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Review article

Role of UHRF1 in malignancy and its function as a therapeutic target for molecular docking towards the SRA domain

Sravani Polepalli[a], Sophia M George[a], R Valli Sri Vidya[a], Gabriel Sunil Rodrigues[b], L. Ramachandra[b], Raghu Chandrashekar[c], Deepak Nayak M[d], Praveen P.N. Rao[e], Richard G. Pestell[f,**], Mahadev Rao[a,*]

[a] Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India
[b] Department of General Surgery, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India
[c] Department of Pharmaceutical Biotechnology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India
[d] Department of Pathology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India
[e] School of Pharmacy, Health Sciences Campus, University of Waterloo, Waterloo, ON, N2L 3G1, Canada
[f] Pennsylvania Cancer and Regenerative Medicine Research Center, Baruch S. Blumberg Institute, Pennsylvania Biotechnology Center, Wynnewood, Pennsylvania, 19096; Chief Medical Officer, CytoDyn Inc, 111 Main St, Suite 660, Vancouver, WA, 98660, USA

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ABSTRACT

The figure describes the location of UHRF1 (Ubiquitin-like with containing PHD and RING Finger domains 1) gene, mRNA and protein synthesis in the tumor cell and its structural domains with a focus on the docking of Naphthazarin on the SRA domain of UHRF1, resulting in reduction of tumor size.

1. Introduction

Cancer is a malignant disorder arising as a result of unregulated genetic and epigenetic events governing cellule metabolism, survival and proliferation. This disruption results in the downregulation of tumour suppressor genes (TSGs). Globally 18.1 million new cancer cases and about 9.6 million cancer related deaths occur per year (Bray et al., 2018). This rising mortality calls for the attention of health care professionals and basic scientists to probe into the underlying epigenetic factors which could not only tackle resistance and failure to conventional chemotherapy but also bring personalized medicine to the bedside. A heritable alteration in the gene expression without modifying the primary DNA sequence precisely defines epigenetics (Dupont et al., 2009). UHRF1 is regarded as a 'Universal Oncogene' due to its increased expression in numerous malignancies, as reported by multiple studies conducted across the globe over the past decade (Jin et al., 2010a, 2010b; Yang et al., 2012; Kim et al., 2017; Zhu et al., 2015; Babbio et al., 2012; Unoki et al., 2009a; Ge et al., 2016; Yan et al., 2015; Azam et al., 2016; Pita et al., 2009; Qin et al., 2014; Zhang et al., 2016; Pi et al., 2013; Yang et al., 2013). UHRF1 has emerged as a potential biomarker and a promising target for cancer therapy. Recently Xue et al has documented about the oncogenic and therapeutic potential of UHRF1 in numerous cancers and the drugs that are effective at several points of the gene or the cell cycle (Xue et al., 2018). Ubiquitin-

Abbreviations: TSGs, Tumour suppressor genes; ICBP90, Inverted CCAAT box binding protein 90; UHRF1Ubiquitin-like, with containing PHD and RING Finger domains 1; UBL, Ubiquitin-like domain; TTD, Tandem Tudor domain; PHD, Plant Homeo Domain; SRA, Set and Ring Associated; RING, Really Interesting New Gene; NIRF_N, Novel Np95/ ICBP90-like RING finger protein N-terminus; DNMT1, DNA methyltransferase 1; HDAC1, Histone deacetylase 1; EMT, Endothelial to mesenchymal transition; LSCC, Laryngeal squamous cell carcinoma; ESCC, Esophageal squamous cell carcinoma; OS, Overall survival; PFS, Progression-free survival; EGCG, Epigallocatechin-3-gallate; TQ, Thymoquinone; NASTRP, Naphthol AS-TR phosphate; DHA, Dihydroartemisinin; MDR, Multidrug resistance

* Corresponding author at: Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India.

** Corresponding author at: President Pennsylvania Cancer and Regenerative Medicine Center, Institute Distinguished Professor, Baruch S. Blumberg Institute, Pennsylvania Biotechnology Center, Wynnewood, Pennsylvania, 19096, USA.

E-mail addresses: sravani.polepalli2894@gmail.com (S. Polepalli), sophiamgeorge.108@gmail.com (S.M. George), vallisrividyar@gmail.com (R. Valli Sri Vidya), gaby.rodrigues@manipal.edu (G.S. Rodrigues), ramachandra.l@manipal.edu (L. Ramachandra), raghu.c@manipal.edu (R. Chandrashekar), deepak.nayak@manipal.edu (D.N. M), praoperampallinekkar@uwaterloo.ca (P.P.N. Rao), richard.pestell@gmail.com (R.G. Pestell), mahadev.rao@manipal.edu (M. Rao).

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like with containing PHD and RING Finger domains 1 (UHRF1) also known as inverted CCAAT box binding protein 90 (ICBP90) is one of the key epigenetic regulators involved in the process of cell division. It is highly expressed in the proliferating cells and is essential for G1/S transition (Mousli et al., 2003). It plays a vital role in the silencing of tumour supressor genes (TSGs) (Babbio et al., 2012) and in the epigenetic inheritance (Unoki et al., 2009b), resulting in cell proliferation and consequent development of cancer. UHRF1 has gained significant recognition over the past few years due to its unique ability to link epigenetic pathways such as DNA methylation and histone modification (Bronner et al., 2013). Elevated levels of UHRF1 correlated with silencing of tumour suppressor genes through DNA methylation and histone modifications and poor prognosis of cancer (Ashraf et al., 2017; Boukhari et al., 2015; Wang et al., 2012). UHRF1 over expression was found to cause epigenetic silencing of tumour suppressor gene and subsequent UHRF1 knockdown resulted in transcriptional reactivation (Beck et al., 2018). Knockdown of UHRF1 was found to decrease the methylation level in the promoter of a key regulator of necroptosis-receptor-interacting kinase-3 (RIP3), resulting in their increased expression and consequently leading to decreased tumour growth (Yang et al., 2017). UHRF1 knockdown was found to lower cancer cell growth, migration, invasion and increase apoptosis (Zhang et al., 2018a, 2018b). Disruption of the PHD or SRA domain functions was found to reverse the DNA hypermethylation and reactivate the silenced TSG in the cancer cells (Kong et al., 2019). Multiple studies have shown UHRF1 to be a potential regulator of cell proliferation and progression of cancer by multiple signalling pathways (Chen et al., 2019; Gao., 2017; Wei et al., 2018; Wan et al., 2016; Ma et al., 2012). These studies have established the potential role of UHRF1 in the genesis of cancers.

In response to the emerging significance of this gene in the domain of medicine and research, the current study focuses on the role of UHRF1 in several cancers and its significance as a druggable target. The study was formulated after extensive literature search from 97 articles collected through PubMed and Google Scholar using the keywords - 'UHRF1', 'Cancer', 'UHRF1 Inhibitors', 'Docking of SRA domain' and 'target' using Boolean operators "AND/OR."

2. Structure and functions of UHRF1

Initially, UHRF1 was assumed to regulate the expression of topoisomerase IIα by binding to an inverted CCAAT box located in its promoter (Hopfner et al., 2000). Subsequent studies determined that UHRF1 plays a prominent role in several epigenetic pathways using its structural domains. The UHRF1 gene consists of 59,075 bases and is located on the 19th chromosome in the cytogenetic band of 19p13.3. (Fig. 1) ("UHRF1 Gene - GeneCards | UHRF1 Protein | UHRF1 Antibody," n.d.) It consists of an Ubiquitin-like domain (UBL) at the N-terminal followed by Tandem Tudor domain (TTD), Plant Homeodomain (PHD), Set and Ring Associated (SRA) domain and the Really Interesting New Gene (RING) finger domain which is located at the C-terminal (Bronner et al., 2013). UBL synonymously called as NIRF_N (novel Np95/ ICBP90-like RING finger protein N-terminus) is a structural unit that consists of conserved surface lysine's K31 and K50, which are targets of mono- or poly-ubiquitination. (pdb entry 2FAZ, unpublished structure) TTD with the help of its two subdomains; namely TTD_N and TTD_C senses di- and tri- methylated lysine 9 of histone H3 (H3K9me2/ H3K9me3) (Nady et al., 2011). It confers a unique property to this multidomain ubiquitin ligase to interlink DNA methylation and histone modifications (Bronner et al., 2013). The adjoining PHD is a Zn-finger domain which identifies the N-terminus of the H3-tail only when unmodified (H3unmod (N-term)) (Rajakumara et al., 2011). SRA domain plays a major role in recognizing the hemimethylated DNA (Avvakumov et al., 2008) and permits UHRF1 to recruit DNA methyltransferase 1 (DNMT1), a maintenance methyltransferase, to the replication foci in a cell-cycle dependent manner (Greiner et al., 2015; Kilin et al., 2017). The sensitivity and specificity of UHRF1 to DNMT1 is revealed in the fact that UHRF1 and DNMT1 intercommunication resulted in a rise in the affinity of DNMT1 to target hemimethylated DNA (Bashtrykov et al., 2014). RING domain has an E3 ubiquitin ligase activity (Freemont, 2000; Pickart, 2001). This domain plays an important role in histone H3 Lysine 23 (H3K23) monoubiquitination during the S-phase (Nishiyama et al., 2013). The RING domain serves as an essential pre-requirement for recruiting DNMT1 to the target sites of the gene (Nishiyama et al., 2013). Hence it can be generalised that multiple domains of this gene do not work independent of each other to achieve the functional outcome. In an overview, UHRF1 regulates and preserves DNA methylation pattern inheritance during the cell cycle, it acts as a sensor of DNA crosslinks and it facilitates DNA demethylation during development. Thus, any disruption in one or more of these functions would lead to significant genomic alterations, resulting in cancer.

3. Role of UHRF1 as an epigenetic marker

Lately, epigenetic research has found that the presence of constitutional domains confers UHRF1 with the ability to simultaneously detect both methylated DNA and the histone code. Reader motifs that synchronize signal transduction and direct epigenetic signals allow the modification of histone and non-histone proteins (Powell et al., 2011). Malignant cells undergo global DNA hypomethylation while, few regions, such as the promoters of TSGs in contrast, undergo hypermethylation (Jones and Baylin, 2007). Nε -acetylation of lysine residues is a key histone modification involved in transcription and DNA repair (Dawson and Kouzarides, 2012). This epigenetic pathway is regulated by lysine acetyltransferases (KATs) and protein deacetylases such as HDACs (Dawson and Kouzarides, 2012). Histone deacetylation is carried out by HDACs, a cluster of enzymes that reverse lysine acetylation and restores the positive charge on the side chain (Dawson and Kouzarides, 2012). HDAC inhibitors are capable of reversing some of the atypical gene repressions and subsequently induce cell cycle arrest and/or programmed cell death of the malignant cells (Federico and Bagella, 2011).

4. UHRF1 expression in several cancers

4.1. Breast cancer

Breast cancer is one of the most prevalent malignancies globally, in the spectrum of cancer among women. It was found to have an incidence of approximately 1.7 million cases in 2012 and a noteworthy risk of recurrence was experienced by 1/3rd of the diagnosed patients. The disease therefore, accounts for unfavourable clinical outcomes in addition to a heightened mortality associated with the advancement of the disease. Global statistical data account breast cancer to constitute 25% of all cancers in women (Ferlay et al., 2014). The elevated levels of UHRF1 were found to be responsible for regulating BRCA1 transcription by deacetylation of H3 and H4, which facilitated the recruitment of histone deacetylase1 [HDAC1], DNA methyltransferase1 [DNMT1] and histone lysine methyltransferase G9a to BRCA1 promotor in sporadic breast cancer cells (Jin et al., 2010a). Thus it can be concluded that BRCA1 transcription is regulated by UHRF1. Elevated levels of UHRF1 expression causes silencing of the expression of KLF17 through CpG island methylation on its promoter resulting in breast cancer cell proliferation and migration (Gao et al., 2017). The results from the cBioPortal for Cancer Genomics revealed that UHRF1 was overexpressed in breast invasive carcinomas. TCGA Pan Cancer atlas statistics reveal that UHRF1 mRNA was overexpressed in 76 samples out of the 1082 breast cancer samples (Gao et al., 2013; Cerami et al., 2012; Berger et al., 2018).

4.2. Bladder cancer

UHRF1 overexpression increases the methylation of CpG

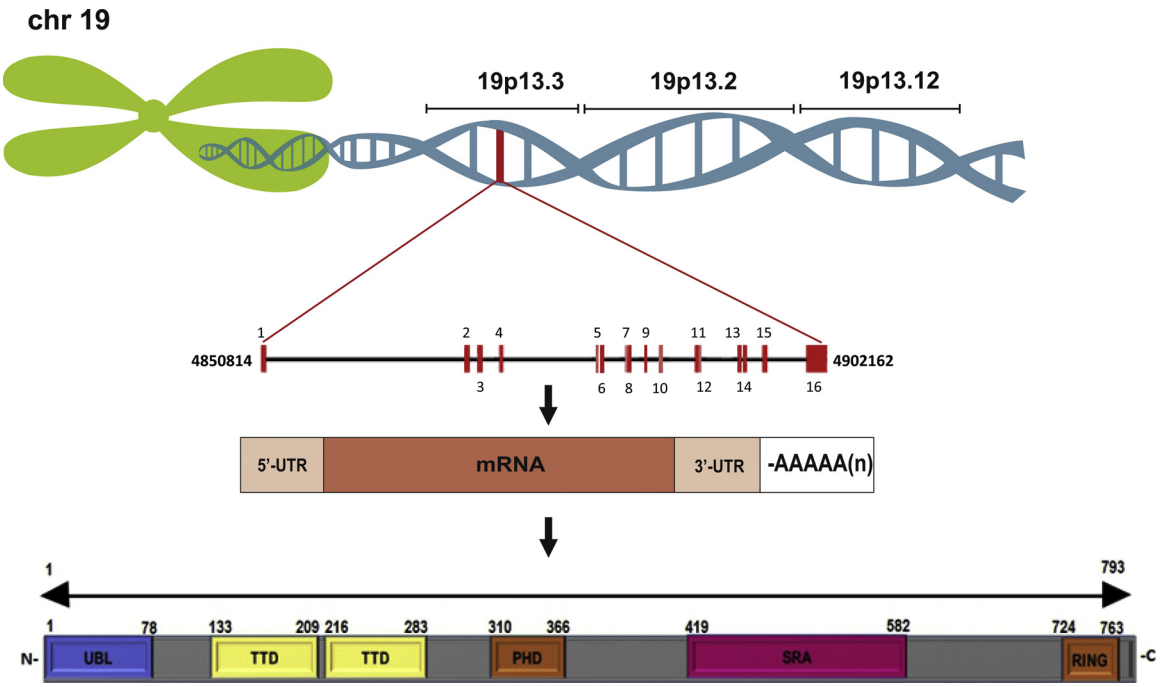

Fig. 1. Transcription and Translation of UHRF1. An overview of the pathway of UHRF1 mRNA and protein synthesis through transcription and translation respectively.

dinucleotides causing epigenetic silencing of the KISS1 gene leading to an increase in the bladder cancer cell invasion, whereas forced expression of KISS1 partially abrogated UHRF1 induced cell invasion (Zhang et al., 2014). Elevated levels of UHRF1 was associated with increased risk of progression and correlated with the stage and grade of the cancer (Unoki et al., 2009b) and the knockdown of the UHRF1 reduced the cell invasion (Zhang et al., 2014). A study by Saidi et al demonstrated that UHRF1 gene expression was found to be approximately 2.5 times higher in samples of TCC (Transitional Cell Carcinoma) in comparison with normal epithelium of control group patients (Saidi et al., 2017). A study conducted by Yang et al shows that patients having higher UHRF1 expression had poorer survival and prognosis than in patients with lower UHRF1 levels. In comparison to normal cells, UHRF1 expression was found to be higher in non- muscle invasive cancers and directly correlated with tumour malignancy (Yang et al., 2012).

4.3. Lung cancer

Elevated levels of UHRF1 was observed in all the histological variants of lung cancer, particularly in non-adenocarcinomas (non-ADCs). About 50% of the lung cancer patients showed an increased level of UHRF1 in the early pathological stage (T0 – T1) which confirms the reliability and sensitivity of this epigenetic marker (Unoki et al., 2010). A study conducted in non-small cell lung cancer patients documented UHRF1 to be a fundamental epigenetic factor, which regulates the cell cycle by means of its characteristic ability to sustain the transcriptional silencing of TSGs by maintaining their promoters in a hyper methylated status. Elevated expression of UHRF1 in tumour tissues was proportional with the hyper methylation of CDKN2A and RASSF1, while the knockdown of UHRF1 prevented these genes from hypermethylation. UHRF1 overexpression was a reliable predictor of the aggressiveness of lung cancer resulting in a poor prognosis.

4.4. Liver cancer

UHRF1 overexpression regulates the tumour suppressive long non coding RNA maternally expressed gene3 [MEG3] via promoter hyper methylation by induction of p53 (Chang et al., 2016). Knockdown of UHRF1 inhibited the tumour growth by inducing cell cycle arrest at G2/M phase. A study found that targeting UHRF1 decreased the migration and invasion of cancer cells by hampering the endothelial to mesenchymal transition [EMT] (Kim et al., 2017). Another study conducted on hepatoblastoma cells by Beck et al have proven that UHRF1 up regulation led to silencing of TSGs thereby sustaining the growth of cells. Thus, UHRF1 can be used as prognostic biomarker marker for assessing the cancer progression (Beck et al., 2018). UHRF1 deficiency is found to trigger upregulation of CXCR4, thereby leading to the activation of AKT and JNK and consequently resulting in an increased secretion of IL-6 (Kim et al., 2017).

4.5. Colorectal cancer

UHRF1 over expression negatively regulates peroxisome proliferator-activated receptor gamma (PPARG), through epigenetic-dependent mechanisms (Sabatino et al., 2012). An advanced clinical stage, proliferation and migration accompanied by the silencing of $p16^{INK4a}$ were attributed to the upregulation of UHRF1 (Wang et al., 2012). Elevated levels of UHRF1 was associated with a poor clinical staging and reduced survival rate and was inversely related to the levels of regulatory miRNA-9 (Zhu et al., 2015).

4.6. Prostate cancer

An immunohistochemical analysis done on 266 prostate cancer samples demonstrated a correlation of increased UHRF1 levels in the progression of malignancy and fatality (Babbio et al., 2012). Consistent results were obtained by another study conducted by Wan X et al. on prostate cancer cell lines. This study documented UHRF1 as one of the oncogenes in prostate cancer and its utility in the determination of the risk of biochemical recurrence in patients after radical prostatectomy (Wan et al., 2016). UHRF1 was also identified to be an independent factor of prognosis and survival determination in prostate cancer (Wan et al., 2016). Elevated levels of UHRF1 has been observed in prostate cancer cell lines. Shijuan et al has shown that UHRF1 knockdown resulted in G1 cell arrest, demethylation of p16INK4A, and apoptosis in

prostate cancer cells (Du et al., 2017). An analysis of prostate cancer samples has shown that UHRF1 was overexpressed in significant portion of tumours (Jazirehi et al., 2014).

4.7. Renal cell carcinoma

UHRF1 mRNA levels were associated with stages ($p = 0.0005$) and grades ($p = 0.0093$) in renal cell carcinoma (RCC) patients (Unoki et al., 2009a). Patients who had high UHRF1 levels showed a significantly poor survival rate when compared to patients with low UHRF1 expression ($p = 0.0096$) (Unoki et al., 2009a). A systematic investigation conducted by Ma et al showed an overexpression of UHRF1 in 70% of RCC tissues, that was achieved through suppression of the p53-dependent activation and apoptosis in ccRCC cell (Ma et al., 2015). Goto Y et al has demonstrated that the knockdown of UHRF1 resulted in the downregulation of pathways such as cell cycle progression, DNA replication, RNA degradation, mismatch repair, RNA transport and nucleotide excision repair, resulting in cell death (Goto et al., 2016). An increased level of UHRF1 mRNA was observed in RCC patients diagnosed with metastasis when compared to that of patients who are not diagnosed with metastasis and in healthy individuals (Wotschofsky et al., 2016). Varol et al has reported that UHRF1 was overexpressed in renal cancer cell lines (Varol et al., 2015).

4.8. Cervical cancer

UHRF1 levels were found to be markedly elevated in cervical squamous cell carcinoma tissues compared to the adjacent normal tissues and UHRF1 knockdown inhibited cancer progression (Ge et al., 2016) Zhang Q et al has reported higher levels of UHRF1 in HPV oncogene E7 expressing cells and HPV-positive cervical cancer cells (Zhang et al., 2018a,2018b). Li et al has shown the role of UHRF1 as a negative regulator of radio-resistance in cervical cancer patients (Li et al., 2009). Yim EK et al has proposed UHRF1 to be one of the genes involved in tumour progression (Yim et al., 2009).

4.9. Ovarian cancer

Research conducted on 80 paired tissue samples has revealed that UHRF1 expression was considerably higher in ovarian tumour cells in comparison to their normal counterparts and UHRF1 inhibition lead to apoptosis initiation (Yan et al., 2015). UHRF1 was reported to be one of the differently expressed gene in the pathogenesis of epithelial ovarian cancer (Shi and Zhang, 2017). Francis et al has demonstrated that the amplification of UHRF1 levels were high in the poorly differentiated lesions in ovarian cancer patients (Enane et al., 2018). Reduction of UHRF1 levels by CDDO-Me resulted in the suppression of ovarian cancer tumour growth (Qin et al., 2016).

4.10. Gastric cancer

A 2- fold rise in UHRF1 levels was observed in gastric cancerous tissues when compared to normal tissues and the levels could be correlated to stage IV and grade III of cancer ($p < 0.05$). Similar correlation between stage and grade has been reported by Soleimani A et al. (Soleimani et al., 2016) UHRF1 was found to be an independent predictor of prognosis in GC patients (Zhou et al., 2015). UHRF1 was associated with the promotion of growth, invasion and migration of MGC803 and SGC7901 cells (Zhang et al., 2018a, 2018b). Conversely Babacan et al reported that UHRF1 had no role as a prognostic marker in gastric cancer (Babacan et al., 2016). UHRF1 DNA levels were elevated in gastric cancer patients and the levels correlated with the age and lymph node metastasis (Ge et al., 2015).

4.11. Other cancers

UHRF1 showed overexpression in a broad array of malignancies such as thyroid cancer (Pita et al., 2009), gallbladder cancer (Qin et al., 2014), medulloblastoma (Zhang et al., 2016), laryngeal squamous cell carcinoma (LSCC) (Pi et al., 2013), esophageal squamous cell carcinoma (ESCC) (Yang et al., 2013) and pancreatic cancer (Crnogorac-Jurcevic et al., 2005).

5. UHRF1- prognosis and survival

Several studies have shown that the rate of proliferation of the cells was directly proportional to the levels of UHRF1 in most of the cancers like breast (Gao et al., 2017), bladder (Zhang et al., 2014; Yang et al., 2012), gastric (Azam et al., 2016), lung (Unoki et al., 2010), liver (Mudbhary et al., 2014), colorectal (Zhu et al., 2015), and prostate cancers. (Wan et al., 2016) A multivariate analysis elucidated that UHRF1 expression was an independent prognostic factor that influenced the overall survival (OS) and progression-free survival (PFS) (OS $p = 0.038$, PFS $p = 0.014$). (Yao et al., 2012) Therefore, patients who displayed elevated levels of the gene were observed to have encountered endpoints of a decreased OS and PFS in comparison to that of normal or nil expression of the same. (Chen et al., 2002) From the above evidence, it can be putatively described that high levels of UHRF1 also correlated with the size of the tumour, stage, metastasis and poor survival rate. Hence, targeting UHRF1 would not only mean a halt in tumorigenesis, angiogenesis, and cell cycle progression but also an improved prognosis, survival and response to radiotherapy (Yang et al., 2013) and chemotherapy. (He et al., 2018) As a result, this newly discovered gene could turn out to be an efficient biomarker for prognosis and survival.

6. Significance of UHRF1 as a therapeutic target

As UHRF1 upregulation leads to proliferation of cancer cells, several studies have been conducted to test its significance as a druggable target (Jin et al., 2010a; Yang et al., 2012; Kim et al., 2017; Zhu et al., 2015; Babbio et al., 2012; Unoki et al., 2009a; Ge et al., 2016; Yan et al., 2015; Pita et al., 2009; Qin et al., 2014; Zhang et al., 2016; Pi et al., 2013; Yang et al., 2013; Crnogorac-Jurcevic et al., 2005). Each domain of UHRF1 can be targeted as a therapeutic target owing to their role in the cell cycle progression. Ubiquitin ligases play a major role in regulating the cell cycle. Hence, they are being investigated to identify next generation therapeutic opportunities for cancer therapy (Senft et al., 2018). As mentioned above, SRA domain of UHRF1 plays a key role in the identification of hemimethylated DNA (Avvakumov et al., 2008). Hence, using small molecules to prevent interaction of SRA domain and hemimethylated DNA might result in the prevention of aberrant DNA methylation (Bashtrykov et al., 2014). The PHD and TTD domains play a major role in the recognition of H3K9me3 (Cheng et al., 2013). The polybasic linker between the SRA and RING domains control the transition between PHD and TTD mediated histone reader states by reversible binding to TTD grove or the phospholipid PI5P (Houliston et al., 2017). Hence preventing the interaction between UHRF1 domains using small molecules might result in the prevention of cell cycle progression (Houliston et al., 2017). Apart from inhibition of UHRF1, HDAC and DNMT1 inhibition has also been studied (Arzenani et al., 2011). However, targeting UHRF1 for anticancer effects may provide drugs with lesser side effects and better efficacy when compared to DNMT1 and HDAC inhibitors as its levels are significantly low in non-cancerous cells (Jenkins et al., 2005; Unoki et al., 2004; Unoki et al., 2009a). However, the development of novel selective UHRF1 inhibitors is challenging.

Repurposing existing drugs to target UHRF1 can decrease the time, cost and labour involved in developing a potential drug. Several natural compounds have been proven to be efficacious in inhibiting the cancer

Table 1
Drugs targeting the domains of UHRF1. A summary of several natural and synthetic compounds that act on UHRF1 or the domains of the gene directly or indirectly.

SNO	DRUGS	TARGETS	REFERENCE
1	Epigallocatechin-3-gallate	UHRF1, DNMT1	(Achour et al., 2013)
2	Thymoquinone	UHRF1, DNMT1, HDAC1	(Alhosin et al., 2010)
3	Luteolin	UHRF1, DNMT1	(Krifa et al., 2014)
4	Limoniastrumguyonianum aqueous gall extract	UHRF1, DNMT1	(Krifa et al., 2013)
5	Aroniamelanocarpa juice	UHRF1	(Sharif et al., 2012)
6	Red wine polyphenolic extract	UHRF1	(Sharif et al., 2010)
7	Naphthol AS-TR phosphate	UHRF1	(Park et al., 2015)
8	Dihydroartemesinin	UHRF1, DNMT1	(Du et al., 2017)
9	Adriamycin	UHRF1, DNMT1	(Arima et al., 2004)
10	Propranolol	UHRF1	(STILES et al., 2012)
11	Cisplatinum	UHRF1	(Jenkins et al., 2005)
12	Etoposide	UHRF1	(Jenkins et al., 2005)
13	Bleomycin	UHRF1	(Jenkins et al., 2005)
14	Nocodazole	UHRF1	(Jenkins et al., 2005)
15	Hydroxyurea	UHRF1	(Jenkins et al., 2005)
16	Paclitaxol	UHRF1	(Jenkins et al., 2005)

cell proliferation by downregulating the levels of UHRF1 (Table 1). Epigallocatechin-3-gallate (EGCG) downregulates UHRF1 and DNMT1 expression in Jurkat cells and up-regulates p73 and $p16^{INK4A}$ (Achour et al., 2013). Another drug thymoquinone (TQ) inhibits Jurkat cell proliferation and causes a halt in the cell cycle at the G1 phase in a concentration-dependent fashion (Alhosin et al., 2010). Treatment with TQ triggered programmed cell death (Alhosin et al., 2010). It induced a re-expression of p53 which led to the down-regulation of UHRF1, DNMT1 and HDAC1 (Alhosin et al., 2010). Luteolin, a 3′,4′,5,7-tetrahydroxyflavone was found to downregulate UHRF1 and caused apoptosis in colorectal cancer cells (Krifa et al., 2014) by inducing cell cycle arrest in G2/M phase and re-expression of TSGs $p16^{INK4A}$. Inhibition of UHRF1/DNMT1 and up-regulation of $p16^{INK4A}$ by Limoniastrumguyonianum aqueous gall extract and luteolin led to the arrest of HeLa cells in G2 phase thereby, leading to their apoptosis (Krifa et al., 2013). Aronia melanocarpa juice (Sharif et al., 2012) and red wine polyphenolic extracts (Sharif et al., 2010) are found to downregulate UHRF1. Naphthol AS-TR phosphate (NASTRP) was found to significantly reduce E2F8 (E2F Transcription Factor 8) consequently leading to UHRF1 downregulation (Park et al., 2015). Dihydroartemisinin (DHA) downregulates UHRF1/DNMT1 and upregulates $p16^{INK4A}$ in a dose-dependent fashion at G1/S phase (Du et al., 2017). A study showed significant p53-dependent reduction in UHRF1 mRNA and protein in HCT116 cells when treated with adriamycin (Arima et al., 2004). Another natural compound naphthazarin, an anti-inflammatory, antitumor, an antioxidant and antibacterial agent, targets UHRF1 in MCF-7 breast cancer cells (Kim et al., 2015). A study conducted on human infantile hemangioma endothelial cells (HemECs) showed that the synthetic β-blocker drug propranolol led to two-fold the downregulation of UHRF1 ($P < 0.05$) (Stiles et al., 2012). Downregulation of UHRF1 has been observed to be higher upon treatment with genotoxic agents like cisplatin, etoposide and bleomycin when compared to taxols, nocodazole and hydroxyurea (Jenkins et al., 2005).

Resistance to chemotherapy is a commonly encountered problem during the treatment of cancer. A study demonstrated that RCC patients undergoing treatment with sunitinib had an overexpression of UHRF1 which was associated with decreased overall survival (OS) following surgery (Goto et al., 2016). The sensitivity of retinoblastoma cells to chemotherapeutic agents such as etoposide and camptothecin dramatically increased with the knockdown of UHRF1 (He et al., 2018). Another study demonstrated that the knockdown of MDR1 expression by UHRF1 might offer potential means to combat multidrug resistance (MDR) in breast cancer therapy (Jin et al., 2010b). From the above studies, it can be concluded that UHRF1 can be a desirable target to develop novel anticancer agents (Fig. 2).

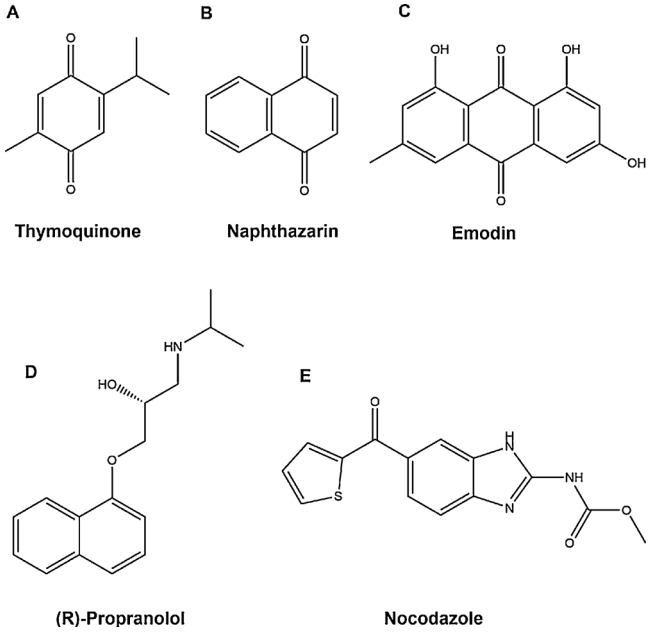

Fig. 2. Chemical structures of thymoquinone, naphthazarin, emodin, (*R*)-propranolol and nocodazole.

7. Molecular docking studies on the SRA domain of UHRF1

The SRA domain of UHRF1 is known to recognize the methylated cytosine of DNA strands and is an emerging target to design small molecule inhibitors of UHRF1 (Myrianthopoulos et al., 2016; Patnaik et al., 2018). The solved X-ray crystal structure of human UHRF1 SRA domain shows that 5-methylcytosine (5 mC) binding cavity is narrow and is lined by amino acids such as Gly448, Ala463, Gly464, Tyr466, Asp469 and Tyr478 (Avvakumov et al., 2008). In this regard, we investigated the potential of some natural (thymoquinone, naphthazarin, emodin) and synthetic compounds ((*R*)-propranolol and nocodazole, Fig. 3) as UHRF1 inhibitors by conducting molecular docking studies within the 5 mC binding site of SRA. The LibDock algorithm available in the computational software Discovery Studio *Structure-Based-Design* (Biovia Inc.) was used (Rao et al., 2015).

The x-ray crystal structure of human UHRF1 SRA domain was obtained from RCSB protein data bank (pdb id: 3BI7, Avvakumov et al., 2008) and prepared using the *macromolecules* module in Discovery Studio (DS) using CHARMm force field. Then a 10 Å sphere was created by selecting Asp469 around the 5 mC binding site. The ligands thymoquinone, naphthazarin, emodin, (*R*)-propranolol and nocodazole

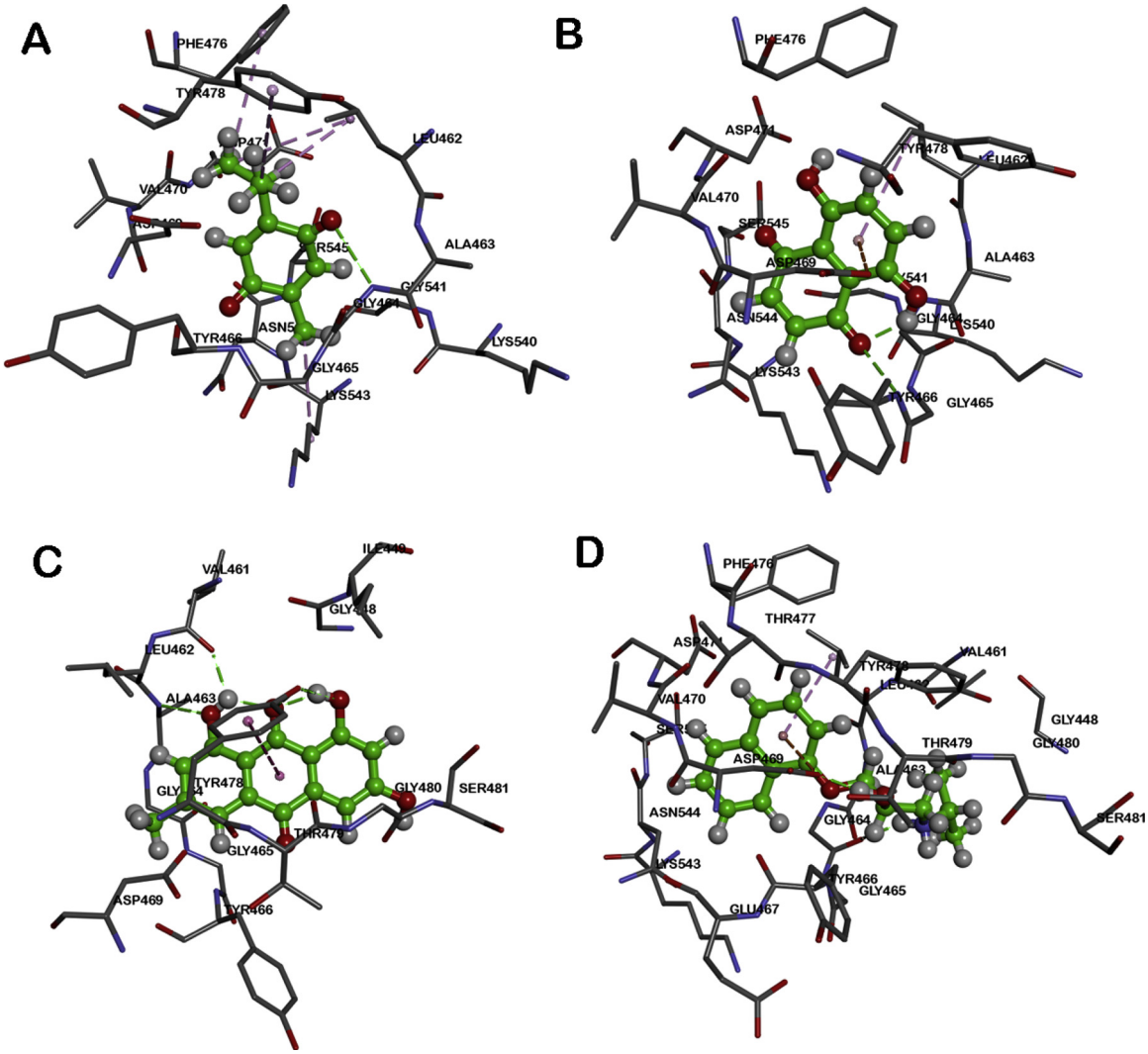

Fig. 3. The binding modes of thymoquinone (Panel A), naphthazarin (Panel B), emodin (Panel C) and (*R*)-propranolol (Panel D) in a ball and stick cartoon within the SRA domain of human UHRF1 (pdb id: 3BI7). Hydrogen atoms are removed to enhance clarity. Polar and nonpolar interactions are color-coded and details are provided in the text.

were built and prepared using the *small molecules* module in DS. They were energy minimized using the *smart minimizer* protocol (200 steps, RMS gradient 0.1 kcal/mol), CHARMm force field and a distance depended dielectric constant. These ligands were docked using the LibDock algorithm by employing 100 hotspots and a docking tolerance of 0.25 Å. Docked poses were subjected to smart minimizer algorithm (0.001 kcal/mol, 1000 steps) using CHARMM force field and distance depended dielectric constant. The binding modes were analyzed by LibDock scoring function and polar/nonpolar contacts in the 5 mC binding site.

The docking studies of the natural phytochemical thymoquinone, on the SRA domain, shows that the planar benzoquinone ring was oriented in the 5 mC binding cavity and one of the quinone ketones, was forming a hydrogen bond with the backbone NH of Gly464 (distance ˜ 2.03 Å, Fig. 3A). The C2 isopropyl substituent was oriented in a hydrophobic region comprised of Leu462, Val470, Phe476 and Tyr478 and underwent π–alkyl and alkyl–alkyl interactions with side chains of Leu462 and Phe476 (distance < ; 5.0 Å). The C5 methyl substituent was closer to the entrance of the cavity near Gly464, Gly541, Lys543 and Asn544. It underwent hydrophobic contact with methylene groups of Lys543 (distance < ; 5.0 Å, Fig. 3A). Molecular docking studies of another natural compound naphthazarin, which is a quinone derivative similar to thymoquinone, shows that it was able to bind in the 5 mC binding cavity and exhibited favourable interactions (Fig. 3B). Both quinone ketone substituents were forming multiple hydrogen bonds with backbone NHs of Gly465, Tyr466 and Asp471 (distance < ; 2.70 Å). One of the hydroxyl groups was in contact with the backbone ketone of Gly464 via a hydrogen bond (distance =2.16 Å). The naphthyl ring itself underwent π–alkyl interaction with side chains of Leu462 (distance < ; 5.0 Å). Interestingly, the naphthyl aromatic ring also underwent π–anion interactions with carboxylate side chain of Asp469 (distance < ; 4.0 Å). Furthermore, we conducted molecular docking studies of another natural compound emodin, which is also a quinone derivative. Interestingly, the central aromatic ring of emodin was stacked against Tyr478 where it underwent π–π T-shaped interactions (distance < ; 5.0 Å) and one of the quinone ketones underwent hydrogen bonding interactions with OH of Tyr478 (distance < ; 3.0 Å, Fig. 3C).

In addition, both C1 and C8 hydroxyl groups were in contact with Gly448, Val461 and Ala463 via hydrogen bonds (distance < ; 3.0 Å). These studies show that planar monocyclic, bicyclic and tricyclic quinones are capable of binding in the 5 mC binding cavity of SRA. A similar docking study of (*R*)-propranolol, which is marketed as a β-blocker to treat cardiovascular conditions, was carried out using the SRA domain of UHRF1. This study shows that the aromatic bicyclic naphthyloxy substituent of (*R*)-propranolol was in contact with Leu462, Asp469, Val470, and Phe476 (Fig. 3D). The naphthyloxy aromatic ring

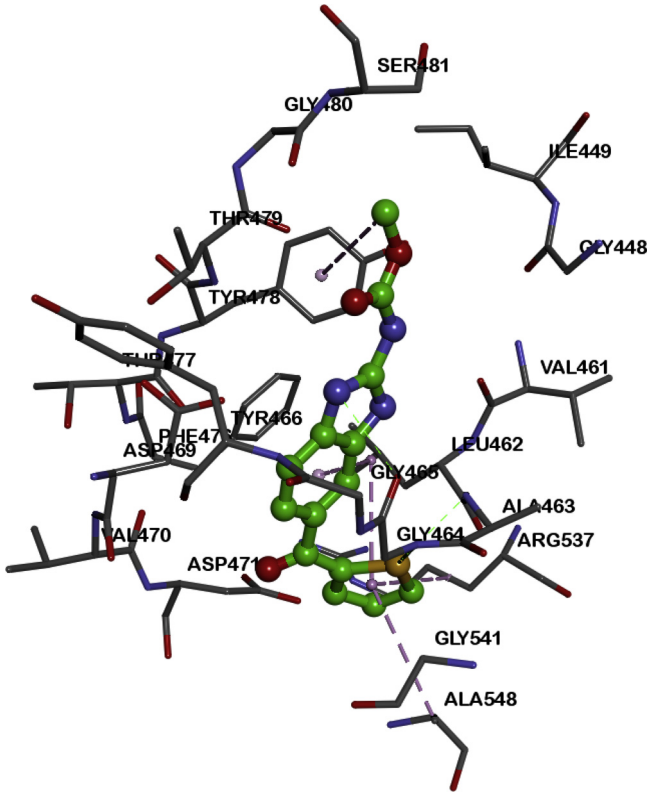

Fig. 4. The binding mode of nocodazole (ball and stick cartoon) within the SRA domain of human UHRF1 (pdb id: 3BI7). Hydrogen atoms are removed to enhance clarity. Polar and nonpolar interactions are color-coded and details are provided in the text.

underwent π–anion contact with carboxylate group of Asp469 (distance = 3. 41 Å) and π–alkyl interaction with Leu462 (distance = 4.62 Å). The naphthyloxy oxygen atom formed a hydrogen bond with the backbone NH of Tyr466 (distance = 2.78 Å) whereas the protonated amine was in contact with Gly464 via hydrogen bonding (distance = 1.87 Å). The isopropyl group of isopropylaminopropan-2-ol substituent was in contact with hydrophobic amino acids Val461 and Ala463 via alkyl–alkyl interactions (distance < ; 5.0 Å) whereas the isopropanol OH was in contact with the carboxylate of Asp469 (distance = 1.87 Å). This shows that (*R*)-propranolol can undergo multiple favourable interactions within the SRA domain and has the potential to be repurposed as a UHRF1 inhibitor in cancer therapy. Next, we investigated the interaction of a synthetic compound nocodazole, a known inhibitor of microtubule polymerization by conducting molecular docking studies within the SRA domain of UHRF1. Nocodazole adopts an L-shaped conformation in the 5 mC binding cavity with the central benzimidazole ring surrounded by Leu462, Gly464, Gly465 and Tyr466 (Fig. 4). The aromatic benzene ring underwent π–alkyl interaction with Leu462 side chain (distance < ; 5.0 Å) whereas one of the imidazole nitrogens was in contact with backbone ketone of Gly465 (distance < ; 2.0 Å). Interestingly, the thiophene ring underwent polar contact with NH backbone of Gly464 (distance < ; 2.0 Å) and π–alkyl hydrophobic interactions with Leu462 (distance < ; 5.0 Å, Fig. 4). The carbamate substituent was oriented closer to Ile449, Tyr478, Thr479, Gly480 and Ser481 where the methoxy group underwent π–alkyl interactions with Tyr478 (distance < ; 5.0 Å). These observations suggest the potential of nocodazole as a UHRF1 inhibitor.

8. Conclusion

Multiple studies have documented on UHRF1 overexpression in several cancers and the potential clinical utility of UHRF1 as a diagnostic and prognostic biomarker in the determination of the presence and progression of malignancy. Its expression levels can also be used to predict the OS and PFS of patients and subsequently employed for selection of a suitable treatment regimen. Even though there is enough evidence proving UHRF1 overexpression, more trials in larger cohorts need to be conducted to establish its potential as a biomarker. One of the fundamental concepts to be considered in the field of precision medicine is the heterogeneity of tumours. This calls for population-specific studies to be conducted in the Indian subcontinent that may or may not show an overexpression of the UHRF1. Hence, the results of studies that show efficacy or expression of new anticancer drugs or targets cannot be extrapolated to the Indian population due to their differences in genetic makeup. This reinforces the need to look for the overexpression of this “Universal gene” in the Indian subcontinent following which new drugs or existing drugs can be developed or repurposed to bring remission or cure.

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