High *PTPRQ* Expression and Its Relationship to Expression of *PTPRZ1* and the Presence of *KRAS* Mutations in Colorectal Cancer Tissues

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Abstract. Background: The risk of sporadic colorectal cancer (CRC) is strongly influenced by lifestyle, environmental and genetic factors. Protein tyrosine phosphatases belong to a group of enzymes whose role in CRC has not yet been intensively studied. They play an important role in activation/de-activation of many enzymes, influencing cell biology by catalyzing reactions opposing those catalyzed by kinases. Protein tyrosine phosphatase receptor-like type Q (*PTPRQ*) and protein tyrosine phosphatase receptor-like type Z polypeptide 1 (*PTPRZ1*) have both been shown to be important in development of many cancer types including CRC. Materials and Methods: The expression level of *PTPRQ* and *PTPRZ1* was determined by real-time polymerase chain reaction in 16 CRC tissues obtained from patients diagnosed with adenocarcinoma coli. Results: We revealed a high level of *PTPRQ* expression (*p* = 0.0080), as well as an association between expression levels of *PTPRQ* and *PTPRZ1* (*p* < 0.0001). Moreover *PTPRQ* expression was higher in tissues presenting with Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation (*p* = 0.0293). Conclusion: We confirmed the contribution of *PTPRZ1* and especially *PTPRQ* in CRC development, supporting the hypothesis that *PTPRQ* is a candidate oncogene, playing a crucial role in phosphorylation/dephosphorylation signaling pathways.

Colorectal cancer (CRC) is one of the most common cancer types worldwide and, despite immense progress in CRC diagnosis and therapy, the prognosis in late-stage cases is poor. Approximately 70-85% of cases are sporadic and no genetic risk factors have been identified (1, 2). However, the risk of sporadic CRC is strongly influenced by diet, physical activity, the environment, and genetic changes accumulated during life (1, 2). Accumulation of somatic mutations and alterations in epigenetic mechanisms influencing expression of proto-oncogenes, tumor suppressors and mismatch repair (MMR) genes, along with genomic instability [chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype of (CIMP)], play a key role in CRC development and progression (2). Moreover, genetic heterogeneity of CRC strongly suggests the importance of alteration and deregulation of many other genes important for cellular metabolism (2).

Protein tyrosine phosphatases (PTPs) as candidate tumor-suppressor genes belong to the group of the principal genes examined recently in many types of cancer, including CRC. In contrast, genes encoding protein kinases that catalyze the opposite reaction by activating many enzymes were shown to be proto-oncogenes (3-5). The role of PTPs in dephosphorylation of many proteins makes them essential for homeostasis, cell signaling, apoptosis and other processes, alterations of which are crucial for carcinogenesis (4, 6). However, several studies demonstrate an oncogenic potential of PTPs, suggesting their pleiotropic and crucial biological role in this process (3, 7, 8). A key role in CRC development was recently revealed for alterations in the following PTPs: *PTPRA, PTPRE, PTPRH, PTPF, PTPG, PTPRJ, PTPRM, PTPRT, PTPRQ, PTPRZ1, PTP4A3, PTPN5, PTPN13, PTPN14, PTPN21, PTPN23* and others (3-5).

Protein tyrosine phosphatase receptor-like type Q (*PTPRQ*) [OMIM *603317*] and protein tyrosine phosphatase receptor-like type Z polypeptide 1 (*PTPRZ1*) [OMIM *176891*] both have been described as being active in the cellular protein phosphorylation network that is...
crucial for carcinogenesis (9-13). *PTPRQ* (located in 12q21.2) is expressed as a cytosolic or receptor-like protein depending on an alternative promoter usage or alternative splicing (14). Their potential growth-regulatory functions have been described in *in vitro* experiments (15). Moreover, epigenetic silencing of *PTPRQ* and *PTPRZ1* has been described in CRC (16). In contrast, an amplification of regions encoding these enzymes (12q21.2 and 7q31.3 for *PTPRQ* and *PTPRZ1*, respectively) was reported in CRC (11, 17). *PTPRZ1* (located in 7q31.3) has also been shown to be expressed in oral squamous cell, cervical and small-cell lung carcinoma and in colorectal cancer (9, 10, 12, 13).

Thus, the aim of our investigation was to examine the expression of *PTPRQ* and *PTPRZ1*. An amplification of the respective coding loci (12q21.2 and 7q31.3) along with *PTPRZ1* protein over-production was revealed in our previous study (9, 11).

### Materials and Methods

Analyses were performed on 16 CRC tissues in comparison to healthy ones derived from the same individuals. All samples were obtained from patients of the First Department of Surgical Oncology of the Lower Silesian Oncology Centre in Wroclaw, diagnosed with adenocarcinoma coli, before chemo- and radiotherapy. All the patients included in study group had a negative family history in regard to both accumulation of cancer in their family and hereditary cancer syndromes. The group consisted of 10 females and six males, with a mean age of 67.25±11 years (Table I).

Table I. *The clinical and molecular characteristic of colorectal cancer tissues.*

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F: Female; M: male; mutation: 0: lack of mutation, 1: presence of mutation; R: right; L: left; T: tumor; N: nodules; M: metastasis; MSI: microsatellite instability; MSS: microsatellite stability; MLH1: MutL homolog 1; HME: high methylation epigenotype; LME: low methylation epigenotype; *BRAF*: B-Raf proto-oncogene; *KRAS*: Kirsten rat sarcoma viral oncogene homolog; nd: no data.

Molecular characterization of tumors included analysis of: the B-Raf proto-oncogene (*BRAF*) V600E (exon 15) and *KRAS* (codon 12 and codon 13) mutations, methylator phenotype (CIMP), MSI and CIN (18). The clinical and molecular data of all CRC tissues are presented in Table I.

The study design was accepted by the Wroclaw Medical University Ethical Committee (Approval number 328/2009).

**RNA isolation.** Fresh cancer and healthy tissues were collected during surgery in RNAlater (Qiagen, Hilden, Germany). Total RNA was extracted from homogenized frozen samples using TriPure Isolation Reagent (Roche, Basel, Switzerland) according to the producer’s protocol based on a published method (19). The RNAs were examined using Experion RNA StdSens Chips (Bio-Rad, Hercules, CA, USA) for Experion Automated Electrophoresis System (Bio-Rad). For further analysis, RNA samples with concentration over 100 ng/μl and RNA quality indicator (RQI) over 5 were qualified.

**Reverse transcription and expression analysis.** cDNA synthesis was performed using Transcriptor First-Strand cDNA Synthesis kit (Roche) according to the producer’s protocol. cDNA was diluted to 100 ng/μl. Relative quantification of *PTPRZ1* and *PTPRQ* and the reference gene beta actin (*ACTB*) were measured with the Precision 2X qPCR mastermix SG (Primer Design, Southampton, Hampshire, UK) using RotorGene RT-PCR (Qiagen). Primer sequences are described in Table II.

The following PCR conditions were used: initial denaturation at 95°C for 2 min, and then 50 cycles of denaturation at 95°C for 15 s, with data collection at 60°C for 60 s. qPCR normalization was carried out according to The Minimum Information for Publication of Quantitative Real-Time PCR Experiments standards (20). Results are shown in Table III.
Statistical analysis. Each set of analyses was repeated three times. Fold changes (FC) in the expression of each gene was calculated using the ΔΔCt method. Non-parametric Wilcoxon signed-rank test was used for the statistical analysis of differences in data for healthy and cancerous tissue. p-value less than 0.05.

Results

We observed a high level of expression of both examined genes (FC>2), but only for PTPRQ was the difference between cancerous and healthy tissue statistically significant (p=0.0080) (Table IV).

Analyzing expression and the clinical pattern by multiple regression revealed that PTPRZ1 expression was associated with PTPRQ expression: an increase of PTPRZ1 expression by 1 unit raised the PTPRQ expression by 11.3 times (p<0.0001). PTPRQ expression was also found to rise 121-fold in tissues with KRAS mutation in comparison to tissues without this alteration (p=0.0293) (Table V).

We did not observe any correlation between the expression level of either of the examined genes and other genetic or clinical variables.

Discussion

PTPs, catalyzing dephosphorylation, act in close cooperation with protein kinases and thus control signaling pathways of crucial processes in the cell (3). Therefore, different PTPs have been recently examined in many types of cancer and their dual role as either tumor suppressors or oncogenes has been demonstrated (3, 4). PTPRZ1 was firstly described as being expressed in the central nervous system, but recently its expression in a variety of cancer tissue such as gastric (8), oral squamous cell (10), colorectal and cervical carcinoma (12), as well as...
in normal colonic tissue was also reported (9). Moreover, in an in vitro experiment, invasion of glioblastoma cells was inhibited by *PTPRZ1* expression (21).

*PTPRQ* is described as a phosphatase with double protein-tyrosine phosphatase and phosphatidylinositol phosphatase activity. In cultured cells, *PTPRQ* overexpression inhibits proliferation and promotes apoptosis (15).

In our previous study, comparative genomic hybridization analysis indicated an amplification of regions containing both *PTPRZ1* (7q31.2) and *PTPRQ* (12q21.2), along with *PTPRZ1* protein overexpression (using immunohistochemical and western blot methods), thus suggesting that both *PTPRZ1* and *PTPRQ* may act as oncogenes and therefore promote tumorigenesis. While analyzing chromosomal instability in CRC, we recorded amplification of the 12q21.2 region containing *PTPRQ* (9, 11).

Our present study aimed at expanding previous observations. Here we observed not only an increase in *PTPRQ* expression in CRC when compared to matched healthy tissues (*p*=0.0080), but also an association between the levels of *PTPRQ* and *PTPRZ1* expression. Moreover, in CRC tissues carrying *KRAS* codon 12 mutation expression of *PTPRZ1* was far higher than in CRC tissues without this alteration. *RAS* genes belong to the group of oncogenes most frequently mutated (30-50% of all CRCs). Mutations are mainly located at the first exon, codons 12 (80%) and 13 (15-20%) (22). As *KRAS* acts on signal transduction pathway for cellular growth and differentiation downstream of epidermal growth factor receptor (*EGFR*), mutations can result in resistance to anti-EGFR therapy (22). An association between an increase in *PTPRQ* expression and *KRAS* mutations may suggest their interrelations in the molecular background of CRC.

In our study, an increase of *PTPRZ1* expression in CRC tissues versus healthy tissues was not statistically significant but does appear to be associated with *PTPRQ* expression. From our results and those of others, the data seem to suggest a role of *PTPRZ1* in carcinogenesis (9-12, 21).

In conclusion, we confirmed the contribution of *PTPRZ1*, and especially *PTPRQ*, in CRC carcinogenesis and demonstrated that *PTPRQ* expression is correlated with *KRAS* mutation. Despite the fact that further studies are needed to evaluate the role of elevated expression of *PTPRQ* in CRC, we hypothesize that *PTPRQ* is a candidate oncogene playing an important role in phosphorylation/dephosphorylation signaling pathway in CRC carcinogenesis.

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References


Laczmanska et al: Expression of PTPRZ1 and PTPRQ in CRC

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