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A missense variant in *PTPN12* associated with the risk of colorectal cancer by modifying Ras/MEK/ERK signaling

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ABSTRACT

Background: The classical protein tyrosine phosphatases (PTPs) have been widely reported to be associated with various human malignancies including colorectal cancer (CRC). However, there are few comprehensive analyses of the association between the classical *PTP* genes and CRC risk.
Methods: First, a bioinformatics analysis was performed to identify missense variants within the classical *PTP* gene family. Second, exome-wide association data and an independent population study were conducted to evaluate effects of candidate variants on CRC risk. Finally, functional assays based on signaling pathways were applied to uncover the potential pathogenic mechanism.
Results: We identified that *PTPN12* rs3750050 G allele presented a 19% increase the risk of CRC, with an OR of 1.19 (95% CI = 1.09–1.30, $P = 1.015 \times 10^{-4}$) under an additive model in the combined analysis. Furthermore, biochemical assays illustrated that rs3750050 could impair the inhibitory effect of PTPN12 on Ras/MEK/ERK signaling by impeding SHC dephosphorylation, increase the expression of cyclin D1 and ultimately lead to aberrant cell proliferation, thus contributing to CRC pathogenesis.
Conclusion: Our study highlights that *PTPN12* rs3750050 could increase CRC risk by modifying Ras/MEK/ERK signaling. This work provides a novel insight into the roles of genetic variants within *PTP* genes in the pathogenesis of CRC.

1. Introduction

Colorectal cancer (CRC) is the fourth common malignancy and a leading cause of cancer-related death worldwide [1]. In China, CRC is the fifth frequently diagnosed cancer and its incidence has been rising these years [1,2]. Although lifestyles and environmental exposures are contributed to CRC [3–5], genetic factors also play an important role in the pathogenesis of this cancer [6]. Nowadays there are many CRC susceptible loci identified by genome-wide association studies (GWAS) and candidate gene researches, but these findings can only explain a small part of CRC risk [7–10]. Hence, it is desiderated to excavate more functional loci through multiple viewpoints to construct the CRC's genetic architecture.

Protein tyrosine phosphatases (PTPs) dephosphorylate specific target proteins, antagonize activities of protein tyrosine kinases (PTKs), and play a crucial role in cell growth, proliferation and motility [11]. Genetic alterations in *PTP* genes might have adverse effects on cell life activities and ultimately involve in tumorgenesis [12–14]. As an earliest found and most studied subfamily, classical *PTPs* consist of 38 members including 21 receptor *PTPs* (*PTPRs*) and 17 non-receptor *PTPs* (*PTPNs*), which have been reported to be associated with various human cancers such as CRC [11,13,14]. In 2002, Ruivenkamp screened for cancer-related susceptible loci by mapping and cloning quantitative trait loci in mice, and remarkably found frequent deletions of *PTPRJ* in human cancer samples [15]. Later, Q276 P and R326Q variants of this gene were reported to modify the risk of thyroid carcinomas, esophageal squamous cell carcinoma and CRC [16,17]. Another famous example was the large-scale mutational analysis performed by Wang and colleagues [18]. They used high-throughput sequencing to explore somatic mutations in *PTP* gene superfamily and identified six frequently

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mutated classical *PTPs* (*PTPN3*, *PTPN13*, *PTPN14*, *PTPRF*, *PTPRG* and *PTPRT*) [18]. In addition, there have also been several studies about the association between genetic variants in other classical *PTP* members and human cancers, including *PTPN12* and breast cancer [19], *PTPN23* and CRC [20], and *PTPRG* and lung cancer [21].

Increasing evidence has strongly suggested that genetic variants in classical *PTP* gene family could play an important role in carcinogenesis. However, previous studies often explored effects of single or several classical *PTPs*, rare systematic analysis has investigated the association of classical *PTP* gene family with CRC risk. Hence, we hypothesized that missense variants in classical *PTP* gene family could modulate the susceptibility to CRC by influencing protein function. A bioinformatics analysis was initially applied to systematically search genetic missense variants in classical *PTP* genes, and then a two-stage case-control study was performed to examine the association of candidate variants with CRC risk in a Chinese population. Finally, we explored the potentially pathogenic mechanism of verified variants by functional assays.

2. Subjects and methods

2.1. Variants selection and genotyping

A flow chart of variants selection is shown in Supplementary Fig. 1. We got location information of classical *PTP* gene family in the Ensembl database (GRCh37, available at http://www.ensembl.org/index.html, listed in Supplementary Table 1). Based on gene location, a total of 53 840 variants within these 38 classical *PTP* genes were acquired from UCSC Table Browser (available at http://genome.ucsc.edu/cgi-bin/hgTables?command=start). Then we acquired the genotyping data of candidate variants from the previous CRC exome chip by the following filters [22]: (1) missense, (2) minor allele frequency (MAF) ≥ 0.05, (3) allele count = 2. The criteria above were based on the following considerations: (1) for rare variants with MAF < 0.05, there are less than 3 homozygotes per 1000 subjects averagely. Our study only included 1062 CRC patients and 2184 controls for the first stage, so the sample size was insufficient to evaluate those variants with MAF < 0.05; (2) TaqMan assay used in our study is an effective genotyping method in molecular epidemiology, but the probes are suitable to differentiate biallelic variants. Finally, we obtained 18 *PTP* variants matched on the criteria (Supplementary Table 2). In replication stage, the promising variants were genotyped by the TaqMan assay (Life technologies, CA, USA) via ABI 7900 H T real-time PCR system (Life technologies, CA, USA). We chose 5% duplicates and negative controls as quality controls, and found 100% concurrence rate of the duplicates.

2.2. Study subjects

The study was an extension of our previous exome chip on CRC comprising 1062 CRC patients and 2184 controls, and the detailed characteristics of the study subjects were described previously [22]. The replication stage consisted of 767 CRC cases and 1215 controls. Cases were enrolled in September 2010 from the First Affiliated Hospital and Affiliated Nanjing First Hospital of Nanjing Medical University (NMU). Controls were cancer-free individuals from a large cohort of health screenings. All these participants were genetically unrelated Chinese. Cases were pathologically confirmed while controls were frequency-matched to cases by gender and age (± 5 years). We collected individual information (age, gender, smoking, drinking, etc.) through personal interviews by trained workers. Non-smokers were defined as individuals who had never smoked or who had smoked < 100 cigarettes before the date of cancer diagnosis for patients, or before the date of interview for controls. Otherwise, subjects were defined as smokers, including ex-smokers and current smokes [23–26]. At the recruitment, we also collected blood samples from participants based on their written informed consents. Our study was approved by the institutional review boards of Tongji Medical College of Huazhong University of Science and Technology and NMU. All the methods were performed in accordance with the approved guidelines.

2.3. Functional assays

Human colon adenocarcinoma cell line SW480 was purchased from the cell bank of the committee on type culture collection of Chinese Academy of Science (Shanghai, China) in October 2016, and maintained in Dulbecco's modified Eagle medium (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA). This cell line was authenticated by short tandem repeat profiling (Applied Biosystems, USA), which was tested for non-contamination of mycoplasma (MycoAlert, USA) in October 2016. Main functional assays included cell proliferation assay, caspase-3 activity detection, cell cycle distribution, real-time polymerase chain reaction (PCR) and Western Blot Analysis. Reagents and procedures were detailed in Supplementary materials.

2.4. Statistical analysis

Hardy-Weinberg equilibrium (HWE) in controls was examined by a goodness-of-fit χ^2 test. Differences in distributions of demographic characteristics including age, gender, smoking and drinking were assessed by independent-samples *t*-test or Pearson's χ^2 test between cases and controls. We used the odds ratio (OR) and 95% confidence interval (95% CI) to evaluate the association between candidate variants and the CRC risk under the unconditional logistic regression analysis with adjusting for age, gender, smoking and drinking. A multiplicative interaction model was applied to investigate the potential gene-environment interaction of candidate variants with smoking or drinking exposure. The false discovery rate (FDR) was calculated by Benjamini and Hochberg method for multiple testing adjustment [27]. In Stage one of variants selection, we appropriately relaxed the screening restriction to set $FDR_{-adjusted} < 0.1$ as the significant criteria, aiming to excavate more potential candidate variants. In other cases, a two-sided $P < 0.05$ was considered as statistically significant. For stage one, the PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) was applied for batch computing of multiple loci. For stage two, Statistic Analysis System software (version 9.4, SAS Institute, Cary, NC) was used for calculation of single locus. For western blot analysis, GeneSnap was applied to scan exposured protein bands and image J (http://imagej.nih.gov/ij/download.html) was used to analyze the intensities of the protein scans. In addition, we evaluated the statistical power for the population association study via Power 3.0 [28]. Given our sample size to detected an OR = 1.50, an estimated power was greater than 0.72 for variants with MAF ≥ 0.05 in stage one, and in stage two, a power of 0.98 was estimated for candidate variants (MAF > 0.30).

3. Results

3.1. Characteristics of subjects

The detailed demographic characteristics of CRC exome chip were described previously. In replication stage, we recruited 767 CRC cases and 1215 cancer-free controls in the Nanjing area. Cases and controls were well matched in the distribution of age (59.1 ± 13.3 versus 58.4 ± 14.3, $P = 0.230$) and gender (Male percentage: 52.0% versus 49.9%, $P = 0.352$). However, there was marginally significant between the two group in smoking ($P = 0.082$) or drinking ($P = 0.060$).

3.2. Association between candidate variants and CRC risk

1062 cases and 2184 control were retained after quality control of the previous exome-wide association study [22]. A total of 18 candidate variants (Supplementary Table 2) were identified by integrating the bioinformatic approaches and the epidemiological data from CRC

Table 1
Association analyses between *PTPN12* rs3750050 and CRC risk in both stages.

	Stage one			Stage two			Combination		
	Cases/Controls	OR (95% CI)[a]	*P*[a]	Cases/Controls	OR (95% CI)[a]	*P*[a]	Cases/Controls	OR (95% CI)[a]	*P*[a]
rs3750050									
AA	90/229	reference		69/157	reference		159/386	reference	
AG	442/959	1.18 (0.90, 1.54)	0.240	329/542	1.37(1.00,1.88)	0.050	771/1501	1.25(1.02, 1.53)	0.033
GG	530/996	1.36 (1.04, 1.77)	0.025	351/476	1.67(1.22, 2.29)	0.001	881/1472	1.46(1.19, 1.78)	3.036×10^{-4}
Dominant model		1.27 (0.98, 1.64)	0.069		1.51(1.12, 2.04)	0.007		1.35(1.11, 1.64)	0.003
Additive model		1.16 (1.04, 1.30)	0.010		1.27(1.10, 1.45)	0.001		1.19(1.09, 1.30)	1.015×10^{-4}
rs6780013									
GG	449/810	reference		285/504	reference		734/1314	reference	
GA	485/1037	0.84 (0.72, 0.99)	0.034	338/519	1.16 (0.95, 1.42)	0.151	823/1556	0.94(0.83, 1.07)	0.362
AA	128/336	0.68 (0.54, 0.87)	0.002	109/166	1.16 (0.88, 1.54)	0.302	237/502	0.85(0.71, 1.01)	0.067
Dominant model		0.80 (0.69, 0.93)	0.004		1.16 (0.96, 1.40)	0.126		0.92(0.82, 1.03)	0.163
Additive model		0.83 (0.75, 0.93)	0.001		1.10 (0.96, 1.25)	0.176		0.93(0.85, 1.01)	0.071

[a] Adjusted for confunding factors including age, gender, smoking and drinking.

exome chip. As shown in Supplementary Table 3, these 18 variants distributed across multiple chromosomes, of which 13 candidates belonged to *PTPRs* and 4 belonged to *PTPNs*. All the genotyping call rates of the candidate SNPs were > 99%. After adjustment for age, gender, smoking and drinking, 2 variants rs3750050 and rs6780013 within *PTPNs* were found to be related with the susceptibility to CRC (Supplementary Table 4); the associations were still significant after multiple testing adjustment ($FDR_{\text{-adjusted}} < 0.1$). Interestingly, the major allele of *PTPN12* rs3750050 differs among different population that A allele is more frequent in Caucasians while G allele is predominant in Asians. Thus we considered the ancestral allele of this variant, A allele, as the reference in this study. The *PTPN12* rs3750050 G allele was associated with an increased risk of CRC compared with A allele, with OR of 1.16 (95%CI = 1.04–1.30, $P = 0.010$) under additive model. Besides, individuals carrying *PTPN23* rs6780013 GA or AA genotype had an OR of 0.80 (95% CI = 0.69-0.93, $P = 0.004$) compared with individuals with the GG genotype.

In stage two, we performed a case-control study in another independent population to further verify the association between the two promising variants and CRC risk. As shown in Table 1, the consistent effects on CRC risk for rs3750050 AG/GG genotypes were observed in stage 2, with OR of 1.51 (95% CI = 1.12–2.04, $P = 0.007$) under dominant model. Moreover, individuals carrying AG or GG genotype presented increased CRC risk compared with individuals carrying AA genotype (adjusted OR = 1.35, 95% CI = 1.11–1.64, $P = 0.003$) in combined analyses. In addition, we also summarized the effects of *PTPN23* rs6780013 on the susceptibility to CRC. Unfortunately, it failed to be successfully verified in stage two. We combined data of the both stages and found that nearly all the models suggested a non-significant result.

Moreover, we analyzed the interactions of gene-environment and displayed results in Supplementary Table 5. We did not found any significant interaction of *PTPN12* variants with smoking or drinking exposure.

3.3. Rs3750050 impaired the anti-proliferative effects of PTPN12 by accelerating G1/S transition

Protein PTPN12 (also known as PTP-PEST) is reported to suppress cell proliferation and tumorigenicity. Thus, we speculated the missense variant rs3750050 may impair the anti-proliferative effect of PTPN12 by leading to a substitution of amino acid residue T573 to A573. Therefore, a CCK-8 assay was conducted to examine the effect of rs3750050 on cell proliferation. As shown in Fig. 1a, the constructs containing rs3750050-A allele (PTPN12 wildtype) showed significantly reduced cell viability compared with counterpart rs3750050-G allele (PTPN12 mutant T573 A) ($P = 0.008$). Besides, the different effect on cell proliferation between the constructs containing rs3750050-A allele and counterpart rs3750050-G allele was more evident after knockdown of PTPN12 ($P < 0.001$).

To rule out the possibility that the differences of cell viability between cells ectopically expressing GFP-PTPN12 and GFP-T573 A was caused by cell death, a caspase-3 activity assay was performed to determine the effects of *PTPN12* variants on cell apoptosis. Fig. 1b showed that no obvious apoptosis could be detected in wildtype or T573 A -expressing cells after IGF1 treatment.

To further explore how T573 A impaired the anti-proliferative effects of *PTPN12*, we analyze cell cycle distribution by flow cytometry. As shown in Fig. 1c and Supplementary Fig. 2, knockdown of *PTPN12* substantially promoted cell cycle progression in SW480 cells. Moreover, cells overexpressing rs3750050-G had significantly enhanced cell cycle procession in comparison to cells overexpressing rs3750050-A. Taken together, these findings suggested that the rs3750050 impaired the anti-proliferative effects of *PTPN12* by accelerating G1/S transition.

3.4. Rs3750050 attenuated the inhibitory effect of PTPN12 on Ras/MEK/ERK signaling by impeding SHC dephosphorylation

Numerous studies indicated that PTPN12, a scaffold protein tyrosine phosphatase, exerted anti-tumor activity by targeting a set of substrates, including Shc, Pyk2, Fak, Cas and so on. Interestingly, we found that T573 A mutation located near the motif required for PTPN12-SHC interaction (576~613aa). PTPN12 could bind to p52 SHC and suppress the signal transduction of RTKs/Ras/Raf/MEK/ERK pathways, which plays a critical role in cell cycle regulation and tumorigenesis. Thus, the effect of rs3750050 on Ras/MEK/ERK signaling was examined. We observed that up-regulation of phosphor-p52SHC level induced by IGF1 stimulus could be sharply suppressed by overexpressing PTPN12, while this inhibition was significantly attenuated in cells transfected with PTPN12 T573 A mutant (Fig. 2a and b). IGF1 stimulation led to a rise in phospho-ERK protein level, which could be also suppressed by overexpressing PTPN12. Notably, we found significant differences in the phospho-ERK level for cells expressing PTPN12 and PTPN12 T573 A. Cells overexpressing PTPN12 T573 A significantly reduced the inhibition phospho-ERK protein level.

As an important effector of Ras/MEK/ERK signaling, *CCND1* is required for cell cycle G1/S transition. Thus, we performed real-time PCR to examine the influence of rs3750050 on *CCND1* gene expression. As expected, T573A-transfected cells demonstrated significantly higher *CCND1* expression than *PTPN12* wildtype-transfected cells (Fig. 2c).

All these consistent results strongly suggested that rs3750050 attenuated the inhibitory effect of *PTPN12* on Ras/MEK/ERK signaling by impeding SHC dephosphorylation, then up-regulated the expression of *CCND1* to accelerate G1/S transition and led to the aberrant cell

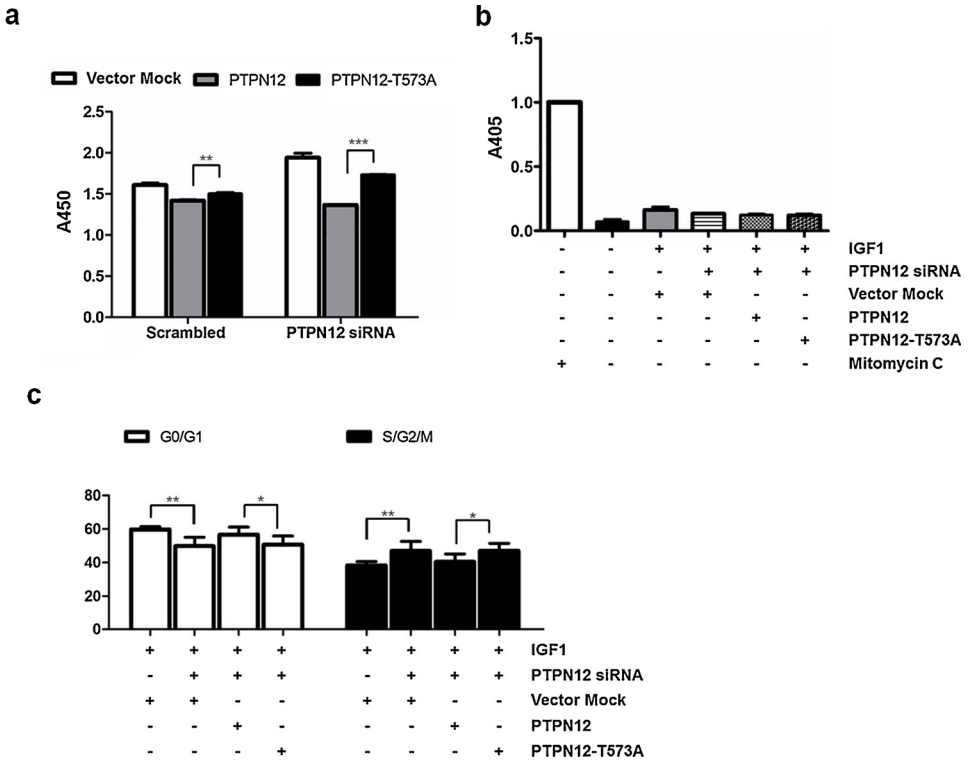

Fig. 1. A common variant T573 A impaired the anti-proliferative effects of *PTPN12* by accelerating G1/S transition. The endogenous PTPN12 expression was knocked down by specific siRNA in Sw480 cells. Then, wildtype (wt) PTPN12 and T573 A mutant were ectopically expressed in PTPN12 silencing cells. **(a)** The effects of wt PTPN12 and mutant on the cell viability of SW480 were evaluated using CCK-8 assay after IGF1(100 ng/ml) treatment. **(b)** The apoptosis levels of Sw480 cells transfected with wt PTPN12 or mutant were determined using caspase-3 activity assay after IGF1 stimulation. Sw480 cell lysate treated with mitomycin C (100 μg/ml) was used as a positive control. **(c)** Cells transfected with wt PTPN12 or mutant were synchronized by serum starvation and then treated with IGF1. Cell cycle distribution were monitored by flow cytometry. All of the experiments were triplicated and data were shown in mean ± SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

proliferation in CRC cells (Fig. 3).

4. Discussion

In this study, we integrated bioinformatic analyses, exome-wide association data and biochemical assays to investigate the association of missense variants in the classical *PTP* gene family with CRC risk. Population study revealed that *PTPN12* rs3750050 significantly modified the risk of CRC. Further biochemical assays indicated that rs3750050 G (T573 A) significantly impaired PTPN12's

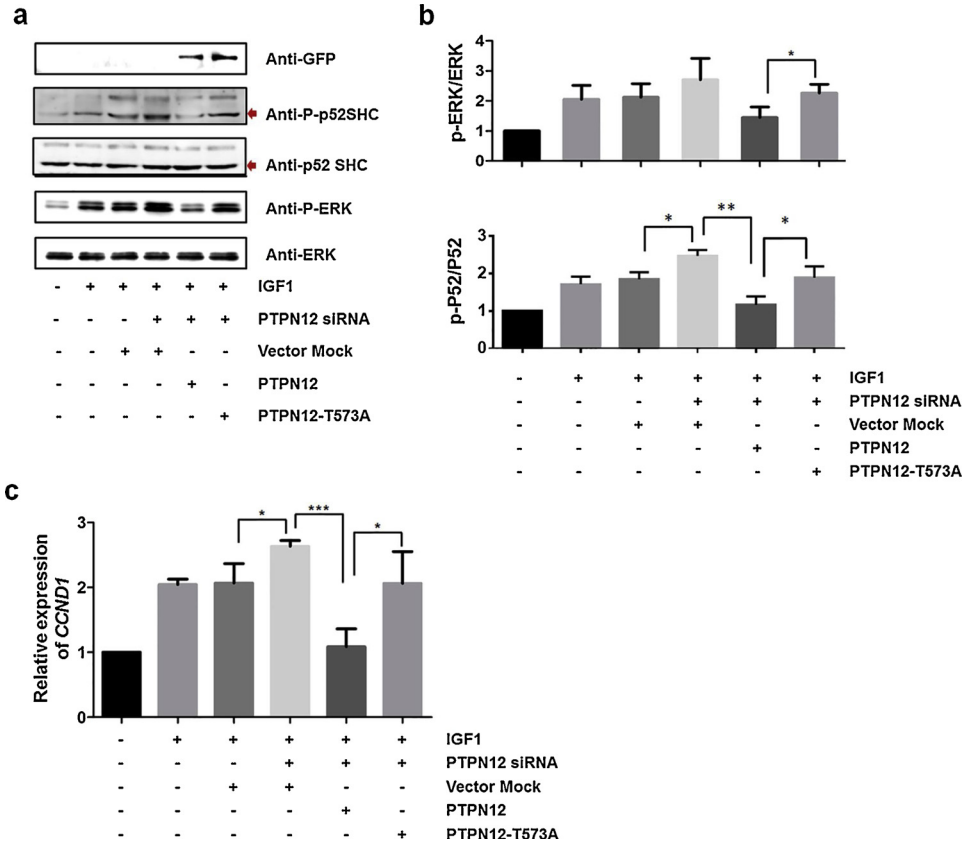

Fig. 2. The variant T573 A attenuated the inhibitory effect of *PTPN12* on Ras/MEK/ERK signaling by impeding SHC dephosphorylation. The endogenous PTPN12 expression was knocked down by specific siRNA in Sw480 cells. Then, wildtype (wt) PTPN12 and T573 A mutant were ectopically expressed in PTPN12 silencing cells. The effects of ectopically expressed wt PTPN12 and mutant on SHC and ERK activation induced by IGF1 administration was analyzed by Western blotting using anti-phosph-SHC and anti-phosph-ERK antibodies. Representative Western blotting image was shown **(a)**. Semi-quantitative results of immunoblots in *a* which were obtained using software Image J v2.1.4.7 **(b)**. SW480 cells were transfected with constructions encoding wt PTPN12 or mutant and the relative expression of *CCND1* were assayed by RT-PCR after 8 h IGF1 stimulation **(c)**.

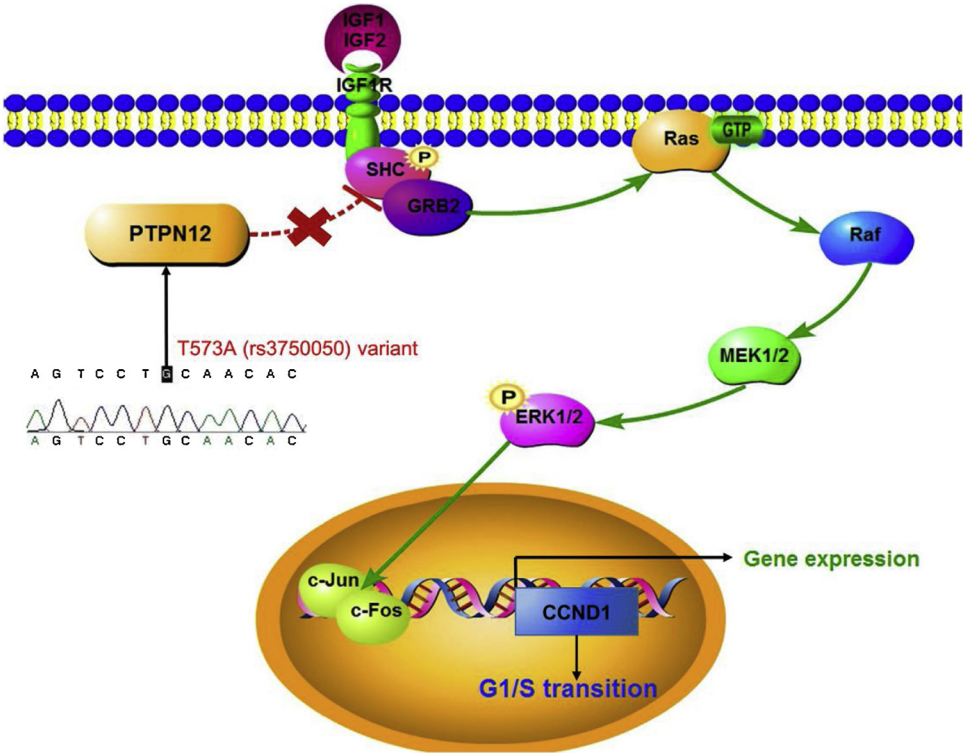

Fig. 3. The proposed role *PTPN12* T573 A mutant in IGF1- induced cell proliferation. IGF1 stimulation initiates the signaling transduction of Ras/Raf/ERK pathway by recruiting and phosphorylating the adaptor protein p52SHC. Activated ERK phosphorylates AP-1 to upregulate regulator molecules including *CCND1*, and accelerates G1 to S phase transition. *PTPN12* functions as a negative regulator for Ras/Raf/ERK signaling cascade by dephosphorylating activated–SHC protein. **However, PTPN12 T573 A mutant fails to dephosphorylate activated-SHC, increases *CCND1*, and ultimately leads to aberrant cell proliferation.**

dephosphorylation on Ras/MEK/ERK signaling, then accelerate G1/S transition via up-regulation of *CCND1* expression, thus making effect on the pathogenesis of CRC.

In stage one, we identified two promising variants. One was *PTPN23* rs6780013, but it failed to pass the verification of stage two. This variant is located in a susceptible region 3p21.31 reported by a recent CRC GWAS [20]. Unfortunately, evidence from our study was insufficient to support the association of *PTPN23* and CRC risk. Another was *PTPN12* rs3750050, which was successfully validated in both stages and subsequent functional assays. Protein PTPN12 is a cytoplasmic tyrosine phosphatase expressed ubiquitously that efficiently dephosphorylate specific tyrosine substrates, thus playing a part in in regulating cell growth, proliferation and migration [29–32]. In 2011, Sun first showed *PTPN12* as a new tumor suppressor gene and showed that loss of this protein resulted in activation of multiple PTKs in breast cancer [19]. Recently, Kwiatkowski also reported that *PTPN12* is a novel candidate gene for early-onset CRC susceptibility [33]. Our study not only supported the contributing role of *PTPN12* in CRC susceptibility, but also further revealed how the functional variant impaired the tumor-suppressor effect of *PTPN12* in CRC susceptibility.

PTPN12 rs3750050 is located within the proline-rich region adjacent to C-terminal of this protein (573aa). Charest A reported that the motif (576˜613aa) of mouse PTPN12 is necessary for SHC-PTPN12 interaction [34]. Comparing nucleotide sequences of the human, mouse, rat, yak and Pteropus PTPN12 protein, we find the motif (576˜613aa) is high conservative. Moreover, Tyr573 which is located near the motif also show extreme evolutionary conservation. So, we speculated that although this variant does not occur in the catalytic domain (28-293aa), the variant might impair the regulation of SHC by PTPN12. Our experiments provided a concrete evidence line to illustrate a proposed oncogenic role of rs3750050 in CRC (Fig. 3). In normal condition, IGF1 stimulates the initiation of Ras/Raf/ERK signaling pathway through phosphorylating the adaptor protein p52 SHC. Then SHC recruits GRB2 to constitute complex adaptors, which relay and amplify an exquisitely fine-tuned regulation of multiple downstream effectors, including Ras, Raf, MEK and ERK. Activated ERK phosphorylates AP-1 to upregulate cell cycle regulators, and eventually accelerates G1 to S phase transition. At the end of G1 phase, low-phosphorylated Rb protein binds to E2F–HDAC complex to silent its transcriptional activity, inducing cell into checkpoint. *CCND1* encodes cyclin D1, a crucial subunit of cyclin D1-CDK4/6 complex, which could phosphorylate Rb, dissociate the silent complex and then promote G1/S transition. Recent studies have revealed that cyclin D1 induction is a major way for Ras/MEK/ERK signaling to promote G1/S transition [35]. Thus we selected *CCND1* as the testing effector. Faisal A and Habib T' studies both suggest that *PTPN12* negatively regulates Ras/Raf/ERK signaling cascade by dephosphorylating activated-SHC protein [36,37]. When a functional damage (e.g. rs3750050) occurs in *PTPN12*, the mutant PTPN12 fails to dephosphorylate activated-SHC, impairs its inhibitory regulation on Ras/Raf/ERK pathway, then increases the expression of *CCND1*, and ultimately leads to aberrant cell proliferation. Our findings might suggest tightly regulation of RTKs by PTPN12 might be critical, whose dysregulation contributes to colorectal carcinogenesis.

However, some limitations should be acknowledged. First, only missense variants with MAF ≥ 0.05 were inspected in this study. Other polymorphisms within *PTP* genes including copy number variant and rare variants should also be investigated in future researches. Second, for the scope of designed probes on the CRC exome chip, we only analyzed 18 candidate variants identified by the bioinformatic analysis. The remaining candidate variants not designed on the exome chip should be further explored. Third, our study was a retrospective observational research based on hospital population, which was inevitably influenced by selection bias and recall bias. At last, other confounders such as body mass index (BMI) and red meat intake were not evaluated in this study. Independent studies with better design and larger-scale are desiderated to confirm our findings.

In conclusion, we highlighted that *PTPN12* rs3750050 significantly increased the risk of CRC via a two-stage case-control study. Following functional assays revealed that this variant could attenuate *PTPN12*'s inhibitory effect on Ras/MEK/ERK signaling, enhance cell cycle procession and proliferation, thus participating in CRC development. Our study provides a novel insight into the roles of variants within *PTP* genes in the pathogenesis of CRC.

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Conflicts of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Na Shen: Data curation, Methodology, Writing - original draft. **Lu Li:** Data curation, Methodology, Writing - original draft. **Wang Xu:** Data curation, Methodology. **Jianbo Tian:** Data curation, Methodology. **Yang Yang:** Data curation, Methodology. **Ying Zhu:** Data curation, Methodology. **Yajie Gong:** Data curation, Methodology. **Juntao Ke:** Data curation, Methodology. **Jing Gong:** Data curation, Methodology. **Jiang Chang:** Data curation, Methodology. **Rong Zhong:** Conceptualization, Project administration, Supervision, Writing - review & editing. **Xiaoping Miao:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.canep.2019.01.013.

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