Chapter 14
Signaling of Tumor-Induced Immunosuppression of Dendritic Cells

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Abstract Dendritic cells (DCs) are professional antigen-presenting cells that regulate the immune system. In cancers, they uptake tumor-associated antigens, deliver them to T cells, and induce tumor-specific T cell responses. However, tumor cells develop mechanisms to evade the immune system, partly by impairing DC differentiation and function. Functionally deficient DCs may associate with acquisition of tolerogenic/immunosuppressive activities that actively block the development of antitumor immunity, and there is strong evidence supporting the presence of regulatory DCs in different DC subsets. Mechanistic studies reveal that intracellular signaling pathways, such as MAP kinases (MAPKs), JAK/STAT3, PI3 K/Akt, and NF-κB, which are critical to the regulation of DC differentiation, survival, and activity, are found to be hyperactivated both in tumor cells and in DCs in malignancies. The constitutive activation of these pathways in cancer cells leads to tumor cell secretion of cytokines that activate intracellular signaling pathways, particularly p38 MAPKs, in DCs or their progenitor cells and impair DC differentiation and function. In this chapter, we will discuss the dysfunction of DCs and the presence of regulatory DCs in cancer settings. We will focus on the signaling pathways that mediate DC dysfunction, particularly p38 MAPKs, in negatively regulating DC differentiation and function in cancers.

Keywords Dendritic cells · Signaling pathways · Regulatory dendritic cells · Tolerogenic dendritic cells · ERK · MAPK · NF-κB · STAT

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14.1 Introduction

Dendritic cells (DCs) are populations of professional antigen-presenting cells that regulate the immune system (Evel-Kabler and Chen 2006; Santini and Belardelli 2003; Sheng et al. 2005). They originate from $CD34^+$ bone-marrow stem cells, and have high plasticity and common morphological and functional characteristics (Sheng et al. 2005; Gabrilovich et al. 1996). During their development, DCs are classified as immature, semimature and mature cells after migration into the peripheral tissues from their bone marrow precursor cells. In the immature stage, DCs are primarily localized in the peripheral tissues and perform specialized functions of antigen uptake and processing, in which they capture and carry antigens to the lymph nodes. In the lymphoid organs, DCs become mature and subsequently, interact with antigen-specific T cells and initiate immune responses (Di Nicola and Lemoli 2000; Sinkovics and Horvath 2000; Aloysius et al. 2006).

One of the most important findings about DCs is that these cells are endowed with two critical features: subset and functional plasticity (Steinman and Banchereau 2007). This diversity permits the adaptive immune system to mount functionally distinct types of responses. The two major DC subsets are the classic DCs (cDCs) and the plasmacytoid DCs (pDCs). pDCs are the frontline in antiviral immunity because of their capacity to rapidly produce high amounts of type I interferon (IFN) in response to viruses (Liu 2005). In contrast, cDCs are efficient phagocytic cells that reside within lymphoid and nonlymphoid organs. In mice, certain cDC populations such as lymphoid organ $CD11c^{hi}CD11b^{lo}MHCII^+CD4^-CD8^+$ DCs ($CD8^+$ DCs) and tissue-resident $CD11c^{hi}CD11b^{lo}MHCII^+CD103^-$ DCs ($CD103^+$ DCs) demonstrate efficient antigen cross-presentation ability by using MHC class I to present exogenously derived antigens (den Haan and Bevan 2002; Belz et al. 2004; Beauchamp et al. 2010; Bedoui et al. 2009). Accordingly, $CD8^+$ DCs and $CD103^+$ DCs have important roles in antiviral and antitumor immune responses. Because of their effective antigen presentation properties to deliver tumor-associated MHC class I antigens to $CD8^+$ T cells, human equivalents to murine cDCs are being used for anticancer therapy (Koski et al. 2008).

It is widely accepted that functional properties of DCs are maturation-dependent (Steinbrink et al. 2009). However, recent evidence suggests that both phenotypically immature and mature DCs may be conditioned by the microenvironment to display immune tolerant and/or immunosuppressive functions (Lin et al. 2010; Gregori 2011; Manicassamy and Pulendran 2011). Nonetheless, DCs should be considered to be a specialized group of antigen-presenting cells with high functional plasticity. This plasticity of DCs, including immunostimulating or immunosuppressive potential, or both, depends on the consequence and combination of microenvironmental stimuli affecting DC differentiation, activation and polarization.

14.2 Dendritic Cells in Cancer: Immunosurveillance Versus Tumor Evasion

Cancer immunosurveillance is the inflammatory process whereby the immune system recognizes and eliminates an early developing tumor (Sheng et al. 2011). It is evident that innate leukocytes like DCs, macrophages and natural killer (NK) cells can sense early tissue stress and matrix alteration during cellular transformation (Sheng et al. 2011). Additionally, studies in both animal models and in clinical settings have clearly supported the idea of spontaneous tumor immune surveillance by T cells (Galon et al. 2006; Zhang et al. 2003). The increased susceptibility to spontaneously arising and/or chemically induced murine tumors in IFN-γ, perforin or interleukin (IL)-12 knockout mice is suggestive of the ability of DCs to mature in the tumor microenvironment, effectively uptake tumor-associated antigens for delivery to T cells, and induce tumor-specific T cell responses (Liu et al. 2004; Shankaran et al. 2001; van den Broek et al. 1996).

The cancer "immunoediting" hypothesis implies that all symptomatic tumors represent a failure of the immune system (Schreiber et al. 2011). Tumors can be kept in check for long periods, through a dynamic balance that results in the progressive loss of immunogenicity by tumor cells (Schreiber et al. 2011). In addition, the cancer immunoediting hypothesis has recently evolved to include a role for tumor-induced immunosuppression in accelerated tumor growth, because clinical trials using blocking common immunosuppressive checkpoints (such as CTLA4 or PD-1) demonstrated that preventing tumor-induced T cell paralysis restores protective immunity against established cancers, suggesting that advanced tumors remain somewhat immunogenic (Simeone and Ascierto 2012). However, the role of DCs in the "elimination, equilibrium and escape" stages suggested by immunoediting is still elusive.

Perhaps one of the most successful models to recapitulate the multitude of functional states of DCs in tumor initiation, equilibrium and escape stages is described by Scarlett et al., using a new inducible p53-dependent model of aggressive ovarian carcinoma, which is different from other models that initiate tumors before the development of a mature immune system or use transplantable tumor cell lines (Scarlett et al. 2012). In this model, measurable antitumor immunity from very early stages was driven by infiltrating DCs and prevented steady tumor growth for prolonged periods, indicating a protective role played by DCs in the induction of antitumor T cell-mediated immune responses. However, tumors aggressively progressed to terminal disease in a comparatively short time during which a phenotypic switch in expanding DC infiltrates could be detected. In the escape phase, tumor cells remained immunogenic at advanced stages, whereas antitumor T cells became less responsive and their enduring activity was abrogated by immunosuppressive DCs within the advanced tumor microenvironment. Notably, depleting DCs early in the disease course accelerated tumor expansion, but DC depletion at advanced stages significantly delayed aggressive malignant progression. These results clearly demonstrated that DCs in the tumor microenvironment serve as a double-edged sword: phenotypically divergent DCs drive both immunosurveillance and accelerate malignant growth.

14.3 Dysfunction of Dendritic Cells in Cancer

DCs have been used as biological adjuvants in tumor vaccinations due to their key role in tumor immunity (Steinman and Banchereau 2007; Koido et al. 2010; Palucka et al. 2009; Gabrilovich 2002; Kusmartsev and Gabrilovich 2002). However, antitumor immune responses are often deficient and unsatisfactory, and suppression and re-polarization of DC function in cancer patients are thought to contribute to the failure of antitumor immune responses and consequent disease progression (Palucka et al. 2010a, b; 2011). Subversion of tumor immunity by manipulating the tumor microenvironment and DC subset distribution and/or function is mediated by various tumor-derived and/or stromal factors, many of which remain to be identified (Ma et al. 2012).

Thus far, the abnormalities of DC differentiation and function are considered one of the major factors limiting the success of cancer vaccines in clinical trials. Therefore, studies of the mechanisms of tumor-induced DC dysfunction may be a key point to improve antitumor immune responses in cancer patients. The most common dysfunction of cDCs in the tumor microenvironment is demonstrated as unable to stimulate allogeneic and/or syngeneic T cell proliferation, reduced expression of costimulatory molecules, decreased uptake, processing and presentation of antigens, inefficient motility and migration towards specific chemokines and decreased production of IL-12 (Vicari et al. 2002; Yang and Carbone 2004). This type of functionally deficient DCs is usually not immunosuppressive. However, in specific tumor microenvironment conditions, the loss of function in DCs may, at least in part, be associated with acquisition of tolerogenic/immunosuppressive activities, including actively blockade of antitumor immunity, recruitment and expansion of regulatory Treg (T) cells and support of tumor progression by promoting intratumoral neoangiogenesis and metastases (Lin et al. 2010; Pinzon-Charry et al. 2005; Cools et al. 2007). This type of DCs with tolerogenic properties is termed regulatory DCs (regDCs) or tolerogenic DCs (Shurin et al. 2012). In most cases, the capacity of DCs to coordinate the immune response is not an intrinsic quality of the cell but is rather the result of specific microenvironmental signals for repolarization and/or recruitment, including the local cytokine/chemokine network and the milieu of soluble factors from the neighboring cells. For instance, tumor-derived IL-10, tumor growth factor (TGF)-β, IL-6, vascular endothelial growth factor (VEGF), macrophage colony-stimulating factor (M-CSF), and prostaglandin-E2 (PGE2) can render DCs to acquire regulatory instead of stimulatory capacities (Shurin et al. 2006; Kusmartsev and Gabrilovich 2006a; Lin and Karin 2007).

Strong evidence supports the presence of regDCs in different subsets, including immature and mature myeloid cells, conventional DCs, and pDCs (Gregori 2011; Manicassamy and Pulendran 2011; Shurin et al. 2012). To date, many types of regDCs with different phenotypes have been described. For instance, regDCs have been reported as DCs expressing high levels of CD80 and CD86, producing IL-10 and inducing differentiation of $CD4^+$ Treg cells (Akbari and Umetsu 2005). Other groups suggested that regDCs expressed exceptionally low levels of costimulatory

molecules, supported the generation of $CD4^+$ and $CD8^+$ Treg cells and prevented graft-versus-host disease (GVHD) (Isomura et al. 2008). In contrast, it has been also reported that regDCs expressed high levels of costimulatory/inhibitory B7-H1, B7-DC, and B7-H3 molecules and were capable of blocking DTH induction (Zhang et al. 2004). Furthermore, regDCs may be demonstrated by production of IL-10 and nitric oxide (NO), IL-10, TGF-β, cyclooxygenase 2 (COX-2), and indoleamine 2, 3-dioxygenase (IDO) (Kwon et al. 2010). However, many of tumor-associated regDCs were induced in vitro by culturing DCs in the immunosuppressive cytokines or drugs. Importantly, it becomes clear that the normal stromal microenvironment of the spleen, lung, and liver can drive DCs and hemopoietic progenitors to differentiate into regDCs with the phenotype of $CD11c^{low}CD11b^{high}Ia^{low}$ and high secretion of IL-10, NO, and IP-10 but less IL-12 (Zhang et al. 2004; Tang et al. 2006; Xia et al. 2008; Li et al. 2008a). These $CD11^{clow}CD11b^{high}Ia^{low}$ regDCs can be considered as "natural occurring regDCs", favor Th2 type immune responses and also induce Treg cell generation/expansion, and thus suppress type-1 T cell-mediated antitumor immunity and autoimmune diseases (Li et al. 2008b; Liu et al. 2009). We also observed that this type of "natural occurring regDCs" was the majority DC subset in murine lung tumor tissues (Lu et al. 2012). However, under some specific therapy, the recruitment of $CD8\alpha^+$ DCs, which are specialized for cross-presentation of antigen by MHC class I molecules to $CD8^+$ T cells, was associated with significant antitumor CTL responses (Lu et al. 2012).

14.4 Inhibitory Pathways of Immunosuppression Mediated by Cancer-Educated Regulatory Dendritic Cells

As discussed above, dysfunction of DCs within the cancer microenvironment may also associate with tolerogenic and immunosuppressive properties. Such immunosuppressive regDCs can mediate either direct effects on effector T cells or indirect effects on the T cells by induction and/or activation of other immune regulatory cells, such as Treg cells and myeloid-derived suppressor cells (MDSCs). Several soluble regDC-derived factors and membrane-bound or intracellular molecules are also responsible for these regDC-mediated immunosuppressive activities.

Tumor microenvironment-subverted DCs lack effector T cell stimulatory capacity but might be endowed with the ability to promote suppressive Treg cells (Steinman et al. 2003; Hubert et al. 2007; Stoitzner et al. 2008). Several studies provide evidence for the different subsets of regDCs capable of promoting Treg cell expansion and/or function (Hartmann et al. 2003; Wei et al. 2005). In addition to tumor-derived factors which directly induce Treg cell proliferation and/or generation of Treg cells from naive T cells, regDCs that are educated by the tumor microenvironment provide essential signals that contribute to Treg cell expansion and suppressive activity, which include IDO activity, PD-L1, TGF-β, IL-10 and so

on (Ni et al. 2012; Ramos et al. 2012; Sharma et al. 2007). Induction of Treg cells by regDCs thus appears to be one of the essential mechanisms employed by tumor cells to generate immunosuppressive Treg cells. Reciprocally, cancer/regDC-induced Treg cells, by restraining DC maturation and by inducing regDC expression and production of immunosuppressive molecules, may further skew DC differentiation towards an inhibitory cell population (Janikashvili et al. 2011). This positive feedback loop by which regDCs induce Treg cells that in turn enhance DC immunosuppressive function may significantly contribute to the persistence of the immune tolerance to cancer, and therefore, targeting the generation and function of these two suppressive cell populations is a desirable goal in immunotherapeutic approaches.

MDSCs are a mixed cell population of myeloid cells including immature granulocytes, macrophages, DCs, and myeloid progenitors (Kusmartsev and Gabrilovich 2006b). In mice, phenotypic $Gr1^+CD11b^+$ MDSCs were detected in all tested tumor models. Significant accumulation of this cell population has been found in patients with various types of cancer (Almand et al. 2001). MDSCs express high levels of immunosuppressive factors such as IDO, IL-10, arginase, inducible nitric oxide synthase (NOS2), NO, and reactive oxygen species, and use these molecules to suppress T cell-mediated immunity, DC function as well as induce regDCs (Marigo et al. 2008). Tumor/regDC-derived PGE2, in combination with lipopolysaccharide (LPS), IL-1β and IFN-γ induced production of COX-2 by monocytes, and redirected the development of $CD1a^+$ DCs to $CD14^+CD33^+CD34^+$ monocytic MDSCs (Obermajer et al. 2011). DCs/regDCs contribute to the induction and persistence of MDSCs, highlighting the potential for its manipulation to enhance immune responses in cancer.

Production of IL-10 and TGF-β by regDCs has been well established, and the role of these two cytokines in polarization of Treg cells has been repeatedly confirmed (Lin et al. 2010; Janikashvili et al. 2011). IL-10 and TGF-β are identified as anti-inflammatory cytokines with immunosuppressive properties and have crucial roles in preventing autoimmunity. They suppress antigen presentation and subsequent T cell proliferation, inhibit Th1 cytokine production and DC maturation. IL-10 and TGF-β expression has been shown to correlate with poor prognoses in many cancers. IL-10 also confers resistance of tumor cells to apoptosis and increases metastatic potential, and promotes angiogenesis by regulating VEGF production by myeloid cells (Zeng et al. 2010; Riboldi et al. 2005). Importantly, IL-10-conditioned tumor cells exhibit decreased expression of MHC class I and are resistant to $CD8^+$ CTL-mediated cytotoxicity (Kurte et al. 2004).

The enzyme arginase metabolizes L-arginine to L-ornithine and urea. Besides its fundamental role in the hepatic urea cycle, arginase is also expressed by the immune cells (Munder 2009). L-arginine depletion by arginase profoundly suppresses T cell-mediated immune responses, which has been considered as one of the fundamental mechanisms of inflammation-associated immunosuppression (Munder 2009). However, evidence of arginase expression by tumor-associated regDCs has been obtained recently showing that tumor-infiltrating regDCs can induce $CD8^+$ T cell exhaustion via L-arginine metabolism (Norian et al. 2009).

These regDCs are reported to display $CD11c^{+}CD11b^{high}Ia^{low}$ phenotype, and might be educated by tumor-derived factors such as TGF-β and PGE2 (Scarlett et al. 2012; Liu et al. 2009).

IDO catalyzes the degradation of the essential amino acid tryptophan into kynurenine, and can mediate tryptophan deprivation in the T cell microenvironment (Munn et al. 2002). IDO activity has been shown to downregulate the expression of TCR-ζ-chain and lead to the activation of the GCN2 (general control non-repressed 2) kinase pathway that results in T cell G1-phase arrest and apoptosis (Munn et al. 2005). In addition, the byproducts of the tryptophan catabolism such as L-kynurenine, 3-hydroxykynurenie, or 3-hydroxyanthranilic acid may be endowed with inherent suppressive activity (Fallarino et al. 2003). IDO activity can be detected in different subsets of DCs in mouse and humans, and the expression of IDO in DCs was associated with DC-induced immunosuppression (Ghahary et al. 2004; O'Neill et al. 2004). Tryptophan depletion by IDO has been identified as a possible factor involved in regDC-induced Treg cell expansion and activation (Sharma et al. 2007; Fallarino et al. 2006). Treg cell induction and activation by IDO^{+} regDCs require the GCN2 pathway and can be partially prevented by CTLA-4 blockade (Sharma et al. 2007). IDO^{+} regDCs also suppress the conversion of $CD4^{+}Foxp3^{+}$ Tregs cells to Th17-like effector cells in tumor-draining lymph nodes (Sharma et al. 2009). These studies suggested that IDO-expressing regDCs found at the tumor sites and in tumor-draining lymph nodes might help suppress the initiation of immune responses to tumor-associated antigens and create systemic tolerance to tumor cells.

PD-1 and PD-L1 belong to the B7 family of costimulatory molecules and are expressed on activated DCs, monocytes/macrophages, T cells, B cells, as well as tumor cells (Keir et al. 2008; Dong et al. 2002). PD-L1 promotes differentiation and maintains the function of induced Treg cells (Francisco et al. 2009). Blockage of PD-1/PD-L1 interaction increases infiltration of $CD8^{+}$ T cells to tumors, suggesting that PD-L1 induces tumor-specific CTL exhaustion (Zou 2005). PD-L1 and/or PD-1 expression levels on myeloid DCs correlate with poorer cancer prognosis (Zou 2005; Thompson et al. 2007). For instance, it has been reported that ovarian cancer-infiltrating DCs progressively expressed upregulated PD-1 and PD-L1 molecules, and were immunosuppressive to T cell immunity and blocked their infiltration into advanced tumors (Zou 2005).

14.5 Signal Pathways Involved in Dendritic Cell Dysfunction in Cancer

Tumor microenvironment is well known to be immunosuppressive (Kim et al. 2006a; Rabinovich et al. 2007). Tumor cells consistently release many kinds of immunosuppressive and proinflammatory factors such as VEGF, TGF-β, IL-10, PGE2, M-CSF and IL-6, which facilitate tumor immune escape and tumor growth, partially by actively reprogramming DC dysfunction for tumor cell escape of

immunological attack (Zou 2005; Bennaceur et al. 2009). Although the list of tumor-derived and stromal factors involving the impaired or repolarized DC function might be getting longer, many of them may utilize similar transcription factors and signaling pathways.

Numerous recent studies have reported that tumor-induced activation of intracellular signaling pathways, such as mitogen-activated protein kinases (MAPKs), JAKs/STATs, and NF-κB, contributes to various defects of the immune system, particularly through compromising DC differentiation and function. Even though these signaling pathways are important for the development of normal hematopoietic cells, activation of these pathways is usually present in both tumor cells and abnormal DCs to support tumor growth and survival (Ade et al. 2007; Nefedova et al. 2004; Philpott et al. 2004). In this section we will discuss signaling pathways mediating DC dysfunction, particularly p38 MAPKs, in negatively regulating DC differentiation and function in cancers.

14.5.1 MAPK Signaling Pathways

MAPKs are proline-directed serine and threonine protein kinases, and are activated by dual-specificity kinases with phosphorylation of threonine and tyrosine in a Thr-Xaa-Tyr motif. Activation of MAPK signaling pathways is through a MAPK-activating phosphorylation cascade, in which upstream kinases phosphorylate their downstream kinases on threonine and tyrosine residues, starting from MAPK kinase kinases (MAPKKKs), to MAPK kinases (MAPKKs), and finally to MAPKs. The activated MAPKs then interact with their cytoplasmic substrates and translocate into the nucleus, where they act as transcription factors and regulate target gene transcription (Nakamura et al. 1996; Ichijo 1999).

MAPK signaling pathways are crucial for diverse cellular functions, including proliferation, differentiation, and apoptosis (Aplin et al. 2002; Budagian et al. 2003; Kawakami et al. 2003; Sigaud et al. 2005). There are three types of MAPKs, extracellular signal-regulated kinases (ERKs), c-jun N-terminal kinases (JNKs), and p38 MAPKs, which are identified by the intervening amino acid. The ERK pathway, activated by polypeptide growth factors through their tyrosine kinase receptors, regulates cellular growth and survival. JNK and p38 signaling pathways are activated by stress stimuli and inflammatory cytokines, and are involved in cellular differentiation, cytokine production, and apoptosis. MAPK signaling pathways have been shown to be frequently activated in cancers, and may contribute to malignant phenotypes and uncontrolled cell growth. In addition, MAPK signaling pathways are involved in the regulation of immune responses, including the initiation phase of innate immunity, activation of adaptive immunity, and cell death after completing immune function (Nakahara et al. 2004; Canesi et al. 2005; Kim et al. 2005; Zou and Hu 2005). Notably, recent studies have indicated that MAPK signaling pathways differentially regulate all aspects of DC phenotypic maturation, cytokine production, and DC functional development (Nakahara et al. 2004; Cruz et al. 1999;

Xie et al. 2005; Wang et al. 2006a). Stimuli such as LPS, TNF-α, haptens, or ultraviolet-B (UVB) induce maturation of DCs via MAPK signaling pathways (Nakahara et al. 2004; Cruz et al. 1999; Tassiulas et al. 2007). On the other hand, tumor-induced abnormalities of DC differentiation and function are also associated with hyperactivation of MAPK signaling pathways (Wang et al. 2006a, b).

14.5.2 p38 MAPKs in Dendritic Cell Differentiation, Maturation, and Activity

There are four p38 MAPKs; α and β, which are 75 % homologous, and γ and δ, which are more distant relatives. All p38 MAPKs can be activated by the same upstream MAP kinase kinases, such as MKK3 or MKK6, upon the stimulation of inflammatory cytokines or stress (Ichijo 1999; Lee et al. 2006). p38 MAPK signaling induces the activation of MAPK-activated protein kinase (MAPKAPK)-2 (Zaru et al. 2007), synthesis of TNF-α (Lee et al. 2006; Park et al. 1999), and phosphorylation of transcription factors such as activating-transcription-factor-2 (ATF-2), Elk-1 and SAP-1.

The p38 MAPK signaling pathway is essential for normal DC maturation and activity (Xie et al. 2005; Ardeshna et al. 2000; Matos et al. 2005a, b; Osawa et al. 2006). LPS-induced maturation and upregulation of surface antigens on DCs such as CD40, CD80, CD83, CD86, and MHC class II molecules require p38 MAPKs (West et al. 2004; Bharadwaj et al. 2005). The p38 MAPK inhibitor SB203580 abrogates the upregulation of surface antigens in the process of DC maturation induced by LPS, $NiCl_2$, $NiSO_4$, and CD40L. Furthermore, LPS-induced DC secretion of cytokines such as TNF-α, IL-6, and IL-12, also depends on the activation of p38 MAPKs, because SB203580 has been shown to inhibit DC secretion of these cytokines (Lee et al. 2006; Randolph et al. 2005; Saito et al. 2006). In addition, LPS-enhanced allostimulatory activity of DCs is abrogated by SB203580 treatment, indicating that p38 MAPKs are required for the endocytotic and allostimulatory functions of DCs (Kang et al. 2004).

However, we have shown that the importance of p38 MAPK signaling pathways in DCs is stage-dependent. While crucial for immature DCs to mature and secrete cytokines, activation of p38 MAPKs is detrimental to the generation and differentiation of DCs from monocytes. During the differentiation of monocytes to immature DCs, p38 MAPK activation induced by LPS impaired DC differentiation and p38 MAPK inhibitor SB203580 restored generation of functional DCs in culture with LPS. Moreover, addition of SB203580 to cultures of normal monocytes accelerated the differentiation of the cells into immature DCs. These results could be explained by the findings that inhibition of p38 MAPKs enhances the phosphorylation of ERK and NF-κB activity and leads to enhanced upregulation of expression of DC-related adhesion and costimulatory molecules and antigen-presentation capacity (Xie et al. 2005; Lee et al. 2006; Ardeshna et al. 2000; Osawa et al. 2006).

14.5.3 p38 MAPKs in Tumor-Induced Dendritic Cell Dysfunction

DCs from cancer patients are functionally defective, however, the underlying molecular mechanisms are poorly understood at the present time. We have used the murine 5TGM1 myeloma model to examine the effects and mechanism of tumor-derived factors on the differentiation and function of DCs. Myeloma cells or tumor culture conditioning medium (TCCM) were shown to inhibit differentiation and function of bone marrow-derived DCs (BMDCs), as evident by the down-regulated expression of DC-related surface molecules, decreased IL-12 secretion, and compromised capacity of the cells to activate allospecific T cells. Moreover, TCCM-treated BMDCs were inferior to normal BMDCs at priming tumor-specific immune responses in vivo. Neutralizing antibodies against IL-6, IL-10, and TGF-β partially abrogated the effects. Our results showed that TCCM treatment activated p38 MAPK and JNK but inhibited ERK. Inhibiting p38 MAPK restored the phenotype, cytokine secretion, and function of TCCM-treated BMDCs. BMDCs from cultures with both TCCM and p38 inhibitor were as efficacious as normal BMDCs at inducing tumor-specific antibody, type-1 T cell, and CTL responses, and prolonging mouse survival. Thus, our results suggest that tumor-induced p38 MAPK activation and ERK inhibition in DCs may be a new mechanism for tumor evasion, and regulating these pathways during DC differentiation provides new strategies for generating potent DC vaccines for immunotherapy in cancer patients (Wang et al. 2006a).

Next, we examined whether the defects can be observed in DCs from patients with myeloma. Previous studies have demonstrated that circulating DCs in myeloma patients are functionally abnormal (Ratta et al. 2002). However, no study had been performed to examine monocyte-derived DCs (MoDCs), which are commonly used for immunotherapy in patients. We found that patient-derived MoDCs are phenotypically and functionally defective. Compared with their normal counterpart, patient-derived mature MoDCs expressed significantly lower levels of CD1a, CD40, CD80, and HLA-DR, and were deficient at activating alloreactive T cells, presenting recall antigen, and activating autologous antigen-specific T cells. These abnormalities may be attributed to elevated production of autocrine cytokines such as IL-6, activated p38 MAPK and STAT3, and inhibited MEK/ERK signaling pathways in the progenitor cells. Treatment with neutralizing IL-6-specific antibody and more importantly, p38 MAPK inhibitor, or both, could correct these abnormalities. Treating patient-derived cells with these agents not only significantly increased cell yield, but also produced MoDCs that were as functional as their normal counterpart (Wang et al. 2006b). Thus, our studies have delineated the mechanistic defects of MoDCs from myeloma patients, and identified ways for restoring the function of the cells to improve the efficacy of DC-based immunotherapy in this disease.

In line with our findings, others showed that constitutive activation of p38 MAPK is responsible for turning off DCs to display a tolerogenic profile during

melanoma progression, and suppression of p38 MAPK activity in DCs from tumor-bearing mice could reconstitute their impaired function as shown by normalization of cytokine secretion pattern and T cell stimulation capacity (Zhao et al. 2009). Another recent study also showed that inhibiting p38 MAPK signaling in DCs attenuates Treg cell induction in response to Toll-like receptor (TLR) agonists and enhances their efficacy as vaccine adjuvants and cancer immunotherapeutics (Jarnicki et al. 2008). TLR ligands are commonly used adjuvants that promote type-1 T cell responses against tumor antigens. However, TLR ligands also promote the induction of IL-10-secreting Treg cells through p38 MAPK-induced IL-10 production by DCs. Inhibition of p38 MAPKs by SB203580 suppressed TLR-induced IL-10 and PGE_2 and enhanced IL-12 production in DCs. Inhibition of p38 MAPKs enhanced the antitumor therapeutic efficacy of DCs pulsed with antigen and CpG, which was associated with an enhanced frequency of IFN-γ-secreting T cells and a reduction of $Foxp3^+$ Treg cell infiltration of the tumors. Taken together, these findings indicate that p38 is an important therapeutic target and inhibiting p38 activity in DCs obtained from cancer patients or DCs pulsed with tumor antigens and TLR agonists will enhance the immunogenicity of the cells.

14.5.4 ERK and Dendritic Cell Dysfunction in Cancer

Recent studies have demonstrated that the ERK and p38 MAPK signaling pathways differentially regulate DC maturation and modulate the initial commitment of naïve T-helper (Th) cells toward Th1 or Th2 subsets (Aplin et al. 2002; Lee et al. 2006; Kandilci and Grosveld 2005). The p38 MAPK inhibitor SB203580 suppressed DC maturation, whereas the presence of ERK inhibitors PD98059 or U0126 enhanced LPS-induced phenotypic and functional maturation of DCs, and increased the expression of MHC complex and costimulatory molecules. In a recent study, cDCs derived in vitro from murine $ERK1^{-/-}$ bone marrow progenitors were demonstrated with increased surface expression of activation markers and enhanced T cell stimulation, suggesting that ERK1 negatively regulated functional differentiation of DCs (Bendix et al. 2010). Importantly, ERK signaling in DCs has been shown to suppress the immune response and stimulate the expansion of Treg cells (Escors et al. 2008). Selective ERK activation in both mouse and human DCs generated regDCs with immunosuppressive capacity, leading to Treg cell expansion by secreting bioactive TGF-β1 and IL-10 (Escors et al. 2008; Arce et al. 2011).

However, MAPK pathways, which are frequently activated in cancers, have active roles in immune evasion in cancer. Tumor lysate has been shown to markedly suppress TLR-4-dependent IL-12p40 and p70 production from DCs by hyperactivating ERK signaling in DCs, and these tumor lysate-treated DCs were less able to generate Th1-responses from naïve T cells (Jackson et al. 2008). Blockade of MEK1/2, the upstream kinase for ERK, with U0126 prevented ERK

activation, restored IL-12p70 production, and permitted effective generation of Th1-responses (Jackson et al. 2008). In addition, by using ERK inhibitor U0126 and lentiviral $BRAF^{V600E}$ RNA interference, Sumimoto et al. demonstrated that the ERK signaling pathway is essential for production of immunosuppressive factors by human melanoma cells that have constitutively activated ERK due to the $BRAF^{V600E}$ mutation, which can be detected in the majority of patients with melanomas (Sumimoto et al. 2004, 2006; Tanami et al. 2004). These findings indicate that pharmacological intervention in the MEK-ERK axis may be used to render DC resistant to the suppressive effects of tumor microenvironment and may become part of a combination immunotherapy.

14.5.5 Role of JAKs/STATs Signaling in Tumor-Induced Dendritic Cell Dysfunction

Over the past several years, investigators have been working on the JAK/STAT signaling pathways in the context of cancer-mediated evasion of the immune system (Kortylewski et al. 2005a; Nefedova et al. 2005; Kim et al. 2006b). JAK mutations and/or STAT abnormal activation are found in many types of cancers, such as myeloproliferative disorders with acquired JAK2 mutations (Taki and Taniwaki 2006; Jost 2007; Mata et al. 2007), T cell acute lymphoblastic leukemia (Taki and Taniwaki 2006), and leukemia or lymphoma with constitutive phosphorylation of JAK3, STAT1, STAT3, and STAT5 (Aboudola et al. 2007). Among them, constitutive activation of STAT3 is common in a variety of lymphoid or myeloid malignancies and solid tumors, in human tumor cell lines and primary tumor cells from patients, and in virus-transformed cells (Yu et al. 1995; Campbell et al. 1997; Cheng et al. 2004; Park et al. 2005). Recent studies showed that hyperactivation of STAT3 is found in multiple myeloma, breast cancer, and prostate cancer (Wang et al. 2004a).

In addition to STAT3-induced intrinsic oncogenic activities, studies have shed light on STAT3-mediated cancer cell-initiated immune evasion signals in various immune cells (Yu et al. 2007). Soluble factors released from tumor cells, such as IL-10, IL-6, VEGF or M-CSF, induced activation of STAT3 in myeloid cells, leading to systemic accumulation and activation of MDSCs and inhibition of DC differentiation toward immunogenic status (Wang et al. 2004b; Nefedova et al. 2004). Since JAK/STAT3 signaling pathway is a major signaling pathway that can be activated by cytokines binding to their membrane receptors, tumor-derived factors inhibit DC differentiation and function mainly via JAK/STAT3 activation (Li et al. 2007). For instance, treatment of DCs with tumor-conditioned medium reduced expression of IL-12 and MHC II and costimulatory molecules, and promoted transcription of IDO due to activated STAT3-induced inhibition of canonical NF-κB activity (Hoentjen et al. 2005; Kitamura et al. 2005; Nefedova et al. 2005; Sun et al. 2009). In addition, Treg cells hamper DC function by activating STAT3 signaling pathway in DCs (Larmonier et al. 2007). Inactivation

of STAT3 signaling in hematopoietic cells by pharmacological inhibitors, such as JSI-124 or CPA-7, demonstrated enhanced antitumor immune responses through the activation of various immune cells, especially DCs, and inactivation of immune suppressor cells, such as MDSCs and Treg cells (Nefedova et al. 2005; Kortylewski et al. 2005b). Similarly, $STAT3^{-/-}$ bone marrow progenitor cells were also refractory to tumor-derived inhibitory factor-mediated suppression of DCs differentiation (Wang et al. 2004b). Importantly, DCs derived from $STAT3^{-/-}$ mice displayed higher cytokine production in response to TLR stimulation, and induced effective antitumor effects when used as vaccine through systemic Th1 immune responses (Iwata-Kajihara et al. 2011). Since inhibition of STAT3 abrogated the negative effects of the tumor-derived factors on myeloid cell differentiation, these observations suggest that JAK/STAT signaling pathways may be negative regulators of DC differentiation and function in malignancies.

14.5.6 Other Signaling Pathways in Tumor-Induced Dendritic Cell Dysfunction

It is well known that activation of NF-κB plays an important role in DC maturation and function (Ade et al. 2007; Zou and Hu 2005; Osawa et al. 2006). JAK/STAT, p38 MAPK, and ERK signaling pathways crosstalk with the NF-κB pathway, and factors activating STATs or MAPKs also stimulate NF-κB, which includes members of p50, p52, RelA, RelB, and cRel. The proteins form active hetero- or homodimers, translocate to the nuclei, and initiate the transcription of target genes. NF-κB activity has been shown to be high in DCs, and upregulation of IL-12 expression requires activation of both p38 MAPK and NF-κB (Ade et al. 2007). Our and others previous studies showed that differentiation of immature DCs is accompanied by increased NF-κB activity and that inhibiting p38 MAPK enhances the activity of NF-κB in immature DCs (Ade et al. 2007; Wang et al. 2006a, b). Because high levels of NF-κB activity are frequently found in many types of cancers, NF-κB signaling pathways may also contribute to tumor-induced DC dysfunction in cancer patients.

14.6 Conclusion

DCs play important roles in initiating innate and adaptive immune responses, which are critical for the antitumor immune response. However, hyperactivation of signaling pathways such as JAKs/STATs, MAPKs, and NF-κB in both tumor cells and tumor-infiltrating DCs is critical for tumor-induced immunosuppression of tumor-bearing hosts. The activation of multiple signaling pathways in tumor cells mediates the expression and secretion of tumor-derived factors to the tumor microenvironment. Subsequently, these tumor-derived factors impair DC differentiation and

impair their function, resulting in DC-mediated immune tolerance. The concept for revitalizing the capacity of immunogenic DCs to stimulate CTLs is widely accepted to be a critical step to enhancing antitumor immunity.

Blockage of tumor-induced DC dysfunction by targeting signaling molecules or pathways may restore DC function. Inhibitors to signaling molecules are already under investigation in clinical trials as therapeutic agents to treat cancers (Barclay et al. 2007; Chou et al. 2005; Demuth et al. 2007; Do et al. 2004; Jing et al. 2006; Jiang et al. 2007; Kirkwood et al. 2007; McKay et al. 2000; Yoshikawa et al. 2001). These antagonists as anti-cancer drugs are expected to not only improve DC function, but also and more importantly, may boost antitumor immunity in cancer patients. Even though these signaling pathways are pivotally important for normal cell proliferation and survival and blockade of them may possibly lead to toxicity in patients, some encouraging preliminary results have already been obtained from clinical trials that examine the efficacy of the specific inhibitors. In future studies, it will be important to identify novel and specific targets in these signaling pathways for cancer therapy.

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