ELSEVIER

European Journal of Cancer 41 (2005) 2569–2576

European Journal of Cancer

www.ejconline.com

Transcription inhibition: A potential strategy for cancer therapeutics

Frederick A. Derheimer [a,b], Ching-Wei Chang [a], Mats Ljungman [a,b,c,*]

[a] *Department of Radiation Oncology, Division of Radiation and Cancer Biology, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI 48109, USA*

[b] *Program in Molecular and Cellular Biology, University of Michigan Medical School, Ann Arbor, MI 48109, USA*

[c] *Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, MI 48109, USA*

Available online 5 October 2005

Abstract

Interference with transcription triggers a stress response leading to the induction of the tumour suppressor p53. If transcription is not restored within a certain time frame cells may undergo apoptosis in a p53-dependent and independent manner. The mechanisms by which blockage of transcription induces apoptosis may involve diminished levels of anti-apoptotic factors, inappropriate accumulation of proteins in the nucleus, accumulation of p53 at mitochondria or complications during replication. Many chemotherapeutic agents currently used in the clinic interfere with transcription and this interference may contribute to their anti-cancer activities. Future efforts should be directed towards exploring whether interference of transcription could be used as an anti-cancer therapeutic strategy.

Keywords: RNA polymerase II; p53; Apoptosis; Chemotherapy

1. Introduction

The goal of most cancer therapeutic approaches is to kill off the cancer cells in tumours. Cancer cells can die by programmed cell death, such as apoptosis or autotrophy, or by necrosis or mitotic catastrophe [1]. One possible mechanism that makes tumour cells more sensitive to certain cancer therapeutic agents than normal tissues is that they have various defects in their DNA damage responses. For example, tumours often have mutations in genes that code for proteins involved in regulating cell cycle checkpoints such as p53 and the retinoblastoma protein. However, cancer cells often over express anti-apoptotic factors to suppress signals ordering the cells to undergo apoptosis [2]. This could lead to resistance of the tumour to anti-cancer treatment.

It has been shown that inhibition of transcription results in a time-dependent induction of apoptosis. Importantly, transformed cells appear to be more sensitive to disruption of RNA synthesis than corresponding normal cells [3]. It is possible that the acquired over expression of anti-apoptotic factors in cancer cells is dependent on ongoing transcription, so by inhibiting transcription, cancer cells will not be able to sustain high levels of these factors and may die. Thus, approaches to target transcription may be useful in cancer treatment. In this review we will discuss the transcription stress response, possible mechanisms of how transcription blockage results in cell death and how transcription may be a useful target for inactivation by anti-cancer therapies.

2. The transcription stress response

Our lab and others have shown that treatment of cells with agents that cause DNA damage and block transcription results in the nuclear accumulation and phosphory-

* Corresponding author. Present address: 4306 CCGC, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0936, USA. Tel.: +1 734 764 3330; fax: +1 734 647 9654.

E-mail address: ljungman@umich.edu (M. Ljungman).

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.08.012

lation of p53 [4–6]. Furthermore, agents that do not cause DNA damage but arrest RNA polymerase II transcription, such as 5,6-dichloro-1-b-D-ribofuranosylbenzimidazole (DRB) [6,7], siRNA against RNA polymerase II [8] or nuclear microinjection of anti-RNA polymerase II antibodies [9], induce the nuclear accumulation of p53 suggesting that blockage of transcription is sufficient for the induction of p53. Interestingly, agents that block RNA polymerase II prior to entering the elongation phase accumulate p53 proteins that lacks phosphorylation at the ser15 site while agents that preferentially block the elongating form of RNA polymerase trigger the phosphorylation and accumulation of p53 [7–9].

RNA polymerases continuously traverse selected regions of the genome as they transcribe genes. This translocation machinery could also be used by cells as a "scanning mechanism" to monitor the DNA and to alert the cell if transcription has stalled at sites of DNA lesions [10]. In fact, a blocked RNA polymerase II transcription complex can recruit DNA repair proteins to help remove the blocking lesion and promote recovery of RNA synthesis through transcription-coupled repair (TCR) [11–13]. Furthermore, blockage of transcription is also linked to the induction of p53 [4–6,10,14] and apoptosis [5]. Through the coupling of p53 activation to blocked transcription the cell has a method to activate cell cycle checkpoints, apoptosis, or stimulate DNA repair to allow the recovery of transcription. Recent results from our laboratory suggest that the ATR kinase may link blocked RNA polymerases with activation of p53 [9]. The transcription stress response may have evolved to aid cells in recognising DNA damage and to activate signal transduction pathways [10].

3. Blockage of transcription and apoptosis

Transcription is an essential process and its inhibition over a certain period of time will lead to apoptosis by both p53-dependent and p53-independent mechanisms. Following insult by UV-irradiation, the ability of cells to remove the transcription-blocking DNA lesions and recover RNA synthesis is crucial for the survival of the cells [5,15,16]. Here we will discuss four potential mechanisms by which blockage of transcription may results in apoptosis (Fig. 1).

3.1. Balance of anti- and pro-apoptotic factors

Experiments in our laboratory using the reversible transcription inhibitor DRB have suggested that there appears to be a "point of no return" following transcription inhibition at which reversal of the transcription blockage does not save the cells. We suggest that each cell has an "apoptotic clock" governing the time it takes to tip the balance of anti- and pro-apoptotic factors in favour of apoptosis. Evidence of an apoptotic clock also exists from experiments using UV light where full recovery of RNA synthesis within 6 h correlates with good survival while lack of recovery within 6 h correlates with significant induction of apoptosis [15,17–19].

The balance between cell survival and cell death is tightly regulated in all cells. Over expression of apoptosis-promoting factors such as bax or reduced expression of anti-apoptotic factors such as Bcl-2 or Mcl-1 can tip the balance in favour of apoptosis [20,21]. It is possible that inhibition of transcription preferentially reduces the level of anti-apoptotic factors relative to pro-apoptotic factors and that this is sufficient to tip the balance in favour of apoptosis. Following inhibition of transcription, the rate at which a particular protein is lost depends on the half-life of the mRNA and of the protein as well as the rate of translation. The average half-life of mRNAs is about 10 h but some transcripts are turned over in 10 min [22]. Protein half-life can range from minutes to over 100 h.

Table 1 summarises mRNA half-life for some genes encoding pro- and anti-apoptotic proteins. From this

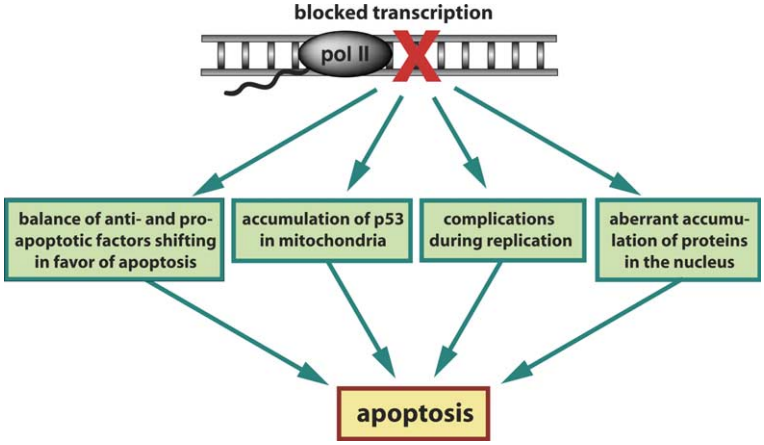

Fig. 1. Possible mechanisms by which blockage of transcription induces apoptosis in cells. See text for details.

Table 1
Estimated half-lives of some mRNAs encoding pro- and anti-apoptotic factors

Name	Half-life[a] (min)	Cell line	Inhibitor	References
Pro-apoptotic				
TNFRSF-6/Fas/Apo-1	44	T lymphocytes	Act D	[72]
	218[b,c]	HepG2	Act D	[22]
TNFSF-2/TNF-alpha	<120	OCI-Ly3	flavopiridol	[23]
	>360	T lymphocytes	Act D	[72]
TNFSF-6/FasL	<120	OCI-Ly3	flavopiridol	[23]
Bcl-2-binding protein Nip3	125[e]	T lymphocytes	Act D	[72]
	>360[e]	T lymphocytes	Act D	[72]
	556[b]	HepG2	Act D	[22]
MADD	127	T lymphocytes	Act D	[72]
Caspase-7 isoform beta precursor	128	T lymphocytes	Act D	[72]
	411[b]	HepG2	Act D	[22]
TNFRSF1A	147[b]	HepG2	Act D	[22]
TNFRSF21	359[b]	HepG2	Act D	[22]
Caspase-6 isoform beta	>360	T lymphocytes	Act D	[72]
	723[b]	HepG2	Act D	[22]
Programmed cell death 8/AIF	>360	T lymphocytes	Act D	[72]
	1111[b]	HepG2	Act D	[22]
Bax	460[b,d]	HepG2	Act D	[22]
Bid	896[b]	HepG2	Act D	[22]
TNFSF-10/TRAIL	896[b]	HepG2	Act D	[22]
PIG3	682[b]	HepG2	Act D	[22]
PIG8	15000[b]	HepG2	Act D	[22]
PIG11	2143[b]	HepG2	Act D	[22]
Anti-apoptotic				
NAIP	short	OCI-Ly3	flavopiridol	[23]
MCL-1	64[b]	HepG2	Act D	[22]
	<120	OCI-Ly3	flavopiridol	[23]
TNFSF-5/CD40 ligand	74	T lymphocytes	Act D	[72]
c-IAP1	<120	OCI-Ly3	flavopiridol	[23]
	163[b]	HepG2	Act D	[22]
c-IAP2	<120	OCI-Ly3	flavopiridol	[23]
TNFAIP3/A20	<120	OCI-Ly3	flavopiridol	[23]
A1/bfl-1	<180	OCI-Ly3	flavopiridol	[23]
FLIP	331[b]	HepG2	Act D	[22]
Bcl-xL	706[b]	HepG2	Act D	[22]
NF-kB1	268[b]	HepG2	Act D	[22]
MAP4	541[b]	HepG2	Act D	[22]

[a] Different normalization factors for the arrays might be used; thus absolute stability comparison might not be valid between references.
[b] The half-life values were calculated from 1/decay rate as described in [22].
[c] Average of three determinations for the same gene using three different accession numbers.
[d] Average of two determinations for the same gene using two different accession numbers.
[e] Values obtained using different probes for the same gene.

table it appears that the half-lives of mRNAs encoding pro-apoptotic components in general are longer than for genes encoding anti-apoptotic proteins; supporting a model in which induction of apoptosis following blockage of transcription may be solely regulated by differential stability of mRNAs [10,23]. A change in the ratio of pro- and anti-apoptotic factors following UV-irradiation may in addition to their individual stabilities be differentially regulated by their gene sizes [24,25]. In fact, many of the genes encoding pro-apoptotic proteins are on average smaller in size than the genes encoding anti-apoptotic proteins [25]. It is possible that the particular sizes of introns in these genes may have been selected during evolution as a mechanism to regulate apoptosis following UV light exposure, where genes with large introns would be preferentially inhibited by UV light compared to smaller genes due to their larger target size.

3.2. Role of p53 in mediating transcription block-induced apoptosis

Since p53 proteins accumulate following blockage of transcription, it is possible that p53 may contribute to the induction of apoptosis following inhibition of transcription. The role of p53 in mediating the induction of apoptosis following inhibition of transcription is not clear. Studies using human colon cancer cells have

shown a clear trend favouring apoptosis in cells expressing wild-type p53 compared to cancer cells expressing mutant p53 following inhibition of RNA polymerase II-mediated transcription with DRB [26]. Furthermore, human colon cancer cells with both p53 genes deleted were found to be significantly more resistant to the induction of apoptosis following inhibition of transcription by α-amanitin or siRNA against RNA polymerase II than were parental cells with wtp53 [8]. Thus, it appears that p53 enhances the induction of apoptosis following RNA polymerase II inhibition at least in some cell types. However, contrasting results have been reported from other studies using murine embryonic fibroblasts (MEFs) which showed that inhibition of transcription with DRB or α-amanitin efficiently induce apoptosis in both p53+/+ and p53−/− MEF cells [3].

The transactivation activity of p53 is not expected to be involved in the induction of apoptosis following inhibition of transcription since p53 would not be able to induce expression of downstream target genes. Importantly, it has been shown that expression of a transactivation-deficient truncated p53 gene in p53−/− colon cancer cells restores the apoptosis sensitivity of these cells to inhibition of transcription, suggesting that p53 has a transactivation-independent function in enhancing the induction of apoptosis following inhibition of transcription [8]. One transactivation-independent function of p53 in stimulating the induction of apoptosis is its ability to translocate to mitochondria and bind to anti-apoptotic Bcl-2 family proteins [27–29]. In fact, p53 was found to accumulate in mitochondria following blockage of transcription. This correlated to the induction of apoptosis and suggests that p53 may contribute to the induction of apoptosis following sustained blockage of transcription by triggering mitochondria-dependent induction of apoptosis [8].

The role of p53 in the induction of apoptosis in human fibroblasts following exposure to UV light or cisplatin is not clear. In fact, studies have shown that p53 actually protects human fibroblasts and colon cancer cells form the induction of apoptosis by these agents [17–19]. Thus, it appears that the protecting effects of p53 in DNA repair and activation of cell cycle checkpoints overrides its roles in inducing apoptosis. In addition, we have found that cells with wild-type 53 recover RNA synthesis faster after UV-irradiation than cells with compromised p53 function [17–19]. Depletion of p53 by expression of the HPV16 ubiquitin ligase E6 in human cell lines with inherent defects in DNA repair revealed that loss of p53 significantly sensitised transcription-coupled repair-proficient XP-C cells [19]. These results suggest that the role of p53 in the protection against apoptosis involves enhanced recovery of transcription and that this enhancement is only operational in TCR-proficient cells.

3.3. Complications during replication by stalled transcription complexes

DNA-damaging agents, such as UV light or cisplatin, induce DNA lesions that can block the elongation of RNA polymerase complexes. In order to resume RNA synthesis, both the lesion and the stalled RNA polymerase complex must be removed. It has been shown that the largest subunit of RNA polymerase II is targeted for ubiquitylation and degradation shortly after cells are exposed to UV light or cisplatin [30–32]. The BRCA1/BARD1 complex [33] and the von Hippel-Lindau protein [34] may target RNA polymerase II for ubiquitylation and degradation. The degradation of stalled RNA polymerases appears to be dependent on the Cockayne's syndrome factors CSA and CSB since ubiquitylation and degradation of the largest subunit of RNA polymerase is reduced in CS-A and CS-B cell lines [31,35].

It has been shown that UV-induced apoptosis is associated with the S-phase of the cell cycle [36,37]. The mechanism for this is not clear but if the stalled RNA polymerase complex is not removed prior to entry into S-phase, it is possible that the replication machinery may collide with stalled RNA polymerases [10]. Interestingly, studies using the RNA polymerase II inhibitor α-amanitin, which is thought to result in the targeting of RNA polymerase for ubiquitin-dependent degradation [38], have also shown a connection between transcription inhibition and passage through the S-phase [8]. In this case apoptosis does not seem to occur as a consequence of collision between stalled RNA polymerases and the replication machinery since the inhibition of transcription is due to the loss rather than stalling of RNA polymerases. It is possible that RNA polymerase II plays a supporting role in replication either directly or indirectly through the transcription of genes important for the replication process.

3.4. Aberrant accumulation of proteins in the cell nucleus

Nuclear import and export of proteins through nuclear pore complexes are mediated by nuclear localisation signals (NLS) and nuclear export signals (NES) [39–41]. Proteins containing leucine-rich NES domains are exported by the aid of CRM1, a member of the importin-β family of proteins [42–46]. It has been estimated that there are at least 75 proteins that have been confirmed containing NES domains [47] (Table 2).

We recently reported that nuclear export of NES-containing proteins requires ongoing synthesis and export of mRNAs [48]. In these studies, a fusion protein consisting of a NES domain fused to the green fluorescent protein (GFP) was microinjected into the nucleus of cells and the ability of this protein to export to the cytoplasm was measured within 20 min of injection. It

Table 2
List of NES-containing proteins that may affect the regulation of apoptosis

Protein	Functional class	NES domains	Nuclear accumulation after blockage of transcription	Effects of nuclear accumulation by blocking export
Cyclin B1	Cell cycle	Yes	?	Induces apoptosis [73]
NF-kB p65 subunit	Transcription factor	Yes	?	Inhibits apoptosis [74,75]
p53	Transcription factor, DNA repair, cell cycle regulation, apoptosis	Yes	Yes [4–6]	Enhances DNA repair, induces cell cycle arrest or apoptosis [76]
p73	Transcription factor	Yes	?	Induces apoptosis [77]
Mdm2	DNA repair, cell cycle, apoptosis	Yes	?	Inhibits p53 and retains it in the nucleus [76]
Cdc25B	Cell cycle	Yes	?	Promotes entry into mitosis [78]
BRCA1	DNA repair, DNA, damage signalling	Yes	?	May enhance DNA repair, activate cell cycle checkpoints and may together with BARD1 target RNA polymerase II for ubiquitylation [79,80]
VHL	Transcription, ubiquitin ligase complex	No [56]	Yes [56]	May suppress transcription elongation and promote ubiquitylation of target molecules (HIF-1 and RNA pol II) [34,81,82]
p32	Mitochondrial protein [55]	Not in single molecule [55]	Yes [55]	?
HIF-alpha	Transcription factor	Partially conserved [57,83]	Yes [57]	May promote angiogenesis, cell survival, cell invasion, and drug resistance [84]
Dsh	Signal transduction	Yes [85]	?	Affects the Wnt pathway [85] and promotes apoptosis [86]
c-Abl	Protein tyrosine kinase	Yes	?	Promotes apoptosis in a p73-dependent manner [87,88]
HDAC4&5	Histone deacetylases	Yes	?	May suppress gene expression by modulating chromatin structure [89,90]

was found that when transcription was inhibited in cells either with drugs or microinjection of anti-RNA polymerase II antibodies, the nuclear export of the microinjected NES-tagged GFP proteins was significantly reduced [48]. Furthermore, targeting either Tap or Nup160, two components required for mRNA export, also blocked nuclear export of the fusion protein. Thus, these results suggest that general NES-dependent protein nuclear export requires ongoing nuclear export of mRNAs [48].

The p53 protein is a nucleocytoplasmic shuttling protein that contains multiple NLS and NES sequences [49–51]. The MDM2 ubiquitin ligase is important in regulating the level of p53 in cells by directing the nuclear export and degradation of p53 [52,53]. Interestingly, when transcription is inhibited by DRB, p53 accumulates in the nucleus despite the fact that MDM2 can bind to p53 and p53 proteins are ubiquitylated [54]. This suggests that inhibition of transcription blocks the ability of p53 to shuttle to the cytoplasm in an MDM2-independent manner. One possibility is that efficient nuclear export of p53 is somehow linked to the nuclear export of mRNA. In fact, similarly to the NES-GFP fusion protein mentioned above, p53 accumulates in the nucleus following specific inhibition of either RNA polymerase II-mediated transcription or Tap and Nup160-dependent nuclear export of mRNAs [9]. Furthermore, several other nucleocytoplasmic shuttling proteins such as p32 [55], VHL [56] and HIF-α [57] have been shown to accumulate in the nucleus following blockage of transcription. Thus, it appears that the nuclear export of NES-containing proteins in general depends on the continuous flow of mRNAs from the nucleus to the cytoplasm and that this protein export is blocked by inhibition of either mRNA synthesis or mRNA export. Further studies are needed to determine whether the aberrant accumulation of nucleocytoplasmic shuttling proteins in the nucleus following inhibition of transcription contributes to apoptosis.

4. Transcription blockage and anti-cancer strategies

Since inhibition of transcription induces cell death in a number of cancer cell lines [26,58] and that the induction of apoptosis has been shown to be more pronounced in transformed cells compared to their non-transformed counterparts [3], it is of interest to evaluate the potential usefulness of transcription as a cancer therapeutic target. Caution must be taken though

since long-term non-selective inhibition of transcription most likely would produce severe side effects and therefore targeted delivery of transcription inhibitors to the tumour would be preferable. One strategy that should be explored is whether transient inhibition of transcription may selectively kill tumour cells because they generally enter S-phase more frequently, a phase especially sensitive to transcription blockage [8,36,37]. Alternatively, cancer cells may be preferentially sensitive to transcription blockage because they may have less time allotted on their "apoptotic clocks" compared to normal cells before the balance of anti- and pro-apoptotic proteins will tip in favour of apoptosis.

There are many currently used chemotherapeutic agents that are known to have inhibitory effects on transcription. The topoisomerase inhibitors camptothecin and doxorubicin, the DNA-damaging agent cisplatin, the cyclin-dependent kinase-inhibitory agents flavopiridol and roscovitine and the nucleotide analogs 5-fluorouracil and fludarabine are thought to exert some of their anti-cancer activity by inhibiting transcription [6,23,59–66]. Interestingly, the combination of transcriptional inhibition with other agents such as ionising radiation [67–69] or the nuclear export inhibitor leptomycin B [70,71] have shown synergistic effects on cancer cells. These findings warrant further explorations of the potential usefulness of the transcription machinery as a therapeutic target in cancer treatment.

Conflict of interest statement

None declared.

Acknowledgements

We thank all the current and former members of the Ljungman lab for their contributions in forming some of the concepts discussed in this article. The work relevant to this article was supported by a research grant from the National Institute of Health (CA-82376), the Cell and Molecular Biology Graduate Program at the University of Michigan and from funds from the Department of Radiation Oncology at the University of Michigan.

References

1. Brown J, Attardi LD. The role of apoptosis in cancer and treatment response. *Nat Rev Cancer* 2005, **5**, 231–237.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000, **100**(1), 57–70.
3. Koumenis C, Giaccia A. Transformed cells require continuous activity of RNA polymerase II to resist oncogene-induced apoptosis. *Mol Cell Biol* 1997, **17**(12), 7306–7316.
4. Yamaizumi M, Sugano T. UV-induced nuclear accumulation of p53 is evoked through DNA damage of actively transcribed genes independent of the cell cycle. *Oncogene* 1994, **9**(10), 2775–2784.
5. Ljungman M, Zhang F. Blockage of RNA polymerase as a possible trigger for uv light-induced apoptosis. *Oncogene* 1996, **13**(4), 823–831.
6. Ljungman M, Zhang FF, Chen F, *et al*. Inhibition of RNA polymerase II as a trigger for the p53 response. *Oncogene* 1999, **18**(3), 583–592.
7. Ljungman M, O'Hagan HM, Paulsen MT. Induction of ser15 and lys382 modifications of p53 by blockage of transcription elongation. *Oncogene* 2001, **20**(42), 5964–5971.
8. Arima Y, Nitta M, Kuninaka S, *et al*. Transcriptional blockade induces p53-dependent apoptosis associated with translocation of p53 to mitochondria. *J Biol Chem* 2005, **280**, 19166–19176.
9. Derheimer FA, O'Hagan HM, Ljungman M. The ATR kinase link transcriptional stress to p53 submitted.
10. Ljungman M, Lane DP. Opinion: transcription – guarding the genome by sensing DNA damage. *Nat Rev Cancer* 2004, **4**(9), 727–737.
11. Mellon I, Spivak G, Hanawalt PC. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell* 1987, **51**, 241–249.
12. Svejstrup JQ. Mechanisms of transcription-coupled DNA repair. *Nat Rev Mol Cell Biol* 2002, **3**(1), 21–29.
13. van Hoffen A, Balajee AS, van Zeeland AA, *et al*. Nucleotide excision repair and its interplay with transcription. *Toxicology* 2003, **193**(1–2), 79–90.
14. Ljungman M. Dial 9-1-1 for p53: Mechanisms of p53 activation by cellular stress. *Neoplasia* 2000, **2**(3), 208–225.
15. Queille S, Drougard C, Sarasin A, *et al*. Effects of XPD mutations on ultraviolet-induced apoptosis in relation to skin cancer-proneness in repair-deficient syndromes. *J Invest Derm* 2001, **117**(5), 1162–1170.
16. Billecke CA, Ljungman ME, McKay BC, *et al*. Lack of functional pRb results in attenuated recovery of mRNA synthesis and increased apoptosis following UV radiation in human breast cancer cells. *Oncogene* 2002, **21**(29), 4481–4489.
17. McKay B, Ljungman M. Role for p53 in the recovery of transcription and protection against apoptosis induced by ultraviolet light. *Neoplasia* 1999, **1**, 276–284.
18. McKay BC, Chen F, Perumalswami CR, *et al*. The tumour suppressor p53 can both stimulate and inhibit ultraviolet light-induced apoptosis. *Mol Biol Cell* 2000, **11**(8), 2543–2551.
19. McKay BC, Becerril C, Ljungman M. P53 plays a protective role against UV- and cisplatin-induced apoptosis in transcription-coupled repair proficient fibroblasts. *Oncogene* 2001, **20**(46), 6805–6808.
20. Harada H, Grant S. Apoptosis regulators. *Rev Clin Exp Hematol* 2003, **7**(2), 117–138.
21. MacCallum DE, Melville J, Frame S, *et al*. Seliciclib (CYC202, R-Roscovitine) induces cell death in multiple myeloma cells by inhibition of RNA polymerase II-dependent transcription and down-regulation of Mcl-1. *Cancer Res* 2005, **65**(12), 5399–5407.
22. Yang E, van Nimwegen E, Zavolan M, *et al*. Decay rates of human mRNAs: correlation with functional characteristics and sequence attributes. *Genome Res* 2003, **13**(8), 1863–1872.
23. Lam LT, Pickeral OK, Peng AC, *et al*. Genomic-scale measurement of mRNA turnover and the mechanisms of action of the anti-cancer drug flavopiridol. *Genome Biol* 2001, **2**(10), 1–11.
24. Sauerbier W, Hercules K. Gene and transcription mapping by radiation effects. *Annu Rev Genet* 1978, **12**, 329–363.
25. McKay BC, Stubbert LJ, Fowler CC, *et al*. Regulation of ultraviolet light-induced gene expression by gene size. *Proc Natl Acad Sci USA* 2004, **101**(17), 6582–6586.
26. Poele RHT, Okorokov AL, Joel SP. RNA synthesis block by 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazoleDRB (DRB) trig-

gers p53-dependent apoptosis in human colon carcinoma cells. *Oncogene* 1999, **18**(42), 5765–5772.
27. Erster S, Mihara M, Kim RH, *et al*. *In vivo* mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA damage that can precede p53 target gene activation. *Mol Cell Biol* 2004, **24**(15), 6728–6741.
28. Mihara M, Erster S, Zaika A, *et al*. p53 Has a direct apoptogenic role at the mitochondria. *Mol Cell* 2003, **11**(3), 577–590.
29. Marchenko ND, Zaika A, Moll UM. Death signal-induced localisation of p53 protein to mitochondria. A potential role in apoptotic signaling. *J Biol Chem* 2000, **275**(21), 16202–16212.
30. Ratner JN, Balasubramanian B, Corden J, *et al*. Ultraviolet radiation-induced ubiquitination and proteasomal degradation of the large subunit of RNA polymerase II – implications for transcription-coupled DNA repair. *J Biol Chem* 1998, **273**(9), 5184–5189.
31. McKay BC, Chen F, Clarke ST, *et al*. UV light-induced degradation of RNA polymerase II is dependent on the Cockayne's syndrome A and B proteins but not p53 or MLH1. *Mutat Res* 2001, **485**(2), 93–105.
32. Yang LY, Jiang H, Rangel KM. RNA polymerase II stalled on a DNA template during transcription elongation is ubiquitinated and the ubiquitination facilitates displacement of the elongation complex. *Int J Oncol* 2003, **22**(3), 683–689.
33. Kleiman FE, Wu-Baer F, Fonseca D, *et al*. BRCA1/BARD1 inhibition of mRNA 3′ processing involves targeted degradation of RNA polymerase II. *Genes Dev* 2005, **19**(10), 1227–1237.
34. Kuznetsova AV, Meller J, Schnell PO, *et al*. von Hippel-Lindau protein binds hyperphosphorylated large subunit of RNA polymerase II through a proline hydroxylation motif and targets it for ubiquitination. *Proc Natl Acad Sci USA* 2003, **100**(5), 2706–2711.
35. Bregman DB, Halaban R, Vangool AJ, *et al*. UV-induced ubiquitination of RNA polymerase II: A novel modification deficient in cockayne syndrome cells. *Proc Natl Acad Sci USA* 1996, **93**(21), 11586–11590.
36. Dunkern TR, Kaina B. Cell proliferation and DNA breaks are involved in ultraviolet light-induced apoptosis in nucleotide excision repair-deficient Chinese hamster cells. *Mol Biol Cell* 2002, **13**(1), 348–361.
37. McKay B, Becerril C, Spronck J, *et al*. Ultraviolet light-induced apoptosis is associated with S-phase in primary human fibroblasts. *DNA Repair* 2002, **1**, 811–820.
38. Nguyen V, Giannoni F, Dubois M-F, *et al*. *In vivo* degradation of RNA polymerase II largest subunit triggered by α-amanitin. *Nucleic Acids Res* 1996, **24**, 2924–2929.
39. Gorlich D, Kutay U. Transport between the cell nucleus and the cytoplasm. *Annu Rev Cell Dev Biol* 1999, **15**, 607–660.
40. Weis K. Nucleocytoplasmic transport: cargo trafficking across the border. *Curr Opin Cell Biol* 2002, **14**(3), 328–335.
41. Fabbro M, Henderson BR. Regulation of tumour suppressors by nuclear-cytoplasmic shuttling. *Exp Cell Res* 2003, **282**(2), 59–69.
42. Fischer U, Huber J, Boelens WC, *et al*. The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell* 1995, **82**(3), 475–483.
43. Fornerod M, Ohno M, Yoshida M, *et al*. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell* 1997, **90**(6), 1051–1060.
44. Kudo N, Khochbin S, Nishi K, *et al*. Molecular cloning and cell cycle-dependent expression of mammalian CRM1, a protein involved in nuclear export of proteins. *J Biol Chem* 1997, **272**(47), 29742–29751.
45. Mattaj IW, Englmeier L. Nucleocytoplasmic transport: the soluble phase. *Annu Rev Biochem* 1998, **67**, 265–306.
46. Stade K, Ford CS, Guthrie C, *et al*. Exportin 1 (Crm1p) is an essential nuclear export factor. *Cell* 1997, **90**(6), 1041–1050.
47. la Cour T, Gupta R, Rapacki K, *et al*. NESbase version 1.0: a database of nuclear export signals. *Nucleic Acids Res* 2003, **31**(1), 393–396.
48. O'Hagan HM, Ljungman M. Efficient NES-dependent protein nuclear export requires ongoing synthesis and export of mRNAs. *Exp Cell Res* 2004, **297**(2), 548–559.
49. Stommel JM, Marchenko ND, Jimenez GS, *et al*. A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localisation and p53 activity by NES masking. *EMBO J* 1999, **18**(6), 1660–1672.
50. Zhang YP, Xiong Y. A p53 amino-terminal nuclear export signal inhibited by DNA damage-induced phosphorylation. *Science* 2001, **292**(5523), 1910–1915.
51. Liang SH, Clarke MF. The nuclear import of p53 is determined by the presence of a basic domain and its relative position to the nuclear localisation signal. *Oncogene* 1999, **18**(12), 2163–2166.
52. Geyer RK, Yu ZK, Maki CG. The MDM2 RING-finger domain is required to promote p53 nuclear export. *Nat Cell Biol* 2000, **2**(9), 569–573.
53. Gu JJ, Nie LH, Wiederschain D, *et al*. Identification of p53 sequence elements that are required for MDM2-mediated nuclear export. *Mol Cell Biol* 2001, **21**(24), 8533–8546.
54. O'Hagan HM, Ljungman M. Nuclear accumulation of p53 following inhibition of transcription is not due to diminished levels of MDM2. *Oncogene* 2004, **23**(32), 5505–5512.
55. Brokstad KA, Kalland KH, Russell WC, *et al*. Mitochondrial protein p32 can accumulate in the nucleus. *Biochem Biophys Res Commun* 2001, **281**(5), 1161–1169.
56. Lee S, Neumann M, Stearman R, *et al*. Transcription-dependent nuclear-cytoplasmic trafficking is required for the function of the von Hippel-Lindau tumour suppressor protein. *Mol Cell Biol* 1999, **19**(2), 1486–1497.
57. Groulx I, Lee S. Oxygen-dependent ubiquitination and degradation of hypoxia-inducible factor requires nuclear-cytoplasmic trafficking of the von Hippel-Lindau tumour suppressor protein. *Mol Cell Biol* 2002, **22**(15), 5319–5336.
58. Chang TC, Tsai LC, Hung MW, *et al*. Effects of transcription and translation inhibitors on a human gastric carcinoma cell line - Potential role of Bcl-X-s in apoptosis triggered by these inhibitors. *Biochem Pharmacol* 1997, **53**(7), 969–977.
59. Ljungman M, Hanawalt PC. The anti-cancer drug camptothecin inhibits elongation but stimulates initiation of RNA polymerase II transcription. *Carcinogenesis* 1996, **17**(1), 31–35.
60. Collins I, Weber A, Levens D. Transcriptional consequences of topoisomerase inhibition. *Mol Cell Biol* 2001, **21**(24), 8437–8451.
61. Furuta T, Ueda T, Aune G, *et al*. Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. *Cancer Res* 2002, **62**(17), 4899–4902.
62. Geoffroy FJ, Allegra CJ, Sinha B, *et al*. Enhanced cytotoxicity with interleukin-1 alpha and 5-fluorouracil in HCT116 colon cancer cells. *Oncol Res* 1994, **6**(12), 581–591.
63. Pritchard DM, Watson AJM, Potten CS, *et al*. Inhibition by uridine but not thymidine of p53-dependent intestinal apoptosis initiated by 5-fluorouracil: Evidence for the involvement of RNA perturbation. *Proc Natl Acad Sci USA* 1997, **94**(5), 1795–1799.
64. Huang P, Sandoval A, Van Den Neste E, *et al*. Inhibition of RNA transcription: a biochemical mechanism of action against chronic lymphocytic leukemia cells by fludarabine. *Leukemia* 2000, **14**(8), 1405–1413.
65. Ljungman M, Paulsen MT. The cyclin-dependent kinase inhibitor roscovitine inhibits RNA synthesis and triggers nuclear accumulation of p53 that is unmodified at Ser15 and Lys382. *Mol Pharm* 2001, **60**(4), 785–789.
66. Gojo I, Zhang B, Fenton RG. The cyclin-dependent kinase inhibitor flavopiridol induces apoptosis in multiple myeloma cells through transcriptional repression and down-regulation of Mcl-1. *Clin Cancer Res* 2002, **8**(11), 3527–3538.

67. Blaydes JP, Craig AL, Wallace M, *et al*. Synergistic activation of p53-dependent transcription by two cooperating damage recognition pathways. *Oncogene* 2000, **19**(34), 3829–3839.
68. O'Hagan HM, Ljungman M. Phosphorylation and nuclear accumulation are distinct events contributing to the activation of p53. *Mutat Res* 2004, **546**(1–2), 7–15.
69. Maggiorella L, Deutsch E, Frascogna V, *et al*. Enhancement of radiation response by roscovitine in human breast carcinoma *in vitro* and *in vivo*. *Cancer Res* 2003, **63**(10), 2513–2517.
70. Hietanen S, Lain S, Krausz E, *et al*. Activation of p53 in cervical carcinoma cells by small molecules. *Proc Natl Acad Sci USA* 2000, **97**(15), 8501–8506.
71. Lain S, Lane D. Improving cancer therapy by non-genotoxic activation of p53. *Eur J Cancer* 2003, **39**(8), 1053–1060.
72. Raghavan A, Ogilvie RL, Reilly C, *et al*. Genome-wide analysis of mRNA decay in resting and activated primary human T lymphocytes. *Nucleic Acids Res* 2002, **30**(24), 5529–5538.
73. Porter LA, Cukier IH, Lee JM. Nuclear localisation of cyclin B1 regulates DNA damage-induced apoptosis. *Blood* 2003, **101**(5), 1928–1933.
74. Kau TR, Way JC, Silver PA. Nuclear transport and cancer: from mechanism to intervention. *Nat Rev Cancer* 2004, **4**(2), 106–117.
75. May MJ, Ghosh S. Signal transduction through NF-kappa B. *Immunol Today* 1998, **19**(2), 80–88.
76. Xu L, Massague J. Nucleocytoplasmic shuttling of signal transducers. *Nat Rev Mol Cell Biol* 2004, **5**(3), 209–219.
77. Jost CA, Marin MC, Kaelin WG. p73 is a human p53-related protein that can induce apoptosis. *Nature* 1997, **389**(6647), 191–194.
78. Smits VA, Medema RH. Checking out the G(2)/M transition. *Biochim Biophys Acta* 2001, **1519**(1–2), 1–12.
79. Feng Z, Kachnic L, Zhang J, *et al*. DNA damage induces p53-dependent BRCA1 nuclear export. *J Biol Chem* 2004.
80. Yoshida K, Miki Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci* 2004, **95**(11), 866–871.
81. Tanimoto K, Makino Y, Pereira T, *et al*. Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumour suppressor protein. *Embo J* 2000, **19**(16), 4298–4309.
82. Duan DR, Pause A, Burgess WH, *et al*. Inhibition of transcription elongation by the VHL tumour suppressor protein. *Science* 1995, **269**(5229), 1402–1406.
83. Pollenz RS, Barbour ER. Analysis of the complex relationship between nuclear export and aryl hydrocarbon receptor-mediated gene regulation. *Mol Cell Biol* 2000, **20**(16), 6095–6104.
84. Yeo EJ, Chun YS, Park JW. New anticancer strategies targeting HIF-1. *Biochem Pharmacol* 2004, **68**(6), 1061–1069.
85. Itoh K, Brott BK, Bae GU, *et al*. Nuclear localisation is required for Dishevelled function in Wnt/beta-catenin signaling. *J Biol* 2005, **4**(1), 3.
86. You L, He B, Xu Z, *et al*. An anti-Wnt-2 monoclonal antibody induces apoptosis in malignant melanoma cells and inhibits tumour growth. *Cancer Res* 2004, **64**(15), 5385–5389.
87. Agami R, Blandino G, Oren M, *et al*. Interaction of c-Abl and p73 alpha and their collaboration to induce apoptosis. *Nature* 1999, **399**(6738), 809–813.
88. Vella V, Zhu J, Frasca F, *et al*. Exclusion of c-Abl from the nucleus restrains the p73 tumour suppression function. *J Biol Chem* 2003.
89. Chawla S, Vanhoutte P, Arnold FJ, *et al*. Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. *J Neurochem* 2003, **85**(1), 151–159.
90. Wang AH, Bertos NR, Vezmar M, *et al*. HDAC4, a human histone deacetylase related to yeast HDA1, is a transcriptional corepressor. *Mol Cell Biol* 1999, **19**(11), 7816–7827.