

Review Article

Calprotectin functions in the skin: an overview

鈣衛蛋白在皮膚中的功能之概述

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S100A8/A9 is a heterodimer of calcium-binding proteins that displays a broad range of cell-type-dependent functions. It enhances the innate immunity through Toll-like receptor 4, the receptor for advanced glycation end-products (AGE), or by promoting the release of inflammatory cytokines. It is highly expressed during all phases of wound healing, coordinating the healing processes in different cell types. As an antimicrobial peptide (AMP), it attacks the bacteria directly by arresting their growth through sequestration of manganese and zinc inside the bacterial cells. S100A8/A9 overexpression suppresses keratinocyte proliferation and promotes their differentiation. This leads to a form of immune reservoir against UV-induced carcinogenesis, where S100A8/A9 is overexpressed. Pathway interactions of S100A8/A9 in different skin diseases are still to be investigated.

S100A8 / A9 蛋白是一種鈣結合蛋白的異二聚體，顯示出廣泛的細胞依賴型功能。它通過第四型類鐸受體（晚期糖基化終產物的受體）或通過促進炎性細胞因子的釋放來增強先天免疫力。在傷口癒合的所有階段它都高度表達，以協調不同細胞類型的癒合過程。作為抗微生物肽，它通過在細菌細胞內隔離錳（ Mn^{2+} ）和鋅（ Zn^{2+} ）來直接中止細菌的生長。S100A8 / A9 蛋白的超量表達會抑制角質細胞增殖並促進其分化；這促成一種免疫儲備的模式來抵抗紫外線誘發的癌變，需要時S100A8 / A9蛋白便會超量表達。S100A8 / A9蛋白在不同皮膚疾病中的相互作用通路，仍有待研究。

Keywords: AMPs, calprotectin, inflammatory skin diseases, keratinocyte proliferation, S100A8/A9

關鍵詞： 抗微生物肽、鈣衛蛋白、炎症性皮膚病、角質細胞增殖、S100A8 / A9 蛋白

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Introduction

Located on chromosome 1q21 in a gene cluster known as epidermal differentiation complex (EDC),¹ the S100 proteins constitute a family of low molecular weight calcium-binding proteins of the human epithelium (Figure 1).^{2,3} First, they were found to be overexpressed in psoriasis,⁴ and upregulated by retinoic acid,^{5,6} and seem to be important epithelial constituents. They are encoded by a family of genes whose symbols use the S100 prefix and perform diverse functions in the human skin.^{2,3} S100 proteins are also considered as environmentally relevant damage-associated molecular pattern molecules (DAMPs), since knockdown of aryl hydrocarbon receptor downregulates their expression.⁷

The two myeloid-related protein-14 and 8 (MRP14 and MRP8), also called S100A9 and S100A8 respectively, are members of the S100 protein family and form together a functioning heterodimer called calprotectin (Figure 2).⁸ This complex is involved in a variety of biological processes, including calcium-dependent signaling pathways and cell differentiation. Moreover, it is a very sensitive local and systemic inflammatory biomarker reflecting inflammatory activities even in subclinical stages,⁸ which may give S100A8/A9 a clinical significance in many diseases. For instance, its concentration in faeces and serum was found to be directly correlated with the disease activity in patients with ulcerative colitis and Crohn's disease.^{9,10} The serum calprotectin has also been found to be a good indicator for psoriasis and arthritis severity.¹¹ However, calprotectin is not yet a suitable substitute for the well-established inflammatory markers (e.g., erythrocyte sedimentation rate or c-reactive protein [CRP]) for three reasons. First: the levels of S100A8/A9 have been found not to correlate to the course of certain inflammatory diseases, as discussed below. Second, in comparison to CRP, they are non-standardised and cost-effective measurement tools. Third, S100A8/A9 expression

and the clinical significance in the majority of inflammatory diseases remain to be investigated.

Expression of S100A8/A9 in inflammatory skin diseases

The contribution of calprotectin has only been considered in a few dermatoses. In psoriasis, S100A8/A9 is highly overexpressed in the lesions and regulated by oncostatin M through the STAT3 pathway.¹² While S100A8/A9 is a potent suppressor for keratinocyte proliferation and an inducer for differentiation, this mechanism is obviously ineffective in psoriasis where keratinocytes undergo excessive hyperproliferation. In contrast, S100A8/A9 in atopic dermatitis is undetectable. Remarkably, the treatment of atopic dermatitis with pimecrolimus induces the expression of S100A8/A9 and enhances barrier function.¹³ S100A8/A9 is strongly overexpressed in the affected skin with hidradenitis suppurativa/acne inversa (HS) in comparison to healthy looking skin from the same patient.¹⁴ Unlike psoriasis, where the serum levels of S100A9/A8 correlate to the disease severity, recent studies involving 29 patients with HS have shown no correlation between disease severity and the serum level of S100A9/A8.^{4,15} However, the functional pathways and interactions of S100A8/A9 in HS still need to be studied.

Role of S100A8/A9 in the innate immunity

At the cellular level, the expression of calprotectin under normal conditions can be seen predominantly in the myeloid cells (neutrophils, monocytes, macrophages). It is expressed in the epidermal keratinocytes and endothelial cells only under stress, suggesting involvement of S100A8/A9 in the intercellular biological processes within the non-myeloid cells.¹⁶ S100A8/A9 alone comprises 30-60% of the cytosolic proteins of neutrophils. This observation has always indicated a central role of S100A8/A9 in the innate immune response. Although this fact was discovered more

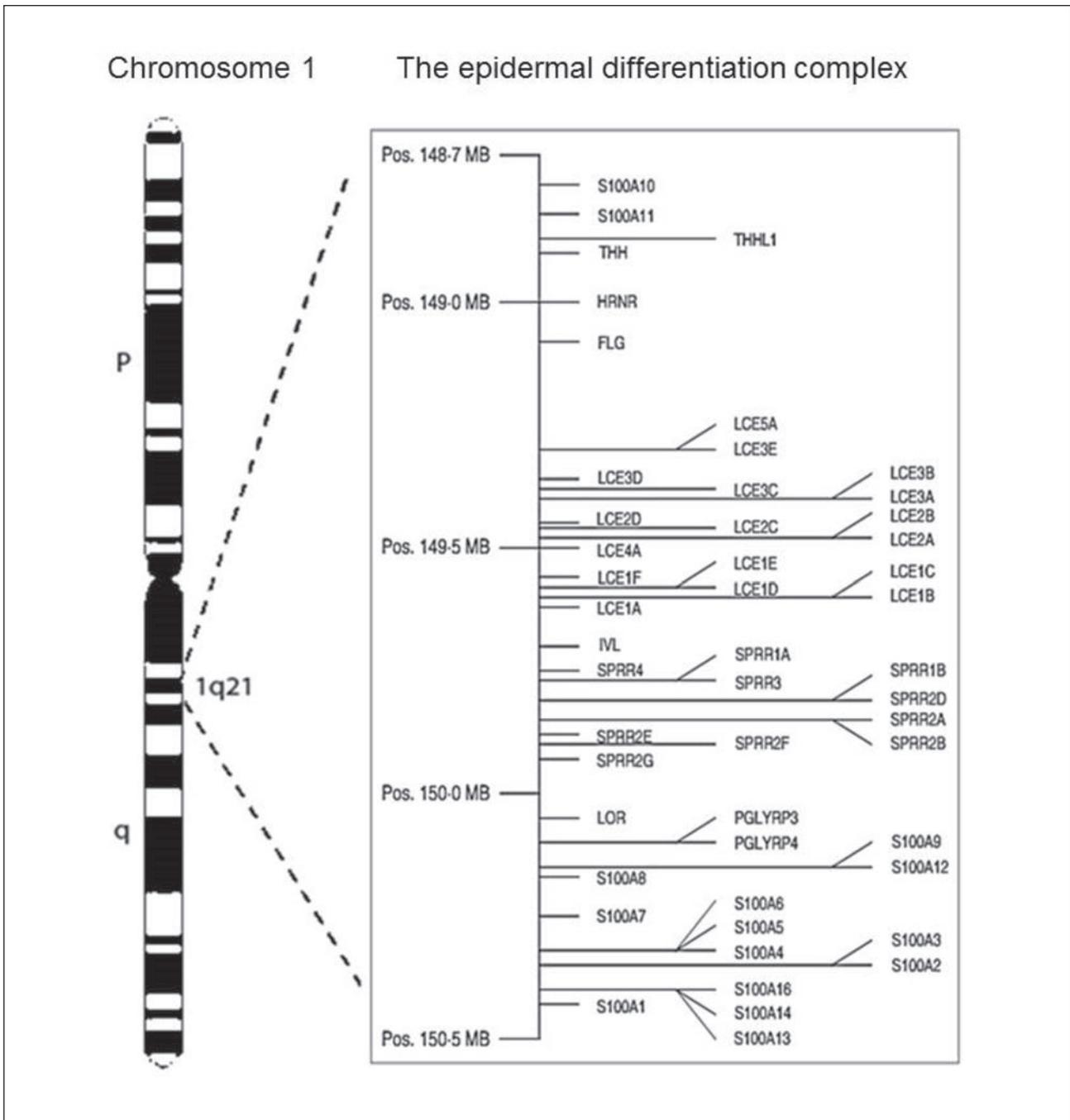


Figure 1. S100A8/A9 encoding genes are located within the epidermal differentiation complex on the long arm of chromosome 1, where a coordinately local regulation with other S100 proteins and the proteins of the cornified cell envelope (involucrin, lorcin, and filaggrin) can be seen. Source: UCSC Genome Browser database (<http://genome.ucsc.edu/>).

than 20 years ago, the exact mechanism was not known until the recent evidence of its role as endogenous damage-associated molecular pattern molecule. It promotes the inflammatory response to lipopolysaccharides by endogenous activation of the Toll-like receptor 4 or the receptors for the advanced glycation end products (AGE). This kind of response was seen in cancer cells as well as in autoimmunity and infection.¹⁷⁻¹⁹ S100A8/A9 is not only a strong inducer of chemotaxis and adhesion of neutrophils,²⁰ but also a potent amplifier of the inflammatory process, producing tumor necrosis growth (TNF)- α and other cytokines. It is also essential for development of autoreactive CD8+ T cells.

During wound healing, S100A8/9 is expressed by inflammatory cells as early as the first three hours after injury (inflammatory phase). It is involved in the release of many inflammatory mediators from monocytes and macrophages and promotes their migration.²¹

S100A8/A9 functions in keratinocytes

S100A8/A9 coding genes are located in the EDC locus, where coordinated local regulation among the locus genes can be seen.²² As mentioned earlier, calprotectin induces migration of keratinocytes and suppresses their proliferation under inflammatory stimuli in-vitro.²³ As a result,

the overexpressed S100A8/A9 arrests the growth of the damaged cells, induces apoptosis and prevents UV-induced carcinogenesis. The same mechanism is also an effective response against viral infections.²⁴

During the late phases of wound healing (the proliferative phase and the remodeling phase), S100A8/A9 induces proliferation, migration, and differentiation of keratinocytes and fibroblasts. It also endorses the release of their proteases and growth factors.²⁵ S100A8/A9 promotes keratinocyte differentiation through activation of the NF- κ B pathway by activation of epithelial nicotinamide adenine dinucleotide phosphate (NADPH).²³ Additionally, it creates a positive feedback loop, stimulating the growth of the keratinocytes involving the cytokines: Interleukin (IL)1 α , IL6, IL8, TNF- α , interferon (IFN)- α , IFN- γ , CXCL1, CXCL2, CXCL3 and chemokine ligand 20.²⁶ S100A8/9 overexpression impairs cell growth and differentiation.²⁷ Interestingly, the bacterial flagellin of *Escherichia coli* (*E. coli*) upregulates the expression of S100A8/9 in the keratinocytes.²⁸ In addition to the indirect role of S100A8/A9 in innate immunity by promoting production of cytokines, it acts directly against *E. coli*, *Staphylococcus aureus* (*S. aureus*), *S. epidermidis*, *Klebsiella* and fungal infections, as a member of the antimicrobial peptides. The

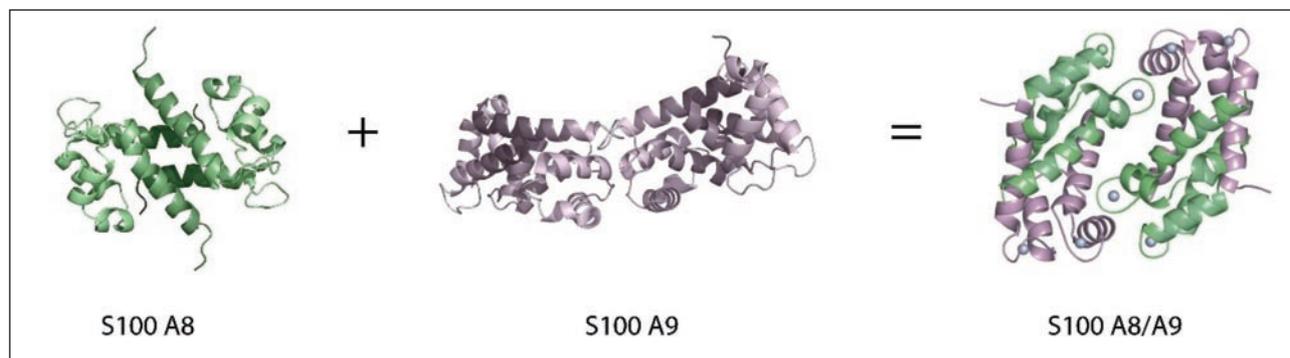


Figure 2. Crystal structure of S100A8 (green), S100A9 (purple), and S100A8/A9 heterodimer. Ca²⁺ ions (shown in blue) are essential for forming the chemically stable and physiologically active calprotectin heterodimer [Source of the S100A8/A9 structure: from Vogl T et al. Int J Mol Sci 2012;13:2893-2917].³⁴

mechanism includes the sequestration of manganese and zinc inside the bacterial cells, which inhibits their growth.²⁹

Unexpectedly, S100A8/A9 shows an anti-inflammatory phenotype in dendritic cells.³⁰ The non-inflammatory functions of S100A8/9 in non-myeloid cells (e.g. keratinocytes, endothelial cells, vascular smooth muscle cells) have received more attention as a potential mediator in the ischaemic heart diseases and cancers.³¹ A high serum level of S100A8/A9 in smokers and obese individuals has been linked significantly to the other risk factors of coronary artery disease and the prognosis of patients with ischaemic heart disease.³² The involvement of this heterodimer in the pathogenesis of many inflammatory diseases (e.g. rheumatoid arthritis, inflammatory bowel disease, cystic fibrosis, diabetes mellitus I, and

inflammation-associated cancers) has also received further attention in recent years.

Conclusion

Though S100A8 and S100A9 are functionally and structurally comparable, they have distinctive intra- and extracellular functions.³³ Their contribution in the innate immune response, not only through the myeloid cells, but also by modifying the proliferation and growth of skin cells, and especially keratinocytes, plays a central role in many inflammatory responses including wound healing (Figure 3). The functions of S100A8/A9 as an anti-microbial peptide in addition to its regulatory effects on keratinocyte differentiation may help us better understand the pathogenesis of several skin diseases.

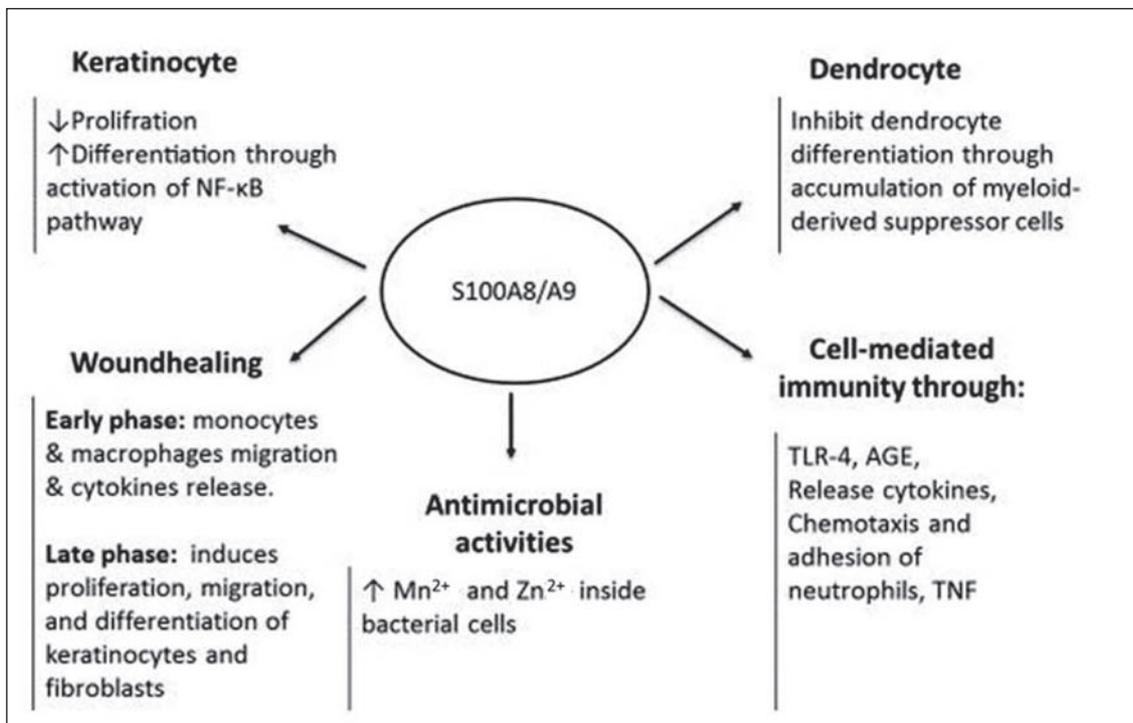


Figure 3. In addition to its role in the innate immunity and wound healing process, calprotectin inhibits carcinogenesis by promoting keratinocyte differentiation but also impairs antitumor immune responses by inhibition of dendritic cell differentiation.

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Conflict of interest

The authors declare no conflict of interest.

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