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RESEARCH

Microchimeric Cells, Sex Chromosome Aneuploidies and Cancer

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Abstract The phenomenon of feta-maternal microchimerisms inspires numerous questions. Many questions remain to be answered regarding this new avenue of genetics. The X and Y chromosomes have been associated with malignancy in different types of human tumors. We aimed to investigate the numerical aberrations of chromosomes X and Y in lung cancer (LC) and bladder cancer (BC) and review recent evidence for possible roles of microchimeric cells (McCs) in these cancers. We carried out cytogenetic analysis of the tumor and blood sampling in 52 cases of people with BC and LC, and also with 30 healthy people. A total of 48 (92.3 %) of the patients revealed sex chromosome aneuploidies (SCAs). A total SCAs was found in 9.8 % of 2282 cells that were analyzed as one or more cells in each case. The 68 and 95 SCAs were found in the 1952 (8.4 %) cells in peripheral blood, and 41 and 19 SCAs in the 330 (18.2 %) cells in the tumoral tissues respectively. There was a significant difference in the frequencies of SCAs between the patients and the control groups determined by the Fischer's Exact Test ($p<0.0001$). The frequencies of SCAs were higher in the tumoral tissues than in the blood ($p<0.0001$). There was a significant difference in the frequencies of SCAs between the tumor and blood tissues, and this was higher in the tumor tissue ($p<0.0001$). In general, 78.9 % (41) of the 52 patients with LC and BC had X and Y chromosome monosomies. Largely a Y chromosome loss was present in 77.8 % of the men, and the 47, XXY karyotype was found in 33.3 % of them. The second most common SCA was monosomy X, and was found in 71.4 % of the women. McCs were observed in 26.9 % of the 52 patients, and the frequencies of McCs were higher in the blood than in the tissues ($p<0.0001$). XY cells were identified in the lung and bladder tissues of the women who had been pregnant with boys, but not in those who had not. There was a significant difference in the frequencies of McCs between the LC and BC patients ($p<0.0005$). We speculate that the microchimerism could have a general beneficial role in cancer, in which some sites may not be evident because of an allogeneic maternal immune reaction that hastens cancer development. A further understanding of McCs may help in anticipating its implications in cancer. Our results may suggest that SCAs may be contributing factors in the development of LC and BC, and aneuploidies of X and Y chromosomes play a role in the pathogenesis of cancers.

Keywords Sex chromosome aneuploidies · Microchimeric cells · Lung cancer · Bladder cancer

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Introduction

CAs are one of the hallmarks of neoplastic cells, and the persistent presence of chromosome instability has been demonstrated in human cancers. Raised cancer risks have been

reported in association with particular SCAs in breast cancer, and in gonadal cancer in women with Turner's syndrome with Y chromosome material present [1,2]. There have also been case-reports suggesting the possibility of increased risks of many other cancers. The loss and gain of the X chromosome in women with aging is much more frequent than that of the Y chromosome in males. However, paradoxically, X chromosome aneuploidy is rarely seen in the dividing cells of the bone marrow in females [3]. A loss of the Y chromosome is frequently observed in myeloproliferative diseases and myelodysplastic syndromes, and can also be seen in lymphoproliferative disorders such as lymphomas [4]. A loss of the Y chromosome in contrast, is a common secondary change in both cancer cells and in a few leukemias. SCAs in LC may provide a valuable clue to the identification of target loci and culminate in a successful search for the major genes. BC is one of the most common malignancies, and has many known risk factors. Many cytogenetic alterations are known to occur in BC, but the significance of most of them is poorly understood.

The presence of a small population of cells within one individual from another genetically distinct individual is referred to as microchimerism (Mc). Because of this, analyzing the Y-chromosome specific genes is the more sensitive method and allows for the detection of one male cell per 100,000 female cells [5]. The phenomenon of feta-maternal McCs poses numerous questions. Many questions remain to be answered regarding this new avenue of genetics. The fetal microchimeric cells (FMcCs) may be deleterious for the mother when implicated in the induction of autoimmune diseases and of repeated abortion. Usually, FMcCs are beneficial for the mothers, and, can repair damaged tissues, transmit paternal resistance alleles, and improve the directory of T cell receptors. In cancer, the effects are more contrasted, beneficial and protective for certain cancers, but harmful and favouring the development for the others. FMcCs have been shown to repair damage to some tissues, but do they also fight cancer? Male cells in lungs were clustered in tumor rather than the surrounding healthy tissues. In conclusion, male presumed-FMcCs were identified in pathological post-reproductive tissues, where they were more likely to be located in diseased tissues at several-fold higher frequency than normal tissues. It is suggested that FMcCs are present in sites of tissue injury, and may be stem cells, either recruited from marrow or having proliferated locally [6]. Sawicki hypothesized that fetal cells have tumorigenic potential and can act as cancer stem cells [7]. She described a case in which FCs constituted the majority of the lung tumors in a murine model. Based on these studies, it is unclear whether FCs are involved in tissue repair or contribute to tumor growth. Further research is required to characterize this potentially protective subset of FMcCs. The phenomenon of FMcCs and maternal microchimeric cells (MMcCs) inspires numerous questions and offers new perspectives on the biology of cancer. More evidence is needed to clarify the role of Mcs in autoimmunity and cancer. Here, we aimed to evaluate the numerical aberrations of chromosomes X and Y in LC and BC by cytogenetic techniques, and then review recent evidence for possible roles of McCs in these cancers. While our aim was to find SCAs, we in fact found Mcs in the patients.

Materials and Methods

Study Population

This study involved 33 patients with LC who were referred from the Clinics for Chest Diseases, at the Balcali Hospital, Adana, Turkey. The patient group consisted of 30 males and 3 females. Their ages ranged from 33 to 73 years with a mean age of 56.5±10.005 years. Collective data was taken for each patient (age, smoking habits, the type of tumor, and the family cancer history). The tobacco consumption of these patients ranged from 10 to 80 packets/year and the average tobacco consumption was 41.8±17.7 packets/year. Four patients had never smoked tobacco. Twenty nine (29) patients had non-small lung carcinoma (NSCLC) whereas 4 had small cell lung carcinoma (SCLC). Not all patients received chemotherapy or radiotherapy before the present analysis (Table 1).

The study also involved 27 smokers and 7 nonsmokers with BC, referred from the Clinics of Urology, Balcali Hospital, in Adana, Turkey. The group of patients included 30 males and 4 females. Their ages ranged from 26 to 81 years, with a mean age of 60.6±14.2 years. For each patient, the collective data was included retrospectively to assess the diagnosis in relation to the smoking habits of each patient. The collective data included the patient's smoking habits and the family's cancer history. The tobacco consumption of these patients ranged from 2 to 60 packets/year and the average tobacco consumption was 23.5±18.5 packets/year, with 7 patients who had never smoked. None of the patients had received any preoperative radiotherapy or chemotherapy. A histological study showed that 22 (64.7 %) patients had high-grade (HG) invasive, 11 (32.3 %) had low-grade (LG) noninvasive tumors, and one patient had carcinoma in situ (CIS). The patient with CIS was added to the HG tumor group (Table 1).

The control group consisted of 30 healthy individuals (29 males and 1 females). Their ages ranged from 37 to 71 years, with a mean age of 53,4±10,4 years. The average tobacco concumption was 36,1±19,2 packets/years (Table 1).

Cytogenetic Examination from Blood

The peripheral blood samples from each subject with LC, BC, and the control groups were taken for culture. The expression of the cytogenetic anomalies in each sample was examined in the genetic laboratory of the Department of Medical Biology

Table 1 The demographic information of study population

Characteristics	Patients		Control
	Lung cancer	Bladder cancer	
Gender			
Males	30	30	29
Females	3	4	1
Age (Mean±SD)	56,5±10,005 years	60,6±14,2 years	53,4±10,4 years
Tobacco consumption	41,8±17,7 packets/year	23.5±18,5 packets/year	36,1±19,2 packets/year
Smoking status			
Never	4	7	–
Former	–	–	–
Current	29	27	30
Tumor type			
NSCLC	29	–	–
SCLC	4	–	–
BC-HG	–	23	–
BC-LG	–	11	–
Family cancer history	16.7 % (LC) 35 % (other cancers)	Unknown	–
Chemotherapy/Radiotherapy			
Yes	–	–	–
No	All	All	–

and Genetics, Faculty of Medicine, Çukurova University, Adana, Turkey. A sample of 0.3 ml blood was incubated at 37 °C for 72 h in RPMI-1640 medium. Standard cytogenetic techniques were used for harvesting and slide preparation in order to detect the SCA's in each sample. All the slides were dyed with GTG-banding, and at least 25 metaphases were analyzed.

Cytogenetic Examination from Tumoral Tissues

The lung tumor and nonmalignant bronchial epithelial samples were obtained from the patients by bronchoscopy. The normal bronchial epithelium was taken from the other lung section considered as the tumor-free area. The bladder tumor samples were obtained from the patients surgically. All samples were mechanically minced and enzimatically disaggregated by the Trypsin-EDTA (Biological Industries) for 1 h. After digestion, the BioAMF1 medium (Biological Industries) supplemented with the supplement, penicillin-streptomycin (Biological Industries), and gentamycin (Biological Industries) were used for the culture. A long-term cell culturing method was performed for the proliferation of tumor and normal cells. After sufficient proliferation (average 10 days), standard cytogenetic techniques were used for harvesting and slide preparation. After the GTG-banding, a minimum of 25 metaphases from the normal and tumor tissues for each individual were analyzed. However, in some cases, an insufficient amount of metaphase was obtained.

Statistical Analysis

The statistical analysis was performed using the statistical package for social sciences (SPSS/PC 19 version, 2010). In the statistical analysis, the Fischer's exact test was used to determine the significance of the differences between the blood and tissues, and between the patients and the controls in terms of the number of SCAs and microchimeric cells. The differences were considered significant at $p<0,05$.

Results

The 52 of 67 patients with LC and BC were analyzed as cytogenetic. A total of 48 (52 cases, 92.3 %) patients revealed SCAs. A total SCAs was found in 9.8 % of the 2282 cells analyzed as one or more cell in each case. The 68 and 95 SCAs were found in the 1952 (8.4 %) cells in the peripheral blood, and 41 and 19 SCAs in the 330 (18.2 %) cells in the tumoral tissues respectively. The frequencies of SCAs were higher in the tumoral tissues than in the blood, and this difference was found statistically significant ($p<0.0001$). In the control group, SCAs were found in 5.9 % of the 34 individuals (2/34), in 2(0.2 %) out of the cells among 980 cells analyzed. There was a significant difference in the frequencies of SCAs between the patient and control groups determined by the Fischer's exact test ($p<0.0001$). Thus far, research involving FMcCs and cancer has primarily used peripheral blood and

neoplastic tissues. We also found the cells carrying different sex chromosomes in men and women with LC and BC. These cells were considered as microchimeric cells (McCs). The male cells were identified in the lung and bladder tissues from the women who had been pregnant with a male children, but not in those who had not. These McCs were observed in 26.9 % of the 52 patients analyzed and in 20 (0.9 %) of 2282 cells analyzed. The frequencies of McCs were higher in blood than tissues ($p<0.0001$) (Table 2).

In the LC patients, a total of 33 (100 %) patients revealed SCAs in one or more cells (in both blood and tissue). A total 109 SCAs were found in 6.5 % of the 1668 cells analyzed. The 68 and 41 SCAs were found in the 1509 (4.5 %) cells in the peripheral blood and the 159 (25.8 %) cells in tissues respectively. There was a significant difference in the frequencies of SCAs between the tumor and blood tissues, and was higher in the tumor tissue ($p<0.0001$) (Table 2). The most common karyotype seen among the male patients was the loss of the Y chromosome (45,X-Y) [in 24 of 30 males (80 %)]. The sex chromosome trisomies were present in one or more cells in 16 cases (48.5 % of 33 cases), including 47,XXY (in 14 cases); 47,XXX (in 1 case) and 48,XXYY (in 1 case). McCs was identified in 7 (21.2 %) of 33 patients with LC. The McCs were found in the blood cells, but not in the tissue (Table 3).

The cells in the BCs had a higher incidence of aneuploidies. The SCAs were found in 18.6 % (114 cells) of 614 cells analyzed in 19 patients. The 95 and 19 SCAs were found in the 443 (21.4 %) cells of peripheral blood, and in the 171(11.1 %) cells in the tumoral tissues respectively (Table 2). Specifically, chromosome Y aneuploidies were observed to be most frequent in our patients [in 29 cells and in 12 patients (63.2 %)]. X-aneuploidies were observed in only 26.3 % (5 cases) of patients as findings in 13.8 % (85) cells (Table 3). Among these patients, one female had Turner mozaisizm [46,XX/45,X(80 %)]. Poliploidic numerical changes were present at 0.5 % of the cells in 2 (10.5 %) patients, including 69,XXY and 92,XXYY (Table 3). McCs observed in 7 (36.8 %) of 19 patients (36.8 % in blood and 5.3 % in tissues), was found in 2.1 % of the 614 cells analyzed. There was a significant difference in the frequencies of McCs between the LC and BC patients ($p<0.0005$) (Table 2).

In general, 78.9 % (41) of the 52 patients with LC and BC had X and Y chromosome monosomies. Monosomy Y was the most common karyotype among the patients, and was found in 35 of the 45 males (77.8 %). The second most common karyotype seen among 45 males was the 47,XXY seen in 15 patients (33.3 %). The other common karyotype seen among 7 females was the loss of one X chromosome (monosomy X), which was observed in only 5 patients (71.4 %). 48, XXXX and 69,XXY karyotypes were found in one cell of each of one of the cases. The 92,XXXX karyotype was observed in one female. Also in one patient, the 47,XYY karyotype was found in four of the 11 cells (%36.4) (Table 3).

Discussion

Most human cancers display structural and numerical CAs, and there is growing evidence that at least some of these aberrations play an important role in the development of all cancer types [8]. Aneuploidies are a commonly observed feature in BC, and it has been suggested that is a driving force in tumor development by enhancing genomic instability. One of the main results in our patients was the numerical sex-chromosome changes; except for structural CAs. Approximately 96 % of the patients revealed SCAs. The frequencies of SCAs was higher in the tumoral tissues ($p<0.0001$), and may be the affect of the susceptibility to the tumors. Several studies have shown that aneusomies in different chromosomes are associated with aggressive tumor behavior [9,10]. Just as, the X chromosome was found to be involved in carcinogenesis and the malignant progression of different types of tumors,

Table 2 The frequencies of sex chromosome aneuploidies and microchimeric cells in patients with lung and bladder cancer and controls

	LC Patients			BC Patients			LC + BC Patients			Control	
	Blood	Tissue	*p*-value	Blood	Tissue	*p*-value	Blood	Tissue	*p*-value	Blood	*p*-value
The number of cells with SCAs/ The number of cells analysed	68/1509	41/159	<0.0001[d]	95/443	19/171	<0.05[e]	163/1952	60/330	<0.0001[a]	2/980	<0.0001[b]
The number of microchimeric cells/ The number of cells analysed	7/1509	0/159	–	12/443	1/171	<0.0005[f]	19/1952	1/330	<0.0001[c]	–	–

[a] In the patient group when the frequencies of SCAs in tumoral tissues were compared with in bloods, there was a significant differences ($p<0.0001$)

[b] When the frequencies of SCAs in patients were compared with controls, there was a significant differences ($p<0.0001$)

[c] In the patient group when the frequencies of McCs in blood were compared with in tumoral tissues, there was a significant differences ($p<0.0001$)

[d] In the LC patient group when the frequencies of SCAs in tumoral tissues were compared with in bloods, there was a significant differences ($p<0.0001$)

[e] In the BC patient group when the frequencies of SCAs in blood were compared with in tissues, there was a significant differences ($p<0.05$)

[f] When the frequencies of McCs in BC patients were compared with LC patients, there was a significant differences ($p<0.0005$)

Table 3 Microchimeric cells and sex chromosomal changes in blood and malignant tissue of patients with lung cancer and bladder cancer

Case no.	Age/Sex	Tumortype	Sex chromosomal changes (number of cell with anomaly/total number of cell analyzed)		Microchimeric cells (number of cell with anomaly/total number of cell analyzed)	
			Blood	Tissue	Blood	Tissue
				LUNG CANCER (LC)		
C1	59/M	NSCLC	–	–	46,XX (1/50)	
C2	53/M	SCLC	–	45,X -Y (10/20)	–	–
C3	50/M	SCLC	45,X -Y (1/25)	–	–	–
C4	45/M	NSCLC	45,X -Y (1/25)	–	–	–
C5	73/M	NSCLC	45,X -Y (2/25), 47,XXY (1/25)	–	48,XXXX (1/25)	–
C6	59/M	NSCLC	45,X -Y (1/50), 47,XXY (1/50)	–	–	–
C7	53/M	NSCLC	45,X -Y (3/25)	–	–	–
C8	33/M	NSCLC	45,X -Y (2/50)	–	–	–
C9	55/M	NSCLC	47,XXY (1/50)	–	–	–
C10	63/M	SCLC	45,X -Y (3/35), 47,XXY(1/25)	45,X -Y (2/11), 47,XYY (4/11) 48,XXYY (1/11)	–	–
C11	58/F	NSCLC		45,X -X (1/21)	46,XY(1/25)	–
C12	65/M	NSCLC	45,X -Y (1/25), 47,XXY (1/50)	45,X -Y (1/25)	–	–
C13	61/M	NSCLC		–	46,XX (1/50)	–
C14	51/M	SCLC	45,X -Y (1/25)	–	–	–
C15	56/M	NSCLC	47,XXY (2/100)	–	–	–
C16	57/M	NSCLC	45,X -Y (7/25), 47,XXY (1/50)	–	–	–
C17	67/M	NSCLC	45,X -Y (1/50), 47,XXY (1/25)	–	46,XX (1/50)	–
C18	61/M	NSCLC	45,X -Y (3/25), 47,XXY (1/50)	–	–	–
C19	66/F	NSCLC	45,X -X (3/50)	45,X -X (20/25)	–	–
C20	56/M	NSCLC	45,X -Y (1/50)	–	–	–
C21	52/M	NSCLC	45,X -Y (1/25), 47,XXY (1/25)	–	–	–
C22	69/M	NSCLC	45,X -Y (1/25), 47,XXY (1/25)	–	48,XXXX (1/25)	–
C23	57/M	NSCLC	45,X -Y (1/50)	–	–	–
C24	68/M	NSCLC	45,X -Y (1/50)	–	–	–
C25	70/M	NSCLC	45,X -Y (1/50)	–	–	–
C26	71/M	NSCLC	45,X -Y (2/50), 48,XXYY(1/50)	–	–	–
C27	65/M	NSCLC	45,X -Y (1/50)	–	–	–
C28	59/F	NSCLC	45,X -X (6/35), 47,XXX (1/25)	–	46,XY (1/25)	–
C29	66/M	NSCLC	45,X -Y (3/25), 47,XXY (1/50)	–	–	–
C30	59/M	NSCLC	45,X -Y (3/50)	–	–	–
C31	47/M	NSCLC	47,XXY (1/50)	–	–	–
C32	54/M	NSCLC	47,XXY (1/50)	–	–	–
C33	51/M	NSCLC	45,X -Y (1/50)	–	–	–
		Grade/stage		BLADDER CANCER (BC)		
C1	61/M	BC-H/T2a		45,X -Y (2/20)	46,XX (2/50)	–
C2	53/F	BC-H/T1	45,X –X (1/50)	–	–	–
C3	81/M	BC-L/T1	45,X -Y (2/50)	–	–	–
C4	42/M	BC-L/T1	45,X -Y (2/25)	45,X -Y (1/3)	–	–
C5	73/M	BC-H/T3b	–	47,XXY (1/12)	46,XX (2/35)	46,XX (1/12)
C6	73/M	BC-H/T1	–	45,X -Y (1/25)	–	–
C7	38/M	BC-L/T1	–	–	46,XX (1/50),47,XXX (1/50)	–
C8	79/M	BC-L/Ta	–	45,X -Y (1/10)	–	–
C9	74/F	BC-H/T1	–	92,XXXX (1/17)	92,XXYY (1/17), 46,XY (1/17)	–
C10	26/M	BC-L/Ta	45,X -Y (1/33)	45,X -Y (9/14)	–	–
C11	56/M	BC-L/T1	45,X -Y (1/50)	–	–	–
C12	44/M	BC-H/T3b	45,X -Y (2/50)	–	–	–
C13	60/M	BC-H/T2a	–	–	46,XX (2/50)	–
C14	65/M	BC-H/T4	–	45,X -Y (1/30), 69,XXY (2/30)	–	–

Table 3 (continued)

Case no.	Age/Sex	Tumortype	Sex chromosomal changes (number of cell with anomaly/total number of cell analyzed)		Microchimeric cells (number of cell with anomaly/total number of cell analyzed)	
			Blood	Tissue	Blood	Tissue
C15	65/F	BC-L/T1	–	–	46,XX (1/10)	–
C16	72/M	BC-H/T4a	45,X -Y (1/25)	–	–	–
C17	72/M	BC-H/T3b	45,X -Y (1/50)	–	–	–
C18	79/F	BC-H/T1	46,XX/45,X -X(80/100)	–	–	–
C19	43/M	BC-H/T1	45,X -Y (4/20)	–	46,XX (1/20)	–

an increasing number of potentially responsible genes have been identified [11]. In particular, chromosomal gains or deletions have been associated with tumoral progression, the presence of metastases, and the worse prognosis in tumors of the breast, ovaries, and uterine cervix [12–14]. The addition of one X chromosome is relatively common in leukemias, lymphomas, and prostate cancer, and generally occurs in association with other karyotypic changes [15,16]. It is really not known whether this addition involves the active or the inactive X chromosome. Although there are numerous X-linked genes that may be involved in neoplasia, including the MAGE tumor-specific antigen loci, the pseudoautosomal GM-CSFR gene possibly escapes X chromosome inactivation, and the ARAF1, ELK1, and MCF2 oncogenes [17–21]. With regard to the Y chromosome, deletions have been shown to be involved in prostate cancer, male breast carcinomas, and pancreatic adenocarcinomas [22–27]. Our study also shows that there is an association between malignancy and SCAs. Therefore, it seems that sufficient conclusions can be derived from the clinical significance of SCAs in cancers and correlate genetic changes with disease aggressiveness.

In the present study, 78.9 % of the patients had monosomy X and Y. The Y aneuploidies were observed as common. In particular, the Y chromosome losses were found in 77.8 % of the male patients, with the second most common karyotype seen among males being the XXY, XXYY and XYY chromosome structures. There are also several reports that suggest that structural and numerical sex chromosome changes were seen frequently in LC patients [4,8]. Powell et al. suggested a prognostic relevance of Y chromosome losses in a study analyzing approximately 100 cases by cytogenetic [28]. Such an association was also confirmed from our patients. Furthermore, Mohanty stated that the loss of the Y chromosomes was a common secondary change in cancer cells and in a few leukemias, and common in many tumor types including papillary renal cell cancer [29]. The applied for losses of the Y chromosome has been determined in 10–40 % of BC [30,31]. The clinical significance of the Y chromosome losses is largely unknown, since relatively few sets of male BC patients have been evaluated. Previous studies have shown that there is a relationship between the Y chromosome loss and BC [32]. Thus, cells in the BCs had a higher incidence of aneuploidies in our patients, and specifically chromosome Y aneuploidies were observed to be the most frequent (84.2 %). The cause and relevance of the Y chromosome losses remain unclear, because of the associations between the Y chromosome losses and the clinical outcome. Later studies have supported the theory that the Y chromosome loss is a nonphenotypic event associated with the aging process in males [33–35]. However, other studies have shown that age is not clearly related and the X and Y chromosomes that are lost reappeared after therapy and during clinical remission. Therefore, it supports the hypothesis that the loss of sex chromosome is due to the evolution of a malignant clone. In which way it has influenced the malignant process; however, is difficult to say. These findings suggest that the Y chromosomes play a role in the pathogenesis of LC and BC, and may be important in the detection of cancer development at an early stage, thus it may be a diagnostic criterion for LC and BC.

X chromosome aberrations have been found in the neoplasms of different organs. In particular, the loss of the X chromosome, with a frequency ranging from 20 to 30 %, has been reported through cytogenetic analysis for a wide variety of tumors; including those of the lung, ovaries, testis, and nervous system, as well as melanomas [36,37]. These changes may be important for detecting cancer development at an early stage; thus, it may be a diagnostic criterion for LC. The X chromosome abnormalities have been reported at a lower frequency in leiomyomata [38–40]. However, case reports have suggested the possibility of the associations of X polysomy with several malignancies [41,42]. In our study, the second most common karyotype seen among the patients was the 47,XXY (33.3 %). This is not dissimiliar to the highly significantly raised risk of non-Hodgkin lymphoma based in male patients with 47,XXY [43]. It thus appears that a gene (s) raising the risk of non-Hodgkin lymphoma is located on the X chromosome, and is overexpressed because it escapes the X-inactivation; both in males and females with extra X chromosomes [44]. Sex chromosome losses are frequent in urothelial

bladder neoplasms of all grades and stages. This argues for the early occurrence of sex chromosomes losses in a subset of urothelial cancers. It appears that the numerical changes of the X chromosome may be preferentially involved and important in detecting cancer development. This amplification might suggest that females with X chromosome allelic imbalance might be more prone to developing cancers.

Microchimerism is the existence of small amounts of DNA in the body coming from a genetically different person. During pregnancy, a small number of fetal stem cells cross the placenta into the mother's bloodstream and can survive for decades in her skin, liver, brain, and spleen; a phenomenon called fetal microchimerism (FMc). FMcCs have been shown to repair the damage of some tissues, but do they also fight cancer? Thus far, research involving FMc and cancer has primarily used peripheral blood and neoplastic tissue [43–46]. The main results in our patients were the existence of small amounts of DNA in the body coming from a genetically different person. These McCs were observed in 26.9 % of the patients, and in 0.9 % of the cells analyzed. Male cells were identified in lung and bladder tissue from women with known pregnancies with a male child only. Unfortunately, we were not able to determine the nature of these cells. Why were McCs observed in patients with cancer, but not in the controls? McCs were frequently seen in the tissues of patients. This poses numerous questions: Does microchimerism have a role in the etiology of cancer? Are McCs beneficial for the fetus or the mother or McCs as cancer stem cells?

FMcCs have been hypothesized to be involved in chronic inflammatory responses leading to tissue damage or to participate in the repair of damaged tissue and in resistance to infections. However, it cannot be excluded that it may not have any biological significance. It is suggested that FMcCs are present at sites of tissue injury and may be stem cells; either recruited from marrow or having proliferated locally. Their frequency in tumors was several-folds higher in lung tumors than in the surrounding healthy lung tissue. FMcCs have also been shown to cluster in lung tumors in women decades after pregnancy [45]. Microchimerism may cause a protective effect by causing our immune system to be alert for malignant cells to destroy. We have known for some time that pregnancy can be protective from breast cancer. However, Gadi shows that FMcCs may help reduce risk of breast cancer [46]. Fetal microchimerism (FMc) may cause a protective effect by causing the immune system to be alert for malignant cells to destroy. Recently, there has been an increase in studies on FMcCs and cancer in both humans and mice. The proposed hypotheses concerning the function of FCs in the pathogenesis of maternal cancer include the promotion of tumorigenesis, the protection by providing immunosurveillance, and the participation in tissue repair. In contrast, Sawicki hypothesized that FMcCs have a tumorigenic potential and can act as cancer stem cells [47]. She described a case in which FMcCs constituted the majority of lung tumors in a murine model. The ability to self-renew, a characteristic shared by cancer stem cells and normal stem cells residing in somatic tissues, and responsible for their maintenance, has led to some suggestions that tumorigenesis may be initiated in normal stem cells [7]. As a result of the mutation or microenvironmental influences, these cells may lose regulatory controls that normally keep cell proliferation and differentiation in check and thus, cancer may develop. Given the stem cell-like potential of FMcCs to differentiate into the cells of the multiple lineages and their persistent presence in women following pregnancy, it is intriguing to speculate that, as a consequence of genetic alterations or changes in their microenvironmental niche, these cells may act like cancer stem cells and give rise to tumors. The results of one study suggest that fetal microchimerism may be involved in the pathogenesis or progression of cervical cancer [48]. Nelson and collaborators detected fetal microchimerism in significantly fewer women who had breast cancer than in healthy women [49]. While this result suggests that the circulating cells of the fetal origin may have differentiated and contributed to the development of cervical tumors, the number of tumors examined was very small, and further work needs to be done to draw firm conclusions. The overall results of the previously cited breast cancer study clearly show a protective role for FMcCs [46]. In light of the possibility that these cells may sometimes give rise to tumors, it is noteworthy that the incidence of fetal cells in the peripheral blood buffy coat of two breast cancer cases in this study was very high relative to the incidence in other breast tumors and in the normal control women. Thus, the two outlying cases had values of 277 and 374 fetal genomes per 106 maternal genomes, whereas the median concentrations for cases and controls were 2, with ranges of (0–375) and (0–78) respectively. Perhaps these two outlier cases are rare ones where FMcCs have transformed and actually contributed to the growth of the tumor. Based on these studies, it is unclear whether FMcCs are involved in the tissue repair or if they contribute to the tumor growth.

In the present study, there was a significant difference in the frequencies of McCs between patients with LC and BC ($p<0.0005$). The concentration of FMcCs had approximately a four-fold increased risk for BC when compared with LC. This result suggested that BC might be associated with McCs. However, why were McCs frequently seen in patients with BC? Similarly, Kamper-Jørgensen et al. recently found a male microchimerism presence to be associated with a 70 % reduced odds for developing breast cancer, and a 4-fold increased odds for developing colon cancer [50]. In another study, FMcCs were identified in 50 % of papillary thyroid tumors [51]. Women harboring FMcCs were less likely to have had breast cancer and FMcCs concentrations were higher in the controls versus the cases [49]. Perhaps the microenvironment of normal lung stem cells contributes to the

propensity of the A/J strain to develop lung tumors, and serves in a similar way to convert FMcCs lodged in the lung into the cancer stem cells. The frequency of Mc appears to be increased when fetal cytogenetic abnormalities occur. In a study by Bianchi et al. the mean number of the male fetal cells present in the mother when the fetus has Down's syndrome was elevated 6–fold compared to women with normal male fetuses. Furthermore, in a study regarding FMc in newborn tissues reported by Srivatsa et al., it was found that FMcCs levels were highest in the thymus of a child with Down's syndrome [52,53]. These results suggested that LC and BC might be associated with McCs.

Conclusion

Our results may suggest that SCAs may be contributing factors in the development of LC and BC, and aneuploidies of the X and Y chromosomes play a role in the pathogenesis of cancers. The large interindividual variability of these results and an increased relative risk has been described in all studies published to date. We consider that a high level of SCAs in tumoral tissues and peripheral blood lymphocytes may be an early marker of LC and BC. We are only just beginning to understand the role that fetal-maternal McCs play in cancer. Presumed-MCs in the lung and bladder were clustered in the tumor rather than blood, and were four times greater in the bladder than in the lung tissue, where they were more likely to be located in diseased tissues. It is suggested that McCs can be present at sites of tissue injury and may be stem cells, either recruited from marrow or having proliferated locally. We speculated whether Mc could have a general beneficial role in cancer, which in some sites may not be evident because an allogeneic maternal immune reaction hastens cancer development. It is my hope that future studies associating Mc and cancer at various sites will help to clarify this. Now it seems that McCs survive in a mother's tissues. If fetal cell levels can be boosted, it may also aid cancer treatments.

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