HAUSP as Epigenetic Regulator for Chromatin Effector Proteins

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HAUSP (herpes virus-associated ubiquitin-specific protease), also known as Ubiquitin Specific Protease 7, plays critical roles in cellular processes, such as chromatin biology and epigenetics, through the regulation of different signaling pathways. HAUSP is a main partner of the “Epigenetic Code Replication Machinery,” ECREM, a large protein complex that includes several epigenetic players, such as the ubiquitin-like containing plant homeodomain (PHD) and an interesting new gene (RING), finger domains 1 (UHRF1), as well as DNA methyltransferase 1 (DNMT1), histone deacetylase 1 (HDAC1), histone methyltransferase G9a, and histone acetyltransferase TIP60. Due to its deubiquitinase activity and its ability to team up through direct interactions with several epigenetic regulators, mainly UHRF1, DNMT1, TIP60, the histone lysine methyltransferase EZH2, and the lysine-specific histone demethylase LSD1, HAUSP positions itself at the top of the regulatory hierarchies involved in epigenetic silencing of tumor suppressor genes in cancer.

HAUSP UHRF1 epigenetic cancer

1. Introduction

Ubiquitination, the addition of a small protein called ubiquitin (76 amino acids) to other target proteins is a post-translational protein modification that controls almost all processes in the cell and leads to different outcomes, ranging from proteasomal protein degradation and cellular trafficking to cell proliferation, apoptosis, autophagy, DNA repair, and epigenetic modulation of gene expression [1][2][3][4]. Ubiquitination results from a successful collaboration between multienzyme cascades that involve E1 activating enzymes, E2 conjugating enzymes, and E3 ubiquitin ligases [1][5][6][7].

Deubiquitinases, or deubiquitinating enzymes (DUBs), are enzymes that protect many proteins from ubiquitination and are active in various pathologies, including cancer [8][9][10][11]. One of the well-documented cancer-associated DUBs is HAUSP (herpes virus-associated ubiquitin-specific protease), also known as Ubiquitin Specific Protease 7 (USP7), an enzyme that is overexpressed in many solid and blood malignancies [12][13][14][15][16]. The structure of HAUSP includes seven domains: the N-terminal TRAF-like (Tumor necrosis factor Receptor–Associated Factor) domain, the intermediate catalytic core domain, and UBL1, 2, 3, 4, and 5 (C-terminal ubiquitin-like domains) [17][18] (**Figure 1**). Through its deubiquitinase activity, HAUSP has been reported to control the activity of several oncogenic transcription factors, including NOTCH1 in T-cell acute lymphoblastic leukemia [19][20], N-Myc in neuroblastoma [16], β-catenin in colorectal cancer [21], and NEK2 in multiple myeloma [22], indicating that HAUSP has an oncogenic role in cancer. In several tumors, HAUSP was shown to bind to and deubiquitinate the E3

ubiquitin ligases, MDM2 [23][24][25] and MDMX [26], which are known negative regulators of the tumor suppressor gene *p53* [27][28][29][30]. Blocking the deubiquitination function of HAUSP enabled the ubiquitination and proteasomal degradation of both MDM2 and MDMX, which subsequently stabilized and restored p53 protein levels to induce cell death [23][24][26].

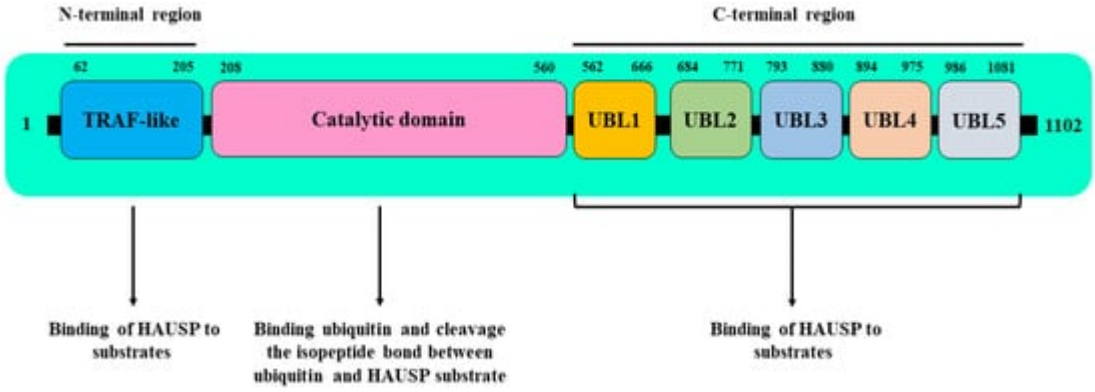

Figure 1. Schematic representation of the domain structure of HAUSP (herpes virus-associated ubiquitin-specific protease). The structure of HAUSP includes seven domains, N-terminal TRAF-like (Tumor necrosis factor Receptor–Associated Factor) domain, intermediate catalytic core and five consecutive C-terminal ubiquitin-like domains (Ubiquitin-like domains), UBL1-5. The N-terminal TRAF-like domain and the five UBL domains are sites for the binding of HAUSP to many proteins. Through the catalytic core domain, HAUSP binds ubiquitin and cleave the is peptide bond between ubiquitin and HAUSP substrate [17][18][31].

HAUSP, via its different domains, interacts with several proteins implicated in coordinating various signaling pathways. HAUSP is found in many protein complexes with different functions, including the “Epigenetic Code Replication Machinery,” ECREM. Indeed, HAUSP is a main partner of ECREM, a large macromolecular complex that includes several epigenetic players, such as the ubiquitin-like containing plant homeodomain (PHD) and an interesting new gene (RING) finger domains 1 (UHRF1), as well as DNA methyltransferase 1 (DNMT1), histone deacetylase 1 (HDAC1), histone methyltransferase G9a, and histone acetyltransferase TIP60 [32][33][34][35][36][37][38]. Growing evidence indicates that the silencing of tumor suppressor genes (TSGs) in tumors is the result of a coordinated in-depth dialogue between DNA methylation and various histone post-translational modifications (PTMs) [39][40][41][42].

Several reports have shown that a faithful inheritance of the epigenetic patterns (DNA methylation and histone PTMs) during cell division involves temporal and spatial control of the chromatin effector proteins mainly UHRF1 and DNMT1, which govern various epigenetic events [34][39][43][44][45][46][47]. Understanding the major factors regulating the expression and activity of chromatin effector proteins UHRF1 and DNMT1 could therefore unlock new secrets regarding the transmission of epigenetic patterns during cell division. HAUSP positions itself at the top of the regulatory hierarchies involved in the epigenetic silencing of TSGs in cancer due to its deubiquitinase activity and ability to team up through direct interactions with several epigenetic players (**Table 1**) mainly the epigenetic reader UHRF1 [38][48]; DNMT1 [38][49]; the histone acetyltransferases TIP60 [50] and CBP (CREB binding protein) [51]; the histone lysine methyltransferases EZH2 (Enhancer of Zeste 2) [52] and MLL5 (Mixed-lineage leukemia 5)

[53], and the lysine specific histone demethylases LSD1 (Lysine-specific demethylase 1) [54][55] and PHF8 (PHD finger protein 8) [56] (**Figure 2**).

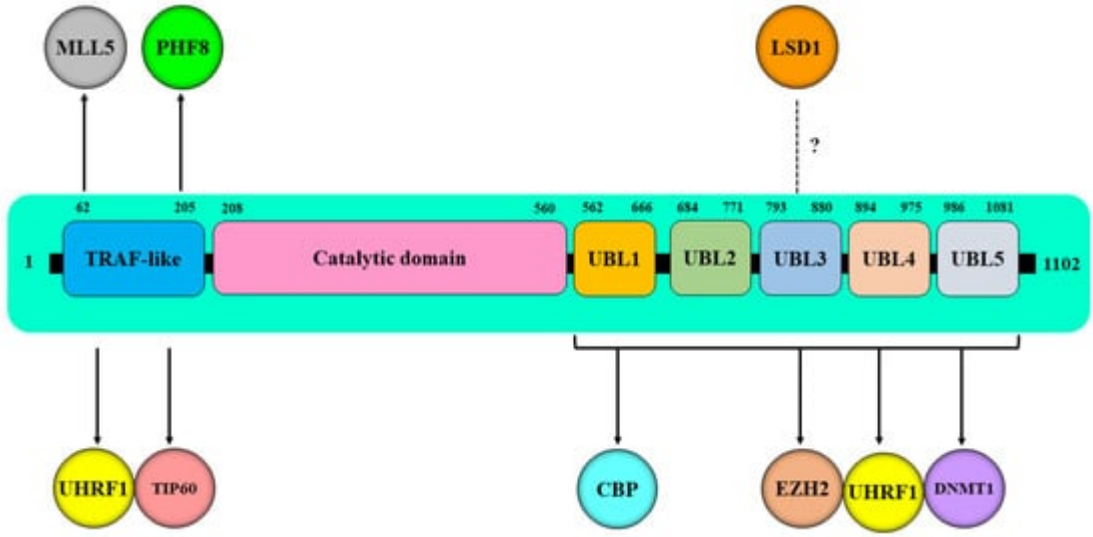

Figure 2. Schematic representation of interactions of HAUSP domains with various epigenetic players. HAUSP via its TRAF-like domain interacts with the ubiquitin-like containing plant homeodomain (PHD) and an interesting new gene (RING) finger domains 1 (UHRF1) [38], histone acetyltransferase TIP60 [50], the histone lysine methyltransferase MLL5 (Mixed-lineage leukemia 5) [53] and the lysine specific histone demethylase PHF8 (PHD finger protein 8) [56]. HAUSP through the C-terminal region which covers its five UBL domains can interact also with UHRF1 [48], histone acetyltransferase CBP (CREB binding protein) [51], histone methyltransferase EZH2 [52] and DNA methyltransferase 1 (DNMT1) [38]. HAUSP can also bind to LSD1 but the interaction site is not yet known [54].

Table 1. Role of HAUSP in the regulation of epigenetic players and related events.

Epi-Partner	Role of Epi-Partner	HAUSP Interaction Site	Epi-Partner Interaction Site	Related Epigenetic Events	Refs.
UHRF1	Reader of both epigenetic marks (DNA methylation and histone code)	UBL1 domain (residues 560–664)	A linker region encompassing amino acids 600–687 between the SRA and RING finger domains of UHRF1	Promoting the stability of UHRF1 through HAUSP-dependent deubiquitination	[48]
		TRAF-like domain	SRA domain	Promoting the stability of UHRF1 through HAUSP-dependent deubiquitination	[38]
		(UBL1-2) domains	A polybasic region (PBR) in the C terminus	Promoting the association of the TTD-PHD domains of UHRF1 with chromatin	[57]

Epi-Partner	Role of Epi-Partner	HAUSP Interaction Site	Epi-Partner Interaction Site	Related Epigenetic Events	Refs.
				and, hence, efficient H3K9me3 binding	
DNMT1	DNA methyltransferase 1	C-terminal domain	Targeting sequence (TS) domain	Promoting the stability of DNMT1 through HAUSP-dependent deubiquitination	[38]
		UBL domains (residues 560–1102)	KG linker (residues 1109–1119)	Promoting the stability of DNMT1 through acetylation of KG linker of DNMT1	[49]
TIP60	Histone acetyltransferase	TRAF-like domain		Increased levels of H2AK 5 and H4K5	[50]
CBP	Histone acetyltransferase	Region encompassing amino acids 600–687	CH3 domain	Increased levels of H3K56Ac	[51]
MLL5	Histone lysine methyltransferase	TRAF domain	Multiple domains	Increased levels of H3K4m3	[53]
EZH2	Histone lysine N-methyltransferase	C-terminal region	^{489}PRKKKRK^{495} region	Increased levels of H3K27m3	[52]
					[54]
LSD1	Lysine specific demethylase 1		A region encompassing amino acids 600–687	Demethylation of H3K4me2 and H3K9me2	[55]
PHF8	Histone lysine demethylase	TRAF-like domain	The C-terminal region	Demethylation of H3K9me1,2, H3K27me2, and H4K20me1	[56]

1. Rawat, R.; Starczynowski, D.T.; Ntziachristos, P. Nuclear deubiquitination in the spotlight: The multifaceted nature of USP7 biology in disease. Curr. Opin. Cell Biol. 2019, 58, 85–94.
2. Uckelmann, M.; Sixma, T.K. Histone ubiquitination in the DNA damage response. DNA Repair 2017, 56, 92–101.
3. Liu, J.; Shaik, S.; Dai, X.; Wu, Q.; Zhou, X.; Wang, Z.; Wei, W. Targeting the ubiquitin pathway for cancer treatment. Biochim. Biophys. Acta 2015, 1855, 50–60.
4. Yerlikaya, A.; Kanbur, E.; Stanley, B.A.; Tümer, E. The Ubiquitin-Proteasome Pathway and Epigenetic Modifications in Cancer. Anti-Cancer Agents Med. Chem. 2021, 21, 20–32.
5. Komander, D.; Rape, M. The ubiquitin code. Annu. Rev. Biochem. 2012, 81, 203–229.

2. Role of HAUSP in the Maintenance of DNA Methylation

2.1. HAUSP-Dependent Regulation of DNMT1 by Ubiquitination

The deubiquitinase activity of HAUSP regulates the stability and enzymatic activity of DNMT1 through several mechanisms. HAUSP can bind to DNMT1 and stabilize it through HAUSP-mediated deubiquitination [58]. High expression levels of DNMT1 protein have been found in human colon cancers, and this overexpression was correlated with HAUSP protein expression. Interestingly, the depletion of HAUSP in both human embryonic kidney

cells and mediated cancer cells resulted in ubiquitin-dependent DNMT1 ubiquitination and reduction in DNMT1 protein expression. A similar increase in DNMT1 ubiquitination levels and a decrease in its protein expression were reported when HAUSP was knocked out in colorectal cancer cells [58]. By contrast, HAUSP overexpression in HAUSP knockout cells led to deubiquitination of DNMT1 and restoration of DNMT1 protein expression, suggesting that the deubiquitinase activity of HAUSP protects DNMT1 from proteasomal degradation, thereby promoting the stability and enzymatic activity of DNMT1 and the maintenance of DNA methylation [58].

One of the well-documented epigenetic regulators of the DNA methylation maintenance machinery is the epigenetic reader UHRF1, which has multiple functional domains [59][60][61]. Through its SRA domain, UHRF1 recognizes and binds hemi-methylated CpG islands, and via the same SRA domain, UHRF1 recruits DNMT1 to its correct position on chromatin to ensure faithful transmission of DNA methylation patterns during DNA replication [62][63][64].

HAUSP binds to UHRF1 by its TRAF domain, but it also binds via its UBL domain (residues 560–664) to a linker region encompassing amino acids 600–687 between the SRA and RING finger domains of UHRF1, and this direct interaction is required for UHRF1 stability [48]. The downregulation of HAUSP decreased the expression levels of UHRF1 protein, whereas the overexpression of wild-type HAUSP, but not the catalytically inactive mutant HAUSP (C223S), significantly decreased the ubiquitination of UHRF1 and promoted its stability [48].

Obtaining a deep insight into how HAUSP deubiquitinase activity is involved in the stabilization of UHRF1 protein will help in understanding the regulatory role of HAUSP in the UHRF1-dependent maintenance of DNA methylation. In this regard, HAUSP was shown to regulate the stability of UHRF1 protein by targeting the ubiquitin ligase activity of the UHRF1 RING domain [38][59][65], which is used by UHRF1 to ubiquitinate itself (autoubiquitination) [59][60] or to ubiquitinate other substrates, mainly histone 3 [65][66][67]. Indeed, HAUSP, via its deubiquitinase activity, was shown to interfere with the E3 ubiquitin ligase activity of the RING domain of UHRF1, thereby eliminating the autoubiquitination of UHRF1 via the removal of ubiquitin adducts [38]. This led to the stabilization of UHRF1, indicating that HAUSP regulates the maintenance of DNA methylation through a direct association with UHRF1 [38].

2.2. HAUSP-Dependent Regulation of DNMT1 by Acetylation

HAUSP can also regulate DNMT1 stability through an acetylation process [36][38][49][58][68]. HAUSP, through its UBL domains (residues 560–1102), was found to stabilize DNMT1 by binding to the KG linker (KG^{DNMT1}) of DNMT1 (residues 1109–1119) promoting the stability of DNMT1 [49]. The depletion of HAUSP in several cancer cell lines resulted in a significant decrease in DNMT1 expression at the protein level, but not at the mRNA level. Similar findings were observed when HAUSP was knocked out in colon cancer cells, supporting the concept that HAUSP deubiquitinates and protects DNMT1 from proteasomal degradation, thereby promoting DNMT1 stability [49].

3. Role of HAUSP in the Regulation of Histone Post-Translational Modifications

6. Schulman, B.A.; Harper, J.W. Ubiquitin-like protein activation by E1 enzymes: The apex for downstream signalling pathways. Nat. Rev. Mol. Cell Biol. 2009, 10, 319–319.
7. Ye, Y.; Rape, M. Building ubiquitin chains: E2 enzymes at work. Nat. Rev. Mol. Cell Biol. 2009, 10, 755–764.
8. Mennerich, D.; Kubaichuk, K.; Kietzmann, T. DUBs, Hypoxia, and Cancer. Trends Cancer 2019, 5, 632–653.
9. Poondla, N.; Chandrasekaran, A.P.; Kim, K.S.; Ramakrishna, S. Deubiquitinating enzymes as cancer biomarkers: New therapeutic opportunities? BMB Rep. 2019, 52, 181–189.
10. Farshi, P.; Deshmukh, R.R.; Nwankwo, J.O.; Arkwright, R.T.; Cvek, B.; Liu, J.; Dou, Q.P. Deubiquitinases (DUBs) and DUB inhibitors: A patent review. Expert Opin. Ther. Pat. 2015, 25, 1191–1208.
11. McClurg, U.L.; Robson, C.N. Deubiquitinating enzymes as oncotargets. Oncotarget 2015, 6, 9657–9668.
12. Song, M.S.; Salmena, L.; Carracedo, A.; Egia, A.; Lo-Coco, F.; Teruya-Feldstein, J.; Pandolfi, P.P. The deubiquitinylation and localization of PTEN are regulated by a HAUSP-PML network. Nature 2008, 455, 813–817.
13. Zhou, J.; Wang, J.; Chen, C.; Yuan, H.; Wen, X.; Sun, H. USP7, Target Validation and Drug Discovery for Cancer Therapy. Med. Chem. 2018, 14, 3–18.
14. Bhattacharya, S.; Chakraborty, D.; Basu, M.; Ghosh, M.K. Emerging insights into HAUSP (USP7) in physiology, cancer and other diseases. Signal Transduct. Target. Ther. 2018, 3, 1–12.
15. Cheng, C.D.; Dong, Y.F.; Niu, W.X.; Niu, C.S. HAUSP promoted the growth of glioma cells in vitro and in vivo via stabilizing NANOG. Pathol. Res. Pract. 2020, 216, 152883.
16. Tavana, O.; Li, D.; Dai, C.; Lopez, G.; Banerjee, D.; Kon, N.; Chen, C.; Califano, A.; Yamashiro, D.J.; Sun, H.; et al. HAUSP deubiquitinates and stabilizes N-Myc in neuroblastoma. Nat. Med. 2016, 22, 1180–1186.
17. Pozhidaeva, A.; Bezsonova, I. USP7, Structure, substrate specificity, and inhibition. DNA Repair 2019, 76, 30–39.
18. Holowaty, M.N.; Sheng, Y.; Nguyen, T.; Arrowsmith, C.; Frappier, L. Protein interaction domains of the ubiquitin-specific protease, USP7/HAUSP. J. Biol. Chem. 2003, 278, 47753–47761.
19. Jin, Q.; Martinez, C.A.; Arcipowski, K.M.; Zhu, Y.; Gutierrez-Diaz, B.T.; Wang, K.K.; Johnson, M.R.; Volk, A.G.; Wang, F.; Wu, J.; et al. USP7 Cooperates with NOTCH1 to Drive the Oncogenic Transcriptional Program in T-Cell Leukemia. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 2019, 25, 222–239.

3.1. Role of HAUSP in the Regulation of Histone Monoubiquitination

Monoubiquitination of histones, particularly H2A and H2B, is a common type of histone PTM that controls gene expression [69][70][71]. Monoubiquitination of histone H2A on lysine 119 (H2AK119ub1) is a well-documented epigenetic modification linked to the Polycomb Repressor Complex 1 (PRC1) and is frequently associated with gene silencing [71][72][73][74]. This histone mark (H2AK119ub1) is catalyzed by the E3 ubiquitin ligase RING1B, which is overexpressed in many human tumors [75][76][77][78] and plays an important role in PRC1-mediated gene silencing through monoubiquitination of histone H2A [79][80]. HAUSP can directly interact with and specifically deubiquitinate RING1B, thereby modulating the level of ubiquitinated H2A [79]. The same effects on H2AK119ub1 were found when HAUSP was downregulated or knocked out in human colorectal carcinoma cells HCT116 [81].

3.2. Role of HAUSP in the Regulation of Histone Acetylation

The histone acetyltransferase TIP60 is a member of the ECREM complex that also includes HAUSP, UHRF1, and DNMT1, and each member has regulatory effects on the other partners (35, 49, 61, 70, 71, 73). TIP60, through its acetyltransferase, was shown to acetylate, destabilize, and trigger the ubiquitination of DNMT1 through the E3 ligase activity of UHRF1 [58]. TIP60 is also required for acetylation of histones [82][83][84] and of nonhistone proteins, such as the tumor suppressor p53 [85][86]. Thus, deciphering the mechanisms of TIP60 regulation is crucial for unraveling the complex role of this histone acetyltransferase in tumors. TIP60 is a target of the deubiquitinase activity of HAUSP [50], which interacts through its TRAF domain to deubiquitinate and stabilize TIP60, leading to the subsequent induction of p53-dependent apoptosis [50].

3.3. Role of HAUSP in the Regulation of Histone Methylation

Histone lysine methyltransferases (KMTs), such as EZH2 [87] and MLL5, [53] and lysine specific histone demethylase (KDMs), including LSD1 [88][89] and PHF8 [90][91], are two families of enzymes involved in histone methylation and demethylation, respectively. Specifically, the trimethylation of lysine 27 of histone 3 (H3K27me3), mediated by EZH2, is considered one of histone changes that silence target genes, thereby promoting tumor growth and metastasis in many tumors [87][92][93][94]. EZH2 is a core subunit of the Polycomb Repressive Complex 2 (PRC2), which is associated with cancer [95][96].

3.4. Role of HAUSP in the UHRF1-Mediated Readout of Histone H3 Lysine 9 Methylation

During DNA replication in the S phase of the cell cycle, UHRF1 is loaded onto replication forks via its binding affinity to hemi-methylated DNA through its SRA domain [62][63][64] and by its ability to bind to and ubiquitinate di-or tri-methylated histone H3K9 (H3K9me2,3) through a coordinated cooperation between both its TTD and PHD domains [97][98]. However, in-depth investigation of how UHRF1-mediated ubiquitination of methylated histone H3K9 is regulated is of great importance for understanding the regulation of this maintenance machinery axis.

20. Shan, H.; Li, X.; Xiao, X.; Dai, Y.; Huang, J.; Song, J.; Liu, M.; Yang, L.; Lei, H.; Tong, Y.; et al. USP7 deubiquitinates and stabilizes NOTCH1 in T-cell acute lymphoblastic leukemia. Signal Transduct. Target. Ther. 2018, 3, 29.
21. Novellasdemunt, L.; Foglizzo, V.; Cuadrado, L.; Antas, P.; Kucharska, A.; Encheva, V.; Snijders, A.P.; Li, V.S.W. USP7 Is a Tumor-Specific WNT Activator for APC-Mutated Colorectal Cancer by Mediating β-Catenin Deubiquitination. Cell Rep. 2017, 21, 612–627.
22. Franqui-Machin, R.; Hao, M.; Bai, H.; Gu, Z.; Zhan, X.; Habelhah, H.; Jethava, Y.; Qiu, L.; Frech, I.; Tricot, G.; et al. Destabilizing NEK2 overcomes resistance to proteasome inhibition in multiple myeloma. J. Clin. Investig. 2018, 128, 2877–2893.
23. Tavana, O.; Sun, H.; Gu, W. Targeting HAUSP in both p53 wildtype and p53-mutant tumors. Cell Cycle 2018, 17, 823–828.
24. Brooks, C.L.; Li, M.; Hu, M.; Shi, Y.; Gu, W. The p53–Mdm2–HAUSP complex is involved in p53 stabilization by HAUSP. Oncogene 2007, 26, 7262–7266.
25. Bhattacharya, S.; Ghosh, M.K. HAUSP, a novel deubiquitinase for Rb-MDM2 the critical regulator. FEBS J. 2014, 281, 3061–3078.
26. Meulmeester, E.; Pereg, Y.; Shiloh, Y.; Jochemsen, A.G. ATM-mediated phosphorylations inhibit Mdmx/Mdm2 stabilization by HAUSP in favor of p53 activation. Cell Cycle 2005, 4, 1166–1170.
27. Harris, S.L.; Levine, A.J. The p53 pathway: Positive and negative feedback loops. Oncogene 2005, 24, 2899–2908.
28. Karni-Schmidt, O.; Lokshin, M.; Prives, C. The Roles of MDM2 and MDMX in Cancer. Annu. Rev. Pathol. 2016, 11, 617–644.
29. Marine, J.C.; Jochemsen, A.G. Mdmx as an essential regulator of p53 activity. Biochem. Biophys. Res. Commun. 2005, 331, 750–760.
30. Melo, A.N.; Eischen, C.M. Protecting the genome from mdm2 and mdmx. Genes Cancer 2012, 3, 283–290.
31. Li, Y.; Reverter, D. Molecular Mechanisms of DUBs Regulation in Signaling and Disease. Int. J. Mol. Sci. 2021, 22, 986.
32. Alhosin, M.; Sharif, T.; Mousli, M.; Etienne-Selloum, N.; Fuhrmann, G.; Schini-Kerth, V.B.; Bronner, C. Down-regulation of UHRF1, associated with re-expression of tumor suppressor genes, is a common feature of natural compounds exhibiting anti-cancer properties. J. Exp. Clin. Cancer Res. 2011, CR 30, 41.
33. Xue, B.; Zhao, J.; Feng, P.; Xing, J.; Wu, H.; Li, Y. Epigenetic mechanism and target therapy of UHRF1 protein complex in malignancies. OncoTargets Ther. 2019, 12, 549–559.

A structural characterization of the HAUSP/UHRF1 complex showed that HAUSP, through its first two UBL domains (UBL1-2), binds to a polybasic region (PBR) in the C terminus of UHRF1 [57], a region responsible for blocking the interaction of the TTD domain of UHRF1 with the tri-methylation of lysine 9 of histone 3 (H3K9me3) [99]. The binding of HAUSP to the PBR region of UHRF1 induces an allosteric regulation of UHRF1 by disrupting the intramolecular interaction between the TTD and PBR domains of UHRF1, thereby releasing the TTD domain from its association with PBR and thus from the "TTD-occluded state" and allowing it to adopt an "open state" that promotes the association of the TTD-PHD domains of UHRF1 with chromatin and, hence, efficient H3K9me3 binding [57].

4. HAUSP Inhibitors as Promising Anticancer Agents

The important role of HAUSP in modulating several pro-proliferative and anti-apoptotic genes has led to extensive efforts to develop HAUSP-selective inhibitors [100][101][102]. Moreover, the solved X-ray structure of HAUSP in complex with those molecules further advanced the structure-based design potent and reversible and irreversible inhibitors [103]. Irreversible HAUSP small molecules inhibitors work via alkylating the catalytic Cys223, blocking its interaction with ubiquitin. However, the similarity of the catalytic site of HAUSP with other deubiquitinases reduces the selectivity of such inhibitors. On the other hand, reversible inhibitors that bind noncovalently to the site adjacent to the catalytic domain displayed better selectivity vis-à-vis other deubiquitinases [104].

34. Abdullah, O.; Omran, Z.; Hosawi, S.; Hamiche, A.; Bronner, C.; Alhosin, M. Thymoquinone Is a Multitarget Single Epidrug That Inhibits the UHRF1 Protein Complex. Genes 2021, 12, 622.

35. Unoki, M. Current and potential anticancer drugs targeting members of the UHRF1 complex including epigenetic modifiers. Recent Pat. Anti-Cancer Drug Discov. 2011, 6, 116–130.

36. Ahmad, T.; Ashraf, W.; Ibrahim, A.; Zaayter, L.; Muller, C.D.; Hamiche, A.; Mély, Y.; Bronner, C.; Mousli, M. TIP60 governs the auto-ubiquitination of UHRF1 through USP7 dissociation from the UHRF1/USP7 complex. Int. J. Oncol. 2021, 59, 89.

37. Choudhry, H.; Zamzami, M.A.; Omran, Z.; Wu, W.; Mousli, M.; Bronner, C.; Alhosin, M. Targeting microRNA/UHRF1 pathways as a novel strategy for cancer therapy. Oncol. Lett. 2018, 15, 3–10.

38. Felle, M.; Joppien, S.; Németh, A.; Diermeier, S.; Thalhammer, V.; Dobner, T.; Kremmer, E.; Kappler, R.; Längst, G. The USP7/Dnmt1 complex stimulates the DNA methylation activity of Dnmt1 and regulates the stability of UHRF1. Nucleic Acids Res. 2011, 39, 8355–8365.

39. Bronner, C.; Alhosin, M.; Hamiche, A.; Mousli, M. Coordinated Dialogue between UHRF1 and DNMT1 to Ensure Faithful Inheritance of Methylated DNA Patterns. Genes 2019, 10, 65.

40. Du, J.; Johnson, L.M.; Jacobsen, S.E.; Patel, D.J. DNA methylation pathways and their crosstalk with histone methylation. Nat. Rev. Mol. Cell Biol. 2015, 16, 519–532.

41. Ren, W.; Fan, H.; Grimm, S.A.; Guo, Y.; Kim, J.J.; Yin, J.; Li, L.; Petell, C.J.; Tan, X.F.; Zhang, Z.M.; et al. Direct readout of heterochromatic H3K9me3 regulates DNMT1-mediated maintenance DNA methylation. Proc. Natl. Acad. Sci. USA 2020, 117, 18439–18447.

42. Cedar, H.; Bergman, Y. Linking DNA methylation and histone modification: Patterns and paradigms. Nat. Rev. Genet. 2009, 10, 295–304.

43. Bronner, C.; Krifa, M.; Mousli, M. Increasing role of UHRF1 in the reading and inheritance of the epigenetic code as well as in tumorogenesis. Biochem. Pharmacol. 2013, 86, 1643–1649.

44. Kilin, V.; Gavvala, K.; Barthes, N.P.; Michel, B.Y.; Shin, D.; Boudier, C.; Mauffret, O.; Yashchuk, V.; Mousli, M.; Ruff, M.; et al. Dynamics of Methylated Cytosine Flipping by UHRF1. J. Am. Chem. Soc. 2017, 139, 2520–2528.

45. Bronner, C.; Fuhrmann, G.; Chédin, F.L.; Macaluso, M.; Dhe-Paganon, S. UHRF1 Links the Histone code and DNA Methylation to ensure Faithful Epigenetic Memory Inheritance. Genet. Epigenet. 2010, 2009, 29–36.

46. Li, T.; Wang, L.; Du, Y.; Xie, S.; Yang, X.; Lian, F.; Zhou, Z.; Qian, C. Structural and mechanistic insights into UHRF1-mediated DNMT1 activation in the maintenance DNA methylation. Nucleic Acids Res. 2018, 46, 3218–3231.

47. Liu, X.; Gao, Q.; Li, P.; Zhao, Q.; Zhang, J.; Li, J.; Koseki, H.; Wong, J. UHRF1 targets DNMT1 for DNA methylation through cooperative binding of hemi-methylated DNA and methylated H3K9.

Nat. Commun. 2013, 4, 1563.

48. Ma, H.; Chen, H.; Guo, X.; Wang, Z.; Sowa, M.E.; Zheng, L.; Hu, S.; Zeng, P.; Guo, R.; Diao, J.; et al. M phase phosphorylation of the epigenetic regulator UHRF1 regulates its physical association with the deubiquitylase USP7 and stability. Proc. Natl. Acad. Sci. USA 2012, 109, 4828–4833.
49. Cheng, J.; Yang, H.; Fang, J.; Ma, L.; Gong, R.; Wang, P.; Li, Z.; Xu, Y. Molecular mechanism for USP7-mediated DNMT1 stabilization by acetylation. Nat. Commun. 2015, 6, 7023.
50. Dar, A.; Shibata, E.; Dutta, A. Deubiquitination of Tip60 by USP7 determines the activity of the p53-dependent apoptotic pathway. Mol. Cell. Biol. 2013, 33, 3309–3320.
51. Wu, H.T.; Kuo, Y.C.; Hung, J.J.; Huang, C.H.; Chen, W.Y.; Chou, T.Y.; Chen, Y.; Chen, Y.J.; Chen, Y.J.; Cheng, W.C.; et al. K63-polyubiquitinated HAUSP deubiquitinates HIF-1α and dictates H3K56 acetylation promoting hypoxia-induced tumour progression. Nat. Commun. 2016, 7, 13644.
52. Gagarina, V.; Bojagora, A.; Lacdao, I.K.; Luthra, N.; Pfoh, R.; Mohseni, S.; Chaharlangi, D.; Tan, N.; Saridakis, V. Structural Basis of the Interaction Between Ubiquitin Specific Protease 7 and Enhancer of Zeste Homolog 2. J. Mol. Biol. 2020, 432, 897–912.
53. Ding, X.; Jiang, W.; Zhou, P.; Liu, L.; Wan, X.; Yuan, X.; Wang, X.; Chen, M.; Chen, J.; Yang, J.; et al. Mixed Lineage Leukemia 5 (MLL5) Protein Stability Is Cooperatively Regulated by O-GlcNac Transferase (OGT) and Ubiquitin Specific Protease 7 (USP7). PLoS ONE 2015, 10, e0145023.
54. Yi, L.; Cui, Y.; Xu, Q.; Jiang, Y. Stabilization of LSD1 by deubiquitinating enzyme USP7 promotes glioblastoma cell tumorigenesis and metastasis through suppression of the p53 signaling pathway. Oncol. Rep. 2016, 36, 2935–2945.
55. Liu, J.; Feng, J.; Li, L.; Lin, L.; Ji, J.; Lin, C.; Liu, L.; Zhang, N.; Duan, D.; Li, Z.; et al. Arginine methylation-dependent LSD1 stability promotes invasion and metastasis of breast cancer. EMBO Rep. 2020, 21, e48597.
56. Wang, Q.; Ma, S.; Song, N.; Li, X.; Liu, L.; Yang, S.; Ding, X.; Shan, L.; Zhou, X.; Su, D.; et al. Stabilization of histone demethylase PHF8 by USP7 promotes breast carcinogenesis. J. Clin. Investig. 2016, 126, 2205–2220.
57. Zhang, Z.M.; Rothbart, S.B.; Allison, D.F.; Cai, Q.; Harrison, J.S.; Li, L.; Wang, Y.; Strahl, B.D.; Wang, G.G.; Song, J. An Allosteric Interaction Links USP7 to Deubiquitination and Chromatin Targeting of UHRF1. Cell Rep. 2015, 12, 1400–1406.
58. Du, Z.; Song, J.; Wang, Y.; Zhao, Y.; Guda, K.; Yang, S.; Kao, H.Y.; Xu, Y.; Willis, J.; Markowitz, S.D.; et al. DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. Sci. Signal. 2010, 3, ra80.

59. Jenkins, Y.; Markovtsov, V.; Lang, W.; Sharma, P.; Pearsall, D.; Warner, J.; Franci, C.; Huang, B.; Huang, J.; Yam, G.C.; et al. Critical role of the ubiquitin ligase activity of UHRF1, a nuclear RING finger protein, in tumor cell growth. Mol. Biol. Cell 2005, 16, 5621–5629.

60. Ibrahim, A.; Alhosin, M.; Papin, C.; Ouararhni, K.; Omran, Z.; Zamzami, M.A.; Al-Malki, A.L.; Choudhry, H.; Mély, Y.; Hamiche, A.; et al. Thymoquinone challenges UHRF1 to commit auto-ubiquitination: A key event for apoptosis induction in cancer cells. Oncotarget 2018, 9, 28599–28611.

61. Hashimoto, H.; Horton, J.R.; Zhang, X.; Bostick, M.; Jacobsen, S.E.; Cheng, X. The SRA domain of UHRF1 flips 5-methylcytosine out of the DNA helix. Nature 2008, 455, 826–829.

62. Avvakumov, G.V.; Walker, J.R.; Xue, S.; Li, Y.; Duan, S.; Bronner, C.; Arrowsmith, C.H.; Dhe-Paganon, S. Structural basis for recognition of hemi-methylated DNA by the SRA domain of human UHRF1. Nature 2008, 455, 822–825.

63. Bostick, M.; Kim, J.K.; Estève, P.O.; Clark, A.; Pradhan, S.; Jacobsen, S.E. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. Science 2007, 317, 1760–1764.

64. Sharif, J.; Muto, M.; Takebayashi, S.; Suetake, I.; Iwamatsu, A.; Endo, T.A.; Shinga, J.; Mizutani-Koseki, Y.; Toyoda, T.; Okamura, K.; et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature 2007, 450, 908–912.

65. Nishiyama, A.; Yamaguchi, L.; Sharif, J.; Johmura, Y.; Kawamura, T.; Nakanishi, K.; Shimamura, S.; Arita, K.; Kodama, T.; Ishikawa, F.; et al. Uhrf1-dependent H3K23 ubiquitylation couples maintenance DNA methylation and replication. Nature 2013, 502, 249–253.

66. Misaki, T.; Yamaguchi, L.; Sun, J.; Orii, M.; Nishiyama, A.; Nakanishi, M. The replication foci targeting sequence (RFTS) of DNMT1 functions as a potent histone H3 binding domain regulated by autoinhibition. Biochem. Biophys. Res. Commun. 2016, 470, 741–747.

67. Qin, W.; Wolf, P.; Liu, N.; Link, S.; Smets, M.; La Mastra, F.; Forné, I.; Pichler, G.; Hörl, D.; Fellinger, K.; et al. DNA methylation requires a DNMT1 ubiquitin interacting motif (UIM) and histone ubiquitination. Cell Res. 2015, 25, 911–929.

68. Qin, W.; Leonhardt, H.; Spada, F. Usp7 and Uhrf1 control ubiquitination and stability of the maintenance DNA methyltransferase Dnmt1. J. Cell. Biochem. 2011, 112, 439–444.

69. Marsh, D.J.; Ma, Y.; Dickson, K.A. Histone Monoubiquitination in Chromatin Remodelling: Focus on the Histone H2B Interactome and Cancer. Cancers 2020, 12, 3462.

70. Yan, Q.; Chen, B.J.; Hu, S.; Qi, S.L.; Li, L.Y.; Yang, J.F.; Zhou, H.; Yang, C.C.; Chen, L.J.; Du, J. Emerging role of RNF2 in cancer: From bench to bedside. J. Cell. Physiol. 2021, 236, 5453–5465.

71. Marsh, D.J.; Dickson, K.A. Writing Histone Monoubiquitination in Human Malignancy—The Role of RING Finger E3 Ubiquitin Ligases. Genes 2019, 10, 67.

72. Cohen, I.; Bar, C.; Ezhkova, E. Activity of PRC1 and Histone H2AK119 Monoubiquitination: Revising Popular Misconceptions. BioEssays News Rev. Mol. Cell. Dev. Biol. 2020, 42, e1900192.
73. Benitz, S.; Regel, I.; Reinhard, T.; Popp, A.; Schäffer, I.; Raulefs, S.; Kong, B.; Esposito, I.; Michalski, C.W.; Kleeff, J. Polycomb repressor complex 1 promotes gene silencing through H2AK119 mono-ubiquitination in acinar-to-ductal metaplasia and pancreatic cancer cells. Oncotarget 2016, 7, 11424–11433.
74. Sarkari, F.; Wheaton, K.; La Delfa, A.; Mohamed, M.; Shaikh, F.; Khatun, R.; Arrowsmith, C.H.; Frappier, L.; Saridakis, V.; Sheng, Y. Ubiquitin-specific protease 7 is a regulator of ubiquitin-conjugating enzyme UbE2E1. J. Biol. Chem. 2013, 288, 16975–16985.
75. Zhang, Y.; Chan, H.L.; Garcia-Martinez, L.; Karl, D.L.; Weich, N.; Slingerland, J.M.; Verdun, R.E.; Morey, L. Estrogen induces dynamic ERα and RING1B recruitment to control gene and enhancer activities in luminal breast cancer. Sci. Adv. 2020, 6, eaaz7249.
76. Chan, H.L.; Beckedorff, F.; Zhang, Y.; Garcia-Huidobro, J.; Jiang, H.; Colaprico, A.; Bilbao, D.; Figueroa, M.E.; LaCava, J.; Shiekhattar, R.; et al. Polycomb complexes associate with enhancers and promote oncogenic transcriptional programs in cancer through multiple mechanisms. Nat. Commun. 2018, 9, 3377.
77. Benitz, S.; Straub, T.; Mahajan, U.M.; Mutter, J.; Czemmel, S.; Unruh, T.; Wingerath, B.; Deubler, S.; Fahr, L.; Cheng, T.; et al. Ring1b-dependent epigenetic remodelling is an essential prerequisite for pancreatic carcinogenesis. Gut 2019, 68, 2007–2018.
78. Sánchez-Molina, S.; Figuerola-Bou, E.; Blanco, E.; Sánchez-Jiménez, M.; Táboas, P.; Gómez, S.; Ballaré, C.; García-Domínguez, D.J.; Prada, E.; Hontecillas-Prieto, L.; et al. RING1B recruits EWSR1-FLI1 and cooperates in the remodeling of chromatin necessary for Ewing sarcoma tumorigenesis. Sci. Adv. 2020, 6, eaba3058.
79. de Bie, P.; Zaaroor-Regev, D.; Ciechanover, A. Regulation of the Polycomb protein RING1B ubiquitination by USP7. Biochem. Biophys. Res. Commun. 2010, 400, 389–395.
80. Wang, H.; Wang, L.; Erdjument-Bromage, H.; Vidal, M.; Tempst, P.; Jones, R.S.; Zhang, Y. Role of histone H2A ubiquitination in Polycomb silencing. Nature 2004, 431, 873–878.
81. Wheaton, K.; Sarkari, F.; Johns, B.S.; Davarinejad, H.; Egorova, O.; Kaustov, L.; Raught, B.; Saridakis, V.; Sheng, Y. UbE2E1/UBCH6 Is a Critical In Vivo E2 for the PRC1-catalyzed Ubiquitination of H2A at Lys-119. J. Biol. Chem. 2017, 292, 2893–2902.
82. Squatrito, M.; Gorrini, C.; Amati, B. Tip60 in DNA damage response and growth control: Many tricks in one HAT. Trends Cell Biol. 2006, 16, 433–442.
83. Judes, G.; Rifaï, K.; Ngollo, M.; Daures, M.; Bignon, Y.J.; Penault-Llorca, F.; Bernard-Gallon, D. A bivalent role of TIP60 histone acetyl transferase in human cancer. Epigenomics 2015, 7, 1351–

1363.

84. Judes, G.; Dubois, L.; Rifaï, K.; Idrissou, M.; Mishellany, F.; Pajon, A.; Besse, S.; Daures, M.; Degoul, F.; Bignon, Y.J.; et al. TIP60, an actor in acetylation of H3K4 and tumor development in breast cancer. Epigenomics 2018, 10, 1415–1430.
85. Tang, Y.; Luo, J.; Zhang, W.; Gu, W. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. Mol. Cell 2006, 24, 827–839.
86. Sykes, S.M.; Mellert, H.S.; Holbert, M.A.; Li, K.; Marmorstein, R.; Lane, W.S.; McMahon, S.B. Acetylation of the p53 DNA-binding domain regulates apoptosis induction. Mol. Cell 2006, 24, 841–851.
87. Yamagishi, M.; Uchimaru, K. Targeting EZH2 in cancer therapy. Curr. Opin. Oncol. 2017, 29, 375–381.
88. Lim, S.; Janzer, A.; Becker, A.; Zimmer, A.; Schüle, R.; Buettner, R.; Kirfel, J. Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology. Carcinogenesis 2010, 31, 512–520.
89. Lan, F.; Nottke, A.C.; Shi, Y. Mechanisms involved in the regulation of histone lysine demethylases. Curr. Opin. Cell Biol. 2008, 20, 316–325.
90. Tsukada, Y.; Ishitani, T.; Nakayama, K.I. KDM7 is a dual demethylase for histone H3 Lys 9 and Lys 27 and functions in brain development. Genes Dev. 2010, 24, 432–437.
91. Feng, W.; Yonezawa, M.; Ye, J.; Jenuwein, T.; Grummt, I. PHF8 activates transcription of rRNA genes through H3K4me3 binding and H3K9me1/2 demethylation. Nat. Struct. Mol. Biol. 2010, 17, 445–450.
92. Kim, K.H.; Roberts, C.W. Targeting EZH2 in cancer. Nat. Med. 2016, 22, 128–134.
93. Duan, R.; Du, W.; Guo, W. EZH2, a novel target for cancer treatment. J. Hematol. Oncol. 2020, 13, 104.
94. Varambally, S.; Dhanasekaran, S.M.; Zhou, M.; Barrette, T.R.; Kumar-Sinha, C.; Sanda, M.G.; Ghosh, D.; Pienta, K.J.; Sewalt, R.G.; Otte, A.P.; et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 2002, 419, 624–629.
95. Kirmizis, A.; Bartley, S.M.; Kuzmichev, A.; Margueron, R.; Reinberg, D.; Green, R.; Farnham, P.J. Silencing of human polycomb target genes is associated with methylation of histone H3 Lys 27. Genes Dev. 2004, 18, 1592–1605.
96. Lee, T.I.; Jenner, R.G.; Boyer, L.A.; Guenther, M.G.; Levine, S.S.; Kumar, R.M.; Chevalier, B.; Johnstone, S.E.; Cole, M.F.; Isono, K.; et al. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 2006, 125, 301–313.

97. Houliston, R.S.; Lemak, A.; Iqbal, A.; Ivanochko, D.; Duan, S.; Kaustov, L.; Ong, M.S.; Fan, L.; Senisterra, G.; Brown, P.J.; et al. Conformational dynamics of the TTD-PHD histone reader module of the UHRF1 epigenetic regulator reveals multiple histone-binding states, allosteric regulation, and druggability. J. Biol. Chem. 2017, 292, 20947–20959.
98. Cheng, J.; Yang, Y.; Fang, J.; Xiao, J.; Zhu, T.; Chen, F.; Wang, P.; Li, Z.; Yang, H.; Xu, Y. Structural insight into coordinated recognition of trimethylated histone H3 lysine 9 (H3K9me3) by the plant homeodomain (PHD) and tandem tudor domain (TTD) of UHRF1 (ubiquitin-like, containing PHD and RING finger domains, 1) protein. J. Biol. Chem. 2013, 288, 1329–1339.
99. Gelato, K.A.; Tauber, M.; Ong, M.S.; Winter, S.; Hiragami-Hamada, K.; Sindlinger, J.; Lemak, A.; Bultsma, Y.; Houliston, S.; Schwarzer, D.; et al. Accessibility of different histone H3-binding domains of UHRF1 is allosterically regulated by phosphatidylinositol 5-phosphate. Mol. Cell 2014, 54, 905–919.
100. Colland, F.; Formstecher, E.; Jacq, X.; Reverdy, C.; Planquette, C.; Conrath, S.; Trouplin, V.; Bianchi, J.; Aushev, V.N.; Camonis, J.; et al. Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells. Mol. Cancer Ther. 2009, 8, 2286–2295.
101. Kategaya, L.; di Lello, P.; Rougé, L.; Pastor, R.; Clark, K.R.; Drummond, J.; Kleinheinz, T.; Lin, E.; Upton, J.P.; Prakash, S.; et al. USP7 small-molecule inhibitors interfere with ubiquitin binding. Nature 2009, 550, 534–538.
102. Pozhidaeva, A.; Valles, G.; Wang, F.; Wu, J.; Sterner, D.E.; Nguyen, P.; Weinstock, J.; Kumar, K.G.S.; Kanyo, J.; Wright, D.; et al. USP7-Specific Inhibitors Target and Modify the Enzyme's Active Site via Distinct Chemical Mechanisms. Cell Chem. Biol. 2017, 24, 1501–1512.
103. Nininahazwe, L.; Liu, B.; He, C.; Zhang, H.; Chen, Z.S. The emerging nature of Ubiquitin-specific protease 2017, 7 (USP7): A new target in cancer therapy. Drug Discov. Today 2021, 26, 490–502.
104. Altun, M.; Kramer, H.B.; Willems, L.I.; McDermott, J.L.; Leach, C.A.; Goldenberg, S.J.; Kumar, K.G.; Konietzny, R.; Fischer, R.; Kogan, E.; et al. Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. Chem. Biol. 2011, 18, 1401–1412.