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Review

Review

The role of macrophages in the formation of hypertrophic scars and keloids

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Abstract

Numerous studies have shown that macrophages can orchestrate the microenvironment from the early stage of wound healing to the later stages of scar formation. However, few reviews have highlighted the significance of macrophages during the formation of abnormal scars. The purpose of this review was to outline the polarization of macrophages from early to late stage of pathological scar formation, focusing on spatiotemporal diversity of M1 and M2 macrophages. In this review, the role of macrophages in the formation of hypertrophic scars and keloids is summarized in detail. First, an increased number of M2 cells observed before injuries are significantly associated with susceptibility to abnormal scar pathogenesis. Second, decreased expression of M1 at the early stage and delayed expression of M2 at the late stage results in pathological scar formation. Third, M2 cells are highly expressed at both the margin and the superficial region, which is consistent with the invasive property of keloids. Finally, this review helps to characterize strategies for the prediction and prevention of pathological scar formation.

Key words: Hypertrophic scar, Keloid, Macrophages, Predisposition, Wound healing

Background

The normal wound healing response can be categorized into haemostasis, inflammation, proliferation and remodeling, and can result in scar formation. This delicate balance of healing processes can be impaired dramatically, resulting in a chronic wound or excessive abnormal scar formation. A persistent inflammatory phase and delayed wound healing lead to the formation of hypertrophic scars (HTS) [1,2]. Keloids may appear directly after wound injury or grow some years later from a mature scar [3]. The complexity of scar formation makes it difficult to summarize the process with a single explanation. The immune system has been shown to regulate atypical fibroblast proliferation, myofibroblast transformation [4] and collagen I accumulation [5] during abnormal scar formation.

The crucial roles of macrophages during skin repair and different healing stages have been well described [6]. Numerous studies have shown that macrophages can orchestrate the microenvironment from the early stage of wound healing to the late stage of scar formation [7]. The depletion of macrophages during different wound healing stages revealed that macrophages have intense impacts on stage-specific healing mechanisms [6, 8]. In a mouse model, macrophage influx at the early stage (0–4 days) of skin repair induces robust vascularized granulation tissue, myofibroblast differentiation and wound contraction [9]. During the intermediate stage

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(4–8 days) of healing response, macrophages can not stabilize vascular structures and transfer granulation tissue into scar tissue [6]. There is no impact of macrophages at the late stage (8–4 days) of the wound healing process [6].

The classical monocytes, which are CD14++CD16- [8], are derived from bone marrow and circulate in the blood [10]. In response to damage-associated molecular pattern molecules (DAMPs) or pathogen-associated molecular patterns (PAMPs), interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α) and chemokine C-C motif ligand (CCL2) [11], circulating monocytes are recruited into tissues. When there is an injury, the presence of inflammatory cytokines, such as TNF-α and interferon-γ (IFN-γ), facilitates the recruitment and adhesion of circulating monocytes to endothelium and translocation into the tissue space [12]. After entering the wound space, CD14+ monocytes transform into macrophages, which not only engulf the pathogens and cellular debris but also produce cytokines and stimulate collagen production and angiogenesis to initiate the healing processes [13, 14].

Depending on different microenvironments, macrophages can polarize into two major phenotypes. Monocytes polarize into classically activated M1 macrophages in the presence of IFN-γ, TNF-α, DAMPs and lipopolysaccharide (LPS) [15, 16]. These pro-inflammatory macrophages secrete cytokines, such as IL-1β, IL-6 and TNF-α [4], which are responsible not only for participating in immune reactions but also stimulating proliferation of fibroblasts and keratinocytes. The function of these M1 macrophages is to remove cellular debris from the wound [17]. Macrophages are considered plastic cells; they can change their phenotypes according to their local cytokine/chemokine microenvironments [18–20]. Generally, activated M2 macrophages can be produced by the stimulation of IL-4, IL-13 or apoptotic neutrophils. After activation, these M2 macrophages produce cytokines—such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF)—and stimulate proliferation of keratinocytes and fibroblasts [21]. Specifically, M2 macrophages are now classified into four subgroups: M2a, M2b, M2c and M2d [22]. M2a macrophages, also known as wound healing macrophages, are stimulated by IL-4 and/or IL-13 and produce high levels of arginase-1 (Arg-1), PDGF, IGF-1 and other cytokines [23]. The M2a macrophages can produce collagen precursors to stimulate fibroblasts during tissue repair [12, 24]. They are also involved in the extracellular matrix (ECM) formation and angiogenesis [12, 24]. M2b macrophages, also known as regulatory macrophages, can be stimulated by toll-like receptors or IL-1 receptor ligands [12]. M2b macrophages produce high levels of IL-10 to suppress inflammation and secrete IL-6, TNF and different matrix metalloproteinases (MMPs) [7]. M2c macrophages, also known as pro-resolving macrophages due to their matrix remodeling ability [12], are stimulated by glucocorticoids, IL-10 and TGF-β [25]. They can secrete IL-10, IL-1β, MMP9 and TGF-β. M2d macrophages are activated by IL-6 and adenosine receptors and produce high levels of IL-10, TGF-β and VEGF [26]. They can also inhibit pro-inflammatory M1 macrophages by down-regulating TNF-α and IL-12 [27] (Fig. 1).

Previous studies have focused on the different M1/M2 distributions during abnormal scar formation [28, 29]. Increasing evidence shows that M1 is the predominant macrophage population in the early stage of scar formation (inflammatory and early proliferative stage), whereas M2 is the main population in the late stage of scar formation (late proliferative and remodeling stage) [30]. The temporal changes in M1/M2 distribution occur in various tissue repair processes and fibrosis, including skin [30], kidney [31] and liver [32]. After skin injury, Jin *et al*. demonstrated that M1-associated genes and proteins were less elevated in keloid tissues than M2-associated genes and proteins [33]. Additionally, Li *et al*. suggested that infiltrated M2 was more commonly present than M1 in keloid tissues [34]. However, simply considering the polarization of M1 to M2 macrophages does not explain the role of macrophages in abnormal scar formation. It is important to consider other characteristics that macrophages show during the formation of abnormal scars, which, in turn, may lead to various clinical strategies.

In this article, we review the characteristics of macrophages during the formation of HTS and keloid. In addition, the significant roles of macrophages in scar predisposition are described. Finally, the polarization of macrophages from the early stage to the late stage of HTS formation and the recent studies examining spatial variances of keloids are summarized.

Review

Role of macrophages in scar predisposition

The formation of abnormal scars is promoted by systemic factors, including genetics, sex hormones, hypertension and smoking; and by local stimuli, such as mechanical tension and inflammation [35]. Genetics has a strong relation to keloid predisposition [36]. For example, people of darker skin complexion and those with a family history of keloid have a higher predisposition for keloid occurrence [37]. Inflammation plays a critical role in scar formation, which is not only affected by the post-wound microenvironment but also by the number and subtypes of macrophages presenting in the tissue before the injury. In a prospective study [38], the authors took biopsies immediately after the incision and investigated baseline M2 macrophages in the local wound healing milieu. During the follow-up period, the group of patients who developed HTS had higher baseline M2 macrophages (CD68+, CD206+) compared with patients who developed normal scars [38] (Fig. 2a). While studies on preoperative macrophages in keloid formation are still insufficient, infiltrated M2 macrophages have been primarily found in keloid tissues compared to normal skin [29]. The possible explanation for the association between increased preinjury M2 macrophages and HTS

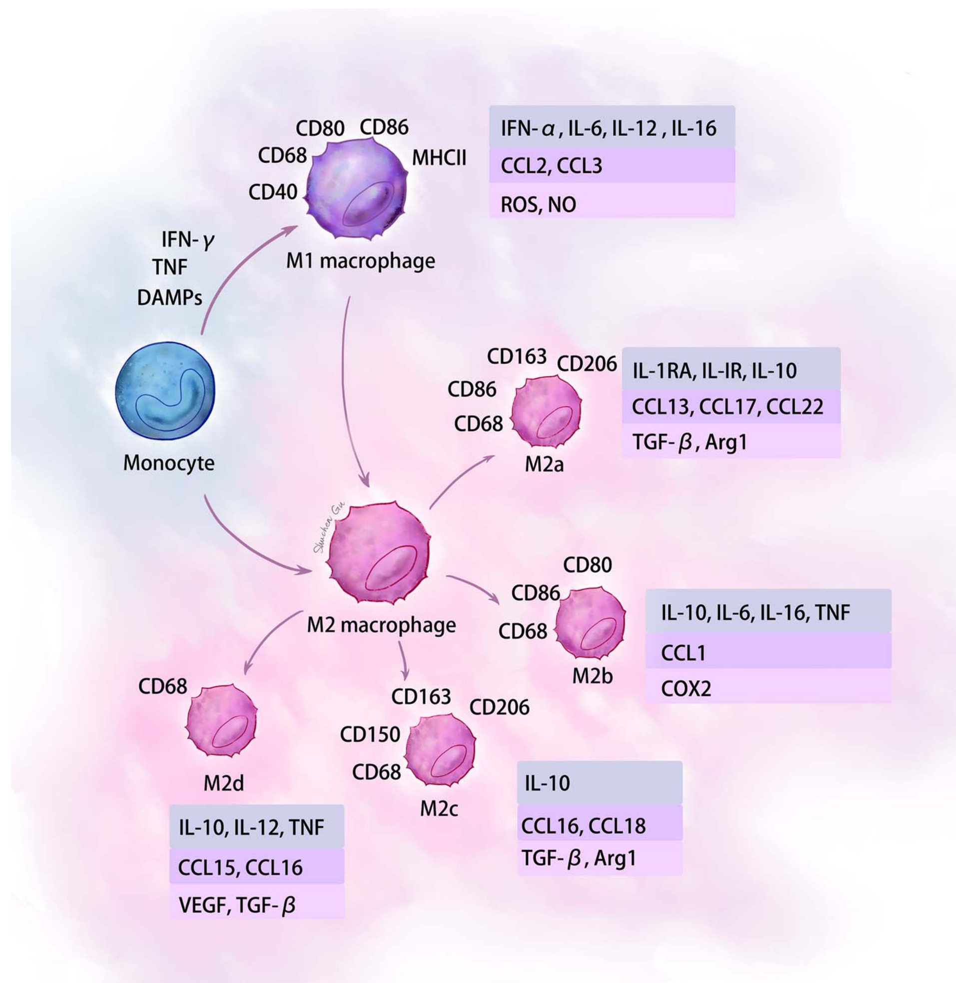

Figure 1. Summary of the polarization states and major cytokines and chemokines produced by macrophages. *IFN-γ* interferon gamma, *DAMPs* damage-associated pattern molecules, *TNF* tumor necrosis factor, *IL* interleukin, *VEGF* vascular endothelial growth factor, *TGF-β* transforming growth factor-beta, *MHC* major histocompatibility complex, *NO* nitric oxide, *Arg* arginase

formation could be that tissue-resident macrophages altered the immune microenvironment to suppress adaptive immune responses, including M1 macrophages, to favour HTS formation [39]. Tissue-resident macrophages are extremely heterogeneous, which are determined by tissue-specific niche through paracrine signaling, cell-to-cell interaction and local factors, such as inflammation [40]. During wound healing, the fundamental role of tissue-resident macrophages in immune surveillance and induction of inflammation has been well described. However, the heterogeneity of tissue-resident macrophages pre- and post-injury, as well as their corresponding contributions to abnormal scar formation, should be well investigated in the future.

Furthermore, the incidence of keloid in different body sites is related to the number and subtypes of macrophages. Previously, Butzelaar et al. measured the macrophages of skin samples from both predilection sites (such as earlobes, mandible, neck and shoulders) and non-predilection sites (such as the upper eyelid, cheek and abdomen) [41]. The results demonstrated that significantly lower numbers of M1 macrophages (CD40+) were observed at the predilection sites of keloid formation, but equal numbers of M2 macrophages (CD163+) were observed at the predilection sites and non-predilection sites [41]. The existence of an anti-inflammatory microenvironment before an injury is one major distinction of predilection and non-predilection sites of keloid formation. Therefore, although the mechanisms underlying scar predisposition have not been thoroughly elucidated, it seems that the increased number of M2 macrophages and decreased/equal number of M1 macrophages play a vital role in scar susceptibility [7, 42, 43]. In the future, the parameters of M1/M2 macrophage balance in normal skin should be established and these parameters could be used as potential predicting factors for risk evaluation for pathological scar formation.

To prevent the formation of pathological scars, changing the polarization of macrophages before or after injuries may be a novel clinical strategy. In malignant tumors, one of the characteristics is the polarization of tumor-associated macrophages from M1-like macrophages (pro-immune) to M2-like macrophages (immune-suppressive). In tumor immunotherapy, macrophages can be polarized into M1

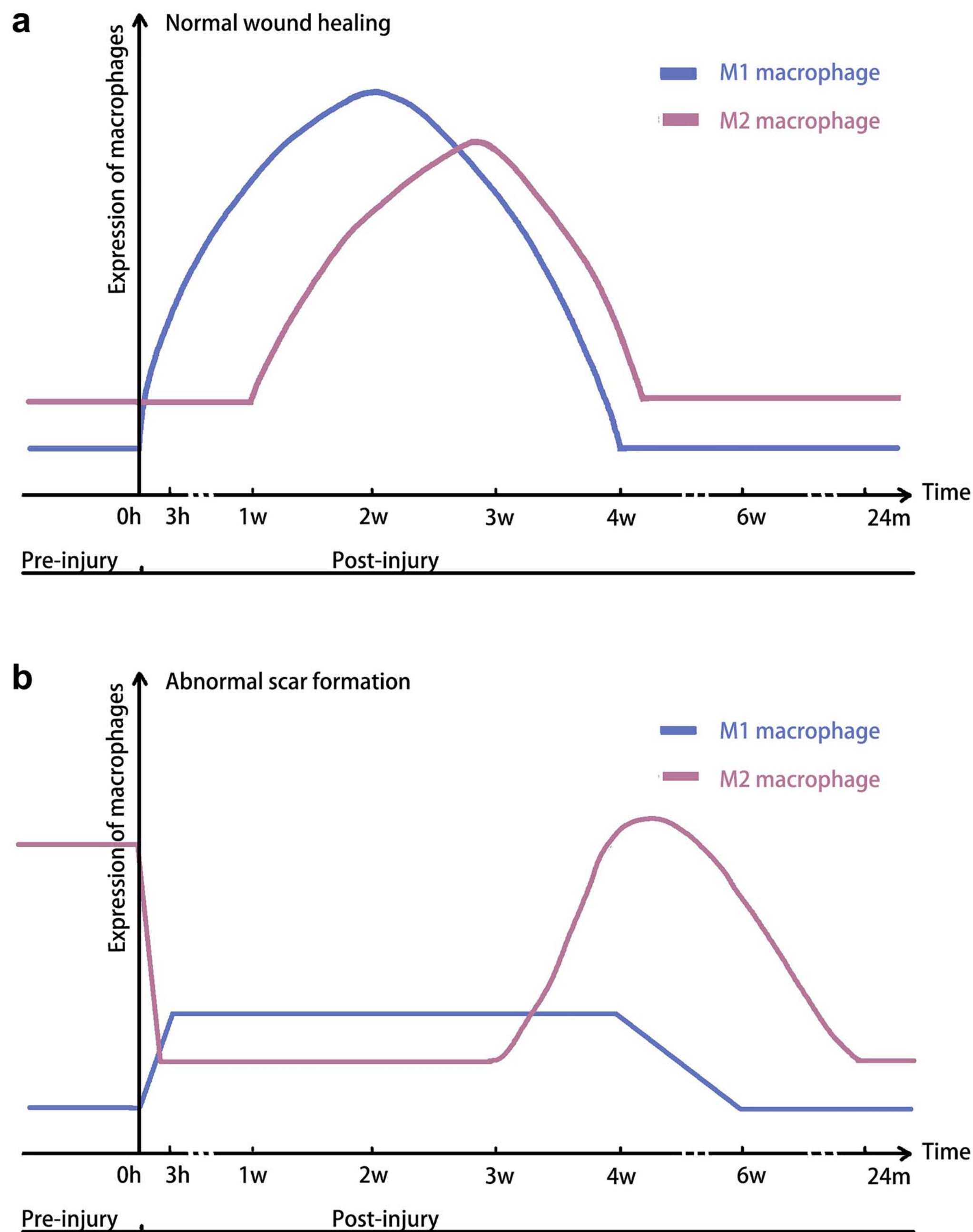

Figure 2. Comparison of macrophage polarization during the wound healing and abnormal scar formation. (**a**) The number of M1 and M2 macrophages expressed during normal wound healing. (**b**) The number of M1 and M2 macrophages expressed during abnormal scar formation

phenotype by reprogramming M0- or M2-like tumor-associated macrophages [44], by targeting micro RNAs that are relevant in macrophage activation and function [45, 46] or by promoting the expression of M1 cytokines which could regulate polarization thorough a feedback loop [47].

The previous study has shown that there were some similarities between tumors, wound and scars [48]. Currently, there is still a lack of clinical trials focusing on converting the polarization of macrophages to interfere with the formation of abnormal scars. In the future, converting the polarization of macrophages from M2 to M1 before surgery may transform the anti-inflammatory microenvironment into a pro-inflammatory milieu to prevent abnormal scar formation.

Role of macrophages in the early stage of pathological scar formation

In the process of wound healing, M1 macrophages are necessary for initiating inflammatory phases by killing pathogens and scavenging debris, while M2 macrophages participate in the proliferation and remodeling stage [49].

During normal tissue repair, the early stage of normal scar formation is marked by high expression of M1 cytokines

and biomarkers. In a study, biopsies were taken from human traumatic and burn injury tissues and the number of M1 macrophages started to increase at 0–2 days, peaked at 7–14 days and declined significantly at 14–28 days post-injury [50] (Fig. 2a). However, the imbalance of macrophage polarization during wound healing results in HTS formation [51]. Within the first 3 hours after surgical incision, the levels of M1 inflammatory proteins in HTS, including IL-6 and CCL2, were significantly lower compared to those in normal scar tissue [38]. Additionally, the pro-inflammatory cytokine messenger RNA levels, such as TNF-α, CCL-2 and IL-1β, remained low during HTS formation [52]. Therefore, due to the decreased expression of M1 cytokines, a reduced early inflammation stage may result in the formation of HTS, rather than a normal scar (Fig. 2b).

Role of macrophages in the later stage of pathological scar formation

Considering the different properties of M1 and M2 macrophages, the phases of HTS formation may be divided into the early stage, which is characterized by a low number of M2 macrophages, and the late stage, in which the tissue is heavily infiltrated by M2 macrophages [38, 52].

Previously, one study has shown that the density of M2 macrophages in normal scarring remained low at the early stage (0–14 days) and peaked at 14–28 days [50] (Fig. 2a). Compared to normal scar formation, HTS progression is related to the delayed and prolonged expression of both M2 macrophages and anti-inflammatory cytokines produced by them. Compared with healthy individuals, Liu *et al*. found that the peripheral blood mononuclear cells, which expressed immature M2 marker (CD204), were highly elevated in the blood of burn patients at 2 weeks [53]. Although these cells can upregulate pro-fibrotic factors, their ability may still differ from mature M2 macrophages. Additionally, van den Broek *et al*. found that CD163+ M2 macrophages could only be detected at 4–6 weeks post-injury in HTS-forming patients compared to 2 weeks in normal-scar-forming patients [52]. Moreover, as HTS formation progressed, the concentrations of IL-10 and IL-1RN continually reduced and reached normal levels in 6 months. The expression of Arg-1 and CD206 decreased, and the number of M2 macrophages (CD68+ and CMAF+) returned to baseline 24 months after injury [50] (Fig. 2b). These findings are consistent with those of previous studies in animal models. Zhu *et al*. established a human HTS-like nude mouse model by grafting human skin on the mice [54]. In the xenografted mice, M2 macrophages (F4/80+ and Arg-1+) and the specific cytokines, IL-10 and IL-1α, showed delayed expression compared to the autograft group.

Generally, M2 macrophages significantly increase at 4 weeks post-injury and return to baseline at 8 weeks without further recurrence [55]. However, if the number of M2 macrophages remains at a high level, it will lead to either HTS or keloid. Previously, some researchers have held that the concept of HTS and keloids may be the same pathological disease at different temporal points [56]. However, through systematically comparing clinical, histopathologic, biochemical and molecular differences between keloid and HTS, it is becoming more important to recognize differences between keloid and HTS as well as to treat keloid as a separate entity different from HTS [57]. The difference in spatiotemporal manners of macrophages in HTS and keloid need to be further elucidated.

TGF-β, one of the major growth factors produced by M2 macrophages, has been reported to play a significant role in HTS formation. However, the expression of TGF-β did not consistently decrease in parallel with the decrease of M2 macrophages, indicating that M2 macrophages were not the only source of TGF-β secretion [54].

Role of macrophages in different spatial variables of pathological scars

Instead of over-proliferation at the wound center, the keloid is characterized by invasive behavior from the wound margin into surrounding normal skin tissue [58]. Various spatial variables highlight the diversity within keloid. From the macroscopic perspective, the center of keloid tissue is paler and more shrunken compared to its erythematous and swollen margin site [59]. Microscopically, epidermal thickness, collagen ratios and distribution, fibroblast density and the infiltration of inflammatory cells all differ within keloid [60, 61]. Bagabir *et al*. suggested that keloid should be horizontally divided into three lesional sites: intralesional (center), perilesional (margin) and extralesional (adjacent normal skin) [29]. Vertically, according to histology, keloid was composed of epidermis, superficial dermis, mid-dermis and deep dermis [62]. However, the spatial role of macrophages in different sites both horizontally and vertically should be further studied.

M2 macrophages are thought to secrete profibrotic factors, such as TGF-β, to promote wound fibrosis. Bagabir *et al*. presented that M2 macrophages (CD163+) were markedly increased in perilesional sites, which was also consistent with the invasive behavior of the keloid margin [29, 63]. In the superficial dermis region, a heavy cellular infiltrate was found, including active fibroblasts, T cells, CD68+ macrophages and CD163+ macrophages [28, 29], together with an increased number of horizontal collagen fibers and microvessels [64]. Perivascular inflammation was observed around the microvessels of the subpapillary and papillary dermis in the keloid lesion [65].

In summary, current studies mainly focus on the distribution and quantity of macrophages. Further exploration of macrophage subtypes is still needed. To find an effective treatment for keloid, future studies should further investigate the M1/M2 macrophage distribution and corresponding cytokine changes in keloids.

Conclusions

This review demonstrated that the number of M2 macrophages presenting in the tissue pre-injury could serve as a local prognostic factor for the formation of pathological scars.

This M2-favouring microenvironment before injuries inhibits adaptive immune responses and results in HTS or keloids. After injury occurred, the reduced expression of M1 cytokines in early stage, and the delayed and prolonged expression of both M2 and anti-inflammatory cytokines in later stage may result in the formation of HTS, rather than a normal scar. Other immune cells, such as T cells, B cells, mast cells and neutrophils, were not summarized in this review. There have been few pieces of research focused on the spatiotemporal diversity of these immune cells. Besides, different immune cells might collaborate during abnormal scar formation, which needs to be addressed in future studies. Therefore, future studies should concentrate on the interaction of different immune cells during pathological scar formation.

Abbreviations

HTS, hypertrophic scar; DAMP, damage-associated molecular pattern; PAMP, pathogen-association molecular pattern; IL, interleukin; TNF-α, tumor necrosis factor-α; CCL2, chemokine C-C motif ligand; IFN-γ, interferon-γ; LPS, lipopolysaccharide; PDGF, Platelet-derived growth factor; TGF-β, transforming growth factor-beta; IGF-1, insulin-like growth factors-1; VEGF, vascular endothelial growth factor; Arg-1, argnase-1; ECM, extracellular matrix; MMP, mitochondrial membrane potential

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Authors' contributions

All authors read and approved the final manuscript.
Xiangwen Xu and Shuchen Gu contributed equally to the review, and should be viewed as co-first authors.

Conflicts of interest

None declared.

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