Research Article

Altered Peroxisome-Proliferator Activated Receptors Expression in Human Endometrial Cancer

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1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors of the nuclear hormone receptor superfamily [1, 2]. Three distinct PPAR isoforms termed α, β/δ, and γ have been identified [3–5]. They share several structural common features, but each is distinctly expressed in different tissues. PPARα and γ are predominantly expressed in heart, muscles, liver, and in adipocytes [3–5]. PPARβ is more ubiquitously expressed, but shares certain common downstream effects with PPARα [4, 5]. For both PPARα and β, it has been shown that their activation is responsible for the enhancements in energy substrate utilization [4]. This ability of PPARs to regulate cellular metabolism leads to the question, whether the tumor cells have altered PPAR expression. It is tempting to speculate so, since high energy substrate consumption is a well-known feature of neoplastic cells, especially those with high rates of cell proliferation. Indeed, a growing number of researches begin to suggest an important role of PPAR activation in the biology of the neoplastic process. Furthermore, some studies offer the prospect of using PPAR as a destination point of action for both prevention and treatment of cancers [6–9].

Endometrial cancer (EC) is one of the most widespread gynecologic cancers in Europe, and according to FIGO classification (International Federation of Gynecology and Obstetrics) as well as Bokhman theory [10] endometrial cancer—endometrioid type (FIGO stage I, type I) is the most frequent. In addition, EC is usually present with well-differentiated morphology (G1) along with an endometrioid features [10]. Based on that we sought to investigate PPARs expression in this type of cancer. We examined different PPAR (α, β/δ, γ) isoforms expression at the level of transcription (mRNA) and proteins (by immunohistochemistry and Western Blot technique).

2. Material and Methods

The present study conforms with the principles outlined in the Declaration of Helsinki and was approved by the
the following conditions: 5 min at 95 °C.

2.1. RNA Extraction and cDNA Synthesis. Total RNA was extracted from frozen endometrial malignant and normal tissues according to Chomczynski and Sacchi method [11]. RNA integrity was verified by elecrophoresis in 1.5% agarose gel and staining with ethidium bromide, and by RNA integrity was verified by elecrophoresis in 1.5% agarose gel, extracted and purified from agarose slices using DNA Gel Extraction Kit (Millipore, USA), quantified by the use of One Dscan/Zero Dscan software (Scanalytics Inc., USA), and then diluted in sterile water [12].

2.2. Western Blot Analysis. Tissue samples (control and endometrial cancer) were homogenized in RIPA buffer (1:10 v/w, ice-cold, pH 7.4), with the addition of protease inhibitors cocktail (1 mM EDTA, 1 mM PMSF, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin). Then samples were centrifuged at 10,000 g for 30 min at 4°C and the supernatant was analyzed further. Protein content was measured with the BCA protein assay kit (Sigma). Bovine serum albumin was used as a standard. Proteins (50 μg) were separated by SDS PAGE on 10% gel. Separated proteins were transferred on nitrocellulose membranes (BioRad) in transfer buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycin, 20% methanol) at 14 V in 4°C. An equal sample loading was confirmed by Ponceau S stain. Then nitrocellulose blots were placed in blocking buffer (5% nonfat milk in TBS-T) for 1 h. The membranes were incubated with primary antibodies against PPARα, PPARβ, PPARγ (ab8934, ab23673, ab19481 Abcam, UK) or β-actin (ab3280, Abcam, UK), for 2 h at 4°C. After three washings in TBS-T, membranes were incubated with an alkaline phosphatase-conjugated secondary antibody (Sigma). Protein bands were scanned and quantified using a Gel Doc EQ system (Bio-Rad). The total content of PPARs in homogenate was normalized to the β-actin expression and presented in arbitrary units (ODU-optical density units).

2.3. Immunohistochemical Tissue Staining. For immunohistochemical studies 2-3 representative sections from each case of the endometrial cancer and normal tissues were selected. PPARs immunoreactivity was evaluated by the use of the polyclonal antibody (Santa Cruz Biotechnology, USA) as recommended by the producer. Briefly, the sections were deparaffinized in xylens and hydrated through graded alcohols. Antigen unmasking was performed using heat treatment in a microwave oven at 750 W for 7 minutes in a container with 10 mM sodium citrate buffer, pH 6.0. Sections were allowed to cool in the buffer at room temperature for 30 minutes and were rinsed in deionized H2O three times for 2 minutes each. The endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 20 minutes. After rinsing in PBS, the sections were incubated for 1 hour with 1.5% normal blocking serum in PBS. The blocking reagent was removed, and then the sections were incubated with primary antibody at 4°C overnight using staining chamber (The Binding Site, United Kingdom). Primary antibodies were diluted in PBS with 1.5% normal blocking serum. Omitting primary antibodies served as negative control. After rinsing in three changes of PBS, a streptavidin-biotin-peroxidase complex technique was used to reveal antibody-antigen reactions (EnVision kit, Dako, Denmark). Staining was routinely developed using 3,3′-diaminobenzidine as a chromogen (Dako, Denmark). Sections were counterstained with hematoxylin. Two independent pathologists, who were
blinded to the clinicopathological data of the patients, evaluated immunostainings with the use of light microscopy (20x and 40x objectives). The evaluation of PPAR expression was analyzed in 10 different tumor fields, and the mean percentage of tumor cells displaying positive staining was scored. No staining of the cells was observed in any of the tumor sections after omitting the first antibody. Immunohistochemical expression for each PPAR isoform is presented as the percentage (%) of the immunopositive cells present in healthy endometrium or endometrial cancer tissue.

2.4. Statistical Analysis. Statistical comparisons were made by using appropriate tests (Mann-Whitney U test, t-Student test, and/or one-way ANOVA followed by the Newman-Keul post hoc test). P < 0.05 was considered statistically significant.

3. Results

All selected patients (n = 10) in the control group with normal endometrial mucosa had no hypertension or diabetes nor obesity (BMI was 28.2), with the average age around 48.5 (range 35–54). The average age in the group of patients with endometrial cancer was around 59 (range 43–73), and there was no evidence either of obesity (BMI was 30.8) or hypertension nor diabetes (data not shown).

3.1. Immunohistochemical Expression. Positive PPAR (α, β, γ) immunohistochemical staining was detected in normal endometrial mucosa (for PPAR α: 57%, PPAR β: 55%, and PPARγ: 61%) as well as in endometrial cancers (for PPARα: 78%; PPARβ: 77%; PPARγ: 84%) (Figure 1). With respect to intensity of the staining, we observed more frequently a stronger positive reaction in cancer cells than in healthy mucosa, but not in all cases (data not shown). A trend towards higher expression of PPAR α, β in endometrial cancers was noticed (+21% and +22%, P = 0.067, resp.). In opposite, EC cells showed lower expression for PPARγ (−23%, P < 0.05). Localization of the staining was similar for all the PPARs isoforms. Immunopositive cells in normal and endometrial cancer tissue were found broadly in nuclear and perinuclear region (Figure 1).

3.2. PPARs mRNA Content. The mRNA content of all PPARs was examined in normal endometrial mucosa (n = 10) as well as in endometrial cancers (n = 35). The mRNA expression of each PPAR isoform was significantly higher in normal endometrial tissue comparing with EC (PPARα: +3.1-fold; PPARβ: +3.8-fold; PPARγ: +4.1-fold; P < 0.05; Table 1).

3.3. PPARs Protein Expression. Western blots analyses confirmed greater immunohistochemical expression of PPARα...
and PPARβ isoforms in endometrial cancer tissue comparing with normal mucosa (PPARα: +0.7-fold; PPARβ: +2.0-fold; P < 0.05; Figure 2). An opposite effect was observed for the expression of PPARγ, which was significantly lower in EC (PPARγ: −1.5-fold; P < 0.05; Figure 2).

4. Discussion

The present study was undertaken to characterize the expression of PPARs in endometrial cancers (EC) at the transcriptional (mRNA) and posttranscriptional (proteins) levels. Immunohistochemistry was applied for the evaluation of the PPARs immunoexpression and subcellular distribution. Protein expression was further quantified by Western Blot technique. To the best of our knowledge, this is the first study to report that only the expression of PPARα and β is relatively higher in EC, but not PPARγ. The disassociation of mRNA content and respective protein product were also found in endometrial cancers. This discrepancy of the mRNA content and the expression of respective protein is commonly observed in neoplastic tissues [12, 13].

4.1. PPARγ Expression. We found reduced immunohistochemical PPARγ expression in EC, which is consistent with other reports showing rather moderate immunoreactivity of PPARγ expression in endometrial carcinoma cells [14, 15]. This relatively low PPARγ expression was also found in other tumor cells, and some studies begin to suggest that PPARγ agonists may inhibit cell proliferation in the neoplastic cell lines [16–18]. It seems highly possible, since several in vitro studies have revealed that pioglitazone, a PPAR-γ agonist, induces cell differentiation [19] and several clinical studies have demonstrated that the activation PPAR-γ increases the degree of histopathological differentiation of liposarcoma [19, 20]. However, it is not the case for all neoplastic transformation, as others demonstrated that highly malignant cancer cell lines are characterized by higher expression of PPARγ and these data suggest that PPARγ may act in a cancer-permissive fashion [21, 22].

4.2. PPARα and/or PPARβ Expression. In our study we found increased expression (immunoreactivity further quantified by Western Blot) of both PPARα and PPARβ isoforms in EC compared to healthy endometrium. This finding may imply a possible role for both PPARs (α and β) in neoplastic transformation of endometrial cells. However, we are aware of the limitations of our study. First the study was limited to one type of endometrial cancers (FIGO 1), and an open question remains whether there is a progression of the PPARs expression in more advanced endometrial cancers. Secondly, some reports suggest that there is a considerable background immunoreactivity when PPARs expression is measured by immunohistochemistry [23, 24]. This is important as there are a number of reports showing either an increase or decrease in PPAR (α and β) immunohistochemical expression, even in the same tissue [23, 25]. Nonetheless, the speculations concerning PPARα/β relative expression and function in cancer cells are further based on their opposite to PPARγ physiological roles. PPARγ is thought to be primarily involved in processes that augment differentiation of the cells and/or storage of energy, and PPARα/β activation presumably enhances the processes related to the fuel expenditure. This suggests a possibility that neoplastic cells may have greater PPARα/β expression/activity, which should activate genes controlling cellular metabolism and result in faster metabolic rates of cancer cells. From rodent studies it is becoming evident that chronic treatment with PPARα agonist induces incidences of liver tumors through a mechanism, that results in an increase of both cellular proliferation and oxidative stress [26]. However, such a tumorigenic influence of PPARα activation was found only in animal studies. As far there is no evidence that PPARα agonists such as fibrates are associated with elevated risk of cancer in humans [27, 28].

In summary, we provide evidence for altered expression of different PPAR isoforms in endometrial cancer cells,
namely, greater expression of PPARα and PPARβ, with concomitant reduction of PPARγ in EC.

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References


