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Commentary

Drug discovery targeting epigenetic codes: The great potential of UHRF1, which links DNA methylation and histone modifications, as a drug target in cancers and toxoplasmosis

Motoko Unoki [a,*], Julie Brunet [b], Marc Mousli [b]

[a] Laboratory for Biomarker Development, The Institute of Physical and Chemical Research, Center for Genomic Medicine, RIKEN, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

[b] Institut de Parasitologie et de Pathologie Tropicale de Strasbourg, UPRES E.A. 3950: Interactions Cellulaires et Moléculaires Hôte-Parasite, Faculté de Médecine, Université de Strasbourg, Strasbourg 67000, France

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ABSTRACT

UHRF1 plays a central role in transferring methylation status from mother cells to daughter cells. Its SRA domain recognizes hemi-methylated DNA that appears in daughter DNA strands during duplication of DNA. UHRF1 recruits DNMT1 to the site and methylates both strands. UHRF1 also binds to HDAC1 and di- and tri-methyl K9 histone H3, ubiquitinates histone H3, and associates with heterochromatin formation, indicating that UHRF1 links histone modifications, DNA methylation, and chromatin structure. UHRF1 is a direct target of E2F1 and promotes G1/S transition. The tumor suppressor p53, which is deficient in 50% of cancers, down-regulates UHRF1 through up-regulation of p21/WAF1 and subsequent deactivation of E2F1. The expression levels of UHRF1 are up-regulated in many cancers, probably partially because of the absence of wild type p53, but it is probably regulated by several other factors. Knockdown of UHRF1 expression in cancer cells suppressed cell growth, suggesting that UHRF1 can be a useful anticancer drug target. Recently, it was revealed that UHRF1 plays important roles not only in carcinogenesis, but also in toxoplasmosis, which is occasionally fatal to people with a weakened immune system, and can cause blindness in the major pathology of ocular toxoplasmosis. *Toxoplasma gondii*, which causes toxoplasmosis, utilizes UHRF1 to control the cell cycle phase and enhance its proliferation. Thus, knockdown of UHRF1 can be effective at stopping the proliferation of the parasites in infected cells. In this review, we discuss several possible methods that can inhibit the multiple unique functions of UHRF1, which can be utilized for treating cancers and toxoplasmosis.

1. Introduction

Ubiquitin-like with PHD and ring finger domains 1 (UHRF1), also known as ICBP90 or Np95, plays a central role in transferring methylation status from mother cells to daughter cells [1–4]. Its SRA domain recognizes hemi-methylated cytosines that appear in daughter DNA strands during replication of DNA through the S phase or during the process of DNA repair. UHRF1 physically interacts with proliferating cell nuclear antigen (PCNA), which plays an essential role in nucleic acid metabolism as a component of the replication and repair machinery, and also recruits DNA (cytosine-5-)-methyltransferase 1 (DNMT1) to the sites to methylate the newly synthesized strands [4]. Because UHRF1 promotes G1/S transition [5], which is facilitated by *T. gondii* for its proliferation [6], UHRF1 has a critical role in cell cycle progression. Accordingly, UHRF1 is overexpressed in various cancers [7–11], suggesting that UHRF1 can be utilized for molecular targeted therapies and also for vaccination of cancer patients.

Molecular targeted therapies are a new generation of anticancer therapies that inhibit the functions of specific oncogenic proteins, which are predominantly expressed in cancer cells and essential for cancer progression and/or malignancy. Compared with current standard anticancer drugs, molecular targeted therapies are expected to have fewer side effects because of enhanced specificity to cancer cells. The therapies include monoclonal humanized antibodies, small interference RNAs (siRNAs), small molecular compounds, and permeable dominant negative peptides. Because

Abbreviations: UHRF1, ubiquitin-like with PHD and ring finger domains 1; DNMT1, DNA (cytosine-5-)-methyltransferase 1; PCNA, proliferating cell nuclear antigen; HDAC, histone deacetylase; H3K9, histone H3 lysine 9; SRA domain, SET and RING finger associated domain; PHD finger, plant homeodomain finger; *T. gondii*, *Toxoplasma gondii*; STAT, Signal Transducers and Activators of Transcription.

* Corresponding author. Tel.: +81 3 5449 5233; fax: +81 3 5449 5124.
E-mail addresses: unokim@ims.u-tokyo.ac.jp, icbp90pink1@mac.com (M. Unoki).

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Table 1
Binding partners of UHRF1 and UHRF2.

Name	Interacting proteins	Description	Refs.
UHRF1	HDAC1	Histone deacetylase.	[9,15]
	UHRF1BP1	Unknown.	[9]
	Histone H1, H2B, H3	Histones.	[17]
	RB	Suppresses tumorigenesis by inhibiting cell cycle progression at the G1/S transition.	[63]
	DNMT1	DNA methyltransferase.	[14,15,64]
	PCNA	Plays important roles at DNA replication fork and repair site.	[4]
	H3K9me2, me3	Methylated histone H3 lysine 9.	[13]
	G9a/EHMT2	Methyltransferase.	[14]
	Eme1	Endonuclease that is critical for genomic stability.	[65]
UHRF2	PCNP	Unknown.	[32]
	Phosphorylated Cdk2	G1/S transition.	[33]
	Cyclin E	G1/S transition.	[33]

UHRF1 is essential for both cancer progression and proliferation of *Toxoplasma gondii* (*T. gondii*) in infected cells, these therapies can be effective for treating these diseases. Cancer vaccine is also an emerging cancer treatment utilizing the intrinsic immune response in cancer patients [12]. Because the characteristics of UHRF1 meet the criteria to be the target of molecular targeted therapies and also for a cancer vaccine, in this review, we discuss possible applications of UHRF1 in clinical use.

2. UHRF1 and epigenetic codes

UHRF1 is a unique molecule, which links DNA methylation, histone methylation, histone deacetylation, histone ubiquitination, and heterochromatin formation through interaction with methyl-CpG in DNA strands, methylated histone H3 lysine 9 (H3K9), HDAC1, DNMT1, PCNA, and G9a/EHMT2 (Table 1 and Fig. 1B) [1–4,9,13–15]. There have not been any anticancer drugs or

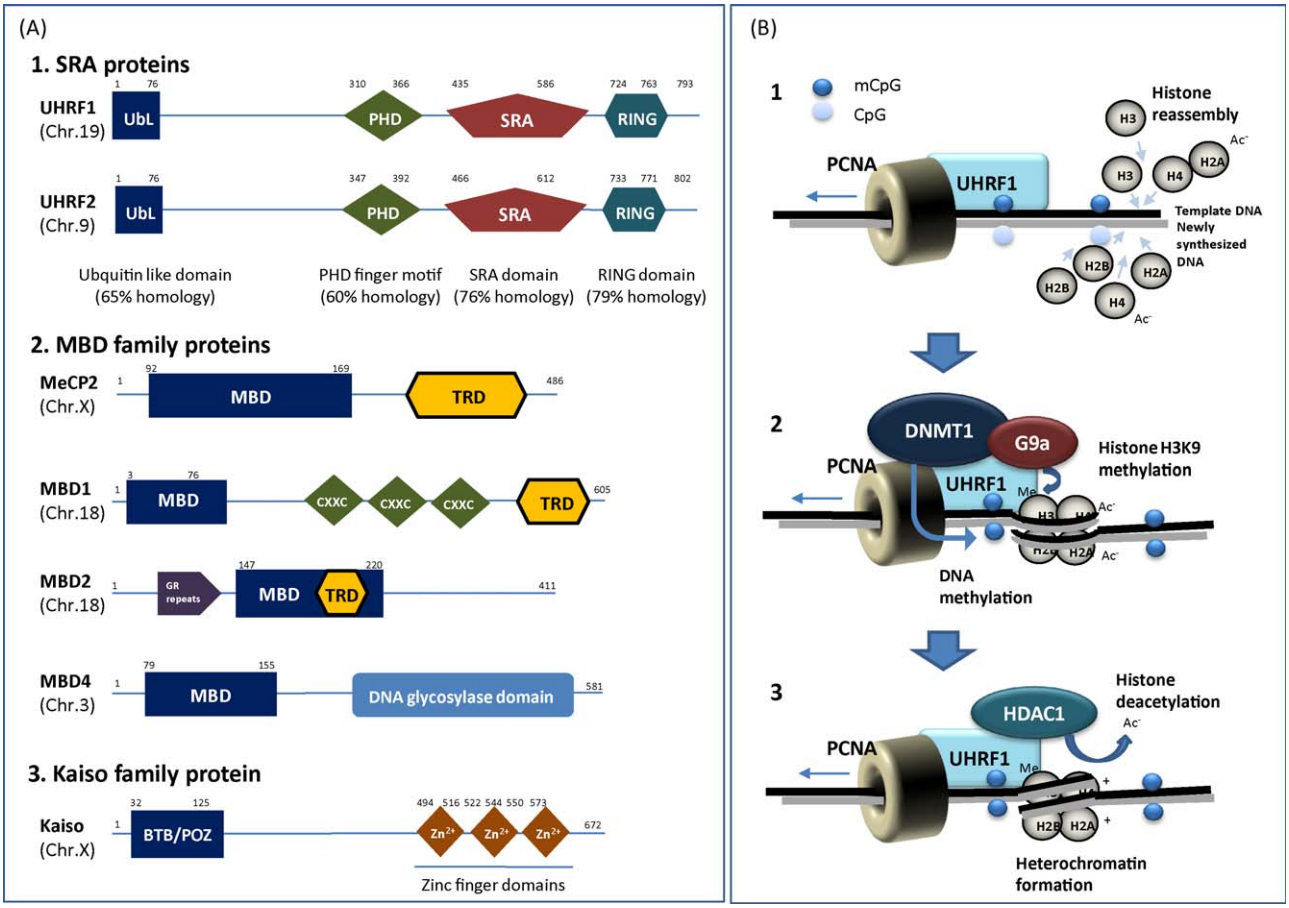

Fig. 1. Molecular characteristics and functions of UHRF1. (A) Methyl-CpG binding proteins. (1) SRA domain proteins. Amino acid structures of UHRF1 and UHRF2. Both proteins possess an ubiquitin-like domain (UbL), a PHD domain, an SRA domain, and a RING domain. Homology of each domain between UHRF1 and UHRF2 is 65%, 60%, 76%, and 79%, respectively. (2) MBD family proteins. MBD3 does not possess binding affinity to methyl-CpG, thus, it is omitted from this figure. Abbreviations are; MBD, methyl-binding domain; TRD, transcription repression domain; GR repeats, glysine and arginine repeats; BTB/POZ, broad complex, tramtrack, bric a brac/pox virus zinc finger. (3) Kaiso family protein. (B) Proposed mechanism of heterochromatin formation through UHRF1 at DNA replication fork or DNA repair site. (1) UHRF1 binds to PCNA and the SRA domain of UHRF1 recognizes hemi-methylated CpG on newly synthesized DNA. Then histones are reassembled. (2) UHRF1 recruits DNMT1 to methylate both DNA strands to transfer methylation status. UHRF1 also recruits G9a to methylate histone H3K9. Methylated histone H3K9 binds to the PHD domain of UHRF1. (3) UHRF1 recruits HDAC1 to the site and deacetylates histones. Then, histones become charged positively and bind to negatively charged DNA tightly, causing heterochromatin formation.

any drugs for toxoplasmosis targeting such a protein which links variety of epigenetic modifications.

2.1. How does UHRF1 link DNA methylation, histone modifications, and heterochromatin formation?

UHRF1 has multiple domains which confer a variety of functions to this protein (Fig. 1A-1). The SRA domain of UHRF1 recognizes hemi-methylated DNA [1–3]. On the other hand, the PHD domain of UHRF1 recognizes methylated H3K9 [13], which is known to be tightly associated with heterochromatin formation, and subsequent transcriptional suppression [16]. Although the precise order of the series of processes at DNA replication forks and DNA repair sites is still unclear, we propose a possible model described in Fig. 1B. First, UHRF1 may bind to PCNA and recognize hemi-methylated DNA through its SRA domain (Fig. 1B-1) [1–3,9]. Histones are reassembled immediately after DNA replication (Fig. 1B-1). Next, UHRF1 may recruit G9a, which methylates H3K9, and then the methylated H3K9 binds tightly to the PHD domain of UHRF1 [14]. Simultaneously, UHRF1 may recruit DNMT1 and methylate both DNA strands to transfer methylation status (Fig. 1B-2). Finally, UHRF1 may recruit histone deacetylase 1 (HDAC1) to deacetylate histones which in turn facilitate heterochromatin formation (Fig. 1B-3) [9]. Histone deacetylation also correlates with transcriptional suppression. Histone tails are positively charged when they are deacetylated, and histone acetylation neutralizes the positive charge. In this context, histone deacetylation increases the binding force between histone tails, which are positively charged, and DNA, which is negatively charged, causing subsequent heterochromatin formation. Thus, UHRF1 can be a transcriptional repressor. Additionally, UHRF1 ubiquitinates histone H3 through its E3 ubiquitin ligase activity [13,17]. Histone ubiquitination is known to be required for transcriptional initiation and elongation, silencing, and DNA repair [18]. UHRF1 may have different roles other than those involved in the DNA replication and DNA repair process.

2.2. Other methyl-CpG binding proteins also link epigenetic modifications

There have been only three reported domains which confer recognition of methyl-CpG [19]. These are the methyl-CpG binding domain (MBD) of MBD proteins (MBD1, MBD2, MBD4, and MeCP2) (Fig. 1A-2), the zinc finger motifs of Kaiso (Fig. 1A-3), and the SRA domain of UHRF1 and UHRF2 (Fig. 1A-1) [19–21]. Even though these three domains recognize methyl-CpG, only the SRA domain recognizes hemi-methylated cytosine. The MBD of each MBD protein has differential affinity to different methyl-CpG density; some of these proteins prefer a low density of methyl-CpG, and some of them prefer a high density of methyl-CpG [22]. Kaiso/ZBTB33 is also a unique protein, which has a BTB/POZ domain and three zinc finger motifs. Kaiso recognizes methyl-CpG and the specific consensus sequence, TCCTGCNA, through the same zinc finger motifs [23]. Among these proteins, MBD1, MBD2, and MeCP2 are involved in an HDAC complex. MBD4 possesses mismatch repair activity, which is unique among the methyl-CpG binding proteins. Thus, some of their roles may overlap, and some of them may be unique. Nevertheless, all of these proteins are certainly important for transferring and decoding epigenetic codes, not only those encoded by DNA methylation status and local chromatin structure, but also those encoded by various histone modifications.

Recently, it was reported that one of the methyl-CpG binding proteins, MeCP2, which is well known for recruiting histone deacetylase activity, facilitates H3K9 methylation through recruitment of methyltransferase activity [24]. MBD1, which is also one of the methyl-CpG binding proteins, forms a stable complex with histone H3K9 methyltransferase SETDB1 [25]. During DNA replication, MBD1 recruits SETDB1 to the large subunit of chromatin assembly factor CAF-1 to form an S phase-specific CAF-1/MBD1/SETDB1 complex that facilitates methylation of H3K9 during replication-coupled chromatin assembly. It was also reported that SUV39H1, which is an H3K9 histone methyltransferase, binds to DNA methyltransferases, Dnmt3a and Dnmt1, and also HP1β/CBX1, which contributes to heterochromatin formation [26], suggesting that histone methylation and DNA methylation are closely related by methyl-CpG binding proteins. These emerging results suggest that not only UHRF1, but also other methyl-CpG binding proteins connect DNA methylation and histone methylation, contributing to heterochromatin formation. DNA methylation and histone methylation have been known to be closely and dynamically correlated for more than a decade, but the precise mechanism remains unclear. The methyl-CpG binding proteins including UHRF1 may help fill in the missing link.

3. Roles of UHRF1 in cancers

UHRF1 is significantly overexpressed in various cancers [7–11], probably partially because of the rapid cell cycle progression and the high incidence of DNA repair processes in cancer cells. In about 50% of cancers, expression and/or the functions of tumor suppressor p53 are abrogated [27]. Because p53 down-regulates UHRF1 through deactivation of E2F1, which is a direct upstream regulator of UHRF1 [5,8,9], absence of intact p53 in cancer cells can be another cause of the UHRF1 overexpression in the various cancers (Fig. 2A and B). In cancer cells, CpG islands located in tumor suppressor genes' promoters are often methylated, causing down-regulation of these tumor suppressors. Unoki et al. found that UHRF1 localizes on these methylated tumor suppressor promoters including promoters of $p16^{INK4A}$, $p14^{ARF}$, *FHIT*, *RARβ*, *APC*, and *DAPK*, and recruits HDAC1 to the site to suppress expression of tumor suppressor genes through deacetylation of histones and subsequent heterochromatin formation [9]. Because the recent progress of UHRF1 research revealed that it binds to hemi-methylated DNA, localization of UHRF1 on tumor suppressor promoters may not be specific. However, it is certainly important that UHRF1 contributes to maintaining DNA methylation status including the promoters of tumor suppressor genes. If this maintenance is disrupted, random expression of various genes can be triggered, disturbing cellular homeostasis and causing apoptosis or cell cycle arrest. Accordingly, knockdown of UHRF1 interferes with cancer cell proliferation, suggesting that UHRF1 is essential for supporting rapid expansion of cancer cells [9].

4. Impact of UHRF1 overexpression on cancers

Expression levels of UHRF1 in several cancers have been examined. Here we overview these reports.

4.1. UHRF1 expression in breast cancer

High expression of UHRF1 in breast cancer has been reported from several laboratories [7–9]. Overexpression of UHRF1 was observed in 68% of moderately and poorly differentiated breast cancers (histological types a2 and a3), and only in 24% of well-differentiated cancer (histological types a1 and 1a) [9]. UHRF1 is overexpressed even in 88% of triple negative breast cancer (our microarray data [28]), which does not express the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2). This subtype of breast cancer is clinically characterized as significantly aggressive and unresponsive to standard treatments, and is thus associated with a poor prognosis. The standard treatments target hormone receptors such

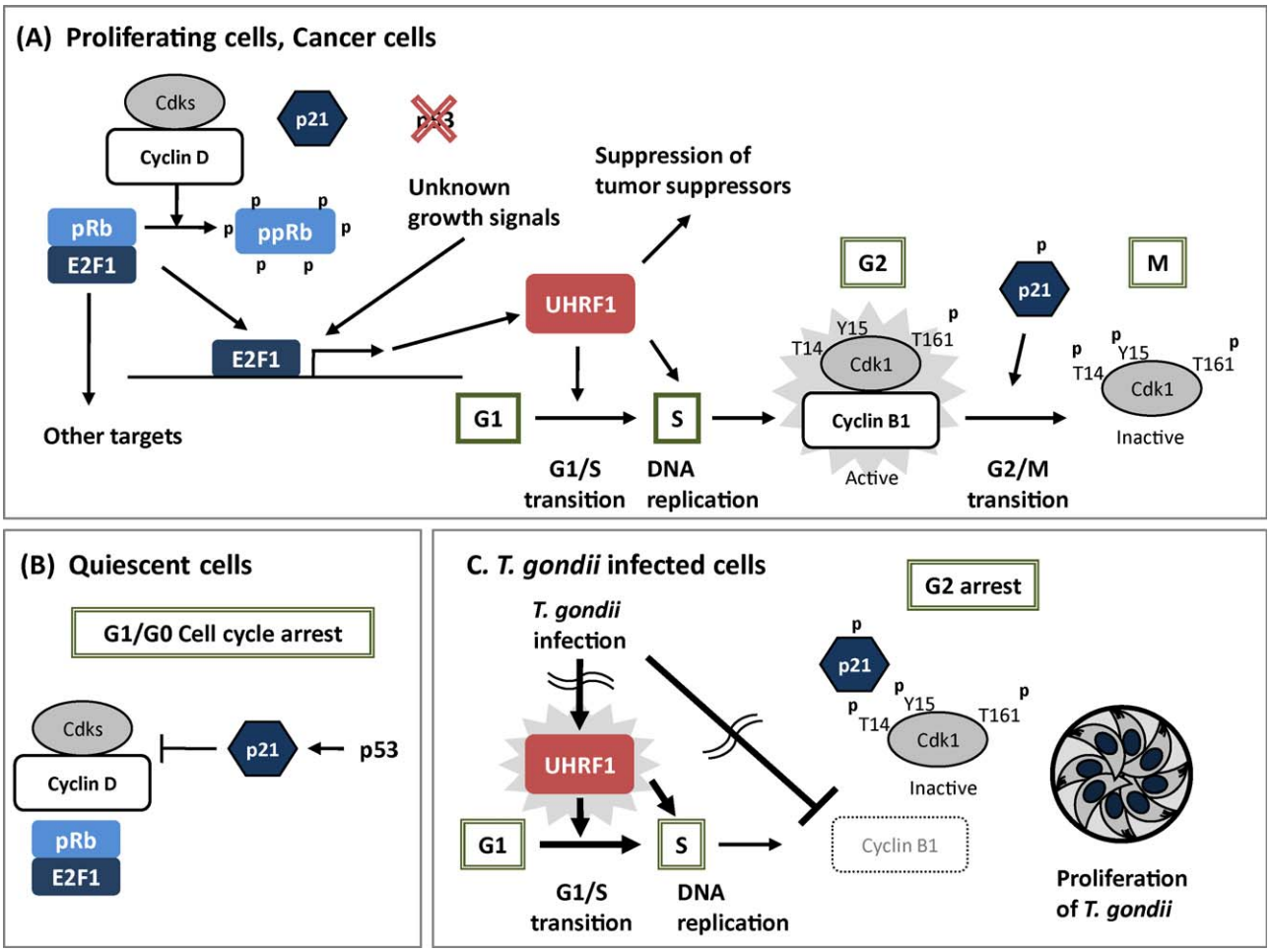

Fig. 2. A schematic model of the UHRF1 pathway in proliferating cells, quiescent cells, and Toxoplasma infected cells. (A) In proliferating cells or cancer cells, the functions of p53 are abrogated, leading to subsequent activation of cyclin D/Cdks. Activated cyclin D/Cdk complex phosphorylates pRb, causing activation of E2F1. Activated E2F1 binds to the promoter of UHRF1, and transactivates UHRF1. In proliferating cells, rapid DNA replication occurs. UHRF1 binds to newly synthesized DNA with PCNA and transfer methylation status from mother cells to daughter cells. Although still detailed molecular mechanism is unknown, UHRF1 also promotes G1/S transition, and then the cyclin B1/Cdk1 complex is activated in the G2/M phase. Hyperphosphorylated p21 can activate the kinase activity of the complex. (B) In quiescent cells, the p53-p21 pathway is dominant and suppresses cell cycle progression through suppression of cyclin D and Cdks (Cdk2, -4, and -6). (C) In *T. gondii*-infected cells, the parasite utilizes UHRF1 and promotes the G1/S transition. The parasite next stops the cell cycle at the G2 phase, which is a suitable state for its proliferation, through down-regulation of cyclin B1 by an unknown mechanism. This figure was modified from our previous article [6]. The definitive version is available at www.wileyinterscience.com.

as ER and PR using hormone-based drugs such as tamoxifen and aromatase inhibitors. These drugs are effective for breast cancer expressing these hormone receptors. Recently, Trastuzumab (Herceptin), which is a therapeutic antibody targeting Her2, was added to the standard treatments as an option. This drug is effective for Her2 positive breast cancer. Because triple negative breast cancer does not express these three receptors but expresses UHRF1, targeting UHRF1 may be effective and improve the prognosis of this type of breast cancer.

4.2. UHRF1 expression in bladder and kidney cancer

Recently we found that *UHRF1* is overexpressed in kidney and bladder tumors, including tumors occurring in upper tract, at the mRNA level [29]. Although overexpression of *UHRF1* in kidney cancer at the mRNA level was associated with several characteristics of kidney tumor patients including 5-year survival rates, pathological staging and histological grade, the overexpression was not detected at the protein level by immunohistochemistry because of the sensitivity differences between the methodologies for detecting mRNA and protein or of the different stability between *UHRF1* mRNA and UHRF1 protein. Thus, detection of *UHRF1* mRNA overexpression in surgical specimens might be useful as a prognosis tool in kidney cancer, but immunohistochemical staining of UHRF1 in the cancer may not be useful. On the other hand, overexpression of UHRF1 in bladder cancer was verified at the protein level. Bladder cancer is the second most common cancer of the urinary system. Early diagnosis of this tumor and estimation of risk of future progression after initial transurethral resection have a significant impact on prognosis. Although there are several molecular markers for diagnosis and prognosis of this tumor including nuclear matrix protein-22 and human complement factor H related protein, their accuracy is still not ideal. Further analysis revealed that overexpression of UHRF1 in bladder cancer was significantly correlated with stage and grade. Although UHRF1 expression in muscle-invasive cancer was greater than that in non-invasive (pTa) or superficially invasive (pT1) cancers, UHRF1 could still be detected by immunohistochemistry in these cancers. Because *UHRF1* was overexpressed especially in transitional cell carcinomas (TCCs) occurring in the upper tract, which are also categorized as bladder tumors, UHRF1 might be a very useful diagnostic marker for this type of tumor. Upper tract TCCs are often very malignant when diagnosed, partially because they are relatively difficult to find at an early stage. But the high expression of UHRF1 was detected even in upper tract TCCs at a relatively early stage (pT1), suggesting that

the overexpression is correlated not only with stages and grades, but also with anatomic site. If the cancer is found at an early stage, the prognosis of patients can be improved. Thus, the development of a sensitive urine based detection marker is still being sought. Examination of voided urine or bladder barbotage for exfoliated cancer cells is useful for diagnosis of urothelial tumor anywhere in the urinary tract, from the calyx, through the ureters, into bladder and urethra. However, cytological interpretation can be problematic; low cellular yields, atypia, degenerative changes, urinary tract infections, stones and intravesical instillations hamper a correct diagnosis. Because the expression of *UHRF1* in peripheral blood mononuclear cells (PBMCs) was low, the presence of these cells in urine would not impede the diagnosis. Additionally, overexpression of UHRF1 was also associated with increased risk of progression after TURBT. Thus, detection of UHRF1 in urine sediments or surgical specimens will be a useful marker for diagnosis and prognosis of bladder cancer.

4.3. UHRF1 expression in other cancers

4.3.1. Lung cancer

It was reported that *UHRF1* is overexpressed in lung cancer [7]. Lung cancer is the top leading worldwide cause of cancer-related death. The prognosis of lung cancer patients is generally poor. It is acknowledged that prognosis and treatment outcomes in lung cancer might be improved by increasing the effectiveness of early-stage diagnosis. Lung cancer is derived from accumulation of various genetic and epigenetic abnormalities in genomes such as activation of oncogenes including *EGFR*, *MYC*, *KRAS*, *KIT*, *MET*, *ERBB2*, and *CCND1* and inactivation of tumor suppressor genes including *p53*, *p16*/*p14*, *RB*, *PTEN*, *FHIT*, and *RASSF1A*. Abnormal expression of UHRF1 can be another cause of lung cancer malignancy. Because the precise cause of lung cancer varies among individuals, development of various diagnosis- and prognosis-markers and therapeutic tools are necessary. UHRF1 can be one of these diagnosis, prognosis, and therapeutic tools for lung cancer.

4.3.2. Pancreatic cancer

Pancreatic cancer is one of the most malignant tumors, and is called a ''silent killer'' because early pancreatic cancer often does not cause any symptoms, and the later symptoms are usually non-specific and varied, leading to locally advanced- or metastatic-disease at time of diagnosis. Patients diagnosed with pancreatic cancer typically have a poor prognosis; 5-year survival rate after diagnosis is less than 5%. UHRF1 was identified as a protein which was the most overexpressed in pancreatic cancer among 900 well-characterized proteins by proteomic analysis [11]. In their result, UHRF1 was overexpressed in approximately 80% of pancreatic ductal adenocarcinoma (PDAC), in most pancreatic intraepitherial neoplasia, and in all liver metastasis lesions. In contrast, UHRF1 was not overexpressed in chronic pancreatitis or normal specimens. They also found that two other proteins, ATP7A and aldehyde oxidase 1 (AOX1), are differentially expressed in PDAC, concluding that combination of UHRF1, ATP7A, and AOX1 analysis could potentially provide a useful additional diagnostic tool for fine-needle aspirated or cytological specimens obtained during endoscopic investigations. In addition to its potential as a diagnostic marker, UHRF1 can be a therapeutic tool, if inhibition of UHRF1 effectively suppresses growth of pancreatic cancer.

4.3.3. Astrocytomas

Gliomas are primary central nervous system tumors arising from astrocytes, oligodendrocytes, or their precursors, with astrocytomas being the most common among them. UHRF1 was overexpressed in astrocytomas determined by representational difference analysis (RDA) subtractive technology [10]. The overexpression significantly correlated with WHO grading [10]; grade I is pilocytic astrocytoma, grade II is low-grade astrocytoma, grade III is anaplastic astrocytoma, and grade IV is glioblastoma multiforme. The latter three grades are highly infiltrating and are hence named diffuse astrocytomas. Grade II astrocytoma has an intrinsic tendency to progression to grades III and IV as a consequence of sequential acquisition of genetic alterations. High expression of UHRF1 was detected in grades II–IV. Thus, UHRF1 may associate with a diffuse phenotype. It is mentioned in the article that UHRF1 and other four genes that they identified have a potential relevance for diagnosis and prognosis assessment and can also be useful in future therapeutic intervention.

4.3.4. Prostate cancer

In prostate cancer, moderate overexpression of UHRF1 was detected. But the magnitude of the change was not as dramatic as that observed in the other cancers [7]. Although further analysis is required, UHRF1 may not be a perfect molecular target for treating prostate cancer.

4.3.5. Cervical cancer

High-risk human papilloma virus (HR-HPV) persistent infection is significantly associated with development of high-grade squamous intraepithelial lesions (HSILs) from low-grade squamous intraepithelial lesions (LSILs) and subsequent carcinogenesis of cervical cancer. Cervical cancer is a unique cancer in the sense that it can be prevented, because routine health checks of cervical smears enabled early detection of the cancer. Lorenzato et al. screened potential markers for detecting the HSILs [30]. One of their candidate markers was UHRF1. In their result, the presence of UHRF1- or MIB-1-positive cells in the upper two thirds of the epithelium was a very accurate feature to select HSILs; 97.6% of HSILs were positive for UHRF1 and 100% for MIB-1. They concluded that the most accurate test to distinguish an LSIL from an HSIL was achieved by analyzing the association of a suspect DNA ploidy profile and the presence of UHRF1- or MIB-1-positive cells in the upper two thirds of the epithelium.

4.3.6. Possible other cancers

The Oncomine database (http://www.oncomine.org/main/mainx.jsp) is a useful tool for reviewing hundreds of microarray results [31]. The database implicates overexpression of *UHRF1* in astrocytoma, oligodendroglioma, glioblastoma multiforme, cervical cancer, breast cancer, lung cancer, and renal cancer. As we described above, overexpression of *UHRF1* in these tumors has been verified by other researchers and various methods. Thus, the database is worth referring to, although verification of the data by means other than microarray is necessary. According to the database, *UHRF1* may be up-regulated in hepatocellular carcinoma and ovarian cancer, and the *UHRF1* overexpression in ovarian cancer may correlate with grade of the cancer.

5. UHRF1 as a target of molecular targeted therapy of cancers

UHRF1 can be utilized as a diagnosis- and prognosis-marker or a molecular target in certain types of cancers as we described above. Here we describe UHRF1's characteristics as a therapeutic tool (Fig. 3).

5.1. Antibody therapy cannot be applied for UHRF1 molecular targeted therapy

Target proteins for antibody therapy should be membrane proteins that are recognized by specific antibodies. Because

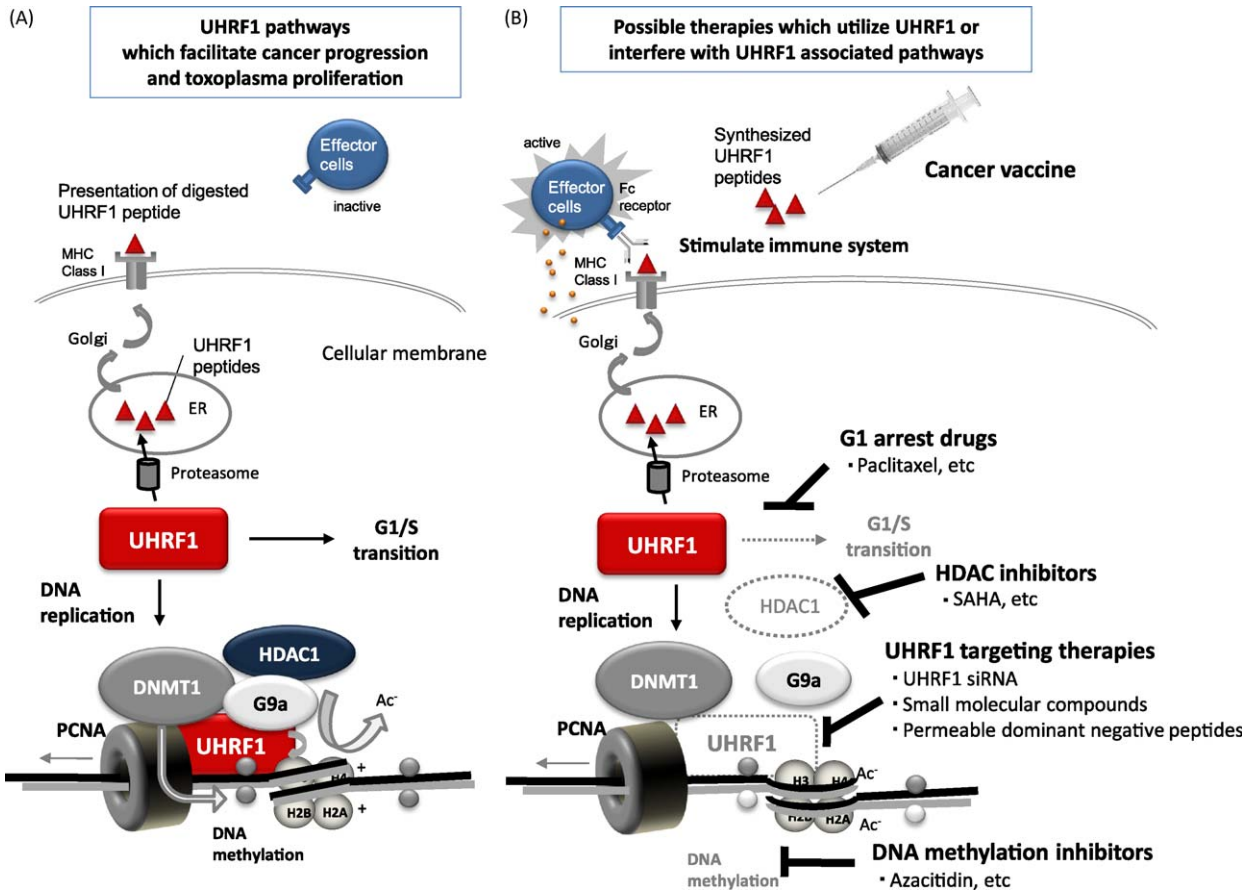

Fig. 3. Possible molecular targeting therapies utilizing UHRF1 products or interfering UHRF1 associated pathways, which are introduced in this article. (A) UHRF1 pathways, which facilitate cancer progression and Toxoplasma proliferation, and a general process of protein degradation through proteasome. (B) Possible therapies which utilize UHRF1 or interfere with UHRF1 associated pathways. Cancer vaccine using UHRF1 synthesized UHRF1 peptides can be used for cancer therapy. UHRF1 targeting therapies including UHRF1-siRNA, small molecular compounds and permeable dominant negative peptides can be used for cancer and also Toxoplasma therapies. DNA methylation inhibitors, HDAC inhibitors, G1 arrest drugs are now in clinical use for treating cancer. These drugs can be used for Toxoplasma treatment.

subcellular localization of UHRF1 is to the nucleus, antibody therapy cannot be directly applied.

5.2. Small molecular compounds targeting the SRA domain or the other domains

Because the structure of the SRA domain was determined by X-ray crystallography recently [1–3], the design of small molecular compounds that fit the domain *in silico* may be relatively easy. The 5-methylcytosine base at the hemi-methylated site is flipped out from the DNA helix in the SRA–DNA complex and fits tightly into a protein pocket on the concave surface. Thus, a 5-methylcytosine-like small molecular compound might be an antagonist of UHRF1, although it is still unclear whether the inhibition of the SRA domain's function is enough to interfere with the entirety of the oncogenic functions of UHRF1. When a small molecular compound targeting the SRA domain of UHRF1 is developed, specificity should be considered, because another UHRF1 family protein, UHRF2/NIRF, also possesses binding affinity to methylated DNA [9] and may have a tumor suppressive role unlike UHRF1 (Fig. 1A-1). Expression levels of endogenous *UHRF2* increased in proliferating cells compared with quiescent cells [32], suggesting that UHRF2 may play roles similar to UHRF1. Nevertheless, overexpression of UHRF2 induced cell cycle arrest at the G1 phase [33]. Homology of the SRA domain between UHRF1 and UHRF2 is 76% (Fig. 1A-1), suggesting that design of UHRF1 specific-small molecular compounds may be possible. Other domains of UHRF1 also can be targets of small molecular compounds, although the structures of these domains have not been determined by X-ray crystallography yet. As we mentioned above, because the PHD domain and the RING domain of UHRF1 possess binding activity to methylated H3K9, and E3 ubiquitin ligase activity, respectively, targeting these domains may abrogate the oncogenic functions of UHRF1. Even if a compound targeting a domain does not have any effects on cell growth, the combination of several compounds targeting different domains may inhibit the functions of UHRF1.

5.3. siRNAs against UHRF1

Because siRNAs against UHRF1 suppressed cell growth [9], UHRF1-siRNA therapy can be applied to the treatment of cancer patients. siRNA is very unstable in blood because of rapid degradation by serum nucleases, and is excreted from blood into urine from glomera quite rapidly, probably because of its small molecular weight, linear structure, negative electric charge, and solubility. Their negative electric charge prevents siRNAs from entering target cells, as well. To deliver siRNA to a targeted location is also an area of active research. Thus, establishment of good DDSs is the biggest hurdle to overcome prior to using siRNA as a therapeutic strategy. Although there are many hurdles, significant progress has been made in recent years in the delivery of siRNA to tumors, and several promising siRNA delivery platforms have begun to emerge utilizing the Enhanced Permeability and Retention (EPR) effect. The effect utilizes the property by which certain sizes of molecules tend to accumulate in tumor tissue much more than they do in normal tissues, because of the vascular

hyperpermeability in tumor lesion. These platforms include liposomes, in which siRNA is encapsulated in a lipid vesicle; polyplexes, in which a cationic carrier is used to bind siRNA to form siRNA-containing nanoparticles; liposome-polycation-DNA (LPD) complexes, in which an siRNA-containing polyplex is encapsulated in a lipid vesicle; and siRNA conjugates, in which siRNA is coupled to a targeting moiety that carries the siRNA into target cells via receptor-mediated endocytosis [34]. A number of groups have been investigating chemical modifications and alternative backbones, which improve the stability of siRNAs [35]. As a result, the stability of siRNAs has been increasing. Once these hurdles are overcome, the siRNA therapy will bring tremendous benefits to cancer patients.

5.4. Permeable dominant negative peptides that interfere with interaction between UHRF1 and its binding partners

A permeable dominant negative peptide, which is partial region of UHRF1 or that of its binding proteins, can also be utilized for interfering with UHRF1's bindings to its partners. As an example, a peptide derived from AMAP1 specifically blocked AMAP1/cortactin binding and effectively inhibited breast cancer invasion and metastasis [36]. Because UHRF1 interacts with various proteins including Rb, HDAC1, UHRF1BP1, DNMT1, PCNA, G9a, and methylated H3K9 [9,13–15], if inhibition of some of these bindings can cause apoptosis or cell cycle arrest, peptide therapy can be applied. The biggest problem is stability of the peptide. A DDS that provides cancer specific delivery and also increases stability of the peptide is required.

5.5. Cancer vaccines utilizing UHRF1 peptides

Although UHRF1 is moderately expressed in bone marrow, overexpression of UHRF1 is more significant in some types of cancers, as we described above. Cancer antigens, which are also called oncoantigens and overexpressed in tumor cells, are digested into peptides in cells and often presented on cytoplasmic membrane with HLA-class I molecules, which are recognized by T cells. Thus, UHRF1 may be such a cancer antigen. If so, UHRF1 peptides can be used as cancer vaccines to stimulate the immune systems of cancer patients. Cancer vaccines have had limited success so far. One of the reasons is that the therapy has been applied to cancer patients in the late stage. In these patients, tumor burden is much larger than the ability of the innate immune cells of patients, causing difficulty in removing all cancer cells. Now cancer vaccine protocols are being revised concerning the treatment of cancer patients. When a cancer vaccine is used for cancer patients at an early stage or for patients in the setting of minimal residual disease states, more effect is expected. There is a potential concern regarding the generation of autoimmunity when the immune system does not shut down appropriately after vaccine stimulation. Many clinical trials are now ongoing worldwide to evaluate the effect and the concern of this therapy.

6. Possible side effects

Expression of UHRF1 is increased in proliferating cells, and almost no expression is detected in quiescent cells [9,37]. Recently our results showed that UHRF1 is not expressed in vital organs including heart, lungs, liver, kidneys, and bladder at the protein level [29]. However, *UHRF1* is expressed in thymus and bone marrow at relatively high levels among normal tissues [37]. The thymus atrophies after puberty during a process directed by the high levels of circulating sex hormones, and is replaced with fat. Thus, interference with UHRF1 functions in thymus by molecular targeted therapies will not cause any side effects in adult cancer patients. However, expression of UHRF1 in bone marrow may cause moderate myelosuppression. There is a small molecular compound named suberoylanilide hydroxamic acid (SAHA) which interferes with HDAC1, -3, and -6 activities. Expression levels of HDAC1, -3, and -6 in bone marrow are 1.3–1.6 times higher than that of UHRF1 in bone marrow according to the GNF SymAtlas (http://symatlas.gnf.org/SymAtlas/). SAHA was approved for treating cutaneous T cell lymphoma (CTCL) by the US Food and Drug Administration (FDA) in 2006. So far, no report of myelosuppression caused by the drug has been reported. Thus, expression of UHRF1 in bone marrow may not cause myelosuppression.

7. Role of UHRF1 in toxoplasmosis

Recently, it was reported that UHRF1 has significant roles not only in carcinogenesis, but also in toxoplasmosis [6]. Here we discuss roles of UHRF1 in toxoplasmosis and also possible therapies targeting UHRF1 or its pathway (Fig. 3).

7.1. T. gondii and toxoplasmosis

T. gondii is an obligate intracellular parasite estimated to infect about one-third of the world's human population [38]. *T. gondii* is able to modulate host cell functions to support its multiplication and growth. It affects many physiological phenomena such as apoptosis and cell proliferation [6,39]. The parasite is autonomous for most of its needs regarding synthesis and transport, but it must be intracellular because it is auxotrophic for several metabolites such as tryptophan [40], arginine [41], polyamines [42], purines [42], cholesterol [44], iron [45] and other essential nutrients. *T. gondii* can efficiently access these metabolites through pores in the parasitophorous vacuole membrane [46] or, for cholesterol, from host lysosomal compartments by an active mechanism. The host endocytic structures are translocated along host microtubules to the parasite [47].

Human infection with *T. gondii* occurs transplacentally or by ingesting uncooked infected meat, contaminated vegetables, and/or contaminated water. Primary infection in pregnant women results in transplacental transmission of the parasite and causes congenital toxoplasmosis. The consequence of congenital toxoplasmosis may provoke abortion, fetal death, hydrocephalus, mental retardation, and retinochoroiditis [43]. The acute phase of *T. gondii* infection is usually asymptomatic. It can result in a flu-like syndrome accompanied by lymphadenopathy [44]. During the acute phase, the host immune response limits the spread of the parasite and leads to the formation of cysts in organs like eye, brain, and muscles [45]. The formation of cysts is characteristic of the chronic phase of infection. They persist for the entire host-life and are usually well tolerated by the body. However, recent studies show that infection by *T. gondii* could be an important factor promoting the onset of neurological disorders such as schizophrenia [46]. In immunocompromised patients, such as HIV infected patients, and fetuses, *T. gondii* infection can be severe and lethal. Ocular toxoplasmosis occurs during acute acquired infection, during active congenital infection and in immune compromised persons usually as reactivation disease. Depending on the patient's age, ocular symptoms vary presenting with reduced visual acuity, strabismus, nystagmus, and retinochoroiditis in young children [44]. The brain damage called toxoplasmosis encephalitis is a major complication of toxoplasmosis in immunocompromised patients. It occurs in a primary infection or in cyst reactivation [47]. At the present time, there are no vaccines or drugs eliminating the intracellular parasite.

7.2. The role of UHRF1 in T. gondii-infected cells

Recent data demonstrated that UHRF1 is significantly overexpressed in *T. gondii*-infected cells [6]. Moreover, a dysregulation of the host cell cycle after infection has recently been reported [6,48]. *T. gondii* induces G1/S transition in infected cells, followed by G2 arrest probably caused by cyclin B down-regulation (Fig. 2C). Thus, UHRF1 may be exploited by the parasite to control host *cyclin B* gene expression. Moreover, while parasite proliferation was normal in cells that were in G2 phase, it was suppressed in G1-arrested cells induced by UHRF1-siRNA, indicating that *T. gondii* prefers G2 phase for its proliferation. This suggests that *T. gondii* creates a suitable environment for its own proliferation by controlling UHRF1 expression [6]. Targeting UHRF1 may be a new way to prevent not only their proliferation but also parasitic intracellular persistence. Other recent data showed that *T. gondii* manipulates the host epigenetic machinery to control the host cell genome [49]. Because UHRF1 links epigenetic modifications including DNA methylation, histone methylation, and heterochromatin formation (see Section 2), *T. gondii* may exploit UHRF1 to control host cell epigenetic machinery dynamically. Because UHRF1 recruits a DNA methylase and also a histone methylase [4,14], aberrant overexpression of UHRF1 may cause hypermethylation of host genome including the *cyclin B* promoter region which contains many CpG islands [50], although probably *cyclin B* is not the only gene controlled by UHRF1. In any case, these epigenetic changes may contribute to a suitable environment for parasite intracellular growth.

7.3. Possible involvement of UHRF1 in regulation of proinflammatory cytokines by T. gondii

T. gondii modifies host signaling pathways to establish an anti-apoptotic environment, subverts immune cells, and ensures its dissemination [51]. The parasite developed a strategy to bypass apoptosis in order to conserve the integrity of the host cells by blocking apoptotic mitochondrial pathways of the host cells [52], and by preventing overinduction of proinflammatory mediators produced by the innate immune system. Activation of the phosphoinositol 3 kinase pathway in *T. gondii*-infected cells leads to inactivation of the pro-apoptotic factor Bad and to activation of the anti-apoptotic NF-κB pathway [53]. A parasite kinase released from *T. gondii* is suggested to be responsible for phosphorylation of IκB, which is the inhibitor of NF-κB [54]. *T. gondii* also exploits STAT3 to down-regulate IL-12 and TNF-α, which are proinflammatory mediators required for macrophage activation and nitric oxide production [55]. *T. gondii* also interferes with histone H3 phosphorylation and acetylation to prevent chromatin remodeling at the *TNF-α* promoter region, leading to down-regulation of several proinflammatory mediators, which are regulated by TNF-α [49]. Because *T. gondii* induces UHRF1, which interacts with HDAC1, G9a, methyl-CpG, and also methylated H3K9 [9,13,14], *T. gondii* may utilize UHRF1 to interfere with the chromatin remodeling at the *TNF-α* promoter region.

8. Possible molecular targeted therapies of toxoplasmosis by interfering with the parasite–host interactions

Intracellular cysts are resistant to current drug treatments. Identification of cell pathways co-opted by *T. gondii* is important to discover novel drug targets to treat and prevent toxoplasmosis. There are several drugs that are used for toxoplasmosis treatment such as Pyrimethamine, Sulfadiazine, and Spiramycin. These drugs, which target *T. gondii* itself, are still the main treatments for *T. gondii* infection in pregnancy, infants with congenital toxoplasmosis, and ocular toxoplasmosis [56]. But there are no drugs that target host cell circumstance. Here we propose a new class of medicines that target the host cell state to prevent further expansion and persistence of *T. gondii*. These medicines should be used with the current drugs targeting *T. gondii* itself. Although targeting UHRF1 can be effective, we also consider that drugs, which can interfere with the UHRF1 pathway, can be effective as well.

8.1. Molecular therapies utilizing drugs currently in clinical use for different purposes

8.1.1. Molecular therapy targeted cell cycle status of host cells

It was shown that cells parasitized by *T. gondii* release a factor capable of inducing uninfected cells to enter the S phase of the cell cycle. This allows *T. gondii* to more readily invade new cells in the S phase [57]. In addition, *T. gondii* exploits UHRF1 and facilitates G1/S transition [6]. Therefore, the use of a drug modifying the status of the host cell cycle may disrupt the proliferation of the parasite. Paclitaxel, which is one of the G1 arrest drugs in clinical use, was reported to suppress intracellular *T. gondii* proliferation in drug-pretreated confluent fibroblast cells, although the infection ratio was not different between treated and untreated cells, indicating that the drug cannot prevent infection, but can prevent proliferation of the parasite [58].

8.1.2. Molecular therapy targeted nutriments acquisition

UHRF1 downregulation can stop the cell cycle at the G1 phase. If G1 arrest lingers, cells may enter the G0 phase, which provides poor nutrients to *T. gondii*. Thus, down-regulation of UHRF1 may suppress proliferation of *T. gondii* through lack of nutrients also. *T. gondii* utilizes the host microtubule network to acquire nutrients. A major role for microtubules is to support the membrane vesicle movements involved in cellular membrane trafficking [59]. To interfere with the dynamics of the microtubular network, Taxol, which acts on the microtubules' hyperstabilization and impairs their dynamic activity [60], can stop the sequestration of host lysosomes by the parasite, thus denying them of cholesterol. Thus, Taxol may inhibit proliferation of *T. gondii*.

8.1.3. Molecular therapy targeted host chromatin modulation

Another strategy could be to reverse the DNA methylation-induced silencing of several genes manipulated by the parasite, which is suggested by our preliminary data (not shown). A cytidine analog, azacitidine, is a DNA methylation inhibitor, which inhibits activity of DNA methyltransferases in replicating cells. Azacitidine might be an effective drug against toxoplasmosis. Azacitidine is mainly used in the treatment of myelodysplastic syndrome (MDS) with minor adverse reactions, for which it received approval by the U.S. FDA in 2004. Other epigenetic changes also have potential to be targeted in the development of novel therapeutic approaches. Because *T. gondii* targets the histone modification machinery, probably partially through the association with UHRF1, which recruits HDAC1 [9], HDAC inhibitors such as SAHA (see Section 6) may have a potential to treat toxoplasmosis.

8.2. Molecular therapies which target UHRF1 directly

Compared with these drugs which are currently in clinical use for different purposes, development of molecular therapies targeting UHRF1 may take a longer time before clinical trials. However, as UHRF1 is specifically induced in *T. gondii*-infected cells and expresses at very low level in uninfected cells, UHRF1 targeting therapies may have smaller side effects compared with therapies utilizing the drugs currently in clinical use for different purposes.

As described above for cancer therapy (see Section 5), the use of UHRF1 inhibitors such as UHRF1-siRNA or small molecular

compounds might prevent parasite proliferation and persistence in host cells by inducing G1 arrest. The persistence of the parasite particularly occurs in ocular toxoplasmosis, which is one of the major complications of congenital toxoplasmosis. Congenital ocular toxoplasmosis is a disease dreaded due to the risk of permanent blindness. It modulates the cytokines' profile in the aqueous humor of ocular toxoplasmosis patients [61]. Study of the role of UHRF1 regulation in eyes, which is an immunologically privileged organ, may bring a new insight for developing drugs. If UHRF1 plays a significant role in ocular toxoplasmosis, intraocular injection of UHRF1-siRNA can be applied in combination with conventional anti-infectives, because the intraocular injection of siRNA has already been tested in humans for the treatment of choroidal neovascularization [62]. If effective small molecular compounds which interfere with UHRF1 functions are developed, these compounds can also be applied to treat ocular toxoplasmosis as eye-drops.

9. Conclusion

UHRF1 links many epigenetic modifications through its multiple domains and also various binding partners. UHRF1 is essential for cancer progression and also for G1/S transition, which is required for the proliferation of *T. gondii*, which prefers G2 phase for its proliferation. Expression of UHRF1 is up-regulated in various cancers, and thus UHRF1 may be a cancer antigen, which can be used for cancer vaccination. Knockdown of UHRF1 suppressed cancer cell proliferation, indicating that UHRF1 is a very attractive molecule for molecular targeted therapies, and also a diagnosis- and prognosis-marker of cancers. Because knockdown of UHRF1 also inhibited proliferation of *T. gondii*, the molecular targeted therapies can be applied for treating toxoplasmosis to disrupt the parasite–host interaction, with a combination of current drugs targeting *T. gondii* itself.

Conflict of interest statement

None declared.

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