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REVIEW ARTICLE

Tumor-targeted fluorescence labeling systems for cancer diagnosis and treatment

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Abstract

Conventional imaging techniques are available for clinical identification of tumor sites. However, detecting metastatic tumor cells that are spreading from primary tumor sites using conventional imaging techniques remains difficult. In contrast, fluorescence-based labeling systems are useful tools for detecting tumor cells at the single-cell level in cancer research. The ability to detect fluorescent-labeled tumor cells enables investigations of the biodistribution of tumor cells for the diagnosis and treatment of cancer. For example, the presence of fluorescent tumor cells in the peripheral blood of cancer patients is a predictive biomarker for early diagnosis of distant metastasis. The elimination of fluorescent tumor cells without damaging normal tissues is ideal for minimally invasive treatment of cancer. To capture fluorescent tumor cells within normal tissues, however, tumor-specific activated target molecules are needed. This review focuses on recent advances in tumor-targeted fluorescence labeling systems, in which indirect reporter labeling using tumor-specific promoters is applied to fluorescence labeling of tumor cells for the diagnosis and treatment of cancer. Telomerase promoter-dependent fluorescence labeling using replication-competent viral vectors produces fluorescent proteins that can be used to detect and eliminate telomerase-positive tumor cells. Tissue-specific promoter-dependent fluorescence labeling enables identification of specific tumor cells. Vimentin promoter-dependent fluorescence labeling is a useful tool for identifying tumor cells that undergo epithelial–mesenchymal transition (EMT). The evaluation of tumor cells undergoing EMT is important for accurately assessing metastatic potential. Thus, tumor-targeted fluorescence labeling systems represent novel platforms that enable the capture of tumor cells for the diagnosis and treatment of cancer.

Abbreviations: Ad5, adenovirus type 5; Ad35, adenovirus type 35; AFP, alpha-fetoprotein; CAR, coxsackievirus and adenovirus receptor; CEA, carcinoembryonic antigen; CTC, circulating tumor cell; ctDNA, cell-free tumor DNA; EGFP, enhanced green fluorescent protein; EMT, epithelial–mesenchymal transition; EV, extracellular vesicle; FSP1, fibroblast-specific protein 1; GFP, green fluorescent protein; HCC, hepatocellular carcinoma; HSV1, human simplex virus type-1; hTERT, human telomerase reverse transcriptase; Id1, inhibitor of DNA binding 1; IL-1β, interleukin-1β; miRNA, microRNA; PDAC, pancreatic ductal adenocarcinoma; PDT, photodynamic therapy; PSA, prostate-specific antigen; PSES, prostate-specific enhancer sequence; PSMA, prostate-specific membrane antigen; RFP, red fluorescent protein; ROS, reactive oxygen species; SEAP, secreted embryonic alkaline phosphatase; TME, tumor microenvironment.

KEYWORDS
adenovirus, EMT, survivin, telomerase, vimentin

1 | INTRODUCTION

Tumor cells exhibit characteristics that increase the potential for malignancy, such as unlimited cell proliferation, chemoresistance, migratory and invasive abilities, and metastasis, which can lead to poor prognosis in cancer patients. Recent advances in antitumor techniques such as surgery, chemotherapy, radiotherapy, and immunotherapy have improved the clinical outcome of cancer patients. However, advanced cancers are often refractory to antitumor therapies and exhibit tumor recurrence, distant metastasis, and poor prognosis. Therefore, early diagnosis of cancer is one of the most prominent strategies to cure cancer patients.

A variety of medical imaging techniques are available to detect tumor sites in clinical practice, including X-rays, ultrasound, computed tomography, and magnetic resonance imaging. These techniques enable the detection of primary and metastatic tumors throughout the body.[1] Nuclear medicine techniques, including PET and single photon emission computed tomography, can be used in supportive fashion to confirm the malignant potential of tumor tissue using biomolecule-targeted radioactive isotopes.[1] These imaging techniques are useful for determining the anatomic localization of tumors and their invasiveness into surrounding normal tissues. However, detecting invasive and metastatic tumor cells that are spreading from primary tumors remains difficult using conventional imaging techniques. Therefore, novel imaging techniques useful for evaluating the biodistribution of metastatic tumor cells are needed.

Fluorescence-based labeling systems are powerful tools for detecting tumor cells at the single-cell level in cancer research. Various fluorescent proteins, including GFP and RFP, are widely used to visualize the behavior of tumor cells using intravital imaging techniques in animal experiments.[2] The detection of tumor cells using these fluorescence labeling systems enables investigations of the biodistribution of tumor cells for the diagnosis and treatment of cancer. For example, the presence of CTCs in the peripheral blood of cancer patients is a predictive biomarker for early diagnosis of distant metastasis. Liquid biopsy has recently emerged as a minimally invasive procedure for the detection of CTCs,[3] ctDNA,[3] miRNA,[4] and EVs[5] in body fluids. In contrast, the ability to eliminate tumor cells without damaging normal tissues would be ideal in minimally invasive treatment of cancer. Oncolytic virotherapy using tumor-specific replication-competent viruses has recently emerged as a novel antitumor technique to induce tumor-specific cell death without harming normal cells.[6] Replication-competent viral vectors carrying tumor-specific gene promoters can be further applied to the fluorescence labeling of CTCs in the peripheral blood of cancer patients.[7] Thus, fluorescence labeling systems are useful options for identifying tumor cells in the diagnosis and treatment of cancer.

2 | TUMOR-TARGETED FLUORESCENCE LABELING SYSTEMS

Indirect reporter labeling is one of the leading tumor-targeted fluorescence labeling systems that contribute to the diagnosis and treatment of tumors (Figure 1). Indirect reporter labeling induces the expression of genes encoding fluorescent proteins in tumor cells using tumor-specific gene promoters, which is useful for the diagnosis of cancer. Tumor-specific activated molecules are used to identify tumor cells in tumor-targeted fluorescence labeling systems. There are several types of intrinsic factors that are activated in a tumor-specific manner and related to malignancy potential. Unlimited cell proliferation is the most common feature of the vast majority of tumor cells, in which telomerase activity plays a central role in preventing replicative senescence through elongation of telomeres.[8] Activation of antiapoptotic survival pathways, such as survivin[9] and Id1,[10] inevitably contributes to chemoresistance. Detecting tumor markers such as PSA, CEA, and AFP in the serum of cancer patients is useful in the assessment of tissue-specific tumors.[11] Migration and invasiveness are well-known characteristics of metastatic tumor cells in association with EMT, which is a fundamental process that induces the mesenchymal phenotype in epithelial tumor cells.[12] Epithelial–mesenchymal signatures in tumor tissues are highly associated with

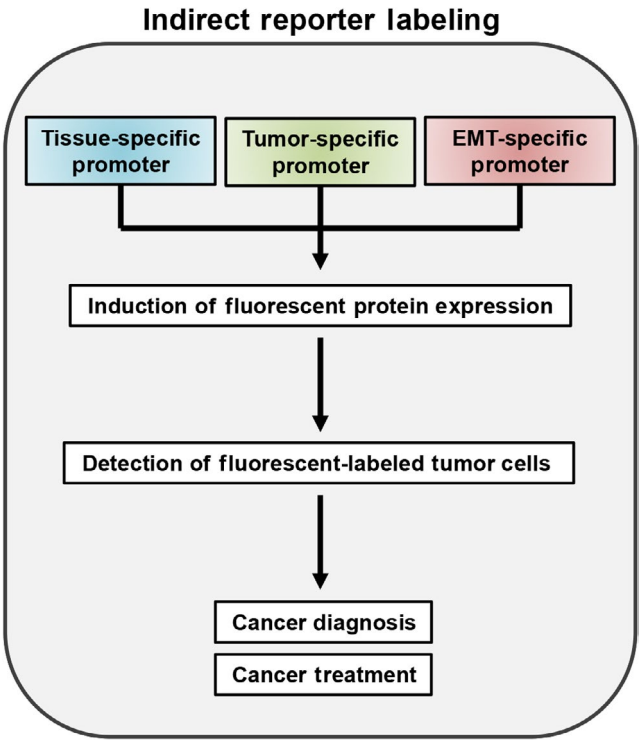

FIGURE 1 Tumor-targeted fluorescence labeling systems using indirect reporters. EMT, epithelial–mesenchymal transition

poor prognosis in patients with various types of cancers.[13–16] Given the tumor-promoting role of various intrinsic factors, these factors could be exploited as tumor-specific target molecules to identify tumor cells in the diagnosis and treatment of cancer.

Optical imaging techniques are needed to detect fluorescently labeled tumor cells in vivo. As several other reviews have summarized in vivo optical imaging techniques,[17,18] we focus here on fluorescence-based labeling systems that enable identification of tumor cells through the targeting of tumor-specific molecules. We summarize various types of fluorescence labeling systems based on tumor-specific activated molecules, such as telomerases, survivin, tumor markers, and the EMT process. Moreover, we discuss future perspectives regarding the clinical application of tumor-targeted fluorescence labeling systems for the diagnosis and treatment of cancer.

3 | TUMOR-SPECIFIC PROMOTER-DEPENDENT FLUORESCENCE LABELING

Indirect reporter labeling induces the expression of fluorescent proteins in tumor cells using tumor-specific promoters (Figure 1). There are several types of tumor-specific gene promoters that are used to regulate the expression of therapeutic transgenes in cancer gene therapy.[19] These tumor-specific gene promoters can be applied to the fluorescent labeling of tumor cells. Replication-competent viral vectors carrying tumor-specific gene promoters are useful tools for inducing the expression of fluorescent proteins in tumor cells.[20]

3.1 | Telomerase promoter-dependent fluorescence labeling

Telomerase is an enzyme that elongates the length of telomeres by repeated TTAGGG nucleotide sequences at the ends of a chromosome.[21] Most tumor cells exhibit high telomerase activity in association with unlimited cell proliferation, whereas most types of normal somatic cells exhibit no telomerase activity. Telomerase is a ribonucleoprotein complex consisting of two different subunits, a catalytic subunit (hTERT and telomerase-associated protein 1)[22,23] and an RNA subunit (human telomerase RNA component).[24] As hTERT expression is highly associated with telomerase activity in tumor cells,[25,26] we developed a telomerase-dependent replicative adenovirus, OBP-401 (TelomeScan), in which the *hTERT* gene promoter is inserted into the Ad5 genome to drive the expression of the adenoviral *E1A* and *E1B* genes for tumor cell-specific viral replication[27] (Figure 2 and Table 1). OBP-401 harbors the GFP expression cassette in the E3 region, enabling visualization of tumor cells as GFP-positive cells (Figure 2). The OBP-401-based GFP reporter labeling system is useful for the detection of CTCs in the peripheral blood.[28] When GFP-positive CTCs isolated by flow cytometry were analyzed using direct sequencing and allele-specific PCR, the mutation status in the *KRAS/BRAF* oncogenes was similarly detectable between primary tumor tissues and CTCs in patients with colorectal cancers.[29] Moreover, when intraoperative peritoneal wash collected for conventional cytology was infected with OBP-401, OBP-401 induced GFP-positive peritoneal tumor cells, which are associated with poor prognosis, especially in cytology-positive patients with gastric cancers.[30]

Although the OBP-401-based fluorescence labeling system is useful for detecting telomerase-positive tumor cells, there are some limitations to this labeling system. One limitation is the possibility of false-positive detection of populations of hematopoietic cells, such as $CD13^+$ $CD14^+$ monocytes, which are detected as GFP-positive cells in the peripheral blood.[31] Another limitation is false-negative results due to missed detection of populations of tumor cells lacking CAR expression. To address these limitations, Sakurai et al.[32] developed a modified OBP-401 (OBP-1101, TelomeScan F35), in which the target sequences of miR-142-3p are inserted into the E1 and E3 regions and the fiber of Ad5 is replaced with that of Ad35 (Figure 2 and Table 1). As miR-142-3p is ubiquitously expressed in various types of hematopoietic cells, including monocytes,[33,34] OBP-1101-mediated E1 and GFP expression is attenuated in miR-142-3p-positive hematopoietic cells,[32] leading to an improvement in tumor-specific fluorescent labeling. Although Ad5 can bind to CAR on the surface of target cells,[35] Ad35 binds with greater affinity to CD46, which is expressed on a variety of tumor cells more ubiquitously than CAR. OBP-1101 with Ad35 fibers can infect CAR-negative tumor cells by binding to CD46,[32] leading to an improvement in viral infectivity. Togo et al.[36] reported that the OBP-1101-based fluorescence labeling system is a promising tool for detecting highly malignant CTCs expressing the mesenchymal marker vimentin in non-small-cell lung cancer patients. OBP-1101 thus appears to be superior to OBP-401 in terms of inducing tumor-specific GFP expression.

Herpes simplex virus type-1 can be also utilized as a tool to induce tumor-specific GFP expression using the hTERT promoter. Zhang et al.[37] developed a telomerase-dependent replicative herpes simplex virus, oHSV1-hTERT-GFP, in which the endogenous *ICP4* promoter is replaced with the *hTERT* gene promoter to drive the expression of *ICP4* for tumor-specific viral replication (Figure 2 and Table 1). oHSV1-hTERT-GFP contains the GFP expression cassette in the ICP34.5 region, thus enabling visualization of tumor cells as GFP-positive cells. The oHSV-hTERT-GFP-based GFP induction system is useful for detecting CTCs in the peripheral blood of cancer patients.[38]

Human telomerase reverse transcriptase promoter-driven replicative viruses have emerged as not only novel diagnostic tools but also novel antitumor methods for cancer treatment.[39] OBP-301 (suratadenoturev), the original virus of OBP-401, exhibits a broad spectrum of antitumor efficacy against malignant tumor cells with telomerase activity.[40] OBP-401 also exhibits antitumor effects against a variety of cancers, including lung cancer,[41] colon cancer,[41] breast cancer,[42] and gastric cancer.[43] Intraperitoneal injection of OBP-401 was shown to enhance the therapeutic efficacy of chemotherapeutic agents in mice with intraperitoneal metastasis of gastric cancer.[43] In contrast, PDT is a minimally invasive antitumor

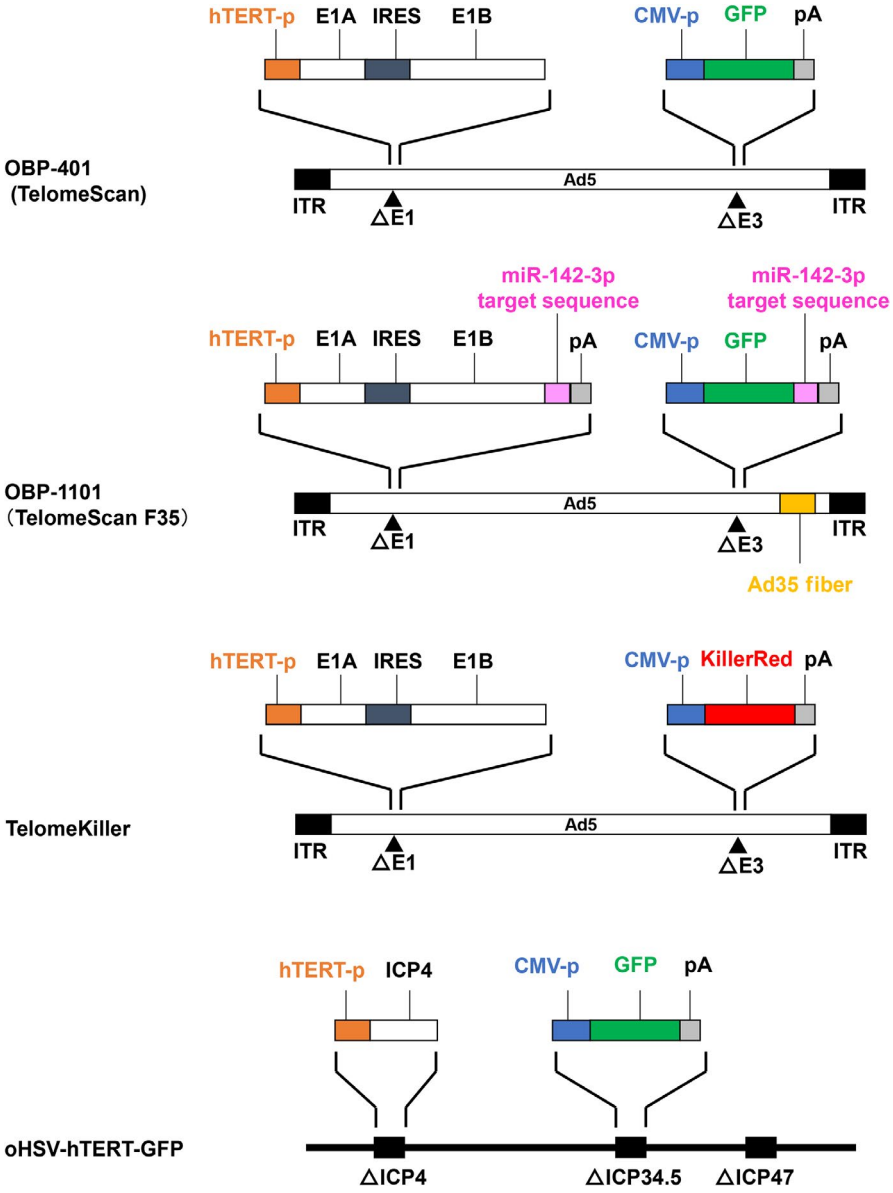

FIGURE 2 Fluorescence labeling systems using telomerase promoter-dependent replicative viruses expressing fluorescent proteins. Ad5, adenovirus type 5; Ad35, adenovirus type 35; CMV, cytomegalovirus; GFP, green fluorescent protein; hTERT, human telomerase reverse transcriptase; IRES, internal ribosome entry site; ITR, inverted terminal repeat; miR, microRNA

technique for inducing tumor-specific cytotoxicity through the induction of photosensitizer-mediated generation of ROS upon light irradiation.[44,45] For the application of hTERT promoter-driven replicative viruses to PDT, we generated a telomerase-specific replicative photodynamic viral agent known as TelomeKiller, which harbors the KillerRed expression cassette in the E3 region, enabling elimination of tumor cells through KillerRed-mediated generation of ROS upon green-light irradiation[46,47] (Figure 2 and Table 1). Thus, hTERT promoter-driven viral vectors are useful tools for detecting and eradicating telomerase-positive tumor cells.

3.2 | Survivin promoter-dependent fluorescence labeling

Survivin is an inhibitor of apoptosis and plays a central role in the survival of cancer cells by inhibiting therapy-induced cell death.[9] Survivin expression is highly upregulated in a variety of tumor cells at the transcriptional level,[9] suggesting that the survivin gene promoter is a universal tumor-specific promoter, similar to the *hTERT* gene promoter. Kamizono et al.[48] showed that the survivin promoter is useful for the tumor-specific replication of oncolytic adenoviruses. For the fluorescent labeling of survivin-positive tumor cells, Seo et al.[49] developed a survivin promoter-specific replicative adenovirus, Ad5/35E1apsurvivinE4, in which the survivin promoter is inserted into the Ad5 genome to drive expression of the adenoviral *E1A* and *E4* genes for survivin-specific virus replication (Figure 3 and Table 1). Ad5/35E1apsurvivinE4 encodes the GFP expression cassette and Ad35 fiber gene, thus enabling visualization of survivin-positive cancer cells independent of CAR expression (Figure 3). In contrast, Yamamoto et al.[50] generated a survivin promoter-driven replicative adenovirus, AdSur-SYE, in which the E1 promoter is replaced with survivin promoter for survivin-specific virus replication (Figure 3 and Table 1). The fiber

TABLE 1 Summary of tumor-specific or tissue-specific promoter-dependent adenoviruses expressing fluorescent proteins

No.	Promoter	Virus	Origin	Fluorescent protein	Fiber	Application	References
1	hTERT	OBP-401 (TelomeScan)	Ad5	GFP	Ad5	Detection of CTC and DTC	25-28
2	hTERT	OBP-1101 (TelomeScan F35)	Ad5	GFP	Ad35	Detection of CTC	30, 34
3	hTERT	TelomeKiller	Ad5	KillerRed	Ad5	Elimination of tumor cell	44, 45
4	Survivin	Ad5/35E1apsurvivinE4	Ad5	GFP	Ad35	Detection of CTC	47
5	Survivin	AdSur-SYE	Ad5	EGFP	SYENFSA ligand	Detection of PDAC cell	48, 49
6	Survivin	CRAd5/11-Sp-eGFP	Ad5	EGFP	Ad5/11	Elimination of glioma cell	50
7	Id1	Ad5/3-Id1-SEAP-Id1-mCherry	Ad5	mCherry	Ad5/3	Detection of tumor cell	53, 54
8	PSES	Ad5/35E1aPSESE4	Ad5	GFP	Ad35	Detection of PC cell	55, 56
9	CEA	AdCEAp-EGFP	Ad5	EGFP	Ad5	Detection of PDAC cell	59
10	AFP	Ad/Ha2bm-GFP	Ad5	GFP	Ad5	Detection of HCC cell	63

Abbreviations: Ad5, adenovirus type 5; Ad35, adenovirus type 35; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; CTC, circulating tumor cell; DTC, disseminated tumor cell; EGFP, enhanced green fluorescent protein; GFP, green fluorescent protein; HCC, hepatocellular carcinoma; hTERT, human telomerase reverse transcriptase; Id1, inhibitor of DNA binding 1; PC, prostate cancer; PDAC, pancreatic ductal adenocarcinoma; PSES, prostate-specific enhancer sequence.

of AdSur-SYE contains the SYENFSA ligand, which is sequence identified to bind with greater affinity to human pancreatic cancer cells.[51] As AdSur-SYE harbors the EGFP expression cassette in the E3 region, the AdSur-SYE-based EGFP induction system is highly effective for targeting pancreatic cancer cells. In addition, Li et al.[52] developed a survivin promoter-specific replicative adenovirus, CRAd5/11-Sp-eGFP, which expresses EGFP and chimeric Ad5/11 fiber consisting of an Ad5 tail and an Ad11 shaft and knob (Figure 3 and Table 1). CRAd5/11-Sp-eGFP efficiently detects and eliminates survivin-positive glioma cells. Survivin promoter-driven viral vectors are thus useful tools for the detection and elimination of survivin-positive tumor cells.

3.3 | Inhibitor of DNA binding 1 promoter-dependent fluorescence labeling

Inhibitor of DNA binding 1 is a member of the helix–loop–helix transcription factor family of proteins that control a variety of cellular processes, including cell proliferation and cell cycle regulation.[53] As expression of Id1 mRNA and protein is increased in multiple types of cancer,[54] the Id1 promoter is a promising candidate for a universal tumor-specific promoter. Warram et al.[55] developed a dual reporter adenoviral vector, Ad5/3-Id1-SEAP-Id1-mCherry, in which the Id1 promoter is inserted into the E1- and E3-deleted Ad5 genome to drive expression of the SEAP enzyme for blood-based screening and the fluorescent reporter mCherry for detecting Id1-positive tumor cells (Table 1). Ad5/3-Id1-SEAP-Id1-mCherry expresses a hybrid Ad5/3 fiber for improved infectivity of CAR-negative tumor cells by binding to CD46. Infection with Ad5/3-Id1-SEAP-Id1-mCherry results in the secretion of SEAP and mCherry by human breast and prostate cancer cells.[55,56] This dual reporter system is a unique and useful approach for detecting Id1-positive tumor cells using blood-based screening and tumor visualization.

4 | TISSUE-SPECIFIC PROMOTER-DEPENDENT FLUORESCENCE LABELING

Tissue-specific promoters are useful for inducing the expression of fluorescent proteins in some tumor cells (Figure 1). Tissue-specific gene promoters are used to drive the expression of therapeutic transgenes in cancer gene therapy.[19] Replication-competent viral vectors with tissue-specific gene promoters are also useful tools for inducing the expression of fluorescent proteins in tumor cells.[20]

4.1 | Prostate-specific promoter-dependent fluorescence labeling

Prostate-specific antigen and PSMA are highly sensitive markers for prostate cancer. To target prostate cancer cells in gene therapy, Lee

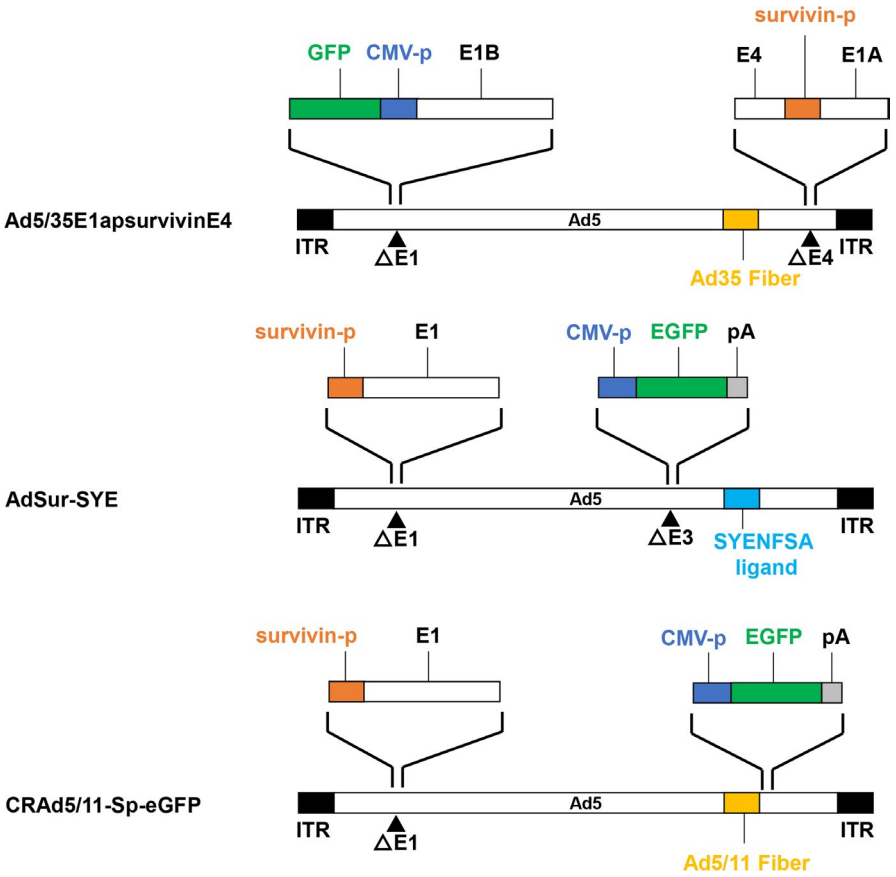

FIGURE 3 Fluorescence labeling systems using survivin promoter-dependent replicative viruses expressing fluorescent proteins. Ad5, adenovirus type 5; Ad35, adenovirus type 35; CMV, cytomegalovirus; EGFP, enhanced green fluorescent protein; GFP, green fluorescent protein; hTERT, human telomerase reverse transcriptase; IRES, internal ribosome entry site; ITR, inverted terminal repeat; miR, microRNA

et al.[57] developed a PSES, which is a chimeric enhancer derived from the promoter of the *PSA* and *PSMA* genes. For the fluorescent labeling of prostate cancer cells, Hwang et al.[58] developed a prostate-specific replicative adenovirus, Ad5/35E1aPSESE4, in which the *PSES* gene is inserted into the Ad5 genome to drive expression of the adenoviral *E1A* and *E4* genes for prostate-specific virus replication (Table 1). Ad5/35E1aPSESE4 encodes the GFP expression cassette and Ad35 fiber gene, thereby enabling visualization of prostate cancer cells independent of CAR expression (Table 1). The Ad5/35E1aPSESE4-based GFP induction system is useful for detecting CTCs in the peripheral blood of prostate cancer patients.[58] Prostate promoter-driven viral vectors are thus useful tools for detecting prostate tumor cells.

4.2 | Carcinoembryonic antigen promoter-dependent fluorescence labeling

Pancreatic ductal adenocarcinoma is a highly lethal disease, with a 5-year survival rate of less than 10%.[59] Tools enabling early diagnosis of PDAC are needed to improve the clinical outcome of PDAC patients. Carcinoembryonic antigen is the standard serum tumor marker for assessing a variety of gastrointestinal cancers, including pancreatic cancer.[60] The secretion of CEA to the extracellular environment is highly associated with CEA promoter activity in PDAC cells.[61]

To target CEA-positive PDAC cells, Xu et al.[61] developed CEA promoter-specific replicative adenoviruses expressing EGFP (AdCEAp-EGFP) or heat shock protein 70 (AdCEAp-Hsp70), in which the CEA promoter-driven E1A expression cassettes are inserted into the Ad5 genome (Table 1). AdCEAp-EGFP induces EGFP expression to enable visualization of CEA-positive PDAC cells, whereas AdCEAp-Hsp70 induces cytopathic effects useful in the treatment of CAE-positive PDAC cells. Thus, CEA promoter-driven viral vectors are promising tools for detecting and eliminating CEA-positive PDAC cells.

4.3 | Alpha-fetoprotein promoter-dependent fluorescence labeling

Hepatocellular carcinoma is the most common type of liver cancer.[62] Alpha-fetoprotein is widely used as serum tumor marker for the diagnosis of HCC.[63] Alpha-fetoprotein expression is highly associated with clinical stage, early recurrence, and poor prognosis in HCC patients.[64] For the fluorescence labeling of AFP-positive HCC cells, Yoon et al.[65] developed an AFP promoter-specific non-replicative adenovirus carrying the *GFP* gene (Ad/Ha2bm-GFP) (Table 1). Ad/Ha2bm-GFP encodes the GFP expression cassette under the control of a modified AFP promoter containing two types of enhancer regions and hypoxia-responsive elements. Ad/Ha2bm-GFP efficiently induces GFP expression in AFP-positive HCC cells even under hypoxic conditions. Thus, AFP promoter-driven viral vectors are useful for detecting AFP-positive HCC cells.

5 | EPITHELIAL–MESENCHYMAL TRANSITION-DEPENDENT FLUORESCENCE LABELING

Invasion and metastasis are hallmarks of cancer. The processes of invasion and metastasis are highly associated with the EMT program, which is a fundamental process by which epithelial tumor cells acquire mesenchymal characteristics with invasive and metastatic potential.[12] In tumor cells undergoing EMT, mesenchymal markers such as N-cadherin and vimentin are upregulated, whereas epithelial markers such as E-cadherin and cytokeratin are downregulated. The EMT program is cooperatively regulated by several intrinsic factors, including EMT-activating transcription factors and EMT-suppressive miRNAs.[66] As EMT signatures are highly associated with poor prognosis in patients with a variety of cancers,[13–16] the evaluation of tumor cells undergoing EMT is important to accurately assess metastatic potential. Indirect reporter labeling systems based on EMT-dependent promoters are available to induce the expression of fluorescent proteins in tumor cells undergoing EMT (Figure 1).

5.1 | Irreversible EMT-dependent fluorescence labeling

For the fluorescence labeling of tumor cells undergoing EMT, Fischer et al.[67] developed a transgenic mouse model using a fibroblast-specific marker-dependent fluorescence switching system, in which the FSP1 promoter drives the Cre-lox recombinase system to induce the RFP-to-GFP conversion in tumor cells undergoing EMT (Figure 4). Zhao et al.[68] used transgenic mice with the FSP1-dependent fluorescence switching system to demonstrate that tumor cells undergoing EMT localize in the area of tumors close to blood vessels. In contrast, in experiments using the FSP1-dependent fluorescence switching system, Bornes et al. reported that a subpopulation of pre-EMT RFP-positive cells with low epithelial marker expression exhibited greater metastatic potential than post-EMT GFP-positive cells.[69] Lourenco et al.[70] also showed that pre-EMT RFP-positive cells play a more dominant role in lung metastasis than post-EMT GFP-positive cells. Recent evidence also implicates partial/hybrid EMT cells that possess both epithelial and mesenchymal characteristics due to EMT plasticity in the metastatic process.[66,71] As Cre-lox recombinase-based fluorescence labeling is irreversible, this technique might be inadequate for the real-time labeling of EMT plasticity in tumor cells.

5.2 | Reversible EMT-dependent fluorescence labeling

The plasticity of the EMT program makes it difficult to detect tumor cells undergoing EMT during the metastasis process. Therefore, fluorescence labeling systems that enable reversible visualization of tumor cells undergoing EMT are needed. To investigate the plasticity of the EMT program in tumor cells, we developed a novel fluorescence labeling system using the VRV3 vector, in which the vimentin promoter-dependent RFP expression cassette encodes the 3′-UTR of vimentin[72] (Figure 4). The vimentin promoter-dependent RFP expression vector lacking the 3′-UTR of vimentin induces RFP expression in non-EMT tumor cells, whereas addition of the 3′-UTR of vimentin enables reversible visualization of EMT-dependent RFP expression in human colorectal cancer HCT116 and RKO cells.[72] Treatment with inflammatory cytokines, tumor necrosis factor-α, and IL-1β induced EMT-related RFP expression in HCT116-VRV3 and RKO-VRV3 cells (Figure 4). Conversely, removal of inflammatory cytokines reduced RFP expression in HCT116-VRV3 and RKO-VRV3 cells, indicating that this labeling system is reversible. In vivo experiments using HCT116-VRV3 cells indicated that RFP-positive tumor cells undergoing EMT localized within primary and metastatic tumor areas harboring IL-1β-positive inflammatory macrophages.[72] Consistent with HCT116-VRV3 and RKO-VRV3 cells, the ATCC recently established several EMT-reporter cell lines, including A549 VIM RFP (CCL-185EMT), HCT116 VIM RFP (CCL-247EMT), and MD-MB-231 VIM RFP (HTB-26EMT). These cells were generated by inserting the RFP reporter gene before the stop codon in the last exon of the *vimentin* gene using genome editing[73] (Figure 4). Treatment with miR-200 inhibitor induced expression of the VIM-RFP fusion protein in HCT116 VIM RFP cells (Figure 4). These vimentin promoter-dependent fluorescence labeling systems are useful tools for exploring the underlying mechanism of EMT regulation in tumor cells.

6 | FUTURE PERSPECTIVES

Telomerase-targeted fluorescence labeling systems using viral vectors are useful tools for detecting tumor cells in the peripheral blood of cancer patients. Although the presence of CTCs is a predictive biomarker for the early diagnosis of distant metastasis,[3] it can be difficult to detect the small population of tumor cells in all cancer patients. Several types of biomarkers, such as ctDNA,[3] miRNA,[4] and EVs,[5] have recently emerged as liquid biopsy markers to evaluate the malignant potential of cancer patients. Recent reports have suggested that the premetastatic niche, which is a metastasis-supportive microenvironment, is established by primary tumor-derived EVs in secondary organs before the metastatic colonization by CTCs.[74] Assessing both CTCs and EVs could be more useful for the surveillance of metastatic potential in all cancer patients.

Tumor-targeted replicative viruses expressing fluorescent proteins enable the visualization of tumor cells within normal tissues, which enhances the eradication of tumor cells when combined with various antitumor therapies. Intratumoral injection of the telomerase-specific replicative adenovirus OBP-401 induces the fluorescence labeling of primary tumors and tumor cells metastasized to lymph nodes, which facilitates the surgical resection of tumor areas using fluorescence-guided surgery, thereby preventing

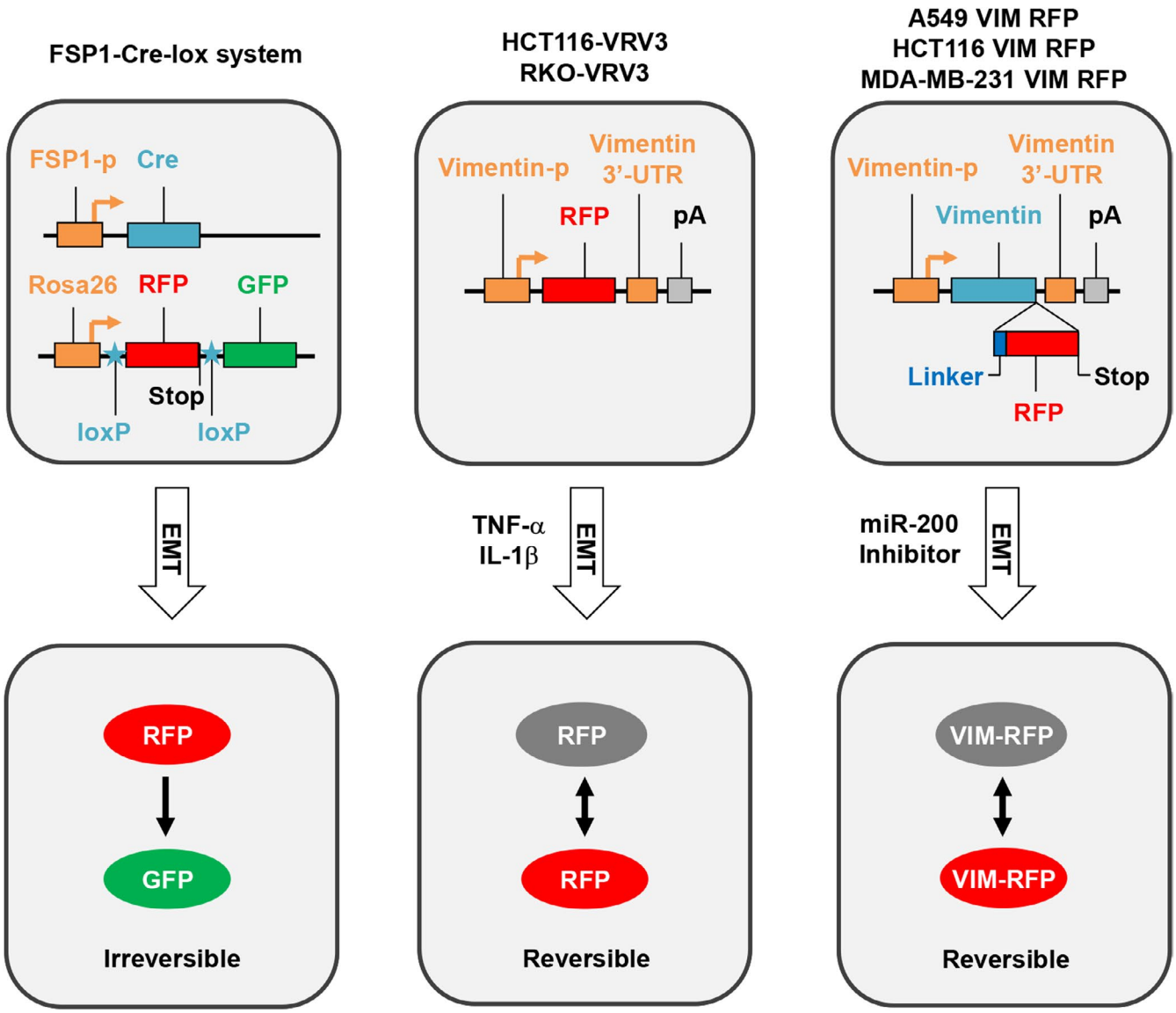

FIGURE 4 Fluorescence labeling systems using epithelial–mesenchymal transition-specific promoter-dependent fluorescent probes. EMT, epithelial-mesenchymal transition; FSP1, fibroblast-specific protein 1; GFP, green fluorescent protein; IL-1β, interleukin-β; miR, microRNA; RFP, red fluorescent protein; TNF-α, tumor necrosis factor-α; UTR, untranslated region; VIM, vimentin

tumor recurrence.[75,76] In contrast, intraperitoneal administration of OBP-401 enables the fluorescence labeling of intraperitoneally disseminated tumor cells, which enhances the sensitivity to chemotherapeutic agents by induction of mitotic catastrophe.[43] Therefore, tumor-specific replicative viruses expressing fluorescent proteins could provide promising options to treat fluorescent-labeled tumor cells by combining antitumor techniques.

Vimentin-targeted fluorescence labeling systems offer opportunities to investigate the behavior of tumor cells undergoing EMT during tumor progression. The detection of tumor cells undergoing EMT is suggestive of the involvement of EMT-inducing factors within surrounding normal tissues. Takahashi et al.[77] recently reported a tissue-clearing technology that is useful for visualizing the cell cycle status of primary and metastatic tumor cells in nude mice using human cancer cells stably expressing a fluorescent ubiquitination-based cell cycle indicator. This tissue-clearing method could be useful for analyzing the biodistribution of metastatic tumor cells undergoing EMT in mice using vimentin-targeted fluorescence labeling systems. In contrast, although EMT features are highly associated with poor prognosis in cancer patients, efforts to develop anti-EMT therapies have thus far been unsuccessful. Zhao et al.[78] recently reported that 3D organoid culture systems using mesenchymal breast cancer are useful for the screening of EMT-reversing drugs by evaluating the resulting morphological changes. Using vimentin promoter-dependent fluorescence labeling systems, it is possible to identify EMT-inducing factors within the TME and thereby candidate reagents for anti-EMT therapy. Vimentin promoter-dependent fluorescence labeling systems could be useful options for evaluating the potential of both EMT-inducing factors and EMT-inhibiting drugs.

7 | CONCLUSIONS

Fluorescence-based labeling systems using several types of tumor-specific target molecules enable the visualization of tumor cells as a means of investigating their biodistribution within normal tissues. The detection of metastatic tumor cells in the peripheral blood of cancer patients is a predictive biomarker for the surveillance of early metastasis. The characterization of EMT tumor cells could facilitate elucidation of the underlying mechanism of metastatic potential acquisition in association with the TME. The development of novel antitumor techniques using tumor-specific target molecules is an ideal minimally invasive strategy to treat cancer patients without affecting normal tissues. Although a number of obstacles remain in investigating the biodistribution of tumor cells in the human body using optical imaging techniques, tumor-targeted fluorescence labeling systems are promising novel platforms for detecting tumor cells in the diagnosis and treatment of cancer.

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CONFLICTS OF INTEREST

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