

through interaction with Atg5 and FADD [31]. In contrast, the adenoviral E4 protein inhibits autophagy. E4 protein activates the mammalian target of rapamycin (mTOR) signaling pathway [32], which suppresses autophagy through suppression of unc-51-like autophagy activating kinase 1 (ULK1) activity by phosphorylation [33]. In fact, E4 protein inhibits the oncolytic potential of the E1B-55K-deleted oncolytic adenovirus ONYX-015 through activation of the mTOR signaling pathway [34]. Suppression of the mTOR signaling pathway by rapamycin and everolimus enhances the autophagic cell death induced by oncolytic adenoviruses (Table 1). Thus, oncolytic adenoviruses may strictly induce autophagy through regulation of the E1-dependent activation signaling pathway and the E4-dependent inhibitory signaling pathway.

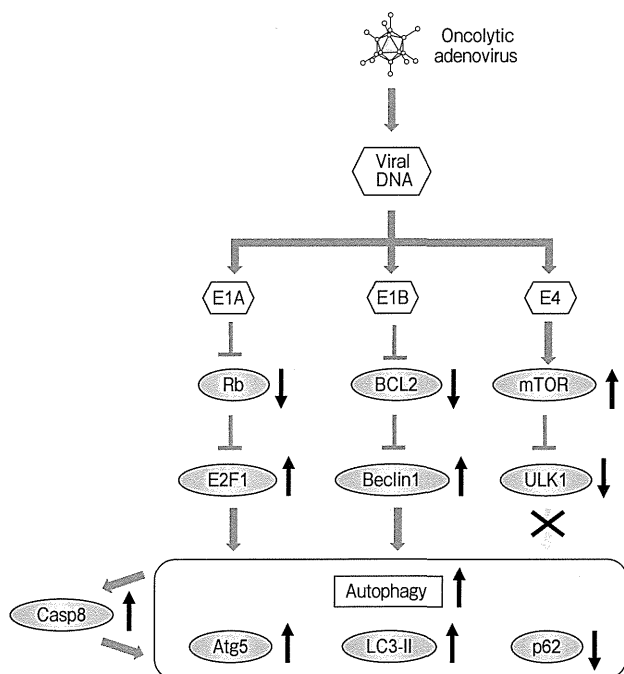


Fig. 2 Schematic diagram of the molecular mechanism of oncolytic adenovirus-mediated autophagy induction. Oncolytic adenovirus induces the accumulation of adenoviral DNA-derived E1A, E1B, and E4 proteins. E1A protein upregulates E2F1 expression by suppressing Rb activity. E1B protein upregulates Beclin1 expression through the dissociation of the Beclin1-BCL2 complex. In contrast, E4 protein downregulates ULK1 expression by activating the mTOR signaling pathway. E1A and E1B proteins activate, but E4 protein inhibits, autophagy in association with Atg5 upregulation, LC3-II upregulation, and p62 downregulation. Moreover, oncolytic adenovirus-mediated autophagy can be enhanced by caspase-8 (Casp8) activation.

Small Noncoding RNAs Regulated by Oncolytic Adenovirus

Infection with wild-type Ad5 regulates 2 different types of small noncoding RNAs in the infected cells, viral miRNAs (mivaRNAs) and cellular miRNAs, which are respectively derived from virus-associated RNA (VA RNA) and cellular primary miRNA transcripts (Fig. 3) [35]. Recently, these 2 types of small noncoding RNAs have been suggested to be involved in antiviral defense and viral replication. VA RNAs consist of 2 types of 160-nucleotide-noncoding RNAs, VA RNAI and VA RNAII, which are transcribed by cellular RNA polymerase III from the adenoviral genome. VA RNA induces the expression of type I interferon (IFN), which is associated with innate

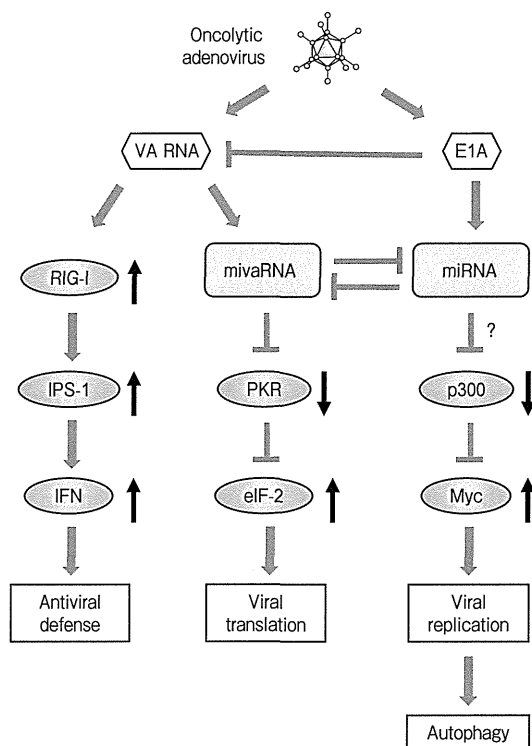


Fig. 3 Schematic diagram of antiviral and proviral processes regulated by small noncoding RNAs. When infected with oncolytic adenovirus, two types of small noncoding RNAs, viral microRNA (mivaRNA) and cellular microRNA (miRNA), are induced by virus-associated RNA (VA-RNA) and E1A, respectively. VA RNA regulates antiviral defense and viral translation through the RIG-I-IPS-1-IFN signaling pathway and mivaRNA-PKR-eIF-2 signaling pathway, respectively. E1A regulates viral replication and autophagy through the miRNA-p300-Myc signaling pathway.

immune responses [36–38]. In terms of the molecular mechanism of VA RNA-mediated IFN induction, the involvement of IFN- β promoter stimulator-1 (IPS-1) and retinoic acid-inducible gene I (RIG-I) has been suggested [37–39], whereas the role of VA RNA-derived miRNAs remains to be elucidated. VA RNA further activates the eukaryotic translation initiation factor 2 α (eIF-2) through suppression of protein kinase R (PKR), contributing to the translation of viral mRNA [40–42]. However, a recent report has suggested that VA RNA-derived miRNAs are not involved in adenovirus-mediated cell lysis [43]. In contrast to this report, evidence also supports the utility of VA RNA-deleted oncolytic adenovirus to induce more profound antitumor effects in tumor cells and lower cytotoxicity in normal cells than wild-type adenovirus [44–46]. In contrast, adenoviral E1A protein modulates the expression of cellular miRNAs in tumor cells (Fig. 3) [47]. We also demonstrated the modulation of cellular miRNAs in oncolytic adenovirus-infected tumor cells [13, 14]. These miRNAs are mainly transcribed by RNA polymerase II from the cellular DNA genome. E1A protein is necessary for viral replication because E1A-deleted adenovirus lacks the ability to replicate. E1A protein induces DNA synthesis and viral replication followed by S phase entry through upregulation of c-Myc [48–50], which is caused by suppression of the p300 transcriptional co-activator through an unknown mechanism [51, 52]. Since miR-132 regulates the antiviral defense and viral replication through suppression of p300 after infection with other types of viruses [53, 54], viral replication of oncolytic adenovirus may also be regulated by an E1A-dependent miRNA network through suppression of p300 (Fig. 3). Recent reports have suggested that both p300 knockdown [55] and c-Myc overexpression [56] induce autophagy; thus, viral replication induced by p300 downregulation and c-Myc upregulation may contribute to autophagy induction. These findings suggest that VA RNA-derived miRNAs mainly induce antiviral defense in the innate immune response, whereas E1A protein supports viral replication in the adaptive immune response. E1A-mediated viral replication may further promote the autophagic machinery. Moreover, VA RNA-derived miRNAs and cellular miRNAs competitively inhibit the RNAi machinery [57–59], whereas E1A protein suppresses the accumulation of VA RNAs through

suppression of RNA polymerase III [60]. Therefore, the crosstalk between VA RNA and E1A protein may finally decide the cellular fate with respect to cell survival or cell death in oncolytic adenovirus-infected cells.

E2F1-Regulated miRNAs in Oncolytic Adenovirus-Induced Cell Death

Adenovirus infection activates E2F1 expression through the E1A-dependent suppression of Rb protein [27]. Recent evidence in tumor biology has shown the presence of the E2F1-regulated small noncoding miRNAs as tumor-suppressive factors [61] or oncogenic factors [62]. The crosstalk between E2F1 and the E2F1-regulated miRNA network has been suggested to be critical for determining the prosurvival and antiproliferative effects of E2F1 in cancer [63]. We recently demonstrated the involvement of E2F1-regulated miRNAs in oncolytic adenovirus-induced cell death (Fig. 4) [13, 14]. When human cancer cells were infected with the hTERT promoter-driven oncolytic adenovirus OBP-301, miR-7 expression was upregulated in a time- and dose-dependent manner [13]. Overexpression of exogenous E2F1 induced miR-7 expression, whereas pretreatment with E2F1 siRNA attenuated the OBP-301-induced miR-7 upregulation in human cancer cells, suggesting the E2F1-dependent regulation of miR-7 expression. Interestingly, ectopic expression of exogenous miR-7 induced autophagy-related cell death through suppression of oncogenic epidermal growth factor receptor (EGFR) [13]. Another report also supports that suppression of EGFR expression by EGFR siRNA induces autophagic cell death in human cancer cells [64]. The EGFR-downstream phosphatidylinositol 3'-kinase-Akt-mTOR signaling pathway suppresses the autophagy machinery [65]. We reported that OBP-702 in p53-expressing OBP-702-infected tumor cells suppressed the expression of p21, which functions as both an anti-apoptotic [66] and anti-autophagic factor [67], through upregulation of miR-93 and miR-106 [14, 68]. miR-93/106-mediated p21 downregulation enhanced the apoptotic and autophagic cell deaths induced by p53-mediated pro-apoptotic BCL2-associated X protein (BAX) and pro-autophagic DNA-damage regulated autophagy modulator (DRAM) proteins [14]. miR-93 and miR-106 are upregulated by E2F1

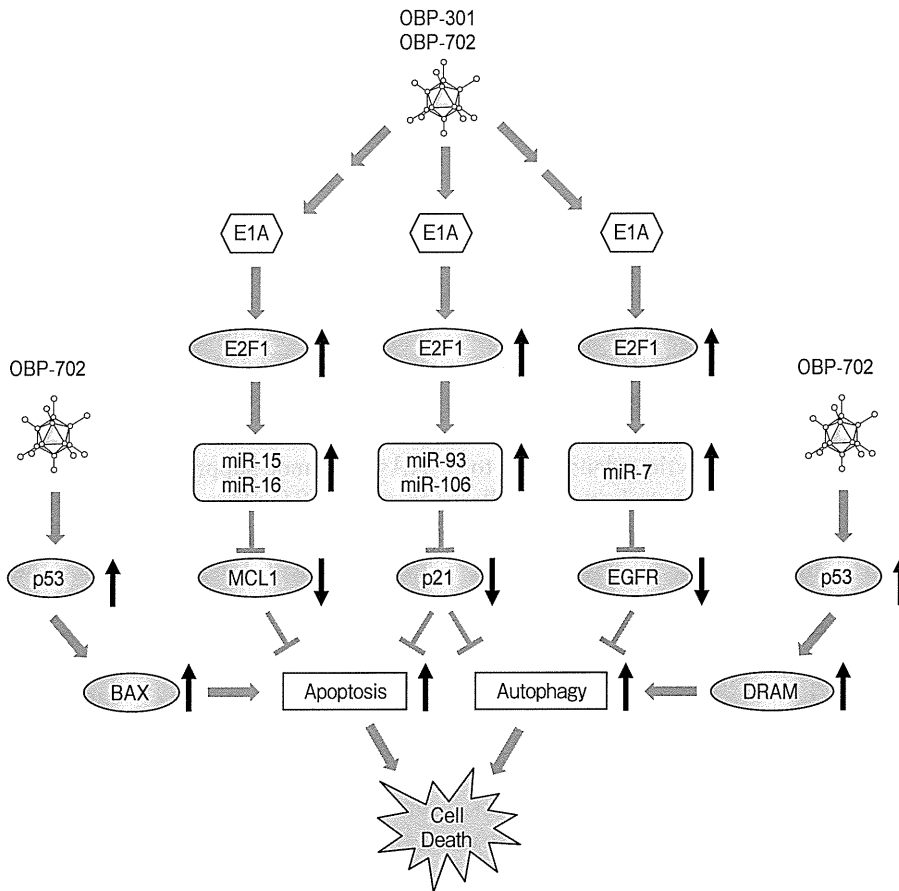


Fig. 4 Schematic diagram of E1A-dependent cell death pathways through the E2F1-regulated miRNA network. Oncolytic adenoviruses, OBP-301 and OBP-702, induce E1A-dependent cell death pathways through regulation of the E2F1-inducible miRNA network. E2F1-inducible miRNAs (miR-7, miR-15, miR-16, miR-93, miR-106) induce apoptotic and autophagic cell death through suppression of anti-apoptotic and anti-autophagic factors (MCL1, p21, EGFR). Overexpression of tumor suppressor p53 also induces apoptotic and autophagic cell death via upregulation of p53-downstream targets, BAX and DRAM, respectively, in OBP-702-infected tumor cells.

and suppress the expression of p21 [69]. We recently observed that, in human tumor cells co-treated with OBP-301 and chemotherapy, OBP-301 downregulated the expression of the anti-apoptotic BCL2 family myeloid cell leukemia sequence 1 (MCL1) through upregulation of miR-15 and miR-16, resulting in the enhancement of chemotherapy-induced apoptotic cell death (our unpublished data). Other types of oncolytic adenoviruses downregulate the expression of MCL1 in infected tumor cells [70, 71]. Moreover, miR-15 and miR-16 can be upregulated by E2F1 [72] and suppress the expression of MCL1 [73]. A recent report also supports the modulation of cellular miRNAs in adenoviral E1A-overexpressed human cancer cells [47]. Downregulation of miR-520h by E1A activation

was associated with E1A-dependent antitumor effects [47]. In this report, more interestingly, some E2F1-inducible miRNAs (miR-92, miR-18, miR-15, miR-93, miR-25) were upregulated in the E1A-expressing human cancer cells [47], which was consistent with our recent experimental observations (Fig. 4). These findings suggest that the E2F1-regulated miRNA network is a critical signaling pathway for fine-tuning the oncolytic adenovirus-induced autophagic and apoptotic cell deaths through the suppression of anti-autophagic and anti-apoptotic factors.

Conclusion

Oncolytic virotherapy is a promising antitumor

strategy for eliminating tumor cells without adversely affecting normal cells. Various types of oncolytic adenoviruses have been developed, and these viruses mainly induce autophagy-related cell death in tumor cells. However, the molecular mechanisms underlying the oncolytic adenovirus-induced autophagic cell death remains unclear. Some adenoviral proteins, including E1A, E1B, and E4, have been suggested to be implicated in the regulation of autophagic machinery. Moreover, following E1A accumulation, the E2F1-regulated microRNA network may be involved in fine-tuning of the oncolytic cell death associated with autophagy. An exploration of the functional role and molecular basis of oncolytic adenovirus-mediated autophagy induction would help to improve the therapeutic potential of oncolytic virotherapy.

Acknowledgments. This study was supported by grants from the Ministry of Health, Labour, and Welfare of Japan and from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- Mizushima N and Komatsu M: Autophagy: renovation of cells and tissues. *Cell* (2011) 147: 728–741.
- Sato K, Tsuchihara K, Fujii S, Sugiyama M, Goya T, Atomi Y, Ueno T, Ochiai A and Esumi H: Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation. *Cancer Res* (2007) 67: 9677–9684.
- Hu YL, DeLay M, Jahangiri A, Molinaro AM, Rose SD, Carbonell WS and Aghi MK: Hypoxia-induced autophagy promotes tumor cell survival and adaptation to antiangiogenic treatment in glioblastoma. *Cancer Res* (2012) 72: 1773–1783.
- Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T and Thompson CB: Growth factor regulation of autophagy and cell survival in the absence of apoptosis. *Cell* (2005) 120: 237–248.
- Kudchodkar SB and Levine B: Viruses and autophagy. *Rev Med Virol* (2009) 19: 359–378.
- Oh JE and Lee HK: Modulation of pathogen recognition by autophagy. *Front Immunol* (2012) 3: 44.
- Russell SJ, Peng KW and Bell JC: Oncolytic virotherapy. *Nat Biotechnol* (2012) 30: 658–670.
- Ito H, Aoki H, Kuhnel F, Kondo Y, Kubicka S, Wirth T, Iwado E, Iwamaru A, Fujiwara K, Hess KR, Lang FF, Sawaya R and Kondo S: Autophagic cell death of malignant glioma cells induced by a conditionally replicating adenovirus. *J Natl Cancer Inst* (2006) 98: 625–636.
- Jiang H, Gomez-Manzano C, Aoki H, Alonso MM, Kondo S, McCormick F, Xu J, Kondo Y, Bekele BN, Colman H, Lang FF and Fueyo J: Examination of the therapeutic potential of Delta-24-RGD in brain tumor stem cells: role of autophagic cell death. *J Natl Cancer Inst* (2007) 99: 1410–1414.
- Ulasov IV, Tyler MA, Zhu ZB, Han Y, He TC and Lesniak MS: Oncolytic adenoviral vectors which employ the survivin promoter induce glioma oncolysis via a process of beclin-dependent autophagy. *Int J Oncol* (2009) 34: 729–742.
- Kawashima T, Kagawa S, Kobayashi N, Shirakiya Y, Umeoka T, Teraishi F, Taki M, Kyo S, Tanaka N and Fujiwara T: Telomerase-specific replication-selective virotherapy for human cancer. *Clin Cancer Res* (2004) 10: 285–292.
- Endo Y, Sakai R, Ouchi M, Onimatsu H, Hioki M, Kagawa S, Uno F, Watanabe Y, Urata Y, Tanaka N and Fujiwara T: Virus-mediated oncolysis induces danger signal and stimulates cytotoxic T-lymphocyte activity via proteasome activator upregulation. *Oncogene* (2008) 27: 2375–2381.
- Tazawa H, Yano S, Yoshida R, Yamasaki Y, Sasaki T, Hashimoto Y, Kuroda S, Ouchi M, Onishi T, Uno F, Kagawa S, Urata Y and Fujiwara T: Genetically engineered oncolytic adenovirus induces autophagic cell death through an E2F1-microRNA-7-epidermal growth factor receptor axis. *Int J Cancer* (2012) 131: 2939–2950.
- Hasei J, Sasaki T, Tazawa H, Osaki S, Yamakawa Y, Kunisada T, Yoshida A, Hashimoto Y, Onishi T, Uno F, Kagawa S, Urata Y, Ozaki T and Fujiwara T: Dual programmed cell death pathways induced by p53 transactivation overcome resistance to oncolytic adenovirus in human osteosarcoma cells. *Mol Cancer Ther* (2013) 12: 314–325.
- Yokoyama T, Iwado E, Kondo Y, Aoki H, Hayashi Y, Georgescu MM, Sawaya R, Hess KR, Mills GB, Kawamura H, Hashimoto Y, Urata Y, Fujiwara T and Kondo S: Autophagy-inducing agents augment the antitumor effect of telomerase-sense oncolytic adenovirus OBP-405 on glioblastoma cells. *Gene Ther* (2008) 15: 1233–1239.
- Ulasov IV, Sonabend AM, Nandi S, Khramtsov A, Han Y and Lesniak MS: Combination of adenoviral virotherapy and temozolomide chemotherapy eradicates malignant glioma through autophagic and apoptotic cell death in vivo. *Br J Cancer* (2009) 100: 1154–1164.
- Alonso MM, Jiang H, Yokoyama T, Xu J, Bekele NB, Lang FF, Kondo S, Gomez-Manzano C and Fueyo J: Delta-24-RGD in combination with RAD001 induces enhanced anti-glioma effect via autophagic cell death. *Mol Ther* (2008) 16: 487–493.
- Jiang H, White EJ, Rios-Vicil CI, Xu J, Gomez-Manzano C and Fueyo J: Human adenovirus type 5 induces cell lysis through autophagy and autophagy-triggered caspase activity. *J Virol* (2011) 85: 4720–4729.
- Piya S, White EJ, Klein SR, Jiang H, McDonnell TJ, Gomez-Manzano C and Fueyo J: The E1B19K oncoprotein complexes with Beclin 1 to regulate autophagy in adenovirus-infected cells. *PLoS One* (2011) 6: e29467.
- Rajecki M, af Hallstrom T, Hakkarainen T, Nokisalmi P, Hautaniemi S, Nieminen AI, Tenhunen M, Rantanen V, Desmond RA, Chen DT, Guse K, Stenman UH, Gargini R, Kapanen M, Klefstrom J, Kanerva A, Pesonen S, Ahtiainen L and Hemminki A: Mre11 inhibition by oncolytic adenovirus associates with autophagy and underlies synergy with ionizing radiation. *Int J Cancer* (2009) 125: 2441–2449.
- Baird SK, Aerts JL, Eddaoudi A, Lockley M, Lemoine NR and McNeish IA: Oncolytic adenoviral mutants induce a novel mode of programmed cell death in ovarian cancer. *Oncogene* (2008) 27: 3081–3090.
- Botta G, Passaro C, Libertini S, Abagnale A, Barbato S, Maione AS, Hallden G, Beguinot F, Formisano P and Portella G: Inhibition of autophagy enhances the effects of E1A-defective oncolytic adenovirus dl922-947 against glioma cells in vitro and in vivo. *Hum Gene Ther* (2012) 23: 623–634.
- Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M and Ohsumi Y: A protein conjugation

- system essential for autophagy. *Nature* (1998) 395: 395–398.
24. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y and Yoshimori T: LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosomal membranes after processing. *EMBO J* (2000) 19: 5720–5728.
 25. Bjorkoy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H and Johansen T: p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol* (2005) 171: 603–614.
 26. Rodriguez-Rocha H, Gomez-Gutierrez JG, Garcia-Garcia A, Rao XM, Chen L, McMasters KM and Zhou HS: Adenoviruses induce autophagy to promote virus replication and oncolysis. *Virology* (2011) 416: 9–15.
 27. Bagchi S, Raychaudhuri P and Nevins JR: Adenovirus E1A proteins can dissociate heteromeric complexes involving the E2F transcription factor: a novel mechanism for E1A trans-activation. *Cell* (1990) 62: 659–669.
 28. Polager S, Ofir M and Ginsberg D: E2F1 regulates autophagy and the transcription of autophagy genes. *Oncogene* (2008) 27: 4860–4864.
 29. Garcia-Garcia A, Rodriguez-Rocha H, Tseng MT, Montes de Oca-Luna R, Zhou HS, McMasters KM and Gomez-Gutierrez JG: E2F-1 lacking the transcriptional activity domain induces autophagy. *Cancer Biol Ther* (2012) 13: 1091–1101.
 30. Kuroda S, Fujiwara T, Shirakawa Y, Yamasaki Y, Yano S, Uno F, Tazawa H, Hashimoto Y, Watanabe Y, Noma K, Urata Y, Kagawa S and Fujiwara T: Telomerase-dependent oncolytic adenovirus sensitizes human cancer cells to ionizing radiation via inhibition of DNA repair machinery. *Cancer Res* (2010) 70: 9339–9348.
 31. Pyo JO, Jang MH, Kwon YK, Lee HJ, Jun JI, Woo HN, Cho DH, Choi B, Lee H, Kim JH, Mizushima N, Ohsumi Y and Jung YK: Essential roles of Atg5 and FADD in autophagic cell death: dissection of autophagic cell death into vacuole formation and cell death. *J Biol Chem* (2005) 280: 20722–20729.
 32. O'Shea C, Klupsch K, Choi S, Bagus B, Soria C, Shen J, McCormick F and Stokoe D: Adenoviral proteins mimic nutrient/growth signals to activate the mTOR pathway for viral replication. *EMBO J* (2005) 24: 1211–1221.
 33. Kim J, Kundu M, Viollet B and Guan KL: AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* (2011) 13: 132–141.
 34. Thomas MA, Broughton RS, Goodrum FD and Ornelles DA: E4orf1 limits the oncolytic potential of the E1B-55K deletion mutant adenovirus. *J Virol* (2009) 83: 2406–2416.
 35. Carnero E, Sutherland JD and Fortes P: Adenovirus and miRNAs. *Biochim Biophys Acta* (2011) 1809: 660–667.
 36. Weber F, Wagner V, Kessler N and Haller O: Induction of interferon synthesis by the PKR-inhibitory VA RNAs of adenoviruses. *J Interferon Cytokine Res* (2006) 26: 1–7.
 37. Yamaguchi T, Kawabata K, Kouyama E, Ishii KJ, Katayama K, Suzuki T, Kurachi S, Sakurai F, Akira S and Mizuguchi H: Induction of type I interferon by adenovirus-encoded small RNAs. *Proc Natl Acad Sci U S A* (2010) 107: 17286–17291.
 38. Minamitani T, Iwakiri D and Takada K: Adenovirus virus-associated RNAs induce type I interferon expression through a RIG-I-mediated pathway. *J Virol* (2011) 85: 4035–4040.
 39. Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O and Akira S: IPS-1, an adaptor triggering RIG-I and Mda5-mediated type I interferon induction. *Nat Immunol* (2005) 6: 981–988.
 40. Thimmappaya B, Weinberger C, Schneider RJ and Shenk T: Adenovirus VAI RNA is required for efficient translation of viral mRNAs at late times after infection. *Cell* (1982) 31: 543–551.
 41. Kitajewski J, Schneider RJ, Safer B, Munemitsu SM, Samuel CE, Thimmappaya B and Shenk T: Adenovirus VAI RNA antagonizes the antiviral action of interferon by preventing activation of the interferon-induced eIF-2 alpha kinase. *Cell* (1986) 45: 195–200.
 42. Aparicio O, Razquin N, Zaratigui M, Narvaiza I and Fortes P: Adenovirus virus-associated RNA is processed to functional interfering RNAs involved in virus production. *J Virol* (2006) 80: 1376–1384.
 43. Kamel W, Segerman B, Oberg D, Punga T and Akusjarvi G: The adenovirus VA RNA-derived miRNAs are not essential for lytic virus growth in tissue culture cells. *Nucleic Acids Res* (2013) 41: 4802–4812.
 44. Wang Y, Xue SA, Hallden G, Francis J, Yuan M, Griffin BE and Lemoine NR: Virus-associated RNA I-deleted adenovirus, a potential oncolytic agent targeting EBV-associated tumors. *Cancer Res* (2005) 65: 1523–1531.
 45. Cascallo M, Gros A, Bayo N, Serrano T, Capella G and Alemany R: Deletion of VAI and VAIL RNA genes in the design of oncolytic adenoviruses. *Hum Gene Ther* (2006) 17: 929–940.
 46. Machitani M, Katayama K, Sakurai F, Matsui H, Yamaguchi T, Suzuki T, Miyoshi H, Kawabata K and Mizuguchi H: Development of an adenovirus vector lacking the expression of virus-associated RNAs. *J Control Release* (2011) 154: 285–289.
 47. Su JL, Chen PB, Chen YH, Chen SC, Chang YW, Jan YH, Cheng X, Hsiao M and Hung MC: Downregulation of microRNA miR-520h by E1A contributes to anticancer activity. *Cancer Res* (2010) 70: 5096–5108.
 48. Baluchamy S, Sankar N, Navaraj A, Moran E and Thimmappaya B: Relationship between E1A binding to cellular proteins, c-myc activation and S-phase induction. *Oncogene* (2007) 26: 781–787.
 49. Chakraborty AA and Tansey WP: Adenoviral E1A function through Myc. *Cancer Res* (2009) 69: 6–9.
 50. Singhal G, Leo E, Setty SK, Pommier Y and Thimmappaya B: Adenovirus E1A Oncogene Induces Rereplication of Cellular DNA and Alters DNA Replication Dynamics. *J Virol* (2013) 87: 8767–8778.
 51. Kolli S, Buchmann AM, Williams J, Weitzman S and Thimmappaya B: Antisense-mediated depletion of p300 in human cells leads to premature G1 exit and up-regulation of c-MYC. *Proc Natl Acad Sci U S A* (2001) 98: 4646–4651.
 52. Sankar N, Kadeppagari RK and Thimmappaya B: c-Myc-induced aberrant DNA synthesis and activation of DNA damage response in p300 knockdown cells. *J Biol Chem* (2009) 284: 15193–15205.
 53. Lagos D, Pollara G, Henderson S, Gratrix F, Fabani M, Milne RS, Gotch F and Boshoff C: miR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. *Nat Cell Biol* (2010) 12: 513–519.
 54. Chiang K, Liu H and Rice AP: miR-132 enhances HIV-1 replication. *Virology* (2013) 438: 1–4.
 55. Lee IH and Finkel T: Regulation of autophagy by the p300 acetyltransferase. *J Biol Chem* (2009) 284: 6322–6328.
 56. Tsuneoka M, Umata T, Kimura H, Koda Y, Nakajima M, Kosai K, Takahashi T, Takahashi Y and Yamamoto A: c-myc induces autophagy in rat 3Y1 fibroblast cells. *Cell Struct Funct* (2003) 28: 195–204.
 57. Lu S and Cullen BR: Adenovirus VA1 noncoding RNA can inhibit small interfering RNA and MicroRNA biogenesis. *J Virol* (2004) 78: 12868–12876.

58. Andersson MG, Haasnoot PC, Xu N, Berenjian S, Berkhout B and Akusjarvi G: Suppression of RNA interference by adenovirus virus-associated RNA. *J Virol* (2005) 79: 9556-9565.
59. Xu N, Segerman B, Zhou X and Akusjarvi G: Adenovirus virus-associated RNAI-derived small RNAs are efficiently incorporated into the rna-induced silencing complex and associate with polyribosomes. *J Virol* (2007) 81: 10540-10549.
60. Sollerbrant K, Akusjarvi G and Svensson C: Repression of RNA polymerase III transcription by adenovirus E1A. *J Virol* (1993) 67: 4195-4204.
61. Lize M, Pilarski S and Dobbelstein M: E2F1-inducible microRNA 449a/b suppresses cell proliferation and promotes apoptosis. *Cell Death Differ* (2010) 17: 452-458.
62. Woods K, Thomson JM and Hammond SM: Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors. *J Biol Chem* (2007) 282: 2130-2134.
63. Emmrich S and Putzer BM: Checks and balances: E2F-microRNA crosstalk in cancer control. *Cell Cycle* (2010) 9: 2555-2567.
64. Weihua Z, Tsan R, Huang WC, Wu Q, Chiu CH, Fidler IJ and Hung MC: Survival of cancer cells is maintained by EGFR independent of its kinase activity. *Cancer Cell* (2008) 13: 385-393.
65. Wu YT, Tan HL, Huang Q, Ong CN and Shen HM: Activation of the PI3K-Akt-mTOR signaling pathway promotes necrotic cell death via suppression of autophagy. *Autophagy* (2009) 5: 824-834.
66. Gorospe M, Cirielli C, Wang X, Seth P, Capogrossi MC and Holbrook NJ: p21(Waf1/Cip1) protects against p53-mediated apoptosis of human melanoma cells. *Oncogene* (1997) 14: 929-935.
67. Fujiwara K, Daido S, Yamamoto A, Kobayashi R, Yokoyama T, Aoki H, Iwado E, Shinjima N, Kondo Y and Kondo S: Pivotal role of the cyclin-dependent kinase inhibitor p21WAF1/CIP1 in apoptosis and autophagy. *J Biol Chem* (2008) 283: 388-397.
68. Yamasaki Y, Tazawa H, Hashimoto Y, Kojima T, Kuroda S, Yano S, Yoshida R, Uno F, Mizuguchi H, Ohtsuru A, Urata Y, Kagawa S and Fujiwara T: A novel apoptotic mechanism of genetically engineered adenovirus-mediated tumour-specific p53 overexpression through E1A-dependent p21 and MDM2 suppression. *Eur J Cancer* (2012) 48: 2282-2291.
69. Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, Iliopoulos D, Pilozi E, Liu CG, Negrini M, Cavazzini L, Volinia S, Alder H, Ruco LP, Baldassarre G, Croce CM and Vecchione A: E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer Cell* (2008) 13: 272-286.
70. Wirth T, Kuhnel F, Fleischmann-Mundt B, Woller N, Djojotubro M, Rudolph KL, Manns M, Zender L and Kubicka S: Telomerase-dependent virotherapy overcomes resistance of hepatocellular carcinomas against chemotherapy and tumor necrosis factor-related apoptosis-inducing ligand by elimination of Mcl-1. *Cancer Res* (2005) 65: 7393-7402.
71. You L, Wang Y, Jin Y and Qian W: Downregulation of Mcl-1 synergizes the apoptotic response to combined treatment with cisplatin and a novel fiber chimeric oncolytic adenovirus. *Oncol Rep* (2012) 27: 971-978.
72. Ofir M, Hacoen D and Ginsberg D: MiR-15 and miR-16 are direct transcriptional targets of E2F1 that limit E2F-induced proliferation by targeting cyclin E. *Mol Cancer Res* (2011) 9: 440-447.
73. Calin GA, Cimmino A, Fabbri M, Ferracin M, Wojcik SE, Shimizu M, Taccioli C, Zanesi N, Garzon R, Aqeilan RI, Alder H, Volinia S, Rassenti L, Liu X, Liu CG, Kipps TJ, Negrini M and Croce CM: MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc Natl Acad Sci U S A* (2008) 105: 5166-5171.

