ANTICANCER RESEARCH *34*: 1079-1086 (2014)

Review

Glycodelin A – A Famous Lipocalin and its Role in Breast Cancer

ALEXANDRA C. KÖLBL, UDO JESCHKE, DARIUS DIAN, KLAUS FRIESE and ULRICH ANDERGASSEN

Department of Obstetrics and Gynaecology, Ludwig Maximilian University of Munich, Munich, Germany

Abstract. *Lipocalins are a large protein family with only little sequence homology but highly conserved structural similarity. Many lipocalins play crucial roles in the generation of epithelial cancer, influencing pathways which regulate cell motility, cell differentiation and neovascularisation. Thereby they can be used as biomarkers of cancer, in most cases for a rather good prognosis. Glycodelin is a lipocalin existing in three isoforms which differ only by glycosylation, but which have different functions. In breast cancer, glycodelin A is known to contribute to a more differentiated cell morphology and is a biomarker for a favourable prognosis, but also plays a role in angiogenesis. Glycodelin A is a useful prognostic marker as it can be detected in serum samples, but is also a target for therapeutical interventions.*

The Lipocalin Family – Structure and Function

Lipocalins are small secreted proteins, belonging to the calycin-protein family and can be found in nearly all organisms from eubacteria to eukaryotic cells (1). Interestingly the members of the lipocalin family exhibit only little sequence identity. They are classified as 'kernel' lipocalins, sharing three conserved regions, or as 'outlier' lipocalins, sharing less than these three conserved regions (2). Nevertheless, they are characterized by a high structural similarity, consisting of eight anti-parallel β-sheets connected by seven short loops, thereby forming a cup-like structure with loop 1 as a lid (3). Lipocalins bind lipophilic molecules within this cup-like structure; binding specifity is thereby influenced by cup size and conformation, as well as amino acid composition. Apart from molecular binding and transport, a variety of functions of the different members of the lipocalin family are known: binding of cell surface receptors, complex formation, invertebrate coloration, olfaction and pheromone transport, prostaglandin synthesis, regulation of cell homeostasis and modulation of immune response (3).

Correspondence to: Professor Dr. Udo Jeschke, Department of Obstetrics and Gynaecology, Ludwig Maximilian University of Munich, Maistrasse 11, D-80337 Munich, Germany. Tel: +49 8951604266, Fax: +49 8951604916, e-mail: udo.jeschke@med.uni-muenchen.de

Key Words: Glycodelin, lipocalin, immunosuppression, breast cancer, review.

Glycodelin

General facts. The human glycodelin gene Human Genome Organisation (HUGO) gene symbol: *PAEP,* progesterone-associated endometrial protein (4); is located on chromosome 9q34 (5), in a region which comprises of many other genes of the lipocalin family (6-8). The gene consists of seven exons and six introns (9). From the primary gene sequence, a 180-amino-acid protein is made, which includes an 18-amino-acid signal sequence and three *N*-glycosylation sites at Asn 28, 63 and 85 (10). As these glycosylation sites can be differentially glycosylated, influencing functions of the molecule, it was called glycodelin (10-12), although many different names exist for the protein, depending on the tissue in which it was described (10, 13-17). Glycodelin, which is the major lipocalin of the reproductive axis, involved in cell recognition and differentiation (18), shows high structural similarity to β-lactoglobin, the major constituent of whey (19, 20), and has a retinol-binding motif (21), although glycodelin was never found to bind retinol (22).

Glycodelin isoforms and non-oncogenetic functions. Three isoforms of glycodelin are described in the literature, differing only in glycosylation patterns and in their function (23). An important point is that different glycosylation does not influence protein folding (22).

Glycodelins are synthesized in glandular epithelial cells of the secretory endometrium (24), in the Fallopian tubes (10, 25), in the seminal gland and ampulla of the ductus deferens

(23, 26), in ovarian tumors and healthy ovaries (27, 28), in bone marrow (16), and in healthy breast tissue and breast tumors (29). It can also be detected in the epithelial cells of the umbilical cord vein (30).

First of all Glycodelin A is found in the amniotic fluid, the endometrium, the decidua and the serum of pregnant women (22). Glycodelin A has a molecular weight of 18.78 kDa determined from the cDNA sequence (20), but the molecular mass determined by gel electrophoresis is significantly higher (28 kDa) due to glycosylation (31), as carbohydrates account for 17.5% of the protein (14). Its expression is stimulated by progesterone (18). Glycodelin A exhibits anti-fertilisation activity by binding spermatozoa and thereby inhibiting the binding of the sperm to the *zona pellucida* of the oocyte (32). Binding of the sperm to the oocyte is only possible during the so called 'fertilisation window', in which the absence of glycodelin A is regulated by oestrogen. While the anti-fertilisation activity is glycosylation-dependent (33), its immunosuppressive activity is not dependent on the state of glycosylation of the molecule. The role of glycodelin A in the regulation of the immune system is inhibition of lymphocyte proliferation (34), natural killer cell cytotoxicity (35), T-cell proliferation and Th1-cytokine response (36), induction of T-cell apoptosis (37) and even regulation of B-cell response (38) by binding the human B-cell receptor CD22 (39). Furthermore, glycodelin A blocks E-selectin-mediated cell adhesion *in vitro* much more strongly than sialyl-Lewis^x (40). An intriguing fact is that the inhibition of natural killer cell activity by glycodelin A permits the implantation of the embryo in the maternal placenta by counteracting the maternal defence against implantation. Glycodelin A is therefore found to be increased during the implantation phase (10, 41). In other words, glycodelin A is expressed during the first two to three days post-menstruation by the glandular cells of the endometrium, then, around the time of ovulation, glycodelin is no longer detectable (42, 43); expression returns around the fifth post-ovulatory day (44-46). In the case of pregnancy, glycodelin A secretion increases profoundly (13, 34, 47); post-menopausally, only very low glycodelin A levels are measured (48, 49).

Glycodelin F, which is mainly expressed in the ovary, and synthesised in the granulosa cells (50), has a function in principle similar to that of glycodelin A (51). It also binds the sperm head, thereby inhibiting acrosome reaction and sperm-egg binding. Upon de-glycosylation, glycodelin F dissociates from the sperm and sperm-egg binding is possible. The de-glycosylation takes place during the passage of the sperm through the corona cell layer. Glycodelin F is thereby important to prevent a premature acrosome reaction.

Glycodelin S is mainly found in the seminal fluid and is differentially glycosylated from glycodelin A, being rich in fucose, with Lewis^x/Lewis^y antennae (33). It does not inhibit sperm-egg binding, although it also has two binding sites on spermatozoa (52, 53). The binding of glycodelin S is not in competition with glycodelin A/F. Glycodelin S reduces cholesterol efflux from spermatozoa, thereby suppressing capacitation. During the passage of the sperm through the cervix (52), glycodelin S is de-glycosylated and dissociates from the sperm, allowing the sperm to mature.

Glycodelins in cancer. Expression of glycodelins was found in some types of breast cancer (16), ovarian serous carcinoma (54) and bi-phasic synovial carcinoma (55). The presence of glycodelin in cancerous tissue is mostly linked to a better prognosis than is attributed to glycodelin-negative tissue (54), as glycodelin is a protein typical of differentiated tissue. Furthermore, experiments demonstrated that the presence of glycodelin reduces cell proliferation and reverses malignant features (18, 29, 56, 57). *In vitro* as well as *in vivo* models of tumorigenesis found that glycodelin up-regulates the expression of tumour-suppressor genes, while it reduces the expression of oncogenes, thus it could be concluded that glycodelin is itself a tumor-suppressor (58). Furthermore expression of glycodelin correlates with tumour grading, meaning it is reduced in G2/FIGO (International Federation of Gynecology and Obstetrics) III-IV tumours (59). On the other hand, there are indications that glycodelin might play a role in neovascularisation, thereby promoting tumour growth (60).

Role of Glycodelin A in Breast Cancer

When it became known that glycodelin A was expressed in tissues outside the reproductive tract, for example in normal and neoplastic glandular epithelia of the breast, its role in these tissues was studied. As glycodelin A was found to be localized in highly differentiated acinar epithelia and a role in organization of epithelial tissue was presumed. To further clarify this finding, cDNA of glycodelin A was transfected into MCF-7 cells (29). After transfection, MCF-7 cells showed an altered growth behaviour, including the formation of acinar configurations, growth instability in semisolid media because of apoptosis, expression of markers of organized epithelia such as cytokeratins (CK) 8 and 18, and E-cadherin, changes in the intracellular distribution of β-catenin and decreased proliferation; after transfection with glycodelin cDNA the cells exhibit a phenotype of organized epithelium (29). The findings of Hautala *et al*. fit these results: They introduced MCF-7 cells transfected with glycodelin cDNA into mouse mammary fat pads. The result was a formation of smaller tumors with a more differentiated phenotype in comparison to implantation of non-transfected MCF-7 cells (61). It was concluded that glycodelin induces differentiation, reduces the expression of oncogenes, and similarly increases the expression of tumor-suppressor genes, thereby contributing to a more favourable prognosis (58).

Nevertheless, these results obtained in model systems have to be treated with caution. It was recently shown by tissue microarray experiments analyzing gene expression that glycodelin expression is associated with a low proliferation rate and well-differentiated cell forms in sporadic cases of breast cancer. In contrast, in familial non-BRCA (breast cancer associated genes)1/2 tumours, glycodelin expression is linked to a worse prognosis, with positive lymph nodal state, expression of human epidermal growth factor receptor 2 (HER2) and increased risk for distant metastasis. There seem to be different gene expression profiles in familial and sporadic breast cancer, resulting in different pathways of disease progression (62), which need to be taken in account when evaluating glycodelin expression and its meaning for the patient.

In regard to sporadic breast cancer, which comprises the largest group within breast cancer, it was found that the expression of glycodelin A is independent of different histological forms (63) and of grading (64), but a slight correlation to steroid receptor expression was found, meaning that glycodelin A could be a marker for differentiation. In 2010, an increase in glycodelin expression was found in estrogen receptor/progesterone receptor (ER/PR)-positive tumor samples which had a lymph node involvement (65), so in addition to being a marker for breast cancer differentiation, there might be some role for glycodelin A in lymph node metastasis. Furthermore, the expression of glycodelin A could be regarded as a prognostic marker, as it decreases with increasing malignancy and exhibits higher expression in tissues from patients with good prognosis (66). However, it should be borne in mind that the presence and expression of the glycodelin protein is the decisive factor, not the expression of its mRNA, which does not decrease with increasing malignancy (67). This fact has to be considered for evaluation of the study of Kostadima *et al*., who extracted mRNA from paraffin-embedded breast cancer tissues and correlated the mRNA expression to clinicopathological and molecular parameters, and to patient outcome. They found no prognostic utility for glycodelin mRNA expression for overall survival or disease-free survival (68).

The experiments of Song *et al*. represent a strong contrast to the data above. As it was known that glycodelin A was found in endothelial cells of the umbilical cord and tumor blood vessels, they examined the tube formation and migration of human umbilical vein endothelial cells upon addition of amniotic fluid, which is rich in glycodelin, or of a synthetically-produced protein mimicking glycodelin. An increase in tube formation and cell migration was found, indicating a promotion of angiogenesis *in vivo*. This effect was blocked by antibodies against the synthetic peptide or vascular epidermal growth factor (VEGF). It could, therefore, be supposed that the effect of glycodelin is mediated by VEGF, as glycodelin increases the release of both VEGF protein and mRNA expression, as well as mRNA expression of VEGF receptor. From all these results, it could be concluded that glycodelin A plays a role in neovascularisation of tumors (60).

A point for therapeutical intervention comes from the group of Ramachandran *et al*., who found that the expression of glycodelin A is induced by lipophosphatidic acid (LPA) in a dose-dependent manner. LPA is rather similar to phorbol-myristate acid (PMA), which is increased in the serum of patients with cancer. Controlling the amount of LPA/PMA could help prevent glycodelin A expression and thereby reduce neoangiogenesis (69).

Other Lipocalins and their Associations with Breast Cancer

Prolactin. Prolactin is known to induce the proliferation of normal alveoli in pregnancy, especially during lactation, and abnormal alveoli in hormonally-dependent breast cancer (70) and especially increases the risk for male breast cancer by a transforming growth factor α-mediated pathway (71). An intervention into this pathway could be the basis for a therapeutic strategy directed against male breast cancer.

Apolipoprotein D. Apolipoprotein D is a small (24 kDa) glycoprotein, regulated by retinoic acid. The presence of retinoic acid leads to an accumulation of apolipoprotein D, especially in ER-positive breast cancer cell lines, and inhibits proliferation and tumor progression by establishment of a more differentiated phenotype (72). Furthermore it is involved in intracellular ligand binding and inhibits translocation of phosphorylated mitogen-activated protein kinases into the nucleus. Thereby it is associated with a favourable histology and grading in breast cancer. However, apolipoprotein D is associated with an adverse prognosis, if it is found in the tumor stroma, and ER-positive/ apolipoprotein D-positive cells have a non-functional ER pathway, hence the cancer cells would not react to a tamoxifen-based therapy (73). Moreover, apolipoprotein D seems to correlate with lymph node metastasis, therefore it also has a prognostic relevance (74).

Lipocalin 2. Also known as neutrophil gelatinase-associated lipocalin (NGAL), lipocalin 2 is a small secreted protein and is known by many different names in the literature. Lipocalin 2 is in fact mostly used for the mouse homolog of NGAL (2).

LCN2 stabilizes matrix metalloproteinase-9 from auto-degradation. If Lipocalin 2 is added to matrix metallo-proteinase-9-positive breast cancer cells, they exhibit a more aggressive phenotype, and when secreted by macrophages, lipocalin 2 induces cellular growth of MCF-7 cells (75). Up-regulation of lipocalin 2 results in increased tumor growth and angiogenesis (76) and is hence

involved in metastasis formation (77). But as it can be detected in patients' urine, it seems to be a useful marker for the determination of tumor stage. Furthermore lipocalin 2 is correlated with the disease severity score (78), a negative hormone receptor status, HER2 overexpression, poor grading, lymph node metastasis, and a high proliferation index (Ki-67), and is generally a predictor of poor prognosis (79). However, it has to be distinguished between the low risk groups on one hand, where it is a marker of pathological complete remission after neoadjuvant chemotherapy, and on the other hand primary breast cancers, where it can be regarded as a prognostic factor for reduced disease-free survival (80). One therapeutic strategy directed against the lipocalin 2 pathway uses the transcription factor C/enhancer binding protein ζ, which inhibits the transcription of LCN2 and thereby inhibits migration and invasion in breast cancer (81). Lipocalin 2 is also involved in red blood cell production, leading to anaemia, which is frequently found in patients with cancer. As up-regulation of HER2 results in up-regulation of lipocalin 2 *via* the nuclear factor κB-pathway, this could be another starting point for therapeutical intervention (45). The most recent research in the field of lipocalin 2 is on micro RNAs, regulating it´s expression. If micro RNA miR-138 is present, then the expression of lipocalin 2 is significantly reduced, as is cell migration. Therefore miR-138 can be regarded as a tumor suppressor, preventing tumor formation and metastasis growth (82).

Similar to the literature on human lipocalin 2 are the findings for lipocalin 2 in mouse. It also seems to be regulated by HER2 *via* nuclear factor κB-pathways, and is up-regulated in epithelial cancer. Up-regulation of lipocalin 2 is accompanied by an increased expression of mesenchymal markers and a decrease in epithelial markers (83, 84), hence promoting tumor progression by epithelial–mesenchymal transition. Like lipocalin 2 in humans, it is known to stabilize matrix metalloproteinase-9 (85) and to regulate angiogenesis *via* the VEGF pathway, promoting angiogenesis and tumor progression (84), as well as breast cancer cell invasion in mice (86). Lipocalin 2 double-knockout mice have a delayed onset of mammary tumors, a decreased tumor burden, and a reduced matrix metalloproteinase-9 activity, but no reduction of lung metastasis was found (87). Lipocalin 2 can also be regarded as a biomarker: elevated levels in serum samples indicate a reduced disease-free survival (88). In contrast to that are results from Cramer *et al*., who found no correlation of Lipocalin 2 with tumor size, tumor appearance, tumor volume and number of metastases, and thus call the role of Lipocalin 2 in tumor development into question (89).

As a conclusion, most kinds of lipocalins play a role in tumor development, progression, angiogenesis and formation of distant metastases, and therefore have a significant impact on cancer diagnosis. Much research will be required to exploit all the therapeutical possibilities which exist on the basis of these molecules.

References

1 Ganfornina MD, Gutierrez G, Bastiani M and Sanchez D: A phylogenetic analysis of the lipocalin protein family. Mol Biol Evol *17(1)*: 114-126, 2000.

2 Chakraborty S, Kaur S, Guha S and Batra SK: The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. Biochim Biophys Acta *1826(1)*: 129-169, 2012.

3 Flower DR: The lipocalin protein family: structure and function. Biochem J *318(Pt 1)*: 1-14, 1996.

4 Kamarainen M, Julkunen M and Seppala M: HinfI polymorphism in the human progesterone associated endometrial protein (PAEP) gene. Nucleic Acids Res *19(18)*: 5092, 1991.

5 Van Cong N, Vaisse C, Gross MS, Slim R, Milgrom E and Bernheim A: The human placental protein 14 (PP14) gene is localized on chromosome 9q34. Hum Genet *86(5)*: 515-518, 1991.

6 Chan P, Simon-Chazottes D, Mattei MG, Guenet JL and Salier JP: Comparative mapping of lipocalin genes in human and mouse: the four genes for complement C8 gamma chain, prostaglandin-D-synthase, oncogene-24p3, and progestagen-associated endometrial protein map to HSA9 and MMU2. Genomics *23(1)*: 145-150, 1994.

7 Glasgow BJ, Heinzmann C, Kojis T, Sparkes RS, Mohandas T and Bateman JB: Assignment of tear lipocalin gene to human chromosome 9q34-9qter. Curr Eye Res *12(11)*: 1019-1023, 1993.

8 White DM, Mikol DD, Espinosa R, Weimer B, Le Beau MM, Stefansson K: Structure and chromosomal localization of the human gene for a brain form of prostaglandin D2 synthase. J Biol Chem *267(32)*: 23202-23208, 1992.

9 Vaisse C, Atger M, Potier B and Milgrom E: Human placental protein 14 gene: sequence and characterization of a short duplication. DNA Cell Biol *9(6)*: 401-413, 1990.

10 Julkunen M, Wahlstrom T and Seppala M: Human fallopian tube contains placental protein 14. Am J Obstet Gynecol *154(5)*: 1076-1079, 1986.

11 Dell A, Morris HR, Easton RL, Panico M, Patankar M, Oehniger S *et al*: Structural analysis of the oligosaccharides derived from glycodelin, a human glycoprotein with potent immunosuppressive and contraceptive activities. J Biol Chem *270(41)*: 24116-24126, 1995.

12 Seppala M, Bohn H and Tatarinov Y: Glycodelins: Tumour Biol *19(3)*: 213-220, 1998.

13 Bell SC and Bohn H: Immunochemical and biochemical relationship between human pregnancy-associated secreted endometrial alpha 1- and alpha 2-globulins (alpha 1- and alpha 2-PEG) and the soluble placental proteins 12 and 14 (PP12 and PP14). Placenta *7(4)*: 283-294, 1986.

14 Bohn H, Kraus W and Winckler W: New soluble placental tissue proteins: their isolation, characterization, localization and quantification. Placenta Suppl *4*: 67-81, 1982.

15 Joshi SG, Henriques ES, Smith RA and Szarowski DH: Progestogen-dependent endometrial protein in women: tissue concentration in relation to developmental stage and to serum hormone levels. Am J Obstet Gynecol *138(8)*: 1131-1136, 1980.

16 Kamarainen M, Riittinen L, Seppala M, Palotie A and Andersson LC: Progesterone-associated endometrial protein – a constitutive marker of human erythroid precursors. Blood *84(2)*: 467-473, 1994.
17 Petrunin DD, Griaznova IM, Petrunina Iu A and Tatarinov Iu S: Immunochemical identification of human placental organ specific alpha2-globulin and its concentration in amniotic fluid. Biull Eksp Biol Med *82(7)*: 803-804, 1976.
18 Seppala M, Taylor RN, Koistinen H, Koistinen R and Milgrom E: Glycodelin: a major lipocalin protein of the reproductive axis with diverse actions in cell recognition and differentiation. Endocr Rev *23(4)*: 401-430, 2002.
19 Huhtala ML, Seppala M, Narvanen A, Palomaki P, Julkunen M and Bohn H: Amino acid sequence homology between human placental protein 14 and beta-lactoglobulins from various species. Endocrinology *120(6)*: 2620-2622, 1987.
20 Julkunen M, Seppala M and Janne OA: Complete amino acid sequence of human placental protein 14: a progesterone-regulated uterine protein homologous to beta-lactoglobulins. Proc Natl Acad Sci USA *85(23)*: 8845-8849, 1988.
21 Papiz MZ, Sawyer L, Eliopoulos EE, North AC, Findlay JB, Sivaprasadarao R *et al*: The structure of beta-lactoglobulin and its similarity to plasma retinol-binding protein. Nature *324(6095)*: 383-385, 1986.
22 Koistinen H, Koistinen R, Seppala M, Burova TV, Choiset Y and Haertle T: Glycodelin and beta-lactoglobulin, lipocalins with a high structural similarity, differ in ligand binding properties. FEBS Lett *450(1-2)*: 158-162, 1999.
23 Koistinen H, Koistinen R, Kamarainen M, Salo J and Seppala M: Multiple forms of messenger ribonucleic acid encoding glycodelin in male genital tract. Lab Invest *76(5)*: 683-690, 1997.
24 Bell SC, Keyte JW and Waites GT: Pregnancy-associated endometrial alpha 2-globulin, the major secretory protein of the luteal phase and first trimester pregnancy endometrium, is not glycosylated prolactin but related to beta-lactoglobulins. J Clin Endocrinol Metab *65(5)*: 1067-1071, 1987.
25 Saridogan E, Djahanbakhch O, Kervancioglu ME, Kahyaoglu F, Shrimanker K and Grudzinskas JG: Placental protein 14 production by human Fallopian tube epithelial cells *in vitro*. Hum Reprod *12(7)*: 1500-1507, 1997.
26 Julkunen M, Wahlstrom T, Seppala M, Koistinen R, Koskimies A, Stenman UH *et al*: Detection and localization of placental protein 14-like protein in human seminal plasma and in the male genital tract. Arch Androl *12 Suppl*: 59-67, 1984.
27 Kamarainen M, Leivo I, Koistinen R, Julkunen M, Karvonen U, Rutanen EM *et al*: Normal human ovary and ovarian tumors express glycodelin, a glycoprotein with immunosuppressive and contraceptive properties. Am J Pathol *148(5)*: 1435-1443, 1996.
28 Riittinen L: Serous ovarian cyst fluids contain high levels of endometrial placental protein 14. Tumour Biol *13(3)*: 175-179, 1992.
29 Kamarainen M, Seppala M, Virtanen I and Andersson LC: Expression of glycodelin in MCF-7 breast cancer cells induces differentiation into organized acinar epithelium. Lab Invest *77(6)*: 565-573, 1997.
30 Zhou HM, Ramachandran S, Kim JG, Raynor DB, Rock JA and Parthasarathy S: Implications in the management of pregnancy: II. Low levels of gene expression but enhanced uptake and accumulation of umbilical cord glycodelin. Fertil Steril *73(4)*: 843-847, 2000.
31 Riittinen L, Narvanen O, Virtanen I and Seppala M: Monoclonal antibodies against endometrial protein PP14 and their use for purification and radioimmunoassay of PP14. J Immunol Methods *136(1)*: 85-90, 1991.
32 Oehninger S, Coddington CC, Hodgen GD and Seppala M: Factors affecting fertilization: endometrial placental protein 14 reduces the capacity of human spermatozoa to bind to the human *zona pellucida*. Fertil Steril *63(2)*: 377-383, 1995.
33 Morris HR, Dell A, Easton RL, Panico M, Koistinen H, Koistinen R *et al*: Gender-specific glycosylation of human glycodelin affects its contraceptive activity. J Biol Chem *271(50)*: 32159-32167, 1996.
34 Bolton AE, Pockley AG, Clough KJ, Mowles EA, Stoker RJ, Westwood OM *et al*: Identification of placental protein 14 as an immunosuppressive factor in human reproduction. Lancet *1(8533)*: 593-595, 1987.
35 Okamoto N, Uchida A, Takakura K, Kariya Y, Kanzaki H, Riittinen L *et al*: Suppression by human placental protein 14 of natural killer cell activity. Am J Reprod Immunol *26(4)*: 137-142, 1991.
36 Mishan-Eisenberg G, Borovsky Z, Weber MC, Gazit R, Tykocinski ML and Rachmilewitz J: Differential regulation of Th1/Th2 cytokine responses by placental protein 14. J Immunol *173(9)*: 5524-5530, 2004.
37 Mukhopadhyay D, Sundereshan S, Rao C and Karande AA: Placental protein 14 induces apoptosis in T cells but not in monocytes. J Biol Chem *276(30)*: 28268-28273, 2001.
38 Yaniv E, Borovsky Z, Mishan-Eisenberg G and Rachmilewitz J: Placental protein 14 regulates selective B cell responses. Cell Immunol *222(2)*: 156-163, 2003.
39 Powell LD, Jain RK, Matta KL, Sabesan S and Varki A: Characterization of sialyloligosaccharide binding by recombinant soluble and native cell-associated CD22. Evidence for a minimal structural recognition motif and the potential importance of multisite binding. J Biol Chem *270(13)*: 7523-7532, 1995.
40 Jeschke U, Wang X, Briese V, Friese K and Stahn R: Glycodelin and amniotic fluid transferrin as inhibitors of E-selectin-mediated cell adhesion. Histochem Cell Biol *119(5)*: 345-354, 2003.
41 Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A *et al*: Global gene profiling in human endometrium during the window of implantation. Endocrinology *143(6)*: 2119-2138, 2002.
42 Julkunen M, Koistinen R, Suikkari AM, Seppala M and Janne OA: Identification by hybridization histochemistry of human endometrial cells expressing mRNAs encoding a uterine beta-lactoglobulin homologue and insulin-like growth factor-binding protein-1. Mol Endocrinol *4(5)*: 700-707, 1990.
43 Seppala M, Riittinen L, Julkunen M, Koistinen R, Wahlstrom T, Iino K *et al*: Structural studies, localization in tissue and clinical aspects of human endometrial proteins. J Reprod Fertil Suppl *36*: 127-41, 1988.
44 Julkunen M, Apter D, Seppala M, Stenman UH and Bohn H: Serum levels of placental protein 14 reflect ovulation in nonconceptional menstrual cycles. Fertil Steril *45(1)*: 47-50, 1986.
45 Li SH, Hawthorne VS, Neal CL, Sanghera S, Xu J, Yang J *et al*: Up-regulation of neutrophil gelatinase-associated lipocalin by ErbB2 through nuclear factor-kappaB activation. Cancer Res *69(24)*: 9163-9168, 2009.

46 Li TC, Dalton C, Hunjan KS, Warren MA and Bolton AE: The correlation of placental protein 14 concentrations in uterine flushing and endometrial morphology in the peri-implantation period. Hum Reprod *8(11)*: 1923-1927, 1993.

47 Julkunen M, Rutanen EM, Koskimies A, Ranta T, Bohn H and Seppala M: Distribution of placental protein 14 in tissues and body fluids during pregnancy. Br J Obstet Gynaecol *92(11)*: 1145-1151, 1985.

48 Seppala M, Alfthan H, Vartiainen E and Stenman UH: The post-menopausal uterus: the effect of hormone replacement therapy on the serum levels of secretory endometrial protein PP14/beta-lactoglobulin homologue. Hum Reprod *2(8)*: 741-743, 1987.

49 Seppala M, Julkunen M, Koskimies A, Laatikainen T, Stenman UH and Huhtala ML: Proteins of the human endometrium. Basic and clinical studies toward a blood test for endometrial function. Ann NY Acad Sci *541*: 432-444, 1988.

50 Tse JY, Chiu PC, Lee KF, Seppala M, Koistinen H, Koistinen R *et al*: The synthesis and fate of glycodelin in human ovary during folliculogenesis. Mol Hum Reprod *8(2)*: 142-148, 2002.

51 Chiu PC, Koistinen R, Koistinen H, Seppala M, Lee KF and Yeung WS: Zona-binding inhibitory factor-1 from human follicular fluid is an isoform of glycodelin. Biol Reprod *69(1)*: 365-372, 2003.

52 Chiu PC, Chung MK, Tsang HY, Koistinen R, Koistinen H, Seppala M *et al*: Glycodelin-S in human seminal plasma reduces cholesterol efflux and inhibits capacitation of spermatozoa. J Biol Chem *280(27)*: 25580-25589, 2005.

53 Yeung CH, Barfield JP and Cooper TG: Chloride channels in physiological volume regulation of human spermatozoa. Biol Reprod *73(5)*: 1057-1063, 2005.

54 Mandelin E, Lassus H, Seppala M, Leminen A, Gustafsson JA, Cheng G *et al*: Glycodelin in ovarian serous carcinoma: association with differentiation and survival. Cancer Res *63(19)*: 6258-6264, 2003.

55 Kamarainen M, Miettinen M, Seppala M, von Boguslawsky K, Benassi MS, Bohling T *et al*: Epithelial expression of glycodelin in biphasic synovial sarcomas. Int J Cancer *76(4)*: 487-490, 1998.

56 Arnold JT, Lessey BA, Seppala M and Kaufman DG: Effect of normal endometrial stroma on growth and differentiation in Ishikawa endometrial adenocarcinoma cells. Cancer Res *62(1)*: 79-88, 2002.

57 Koistinen H, Seppala M, Nagy B, Tapper J, Knuutila S and Koistinen R: Glycodelin reduces carcinoma-associated gene expression in endometrial adenocarcinoma cells. Am J Obstet Gynecol *193(6)*: 1955-1960, 2005.

58 Koistinen H, Hautala LC, Seppala M, Stenman UH, Laakkonen P and Koistinen R: The role of glycodelin in cell differentiation and tumor growth. Scand J Clin Lab Invest *69(4)*: 452-459, 2009.

59 Tsviliana A, Mayr D, Kuhn C, Kunze S, Mylonas I, Jeschke U *et al*: Determination of glycodelin-A expression correlated to grading and staging in ovarian carcinoma tissue. Anticancer Res *30(5)*: 1637-1640, 2010.

60 Song M, Ramaswamy S, Ramachandran S, Flowers LC, Horowitz IR, Rock JA *et al*: Angiogenic role for glycodelin in tumorigenesis. Proc Natl Acad Sci USA *98(16)*: 9265-9270, 2001.

61 Hautala LC, Koistinen R, Seppala M, Butzow R, Stenman UH, Laakkonen P *et al*: Glycodelin reduces breast cancer xenograft growth *in vivo*. Int J Cancer *123(10)*: 2279-2284, 2008.

62 Hautala LC, Greco D, Koistinen R, Heikkinen T, Heikkila P, Aittomaki K *et al*: Glycodelin expression associates with differential tumour phenotype and outcome in sporadic and familial non-BRCA1/2 breast cancer patients. Breast Cancer Res Treat *128(1)*: 85-95, 2011.

63 Kamarainen M, Halttunen M, Koistinen R, von Boguslawsky K, von Smitten K, Andersson LC *et al*: Expression of glycodelin in human breast and breast cancer. Int J Cancer *83(6)*: 738-742, 1999.

64 Scholz C, Toth B, Barthell E, Mylonas I, Weissenbacher T, Friese K *et al*: Immunohistochemical expression of glycodelin in breast cancer correlates with estrogen-receptor alpha and progesterone-receptor A positivity. Histol Histopathol *24(4)*: 467-471, 2009.

65 Scholz C, Toth B, Barthell E, Mylonas I, Weissenbacher T, Friese K *et al*: Glycodelin expression in correlation to grading, nodal involvement and steroid receptor expression in human breast cancer patients. Anticancer Res *30(5)*: 1599-1603, 2010.

66 Shabani N, Mylonas I, Kunert-Keil C, Briese V, Janni W, Gerber B *et al*: Expression of glycodelin in human breast cancer: immunohistochemical analysis in mammary carcinoma in situ, invasive carcinomas and their lymph node metastases. Anticancer Res *25(3A)*: 1761-1764, 2005.

67 Jeschke U, Mylonas I, Kunert-Keil C, Dazert E, Shabani N, Werling M *et al*: Expression of glycodelin protein and mRNA in human ductal breast cancer carcinoma in situ, invasive ductal carcinomas, their lymph node and distant metastases, and ductal carcinomas with recurrence. Oncol Rep *13(3)*: 413-419, 2005.

68 Kostadima L, Pentheroudakis G, Fountzilas G, Dimopoulos M, Pectasides D, Gogas H *et al*: Survivin and glycodelin transcriptional activity in node-positive early breast cancer: mRNA expression of two key regulators of cell survival. Breast Cancer Res Treat *100(2)*: 161-167, 2006.

69 Ramachandran S, Ramaswamy S, Cho C and Parthasarathy S: Lysophosphatidic acid induces glycodelin gene expression in cancer cells. Cancer Lett *177(2)*: 197-202, 2002.

70 Rose-Hellekant TA, Arendt LM, Schroeder MD, Gilchrist K, Sandgren EP and Schuler LA: Prolactin induces ERalpha-positive and ERalpha-negative mammary cancer in transgenic mice. Oncogene *22(30)*: 4664-4674, 2003.

71 Arendt LM and Schuler LA: Prolactin drives estrogen receptor-alpha-dependent ductal expansion and synergizes with transforming growth factor-alpha to induce mammary tumors in males. Am J Pathol *172(1)*: 194-202, 2008.

72 Lopez-Boado YS, Tolivia J and Lopez-Otin C: Apolipoprotein D gene induction by retinoic acid is concomitant with growth arrest and cell differentiation in human breast cancer cells. J Biol Chem *269(43)*: 26871-26878, 1994.

73 Soiland H, Soreide K, Janssen EA, Korner H, Baak JP and Soreide JA: Emerging concepts of apolipoprotein D with possible implications for breast cancer. Cell Oncol *29(3)*: 195-209, 2007.

74 Soiland H, Janssen EA, Korner H, Varhaug JE, Skaland I, Gudlaugsson E *et al*: Apolipoprotein D predicts adverse outcome in women >or=70 years with operable breast cancer. Breast Cancer Res Treat *113(3)*: 519-528, 2009.

75 Jung M, Weigert A, Tausendschon M, Mora J, Oren B, Sola A *et al*: Interleukin-10-induced neutrophil gelatinase-associated lipocalin production in macrophages with consequences for tumor growth. Mol Cell Biol *32(19)*: 3938-3948, 2012.

76 Fernandez CA, Yan L, Louis G, Yang J, Kutok JL and Moses MA: The matrix metalloproteinase-9/neutrophil gelatinase-associated lipocalin complex plays a role in breast tumor growth and is present in the urine of breast cancer patients. Clin Cancer Res *11(15)*: 5390-5395, 2005.
77 Chappell WH, Abrams SL, Franklin RA, LaHair MM, Montalto G, Cervello M *et al*: Ectopic NGAL expression can alter sensitivity of breast cancer cells to EGFR, Bcl-2, CaM-K inhibitors and the plant natural product berberine. Cell Cycle *11(23)*: 4447-4461, 2012.
78 Provatopoulou X, Gounaris A, Kalogera E, Zagouri F, Flessas I, Goussetis E *et al*: Circulating levels of matrix metalloproteinase-9 (MMP-9), neutrophil gelatinase-associated lipocalin (NGAL) and their complex MMP-9/NGAL in breast cancer disease. BMC Cancer *9*: 390, 2009.
79 Bauer M, Eickhoff JC, Gould MN, Mundhenke C, Maass N and Friedl A: Neutrophil gelatinase-associated lipocalin (NGAL) is a predictor of poor prognosis in human primary breast cancer. Breast Cancer Res Treat *108(3)*: 389-397, 2008.
80 Wenners AS, Mehta K, Loibl S, Park H, Mueller B, Arnold N *et al*: Neutrophil gelatinase-associated lipocalin (NGAL) predicts response to neoadjuvant chemotherapy and clinical outcome in primary human breast cancer. PLoS One *7(10)*: e45826, 2012.
81 Wang L, Li H, Wang J, Gao W, Lin Y, Jin W *et al*: C/EBP zeta targets to neutrophil gelatinase-associated lipocalin (NGAL) as a repressor for metastasis of MDA-MB-231 cells. Biochim Biophys Acta *1813(10)*: 1803-1813, 2011.
82 Lee YC, Tzeng WF, Chiou TJ and Chu ST: MicroRNA-138 suppresses neutrophil gelatinase-associated lipocalin expression and inhibits tumorigenicity. PLoS One *7(12)*: e52979, 2012.
83 Shi H, Gu Y, Yang J, Xu L, Mi W and Yu W: Lipocalin 2 promotes lung metastasis of murine breast cancer cells. J Exp Clin Cancer Res *27*: 83, 2008.
84 Yang J, Bielenberg DR, Rodig SJ, Doiron R, Clifton MC, Kung AL *et al*: Lipocalin 2 promotes breast cancer progression. Proc Natl Acad Sci USA *106(10)*: 3913-3918, 2009.
85 Leng X, Ding T, Lin H, Wang Y, Hu L, Hu J *et al*: Inhibition of lipocalin 2 impairs breast tumorigenesis and metastasis. Cancer Res *69(22)*: 8579-8584, 2009.
86 Gaudineau B, Fougere M, Guaddachi F, Lemoine F, de la Grange P and Jauliac S: Lipocalin 2, the TNF-like receptor TWEAKR and its ligand TWEAK act downstream of NFAT1 to regulate breast cancer cell invasion. J Cell Sci *125(Pt 19)*: 4475-4486, 2012.
87 Berger T, Cheung CC, Elia AJ and Mak TW: Disruption of the Lcn2 gene in mice suppresses primary mammary tumor formation but does not decrease lung metastasis. Proc Natl Acad Sci USA *107(7)*: 2995-3000, 2010.
88 Sung H, Choi JY, Lee SA, Lee KM, Han S, Jeon S *et al*: The association between the preoperative serum levels of lipocalin-2 and matrix metalloproteinase-9 (MMP-9) and prognosis of breast cancer. BMC Cancer *12*: 193, 2012.
89 Cramer EP, Glenthoj A, Hager M, Juncker-Jensen A, Engelholm LH, Santoni-Rugiu E *et al*: No effect of NGAL/lipocalin-2 on aggressiveness of cancer in the MMTV-PyMT/FVB/N mouse model for breast cancer. PLoS One *7(6)*: e39646, 2012.

Received December 11, 2013
Revised January 7, 2014
Accepted January 9, 2014