The ubiquitin-proteasome system in cancer, a major player in DNA repair. Part 2: transcriptional regulation

Panagiotis J. Vlachostergios *, Anna Patrikidou, Danai D. Daliani, Christos N. Papandreou

Division of Medical Oncology, University Hospital of Larissa, Larissa, Greece

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Abstract

DNA repair is an indispensable part of a cell’s defence system against the devastating effects of DNA-damaging conditions. The regulation of this function is a really demanding situation, particularly when the stressing factors persist for a long time. In such cases, the depletion of existing DNA repair proteins has to be compensated by the induction of the analogous gene products. In addition, the arrest of transcription, which is another result of many DNA-damaging agents, needs to be overcome through regulation of transcription-specific DNA repair pathways. The involvement of the ubiquitin-proteasome system (UPS) in cancer- and chemotherapy-related DNA-damage repair relevant to the above transcriptional modification mechanisms are illustrated in this review. Furthermore, the contribution of UPS to the regulation of localization and accessibility of DNA repair proteins to chromatin, in response to cellular stress is discussed.

Keywords: DNA repair • DNA-damage response • transcription • ubiquitin-proteasome system • SUMO • NEDD8 • MGMT • mismatch repair • base-excision repair • nucleotide-excision repair • double-strand break repair • Fanconi anaemia pathway • post-replication repair

Introduction

The ‘reaction’ of a cell in the face of a DNA-damaging situation is an essential condition for its survival. There are five main DNA repair pathways that can be activated, depending on the type of induced damage: direct or reversion repair by O\textsubscript{6}-methylguanine DNA methyltransferase (MGMT), mismatch repair (MMR), base-excision repair (BER), nucleotide-excision repair (NER), separated into global genomic repair (GGR) and transcription-coupled repair (TCR) and double-strand breaks repair (DSBR), which involves homologous recombination (HR) and non-homologous end joining (NHEJ) repair sub-pathways [1–6]. Additionally, there exist a DNA replication block bypassing repair mechanism termed post-replication repair (PRR), consisting of both error-prone translesion synthesis (TLS) and error-free damage avoidance [7], as well as a DNA-crosslink repair pathway, combining HR and TLS, the Fanconi anaemia pathway (FA) [8]. The aberration of one or more of these DNA repair pathways is a common event in cancer cells that may be, at least partially, responsible for genomic instability and increased sensitivity to radiation or DNA-damaging agents. In fact, abnormalities or deficiencies in the expression of a great number of DNA repair factors such as breast cancer protein 1 (BRCA1), BRCA2, FA genes or/and crucial cell cycle and DNA-damage response regulators such as p53 not only have been correlated with carcinogenesis but they also reflect a disturbed, unbalanced network of existing DNA repair mechanisms and probably unidentified back-up systems that could be well studied and exploited for the development of novel potential cancer treatment options [9].

The ubiquitin-proteasome system (UPS) is long known as a cellular tool for the marking and proteolytic degradation of proteins
involved in a wide variety of structural and functional roles inside
the cell. The UPS includes the ‘ubiquitously’ expressed 76-amino
acid protein ubiquitin (Ub), the multi-subunit protein organelle
26S proteasome, consisting of one 20S catalytic and two 19S reg-
ulatory subunits, and finally, a three-step enzymatic cascade of
Ub-activating (E1), Ub-conjugating (E2) and Ub-ligase (E3)
enzymes that attach Ub to the target protein [10–13]. In an
expanding outlook of the ubiquity protein family there are several
Ub-like proteins, sharing similarities in both structure and activa-
tion process, mainly represented by the small ubiquitin-like mod-
ifier (SUMO) protein and neural precursor cell expressed, develop-
mentally down-regulated protein 8 (NEDD8) [14].

An increasing amount of evidence supports the involvement of
UPS in neoplastic formation and oncogenesis, via dysregulation
of either proteasome-dependent degradation or/and Ub- and Ub
family-related signalling. There are many examples demonstrating
this causative relation, as a great number of cellular proteins with
various roles have a close structural or functional connection with
abnormally Ub- or Ub-like ligases, deubiquitinating enzymes and
UPS-regulated signalling factors and pathways (such as p53 and
NF-κB) [15]. In this context, it is reasonable to expect a prominent
effect of UPS dysregulation on a cellular function so vital as DNA
repair, further considering that a DNA-damaging stimulus may act
not only on the real-time protein reservoir of cells but also on the
dynamically inducible transcriptome. Indeed, recent evidence is
supportive of a complicated connection between the UPS and DNA
repair pathways that does not confine to post-translational modi-
fications alone. The UPS-related DNA repair processes (Fig. 1) are
reviewed here in the context of transcriptional modifications
induced by UPS. This transcriptional regulation, as described
herein, is mainly demonstrated in response to DNA damage and
includes not only the UPS-dependent induction of some DNA
repair genes, but also the facilitation of transcription-specific DNA
repair pathways, such as TCR (which is a part of NER) and DNA-
protein adducts reversal. Finally, an indirect effect of UPS on both
DNA repair and transcription in general is associated with the
availability of nuclear Ub-reservoir as well as with the sub-nuclear
localization and distribution of DNA repair factors and DNA dam-
age (Figs. 2 and 3, Table 1).

**Transcriptional modifications of DNA repair by UPS**

The UPS has recently been proven to be an indispensable cellular
regulatory factor for transcription in general, with various actions
ranging from chromatin remodelling, transcription activator-
turnover, co-activator and co-repressor exchange, to RNA poly-
merase recruitment, elongation progression and transcription termi-
nation [16–19]. Remarkably, even a feedback mechanism between
protein synthesis and degradation via transcriptional regulation of
ribosomal protein genes by the proteasome has been implied
[20, 21]. It would be reasonable to expect that DNA repair proteins
may not be excluded from this regulation, either at basal gene-
expression level or as part of transcriptional response to DNA dam-
age, and in both proteolytic and non-proteolytic ways. Focusing on
specific descriptions of the UPS-DNA repair connection at transcrip-
tional level, there are a few outstanding examples in recent literature.

**TCR pathway (NER)**

The proteolytic regulation of repair of actively transcribed genes
(TCR) by the UPS involves the poly-ubiquitination of RNA poly-
merase II (pol II) at stalled sites of bulky lesions, which can either
be bypassed or degraded after prolonged elongation-discontinua-
tion of transcription. The first case is accomplished by the TCR
proteins termed Cockayne Syndrome A and B (CSA, CSB), how-
ever, if pol II is irreversibly trapped, the 26S proteasome takes
over [16, 22–26]. A unique status of phosphorylation of the car-
boxy-terminal domain (CTD) of the largest subunit of pol II, Rpb1,
is associated with adequacy for ubiquitylation and the latter is
effected by an Ub ligase, of which the yeast Elc1 protein is a com-
ponent in this process. This ligase is supported to be the same
with the one involved in Rad7-Rad16-dependent NER of lesions
from the non-transcribed regions of the genome that were previ-
ously described [27]. Moreover, UV irradiation induces the degra-
dation of CSB via the UPS in a CSA-dependent manner, after the
completion of repair and this is considered a crucial step in the
post-TCR resumption of transcription at a normal rate [28]. Reversal of yeast RNA pol II ubiquitylation was recently found to
be effected by the Ub protease Ubp3 [29]. Most recent data are
suggestive of a timely regulation of the entire process of TCR,
Fig. 2: Transcriptional modifications of DNA repair by UPS in response to cellular stressing conditions. The regulatory roles of UPS exerted on DNA repair on the transcriptional level are exerted (A) directly through binding of the proteasome-associated protein Rpn4p on promoter of MAG1 and Rad23 genes, encoding repair proteins of BER and NER pathway, respectively, (B) on transcription-coupled DNA repair of bulky lesions via degradation of the largest subunit of RNA pol II and subsequent cleavage of the lesion-containing DNA strand, followed by resynthesis and resumption of transcription, (C) on protein-DNA adducts removal, via degradation of topoisomerases, making the underlying lesion recognizable and subject to further procession by different DNA repair pathways according to the type of the lesion, (D) on modulation of chromatin compaction and accessibility of DNA repair proteins by Ub de-conjugation from histones, (E) on inducible by PARP, removal of damaged histones, (F) on availability of DNA repair proteins to different sites of DNA damage after dispersal of PML-NBs, (G) on assembly of BRCA1 after MDC1 ubiquitination and subsequent degradation.
Fig. 3 Transcriptional activation of DNA repair mediated by UPS-dependent pathways. The induction of DNA repair genes in response to DNA damage involves the activation (NF-κB) or stabilization (p53) of regulatory proteins in a UPS-controlled process. The activation of the NF-κB pathway results in upregulation of MGMT, Ku70 and Ku80 expression. The stabilization of p53 coordinates the induction of BER, NER, MMR, DR, HR, NHEJ, FA genes and accessory (GADD45, p53R2, PML) or suspending (PPM1D) factors, acting both independently and downstream of BRCA1.

Abbreviations: NEMO, NF-κB essential modulator; IκB, inhibitor of κB; NF-κB, nuclear factor kappa B; MGMT, O6-methylguanine-DNA methyltransferase; NHEJ, non-homologous end-joining; DR, direct repair; NER, nucleotide-excision repair; GGR, global genomic repair; TCR, transcription-coupled repair; CSA, Cockayne syndrome protein A; CSB, Cockayne syndrome protein B; RNA pol II, RNA polymerase II; XPG, Xeroderma pigmentosum complementation group G; XPF, Xeroderma pigmentosum complementation group F; ERCC1, excision repair cross-complementing rodent repair deficiency complementation group 1; PCNA, proliferating cell nuclear antigen protein; Pol α, ε, DNA polymerases α, ε; Lig 1, DNA-ligase 1; Top1, topoisomerase 1; Top2α, topoisomerase 2α; Top2β, topoisomerase 2β; SSB, single-strand break; PARP, poly-ADP-ribose polymerase; XRCC1, X-ray repair complementing defective repair in Chinese hamster cells 1; BER, base-excision repair; DSB, double-strand break; HR, homologous recombination; NHEJ, non-homologous end-joining; Rpn4p, proteasome-related protein 4p; MPG, 3-methyladenine-DNA glycosylase; HR23, homologue of Rad23; COX-2, cyclooxygenase (COX) 2; GADD45, growth arrest and DNA damage 45; FANCC, Fanconi anemia, complementation group C; pS3R2, ribonucleotide reductase small subunit; UNG2, uracil DNA glycosylase; MDC1, mediator of DNA-damage checkpoint 1.

which depends on an Ub-clock countdown that provides enough time for repair until the signal for degradation emanating from poly-ubiquitylation gets more enhanced [30].

An important piece of information regarding the regulation of TCR by UPS concerns the physical interaction of pol II with BCRA1 protein, which is only part of a more deep involvement of the latter in the process of transcription regulation, being a structural component of the pol II holoenzyme [31, 32]. BRCA1, apart from being a major DNA repair protein involved in DSBR, it has a structure of an E3 Ub-ligase as a result of its RING-finger N-terminal domain. In complex with BARD1 (BRCA1-associated RING domain protein 1), it targets Rpb1 for ubiquitylation and subsequent proteasome-mediated degradation at sites of stalled pol II and transcription machinery [31, 32]. The BRCA1-TCR connection further involves the negative effect of the former on the phosphorylation status of the CTD of pol II, via inhibition of the Cdk-activating kinase (CAD) subcomplex of TFIIH in an ATP-dependent manner [32, 33].
### Table 1

**UPS-mediated transcriptional regulation of DNA repair (directly or via UPS-dependent transcription factors)**

| Target gene | DNA repair pathway | Modifier | Result |
|-------------|--------------------|----------|--------|
| MGMT       | DR                 | IFN-κB   | Induction |
|            |                    | p53      | Induction (upon DNA damage) |
| MAG1 (MPG) | BER                | 26S proteasome (Rpn4p) | Induction |
|            |                    | p53      | Repression (of basal expression) |
| DDB2       | NER                | p53      | Induction |
| XPC         | NER                | p53      | Induction |
| Rad23 (HR23)| NER               | 26S proteasome (Rpn4p) | Induction |
|            |                    | p53      | Induction |
| GADD45     | BER, NER           | p53      | Induction |
| PPM1D      | BER                | p53      | Induction |
| p53R2      | All pathways       | p53      | Induction |
| PCNA       | BER, NER, MMR, DSBR, PRR | p53 | Induction |
| MSH2       | MMR                | p53      | Induction |
| PMS2       | MMR                | p53      | Induction |
| MLH1       | MMR                | p53      | Induction |
| KARP-1     | NHEJ               | p53      | Induction |
| Rad51      | HR                 | p53      | Induction |
| FAC (FANCC)| FA                 | p53      | Induction |
| Ku70       | NHEJ               | NF-κB    | Induction |
| Ku80       | NHEJ               | NF-κB    | Induction |
| ERCC1      | NER                | 26S proteasome | No induction after proteasome inhibition preceding cisplatin-treatment |
| PML        |                    | p53      | Induction |

| Target protein | DNA repair pathway | Modifier | Result |
|---------------|--------------------|----------|--------|
| RNA pol II    | TCR (NER)          | Poly-Ub & 26S proteasome | Degradation of Rpb1 |
|               |                    | NEDD8    | Unknown |

**Table 1 Continued**

|                      | SUMO | BRCA1 | Unknown |
|----------------------|------|-------|---------|
|                      | Ubiquitylation for degradation of Rpb1 | Inhibition of phosphorylation of CTD |
| CSB                  |      |       |         |
| Top1                 |      |       |         |
| Top2                 |      |       |         |
| PML                  |      |       |         |
| MDC1                 |      |       |         |
| H2A, H2B             |      |       |         |

Abbreviations: MGMT, O<sup>6</sup>-methylguanine-DNA methyltransferase; DR, direct repair; IFN-κB, nuclear factor kappa B; MAG1 (MPG), 3-methyladenine-DNA glycosylase; Rpn4p, proteasome-related protein 4p; BER, base-excision repair; NER, nucleotide-excision repair; HR23, homologue of Rad23; ERCC1, excision repair cross-complementing rodent repair deficiency complementation group 1; NHEJ, non-homologous end-joining; RNA pol II, RNA polymerase II; TCR, transcription-coupled repair; NEDD8, nuclear precursor cell expressed developmentally down-regulated 8; CSB, Cockayne syndrome protein B; Top1, topoisomerase 1; Top2, topoisomerase 2; SUMO, small ubiquitin-like modifier; PML, promyelocytic leukaemia protein; NBs, nuclear bodies; MDC1, mediator of DNA-damage checkpoint 1; BRCA1, breast cancer protein 1; H2A, histone 2A; H2B, histone 2B; DDB2, damage-specific DNA binding protein 2; XPC, Xeroderma pigmentosum, complementation group C; GADD45, growth arrest and DNA damage 45; PPM1D, protein phosphatase 1D magnesium-dependent, delta isofrom; PCNA, proliferating cell nuclear antigen; MSH2, MutS homolog 2; PMS2, PMS2 post-mitotic segregation increased 2; MLH1, MutL homolog 1; KARP-1, Ku86 autoantigen-related protein-1; FANCC, Fanconi anemia, complementation group C; Rpb1 (POLR2A), polymerase (RNA) II (DNA directed) polypeptide A.
Also, there are intriguing data that possibly implicate UPS in TCR regulation via a yet unidentified mechanism involving the SUMOylation of pol II at its first subunit, Rpb1. This was demonstrated in a proteome-wide approach-based study in yeast, which identified all three RNA polymerases as Smt3-substrates (the yeast homologue of SUMO1) [34]. Finally, pol II as well as topoisomerases 2α and 2β (which are discussed below) belong to a group of transcriptional and repair factors that have been found to interact with NEDD8 in proteomic analyses with the use of affinity purification and tandem mass spectrometry [35, 36].

Protein-DNA adducts repair

The repair of a newly discovered type of DNA damage, consisting of protein-DNA adducts, has also been linked to UPS by the establishment of a UPS proteolytic involvement in the transcription-dependent removal of transient topoisomerase I (Top1) – DNA cleavage complexes, induced by camptothecins and other sources of DNA damage. Topoisomerases catalyze and guide the unknotting of DNA by creating transient breaks in the DNA. A proposed model for transcription-dependent processing of Top1-DNA covalent complexes into strand breaks involves CPT stabilization of reversible Top1 cleavage complexes on actively transcribed regions, followed by transcription arrest of elongating RNA pol, formation of poly-ubiquitin chain on Top1 and subsequent degradation by the 26S proteasome, exposing the otherwise Top1-concealed single-strand break (SSB) to further repair through a poly-ADP-ribose polymerase (PARP)1-dependent pathway (BER) [37]. Also, results from a previous study suggested that degradation of both Top1 and the large subunit of RNA polymerase II precede repair of the exposed single-strand breaks by TCR [38]. A similar mechanism of damage repair process defines the regulation of Top2 cleavable complexes, induced by drugs such as etoposide and doxorubicin. The entrapment of Top2-DNA complexes is reversed by Ub-mediated proteolytic degradation of Top2α, Top2β by 26S proteasome and enables the exposure of the underlying DSB to processing by HR or NHEJ [39, 40].

The consideration of a non-proteolytic involvement of UPS in topoisomerase-mediated DNA-damage repair has brought more light to the understanding of its role. Studies in yeast have suggested that hTop1 and hTop2α or hTop2β conjugates are primarily hTop-SUMO-1 conjugates rather than hTop-Ub conjugates. The signal that triggers SUMO-1 conjugation of hTOP is most likely the hTOP-CPT-DNA cleavable complex and the conjugation of SUMO-1 is effected by its E2 ligase, Ubc9. The roles of this SUMOylation, although speculative, could involve either the targeting of repair proteins to the nuclear pore and bodies or the binding of proteins to the site of DNA damage [41].

BER, NER pathways

Results from a computational gene analysis study in yeast revealed a DNA-damage (MMS-induced) responsive BER gene, 3-methyladenine DNA glycosylase (MAG1) which is partly regulated by the proteasome-associated protein Rpn4p through its binding to MAG1 upstream repressor sequence two-like elements [42]. The great similarity of this sequence motif to the recently identified proteasome-associated control element (PACE), found upstream of genes encoding proteins that are involved in Ub-mediated proteolytic degradation [43], demonstrates a direct, non-proteolytic link between UPS and induction of a DNA repair gene. Moving forward to NER regulation of expression, the same link was established for Rad23, as loss of inducibility was observed in Rpn4p deleted strains upon MMS exposure [42]. These data are even more intriguing, given the information that the human Rad23 proteins, hHR23A and B interact with 3-methyladenine DNA glycosylase protein (MPG) and can serve as accessory proteins for DNA-damage recognition in BER, elevating the rate of MPG protein-catalyzed excision from hypoxanthine-containing substrates [44].

Moreover, another connection between the transcriptionally induced parts of BER, NER pathways and UPS seems to exist, which is effected via intervention of the p53 protein. p53 protein levels are regulated by UPS in a switch-like manner in both normal conditions and DNA damage. This is an established circuit involving, in brief, the interaction between p53 and the E3 Ub ligase Mdm2, which keeps the former inactive in unstressed cells by targeting it for proteasome-mediated degradation. When DNA-damage conditions occur, Mdm2 is subjected to accelerated auto-ubiquitination and proteasome-dependent degradation, enabling p53 stabilization and consequent exertion of its post-translational and transcriptional roles in determining cells' fate towards cell cycle arrest and DNA repair, or apoptotic death [45, 46]. Among different p53-target genes, a few DNA repair genes that are part of the BER and NER machineries are directly regulated. 3-Methyladenine (3-MeAde) DNA glycosylase, the first step of BER (described above) was found to be transcriptionally repressed by p53 following exposure of cells to nitrous oxide (NO); this was a late event, in accordance with down-regulation of measured 3-MeAde DNA glycosylase activity, preceded by a temporary early increase that was observed before the accumulation of p53 [47]. Based on these data, the authors provide a plausible model in which p53 exerts a possible antimutagenic role by preventing the creation of a mutator phenotype that would emerge in its absence, given that the resulting elevation of 3-MeAde DNA glycosylase activity followed by the increased generation of apurinic/apyrimidinic (AP) sites could not be compensated by AP endonuclease activity (APE) activity (downstream BER enzyme), as the latter remains unaffected by nitric oxide treatment [47].

With respect to NER, the GGR-sub-pathway human damage-specific DNA binding protein 2 (DDB2) gene was demonstrated to be directly activated by p53 in response to DNA damage by identifying a consensus p53-binding site at the 5’ untranslated region (UTR) of DDB2 and confirming the activation of the gene by using luciferase reporter assays [48]. This piece of information is complementary to previous relevant reports that had initially correlated the activation of DDB2 gene transcription with...
accumulation of p53, either transfected or UV and ionising radia-
tion (IR)-induced [49]. Another crucial GGR-damage recognition
factor that was proven to be regulated by p53 in both baseline
and DNA-damage inducible mRNA and protein levels is
Xeroderma pigmentosum, complementation group C (XPC). Simi-
larly to DDB2, a strong candidate for a p53 response ele-
ment in the promoter of the XPC genomic sequence was also
identified and further confirmed in electrophoretic mobility-shift
assay [50]. A wider search for p53 targets based on chromatin
immunoprecipitation (ChIP) analysis under normal and DNA
damage-induced conditions has revealed another important NER
gene, Rad23, to be under p53 control. Rad23, however, exhibits
quite complex kinetics, following a pattern of rapid repression
and later reactivation with a peak between 8/16 hrs and repres-
sion again by 24 hrs. [51]. This is in line with a feedback loop
acting on p53 (clearly justified, taking into account that Rad23
has been shown to promote p53-degradation). [52]. This is also
the case for BRCA1 [52], however, the above-mentioned data
only slightly illustrate its contribution in transcriptional DNA
repair regulation, which is best described below.

A more deep insight into the potential mechanisms of p53-
mediated interference of UPS with DNA repair was offered by a
study of Zhu et al (2007). The authors’ objective was to investigate
whether the turnover of p53 by UPS plays a role in gene expres-
sion, by examining certain p53-target genes like DDB2, Mdm2 and
p21 in normal (non-cancerous) cells. Their results indicate that
proteasome function is required for efficient p53-mediated tran-
scription as, in the presence of a proteasome inhibitor, mRNAs of
the above-mentioned genes were attenuated both in baseline and
UV conditions. Furthermore, ubiquitylation is also required, as dis-
ruption of function of an E1 enzyme caused a decrease in p53-
mediated transcription. More importantly, Sug1, a component of
19S proteasome was found to physically interact with p53 in vitro
and in vivo, and their recruitment to p21 promoter was visualised
in a ChIP assay. The exact mechanism that governs this UPS-
dependent process is not fully understood but it is likely that ubiqu-
itylation and proteasome function might be indispensable for the
ability of the promoter-bound p53 to fire of new rounds of trans-
cription [53].

Additionally, apart from being ubiquitinated and subjected to
proteasome-mediated degradation, p53 is also a target for
SUMOylation and NEDDylation. SUMO-1 conjugation is effec-
ted via the coordinated collaboration of Mdm2 and the tumour
suppressor ARF, which are both required in a p53-Mdm2-ARF
complex to stimulate p53 SUMOylation via SUMOylation and
nucleolar targeting of Mdm2. The de-SUMOylating enzyme SEP1
ensures the reversibility of the process [54]. Based on most recent
data, the functional relevance of this p53 modification with relation
to the protein’s transcriptional activity is ambiguous, and surely
unknown as far as induction of DNA repair is concerned. It might
be that SUMOylation may integrate both negative and positive reg-
ulatory functions on p53, featuring differential regulation of
selected p53-target genes [55]. With regard to p53 NEDDylation,
Xirodimas et al. demonstrated that it is an Mdm2-dependent
procedure, preceded by Mdm2 auto-NEDDylation, with great
similarity to the auto-ubiquitination activity of Mdm2. Also, the
different kinetics of p53-NEDDylation and ubiquitination in response
to UV and IR as well as the observation of a suppressive effect of
NEDDylation on p53 transcriptional activity (tested in transfected
cells by luciferase assays and temperature shift) led the authors to
suggest a model for the role of the NEDD8 conjugation pathway as
a negative regulator of p53 and Mdm2 function. According to that,
Mdm2 protein promotes NEDDylation of p53 and itself. The
NEDD8 conjugation pathway inhibits both the transcriptional
activity of p53 and the suppressive effect of Mdm2 on p53 func-
tion. In this way, Mdm2 negatively controls the function of p53
and itself through the same pathway [56].

The above findings might substantiate a direct regulatory role of
UPS in the transcription of those DNA repair factors that are p53-
target genes. (Most DNA repair genes that are transcriptionally p53-
dependent have been described very well by Gatz and Wieselmuller
[57]). This suggestion may also apply to p53-target genes that do
not strictly belong to the DNA repair machinery, but contribute to
the initialization or facilitation of DNA repair processes. This is
the case for growth arrest and DNA-damage 45 (GADD45), which is a
p53-responsive DNA damage-inducible protein [58, 59] that causes
cell cycle arrest and functions in excision repair pathways (BER,
NER) via binding to proliferating cell nuclear antigen protein (PCNA)
[58–61]. A further contribution of GADD45 to the enhancement
of GGR sub-pathway of NER might be mediated by facilitation of chro-
matin-DNA repair protein interactions [62] or and negative regula-
tion of basal levels of p21 [63]. Additionally to the p53-dependent
transcriptional induction of GADD45, a p53 independent mecha-
nism that is regulated by BRCA1 has also been suggested, via phys-
ical association of the latter with a transcriptional domain on the
GADD45 promoter and specific transcription factors [31]. BRCA1
has been found to play several regulatory roles in DNA-damage
repair, transcription and cell cycle control, part of which are accom-
plished through stimulation of the p53-transcription activity [31, 32],
with DDB2 gene upregulation being a striking, well-described exam-
ple [64]. More importantly, the final effect of this BRCA1 involve-
ment is a redirection of the profile of p53-transactivated genes from
proapoptotic to DNA repair- and growth arrest-related [31, 65, 66].
Furthermore, the implication of UPS in BRCA1-mediated DNA repair
transcriptional regulation may be further enriched by the evidence
that interaction of BRCA1 with SUMO1 suppresses transcription of
BRCA1-target genes (including GADD45) in a SUMOylation inde-
dependent, histone deacetylase 1 (HDAC1)-dependent manner. This is
best illustrated in a proposed model where SUMO1 modulates the
occupancy of the promoter of BRCA1-target genes by causing dis-
assembly of BRCA1 and assembly of HDAC1, thus reducing the
level of acetyl-histones. When DNA damage occurs, this model is
reversed and BRCA1 is recruited whereas SUMO1 and HDAC1 are
released from the promoter [67].

Moving back to our previous report on p53-regulated genes of
NER and BER pathways, (protein phosphatase magnesium-
dependent 1 delta (PPM1D) is a recently identified p53-transcrip-
tional target that was surprisingly found to directly interact with the
activated BER enzyme Uracil DNA glycosylase 2 (UNG2) causing its dephosphorylation and subsequent decrease in UNG2-associated BER activity [68]. The accumulation of PPM1D after transactivation by p53 also results in inhibition of the latter, thus proposed to enable the cell to re-enter the cell cycle and render the DNA repair procedures less active [68].

Finally, the identification of a new p53-responsive DNA-damage-induced gene, ribonucleotide reductase small subunit (p53R2), encoding a ribonucleotide reductase, has revealed another mode of p53 (and ultimately UPS) involvement in enhancing DNA repair in general, in this case via supplying critical precursors for DNA synthesis from ribonucleotide diphosphates to repair-damaged DNA [59]. Of a similar generalised impact on most DNA repair pathways (BER, NER, MMR, DSBR, PRR) is the contribution of p53-mediated induction of PCNA. Interestingly, the reverse relationship of cellular levels of these two proteins determines the occurrence of DNA replication (high PCNA and low p53) or apoptosis (low or absent PCNA and high p53), whereas DNA repair is favoured when both protein levels are elevated [69].

**MMR pathway**

Transcriptional regulation of MMR by UPS in a p53-inducible manner has been demonstrated with regard to MutS homolog 2 (MSH2), PMS2 post-meiotic segregation increased 2 (PMS2) and MutL homolog 1 (MLH1) genes via identification of p53-response elements in the promoter regions of the respective genes [70, 71] and serial analysis of binding elements technology [72]. Subsequent confirmation of induction was performed with forced p53 expression using transfection models [70, 71] and p53-activating DNA-damaging drugs [72].

**MGMT repair pathway**

There are a couple of potential mechanisms of transcriptional regulation of the direct repair (MGMT) pathway orchestrated by UPS. MGMT follows an inducible pattern similar to the p53-regulatory model described above. In specific, it was found that MGMT mRNA and protein are induced by IR only in wild-type p53-expressing cells of mice and rat and this effect is mediated by MGMT promoter activation based on transfection of MGMT-promoter constructs into p53-wild-type, mutant and deficient cells. However, the effect of p53 on MGMT expression appears to be dual, as the former suppresses basal MGMT promoter activity when overexpressed in cells upon transfection with p53 expression vector [73, 74], possibly without binding of p53 to MGMT promoter [74]. A later study in human tumour cell lines is also in line with this argument, concluding that overproduction of wild-type p53 protein in human tumours curtails the transcription of the MGMT gene and confers a MGMT-deficient phenotype [75]. More recent data from experiments on astrocytic cells confirm the direct interaction between p53 and the MGMT promoter, further showing that disruption of p53 in a glioblastoma cell line resulted in significant reduction of MGMT expression without affecting promoter methylation that is a common incidence in these tumours [76].

According to another model, DNA damage, induced by alkylating agents, causes increased MGMT expression via induction of the transcription factor Nuclear factor κB (NF-κB) p65, which was demonstrated to interact with two putative NF-κB binding sites within the MGMT promoter [77]. But where does the UPS fit in and how can such a speculation be justified? Following genotoxic stress, the regulatory subunit of cytoplasmic IkB kinase complex, IkKα or NEMO (NF-κB essential modulator) is translocated to the nucleus and consecutively subjected to SUMOylation, ATM (ataxia-telangiectasia mutated, a signal transducing kinase)-dependent phosphorylation, deconjugation of SUMO and ubiquitylation, thus facilitating its cytoplasmic re-localization, in order to activate NF-κB. This activation is effected through phosphorylation and ubiquitination of the NF-κB inhibitory proteins IkB-α and IkB-β. These proteins are substrates of 26S proteasome in a way that proteosomal degradation releases NF-κB from its complex, facilitating its transport to the nucleus [78, 79]. Another way of NF-κB activation from UPS is the 26S proteasome-mediated cleavage of NF-κB p30 and p52, respectively [80–83]. Among the various different NF-κB heterodimer p50-p65-target genes, MGMT promoter becomes activated and the transcribed gene product repairs the DNA damage, O6-methylguanine [77]. This could be a possible explanation, featuring both proteolytic and non-proteolytic roles of UPS.

In the case of MGMT gene-induction, as well as for any DNA repair gene that is transcriptionally modulated by both p53 and NF-κB, the plot gets thicker and more difficult to predict, considering data on the existence of interplay between these two major transcription factors. Both antagonistic and synergistic interactions exist, largely varying according to induced conditions and cell types [84–89].

**DSBR pathway**

Transcriptional induction of other DNA repair factors by NF-κB at a constitutive level has also been implicated for *NHEJ proteins Ku70 and Ku80, in a cyclooxygenase-2 (COX-2)-dependent mechanism* [90]. This suggestion was based on the observation that acinar gastric cells with low constitutive NF-κB p50 levels had lower expression of both Ku70 and Ku80. Furthermore, the use of COX-2 a well-known NF-κB-target protein inhibitors suppressed while prostaglandin E2 (PGE2)-enhanced Ku70 and Ku80 expression in cells with low constitutive NF-κB level. The authors have also revealed a reverse pattern of NF-κB/Ku interaction in which Ku (Ku70-Ku80 heterodimer) acts as an upregulator of p50 transcription by interacting with the recombination signalling protein RBP-Jκ (which binds to p50 promoter) [90].

With respect to the question whether a link between p53 and transcriptional modification of DSBR genes exists, most data are compatible with a direct involvement of p53 in DSBR pathways at the level of protein-protein interactions. However, the transactivation of
Ku86 autoantigen-related protein-1 (KARP-1) gene by p53 following DNA damage is an exception. This was proved by identification of a functional p53 binding site within the second intron of the gene and observation of increased mRNA and protein levels in a p53- and ATM-dependent way [91]. This finding is of great importance, considering previous data about KARP-1 involvement in the regulation of DNA-PK activity [92]. More recently, Rad51 was added in the list of p53-dependent DSBR genes, revealed in an oligo-based array containing several promoters [Ceribelli 2006], and its expression was repressed as a response to DNA damage via a direct binding of p53 in the Rad51 promoter [93].

FA pathway

The FA complementation group C (FAC or FANCC) gene could also be enlisted in the group of p53-inducible DNA repair factors, the transcription of whom might be indirectly affected by the UPS (as a result of the existence of the p53-UPS connection, described above). The establishment of p53 contribution to the expression of FAC was based on the description of two p53-binding sites in the promoter and coding region of the gene, respectively. Also, p53 overexpression led to significant enhancement of transcription of FAC gene, although luciferase assays disclosed no modulation of the promoter activity (leading to the conclusion that p53-binding is not directly responsible for activation of FAC transcription) [94].

DNA repair as part of UPS-related stress response

DNA damage-inducing cellular stress generates the activation of a really complex but well-coordinated signal transduction network of sensors, mediators and downstream effectors of the biochemical and physiological consequences – modifications of various interconnected cellular processes, involving cell cycle progression, survival and apoptotic regulatory pathways, DNA repair, transcriptional and translational procedures [95]. In the incidence of proteotoxic stress induced by proteasome inhibition, there could be a possible association of UPS-related, proteolysis-dependent functions with an indirect impact on DNA repair. It is well known that the main pool of cellular Ub is present in conjugates of mono- and poly-ubiquitinated proteins, with only a small fraction free. The use of proteasome inhibitors results in accumulation of poly-ubiquitinated proteins and redistribution of Ub from nucleus mono-Ub sources to the cytosol, given that Ub neosynthesis cannot compensate the acute needs for Ub. Consequently, Ub-dependent processes such as transcription, chromatin remodelling and DNA repair are largely affected, particularly via de-ubiquitylation of histones H2A and H2B, which are a major pool of nuclear Ub [96, 97]. The final outcome is a significant decrease in DNA replication and RNA transcription, explained by a subsequent condensation of nucleosomes but also by the ubiquitination of transcription factors and other proteins involved in replication (such as co-activators and co-repressors), which possibly inactivates them or prevents their nuclear translocation [98]. The indirect effect of chromatin condensation on DNA repair by limiting the accessibility of repair proteins to damaged sites is best illustrated in the results of a study measuring the efficacy of NER in ovarian cancer cells treated with a combination of proteasome inhibitor followed by cisplatin. Proteasome inhibition had a profound sensitizing effect by inducing deubiquitination of ubiquitinated histone H2A (uH2A), increasing the extent of cisplatin-DNA adducts, and diminishing NER-dependent repair of cisplatin-DNA lesions [99]. Proteasome inhibitor also prevented the increase in ERCC-1 mRNA expression that occurs in cells exposed to cisplatin [100, 101]. ERCC-1 protein is responsible for the excision of lesions processed by NER [2]. Finally, cells treated with the combination of proteasome inhibitor and cisplatin underwent apoptosis more quickly than cells treated with either agent alone [99–101].

It appears that the degree of chromatin compaction may, at least partially, determine both the likelihood of a DNA sequence to be assaulted by damage and the feasibility of DNA repair. This suggestion is based on the observation of similarities of chromatin recruitment of DNA repair proteins between both FA and DSBR pathways, which is effected by ubiquitination (of FANCD2-FANCI and histone H2AX, respectively), interaction with Ub-binding proteins, assembly of a multisubunit DNA repair complex and finally a deubiquitination event after the completion of repair [102].

The management of oxidative stress is another demanding situation for cells, partly dealt with PARP-mediated activation of nuclear 20S proteasome activity, leading to proteolytic removal of oxidatively damaged histones. The adaptation to oxidative stress through poly(ADP)-ribosylation of the proteasome was shown by inhibitor experiments, 14C-ADP-ribose incorporation assays, immunoblotting, in vitro reconstitution experiments and immunoprecipitation of (activated) proteasome with anti-poly-ADP-ribose polymerase antibodies in human myelogenous leukaemia cells [103]. This PARP-dependent enhancement of proteolytic activity of the proteasome is an example of how the concerted action of two secondary antioxidant defence systems might lead to or support the restitution of the native chromatin structure, as well as an important element in the development of long-term resistance to many chemotherapeutic drugs [103, 104].

With respect to genotoxic stressors, a more general role of UPS mobilization on the adjustment of the DNA repair machinery is summarized in the regulation of Promyelocytic leukaemia protein (PML) nuclear bodies (NBs) dispersal in response to alkylating agents. PML NBs, present in the majority of human cell types, are responsible, among other functions, for the assembling of DNA repair proteins in sub-nuclear depots, under normal conditions [105]. The sumoylation of PML was proved to be indispensable for the assembly of PML NBs as a PML mutant that could no longer be modified by SUMO-1 failed to achieve NB assembly, and displayed an aberrant nuclear localization pattern [106, 107]. SUMO-2 and SUMO-3 can play a similar role in this process, compensatory in cases of lack of SUMO-1 [108].
When DNA damage occurs, cells orchestrate the redistribution of these nuclear foci in order to achieve the release and accessibility of DNA repair proteins to sites of damaged DNA across the genome. However, the use of a proteasome inhibitor in MMS-treated cells led to stabilization of PML NBs, demonstrating that this process is proteasome-dependent [105]. This finding is only part of general evidence indicating that global protein degradation that is dependent on proteasome function increases upon DNA damage. Furthermore, optimal proteasome function is a prerequisite condition for long-term cell growth after acute exposure to alkylating agents [109]. These data are consistent with the idea that an increase in the intracellular concentration of damaged proteins, resulting from protein alkylation coupled with inhibition of the mechanism to purge the damaged proteins results in a growth defect [109]. This is a rationale for combing the use of alkylating agents with that of proteasome inhibitors in cancer therapy. Moreover, a less direct involvement of UPS in the PML-related response is effectuated via p53; the former has been identified (via mRNA expression, identification of p53 consensus binding sites in PML promoter and luciferase reporter assays) as a p53-target gene that really contributes to its tumour-suppressor functions in cell cycle arrest, senescence and apoptosis by functioning both downstream and upstream of p53 [110].

The contribution of SUMO to the sub-nuclear organization of DNA repair processes was also, very recently, demonstrated to involve the functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent Ub ligase. First, epistasis analysis (E-MAP) using DNA repair factors in yeast indicated a functional relationship and physical interaction between a nuclear pore subcomplex and Sis5/Sis8, a SUMO-dependent Ub ligase. Second, real-time imaging and ChIP confirmed stable recruitment of damaged DNA to nuclear pores. Spontaneous gene conversion was enhanced in a Sis6-dependent manner by tethering donor sites at the nuclear periphery. This suggests that strand breaks are shunted to nuclear pores for a repair pathway controlled by a conserved SUMO-dependent E3 ligase [111].

Similarly, to the mechanism of PML-NBs proteasome-dependent dispersal in response to DNA damage, the disassembly of mediator of DNA-damage checkpoint protein 1 (MDC1), which is an early and key component of the genome surveillance network, activated by DNA double-strand breaks (DSBs), was found to be Ub- and proteasome-dependent. Increased MDC1 ubiquitylation was observed in response to irradiation, which was correlated with a reduction in total MDC1 protein levels. Blocking MDC1 degradation by proteasome inhibitors led to a persistence of MDC1 foci. Interestingly, the persistence of MDC1 foci was associated with an abrogated recruitment of the downstream factor BRCA1. Consequently, this novel mechanism for the disassembly of MDC1 foci via UPS-dependent degradation appears to be a key step for the efficiency of an important part of DNA repair, the assembly of BRCA1 foci [112].

Conclusions and future perspectives

It is clear that the effect of UPS on transcriptional regulation of DNA repair is mainly represented by the existence of direct interactions between proteasome units or UPS-dependent transcription factors and promoters of DNA repair genes. However, in an effort to illustrate a less prominent connection between transcription of DNA repair and UPS, we have included data concerning the post-translational regulation of transcription-specific DNA repair pathways by UPS. This correlation might seem unlikely at a first glance, it is nevertheless reasonable to expect an indirect impact of delay or suspension of transcription elongation during the expression of DNA repair genes, given that the DNA-damaged sites could be located anywhere across the genome and transcriptome and thus, affects any coding region.

Furthermore, an extensive description is made of how different types of cellular stress can influence the effectiveness of DNA repair by dealing with chromatin recruitment and sub-nuclear compartmentalization of DNA repair proteins and complexes. Here, as well, may lay implications about UPS-dependent transcriptional modifications of DNA repair, as dense chromatin – induced by proteotoxic stress – is unsuitable for both access by DNA repair proteins and gene transcription in general (including DNA repair genes). Finally, the contribution of proteasome-dependent dispersal of DNA repair factors foci or DNA-damage response factors foci in their proper access to DNA and efficiency of DNA repair might concern various DNA repair processes, including transcription-specific repair.

As a whole, UPS is proved to be a determinant of cellular defensive mechanisms against various effectors of stress and DNA damage. No DNA repair pathway, known until now, seems to remain unaffected by this multifunctional sub-cellular entity, either at the transcriptional or at the post-translational level (which was the subject of part I of this review) [113]. As more data come to light, the interconnection between UPS and DNA repair is expected to become even more profound and firmly established. Already at this point, the benefit of this correlation on a clinical basis of cancer therapeutics is evident. The use of proteasome-inhibitors together with DNA-damaging agents is a promising chemotherapy combination, already being tested in phase I, II, III clinical trials [114–118]. The knowledge of molecular mechanisms underlying this combination treatment is the key to interpret and manipulate pharmacological knowledge and apply targeted drug therapies in the most beneficial way possible for patients.

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