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Aberrant pre-receptor regulation of estrogen and progesterone action in endometrial cancer

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Abstract

Endometrial cancer is related to estrogen stimulation not opposed by progesterone. We have examined expression of the pre-receptor regulatory enzymes aromatase, 17 β -hydroxysteroid dehydrogenases (17 β -HSDs), 20 α -hydroxysteroid dehydrogenases (20 α -HSDs), sulfatase and sulfotransferase, and estrogen (ERs) and progesterone (PRs) receptors in samples of endometrial cancer and adjacent normal endometrium. No significant gene up-regulation was seen, although aromatase, AKR1C3, a 17 β -HSD and 20 α -HSD, and AKR1C1, the major 20 α -HSD, were up-regulated in 50% of samples. Significant down-regulation was seen for 17 β -HSD types 1 and 7, sulfotransferase, ER α , ER β , PR-AB. Western blotting revealed higher levels of AKR1C3 and PR-B and lower levels of ER α in cancerous endometrium, and immunohistochemistry confirmed expression of AKR1C3, PR-B and ER α at the cellular level. Up-regulation of aromatase in concert with AKR1C3 can lead to increased levels of estradiol, which acts via ER α . Up-regulation of AKR1C1 and AKR1C3 can result in lower levels of the protective progesterone, which acts mainly via PR-B.

Key words: pre-receptor regulation, hydroxysteroid-dehydrogenases, aromatase, sulfatase, sulfotransferase, ER, PR

Introduction

Endometrial cancer (EC) is the most common gynaecological malignancy and the fourth-most-common cancer in women in Western Europe and the USA (International Agency for Research on Cancer, 2005). Most EC cases are sporadic, with only 10% considered familial (Ryan et al., 2005; Amant et al., 2005). The sporadic cases can be divided into two subgroups: type 1, estrogen-dependent endometrioid carcinomas (80%); and type 2, non-estrogen-dependent EC with serous papillary or clear-cell morphology (20%) (Ryan et al. 2005; Amant et al., 2005). Type 1 EC cases occur in pre-menopausal women (21% in women younger than 45 years) and post-menopausal women and are usually of low grade; they are frequently preceded by endometrial hyperplasia and commonly associated with abnormalities in DNA mismatch repair genes, k-Ras, PTEN and β -catenin (Inoue, 2001; Lax, 2004; Schindler, 2004; Ryan et al., 2005). Type 2 EC occurs mainly in post-menopausal women, and they are usually high-grade tumours that develop *de novo* or through metaplasia and that are associated with abnormalities of p53 and HER2/neu (Inoue, 2001; Lax 2004; Ryan et al., 2005).

Type 1 ECs have been studied in the greatest detail. Clinicopathological findings have indicated that type 1 ECs arise through a series of precursor lesions, in the form of simple, complex and atypical hyperplasia, through unopposed and prolonged estrogen stimulation (Inoue, 2001 Sonoda, 2005). This exposure to estrogens (of endogenous or exogenous origin) that is not opposed by progesterone (P) or synthetic progestins increases the mitotic activity of endometrial cells and the number of DNA replication errors, leading to somatic mutations that can result in a malignant phenotype (Inoue, 2001; Sonoda, 2005). Increased local estradiol (E2) concentrations have been detected in cancerous endometrium (Berstein et al., 2003). In premenopausal women E2 can be formed in the ovaries as well as in peripheral sites; while in postmenopausal women E2 can only be produced in peripheral sites, such as adipose tissue, bone, the vascular endothelium, aortic smooth muscle cells, numerous sites in the brain (Simpson, 2003), and also in cancerous endometrium from inactive precursors of adrenal (dehydroepiandrosterone [DHEA], DHEA sulfate) or ovarian (androstenedione (A)) origin, or from circulating estrone (E1) sulfate (Fig. 1). In peripheral sites, E2 can be formed via the aromatase pathway from DHEA sulfate and DHEA via A or testosterone (T) by the action of sulfatase, 3β -HSD, aromatase and the reductive 17β -HSDs, or from E1-sulfate via the sulfatase pathway by the action of sulfatase and reductive 17β -HSDs (Fig. 1).

The 17β -HSDs that are directly or indirectly involved in E2 formation can be divided into estrogenic and androgenic enzymes, according to their substrate specificities. Among the estrogenic enzymes, 17β -HSDs types 1, 7 and 12 are the reductive enzymes, which can form the potent E2 (K_d , 10^{-10} M, for its corresponding receptors) from less potent E1; 17β -HSD types 2, 4 and 8, catalyze the reverse reaction (Kuiper et al., 1997; Mindnich et al., 2004; Lukacik et al., 2006; Möller et al., 2006). Previous studies have shown that all of the estrogenic 17β -HSDs are expressed in the human uterus and could thus be involved in E2 formation. The kinetic parameters for the reductive estrogenic enzyme isoforms show that 17β -HSD type 1 converts E1 to E2 with a high catalytic efficiency (Puranen et al., 1997; Gangloff et al., 2001), and 17β -HSD type 7 has a much lower efficiency (Törn et al., 2003); 17β -HSD type 12 has not yet been fully characterized and only a K_m has been reported (Luu-The et al., 2006). The kinetic parameters of some of the oxidative 17β -HSDs show that 17β -HSD type 2 is the major oxidative 17β -HSD, while 17β -HSD types 4 and 8 have less important roles (Adamski et al., 1995; Lu et al., 2002; Ohno et al., 2008).

In addition to the estrogenic 17β -HSDs, the androgenic 17β -HSDs can also promote E2 production via the aromatase pathway. There are two reductive androgenic 17β -HSDs, 17β -HSD type 3 and AKR1C3 (also know as 17β -HSD type 5), but only AKR1C3 is

expressed in human endometrium (Penning et al., 2000; Lanišnik Rižner et al., 2006). The kinetic parameters of AKR1C3 show a relatively low catalytic efficiency for T formation (Penning et al., 2006). Among the oxidative androgenic isoenzymes, 17 β -HSD type 2 has the major role in T inactivation (Lu et al., 2002). All 17 β -HSDs except for 17 β -HSD types 1 and 2 catalyze in addition to oxidations/reductions of steroids also conversions of other substrates and have thus also other putative roles.

In addition to the 17 β -HSDs, other enzymes of the aromatase and sulfatase pathways can take part in the production of increased levels of E2. These enzymes are aromatase, a member of the CYP P450 superfamily, and sulfatase and sulfotransferase, members of their respective superfamilies (Sasano and Harada, 1998; Utsunomiya et al., 2004). Aromatase converts A and T to E1 and E2, respectively, with nM K_m values (Krekels et al., 1991; Zhang et al., 2002); sulfatase converts E1-sulfate with a μ M K_m value, while sulfotransferase inactivates E1 and E2 with nM K_m values (Falany et al., 1995; Hernandez-Guzman et al., 2001). All of these enzymes are expressed in human endometrium (Ito et al., 2007).

P counteracts the effects of estrogens by inducing differentiation of the endometrium, and a lack or absence of P or progestagens in an estrogen-stimulated endometrium results in the development of a variety of hyperplastic conditions, from simple, complex and severe hyperplasia, to carcinoma *in situ* (Sasaki et al., 2001; Schindler, 2004). The main sites of P synthesis are the ovaries and the adrenal gland. After menopause, when the ovaries cease to function, the levels of protective P drop, although some P may still be formed in the adrenal gland and at some other peripheral sites, including the endometrium, which has all of the necessary enzymes, including steroidogenic acute regulatory protein STAR, P450 side chain cleavage enzyme P450ssc and 3 β -HSD (Rhee et al., 2003). P can be metabolized by the action of 20 α -HSDs, 5 α -reductases, 5 β -reductases, 3 α -HSDs and 3 β -HSDs to form less potent derivatives that have lower affinities towards the progesterone receptors (PR-A, PR-B) (Fig. 2) (Pollow et al., 1975; Arici et al., 1999). 20 α -HSDs catalyze the reduction of P to 20 α -hydroxyprogesterone (20 α -OHP), a weak progestagen, and the oxidation of 20 α -OHP to P. 20 α -HSD activity has been reported for the aldo-keto isozymes AKR1C1 and AKR1C3, and 17 β -HSD types 1 and 2 (Penning et al., 2000). AKR1C1, AKR1C3 and 17 β -HSD type 1 are reductases and inactivate P, and 17 β -HSD type 2 acts as an oxidase and converts inactive 20 α -OHP back to the active P. All of these enzymes are expressed in normal and diseased endometrium (Lanišnik Rižner et al., 2006).

The goals of the present study were to examine the pre-receptor regulation of estrogen and P action within cancerous endometrium, to correlate the expression of the pre-receptor regulatory enzymes with the expression of ERs and PRs, and to propose a hypothesis regarding the mechanisms of aberrant regulation of estrogen and P action.

Methods

Endometrial tissue

The 25 endometrial cancer specimens and adjacent normal endometrium were obtained from postmenopausal patients undergoing hysterectomies (Table 1). The study was approved by the National Medical Ethics Committee of the Republic of Slovenia. Expression analysis of 17 β -HSD types 4, 8 and 12, and AKR1C3 was performed on the 25 samples, and for another 10 genes on 16 samples of endometrial cancer.

RNA isolation

Total RNA was isolated from the tissue samples using Tri Reagent (Sigma), according to the manufacturer instructions. The quality of the RNA samples was determined using an Agilent 2100 Bioanalyzer. The total RNA was reverse transcribed using random hexamer primers (Invitrogen), as described previously (Lanišnik Rižner et al., 2006).

Real-time PCR

The expression of 14 genes, and cyclophilin A as the house-keeping gene, was examined using real-time Taq Man PCR assays. The primers and fluorescent TaqMan[®] MGB probes for the amplification of AKR1C1 and AKR1C3 were designed as described previously (Lanišnik Rižner et al., 2006). All of the primers and minor groove-binder probes (FAM or VIC[®] dye-labelled) were obtained from Applied Biosystems. The RT-PCR samples were run in duplicate, and the relative quantifications of gene expression were performed using the standard curve method (for AKR1C1 and AKR1C3) or the comparative Ct method (other genes). Expression levels of all genes except AKR1C1, AKR1C3 and PR-AB were multiplied by 10⁵ and logarithmically transformed.

Statistical evaluation

The differences in the expression levels of the selected genes in the cancerous endometrium, as compared to the adjacent normal endometrium, were analyzed using the Wilcoxon test. The statistical tests were two-tailed, and differences in p values of less than 0.05 were considered to be significant. The statistical calculations and tests were performed using the SPSS software (SPSS Inc, USA).

Western blotting

Protein fractions were isolated from samples of cancerous endometrium and the adjacent normal endometrium previously used for RNA isolation (following the Tri Reagent instructions). Protein aliquots of 70 μ g were separated by SDS PAGE on 12% gels. The proteins were transferred from gels to PVDF membranes and incubated with 5% non-fat milk in TTBS for 1 h. The membranes were then incubated overnight at 4 °C with primary mouse monoclonal antibodies: anti-AKR1C3 (1:1000, Sigma-Aldrich, USA) anti-PRB (1:500, Santa Cruz USA), anti-ER α (1:200, DakoCytomation, Denmark), or anti- β -actin (1:2500, Sigma-Aldrich, USA) antibodies in TTBS with 1% non-fat milk powder. The next day, the membranes were incubated with the secondary antibodies (peroxidase-conjugated goat anti-mouse IgG+IgM (H+L)) dilutions 1:3000; 1:10000 and 1: 3000, respectively (Jackson ImmunoResearch Laboratories Inc., USA) for 1 h. Following the washing steps, Supersignal West Pico Chemiluminescence Substrate (Pierce Biotechnology) was used for the detection of the bound antibodies, according to the manufacturer instructions. Quantification of Western Blots was done with UVIband software (Uvitec, Cambridge, UK).

Immunohistochemistry

The formalin-fixed, paraffin-embedded endometrium samples were de-waxed in xylene and rehydrated. The sections were incubated in H₂O₂ to block endogenous peroxidase. After antigen retrieval in sodium citrate buffer, the sections were incubated with monoclonal mouse antibodies: anti-AKR1C3 (1:500, Sigma-Aldrich, USA), anti-aromatase (1:20, AbD Serotec, Germany) and anti-ER α (1:20, DakoCytomation, Denmark). The peroxidase–antiperoxidase complex with substrate DAB was used to detect bound antibodies.

Results and Discussion

1. Estradiol production in cancerous endometrium

The aromatase pathway

We first examined the expression of enzymes involved in the aromatase pathway (Fig. 1). The data on aromatase expression are rather controversial: Bulun et al. (2005) have shown aromatase transcripts in endometriosis tissue, but not in endometrial tissue from disease-free women, while Jongen et al. (2005) detected significant aromatase activities in both benign and malignant endometrial tissue, and Watanabe et al. (1995) even reported on increased aromatase activity in neoplastic endometrium. Our data here showed no significant differences in expression levels between the cancerous and the adjacent normal endometrium, and they are in agreement with similar studies on aromatase expression in cancerous endometrium in comparison with normal endometrium (Pathirage, et al., 2006). However, in the present study, a pair-wise comparison revealed an increased mRNA ratio of tumour:normal tissue in seven of the 16 samples, with the expression in the cancerous endometrium in three samples being three-fold higher than that in the normal endometrium (Fig. 3). The expression of aromatase was also confirmed at the cellular level by immunohistochemical staining, showing a weak signal in glandular epithelial cells (Fig. 3).

Next, we examined the expression of reductive 17 β -HSDs, and surprisingly we detected significantly lower expression levels of 17 β -HSD types 1 and 7 in the cancerous endometrium, 2.2-fold ($p = 0.015$) and 1.2-fold ($p = 0.004$), respectively there was no significant difference in the expression of 17 β -HSD type 12 (Fig. 4). These data thus suggest a decreased local production of E2. The expression of the oxidative 17 β -HSDs can also affect local E2 concentrations. Utsonomiya et al. (2003) detected the expression of 17 β -HSD type 2 in only 50% of their endometrial cancer specimens at the protein level. In our specimens here, 17 β -HSD type 2 was not expressed in one of 16 cancerous samples, and we did not detect significant differences in its expression levels in the cancerous compared to the normal endometrium. We also did not detect significant differences in the expression of 17 β -HSD types 4 and 8 (Fig. 4). The expression of 17 β -HSD types 8 and 12 has not yet been examined in human endometrium, and we are here the first to show their expression in cancerous endometrium.

We also examined the expression of AKR1C3, the last enzyme of the aromatase pathway. As we have shown previously (Lanišnik Rižner et al., 2006), the median AKR1C3 expression levels did not differ significantly between cancerous and adjacent normal endometrium, although the pair-wise comparison revealed an increased ratio of cancerous versus normal tissue in 7 samples, out of 16 tested (Lanišnik Rižner et al., 2006). Here, we examined AKR1C3 expression in a larger group of 25 patients with endometrial cancer, with higher mRNA levels seen in 12 samples of cancerous endometrium (Fig. 5). These results led us to investigate AKR1C3 expression also at the protein level (Fig. 5). We isolated the protein fractions of the same 16 samples of cancerous and adjacent normal endometrium that had previously been analyzed by real-time PCR, and we performed Western blotting with ECL detection. In eight of the 16 cases, we detected higher AKR1C3 levels, and in 3 cases lower levels, in the cancerous than the normal endometrium; in 2 cases there were no differences in expression, and in 3 samples we did not detect AKR1C3 (Fig. 5). Comparing the means, we saw a 2.5-fold up-regulation, which was not statistically significant. Expression of AKR1C3 was confirmed also at the cellular level by immunohistochemistry: monoclonal anti-AKR1C3 antibodies stained the glandular and luminal epithelial cells in cancerous endometrium in all of the 10 samples examined (Fig. 6).

Our data thus suggest that in some patients, the aromatase pathway via T has an important role in E2 production within cancerous endometrium. The increased expression of AKR1C3 can lead to increased levels of T, which can be converted to E2 by aromatase. Also, the aromatase pathway via A can have a certain role, as AKR1C3 can act also as an estrogenic 17 β -HSD and may be involved in E2 formation from E1. However, AKR1C3 has the highest catalytic efficiency when acting as a 20 α -HSD, which converts P into the less active 20 α -OHP; therefore, AKR1C3 may have an even more important role in decreasing the concentrations of protective P.

The sulfatase pathway

The data regarding the expression of sulfatase and sulfotransferase in endometrial cancer are also controversial. Utsunomiya et al. (2004) have shown increased sulfatase and decreased sulfotransferase immunoreactivities, suggesting increased availability of the biologically active estrogens, while Tanaka et al. (2003) have shown lower sulfatase activity in cancerous tissue than in normal endometrium. In the present study, there were no significant differences in the expression of sulfatase, while there was a significantly decreased (nine-fold; $p = 0.041$) expression of sulfotransferase in the cancerous endometrium, thus leading to a six-fold increase in the sulfatase:sulfotransferase ratio that affects E1 and E2 production in the cancerous endometrium (Fig. 7). Our data thus suggest that the sulfatase pathway has an important role in E2 formation within the cancerous endometrium; however, sulfatase should also be acting in concert with the reductive 17 β -HSDs.

2. Estradiol production in correlation with the expression of estrogen receptors

Previously, significantly higher levels of ER α than ER β have been reported in the specimens of normal and diseased endometrium (Utsunomiya, et al., 2000; Sakaguchi et al., 2002). The ratio of ER α :ER β has differed across normal and cancerous tissues, with both decreases and increases seen in this ratio (Saegusa et al., 2000; Utsunomiya et al., 2000; Sakaguchi et al., 2002). Our real-time PCR analysis showed both ER α and ER β to be significantly down-regulated in cancerous endometrium (Fig. 8), thus agreeing with the study of Hu et al. (2005). However, in the present study, the ER α :ER β ratio did not change significantly. Decreased expression of ER β has previously been seen in many estrogen-dependent tumours (Bardin et al., 2004). ER β acts as a modulator of ER α and has an antiproliferative and pro-apoptotic role (Lindberg et al., 2003). Since ER β is considered to be a repressor of ER α , its lower expression in the cancerous endometrium should lead to an enhanced E2 action through ER α . We confirmed the expression of ER α also at the protein and the cellular levels (in all samples examined) (Figs. 6 and 8), suggesting that E2 formed locally would act through ER α .

3. Progesterone metabolism in correlation with expression of progesterone receptors

We have examined the expression of the human 20 α -HSDs, the reductive AKR1C1, AKR1C3 and 17 β -HSD type 1, and the oxidative 17 β -HSD type 2. There were no significant differences in the expression of the major reductive enzymes AKR1C1 and AKR1C3, which convert potent P to less active 20 α -OHP, in both cancerous endometrium and adjacent normal endometrium (Fig. 5). There was also no significant difference in expression of the oxidative enzyme. The reductive 17 β -HSD type 1, which has the lower catalytic efficiency for P inactivation, was significantly down-regulated (Fig. 4). Previously we reported higher levels of AKR1C1 and AKR1C3 mRNA in 9 and 7 samples of cancerous endometrium, out of 16 examined, respectively (Lanišnik Rižner et al., 2006), and now we have re-examined AKR1C3 expression in a larger group of 25 patients with endometrial cancer (Fig. 5). There were still no significant differences detected between the median expression levels, although

higher levels of AKR1C3 were seen in 12 endometrial cancer samples out of 25. Up-regulation of AKR1C1 and AKR1C3, which was observed in the same specimens, would lead to decreased levels of protective progesterone in these patients.

We also examined the expression of the progesterone receptors PR-AB, with real-time PCR revealing significantly lower levels of PR-AB (Fig. 5), while further Western blotting showed significantly higher levels of PR-B protein in samples of endometrial cancer, as compared to normal endometrium (Fig. 5), suggesting that the lower levels of PR-AB arises as a result of down-regulation of PR-A. Expression of PR-B was confirmed also at the cellular level by immunohistochemical staining, where epithelial glandular cells were positive in all 8 cases examined (Fig. 6). The major role of PR-A in the endometrium is to oppose estrogen action by preventing ER α transactivation and estrogen-induced endometrial proliferation, partly by limiting PR-B effects (Ryan et al., 2005). In contrast, PR-B acts as an endometrial estrogen agonist (Ryan et al., 2005). Aberrant ratios of these PR isoforms have been reported in endometrial hyperplasia, complex atypical hyperplasia, and endometrial cancer (Arnett-Mansfield et al., 2001; Ryan et al., 2005), and dominant expression of PR-B has been reported in all metastatic lesions of uterine endometrial cancers (Fujimoto, et al. 2000), in agreement with our data here. Shabani et al. (2007) examined the expression of PR-A and PR-B in 293 endometrial cancer cases by immunohistochemistry, and reported an association between the lack of PR-A expression and poor differentiation in endometrial cancer patients. Also, a functional polymorphism in the promoter of PR that results in increased transcription of PR-B, an altered PR-A/PR-B ratio, and is associated with increased risk of EC, shows the important role of PR-B in EC development (De Vivo, 2002). Our data thus suggest that the lower levels of protective P act mainly via PR-B.

4. Proposed mechanisms of disturbed estrogen and progesterone metabolism and action in cancerous endometrium

In some endometrial cancer patients, it appears that up-regulation of AKR1C3 (in 12 out of 25 specimens) in concert with up-regulation of aromatase (in seven out of 16) might be responsible for an increased local E2 production. Also, an increased ratio of sulfatase:sulfotransferase (six-fold) might result in higher E2 levels. However, sulfatase should act in concert with the reductive 17 β -HSD, and the reductive estrogenic 17 β -HSD, which would be up-regulated in cancerous endometrium, has not yet been identified (Fournier and Poirier, 2008). This may be the role of AKR1C3, which also acts as an estrogenic 17 β -HSD. The E2 formed in the cancerous endometrium would then exert its action through ER α , an action that can be further enhanced by the down-regulation of ER β (2.6-fold down-regulated). However, estrogens may also be metabolized to genotoxic, mutagenic metabolites that can initiate carcinogenesis (Bolton and Thatcher, 2008). Both E1 and E2 can be hydroxylated by different cytochrome P450 enzymes (CYP1A1, CYP1A2, CYP1B1) to form catechols, and further oxidised to estrogen o-quinones, which can form DNA adducts or cause oxidative damage to DNA (Bolton and Thatcher, 2008).

In some endometrial cancer patients, up-regulation of AKR1C1 (in 9 cases out of 16) and AKR1C3 (in 12 cases out of 25) can lead to decreased levels of protective P. The action of P may be further disturbed by down-regulation of PR-AB and up-regulation of PR-B, which can result in lower levels of PR-A and a changed ratio of PR-B/PR-A. Our data suggest that levels of PR-A may be insufficient to oppose estrogen action, by preventing ER α transactivation and endometrial proliferation.

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References

- Adamski, J., Normand, T., Leenders, F., Monte, D., Begue, D., Stehelin, D., Jungblut, P.W., De Launoit, Y. 1995. Molecular cloning of a novel widely expressed human 80 kDa 17 β -hydroxysteroid dehydrogenase IV. *Biochem. J.* 311, 437-443.
- Arici, A., Marshburn, P.B., MacDonald, P.C., Dombrowski, R.A. 1999. Progesterone metabolism in human endometrial stromal and gland cells in culture. *Steroids* 64, 530-534.
- Armett-Mansfield, R-L-, deFazio, A., Wain, G.V., Jaworski, R.C., Byth, K., Mote, P.A., Clarke, C.L. 2001. Relative expression of progesterone receptors A and B in endometrioid cancers of the endometrium. *Cancer Res.* 61, 4576-4582.
- Amant, F., Moerman, P., Neven, P., Timmerman, D., Van Limbergen, E., Vergote, I. 2005. Endometrial cancer. *Lancet* 366, 491-505.
- Bardin, A., Boulle, N., Lazennec, G., Vignon, F., Pujol, P. 2004. Loss of ER β expression as a common step in estrogen-dependent tumour progression. *Endocrine-Related Cancer*, 11, 537-551.
- Berstein, L.M., Tchernobrovkina, A.E., Gamajunova, V.B., Kovalevskij, A.J., Vasilyev, D.A., Chepik, O.F., Turkevitch, E.A., Tsyrlina, E.V., Maximov, S.J., Ashrafian, L.A., Thijssen, J.H.H. 2003. Tumour estrogen content and clinico-morphological and endocrine features of endometrial cancer. *J. Cancer. Res. Clin. Oncol.* 129, 245-249.
- Bolton, J.L., Thatcher, G.R.J. 2008. Potential mechanisms of estrogen quinone carcinogenesis. *Chem. Res. Toxicol.* 21, 93-101.
- Bulun, S.E., Imir, G., Utsunimiya, H., Thung, S., Gurates, B., Tamura, M. 2005. Aromatase in endometriosis and uterine leiomyomata. *J. Steroid Biochem. Mol. Biol.*, 95, 57-62.
- De Vivo, I., Huggins, G.S., Hankinson, S.E., Lescault, P.J., Boezen, M., Colditz, G.A., Hunter, D.J. 2002. A functional polymorphism in the promoter of the progesterone receptor gene associated with endometrial cancer risk. *Proc. Natl. Acad. Sci. USA* 99, 12263-12268.
- Falany, C.N., Krasnykh, V., Falany, J.L. 1995. Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. *J. Steroid Biochem. Mol. Biol.* 52, 529-539.
- Fournier, M.A., Poirier, D. 2008. Estrogen formation in endometrial and cervix cancer cell lines: Involvement of aromatase, steroid sulfatase and 17 β -hydroxysteroid dehydrogenases types 1, 5, 7 and 12. *Mol. Cell. Endocrinol.* (this issue)
- Fujimoto, J., Sakaguchi, H., Aoki, I., Khatun, S., Toyoki, H., Tamaya, T. 2000. Steroid receptors and metastatic potential in endometrial cancers. *J. Steroid Biochem. Mol. Biol.* 209-212.
- Gangloff, A., Garneau, A., Huang, Y.-W., Yang, F., Lin, S.-X. 2001. Human oestrogenic 17 β -hydroxysteroid dehydrogenase specificity: enzyme regulation through an NADPH-dependent substrate inhibition towards the highly specific oestrone reduction. 356. 269-276.
- Hernandez-Guzman, F.G., Higashiyama, T., Osawa, Y., Ghosh, D. 2001. Purification, characterization and crystallization of human placental estrone/dehydroepiandrosterone sulfatase, a membrane-bound enzyme of the endoplasmic reticulum. *J. Steroid Biochem. Mol. Biol.* 78, 441-450.
- Hu, K., Zhong, G., He, F. 2005. Expression of estrogen receptors ER α and ER β in endometrial hyperplasia and adenocarcinoma. *Int. J. Gynecol. Cancer*, 15, 537-541.
- Inoue, M. 2001. Current molecular aspects of the carcinogenesis of the uterine endometrium. *Int. J. Gynecol. Cancer*, 11, 339-348.
- International Agency for Research on Cancer, 2005. Globocan 2002.

- Ito, K., Utsunomiya, H., Yaegashi, N., Sasano, H. 2007. Biological roles of estrogen and progesterone in human endometrial carcinoma – new developments in potential endocrine therapy for endometrial cancer. *Endocrine J.* 54, 667-679.
- Jongen, V.H., Thijssen, J.H., Hollema, H., Donker, G.H., Santema, J.G., Van der Zee, A.G., Heineman, M.J. 2005. Is aromatase cytochrome P450 involved in the pathogenesis of endometrioid endometrial cancer? *Int. J. Gynecol. Cancer*, 15, 529-536.
- Krekels, M.D., Wouters, W., De Coster, R., Van Ginckel, R., Leonaers, A., Janssen, P.A. 1991. Aromatase in the human choriocarcinoma JEG-3: inhibition by R76713 in cultured cells and in tumours grown in nude mice. *J. Steroid Biochem. Mol. Biol.* 38, 415-422.
- Kuiper, G.J.M., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J.-Å. 1997. Comparison of the ligand-binding specificity and transcript tissue distribution of estrogen receptor α and β . *Endocrinology*, 138, 863-870.
- Lanišnik Rižner, T., Šmuc, T., Ruprecht, R., Šinkovec, J., Penning, T.M. 2006. AKR1C1 and AKR1C3 may determine progesterone and estrogen ratios in endometrial cancer. *Mol. Cell. Endocrinol.*, 248, 125-135.
- Lax, S.F. 2004. Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. *Virchows. Arch.* 444, 213-223.
- Lindberg, M.K., Moverare, S., Skrtic, S., Gao, H., Dahlman-Wright, K., Gustafsson, J.-Å., Ohlsson, C. 2003. Estrogen receptor ER β reduces ER α regulated gene transcription, supporting a ying-yang relationship between ER α and ER β . *Mol. Endocrinol.*, 17, 203-208.
- Lu, M.-L., Huang, Y.-W., Lin, S.-X. 2002. Purification, reconstitution, and steady-state kinetics of the trans-membrane 17 β -hydroxysteroid dehydrogenase 2. *J. Biol. Chem.*, 277, 22123-22130.
- Lukacik, P., Kavanagh, K.L., Oppermann, U. 2006. Structure and function of 17 β -hydroxysteroid dehydrogenase. *Mol. Cell. Endocrinol.*, 248, 61-71.
- Luu-The, V., Tremblay, P., Labrie, F. 2006. Characterization of type 12 17 β -hydroxysteroid dehydrogenase, an isoform of type 3 17 β -hydroxysteroid dehydrogenase responsible for estradiol formation in women. *Mol. Endocrinol.*, 10, 437-443.
- Mindnich, R., Möller, G., Adamski, J. 2004. The role of 17 β -hydroxysteroid dehydrogenases. *Mol. Cell. Endocrinol.*, 215, 19-30.
- Möller, G., Adamski, J. 2006. Multifunctionality of human 17 β -hydroxysteroid dehydrogenase. *Mol. Cell. Endocrinol.*, 248, 47-55.
- Ohno, S., Nishikawa, K., Honda, Y., Nakajin, S. 2008. Expression in *E. coli* and tissue distribution of the human homologue of the mouse Ke6 gene, 17 β -hydroxysteroid dehydrogenase type 8. *Mol. Cell. Biochem.* 309, 209-215.
- Pathirage, N., Nezza, L.A., Salmonsens, L.A., Jobling, T., Simpson, E.R., Clyne, C.D. 2006. Expression of aromatase, estrogen receptors, and their coactivators in patients with endometrial cancer. *Fertil. Steril.* 86, 469-472.
- Penning, T.M., Burczynski, M.E., Jez, M.E., Hung, C.F., Lin, H.K., Ma, H., Moore, M., Palackal, N., Ratnam K. 2000. Human 3 α -hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex steroids. *Biochem. J.*, 351, 67-77.
- Penning, T.M., Steckelbroeck, S., Bauman, D.R., Miller, M.W., Jin, Y., Peehl, D.M., Fung, K.-M., Lin, H.-K. 2006. Aldo-keto reductase (AKR) 1C3: Role in prostate disease and the development of specific inhibitors. *Mol. Cell. Endocrinol.* 248, 182-191.

- Pollow, K., Luebbert, H., Boquoi, E., Pollow, B. 1975. Progesterone metabolism in normal human endometrium during the menstrual cycle and in endometrial carcinoma. *J. Clin. Endocrinol. Metab.* 41, 729-737.
- Puranen, T., Poutanen, M., Ghosh, D., Vihko, P., Vihko, R. 1997. Characterization of structural and functional properties of human 17 β -hydroxysteroid dehydrogenase type 1 using recombinant enzymes and site-directed mutagenesis. *Mol. Endocrinol.* 11, 77-86.
- Rhee, H.S., Oh, S.H., Ko, B.J., Han, D.M., Jeon, B.H., Park, H., Moon, H.B., Kim, W.S. 2003. Expression of 3 β -hydroxysteroid dehydrogenase and P450 side chain cleavage enzyme in the human uterine endometrium. *Exp. Mol. Med.* 35:160-166.
- Ryan, A.J., Susil, B., Jobling, T.W., Oehler, M.K. 2005. Endometrial cancer. *Cell Tissue Res.* 322, 53-61.
- Sakaguchi, H., Fujimoto, J., Aoki, I., Toyoki, H., Khatun, S., Tamaya, T., 2002. Expression of oestrogen receptors α and β in uterine endometrial and ovarian cancers. *Eur. J. Cancer.*, 38, S74-S75.
- Sasaki, M., Dharia, A., Oh, B.R., Tanaka, Y., Fujimoto, S., Dahia, R. 2001. Progesterone receptor B gene inactivation and CpG hypermethylation in human uterine endometrial cancer. *Cancer Res.* 61, 97-102.
- Sasano, H., Harada, N. 1998. Intratumoral aromatase in human breast, endometrial and ovarian malignancies. *Endocrine Rev.*, 19, 593-607.
- Sasano, H., Suzuki, T., Takeyama, J., Utsunomiya, H., Ito, K., Ariga, N., Moriya, T. 2000. 17 β -hydroxysteroid dehydrogenase in human breast and endometrial carcinoma. *Oncology*, 59, 5-12.
- Schindler A.E. 2004. Progestogens, endometrial hyperplasia and endometrial cancer. *Gynecol. Forum.* 9, 31-33.
- Shabani, N., Kuhn, C., Kunze, S., Schulze, S., Mayr, D., Dian, D., Gingelmaier, A., Schindlbeck, C., Willgeroth, F., Sommer, H., Jeschke, U., Friese, K., Mylonas, I. 2007. Prognostic significance of oestrogen receptor alfa (ER α) and beta (ER β), progesterone receptor A (PR-A) and b (PR-b) in endometrial carcinomas. *Eur. J. Cancer* 43, 2434-2444.
- Simpson, E.R. 2003. Sources of estrogen and their importance. *J. Steroid Biochem. Mol. Biol.* 86, 225-230.
- Sonoda, Y., Brakat, R.R. 2006. Screening and the prevention of gynecologic cancer. Endometrial cancer. *Best Pract. Res. Clin. Obstet. Gynaecol.* 20. 363-377.
- Tanaka, K., Kubushiro, K., Iwamori, Y., Okairi, Y., Kiguchi, K., Ishiwata, I., Tsukazaki, K., Nozawa, S., Iwamori, M. 2003. Estrogen sulfotransferase and sulfatase: roles in the regulation of estrogen activity in human uterine endometrial carcinomas. *Cancer Sci.*, 94, 871-876.
- Törn, S., Nokelainen, P., Kurkela, R., Pulkka, A., Menjivar, M., Ghosh, S., Coca-Prados, M., Peltoketo, H., Isomaa, V., Vihko, P. 2003. Production, purification, and functional analysis of recombinant human and mouse 17 β -hydroxysteroid dehydrogenase type 7. *Biochem. Biophys. Res. Comm.*, 305, 37-45.
- Utsunomiya, H., Suzuki, T., Harada, N., Ito, K., Matsuzaki, S., Konno, R., Sato, S., Yajima, A., Sasano H. 2000. Analysis of estrogen receptor alpha and beta in endometrial carcinomas: correlation with ER beta and clinicopathologic findings in 45 cases. *Int. J. Gynecol. Pathol.*, 19, 355-341.
- Utsunomiya, H., Suzuki, T., Ito, K., Moriya, T., Konno, R., Sato, S., Yaegashi, N., Okamura, K. and Sasano, H. 2003. The correlation between the response to progestogen treatment and the expression of progesterone receptor B and 17 β -hydroxysteroid dehydrogenase type 2 in human endometrial carcinoma. *Clin. Endocrinol.*, 58, 696-703.

- Utsunomiya, H., Ito, K., Suzuki, T., Kitamura, T., Kaneko, C., Nakata, T., Niikura, H., Okamura, K., Yaegashi, N., Sasano, H. 2004. Steroid sulfatase and estrogen sulfotransferase in human endometrial carcinoma. *Clin. Cancer Res.*, 10, 5850-5856.
- Watanabe, K., Sasano, H., Harada, N., Ozaki, M., Niikura, H., Sato, S., Yajima, A. 1995. Aromatase in human endometrial carcinoma and hyperplasia. *Am. J. Pathol.* 146, 491-500.
- Zhang, F., Zhou, D., Kao, Y.C., Ye, J., Chen, S. 2002. Expression and purification of a recombinant form of human aromatase from *Escherichia coli*. *Biochem. Pharmacol.* 64, 1317-1324.

Figure Legends

Figure 1. Local production of estradiol.

Estradiol formation in human endometrium, showing the aromatase pathway from DHEA sulfate and DHEA via androstenedione and testosterone, and the sulfatase pathway from estrone sulfate. DHEA, dehydroepiandrosterone; 17 β -HSDs, 17 β -hydroxysteroid dehydrogenases.

Figure 2. Metabolism of progesterone

Metabolism of progesterone by 20 α -hydroxysteroid dehydrogenases (20 α -HSDs), 5 α -reductase, 3 α - and 3 β -hydroxysteroid dehydrogenases (3 α - and 3 β -HSDs), with the formation of derivatives with lower affinities towards PRs.

Figure 3. Expression of aromatase

A) Relative expression levels (normalized to cyclophilin A) in paired samples of endometrial cancer and normal endometrium. B) Immunohistochemical staining in cancerous endometrium (a) and control staining without the primary antibodies (b).

Figure 4. Expression of reductive and oxidative 17 β -HSDs

Relative expression levels (to cyclophilin A) in paired samples of endometrial cancer and normal endometrium of 17 β -HSD types 1 (A), 7 (B), 12 (C), 2(D), 4 (E) and 8 (F).

Figure 5. Expression of AKR1C3, AKR1C1, PR-A/B and PR-B

Relative expression levels (to cyclophilin A) of AKR1C1 (A), AKR1C3 (B), and PR-AB (C) genes in normal endometrium and endometrial cancer. AKR1C3 (D) and PR-B (E) expression at the protein level. Mean protein levels of AKR1C3 and PR-B normalized to β -actin $\pm$ standard error of means (S.E.M) are shown.

Figure 6. Expression of AKR1C3, ER α and PR-B at the cellular level

Immunohistochemical staining of AKR1C3, ER α , and PR-B in four specimens (8, 19, 22 and 23) of cancerous endometrium, and control staining without the primary antibodies.

Figure 7. Expression of sulfatase and sulfotransferase

Relative expression levels (to cyclophilin A) in paired samples of endometrial cancer and normal endometrium of sulfatase (A), and sulfotransferase (B).

Figure 8. Expression of ER α , ER β

Relative expression levels (to cyclophilin A) of ER α (A) and ER β (B) genes in normal endometrium (normal) and endometrial cancer (cancer). C) ER α expression at the protein level. Mean protein levels of ER α normalized to β -actin $\pm$ standard error of mean (S.E.M) is shown.

Table 1: Patients details for the cancer group.

Number	Sample**	Age	Diagnosis
1	5	37	G2/3
2	7	50	G1
3	8	83	G2/3
4	10	53	G1
5	11	60	G1
6	13	54	G2/S
7	18	79	G1
8	19	74	G1/2*
9	20	76	G2/3
10	21	53	G2
11	22	36	G1/2*
12	23	45	G1/2*
13	24	69	G2/3*
14	25	54	G3/CC*
15	26	72	G1/2
16	27	62	S*
17	30	54	G1
18	32	52	G1
19	33	77	G3
20	34	57	G1
21	35	61	G2
22	38	78	G2/3
23	40	71	G1
24	42	81	G1
25	44	73	G1

Mean age 62.4 ± 13.7 years, G1-G3: grading according to histological differentiation, S: serous adenocarcinoma, CC: clear cell adenocarcinoma, *atypical hyperplasia. **The specimen numbers are arbitrary, for the convenience of the study.

Figure 1

Figure 2

B)