制に対して抵抗性を獲得することを見出した[22]。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[1]。

このように，癌の悪性形質の根本である癌シグナル伝達異常は，従来研究されてきた癌細胞の増殖や浸潤などに関わる他に，多数の免疫抑制分子の発現にも関与し，腫瘍免疫回避の根本的原因となり，よい治療標的となり得る（図1）。例えば，最近新しく開発され，悪性黒色腫の臨床試験にて効果が実証されつつある変異型 BRAF 阻害剤（PLX4032, GSK2118436）などは，正常免疫細胞への副作用が少なく，かつ前述の免疫回避解除の目的でも使用できそうで，我々はその可能性を検討している。実際，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[23]。STAT3 阻害薬や MEK 阻害薬（MAPK 経路）も数種類が開発され，米国では臨床試験が進行中である。さらに，STAT3 や Wnt/β-catenin などのいくつかのシグナル経路は，前述のように抑制性の免疫細胞でも重要な働きを持っており，これらのシグナル分子に対する分子標的薬は，癌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

担癌生体が免疫抑制的であることは以前から知られていたが，最近，その責任分子，細胞，メカニズムが少しずつ解明され，本稿で見たように，それらを標的とした治療薬の開発も進んでいる。変異型 BRAF 阻害薬や gemcitabine などの免疫抑制解除を目的として使用できる既存の分子標的薬，抗がん剤と，既存の免疫療法との併用治療は，倫理的問題も軽く，Translational Research で様々なヒントが得られるかもしれない。今後はより強力な免疫誘導増強法と免疫抑制解除法を免疫療法といかに併用していくかが，癌免疫療法の臨床効果を改善するのに重要である。

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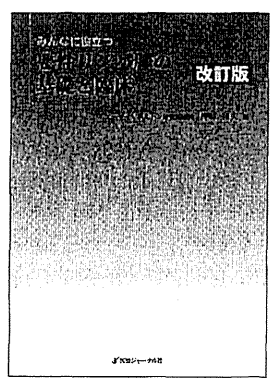

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Ⅲ 基礎研究

分子標的薬の作用機序・薬理作用
がん関連標的分子・標的経路

がん免疫療法開発における制御ポイント

Molecular targets for development of cancer immunotherapy

河上　裕

Key words：免疫療法，腫瘍抗原，樹状細胞，T細胞，免疫抑制

はじめに

臨床でみられるがん細胞は，長年にわたるがんの発生過程で，免疫防御機構に排除されずに増殖してきた細胞である．発がん物質投与後に発生するマウス腫瘍の経時的な観察によると，がん組織では，がん細胞，免疫細胞，その他の間質細胞との相互作用が起こり，マクロファージや肥満細胞などの自然免疫系細胞(innate immunity)，線維芽細胞や間葉系幹細胞などの間質細胞は，むしろ，がん細胞の増殖浸潤の促進にかかわること，NK細胞やT細胞などはがん細胞の排除に働くこと(免疫監視：immunosurveillance)，しかし，遺伝子不安定性を基本性質としてもつがん細胞は変異により，免疫抵抗性や免疫抑制性を獲得し，そのようながん細胞が選択的に増殖する(immune-editing)ことがわかっている．実際，ヒトでも患者から得たがん細胞は，低免疫原性な腫瘍抗原，抗原提示HLAの異常，多様な免疫抑制活性など，様々な機序による免疫抵抗性や免疫抑制性をもつので，ヒトでもがん形成過程で，同様なimmune-editingが起こっていると考えられる．ヒトでも免疫不全状態で発生するがんは免疫感受性であることもこれを示唆している．したがって，効果的ながん免疫療法の開発のためには，本稿で紹介する抗腫瘍免疫ネットワークを総合的に強力に制御することが重要である．

1 抗腫瘍免疫増強のために重要な制御ポイント

同定したヒト腫瘍抗原とHLAテトラマー技術などを用いて，以前はブラックボックスであった患者生体内での抗腫瘍免疫応答の定量的・定性的な解析が進み，がん免疫療法が効く場合の機序，また効かない場合の理由，すなわち免疫療法後，がん細胞排除に至る各段階での問題点が少しずつ明らかになってきた[1]．著者らも，免疫療法臨床試験の解析結果から，効果的な免疫療法の開発のためには，抗腫瘍免疫ネットワークにおいて，以下に詳述するポイントの改良が必要だと考えている(**図1**)．これらのポイントの制御法を開発改良して，適切に併用することにより，総合的に抗腫瘍免疫ネットワークを制御することが効果的な免疫療法開発のキーと考えられる[2]．

1）内在性腫瘍抗原に対する免疫誘導を促進する生体内腫瘍破壊法

腫瘍抗原ワクチンの臨床試験で腫瘍縮小を認

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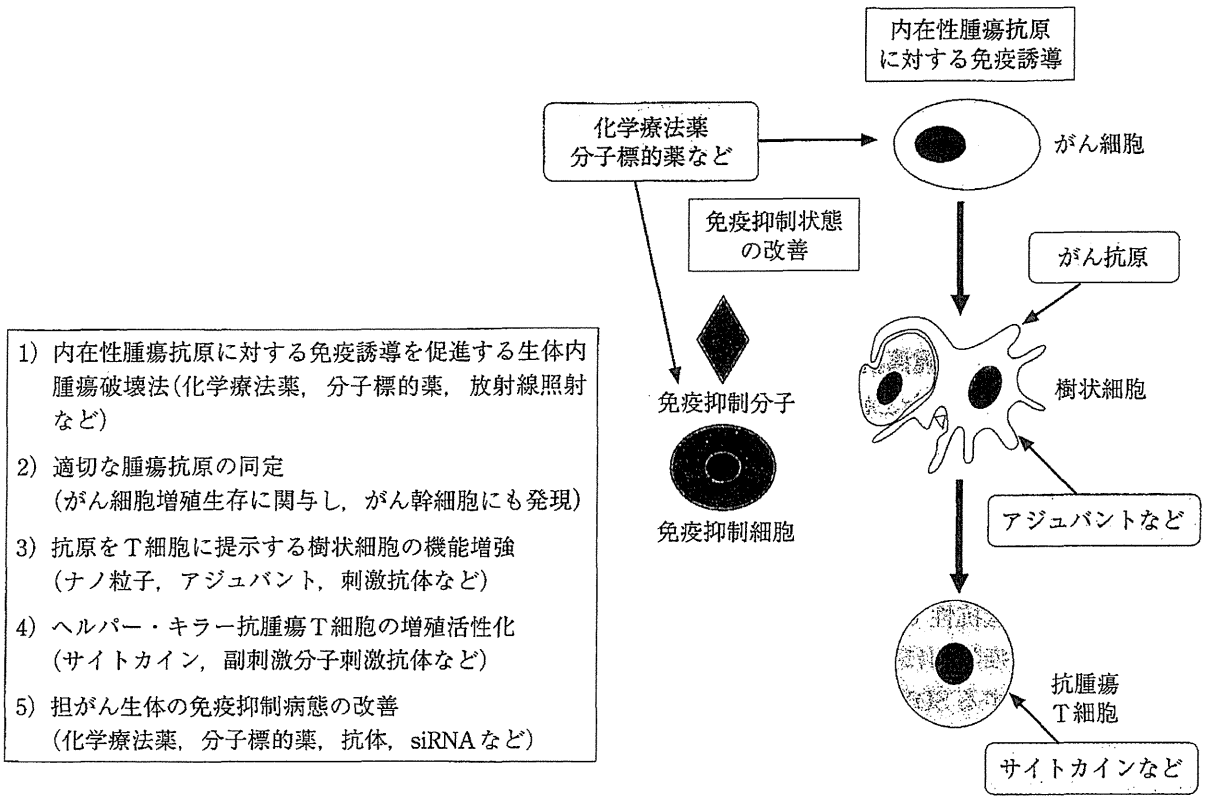

図1 効果的ながん免疫療法開発のための抗腫瘍免疫ネットワークの制御ポイント

効果的ながん免疫療法の開発のためには，①適切な生体内腫瘍破壊，②適切な腫瘍抗原の使用，③適切なDCの抗原取り込み・プロセシング・成熟化の促進，④適切なヘルパーT細胞サブセットの増殖活性化，⑤適切な抗腫瘍エフェクター免疫細胞の増殖活性化，⑥適切な免疫抑制状態の改善などの総合的な免疫制御が重要である．

めた症例のがん組織を詳細に解析すると，免疫に用いた腫瘍抗原に特異的なT細胞ががん細胞を排除しているのではなく，ワクチンという免疫操作がトリガーとなって内在性の複数の腫瘍抗原に対するT細胞を誘導し(抗原スプレッディング)，がん組織内でがん細胞の排除に働く場合があることがわかった[3]．興味深いことに，内在性腫瘍抗原に対するT細胞は，メモリーT細胞からではなく，ナイーブT細胞から誘導された可能性も示唆されている．症例ごとにがん細胞の遺伝子変異も，免疫原性が高い抗原も異なるので，症例ごとに異なる適切な内在性腫瘍抗原を免疫誘導が起こりやすいように(immunogenic cancer cell death)樹状細胞(dendritic cell: DC)に提供しT細胞を活性化させる生体内腫瘍破壊法の開発が重要である．

生体内腫瘍破壊法としては，放射線照射，凍結融解法，熱凝固法，光線力学法などの物理的方法による破壊，化学療法薬，分子標的薬，抗腫瘍抗体，オンコウイルスなどによる破壊がある．どの方法が最も優れているかはまだ十分に比較検討されていない．放射線照射はがん細胞にアポトーシスを誘導して抗腫瘍免疫誘導を起こし，それがAbscopal効果の一因と考えられている．ラジオ波などの熱凝固法は，熱ショックタンパク質の誘導により免疫誘導作用を示すが，凍結融解法も同等の免疫誘導効果を示すことが報告されている．化学療法では，アントラサイクリン系薬剤などの特定の薬剤は腫瘍破壊時にカルレクチンのがん細胞膜への移動を起こし，その受容体をもつDCによる腫瘍抗原取り込みの促進，HMGB-1放出によるDC上のTLR4などの刺激によるDC成熟化の促進，ATP放出によるプリン受容体を介したDCのIL1産生など

の分子機序により，抗腫瘍免疫誘導作用が強い(immunogenic cancer cell death)との報告がある[4]．著者らは，マウス腫瘍モデルで，凍結融解法や単純ヘルペスウイルス(HSV)などを用いた腫瘍前処置による内在性腫瘍抗原に対する免疫誘導増強作用を検討し，凍結融解法はDC腫瘍内投与による免疫療法の臨床試験でも併用した[5]．

2）がん幹細胞にも発現し，がん細胞の増殖生存に関与する腫瘍抗原の同定

がん細胞の増殖生存などに関係しない腫瘍抗原では，免疫療法後に抗原を消失したがん細胞が選択的に増殖する場合があるが，がん細胞の増殖生存に直接関与する腫瘍抗原は抗原消失によるがん細胞の免疫回避が少ないと考えられる．また，化学療法抵抗性でがん再発の原因になるがん幹細胞を免疫学的に排除するために，がん幹細胞にも発現するSOX2やWT-1などの腫瘍抗原の同定も進められている．著者らも，ヒト脳腫瘍幹細胞に発現するSOX6や悪性黒色腫幹細胞での発現が考えられる共通変異BRAF抗原を見いだし臨床応用を検討している[6]．一方，著者らは，T細胞免疫療法で完全寛解になった後で，3年ごとに再発する症例を経験しており，がん幹細胞は化学療法だけでなく，免疫療法にも抵抗性をもつ場合もあることが示唆されており，今後の更なる研究が必要である．

3）樹状細胞の抗原処理提示能とT細胞活性化能の増強

DCの抗原処理提示能およびT細胞活性化能を増強するために，様々なことが試みられている．Fc受容体やスカベンジャー受容体などのDCに発現する分子に腫瘍抗原を標的化する方法がマウスモデルで検討されている．DCのクロスプライミングなどの抗原プロセス機能を増強する方法として，熱ショックタンパク質(Hsp)や各種ナノビーズなどを用いた抗原付加が試みられている．DCを適切に成熟活性化するために，抗CD40アゴニストや天然・人工のtoll様受容体(TLR)などの危険センサー(微生物タンパク，脂質，核酸などに対する)を刺激する分子(アジュバント)が探索され，臨床試験で検討されている．著者らも新規BCG-CWS製剤，HSVタンパク質，新規TLR刺激低分子化合物などを検討している．最近の肺がん手術後のMAGE-A3タンパクワクチンでは，新規アジュバントが併用され，第2相臨床試験では27％の再発予防効果が認められ，現在，2,000人以上を対象とした世界規模の第3相臨床試験が進行中である．

4）生体内での抗腫瘍エフェクター細胞やヘルパーT細胞の増殖活性化

抗腫瘍細胞傷害性T細胞(CTL)などのエフェクター細胞やヘルパーT細胞を生体内で十分に増殖活性化する方法として，前記のDC機能の増強に加えて，IL2，IL15，IL21などのT細胞増殖性サイトカイン，T細胞の副刺激分子に対する刺激抗体などが検討されている．免疫抑制活性をもつ制御性T細胞(Treg)の増殖活性がなく，メモリー$CD8^+$T細胞の増殖活性が強いIL15が期待され，最近，臨床試験で検討されている．ヘルパーT細胞の活性化では，抗腫瘍免疫応答に適切なヘルパーT細胞サブセット(Th1やTh17など)の誘導が重要である．CD28などのT細胞副刺激分子に対する刺激抗体は，抗CD28抗体投与で認められたサイトカインストームなどの重篤な副作用を起こす可能性もあり，慎重な臨床試験の実施が必要である．

後述する培養T細胞を投与する養子免疫療法では，リンパ球抑制薬剤(cyclophosphamideやfludarabine)投与や全身性放射線照射(TBI)などの前処置により，体内の免疫細胞を減少させておくと，Tregなどの免疫抑制性細胞の減少に加えて，投与T細胞以外の免疫細胞によるIL7やIL15の消費が抑えられる結果，投与T細胞が十分に利用できるようになり(サイトカインシンク)，homeostatic expansion機構が作動して，投与T細胞が生体内で強力に増殖する．同時に，投与後に生体内での増殖能が高い培養T細胞の調製法(短期間培養，IL2/IL12遺伝子導入，セントラルメモリー・ナイーブタイプT細胞など)が研究されている[7]．

5）がん関連微小環境における免疫抑制状態の改善

がん細胞は腫瘍抗原や，HLAなどの抗原提示

にかかわる分子の異常により免疫抵抗性を示すことがある．腫瘍抗原は多数あるので，抗原消失は根本的な問題にはならないが，HLA消失は抗腫瘍T細胞にとっては重大な問題である[8]．がんによっては，IFNやHDAC阻害剤などのエピジェネティック作動薬によりHLA発現の回復が可能である．また，がん細胞はTGF-βなどの免疫抑制分子を産生して抗腫瘍免疫を抑制するだけでなく，様々な免疫抑制性細胞(Treg，寛容性DC，骨髄由来免疫抑制細胞(MDSC)，M2マクロファージ，好中球，免疫抑制性γΔT細胞やNKT細胞など)を誘導して，免疫抑制的ながん関連微小環境(がん組織，センチネルリンパ節，骨髄など)を構築している[9]．これらの抗腫瘍免疫抑制系は，本来，自己免疫の抑制や異物排除後に活性化した免疫応答を元に戻すネガティブフィードバック機序などの免疫系の恒常性を保つための仕組みであり，がん細胞はそれを悪用している．したがって，これらの抑制機構の阻害は，自己免疫反応や過剰免疫反応による副作用を起こす可能性もある．

免疫抑制状態の改善法として，免疫抑制エフェクターであるTGF-β，Treg，PD-1/PD-L1，IDO，Cox2などの除去や阻害が試みられている．Treg抑制活性の阻害やT細胞活性化ネガティブフィードバック機構の阻害による抗腫瘍T細胞増強作用が期待される抗CTLA4抗体投与の臨床試験では，自己免疫反応との相関がみられる抗腫瘍効果が認められ，悪性黒色腫では4カ月の延命効果や一部の症例での明確な腫瘍縮小効果が認められ，米国FDAに承認されている[10]．抗PD-1抗体や抗PD-L1抗体投与の臨床試験でも同様な抗腫瘍効果が認められ，大変期待されている．抗PD-1抗体治療では，長期にわたる抗腫瘍効果がみられており，がん組織において，がん細胞がPD-L1を発現し，かつ$CD8^+$T細胞の浸潤がみられる症例では，抗腫瘍効果が高率に認められている[11]．

著者らは　がん細胞の遺伝子シグナル異常を起点とする複数の免疫誘導カスケードの上流での遮断を試みている．がん細胞のがん遺伝子による恒常的シグナル活性化(MAPK，STAT3，NF-κB，Wnt/β-catenin，Snailなど)をシグナル阻害剤などの分子標的治療薬やsiRNAで抑えることにより，複数の免疫抑制分子の産生やその後に起こる複数の免疫抑制性細胞の誘導を抑制できる[12]．また，STAT3などの阻害は，がん細胞だけでなく，DCやマクロファージなどの免疫細胞にも直接作用して，免疫増強作用(IL12などのサイトカイン産生亢進，がん細胞由来免疫抑制分子に対する抵抗性など)を示す[13]．化学療法薬の中にはgemcitabineのように，免疫抑制作用が比較的弱く，MDSC減少作用を示し，抗腫瘍免疫増強作用が期待されている薬剤もある．

6) 総合的な免疫制御法としての養子免疫療法

前記の免疫制御技術の適切な併用が効果的な免疫療法の開発に重要であるが，一例として，薬剤やTBIなどの前処置後に，腫瘍浸潤リンパ球から培養した抗腫瘍T細胞を投与し，腫瘍抗原ワクチンや高用量IL2投与を併用する総合的な養子免疫療法がある．この方法により，多発転移巣をもつ進行悪性黒色腫に対して，RECIST基準で70％以上の奏効率と約20％の完全寛解(CR)例が得られており，CR症例では，3-8年間の経過観察期間中にまだ1例しか再発しておらず，治癒した可能性もある．

更に，悪性黒色腫以外のがんにも本法を実施するために，腫瘍抗原を認識するT細胞受容体(TCR)遺伝子をレトロウイルスベクターで末梢血リンパ球に導入して作製した人工的な抗腫瘍T細胞を用いた養子免疫療法が開発され，悪性黒色腫だけでなく，滑膜肉腫に対しても強力な抗腫瘍効果が得られている．また抗体の可変領域をTCR定常領域と融合し，更に副刺激因子であるCD28や4-1BBなどの細胞内領域を融合して十分なT細胞活性化シグナルを伝達可能にした人工的な抗腫瘍受容体(キメラ抗原受容体(chimeric antigen receptor: CAR))を導入したT細胞を用いた養子免疫療法も開発されている．化学療法抵抗性B細胞悪性リンパ腫や慢性リンパ性白血病に対してCD19特異的CAR導入T細胞を用いた養子免疫療法が実施され，強力

な治療効果が得られている.

おわりに

単純ながんワクチンだけでは，進行がんに対しては十分な治療効果が認められていないが，今後，標準治療後の再発予防を目的としたアジュバント設定での治療や，前記の免疫制御技術を適切に併用した総合的な免疫療法により，免疫療法の効果を明確にできる可能性が高い．また，がん細胞の免疫原性，および宿主免疫応答能には個人差も大きく，免疫療法の効果が期待できる症例の選択を可能にする診断法や，効果が期待できる状態に改善する方法の開発も重要である．著者らも症例選択や効果予測のための血液・がん組織バイオマーカーの探索を進めている[14]．最近，大腸がん，卵巣がん，悪性黒色腫などでは，治療前のがん組織の免疫状態，例えばメモリー $CD8^+$T細胞の浸潤は，その後の予後および免疫療法や化学療法の治療効果と相関するとの報告があり，抗腫瘍免疫応答は免疫療法を越えて，がん治療にとって重要である可能性が示唆されている[14]．したがって，がん免疫療法開発のためには，免疫制御技術の開発に加えて，バイオマーカーや免疫評価法の開発も必須である[15]．

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The mechanisms of cancer immunoescape and development of overcoming strategies

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Abstract Cancer-induced immunosuppression is a major problem as it reduces the anti-tumor effects of immunotherapies. In cancer tissues, cancer cells, immune cells, and other stromal cells interact and create an immunosuppressive microenvironment through a variety of immunosuppressive factors. Some cancer subpopulations such as cancer cells undergoing epithelial–mesenchymal transition and cancer stem-like cells have immunosuppressive and immunoresistant properties. The production of immunosuppressive factors by cancer cells is mechanistically attributed to oncogenic signals frequently activated in cancer cells, including the STAT3, MAPK, NF-κB, and Wnt/β-catenin signals, which are upstream events leading to immunosuppressive cascades. Moreover, some of these signals are also activated in immunosuppressive immune cells stimulated by cancer-derived factors and contribute to their immunosuppressive activities. Therefore, targeting these signals both in cancer cells and immunosuppressive immune cells may result in the restoration of immunocompetence in cancer patients and improve current immunotherapy.

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Keywords Cancer immunotherapy · Immunosuppression · EMT · Cancer stem cell · Mesenchymal stem cell · Oncogenic signal

1 Introduction

Immunoescape via immunosuppression and immunoresistance is one of the major malignant phenotypes of cancer cells as well as altered proliferation, survival, and invasion/metastasis. During the tumorigenesis, cancer cells interact with various immune cells and other stromal cells. It has recently been shown that these immune cells sometimes promote cancer cell proliferation and invasion during tumor development. In addition, cancer cells are shown to be eliminated by immune system in mouse tumor models (immunosurveillance). However, selective growth of cancer cells which acquired immunosuppressive and immunoresistant abilities due to their genetic instability occurs. The process is called cancer immunoediting. Finally, clinically apparent cancer cells are believed to have acquired immunoresistant features through a variety of mechanisms such as reduced immunogenecities and inducing immunosuppressive molecules and cells (Fig. 1). In fact, many immunotherapies such as cancer vaccines often showed insufficient anti-tumor effects partly because of these immunoescaping mechanisms in patients [1], although T cell-based adoptive immunotherapies following lymphodepletive treatment, which is believed to reduce immunosuppressive conditions, had dramatic impacts on the advanced large tumors [2, 3]. In this mini-review, we discuss the mechanisms involved in the cancer cell-induced immunosuppression and possible strategies to overcome it.

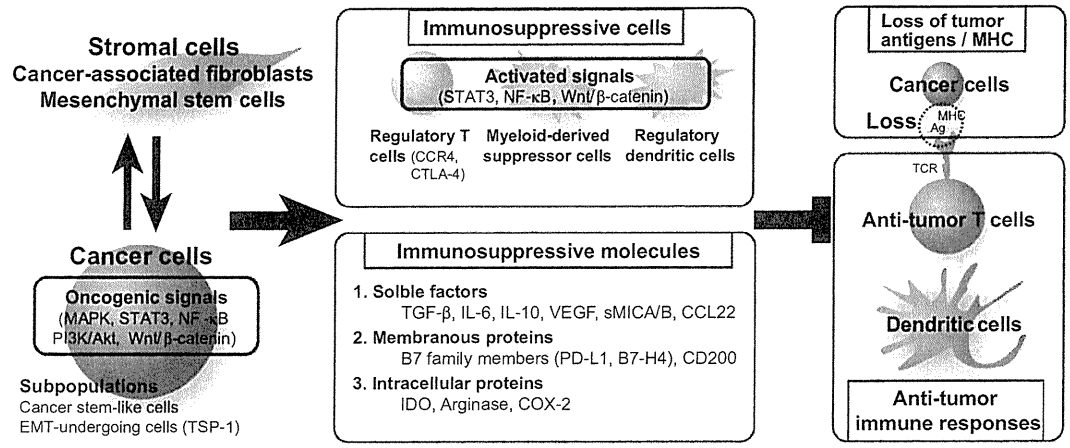

Fig. 1 In cancer tissues, cancer cells, immune cells, and other stromal cells interact with each other and built an immunosuppressive microenvironment through the production of immunosuppressive molecules and the induction of immunosuppressive immune cells. Cancer cells directly escape from T cell recognition by loss of tumor antigens and MHC. Some cancer subpopulations such as EMT-undergoing cancer cells or cancer stem-like cells have immunosuppressive and immunoresistant properties. Some oncogenic signals are activated and contribute to immunosuppressive activities both in cancer cells and in immunosuppressive immune cells. Thus, these signaling pathways are ideal targets for restoration of immunocompetence of cancer patients. *TCR* T cell receptor, *Ag* antigen, *MHC* major histocompatibility complex

2 Problems in the processing and presentation of tumor antigens

Cancer cells escape from T cell recognition by various mechanisms, including loss of tumor antigens, human leukocyte antigen (HLA), and other antigen-processing molecules such as TAP (transporter associated with antigen processing). Because cancer cells generally express multiple tumor antigens, the loss of some tumor antigens dose not result in loss of antigenicity. The molecules associated with tumor survival and growth can be ideal tumor antigens because antigenic modulation (down-regulation) rarely occurs. The causes of HLA down-regulation include gene defects or mutations of HLA heavy chain and $\beta 2$-microglobulin, their low transcription, and the dysfunction of antigen-processing molecules. In human melanoma, loss of all HLA class I often occurs due to mutations of $\beta 2$-microglobulin [4]. Although recovery of HLA expression is difficult in the cases with the mutational changes, HLA down-regulation via epigenetic changes observed in breast and prostate cancers can be reversed with pharmacologic agents such as histone deacetylase (HDAC) inhibitors.

3 Immunosuppressive and immunoresistant properties of cancer cell subpopulations

Cancer tissues consist of heterogenous cancer subpopulations. From the immunological aspects, we have been particularly interested in cancer stem cells (CSCs) and EMT (epithelial–mesenchymal transition)-undergoing cancer cells. CSCs or cancer-initiating cells are small subpopulations which have the strong ability to initiate tumor by self-renewal and the multi-lineage differentiation ability [5]. CSCs are thought to be resistant to chemotherapies partly due to expression of ABC transporters which pump out the chemotherapeutic agents. Thus, possibility of immunotherapy to eliminate CSCs has been exploited. Cytotoxic T lymphocytes (CTLs) specific for SOX2, which is reported to be expressed in CSCs, may eliminate SOX2-positive cancer stem-like cells in patients with monoclonal gammopathy of undetermined significance (MGUS), a precursor lesion to multiple myeloma [6]. We have identified that SOX6 might be expressed in human glioma stem-like cells enriched by sphere forming methods, which had high tumor-initiating ability and differentiation ability to both glial cells and neurons, and SOX6-specific CTL could lyse the glioma stem-like cells [7]. However, CSCs may also have immunoresistant property in some cases. We had experienced a patient with malignant melanoma who suffered from repeated recurrences even after complete remission by T cell-based immunotherapies over a decade [8]. In this case, dormant cancer stem-like cells might be resistant to the immunotherapy and repeatedly relapsed. We also found that some cancer stem-like cells enriched by the side population method defined by Hoechst dye exclusion showed high tumorigenicity in immunodeficient and relatively strong resistance to CTLs.

Recently, human breast cancers under EMT were reported to have the similar phenotypes to CSCs [9]. EMT is involved in cancer cell invasion and metastasis through

loss of E-cadherin-dependent cell–cell adhesion and increased invasion ability induced by EMT-related transcriptional factors such as Snail, Slug, Twist, and ZEB. We then evaluated immunologic properties of snail-induced EMT cancer cells and found that EMT melanoma were relatively resistant to CTLs [10]. In addition, the snail-induced EMT cancer cells induced immunosuppression via multiple mechanisms, including the production of immunosuppressive cytokines such as TGF-β, IL10, and TSP-1, impairment of dendritic cells (DCs), and induction of regulatory T cells (Treg). Intratumoral administration of Snail-1-specific siRNA or anti-TSP-1 Ab resulted in tumor growth inhibition accompanied by the recovery of DC function, the reduction of Tregs, and the induction of tumor-specific CTL. These observations suggest that cancer cell subpopulations, CSC, and EMT-undergoing cancer cells have immunoresistant and immunosuppressive properties and that we may need additional interventions to eliminate these cancer subpopulations.

4 Expression of immunosuppressive molecules by cancer cells

4.1 Immunosuppressive molecules expressed by cancer cells

Cancer cells express a variety of immunosuppressive cytokines and chemokines such as TGF-β, IL6, IL10, and VEGF, which directly suppress DCs or effector T cells [11]. Moreover, cytokines such as TGF-β and IL10 can induce immunosuppressive immune cells such as regulatory DCs (DCregs) and Tregs. DCregs can also induce Tregs, which amplifies the loop cascade in building an immunosuppressive cancer microenvironment. These immunosuppressive cytokines and chemokines are produced not only by cancer cells but also by tumor-infiltrating immune cells. Soluble MICA/B (MHC class I chain-related A, B), a membrane ligand of NKG2D, are shed from some cancer cells, down-regulate NKG2D expression on NK cells and T cells and suppress their functions [12]. A variety of human cancers ectopically express inhibitory B7 family members, PD-L1 and B7-H4, and inhibit anti-tumor T cells [13]. A clinical trial using MDX-1106, a humanized antibody against PD-1, a receptor for PD-L1, was conducted for patients with various cancers, and some partial response (PR) and complete response (CR) cases were observed [14].

Ectopic expression of indolamine 2,3-dioxygenase (IDO) which suppresses the T cell function through catabolizing tryptophan, an essential amino acid for T cell proliferation and differentiation, has been reported in various cancers and the tumor-infiltrating DCs. Administration of IDO inhibitors, methyl-tryptophan (1-MT) and methylthiohydantoin-tryptophan (MTH-Trp) [11], combined with paclitaxel induced the regression of mouse breast carcinomas, and a phase I clinical trial using 1-MT is currently being carried out. Arginase (ARG), an enzyme catabolizing arginine important for immune cell function, is also over-expressed in cancer microenvironment by cancer cells and tumor-infiltrating myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) and is involved in immunosuppression.

Expression of CD200, a type-1 membrane glycoprotein, which was reported to have multiple immunosuppressive abilities, including reduction of Th1 cytokine production and T cell activation, and induction of DCregs and Tregs [15], has been found in several human cancer cells, including ovarian cancers, melanoma, and leukemia. CD200 is implicated as a prognostic factor in multiple myeloma [15] and acute myeloid leukemia [16]. Since CD200 is expressed in hair follicle bulge stem cells [17] and embryonic stem cells [18], and possibly in cancer stem cells of prostate, breast, brain, and colon cancers [19], CD200 may be involved in immune evasion mechanisms of cancer stem cells.

4.2 Oncogenic signals inducing production of immunosuppressive molecules

Recently, we and others have reported that the production of immunosuppressive molecules such as TGF-β, IL10, VEGF, and IL6 were induced by constitutively activated oncogenic signals in human cancer cells and that inhibition of these signals by small molecule inhibitors or specific RNAi could reduce the production of multiple immunosuppressive cytokines simultaneously [20, 21]. MAPK signals, which are frequently activated by mutated $BRAF^{V600E}$ in human melanoma, were involved in the production of IL6, IL10, and VEGF, and treatment of $BRAF^{V600E}$-specific RNAi or MEK inhibitors inhibited these cytokine productions [20]. Immunization with MEK-depleted cancer cells could induce anti-tumor cytotoxic T cells in vivo compared with control cancer cells in the mouse tumor model. CD200 has been reported to be induced by the activated MAPK signal in human melanoma, and it may be one of the immunosuppressive effector molecules in the MAPK-activated cancer cells [22].

Inhibition of STAT3, which are frequently activated in various human cancers, also showed reduced immunosuppressive cytokine productions [20, 21]. Systemic administration of STAT3 inhibitors not only inhibited tumor growth and the production of immunosuppressive cytokines but also induced the production of inflammatory cytokines and chemokines, leading to augmentation of DC function and CTL induction [23]. We found that Wnt/β-catenin signal was frequently activated in human

melanoma and involved in the production of immunosuppressive cytokines (Yaguchi et al., manuscript in submission). In human glioma, PI3K/Akt signal is involved in PD-L1 expression [24]. To identify signaling pathways associated with the production of immunosuppressive molecules in human cancer cells, we performed a comprehensive screening using RNAi libraries and found a novel kinase involved in TGF-β and IL10 production. New potential signaling targets for restoring immunocompetence may be identified by this technique.

Some signaling pathways mentioned above are also activated in immunosuppressive immune cells. Cancer-derived factors present in cancer tissue microenvironment such as VEGF, IL6, and IL10 activate STAT3 in tumor-infiltrating DC and MDSC, and induced their immunosuppressive functions [21]. Inhibition of STAT3 in these immune cells renders them resistant to cancer-derived factors as well as enhancing production of cytokines such as IL12 due to blockade of negative feedback mechanism of cytokine production by DC (Iwata et al., manuscript in revision). Wnt/β-catenin is also reported to be involved in the generation of DCregs [25] and the enhancement of Treg survivals [26]. Thus, targeting STAT3 or Wnt/β-catenin both in cancer cells and these immunosuppressive immune cells may result in restoration of immunocompetence of cancer patients. These observations indicate that inhibitors on these signaling molecules upstream of immunosuppressive cascades may be useful for improving current immunotherapies by simultaneous inhibition of multiple immunosuppressive mechanisms, in addition to their direct inhibiting effects on cancer cells (Fig. 1).

When signal inhibitors are used for cancer treatment, their adverse effects on normal cells including T cells need to be considered. For example, as MAPK signal is also utilized by T cell proliferation, the MAPK signal inhibition may also reduce the anti-tumor immune T cell responses. However, cancer cells harboring $BRAF^{V600E}$ are more depending on MAPK signaling than normal cells, and more sensitive to MEK inhibitors [27], indicating possible use of appropriate dose of MAPK inhibitors without inhibiting anti-T cell responses. In addition, new BRAF inhibitors such as PLX4032 and GSK2118436 which have higher inhibitory activity on the mutant $BRAF^{V600E}$ over wild-type BRAF have recently been developed, and their administration demonstrated significant anti-tumor activity on patients with the mutant $BRAF^{V600E}$ in clinical trials [28, 29]. Thus, the mutant BRAF-selective inhibitors are attractive reagents for combined use with immunotherapy. Administration of multi-kinase inhibitor, sunitinib, which has direct anti-tumor effects on RCC cancer cells, has shown to induce Th1 responses accompanied by reduction of MDSC and Treg [30, 31]. Therefore, signal inhibitors may be useful for reversal of cancer-induced immunosuppressive condition without inhibiting anti-tumor T cell responses.

5 Cells responsible for immunosuppression in cancer microenvironment

5.1 Immunosuppressive immune cells

A variety of immunosuppressive immune cells, including Tregs, MDSCs, TAMs, and DCregs, are involved in generation of cancer microenvironment [11]. Tregs include CD4+ CD25+ FOXP3+ Tregs, which are divided into two groups, natural occurring Tregs and peripherally induced Tregs, IL10-producing Tr1 cells, and TGF-β-producing Th3 cells. In both mouse experiments and cancer patients, Tregs increased in tumor tissues, draining lymph nodes, and peripheral blood. High ratio of FOXP3+ Tregs versus CD8+ effector T cells in tumor tissues was reported to be correlated with poor prognosis of cancer patients [32]. TGF-β and IL10 produced by cancer cells and DCregs induce Tregs. We observed that Treg is involved in the immunosuppression during snail-induced EMT [10] and that depletion of Treg is important for DC-based immunotherapy [33]. Depletion of Tregs or inhibition of their suppressive activity has been attempted by targeting cell surface molecules predominantly expressed in Tregs, such as CD25, CTLA4, GITR, and OX40 [34]. Recently, it has been showed in a phase III clinical study that patients with metastatic melanoma who received an antibody to CTLA-4 (Ipilimumab) with or without a gp100 vaccine survived nearly 4 months longer than those who received the gp100 vaccine alone [35]. CCL22 produced by cancer cells and macrophages plays an important role in the recruitment of CCR4-expressing Tregs [36]. A humanized anti-CCR4 Ab has recently been developed and shown anti-tumor effects on CCR4-expressing ATL (adult T cell leukemia) in clinical trials [37]. Considering that CCR4 is also expressed on Tregs and Th2 cells, both of which have negative impacts on anti-tumor immune responses, we are currently evaluating whether the anti-CCR4 Ab is useful for reversal of immunosuppressive condition in tumor-bearing hosts.

The MDSCs are heterogeneous populations of the cells derived from immature myeloid cells and show abilities to suppress the T cell function. In mice, MDSCs are identified as Gr1+ CD11b+ cells. But, in humans, specific markers have not been identified, and combinations of several markers are suggested, such as Lin− HLA-DR− CD33+ or CD14− CD11b+ CD33+ [38]. MDSCs are induced in peripheral blood, spleens, and tumor tissues in both cancer patients and tumor-bearing mice. T cell dysfunction is induced by MDSCs via various molecules, including IL10, TGF-β, reactive oxygen species (ROS), arginase, and

nitric-oxide synthase (NOS). We observed that STAT3 and NF-kB contribute to MDSC's expansion and immunosuppressive function (Nishio et al., manuscript in preparation; Iwata et al., manuscript in preparation). Administration of a NF-kB inhibitor to tumor-bearing mice inhibited the immunosuppressive function of MDSC. A variety of therapeutic strategies to reduce immunosuppression by MDSC are currently explored. The mechanisms of the MDSCs depletion include promoting myeloid-cell differentiation, inhibiting MDSC expansion, and inhibiting MDSC function using diverse reagents including all-*trans* retinoic acid (ATRA), vitamin D3, anti-VEGF Ab, chemotherapies such as gemcitabine and doxorubicin, COX-2 inhibitor, sunitinib (tyrosine kinase inhibitor), and sidenafil (phosphodiesterase-5) [38].

Dendritic cells are professional antigen-presenting cells that are indispensable for the induction of antigen-specific anti-tumor immune responses. But DCs become functionally defective both in tumor sites and peripheral blood due to tumor-derived immunosuppressive factors such as IL6, IL10, VEGF, TGF-β, and PGE2. Moreover, we and others found that DCs showing immunosuppressive phenotypes including Treg induction are induced in tumor-bearing hosts, such as high IL10-producing DCs, IDO-expressing DCs, and PD-L1-expressing DCs [10, 11]. As for plasmacytoid DCs (pDCs), they also show immunosuppressive phenotypes in tumor sites, such as low Type I interferon (IFN) production and the ability to induce Treg. We found that ILT-7 ligands expressed on human cancer cells suppressed the pDC function to produce type I IFN [39].

5.2 Other immunosuppressive stromal cells

Tumor tissues consist of cancer cells and a variety of stromal cells including immune cells, fibroblasts, adipocytes, vascular endothelial cells, and mesenchymal stem cells. These cancer-associated stromal cells may contribute to the malignant phenotypes of cancer cells, such as transformation, tumor growth and survival, invasion, and immunosuppression. Major stromal cells other than immune cells in cancer microenvironment are fibroblasts and mesenchymal stem cells (MSCs), which originate from surrounding normal tissues and bone marrow [40, 41]. The fibroblasts from tumor tissues show activated phenotypes similar to fibroblasts involved in wound healing and have been termed cancer-associated fibroblasts (CAFs). TGF-β, PDGF, and FGF2 secreted from cancer cells activate fibroblasts, which have generally been considered as the major source of CAFs [42].

The CAFs produce a variety of soluble factors, including classical growth factors such as HGF, EGF, and TGF-β and angiogenesis stimulators such as VEGF, FGF, and CTFG, all of which directly and indirectly enhance the ability of tumor progression [40, 43]. Recently, CAFs are reported to produce chemokines such as CXCL12 (SDF-1) [44] or CXCL14 [45], which recruit bone marrow-derived endothelial precursor cells or macrophages to cancer tissues. Additionally, it has recently been reported that CAFs themselves harbored gene mutations in p53 [46]. Altogether, through production of these molecules, CAFs have strong influences on building cancer microenvironment. Considering CAFs produce immunosuppressive molecules such as TGF-β and VEGF, CAFs may also be associated with cancer immunoescape. Actually, CAF depletion by a DNA vaccination targeted to fibroblast activation protein resulted in a shift of the immune microenvironment from a Th2 to Th1 polarization accompanied by up-regulation of IL2 and IL7 and decrease of MDSCs, TAMs, and Tregs [47]. However, the molecular mechanism leading to CAF-induced immune suppression still remains to be elucidated.

Mesenchymal stem cells are heterogeneous subsets of stromal stem cells that can differentiate into a variety of mesenchymal cells such as adipocytes, chondrocytes, and osteocytes. Although the major source of the MSCs is a bone marrow, MSCs can be isolated from many adult tissues and can be recruited at sites of injury and inflammation, where they contribute to tissue remodeling. Recently, MSCs are reported to migrate into tumor tissues from bone marrow and can be one of the major sources of CAF [48–50]. A variety of growth factors, cytokines, and chemokines in cancer microenvironment such as EGF, HGF, bFGF, PDGF, VEGF, and IL-8 can recruit MSC into tumors [51]. When incorporated into tumor tissues, MSCs have anti-apoptotic effects on cancer cells, promote tumor vascularization by producing VEGF [51], and promote their invasion and metastases by producing CCL5 [52]. Moreover, MSCs may also be involved in immunosuppression during the tumor development. By producing immunosuppressive molecules such as TGF-β, IDO, IL10, IL6, and prostaglandin E2, MSCs show immunosuppressive effects on a variety of immune cells, including inhibition of T cell proliferation, dendritic cell maturation, NK cell activation, and induction of Tregs [53]. Clinical trials of MSC administration for patients with steroid-resistant severe graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation have been performed, and it reduced occurrence of GVHD [54]. Co-injection of MSC cell lines allowed cancer cells grow in allogeneic recipients along with the inhibition of lymphocyte infiltration into the tumor tissues [55]. We have also confirmed the MSC infiltration into tumor tissues and are currently investigating the molecular mechanisms for the MSC-induced immunosuppression in tumor microenvironments.

6 Concluding remarks

Immunoescaping ability of cancer cells is now believed to be one of the important malignant phenotypes of cancer cells. Correction of the immunosuppressive microenvironment of cancer patients may lead to better anti-tumor effects of cancer treatment, particularly immunotherapy. Modification of anti-tumor immune responses in patients by targeting the immunoregulatory molecules, including CTLA-4, PD-1, and IDO, has recently been in progress, and some promising results have already been observed. Therefore, appropriate combination of immunotherapy and the modification of immunosuppressive condition is a really promising strategy. In addition, personalized immunotherapies may also be ideal where appropriate combinations can be chosen for each patient because of the differentials mechanisms of immunosuppression among patients. Further understanding of the mechanisms for the cancer-induced immunosuppression and development of methods to overcome them is critical to improve current cancer treatment.

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