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The Occurrence of Fetal Microchimeric Cells in Endometrial Tissues Is a Very Common Phenomenon in Benign Uterine Disorders, and the Lower Prevalence of Fetal Microchimerism Is Associated with Better Uterine Cancer Prognoses

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This is the first study carried out to describe the role of fetal microchimerism (FM) in the pathogenesis of uterine cancer. The prevalence and concentration of male fetal microchimeric cells (FMCs) were examined in endometrial tissues in relation to subtypes of uterine cancer, and the histological grade and stage of the tumor. FM occurrence was analyzed in relation to risk factors, including hypertension, obesity, type 2 diabetes, dyslipidemia, age at cancer diagnosis, and patient pregnancy history. The prevalence and concentration of FMCs were examined in endometrial tissues using real-time polymerase chain reaction, *SRY* and β-globin sequences as markers for male fetal FMCs and total DNA. The studied group involved 47 type 1 endometrial cancers, 28 type 2 endometrial cancers, and 41 benign uterine diseases. While the prevalence of FM was decreased only in type 1 endometrial cancer, compared with benign uterine disorders (38.3% vs.70.7%; odds ratio [OR] = 0.257, 95% confidence interval [CI]: 0.105 to 0.628, $p = 0.003$), FMC concentrations did not differ within examined groups. The lower FM prevalence was detected in low-grade (grade 1 and grade 2) endometrioid cancer (38.3% vs. 70.7%, OR = 0.256, 95% CI: 0.105 to 0.627, $p = 0.003$) and in FIGO 1 tumors (40.7% vs. 70.7%, OR = 0.285, 95% CI: 0.120 to 0.675, $p = 0.004$). No correlation between FM prevalence or FMC concentrations and risk factors was demonstrated. A lower prevalence of male FM seemed to be associated with better prognoses in uterine cancer based on tumor subtype, histological grade, and stage of the tumor.

Introduction

Fetal microchimerism (FM) is defined as the long-term persistence of small numbers of fetal-derived allogeneic cells in maternal organs and circulation. FM is a naturally occurring phenomenon accompanying each gestation (Artlett, 2005; Yan *et al.*, 2005; Fleta *et al.*, 2006; Lapaire *et al.*, 2007). Fetal cells cross the placenta throughout gestation. While the majority of fetal cells (nucleated erythrocytes, leukocytes, and trophoblasts) are present in the maternal circulation throughout gestation, some fetal cells cross the placenta exclusively during certain stages of pregnancy. For instance, mesenchymal stem cells can be detected in maternal circulation only during the 7th and 14th weeks of gestation (Clayton *et al.*, 1964; Walknowska *et al.*, 1969; Mueller *et al.*, 1990; Campagnoli *et al.*, 2001). Stem and progenitor cells of fetal origin (hematopoietic and mesenchymal stem cells, endothelial progenitor cells) can engraft and proliferate in maternal bone marrow. Afterward, they settle in target maternal tissues, where, under appropriate micro-environmental stimuli, they can differentiate into cells expressing tissue-specific markers and carry out a variety of functions (Bianchi *et al.*, 1996; Campagnoli *et al.*, 2000; Guetta *et al.*, 2003; O'Donoghue *et al.*, 2003; Bayes-Genis *et al.*, 2005; Khosrotehrani and Bianchi, 2005; Nguyen *et al.*, 2006; O'Donoghue and Chan 2006; Buemi *et al.*, 2007; Savvidou *et al.*, 2008; Parant *et al.*, 2009; Luppi *et al.*, 2010). Fetal microchimeric cells (FMCs) have been recently shown to have diverse and controversial affects. FMCs can be involved in tissue repair or take part in inducement of chronic inflammation, leading to autoimmunity and cancer (Nassar *et al.*, 2012). With regard to gynecologic malignancies, only a limited number of studies are available. A study by Cha *et al.* (2003) was the first to report the presence of male microchimeric cells in cervical tissues derived from patients with cervical cancer. Initial studies by Gadi and Nelson (2007) and Gadi *et al.* (2008) demonstrated that the presence of allogeneic FM in peripheral blood mononuclear

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cells in women, who had given birth to a son, contributed significantly to the reduction in the risk of breast cancer. They reported an FM prevalence of 56% in controls and 26% in patients with breast cancer. Their results suggested that the enigma of why some parous women are not afforded protection from breast cancer by pregnancy, might, in part, be explained by the absence of FM (Gadi *et al.*, 2008). A contemporary study by Gilmore *et al.* (2008) also showed a higher prevalence of male cells (57% vs. 34%) in the maternal circulation of normal parous women compared with those with various malignant diseases, including, but not limited to, gynecologic malignancies, indicating that the absence of male microchimerism might be a risk factor for developing breast cancer. A study by Kamper-Jørgensen *et al.* (2012) highlighted the opposite effects of FM in blood samples on later development of breast and colon cancer. While the detection of male microchimerism was strongly associated with a reduced risk of developing breast cancer (70% vs. 40%), they found an increased risk of developing colon cancer (70% vs. 90%). Gadi (2010) found a protective association between FM in breast tissue and breast cancer. They reported a higher prevalence of FM in breast tissues from cancer-free women compared with unaffected breast tissues from patients with an invasive breast cancer diagnosis (63% vs. 26%, respectively). The latest study by Dhimolea *et al.* (2013) reported decreased FM prevalence in ductal invasive breast cancer compared with controls (21.0% vs. 56.0%, $p < 0.001$).

Uterine cancer is defined as any invasive neoplasm of the uterine corpus. Invasive neoplasms of female pelvic organs account for almost 15% of all cancers in women. The most common of these malignancies, in the United States and Europe, is endometrial cancer (Schottenfeld, 1995; Olson *et al.*, 2009). There are two forms of endometrial cancer. Type 1 endometrial cancer represented by low-grade endometrioid adenocarcinoma, which is thought to be primarily related to imbalances in reproductive hormones, usually constitutes more than 80% of all endometrial cancers (Milne *et al.*, 2011) and has a favorable prognosis. Type 2 endometrial cancer, considered nonestrogen dependent, represented by serous carcinoma and cell clear carcinoma, is highly aggressive and is associated with a worse prognosis. Recently, it has been reported that grade 3 endometrioid carcinomas shared a molecular pathway with type 2 endometrial carcinomas (Alvarez *et al.*, 2012). It has also been shown that grade 3 endometrioid carcinomas had a clinical behavior close to that reported in type 2 endometrial cancer (Alektiar *et al.*, 2002; Soslow *et al.*, 2007). That is why some authors start classifying high-grade endometrioid carcinomas (grade 3) as type 2 endometrial cancers (Amant *et al.*, 2005; Bakkum-Gamez *et al.*, 2008).

Since it is now universally accepted that carcinosarcomas are not uterine sarcomas but carcinomas with a sarcomatoid phenotype, they can also be grouped with high-grade (type 2) uterine carcinomas (Kurman *et al.*, 2011).

Known risk factors for type 1 endometrial cancer include early menarche, late menopause, anovulation, infertility and/or nulliparity, obesity, diabetes, and higher lifetime estrogen exposure (Sherman, 2000; Purdie and Green, 2001; Kaaks *et al.*, 2002; Akhmedkhanov *et al.*, 2006; Trentham-Dietz *et al.*, 2006; Wernli *et al.*, 2006; Zagouri *et al.*, 2009). The only known risk factor for type 2 endometrial cancer is age (>60 years), so most of these tumors occur after menopause.

To our knowledge, no study describing the role of FM in the pathogenesis of uterine cancer has been carried out. For that reason, we evaluated the prevalence of FM and FMC concentrations in tumor and control endometrial tissues in a population of Czech women. Further, we investigated the relationship between FM and the severity of the disease with regard to the uterine cancer subtype, the histological stage and grade of the tumor. The association part of the study focused on the risk factors, including patient age at diagnosis, patient pregnancy history, obesity, hypertension, dyslipidemia, and type 2 diabetes.

Materials and Methods

The study examined the presence of FM in frozen and/or fresh endometrial tissue specimens. The studied control group involved 41 patients (dysfunctional uterine bleeding, leiomyomas, endometrial polyps, benign ovarian cysts, prolapsed uterus, and benign endometrial hyperplasia) aged 36–75 years (mean 52.4 years). The patients in the control group suffered from type 2 diabetes (10.3%), hypertension (20.5%), and dyslipidemia (2.4%). Relative to BMI, the control group had the following distribution (38.9% normal range, 16.7% overweight, and 44.4% obese). The history of pregnancy in the control group was as follows: 5 - no pregnancies, 7 - one pregnancy, 8 - two pregnancies, 9 - three pregnancies, 7 - four pregnancies, and 5 - five or more pregnancies.

The cancer patient group enrolled 75 patients with uterine cancer. The group included 47 women with type 1 endometrial cancer (17 grade 1 endometrioid adenocarcinomas and 30 grade 2 endometrioid adenocarcinomas) and 28 women with type 2 endometrial cancer (19 grade 3 endometrioid adenocarcinomas, 2 clear cell endometrial carcinomas, 3 serous carcinomas, and 4 metaplastic carcinomas). The patients were diagnosed with uterine cancer at the mean age of 65.5 years (range 44–90 years). Tumor stages according to the FIGO 2009 classification (54 FIGO 1, 7 FIGO 2, 12 FIGO 3 and 2 FIGO 4) and tumor histological grades (17 G1, 30 G2 and 19 G3) were assessed. The prevalence of type 2 diabetes was 28.2%. The prevalence of hypertension and dyslipidemia in the uterine cancer group was 56.3% and 16.0%, respectively.

The cancer patients were subdivided according to BMI (11.3% BMI ≤ 25, 41.9% BMI >25, and 46.8% BMI >30). The pregnancy history of the uterine cancer group was as follows: 3 - no pregnancies, 11 - one pregnancy, 28 - two pregnancies, 19 - three pregnancies, 6 - four pregnancies, and 8 - five or more pregnancies. The study was performed in a retrospective manner using biological samples collected from January 2009 to December 2010. All patients who participated in this study provided written informed consent. The study was approved by the local ethics committee.

Processing of samples and real-time PCR analysis

DNA was extracted from 25 mg of endometrial tissue using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany). In fresh tissue samples, DNA was eluted in 200 μL of AE buffer. To enrich DNA from frozen tissues, DNA was eluted using 60 μL of AE buffer. These two approaches were selected on the base of previous testing, yielding approximately the same quantity of fetal cells in identical tissues. The real-time PCR analysis was performed using a 7500 Real-Time PCR system (Applied Biosystems, Branchburg, NJ) as previously described. Two protocols produced the best results, identifying small numbers of male FMCs in uterine tissues.

15 μL and/or 47 μL were used as a template for the *SRY*-specific polymerase chain reaction. TaqMan amplification reactions were set up in a reaction volume of 50 μL and/or 100 μL using TaqMan Universal PCR Master Mix (Applied Biosystems). Each sample was analyzed in three replicate settings for the *SRY* gene. A patient's specimen was considered positive if the amplification signal occurred on a threshold cycle < 50. The calibration curves for the *SRY* and β-globin (*GLO)* genes were run parallel to each analysis. The standard curves were prepared using two approaches. First, DNA from the peripheral blood of a healthy male donor was isolated, its concentration was measured using a spectrophotometer (NanoDrop-1000; Witec AG, Switzerland) and converted to the number of cells using a conversion factor (one diploid genome being equivalent to 6.6 pg of DNA). Second, DNA derived from a healthy male donor of known concentration (converted to the number of cells using a conversion factor) was spiked to the known concentration of female DNA. FM was expressed as the number of FMCs per 10^5 total cells. Five microliter of DNA was used as a template for the *GLO* PCR reaction.

Statistical analysis

The Chi-square test and univariate logistic regression model were used to compare the absence or the presence of FM across the groups. One-way analysis of variance (ANOVA) was used to test possible differences in mean concentrations of fetal-derived cells in endometrial tissues between groups. A series of multiple logistic regression analyses were performed to evaluate the effect of putative risk factors on the prevalence of FM in the groups of patients with uterine cancer and controls. Similarly, two-way ANOVA analyses were performed to compare the differences in concentrations of FMCs in endometrial tissues between the uterine cancer group and the control group in relation to particular risk factor.

In addition, the relationship between FM prevalence or FMC concentrations in endometrial tissue and the age of the patient at the time of diagnosis, patient pregnancy history (total number of pregnancies, including both completed and uncompleted pregnancies), and the body mass index was studied using linear regression models.

The significance level was established at a *p*-value of $p < 0.05$. If there was statistical significance with the ANOVA test, then the Bonferroni's *post-hoc* analysis was applied.

Results

The prevalence of male FM in uterine cancer

Overall, a significantly decreased prevalence of male FM was observed in women who developed uterine cancer compared with uterine cancer-free controls (44.0% vs. 70.7%; odds ratio [OR] = 3.076, 95% confidence interval [CI]: 1.365 to 6.933, $p = 0.007$). However, the difference in concentrations of fetal-derived cells between uterine cancer and cancer free-groups did not achieve statistical significance (F = 0.013, df = 1,114, $p = 0.910$). Fetal cells were detected at mean concentrations of 0.090 and 0.050 per 10^5 total cells in uterine cancer cases and controls, respectively.

A significant difference in the prevalence of male FM was found between uterine cancer patients and the control group. While the prevalence of male FM between the control group and type 2 endometrial cancer (70.7% vs. 53.6%; OR = 0.478, 95% CI: 0.175 to 1.301, $p = 0.148$) did not differ, a lower prevalence was detected in patients with type 1 endometrial cancer (70.7% vs. 38.3%; OR = 0.257, 95% CI: 0.105 to 0.628, $p = 0.003$), (Fig. 1A). However, the number of chimeric cells did not differ within the examined groups (type 1 endometrial cancer: mean 0.146 per 10^5 total cells vs. type 2 endometrial cancer: mean 0.023 per 10^5 total cells vs. controls: mean 0.050 per 10^5 total cells), (F = 0.429, df = 2,113, $p = 0.652$), (Fig. 1B).

Association between male FM and the histological grade of endometrioid cancer

A significant effect of the histological grade of the tumor on the prevalence of male FM in examined endometrioid cancer tissues was revealed. A significantly lower prevalence of male FM was observed in low-grade (grade 1 and grade 2)

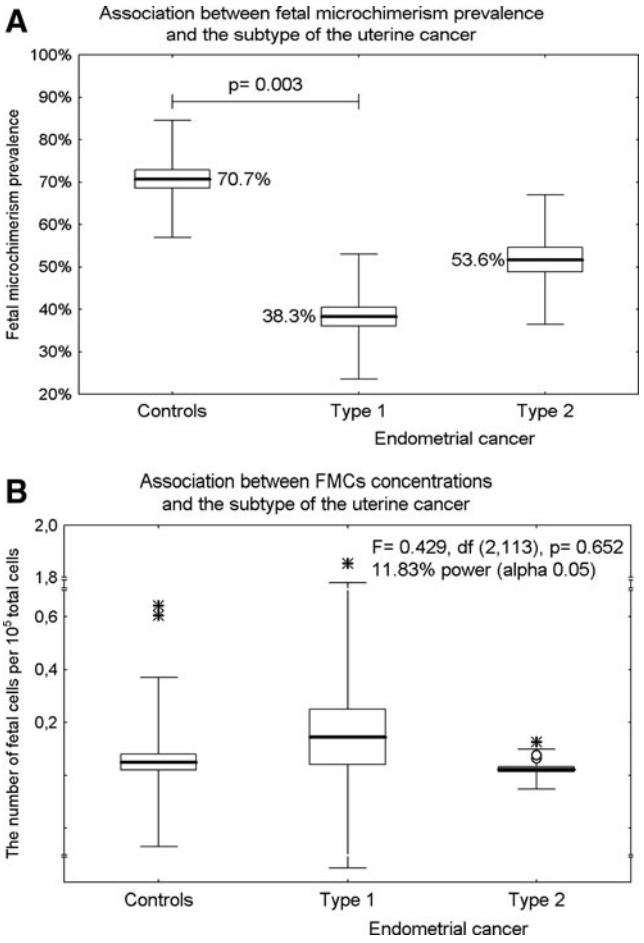

FIG. 1. The prevalence of male fetal microchimerism (FM) in uterine cancer. Results expressed as box plots; upper and lower limits of the boxes represent the mean ± SE (standard error), upper and lower whiskers represent the mean ± 2SD (standard deviations), mean is indicated by the line in each box; outliers are indicated by circles and extremes by asterisks. The significance level was established at a *p*-value of $p < 0.05$. **(A)** While the prevalence of male FM between the control group and type 2 endometrial cancer did not differ, a lower prevalence was detected in patients with type 1 endometrial cancer. **(B)** The number of chimeric cells did not differ within the examined groups (type 1 endometrial cancer vs. type 2 endometrial cancer vs. controls).

endometrioid cancer (38.3% vs. 70.7%, OR = 0.256, 95% CI: 0.105 to 0.627, $p = 0.003$). The difference in prevalence of male FM between high-grade (grade 3) endometrioid tumors and the control group did not reach statistical significance (42.1% vs. 70.7%, OR = 0.301, 95% CI: 0.097 to 0.934, $p = 0.138$), (Fig. 2A).

Mean FMC concentrations in endometrial tissues of patients with grade 1 endometrioid carcinomas (0.232 per 10^5 total cells), grade 2 endometrioid carcinomas (0.076 per 10^5 total cells), and grade 3 endometrioid carcinomas (0.010 per 10^5 total cells) were assessed. Statistical analyses showed no association between concentrations of male FMCs and the histological grade of the tumor within the endometrioid cancer group (F = 0.579, df = 4,149, $p = 0.678$), (Fig. 2B).

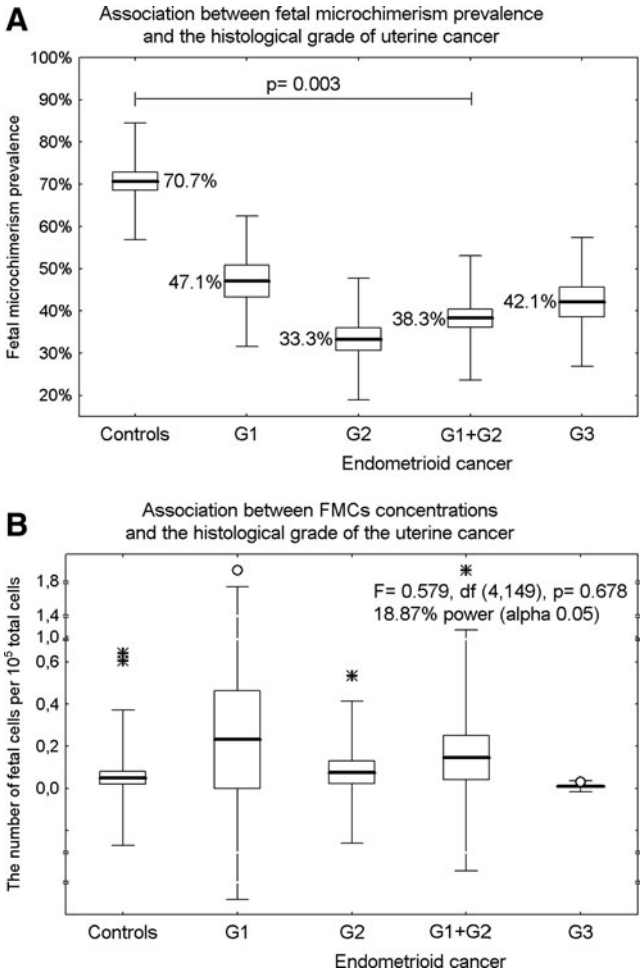

FIG. 2. Association between male FM and the histological grade of endometrioid cancer. Results expressed as box plots; upper and lower limits of the boxes represent the mean ± SE, upper and lower whiskers represent the mean ± 2SD, mean is indicated by the line in each box; outliers are indicated by circles and extremes are indicated by asterisks. The significance level was established at a p-value of $p < 0.05$. **(A)** While the difference in prevalence of male FM between high grade (grade 3) endometriod tumors and the control group did not reach statistical significance, a significantly lower prevalence of male FM was observed in low-grade (grade 1 and grade 2) endometrioid cancer. **(B)** No association between concentrations of male FMCs and the histological grade of the tumor within the endometrioid cancer group was found.

Association between male FM and the stage of the uterine cancer according to the FIGO classification

FM prevalence in endometrial tissues differed significantly depending on the stage of uterine cancer. While the lowest prevalence of FM was detected in FIGO 1 tumors compared with the control group (40.7% vs. 70.7%, OR = 0.285, 95% CI: 0.120 to 0.675, $p = 0.004$), no difference between uterine cancer-free tissue samples and FIGO 2 (70.7% vs. 57.1%, OR = 0.552, 95% CI: 0.107 to 2.848, $p = 0.478$) or FIGO 3 and FIGO 4 tumors (70.7% vs. 50.0%, OR = 0.414, 95% CI: 0.119 to 1.437, $p = 0.165$) was observed (Fig. 3A).

The concentrations of FMCs showed no difference between groups of patients with uterine cancer (F = 0.324, df = 3,112, $p = 0.808$).The mean number of fetal cells per 10^5 total cells tested for microchimerism was 0.093 in FIGO 1, 0.180 in FIGO 2, and 0.028 in FIGO 3 and FIGO 4 (Fig. 3B).

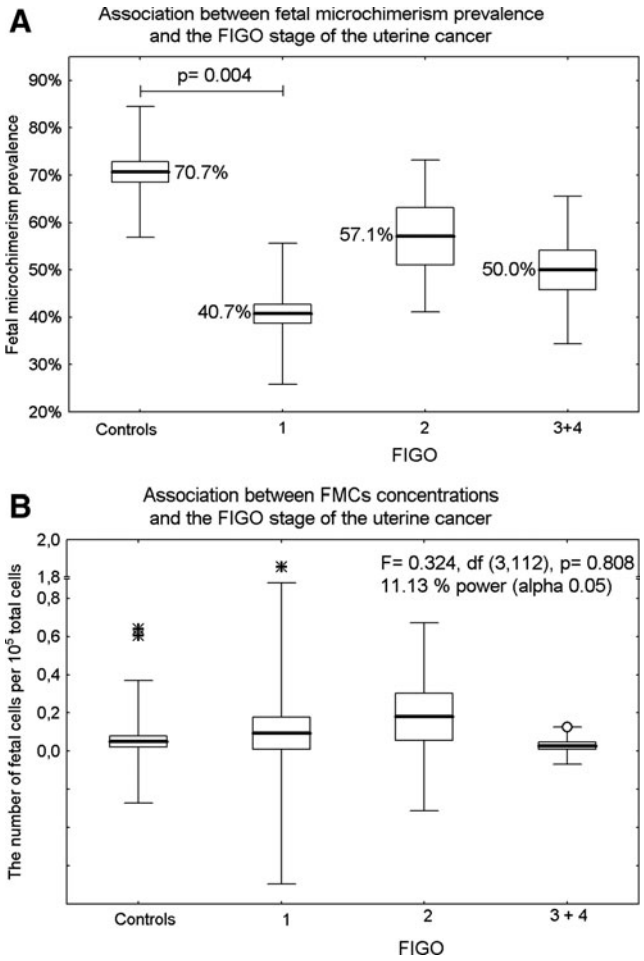

FIG. 3. Association between male FM and the stage of the uterine cancer according to the FIGO classification. Results expressed as box plots; upper and lower limits of the boxes represent the mean ± SE, upper and lower whiskers represent the mean ± 2SD, mean is indicated by the line in each box; outliers are indicated by circles and extremes are indicated by asterisks. The significance level was established at a p-value of $p < 0.05$. **(A)** While the lowest prevalence of FM was detected in FIGO 1 tumors compared to the control group, no difference between uterine cancer-free tissue samples and FIGO 2 or FIGO 3 and FIGO 4 tumors was observed. **(B)** The concentrations of FMCs showed no difference between groups of patients with uterine cancer.

The association study of FM and putative risk factors for uterine cancer

No relation between hypertension and FM prevalence was observed (OR = 1.280, 95% CI: 0.561 to 2.918, $p = 0.558$). Similarly, there was no difference in concentrations of FMCs ($F = 0.075$, df = 1,102, $p = 0.785$) between hypertensive and normotensive subjects ($F1 = 2.344$, df = 1,102, $p = 0.129$) within the uterine cancer group and the control group ($F2 = 0.033$, df = 1,102, $p = 0.785$).

FM prevalence (OR = 1.046, 95% CI: 0.405 to 2.702, $p = 0.925$) and FMC concentrations ($F = 0.187$, df = 1,102, $p = 0.666$; $F1 = 0.013$, df = 1,102, $p = 0.908$; $F2 = 0.196$, df = 1,102, $p = 0.659$) were approximately equal in the uterine cancer group and the control group, relative to type 2 diabetes.

The results indicate no association between the presence of dyslipidemia and the prevalence of FM (OR = 2.465, 95% CI: 0.702 to 8.652, $p = 0.159$) or FMC concentrations in endometrial tissues ($F = 0.003$, df = 1,112, $p = 0.959$; $F1 = 0.004$, df = 1,112, $p = 0.953$; $F2 = 0.158$, df = 1,112, $p = 0.692$) in groups of patients with or without uterine cancer.

There was no significant effect of body mass index, when patients were subdivided into particular categories involving norm and overweight/obese patients, on FM prevalence (OR = 0.473, 95% CI: 0.155 to 1.446, $p = 0.189$) or FMC concentrations in endometrial tissues ($F = 1.384$, df = 1,90, $p = 0.243$; $F1 = 0.143$, df = 1,90, $p = 0.706$; $F2 = 0.085$, df = 1,90, $p = 0.771$). In addition, no relationship between both FM prevalence and the concentration of FMCs in endometrial tissues and BMI was found in the uterine cancer group ($R^2 = 0.0194$, $p = 0.426$; $R^2 = 0.0092$, $p = 0.385$) or cancer-free controls ($R^2 = 0.0522$, $p = 0.191$; $R^2 = 0.0129$, $p = 0.239$).

Although uterine cancer is more common in patients older than 60 years of age, the association part of the study focused on the comparison of FM prevalence and FMC concentrations in endometrial tissues between age-matched groups. The control group and the uterine cancer group below the age of 60 also showed the difference in FM prevalence (73.3% vs. 41.6%; OR = 3.385, 95% CI: 1.137 to 10.077, $p = 0.029$); while no differences in FMC concentrations ($F = 1.148$, df = 1,57, $p = 0.289$) were observed. Likewise, the benign uterine disorder group and the uterine cancer group older than 60 years of age differed in FM prevalence (63.5% vs. 45.1%; OR = 2.275, 95% CI: 0.584 to 8.862, $p = 0.049$) but did not differ in FMC concentrations ($F = 1.228$, df = 1,55, $p = 0.20$). No difference in FM prevalence (controls: OR = 1.571, 95% CI: 0.361 to 6.842, $p = 0.547$; cancer patients: OR = 1.056, 95% CI: 0.414 to 2.692, $p = 0.909$) and in FMC concentrations (controls: $F = 1.352$, df = 1,39, $p = 0.259$; cancer patients: $F = 0.935$, df = 1,73, $p = 0.337$) was observed within individual groups with regard to age (below 60 yrs vs. over 60 yrs). Similarly, a linear regression analysis showed no association between testing positive for FM and concentrations of FMCs in endometrial tissues versus age at diagnosis in women who developed uterine cancer ($R^2 = 0.00047$, $p = 0.853$; $R^2 = 0.00053$, $p = 0.844$) or benign uterine disorders ($R^2 = 0.06941$, $p = 0.126$; $R^2 = 0.07944$, $p = 0.074$).

FM prevalence and FMC concentrations in endometrial tissues were also analyzed in relation to the patients' pregnancy history (i.e., number of childbirths, spontaneous abortions and miscarriages). No association between either FM prevalence or FMC concentrations and the number of pregnancies in the uterine cancer group ($R^2 = 0.0065$, $p = 0.496$; $R^2 = 0.000068$, $p = 0.944$) and the control group ($R^2 = 0.0013$, $p = 0.838$; $R^2 = 0.040$, $p = 0.247$) was revealed.

Discussion

We previously reported considerable inter-individual variation in the *DYS-14* copy number in a group of healthy men; therefore, we believed that the *DYS-14* sequence is not an optimal marker for male fetal DNA quantification (Hromadnikova *et al.*, 2008, 2009). That is why our studies to assess the prevalence of FM were done preferentially using the *SRY* gene as the marker. Unfortunately, a recently discovered sex-independent fetal-specific marker, the hypermethylated *RASSF1A* sequence, cannot be used for fetal DNA quantification in cancer patients, because hypermethylation of the promoter, associated with the inactivation of the tumor suppressor gene, has been frequently observed in various tumors (van der Weyden and Adams, 2007; Pallarés *et al.*, 2008; Banno *et al.*, 2012).

Overall, FM prevalence is more common in patients with benign diseases of the uterus than in uterine cancer. With regard to the individual subtypes of uterine cancer, the prevalence of FM was different between cancer patients and controls. Patients with type 1 endometrial cancer were less likely to be FM positive than uterine cancer-free controls. On the other hand, the prevalence of FM in those patients with a more aggressive, faster-growing form of cancer that tends to have a poorer prognosis, such as type 2 endometrial cancer, was the same relative to the control group.

The analysis conducted to examine the association between the prevalence of FM and the histological tumor grade score revealed significantly decreased prevalence of FM in low-grade endometrioid adenocarcinomas compared with controls. Interestingly, no difference in FM prevalence between high-grade uterine tumors and uterine cancer-free controls was observed.

Similarly, we observed a lower prevalence of FM in patients in whom the cancer was restricted to the body of the uterus (FIGO 1) compared with the control group with benign diseases of the uterus. The results also suggested that there was no difference in FM prevalence in endometrial tissues within women in whom the cancer had spread from the body of the uterus to the supporting connective tissue of the cervix (FIGO 2) and/or outside the uterus (FIGO 3+4) and the control group with benign diseases of the uterus. Overall, the present study suggests that better prognoses of uterine cancer are usually associated with lower prevalence of FM in tumor tissues compared with control tissues derived from benign uterine disorders. Interestingly, the concentration of FMCs in endometrial tissues did not differ between the examined groups.

It is evident that most women with nonmalignant uterine diseases harbor FMCs in very low concentrations. This phenomenon may be associated with ongoing disorders that are accompanied by local inflammation for which control patients underwent surgery. Besides hormonal regulation, a number of other factors involving inflammatory processes have been reported to regulate uterine myoma (Miura *et al.*, 2006; Khan *et al.*, 2010). On the other hand, the occurrence of FM in endometrial tissue may be linked with protection

against the development of uterine cancer. However, the occurrence of FM in women without these reproductive diseases, who could not be used as controls in the study, remains unclear. The endometrial biopsy is usually performed in women older than the age of 35 to determine the cause of abnormal menstrual periods, bleeding after menopause, bleeding associated with taking hormone replacement medications, or to screen for endometrial cancer. Normal endometrial tissue is described as proliferative or secretory endometrium that has the thickness of the uterine lining comparable to that of a healthy uterus and lacks the presence of precancerous and cancerous cell growth. However, even patients with abnormal vaginal bleeding have normal endometrial tissue on biopsy. Moreover, an endometrial biopsy does not provide a sufficient amount of biological material to study the occurrence of FM. The only option enabling study is utilization of biological material from surgery, indicated for uterine abnormalities identified with an endometrial biopsy.

The occurrence of male microchimeric cells in tumor tissues may be related to their active involvement in the complex process of tumorigenesis, involving tumor initiation and propagation inclusive of integration into the tumor stroma (Dubernard *et al.*, 2008), neoangiogenesis, facilitation of metastasis (Nguyen *et al.*, 2009), inducement of immune responses (Sawaya *et al.*, 2004; Gadi, 2009) followed by reparation of inflammation damaged tissues, as previously suggested for certain autoimmune diseases and malignancies (Lee *et al.*, 2010).

Since very low concentrations of FMCs are present in uterine cancer tissues and benign uterine disorders, the identification of their origin, phenotype, and role in maternal tissues, using the most sensitive currently available techniques, is relatively unfeasible. Nevertheless, an investigation of the biological consequences of pregnancy-associated FM is fundamental. Recently, reported developments of highly sensitive symptomatic qPCR assays have opened up the possibility of analyzing paraffin-embedded tissues that were previously unusable for chimerism studies (Dhimolea *et al.*, 2013). This discovery represents the first step toward quantification of allogeneic cells in more accessible biological material for most research centers. The next steps should be directed toward the development of more precise techniques for visualization of rare fetal cells in paraffin-embedded tissues that can be dissected for consecutive single cell analysis. Incorporation of live cell imaging techniques, to obtain a better understanding of biological function, through the study of cellular dynamics, would be desirable as well.

With these types of advancements, the origin of allogeneic cells in maternal tissues might be definitely confirmed. Although pregnancy is the most common setting, in which FM is encountered, microchimerism can also occur after allogeneic blood transfusion (Vervoordeldonk *et al.*, 1998; Lapierre *et al.*, 2007). Microchimeric cells can also be transferred *in utero* from a twin and potentially from an unrecognized (vanished) twin, which is relatively common in healthy pregnancies (de Bellefon *et al.*, 2010). Sexual transmission and needle sharing have also been suggested as possible mechanisms for microchimerism; however, to date, they have not been well documented (Bloch *et al.*, 2013).

Some health-related lifestyle factors, such as type 2 diabetes or insulin resistance, obesity, hypertension, dyslipidemia, and increasing age, may be highly relevant to later uterine cancer development (Grossman *et al.*, 2002; Amant *et al.*, 2005; Rapp *et al.*, 2005; Friberg *et al.*, 2007; Lucenteforte *et al.*, 2007; Pallarés *et al.*, 2008; Schmandt *et al.*, 2011; von Gruenigen *et al.*, 2011; Seth *et al.*, 2012; Chen *et al.*, 2013; Edlinger *et al.*, 2013). The lower prevalence of male FM (1.6 times) was also observed as affecting the development of uterine cancer.

We studied the association between FM prevalence and FMC concentrations in endometrial tissues and putative risk factors for uterine cancer. However, the association analyses pointed to no relationship between FM prevalence or FMC concentrations and appropriate risk factors. It is well known that multiparous women have a lower risk of developing hormone-dependent cancers. Several studies have provided evidence that multiparity might confer a protective effect on the risk of death from endometrial cancer (Chan *et al.*, 2011; Cramer, 2012). We tested the possibility that the prevalence of FM and concentrations of FMCs increased with increasing numbers of pregnancies. However, the analysis indicated no trend of increasing FM prevalence and FMC concentrations associated with higher numbers of pregnancies. On the basis of these findings, we hypothesized that the protective effect of multiparity, relative to the onset of uterine cancer, is not associated with FM.

Conclusion

Low concentrations of FMCs are very common in endometrial tissues derived from patients treated for benign uterine disorders. In cases of uterine cancer, a lower prevalence of FM was demonstrated. A lower prevalence of FM seems to be associated with better prognoses in uterine cancer based on tumor subtype, histological grade, and stage of the tumor. A lower prevalence of FM was observed in low-grade type 1 endometrial cancer and pT1 tumors. No relationship between FM prevalence or FMC concentrations in endometrial tissues and the prevalence of hypertension, type 2 diabetes, dyslipidemia, overweight and obesity, age of patients, and total number of previous pregnancies was demonstrated.

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Disclosure Statement

This work is original, unpublished, and not under consideration by another journal. No competing financial interests exist.

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