UHRF1: A diagnostic and prognostic marker of cancer

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Introduction

There are many members of the UHRF family including UHRF1, UHRF2, UHRF3, and UHRF4 having different functions. In this review, we mainly study the activity of UHRF1. It is a multi-domain protein associated with epigenetic mechanisms of cell regulation and proliferation. UHRF1 stands for Ubiquitin-like PHD Ring finger 1 having a location in the chromosomal region 19p13.3 [1]. It plays a key role in transferring methylation from mother to daughter DNA strands [2-6]. mUHRF1 was discovered against murine thymic lymphoma by engineering antibodies. hUHRF1 has activity of E3 ligases for histones H3. Expression of UHRF1 increases in breast cancer, cervical lesions, rhabdomyosarcoma, pancreatic adenocarcinoma, prostate cancer, and lung cancer [7]. Increased expression of UHRF1 in human pulmonary fibroblasts causes increased topoisomerase IIα expression and hence increased cell proliferation. Similarly, depletion of UHRF1 causes DNA damage response, G2/M phase arrest, and apoptosis formation [8-10]. UHRF1 by interacting with DNMT1 and HDAC1 induces heterochromatin structure.

Structure of UHRF1

It possesses a UBL (Ubiquitin-like domain), TTD (cryptic Tandem Tudor domain), PHD (plant homeodomain), SRA (Set and Ring associated), and RING (Really Interesting New Gene) domain as shown in figure 1. UBL domain contains α/β ubiquitin folds with surface Lysine residues i.e. 35% similar to ubiquitin, consisting of 76 amino acids and has an important role in cell cycle progression, protein degradation, and gene transcription [11]. PH Domain recognizes di and tri-methylation of histone h3 lysine 9 (H3K9), associated with heterochromatin formation, transcriptional processes and also with downregulation of UHRF1 both in human and mouse causes disrupt H3K9 distribution. PHD promotes gene activation and inactivation by interacting with tri-methylated H3K4. Various studies have shown that PHD has the ability to read the histone code as well [12]. The SRA domain contains 150-170 amino acids directly involved in DNA methylation to the target sequences by recognizing hemimethylated Cytosine of new daughter DNA strand binds with DNMT1 and involved in heterochromatin formation along with PH domain. It sets a
bridge between DNA methylation and histone code by allowing UHRF1 to bind with HDAC1, methylated DNA and DNMT1. RING is 76 amino acid long polypeptide domain, attached with Lysine on cellular protein by its C-terminal. Ubiquitination is mediated by E1/E2/E3 enzymes; especially E3 catalyzes the binding of the C-terminal with Lysine on targeted protein. There are two main classes of E3 ligases, HECT and Ring class having Ring finger domain [13]. UHRF family has auto-ubiquitinating activity [9] like many ligases which contain Ring finger domain [14]. UHRF1 ubiquitinates histone H3 and these are substrates for the UHRF family.

**Figure 1:** Construction of UHRF1: UBL (Ubiquitin-like domain), TTD (cryptic Tandem Tudor domain), PHD (plant homeodomain), SRA (Set and Ring associated) and RING (Really Interesting New Gene) domains interact with DNMT1, HDAC1, H3K9 and Histone H3 proteins that lead to epigenetics code of inheritance.

**Mechanism of UHRF1 in heterochromatin formation**

1- First UHRF1 binds to PCNA and recognizes hemimethylated DNA through its SRA domain. UHRF1 recruits the G9a, DNMT1, and HDAC1 which methylates the h3k9 and binds with PHD domain, both DNA strands and transfer the methylation status from mother to daughter DNA strand.

2- Secondly UHRF1 recruits the G9a which methylates the h3k9 (histone h3 lysine9) and methylated h3k9 binds with the PHD domain of the UHRF1 step 2 in figure 7 [15] then it recruits the DNMT1 which methylates both DNA strands and transfers the methylation status from mother to daughter DNA strand.

3- Finally UHRF1 recruits the histone deacetylase 1 (HDAC1) which deacetylates the histone proteins and helps in heterochromatin formation and transcriptional suppression Step 3 in figure 2 [15].

**UHRF1 and epigenetic codes**

UHRF members directly influence the histone code by their enzymatic activity (E3 ligase) via RING, maintaining the epigenetic code (DNA methylation and histone code) and genomic integrity. It might be a tumor suppressor gene [16].

UHRF1 interacts with the methyl-CpG region in DNA strand, methylated H3K9, DNMT1, HDAC1, PCNA and G9a, links with DNA methylation and histone methylation, deacetylation and ubiquitination with heterochromatin formation as shown in figure 3 [4,6,2,1,17,15,18]. DNA methylation and histone modification both act together, changing the gene expression and heterochromatin structure [4,19,20].

**Up regulation of UHRF1**

UHRF1 is significantly over-expressed in various cancers and tumor cells [9,21]. Upregulation of UHRF1 is associated with high levels of p73, SIRT1, Caspase 3, DNMT1, and HDAC1 which methylates the h3k9 and binds with PHD domain, both DNA strands and transfer the methylation status from mother to daughter DNA strand and deacetylates the histone proteins respectively which leads to heterochromatin formation.

**Downregulation of UHRF1**

Downregulation of UHRF1 causes DNA damage by breaking DNA strands [26,27], cell cycle arrest in the G2/M stage by inhibiting Cyclin-dependent kinases 1 (cdk-1), a regulator of cell cycle progression in G2 phase during mitosis and apoptosis by activating Caspase 8 [10] as shown in figure 5. chk 1 and chk 2 are activated by phosphorylation in DNA damage response. Loss of chk 2 occurs in UHRF1-depleted cells and inhibits the
Upstream regulation of UHRF1: Upregulation of UHRF1 is associated with high levels of p73, SIRT1, Caspase 3, DNMT1, and HDAC1 level and ultimately leads to cancer.

Phosphorylation of chk1, it leads to cell death by cell cycle arrest. Apoptosis results from depletion of UHRF1 are p53 independent and this pathway regulates Caspase 8 functions, which causes activation of Caspase 3 and ultimately apoptosis [28]. Various studies have shown that depletion of UHRF1 prevents cell cycle progression so it is useful to prevent the growth of tumor cells in cancer treatment [29], and UHRF1 depleted cells become more sensitive to DNA damaging agents [16,30].

Depletion of UHRF1: Down-regulation of UHRF1 causes DNA damage by breaking DNA strands, cell cycle arrest in the G2/M stage by inhibiting CDK-1, and apoptosis by activating Caspase 8.

Role of UHRF1 in cancers

UHRF1 is downregulated by p53 via the up-regulation of p21 and deactivation of E2F1, an up-regulator of UHRF1 [8,21,30]. As p53 is deficient in 50% of cancers [31], So UHRF1 is upregulated in many cancers by the following mechanism. In p53 deficient cancers, the cyclin D/cdk complex become activated, causing phosphorylation of PRB and phosphorylated PRB activates the E2F1 which binds with UHRF1 and upregulates its level which leads to cancer as shown in figure 6. UHRF1 is upregulated by rapid cell cycle progression as well [21,8,9,32,33]. In the rapid cell cycle, UHRF1 binds with newly synthesized DNA with PCNA, DNMT1, and HDAC1 and hastily transfers the CH3 group from mother to daughter progeny. It activates the G1/S phase and the cyclin B/cdk1 complex becomes activated in the G2/M phase and p21 inactivates this cdk1 [34].

UHRF1 as a diagnostic and prognostic marker

Up-regulation of UHRF1 has been associated with various cancers e.g, including breast cancer, lungs cancer, bladder, prostate, cervical, and pancreatic cancers [21,33,9,35,32,36].

UHRF1 as a potential therapeutic target

All UHRF1 members have ubiquitin ligase E3 activity, targeting E3 ligases proved beneficial as it is an ideal drug target in anticancer therapy [13]. Expression of E3 ligases along with UHRF1 increases in cancer cells so when they are inhibited, growth arrest and apoptosis occur [13,11,37]. So, targeting UHRF1 is selective anticancer therapy.

DNA methylation pattern in UHRF1

The most important domain involved in DNA methylation is the UHRF1-SRA domain. UHRF1 binds with hemimethylated DNA with the SRA domain which is involved in the proper setting of DNMT1 on the DNA strand [22,4]. DNMT1 and UHRF1 are two proteins that have affinity and selectivity for hemimethylated DNA on their own and both are essential for performing their functions [6]. It is supposed that UHRF1 moves along DNA strands, recognizes hemimethylated CpG region [6,3,4] and it dictates DNMT1 through an unknown mechanism to catalyze methylation at the target site. Another model states that when DNMT1 starts acting, UHRF1 gets separated from the CpG region, allowing DNMT1 to work properly. It is widely researched how DNA hypermethylation makes the gene transcriptionally silent. DNA methylation behaves as a signal for the recruitment of CpG methyl binding domain (MBD) [38] which further recruits the histone deacetylase (HDAC) and form many genes silencing complexes as shown in Figure 7.

Factor influencing DNA methylation

Many factors have a direct impact on the extent of DNA methylation pattern.

Aging: As the tissue becomes aged, there is more possibility that the genome will become hypomethylated and certain CpG islands become hypermethylated. But it is not known whether this change makes the person more susceptible to cancer or not [39].

Diet: DNA methylation requires methyl group which comes from folate and methionine components of the diet. As mammals lack the ability to produce folate and methionine them-
and/or over-expression of UHRF1 in cancer cells results from an alteration of the p53 tumor suppressor gene.

When interferes with the ubiquitin ligase activity of UHRF1 (by overexpression of RING domain mutant sound) increases the sensitivity of cancer cells to chemotherapeutic agents [9]. In addition, it was reported that inhibition of the expression of UHRF1 causes a reduction of ribonucleotide reductase, an enzyme essential for the synthesis of deoxy nucleotides [28]. The concomitant decrease in the expression of both proteins leads to increased cell sensitivity to hydroxyurea (HydreaTM), which can be particularly effective in the treatment of leukemia. It was suggested that the anti-transcriptional targeting of UHRF1 might be interesting in the case of cancers resistant to hydroxyurea without resorting to the increase in therapeutic doses [28].

All these studies seem to converge on the fact that UHRF1 is an attractive target to develop new anti-cancer.

References

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