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Minireview

Tyrosine phosphatases as a superfamily of tumor suppressors in colorectal cancer

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Phosphorylation and dephosphorylation processes catalyzed by numerous kinases and phosphorylases are essential for cell homeostasis and may lead to disturbances in a variety of vital cellular pathways, such as cell proliferation and differentiation, and thus to complex diseases including cancer. As over 80% of all oncogenes encode protein tyrosine kinases (PTKs), protein tyrosine phosphatases (PTPs), which can reverse the effects of tyrosine kinases, are very important tumor suppressors. Alterations in tyrosine kinase and phosphatase genes including point mutations, changes in epigenetic regulation, as well as chromosomal aberrations involving regions critical to these genes, are frequently observed in a variety of cancers. Colorectal cancer (CRC) is one of the most common cancers in humans. CRCs occur in a familial (about 15% of all cases), hereditary (about 5%) and sporadic (almost 75–80%) form. As genetic-environmental interrelations play an important role in the susceptibility to sporadic forms of CRCs, many studies are focused on genetic alterations in such tumors. Mutational analysis of the tyrosine phosphatome in CRCs has identified somatic mutations in *PTPRG, PTPRT, PTPN3, PTPN13* and *PTPN14*. The majority of these mutations result in a loss of protein function. Also, alterations in the expression of these genes, such as decreased expression of *PTPRR, PTPRO, PTPRG* and *PTPRD*, mediated by epigenetic mechanisms have been observed in a variety of tumors. Since cancer is a social and global problem, there will be a growing number of studies on alterations in the candidate cancer genes, including protein kinases and phosphatases, to determine the origin, biology and potential pathways for targeted anticancer therapy.

Keywords: phosphatases, colorectal cancer, mutations, epigenetics

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INTRODUCTION

Cell homeostasis should be tightly controlled. One of the key mechanisms in this control is the process of phosphorylation and dephosphorylation catalyzed by numerous kinases and phosphatases. Phosphorylation/dephosphorylation of a protein switches it between an active and inactive form, which is connected with conformational changes. The reversibility and rapidity of this process make it one of the main mechanisms in cell homeostasis (Arena *et al.,* 2005; Tabernero *et al.,* 2008).

Phosphorylation and dephosphorylation affect the formation of protein complexes by altering protein conformation and therefore are crucial in the regulation of receptors, ion channels, signaling proteins and transcriptional factors (Arena *et al.,* 2005).

TYROSINE KINASES AND PHOSPHATASES — GENERAL CHARACTERISTICS

Kinases catalyze the transfer of a phosphate group from the coenzyme adenosine-5'-triphosphate (ATP) to specific molecules, e.g. proteins or lipids. Phosphorylases catalyze a reverse process, i.e. the removal of a phosphate group from a substrate (Arena *et al.,* 2005; Tabernero *et al.,* 2008). Phosphorylation is a process in which a negatively charged, hydrophilic phosphate (PO_4) group is usually added to a hydroxyl group of a serine, threonine or tyrosine residue in a protein through a phosphoester bond (O-phosphate), but can be also added to an aspartate, histidine and arginine residue or to some lipids. Both groups of enzymes are usually characterized by a conserved catalytic domain (Arena *et al.,* 2005; Tabernero *et al.,* 2008).

A non-enzymatic pathway of protein phosphorylation is also active in mammals. In this process, which plays a crucial role in the regulation of endocytosis, chemotaxis and apoptosis, pyrophosphorylation is mediated by inositol pyrophosphates (Saiardi *et al.,* 2004).

Because of the huge number of kinases and phosphatases and their crucial role in cell homeostasis, the set of genes encoding kinases is named the *kinome*, while the one encoding phosphatases is called the *phosphatome* (Arena *et al.,* 2005).

Tyrosine kinases and phosphatases are either receptor protein kinases/phosphatases or soluble non-receptor (intracellular) enzymes (Arena *et al.,* 2005). According to their catalytic mechanism PTPs belong to the following two groups: Cys-based (3 classes in humans, 103 genes) and Asp-based PTPs (4 genes in humans). Class I Cys-based PTPs comprise 99 enzymes, wherein classical PTPs are represented by 38 proteins i.e., 21 classical transmembrane PTPs and 17 non-receptor PTPs (NRPTPs), while dual specific phosphatases (DSPs) are represented by 61 enzymes (Alonso *et al.,* 2004). Cytoplasmic NRPTPs consist of catalytic domains, special non-catalytic domains or domains that are involved in subcellular directing, ligand binding or activity regulation. Receptor-

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Abbreviations: ATP, adenosine-5′-triphosphate; CAN genes, candidate cancer genes; CRC, colorectal cancer; DSP, dual-specific phosphatase; LMW, low molecular weight; PANTHER, **P**rotein **AN**alysis **TH**rough **E**volutionary **R**elationships; PTK, protein tyrosine phosphatase; PTP, protein tyrosine kinase; NRPTP, non-receptor protein tyrosine phosphatase; RPTP, receptor protein tyrosine phosphatase; SCRC, sporadic colorectal cancer.

like PTPs (RPTPs or PTPRs) are transmembrane proteins and are usually composed of an N-terminal extracellular domain, a single transmembrane domain and one or two highly conserved intracellular catalytic domains. PTPRs are employed in transmembrane signal transduction. Dual specific protein phosphatases (DSPs) catalyze the dephosphorylation of serine and threonine in addition to tyrosine residues (Alonso *et al.,* 2004; Tabernero *et al.*, 2008).

Class II Cys-based LMW-PTPs (low molecular weight; 1 gene in humans), whose expression has been observed in the majority of human tissues, are involved in controlling cytoskeleton, cell growth and adhesion. LMW-PTPs present a high structural, but not sequence, homology to class I PTPs (Alonso *et al.,* 2004; Tabernero *et al.,* 2008; Wang *et al.,* 2004). Class III consists of 3 genes encoding cell cycle regulators (CDC25A, B and C) (Alonso *et al.,* 2004).

As PTPs are involved in the regulation of so many biological pathways, there has been an increasing number of studies published recently on their role in normal and pathological processes (Alonso *et al.,* 2004).

PTP MUTATIONS AND COLORECTAL CANCER

More than 300 genes have been identified up to now as being involved in carcinogenesis. The major impact cancer genes are oncogenes and tumor suppressor genes (Arena *et al.,* 2005; Jacob *et al.,* 2005; Julien *et al.,* 2011). Approximately 80% of all oncogenes code for protein kinases (PTKs). Some tyrosine phosphatases (PTPs) may act as tumor suppressors reversing the negative effects of PTKs, while others may act as oncoproteins exerting a positive effect on signaling processes by dephosphorylation and activation of PTKs (Jacob *et al.,* 2005; Julien *et al.,* 2011).

Colorectal cancer (CRC) is one of the most common human cancers, affecting both women and men. Colorectal cancers occur in a sporadic, familial and hereditary form (Cheah 2009; Wicki *et al.,* 2010). Differences in the molecular pathways underlying the development of various CRC subgroups led to the hypothesis that they constituted different disease entities (Samowitz 2008).

The majority of CRCs are sporadic (about 80%), but studies on monozygotic twins showed that both genetic and environmental factors modulate individual susceptibility to CRC (Cheah, 2009). Thus, an individual's susceptibility to sporadic CRC (SCRC) depends on the combination of environmental exposure to potentially carcinogenic agents (e.g. diet, smoking) and low penetrant genes that modulate the response to these environmental factors (Toland *et al.,* 2008).

The most frequent genetic alterations in SCRC are *APC* somatic mutations detected in up to 80% of sporadic cases. Loss of APC function results in an accumulation of nuclear beta-catenin and thus in the activation of the Wnt signaling pathway (Samowitz 2008; Cheah 2009; Wicki *et al.,* 2010). Recent studies on anti-EGFR therapy in patients with metastatic CRCs revealed that mutations in genes such as *BRAF*, *KRAS* and *PIK3C* result in resistance to this therapy (Lurkin *et al.,* 2010).

Genome-wide studies employing microarray technology have revealed a variety of candidate genes suspected of being important in the development, progression and metastasis of SCRC (Wang *et al.,* 2004; Toland *et al.,* 2008; Mokarram *et al.,* 2009; Kim *et al.,* 2011; van Roon *et al.,* 2011). Many of them code for kinases and phosphatases. A variety of genetic, as well as epigenetic, alterations affecting their function and/or expression have been identified recently (Wang *et al.,* 2004; Arena *et al.,* 2005; Jacob *et al.,* 2005; Toland *et al.,* 2008).

Mutational analysis of the tyrosine phosphatome in CRC performed in an elegant experiment by Wang *et al.* (2004) allowed them to identify 83 somatic mutations in the following six genes: three members of the RPTP subfamily (*PTPRF*, *PTPRG*, *PTPRT*) and three members of the NRPTPs (non-receptor) subfamily (*PTPN3*, *PTPN13, PTPN14*) in 26% of the examined CRCs. Moreover, the authors identified six other *PTP* mutations in lung, gastric and breast cancer cells. The majority of the mutations detected were related to a loss of enzyme function (Wang *et al.,* 2004).

Studies on the function of *PTPRT*, which was reported to be most frequently mutated among the analyzed phosphatases in CRC (Wang *et al.,* 2004), have showed that paxillin is a direct substrate of PTPRT (Zhao *et al.,* 2010). Phosphorylation of paxillin was observed at multiple sites including 8 different tyrosine residues. The oncogenic function of phospho-paxillin is exerted by deregulation of cell adhesion, migration, proliferation and apoptosis (Zhao *et al.,* 2010). However, PTPRT also acts as a tumor suppressor by inactivating STAT3 (signal transducer and activator of transcription 3), since dephosphorylation of STAT3 Y705 residue results in a decrease in its activity (Zhang *et al.,* 2007).

Tumor suppressor effect in colon cancer has also been observed for the following protein tyrosine phosphatases: DEP-1 (density enhanced phosphatase), which inhibits proliferation and migration of cancer cells and PTPRD which is involved in cell adhesion and suppression of cell migration (Balavenkatraman *et al.,* 2006; Funato *et al.,* 2011).

Frameshift mutations in six phosphatases (*PTPN21*, *PTPN23*, *PTPN5*, *PTPRA*, *PTPRE* and *PTPRS*) in colorectal cancers were also reported by Korff *et al.* (2008). However, these authors did not observe any impact of *PTP* mutations on CRC tumorigenesis. The difference between the results of Korff *et al.* (2008) and Wang *et al.* (2004) may result from different molecular characteristics of the tumors studied, as Korff *et al.* (2008) analyzed CRCs showing microsatellite instability (MSI), while Wang *et al.* (2004) did not specially select such CRCs (Wang *et al.,* 2004; Korff *et al.,* 2008; Julien *et al.,* 2011). In *in vivo* studies, the gene coding for *PTPRJ* has been identified as a gene associated with susceptibility to colon cancer (Ruivenkamp *et al.,* 2003). Moreover, frequent LOH (loss of heterozygosity) at position 11p11 (the *PTPRJ* locus) has been frequently observed in SCRCs, suggesting involvement of this gene in the carcinogenesis of CRCs (Ruivenkamp *et al.,* 2003; Puijenbroek *et al.,* 2005).

Studies on the role of dual specific phosphatase (DPTP) genes in gastric and colorectal cancer have been performed on tumors with and without MSI. Deletions and duplications of one nucleotide (frameshift mutations) were detected in the nucleotide repeats in *CDC14A* and *MTMR3*, but only in the MSI-H cancer group. This may suggest that alterations in these two genes may contribute to the oncogenesis of colon and gastric cancer with MSI (Song *et al.,* 2010).

EPIGENETIC REGULATION OF *PTP* ACTIVITY IN CRCs

Alterations in DNA methylation leading to chromatin modification and thus to changes in gene expression be-

long to the most important molecular mechanisms in the development and progression of tumors (Motiwala *et al.,* 2003; Kim *et al.,* 2011).

Hypomethylation leading to the activation of proto-oncogenes, as well as hypermethylation resulting in the inactivation of tumor suppressors, have been frequently observed in cancers (Motiwala *et al.,* 2003; Menigatti *et al.,* 2009; Kim *et al.,* 2011). Changes in PTP expression have been frequently observed among other genetic alterations in CRCs. *PTPRR* inactivation, leading to a reduction in the mRNA level has been observed in precancerous colorectal lesions, colorectal tumors, colorectal cell lines and also in liver metastases. *PTPRR* encodes the transmembrane protein tyrosine phosphatase, a receptor-type phosphatase, which is employed in the RAS/RAF/MAPK/ERK pathway (Menigatti *et al.,* 2009).

Hypermethylation, and thus a decrease in the expression of PTPs, has been observed in a variety of tumors, such as hepatocellular rat carcinomas (*PTPRO*), cutaneous T-cell lymphomas (*PTPRG)* and glioblastomas (*PTPRD)* (Motiwala *et al.,* 2003; Wang *et al.,* 2004; van Doorn *et al.,* 2005; Veeriah *et al.,* 2009). *PTPRG* and *PTPRO*, which play a role in apoptosis, terminal differentiation and cell cycle, are candidates for tumor suppressor genes in colon, renal and lung carcinomas (Motiwala *et al.,* 2003; Wang *et al.,* 2004; van Doorn *et al.,* 2005). PTPRD dephosphorylates the oncoprotein STAT3 and thus regulates the STAT3 pathway. Because of its proximity to *CDKN2A* on chromosome 9p, the loss of *PTPRD* was thought to be associated with the loss of the *CDKN2A* gene. Subsequent analysis proved that homozygous deletion of *PTPRD* without the deletion of neighboring genes is frequently observed in a variety of tumors (Veeriah *et al.,* 2009).

Studies of candidate cancer genes (CAN genes) in CRCs revealed promoter hypermethylation in over 65% of those genes (Mokarram *et al.,* 2009). It has been proved that a loss of PTPRD activity can be caused by classical mechanisms, which are critical for tumor suppressor genes (Veeriah *et al.,* 2009).

Array-based technology is one of the most powerful tools in cancer research enabling a comprehensive analysis of a huge number of biological factors in a short time.

Microarray studies of methylation in colorectal cancer analyzing 27 578 CpG sites in 14 000 genes, revealed hypermethylation of 621 CpG sites located in the promoter regions of a variety of genes (Kim *et al.,* 2011). Downregulation of the expression of hypermethylated genes has been observed. However, these results were not statistically significant. Functional analysis of these genes using the PANTHER (**P**rotein **AN**alysis **TH**rough **E**volutionary **R**elationships) classification system revealed their involvement in signal transduction, mRNA transcription, cell-cell communication and surface-receptor mediated signal transduction, as well as cell adhesion, proliferation and differentiation (Kim *et al.,* 2011). Kinases and phosphorylases are involved in most of these processes (Arena *et al.,* 2005).

Aberrant methylation of the CpG-rich region in intron 1 of *PTPRG* has been detected in colon adenomas developing into carcinomas in both hereditary nonpolyposis colon cancer and in sporadic colorectal tumors. Despite the fact that this alteration has not been connected with deregulation of *PTPRG* expression, the high specificity (96%) and sensitivity (94%) of the status of *PTPRG* methylation open the possibility of its application as a diagnostic marker in the early detection of CRC (van Roon *et al.,* 2011).

CONCLUSION

The important role of phosphatases and kinases as both oncogenes and tumor suppressors, as well as the apparent reversibility of their oncogenic effects in phosohorylation/defosphorylation processes make these genes/proteins interesting potential targets in anticancer therapy. Therefore, an increasing number of studies on their role in cancerogenesis have been published recently.

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