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

Received: February 1, 2009; Accepted: June 3, 2009

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^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

*Correspondence to: Panagiotis J. VLACHOSTERGIOS,
Division of Medical Oncology, University Hospital of Larissa,
Larissa 41110, Greece.
Tel.: 0030-2410-682785
Fax: 0030-2410-672467
E-mail: pvlacho@med.uth.gr

doi:10.1111/j.1582-4934.2009.00825.x

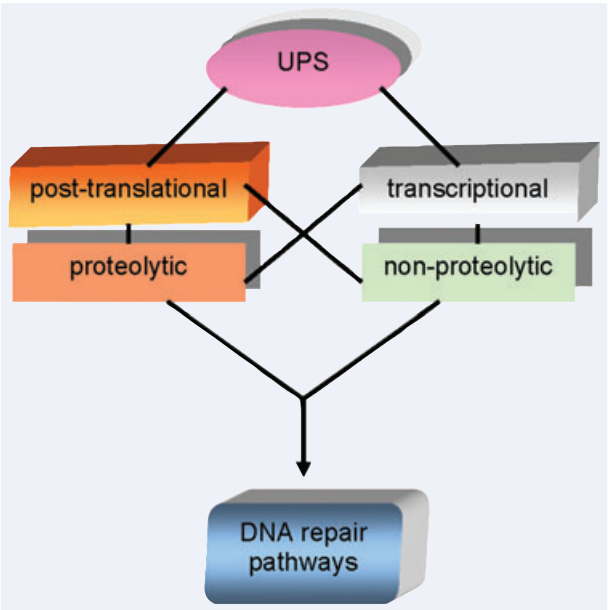

Fig. 1 Modes of UPS involvement in regulation of DNA repair.

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 regulatory 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 activation process, mainly represented by the small ubiquitin-like modifier (SUMO) protein and neural precursor cell expressed, developmentally 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 modifications 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 damage (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 polymerase recruitment, elongation progression and transcription termination [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 damage, and in both proteolytic and non-proteolytic ways. Focusing on specific descriptions of the UPS-DNA repair connection at transcriptional 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 polymerase II (pol II)* at stalled sites of bulky lesions, which can either be bypassed or degraded after prolonged elongation-discontinuation of transcription. The first case is accomplished by the TCR proteins termed *Cockayne Syndrome A and B (CSA, CSB)*, however, if pol II is irreversibly trapped, the 26S proteasome takes over [16, 22–26]. A unique status of phosphorylation of the carboxy-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 component 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 previously described [27]. Moreover, UV irradiation induces the degradation 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,

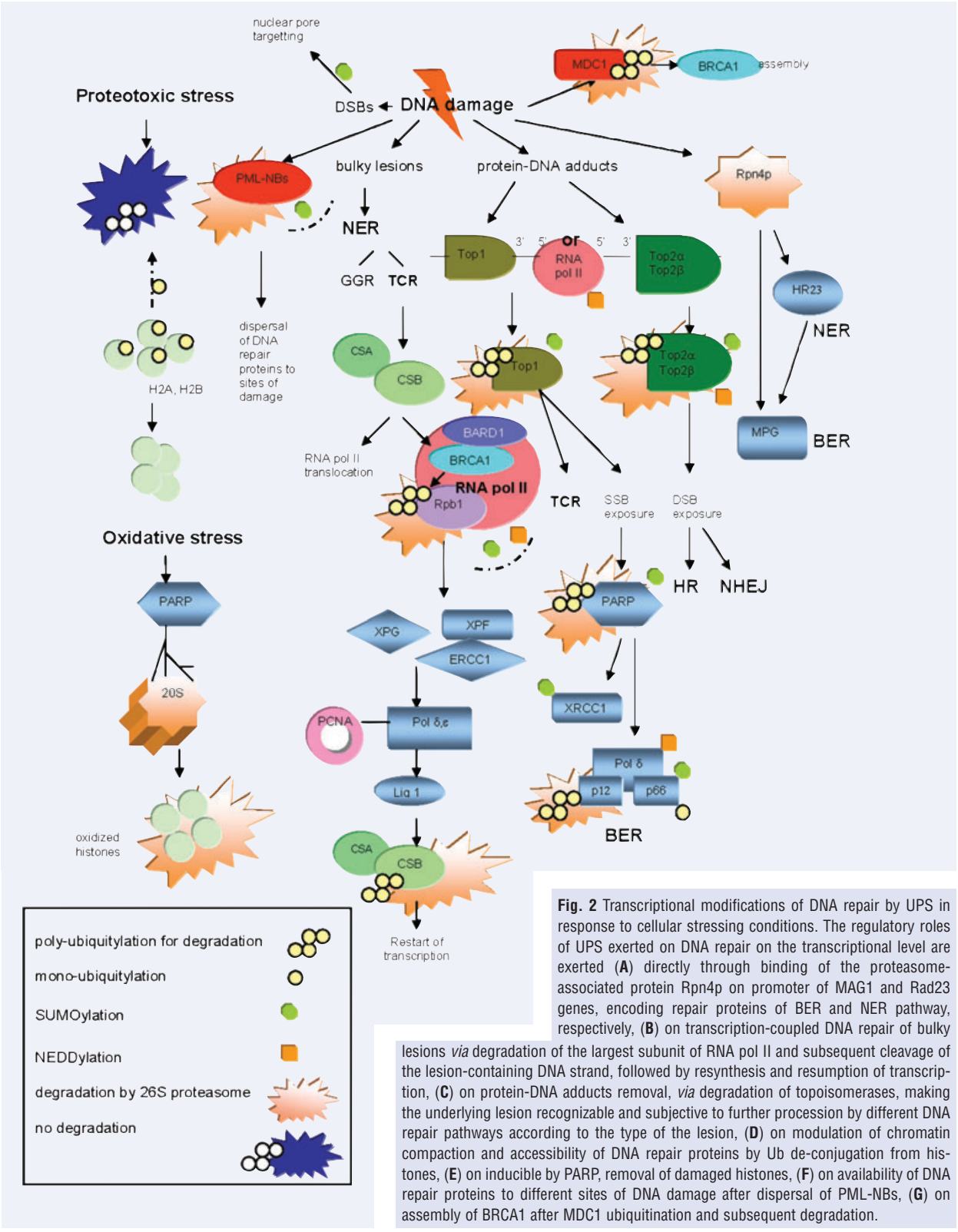

Fig. 2 Transcriptional modifications of DNA repair by UPS in response to cellular stressing conditions. The regulatory roles of UPS exerted on DNA repair on the transcriptional level are exerted (**A**) directly through binding of the proteasome-associated protein Rpn4p on promoter of MAG1 and Rad23 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 subjective 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.

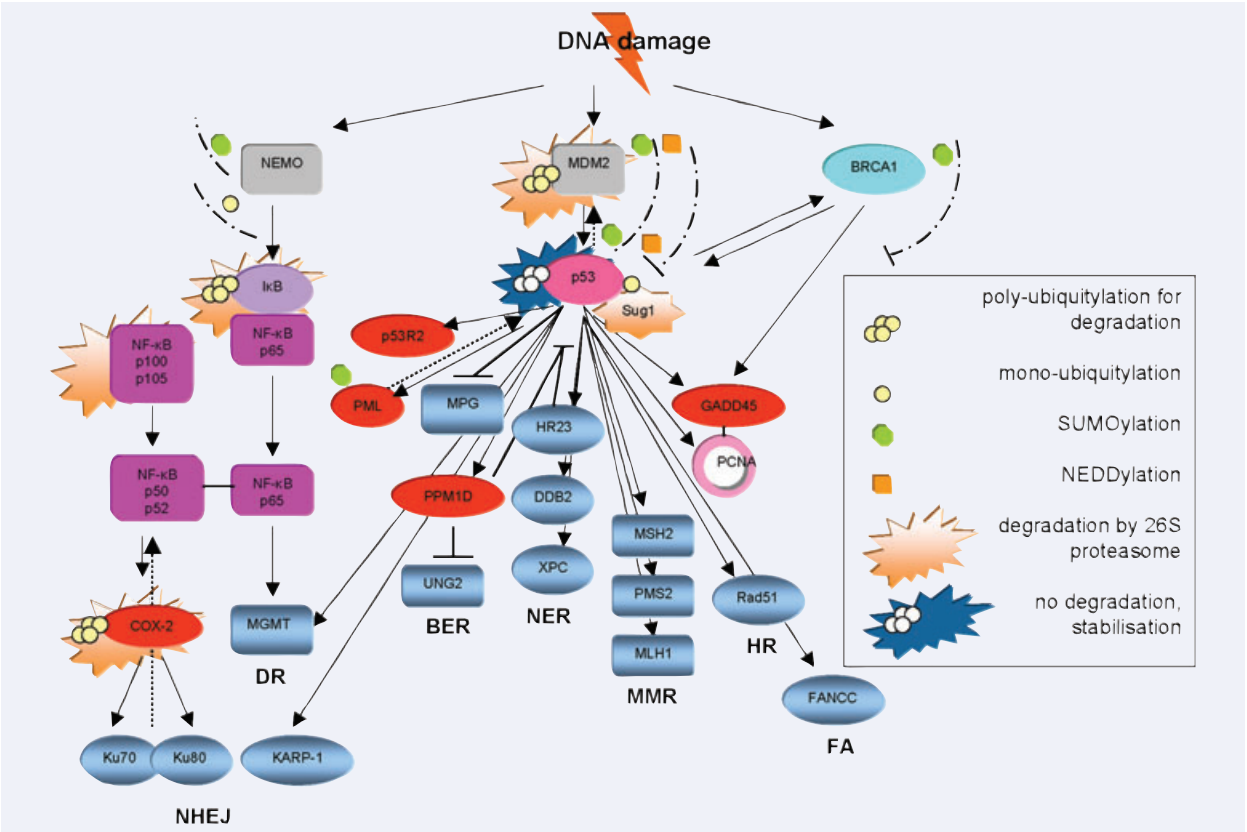

Fig. 3 Transcriptional activation of DNA repair mediated by UPS-dependent pathways. The induction of DNA repair genes in response to DNA damage involves the activation (NF-κB) or stabilization (p53) of regulatory proteins in a UPS-controlled process. The activation of the NF-κB pathway results in upregulation 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, O^6-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 δ, ε, DNA polymerases δ, 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; PPM1D, protein phosphatase 1D magnesium-dependent, delta isoform; MSH2, MutS homolog 2; PMS2 post-meiotic segregation increased 2; MLH1, MutL homolog 1; KARP-1, Ku86 autoantigen-related protein-1; FANCC, Fanconi anemia, complementation group C; p53R2, 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	NF-κB	Induction
		p53	Induction (upon DNA damage)
			Repression (of basal expression)
MAG1 (MPG)	BER	26S proteasome (Rpn4p)	Induction
		p53	Repression
DDB2	NER	p53	Induction
XPC	NER	p53	Induction
Rad23 (HR23)	NER	26S proteasome (Rpn4p)	Induction
		p53	Induction
GADD45	BER, NER	p53	Induction
		BRCA1	Induction
PPM1D	BER	p53	Induction
		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
		SUMO	Unknown
		BRCA1	Ubiquitylation for degradation of Rpb1
			Inhibition of phosphorylation of CTD
CSB	TCR (NER)	Poly-Ub & 26S proteasome	Degradation
Top1	Protein-DNA adducts repair	Poly-Ub & 26S proteasome	Degradation
		SUMO	Nuclear pore targeting or protein binding
Top2	Protein-DNA adducts repair	Poly-Ub & 26S proteasome	Degradation
		SUMO	Nuclear pore targeting or protein binding
		NEDD8	Unknown
PML		26S proteasome	Dispersal of NBs
		SUMO	Assembly of NBs
MDC1		Poly-Ub & 26S proteasome	Degradation, BRCA1 assembly
H2A, H2B		Mono-Ub	Deubiquitylation of H2A/H2B after proteasome inhibition, dense chromatin

Abbreviations: MGMT, O^6-methylguanine-DNA methyltransferase; DR, direct repair; NF-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, neural 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 isoform; PCNA, proliferating cell nuclear antigen; MSH2, MutS homolog 2; PMS2, PMS2 post-meiotic 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 and 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 (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 radiation (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)*. Similarly to DDB2, a strong candidate for a p53 response element 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 repression 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 expression, 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 transcription 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 disruption 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 ubiquitylation and proteasome function might be indispensable for the ability of the promoter-bound p53 to fire off new rounds of transcription [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 effected *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 regulatory 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 function. 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 chromatin-DNA repair protein interactions [62] or/and negative regulation of basal levels of p21 [63]. Additionally to the p53-dependent transcriptional induction of GADD45, a p53 independent mechanism that is regulated by BRCA1 has also been suggested, *via* physical association of the latter with a transactivation 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 accomplished through stimulation of the p53-transcription activity [31, 32], with DDB2 gene upregulation being a striking, well-described example [64]. More importantly, the final effect of this BRCA1 involvement 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 independent, 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 disassembly 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-transcriptional 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 IκB kinase complex, IKKγ 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 IκB-α and IκB-β. These proteins are substrates of 26S proteasome in a way that proteasomal 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 prodromal forms p105 and p100, to generate p50 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, O^6-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 [Ceribeli 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, ^{14}C-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 generic 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 effected *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 Slx5/Slx8, 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 Slx8-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.

Acknowledgements

The authors declare that we have no conflict of interest related to this article.

References

1. **Hansen WK, Kelley MR.** Review of mammalian DNA repair and translational implications. *J Pharmacol Exp Ther.* 2000; 295: 1–9.
2. **Christmann M, Tomicic MT, Roos WP, *et al.*** Mechanisms of human DNA repair: an update. *Toxicology.* 2003; 193: 3–34.
3. **Wood RD, Mitchell M, Lindahl T.** Human DNA repair genes. *Mutat Res.* 2005; 577: 275–83.
4. **Pourquier P.** La réparation de l' ADN, cible potentielle d'un développement thérapeutique en cancérologie. *Bull Cancer.* 2006; hors série: 124–44.
5. **Sánchez-Pérez I.** DNA inhibitors in cancer treatment. *Clin Transl Oncol.* 2006; 8: 642–6.
6. **Damia G, D'Incalci M.** Targetting DNA repair as a promising approach in cancer therapy. *Eur J Cancer.* 2007; 43: 1791–801.
7. **Barbour L, Xiao W.** Regulation of alternative replication bypass pathways at stalled replication forks and its effects on genome stability: a yeast model. *Mutat Res.* 2003; 532: 137−55.
8. **Kennedy RD, D' Andrea AD.** The Fanconi Anemia/BRCA pathway: new faces in the crowd. *Genes Dev.* 2005; 19: 2925–40.
9. **Lieberman HB.** DNA damage repair and response proteins as targets for cancer therapy. *Curr Med Chem.* 2008; 15: 360–7.
10. **Baumeister W, Walz J, Zuhl F, *et al.*** The proteasome: paradigm of a self-compartmentalizing protease. *Cell.* 1998; 92: 367–80.
11. **Ciechanover A, Orian A, Schwartz AL.** The ubiquitin-mediated proteolytic pathway: mode of action and clinical implications. *J Cell Biochem Suppl.* 2000; 34: 40–51.
12. **Adams J.** The proteasome: structure, function, and role in the cell. *Cancer Treat Rev.* 2003; 29 Suppl 1: S3–9.
13. **Behuliak M, Celec P, Gardlik R, *et al.*** Ubiquitin – the kiss of death goes Nobel. Will you be quitting? *Bratisl Lek Listy* 2005; 106: 93–100.
14. **Hermann J, Lerman LO, Lerman A.** Ubiquitin and ubiquitin-like proteins in protein regulation. *Circ Res.* 2007; 100: 1276–91.
15. **Hoeller D, Hecker CM, Dikic I.** Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. *Nat Rev Cancer.* 2006; 6: 776–88.
16. **Muratani M, Tansey WP.** How the ubiquitin-proteasome system controls transcription. *Nat Rev Mol Cell Biol.* 2003; 4: 192–201.
17. **Collins GA, Tansey WP.** The proteasome: a utility tool for transcription. *Curr Opin Genet Dev.* 2006; 16: 197–202.
18. **Kodadek T, Sikder D, Nalley K.** Keeping transcriptional activators under control. *Cell.* 2006; 127: 261–4.
19. **O'Connell BC, Harper JW.** Ubiquitin proteasome system (UPS): what can chromatin do for you?. *Curr Opin Cell Biol.* 2007; 19: 206–14.
20. **Auld KL, Brown CR, Casolari JM, *et al.*** Genomic association of the proteasome demonstrates overlapping gene regulatory activity with transcription factor substrates. *Mol Cell.* 2006; 21: 861–71.
21. **Auld KL, Silver PA.** Transcriptional regulation by the proteasome as a mechanism for cellular protein homeostasis. *Cell Cycle.* 2006; 5: 1503–5.
22. **Huang TT, D' Andrea AD.** Regulation of DNA repair by ubiquitylation. *Nat Rev Mol Cell Biol.* 2006; 7: 323–34.
23. **Bergink S, Jaspers NG, Vermeulen W.** Regulation of UV-induced DNA damage response by ubiquitylation. *DNA Repair.* 2007; 6: 1231–42.
24. **Lee KB, Wang D, Lippard SJ, *et al.*** Transcription-coupled and DNA damage-dependent ubiquitination of RNA polymerase II in vitro. *PNAS.* 2002; 99: 4239–44.
25. **Svejstrup JQ.** Rescue of arrested RNA polymerase II complexes. *J Cell Sci.* 2003; 116: 447–51.
26. **Gillette TG, Gonzalez F, Delahodde A, *et al.*** Physical and functional association of RNA polymerase II and the proteasome. *PNAS.* 2004; 101: 5904–9.
27. **Ribar B, Prakash L, Prakash S.** Requirement of ELC1 for RNA polymerase II polyubiquitylation and degradation in response to DNA damage in Saccharomyces cerevisiae. *Mol Cell Biol.* 2006; 26: 3999–4005.
28. **Groisman R, Kuraoka I, Chevallier O, *et al.*** CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. *Genes Dev.* 2006; 20: 1429–34.
29. **Kvint K, Uhler JP, Taschner MJ, *et al.*** Reversal of RNA polymerase II ubiquitylation by the ubiquitin protease Ubp3. *Mol Cell.* 2008; 30: 498–506.
30. **Daulny A, Tansey WP.** Damage control: DNA repair, transcription, and the ubiquitin-proteasome system. *DNA Repair.* 2009 (Epub ahead of print).
31. **Mullan PB, Quinn JE, Harkin DP.** The role of BRCA1 in transcriptional regulation and cell cycle control. *Oncogene.* 2006; 25: 5854–63.
32. **Yoshida K, Miki Y.** Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cell Sci.* 2004; 95: 866–71.
33. **Moisan A, Larochelle C, Guillemette B, *et al.*** BRCA1 can modulate RNA polymerase II carboxy-terminal domain phosphorylation levels. *Mol Cell Biol.* 2004; 24: 6947–56.
34. **Panse VG, Hardeland U, Werner T, *et al.*** A proteome-wide approach identifies sumoylated substrate proteins in yeast. *J Biol Chem.* 2004; 279; 41346–51.
35. **Jones J, Wu K, Yang Y, *et al.*** A targeted proteomic analysis of the ubiquitin-like modifier nedd8 and associated proteins. *J Proteome Res.* 2008; 7: 1274–87.
36. **Norman JA, Shiekhattar R.** Analysis of Nedd8-associated polypeptides: a model for deciphering the pathway for ubiquitin-like modifications. *Biochemistry.* 2006; 45: 3014–9.
37. **Lin CP, Ban Y, Lyu YL, *et al.*** A ubiquitin-proteasome pathway for the repair of Top1-DNA covalent complexes. *J Biol Chem.* 2008; 283: 21074–83.
38. **Desai SD, Zhang H, Rodriguez-Bauman A, *et al.*** Transcription-dependent degradation of Topoisomerase I-DNA covalent complexes. *Mol Cell Biol.* 2003; 23: 2341–50.
39. **Mao Y, Desai SD, Ting CY, *et al.*** 26S proteasome-mediated degradation of Topoisomerase II-DNA cleavable complexes. *J Biol Chem.* 2001; 276: 40652–8.
40. **Zhang A, Lyu YL, Lin CP, *et al.*** A protease pathway for the repair of Topoisomerase II-DNA covalent complexes. *J Biol Chem.* 2006; 281: 35997–6003.
41. **Mao Y, Sun M, Desai SD, *et al.*** SUMO-1 conjugation to Topoisomerase I: a possible repair response to topoisomerase-mediated DNA damage. *PNAS.* 2000; 97: 4046–51.
42. **Jelinsky SA, Estep P, Church GM, *et al.*** Regulatory networks revealed by transcriptional profiling of damaged Saccharomyces cerevisiae cells: Rpn4 links base excision repair with proteasomes. *Mol Cell Biol.* 2000; 20: 8157–67.
43. **Mannhaupt G, Schnall R, Karpov V, *et al.*** Rpn4p acts as a transcription factor by binding to PACE, a nonamer box found upstream of 26S proteasomal and other genes in yeast. *FEBS Lett.* 1999; 450: 27–34.

44. **Miao F, Bouziane M, Dammann R, *et al.*** 3-methyladenine-DNA glycosylase (MPG protein) interacts with human RAD23 proteins. *J Biol Chem.* 2000; 275: 28433–8.
45. **Stommel JM, Wahl GM.** A new twist in the p53-feedback loop: stress-activated MDM2 destabilization is required for p53 activation. *Cell Cycle.* 2005; 4: 411–7.
46. **Yang Y, Li CC, Weissman AM.** Regulating the p53 system through ubiquitination. *Oncogene.* 2004; 23: 2096–106.
47. **Zurer I, Hofseth LJ, Cohen Y, *et al.*** The role of p53 in base excision repair following genotoxic stress. *Carcinogenesis.* 2004; 25: 11–9.
48. **Tan T, Chu G.** p53 binds and activates the xeroderma pigmentosum DDB2 gene in humans but not mice. *Mol Cell Biol.* 2002; 22: 3247–54.
49. **Hwang BJ, Ford JM, Hanawalt PC, *et al.*** Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *Proc Natl Acad Sci USA.* 1999; 96: 424–8.
50. **Adimoolam S, Ford JM.** p53 and DNA damage-inducible expression of the xeroderma pigmentosum group C gene. *Proc Natl Acad Sci USA.* 2002; 99: 12985–90.
51. **Ceribeli M, Alcalay M, Viganò MA, *et al.*** Repression of new p53 targets revealed by ChIP on chip experiments. *Cell Cycle.* 2006; 5: 1102–10.
52. **Glockzin S, Ogi FX, Hengstermann A, *et al.*** Involvement of the DNA repair protein hHR23 in p53 degradation. *Mol Cell Biol.* 2003; 23: 8960–9.
53. **Zhu Q, Wani G, Yao J, *et al.*** The ubiquitin-proteasome system regulates p53-mediated transcr iption at p21waf1 promoter. *Oncogene.* 2007; 26: 4199–208.
54. **Chen L, Chen J.** MDM2-ARF complex regulates p53 sumoylation. *Oncogene.* 2003; 22: 5348–57.
55. **Stehmeier P, Muller S.** Regulation of p53 family members by the ubiquitin-like SUMO system. *DNA Repair.* 2009.
56. **Xirodimas DP, Saville MK, Bourdon JC, *et al.*** Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. *Cell.* 2004; 118: 83–97.
57. **Gatz SA, Wiesmüller L.** p53 in recombination and repair. *Cell Death Differ.* 2006; 13: 1003–16.
58. **Tanaka H, Arakawa H, Yamaguchi T, *et al.*** A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature.* 2000; 404: 42–9.
59. **Levine AJ.** p53, the cellular gatekeeper for growth and division. *Cell.* 1997; 88: 323–31.
60. **Liebermann DA, Hoffman B.** Gadd45 in stress signaling. *J Mol Signal.* 2008; 3: 15.
61. **Smith ML, Chen IT, Zhan Q, *et al.*** Interaction of the p53-regulated protein Gadd45 with prol iferating cell nuclear antigen. *Science.* 1994; 266: 1376–80.
62. **Smith ML, Ford JM, Hollander MC, *et al.*** p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol Cell Biol.* 2000; 20: 3705–14.
63. **Maeda T, Espino RA, Chomey EG, *et al.*** Loss of p21WAF1/Cip1 in Gadd45-deficient keratinocytes restores DNA repair capacity. *Carcinogenesis.* 2005; 26: 1804–10.
64. **El-Deiry WS.** Transactivation of repair genes by BRCA1. *Cancer Biol Ther.* 2002; 1: 490–1.
65. **MacLachlan TK, Takimoto R, El-Deiry WS.** BRCA1 directs a selective p53-dependent transcriptional response towards growth arrest and DNA repair targets. *Mol Cell Biol.* 2002; 22: 4280–92.
66. **Starita LM, Parvin JD.** The multiple nuclear functions of BRCA1: transcription, ubiquitination and DNA repair. *Curr Opin Cell Biol.* 2003; 15: 345–50.
67. **Park MA, Seok YJ, Jeong G, *et al.*** SUMO1 negatively regulates BRCA1-mediated transcription, via modulation of promoter occupancy. *Nucleic Acids Res.* 2008; 36: 263–83.
68. **Lu X, Nguyen TA, Appella E, *et al.*** Homeostatic regulation of base excision repair by a p53-induced phosphatase: linking stress response pathways with DNA repair proteins. *Cell Cycle.* 2004; 3 : 1363–6.
69. **Paunesku T, Mittal S, Protić M, *et al.*** Proliferating cell nuclear antigen (PCNA): ringmaster of the genome. *Int J Radiat Biol.* 2001; 77: 1007–21.
70. **Scherer SJ, Maier SM, Seifert M, *et al.*** p53 and c-Jun functionally synergize in the regulation of the DNA repair gene hMSH2 in response to UV. *J Biol Chem.* 2000; 275: 37469–73.
71. **Warnick CT, Dabbas B, Ford CD, *et al.*** Identification of a p53 response element in the promoter region of the hMSH2 gene required for expression in A2780 ovarian cancer cells. *J Biol Chem.* 2001; 276 : 27363–70.
72. **Chen J, Sadowski I.** Identification of the mismatch repair genes PMS2 and MLH1 as p53 target genes by using serial analysis of binding elements. *Proc Natl Acad Sci USA.* 2005; 102: 4813–8.
73. **Harris LC, Remack JS, Houghton PJ, *et al.*** Wild-type p53 suppresses transcription of the human O6-methylguanine-DNA methyltransferase gene. *Cancer Res.* 1996; 56: 2029–32.
74. **Grombacher T, Eichhorn U, Kaina B.** p53 is involved in regulation of the DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) by DNA damaging agents. *Oncogene.* 1998; 17: 845–51.
75. **Srivenugopal KS, Shou J, Mullapudi SR, *et al.*** Enforced expression of wild-type p53 curtails the transcription of the O(6)-methylguanine-DNA methyltransferase gene in human tumor cells and enhances their sensitivity to alkylating agents. *Clin Cancer Res.* 2001; 7: 1398–409.
76. **Blough MD, Zlatescu MC, Cairncross JG.** O6-methylguanine-DNA methyltransferase regulation by p53 in astrocytic cells. *Cancer Res.* 2007; 67: 580–4.
77. **Lavon I, Fuchs D, Zrihan D, *et al.*** Novel mechanism whereby nuclear factor κB mediates DNA damage repair through regulation of O6-methylguanine-DNA-methyltransferase. *Cancer Res.*2007; 67: 8952–9.
78. **Huang TT, Wuerzberger-Davis SM, Wu ZH, *et al.*** Sequential modification of NEMO/IKKγ by SUMO-1 and ubiquitin mediates NF-κB activation by genotoxic stress. *Cell.* 2003; 115: 565–76.
79. **Janssens S, Tschopp J.** Signals from within: the DNA-damage-induced NF-κB response. *Cell Death Differ.* 2006; 13: 773–84.
80. **Beinke S, Ley SC.** Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. *Biochem J.* 2004; 382: 393–409.
81. **Magnani M, Crinelli R, Bianchi M, *et al.*** The ubiquitin-dependent proteolytic system and other potential targets for the modulation of nuclear factor-κB (NF-κB). *Curr Drugs Targets.* 2000; 1: 387–99.
82. **Lin L, DeMartino GL, Greene WC.** Cotranslational biogenesis of NF-κB p50 by the 26S proteasome. *Cell.* 1998; 92: 819–28.
83. **Palombella VJ, Rando OJ, Goldberg AL, *et al.*** The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB. *Cell.* 1994; 78: 773–85.
84. **Webster GA, Perkins ND.** Transcriptional cross talk between NF-kappaB and p53. *Mol Cell Biol.* 1999; 19: 3485–95.
85. **Benoit V, Hellin AC, Huygen S, *et al.*** Additive effect between NF-kappaB subunits and p53 protein for transcriptional activation of human p53 promoter. *Oncogene.* 2000; 19: 4787–94.

86. **Shao J, Fujiwara T, Kadowaki Y, *et al.*** Overexpression of the wild-type p53 gene inhibits NF-kappaB activity and synergizes with aspirin to induce apoptosis in human colon cancer cells. *Oncogene.* 2000; 19: 726–36.
87. **Jiang XH, Wong BC, Lin MC, *et al.*** Functional p53 is required for triptolide-induced apoptosis and AP-1 and nuclear factor-kappaB activation in gastric cancer cells. *Oncogene.* 2001; 20: 8009–18.
88. **Tergaonkar V, Pando M, Vafa O, *et al.*** p53 stabilization is decreased upon NFkappaB activation: a role for NFkappaB in acquisition of resistance to chemotherapy. *Cancer Cell.* 2002; 1: 493–503.
89. **Tergaonkar V, Perkins ND.** p53 and NF-kappaB crosstalk: IKKalpha tips the balance. *Mol Cell.* 2007; 26: 158–9.
90. **Kim H.** DNA repair Ku proteins in gastric cancer cells and pancreatic acinar cells. *Amino Acids.* 2008; 34: 195–202.
91. **Myung K, Braastad C, He DM, *et al.*** KARP-1 is induced by DNA damage in a p53- and ataxia telangiectasia mutated-dependent fashion. *Proc Natl Acad Sci U S A.* 1998; 95: 7664–9.
92. **Myung K, He DM, Lee SE, *et al.*** KARP-1: a novel leucine zipper protein expressed from the Ku86 autoantigen locus is implicated in the control of DNA-dependent protein kinase activity. *EMBO J.* 1997; 16: 3172–84.
93. **Arias-Lopez C, Lazaro-Trueba I, Kerr P, *et al.*** p53 modulates homologous recombination by transcriptional regulation of the RAD51 gene. *EMBO Rep.* 2006; 7: 219–24.
94. **Liebetrau W, Budde A, Savoia A, *et al.*** p53 activates Fanconi anemia group C gene expression. *Hum Mol Genet.* 1997; 6: 277–83.
95. **Harper JW, Elledge SJ.** The DNA damage response: ten years after. *Mol Cell.* 2007; 28: 739–45.
96. **Dantuma NP, Groothuis TA, Salomons FA, *et al.*** A dynamic ubiquitin equilibrium couples proteasomal activity to chromatin remodelling. *J Cell Biol.* 2006; 173: 19–26.
97. **Groothuis TA, Dantuma NP, Neefjes J, *et al.*** Ubiquitin crosstalk connecting cellular processes. *Cell Div.* 2006; 1: 21.
98. **Mimnaugh EG, Chen HY, Davie JR, *et al.*** Rapid deubiquitination of nucleosomal histones in human tumor cells caused by proteasome inhibitors and stress response inducers: effects on replication, transcription, translation, and the cellular stress response. *Biochemistry.* 1997; 36: 14418–29.
99. **Mimnaugh EG, Yunmbam MK, Li Q, *et al.*** Prevention of cisplatin-DNA adduct repair and potentiation of cisplatin-induced apoptosis in ovarian carcinoma cells by proteasome inhibitors. *Biochem Pharmacol.* 2000; 60: 1343–54.
100. **Li QQ, Ding L, Reed E.** Proteasome inhibition suppresses cisplatin-dependent ERCC-1 mRNA expression in human ovarian tumor cells. *Res Commun Mol Pathol Pharmacol.* 2000; 107: 387–96.
101. **Li QQ, Yunmbam MK, Zhong X, *et al.*** Lactacystin enhances cisplatin sensitivity in resistant human ovarian cancer cell lines via inhibition of DNA repair and ERCC-1 expression. *Cell Mol Biol (Noisy-le-grand).* 2001; 47: OL61–72.
102. **Cohn MA, D' Andrea AD.** Chromatin recruitment of DNA repair proteins: lessons from the Fanconi anemia and double strand break repair pathways. *Mol Cell.* 2008; 32: 306–12.
103. **Ullrich O, Reinheckel T, Sitte N, *et al.*** Poly-ADP ribose polymerase activates nuclear proteasome to degrade oxidatively damaged histones. *Proc Natl Acad Sci USA.* 1999; 96: 6223–8.
104. **Arnold J, Grune T.** PARP-mediated proteasome activation: a co-ordination of DNA repair and protein degradation?. *Bioessays.* 2002; 24: 1060–5.
105. **Conlan LA, McNees CJ, Heierhorst J.** Proteasome-dependent dispersal of PML nuclear bodies in response to alkylating DNA damage. *Oncogene.* 2004; 23: 307–10.
106. **Zhong S, Müller S, Ronchetti S, *et al.*** Role of SUMO-1 modified PML in nuclear body formation. *Blood.* 2000; 95: 2748–53.
107. **Zhao J.** SUMO regulates diverse biological processes. *Cell Mol Life Sci.* 2007; 64: 3017–33.
108. **Evdokimov E, Sharma P, Lockett SJ, *et al.*** Loss of SUMO1 in mice affects RanGAP1 localization and formation of PML nuclear bodies, but is not lethal as it can be compensated by SUMO2 or SUMO3. *J Cell Sci.*2008; 121: 4106–13.
109. **Burgis NE, Samson LD.** The protein degradation response of Saccharomyces cerevisiae to classical DNA-damaging agents. *Chem Res Toxicol.* 2007; 20: 1843–53.
110. **de Stanchina E, Querido E, Narita M, *et al.*** PML is a direct p53 target that modulates p53 effector functions. *Mol Cell.* 2004; 13: 523–35.
111. **Nagai S, Dubrana K, Tsai-Pflugfelder M, *et al.*** Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science.* 2008; 322: 597–602.
112. **Shi W, Ma Z, Willers H, *et al.*** Disassembly of MDC1 Foci is controlled by ubiquitin-proteasome-dependent degradation. *J Biol Chem.* 2008; 283: 31608–16.
113. **Vlachostergios PJ, Patrikidou A, Daliani DD, *et al.*** The ubiquitin-proteasome system in cancer, a major player in DNA repair. Part I: post-translational regulation. *J Cell Mol Med.* 2009; 13: 2026–38.
114. **Orlowski RZ, Nagler A, Sonneveld P, *et al.*** Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: combination therapy improves time to progression. *J Clin Oncol.* 2007; 25: 3892–901.
115. **Berenson JR, Yang HH, Sadler K, *et al.*** Phase I/II trial assessing bortezomib and melphalan combination therapy for the treatment of patients with relapsed or refractory multiple myeloma. *J Clin Oncol.* 2006; 24: 937–44.
116. **Aghajanian C, Dizon DS, Sabbatini P, *et al.*** Phase I trial of bortezomib and carboplatin in recurrent ovarian or primary peritoneal cancer. *J Clin Oncol.* 2005; 23: 5943–9.
117. **Reece DE, Rodriguez GP, Chen C, *et al.*** Phase I-II trial of bortezomib plus oral cyclophosphamide and prednisone in relapsed and refractory multiple myeloma. *J Clin Oncol.* 2008; 26: 4777–83.
118. **Kubicek GJ, Werner-Wasik M, Machtay M, *et al.*** Phase I trial using the proteasome inhibitor bortezomib and concurrent temozolomide and radiotherapy for central nervous system malignancies. *Int J Radiat Oncol Biol Phys.* 2008; 74: 433–9.