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Expression of the epidermal growth factor system in endometrioid endometrial cancer

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Abstract

Objective. The Epidermal Growth Factor (EGF) system is expressed in healthy premenopausal endometrium. We describe the expression of the four receptors, HER1, HER2, HER3, HER4 and the six ligands amphiregulin, transforming growth factor α (TGF-α), heparin binding EGF like growth factor (HB-EGF), betacellulin, epiregulin and EGF in endometrioid endometrial cancer.

Methods. We have uterine samples from 45 women with endometrioid endometrial cancer. As normal counterparts, endometrial samples from thirteen postmenopausal women, and previous data on fourteen premenopausal women were employed. Extracted RNA was analyzed by real-time PCR; the receptors and ligands were localized by immunohistochemistry.

Results. Three receptors (HER1, HER2 and HER4) and two detectable ligands (TGF-α and HB-EGF) are expressed significantly higher in endometrial cancer than in healthy postmenopausal endometrium. Cancer tissue show significantly lower expression of HER1 and HER3, and higher expression of HER4, amphiregulin, TGF-α and HB-EGF compared to premenopausal endometrium; no difference is seen in HER2. EGF is undetectable in all of the samples. Immunohistochemically the receptors locate to the epithelium and/or glands while the ligands locate to the stroma (amphiregulin), the stroma and the epithelium (TGF-α, epiregulin), the epithelium (betacellulin) or are not detectable (HB-EGF, EGF).

Conclusions. mRNA of all receptors and five ligands are present in endometrioid endometrial cancer, and the protein of all receptors and four ligands are identified by immunohistochemistry. The expression pattern in endometrioid endometrial cancer differs from the pattern in pre- and postmenopausal endometrium. The most remarkable finding is an increased level of HER4, a receptor which correlates to a favorable prognosis in other types of cancers.

Keywords: Human endometrium; Endometrial cancer; Endometrioid; HER1; HER2; HER3; HER4; Amphiregulin; TGF-α; HB-EGF; Betacellulin; Epiregulin; EGF; Immunohistochemistry; Real-time PCR

Introduction

The epidermal growth factor (EGF) system consists of four receptors, Human Epidermal growth factor Receptor 1 (HER1) (also called EGF receptor/EGFr/ErbB1), HER2 (also called ErbB2), HER3 and HER4 and numerous ligands or EGF-related peptide growth factors including amphiregulin (AR), transforming growth factor-α (TGF-α), heparin binding EGF like growth factor (HB-EGF), betacellulin (BCL), epiregulin (EPI) and EGF. The receptors are trans-membrane glycoproteins with an extracellular ligand-binding domain, a trans-membrane region and an intracellular domain with tyrosine kinase activity. Extracellular ligand binding induces dimerization and consequently activation of the intra-cellular tyrosine kinase.

The ligands mentioned binds to HER1 and HB-EGF, EPI and BCL also bind to HER4. HER2 has no ligands of its own, but

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works as the preferred co-factor for the other receptors, HER3 functions solely in heterodimerization due to impaired tyrosine kinase while HER1 and HER4 form both hetero- and homodimers.

The EGF system is ubiquitous in human organs and play fundamental roles in different processes such as embryogenesis, development, proliferation and differentiation [1–3].

In cancer, the EGF system participates in proliferation, migration and invasion, differentiation and angiogenesis. The impact is driven by interaction with other membrane bound receptors and intra-cellular compounds [4].

Previously, we have described the expression of the EGF system in healthy human endometrium [5]. We found a cyclical variation in mRNA expression of all four receptors and two of the six investigated ligands, and concluded that the EGF system possibly could be involved in normal cyclical growth of human endometrium.

Endometrial cancer is the seventh most common cancer in women worldwide [6]. In Denmark, there is approximately 600 new cases per year, thereby making Denmark one of the countries in Europe with the highest incidence [7]. The overall 5 year survival is 70% and the 5 year relative survival is 81%, pointing to a highly curable cancer. The survival depends on stage at time of diagnosis, grading and histology of the tumor. These factors are also the basis for classifying the women in groups of low or high risk of recurrent disease. Low risk or type I cancers comprise of endometrioid cancer grade 1 and 2, and grade 3 with less than 50% invasion of the myometrium, while high risk or type II cancers are grade 3 endometrioid cancers with more than 50% invasion of the myometrium and serous papillary, clear cell and more rare histological types (University of Bonn, http://www.meb.uni-bonn.de/cancer.gov) [8]. The primary treatment includes a total hysterectomy and bilateral salpingo-oophorectomy which in high risk patients are followed by radiotherapy and in some cases chemotherapy.

In a formerly published Danish study on endometrial cancer, the majority (89%) of the endometrial cancers belonged to the low risk group who had an overall recurrence free survival of 93% [9]; 7% experienced recurrent disease and 4% died of their low risk cancer. In number these 4% comprised 28 of 102 deaths due to all types of endometrial cancer. These figures and other results [6] argue that adjuvant radiation therapy as standard treatment to low risk patients is not necessary. The figures also raise the question of tools for identification of misclassified low risk patients that is patients who do not survive despite being classified as having a favorable prognosis.

In the present investigation, we focus on endometrioid endometrial cancer, the most common endometrial malignancy. To the best of our knowledge, this publication is the first to describe the expression of the EGF system at mRNA level combined with its immunohistochemical localization in endometrioid endometrial cancer. Furthermore, the expression of the receptors and ligands of the EGF system in the malignant endometrium is compared with the endometrium from healthy pre- and postmenopausal women.

Material and methods

Forty-five women undergoing surgery because of endometrial cancer and 13 healthy postmenopausal women were included from January 2002 to April 2005. The inclusion criteria for women with endometrial cancer were endometrioid endometrial cancer, age over 18; Caucasian and mother tongue Danish. The women were selected consecutively. The women had their diagnosis of cancer within a fortnight prior to surgery, and due to the psychological stress about half chose not to participate. The diagnosis of endometrial cancer was confirmed by a senior pathologist specialized in gynecological cancer. Healthy postmenopausal endometrial samples were taken from women allocated to the gynecological department who were included due to benign vaginal surgery for cysto- or rectocele. They were all at least one year after their last menstrual bleeding and they had to be Caucasian, 2 months after cessation of systemically or local sex hormone treatment, to have no former history of endometrial disease, a BMI between 20 and 30 and Danish as mother tongue.

Endometrial cancer samplings were performed under general anesthetics and the postmenopausal women were under general or spinal anesthetics during sampling.

The women with endometrial cancer had samples removed twice; first with an uterine explora curette® (Milex Products, Inc., Chicago, USA) prior to laparotomy, secondly as a knife biopsy after removal of the uterus. Healthy women had an endometrial sample removed with a uterine explora curette®. Half of the tissue was snap frozen in liquid nitrogen and stored at − 80°C, the other half was kept in Lillie's liquid®, a formaldehyde buffer 4% neutral and with pH 7.0 (Merck Eurolab A/S, Denmark). The samples were investigated by real-time PCR and immunohistochemistry. All women had a peripheral blood sample taken. A short gynecological anamnesis was taken.

All investigations followed the Declaration of Helsinki, and all participants gave informed consent prior to participation. The local scientific ethic committee approved the study (number 2001.0253).

RNA extraction and Reverse transcription were performed as described previously [5] employing 0.1 μg RNA per sample. All samples were handed by the same technician. The adequacy of the samples was ensured by investigating the content of β_2 microglobulin based on mRNA. The reverse transcription of the samples was made successively in two runs.

Real-time PCR

Real-time PCR for quantification of the receptors, HER1, HER2, HER3, HER4, and the ligands, AR, TGF-α, HB-EGF, BCL, EPI, EGF, was performed by use of a LightCycler (Roche A/S, Denmark). All analysis except HER2 employed SYBRgreen whereas HER2 employed hydrolysis probes. Further information is given in [5] including information of imprecision, intra- and interassay variation. Forty-five tumor samples and four to thirteen postmenopausal samples were analyzed for the mRNA expression of all receptors and ligands.

The results are presented as absolute values relative to the mRNA content in the calibrator used for generating the calibration curve. One of the calibrators is routinely used as positive control, as negative control water was added instead of RNA. The levels of expression of each component are not comparable, since the absolute amount of mRNA for each specimen examined is unknown.

Initially, we wanted to use a housekeeping gene as a reference, but found significant cyclic variation during the menstrual cycle for both β_2 microglobulin and 18SRNA (data not presented). From the literature, it has been shown that β-actin also display this cyclic variation [10].

Immunohistochemical analysis

The primary antisera used are: HER1 (M7239, Clone E30, monoclonal; Oncogene, Merck KGaA, Germany), HER2 (A0485, polyclonal, rabbit; Dako, Denmark), HER3 (AB10, polyclonal, rabbit; Neomarkers, California) and HER4 (Ab-4 Clone HFR-1; Neomarkers, California), AR (Ab 1, polyclonal, rabbit; Neomarkers, California), TGF-α (Ab 1, GF10, monoclonal; Oncogene, Merck KGaA, Germany), HB-EGF (AF-259-NA, polyclonal, goat; R&D, USA), BCL (AB-261-NA, polyclonal, goat; RD, Oxon, UK), and EPI