Biomedicine & Pharmacotherapy 99 (2018) 402–410

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

NF-κB-induced WIP1 expression promotes colorectal cancer cell proliferation through mTOR signaling

Fei Bai[a], Huijun Zhou[b], Zhongping Fu[a], Jiangbo Xie[a], Yingbin Hu[a], Shaolin Nie[a,*]

[a] Department of Colorectal Surgery, Hunan Cancer Hospital & The Affiliated Hospital of Xiangya School of Medicine, Central South University, PR China
[b] Department of Gastroenterology and Urology, Hunan Cancer Hospital&The Affiliated Hospital of Xiangya School of Medicine, Central South University, PR China

ARTICLE INFO

Keywords:
Colorectal cancer (CRC)
Cell proliferation
Wild-type p53-induced protein 1 (WIP1)
NF-κB
mTOR
p21

ABSTRACT

Colorectal cancer (CRC) is one of the major causes of cancer deaths worldwide. Wild-type p53-induced protein 1 (WIP1) is overexpressed in multiple human cancers and acted as an oncogene. This study was aimed to investigate the effect of WIP1 in colorectal cancer growth and analyzed underlying mechanisms. Herein, we determined WIP1 expression in CRC tissues and cell lines, as well as evaluated its detailed function in CRC cell proliferation. Several factors have been reported to mediate WIP1 effects; herein, we examined the involvement of mTOR and p21 in WIP1 regulation of CRC cell proliferation. Moreover, NF-κB has been regarded as a positive transcriptional regulator of WIP1 to activate its expression. NF-κB knockdown suppressed CRC cell proliferation, which could be reversed by WIP1 overexpression, through p21 and mTOR. Further, we examined the binding of NF-κB to the promoter region of WIP1. In CRC tissues, NF-κB expression was significantly up-regulated, and positively correlated with WIP1 expression, suggesting that inhibiting NF-κB expression to attenuate its activating effect on WIP1 expression presented a promising strategy of controlling excess proliferation of CRC cell. In summary, WIP1 promotes CRC proliferation through p21 and mTOR, both downstream targets of p53; NF-κB served as a positive transcriptional regulator of WIP1 to activate its expression and affect its function in CRC cells. Our finding provided a novel strategy for treatment for CRC.

1. Introduction

Colorectal cancer (CRC) is a fatal cancer resulting in huge economic losses and health damage worldwide. Its prognosis is closely related to the disease stage at the time of diagnosis [1]. Five-year survival can reach up to 90% in patients with localized disease but falls to 68% for patients with lymph node involved; moreover, this indicator will drop to 10% in patients with distant metastases [2]. Diagnosis in advanced stages may result in poor survival. Thus, early detection of symptomless CRC could reduce CRC mortality since removal of these precursors during colonoscopy reduces the incidence of CRC [3]. Identifying reliable early-stage prognostic markers and personalized targeted therapy may improve the outcome and survival in patients with CRC.

Wild-type p53-induced protein (phosphatase) 1 (WIP1), a member of serine/threonine phosphatases of PP2C family, is widely expressed at the basal level and can be dramatically induced by exposing cells to genotoxic stress [5]. RNAi-induced WIP1 knockdown results in prolonged G2 checkpoint whereas WIP1 overexpression leads to checkpoint reload [6]. WIP1 phosphatase overexpression is commonly observed in many kinds of cancers and serves as an oncogene. In contrast, WIP1 knockdown can slow the progress of cancer in the mouse model [7–9]. Similarly, RNAi-mediated WIP1 depletion suppresses cancer cell proliferation, indicating that WIP1 can serve as a potential pharmacological target [9]. The above findings, therefore, suggest that WIP1 might have potential in CRC by serving as a biomarker and/or novel target. Herein, we first monitored WIP1 expression in CRC tissues and cells, and then evaluated the effect of WIP1 on CRC cell proliferation to assess the possibility of WIP1 being a potential diagnosis marker and target.

Regarding the molecular mechanism, WIP1 exerts its function in the hematopoietic system through regulating several downstream targets, such as p53, p38MAPK kinase, NF-κB, mTOR, ATM and p21 [10–12]. WIP1 contribute to two different haematopoietic stem cell aging phenotypes through two distinct downstream pathways: the WIP1–mTORC1 axis and the WIP1–p53 axis [13]. GSK2830371 (an inhibitor of WIP1)-mediated WIP1 knockdown increased the expression of p21 in breast cancer cells [11]; treating breast cancer cells with GSK2830371 leads to accumulation of the cell cycle in G1/2 phases in a p21-dependent manner [11]. After evaluation of WIP1 function in CRC cell proliferation, we also investigated whether mTOR and p21 were

* Corresponding author at: No.283 Tongzipo Road, Yuelu District, Changsha, Hunan, 410013, PR China.
E-mail address: mykj1230@163.com (S. Nie).

https://doi.org/10.1016/j.biopha.2018.01.075
Received 24 October 2017; Received in revised form 3 January 2018; Accepted 12 January 2018

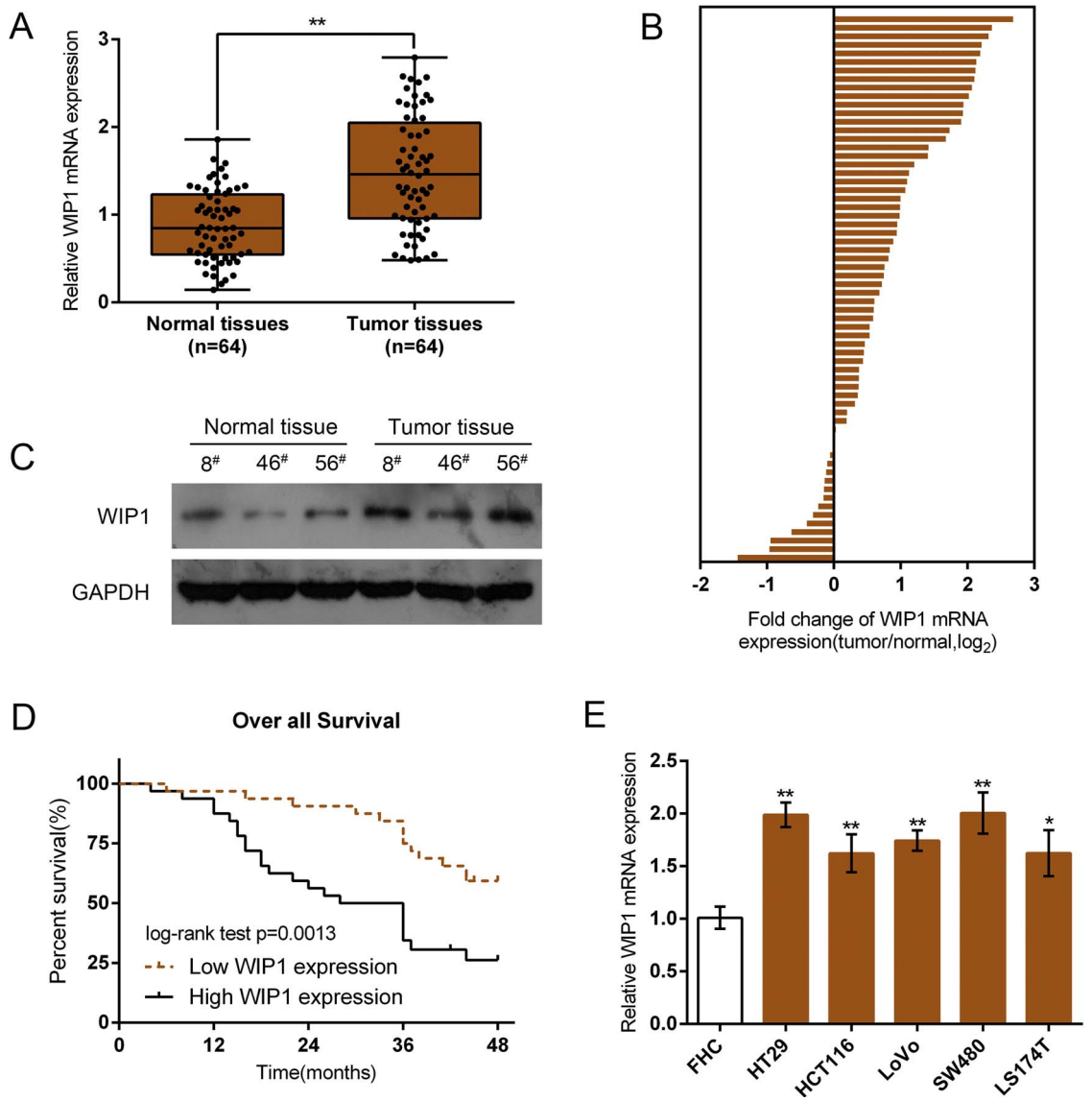

Fig. 1. WIP1 expression was up-regulated in CRC tissues and cell lines and correlated with poorer prognosis of patients with CRC. (A) Expression of WIP1 in 64 paired CRC and adjacent normal tissues was determined using real-time PCR assays. (B) The fold-change of WIP1 expression in 64 paired CRC and non-tumor tissue samples was shown as tumor/normal (log_2). (C) WIP1 protein in three paired randomly selected CRC and normal tissues was determined using Western blot assays. (D) Kaplan-Meier overall survival curves for 64 patients with CRC classified according to relative WIP1 expression level. (D) WIP1 expression in CRC cell lines, HT29, HCT116, LoVo, SW480, LS174T and a normal cell line, FHC (human colon epithelial cell) using real-time PCR assays. The data are presented as mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

involved in WIP1 regulation of cancer cell proliferation.

WIP1 knockdown can slow the progress of cancer in the mouse model [7–9], suggesting that hindering excessive WIP1 expression might affect carcinogenesis; thus, we also explored the possible regulatory mechanism of WIP1 expression attempting to figure out the way of correcting WIP1 dysregulation. As we mentioned, the effect of WIP1 could be mediated by several factors, including NF-κB, a transcription factor playing a key role in inflammation and augments the initiation, promotion, and progression of cancer [14,15]. According to Lowe et al., NF-κB is a positive transcription factor of WIP1 [16]. Herein, we hypothesized that NF-κB might activate WIP1 in CRC, investigated the combined effect of NF-κB and WIP1 on CRC cell growth, as well as confirmed the interaction between NF-κB and WIP1. We evaluated NF-κB expression in CRC tissues and the correlation with WIP1 expression to further confirm the above findings.

Taken together, we evaluated the expression and detailed function of WIP1 in CRC cell lines to assess the possibility of WIP1 being a biomarker and treatment target in CRC. We demonstrated that WIP1 overexpression promoted CRC cell proliferation; mTOR and p21 could be regulated by WIP1 in CRC cell lines; NF-κB served as a positive transcriptional regulator of WIP1 to activate its expression and affect its function in Colorectal cancer cell lines. We provided novel biomarkers and targets for diagnosis and treatment for CRC.

2. Materials and methods

2.1. Tissue samples, cell lines and cell transfection

We collected 64 paired colorectal cancer tissues and the matched adjacent normal tissues from patients who underwent surgical resection at Hunan Cancer Hospital, Central South University (Changsha, China). The tissues were snap-frozen in liquid nitrogen, and then stored at −80 °C. This project was approved by the Ethic Committee of Hunan Cancer Hospital, Central South University. All patients' informed consents were obtained.

We purchased the colorectal cancer cell lines, HT29, HCT116, LoVo, SW480, LS174T and a normal cell line, FHC (human colon epithelial cell), from the American Type Culture Collection (ATCC, Manassas, VA,