Interleukin (IL)-12 and IL-23 and Their Conflicting Roles in Cancer

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The balance of proinflammatory cytokines interleukin (IL)-12 and IL-23 plays a key role in shaping the development of antitumor or protumor immunity. In this review, we discuss the role IL-12 and IL-23 plays in tumor biology from preclinical and clinical data. In particular, we discuss the mechanism by which IL-23 promotes tumor growth and metastases and how the IL-12/IL-23 axis of inflammation can be targeted for cancer therapy.

The recognized interleukin (IL)-12 cytokine family currently consists of IL-12, IL-23, IL-27, and IL-35 and these cytokines play important roles in the development of appropriate immune responses in various disease conditions (Vignali and Kuchroo 2012). They act as a link between the innate and adaptive immune system through mediating the appropriate differentiation of naïve CD4⁺ T cells into various T helper (Th) subsets and regulating the functions of different effector cell types. IL-12, IL-23, and IL-27 are secreted by activated antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages (Vignali and Kuchroo 2012), whereas IL-35 is generally thought to be produced by regulatory T (Treg) and B cells, although they were recently detected in human tolerogenic DCs (Pylayeva-Gupta 2016). A unique feature of these cytokines is their heterodimeric subunit

composition whereby the α -subunit (p19, p28, p35) and β -subunit (p40, Ebi3) are differentially shared to generate IL-12 (p40-p35), IL-23 (p40-p19), IL-27 (Ebi3-p28), and IL-35 (p40-p35) (Fig. 1A). Given their ability to share α - and β -subunits, it has been predicted that combinations such as Ebi3-p19 and p28-p40 could exist and serve physiological function (Fig. 1B) (Wang et al. 2012; Flores et al. 2015; Ramnath et al. 2015). Similarly, the subunit-sharing feature of the IL-12 cytokine family also extends to its receptor chain usage (Fig. 1B). Although they share structural similarities and downstream signaling components, members of the IL-12 cytokine family mediate distinct biological functions. IL-12 and IL-23 are proinflammatory cytokines and are required for the development of Th1 and Th17 cells (Vignali and Kuchroo 2012). In contrast, IL-27 has im-

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Figure 1. Schematic representation of the interleukin (IL)-12 cytokine family and their receptors, and associated Janus kinase and signal transducers and activators of transcription (JAK-STAT) signaling partners. (A) Current members of the IL-12 family with defined physiological function. (B) Potential new members of the IL-12 cytokine family that can be generated following subunit pairing. Ebi3, Epstein–Barr virus-induced gene 3 protein (also known as IL-27β); IL-12R, IL-12 receptor; IL-23R, IL-23 receptor; TYK2, tyrosine kinase 2.

munosuppressive function and can curtail different classes of inflammation through its ability to directly modify $CD4^{+}$ and $CD8^{+}$ T-cell effector functions, to induce IL-10, and to promote specialized Treg cell responses (Yoshida and Hunter 2015). Likewise, IL-35 has an immunosuppressive function as shown by its ability to induce the development of IL-35-producing induced Tregs and the suppression of T-cell effector function and proliferative capacity in a variety of in vitro and in vivo systems (Vignali and Kuchroo 2012; Pylayeva-Gupta 2016).

In cancer, inflammation has been shown to play a critical role in tumor initiation, growth, and metastasis (Grivennikov et al. 2010; Elinav et al. 2013). It is now appreciated that the balance between the proinflammatory cytokine IL-12

and IL-23 in tumors can shape the development of antitumor or protumor immunity. Given that the importance of IL-12 in promoting antitumor immunity is well recognized and recently reviewed (Tugues et al. 2015), this review will particularly focus on discussing the role of IL-23 in tumor biology and its mechanism of action in promoting tumor growth and metastases. Finally, we discuss how IL-12 and IL-23 are cross-regulated and how the IL-12/IL-23 axis of inflammation can be targeted for cancer therapy.

ROLE OF IL-12 AND IL-23 IN TUMOR BIOLOGY

APCs, such as DCs and macrophages, are thought to be the predominant source of IL-

IL-12 and IL-23 (Hunter 2005). A major role of IL-12 is to promote differentiation of Th1 cells and to induce type II interferon (IFN)- γ production. The requirement for host IL-12 and its downstream cytokine in activating antitumor immunity is well recognized and has been previously reviewed extensively (Colombo and Trinchieri 2002; Dunn et al. 2006; Tugues et al. 2015). A number of studies have clearly illustrated the importance of endogenous IL-12 and IFN- γ in preventing cancer initiation, growth, and metastasis. In contrast, IL-23 plays an important role in promoting the proliferation and effector function of Th17 cells, which are characterized by expression of the IL-17 family cytokines (Aggarwal et al. 2003; Langrish et al. 2004; Langrish et al. 2005). Mice that were deficient for IL-12/23p40 or IFN- γ and challenged with methylcholanthrene-A (MCA), a chemical carcinogen, displayed increased rate and frequency of tumor growth compared to wild-type (WT) controls (Kaplan et al. 1998; Smyth et al. 2000). In contrast, mice deficient in IL-23p19 were strongly protected from developing MCA-induced fibrosarcomas (Teng et al. 2010). In another study, using a mouse model of dimethylbenz[a]anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted two-stage skin carcinogenesis, IL-12/23p40-deficient and IL-23-p19-deficient mice displayed significantly decreased numbers of carcinogen-induced papillomas compared to WT mice, whereas the opposite was observed in IL-12p35-deficient mice (Langowski et al. 2006). Similarly, mice lacking IL-12p35 and IFN- γ were more susceptible to mortality caused by *N*-methyl-*N*-nitrosourea (MNU)-induced lymphoma compared to IL-12/23p40-deficient mice (Liu et al. 2004). Although naïve IL-12/23p40-deficient mice did not display any increase in tumor development compared to WT mice when monitored over their normal life span (Street et al. 2002), a proportion of aged mice deficient for IL-12R β 2 and, hence, nonresponsive to IL-12, developed plasmacytoma and lung epithelial tumors (Airolidi et al. 2005). In addition to directly activating T-cell and natural killer (NK) cell effector function, IL-12 and IFN- γ can modulate the tumor mi-

croenvironment to be more conducive to anti-tumor immunity by inhibiting angiogenesis and expanding intratumoral Tregs (Cao et al. 2009; Tugues et al. 2015). Nevertheless, IL-12 may have IFN- γ -independent tumor-suppressive properties. A study using IL-12-producing B16 melanomas, in which innate lymphoid tissue-inducer cells but not T and NK cells induced tumor suppression (Eisenring et al. 2010), showed the pleiotropic ability of IL-12 to activate multiple arms of antitumor immunity.

The data that support the tumor-promoting effect of host IL-23 is also strong. A seminal paper by Langowski et al. (2006) provided the first demonstration that mice deficient in IL-23p19 were resistant to DMBA/TPA-induced skin papillomas and this resistance correlated with a significant increase in CD8⁺ T cells infiltrating the skin and a reduction in IL-17A, matrix metalloproteinase 9 (MMP9), CD31, granulocytes (Gr-1⁺), and macrophages (CD11b⁺, F4/80⁺). A study by Teng et al. (2010) further confirmed the resistance phenotype of these mice to DMBA/TPA-induced skin papillomas and also highlighted their resistance to MCA-induced fibrosarcomas. Interestingly, IL-17A did not promote the formation of MCA-induced fibrosarcomas, showing that IL-23p19 had tumor-promoting properties independent of IL-17A (Teng et al. 2010). In contrast to the MCA model, mice deficient for IL-12/IL-23p40 were still protected from tumor development following DMBA/TPA treatment, suggesting in this model that the loss of IL-23 was more important than the loss of IL-12 (Teng et al. 2010). In this model, *Il17a*-deficient mice displayed a protective phenotype albeit weaker than *Il23a*-deficient mice. Furthermore, this study also showed that, not only did IL-23 suppress the antitumor function of T cells as first uncovered by Langowski et al. 2006, it also suppressed the antimetastatic function of NK cells (Teng et al. 2010). In two other *de novo* mouse models of colon carcinogenesis (CPC-APC, *Apc*^{Min/+}), IL-23 and IL-17A both had tumor-promoting effects as loss of IL-23/IL-23R/IL-17R or IL-23R/IL-17A blockade resulted in reduced tumor load (Wu et al. 2009; Grivennikov et al. 2012). Interestingly, one study re-

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ported that expression of IL-23 alone in mice was sufficient to induce rapid (3–4 wk) de novo development of intestinal adenomas with 100% incidence. Tumorigenesis was mediated by type 3 innate lymphoid cells (ILC3) but independent of exogenous carcinogens, *Helicobacter* colonization, or preexisting tumor-suppressor gene mutations (Chan et al. 2014). Similarly, a role for IL-23 has also been shown for the T-cell-mediated tumor dormancy phase (equilibrium) of cancer immunoediting (Schreiber et al. 2011), best characterized in the MCA-induced fibrosarcoma model (Koebel et al. 2007). In mice bearing dormant tumors induced by MCA, anti-IL-23 resulted in the elimination of the residual tumor cells, whereas anti-IL-12p40 (IL-12 and IL-23) led to their outgrowth (Teng et al. 2012), thus showing that loss of IL-23 was not sufficient to compensate for loss of IL-12 in this setting. Overall, these studies suggest the hierarchy of dominance between IL-12 in tumor suppression and IL-23 in tumor promotion in de novo models of inflammation-induced carcinogenesis varies, and this can be because of the type and location of the tumor and the immune cell infiltrates.

On the contrary, there have been a number of studies suggesting that IL-23 can have tumor suppressing effects when it is overexpressed in different tumor cell lines and implanted into mice (Lo et al. 2003; Chiyo et al. 2004; Hu et al. 2006, 2009; Oniki et al. 2006; Shan et al. 2006; Yuan et al. 2006; Kaiga et al. 2007; Reay et al. 2012; Ngiow et al. 2013). A recent study also showed that injection of IL-23 in combination with a transforming growth factor β (TGF- β) receptor inhibitor could suppress the progression of premalignant lesions to cancer in a 4-nitroquinoline-1-oxide (4NQO) carcinogen-induced mouse model of oral cancer due to maintenance of Th17 cells and preventing their shift to a Treg phenotype (Young et al. 2016). In an acute UV-induced immunosuppression model, IL-23 was shown to be important in reducing UV-induced DNA damage and inhibiting UV-induced Tregs (Majewski et al. 2010). Similarly, a later study reported that mice deficient for IL-23p19 and chronically exposed to UVB were found to have a higher likelihood of

developing tumors, particularly nonepithelial sarcomas, compared to the WT controls (Jantschitsch et al. 2012). One explanation could be that Th17 cells or IL-17A may have a role in tumor suppression similar to the DMBA/TPA model, although the role of Th17/IL-17A was not examined in this study. Although these data appear contradictory at first, the caveat of most of these experiments is that the expression of IL-23 is in a nonphysiological manner.

CLINICAL RELEVANCE OF IL-23 EXPRESSION IN HUMAN CANCERS

In agreement with the role of endogenous IL-23 in promoting mouse tumor growth, IL-23 was found to be overexpressed in many human cancers (Table 1). Similarly, two functional genetic variants of the IL-23R (IL-23R rs1884444 T>G and rs6682925 T>C) have been found to contribute to susceptibility to solid cancer and blood malignancies. However, the functional consequences of these variants in modulating IL-23R signaling are not clear (Chu et al. 2012; Qian et al. 2013a; Xu et al. 2013). In contrast, there has only been one clinical study in ovarian cancer in which a higher level of intratumoral IL-23p19 transcript correlated with improved patient overall survival. However, higher levels of intratumoral IL-12p35 transcripts were also measured in this study (Wolf et al. 2010). Overall, these studies provide compelling evidence that supports the involvement of IL-23 in the pathogenesis of different cancers, particularly those that are of inflammation-induced origin (Elinav et al. 2013).

CELLULAR SOURCES OF IL-23 IN TUMORS

In mice and humans, the main sources of IL-23 are thought to be produced by myeloid cells in response to exogenous or endogenous signals, such as damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), or tumor-secreted factors such as prostaglandin E2 (PGE₂) (Qian et al. 2013b; von Scheidt et al. 2014; Chang et al. 2015; Teng et al. 2015; Kvedaraite et al. 2016). However, it remains unclear which myeloid subsets in tu-

Table 1. Overexpression of interleukin (IL)-23 in different human cancers

Cancer	Correlation	IL-23 levels in patients	IL-23 levels in control	Sample size	Method of detection	References
NSCLC	Increased levels associated with increased risk of NSCLC	0.27 (0.15–0.73) pg/mL	0.17 (0.10–0.28) pg/mL	218	CBA	Liao et al. 2015
NSCLC	N/A	Higher compared to controls		53	qPCR	Baird et al. 2013
Lung (NSCLC and SCLC)	N/A	491.27 ± 1263.38 pg/mL (serum)	240.51 ± 233.18 pg/mL (serum)	46	ELISA	Cam et al. 2016
CRC	N/A	Higher compared to normal tissue		7	qPCR	Grivennikov et al. 2012
CRC	N/A	~5000 ng/mL from homogenized tumors	~2000 ng/mL normal tissues	13	qPCR ELISA	Lan et al. 2011
CRC	Significantly higher at all tumor stages (I–IV) compared with healthy donors	31.5 ± 10.5 pg/mL (serum)	8.48 ± 13.3 pg/mL (serum)	48	ELISA	Stanilov et al. 2010
CRC	Elevated expression with concomitant VEGF overexpression positively correlated with histological grade 2	189.46 pg/mL (serum)	34.77 pg/mL (serum)	40	ELISA	Ljubic et al. 2010
HCC	Positively correlated with metastasis	Higher compared to normal tissue		81 49 112	qPCR and IHC qPCR	Li et al. 2012 Wolf et al. 2010
Ovarian cancer	Higher levels positively correlated with OS	Higher compared to normal tissue				
Breast cancer	Higher levels negatively correlated with OS	14.522 ± 11.39 pg/mL (serum)	6.34 ± 4.6 pg/mL (serum)	50	ELISA	Gangemi et al. 2012
Pancreatic cancer	N/A	266.5 ± 98.1 pg/mL (serum)	95.1 ± 37.2 pg/mL (serum)	20	ELISA	He et al. 2011
MM	N/A	~300 pg/mL (bone marrow)	~50 pg/mL (bone marrow)	5	ELISA	Prabhala et al. 2010
Bladder cancer	N/A	Higher IL-23 producing CD123 ⁺ pDCs compared to healthy volunteers		20	IHC	Wang et al. 2016
Melanoma	N/A	Higher than normal tissue, benign melanocytic nevi, and Spitz nevi		35	IHC	Ganzetti et al. 2015
Advanced gastric cancer	N/A	293 ± 132 pg/mL (serum)	102 ± 55 pg/mL (serum)	36	qPCR ELISA	Zhang et al. 2008

NSCLC, Non-small-cell lung carcinoma; SCLC, small-cell lung carcinoma; CRC, colorectal carcinoma; HCC, hepatocellular carcinoma; MM, multiple myeloma; OS, overall survival; pDC, plasmacytoid dendritic cells; CBA, cytometric bead array; qPCR, real-time polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; VEGF, vascular endothelial growth factor.

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mors secrete IL-23, and questions remain as to whether tumors themselves can also secrete IL-23. In mice, most studies have quantified IL-23p19 messenger RNA (mRNA) expression, given the lack of current reagents that can robustly detect IL-23 protein levels by ELISA or intracellular staining. However, a caveat is that increased expression of p19 mRNA may not correlate with the translation of p19 protein and/or formation of secreted bioactive IL-23, which requires coexpression and disulfide bond formation between the IL-23p19 and IL-12p40 subunits in the same cell (Waibler et al. 2007; Brentano et al. 2009; Floss et al. 2015). IL-23 has been measured in the supernatant from tumor, infiltrating CD11c⁺DCs and CD11b⁺CD11c⁻ macrophages sorted from mice bearing subcutaneous B16 melanoma tumors (Kortylewski et al. 2009). In CPC-APC/IL-23^{-/-} mice harboring the green fluorescent protein (GFP) gene in the IL-23p19 locus, GFP was detected in both CD11b⁺ or CD11b⁻ cells derived from the mesenteric lymph node or tumor, although this was not quantitated and control staining was not shown (Grivennikov et al. 2012). In B16F10 and RM-1 tumor-bearing lungs, expression of IL-23, as measured by flow cytometry, was found to be restricted to MHC-II⁺CD11b⁺CD11c⁺ cells rather than MHC-II⁺CD11c⁺CD11b⁻ cells, following lipo-

polysaccharide (LPS) restimulation (von Scheidt et al. 2014). Overall, this indicates that mouse IL-23 production can be restricted to specific immune cell subsets and depends on the context and environment in which it is measured.

To date, there have been no convincing studies showing that mouse tumor cells themselves produce IL-23. IL-23 (as measured by ELISA) was not detected in in vitro culture of various experimental mouse tumor cell lines such as EG7, B16F10, and RM-1 (von Scheidt et al. 2014). Similarly, in another study, very little IL-23 was detected in in vitro cultures of B16 and C4 melanoma tumor cell lines (Kortylewski et al. 2009). However, whether these cell lines can produce IL-23 following exposure to various metabolites or cytokine stimulation or following in vivo inoculation remains to be examined. Similarly, only a few studies have reported that human tumor cell lines themselves could secrete IL-23 (Table 2). However, these studies generally used real-time polymerase chain reaction (qPCR) and/or Western blotting to measure the presence of IL-23p19; whether bioactive IL-23 is secreted remains to be validated.

EXPRESSION PATTERN OF IL-12R AND IL-23R

The IL-12 and IL-23 receptors are made up of IL-12Rβ1 and IL-12Rβ2 or IL-12Rβ1 and IL-

Table 2. Interleukin (IL)-23-producing human tumor cell lines

Human tumor cell lines	IL-23 detection method	References
Human lung carcinoma cell lines	RT-PCR	Baird et al. 2013
A549 (adenocarcinoma)		
SK-MES-1 (squamous-cell carcinoma)		
H1299, H460, and H647 (large-cell carcinoma)		
BEAS2B (SV40 transformed normal bronchoepithelia)		
HBEC3, HBEC4, and HBEC5 (normal bronchial epithelial cell lines immortalized in the absence of viral oncoproteins)		
Human oral squamous cell carcinoma	qPCR	Fukuda et al. 2010a,b
HSC-2, HSC-3, HSC-4, and Ca9-22		
Human hepatocellular carcinoma	WB	Li et al. 2012
HepG2, PLC8024, QGY7703, H2P, H2M, Huh7, and MHCC-97L		
Human pancreatic carcinoma cell line	qPCR, cytokine array	Chang et al. 2015
PANC-1		

RT-PCR, Reverse transcription polymerase chain reaction; qPCR, real-time polymerase chain reaction; WB, Western blot.

23R, respectively (Fig. 1A). Signaling by IL-12 stimulates nonreceptor Janus kinase 2 (JAK2) and tyrosine kinase 2 (TYK2) activity resulting in the phosphorylation of signal transducers and activators of transcription (STAT) family members, particularly STAT4 (Zundler and Neurath 2015), whereas STAT3 is preferentially activated following IL-23 stimulation (Floss et al. 2015). In mice and humans, a range of innate and adaptive immune cells have been shown to express IL-12 and IL-23 receptors constitutively as measured by qPCR, flow cytometry, or using an IL-23R GFP KI reporter mouse (Tables 3 and 4), although they appear to be expressed at varying levels, which can be further up-regulated following activation (Ivanov et al. 2006; Ghoreschi et al. 2010; Gaffen et al. 2014). In addition, it was suggested that the IL-12 and IL-23 receptors may not be expressed on the same cell population (Chognard et al. 2014). Therefore, it will be interesting to assess the IL-23R-expressing cells present in tumors and whether their composition differs in different tumor microenvironments. Although mouse tumor cell lines generally have not been reported to express IL-23R, a number of studies have reported that some human tumor cell lines expressed IL-23R and could respond to IL-23 (Table 5). Interestingly, varying levels of IL-23R (as measured by flow cytometry) were also detected on primary tumors, such as pediatric B-ALL cell samples compared to their normal counterparts (Cocco et al. 2010) in diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) (Cocco et al. 2012) and in a proportion of colorectal carcinoma (CRC) (Suzuki et al. 2012).

MECHANISM OF IL-23 IN PROMOTING TUMOR GROWTH AND METASTASES

The antitumor and antimetastatic activities of IL-12 are thought to be mediated by STAT4 activation of IFN- γ (Colombo and Trinchieri 2002; Trinchieri 2003; Zundler and Neurath 2015). In contrast, the mechanism of action of IL-23 is not fully elucidated. Although IL-23 has been linked with Th17 cells and is crucial for their function and cytokine production (such as IL-17A) in vivo (Muranski and Restifo

2013), IL-23 can have tumor-promoting function independent of IL-17A (as discussed above). Interestingly, a recent study reported that mice lacking IL-17A had reduced lung metastases (Kulig et al. 2016), although mice lacking IL-23 were not examined in the same assay. Nevertheless, it is likely that IL-17A will be involved in the protumor activity of IL-23 because, in some models, anti-IL-17A alone or in combination with anti-IL-23R could reduce bacterial-induced colon carcinogenesis (Wu et al. 2009). Similarly, in a mouse model of *Apc*-driven colorectal cancer model, IL-23 signaling was shown to promote tumor growth and progression and development of an intratumoral IL-17 response (Grivennikov et al. 2012). However, Th17 cells and IL-17A have also been reported to have tumor-suppressing function in some mouse models of cancer and in certain human cancers (Zou and Restifo 2010; Wilke et al. 2011b). It would appear that the requirement of IL-17 for the tumor-promoting activity of IL-23 may be tumor-dependent. In one study, it was reported that tumor-infiltrating Tregs expressed IL-23R and that blocking IL-23R signaling could reduce Treg numbers and their capacity to secrete IL-10 in a number of experimental mouse tumor models (Kortylewski et al. 2009). However, expression of IL-23R on Tregs has not been reported elsewhere. Nevertheless, this study also showed that STAT3 enhanced the expression of IL-23 in macrophages but inhibited IL-12 in DCs in the tumor microenvironment (Kortylewski et al. 2009). Thus, one mechanism by which IL-23 promotes tumorigenesis may be through driving protumor inflammation to suppress antitumor effector cells.

In addition to IL-17A and IL-10, other Th17 cytokines that have been reported to be regulated by IL-23, including other IL-17 isoforms and IL-22 (Eyerich et al. 2010; Cornelissen et al. 2011). Similar to IL-17A, IL-22 has been shown in different mouse models of inflammation/carcinogen-induced cancer to mediate both tumor-promoting and -suppressing functions (Blake and Teng 2014). Importantly, it is clear that the function of Th17 cells in tumor immunity cannot be linked just to the function of the IL-17A given that other cells of the innate and

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Table 3. IL-23R expression on immune cells in mice

Immune cells	Organ	Mouse	Methods	References
$\gamma\delta$ T ($\gamma\delta^+$)	Spleen, LP	Naïve IL-23R GFP B6	qPCR, FC	Chognard et al. 2014
$\gamma\delta$ T ($\gamma\delta^+$)	Lung	Naïve IL-23R GFP B6	FC	Paget et al. 2015
$\gamma\delta$ T ($\gamma\delta^+$) in vitro cultured	Spleen	Naïve B6	qPCR, FC	Sutton et al. 2009
$\gamma\delta$ T ($\gamma\delta^+$)	LN, LP	Naïve IL-23R GFP B6	FC	Awasthi et al. 2009
$\gamma\delta$ T ($CD3^+$ TCR δ^+)	Spleen, LN	EAE B6 mice	qPCR, FC	Raverdeau et al. 2016
Memory T cells (DX5 $^-$ TCR β^+ CD62L $^-$)	Spleen	Naïve B6	qPCR	Rachitskaya et al. 2008
CD4 $^+$ T cells (CD3 $^+$ CD4 $^+$)	LP	Naïve IL-23R GFP B6	FC	Chan et al. 2014
CD4 $^+$ T (CD4 $^+$ TCR β^+)	Spleen, LN	Naïve IL-23R GFP B6	FC	Awasthi et al. 2009; Chognard et al. 2014
Th17 cells (CD4 $^+$) cultured in in vitro Th17 conditions	Spleen	Naïve B6	qPCR	Ciric et al. 2009; El-Behi et al. 2011
LTi cells (CD3 $^-$ CD4 $^+$)	LP	Naïve IL-23R GFP B6	FC	Awasthi et al. 2009
Treg (CD4 $^+$ FOXP3 $^+$)	Tumor, DLN	B16, MC38 and MB49 tumor-bearing B6	FC	Kortylewski et al. 2009
Tc17 (CD8 $^+$) Cultured in in vitro Tc17 conditions	Spleen	Naïve B6	qPCR	Ciric et al. 2009
B cells (CD19 $^+$ B220 $^+$)	Spleen, LP	Naïve IL-23R GFP B6	FC	Chognard et al. 2014
NKT (DX5 $^+$ TCR β^+)	Spleen	Naïve B6	qPCR	Rachitskaya et al. 2008
NKT (α -GC/CD1d tetramer $^+$ TCR β^+ NK1.1 $^-$) stimulated with anti-CD3 and anti-CD28	Thymus	Naïve B6	qPCR	Coquet et al. 2008
DC (CD11c $^+$)	Spleen, LN	Naïve B6, naïve IL-23R GFP B6	qPCR, FC	Awasthi et al. 2009, El-Behi et al. 2011
Macrophage (CD11b $^+$)	LN, LP	Naïve IL-23R GFP B6	FC	Awasthi et al. 2009
Inflammatory macrophage (CD4 $^-$ CD11b $^+$ CD45 $^+$)	CNS	EAE B6	qPCR	Cua et al. 2003
Neutrophil thioglycollate induced	Peritoneal cavity	Naïve B6	qPCR	Chen et al. 2016
Neutrophil (Ly6G $^+$)	BM	Naïve B6	qPCR, FC	Taylor et al. 2014
Neutrophils (stimulated)	Colon	DSS-treated B6	qPCR	Zindl et al. 2013
LT β R $^+$ cells	LP	DSS-treated mice	qPCR	Macho-Fernandez et al. 2015
ILC3 (NKp46 $^+$ CD127 $^+$ CD117 $^+$ CD49b $^-$)	Spleen	Naïve IL-23R GFP B6	FC	Chognard et al. 2014
ILC3 (Thy1 $^+$ NKp46 $^+$ CD3 $^-$)	LP	Naïve IL-23R GFP mice	FC	Chan et al. 2014
LTi-like cells (CD3 $^-$ CD127 $^+$ CD117 $^+$)	Spleen	Naïve IL-23R GFP mice	FC	Chognard et al. 2014
LTi (Thy1 $^+$ cKIT $^+$ NKp46 $^-$ CD3 $^-$)	LP	Naïve IL-23R GFP mice	FC	Chan et al. 2014
Thymocytes (Thy1.2 $^+$)	Thymus	Naïve B6	qPCR, FC	Li et al. 2014
Thymic epithelial cells	Thymus	Naïve B6	qPCR	Li et al. 2014

FC, Flow cytometry; DLN, draining lymph node; LN, lymph node; LP, lamina propria; EAE, experimental autoimmune encephalomyelitis; B6, C57BL/6 mice; Treg, T regulatory cell; LTi, lymphoid tissue inducer cell; CNS, central nervous system; DSS, dextran sodium sulfate; LT β R, lymphotoxin β receptor; ILC3, type 3 innate lymphoid cells; BM, bone marrow; NKT, natural killer T cell; DC, dendritic cell; GFP, green fluorescent protein; IL, interleukin; qPCR, real-time polymerase chain reaction.

Table 4. Interleukin (IL)-23R expression on human immune cells

Immune cells	Organ cells were isolated from	Method of detection	References
$\gamma\delta$ T cells ($CD3^+ TCR\delta^+$)	PBMC	RT-PCR	Chognard et al. 2014
$\gamma\delta$ T cells ($CD3^+ V\gamma9^+$)	PBMC, cord blood	FC	Moens et al. 2011
$CD4^+$ T cells ($CD3^+ CD4^+$)	PBMC	RT-PCR	Chognard et al. 2014
$CD4^+$ memory T cells ($CD4^+ CD45RO^+$)	PBMC	FC	Sarin et al. 2011
Memory T cells ($DX5^- TCR^+ CD62L^-$)	PBMC	qPCR	Rachitskaya et al. 2008
Tc17 cells ($CD8^+$)	PBMC	FC	Sarin et al. 2011
$CD8^+$ T cells ($CD3^+ CD8^+$)	PBMC	RT-PCR	Chognard et al. 2014
ILC3 ($Lin^- CD127^+ c-Kit^+ NKp44^+$)	Tonsil	qPCR	Bernink et al. 2013
Neutrophil	PBMC	IF	Taylor et al. 2014
NKT cells ($DX5^+ TCR\beta^+$)	PBMC	qPCR	Rachitskaya et al. 2008
MAIT cells ($CD3^+ CD161^{high} V\alpha7.2^+ CD4^- TCR\gamma\delta^-$)	Liver blood	Nanostring	Tang et al. 2013

PBMC, Peripheral blood mononuclear cells; MAIT, mucosal-associated invariant T cells; ILC3, type 3 innate lymphoid cells; FC, flow cytometry; IF, immunofluorescence; RT-PCR, reverse transcription polymerase chain reaction; qPCR, real-time polymerase chain reaction; NKT, natural killer T cell.

adaptive immune system, such as $\gamma\delta$ T cells, NK-T cells, and ILCs, also produce substantial quantities of this and other cytokines such as IL-22 (Wilke et al. 2011a,b; Sabat et al. 2013). Other cytokines, including IL-6 and granulocyte macrophage colony-stimulating factor (GM-CSF), can also be induced downstream from IL-23/IL-23R signaling and have also been implicated in the pathogenesis of several autoinflammatory and autoimmune diseases (Yen et al. 2006; Lindroos et al. 2011; Wu et al. 2016). Whether these cytokines contribute to the protumor effect of IL-23 remains to be investigated. IL-23 is also thought to negatively regulate the functions and infiltration of $CD8^+$ T cells into tumor tissue. IL-23p19-deficiency or anti-IL-23p19 antibody treatment has been shown to increase $CD8^+$ T-cell infiltration into DMBA/TPA-treated skin (Langowski et al. 2006), as measured by immunohistochemistry (IHC). Indeed, cytotoxic markers such as FasL, perforin, and granzymes were shown to be up-regulated in carcinogen-treated skin of IL-23p19-deficient mice (Langowski et al. 2006). Importantly, within tumors, $CD8^+$ T cells have been shown to be required for tumor suppression mediated by ablation of IL-23/IL-23R signaling (Langowski et al. 2006; Teng et al. 2011; von Scheidt et al. 2014). However, so far, there is no clear evidence that IL-23R is expressed by tumor-infiltrating $CD8^+$ T cells, and it is most

likely that IL-23 suppresses the antitumor activity of $CD8^+$ T cells indirectly. In addition to their effects on immune cells, IL-23R expression on human tumor cell lines has been reported. Coculture with IL-23 generally promoted their proliferation except in the case of B-cell malignancies in which a high dose of IL-23 was used (Table 5). Interestingly, it has also been shown that different concentrations of IL-23 can exert opposite effects on the capability of IL-23R expressing human lung cancer cells to proliferate (Li et al. 2013).

A key function of IL-23 appears to be its ability to promote tumor metastases through up-regulation of proangiogenic factors. Evidence suggests that IL-23 overexpression can induce metastasis of hepatocellular carcinoma (HCC), CRC, melanoma, and esophageal and thyroid cancer (Li et al. 2012; Suzuki et al. 2012; Zhang et al. 2014; Chen et al. 2015; Ganzetti et al. 2015; Klein et al. 2015; Mei et al. 2015). IL-23 was reported to directly up-regulate expression of MMP9 and vascular endothelial growth factor (VEGF)-C in human esophageal cancer cell lines to facilitate epithelial–mesenchymal transition and migratory ability in vitro (Chen et al. 2015). In the same study, patients with esophageal cancer ($n = 23$) who had lymphatic and distant metastasis compared to those who did not had significantly higher IL-23p19 expression (although the source of the anti-IL-

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Table 5. Interleukin (IL)-23R expressing human tumor cell lines

Human tumor cell lines expressing IL-23R	IL-23R detection method	Effect of coculture with IL-23	References
Lung adenocarcinoma: A549, SPCA-1	IHC, IF WB	Low concentration promoted proliferation, whereas high concentration suppressed proliferation	Li et al. 2013 Baird et al. 2013
Oral squamous cell carcinoma: HSC-2, HSC-3, HSC-4, Ca9-22	WB, RT-PCR	Promoted proliferation of HSC-3	Fukuda et al. 2010b
Pro-B-ALL cell line: RS4;11 Pre-B-ALL cell line: Nalm-6, Nalm-697	FC	High concentration suppressed proliferation and promoted apoptosis	Cocco et al. 2010
B-cell lymphoma: SU-DHL-4, DOHH-2, OCI-LY8 cell	FC	Suppressed proliferation of SU-DHL-4	Cocco et al. 2012
Colon carcinoma: SW480	RT-PCR, IHC	Not performed	Lan et al. 2011
Colon carcinoma: SW480, HCT116, and HT29	ND	Promoted proliferation of HCT116, HT29, but not SW480	Shapiro et al. 2016
Colon carcinoma: MIP101, DLD-1, and KM12c	WB	Promoted proliferation and invasive capability of DLD-1 only	Suzuki et al. 2012
Hepatocellular carcinoma: PLC8024 and QGY-7703	ND	Promoted invasion and migration and production of MMP9	Li et al. 2012

WB, Western blot; IHC, immunohistochemistry; RT-PCR, reverse transcription polymerase chain reaction; MMP9, matrix metalloproteinase 9; FC, flow cytometry; IF, immunofluorescence; ND, not detected; B-ALL, B-acute lymphoblastic leukemia.

23 antibody used for IHC was not listed [Chen et al. 2015]). Similarly, another study also showed that IL-23 could promote the migration and invasive ability of HCC cell lines through up-regulation of MMP9 expression via activation of NF- κ B/p65 (Li et al. 2012). Higher IL-23p19 levels, as measured by mRNA expression and IHC, were also detected in primary HCC tumor tissues with metastasis compared with paired nontumor tissue (Li et al. 2012). IL-23 was also reported to correlate with IL-17A and MMP9 expression in these clinical samples. Similar findings were also reported for IL-23 in facilitating the migration and invasive ability of human thyroid cancer cell lines to migrate and this was mediated via an miR-25/SOCS4 signaling pathway (Mei et al. 2015). Additionally, higher IL-23p19 mRNA expression was present in thyroid cancer patients who had lymphatic and distant metastasis. It was also reported that IL-23 promoted the metastasis of CRC with impaired SOCS3 expression via a STAT5 pathway (Zhang et al. 2014). In melanoma, astrocytes (glial cells) were reported to fa-

cilitate human melanoma brain metastasis via secretion of IL-23 in an orthotopic brain melanoma metastases mouse model (Klein et al. 2015). Using IHC, Ganzetti et al. (2015) found that the intensity and percentage of IL-17 and IL-23 was significantly higher in malignant melanomas than in benign melanocytic or Spitz nevi. Overall, it appears that the direct effects of IL-23 are most likely mediated through induction of cell-cycle pathways (cyclin-dependent kinases and cyclin D) and oncogenic and associated genes that regulate growth-factor-induced signaling (AKT, NF- κ B, AP-1) (Kortylewski et al. 2009; Chan et al. 2014).

CROSS-REGULATION OF IL-12 AND IL-23

In tumors, the ratio of IL-12 and IL-23 produced by DCs and macrophages will be determined by the balance of endogenous Toll-like receptor (TLR) agonists, danger signals, and/or tumor-derived mediators in the tumor micro-environment, leading to activation of their respective downstream pathways, which ulti-

mately dictate tumor growth outcome (Gerosa et al. 2008; Ngiow et al. 2013). The mechanism by which IL-12 and IL-23 regulate each other is not well elucidated and may be quite complex. Some studies have reported that IFN- γ can negatively regulate IL-23 production by inhibiting mouse *Il23a* gene-promoter activity (Sheikh et al. 2010, 2011). Conversely, another study showed that IL-23 antagonized IL-12-induced secretion of IFN- γ (Sieve et al. 2010). Adding to the complexity, a recent study in mice suggested signaling through IL-23R may potentially promote IL-12 production, whereas signaling through IL-12R β 2 suppresses IL-1 β and IL-23 (Chognard et al. 2014). Furthermore, a dichotomous pattern of expression for IL-12 and IL-23 receptors in both mouse and humans was reported in this study, suggesting that immune cells involved in antitumor responses may be quite distinct from those that are tumor promoting. Finally, IL-12 and IL-23 can also be regulated both genetically and epigenetically. It was reported that conventional DCs (cDCs) and plasmacytoid DCs (pDCs) from *Grm4*^{-/-} mice produced higher amounts of IL-6 and IL-23, but less IL-12 and IL-27 compared to their WT counterparts in response to LPS or CpG-ODN, respectively (Fallarino et al. 2010). In addition, phosphatase 2A has been shown to negatively regulate IL-23 but not IL-12 in LPS stimulated DC by suppressing *IL-23p19* gene expression (Chang et al. 2010). In contrast, TRABID, a deubiquitinase, was reported to mediate epigenetic regulation of both *Il12* and *Il23* gene expression (Balhara et al. 2016), while deubiquitinases ADAM10 and ADAM17 have been reported to mediate IL-23R ectodomain shedding (Franke et al. 2016). In mice and humans, differential splicing of the *IL-23R* gene has also been reported to generate antagonistic soluble IL-23R variants (Zhang et al. 2006; Kan et al. 2008; Mancini et al. 2008; Floss et al. 2015). These soluble IL-23R may potentially reduce the cellular responsiveness of IL-23R expressing cells toward IL-23 and can also bind to IL-23 and act as competitive antagonist of IL-23 signaling (Franke et al. 2016).

Recently, it was reported that IL-4 had opposing effects on the production of either IL-12

or IL-23 in which it promoted the IL-12-producing capacity of DCs while abrogating IL-23 production (Guenova et al. 2015). It is also possible that the IL-12 and IL-23 pathways can be regulated by their shared IL-12p40, p35, p19 subunits, and corresponding receptors. Free IL-12p40 exists in either homodimeric IL-12p80 or monomeric IL-12p40 forms in mice and human, and can act as natural antagonists of IL-12 and IL-23 by competing for binding to IL-12R β 1 (Mattner et al. 1993; Gillesen et al. 1995; Ling et al. 1995; Trinchieri 2003; Shimo-zato et al. 2006). Similarly, in mice, it has been reported that IL-12p80 suppressed splenic Tregs via induction of nitric oxide from APCs, and this suppression was dependent on IL-12R β 1 rather than IL-12R β 2 in vitro (Brahmachari and Pahan 2009). Given that IL-12p35 is also used as a shared subunit by the inhibitory cytokine IL-35 (IL-12p35/Ebi3 [IL-27p28]) produced by Tregs and B cells (Collison et al. 2007; Shen et al. 2014), one must also consider how the balance between IL-12 and IL-35 impacts on tumorigenesis (Banchereau et al. 2012). Similarly, a recent study showing that IL-23p19 can interact with Ebi3 (Ramnath et al. 2015) raises new questions regarding its physiological function and how it may regulate the other IL-12 family cytokines in which common subunits are shared.

Interestingly, a number of papers have also reported that immune checkpoint receptors such as TIM-3 and PD-1 expressed on monocytes/macrophages can regulate the balance of IL-12/IL-23 in viral infections such as hepatitis C (Zhang et al. 2011a,b; Wang et al. 2013). Similarly, a recent study reported that agonistic anti-BTLA inhibited DC-induced Th17- and Th1-cell responses because of decreased production of the Th17- and Th1-related cytokines IL-1 β , IL-6, IL-23, and IL-12p70 and reduced CD40 expression in DCs (Ye et al. 2016). Given that checkpoint receptors are a major pathway of tumor-induced immune suppression (Pardoll 2012), this represents another mechanism by which the IL-12/IL-23 balance can be affected. Overall, the subunit sharing between the IL-12 family of cytokines makes it difficult to definitively delineate the

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function of each cytokine. Generating antibodies that can specifically neutralize the specific cytokine rather than the shared subunits might be a better approach for dissecting out the endogenous role these family members play in promoting or suppressing tumorigenesis.

TARGETING THE IL-12/IL-23 AXIS OF INFLAMMATION FOR CANCER THERAPY

It is now recognized that the immune system is actively suppressed in the tumor microenvironment and that lymphoid, myeloid, and granulocytic cells contribute to this suppression. In the past 5 years, therapies targeting T-cell immune checkpoint receptors have achieved remarkable success in the clinic, particularly in combination, and will increasingly become incorporated into standard-of-care for many cancer types (Smyth et al. 2016). Nevertheless, not all cancer types respond to checkpoint blockade even when targeted in combination. Given that human tumors are extremely heterogeneous with respect to their proportions of immune cells, blocking immunosuppressive pathways mediated by both T and myeloid cells may be required in certain cancer types to release full endogenous antitumor immunity.

A number of preclinical mouse studies have shown that changing the balance of IL-12 and IL-23 can promote tumor progression or suppression and thus targeting this axis may be beneficial particularly in combination immunotherapies. In one study, intratumoral IL-12 application in combination with systemic checkpoint blockade of CTLA-4 resulted in eradication of mice with orthotopic gliomas (Vom Berg et al. 2013). Similarly, another study showed a combination of agonistic anti-CD40 monoclonal antibodies (mAbs) to drive IL-12 production and anti-IL-23 mAbs to counter the tumor-promoting effects of IL-23 had greater antitumor activity than either agent alone (von Scheidt et al. 2014). The efficacy of this combination may potentially be effective in patients whose cancer display rich myeloid infiltrates and up-regulated IL-23 (e.g., sarcomas), where neutralization of IL-23 in

the tumor microenvironment may allow the agonistic activity of anti-CD40 to be fully maximized. More recently, agonistic CD40 mAb-driven IL-12 was shown to reverse resistance to anti-PD-1 therapy in T-cell-rich tumors (Ngiow et al. 2016). Similarly, in a preclinical murine model of bladder cancer, Tasquiminod, a small molecule that binds S100A9 increased tumor mRNA expression levels of *Il12b*, *Tbet*, and *Ifng* and synergized with anti-PD-L1 to suppress tumor growth (Nakhle et al. 2016).

Prophylactic neutralization of IL-23 has been shown to significantly suppress experimental lung metastases of B16F10 melanoma, RM-1 prostate carcinoma, and 3LL lung carcinoma, which are controlled by host NK cells rather than CD8⁺ T cells (Teng et al. 2010, 2011). In mice bearing established lung metastases, IL-2 immunotherapy was enhanced in mice deficient for IL-23p19, suggesting neutralizing anti-IL-23 mAbs may synergize with immunotherapies that activate NK cells such as IL-2 (Teng et al. 2010). Neutralization of IL-23 also synergized with anti-ERBB2 mAb in suppressing subcutaneous growth of established Her-2/neu-positive breast tumors in mice (Teng et al. 2010). In addition, targeting of activating receptors such as CD137 or CD226 (DNAM-1) (Kohrt et al. 2012; Blake et al. 2016) or inhibitory receptors such as CD96 and TIGIT may also synergize with IL-23 neutralization to suppress metastases (Blake et al. 2016). Alternatively, anti-IL-23p19 mAbs can be combined with chemotherapy, such as gemcitabine, that has been shown to induce IL-23p19 and IL-23R mRNA expression in non-small-cell lung carcinoma (NSCLC) cell lines (Baird et al. 2013).

CONCLUSIONS

In this review, we have discussed how the balance of proinflammatory cytokines IL-12 and IL-23 plays a key role in shaping the development of antitumor or protumor immunity, respectively. Although the antitumor efficacy of IL-12 is generally mediated via downstream activation of IFN- γ , IL-23 can have a tumor-promoting function independent of IL-17A, such as directly up-regulating proangiogenic factors

to facilitate the epithelial–mesenchymal transition and migratory ability of tumor cells. Preclinically, strategies to alter the ratio of IL-12 and IL-23 in the tumor microenvironment have been shown to synergize in combination with other anticancer therapies. Clinically, IL-23 is overexpressed in a number of cancer types, which could potentially be targeted, particularly in those that display rich myeloid infiltrates. Although there are obvious caveats in the interpretation of mouse studies on the role of IL-12 and IL-23 in tumor biology, clinical studies have reported the favorable safety profile of anti-IL-12/23p40 mAbs (Ustekinumab). However, there was an increased risk in developing malignancies in psoriasis patients treated when high doses of anti-IL-12/23p40 mAbs were administered (Young and Czarnecki 2012). In contrast, major adverse cardiac events were observed in psoriasis patients treated with another anti-IL-12/23p40 mAb (Briakinumab) (Teng et al. 2015). To date, a number of IL-23-specific antagonists have showed efficacy and safety in the treatment of psoriasis patients in late-stage clinical trials (Gordon et al. 2015; Teng et al. 2015). Should larger and longer-term trials show that neutralization of IL-23 impacts minimally on the risk of malignancies and infection development, anti-IL-23 could potentially be repositioned for use in immuno-oncology in combination with other immunotherapies.

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