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REVIEW ARTICLE

Ki67 targeted strategies for cancer therapy

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Abstract Ki67 is a well-known proliferation marker for the evaluation of cell proliferation. Numerous studies have indicated that Ki67 index independently predicts cancer progression. Moreover, because Ki67 is highly expressed in malignant cells but almost could not be detected in normal cells, it has become a promising target for cancer therapy. In this review, we summarize recent advances in Ki67 targeted cancer therapy. In particular, we highlight recent development on the exploitation of Ki67 promoter to drive the expression of siRNAs or therapeutic genes in cancer cells specifically. The use of Ki67 as an attractive target opens a new avenue for cancer therapy.

Keywords Ki67 · Gene therapy · Renal cancer · siRNA · Target therapy · Proliferation

Introduction

The nuclear Ki67 protein is generally expressed only in proliferating cells [1]. Ki67 is mainly located in the nucleolar cortex during interphase, and is recruited to condensed chromosomes during mitosis [2, 3]. Ki67 gene is located on chromosome 10q25-ter and encodes two Ki67 isoforms of 345 and 395 kDa, respectively [4–6].

Ki67 expression level increases from G1 phase to mitosis, and then rapidly decreases immediately after mitosis. Ki67 protein is detected in the nuclei of cells at G1, S, G2 phase and mitosis, but not in the nuclei of quiescent cells at G0 phase [7, 8]. Therefore, Ki67 expression level indicates the status of cell proliferation. Indeed, Ki67 is highly overexpressed in cancer cells and has been proposed as a prognostic marker of cancer [9, 10].

In this review, we first summarize recent progress regarding the prognostic value of Ki67, and then focus on rational design of Ki67 as a therapeutic target for cancer therapy in preclinical and clinical studies.

Ki67 as a prognostic factor of cancer

Ki67 has been widely investigated as a potential prognostic marker of proliferation in retrospective studies of malignant diseases [11, 12]. Accumulating clinical studies have shown the promise of Ki67 as a tool for cancer diagnosis [13–16]. Immunostaining for Ki67 expression is the gold standard, and a cutoff level of 10–14% positive staining is used to judge high risk of prognosis [17–19]. Ki67 index has become an independent prognostic factor for prostate cancer patients [20].

To investigate clinical value of p53 and Ki67 expression in renal cell carcinoma (RCC), a retrospective analysis of clinical data from 1239 patients with RCC was performed and the results indicated that the combined detection of p53 and Ki67 was superior to any single marker to improve the accuracy of the prognosis of RCC patients [21].

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Ki67 as a therapeutic target for cancer therapy

Given the important role of Ki67 in cell proliferation, the inhibition of Ki67 might be considered when designing novel strategies for cancer therapy. Ki67 is proposed as an attractive therapeutic target for cancer because it is highly expressed in most malignant cells but is rarely detected in normal cells [22]. Below we will discuss different approaches for Ki67 targeted cancer therapy (Fig. 1).

Ki67-Antisense nucleotide

Systemically delivered unformulated antisense oligonucleotides (ASOs) have proven to be effective as human therapeutics in several non-oncology diseases [23–27]. Several ASOs have been used in clinical studies to cure carcinoma [28–30]. Schlüter et al. found that Ki67 specific antisense oligonucleotides (ASODNs) inhibited the proliferation of human myeloma cells [4]. Kausch et al. reported that Ki67 ASOs inhibited cancer cell proliferation and tumor growth in vitro and in vivo [31]. Furthermore, Ki67 ASODNs have been used in phase I clinical study in patients with bladder cancer [32]. Recently, we demonstrated that methylated oligonucleotide targeting Ki67 could effectively inhibit the proliferation while induce the apoptosis of renal carcinoma cells [33].

Anti-Ki67 peptide nucleic acid

Unfortunately, clinical application of ASOs is limited due to several disadvantages such as low affinity, susceptibility to nuclease degradation and non-specific binding. Peptide nucleic acids (PNAs) are synthetic DNA analogs in which phosphodiester backbone is substituted with unchanged 2-N-aminoethylglycine units. Importantly, PNA backbone provides good and specific hybridization property with complementary targets [34]. Recently, PNAs have been developed as antisense (targeting mRNA or microRNAs) and antigene agents (targeting genomic DNA) to regulate gene expression [35–37]. For example, PNAs were more efficient than analogous ASOs to inhibit human telomerase activity [38]. Moreover, we treated human renal carcinoma 786-0 cells with the lipid-delivered PNAs against Ki67 and Ki67 ASOs, and found that anti-Ki67 PNA had stronger effects to inhibit the proliferation and induce the apoptosis of renal carcinoma cells than ASO. Thus PNA against Ki67 is a promising agent for the treatment of renal cancer [39].

RNA interference (RNAi) targeting Ki67

RNAi emerges as a powerful tool for cancer therapy [40]. siRNAs could inhibit multiple targets simultaneously and maximize antitumor efficacy [41]. Recently, we investigated the effects of siRNA against Ki67 on Ki67 expression and the proliferation of human RCCs, and found that

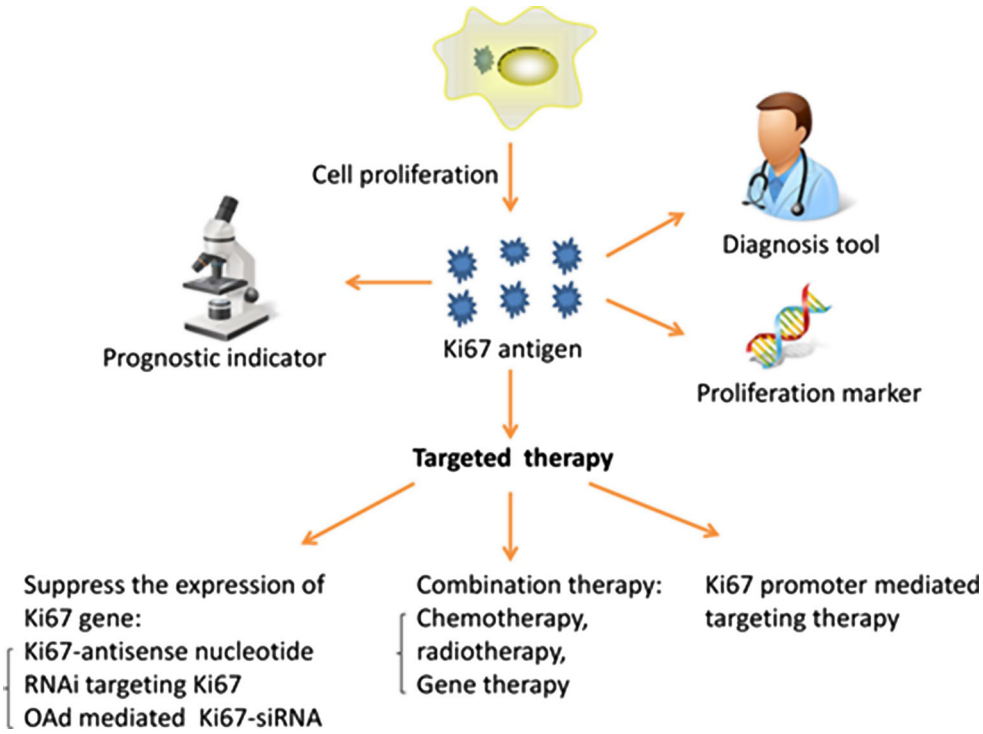

Fig. 1 Different approaches for Ki67 targeted cancer therapy

siRNA-mediated knockdown of Ki67 resulted in efficient and specific inhibition of in vitro cell proliferation compared to antisense technologies [42].

To circumvent the drawback that siRNA-mediated effects are transient, we established pSilencerKi67 construct that contained short hairpin RNAs (shRNAs) against Ki67. We demonstrated that pSilencerKi67 demonstrated better inhibition of the proliferation and induction of the apoptosis of 786-O human renal carcinoma cells than synthetic siRNAs [43].

Oncolytic adenoviral mediated Ki67-siRNA

The off-target effects and immune response via the activation of toll-like receptor (TLR) have hampered preclinical and clinical application of siRNAs [44–53]. To overcome these obstacles, numerous methods including modification of RNAs, optimization of delivery systems, and proper in vivo administration have been exploited intensively [54–56]. One of the promising delivery systems is oncolytic adenovirus.

Oncolytic viruses can replicate selectively in tumor cells, leading to intratumoral virus spread. Several clinical trials have tested a variety of conditionally replicative viruses such as conditionally replicative adenovirus (CRAds), vaccinia virus, herpes simplex virus and Newcastle disease virus. To selectively target CRAds to tumor cells, we could employ two strategies: one is to delete viral element necessary for viral replication in normal cells but not in tumor cells such as ONYX-015 [57, 58]; the other is to use a tumor-specific promoter to drive the gene necessary for viral replication [59].

In preclinical models and clinical trials CRAds have shown varying degree of success [60]. In particular, CRAds based on Ad5 have demonstrated good efficacy and safety for cancer gene therapy [61–69]. We constructed ZD55-Ki67 to keep lytic ability of oncolytic adenovirus and deliver shRNA against Ki67. Silencing of Ki67 induced apoptosis in renal cancer cells in vitro and inhibited renal cancer growth in nude mice [70]. Our data indicate that the armed oncolytic adenovirus ZD55–Ki67 could be used for renal cancer gene therapy.

To further increase the safety of CRAds, it is necessary to modify them to restrict adenovirus replication only in tumor cells [71, 72]. We developed an oncolytic virus G250–Ki67, in which E1A gene (essential early viral genes for replication) was controlled by renal cancer specific G250 promoter. Our data indicate that this vector efficiently replicated in renal cancer cells only, and mediated knockdown of Ki67 to inhibit the proliferation while induce the apoptosis of renal cancer cells. Therefore, G250-specific CRAds carrying Ki67-siRNA show promise for renal clear cell cancer therapy [73].

Oncolytic adenovirus targeting both Ki67 and telomerase

Cancer is a complex disease resulting from the accumulation of multiple mutations. Thus, multiple siRNAs are frequently used to silence multiple oncogenes [74]. Telomerase is composed of an ubiquitously expressed RNA component (hTR) and a catalytic subunit human telomerase reverse transcriptase (hTERT) [75]. Telomerase becomes an attractive target for cancer therapy because telomerase activity is very high in cancer cells but is either reduced or absent in normal cells. We recently constructed an oncolytic adenovirus that contains Ki67 promoter to control E1A expression, and double siRNAs to target Ki67 and hTERT. This vector effectively inhibited the growth of renal cancer cells both in vitro and in animal models, and provides a promising strategy by silencing different oncogenes for renal cancer therapy [76].

Ki67 promoter controlled cancer gene therapy

To understand the mechanism that regulates Ki67 expression, we characterized Ki67 core promoter which is TATA less and GC rich region containing putative Sp1 binding sites. Overexpression of Sp1 enhanced Ki67 promoter activity, while downregulation of Sp1 expression effectively inhibited Ki67 transcription. Thus, Sp1 is essential to Ki67 promoter activity [77, 78]. Furthermore, we showed that interferon regulatory factor 1 (IRF1) repressed Ki67 transcription in human renal carcinoma cells in a dose dependent manner, thus Ki67 is a target of IRF1 [9, 79]. In addition, we found that p53 inhibited Ki67 promoter activity dose dependently and identified Sp1-binding sites responsible for p53 mediated repression of Ki67 transcriptional [80].

Notably, Ki67 promoter keeps the specificity after integration into adenovirus genome [81]. A novel double regulated oncolytic adenovirus Ki67-ZD55-IL-24 was constructed in which both E1A and interleukin (IL)–24 expression is driven by Ki67 promoter, and it showed specific anti-tumor effects against melanoma [82]. Ki67-ZD55-IL-24 also caused significant inhibition of melanoma cell migration and invasion, and induced apoptosis effectively in melanoma xenografts in nude mice [83].

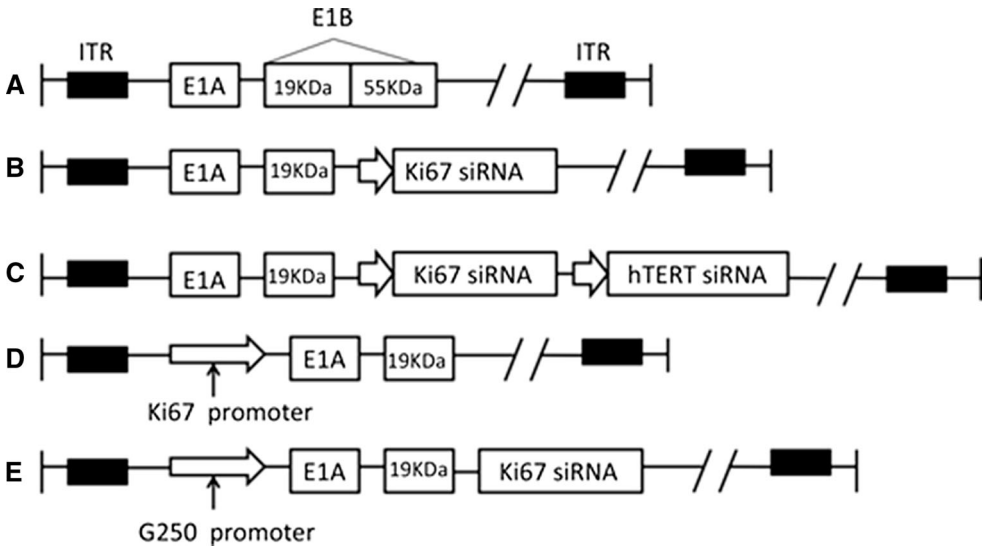

Fig. 2 Schematic structures of CRAd vectors. (A) Wild-type adenovirus. (B) Recombinant adenovirus. E1B55 KDa gene was substituted with a shRNA sequence cassette to knockdown Ki67. (C) CRAd vector containing double-cistronic shRNA construct. (D) CRAds armed with Ki67 promoter. (E) CRAds armed with G250 promoter and a shRNA sequence cassette targeting Ki67

Combining CRAd therapy with chemotherapy

Several clinical studies have reported good safety and moderate anti-tumor efficacy of Onyx-015 whether administered systemically or locally [84–86]. However, combination of CRAds with chemotherapy achieved much better anti-tumor efficacy than either treatment alone, perhaps due to synergistic or complementary effects [87–90]. We demonstrated that Ki67-ZD55-IL-24 significantly enhanced anti-tumor efficacy of alkylating agent temozolomide (TMZ) against melanoma [82]. In addition, Ki67-ZD55-IL-24 conjugated with TMZ exhibited high efficacy to kill melanoma cells [91].

Combining CRAd therapy with radiotherapy

The regimens combining oncolytic adenoviruses with radiotherapy have shown greater anti-tumor efficacy than either therapy alone [92–94]. For example, Ki67-ZD55-IL-24 significantly enhanced anti-tumor efficacy of radiotherapy by the induction of apoptosis in renal cells, and radiotherapy did not interfere with the replication of CRAds. Therefore, the novel strategy of combing CRAds with radiotherapy has the potential for effective treatment of renal cell cancer [70].

Conclusions

Increased proliferation is a hallmark of malignant tumors, and thus nuclear protein Ki67 has been regarded as a valuable cancer biomarker. Clinically, Ki67 expression is correlated to clinical stage and metastasis of tumors. In addition, Ki67 expression is particularly high in poorly differentiated cancer tissues. Ki67 is an attractive biomarker for the diagnosis and prognosis of solid tumors. More importantly, various studies have indicated that the strategy of inhibiting Ki67 holds promise for renal cancer therapy. In particular, the utilization of Ki67 promoter in the design of CRAds vectors enriches our power to specially and effectively destroy cancer cells (Fig. 2). It is expected that Ki67 target cancer therapy will be applied in the clinical in the near future.

Compliance with ethical standards

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Conflict of interest All authors declare no conflict of interest.

Research involving human participants and/or animals Not applicable.

Informed consent Not applicable.

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