(54) Title: CANCER METASTASIS INTERFERING VACCINE

(57) Abstract: A vaccine that has been synthesized by a polyvalent substrate and the idiotype parts of antibodies that recognize antineoplastic peptides. The vaccine induces the production of antidiotype antibodies that inhibit metastasis formation. It is known that the ability of a cancer cell to metastasize is primary dependent by its property to bind with extracellular matrix proteins such as laminin and fibronectin. On these proteins the antineoplastic sequences (peptides) YIGSR and RGD have been discovered since 1991. Attempts to use these peptides either per se or as constituents of polymers in order to inhibit spontaneous metastasis in experimental animals had only limited results because of the limited life time of this preparation in the circulation. Using the described vaccine the immune system is led to the production of antidiotype antibodies that have shape and properties similar to the antineoplastic peptides YIGSR and RGD. Pilot experiments indicated that these antibodies can inhibit the formation of spontaneous metastasis in experimental animals.
Cancer metastasis interfering vaccine

The present invention report describes a novel molecule that has been synthesized by a multivalent substrate and the idiotype parts of antibodies that recognize and bind antimetastatic peptides.

The molecule is used for the production by the living organism of antidiotype antibodies \(^{1-4}\) with antimetastatic properties.

The ability of cancer to metastasize is the most lethal characteristic of this disease \(^{5}\), that remains mainly incurable and one of the most common causes of mortality in well developed countries\(^{5}\). Therefore the suppression and eradication of metastases is a major goal of alternative treatment strategies for cancer \(^{6}\).

The ability of a cancer cell to metastasize depends by several properties, however high affinity binding with extracellular matrix molecules is currently considered as necessary. \(^{7-12}\).

This ability is attributed to the enrichment of specific metastatic cell surface binding sites. These binding sites recognize specific aminoacid sequences situated on some extracellular matrix proteins such as laminin and fibronectin\(^{13}\).

It has been reported that proteolytic fragments of laminin and fibronectin occupy metastatic cell surface binding sites and thus inhibit experimental metastasis \(^{14}\). The aminoacid sequences that are immediately related with this property have been discovered and described\(^{10,15-29}\). The sequence (peptide) YIGSR that recognizes a metastasis associated high affinity laminin receptor has been discovered on laminin \(^{30}\) and the sequence RGDS, that recognizes a family of extracellular matrix receptors called integrins has been discovered on fibronectin \(^{22,26,31-34,35,36}\).

It is presently well known that the metastatic ability of cancer cells can be experimentally inhibited if the binding sites described above are covered by the synthetic peptide YIGSR \(^{37}\). The same peptide has been also used for the in vitro selection of melanoma cell lines with high metastatic potential \(^{38}\). Moreover the radiolabeled peptides YIGSR and RGDS have been used in vivo for the detection of metastatic sites \(^{28,29,39}\).

It was thus obvious since 1991 \(^{40}\) that polypeptides which contain RGD and/or YIGSR sequences could provide a promising approach for the control and prevention of cancer metastasis.

However, despite of the above evidence, the peptides RGD or YIGSR, had no effect in the spontaneous metastasis model, and only multiple intravenous
administrations of polymers containing these sequences may result in a reduction of metastatic sites \(^{40}\).

Several polymers containing these sequences have been proposed as antimitastatic agents in the past \(^{35,40,41,58}\). Unfortunately these efforts had minimum success because of the limited life span of these molecules in plasma \(^{47,57,59-61}\).

The advantage of the present invention is that after vaccination with the molecule we describe, the immunological system is directed to produce specific antibodies with idioype sequences that are similar to the antimitastatic peptides. Thus the living organism produces antibodies with properties comparable with the described peptide properties. As our pilot experiments indicated these antibodies offer significant defense against cancer metastasis.

During these experiments, serum derived from rabbits immunized with the peptide YIGSR has been used for the vaccination of mice, while other mice (controls) were immunized with non specific rabbit serum. After vaccination all mice were inoculated with the same amount of Lewis lung carcinoma cells 3LL. The mice were sacrificed after 20 days and the lungs were observed for the evidence of metastasis macroscopically and microscopically.

The immunized mice had significantly smaller tumors and less micrometastases around the lung vessels in comparison to controls. Macroscopically lung metastases were obvious only in control mice.

According to the present innovation for the preparation of the vaccine molecules of polylysine, polyethylene glycol or any other polyvalent molecule can be used. On these molecules multiple Fab fragments or V regions (idiotypes) of gamma globulins (antibodies) are covalently attached. These parts have been prepared by polyclonal or monoclonal antibodies or by bio-engineering methods. The antibodies have been raised against the antimitastatic peptides YIGSR and/or RGD or other molecules containing these sequences. Thus novel polyvalent antigenic molecules are synthesized that can be used as antigens (vaccines). The multiple antigen recognition sequences (idiotypes) that are included in this molecule, have been raised against antimitastatic peptides YIGSR and/or RGD and for this reason have a shape complimentary to these peptides. Thus this molecule will lead the immune system to produce antiidiotype antibodies with a shape and properties that are analog to the original molecules (peptides).
Reference List


Claims.

1. A vaccine that is composed by a polyvalent substrate (polylsine, polyethylenglucole, or any other polyvalent molecule). On this molecule multiple Fab parts or V regions (idiotypes) of gamma globulins (antibodies) have been covalently bound. These parts have been prepared by polyclonal or monoclonal antibodies or by other biotechnological or by direct biochemical synthesis. These antibodies have been raised against synthetic peptides with known antimetastatic properties.

These molecules are used alone or in combination with other molecules for the vaccination of humans or other living organisms aiming to inhibit the formation of cancer metastasis.

2. A vaccine according to the claim 1, that is not composed by a polyvalent substrate. Instead the multiple Fab parts or V regions of gamma globulins are directly bound to each other. By this way the composed molecule consists by multiple parts or multiple synthetic analogs of Fab parts or V regions of gamma globulins.


INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER


According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>EP 0 657 471 A (CENTRO DE INMUNOLOGIA MOLECULAR) 14 June 1995 (1995-06-14) examples table 2, line 12 claims</td>
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

4 September 2002

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Name and mailing address of the ISA

European Patent Office, P.B. 5618 Patentlaan 2 NL-2280 HV Rijswijk
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