92

## MUTANT E-CADHERIN AS A NEW TARGET FOR DIAGNOSIS AND THERAPY OF DIFFUSE-TYPE GASTRIC CANCER

Karl-Friedrich Becker[1,3], Ingrid Becker[1], Christoph Schuhmacher[2], Elisabeth Kremmer[4], Manfred Eulitz[5], Heinz Höfler[1,3].
Technische Universität, Klinikum rechts der Isar, [1]Institut für Pathologie and [2]Chirurgische Klinik, Ismaninger Strasse 22, D-81675 München, Germany; GSF-Forschungszentrum, [3]Institut für Pathologie, [4]Institut für Immunologie, [5]Institut für Klinische Molekularbiologie, Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany.

Gastric cancer is the second most common cancer world-wide with approximately 670 000 new cases per year. Despite recent advances in the molecular pathology of gastric cancer, translation into the clinic for earlier diagnosis or more specific therapeutic purposes has been less forthcoming. In 50% of diffuse-type gastric carcinomas we detected mutated forms of the homophilic cell adhesion molecule E-cadherin. Since these mutations generally affect the extracellular portion of the transmembrane molecule and do not interrupt the reading frame, altered E-cadherin protein may be an excellent tumor marker. In-frame deletions of exon 9 from the E-cadherin messenger RNA, due to different somatic splice-site gene mutations, were identified as a mutational hot spot. Here we report the characterization of a rat monoclonal antibody, delta 9-1, that specifically reacts with mutant E-cadherin lacking exon 9 but not with the wild-type protein. This extraordinary high specificity was demonstrated using fluorescence activated cell sorting, immuno-fluorescence and Western blot analysis of E-cadherin negative cells that had been transfected with either mutant or wild-type E-cadherin complementary DNA. More importantly, in gastric carcinoma specimens known to express mutant E-cadherin messenger RNA lacking exon 9, monoclonal antibody delta 9-1 targets exclusively tumor cells in routine formalin fixed and paraffin embedded material. Non-tumorous cells, including normal gastric epithelium expressing wild-type E-cadherin, are not stained. Other non-tumorous tissues examined did not react with delta 9-1. Thus, E-cadherin mutation-specific monoclonal antibodies should be attractive candidates for diagnosis of malignant cells. For therapeutic purposes, these apparently tumor cell-specific antibodies could be used for gene therapy, for immunotherapy, and as immunotoxins to treat small tumor deposits for adjuvant-, neoadjuvant- and additive therapy.

93

## Isolation and characterization of an Ewing's tumor specific antigen recognized by a mAb

A. Borowski, I. Vogt, L. R. Shi, J. Wei, E. M. Schneider
Immunology Laboratory, Institute of Hemostasis and Transfusion Medicine
Heinrich-Heine-University of Düsseldorf

The Ewing's tumors (ET) are a family of primitive neuroectodermal tumors (PNET), containing the Ewing's sarcoma and the PNET, sharing the same translocation (11;22)(q24;q12). The translocation fuses the *EWS* gene to the *ets*-related *FLI*-1 gene. This fusionprotein shows properties which are different from the related native *ews* and *fli*-1 proteins. *EWS/FLI*-1 is apparently related to the transformation of the primitive neuroectodermal cells and possibly encodes for a tumor specific antigen. To examine this possibility, we further characterized 16 specific monoclonal antibodies (mAb) with tumor specificity, two of which little crossreacted with either T cells (ab No. 9 recognizing the antigen MIC2) and/or cultured monocytes (ab No 16) by immuno-histochemical and flowcytometric analyses [1]. To characterize the antigens recognized by the ET specific mAb, a cDNA library of the ET cell line VH-64 was constructed in the lambda ZAP Express™ vector. The primary library has a titer of $5x10^5$ phages. The library was checked with 30 randomly picked clones containing inserts of more than 1kb. Five $x10^5$ phages of the amplified cDNA library were screened with a pool of the 16 ET specific mAb. Four clones were isolated which are recognized by the mAb No. 16 SN85/1857.15.30. These clones represent an insert of around 3,5 kb which corresponds to a deduced protein of around 130 kDa. Immuneprecipitation performed with radiolabeled cell lysate revealed a MW of 140 kDa for this antigen. Until now, the first 300 bp of the 3' and 5' site leave no sequence homology to a known transmembrane protein expressed in human tissues. Analyses imply that the antigen defined by ab No 16 plays a role in the recognition of NK cells, since mAb SN85/1857.15.30 inhibits the lysis by NK cells. Thus, we apparently succeeded to clone an ET specific surface antigen recognized by a mAb displaying an immunemodulatory function.

[1]L. R. Shi et al. *Cancer Immunol Immunother* (1994) **38**: 208-213

94

## GENERATION OF ANTI-TUMOR ACTIVITY BY BISPECIFIC ANTIBODIES IN NON-HODGKIN LYMPHOMA: *IN VITRO* INHIBITION OF AUTOLOGOUS HAEMATOPOIESIS

M. Brandl[1], W.Wilmanns[1,2], G.Jung[1]
[1]Medizinische Klinik III der LMU München; [2]GSF-Research Center for Enviroment and Health, D-81377 München

We have recently reported that a combination of two bispecific antibody fragments [$F(ab')_2$] directed against CD3 and CD28 on the T cell side and CD20 on the Non-Hodgkin lymphoma cell side is able to induce efficient T cell activation and proliferation *in vitro*. This autologous T cell activation always results in cytotoxicity against the tumor cells.
To investigate if this combination of bispecific antibody fragments provides a new strategy for *ex vivo* purging of autologous bone marrow it was necessary to look for the effect of antibody induced T cell activation *in vitro* on autologous haematopoietic progenitor cells. Therefore we carried out long-term bone marrow cultures (LTC) for up to 4 weeks. The weekly output of clonogenic cells was evaluated in a colony forming unit assay. Colony formation was highly suppressed if LTC were incubated with both bispecific antibody fragments ($\alpha$CD3x$\alpha$CD20 and $\alpha$CD28x$\alpha$CD20). This toxic effect is partly due to a toxicity of TNF$\alpha$, a cytokine that is produced during T cell activation. Besides a direct cytotoxic effect on haematopoietic progenitor cells TNF$\alpha$ inhibits the growth of autologous fibroblasts neccessary for the survival of stem cells *in vitro*.
Hence in case of *ex vivo* bone marrow purging of highly contaminated bone marrow with bispecific antibody fragments an inhibition of the reconstitution capacity of the autologous bone marrow transplant is to be expected.

95

## GLYCOTOPES OF THE THOMSEN-FRIEDENREICH TYPE AS PROMISING TARGETS FOR BIOLOGICAL TUMOUR THERAPIES

Y.Cao[1], P.Stosiek[2], B.M.Ghadimi[3], G.Pecher[3], H.Shigeoka[4], and U.Karsten[1], [1]Max Delbrück Centre for Molecular Medicine, Berlin-Buch, [2]Karl Thiem Klinikum, Cottbus, [3]Virchow Klinikum of the Humboldt University, Berlin, Germany, and [4]Kinki University School of Medicine, Osaka, Japan

Biological tumour therapies critically depend on suitable target molecules. Since it is well established that the glycosylation of membrane glycoconjugates is oncodevelopmentally regulated, it is no wonder that tumour-associated epitopes are often glycotopes. Recent evidence is confirming that carbohydrate epitopes are presented to the immune system in a manner similar to peptides, and that they evoke humoral as well as cellular responses. We have chosen the long-known Thomsen-Friedenreich antigen (TF, core 1 structure of O-glycans, Galß1-3GalNAc$\alpha$1-) and the closely related Tn and sialosyl-Tn antigens, for our studies. With a newly developed panel of monoclonal antibodies to different sub-specificities of TF, we have reexamined the expression of TF in normal, benign and malignant human tissues by immunohistochemistry. This was extended by antibodies to Tn, sialosyl-Tn, and to MUC1, the carrier molecule of these glycotopes, as well as by the lectins Jacalin and Amaranthin. The results strongly suggest that TF is highly tumour-specific, with only a few exceptions in non-malignant tissues, which, in addition, represent immunologically priviledged sites. In the gastrointestinal tract, TF can already be detected in late (highly dysplastic) adenomas. Whereas the specificity of TF is excellent, its sensitivity in terms of per cent positive cells within the tumour is not sufficiently high. With a combination of antigenic determinants, including TF, Tn and MUC1, this can be overcome. Such a combination is a promising target for immune intervention, eg for the development of a tumour vaccine against minimal residual cancer.

Furthermore, we have found a correlation between the expression of TF on primary colorectal carcinomas and the development of liver metastases within one year. In an animal metastasis model based on a similar mechanism, we were able to suppress the development of liver metastases by application of the anti-TF antibody, A78-G/A7.