CANCER GENOMICS & PROTEOMICS *1*: 241-248 (2004)

The Carcinoma-associated Antigen EpCAM Induces Glyoxalase 1 Resulting in Enhanced Methylglyoxal Turnover

MARKUS MÜNZ[1*], TANJA HOFMANN[2*], BURGHARDT SCHEIBE[3], MATTHIAS GÄNGE[3], KAY T. JUNGHANNS[3], REINHARD ZEIDLER[4] and OLIVIER GIRES[1,2]

[1]*Clinical Cooperation Group Molecular Oncology, GSF-Research Center for Environment and Health, and Head and Neck Research Department Munich;*
[2]*Head and Neck Research Department, University Clinics Großhadern, Marchioninistr. 15, 81377 Munich;*
[3]*Amersham Biosciences Europe GmbH, Münzingerstr. 9, 79111 Freiburg;*
[4]*Vaecgene Biotech. Inc., Marchioninistr. 25, D-81377 Munich, Germany*

Abstract. *Background: The epithelial cell adhesion molecule (EpCAM) is a homophilic adhesion molecule expressed de novo on a variety of epithelial tumors. Overexpression of EpCAM results in enhanced proliferation and rapid induction of the proto-oncogene c-myc. Materials and Methods: The novel proteomics-based fluorescence difference gel electrophoresis (DIGE technology) was used to study EpCAM effects on the proteome of human epithelial cells. Results: DIGE analysis resulted in the identification of five proteins with a significantly changed regulation ranging from –1.3 to +5.8-fold. One of the identified proteins, namely glyoxalase 1, experienced a shift in the isoelectric point from pH 5.2 to 5.0 upon EpCAM expression. This shift correlated with a gain of enzymatic activity of glyoxalase 1 resulting in an enhanced methylglyoxal turnover. Conclusion: We show the potential of the DIGE technology to rapidly and quantitatively analyze proteomes for changed expression levels and, importantly, posttranslational modifications. Furthermore, we describe new targets of the carcinoma antigen EpCAM including glyoxalase1.*

The epithelial cell adhesion molecule (EpCAM) is a homophilic, Ca^{2+}-independent adhesion molecule, that is absent or only weakly expressed in healthy squamous epithelia but, in contrast, strongly expressed in squamous cell carcinomas (SCC) (1). *De novo* expression of EpCAM in SCC correlated with increased proliferation as well as decreased differentiation (2). Thus, EpCAM is an interesting diagnostic and therapeutic target, that has also been used as an anchor molecule for antibody-based immunotherapy and retroviral targeting of tumor cells (3-7). However, understanding of the biological function of EpCAM in carcinogenesis is poor and incomplete. Litvinov and colleagues demonstrated that EpCAM is a homo- and heterophilic transmembrane protein involved in intercellular adhesion (2,8). Correct localization of EpCAM at the plasma membrane was dependent upon its interaction with the cytoskeleton *via* the direct binding of α-actinin (9). The lymphocyte-associated inhibitory receptors 1 and 2 (LAIR1/2) were identified as extracellular ligands for EpCAM (10). LAIR1 is expressed on lymphocytes, where its activation imposes inhibitory signals on these cells. The molecular effects induced following EpCAM/LAIR1 interaction are so far unknown. Additionally, a tumor-specific glycosylation of the extracellular portion of EpCAM was demonstrated in SCC of the head and neck (11), which may influence the binding affinity and function of EpCAM.

In order to address open questions concerning the molecular functions of EpCAM experimentally, we have established cellular systems that allow the molecular analysis of EpCAM-mediated effects. Using a phenotypic and transcriptome analysis, we demonstrated that EpCAM triggers the proliferation and metabolism of epithelial cells along with the rapid induction of the proto-oncogene *c-myc* (12). EpCAM-expressing cells were less dependent upon growth factor supplementation and formed colonies when grown in soft agarose. Further, the aggregated 26 amino acids encompassing the intracellular domain of EpCAM were necessary and sufficient for EpCAM signalling to c-myc and growth promoting effects. In the present study, we performed a proteome analysis in human embryonic

*Both authors contributed equally to this work

Correspondence to: Markus Münz, GSF-Research Center for Environment and Health, and Head and Neck Research Dept. Munich, Germany. Tel: +49-89-70953895, Fax: +49-89-70956896, e-mail: markus.muenz@med.uni-muenchen.de

Key Words: EpCAM, glyoxalase 1, DIGE, squamous cell carcinoma.

Table I. *Accession number, protein name and identification scores of the isolated EpCAM regulated proteins. A score ≥ 62 represents a p-value < 0.05. Mw: molecular weight, pI: isoelectric point, Reg.: X-fold up- (+) or down-regulation (-), Seq. cov.: sequence coverage upon MALDI-TOF analysis. Occur.: occurence in number of gels.*

Acc. N°	Protein	Mw	pI	Mowse score	Reg.	T-test	Ref.	Matched pept.	Seq. cov.	Occur.
Q04760	Lactoylglutathione lyase/ glyoxalase 1	20.8	5.25	79	+3.9	0.005	(33)	8	37%	4/4
Q04760	Lactoylglutathione lyase / glyoxalase 1	20.8	5.25	110	-1.3	0.001	(34)	8	53%	4/4
P14854	Cytochrome c oxidase polypeptide Vib	10.3	6.78	93	+5.8	$4x10^{-6}$	(35)	6	75%	4/4
P29373	Retinoic-acid binding protein II, cellular (CRABP-II)	15.7	5.43	105	+3.7	$6x10^{-5}$	(36)	7	59%	3/4
P30041	Peroxiredoxin 6	25.0	6.02	73	+1.4	0.004	(37)	6	34%	4/4

kidney cell (HEK293) transfectants using the novel fluorescence difference in gel electrophoresis technology DIGE. This technique allows the separation of two independent protein lysates labelled with different fluorescence dyes in one sample, and the use of internal standards to quantify differences in protein expression levels thoroughly. Here, we focussed on the induction of a post-translational modification of the glyoxalase 1 protein in EpCAM-positive cells, which resulted in an increased enzymatic activity in HEK293 transfectants and in the carcinoma cell line FaDu. Glyoxalase 1 is part of an enzymatic system necessary to metabolize methylglyoxal, a cytotoxic by-product enriched during glycolysis. In summary, glyoxalase 1 is a new target protein of the pan-carcinoma antigen EpCAM, which undergoes posttranslational modifications leading to an enhanced enzymatic activity.

Materials and Methods

Cell lines and plasmids. 293-ΔEpCAM and 293-EpCAM cells were established by stable transfection of HEK293 with the indicated expression plasmids using Lipofectamine (Gibco, Karlsruhe, Germany) according to the manufacturer's protocol. EpCAM pCEP4 and pMEP expression plasmids were kind gifts from Dr. S. Litvinov. FaDu is a squamous cell carcinoma line derived from a hypopharynx carcinoma. Stable transfectants were grown in standard medium (DMEM) containing 10% FCS and 200μg/ml Hygromycin (Calbiochem, Schwalbach, Germany) and propagated three times a week.

Standard 2D-PAGE and DIGE analysis. For 2D-PAGE analysis cells ($5x10^6$) were washed once in PBS and once in PBS/H_2O (1:1). Thereafter, cells were lysed in 2D-lysis buffer containing 9M urea, 4% CHAPS, 65mM DTE, 1mM EDTA. DNA was shredded using QIAshredder (Qiagen, Hilden, Germany) and pelleted at 42,000g for 1h. Proteins were separated using linear immobiline pH gradients (IPG strips pH3-10 or pH4-7; Amersham Biosciences) followed by SDS-PAGE. Differentially-expressed proteins were excised from a Coomassie- and silver-stained gel, respectively, and analyzed by MALDI-ToF. The resulting peptide mass fingerprints were used for a database search in the Matrix science mascot data bank (http://www.matrix-science.com). Standard search parameters were used – taxonomy: homo sapiens, enzyme: trypsin, fixed modifications: carbamidomethyl (C), variable modifications: oxidation (M), peptide tolerance: 150ppm. Four independent DIGE experiments were performed and carried out as recommended by the manufacturer (Amersham Biosciences). Briefly, 50 μg of each protein sample (293-ΔEpCAM and 293-EpCAM, in total 8 samples) were labelled with 400pmol of the respective Cy-Dye (Cy3 and 5). In parallel, equal amounts of the 8 samples (25 μg each; 4x 293-ΔEpCAM and 4x 293-EpCAM samples) were mixed and labelled with Cy2 (1600 pmol) to generate an internal standard (200μg). Fifty μg of this internal standard was added to each experiment. All three labelled samples (Cy3, Cy5 and the internal standard) of each experiment were pooled and separated by 2D-PAGE, visualized by fluorescence scanning and processed as described above.

GLOX 1 enzymatic activity assay. Glyoxalase 1 activity was measured according to Oray and Norton (13). The assay was performed in 200mM imidazole-HCl (pH 7) and 16mM $MgSO_4$. Final concentrations of methylglyoxal and gluthathione were 7.9 mM and 1mM, respectively, per reaction. The increase in absorbance at 240nm due to the formation of S-D-lactoylgluthathione was measured and expressed as μmol/min/mg protein input. One unit of glyoxalase 1 catalyzes the formation of 1μmol of S-D-lactoylgluthathione.

Results

DIGE analysis of EpCAM-induced effects on the proteome. Human embryonic kidney cells (HEK293) were stably transfected with an expression vector for the carcinoma-associated antigen EpCAM, or the corresponding empty vector only. The expression of EpCAM was assessed by immunoblotting and flow cytometry analysis in the resulting stable transfectants. EpCAM was absent in 293-ΔEpCAM cells harboring the empty vector, whereas 293-EpCAM cells expressed this molecule at levels comparable with head and neck carcinoma cell lines (data not shown and Figure 2 right panel). In order to analyze the effects of EpCAM on protein expression levels and posttranslational modifications in 293-ΔEpCAM and 293-EpCAM cells, a proteomics-based analysis was performed using the DIGE-technology. To do

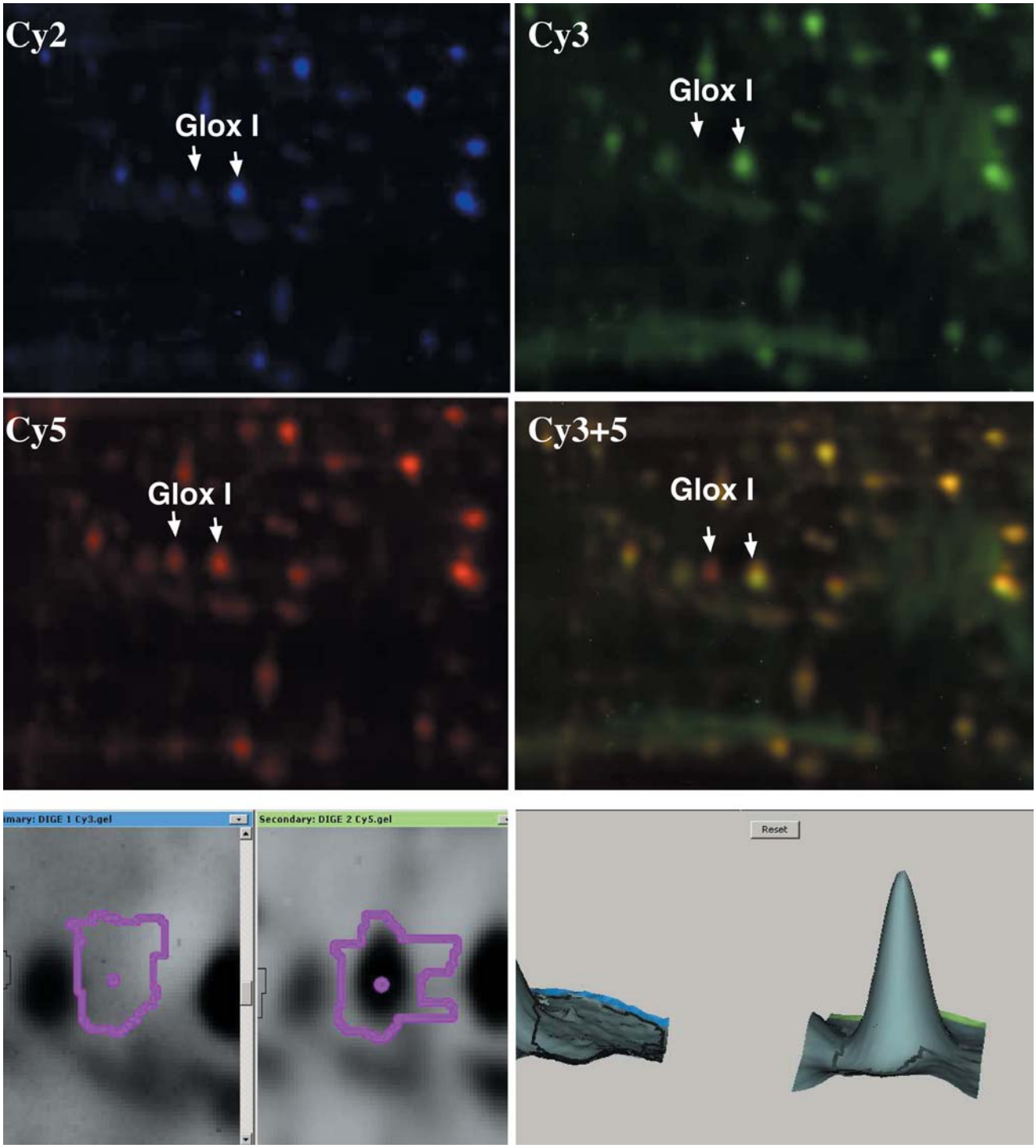

Figure 1. *DIGE analysis of EpCAM-mediated effects in HEK transfectants. 293-ΔEpCAM and 293–EpCAM cells were lysed and labelled with Cy2 (internal standard), Cy3 (293-ΔEpCAM) and Cy5 (293–EpCAM) according to the manufacturer's protocol (see Materials and Methods). Labelled proteins were pooled and separated by 2D-PAGE in a linear range of pH 4-7 and 12% SDS-PAGE. Shown are the representative results of one out of four DIGE gels in single fluorescences and overlay images. The quantification of the protein expression pattern was performed with the DeCyder™ software. Shown is the result for the Glox 1 protein (lower panel).*

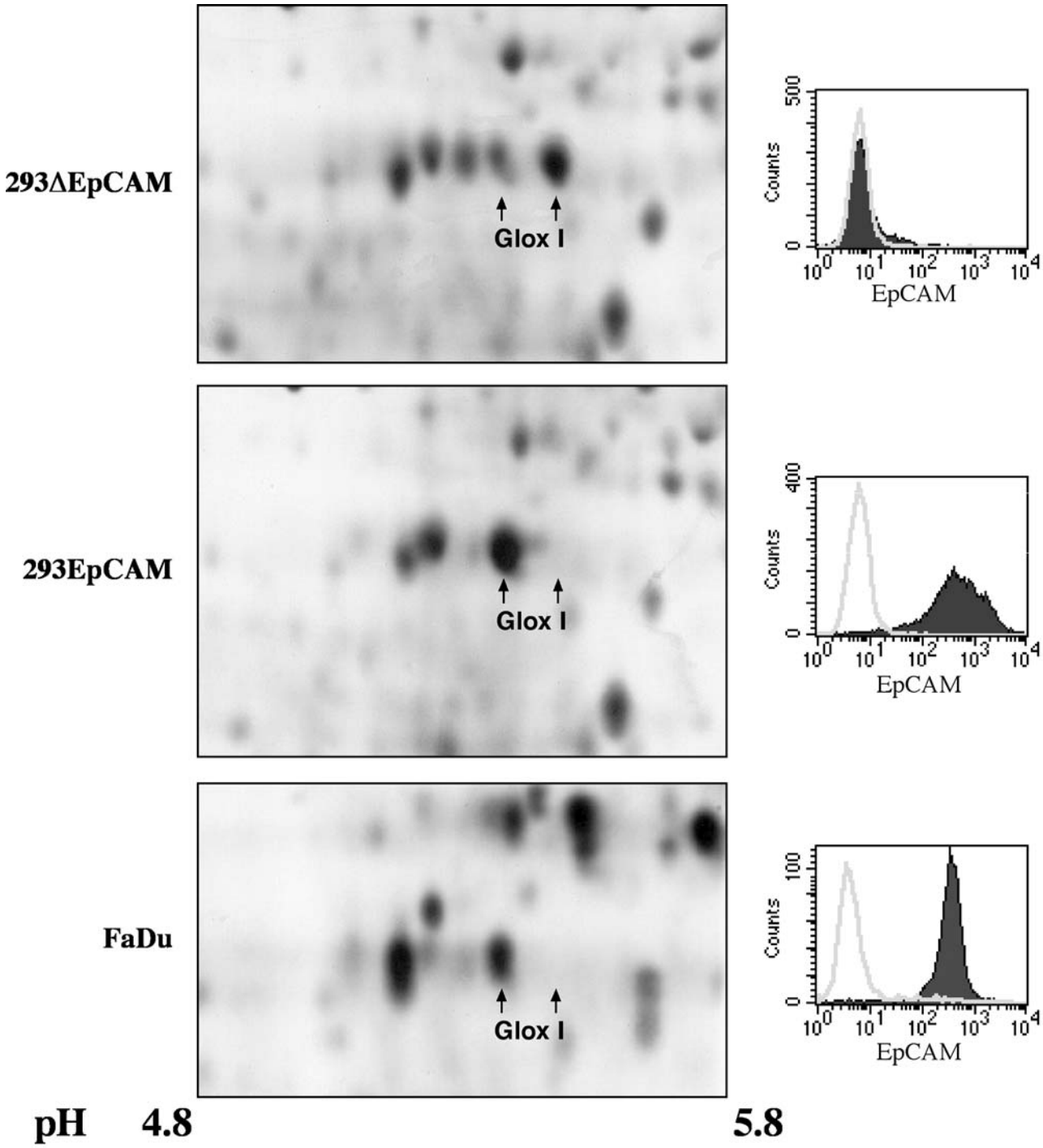

Figure 2. *Two-dimensional analysis of Glox 1 expression in HEK293 transfectants and FaDu carcinoma cells. Protein lysates of the indicated cell lines were separated by 2D-PAGE and stained with silver nitrate. Sections of the resulting gels display different variants of Glox 1 as identified by MALDI-ToF mass spectrometry and Mascot Science database search. The representative cell surface expression of EpCAM, as measured by flow cytometry using specific antibodies, is shown in the right panel.*

so, protein lysates of 293-ΔEpCAM and 293-EpCAM cells were labelled with the fluorescent Dyes Cy3 and Cy5, respectively, and with Cy2 as an internal standard. Labelled samples were pooled and separated subsequently by 2D-PAGE (see Materials and Methods). The quantification of the changes in protein expression was performed with the

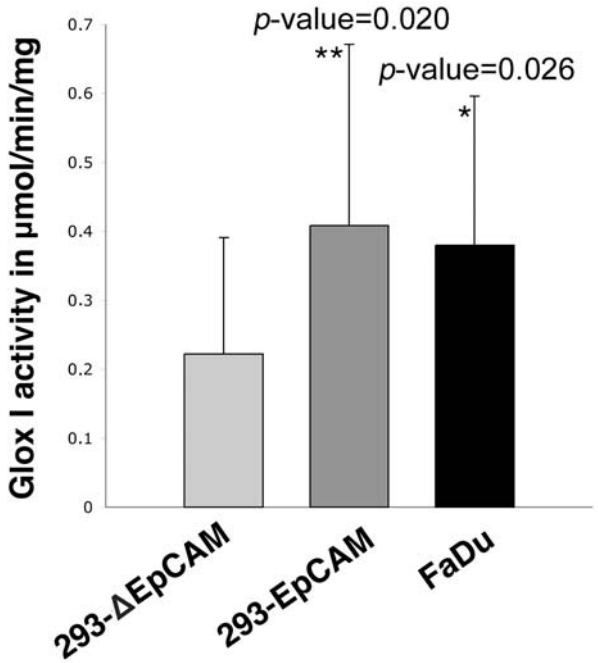

Figure 3. *Enzymatic Activity of Glox 1 in HEK293 transfectants and FaDu carcinoma cells. Protein lysates of the indicated cell lines were generated and the enzymatic activity of Glox 1 determined. Shown are the representative results of three individual experiments with standard deviation and T-test p-values. Results are given in µmol methylglyoxal metabolised per min and mg protein lysate.*

DeCyder™ software. In the present study, we concentrated strictly on proteins regulated with a significance better than $p<0.005$. The corresponding protein spots were excised from the gel and subjected to an in-gel tryptic digest. The resulting tryptic peptides were analyzed by MALDI-ToF mass spectrometry (MS) and the obtained peptide mass fingerprints (PMFs) used to search protein databases at www.matrix-science.com. The analysis yielded five proteins with a regulation ranging from –1.3 to +5.9 and high statistical significance (p-values from $4x10^{-6}$ to 0.005). Two protein spots represented glyoxalase 1, and the others were identified as cytochrome C oxidase polypeptide Vib, retinoic-acid binding protein and peroxiredoxin 6 (Table I).

EpCAM induced a changed migration pattern of glyoxalase 1. As mentioned above, we identified two isoforms of glyoxalase 1 (Glox 1) with a reversed regulation pattern in HEK293 transfectants. In 293-EpCAM cells an acidic form of Glox 1 was significantly up-regulated by a mean factor of 3.92, whereas the basic form was down-regulated by a mean factor of –1.3 as compared with 293-ΔEpCAM cells (Figure 1 and Table I). The metabolic enzyme Glox 1 catalyzes the turn-over of methylglyoxal, which is a cytotoxic by-product of glycolysis, to S-D-lactoyl-glutathione in the presence of glutathione (14). Since EpCAM expression results in an increased metabolism (12), we focussed on the analysis of Glox 1 regulation by EpCAM. To do so, we compared the expression pattern of Glox 1 in HEK293 transfectants and the EpCAM-positive head and neck carcinoma cell line FaDu in standard 2D-PAGE experiments. The comparison of the Glox 1 patterns in these three cell lines showed clear similarities between 293-EpCAM and FaDu cells: the predominant form of Glox 1 in these cells migrated to an estimated pI of 5.0, while the majority of Glox 1 in 293-ΔEpCAM cells migrated to an estimated pI of 5.2 (Figure 2). Additionally, a slight increase in molecular weight was observable for the Glox 1 variant migrating to a pH of 5.0. Thus, the ectopic expression of EpCAM in HEK293 cells, but also the endogenous EpCAM expression in carcinoma cells, resulted in a modified migration property of Glox 1 with respect to the isoelectric point and the molecular weight of this protein.

EpCAM induces Glox 1 resulting in enhanced methylglyoxal turn-over. The observed shift in migration of Glox 1 from pI 5.2 to 5.0 was reported to be associated with an increase in the enzymatic activity of this protein (15). Therefore, we analyzed the enzymatic activity of Glox 1 in 293-ΔEpCAM, 293-EpCAM and FaDu cell lysates. The turn-over rate of methylglyoxal was assessed in the presence of glutathione as the increase of absorbance at 240nm, and expressed as µmol/min/mg protein input. Cells expressing EpCAM (293-EpCAM and FaDu) had a 2-fold increase in Glox 1 activity (Figure 3), clearly correlating with the Glox 1 modifications.

Discussion

The tumor-associated antigen EpCAM is over- or *de novo* expressed in the great majority of carcinomas (1). The number of cells expressing EpCAM, as well as the level of expression, were reported to increase during carcinogenesis (2). Further, EpCAM expression negatively correlates with the clinical prognosis of patients suffering from prostatic cancer (16), breast cancer (17,18), lung cancer (19) and HNSCC (20). Despite the usage of EpCAM in various diagnostic and therapeutic approaches (3-5), its function in the process of carcinogenesis remains somewhat unclear and understanding of the molecular mechanisms is sparse. In order to assess these open questions experimentally, we have established a cellular system for the direct analysis of EpCAM-mediated effects in cells with similar genetic background. This cellular system allowed the demonstration of the direct effects of EpCAM on proliferation and metabolism upon phenotypic and transcriptome analysis. The genes identified included the oncogene *c-myc*, cyclin A and E, whose up-regulation may at least partly explain the enhanced proliferation capacity of

EpCAM–expressing cells (12). Although, the identification of regulated genes using transcriptome analysis is of great value regarding low abundant mRNAs, the knowledge of protein regulation and post-translational modification is just as relevant. Therefore, we complemented our screening procedures using the recently developed proteomics-based DIGE technology. DIGE has several advantages over standard two-dimensional gel electrophoresis: (i) making use of fluorescence-labelled proteins ensures a higher sensitivity and, importantly, a highly improved dynamic range, as compared with standard staining protocols such as Coomassie or silver, (ii) running the samples stained with spectrally resolvable dyes in one single two-dimensional gel electrophoresis avoids changes in the migration pattern of proteins due to gel to gel variation, and (iii) the usage of an internal standard allows, for the first time, a thorough and reliable quantification even of minute changes in protein expression.

Here, we show that the *de novo* expression of EpCAM in epithelial human cells (HEK293) and in head and neck carcinoma cells resulted in a modification of the glycolysis-related enzyme Glox 1. On the one hand this modification leads to an increased negative net charge of the protein, resulting in a shift of the isolelctric point of Glox 1 from pH 5.2 to 5.0. On the other hand, the modified Glox 1 variant experiences a slight increase in molecular weight. Both changes are reminiscent of a phosphorylation of the protein, although this remains to be proven experimentally. Changes in Glox 1 migration properties have been reported by Mitsumoto and colleagues in the context of nitric oxid (NO) signalling in cultured human endothelial cells (15). NO treatment of endothelial cells resulted in a shift of the isoelectric point of Glox 1 from pH 5.0 to 5.2 in the absence of DTT in the lysis buffer used. This shift was attributed to oxidation processes induced by NO and correlated with a decrease in Glox 1 activity. This is in contrast to our own findings, since we observed Glox 1 modifications in the presence of reducing agents, *i.e.* 65mM DTE. Thus, additional mechanisms seem to mesh into these regulatory processes which remain to be described.

The primary task of Glox enzymes (Glox 1 and 2) is the detoxification of methylglyoxal (21). The major source for methylglyoxal in physiological systems is the formation by non-enzymatic and/or enzymatic elimination of phosphate from dihydroxyacetone phosphate und glyceraldehyde phosphate, *via* the phospho-enediolate intermediate during glycolysis (22,23). It modifies guanylate residues in DNA and RNA, targets them for degradation, and induces apoptosis. Furthermore, methylglyoxal modifies arginine residues in proteins, which then undergo receptor-mediated endocytosis and lysosomal degradation in monocytes/macrophages, and induce cytokine synthesis and secretion (24). Detoxification of methylglyoxal is performed by Glox 1 in combination with Glox 2, and represents an important step for carcinoma cells as they display enhanced glycolysis and metabolism, a phenomenon also known as the Warburg effect (25). Enhanced expression of Glox 1 and 2 was reported in different cancer entities, such as bladder carcinomas (26), prostate carcinomas, where it played a role in cell viability (27), in ovarian (28) and breast carcinomas (29), and is often associated with resistance to chemotherapeutica (30). Thus, the inhibition of the Glox enzymatic system has gained renewed interest in the context of antitumoral therapy modalities (31). Interestingly, the induction of plant-derived Glox 1 enzymatic activity was demonstrated to be Ca^{2+}/CaM I-dependent (32). The study of differences induced by EpCAM expression at the transcriptome level revealed the robust up-regulation of CaM I in 293-EpCAM (data not shown). It is therefore tempting to speculate that the observed induction of Glox 1 enzymatic activity in the presence of EpCAM is mediated by the enhanced expression of CaM I in these cells.

Taken together, the results presented identify a novel target of the tumor-associated antigen EpCAM, namely Glox 1, which plays an important role in cellular detoxification, which is crucial in rapidly growing cells. Therefore, these findings give further insights into the molecular basis of EpCAM-mediated effects, such as the enhanced metabolism and proliferation of EpCAM-expressing cells. Additionally, the experiments underline the potential of the DIGE technology to quantify precisely changes in protein levels and protein modifications.

References

1 Balzar M, Winter MJ, de Boer CJ and Litvinov SV: The biology of the 17-1A antigen (Ep-CAM). J Mol Med *77*: 699-712, 1999.

2 Litvinov SV, van Driel W, van Rhijn CM, Bakker HA, van Krieken H, Fleuren GJ and Warnaar SO: Expression of Ep-CAM in cervical squamous epithelia correlates with an increased proliferation and the disappearance of markers for terminal differentiation. Am J Pathol *148*: 865-75, 1996.

3 Riethmüller G, Schneider Gadicke E, Schlimok G, Schmiegel W, Raab R, Hoffken K, Gruber R, Pichlmaier H, Hirche H, Pichlmayr R *et al*: Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. Lancet *343*: 1177-83, 1994.

4 Zeidler R, Reisbach G, Wollenberg B, Lang S, Chaubal S, Schmitt B and Lindhofer H: Simultaneous activation of T cells and accessory cells by a new class of intact bispecific antibody results in efficient tumor cell killing. J Immunol *163*: 1246-52, 1999.

5 Zeidler R, Mysliwietz J, Csanady M, Walz A, Ziegler I, Schmitt B, Wollenberg B and Lindhofer H: The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumour cells. Br J Cancer *83*: 261-6, 2000.

6 Zaloudik J, Li W, Jacob L, Kieny MP, Somasundaram R, Acres B, Song H, Zhang T, Li J and Herlyn D: Inhibition of tumor growth by recombinant vaccinia virus expressing GA733/CO17-1A/EpCAM/KSA/KS1-4 antigen in mice. Cancer Gene Ther *9*: 382-9, 2002.

7 Naundorf S, Preithner S, Mayer P, Lippold S, Wolf A, Hanakam F, Fichtner I, Kufer P, Raum T, Riethmuller G, Baeuerle PA and Dreier T: *In vitro* and *in vivo* activity of MT201, a fully human monoclonal antibody for pancarcinoma treatment. Int J Cancer *100*: 101-10, 2002.
8 Litvinov SV, Balzar M, Winter MJ, Bakker HA, Briaire-de Bruijn IH, Prins F, Fleuren GJ and Warnaar SO: Epithelial cell adhesion molecule (Ep-CAM) modulates cell-cell interactions mediated by classic cadherins. J Cell Biol *139*: 1337-48, 1997.
9 Balzar M, Bakker HA, Briaire-de-Bruijn IH, Fleuren GJ, Warnaar SO and Litvinov SV: Cytoplasmic tail regulates the intercellular adhesion function of the epithelial cell adhesion molecule. Mol Cell Biol *18*: 4833-43, 1998.
10 Meyaard L, van der Vuurst de Vries AR, de Ruiter T, Lanier LL, Phillips JH and Clevers H: The epithelial cellular adhesion molecule (Ep-CAM) is a ligand for the leukocyte-associated immunoglobulin-like receptor (LAIR). J Exp Med *194*: 107-12, 2001.
11 Pauli C, Munz M, Kieu C, Mack B, Breinl P, Wollenberg B, Lang S, Zeidler R and Gires O: Tumor-specific glycosylation of the carcinoma-associated epithelial cell adhesion molecule EpCAM in head and neck carcinomas. Cancer Lett *193*: 25-32, 2003.
12 Münz M, Kieu C, Mack B, Schmitt B, Zeidler R and Gires O: The carcinoma associated antigen EpCAM up-regulates c-Myc and induces cell proliferation. Oncogene in press, 2004.
13 Oray B and Norton SJ: Glyoxalase I from mouse liver. Methods Enzymol *90 Pt E*: 542-6, 1982.
14 Thornalley PJ: The glyoxalase system in health and disease. Mol Aspects Med *14*: 287-371, 1993.
15 Mitsumoto A, Kim KR, Oshima G, Kunimoto M, Okawa K, Iwamatsu A and Nakagawa Y: Glyoxalase I is a novel nitric-oxide-responsive protein. Biochem J *344 Pt 3*: 837-44, 1999.
16 Poczatek RB, Myers RB, Manne U, Oelschlager DK, Weiss HL, Bostwick DG and Grizzle WE: Ep-Cam levels in prostatic adenocarcinoma and prostatic intraepithelial neoplasia. J Urol *162*: 1462-6, 1999.
17 Gastl G, Spizzo G, Obrist P, Dunser M and Mikuz G: Ep-CAM overexpression in breast cancer as a predictor of survival. Lancet *356*: 1981-2, 2000.
18 Spizzo G, Obrist P, Ensinger C, Theurl I, Dunser M, Ramoni A, Gunsilius E, Eibl G, Mikuz G and Gastl G: Prognostic significance of Ep-CAM and Her-2/neu overexpression in invasive breast cancer. Int J Cancer *98*: 883-8, 2002.
19 Kubuschok B, Passlick B, Izbicki JR, Thetter O and Pantel K: Disseminated tumor cells in lymph nodes as a determinant for survival in surgically resected non-small-cell lung cancer. J Clin Oncol *17*: 19-24, 1999.
20 Piyathilake CJ, Frost AR, Weiss H, Manne U, Heimburger DC and Grizzle WE: The expression of Ep-CAM (17-1A) in squamous cell cancers of the lung. Hum Pathol *31*: 482-7, 2000.
21 Thornalley PJ: The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J *269*: 1-11, 1990.
22 Phillips SA and Thornalley PJ: The formation of methylglyoxal from triose phosphates. Investigation using a specific assay for methylglyoxal. Eur J Biochem *212*: 101-5, 1993.
23 Richard JP: Mechanism for the formation of methylglyoxal from triosephosphates. Biochem Soc Trans *21*: 549-53, 1993.
24 Thornalley PJ: Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification--a role in pathogenesis and antiproliferative chemotherapy. Gen Pharmacol *27*: 565-73, 1996.
25 Racker E and Spector M: Warburg effect revisited: merger of biochemistry and molecular biology. Science *213*: 303-7, 1981.
26 Mearini E, Romani R, Mearini L, Antognelli C, Zucchi A, Baroni T, Porena M and Talesa VN: Differing expression of enzymes of the glyoxalase system in superficial and invasive bladder carcinomas. Eur J Cancer *38*: 1946-50, 2002.
27 Davidson SD, Milanesa DM, Mallouh C, Choudhury MS, Tazaki H and Konno S: A possible regulatory role of glyoxalase I in cell viability of human prostate cancer. Urol Res *30*: 116-21, 2002.
28 Jones MB, Krutzsch H, Shu H, Zhao Y, Liotta LA, Kohn EC and Petricoin EF 3rd: Proteomic analysis and identification of new biomarkers and therapeutic targets for invasive ovarian cancer. Proteomics *2*: 76-84, 2002.
29 Rulli A, Carli L, Romani R, Baroni T, Giovannini E, Rosi G and Talesa V: Expression of glyoxalase I and II in normal and breast cancer tissues. Breast Cancer Res Treat *66*: 67-72, 2001.
30 Thornalley PJ: Protecting the genome: defence against nucleotide glycation and emerging role of glyoxalase I overexpression in multidrug resistance in cancer chemotherapy. Biochem Soc Trans *31*: 1372-7, 2003.
31 Creighton DJ, Zheng ZB, Holewinski R, Hamilton DS and Eiseman JL: Glyoxalase I inhibitors in cancer chemotherapy. Biochem Soc Trans *31*: 1378-82, 2003.
32 Deswal R and Sopory SK: Glyoxalase I from Brassica juncea is a calmodulin stimulated protein. Biochim Biophys Acta *1450*: 460-7, 1999.
33 Kim NS, Umezawa Y, Ohmura S and Kato S: Human glyoxalase I. cDNA cloning, expression, and sequence similarity to glyoxalase I from *Pseudomonas putida*. J Biol Chem *268*: 11217-21, 1993.
34 Ranganathan S, Walsh ES, Godwin AK and Tew KD: Cloning and characterization of human colon glyoxalase-I. J Biol Chem *268*: 5661-7, 1993.
35 Carrero-Valenzuela RD, Quan F, Lightowlers R, Kennaway NG, Litt M and Forte M: Human cytochrome c oxidase subunit VIb: characterization and mapping of a multigene family. Gene *102*: 229-36, 1991.
36 Eller MS, Oleksiak MF, McQuaid TJ, McAfee SG and Gilchrest BA: The molecular cloning and expression of two CRABP cDNAs from human skin. Exp Cell Res *198*: 328-36, 1992.
37 Kim TS, Sundaresh CS, Feinstein SI, Dodia C, Skach WR, Jain MK, Nagase T, Seki N, Ishikawa K, Nomura N and Fisher AB: Identification of a human cDNA clone for lysosomal type Ca^{2+}-independent phospholipase A2 and properties of the expressed protein. J Biol Chem *272*: 2542-50, 1997.

Received April 27, 2004
Accepted May 19, 2004