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(54)【発明の名称】肝臓疾患用バイオマーカーおよびその使用方法

(57)【要約】
【課題】　肝臓疾患用のバイオマーカーおよびその使用方法を提供する。
【解決手段】　SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせあるいは該アミノ酸配列に対する抗体の何れかから選択される、肝臓疾患用のバイオマーカー。該バイオマーカーを含むことを特徴とする、肝臓疾患用の検出キット。試料を調製する工程と；上記バイオマーカーを用いて、該試料中の自己抗体を同定し、かつ捕獲する工程と；該自己抗体を検出する工程を含むスクリーニング法。
【選択図】　なし

【特許請求の範囲】
【請求項１】
SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせあるいは該アミノ酸配列に対する抗体の何れかから選択される、肝臓疾患用バイオマーカー。
【請求項２】
該肝臓疾患が肝硬変または肝臓癌である、請求項1記載のバイオマーカー。
【請求項３】
該変異型と、SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列の何れか1種とが、80％を越える配列相同性を示す、請求項1記載のバイオマーカー。
【請求項４】
SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせの何れかから選択される、バイオマーカーを含むことを特徴とする、肝臓疾患用の検出キット。
【請求項５】
該肝臓疾患が肝硬変または肝臓癌である、請求項4記載の検出キット。
【請求項６】
該検出キットが、更にSEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせの何れか1種に対する自己抗体を認識できる、二次抗体をも含む、請求項4記載の検出キット。
【請求項７】
肝臓疾患をスクリーニングする方法であって、試料を調製する工程と；SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせの何れか1種から選択されるバイオマーカーを用いて、該試料中の自己抗体を同定し、かつ捕獲する工程と；該自己抗体を検出する工程を含むことを特徴とする、上記スクリーニング方法。
【請求項８】
該試料が、全血または血清を含む、請求項7記載の方法。
【請求項９】
該試料が血清である、請求項8記載の方法。
【請求項１０】
該バイオマーカーを、検出用のキットとすることができる、請求項7記載の方法。
【請求項１１】
該バイオマーカーを、まず基板上に固定化する、請求項7記載の方法。
【請求項１２】
該基板が、イムノアッセイ用プレートまたはバイオチップである、請求項11記載の方法。
【請求項１３】
該試料を、まず蛍光マーカーで標識する、請求項7記載の方法。
【請求項１４】
該方法が、更に該自己抗体を認識し、かつ吸着する二次抗体を使用する工程を含む、請求項7記載の方法。
【請求項１５】
該二次抗体が変性されており、かつ発色反応、放射能または蛍光によって検出できる特別な官能基を持つ、請求項14記載の方法。
【請求項１６】
該自己抗体の検出を、蛍光スキャナーを用いて、蛍光標識された自己抗体を検出することにより達成する、請求項7記載の方法。
【請求項１７】
該自己抗体の検出を、酵素結合イムノソーベントアッセイ(ELISA)、ラジオイムノアッ

セイ(RIA)または免疫蛍光により、該二次自己抗体を検出することにより達成する、請求項7記載の方法。

【請求項１８】

SEQ ID NO:1～SEQ ID NO:24に記載された、何れか1種のアミノ酸配列に対する、一連の抗体を含む、肝臓疾患用の検出キット。

【請求項１９】

該肝臓疾患が肝硬変または肝臓癌である、請求項18記載の検出キット。

【請求項２０】

肝臓疾患をスクリーニングする方法であって、試料を調製する工程と；SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列の何れか1種に対する抗体を使用して、該試料中の抗原を捕獲する工程と；該抗体-抗原錯体を検出する工程を含むことを特徴とする、上記スクリーニング方法。

【請求項２１】

該試料が、全血または血清を含む、請求項20記載の方法。

【請求項２２】

該試料が血清である、請求項21記載の方法。

【発明の詳細な説明】

【技術分野】

【０００１】

本発明は、肝臓疾患用バイオマーカーおよびその使用方法に関連し、そこでは、自己抗原をスクリーニングする方法を使用して、肝臓疾患を検出するために使用できる、バイオマーカーを同定する。これらの同定されたバイオマーカーは、更に肝臓疾患のスクリーニングのために、試料(検体)における自己抗体または自己抗原の存在を検出するための、検出用キットへと発展させられる。

【背景技術】

【０００２】

損なわれた免疫機能を持つ人々は、免疫疾患を発症する傾向がある。多くのヒト疾患の病因学は、以下に記載する3つの状態の何れかにおいて、その原因を、我々の免疫系にまで遡ることを可能とする。その第一の状態は、低い免疫性、免疫細胞のより低い活性、または免疫細胞数の低下であり、例えばヒトの身体が、侵入してくる細菌、ウイルスまたは真菌を撃退することができず、かつ伝染性の疾患、例えば感冒、流行性感冒、肺炎、腸炎、または肝炎およびAIDSに対しても罹り易くなる。その第二の状態は、免疫不全または免疫系の過剰反応であり、そこでは該侵入する物質は微生物ではなく、些細な花粉または摂取した食物における巨大タンパクであり、それらに対して該免疫系が大量の抗体を放出する。このような攻撃および防御は、我々の細胞内で起こり、連鎖反応を生じ、これはまたアレルギーとも呼ばれる。真の病原体、例えば細菌、ウイルスまたは真菌が、この時点で人の身体を攻撃した場合には、該免疫系は最早抵抗性を高めることは不可能である。該損なわれた免疫系の第三の状態は、ヒトの身体内で免疫細胞が正常な細胞を攻撃することであり、これはリウマトイド関節炎、紅斑性狼ソウ(エリテマトーデス)およびヘルペスの場合におけるように、自己免疫疾患と呼ばれる。このような免疫疾患は、自己抗体が、ヒト身体自体の細胞に対して生成され、組織の損傷および疾病をもたらすという、識別、確認の問題を含む我々自身の免疫系に起因する。

【０００３】

現在では、自己抗体が自己免疫疾患だけに存在する訳ではないことは、公知である。益々多数の研究が、癌に対する免疫応答において、自己抗原(腫瘍由来)および自己抗体(身体由来)が、幾つかの場合において存在することを示している。従って、身体の応答を引出す、腫瘍自己抗原の検出は、癌のテスト、診断、または予後、および更に疾患の治療のために行い、かつこれらにおいて適用することができる。

米国特許第6,631,330号、同第5,137,807号、同第5,830,667号、同第6,264,949号および同第5,985,542号は、硬変、線維症または自己免疫性肝炎(AIH)の診断における、バイオマ

ーカーの使用を開示しており、米国特許第4,994,374号および同第5,175,084号は、肝細胞癌の診断における、バイオマーカーの使用を開示しており、米国特許第6,410,724号は、診断手段として、肝細胞癌に関連するDNAプライマーを使用している。しかし、これらの特許に記載されているバイオマーカーは、正確さに劣り、あるいはある程度まで、干渉に影響され易い。

【０００４】

米国特許第5,891,436号および公開No.20030138860は、原発性胆汁性肝硬変または肝細胞癌に対する診断手段として、ヒト血清中の自己抗体の存在を検出するために、バイオマーカーを用いることを開示している。これらの特許は、癌患者における自己抗体の存在を明らかにし、またその結果として、癌のスクリーニングにおけるバイオマーカーの使用に関する、合理性を確立している。

癌は、1982年以来、台湾における主な死因となっており、また肝癌は、男性、女性両者における第一の死因としてランク付けされている。従って、早期診断および早期治療が、その死亡率を下げるのに役立つことを期待しつつ、高い正確性を持ち、干渉に影響され難いバイオマーカーを見出すことが重要であり、またこれらのバイオマーカーを使用して、肝硬変および癌用の検出キットを開発し、肝臓疾患に罹患した患者を効率よくスクリーニングすることが重要である。

【発明の開示】

【０００５】

上記公知技術の諸欠点を処理するために、本発明は、肝臓疾患用のバイオマーカーを提供し、該マーカーは、更に発展させて、自己抗体の存在に係る知見に基いて、肝硬変および肝臓癌を診断するための、検出キットとすることができる。

本発明の目的は、SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせあるいは該アミノ酸配列に対する抗体の何れかから選択される、肝硬変および肝臓癌を検出するためのバイオマーカーを提供することにある。

本発明によれば、上記変異型は、1種以上のアミノ酸を、該バイオマーカーのアミノ酸配列におけるアミノ酸と置換し、該アミノ酸配列から削除し、そこに挿入し、および/または付加することにより得られ、この変異型のアミノ酸配列と、該バイオマーカーのアミノ酸配列とは、80％を越える配列相同性を持つ。

本発明の他の目的は、SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせの何れかから選択される、一組のバイオマーカーを含む、肝臓疾患用の検出キットを提供することにある。

【０００６】

本発明の一態様において、上記検出キットは、更にSEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型の何れか1種に対する抗体を認識できる、二次抗体をも含むことができる。

本発明の、更なる目的は、肝臓疾患をスクリーニングするための方法を提供することにあり、この方法は、試料(または検体)を調製する工程；SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせの何れか1種から選択されるバイオマーカーを用いて、該試料中の自己抗体を捕獲する工程；および該自己抗体を検出する工程、を含む。

更に別の本発明の目的は、SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列の何れか一つに対する一連の抗体を含む、肝臓疾患用の検出キットを提供することにある。

本発明の更なる目的は、肝臓疾患をスクリーニングするために、上記検出キットを使用する方法を提供することにあり、該方法は、試料を調製する工程；SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列の何れか1種に対する抗体を使用して、該試料中の抗原を捕獲する工程；および該抗体-抗原錯体を検出する工程を含む。

【０００７】

本発明は、自己抗原スクリーニング法の利用に基くものであり、この方法は、まず正常なヒト、肝硬変患者および肝癌患者由来の抗体各々を精製する工程およびこれら抗体を異なるカラム内に固定化する工程；肝臓疾患関連細胞系(HepG2 C3AおよびSNU-387)由来の細胞抽出物を、順次該正常抗体カラムおよび患者抗体カラムに通して、肝硬変および肝癌と関連する自己抗原を得る工程；これらの自己抗原を、酵素結合イムノソーベントアッセイ(ELISA)、ラジオイムノアッセイ(RIA)または免疫蛍光と組み合わせた、バイオマーカーキットとして使用して、該スクリーニングした試料における該自己抗原に対する自己抗体の存在を検出し、かつこの検出結果に基いて、該患者が、肝硬変または肝癌に罹患しているか否かを決定する工程を含む。これらのバイオマーカーは、既存の自己抗体に基いて同定されるので、これらを診断キットに発展させ、該バイオマーカーに対する自己抗体の存在に基いて、該患者が、このような疾患に罹っているか否かを決定することができる。このような方法は、該抗原の直接的なスクリーニングよりも著しく容易であり、かつより高い精度および感度をもたらす。

【発明を実施するための最良の形態】

【０００８】

本発明は、肝臓疾患、例えば肝硬変および肝癌の検出において使用できる、バイオマーカーを同定するための、自己抗原スクリーニング法の利用に関する。図1に示すような、該自己抗原スクリーニング法は、以下のような諸工程を含む：まず正常なヒトおよび患者由来の血清サンプルを得、かつ抗体を捕獲して、該血清サンプル中に含まれる該抗体を精製することのできる、アフィニティーカラムに、該各サンプルを通す工程；次いで、得られる精製された正常抗体および患者抗体各々をカラムに充填して、正常なヒト由来の抗体を含むカラム(正常抗体カラム)および患者由来の抗体を含むカラム(患者抗体カラム)を得る工程、ここで抗体は、該抗体と該カラム内の化学的な官能基との間に形成される、化学的な結合を介して固定化される；および疾患関連細胞系または病理学的な組織からの抽出物であり得る、サンプルを得る工程。ここで、上記血清サンプルは、単一の患者のサンプルまたは複数の患者由来の血清を含む、混合サンプル何れであっても良い。

【０００９】

この手順を継続するために、疾患関連細胞系または病理学的な組織からの抽出物由来のサンプルが、該正常抗体カラムに通される。該カラムでは、正常抗体の特異的なアフィニティーを介して、非-特異的な抗原が捕獲され、かつ該カラム内に保持される。この工程は、該患者抗体カラムを使用して、該サンプル中の自己抗原をスクリーニングする前の、該サンプルの予備処理であると考えることができる。非-特異的な抗原を除去した後、該サンプルは、特異的な抗原のみで構成されることになる。次いで、該サンプルを、患者抗体を充填したカラムに通して、疾患関連抗原をスクリーニングする。非-特異的な抗原が、正常血清抗体によって除去されているので、患者の抗体により同定されることになる該自己抗原は、更に一層特異的なものとなる。

最後に、該患者抗体カラムから置換された自己抗原は、マススペクトル技術による測定にかけられ、この測定手順では、マススペクトル図からのシグナルと、データベースとの比較を行って、該自己抗原に関する情報を得る。

【００１０】

肝臓疾患関連細胞系内の自己抗原は、上記方法に従って精製され、かつ同定される。これらの自己抗原が、患者血清中の抗体によって同定された場合には、該自己抗原またはその誘導体またはフラグメントもしくは変異型またはそれらの組み合わせを、バイオマーカーとして利用し、かつ検出用キットへと発展させることができる。スクリーニングすべき検体における自己抗体の存在を検出することにより、該患者が肝硬変または他の肝臓疾患に罹患しているか否かを、決定することができる。バイオマーカーに加えて、該検出キットは、更に該バイオマーカーに対する自己抗体を認識して、この検出法の適用を容易にすることのできる、二次抗体を含むことができる。

図2に示したように、上記検出キットを利用するこの方法は、以下のような諸工程を含む：試料を調製する工程；およびバイオマーカーを使用して、該試料中の自己抗体を検出

する工程。該バイオマーカーは、該自己抗原スクリーニング法によりスクリーニングされた自己抗原またはその誘導体またはフラグメントもしくは変異型またはそれらの組み合わせから選択される。上記試料は、全血または血清である。

【００１１】

この検出を容易にするために、上記のバイオマーカーは、任意の形状であり得、検出キットまたは基板上に予め固定化したものを含むが、これらに限定されず、該基板は、イムノアッセイプレートまたはバイオチップであり得る。該バイオマーカーにより捕獲された、該試料中の自己抗体を、該二次抗体によって認識し、かつ吸着でき、該二次抗体は発色反応、放射性検出または蛍光検出用の、特殊な官能基を持つ、変性された抗体である。

自己抗体を該二次抗体によって吸着した後、特別な試薬を添加して、発色反応させ、また酵素結合イムノソーベントアッセイ(ELISA)を利用して、該二次抗体の存在を決定し、その結果から、該患者が肝癌または肝硬変に罹っているか否かを決定するための基礎として、該自己抗体の存在を突き止める。該二次抗体の存在およびその結果としての該自己抗体の存在は、またラジオイムノアッセイ(RIA)または免疫蛍光法により決定することも可能である。

【００１２】

上記スクリーニング法が、該二次抗体を含まない場合、該試料は、該バイオマーカーと反応させる前に、蛍光マーカー(例えば、cy3またはCy5)で標識することができる。該バイオマーカーによりスクリーニングされた、この蛍光-標識を施した自己抗体は、次いで該二次抗体を使用すること無しに、蛍光スキャナーにより検出できる。

該自己抗体存在の検出に加えて、該抗原の検出も、患者が肝硬変または肝臓癌に罹っているか否かを決定するための基礎として利用することができる。この目的を達成するために、本発明は抗体を含む検出用キットをも提供し、該抗体は、肝臓疾患をスクリーニングするための、該自己抗原スクリーニング法により同定された自己抗原を認識することができる。

肝硬変または肝癌をスクリーニングするための、上記検出キットを使用する方法は、以下の工程を含む：血清試料を調製する工程；上記抗体を用いて、該血清中の該抗原を認識し、かつ捕獲する工程；および生成する抗体-抗原錯体を検出する工程。

【実施例】

【００１３】

本発明の利点は、更に実際の例を示すことにより説明されるが、以下の実施例における説明は、本発明の実際の適用に、何等制限を加えるものではない。

実施例1：肝臓疾患に罹っている患者の血清中の自己抗体を用いた、自己抗原のスクリーニング、該血清サンプル中の自己抗体の精製

まず、肝硬変または肝癌に罹っている患者の血清を得、該血清を結合バッファー(20mMのPBS、pH 7.0)で1:10の割合にて希釈し、次いで0.45μmのメンブランフィルタを用いて、この希釈された血清を濾過し、後の段階におけるカラムの詰まりを回避し、次いでプロテインG(Protein G)アフィニティーカラムを、1ml/分なる流速で、該カラム体積の10倍量の上記結合バッファーで洗浄し、次に該濾過した血清サンプルを、0.2ml/分なる流量で該プロテインGアフィニティーカラムに通して、そのアフィニティーにより該抗体を該カラム内に保持し、再度該プロテインGアフィニティーカラムを、1ml/分なる流速で、該カラム体積の5-10倍量の上記結合バッファーで洗浄して、該血清サンプル中の、該カラムとアフィニティー結合を形成しない物質を除去する。1ml/分なる流速で、該カラム体積の2-5倍量の溶出バッファー(0.1Mグリシン-HCl、pH 2.7)を用いて、該カラムから抗体を溶出し、予め60-200μlのTris-HCl溶液(1M、pH 9.0)を加えた試験管に、該溶出された抗体を集めた。最後に、該サンプルを、結合バッファー(0.2M $NaHCO_3$、0.5M NaCl、pH 8.3)に移して、該血清サンプル中の自己抗体(IgG)の精製を完了する。

【００１４】

本発明の方法は、各々1種の正常なIgGおよび患者IgGカラムを必要とする。従って、正常なヒトおよび患者由来の血清を得、これらを上記の精製段階に掛ける必要がある。

自己抗体を含むカラムの調製
酸性化溶液(1mM HCl、氷浴内)1滴を、ピペットでNHS-活性化カラムに滴下し、泡の生成を阻止する。このカラムの上端部に注射器またはポンプを接続した後、該カラムの底部におけるアダプタを取外す。該カラム体積の2倍量の該酸性化溶液で、該カラム内のイソプロパノールを洗い流す。この洗浄段階を3回繰り返した後、自己抗体を含む該サンプルを、該カラムに注入する。精製された自己抗体を含む上記結合バッファーを、該カラム体積と等価な体積および0.5-10mg/mlなる濃度を持つ溶液とした。自己抗体を含む上記サンプルを、該カラムに通した後に、このカラムを封止し、かつこの反応を25℃にて15-30分間、または4℃にて4時間に渡り行って、化学結合により、該抗体を該カラムに固定化させた。

【００１５】
該自己抗体と該カラムとを結合させた後、該カラムを、その体積の2倍量の、遮断バッファー(0.5Mのエタノールアミン、0.5MのNaCl、pH 8.3)で溶出し、これら段階を3回繰り返した。次に、このカラムを、その体積の2倍量の、洗浄バッファー(0.1Mのアセテート、0.5MのNaCl、pH 4)で洗浄し、同様にこれら段階を3回繰り返した。再度、各々該カラム体積の2倍量の、上記遮断バッファーを用いて、該カラムを3回溶出し、次にこのカラムを15-30分間反応させて、自己抗体と結合しない、該カラム内の官能基を遮断し、かつ不活性化した。この遮断反応の完了後、各々該カラム体積の2倍量の、上記洗浄バッファーを用いて、該カラムを3回洗浄し、次に該カラム体積の2倍量の、上記遮断バッファーを用いて、該カラムを3回溶出して、自己抗体と結合しない全ての官能基を、確実に遮断した。再度、各々該カラム体積の2倍量の、上記洗浄バッファーを用いて、該カラムを3回洗浄した。最後に、該カラム体積の2-5倍量の、中性pHを持つバッファーで溶出して、該自己抗体を充填したカラムの調製を完了した。

【００１６】
肝臓疾患関連細胞系の抽出物由来の、自己抗原の同定
まず、培地を除去した、2.68mgのHepG2 C3A細胞を、氷浴で処理したTrisの塩溶液(50mM Tris、pH 7.5、150mM NaCl、1.5mM PMSF、ホスファターゼ阻害剤)で2度洗浄し、次いで1mlのトライトン(Triton)抽出液(15mM Tris、pH 7.5、120mM NaCl、25mM KCl、2mM EGTA、0.1mM DTT、0.5% Triton X-100、10μg/mlのロイペプチン、0.5mM PMSF、およびホスファターゼ阻害剤)に添加し、4℃にて30分間放置した。この時点で、細胞は分解し、かつタンパク質を遊離し始めた。この溶液を、4℃にて15分間、(テーブルトップ式遠心機で)14,000rpmにて遠心分離処理して、固形分、不溶性細胞構成物を除去した。その上澄みを集めて、イムノアフィニティークロマトグラフィーにかけた。

【００１７】
回収した細胞抽出物を、1:10の割合で、上記結合バッファーで希釈し、0.45μmのメンブランフィルタに通して、後の段階における該カラムの閉塞を防止した。該サンプルを、該IgGカラムに注入する前に、1ml/分なる流速で、該カラム体積の10倍量の上記結合バッファーで、該正常および患者抗体カラムを洗浄した。次いで、濾過した細胞抽出物を、0.2ml/分なる流速で、該正常抗体カラムに通した。該正常抗体カラムを、1ml/分なる流速で、該カラム体積の5-10倍量の結合バッファーで溶出した。この時点において、該正常抗体により同定され、かつ捕獲された、該細胞抽出物中の抗原は、該カラム内に保持されるはずである。この段階の目的は、該HepG2 C3A細胞内の非-特異的な抗原を除去することである。結果として、該カラムを通過する該細胞抽出物は、非-特異的な抗原を含まない。得られた細胞抽出物を、該患者抗体カラムに注入した。このカラムを、1ml/分なる流速で、該カラム体積の5-10倍量の結合バッファーで溶出した。この時点において、該細胞抽出物中に存在する自己抗原は、該患者由来の自己抗体により捕獲され、かつ該カラム内に保持されるであろう。該細胞抽出物を該正常抗体カラムに通した場合、該正常抗体により捕獲される抗原は、該カラム内に保持され、一方抗原を含まない細胞抽出物は、該正常抗体により同定し、かつ捕獲することができ、該患者抗体により同定し、かつ捕獲できる抗原のみが、該カラムに保持されるであろう。該患者抗体カラム内に保持された抗原を、1ml/分

なる流速で、該カラム体積の2-5倍量の溶出バッファーを用いて溶出し、かつ捕集した。該カラムを通過した部分を、トリプシンを用いてタンパク加水分解にかけ、得られたペプチドをマススペクトル技術を利用して検定した。得られたスペクトル図を、データベースと比較して、該タンパクに関する情報を得た。

【００１８】

肝臓疾患関連細胞系を、肝硬変または肝癌に罹っている患者の血清中の自己抗体によってスクリーニングすることにより、以下の自己抗原を得た：

1. ヌクレオシドジホスフェートキナーゼ(gi|1421609, SEQ ID NO:1)
2. NM23タンパク(gi|35068, SEQ ID NO:2)
3. ATPシンターゼβ-鎖、ミトコンドリア[プリカーサ] (gi|28940, SEQ ID NO:3)
4. 14-3-3ζ-タンパク(チロシン3/トリプトファン5-モノオキシゲナーゼ活性化タンパク) (gi|4507953, SEQ ID NO:4)
5. 14-3-3ε-タンパク(チロシン3/トリプトファン5-モノオキシゲナーゼ活性化タンパク) (gi|4507953, SEQ ID NO:5)
6. タンパクジスルフィドイソメラーゼ-関連タンパク5(gi|1710248, SEQ ID NO:6)
7. 命名されていないタンパク製品(gi|21750187, SEQ ID NO:7)
8. トロポミオシンα3 (gi|37403, SEQ ID NO:8)
9. トリポミオシン(trypomyosin)α4 (gi|10435300, SEQ ID NO:9)
10. カルレティキュリンプリカーサ(gi|4757900, SEQ ID NO:10)
11. ヒトプレ-mRNAスプライシング因子SF2p32 (gi|338043, SEQ ID NO:11)

【００１９】

12. 腫瘍壊死因子タイプIレセプタ関連タンパクTRAP-1 (gi|1082886, SEQ ID NO:12)
13. 腫瘍拒絶抗原(gp96) 1; グルコース調節タンパク(gi|4507677, SEQ ID NO:13)
14. 熱ショックタンパク質90-β(gi|72222, SEQ ID NO:14)
15. 熱ショックタンパク質90-α (gi|123678, SEQ ID NO:15)
16. 熱ショック60kDaタンパク質 1 (gi|31542947, SEQ ID NO:16)
17. HMG-1 (gi|968888, SEQ ID NO:17)
18. KIAA0144遺伝子生成物(NICE-4タンパク質) (gi|13111995, SEQ ID NO:18)
19. バロシン(Valosin)含有タンパク質(p97); 転移性小胞体ATPアーゼ (gi|6005942, SEQ ID NO:19)
20. グリセロアルデヒド3-ホスフェートデヒドロゲナーゼ、肝臓(gi|30157565, SEQ ID NO:20)
21. サイトケラチン(gi|1419564, SEQ ID NO:21)
22. IGF-II mRNA-結合タンパク質 1 (gi|4191608, SEQ ID NO:22)
23. NADPH: キノンレダクターゼ(gi|13236495, SEQ ID NO:23)
24. ヒトコ-シャペロンP23の結晶構造(hsp-90コ-シャペロン) (gi|9257073, SEQ ID NO:24)

【００２０】

肝臓疾患関連細胞系由来の抗体で同定した自己抗原を以下の表1に示す。表1の左側には、GI番号およびそのタンパク質名を、およびその右側には、肝硬変または肝癌に罹っている患者の血清を用いて、細胞系から同定できる自己抗原を示す。示されたように、これらの自己抗原は、単一の肝臓疾患においてのみ存在するのではなく、これらは繰返し、起源を異にする血清中の自己抗体を用いて、様々な細胞系において同定されており、このことは肝臓疾患との密接な相関関係の存在を示している。表1に掲載したタンパク質の幾つかは、2つのGI番号を持つ。これは、該タンパク質およびその変異型が、マススペクトルにおいて同様な結果を示すからである。

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【００２１】

【表１】
表1：肝臓疾患関連細胞系からスクリーニングされた自己抗原

GI番号	タンパク質名	肝硬変血清vs. HepG2 C3A	肝癌血清vs. HepG2 C3A	肝硬変血清vs. SNU-387	肝癌血清vs. SNU-387
1421609	ヌクレオシドジホスフェートキナーゼ(=NM23タンパク)	●	●	●	●
28940	ATPシンターゼβ-鎖、ミトコンドリア[プリカーサ]	●		●	
4507953, 5803225	14-3-3タンパク	●		●	
1710248	タンパクジスルフィドイソメラーゼ-関連タンパク5		●		●
21750187	gi|21750187命名されていないタンパク製品(RAN rec mot.)		●		
37403, 10435300	トロポミオシン			●	●
4757900	カルレティキュリンプリカーサ	●	●		
338043	ヒトプレ-mRNAスプライシング因子SF2p32、完全配列	●	●		
1082886	腫瘍壊死因子タイプIレセプタ関連タンパクTRAP-1	●	●		
4507677	腫瘍タンパク抗原(gp96) 1;グルコース調節タンパク	●	●		
72222, 123678	熱ショックタンパク質90	●	●		
31542947	熱ショック60kDaタンパク質 1(シャペロニン); ミトコンドリアマトリックスタンパクP1	●			
968888	HMG-1(高-移動度群-1)	●			
13111995	KIAA0144遺伝子生成物(NICE-4タンパク質)	●			
6005942	バロシン含有タンパク質(p97); 転移性小胞体ATPアーゼ	●			
30157565	グリセロアルデヒド3-ホスフェートデヒドロゲナーゼ、肝臓	●			
1419564	サイトケラチン	●			
4191608	IGF-II mRNA-結合タンパク 1	●			
13236495	NADPH: キノンレダクターゼ		●		
9257073	ヒトコ-シャペロンP23の結晶構造(hsp-90コ-シャペロン)		●		

【００２２】
実施例2：上記自己抗原スクリーニング法により同定される自己抗原の入手性の測定
実施例1で同定された24種の自己抗原の入手性を立証するために、正常なヒト、肝硬変

患者および肝癌患者由来の血清サンプルに関するアッセイを、イムノアッセイ(ELISA、RIAまたは免疫蛍光法)および上記24種のバイオマーカーを使用して、実施した。このアッセイ法は、図2に示すように、以下に説明する段階を含む。試料を調製する工程；SEQ ID NO:1～SEQ ID NO:24に記載されたアミノ酸配列またはその誘導体またはフラグメントまたはその変異型もしくはこれらの組み合わせの何れか1種から選択されるバイオマーカーを用いて、該試料中の自己抗体を捕獲する工程；および該自己抗体を検出する工程。

酵素結合イムノソーベントアッセイ(ELISA)の例においては、以下の段階を採用する：まず、該バイオマーカーを被覆バッファー(a. 50mMのNa_2HCO_3, pH=9.6;またはb. 20mMのTris-HCl, pH=8.5;またはc. 10mMのPBS, pH=7.4から選択)で、0.5～10μg/mlなる濃度まで希釈し、ここで該被覆バッファーは、該バイオマーカーのPI値に従って選択され、好ましくはpIよりも高いpH1～2を持つバッファーを選択する。100μl/ウエルのバイオマーカー液をELISAプレートに添加し、4℃にて一夜これを放置して、固定化させた。

【００２３】

手順を継続するために、該プレートを、PBSTバッファー(PBSTバッファー：PSBバッファー+0.05%Tween-20)で2回洗浄することにより、未結合のバイオマーカーを除去し、次いで200μl/ウエルの遮断バッファー(a. ゼラチン-NET: 0.5%ゼラチン、0.15MのNaCl、5mMのEDTA・2Na、0.05%のTween-20、50mMのTris塩基、またはb. 1% BSA-PBS, pH=7.4、またはc. 5%の脱脂ミルク-PBS、pH=7.4から選択)を添加し、周囲温度下で、少なくとも2時間遮断反応を行い；この反応の完了後、PBSTバッファーで3回洗浄し、100μl/ウエルの血清溶液をアッセイに供した(血清溶液は、該血清サンプルを、該遮断バッファーで1000倍に希釈することにより得た)。この時点において、該血清中の自己抗体は、固定化したバイオマーカーと反応するであろう。周囲温度での少なくとも2時間の反応の後、該プレートをPBSTバッファーで4回洗浄し、次に100μl/ウエルの二次抗体(該遮断バッファーで5000倍に稀釈)を添加した。この時点で、該二次抗体は、該自己抗体を認識し、かつ吸着するであろう。周囲温度での少なくとも1時間の反応の後、該プレートをPBSTバッファーで5回洗浄した。次いで、100μl/ウエルのTMBを添加し、30分間に渡り発色反応を誘発させた。その後、100μl/ウエルの0.5M H_2SO_4を添加し、また450nmにおける吸光度を測定した。

【００２４】

該自己抗体の発現を、肝硬変および/または肝癌の診断において、確実に使用可能とするために、ELISAを使用して、夫々の自己抗原によって同定された如き、正常なヒト、肝硬変患者および肝癌患者由来の血清中の自己抗体の吸光度値を得る。5種のタンパク質、即ちGADPH、NADPH、HMG-1、NM23およびサイトケラチンから導かれたデータを、生物統計学的解析およびウイルコクソン/マン/ホイットニーテスト(Wilcoxon-Mann-Whitney Test)に掛けた。以下の表に示すように、95%の信頼度で、以下のような結果を得た。

【００２５】

【表２】

	GADPH	NADPH	HMG-1	NM23	サイトケラチン
正常なヒトvs.肝硬変患者	p=0.001	p=0.001	p=0.00006	p=0.0001	p=0.001
正常なヒトvs. 肝癌患者	p=0.017	p=0.016	p=0.015	p=0.002	p=0.016
肝硬変患者vs. 肝癌患者	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

正常なヒト：N=10；肝硬変患者：N=15；肝癌患者：N=21(p<0.05なる仮説は妥当である)。

【００２６】

正常なヒト、肝硬変患者および肝癌患者におけるバイオマーカーにより検出される自己抗体の発現間には差異があるものと仮定すると、上記表は、このような仮定が、正常なヒトvs.肝硬変患者および正常なヒトvs. 肝癌患者において妥当であることを示しており、このことは、正常なヒトと肝硬変患者との間および正常なヒトと肝癌患者との間において見られる、バイオマーカーにより検出される自己抗体の発現レベルにおける差が、統計的

に有意であることを意味している。

【００２７】

統計処理は、正常なヒトおよび肝硬変患者における、GADPH-検出自己抗体の発現レベルが、8.375倍異なっており、一方正常なヒトおよび肝癌患者におけるそれは、4.86倍異なっていることを示し、また正常なヒトおよび肝硬変患者における、HMG-1-検出自己抗体の発現レベルは、74倍異なっており、かつ正常なヒトおよび肝硬変患者における、NM23-検出自己抗体の発現レベルは、24倍異なっており、一方で正常なヒトおよび肝癌患者におけるそれは、8.545倍異なっていることを示している。これらの結果は、ここに与えられた24種の自己抗原によって検出されるような、肝硬変および肝臓癌患者の、抗体発現レベルが、正常なヒトにおけるこれらの値よりも高いことを証明している。従って、イムノアッセイと組み合わせた、これら24種の自己抗原を使用する検出キットは、スクリーニングされる検体における自己抗体の発現レベルに基いて、肝硬変および肝癌をスクリーニングすることを可能とする。

上記のような好ましい本発明の態様は、本発明を限定することを意味するものではない。本発明の精神および添付した特許請求の範囲を逸脱すること無しに、当業者によってなし得るあらゆる改良並びに変更は、本発明の保護されるべき範囲および特許請求の範囲に入るはずである。

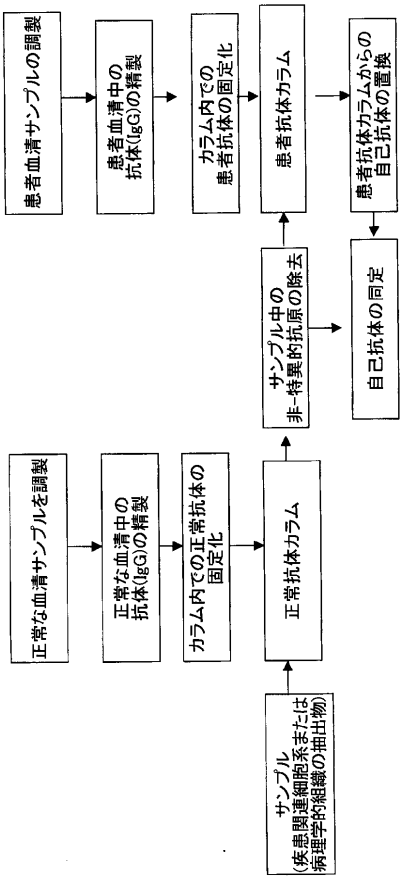
【図面の簡単な説明】

【００２８】

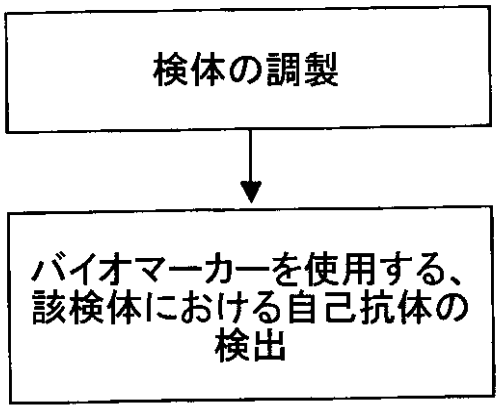
【図１】本発明による自己抗原スクリーニング法の工程系統図である。

【図２】本発明により、自己抗体をスクリーニングするための、バイオマーカーの利用に関する工程系統図である。

【図１】



【図２】



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【外国語明細書】

Biomarkers for liver diseases and method for using the same

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention is related to biomarkers for liver diseases and method for using the same, in which a method for screening autoantigens is employed to identify biomarkers that can be used in detecting liver diseases. The identified biomarkers are further developed into detection kits to detect the presence of autoantibodies or autoantigens in specimens for screening of liver diseases.

DESCRIPTION OF RELATED ART

People with impaired immune functions are prone to develop immune diseases. The etiology of many human diseases may be traced to our immune system in any of the three conditions described below. The first is reduced immunity, lower activity of immune cells, or reduced quantity of immune cells, such that the human body cannot fight off the invading bacteria, virus or mold, and becomes susceptible to contagious diseases, such as common cold, flu, pneumonia, enteritis, or even hepatitis and AIDS. The second condition is immunodeficiency or over-reaction of the immune system where the invading substances are not germs, but tiny pollens or macromolecular proteins in the food ingested, against which the immune system releases a large amount of antibodies. Such attack and defense occur in our cells, causing a chain of reactions which is also called allergy. When

real pathogens such as bacteria, virus or mold attack the human body at this time, the immune system is no longer able to put up resistance. The third condition of impaired immune system is the immune cells attack normal cells in the human body, called autoimmune disorder as in the case of rheumatoid arthritis, lupus erythematous, and herpes. Such immune diseases arise from our own immune system having an identification problem that autoantibodies are produced against human body's own cells, resulting in tissue damage and illnesses.

It is now known that autoantibodies are present not just in autoimmune diseases. More and more studies indicate that in the immune response to cancer, autoantigen (from the tumor) and autoantibody (from the body) exist in some cases. Thus the detection of tumor autoantigen that elicits body response may be directed towards and applied in the testing, diagnosis, or prognosis of cancer, and furthermore, in the treatment of disease.

U.S. Patents No.6,631,330, 5,137,807, 5,830,667, 6,264,949, and 5,985,542 disclose the use of biomarkers in the diagnosis of cirrhosis, fibrosis or autoimmune hepatitis (AIH); U.S. Patents No.4,994,374 and 5,175,084 disclose the use of biomarkers in the diagnosis of hepatocellular carcinoma; U.S. Patent No.6,410,724 uses DNA primer associated with hepatocellular carcinoma as a diagnostic tool. But the biomarkers disclosed in those patents lack accuracy or are susceptible to interference to a certain extent.

U.S. Patent No.5,891,436 and Publication No.20030138860 disclose the use of biomarkers to detect the presence of autoantibodies in human serum as a diagnostic tool for primary biliary cirrhosis or hepatocellular carcinoma. Those patents confirm the

existence of autoantibodies in cancer patients and thereby establish the rational for using biomarkers in cancer screening.

Cancer has been the leading cause of death in Taiwan since 1982, whereas liver cancer is ranked among the top as the cause of death in both men or women. Thus it is important to find biomarkers with high accuracy and not susceptible to interference and use those biomarkers to develop detection kits for liver cirrhosis and cancer to effectively screen patients with liver diseases in the hope that early diagnosis and early treatment can help lower the mortality rate.

SUMMARY OF THE INVENTION

In addressing the drawbacks of prior arts, the present invention provides biomarkers for liver diseases, which can be developed into detection kits for diagnosis of liver cirrhosis and liver cancer based on the knowledge of the existence of autoantibodies.

An objective of the present invention is to provide biomarkers for detecting liver cirrhosis and liver cancer, which are selected from any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof or the antibodies against the amino acid sequences.

According to the present invention, the aforesaid variants are obtained by substituting, deleting, inserting and/or adding to the amino acid in the amino acid sequences of the biomarker with one or more amino acids; the amino acid sequence of the variant and that of the biomarker have sequence homology greater than 80%.

Another objective of the present invention is to provide a detection kit for liver

diseases, comprising a set of biomarkers selected from any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof.

In one embodiment of the present invention, the aforesaid detection kit may further include secondary antibodies that can recognize the antibodies against any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants thereof.

A further objective of the present invention is to provide a method for screening liver diseases, comprising the steps of: providing a specimen; using biomarkers selected from any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof to capture the autoantibody in the specimen; and detecting the autoantibody.

Yet another objective of the present invention is to provide a detection kit for liver diseases, comprising a set of antibodies against any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24.

A further objective of the present invention is to provide a method using the aforesaid detection kit to screen liver diseases, comprising the steps of: providing a specimen; using the antibody against any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 to capture the antigen in the specimen; and detecting the antibody-antigen complex.

This invention is based on the use of autoantigen screening method, comprising the steps of: firstly purifying antibodies from normal persons, liver cirrhosis patients, and

liver cancer patients respectively and immobilizing them in different columns; passing the cell extracts from liver disease related cell lines (HepG2 C3A & SNU-387) in sequence through the normal antibody column and patient antibody column to obtain autoantigens associated with liver cirrhosis and liver cancer; using those autoantigens as biomarker kits coupled with enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or immunofluorescence to detect the presence of autoantibodies against said autoantigens in the screened specimen, and based on which, to determine whether the patient has liver cirrhosis or liver cancer. Since those biomarkers are identified based on existing autoantibodies, they can be developed into diagnostic kits to determine if the patient has such diseases based on the presence of autoantibodies against the biomarkers. Such method is much easier than direct screening of the antigen and offers greater accuracy and sensitivity.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a flow chart of an autoantigen screening method according to the present invention.

FIG. 2 shows a flow chart of using biomarkers for screening autoantibody according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of an autoantigen screening method to

identify biomarkers that may be used in the detection of liver diseases, such as liver cirrhosis and liver cancer. Said autoantigen screening method as shown in Fig. 1 comprises the following steps: firstly obtaining serum samples from normal persons and patients and passing the respective samples over affinity columns that can capture antibodies to purify the antibodies contained in the serum samples; next packing respectively the resulting purified normal antibodies and patient antibodies into columns to obtain a column containing antibodies from normal persons (normal antibody column) and a column containing antibodies from patients (patient antibody column) in which antibodies are immobilized through the chemical bonding formed between the antibodies and chemical functional groups in the column; obtaining a sample which may be the extract of disease related cell lines or pathological tissues; the aforesaid serum sample may be that of a single patient or a mixture sample containing the sera of a plurality of patients.

To continue the procedure, passing the sample from the extract of disease related cell lines or pathological tissues over the normal antibody column where non-specific antigens are captured and retained in the column through the specific affinity of normal antibodies; this step may be viewed as pre-treatment of the sample before the patient antibody column is used to screen autoantigens in the sample. After non-specific antigens are removed, the sample constitutes only specific antigens. Next, passing the sample over the column packed with patient antibodies to screen disease related autoantigen. Since non-specific antigens have been removed by normal serum antibodies, the autoantigens as identified by patient's autoantibodies are more specific.

Finally, the autoantigens displaced from the patient antibody column are subjected to determination by the mass spectrum technology; the aforesaid determination procedure involves comparing the signals from mass spectrograph with the database to obtain the information on the autoantigens.

Autoantigens in liver disease related cell lines are purified and identified according to the method described above. Given that those autoantigens are identified by the antibodies in patient sera, the autoantigens or derivatives or fragments or variants or combinations thereof can be utilized as biomarkers and developed into detection kits. By detecting the presence of autoantibodies in screened specimen, it can be determined whether the patient has liver cirrhosis or other liver diseases. In addition to biomarkers, the detection kits can further include secondary antibodies that can recognize the autoantibodies against the biomarkers to facilitate the application of the detection method.

As shown in Fig. 2, the method utilizing the detection kits described above comprises the steps of: providing a specimen; using biomarkers to detect the autoantibody in the specimen. Said biomarkers are selected from autoantigens screened by the autoantigen screening method or its derivatives or fragments or variants or the combination thereof. The aforesaid specimen is whole blood or serum, preferably serum.

To facilitate the detection, the aforesaid biomarker may come in any form, including but not limited to, a detection kit or pre-immobilized on a substrate, said substrate may be an immunoassay plate or a biochip, said substrate may be an immunoassay plate or a biochip. The autoantibodies in the specimen captured by the biomarkers can be recognized and adsorbed by the secondary antibodies, which are

modified antibodies having special functional groups for color reaction, radio detection or fluorescence detection.

After autoantibody is adsorbed by the secondary antibody, a special reagent is added to undergo color reaction and enzyme-linked immunosorbent assay (ELISA) is employed to determine the presence of the secondary antibody, and from which to learn the presence of the autoantibody as a basis for determining if the patient has liver cancer or liver cirrhosis. The presence of the secondary antibody and thereby the presence of the autoantibody can also be determined by radioimmunoassay (RIA) or immunofluorescence.

If the screening method does not include the secondary antibody, the specimen may be labeled with a fluorescence marker (e.g. cy3 or Cy5) prior to reacting with the biomarkers. The fluorescence-labeled autoantibodies screened by the biomarkers can then be detected by a fluorescence scanner without the use of the secondary antibody.

Besides detecting the presence of the autoantibody, detection of the antigen may also be used as a basis for determining whether a patient has liver cirrhosis or liver cancer. To achieve this purpose, the present invention also provides a detection kit containing antibodies that can recognize autoantigens identified by the autoantigen screening method for the screening of liver diseases.

The method of using the aforesaid detection kit for screening liver cancer and liver cirrhosis comprises the steps of: providing a serum specimen; using the aforesaid antibody to recognize and capture the antigen in the serum; and detecting the antibody-antigen complex.

The advantages of the present invention are further depicted with the illustration

of an example, but the descriptions made in the example should not be construed as a limitation on the actual application of the present invention.

Example 1– Screening of autoantigens using autoantibodies in sera of patients with liver diseasesPurification of autoantibodies in the serum sample

Firstly obtaining a serum of a patient with liver cirrhosis or liver cancer, diluting the serum with a binding buffer (20mM PBS, pH 7.0) at the ratio of 1:10, and then filtering the diluted serum using a 0.45μm filter membrane to prevent the blockage of column in subsequent steps; next rinsing a Protein G affinity column with the binding buffer ten times the column volume at the rate of 1 ml/min, and then passing the filtered serum sample over the Protein G affinity column at the rate of 0.2 ml/min to retain the antibodies in the column through affinity; rinsing the Protein G affinity column again using the binding buffer 5-10 times the column volume at the rate of 1 ml/min to remove substances in the serum sample that do not form affinity bonding with the column. Eluting antibodies from the column using an elution buffer (0.1 M Glycine-HCl, pH 2.7) 2-5 times the column volume at the rate of 1 ml/min and collecting the eluted antibodies in a test tube which is added beforehand with 60-200 μl Tris-HCL solution (1M, pH 9.0). Finally displacing the sample in a coupling buffer (0.2M $NaHCO_3$, 0.5M NaCl, pH 8.3) to complete the purification of autoantibodies (IgG) in the serum sample.

The method according to the present invention requires one normal IgG and patient IgG column each. Thus sera from normal persons and patients should be obtained and subject to the purification steps described above.

Preparation of columns containing autoantibodies

Pipette one drop of an acidification solution (1mM HCl, ice bathed) into a NHS-activated column to prevent the formation of bubbles. After connecting the upper end of the column with a syringe or pump, removing the adapter at the- bottom of the column. Rinsing out isopropanol in the column using the acidification solution two times the column volume. After repeating the wash step three times, injecting the sample containing autoantibodies into the column. Preparing the aforesaid coupling buffer containing purified autoantibodies into a solution with a volume equivalent to one time the column volume and a concentration of 0.5-10 mg/ml. After passing the aforesaid sample containing autoantibodies over the column, sealing the column and let the reaction go on for 15-30 minutes under 25°C or 4 hours under 4°C to immobilize the antibodies in the column through chemical bonding.

After the bonding between the autoantibodies and the column, eluting the column with a blocking buffer (0.5M ethanolamine, 0.5M NaCl, pH 8.3) two times the column volume, and repeating the steps three times. Then rinsing the column with a washing buffer (0.1M acetate, 0.5M NaCl, pH 4) two times the column volume and also repeating the steps three times. Again eluting the column three times using the aforesaid blocking buffer two times the column volume each time, and then let the column react 15-30 minutes to block and inactivate the functional groups in the column that are not bound with autoantibodies. After completing the blocking reaction, rinsing the column three times using the aforesaid washing buffer two times the column volume each time, followed by eluting the column three times using the aforesaid blocking buffer two times

the column volume to make sure all functional groups not bound with autoantibodies are blocked. Again rinsing three times the column using the washing buffer two times the column volume each time. Finally eluting the column with a pH neutral buffer 2-5 times the column volume to complete the preparation of the column packed with the autoantibodies.

Identification of autoantigens from extract of liver disease related cell lines

Firstly rinsing 2.68 mg of HepG2 C3A cells with culture medium removed with an ice-bathed Tris saline solution (50mM Tris pH 7.5, 150 mM NaCl, 1.5 mM PMSF, phosphatase inhibitors) twice, then adding in 1ml of Triton Extraction solution (15mM Tris pH 7.5, 120mM NaCl, 25mM KCl, 2mM EGTA, 0.1mM DTT, 0.5% Triton X-100, 10μg/ml leupeptin, 0.5mM PMSF, and phosphatase inhibitors) and let it stand for 30 minutes under 4°C. At this time, cells start to decompose and release proteins. Centrifuging (with a tabletop centrifuge) the solution at 14,000 rpm under 4°C for 15 minutes to remove solid, insoluble cell structures. Collecting the supernatants to carry on immunoaffinity chromatography.

After diluting the cell extract collected with the binding buffer at the ratio of 1:10, passing it through a 0.45μ filter membrane to prevent the blockage of the column in subsequent steps. Prior to injecting the sample into the IgG column, rinsing the normal and patient antibody columns with the binding buffer ten times the column volume at the rate of 1 ml/min. Then passing the filtered cell extract over the normal antibody column at the rate of 0.2 ml/min. Eluting the normal antibody column with the binding buffer 5-10 times the column volume at the rate of 1ml/min. At this time, antigens in the cell extract

that are identified and captured by the normal antibodies will be retained in the column. The purpose of this step is to remove non-specific antigens in the HepG2 C3A cells. As a result, the cell extract that has passed through the column is free of non-specific antigens. Injecting the resulting cell extract into the patient antibody column. Eluting the column with the binding buffer 5-10 times the column volume at the rate of 1 ml/min. At this time, the autoantigens present in the cell extract will be captured by the autoantibodies from the patients and retained in the column. When the cell extract passes over the normal antibody column, the antigens captured by the normal antibodies are retained in the column, whereas the cell extract free of antigens can be identified and captured by the normal antibodies, only antigens that can be identified and captured by the patient antibodies will be retained by the column. The antigens retained in the patient antibody column are eluted and collected using the elution buffer 2-5 times the column volume at the rate of 1ml/minl. Subjecting the flow-through to protein hydrolysis using trypsin and the resulting peptides are assayed using the mass spectrum technology. The resulting spectrographs are compared with the database to obtain the information on the proteins.

By screening liver disease related cell lines with autoantibodies in the serum of the patients with liver cirrhosis or liver cancer, the following autoantigens are obtained:

1. Nucleoside diphosphate kinase (gi | 1421609, SEQ ID NO.1).
2. NM23 protein (gi | 35068, SEQ ID NO.2).
3. ATP synthase beta chain, mitochondrial [precursor] (gi | 28940, SEQ ID NO.3).
4. 14-3-3 zeta protein (tyrosine 3/tryptophan 5-monooxygenase activation protein) (gi | 4507953, SEQ ID NO.4).

5. 14-3-3 epsilon protein (tyrosine 3/tryptophan 5-monooxygenase activation protein) (gi|4507953, SEQ ID NO.5).

6. Protein disulfide isomerase-related protein 5 (gi|1710248, SEQ ID NO.6).

7. Unnamed protein product (gi|21750187, SEQ ID NO.7).

8. Tropomyosin alpha 3 (gi|37403, SEQ ID NO.8).

9. Trypomyosin alpha 4 (gi|10435300, SEQ ID NO.9).

10. Calreticulin precursor (gi|4757900, SEQ ID NO.10).

11. Human pre-mRNA splicing factor SF2p32 (gi|338043, SEQ ID NO.11).

12. Tumor necrosis factor type I receptor associated protein TRAP-1 (gi|1082886, SEQ ID NO.12)..

13. Tumor rejection antigen (gp96) 1; glucose regulated protein (gi|4507677, SEQ ID NO.13).

14. Heat shock protein 90-beta (gi|72222, SEQ ID NO.14).

15. Heat shock protein 90-alpha (gi|123678, SEQ ID NO.15).

16. Heat shock 60kDa protein 1 (gi|31542947, SEQ ID NO.16).

17. HMG-1 (gi|968888, SEQ ID NO.17).

18. KIAA0144 gene product (NICE-4 protein) (gi|13111995, SEQ ID NO.18).

19. Valosin-containing protein (p97); transitional endoplasmic reciculum ATPase (gi|6005942, SEQ ID NO.19).

20. Glyceraldehyde 3-phosphate dehydrogenase, liver (gi|30157565, SEQ ID NO.20).

21. Cytokeratin (gi | 1419564, SEQ ID NO.21).

22. IGF-II mRNA-binding protein 1 (gi | 4191608, SEQ ID NO.22).

23. NADPH: quinone reductase (gi | 13236495, SEQ ID NO.23).

24. Crystal Structure of The Human Co-Chaperone P23 (hsp-90 co-chaperone) (gi | 9257073, SEQ ID NO.24).

The autoantigens identified with the antibodies from liver disease related cell lines are shown in Table 1; the left side of the Table 1 lists the GI number and name of the proteins and the right side indicates the autoantigens that may be identified from cell lines using sera of patients with liver cirrhosis or liver cancer. As shown, those autoantigens are not just present in one liver disease, they are repeatedly identified in different cell lines using autoantibodies in sera of different sources, indicating their close correlation with liver diseases. Some proteins listed in Table 1 have two GI numbers. That is because the protein and its variant had similar results in the mass spectrometry.

Table 2 Autoantigens screened from liver disease related cell lines

GI number	Name of protein	Liver cirrhosis serum vs. HepG2 C3A	Liver cancer serum vs. HepG2 C3A	Liver cirrhosis serum vs. SNU-387	Liver cancer serum vs. SNU-387
1421609	Nucleoside Diphosphate Kinase (=NM23 protein)	●	●	●	●
28940	ATP synthase beta chain, mitochondrial [Precursor]	●		●	
4507953, 5803225	14-3-3 protein	●		●	
1710248	Protein disulfide isomerase-related protein 5		●		●
21750187	Gi|21750187 Unnamed protein product (RAN_rec_mot.)		●		
37403, 10435300	Tropomyosin			●	●
4757900	Calreticulin precursor	●	●		
338043	Human pre-mRNA splicing factor SF2p32, complete sequence	●	●		
1082886	Tumor necrosis factor type 1 receptor associated protein TRAP-1	●	●		
4507677	Tumor protein antigen (gp96)1; glucose regulated protein	●	●		
72222, 123678	Heat shock protein 90	●	●		
31542947	Heat shock 60kDa protein 1 (chaperonin); mitochondrial matrix protein P1	●			
968888	HMG-1 (high-mobility group-1)	●			
13111995	KIAA0144 gene product (NICE-4 protein)	●			
6005942	Valosin-containing protein (p97); transitional endoplasmic reticulum ATPase	●			
30157565	Glyceraldehyde 3-phosphate dehydrogenase, liver	●			
1419564	Cytokeratin	●			
4191608	IGF-II mRNA-binding protein 1	●			
13236495	NADPH-quinone reductase		●		
9257073	Crystal Structure of The Human Co-Chaperone P23 (hsp-90 co-chaperone)		●		

Example 2–Determining the availability of autoantigens identified by the autoantigen screening method

To demonstrate the availability of 24 autoantigens identified in Example 1, further assay of serum samples from normal persons, liver cirrhosis patients and liver cancer patients using immunoassay (ELISA, RIA or immunofluorescence) and the aforesaid 24 biomarkers is carried out. The assay method includes the following steps as shown in Fig. 2: providing a specimen; using the biomarker selected from any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof to capture the autoantibody in the specimen; and detecting the autoantibody.

In the example of enzyme-linked immunosorbent assay (ELISA), the following steps are taken: firstly diluting the biomarker with a coating buffer (choice of a. 50mM Na_2HCO_3, pH=9.6, or b. 20mM Tris-HCl, pH=8.5, or c. 10mM PBS, pH=7.4) to a concentration of 0.5 ~ 10 μg/ml, where the coating buffer is selected according to the PI value of the biomarker, preferably a buffer having pH 1 ~ 2 higher than pI. Adding 100 μl/well biomarker solution to ELISA plate and let it stand overnight under 4°C for immobilization.

To continue the procedure, removing an unattached biomarker by washing the plate with a PBST buffer twice (PBST buffer: PSB buffer + 0.05% Tween-20), then adding a 200 μl/well blocking buffer (choice of a. Gelatin-NET: 0.5% Gelatin, 0.15M NaCl, 5mM EDTA•2Na, 0.05% Tween-20, 50mM Tris base, or b. 1% BSA-PBS, pH=7.4, or c. 5% non-fat milk-PBS, pH=7.4) and let blocking reaction go on for at least 2 hours

under ambient temperature; after the reaction is completed, washing with a PBST buffer three times and then depositing a 100 μl/well serum solution to be assayed (a serum solution is obtained by diluting the serum sample 1000 times with the blocking buffer). At this time, the autoantibodies in the serum will react with immobilized biomarkers. After reaction for at least 2 hours under ambient temperature, washing the plate four times with the PBST buffer and then adding in a 100 μl/well secondary antibody (diluted 5000 times with the blocking buffer). At this time, the secondary antibody would recognize and adsorb the autoantibody. After reaction for at least 1 hour under ambient temperature, washing the plate five times with the PBST buffer. Then adding in a 100 μl/well TMB to elicit color reaction for 30 minutes. Afterwards, adding a 100 μl/well 0.5M H_2SO_4 and detecting absorbance at 450nm.

To make sure the expression of the autoantibody can be used for diagnosis of liver cirrhosis and/or liver cancer, ELISA is employed to obtain the absorbance values of autoantibodies in the sera of normal persons, liver cirrhosis patients and liver cancer patients as identified by respective autoantigens. The data derived from five proteins-GADPH, NADPH, HMG-1, NM23 and Cytokeratin are subject to biostatistical analysis and Wilcoxon-Mann-Whitney Test. The following results at a 95% confidence level as shown in the table below are obtained:

	GADPH	NADPH	HMG-1	NM23	Cytokeratin
Normal person vs. Liver cirrhosis patient	p =0.001	p =0.001	p =0.00006	p =0.0001	p =0.001
Normal person	p =0.017	p =0.016	p =0.015	p =0.002	p =0.016

vs. Liver cancer patient					
Liver cirrhosis patient vs. Liver cancer patient	p>0.05	p>0.05	p>0.05	p >0.05	p >0.05

Normal person: N=10; liver cirrhosis patient: N=15; liver cancer patient: N=21 (the assumption of $p<0.05$ is valid)

Assuming there are differences between the expressions of biomarker-detected autoantibodies in normal persons, liver cirrhosis patients and liver cancer patients, the table above shows that such assumption was valid in normal persons versus liver cirrhosis patients and normal persons versus liver cancer patients, meaning the differences in the expression levels of biomarker-detected autoantibodies between normal persons and liver cirrhosis patients and between normal persons and liver cancer patients are statistically significant.

Statistics shows that the expression levels of GADPH-detected autoantibodies in normal persons and liver cirrhosis patients differed by 8.375 folds, while that in normal persons and liver cancer patients differed by 4.86 folds; the expression levels of HMG-1 - detected autoantibodies in normal persons and liver cirrhosis patients differed by 74 folds; the expression levels of NM23-detected autoantibodies in normal persons and liver cirrhosis patients differed by 24 folds, while that in normal persons and liver cancer patients differed by 8.545 folds. These results demonstrate that the expression levels of the antibodies in liver cirrhosis and liver cancer patients as detected by the 24 autoantigens provided herein were higher than those in normal persons. Thus a detection kit using those 24 autoantigens coupled with immunoassay may be applied in the

screening of liver cirrhosis and liver cancer based on the expression levels of autoantibodies in the screened specimens.

The preferred embodiment of the present invention as disclosed above is not meant to limit this invention. All modifications and alterations made by those familiar with the skill without departing from the spirits of the invention and appended claims shall remain within the protected scope and claims of the invention.

SEQUENCE LISTING

<110> Industrial Technology Research Institute

<120> Biomakers for Liver Diseases and Method for Using The Same

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<160> 24

<170> PatentIn version 3.2

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Ala Asn Leu Glu Arg Thr Phe Ile Ala Ile Lys Pro Asp Gly Val Gln
1 5 10 15

Arg Gly Leu Val Gly Glu Ile Ile Lys Arg Phe Glu Gln Lys Gly Phe
20 25 30

Arg Leu Val Ala Met Lys Phe Leu Arg Ala Ser Glu Glu His Leu Lys
35 40 45

Gln His Tyr Ile Asp Leu Lys Asp Arg Pro Phe Phe Pro Gly Leu Val
50 55 60

Lys Tyr Met Asn Ser Gly Pro Val Val Ala Met Val Trp Glu Gly Leu
65 70 75 80

Asn Val Val Lys Thr Gly Arg Val Met Leu Gly Glu Thr Asn Pro Ala
85 90 95

Asp Ser Lys Pro Gly Thr Ile Arg Gly Asp Phe Cys Ile Gln Val Gly
100 105 110

Arg Asn Ile Ile His Gly Ser Asp Ser Val Lys Ser Ala Glu Lys Glu
115 120 125

Ile Ser Leu Trp Phe Lys Pro Glu Glu Leu Val Asp Tyr Lys Ser Cys
130 135 140

Ala His Asp Trp Val Tyr Glu
145 150

<210> 2
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Cys Cys Glu Pro Arg Gly Ser Arg Ala Arg Phe Gly Cys Trp Arg Leu
1 5 10 15

Gln Pro Glu Phe Lys Pro Lys Gln Leu Glu Gly Thr Met Ala Asn Cys
20 25 30

Glu Arg Thr Phe Ile Ala Ile Lys Pro Asp Gly Val Gln Arg Gly Leu
35 40 45

Val Gly Glu Ile Ile Lys Arg Phe Glu Gln Lys Gly Phe Arg Leu Val
50 55 60

Gly Leu Lys Phe Met Gln Ala Ser Glu Asp Leu Leu Lys Glu His Tyr
65 70 75 80

Val Asp Leu Lys Asp Arg Pro Phe Phe Ala Gly Leu Val Lys Tyr Met
85 90 95

His Ser Gly Pro Val Val Ala Met Val Trp Glu Gly Leu Asn Val Val
100 105 110

Lys Thr Gly Arg Val Met Leu Gly Glu Thr Asn Pro Ala Asp Ser Lys
115 120 125

Pro Gly Thr Ile Arg Gly Asp Phe Cys Ile Gln Val Gly Arg Asn Ile
130 135 140

Ile His Gly Ser Asp Ser Val Glu Ser Ala Glu Lys Glu Ile Gly Leu
145 150 155 160

Trp Phe His Pro Glu Glu Leu Val Asp Tyr Thr Ser Cys Ala Gln Asn
165 170 175

Trp Ile Tyr Glu

180

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1 5 10 15

Arg Val Ala Ala Ala Pro Ala Ser Gly Ala Leu Arg Arg Leu Thr Pro
20 25 30

Ser Ala Ser Leu Pro Pro Ala Gln Leu Leu Leu Arg Ala Val Arg Arg
35 40 45

Arg Ser His Pro Val Arg Asp Tyr Ala Ala Gln Thr Ser Pro Ser Pro
50 55 60

Lys Ala Gly Ala Ala Thr Gly Arg Ile Val Ala Val Ile Gly Ala Val
65 70 75 80

Val Asp Val Gln Phe Asp Glu Gly Leu Pro Pro Ile Leu Asn Ala Leu
85 90 95

Glu Val Gln Gly Arg Glu Thr Arg Leu Val Leu Glu Val Ala Gln His
100 105 110

Leu Gly Glu Ser Thr Val Arg Thr Ile Ala Met Asp Gly Thr Glu Gly
115 120 125

Leu Val Arg Gly Gln Lys Val Leu Asp Ser Gly Ala Pro Ile Lys Ile
130 135 140

Pro Val Gly Pro Glu Thr Leu Gly Arg Ile Met Asn Val Ile Gly Glu
145 150 155 160

Pro Ile Asp Glu Arg Gly Pro Ile Lys Thr Lys Gln Phe Ala Pro Ile
165 170 175

His Ala Glu Ala Pro Glu Phe Met Glu Met Ser Val Glu Gln Glu Ile
180 185 190

Leu Val Thr Gly Ile Lys Val Val Asp Leu Leu Ala Pro Tyr Ala Lys
195 200 205

Gly Gly Lys Ile Gly Leu Phe Gly Gly Ala Gly Val Gly Lys Thr Val
210 215 220

Leu Ile Met Glu Leu Ile Asn Asn Val Ala Lys Ala His Gly Gly Tyr
225 230 235 240

Ser Val Phe Ala Gly Val Gly Glu Arg Thr Arg Glu Gly Asn Asp Leu
245 250 255

Tyr His Glu Met Ile Glu Ser Gly Val Ile Asn Leu Lys Asp Ala Thr
260 265 270

Ser Lys Val Ala Leu Val Tyr Gly Gln Met Asn Gln Pro Pro Gly Ala
275 280 285

Arg Ala Arg Val Ala Leu Thr Gly Leu Thr Val Ala Glu Tyr Phe Arg
290 295 300

Asp Gln Glu Gly Gln Asp Val Leu Leu Phe Ile Asp Asn Ile Phe Arg
305 310 315 320

Phe Thr Gln Ala Gly Ser Glu Val Ser Ala Leu Leu Gly Arg Ile Pro
325 330 335

Ser Ala Val Gly Tyr Gln Pro Thr Leu Ala Thr Asp Met Gly Thr Met
340 345 350

Gln Glu Arg Ile Thr Thr Thr Lys Lys Gly Ser Ile Thr Ser Val Gln
355 360 365

Ala Ile Tyr Val Pro Ala Asp Asp Leu Thr Asp Pro Ala Pro Ala Thr
370 375 380

Thr Phe Ala His Leu Asp Ala Thr Thr Val Leu Ser Arg Ala Ile Ala
385 390 395 400

Glu Leu Gly Ile Tyr Pro Ala Val Asp Pro Leu Asp Ser Thr Ser Arg
405 410 415

Ile Met Asp Pro Asn Ile Val Gly Ser Glu His Tyr Asp Val Ala Arg
420 425 430

Gly Val Gln Lys Ile Leu Gln Asp Tyr Lys Ser Leu Gln Asp Ile Ile
435 440 445

Ala Ile Leu Gly Met Asp Glu Leu Ser Glu Glu Asp Lys Leu Thr Val
450 455 460

Ser Arg Ala Arg Lys Ile Gln Arg Phe Leu Ser Gln Pro Phe Gln Val
465 470 475 480

Ala Glu Val Phe Thr Gly His Met Gly Lys Leu Val Pro Leu Lys Glu
485 490 495

Thr Ile Lys Gly Phe Gln Gln Ile Leu Ala Gly Glu Tyr Asp His Leu
500 505 510

Pro Glu Gln Ala Phe Tyr Met Val Gly Pro Ile Glu Glu Ala Val Ala
515 520 525

Lys Ala Asp Lys Leu Ala Glu Glu His Ser Ser
530 535

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Met Asp Lys Asn Glu Leu Val Gln Lys Ala Lys Leu Ala Glu Gln Ala
1 5 10 15

Glu Arg Tyr Asp Asp Met Ala Ala Cys Met Lys Ser Val Thr Glu Gln
20 25 30

Gly Ala Glu Leu Ser Asn Glu Glu Arg Asn Leu Leu Ser Val Ala Tyr
35 40 45

Lys Asn Val Val Gly Ala Arg Arg Ser Ser Trp Arg Val Val Ser Ser
50 55 60

Ile Glu Gln Lys Thr Glu Gly Ala Glu Lys Lys Gln Gln Met Ala Arg
65 70 75 80

Glu Tyr Arg Glu Lys Ile Glu Thr Glu Leu Arg Asp Ile Cys Asn Asp
85 90 95

Val Leu Ser Leu Leu Glu Lys Phe Leu Ile Pro Asn Ala Ser Gln Ala
100 105 110

Glu Ser Lys Val Phe Tyr Leu Lys Met Lys Gly Asp Tyr Tyr Arg Tyr
115 120 125

Leu Ala Glu Val Ala Ala Gly Asp Asp Lys Lys Gly Ile Val Asp Gln
130 135 140

Ser Gln Gln Ala Tyr Gln Glu Ala Phe Glu Ile Ser Lys Lys Glu Met

145 150 155 160

Gln Pro Thr His Pro Ile Arg Leu Gly Leu Ala Leu Asn Phe Ser Val
165 170 175

Phe Tyr Tyr Glu Ile Leu Asn Ser Pro Glu Lys Ala Cys Ser Leu Ala
180 185 190

Lys Thr Ala Phe Asp Glu Ala Ile Ala Glu Leu Asp Thr Leu Ser Glu
195 200 205

Glu Ser Tyr Lys Asp Ser Thr Leu Ile Met Gln Leu Leu Arg Asp Asn
210 215 220

Leu Thr Leu Trp Thr Ser Asp Thr Gln Gly Asp Glu Ala Glu Ala Gly
225 230 235 240

Glu Gly Gly Glu Asn
245

<210> 5
<211> 255
<212> PRT
<213> Human

<400> 5

Met Asp Asp Arg Glu Asp Leu Val Tyr Gln Ala Lys Leu Ala Glu Gln
1 5 10 15

Ala Glu Arg Tyr Asp Glu Met Val Glu Ser Met Lys Lys Val Ala Gly
20 25 30

Met Asp Val Glu Leu Thr Val Glu Glu Arg Asn Leu Leu Ser Val Ala
35 40 45

Tyr Lys Asn Val Ile Gly Ala Arg Arg Ala Ser Trp Arg Ile Ile Ser
50 55 60

Ser Ile Glu Gln Lys Glu Glu Asn Lys Gly Gly Glu Asp Lys Leu Lys
65 70 75 80

Met Ile Arg Glu Tyr Arg Gln Met Val Glu Thr Glu Leu Lys Leu Ile
85 90 95

Cys Cys Asp Ile Leu Asp Val Leu Asp Lys His Leu Ile Pro Ala Ala
100 105 110

Asn Thr Gly Glu Ser Lys Val Phe Tyr Tyr Lys Met Lys Gly Asp Tyr
115 120 125

His Arg Tyr Leu Ala Glu Phe Ala Thr Gly Asn Asp Arg Lys Glu Ala
130 135 140

Ala Glu Asn Ser Leu Val Ala Tyr Lys Ala Ala Ser Asp Ile Ala Met
145 150 155 160

Thr Glu Leu Pro Pro Thr His Pro Ile Arg Leu Gly Leu Ala Leu Asn
165 170 175

Phe Ser Val Phe Tyr Tyr Glu Ile Leu Asn Ser Pro Asp Arg Ala Cys
180 185 190

Arg Leu Ala Lys Ala Ala Phe Asp Asp Ala Ile Ala Glu Leu Asp Thr
195 200 205

Leu Ser Glu Glu Ser Tyr Lys Asp Ser Thr Leu Ile Met Gln Leu Leu
210 215 220

Arg Asp Asn Leu Thr Leu Trp Thr Ser Asp Met Gln Gly Asp Gly Glu
225 230 235 240

Glu Gln Asn Lys Glu Ala Leu Gln Asp Val Glu Asp Glu Asn Gln
245 250 255

<210> 6
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Leu Tyr Ser Ser Ser Asp Asp Val Ile Glu Leu Thr Pro Ser Asn Phe
1 5 10 15

Asn Arg Glu Val Ile Gln Ser Asp Ser Leu Trp Leu Val Glu Phe Tyr
20 25 30

Ala Pro Trp Cys Gly His Cys Gln Arg Leu Thr Pro Glu Trp Lys Lys

35 40 45

Ala Ala Thr Ala Leu Lys Asp Val Val Lys Val Gly Ala Val Asp Ala
50 55 60

Asp Lys His His Ser Leu Gly Gly Gln Tyr Gly Val Gln Gly Phe Pro
65 70 75 80

Thr Ile Lys Ile Phe Gly Ser Asn Lys Asn Arg Pro Glu Asp Tyr Gln
85 90 95

Gly Gly Arg Thr Gly Glu Ala Ile Val Asp Ala Ala Leu Ser Ala Leu
100 105 110

Arg Gln Leu Val Lys Asp Arg Leu Gly Gly Arg Ser Gly Gly Tyr Ser
115 120 125

Ser Gly Lys Gln Gly Arg Ser Asp Ser Ser Ser Lys Lys Asp Val Ile
130 135 140

Glu Leu Thr Asp Asp Ser Phe Asp Lys Asn Val Leu Asp Ser Glu Asp
145 150 155 160

Val Trp Met Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Asn
165 170 175

Leu Glu Pro Glu Trp Ala Ala Ala Ala Ser Glu Val Lys Glu Gln Thr
180 185 190

Lys Gly Arg Val Lys Leu Ala Ala Val Asp Ala Thr Val Asn Gln Val
195 200 205

Leu Ala Ser Arg Tyr Gly Ile Arg Gly Phe Pro Thr Ile Lys Ile Phe
210 215 220

Gln Lys Gly Glu Ser Pro Val Asp Tyr Asp Gly Gly Arg Thr Arg Ser
225 230 235 240

Asp Ile Val Ser Arg Ala Leu Asp Leu Phe Ser Asp Asn Ala Pro Pro
245 250 255

Pro Glu Leu Leu Glu Ile Ile Asn Glu Asp Ile Ala Lys Arg Thr Cys
260 265 270

Glu Glu His Gln Leu Cys Val Val Ala Val Leu Pro His Ile Leu Asp
275 280 285

Thr Gly Ala Ala Gly Arg Asn Ser Tyr Leu Glu Val Leu Leu Lys Leu
290 295 300

Ala Asp Lys Tyr Lys Lys Lys Met Trp Gly Trp Leu Trp Thr Glu Ala
305 310 315 320

Gly Ala Gln Ser Glu Leu Glu Thr Ala Leu Gly Ile Gly Gly Phe Gly
325 330 335

Tyr Pro Ala Met Ala Ala Ile Asn Ala Arg Lys Met Lys Phe Ala Leu

340 345 350

Leu Lys Gly Ser Phe Ser Glu Gln Gly Ile Asn Glu Phe Leu Arg Glu
355 360 365

Leu Ser Phe Gly Arg Gly Ser Thr Ala Pro Val Gly Gly Gly Ala Phe
370 375 380

Pro Thr Ile Val Glu Arg Glu Pro Trp Asp Gly Arg Asp Gly Glu Leu
385 390 395 400

Pro Val Glu Asp Asp Ile Asp Leu Ser Asp Val Glu Leu Asp Asp Leu
405 410 415

Gly Lys Asp Glu Leu
420

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<211> 687
<212> PRT
<213> Human

<400> 7

Met Val Lys Leu Ala Lys Ala Gly Lys Asn Gln Gly Asp Pro Lys Lys
1 5 10 15

Met Ala Pro Pro Pro Lys Glu Val Glu Glu Asp Ser Glu Asp Glu Glu
20 25 30

Met Ser Glu Asp Glu Glu Asp Asp Ser Ser Gly Glu Glu Val Val Ile
35 40 45

Pro Gln Lys Lys Gly Lys Lys Ala Ala Ala Thr Ser Ala Lys Lys Val
50 55 60

Val Val Ser Pro Thr Lys Lys Val Ala Val Ala Thr Pro Ala Lys Ala
65 70 75 80

Val Thr Thr Pro Gly Lys Lys Gly Ala Thr Pro Gly Lys Ala Leu Val
85 90 95

Ala Thr Pro Gly Lys Lys Gly Ala Ala Ile Pro Ala Lys Gly Ala Lys
100 105 110

Asn Gly Lys Asn Ala Lys Lys Glu Asp Ser Asp Glu Glu Glu Asp Asp
115 120 125

Asp Ser Glu Glu Asp Glu Glu Asp Asp Glu Asp Glu Asp Glu Asp Glu
130 135 140

Asp Glu Ile Glu Pro Ala Ala Met Lys Ala Ala Ala Ala Ala Pro Ala
145 150 155 160

Ser Glu Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Asp
165 170 175

Asp Asp Glu Glu Asp Asp Ser Glu Glu Glu Ala Met Glu Thr Thr Pro
180 185 190

Ala Lys Gly Lys Lys Ala Ala Lys Val Val Pro Val Lys Ala Lys Asn
195 200 205

Val Ala Glu Asp Glu Asp Glu Glu Glu Asp Asp Glu Asp Glu Asp Asp
210 215 220

Asp Asp Asp Glu Asp Asp Glu Asp Asp Asp Asp Glu Asp Asp Glu Glu
225 230 235 240

Glu Glu Glu Glu Glu Glu Glu Glu Pro Val Lys Glu Ala Pro Gly Lys
245 250 255

Arg Lys Lys Glu Met Ala Lys Gln Lys Ala Ala Pro Glu Ala Lys Lys
260 265 270

Gln Lys Val Glu Gly Thr Glu Pro Thr Thr Ala Phe Asn Leu Phe Val
275 280 285

Gly Asn Leu Asn Phe Asn Lys Ser Ala Pro Glu Leu Lys Thr Gly Ile
290 295 300

Ser Asp Val Phe Ala Lys Asn Asp Leu Ala Val Val Asp Val Arg Ile
305 310 315 320

Gly Met Thr Arg Lys Phe Gly Tyr Val Asp Phe Glu Ser Ala Glu Asp
325 330 335

Leu Glu Lys Ala Leu Glu Leu Thr Gly Leu Lys Val Phe Gly Asn Glu
340 345 350

Ile Lys Leu Glu Lys Pro Lys Gly Lys Asp Ser Lys Lys Glu Arg Asp
355 360 365

Ala Arg Thr Leu Leu Ala Lys Asn Leu Pro Tyr Lys Val Thr Gln Asp
370 375 380

Glu Leu Lys Glu Val Phe Glu Asp Ala Ala Glu Ile Arg Leu Val Ser
385 390 395 400

Lys Asp Gly Lys Ser Lys Gly Ile Ala Tyr Ile Glu Phe Lys Thr Glu
405 410 415

Ala Asp Ala Glu Lys Thr Phe Glu Glu Lys Gln Gly Thr Glu Ile Asp
420 425 430

Gly Arg Ser Ile Ser Leu Tyr Tyr Thr Gly Glu Lys Gly Gln Asn Gln
435 440 445

Asp Tyr Arg Gly Gly Lys Asn Ser Thr Trp Ser Gly Glu Ser Lys Thr
450 455 460

Leu Val Leu Ser Asn Leu Ser Tyr Ser Ala Thr Glu Glu Thr Leu Gln
465 470 475 480

Glu Val Phe Glu Lys Ala Thr Phe Ile Lys Val Pro Gln Asn Gln Asn
485 490 495

Gly Lys Ser Lys Gly Tyr Ala Phe Ile Glu Phe Ala Ser Phe Glu Asp
500 505 510

Ala Lys Glu Ala Leu Asn Ser Cys Asn Lys Arg Glu Ile Gly Gly Arg
515 520 525

Ala Ile Arg Leu Glu Leu Gln Gly Pro Arg Gly Ser Pro Asn Ala Arg
530 535 540

Ser Gln Pro Ser Lys Thr Leu Phe Val Lys Gly Leu Ser Glu Asp Thr
545 550 555 560

Thr Glu Glu Thr Leu Lys Glu Ser Phe Asp Gly Ser Val Arg Ala Arg
565 570 575

Ile Val Thr Asp Arg Glu Thr Gly Ser Ser Lys Gly Phe Gly Phe Val
580 585 590

Asp Phe Asn Ser Glu Glu Asp Ala Lys Ala Ala Lys Glu Ala Met Glu
595 600 605

Asp Gly Glu Ile Asp Gly Asn Lys Val Thr Leu Asp Trp Ala Lys Pro
610 615 620

Lys Gly Glu Gly Gly Phe Gly Gly Arg Gly Gly Gly Arg Gly Gly Phe
625 630 635 640

Gly Gly Arg Gly Gly Gly Arg Gly Gly Arg Gly Gly Phe Gly Gly Arg
645 650 655

Gly Arg Gly Gly Phe Gly Gly Arg Gly Gly Phe Arg Gly Gly Arg Gly
660 665 670

Gly Gly Gly Asp His Lys Pro Gln Gly Lys Lys Thr Lys Phe Glu
675 680 685

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Met Ala Gly Ile Thr Thr Ile Glu Ala Val Lys Arg Lys Ile Gln Val
1 5 10 15

Leu Gln Gln Gln Ala Asp Asp Ala Glu Glu Arg Ala Glu Arg Leu Gln
20 25 30

Arg Glu Val Glu Gly Glu Arg Arg Ala Arg Glu Gln Ala Glu Ala Glu
35 40 45

Val Ala Ser Leu Asn Arg Arg Ile Gln Leu Val Glu Glu Glu Leu Asp
50 55 60

Arg Ala Gln Glu Arg Leu Ala Thr Ala Leu Gln Lys Leu Glu Glu Ala
65 70 75 80

Glu Lys Ala Ala Asp Glu Ser Glu Arg Gly Met Lys Val Ile Glu Asn
85 90 95

Arg Ala Leu Lys Asp Glu Glu Lys Met Glu Leu Gln Glu Ile Gln Leu
100 105 110

Glu Glu Ala Lys His Ile Ala Glu Glu Ala Asp Arg Lys Tyr Glu Glu
115 120 125

Val Ala Arg Lys Leu Val Ile Ile Glu Gly Asp Leu Glu Arg Thr Glu
130 135 140

Glu Arg Ala Glu Leu Ala Glu Ser Arg Cys Arg Glu Met Asp Glu Gln
145 150 155 160

Ile Arg Leu Met Asp Gln Asn Leu Lys Cys Leu Ser Ala Ala Glu Glu
165 170 175

Lys Tyr Ser Gln Lys Glu Asp Lys Tyr Glu Glu Glu Ile Lys Ile Leu
180 185 190

Thr Asp Lys Leu Lys Glu Ala Glu Thr Arg Ala Glu Phe Ala Glu Arg
195 200 205

Ser Val Ala Lys Leu Glu Lys Thr Ile Asp Asp Leu Glu Asp Thr Asn
210 215 220

Ser Thr Ser Gly Asp Pro Val Glu Lys Lys Asp Glu Thr Pro Phe Gly

225 230 235 240

Val Ser Val Ala Val Gly Leu Ala Val Phe Ala Cys Leu Phe Leu Ser
245 250 255

Thr Leu Leu Leu Val Leu Asn Lys Cys Gly Arg Arg Asn Lys Phe Gly
260 265 270

Ile Asn Arg Pro Ala Val Leu Ala Pro Glu Asp Gly Leu Ala Met Ser
275 280 285

Leu His Phe Met Thr Leu Gly Gly Ser Ser Leu Ser Pro Thr Glu Gly
290 295 300

Lys Gly Ser Gly Leu Gln Gly His Ile Ile Glu Asn Pro Gln Tyr Phe
305 310 315 320

Ser Asp Ala Cys Val His His Ile Lys Arg Arg Asp Ile Val Leu Lys
325 330 335

Trp Glu Leu Gly Glu Gly Ala Phe Gly Lys Val Phe Leu Ala Glu Cys
340 345 350

His Asn Leu Leu Pro Glu Gln Asp Lys Met Leu Val Ala Val Lys Ala
355 360 365

Leu Lys Glu Ala Ser Glu Ser Ala Arg Gln Asp Phe Gln Arg Glu Ala
370 375 380

Glu Leu Leu Thr Met Leu Gln His Gln His Ile Val Arg Phe Phe Gly
385 390 395 400

Val Cys Thr Glu Gly Arg Pro Leu Leu Met Val Phe Glu Tyr Met Arg
405 410 415

His Gly Asp Leu Asn Arg Phe Leu Arg Ser His Gly Pro Asp Ala Lys
420 425 430

Leu Leu Ala Gly Gly Glu Asp Val Ala Pro Gly Pro Leu Gly Leu Gly
435 440 445

Gln Leu Leu Ala Val Ala Ser Gln Val Ala Ala Gly Met Val Tyr Leu
450 455 460

Ala Gly Leu His Phe Val His Arg Asp Leu Ala Thr Arg Asn Cys Leu
465 470 475 480

Val Gly Gln Gly Leu Val Val Lys Ile Gly Asp Phe Gly Met Ser Arg
485 490 495

Asp Ile Tyr Ser Thr Asp Tyr Tyr Arg Val Gly Gly Arg Thr Met Leu
500 505 510

Pro Ile Arg Trp Met Pro Pro Glu Ser Ile Leu Tyr Arg Lys Phe Thr
515 520 525

Thr Glu Ser Asp Val Trp Ser Phe Gly Val Val Leu Trp Glu Ile Phe

530 535 540

Thr Tyr Gly Lys Gln Pro Trp Tyr Gln Leu Ser Asn Thr Glu Ala Ile
545 550 555 560

Asp Cys Ile Thr Gln Gly Arg Glu Leu Glu Arg Pro Arg Ala Cys Pro
565 570 575

Pro Glu Val Tyr Ala Ile Met Arg Gly Cys Trp Gln Arg Glu Pro Ser
580 585 590

Asn Ala Thr Ala Ser Arg Met Cys Thr Pro Gly Cys Lys Pro Trp Pro
595 600 605

Arg His Leu Leu Ser Thr Trp Met Ser Trp Ala Arg Gly Pro Ala Gln
610 615 620

Gly Leu Gly Val Val Ser Arg Asn Thr Gly Ala Cys Pro Gln His Pro
625 630 635 640

Pro

<210> 9
<211> 284
<212> PRT
<213> Human

<400> 9

Met Glu Ala Ile Lys Lys Lys Met Gln Met Leu Lys Leu Asp Lys Glu
1 5 10 15

Asn Ala Ile Asp Arg Ala Glu Gln Ala Glu Ala Asp Lys Lys Ala Ala
20 25 30

Glu Asp Lys Cys Lys Gln Val Glu Glu Glu Leu Thr His Leu Gln Lys
35 40 45

Lys Leu Lys Gly Thr Glu Asp Glu Leu Asp Lys Tyr Ser Glu Asp Leu
50 55 60

Lys Asp Ala Gln Glu Lys Leu Glu Leu Thr Glu Lys Lys Ala Ser Asp
65 70 75 80

Ala Glu Gly Asp Val Ala Ala Leu Asn Arg Arg Ile Gln Leu Val Glu
85 90 95

Glu Glu Leu Asp Arg Ala Gln Glu Arg Leu Ala Thr Ala Leu Gln Lys
100 105 110

Leu Glu Glu Ala Glu Lys Ala Ala Asp Glu Ser Glu Arg Gly Met Lys
115 120 125

Val Ile Glu Asn Arg Ala Met Lys Asp Glu Glu Lys Met Glu Ile Gln
130 135 140

Glu Met Gln Leu Lys Glu Ala Lys His Ile Ala Glu Glu Ala Asp Arg
145 150 155 160

Lys Tyr Glu Glu Val Ala Arg Lys Leu Val Ile Leu Glu Gly Glu Leu
165 170 175

Glu Arg Ala Glu Glu Arg Ala Glu Val Ser Glu Leu Lys Cys Gly Asp
180 185 190

Leu Glu Glu Glu Leu Lys Asn Val Thr Asn Asn Leu Lys Ser Leu Glu
195 200 205

Ala Ala Ser Glu Lys Tyr Ser Glu Lys Glu Asp Lys Tyr Glu Glu Glu
210 215 220

Ile Lys Leu Leu Ser Asp Lys Leu Lys Glu Ala Glu Thr Arg Ala Glu
225 230 235 240

Phe Ala Glu Arg Thr Val Ala Lys Leu Glu Lys Thr Ile Asp Asp Leu
245 250 255

Glu Glu Lys Leu Ala Gln Ala Lys Glu Glu Asn Val Gly Leu His Gln
260 265 270

Thr Leu Asp Gln Thr Leu Asn Glu Leu Asn Cys Ile
275 280

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<211> 417
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<400> 10

Met Leu Leu Ser Val Pro Leu Leu Leu Gly Leu Leu Gly Leu Ala Val
1 5 10 15

Ala Glu Pro Ala Val Tyr Phe Lys Glu Gln Phe Leu Asp Gly Asp Gly
20 25 30

Trp Thr Ser Arg Trp Ile Glu Ser Lys His Lys Ser Asp Phe Gly Lys
35 40 45

Phe Val Leu Ser Ser Gly Lys Phe Tyr Gly Asp Glu Glu Lys Asp Lys
50 55 60

Gly Leu Gln Thr Ser Gln Asp Ala Arg Phe Tyr Ala Leu Ser Ala Ser
65 70 75 80

Phe Glu Pro Phe Ser Asn Lys Gly Gln Thr Leu Val Val Gln Phe Thr
85 90 95

Val Lys His Glu Gln Asn Ile Asp Cys Gly Gly Gly Tyr Val Lys Leu
100 105 110

Phe Pro Asn Ser Leu Asp Gln Thr Asp Met His Gly Asp Ser Glu Tyr
115 120 125

Asn Ile Met Phe Gly Pro Asp Ile Cys Gly Pro Gly Thr Lys Lys Val
130 135 140

His Val Ile Phe Asn Tyr Lys Gly Lys Asn Val Leu Ile Asn Lys Asp
145 150 155 160

Ile Arg Cys Lys Asp Asp Glu Phe Thr His Leu Tyr Thr Leu Ile Val
165 170 175

Arg Pro Asp Asn Thr Tyr Glu Val Lys Ile Asp Asn Ser Gln Val Glu
180 185 190

Ser Gly Ser Leu Glu Asp Asp Trp Asp Phe Leu Pro Pro Lys Lys Ile
195 200 205

Lys Asp Pro Asp Ala Ser Lys Pro Glu Asp Trp Asp Glu Arg Ala Lys
210 215 220

Ile Asp Asp Pro Thr Asp Ser Lys Pro Glu Asp Trp Asp Lys Pro Glu
225 230 235 240

His Ile Pro Asp Pro Asp Ala Lys Lys Pro Glu Asp Trp Asp Glu Glu
245 250 255

Met Asp Gly Glu Trp Glu Pro Pro Val Ile Gln Asn Pro Glu Tyr Lys
260 265 270

Gly Glu Trp Lys Pro Arg Gln Ile Asp Asn Pro Asp Tyr Lys Gly Thr
275 280 285

Trp Ile His Pro Glu Ile Asp Asn Pro Glu Tyr Ser Pro Asp Pro Ser

290 295 300

Ile Tyr Ala Tyr Asp Asn Phe Gly Val Leu Gly Leu Asp Leu Trp Gln
305 310 315 320

Val Lys Ser Gly Thr Ile Phe Asp Asn Phe Leu Ile Thr Asn Asp Glu
325 330 335

Ala Tyr Ala Glu Glu Phe Gly Asn Glu Thr Trp Gly Val Thr Lys Ala
340 345 350

Ala Glu Lys Gln Met Lys Asp Lys Gln Asp Glu Glu Gln Arg Leu Lys
355 360 365

Glu Glu Glu Glu Asp Lys Lys Arg Lys Glu Glu Glu Glu Ala Glu Asp
370 375 380

Lys Glu Asp Asp Glu Asp Lys Asp Glu Asp Glu Glu Asp Glu Glu Asp
385 390 395 400

Lys Glu Glu Asp Glu Glu Glu Asp Val Pro Gly Gln Ala Lys Asp Glu
405 410 415

Leu

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Leu Arg Cys Val Pro Arg Val Leu Gly Ser Ser Val Ala Gly Leu Arg
1 5 10 15

Ala Ala Ala Pro Ala Ser Pro Phe Arg Gln Leu Leu Gln Pro Ala Pro
20 25 30

Arg Leu Cys Thr Arg Pro Phe Gly Leu Leu Ser Val Arg Ala Gly Ser
35 40 45

Glu Arg Arg Pro Gly Leu Leu Arg Pro Arg Gly Pro Cys Ala Cys Gly
50 55 60

Cys Gly Cys Gly Ser Leu His Thr Asp Gly Asp Lys Ala Phe Val Asp
65 70 75 80

Phe Leu Ser Asp Glu Ile Lys Glu Glu Arg Lys Ile Gln Lys His Lys
85 90 95

Thr Leu Pro Lys Met Ser Gly Gly Trp Glu Leu Glu Leu Asn Gly Thr
100 105 110

Glu Ala Lys Leu Val Arg Lys Val Ala Gly Glu Lys Ile Thr Val Thr
115 120 125

Phe Asn Ile Asn Asn Ser Ile Pro Pro Thr Phe Asp Gly Glu Glu Glu
130 135 140

Pro Ser Gln Gly Gln Lys Val Glu Glu Gln Glu Pro Glu Leu Thr Ser
145 150 155 160

Thr Pro Asn Phe Val Val Glu Val Ile Lys Asn Asp Asp Gly Lys Lys
165 170 175

Ala Leu Val Leu Asp Cys His Tyr Pro Glu Asp Glu Val Gly Gln Glu
180 185 190

Asp Glu Ala Glu Ser Asp Ile Phe Ser Ile Arg Glu Val Ser Phe Gln
195 200 205

Ser Thr Gly Glu Ser Glu Trp Lys Asp Thr Asn Tyr Thr Leu Asn Thr
210 215 220

Asp Ser Leu Asp Trp Ala Leu Tyr Asp His Leu Met Asp Phe Leu Ala
225 230 235 240

Asp Arg Gly Val Asp Asn Thr Phe Ala Asp Glu Leu Val Glu Leu Ser
245 250 255

Thr Ala Leu Glu His Gln Glu Tyr Ile Thr Phe Leu Glu Asp Leu Lys
260 265 270

Ser Phe Val Lys Ser Gln
275

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Arg Ala Leu Arg Arg Ala Pro Ala Leu Ala Ala Val Pro Gly Gly Lys
1 5 10 15

Pro Ile Leu Cys Pro Arg Arg Thr Thr Ala Gln Leu Gly Pro Arg Arg
20 25 30

Asn Pro Ala Trp Ser Leu Gln Ala Gly Arg Leu Phe Ser Thr Gln Thr
35 40 45

Ala Glu Asp Lys Glu Glu Pro Leu His Ser Ile Ile Ser Ser Thr Glu
50 55 60

Ser Val Gln Gly Ser Thr Ser Lys His Glu Phe Gln Ala Glu Thr Lys
65 70 75 80

Lys Leu Leu Asp Ile Val Ala Arg Ser Leu Tyr Ser Glu Lys Glu Val
85 90 95

Phe Ile Arg Glu Leu Ile Ser Asn Ala Ser Asp Ala Leu Glu Lys Leu
100 105 110

Arg His Lys Leu Val Ser Asp Gly Gln Ala Leu Pro Glu Met Glu Ile
115 120 125

His Leu Gln Thr Asn Ala Glu Lys Gly Thr Ile Thr Ile Gln Asp Thr
130 135 140

Gly Ile Gly Met Thr Gln Glu Glu Leu Val Ser Asn Leu Gly Thr Ile
145 150 155 160

Ala Arg Ser Gly Ser Lys Ala Phe Leu Asp Ala Leu Gln Asn Gln Ala
165 170 175

Glu Ala Ser Ser Lys Ile Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser
180 185 190

Ala Phe Met Val Ala Asp Arg Val Glu Val Tyr Ser Arg Ser Ala Ala
195 200 205

Pro Gly Ser Leu Gly Tyr Gln Trp Leu Ser Asp Gly Ser Gly Val Phe
210 215 220

Glu Ile Ala Glu Ala Ser Gly Val Arg Thr Gly Thr Lys Ile Ile Ile
225 230 235 240

His Leu Lys Ser Asp Cys Lys Glu Phe Ser Ser Glu Ala Arg Val Arg
245 250 255

Asp Val Val Thr Lys Tyr Ser Asn Phe Val Ser Phe Pro Leu Tyr Leu
260 265 270

Asn Gly Arg Arg Met Asn Thr Leu Gln Ala Ile Trp Met Met Asp Pro

275 280 285

Lys Asp Val Gly Glu Trp Gln His Glu Glu Phe Tyr Arg Tyr Val Ala
290 295 300

Gln Ala His Asp Lys Pro Arg Tyr Thr Leu His Tyr Lys Thr Asp Ala
305 310 315 320

Pro Leu Asn Ile Arg Ser Ile Phe Tyr Val Pro Asp Met Lys Pro Ser
325 330 335

Met Phe Asp Val Ser Arg Glu Leu Gly Ser Ser Val Ala Leu Tyr Ser
340 345 350

Arg Lys Val Leu Ile Gln Thr Lys Ala Thr Asp Ile Leu Pro Lys Trp
355 360 365

Leu Arg Phe Ile Arg Gly Val Val Asp Ser Glu Asp Ile Pro Leu Asn
370 375 380

Leu Ser Arg Glu Leu Leu Gln Glu Ser Ala Leu Ile Arg Lys Leu Arg
385 390 395 400

Asp Val Leu Gln Gln Arg Leu Ile Lys Phe Phe Ile Asp Gln Ser Lys
405 410 415

Lys Asp Ala Glu Lys Tyr Ala Lys Phe Phe Glu Asp Tyr Gly Leu Phe
420 425 430

Met Arg Glu Gly Ile Val Thr Ala Thr Glu Gln Glu Val Lys Glu Asp
435 440 445

Ile Ala Lys Leu Leu Arg Tyr Glu Ser Ser Ala Leu Pro Ser Gly Gln
450 455 460

Leu Thr Ser Leu Ser Glu Tyr Ala Ser Arg Met Arg Ala Gly Thr Arg
465 470 475 480

Asn Ile Tyr Tyr Leu Cys Ala Pro Asn Arg His Leu Ala Glu His Ser
485 490 495

Pro Tyr Tyr Glu Ala Met Lys Lys Lys Asp Thr Glu Val Leu Phe Cys
500 505 510

Phe Glu Gln Phe Asp Glu Leu Thr Leu Leu His Leu Arg Glu Phe Asp
515 520 525

Lys Lys Lys Leu Ile Ser Val Glu Thr Asp Ile Val Val Asp His Tyr
530 535 540

Lys Glu Glu Lys Phe Glu Asp Arg Ser Pro Ala Ala Glu Cys Leu Ser
545 550 555 560

Glu Lys Glu Thr Glu Glu Leu Met Ala Trp Met Arg Asn Val Leu Gly
565 570 575

Ser Arg Val Thr Asn Val Lys Val Thr Leu Arg Leu Asp Thr His Pro

580 585 590

Ala Met Val Thr Val Leu Glu Met Gly Ala Ala Arg His Phe Leu Arg
595 600 605

Met Gln Gln Leu Ala Lys Thr Gln Glu Glu Arg Ala Gln Leu Leu Gln
610 615 620

Pro Thr Leu Glu Ile Asn Pro Arg His Ala Leu Ile Lys Lys Leu Asn
625 630 635 640

His Cys Ala Gln Ala Ser Leu Ala Trp Leu Ser Cys Trp Trp Ile Arg
645 650 655

Tyr Thr Arg Thr Pro
660

<210> 13
<211> 803
<212> PRT
<213> Human

<400> 13

Met Arg Ala Leu Trp Val Leu Gly Leu Cys Cys Val Leu Leu Thr Phe
1 5 10 15

Gly Ser Val Arg Ala Asp Asp Glu Val Asp Val Asp Gly Thr Val Glu
20 25 30

Glu Asp Leu Gly Lys Ser Arg Glu Gly Ser Arg Thr Asp Asp Glu Val
35 40 45

Val Gln Arg Glu Glu Glu Ala Ile Gln Leu Asp Gly Leu Asn Ala Ser
50 55 60

Gln Ile Arg Glu Leu Arg Glu Lys Ser Glu Lys Phe Ala Phe Gln Ala
65 70 75 80

Glu Val Asn Arg Met Met Lys Leu Ile Ile Asn Ser Leu Tyr Lys Asn
85 90 95

Lys Glu Ile Phe Leu Arg Glu Leu Ile Ser Asn Ala Ser Asp Ala Leu
100 105 110

Asp Lys Ile Arg Leu Ile Ser Leu Thr Asp Glu Asn Ala Leu Ser Gly
115 120 125

Asn Glu Glu Leu Thr Val Lys Ile Lys Cys Asp Lys Glu Lys Asn Leu
130 135 140

Leu His Val Thr Asp Thr Gly Val Gly Met Thr Arg Glu Glu Leu Val
145 150 155 160

Lys Asn Leu Gly Thr Ile Ala Lys Ser Gly Thr Ser Glu Phe Leu Asn
165 170 175

Lys Met Thr Glu Ala Gln Glu Asp Gly Gln Ser Thr Ser Glu Leu Ile
180 185 190

Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Phe Leu Val Ala Asp Lys
195 200 205

Val Ile Val Thr Ser Lys His Asn Asn Asp Thr Gln His Ile Trp Glu
210 215 220

Ser Asp Ser Asn Glu Phe Ser Val Ile Ala Asp Pro Arg Gly Asn Thr
225 230 235 240

Leu Gly Arg Gly Thr Thr Ile Thr Leu Val Leu Lys Glu Glu Ala Ser
245 250 255

Asp Tyr Leu Glu Leu Asp Thr Ile Lys Asn Leu Val Lys Lys Tyr Ser
260 265 270

Gln Phe Ile Asn Phe Pro Ile Tyr Val Trp Ser Ser Lys Thr Glu Thr
275 280 285

Val Glu Glu Pro Met Glu Glu Glu Glu Ala Ala Lys Glu Glu Lys Glu
290 295 300

Glu Ser Asp Asp Glu Ala Ala Val Glu Glu Glu Glu Glu Glu Lys Lys
305 310 315 320

Pro Lys Thr Lys Lys Val Glu Lys Thr Val Trp Asp Trp Glu Leu Met
325 330 335

Asn Asp Ile Lys Pro Ile Trp Gln Arg Pro Ser Lys Glu Val Glu Glu
340 345 350

Asp Glu Tyr Lys Ala Phe Tyr Lys Ser Phe Ser Lys Glu Ser Asp Asp
355 360 365

Pro Met Ala Tyr Ile His Phe Thr Ala Glu Gly Glu Val Thr Phe Lys
370 375 380

Ser Ile Leu Phe Val Pro Thr Ser Ala Pro Arg Gly Leu Phe Asp Glu
385 390 395 400

Tyr Gly Ser Lys Lys Ser Asp Tyr Ile Lys Leu Tyr Val Arg Arg Val
405 410 415

Phe Ile Thr Asp Asp Phe His Asp Met Met Pro Lys Tyr Leu Asn Phe
420 425 430

Val Lys Gly Val Val Asp Ser Asp Asp Leu Pro Leu Asn Val Ser Arg
435 440 445

Glu Thr Leu Gln Gln His Lys Leu Leu Lys Val Ile Arg Lys Lys Leu
450 455 460

Val Arg Lys Thr Leu Asp Met Ile Lys Lys Ile Ala Asp Asp Lys Tyr
465 470 475 480

Asn Asp Thr Phe Trp Lys Glu Phe Gly Thr Asn Ile Lys Leu Gly Val
485 490 495

Ile Glu Asp His Ser Asn Arg Thr Arg Leu Ala Lys Leu Leu Arg Phe
500 505 510

Gln Ser Ser His His Pro Thr Asp Ile Thr Ser Leu Asp Gln Tyr Val
515 520 525

Glu Arg Met Lys Glu Lys Gln Asp Lys Ile Tyr Phe Met Ala Gly Ser
530 535 540

Ser Arg Lys Glu Ala Glu Ser Ser Pro Phe Val Glu Arg Leu Leu Lys
545 550 555 560

Lys Gly Tyr Glu Val Ile Tyr Leu Thr Glu Pro Val Asp Glu Tyr Cys
565 570 575

Ile Gln Ala Leu Pro Glu Phe Asp Gly Lys Arg Phe Gln Asn Val Ala
580 585 590

Lys Glu Gly Val Lys Phe Asp Glu Ser Glu Lys Thr Lys Glu Ser Arg
595 600 605

Glu Ala Val Glu Lys Glu Phe Glu Pro Leu Leu Asn Trp Met Lys Asp
610 615 620

Lys Ala Leu Lys Asp Lys Ile Glu Lys Ala Val Val Ser Gln Arg Leu
625 630 635 640

Thr Glu Ser Pro Cys Ala Leu Val Ala Ser Gln Tyr Gly Trp Ser Gly
645 650 655

Asn Met Glu Arg Ile Met Lys Ala Gln Ala Tyr Gln Thr Gly Lys Asp
660 665 670

Ile Ser Thr Asn Tyr Tyr Ala Ser Gln Lys Lys Thr Phe Glu Ile Asn
675 680 685

Pro Arg His Pro Leu Ile Arg Asp Met Leu Arg Arg Ile Lys Glu Asp
690 695 700

Glu Asp Asp Lys Thr Val Leu Asp Leu Ala Val Val Leu Phe Glu Thr
705 710 715 720

Ala Thr Leu Arg Ser Gly Tyr Leu Leu Pro Asp Thr Lys Ala Tyr Gly
725 730 735

Asp Arg Ile Glu Arg Met Leu Arg Leu Ser Leu Asn Ile Asp Pro Asp
740 745 750

Ala Lys Val Glu Glu Glu Pro Glu Glu Glu Pro Glu Glu Thr Ala Glu
755 760 765

Asp Thr Thr Glu Asp Thr Glu Gln Asp Glu Asp Glu Glu Met Asp Val
770 775 780

Gly Thr Asp Glu Glu Glu Glu Thr Ala Lys Glu Ser Thr Ala Glu Lys
785 790 795 800

Asp Glu Leu

<210> 14
<211> 724
<212> PRT
<213> Human

<400> 14

Met Pro Glu Glu Val His His Gly Glu Glu Glu Val Glu Thr Phe Ala
1 5 10 15

Phe Gln Ala Glu Ile Ala Gln Leu Met Ser Leu Ile Ile Asn Thr Phe
20 25 30

Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu Leu Ile Ser Asn Ala Ser
35 40 45

Asp Ala Leu Asp Lys Ile Arg Tyr Glu Ser Leu Thr Asp Pro Ser Lys
50 55 60

Leu Asp Ser Gly Lys Glu Leu Lys Ile Asp Ile Ile Pro Asn Pro Gln
65 70 75 80

Glu Arg Thr Leu Thr Leu Val Asp Thr Gly Ile Gly Met Thr Lys Ala
85 90 95

Asp Leu Ile Asn Asn Leu Gly Thr Ile Ala Lys Ser Gly Thr Lys Ala

100 105 110

Phe Met Glu Ala Leu Gln Ala Gly Ala Asp Ile Ser Met Ile Gly Gln
115 120 125

Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu Val Ala Glu Lys Val Val
130 135 140

Val Ile Arg Lys His Asn Asp Asp Glu Gln Tyr Ala Trp Glu Ser Ser
145 150 155 160

Ala Gly Gly Ser Phe Thr Val Arg Ala Asp His Gly Glu Pro Ile Gly
165 170 175

Met Gly Thr Lys Val Ile Leu His Leu Lys Glu Asp Gln Thr Glu Tyr
180 185 190

Leu Glu Glu Arg Arg Val Lys Glu Val Val Lys Lys His Ser Gln Phe
195 200 205

Ile Gly Tyr Pro Ile Thr Leu Tyr Leu Glu Lys Glu Arg Glu Lys Glu
210 215 220

Ile Ser Asp Asp Glu Ala Glu Glu Glu Lys Gly Glu Lys Glu Glu Glu
225 230 235 240

Asp Lys Asp Asp Glu Glu Lys Pro Lys Ile Glu Asp Val Gly Ser Asp
245 250 255

Glu Glu Asp Asp Ser Gly Lys Asp Lys Lys Lys Lys Thr Lys Lys Ile
260 265 270

Lys Glu Lys Tyr Ile Asp Gln Glu Glu Leu Asn Lys Thr Lys Pro Ile
275 280 285

Trp Thr Arg Asn Pro Asp Asp Ile Thr Gln Glu Glu Tyr Gly Glu Phe
290 295 300

Tyr Lys Ser Leu Thr Asn Asp Trp Glu Asp His Leu Ala Val Lys His
305 310 315 320

Phe Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Leu Leu Phe Ile Pro
325 330 335

Arg Arg Ala Pro Phe Asp Leu Phe Glu Asn Lys Lys Lys Lys Asn Asn
340 345 350

Ile Lys Leu Tyr Val Arg Arg Val Phe Ile Met Asp Ser Cys Asp Glu
355 360 365

Leu Ile Pro Glu Tyr Leu Asn Phe Ile Arg Gly Val Val Asp Ser Glu
370 375 380

Asp Leu Pro Leu Asn Ile Ser Arg Glu Met Leu Gln Gln Ser Lys Ile
385 390 395 400

Leu Lys Val Ile Arg Lys Asn Ile Val Lys Lys Cys Leu Glu Leu Phe

405 410 415

Ser Glu Leu Ala Glu Asp Lys Glu Asn Tyr Lys Lys Phe Tyr Glu Ala
420 425 430

Phe Ser Lys Asn Leu Lys Leu Gly Ile His Glu Asp Ser Thr Asn Arg
435 440 445

Arg Arg Leu Ser Glu Leu Leu Arg Tyr His Thr Ser Gln Ser Gly Asp
450 455 460

Glu Met Thr Ser Leu Ser Glu Tyr Val Ser Arg Met Lys Glu Thr Gln
465 470 475 480

Lys Ser Ile Tyr Tyr Ile Thr Gly Glu Ser Lys Glu Gln Val Ala Asn
485 490 495

Ser Ala Phe Val Glu Arg Val Arg Lys Arg Gly Phe Glu Val Val Tyr
500 505 510

Met Thr Glu Pro Ile Asp Glu Tyr Cys Val Gln Gln Leu Lys Glu Phe
515 520 525

Asp Gly Lys Ser Leu Val Ser Val Thr Lys Glu Gly Leu Glu Leu Pro
530 535 540

Glu Asp Glu Glu Glu Lys Lys Lys Met Glu Glu Ser Lys Ala Lys Phe
545 550 555 560

Glu Asn Leu Cys Lys Leu Met Lys Glu Ile Leu Asp Lys Lys Val Glu
565 570 575

Lys Val Thr Ile Ser Asn Arg Leu Val Ser Ser Pro Cys Cys Ile Val
580 585 590

Thr Ser Thr Tyr Gly Trp Thr Ala Asn Met Glu Arg Ile Met Lys Ala
595 600 605

Gln Ala Leu Arg Asp Asn Ser Thr Met Gly Tyr Met Met Ala Lys Lys
610 615 620

His Leu Glu Ile Asn Pro Asp His Pro Ile Val Glu Thr Leu Arg Gln
625 630 635 640

Lys Ala Glu Ala Asp Lys Asn Asp Lys Ala Val Lys Asp Leu Val Val
645 650 655

Leu Leu Phe Glu Thr Ala Leu Leu Ser Ser Gly Phe Ser Leu Glu Asp
660 665 670

Pro Gln Thr His Ser Asn Arg Ile Tyr Arg Met Ile Lys Leu Gly Leu
675 680 685

Gly Ile Asp Glu Asp Glu Val Ala Ala Glu Glu Pro Asn Ala Ala Val
690 695 700

Pro Asp Glu Ile Pro Pro Leu Glu Gly Asp Glu Asp Ala Ser Arg Met

705 710 715 720

Glu Glu Val Asp

<210> 15
<211> 732
<212> PRT
<213> Human

<400> 15

Met Pro Glu Glu Thr Gln Thr Gln Asp Gln Pro Met Glu Glu Glu Glu
1 5 10 15

Val Glu Thr Phe Ala Phe Gln Ala Glu Ile Ala Gln Leu Met Ser Leu
20 25 30

Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu Leu
35 40 45

Ile Ser Asn Ser Ser Asp Ala Leu Asp Lys Ile Arg Tyr Glu Thr Leu
50 55 60

Thr Asp Pro Ser Lys Leu Asp Ser Gly Lys Glu Leu His Ile Asn Leu
65 70 75 80

Ile Pro Asn Lys Gln Asp Arg Thr Leu Thr Ile Val Asp Thr Gly Ile
85 90 95

Gly Met Thr Lys Ala Asp Leu Ile Asn Asn Leu Gly Thr Ile Ala Lys
100 105 110

Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Gln Ala Gly Ala Asp Ile
115 120 125

Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu Val
130 135 140

Ala Glu Lys Val Thr Val Ile Thr Lys His Asn Asp Asp Glu Gln Tyr
145 150 155 160

Ala Trp Glu Ser Ser Ala Gly Gly Ser Phe Thr Val Arg Thr Asp Thr
165 170 175

Gly Glu Pro Met Gly Arg Gly Thr Lys Val Ile Leu His Leu Lys Glu
180 185 190

Asp Gln Thr Glu Tyr Leu Glu Glu Arg Arg Ile Lys Glu Ile Val Lys
195 200 205

Lys His Ser Gln Phe Ile Gly Tyr Pro Ile Thr Leu Phe Val Glu Lys
210 215 220

Glu Arg Asp Lys Glu Val Ser Asp Asp Glu Ala Glu Glu Lys Glu Asp
225 230 235 240

Lys Glu Glu Glu Lys Glu Lys Glu Glu Lys Glu Ser Glu Asp Lys Pro
245 250 255

Glu Ile Glu Asp Val Gly Ser Asp Glu Glu Glu Glu Lys Lys Asp Gly
260 265 270

Asp Lys Lys Lys Lys Lys Lys Ile Lys Glu Lys Tyr Ile Asp Gln Glu
275 280 285

Glu Leu Asn Lys Thr Lys Pro Ile Trp Thr Arg Asn Pro Asp Asp Ile
290 295 300

Thr Asn Glu Glu Tyr Gly Glu Phe Tyr Lys Ser Leu Thr Asn Asp Trp
305 310 315 320

Glu Asp His Leu Ala Val Lys His Phe Ser Val Glu Gly Gln Leu Glu
325 330 335

Phe Arg Ala Leu Leu Phe Val Pro Arg Arg Ala Pro Phe Asp Leu Phe
340 345 350

Glu Asn Arg Lys Lys Lys Asn Asn Ile Lys Leu Tyr Val Arg Arg Val
355 360 365

Phe Ile Met Asp Asn Cys Glu Glu Leu Ile Pro Glu Tyr Leu Asn Phe
370 375 380

Ile Arg Gly Val Val Asp Ser Glu Asp Leu Pro Leu Asn Ile Ser Arg
385 390 395 400

Glu Met Leu Gln Gln Ser Lys Ile Leu Lys Val Ile Arg Lys Asn Leu
405 410 415

Val Lys Lys Cys Leu Glu Leu Phe Thr Glu Leu Ala Glu Asp Lys Glu
420 425 430

Asn Tyr Lys Lys Phe Tyr Glu Gln Phe Ser Lys Asn Ile Lys Leu Gly
435 440 445

Ile His Glu Asp Ser Gln Asn Arg Lys Lys Leu Ser Glu Leu Leu Arg
450 455 460

Tyr Tyr Thr Ser Ala Ser Gly Asp Glu Met Val Ser Leu Lys Asp Tyr
465 470 475 480

Cys Thr Arg Met Lys Glu Asn Gln Lys His Ile Tyr Tyr Ile Thr Gly
485 490 495

Glu Thr Lys Asp Gln Val Ala Asn Ser Ala Phe Val Glu Arg Leu Arg
500 505 510

Lys His Gly Leu Glu Val Ile Tyr Met Ile Glu Pro Ile Asp Glu Tyr
515 520 525

Cys Val Gln Gln Leu Lys Glu Phe Glu Gly Lys Thr Leu Val Ser Val
530 535 540

Thr Lys Glu Gly Leu Glu Leu Pro Glu Asp Glu Glu Glu Lys Lys Lys
545 550 555 560

Gln Glu Glu Lys Lys Thr Lys Phe Glu Asn Leu Cys Lys Ile Met Lys
565 570 575

Asp Ile Leu Glu Lys Lys Val Glu Lys Val Val Val Ser Asn Arg Leu
580 585 590

Val Thr Ser Pro Cys Cys Ile Val Thr Ser Thr Tyr Gly Trp Thr Ala
595 600 605

Asn Met Glu Arg Ile Met Lys Ala Gln Ala Leu Arg Asp Asn Ser Thr
610 615 620

Met Gly Tyr Met Ala Ala Lys Lys His Leu Glu Ile Asn Pro Asp His
625 630 635 640

Ser Ile Ile Glu Thr Leu Arg Gln Lys Ala Glu Ala Asp Lys Asn Asp
645 650 655

Lys Ser Val Lys Asp Leu Val Ile Leu Leu Tyr Glu Thr Ala Leu Leu
660 665 670

Ser Ser Gly Phe Ser Leu Glu Asp Pro Gln Thr His Ala Asn Arg Ile
675 680 685

Tyr Arg Met Ile Lys Leu Gly Leu Gly Ile Asp Glu Asp Asp Pro Thr
690 695 700

Ala Asp Asp Thr Ser Ala Ala Val Thr Glu Glu Met Pro Pro Leu Glu
705 710 715 720

Gly Asp Asp Asp Thr Ser Arg Met Glu Glu Val Asp
725 730

<210> 16
<211> 573
<212> PRT
<213> Human

<400> 16

Met Leu Arg Leu Pro Thr Val Phe Arg Gln Met Arg Pro Val Ser Arg
1 5 10 15

Val Leu Ala Pro His Leu Thr Arg Ala Tyr Ala Lys Asp Val Lys Phe
20 25 30

Gly Ala Asp Ala Arg Ala Leu Met Leu Gln Gly Val Asp Leu Leu Ala
35 40 45

Asp Ala Val Ala Val Thr Met Gly Pro Lys Gly Arg Thr Val Ile Ile
50 55 60

Glu Gln Ser Trp Gly Ser Pro Lys Val Thr Lys Asp Gly Val Thr Val
65 70 75 80

Ala Lys Ser Ile Asp Leu Lys Asp Lys Tyr Lys Asn Ile Gly Ala Lys
85 90 95

Leu Val Gln Asp Val Ala Asn Asn Thr Asn Glu Glu Ala Gly Asp Gly
100 105 110

Thr Thr Thr Ala Thr Val Leu Ala Arg Ser Ile Ala Lys Glu Gly Phe
115 120 125

Glu Lys Ile Ser Lys Gly Ala Asn Pro Val Glu Ile Arg Arg Gly Val
130 135 140

Met Leu Ala Val Asp Ala Val Ile Ala Glu Leu Lys Lys Gln Ser Lys
145 150 155 160

Pro Val Thr Thr Pro Glu Glu Ile Ala Gln Val Ala Thr Ile Ser Ala
165 170 175

Asn Gly Asp Lys Glu Ile Gly Asn Ile Ile Ser Asp Ala Met Lys Lys
180 185 190

Val Gly Arg Lys Gly Val Ile Thr Val Lys Asp Gly Lys Thr Leu Asn
195 200 205

Asp Glu Leu Glu Ile Ile Glu Gly Met Lys Phe Asp Arg Gly Tyr Ile
210 215 220

Ser Pro Tyr Phe Ile Asn Thr Ser Lys Gly Gln Lys Cys Glu Phe Gln
225 230 235 240

Asp Ala Tyr Val Leu Leu Ser Glu Lys Lys Ile Ser Ser Ile Gln Ser

245 250 255

Ile Val Pro Ala Leu Glu Ile Ala Asn Ala His Arg Lys Pro Leu Val
260 265 270

Ile Ile Ala Glu Asp Val Asp Gly Glu Ala Leu Ser Thr Leu Val Leu
275 280 285

Asn Arg Leu Lys Val Gly Leu Gln Val Val Ala Val Lys Ala Pro Gly
290 295 300

Phe Gly Asp Asn Arg Lys Asn Gln Leu Lys Asp Met Ala Ile Ala Thr
305 310 315 320

Gly Gly Ala Val Phe Gly Glu Glu Gly Leu Thr Leu Asn Leu Glu Asp
325 330 335

Val Gln Pro His Asp Leu Gly Lys Val Gly Glu Val Ile Val Thr Lys
340 345 350

Asp Asp Ala Met Leu Leu Lys Gly Lys Gly Asp Lys Ala Gln Ile Glu
355 360 365

Lys Arg Ile Gln Glu Ile Ile Glu Gln Leu Asp Val Thr Thr Ser Glu
370 375 380

Tyr Glu Lys Glu Lys Leu Asn Glu Arg Leu Ala Lys Leu Ser Asp Gly
385 390 395 400

Val Ala Val Leu Lys Val Gly Gly Thr Ser Asp Val Glu Val Asn Glu
405 410 415

Lys Lys Asp Arg Val Thr Asp Ala Leu Asn Ala Thr Arg Ala Ala Val
420 425 430

Glu Glu Gly Ile Val Leu Gly Gly Gly Cys Ala Leu Leu Arg Cys Ile
435 440 445

Pro Ala Leu Asp Ser Leu Thr Pro Ala Asn Glu Asp Gln Lys Ile Gly
450 455 460

Ile Glu Ile Ile Lys Arg Thr Leu Lys Ile Pro Ala Met Thr Ile Ala
465 470 475 480

Lys Asn Ala Gly Val Glu Gly Ser Leu Ile Val Glu Lys Ile Met Gln
485 490 495

Ser Ser Ser Glu Val Gly Tyr Asp Ala Met Ala Gly Asp Phe Val Asn
500 505 510

Met Val Glu Lys Gly Ile Ile Asp Pro Thr Lys Val Val Arg Thr Ala
515 520 525

Leu Leu Asp Ala Ala Gly Val Ala Ser Leu Leu Thr Thr Ala Glu Val
530 535 540

Val Val Thr Glu Ile Pro Lys Glu Glu Lys Asp Pro Gly Met Gly Ala

545 550 555 560

Met Gly Gly Met Gly Gly Gly Met Gly Gly Gly Met Phe
565 570

<210> 17
<211> 215
<212> PRT
<213> Human

<400> 17

Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Arg Lys Met Ser Ser Tyr
1 5 10 15

Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
20 25 30

Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
35 40 45

Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
50 55 60

Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
65 70 75 80

Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
85 90 95

Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
100 105 110

Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
115 120 125

Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
130 135 140

Glu Lys Lys Ala Glu Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
145 150 155 160

Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
165 170 175

Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Gly Glu Glu
180 185 190

Asp Glu Glu Asp Glu Glu Glu Glu Glu Asp Glu Glu Asp Glu Asp Glu
195 200 205

Glu Glu Asp Asp Asp Asp Glu
210 215

<210> 18
<211> 1087
<212> PRT
<213> Human

<400> 18

Met Met Thr Ser Val Gly Thr Asn Arg Ala Arg Gly Asn Trp Glu Gln
1 5 10 15

Pro Gln Asn Gln Asn Gln Thr Gln His Lys Gln Arg Pro Gln Ala Thr
20 25 30

Ala Glu Gln Ile Arg Leu Ala Gln Met Ile Ser Asp His Asn Asp Ala
35 40 45

Asp Phe Glu Glu Lys Val Lys Gln Leu Ile Asp Ile Thr Gly Lys Asn
50 55 60

Gln Asp Glu Cys Val Ile Ala Leu His Asp Cys Asn Gly Asp Val Asn
65 70 75 80

Arg Ala Ile Asn Val Leu Leu Glu Gly Asn Pro Asp Thr His Ser Trp
85 90 95

Glu Met Val Gly Lys Lys Lys Gly Val Ser Gly Gln Lys Asp Gly Gly
100 105 110

Gln Thr Glu Ser Asn Glu Glu Gly Lys Glu Asn Arg Asp Arg Asp Arg
115 120 125

Asp Tyr Ser Arg Arg Arg Gly Gly Pro Pro Arg Arg Gly Arg Gly Ala
130 135 140

Ser Arg Gly Arg Glu Phe Arg Gly Gln Glu Asn Gly Leu Asp Gly Thr

145 150 155 160

Lys Ser Gly Gly Pro Ser Gly Arg Gly Thr Glu Arg Gly Arg Arg Gly
165 170 175

Arg Gly Arg Gly Arg Gly Gly Ser Gly Arg Arg Gly Gly Arg Phe Ser
180 185 190

Ala Gln Gly Met Gly Thr Phe Asn Pro Ala Asp Tyr Ala Glu Pro Ala
195 200 205

Asn Thr Asp Asp Asn Tyr Gly Asn Ser Ser Gly Asn Thr Trp Asn Asn
210 215 220

Thr Gly His Phe Glu Pro Asp Asp Gly Thr Ser Ala Trp Arg Thr Ala
225 230 235 240

Thr Glu Glu Trp Gly Thr Glu Asp Trp Asn Glu Asp Leu Ser Glu Thr
245 250 255

Lys Ile Phe Thr Ala Ser Asn Val Ser Ser Val Pro Leu Pro Ala Glu
260 265 270

Asn Val Thr Ile Thr Ala Gly Gln Arg Ile Asp Leu Ala Val Leu Leu
275 280 285

Gly Lys Thr Pro Ser Thr Met Glu Asn Asp Ser Ser Asn Leu Asp Pro
290 295 300

Ser Gln Ala Pro Ser Leu Ala Gln Pro Leu Val Phe Ser Asn Ser Lys
305 310 315 320

Gln Thr Ala Ile Ser Gln Pro Ala Ser Gly Asn Thr Phe Ser His His
325 330 335

Ser Met Val Ser Met Leu Gly Lys Gly Phe Gly Asp Val Gly Glu Ala
340 345 350

Lys Gly Gly Ser Thr Thr Gly Ser Gln Phe Leu Glu Gln Phe Lys Thr
355 360 365

Ala Gln Ala Leu Ala Gln Leu Ala Ala Gln His Ser Gln Ser Gly Ser
370 375 380

Thr Thr Thr Ser Ser Trp Asp Met Gly Ser Thr Thr Gln Ser Pro Ser
385 390 395 400

Leu Val Gln Tyr Asp Leu Lys Asn Pro Ser Asp Ser Ala Val His Ser
405 410 415

Pro Phe Thr Lys Arg Gln Ala Phe Thr Pro Ser Ser Thr Met Met Glu
420 425 430

Val Phe Leu Gln Glu Lys Ser Pro Ala Val Ala Thr Ser Thr Ala Ala
435 440 445

Pro Pro Pro Pro Ser Ser Pro Leu Pro Ser Lys Ser Thr Ser Ala Pro

450 455 460

Gln Met Ser Pro Gly Ser Ser Asp Asn Gln Ser Ser Ser Pro Gln Pro
465 470 475 480

Ala His Gln Lys Leu Lys Gln Gln Lys Lys Lys Ala Ser Leu Thr Ser
485 490 495

Lys Ile Pro Ala Leu Ala Val Glu Met Pro Gly Ser Ala Asp Ile Ser
500 505 510

Gly Leu Asn Leu Gln Phe Gly Ala Leu Gln Phe Gly Ser Glu Pro Val
515 520 525

Leu Ser Asp Tyr Glu Ser Thr Pro Thr Thr Ser Ala Ser Ser Ser Gln
530 535 540

Ala Pro Ser Ser Leu Tyr Thr Ser Thr Ala Ser Glu Ser Ser Ser Thr
545 550 555 560

Ile Ser Ser Asn Gln Ser Gln Glu Ser Gly Tyr Gln Ser Gly Pro Ile
565 570 575

Gln Ser Thr Thr Tyr Thr Ser Gln Asn Asn Ala Gln Gly Pro Leu Tyr
580 585 590

Glu Gln Arg Ser Thr Gln Thr Arg Arg Tyr Pro Ser Ser Ile Ser Ser
595 600 605

Ser Pro Gln Lys Asp Leu Thr Gln Ala Lys Asn Gly Phe Ser Ser Val
610 615 620

Gln Ala Thr Gln Leu Gln Thr Thr Gln Ser Val Glu Gly Ala Thr Gly
625 630 635 640

Ser Ala Val Lys Ser Asp Ser Pro Ser Thr Ser Ser Ile Pro Pro Leu
645 650 655

Asn Glu Thr Val Ser Ala Ala Ser Leu Leu Thr Thr Thr Asn Gln His
660 665 670

Ser Ser Ser Leu Gly Gly Leu Ser His Ser Glu Glu Ile Pro Asn Thr
675 680 685

Thr Thr Thr Gln His Ser Ser Thr Leu Ser Thr Gln Gln Asn Thr Leu
690 695 700

Ser Ser Ser Thr Ser Ser Gly Arg Thr Ser Thr Ser Thr Leu Leu His
705 710 715 720

Thr Ser Val Glu Ser Glu Ala Asn Leu His Ser Ser Ser Ser Thr Phe
725 730 735

Ser Thr Thr Ser Ser Thr Val Ser Ala Pro Pro Pro Val Val Ser Val
740 745 750

Ser Ser Ser Leu Asn Ser Gly Ser Ser Leu Gly Leu Ser Leu Gly Ser

755 760 765

Asn Ser Thr Val Thr Ala Ser Thr Arg Ser Ser Val Ala Thr Thr Ser
770 775 780

Gly Lys Ala Pro Pro Asn Leu Pro Pro Gly Val Pro Pro Leu Leu Pro
785 790 795 800

Asn Pro Tyr Ile Met Ala Pro Gly Leu Leu His Ala Tyr Pro Pro Gln
805 810 815

Val Tyr Gly Tyr Asp Asp Leu Gln Met Leu Gln Thr Arg Phe Pro Leu
820 825 830

Asp Tyr Tyr Ser Ile Pro Phe Pro Thr Pro Thr Thr Pro Leu Thr Gly
835 840 845

Arg Asp Gly Ser Leu Ala Ser Asn Pro Tyr Ser Gly Asp Leu Thr Lys
850 855 860

Phe Gly Arg Gly Asp Ala Ser Ser Pro Ala Pro Ala Thr Thr Leu Ala
865 870 875 880

Gln Pro Gln Gln Asn Gln Thr Gln Thr His His Thr Thr Gln Gln Thr
885 890 895

Phe Leu Asn Pro Ala Leu Pro Pro Gly Tyr Ser Tyr Thr Ser Leu Pro
900 905 910

Tyr Tyr Thr Gly Val Pro Gly Leu Pro Ser Thr Phe Gln Tyr Gly Pro
915 920 925

Ala Val Phe Pro Val Ala Pro Thr Ser Ser Lys Gln His Gly Val Asn
930 935 940

Val Ser Val Asn Ala Ser Ala Thr Pro Phe Gln Gln Pro Ser Gly Tyr
945 950 955 960

Gly Ser His Gly Tyr Asn Thr Gly Val Ser Val Thr Ser Ser Asn Thr
965 970 975

Gly Val Pro Asp Ile Ser Gly Ser Val Tyr Ser Lys Thr Gln Gln Ser
980 985 990

Phe Glu Lys Gln Gly Phe His Ser Gly Thr Pro Ala Ala Ser Phe Asn
995 1000 1005

Leu Pro Ser Ala Leu Gly Ser Gly Gly Pro Ile Asn Pro Ala Thr
1010 1015 1020

Ala Ala Ala Tyr Pro Pro Ala Pro Phe Met His Ile Leu Thr Pro
1025 1030 1035

His Gln Gln Pro His Ser Gln Ile Leu His His His Leu Gln Gln
1040 1045 1050

Asp Gly Gln Thr Gly Ser Gly Gln Arg Ser Gln Thr Ser Ser Ile

1055 1060 1065

Pro Gln Lys Pro Gln Thr Asn Lys Ser Ala Tyr Asn Ser Tyr Ser
1070 1075 1080

Trp Gly Ala Asn
1085

<210> 19
<211> 806
<212> PRT
<213> Human

<400> 19

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Leu Lys Gln Lys Asn Arg Pro Asn Arg Leu Ile Val Asp Glu Ala Ile
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Asn Glu Asp Asn Ser Val Val Ser Leu Ser Gln Pro Lys Met Asp Glu
35 40 45

Leu Gln Leu Phe Arg Gly Asp Thr Val Leu Leu Lys Gly Lys Lys Arg
50 55 60

Arg Glu Ala Val Cys Ile Val Leu Ser Asp Asp Thr Cys Ser Asp Glu
65 70 75 80

Lys Ile Arg Met Asn Arg Val Val Arg Asn Asn Leu Arg Val Arg Leu
85 90 95

Gly Asp Val Ile Ser Ile Gln Pro Cys Pro Asp Val Lys Tyr Gly Lys
100 105 110

Arg Ile His Val Leu Pro Ile Asp Asp Thr Val Glu Gly Ile Thr Gly
115 120 125

Asn Leu Phe Glu Val Tyr Leu Lys Pro Tyr Phe Leu Glu Ala Tyr Arg
130 135 140

Pro Ile Arg Lys Gly Asp Ile Phe Leu Val Arg Gly Gly Met Arg Ala
145 150 155 160

Val Glu Phe Lys Val Val Glu Thr Asp Pro Ser Pro Tyr Cys Ile Val
165 170 175

Ala Pro Asp Thr Val Ile His Cys Glu Gly Glu Pro Ile Lys Arg Glu
180 185 190

Asp Glu Glu Glu Ser Leu Asn Glu Val Gly Tyr Asp Asp Ile Gly Gly
195 200 205

Cys Arg Lys Gln Leu Ala Gln Ile Lys Glu Met Val Glu Leu Pro Leu
210 215 220

Arg His Pro Ala Leu Phe Lys Ala Ile Gly Val Lys Pro Pro Arg Gly
225 230 235 240

Ile Leu Leu Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu Ile Ala Arg
245 250 255

Ala Val Ala Asn Glu Thr Gly Ala Phe Phe Phe Leu Ile Asn Gly Pro
260 265 270

Glu Ile Met Ser Lys Leu Ala Gly Glu Ser Glu Ser Asn Leu Arg Lys
275 280 285

Ala Phe Glu Glu Ala Glu Lys Asn Ala Pro Ala Ile Ile Phe Ile Asp
290 295 300

Glu Leu Asp Ala Ile Ala Pro Lys Arg Glu Lys Thr His Gly Glu Val
305 310 315 320

Glu Arg Arg Ile Val Ser Gln Leu Leu Thr Leu Met Asp Gly Leu Lys
325 330 335

Gln Arg Ala His Val Ile Val Met Ala Ala Thr Asn Arg Pro Asn Ser
340 345 350

Ile Asp Pro Ala Leu Arg Arg Phe Gly Arg Phe Asp Arg Glu Val Asp
355 360 365

Ile Gly Ile Pro Asp Ala Thr Gly Arg Leu Glu Ile Leu Gln Ile His
370 375 380

Thr Lys Asn Met Lys Leu Ala Asp Asp Val Asp Leu Glu Gln Val Ala
385 390 395 400

Asn Glu Thr His Gly His Val Gly Ala Asp Leu Ala Ala Leu Cys Ser
405 410 415

Glu Ala Ala Leu Gln Ala Ile Arg Lys Lys Met Asp Leu Ile Asp Leu
420 425 430

Glu Asp Glu Thr Ile Asp Ala Glu Val Met Asn Ser Leu Ala Val Thr
435 440 445

Met Asp Asp Phe Arg Trp Ala Leu Ser Gln Ser Asn Pro Ser Ala Leu
450 455 460

Arg Glu Thr Val Val Glu Val Pro Gln Val Thr Trp Glu Asp Ile Gly
465 470 475 480

Gly Leu Glu Asp Val Lys Arg Glu Leu Gln Glu Leu Val Gln Tyr Pro
485 490 495

Val Glu His Pro Asp Lys Phe Leu Lys Phe Gly Met Thr Pro Ser Lys
500 505 510

Gly Val Leu Phe Tyr Gly Pro Pro Gly Cys Gly Lys Thr Leu Leu Ala
515 520 525

Lys Ala Ile Ala Asn Glu Cys Gln Ala Asn Phe Ile Ser Ile Lys Gly
530 535 540

Pro Glu Leu Leu Thr Met Trp Phe Gly Glu Ser Glu Ala Asn Val Arg
545 550 555 560

Glu Ile Phe Asp Lys Ala Arg Gln Ala Ala Pro Cys Val Leu Phe Phe
565 570 575

Asp Glu Leu Asp Ser Ile Ala Lys Ala Arg Gly Gly Asn Ile Gly Asp
580 585 590

Gly Gly Gly Ala Ala Asp Arg Val Ile Asn Gln Ile Leu Thr Glu Met
595 600 605

Asp Gly Met Ser Thr Lys Lys Asn Val Phe Ile Ile Gly Ala Thr Asn
610 615 620

Arg Pro Asp Ile Ile Asp Pro Ala Ile Leu Arg Pro Gly Arg Leu Asp
625 630 635 640

Gln Leu Ile Tyr Ile Pro Leu Pro Asp Glu Lys Ser Arg Val Ala Ile
645 650 655

Leu Lys Ala Asn Leu Arg Lys Ser Pro Val Ala Lys Asp Val Asp Leu
660 665 670

Glu Phe Leu Ala Lys Met Thr Asn Gly Phe Ser Gly Ala Asp Leu Thr
675 680 685

Glu Ile Cys Gln Arg Ala Cys Lys Leu Ala Ile Arg Glu Ser Ile Glu
690 695 700

Ser Glu Ile Arg Arg Glu Arg Glu Arg Gln Thr Asn Pro Ser Ala Met
705 710 715 720

Glu Val Glu Glu Asp Asp Pro Val Pro Glu Ile Arg Arg Asp His Phe
725 730 735

Glu Glu Ala Met Arg Phe Ala Arg Arg Ser Val Ser Asp Asn Asp Ile
740 745 750

Arg Lys Tyr Glu Met Phe Ala Gln Thr Leu Gln Gln Ser Arg Gly Phe
755 760 765

Gly Ser Phe Arg Phe Pro Ser Gly Asn Gln Gly Gly Ala Gly Pro Ser
770 775 780

Gln Gly Ser Gly Gly Gly Thr Gly Gly Ser Val Tyr Thr Glu Asp Asn
785 790 795 800

Asp Asp Asp Leu Tyr Gly
805

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<211> 260
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<213> Human

<400> 20

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1 5 10 15

Leu Val Thr Arg Ala Ala Phe Asn Ser Gly Lys Val Asp Ile Val Thr
20 25 30

Ile Asn Asp Pro Phe Ile Asp Leu Asn Tyr Pro Phe Ile Asp Leu Asn
35 40 45

Tyr Met Ile Tyr Met Phe Gln Tyr Asp Ser Met Ala Asn Ser Met Ala
50 55 60

Pro Ser Arg Leu Arg Met Gly Ser Leu Ser Ser Arg Glu Ile Pro Ser
65 70 75 80

Pro Ser Ser Arg Ser Glu Ile Pro Pro Lys Ser Asn Gly Gly Glu Ala
85 90 95

Lys Arg Ile Ile Ile Ser Ala Pro Ser Ala Asp Ala Pro Met Phe Met
100 105 110

Met Gly Ile Asn Arg Glu Lys Tyr Asp Asn Ser Leu Glu Ile Ile Ser
115 120 125

Asn Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Leu Ala Lys Val Ile
130 135 140

His Asp Asn Phe Gly Ile Met Glu Gly Leu Met Thr Thr Val His Ala

145 150 155 160

Ile Ala Ala Thr Gln Lys Thr Val Asp Ser Pro Ser Gly Lys Leu Trp
165 170 175

Cys Asp Gly Cys Arg Ala Leu Gln Asn Ile Ile Pro Ala Ser Thr Gly
180 185 190

Ser Ser Leu Ala Lys Pro Ser Val Val Pro Thr Thr Asn Val Ser Val
195 200 205

Val Asp Leu Thr Cys His Leu Glu Lys Pro Ala Lys Tyr Asp Asp Ile
210 215 220

Lys Lys Trp Cys Ser Arg His Gln Lys Ala Pro Ser Arg Ala Ser Trp
225 230 235 240

Ala Thr Leu Ser Thr Cys Asn Arg Val Val Asp Leu Met Ala His Met
245 250 255

Ala Ser Lys Glu
260

<210> 21
<211> 279
<212> PRT
<213> Human

<400> 21

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Ile Asn Phe Leu Arg Gln Leu Tyr Glu Glu Glu Leu Arg Glu Leu Gln
20 25 30

Ser Gln Ile Ser Asp Thr Ser Val Val Leu Ser Met Asp Asn Ser Arg
35 40 45

Ser Leu Asp Met Asp Ser Ile Ile Ala Glu Val Lys Ala Gln Tyr Glu
50 55 60

Asp Ile Ala Asn Arg Ser Arg Ala Glu Ala Glu Ser Met Tyr Gln Ile
65 70 75 80

Lys Tyr Glu Glu Leu Gln Ser Leu Ala Gly Lys His Gly Asp Asp Leu
85 90 95

Arg Arg Thr Lys Thr Glu Ile Ser Glu Met Asn Arg Asn Ile Ser Arg
100 105 110

Leu Gln Ala Glu Ile Glu Gly Leu Lys Gly Gln Arg Ala Ser Leu Glu
115 120 125

Ala Ala Ile Ala Asp Ala Glu Gln Arg Gly Glu Leu Ala Ile Lys Asp
130 135 140

Ala Asn Ala Lys Leu Ser Glu Leu Glu Ala Ala Leu Gln Arg Ala Lys
145 150 155 160

Gln Asp Met Ala Arg Gln Leu Arg Glu Tyr Gln Glu Leu Met Asn Val
165 170 175

Lys Leu Ala Met Asp Ile Glu Ile Ala Thr Tyr Arg Lys Leu Leu Glu
180 185 190

Gly Glu Glu Ser Arg Leu Glu Ser Gly Met Gln Asn Met Ser Ile His
195 200 205

Thr Lys Thr Thr Gly Gly Tyr Ala Gly Gly Leu Ser Ser Ala Tyr Gly
210 215 220

Gly Leu Ala Ser Pro Gly Leu Ser Tyr Ser Leu Gly Ser Ser Phe Gly
225 230 235 240

Ser Gly Ala Gly Ser Ser Ser Phe Ser Arg Thr Ser Ser Ser Arg Ala
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Val Val Val Lys Lys Ile Glu Thr Arg Asp Gly Lys Leu Val Ser Glu
260 265 270

Ser Ser Asp Val Leu Pro Lys
275

<210> 22
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<212> PRT
<213> Human

<400> 22

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Asp Leu Glu Lys Val Phe Ala Glu His Lys Ile Ser Tyr Ser Gly Gln
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Phe Leu Val Lys Ser Gly Tyr Ala Phe Val Asp Cys Pro Asp Glu His
        35                  40                  45

Trp Ala Met Lys Ala Ile Glu Thr Phe Ser Gly Lys Val Glu Leu Gln
    50                  55                  60

Gly Lys Arg Leu Glu Ile Glu His Ser Val Pro Lys Lys Gln Arg Ser
65                  70                  75                  80

Arg Lys Ile Gln Ile Arg Asn Ile Pro Pro Gln Leu Arg Trp Glu Val
                85                  90                  95

Leu Asp Ser Leu Leu Ala Gln Tyr Gly Thr Val Glu Asn Cys Glu Gln
            100                 105                 110

Val Asn Thr Glu Ser Glu Thr Ala Val Val Asn Val Thr Tyr Ser Asn
        115                 120                 125

Arg Glu Gln Thr Arg Gln Ala Ile Met Lys Leu Asn Gly His Gln Leu
    130                 135                 140
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Glu Asn His Ala Leu Lys Val Ser Tyr Ile Pro Asp Glu Gln Ile Ala
145 150 155 160

Gln Gly Pro Glu Asn Gly Arg Arg Gly Gly Phe Gly Ser Arg Gly Gln
165 170 175

Pro Arg Gln Gly Ser Pro Val Ala Ala Gly Ala Pro Ala Lys Gln Gln
180 185 190

Gln Val Asp Ile Pro Leu Arg Leu Leu Val Pro Thr Gln Tyr Val Gly
195 200 205

Ala Ile Ile Gly Lys Glu Gly Ala Thr Ile Arg Asn Ile Thr Lys Gln
210 215 220

Thr Gln Ser Lys Ile Asp Val His Arg Lys Glu Asn Ala Gly Ala Ala
225 230 235 240

Glu Lys Ala Ile Ser Val His Ser Thr Pro Glu Gly Cys Ser Ser Ala
245 250 255

Cys Lys Met Ile Leu Glu Ile Met His Lys Glu Ala Lys Asp Thr Lys
260 265 270

Thr Ala Asp Glu Val Pro Leu Lys Ile Leu Ala His Asn Asn Phe Val
275 280 285

Gly Arg Leu Ile Gly Lys Glu Gly Arg Asn Leu Lys Lys Val Glu Gln

290 295 300

Asp Thr Glu Thr Lys Ile Thr Ile Ser Ser Leu Gln Asp Leu Thr Leu
305 310 315 320

Tyr Asn Pro Glu Arg Thr Ile Thr Val Lys Gly Ala Ile Glu Asn Cys
325 330 335

Cys Arg Ala Glu Gln Glu Ile Met Lys Lys Val Arg Glu Ala Tyr Glu
340 345 350

Asn Asp Val Ala Ala Met Ser Leu Gln Ser His Leu Ile Pro Gly Leu
355 360 365

Asn Leu Ala Ala Val Gly Leu Phe Pro Ala Ser Ser Ser Ala Val Pro
370 375 380

Pro Pro Pro Ser Ser Val Thr Gly Ala Ala Pro Tyr Ser Ser Phe Met
385 390 395 400

Gln Ala Pro Glu Gln Glu Met Val Gln Val Phe Ile Pro Ala Gln Ala
405 410 415

Val Gly Ala Ile Ile Gly Lys Lys Gly Gln His Ile Lys Gln Leu Ser
420 425 430

Arg Phe Ala Ser Ala Ser Ile Lys Ile Ala Pro Pro Glu Thr Pro Asp
435 440 445

Ser Lys Val Arg Met Val Ile Ile Thr Gly Pro Pro Glu Ala Gln Phe
450 455 460

Lys Ala Gln Gly Arg Ile Tyr Gly Lys Leu Lys Glu Glu Asn Phe Phe
465 470 475 480

Gly Pro Lys Glu Glu Val Lys Leu Glu Thr His Ile Arg Val Pro Ala
485 490 495

Ser Ala Ala Gly Arg Val Ile Gly Lys Gly Gly Lys Thr Val Asn Glu
500 505 510

Leu Gln Asn Leu Thr Ala Ala Glu Val Val Val Pro Arg Asp Gln Thr
515 520 525

Pro Asp Glu Asn Asp Gln Val Ile Val Lys Ile Ile Gly His Phe Tyr
530 535 540

Ala Ser Gln Met Ala Gln Arg Lys Ile Arg Asp Ile Leu Ala Gln Val
545 550 555 560

Lys Gln Gln His Gln Lys Gly Gln Ser Asn Gln Ala Gln Ala Arg Arg
565 570 575

Lys

<210> 23

<211> 329
<212> PRT
<213> Human

<400> 23

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1 5 10 15

Gly Gly Pro Glu Val Leu Lys Leu Arg Ser Asp Ile Ala Val Pro Ile
20 25 30

Pro Lys Asp His Gln Val Leu Ile Lys Val His Ala Cys Gly Val Asn
35 40 45

Pro Val Glu Thr Tyr Ile Arg Ser Gly Thr Tyr Ser Arg Lys Pro Leu
50 55 60

Leu Pro Tyr Thr Pro Gly Ser Asp Val Ala Gly Val Ile Glu Ala Val
65 70 75 80

Gly Asp Asn Ala Ser Ala Phe Lys Lys Gly Asp Arg Val Phe Thr Ser
85 90 95

Ser Thr Ile Ser Gly Gly Tyr Ala Glu Tyr Ala Leu Ala Ala Asp His
100 105 110

Thr Val Tyr Lys Leu Pro Glu Lys Leu Asp Phe Lys Gln Gly Ala Ala
115 120 125

Ile Gly Ile Pro Tyr Phe Thr Ala Tyr Arg Ala Leu Ile His Ser Ala
130 135 140

Cys Val Lys Ala Gly Glu Ser Val Leu Val His Gly Ala Ser Gly Gly
145 150 155 160

Val Gly Leu Ala Ala Cys Gln Ile Ala Arg Ala Tyr Gly Leu Lys Ile
165 170 175

Leu Gly Thr Ala Gly Thr Glu Glu Gly Gln Lys Ile Val Leu Gln Asn
180 185 190

Gly Ala His Glu Val Phe Asn His Arg Glu Val Asn Tyr Ile Asp Lys
195 200 205

Ile Lys Lys Tyr Val Gly Glu Lys Gly Ile Asp Ile Ile Ile Glu Met
210 215 220

Leu Ala Asn Val Asn Leu Ser Lys Asp Leu Ser Leu Leu Ser His Gly
225 230 235 240

Gly Arg Val Ile Val Val Gly Ser Arg Gly Thr Ile Glu Ile Asn Pro
245 250 255

Arg Asp Thr Met Ala Lys Glu Ser Ser Ile Ile Gly Val Thr Leu Phe
260 265 270

Ser Ser Thr Lys Glu Glu Phe Gln Gln Tyr Ala Ala Ala Leu Gln Ala
275 280 285

Gly Met Glu Ile Gly Trp Leu Lys Pro Val Ile Gly Ser Gln Tyr Pro
290 295 300

Leu Glu Lys Val Ala Glu Ala His Glu Asn Ile Ile His Gly Ser Gly
305 310 315 320

Ala Thr Gly Lys Met Ile Leu Leu Leu
325

<210> 24
<211> 125
<212> PRT
<213> Human

<400> 24

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1 5 10 15

Ile Glu Phe Cys Val Glu Asp Ser Lys Asp Val Asn Val Asn Phe Glu
20 25 30

Lys Ser Lys Leu Thr Phe Ser Cys Leu Gly Gly Ser Asp Asn Phe Lys
35 40 45

His Leu Asn Glu Ile Asp Leu Phe His Cys Ile Asp Pro Asn Asp Ser
50 55 60

Lys His Lys Arg Thr Asp Arg Ser Ile Leu Cys Cys Leu Arg Lys Gly

65 70 75 80

Glu Ser Gly Gln Ser Trp Pro Arg Leu Thr Lys Glu Arg Ala Lys Leu
85 90 95

Asn Trp Leu Ser Val Asp Phe Asn Asn Trp Lys Asp Trp Glu Asp Asp
100 105 110

Ser Asp Glu Asp Met Ser Asn Phe Asp Arg Phe Ser Glu
115 120 125

60

What is claimed is:

1. A Biomarker for liver diseases selected from any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof or the antibodies against said amino acid sequences.

2. The biomarker according to Claim 1, wherein said liver disease is liver cirrhosis or liver cancer.

3. The biomarker according to Claim 1, wherein said variant and any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 show sequence homology greater than 80%.

4. A detection kit for liver diseases, comprising biomarkers selected from any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof.

5. The detection kit according to Claim 4, wherein said liver disease is liver cirrhosis or liver cancer.

6. The detection kit according to Claim 4, wherein the detection kit can further include secondary antibodies that can recognize autoantibody against any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof.

7. A method for screening liver diseases, comprising the steps of:

providing a specimen;

using a biomarker selected from any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof to identify and capture autoantibodies in the specimen; and

detecting the autoantibodies.

8. The method according to Claim 7, wherein said specimen includes whole blood or serum.

9. The method according to Claim 8, wherein said specimen is serum.

10. The method according to Claim 7, wherein said biomarker may be made into detection kits.

11. The method according to Claim 7, wherein said biomarker is firstly immobilized on a substrate.

12. The method according to Claim 11, wherein said substrate is a immunoassay plate or a biochip.

13. The method according to Claim 7, wherein said specimen is firstly labeled with a fluorescence marker.

14. The method according to Claim 7, wherein the method further include a step of using a secondary antibody to recognize and adsorb the autoantibody.

15. The method according to Claim 14, wherein said secondary antibody is modified and has a special functional group detectable by means of color reaction, radioactivity or fluorescence.

16. The method according to Claim 7, wherein the detection of the autoantibody is achieved by using a fluorescence scanner to detect a fluorescence-labeled autoantibody.

17. The method according to Claim 7, wherein the detection of the autoantibody is achieved by detection of the secondary autoantibody with enzyme –linked immunosorbent assay (ELISA), radioimmunoassay (RIA) or immunofluorescence.

18. A detection kit for liver diseases, comprising a set of antibodies against any one of amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24.

19. The detection kit according to Claim 18, wherein said liver disease is liver

cirrhosis or liver cancer.

20. A screening method for liver diseases, comprising the steps of:

providing a specimen;

using an antibody against any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 to capture an antigen in the specimen; and

detecting the antibody-antigen complex.

21. The screening method according to Claim 20, wherein said specimen is whole blood or serum.

22. The screening method according to Claim 21, wherein said specimen is serum.

ABSTRACT

Biomarkers for liver diseases and method for using the same are provided. For detecting liver cirrhosis and liver cancer, the biomarkers are selected from any one of the amino acid sequences with SEQ ID NO:1 to SEQ ID NO:24 or derivatives or fragments or variants or the combination thereof or the antibodies against the amino acid sequences. Then the biomarkers are further developed into detection kits, such that by detecting the existence of autoantibodies or autoantigens in screened specimens, liver diseases are detected with higher accuracy and sensitivity.

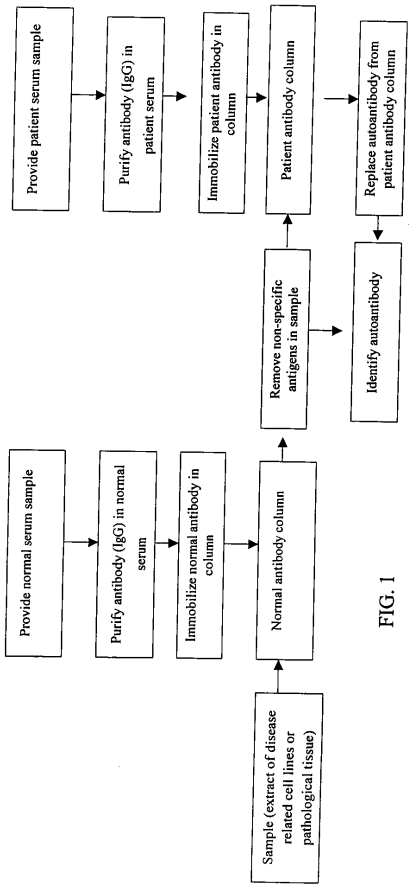

FIG. 1

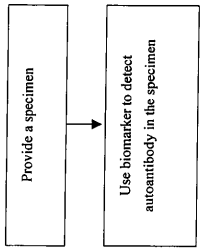

FIG. 2

专利名称(译)	用于肝病的生物标志物及其使用方法		
公开(公告)号	JP2005181342A	公开(公告)日	2005-07-07
申请号	JP2004382611	申请日	2004-12-17
[标]申请(专利权)人(译)	财团法人工业技术研究院		
申请(专利权)人(译)	财团法人工业技术研究院		
[标]发明人	ツェンツーリン 鄭平福		
发明人	ツェン ツー リン 鄭平福		
IPC分类号	G01N33/53 C07K14/47 C07K16/18 G01N21/78 G01N33/543 G01N33/567 G01N33/574 G01N33/68		
CPC分类号	G01N33/57438 G01N33/6893 G01N2800/08 G01N2800/085		
FI分类号	G01N33/53.D G01N33/53.N G01N21/78.C G01N21/78.Z G01N33/543.541.B G01N33/543.575 G01N33/574.A C07K14/47.ZNA C07K16/18 G01N33/574.Z		
F-TERM分类号	2G054/AA07 2G054/AB04 2G054/CE02 2G054/EA03 2G054/EA06 4H045/AA30 4H045/BA10 4H045/CA40 4H045/CA41 4H045/DA75 4H045/EA50 4H045/EA51 4H045/FA71 4H045/GA26		
代理人(译)	小川伸男		
优先权	092136309 2003-12-19 TW		
其他公开文献	JP4105156B2		
外部链接	Espacenet		

摘要(译)

要解决的问题：为肝脏疾病提供生物标志物及其使用方法。 解决方案：选自SEQ ID NO：1至SEQ ID NO：24中描述的氨基酸序列或其衍生物或片段或其变体，其组合或针对氨基酸序列的抗体的肝病生物标志物。一种用于肝病的检测试剂盒，其特征在于包含所述生物标志物。准备样品;使用生物标记物鉴定和捕获样品中的自身抗体;并检测自身抗体。【选择图】无

【表１】
表1：肝臓疾患関連細胞系からスクリーニングされた自己抗原

GI番号	タンパク質名	肝硬変血清vs. HepG2 C3A	肝癌血清vs. HepG2 C3A	肝硬変血清vs. SNU-387	肝癌血清vs. SNU-387
1421609	ヌクレオシドジホスフェートキナーゼ(=NM23タンパク)	●	●	●	●
28940	ATPシンターゼβ-鎖、ミトコンドリア[プリカーサー]	●		●	
4507953, 5803225	14-3-3タンパク	●		●	
1710248	タンパクジスルフィドイソメラーゼ-関連タンパク5		●		●
21750187	gi|21750187命名されていないタンパク製品(RAN rec mot.)		●		
37403, 10435300	トロポミオシン			●	●
4757900	カルレティキュリンプリカーサ	●	●		
338043	ヒトプレ-mRNAスプライシング因子SF2p32、完全配列	●	●		
1082886	腫瘍壊死因子タイプIレセプタ関連タンパクTRAP-1	●	●		
4507677	腫瘍タンパク抗原(gp96) 1; グルコース調節タンパク	●	●		
72222, 123678	熱ショックタンパク質90	●	●		
31542947	熱ショック60kDaタンパク質 1(シャペロニン); ミトコンドリアマトリックスタンパクP1	●			
968888	HMG-1(高-移動座群-1)	●			
13111995	KIAA0144遺伝子生成物(NICE-4タンパク質)	●			
6005942	パロシン含有タンパク質(p97); 転移性小胞体ATPアーゼ	●			
30157565	グリセロアルデヒド3-ホスフェートデヒドロゲナーゼ、肝臓	●			
1419564	サイトケラチン	●			
4191608	IGF-II mRNA-結合タンパク 1	●			
13236495	NADPH: キノンレダクターゼ		●		
9257073	ヒトニーシャペロンP23の結晶構造(hsp-90ニーシャペロン)		●		