REVIEW

Glycodelin in reproductive endocrinology and hormone-related cancer

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Abstract

Glycodelin is an endocrine-regulated glycoprotein that has significant effects on immune cells, apoptosis, reproduction, cell adhesion, differentiation and cancer. In reproduction, glycodelin contributes to capacitation and immunoprotection of spermatozoa, and it modulates sperm–oocyte binding, acrosome reaction and implantation. In endocrine-related cancer, the differentiation inducing effects of glycodelin are accompanied by growth restriction of malignant cells, decreased expression of oncogenes, increased expression of tumour suppressor genes and morphological reversion of the malignant phenotype. This review features these properties and clinical connections, highlighting the role of glycosylation in biological actions.

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Background

After the primary structure (1), major complex type N-glycan structures (2, 3) and various biological actions (2–6) of a glycoprotein named PP14 had been resolved, the name ‘glycodelin’ was suggested by the Helsinki team to their collaborators (2, 3, 7) and then accepted by the pioneers who had used other names, such as human placental organ specific α 2-globulin, progesterone-associated endometrial protein (PAEP) and zona-binding inhibitory factor for the same glycoprotein (8–10). In addition, immunological and/or sequence similarity has been reported for PP14, α 2-pregnancy-associated endometrial globulin (11) and progesterone-dependent endometrial protein (12). The gene sequence of glycodelin has been documented (13), and the Human Genome Organization has registered PAEP as the official symbol of the glycodelin gene (9). Four glycoforms, namely glycodelin-S, -A, -F and -C have been characterised in reproductive tissues. They all share a common protein backbone but differ in glycosylation and biological activity (14, 15).

Sites of synthesis

In men, glycodelin-S is secreted from seminal vesicle glands to seminal fluid (16–18).

In women, secretory and decidualised endometrium are the major sites of glycodelin synthesis (Fig. 1) (19–22). Glycodelin is mainly secreted into uterine

fluid (23) and amniotic fluid (24, 25), less to serum. Glycodelin has also been found in many other sites of the body, but not all of them actually synthesise the protein as evidenced by the absence of mRNA (Table 1). For instance, during ovarian follicle development, glycodelin-F immunoreactivity becomes detectable in the granulosa and theca cells of late secondary follicles, but only the granulosa cells express glycodelin mRNA (26). Glycodelin-F is secreted from the granulosa cells into follicular fluid and is taken up by the cumulus cells in which partial deglycosylation takes place to yield glycodelin-C (cumulus cell glycodelin) (14, 27).

Regulation of synthesis

Hormonal regulation of glycodelin-S synthesis in men is unknown.

Progesterone and progestins

In normal ovulatory cycles, progesterone secretion is followed by endometrial glycodelin-A synthesis in epithelial glands from 4 to 5 postovulatory days onwards (15, 38). Glycodelin mRNA is markedly upregulated in the mid and late secretory endometrium (Fig. 1). Glycodelin protein remains detectable in deep basal glands and the serum concentration remains elevated until days 1–3 of the next period (36). In anovulatory cycles, glycodelin serum concentration remains low throughout the cycles. In the oviduct,

Figure 1 (A) Expression of glycodeilin in human secretory endometrium and (B) its circulating levels in a normal ovulatory cycle in respect of progesterone and relaxin concentrations. Data adapted from Refs (19, 36, 37).

glycodeilin synthesis is stimulated by estrogen and progesterone (Table 2).

In early pregnancy, the rise of glycodeilin-A concentration in serum and amniotic fluid peaks at 6–12 weeks gestation (24, 25), whereas in women with premature ovarian failure who conceived after ovum donation and embryo transfer the glycodeilin levels are much lower than in the women with normal ovarian function (39). This remarkable difference suggests that the ovary itself or factors under ovarian control are involved in glycodeilin secretion. More direct evidence for progesterone involvement comes from *in vitro* studies (Table 2). However, it seems that the effect of progesterone

on glycodeilin expression involves multiple factors rather than a simple pathway (see below).

Human chorionic gonadotrophin

Besides, the classic stimulatory effect of trophoblastic hCG on progesterone secretion by the corpus luteum, hCG has receptors in the endometrium where it stimulates glycodeilin secretion (40, 41).

Relaxin

Relaxin has been suggested to be another ovarian factor contributing to glycodeilin secretion. Close temporal relationship exists between relaxin and glycodeilin serum profiles in late luteal phase and pregnancy. Because the glycodeilin-inducing effect of progesterone is slow it has been suggested its action is based on the local inducers, such as relaxin or transcription factors that control the glycodeilin promoter activity (42) (Table 2).

Epigenetic regulation

Histone acetyltransferases and histone deacetylases (HDACs) regulate gene expression through chromatin modification by catalysing transfer or removal of acetyl groups respectively. Histone deacetylase inhibitors (HDACIs), e.g. suberoylanilide hydroxamic acid (SAHA) and trichostatin (TSA) increase the level of histone acetylation, thereby turning on specific genes, including glycodeilin, whose expression products induce cell growth arrest, differentiation and apoptosis (45–48). In baboon, the site of TSA action is localised to the region in the glycodeilin promoter that contains the proximal Sp1 site (48). Deletions of this region have no effect on progestogen responsiveness, suggesting existence of at least two regions in the glycodeilin promoter that are important for the induction of glycodeilin expression. Maximal expression of the glycodeilin gene

Table 1 Sites of glycodeilin expression/synthesis.

Tissue	Presence of protein	Presence or absence of mRNA or other evidence ⁽ⁱ⁾	References
Secretory endometrium	+	+	(19) ⁽ⁱ⁾ , (20)
Decidualised endometrium	+	+	(20, 22, 24)
Seminal vesicle	+	+	(16–18)
Fallopian tube	+	+	(28–30) ⁽ⁱ⁾
Breast, breast cancer	+	+	(31, 32)
Bronchus epithelium	+	nd	(32)
Ovary, ovarian cancer	+	+	(26, 33)
Cumulus cells	+	–	(26, 27)
Ecocrine sweat glands	+	nd	(32)
Bone marrow	+	+	(34, 35)

nd, not done; (i), labelled amino acid incorporation.

Table 2 Regulation of glycodelin synthesis.

Substance or factor	Type of evidence	References
Progesterone and progestins	Endometrial Gd expression induced under progesterone influence Progestins and antiprogestins upregulate Gd production <i>in vivo</i> MPA-activated PR modulates Gd promoter through the Sp1 site PREs present in glycodelin gene (<i>PAEP</i>) promoter Oviductal synthesis stimulated by E and P	(19–21, 36, 38) (43) (44) (13) (29, 30)
HCG	Stimulation of baboon glycodelin secretion by hCG <i>in vivo</i> Similar serum concentration profiles during human pregnancy, peaking around 10 weeks	(41) (24)
Relaxin	Close temporal relationship between relaxin and glycodelin serum profiles in late luteal phase, with the onset of relaxin preceding glycodelin secretion by 1–2 days in non-conceptive ovulatory cycles and during pregnancy In fertile-aged women injected with recombinant human relaxin for 28 days, only the subjects demonstrating ovarian cyclicity show elevated glycodelin in response to recombinant relaxin administration, whereas no elevation is seen in placebo-treated subjects or in the subjects without ovarian cyclicity Continuous relaxin administration stimulates glycodelin secretion throughout the menstrual cycle, including the periovulatory nadir In cultured endometrial cells, relaxin increases the glycodelin production rate two- to six-fold, and the glycodelin mRNA concentration is increased 2- to 11-fold	(37) (37) (37) (42)
Histone deacetylase inhibitors	SAHA and TSA increase the level of histone acetylation, thereby turning on glycodelin expression that induces cell growth arrest and differentiation, attenuated by glycodelin siRNA	(45–47)

may depend also on factors regulated by progestogen and inhibited by HDAC (48).

Biochemical properties: glycosylation dictates function

Immunosuppression and apoptosis

Protection of the human embryo/foetus from maternal immune response is essential for successful reproduction. Glycodelin plays a role here in many ways. After the first report (4), the immunosuppressive nature of glycodelin-A has become well established (Table 3). This is important because decidualised endometrium is replete with immune cells, e.g. lymphocytes, uterine natural killer (NK) cells and monocytes, and the foetal semiallograft is their potential target. Through its actions on T cells, B cells and NK cells glycodelin is involved in foeto-embryonic defence mechanisms (49). Glycodelin is selectively expressed in decidual NK cells (50). It is also found on the sperm head (26, 27, 51, 52).

Table 3 Immunosuppressive effects of glycodelin-A.

Effect	References
Suppresses lymphocyte proliferation	(56)
Induces tolerogenic phenotype in monocyte-derived dendritic cells	(57)
Inhibits T cell activity/proliferation, induces apoptosis	(53, 58, 59)
Inhibits B cell activity/proliferation	(60)
Induces apoptosis in monocytes	(64)

The sperm-bound glycodelin may protect spermatozoa against maternal lymphocytes by glycosylation-dependent actions (15).

Glycodelin appears to desensitise T-cell receptor (TCR) signalling by its association with the tyrosine phosphatase receptor, CD45 (53). Other mechanisms may also be involved because glycodelin-A is inhibitory to T-cells upon phorbol ester and ionophore stimulation that bypass the TCR-proximal signalling events (54). The IC₅₀ for glycodelin-A activity on T-cells is around 200 nM (5.6 µg/l) (54). This is within the concentration range of glycodelin-A in the first trimester amniotic fluid and decidualised endometrium (19, 25). Thus, the glycodelin concentration at the foeto-maternal interface is sufficiently high to contribute to protection of the foetal semiallograft against maternal immune cells, without generalised immunocompromise in pregnant women whose blood concentration of glycodelin is too low to produce detectable immunosuppression (24, 54, 55).

Apoptosis Glycodelin-A induces apoptosis both in T-cells and monocytes. This activity is caspase-dependent and is related to immunosuppressive activity (59). The effect is glycosylation dependent, which may explain some of the variable results (54, 59, 61–64). Specially, the terminal sialic acid residues and the size of the glycans appear important. Recombinant glycodelin from Chinese hamster ovary (CHO) cells with the same protein backbone as in glycodelin-A shows no apoptotic activity, but it can be rendered apoptotic by mannosidase treatment (61). This may result from the high proportion of oligomannoside glycans in the proteins

produced in CHO cells (65) and from the fact that the major complex type *N*-glycans in glycodeclin produced in CHO cells are devoid of the typical lacdiNAc glycans present in glycodeclin-A (66). Accessibility to the apoptotic region of glycodeclin may modulate apoptotic activity either by masking or unmasking the functional region in the glycodeclin molecule (61).

In peripheral blood T cells treated with glycodeclin-A, mitochondrial depolarisation precedes the onset of DNA fragmentation in apoptosis (54). When a human T-cell leukaemia cell line and its derivative overexpressing antiapoptotic Bcl-2 were treated with glycodeclin-A, the Bcl-2 overexpressing cells were resistant to mitochondrial membrane permeabilisation and apoptosis. This indicates that overexpression of Bcl-2 is sufficient to rescue these cells from glycodeclin-A induced death (54). In addition to, the NK cell activity in the innate immune system, glycodeclin also inhibits proliferation of several monocytic cell lines and induces apoptosis in these cell lines as well as in primary monocytes through caspase-independent intrinsic mitochondrial pathway (64). These results exemplify the mechanisms by which glycodeclin exhibits its apoptotic action. They also suggest that, at the foeto-maternal interface, glycodeclin plays a protective role by depleting the monocytes that could become proinflammatory, leaving the macrophages untouched to carry on with efficient clearance of the apoptotic cells (64).

Fertilisation

Glycodeclin regulates sperm function during critical steps of the fertilisation process (Fig. 2). These activities depend on the specific glycosylation patterns of glycodeclin that vary in different parts of the reproductive tract. Glycodeclin binding to ejaculated spermatozoa is positively correlated with sperm morphology (67). Glycodeclin-S binds on the sperm head, thereby inhibiting cholesterol efflux and capacitation of the spermatozoa (52). Glycodeclin-S is removed from spermatozoa as they migrate through the uterine cervix to reach the oocyte in the fallopian tube. In the uterine cavity, sperm-bound glycodeclin-A protects the spermatozoa against maternal lymphocytes (15).

Fertilisation depends on successful binding of the spermatozoa to the zona pellucida, followed by acrosome reaction. Glycodeclin-A inhibits sperm-oocyte binding (6). Therefore, absence or low abundance of glycodeclin-A in endometrium during the periovulatory fertile window is biologically meaningful (55). Certain progestin-based contraceptives increase endometrial glycodeclin-A secretion over the fertile midcycle (68), possibly contributing to the contraceptive effect.

After ovulation, follicular fluid with the oocyte-cumulus complex is transported to the oviduct where fertilisation takes place. The spermatozoa have to migrate through the uterus, follicular fluid and eventually the cumulus oophorus in order to bind to

the zona pellucida. Follicular fluid glycodeclin-F binds to the sperm head and shares the zona-binding inhibitory activity with glycodeclin-A (14, 26). In addition, glycodeclin-F inhibits progesterone-induced acrosome reaction (26). This activity may prevent premature acrosome reaction in the fertilising spermatozoon before it has attached to the zona pellucida.

Both glycodeclin-A and -F compete with zona pellucida glycoproteins for receptors on the sperm membrane. The receptor involved in this binding has been identified as fucosyltransferase 5 (FUT5) (70). The receptor binds competitively to both glycodeclin-A and the zona pellucida glycoproteins. Therefore, prior binding of glycodeclin-A to FUT5 on a spermatozoon may occupy its binding site(s) of the zona pellucida glycoproteins and inhibit fertilisation unless glycodeclin-A is transformed or removed (70) (Fig. 2).

The cumulus cells can transform the inhibitory isoforms glycodeclin-A and -F to glycodeclin-C (27). This glycoform can displace the inhibitory glycoforms from spermatozoa, thereby enhancing their zona-binding capacity. Taken together, the changes in glycodeclin glycosylation can provide a powerful posttranslational mechanism to regulate biological activity of glycodeclin that affects fertilisation.

Endometrial receptivity and implantation

Endometrial receptivity is a prerequisite for successful implantation. The receptivity is maximal when the endometrium is in mid-secretory phase, between cycle days 20 and 24 or 6 and 10 days after ovulation. Considering the steep daily rise in glycodeclin-A expression during the receptive phase, precise dating of the samples in respect of ovulation (e.g. preovulatory LH surge or hCG administration, or oocyte retrieval) is critical for clinical studies. Glycodeclin is abundant in endometrium during the window of implantation (19–21), when the apical membranes lining the uterine cavity develop large membrane projections called pinopodes (71). Glycodeclin is localised on the pinopodes, but it is also secreted from luminal epithelial glands regardless of pinopode formation (72). Temporal associations have been found between glycodeclin and other markers of endometrial receptivity, such as progesterone receptor B (PRB; inverse correlation), leukaemia inhibitory factor receptor (72), $\alpha\text{v}\beta 3$ integrin (vitronectin receptor) (73) and MUC-1 (74), the major cell surface mucin. During implantation, the human blastocyst increases endometrial anti-adhesive MUC-1 protein during the apposition phase, whereas it induces paracrine cleavage in MUC-1 at the implantation site (75). Reduction of MUC1 with increasing glycodeclin concentration at the implantation site may have a connection because glycodeclin transfection has been found to downregulate MUC-1 expression in endometrial carcinoma cells. The significance of the inverse relationship between glycodeclin and MUC-1 in the human implantation remains to be investigated.

Figure 2 Biological actions of glycodelin isoforms in human fertilisation. Data compiled from data in Refs (2, 3, 6, 14, 15, 26, 27, 52, 69, 70).

In regards to endometrial receptivity following controlled ovarian hyperstimulation (COH), glandular/stromal dyssynchrony is common on cycle day 21–23 histology, accelerated maturation being more common in the stroma than in the glands (73). Due to variations in treatment modalities and study designs, results on glycodelin serum levels as predictors of implantation have given mixed results (38, 76). But, in concordance with the advancement of endometrial maturation morphology, endometrial glycodelin-A expression is significantly increased throughout the implantation phase of the cycles with COH when compared with normal menstrual cycles (77). Results of microarray profiling of endometrial genes have confirmed the upregulation of glycodelin at the implantation phase (21) but, more than 200 genes showed more than threefold differential gene expression when the biopsies taken at LH +7 of the previous natural cycle were compared with the biopsies taken at hCG +7 of the COH cycle. The large number of gene expression

disturbance highlights the need for efforts to optimise the COH protocols (78).

Other reasons for variable results arise from technical differences. For instance, some glycodelin peptide antibodies differ from those generated using native glycosylated glycodelin, producing variable results on proliferative versus secretory phase endometrium (15, 79–81). Furthermore, endometrial cells expressing full-length glycodelin protein have shown upregulation of adhesion ability, whereas those expressing a splicing variant that lacks part of glycodelin encoded by exon 4 have shown downregulation of the same (47). Furthermore, there are ethical constraints to study human implantation directly. Therefore, a step forward is the use of an *in vitro* implantation assay (47). It employs attachment of choriocarcinoma cells to endometrial adenocarcinoma cells, mimicking trophoblast-to-endometrium attachment in implantation. Here, induction of glycodelin expression in endometrial cells by either steroid hormones or SAHA has been

found to enhance trophoblast-to-endometrium attachment that can be abrogated by downregulation of the glycodeclin gene with siRNA (47). The dramatic effects brought about by glycodeclin gene silencing demonstrate the important role glycodeclin plays in the trophoblast-to-endometrium attachment. The results also suggest that histone deacetylase inhibitors have the potential to supplant steroids in the treatment of implantation failure (47).

Conditions related to implantation and placentation failure

Unexplained infertility and early pregnancy loss

Unexplained infertility or recurrent miscarriage may result from inadequate implantation and/or placentation. In such conditions, subnormal glycodeclin concentrations have been reported in endometrium, uterine flushings and serum at the time of expected endometrial receptivity (82–85). Both glycodeclin mRNA and protein are downregulated in human first trimester miscarriage (86), and pregnant women with polycystic ovary syndrome (PCOS), who subsequently miscarry show subnormal rise of glycodeclin serum concentration during the first trimester, compatible with weaker placentation (87). As women with PCOS may have insulin resistance, it is not surprising that treatment with metformin has been found to increase glycodeclin serum concentration in non-pregnant women with PCOS (88). However, in non-pregnant women, hyper-insulinaemic clamp causes only small fluctuations within the normal range of serum glycodeclin concentration, demonstrating that insulin has no significant acute effect on serum glycodeclin concentration (89).

Endometriosis Here, endometrial tissue is found outside the uterus, and the condition is associated with pelvic pain and infertility. Implantation failure due to progesterone resistance has been suggested to underlie endometriosis-related infertility (90). This is supported by meta-analysis data showing that pregnancy rates from IVF treatment of women with endometriosis and infertility are only 56% of those in women who undergo IVF treatment for tubal factor infertility (91). Endometriotic lesions are histologically similar to the eutopic endometrium, but they are genetically and biochemically different (92, 93). For instance, implantation failure due to endometrial factors is indicated by paralleled gene expression profiling studies using oligonucleotide microarrays that uncover differentially regulated genes in eutopic endometrium from women with versus without endometriosis (92). Among the genes that are upregulated during the normal window of implantation, many genes were significantly decreased in women with endometriosis. These included glycodeclin. On the other hand, many other genes that are normally downregulated during the window of implantation were significantly upregulated with

endometriosis (92, 93). Although epithelial cells of pelvic and ovarian endometriotic implants express glycodeclin, its cyclic expression is more irregular than in eutopic endometrium (94). Glycodeclin is shed from endometriotic lesions into the peritoneal fluid and/or serum, depending on the type and penetration of the lesions (95, 96). In view that peritoneal fluid from women with endometriosis decreases sperm binding to the zona pellucida in hemizona assay (97), glycodeclin in peritoneal fluid may contribute to this activity. Significantly, eutopic endometrium from women with and without endometriosis shows biochemical differences in many markers that have PRE(s) in their promoter region (98). One of these aberrancies is reduced peri-implantation phase glycodeclin expression in eutopic endometrium, found in women with mild endometriosis. This suggests the presence of endometrial abnormalities that are intrinsic to the disorder rather than a late manifestation (93, 98).

Hormone-related cancer

Breast cancer

Both oestrogen and progesterone are involved in breast carcinogenesis. Evidence from large studies on postmenopausal hormone replacement therapy shows an increased incidence of breast cancer in women taking combined treatment with E and P, but not with E alone (99, 100). The reports have raised concern and criticism because retrospective and observational studies had suggested even protection or no increased risk (101, 102). Notwithstanding this, the reports have led to reduction of long-term hormone replacement therapy in postmenopausal women.

In addition to the occurrence of estradiol receptors (ER) and progesterone receptors (PR) in the normal breast and breast cancer tissue, both glycodeclin mRNA and protein are expressed. In breast cancer tissue, glycodeclin expression has been observed in 69% of ER/PR-negative and 74% of the receptor-positive tumours (31). This irrespective, glycodeclin expression may be significant because of its relationship with growth restriction and cell differentiation in breast cancer cells *in vitro*, and the results showing that glycodeclin transfection into breast cancer cells can lead to reversion of the malignant phenotype (32). In addition, glycodeclin has been shown to reduce breast cancer xenograft growth *in vivo* (Fig. 3). In hormone-responsive tumours, glycodeclin-induced differentiation is related to the decreased expression of oncogenes and an increased expression of tumour suppressor genes, emphasising the tumour suppressor nature of glycodeclin (Table 4). The role of glycodeclin glycosylation in the transfected tumour cells is still an unresolved issue that may account for the diverse responses in the cell lines studied (104). But interestingly, glycodeclin-inducing SAHA has been reported to

act as a trans-differentiation agent in MCF-7 breast cancer cells (105). Therefore, the recent approval of SAHA by the US Food and Drug Administration for the treatment of malignant cutaneous T-cell lymphoma (106) may be of interest for clinical trials also in breast cancer and endometrial cancer (see below). The significance of glycodelin expression in breast cancer patients is further indicated by clinical results showing that glycodelin expression varies according to the clinical stage: it is detected in 100% of carcinoma *in situ* tumours, 90% of invasive carcinomas without metastases, 50% of tumours with lymph node metastases, 40% of those with distant metastases and 38% of recurrent tumours, suggesting prognostic significance of glycodelin expression in cancer tissue (107).

Endometrial cancer

Cancer of the endometrium is the most common gynaecologic malignancy that accounts for 6% of all cancers in women, the incidence rising after 50 years of age. The disease is associated with obesity,

hyperinsulinaemia, unopposed oestrogen exposure and hyperandrogenism which are risk factors for endometrial carcinoma. For instance, prolonged exposure to unopposed oestrogen may result in endometrial hyperplasia and eventually carcinoma (108), and progesterone counteracts this development (109). Likewise, prolonged anovulation in women with PCOS may eventually lead to endometrial cancer, not only through unopposed oestrogen, but also through hyperandrogenaemia, hyperinsulinaemia and insulin resistance that are common in this disorder (110). The most common presenting symptom is abnormal uterine bleeding. Endometrial carcinoma may or may not contain ER and PR, and their presence correlates with the FIGO grade and survival. For detailed guidelines of diagnosis, classification and treatment the readers are referred to the FIGO guidelines (111).

In addition to the alterations in the malignant epithelial cells, endometrial cancer is characterised by alterations in the stromal cells and the supporting extracellular matrix. The basement membrane plays an important part in stromal–epithelial interactions.

Figure 3 Glycodelin transfected human MCF-7 breast cancer cells show morphologic changes towards the normal phenotype in different growth substrata and reduced xenograft tumour growth associated with reduced expression of Ki-67 cell proliferation marker, and increased β -catenin, a sign of favourable prognosis (103). Data adapted from Ref. (104).

Table 4 Glycodelin transfection into hormone related cancer cells regulates expression of genes associated with tumour growth and/or metastasis.

Glycodelin induced changes	Significance ^a	References
Tumour growth/metastasis promoting genes		
Reduces MUC1 and Bcl-X _L ^b	Decreases chemoresistance	(114)
Reduces cyclin-dependent kinase 2 ^c	Reduces tumour growth	(104)
Reduces matrix metalloproteinase 1 ^c	Decreases metastatic propensity	(104)
Reduces cyclin D1 ^d	Reduces tumour growth	(104)
Reduces transglutaminase 2 ^d	Decreases chemoresistance	(104)
Tumour growth/metastasis suppressing genes		
Increases caveolin-1 and FGF2 ^c	Reduces tumour growth	(104)
Increases membranous β -catenin ^{c,d}	Better prognosis	(104)
Increases cyclin dependent kinase inhibitors p21, p27 and p16 ^e	Reduces tumour growth	(115)

^aSuggested cancer related significance of the observed gene expression changes.^bHEC-1B endometrial carcinoma cells.^cMCF-7 breast cancer cells.^dMCF-7 breast cancer xenograft tumours.^eIshikawa endometrial adenocarcinoma cells.

Whereas glycodelin is a major bioactive protein in the normal secretory endometrium, it has not been detected in endometrial adenocarcinoma tissue (112). But, glycodelin can be induced in endometrial adenocarcinoma cells. At the same time as glycodelin becomes expressed the phenotype of carcinoma cells differentiates to more closely resemble normal endometrial epithelium. This has been achieved by reintroduction of stromal factors and appropriate extracellular matrix components to the carcinoma cell culture (113). Interestingly, E and P show little growth effect on Ishikawa carcinoma cells in the absence of stromal-induced factors in conditioned medium, whereas the presence of E- and P-treated stromal medium with basement membrane extract brings about phenotypic reversion and glycodelin expression (113). Of note, overexpression of glycodelin in endometrial adenocarcinoma cells reduces cell proliferation, MUC1 staining, and the antiapoptotic Bcl-X_L gene expression (114). A similar effect can be obtained with HDACIs (46, 47). Importantly, both the HDACI-induced inhibition of cell growth and differentiation and the HDACI-induced glycodelin expression can be attenuated by glycodelin gene silencing with siRNA. This demonstrates the key role glycodelin plays in these changes (46, 47). The results also suggest that, in addition to cancer, HDACI or progesterone induced expression of glycodelin contributes to the suppression of endometrial epithelial growth observed during the secretory phase of the cycle (115). Indeed, overexpression of glycodelin has been found to block G1/S progression in cell proliferation, with concomitant upregulation of CDKIs (115) that are known to inhibit cell cycle progression and retard cell growth (116).

Given that progesterone is involved in endometrial glycodelin secretion and differentiation, one would expect some survival benefit from adjuvant progesterone/progestin treatment of women with endometrial cancer. However, meta-analysis of randomised studies and a subsequent large randomised study have shown

that this is not the case (117, 118). Therefore, progestin treatment is no longer recommended as an adjuvant therapy for endometrial cancer, but the effects on survival, if any, of glycodelin-inducing HDACIs, e.g. SAHA and TSA remain to be examined.

Ovarian cancer

Ovarian cancer accounts for 23% of all gynaecologic cancers and 47% of all deaths from gynaecologic cancer. The largest number of patients with epithelial ovarian cancer is found in the age group 60–64 years (111). The success of treatment depends on early diagnosis. But, early symptoms are rare and there is no effective screening for early disease. Therefore, ovarian cancer is often advanced when detected for the first time. Risk factors for ovarian cancer are essentially reproductive and genetic in nature. Women with no children are at greater risk, and repeated rupture and repair (ovulation) of the surface epithelium provides an opportunity for genetic aberrations to take place. On the other hand, first pregnancy at an early age, early menopause and the use of oral contraceptives are associated with a lower risk (119).

The best biomarker in the management of epithelial ovarian cancer is CA 125 (120). It is better suited for monitoring of treatment than early detection of disease for the first time. Glycodelin is expressed in the normal ovary (26, 33). Glycodelin mRNA and protein have also been found in various types of ovarian tumours, from benign cysts to serous, endometrioid and mesonephric carcinomas, as well as carcinosarcoma (33, 121, 122). In immunohistochemistry, glycodelin is localised to the cancer cells, but staining of various parts of the tissue varies according to the antibodies used. For instance, glycodelin peptide antibodies show wider immunoreactivity extending into vascular endothelium (79, 123) compared with the antibodies against native glycosylated glycodelin that show no vascular or angiogenic connection (15, 38, 122). Moreover, antibodies against

Table 5 Five- and 10-year overall survival rates of patients with glycodelin-positive and -negative ovarian serous carcinoma.

Subjects	n	5-year overall survival (%)	10-year overall survival (%)
All	460	50	40
Gd +ve	301	55*	47 [†]
Gd -ve	159	39*	26 [†]

* $P < 0.001$, [†] $P < 0.0001$. Adapted from Ref. (122).

native glycosylated glycodelin show more frequent expression in well differentiated than in poorly differentiated ovarian serous carcinomas, and it is more common in early stage compared with advanced-stage carcinomas (122). Importantly, the difference remains within the patients of the same grade and the same clinical stage. This was related to survival so that the patients with glycodelin-expressing tumours showed a higher 5- and 10-year overall survival compared with those with glycodelin-negative tumours (Table 5). These results corroborate the findings that glycodelin expression in tumour tissue indicates a favourable prognostic sign, likely due to its differentiation-related actions.

Concluding remarks

Glycodelin provides a well-documented example of the role protein glycosylation plays in human reproduction and immune cell reactivity. The biological actions depending on the carbohydrate moieties of glycodelin isoforms show that the recognition systems between the gametes and the immune cells have converged – they are both based on similar oligosaccharide structures attached to the same protein backbone. While much of the details still remain uncovered, the key role of glycosylation for biological actions has become even more obvious in view that the unique N-glycans alone, derived from glycodelin-A, can modulate hormone production in trophoblast tumour cells (124). The differentiation-inducing propensity of glycodelin is associated not only with restriction of cell growth and reversion of the malignant phenotype *in vitro*, but it also has a connection with clinical oncology – glycodelin expression in tumour tissue is a sign of better prognosis. Finally, the studies employing downregulation of glycodelin by siRNA have shown that glycodelin is actually the molecule by which HDACs execute the differentiation- and cell attachment-enhancing actions. As a HDACi (SAHA) was recently approved by FDA for treatment of a malignant disease, research expanding this propensity into treatment of the hormone-related cancers remains a challenge for future studies.

Declaration of interest

The authors declare that there is no conflict of interest that prejudice the impartiality of this scientific work.

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