Implications of the USP10-HDAC6 axis in lung cancer - A path to precision medicine

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Abstract

Lung cancer is the leading cause of cancer death among both men and women in the United States. Because lung cancer is genetically heterogeneous, tailored therapy alone or in combination with chemotherapy would increase patient overall survival as compared with the one-size-fits-all chemotherapy. TP53-mutant lung cancer accounts for more than half of all lung cancer cases and is oftentimes more aggressive and resistant to chemotherapy. Directly targeting mutant p53 has not yet been successful, so identification of novel therapy targets and biomarkers in the TP53-mutant lung cancer is urgently needed to increase the overall survival in this subgroup. Deubiquitinating enzymes (DUBs) regulate a vast majority of proteins (DUBs’ substrates) via removal of ubiquitin moieties or ubiquitin chains from these proteins, thereby altering the stability and/or functions of these substrates. In this review, we will focus on a DUB, referred to as ubiquitin-specific peptidase 10 (USP10) whose substrates include both oncogenic proteins and tumor suppressors. Therefore, targeting USP10 in cancer is highly context-dependent. Here, we will discuss USP10’s functions in cancer by examining its various known substrates. In particular, we will elaborate our recent findings in the oncogenic role of USP10 in the TP53-mutant subgroup of lung cancer, focusing on USP10’s function in the DNA damage response (DDR) via histone deacetylase 6 (HDAC6). Overall, these findings support the notion that targeting USP10 in the TP53-mutant subgroup of NSCLC would sensitize patients to cisplatin-based chemotherapy. Generating potent and specific clinically relevant USP10 inhibitors would benefit the TP53-mutant subgroup of NSCLC patients.

Introduction

Post-translational modifications (PTMs) participate in virtually all biological processes; as such, the enzymes responsible for these PTMs are pivotal for normal cellular functions. One of these PTMs, ubiquitination, is regarded as a key regulator of a myriad of complex cellular processes; it may rival phosphorylation in scope and exceed it in complexity. As a reversible PTM, ubiquitination is governed by two groups of enzymes that either attach or remove the modification. The first group is responsible for adding the ubiquitin
moieties or polyubiquitin chains to the target proteins or substrates. In doing so, three
types of enzymes—E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes,
and E3 ubiquitin ligases—are required to coordinate the completion of this task [1]. The
second group, deubiquitinating enzymes (DUBs), catalyze the reverse reaction—removing
the ubiquitin moieties or polyubiquitin chains from the substrates [2]. One of the major
functions of E3 ubiquitin ligases and DUBs is governing protein homeostasis—the former
promotes protein degradation mainly via the ubiquitin-proteasome pathway, and the latter
stabilize proteins via removal of the polyubiquitin chains from the target proteins. The
human genome encodes ~600 E3 ubiquitin ligases and ~100 DUBs [3]. Mounting evidence
indicates that dysregulation or aberrant expression of E3 ubiquitin ligases or DUBs results
in the development of a plethora of diseases, including cancer [4]. Based on their catalytic
mechanism, DUBs fall into two groups [3]. The first group utilizes the cysteine amino
acid in the catalytic triad, hence why these DUBs are referred to as thiol proteases or
cysteine proteases. They can be further classified into five families: ubiquitin-specific
proteases (USPs), ubiquitin C-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs),
Machado-Joseph disease proteases (MJD), and the newly identified motif interacting with
Ub-containing novel DUB family proteases (MINDYs) [3]. The second group, whose
activation requires a zinc ion, only contains one family: JAB1/MPN/Mov34 metalloenzymes
(JAMMs) [2].

USP10 belongs to the USP family, which consists of 54 members and is the largest family
in the DUB superfamily [5]. USP10’s role in cancer has been intensively studied in the
past decade. Numerous tumor suppressors and oncogenic proteins have surfaced as USP10’s
substrates [6–17], and the list of these substrates is still rapidly growing (Table 1). Of note,
the most famous tumor suppressor, p53 protein, was identified as USP10’s substrate in 2010
[6]. Therefore, it is conceivable to imagine that like p53 USP10 is a tumor suppressor as
well, since USP10 stabilizes p53 to enhance p53’s tumor suppressor function. However, as
a DUB, USP10 may also regulate the stability of mutant p53 (mutp53) and USP10 could
serve an oncogenic role in TP53-mutant cancers. In an indirect scenario, USP10 could
either exacerbate or dampen the oncogenic function of mutant p53 through its substrates.
In support of the second scenario, we recently discovered that the depletion or inhibition
of USP10 in TP53-null or TP53-mutant non-small cell lung cancer (NSCLC) reduces
tumor burden in a xenograft mouse model and sensitizes tumors to cisplatin, highlighting
USP10’s chemo-resistant role in this subset of NSCLC. Mechanistically, we have shown that
USP10 regulates the DNA damage response (DDR) via histone deacetylase 6 (HDAC6) to
confer cisplatin resistance, as HDAC6 regulates multiple DDR proteins. In this review, we
will further discuss the above findings and ask the following open question: Why USP10
functions differently in TP53-wild-type and TP53-mutant subgroups of lung cancer?

**USP10’s Function Differs in Wild-Type TP53 and Mutant TP53 NSCLC Cell Lines**

In 2014, we reported that HDAC6 serves as a ubiquitin E3 ligase to promote degradation
of a key DNA mismatch repair protein MSH2 [18]. Logically, we were curious about the
enzyme, which counteracts HDAC6 to stabilize MSH2 and employed a protein purification
approach to examine the proteins associated with MSH2. We immuno-precipitated MSH2
and identified MSH2-associated proteins by mass spectrometry analysis (LC-MS/MS). As
expected, we found that most of the peptides detected in the MSH2 complex belong to MSH6—a well-known binding partner of MSH2—which forms a heterodimer with MSH2 referred to as MutSa [19] Interestingly, we indeed found that abundant peptides in the complex belong to a DUB, USP10. We then set out to investigate whether USP10 stabilizes MSH2, leading to an increased level of the MSH2-MSH6 complex (MutSa), which could sense various DNA adducts and trigger the downstream signaling to promote apoptosis or cell cycle arrest. We published our findings in 2016 showing that the depletion of USP10 confers cellular resistance to both drugs, 6-TG (6-thioguanine) and MNNG (N-methyl-N’nitro-N-nitrosoguanidine), in NSCLC cells due to the decreased level of MSH2 [20]. As we further tested the role of USP10 in cisplatin sensitivity in a large panel of NSCLC cell lines, surprisingly, we discovered that upon cisplatin treatment the depletion or inhibition of USP10 could lead to two entirely different outcomes: cell survival or apoptosis, depending on the cellular status of TP53—USP10 promotes cisplatin sensitivity in wild-type p53 cells, while USP10 confers cisplatin resistance in mutant p53 cells. This unexpected finding prompted us to study the underlying mechanism by which USP10 confers cisplatin resistance in mutant p53 cells.

The Role of HDAC6 in Cancer and the DNA Damage Response

HDAC6 belongs to the class IIb HDAC family and is a well-known oncogenic protein [21,22]. HDAC6 is overexpressed in numerous cancers, including gastric cancer [23], glioblastoma [24], and melanoma [25]. Our current publication has shown that HDAC6 protein is overexpressed in all three histological subtypes of NSCLC patient samples—adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [26]-suggesting the oncogenic role of HDAC6 in NSCLC. Recently, the role of HDAC6 in chemotherapy efficacy in several cancers, including lung cancer, has been explored by our group and others [27,28]. For example, HDAC6 confers resistance to temozolomide in glioblastoma [24], and the HDAC6-selective inhibitor ACY-1215 accelerates vemurafenib-induced cell death of BRAF-mutant melanoma cells [25]. Our group is among the first to show that HDAC6 is associated with cisplatin resistance in NSCLC cells [28]. However, the mechanism by which HDAC6 confers chemo-resistance is not fully revealed.

HDAC6 has been well-established as a master regulator of cellular stress responses, including ubiquitinated/misfolded protein stress [29], ER stress [25], and genotoxic stress [27,28]. Our group is a pioneer in defining the role of HDAC6 in the DDR. Structurally, HDAC6 is unique among the members of the HDAC family in that it contains two tandem repeats of deacetylase domains (referred to as DAC1 and DAC2), an SE14 motif [30], and a ZnF-UBP domain [31] that binds to mono-, poly-ubiquitin chains and ubiquitinated proteins. We are the first to reveal that DAC1 harbors ubiquitin E3 ligase activity toward a key DNA mismatch repair protein, MSH2, thereby regulating the homeostasis of MutSa and the DDR [18]. Our follow-up study has revealed that HDAC6 could also deacetylate MLH1 and decrease the formation of the MutSa·MutLa complex, leading to 6-TG resistance [32]. Most recently, we reported that HDAC6 ubiquitinates a crucial cell cycle checkpoint kinase 1 (Chk1) and confers radioresistance in NSCLC [33]. Because the DNA mismatch repair (MMR) complex (MutSa·MutLa) interacts with the protein components in the ATR-Chk1 pathway, we hypothesize that HDAC6 governs DDR via the MMR complex as well as
ATR-Chk1 signaling. As aforementioned both HDAC6 and USP10 regulate MSH2, we then tested whether USP10 and HDAC6 interact. Our investigations then revealed that HDAC6 interacts with USP10, and it is a substrate of USP10. Therefore, we suspect that USP10 regulates DDR via HDAC6.

The USP10-HDAC6 Axis in a mutp53 Background

In general, mutp53 proteins promote cancer cell proliferation by acting as homeostatic factors that sense and protect cancer cells from transformation-related stress stimuli, including DNA lesions, oxidative and proteotoxic stress, metabolic imbalance, interaction with the tumor microenvironment, and the immune system [34]. Molecules, which dampen mutp53’s oncogenic function, could be exploited as targets for cancer treatment. Previous investigation has shown that HDAC6 deacetylates HSP90, a heat shock protein, which stabilizes mutp53 [35]. Therefore, USP10 could contribute to mutp53 stabilization via stabilizing HDAC6. However, it is unclear whether USP10 could also directly stabilize mutp53. Thus, we suspect that USP10 could reduce the DDR and stabilize mutp53 to promote tumor progression, and confers cisplatin resistance via HDAC6 in the mutp53 subset of NSCLC (Figure 1).

Perspectives

As both USP10 and HDAC6 are enzymes and each has numerous substrates, using proteomics approaches to identify their substrates would provide a big picture of their roles in NSCLC. In addition, since both could play roles in the nucleus, genomics studies on USP10 and HDAC6 would provide evidence on how they affect gene transcription. Overall, we will gain a better understanding of the USP10-HDAC6 axis by an integrated genomics and proteomics approach. Translationally, developing potent and specific USP10 inhibitors with medicinal chemists would further establish USP10 as an important target in the mutp53 subset of lung cancer. Finally, as the current landscape for the treatment of advanced lung cancer has shifted towards anti-PD-1/PD-L1-based immunotherapy [36], further investigations to connect the USP10-HDAC6 axis to immunotherapy are certainly warranted.

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References

1. Pickart CM. Mechanisms underlying ubiquitination. Annual Review of Biochemistry. 2001;70.
2. Reyes-Turcu FE, Ventii KH, Wilkinson KD. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. Annual Review of Biochemistry. 2009 7 7;78:363–97.
3. Mevissen TE, Komander D. Mechanisms of deubiquitinase specificity and regulation. Annual Review of biochemistry. 2017 6 20;86:159–92.
4. Wilkinson KD. DUBs at a glance. Journal of Cell Science. 2009 7 15;122(14):2325–9. [PubMed: 19571111]
5. Ventii KH, Wilkinson KD. Protein partners of deubiquitinating enzymes. Biochemical Journal. 2008 9 1;414(2):161–75.
6. Yuan J, Luo K, Zhang L, Cheville JC, Lou Z. USP10 regulates p53 localization and stability by deubiquitinating p53. Cell. 2010 2;5(10):384–96. [PubMed: 20096447]
7. Sun J, Li T, Zhao Y, Huang L, Sun H, Wu H, et al. USP10 inhibits lung cancer cell growth and invasion by upregulating PTEN. Molecular and Cellular Biochemistry. 2018 4;144(1–2):1–7. [PubMed: 28852924]
8. Ko A, Han SY, Choi CH, Cho H, Lee MS, Kim SY, et al. Oncogene-induced senescence mediated by c-Myc requires USP10 dependent deubiquitination and stabilization of p14ARF. Cell Death & Differentiation. 2018 6;25(6):1050–62. [PubMed: 29472714]
9. Wang X, Xia S, Li H, Wang X, Li C, Chao Y, et al. The deubiquitinase USP10 regulates KLF4 stability and suppresses lung tumorigenesis. Cell Death & Differentiation. 2020 6;27(6):1747–64. [PubMed: 31748695]
10. Lin Z, Yang H, Tan C, Li J, Liu Z, Quan Q, et al. USP10 antagonizes c-Myc transcriptional activation through SIRT6 stabilization to suppress tumour formation. Cell Reports. 2013 12;6(5):1639–49. [PubMed: 24332849]
11. Ouyang SW, Liu TT, Liu XS, Zhu FX, Zhu FM, Liu XN, et al. USP10 regulates Musashi-2 stability via deubiquitination and promotes tumour proliferation in colon cancer. FEBS Letters. 2019 2;593(4):406–13. [PubMed: 30604502]
12. Chen Q, Hang Y, Zhang T, Tan L, Li S, Jin Y. USP10 promotes proliferation and migration and inhibits apoptosis of endometrial stromal cells in endometriosis through activating the Raf-1/MEK/ERK pathway. American Journal of Physiology-Cell Physiology. 2018 12;315(6):C863–72. [PubMed: 30281322]
13. Weisberg EL, Schauer NJ, Yang J, Lamberto I, Doherty L, Bhatt S, et al. Inhibition of USP10 induces degradation of oncogenic FLT3. Nature Chemical Biology. 2017 12;13(12):1207–15. [PubMed: 28967922]
14. Takayama KI, Suzuki T, Fujimura T, Takahashi S, Inoue S. Association of USP10 with G3BP2 inhibits p53 signaling and contributes to poor outcome in prostate cancer. Molecular Cancer Research. 2018 5;16(5):846–56. [PubMed: 29378906]
15. Ouchida AT, Kacal M, Zheng A, Ambroise G, Zhang B, Norberg E, et al. USP10 regulates the stability of the EMT-transcription factor Slug/SNAI2. Biochemical and Biophysical Research Communications. 2018 8;502(4):429–34. [PubMed: 29803676]
16. Yang J, Meng C, Weisberg E, Case A, Lamberto I, Magin RS, et al. Inhibition of the deubiquitinase USP10 induces degradation of SYK. British Journal of Cancer. 2020 4;122(8):1175–84. [PubMed: 32015510]
17. Zhu H, Yan F, Yuan T, Qian M, Zhou T, Dai X, et al. USP10 Promotes Proliferation of Hepatocellular Carcinoma by Deubiquitinating and Stabilizing YAP/TAZ. Cancer Research. 2020 6;80(11):2204–16. [PubMed: 32217697]
18. Zhang M, Xiang S, Joo HY, Wang L, Williams KA, Liu W, et al. HDAC6 deacetylates and ubiquitinates MSH2 to maintain proper levels of MutSβ. Molecular Cancer Cell. 2014 7;35(5):31–46. [PubMed: 24882211]
19. Drummond JT, Li GM, Longley MJ, Modrich P. Isolation of an hMSh2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science. 1995 6;30(5219):1909–12. [PubMed: 7604264]
20. Zhang M, Hu C, Tong D, Xiang S, Williams K, Bai W, et al. Ubiquitin-specific peptidase 10 (USP10) deubiquitimates and stabilizes MutS homolog 2 (MSH2) to regulate cellular sensitivity to DNA damage. Journal of Biological Chemistry. 2016 5;291(20):10783–91.
21. Grozinger CM, Hassig CA, Schreiber SL. Three proteins define a class of human histone deacetylases related to yeast Hda1p. Proceedings of the National Academy of Sciences. 1999 4;27(9):4868–73.
22. Aldana-Masangkay GI, Sakamoto KM. The role of HDAC6 in cancer. Journal of Biomedicine and Biotechnology. 2010 11;7:2011.
23. Park SJ, Kim JK, Bae HJ, Eun JW, Shen Q, Kim HS, et al. HDAC6 sustains growth stimulation by prolonging the activation of EGF receptor through the inhibition of rapabptin-5-mediated early endosome fusion in gastric cancer. Cancer Letters. 2014 11;354(1):97–106. [PubMed: 25111897]
24. Wang Z, Hu P, Tang F, Lian H, Chen X, Zhang Y, et al. HDAC6 promotes cell proliferation and confers resistance to temozolomide in glioblastoma. Cancer Letters. 2016 8 28;379(1):134–42. [PubMed: 27267806]

25. Peng U, Wang Z, Pei S, Ou Y, Hu P, Liu W, et al. ACY-1215 accelerates vemurafenib induced cell death of BRAF-mutant melanoma cells via induction of ER stress and inhibition of ERK activation. Oncology Reports. 2017 2 1;37(2):1270–6. [PubMed: 28035401]

26. Hu C, Zhang M, Moses N, Hu CL, Polin L, Chen W, et al. The USP10-HDAC6 axis confers cisplatin resistance in non-small cell lung cancer lacking wild-type p53. Cell Death & Disease. 2020 5 7;11(5):1–8. [PubMed: 31911576]

27. Namdar M, Perez G, Ngo L, Marks PA. Selective inhibition of histone deacetylase 6 (HDAC6) induces DNA damage and sensitzes transformed cells to anticancer agents. Proceedings of the National Academy of Sciences. 2010 11 16;107(46):20003–8.

28. Wang L, Xiang S, Williams KA, Dong H, Bai W, Nicosia SV, et al. Depletion of HDAC6 enhances cisplatin-induced DNA damage and apoptosis in non-small cell lung cancer cells. PloS One. 2012 9 5;7(9):e44265.

29. Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell. 2003 12 12;115(6):727–38. [PubMed: 14675537]

30. Bertos NR, Gilquin B, Chan GK, Yen TJ, Khochbin S, Yang XJ. Role of the tetradecapeptide repeat domain of human histone deacetylase 6 in cytoplasmic retention. Journal of Biological Chemistry. 2004 11 12;279(46):48246–54.

31. Hook SS, Orian A, Cowley SM, Eisenman RN. Histone deacetylase 6 binds polyubiquitin through its zinc finger (PAZ domain) and copurifies with deubiquitinating enzymes. Proceedings of the National Academy of Sciences. 2002 10 15;99(21):13425–30.

32. Zhang M, Hu C, Moses N, Haakenson J, Xiang S, Quan D, et al. HDAC6 regulates DNA damage response via deacetylating MLH1. Journal of Biological Chemistry. 2019 4 12;294(15):5813–26.

33. Moses N, Zhang M, Wu JY, Hu C, Xiang S, Malysa A, et al. HDAC6 Regulates Radiosensitivity of Non-Small Cell Lung Cancer by Promoting Degradation of Chk1. bioRxiv. 2020 1 1.

34. Mantovani F, Collavin L, Del Sal G. Mutant p53 as a guardian of the cancer cell. Cell Death & Differentiation. 2019 2;26(2):199–212. [PubMed: 30538286]

35. Li D, Marchenko ND, Moll UM. SAHA shows preferential cytotoxicity in mutant p53 cancer cells by destabilizing mutant p53 through inhibition of the HDAC6-Hsp90 chaperone axis. Cell Death & Differentiation. 2011 12;18(12):1904–13. [PubMed: 21637290]

36. Hendriks LE, Besse B. CLINICAL ONCOLOGY Windows open for cancer immunotherapy. Nature. 2018:558(7710):376–7. [PubMed: 29907821]
**Figure 1:**
USP10 functions distinctly in wild-type p53 and mutant p53 lung cancer cells upon DNA damage. **A)** In wild-type p53 cells, USP10 stabilizes and translocates p53 in the nucleus and sensitizes cells to DNA damage, such as ionizing radiation, **B)** whereas in the mutant p53 cells, USP10 stabilizes HDAC6. There are two ways HDAC6 could then contribute to chemo- and radio- resistance: HDAC6 exerts its deacetylase activity to deacetylate HSP90 and promote binding to mutant p53, leading to hyperstability of mutant p53. Or HDAC6 exerts its ubiquitin E3 ligase activity and deacetylase activity to promote degradation of the
DNA mismatch repair complex (MutS\textalpha-MutL\textalpha) and cell cycle checkpoint kinase 1 (Chk1), contributing to chemo- and radio- resistance. Our model suggests that developing USP10 inhibitors will benefit lung cancer patients harboring mutant p53.
Table 1:

USP10 substrates.

| Tumor suppressors       | Cancer types            | References          |
|-------------------------|-------------------------|---------------------|
| p53                     | Renal cell carcinoma    | Yuan et al., 2010 [6] |
| PTEN                    | Lung cancer             | Sun et al., 2018 [7]  |
| P14ARF                  | Non-small cell lung cancer | Ko et al., 2018 [8] |
| KLF4                    | Lung cancer             | Wang et al., 2020 [9] |
| SirT6                   | Colon cancer            | Lin et al., 2013 [10] |

| Oncogenic proteins      |                        |                     |
|-------------------------|-------------------------|---------------------|
| Musashi 2               | Colon cancer            | Ouyang et al., 2019 [11] |
| Raf-MEK-ERK             | Endometrial cancer      | Chen et al., 2018 [12] |
| FLT3-ITD                | Acute myeloid leukaemia | Weisberg et al., 2017 [13] |
| Spleen tyrosine kinase (SYK) | Acute myeloid leukaemia | Yang et al., 2020 [16] |
| G3BP2                   | Prostate cancer         | Takayama et al., 2018 [14] |
| Slug/Snai2              | Epithelial-mesenchymal transition | Ouchida et al., 2018 [15] |
| YAP/TAZ                 | Liver cancer            | Zhu et al., 2020 [17] |
| HDAC6                   | Non-small cell lung cancer | Hu et al., 2020 [26] |