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Act. 523 RESPUESTA Folios: 13

As. 101.507

Señor

DIRECTOR DIVISIÓN DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E.

S.

D.

No. correcto 06-68312 ver radicación del
24/04/12

REF: Expediente No. 07 015795 solicitud de patente de invención a favor
de **PIERRE FABRE MEDICAMENT**. Título **NOVEDOSOS
ANTICUERPOS DE RECEPTOR HIBRIDO ANTI-INSULINA/IGF-I
O DE RECEPTOR HIBRIDO ANTI-INSULINA/IGF-I E IGF-IR Y SUS
USOS**

RESPUESTA A EXAMEN DE FONDO

JUAN PABLO CADENA SARMIENTO, portador de la tarjeta profesional No. 57.492 del C.S.J., obrando como apoderado de la sociedad solicitante me permito dar respuesta al oficio número 000547 relacionado con la patentabilidad de la presente invención y notificado por fijación en lista el 19 de enero de 2012.

Claridad

Presento a consideración del señor examinador un nuevo pliego reivindicatorio de 1-16 reivindicaciones en el cual consideramos se han superado todas y cada una de las objeciones del señor examinador. Específicamente, al anticuerpo se encuentra reivindicado por sus 6 CDR correspondientes a las cadenas ligera y pesada. De esta forma, el anticuerpo se encuentra definido por sus cadenas ligera y pesada tal como lo solicitó el señor examinador.

Por otra parte, aunque no se reivindica el hibridoma murino, adjuntamos a la presente respuesta copia del certificado de depósito del hibridoma murino CNCM I.2717 requerido por su Despacho.

Novedad

De acuerdo a la nueva definición del anticuerpo de la reivindicación 1, es claro que debido a su nueva configuración, este no se encuentra revelado en el documento D1.

En cuanto al nivel inventivo de la invención, deseamos resaltar que el anticuerpo reivindicado tiene la habilidad de unirse al receptor híbrido de insulina humana IGF-I (híbrido R) así como también al receptor R-híbrido y el receptor del factor de crecimiento insulinoide tipo I (IGF-IR) y el híbrido R de manera conjunta. Adicionalmente, otra de las ventajas del anticuerpo es que tiene la capacidad de inhibir la fijación de los ligandos nativos de estos receptores como el ligando IGF1, el ligando IGF2 o la insulina. En el ejemplo 26 de la descripción, el señor examinador podrá observar que efectivamente, el anticuerpo reivindicado tiene la capacidad de unirse a estos receptores. Específicamente, el anticuerpo 7C10 y el h/C10 inhiben la actividad tirosin kinasa de los receptores híbrido-R e IGF-IR evitando la sobreexpresión de tumores o la activación anormal del receptor híbrido-R.

En este sentido, teniendo en cuenta la alta afinidad del anticuerpo por el receptor IGF-IR así como el híbrido R y su capacidad de bloquear la actividad tirosinquinasa del mismo la cual es causante de crecimiento celular irregular lo hace un anticuerpo especialmente efectivo en el tratamiento de cáncer.

El anticuerpo reivindicado entonces es capaz de inhibir el crecimiento de tumores que expresan o que presentan una activación anormal de los receptores híbrido-R o tanto el híbrido R como el receptor IGF-IR.

Por lo tanto, el anticuerpo reivindicado tiene la capacidad de bloquear de manera conjunta ambos receptores debido a la alta afinidad del anticuerpo de unirse a los mismos y al mismo tiempo bloquear la activación de sus ligandos nativos IGF1, IGF2 o insulina.

Es importante tener en cuenta también que en algunos tejidos como el hígado o la placenta a medida que el tumor se sobre expresa o presenta una activación anormal, tanto el receptor IGF-IR como el IR-A tienden a sobreexpresarse en otro tipo de tejidos tal como se demuestra en los artículos adjuntos al presente memorial.

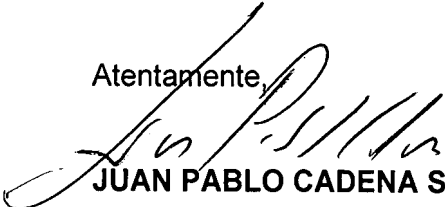
De acuerdo a lo anterior, la invención es una nueva herramienta terapéutica donde el anticuerpo es capaz de bloquear al mismo tiempo estos receptores impidiendo la activación anormal en un mismo tumor del híbrido-R o tanto el híbrido-R como el receptor IGF-IR.

Así, el problema técnico de evitar los fenómenos de escape mediados por la expresión o activación anormal en un mismo tumor de los receptores híbrido-R o híbrido R e IGF-IR en conjunto se resuelve mediante el anticuerpo reivindicado.

Esta capacidad del anticuerpo de unirse a dos receptores distintos e inhibir la actividad tirsinkinasa de los mismos no era evidente a partir de las enseñanzas de D1 y por lo tanto consideramos que esta ventaja del anticuerpo reivindicado debe considerarse como inventiva.

Finalmente, teniendo en cuenta la amplia restricción realizada al pliego reivindicatorio, solicitamos al señor examinador, si es posible, emitir una segunda acción oficial en caso de existir objeciones adicionales a la patentabilidad del anticuerpo.

Atentamente,


JUAN PABLO CADENA SARMIENTO

T.P. No. 57.492 del C.SJ.

C.C. 80.410.337 de Usaquén

Calle 70 A No. 4 - 41

REIVINDICACIONES

1. Un anticuerpo aislado, o uno de sus fragmentos funcionales, caracterizándose dicho anticuerpo, o uno de sus dichos fragmentos, porque es capaz de unirse al híbrido-R, isoforma(s) A y/o B, e inhibir la unión de al menos uno de sus ligandos nativos, seleccionado del grupo conformado por IGF1, IGF2 e insulina, comprendiendo dicho anticuerpo aislado una cadena ligera que comprende las regiones determinantes complementarias CDRs de secuencia SEQ ID No. 2, 4 y 6, y comprendiendo una cadena pesada que comprende las CDRs de secuencia SEQ ID No. 8, 10 y 12.

2. El anticuerpo como se reivindica en la reivindicación 1, caracterizado porque dicho fragmento funcional se selecciona de los fragmentos Fv, Fab, F(ab')₂, Fab', scFv, scFv-Fc, scFv-CH3 y los diacuerpos.

3. El anticuerpo, o uno de sus fragmentos funcionales, como se reivindica en la reivindicación 1, caracterizado porque dicho anticuerpo comprende una cadena ligera de secuencia que comprende la secuencia de aminoácidos SEQ ID No. 54, y porque comprende una cadena pesada de secuencia que comprende la secuencia de aminoácidos SEQ ID No. 69.

4. El anticuerpo, o uno de sus fragmentos funcionales, como se reivindica en la reivindicación 3, caracterizado porque dicho anticuerpo es un anticuerpo quimérico y, por que además comprende las regiones constantes de cadena ligera y cadena pesada, derivadas de un anticuerpo de una especie heteróloga al ratón.

5. El anticuerpo quimérico, o uno de sus fragmentos funcionales, como se reivindica en la reivindicación 4, caracterizado porque dicha especie heteróloga es el hombre.

6. El anticuerpo quimérico, o uno de sus fragmentos funcionales, como se reivindica en la reivindicación 5, caracterizado porque las regiones constantes de cadena ligera y cadena pesada, derivadas de un anticuerpo humano, son respectivamente la región kappa o lambda y gamma-1, gamma-2 o gamma-4.

7. El anticuerpo, o uno de sus fragmentos funcionales, como se reivindica en la reivindicación 1, caracterizado porque dicho anticuerpo es un anticuerpo humanizado y contiene una cadena ligera y una cadena pesada, en la cual los segmentos del esqueleto FR1 a FR4 de dicha cadena ligera y cadena pesada se derivan respectivamente de segmentos del esqueleto FR1 a FR4 de cadena ligera y cadena pesada de anticuerpo humano.

8. El anticuerpo humanizado, o uno de sus fragmentos funcionales, como se reivindica en la reivindicación 7, caracterizado porque dicho anticuerpo comprende una cadena ligera que comprende la secuencia de aminoácidos SEQ ID No. 61 ó 65, y porque comprende una cadena pesada que comprende la secuencia de aminoácidos SEQ ID No. 75, 79 u 83.

9. El anticuerpo humanizado, o uno de sus fragmentos funcionales, como se reivindica en la reivindicación 8, caracterizado porque dicho anticuerpo comprende una cadena ligera que comprende la secuencia de aminoácidos SEQ ID No. 65, y porque comprende una cadena pesada de secuencia que comprende la secuencia de aminoácidos SEQ ID No. 79 u 83, preferiblemente SEQ ID No. 83.

10. El anticuerpo, o uno de sus fragmentos funcionales, como se reivindica en una de las reivindicaciones 1 y 3 a 9 como un medicamento.

11. Un método de diagnóstico in vitro de enfermedades inducidas por una sobre-expresión o una subexpresión del híbrido-R y, a partir de una muestra biológica, en la cual se sospecha la presencia anormal de híbrido-R, caracterizado porque dicha muestra biológica se pone en contacto con un anticuerpo como se reivindica en una de las reivindicaciones 1 y 3 a 9, siendo posible marcar dicho anticuerpo, si es necesario.

12. Un kit para la detección y/o la cuantificación de una sobre-expresión o de una subexpresión del híbrido-R en una muestra biológica, preferiblemente una sobre-expresión de dicho receptor, caracterizado porque dicho kit comprende los siguientes elementos:

a) un anticuerpo, o uno de sus fragmentos funcionales, como se reivindica en una de las reivindicaciones 1 y 3 a 9;

b) en forma opcional, los reactivos para la formación del medio favorable a la reacción inmunológica;

c) en forma opcional, los reactivos que permiten la demostración de complejos híbrido-R/anticuerpo producidos por la reacción inmunológica.

13. Un método de identificar un modulador de híbrido-R, que comprende:

a) poner en contacto el híbrido-R con el anticuerpo, como se reivindica en una de las reivindicaciones 1 y 3 a 9;

b) poner en contacto el complejo de (a) con una biblioteca de compuestos;

c) identificar un compuesto que desestabiliza el complejo de (a); y

d) determinar si el compuesto exhibe actividad agonista o antagonista en el híbrido-R, en donde esta actividad indica identificación de un modulador de híbrido-R.

14. Un método de identificar un modulador de híbrido-R, que comprende:

a) cribar una biblioteca de secuencias de péptidos, en donde dicha biblioteca de secuencias de péptidos se une a híbrido-R, y en donde la biblioteca se deriva de una secuencia de péptidos que contiene por lo menos una secuencia seleccionada del grupo conformado por SEQ ID No. 2, 4, 6, 8, 10 y 12; y

b) determinar si la secuencia de aminoácidos aislada en (a) exhibe actividad agonista o antagonista en células sensibles a híbrido-R, seleccionadas del grupo conformado por estirpes celulares que presentan híbrido-R, tales como, por ejemplo, MDA-MB-231, MDA-MB-157, MDA-MB-468, MDA-MB-453 y ZR-75 para células de mama y BC-PAP para células de tiroides, en donde esta actividad indica identificación de un modulador de híbrido-R.

15. Un método de detectar parejas de unión para el anticuerpo de la reivindicación 1 en una muestra, método que comprende:

a) incubar el anticuerpo, como se reivindica en una de las reivindicaciones 1 y 3 a 9, con una muestra biológica obtenida de un paciente con un trastorno proliferativo celular, caracterizado por un nivel anormal de expresión y/o activación de híbrido-R humano, bajo condiciones suficientes para permitir unión específica del anticuerpo a su pareja de unión, y

b) detectar unión específica, en donde unión específica indica la presencia de una pareja de unión en la muestra.

16. Un método de purificar un híbrido-R de una muestra, método que comprende:

a) incubar el anticuerpo, como se reivindica en la reivindicaciones 1 y 3 a 9, con una muestra bajo condiciones que permitan unión específica del anticuerpo y el receptor; y

b) separar el anticuerpo de la muestra y obtener el receptor purificado.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO Jean-Yves BONNEFOY Institut de Recherche Pierre Fabre Centre d'Immunologie Pierre Fabre 5, Av Napoléon III – BP 497 74164 Saint-Julien-en-Genevois CEDEX NAME AND ADDRESS OF DEPOSITOR	RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page Copy of the Original Receipt Certified by Dr Georges Wagener CNCM, Executive Head <signature> May 18, 2006
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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 7 C 10	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: I-2717
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☒ a scientific description ☒ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on September 19, 2001 (date of the original deposit) ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on 1 (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES (CNCM) Address: Institut Pasteur 25, rue du Docteur Roux F-75724 Paris Cedex 15 (France)	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: <signature> Georges WAGENER Paris, November 08, 2001

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.
Form BP/4 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO Madame Liliane GOETSCH, Monsieur Jean-Yves BONNEFOY Institut de Recherche Pierre Fabre Centre d'Immunologie PIERRE FABRE 5, Avenue Napoléon III – B.P. 497 74164 SAINT-JULIEN-EN-GENEVOIS NAME AND ADDRESS OF THE PARTY TO WHOM THE VIABILITY STATEMENT IS MADE	VIABILITY STATEMENT issued pursuant to Rule 10.2 by the INTERNATIONAL DEPOSITARY AUTHORITY identified on the following page Copy of the Last Viability Statement Certified by Dr Georges Wagener CNCM, Executive Head <signature> May 18, 2006
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I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Jean-Yves BONNEFOY Institut de Recherche Pierre Fabre Address: Centre d'Immunologie Pierre Fabre 5, Av Napoléon III – BP 497 74164 Saint-Julien-en-Genevois CEDEX	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: I-2717 Date of the deposit or of the transfer ¹ September 19, 2001
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on September 19, 2001 . On that date the said microorganism was ☒ ³ viable ☐ ³ no longer viable	

¹ Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED ⁴	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES (CNCM) Address: Institut Pasteur 25, rue du Docteur Roux F-75724 Paris Cedex 15 (France)	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Georges Wagener <signature> Date: Paris, November 08, 2001 Simona OZDEN <signature>

⁴ Fill in if the information has been requested and if the results of the test were negative.

Abstract of Cited References in the Instruction Letter dated 23 April 2012

Biochem J. 1997 Oct 1;327 (Pt 1):209-15.

Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting.

Baillyes EM, Navé BT, Soos MA, Orr SR, Hayward AC, Siddle K.

Source

University of Cambridge, Department of Clinical Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR, U.K.

Abstract

The insulin receptor (IR) and type 1 insulin-like growth factor (IGF-I) receptor (IGFR) are both widely expressed in mammalian tissues, and are known to be capable of heteromeric assembly as insulin/IGF hybrid receptors, in addition to the classically described receptors. By selective immunoadsorption of radioligand/receptor complexes and by immunoblotting we have determined the fraction of insulin receptors and IGF receptors occurring as hybrids in different tissues. Microsomal membranes were isolated from tissue homogenates and solubilized with Triton X-100. Solubilized receptors were incubated with ¹²⁵I-IGF-I, and radioligand/receptor complexes bound by IR-specific and IGFR-specific monoclonal antibodies were quantified. The fraction of IGF-I binding sites behaving as hybrids (anti-IR-bound/anti-IGFR-bound) was approx. 40% in liver and spleen, 70% in placenta, and 85-90% in skeletal muscle and heart, similar results being obtained in rabbit and human tissues. There was no correlation between the proportion of hybrids and the ratio of ¹²⁵I-insulin/¹²⁵I-IGF-I binding in different tissues. The fraction of ¹²⁵I-insulin bound to hybrids was too low for accurate quantification, because of the relatively low affinity of hybrids for insulin. The fraction of insulin receptors present in hybrids was therefore determined by immunoblotting. Receptors in solubilized human placental microsomal membranes were precipitated with IR-specific or IGFR-specific monoclonal antibodies, and after SDS/PAGE, blots were prepared and probed with IR-specific and IGFR-specific antisera. It was found that 15% of IR and 80% of IGFR were present in hybrids. Consistent with these figures, the overall level of IR was estimated, by blotting with the respective antibodies at concentrations shown to give equal signals with equal amounts of receptor, to be 4-fold greater than IGFR. Overall it was concluded that a significant fraction of both IR and IGFR occurs as hybrids in most mammalian tissues, including those that are recognized targets of insulin and IGF action. The fraction of hybrids in different tissues was not a simple function of the relative levels of IR and IGFR, possibly because of heterogeneity of receptor expression in different cell types. However, in placenta the proportions of IR, IGFR and hybrids were consistent with a process of random assembly reflecting the molar ratio of IR and IGFR half-receptors.

Mol Cell Biol. 1999 May;19(5):3278-88.

Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells.

Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A, Vigneri R.

Source

Istituto di Medicina Interna, Malattie Endocrine e del Metabolismo, University of Catania, Ospedale Garibaldi, 95123 Catania, Italy.

Abstract

Insulin-like growth factor II (IGF-II) is a peptide growth factor that is homologous to both insulin-like growth factor I (IGF-I) and insulin and plays an important role in embryonic development and carcinogenesis. IGF-II is believed to mediate its cellular signaling via the transmembrane tyrosine kinase type 1 insulin-like growth factor receptor (IGF-I-R), which is also the receptor for IGF-I. Earlier studies with both cultured cells and transgenic mice, however, have suggested that in the embryo the insulin receptor (IR) may also be a receptor for IGF-II. In most cells and tissues, IR binds IGF-II with relatively low affinity. The IR is expressed in two isoforms (IR-A and IR-B) differing by 12 amino acids due to the alternative splicing of exon 11. In the present study we found that IR-A but not IR-B bound IGF-II with an affinity close to that of insulin. Moreover, IGF-II bound to IR-A with an affinity equal to that of IGF-II binding to the IGF-I-R. Activation of IR-A by insulin led primarily to metabolic effects, whereas activation of IR-A by IGF-II led primarily to mitogenic effects. These differences in the biological effects of IR-A when activated by either IGF-II or insulin were associated with differential recruitment and activation of intracellular substrates. IR-A was preferentially expressed in fetal cells such as fetal fibroblasts, muscle, liver and kidney and had a relatively increased proportion of isoform A. IR-A expression was also increased in several tumors including those of the breast and colon. These data indicate, therefore, that there are two receptors for IGF-II, both IGF-I-R and IR-A. Further, they suggest that interaction of IGF-II with IR-A may play a role both in fetal growth and cancer biology.

Oncogene. 1999 Apr 15;18(15):2471-9.

Insulin receptor activation by IGF-II in breast cancers: evidence for a new autocrine/paracrine mechanism.

Sciacca L, Costantino A, Pandini G, Mineo R, Frasca F, Scalia P, Sbraccia P, Goldfine ID, Vigneri R, Belfiore A.

Source

Istituto di Medicina Interna, Malattie Endocrine e del Metabolismo, Università di Catania, Ospedale Garibaldi, Italy.

Abstract

IGF-II, produced by breast cancer epithelial and stromal cells, enhances tumor growth by activating the IGF-I receptor (IGF-I-R) via autocrine and paracrine mechanisms. Previously we found that the insulin receptor (IR), which is related to the IGF-I-R, is overexpressed in breast cancer cells. Herein, we find that, in breast cancer the IR is activated by IGF-II. In eight human breast cancer cell lines studied there was high affinity IGF-II binding to the IR, with subsequent IR activation. In these lines, IGF-II had a potency up to 63% that of insulin. In contrast, in non malignant human breast cells, IGF-II was less than 1% potent as insulin. Via activation of the IR tyrosine kinase IGF-II stimulated breast cancer cell growth. Moreover, IGF-II also activated the IR in breast cancer tissue specimens; IGF-II was 10-100% as potent as insulin. The IR occurs in two isoforms generated by alternative splicing of exon 11; these isoforms are IR-A (Ex11-) and IR-B (Ex11+). IR-A was predominantly expressed in breast cancer cells and specimens and the potency of IGF-II was correlated to the expression of this isoform ($P < 0.0001$). These data indicate, therefore, that the IR-A, which binds IGF-II with high affinity, is predominantly expressed in breast cancer cells and represents a new autocrine/paracrine loop involved in tumor biology.

Mol Pathol. 2001 Jun;54(3):121-4.

The IGF system in thyroid cancer: new concepts.

Vella V, Sciacca L, Pandini G, Mineo R, Squatrito S, Vigneri R, Belfiore A.

Source

Cattedra di Endocrinologia, Istituto di Medicina Interna, Malattie Endocrine e del Metabolismo, University of Catania, Ospedale Garibaldi, Catania, Italy.

Abstract

In recent years, the activation of the insulin-like growth factor (IGF) system in cancer has emerged as a key factor for tumour progression and resistance to apoptosis. Therefore, a variety of strategies have been developed to block the type I IGF receptor (IGF-I-R), which is thought to mediate the biological effects of both IGF-I and IGF-II. However, recent data suggest that the IGF signalling system is complex and that other receptors are involved. To unravel the complexity of the IGF system in thyroid cancer, IGF-I and IGF-II production, and the expression and function of their cognate receptors were studied. Both IGFs were found to be locally produced in thyroid cancer: IGF-I by stromal cells and IGF-II by malignant thyrocytes. Values were significantly higher in malignant tissue than in normal tissue. IGF-I-Rs were overexpressed in differentiated papillary carcinomas but not in poorly differentiated or undifferentiated tumours, whereas insulin receptors (IRs) were greatly overexpressed in all tumour histotypes, with a trend for higher values in dedifferentiated tumours. As a consequence of IR overexpression, high amounts of IR/IGF-I-R hybrids (which bind IGF-I with high affinity) were present in all thyroid cancer histotypes. Because of recent evidence that isoform A of IR (IR-A) is a physiological receptor for IGF-II in fetal life, the relative abundance of IR-A in thyroid cancer was measured. Preliminary data indicate that overexpressed IRs mainly occur as IR-A in thyroid cancer. These data indicate that both IR/IGF-I-R hybrids and IR-A play an important role in the overactivation of the IGF system in thyroid cancer and in IGF-I mitogenic signalling in these tumours.

Clin Cancer Res. 1999 Jul;5(7):1935-44.

Insulin and insulin-like growth factor-I (IGF-I) receptor overexpression in breast cancers leads to insulin/IGF-I hybrid receptor overexpression: evidence for a second mechanism of IGF-I signaling.

Pandini G, Vigneri R, Costantino A, Frasca F, Ippolito A, Fujita-Yamaguchi Y, Siddle K, Goldfine ID, Belfiore A.

Source

Istituto di Medicina Interna, Malattie Endocrine e del Metabolismo, Università di Catania, Ospedale Garibaldi, Italy.

Abstract

The insulin receptor (IR) form hybrids with the closely related insulin-like growth factor-I (IGF-I) receptor (IGF-I-R). Because most human breast carcinomas overexpress both the IR and the IGF-I-R, we evaluated whether the insulin/IGF-I hybrid receptor (Hybrid-R) is also overexpressed in these tumors and what role it plays in breast cancer biology. Using specific ELISAs and Western blots, we measured Hybrid-R content and function in 8 human cultured breast cancer cell lines and 39 human breast cancer specimens. Hybrid-R content and function were also compared to the content and function of the IR and the IGF-I-R. Hybrid-R content exceeded the IGF-I-R content in >75% of breast cancer specimens and was directly related to the molar ratio of both the IR and IGF-I-R content, suggesting that Hybrid-R formation occurred by random assembly of IR and IGF-I-R half-receptors. Hybrid-Rs became tyrosine autophosphorylated when breast cancer cells were exposed to IGF-I but not when they were exposed to insulin. In cells with an elevated Hybrid-R content, Hybrid-R autophosphorylation in response to IGF-I exceeded IGF-I-R autophosphorylation, suggesting that most of the IGF-I effect occurred via the Hybrid-R.

Furthermore, Hybrid-Rs mediated growth in response to IGF-I, as indicated by experiments with blocking antibodies to the IGF-I-R. These data indicated therefore that: (a) Hybrid-Rs are present and play a major role in mediating the IGF-I signal in breast cancer; (b) their expression is directly related to IR overexpression; and (c) potential therapies designed to block IGF-I actions in breast cancer must take into account the role of these Hybrid-Rs.

Biochimie. 1999 Apr;81(4):403-7.

Insulin/IGF-I hybrid receptors play a major role in IGF-I signaling in thyroid cancer.

Belfiore A, Pandini G, Vella V, Squatrito S, Vigneri R.

Source

Istituto di Medicina Interna, Malattie Endocrine e del Metabolismo, Università di Catania, Ospedale Garibaldi, Italy.

Abstract

The insulin-like growth factor-I (IGF-I) plays an important role in determining the biological behavior of a variety of malignancies. We measured IGF-I, its receptor and related receptors in thyroid cancer. IGF-I was present both in normal thyroid tissue and in thyroid cancer tissue and it was produced by stromal cells but not by thyrocytes. Values were significantly higher in malignant than in normal tissue. IGF-I receptors (IGF-I-Rs) and the homologous insulin receptors (IRs) were found overexpressed in both thyroid cancer cell lines (n = 4) and specimens (n = 17) as compared to normal values. In addition, high levels of hybrid IGF-I/insulin receptors (IR/IGF-I-Rs) were present in both thyroid cancer specimens and cell lines. IR/IGF-I-R hybrids were the most represented type of receptor in 14/17 specimens and exceeded the IGF-I-R content in all cases. Hybrid content correlated with the IR and IGF-I-R content, suggesting that in thyroid tissue hybrid formation occurs by random assembly of IR and IGF-I-R half receptors. Hybrid receptor autophosphorylation was stimulated by IGF-I with high affinity. In cells with a high IR/IGF-I-Rs content, blocking antibodies specific to these receptors substantially inhibited IGF-I induced cell growth. These data indicate that the IGF-I system is overactivated in thyroid cancer and that IR/IGF-I-R hybrid receptors play an important role in IGF-I mitogenic signaling in these tumors.