Published in final edited form as:

Biol Chem. 2008 November ; 389(11): 1441–1445. doi:10.1515/BC.2008.157.

Immunohistochemical evidence for ubiquitous distribution of metalloendoprotease insulin-degrading enzyme (IDE; insulysin) in human non-malignant tissues and tumor cell lines

Gregor Weirich¹, Karin Mengele², Christina Yfanti², Apostolos Gkazepis², Daniela Hellmann², Anita Welk², Cecylia Giersig³, Wen-Liang Kuo⁴, Marsha Rich Rosner⁴, Wei-Jen Tang⁴, and Manfred Schmitt^{2,*}

¹Institute of Pathology, Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technical University of Munich, Ismaninger Str. 22, D-81675 Munich, Germany

²Clinical Research Unit, Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technical University of Munich, Ismaninger Str. 22, D-81675 Munich, Germany

³Federal Institute for Drugs and Medical Devices (BfArM), Kurt-Georg-Kiesinger-Allee 3, D-53175 Bonn

⁴Ben May Institute for Cancer Research, Center for Integrative Sciences, 929 East 57th Street, W421, Chicago, IL 60637, USA

Abstract

Immunohistochemical evidence for ubiquitous distribution of metalloprotease insulin-degrading enzyme (IDE; insulysin) in human non-malignant tissues and tumor cells is presented. Immunohistochemical staining was performed on a multi-organ tissue microarray (pancreas, lung, kidney, central/peripheral nervous system, liver, breast, placenta, myocardium, striated muscle, bone marrow, thymus, spleen) and on a cell microarray encompassing 31 tumor cell lines of different origin plus trophoblast cells, and normal blood lymphocytes and granulocytes. IDE protein is expressed by all of the tissues assessed and in all of the tumor cell lines except Raji and HL-60; trophoblast cells and granulocytes but not normal lymphocytes are also IDE-positive.

Keywords

Antibody; immunohistochemistry; insulinase; inverzincin, normal tissue; tumor cell line

Degradation of insulin is mediated by the Zn²⁺-dependent metalloendopeptidase insulin-degrading enzyme (IDE, insulysin, insulinase; EC 3.4.22.11, MW=110 kDa) belonging to the M16A pitrilysin subfamily, although protein disulfide isomerase and cathepsin D are also involved in insulin metabolism (Duckworth et al., 1998; Valera Mora et al., 2003; Barrett and Rawlings, 2004). Increased expression of IDE is correlated with decreased circulating levels of insulin and Aβ peptide, while IDE deficiency is correlated with increased risks of Alzheimer's disease and Type 2 diabetes mellitus (Bertram et al., 2000; Bennett et al., 2003; Sladek et al., 2007).

Although insulin clearance predominantly occurs in the liver and the kidney, other tissues and cells types may participate in insulin degradation, as well, especially striated muscle,

*Corresponding author Prof. Dr. Manfred Schmitt, Clinical Research Unit, Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technical University of Munich, Ismaninger Str. 22, D-81675 Munich, Germany. manfred.schmitt@lrz.tum.de.

adipocytes, gastrointestinal epithelia, fibroblasts, monocytes and lymphocytes (Valera Mora et al., 2003). Apart from insulin, other growth factors were also found to be substrates for IDE such as transforming growth factor α (Gehm et al. 1991) conveying to IDE the potential of a ubiquitous growth factor degrader.

IDE is ubiquitously expressed (www.genecards.org/cgi-bin/carddisp.pl?gene=IDE) and present in bacteria, fungi, animals, plants, and human beings (Kuo et al., 1993; Adames et al., 1995; Becker and Roth, 1995; Bertram et al., 2000; Bennett 2003; Sladek et al., 2007; also see distribution within family M16.002: insulysin at <http://merops.sanger.ac.uk/>). Kuo et al. (1993) examined presence of IDE in rat tissues at the gene and protein level; expression was demonstrated in the testis, tongue, brain, kidney, prostate, heart, muscle, liver, intestine, skin, spleen, lung, thymus, and uterus. Less, however, is known about distribution of IDE protein in human tissues which was demonstrated to be present in the liver, adipocytes (Valera Mora et al., 2003), muscle cells (Neal and Kitabchi, 1982), erythrocytes (Shii et al., 1986), breast and kidney (Duckworth 1998; Radulescu et al., 2007).

Besides its predominant presence in the cytosol (Authier et al., 1996; Chang et al., 1997), IDE is also found in small but significant amounts in subcellular compartments of cultured cells, e.g. on the plasma membrane (Hersh, 2006), in endosomes (Hamel et al., 1991), peroxisomes (Authier et al., 1995), and mitochondria (Leissring et al., 2004). So far, human IDE expression and function have been studied mainly in the context of diabetes mellitus and Alzheimer's disease (Farris et al., 2005), yet not with respect to cancer, except for some reports on IDE presence in tumor cell lines (Harada et al., 1993; Authier et al., 1996; Melino et al., 1996; Chang et al., 1997).

We recently, for the first time, explored protein expression of IDE in human normal and neoplastic breast tissues by immunohistochemistry (Radulescu et al. 2007) and reported that IDE expression is elevated in human breast cancer tissues compared to normal breast glands. Furthermore, we detected insulin protein in high-grade breast cancer employing an immunoassay, a result that is in line with early findings by other groups (Castro et al., 1980; Spring-Mills et al., 1984).

To better understand whether IDE acts as a local or a global player in human tissues, we now assessed IDE protein expression patterns in normal human cells and tissues and in cultured tumor cells by applying the IDE-directed antibody UCG 43/6. This polyclonal antibody was generated by injecting rabbits with recombinant human wild type IDE tagged with six histidine residues at the N-terminus, as outlined by Chesneau et al., 2000, and Shen et al., 2006. The antibody was applied to normal tissues, normal cells and tumor cells; for this purpose, tissue microarrays and cell microarrays were prepared encompassing 13 normal tissues (Table 1, Figure 1–3), several normal cells and 31 tumor cell lines (Table 2, Figure 4–6).

IDE was detected in the cytoplasm of a wide range of cells within the 13 normal human tissues investigated (Table 1, Figure 1–3), matching with findings published for rat IDE by Kuo et al. (1993). For instance, moderate to strong cytoplasmic expression of IDE was observed in hepatocytes and renal tubule cells whereas glomeruli did not stain with the IDE-directed antibody. This fact is not surprising since both organs are known for their high insulin degradation rates (Valera Mora et al, 2003). Although in most of the tissues investigated IDE was distributed homogeneously within the cells, striated muscle and cardiomyocytes of the heart displayed intense cytoplasmic but subcellular compartmentalization of IDE expression (Figure 3). In this context it is worth mentioning that insulin can be cleared by muscle cells which contain insulin receptors and regulation mechanism for insulin internalization and metabolism (Valera Mora et al., 2003).

The observed tissue-wide distribution of IDE protein does point to the fact that a wide range of tissues may be capable of insulin uptake and degradation, a fact that pinpoints the essential metabolic role of insulin and its control by insulin degrading enzymes such as IDE (Hamel et al., 1991, Fawcett et al., 2007). On the background of the ubiquitous IDE expression in normal tissues and cells it is not surprising that 29 of the 31 human tumor cell lines tested exhibited some degree of cytosolic IDE protein expression (Table 2, Figure 4–6) whereas Raji lymphoma cells and HL-60 leukemia cells did not stain with the IDE-directed antibody. Peripheral blood lymphocytes were negative as well. In addition, we confirm the findings of Chang et al. (1997) who reported expression of IDE protein by the colon cancer cell line Caco-2. Interestingly, the breast cancer cell lines investigated showed moderate to strong IDE expression, a finding linked to results obtained by immunostaining of high-grade breast cancer specimens for presence of IDE protein (Radulescu et al., 2007).

Since insulin is widespread in the human organism by binding to receptors on almost every cell type, it is not surprising that IDE, the most important enzyme to metabolize insulin and an important degrader of other small proteins, is detected in our study in almost all tissues and cell types matching up with IDE RNA expression data supplied by Su et al. (2004) as part of the gene atlas (symatlas) of the human encoding transcriptome.

Clinical and epidemiological studies have shown that type 2 diabetes, and hyperinsulinaemia, increased the risk of developing Alzheimer's disease in the elderly. The link between hyperinsulinemia and Alzheimer's disease may be IDE (Qiu and Folstein, 2006). Besides insulin, IDE also degrades amylin, peptides related to the pathology of type 2 diabetes, along with amyloid- β peptide, which is found in the brain of Alzheimer patients (Qiu and Folstein, 2006). Furthermore, functional *in vitro* studies have shown that IDE and insulin are key regulators of the proteasome, a ubiquitous protein complex meant for degradation of unneeded or damaged proteins by proteolysis. In this respect, as a consequence of insulin-dependent diabetes mellitus, the loss of lean muscle mass is a result of accelerated proteolysis by the proteasome since insulin inhibits protein degradation by interaction with IDE (Bennett et al., 2003; Fawcett et al., 2007). On the other hand, the regulation of IDE expression is not fully elucidated but ATP seems to act as a negative regulator of IDE (Camberos et al. 2001). While studies of the role of IDE in the pathogenesis of diabetes mellitus and Alzheimer's disease are in focus of ongoing clinical research, such studies have not been reported for the role of IDE in malignancy yet.

Acknowledgments

This study was supported in part by a grant from the Federal Institute for Drugs and Medical Devices, Bonn, Germany.

References

1. Adames N, Blundell K, Ashby MN, Boone C. Role of yeast insulin-degrading enzyme homologs in propheromone processing and bud site selection. *Science*. 1995; 270(5235):46446–46447.
2. Authier F, Bergeron JJ, Ou WJ, Rachubinski RA, Posner BI, Walton PA. Degradation of the cleaved leader peptide of thiolase by a peroxisomal proteinase. *Proc. Natl. Acad. Sci. USA*. 1995; 92:3859–3863. [PubMed: 7731996]
3. Authier F, Cameron PH, Taupin V. Association of insulin-degrading enzyme with a 70 kDa cytosolic protein in hepatoma cells. *Biochem. J*. 1996; 319(Pt 1):149–158. [PubMed: 8870662]
4. Barrett, AJ.; Rawlings, ND. Introduction: metallopeptidases and their clans. In: Barrett, AJ.; Rawlings, ND.; Woessner, JF., editors. *Handbook of Proteolytic Enzymes*. London: Elsevier Academic Press; 2004. p. 231–268.
5. Becker AB, Roth RA. Insulysin and pitrilysin: insulin-degrading enzymes of mammals and bacteria. *Methods Enzymol*. 1995; 248:693–703. [PubMed: 7674956]

6. Bennett RG, Fawcett J, Kruer MC, Duckworth WC, Hamel FG. Insulin inhibition of the proteasome is dependent on degradation of insulin by insulin-degrading enzyme. *J. Endocrinol.* 2003; 177:399–405. [PubMed: 12773120]
7. Bertram L, Blacker D, Mullin K, Keeney D, Jones J, Basu S, Yhu S, McInnis MG, Go RC, Vekrellis K, et al. Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science.* 2000; 290:2302–2303. [PubMed: 11125142]
8. Camberos MC, Pérez AA, Udrișar DP, Wanderley MI, Cresto JC. ATP inhibits insulin-degrading enzyme activity. *Exp. Biol. Med.* 2001; 226:334–341.
9. Castro A, Ziegels-Weissman J, Buschbaum P, Voigt W, Morales A, Nadji M. Immunochemical demonstration of immunoreactive insulin in human breast cancer. *Res. Commun. Chem. Pathol. Pharmacol.* 1980; 29:171–182. [PubMed: 6996061]
10. Chang LL, Stout LE, Wong WD, Buls JG, Rothenberger DA, Shier WT, Sorenson RL, Bai JP. Immunohistochemical localization of insulin-degrading enzyme along the rat intestine, in the human colon adenocarcinoma cell line (Caco-2), and in human ileum. *J. Pharm. Sci.* 1997; 86:116–119. [PubMed: 9002470]
11. Chesneau V, Rosner MR. Functional human insulin-degrading enzyme can be expressed in bacteria. *Protein Expr Purif.* 2000; 19:91–98. [PubMed: 10833395]
12. Duckworth WC, Bennett RG, Hamel FG. Insulin Degradation: Progress and Potential. *Endocr. Rev.* 1998; 19:608–624. [PubMed: 9793760]
13. Farris W, Leissring MA, Hemming ML, Chang AY, Selkoe DJ. Alternative splicing of human insulin-degrading enzyme yields a novel isoform with a decreased ability to degrade insulin and amyloid beta-protein. *Biochemistry.* 2005; 44:6513–6525. [PubMed: 15850385]
14. Fawcett J, Permana PA, Levy JL, Duckworth WC. Regulation of protein degradation by insulin-degrading enzyme: analysis by small interfering RNA-mediated gene silencing. *Arch. Biochem. Biophys.* 2007; 468:128–133. [PubMed: 17964527]
15. Gehm BD, Rosner MR. Regulation of insulin., epidermal growth factor., transforming growth factor- α levels by growth factor-degrading enzymes. *Endocrinology.* 1991; 128:1603–1610. [PubMed: 1847863]
16. Hamel FG, Mahoney MJ, Duckworth WC. Degradation of intraendosomal insulin by insulin degrading enzyme without acidification. *Diabetes.* 1991; 40:436–443. [PubMed: 2010043]
17. Harada S, Smith RM, Smith JA, Jarett L. Inhibition of insulin-degrading enzyme increases translocation of insulin to the nucleus in H35 rat hepatoma cells- evidence of a cytosolic pathway. *Endocrinology.* 1993; 132:2293–2298. [PubMed: 8504733]
18. Hersh LB. The insulysin (insulin degrading enzyme) enigma. *Cell. Mol. Life Sci.* 2006; 63:2432–2434. [PubMed: 16952049]
19. Kuo WL, Montag AG, Rosner MR. Insulin-degrading enzyme is differentially expressed and developmentally regulated in various rat tissues. *Endocrinology.* 1993; 132:604–611. [PubMed: 7678795]
20. Leissring MA, Farris W, Wu X, Christodoulou DC, Haigis MC, Guarente L, Selkoe DJ. Alternative translation initiation generates a novel isoform of insulin-degrading enzyme targeted to mitochondria. *Biochem. J.* 2004; 383(Pt. 3):439–446. [PubMed: 15285718]
21. Melino G, Draoui M, Bernardini S, Bellincampi L, Reichert U, Cohen P. Regulation by retinoic acid of insulin-degrading enzyme and of a related endoprotease in human neuroblastoma cell lines. *Cell Growth Differ.* 1996; 7:787–796. [PubMed: 8780892]
22. Neal GW, Kitabchi AE. Insulin degradation by human skeletal muscle. *Biochim. Biophys. Acta.* 1982; 719:259–266. [PubMed: 6758860]
23. Qiu WQ, Folstein MF. Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. *Neurobiol. Aging.* 2006; 27:190–198. [PubMed: 16399206]
24. Radulescu RT, Hufnagel C, Luppa P, Hellebrand H, Kuo WL, Rosner MR, Harbeck N, Giersig C, Meindl A, Schmitt M, Weirich G. Immunohistochemical demonstration of the zinc metalloprotease insulin-degrading enzyme in normal and malignant human breast: Correlation with tissue insulin levels. *Int. J. Oncol.* 2007; 30:73–80. [PubMed: 17143514]
25. Shen Y, Joachimiak A, Rosner MR, Tang WJ. Structures of human insulin-degrading enzyme reveal a new substrate recognition mechanism. *Nature.* 2006; 443:870–874. [PubMed: 17051221]

26. Shii K, Yokono K, Baba S, Roth RA. Purification and characterization of insulin-degrading enzyme from human erythrocytes. *Diabetes*. 1986; 35:675–683. [PubMed: 3519322]
27. Simon R, Mirlacher M, Sauter G. Tissue microarrays in cancer diagnosis. *Expert Rev. Mol. Diagn.* 2003; 3:421–430. [PubMed: 12877382]
28. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*. 2007; 445(7130):881–885. [PubMed: 17293876]
29. Spring-Mills EJ, Stearns SB, Numann PJ, Smith PH. Immunocytochemical localization of insulin- and somatostatin-like material in human breast tumors. *Life Sci.* 1984; 35:185–190. [PubMed: 6376992]
30. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc. Natl. Acad. Sci. USA*. 2004; 101:6062–6067. [PubMed: 15075390]
31. Valera Mora ME, Scarfone A, Calvani M, Greco AV, Mingrone G. Insulin clearance in obesity. *J. Am. Coll. Nutr.* 2003; 22:487–493. [PubMed: 14684753]

Figure 1.

Immunohistochemical analysis of normal human tissues employing rabbit polyclonal antibody UCG 43/6 directed to recombinant full-length IDE (Shen et al., 2006), stained in red. Nuclei are counterstained with hematoxylin (blue color). Microscopic magnification: $\times 400$. Staining intensity: **A) Liver**: strong immunoreactivity (3+) present in hepatocytes. **B) Kidney**: strong immunoreactivity (3+) present in tubular cells surrounding a negative glomerulum (0). **C) Pancreas**: moderate staining (2+) present in islet cells (left). Strong staining (3+) is observed in acinar cells; no staining (0) is seen in the central ductal epithelia of the pancreas (right). **D) Lung**: respiratory epithelia show strong staining (3+, left). Alveolar macrophages show strong staining (3+) as well; pneumocytes exhibit a moderate staining (2+, right). Immunoreactive score: 0 = negative, 1+ = weak, 2+ = moderate, 3+ = strong.

Figure 2. Immunohistochemical analysis of normal human tissues employing rabbit polyclonal antibody UCG 43/6 directed to full-length IDE, stained in red. Nuclei are counterstained with hematoxylin (blue color). Microscopic magnification: $\times 400$. Staining intensity: **A)** Breast: moderate but heterogeneous immunoreactivity (2+) present in duct epithelia of the mammary gland. **B)** Placenta: moderate but heterogeneous immunoreactivity (2+) present in trophoblast cells. **C)** Bone marrow: strong staining (3+) present in hematopoietic cells. **D)** Thymus: weak staining (1+) present in lymphocytes and epithelial cells.

Figure 3.

Immunohistochemical analysis of normal human tissues employing rabbit polyclonal antibody UCG 43/6 directed to full-length IDE, stained in red. Nuclei are counterstained with hematoxylin (blue color). Microscopic magnification: $\times 400$. Staining intensity: **A)** Striated muscle: Weak immunoreactivity (1+). **B)** Cardiomyocytes of the heart: Moderate immunoreactivity (2+). **C)** Nerve ganglions of the peripheral nervous system: Moderate immunoreactivity (2+). **D)** Peripheral blood granulocytes: Moderate immunoreactivity (2+).

Figure 4.
Immunocytochemical analysis of tumor cell lines employing polyclonal rabbit antibody UCG 43/6 directed to full-length IDE, stained in red. Nuclei are counterstained with hematoxylin (blue color). Microscopic magnification: $\times 400$. Staining intensity: **A)** Breast: SK-BR-3 (3+), **B)** Breast: MDA-MB-435 (2+), **C)** Ovary: OVCAR3 (2+), **D)** Ovary: OVMZ10 (2+).

Figure 5.
Immunocytochemical analysis of tumor cell lines employing polyclonal rabbit antibody UCG 43/6 directed to full-length IDE, stained in red. Nuclei are counterstained with hematoxylin (blue color). Microscopic magnification: $\times 400$. Staining intensity: **A)** Bladder: EJ 28 (2+), **B)** Prostate: LNCaP (2+), **C)** Colon: SW480 (1+), **D)** Pancreas: MIA PaCa-2 (2+).

Figure 6.
Immunocytochemical analysis of tumor cell lines employing polyclonal rabbit antibody UCG 43/6 directed to full-length IDE, stained in red. Nuclei are counterstained with hematoxylin (blue color). Microscopic magnification:×400. Staining intensity: **A) Oral cavity:** BHY (2+), **B) Muscle:** HT-1080 (2+), **C) Brain:** SNB19 (3+), **D) Bone:** Saos-2 (1+).

Table 1

IDE reactivity scores.

organ	IDE reactivity
Pancreas: islet cells	2+
Pancreas: acinar cells	3+
Pancreas: ductal epithelia	0
Lung: respiratory epithelia	3+
Lung: alveolar macropages	3+
Lung: pneumocytes	2+
kidney: tubler cells	3+
kidney: glomerulum	0
Peripheral nervous system	2+
Central nervous system	1+
Liver	3+
Breast,female	2+
Placenta	2+
Myocardium	2+
Striated muscle	1+
Bone marrow	3+
Thymus	1+
Spleen	1+

Tissue microarrays were constructed using a 1 mm punch-core needle instrument (MTA I Personal Tissue Arrayer, Beecher Instruments, Sun Prairie, USA) as previously described (Simon et al., 2003). To compensate for individual differences in tissue heterogeneity and staining intensity, specimens from three different individuals were sampled per organ. Tissue microarrays were deparaffinized and hydrated by passing them through xylene and a graded ethanol series. Antigen retrieval was performed by boiling the slides in 10 mM citrate buffer, pH 6.0 for 4 min. Endogenous peroxidase activity was quenched by incubating the sections with 3% H₂O₂ in distilled H₂O for 20 min at room temperature (RT). Sections were incubated overnight at 4°C with 5 µl rabbit polyclonal antibody UCG 43/6 against recombinant full-length human IDE (Shen et al., 2006) per ml of antibody diluent S2022 (Dako, Hamburg, Germany), washed with TBS and then peroxidase-labeled mouse-anti-rabbit IgG (Jackson ImmunoResearch, Dianova, Hamburg, Germany) was added (30 min, RT). Control tissue microarrays contained no primary antibody. Sections were then washed with TBS, followed by addition of peroxidase substrate 3-amino-9-ethylcarbazole (K3464, Dako). Stained sections were photographed using a SONY 3CCD color video camera attached to a Zeiss (Göttingen, Germany) Axioplan 2 microscope using Zeiss AxioVision software 4.5 SP1. A four-grade scoring system was used to assess the intensity of immunoreactivity: 0, negative; 1+, weak; 2+, moderate; and 3+, strong. Punch core biopsies of normal human kidney known to express IDE protein (Su et al., 2004) were used as an internal positive control; for negative controls the primary antibody was omitted.

Table 2

Cell lines used in this study.

Cell line	Origin	Supplier	IDE reactivity
SK-BR-3	Breast ductal carcinoma(pleural effusion)	ATCC, Manassas, USA	3+
BT-20	Breast cancer	ATCC	2+
MCF7	Breast ductal carcinoma(pleural effusion)	ATCC	2+
MBA-MB-435	Breast ductal carcinoma(pleural effusion)	ATCC	2+
ZR-75-1	Breast ductal carcinoma(ascites)	ATCC	1+
OVCAR-3	Ovarian adenocarcinoma	ATCC	2+
OVMZ6	Ovarian cancer	V. Moebus, Frankfurt, Germany	1+
OVMZ10	Ovarian cancer	V. Moebus	2+
OVMZ15	Ovarian cancer	V. Moebus	1+
OVMZ19	Ovarian cancer	V. Moebus	1+
HeLa	Cervix carcinoma	ATCC	1+
EJ 28	Bladder cancer	A. Lehmer, Munich, Germany	2+
RT-112	Urinary bladder transitional cell carcinoma	DSMZ, Braunschweig, Germany	2+
Caki-1	Kidney carcinoma(clear all)	CLS, Eppelheim, Germany	2+
DU 145	Prostate cancer (brain metastasis)	ATCC	2+
LNCaP	Prostate adenocarcinoma	ATCC	2+
PC-3	Prostate adenocarcinoma	ATCC	1+
Caco-2	Colorectal adenocarcinoma	ATCC	1+
SW480	Colorectal adenocarcinoma	ATCC	1+
MIA PaCa-2	Pancreatic cancer	ATCC	2+
PaTu-II	Pancreatic cancer	D. Saur, Munich, Germany	1+
CAL 27	Squamous cell carcinoma, tongue	DSMZ	1+
BHY	Squamous carcinoma, oral	DSMZ	2+
FaDu	Squamous carcinoma, pharynx	ATCC	1+
U-2OS	Osteosarcoma	ATCC	1+
Saos-2	Osteosarcoma	ATCC	1+
HT-1080	Fibrosarcoma	ATCC	2+
SNB19	Glioma	ATCC	3+
Raji	Burkitt's lymphoma	ATCC	0
U-937	Histiocytic lymphoma	ATCC	1+
HL-60	Acute promyelocytic leukemia	ATCC	0
Granulocytes	Normal blood	TUM ^a , Munich, Germany	2+
Lymphocytes	Normal blood	TUM	0
Trophoblast cells	Normal placenta	TUM	2+

All cell lines were cultivated in DMEM-supplemented with 10% fetal calf serum, 0.2% arginine/asparagine and 1% HEPES or RPMI supplemented with 10% fetal calf serum and 1% glutamine. For the construction of cell microarrays, cultured cells (3×10^7) were fixed with 5 ml of 10% formalin in phosphate-buffered saline (PBS) for 30 min at 20°C, centrifuged (800 g, 5 min, 20°C), washed with Tris-buffered saline at 20°C, then with PBS, and centrifuged again. Subsequently, 150 µl thrombin (10 U/ml in H₂O; Sigma-Aldrich, Munich, Germany), 750 µl casein (Sigma-Aldrich; 10 mg/ml 0.04 M Tris-HCl, pH 8.0) and 600 µl fibrinogen (25 mg/ml H₂O; Sigma-Aldrich), were added to the cell pellet and left overnight at 4°C to solidify. Cell clots were fixed in 4% formalin in PBS, embedded, and paraffinized according to standard procedures. Since cells are

formalin-fixed and paraffin-embedded, the procedure for construction of cell microarrays was identical to that for tissue microarrays (see legend to Table 1). Cell microarrays were stained for IDE protein expression using polyclonal rabbit antibody UCG 43/6 and a streptavidin-biotin detection kit (LSAB, K5003; Dako) as described by Radulescu et al. (2007). Punch cores of a cell clot of the colon cancer cell line Caco-2 known to express IDE protein (Chang et al., 1997) were used as an internal positive control; for negative controls the primary antibody was omitted.

^aTUM, Department of Obstetrics and Gynecology, Technical University of Munich.