Review

Cell polarization, migration and tissue repair: A promising field for future melatonin research

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ABSTRACT

Melatonin has been shown to support the repair of various tissue injuries. Wound healing is a complicated process that comprises several different cellular activities and regulation mechanisms, such as activation and programming of stem cells, interaction of different cell types, polarization of cells, especially concerning the alternative of pro- vs. anti-inflammatory behavior, migration of cells to the site of replacement, with guidance by other cells and modified extracellular matrix, as observed in the formation of biobridges. In most of these processes, melatonin acts as a decisive modulator, but details depend on tissue and cell types and have not been completely identified. Many aspects will require a considerable amount of work for understanding, in this context, the role of melatonin on a comprehensive basis. Moreover, the modulation of important cell properties has remained partially unknown or has only poorly considered in recent work. For instance, pro- or anti-inflammatory polarization of cells has been described in various cell types, not only in macrophages, in which melatonin is a major regulator, but also in microglia, in astrocytes and in neutrophils. Even in fibroblasts, polarization has been observed and concerns the alternative of inflammatory or fibrotic behavior. Notably, polarized cells that support healing in normal tissue seem to also protect tumors, whereas inflammatory phenotypes show antitumor activities. With regard to antitumor properties of melatonin, it seems necessary to clarify whether melatonin polarizes cells differently in the tumor microenvironment, compared to normal tissue, in which it promotes healing.

Key words: Biobridge, cell migration, cell polarization, healing, inflammation, melatonin, sirtuins, stem cells, tissue repair

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1. INTRODUCTION

During the last decades, melatonin research had mainly focused on two important functions, melatonin's chronobiological role and the multiply documented protective actions of this fascinating molecule. The latter aspect mainly concerned its capacity of defending against free radicals, by both direct scavenging and reducing their formation via upregulating antioxidant enzymes. Additionally, melatonin was shown to be protective by contributing to the avoidance of physiological conditions that favor radical formation (1, 2). A more recent example for this latter capacity had emerged when melatonin was shown to modulate the polarization of

macrophages (3). This remarkable effect of favoring the polarization to anti-inflammatory M2 macrophages instead of the proinflammatory M1 phenotype resolved the previous paradox that melatonin can act in either pro- or anti-inflammatory ways, depending on conditions (4). The changes in polarization can be easily followed by determining the M1 marker iNOS (inducible NO synthase) and the M2 marker Arg-1 (arginase 1) (3). As far as melatonin acts on macrophages that had been polarized before to the M1 type or on stable cell lines derived from them, it will act in a proinflammatory way (5). However, this will not take place, if the cells had previously been polarized to M2. This kind of functional differentiation towards M2 cells may explain why melatonin was frequently shown to act *in vivo* as an anti-inflammatory agent (4, 6-8). However, the development of M2 macrophages bears an additional complexity, as several subtypes have to be distinguished, which differ with regard to their functional context. The subtype M2a is typically found to be concomitantly active with M1 and, therefore, seems to serve as a preventing agent against overshooting M1 activity. The other subtypes are associated with wound healing, in which M2b is typical for the proliferative phase after injury, whereas M2c appears in a later phase of remodeling or regeneration (9). An additional subtype, M2d, was found to be induced by the typically but not generally proinflammatory IL-6 via STAT3 (10). Moreover, the abundance of subtypes is, to a certain extent, tissue-specific and variable with regard to diseases (11, 12). It should also be noted that, in later stages of wound healing, melatonin may also increase iNOS again, in conjunction with an upregulation of VEGF (vascular endothelial growth factor) (13). The increase in iNOS may not imply a reversal of M2 polarization, but rather a replacement of macrophage subpopulations. The rise in VEGF can be interpreted as an angiogenic stimulus. Importantly, the different M2 subtypes have not generally to be seen as the friendly factors as they may appear because of their anti-inflammatory properties. In the context of cancer biology, they have turned out to be tumor promoting, contrary to the anti-tumor activities of M1 macrophages (12, 14). With regard to melatonin, which also acts differently in tumor and nontumor cells (15), though in an opposite way, it might be of interest to find out whether it may not favor M2 polarization in cancer cells.

Regardless of these details, the association of M2 macrophages with healing indicates another possible role of melatonin in processes of tissue repair. In fact, melatonin's potential in tissue repair has been observed and discussed in various organs (16-18), either in terms of direct effects, or via modulation of other signaling molecules, or being mediated by exosomes from melatonin-treated cells (17). Not surprisingly, these observations also included the participation of stem cells that are needed for the repair processes. The modulation of stem cell programming by melatonin has been recently reviewed (19).

Another important aspect of cell polarization, again with consequences to tissue repair, can be found in the central nervous system, insofar as this phenomenon also exists in microglia, in a similar way as in macrophages, and may exceed to other cell types that are usually not discussed in the context of polarization (20).

Tissue repair frequently comprises the necessity of cell migration in order to place the newly differentiated cells to the sites of requirement. This aspect, which is tightly associated with differential stem cell programming and also influenced by polarization of regulator cells, is of particular interest for repair in the central nervous system (19). Initial findings indicate that melatonin participates in these intertwined mechanisms.

This short review does not primarily focus on the contributions of melatonin released from numerous different cell types including immune cells (2, 21) and interactions between its different sources (22-24), but rather on aspects of applicability, either by administration of melatonin or exosomes from melatonin-treated macrophages.

2. PROGRAMMING OF STEM CELLS

Melatonin has been repeatedly shown to participate in the programming of various types of stem cells, as recently summarized (19). Comparisons of the different types of stem cells lead to the necessity of distinguishing between specific modes of action, not only with regard to the varying differentiation factors involved. In particular, the spectrum of effects differs profoundly between unipotent and pluripotent stem cells. Pluripotent stem cells can be selectively driven by external regulators such as melatonin towards specific developmental directions, as has been shown in the classic case of mesenchymal stem cells (MSCs) that can switch between adipogenic, chondrogenic and osteogenic routes. Depending on the cellular environment, high or low tissue-specific expression of transcription factors such as Pax2 (paired box 2) and Egr1 (early growth response protein 1), of differentiation factors such as Wnt (a name derived from the *Drosophila* gene *wingless* and the murine virus integration gene *Int-1*) and its downstream mediators, especially β-catenin, melatonin can promote any of these routes, but, in the presence of these regulators, it typically favors osteogenicity (19, 25-30). Additionally, differential epigenetic modifications of CpG islands in *Runx2* and *PPARγ* promoters seem to be relevant to the allowance of the respective alternate route (31).

However, it is important to be aware that these three developmental routes of the MSC fate mainly represent examples devoid of exclusiveness. These findings are mainly based on experiments using adipose tissue-derived and bone marrow MSCs. MSCs from other sources, such as inflamed dental pulp or umbilical cord, and even myoblasts can also be directed towards osteogenesis, if Wnt4 or other Wnt subforms are upregulated independently of melatonin (19). On the other hand, MSCs from adipose tissue, bone marrow, amniotic fluid and dental pulp can be transdifferentiated to neuronal precursor cells in an alternate ectopic environment, presumably based on noncanonical modes of Wnt signaling (19).

Contrary to the potential of multilateral differentiation in pluripotent stem cells, unipotent stem cells can only develop in a unilateral mode. Nevertheless, regulator molecules such as melatonin can have a substantial impact on the fate of these cells. Melatonin has been shown to act in multiple ways on such cells, as observed in spermatogonial stem cells. Apart from protecting against chemical insults caused, e.g., by oxidative stress, enhanced mitochondrial activity and its preservation have been recently shown to be decisive for stem cell proliferation, which is impaired under conditions of mitochondrial stress (32). With regard to melatonin, two aspects should be, in this context, of particular interest. First, the multiply demonstrated protective actions of melatonin on mitochondria (8, 33-39) might facilitate or contribute to stem cell activation. Second, melatonin was shown to upregulate, in nontumor cells, several sirtuins, especially SIRT1 and SIRT3 (40-42), while functionality of these sirtuins has been found to be a prerequisite for the avoidance of mitochondrial stress (32). Moreover, melatonin can promote differentiation of spermatogonial stem cells to sperm-like cells and enhance their proliferation rates *in vivo*, in particular, via stimulation of GDNF (glial cell line-derived neurotrophic factor) release by Sertoli cells (43). As far as asymmetrical cell division of stem cells is stimulated, melatonin also supports the self-renewal of the stem cell population and, thereby, antagonizes their age-dependent declines. Moreover, self-renewal is typically associated with the conservation of stemness, as usually demonstrated by nestin expression levels (19, 44, 45). Such effects have been observed in both unipotent and pluripotent stem cells and are, therefore, independent of programming by melatonin (19).

3. POLARIZATION OF CELLS

As already mentioned in the introduction, polarization to cell subtypes with partially or completely different properties is not restricted to macrophages, but also occurs in microglia

(19). However, similar phenomena exist in other cells (Figure 1), but are only rarely considered. For instance, astrocytes can exist as two functionally different subtypes, known as neurotoxic A1 and neuroprotective A2 cells (46-48). Like M1 microglia, A1 astrocytes are believed to release NO, TNFα, IL-6, IL-1β, the same CCL and CXCL chemokines, and additionally IL-1α, GM-CSF and the complement protein C1q, whereas A2 cells secrete the anti-inflammatory cytokines IL-4, IL-10, IL-13 and TGFβ (46). The signals driving to A1 or A2 are partially related to microglial activities, whereas anti-inflammatory polarization to A2 can be also achieved via ER (estrogen receptor) signaling, otherwise known for its neuroprotective effects (49). According to actual knowledge, it seems that polarizations to proinflammatory M1 and A1 or, alternately, to the anti-inflammatory M2 and A2 cells are jointly regulated and mechanistically interconnected, depending on local requirements. This may be favorable, as the same tissue or parts of it can be influenced towards the same direction. A presumably important aspect of the interconnections concerns the polarizing effects of cytokines that are released from other already polarized cells. For instance, TGFβ released by M2 macrophages or M2 microglia favors polarization of other cell types to the anti-inflammatory phenotype. Similar effects may be assumed for other cytokines. Insofar, melatonin can be expected to mediate polarization by additional indirect effects, as a direct influence that leads to M2 macrophages or M2 microglia should be able to induce anti-inflammatory phenotypes in the other cell types, too (cf. Figure 1).

The existence of pro- and anti-inflammatory cell types that are generated by polarization has been also described in neutrophils, which primarily act in the periphery. Correspondingly, they are named N1 and N2 (50). The properties of the two cell types seem to partially differ with regard to organs and microenvironment. In the oral cavity, their polarization seems to be influenced by the local microbiota (50). As indicated above, polarization to anti-inflammatory N2 cells is favored by TGFβ (51, 52), whereas the spectrum of N1 inducing cytokines awaits further investigation. With regard to cancer biology, N1 and N2 exhibit properties reminiscent of M1 and M2 macrophages (cf. Introduction). Tumor-associated neutrophils (TANs) polarized to the proinflammatory N1 type (N1 TANs) are anti-tumorigenic, in particular, by expressing immune-activating cytokines and chemokines, whereas anti-inflammatory N2 TANs have proved to be protumorigenic by suppressing tumor-directed immune responses and releasing angiogenic factors (50). The spectrum of neutrophil subtypes seems to exceed the N1/N2 polarity. Subsets that are preferentially recruited to sites of inflammation may be partially, but not completely identical with N1 cells.

A typical difference between cells that are polarized to either a proinflammatory or an anti-inflammatory phenotype concerns the expression of either inducible NO synthase (iNOS) or arginase 1 (Arg1) (3), observations that are not only applicable to monocyte-derived cell types. In addition to its involvement in oxidative and nitrosative stress, NO acts as an inflammatory signaling molecule (1-4, 7). Therefore, NO release by M1, A1, and N1 cells is the consequence of iNOS activation, whereas its lack in M2, A2 and N2 results from the consumption of the NOS substrate, arginine, by Arg1. Thereby, both oxidative/nitrosative stress and inflammatory signaling are strongly reduced. Moreover, arginine degradation is the starting point of polyamine synthesis, i.e., formation of another group of anti-inflammatory molecules (53).

Generally, polarization of cells to generate functionally different subsets should not be regarded as an exception, but seems to be a rather common phenomenon. This may even concern cells that are, from a conventional point of view, not considered as major players in inflammation control and redox biology. Fibroblasts may serve as an example. These cells are mostly perceived as structural components of tissues, including the stroma of tumors.

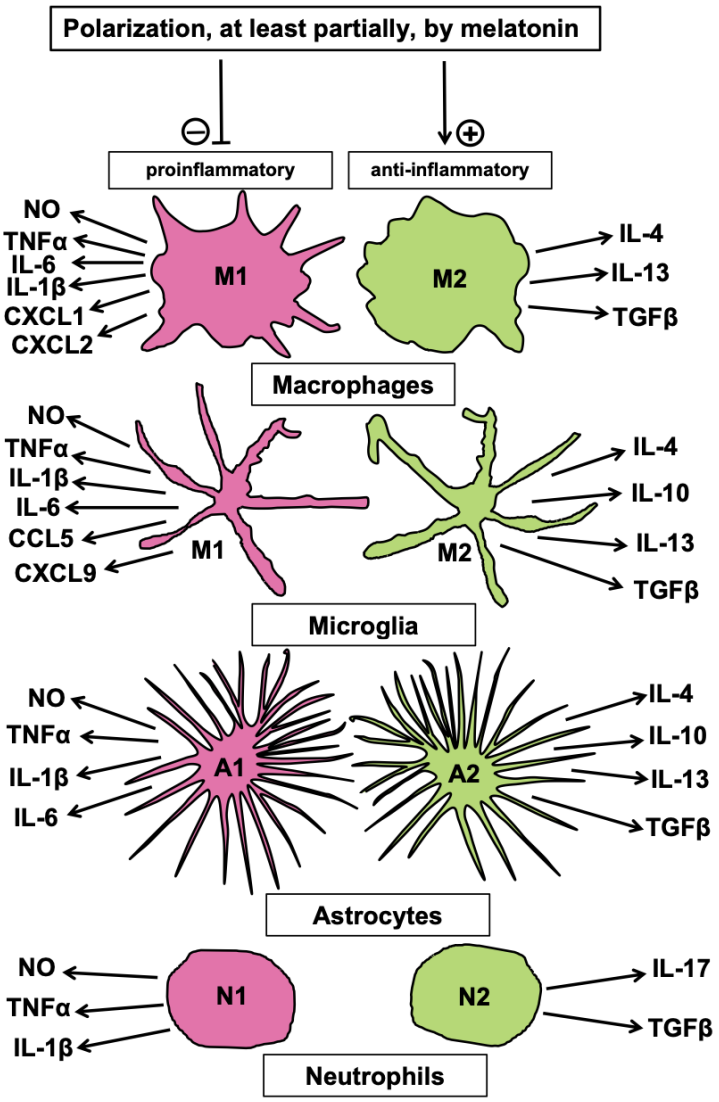

Fig. 1. Proinflammatory vs. anti-inflammatory polarization in four different cell types.

IL, interleukin; NO, nitric oxide; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor α; CCL, CXCL, chemokines. The numbers of cytokines and chemokines released from the respective polarized phenotypes may be even larger, but this would require further investigation or confirmation.

The tissue-specific microenvironment can exert different influences on fibroblasts by exposing them to locally specific factors released by neighboring cells or to components of the extracellular matrix. Such differences between induced subtypes become particularly evident when comparing tumor and nontumor tissues. Changes in stromal properties between tumor suppression and tumor promotion can be observed, in particular, in the fibroblasts, which represent a substantial quantity of the tumor stroma. Under the influence of cancer-derived cytokines, normal fibroblasts turn to cancer-associated fibroblasts (CAFs). Under these conditions, an immunosuppressive microenvironment is generated that favors tumor growth and has been reported to be explainable by the expression of the tumor-promoting gene LOXL2 (54). It was also reported that the expression of HIF-2 in CAFs contributes to the immunosuppressive microenvironment by polarizing macrophages to M2 in pancreatic cancer (55). Again, this conclusion underlines the importance of interplays between polarized cells of

different origin. In normal tissue, fibroblasts can be polarized to two main phenotypes, an inflammatory and a fibrotic one. In mouse fibroblasts, this was achieved by exposure to TNFα or TGFβ1, respectively (56). As either of these directions of polarity can be problematic in pathological terms, the two phenotypes should not be regarded as a usual alternative in healthy tissues, but rather as possible consequences of fibroblast activation.

Presumably, the list of polarizable cells will become much longer upon progressing research in this field. However, polarization does not necessarily concern the alternative of pro- vs. anti-inflammatory properties. In a more general understanding, any induced, at least moderately stable functional diversification of cells may be considered as a result of a kind of polarization, Especially the immune system, with its countless cellular subspecies and cytokine-induced phenotypes, will reveal many cases in which polarization, in the broader sense, takes place.

4. CELL MIGRATION

Stem cell activation and polarization of differentiating cells are frequently associated with migration. In particular, this is a requirement for the repair of injured tissues, since cells need to be directed to the site of damage for purposes of replacement. Cell migration can be observed in different cells during repair of various tissues. In the skin, keratinocytes, hair follicle cells and fibroblasts can be shown to migrate (57-59). Migratory fibroblasts are discernable from their resting stages by changes in the intracellular position of the nucleus (60). With regard to the involvement of SIRT1 in these cases (59, 60), effects via melatonin may be assumed. However, even under ectopic conditions, melatonin stimulated migration of endothelial stem cells in a study on renal ischemia, in addition to maturation effects (61). A recent review addressed the relationships between differentiation, proliferation and migration of cells after a preceding inflammatory phase with autophagy, followed by new formation of extracellular matrix and macrophage polarization to M2 (62). According to this view, inflammation and autophagy are required for local pathogen clearance, but also provide signals for activation and migration of cells as well as healing processes including M2 polarization. Another study interpreted the stimulation of migration in adipose-derived stem cells by melatonin as a consequence of downregulation of proinflammatory cytokines (63).

An important aspect of cell migration is the role of the extracellular matrix, whose either intact or defective local status as well as its composition may inhibit or stimulate migratory behavior (64). A highly impressive example for the role of the extracellular matrix in cell migration has become evident in studies on the application of stem cells in brain injury (65-71). The application of neuronal or even mesenchymal stem cells, with the original intention of replacing lost neurons, led to the remarkable finding that the transferred stem cells induced a local remodeling of the extracellular matrix, which formed a so-called biobridge that directed endogenous stem cells towards the site of injury. With regard to the numerous studies on the protective and healing effects of melatonin in various forms of brain injury (8, 72-76), it would be of considerable interest to study whether melatonin also participates in biobridge formation.

5. ROLE OF THE MELATONIN – SIRTUIN CONNECTION

The actions of melatonin are mostly explained by canonical signal transduction pathways transmitted via the MT_1 and MT_2 receptors. Even the proximate pathways prove to be complex in their details, as they contain several alternatives and are additionally modulated by receptor-binding proteins (75). Meanwhile, melatonin has been shown to influence the expression and activities of several other regulation factors, properties that expand melatonin's spectrum of effects (40, 76, 77). These factors include redox regulators, pro- and anti-inflammatory cytokines, numerous microRNAs and other noncoding RNAs, and, in particular, several

sirtuins, among which SIRT1 has been most often studied (42). Thus, melatonin's modes of signaling have been substantially broadened by the additional secondary routes. With regard to SIRT1, the relationship to melatonin is important under two aspects. First, in nontumor cells, SIRT1 is typically upregulated by melatonin, but downregulated in cancer cells (42), a surprising difference, which has been explained by the dysregulation of the sirtuin in cancer that is caused by silencing of tumor-suppressing components of cellular circadian oscillators (78). Second, melatonin and SIRT1 act, in many cases, in the same way, which has become particularly evident in the control of inflammation. Moreover, melatonin's anti-inflammatory effects were shown to be suppressed, in these cases, by sirtuin inhibitors such as sirtinol or EX527 as well as by *Sirt1* siRNA, findings that lead to the conclusion that SIRT1 transmits such actions of melatonin (20, 40, 42, 79, 80).

In addition to the effects previously summarized (20, 40, 42), other recent data on actions by sirtuins have revealed various new insights. These concern several aspects that are addressed in this review, such as programming (81, 82) and proliferation (82, 83) of stem cells, in particular, MSCs, differentiation (59), wound healing and tissue repair (58, 59, 84-87), inflammation (63, 91, 93, 94), as well as keratinocyte and fibroblast migration (63, 64, 95). Although the relationship to melatonin was only occasionally addressed in these papers (89), the overlap with melatonin's known spectrum of actions is so evident that future studies should use corresponding experimental approaches to re-investigate the possible, and presumably likely, involvement of melatonin in these systems.

Notably, the relatively strong relationship between melatonin and SIRT1 should be taken as a reason for expanding such studies to other sirtuin subforms. With regard to mitochondrial effects of melatonin, the mitochondrially located SIRT3 should be of particular interest. A few respective findings had been previously discussed (42). The importance of SIRT3 in mitochondria of stem cells has been recently addressed (91) and should be worth further efforts to connect these findings with melatonin research. Another possible connection may be assumed for melatonin and SIRT6. This subform is strongly associated with chromatin and driven in the same way by the NAD^+ cycle as known for SIRT1 (42). In functional terms, SIRT6 was recently shown to be involved in processes that are reminiscent of actions by melatonin concerning healing of nerve injury, cell migration and M2 polarization of macrophages (92).

6. CONCLUSIONS

Melatonin's remarkable potential of protecting cells against oxidative damage and various other forms of stress has been documented in many hundreds of publications. However, defense against damage is only one side of the coin, especially as protection rarely leads to a fully complete prevention of damage. Moreover, sudden events such as traumatic injuries, ischemic or hemorrhagic insults, and injuries by accidents are poorly or not prevented by preceding cautious measures. Therefore, the healing of unavoided damage including the suppression of its undesired consequences, such as long-lasting inflammation, is likewise important for recovery to health. Although melatonin has been shown to be highly valuable in terms of reduction of post-injury damage, the aspect of tissue repair has been to date of rather secondary interest. From this author's point of view, this gap needs more attention. Moreover, it may turn out to become a promising field of melatonin research, which might have the potential for covering the entire spectrum of the health-promoting role and value of melatonin. Additionally, the aspect of tissue repair includes novel strategies of treating losses of cells by stem cell transfer. Several studies have already demonstrated the usefulness of melatonin and its downstream factor, SIRT1, in such approaches (19, 58, 59, 61, 63, 72, 83, 94-101). In the

future, the concept of biobridges (65-70) would merit additional attention in investigations on brain tissue repair.

The main aspects of this short review on preconditions of tissue repair should be regarded as interconnected phenomena, although the respective processes largely occur in a sequential order. Therefore, activation and programming of stem cells, polarization of effector cells, and controlled migration to the site of injury should ideally be investigated from an integrating point of view. Additional aspects such as self-renewal with maintenance of stemness, regulation of inflammation and of autophagy, modification of the extracellular matrix, protection against oxidative damage, downstream signaling including noncoding RNAs, sirtuins, and exosomal transmission are intertwined with the aforementioned phenomena. Some of these details concern the classic protective role of melatonin, whereas others have been rather recently added to the melatonin field. Numerous novel findings should attract the interest of researchers working on melatonin and may promise substantial insights, especially concerning wound healing, repair of the injured brain and practicability of stem cell transplantation.

On the other hand, the remarkable spectrum of protective actions should not mislead to precocious conclusions, especially by assuming that melatonin's actions are generally identical. In particular, the differences between tumor and nontumor tissues have to remain a matter of awareness. In the tumor microenvironment, actions by effector cells such as macrophages and neutrophils can be just opposite to those found in healthy tissues. This has become particularly evident concerning cell polarization, as proinflammatory phenotypes display antitumor activities. With regard to antitumor properties of melatonin, it seems to be an urgent task to clarify whether melatonin causes, in tumors, anti-inflammatory polarization as in healthy tissue or, instead, proinflammatory polarization, which would conform to its antitumor activities. In this regard, it would be also of interest to find out whether differences between tumors may exist that relate to their sensitivity to melatonin treatment.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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