Biochemical and Biophysical Research Communications 422 (2012) 169–173

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Proteasomal insensitivity of apoptin in tumor cells

Henriëtte L. Lanz, Johnny Suijker, Mathieu H.M. Noteborn, Claude Backendorf *

Department of Molecular Genetics, Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands

A R T I C L E I N F O

Article history:
Received 19 April 2012
Available online 30 April 2012

Keywords:
Protein degradation
Proteasome
Apoptin
Bortezomib
Ada-Ahx$_3$L$_3$VS
Tumor-selective apoptosis

A B S T R A C T

The small viral protein apoptin is capable of inducing apoptosis selectively in human tumor cells. In normal cells apoptin localizes in the cytoplasm where it forms aggregates, becomes epitope-shielded and eventually degraded. By inhibiting the proteasome activity with the chemical inhibitors bortezomib and Ada-Ahx$_3$L$_3$VS apoptin levels can be stabilized in normal cells similar to the tumor suppressor p53 protein. In contrast, proteasome inhibition in tumor cells did not affect the apoptin stability while it still stabilized p53 levels. Apparently, apoptin is degraded by proteasomal activity in normal human cells, a process that no longer takes place in tumor cells. This loss of proteasomal susceptibility appears to be specific for apoptin.

1. Introduction

Apoptin is a small protein produced by Chicken Anemia Virus (CAV) that is capable of inducing apoptosis in human tumor cells while leaving normal cells intact [1]. In tumor cells apoptin is phosphorylated on T108 and translocates to the nucleus whereas in normal cells it stays in the cytoplasm in a non-phosphorylated fashion [2,3]. In normal cells, microinjected bacterially expressed MBP–apoptin forms in time large aggregates that after 24 h can no longer be detected in an immuno-fluorescence assay. The protein can still be detected by the use of stringent buffers and Western blot, showing that it becomes epitope-shielded. At later time points the protein could no longer be detected in normal cells and was eventually degraded [4]. This particular behavior was not observed in tumor cells.

Zhang et al. [4] showed that the disappearance of MBP–apoptin was not caused by lysosomal degradation prompting us to investigate the role of the proteasome in the degradation of apoptin in both normal and tumor cells. The proteasome is a multi-subunit protease, which is composed of a proteolytic 20S core with regulatory 19S caps. The proteolytic activity is contained in the β1, β2 and β5 subunits, all with their own substrate specificity [5,6].

To study the role of the proteasome in the degradation of apoptin we used the proteasome inhibitors bortezomib [7] and Ada-Ahx$_3$L$_3$VS [8] to block proteasomal degradation in cells expressing apoptin. We show that inhibition of the proteasome in normal cells leads to stabilization of apoptin and p53 levels, while in tumor cells both proteasome inhibitors affect p53 protein levels but have no influence on the amount of apoptin protein.

2. Material and methods

2.1. Cell culture

The normal human diploid foreskin F44 fibroblasts, isolated from neonatal foreskin, were obtained in the late 1980s from Dr. M. Ponec (Dept. Dermatology, Leiden University Medical Center). Cells were batch-frozen after careful morphological inspection. At subsequent passages cells were regularly screened for their typical fibroblast-like morphological appearance. F44 cells were used below passage 15 and cultured in 1:1 Dulbecco's Modified Eagle's Medium: Ham's F12 (DMEM/F12) (PAA, Colbe, Germany) containing 10% fetal calf serum (Thermo Scientific, Geel, Belgium), 100 μg/mL penicillin, 100 μg/mL streptomycin (Duchefa, Biochemie, Haarlem, The Netherlands) and 2 mM glutaMAX (PAA). The human osteosarcoma cell line U2Os was selected as it expresses wild-type p53 [15]. U2Os cells were purchased from the American Type Culture Collection (ATCC, Wesel, Germany) and cultured in DMEM (PAA) containing 10% newborn calf serum (Thermo Scientific), 100 μg/mL penicillin, 100 μg/mL streptomycin (Duchefa) and 2 mM glutaMAX (PAA). Cells were cultured at 37 °C in a humidified 5% CO_2 incubator. Cell morphology was regularly monitored to control the absence of cross-contamination.

Abbreviations: Apoptin, apoptosis inducing protein; MBP, maltose binding protein; APC/C, anaphase promoting complex/cyclosome.

* Corresponding author. Fax: +31 (0) 71 527 4357.

E-mail addresses: h.lanz@chem.leidenuniv.nl (H.L. Lanz), johnny_suijker@hotmail.com (J. Suijker), m.noteborn@chem.leidenuniv.nl (M.H.M. Noteborn), backendo@chem.leidenuniv.nl (C. Backendorf).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.132

2.2. Plasmids

The construction and expression of pcDNA3.1(+)flag-apoptin was previously described [9]. In short, the DNA sequence encoding apoptin was synthesized by Baseclear (Leiden, The Netherlands) according to the apoptin sequence published by Noteborn et al. [10] and cloned into the mammalian expression vector pcDNA3.1(+) (Invitrogen, Breda, The Netherlands). The oligonucleotide fragment encoding the flag-tag (Invitrogen) was inserted to create the pcDNA3.1(+)flag-apoptin plasmid encoding apoptin fused with a flag-tag at its N-terminus. The phosphorylation-negative flag-apoptin(5Ala)106 mutant was created as described in Danen-van Oorschot et al. [11]. The flag-tagged apoptin(5Ala)106 mutant plasmid was constructed by replacing the five amino acid stretch from position 106–110 by alanines in the pcDNA3.1(+)flag-apoptin construct background.

2.3. Transfection

Cells were transfected with plasmids by using Amaxa nucleofection (Lonza AG, Cologne, Germany) according to the adapted manufacturer's protocol. 10^6 cells were taken up in 120 µL nucleofector buffer (F44: 140 mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 5 mM KCl, 10 mM $MgCl_2$ and U2Os: 90 mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 5 mM KCl, 10 mM $MgCl_2$, 20 mM Hepes-KOH pH 7.2), mixed with DNA, transferred to a transfection cuvet (VWR, Amsterdam, The Netherlands) and transfected with program U-20 or X-01 for F44 or U2Os, respectively. After addition of medium, cells were seeded on 6 cm dishes.

2.4. Inhibitor treatment

The proteasome inhibitors bortezomib and Ada-Ahx$_3$L$_3$VS (a kind gift from Dr. Bobby Florea, Leiden University, Leiden, the Netherlands) were used to block proteasomal protein degradation [12]. The proteasome inhibitors were added to cells 16 h after transfection. Increasing concentrations of inhibitors were mixed with medium before addition to cells. After 24 h, unless otherwise indicated, cells were lysed and subjected to Western blot analysis.

2.5. Immune fluorescence assay

Cells grown on glass coverslips were first washed with phosphate buffered saline (PBS) at indicated time points after transfection and subsequently fixed at room temperature for 10 min with 1% formaldehyde, 5 min with 100% methanol and 2 min with 80% acetone. After air-drying the slides were used for immunocytochemical staining. For antibody staining, the cells were first incubated with PBS plus 0.05% Tween 20 (PBS-Tween; Sigma Aldrich, Zwijndrecht, The Netherlands) plus 5% normal goat serum (NGS) (Sigma Aldrich) for 1 h. Next, the cells were incubated with the first antibody (1:150 monoclonal mouse-anti-flag antibody, Sigma–Aldrich) in PBS-Tween plus 5% NGS for 2 h, washed with PBS-Tween, and incubated with the second antibody (1:100 rhodamine-conjugated goat–anti-rabbit antibody, Sanbio, Uden, the Netherlands) in PBS-Tween plus 5% NGS for 1 h. After washing with PBS-Tween cells were incubated 15 min with Hoechst 33,358 (2 µg/mL). Stained sections were mounted using PolyMount Mounting Media (Tebu-Bio, Heerhugowaard, The Netherlands) and analyzed with a fluorescence microscope (Olympus, Zoeterwoude, The Netherlands) with the Cell P software version 2.8 (Olympus).

2.6. Western blot

Protein samples were separated on 15% SDS–PAGE gels and transferred to polyvinylidene fluoride membranes (Hybond-P, GE Healthcare, Hoevelaken, The Netherlands). The membranes were blocked with 5% non-fat milk in Tris–HCl-buffered saline containing 0.1% Tween-20 (Sigma Aldrich, Zwijndrecht, the Netherlands) for 1 h at room temperature. Primary antibodies directed against flag (mouse monoclonal M2, 1:1000, Sigma Aldrich), p53 (mouse monoclonal DO-1, 1:1000, Santa Cruz Biotechnology, Heidelberg, Germany) or actin (goat polyclonal I-19, 1:1000, Santa Cruz Biotechnology) were incubated in blocking buffer for 1 h at room temperature. Peroxidase-coupled secondary antibodies (1:10,000) were obtained from Jackson ImmunoResearch Laboratories, Suffolk, UK, and membranes were incubated for 1 h at room temperature. Detection was achieved with enhanced chemiluminescence. Films were quantified using Quantity One Analysis Software (Biorad, Veenendaal, The Netherlands).

3. Results

3.1. Apoptin is degraded in normal human fibroblasts

In order to analyze a possible role of proteasomal degradation of apoptin we first examined the fate of apoptin levels in normal human fibroblasts ectopically expressing apoptin. After transfection of normal human fibroblasts with plasmid encoding flag-tagged apoptin, cells were fixed at various time-points and stained with antibody against the flag-tag. At 8 h post transfection a diffuse apoptin distribution is observed throughout the cytoplasm. At later time-points apoptin starts to form granules and eventually can no longer be detected (Fig. 1A). Transfected flag-apoptin shows the same cytoplasmic localization as microinjected MBP–apoptin [4]. Apoptin protein levels were also analyzed by Western blot. Already 40 h after transfection a drop in protein can be clearly observed, with hardly any to be detected 3 days after transfection (Fig. 1B). This result indicates that apoptin likely gets degraded.

To analyze whether this reduction in protein was due to degradation of the apoptin protein, the proteasome was inhibited with the proteasome inhibitor bortezomib. Stabilization and an actual increase in apoptin protein (1.5×) could be observed after several days (Fig. 1B and C) suggesting that the decrease in apoptin levels in normal cells is due to proteasomal degradation.

3.2. Bortezomib and Ada-Ahx$_3$L$_3$VS inhibit apoptin and p53 degradation equally in normal human fibroblasts

Next, we analyzed whether besides apoptin a protein known to be degraded by the proteasome, i.e. tumor suppressor protein p53, can be stabilized by bortezomib with similar kinetics as apoptin in normal human fibroblasts. To that end, 16 h after transfection increasing amounts of bortezomib were added to human normal fibroblasts expressing ectopic apoptin and endogenous p53. Twenty-four hours after the addition of bortezomib the cells were lysed and analyzed for apoptin and p53 levels by means of Western blot. A gradual raise of apoptin protein levels could be observed as the concentration of bortezomib increased (Fig. 2A, up to ~7.5x increase compared to control). The increase in the amount of apoptin was mimicked by the level of p53 (Fig. 2B, up to ~7.5x increase compared to control).

As bortezomib mainly inhibits the β5 subunit of the proteasome [13] we also examined the broad-spectrum proteasome inhibitor Ada-Ahx$_3$L$_3$VS [8] in a similar experiment. This led to a stabilization of both apoptin and p53 levels (Fig. 2), however, not to higher

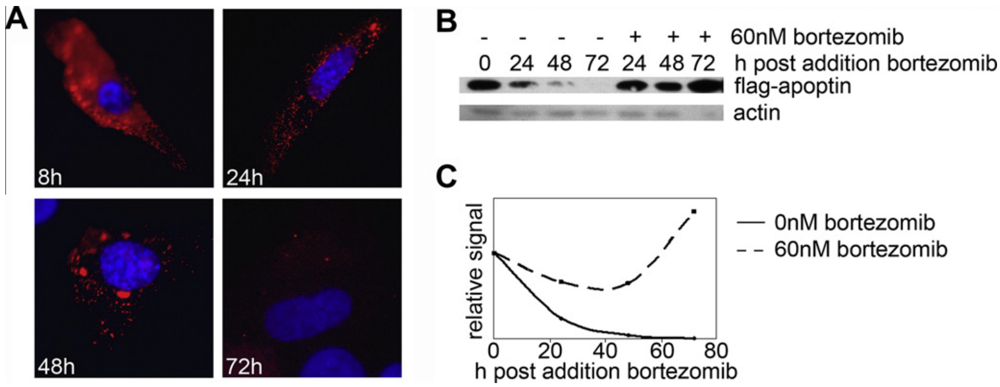

Fig. 1. Apoptin is degraded in time in normal cells, a process that can be blocked by bortezomib. Normal human fibroblasts F44 were transfected with plasmid encoding flag-tagged apoptin. (A) Cells were fixed 8, 24, 48 and 72 h post transfection. Flag-tagged apoptin was stained with a flag-specific antibody conjugated to rhodamine (red) and DNA was detected with Hoechst (blue). Apoptin appears in the cytoplasm as fine particles (8 h), gradual clusters (24 h), increased aggregates (48 h) and eventually becomes degraded (72 h). (B) Sixteen hours post transfection 0 or 60 nM bortezomib was added to the cells and at three time points after addition cells were lysed. Total apoptin levels were determined by Western blot analysis with anti-flag antibody. (C) The relative amount of apoptin (as compared to $t = 0$) with and without bortezomib was quantified and plotted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

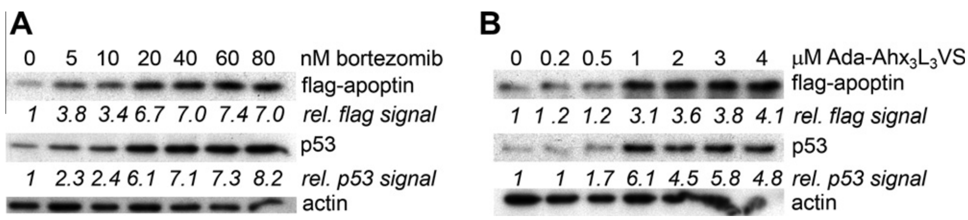

Fig. 2. Proteasome inhibitors stabilize apoptin and p53 in normal cells. Normal human F44 fibroblasts were transfected with plasmid encoding flag-tagged apoptin. (A) Sixteen hours post transfection increasing concentrations of bortezomib or (B) Ada-Ahx$_3$L$_3$VS were added and 24 h later cells were lysed. Total levels of apoptin and p53 were determined by Western blot analysis. The relative amounts of apoptin and p53 (relative to the untreated sample) were quantified and are indicated below the corresponding panels.

levels than observed with bortezomib (compare Fig. 2A/B, ~7.5x increase with bortezomib compared to a ~4x increase of flag-apoptin signal with Ada-Ahx$_3$L$_3$VS).

Our results based on two different proteasomal inhibitors indicate that apoptin is degraded in normal human cells via the proteasomal pathway.

3.3. Bortezomib inhibits phosphorylation-deficient apoptin mutant protein with similar kinetics as p53 in normal human cells

Apoptin induces apoptosis in tumor cells. This complicates the study of proteasomal breakdown in tumor cells. The apoptotic potential of apoptin in cancer cells is closely linked to its phosphorylation. Removal of the phosphorylation site T108 and the adjoining threonines greatly reduces the cell death potential of apoptin [14]. Therefore, we used the flag-apoptin(5Ala)106 mutant in which the amino acid stretch from position 106 to 110 has been replaced by alanines [11].

Normal human fibroblasts transfected with plasmid encoding this apoptin(5Ala)106 mutant showed high protein expression at early time points, but as for the wild-type apoptin (Fig. 1B and C), the apoptin(5Ala)106 mutant protein level diminished in time (Fig. 3A). On the contrary, upon addition of bortezomib the amount of apoptin(5Ala)106 stayed high (Fig. 3A). Increasing concentrations of bortezomib resulted in more apoptin(5Ala)106 protein (Fig. 3B, almost 3x increase).

The similar kinetics of degradation of the phosphorylation-deficient mutant compared to wild-type apoptin in normal cells makes it an appropriate construct to study the degradation characteristics of apoptin in human tumor cells. P53 stabilization showed similar kinetics as in the wild-type apoptin experiment.

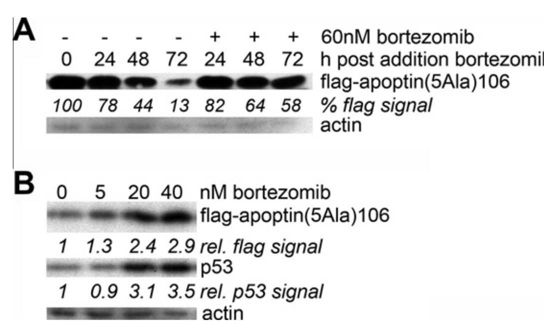

Fig. 3. Apoptin(5Ala)106 is degraded in normal cells in a similar fashion as p53 and apoptin. Normal human fibroblasts F44 were transfected with plasmid encoding flag-tagged apoptin(5Ala)106 mutant. Sixteen hours post transfection bortezomib was added. (A) At various time points cells were lysed and total apoptin was determined by Western blot analysis. (B) Twenty-four hours after addition of increasing concentrations of bortezomib total apoptin and p53 were determined by Western blot. The relative amounts of apoptin and p53 (relative to the untreated sample) were quantified and are indicated below the corresponding panels.

3.4. Bortezomib and Ada-Ahx$_3$L$_3$VS stabilize p53 but not apoptin(5Ala)106 in human osteosarcoma cells

To examine the behavior of apoptin protein upon bortezomib or Ada-Ahx$_3$L$_3$VS treatment in tumor cells, human osteosarcoma U2Os cells were used because they express wild-type p53 and are sensitive for apoptin-induced apoptosis [15,16]. U2Os cells were transfected with plasmids encoding flag-tagged apoptin(5Ala)106 protein. Sixteen hours after transfection, the p53-positive human osteosarcoma cells were treated with increasing concentrations of bortezomib or Ada-Ahx$_3$L$_3$VS and the cells were lysed 24 h later.

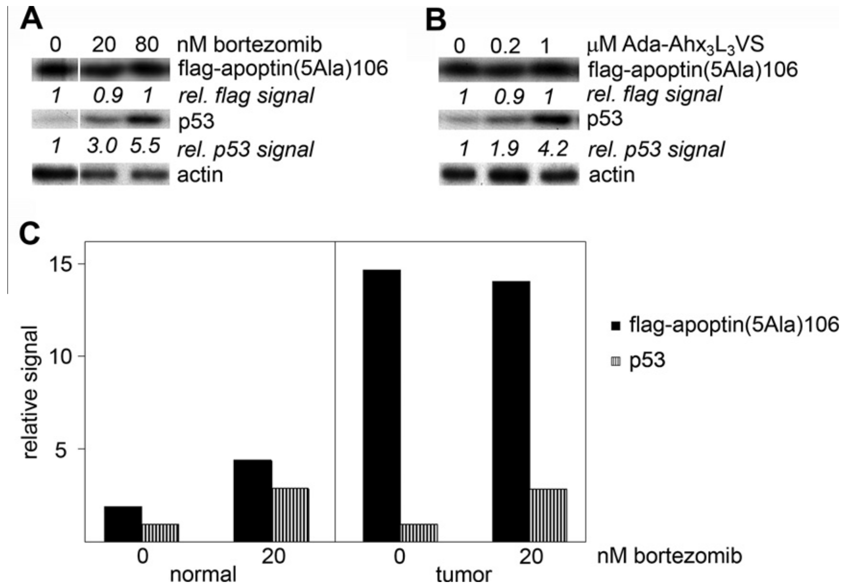

Fig. 4. Proteasome inhibitors influence p53 but not apoptin(5Ala)106 stability in tumor cells. Human osteosarcoma cells U2Os were transfected with plasmid encoding flag-tagged apoptin(5Ala)106. (A) Sixteen hours post transfection cells were treated with increasing concentrations of bortezomib or (B) Ada-Ahx$_3$L$_3$VS and 24 h later cells were lysed. Total amounts of apoptin and p53 were determined by Western blot analysis. The relative amounts of apoptin and p53 (relative to the 0 nM bortezomib signal) were quantified and are indicated below the corresponding panels. (C) In order to make a direct comparison between the apoptin protein level in normal and tumor cells two representative experiments, one in normal and one in tumor cells, are visualized in the same chart. The relative amounts of apoptin and p53 in Figs. 3B and 4A were quantified relative to the untreated p53 levels and are plotted.

Increasing amounts of both bortezomib and Ada-Ahx$_3$L$_3$VS led to an increase in total p53 (Fig. 4A/B). The p53 levels changed similarly as in normal cells as they increased 5x with bortezomib and 4x with Ada-Ahx$_3$L$_3$VS. This shows that the proteasome and the proteasomal inhibitors function in the U2Os tumor cell line. Remarkably, the amount of apoptin(5Ala)106 protein was not influenced by inhibition of the proteasome by increasing the concentrations of both bortezomib and Ada-Ahx$_3$L$_3$VS (Fig. 4). In accordance with this finding is the observation that the basal level of apoptin protein in the U2Os cells without addition of inhibitor is 15x higher than in normal cells (Fig. 4C). Altogether, these results show that apoptin(5Ala)106 can not become degraded by the proteasome in human U2Os tumor cells.

4. Discussion

By inhibiting the proteasome activity with the known proteasome inhibitors bortezomib and Ada-Ahx$_3$L$_3$VS, which both clearly increased the stability of the tumor suppressor protein p53 in normal and cancer cells, we revealed that apoptin protein is degraded in normal but not in tumor cells through the process of proteasomal degradation.

Apoptin is a potential therapeutic agent with very distinct behavioral differences between normal and tumor cells. The differential proteasomal degradation of apoptin between normal and tumor cells will likely contribute to its selectivity.

The tumor selective induction of apoptosis is the main feature of apoptin. This characteristic is preceded by the nuclear localization in cancer cells [3]. In tumor cells apoptin is phosphorylated on T108, a modification that is not observed in normal cells [2]. To circumvent that apoptin-induced apoptosis interferes with our protein degradation or stabilization measurements we developed a flag-tagged phosphorylation-deficient apoptin(5Ala)106 mutant that has a greatly reduced cell death inducing ability [14]. In normal cells this mutant showed the same response to inhibition of the proteasome as did wild-type apoptin. In tumor cells, however, both the proteasome inhibitor bortezomib and Ada-Ahx$_3$L$_3$VS had no influence on the level of apoptin(5Ala)106, whereas the positive control p53 was clearly stabilized with similar kinetics as in normal cells.

Nuclear translocation is a characteristic of apoptin in tumor cells. P53 can also localize to both the nucleus and cytoplasm and is degraded in both compartments [17]. This raised the question whether the difference in degradation of apoptin is due to a different proteasomal activity in the nucleus. Although several different catalytic subunit compositions are known between cell types as described above, no distinction has yet been established between the subunit composition of the proteasome in the nucleus and the cytoplasm.

In normal cells the highest levels of apoptin and p53 are reached with the inhibitor bortezomib as compared to Ada-Ahx$_3$L$_3$VS. Bortezomib has a high affinity for the β5 enzymatic subunit of the proteasome relative to Ada-Ahx$_3$L$_3$VS which has an equal effect on all three β subunits. Apparently it is the β5 subunit that plays a major role in both apoptin and p53 degradation.

Although elevated levels of proteasome subunits have been found in some cancers, and proteasome inhibitors are used in the treatment of certain cancer types, a clear molecular difference between tumor and normal proteasome subunits has not yet been reported [18,19].

Possibly, it is not a difference in proteasome assembly that leads to the higher stability of apoptin in tumor cells. Some viral proteins are known to modulate (viral) protein stability through the proteasome [20]. In this respect it is interesting to mention that apoptin influences the anaphase promoting complex or cyclosome (APC/C) complex. Teodoro et al. [23] showed that interaction of apoptin with the APC1 subunit results in destabilisation of the APC/C complex. The APC/C is an upstream effector of the proteasome that is responsible for correct progression of the cell cycle by targeting cell cycle related proteins for degradation [21]. APC/C is the main E3 ligase in the nucleus targeting proteins for degradation. Subunits of the APC/C are mutated in several cancers and are a popular target for viral proteins [22]. It is tempting to hypothesise that by disrupting the APC/C E3 ligase activity apoptin itself creates the situation that enables its stabilization. More research will, however, be needed to fully comprehend the molecular basis of the stable apoptin protein levels in tumor cells.

The tumor-selective apoptosis induction by apoptin makes it a very promising anti-cancer agent. Here, we show a novel characteristic of apoptin. The differential proteasomal sensitivity likely will contribute to the application of apoptin as a safe and efficient therapeutic agent.

Acknowledgments

This work was supported by a Grant from the Dutch Royal Society of Arts and Sciences (KNAW) and by the Leiden Institute of Chemistry.

References

[1] A.A.A.M. Danen-van Oorschot, D.F. Fischer, J.M. Grimbergen, B. Klein, S. Zhuang, J.H. Falkenburg, C. Backendorf, P.H. Quax, A.J. van der Eb, M.H.M. Noteborn, Apoptin induces apoptosis in human transformed and malignant cells but not in normal cells, Proc. Natl. Acad. Sci. USA 94 (1997) 5843–5847.
[2] J.L. Rohn, Y.-H. Zhang, R.I. Aalbers, N. Otto, J. Den Hertog, N.V. Henriquez, C.J. van de Velde, P.J. Kuppen, D. Mumberg, P. Donner, M.H.M. Noteborn, A tumor-specific kinase activity regulates the viral death protein apoptin, J. Biol. Chem. 277 (2002) 50820–50827.
[3] C. Backendorf, A.E. Visser, A.G. de Boer, R.M.E. Zimmerman, M. Visser, P. Voskamp, Y.-H. Zhang, M.H.M. Noteborn, Apoptin: therapeutic potential of an early sensor of carcinogenic transformation, Annu. Rev. Pharmacol. Toxicol. 48 (2008) 143–169.
[4] Y.-H. Zhang, S.R. Leliveld, K. Kooistra, C. Molenaar, J.L. Rohn, H.J. Tanke, J.-P. Abrahams, M.H.M. Noteborn, Recombinant apoptin multimers kill tumor cells but are nontoxic and epitope-shielded in normal-cell-specific fashion, Exp. Cell Res. 289 (2003) 36–46.
[5] A. Hershko, A. Ciechanover, The ubiquitin system, Annu. Rev. Biochem. 67 (1998) 425–479.
[6] S. Murata, H. Yashiroda, K. Tanaka, Molecular mechanisms of proteasome assembly, Nat. Rev. Mol. Cell Biol. 10 (2009) 104–115.
[7] J.C. Cusack Jr, R. Liu, M. Houston, K. Abendroth, P.J. Elliott, J. Adams, A.S. Baldwin Jr, Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-B inhibition, Cancer Res. 61 (2001) 3535–3540.
[8] B.M. Kessler, D. Tortorella, M. Altun, A.F. Kisselev, E. Fiebiger, B.G. Hekking, H.L. Ploegh, H.S. Overkleeft, Extended peptide-based inhibitors efficiently target the proteasome and reveal overlapping specificities of the catalytic β-subunits, Chem. Biol. 8 (2001) 913–929.
[9] R.M.E. Zimmerman, D.-J. Peng, H.L. Lanz, Y.-H. Zhang, A.A.A.M. Danen-van Oorschot, S. Qu, C. Backendorf, M.H.M. Noteborn, PP2A inactivation is a crucial step in triggering apoptin-induced tumor-selective killing, Cell Death Dis. 3 (2012) e291.
[10] M.H.M. Noteborn, G.F. de Boer, D.J. van Roozelaar, C. Karreman, O. Kranenburg, J.G. Vos, S.H.M. Jeurissen, R.C. Hoeben, A. Zantema, G. Koch, H. van Ormondt, A.J. van der Eb, Characterization of cloned chicken anemia virus DNA that contains all elements for the infectious replication cycle, J. Virol. 65 (1991) 3131–3139.
[11] A.A.A.M. Danen-van Oorschot, P. Voskamp, M. Seelen, M. van Miltenburg, M. Bolk, S. Tait, J. Boesen-de Cock, J. Rohn, J. Borst, M.H.M. Noteborn, Human death effector domain-associated factor interacts with the viral apoptosis agonist apoptin and exerts tumor-preferential cell killing, Cell Death Dif. 11 (2004) 564–573.
[12] B.I. Florea, M. Verdoes, N. Li, W.A. van der Linden, P.P. Geurink, H. van den Elst, T. Hofmann, A. de Ru, P.A. van Veelen, K. Tanaka, K. Sasaki, S. Murata, H. den Dulk, J. Brouwer, F.A. Ossendorp, A.F. Kisselev, H.S. Overkleeft, Activity-based profiling reveals reactivity of the murine thymoproteasome-specific subunit beta5t, Chem. Biol. 17 (2010) 795–801.
[13] A.F. Kisselev, A. Callard, A.L. Goldberg, Importance of the different proteolytic sites of the proteasome and the efficacy of inhibitors varies with the protein substrate, J. Biol. Chem. 281 (2006) 8582–8590.
[14] J.L. Rohn, Y.-H. Zhang, S.R. Leliveld, A.A.A.M. Danen-van Oorschot, N.V. Henriquez, J.-P. Abrahams, N.H.M. Noteborn, Relevance of apoptin's integrity for its functional behaviour, J. Virol. 79 (2005) 1337–1338.
[15] L. Diller, J. Kassel, C.E. Nelson, M.A. Gryka, G. Litwak, M. Gebhardt, B. Bressac, M. Ozturk, S.J. Baker, B. Vogelstein, P53 functions as a cell cycle control protein in osteosarcomas, Mol. Cell Biol. 10 (1990) 5772–5781.
[16] S.M. Zhuang, A. Shvarts, H. van Ormondt, A.G. Jochemsen, A.J. van der Eb, M.H.M. Noteborn, Apoptin, a protein derived from chicken anemia virus, induces p53-independent apoptosis in human osteosarcoma cells, Cancer Res. 55 (1995) 486–489.
[17] J. Yuan, K. Luo, L. Zhang, J.C. Cheville, Z. Lou, USP10 regulates p53 localization and stability by deubiquitinating p53, Cell 140 (2010) 384–396.
[18] D. Hoeller, I. Dikic, Targeting the ubiquitin system in cancer therapy, Nature 458 (2009) 438–444.
[19] M.P. Bousquet-Dubouch, B. Fabre, B. Monsarrat, O. Burlet-Schiltz, Proteomics to study the diversity and dynamics of proteasome complexes: from fundamentals to the clinic, Expert Rev. Proteomics 8 (2011) 459–481.
[20] Z. Hu, Z. Zhang, E. Doo, O. Coux, A.L. Goldberg, T.J. Liang, Hepatitis B virus X protein is both a substrate and a potential inhibitor of the proteasome complex, J. Virol. 73 (1999) 7231–7240.
[21] I. Garcia-Higuera, E. Manchado, P. Dubus, M. Canamero, J. Mendez, S. Moreno, M. Malumbres, Genomic stability and tumour suppression by the APC/C cofactor Cdh1, Nat. Cell Biol. 10 (2008) 802–811.
[22] L. Smolders, J.G. Teodoro, Targeting the anaphase promoting complex: common pathways for viral infection and cancer therapy, Expert Opin. Ther. Targets 15 (2011) 767–780.
[23] J.G. Teodoro, D.W. Heilman, A.E. Parker, M.R. Green, The viral protein apoptin associates with the anaphase-promoting complex to induce G2/M arrest and apoptosis in the absence of p53, Genes Dev. 18 (2004) 1952–1957.