

Table 1. miRNAs commonly deregulated in human gastrointestinal cancers.

Function of miRNA	miRNA	Expression level in GI tumors	Transcriptional regulators	Promoter methylation	Chromosomal region	Chromosomal alteration	Target genes	Ref.
Tumor-suppressive miRNA	miR-34a	Downregulation	p53	+	Ch1p36	Loss	E2F3, BCL2, CDK6, SIRT1	[12,26,34,37,39-41]
	miR-34b/34c	Downregulation	p53	+	Ch11q23	Unknown	E2F3, CDK6	[35,38,42]
	miR-143/145	Downregulation	p53	Unknown	Ch5q33	Unknown	KRAS, DNMT3A, IRS1, MUC1, STAT1	[43-55]
Oncogenic miRNA	miR-21	Upregulation	IL-6, STAT3	-	Ch17q23	Unknown	PDCD4, CDC25A	[56-66]
	miR-17-92 cluster	Upregulation	c-Myc, E2F3, p53	-	Ch13q31	Amplification	CDKN1A(p21), PTEN, BIM	[67-74]

c-Myc [49], insulin receptor substrate (*IRS*)-1 [52,53], mucin 1 (*MUC1*) [54] and signal transducer and activator of transcription1 (*STAT1*) [55] genes in human colon cancer cells (Figure 3). Subsequently, overexpression of *miR-143/145* induces the suppression of cell proliferation, cell invasion and tumor growth [50-55].

3.2 Oncogenic miRNAs

3.2.1 miR-21

miR-21 is located on human chromosome 17q23. Upregulation of *miR-21* is frequently observed in a variety of human cancers [3]. *miR-21* is overexpressed in human gastric cancers [56-60] and this expression was significantly associated with overall survival and with relapse-free survival of gastric cancer patients [59]. Furthermore, *miR-21* expression was significantly higher in *Helicobacter pylori*-infected gastric mucosa [58]. Human colon cancers also showed *miR-21* overexpression, which was associated with poor survival of colon cancer patients [61]. High *miR-21* expression has been associated with the expression of inflammatory cytokines [62]. Regarding the transcriptional regulation of *miR-21* (Figure 4), *miR-21* is upregulated by IL-6-dependent induction of STAT3 in human colon cancer cells [63]. In contrast, STAT3 activation is suppressed by tumor suppressor p53 in human breast cancer cells [64]. These results indicate that inflammatory stimuli and loss of p53 function induce *miR-21* overexpression, thereby contributing to the progression of gastrointestinal tumors. Regarding the molecular mechanism of *miR-21* oncogenic function (Figure 4), *miR-21* overexpression promotes cell transformation through suppression of the programmed cell death 4 (*PDCD4*) gene [65], whose expression was inversely correlated with *miR-21* expression in human gastric cancers [60]. Furthermore, *miR-21* suppresses *CDC25A* expression and subsequently induces cell cycle progression in human colon cancer cells [66].

3.2.2 The miR-17-92 cluster

Overexpression of the *miR-17-92* cluster has been reported in colon cancers [67,68]. Although the human chromosome 13q31, on which the *miR-17-92* cluster is located, is amplified in gastrointestinal tumors [69,70], the relationship between *miR-17-92* overexpression and chromosomal alterations in gastrointestinal tumors remains unclear. The *miR-17-92* cluster is overexpressed in human embryonic colon tissues as well as in colon cancers [68], suggesting that the *miR-17-92* cluster is involved in cell proliferation of the normal colon and in tumor development. The *miR-17-92* cluster is upregulated by oncogenic c-Myc [71] and by E2F3 [72]. In contrast, the tumor suppressor p53 represses expression of the *miR-17-92* cluster [73]. Thus, the balance between the expression of oncogenic and tumor-suppressive genes in human colon epithelium may induce *miR-17-92* cluster overexpression, thereby contributing to colon carcinogenesis. Regarding the oncogenic

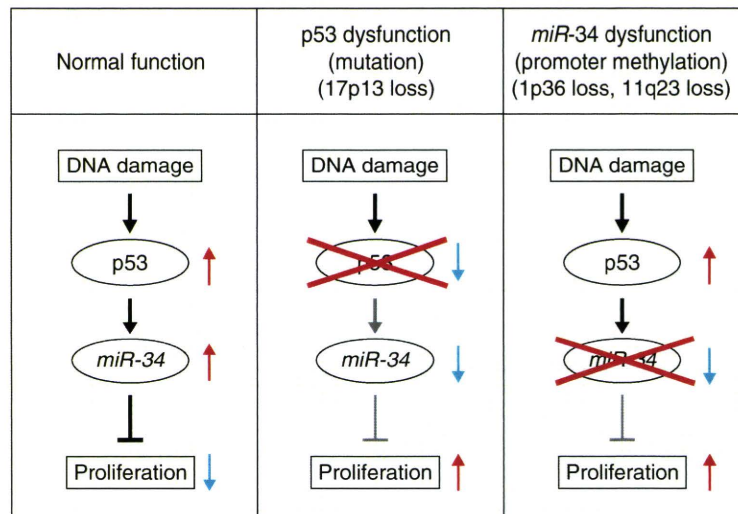


Figure 2. Possible involvement of *miR-34* regulation by p53 or of *miR-34* dysfunction in aberrant cell proliferation of human cancer cells. Left panel: DNA damage induces p53 activation, leading to *miR-34* upregulation and suppression of cell proliferation in human cancer cells with normal p53 and *miR-34* function. Middle panel: p53 dysfunction caused by mutation or by loss of chromosome 17p13 contributes to *miR-34* downregulation and aberrant cell proliferation in human cancer cells after DNA damage. Right panel: *miR-34* dysfunction caused by promoter methylation or by loss of chromosome 1p36 or 11q23 contributes to aberrant cell proliferation in human cancer cells, even if DNA damage induces p53 activation.

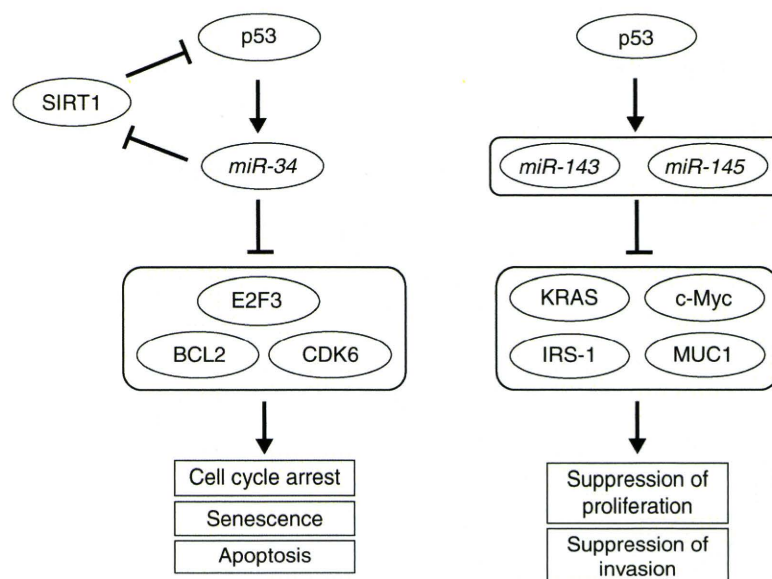


Figure 3. Tumor-suppressive roles of *miR-34* and *miR-143/145* in gastrointestinal cancer cells. p53-induced *miR-34* overexpression downregulates target genes (*E2F3*, *BCL2* and *CDK6*), leading to induction of cell cycle arrest, senescence and apoptosis in human cancer cells. Furthermore, *miR-34*-mediated suppression of SIRT1 expression can induce p53 activation, thereby contributing to a positive-feedback loop that results in strong induction of *miR-34* expression. In contrast, p53-induced *miR-143/145* overexpression downregulates target genes (*KRAS*, *c-Myc*, *IRS-1* and *MUC1*), leading to suppression of cell proliferation and invasion in human cancer cells.

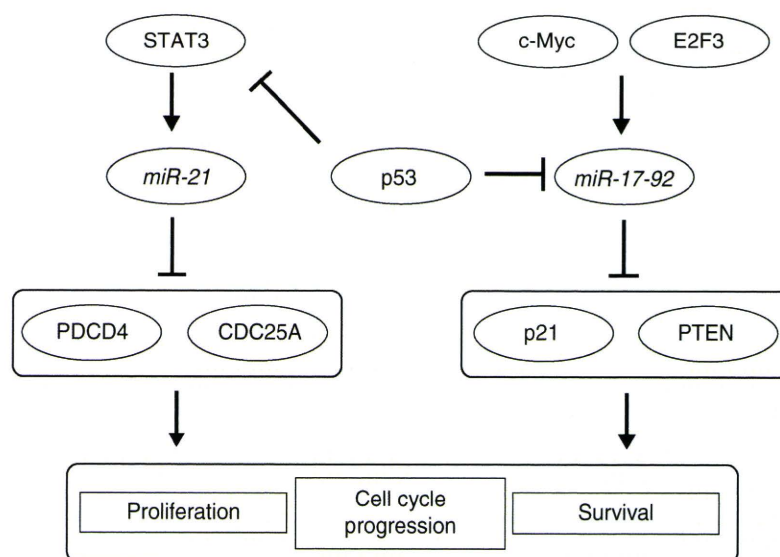


Figure 4. Oncogenic roles of *miR-21* and *miR-17-92* cluster in gastrointestinal cancer cells. STAT3-induced *miR-21* overexpression downregulates target genes (*PDCD4* and *CDC25A*), leading to proliferation, cell cycle progression and survival in human cancer cells. In contrast, c-Myc or E2F3 induction of the *miR-17-92* cluster downregulates target genes (*p21* and *PTEN*), leading to cell proliferation, cell cycle progression and survival in human cancer cells. p53 can suppress expression of *miR-21* and the *miR-17-92* cluster.

mechanism of the *miR-17-92* cluster (Figure 4), the *miR-17-92* cluster suppresses many target genes including *CDKN1A* (*p21*), phosphatase and tensin homologue (*PTEN*) and bcl-2 interacting mediator of cell death (*BIM*), thereby enhancing cell proliferation, cell cycle progression and cell survival [74].

4. miRNAs as novel biomarkers in gastrointestinal tumors

It has recently been predicted that it may be possible to detect aberrant miRNA expression in plasma, which could function as a novel biomarker for the early detection of various human cancers [75]. Indeed, the expression levels of oncogenic *miR-21* and the *miR-17-92* cluster in plasma were significantly higher in patients with gastrointestinal cancers compared with healthy controls [76-78]. Furthermore, overexpression of *miR-21* was detected in fecal miRNAs from patients with colorectal tumors including adenoma and adenocarcinoma [79]. These reports suggest that detection of oncogenic miRNAs that are highly expressed in the blood and stools of patients with gastrointestinal tumors is a promising screening system for early diagnosis of these tumors. Furthermore, the expression levels of *miR-17-3p* and *miR-92* among the *miR-17-92* cluster was significantly reduced after surgery in plasma of patients with colorectal cancers [78]. Thus, re-overexpression of oncogenic miRNAs in blood and stool may be also useful biomarker for the early detection of tumor recurrence after surgical resection in gastrointestinal cancer patients.

5. Potential role of miRNAs in cancer gene therapy for gastrointestinal tumors

5.1 Upregulation of tumor-suppressive *miR-34* expression

5.1.1 Conventional therapy

Conventional anticancer therapy, such as chemotherapy and radiation, induces *miR-34* expression in human cancer cells that have normal p53 and *miR-34* function (Figure 5) [12,24-27]. However, since more than 50% of human gastrointestinal tumors lack normal p53 function [28-33] and are therefore deficient in p53-induced *miR-34* expression, novel anticancer therapy that can induce *miR-34* expression in these tumors needs to be developed.

5.1.2 p53-expressing adenovirus

One possible method that might be effective for induction of *miR-34* in human cancer cells that have no functional p53 expression due to mutation or to chromosome 17p13 loss (Figure 2) is to infect the tumor cells with a p53-expressing adenovirus vector (Ad-p53) (Figure 5). Previous studies have shown that adenovirus-mediated overexpression of the p53 gene suppresses cell proliferation and tumor growth through induction of apoptotic cell death in human gastric cancer cells [80,81]. We previously reported that adenovirus-mediated wild-type p53 transfer efficiently suppressed cell proliferation, tumor growth and angiogenesis in human colon cancer cells [82,83]. Furthermore, induction of p53 overexpression by the Ad-p53 vector, in combination with aspirin treatment, enhances apoptotic cell

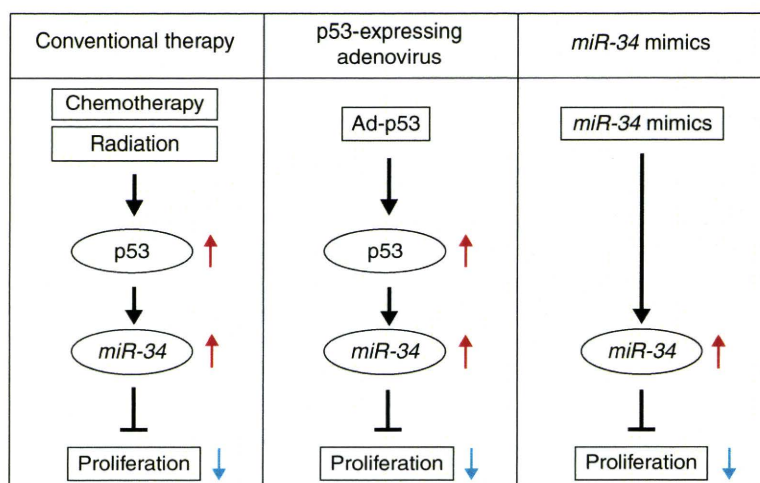


Figure 5. Suppression of aberrant cell proliferation through *miR-34* upregulation by conventional therapy, by a p53-expressing adenovirus or by *miR-34* mimics in human cancer cells. Left panel: conventional therapy, such as chemotherapy and radiation, upregulates *miR-34* expression through p53 activation in human cancer cells that have normal p53 and *miR-34* function. Middle panel: a p53-expressing adenovirus results in exogenous p53 expression, leading to *miR-34* expression, in human cancer cells that lack normal p53 function. Right panel: *miR-34* mimics result in *miR-34* expression in human cancer cells that lack normal *miR-34* function.

death through inhibition of NF- κ B expression in human colon cancer cells [84]. These findings suggest that adenovirus-mediated p53 overexpression is a promising anti-tumor therapy for gastrointestinal tumors. Adenovirus-mediated p53 overexpression may induce *miR-34* expression, thereby contributing to the suppression of tumor growth in gastrointestinal tumors.

Several Phase I clinical trials have shown that treatment with recombinant, replication-deficient Ad-p53 was well tolerated in patients with NSCLC [85-87]. However, the low transduction rate of p53 gene transfer by replication-deficient Ad-p53 is major problem that needs to be overcome in order to improve the clinical outcome in patients with advanced cancers. We recently reported that combination therapy of Ad-p53 with a replication-competent oncolytic adenovirus enhances and sustains the expression level of p53, leading to enhanced apoptotic cell death of human cancer cells [88]. Furthermore, a conditionally replication-competent p53-expressing adenovirus also enhances and sustains p53 gene expression [89], which probably leads to strong *miR-34* expression in human cancer cells.

5.1.3 *miR-34* mimics

In the case of human cancer cells that have no functional *miR-34* expression due to promoter methylation and/or loss of chromosome 1p36 or 11q23 (Figure 2), direct *miR-34* upregulation by *miR-34* mimics should be attempted (Figure 5). We previously reported that ectopic expression of *miR-34a* suppressed cell viability and induced subsequent senescence-like growth arrest in human colon cancer cells that

expressed either wild-type or mutated *p53* genes [12]. Furthermore, *miR-34a* overexpression was recently reported to suppress tumor sphere formation of *p53*-mutated human gastric cancer cells [90]. Since tumor sphere formation is one of the characteristics of cancer stem cells [91], restoration of *miR-34a* expression may be a promising antitumor therapy against cancer stem cells in gastrointestinal tumors. Indeed, an antitumor effect of *miR-34a* overexpression has been recently shown in human cancer stem cells in the pancreas [92] and the brain [93]. Exploration of the antitumor effect of *miR-34a* mimics against cancer stem cells in gastrointestinal tumors is warranted.

5.2 Downregulation of oncogenic *miR-21* expression

Since a variety of human cancer cells including gastrointestinal tumors have been shown to overexpress *miR-21* [53-59], development of a cancer gene therapy that would suppress oncogenic *miR-21* overexpression would be a promising antitumor therapy against many human cancers. Several strategies, such as anti-inflammatory drugs, antisense oligonucleotides and miRNA sponges, have been suggested to efficiently suppress oncogenic miRNA expression in human cancer cells.

5.2.1 Anti-inflammatory drugs

The anti-inflammatory drug, Curcumin, has been recently shown to downregulate *miR-21* expression in human pancreatic cancer cells [94]. Since Curcumin can inhibit IL-6-mediated STAT3 activation [95], which induces *miR-21* expression in human colon cancer cells [63], Curcumin treatment may downregulate *miR-21* expression in human colon cancers.

5.2.2 Antisense oligonucleotides

Antisense oligonucleotides have been frequently used in *in vitro* experiments to directly suppress the expression of oncogenic miRNAs. A *miR-21* antisense oligonucleotide has been shown to suppress *miR-21* expression in human gastric cancer cells, resulting in suppression of cell proliferation and induction of apoptotic cell death [58]. In *in vivo* settings, a *miR-21* antisense oligonucleotide efficiently suppresses the tumor growth of human breast cancer cells [9] and human glioma cells [96]. These results suggest that the use of *miR-21* antisense oligonucleotides is a promising antitumor therapy against gastrointestinal tumors.

5.2.3 miRNA sponges

Overexpression of a miRNA sponge, which contains multiple binding sites for a specific miRNA, has been shown to down-regulate the inhibitory effect of endogenous miRNA against many target genes [97]. Recently, it has been shown that a miRNA sponge for *miR-10b*, whose expression is significantly associated with breast cancer metastasis, can suppress *miR-10b* expression as efficiently as an antisense oligonucleotide and contributes to the suppression of lung metastasis in an orthotopic breast tumor animal model [98]. Thus, miRNA sponges may also be a promising antitumor therapy for the suppression of oncogenic *miR-21* overexpression in human gastrointestinal tumors.

5.3 Delivery of miRNA-based cancer gene therapy in gastrointestinal tumors

Establishment of delivery systems to induce efficient antitumor effect without normal tissue damage is an important issue for the miRNA-based cancer gene therapy. If gastrointestinal cancers are observed under gastroendoscopy or colonoscopy, the intratumoral injection of miRNA mimics, adenoviral vectors, antisense oligonucleotide and miRNA sponges can be performed. However, if the gastrointestinal tumors are with distal organ metastasis, systemic delivery of miRNA-based cancer gene therapy should be considered.

6. Conclusions

Diverse genetic alterations have been shown by many cancer researchers to play a role in the pathogenesis of gastrointestinal tumors and a 'multi-step colon carcinogenesis theory' has been established by Vogelstein's group [99] since the 1990s. However, since non-coding miRNAs have been shown to be deregulated in a variety of human cancers including gastrointestinal tumors [2,3], in order to understand the pathogenesis of gastrointestinal tumors it will be necessary to determine the molecular mechanism of the interaction

between protein-coding genes and non-coding miRNA genes [100]. Thus, an understanding at the molecular level of miRNA-related cancer progression would provide a novel platform for the development of miRNA-based tumor diagnosis and cancer gene therapy for the treatment of patients with gastrointestinal tumors.

7. Expert opinion

Recent advances in molecular biology have revealed the aberrant expression of many miRNAs in a variety of human cancers including gastrointestinal tumors, suggesting a potential role of miRNAs in tumor initiation, progression and metastasis. Indeed a number of reports have indicated that miRNAs can regulate diverse cell fates in human normal and cancer cells. The miRNAs *miR-192/194/215* and *miR-7* have recently been shown to play functional roles during differentiation of human intestinal epithelial cells. In contrast, human gastrointestinal tumors show downregulation of tumor suppressive miRNAs (*miR-34* and *miR-143/145*) and upregulation of oncogenic miRNAs (*miR-21* and *miR-17-92*). Interestingly, the tumor suppressor p53 can induce both differentiation-related and tumor-suppressive miRNAs, whereas it can further suppress oncogenic miRNAs in gastrointestinal epithelium and tumors. These data suggest that restoration of p53 expression is a promising cancer gene therapy against gastrointestinal tumors. However, the low transduction rate of p53 gene transfer by a replication-deficient Ad-p53 is a major problem that needs to be overcome in order to improve the clinical outcome in patients with advanced cancers. In human cancers with *miR-34* dysfunction, restoration of *miR-34* rather than of p53 may be effective for induction of *miR-34* expression. Furthermore, suppression of oncogenic miRNA expression in combination with *miR-34* restoration may be a more effective therapy than restoration of p53. Thus, understanding of the molecular mechanism of miRNA-related cancer progression would provide a novel platform for the development of miRNA-based cancer gene therapy for the treatment of patients with gastrointestinal tumors. Furthermore, the development of an early detection system for oncogenic miRNAs that are highly expressed in blood and stool would improve the clinical outcome of patients with gastrointestinal tumors.

Declaration of interest

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

Bibliography

- ▶ 1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116:281-97
- ▶ 2. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature* 2005;435:834-8
- ▶ 3. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006;103:2257-61
- ▶ 4. Aguo J, Miao Y, Xiao B, et al. Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues. *J Gastroenterol Hepatol* 2009;24:652-7
- ▶ 5. Katada T, Ishiguro H, Kuwabata Y, et al. microRNA expression profile in undifferentiated gastric cancer. *Int J Oncol* 2009;34:537-42
- ▶ 6. Michael MZ, O'Connor SM, van Holst Pellekaan NG, et al. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 2003;1:882-91
- ▶ 7. Bandres E, Cubedo E, Agirre X, et al. Identification by real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. *Mol Cancer* 2006;5:29
8. Koturbash I, Zemp FJ, Pogribny I, Kovalchuk O. Small molecules with big effects: the role of the microRNAome in cancer and carcinogenesis. *Mut Res* 2010: published online 21 ma 2010, doi:10.1016/j.mrgentox.2010.05.006
- ▶ 9. Si ML, Zhu S, Wu H, et al. miR-21-mediated tumor growth. *Oncogene* 2007;26:2799-803
- ▶ 10. Gregory PA, Bert AG, Paterson EL, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 2008;10:593-601
- ▶ 11. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA* 2005;102:13944-9
- ▶ 12. Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. Tumor-suppressive miR-34 induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. *Proc Natl Acad Sci USA* 2007;104:15472-7
- ▶ 13. McLin VA, Henning SJ, Jamrich M. The role of the visceral mesoderm in the development of the gastrointestinal tract. *Gastroenterology* 2009;136:2074-91
- ▶ 14. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. *Development* 2005;132:4653-62
- ▶ 15. Hino K, Tsuchiya K, Fukao T, et al. Inducible expression of microRNA-194 is regulated by HNF-1alpha during intestinal epithelial cell differentiation. *RNA* 2008;14:1433-42
- ▶ 16. McKenna LB, Schug J, Vourekas A, et al. MicroRNAs control intestinal epithelial differentiation, architecture, and barrier function. *Gastroenterology* 2010;39:1654-64
- ▶ 17. Braun CJ, Zhang X, Savelyeva I, et al. P53-responsive microRNAs 192 and 215 are capable of inducing cell cycle arrest. *Cancer Res* 2008;68:10094-104
- ▶ 18. Geoges SA, Biery MC, Kim SY, et al. Coordinated regulation of cell cycle transcripts by p53-inducible microRNAs, miR-192 and miR-215. *Cancer Res* 2008;68:10105-12
- ▶ 19. Song B, Wang Y, Kudo K, et al. miR-192 regulates dihydrofolate reductase and cellular proliferation through the p53-microRNA circuit. *Clin Cancer Res* 2008;14:8080-6
- ▶ 20. Gartel AL, Serfas MS, Gartel M, et al. p21 (WAF1/CIP1) expression is induced in newly nondividing cells in diverse epithelia and during differentiation of the Caco-2 intestinal cell line. *Exp Cell Res* 1996;227:171-81
- ▶ 21. Nguyen HT, Dalmasso G, Yan Y, et al. MicroRNA-7 modulates CD98 expression during intestinal epithelial cell differentiation. *J Biol Chem* 2010;285:1479-89
- ▶ 22. Yan Y, Vasudevan S, Nguyen HT, Merlin D. Intestinal epithelial CD98: an oligomeric and multifunctional protein. *Biochim Biophys Acta* 2008;1780:1087-92
- ▶ 23. Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet* 2009;10:704-14
- ▶ 24. He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. *Nature* 2007;447:1130-4
- ▶ 25. Raver-Shapira N, Marciano E, Meiri E, et al. Transcriptional activation of miR-34 contributes to p53-mediated apoptosis. *Mol Cell* 2007;26:731-43
- ▶ 26. Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol Cell* 2007;26:745-52
- ▶ 27. Bommer GT, Gerin I, Feng Y, et al. P53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr Biol* 2007;17:1298-307
- ▶ 28. Tamura G, Kihana T, Nomura K, et al. Detection of frequent p53 gene mutations in primary gastric cancer by cell sorting and polymerase chain reaction single-strand conformation polymorphism analysis. *Cancer Res* 1991;51:3056-8
- ▶ 29. Renault B, van den Broek M, Fodde R, et al. Base transitions are the most frequent genetic changes at p53 in gastric cancer. *Cancer Res* 1993;53:2614-17
- ▶ 30. Kastrinakis WV, Ramchurren N, Rieger KM, et al. Increased incidence of p53 mutations is associated with hepatic metastasis in colorectal neoplastic progression. *Oncogene* 1995;11:647-52
- ▶ 31. Sano T, Tsujino T, Yoshida K, et al. Frequent loss of heterozygosity on chromosome 1q, 5q, and 17p in human gastric carcinomas. *Cancer Res* 1991;51:2926-31
- ▶ 32. Khine K, Smith DR, Goh HS. High frequency of allelic deletion on chromosome 17p in advanced colorectal cancer. *Cancer* 1994;73:28-35
- ▶ 33. Risio M, Casorzo L, Chiecchio L, et al. Deletions of 17p are associated with transition from early to advanced colorectal cancer. *Cancer Genet Cytogenet* 2003;147:44-9
- ▶ 34. Lodygin D, Tarasov V, Epanchintsev A, et al. Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. *Cell Cycle* 2008;7:2591-600
- ▶ 35. Toyota M, Suzuki H, Sasaki Y, et al. Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is

Role of microRNAs in gastrointestinal tumors

- associated with CpG island methylation in colorectal cancer. *Cancer Res* 2008;68:4123-32
- ▶36. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 2004;101:2999-3004
- ▶37. Ragnarsson G, Eiriksdottir G, Johannsdottir JT, et al. Loss of heterozygosity at chromosome 1p in different solid human tumours: association with survival. *Br J Cancer* 1999;79:1468-74
- ▶38. Tenesa A, Farrington SM, Prendergast JG, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus 11q23 and replicates risk loci at 8q24 and 18q21. *Nat Genet* 2008;40:631-7
- ▶39. Welch C, Chen Y, Stallings RL. MicroRNA-34 functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene* 2007;26:5017-22
40. Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. *Proc Natl Acad Sci USA* 2008;105:13421-6
- ▶41. Sun F, Fu H, Liu Q, et al. Downregulation of CCND1 and CDK6 by miR-34a induces cell cycle arrest. *FEBS Lett* 2008;582:1564-8
- ▶42. Hermeking H. The miR-34 family in cancer and apoptosis. *Cell Death Differ* 2010;17:193-9
- ▶43. Iio A, Nakagawa Y, Hirata I, et al. Identification of non-coding RNAs embracing microRNA-143/145 cluster. *Mol Cancer* 2010;9:136
- ▶44. Takagi T, Iio A, Nakagawa Y, et al. Decreased expression of microRNA-143 and -145 in human gastric cancers. *Oncology* 2009;77:12-21
- ▶45. Michael MZ, O'Connor SM, van Holst Pellekaan NG, et al. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 2003;1:882-91
- ▶46. Akao Y, Nakagawa Y, Naoe T. MicroRNA-143 and -145 in colon cancer. *DNA Cell Biol* 2007;26:311-20
- ▶47. Wang CJ, Zhou ZG, Wang L, et al. Clinicopathological significance of microRNA-31, -143 and -145 expression in colorectal cancer. *Dis Markers* 2009;26:27-34
- ▶48. Suzuki HI, Yamagata K, Sugimoto K, et al. Modulation of microRNA processing by p53. *Nature* 2009;460:529-33
- ▶49. Sachdeva M, Zhu S, Wu F, et al. p53 represses c-Myc through induction of the tumor suppressor miR-145. *Proc Natl Acad Sci USA* 2009;106:3207-12
- ▶50. Chen, X, Guo X, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. *Oncogene* 2009;28:1385-92
- ▶51. Ng EK, Tsang WP, Ng SS, et al. MicroRNA-143 targets DNA methyltransferases 3A in colorectal cancer. *Br J Cancer* 2009;101:699-706
- ▶52. Shi B, Sepp-Lorenzino L, Prisco M, et al. MicroRNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. *J Biol Chem* 2007;282:32582-90
- ▶53. La Rocca G, Badin M, Shi B, et al. Mechanism of growth inhibition by microRNA 145: the role of the IGF-I receptor signaling pathway. *J Cell Physiol* 2009;220:485-91
- ▶54. Sachdeva M, Mo YY. MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1. *Cancer Res* 2010;70:378-87
- ▶55. Gregersen LH, Jacobsen AB, Frankel LB, et al. MicroRNA-145 targets YES and STAT1 in colon cancer cells. *PLoS One* 2010;5:e8836
- ▶56. Chan SH, Wu CW, Li AF, et al. miR-21 microRNA expression in human gastric carcinomas and its clinical association. *Anticancer Res* 2008;28:907-11
- ▶57. Guo J, Miao Y, Xiao B, et al. Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues. *J Gastroenterol Hepatol* 2009;24:652-7
- ▶58. Zhang Z, Li Z, Gao C, et al. miR-21 plays a pivotal role in gastric cancer pathogenesis and progression. *Lab Invest* 2008;88:1358-66
- ▶59. Li X, Zhang Y, Zhang Y, et al. Survival prediction of gastric cancer by a seven-microRNA signature. *Gut* 2010;59:579-85
- ▶60. Motoyama K, Inoue H, Mimori K, et al. Clinicopathological and prognostic significance of PDCD4 and microRNA-21 in human gastric cancer. *Int J Oncol* 2010;36:1089-95
- ▶61. Schetter AJ, Leung SY, Sohn JJ, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA* 2008;299:425-36
- ▶62. Schetter AJ, Nguyen GH, Bowman ED, et al. Association of inflammation-related and microRNA gene expression with cancer-specific mortality of colon adenocarcinoma. *Clin Cancer Res* 2009;15:5878-87
- ▶63. Iliopoulos D, Jaeger SA, Hirsch HA, et al. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. *Mol Cell* 2010;39:493-506
- ▶64. Lin J, Jin X, Rothman K, et al. Modulation of signal transducer and activator of transcription 3 activities by p53 tumor suppressor in breast cancer cells. *Cancer Res* 2002;62:376-80
- ▶65. Lu Z, Liu M, Stribinskis V, et al. MicroRNA-21 promotes cell transformation by targeting the programmed cell death 4 gene. *Oncogene* 2008;27:4373-9
- ▶66. Wang P, Zou F, Zhang X, et al. microRNA-21 negatively regulates Cdc25A and cell cycle progression in colon cancer cells. *Cancer Res* 2009;69:8157-65
- ▶67. Lanza G, Ferracin M, Gafa R, et al. mRNA/microRNA gene expression profile in microsatellite unstable colorectal cancer. *Mol Cancer* 2007;6:54
- ▶68. Monzo M, Navarro A, Bandres E, et al. Overlapping expression of microRNAs in human embryonic colon and colorectal cancer. *Cell Res* 2008;18:823-33
- ▶69. Koizumi Y, Tanaka S, Mou R, et al. Changes in DNA copy number in primary gastric carcinomas by comparative genomic hybridization. *Clin Cancer Res* 1997;3:1067-76
- ▶70. Neklason DW, Tuohy TM, Stevens J, et al. Colorectal adenomas and cancer link to chromosome 13q22.1-13q31.3 in a large family with excess colorectal cancer. *J Med Genet* 2010;47:692-9
- ▶71. O'Donnell KA, Wentzel EA, Zeller KI, et al. c-Myc-regulated microRNAs

- modulate E2F1 expression. *Nature* 2005;435:839-43
- ▶72. Woods K, Thomson JM, Hammond SM. Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors. *J Biol Chem* 2007;282:2130-4
- ▶73. Yan HL, Xue G, Mei Q, et al. Repression of the miR-17-92 cluster by p53 has an important function in hypoxia-induced apoptosis. *EMBO J* 2009;28:2719-32
- ▶74. Olive V, Jiang I, He L. miR-17-92, a cluster of miRNAs in the midst of the cancer network. *Int J Biochem Cell Biol* 2010;42:1348-54
- ▶75. Cortez MA, Calin GA. MicroRNA identification in plasma and serum: a new tool to diagnosis and monitor diseases. *Exp Opin Biol Ther* 2009;9:703-11
- ▶76. Tsujiura M, Ichikawa D, Komatsu S, et al. Circulating microRNAs in plasma of patients with gastric cancers. *Br J Cancer* 2010;102:1174-9
- ▶77. Zhou H, Guo JM, Lou YR, et al. Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using microRNA as a marker. *J Mol Med* 2010;88:709-17
- ▶78. Ng EK, Chong WW, Jin H, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut* 2009;58:1375-81
- ▶79. Link A, Balaguer F, Shen Y, et al. Fecal microRNAs as novel biomarkers for colon cancer screening. *Cancer Epidemiol Biomarkers Prev* 2010;19:1766-74
- ▶80. Ohashi M, Kanai F, Ueno H, et al. Adenovirus mediated p53 tumour suppressor gene therapy for human gastric cancer cells in vitro and in vivo. *Gut* 1999;44:366-71
- ▶81. Tatebe S, Matsuura T, Endo K, et al. Adenovirus-mediated transfer of wild-type p53 gene results in apoptosis or growth arrest in human cultured gastric carcinoma cells. *Int J Oncol* 1999;15:229-35
- ▶82. Spitz FR, Nguyen D, Skibber JM, et al. In vivo adenovirus-mediated p53 tumor suppressor gene therapy for colorectal cancer. *Anticancer Res* 1996;16:3415-22
- ▶83. Bouver M, Ellis LM, Nishizaki M, et al. Adenovirus-mediated wild-type p53 gene transfer down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in human colon cancer. *Cancer Res* 1998;58:2288-92
- ▶84. Shao J, Fujiwara T, Kadowaki Y, et al. Overexpression of the wild-type p53 gene inhibits NF- κ B activity and synergizes with aspirin to induce apoptosis in human colon cancer cells. *Oncogene* 2000;19:726-36
- ▶85. Fujiwara T, Tanaka N, Kanazawa S, et al. Multicenter phase I study of repeated intratumoral delivery of adenoviral p53 in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2006;24:1689-99
- ▶86. Nemunaitis J, Swisher SG, Timmons T, et al. Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non-small-cell lung cancer. *J Clin Oncol* 2000;18:609-22
- ▶87. Swisher SG, Roth JA, Nemunaitis J, et al. Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1999;91:763-71
- ▶88. Sakai R, Kagawa S, Yamasaki Y, et al. Preclinical evaluation of differentially targeting dual virotherapy for human solid cancer. *Mol Cancer Ther* 2010;9:1884-93
- ▶89. Van Beusechem VW, van den Doel PB, Grill J, et al. Conditionally replicative adenovirus expressing p53 exhibits enhanced oncolytic potency. *Cancer Res* 2002;62:6165-71
- ▶90. Ji Q, Hao X, Meng Y, et al. Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumorspheres. *BMC Cancer* 2008;8:266
- ▶91. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008;8:755-68
- ▶92. Ji Q, Hao X, Zhang M, et al. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. *PLoS One* 2009;4:e6816
- ▶93. Guessous F, Zhang Y, Kofman A, et al. microRNA-34a is tumor suppressive in brain tumors and glioma stem cells. *Cell Cycle* 2010;9:1031-6
- ▶94. Ali S, Ahmad A, Banerjee S, et al. Gemcitabine sensitivity can be induced in pancreatic cancer cells through modulation of miR-200 and miR-21 expression by curcumin or its analogue CDF. *Cancer Res* 2010;70:3606-17
- ▶95. Bharti AC, Donato N, Aggarwal BB. Curcumin (diferuloylmethane) inhibits constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells. *J Immunol* 2003;171:3863-71
- ▶96. Corsten MF, Miranda R, Kasmieh R, et al. MicroRNA-21 knockdown disrupts glioma growth in vivo and displays synergistic cytotoxicity with neural precursor cell delivered S-TRAIL in human gliomas. *Cancer Res* 2007;67:8994-9000
- ▶97. Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods* 2007;4:721-6
- ▶98. Ma L, Reinhardt F, Pan E, et al. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. *Nat Biotechnol* 2010;28:341-7
- ▶99. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759-67
- ▶100. Slaby O, Svoboda M, Michalek J, Vyzula R. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. *Mol Cancer* 2009;8:102

Affiliation

Hiroshi Tazawa^{1,2}, Shunsuke Kagawa² & Toshiyoshi Fujiwara^{†2}

[†]Author for correspondence

¹Okayama University Hospital, Center for Gene and Cell Therapy, Okayama, Japan

²Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Department of Gastroenterological Surgery, 2-5-1 Shikata-cho, Okayama 700-8558, Japan

Tel: +81 86 235 7997;

Fax: +81 86 235 7884;

E-mail: toshi_f@md.okayama-u.ac.jp

For reprint orders, please contact reprints@expert-reviews.com

EXPERT
REVIEWS

Telomerase-specific oncolytic virotherapy for human gastrointestinal cancer

Expert Rev. Anticancer Ther. 11(4), 525–532 (2011)

Toshiyoshi Fujiwara¹,
Yasuhiro Shirakawa¹
and Shunsuke Kagawa¹

¹Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan
^{*}Author for correspondence:
Tel.: +81 862 357 257
Fax: +81 862 218 775
toshi_f@md.okayama-u.ac.jp

Replication-selective tumor-specific viruses present a novel approach for treatment of neoplastic disease. These vectors are designed to induce virus-mediated lysis of tumor cells after selective viral propagation within the tumor. Human telomerase is highly active in more than 85% of primary cancers, regardless of their tissue origins, and its activity correlates closely with human telomerase reverse transcriptase (hTERT) expression. We constructed an attenuated adenovirus 5 vector (OBP-301), in which the hTERT promoter element drives the expression of *E1* genes. Since only tumor cells that express telomerase activity are able to activate this promoter, the hTERT proximal promoter allows for preferential expression of viral genes in tumor cells, leading to selective viral replication and oncolytic cell death. Lymphatic invasion is a major route for cancer cell dissemination, and adequate treatment of locoregional lymph nodes is required for curative treatment in patients with gastrointestinal tumors. In this article we show that intratumoral injection of OBP-301 mediates effective *in vivo* purging of metastatic tumor cells from regional lymph nodes, which may help optimize treatment of human gastrointestinal malignancies.

KEYWORDS: adenovirus • colorectal cancer • lymph node • metastasis • telomerase

Viruses are the simplest form of life, carry genetic material and are capable of entering host cells efficiently. Because of these properties, many viruses have been adapted as gene-transfer vectors [1–3]. Adenoviruses have been studied extensively and are well-characterized. Adenoviruses are large dsDNA viruses with tropism for many human tissues such as bronchial epithelia, hepatocytes and neurons. Furthermore, they are capable of transducing nonreplicating cells and can be grown to high titers *in vitro*, which allows for their potential clinical use. High titers of replication-defective adenoviruses can be produced and have been successfully used in eukaryotic gene expression [1,4,5]. Numerous studies using *in vitro* and animal models have tested a wide variety of adenoviral gene-therapy agents and have reported potential beneficial effects for different target diseases, including tolerability and safety [6–9].

Oncolytic viruses that can selectively replicate in tumor cells and lyse infected cells have been extensively investigated as novel anticancer agents [3,10,11]. These vectors are designed to induce virus-mediated lysis of tumor cells after selective viral propagation within the tumor cell while remaining innocuous to normal

tissues [12]. Clinical trials of intratumoral injection of Onyx-015, which is an adenovirus with the *E1B* 55-kDa gene deleted and engineered to selectively replicate in and lyse p53-deficient cancer cells [13], alone or in combination with cisplatin/5-fluorouracil, have been conducted in patients with recurrent head and neck cancer [14,15]. However, subsequent studies have clarified that the capacity of Onyx-015 to replicate independently of the cell cycle does not correlate with the status of p53 [16], but is determined by late viral RNA export [17].

The optimal treatment of human cancer requires improvement of the therapeutic ratio to increase the cytotoxic efficacy on tumor cells and decrease that on normal cells. This may not be an easy task because the majority of normal cells surrounding tumors are sensitive to cytotoxic agents. Thus, to establish reliable therapeutic strategies for human cancer, it is important to seek genetic or epigenetic targets present only in cancer cells. One of the targeting strategies has involved the use of tissue-specific promoters to restrict gene expression or viral replication in specific tissues. A large number of different tissue-specific promoters have been used for virotherapy applications for targeting tumors derived

from various tissues; however, tumor-specific, rather than tissue-specific, promoters would be more advantageous. For example, the promoter of human telomerase reverse transcriptase (hTERT) is highly active in most tumor cells but inactive in normal somatic cell types.

This article highlights some very promising advances in cancer therapeutic technologies using the hTERT promoter against human gastrointestinal cancer, especially for regional lymph node metastasis.

Lymph node metastasis in human gastrointestinal cancer

Lymph node status provides important information for both the diagnosis and treatment of human gastrointestinal cancer. Lymphatic invasion is a major route for cancer cell dissemination, and lymph node metastases represent an aggressive tumor behavior and are associated with a high rate of regional recurrence, which portends a poor outcome and may produce marked morbidity [18–20]. Therefore, adequate resection of the locoregional lymph nodes is required for curative treatment in patients with gastrointestinal malignancies such as esophageal, gastric and colorectal cancers [21,22]. Extended lymphadenectomy, however, may greatly impair quality of life, especially for patients with early-stage epithelial neoplasms of the GI tract [23]. These primary tumors can be removed by new endoluminal therapeutic techniques such as endoscopic submucosal dissection; however, patients with submucosal invasion, lymphovascular infiltration of cancer cells, or undifferentiated histology often become candidates for surgical organ resection with lymphadenectomy because there is a risk of regional lymph node metastasis, although the frequency is relatively low [24]. For example, resection of upper gastrointestinal organs such as gastrectomy and esophagectomy may result in weight loss and microgastric. Thus, a less invasive procedure to selectively treat lymph node metastasis would benefit these patients by allowing them to avoid prophylactic surgery.

Telomerase activity for transcriptional cancer targeting

One of the hallmarks of cancer is unregulated proliferation of a certain cell population, which eventually affects normal cellular function in the human body, and this almost universally correlates with the reactivation of telomerase. Tumor cells can maintain telomere length predominantly due to telomerase, and its activity is detected in approximately 85% of malignant tumors [25], whereas telomerase is absent in most normal somatic tissues [26], with a few exceptions, including peripheral blood leukocytes and certain stem cell populations [27,28]. The strong association between telomerase activity and malignant tissue suggests that telomerase can be a plausible target for the diagnosis and treatment of cancer [29].

The enzyme telomerase is a ribonucleoprotein complex responsible for the addition of TTAGGG repeats to the telomeric ends of chromosomes and contains three components: a RNA subunit (known as hTR, hTER or hTERC) [30], telomerase-associated protein (hTEP)1 [31], and a catalytic subunit (hTERT) [32,33]. Both hTR and hTERT are required for the reconstitution of telomerase activity *in vitro* [34] and, therefore, represent the minimal

catalytic core of telomerase in humans [35]. Both hTR and hTERT transcripts are easily detectable in cancer cells but are either absent or exist in low levels in normal cells [36]. However, the hTR promoter is always stronger than hTERT, with presumably more background [37]. Thus, the hTERT promoter region can be substantially used as a fine-tuning molecular switch that works exclusively in tumor cells.

hTERT promoter-driven telomerase-specific oncolytic adenovirus

The use of modified adenoviruses that replicate and complete their lytic cycle preferentially in cancer cells is a promising strategy for the treatment of cancer. One approach to achieve tumor specificity of viral replication is based on the transcriptional control of genes that are critical for virus replication, such as *E1A* or *E4*. As described earlier, telomerase, especially its catalytic subunit hTERT, is expressed in the majority of human cancers and the hTERT promoter is preferentially activated in human cancer cells [25]. Thus, the broadly applicable hTERT promoter might be a suitable regulator of adenoviral replication. Indeed, it has been reported previously that transcriptional control of *E1A* expression via the hTERT promoter could restrict adenoviral replication to telomerase-positive tumor cells and efficiently lyse tumor cells [38–43]. Furthermore, Kuppuswamy *et al.* have recently developed a novel oncolytic adenovirus (VRX-011), in which the replication of the vector targets cancer cells by replacing the adenovirus *E4* promoter with the hTERT promoter [44]. VRX-011 is also able to overexpress the adenovirus death protein (also known as E3–11.6K), which is required for efficient cell lysis and the release of virions from cells at late stages of infection.

The adenovirus *E1B* gene is expressed early in viral infection and its gene product inhibits E1A-induced p53-dependent apoptosis, which in turn promotes the cytoplasmic accumulation of late viral mRNA, leading to a shutdown of host cell protein synthesis. In most vectors that replicate under the transcriptional control of the *E1A* gene including hTERT-specific oncolytic adenoviruses, the *E1B* gene, is driven by the endogenous adenovirus E1B promoter. However, Li *et al.* have demonstrated that transcriptional control of both *E1A* and *E1B* genes by the α -fetoprotein promoter with the use of the internal ribosome entry site significantly improved the specificity and therapeutic index in hepatocellular carcinoma cells [45]. Based on the aforementioned information, we developed telomelysin (OBP-301), in which the tumor-specific hTERT promoter regulates both the *E1A* and *E1B* genes (FIGURE 1). Telomelysin is expected to control viral replication more stringently, thereby providing better therapeutic effects in tumor cells, as well as attenuated toxicity in normal tissues [46].

In vitro & *in vivo* anti-tumor efficacy of telomelysin in human gastrointestinal cancer

The majority of human cancer cells acquire immortality and unregulated proliferation by the expression of hTERT [25], and therefore it has been hypothesized that hTERT-specific telomelysin possesses a broad-spectrum antineoplastic activity against a variety of human tumors [46,47]. Telomelysin induced selective

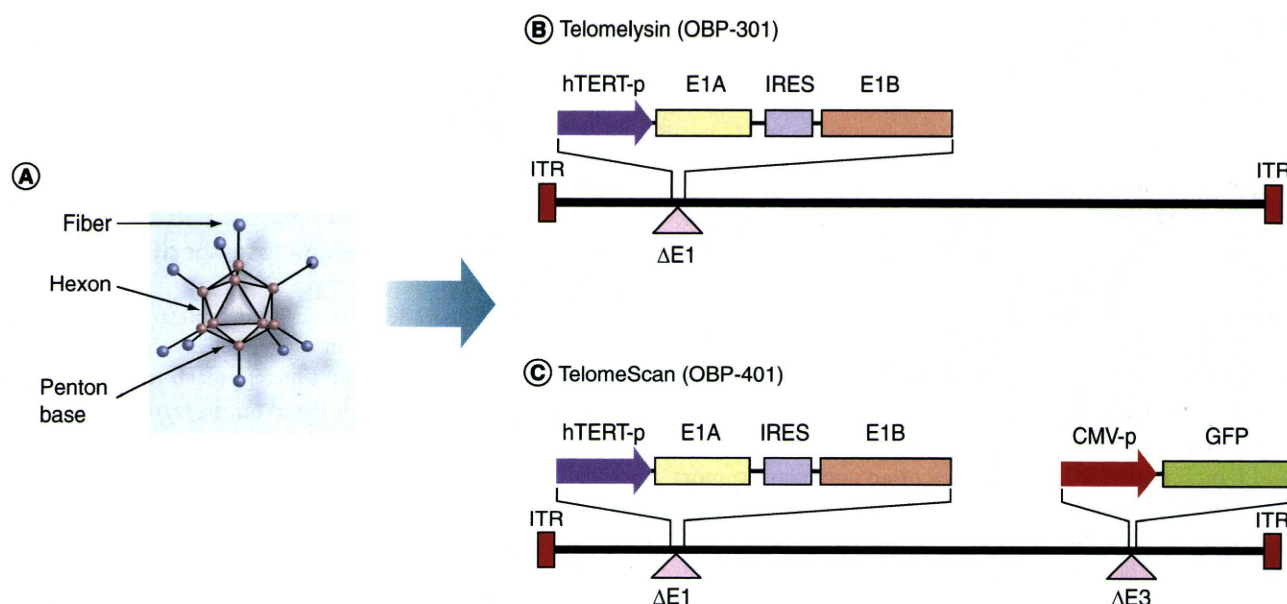


Figure 1. Structures of telomerase-specific oncolytic adenoviruses. (A) Schematic representation depicting the major structural components of the adenovirus (hexon, penton base and fiber). (B) Telomelysin (OBP-301), in which the promoter element drives the expression of *E1A* and *E1B* genes linked with an internal ribosome entry site. (C) TelomeScan (OBP-401) is a telomerase-specific replication-competent adenovirus variant, in which the *GFP* gene is inserted under a CMV-p into the E3 region for monitoring viral replication.

CMV-p: Cytomegalovirus promoter; GFP: Green fluorescent protein; hTERT-p: Human telomerase reverse transcriptase promoter; IRES: Internal ribosome entry site; ITR: Inverted terminal repeat.

E1A and *E1B* expression in cancer cells, which resulted in 5–6-log viral replication 3 days after infection; on the other hand, telomelysin replication was attenuated up to 2 logs in cultured normal cells [46,47].

In vitro cytotoxicity assays demonstrated that telomelysin could efficiently kill various types of human gastrointestinal cancer cell lines including esophageal cancer, gastric cancer and colorectal cancer in a dose-dependent manner [48]. These data clearly demonstrate that telomelysin exhibits desirable features for use as an oncolytic therapeutic agent, as the proportion of cancers potentially treatable by telomelysin is extremely high.

The *in vivo* anti-tumor effect of telomelysin was also investigated by using athymic mice carrying xenografts, because most murine tumor cells are known to express low levels of coxsackie and adenovirus receptor (CAR). Intratumoral injection of telomelysin into human colorectal tumor xenografts resulted in a significant inhibition of tumor growth and enhancement of survival [46,47]. Macroscopically, massive ulceration was noted on the tumor surface after injection of high-dose telomelysin, indicating that telomelysin induced intratumoral necrosis due to direct lysis of tumor cells by viral replication *in vivo* [49].

***In vivo* lymphatic spread of virus on regional lymph nodes**

The therapeutic potential of viral agents against primary tumors as well as their systemic biodistribution targeting distant metastases has been intensively investigated [3,10,50]. However, few studies

have examined the ability of the virus to traffic to the regional draining lymph nodes. Recently, Burton *et al.* showed that replication-deficient adenovirus could be successfully transported to the regional lymph nodes and noninvasively detect metastasis by expressing the prostate-specific reporter gene in an orthotopic prostate xenograft [51].

To verify that oncolytic adenoviruses traffic through the lymphatics to the regional lymph nodes, we used an orthotopic mouse model of human rectal cancer with spontaneous lymph node metastasis. We have demonstrated that intratumoral injection of the telomerase-specific, replication-selective, green fluorescent protein (GFP)-expressing adenovirus TelomeScan (OBP-401) (FIGURE 1) could efficiently visualize metastatic lymph nodes with GFP fluorescence signals in human cancer xenograft models [52,53]. These studies suggest the possible application of the adenovirus vectors as a lymphotropic agent for the treatment of lymph node metastasis.

***In vitro* purging of human colorectal cancer cells by telomelysin**

In vitro purging experiments demonstrated that telomelysin infection could selectively eliminate human tumor cells in the presence of human or mouse lymphocytes [54]. We used TelomeScan to visualize viable human tumor cells after purging with telomelysin, as we have previously shown the high sensitivity and specificity of this molecular-imaging method [52,53]. It has been reported that the fiber-modified adenovirus serotype 5 (Ad5) and the

adenovirus vector based on another serotype such as Ad35 is able to efficiently transduce exogenous genes into hematopoietic cells, including stem cells. The unmodified Ad5, however, can rarely infect these cells because of the lack of CAR expression [55]. Indeed, Ad5-based telomelysin had no apparent effects on the viability of lymphocytes *in vitro*. These results suggest that normal lymphocytes in the regional lymph nodes could be strictly protected from telomelysin-induced oncolysis, because lymphocytes are not permissive for telomelysin infection and viral replication is also unlikely to occur in normal cells due to their low telomerase activity [27].

In vivo anti-tumor effect of telomelysin on lymph node metastasis

Mice bearing orthotopic human colorectal tumors received three courses of telomelysin intratumoral injections every 2 days, starting 2 weeks after tumor inoculation. Histopathological examination of the excised total lymph nodes showed that telomelysin treatment considerably reduced the metastatic rates. We also used a simple real-time *Alu* PCR assay to quantify the few metastatic human tumor cells in a background of large numbers of mouse host cells [54]. This human-specific amplification method enabled us to detect human tumor cells in a linear range of 10^3 – 10^8 cells/sample and monitor the time-dependent exponential growth of spontaneous lymph node metastasis from orthotopic colorectal tumor xenografts. In accordance with the histologically confirmed results, the *Alu* PCR assay indicated that intratumoral injection of telomelysin into the primary tumors significantly inhibited lymph node metastasis with high levels of viral replication.

We also used TelomeScan and a 3D optical detection system (IVIS® 200). After 2 weeks of orthotopic implantation of human colorectal tumor cells, telomelysin was administered intratumorally for five cycles. We then used the IVIS 200 imaging system to explore the abdominal cavity at laparotomy following a single injection of TelomeScan into the tumors. The number of GFP-positive lymph nodes and the GFP signal levels of individual lymph nodes were much higher in mock-treated control mice than in telomelysin-treated mice. Indeed, the sum of GFP fluorescence intensity in the abdominal cavity was significantly lower in mice treated with telomelysin, confirming the *in vivo* biological purging effect of telomelysin. The fact that two independent and highly sensitive approaches showed comparable results suggests a potent *in vivo* purging effect of oncolytic virotherapy on regional lymph nodes.

For effective treatment of metastatic tumors, intravenously infused chemotherapeutic drugs must be distributed in sufficient concentrations into the tumor sites; oncolytic viruses, however, are still able to replicate in the tumor, cause oncolysis and then release virus particles that could reach the distant metastatic lesions. Moreover, intratumoral injection can avoid hepatotoxicity that may be induced by systemic adenoviral administration. Therefore, intratumoral administration that causes the release of newly formed virus from infected tumor cells is theoretically suitable for oncolytic virus rather than systemic administration.

Preoperative intratumoral administration of telomelysin against lymph node metastasis

Currently, surgery and radiation are the most effective and clinically reliable local management strategies for human malignancies, including lymphatic metastases. Indeed, ionizing radiation targeting the lower half of the mouse body, including primary tumors and the para-aortic lymphatic area, significantly inhibited lymph node metastasis, although systemic toxicity such as weight loss was remarkable in irradiated mice compared with mice treated with telomelysin. In fact, total-body irradiation at a dose of 10 Gy has been reported to be lethal in mice because of acute radiation syndromes involving the hematopoietic system and GI tract [56]. We demonstrated that regional injection of telomelysin might be more simple and safe than radiotherapy as a treatment for metastatic lymph nodes [54].

We also assessed the effect of surgical resection of primary rectal tumors on lymph node metastasis. Unexpectedly, metastatic tumor cells in the lymph nodes considerably increased after surgical removal of primary rectal tumors, presumably due to the spread of tumor cells into the lymphatic circulation during the surgical procedure. Another possible explanation of this phenomenon includes a decrease in angiogenic inhibitors such as angiostatin and endostatin secreted from the primary tumor mass [57]. By contrast, intratumoral injection of telomelysin prior to surgical resection significantly inhibited lymph node metastasis. Telomelysin causes viral spread into the regional lymphatic area and selectively replicates in neoplastic lesions, resulting in eradication of lymph node metastasis. Tumor cells infected with telomelysin in the primary tumors are also unable to metastasize to the regional lymph nodes. Therefore, although the surgical procedure itself has the potential to promote regional metastasis, the preoperative treatment with telomelysin may prevent this undesirable event.

Clinical application of telomelysin

Preclinical models suggest that telomelysin could selectively kill a variety of human cancer cells *in vitro* and *in vivo* via intracellular viral replication regulated by hTERT transcriptional activity. Pharmacological and toxicological studies in mice and cotton rats have demonstrated that none of the animals treated with telomelysin showed signs of viral distress (e.g., ruffled fur, weight loss, lethargy or agitation) or histopathological changes in any organs at autopsy. These promising data led us to design a Phase I clinical trial of telomelysin as a monotherapy.

The protocol "A Phase I injection study of intratumoral injection with telomerase-specific replication-competent oncolytic adenovirus, telomelysin (OBP-301) for various solid tumors", sponsored by Oncolys BioPharma, Inc., is an open-label, Phase I, three-cohort dose-escalation study [58,59]. The trial commenced following approval from the US FDA in October 2006. The study has been completed to assess the safety, tolerability and feasibility of intratumoral injection of the agent in patients with advanced solid cancer. The doses of telomelysin were escalated from low to high virus particles in one log increment. In total, 16 patients with a variety of solid tumors such as melanoma, head

and neck cancer, breast cancer, lung cancer, and sarcomas were treated with a single-dose intratumoral injection of telomelysin and then monitored over 1 month.

All patients received telomelysin without dose-limiting toxicity. Common grade 1 and 2 toxicities included injection-site reactions (pain, induration) and systemic reactions (fever, chills). The data of pharmacokinetics and biodistribution of telomelysin may be of interest. Clinical trials of intratumoral and intravenous administration of CG7870, a replication-selective oncolytic adenovirus genetically engineered to replicate preferentially in prostate tissue, demonstrated a second peak of the virus genome in the plasma [60,61], suggesting active viral replication and shedding into the bloodstream. In fact, circulating viral DNA was transiently (<6 h after injection) detected in plasma in 13 out of 16 patients within 24 h of injection. This dose-dependent initial peak in circulating virus was followed by a rapid decline; however, three patients demonstrated evidence of prolonged viral replication through the detection of plasma viral DNA at days 7 and 14, suggesting telomelysin replication in primary tumors. One of these three patients had disappearance of the injected malignant lesion and locoregional uninjected satellite nodules, fulfilling a definition of complete response at day 28. Seven patients fulfilled the Response Evaluation Criteria In Solid Tumors (RECIST) definition for stable disease 56 days after treatment, although six patients showed a 6.6–43% reduction in tumor size. Thus, telomelysin is well-tolerated and warrants further clinical studies for solid cancer.

Expert commentary

There have been very impressive advances in our understanding of the molecular aspects of human gastrointestinal cancer and in the development of technologies for the genetic modification of viral genomes. Transcriptional targeting is a powerful tool for tumor selectivity in cancer therapy, and the hTERT-specific oncolytic adenovirus achieves a more strict targeting potential due to the amplified effect of viral replication. Several independent studies that used different regions of the hTERT promoter and different sites of adenoviral genome responsible for viral replication have shown that the hTERT promoter allows adenoviral replication as a molecular switch and induces selective cytopathic effects in a variety of human tumor cells [38–40,46–48]. Among these viral constructs, to the best of our knowledge telomelysin seems to be the first hTERT-dependent oncolytic adenovirus that has been used in a clinical trial based on preclinical pharmacological and toxicological studies. Thus, telomerase-specific targeted oncolytic adenovirus holds promise for the treatment of human cancer.

Nevertheless, many ethical and technical hurdles remain to be tackled and must be solved before virotherapy ever reaches routine clinical application. Safety considerations in the manufacture of the virus and clinical protocols are among the most important issues to be studied. Another important issue is to find ways to improve virus cell binding and entry. Although telomelysin showed a broad and profound anti-tumor effect in human cancer originating from various organs, one weakness of telomelysin is that virus infection efficiency depends upon CAR expression, which may not be highly expressed on the cell surface of some

types of human cancer cells. Thus, tumors that have lost CAR expression may be refractory to infection with telomelysin. Since modification of fiber protein is an attractive strategy for overcoming the limitations imposed by CAR's dependence on telomelysin infection, we modified the telomelysin fiber to contain the Arg–Gly–Asp (RGD) peptide, which binds with high affinity to integrins ($\alpha v \beta 3$ and $\alpha v \beta 5$) on the cell surface, on the HI loop of the fiber protein. The resultant adenovirus, termed telomelysin–RGD or OBP-405, mediated not only CAR-dependent virus entry but also CAR-independent, RGD-integrin-dependent virus entry [47,62]. Telomelysin–RGD has an apparent oncolytic effect on human cancer cell lines with extremely low CAR expression. These data suggest that fiber-modified telomelysin–RGD exhibits a broad target range by increasing infection efficiency, although one needs to be cautious regarding increased toxicity since hematopoietic cell populations such as dendritic cells can be efficiently infected with RGD-modified adenovirus [63].

It has been shown that telomelysin delivered to the primary tumor site is able to spread into the regional draining lymphatics, selectively replicate in neoplastic foci, and then reduce the number of tumor cells in metastatic lymph nodes in an orthotopic human colorectal cancer xenograft model [54]. This virus-mediated molecular surgery for lymph node metastasis mimics the clinical scenario of lymphadenectomy; the technique, however, seems to be safer and less invasive. Moreover, we have demonstrated that preoperative delivery of telomelysin into primary tumors prevented the exacerbation of lymph node metastasis by surgical procedures. Telomelysin may offer advantages over other oncolytic viruses targeting lymphatic metastasis, as its safety profile as well as biodistribution pattern after intratumoral delivery have already been confirmed in a Phase I clinical trial for various types of solid tumors [58,59]. Our study provides evidence for the *in vivo* purging effect of telomelysin in regional lymph nodes that is sufficiently reliable to support this approach. Thus, Phase II studies of telomerase-specific virotherapy targeting lymph node metastasis in human cancer patients are warranted.

Five-year view

A possible future direction for telomelysin includes combination therapy with conventional therapies such as chemotherapy, radiotherapy, surgery, immunotherapy, and new modalities such as antiangiogenic therapy. Since the results of a Phase I clinical trial demonstrated that even partial elimination of the tumor induced by intratumoral injection of telomelysin could be clinically beneficial, the combination approaches may lead to the development of more advanced biological therapy for human cancer. The combination of systemic chemotherapy and local injection of telomelysin has previously been shown to be effective [64–66]. As a replication-deficient adenovirus could replicate in cancer cells and enhance the anticancer effect when cotransfected with telomelysin that could produce E1 protein, we demonstrated the synergistic effects of telomelysin combined with an E1-deleted replication-deficient adenoviral vector expressing human wild-type *p53* tumor-suppressor gene (Ad5CMV-p53; Advexin) [67,68]. Telomelysin is also synergistic with ionizing radiation against

human esophageal cancer cells, and we clarified the E1B 55 kDa-mediated mechanism used by telomelysin to inhibit DNA repair. Peri- or post-operative administration of telomelysin may be also valuable as adjuvant therapy in areas of microscopic residual disease at tumor margins to prevent recurrence or regrowth of tumors.

The field of targeted oncolytic virotherapy is progressing considerably and is rapidly gaining medical and scientific acceptance. Although many technical and conceptual problems remain to be solved, ongoing and future clinical studies will no doubt continue

to provide important clues that may allow substantial progress in human gastrointestinal cancer therapy.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Adenoviruses are capable of transducing nonreplicating cells and can be grown to high titers *in vitro*, which allows for their potential use clinically as gene-transfer vectors.
- Adequate resection of the locoregional lymph nodes is required for curative treatment in patients with gastrointestinal malignancies; however, a less invasive method to selectively treat lymph node metastasis would benefit patients by allowing them to avoid a prophylactic surgery.
- Our team has developed telomelysin (OBP-301), in which the tumor-specific hTERT promoter controls viral replication stringently, thereby providing better therapeutic effects in tumor cells, as well as attenuated toxicity in normal tissues.
- Telomelysin delivered to the primary tumor site is able to spread into the regional draining lymphatics, selectively replicate in neoplastic foci, and then reduce the number of tumor cells in metastatic lymph nodes.
- Preoperative delivery of telomelysin into primary tumors prevents the exacerbation of lymph node metastases by surgical procedures.
- Phase II studies of telomerase-specific virotherapy targeting lymph node metastasis in human cancer patients are warranted.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- ▶1 Kaplan JM. Adenovirus-based cancer gene therapy. *Curr. Gene Ther.* 5(6), 595–605 (2005).
- ▶2 Guo ZS, Thorne SH, Bartlett DL. Oncolytic virotherapy: molecular targets in tumor-selective replication and carrier cell-mediated delivery of oncolytic viruses. *Biochim. Biophys. Acta* 1785(2), 217–231 (2008).
- ▶3 Kirn DH, Thorne SH. Targeted and armed oncolytic poxviruses: a novel multi-mechanistic therapeutic class for cancer. *Nat. Rev. Cancer* 9(1), 64–71 (2009).
- ▶4 Mizuguchi H, Kay MA. Efficient construction of a recombinant adenovirus vector by an improved *in vitro* ligation method. *Hum. Gene Ther.* 9(17), 2577–2583 (1998).
- ▶5 Stone D, Lieber A. New serotypes of adenoviral vectors. *Curr. Opin. Mol. Ther.* 8(5), 423–431 (2006).
- ▶6 Wilson JM, Engelhardt JF, Grossman M *et al.* Gene therapy of cystic fibrosis lung disease using E1 deleted adenoviruses: a Phase I trial. *Hum. Gene Ther.* 5(4), 501–519 (1994).
- ▶7 Crystal RG, McElvaney NG, Rosenfeld MA *et al.* Administration of an adenovirus containing the human CFTR cDNA to the respiratory tract of individuals with cystic fibrosis. *Nat. Genet.* 8(1), 42–51 (1994).
- ▶8 Crystal RG, Hirschowitz E, Lieberman M *et al.* Phase I study of direct administration of a replication deficient adenovirus vector containing the *E. coli* cytosine deaminase gene to metastatic colon carcinoma of the liver in association with the oral administration of the pro-drug 5-fluorocytosine. *Hum. Gene Ther.* 8(8), 985–1001 (1997).
- ▶9 Serman DH, Treat J, Litzky LA *et al.* Adenovirus-mediated herpes simplex virus thymidine kinase/ganciclovir gene therapy in patients with localized malignancy: results of a Phase I clinical trial in malignant mesothelioma. *Hum. Gene Ther.* 9(7), 1083–1092 (1998).
- ▶10 Liu TC, Galanis E, Kirn D. Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. *Nat. Clin. Pract. Oncol.* 4(2), 101–117 (2007).
- ▶11 Fujiwara T. Telomerase-specific virotherapy for human squamous cell carcinoma. *Expert Opin. Biol. Ther.* 9(3), 321–329 (2009).
- ▶12 Hawkins LK, Lemoine NR, Kirn D. Oncolytic biotherapy: a novel therapeutic platform. *Lancet Oncol.* 3(1), 17–26 (2002).
- Review describing the concept of oncolytic virotherapy.
- ▶13 Bischoff JR, Kirn DH, Williams A *et al.* An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 274(5286), 373–376 (1996).
- ▶14 Khuri FR, Nemunaitis J, Ganly I *et al.* A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat. Med.* 6(8), 879–885 (2000).
- ▶15 Nemunaitis J, Khuri F, Ganly I *et al.* Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J. Clin. Oncol.* 19(2), 289–298 (2001).
- ▶16 Goodrum FD, Ornelles DA. p53 status does not determine outcome of E1B 55-kilodalton mutant adenovirus lytic infection. *J. Virol.* 72(12), 9479–9490 (1998).
- ▶17 O'Shea CC, Johnson L, Bagus B *et al.* Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. *Cancer Cell* 6(6), 611–623 (2004).
- ▶18 Maehara Y, Oshiro T, Endo K *et al.* Clinical significance of occult micrometastasis lymph nodes from patients with early gastric cancer who died of recurrence. *Surgery* 119(4), 397–402 (1996).

- ▶ 19 Rivadeneira DE, Simmons RM, Christos PJ *et al.* Predictive factors associated with axillary lymph node metastases in T1a and T1b breast carcinomas: analysis in more than 900 patients. *J. Am. Coll. Surg.* 191(1), 1–6 (2000).
- ▶ 20 Chang GJ, Rodriguez-Bigas MA, Skibber JM *et al.* Lymph node evaluation and survival after curative resection of colon cancer: systematic review. *J. Natl Cancer Inst.* 99(6), 433–441 (2007).
- ▶ 21 Volpe CM, Koo J, Miloro SM *et al.* The effect of extended lymphadenectomy on survival in patients with gastric adenocarcinoma. *J. Am. Coll. Surg.* 181(1), 56–64 (1995).
- ▶ 22 Harrison LE, Karpeh MS, Brennan MF. Extended lymphadenectomy is associated with a survival benefit for node-negative gastric cancer. *J. Gastrointest. Surg.* 2(2), 126–131 (1998).
- ▶ 23 Sasako M, Sano T, Yamamoto S *et al.* D2 lymphadenectomy alone or with para-aortic nodal dissection for gastric cancer. *N. Engl. J. Med.* 359(5), 453–462 (2008).
- ▶ 24 Gotoda T, Sasako M, Ono H *et al.* Evaluation of the necessity for gastrectomy with lymph node dissection for patients with submucosal invasive gastric cancer. *Br. J. Surg.* 88(3), 444–449 (2001).
- ▶ 25 Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur. J. Cancer* 33(5), 787–791 (1997).
- ▶ 26 Dong CK, Masutomi K, Hahn WC. Telomerase: regulation, function and transformation. *Crit. Rev. Oncol. Hematol.* 54(2), 85–93 (2005).
- ▶ 27 Hiyama K, Hirai Y, Kyoizumi S *et al.* Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J. Immunol.* 155(8), 3711–3715 (1995).
- ▶ 28 Tahara H, Yasui W, Tahara E *et al.* Immuno-histochemical detection of human telomerase catalytic component, hTERT, in human colorectal tumor and non-tumor tissue sections. *Oncogene* 18(8), 1561–1567 (1999).
- ▶ 29 Shay JW, Wright WE. Telomerase: a target for cancer therapeutics. *Cancer Cell* 2(4), 257–265 (2002).
- ▶ 30 Feng J, Funk WD, Wang SS *et al.* The RNA component of human telomerase. *Science* 269(5228), 1236–1241 (1995).
- ▶ 31 Harrington L, McPhail T, Mar V *et al.* A mammalian telomerase-associated protein. *Science* 275(5302), 973–977 (1997).
- ▶ 32 Meyerson M, Counter CM, Eaton EN *et al.* hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 90(4), 785–795 (1997).
- ▶ 33 Nakamura TM, Morin GB, Chapman KB *et al.* Telomerase catalytic subunit homologs from fission yeast and human. *Science* 277(5328), 955–959 (1997).
- ▶ 34 Nakayama J, Tahara H, Tahara E *et al.* Telomerase activation by hTERT in human normal fibroblasts and hepatocellular carcinomas. *Nat. Genet.* 18(1), 65–68 (1998).
- ▶ 35 Beattie TL, Zhou W, Robinson MO *et al.* Reconstitution of human telomerase activity *in vitro*. *Curr. Biol.* 8(3), 177–180 (1998).
- ▶ 36 Keith WN, Sarvesvaran J, Downey M. Analysis of telomerase RNA gene expression by *in situ* hybridization. *Methods Mol. Biol.* 19165–19181 (2002).
- ▶ 37 Bilsland AE, Merron A, Vassaux G *et al.* Modulation of telomerase promoter tumor selectivity in the context of oncolytic adenoviruses. *Cancer Res.* 67(3), 1299–1307 (2007).
- ▶ 38 Wirth T, Zender L, Schulte B *et al.* A telomerase-dependent conditionally replicating adenovirus for selective treatment of cancer. *Cancer Res.* 63(12), 3181–3188 (2003).
- ▶ 39 Lanson NA Jr, Friedlander PL, Schwarzenberger P *et al.* Replication of an adenoviral vector controlled by the human telomerase reverse transcriptase promoter causes tumor-selective tumor lysis. *Cancer Res.* 63(22), 7936–7941 (2003).
- ▶ 40 Irving J, Wang Z, Powell S *et al.* Conditionally replicative adenovirus driven by the human telomerase promoter provides broad-spectrum antitumor activity without liver toxicity. *Cancer Gene Ther.* 11(3), 174–185 (2004).
- ▶ 41 Kim E, Kim JH, Shin HY *et al.* Ad-mTERT- δ 19, a conditional replication-competent adenovirus driven by the human telomerase promoter, selectively replicates in and elicits cytopathic effect in a cancer cell-specific manner. *Hum. Gene Ther.* 14(15), 1415–1428 (2003).
- ▶ 42 Huang TG, Savontaus MJ, Shinozaki K *et al.* Telomerase-dependent oncolytic adenovirus for cancer treatment. *Gene Ther.* 10(15), 1241–1247 (2003).
- ▶ 43 Zou W, Luo C, Zhang Z *et al.* A novel oncolytic adenovirus targeting to telomerase activity in tumor cells with potent. *Oncogene* 23(2), 457–464 (2004).
- ▶ 44 Kuppuswamy M, Spencer JF, Doronin K *et al.* Oncolytic adenovirus that overproduces ADP and replicates selectively in tumors due to hTERT promoter-regulated E4 gene expression. *Gene Ther.* 12(22), 1608–1617 (2005).
- ▶ 45 Li Y, Yu DC, Chen Y *et al.* A hepatocellular carcinoma-specific adenovirus variant, CV890, eliminates distant human liver tumors in combination with doxorubicin. *Cancer Res.* 61(17), 6428–6436 (2001).
- ▶ 46 Kawashima T, Kagawa S, Kobayashi N *et al.* Telomerase-specific replication-selective virotherapy for human cancer. *Clin. Cancer Res.* 10(1 Pt 1), 285–292 (2004).
- **First description of the development of telomelysin.**
- ▶ 47 Taki M, Kagawa S, Nishizaki M *et al.* Enhanced oncolysis by a tropism-modified telomerase-specific replication-selective adenoviral agent OBP-405 ('Telomelysin-RGD'). *Oncogene* 24(19), 3130–3140 (2005).
- ▶ 48 Hashimoto Y, Watanabe Y, Shirakiya Y *et al.* Establishment of biological and pharmacokinetic assays of telomerase-specific replication-selective adenovirus. *Cancer Sci.* 99(2), 385–390 (2008).
- ▶ 49 Fujiwara T, Urata Y, Tanaka N. Telomerase-specific oncolytic virotherapy for human cancer with the promoter. *Curr. Cancer Drug Targets* 7(2), 191–201 (2007).
- ▶ 50 Liu TC, Kirn D. Systemic efficacy with oncolytic virus therapeutics: clinical proof-of-concept and future directions. *Cancer Res.* 67(2), 429–432 (2007).
- ▶ 51 Burton JB, Johnson M, Sato M *et al.* Adenovirus-mediated gene expression imaging to directly detect sentinel lymph node metastasis of prostate cancer. *Nat. Med.* 14(8), 882–888 (2008).
- ▶ 52 Kishimoto H, Kojima T, Watanabe Y *et al.* *In vivo* imaging of lymph node metastasis with telomerase-specific replication-selective adenovirus. *Nat. Med.* 12(10), 1213–1219 (2006).
- **Important article reporting on viral spread into locoregional lymphatics.**
- ▶ 53 Kurihara Y, Watanabe Y, Onimatsu H *et al.* Telomerase-specific virotheranostics for human head and neck cancer. *Clin. Cancer Res.* 15(7), 2335–2343 (2009).
- ▶ 54 Kojima T, Watanabe Y, Hashimoto Y *et al.* *In vivo* biological purging for lymph node metastasis of human colorectal cancer by telomerase-specific oncolytic virotherapy. *Ann. Surg.* 251(6), 1079–1086 (2010).

- **Important article describing the anti-tumor effects of oncolytic virotherapy for lymph node metastasis.**
- ▶55 Kawabata K, Sakurai F, Koizumi N *et al.* Adenovirus vector-mediated gene transfer into stem cells. *Mol. Pharm.* 3(2), 95–103 (2006).
- ▶56 Burdelya LG, Krivokrysenko VI, Tallant TC *et al.* An agonist of Toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science* 320(5873), 226–230 (2008).
- ▶57 Folkman J. Role of angiogenesis in tumor growth and metastasis. *Semin. Oncol.* 29(6 Suppl. 16), 15–18 (2002).
- 58 Fujiwara T, Tanaka N, Numunaitis JJ *et al.* Phase I trial of intratumoral administration of OBP-301, a novel telomerase-specific oncolytic virus, in patients with advanced solid cancer: evaluation of biodistribution and immune response. *J. Clin. Oncol.* 26(15), 3572 (2008).
- ▶59 Nemunaitis J, Tong AW, Nemunaitis M *et al.* A Phase I study of telomerase-specific replication competent oncolytic adenovirus (telomelysin), for various solid tumors. *Mol. Ther.* 18(2), 429–434 (2010).
- **First report of a clinical trial of telomelysin.**
- ▶60 DeWeese TL, van der Poel H, Li S *et al.* A Phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. *Cancer Res.* 61(20), 7464–7472 (2001).
- ▶61 Small EJ, Carducci MA, Burke JM *et al.* A Phase I trial of intravenous CG7870, a replication-selective, prostate-specific antigen-targeted oncolytic adenovirus, for the treatment of hormone-refractory, metastatic prostate cancer. *Mol. Ther.* 14(1), 107–117 (2006).
- ▶62 Yokoyama T, Iwado E, Kondo Y *et al.* Autophagy-inducing agents augment the antitumor effect of telomerase-selective oncolytic adenovirus OBP-405 on glioblastoma cells. *Gene Ther.* 15(17), 1233–1239 (2008).
- ▶63 Okada N, Tsukada Y, Nakagawa S *et al.* Efficient gene delivery into dendritic cells by fiber-mutant adenovirus vectors. *Biochem. Biophys. Res. Commun.* 282(1), 173–179 (2001).
- ▶64 Fujiwara T, Kagawa S, Kishimoto H *et al.* Enhanced antitumor efficacy of telomerase-selective oncolytic adenoviral agent OBP-401 with docetaxel: preclinical evaluation of chemovirotherapy. *Int. J. Cancer* 119(2), 432–440 (2006).
- ▶65 Watanabe T, Hioki M, Fujiwara T *et al.* Histone deacetylase inhibitor FR901228 enhances the antitumor effect of telomerase-specific replication-selective adenoviral agent OBP-301 in human lung cancer cells. *Exp. Cell Res.* 312(3), 256–265 (2006).
- ▶66 Liu D, Kojima T, Ouchi M *et al.* Preclinical evaluation of synergistic effect of telomerase-specific oncolytic virotherapy and gemcitabine for human lung cancer. *Mol. Cancer Ther.* 8(4), 980–987 (2009).
- ▶67 Fujiwara T, Tanaka N, Kanazawa S *et al.* Multicenter Phase I study of repeated intratumoral delivery of adenoviral p53 in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* 24(11), 1689–1699 (2006).
- ▶68 Sakai R, Kagawa S, Yamasaki Y *et al.* Preclinical evaluation of differentially targeting dual virotherapy for human solid cancer. *Mol. Cancer Ther.* 9(6), 1884–1893 (2010).

