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ORIGINAL ARTICLE

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Xq25 and Xq26 identify the common minimal deletion region in malignant gastroenteropancreatic endocrine carcinomas

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Abstract Loss of heterozygosity (LOH) for markers on X chromosome are associated with malignancy in endocrine tumors of the stomach and pancreas. The aim of this work is to investigate low-grade, well-differentiated endocrine carcinomas (WDEC) vs high-grade, poorly differentiated endocrine carcinomas (PDEC) of the gastroenteropancreatic (GEP) tract for common deletion regions on X chromosome. We performed a comparative allelotyping analysis with 24 highly polymorphic markers for the X chromosome in 12 WDECs and 5 PDECs. Overall, the LOH frequency in all informative loci investigated was 59% in primary and 61% in metastasis, with a significantly higher rate in PDECs than in WDECs ($p<0.015$ for primary and $p<0.00005$ for metastasis). In both WDECs and PDECs, the small Xq25 region as defined by DXS8059, DXS8098, and DXS8009 markers showed higher LOH rate as compared to the rest of the chromosome markers ($p<0.04$). In addition, LOH was very frequently elevated also in DXS294 and in DXS102 loci mapping the chromosomal region Xq26. In no instances differences were found between primary tumors and metastases. Methylation analysis revealed that Xq25 loss preferentially occurred on the inactive X chromosome, a feature in agreement with findings from other human cancers suggesting escape of tumor suppressor genes to X chromosome inactivation at this region. Overall, our data indicate that the two chromosomal regions, Xq25 and Xq26, may participate to the malignant progression of GEP endocrine carcinomas.

Keywords X chromosome · Gastroenteropancreatic endocrine carcinomas · Loss of heterozygosity · Tumor suppressor genes

Introduction

Molecular and cytogenetic studies indicate that X chromosome is involved in the carcinogenesis and malignant progression of different types of human tumor. An increasing number of genes have been isolated as potentially responsible. Comparative genomic hybridization (CGH) analysis has demonstrated frequent gains on X chromosome that correlated with tumor progression in liver [38], stomach [33], and adrenal gland [32]. Conversely, X chromosome deletions have frequently been associated with the presence of metastases and/or worse prognosis in cancer of the breast [28], the uterine cervix [18], the ovary [6], and in papillary renal cell carcinoma [16]. These deletions are regarded as strong evidence for the occurrence of tumor suppressor genes (TSG) on this chromosome [6, 9, 16, 28, 29].

In our previous studies, allelic losses [loss of heterozygosity (LOH)] for just three microsatellite markers on X chromosome (DXS989 on p arm and DXS1003 and DXS1192 on q arm) were found to be restricted to foregut malignant endocrine tumors [9, 29]. These data were subsequently confirmed by Missiaglia et al. [25] who demonstrated a strong association between X chromosome markers LOH, aggressiveness, and shorter survival rates in 20 nonfunctioning pancreatic endocrine tumors (PETs). More recently, on this line, Chen et al. [4] found that X chromosome LOH frequently occurred in gastrinomas and was significantly associated with aggressive tumor growth and larger tumor size. On this basis, X chromosome LOH has been regarded as a potentially useful prognostic factor for aggressive growth in endocrine tumors of the upper

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gastrointestinal tract and pancreas. Conversely, we and others demonstrated that poorly differentiated endocrine carcinomas (PDEC) of the stomach and the colon display similar genetic alterations indicating a site-independent genetic background for undifferentiated endocrine carcinomas at variance with well-differentiated endocrine carcinomas (WDECs) [12, 30].

In this work, we performed a high-resolution comparative allelotyping on X chromosome on WDECs vs PDECs of the gastroenteropancreatic (GEP) tract. Methylation analysis for CpG islands on Xq25 and immunohistochemistry for metalloproteinases 2 (MMP-2) and 9 (MMP-9) regulated by the myeloid elf-1 like factor gene (MEF) located at Xq26 were also performed. Our main aim was to identify minimal common deletion regions, ultimately indicating TSG possibly involved in the mechanism of malignancy in tumors of the GEP endocrine system.

Materials and methods

Patients and tissue specimens

Matched tumor and adjacent normal tissues were obtained from 17 female patients with malignant endocrine tumors of the pancreas and gastrointestinal tract collected in the files of the Department of Anatomic Pathology, University of Parma. According to the World Health Organization (WHO) criteria [36], in 12 patients, the tumors were WDECs (low grade malignant tumors, WHO class 2), and in five patients, the tumors were PDECs (high grade malignant tumors, WHO class 3). The age range of patients with WDEC was 43–73 years (mean 58.6), and with PDEC, 45–83 years (mean 70).

The main clinicopathological data of patients are summarized in Table 1. In eight cases, metastases were also analyzed (lymph node, cases #1, #2, #4, #6, #13, #14; liver, case #16, and both lymph node and liver, case #17). In two cases (#3 and #12), only liver metastases were available for the study. PDECs were either solitary (#13, #14, #15) or associated with adenocarcinoma (#16) or adenoma (#17). The primary tumor of patient #17 was not available for study, and DNA from liver and lymph node endocrine carcinoma metastases was used. In the two biphenotypic cases, the endocrine and nonendocrine tumor components occurred as separate masses with distinct morphology and were microdissected separately.

All tissue specimens were routinely formalin fixed and paraffin embedded. For 80% tumor cell enrichment, both normal and tumor tissues were microdissected from 5-μm sections stained with hematoxylin and DNA extracted using the QIAamp Tissue Kit (Qiagen GmbH, Hilden, Germany).

Polymerase chain reaction

DNA samples were microallelotyped for 24 polymorphic microsatellite markers covering the whole X chromosome (Table 2) selected from the Genome databases (http://www.gdb.org and http://www.geneatlas.org). Moreover, four microsatellite markers (D3S1621, D3S1100, D3S1478,

Table 1 Clinicopathological data of 17 female patients affected by malignant gastroenteropancreatic endocrine carcinomas

Type	Case	Age	Location	Metastases	Hormonal production	Syndrome
WDEC	1	47	Pancreas	N, L		NO
	2	63	Pancreas	N	Gastrin	NO
	3	51	Pancreas	L	PP	NO
	4	73	Pancreas	B, L	Somatostatin, glucagon	NO
	5	51	Pancreas	N, P	PP	NO
	6	43	Pancreas	N	Glucagon	NO
	7	75	Pancreas	P	PP	NO
	8	73	Pancreas	N		NO
	9	63	Pancreas	N		NO
	10	47	Stomach	N		NO
	11	70	Stomach	L	Serotonin	NO
	12	47	Duodenum	N, L	Gastrin	ZES/MEN-1
PDEC	13	83	Stomach	N		NO
	14	63	Colon	N		NO
	15	79	Colon	N, L		NO
	16[a]	80	Colon	N, L		NO
	17[b]	45	Colon	N, L		NO

WDEC Well-differentiated endocrine carcinoma, *PDEC* poorly-differentiated endocrine carcinoma, *N* lymph node, *L* liver, *P* peritoneum, *B* bone, *PP* pancreatic polypeptide, type of associated clinical syndrome: *ZES/MEN-1* Zollinger–Ellison syndrome/multiple endocrine neoplasia type 1, *NO* absence of associated syndrome (non functioning tumor)

[a]Associated with adenocarcinoma

[b]Associated with tubulo-villous adenoma

Table 2 Microsatellite marker set used for the allelotyping analysis on X chromosome and cytogenetic location

Microsatellite markers	Cytogenetic band	Geneatlas position[a] (bp)	UniSTS (NCBI)[a] position (bp)
DXS996	Xp22.3–p22.3	4.579.779	5.718.887
DXS207	Xp22.2–p22.2	14.112.607	15.188.763
DXS989	Xp22.13	21.564.927	22.944.099
DXS1237	Xp21.3–p21.2	30.390.426	31.745.042
MAOA.PCR1	Xp11.4–p11.3	41.474.541	43.359.613
DXS1003	Xp11.23	44.475.054	46.290.665
DXS1367	Xp11.3–p11.23	45.629.747	47.445.359
DXS1111	Xq12	61.995.658	67.811.398
DXS56	Xq13.2–q13.3	70.359.906	76.754.441
DXS738	Xq21.1–q21.1	–	–
DXS990	Xq21.33	86.401.205	89.918.106
DXS1153	Xq22.1–q22.3	–	98.452.635
DXS1220	Xq22.3–q23	107.802.910	114.431.063
DXS1001	Xq24	112.852.213	119.618.571
DXS8059	Xq24–q25	115.186.425	121.988.946
DXS8098	Xq24–q25	115.837.288	122.639.810
DXS8009	Xq25–q26	119.039.778	125.899.570
DXS1047	Xq25–q26	121.941.047	128.800.855
DXS692	Xq25–q26.2	124.850.440	131.710.249
DXS730	Xq26	130.696.964	137.556.978
DXS294	Xq26	–	–
DXS1192	Xq26	131.233.614	138.093.629
DXS102	Xq26	131.248.873	138.108.888
DXS731	Xq27–q28	139.986.141	146.878.018

[a]See "Materials and methods"

D3S1481) located on 3p chromosome were analyzed in the study. Forward primers were synthesized with a fluorescent tag (WellRed dyes from Research Genetics, Huntsville, AL, USA). DNA was amplified in a 25-μl reaction solution containing 2.5 μl of 10× buffer (Promega, Madison, WI, USA), 1 to 2 mM $MgCl_2$, 0.4 μmol/l primer pairs, 200 μM dNTPs, and 1.25 U Taq polymerase (Promega). Amplifications were performed using a 5-min initial denaturation at 95°C, followed by 35 cycles of 30″ at 94°C, 30″ at 53–64°C, and 1′ at 72°C; or by 40 cycles of 1′ at 94°C, 1′ at 55°C, and 2′ at 72°C (Table 2). The fluorescent-labeled polymerase chain reaction (PCR) products underwent electrophoresis on an automated DNA sequencer CEQ 2000XL (Beckman Coulter Inc., Fullerton, CA), and signals were analyzed using CEQ 2000XL analysis software (Beckman Coulter).

Definition of loss of heterozygosity

Allelic loss was defined as the ratio of relative allelic peak height in the tumor DNA to relative allelic peak height in the corresponding normal DNA. The formula employed for the calculation was (T2/T1)/(N2/N1), where T1 and N1 are the height values for the smaller allele, and T2 and N2 are the height values for the larger allele of the tumor (T) and normal (N) samples, respectively. For informative markers, LOH was scored when signal reduction for one allele was of 40% or more [2, 29].

Methylation-specific polymerase chain reaction

The methylation status of a CpG site located at position 225–226 of the DXS8059 microsatellite sequence was determined by methylation-specific PCR (MS-PCR) [28]. Genomic DNA (3 μg) was modified with sodium bisulfite as originally described by Herman et al. [14]. DNA modification was performed with the CpGenome DNA modification kit according to the manufacturer's instructions (Intergen Company, Oxford, UK). Primers for PCR amplification of the methylated (MA) allele were 59M2CF, 5′-TGTAGTTAGTTTGGTGTTTGTT-3′ and 8059MR, 5′-CAAACCAAAAATTCCCTCCG-3′, yielding a 97-bp fragment, whereas the primers for amplification of the unmethylated (UMA) allele were 59M2CF, 5′-TGTAGT TAGTTTGGTGTTTGTT-3′ and 8059N4R, 5′-AACCA CAAACCAAAAAATTCCCTCCA-3′, yielding a fragment of 102 bp. Genomic DNA positive controls were unmethylated DNA from normal lymphocytes for UMA allele and CpGenomeUniversal Methylated DNA for the MA allele (Intergen Company). Multiplexed PCR with all four primers was performed in a final volume of 25 μl in a mixture containing 125 μM each dNTP, 1.5 mM $MgCl_2$, 1 μM each of the primers, 0.05 U/μl of Thermo-Start DNA Polymerase (ABgene, Surrey, UK), and 50 ng of the bisulfite-modified DNA. MS-PCR cycling conditions were as follows: 15′ at 95°C, for DNA polymerase activation; 35 cycles each consisting of denaturation at 95°C for 30″, primer annealing at 54°C for 30″, and elongation at 72°C for

30″. MS-PCR products were analyzed by 3% metaphore gel electrophoresis. To determine whether the MA or UMA sequence of the DXS8059 marker was lost, normal ad tumoral tissues were compared for the intensity of MS-PCR bands corresponding to the methylated and unmethylated CpG sites using the Gel Doc software (Biorad). The following formula was employed: $(\text{alleleUMA/alleleMA})_{\text{NORM}}/(\text{alleleUMA/alleleMA})_{\text{TUM}}$. Values less than 1 were indicative of LOH on MA allele, and values greater than 1 were for LOH on UMA allele.

Immunohistochemistry

The following primary monoclonal antibodies were used: anti-metalloproteinase 2 (MMP-2, clone 1B4, Novocastra, Newcastle, UK; working dilution, 1/50); anti-metalloproteinase 9 (MMP-9, clone 2C3, Novocastra; working dilution, 1/50). Sections were treated with 10 mM citrate, pH 6.0, in a 750-W microwave oven for three 5′ cycles for antigen retrieval before immunostaining. Immunostaining was done with the streptavidin-biotin kit (LSAB2, Dakocytomation, Glostrup, Denmark) according to the manufacturer's specifications. Slides were counterstained with hematoxylin. Colon carcinomas served as positive control for MMP-2 and liver for MMP-9. Negative controls consisted of substituting normal mouse serum for the primary antibodies. The staining was scored as follows: no positive cells (−); <20% of the tumor cells staining positive (+); 20–50% of the tumor cells staining positive (++); >50% of the tumor cells staining positive (+++) [37].

Statistics

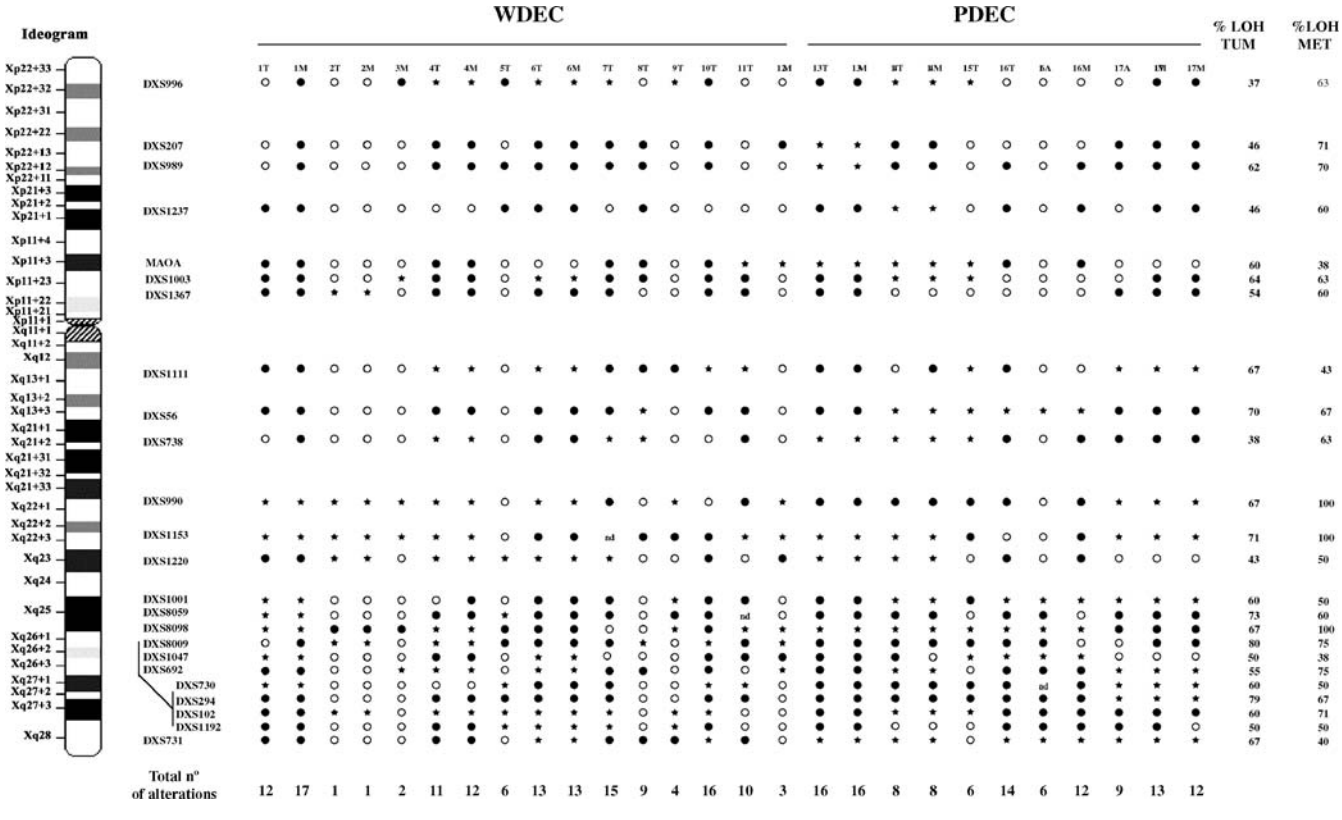
The data obtained in the primary tumors and metastases were evaluated separately and examined with χ^2 analysis and Fisher's exact test with the SPSS 11.5 statistical software package (SPSS, Inc., Chicago, IL). *P* values less than 0.05 were considered statistically significant, and two-sided *P* values were used.

Results

Microallelotyping analysis on X chromosome

The results of the LOH analysis are summarized in Table 3. In all 17 patients, LOH was detected for at least 1 of the 24 tested polymorphic markers mapping at the whole X chromosome. In one case (#13), LOH was identified with all microsatellites studied. Metastases showed the same

Table 3 Allelotyping results of 17 female patients with digestive malignant endocrine tumors (cases #1–12 WDEC, cases #13–17 PDEC)



• LOH, ○ retention of heterozygosity, ★ noninformative, *nd* not determined, *T* primary tumor, *M* metastasis, *A* esocrine component

pattern of alterations as the primary tumors except in cases #1 and #4 in which allelic losses were found for additional loci (five in case #1; one in case #3). Overall, the LOH frequency for all informative loci investigated was 59% for primaries and 61% for metastases, with higher rates for PDECs as compared to WDECs primaries (72 vs. 55%, $p<0.015$) and metastases (81 vs. 48%, $p<0.00005$). The frequency of LOH at the individual 24 microsatellite loci varied from 39% in DXS996 locus to 82% in DXS8098 locus. The total degree of informativeness for the microsatellite markers used in the study was of 70% (38–93%), without any difference between WDEC (72%) and PDEC (66%).

Two common regions of deletion were identified on the long arm of X chromosome. Overall, the most frequent losses were observed at Xq25 region with a LOH frequency for DXS8059, DXS8098, and DXS8009 loci as high as 73, 67, and 80%, respectively, in primary tumors and 60, 100, and 75%, respectively, in metastases. No statistical significant difference was observed at any level between primary and metastatic tissues.

In particular, the LOH rate for primary carcinomas in the small region (of approximately 4.6 Mbp in length) defined by DXS8059 and DXS8009 was significantly higher than in the rest of the chromosome ($p<0.04$). No statistically significant differences were observed between WDECs and PDECs both in primary (68 vs. 87%, respectively) and metastatic tissues (64 vs. 83%, respectively). Moreover, DXS8098 was the only locus lost in WDEC case #2, whereas in case #3, its deletion was associated with a loss of only one of the other p-ter markers. Finally, all informative markers in this region were lost in nine cases (#1mt, #4, #5, #6, #10, #13, #14, #16, #17mt).

The overall frequency of LOH was elevated also for DXS294 (primary, 79% vs metastases, 67%) and DXS102 (primary, 60% vs metastases 71%) loci mapping the chromosomal region Xq26 (of approximately 1 Mbp in length). The rate of losses at these loci was significantly higher in PDEC (primary and metastasis 100%) than in WDEC (primary 62%, metastasis, 49%). For all cases investigated, the degree of markers' informativeness in the chromosomal regions at Xq25 and Xq26 was of 69 and 70% respectively, with no difference between WDECs and PDECs.

Other microsatellite markers with high LOH rate in WDECs and PDECs both in primary and metastatic tissues were DXS989 (62 and 70%), DXS56 (70 and 67%), DXS990 (67 and 100%), and DXS1153 (71 and 100%) located on Xp22.13, Xq13.2–13.3, Xq21.33, and Xq22.1–22.3 respectively. No statistically significant difference was observed in the LOH frequency of these microsatellite markers between WDECs and PDECs.

To evaluate the relation of allelic losses on X chromosome with those on 3p, reported by several studies [8, 15, 27, 31] to be associated with malignant progression, we have investigated four microsatellite markers of 3p (D3S1621, D3S1100, D3S1478, D3S1481). In our case, the overall 3p LOH rate was 48% in primary tumor and 37.5% in metastases with significant difference between primary PDECs (90%) and WDECs (19%) ($p<0.0003$). When the LOH rates at Xq25 and Xq26 were compared with those at 3p, an inverse correlation was found for WDECs (Xq25, $p<0.0007$; Xq26, $p<0.002$). Conversely, no differences were found for PDECs.

The nonendocrine component of the two biphenotypic tumors (#16, #17) consistently showed a lower number of LOH than its endocrine counterpart (Table 3).

Methylation analysis on X chromosome

Methylation analysis of CpG islands in microsatellite loci may provide information concerning chromosome inacti-

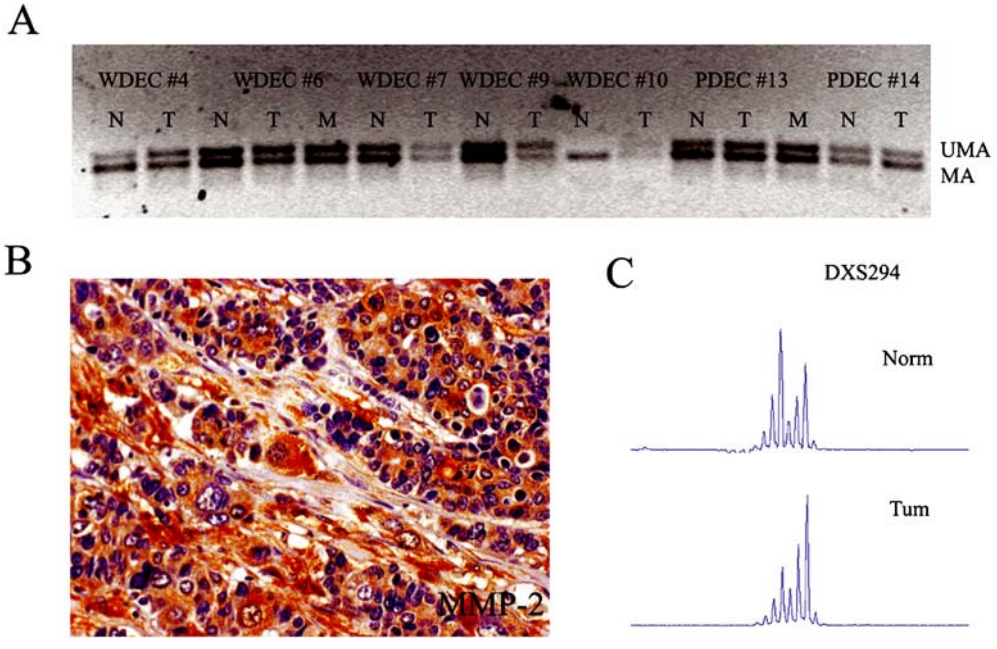


Fig. 1 **a** Results of methylation-specific PCR of a CpG site located at position 225–226 of the DXS8059 marker in malignant gastroenteropancreatic endocrine tumors. Analysis of the intensity of the bands using the Gel Doc software showed LOH of the methylated allele in all samples except the PDEC #14 (*N* normal, *T* tumor tissue, *M* metastatic tissue, *UMA* unmethylated allele, *MA* methylated allele). **b** Intense immunohistochemical expression of MMP-2 in a case of PDEC (#13). **c** Electropherograms of the same tumor showing LOH for DXS294 marker in tumoral tissue

Table 4 Immunohistochemical expression and LOH status in 10 female patients with gastroenteropancreatic endocrine carcinomas

Case #	Type	Xq25LOH	Methylation in Xq25	Xq26LOH	Immunohistochemical expression	
					MMP-2	MMP-9
2	Primary	Present	MA	Absent	+	−
3	Metastasis	Present	MA	Absent	−	−
6	Primary	Present	MA	Present	−	−
7	Primary	Present	MA	Present	+	−
9	Primary	Present	MA	Absent	+	−
11	Primary	Present	MA	Present	−	−
13	Primary	Present	MA	Present	+++	−
14	Primary	Present	UMA	Present	+	−
	Metastasis	Present	UMA	Present	−	−
15	Primary	Present	MA	Present	+++	+
16	Primary	Present	UMA	Present	−	+
	Metastasis	Present	UMA	Present	+	−

MA Methylated, *UMA* unmethylated

vation. To determine if the X copy undergoing LOH in our series of GEP carcinomas was active or inactive, we selected DXS8059 marker containing two CpG sites available for methylation analysis (Fig. 1) because the DXS8098 marker, which showed the highest rate of LOH in our series, does not contain any CpG site. The five WDECs (#4, #6, #7, #9, and #10) and the four PDECs (#13, #14, #16, and #17) which exhibited LOH at DXS8059 marker were analyzed (see Table 4). Our results showed that Xq25 LOH occurred in the active (unmethylated) allele of cases #14 and #16 and in the inactive allele of the remaining cases.

Immunohistochemistry

To determine the potential implication of myeloid elf-1 like factor (MEF), a putative tumor suppressor gene located on Xq26, tumor samples from 10 cases were investigated by immunohistochemistry for MMP-2 and MMP-9 that are negatively regulated by MEF. The immunohistochemical results are summarized in Table 4. The expression of MMP-2 was detected in 5/9 (56%) primary tumors, with strong positivity (+++) in two cases and weak and focal positivity (+) in three cases. The expression of MMP-9 was detected in 2/9 (22%) primary tumors, one of these showing moderate positivity (++), the other weak staining. No relation between MMP-2 or MMP-9 and Xq26 LOH was found (Table 4). It is worth noting, however, that the two cases with strong MMP-2 positivity showed Xq26 LOH (Fig. 1b).

Discussion

Different regions of X chromosome were shown to be deleted in malignant tumors of different organs including breast [28], uterine cervix [18], ovary [6], prostate [19], and kidney [16]. The present work shows high frequency of LOH on X chromosome in a series of malignant GEP endocrine primaries (59%) and metastases (61%), with a significantly higher LOH rate in PDEC (72% primaries and 81% metastases) than in WDEC (55% and 48%). This latter finding is in accordance with the results of a recent work by Furlan et al. [12] based on a microallelotyping analysis of chromosomes 1, 3, 5q, 6, 11, and 18, showing a significantly higher frequency of chromosomal derangements in PDECs than in WDECs of the GEP tract. Moreover, no recurrent allelic imbalance at specific chromosomal regions was detected in PDECs according to the primary anatomic site. Similar findings were also reported in a previous study from our laboratory [30]. The present results demonstrate that X chromosome is heavily involved in the chromosomal instability of PDECs.

Our study also aimed at assessing common deletion regions on X chromosome in an attempt to focus on potential TSGs implicated in endocrine tumor pathogenesis and malignancy. The existence of such genes is supported by the X chromosome involvement in the establishment of immortality and in the control of cell proliferation in vitro [21, 34].

Frequent X chromosome LOH has been detected in carcinomas of gallbladder [40], breast [28], ovary [6], and in renal oncocytomas [39]. In neuroendocrine tumors, X chromosome deletions have been reported in parathyroid [11], pancreatic, and gastric tumors [9, 25, 29]. Such losses were associated with malignancy in endocrine tumors of the stomach and pancreas, whereas they were virtually absent in benign neoplasms. However, the small number of markers tested in these studies did not allow to identify a common minimal chromosomal region of deletions [9, 29].

The present study of malignant GEP endocrine tumors relied on a panel of 24 polymorphic microsatellite markers on the whole X chromosome and demonstrated that the two chromosomal regions Xq25 and Xq26 were most frequently lost. The highest frequency of LOH (67% in primaries and 100% in metastases) was found for DXS8098 marker mapping on Xq25 between the two

other frequently deleted loci, DXS8059 and DXS8009 (73 and 80% in primaries and 60 and 75% in metastases, respectively). The same LOH pattern with the highest frequency of losses for DXS8098 and high LOH rates for DXS8009 and DXS8059 was reported in breast carcinomas and associated with larger tumor size, higher histological grade, and axillary lymph node metastasis [28].

For the development of neoplasia, complete inactivation of the tumor suppressor function is necessary in autosomal chromosomes requiring the inactivation of both alleles [22]. In the X chromosome, one of the two copies is almost entirely inactivated by DNA methylation during early development [28]. In the present study, we determined which copy, active or inactive, underwent loss of the Xq25 region. Our results showed that the loss occurs preferentially on the inactive X chromosome. Similar prevalence of Xq25 losses of the inactive Xq has been also reported in ovarian and breast carcinomas [5, 6, 28]. The most likely explanation for this phenomenon is that the putative tumor suppressor gene(s) in Xq25 escapes X inactivation and is expressed from both chromosomes or from the inactive X chromosome alone [28]. In addition, this hypothesis implies that if the putative TSG is expressed on the active X chromosome it may be inactivated by point mutations [28].

There are several putative tumor suppressor genes within the Xq25 chromosome region, here identified as frequently deleted. Among them, ODZ1 and SH2D1A appear as good candidates. ODZ1 encodes for Tenascin, an oligomeric glycoprotein of the extracellular matrix involved in morphogenetic movements, tissue patterning, repair, and tumor invasion [17, 24, 26]. SH2D1A was found mutated in the X-linked lymphoproliferative disease [23] and in associated non-Hodgkin lymphomas [3].

Additional X markers were also found frequently deleted in the present study, including DXS294 (79% in primaries and 67% in metastases) and DXS102 (60% in primaries and 71% in metastases), both mapping at Xq26. This chromosomal region appears to be involved in the development of different tumors including ovarian [6] and breast carcinomas [7] and endocrine tumors of the lung [10]. In ovarian carcinomas, Xq25–26.1 LOH has been associated with higher histological grade and advanced tumor stage [6].

Candidate tumor suppressor genes mapping at Xq26 are myeloid elf-1 like factor MEF and Glypican-3 (GPC-3). MEF (ELF4) is an ETS-transcription factor with tumor suppressive activity as demonstrated in cell lines of human nonsmall cell lung carcinoma [35]. MEF may have an important role in tumor differentiation and angiogenesis by suppressing the transcription and promoter activities of the genes encoding for the matrix metalloproteinases, MMP-2 and MMP-9, and interleukin-8 [13, 35]. In our study, the immunohistochemical expression of MMP-2 was intense in two PDECs only, both with Xq26 LOH, whereas MMP-9 was consistently negative with the exception of two cases showing weak positivity (Table 3). These results do not support the involvement of the tumor suppressor gene MEF in most GEP endocrine carcinomas. GPC-3 is a heparan sulfate proteoglycan linked to the cell membrane by a glycosyl–phosphatidylinositol anchor and is involved in the progression of several types of malignant tumors, including mesotheliomas, ovarian, and lung carcinomas [20].

Finally, several studies pointed out the significant association of 3p LOH with malignancy in GEP endocrine tumors [8, 15, 27, 31]. In the present study, 3p LOH rates were found inversely correlated with those at Xq25–26 in WDECs, indicating an independent involvement of the two chromosome regions in the pathogenesis of well-differentiated malignant endocrine neoplasms. On the contrary, no differences were found in PDECs in keeping with the high chromosomal instability of this type of tumors. In this study, 3p LOH rates in PDECs were higher than those found in previous investigations from our laboratory [30, 31]. The use of a larger number of 3p markers in the present study may justify such difference, indicating that PDECs have more extended allelic losses on 3p. As previously demonstrated, in fact, the extension of 3p LOH increases with tumor aggressiveness [1].

In conclusion, our data suggest that LOH on X chromosome is an important event in the carcinogenesis of GEP endocrine carcinomas and reveal the existence of two common chromosomal deletion regions, mapping at Xq25 and at Xq26 and harboring candidate tumor suppressor genes potentially involved in the progression and malignant behavior of these tumors.

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