Extra View

WWP2 ubiquitin ligase and it's isoforms: new biological insight and promising disease targets

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ABSTRACT

A number of recent papers on the WWP2 E3 ubiquitin ligase together with two novel WWP2 isoforms have revealed important biological insight and disease-specific functions, and also impacted on our understanding of ubiquitin ligases in cell cycle regulation, apoptosis and differentiation. Gene knockout studies suggest a developmental role for WWP2 in chondrogenesis via mechanisms involving cartilage-specific transcription factors. Furthermore, WWP2 isoforms have been shown to selectively target oncogenic signaling pathways linked to both the pTEN tumour suppressor and the TGFβ/Smad signaling pathway. Here, it is suggested that WWP2 isoforms have now emerged as central physiological regulators as well as promising new disease targets, and that the challenge ahead is to now develop highly selective WWP2 inhibitors with utility in cartilage disease such as osteoarthritis and as new anticancer strategies.

Protein ubiquitination represents a post-translational modification that regulates a variety of cellular processes including protein turnover, trafficking, sub-cellular localisation, transcriptional function and DNA repair mechanisms [1, 2]. Ubiquitin is a 76 amino acid protein and attaches covalently to target proteins via an isopeptide bond between the C-terminal Gly76 carboxyl group on ubiquitin and the free amino group on internal lysine's within the substrate. Ubiquitin conjugation involves a cascade of events catalyzed sequentially by E1 (ubiquitin-activating), E2 (ubiquitin-conjugating), and E3 (ubiquitin ligase) enzymes. There are approximately 600 E3 ubiquitin ligases in the mammalian genome [3], and they are key to providing target substrate specificity. E3 ligases can be further sub-divided primarily into RING type representing around 95% of the total and HECT domain ligases, of which there are 28 family members. The RING type E3s have no intrinsic ligase activity, and act as scaffolds that recruit specific substrates to an associated E2 ligase that is then responsible for ubiquitin transfer. In contrast, the HECT E3s have intrinsic ligase activity, and they lock onto specific substrates as well as directly mediating ubiquitination.

Within the HECT E3s is a small group (9 in total) referred to as the Nedd4 superfamily. The Nedd4 E3s have a unique structural organization that includes the so-called HECT ligase catalytic domain, but also a membrane-targeting Ca^{2+}/phospholipid-binding C2 domain and up to four WW (double tryptophan) domains. The WW domains interact with PPXY, phospho-Ser-Pro and Pro-Arg motifs and are primarily responsible for recruitment of specific target substrates. These unique structural and architectural features of the Nedd4 E3s not only makes an important contribution to their functional diversity, but also singles out this small group of E3 ligases for selective therapeutic targeting strategies, perhaps focused based on disrupting specific substrate recruitment. A similar strategy has been successfully applied to the selective cancer cell blockade of interactions between mdm2 ubiquitin ligase and p53 using nutlins [4].

Whilst a number of specific substrates have been identified for individual Nedd4 family members [5], their normal biological as well as pathological roles remains poorly understood. Recently, there have been a number of reports highlighting novel functional roles for one member of the Nedd4 family, the WWP2 E3 ligase. Gene knockout studies reveal key developmental roles for WWP2 in craniofacial development [6] and chondrogenesis [7], and cell/molecular approaches reveal two new substrates for WWP2, pTEN [8] and Smads [9], that link to critical oncogenic signaling pathways responsible for cell survival and epithelial-to-mesenchymal transition (EMT), respectively. Furthermore, the characterization of two new isoforms generated from the WWP2 gene locus, WWP2-N and WWP2-C [9], highlights novel intra-molecular mechanisms for controlling WWP2 biological activity.

The first WWP2 knockout mouse was recently reported by Zou *et al* [6], and clearly demonstrates an important developmental role for WWP2 in craniofacial patterning, that is likely to be linked to its abundant expression in cartilage tissue, and driven by the cartilage-specific transcription factor Sox9. Furthermore, biochemical studies identified Goosecoid (Gsc), a homeobox transcription factor that is already known to be important for craniofacial development, as a direct substrate for WWP2. In this instance, WWP2 was found to mono-ubiquitinylate Gsc and thereby enhance its transcriptional activity, to increase expression of another key cartilage regulatory protein Sox6. In a separate study by Nakamura *et al* [7], the transcriptional regulation of WWP2 by Sox9 was confirmed, but WWP2 was also found to complex with Sox9 and encourage its transcriptional activity by facilitating its nuclear translocation. Once in the nucleus, WWP2 further recruits the transcription enhancer Med25 into to the WWP2/Sox9 complex to augment Sox9 transcriptional activity, although this activity did not appear to be ubiquitinylation-dependent. WWP2 is highly expressed in limb buds at E12.5 in mice and then in peri-articular chondrocytes at E16.5. Instead of using mouse knockouts, Nakamura et al [7] utilised specific morpholinos and a developing zebrafish model system, and again noticed defects in palatogenesis linked to aberrant cartilage function.

Whilst both these reports highlight an important developmental role for WWP2 allied to cartilage and chondrocyte biology, two other recent reports suggest that WWP2 can also control the function of key oncogenic signalling pathways linked to cancer cell survival and tumour spread. PTEN is a lipid phosphatase that is frequently mutated in human cancer, and Maddika et al recently reported that PTEN can complex with WWP2 and undergo polubiquitinated mediated proteasomal turnover [8]. WWP2-mediated depletion of PTEN, which is also an important negative regulator of the PI3K-AKT pathway, consequently elevated AKT signalling activity and rendered prostate cancer cell lines resistant to stress-induced cell death. Following on from this, stable expression of WWP2 enhanced transformation of prostate cancer cells based soft-agar colony formation assays, an effect that was further supported using *in vivo* xenograft experiments.

The oncogenic potential of WWP2 is further supported by the study of Soond and Chantry [9]. Here, WWP2 was found to interact with Smad proteins that are responsible for canonical signalling activity through the transforming growth factor-β (TGFβ) signalling pathway. TGFβ, acting through Smad transcription factors, has a multifunctional role in cancer and in late-stage tumours it is responsible for driving the differentiation programme known as EMT that converts static epithelial cells into highly invasive mesenchymal cells, a necessary pre-requisite for tumour cell metastasis. Intriguingly, this study revealed for the first time two new isoforms generated from the WWP2 gene locus, an N-terminal WWP2-N isoform containing WW1 domain, and a C-terminal WWP2-C isoform harbouring WW4 domain and the HECT E3 ligase domain. Furthermore, these isoforms displayed differentially binding activity towards individual Smad proteins. The full-length WWP2 (WWP2-FL) bound to TGFβ receptor regulated R-Smads (Smads 2/3) and also to inhibitory I-Smad7, although it has a substrate preference for I-Smad7 which is polyubiquitinated and rapidly degraded. However, the truncated isoforms displayed differential binding activities, and WWP2-N bound onto Smads 2/3 selectively,

whereas WWP2-C interacted 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 causing degradation of unstimulated Smads 2/3. Consequently, it was suggested that WWP2-FL has a role to play in TGFβ-induced cancer cell metastasis based on its preferred substrate preference for inhibitory Smad7, and this was supported by cell based EMT experiments in which expression of an isolated Smad7-binding WW4 domain caused selective disruption of the Smad7:WWP2 complex, and stabilised Smad7 protein levels to thereby prevent TGFβ-induced EMT. Furthermore, it was suggested that one role of WWP2-N might in fact be to suppress TGFβ-induced EMT, by virtue of its unique ability to limit the levels of receptor regulated R-Smads 2/3. Significantly, this study also highlighted for the first time an interdependent role for distinct WWP2 isoforms, that could impact on both the *in vivo* and cell based studies of WWP2-FL function highlighted above.

Although from these recent studies it is clear that WWP2 is important for cartilage development and function, as well as being a potential proto-oncogene, it remains to be established how the individual WWP2 isoforms contribute to these normal biological and disease-specific activities. For example, the WWP2 mouse knockout was generated using a targeting constructs inserted into introns 3-4 of the WWP2 locus. Since the WWP2-C isoform is likely to be generated from an internal promoter within intron 10-11, then it is possible, although not confirmed, that WWP-C is still expressed in these knockout mice. Since the WWP2-C transcript has thus far only been detectable in cartilage-derived cell lines, then it may be that knockout of the WWP2-C isoform, perhaps also within the WWP2-FL$^{-/-}$ background, would lead to a more severe skeletal phenotype. In addition, factors that regulate WWP2-C promoter activity remain to be determined and could provide further insight into the chondrocytic-specific functional roles for WWP2-C isoform, particularly in the context of TGFβ/Smad signalling activity that is itself already known to be necessary for normal chondrocyte function [10], and is

also implicated in major cartilage diseases such as osteoarthritis [11]. Furthermore, the WWP2-N isoform and its role in cartilage biology and oncogenesis warrants further investigation, particularly with regard to Smad regulation, but also other emerging WWP2 substrates including Gsc, Sox9, and PTEN, as well as the stem cell factor Oct4 [12, 13, 14]. In addition, although WWP2-N and WWP2-FL expression is likely to be co-regulated since they share a common promoter, WWP2-N is generated from a splicing event involving as yet unknown RNA splicing factors that leads to retention of intron 9-10 and generates a neo-transcript harbouring a premature stop-codon. Moreover, the utility of WWP2 and their isoforms as prognostic biomarkers and novel drug targets in cancer and/or cartilage diseases needs to be addressed. Whilst WWP2 has now stood out from the E3 ligase crowd, the challenge ahead is to capitalize on this functional insight and to now develop highly selective WWP2 and WWP2 isoform inhibitory strategies, based either on small molecule approaches against the ligase activity of WWP2-FL and/or WWP2-C, or selective disruption of substrate-specific WWP2 WW domain interactions. The 3D structure of the related WWP1 E3 ubiquitin ligase has reported and, in the absence of a WWP2 structure, this could be used for structure-based design approaches perhaps combined with high-throughput screening approaches as highlighted for the Siah2 ubiquitin ligase in prostate cancer [15]. Certainly, it is encouraging that recent studies now strengthen the case for moving forward in a similar manner with WWP2, and it's isoforms, as promising disease targets in the future.

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References

1. Kerscher O, Felberbaum R, Hochstrasser M. Modification of proteins by ubiquitin and ubiquitin-like proteins. Ann Rev Cell Dev Biol 2006; 22:159-180.
2. Navon A, Ciechanover A. The 26S Proteasome: From Basic Mechanisms to Drug Targeting. J Biol Chem 2009; 284:33713-33718.
3. Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A, Chanda SK, Batalov S, Joazeiro CA. Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. PLoS ONE 2008; 3(1):e1487.
4. van Leeuwen IM, Laín S. Pharmacological manipulation of the cell cycle and metabolism to protect normal tissues against conventional anticancer drugs. Oncotarget 2011; 2(4):274-276.
5. Rotin D, Kumar S. Physiological functions of the HECT family of ubiquitin ligases. Nat Rev Mol Cell Biol 2009; 10:398-409.
6. Zou W, Chen X, Shim JH, Huang Z, Brady N, Hu D, Drapp R, Sigrist K, Glimcher LH, Jones D. The E3 ubiquitin ligase Wwp2 regulates craniofacial development through mono-ubiquitylation of Goosecoid. Nat Cell Biol 2011; 13:59-65.
7. Nakamura Y, Yamamoto K, He X, Otsuki B, Kim Y, Murao H, Soeda T, Tsumaki N, Deng JM, Zhang Z, Behringer RR, Crombrugghe B, Postlethwait JH, Warman ML, Nakamura T, Akiyama H. Wwp2 is essential for palatogenesis mediated by the interaction between Sox9 and mediator subunit 25. Nat Commun 2011; 2:251.

8. Maddika S, Kavela S, Rani N, Palicharla VR, Pokorny JL, Sarkaria JN, Chen J. WWP2 is an E3 ubiquitin ligase for PTEN. Nat Cell Biol 2011; May 1. [Epub ahead of print].

9. Soond, S., and Chantry, A. Selective targeting of activating and inhibitory Smads by distinct WWP2 ubiquitin ligase isoforms differentially modulates TGFβ signalling and EMT, Oncogene 2011; 30:2451-2462.

10. van der Kraan PM, Blaney Davidson EN, Blom A, van den Berg WB. TGFβ signaling in chondrocyte terminal differentiation and osteoarthritis: modulation and integration of signaling pathways through receptor-Smads. Osteoarthritis Cartilage 2009; 17:1539-1545.

11. Blaney Davidson EA, van der Kraan PM, van den Berg WB. TGFβ and osteoarthritis. Osteoarthritis and Cartilage 2007; 15:597-604.

12. Xu H, Wang W, Li C, Yu H, Yang A, Wang B, Jin Y. WWP2 promotes degradation of transcription factor OCT4 in human embryonic stem cells. Cell Res 2009; 19:561-73.

13. Liao B, Jin Y. Wwp2 mediates Oct4 ubiquitination and its own auto-ubiquitination in a dosage-dependent manner. Cell Res 2010; 20:332-344.

14. Stefanovic S, Pucéat M. Oct-3/4: not just a gatekeeper of pluripotency for embryonic stem cell, a cell fate instructor through a gene dosage effect. Cell Cycle 2007; 6(1):8-10.

15. Qi J, Pellecchia M, Ronai ZA. The Siah2-HIF-FoxA2 axis in prostate cancer – new markers and therapeutic opportunities. Oncotarget 2010; 1(5):379-385.