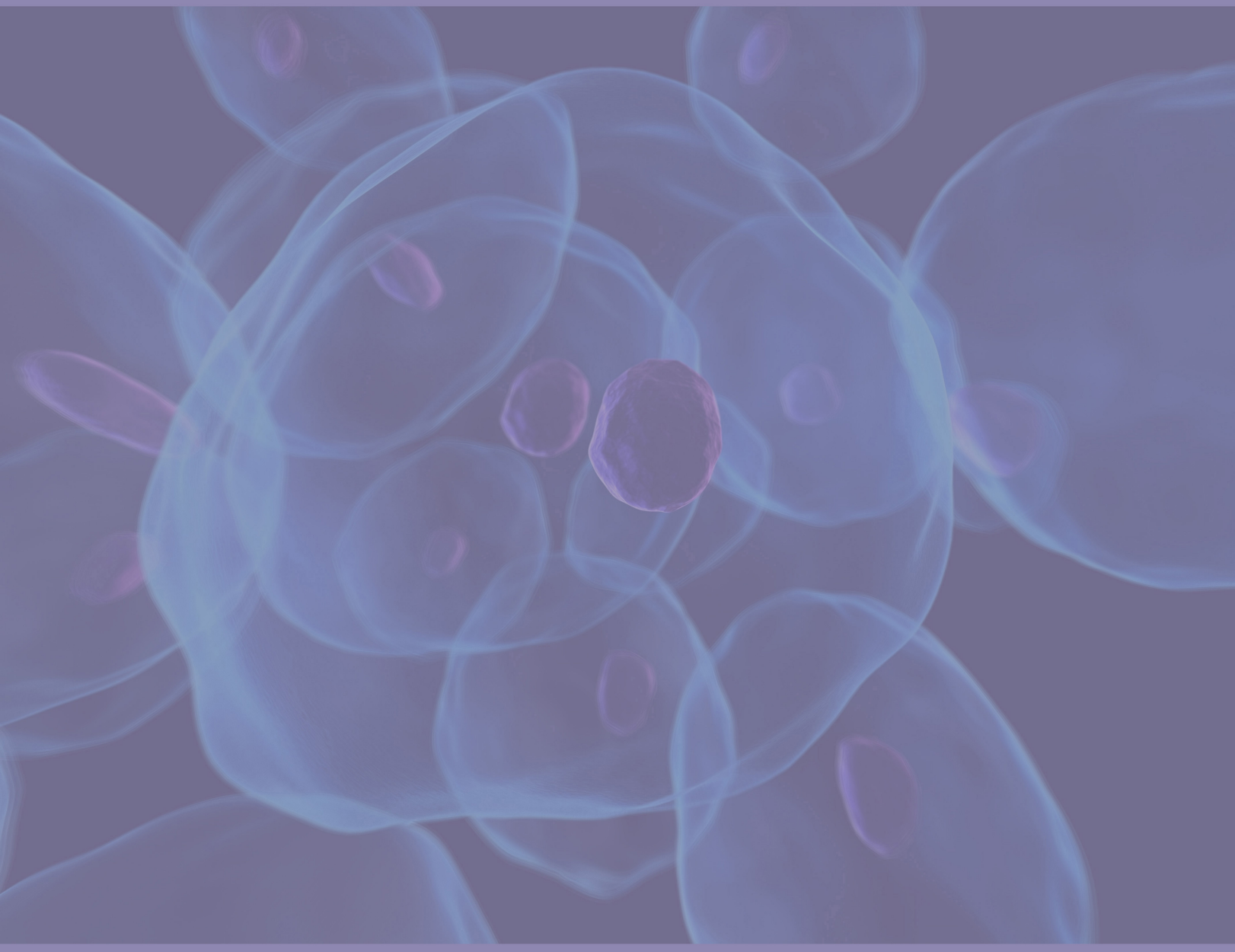

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