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Allelic Losses from Chromosome 17 in Human Osteosarcomas+

Marianna SZTÁN[1], Zsuzsa PÁPAI[2], Miklós SZENDRŐI[3], Marco van der LOOIJ[1] and Edith OLÁH[1,4]

Departments of [1]Molecular Biology and [2]Internal Medicine, National Institute of Oncology, Budapest, Hungary, [3]Institute of Orthopedics, Semmelweis Medical University, Budapest, Hungary

Genetic alterations of chromosome 17 have been reported to occur frequently both in human sporadic and familial malignancies. The present study was undertaken to explore the possible involvement of chromosome 17 genes including TP53 and the breast cancer susceptibility BRCA1 tumor suppressor genes in the development of sporadic osteogenic sarcoma. Fifteen patients were screened by polymerase chain reaction (PCR) for loss of heterozygosity (LOH) using four highly polymorphic markers. Loss of heterozygosity at the TP53 locus was detected in 40% (6/15) of informative cases while it was 14% (2/14) at the locus of thyroid hormone receptor alpha (THRA1), 21% (3/14) at the D17S855 locus intragenic to BRCA1 and 27% (4/15) at the D17S579 locus. In 53% of the cases studied at least one locus on chromosome 17 was affected by LOH. In our panel, the overall LOH frequency on 17p and 17q was observed to be 40% (6/15) and 27% (4/15), respectively. Comparison of LOH frequencies with clinical and prognostic features revealed significant correlation only with tumor recurrence. Our results confirm that the role of the TP53 tumor suppressor gene is important in the pathogenesis of sporadic osteosarcoma and suggest that 17q12-21 region abnormalities may be involved in the development and/or progression of this tumor. (Pathology Oncology Research Vol 3, No 2, 115–120, 1997)

Keywords: chromosome 17; TP53; tumor suppressor gene; loss of heterozygosity; osteosarcoma

Introduction

Osteosarcoma is the most common primary tumor of bone and occurs both in sporadic and familial forms. Clinically, the tumor is characterized by its occurrence in young people, aggressive growth, and frequent metastases to lung. Genetic changes underlying initiation and progression of osteosarcoma are poorly understood.[17]

Studies on genetic alterations in osteosarcoma revealed a spectrum of allelic losses on many different chromosomes which indicates a very complex pattern of genetic changes in this type of tumors.[25,29] Mutation of the TP53 tumor suppressor gene is one of the most common genetic changes found in human cancer.[19] Abnormalities of TP53 gene such as allelic loss, gross rearrangement, mutation and deletion have indeed been observed in a large fraction of osteosarcomas.[13,18] In Li-Fraumeni syndrome, mutation of TP53 also confers an elevated risk for various malignant diseases, including osteosarcoma.[10]

Experimental evidence implicates BRCA1 as a tumor suppressor gene that has a major role in the development of inherited breast and ovarian cancer. Moreover, BRCA1 is affected by LOH in a high proportion of sporadic breast and ovarian tumors and the gene is known to be affected in other cancers such as squamous cell carcinoma of the esophagus, prostate and colon cancer.[3,5,7,8,9,11,20] It has recently been shown that BRCA1 may function as a transcription factor, although the function of the protein product is not known and is still subject to strong arguments.[30]

Suggestion of possible involvement of the BRCA1 region in bone disease came from the observation that a patient with osteogenesis imperfecta developed breast

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Correspondence: Prof. Dr. Edith OLÁH, National Institute of Oncology, Department of Molecular Biology, Ráth György u. 7-9, H-1525 Budapest, Hungary,Fax: +(36-1) 156-2402; E-mail: c.olah@oncol.hu

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cancer. The gene responsible for inherited disorder of osteogenesis imperfecta, Collagen Type I, is located on chromosome 17q21-22, in the vicinity of the BRCA1.[4,23]

In the present study we determined the LOH frequency at the TP53 tumor suppressor gene locus and in the 17q12-21 region including BRCA1 gene. Furthermore, the clinicopathological significance of LOHs and possible association with overall survival was investigated in order to evaluate the role of these genes in the pathogenesis of osteosarcomas.

Materials and Methods

Samples

Twenty-one fresh tumor tissue samples were collected at the National Institute of Oncology, Budapest and Orthopedic Institute of Semmelweis Medical University, Budapest from 15 patients, treated before and after surgery for primary osteosarcoma according to COSS 91 protocol.[31] All but two patients survived following an average of 3.5 years follow-up period.

Two of the 15 patients were responders to COSS chemotherapy while others were moderate or non-responders. In addition, all patients had highly malignant tumors (clinical stage II/B and histopathological grade III) at the time of diagnosis. Clinical and pathological records are summarized in *Table 1*. The distribution of the tumor samples was as follows: 14 derived from initial biopsies, 3 from surgical specimens and one from a recurrent lesion. In one case a biopsy, a surgical specimen and also a local recurrence and lymph node metastasis were available for analysis.

DNA extraction, PCR amplification, LOH analysis

DNA was extracted from matched tumor and normal samples, i.e. peripheral lymphocytes, by standard methods. To determine the frequency of LOH four $(CA)_n$ microsatellite repeat markers (TP53CA, THRA1, D17S855 and D17S579) were amplified. The D17S855 locus was to be intragenic to the BRCA1 gene, THRA1 is the thyroid hormone receptor alpha gene and the D17S579 locus is distal to the BRCA1 gene (primer details are shown in *Table 3*).

PCR reactions were performed at a final volume of 40 µl containing 100 ng genomic DNA, 10 pmol of each primer, 1.5 mM $MgCl_2$, 200 µM each dNTPs, 50 mM KCl, 10 mM Tris (pH 8.3) and 1.5 U AmpliTaq DNA Polymerase (Perkin Elmer Cetus). The 5′-primers of markers were end-labeled with [γ^{32}P]ATP using T4 Polynucleotide Kinase B. Samples were amplified in 30-35 cycles, each containing a denaturation step (45 sec at 93°C), an annealing step (1 or 1.5 min at appropriate temperature) and an extension step (70 sec at 72°C).

In all cases, PCR cycles were preceded by an initial denaturation step (4 min at 94°C) and followed by a final elongation step (7 min at 72°C). PCR products were separated on 6% denaturing acrylamide gel. After fixation and drying, gels were autoradiographed for 1-14 days at -80°C. The samples were scored for LOH by laser densitometer (Molecular Dynamics) comparing the autoradiographic signals of the corresponding normal and tumor tissue samples, and defined positive if there was a greater than 50% reduction in the intensity of one allele in the tumor.

Table 1. **Clinicopathological characteristics of osteosarcoma patients**

Patients number	*Age at diagnosis (yr)*	*Sex*	*Initial site*	*Survival (months)*	*Grade*	*Stage*	*Recurrence*	*Histological subtype*
1	10	m	femur dist.	> 42	III	IIB	-	osteoblast. scler.
2	14	m	tibia prox.	42	III	IIB	lung	osteobl. chondrobl.
3	13	m	femur	42	III	IIB	-	osteoblast. teleangiect.
4	13	m	femur dist.	38	III	IIB	-	osteoblast.
5	14	m	tibia	38	III	IIB	lung	osteoblast.
6	10	f	femur dist.	37	III	IIB	lung	osteoblast.
7	20	m	humerus	12	III	IIB	rec+lung	osteoblast. teleangiect.
8	12	f	femur dist.	34	III	IIB	-	mixed cell
9	14	m	humer prox.	33	III	IIB	-	mixed cell. osteoblast.
10	18	m	humer prox.	12	III	IIB	rec+lung	osteoblast. fibroblast.
11	17	m	tibia prox.	32	III	IIB	lung	osteoblast. osteoid like
12	16	f	femur dist.	30	III	IIB	-	(osteoblast.) fibroblast.
13	39	m	tibia	30	III	IIB	lung	osteoblast. fibroblast.
14	20	m	femur	30	III	IIB	lung	mixed cell
15	15	f	femur dist.	28	III	IIB	lung	osteoblast.

humer - humerus, prox - proximal, dist - distal, rec - recidiva, osteoblast. - osteoblastic, scler - sclerotic, chondrobl. - chondroblastic, teleangiect. - teleangiectatic, fibroblast. - fibroblastic; m – male; f – female

Statistical analysis

All statistical analysis was carried out using Fischer's exact t-test. One tailed p values <0.05 were considered statistically significant.

Results

Twenty-one corresponding normal and tumor samples derived from fifteen osteosarcoma patients were screened for allele loss from chromosome 17. Six out of fifteen (40%) tumors analyzed showed LOH at TP53 locus. Fourteen percent (2/14) of the informative cases were detected to be LOH positive for THRA1, while 21% (3/14) of the cases showed LOH for D17S855 and 27% (4/15) for D17S579 (*Table 2*).

All samples were informative for at least one marker. Representative examples of chromosome 17 allele losses at the different loci are shown in *Fig 1*. Chromosomal location of the markers is shown in *Table 3*.

Comparison of LOH frequencies with the location of microsatellite repeat markers revealed significant association between LOH at the BRCA1 locus and the distal D17S579 locus (p=0.009) and between BRCA1 and the proximal THRA1 locus (p=0.029). In contrast, between the two flanking markers no significant correlation was observed for LOH. No association was detected between LOH at TP53 and at any locus of chromosome 17q. The overall LOH frequency was 40% (6/15) on the short arm of chromosome 17 and 27% (4/15) in BRCA1 region. In 53% of the cases at least one locus was affected on chromosome 17.

Four tumors out of fifteen (27%) showed LOH at two or more loci on chromosome 17 (*Table 2*). In two cases the whole BRCA1 region was LOH positive (patient 10 and 15) and in two tumors both the short (the TP53 locus) and the long arm chromosome 17 loci were affected (patients 13 and 14). Small deletions affecting only one marker were observed in 50% of primary osteosarcomas.

In the case of patient 7, where primary, metastatic and recurrent tumor samples were all available, the primary tumors were LOH negative, while deletion of the whole BRCA1 region occurred in the secondary lesion (*Table 4*). The same LOH pattern was detected in patient 10, although no primary lesion was available. In five out of nine patients

Table 2. **LOH from chromosome 17 in human sporadic osteosarcoma**

Patients number	*Samples*	*LOH 17p*		*LOH at 17q loci*	
		TP53	*THRA1*	*D17S855*	*D17S579*
1	P	–	–	–	–
2	P	+	–	–	–
3	P	–	–	–	–
4	P	–	–	0	–
5	P	+	–	–	–
6	P	+	–	–	–
7	P	+	–	–	–
8	P	–	–	–	–
9	P	–	–	–	–
10	R	–	+	+	+
11	P	–	–	–	–
12	P	–	–	–	–
13	P	+	–	+	+
14	P	+	0	–	+
15	P	–	+	+	+
LOHs#		6/15 (40%)	2/14 (14%)	3/14 (21%)	4/15 (27%)
Overall LOH		6/15 (40%)		4/15 (27%)	

LOH# frequency – number of LOH positive cases/number of informative cases
+ loss of heterozygosity; – retention of heterozygosity, 0 uninformative case
P – primary tumor; R – local recurrence

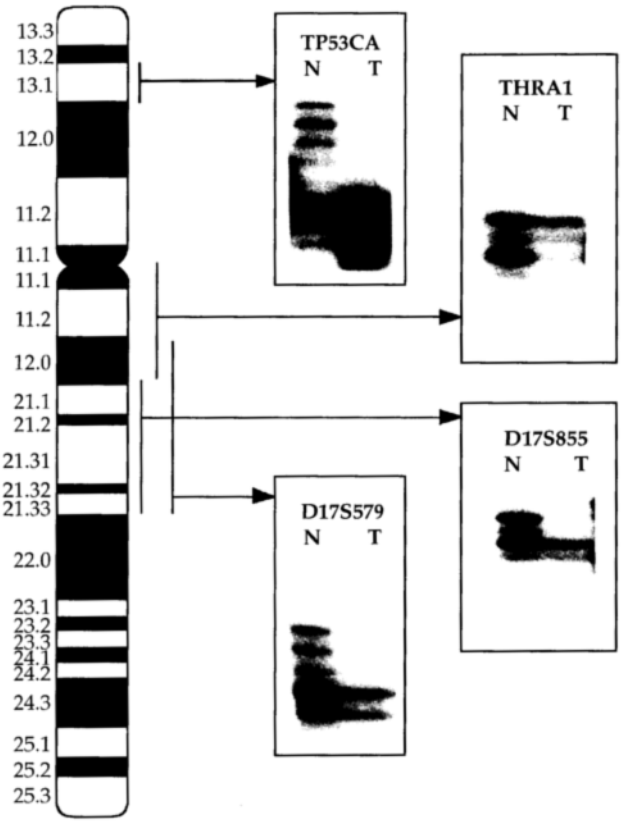

Figure 1. *Representative autoradiograms of losses from chromosome 17 in human osteosarcoma tumor samples. Genomic DNA from matching normal (N) and tumor (T) tissues was amplified by PCR using locus-specific primers (shown in Table 3).*

Table 3. **Primers used in this study**

Locus	*Chromosomal localization*	*Amplicon size (bp)*	*Sequences*
THRA1	17q11–17q12	135–157	GGG CAA AAA TGT CTT AAG C CAG CAT AGC ATT GCC TTC
D17S855	17q12–17q21	143–157	GGA TGG CCT TTT AGA AAG TGG ACA CAG ACT TGT CCT ACT GCC
D17S579	17q12–17q21	111–133	AGT CCT GTA GAC AAA ACC TG CAG TTT CAT ACC AAG TTC CT
TP53CA	17p13.1	103–135	AGG GAT ACT ATT CAG CCC GAG GTG ACT GCC ACT CCT TGC CCC ATT C

who developed secondary lesions, leading to metastasis and/or tumor recurrence in a short period, LOH in the BRCA1 region was observed. Statistical analysis revealed significant correlation (p=0.044) between allele loss and frequency of secondary tumor occurrence. We could not observe correlation between LOH frequencies and any other prognostic features in our set of patients (*Table 1*).

Discussion

Frequent observation of somatic allelic deletion or loss of heterozygosity (LOH) at a specific chromosomal locus in a particular tumor reveals the presence of a tumor suppressor gene.[8] Although a number of tumor suppressor genes have been implicated in the development of primary osteosarcoma only RB1 and TP53 proved to be strong candidates for playing a role in the pathogenesis of this malignancy.[1,19,28]

We observed LOH from chromosome 17p or 17q in more than half (53%) of the informative cases, and deletions affecting larger portions (at least two loci) of chromosome 17 were detected in half of them. Although in two primary lesions concordant deletion was detected on both arms of chromosome 17, our results suggest that LOH at TP53 and BRCA1 occurs independently. This observation is in line with results reported in studies on breast and ovarian cancers.[14,26]

In accordance with earlier studies where LOH rates for TP53 were reported varying between 30% and 75%, the observed LOH frequency at TP53 locus was 40% in our case.[1,27] The status of the TP53 gene was the same in primary cases as in more advanced specimens suggesting that at least in a portion of bone tumors TP53 alteration may be an early event in tumor development. Several observations suggest that alteration of TP53 gene may play a major role in the pathogenesis of osteosarcoma since inactivation of the gene generally occurs at the time of the transition from benign to malignant phenotype.[13,18,21]

Table 4. **Allele loss from chromosome 17 of paired primary and secondary lesions of patient 7**

	Chromosomal markers			
Samples	*TP53*	*THRA1*	*D17S855*	*D17S579*
Biopsy	+	–	–	–
Surgery	+	–	–	–
Recidive	+	+	–	–
Metastasis	+	+	+	+

It has already been proved that regions hosting putative tumor suppressor genes other than the BRCA1 on chromosome 17q are important in the development of several sporadic cancers.[6,11,12,16,20,22,24]

We observed an overall LOH frequency of 27% in the BRCA1 region. The significant correlation between LOH at the BRCA1 gene and at both of the flanking loci suggests that loss of a large portion of the BRCA1 region detected in the majority of our samples is characteristic of primary osteosarcomas. The most commonly deleted locus was D17S579 that is located distal to the BRCA1 gene and in the vicinity of the Collagen Type I gene. These data together support the possible involvement of yet unknown gene(s) located close and probably distal to the BRCA1 gene and suggest that inactivation of BRCA1 itself may play a role in the development in a portion of sporadic osteosarcomas.

Loss of the whole BRCA1 region was characteristic of the secondary lesions in our study. This observation suggests that abnormalities of this region may promote tumor progression and therefore at least in a proportion of tumors may be associated with aggressive behavior of osteosarcoma. This is in agreement with Beckmann et al.[2] who demonstrated that LOH of BRCA1 region correlated significantly with more advanced tumor phenotypes. However, other investigators reported allele losses affecting different 17q regions including 17q12-21 as an early event in carcinogenesis of different sporadic malignancies.[20,26] Regarding the restricted sample size our conclusion should be considered a preliminary result since it is unlikely that our small data set will produce compelling evidence for this hypothesis.

Interestingly, in patient seven, where corresponding primary and secondary tumor samples were available successive occurrence of loss of BRCA1 loci was also noted in the secondary lesions. In order to determine whether this phenomenon is a coincidence or a characteristic of osteosarcoma development, further analysis of representative samples is currently being undertaken in our laboratory.

We could not observe any association between the LOH frequencies and disease outcome, although several reports attributed possible clinical importance to alterations of tumor suppressor genes located on chromosome 17.[2,29] However, studying osteosarcoma e presents intrinsic difficulties because the majority of patients are at an advanced stage at the time of diagnosis (all patients were stage IIB and tumors were of histological grade III) which hampers the study of early events in the development and progression of this cancer. In order to further investigate the significance of allelic losses as a prognostic factor in tumor phenotype, a larger study including early stages of sporadic osteosarcoma tumor samples and longer follow up status is needed.

In conclusion, our results support the idea that inactivation of chromosome 17 regions distal to BRCA1 and TP53, may have an impact on development of osteogenic sarcomas. With future fine deletion mapping around the neighboring Collagen Type I gene (17q21-22) and mutation analysis of the BRCA1 gene may reveal their role in the pathogenesis and/or progression of this disease.

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