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Expression of the energy substrate transporters in uterine fibroids

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ABSTRACT

Proliferating cells exhibit accelerated rates of substrate utilization, favoring glucose over fatty acids (FA's) oxidation. Protein-mediated transport is thought to play a predominant role in facilitating either glucose or FA routing into the cells. In the present study, we examined the expression of glucose transporters (GLUT-1, GLUT-4) and fatty acids transporters (FAT/CD36, FATP-1, FATP-4) at transcript and protein levels as well as cytosolic fatty acid binding proteins (H-FABP, ACBP) in human fibroids (n = 74, size up to 3 cm diameter) and compared with pair-matched healthy myometrium. Additionally lipid content (diacylglycerols, triacylglycerols and ceramide) was estimated by gas liquid chromatography (GLC). Uterine fibroids displayed decreased expression of both FAT/CD36 and FATP-1 proteins along with lower diacylglycerol (DAG) and triacylglycerol (TAG) content as compared to healthy pair-matched myometrium. The expression of glucose transport proteins (GLUT-4 and GLUT-1) remained relatively constant, although the higher expression of GLUT-1 in uterine fibroids did not reach the minimum significance threshold ($p = 0.056$). However, no change in either cytochrome c oxidase (COX IV) or hydroxyacyl-CoA dehydrogenase (HADHSC) was observed and these data confirm a possible metabolic shift favoring glucose utilization over fatty acid oxidation in human uterine fibroids.

1. Introduction

Uterine fibroids (leiomyomas) are the most common benign tumors in women of childbearing age [1–3]. They are characterized by a low mitotic index and generally are considered as slow growing with low neoplastic potential. However, despite the low proliferative index, fibroids can growth very large (20 cm and more) and some could transform to more invasive tumors (i.e., leyomiosarcoma). Thus far, the cell biology of fibroids is not fully explored, especially with respect to the changes in energy of cellular metabolism [4,5].

Healthy myometrium cells (smooth muscle cells) use both glucose and fatty acids (FA) as major energy substrates. It is also well recognized that, a crucial rate-limiting process in cellular glucose utilization, involves the transport of glucose across the cell membrane. Similarly, data indicate an importance of protein-mediated FA transport into the cells [6,7]. Several studies have shown that, membrane proteins such as CD36, FABP, FATPs facilitate fatty acids movement across the plasma membranes in different type of cells (including skeletal and smooth muscles) [8,9].

From recent literature overview it is becoming evident that metabolic switch in atypical cells is an essential feature for the development and progression of abnormal cells. The progression of cancer requires constant energy supply that may be achieved by increasing glucose and/or fatty acid flux into the cells. It has been shown that, accelerated glycolysis is the biochemical characteristics of neoplastic cells (Warburg effect). Our previous study showed also greater expression of GLUT-1, GLUT-3 and GLUT-4 in endometrial cancer tissue comparing to healthy endometrium [10]. While there are numerous studies showing overexpression of glucose transporters and increased glucose metabolism in variety of tumors, there are fewer studies on the role of fatty acid transporters in malignant cells [10]. Given the essential functions of glucose and fatty acids transport proteins and possibly altered expression during metabolic challenges, it is of particular interest to verify whether there is a change in the transporters expression in benign tumors of uterus. In the present study we evaluated the proteins transporters expression: FAT/CD36, FATP-1, FATP-4, GLUT-1, GLUT-4. Additionally, intramuscular lipid content (TAG, DAG,

Abbreviation: FAT, fatty acid translocase; FATP, fatty acid transport protein; GLUT, glucose transporter; FABP, fatty acid binding protein; Cer, ceramide; TAG, triacyloglycerol; DAG, diacyloglycerol; COX IV, cytochrome c oxidase IV; HADHSC, hydroxyacyl-CoA dehydrogenase.

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Table 1
Primer sequences used for real-time PCR.

Name	Accession no.	Primer length (bp)	Direction and site sequence	Amplicon lenght (bp)
GLUT4	NM_001042	19 20	5′- GAC CAA CTA AGG CAA AGA G-3′ 5′- CAA TAG GAT GCT TGT CTT CA-3′	183
GLUT1	NM_006516	20 22	5′-CAC CAC CTC ACT CCT GTT AC-3′ 5′-CCA CTT ACT TCT GTC TCA CTC C-3′	123
GOT2 (FABPpm)	NM_002080	19 24	5′-TAA GTT CAG CCG AGA TGT C-3′ 5′-GTC ATA ATA CCG ATA ACC TTG TAG-3′	103
CD36	NM_001001547	18 21	5′-GGA CGC TGA GGA CAA CAC-3′ 5′-GCC AGA TTG AGA ACT GTG AAG-3′	108
FATP 4	NM_005094.3	32	5′-GGCCCAACGACATCGTCTAT-3′, 5′-CTTCCGAATCACCACCGTCA-3′	107
FATP-1	NM_198580.1	35	5′-CGCCAAATCGGGGAGTTCTA-3′, 5′-TTGAAACCACAGGAGCCGAC-3′	86
β-actin	NM_001101	20 24	5′-AGT CGG TTG GAG CGA GCA TC-3′ 5′-GGA CTT CCT GTA ACA ACG CAT CTC-3′	115

All the Ct values were used for calculation relative expression of genes according to the formula [10].
$R = (E_{target})^{\Delta CPtarget(control-sample)} / (E_{ref})^{\Delta CPref(control-sample)}$
R—relative expression ratio of target gene.
E_{target}—real-time PCR efficiency of target gene transcript.
E_{ref} —real-time PCR efficiency of reference gene transcript.
ΔCP_{target} —the CP deviation of control minus sample of the target gene transcript.
ΔCP_{ref}—the CP deviation of control minus sample of the reference gene transcript.
CP—crossing points = Ct.

Ceramide) and fatty acids composition was also analyzed in uterine fibroids.

2. Materials and methods

2.1. Patients characteristics and tissue collection

The present study conforms with the guidelines delineated in the Declaration of Helsinki and was approved by the Institutional Review Board (IBR) of the Medical University of Bialystok. All patients gave their informed consent prior to their inclusion in the study. Among January 2011 to March 2012 the group of women was investigated in the Department of Gynecology and Gynecologic Oncology, Medical University of Bialystok, Poland. First diagnosis of fibroids was made in out-patient clinic and then confirmed by second, independent sonographer. Normal myometrial specimens and fibroids were obtained from 74 patients, aged 36–52 years old (average age 44.1 years) undergoing simple hysterectomy. Surgical procedures were routinely performed in follicular phase of menstrual cycle. In attempt to make the study cohort homogeneous as possible patients have to meet the following criteria: no hormonal or other medications history treatment for at least six months before surgery; regular menstrual period (27–32 days); no history of internal disease (hypertension, diabetes, or other endocrine disease); no basic blood biochemical examinations abnormalities at those women (Table 2).

The sizes of dissected fibroids ranged from 10 up to 50 mm in diameter. Uterine leiomyomas included for the study was 35–40 mm in diameter and situated within myometrial wall of the uterus. The specimens included for the study was obtained just under the capsule of the tumor in each case. Healthy myometrial tissues were obtained from surrounding normal myometrium situated more than 20 mm away from the fibroid capsule and were used as paired controls. Pathologist to exclude adenomyosis or any malignant changes and/or inflammatory infiltration examined all specimens. The tissue samples were confirmed as histologically ordinary fibroids. All specimens, after dissection, were immediately frozen in liquid nitrogen and were stored at −80 °C until use.

2.2. Real-time quantitative PCR analyses

Total RNA was isolated from 50 mg of frozen tissue (fibroids and pair-matched healthy myometrium) using Total RNA (A&A Biotechnology, Poland) according to the producer's instructions. Following RNA purification, DNase treatment (Ambion) was performed to ensure that there was no contaminating genomic DNA. cDNA was generated using High Capacity cDNA Reverse Transkription Kit (Applied Biosystem, CA,USA). PCR products were obtained by amplification of cDNA using specific primers (see Table 1). PCR was performed with SYBR Green JumpStart Taq ReadyMix (Sigma), using a Bio-Rad Chromo4 system. Specific reaction conditions were determined as described recently [10]. PCR efficiency was examined and a melt curve was performed at the end of each reaction to verify PCR product specificity. Relative expression of genes was calculated and the results were normalized to β-actin expression measured in each sample [10].

2.3. Western blot analyses

Routine Western blotting procedures were used to detect proteins as described previously [11]. Briefly, the expression of selected proteins (FAT/CD36, FATP-1,4; GLUT-1,4 and H-FABP, ACBP, COX IV and HADHSC)) was determined in homogenates from healthy myometrium (n = 74), and fibroids (n = 74). The tissue was homogenized in a lyses buffer (HEPES 20 mmol/L, EDTA 2 mmol/L, EGTA 2 mmol/L, Triton 1%, PMSF 5 μmol/L, Na3VO4 50 μmol/L) containing protease inhibitor cocktail (Sigma, St. Louis, USA). After centrifugation at 10.000*g* for 10 min at 4 °C, protein concentration was determined by use of the BCA protein Assay kit (Pierce). Total proteins (90 μg) were denatured and separated using 10% SDS-polyacrylamide gel electrophoresis and finally transferred to nitrocellulose membrane (Bio-Rad, CA, USA). Equal protein concentrations were loaded in each lane as latter confirmed by Ponceau staining the blot membrane. Membranes were blocked at room temperature for 2 h in Tris-buffered saline/Tween 20 (TBST) (20 mmol/L Tris, pH 7.6; 137 mmol/L NaCl; 0.1% Tween 20) containing 10% nonfat dry milk. After that, the membranes were immunoblotted with selected primary (FAT/CD36, Abcam, UK; FATP-1, FATP-4, GLUT-1, GLUT-4, H-FABP, ACBP, COX IV and HADHSC from Santa Cruz Biotech, MA, USA) and adequate