考察

漢方成分 No. 9 を担がんマウスに投与し、漢方成分 No. 9 の抗腫瘍効果を検討した。実験最終日までの体重減少が顕著ではないことから、著しい毒性はないと推測される。腫瘍細胞と健常マウス脾細胞に対しても細胞傷害性が認められなかった。一方、高濃度領域の漢方成分 No. 9 溶液は CT26 細胞増殖を亢進させる作用があった。腫瘍径の増大を抑える働きがみられたことから、生体内では必ずしも腫瘍の増殖を促進しているとは言えず、むしろ漢方成分 No. 9 の何らかの作用によりがん免疫状態を改善している可能性がある。フローサイトメトリーによる解析結果では顕著な差は見られなかった。しかしながら、腫瘍抗原特異的なT細胞誘導による IFNγの産生量は有意に増加しており、漢方成分 No. 9 の投与により抗原特異的な CTL の誘導が促進されたことを示唆する。今後更に抗腫瘍免疫応答のメカニズムを明らかとすべく、研究を進める必要がある。

漢方成分 No. 16 の抗腫瘍効果を担がんマウスモデルを利用して検討した。実験最終日までの体重減少が顕著ではないことからも、著しい毒性はないといえる。腫瘍細胞と健常マウス脾細胞に対しても細胞傷害性が認められず、細胞増殖に影響を与えるのは 10μM作用時の健常マウス脾細胞だけであった。従って、漢方成分 No. 16 の抗腫瘍効果は腫瘍細胞へ直接的な細胞毒性作用ではないと考えられる。フローサイトメトリーによる解析結果では顕著な差は見られなかったが、腫瘍近傍の鼠径リンパ節中で $CD8^+$T細胞の割合が増加する傾向があった。また、腫瘍抗原特異的なT細胞誘導による IFNγの産生量も増加させており、漢方成分 No. 16 の投与により抗原特異的な応答の誘導が促進されたことを示唆する。

漢方成分 No. 17 を担がんマウスに投与し、漢方成分 No. 17 抗腫瘍効果を検討した。実験最終日までの体重減少が顕著ではないことからも、著しい毒性は作用していないといえる。また、担がん状態による脾腫を回避しており、腫瘍細胞の浸潤が少ないことが示唆される。腫瘍細胞と健常マウス脾細胞に対しても高濃度領域で細胞傷害活性が存在し、細胞増殖に対しても漢方成分 No. 17 溶液の濃度依存的に細胞増殖を有意に抑制した。従って、漢方成分 No. 17 抗腫瘍効果は細胞毒性や、各種細胞に対する細胞増殖抑制による作用を含んでおり、マウスに投与する濃度や腫瘍細胞への直接的作用を考慮する必要がある。フローサイトメトリーによる解析結果では顕著な差は見られなかった。また腫瘍抗原特異的なT細胞誘導による IFNγの産生量は対照群と比較し、むしろ顕著に低下していた。漢方成分 No. 17 はヒトがん細胞からの免疫抑制性サイトカイン産生を抑制において OVCAR3、JHOC5 の産生する IL-6 を顕著に抑制する作用、888mel と 624mel の産生する IL-10 を抑制する作用が確認された。腫瘍細胞の産生するサイトカインに対しては抑制方向に働いており、何らかのシグナル阻害剤として作用している可能性がある。今後更に *in vivo* の実験を行い宿主の免疫担当細胞の活性への影響を検討する必要がある。

漢方成分 No. 23 を担がんマウスに投与し、漢方成分 No. 23 の抗腫瘍効果を検討した。腫瘍細胞に対しては細胞傷害性も細胞増殖抑制も確認されなかったが、健常マウス脾細胞に対しては 3.33-10 μMの濃度範囲で細胞傷害性が確認され、10 μMの濃度範囲で細胞増殖抑制が認められた。健常細胞に対する侵襲性は今後も検討すべきであるが、実験最終日までの体重減少が顕著ではないことから、著しい毒性は作用していないと示唆される。*in vitro* の実験結果で、腫瘍細胞へ直接的作用が確認されなかった。つまり、腫瘍径の増大を抑える働きがみられたことは漢方成分 No. 23 の宿主免疫応答への何らかの作用が影響していると考えられる。フローサイトメトリーによる解析結果では、腫瘍内で $CD4^+$T 細胞頻度の増加、また抗腫瘍免疫を担うＮＫ細胞、ＮＫＴ細胞が局所リンパ節で頻度が増加していた。また腫瘍抗原特異的な T 細胞誘導によって産生する IFNγ産生量が有意に増加したことからも抗腫瘍免疫応答の増強作用が推測される。今後更に抗腫瘍免疫応答のメカニズムを明らかとすべく、研究を進める必要がある。

漢方成分 No. 24 を担がんマウスに投与し、漢方成分 No. 24 抗腫瘍効果を検討した。腫瘍細胞に対しては細胞傷害性も細胞増殖抑制も確認されなかったが、健常マウス脾細胞に対しては 10 μMで細胞傷害性、3.33-10 μMの濃度範囲で細胞増殖抑制が認められた。健常細胞に対する侵襲性は今後も検討すべきであるが、実験最終日までの体重減少が顕著ではないことから、著しい毒性は作用していないと示唆される。*in vitro* の実験結果で、腫瘍細胞へ直接的作用が確認されなかった。つまり、腫瘍径の増大を抑える働きがみられたことは漢方成分 No. 24 の宿主免疫応答への何らかの作用が影響していると考えられる。フローサイトメトリーによる解析結果では、脾臓中で $CD8^+$T 細胞の割合が増加する傾向、NKT 細胞頻度が増加する傾向が見られた。また腫瘍中の Treg 細胞頻度が減少する傾向が見られた。また、腫瘍抗原特異的な T 細胞誘導によって産生する IFNγ産生量が増加していたことからも、T 細胞応答を増強していたことが示唆される。今後更に抗腫瘍免疫応答のメカニズムを明らかとすべく、研究を進める必要がある。

漢方成分 No. 25 を担がんマウスに投与し、漢方成分 No. 25 抗腫瘍効果を検討した。腫瘍細胞に対しては細胞傷害性も細胞増殖抑制も確認されなかったが、健常マウス脾細胞に対しては 10 μMで細胞傷害性、3.33-10 μMの濃度範囲で細胞増殖抑制が認められた。健常細胞に対する侵襲性は今後も検討すべきであるが、実験最終日までの体重減少が顕著ではないことから、著しい毒性は作用していないと示唆される。*in vitro* の実験結果で、腫瘍細胞へ直接的作用が確認されなかった。つまり、腫瘍径の増大を抑える働きがみられたことは漢方成分 No. 25 の宿主免疫応答への何らかの作用が影響していると考えられる。フローサイトメトリーによる解析結果では脾臓で抗腫瘍免疫を担うＮＫＴ細胞が脾臓で頻度が増加する傾向、Treg 細胞頻度は腫瘍において減少傾向が見られた。*in vitro* で漢方成分 No. 25 は Treg 細胞誘導抑制効果を示しており、マウスモデルにおいても効果的に免疫抑制解除に作用しているか、今後更に詳細な実験を行う必要がある。また腫瘍抗原特異的なT細胞誘導による IFNγ の産生量は対照群と比較し、有意に増加した。フローサイトメトリーの結果で示された NKT 細胞の頻度増加と合わせて、どの免疫担当細胞が IFNγ産生増加に寄与するのかを明らかにする必要がある。

漢方成分 No. 35 による抗がん作用の分子機構は、*in vitro* と *in vivo* の両方の実験で明らかとなった。漢方成分 No. 35 の抗がん作用の一つは、がん細胞の増殖阻害であり、CT26 細胞で増殖が抑えられたことを確認した。また、抗がん作用の別の一つは、血管新生を阻害することによって生じた可能性がある。我々は、マウス大腸がん細胞株 CT26 細胞に漢方成分 No. 35 を加えた際、容量依存的に細胞増殖が抑制されることを確認した。これらの結果は腫瘍細胞の増殖や血管新生の抑制が *in vivo* における漢方成分 No. 35 の抗腫瘍作用として、がん細胞へ直接的に作用することを示唆している。また、がん細胞に対する細胞傷害性の実験でコントロールと差がなかったことは、漢方成分 No. 35 の作用に直接的な細胞毒性が伴わないことを示唆している。漢方成分 No. 35 による腸管・全身・腫瘍免疫への効果を検証するために、我々は漢方成分 No. 35 をマウスに経口投与した。脾臓、腫瘍、小腸の免疫細胞の数に大きな変化はなかったが、漢方成分 No. 35 を投与したマウスでは腫瘍抗原特異的 T 細胞の誘導が確認された。この結果は、腫瘍増殖と血管新生に加えて、レスベラトロールが腫瘍抗原特異的な免疫応答を促進する可能性を示唆している。しかし、漢方成分 No. 35 による抗腫瘍作用は複雑であり、*in vivo* での抗腫瘍作用の免疫性メカニズムを解明するためにも、さらなる研究が必要である。

7. 同定した漢方成分の免疫作用の評価法の開発

これまでの様々なスクリーニングから得られた漢方成分の一部は、AhR に対するアンタゴニスト活性を持っている。これらの作用を今後評価するために、今年度、評価系の確立を試みた。

結果

7.1) IL-10 産生性樹状細胞の誘導抑制作用

AhR の発現は樹状細胞にも認められ、その活性化が樹状細胞の機能に影響を及ぼすことが示唆される。マウスの骨髄細胞を採取し AhR アンタゴニスト存在下で樹状細胞の誘導を行ったところ、コントロールの樹状細胞と比較して IL-10 の産生が低下し、IL-12 の産生が上昇した（図７２A)。この結果より、AhR の活性化が樹状細胞を免疫抑制的な形質へと誘導することが示唆される。この系を用いれば、AhR アンタゴニストとしてこれまでに同定した漢方成分が AhR による免疫抑制的な樹状細胞の誘導を阻害できるか評価することが可能になる。

7.2) キヌレニン高産生性 IDO 遺伝子導入マウスがん細胞移植モデルの作成

AhR の内在性リガンドとして働き得るキヌレニンを高産生させ AhR 活性化阻害の影響をよりわかりやすく観察するため、キヌレニンを産生する酵素 IDO を遺伝子導入したマウスがん細胞株を作製した。この細胞株は AhR の活性化が mock control に比べ顕著に誘導されていることを CYP1A1 の発現によって確認している（図７２B)。IDO 高発現マウスがん細胞をマウスに皮下移植し、２週間後に腫瘍内の免疫細胞を解析したところ、$CD8^+$ T 細胞の割合が減少し、$Foxp3^+$ 制御性 T 細胞の割合が増加する傾向が見られた。この結果より、キヌレニンによる AhR の活性化によって免疫抑制的な腫瘍環境が誘導されることが示唆される。この系を用いれば、AhR アンタゴニストとして同定した漢方成分が生体内の抑制的な抗腫瘍免疫環境を改善できるか検証することが可能になる。

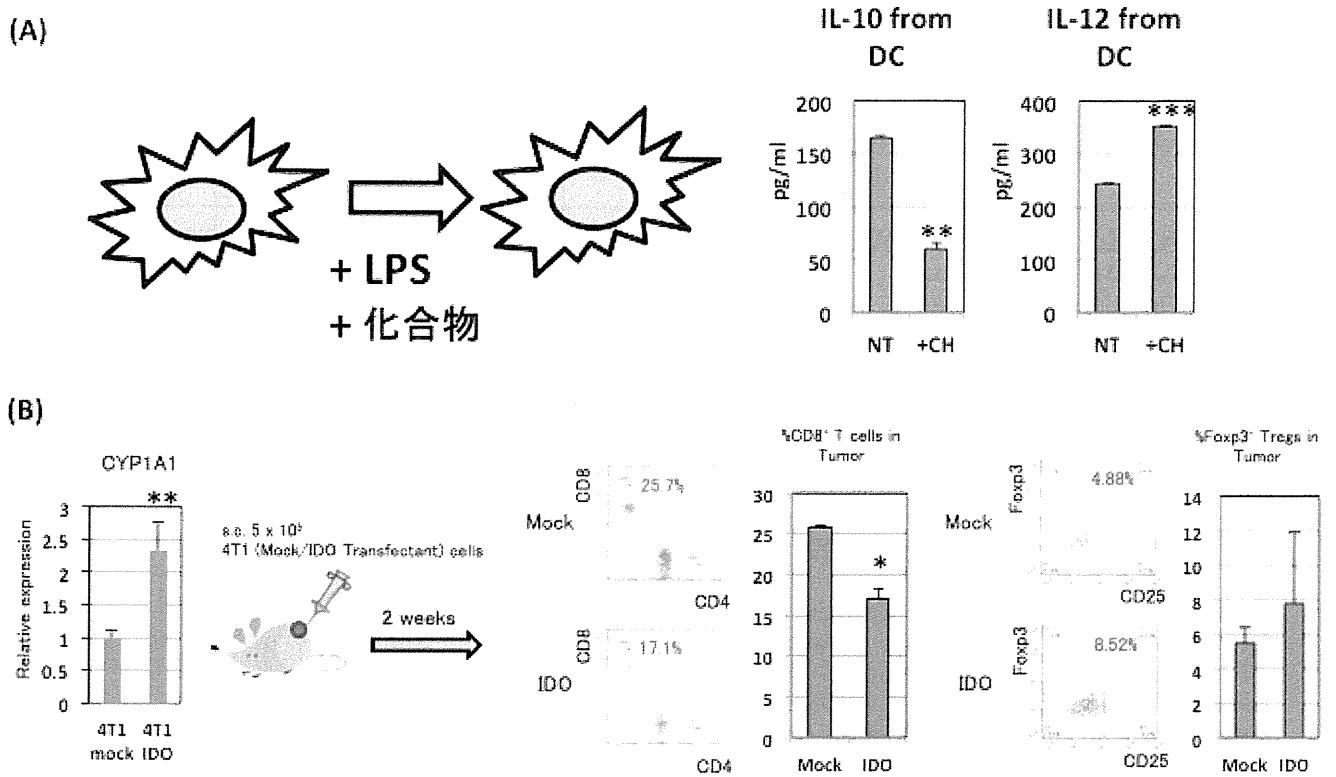

図７２．　同定した漢方成分の免疫作用の評価法の開発

考察

がん細胞において IDO が高発現しトリプトファンの枯渇を引き起こすことが知られている。そして IDO がトリプトファンを代謝することによって産生されるキヌレニンはAhRのアゴニストであることが明らかとなった。そこでこれまでの報告を踏まえ、AhR が免疫抑制的ながん微小環境を引き起こす経路として以下の２つが考えられる。まず IDO を発現しているがん細胞によって産生されたキヌレニンががん細胞自体の AhR の活性化を促し、それによる下流のシグナルや産生される分子が周囲の免疫系細胞に抑制的な影響を及ぼすことが仮定される。がん細胞によって産生されたキヌレニンが、がん細胞自体の AhR を活性化させることを我々は確認しており、また AhR によって IL-8 や IL-10 などの免疫調節分子の産生が制御されていることも分かっている。２つ目は、がん細胞によって産生されたキヌレニンが免疫系細胞の AhR を活性化させ、抑制的な形質へと誘導する可能性である。がんの免疫抑制環境において重要な役割を担っている Foxp3⁺制御性 T 細胞は、キヌレニンによって誘導されることが分かっており、この誘導は AhR を介して起こる。また樹状細胞の免疫抑制的な形質は AhR が重要な役割を担っているとされ、キヌレニンが樹状細胞の AhR に作用しその形質を抑制的に誘導することも考えられる。これらの仮説を証明するための実験を現在進めているが、これまでの結果からもがん細胞、免疫細胞双方での AhR の活性化が免疫抑制的ながん微小環境の構築に深く関わっていることが示唆される。そこで AhR の活性化を制御する方法の開発が重要となり、漢方成分中の AhR アンタゴニストはそのうちの一つとして期待される。

III. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Ueda R, Yaguchi T, Kawakami Y	Human tumor antigens recognized by T cells and their implications for cancer immunotherapy	Wang RF	Innate immune regulation and Cancer immunotherapy	Springer Science /Business Media	New York USA	2011	335-345

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Iwata-Kajihara T, Sumimoto H, Kawamura N, Ueda R, Takahashi T, Mizuguchi H, Miyagishi M, Takeda K, Kawakami Y	Enhanced Cancer Immunotherapy Using STAT3-Depleted Dendritic cells with High Th1-Inducing Ability and Resistance to Cancer Cell-Derived Inhibitory Factors.	J Immunol.	187(1)	27-36	2011
河上裕、小室美紗、小林明日香、梶原岩田知子、宮崎潤一郎、川村直、谷口智憲	ヒトがん細胞に対する免疫応答機構と免疫制御の可能性	腫瘍内科	8(5)	409-416	2011

IV. 研究成果の刊行物・別刷

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Contents

Chapter 19
Human Tumor Antigens Recognized by T Cells and Their Implications for Cancer Immunotherapy

Ryo Ueda, Tomonori Yaguchi, and Yutaka Kawakami

1 Introduction

Recent clinical trials of immunotherapies indicate that tumor reactive autologous T cells are able to regress even advanced, large tumors in melanoma patients. For example, the adoptive transfer of $CD8^+$ cytotoxic T lymphocytes (CTL) specifically targeted for identified tumor antigens following lymphodepletive treatment, such as fludarabine/cyclophosphamide administration and total body irradiation, led to objective tumor responses in more than 70% of patients with melanoma (Dudley et al. 2008). Immunological analyses on these tumor tissues demonstrated that administered T cells may eliminate tumor cells through direct killing and cytokine secretion. Therefore, $CD8^+$ CTLs that recognize MHC class I positive cancer cells are important for in vivo tumor rejection. In addition, $CD4^+$ helper T (Th) cells may also play a role in the induction and maintenance of final effectors including $CD8^+$ T cells and macrophages, the accumulation of $CD8^+$ T cells in tumor tissues, as well as the direct recognition of MHC class II positive malignant cells including most hematological malignancies. Thus, the identification of human tumor antigens recognized by $CD8^+$ and $CD4^+$ T cells is important for the assessment/quantification of in vivo anti-tumor T cell responses and the development of effective immunotherapies. A variety of human tumor antigens recognized by T cells, and their T cell epitopes, have been identified recently using various isolation methods. These studies have led to the understanding of molecular mechanisms underlying human cancer cell recognition by T cells, and to the development of novel immunotherapies (Kawakami et al. 2004). In this chapter, recent progress in human tumor antigen identification and its implication for immunotherapy will be discussed.

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2 Methods for the Identification of Human Tumor Antigens Recognized by T Cells

Human melanoma antigens recognized by T cells were first isolated by expression DNA cloning using tumor reactive T cells, and T cell epitopes were identified by narrowing down the DNA sequences encoding the epitopes (van der Bruggen et al. 1991; Kawakami et al. 1994a). T cell epitope peptides could also be identified directly using mass spectrometry (Cox et al. 1994). However, it is difficult to generate tumor reactive T cells against various cancers other than melanoma. Thus, various strategies not requiring tumor reactive T cells from patients have been attempted. One technique called SEREX (serological analysis of recombinant cDNA expression libraries) involves cDNA expression cloning using serum IgG from cancer patients (Kiniwa et al. 2001). IgG Ab induction indicates that $CD4^+$ helper T cells specific for the target antigens are activated in these patients. In fact, these antigens can induce not only $CD4^+$ helper T cells, but also $CD8^+$ CTL in vitro from PBMC of cancer patients or healthy individuals (Jager et al. 2000) (Reverse immunology). Candidates for T cell antigens such as tissue-specific antigens, cancer-testis antigens, and overexpressed antigens, can also be systematically identified using various genetic analyses, including various cDNA subtraction methods comparing normal tissues and cancer cells, such as classical cDNA subtraction, representational differential analysis (RDA), and differential PCR display. Other methods may involve the comparison of cDNA profiles obtained by SAGE (serial analysis of gene expression), DNA microarray analysis, and EST databases (Brinkmann et al. 1999; Hayashi et al. 2007; Matsuzaki et al. 2005; Goto et al. 2006). Allogeneic antigens such as minor histocompatibility antigen (mHa) may be identified through single nucleotide polymorphism (SNP) searches. Using these techniques, we have identified a variety of human tumor antigens and made catalogs of tumor antigens for various human cancers, which led to the development of novel immunotherapies. The identification of T epitopes revealed the molecular mechanisms by which T cells recognize human cancer cells.

3 The Mechanisms for T Cell Recognition of Human Cancer Cells

The identification of human tumor antigens helped elucidate the events leading to T cell recognition of human cancer cells, including mechanisms for T cell epitope generation (Table 19.1). T cells against tumor-specific mutated peptides (e.g., point mutations, frameshift mutations, and translocations), tissue-specific proteins (e.g., cryptic epitope of self-proteins), overexpressed proteins, and proteins preferentially expressed in germline cells such as testis cells (cancer-testis antigens, cancer-germline antigens, typically expressed by DNA demethylation) were found to be elicited in cancer patients. The identification of T cell epitope peptides also revealed

Table 19.1 Examples of the representative human tumor antigens recognized by T cells

Group	Antigen	Cancer
Mutated peptides	β-Catenin/CDK4	Melanoma
	bcr-abl	CML, ALL
Tissue-specific proteins	gp100/MART-1/melanA	Melanoma
	PSA	Prostate cancer
	Proteinase3	Myelogenous leukemia
Cancer-Testis antigens	MAGE-A3/NY-ESO-1	Various cancers
Oncogenic proteins	WT1/Survivin/hTERT	Various cancers
Oncofetal antigens	CEA/AFP	Colon cancer, hepatoma
Viral proteins	EBV-EBNA	B cell lymphoma
	HPV16-E7	Cervical cancer
Mucin	MUC-1	Breast, ovarian, pancreas cancers
Idiotype	antibody	B cell lymphoma, myeloma
Allogeneic antigen (mHa)	HA1	Various leukemia/lymphoma
Cancer stem cell antigens	SOX2/SOX6	Breast cancer, myeloma, glioma

Table 19.2 Mechanisms for generating T cell epitopes on human cancer cells

Mechanism	Tumor antigens
1. Translation of functional genes	
a. Tissue-specific proteins	Melanosomal proteins (e.g., gp100)
Cryptic epitopes	MART-1, gp100, neo-PAP, CDC27
b. Cancer-testis antigens	MAGEs, NY-ESO-1, CRT2, SOX6
c. Overexpressed antigens	Her2, WT1, Survivin, hTERT
2. Genetic alterations	
a. Pin-point mutation	BRAF, p53, K-ras, MUM-1/2
Acquired MHC binding	β-Catenin, CDK4, MART-2, MUM-3
Extended peptide	Caspase-8
b. Frameshift mutations	$p14^{ARF}$, $p16^{INKa}$, TGFbRII
c. Translocation	bcr-abl, SYT-SSX
3. Translation of alternative ORFs	TRP-1, NY-ESO-1
4. Translation of introns	
a. Incomplete splicing	gp100, TRP-2, MUM-1
b. Cryptic promoter	GnT-V
5. Post-translational modification	
a. Deamidation	Tyrosinase
b. Cysteine oxidation	Tyrosinase
c. Protein splicing	FGF5, gp100
6. Differential processing by proteasomes	MART-1, gp100, MAGE-A1/A3

the causes of low immunogenic responses to tumor antigens, including low HLA binding and low cleavage activity in professional antigen presenting cells (APCs).

In addition, we and others revealed many surprising mechanisms for T cell recognition of human cancer cells (Table 19.2). T cell epitope peptides may be derived from introns (e.g., due to incomplete mRNA splicing or cryptic promoter activation), alternative open reading frames (ORFs), or peptides with post-translational

modifications such as protein splicing, deamidation, and cysteine oxidation. Differential cleavage of T cell epiotopes, by constitutive proteasomes expressed in cancer cells and by immunoproteasomes expressed in professional APCs such as dendritic cells, leads to the differential expression of T cell epitopes between cancer cells and professional APCs. This observation has important implications for clinical applications of tumor antigens. For instance, minimal peptide should be utilized for the immunization of some antigens, instead of whole antigens which require processing within APCs.

4 Implications of the Identified Human Tumor Antigens for the Development of Immunotherapies

The identification of tumor antigens allows for immunizations that can be appropriately controlled for the maximal induction of anti-tumor immune responses by adjusting the amount of administered antigen, immunization methods, and the schedule of administration. In addition, we can modify antigens to increase immunogenicity of the intrinsically low immunogenic tumor antigens (Parkhurst et al. 1996; Rosenberg et al. 1998). We and others have discovered that the induction of immune responses to multiple endogenous tumor antigens may be important for in vivo tumor rejection. Methods which can efficiently induce antigen spreading to endogenous tumer antigen; such as the use of highly immunogenic antigens in combination with appropriate *in situ* tumor destruction to release endogenous tumor antigens in an immunoge manner (Toda et al. 2002; Udagawa et al. 2006), should be developed. One recent technological advancement involves the adoptive transfer of peripheral blood T cells that were retrovirally transduced with T cell receptor (TCR) genes specific for identified tumor antigens including MART-1, gp100, and NY-ESO-1 melanoma antigens (Morgan et al. 2006; Johnson et al. 2009). The adoptive immunotherapy with TCR-transduced T cells has resulted in melanoma regression, suggesting that similar immunotherapy may be developed for patients with other common cancers such as lung cancer, for which the generation of large amounts of anti-tumor T cells is difficult.

In addition to the potential use of tumor antigens as targets for immunotherapy, it is worth emphasizing that the identification of tumor antigens allowed us to assess immune responses in patients both quantitatively and qualitatively during immunotherapy using various methods including ELISPOT, HLA tetramer analysis, and DTH skin tests (Romero et al. 1998). In particular, HLA tetramer analysis allowed for quantitative and qualitative evaluation of the in vivo immune status of tumor-specific T cells by assessing their phenotype (naïve/memory/effector), expression of adhesion/co-stimulatory molecules, production of cytokines and cytotoxic molecules, and anergy status (Lee et al. 1999). Qualitative analysis of T cells in vivo helped us gain a better understanding of anti-tumor immune responses in patients and of tumor escape mechanisms such as tolerance induction, thus leading to improvements in immunotherapy. The identification of tumor antigens or their

immune responses (e.g., tumor antigen-specific IgG in serum) may also be useful as diagnostic biomarkers. They may also be targets for molecular targeting therapies.

Criteria for ideal tumor antigens to develop effective immunotherapy may include (1) high expression in all tumor cells including cancer stem cells (CSC) (no relapse cure), (2) limited expression in normal cells as antigens presented on cell surface by MHCs (no autoimmunity problems), (3) common expression in many patients' cancers (treatment applicable for many patients), (4) high immunogenicity in cancer patients (efficient induction of anti-tumor immune responses), and (5) involvement in tumor cell proliferation and survival (less likely occurrence of antigen loss variants). Although antigens completely satisfying all of these criteria may not be available, the characteristics of representative human tumor antigens have been described using these criteria (Table 19.3).

4.1 Tumor-Specific Antigens Derived from Genetic Alterations in Tumor Cells

Autologous tumor-specific peptides derived from genetic alterations in cancer cells, which are involved in cancer development, are often isolated using T cells and IgG Ab from patients with good prognosis after treatment. For example, we isolated mutated peptides of β-catenin (Robbins et al. 1996) and Ski acyltransferase (MART-2) (Kawakami et al. 2001), using melanoma-reactive T cells derived from tumor infiltrating lymphocytes (TIL). The administration of these peptides along with high doses of IL-2 resulted in tumor regression. An amino acid change from serine to phenylalanine as a result of the β-catenin mutation disrupted a phosphorylation site, resulting in increased β-catenin levels through the prevention of subsequent degradation by proteasomes. Elevated β-catenin levels may be involved in the formation of malignant melanoma phenotypes (Rubinfeld et al. 1997). Mutations in MART-2, which adds palmitate to the N-terminus of the Hedgehog protein, appear to have caused the enzyme to lose GTP binding activity, although MART-2 association with tumorigenesis was not clear (Kawakami et al. 2001; Chamoun et al. 2001). These mutations led to the generation of tumor-specific peptides capable of binding to the patients' HLA. Then, these peptides induced anti-tumor T cell responses. This sequence of events appears to be one of the common mechanisms for the recognition of mutated peptides by autologous T cells, as the same mechanism was found in other tumor antigens such as CDK4 and MUM3 (Kawakami et al. 2005).

We have also isolated a tumor-specific frameshift CDX2 peptide as a colon cancer antigen from a HNPCC patient who had microsatellite instability (MSI) and colon cancer with abundant $CD8^+$ T cell infiltration, and a good prognosis after surgical resection (Ishikawa et al. 2003). This type of colon cancer has unique clinicopathological features, including T cell infiltration, particularly by $CD8^+$ T cells, and relatively good prognosis after treatment despite pathologically malignant undifferentiated type. These observations may indicate that T cell responses to

Table 19.3 Clinical implications of the representative tumor antigens recognized by T cells

Antigens	Expression in		Immunogenicity	Occurrence of antigen loss variants	Autoimmune reaction
	Normal tissues	Cancers			
Tumor-specific unique antigens	None	Relatively homogenous	Intermediate	Low when involved in tumor cell proliferation/survival	None
Tissue-specific antigens	Expressed at low densities on cell surfaces		Intermediate	Relatively high	Relatively high
Cancer-testis antigens	Limited to testis and placenta	Relatively heterogeneous	Intermediate	Low when involved in tumor cell proliferation/survival	Low
Allogeneic antigens	Expressed at high densities on cell surfaces		High	Depending on antigens	GVHD

These characteristics are different among the antigens even in the same category groups

frameshift peptides may contribute to the maintenance of a tumor-free status after treatment. Tumor-specific mutated antigens have also been reported in other cancers, including mutated Caspase-8 with reduced apoptotic activity in head and neck cancer, and bcr-abl oncogenic fusion peptides via translocation in leukemia.

One problem in immunotherapy is the appearance of tumor antigen loss variants sometimes observed in immunotherapy against tissue-specific antigens such as MART-1 and gp100, which are not required for cancer cell survival and proliferation. We have previously shown, using lentiviral siRNA, that mutations in BRAF (V600E) frequently detected in superficial spreading melanoma (SSM) are responsible for the augmented proliferation and invasion of melanoma cells. We could also detect serum IgG for BRAF in some melanoma patients (Sumimoto et al. 2004). Recognition of the mutated peptides by $CD8^+$ CTL and $CD4^+$ helper T cells has been reported (Sharkey et al. 2004; Somasundaram et al. 2006). These tumor-specific antigens involved in the proliferation or survival of cancer cells are attractive targets for immunotherapy, because cancer cells are less likely to lose these antigens. In addition, possible early occurrence of BRAF mutation in melanoma development (presence of mutated BRAF in benign nevi) may also suggest that mutated BRAF are present in melanoma stem cells. Since it is difficult to identify these common mutated antigens, most of which are quite unique to each patient, it may be necessary to develop immunization methods that induce T cells to such endogenous mutated antigens without the need for antigen identification. For example, we have previously reported a technique involving the administration of intratumoral dendritic cells following cryoablative tumor treatment (Udagawa et al. 2006).

4.2 Tissue-Specific Antigens

T cells specific for self peptide antigens derived from tissue-specific antigens, including MART-1, gp100, tyrosinase, TRP1, and TRP2, are frequently detected in melanoma patients (Kawakami et al. 1994a, 1995). Various observations, including the antigens' low HLA binding and the presence of high avidity naïve T cells in healthy individuals, suggest that these peptides are relatively cryptic epitopes that are not presented at high densities on the cell surfaces of melanocytes and professional APCs in healthy individuals (Kawakami and Rosenberg 1996). However, these T cells may be easily induced in patients with memory T cells primed with increased antigens from melanoma cells by immune augmentation methods, e.g., IL-2 administration. One of the mechanisms for the cryptic nature of some epitopes and their low immunogenicity is relatively low HLA binding ability due to misplaced amino acids at critical anchor regions for HLA-peptide binding (typically the second and last portions of the peptides) (Kawakami et al. 1995, 1994b). We have succeeded in generating highly immunogenic peptides by substituting the appropriate amino acids (Parkhurst et al. 1996), and the generated, modified peptides induced anti-tumor immune responses more effectively than the native peptides in vivo (Rosenberg et al. 1998). In other cancers, similar tissue-specific antigens have been identified,

including PSA in prostate cancer and Proteinase 3 in myelogenous leukemia. Although these shared antigens are useful for the treatment of many patients, relatively low immunogenicity, possible autoimmune reactions, and the occurrence of antigen loss variants may be problematic for effective immunotherapy.

4.3 *Cancer-Testis Antigens*

Self peptides derived from cancer-testis (CT) antigens are tumor-specific shared tumor antigens and attractive targets for immunotherapy, because their expression is observed in various cancers and limited to a few cell types in normal tissue, e.g., immunoprivileged testis cells with low HLA expression. Recently, MAGEA3 (Atanackovic et al. 2008) and NY-ESO-1 (Hunder et al. 2008) were extensively studied and some positive results were obtained in a number of clinical trials. Using various methods, including SEREX with testis cDNA library screening, genechip, and RDA analysis comparing testis cDNA profiles, we have identified various CT antigens such as CAGE (Iwata et al. 2005), KU-TES1 (Okada et al. 2006), BORIS, CRT2 (Hayashi et al. 2007), and SOX6 (Ueda et al. 2004), which are recognized by IgG and/or $CD8^+$ CTLs.

SOX6 is an attractive antigen because it may be expressed in glioma stem cells (Ueda et al. 2009). CSC have recently been actively investigated as targets for treatment because of their high tumor initiating ability with resistance to chemotherapy and radiotherapy, thus leading to cancer relapse. SOX6 expression is developmentally regulated and its high expression in the adult was restricted to the testis and glioma tissues (Ueda et al. 2004). SOX6 was expressed in glioma stem cell-like cell lines obtained by the sphere forming method and $CD133^+$ phenotyping, and the giloma stem cell-like cells were lysed by SOX6-specific CTLs (Ueda et al. 2009). Thus, SOX6 may be a useful antigen for the development of immunotherapy for the treatment of glioma.

4.4 *Allogeneic Antigens*

In some circumstances, including allogeneic stem cell transplantations and donor leukocyte infusions (DLI), allogeneic antigens such as minor histocompatibility antigens (mHa) and mismatched MHCs can be tumor antigens for immunotherapy. Donor-derived T cells specific for recipient allogeneic antigens administered to recipient patients could eliminate residual leukemia cells. Compared to the autologous tumor antigens discussed above, the allogeneic antigens are highly immunogenic and are expressed at high densities on cell surfaces, indicating their strong anti-tumor activity. However, to avoid life-threatening graft-versus-host disease (GVHD) caused by the allogeneic immune responses to the recipient's normal tissues, additional strategies are required. We have demonstrated the presence of

cytotoxic CD4+ T cells specific for mismatch HLA-DQ and mHa, which are preferentially expressed in hematopoietic cells including leukemia cells (Matsushita et al. 2006). These CD4+ T cells may induce strong graft vs. leukemia (GVL) effects on the residual MHC class II positive leukemia cells without developing severe GVHD.

5 Concluding Remarks

The identification of human tumor antigens by T cells allowed us to not only develop novel cancer immunotherapies, but also evaluate anti-tumor T cell responses in patients and clarify the problems underlying each step that leads to in vivo immunological tumor rejection. Although simple tumor antigen immunizations along with adjuvants and cytokines showed only limited anti-tumor activity, comprehensive immunotherapy combining various immune-augmenting interventions and methods to correct the immunosuppressive environment in cancer patients may result in the improvement of current immunotherapies.

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