of Hsp90 in cross-presentation is to navigate the associated Ag into static early endosomes within human Mo-DCs. Thus, Hsp90 appears to be a promising natural immunoactivator for use of cancer vaccine development due to its excellent ability to target human DCs and to induce specific CTLs.

Disclosure Statement

The authors have no conflict of interest.

Abbreviations

Ag	antigen
DC	dendritic cell
GM-CSF	granulocyte/macrophage colony-stimulating factor
HSP	heat shock protein
Hsp90	heat shock protein 90
IFN	interferon
IL	interleukin
LDL	low-density lipoprotein
Mo-DC	monocyte-derived dendritic cells
OVA	ovalbumin
pAb	polyclonal antibody
PE	phycoerythrin
PHA	phytohemagglutinin
TAP	transporter associated with antigen processing

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第83回　Metabolomicsの最新情報④

肥満の分子メカニズム—オーバービュー—

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はじめに

『肥満』とは，「肥満組織が過剰に蓄積した状態で，BMI 25 kg/m^2 以上のもの」と定義されている。さらに，肥満と判定（BMI ≧ 25）され，

①肥満に起因あるいは関連し，減量を要する有健康障害者

②健康障害をともないやすいハイリスク肥満者

のいずれかの条件を満たすものを『肥満症』と診断する[1)2)]。現在，日本では男性で約3人に1人，女性で約5人に1人がBMI 25を超えているといわれている[3)]。ここでは肥満と密接に関係のある，摂食調節の分子メカニズムと肥満の脂肪組織におけるグルココルチコイド作用の過剰状態を中心に概説する。

脂肪細胞ホルモン，レプチン

摂食調節の分子メカニズムとして，1994年Friedmanらがレプチンを発見し[4)]，これを機に急速に解明が進んでいる。レプチン遺伝子は，21アミノ酸のシグナルペプチドを含む167アミノ酸のレプチン前駆体をコードしており，脂肪細胞で産生されたレプチン前駆体は血中に分泌され，血中においてはシグナルペプチドが切断された146アミノ酸からなる蛋白質（レプチン）として存在している[4)]。遺伝性肥満のモデルマウスであるob/obマウスでは，レプチン遺伝子の点突然変異により105番目のアミノ酸であるアルギニン残基が終止コドンに置換され，レプチンが産生されないために過食による肥満に至る[4)]。レプチン受容体は5種類のアイソフォームが確認されているが，Ob-Rbのみがレプチンのシグナルを細胞内へ伝達する（図1）[5)6)]。レプチンは脂肪細胞の肥大化にともなって分泌量の増加を認め，血中のレプチン濃度は体脂肪量を鋭敏に反映していることが明らかとなっている。視床下部がレプチンの主たる作用部位で，食欲の制御，交感神経活動の亢進を介した熱産生，褐色脂肪組織や骨格筋における糖利用の促進，脂肪酸燃焼の促進効果を生じる[7)]。しかし肥満状態ではレプチンの作用不全が生じ，血中レプチン濃度の

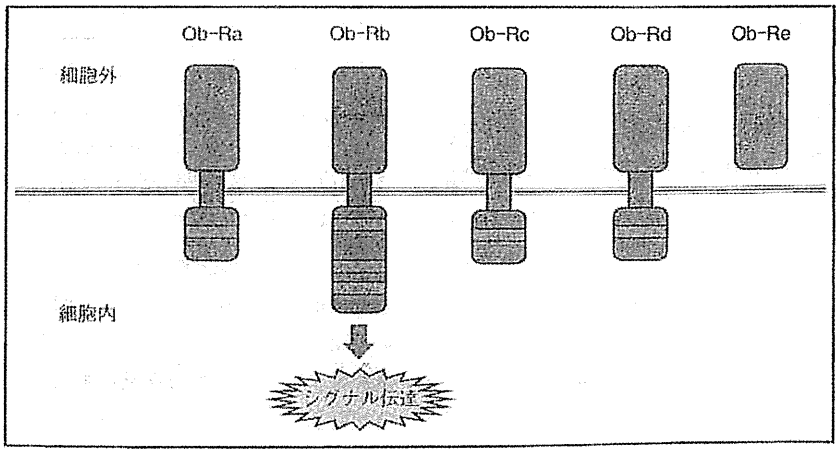

図1　レプチン受容体のアイソフォーム
（文献6より一部改変引用）

Surgery Frontier 21(4)：53-56，2014

上昇にもかかわらず，濃度に見合ったレプチンの効果が発揮されず，レプチン抵抗性を生じている[8]。肥満状態では，可溶型レプチン受容体（Ob-Re）やCRPの血中濃度が上昇し，それらが直接レプチンと結合することによってレプチンのOb-Rbへの結合が阻害される[9][10]。一方で，体重増加にともなって脳脊髄液中ではレプチン濃度が低下し，レプチンの血液脳関門通過障害がレプチン抵抗性を生じる一因とも考えられている[11]。さらにレプチン自体がOb-Rbの発現を抑制することも報告されており，長時間高レプチン濃度状態に曝露されるとOb-Rb発現量が低下すると考えられている[12][13]。また，レプチンによって本来活性化されるSTAT3（signal transducers and activators of transcription 3），PI3K（phosphatidylinositol3）などのシグナル伝達系を傷害する，SOCS（suppressor of cytokine signaling)3，PTP（protein tyrosine phosphatase)1Bの発現の増加も認められ，原因の一端を担っていると考えられる（図2）[14][15]。

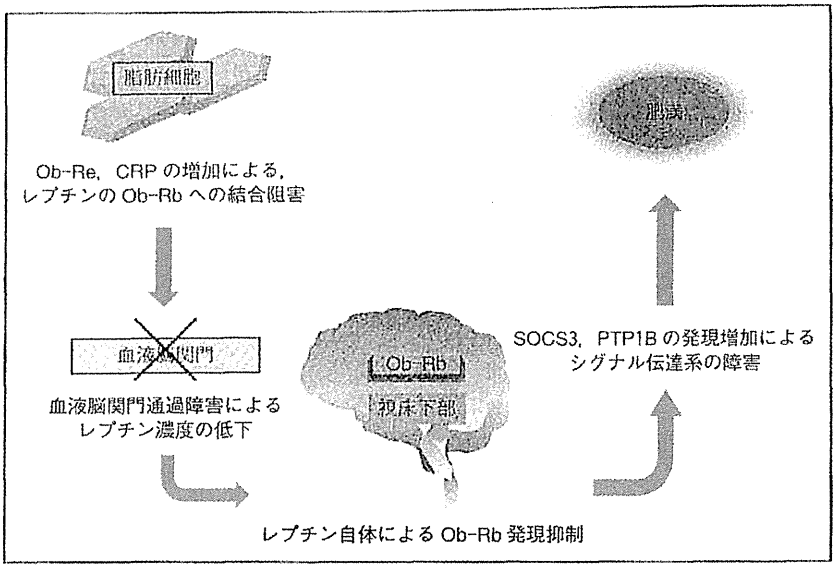

図2　レプチン抵抗性の原因

（文献14，15より引用）

肥満の脂肪組織におけるグルココルチコイド作用の過剰状態

細胞内でグルココルチコイドを活性化する変換酵素である11β-HSD（hydroxysteroid dehydrogenase)1の活性は，肥満脂肪組織内で上昇し，インスリン抵抗性指標を含む種々の代謝パラメーターと強い相関を示すことが報告されている[16]。11β-HSD1は過栄養やストレスによって誘導され，ストレス依存性肥満や肥満脂肪組織の炎症・酸化ストレスの病態に関与している[16]。血中のコルチゾール濃度は視床下部-下垂体-副腎という経路によって制御されているが，個々の細胞におけるコルチゾールの作用強度は細胞内グルココルチコイド活性化酵素，11β-HSD1と不活性化酵素，11β-HSD2のバランスによってコントロールされている[17]。脂肪細胞では11β-HSD2の発現は非常に低く，11β-HSD1が活性化されるとグルココルチコイド作用は抑制されることなく増強を続ける（図3）[18]。元来，内臓脂肪組織の脂肪細胞サイズは皮下脂肪組織に比べ小さく，脂肪組織容積あたりの細胞数も少ない。栄養過多状態では内臓脂肪組織の脂肪細胞が肥大しやすく，機能異常を生じやすい[19]。また，11β-HSD1の発現レベルは皮下脂肪組織よりも内臓脂肪組織で高く，肥満状態ではその差が顕著になる[20]。脂肪細胞で11β-HSD1を過剰発現するトランスジェニックマウスは内臓脂肪蓄積の感受性が高く，インスリン抵抗性，脂質代謝異常，高血圧，脂肪肝をともなう[16]。一方，11β-HSD1ノックアウトマウスではストレスや高脂肪食に対する肝糖新生関連酵素PEPCK（phosphoenolpyruvate carboxykinase）やG6Pase（glucose-6-phosphatase）の誘導を生じないため，糖尿病発症に対して明らかな抵抗性を示し，高脂肪食負荷やob/obマウスとの交配においても内臓脂肪の蓄積は抑制される[21]。また，脂肪組織特異的11β-HSD2トランスジェニックマウス（脂肪組織特異的11β-HSD1ノックア

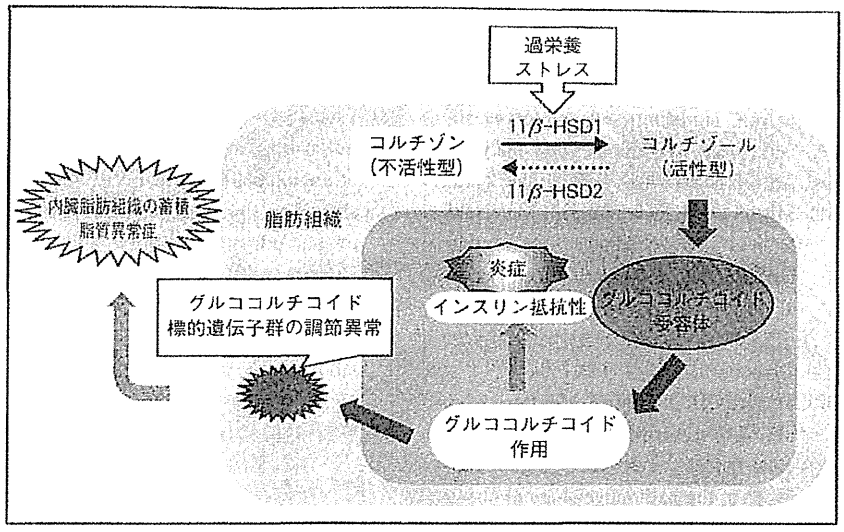

図3　11β-HSD1の活性化によるグルココルチコイド作用の増強

(文献18より引用)

ウトマウス)においても高脂肪食負荷による糖脂質代謝の悪化を認めないことから，脂肪組織で11β-HSD1を抑制することが肥満症治療に有効であることが示唆された[22]。多数例のヒト脂肪組織の解析において，肥満者では脂肪組織での11β-HSD1発現レベルの上昇を認め，ウエスト周囲長，脂質代謝指標やインスリン抵抗性指標と正の相関を示すと報告されている[23)24)]。

おわりに

現在，さまざまな肥満に関する研究が進んでいる。今後肥満における分子メカニズムの全容が解明され，増加の一途をたどっている肥満患者への有効な治療法の開発へとつながることを期待する。

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特集
生体防御における免疫反応の新知見

特集によせて

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高等な生物の免疫機構は，自然免疫（innate immunity）と獲得免疫（acquired immunity）で成立している。脊椎動物においては，これら免疫能を相互に連携作用することにより，有効な生体防御機構が構築されている（図1）。

自然免疫はいわゆる抗原提示機能を有する細胞である樹状細胞（dendritic cell：DC），マクロファージが代表的な役割をになっている。これらの細胞は生来から備わった（子孫へと受け継がれてきた）分子を認識する受容体（pattern-recognition receptors：PRRs）を有し，受容体が異物を識別し（パターン認識機能）それを除去することを可能としている。たとえば，病原体を認識した細胞内ではシグナル伝達によって転写因子が活性化され，サイトカインや炎症反応分子を産生し，その後の一連の免疫機構へとつながる。

一方，獲得免疫は生物発生学的に魚類以上の高等動物が有する各個別の免疫能で，子孫へと受け継がれることのない能力である。リンパ球が多様な抗原を認識する能力を備えていることを背景として，その個体の抗原との人為的あるいは非人為的な接触によって識別するもので，外来（？）のペプチド配列を認識するなど高度で緻密な機能を発揮し生体防御にかかわっている。すなわち，分子を認識するにあたってリンパ球はその受容体遺伝子を再編成させることにより，抗原認識のもと，選択的識別を行うという個別化された生体防御能力である。表1に自然免疫と獲得免疫の違いを概括的に紹介した。

自然免疫とパターン認識機構

リガンドともいうべき微生物の有す

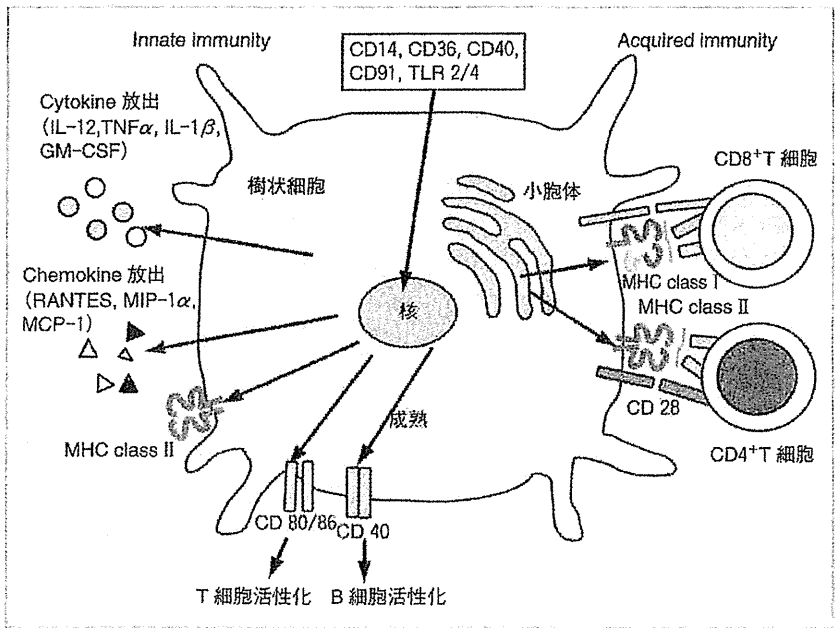

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表1　自然免疫と獲得免疫の比較

比較項目	自然免疫 (innate immunity)	獲得免疫 (adaptive immunity)
担当細胞	抗原提示細胞 (樹状細胞，マクロファージなど)	リンパ球 (T細胞，B細胞)
生物種	昆虫以上の高等進化生物	脊椎動物
反応	早い(数分ないし数時間)	遅い(数日)
受容体形成		
発生時期	生来より	生後の外的侵入後
遺伝子組み換え	不可	可(それ自体が獲得機序)
レパートリー	限定	多様
認識対象	外敵が有する共通な分子 (脂質，核酸，鉱物など)	病原体，腫瘍細胞などの構成成分 (蛋白，ペプチドなど)
個体における記憶	なし	あり

るパターン分子と受容体の解析に関する研究展開はめざましい。

以下に，パターン認識機構の概要と新知見の動向を若干なりとも臨床的視点から紹介し，後述される別稿の論文のための基本的知識として参考にされたい。

1　パターン認識機構のリガンドとパターン認識レセプター

子孫へと遺伝されてきた感染・異物に素早く反応を示すパターン認識機構は，自然免疫の根幹をなす。微生物には特有なパターン分子が存在する。抗原提示細胞のPRRsがそのパターン分子を認識し，外来の微生物に対応する機構の初動を担う。外来のパターン分子をpathogen-associated molecular patterns (PAMPs)と呼ぶ。具体的には，ウイルス由来のRNAパターンやdouble strand (ds) RNAを認識する受容体，細菌由来のmucopeptidesを認識する受容体，ペプチドグリカンのペプチド部分を認識する受容体，あるいは鉱物(尿酸血症，アスベスト，シリカ，アルミニウムなど)を認識する受容体などが次々に明らかにされている。このほか，新しい反応誘発因子として，たとえば微生物パターン分子を認識する代表的受容体として知られるToll-like receptors (TLRs)は，哺乳類ではTLRの分類として1～10が存在するのに対し，マウスではさらにTLR11, 12, 13を有することが知られている(表2)。また，細胞内で菌体成分を認識するnon-TLRsとして，Nodファミリー群，retinoic-acid-inducible gene 1 (RIG-1)，melanoma differentiation-associated gene 5 (MDA5)の存在が知られている。なお，これらについては厳密には受容体の種類が動物種間でその存在数(種類)が異なることも知られるに至っている[1]。一方，病原体により傷害を受けた自己細胞のPRRsの活性分子が遊離すると，それ自体がリガンドとなりうることも知られ，内因性パターン分子(damaged-associated molecular patterns；DAMPs)として注目されてきた。重症感染症や感染合併時の過大侵襲などでは，血中に高頻度で発現してくることが知られ，HMGB1, heat-shock protein, LDLコレステロール，βアミロイドなどが代表的なDAMPsとして知られている[2]。

2　近年注目されてきたパターン認識機構の知見

上記に示したリガンド種別のパターン認識機構の詳細が明白となっていることが注目すべき知見とされている。たとえば，ヒトTLRにおけるリガンドとそのシグナル伝達の相同性が挙げられる。その伝達経路としてMyD88経路やTICAM-1経路が明らかにされるに至っている。その詳細な説明は避けるが，概略を以下に述べたい。TLRはダイマーを形成してリガンド認識とシグナル伝達に預かる。TLR2はTLR1とTLR6がヘテロ複合体を，TLR4はTLR2とMD2が結合して安定化した受容体を形成する。図2にMyD88を“アダプター”とする各種TLRの連関性を示した。最終像としてのTNF, interferon (IFN)-α or βの産生に至るものである。TICAM-1経路はTLR3, TLR4がTICAM-1を“ア

表2 パターン認識機構：受容体とリガンド

受容体	ヒトでの存在	リガンド
TLR1	○	triacyl BLP
TLR2	○	PGN, BLP
TLR3	○	ds RNA
TLR4	○	LPS, Taxol
TLR5	○	flagellin
TLR6	○	diacyl BLP
TLR7	○	ss RNA
TLR8	○	ss RNA
TLR9	○	CpG DNA
TLR10	×（哺乳類）	?
TLR11	×（マウス）	profirin（原虫）
TLR12	×（マウス）	?
TLR13	×（マウス）	?
RIG-1	○	ss RNA, ds RNA
MDA5	○	ds RNA
NOD1	○	G（+）muropeptides
NOD2	○	G（−）muropeptides
NALP3	○	crystals, minerals

BLP：bacterial lipoprotein, PGN：peptideglycan, G：gram

ダプター”とするシグナル伝達で，IRF-3とIFN-βを誘導することが特徴とされている。また，ウイルスRNAを認識する経路として，IRS-1を“アダプター”としてIRF-3活性化を生じる。すなわち，TICAM-1経路に共通する。また，RIP-1を介してNF-$\kappa\beta$活性化経路へ連なる。また，IL-1，IL-18受容体はMyD88経路を活性化し，NF-$\kappa\beta$活性化経路へと連なる。このように紹介した上記経路群については，リガンド種別に研究されてきた歴史はあるが，研究が進むとともに経路間相互に連関性のあることが明らかとなっている。これらのパターン認識機構をどう臨床上へ応用することが可能か，興味深い示唆を得ているがいまだに明確な確証を提案した報告はみられない。

自然免疫から獲得免疫へ

自然免疫から獲得免疫への橋渡しに重要な役割を果たす細胞として，type I IFNを産生するplasmacytoid dendritic cell（pDC）が知られる[3]。このtype I IFNは，CD8Tリンパ球に作用し，キラーT細胞の分化あるいはメモリーT細胞の分化を促すほか，MHC class IあるいはIIの発現上昇など，獲得免疫系への強い影響を有する。このほか，DCの分化の結果，Th1，Th2の分化を促しTh1優位とするなど生体バランスに大きな変化をもたらす（図3）。Th1細胞は，IL-2，IFN-α，TNF-αなどを産生するTh細胞で，細胞障害性T細胞（cytotoxic T cell）やマクロファージを活性化する。その結果，「ウイルス感染細胞」や「細胞内寄生性病原体」の除去や抗腫瘍免疫反応に関与する。Th2細胞は，IL-4，5，6，9，10，13などを産生しB細胞を活性化し，主として細胞外で増殖する微生物の排除に有用となる。このほか，Th17細胞は，IL-17，21，22などを産生し細菌感染，腫瘍免疫に関与している。以上のTh1，Th2，Th17細胞は免疫応答反応として陽に作用し，エフェクターT細胞と総称している。

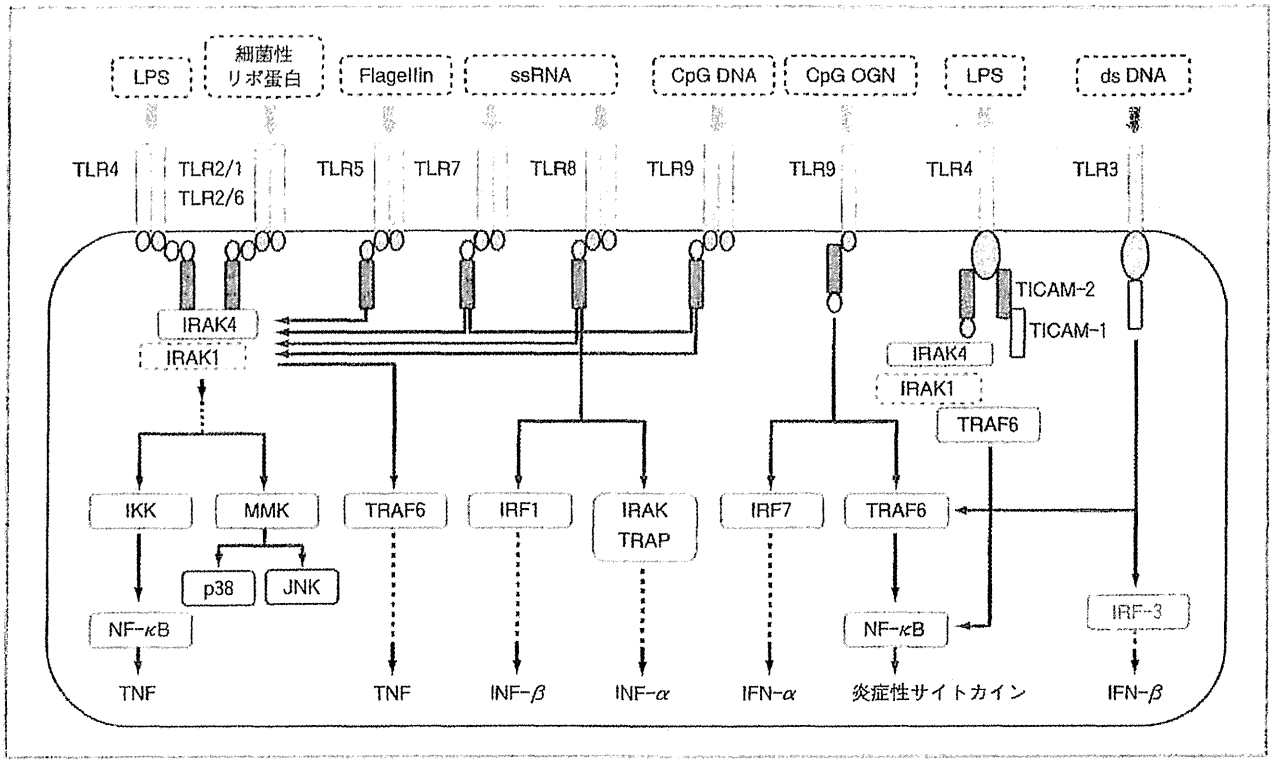

図2　パターン認識機構の主なリガンドと受容体によるシグナル伝達機構

（文献1より改変引用）

一方，負の免疫応答を担うのが抑制性T細胞（regulatory T cell ；Treg）が知られている[4]。これらの活性化プロセスにおいてはIFNやサイトカインなどの産生，あるいはMHCクラスⅡ分子（MHC-Ⅱ）を認識するHLA-DRを介してCD4⁺T細胞に抗原を提示し，T細胞の活性化を誘導する。したがって単球・マクロファージのTLRやHLA-DAの発現については，自然免疫から獲得免疫の橋渡しの役割を担っているといえる。

獲得免疫とその機構

獲得免疫は，腫瘍免疫あるいはウイルスに対するワクチン療法に関する研究により急速な分析が成されている[5]。

1　獲得免疫機構の序論

上記のように自然免疫系の応答は獲得免疫系へと情報が伝搬され，細胞傷害性T細胞，エフェクターT細胞あるいはTregなど各機能別のT細胞に活性化を生じる。T細胞は，キラー（$CD8^+$）T細胞をヘルパー（$CD4^+$T）T細胞の2つのサブセットに大別される。

$CD8^+$T細胞はT細胞受容体（TCR）を介して，MHCクラスⅠ分子（MHC-I）に結合した抗原ペプチドの複合体を認識し，活性化されて細胞傷害性を発揮する（図4）。この際にMHC-Iに結合する複合ペプチドの由来は，ある種の細胞（たとえば，癌細胞，感染細胞など）内の蛋白質がプロテアソームによる分解を受けて生じる。これが細胞表面上のMHC-I上に移送されて，それを$CD8^+$T細胞が特異的に認識し攻撃の標的とするものである。

$CD4^+$T細胞は，抗原ペプチドとMHCクラスⅡ分子（MHC-Ⅱ）の複合

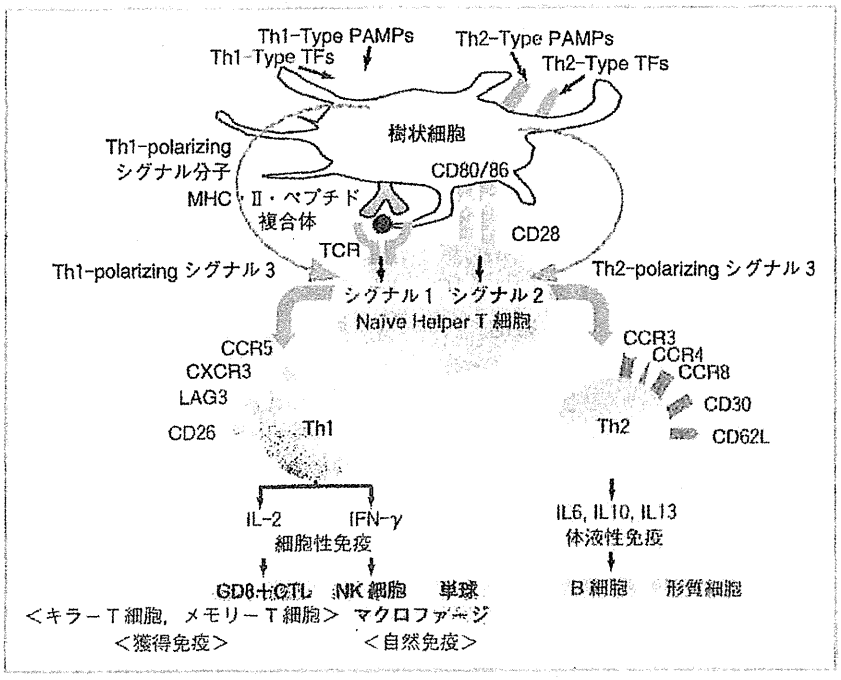

図3 樹状細胞反応とTh1・Th2系細胞反応

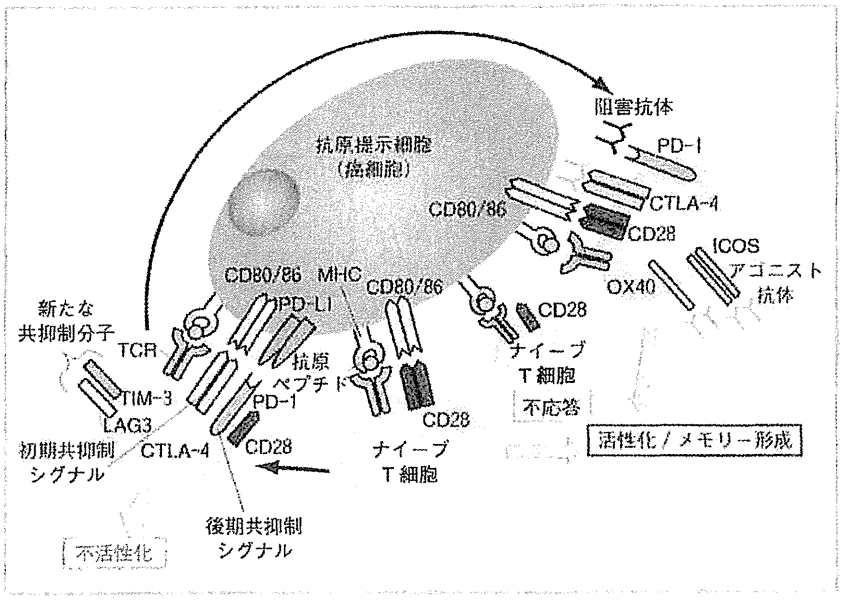

図4 T細胞の活性化と主な細胞間分子と共刺激シグナル

体を認識し活性化する。MHC-Ⅱの発現は樹状細胞，マクロファージ，B細胞などの抗原提示細胞に限定される[6]。今日注目されている腫瘍免疫機構において，腫瘍細胞を特異的に認識して細胞障害性作用を示すのはCD8$^+$T細胞である。その理由としては，ほとんどの腫瘍細胞がMHC-Ⅱを発現しないことに起因している。なお，重要な機序としてクロスプレゼンテーション機構の存在がある。それは樹状細胞にみられる現象で，腫瘍細胞由来のペプチドを取り込み，MHC-Iを介してCD8$^+$T細胞の活性化が生じることが知られている[7]。また，免疫監視機構における初期防御や転換抑制に重要な役割を担う細胞として，natural killer（NK）細胞およびNKT細胞が知られている。前感作なしに癌細胞を傷害しうるリンパ球として知られるNKT細胞がNK細胞の表面マーカーを恒常的に発現していることが，上記のような機能を発揮する機序として明らかになっている。

2 獲得免疫における需要分子

1) MHC-IとTCR

CD8$^+$T細胞のTCRとの結合には，MHC-Iとペプチドのみで可能なことから，co-stimulatory moleculeの存在を問われていない。ただし，樹状細胞にあっては，CD80/86とCD8$^+$細胞のCD28の結合がMHC-Iでも重要となることが明らかになっている（図4）。

2) MHC-ⅡとTCR

MHC-Ⅱと抗原分子をCD4$^+$細胞が認識するにあたり，DCの「CD40」

が CD4$^+$ 細胞の CD40L と，「CD80$^-$/86」が CD4$^+$ 細胞の CD28 と結合することが必須条件となる（図 4）。これは，副刺激分子として重要な存在で相互の結合なくして補助シグナルが細胞内へ伝搬はありえないことから必須の分子となっている。

3）抗原提示細胞，腫瘍細胞あるいは感染細胞とナイーブＴ細胞

ナイーブＴ細胞の活性化には，MHC-Ⅱと CD80/86 に対しての TCR と CD28 分子の関与となるのは原則である（図 4）。一方，ナイーブＴ細胞の不活化には，共抑制分子の存在が知られ，初期共抑制シグナルとして CD80/86 に対応する CTLA-4（cytotoxic T-lymphocyte antigen 4）が後期共抑制シグナルとして PD-L1 と PD-1，そしてナイーブＴ細胞の結合部位以外の細胞膜に TIM-3，LAG3 の発現が左右することも知られるに至っている。また，同様の部位に OX40，ICOS 分子が発現し，それらに対する抗体の使用により CD28 の副刺激のもと活性化およびメモリー形成が成立されることも知られている。

4）Treg 関連分子

Treg は「アポトーシスの誘導」，「IL-2 の消費」抗原提示細胞に対する「抑制性サイトカインの産生」，「アデノシン代謝を介しての IL-2，IFN-γ 産生の抑制」，「APC 成熟化の抑制」などにより免疫反応を抑制する。アポートシス誘導には Treg の FAS-ligand（FasL）の CD39/73，APC 成熟化の抑制には Treg の LAG3 が MHC-Ⅱに，CTLA-4 が CD80/86 へ結合することが知られている（図 4）。

上記に示した以外にも多くの分子の存在，機能分担細胞の種別化に関する研究が進んでいるが，序論として以上の紹介にとどめたい。

おわりに

今回の特集においての免疫に関する知見の紹介としては，自然免疫研究に重点を置いての企画となっている。そのための序論として，免疫反応全体を生体防御として捉えたうえでの自然免疫の位置付けの詳細を学ぶにあたって，本稿ではそのための前知識としての内容を記述した。生体は，「optimal immunity-disease protection」であることが望ましく，「compromized immunity-disease-At risk」に外科医は悩まされている。日頃のエクササイズが，自然免疫能に好影響を与えるとの知見が得られたとの報告はあるが，ヒト臨床上のエビデンスとしては，不十分な状況にある。Ｔ細胞，Ｂ細胞にあっては，エクササイズ終了後 24 時間を経ると元の木阿弥と化すとのことも報告されている。今後どのような研究展開によって，ヒトへの応用が有効となるのであろうか。研究展開に期待したいところである。

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REVIEW ARTICLE

Trials of vaccines for pancreatic ductal adenocarcinoma: Is there any hope of an improved prognosis?

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Abstract Pancreatic tumors are chemoresistant and malignant, and there are very few therapeutic options for pancreatic cancer, as the disease is normally diagnosed at an advanced stage. Although attempts have been made to develop vaccine therapies for pancreatic cancer for a couple of decades, none of the resultant protocols or regimens have succeeded in improving the clinical outcomes of patients. We herein review vaccines tested within the past few years, including peptide, biological and multiple vaccines, and describe the three sets of criteria used to evaluate the therapeutic activity of vaccines in solid tumors.

Keywords Pancreatic cancer · Vaccine · Immunomodulation

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States [1–3] and the fifth most common cause of such deaths in Japan [4]. Although surgical resection is considered to be the only curative therapy for pancreatic cancer, only 20 % of patients have resectable disease at the time of diagnosis [5, 6]. In addition, advanced pancreatic cancer patients exhibit a median survival time (MST) of approximately six months and a 5-year overall survival rate of less than 5 %, despite efforts to manage the tumors with chemotherapy, radiotherapy and other treatments [3, 5–8].

In 1997, Burris et al. reported that gemcitabine monotherapy is superior to fluorouracil (5-FU) monotherapy for

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Table 1 Chemotherapy for advanced pancreatic cancer

	Median survival time (months)	Overall response rate (%)	Trial name	References
Gemcitabine	5.65	5.4		J Clin Oncol 1997;15: 2403–13.
Gemcitabine + erlotinib	6.24	8.6	NCIC CTG PA.3	J Clin Oncol 2007;25: 1960–6.
FOLFIRINOX	11.1	31.6	ACCORD 11	N Engl J Med 2011;364: 1817–25.
Nab-paclitaxel + gemcitabine	8.7	29.2	MPACT trial NCT00844649	N Engl J Med 2013;369: 1691–703.
Gemcitabine +TS-1	10.1	29.3	GEST trial	J Clin Oncol 2013; 31:640–8.

treating pancreatic ductal adenocarcinoma (PDAC) [9]. Gemcitabine monotherapy has subsequently become the standard chemotherapy for PDAC, resulting in an MST of 5.65 months (Table 1). Currently, three protocols have proven to be superior to gemcitabine monotherapy. Combining gemcitabine with erlotinib improved the MST of PDAC to 6.24 months in the NCIC CTG PA3 trial [10], while combining gemcitabine with nab-paclitaxel improved the MST to 8.7 months in the MPACT trial [11]. FOLFIRINOX achieved the longest MST for PDAC (11.1 months) in the ACCORD11 trial [12], and the GEST study obtained similar clinical outcomes. S-1 is an oral fluoropyrimidine derivative that has been shown to be effective against various cancers, and a previous study found that it is at least as effective as gemcitabine against PDAC [13]. In addition, treatment with a combination of gemcitabine + S-1 has been demonstrated to result in an MST of 10.1 months [14]. Although these chemotherapies extend the survival period among PDAC patients, they also result in serious adverse events. Therefore, the optimal chemotherapy regimen for PDAC depends on the patient's performance status.

There have been numerous attempts to develop vaccine therapies for cancer over the past century [2, 3]. Although clinical trials of such vaccines have obtained promising results in specific patients, none of the tested vaccines has exhibited significant improvements in efficacy compared with established therapies. In addition, several issues must be resolved before vaccine therapies can be used in the clinical setting. Tumor-associated antigens (TAA) have been demonstrated to recognize specific human leukocyte antigens (HLA) [15]. Theoretically, the tumor lysate contains all of the antigens expressed by the tumor, and cytotoxic T lymphocytes (CTL) are capable of recognizing some of these antigens [16]. All vaccines for pancreatic cancer are based on the fact that CTL recognize TAA expressed on tumor cells and subsequently attack these cells. The question is how strongly and specifically each TAA stimulates CTL in vivo in the clinical setting. Immune tolerance can develop via various mechanisms, including the downregulation of the major histocompatibility complex (MHC) molecule expression, induction of T cell anergy, reductions in the number of immune effectors and increases in the number of regulatory T cells [17, 18], which may explain why no cancer vaccine therapy has been established as a standard treatment for advanced PDAC. Therefore, in this study, we comprehensively reviewed the clinical outcomes of vaccine therapy against advanced PDAC.

Peptide-based vaccines developed within the past few years

MUC1

Mucin 1, cell surface associated, (MUC1) is a type I transmembrane protein containing multiple tandem repeats of a 20-amino acid sequence. Several MUC1 peptides have been tested as vaccines in the clinical setting; however, most of them have failed to activate CTL [19–21]. Ramanathan et al. [22]; Yamamoto et al. [23] injected pancreatic patients with a vaccine containing a 100-mer extracellular tandem repeat domain of MUC1 and Montanide ISA-51, and both studies obtained similar clinical responses; i.e., the authors detected cytokines (interferon (IFN)-γ or interleukin (IL)-4) and anti-MUC1 antibodies in the patients' sera but did not observe any significant clinical effects. Another recent study involving a vaccine based on a different MUC1 epitope showed similar clinical outcomes, i.e., all seven patients had progressive disease (PD), although some of the patients exhibited immunological responses, such as IFN-γ and granzyme B secretion [24].

K-RAS mutants

K-RAS mutations are frequently found in patients with PDAC. Vaccines targeting mutations in codon 12 of the K-RAS gene have been tested as treatments for advanced [25] or postoperative [26] PDAC in the clinical setting. Gjertsen et al. [[21]] investigated the utility of a K-RAS peptide vaccine containing granulocyte–macrophage colony-stimulating factor (GM-CSF) in 10 patients who had undergone potentially curative

resection (CTN RAS 95002) and 38 patients with advanced disease (CTN RAS 97004). In that study, one patient achieved a partial response (PR), which lasted for 28 months, and the MST of the immunological responders was 4.9 months, compared to 2.0 months for the non-responders.

Human telomerase reverse transcriptase (hTERT)

Human telomerase reverse transcriptase (hTERT) is frequently expressed in cancer cells [27]. hTERT maintains functional telomeres at the end of chromosomes, which protect against cell senescence [28]. A vaccine against pancreatic cancer containing the telomerase peptide GV1001: hTERT (611-626) and GM-CSF was examined by Bernhardt et al. [29], who found the MST of the immunological responders and non-responders to be 7.2 and 2.9 months, respectively.

Vascular endothelial growth factor receptor 2 (VEGFR2)

Vascular endothelial growth factor (VEGF) plays an important role in the progression of PDAC. The type 2 VEGF receptor (VEGFR2) is expressed in PDAC and associated with tumor neovascularization. Miyazawa et al. [[30]] investigated the efficacy of combined treatment consisting of PDAC with a VEGFR2-169 peptide-based vaccine and gemcitabine chemotherapy and reported that one patient achieved a PR, while the disease control rate was 67 %. In addition, the MST was 7.7 months, although 15/18 patients were chemotherapy naive.

G17DT (gastrimmune)

Gastrin is expressed in PDAC and plays a role in regulating the autocrine, paracrine and endocrine systems [31]. The administration of the anti-gastrin immunogen G17DT results in increased serum antibody levels and reduced tumor growth in patients with gastrointestinal malignancies [32]. A randomized, double-blind, placebo-controlled multicenter trial of G17DT was also recently performed [33]. Although, among the intention to treat (ITT) population, no significant differences in MST were detected between the PDAC patients treated with G17DT and those given the placebo, the MST of the two groups differed significantly after excluding major protocol violators and censoring for chemotherapy.

Heat shock protein (HSP)

Heat shock protein (HSP) itself is not an immunogen; however, it acts as a chaperone or carrier of antigenic peptides and possesses a repertoire of cellular peptides for pancreatic cancer [34]. Furthermore, HSPPC-96 (Oncophage) has been tested as a vaccine in the adjuvant setting after complete resection of PDAC [35]. In the latter study, the MST of PDAC was reported to be 2.9 months after surgery; however, this did not result in further clinical studies because only two of 10 patients exhibited increased enzyme-linked immunospot (ELISPOT) reactivity.

Biological vaccines

Fowlpox viral vaccine

Carcinoembryonic antigen (CEA) and MUC1 are highly expressed in PDAC [36]. Viral vectors carrying CEA, MUC1 and TRICOM [a triad of costimulatory molecules: B7.1, intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen 3 (LFA-3)] have been investigated as vaccines against advanced PDAC [37]. In one study, a vaccinia viral vector was used for the initial T cell priming, and a fowlpox viral vector was used for immune boosting. Although this treatment resulted in an MST of 6.3 months (1.5–21.1 months), the five patients who showed T cell responses achieved a longer survival period than the five patients who did not (15.1 and 3.9 months, respectively; $P = 0.002$) [38]. It should be noted that GM-CSF was used as a vaccine adjuvant in the latter trial (Table 2).

Live-attenuated, double-deleted (LADD) Listeria monocytogene vaccine

ANZ-100 is a live-attenuated double-deleted Listeria monocytogene strain (LADD; Lm ΔactA/ΔinlB) found to induce a local proinflammatory response, resulting in the activation of innate and adaptive effector cells [39]. Mesothelin is expressed in PDAC and plays an important role in tumor progression [40]. CRS-207 is a LADD Lm strain that delivers mesothelin antigens into class I and II antigen-processing pathways [41]. In a study examining the utility of CRS-207 as a treatment for advanced cancer, three of the seven subjects with PDAC were long-term survivors, although the detection of a mesothelin-specific T cell response was not correlated with survival [41].

Recent vaccine therapies

WT1

Kobayashi et al. reported a retrospective analysis of 255 advanced PDAC patients who were treated with dendritic

Table 2 Peptide-based vaccines and biological vaccines for advanced pancreatic cancer

Author	Journal	Antigen peptide	Sequences	Combination	Patients	Outcome/MST
Yamamoto	Anticancer Res. 2005;25:3575–9	MUC1	10-mer extracellular tandem repeat domain: $(GVTSAPDTRPAPGSTAPPAH)_5$	Montanide ISA-51	6	1/6 SD
Rong	Clin Exp Med. 2012;12:173–80	MUC1	PDTRPAPGSTAPPAHGVTSA	DC cells	7	All PD
Gjertsen	Int J Cancer. 2001;92:441–50	K-ras	KLVVVGAGGVGKSALTI Asp: D Arg: R Val: V Cyc: C	GM-CSF	38	1 PR 10 SD (10.2 M; 3-23 M) 27 PD 4.9 M responders 2.0 M non-responders
Abou-Alfa	Am J Clin Oncol. 2011;34:321–5	ras12R ras12 V ras12D Wild-type ras	TEYKLVWGARGVGKSALTIQ TEYKLVWGAVGVGKSALTIQ TEYKLVWGADGVGKSALTIQ TEYKLVWGAGGVGKSALTIQ	hGM-CSF	24	Postoperative adjuvant treatment
Bernhardt	Br J Cancer. 2006;95:1474–82	Telomerase hTERT (611–626)	GV1001; EARPALLTSRLRFIPK	GM-CSF	38	7.2 M (24 responders) 2.9 M (14 non-responders)
Miyazawa	Cancer Sci. 2010;101:433–9	VEGFR2-169	RFVPDGNRI	Gemcitabine	18	7.7 M
Gilliam	Pancreas. 2012;41:374–9	Anti-gastrin G17DT Gastrimmune	EGPWLEEEEEAYGWMDF-DT (diphtheria toxoid)	G17DT vs. placebo	152	5.0 M vs 2.8 M
Maki	Dig Dis Sci. 2007;52:1964–72	HSP HSPPC-96 (gp96, Oncophage)			10	Postoperative adjuvant treatment 2.7 Y
Kaufman	J Transl Med. 2007;5:60	MUC1 and CEA	CEA agonist peptide CAP1-6D (YLSGADLNL) MUC-1 agonist peptide P-93L (ALWGQDVTSV)	B7.1, ICAM-1, LFA-3 (TRICOM) Vaccinia virus: PANVAC-V Fowlpox virus: PANVAC-F GM-CSF	10	6.3 M
Le	Clin Cancer Res. 2012;18:858–68	Listeria vaccine ANZ-100, CRS-207			9 vs. 17	NA

Table 3 Recently developed peptide-based vaccines and multiple vaccines for advanced pancreatic cancer

Author	Journal	Antigen peptide	Sequences	Restricted HLA	Combination	Patients	Outcome/MST
Kobayashi	Cancer Immunol Immunother. 2014;63:797–806	WT1 MUC1	CYTWNQMNL RMFPNAPYL TRPAPGSTAPPAHG-VTSAP DTRPAPGSTAP	A24:02 A02:01/02:06 Any A	DC cells OK432	255	9.9 M 10.4 M (erythema)
Nishida	J Immunother. 2014;37:105–14	WT1	CYTWNQMNL	A24:02	Weekly 1000 mg/m^2 GEM	31	8.1 M 10.9 M (DTH)
Asahara	J Translation Res. 2013;11:291	KIF20A-66	KVYLRVRPLL	A2402	Montanide ISA51VG	31	4.7 M 6.1 M (reaction)
Suzuki	J Immunother. 2014;37:36–42	KIF20A-10-66	KVYLRVRPLL	A2402	Montanide ISA51VG	9	5.8 M
Geynisman	J ImmunoThera Cancer. 2013;1:8	CEA CAP1-6D	YLSGADLNL	A2	Montanide/GM-CSF	19	11.1 M
Kameshima	Cancer Sci. 2013;104:124–9	SVN2B	AYACNTSTL	A2402	Montanide/IFN-oc	6	(9.6 M)
Yutani	Oncology Reports. 2013;30:1094–100	31 vaccine peptides		A2, A24, A3, A26	Mono: 8 Chemo: 33	41	7.9 M 9.6 M (chemo)
Kimura	Pancreas. 2012;41:195–205	WT1, Her2, CEA, MUC1, CA125, autologous tumor lysate			DC cells plus LAK plus GEM and S1 OK432	49	S: 8.0 M G: 12.0 M GS + LAK: 16.9 M
Le	J Clin Oncol. 2014;32(suppl 3):Abstract 177	GVAX pancreas and CRS-207 vs. GVAX pancreas alone	Irradiated GM-CSF-secreting allogeneic pancreatic tumor vaccine (GVAX pancreas)		Cyclophosphamide	90	6.1 M vs. 3.9 M 9.7 M (3 or more rounds of vaccine therapy)

cell (DC) vaccines containing Wilms tumor 1 (WT1) and MUC1 after being recruited from seven institutions that followed a unified standard operating procedure. The MST of these patients was 9.9 months [42]. Nishida et al. also examined the utility of chemo-vaccine therapy in which a WT1-based vaccine was used in combination with the administration of 1,000 mg/m^2 of gemcitabine weekly. The latter regimen resulted in an MST of 8.1 months among 31 advanced PDAC patients [43]. In addition, the MST of the immunological responders in these two studies was very similar (10.4 and 10.9 months, respectively) (Table 3).

KIF20A

Kinesin family member 20A (KIF20A) plays an important role in the trafficking of molecules and organelles [44] and is one of the molecules targeted by vaccines against PDAC. A KIF20A vaccine was recently tested using different regimens, including vaccine monotherapy [45] and chemo-vaccine therapy involving gemcitabine [46], and similar MST values were reported in both studies (4.7 and 5.8 months, respectively).

Carcinoembryonic antigen (CEA)

CEA is a 180-kDa immunoglobulin-like molecule expressed on the surface of 90 % of PDAC tumor cells [47]. CAP1-6D, a modified CEA peptide, was combined with Montanide/GM-CSF to produce a vaccine against pancreatic cancer that was subsequently tested in advanced PDAC patients [48]. The MST of the 19 patients was 11.1 months, and one patient, randomized into the 0.01 mg arm, achieved a complete response (CR).

Survivin2B

Survivin is a member of the inhibitors of apoptosis (IAP) family of proteins that protect apoptotic signals by inhibiting the caspase activity [49, 50]. Hence, survivin-expressing cancer cells escape from apoptosis and do not die. Using a peptide-binding assay, we found that the survivin2B 80–88 peptide induces a strong CTL response [51]. We also examined the effects of a survivin2B 80–88 peptide-based vaccine on various cancers in the clinical setting and obtained promising outcomes. In particular, the anti-tumor effect of the survivin2B 80–88 peptide was enhanced by combining it with incomplete Freud's adjuvant and IFN-α injection. Our preliminary clinical study demonstrated a 66.6 % disease control rate in advanced PDAC patients (four of six patients) [52]. Moreover, the PDAC patients in our recent clinical phase I study exhibited an MST of 9.6 months.

Table 4 Evaluation of therapeutic activity in solid tumors

Method	WHO	RECIST	IrRC
	Sum of the products of the two longest perpendicular dimensions (bidimensional)	Sum of the longest dimensions (unidimensional)	Sum of the products of the two longest perpendicular dimensions (SPD) of all index lesions. (bidimensional)
No. of measured lesions	All lesions	Five per organ, 10 in total	Five per organ, 10 in total, and five cutaneous index lesions
CR	Disappearance of all known disease, confirmed at 4 weeks	Disappearance of all known disease, confirmed at 4 weeks	Disappearance of all known disease, confirmed at 4 weeks apart
PR	>50 % decrease in total tumor size, confirmed at 4 weeks	>30 % decrease in total tumor size, confirmed at 4 weeks	>50 % decrease in tumor burden compared with baseline in two observations at least 4 weeks apart
SD	CR, PR, and PD criteria not met	CR, PR, and PD criteria not met	CR, PR, and PD criteria not met
PD	>25 % increase in total tumor size; no CR, PR, or SD documented before increase in tumor size; new lesion (s); > 25 % increase in size of one lesion	>20 % increase in total tumor size; no CR, PR, or SD documented before increase in tumor burden; new lesion (s)	>25 % increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart

Tumor burden = $SPD_{index\ lesions}$ +SPD new, measurable lesions

Multiple vaccines

Personalized peptides

In a previous study, a set of 31 peptides was used to create personalized vaccines for advanced PDAC [53]. A maximum of four peptides were selected from among the 31-peptide set based on the results of HLA typing and the patients' peptide-specific IgG titers. Eight patients received vaccine monotherapy, and 31 patients received chemo-vaccine therapy. In the chemo-vaccine therapy group, gemcitabine was administered in eight patients, S-1 was administered in six patients and gemcitabine + S-1 was given in eight patients. The overall MST was 9.6 months, although that of the patients who underwent monotherapy was 7.9 months. Yanagimoto et al. reported similar clinical outcomes for chemo-vaccine therapy involving personalized vaccines and gemcitabine based on the same regimen [54]. The MST of the patients in the latter study was 9.0 months, although that of the immunological responders was 15.5 months. None of the patients in either study achieved CR (Table 3).

Autologous tumor lysate combined with lymphokine-activated killer cell therapy

Kimura et al. treated 49 PDAC patients with vaccines based on five different peptides and autologous tumor lysate, although the vaccine preparation regimens and anti-tumor therapies varied in each case [16]. Two patients achieved CR after treatment with a combination of DC cell and lymphokine-activated killer cell (LAK) therapy. The MST of the patients treated with LAK + gemcitabine and S-1 was 16.9 months, whereas that of all patients was 12.0 months. It should be noted that the survival time was calculated from the day after the first vaccination, which may have resulted in a shorter survival time (by a couple of months) than would have been obtained using the methods employed in other studies. It is very difficult to evaluate the clinical results of this study due to the effects of the different therapeutic strategies used in each case. However, the fact that multiple patients achieved CR will encourage researchers to pursue this approach further.

GVAX pancreas with CRS-207

GVAX is a series of irradiated GM-CSF-secreting allogeneic pancreatic cell lines that elicit broad antigenic responses. CRS-207 is a LADD Lm strain (Lm ΔactA/ΔinlB) that expresses mesothelin and stimulates the innate and adaptive immune systems. A phase II randomized control trial of GVAX pancreas combined with CRS-207 versus GVAX pancreas alone was presented at the 2014 American Society of Clinical Oncology (ASCO) Gastrointestinal Cancers Symposium [55]. Interestingly, the clinical results demonstrated that both treatments had dose-dependent survival benefits. The MST of the patients who received three or more rounds of vaccine therapy was 9.7 months, and the MST of the GVAX with CRS-207 arm was longer than that of the GVAX-alone arm (6.1 vs. 3.9 months; $P = 0.01$) [56].

Evaluation of therapeutic activity in solid tumors

The response of solid tumors is evaluated using either the WHO [57] or RECIST criteria [58]. The RECIST criteria were developed because the WHO criteria are quite complex and measuring all visible lesions in two dimensions is both time consuming and subject to measuring bias [59]. However, the use of immunotherapeutic agents in cancer patients is associated with the following problems: (a) The measurable anti-tumor activity can take longer to appear during immunotherapy than during cytotoxic therapy; (b) Responses to immunotherapy can occur after the standard criteria for progressive disease (PD) have been met; (c) Discontinuing immunotherapy may not be appropriate in some cases, unless PD is confirmed; (d) Allowing for "clinically insignificant" PD (e.g., small new lesions developing in the presence of other responsive lesions) is recommended; and (e) Durable stable disease (SD) may represent the anti-tumor activity [60]. Therefore, the immune-related response criteria (irRC) were developed to evaluate the immunotherapeutic activity in solid tumors [61]. The most important aspects of the irRC criteria are that (a) new lesions are not classified as PD and (b) two consecutive observations obtained at least four weeks apart are required to diagnose PD. However, the clinical utility of the irRC remains unclear and these criteria may require further optimization [61] (Table 4).

Future research topics

Initial time point for survival assessments

The initial time point for survival assessments should be unified to allow clinical outcomes to be compared between studies. Most PDAC patients already have advanced disease at the time of diagnosis [6]. In addition, the adverse effects of chemotherapies differ markedly among the various regimens [8]. Therefore, the status of PDAC patients at the time point at which they are registered can differ both within and between clinical studies. Kobayashi et al. reported that the MST from the date of diagnosis and the MST from the first vaccination are very different (16.5 vs.

9.9 months) [42]. Therefore, MST data must be interpreted carefully.

Vaccine therapy and chemotherapy

The goal of vaccine therapy for cancer is to increase the native immunity of cancer patients. However, chemotherapy causes irreversible damage to proliferating cancer cells as well as immune cells, including T and B cells. Therefore, there is a conflict between the fundamental principles of these two treatments. Chemotherapy is currently the gold standard treatment for advanced PDAC. Although the biological mechanisms of vaccine therapy and chemotherapy conflict with each other, the anti-cancer activity of vaccine monotherapy or chemo-vaccine combination therapy should be greater than that of chemotherapy alone.

Slow clinical response to vaccine therapy

It is very hard to achieve a complete response (CR) with vaccine therapy alone. We reviewed 19 studies involving a total of 860 patients and found that CR responses were obtained in only three cases. Although none of these studies involved a large number of patients, the poor reported response rates are a concern. One of the patients who achieved a CR was administered CEA CAP1-6D + Montanide/GM-CSF therapy, while the other two were treated with WT1, Her2, CEA, MUC1, cancer antigen 125 and autologous tumor lysate vaccines combined with DC cell-based LAK therapy and chemotherapy. Immunological responses require a long time to control tumor growth and achieve remission. The primary goal of vaccine therapy is to achieve long-term SD [62]. Most previous clinical studies of PDAC involved patients with advanced disease for whom no other therapies were available. Therefore, vaccine therapy may be suitable for patients in other clinical stages or possibly a useful postoperative adjuvant therapy. The main advantage of vaccine therapy is that it has few adverse effects, although it has also demonstrated minimal clinical effects in previous trials. We are currently conducting a phase II study of the survivin2B 80–88 peptide + Montanide + IFN-β as a treatment for PDAC (SUCCESS, Study of Unresectable CanCEr with Survivin-2B peptide vaccine in Sapporo: UMIN000012146), in which half of the required patients have been recruited. The clinical results of the SUCCESS phase II study will be reported by the end of next year.

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