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Original Article

Copy number alterations of chromosomal regions enclosing protein tyrosine phosphatase receptor-like genes in colorectal cancer

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ABSTRACT

Protein tyrosine phosphatases that act in different cellular pathways are described most commonly as tumor suppressors, but also as oncogenes. Their role has previously been described in colorectal cancer, as well as in gastric, breast, thyroid, prostate, ovarian, pancreatic, glioma, liver, leukemia and many other cancers.

In a previous study, we have described protein tyrosine phosphatase receptor type T, M, Z1 and Q genes (*PTPRT*, *PTPRM*, *PTPRZ1* and *PTPRQ*) hypermethylated in sporadic colorectal cancer. Thus, in this study, we examined the relation of unbalanced chromosomal alterations within regions covering these four protein tyrosine phosphatase genes with this cancer.

One hundred and two cancer tissues were molecularly characterized, including analysis of the *BRAF* and *K-ras* mutations and methylator phenotype. The analysis of chromosomal aberrations was performed using Comparative Genomic Hybridization.

We observed amplification of three regions containing genes coding for PTPs, such as PTPRZ1 (7q31.3, amplified in 23.5% of cases), PTPRQ (12q21.2, amplified in 5.9% of cases), PTPRT (20q12, amplified in 29.4% of cases), along with deletions in the region of PTPRM (18p11.2, deleted in 21.6% of cases). These data may suggest that in sporadic colorectal cancer PTPRZ1, PTPRT, PTPRQ probably act as oncogenes, while PTPRM acts as a tumor suppressor gene. Our study also revealed that gains on chromosome 20q12 and losses on chromosome 18p11.2 are connected with the absence of the BRAF mutation and the conventional adenocarcinoma pathway.

Introduction

Protein tyrosine phosphatases (PTPs) are a large and multifunctional family of enzymes that act in different cellular pathways, including cell division, apoptosis or cell differentiation. Their enzymatic activity enables either the turning on or turning off of the activities of many other enzymes. Therefore, the role of receptor-like PTPs (RPTPs), non-receptor PTPs (NRPTPs) and dual-specific protein phosphatases (DSPs) in carcinogenesis is incontrovertible and has been widely described [7]. The fact that protein tyrosine kinases (PTKs) are classified mainly as oncogenes suggests that phosphatases – enzymes catalyzing the opposite reaction – should act as tumor suppressors. At present, among the 107 PTPs that are known, 37 have been described as having tumor suppressor or oncogenic activity [8].

Tumor suppressor genes might be inactivated during tumorigenesis *via* deletion, loss of heterozygosity (LOH), epigenetic silencing or point mutation [8]. It has also been postulated that miRNA, which can cleave mRNAs or inhibit translation, plays a role [17]. Oncogenes are activated by amplifications, translocations, activating point mutations, or also epigenetic regulation. According to their role in cancerogenesis, protein tyrosine phosphatases may be affected by either of these types of events [8].

Chromosomal aberrations involving regions with PTPs, epigenetic alterations, LOH and point mutations have been described in cancers, such as gastric, breast, thyroid, prostate, ovarian, pancreatic, colorectal, glioma, liver, leukemia and many others [8].

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The roles of various protein tyrosine phosphatases have been previously described in colorectal cancer (CRC), one of the most common human sporadic cancers: overexpression of *PTPRA*, *PTPRH*, *PTP4A3* and *PTPN13*, up regulation of *DUSP4* (MKP-2), hypermethylation of *PTPRT*, *PTPRM*, *PTPRZ1* and *PTPRR*. LOH for PTPRJ, as well as point mutations in *PTPRT*, *PTPRF*, *PTPRG*, *PTPN3*, *PTPN13*, *PTPN14*, *PTPN21*, *PTPN23*, *PTPN5*, *PTPRA*, *PTPRS* and *PTPRE* [10,12,14,15]. LOH of *PTPRJ* in the 11p11 chromosomal region has been also observed in CRC [14]. Recent data show that, in a variety of cancers, genes coding for protein tyrosine phosphatases are deleted, while genes coding for four PTPs (*PTP4A3* on chromosome 8q, *DUSP26* on 8p11-12, *PTPN1* on 20q13 and *PTPN7* on 1q32.1) have often been observed to be amplified [7].

In a previous study, we have shown that protein tyrosine phosphatase receptor-like genes *PTPRT*, *PTPRM*, *PTPRZ1* and *PTPRQ* are frequently hypermethylated in sporadic CRC, and thus we postulated their role as tumor suppressors in colorectal carcinogenesis [12]. In this study, we examined alterations in chromosomal regions covering these four protein tyrosine phosphatase genes in CRC tissues.

Materials and methods

The study is based on 102 sporadic CRCs obtained after surgery from the First Department of Surgical Oncology of the Lower Silesian Oncology Centre in Wroclaw. All of them were diagnosed with adenocarcinoma coli. Biological material was collected before chemo- and radiotherapy. All the patients were interviewed regarding their family history of cancer, and those with hereditary cancer syndromes were excluded from the study group.

All cases were molecularly characterized, including analysis of the following: the *BRAF* V600E (exon 15) and *K-ras* (codon 12 and codon 13) mutations, methylator phenotype (CIMP), microsatellite instability (MIN) and chromosomal instability as described earlier [9,11].

The study group consisted of 49 women (48%) and 53 men (52%) of age ranging between 32 and 88 (mean 65.7 ± 11.2). The location of the tumor was proximal and distal in 29 (28.4%) and 73 (71.6%) cases, respectively (Table 1).

The study design was accepted by Wroclaw Medical University Ethical Committee.

The analysis of chromosomal aberrations in the regions encoding *PTPRT* (20q12), *PTPRM* (18p11.2), *PTPRZ1* (7q31.3) and *PTPRQ* (12q21.2) was performed using Comparative Genomic Hybridization (CGH) as previously described by [11] using Isis Software (MetaSystems GmbH, Althusseim, Germany). The normal range of observations was set to be from 0.85 to 1.17. Observations above 1.17 and below 0.85 were defined to correspond to amplifications and deletions, respectively.

Statistical analysis

In order to analyze the associations between the various deletions, amplifications and genotypes studied, Fisher's exact test of association was used, together with Kendall's test for an association between ordered categorical variables (where appropriate). The Benjamini–Hochberg procedure for multiple testing was also applied. The appropriate calculations were carried out with the aid of the IBM SPSS package (version 21) and the R package (version 3.0.2).

Results

Molecular characteristics

The *BRAF* mutation was present in 16 cases (15.7%), the *KRAS* mutation in 30 cases (29,4%), MIN in 14 cases (13.7%) and CIMP Low, Intermediate and High in 40 (39.2%), 40 (39.2%) and 22 (21.6%) cases, respectively.

CGH analysis

When analyzing the chromosomal imbalances in the regions including *PTPRM* (18p11.2), *PTPRT* (20q12), *PTPRQ* (12q21.2), and *PTPRZ1* (7q31.3), we found that (i) gains were more frequent than losses (61/28), (ii) the most frequent gains were on chromosome 20 (30/102), located in 20q, 20q11.1-12, 20q11.2-q13.3 and 20q12-q13.3, (iii) gains on chromosome 7 (24/102) located in 7q, 7q11.1-31.2, 7q11.1-36, 7q21.2-34, 7q31.1-33, (iv) gains on chromosome 12 were located in 12q, 12q14-q21.3 and 12q12-23 (6/102) and (v) there was only one gain on chromosome 18. The most frequent losses occurred on chromosome 18, located in 18p, 18p11.1-11.2 and 18p11.2-11.3 (22/102). We also observed losses on chromosome 20 located in 20q (4/102) and two losses in chromosome 12q21.1-21.3 (2/102) (Table 2).

Table 1
Clinicopathological characteristics of the patients.

Parameter	Value
Age	
Min	32
Max	88
Mean	65.7
St. dev.	11.2
Gender	
Females	49 (48%)
Males	53 (52%)
Localization	
Proximal	29 (28.4%)
Distal	73 (71.6%)
Grading	
G1	11 (10.78%)
G2	21 (20.58%)
G3	5 (4.9%)
B	1 (0.98%)
No data	64 (62.75%)
Staging	
I	11 (10.78%)
II	13 (12.75%)
III	20 (20.4%)
IV	7 (7.14%)
No data	51 (50%)
BRAF mutation	16 (15.7%)
KRAS mutation	30 (29.4%)
MIN	14 (13.7%)
CIMP	
Low	40 (39.2%)
Intermediate	40 (39.2%)
High	22 (21.6%)

Table 2
Gains and losses in regions encoding *PTPRM*, *PTPRT*, *PTPRQ* and *PTPRZ1*.

Total number of tissues	*PTPRM*	*PTPRT*	*PTPRQ*	*PTPRZ1*
102 (100%)	18p11.2	20q12	12q21.2	7q31.3
Gains	1 (0.9%)[a]	30 (29.4%)	6 (5.9%)[a]	24 (23.5%)
Losses	22 (21.6%)	4 (3.9%)[a]	2 (2.0%)[a]	–

[a] Not analysed.