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REVIEW

WWP2 Is One Promising Novel Oncogene

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Abstract
WWP2 is an E3 ubiquitin ligase and plays an important role in regulation of many cellular biological activities through ubiquitination and degradation of its substrates. Recently accumulating evidences indicate that WWP2 plays a crucial part in the pathogenesis in different types of tumors. In this report, the role of this gene especially in tumorigenesis was reviewed. WWP2 is dysregulated in various of tumors, and it promotes carcinogenesis mainly through PTEN/Akt signaling pathway. WWP2 also participates in anti-cancer agents' sensitivity, indicating WWP2 may be a novel target for cancer treatment. WWP2 is one promising novel oncogene.

Keywords WWP2 · Cancer · Akt · Pathogenesis

Introduction

Protein ubiquitination is a process involves E1 (ubiquitin-activating), E2 (ubiquitin-conjugating) and E3 (ubiquitin ligase) enzymes. There are two E1 enzymes in human genomes generated from on gene, about 30 E2 enzymes, and more than 600 E3 enzymes [1]. E3 ubiquitin ligases play key role in substrate specificity and mainly include RING-type and HECT-domain ligases. NEDD4 family is a small sub-group of HECT-domain ligases, including WWP1, WWP2, Smurf1, Smurf2, NEDD4–1 and NEDD4–2. NEDD4 family includes three functional domains, an N-terminal, Ca2+/phospholipid-binding C2 domain for membrane binding, a central region containing up to four WW (double-tryptophan) domains, and a C-terminal HECT domain for ubiquitin protein ligation [2]. The WW domains interact with PPXY (phospho-Ser-Pro and Pro-Arg) containing sequences in target substrates [3, 4].

Among HECT-domain ligases, WWP2 is the least characterized. Human WWP2 (WW domain containing E3 Ub-protein ligase 2) is originally identified in screening for WW domain-containing proteins [4]. By using yeast two-hybrid and in vitro binding assays, Wood and colleagues demonstrated that WWP2 bound to atrophin-1, and designated WWP2 as atrophin-1 interacting protein 2 (AIP2) [5]. WWP2 is ubiquitously expressed in human heart, placenta, lung, liver, muscle, kidney, pancreas and brain [5]. With northern bolt analysis, Huiming Xu et al. identified two WWP2 transcripts, 4.4 and 2.4 kb in length, respectively. The 4.4-kb transcript was the predominant form, and the WWP2 transcript level was highest in skeletal muscle, peripheral leukocytes, placenta, heart, and kidney; higher in the brain, spleen and lung; and lower in the thymus, liver, colon and intestine [6]. Scrutiny of Mammalian Gene Collection (MGC) databases containing sequence-validated full-length protein-coding complementary DNA clones identified a unique and interesting feature of WWP2:there are three WWP2 isoforms generated from the same gene locus: full-length WWP2 (WWP2-FL, 870 aa), an N-terminal isoform (WWP2-N, 336 aa) presumably generated by failure to splice-out intron 9–10, and a C-terminal isoform (WWP2-C, 440 aa) possibly generated from a second promoter within intron10–11. WWP2-N isoform contains C2 domain and only a single WW domain (WW1), and WWP2-C isoform harbors WW4 domain and the HECT E3 ligase

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domain. By using isoform-specific primers and reverse transcriptase–PCR (RT–PCR) methods, SM Soond and A Chantry recently detected these three transcripts in Colo-357 pancreatic carcinoma cells, subsequently confirmed the expression of these there isoforms by Western blotting [7]. However, until now less is known about the specific expression of WWP2 isoforms in human organs. As a E3 ligase, many substrates of WWP2 have been identified until now, including Oct4 [6, 8, 9], ENaC [10], Rpb1 [11], SRG3 [12], TRIF [13], Paip1 [14] and EGR2 [15]. WWP2 regulates the turnover of these proteins by ubiquitination and degradation, then WWP2 takes part in series of cellular activities, such as suppression of translation, maintenance stemness of stem cells, negative regulator of innate immune and inflammatory responses.

The role and clinical implications of HECT-family members in carcinogenesis have been well documented in various types of tumors [2]. And Wei Chen [16] and Chantry A [17] previously reviewed the function of WWP2 though, there is great process about the role of this protein in tumorigenesis. Herein, we reviewed the recent process of WWP2 especially in carcinogenesis.

The Role of WWP2 in Carcinogenesis

It has been well established that E3 ligase genetic alternation, abnormal expression and dysfunction contributes to many diseases pathogenesis, including cancer. And many members of HECT domain E3s have been identified as crucial regulator of cancer development and therapy [2]. However, until recently the role and clinical implications of WWP2 in tumorigenesis began to attract much interest. Accumulating evidences have suggested that WWP2 is abnormally expressed in different types of cancers, including oral cancer, endometrial cancer, liver cancer, glioma and lung cancer [18–24]. Surinder M. and coworkers found that WWP2-N expression was increased in stage II/III prostate cancer, but significantly decreased in stage IIIC melanoma [22]. And WWP2 is also overexpressed in oral cancer, and its expression is correlated with poor prognosis [20]. Sheng-qian Xu et al. reported that both mRNA and protein level of WWP2 were greatly increased in liver cancer tissues compared with adjacent tissues, and WWP2 high expression was correlated with poor prognosis [23]. Compared to paired adjacent normal tissues, WWP2 expression is greatly increased in lung adenocarcinoma tissues, and WWP2 expression is significantly related with tumor differentiation, TNM stage and lymph node metastasis [24]. However, The Cancer Genome Atlas dataset showed WWP2 was homozygously or heterozygously deleted and the expression of WWP2 was decreased in majority of ovarian cancers [25], therefore WWP2 may be a tumor suppressive gene in ovarian cancer. Altogether, the role of WWP2 in different types of cancers may be specific.

Subsequent studies have been carried out to investigate the role of WWP2 in tumorigenesis in various cancers. Suppression of WWP2 expression with siRNA or shRNA significantly inhibited cell proliferation and formation of tumor in prostate cancer, oral cancer, liver cancer and lung cancer in vitro [23, 24, 26]. Furthermore, silence of WWP2 expression induced G1 accumulation [20, 23] and decreased the expression of cell-cycle related genes, including cyclin D1, cyclin E, CDK2, CDK4, and CDK6, meanwhile upregulated p21 and p27 [20]. Consistently, Byeong Hyeok Choi and coworker also found that WWP2 was required for normal cell cycle progression, silence of WWP2 enhanced M phase arrest and accelerated the turnover of cyclin E [18]. Additionally, inhibition of WWP2 also promoted apoptosis in liver cancer cells by downregulating the expression of anti-apoptosis protein Bcl-2 and enhancing the expression of pro-apoptosis proteins, such as Bax, Caspase-7 and Caspase-8 [23]. Moreover, WWP2 also promotes lung cancer and liver cancer cell migration and invasion in vitro by modulating the expression of MMPs [24, 27], consistently high expression of WWP2 is associated with lymph node metastasis in lung cancer [24]. However, WWP2 expression was decreased in the majority of ovarian cancers, ectopic expression of this protein significantly suppressed ovarian cancer cell proliferation in vitro and tumor growth in vivo, indicating WWP2 plays a tumor suppressive role in ovarian cancer [25].

The Role of WWP2 in Anticancer Drug Sensitivity

Besides the oncogenic roles in tumorigenesis, recent studies indicate that WWP2 may also play important part in sensitivity to anticancer agents. Silence the expression of WWP2 with shRNA greatly enhanced Doxorubicin-induced cell death in prostate cancer cells, overexpression of wide type WWP2 not catalytically inactive $WWP2^{C838A}$ restored the sensitivity to Doxorubicin, suggesting that WWP2 ubiquitin ligase activity is necessary for WWP2-mediated chemosensitivity [26]. However, WWP2 is downregulated in ovarian cancer cells, overexpression of this protein resisted platinum drugs-induced resistance by suppressing Notch3 signaling pathway, WWP2 directly binds with Notch3, then promotes Notch3 degradation through ubiqutination-dependent way [25]. Additionally, Gamabufotalin-induced cytotoxicity depends on WWP2-mediated ubiquitination and degradation of c-Myc in melanoma cells, and Gamabufotalin exposure increased WWP2 expression in a JNK-dependent manner [28]. These studies collectively suggested that WWP2 may play a crucial role in anticancer

agents by modulating the expression of tumor-related genes in an ubiquitin-dependent manner. But the above mentioned studies are mainly carried out in vitro, the role of WWP2 in chemoresistance should also be investigated in vivo and in different types of tumors with other anticancer agents.

Signaling Pathways in WWP2-Mediated Tumorigenesis

PTEN/PI3K/Akt signaling pathway is well characterized in WWP2-mediated tumorigenesis. Subbareddy et al. firstly reported WWP2 promoted prostate cancer cell survival by ubiquitination degradation of tumor suppressor PTEN, WWP2 directly interacted with PTEN, then promoted its ubqutination and degradation, then leading to elevated downstream Akt activities, silence the expression of WWP2 leading to increased PTEN expression and decreased Akt activities, resulted in retarded cell proliferation in vitro [26]. Stable expression of wild-type WWP2 not catalytically inactive $WWP2^{C838A}$ downregulated the expression of PTEN and increased the proliferation rate of prostate epithelial cells, further confirming that WWP2 regulates PTEN/PI3K/Akt signaling pathway depending on its E3 ligase activity. The role of PTEN/PI3K/Akt pathway was then subsequently confirmed in ovarian cancer cells and normal cells, silencing of WWP2 resulted in compromised phosphorylation of Akt [18, 20].

Besides PTEN/PI3K/Akt pathway, WWP2 also regulates cancer cell migration and invasion through TGF-β signaling pathway [7]. Soond and Chantry found that WWP2 interacted with Smad proteins which were required for canonical signaling activity via transforming growth factor-β (TGFβ) signaling pathway. TGFβ plays multifunctional role in cancer and is responsible for epithelial–mesenchymal transition (EMT) which promotes static epithelial cells turn into highly invasive mesenchymal cells, a necessary event for cancer metastasis. Moreover, WWP2 isoforms displayed differential binding activity towards individual Smad proteins. WWP2-FL bound to TGFβ receptor regulated R-Smads (Smads 2/3) and also to inhibitory I-Smad7, although it preferentially bound to I-Smad7 which is polyubiquitinated and rapidly degraded. However, WWP2-N associates with Smads 2/3 selectively, whereas WWP2-C interacts with I-Smad7. Unexpectedly, WWP2-N, which lacks a functional HECT ligase domain was also found to complex with WWP2-FL in a TGFβ-regulated manner and activate WWP2-FL ligase activity, leading to degradation of unstimulated Smads 2/3. Consequently, WWP2-FL plays crucial part in TGFβ-induced cancer cell metastasis since it preferentially bound to I-Smad7, further supported by the evidence that cell based EMT experiments in which expression of an isolated Smad7-binding WW4 domain caused selective disruption of the Smad7: WWP2 complex, and stabilized Smad7 protein levels to thereby prevent TGFβ-induced EMT. Furthermore, WWP2-N might suppress TGFβ-induced EMT due to its unique ability to limit the levels of receptor regulated R-Smads 2/3. Subsequent studies further confirmed the role of WWP2 in metastasis in liver cancer and lung cancer [24, 27]. Silence of WWP2 expression remarkably inhibits the migration and invasion capacity of liver cancer and lung cancer cells in vitro, and downregulated the expression of MMPs [24, 27].

Modulation of WWP2 Expression and Activity

Until recently there are rare reports about the modulation of WWP2 expression and its E3 ligase activity. Zhong-yang Ding and colleagues found that HIF-1α knockdown greatly downregulated the expression of WWP2 at both mRNA and protein level in thyroid cancer cell lines [29]. And anticancer drugs may also influence the expression of WWP2, Zhenlong Yu et al. reported that Gamabufotalin exposure increased WWP2 expression in a JNK-dependent manner in multiple myeloma cells, JNK inhibitor SB600125 repressed Gamabufotalin-induced WWP2 expression [28].

As an E3 ligase, the E3 ligase activity is necessary for WWP2-mediated cellular processes. Previous study has suggested WWP2 forms an auto-inhibitory conformation through an intramolecular interaction between its C2 and HECT domains [30]. Bing Liao and Ying Jin also reported that Wwp2 regulates its own ligase activity by poly-ubiquitination via the lysine 63 linkage in a dosage-dependent manner [8]. Subsequently Jia Liu and coworkers found that Cdh1 inhibited the catalytic of WWP2 by directly binding with both its C2 and HECT domains, leading to enhancement of the intramolecular C2-HECT interaction [31]. Mund T et al. reported that Dvl2 binds to the ubiquitin ligase WWP2 and unlocks its ligase activity from autoinhibition, leading to activation of WWP2 activity [32].

Concluding Remarks

The oncogenic role of WWP2 has been well documented in many types of tumors, however, the underlying mechanisms warranted further studies. Moreover, the role of this protein in chemoresistance warranted further investigation. In this report, we reviewed the recent process of WWP2 in carcinogenesis; further research should focus on the specific role of WWP2 isoforms in cancer pathogenesis and progression. Moreover, novel substrates especially oncogenes or tumor suppressive genes should be explored and underlying mechanisms also should be investigated.

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Compliance with Ethical Standards

Conflicts of Interest There is no potential conflict of interest or financial dependence regarding this publication.

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