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TARGETING TUMOR ASSOCIATED MACROPHAGES (TAMS) FOR CANCER TREATMENT: EXPLOITING MELATONIN AS BIOLOGICAL RESPONSE MODIFIER (BRM)

Oncology

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ABSTRACT

Immune system regulates homeostasis of the body during normal and pathological condition where macrophages play very important role. They can be polarized in two phenotypes, either M1 or M2 type, depending on the stimuli present in their microenvironment. In cancer conditions, increased infiltration of leukocyte occurs where macrophages dominate and they promote its growth and are known as tumor-associated macrophages (TAMs). These TAMs resemble with M2 type. For cancer treatment, either infiltration of macrophages at tumor site or repolarization of TAMs towards M1type can be targeted. Biological Response Modifiers (BRMs) are potential immuno-modulators that can be used for targeting TAMs. Melatonin could be used as a BRM as it shows the immuno-modulatory property via affecting different players of immune system. In this review, we summarize properties of TAMs and their role in tumor growth and progression and how they can be targeted for cancer treatment using melatonin as BRM.

KEYWORDS:

Tumor-associated macrophages (TAMs), immuno-modulation, biological response modifiers (BRMs).

1. Introduction

Immune system forms a complex network in the body comprising of immune cells, tissues and organs. It maintains homeostasis of the body by recognizing non-self-cells, altered-self cells and molecules such as pathogens and toxins and then eliminate them.Vertebrates have highly evolved immune system and is categorized in two arms i.e., innate and adaptive (Medzhitov & Janeway, 2000). Innate immunity is the first line of defense comprises of physical and chemical barriers, cells such as macrophages, neutrophils, natural killer cells (NK) and soluble factors like complement proteins, mediators of inflammation including cytokines (Medzhitov & Janeway, 2002). Adaptive immunity is capable of recognizing fine differences between foreign substances and create specific immune response against them. On the basis of function, adaptive immunity is classified in humoral and cellular immunity. Humoral immunity is mediated through secretory molecules, called antibodies, synthesized by B lymphocytes that remains in blood and mucosal secretion that binds with antigen and try to eliminate it from system by various mechanisms. It is generated against extracellular pathogens and their derived toxins. Whereas intracellular pathogens such as virus and bacteria result in alteration in host cells and is recognized by T lymphocytes resulting in cytotoxicity of the target cell and known as cellular immune response (Medzhitov & Janeway, 2002).

Macrophages are important effector cells of innate immune system that also regulate adaptive immune response. Monocytes originate from hematopoietic stem cells which differentiate into macrophages in tissues where they reside and perform diverse function. They not only defend against pathogens but also maintain homeostasis of tissue by resolving inflammation, repairing tissue and wound healing after injury. Inflammation is very tightly regulated process which consists of two phases - initiation and resolution. During inflammation, resident tissue macrophages become activated and start secreting various factors which recruit more immune cells at the site of inflammation that helps in eradicating infection and also lead to resolution of inflammation (Fujiwara & Kobayashi, 2005). Defect in resolution phase leads to accumulation of more immune cells at the site of inflammation where macrophages dominate resulting in prolonged activation of macrophages leading to chronic inflammation.

Chronic inflammation is one of the major cause of cancer. It was observed that there is lympho-reticular infiltration in neoplasm and was suggested that chronic inflammation might be the probable cause for the origin of cancer (Mantovani, Bottazzi, Colotta, Sozzani, & Ruco, 1992). Tumor mass consists of heterogeneous population of cells where neoplastic cells are surrounded by the extracellular matrix, fibroblasts, stromal cells, blood vessels and immune cells (Junttila & de Sauvage, 2013). Cross talk between neoplastic cells and leukocytes has intense role on tumor progression. Leukocytes are dominating cell population found in tumor tissue and among them major subsets are lymphocytes and macrophages which have been implicated in determining the prognosis of the disease. Macrophages found in tumor environment support its growth and thus known as tumor-associated macrophages (TAMs) and density of TAMs is highly correlated with progression of disease in many studies. Studies on various cancers, including Hodgkin's lymphoma, breast carcinoma, endometrial cancer, colorectal cancer and others, showed correlation of poor prognosis of disease with increased density of macrophages (Leek et al., 1996; OHNO et al., 2004; Steidl et al., 2010; S.-Y. Tan et al., 2005). So here we discuss about polarization of macrophages in tumor microenvironment towards TAMs, its pro-tumoral functions and how they could be used as a therapeutic target for cancer treatment by melatonin as the biological response modifier (BRM).

2. Polarization of Macrophages

The important feature of macrophages is their plasticity that means depending upon stimuli present in their surrounding they can polarize into either M1 or M2 phenotype. Also, M1 can be converted back into M2 and vice-versa which is termed as repolarization. Macrophages perform plethora of functions such as elimination of microbes, apoptotic cells and pathogens by activating immune system and are also involved in wound repair in case of injury. These functions are performed by different phenotypes of macrophages. They polarize to M1 phenotype upon exposure to microbes related pathogen associated molecular pattern (PAMP) like LPS, dsRNA, unmethylated CpG motifs, tumor-associated antigens, small synthetic immune modifiers

like R848, some endogenous danger signals like heat shock proteins and in presence of Th1 cytokines like IFN-γ, TNF-α (Medzhitov & Janeway, 2000). Presence of these factors leads to activation of macrophages which synthesize reactive oxygen and nitrogen intermediates and pro-inflammatory cytokines. This is classical activation of macrophages which show antimicrobial activity and can also kill tumor cells and also activate the adaptive immune response (Sawachi et al., 2010). Th2 cytokines like TGF-β, IL-10, IL-4 and others inhibit M1 macrophage activity but they activate macrophages in different manner that is known as alternative activation which defines M2 phenotype of macrophages. M2 macrophages secrete high level of anti-inflammatory cytokines and show high arginase activity. They also suppress immune system which is exploited by pathogens and tumor cells for their survival in host. Stein *et al*., (Stein, Keshav, Harris, & Gordon, 1992) found that IL-4 increases mannose receptor of elicited peritoneal macrophages which increases the clearance of mannosylated ligand and this activation was different from classical thus termed as alternative activation of macrophages. Alternatively activated macrophages differ in morphology with respect to inactivated and classically activated macrophages (Figure 1). Phenotypic differences in classically and alternatively activated macrophages may be defined on the basis of surface marker. Despite having high capacity of phagocytosis, alternatively activated macrophages do not have enhanced capacity to kill tumor cell or microbes(Leidi et al., 2009; Lin, Nguyen, Russell, & Pollard, 2001).

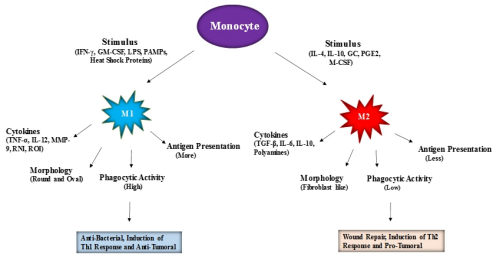

Figure 1: Factors regulating differentiation of monocytes into M1 and M2 phenotype.

Alternatively activated M2 macrophages are linked to many pathological conditions like allergy, cancer, obesity and other diseases. There are several studies which correlate the increasing number of macrophages in tumor environment with poor prognosis of disease and these macrophages show M2 phenotype (Lin et al., 2001; Zeisberger et al., 2006).

3. Polarization of Macrophages into Tumor-Associated Macrophages (TAMs)

Polarization of macrophages depend on the signal or stimuli, present in their surroundings. Tumors secrete various growth factors, chemokines and cytokines like IL-6, TGF-β, IL-10 which leads to polarization of tumor-infiltrating macrophages into M2 phenotype. Various cancer cells secrete IL-6 and high level of serum IL-6 has been accepted as a diagnostic marker for cancer. One report suggest that in *in vitro* condition, IL-6 augments expression of DC206, arginase-1 and ym-1 expression (markers of M2 macrophages) (Fernando, Reyes, Iannuzzi, Leung, & McKay, 2014). Also, cervical cancer cell derived PGE2 and IL-6 polarizes macrophages towards M2 phenotype in *in vitro* condition and thereby increases tumor cell survival (Heusinkveld et al., 2011). M-CSF is well known factor for macrophage differentiation and chemotaxis. Human ovarian and breast cancer cell lines secrete M-CSF and colon cancer cell secrete CSF-1 which direct the monocytes to differentiate into M2 phenotype and also recruit macrophages at tumor site (Tang et al., 1992; Wang et al., 2016). Also, cathelicidin-related antimicrobial peptide, derived from prostate cancer and oncostatin M and eotaxin, derived from hypoxic cancer cells, leads to differentiation of macrophages towards M2 type (Cha et al., 2016; Tripathi et al., 2014).

4. Pro-Tumoral Function of TAMs

TAMs exhibit M2 like phenotype and has pro-tumoral function. They support tumor survival through several mechanisms. They secrete growth factors that help in angiogenesis, tumor metastasis and also help in immune suppression and thus promote tumor growth and survival (Figure 2)(Leidi et al., 2009).

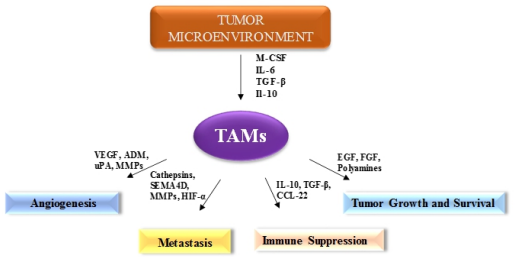

Figure 2: Tumor microenvironment educates macrophages to polarize intoTAMs (M2 phenotype) that show pro-tumoral functions.

4.1. TAMs Mediated Angiogenesis

The pro-angiogenic property of macrophage is regulated by diverse signals originating from tumor and stromal cells. Emerging research suggest that macrophage infiltration is strongly co-related with angiogenic switch in tumor. CSF-1 has been known as chemotactic signal for macrophages infiltration in tumor milieu. Inhibition of CSF-1 in nude tumor mice model decreased the macrophage permeation at tumor site and noticeable decrease in angiogenesis (Lin et al., 2006). Vukanoic *et al*., (Vukanovic & Isaacs, 1995) reported that treatment with linomide impaired tumor angiogenesis via depleting TAMs and impaired secretion of TNF-α in prostrate tumor bearing mice. In accordance with this study Zhang *et al*., (W. Zhang et al., 2010) found that orthotopic HCC tumor bearing mice treated with Zoledronic acid and Clodrolip caused decrease in number of TAMs accompanied with decrease in tumor angiogenesis. Similarly, other reports suggest that Clodronate treatment, encapsulated in liposomes, to human A673 rhabdomyosarcoma tumor bearing mouse resulted in decrease in number of TAMs that was directly associated with decrease in blood vessel density in tumor milieu and also related with increased vascular endothelial growth factor (VEGF) serum level (Zeisberger et al., 2006).

TAMs also secrete factors like adrenomedullin (ADM) and urokinase plasminogen activator (uPA) which help in tumor angiogenesis. ADM shows angiogenic properties via increasing the endothelial cell proliferation and nitric oxide secretion. Tumor microenvironment has characteristically hypoxic area which causes recruitment of TAMs, where they secrete VEGF-A an important angiogenic factor (Murdoch, Giannoudis, & Lewis, 2004). In response to hypoxia, TAMs also secrete various metalloproteinases such as MMP-1, MMP-7, MMP-9 and others. These proteolytic enzymes are actively involved in tumor angiogenesis via inducing extra cellular matrix (ECM) to secrete VEGF. The extracellular metalloproteinase inducer (EMMPRIN) has been reported to be up-regulated in many tumor types, which induces MMPs and VEGF secretion from fibroblast and endothelial cells. Amit-Cohen *et al*., (Amit-Cohen, Rahat, & Rahat, 2013) found that *in vitro* soluble EMMPRIN induces MMP-9 and VEGF secretion in macrophages co-cultured with tumor cells thus augmenting angiogenesis.

4.2. TAMs Mediated Promotion of Tumor Invasion and Metastasis

Metastasis is a phenomenon where tumor cells grow at its primary site, modulate the surrounding for their favorable growth and at advanced stage they evade ECM to come into blood circulation and invade a new site. Metastasis is the leading cause of mortality of tumor patients. TAMs have the ability to support tumor cell proliferation and they also affect tumor metastasis by transforming the tumor milieu. During metastasis, cascade of events takes place like local invasion of tumor cells, survival of tumor cells in blood circulation, extravasation and secondary niche formation. HIF-1α and SEMA4D are elevated in tumor microenvironment and are also associated with tumor angiogenesis. *In situ* experiment of tissue from colon cancer patients revealed that TAMs induces HIF-1α and SEMA4D in tumor cells which markedly increase their invasion and migration (Mu et al., 2013). When THP-1 cells were differentiated into M2 phenotype and co-cultured with human basal cell carcinoma (BCC), they induced COX-2 dependent release of MMP-9 which also increased the invasion of BCC cells (Tjiu et al., 2009). Similarly, macrophages co-cultured with MCF-7 cells caused TNF-α dependent induction of MMPs (MMP-2 and MMP-9) in macrophages and increased the tumor

invasion(Hagemann et al., 2004). Cathepsins are proteases that help in degradation of extracellular matrix. In case of pancreatic tumor bearing mice, TAMs secrete cathepsin S and B which increase angiogenesis and invasion of tumor cells(Gocheva et al., 2010).

4.3. TAMs Mediated Immune Suppression and Survival of Tumor Cells

One of the important phenomena of tumor survival in host is immune suppression where TAMs play major role. They target both innate and adaptive arms of the immune system. TAMs secrete cytokines and chemokines like TGF-β, IL-10, CCL-22 and also express arginase-1.TAMs secrete high TGF-β which support tumor cell proliferation and also help in polarization of infiltrating monocytes into M2 phenotype resulting in decreased tumor cytotoxic activity of macrophages. TGF-β also inhibits differentiation of monocyte into dendritic cells, thus decreases antigen presentation which may affect adaptive immune response. NK cells have cytolytic function and can kill tumor cell but their action may be affected due to high TGF-β level in tumor microenvironment. Thereby high TGF-β down-regulate antitumor function of innate arm. There are reports where increasing number of T cells in tumor microenvironment has been correlated with suppression of disease. Cytotoxic activity of T cells is promoted by activation of Th1 response while Th2 response affects it negatively. TGF-β enhances Th2 response which further differentiates Th cells towards Th2 type, thus provide favorable environment for tumor survival. TGF-β also inhibits expression of certain genes like granzyme, perforin, IFN-γ and Fas ligand in cytotoxic T cells *in vivo (Thomas & Massagué, 2005)*. Further TGF-β promotes Treg cells differentiation and maintenance in tumor microenvironment. TAMs also secrete CCL22 chemokine, which regulate passage of Treg cells in tumor milieu. Programmed cell death -1 (PD-1) and its ligand PD-L1 has immuno-regulatory properties in tumor microenvironment. Increased expression of PD-1 on HCC patients were found to have increased $CD^{8+}PD\text{-}L1^{-}$population that also increased the ratio of infiltrating CD^{8+} cells (Shi et al., 2011). In ovarian carcinoma, PD-L1 expression was high in macrophages that resulted in suppression of tumor-associated antigen (TAA) specific T cell immunity. Blocking of PD-L1 using antibody resulted in restoration of T cell activation and tumor regression(Kryczek et al., 2006). IL-10 expression was found to be high in tumor milieu which inhibits Th1 response through inhibiting of NF-κB activation (Driessler, Venstrom, Sabat, Asadullah, & Schottelius, 2004). IL-10 may impair antigen presenting capacity of APC cells thereby compromises antitumor immune response in tumor milieu. CCL-2 cytokine increases recruitment of macrophages at tumor milieu and thereby support tumor growth(Mizutani et al., 2009).

5. Therapeutic Approach by Targeting TAMs

It is well known that immune-surveillance is hampered in cancer conditions. Also, there is increased infiltration of leukocytes at the tumor site where macrophages dominate. These macrophages or TAMs promote the tumor growth(Hao et al., 2012). Thus TAMs can be a good target to fight against cancer. The major ways to target TAMs could be either by inhibiting TAMs recruitment and survival at tumor microenvironment that would hinder TAMs angiogenic and tissue remodeling characteristic or by repolarizing TAMs into M1 type leading to alteration in immunosuppressive function of TAMs and thereby gaining anti-tumor activity. Since last decade various pathways of TAMs have been targeted to cure tumor. The anti-tumor strategies that are aiming to reprogram the M2 macrophages has been proved to be successful. This require stimuli like cytokines that will repolarize them M1 type like IFN-γ has been shown to reprogram M2 macrophage into M1 on administering in ovarian cancer patients (Colombo et al., 1992).

Biological response modifiers (BRMs) are the substances that modify the immune response. They can be endogenous as well as exogenous. So they could be used in immunotherapy for treating various diseases like cancer where the immune system is suppressed. Various BRMs have been used for cancer treatment (Table 1). One of the endogenous molecule that could be used as BRM is melatonin.There are evidences showing the anti-tumor properties of pineal gland by the secretion of its principal hormone i.e., melatonin. It is known that melatonin shows anti-tumor and immunomodulatory properties (Conti & Maestroni, 1995; Lissoni et al., 1993; Pierpaoli, 1993). So these properties can be exploited for cancer treatment.

Table 1: Clinical implication of various BRMs

BRMs	Action	Clinical Use	References
Endogenous			
Interleukin-2	Cytotoxic T cell and NK cell activation	Melanoma and Kidney Cancer	(Li, Liu, Margolin, & Hwu, 2009; C. Zhang, Zhang, & Chen, 2013)
TNF-α	NK and LAK cell activation, chemotactic recruitment and activation of cells, predominantly of macrophages.	J558L cell line, SA1 Sarcoma, A673 Human rhabdomyosarcoma and HT-29 Human colon adenocarcinoma lines	(Blankenstein et al., 1991; Feinman, Henriksen-DeStefano, Tsujimoto, & Vilcek, 1987; Havell, Fiers, & North, 1988; Ming, Bersani, & Mantovani, 1987; Urban, Shepard, Rothstein, Sugarman, & Schreiber, 1986)
α- interferon	Dendritic cell and NK cell activation	Melanoma, Multiple Myeloma, Carcinoid tumors and some types of Lymphoma and Leukemia	(Herndon et al., 2012; Ravaud & Dilhuydy, 2005; Sutlu & Alici, 2009)
Thymostimulin	Cytotoxic immune response	Hepatocellular carcinoma (HCC)	(Dollinger et al., 2010)
T-activin	T cell activation	Melanoma	(Stanojevic-Bakic, Milosevic, Vuckovic-Dekic, Sasic, & Markovic, 1995)
Exogenous			
Bacillus Calmette-Guerin (BCG)	T cell activation	Melanoma and colorectal cancer	(Mosolits, Nilsson, & Mellstedt, 2005; Ozaki, Okazaki, & Nakao, 1995; Stewart & Levine, 2011)
Thalidomide	Increases NK cell cytotoxicity	Multiple Myeloma	(Davies et al., 2001)
Imiquimod	Agonistic activity towards Toll-like receptor-7 and -8	Basal cell skincancer (BCCs) and Non-melanoma skin cancer (NMSCs)	(Madan, Lear, & Szeimies, 2010; Schon & Schon, 2007; Tillman Jr & Carroll, 2007)
Lenalidomide	T cell activation	Multiple Myeloma	(Chen et al., 2013)
Levamisole	T cell activation	Colon cancer	(Mutch & Hutson, 1991)

5. Melatonin

Melatonin is pineal gland hormone, firstly isolated from bovine pineal tissue in 1958 by Lerner(Lerner, Case, & Takahashi, 1960). Earlier the hormone was thought to be synthesized in vertebrates only but later on it was found to be synthesized by photosynthetic bacteria, unicellular eukaryotes, green plants and fungi also (Hardeland, 1999; Manchester, Poeggeler, Alvares, Ogden, & Reiter, 1994; Poeggeler, Balzer, Hardeland, & Lerchl, 1991; Tilden, Becker, Amma, Arciniega, & McGaw, 1997). It is not only synthesized in the pineal gland but also in retina, bone marrow, gut, thymus, spleen, skin and some innate immune cells like macrophages (Slominski et al., 2002; Stefulj et al., 2001; D. Tan et al., 2002).Its secretion is found to be high at night (Axelrod, Wurtman, & Snyder, 1965) and it is primarily known to regulate circadian rhythm of our body. It is also involved in seasonal reproduction, regulation of immune system, vascular regulation, anti-aging, anti-jetlag and inhibition of cancer (Reiter, 1991).

Melatonin acts on cells through receptor dependent and independent manner,due to its small size and lipophilicity. Its receptors are present on plasma membrane as well as nuclear membrane. The surface

receptors belong to nuclear receptor superfamily ROR/ROZ and nuclear receptors belong to RZR/ROR (Reppert et al., 1995; Reppert, Weaver, & Ebisawa, 1994) orphan receptor family and have three subtypes α, β and γ . (Beckerandre, Andre, & DeLamarter, 1993).

In cancer conditions, melatonin has been reported to inhibit tumor growth and progression via its pro-apoptotic, anti-proliferative (Box 1) and immuno-regulatory mechanisms by various pathways.

BOX-1: Anti-Tumoral Role of Melatonin

I. Pro- Apoptotic

Affects downstream molecules of apoptotic pathway

- Induces membrane depolarization, cytochrome c release and activate caspase-3,-8 and -9 (Rubio et al., 2007).
- Downregulates anti-apoptotic proteins like Bcl -2 and upregulate pro-apoptotic proteins like Bax(Trubiani et al., 2005).
- Increases ROS levels (Fujino, Noguchi, Takeda, & Ichijo, 2006).
- Activates caspase -8 via increasing expression of Fas/FasL.(García-Santos et al., 2012).

II. Anti-Proliferative

- Inhibits or block the cell cycle (Bubenik et al., 1998; Di Bella, Scalera, & Rossi, 1980; Lissoni et al., 1991).
- Activates and increases the p21/WAF1 and p53 levels(Mediavilla, Cos, & Sanchez-Barcelo, 1999).

6.1. Immune Regulation by Melatonin

The direct link between nervous, endocrine and immune system has been established and known for about 40 years (Reiter et al., 2003). Pineal gland and immune system exhibits bidirectional communication and also role of melatonin in neuroendocrine immuno-modulation is well proved in various *in vitro* and *in vivo* studies (Antonio Carrillo-Vico, Guerrero, Lardone, & Reiter, 2005; Antonio Carrillo-Vico, Lardone, Álvarez-Sánchez, Rodríguez-Rodríguez, & Guerrero, 2013; Skwarlo-Sonta, Majewski, Markowska, Oblap, & Olszanska, 2003).

The immuno-modulatory effect of melatonin is supported by presence of its receptor on different immune organs and cells. The nuclear receptor, RORα, has been reported in thymus and spleen of mice(A Carrillo-Vico et al., 2003). In human monocytes, expression of surface receptor, MT1, and nuclear receptors, RORα2 and RZRα, has been reported (García-Mauriño, Pozo, Calvo, & Guerrero, 2000; Pozo, García-Mauriño, Guerrero, & Calvo, 2004). Melatonin balances the function of both innate and adaptive immunity. Immuno-modulatory role of melatonin was highlighted for the first time when decreased cellular and humoral immunity was found in mice. Mice were exposed to continuous light condition and melatonin synthesis was inhibited with the use of propranolol. In this condition mice were unable to respond against SRBC (sheep red blood cells) and impaired autologous mixed lymphocyte reaction was observed and the effect was reversed with melatonin injection (G. J. Maestroni, Conti, & Pierpaoli, 1986). In 1987, the same group reported increased primary and secondary antibody response against SRBC with afternoon melatonin injection in miceShort photoperiods are associated with increased melatonin synthesis that is correlated with increased spleen weight and number of splenic lymphocytes and macrophages(G. Maestroni, Conti, & Pierpaoli, 1987).In humans, increased thymosin and thymulin-1α synthesis was correlated with increased nocturnal melatonin secretion (Molinero, Soutto, Benot, Hmadcha, & Guerrero, 2000). The melatonin immune interaction becomes more interesting after finding of the involvement of melatonin in haematopoiesis. The reports support the fact that melatonin increases precursor cells for granulocyte monocyte (G. Maestroni & Conti, 1996). It regulates hematopoietic cell proliferation by inducing synthesis of opoid peptide in stromal cells of bone marrow (G. J. Maestroni, 1998). Pinealectomized male squirrel showed decrease leukocyte count inperipheral blood and bone marrow which was regained with exogenous melatonin administration (Rai & Haldar, 2003). It also regulates cellular and humoral immunity by altering the immune response towards Th-1 type and thus enhances the level of anti-tumoralcytokines which mainly modulates the macrophage polarization towards M1 type (Srinivasan et al., 2008). It also induces CD^{4+} cells to release IL-2 and IFN-γ and monocytes to release IL-6 (Garcia-Maurino et al., 1997). Italso acts in synergistic manner with LPS to activate the macrophages (Morrey, McLachlan, Serkin, & Bakouche, 1994). It also inhibits iNOS expression in murine macrophages by suppressing NFκB pathway(Gilad et al., 1998). Additionally, it also affects migration of macrophages to the injured site by downregulating ICAM-1 expression thus resulting in good prognosis of diseased condition(Kang et al., 2001).

Thus melatonin could be used to target TAMs which can either inhibit their recruitment at tumor site or can modulate their polarization towards M1 type by modulating the tumor microenvironment.

8. Summary

Macrophages are innate immune cells that maintain homeostasis of body. They are broadly classified as M1 and M2 type. Increased infiltration of leukocyte has been found at tumor site where in macrophage number dominates that promote tumor growth and progression. These are tumor-associated macrophages (TAMs) that show M2 phenotype. So targeting either infiltration of macrophages at tumor site or repolarization of TAMs towards M1 type could serve as an effective approach for cancer treatment. Biological response modifiers (BRMs), potential immuno-modulators, could be used to target them. Melatonin can be used as the BRM as it has been shown to modulate immune system and thus show anti-tumor activity. Similarly, other molecules could also serve as BRM for targeting TAMs as an effective approach for cancer treatment.

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