Published in final edited form as:

Australas J Dermatol. 2018 November ; 59(4): e258–e261. doi:10.1111/ajd.12801.

Activation of RhoA, Smad2, c-Src, PKC- β II/ δ and JNK in atopic dermatitis

Jianyun Lu^{1,*}, Rong Yin^{2,3,*}, Zhibing Fu^{1,2}, Veronica M. Lee², J. Stuart Nelson^{2,4}, Wenbin Tan^{2,&}

¹Department of Dermatology, the Third Xiangya Hospital, Central South University, Changsha, Hunan 412000, China

²Department of Surgery, Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, California 92617, USA

³Department of Dermatology, the Second Hospital of Shanxi Medical University, Taiyuan, Shanxi 030001, China

⁴Department of Biomedical Engineering, University of California, Irvine, Irvine, California 92617, USA

Abstract

Atopic dermatitis (AD) is a multifactorial skin disease characterized by chronic and relapsing inflammations, but the pathogenesis is incompletely understood. We found that the expression of TGF β R1 and the activation of SMAD2, RhoA, JNK, PKC- β II/ δ and c-Src were upregulated in the infiltrated inflammatory cells, fibroblasts and vasculatures in the dermis and epidermis. In addition, increases in the expression of TGF β R1 and phosphorylation levels of JNK and c-Src were positively correlated with the inflammatory progression of AD severity.

Keywords

Atopic dermatitis; SMAD2; RhoA; JNK; PKC; c-Src; Inflammation

Introduction

Atopic dermatitis (AD) is a skin disease associated with chronic and relapsing inflammations and heterogeneous clinic symptoms, which accounts for ~10-20% of children and 1-3% of adults worldwide.¹ Many factors contribute to the development of AD, including immune dysregulation, skin barrier impairment, microbial infection, genetic

&Correspondence: Wenbin Tan, Department of Surgery, Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, California 92612, USA. wenbint@uci.edu, Phone: 949-824-3754, Fax: 949-824-6969.

***Both authors contributed equally to the work.**

Conflict of Interest: Disclosure: None Declared

Disclaimer: Any views expressed here represent personal opinion and do not necessarily reflect those of the U.S. Department of Health and Human Services or the United States federal government.

Prior Publication: None of the material in this manuscript has been published or is under consideration for publication elsewhere, including the Internet.

mutations and pharmacologic, psychological and environmental factors.² The main pathological phenotype of AD is the proliferation and infiltration of inflammatory cells, including mast cells, basophils, eosinophils, T cells and dendritic cells (DC), which results in epidermal hyperplasia and homeostasis abnormalities.² Both innate and adaptive immune systems have been dysregulated in AD, among which the T helper (Th) 2 mediates a predominant inflammatory response in AD.² The Th2 cytokines, such as IL-5, 13, 10, and 31 and chemokines, are elevated in acute and chronic AD.² Th2 hyper-proliferation also leads to the increase of Ig E levels in ADs which subsequently results in degranulation of mast cells upon exposure of allergens.² Present managements of AD include corticosteroids, antihistamines, UV therapy and immunosuppressants. However, the outcome of moderate and severe AD treatment is inadequate due to unsatisfactory efficacy and adverse effects, and there is a medical need for more specific targeted approaches.

In this study we attempted to investigate the key signalling molecules reflecting the progression of inflammation in atopic dermatitis.

Report

Methods

This clinical investigational protocol was approved by the Ethics Committee of the 3rd XiangYa Hospital, Central South University. The standard criteria and guidelines were used for the diagnosis of AD.¹ Eleven patients with moderate and severe AD were enrolled this study after providing informed consent (Table 1). AD severity for each patient was determined by scoring atopic dermatitis (SCORAD). Ten normal skin biopsy specimens retrieved from the skin biopsy tissue bank at our department served as normal controls. All the biopsy samples were de-identified during the study. Punch biopsies (4-mm) were generally obtained from the most active eczematous skin lesion, fixed by buffered 4% formalin and embedded in paraffin. Six µm thick paraffin sections were cut and collected. Antigen retrieval was performed in 10 mM sodium citrate buffer (pH 6.0) at 97°C for 3 hrs. Sections were then incubated in a humidified chamber overnight at 4°C with the following primary antibodies and dilutions: anti-TGFβR1 (Abclonal, 1:500), anti-p-SMAD2 (Abclonal, 1:500), anti-p-RhoA (Santa Cruz Biotech, Dallas, TX, USA; 1:100), anti-p-PKC-βII/d (Santa Cruz Biotech; 1:50), anti-p-JNK (Santa Cruz Biotech; 1:500) and anti-p-c-Src (Y418) (Abclonal; 1:500). Biotinylated anti-mouse and -rabbit secondary antibodies were incubated with the sections for 1 hr. at room temperature after the primary antibodies' reaction. An indirect biotin avidin diaminobenzidine (DAB) system (Dako, Glostrup, Denmark) was used for detection.

The cellular immunoreactivity score was blindly evaluated by two independent researchers from a different research group in the same institute using a system previously reported with minor modifications.³ Briefly, six scales were used to evaluate the immunoreactive (IR) intensity scores: 0 (-), 1 (±), 2(+), 3(++), 4(+++) and 5(++++ and above). Four scales were used to assess the percentage scores of IR cells: 1 (<25%), 2 (25–50%), 3 (51–75%) and 4 (>75%). The final immunoreactivity scores for each antibody were estimated by multiplying the IR intensity and percentage scores. Spearman correlation analysis was used to evaluate the relationship between IHC scores and SCORAD. The IHC scores in AD lesions were

compared with normal skins by Paired t-test. Spearman correlation analysis was used to evaluate the relationship between IHC and SCORAD scores. A p value <0.05 was considered to be significant.

Results

In normal skin, the expression level of TGF β R1 and phosphorylation levels of RhoA, Smad2, c-Src, PKC- β II/8 and JNK were negative or mild in the epidermis and dermal blood vessels (Figure 1). In AD lesions, epidermal hyperplasia and mononuclear cell infiltrate, mainly lymphocytes, were prominently observed (Figure 1). Perivascular inflammatory cell infiltrate was also frequently observed in hyperplastic epidermis (Figure 1). TGF β R1 showed a strong IR signal in the hyperplastic epidermis, dermal blood vessels, fibroblasts and infiltrate (Figure 1b). Smad2 and RhoA showed ubiquitous activation in keratinocytes and infiltrated inflammatory cells, fibroblasts and dermal blood vessels (Figures 1d & f). Activation of JNK, PKC- β II/8 and c-Src were mainly found in infiltrated inflammatory cells, fibroblasts and dermal blood vessels, but were less evident in keratinocytes (Figures 1h, j & l). In addition, PKC- β II/8 was strongly activated in Langerhans cells in the AD epidermis (Figure 1j).

The IHC scores of TGF β R1, p-Smad2, p-RhoA and p-c-Src increased in AD epidermis and dermal infiltrates as compared to normal skins (Figure 2a, $p<0.05$). The IHC scores of p-PKC- β II/8 and p-JNK were upregulated in AD dermal infiltrates as compared to normal skins (Figure 2a, $p<0.05$). In addition, upregulations of TGF β R1 in the AD lesional epidermis and dermal infiltrate were positively correlated with SCORAD scores (Figures 2b & c). The phosphorylation levels of c-Src and JNK in infiltrated inflammatory cells, fibroblasts and dermal blood vessels also showed positive correlations with SCORAD scores (Figures 2d & e). We didn't observe significant correlations of IHC intensities of p-RhoA, p-Smad2 and p-PKC- β II/8 with SCORAD scores.

Discussion

Immune dysregulation is one major cause for the development of AD.² The proliferation and infiltrate of inflammatory cells, including mast cells, basophils, eosinophils, T cells and dendritic cells, result in epidermal hyperplasia, homeostasis abnormalities and skin fibrosis in AD lesions.² TGF β signaling, in interactions with many other signaling systems, shows dichotomous roles in immune suppression or pro-inflammation in the pathogenesis of AD.^{4,5} On one hand, TGF β seems to facilitate AD progression. For example, TGF β is very potent in stimulating migration and infiltration of immune cells and fibroblasts which favor AD development.⁵ In addition, TGF β is required for development and activation of Langerhans cells in skin, the crucial immune cells mediating inflammation in epidermis.⁵ On the other hand, TGF β may exhibit an inhibitory effect on a variety of immune cells during AD development. For example, TGF β signaling can prevent mast cell granulation, thus alleviating AD.⁴ TGF β can regulate the development of T-regulatory (Treg) cells which will subsequently suppress proliferation of effector T cells.⁵ Th cells play critical roles in the pathogenesis of AD. TGF β can inhibit differentiation of Th1 and Th2 cells through suppression of T-bet/Stat4 and GATA-3/NFAT mechanisms.⁶ In addition, TGF β signaling is a crucial mediator for skin fibrosis.^{4,5} The mRNA level of TGF β in AD lesions doesn't show

a significant change as compared to normal skins.⁷ However, its protein level seems more abundant in epidermal keratinocytes and dermal infiltrates in AD lesions than in normal skins,⁸ indicating translational rather than transcriptional mechanism(s) underlying the alteration of TGF β production in AD. Our data also showed that TGF β R1 is upregulated in AD lesions. Together, these data suggest TGF β signaling is enhanced in AD lesions which may play a pivotal role in the immune dysregulation and fibrosis in AD.

TGF β -induced activation of TGF β R1 can result in phosphorylation of SMAD2/3, which subsequently leads to binding SMAD4 and translocates to the nucleus to initiate transcription of target genes.^{6, 9} In addition, activation of TGF β R1 can also stimulate SMAD-independent pathways, including major intracellular signal transducers such as ERK, P38 and JNK.^{6, 9} In our study, the upregulation of TGF β R1 and p-Smad2 is likely a direct result from the dysregulation of TGF β signaling in AD. JNK, PKC- β II/ δ and c-Src are pro-inflammatory kinases which can be directly activated by or cross talk with TGF β signaling. Activations of these kinases lead to the active inflammatory processes observed in AD lesions which are probably associated with, or independent of, TGF β signaling. TGF β signaling can rapidly activate RhoA in a variety of cell types which is critical for the reorganization of the actin cytoskeleton and focal adhesion formation during cell migration.¹⁰ TGF β -stimulated activation of RhoA requires the kinase activity of TGF β R1 but appears to be independent of Smad signaling.¹⁰ Upregulation of p-RhoA may directly facilitate infiltrations of inflammatory cells as well as result in skin fibrosis remodeling in AD.

In summary, our data demonstrated that TGF β R1, p-Smad2, p-RhoA, p-JNK, p-PKC- β II/ δ and p-c-Src were upregulated in AD lesions. Expression of TGF β R1, p-JNK and p-c-Src were positively correlated with the inflammatory progression of AD. Our results suggest TGF β R1, JNK and c-Src can be potential therapeutic targets for treatment of AD.

Acknowledgments

Institutional support was provided by the Arnold and Mabel Beckman Foundation and the David and Lucile Packard Foundation. We greatly appreciate the Sue & Bill Gross Stem Cell Research Center at the University of California, Irvine, for their assistance in acquiring and processing the histology images.

Funding statement:

This work was supported by grants from the National Institutes of Health (AR063766 to WT, AR47551 and AR59244 to JSN), research grants (F03.12 and F01.13 to WT) from the American Society for Laser Medicine and Surgery, and Natural Science Foundation of Hunan Province and Scientific Research Foundation of Department of Health of Hunan Province, China (2015JJ6120, B2015-032 and B2015-034 to JL).

References

1. Eichenfield LF, Tom WL, Chamlin SL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *J Am Acad Dermatol*. 2014; 70:338–51. [PubMed: 24290431]
2. Malik K, Heitmiller KD, Czarnowicki T. An Update on the Pathophysiology of Atopic Dermatitis. *Dermatol Clin*. 2017; 35:317–26. [PubMed: 28577801]
3. Tan W, Chernova M, Gao L, et al. Sustained activation of c-Jun N-terminal and extracellular signal-regulated kinases in port-wine stain blood vessels. *J Am Acad Dermatol*. 2014; 71:964–68. [PubMed: 25135651]

4. Kim HS, Yun JW, Shin TH, et al. Human umbilical cord blood mesenchymal stem cell-derived PGE2 and TGF-beta1 alleviate atopic dermatitis by reducing mast cell degranulation. *Stem Cells*. 2015; 33:1254–66. [PubMed: 25522163]
5. Han G, Li F, Singh TP, et al. The pro-inflammatory role of TGFbeta1: a paradox? *Int J Biol Sci*. 2012; 8:228–35. [PubMed: 22253566]
6. Li MO, Wan YY, Sanjabi S, et al. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol*. 2006; 24:99–146. [PubMed: 16551245]
7. Jeong CW, Ahn KS, Rho NK, et al. Differential in vivo cytokine mRNA expression in lesional skin of intrinsic vs. extrinsic atopic dermatitis patients using semiquantitative RT-PCR. *Clin Exp Allergy*. 2003; 33:1717–24. [PubMed: 14656360]
8. Verhagen J, Akdis M, Traidl-Hoffmann C, et al. Absence of T-regulatory cell expression and function in atopic dermatitis skin. *J Allergy Clin Immunol*. 2006; 117:176–83. [PubMed: 16387603]
9. Esebanmen GE, Langridge WHR. The role of TGF-beta signaling in dendritic cell tolerance. *Immunol Res*. 2017
10. Carthy JM. TGFbeta signaling and the control of myofibroblast differentiation: Implications for chronic inflammatory disorders. *J Cell Physiol*. 2017

Learning points

- Expression of TGF β R1, p-JNK and p-c-Src are positively correlated with the inflammatory progression of atopic dermatitis.
- Our data suggest TGF β R1, JNK and c-Src can be potential therapeutic targets for treatment of atopic dermatitis.

Figure 1.

Upregulation of TGF β R1, p-RhoA, p-Smad2, p-c-Src, p-PKC- β II/ δ and p-JNK in AD lesions. The expression of TGF β R1 (a, b), levels of p-RhoA (c, d), p-Smad2 (e, f), p-c-Src (g, h), p-PKC- β II/ δ (i, j) and JNK (k, l) were determined by IHC in AD lesions as compared to normal skins. The red inset in (h) is the higher magnification from the blue boxed area from the same image. The red inset in (j) shows p-PKC- β II/ δ -IR Langerhans cells (indicated by purple arrows) in epidermis. Scar bar: 50 μ m.

Figure 2.

(a) Relative IHC scores of TGFβRI, p-RhoA, p-Smad2, p-c-Src, p-PKC-βII/δ and p-JNK in AD epidermis and dermal infiltrates. # $p < 0.05$ as compared to normal skins. (b)-(e), The correlations of IHC scores of TGFβRI in epidermis (b) and inflammatory infiltrate (c), p-c-Src (d) and p-JNK (e) in dermal infiltrated cells with patients' SCORAD.

Table 1

Clinical description of AD subjects and biopsy samples

Patient Number	Gender	Age	Diagnosis	Biopsy sites	SCORAD	Serum IgE (IU/ml)
1	M	63	AD, moderate	Trunk	41	1280
2	F	22	AD, severe	Elbow	59.6	n.a.
3	M	33	AD, moderate	Upper middle back	45	n.a.
4	M	13	AD, severe	Lower extremity	63.1	1742
5	M	28	AD, severe	Abdomen	72.1	1849
6	F	21	AD, severe	Popliteal fossa	67	1457
7	M	23	AD, severe	Upper extremity	65.2	417
8	F	28	AD, severe	Lower extremity	64.2	561
9	M	16	AD, severe	Lower extremity	71	1729
10	M	72	AD, severe	Lower extremity	66	2007
11	M	67	AD, severe	Neck	74	1572