The invention concerns variant CD44 surface proteins, antibodies against the variant determinants of these proteins, as well as processes for their preparation, furthermore the DNA sequences which code for these variant protein pieces, as well as the use of these proteins or parts thereof and the antibodies directed against them for the diagnosis and therapy of tumour metastases.

The ability to metastasing forms the actual life-endangering property of malignant tumour cells. The original primary tumour cells inherit this property presumably by a whole series of changes in the course of the tumour progression. As a result of this process, cancer cell variants detach continuously from the primary tumour mass, penetrate through the extracellular matrix and migrate into the lymphatic system or the blood circulation. Often sticking to one another, the metastasing tumour cells are transported in the blood or lymph system, leave the circulatory system at other places in order there to penetrate into secondary tissue to form subsidiary tumours (survey of Hart et al., 1989; Nicolson, 1987). The formation of metastases requires a whole series of interactions of the tumour cells with intercellular matrix and other cells. Almost all of these interactions require cell surface components, such as e.g. the receptors for matrix and lamina, surface-bound proteolytic enzymes, as well as cell adhesion molecules with inclusion of those which cause organ-specific adhesion and thus organ preference of the metastasing, furthermore growth factors and growth factor receptors.

It is known that the membrane proteins of non-metastasing and metastasing tumour cells of the BSp73 rat tumour differ, detected by antibody reaction (Matzku et al., 1983 and 1989).

It has now been found that the metastasing BSp73ASML tumour cells contain a surface protein which corresponds in part to a known glycoprotein participating in the lymphocyte adhesion and cell-cell and cell-matrix exchange action (designations of the normal glycoprotein in the case of humans; CD44, Hermes-1, in the case of the mouse: Ppg-1 and in the case of the rat: HEBFln). However, the new variant CD44 surface protein differs from these known sequences by an extracellular region (ECR) of 154 amino acids which is inserted between the 220th and the 237th amino acid of the human CD44 sequence (or 224th and 239th amino acid of the mouse sequence). This new glycoprotein appears to possess an important role for the cell/matrix or cell/cell binding in the case of the metastasing. Therefore, preparation and characterisation of this protein region (ECR) form one of the tasks of the present invention. By immunisation of mice with membrane proteins which have been obtained from BSp73ASML, spleen cells were produced which form antibodies against the ECR of the variant CD44 surface protein. According to the method of Köhler (1981), these are fused with myeloma cells by polyethylene glycol in order to produce stable cultures. By means of cloning and selection of those cultures which produce antibodies which react with BSp73ASML but not with the non-metastasing parent form BSp73AS and also not with other non-tumorigenic rat cells, specific antibodies against the new protein part ECR can be obtained. For the further investigation, a monoclonal antibody was selected which stained the BSp73ASML cells in the immunofluorescence test especially intensively which has been given the designation mAb1.1ASML (mAb: monoclonal antibody).

In the Western blot test, in a protein hydrolysate from BSp73ASML cells there can be determined 4 protein

bands with molecular weights of 120,000; 150,000; 180,000 and 200,000 with mAb1.1.ASML, whereas extracts from rat fibroblast cells and non-metastasing rat tumour cells give no significant reaction. It could not yet be determined whether these size differences depend upon a different original amino acid sequence or upon a differingly strong subsequent protein modification. In any case, the epitope recognised by the antibody is contained in all 4 protein species but not in the proteins serving for the control from the non-metastasing BSp73AS cells or from normal rat cells.

Isolation of cDNS sequences which code for the ECR of the surface protein.

The monoclonal antibody mAB1.1ASML was used in order to discover the ECR-coding cDNA sequences in a bacterial expression bank. The bank was constructed with the help of PolyA+ RNA from BSp73ASML cells and the pEX vector system (Stanley & Luzio, 1984). The products coded by the cDNA sequences are found as -galactosidase fusion proteins with the help of antibodies. A so-isolated cDNA clone with the name pEX34, positive for the monoclonal antibody 1.1ASML, carries a cDNA sequence fragment of 167 nucleotides. This cDNA fragment was now used in order to scan through a larger cDNA bank in the vector pSP65, again from BSp73ASML RNA. One of these clones isolated therewith, pM66, then served the purpose, with the help of so-called "primer" elongation (starter oligonucleotides) and of the polymerase chain reaction (PCR), to isolate the total length cDNA clone pMeta-1. Evidence of the whole length was obtained with the help of the primer elongation (3207 nucleotides). The colinearity with an RNA from the BSp73ASML tumour was documented by RNase and S1 protection analysis.

The isolation of the cDNA's with the help of the expression in bacteria and the recognition by the antibody demonstrated that the antibody recognises primary amino acid sequences in the ECR of the surface protein.

ECR-coding messenger RNA is expressed in BSp73ASML but not in BSp73AS cells.

Three different sequence samples were produced from the cDNA clones in order to detect specific messenger RNA's by hybridisation. The sample A covers the cDNA region which codes for the ECR (positions: 941 - 1108). The sample B represents sequences between the positions 1403 - 1572 and sample C carries sequences from the start up to the position 794 from pMeta-1 (Fig. 1). Poly A+ RNA of the BSp73 tumour cell lines was separated electrophoretically and analysed with the help of the RNA transfer hybridisation. Four of the cell lines which do not metastase contain no RNA which is homologous to the sample A, whereas RNA's from the metastasing tumour cells BSp73ASML react strongly. In this RNA preparation, the sample A recognises a heterogeneous mixture of various RNA sizes between 2.2 and 3.3 kb and a larger RNA species of 4.9 kb. The exclusive expression of the specific membrane proteins recognised by the monoclonal antibody 1.1ASML in the metastasing tumour cell variant is apparently due to the exclusive expression of the corresponding ECR-coding messenger RNA's. Apparently, the complete cDNA clone pMeta-1 with 3.2 kb cannot represent all sequences of this RNA species. It can only represent one species from the heterogeneous mixture of RNA sizes. The samples B and C give the same hybridisation pattern as sample A in the resolving of the BSp73ASML RNA in any case insofar as one can ascertain in the case of the heterogeneity, i.e. these RNA species carry sequences

which are complementary to all three samples A, B and C. In contradistinction to sample A, samples B and C also recognise messenger RNA species in the non-metastasing cell lines. However, the RNA sizes differ clearly from those in BSp73ASML, there are, namely, detectable four clearly differentiatable messenger RNA species with the sizes 1.5, 2.0, 2.9 and 4.3 kb. Although these RNA species could be concealed in the heterogeneous mixture of the RNA's from BSp73ASML, it is, however, certain that they do not exist in the same amount in the BSp73ASML. It is decisive that RNA sequences with complementarity to sample A are apparently completely absent in the non-metastasing cells. Therefore, we can conservatively conclude that the sequences of the samples A, B and C are contained in the same RNA's in the metastasing tumour, namely, in a manner as though sample A sequences were spliced into the B-C-positive RNA's and as though this alternative splicing procedure only occurred in the metastasing cell line.

In order to demonstrate the colinearity between RNA and cDNA and in order to analyse the differences of the RNA's between the BSp73AS and the BSp73ASML cells, S1 nuclease and RNase protection analyses were carried out. The protected DNA or RNA fragments can only be smaller than the total length because they contain 5'-ends of vector sequences which cannot hybridise with the RNA's from the tumour cells. We first consider the transition to the 3'-side: the transition of sequences with homology to the sample A to those to the sample B. Both techniques indicate a single RNA species in the BSp73AS cells which is co-linear with the samples over a wide range. Further 5' therefrom, cDNA or RNA sample, respectively, differ, which certainly correspond to RNA sequences from

BSp73ASML, from the RNA from the BSp73AS cells. The RNA's from BSp73ASML contain, above all, sequences which protect larger fragments of the sample. The largest fragments correspond to the full length of the DNA or RNA fragments, respectively, which were available for the protection analysis. Small fragments are also detectable. Since the RNA transfer hybridisations have certainly uncovered a heterogeneous mixture of different sizes of RNA's, it is possible that these indicate smaller protected fragments of RNA species which diverge somewhere else from the cDNA, i.e. at places between the previously detected point of divergence and the 5'-end of the available samples. These RNA species are also not detectable in the BSp73AS cells.

We now consider the point of divergence on the 5'-side, thus the transition of sequences which hybridise with the sample C, to that which hybridises with the sample A, thus the ECR-coding sequence. The analysis gives corresponding results for the 5'-cleavage point. RNA's from BSp73AS cells can only protect the available samples over a comparatively small range. Messenger RNA's from BSp73ASML protect longer fragments. They are, namely, colinear over the whole length of the available sample. Thus, one can conclude that the cDNA clone represents pMeta-1 sequences which are pronounced in the metastasing tumour cells BSp73ASML. The 3' and 5' regions are also found in RNA's from BSp73AS. The ECR-coding sequences with the defined transitions, which can be mapped with the help of these above-described techniques, indicate that here an alternative splicing mechanism for the RNA formation must be present. The 5' and 3' cleavage points for the transitions to the ECR sequences are marked in Fig. 1 by arrows.

The monoclonal antibody 1.1ASML identifies a variant form of the glycoprotein CD44.

In order to obtain structural information about the surface protein, all cloned cDNA molecules have been sequenced. The nucleotide sequence of the total length clone pMeta-1 and the amino acid sequences derived therefrom are shown in Figure 2. The total length cDNA clone bridges 3207 nucleotides. The 3' terminus carries a PolyA end, two additional polyadenylation signals lie at positions 2288 and 1743. The first ATG codon follows a consensus initiation sequence and opens a reading frame of 1509 nucleotides corresponding to 503 amino acids. As one should assume for a membrane-standing protein, the first 21 amino acids are hydrophobic and represent a signal peptide. No part of this sequence is hitherto to be found in the data bases. However, we found sequence homology to recently published data about the lymphocyte homing receptor CD44 (or Pgp-1) (Idzerda et al., 1989; Goldstein et al., 1989; Stamenkovic et al., 1989; Nottenburg et al., 1989; Zhou et al., 1989). The homologies are strictly limited to the 5' and 3' parts of the cDNA with inclusion of non-translated regions and they end on the already above-mentioned points of divergence between the BSp73AS and the BSp73ASML sequences. The whole length between the points of divergence (in Figure 2 characterised by colour marking), thus the whole length of the metastasis-specific ECR-coding sequence, is not represented in the Pgp1 or CD44 sequences. The metastasis-specific glycoprotein apparently represents a variant of the CD44 glycoproteins. It carries, namely, an additional extracellular domain of 156 amino acids and thus an expanded extracellular region of 410 amino acids (less 21 amino acid signal peptide), in comparison with 270

amino acids (also less signal peptide) of the unchanged CD44 glycoprotein. In the non-metastasing BSp73AS cells, however, the unchanged forms of this CD44 family are detected. cDNA sequences of these BSp73AS RNA's have also been cloned and the identity with the metastasis-specific clones outside of the extra domains is demonstrated.

The expression of the variant CD44 is correlated with the metastatic potential.

In order to examine whether the expression of the variant CD44 glycoproteins takes place without exception in the BSp73ASML cells and whether they stand in conjunction with the metastatic potential of these cells or the metastatic potential in general, we studied another series of isogeneic rat tumour cell lines, namely, the tumour cell lines of the mammary carcinoma system 13762 NF (Neri et al., 1982). We here compared cell lines which were derived from the parental tumour, namely, the MTPa, MTC, MTF7 and MTA cells (group 1), with cell lines which were established from lymph nodes or lung metastases, namely, MTLy, MTLn2, MTLn3 (group 2). The group 1 cells essentially expressed the normal CD44 pattern similarly to the RNA's from the BSp73AS cells when one hybridises with sample B. On the other hand, with sample A, a smaller amount of a diffuse RNA band is detected, which has approximately the size 2.5 kb. On the other hand, the group 2 cells show a completely different RNA pattern. Both samples A and B hybridise with larger RNA species. The sizes resemble those which are detected in BSp73ASML. The similarity is also documented by RNase and S1 protection analyses. On the basis of these data, we conclude that a change in the splicing pattern of the RNA and the expression of variant CD44 is correlated with the formation of metastases and that

the acquired pattern in these metastasing mammary carcinoma cells correspond very much to those which we have got to know above already for the metastasing BSp73ASML cell line. The high molecular proteins recognised by the antibodies correspond to the two high molecular species on proteins which have been detected in the BSp73ASML extracts. In this mammary tumour series, we thus also detected a metastasis-specific expression of RNS species and of high molecular proteins. That in the group 1, thus the so-called parental cell line, RNA's were found at all which hybridise with the sample A, thus the ECR-coding sequence, and that we also see a weak staining of a protein of 100,000 Dalton with the antibody, we attribute to the fact that the group 1 cells also possess small metastasing ability quite in contra-distinction to our original tumour cell line BSp73AS which shows no metastasing behaviour at all.

<u>The monoclonal antibody 1.1ASML inhibits metastasis formation in the rat</u>.

In a series of experiments for the metastasis formation of the tumour cell line BSp73ASML in isogenic rats, cells were injected subcutaneously and, at different times, the monoclonal antibocy 1.1ASML injected intraperitoneally at intervals of two to three days before and after the tumour administration. In the scope of this immunological protocol, it was also determined how the immune response of the rat towards the injected antibody had taken place. There result, namely, antimouse immunoglobulin antibodies, as well as antiidiotype antibodies. The result of this series of experiments is that the growth and the metastasing of the tumour by injection of 1.1ASML is greatly delayed. This delay permits, in its kinetics, the conclusion that the antibody interferes with a primary

process of the metastasing. The experiment shows us that the protein structure on the surface of the metastasing cells recognised by the antibody has a role in the metastasing process and that therapeutic and diagnostic plans are realistic.

Isolation of the homologous human sequence to the ECR-coding sequence part of the rat cDNA.

For human tumour cells in culture, there naturally does not exist without doubt the possibility to detect corresponding to rat systems whether they also still maintain matastasing properties. Experiments with immune-deficient mice make possible only a very limited prediction regarding the matastasis potential in the case of humans. Therefore, relatively many tumour cell lines which have been placed in cultures anywhere in the world long periods of time ago had to be tested as to whether they express sequences which we could detect for the rat metastases. We have been able to find such a tumour cell line. It originated from a large-cell lung carcinoma of humans and bears the number: LCLC 97. In this tumour cell line, three definite RNA species can be detected (sizes: 5.5, 3.4 and 2.8 kb) which behave quite according to the RNA's which are detectable in the metastasing tumour cell lines of the rat. They hybridise, namely, not only with the sample A but also with the samples B and C, i.e. that also these human RNA species are identical over a wide range with the cDNA pMeta-1 (85%).

However, the monoclonal antibody 1.1ASML does not react with this tumour cell, i.e. the fragment of protein recognised by the antibody must differ in the region of the antigen determinants from the protein which exists on the surface of the human tumour cell. For the non-reactivity, there suffice even the smallest deviations because of the high specificity of the

antibody. The human tumour cell LCLC97 served the purpose to construct a cDNA bank. On the basis of the high agreement between the rat and human sequences, a cDNA clone could be isolated which showed homology with the sample A. The human cDNA was sequenced. In Figure 3 (a,b) is shown the primary sequence and the amino acid sequence derived therefrom. One can recognise that, over a wide range, substantial identity exists between the rat and the human sequence. This human sequence, as well as also the amino acid sequence derived therefrom, is also the subject of this Patent Specification.

Embodimental Examples:

Cells and antibodies:

The following cloned Bsp cell lines were used for the investigations: BSp73 14ASML-1 and 10AS-7 and kept in culture as described by Matzku et al., (1983); furthermore, the mammary carcinoma cell lines described by Neri et al., (1982) monoclonal antibodies against BSp73 ASML membrane proteins were produced by immunisation of Balb c mice. After isolation of the spleen cells of an immunised mouse, these were fusioned with Ag8 myeloma cells for the immortalisation according to the method for the production of monoclonal antibodies of Köhler (1981). The then obtained hybridoma cells were subjected to a screening process in order to find those which produce specific antibodies against BSp73ASML but not against BSp73AS and normal rat fibrinoblast cells. The precise process procedure is described also as in the case of Matzku et al., (1989).

Monoclonal antibody (mAb)-producing hybridoma cells with the corresponding specificity were expanded in the tissue culture and the mAb given off into the medium strongly enriched by ammonium sulphate precipitation and column chromatography (protein A-Sepharose

and MonoQ) and used in this form for the investigations. One of them is mAb1.1ASML.

Immunofluorescence:

For the production of the variant CD44 molecule on various tumour cells, these were taken up in culture, then washed with phosphate-buffered common salt solution (PBS) and incubated with 1.1ASML for 30 minutes at 40°C. As secondary antibody for the detection of the binding, there was used a rhodamine-coupled rabbit anti-mouse IgG and shown in the fluorescence microscope.

Construction of the cDNA expression banks and immuno-screening:

PolyA+ RNA from BSp73ASML was "primed" with oligo (dT) and hexanucleotides of different composition and synthesised with reverse transcriptase from AMV of the first strand of the cDNA. The second strand of the cDNA was produced with E. coli DNA polymerase I, RNaseH and E. coli ligase and subsequently the double-strand cDNA linearised with T4DNA polymerase on the ends. The vectors pEX1, 2 and 3 (Stanley and Luzio, (1984), which make possible the fusioning of the cDNA in 3 different reading frames, were cut out with SmaI restriction endonuclease and ligated with the cDNA (T4 DNA ligase). Competent E. coli DH5 (pCI857) bacteria, which produce a temperature-sensitive repressor, are transfixed with the pEX-cDNA constructs and cultured on nylon filters. The gene for the temperature-sensitive repressor RCI857 lies on the plasmid pCI857, which is compatible with the pEX plasmids. At 28°C, the IP_R promotor, which controls the synthesis of the fusion proteins, is switched off. By temperature increase to 42°C, the CI repressor is inactivated and the synthesis of β-galactosidase/ ASML fusion proteins massively set going. The heat-induced bacteria colonies are subsequently denatured

with chloroform vapour on the filters and these then incubated with PBS which contains 3% dried milk powder, lysozyme and DNase. The bacterial fusion proteins fixed on the nylon filter are now incubated with mAb1.1ASML and, after washing out of non-specifically bound mAb, used for the detection of the binding as secondary antibody 125I-labelled rabbit anti-mouse IgG. After autoradiography, positive clones were isolated from the original bacteria filters and substantially analysed. A clone which synthesised a fusion protein which reacted specifically with 1.1ASML was pEX34. The pEX plasmid contained in the bacteria clone carries 167 nucleotide cDNA which, inter alia, code for the epitope (or the antigen determinants), the specificity of which mAb1.1ASML carries.

The isolation of the total lengths of cDNA mMeta-1 then took place according to standard methods.

Immunisation of the rats with mAb1.1ASML.

BDX rats which are syngenic to the BSp73 tumour cells were injected subcutaneously and intraperitoneally with mAb1.1ASML (coupled to keyhole limpet haemocyanines), together with complete Freund's adjuvant. The first injection took place 10, 7 and 3 days before the injection of the BSpASML cells (in the foot fat pad), the following then 3, 7, 11, 14 and 21 days thereafter. After 28 days, the rats are sacrificed, the various lymph nodes prepared and weighed and macroscopically visible lung matastases counted.

Connection between the expression of variant CD44 surface proteins and metastatic potential

In order to ascertain whether the expression of variant CD44 glucoproteins is only a property of the investigated BSp73ASML cell line or whether the expression can be brought into connection with the

metastatic potential, another series of rat tumour cell lines was investigated which was derived from the 13762NF mammary carcinoma (Neri et al, 1982). Furthermore, cell lines which are derived from primary tumours (MTPa, MTC, MTF7 and MTA (group 1)) were compared with cell lines which are derived from lymph node and lung metastases (MTLy, MTLn2, MTLn3 (group 2)). The pattern of the RNA derived from CD44 is given in Figure 4, whereby samples A, B and D correspond to the samples described on page 4 of the Application, as well as Figure 1. Cells of group 1 all show a normal CD44 pattern with sample B. On the other hand, cells of group 2 show a pattern different therefrom. The RNA is greater than the RNA of group 1 and corresponds to the RNA of BSp73ASML. Smaller RNA's are lost in the case of the hybridisation with sample D. The other patterns show the similarity between the two rat tumour systems.

Also with the sample A, the RNA pattern of the group 2 corresponds to that of BSp73ASML. Whereas sample A does not hybridise with RNA from BSp73AS, there is shown a small diffuse RNA band of about 2.5 kb in the case of cells of group 1. RNase and S1 nuclease protection analysis also show the structural similarity. From these results, a change in the cleavage pattern and the expression of variant CD44 RNA's appears to occur with the formation of metastases.

<u>Transmission of the metastatic potential to non-metastasing BSp73AS cells by over-expression of pMeta-1</u>.

The connection of the expression of variant CD44 species with the metastatic potential in two series of rat tumours indicated a causal role of these glycoproteins in the metastatic process. In order to investigate this, pMeta-1 was transferred into BSp73AS

cells and investigated whether the behaviour of the cells thereby changed. The complete coding region of the pMeta-1 (Fig. 2) was inserted under the SV40 promotor and this formation (diagram in Figure 5), together with PSV2neo, introduced into BSp73AS cells. Individual G418-resistant and pMeta-1-expressing colonies were obtained. The RNA pattern of 2 of these colonies is given in Figure 5. The hybridisation of the variant CD44-specific sample A shows a dominant transcript of about 2.2 kb which in size corresponds to the smallest common RNA which is transcribed in BSp73ASML cells (Figure 5). However, the transfixed cells contain about 10 times as much of this RNA as BSp73ASML.

Other orders of size are observed in one of the transfixed cells (BSp73AS-pSVMeta-1-14) which could be dependent upon the place of the plasmid integration. A pSV2neo simulation transmission clone (not shown) and the BSp73AS receptor cells contain no RNA which is complementary to sample A. In order to discover the endogenic normal CD44 transcriptions (without the extra domains of the pSVMeta-1) in the transfects, the filter was stripped and rehybridised with sample D. This part of the non-transferred 3' sequence is not contained in the expression clone (cf. Figure 5). Sample D demonstrates two main transcripts of 2.9 and 4.9 kb in the RNA of the two transfects (Figure 5, right column), not only in the control BSp73AS but also in the not shown BSp73ASpSVneo.

Approximate quantifications of the different corresponding hybridisations show that the transfects express about 5x as much of the variant CD44 RNA's, which are transcribed by the expression plasmid, as the endogenic gene transcrips.

The over-expressed cDNA is transferred into a protein. The two transfects, which are shown in Figure 5, synthesise mAb1.1ASML-immune stainable proteins of the same apparent size, namely, a main product of 150 kDa and a weaker band at 100 kDa. Since the cDNA sequence codes a primary protein product of only 503 amino acids (corresponds to about 60,000 Dalton), all visible bands must represent modified forms. The 150 kDa band runs together with one of the modified forms of variant CD44 which is expressed in the metastasing cells BSp73ASML. BSp73AS or simulation-transmitting BSp73ASpSVneo cells do not possess this protein. As in BSp73ASML cells, the epitope of the cells expressed by the transfects is free-lying on the cell surface.

In order to demonstrate that the expression of variant CD44 suffices in order to impart to BSp73AS cells a metastatic potential, transfects were injected into syngenic BDX rats (spontaneous metastsis protocol). In earlier experiments, metastatic tumour cells BSp73ASML spread out quickly from the place of the injection and, about 10 days after the injection, were completely dispersed (Matzku, 1984). Therefore, all local tumours were removed by amputations on the 10th day. All carriers of BSp73ASML cells and all animals which had been injected with one of the over-expressed transfects developed lung metastases (Table 1). The course of the metastasis formation was comparably quick within 5 - 8 weeks after the injection. Animals which had received BSp73AS cells or simulation transfects were, after this time, completely healthy (apart from due to the amputation) and even after 5 months no metastases could be ascertained.

In spite of the surprising similarity in the strong metastasis formation, there are some interesting

differences. In all animals, BSp73ASML cells reach the lymph nodes and lead to a massive enlargement of different nodes in the region of the inguinal groin and beside the aorta (Table 1). A transfect (BSp73AS-pSVMetal-14) causes lymph node enlargements in 3 of 8 animals, although all animals develop multiple lung metastases (Table 1). With the other transfect (BSp73AS-pSVMetal-15), no lymph node enlargement is ascertainable. Therefore, the transfects appear to be able to form colonies in the lungs without an obligatory growth phase in the lymph nodes.

Furthermore, the experiment according to Table 1 points to another difference between BSp73AS transfects and BSp73ASML. The individual lung metastases are macroscopically visible whereas those from BSp73ASML are small and numerous but, in a larger series with BSp73ASML (Reber et al., 1990) 11 from 20 animals develop 5 - 20 larger nodes per lung than the transfects.

In order to ascertain that the metastases formed are brought about by the injected transfects and in order to exclude the improbable possibility of a spontaneous mutation which transfers a metastatic potential, the epitope-positive proteins in the total lung extracts and in extracts of recultured, metastasis-producing cells were determined. In the total lung extract, as well as in the extracts of a specific lung node of an animal which had received the BSpAS-pSVMetal-15 transfects, the 15 kDa glycoprotein is detectable. The G418-resistant strain expresses, in the case of the in vitro growth, a protein of the same apparent molecular weight.

Diagnosis and therapy

1. Analysis of human tumour material by in situ hybridisations with the human pMeta-1-sequences present.

These experiments are thought of as preliminary experiments before an Ab is available which recognises the human ECR.

2. Production of antibodies against the human ECR. Cloning of the human pMeta-1 sequences into bacterial expression vectors so that fusions with β-galactosidase or tryptophane E-product result. Immunisation of rabbits with these fusion proteins or with synthesised peptides from the ECR (coupled to carrier molecules). Isolation of the polyvalent or monospecific antibodies.
Possibilities of use:

- Immunohistological investigations of clinical tumour material (diagnosis)
- Detection of soluble ECR in the serum of patients with the help of ELISA tests (diagnosis).
- Construction of toxin-coupled antibodies in order, with the help of the antibody, to bring the toxin into the tumour/metastasis region (therapy).
- Construction of antibodies with two definite antigen binding positions. By means of this double specificity, the attempt is to be made to initiate cytotoxic reactions in the metastasis region (e.g. anti CD2 or CD3 coupling (therapy).

3. Production of hMeta-1 protein by transfection of human or rat cells with an expression vector which carries the complete hMeta-1 cDNA sequence; or purification from LCLC97 cells.
Possibilities of use:

- Injection of the protein or parts thereof in order to block the tissue binding positions of the tumour cells.
- After a characterisation of the binding positions, a use for therapy would also be conceivable which could depend upon the injection of large amounts of binding protein which would then block the migrating tumour cells.

Literature list:

Goldstein, L.A., Zhou, D.F.H., Picker, L.J., Minty, C.N., Bargatze, R.F., Ding, J.F. and Butcher, E.C. (1989) A human lymphocyte homing receptor, the hermes antigen, is related to cartilage proteoglycan core and link proteins. Cell 56: 1063-1072.

Hart, I.R., Goode, N.T. and Wilson, R.E. (1989) Molecular aspects of the metastatic cascade. Biochim. Biophys. Acta 989: 65-84.

Idzerda, R.L., Carter, W.G., Nottenburg, C., Wayner, E.A., Gallatin, W.M. and St. John, T. (1989) Isolation and DNA sequence of a cDNA clone encoding a lymphocyte adhesion receptor for high endothelium. Proc. Natl. Acad. Sci. U.S.A. 86: 4659-4663.

Köhler, G. (1981) In: I. Lefkovits and B. Pernis (eds) Immunological Methods. Vol. 2, p.285. N.Y. Academic Press.

Matzku, S., Komitowski, Mildenberger and Zoller, M. (1983) Characterization of Bsp 73, a spontaneous rat tumor and its in vivo selected variants showing different metastasing capacities. Inv. Met. 3: 109-123.

Matzku, S., Wenzel, A., Liu, S. and Zőller, M. (1989) Antigenic differences between metastatic and nonmetastatic BSp73 rat tumor variants characterized by monoclonal antibodies. Cancer Res. 49: 1294-1299.

Neri, A., Welch, D., Kawaguchi, T. and Nicholson, G.L. (1982) Development and biologic properties of malignant cell sublines and clones of spontaneously metastasizing rat mammary adenocarcinoma. J. Natl. Cancer Inst. 68: 507-517.

Nicholson, G.L. (1987) Tumor cell instability, diversification, and progression to the metastatic phenotype: from oncogene to oncofetal expression. Cancer Res. 47: 1473-1487.

Nottenburg, C., Rees, G. and St. John, T. (1989) Isolation of mouse CD44: structural features are distinct from the primate cDNA. Proc. Natl. Acad. Sci. U.S.A. 86: 8521-8525.

Stamenkovic, I., Amiot, M., Pesando, J.M. and Seed, B. (1989) A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. Cell 56: 1057-1062.

Stanley, K.K. and Luzio, J.P. (1984), Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. EMBO J. 3: 1429-1434.

Wenzel, A. (1986) Charakterisierung von Differenzierungsantigenen auf dem Rattentumor Bsp 73 mit Hilfe monoklonaler Antikörper. Diplomarbeit, Universitat Karlsruhe (Characterisation of differentiating antigens on the rat tumour Bsp 73 with help of monoclonal antibodies. Diploma thesis, University of Karlsruhe).

Zhou, D.F.H., Ding, J.F., Picker, L.F., Bargatze, R.F., Butcher, E.C. and Goeddel, D.V. (1989) Molecular cloning and expression of Pgp-1 - The mouse homolog of the human H-CAM (Hermes) lymphocyte homing receptor. J. Immunol. 143: 3390-3395.

Table 1

Metastatic spreading out of BSp73AS cells which express variant CD44 cDNA pMeta-1 ***

Tumour clone	local occurrence	distribution in the case of metastatic autopsy		
		*LN ing	*LN par	lungs
BSp73ASML	0/8	8/8 ø 1.5-2.5**	8/8 ø 2.5-5.0	8/8 miliary
BSp73AS-pSVMetal-14	0/8	3/8 ø 0.3-1.2	3/8 ø 1.0-4.5	8/8 multiple, ø 0.3-5.0
BSp73AS-pSVMetal-15	0/8	0/8	0/8	8/8 5-20, ø 0.3-10.0
BSp73AS	1/8	0/8	0/8	0/8
BSp73AS-pSVneo	0/8	0/8	0/8	0/8

** average diameter in mm

*** The Table gives the stage 60 days after injection of the indicated cells

* LN = lymph nodes.

Patent Claims

1. DNA-fragment which codes for a part of a surface protein of metastasing tumour cells, whereby this DNA-fragment is selected from

a) the nucleotide sequences

...rMeta-1...

ATT GCA ACT ACT CCA TGG GTT TCT GCC CAC ACA AAA CAG AAC
CAG GAA CGG ACC CAG TGG AAC CCG ATC CAT TCA AAC CCA GAA
GTA CTA CTT CAG ACA ACC ACC AGG ATG ACT GAT ATA GAC AGA
AAC AGC ACC AGT GCT CAT GGA GAA AAC TGG ACC CAG GAA CCA
CAG CCT CCT TTC AAT AAC CAT GAG TAT CAG GAT GAA GAG GAG
ACC CCA CAT GCT ACA AGC ACA ACC TGG GCA GAT CCT AAT AGC
ACA ACA GAA GAA GCA GCT ACC CAG AAG GAG AAG TGG TTT GAG
AAT GAA TGG CAG GGG AAG AAC CCA CCC ACC CCA AGT GAA GAC
TCC CAT GTG ACA GAA GGG ACA ACT - GCC TCA GCC CAC AAC
AAC CAT CCA AGT CAA AGA AGT ACA ACA CAG AGT CAA GAG GAT
GTT TCA TGG ACC GAT TTC TTC GAC CCA ATC TCA CAT CCA ATG
GGA CAA

and

hMeta-1...

AAC CCA AGC CAT TCA AAT CCG GAA GTG CTA CTT CAG ACA ACC
ACA AGG ATG CAT GAT GTA GAC AGA AAT GGC ACC ACT GCT TAT
GAA GGA AAC TGG AAC CCA GAA GCA CAC CCT CCC CTC ATT CAC
CAT GAG CAT CAT GAG GAA GAA GAG ACC CCA CAT TCT ACA AGC
ACA ATC CAG GCA ACT CCT AGT AGT ACA ACG GAA GAA ACA GCT
ACC CAG AAG GAA CAG TGG TTT GGC AAC AGA TGG CAT GAG GGA
TAT CGC CAA ACA CCC AGA GAA GAC TCC CAT TCG ACA ACA GGG
ACA GCT GCA GCC TCA GCT CAT ACC AGC CAT CCA ATG CA

b) nucleotide sequences which are degenerated with regard to the DNA sequences mentioned under a) and/or represent allele variations.

2. DNA fragments according to claim 1, characterised in that they contain nucleotide sequences which hybridise with one of the nucleotide sequences mentioned in claim 1 a) and code for a complete surface glycoprotein of metastasing tumour cells.

3. DNA fragment according to claim 2, characterised in that it hybridises with one of the nucleotide sequences mentioned in claim 1 which has at least 85% homology with the reaction partner.

4. Recombined DNA molecule consisting of a vectorial nucleotide sequence and a DNA fragment according to claims 1, 2 or 3.

5. Recombinant DNA molecule according to claim 4, characterised in that it is an expression vector.

6. Transformed host cell which contains a DNA fragment according to claims 1, 2 or 3 or a recombined DNA molecule according to claims 4 or 5.

7. Polypeptide which is coded by a DNA fragment according to claim 1.

8. Polypeptide producable by a recombinant DNA molecule according to claims 4 or 5 which includes a DNA fragment according to claim 1.

9. Polypeptide according to claims 7 and 8, including the amino acid sequences

...r-protein...

I A T T P W V S A H T K Q N Q E R T Q W N P I H S N P E V L
L Q T T T R M T D I D R N S T S A H G E N W T Q E P Q P P F
N N H E Y Q D E E E T P H A T S T T W A D P N S T T E E A A
T Q K E K W F E N E W Q G K N P P T P S E D S H V T E G T T
A S A H N N H P S Q R M T T Q S Q E D V S W T D F F D P I S
H P M G Q G H Q T E S K

and h-protein

I S S T I S T T P R A F D H T K Q N Q D W T Q W N P S H S N
P E V L L Q T T T R M T D V D R N G T T A Y E G N W N P E A
H P P L I H H E H H E E E E T P H S T S T I Q A T P S S T T
E E T A T Q K E Q W F G N R W H E G Y R Q T P R E D S H S T
T G T A A A S A H T S H P M Q G R T T P S P E D S S W T D F
F N P I S H P M G R G H Q A G R R

and their allele variations and glycosylation products.

10. Poly- and monovalent antibodies which react with an epitope of a polypeptide according to claims 7 to 9.

11. Use of a DNA fragment according to claim 1, its complementary strands,derivatives and parts thereof for the identification, production or isolation of a nucleotide sequence or parts thereof which code for a part of a surface glycoprotein of metastasing tumour cells.

12. Use of an antibody according to claim 10 for the identification, production or isolation of a polypeptide according to claims 7 to 9.

13. Agent for the diagnosis of metastasing tumours and/or metastases which contain at least one antibody according to claim 10.

14. Agent according to claim 12, characterised in that the antibody is coupled with an enzyme, labelled with a coloured material and/or with a radioisotope.

15. Antibody against proteins according to claim 7 produced with the use of DNA according to claim 1 or proteins according to claim 7.

16. Use of antibodies and proteins or parts thereof according to claims 15 and 7 for the production of medicaments which are used in tumour therapy.

17. Use of antibodies according to claim 15 which are coupled with cytotoxic agents for the production of medicaments for tumour therapy.