Periostin in Skin Tissue and Skin-Related Diseases
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ABSTRACT
Extracellular matrix (ECM) is not only involved in the maintenance of normal physiological tissue but also in interactions with other ECM components, tissue remodeling, and modulating immune responses. The skin provides a distinct environment characterized by rich fibroblasts producing various ECM proteins, epithelial-mesenchymal interactions, and immune responses induced by external stimuli.

Recently, periostin—a matricellular protein—has been highlighted for its pivotal functions in the skin. Analysis of periostin null mice has revealed that periostin contributes to collagen fibrillogenesis, collagen cross-linking, and the formation of ECM meshwork via interactions with other ECM components. Periostin expression is enhanced by mechanical stress or skin injury; this is indicative of the physiologically protective functions of periostin, which promotes wound repair by acting on keratinocytes and fibroblasts. Along with its physiological functions, periostin plays pathogenic roles in skin fibrosis and chronic allergic inflammation. In systemic sclerosis (SSc) patients, periostin levels reflect the severity of skin fibrosis. Periostin null mice have shown reduced skin fibrosis in a bleomycin-induced SSc mouse model, indicating a key role of periostin in fibrosis. Moreover, in atopic dermatitis (AD), attenuated AD phenotype has been observed in periostin null mice in a house dust mite extract-induced AD mouse model. Th2 cytokine-induced periostin acts on keratinocytes to produce inflammatory cytokines that further enhance the Th2 response, thereby sustaining and amplifying chronic allergic inflammation. Thus, periostin is deeply involved in the pathogenesis of AD and other inflammation-related disorders affecting the skin. Understanding the dynamic actions of periostin would be key to dissecting pathogenesis of skin-related diseases and to developing novel therapeutic strategies.

KEY WORDS
atopic dermatitis, fibrosis, matricellular protein, periostin, remodeling

INTRODUCTION
The skin is the largest organ of the human body, providing a protective barrier against external pathogens/allergens and thermal and physical injuries; moreover, it plays a role in immunological surveillance and acts as a sensory organ. The extracellular matrix (ECM) is a large component of the dermis and is critical for maintaining normal physiological structure. In the dermis, the predominant ECM is collagen; however, other ECMs—such as fibronectin, laminins, vitronectins, and proteoglycans—are also present. Accumulating evidence has revealed that the ECM is being continuously remodeled as part of the normal homeostasis process in response to mechanical stimuli, angiogenesis, and wound healing. Further, ECM interacts with other ECM tissues, immune cells, and vascular cells, thereby regulating cell proliferation, differentiation and intracellular signaling. Since ECM remodeling is tightly regulated, even a small aberration may disturb its balance, triggering the development of diseases. For instance, fibrosis, which is essentially the excessive accumulation of ECM, can lead to organ dysfunction.

Periostin is an ECM protein composed of a N-terminal EMI domain, four repeated fascinil (Fas I) domains, and a C-terminal domain with a heparin-binding site. Periostin directly binds to type I colla-
gen\textsuperscript{11,12} and fibronectin\textsuperscript{13} via the EMI domain and to tenascin-C\textsuperscript{13} via Fas I domains, likely providing ECM meshwork. Furthermore, as periostin is a matricellular protein,\textsuperscript{14,15} secreted periostin directly acts on various cells as a ligand of integrin receptors to induce cell signaling for proliferation, migration, and differentiation. Interleukin (IL)-4, IL-13, and transforming growth factor-\(\beta\) (TGF-\(\beta\)) are known as major triggers of periostin production.\textsuperscript{11,16} Recent studies have revealed that periostin is prominently expressed during tissue development and ECM remodeling under mechanical stress and injury, playing a role in various pathologies, such as cancers,\textsuperscript{10,17,18} myocardial infarction,\textsuperscript{19,20} valvular heart diseases,\textsuperscript{21,22} fibroproliferative disorders,\textsuperscript{23-26} and chronic allergic inflammations.\textsuperscript{11,27-31}

Since the skin consists of rich ECM produced by fibroblasts and the immune response occurs there, the involvement of periostin in skin remodeling and certain skin-related pathologies has been hypothesized. In fact, periostin has been demonstrated to play a key role in collagen fibrillogenesis and wound repair by analyzing periostin knockout mice.\textsuperscript{9,12,32-36} Interestingly, recent advances in periostin research have shown its functional role in the etiology of diseases, particularly in atopic dermatitis (AD) as a skin-related allergic disease.\textsuperscript{30,31} Clarifying the pathogenic properties of periostin may lead to new therapeutic strategies as well as to novel clinically useful tools, such as biomarkers.

This review focuses on the physiological roles of periostin in the context of cutaneous development, regeneration, and wound repair. Furthermore, it discusses the pathogenic roles of periostin in skin-related diseases, including fibroproliferative disorders and allergic diseases. For additional reviews regarding the role of periostin in lung- or nose-related diseases, please see other articles in this issue.\textsuperscript{37,39}

**PERIOSTIN EXPRESSION DURING SKIN DEVELOPMENT AND AGING**

Periostin is widely expressed in various organs. From a gene expression comparison analysis using normal human tissues, periostin has been shown to be highly expressed in the skin as compared to that in other organs,\textsuperscript{40} probably due to the skin being an environment rich in fibroblasts, which are a major source of periostin. During embryonic development in murine skin, expression patterns of periostin vary with time over the course of development. For instance, peak periostin deposition is observed uniformly and intensely in both the epidermal-dermal junction (EDJ) and dermis at embryonic day 17.5, followed by a gradual decrease, except in hair follicles after birth.\textsuperscript{33} This suggests that periostin may mediate initial skin development and may influence the early interactions of fibroblasts and basal keratinocytes, although the exact mechanisms by which periostin contributes to embryonic skin development in vivo remain unknown.

Typically, periostin is expressed in normal human skin as a mild deposition at the EDJ, papillary dermis, and around hair follicles (Fig. 1). A recent study demonstrated that periostin expression is downregulated during skin aging, which eventually contributes to the phenotype of aged skin.\textsuperscript{41} Further, UV irradiation was shown to reduce periostin expression in dermal fibroblasts; however, periostin knockdown had no effect on collagen expression but resulted in aberrant collagen structures, leading to an increased susceptibility of collagen toward proteases.\textsuperscript{41} Thus, periostin is likely to be involved in skin homeostasis and aging, probably mediated by its functions in collagen assembly, as explained below.

**ROLES OF PERIOSTIN IN COLLAGEN FIBRILLOGENESIS BASED ON THE SKIN PHENOTYPE OF PERIOSTIN KNOCKOUT MICE**

In 2005, Rios et al. first generated periostin null mice and reported the resulting phenotypes.\textsuperscript{42} Periostin deficiency did not exhibit embryonic lethality; however, periostin null mice were severely growth retarded, indicating that periostin is important in postnatal development. The periostin null mice showed a severe periodontal disease-like phenotype with some defects in the adult periesteum, incisor enamel, cartilage, cardiac valves, and periodontal ligament (PDL). In addition, Kii et al. also suggested that periostin may be important in the remodeling of the collagen matrix in the shear zone of the PDL.\textsuperscript{43} Fundamentally, periostin appears to be required for the maintenance of tissue integrity and protection from mechanical stresses in connective tissues.

Subsequently, the skin of periostin null mice was analyzed in detail by Norris et al.,\textsuperscript{12} The skin was apparently normal; however, they demonstrated a reduced diameter of collagen fibrils in the dermis of periostin null mice, resulting in decreased skin stiffness. Collagen cross-linking was significantly reduced in the periostin-deficient mice.\textsuperscript{12} In agreement with previous data that showed interactions of periostin with various ECM proteins,\textsuperscript{11-13} they reported the direct binding of periostin to type I collagen. Thus, periostin was considered to be required for appropriate collagen fibril formation and maturation, i.e., for collagen fibrillogensis. Moreover, the ECM-periostin interaction likely contributes to optimal biomechanical function in connective tissues such as the skin, heart valves, bones, tendons, and ligaments.\textsuperscript{12} Collagen fibrillogensis is the finely regulated process of assembling collagen fibrils wherein several molecules, such as fibronectin and integrins, are involved.\textsuperscript{44} It is likely that matricellular proteins are essential for collagen fibrillogensis, since defective collagen fibrils have also been found in mice with a gene deficiency for another matricellular protein.\textsuperscript{45}
Since periostin null mice exhibit impaired collagen cross-linking, it is hypothesized that periostin likely affects lysyl oxidase (LOX), an enzyme involved in cross-linking collagen that influences the mechanical properties of connective tissues. Recently, Maruhashi et al. revealed that periostin enhanced LOX activity; the levels of active LOX protein, formed by the cleavage of its propeptide by bone morphogenetic protein-1 (BMP-1), were decreased in periostin null mice. Moreover, the overexpression of periostin promoted the proteolytic cleavage of the propeptide mediated by BMP-1, which binds to periostin. Thus, periostin directly supported the BMP-1-mediated activation of LOX, accelerating collagen cross-linking and maintaining the stiffness of connective tissues.

PERIOSTIN IN CUTANEOUS WOUND HEALING

Cutaneous wound healing is a series of physiological events, which involves inflammation, reepithelialization, neovascularization, granulation tissue formation, wound contraction, and ECM reorganization, in that order. After tissue injury, the induction of periostin has been described in various tissues, including the skin. In an experimental murine dermal wound model, periostin upregulation was detected first in the granulation tissue at day 3, followed by a peak level at day 7, and returning to normal levels by 28 days. Therefore, periostin is expressed not in the initial inflammatory stage of wound repair but rather in the granulation and remodeling stages.

Recently, three papers have been published examining the potential roles of periostin in excisional dermal wound repair by analyzing periostin null mice. Indeed, although the underlying mechanisms proposed in these papers differed slightly, similar results were observed all three reports, which demonstrated delayed wound closure between days 3 and 7 in the knockout mice, suggesting consistent and reproducible results. Nishiyama et al. proposed that the delayed wound repair in the gene-deficient model was due to defective reepithelialization and the reduced proliferation of keratinocytes. Periostin-overexpressing HaCaT cells were used to confirm keratinocyte proliferation through phosphorylation of the nuclear factor-xB (NF-xB), which is induced by the proteolytic cleavage of laminin γ2 in association
with periostin. Second, Elliot et al. confirmed a significant reduction in the numbers of α-smooth muscle actin (α-SMA)-positive myofibroblasts in the absence of periostin in the granulation tissue. They proposed that periostin promoted wound contraction by facilitating the differentiation of myofibroblasts. Finally, based on in vitro analysis, Ontsuka et al. showed that the loss of periostin resulted in defects in fibroblasts differentiation and migration. Although the mechanisms underlying the role of periostin in wound healing in vivo remain controversial, partly because in vitro evidences may not always reflect in vivo phenomena, the three reports clearly revealed the crucial role of periostin in cutaneous wound repair.

In dermal wound repair, the proliferation and migration of keratinocytes are essential for skin reepithelialization. Moreover, the activation and differentiation of fibroblasts to myofibroblasts are also critical processes for appropriate skin contraction and remodeling. Recently, using different cell types and experimental methodologies, in vitro studies have examined the actions of periostin on fibroblasts and keratinocytes and their proliferation, migration, and differentiation. As a matricellular protein, periostin interacts with several integrin molecules, such as αvβ3 or αvβ5 on the cell surface, and activates phosphatidylinositol 3-kinase (PI3K)/Akt, focal adhesion kinase (FAK), and mitogen-activated protein (MAP) kinases to exert its cellular functions. However, the molecular mechanism underlying the actions of periostin in the paracrine and/or autocrine loops, i.e., the epithelial-mesenchymal cross-talk through periostin, remains poorly understood. A recent study suggested the necessity of mammalian target of rapamycin (mTOR) signaling in periostin-induced epithelial migration and proliferation, wherein mTOR activation in epithelial cells was shown to be induced not only by fibrolast-derived periostin secreted during wound healing but also by endogenously expressed periostin in epithelial cells under mechanical stress. This result may provide a clue in understanding the dynamic actions and expressions of periostin, both of the variant form as well as the full-length form, since only a variant form of periostin can be secreted.

Using a three-dimensional organotypic air-liquid interface co-culture system, Taniguchi et al. recently demonstrated that periostin regulates keratinocyte proliferation and differentiation in epithelial-mesenchymal interactions through a combination of an autocrine loop of periostin and a paracrine loop composed of IL-1α and IL-6. That is, the release of IL-1α from keratinocytes and IL-6 production from fibroblasts were critical for keratinocyte proliferation and differentiation. Fibroblast-derived periostin was required for IL-1α-induced IL-6 production, which occurred via the activation of the NF-κB pathway. Thus, accumulating evidence demonstrates the importance of epithelial-mesenchymal cross-talk with periostin as a key modulator in wound repair (Fig. 2). Applying these findings to various skin-related pathologies is expected to be of great interest.

PERIOSTIN IN SKIN-RELATED DISEASES

SKIN FIBROSIS

The excessive deposition of ECM components such as collagen and fibronectin in tissues is defined as fibrosis. Organ fibrosis is a final common pathway for many diseases and leads to end-stage organ failure. Unfortunately, no effective therapy for organ fibrosis is yet available. Uncontrollable wound healing responses, including acute and chronic inflammation, angiogenesis, activation of resident cells, and abnormal ECM remodeling, are thought to be involved in the pathogenesis of fibrosis. TGF-β is regarded as a major profibrotic factor, stimulating the synthesis of ECM molecules and activating the differentiation of fibroblasts to myofibroblasts.

Significant periostin expression has been detected during various tissue remodeling processes and in fibrotic conditions, including wound healing, hypertrophic scar and keloid formation, myocardial infarction, sub-epithelial fibrosis in bronchial asthma, bone marrow fibrosis, pulmonary fibrosis, and systemic sclerosis. The previously discussed functions of periostin, i.e., those promoting collagen fibrilogenesis, collagen cross-linking, and binding to other ECM proteins, essentially provide tissue stiffness, which may contribute to fibrosis. Moreover, the matricellular actions of periostin that accelerate the proliferation and differentiation of fibroblasts to myofibroblasts also exacerbate fibrosis. Therefore, periostin appears to be actively involved in the pathogenic process of fibrosis, rather than merely being a deposited ECM component in fibrous tissue (Fig. 3).

SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is a multisystem connective tissue disease characterized by organ fibrosis particularly in the skin and lungs, microvascular abnormalities, and immune dysregulation. We and others have reported the enhanced expression of periostin in the skin of SSc patients. The clinically affected skin of patients with diffuse cutaneous SSc showed extremely intense deposition of periostin with uniform distribution throughout the dermis compared to that in patients with limited cutaneous SSc. Maximum deposition was observed in the extracellular area; moreover, periostin was also noted to be colocalized with fibroblasts, α-SMA-positive myofibroblasts, and endothelial cells. The critical involvement of periostin in the pathogenesis of skin fibrosis in SSc has been confirmed by Yang et al. using a bleomycin (BLM)-induced skin fibrosis model. The dermal
Roles of periostin in wound healing. Wound healing requires reepithelialization, granulation tissue formation, and wound contraction. Induced periostin modulates all of those steps by acting on keratinocytes and fibroblasts. Migrating and proliferating keratinocytes for the reepithelialization are shown as green. Endogenously expressed periostin and/or fibroblasts-derived periostin induce proliferation and/or migration of keratinocytes for the reepithelialization. The keratinocytes-fibroblasts cross-talk in the presence of an autocrine loop of periostin regulates proliferation and differentiation of keratinocytes. Furthermore, periostin promotes differentiation of fibroblasts to myofibroblasts for the wound contraction. Finally, activated fibroblasts and myofibroblasts accelerate ECM production, resulting in fibrosis.

thickness did not initially differ between wild-type mice (WT) and periostin null mice; however, following BLM-induced skin fibrosis, the periostin null mice showed significantly reduced dermal thickness and collagen deposition, indicating a reduction in the fibrotic process.

α-SMA-positive myofibroblasts, which persist in fibrotic connective tissue, are considered to be the final effector cell in all fibrotic conditions. Enhanced myofibroblast deposition is a hallmark of fibrosis in SSc. There is no doubt that TGF-β is a major inducer of myofibroblast differentiation; further, periostin appears to promote this process. In fact, BLM-induced myofibroblast deposition was reduced in periostin null mice. In addition, it is intriguing that TGF-β-induced myofibroblast differentiation was attenuated in periostin null fibroblasts in vitro. Moreover, although stimulation with periostin alone did not induce α-SMA expression in WT fibroblasts in vitro, TGF-β-induced α-SMA expression was enhanced in the presence of periostin. Thus, it is likely that periostin may act as a co-factor or as an enhancer of TGF-β bioactivity. Akin to periostin, another matricellular protein—connective tissue growth factor
The contribution of periostin to fibrosis. Fibrosis is caused as a result of chronic tissue injury and inflammation. Rather than just being a structural ECM protein, periostin is involved in the pathogenic process of fibrosis. Periostin induces proliferation of keratinocytes and fibroblasts, contributes to epithelial-mesenchymal transition (EMT) of keratinocytes, and promotes differentiation of fibroblasts to myofibroblasts. In addition, periostin plays an important role as a co-factor of TGF-β to promote ECM production and differentiation to myofibroblasts. Furthermore, fundamental roles of periostin, i.e., those promoting collagen fibrillogenesis, collagen cross-linking, and binding to other ECM proteins, may contribute to fibrosis by providing tissue stiffness.

(CCN2)—has also been implicated in similar findings. Rather than just being as a downstream mediator of TGF-β, CCN2 appears to be required for optimal TGF-β fibrogenic function. Similarly, periostin may create a favorable environment for the action of fibrogenic factors rather than being a principle cause of fibrosis.

Recently, we reported a significant elevation of serum periostin levels in patients with SSc. The levels were considerably higher in the early stage of diffuse cutaneous type SSc and were intensely correlated with the severity of skin fibrosis, which was determined by the modified Rodnan total skin thickness score. In terms of fibrosis, no clinically useful biomarker relevant to disease activity has been established. In particular, a biomarker of skin fibrosis with minimal interobserver variability is needed. In addition, a previous similar study also showed increased serum levels of periostin in patients with idiopathic interstitial pneumonias (IIPs) and found that the levels reflected histopathological classifications and pulmonary function. Thus, serum periostin levels may be a potential biomarker for fibroproliferative disorders.

**SKIN ALLERGIC INFLAMMATION: ATOPIC DERMATITIS**

Tissue remodeling in allergic inflammation has been implicated in various diseases, including bronchial asthma, allergic rhinitis, eosinophilic esophagitis, and atopic dermatitis. Recurring allergic inflammation, which is predominantly associated with Th2 cytokines such as IL-4/IL-13, induces fibrosis because of continuous tissue repair and remodeling, resulting in a clinically intractable condition. Discovering the mechanisms underlying such remodeling in order to modulate them may lead to novel therapeutic strategies against chronic allergic inflammatory diseases in the near future.

In recent years, the role of periostin in allergic inflammation has been highlighted. The POSTN gene that encodes periostin was identified as a IL-4/IL-13 inducible gene by microarray analysis using human bronchial epithelial cells; further, Takayama et
found that IL-4/IL-13-induced periostin was a novel component of subepithelial fibrosis, which is a cardinal feature of bronchial asthma. As discussed in the other review articles in this issue, it is noteworthy that serum periostin levels appear to be a novel potential clinical biomarker that can predict the efficacy of lebrikizumab, a IL-13 monoclonal antibody, in asthma patients with inadequately controlled disease despite inhaled glucocorticoid therapy.

One of the most important skin-related allergic inflammatory diseases is atopic dermatitis (AD). AD is a chronic inflammatory disorder characterized by eczematous skin lesions with intolerable pruritus. Rubbing or scratching behavior due to pruritus is one of the triggers of chronic skin lesions, especially of lichenification, which pathologically consists of epidermal acanthosis, chronic infiltration by inflammatory cells, and fibrosis. Increased levels of IL-13 in lichenified AD skin indicate the possible importance of IL-13 in chronic inflammation and remodeling in AD.

Although the etiology of AD has not been fully explained thus far, recent studies have well characterized the pathogenesis of AD. A disruption of the skin barrier, which is partly linked to loss-of-function mutations in the gene encoding filaggrin, may allow dermal penetration by external allergens, inducing allergic inflammation. Several analyses using various mouse models have revealed the importance of Th2 cytokines in AD. In addition, epithelial cell-derived molecules, such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, are critical for enhancing the Th2 response. Particularly, TSLP is a key molecule linking keratinocytes with dendritic cells that initiates Th2-polarized inflammation. However, the underlying pathogenic mechanism for persistent TSLP production in the chronic course of AD remains unclear. In other words, the manner in which immune and non-immune cells interact with each other in mediating chronic inflammation in AD has not been well understood. In this regard, Masuoka et al. recently reported a pivotal pathogenic role of periostin in chronic allergic inflammation in AD. Accumulating periostin in the dermis and an AD-like phenotype were observed in house dust mite extract (HDM)-induced AD model mice in BALB-c as well as C57BL6 mice, but not in Rag-2 \( ^{-/-} \) \( \gamma c^{-/-} \) mice. However, genetically periostin-deficient mice exposed to HDM had impaired Th2-induced inflammation and lacked the morphological changes of AD, indicating that periostin is critical for the induction of allergic skin inflammation in this mice model. Furthermore, they found that IL-4 and IL-13 stimulated fibroblasts...
to generate periostin, which acted on keratinocytes to induce NF-κB activation via integrin αv, eventually resulting in the production of proinflammatory cytokines such as TSLP. Consequently, these keratinocyte-derived cytokines amplified the Th2-type immune response, leading to further production of periostin.30 Hence, a vicious loop of Th2 and periostin maintains persistent inflammation in AD (Fig. 4). Considering these findings together, it is greatly intriguing that the ECM protein “periostin” exhibits a prominent role in a linked series of events at all levels, including Th2 inflammation, keratinocyte activation, and possibly skin remodeling (Fig. 2), in the chronic inflammation of AD. Notably, the inhibition of periostin or integrin αvβ3 prevented AD inflammation in their mouse model.30 Thus, blocking the action of periostin or periostin receptors is an attractive therapeutic strategy for AD, although care should be taken regarding the possible risks of blocking periostin’s actions in tissue development, remodeling, and wound healing.

Recently, we analyzed periostin levels in 257 adult AD patients.85 Serum levels of periostin reflected disease severity; patients with the most severe type, i.e., “erythroderma,” had significantly elevated levels of periostin. In addition, periostin was strongly accumulated in the dermis of AD skin lesions with erythroderma as well as lichenification. Furthermore, the levels of thymus and activation-regulated chemokine (TARC) and lactate dehydrogenase (LDH) as well as eosinophil counts correlated with those of serum periostin; serial analysis showed a decrease in periostin levels following treatment.85 These results indicate the possibility of using periostin as a new biomarker for AD.

Clinically, TARC has been shown to be an excellent biomarker, with high correlations to the severity of skin lesions expressed by SCORAD score.86 For biomarkers, it is important to evaluate the clinical utility of the molecule. With respect to periostin and TARC, our preliminary study demonstrated that most cases showing deviations between the levels of TARC and periostin had concurrent viral or bacterial infection or an acute exacerbation of AD because of inappropriate treatment. This indicated that the levels of periostin might not be influenced by infections or by acute events. Periostin levels may thus be a useful novel chronic biomarker for AD, particularly when simultaneously evaluated with TARC. Further studies are needed for evaluating the clinical significance of periostin levels in AD.

CONCLUSIONS

In summary, the functions of periostin in the skin may be divided into two major roles as follows: (i) physiologically protective actions and (ii) pathogenic contributions. Appropriate collagen assembly in the skin requires periostin, and the enhancement of periostin expression under mechanical stress and injury contributes to physiological ECM remodeling and wound repair. On the other hand, as a result of aberrant ECM remodeling, periostin is pathogenically involved in fibrosis-enhancing TGF-β activity. Furthermore, allergic conditions such as AD favor the production of periostin, which maintains and amplifies chronic allergic inflammation via fibroblast-keratinocyte cross-talk. Periostin acts on cells either endogenously or exogenously in a paracrine or autocrine manner. Further research on periostin for analyzing the different characteristics of known periostin variants, may provide a new understanding of this molecule and offer potential therapeutic strategies against skin-related inflammatory diseases.

ACKNOWLEDGEMENTS

I would like to thank Dr. Kenji Izuhara (Saga Medical School, Japan) for his valuable collaboration and Dr. Michiko Aihara (Yokohama City University, Japan) for her support. This work was supported by Grants-in-aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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Allergology International Vol 63, No2, 2014 www.jsaweb.jp/


