Retroviral Vectors for Cancer Gene Therapy

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Abstract

Advances in molecular technologies have led to the discovery of many disease-related genetic mutations as well as elucidation of aberrant gene and protein expression patterns in several human diseases, including cancer. This information has driven the development of novel therapeutic strategies, such as the utilization of small molecules to target specific cellular pathways and the use of retroviral vectors to retarget immune cells to recognize and eliminate tumor cells. Retroviral-mediated gene transfer has allowed efficient production of T cells engineered with chimeric antigen receptors (CARs), which have demonstrated marked success in the treatment of hematological malignancies. As a safety point, these modified cells can be outfitted with suicide genes. Customized gene editing tools, such as clustered regularly interspaced short palindromic repeats–CRISPR-associated nucleases (CRISPR-Cas9), zinc-finger nucleases (ZFNs), or TAL-effector nucleases (TALENs), may also be combined with retroviral delivery to specifically delete oncogenes, inactivate oncogenic signaling pathways, or deliver wild-type genes. Additionally, the feasibility of retroviral gene transfer strategies to protect the hematopoietic stem cells (HSC) from the

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dose-limiting toxic effects of chemotherapy and radiotherapy was demonstrated. While some of these approaches have yet to be translated into clinical application, the potential implications for improved cellular replacement therapies to enhance and/or support the current treatment modalities are enormous.

Keywords

Retrovirus · Gene transfer · Anticancer · CAR · Gene editing · Cytoprotection

Abbreviations

aGVHD	Acute graft-versus-host disease
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AraC	Cytarabine
CARs	Chimeric antigen receptors (CARs)
CLL	Chronic lymphocytic leukemia
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeats–CRISPR-associated nucleases
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
DLBCL	Diffuse large-cell lymphoma
DNA	Deoxyribonucleic acid
EGFP	Enhanced green fluorescent protein
EGFR	Epidermal growth factor receptor
FL	Follicular lymphoma
HDR	Homology-directed repair
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cells
HSPC	HSC and progenitor cells
HSV-tk	Herpes simplex virus thymidine kinase
IFN	Interferon
iPSC	Induced pluripotent stem cells
LTRs	Long-terminal repeats
MCL	Mantle cell lymphoma
MDR1	Multidrug resistance gene 1
MDS	Myelodysplastic syndrome
MGMT	O-6-methylguanine DNA methyltransferase
MPP	Multipotent progenitor
NB	Neuroblastoma
NHEJ	Nonhomologous end-joining
NSG	NOD.cgPrkdc^{scid}IL2rg$^{tm/Wjl}$/Sz
PD-1	Programmed death-1
preTs	T cell-committed progenitors
RD114/TR	Modified feline endogenous retrovirus envelope glycoprotein
RECIST	Response Evaluation Criteria in Solid Tumors

scFv	Single-chain variable fragment
SIN	Self-inactivating
TAA	Tumor-associated antigens
TALENs	TAL-effector nucleases
TCRs	T cell receptors
TERT	Telomerase
Thbd	Thrombomodulin
TNF	Tumor necrosis factor
ZFNs	Zinc-finger nucleases

1 Introduction

Curing cancer remains one of the greatest challenges facing the medical research and biotechnology fields. This task is complicated by the heterogeneity of cancer, with different tumor types exhibiting distinct genetic and phenotypic characteristics. For example, each acute myeloid leukemia (AML) genome is estimated to harbor approximately 13 coding mutations (Klco et al. 2014), while solid tumors of the colon, breast, brain, or pancreas contain 33–66 somatic mutations and melanomas and lung tumors have around 200 mutations (Vogelstein et al. 2013). The actual tumor burden of patients is often composed of different major and minor clones that may differ regarding capacity to engraft, proliferate, differentiate, transit from bone marrow to peripheral blood, and develop resistance (Klco et al. 2014). This makes the identification of the right target(s) critical for successful implementation of molecular-based therapies.

A key factor determining the clinical usefulness of any treatment modality is the successful delivery of the therapeutic agent. In the field of gene therapy, efficient transfer of the therapeutic gene technology to the target cell population is crucial. Retroviral vectors, including lentiviral vectors, are well known for their capacity to efficiently transduce a wide range of cell types and have been successfully used in the clinic (Aiuti et al. 2013; Biffi et al. 2013; Hacein-Bey-Abina et al. 2014; Naldini 2015) (Table 1). As the mechanism of action of retroviral vectors includes incorporation of the viral cargo into the target cell genome, therapies using retroviral vectors carry the risk of insertional mutagenic events in which viral insertion may deregulate the expression of proto-oncogenes or tumor suppressor genes. While some serious adverse events were observed in a subgroup of participants from the early gene therapy trials, these adverse events have not occurred in more recent trials using retroviral vectors with improved biosafety features, such as the use of self-inactivating (SIN) vectors in which viral promoters and enhancers have been deleted from the long-terminal repeats (LTRs), and transgene expression is driven by physiological promoters. Of note, the safety profile of any gene therapeutic

Table 1 Typical uses of retroviral vectors in human diseases in preclinical and clinical settings

Retrovirus	Cell target/topic	References
Foamy virus	HSC/WAS	Uchiyama et al. (2012)
Alpharetrovirus	HSC/X-CGD Pre T cells/leukemia T cells/GvHD NK cells/leukemia	Kaufmann et al. (2013) Hübner et al. (2016) Labenski et al. (2016) Suerth et al. (2016)
Gammaretrovirus	HSC/X-CGD HSC/ADA-SCID HSC/X-SCID (SIN vector) T cells/leukemia NK cells/gene transfer, neuroblastoma	Ott et al. (2006) Aiuti et al. (2002), Gaspar et al. (2011) Hacein-Bey-Abina et al. (2014) Brudno et al. (2016) Guven et al. (2005), Esser et al. (2012)
Lentivirus	HSC/ALD HSC/MLD HSC/WAS T cells/leukemia NK cells/gene transfer, glioblastoma	Cartier et al. (2009) Biffi et al. (2013) Aiuti et al. (2013) Kalos et al. (2011), Grupp et al. (2013), Maude et al. (2014), Porter et al. (2015) Micucci et al. (2006), Zhang et al. (2016)

Abbreviations HSC hematopoietic stem cell, *Pre T cells* precursor T cells, *NK cells* natural killer cells, *X-CGD* X-linked chronic granulomatous disease, *GvHD* graft-versus-host disease, *ADA-SCID* adenosine deaminase-deficient severe combined immunodeficiency, *X-SCID* X-linked severe combined immunodeficiency, *MLD* metachromatic leukodystrophy, *WAS* Wiskott–Aldrich syndrome

approach is also dependent upon the target cell population. For example, more primitive cells such as hematopoietic stem cells and precursor cells exhibit an inherently greater risk of transformation than differentiated cells such as T cells. Interestingly, even the use of gammaretroviral vectors with intact LTRs, which can be considered as one of the riskier retroviral vector configurations, for transduction of T cells has not resulted in any reported transformation events due to insertional mutagenesis (Newzeala et al. 2011, 2012; Heinrich et al. 2013).

Successful delivery and expression of therapeutic genes depend on several variables. For example, the retroviral vector system (i.e., the retroviral genus upon which the vector system is based) strongly influences the site of vector integration. Genomic analyses of murine and human HSC cells following transduction with alpharetroviral SIN vectors demonstrated a neutral genomic insertion site pattern and lower genotoxicity when compared to gammaretroviral or lentiviral SIN vectors (Suerth et al. 2012; Moiani et al. 2014). Gammaretroviral SIN vectors exhibited higher incidences of integrations near transcription start sites, CpG islands, and cancer-associated genes, while lentiviral SIN vectors preferentially integrated into actively transcribed genes. Importantly, retroviral gene delivery can be optimized for particular cell populations by choosing the viral envelope proteins compatible with the receptors expressed by the target cell population (Amirache et al. 2014). Additional factors that affect therapeutic gene transfer and expression in the target

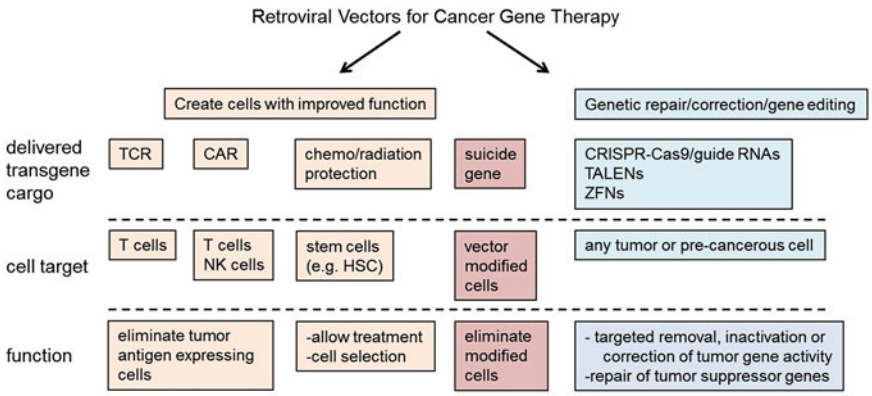

Fig. 1 Overview of retroviral vector use in cancer therapy. Retroviral vectors can be used to improve the immune cell antitumor activity or to protect the sensitive cell populations, such as HSC, from the cytotoxic effects of chemotherapy and radiotherapy (*orange boxes*). In addition to therapeutic genes, target cells can be cotransduced with suicide genes as a safety feature to eliminate the modified cells in case of undesirable effects, such as cell transformation or cytokine storm induction (*red boxes*). Gene editing techniques can be employed for the correction of tumor-specific genetic aberrations (*blue boxes*). *TCR* T cell receptor, *CAR* chimeric antigen receptor, *NK cells* natural killer cells, *HSC* hematopoietic stem cells, *CRISPR-Cas9* clustered regularly interspaced short palindromic repeats–CRISPR-associated nucleases, *TALENs* TAL-effector nucleases, *ZFNs* zinc-finger nucleases

cell include the strength and location of the promoter, transgene properties, the cell culture conditions, and the proliferative status of the cultured cells.

In this chapter, we discuss several available options to employ retroviral vectors in anticancer gene therapy treatment strategies, such as modification of immune cells with chimeric antigen receptors (CARs) or modified T cell receptors (TCRs), gene editing to inhibit oncogenic signals, and cell-protective and replacement approaches (Fig. 1).

2 Anticancer Strategies Based on CARs and TCR

Identification of tumor-associated antigens (TAA) has aided the development of monoclonal antibodies as cancer therapeutics (Scott et al. 2012). This strategy to target cancer cells was further exploited by engineering immune cells (e.g., T cells and NK cells) to express chimeric antigen receptors (CARs), which consist of an extracellular single-chain variable fragment (scFv) that recognizes a specific TAA. The scFv is coupled to intracellular costimulatory signaling domains (e.g., CD28, CD3ζ, 4-1BB, OX40, DAP10, and DAP12) that activate the cytotoxic function of the modified immune cell upon antigen recognition, resulting in specific elimination of cells expressing the TAA (e.g., the cancer cell) (Karlsson et al. 2015; Töpfer et al. 2015). Another strategy to create tumor-specific T cells is to use retroviral vectors to

transfer T cell receptors (TCRs) engineered to exhibit improved binding affinity to a ligand specifically expressed on the target cancer cell population. T cell activation in response to antigen stimulation is modulated by costimulatory molecules, cell adhesion factors, and T cell receptor (TCR) affinity (van der Merwe and Davis 2003). Antigen recognition by CARs or TCRs directs T cell trafficking and accumulation in specific tissues, including tumors. TCRs can also be engineered to guide T cells to tumor tissue via the recognition of specific chemokines or components of the tumor microenvironment. For CAR- and TCR-based strategies, the targeted antigen should ideally be specifically expressed by tumor cells and not by healthy tissues, the antigen should induce an immunogenic response sufficient to eradicate the tumor, and the antigen should be important for the transformed phenotype, such as tumor cell survival, proliferation, metastasis, or resistance (Brentjens et al. 2013; Rapoport et al. 2015; Robbins et al. 2015; Sandri et al. 2016).

2.1 T Cells

Gammaretroviral and lentiviral vectors were used to generate T cells modified with CARs designed to target CD19, and these modified T cells (CART19, CTL019) were evaluated in clinical trials of pediatric and adult B cell malignancies (Kalos et al. 2011; Brentjens et al. 2013; Grupp et al. 2013; Maude et al. 2014; Porter et al. 2015; Brudno et al. 2016). In general, results from these trials were encouraging, with up to a 90 % (27/30 patients) complete response rate with a six-month overall survival rate of 78 % achieved in patients with relapsed or refractory acute lymphoblastic leukemia (ALL) (Maude et al. 2014). In chronic lymphocytic leukemia (CLL) patients, 57 % responded to CTL019 infusion and 29 % of patients achieved a long-lasting complete response (Porter et al. 2015). While CD19 is expressed on malignant B cells in several diseases, including chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), diffuse large-cell lymphoma (DLBCL), follicular lymphoma (FL), and mantle cell lymphoma (MCL), it is also expressed on healthy B cells. Side effects due to CTL019 therapy include B cell aplasia, cytokine-release syndrome, tumor lysis syndrome, and neurological toxicities. The severity of these events varies in different disease settings, but most can be clinically managed (Namuduri and Brentjens 2016).

Successful disease control observed in trials using CD19-targeted T cells in patients with B-cell malignancies led to the development of CAR T cells for other cancers. Retroviral vectors were used to transfer an NY-ESO-1-specific TCR to T cells and tested in clinical trials for metastatic synovial cell sarcoma, melanoma, and myeloma (Robbins et al. 2015; Rapoport et al. 2015). The NY-ESO-1 antigen is expressed in 10–50 % of melanomas, 70–80 % of synovial cell sarcomas, and up to 60 % of advanced myelomas (Atanackovic et al. 2007; van Rhee et al. 2005). The absence of NY-ESO-1 expression on most adult human tissues makes this an interesting target for redirected T cell therapy. Objective clinical responses measured according to RECIST (Response Evaluation Criteria in Solid Tumors)

guidelines were 61 % (11/18) in synovial cell sarcoma patients and 55 % (11/20) in melanoma patients (Robbins et al. 2015). Complete response or near-complete response was achieved in 70 % (14/20) of advanced multiple myeloma patients in a phase I/II trial testing lentiviral-mediated NY-ESO-1-specific TCR expression in T cells (Rapoport et al. 2015). NY-ESO-1-TCR expression was detected up to two years after modified T cell infusion, and loss of engineered T cells was associated with relapse (Rapoport et al. 2015).

It was recently demonstrated that T cells with an HLA-A2-restricted TCR modified to recognize telomerase (TERT) with high affinity successfully controlled human B-CLL progression in an in vivo murine model (Sandri et al. 2016). The main function of TERT is to maintain telomere ends. TERT expression is usually repressed in postnatal somatic cells, which contributes to senescence following a given number of cell divisions. TERT is often highly expressed in rapidly dividing cells such as embryonic stem cells, adult stem cells, and many types of tumor cells. Approximately 80–90 % of primary tumors exhibit high TERT expression, which is thought to contribute to the transformed phenotype. The "on-target, off-tumor" toxicity of TERT-directed T cells was mainly limited to mature granulocytes ($CD45^+CD11b^+CD16^+$), supporting further clinical development of this adoptive immunological anticancer strategy.

The antitumor efficacy of engineered T cells is at least partially a function of adequate expansion and engraftment of the modified cells. A preclinical trial in murine xenograft models of drug-resistant human ALL and CLL demonstrated that the combination of CAR T cell therapy with administration of the Bruton´s tyrosine kinase inhibitor ibrutinib improved CAR T cell engraftment and antileukemic activity (Fraietta et al. 2016). Analysis of $CD8^+$ T cells from CLL patients treated with five to eleven cycles of ibrutinib revealed decreased expression levels of the inhibitory receptor PD-1, suggesting that the reversal of T cell dysfunction also contributed to the enhanced antileukemic activity observed with this combination therapy (Fraietta et al. 2016).

2.2 T Precursor Cells

Retroviral vectors were used to transfer CARs or TCRs to T cell-committed progenitors (preTs) as a strategy to enhance the anticancer effects of preTs. While this approach eliminates the risk of graft-versus-host disease in allogeneic transplantation settings, it is important to use the safest retroviral vector design to limit the potential for serious adverse effects due to insertional mutagenesis, especially considering the inherent risk of transformation of precursor cell populations. A third-generation CAR engineered to target CD123-expressing cells was recently transferred to cord blood-derived $CD34^+$ cells, which were then differentiated in vitro into preTs (Hübner et al. 2016). SIN alpharetroviral vectors pseudotyped with a modified feline endogenous retrovirus envelope glycoprotein (RD114/TR) were used to obtain efficient transduction efficiency with minimized genotoxic risk. As an additional safety feature, the authors incorporated an inducible caspase 9

(iCasp9) cassette and demonstrated apoptosis of the modified cells upon introduction of the AP20187 dimerizing agent. CD123-CAR T cells produced IFNγ when cocultured with $CD123^+$ target cells and specifically lysed $CD123^+$ target cells. Importantly, CD123-CAR preTs engrafted into thymi of NSG (NOD.cgPrkdc^{scid}IL2rg$^{tm/Wjl}$/Sz) mice.

Transplantation of preTs engineered to inducibly express a leukemia-reactive TCR was recently shown to protect mice against repeated leukemia challenges via the generation of memory cells (Hoseini et al. 2015). The modified TCR was delivered via a lentiviral vector, and transduced preTs were enriched by sorting for the enhanced green fluorescent protein (EGFP) reporter. Interestingly, experiments investigating temporal control of TCR expression via doxycycline induction demonstrated that early induction of the modified TCR favored preT cell differentiation into $CD8^+$ T cells and allowed positive selection of the leukemia-reactive T cell subset (Hoseini et al. 2015).

2.3 NK Cells

Gammaretroviral- and lentiviral-mediated transfer of second-generation CARs into NK cell lines has been used to target neuroblastoma (NB) and glioblastoma tumors (Esser et al. 2012; Han et al. 2015; Zhang et al. 2016). GD2-expressing tumor cell lines and primary NB cells were selectively eliminated by GD2-specific-CAR-NK-92 clones in in vitro cocultivation experiments (Esser et al. 2012). The epidermal growth factor receptor (EGFR) is overexpressed in glioblastoma, but not in healthy brain tissue. Intracranial injection of NK-92 cells modified with an EGFR-CAR designed to target wild-type EGFR and the EGFRvIII mutant safely and efficiently eliminated EGFR-expressing glioblastoma tumor cells in an orthotopic xenograft model (Han et al. 2015). Elevated expression of ErbB2 (HER2), a growth factor receptor tyrosine kinase, was found in 41 % of primary glioblastomas and in several established glioblastoma cell lines (Zhang et al. 2016). NK-92 cells engineered via lentiviral gene transfer to express an ErbB2-CAR exhibited potent activity against glioblastoma cells in vitro and in in vivo orthotopic glioblastoma xenograft models (Zhang et al. 2016). These authors are planning a phase I clinical trial to test local application of ErbB2-CAR-NK-92 cells into the resection cavity in patients with recurrent ErbB2-positive glioblastoma.

T cells and NK cells engineered to express CARs may be used to support chemotherapy and radiotherapy treatment strategies to control or eliminate tumor cells. A systematic review combining patient outcome data from six phase I clinical trials testing CD-19 CAR T cells in refractory, relapsed, and advanced B cell malignancies showed that administration of conditioning chemotherapy was a statistically significant favorable factor in multivariate analysis for progression-free survival (Zhu et al. 2016). Increased T cell function, as measured by IFN-γ and TNF-α production, was observed in prostate cancer patients 24 h after a single low-dose pelvic radiation (Spary et al. 2014). In addition to reducing tumor burden,

chemotherapy and radiotherapy may cause increased immunogenicity of tumor cells, thus allowing improved anticancer responses to CAR-modified immune cells.

2.4 Induced Pluripotent Stem Cells (iPSC)

Retroviral transfer of gene cassettes encoding the Yamanaka reprogramming transcription factors is well-established for the generation of induced pluripotent stem cells (iPSC), which exhibit a pluripotent embryonic stem cell-like phenotype (Takahashi and Yamanaka 2006). In principle, iPSC can be derived from any somatic cell type and possess the potential for differentiation into almost any type of tissue. These characteristics make iPSC interesting for disease modeling and regenerative medicine. For example, iPSC technology was used to model glioma tumor-initiating cells, which were then screened with a panel of small molecules to discover new potential treatment options and to improve our understanding of the mechanisms driving tumorigenesis (Sancho-Martinez et al. 2016). The feasibility of using iPSC technology to produce healthy hematopoietic cells devoid of leukemia-specific genetic alterations was recently demonstrated using either skin fibroblasts or bone marrow cells from AML patients (Salci et al. 2015; Hoffmann et al. 2016). These studies highlight the potential of iPSC technology to generate autologous, disease-free cell products for cellular replacement strategies.

3 Gene Therapy

Myelosuppression due to hematopoietic stem cell (HSC) sensitivity is a major dose-limiting toxicity for many chemotherapy and radiotherapy treatment approaches and may even cause interruption of scheduled therapy. One strategy to overcome this obstacle is to use retroviral gene transfer to confer protection to HSC or other desired cell populations. Exploitation of resistance mechanisms discovered in treatment refractory cancer cells, such as increased expression of enzymes important for DNA repair or expression of cellular efflux proteins that remove chemotherapeutic drugs from the cell, has driven the development of chemoresistant HSC.

3.1 O-6-Methylguanine DNA Methyltransferase (MGMT)

Introduction of genetically modified autologous HSC resistant to chemotherapy was used in glioblastoma patients to overcome treatment-related myelosuppression (Adair et al. 2012, 2014). One mechanism that tumor cells can become resistant to alkylating chemotherapies is through increased DNA repair activity. O-6-methylguanine DNA methyltransferase (MGMT) contributes to maintenance of genome stability via repair of the mutagenic DNA lesion O-6-methylguanine back to

guanine (Tano et al. 1990). Intriguingly, decreased survival was observed in glioblastoma patients whose tumor cells lacked methylation of the promoter for the *MGMT* gene, presumably due to the increased MGMT expression and DNA repair activity in the tumor cells (Hegi et al. 2004). MGMT expression level was demonstrated to correspond to the extent of methylation in the *MGMT* gene promoter and coding region (Costello et al. 1994). Retroviral vector delivery of the $MGMT^{P140K}$ mutant protected primary murine bone marrow cells from alkylating agents (Moritz et al. 1995; Maze et al. 1999; Schambach et al. 2006). This therapeutic concept was translated into improved survival in glioblastoma patients (Adair et al. 2012, 2014). $MGMT^{P140K}$ modified HSC exhibited polyclonal engraftment and patients receiving these modified cells tolerated significantly more cycles of temozolomide/O-6-benzylguanine therapy.

3.2 Cytidine Deaminase and the Multidrug Resistance Gene 1 (MDR1)

Myelosuppression is also commonly observed in cancer patients treated with nucleoside analogs such as cytarabine (AraC), gemcitabine, azacytidine, and decitabine. Leukemia cells from patients with refractory acute myeloid leukemia (AML) were found to have elevated activity of cytidine deaminase, an enzyme involved in pyrimidine salvage and that catalyzes hydrolytic deamination of cytidine (Schröder et al. 1998). Increased cytidine deaminase expression and activity in male MDS and AML patients contributed to worse outcome as compared to females treated with azacytidine or decitabine (Mahfouz et al. 2013). Cytidine deaminase inhibitors, such as zebularine, can be used to overcome the tumor cell resistance to clinically used nucleoside analogs (Laliberté et al. 1992). Transfer of cytidine deaminase mutants resistant to zebularine may enhance the chemoprotection of HSC and thus reduce the life-threatening myelosuppression during nucleoside analog treatment (Ruan et al. 2016). Standard cytotoxic chemotherapy for high-risk myelodysplastic syndrome (MDS) and AML consists of a combination of AraC and anthracycline administration (Büchner et al. 2012). P-glycoprotein, a cellular efflux protein coded for by the *MDR1* gene, confers resistance to anthracyclines and other chemotherapeutic agents (Shen et al. 1986). Concomitant lentiviral-mediated gene transfer of cytidine deaminase and the multidrug resistance gene 1 (*MDR1*) protected primary murine hematopoietic stem and progenitor cells from AraC and anthracycline treatment (Brennig et al. 2015).

Lentiviral vector delivery of *MDR1* was also shown to protect human $CD34^+$ HSC and progenitor cells (HSPC) against radiation-induced toxicity (Maier et al. 2008). The mechanism of radioprotection by elevated *MDR1* expression remains to be demonstrated, but may involve upregulation of detoxifying proteins and inhibition of apoptosis by suppression of caspase activity (Maier et al. 2006). A retroviral insertional mutagenesis screen to identify pathways that protect HSPC from irradiation found retroviral vector insertion upstream of thrombomodulin

(*Thbd*), which resulted in elevated *Thbd* expression in radiation-selected cells (Geiger et al. 2012). Lentiviral-mediated thrombomodulin ectopic expression in murine HSPC or endothelial and stromal cells from the bone marrow compartment protected HSPC as demonstrated by an in vivo selection advantage following irradiation (Geiger et al. 2012). Another viral vector integration site discovered in this study was in the gene locus of *Puma*. PUMA mediates p53-induced apoptosis in HSPC and hematopoietic multipotent progenitors (MPP), and repression of PUMA was demonstrated to be important for MPP survival and maintenance of the HSC pool (Belle et al. 2016). Thus, the use of retroviral vectors designed to express cell-protective genes or to downregulate the expression of cell-destructive genes may be useful to combat toxicity to healthy cell compartments.

3.3 Suicide Gene Strategies

The use of retroviral strategies may require some alternative means for removal of the transduced cell populations in the event of an undesired cell transformation event. Several options for this eventuality have been created, including incorporation of suicide genes into the retroviral gene cassette transferred to the therapeutic cells. Herpes simplex virus thymidine kinase (HSV-tk) kills dividing cells by mediating conversion of ganciclovir to the cytotoxic ganciclovir triphosphate. HSV-tk-modified T cells were efficiently eradicated by ganciclovir administration, thus resolving acute graft-versus-host disease (aGVHD) in a clinical trial designed to test this strategy for improved allogeneic bone marrow transplantation safety and efficacy (Bonini et al. 1997). Since HSV-tk is a foreign protein, strategies employing HSV-tk may induce immunogenicity leading to purging of the modified cells earlier than desired (Berger et al. 2006). Another potential challenge is outgrowth of ganciclovir-resistant clones due to silencing of *HSV-tk* gene expression in modified cells (Frank et al. 2004). Additional strategies to remove gene-modified cells in the case of severe adverse events include the use of apoptosis-inducing fusion proteins (e.g., inducible Fas or Caspase 9) or expression of cell surface markers compatible with antibodies for cell depletion (e.g., CD20 can be depleted with rituximab, and truncated epidermal growth factor receptor can be depleted with cetuximab) (Straathof et al. 2005; Vogler et al. 2010; Wang et al. 2011).

3.4 Genome Editing Strategies

Recent advances in molecular biological technologies make specific directed genome editing possible. In principle, after the identification of the genetic lesion causative for tumor cell survival and propagation, techniques employing nucleases such as CRISPR-Cas9, ZFNs, or TALENs can be employed to excise, correct, or silence the respective oncogene(s). DNA double-strand breaks generated by nucleases can be repaired by nonhomologous end-joining (NHEJ) or homology-directed repair (HDR). Insertion and deletion mutations can be generated by NHEJ and may disrupt gene

transcription or translation. Point mutations can be corrected via a dead Cas9 fused to a cytidine deaminase enzyme (Komor et al. 2016). The CRISPR-Cas9 genetic engineering system is based on a bacterial innate immune system, and the genetic editing site can be programmed using an RNA guide sequence to direct cleavage to a specific DNA sequence (Jinek et al. 2012). ZFN and TALEN approaches both use protein–DNA interactions to specifically cleave the genomic DNA at a desired locus.

Retroviral vector technology can be used to deliver CRISPR-Cas9, ZFNs, or TALENs to cancer cells. Lentiviral delivery of a CRISPR-Cas9 knockout library to human melanoma cells was used to discover novel genes important for resistance to vemurafenib, a targeted therapy designed to inhibit activated BRAF mutants (Shalem et al. 2014). Lentivirus-derived particles were used for efficient delivery of ZFN and TALEN proteins into cell lines and primary human keratinocytes (Cai et al. 2014). Efficient delivery of TALENs as mRNA was demonstrated with lentiviral particles containing inactivated reverse transcriptase (Mock et al. 2014). These gene editing technologies can be designed to excise, silence, or correct specific oncogenes/genetic alterations relevant to human cancer. For example, TALEN-mediated disruption of *FLT3*, a commonly mutated gene in AML, was demonstrated in leukemia cell lines (Wang et al. 2015). Lentiviral delivery of CRISPR-Cas9 to mediate genome editing of tumor suppressor genes in an inducible $Kras^{G12D}$ murine lung tumor model allowed identification of cooperating genetic events, such as deletion of *Nkx2-1*, *Pten*, and *Apc* that may contribute to tumorigenesis and progression (Sánchez-Rivera et al. 2014). Genome editing technologies can be used to create disease models based on whole genome sequencing data that are available for many different types of cancers. Additionally, loss of function screening with CRISPR-Cas9 and genome-wide lentiviral guide RNA libraries can be used to create homozygous gene knockouts, which will help create improved disease models by overcoming the problem of incomplete suppression of gene expression common in RNA interference technologies (Koike-Yusa et al. 2014). Targeted gene correction or inactivation of tumor-specific genetic aberrations, including fusion proteins such as BCR-ABL, RUNX1-ETO, PML-RARA, and NPM-ALK, that are causative for cancer may one day be a possible treatment option.

4 Conclusions

Recent advances in available technology have led to greater understanding of the genetic aberrations that cause many types of cancer. Our current challenge is to use this information to design better treatment strategies to control or cure cancer. Retroviral vectors are efficient tools for cell modification, and several types of cells engineered with these vectors are used in clinical trials. Improved retroviral vector designs have increased the safety of gene transfer, and no vector-related serious adverse effects have occurred in any of the patients treated in clinical trials with the

new SIN vector platform (Aiuti et al. 2013; Biffi et al. 2013; Hacein-Bey-Abina et al. 2014; Naldini 2015). As described above, retroviral gene transfer can be used to change cell fate, improve fitness (confer protection), alter proliferation, and to change cell behavior. Development of stable packaging cell lines might solve challenges, such as costs and efforts associated with scaling up viral vector production to meet the increased demand for GMP-grade retroviral vectors suitable for clinical use (Labenski et al. 2016).

The success of recent trials, in which effective control of B cell lymphomas by transfer of CAR-modified T cells was demonstrated, is a driving force behind the search for suitable targets in other hematological and solid cancers. Challenges such as tumor heterogeneity, where expression of the target antigen may be low or even absent on some tumor cells, may require reconsideration of current CAR strategies. For example, three ALL patients who initially responded to CD19 CAR T cell therapy relapsed with $CD19^{-}$ leukemia (Singh et al. 2016). Thus, it might be necessary to generate and deliver immune cells modified to detect more than a single TAA to treat some cancers. Infusion of a pool of immune cells targeting a limited number of TAAs is expected to produce improved disease control, but must be carefully tested to limit the undesired toxicity to healthy tissue. Therapeutic regimens incorporating redirected immune cells may allow improved disease control with decreased doses of chemo- and/or radiotherapies, thus limiting non-specific toxicities. Dose-limiting toxicities, such as suppression of the hematopoietic compartment, associated with chemotherapies and irradiation strategies may also be overcome by the protection of HSC and progenitors with gene therapy.

Effectiveness of T cell therapies can be limited by immunosuppressive pathways, such as expression of the inhibitory immune receptors cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) or programmed death-1 (PD-1) after T cell activation (Pardoll 2012). CAR T cell therapy was improved in a Her-2 transgenic murine breast cancer model by dual treatment with anti-Her-2 T cells and an anti-PD-1 antibody (John et al. 2013). Treatment modalities combining CAR T cells with checkpoint inhibitors designed to target PD-1 and CTLA-4 may be more effective, but the potential adverse events must be carefully monitored.

The use of retroviral gene therapy to deliver genome editing tools represents a major technological advancement that combines efficient gene transfer with the potential for site-specific genetic modification. If we can safely harness the immense power of these molecular tools, cell and gene-specific therapies will become a reality, allowing improved treatment and possibly eradication of many cancers.

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