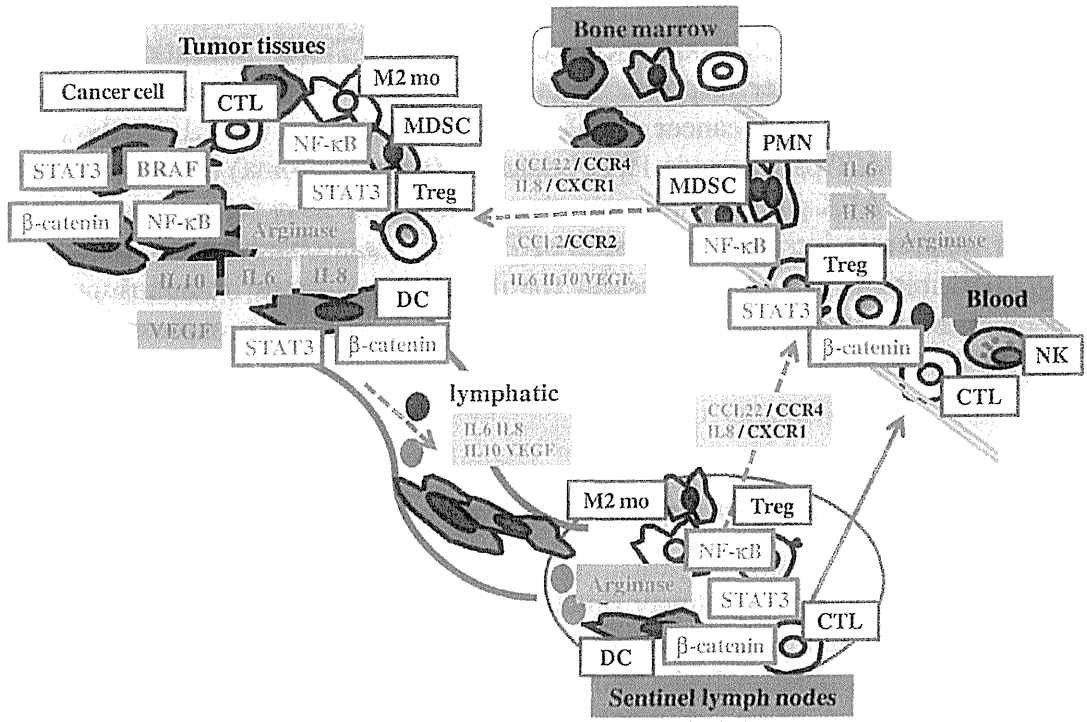

Fig. 12.3 Cancer cell triggered immunosuppressive cascades: MAPK, STAT3, β-catenin, and NF-κB cascades. Alterations of oncogenes and subsequently activated signaling are different among cancer cells even in the same types of cancer. For examples, alterations of MAPK, STAT3, β-catenin, and NF-κB trigger different immunosuppressive cascades via production of immunosuppressive cytokines such as IL-6, IL-8, IL-10, VEGF, etc., and subsequent impairment of DC function, and induction of various immunosuppressive cells such as MDSC and Treg cells

12.5 Clinical Implications of the Immunosuppressive Mechanisms

As described above, multiple immunosuppressive cascades are triggered by gene and signal alternations in human cancer cells and generate immunosuppressive condition particularly in the tumor-associated microenvironments, including the tumor tissues and sentinel lymph nodes (Fig. 12.3). One of the important questions is which molecules and cells, either at upstream or downstream, in the immunosuppressive cascades should be inhibited for the efficient reversal of immunosuppression in patients with cancer. It may depend, at least in part, on cancer types and their genetic alterations.

In general, targeting constitutive active signaling molecules in cancer cells has advantage of direct anti-tumor effects such as inhibition of cancer cell proliferation and direct destruction of malignant cells, which may lead to induction of immune responses to multiple endogenous tumor antigens, including patients' unique antigens (e.g., mutated antigens); this may also lead to simultaneous inhibition of downstream multiple immunosuppressive mechanisms. However, inhibition

of upstream molecules may also cause broad adverse effects, including suppression of anti-tumor immune response, although suppression of anti-tumor immune responses may be avoided by the use of appropriate doses of inhibitors. For instance, as was observed after administration of NF-κB inhibitor or mutated molecule selective inhibitors, such as mutant BRAF selective inhibitors. In contrast, targeting downstream molecules and cells, such as TGF-β, IL-10, PD-L1, IDO, Cox2 or MDSCs and Tregs, by using small molecule inhibitors or antibodies may have advantage of high specificity leading to more efficient blockade with less broad adverse effects. However, inhibition of one molecule or one cell type may not be sufficient to overall reversal of immunosuppression in patients with cancer. The combination of signal inhibitors and blockade of major immuosuppressive molecules or cells (e.g., neutralizing or blocking antibodies for TGF-β or PD-1) may also be attractive strategies for strengthening activity to reverse tumor-associated immunosuppression.

Besides inhibition/blocking of tumor-derived immunosuppressive factors, signal inhibition in immune cells may result in the direct activation of immune cells or inhibition of induction of immunosuppressive cells, including Tregs and MDSCs. Altogether, targeting activated signaling molecules involved in triggering of multiple immunosuppressive cascades may be an attractive strategy for reversal of immunosuppressive conditions in the tumor-associated microenvironments for cancer therapies, particularly immunotherapy (Fig. 12.4). Combination treatments utilizing these molecular targeted drugs and various immunotherapies, including cancer vaccines and check point blockade, are particularly appearing and will be evaluated in future clinical trials. One important point is that an appropriate target may be different among patients, since constitutively activated molecules and signaling pathways vary among patients even with the same type of tumor, indicating necessity of personalized strategy (Table 12.1). In the next 10 years, molecular and cellular basis of cancer-induced immunosuppression in the tumor-associated microenvironments will be further understood, and clinical efficacy of combined immunotherapy with molecular targeted drugs will be clinically evaluated.

In addition to the therapeutic implications of altered gene and signaling involved in cancer-induced immunosuppression, they may play a role in diagnostics. As described above, infiltration of memory $CD8^+$ T cells in tumor mass and serum IL-6/IL-8 levels appear to be prognostic markers and response prediction markers for cancer treatment including immunotherapy. Since the immune status may be a reflection of gene/signal alterations as described above, evaluation of the altered gene/signaling status (e.g., pERK, pSTAT, nuclear translocation of NF-κB, or β-catenin) may also serve as diagnostic biomarkers for cancer patients.

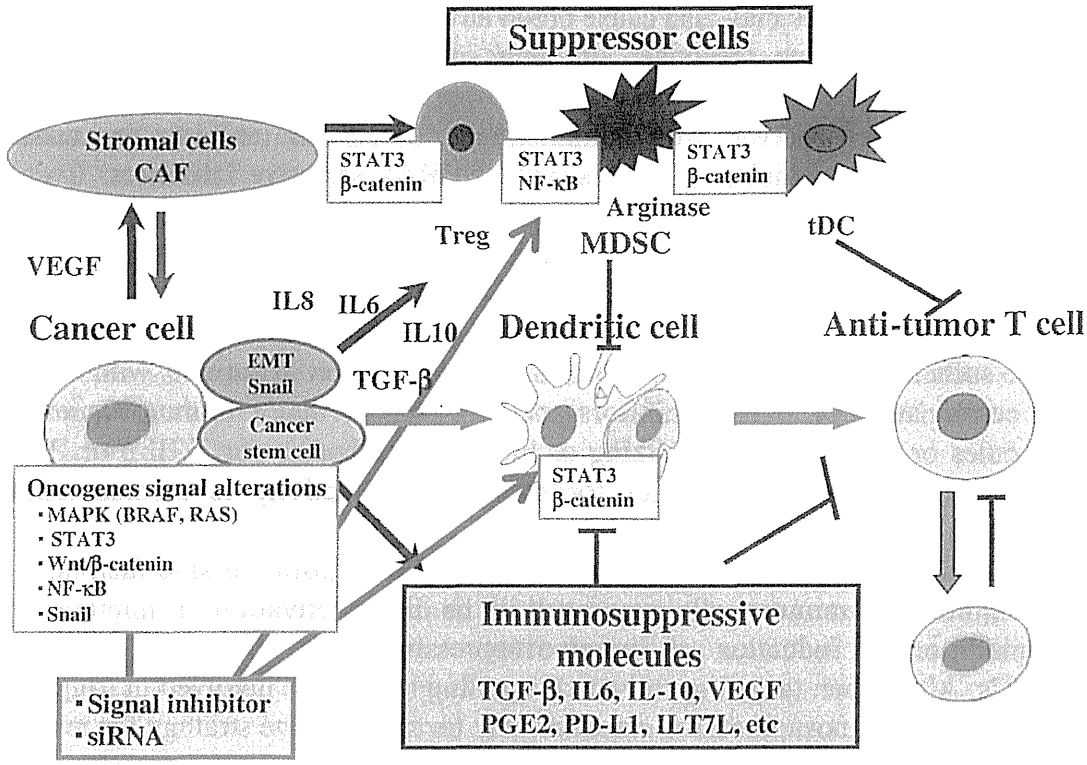

Fig. 12.4 Reversal of immunosuppressive conditions by targeting both cancer cells and immune cells using signal inhibitors. Cancer cell derived factors induce activation of signaling in various immune cells to become immunosuppressive cells. Signal inhibitors (inhibitors for STAT3, β-catenin, NF-κB, etc.) may be useful for reversal of cancer induced immunosuppression by acting on both cancer cells and various immune cells such as DC, MDSC, and Treg cells

Table 12.1 Appropriate targets for reversal of cancer-induced immunosuppression may be different among cancer patients

Active signaling molecules	Immunosuppressive molecules	Cancer type
NRAS/BRAF/MAPK	IL-10, VEGF, IL-6	Melanoma
BRAF/MAPK	IL-10	Colon cancer
KRAS/MAPK	IL-8, VEGF	Pancreatic cancer
MAPK	PD-L1	Ovarian cancer
MAPK	VEGF, IL-8	Renal cell cancer
EGFR/MAPK	IL-6, VEGF	Lung cancer
PI3K/AKT	VEGF, IL-8	Renal cell cancer
PTEN/AKT	PD-L1	Glioma
STAT3	IL-10, IL-6, VEGF	Melanoma
β-catenin	IL-10	Melanoma
β-catenin	IL-10	Colon cancer
NF-κB	IL-6, IL-8, CCL2	Ovarian cancer
NF-κB	IL-6, IL-8, ILT7L	Renal cell cancer
Kinase-X	TGF-β, IL-10, CCL2	Melanoma

12.6 Concluding Remarks

Understating of molecular mechanisms of the immunopathological features of the tumor-associated microenvironments is critical for further development of cancer diagnostics and therapy; not only immunotherapy but also other types of cancer treatments including chemotherapy. In particular, combination therapy utilizing molecular targeted drugs, which are currently used as single agents, and immunotherapy, such as cancer vaccine and check point blockers, is a promising strategy to be exploited in near future clinical trials.

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Cancer-induced immunosuppressive cascades and their reversal by molecular-targeted therapy

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Immunological status in tumor tissues varies among patients. Infiltration of memory-type $CD8^+$ T cells into tumors correlates with prognosis of patients with various cancers. However, the mechanism of the differential $CD8^+$ T cell infiltration has not been well investigated. In general, tumor-associated microenvironments, including tumor and sentinel lymph nodes, are under immunosuppressive conditions such that the immune system is not able to eliminate cancer cells without immune-activating interventions. Constitutive activation of various signaling pathways in human cancer cells triggers multiple immunosuppressive cascades that involve various cytokines, chemokines, and immunosuppressive cells. Signaling pathway inhibitors could inhibit these immunosuppressive cascades by acting on either cancer or immune cells, or both. In addition, common signaling mechanisms are often utilized for multiple hallmarks of cancer (e.g., cell proliferation/survival, invasion/metastasis, and immunosuppression). Therefore, targeting these common signaling pathways may be an attractive strategy for cancer therapy including immunotherapy.

Keywords: immunosuppression; BRAF; STAT3; β-catenin; NF-κB

Introduction

Human tumor antigens recognized by T cells have previously been identified in our studies and applied to various cancer immunotherapies.[1,2] One such melanoma antigen, gp100, was isolated by cDNA expression cloning using tumor infiltrating T cells (TILs).[3–6] In a recent multicenter, randomized clinical trial, gp100 peptide vaccination combined with interleukin 2 (IL-2) resulted in a 16% objective response with 9% complete response (CR).[7] In contrast, adoptive immunotherapy using cultured TILs following myelo-lymphoablative treatment, which depletes various immunosuppressive cells, resulted in more than 70% objective response with 20% durable CR in advanced melanoma patients with multiple metastases.[8] These studies indicate that immunosuppressive conditions, particularly in tumor-associated microenvironments such as tumor and sentinel lymph nodes (SLNs), are one of the major obstacles for the development of effective immunotherapy. Thus, understanding the mechanisms of cancer cell–induced immunosuppression in tumor-associated microenvironments and developing methods to reverse immunosuppression are important for immunotherapy.

Tumor-associated microenvironments

During cancer development, cancer cells, various immune cells, and other stromal cells, such as fibroblasts and mesenchymal stem cells, interact, and immunoediting via immunosurveillance and immunoescape defines immunological characteristics of cancer cells (e.g., loss of highly immunogenic tumor antigens, acquirement of resistance to immune cells, and ability to suppress immune response).[9] Thus, cancer cells seen in the clinic are generally

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immunosuppressive and generate immunosuppressive conditions in tumor-associated microenvironments. Immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs) and regulatory T (T_{reg}) cells, are increased, while dendritic cells (DCs) appear to be impaired in tumors and sentinel lymph nodes in cancer patients.[10] Interestingly, immune status varies among patients, as reflected by recent findings showing that the level of infiltration of memory $CD8^+$ T cells in tumors differs among cancer patients. More $CD8^+$ T cell infiltration correlated with favorable prognosis in various cancers, including colon and ovarian cancer, and also correlated with the response to immunotherapy or chemotherapy in patients with melanoma or colon cancer, respectively.[11,12] However, the mechanisms underlying differences in immune status among cancer patients remain to be investigated.

Gene and signaling alterations in cancer cells

Immune status in tumor microenvironments may be regulated by stimulating factors for antitumor immune response, including expression of immunogenic tumor antigens and human leukocyte antigen (HLA), spontaneous immune response cascades (such as the pathway from tumor DNA to IFN-producing DCs to $CD8^+$ T cell induction),[13] or by immunosuppressive cytokines such as transforming growth factor β (TGF-β) and IL-10. Since TGF-β is produced by most cancer cells and some infiltrating immune and stromal cells, we have evaluated the role of TGF-β in tumor microenvironments. In a mouse tumor model, increased TGF-β expression in tumor microenvironments via implantation of TGF-β cDNA–transfected tumor cells resulted in increased infiltration of immunosuppressive $CD11b^+$ $Gr\text{-}1^+$ MDSCs and $FoxP3^+$ $CD4^+$ T_{reg} cells in both tumors and SLNs. Infiltration of DCs was decreased in tumors; in SLNs the number of DCs was increased compared to non-SLNs in mice with either TGF-β–transduced or mock-transduced tumor cells, but T cell stimulatory activity of DCs was significantly impaired in mice with $TGF\text{-}\beta^+$ tumors. M2-like macrophages producing abundant CCL22, which recruits $CCR4^+$ T_{reg} and Th2 cells, were also increased in both tumors and SLNs.[14] Consequently, induction of tumor-specific T cells in SLNs was significantly reduced, which probably led to decreased infiltration of $CD8^+$ T cells in tumors. Therefore, the mouse tumor model recapitulates some of the immune conditions in tumors and SLNs in cancer patients (work in progress). An increase in immunosuppressive factors such as TGF-β may be one of the mechanisms defining the immune status of tumor microenvironments, such as spontaneous $CD8^+$ T cell responses.

We have also found that the TGF-β-induced transcription factor, Snail, which is known to promote metastasis via epithelial-to-mesenchymal transition (EMT) in cancer cells, also enhances production of multiple immunosuppressive cytokines and chemokines, including TGF-β, IL-10, CCL2, and TSP-1, which cause DC impairment and T_{reg} cell induction. The impaired DCs have less T cell stimulatory activity and induce T_{reg} cells. CCL2 not only impairs DC function but also recruits MDSCs into tumors. Intratumoral administration of Snail-specific siRNA restored immunocompetence of mice implanted with Snail-expressing tumor, and resulted in induction of tumor antigen–specific T cells.[15]

In a more recent work in progress, we have identified an upstream signaling molecule of TGF-β production in human cancer cells by screening immunosuppressive activity in DCs using a kinase siRNA library (unpublished data). The identified kinase is significantly phosphorylated in human cancer cells, and its depletion suppressed TGF-β production by cancer cells (manuscript in preparation). These results indicate that TGF-β, produced by either cancer cells or infiltrated stromal cells in tumor microenvironments, triggers immunosuppressive cascades involving various immunosuppressive cytokines, chemokines, and cells, and reemphasizes that TGF-β cascade is an attractive target for reversal of cancer-induced immunosuppression (Fig. 1).

Multiple immunosuppressive cascades in human cancers

Gene and signal alterations in human cancer cells vary among cancer types, and even within the same type of cancer. Therefore, we have evaluated immunosuppressive mechanisms of various human cancers (Fig. 1).

MAPK signal pathway

We have found that activation of the mitogen-activated protein kinase (MAPK) signal pathway via common BRAF mutation (V600E) not only

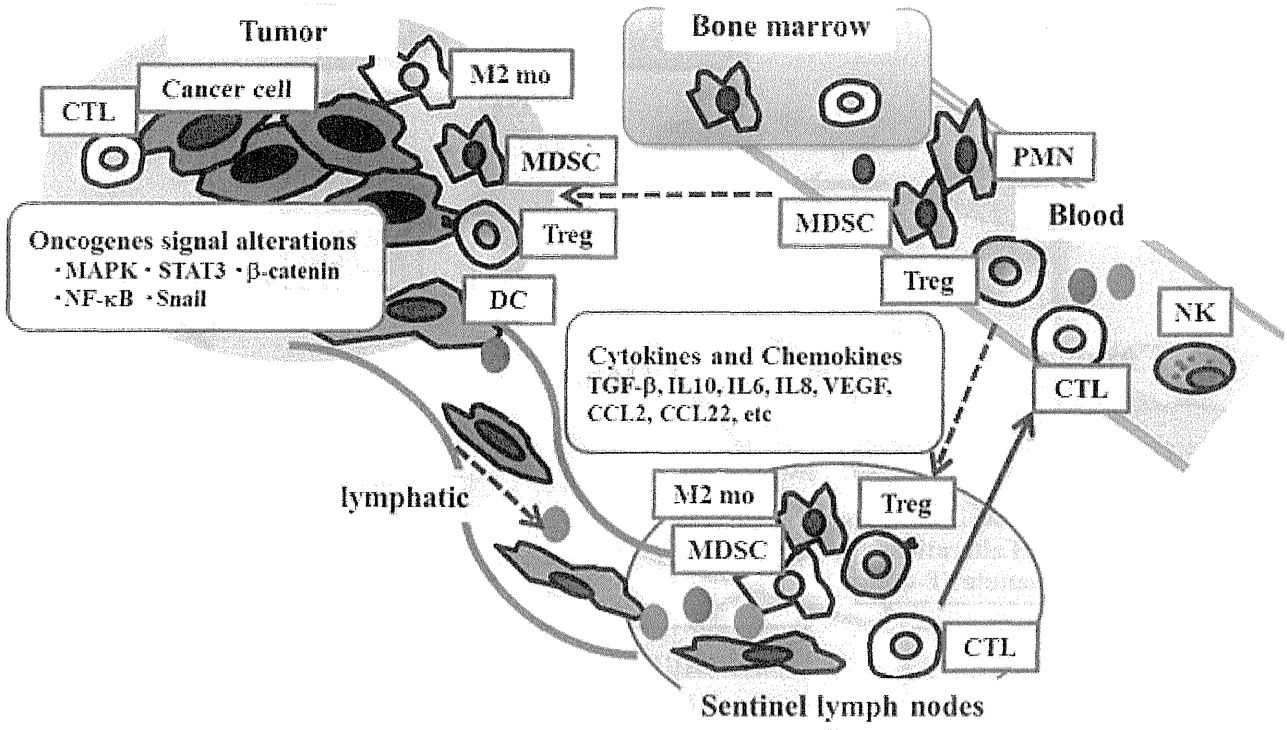

Figure 1. Alterations of genes and signaling in cancer cells trigger multiple immunosuppressive cascades. Alterations of oncogenes and subsequently activated signaling, such as MAPK, STAT3, β-catenin, and NF-κB in human cancer cells, trigger multiple immunosuppressive cascades, including production of multiple immunosuppressive cytokines and chemokines, such as TGF-β, IL-10, CCL2, subsequent impairment of DC function, and induction of various immunosuppressive cells such as MDSCs, M2-like macrophages, and T_{reg} cells.

promotes melanoma cell proliferation and invasion but also promotes production of multiple immunosuppressive cytokines, such as IL-6, IL-10, and VEGF.[16,17] These cytokines suppress T cell stimulatory activity of DCs through decreased IL-12 and TNF-α production and increased IL-10 production. Treatment of human melanoma cells with BRAF (V600E)-specific shRNA or MEK inhibitors resulted in decreased immunosuppressive activity, indicating that the MAPK pathway is involved in DC impairment by melanoma cells.[17] Therefore, the BRAF–MAPK axis is involved in multiple hallmarks of cancer, including cancer cell proliferation, invasion, and immunosuppression. Blockade of the BRAF–MAPK axis not only inhibits cell proliferation and invasion of, but also reverses immunosuppression by melanoma cells, indicating that the MAPK signal pathway might be an attractive target for melanoma treatment (Fig. 2).[16,17]

Although MAPK inhibition may also suppress proliferation of antitumor T cells, recently developed BRAF inhibitors that preferentially inhibit mutant BRAF may have less T cell inhibitory activity. In clinical trials, administration of BRAF inhibitors reduced tumor size in some patients, indicating induction of melanoma cell death *in vivo*.[18] Therefore, the selective mutant BRAF inhibitors may be useful in combination with immunotherapies through the following mechanisms: (1) reduction of tumor volume via cell death and inhibition of proliferation, subsequent decrease of immunosuppressive activity, and increased release of endogenous tumor antigens, including unique mutated antigens, leading to induction of multiple autologous tumor-specific T cells due to less inhibitory activity of the selective BRAF inhibitors for T cell proliferation; (2) restoration of immunocompetence via decreased production of multiple immunosuppressive cytokines, and subsequent simultaneous inhibition of multiple immunosuppressive cascades; (3) increased susceptibility of melanoma cells to cytotoxic T cells (CTLs) due to the reported increased expression of melanosomal tumor antigens;[19] and (4) decreased metastatic ability of melanoma cells. In fact, administration of mutant BRAF–selective inhibitor alone has recently demonstrated increased

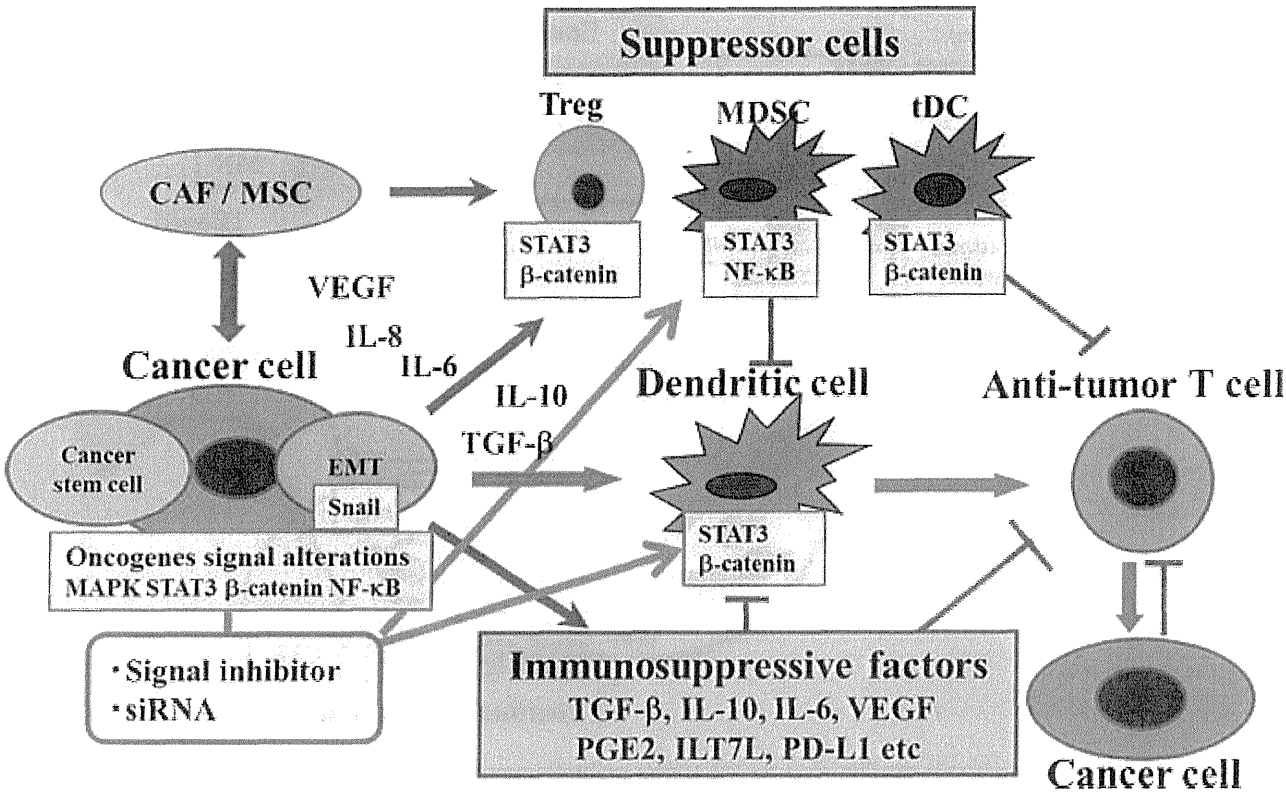

Figure 2. Reversal of cancer-induced immunosuppression by targeting cancer and immune cells with signal inhibitors. Inhibitors for altered signaling molecules, such as BRAF, STAT3, β-catenin, and NF-κB, may restore immunocompetence of cancer patients by acting on both cancer cells and various immune cells, such as DCs, MDSCs, and T_{reg} cells.

infiltration of granzyme-positive $CD8^+$ T cells in tumors, which correlated with tumor reduction.[20]

STAT3 signal pathway

Activation of STAT3 signaling is also observed in human melanoma cells. Similar to BRAF depletion, STAT3 depletion by lentiviral shRNA resulted in inhibition of multiple immunosuppressive cytokines, including IL-6, IL-10, and VEGF.[17] These cytokines activate STAT3 in various immune cells, including DCs, MDSCs, and T_{reg} cells, rendering them immunosuppressive. In a mouse tumor model, STAT3-depleted DCs were found to be resistant to tumor-derived immunosuppressive factors and to have enhanced T cell stimulatory activity via high IL-12 production.[21] Injection of STAT3-depleted DCs into tumors that are immunosuppressed resulted in stronger antitumor effects accompanied by induction of IFN-γ–producing tumor-specific T cells compared to regular DCs.[21] Similarly, subsequent work in progress has indicated that STAT3-depleted macrophages are also resistant to tumor-derived immunosuppressive cytokines, and induction of immunosuppressive MDSCs expressing arginase may be inhibited by STAT3 depletion. These results indicate that activation of STAT3 in cancer cells triggers induction of various immunosuppressive cells, including tolerogenic DCs and MDSCs, partly via STAT3 activation in the immune cells. Therefore, STAT3 inhibitors may also be useful for reversal of cancer-induced immunosuppression by acting on both cancer cells and various immune cells.

In a murine tumor model, various STAT3 inhibitors have been shown to augment antitumor immunity.[22,23] STAT3 inhibitors are currently being evaluated as cancer therapy in clinical trials, and their immunological effects should be evaluated in the future. In addition, inhibitors of upstream molecules of STAT3, including JAK and further upstream molecules such as EGF-R/VEGF-R, are already available in clinic, and may be useful for reversal of immunosuppression and combined use with immunotherapy. JAK inhibitors have been shown to augment antitumor immunity and enhance antitumor effects in combination with immunotherapies, such as IL-12 administration.[24] We have preliminary data indicating that EGF-R inhibitors can suppress production of immunosuppressive cytokines from

human lung cancer cells with EGF-R mutations; administration of EGF-R inhibitors along with cancer vaccines seems to provide synergistic antitumor effects through direct and indirect enhancement of T cell stimulatory activity of DCs in murine tumor models (the indirect enhancement may be via decreased immunosuppressive cytokines from cancer cells). Administration of a multikinase inhibitor, sunitinib, which also suppresses downstream STAT3 signaling, was reported to decrease MDSCs and T_{reg} cells, and increase IFN-γ–producing T cells, in renal cell carcinoma (RCC) patients.[25] Administration of dasatinib, another multikinase inhibitor that also inhibits downstream STAT3 signaling, resulted in increased response rates in some patients with $Ph1^+$ CML and ALL, accompanied by LGL lymphocytosis- and autoimmune-like syndrome.[26] In other work in progress and thus far unpublished, we have found natural compounds in traditional Japanese Kampo medicines that inhibit STAT3 and MAPK pathways, as well as augment antitumor T cell responses when administered in murine tumor models. These observations are consistent with the idea that STAT3 inhibition strategies may be useful for immunotherapy.

NF-κB signal pathway

Some human ovarian cancers produce high amounts of IL-6, IL-8, and CCL2 in a NF-κB–dependent manner. In unpublished preliminary studies, we have found that high plasma levels of IL-6 and IL-8 correlated with poor prognosis of cancer patients and poor response to various immunotherapies. NF-κB inhibitor not only inhibited production of IL-6, IL-8, and CCL2 by cancer cells, but also inhibited differentiation of monocytes to immunosuppressive macrophages in the presence of cancer cell–derived factors. Administration of the NF-κB inhibitor enhanced antitumor T cell responses possibly through reversal of immunosuppressive conditions in a murine tumor model (manuscript in preparation). In human RCC, an NF-κB inhibitor was also found to decrease the intrinsic expression of ILT7L, which inhibits IFN-α production by plasmacytoid DCs.[27] These results indicate that appropriate doses of NF-κB inhibitors may augment antitumor T cell responses by acting on both cancer and immune cells, although high doses of NF-κB inhibitors may also ameliorate induction of antitumor T cells.

Wnt/β-catenin signal pathway

Activation of the Wnt/β-catenin pathway is observed in about 30% of human melanomas and correlates with IL-10 production by melanoma cells. Culture supernatant of melanoma cells generated DCs with high IL-10 and low IL-12 production, less T cell stimulatory activity *in vitro* (partly in an IL-10–dependent manner), and an ability to induce $FOXP3^+$ T_{reg} cells. Pretreatment of melanoma cells with β-catenin-shRNA reduced these immunosuppressive activities. When the β–catenin-activated human melanoma cells were implanted in severe combined immunodeficiency (SCID) mice, mouse DCs in spleen and tumor had impaired T cell stimulatory activity, partly due to IL-10 produced by human melanoma cells.[28] Administration of a β-catenin inhibitor restored the T cell stimulatory activity in splenic DCs of these mice.[28] Since β-catenin was reported to be involved in generation of regulatory DCs and survival of T_{reg}, β-catenin inhibitor may also be useful for reversal of cancer-induced immunosuppression by acting on both cancer and immune cells.

Clinical implications of immunosuppression mechanisms

The specific steps that should be inhibited in these immunosuppressive cascades for effective reversal of immunosuppression in cancer patients have yet to be identified.[10] Targeting activated signaling molecules in cancer cells, upstream of the cascades, may simultaneously inhibit multiple immunosuppressive mechanisms and exert direct antitumor effects, such as inhibition of cancer cell proliferation and destruction of cancer cells, which may lead to further induction of immune responses to endogenous tumor antigens, including patients' unique antigens (e.g., mutated antigens). Signal inhibitors may also have direct effects on immune cells, including direct activation of immune cells or inhibition of generation of immunosuppressive cells, such as T_{reg} cells and MDSCs (Fig. 2). However, upstream inhibition may also cause more broad adverse effects, including suppression of antitumor immune response; although it may be avoided by use of appropriate doses of inhibitors, as shown for NF-κB inhibitor, or by use of mutant molecule-specific inhibitors, such as mutant BRAF-selective inhibitors. In contrast, downstream targeting, including of immunosuppressive effectors such as TGF-β, IL-10,

PD-L1, IDO, Cox2, MDSCs, and T_{reg} cells, by using antibodies or small molecule inhibitors may result in more specific and efficient blockade with less broad adverse effects, although inhibition of one molecule or cell type may be insufficient to reverse immunosuppression overall. The combination of upstream signal inhibitors and downstream blockade of major immuosuppressive factors (e.g., neutralizing or blocking Ab for TGF-β and PD-L1) may also be an attractive strategy for effective restoration of antitumor immune responses.

Altogether, targeting activated signaling molecules involved in triggering multiple immunosuppressive cascades may effectively treat cancer through restoration of antitumor immune responses. Combined treatment with these molecular-targeted drugs and various immunotherapies, including cancer vaccines and check point blockade, should be evaluated in future clinical trials. Since activated signaling molecules are different among patients even with the same type of cancers, using personalized strategies will be important. In addition to their therapeutic implications, evaluation of altered gene/signaling status (e.g., pERK, pSTAT3, nuclear translocation of NF-κB, or β-catenin) may also be useful for diagnosis of cancer patients because altered gene and signaling affect immune status, indicated by, for example, tumor infiltration of memory $CD8^+$ T cells and level of serum cytokines (e.g., IL-6, IL-8), which correlates with prognosis and treatment response in cancer patients.

Conclusion

Understanding the molecular basis of immunopathology in tumor-associated microenvironments is essential for the development of effective cancer diagnostic methods and therapy, not only for immunotherapy, but also for other standard cancer therapies such as chemotherapy. In particular, immunotherapy (e.g., cancer vaccine and check point blockade) combined with molecular-targeted drugs, currently used as single agents, is a promising strategy to be exploited in future clinical trials.

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Conflicts of interest

The authors declare no conflicts of interest.

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化学療法・分子標的薬による免疫応答増強

Activation of the immune system against cancers by chemotherapeutic regimens

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◎近年，がん組織での免疫環境が免疫療法の効果のみならず，その他の治療の効果や予後に影響を与えることがわかり，がん免疫微小環境の制御，改善の重要性が認識されている．一般に，がん組織内はさまざまな機構で免疫抑制状態が誘導されているが，近年その機構が分子レベルで解明され，これらを標的とする治療法が開発されている．さらに既存の抗がん剤や分子標的薬に関しても，がん細胞を直接殺傷する薬効に加えて，がん細胞からの免疫抑制分子の産生を阻害する効果，がん細胞に免疫原性を高めた死を誘導する効果，免疫細胞に対して直接働き免疫増強を誘導する効果によりがん免疫微小環境の改善ができること，免疫療法との併用で効果の増強が可能なことが明らかとなっている．

Key word：がん免疫療法，化学療法，がん免疫回避，がん微小環境

がん細胞の特徴は，さまざまな遺伝子異常により自律的に増殖・生存可能なことと従来考えられてきたが，最近がんの周囲の環境(がん微小環境)もがん細胞に多大な影響を与え，がん固有の特徴の形成に関与していることがわかってきた．そのひとつが宿主の免疫監視機構からの回避(がん免疫回避)である[1]．

腫瘍組織ではさまざまな免疫細胞の浸潤がみられる．一般に臨床で認められるようになったがん組織ではがん細胞自身からの免疫抑制物質の産生，制御性T細胞(regulatory T cell：Treg)に代表される抑制性免疫細胞の誘導など，さまざまな機構で免疫抑制が誘導され，がん免疫回避が成立している(表1)[2]．炎症は発がんの原因，がん細胞の増殖，転移の助長因子として働く一方，Th1応答が強く誘導された場合など，ある局面においては腫瘍拒絶にも働く．最近，腫瘍組織中へのCD8陽性T細胞の浸潤に代表される抗腫瘍免疫応答の有無が，大腸がんなどさまざまながんで予後や化学療法の奏効率と相関しているとの報告がなされている[3]．腫瘍組織の免疫環境が免疫療法の効果のみならず，その他の治療効果や予後に影響を与えていることが示され，腫瘍組織の免疫環境を改善することの重要性が認識されている[4]．

現存の抗がん剤はがん細胞自体を標的として開発されてきたが，最近，これらの薬剤ががん細胞だけでなく周囲の免疫細胞にも影響を与えていることが明らかとなってきた．本稿では，抗がん剤によるがん免疫微小環境の制御法を紹介する．

免疫抑制分子や免疫抑制細胞に対する標的薬

がん微小環境ではTregやMDSC(myeloid-derived suppressor cell)などの免疫抑制性の細胞や分子が誘導され，これらの機能を抗体医薬や低分子化合物を用いて制御し，がん免疫応答を増強させる試みが行われている．もっとも代表的な，PD-1/PD-L1，CTLA-4などの補助刺激分子に対する抗体医薬は臨床試験で効果を認め，なかでも抗CTLA-4抗体(ipilimumab)は2011年にFDAに認可された．これらの薬剤やTregを標的とした治療に関しては本特集，杉山・西川「免疫抑制の克服による抗腫瘍免疫応答増強の可能性――抗腫瘍免疫応答の抑制解除」の稿を参照されたい．

表 1　抗がん剤による抗腫瘍免疫増強の作用機序

作用機序	治療薬
1．がん細胞側の免疫逃避を改善	
A）腫瘍抗原の発現低下を回復	daunorubicin，BRAF 阻害薬
B）HLA の発現低下を回復	gemcitabine，oxaliplatin cyclophosphamide，EGFR-TKI
C）CTL(cytotoxic T lymphocyte)への感受性低下を改善	cisplatin，doxorubicin, paclitaxel 5-FU，CPT-11
D）腫瘍細胞の発現する免疫抑制性分子を抑制(IL-10，IL-6，VEGF，IDO，PD-L1 など)	シグナル阻害薬(STAT3 阻害薬など) 抗 PD-L1 抗体 IDO 阻害薬(1MT，MTH-Trp)，imatinib(IDO 抑制)
2．がん細胞に immunogenic cell death(ICD)を誘導	cyclophosphamide，doxorubicin，mitoxantrone，oxaliplatin，bortezomib，AG490
3．宿主側の免疫逃避を改善	
A）抗腫瘍免疫担当細胞の機能不全を回復(活性化 T 細胞上に発現する免疫抑制性膜分子)	抗 CTLA-4 抗体 抗 PD-1 抗体
B）免疫抑制性細胞の誘導を阻害	
① 制御性 T 細胞(T_{reg})	抗CTLA-4 抗体，cyclophosphamide，paclitaxel，sunitinib，sorasfenib，imatinib
② MDSC(myeloid derived suppressor cell)	gemcitabine，5-FU，docetaxel，cyclophosphmide，sorafenib，sunitinib，PGE5 阻害薬，ATRA，COX2 阻害薬
③ 制御性樹状細胞(IDO の発現)	IDO 阻害薬(1MT，MTH-Trp)
4．その他	
LGL(large granular lymphocyte)の増加 腫瘍量の減少 がん抗原の放出	dasatinib

その他の免疫抑制分子を標的とした，現在新しく開発されている薬剤を以下にまとめる．

1．IDO(indoleamine 2,3-dioxygenase)を標的とした治療

IDO は必須アミノ酸であるトリプトファンの代謝酵素で，周囲にあるトリプトファンを消費し，トリプトファン欠乏に極端に弱い T 細胞の機能を障害する．正常組織では胎盤に発現し，母体による胎児の拒絶を阻止する重要な役割をもつ．多くのがん細胞は IDO を異所性に発現しており，悪性黒色腫や卵巣がんでは予後不良との相関が報告されている．これは IDO の発現により細胞傷害性 T 細胞の活性が阻害され，がん細胞が宿主の免疫監視機構から回避しているためであると考えられる．また，IDO は腫瘍間質細胞，とくに抗原提示細胞にも発現する．乳がん，大腸がんをはじめとするさまざまながんで，IDO を高発現する樹状細胞が腫瘍組織内，リンパ節内で認められており，T 細胞の機能が抑制され，免疫抑制が成立している．IDO に対する阻害薬は，これまでに 1-MT(1-methyl-tryptophan)，MTH-Trp(methylthiohydantoin-tryptophan)などが知られているが，さらに活性の強い阻害薬が複数開発されている[2]．現在 1-MT と，がんワクチンや docetaxel などの抗がん剤を併用した臨床試験が実施され，その臨床効果が検証されている．

トリプトファンは IDO によってキヌレニンに代謝されるが，最近このキヌレニンがダイオキシンレセプターである AhR(aryl hydrocarbon receptor)を活性化し，Treg を誘導することが示された[5]．さらに，グリオーマにおいてはがん細胞が異所性に発現する TDO(tryptophan-2,3-dioxygenase)によってキヌレニンの腫瘍組織における濃度が上がり，AhR を介して腫瘍に対する免疫応答を抑制することが報告された．TDO は IDO と同様キヌレニンの代謝酵素であり，従来はその発現は肝や脳に限られ，腫瘍組織でのトリプトファン代謝はもっぱら IDO が担っていると考えられていたが，TDO も腫瘍で発現し免疫抑制に関与していることが示された．これらの結果よ

り，トリプトファンは欠乏することとその代謝物のキヌレニンの両方で，がん免疫回避に関与していることがわかり，今後さらなる解析が期待されている．

2. MDSC(myeloid-derived suppressor cell)を標的とした治療

MDSCは免疫抑制能をもつ骨髄系細胞の総称であり，マウスではCD11b$^+$，Gr-1$^+$がマーカーとされている．動物モデルにおいてはMDSCががん免疫回避に関与していることを示す報告が数多くあり，その免疫抑制能はおもにMDSCに発現する2つの酵素，NOS2(inducible nitric-oxide synthase 2)とARG1(arginase 1)が担っている．NOS2はNOの産生を介して，T細胞のIL-2レセプターシグナル伝達を抑制することでアポトーシスを誘導し，またARG1はアルギニンの代謝酵素で，アルギニンを周囲の環境から消費することでT細胞の機能を抑制する．ヒトでのMDSCのマーカーは特異的なものがなくLin$^-$HLA-DR$^-$CD33$^+$やCD14$^-$CD11b$^+$CD33$^+$などが提唱され，さまざまながん種で免疫抑制に働いていることが示されている．

Gemcitabineはマウス腫瘍モデルで，MDSCを抑える作用が報告されている．臨床試験では，gemcitabineは比較的免疫抑制作用がない化学療法剤としてがん抗原免疫療法に併用され，がん抗原特異的T細胞の誘導増強の可能性が報告されているが，MDSCの動態などの詳細な解析はされていない．膵がんに対して，gemcitabineを併用したがん抗原ペプチドワクチンの臨床試験が実施され，腫瘍径の減少や腫瘍マーカーの減少などが認められている[6]．

このほかにもさまざまな薬剤でMDSCの抑制が報告されている[7]．多標的チロシンキナーゼ阻害薬(TKI)のsunitinibでは，腎がん患者においてMDSCを減少させる．白血病治療薬であるATRA(all-trans-retinoic acid)は分化誘導をかけることで，MDSCを減少させることがマウスやヒトの臨床試験で報告されている．男性性機能障害治療薬であるPDE-5(phosphodiesterase-5)阻害薬は，NOS2やアルギナーゼの発現を減少させ，MDSCを抑制する．NSAIDsであるCOX-2阻害薬もアルギナーゼの発現を減少させ，MDSCを抑制する機能があると報告されている．

従来の化学療法剤を用いた，がん免疫応答の増強

多くの抗がん剤は免疫抑制作用があり，免疫療法の組合せとして好ましくないと思われがちであるが，投与法によっては免疫増強，免疫抑制解除を目的として使用できる．抗がん剤は，腫瘍細胞および免疫細胞に対して以下の8つの機序で作用し，抗腫瘍免疫応答の増強に働いているかもしれない(表1)．

① がん細胞量減少による，直接的な免疫抑制状態解除．
② がん細胞のアポトーシス，ネクローシスによる，がん抗原の放出．
③ Immunogenic cell death(ICD)の誘導によるがん細胞の免疫原性の増大．
④ TregやMDSCなどの免疫抑制性細胞に対する阻害作用．
⑤ がん細胞の免疫抑制分子産生に対する阻害作用．
⑥ がん細胞上のHLAの発現上昇．
⑦ 腫瘍抗原の発現上昇．
⑧ がん細胞のCTLに対する感受性の増大．

このような観点から免疫抑制作用があまり強くない抗がん剤を適当な用量，タイミングで使い，担がん患者の抗腫瘍免疫応答を増強させる報告が近年増えている．

1. がん細胞を標的とした免疫増強作用

ある種の抗がん剤は腫瘍細胞に対して，immunogenic cell death(ICD)の誘導[4]やHLAの発現，CTLへの感受性を変化させることで，抗腫瘍免疫応答増強に働くことがある．アルキル化剤であるcyclophosphamide，アントラサイクリン系薬剤のdoxorubicinやmitoxantrone，白金製剤のoxaliplatinではがん細胞にICDと呼ばれる免疫原性の高い死を誘導する．マウスがん細胞株を用いた担がんマウスモデルでは，これらの薬剤の抗腫瘍効果は免疫不全マウスで治療するより免疫系が正常な同系マウスで治療する方が優れていることがわかっている[4]．ICDはcalreticulin(CRT)分子の細

胞膜表面への表出，ATPの細胞外への放出，HMGB1(non-histone chromatin binding protein high mobility group box 1)の放出，HSP70やHSP90などのheat shock protein(HSP)の細胞表面への表出および放出によって誘導される[8]．CRTは通常細胞内のERに存在するが，アポトーシスに伴いがん細胞表面に表出し，“eat me”シグナルとして働き，そのレセプターを発現しているDCによるがん細胞貪食が亢進する．細胞外のATPは“find me”シグナルとして働き，免疫細胞を局所に誘導するだけでなく，プリン受容体(P2RX7)に結合しNLRP3インフラマソームを活性化し，IL-1βの分泌を促し，炎症反応を惹起する[9]．HMGB1はTLR4のリガンドとして働きDCなどを活性化する．TLR4やP2RX7のSNP(single nucleotide polymorphism)は大腸がんや乳がんで予後に影響を与えると報告されており[4]，ヒトでもこれらの機構ががん免疫応答に何らかの影響を与えていることが推察される．HSPは腫瘍抗原と複合体を形成し，抗原のDCへの取込みを亢進させる．

上記の抗がん剤に加え，最近，多発性骨髄腫の治療薬で，プロテアソーム阻害薬であるbortezomibやJAK2/STAT3阻害薬であるAG490もICDを誘導することが非Hodgkinリンパ腫の一種であるprimary effusion lymphomaで報告されている[10]．ICD誘導以外の免疫増強の作用機序としてはgemcitabine，oxaliplatin，cyclophosphamideによるHLA class I分子の発現の上昇や，daunorubicinによる腫瘍抗原の発現上昇，cisplatin，doxorubicin，paclitaxel，5-fluorouracil(5-FU)，CPT-11によるCTLに対する感受性の上昇が報告されている[3,11]．

2. 免疫細胞を標的とした免疫増強作用

一般に抗がん剤は，高容量では免疫細胞に対して免疫抑制的に働くが，低用量時や投与のタイミングによっては免疫増強的に働くことがある．Cyclophosphamideは低用量で用いた場合，TregやMDSCの抑制効果，T細胞，NK細胞の機能回復，Th17分化促進などを介して抗腫瘍免疫増強に働く．Doxorubicinは所属リンパ節における腫瘍抗原特異的なCD8$^+$T細胞の増殖やIL-17産生γδ細胞の腫瘍浸潤を亢進させる．このほか，Tregの抑制作用がpaclitaxel，MDSCの抑制作用がgemcitabine，5-FU，docetaxelで報告されている[3,11]．

最近，メラノーマに対してcyclophosphamideとfludarabineの前投与でリンパ球を減少させた後に，培養した腫瘍浸潤T細胞の投与による養子免疫療法をメインに，腫瘍抗原の能動免疫，大量IL-2投与などを併用した総合的免疫治療を行うと，生体内で長期に投与T細胞が増殖することが報告され，50例以上に施行された結果，約50%にCRを含むPR以上の抗腫瘍効果を認めている[12]．この療法ではリンパ球減少処置によって，体内のIL-7やIL-15などのサイトカインを，輸注されたリンパ球が効率よく使用できるようになり，homeostatic proliferationが作動し，投与T細胞が長期に生存できたことが高い奏効率につながったと考えられている．さらにこの処置はTregなどのリンパ球系抑制性細胞も減少させる作用があることがわかっている[13]．このように，特定の化学療法剤の併用で免疫抑制解除および抗腫瘍免疫増強が可能であり，現在，がんワクチンなどの能動免疫療法でも化学療法を併用するさまざまな臨床試験が行われている[8]．

分子標的薬を用いたがん免疫応答の増強

1. EGFR-TKI(gefitinibやerlotinib)

EGFR(epidermal growth factor receptor)特異的チロシンキナーゼ阻害薬(EGFR-TKI)であるgefitinibやerlotinibは，EGFR変異のある非小細胞性肺がんに対して優れた臨床効果を認めている．興味深いことに，肺がんと同時に白血病を発症した症例にEGFR-TKIを投与したところ，EGFRを発現していないにもかかわらず白血病が寛解した事例がある[3]．EGFR-TKIはEGFR以外にもさまざまな標的があることが知られており[14]，これらの事例ではEGFR-TKIのoff-target効果で，抗腫瘍効果が誘導された可能性が考えられる．EGFR-TKIは副作用として間質性肺炎や皮疹など自己免疫応答との関連を示唆するものが多く，EGFR以外の分子を標的として免疫細胞にも影響を与えている可能性が高い．肺胞マクロ

ファージではLPSによる転写因子Fra-1とその標的であるMCP-1の上昇がgefitinibで増強され，上昇したMCP-1によりさらにマクロファージ浸潤が助長され，間質性肺炎の一因となっている可能性がある[15]．また一方で，EGFRシグナルの阻害により細胞上のMHC class IおよびIIの発現が上昇するとの報告もある[16]．これら種々のメカニズムにより，EGFR-TKIが腫瘍免疫応答増強に働いている可能性が考えられる．

2. Sorafenibとsunitinib

SorafenibやsunitinibはVEGFR，PDGFR，KITなどを標的とする多標的チロシンキナーゼ阻害薬で，前者はさらにRafも阻害する．両薬剤はがん細胞自体や血管新生に対して阻害作用をもち，おもに腎がん，肝がんの治療薬として使用されている．これらの薬剤が標的とするMAPKなどのシグナルは免疫細胞でも重要な役割を担っており，最近これらの薬剤の免疫細胞に対しての作用が明らかとなってきた．どちらの薬剤も腎がん患者において，TregやMDSCの浸潤を抑制すると報告されている．しかし実験的には，sorafenibはDCやNK細胞に対して機能抑制的に働く．Sunitinibは，DCやNK細胞に対してはそのような抑制作用を示さず，*in vivo*ではTh2シフトの是正，T細胞の活性化，免疫抑制性サイトカインや分子の減少など，抗腫瘍免疫応答増強に働くとの報告が多い一方で，T細胞に対しては増殖，サイトカイン産生などを抑制するとも報告され，一定の見解が得られていない[17]．これまでの報告からはsorafenibは免疫抑制的であるようであるが，いまのところ臨床における抗腫瘍効果に対する問題点とはなっていない．しかし，免疫療法との併用の際は留意する必要があるであろう．興味深いことに，sorafenibの免疫細胞に対する阻害効果の一部は，直接は標的分子となっていないはずであるNF-κBやPI3Kシグナルの阻害によるものであると報告されている．これらの多標的キナーゼ阻害薬には未知の分子標的がさらに存在する可能性がある．

3. ABL-TKI(imatinibやdasatinib)

ImatinibやdasatinibはABL-チロシンキナーゼ阻害薬(ABL-TKI)であり，慢性骨髄性白血病(CML)，フィラデルフィア染色体陽性急性リンパ性白血病(Ph^+ALL)に対する治療薬である．また，KITなども阻害するため消化管間葉系腫瘍(GIST)などに対しても抗腫瘍効果をもつ．Imatinibは*in vitro*ではT細胞の増殖を抑制するなど，免疫抑制的な作用の報告も散見されるが，*in vivo*ではNK細胞の活性化，STAT3やSTAT5の阻害によるTregの抑制，腫瘍におけるIDOの発現抑制など，腫瘍免疫応答増強に働くことが報告されており，今後さらなるメカニズムの解析が期待されている[3]．

Dasatinibはimatibnib耐性を克服するために開発された第二世代ABL-TKIであり，報告によってはimatinib以上の高い有効性を示すとされている．Dasatinibの主要な標的分子はBCR-ABLキナーゼであるが，そのほかにもSrcをはじめとする数十種類のキナーゼを標的とする．そのなかにはT細胞活性化に必要なLckやFynなども含まれ，dasatinibも*in vitro*では免疫抑制的に働くと考えられていた．しかし，臨床ではむしろ免疫賦活に働くことが示唆されている．

興味深いことに，dasatinib治療患者の数十%が治療後3カ月ほどで，大型顆粒リンパ球(large granular lymphocyte：LGL)増加を伴う，末梢血中のリンパ球数の増加がみられ，この現象はimatinibなどの他のTKIではみられずに，dasatinib特有の現象である．LGLはおもにオリゴクローナルなエフェクターメモリー$CD8^+$細胞，およびNK細胞で構成されており，このLGL増加のみられた患者では腸炎，胸水などの自己免疫反応との関連をうかがわせる副作用が有意に多く，また予後が良好であると報告されている[18-20]．これらの現象は，dasatinibがLGLの増加という免疫の賦活を介して抗腫瘍効果を増大させている可能性を示唆する．しかし，腫瘍抗原特異的T細胞の誘導をdasatinibが亢進させているかに関しては，LGL増加症例で白血病抗原のPR-1特異的T細胞の検出が報告されてはいる[21]ものの，いまだにエビデンスに乏しく不明である．

興味深いことに，LGL増加症例ではCMVの再活性化が有意に多く，さらにCMV特異的$CD8^+$T細胞の増加や，血清中のIP-10，IL-6，MIGなどの増加が報告されている[21]．Dasatinibによる

免疫抑制で，まずCMVが再活性化し，それに伴って上記の炎症性液性因子が産生され，LGLの増加が起こるという仮説が提唱されるが，一方でCMV再活性化とLGL増加に関連はないとの報告[20]もあり，この仮説もさらなる検証が必要である．

また最近，dasatinibは*in vitro*で，T細胞の活性化には抑制的に働くが，NK細胞を増加させることはできるとの報告もなされた．Dasatinibはimatinibなどに比べ標的分子が多く，何らかの特有の作用を免疫細胞に及ぼしていることが考えられる．この現象がCMLやPh^+ALLに限定される現象なのか，または他のがんでもみられ，免疫賦活剤としてより多くのがん種に使用できるのか，今後の解析に期待が寄せられている．

4. がんシグナル伝達分子活性化による免疫抑制

がん細胞は異所性にさまざまな免疫抑制分子を発現しがん免疫回避を誘導するが，最近がん細胞で亢進しているシグナル伝達分子によって，これらの免疫抑制分子の発現が制御されていることがわかってきた．著者らは悪性黒色腫において，変異型BRAF($BRAF^{V600E}$)がMAPKシグナル経路を亢進させ，腫瘍増殖に加えてIL-6，IL-10，VEGFなどの免疫抑制性のサイトカインの産生に関与し，樹状細胞を抑制していること[22]や，Wnt/β-cateninシグナル経路の亢進によりIL-10が産生され，樹状細胞やCTLの機能を抑制していること[23]を見出し，これらのシグナル経路ががん免疫回避の原因となっている可能性を提唱した．そのほかにもがん遺伝子STAT3による免疫抑制性サイトカインの産生，MAPKの亢進によるがん細胞上のCD200の発現亢進とそれによる樹状細胞の抑制，がん遺伝子Aktの活性化によるがん細胞上でのPD-L1の発現，がん抑制遺伝子Bin1の発現低下によるがん細胞でのIDOの発現，などの報告がある[2]．

このように，がんの悪性形質の根本であるがんシグナル伝達異常は，従来研究されてきたがん細胞の増殖や浸潤などにかかわるほかに多数の免疫抑制分子の発現にも関与し，がん免疫回避の根本的原因となり，よい治療標的となりうる．たとえば，悪性黒色腫で使用されている変異型BRAF阻害剤(PLX4032やGSK2118436)などは正常免疫細胞への副作用が少なく，かつ上記の免疫回避解除の目的でも使用できそうである．マウスモデルでは養子免疫療法とPLX4032の併用で抗腫瘍効果の増大が報告されている[24]．また，BRAFの阻害により腫瘍抗原の発現の増加も報告されている[25]．STAT3阻害薬やMEK阻害薬(MAPK経路)も数種類が開発され，アメリカでは臨床試験が進行中である．

興味深いことに，これらのシグナル伝達経路のいくつかは抑制性の免疫細胞においても，その抑制活性を担う重要なシグナルとなっていることがある．がん微小環境中の樹状細胞やTregはSTAT3が亢進しており，それらの細胞の免疫抑制能に関与している[26]．著者らは，DCでSTAT3を阻害しておけばがん細胞による免疫抑制に対して抵抗性を獲得することを見出した[27]．また，Wnt/β-cateninシグナル経路はTregの寿命やDCregの誘導に重要であると報告されている[2]．STAT3やWnt/β-cateninなどの，いくつかのシグナル経路は抑制性の免疫細胞でも重要な働きをもっており，これらのシグナル分子に対する分子標的薬はがん細胞および免疫細胞を同時に標的とすることができ，免疫回避をより効果的に解除できるかもしれない．

おわりに

がん組織中の免疫状態が予後や化学療法の奏効率にも関与していることが示され，がん免疫微小環境の重要性が認識されている．本稿で紹介したように抗がん剤はがん細胞や免疫細胞に作用し，腫瘍免疫応答に影響を与えている．これまでの抗がん剤のスクリーニングは免疫不全マウスにヒトの腫瘍を移植して効果の判定をしており，免疫細胞に対する影響は考慮されていないことがほとんどであった．抗がん作用が顕著にみられなかったために臨床試験でドロップアウトした抗がん剤も，本稿でみたような免疫学的作用を腫瘍，免疫細胞に対してもっている可能性があり，再評価の価値があるかもしれない．また，ヒトの腫瘍，免疫系はマウスと異なる面も多数報告されており，ヒトの腫瘍，免疫系が評価できるマウスモデル