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最終頁に続く

(54) 【発明の名称】 腫瘍細胞の抗癌剤に対する感受性を検定する方法

(57) 【特許請求の範囲】

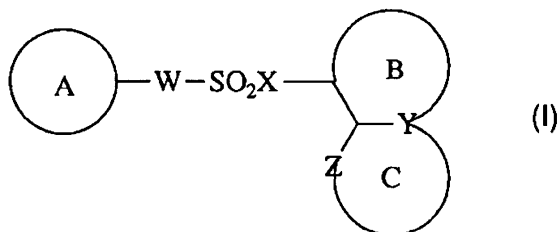
【請求項1】

1). 下記一般式(I)で表される抗癌剤を投与した癌患者より取り出された腫瘍細胞の、下記表A及びBに記載の遺伝子からなる群から選択される1個または複数の遺伝子の発現量を測定し、

2). 該癌患者から抗癌剤投与前に取り出された腫瘍細胞と比較して、表Aに記載の遺伝子の発現量が増加し、または表Bに記載の遺伝子の発現量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する、

工程を含んで成る、該腫瘍細胞の該抗癌剤に対する感受性を検定する方法。

【化1】



(式中、

A環は、シアノ基またはスルホンアミド基で置換されていてもよいベンゼン環を、

B環は、メチル基、エチル基、n-プロピル基またはイソプロピル基で置換されていても

よいベンゼン環を、  
 C環は、シアノ基または塩素原子で置換されていてもよいピロール環を、  
 Wは単結合を、  
 Xは - NH - を、  
 Yは炭素原子を、  
 Zは - NH - を、  
 それぞれ意味する。)

【表 1 A】

表 A

遺伝子名
Ubiquitin-conjugating enzyme
Rep-8
S-adenosylmethionine decarboxylase
p70 Ribosomal S6 kinase alpha-1
c-ErbB3 receptor tyrosine kinase
RNA polymerase II complex component SRB7
Export protein RAE1
Defective mariner transposon Hsmar2

## 【表 1 B - 1】

表 B

遺伝子名	
Serine/threonine protein kinase MNK1	
Putative serine/threonine protein kinase VRK2	
Phospholipid scramblase	
Hydrogen carrier protein, glycine synthase	
KIAA0103	
KIAA0029	
Dihydrolipoamide succinyltransferase	10
Nuclear cap binding protein	
KIAA0077	
KIAA0074	
KIAA0100	
RNA-binding motif protein 10	
PAP39	
KIAA0146	
KIAA0159	
Multifunctional protein CAD	
KIAA0161	
KIAA0169	20
KIAA0197	
Phosphoethanolamine cytidyltransferase	
KIAA0257	
DNA topoisomerase II (top2)	
IEF SSP 9502	
Succinate dehydrogenase flavoprotein subunit	
Lamin B receptor	
Proteasome 26S subunit p45	
Retinoblastoma susceptibility protein (RB1)	
Alpha topoisomerase truncated-form	30
Tropomyosin	
Mitochondrial aspartate aminotransferase	
DNA-binding factor	
Calcineurin A1	
Ubiquitin carboxyl-terminal hydrolase	
Epidermal growth factor receptor HER3	
Mitochondrial NAD(P)+ dependent malic enzyme	
Gamma-tubulin	
Cleavage stimulation factor	
Replication factor C, 40-kDa subunit (A1)	40

## 【表 1 B - 2】

表 B (続き)

遺伝子名	
Medium-chain acyl-CoA dehydrogenase (MCAD)	
Leucine-rich protein	
Monocyte/neutrophil elastase inhibitor	
Basic transcription factor 62kD subunit (BTF2)	
Transcription factor ISGF-3	
APK1 antigen=MAB KI recognized	
PDCD2, Rp8 homolog	10
Inositol 1,4,5-triphosphate receptor type 3	
G-rich sequence factor-1	
Splicesomal protein SAP61	
Cyclin H	
DNA-binding protein NFX1	
CDC16Hs	
Receptor tyrosine kinase DTK	
Chromatin assembly factor-I p150 subunit	
Methylthioadenosine phosphorylase	
Eukaryotic initiation factor 2B-epsilon	
LUCA15	20
Immuno-reactive with anti-PTH Ab	
Gps2	
Splicing factor SRp55-2 (SRp55)	
Glutathione synthetase	
Integrin-linked kinase	
FRAP-related protein (FRP1/ATR)	
Amyloid precursor protein-binding protein 1	
Retinitis pigmentosa GTPase regulator	
Enhancer of zeste homolog 2	
C2f	30
O-linked GlcNAc transferase	
Cdc6-related protein (HsCDC6)	
Clone 23759	
Mitochondrial intermediate peptidase precursor	
CaM kinase II isoform	
Pyridoxal kinase	
Transcription coactivator p75 (DFS70)	
DNA polymerase alpha subunit	
U2 snRNP-specific A protein	
Neuron specific (gamma) enolase	
GARS-AIRS-GART	40
75 kDa subunit NADH dehydrogenase precursor	
S-adenosylmethionine synthetase	
Subunit of coatomer complex	

## 【表 1 B - 3】

表 B (続き)

遺伝子名
PxF
Arginine methyltransferase
Nup88
Carnitine palmitoyltransferase I type I
Transmembrane receptor protein CRF2-4

## 【請求項 2】

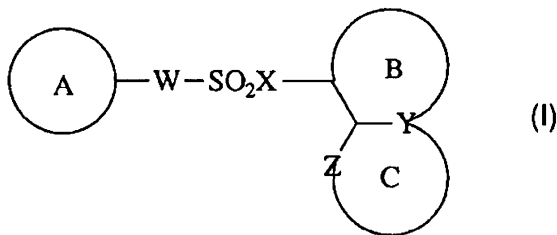
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- 1). 癌患者より取り出された腫瘍細胞に、下記一般式 ( I ) で表される抗癌剤を作用させ、
- 2). 該腫瘍細胞の、下記表 A 及び B に記載の遺伝子からなる群から選択される 1 個または複数の遺伝子の発現量を測定し、
- 3). 無処理の腫瘍細胞と比較して、表 A に記載の遺伝子の発現量が増加し、または表 B に記載の遺伝子の発現量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する、

工程を含んで成る、該腫瘍細胞の該抗癌剤に対する感受性を検定する方法。

## 【化 2】

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(式中、

- A 環は、シアノ基またはスルホンアミド基で置換されていてもよいベンゼン環を、  
 B 環は、メチル基、エチル基、n - プロピル基またはイソプロピル基で置換されていてもよいベンゼン環を、  
 C 環は、シアノ基または塩素原子で置換されていてもよいピロール環を、  
 W は単結合を、  
 X は - NH - を、  
 Y は炭素原子を、  
 Z は - NH - を、  
 それぞれ意味する。)

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## 【表 2 A】

表 A

遺伝子名
Ubiquitin-conjugating enzyme
Rep-8
S-adenosylmethionine decarboxylase
p70 Ribosomal S6 kinase alpha-1
c-ErbB3 receptor tyrosine kinase
RNA polymerase II complex component SRB7
Export protein RAE1
Defective mariner transposon Hsmar2

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【表 2 B - 1】

表 B

遺伝子名	
Serine/threonine protein kinase MNK1	
Putative serine/threonine protein kinase VRK2	
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Hydrogen carrier protein, glycine synthase	
KIAA0103	
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Dihydrolipoamide succinyltransferase	10
Nuclear cap binding protein	
KIAA0077	
KIAA0074	
KIAA0100	
RNA-binding motif protein 10	
PAP39	
KIAA0146	
KIAA0159	
Multifunctional protein CAD	
KIAA0161	
KIAA0169	20
KIAA0197	
Phosphoethanolamine cytidyltransferase	
KIAA0257	
DNA topoisomerase II (top2)	
IEF SSP 9502	
Succinate dehydrogenase flavoprotein subunit	
Lamin B receptor	
Proteasome 26S subunit p45	
Retinoblastoma susceptibility protein (RB1)	
Alpha topoisomerase truncated-form	30
Tropomyosin	
Mitochondrial aspartate aminotransferase	
DNA-binding factor	
Calcineurin A1	
Ubiquitin carboxyl-terminal hydrolase	
Epidermal growth factor receptor HER3	
Mitochondrial NAD(P)+ dependent malic enzyme	
Gamma-tubulin	
Cleavage stimulation factor	
Replication factor C, 40-kDa subunit (A1)	40

## 【表 2 B - 2】

表B (続き)

遺伝子名	
Medium-chain acyl-CoA dehydrogenase (MCAD)	
Leucine-rich protein	
Monocyte/neutrophil elastase inhibitor	
Basic transcription factor 62kD subunit (BTF2)	
Transcription factor ISGF-3	
APK1 antigen=MAB KI recognized	
PDCD2, Rp8 homolog	10
Inositol 1,4,5-triphosphate receptor type 3	
G-rich sequence factor-1	
Splicesomal protein SAP61	
Cyclin H	
DNA-binding protein NFX1	
CDC16Hs	
Receptor tyrosine kinase DTK	
Chromatin assembly factor-I p150 subunit	
Methylthioadenosine phosphorylase	
Eukaryotic initiation factor 2B-epsilon	
LUCA15	20
Immuno-reactive with anti-PTH Ab	
Gps2	
Splicing factor SRp55-2 (SRp55)	
Glutathione synthetase	
Integrin-linked kinase	
FRAP-related protein (FRP1/ATR)	
Amyloid precursor protein-binding protein 1	
Retinitis pigmentosa GTPase regulator	
Enhancer of zeste homolog 2	
C2f	30
O-linked GlcNAc transferase	
Cdc6-related protein (HsCDC6)	
Clone 23759	
Mitochondrial intermediate peptidase precursor	
CaM kinase II isoform	
Pyridoxal kinase	
Transcription coactivator p75 (DFS70)	
DNA polymerase alpha subunit	
U2 snRNP-specific A protein	
Neuron specific (gamma) enolase	
GARS-AIRS-GART	40
75 kDa subunit NADH dehydrogenase precursor	
S-adenosylmethionine synthetase	
Subunit of coatomer complex	

## 【表 2 B - 3】

表B (続き)

遺伝子名
PxF
Arginine methyltransferase
Nup88
Carnitine palmitoyltransferase I type I
Transmembrane receptor protein CRF2-4

## 【請求項 3】

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遺伝子の発現量の測定を、該遺伝子の転写産物であるRNAをDNAマイクロアレイにより定量することにより行う、請求項 1 または請求項 2 に記載の方法。

## 【請求項 4】

遺伝子の発現量の測定を、該遺伝子の転写産物であるRNAを定量的PCRにより定量することにより行う、請求項 1 または請求項 2 に記載の方法。

## 【請求項 5】

請求項 4 に記載の方法において使用するための、該RNAに相補的なオリゴヌクレオチドを構成要素として含む、RNAの定量試薬。

## 【請求項 6】

遺伝子の発現量の測定を、該遺伝子の遺伝子産物である蛋白質をELISAにより定量することにより行う、請求項 1 または請求項 2 に記載の方法。

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## 【請求項 7】

遺伝子の発現量の測定を、該遺伝子の遺伝子産物である蛋白質をウエスタンブロットにより定量することにより行う、請求項 1 または請求項 2 に記載の方法。

## 【請求項 8】

請求項 6 または請求項 7 に記載の方法において使用するための、該蛋白質に対する抗体を構成要素として含む免疫測定試薬。

## 【請求項 9】

抗癌剤がN-(3-クロロ-7-インドリル)-1,4-ベンゼンジスルホンアミドである、請求項 1 ~ 4、6 および 7 のいずれか 1 項に記載の方法。

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## 【発明の詳細な説明】

## 【0001】

## 【発明の属する技術分野】

本発明は、抗癌剤に対し腫瘍細胞が感受性が否かを検定する方法に関する。

## 【0002】

## 【従来の技術】

従来の抗癌剤の臨床治験においては、まず、第一相試験で毒性のプロフィールと最大推奨用量が決められ、次いで第二相試験で腫瘍縮小率を効果判定基準とするレスポンスレート (response rate) により薬剤としての評価が成されてきた。一方、近年の癌生物学の進展に伴い、細胞内情報伝達系や血管新生などを阻害する新しい作用機序の薬剤が、活発な研究開発の途にある。これら新規抗癌剤においては必ずしも毒性用量に近い最大推奨用量が投与される必要性がない可能性が考えられる。さらに、腫瘍の縮小よりもむしろ腫瘍の増殖抑制に伴うQOL (Quality of Life) の改善や延命を指標にした方が薬剤の効果を適正に判定できるものと推測される。この場合、より論理的かつ具体的に薬剤の効果を確かめるためには、腫瘍の増殖抑制メカニズムに密接に関連する生物学的マーカーの変化を、代理 (surrogate) マーカーとして利用することが望まれる。

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## 【0003】

一般的に、抗癌療法において、抗癌剤を投与した際の生体の反応性は、薬剤の標的となる腫瘍細胞の、その薬剤に対する感受性に大きく依存する。この腫瘍細胞の薬剤に対する感受性は、腫瘍細胞毎に大きく異なるものである。このような感受性の差は、その薬剤の標

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的分子もしくはそれに関連する因子の量的もしくは質的な差異、あるいは薬剤耐性の獲得等に起因する。このような背景を踏まえると、標的となる腫瘍細胞が、薬剤に対して感受性を示す際に特異的に引き起こされる腫瘍細胞の変化を、バイオプシ等により取得した腫瘍組織等を用いて測定することができれば、これを代理マーカーとして、早期に薬剤の効果判定、治療法の確立、新たな治療法の選択等が可能となり、非常に有益である。また、治療に先立ってバイオプシ等により取得した腫瘍組織より、常法に従い腫瘍細胞を分離した後薬剤処理を行い、この腫瘍細胞が薬剤感受性であるか否かを上記代理マーカーの変化により測定すれば、予めその薬剤による治療が奏効するか否かを予測することが可能となり、临床上極めて有用である。この代理マーカーは、その変化が抗腫瘍効果に特異的であることが重要であり、かつ高感度に測定可能であればよい。具体的には、薬剤の抗腫瘍効果に特異的な遺伝子発現変動の定量、それら遺伝子発現の変化に伴うタンパク質の量的変動の解析、さらにはその変化に伴う機能変化の解析等何れもこの代理マーカーと成り得る。

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## 【 0 0 0 4 】

E7070 (N-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide) は細胞周期のG1期をターゲットとした抗腫瘍効果を有する化合物であり、現在臨床開発中である (Takashi Owa, Hiroshi Yoshino, Tatsuo Okauchi, Kentaro Yoshimatsu, Yoichi Ozawa, Naoko Hata Sugi, Takeshi Nagasu, Nozomu Koyanagi and Kyosuke Kitoh, J. Med. Chem, 1999, 42, 3789-3799)。

## 【 0 0 0 5 】

この化合物の種々の腫瘍細胞に対する細胞増殖抑制作用の強さのスペクトルは、既存の抗癌剤の何れとも異なり、新たな作用機序を有する抗癌剤としてその効果が期待される。この薬剤の臨床開発を速やかに進行させ臨床治療法を早期に確立するために、さらには確立された治療法に基づき効率よく治療を進め、患者のQOL向上に貢献するために、本薬剤投与時に特異的に使用できる代理マーカーを発見し、応用することが望まれる。

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## 【 0 0 0 6 】

近年、種々のDNAマイクロアレイを用い、多数の遺伝子の発現量を同時に検出する方法が確立され、幅広い目的に応用されている (Schena M, Shalon D, Davis RW, Brown PO, Science 1995, 270, 467-70, Lockhart, D.J., Dong, H., Byrne, M.C., Follettie, M.T., Gallo, M.V., Chee, M.S., Mittmann, M., Wang C., Kobayashi, M., Horton, H. Brown, E.L., Nature Biotechnology, 1996, 14, 1675-1680)。

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## 【 0 0 0 7 】

癌研究の分野においてもこのDNAマイクロアレイを用いた研究は盛んに行われている。例えばDNAマイクロアレイを用いた発現解析によりびまん性大B細胞リンパ腫(diffuse large B-cell lymphoma; DLBCL)を解析した研究においては、DLBCLが遺伝子発現プロファイルの違いにより2つの異なるタイプに分類され、この分類が予後の予測にも繋がること示された (Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J Jr, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Staudt LM, et al, Nature, 2000, 403, 503-11)。また米国National Cancer Instituteの60種類の癌細胞株パネルについて遺伝子発現プロファイルを解析することにより、これら細胞株を再分類し、その特性を検討した報告 (Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, Iyer V, Jeffrey SS, Van de Rijn M, Waltham M, Pergamenschikov A, Lee JC, Lashkari D, Shalon D, Myers TG, Weinstein JN, Botstein D, Brown PO, Nat Genet, 2000, 24, 227-35)、さらにこの60種類の癌細胞株パネルの遺伝子発現プロファイルと、各細胞株の各種抗癌剤に対する感受性との間の関連について考察した報告 (Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, Tanabe L, Kohn KW, Reinhold WC, Myers TG, Andrews DT, Scudiero DA, Eisen MB, Sausville EA, Pommier Y, Botstein D, Brown PO, Weinstein JN, Nat Genet, 2000, 24, 236-44)等がなされている。

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## 【 0 0 0 8 】

また、同様にDNAマイクロアレイ（一部メンブランフィルターを用いたマクロアレイ）を用いて、腫瘍細胞に抗癌剤を作用させた際に起こる遺伝子発現変化を検討した報告もいくつか成されている（Rhee CH, Ruan S, Chen S, Chenchik A, Levin VA, Yung AW, Fuller GN, Zhang W, Oncol Rep, 1999, 6, 393-401. Zimmermann J, Erdmann D, Lalande I, Grossenbacher R, Noorani M, Furst P, Oncogene, 2000, 19, 2913-20. Kudoh K, Ramanna M, Ravatn R, Elkahloun AG, Bittner ML, Meltzer PS, Trent JM, Dalton WS, Chin KV, Cancer Res, 2000, 4161-6）。これらの報告は、遺伝子発現の変動解析が、複数の細胞集団の特性比較や、薬剤の処理等により細胞に引き起こされる生物学的な変化を、分子レベルで包括的に研究する目的で極めて有用であることを示している。

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## 【 0 0 0 9 】

## 【発明が解決しようとする課題】

本発明の課題は、腫瘍細胞に、E7070及びその関連化合物を作用させた際の、該化合物の抗腫瘍効果の代理マーカーを提供することにある。

## 【 0 0 1 0 】

## 【課題を解決するための手段】

本発明者らは、E7070及びその関連化合物を、これら抗癌剤に対し感受性である腫瘍細胞に作用させた際に引き起こされる遺伝子発現の変化を、DNAマイクロアレイ法により解析し、これら抗癌剤により共通に現れる、遺伝子発現の変化を見出した。

## 【 0 0 1 1 】

更に、それら遺伝子の中には3癌種に共通した発現の変化を示すものがあることを見出し、これらの遺伝子の発現変化が、E7070及びその関連化合物の抗腫瘍効果の代理マーカーとして使用できることを見出して、本発明を完成させた。

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すなわち本発明は、以下のものを提供する。

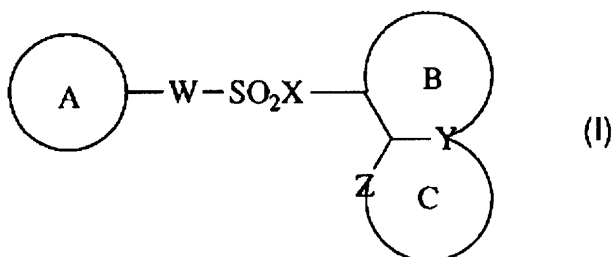
## 【 0 0 1 2 】

1. 1). 下記一般式（I）で表される抗癌剤を投与した癌患者より取り出された腫瘍細胞の、第3表及び第4表に記載の遺伝子からなる群から選択される1個または複数の遺伝子の発現量を測定し、
  - 2). 該癌患者から抗癌剤投与前に取り出された腫瘍細胞と比較して、第3表に記載の遺伝子の発現量が増加し、または第4表に記載の遺伝子の発現量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する、
- 工程を含んで成る、該腫瘍細胞の該抗癌剤に対する感受性を検定する方法。

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## 【 0 0 1 3 】

## 【化3】



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## 【 0 0 1 4 】

（式中、

- A環は、置換基を有していてもよい、単環式または二環式芳香環を、  
 B環は、置換基を有していてもよい、6員環式不飽和炭化水素またはヘテロ原子として窒素原子を1個含む不飽和6員ヘテロ環を、  
 C環は、置換基を有していてもよい、窒素原子を1または2個含む5員ヘテロ環を、  
 Wは単結合または - C H = C H - を、  
 Xは - N ( R <sup>1</sup> ) - または酸素原子を、

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Yは炭素原子または窒素原子を、  
Zは $-N(R^2)-$ または窒素原子を、  
 $R^1$ および $R^2$ は同一または異なって水素原子または低級アルキル基を、  
意味する。)

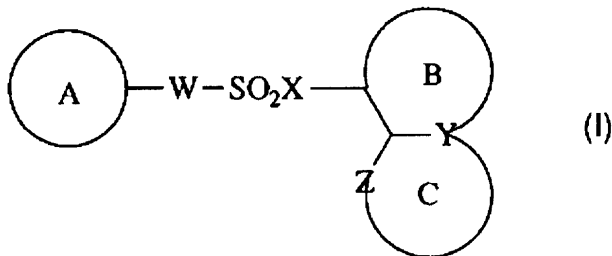
【0015】

2. 1). 癌患者より取り出された腫瘍細胞に、下記一般式(I)で表される抗癌剤を作用させ、  
2). 該腫瘍細胞の、第3表及び第4表に記載の遺伝子からなる群から選択される1個または複数の遺伝子の発現量を測定し、  
3). 無処理の腫瘍細胞と比較して、第3表に記載の遺伝子の発現量が増加し、または第4表に記載の遺伝子の発現量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する、  
工程を含んで成る、該腫瘍細胞の該抗癌剤に対する感受性を検定する方法。

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【0016】

【化4】



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【0017】

(式中、

A環は、置換基を有していてもよい、単環式または二環式芳香環を、  
B環は、置換基を有していてもよい、6員環式不飽和炭化水素またはヘテロ原子として窒素原子を1個含む不飽和6員ヘテロ環を、  
C環は、置換基を有していてもよい、窒素原子を1または2個含む5員ヘテロ環を、  
Wは単結合または $-CH=CH-$ を、  
Xは $-N(R^1)-$ または酸素原子を、  
Yは炭素原子または窒素原子を、  
Zは $-N(R^2)-$ または窒素原子を、  
 $R^1$ および $R^2$ は同一または異なって水素原子または低級アルキル基を、  
意味する。)

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【0018】

3. 遺伝子の発現量の測定を、該遺伝子の転写産物であるRNAをDNAマイクロアレイにより定量することにより行う、1または2に記載の方法。

【0019】

4. 遺伝子の発現量の測定を、該遺伝子の転写産物であるRNAを定量的PCRにより定量することにより行う、1または2に記載の方法。

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【0020】

5. 4に記載の方法において使用するための、該RNAに相補的なオリゴヌクレオチドを構成要素として含む、RNAの定量試薬。

【0021】

6. 遺伝子の発現量の測定を、該遺伝子の遺伝子産物である蛋白質を免疫化学的方法により定量することにより行う、1または2に記載の方法。

【0022】

7. 遺伝子の発現量の測定を、該遺伝子の遺伝子産物である蛋白質をELISAにより定量することにより行う、6に記載の方法。

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## 【 0 0 2 3 】

8. 遺伝子の発現量の測定を、該遺伝子の遺伝子産物である蛋白質をウエスタンブロットにより定量することにより行う、6に記載の方法。

## 【 0 0 2 4 】

9. 6に記載の方法において使用するための、該蛋白質に対する抗体を構成要素として含む免疫測定試薬。

## 【 0 0 2 5 】

10. A環が置換基を有していてもよい、ベンゼンまたはピリジンであり、B環が置換基を有していてもよいベンゼンであり、C環が置換基を有していてもよいピロールであり、Wが単結合であり、かつXおよびZがいずれも - NH - である、1または2に記載の方法。

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## 【 0 0 2 6 】

## 【発明の実施の形態】

以下に本発明の実施の形態について、詳細に説明する。

## 【 0 0 2 7 】

本発明の検定方法は、腫瘍細胞がin vivoまたはin vitroで抗癌剤に暴露されたときの、腫瘍細胞における特定の遺伝子の発現量の変化を、腫瘍細胞の抗癌剤に対する感受性の指標とする点に特徴を有する。

## 【 0 0 2 8 】

従って、本発明の第1の態様の検定方法は、1). 上記一般式(I)で表される抗癌剤を投与した癌患者より取り出された腫瘍細胞の、第3表及び第4表に記載の遺伝子からなる群から選択される1個または複数の遺伝子の発現量を測定し、

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2). 該癌患者から抗癌剤投与前に取り出された腫瘍細胞と比較して、第3表に記載の遺伝子の発現量が増加し、または第4表に記載の遺伝子の発現量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する、  
工程を含んで成る。

## 【 0 0 2 9 】

また、本発明の第2の態様の検定方法は、1). 癌患者より取り出された腫瘍細胞に、上記一般式(I)で表される抗癌剤を作用させ、

2). 該腫瘍細胞の、第3表及び第4表に記載の遺伝子からなる群から選択される1個または複数の遺伝子の発現量を測定し、

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3). 無処理の腫瘍細胞と比較して、第3表に記載の遺伝子の発現量が増加し、または第4表に記載の遺伝子の発現量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する、

工程を含んで成る。

## 【 0 0 3 0 】

本発明における抗癌剤は、上記一般式(I)で表されるスルホンアミド誘導体及びスルホン酸エステル誘導体である。

## 【 0 0 3 1 】

上記一般式(I)において、A環の意味する「置換基を有していてもよい、単環式または二環式芳香環」とは、芳香族炭化水素、または窒素原子、酸素原子および硫黄原子のうち少なくとも1個を含む芳香族ヘテロ環であり、当該環上には置換基1~3個があってもよいものを示す。A環に含まれる主な芳香環を例示すると、ピロール、ピラゾール、イミダゾール、チオフェン、フラン、チアゾール、オキサゾール、ベンゼン、ピリジン、ピリミジン、ピラジン、ピリダジン、ナフタレン、キノリン、イソキノリン、フタラジン、ナフチリジン、キノキサリン、キナゾリン、シンノリン、インドール、イソインドール、インドリジン、インダゾール、ベンゾフラン、ベンゾチオフェン、ベンズオキサゾール、ベンズイミダゾール、ベンゾピラゾール、ベンゾチアゾールなどがある。上記芳香環は置換基1~3個を有していてもよく、置換基が複数個ある場合には、同一または異なってもよい。置換基としては、例えば、低級アルキル基または低級シクロアルキル基で置換され

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ていてもよいアミノ基、低級アルキル基、低級アルコキシ基、水酸基、ニトロ基、メルカプト基、シアノ基、低級アルキルチオ基、ハロゲン基、式 - a - b [ 式中、a は単結合、 $-(CH_2)_k-$ 、 $-O-(CH_2)_k-$ 、 $-S-(CH_2)_k-$  または  $-N(R^3)-(CH_2)_k-$  を、k は 1 ~ 5 の整数を、 $R^3$  は水素原子または低級アルキル基を、b は  $-CH_2-d$  ( 式中、d は低級アルキル基で置換されていてもよいアミノ基、ハロゲン基、水酸基、低級アルキルチオ基、シアノ基または低級アルコキシ基を意味する ) を意味する ] で示される基、式 - a - e - f [ 式中、a は前記と同じ意味を、e は  $-S(O)-$  または  $-S(O)_2-$  を、f は低級アルキル基または低級アルコキシ基で置換されていてもよいアミノ基、低級アルキル基、トリフルオロメチル基、 $-(CH_2)_m-b$  または  $-N(R^4)-(CH_2)_m-b$  ( 式中、b は前記と同じ意味を示し、 $R^4$  は水素原子または低級アルキル基を、m は 1 ~ 5 の整数を意味する ) を意味する ] で示される基、式 - a - g - h [ 式中、a は前記と同じ意味を示し、g は  $-C(O)-$  または  $-C(S)-$  を、h は低級アルキル基で置換されていてもよいアミノ基、水酸基、低級アルキル基、低級アルコキシ基、 $-(CH_2)_n-b$  または  $-N(R^5)-(CH_2)_n-b$  ( 式中、b は前記と同じ意味を示し、 $R^5$  は水素原子または低級アルキル基を、n は 1 ~ 5 の整数を意味する ) を意味する ] で示される基、式 - a - N(R<sup>6</sup>) - g - i [ 式中、a および g は前記と同じ意味を示し、 $R^6$  は水素原子または低級アルキル基を、i は水素原子、低級アルコキシ基または f ( f は前記と同じ意味を示す ) を意味する ] で示される基、式 - a - N(R<sup>7</sup>) - e - f ( 式中、a、e および f は前記と同じ意味を示し、 $R^7$  は水素原子または低級アルキル基を意味する ) で示される基、または式  $-(CH_2)_p-j-(CH_2)_q-b$  ( 式中、j は酸素原子または硫黄原子を意味し、b は前記と同じ意味を示し、p および q は同一または異なって 1 ~ 5 の整数を意味する ) で示される基などを挙げる事ができる。

#### 【 0 0 3 2 】

上記置換基例において、アミノ基が 2 個のアルキル基で置換されている場合には、これらのアルキル基が結合して 5 または 6 員環を形成していてもよい。また、A 環が水酸基またはメルカプト基を有する含窒素ヘテロ環である場合には、これらの基が共鳴構造をとることにより、オキソ基またはチオキソ基の形になっていてもよい。

#### 【 0 0 3 3 】

B 環の意味する「置換基を有していてもよい、6 員環式不飽和炭化水素またはヘテロ原子として窒素原子を 1 個含む不飽和 6 員ヘテロ環」とは、一部が水素化されていてもよい、ベンゼンまたはピリジンであり、当該環上に置換基 1 または 2 個を有していてもよく、置換基が 2 個ある場合には同一または異なっていてもよいものを示す。

#### 【 0 0 3 4 】

C 環の意味する「置換基を有していてもよい、窒素原子を 1 または 2 個含む 5 員ヘテロ環」とは、一部が水素化されていてもよい、ピロール、ピラゾール、イミダゾールであり、当該環上に置換基 1 または 2 個を有していてもよく、置換基が 2 個ある場合には同一または異なっていてもよいものを示す。

#### 【 0 0 3 5 】

B 環および C 環が有していてもよい置換基としては、例えば、ハロゲン基、シアノ基、低級アルキル基、低級アルコキシ基、水酸基、オキソ基、式  $-C(O)-r$  ( 式中、r は水素原子、低級アルキル基で置換されていてもよいアミノ基、低級アルキル基、低級アルコキシ基または水酸基を意味する )、低級アルキル基で置換されていてもよいアミノ基、トリフルオロメチル基などを挙げる事ができる。

#### 【 0 0 3 6 】

上記一般式 ( I ) において、 $R^1$ 、 $R^2$  ならびに A 環、B 環および C 環が有していてもよい置換基の定義中の低級アルキル基は、炭素数 1 ~ 6 の直鎖もしくは分枝状のアルキル基、例えばメチル基、エチル基、n - プロピル基、イソプロピル基、n - ブチル基、イソブチル基、sec - ブチル基、tert - ブチル基、n - ペンチル基 ( アミル基 )、イソペンチル基、ネオペンチル基、tert - ペンチル基、1 - メチルブチル基、2 - メチルブチル基、1, 2 - ジメチルプロピル基、n - ヘキシル基、イソヘキシル基、1 - メチルペンチル基、2

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-メチルペンチル基、3-メチルペンチル基、1,1-ジメチルブチル基、1,2-ジメチルブチル基、2,2-ジメチルブチル基、1,3-ジメチルブチル基、2,3-ジメチルブチル基、3,3-ジメチルブチル基、1-エチルブチル基、2-エチルブチル基、1,1,2-トリメチルプロピル基、1,2,2-トリメチルプロピル基、1-エチル-1-メチルプロピル基、1-エチル-2-メチルプロピル基などを意味する。これらのうち好ましい基としては、メチル基、エチル基、n-プロピル基、イソプロピル基、n-ブチル基、イソブチル基などを挙げる事ができ、これらのうち、最も好ましい基としてはメチル基、エチル基、n-プロピル基、イソプロピル基を挙げる事ができる。

【0037】

A環が有していてもよい置換基の定義中の低級シクロアルキル基は、炭素数3~8のシクロアルキル基を意味し、例えばシクロプロピル基、シクロペンチル基、シクロヘキシル基などを挙げる事ができる。

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【0038】

A環、B環およびC環が有していてもよい置換基の定義中の低級アルコキシ基とは、メトキシ基、エトキシ基、n-プロポキシ基、イソプロポキシ基、n-ブトキシ基、イソブトキシ基、tert-ブトキシ基など上記の低級アルキル基から誘導されるアルコキシ基を意味するが、これらのうち最も好ましい基としてはメトキシ基、エトキシ基を挙げる事ができる。低級アルキルチオ基は、上記の低級アルキル基から誘導されるアルキルチオ基を意味する。またハロゲン原子としてはフッ素原子、塩素原子、臭素原子などが挙げられる。

【0039】

上記一般式(I)で示されるスルホンアミド誘導体またはスルホン酸エステル誘導体は酸または塩基と塩を形成する場合もある。本発明における抗癌剤は一般式(I)で示される化合物の塩をも包含する。酸との塩としては、たとえば塩酸塩、臭化水素酸塩、硫酸塩等の無機酸塩や酢酸、乳酸、コハク酸、フマル酸、マレイン酸、クエン酸、安息香酸、メタンスルホン酸、p-トルエンスルホン酸などの有機酸との塩を挙げる事ができる。また、塩基との塩としては、ナトリウム塩、カリウム塩、カルシウム塩などの無機塩、トリエチルアミン、アルギニン、リジン等の有機塩基との塩を挙げる事ができる。

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【0040】

また、これら化合物の水和物はもちろんのこと光学異性体が存在する場合はそれらすべてが含まれることはいうまでもない。また、本発明における抗癌剤は強い抗腫瘍活性を示すが、生体内で酸化、還元、加水分解、抱合などの代謝を受けて抗腫瘍活性を示す化合物をも包含する。またさらに、本発明における抗癌剤は生体内で酸化、還元、加水分解などの代謝を受けて一般式(I)で示される化合物を生成する化合物をも包含する。

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【0041】

感受性を検定する腫瘍細胞は、一般式(I)で表される抗癌剤に対して感受性を示すものであれば、特に限定されない。例えば、大腸癌、肺癌、乳癌、白血病、膵臓癌、腎臓癌、メラノーマ、悪性リンパ腫、頭頸部癌、胃癌などに由来する腫瘍細胞が挙げられる。

【0042】

癌患者から取り出された腫瘍細胞は、癌患者から摘出された癌組織に含まれる腫瘍細胞も包含される。

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【0043】

第1の態様の検定方法の場合には、癌患者が取り出された腫瘍細胞は、一般式(I)で表される抗癌剤が、腫瘍細胞の感受性が測定可能となる量投与されている癌患者から取り出された腫瘍細胞であればよく、通常には100~1500 mgの用量で1~14日間投与された癌患者から取り出された腫瘍細胞である。

【0044】

第2の態様の検定方法の場合には、癌患者から取り出された腫瘍細胞に、一般式(I)で表される抗癌剤を作用させる条件は、腫瘍細胞の感受性が測定可能となるものであればよく、通常には、培地中0.01~10 μMの抗癌剤濃度で、6~72時間の培養という条件が挙げられる。

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## 【 0 0 4 5 】

遺伝子の発現量の測定は、遺伝子の転写産物であるRNAまたは遺伝子産物である蛋白質を定量することにより行うことができる。RNAまたは蛋白質の定量は、通常には、腫瘍細胞からRNAまたは蛋白質を抽出し、抽出物中のRNAまたは蛋白質を定量することによって行うことができる。以下、1. RNAまたは蛋白質の抽出、2. RNAの定量、3. 蛋白質の定量の順に、それらの例を詳細に説明する。

## 【 0 0 4 6 】

## 1. RNAまたは蛋白質の抽出

1). 一般式 ( I ) で表される抗癌剤を投与された患者の癌組織からの、RNAまたは蛋白質の抽出

一般式 ( I ) で表される抗癌剤を投与された患者より、バイオプシ等で摘出された癌組織から以下に述べる方法で、RNAまたは蛋白質を抽出する。

## 【 0 0 4 7 】

RNAの抽出は、一般的なRNA抽出法に従って行えばよい。例えばTRIZOL試薬 ( ライフテックオリエンタル ) 等を用いて、添付の操作法に従って行えばよい。具体的には以下のとおりである。癌組織50 ~ 100 mgに対して1 mlのTRIZOL試薬を加え、テフロンホモジェナイザーを用いて均一化する。これを遠心し ( 12,000 x g、10分間、4 )、得られた上清を室温で5分間放置後、使用したTRIZOL試薬1 mlに対して0.2 mlの割合でクロロフォルムを添加する。この溶液を15秒間激しく振盪、攪拌し室温で2 ~ 3分間放置後遠心を行う ( 12,000 x g、15分間、4 )。遠心後、水層を新しいチューブに移し、使用したTRIZOL試薬1 mlに対して0.5 mlの割合でイソプロピルアルコールを加え、室温で10分間放置後、遠心を行う ( 12,000 x g、10分間、4 )。得られた沈殿を75%エタノールにて洗浄した後、風乾し、全RNAとして以降の操作に供する。

## 【 0 0 4 8 】

癌組織からの蛋白質の抽出は、Bollag, D. M., Rozycki M. D., Edelstein S. J., Protein Methods, 1996, Wiley-Liss, Inc., New York, U.S.A. Walker, J. M., The protein handbook, 1996, Humana Press, New Jersey, U.S.A.等に記載の方法に従って行えばよい。

## 【 0 0 4 9 】

2). 一般式 ( I ) で表される抗癌剤の存在下で培養した癌細胞からの、RNAまたは蛋白質の抽出

患者よりバイオプシ等で得られた癌組織から、定法に従い癌細胞 ( 腫瘍細胞 ) を分離する。例えばHamburgerら ( Hamburger A., Salmon S. E., Kim M. B., Trent J. M., Soehnle n B. J., Alberts D. S., and Schmidt H. t., Cancer Res., 38, 3438-3443, 1978 ) に従い、得られた組織を無菌的に細切し、その後ステンレスメッシュ、注射針、さらにはナイロンメッシュ等を用いて細胞の懸濁液を調製する。こうして得られた細胞を適当な培地 ( 例えば、10 ~ 15%FCSを含むRPMI-1640、MEM、McCoy培地等 ) に培養する。得られた癌細胞を一般式 ( I ) で表される抗癌剤の存在下に適当な期間、好ましくは3時間・6時間・12時間あるいは24時間、更に好ましくは12時間5%CO<sub>2</sub>条件下37 にて培養し、以下に述べる方法で、RNAまたは蛋白質を抽出する。なお、使用するバイオプシ等で得られた組織中から軟寒天培養法 ( Hamburger A., and Salmon S. E., Science, 197, 461-463, 1977、Hamburger A., and Salmon S. E., J. Clin. Invest., 60, 846-854, 1977、Von Hoff D. D., and Johnson, G. E., Proc. Am. Assoc. Cancer Res., 20, 51, 1979 ) を用いて、癌細胞のみを特異的に分離して用いることも可能である。

## 【 0 0 5 0 】

癌細胞からのRNAの抽出は、癌組織からのRNAの抽出と同様、一般的なRNA抽出法に従って行えばよい。例えばTRIZOL試薬 ( ライフテックオリエンタル ) を用いた場合、添付の操作法に従って行えばよい。具体的には癌細胞5 ~ 10 x 10<sup>6</sup>に対して1 mlのTRIZOL試薬を加え、以下癌組織からのRNAの抽出と同様の操作を行えばよい。

## 【 0 0 5 1 】

癌細胞からの蛋白質の抽出についても、癌組織からの抽出と同様、成書に記載の方法に従えばよい。

【 0 0 5 2 】

## 2 . RNAの定量

RNAはノーザンブロット解析・DNAマイクロアレイ・RT-PCR・定量的PCR等の技術により定量できる。好ましくはDNAマイクロアレイ・定量的PCRであることが好ましい。以下にそれぞれについて説明するが、本発明はこれにより限定されない。

【 0 0 5 3 】

DNAマイクロアレイによる定量は次のように行う。最初に得られたRNAを鋳型としてSuperScrip t Choice System (ライフテックオリエンタル) 及びT7-d(T)<sub>24</sub>プライマーを用いて2本鎖のcDNAを合成し、続いてそのcDNAを鋳型としてビオチン化したcRNAを合成する。

【 0 0 5 4 】

具体的には、先ず得られたRNAよりT7-d(T)<sub>24</sub>プライマーを用いて1本鎖のDNAを合成し、次いでdNTP・DNAリガーゼ・DNAポリメラーゼI・RNase Hを添加して反応後、更にT4 DNAポリメラーゼIを添加して2本鎖cDNAを合成する。得られたcDNAを精製後、RNA Transcript Labeling Kit (Enzo Diagnostics) を用い、ビオチン化UTPならびにCTPを加えてラベル化反応を行う。反応生成物を精製後、200 mM トリス酢酸 pH8.1、150 mM 酢酸マグネシウム、50 mM 酢酸カリウム中で94 にて35分間加熱して、断片化したcRNAを得る。

【 0 0 5 5 】

断片化したcRNAを、例えば100 mM MES、1 M ナトリウム塩、20 mM EDTA、0.01% Tween 20 中、45 にて16時間、GeneChip (Affymetrix) Hu6800あるいは同等の製品にハイブリダイズさせる。ハイブリダイズ後、GeneChipはAffymetrix fluidics stationに添付のプロトコールEukGE-WS2に従い洗浄・染色する。染色にはストレプトアビジン-フィコエリトリンとビオチン化抗ストレプトアビジン山羊抗体を用いる。染色後のGeneChipをHP アルゴンイオンレーザー共焦点顕微鏡 (Hewlett Packard) を用いてスキャンし、蛍光強度を測定する。この蛍光色素の場合、測定は488 nmの励起光を用い570 nmの蛍光を測定する。

【 0 0 5 6 】

定量的データ解析を、好ましくはGeneChip software (Affymetrix) を用いて行う。RNAの定量を行うために、それぞれのプローブファミリー毎に「差 ([完全マッチハイブリダイゼーションシグナル(perfect match hybridization signal)] - [ミスマッチシグナル(mismatch signal)])」の平均 (average difference) を求め、この値が50以上であり、かつ2つの条件間でRNAの定量値が乖離している場合、好ましくは1.8倍以上乖離している場合につき、その遺伝子の発現が有意に「増加」あるいは「減少」したと判断する。

【 0 0 5 7 】

第3表に記載の1個または複数の遺伝子のRNA量が増加し、または第4表に記載の1個または複数の遺伝子のRNA量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する。

【 0 0 5 8 】

また、定量的PCRはSYBR GreenとABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems) を用い、次のように行う。

【 0 0 5 9 】

操作は逆転写反応及びPCR反応の2段階で行う。最初の段階である逆転写反応は、得られたRNAにdNTP・oligo d(T)<sub>16</sub>プライマー・Rnaseインヒビター・Multiscribe Reverse Transcriptase (Perkin-Elmer Applied Biosystems) を加え、25 にて10分間保温後、48 にて30分間加熱することにより行う。反応を95 5分間加熱することにより停止させる。

【 0 0 6 0 】

得られたcDNAを第2段階のPCR反応に供する。PCR反応は、例えば4 ng cDNA、1xSYBR PCRバッファー、3 mM MgCl<sub>2</sub>、各200 μM dATP、dCTP、dGTP、400 μM dUTP、200 nM プライマー対、0.01 U/μl AmpErase UNG、0.025 U/μl AmpliTaq Gold DNA Polymerase (Perkin-Elmer Applied Biosystems) の反応系で行う。反応条件は、例えば50 2分間、95 10

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分間に次いで95 20秒間・55 20秒間・72 30秒間を40サイクルで行う。プライマーとプローブは、例えばPrimer Expression (Perkin-Elmer Applied Biosystems) を用いて設計する。複数検体の比較は、定量値を各検体の転写量に変動の少ないハウスキーピング (house keeping) 遺伝子、好ましくはGAPDHのmRNAレベルにより補正して行う。

【0061】

第3表に記載の1個または複数の遺伝子のRNA量が増加し、または第4表に記載の1個または複数の遺伝子のRNA量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する。

【0062】

本発明は、本発明の検定方法において使用するための、発現量を測定する対称の遺伝子の転写産物であるRNAに相補的なオリゴヌクレオチドを構成要素として含む、RNAの定量試薬も提供する。構成成分となるオリゴヌクレオチドは、定量的PCRに使用されるプライマー及び/またはプローブであり、上述のようにして設計することができる。本発明のRNAの定量試薬は、上記オリゴヌクレオチドに加えて、一般の定量試薬において慣用的な成分を含んでいてもよい。

【0063】

### 3. 蛋白質の定量

蛋白質は、その活性あるいは抗原性を基に定量するが、好ましくは蛋白質一般に適用しやすい抗原性を基にした定量、即ち免疫化学的定量が好ましい。

【0064】

蛋白質に対する抗体は、そのアミノ酸配列よりParkerらの報告 (Parker J. M. R., Guo D., Hodges R. S., Biochemistry, 25, 5425, 1986) あるいはKarplusらの報告 (Karplus P. A., Schulz G. E., Naturwissenschaften, 72, 212, 1985) に基づいて抗原決定基を予測し、ペプチドを合成し、あるいは融合蛋白例えばグルタチオン合成酵素 (GST) との融合蛋白を発現させてグルタチオンカラムで精製して、抗原を作製し、得られた抗原を家兎・マウス等に免疫してポリクローナル、好ましくはモノクローナル抗体 (Harlow E., Lane D., Antibodies: A Laboratory Manual, 1988, Cold Spring Harbor Laboratory Press, New York) を作製することができる。また、市販の抗体が利用可能な場合は、特異性を確かめた上で使うことも許される。

【0065】

得られた抗体を用い、例えば酵素免疫測定法 (石川栄治他、医学書院、1982) またはEnzyme Immunoassay (Ishikawa E., Kawai T., Miyai, K., Igaku-Shoin, Tokyo New York, 1981) に記載の方法によりELISAあるいはRIAを行い蛋白質を定量する。蛋白質が腫瘍細胞外に分泌されるものである場合には、腫瘍細胞から蛋白質を抽出をすることなく培地中の蛋白質を定量することが可能である。

【0066】

第3表に記載の1個または複数の遺伝子の蛋白質量が増加し、あるいは第4表に記載の1個または複数の遺伝子の蛋白質量が減少する場合に、該腫瘍細胞が該抗癌剤に対して感受性であると判定する。

【0067】

本発明は、本発明の検定方法において使用するための、該蛋白質に対する抗体を構成要素として含む免疫測定試薬も提供する。構成成分となる抗体は、上述のようにして得ることができる。本発明の免疫測定試薬は、上記抗体に加えて、一般の免疫測定試薬において慣用的な成分を含んでいてもよい。

【0068】

### 【実施例】

以下に、具体的な例をもって本発明を示すが、本発明はこれに限られるものではない。

【0069】

### 【実施例1】

E7070感受性株及び耐性株の培養とRNAの抽出

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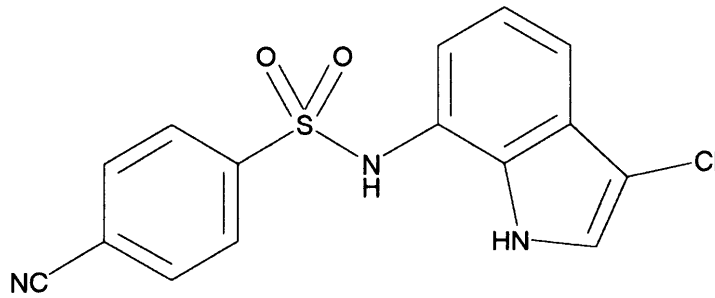
全ての細胞は10%の胎児牛血清、100 units/mlのペニシリン、100  $\mu$ g/mlのストレプトマイシンを添加したRPMI-1640培地を用いて5%CO<sub>2</sub>条件下37℃にて培養した。

【0070】

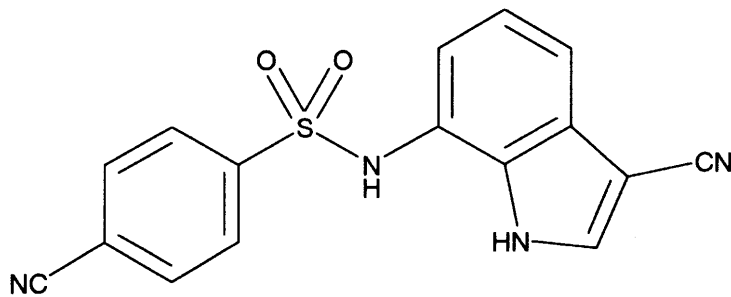
E7070 または下記に構造式を示すER-35748もしくはER-68487をE7070感受性株HCT116-C9及びE7070耐性株HCT116-C9-C1の培地に8  $\mu$ Mの濃度で添加して培養し、0, 3, 6, 12時間後の細胞を回収した。また、薬剤を加えずに12時間培養した細胞も同様に回収した。

【0071】

【化5】



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【0072】

これらの細胞より全RNAを抽出しその後の解析に供した。薬剤添加12時間後の細胞から抽出したRNAならびに薬剤を添加せずに12時間培養した細胞から抽出したRNAを実施例2に示すDNAマイクロアレイによる遺伝子発現解析に用いた。なお、HCT116-C9はヒト大腸癌由来HCT116 (American Type Culture Collection, Manassas, VA, U.S.A.) から分離した垂株であり、このHCT116-C9をE7070存在下培養、E7070濃度を漸次的に上昇させることにより得たE7070耐性垂株がHCT116-C9-C1である。

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【0073】

同様にE7070をE7070感受性株LX-1及びE7070耐性株LX-1-E2の培地に8  $\mu$ Mの濃度で添加して培養し、0, 3, 6, 12時間後の細胞を回収した。これらの細胞より全RNAを抽出しその後の解析に供した。なお、LX-1 (Cancer Chemotherapy Center, Japan Foundation for Cancer Research, Tokyo, Japan) はヒト小細胞性肺癌由来の細胞株であり、このLX-1をE7070存在下培養、E7070濃度を漸次的に上昇させることにより得たE7070耐性垂株がLX-1-E2である。

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【0074】

使用した細胞株HCT116-C9、HCT116-C9-C1、LX-1、LX-1-E2にE7070を添加し72時間培養し、MTT法 (Mosmann T., J. Immunol. Methods, 65, 55, 1983) により測定した細胞増殖抑制曲線を図1に示す。実際の操作はCellTiter 96 Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI) を用いて、添付の操作法に従って行った。

【0075】

回収した細胞からの全RNAの抽出は、TRIZOL試薬 (ライフテックオリエンタル) を用いて添付の操作法に従って行った。

【0076】

【実施例2】

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## DNAマイクロアレイによる遺伝子発現解析

## 1) .cDNA合成とビオチン標識

実施例1で得られたRNAを100  $\mu$ lのジエチルピロカーボネート (DEPC) 処理をした滅菌水に溶解し、さらにRNeasyカラム (QIAGEN) を用いて精製し、SuperScript Choice System (ライフテックオリエンタル) 及びT7-d(T)<sub>24</sub>プライマーを用いて2本鎖のcDNAを合成した。

## 【0077】

まず10  $\mu$ gのRNAに5  $\mu$ MのT7-d(T)<sub>24</sub>プライマー、1x First strand buffer、10 mM DTT、500  $\mu$ MのdNTP mix、20 units/ $\mu$ lのSuperScript II Reverse Transcriptaseを加え、42  $^{\circ}$ Cにて1時間反応させ1本鎖DNAを合成した。続いて1x Second strand buffer、200  $\mu$ MのdNTP mix、67 U/ml DNA ligase、270 U/ml DNAポリメラーゼI、13 U/ml RNase Hを添加して16  $^{\circ}$ Cにて2時間反応させ2本鎖cDNAを合成した。さらに67 U/ml T4 DNAポリメラーゼIを添加して16  $^{\circ}$ Cにて5分間反応させたのち、10  $\mu$ lの0.5 M EDTAを加え反応を停止した。

## 【0078】

得られたcDNAをフェノール/クロロフォルムにて精製し、RNA Transcript Labeling Kit (Enzo Diagnostics) を用い、添付の操作法に従って、ビオチン化UTPならびにCTPによるラベル化反応を行った。反応生成物をRneasyカラムにて精製後、200 mM トリス酢酸 pH8.1、150 mM 酢酸マグネシウム、50 mM 酢酸カリウム中で94  $^{\circ}$ Cにて35分間加熱してcRNAを断片化した。

## 【0079】

## 2) .DNAマイクロアレイ (GeneChip) へのハイブリダイズと測定

断片化したcRNAを、100 mM MES、1 M ナトリウム塩、20 mM EDTA、0.01% Tween 20中、45  $^{\circ}$ Cにて16時間、GeneChip (Affymetrix) Hu6800にハイブリダイズさせた。ハイブリダイズ後、GeneChipはAffymetrix fluidics stationに添付のプロトコールEukGE-WS2に従い洗浄・染色した。染色にはストレプトアビジン-フィコエリトリンとビオチン化抗ストレプトアビジン山羊抗体を用いた。染色後のGeneChipをHP アルゴンイオンレーザー共焦点顕微鏡 (Hewlett Packard) を用いてスキャンし、蛍光強度を測定した。測定は、488 nmの励起光を用い570 nmの蛍光で行った。

## 【0080】

定量的データ解析は全てGeneChip software (Affymetrix) を用いて行った。RNAの定量を行うために、それぞれのプローブファミリー毎に「差 ([完全マッチハイブリダイゼーションシグナル(perfect match hybridization signal)] - [ミスマッチシグナル(mismatch signal)])」の平均 (average difference) を求め、この値が50以上であり、かつ2つの条件間でRNAの定量値が乖離している場合、好ましくは1.8倍以上乖離している場合につき、その遺伝子の発現が有意に「増加」あるいは「減少」したと判断した。

## 【0081】

E7070 またはER-35748、ER-68487をHCT116-C9の培地に8  $\mu$ Mの濃度で添加して培養し、12時間後に細胞から抽出したRNAのGeneChipによる解析結果を、薬剤を加えないで同様の操作により得たHCT116-C9細胞のRNA解析結果と比較した。この結果、E7070、ER-35748、ER-68487の3種の薬剤の処理により、共通してその発現が上昇した遺伝子のリスト (GenBankの登録番号もあわせて示す(以下の表において同じ)) を第1表に、同様に3種の薬剤の処理により共通してその発現が減少した遺伝子のリストを第2表に示す。

## 【0082】

## 【表1】

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第 1 表

登録番号	遺伝子名
D59253	NCBP interacting protein 1
D78514	Ubiquitin-conjugating enzyme
D83767	Rep-8
HG1139-HT4910	FK506-binding protein
HG3484-HT3678	Cdc-like kinase 1 (Clk1)
J04152	Tumor-associated antigen GA733-1, M1S1
M21154	S-Adenosylmethionine decarboxylase
M60724	p70 ribosomal S6 kinase alpha-1
M84349	Transmembrane protein CD59
S61953	c-ErbB3 receptor tyrosine kinase
U52960	RNA polymerase II complex component SRB7
U84720	Export protein RAE1
U92014	Defective mariner transposon Hsmar2
Z18951	Caveolin

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【 0 0 8 3 】

【 表 2 】

第2表

登録番号	遺伝子名	
AB000409	Serine/threonine protein kinase MNK1	
AB000450	Putative serine/threonine protein kinase VRK2	
AB002380	Leukemia-associated Rho GEF	
AB003102	Proteasome 26S subunit p44.5	
AC002045	CIT987SK-A-589H1	
AF002020	Niemann-Pick C disease protein (NPC1)	
AF008445	Phospholipid scramblase	
D00723	Hydrogen carrier protein, glycine synthase	10
D14659	KIAA0103	
D21852	KIAA0029	
D26535	Dihydrolipoamide succinyltransferase	
D28364	Annexin II	
D29677	KIAA0054	
D29810	Unknown product	
D29956	Ubiquitin specific protease 8	
D30756	KIAA0049	
D31883	Actin-binding LIM protein	
D32002	Nuclear cap binding protein	
D38521	KIAA0077	20
D38552	KIAA0073	
D38553	KIAA0074	
D43947	KIAA0100	
D43948	ch-TOG	
D50645	SDF2	
D50663	Dynein (TCTEL1)	
D50912	RNA-binding motif protein 10	
D50916	Ubiquitination factor E4A	
D61391	PAP39	
D63480	KIAA0146	
D63506	Syntaxin-binding protein 3	30
D63875	TPR-containing/SH2-binding protein	
D63880	KIAA0159	
D78586	Multifunctional protein CAD	
D79983	KIAA0161	
D79987	KIAA0165	
D79988	KIAA0166	
D79991	KIAA0169	
D83776	KIAA0191	
D83781	KIAA0197	
D84307	Phosphoethanolamine cytidyltransferase	40
D86981	Amyloid precursor protein-binding protein 2	
D87435	KIAA0248	

【 0 0 8 4 】

【 表 3 】

第2表 (続き)

登録番号	遺伝子名	
D87446	KIAA0257	
D87448	DNA topoisomerase II-binding protein	
D87743	Solute carrier family 9	
HG1869-HT1904	Male enhanced antigen	
HG2379-HT3996	Serine hydroxymethyltransferase, cytosolic	
HG4094-HT4364	Transcription factor Lsf-Id	
J04088	DNA topoisomerase II (top2)	
J04501	Muscle glycogen synthase	10
J04543	Synexin	
L06845	Cysteinyl-tRNA synthetase	
L07033	Hydroxymethylglutaryl-CoA lyase	
L07540	Replication factor C, 36-kDa subunit	
L07597	Ribosomal protein S6 kinase 2	
L07758	IEF SSP 9502	
L21936	Succinate dehydrogenase flavoprotein subunit	
L25444	TAFII70-alpha	
L25931	Lamin B receptor	
L33075	Ras GTPase-activating-like protein IQGAP1	
L33881	Protein kinase C iota isoform	20
L38810	Proteasome 26S subunit p45	
L40395	Eukaryotic initiation factor 2B-beta	
L41870	Retinoblastoma susceptibility protein (RB1)	
L47276	Alpha topoisomerase truncated-form	
M15796	PCNA	
M19267	Tropomyosin	
M22632	Mitochondrial aspartate aminotransferase	
M23379	GTPase-activating protein ras p21	
M24486	Prolyl 4-hydroxylase alpha subunit	
M29204	DNA-binding factor	
M29550	Calcineurin A1	30
M30496	Ubiquitin carboxyl-terminal hydrolase	
M33518	HLA-B-associated transcript 2 (BAT2)	
M34309	Epidermal growth factor receptor HER3	
M37400	Cytosolic aspartate aminotransferase	
M55905	Mitochondrial NAD(P)+ dependent malic enzyme	
M58525	Catechol-O-methyltransferase	
M59911	Integrin alpha-3	
M61764	Gamma-tubulin	
M62994	Filamin B	
M74089	TB1	40
M85085	Cleavage stimulation factor	
M86707	Myristoyl CoA:protein N-myristoyl transferase	

【 0 0 8 5 】

【 表 4 】

第2表 (続き)

登録番号	遺伝子名	
M87338	Replication factor C, 40-kDa subunit (A1)	
M87339	Replication factor C, 37-kDa subunit (RFC4)	
M88163	Global transcription activator (hSNF2/SWI2)	
M91432	Medium-chain acyl-CoA dehydrogenase (MCAD)	
M92439	Leucine-rich protein	
M93056	Monocyte/neutrophil elastase inhibitor	
M95809	Basic transcription factor 62kD subunit (BTF2)	
M95929	Homeobox protein PHOX1	10
M97935	Transcription factor ISGF-3	
S58544	75kDa infertility-related sperm protein	
S59184	RYK=related to receptor tyrosine kinase	
S72904	APK1 antigen=MAB KI recognized	
S78085	PDCD2, Rp8 homolog	
S80343	Arginyl-tRNA synthetase (ArgRS)	
U01062	Inositol 1,4,5-triphosphate receptor type 3	
U02566	Receptor tyrosine kinase tif	
U04285	Lysosomal acid lipase	
U06631	H326	20
U07231	G-rich sequence factor-1	
U07681	Isocitrate dehydrogenase 3 (NAD+) alpha	
U07919	Aldehyde dehydrogenase 6 (ALDH6)	
U08815	Splicesomal protein SAP61	
U10324	Nuclear factor NF90	
U11791	Cyclin H	
U15174	Nip3	
U15306	DNA-binding protein NFX1	
U18291	CDC16Hs	
U18934	Receptor tyrosine kinase DTK	
U20979	Chromatin assembly factor-I p150 subunit	30
U22233	Methylthioadenosine phosphorylase	
U23028	Eukaryotic initiation factor 2B-epsilon	
U23946	LUCA15	
U26648	Syntaxin 5A	
U27459	Origin recognition complex protein 2 homolog	
U27460	Uridine diphosphoglucose pyrophosphorylase	
U28413	Cockayne syndrome complementation group A	
U28811	Cystein-rich fibroblast growth factor receptor	
U28831	Immuno-reactive with anti-PTH Ab	
U28963	Gps2	
U30313	Diadenosine tetraphosphatase	40
U30521	P311 HUM -3.1	
U30827	Splicing factor SRp40-3 (SRp40)	

【 0 0 8 6 】

【 表 5 】

第2表 (続き)

登録番号	遺伝子名	
U30828	Splicing factor SRp55-2 (SRp55)	
U34252	Gamma-aminobutyraldehyde dehydrogenase	
U34683	Glutathione synthetase	
U36341	SLC6A8	
U40282	Integrin-linked kinase	
U46006	Smooth muscle LIM protein (h-SmLIM)	
U49844	FRAP-related protein (FRP1/ATR)	
U50078	Guanine nucleotide exchange factor p532	10
U50939	Amyloid precursor protein-binding protein 1	
U53468	NADH:ubiquinone oxidoreductase subunit B13	
U57629	Retinitis pigmentosa GTPase regulator	
U60808	CDP-diacylglycerol synthase	
U61145	Enhancer of zeste homolog 2	
U61263	Acetolactate synthase homolog	
U63743	Mitotic centromere-associated kinesin (MCAK)	
U65785	Oxygen-regulated protein ORP150	
U72514	C2f	
U72515	C3f	
U76764	CD97	20
U77413	O-linked GlcNAc transferase	
U77949	Cdc6-related protein (HsCDC6)	
U79241	Clone 23759	
U80034	Mitochondrial intermediate peptidase precursor	
U81554	CaM kinase II isoform	
U85611	DNA-PK interaction protein (KIP)	
U89606	Pyridoxal kinase	
U90426	Nuclear RNA helicase	
U94319	Transcription coactivator p75 (DFS70)	
U95740	362G6.1	30
U97188	Putative RNA binding protein KOC	
X06745	DNA polymerase alpha subunit	
X13482	U2 snRNP-specific A protein	
X51956	Neuron specific (gamma) enolase	
X53587	Integrin beta-4	
X54199	GARS-AIRS-GART	
X54867	NKG2-A	
X59871	T cell factor 1	
X61100	75 kDa subunit NADH dehydrogenase precursor	
X66364	Serine/threonine protein kinase PSSALRE	
X68836	S-adenosylmethionine synthetase	40
X70476	Subunit of coatomer complex	
X75535	PxF	

【 0 0 8 7 】

【 表 6 】

第2表 (続き)

登録番号	遺伝子名
X81003	HCG V
X84740	DNA ligase III
X94754	Yeast methionyl-tRNA synthetase homologue
X98248	Sortilin
X99209	Arginine methyltransferase
Y08612	Nup88
Y08682	Carnitine palmitoyltransferase I type I
Y13115	Serine/threonine protein kinase SAK
Z11518	Histidyl-tRNA synthetase
Z17227	Transmembrane receptor protein CRF2-4
Z46629	SOX9
Z68747	Imogen 38

## 【0088】

第1表及び第2表に示した遺伝子に関し、E7070耐性株2株(C9-C1及びC9-C1と同様の方法により得られた別の耐性株(C9-C4))を用い、上記と同様に、8 µMのE7070で12時間処理した後に、遺伝子の変動をGeneChipにより解析した。この結果、1.8倍以上発現が増加または減少する遺伝子は両株において認められなかった。

## 【0089】

以上の結果から、これら3種の抗癌剤の処理により共通に変動する遺伝子は、単独または組み合わせでその変化を測定することによりE7070及びその関連化合物の抗腫瘍効果の代理マーカーとして使用できることが明らかである。

## 【0090】

## 【実施例3】

定量的PCRによる遺伝子発現解析

実施例2で得られた遺伝子の発現変動が、腫瘍細胞のE7070に対する感受性を反映したものであることを確認する目的で、実施例1に示したRNAを用いて、E7070感受性細胞と耐性細胞におけるこれら遺伝子発現の変化をSYBR GreenとABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems) を用いた定量的PCRにより検討した。

## 【0091】

操作は逆転写反応及びPCR反応の2段階で行った。最初の段階である逆転写反応は、1 µgの全RNAに1x TaqMan RT buffer、5.5 mM MgCl<sub>2</sub>、500 µMのdNTP mix、2.5 µM oligo d(T)<sub>16</sub>プライマー、0.4 U/µl RNase Inhibitor、1.25 U/µl Multiscribe Reverse Transcriptase (Perkin-Elmer Applied Biosystems) を加え、25 °Cにて10分間保温後、48 °Cにて30分間加熱することにより行った。反応を95 °C 5分間加熱することにより停止させた。

## 【0092】

得られたcDNAを第2段階のPCR反応に供した。PCR反応は4 ng cDNA、1xSYBR PCRバッファー、3 mM MgCl<sub>2</sub>、各200 µM dATP、dCTP、dGTP、400 µM dUTP、200 nM プライマー対、0.01 U/µl AmpErase UNG、0.025 U/µl AmpliTaq Gold DNA Polymerase (Perkin-Elmer Applied Biosystems) の反応系で行った。反応条件は50 °C 2分間、95 °C 10分間に次いで95 °C 20秒間・55 °C 20秒間・72 °C 30秒間を40サイクルで行った。プライマーはGAPDHは配列番号1及び2、S80343は配列番号3及び4、U07919は配列番号5及び6、U11791は配列番号7及び8、M95809は配列番号9及び10、U18291は配列番号11及び12、U63743は配列番号13及び14、M61764は配列番号15及び16に示す塩基配列を有するオリゴヌクレオチドを用いた。

## 【0093】

各検体中のmRNA量は蛍光強度を測定することにより定量した。なお、複数検体の比較においては、定量値を各検体のGAPDHのmRNA量により補正して行った。薬剤処理0時間における

定量値をコントロール（100%）として、薬剤処理時間と各mRNA量の関係を示したものが図2～図5である。各遺伝子は感受性が最も高いHCT116-C9で最大の発現変動を示し（図2）、感受性株LX-1ではHCT116-C9よりは変動の幅は小さいものの、S80343を除く全ての遺伝子で発現変動を示した（図3）。これに対し耐性株HCT116-C9-C1では、全く発現変動が認められず（図4）、実験に用いた8 $\mu$ Mの薬剤濃度で多少の増殖抑制の効果が認められる耐性株LX-1-E2では、U11791が発現変動を示しM95809およびU18291に僅かな発現変動が認められた（図5）。

【0094】

実施例2で示された遺伝子の発現変動はE7070に対する感受性と相関しており、実施例2で示された遺伝子は、単独または組み合わせでその変化を測定することによりE7070及びその関連化合物の抗腫瘍効果の代理マーカーとして使用できることが確認された。

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【0095】

【実施例4】

ELISAによる解析

実施例2の第2表に示した遺伝子のうち、細胞外に分泌されることが報告されているX51956 (Human ENO2 gene for neuron specific (gamma) enolase) について既に報告されているELISA法 (Duncan M. E., McAleese S. M., Booth N. A., Melvin W. T., and Fothergill J. E., J. of Immunol. Methods, 151, 227-236, 1992、Yamaguchi K., Aoyagi K., Urakami K., Fukutani T., Maki N., Yamamoto S., Otsubo K., Miyake Y., and Kodama T., Jpn. J. Cancer Res., 86, 698-705, 1995) によりその発現レベルを検討した。実際の測定には栄研化学（東京）のNSE ELISA kitを用い、操作は添付の資料に従った。

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【0096】

【実施例5】

3癌種における遺伝子発現解析

HCT116-C9（ヒト大腸癌細胞株）、MDA-MB-435（ヒト乳癌細胞株）及びMOLT-4（ヒトTリンパ芽球系白血病細胞株）の三癌種について、実施例2と同様にして、E7070（8 $\mu$ Mで12h処理）による遺伝子発現変化をGene Chipを用いて調べた。

【0097】

三癌種で共通して1.8倍以上発現が亢進される遺伝子のリストを第3表に示す。また、三癌種で共通して1.8倍以上発現が抑制される遺伝子のリストを第4表に示す。遺伝的背景の全く異なる癌患者から樹立されたこれら三癌種で共通して発現が変動（亢進または減少）する遺伝子は、腫瘍細胞に共通するE7070の抗腫瘍作用メカニズムにより深く関係している可能性が高いので、腫瘍細胞のE7070及びその関連化合物に対する感受性を検定する際に有効なマーカーになり得ると考えられる。

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【0098】

【表7】

第3表

登録番号	遺伝子名	配列番号
D78514	Ubiquitin-conjugating enzyme	17
D83767	Rep-8	18
M21154	S-adenosylmethionine decarboxylase	19
M60724	p70 Ribosomal S6 kinase alpha-1	20
S61953	c-ErbB3 receptor tyrosine kinase	21
U52960	RNA polymerase II complex component SRB7	22
U84720	Export protein RAE1	23
U92014	Defective mariner transposon Hsmar2	24

40

【0099】

【表8】

第4表

登録番号	遺伝子名	配列番号	
AB000409	Serine/threonine protein kinase MNK1	25	
AB000450	Putative serine/threonine protein kinase VRK2	26	
AF008445	Phospholipid scramblase	27	
D00723	Hydrogen carrier protein, glycine synthase	28	
D14659	KIAA0103	29	
D21852	KIAA0029	30	
D26535	Dihydrolipoamide succinyltransferase	31	
D32002	Nuclear cap binding protein	32	10
D38521	KIAA0077	33	
D38553	KIAA0074	34	
D43947	KIAA0100	35	
D50912	RNA-binding motif protein 10	36	
D61391	PAP39	37	
D63480	KIAA0146	38	
D63880	KIAA0159	39	
D78586	Multifunctional protein CAD	40	
D79983	KIAA0161	41	
D79991	KIAA0169	42	20
D83781	KIAA0197	43	
D84307	Phosphoethanolamine cytidyltransferase	44	
D87446	KIAA0257	45	
J04088	DNA topoisomerase II (top2)	46	
L07758	IEF SSP 9502	47	
L21936	Succinate dehydrogenase flavoprotein subunit	48	
L25931	Lamin B receptor	49	
L38810	Proteasome 26S subunit p45	50	
L41870	Retinoblastoma susceptibility protein (RB1)	51	
L47276	Alpha topoisomerase truncated-form	52	
M19267	Tropomyosin	53	30
M22632	Mitochondrial aspartate aminotransferase	54	
M29204	DNA-binding factor	55	
M29550	Calcineurin A1	56	
M30496	Ubiquitin carboxyl-terminal hydrolase	57	
M34309	Epidermal growth factor receptor HER3	58	
M55905	Mitochondrial NAD(P)+ dependent malic enzyme	59	
M61764	Gamma-tubulin	60	
M85085	Cleavage stimulation factor	61	
M87338	Replication factor C, 40-kDa subunit (A1)	62	

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【 0 1 0 0 】

【 表 9 】

第4表 (続き)

登録番号	遺伝子名		
M91432	Medium-chain acyl-CoA dehydrogenase (MCAD)	63	
M92439	Leucine-rich protein	64	
M93056	Monocyte/neutrophil elastase inhibitor	65	
M95809	Basic transcription factor 62kD subunit (BTF2)	66	
M97935	Transcription factor ISGF-3	67	
S72904	APK1 antigen=MAb KI recognized	68	
S78085	PDCD2, Rp8 homolog	69	
U01062	Inositol 1,4,5-triphosphate receptor type 3	70	10
U07231	G-rich sequence factor-1	71	
U08815	Splicesomal protein SAP61	72	
U11791	Cyclin H	73	
U15306	DNA-binding protein NFX1	74	
U18291	CDC16Hs	75	
U18934	Receptor tyrosine kinase DTK	76	
U20979	Chromatin assembly factor-I p150 subunit	77	
U22233	Methylthioadenosine phosphorylase	78	
U23028	Eukaryotic initiation factor 2B-epsilon	79	
U23946	LUCA15	80	20
U28831	Immuno-reactive with anti-PTH Ab	81	
U28963	Gps2	82	
U30828	Splicing factor SRp55-2 (SRp55)	83	
U34683	Glutathione synthetase	84	
U40282	Integrin-linked kinase	85	
U49844	FRAP-related protein (FRP1/ATR)	86	
U50939	Amyloid precursor protein-binding protein 1	87	
U57629	Retinitis pigmentosa GTPase regulator	88	
U61145	Enhancer of zeste homolog 2	89	
U72514	C2f	90	30
U77413	O-linked GlcNAc transferase	91	
U77949	Cdc6-related protein (HsCDC6)	92	
U79241	Clone 23759	93	
U80034	Mitochondrial intermediate peptidase precursor	94	
U81554	CaM kinase II isoform	95	
U89606	Pyridoxal kinase	96	
U94319	Transcription coactivator p75 (DFS70)	97	
X06745	DNA polymerase alpha subunit	98	
X13482	U2 snRNP-specific A protein	99	
X51956	Neuron specific (gamma) enolase	100	40
X54199	GARS-AIRS-GART	101	
X61100	75 kDa subunit NADH dehydrogenase precursor	102	
X68836	S-adenosylmethionine synthetase	103	
X70476	Subunit of coatomer complex	104	

【 0 1 0 1 】

【 表 1 0 】

第4表 (続き)

登録番号	遺伝子名	
X75535	PxF	105
X99209	Arginine methyltransferase	106
Y08612	Nup88	107
Y08682	Carnitine palmitoyltransferase I type I	108
Z17227	Transmembrane receptor protein CRF2-4	109

## 【 0 1 0 2 】

## 【 発明の効果 】

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本発明により、一般式 ( I ) で表される抗癌剤を投与した癌患者より取り出された腫瘍細胞の、第3表及び第4表に記載の遺伝子の発現量を測定することにより、あるいは癌患者より取り出された腫瘍細胞に一般式 ( I ) で表される抗癌剤を作用させ、第3表及び第4表に記載の遺伝子の発現量を測定することにより、該腫瘍細胞の該抗癌剤に対する感受性を調べることが可能となる。

## 【 0 1 0 3 】

## 【 配列表 】

<110> エーザイ株式会社 (Eisai Co., Ltd.)

<120> 腫瘍細胞の抗癌剤に対する感受性を検定する方法

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<151> 2000-11-24

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<213> Homo sapiens

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<211> 2392

<212> DNA

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<213> Homo sapiens

<400> 94

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<221> Unsure

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<223> unsure

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<213> Homo sapiens

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【図面の簡単な説明】

【図 1】 細胞株HCT116-C9、HCT116-C9-C1、LX-1、LX-1-E2のE7070による細胞増殖抑制曲線を示す。

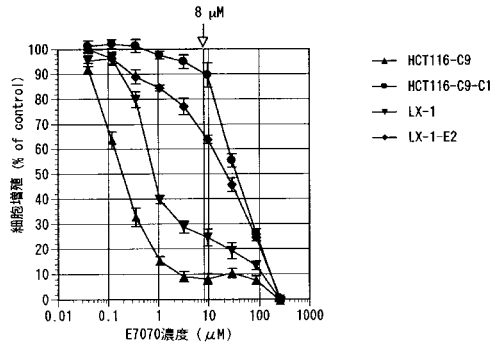
【図 2】 E7070感受性株HCT116-C9における遺伝子発現変動の定量的PCRによる解析の結果を示す。

【図 3】 E7070感受性株LX-1における遺伝子発現変動の定量的PCRによる解析の結果を示す。

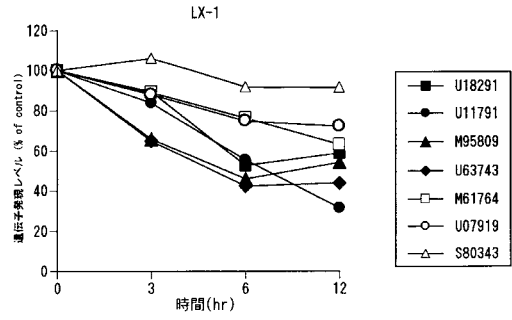
【図 4】 E7070耐性株HCT116-C9-C1における遺伝子発現変動の定量的PCRによる解析の結果を示す。

【図 5】 E7070耐性株LX-1-E2における遺伝子発現変動の定量的PCRによる解析の結果を示す。

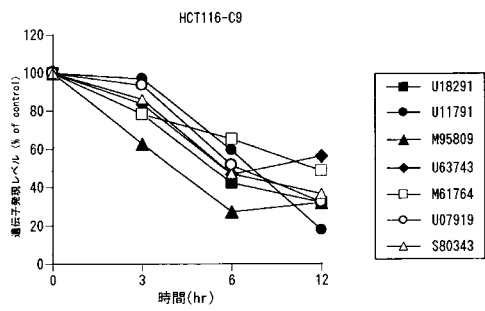
【 図 1 】



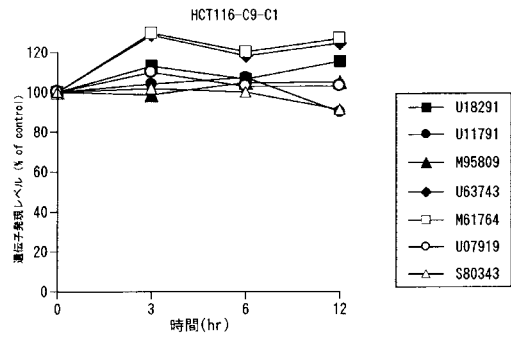
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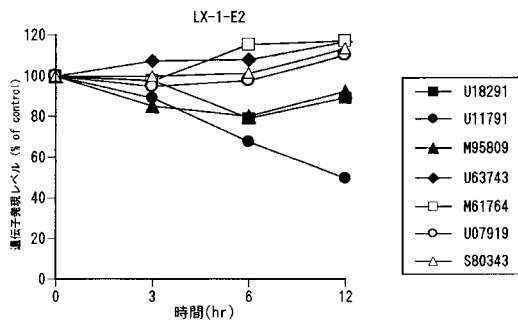
【 図 2 】



【 図 4 】



【 図 5 】



## フロントページの続き

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#### 摘要(译)

要解决的问题：当E7070或相关化合物被允许作用于肿瘤细胞时，提供E7070及其相关化合物的抗肿瘤作用的作用标记物。解决方案：肿瘤细胞对抗癌剂（E7070或其相关化合物）的敏感性如下进行：肿瘤细胞从已经给予抗癌剂的癌症患者中取出，并且基因的表达水平如下所述：确定表3和表4；或者，使抗癌剂作用于从癌症患者取出的肿瘤细胞，测定表3和表4中记载的基因的表达水平。并且，在表3中描述的基因的表达水平增加或表4中描述的基因的表达水平降低的情况下，判断肿瘤细胞对抗癌剂敏感。

