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Immunotherapeutic organoids
A new approach to cancer treatment

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Abbreviations: AAV, adeno-associated virus; bsAb, bispecific antibody; CEA, carcinoembryonic antigen; ECM, extracellular matrix; HSC, hematopoietic stem cells; mAbs, monoclonal antibodies; MSC, mesenchymal stem cells; scFv, single-chain antibody fragment

Therapeutic monoclonal antibodies have revolutionized the treatment of cancer and other diseases. However, several limitations of antibody-based treatments, such as the cost of therapy and the achievement of sustained plasma levels, should be still addressed for their widespread use as therapeutics. The use of cell and gene transfer methods offers additional benefits by producing a continuous release of the antibody with syngenic glycosylation patterns, which makes the antibody potentially less immunogenic. In vivo secretion of therapeutic antibodies by viral vector delivery or ex vivo gene modified long-lived autologous or allogeneic human mesenchymal stem cells may advantageously replace repeated injection of clinical-grade antibodies. Gene-modified autologous mesenchymal stem cells can be delivered subcutaneously embedded in a non-immunogenic synthetic extracellular matrix-based scaffold that guarantees the survival of the cell inoculum. The scaffold would keep cells at the implantation site, with the therapeutic protein acting at distance (immunotherapeutic organoid), and could be retrieved once the therapeutic effect is fulfilled. In the present review we highlight the practical importance of living cell factories for in vivo secretion of recombinant antibodies.

Introduction

Monoclonal antibodies (mAbs) have revolutionized the field of biology and medicine since their first description in 1975.[1] However, the development of therapeutic monoclonal antibodies has been complicated by a number of technical challenges including the appearance of immunogenic responses against murine antibody domains, and their inability to trigger human effector functions.[2] These drawbacks were overcome initially by the generation of chimeric and humanized antibodies and now can be completely avoided by using fully human antibodies.[2]

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However, several limitations hamper native mAb-based treatments, such as low tumor-to-blood ratio, due to long serum half-life and limited tissue penetration, and the need for high doses over a long period of time. There is a wide range of different recombinant antibodies fragments with differences in molecular weight, valence, specificity and format. Thus, half-life and tumor penetration can be fine-tuned by adjusting these parameters.[3] There remain, however, at least two major concerns: the extremely high cost of therapy and the achievement of sustained plasma levels, since the recommended dosage and administration involve repeated bolus injections, with fluctuating plasma concentrations ranging from subtoxic to subtherapeutic.

In Vivo Secretion of Therapeutic Antibodies

Gene therapy has the potential to overcome some of the shortcomings associated with conventional bolus protein therapy by producing a sustained release of the antibody with syngenic glycosylation patterns, that makes the antibody potentially less immunogenic and better tolerated.[4] Two main approaches to gene therapy include in vivo and ex vivo gene transfer methods (**Fig. 1**). In vivo gene therapy implies direct injection of genetic material into the human body, usually by using viral vectors. Ex vivo gene therapy involves modifying target cells, prior to implanting these into the tissues of the living body.

In Vivo Secretion of Full-Length mAbs

Pioneering work by Noel et al.[5] demonstrated that several types of non-lymphoid cells have the ability to secrete full-length IgG antibodies in vitro after retroviral gene transfer. Furthermore, implantation of ex vivo retrovirally-modified myoblasts resulted in detectable mAb serum levels (~1–3 μg/ml) for long periods of time. Four years later, the same group demonstrated that in vivo administration of high doses of a recombinant adenovirus encoding the same antibody gene resulted in a 100- to 200-fold increase in mAb serum levels (~200 μg/ml). However, adenoviral vectors are highly immunogenic and trigger an innate immune response that reduces therapeutic effect and causes inflammation-related side effects.[6,7] On the other hand, adeno-associated virus (rAAV) is a weak innate immunogen and it does not elicit

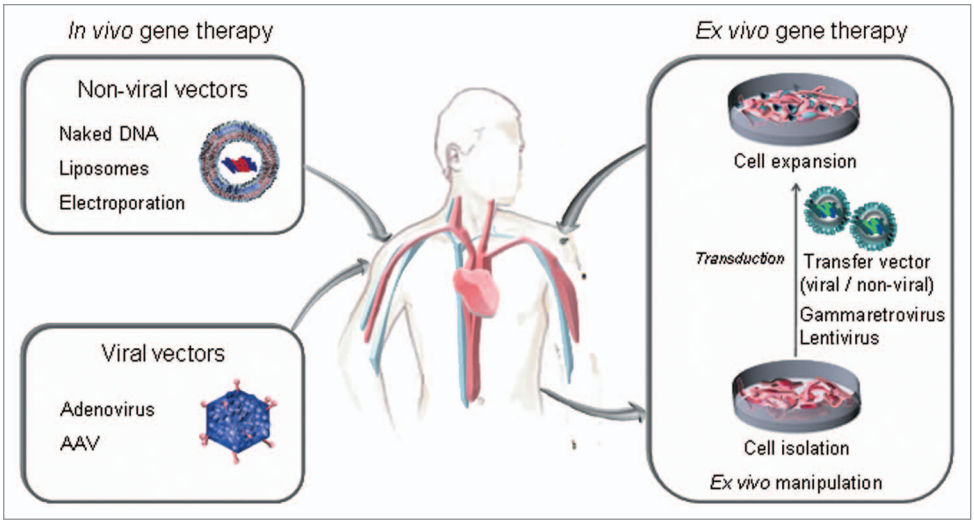

Figure 1. Strategies for in vivo secretion of therapeutic antibodies: direct injection of genetic material using non-viral or viral vectors (in vivo gene therapy), and implantation of genetically modified cells (ex vivo gene therapy).

the immune response observed for adenoviral vectors, although both type of viral vectors share the drawback of prevalence of neutralizing antibodies in the human population.[8] Using this expression system, Fang et al.[9] generated a rAAV serotype 8 encoding a full-length VEGFR-2 neutralizing mAb (DC101). The mAb is expressed from a single open reading frame by linking the heavy and light chains with a self-processing peptide 2A derived from the foot-and-mouth disease virus. A furin cleavage site was introduced to remove 2A-derived residues. A single dose of rAAV8-DC101 resulted in long-term expression of high-levels (> 1,000 μg/ml) of mAb, demonstrating significant anti-tumor efficacy. Watanabe et al.[10] reported that adenoviral vectors and rAAV encoding a full-length anti-VEGF mAb equivalent to bevacizumab (Avastin®) effectively suppresses the growth of human tumors.

Sustained high serum levels of a full-length anti-HER2 (also referred to as HER2/neu or ErbB-2) mAb have also been reported after intramuscular administration of a rAAV vector incorporating the furin/2A technology for monocistronic expression of both heavy and light chains. This strategy achieved significant tumor growth inhibition when rAAV was administered prior to tumor challenge, and demonstrated antitumor efficacy against pre-established tumors when AAV was administered up to 20 d after tumor challenge.[11] Also, long-term therapeutic levels of an anti-HER2 mAb have been documented after a single intravenous injection of an AAV vector based on the non-human primate AAV serotype rh.10 containing the furin/2A expression system, which reduced the growth of HER2 positive tumors and increased the survival of tumor-bearing mice.[12]

A different strategy for cancer therapy used a systemically administered bidirectional lentiviral vector for the in vivo secretion of a full-length anti-Met mAb. This approach resulted in substantial inhibition of tumor growth.[13] Recently, Balazs et al.[14] showed that a single intramuscular injection in mice of a specialized AAV vector containing a self-processing 2A sequence induces lifelong expression of high concentrations of a HIV neutralizing full-length mAb (b12), and it is possible to reach sustained protection against HIV infection.

In Vivo Secretion of Novel Recombinant Antibody Formats

In an attempt to improve tumor penetration, recombinant antibodies with modified properties have been generated. Novel antibody formats, such as the single-chain antibody (scFv), exhibit better pharmacokinetics than intact IgG.[3] However, scFv antibodies exhibit rapid blood clearance and poor retention times on the target owing to small sizes and monovalent binding properties, which results in the necessity of frequent delivery of such fragments.[3] To circumvent these limitations, several gene therapy approaches have been developed to express antibody fragments in vivo.

In 2002, Arafat et al.[15] demonstrated for the first time the therapeutic effect of a scFv secreted by eukaryotic cells. Effective concentrations of scFv were achieved following in vivo administration of an adenoviral vector expressing an anti-erbB2 scFv. Furthermore, in vivo gene transfer via the anti-erbB2 scFv encoding adenovirus resulted in substantial inhibition of tumor growth. A few months later Sanz et al. demonstrated that in vivo secretion of the L36 scFv,[16] that recognizes an angiogenesis-associated laminin epitope,[17] inhibited tumor growth in vivo.[18] In 2006, the same group reported that genetically modified human cells efficiently secreted trivalent and hexavalent antibodies, based on fusion of L36 scFv to different portions of the C-terminal noncollagenous domain of collagen XVIII.[19] In vivo secretion of the multivalent L36 antibodies was more effective in preventing tumor growth than the monomeric scFv counterpart.[19]

Afanasieva et al.[20] demonstrated that a single systemic administration of recombinant adenovirus encoding an anti-VEGF scFv (V65 scFv) or bivalent derivatives (minibody and scFv-Fc) resulted in tumor inhibition and had a therapeutic effect equivalent to that of multiple injections of high amounts of purified V65 scFv. Systemic administration of an adenoviral vector has also been used to deliver in vivo an immunotoxin comprising an anti-HER2 scFv as targeting moiety.[21]

Bispecific antibodies (bsAbs) represent promising approaches to more efficacious antitumor therapy.[22] BsAbs targeting tumor-associated antigens (TAA) and effector cell trigger molecules have been generated and shown to redirect cellular cytotoxicity toward target cells.[2,23,24] The potential of T-cell activating anti-TAA x anti-CD3 bsAbs in cancer therapy has been demonstrated in a variety of in vitro and in vivo models and several bsAbs

have been tested in clinical trials.[22] In fact, an anti-EpCAM x anti-CD3 full-length IgG (catumaxomab) has been approved for intraperitoneal treatment of malignant ascites.[25,26] However, maximum tolerated dose is low due to the toxicity caused by induction of 'cytokine storm', a consequence of cross-linking of T cells with accessory cells bearing Fc receptors, followed by cytokine release-related symptoms.

For these reasons, it is highly recommended using the use of recombinant Fc-lacking bsAbs such as tandem scFv [$(scFv)_2$] and diabodies. Numerous studies have demonstrated the potency of these formats in preclinical and clinical studies.[27,28] However, these recombinant bsAbs present a very short serum half-life and must be administered by continuous infusion using portable minipumps.

In 2003, Blanco et al.[29] demonstrated for the first time therapeutic effect of a recombinant Fc-lacking anti-carcinoembryonic antigen (CEA) x anti-CD3 two-chain diabody secreted by mammalian cells. They generated a bicistronic vector that enable the secretion of functionally active diabody by gene-modified human cells, and promoted unstimulated human primary T cells to proliferate and kill CEA-expressing cancer cells. Importantly, locally produced diabody showed significant cytotoxic activity in vivo against established tumors.[29] Four years later, the same group generated a bicistronic lentiviral vector and demonstrated that primary human lymphocytes can be efficiently transduced to secrete high levels of functional anti-CEA × anti-CD3 diabody. Importantly gene-modified lymphocytes significantly reduced in vivo tumor growth rates in xenograft studies.[30]

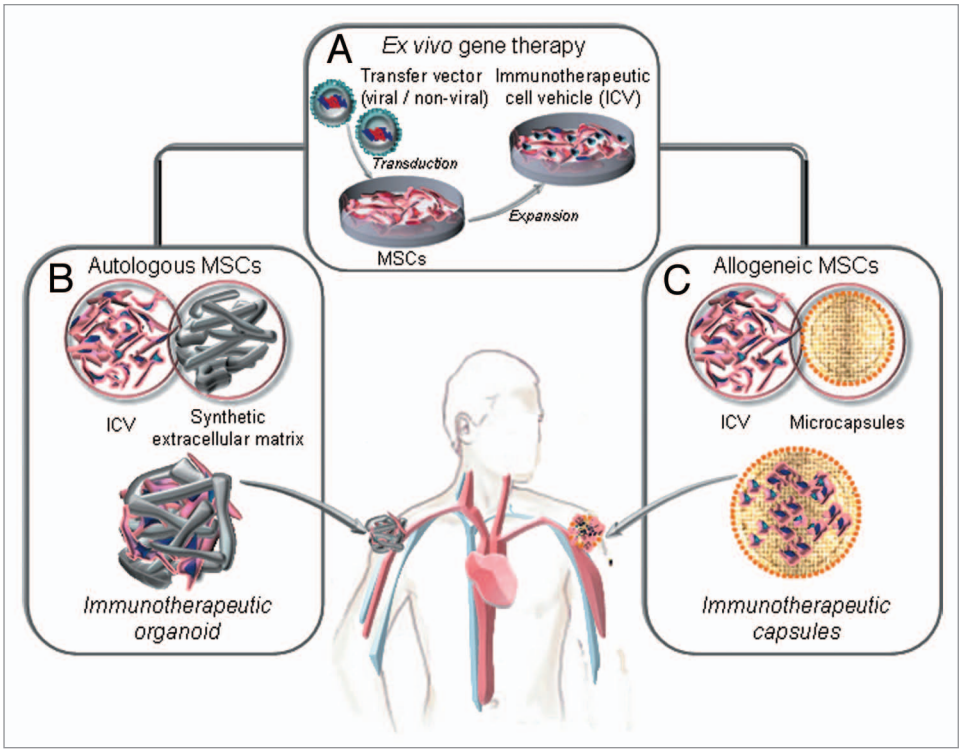

Figure 2. Ex vivo generation of genetically modified mesenchymal stem cells (MSC) as factories for long-term in vivo secretion of immunotherapeutic proteins. (**A**) Ex vivo gene therapy of autologous or allogeneic MSC (isolation, expansion and lentiviral transduction) to generate an immunotherapeutic cell vehicle (ICV). (**B**) The autologous ICV is embedded in a non-immunogenic synthetic extracellular matrix and implanted by subcutaneous injection (immunotherapeutic organoids). The allogenic ICV is microencapsulated and implanted by subcutaneous injection (immunotherapeutic microcapsules).

Ex Vivo Gene-Modified Cells as Factories for Long-Term In Vivo Secretion of Therapeutic Proteins

The application of ex vivo gene-modified human cells for in vivo secretion of therapeutic proteins offers several advantages over viral vector-mediated in vivo gene therapy. Viral vectors are highly efficient as gene delivery vehicles, but raise concerns about safety and limitation of the effect due to immune response. The use of ex vivo gene-modified cells as "factories or biological pumps" eliminates the risk of non-specific diffusion, allows in vitro quantification of protein secretion, and offers the possibility of selection for high-level expression clones prior to administration. Furthermore, cells can be retrieved if administered in certain formats, and/or armed with a "suicide" gene (e.g., HSV thymidine kinase), which can be activated to ensure the destruction of the cell inoculum.

Terminally differentiated mature cells possess a short lifespan, and this implies an obvious limitation to their application in cell-based gene therapy strategies for cancer immunotherapy. In contrast, stem/progenitor cells have greater expansion capacity and constitute a more appropriate cellular source. Since the advent of gene therapy, hematopoietic stem cells (HSC) have been a delivery cell of choice. However, the ex vivo expansion of adult HSC is expensive and time-consuming, and they are difficult to transduce. On the contrary, mesenchymal stem cells (MSC) can be found in virtually all postnatal tissues, are easily transduced and exhibit a unique in vitro expansion capacity using a simple medium formulation.

Local or Systemic Injection of Ex Vivo Gene-Modified MSC for Cancer Immunotherapy

MSC suspended in PBS or solution saline, have been administered in preclinical models of cancer through a wide variety of routes (**Table 1**), in the proximity or not of the tumor. Although MSC are supposed to be endowed with tumor-targeting properties, most studies showing therapeutic effect of gene-modified MSC use one of these two strategies: either coinjection or

Table 1. Ex vivo gene-modified mesenchymal stem cells for cancer immunotherapy

	Gene	MSC source	Route of administration	Disease model	Animal model	Reference
Suspension cells						
	CX3CL1	(M) BM	i.v.	(M) melanoma/colon cancer lung metastasis	(M) C57BL6/BALBc	45
	CX3CL1	(M) BM	intratracheal	(M) colon cancer lung mestatasis	(M) C57BL6/BALBc	46
	IFN-α	(M) BM	i.v.	(M) melanoma lung metastasis	(M) C57BL6	47
	IFN-β	(H) BM	i.v.	(H) melanoma (s.c.), lung metastasis	(M) athymic nude	35
	IFN-β	(H)BM	i.t.	(H) glioma (intracranial)	(M) athymic nude	48
	IFN-β	(H) BM	i.v.	(H) breast cancer lung metastasis	(M) SCID	49
	IFN-β	(M) BM	i.v.	(M) prostate cancer lung metastasis	(M) C57BL6	36
	IFN-β	(H) UCM	i.v.	(H) breast cancer lung metastasis	(M) SCID	50
	IFN-β	(H) UCM	i.v.	(H) bronchioloalveolar cancer (orthotopic)	(M) SCID	51
	IFN-β	(H) BM	i.p.	(H) pancreatic carcinoma (orthotopic)	(M) SCID	52
	IFN-β	(C) AT	s.c.	(M) melanoma (s.c.)	(M) C57BL6	53
	IL-2	(R) BM	i.t.	(R) glioma (intracranial)	(R) Fisher 344	54
	IL-7	(R) BM	i.t.	(R) glioma (intracranial)	(R) Fisher 344	55
	IL-12	(H) BM	i.t./ i.p.	(M) melanoma (s.c.)/lung metastasis	(M) C57BL6	56
	IL-12	(M) BM	p.t.	(M) glioma (intracranial)	(M) C57BL6	57
	IL-12	(M) BM	i.v.	(M) tumors (s.c.), spontaneous metastasis	(M) C57BL6/BALBc	58
	IL-12	(M) BM	i.v.	(H) Ewing's sarcoma (s.c.)	(M) athymic nude	59
	IL-12	(H) BM	i.v.	(H) renal carcinoma (s.c.)	(M) athymic nude	60
	IL-12	(H) UCB	i.t.	(M) glioma (intracranial)	(M) C57BL6	61
	IL-18	(R) BM	i.t.	(R) glioma (intracranial)	(R) Sprague-Dawley	62
	IL-21	(H) UCB	i.v.	(H) ovarian cancer	(M) athymic nude	63
	TNFSF2 (TNF α)	(H) UCB	p.t.	(H) gastric cancer (s.c)	(M) athymic nude	64
	TNFSF10 (TRAIL)	(H) UCB	i.t.	(H) glioma (intracranial)	(M) athymic nude	31
	TNFSF10 (TRAIL)	(H) BM	i.t.	(H) glioma (intracranial)	(M) SCID	32
	TNFSF10 (TRAIL)	(H) BM	p.t.	(H) glioma (intracranial)	(M) athymic nude	65
	TNFSF10 (TRAIL)	(H) BM	i.t./i.v.	(H) breast cancer (s.c.)/lung metastasis	(M) NOD–SCID	66
	TNFSF10 (TRAIL)	(H) AT	i.t./i.v.	(H) cervix carcinoma (s.c.)	(M) NOD–SCID	67
	TNFSF10 (TRAIL)	(H) BM	i.t./i.v.	(H) colorectal carcinoma (s.c.)	(M) athymic nude	34
	TNFSF10 (TRAIL)	(H) BM	i.v.	(H) pancreatic cancer (s.c.)	(M) athymic nude	68
	TNFSF10 (TRAIL)	(H) UCB	i.t.	(H) glioma (intracranial)	(M) athymic nude	69
	TNFSF10 (TRAIL)	(H) BM	i.t.	(H) colorectal carcinoma (s.c.)	(M) athymic nude	70
	TNFSF10 (TRAIL)	(H) AT	i.t.	(R) glioma (intracranial)	(R) Fisher 344	71
	TNFSF10 (TRAIL)	(H) BM	i.t.	(H) glioma (intracranial)	(M) athymic nude	72
	TNFSF14 (LIGHT)	(H) UCB	p.t.	(H) gastric cancer (s.c)	(M) athymic nude	73
	TNFSF14 (LIGHT)	(M) BM	s.c (contralateral)	(M) breast cancer (s.c.)	(M) BALB/c	39
Confined cells						
	anti-CEA x anti-CD3 dAb	(H) BM	Hydrogel-embedded	(H) colorectal carcinoma (s.c.)	(M) athymic nude	40
	IL-2	(M) BM	Matrigel-embedded	(M) melanoma (s.c.)	(M) C57BL6	74
	IL-12	(M) BM	Matrigel-embedded	(M) breast cancer (s.c.)	(M) BALB/c	75

MSC, mesenchymal stem cells; M, mouse; H, human; R, rat; BM, bone-marrow; UCM, umbilical cord matrix; UCB, umbilical cord blood; AT, adipose tissue; dAb, diabody; s.c., subcutaneous; i.v., intravenous; i.t., intratumoral; p.t., peritumoral; i.p., intraperitoneal.

Table 1. Ex vivo gene-modified mesenchymal stem cells for cancer immunotherapy (continued)

	Gene	MSC source	Route of administration	Disease model	Animal model	Reference
Confined cells						
	IL-12	(R) BM	Matrigel-embedded, s.c, i.t., i.v	(M) melanoma (s.c and i.v.)	(M) C57BL6, beige, SCID	76
	PEX	(H) BM	Alginate-PLL microcapsules	(H) glioma (s.c.)	(M) athymic nude	77
	sIGF-I R	(M) BM	Matrigel-embedded	(M/H) colon/lung cancer liver metastases	(M) C57BL6/ athymic nude	43
	TNFSF10 (TRAIL)	(H) BM	Silk scaffold, i.t., i.v.	(H) breast cancer (orthotopic)	(M) NOD–SCID	78

MSC, mesenchymal stem cells; M, mouse; H, human; R, rat; BM, bone-marrow; UCM, umbilical cord matrix; UCB, umbilical cord blood; AT, adipose tissue; dAb, diabody; s.c., subcutaneous; i.v., intravenous; i.t., intratumoral; p.t., peritumoral; i.p., intraperitoneal.

intratumoral/peritumoral injection for localized tumors, or intravenous administration for lung metastasis, taking advantage of the fact that most of the intravenous-inoculated MSC (especially human MSC) are physically retained in the mouse pulmonary filter as a consequence of their size.[31,32]

In fact, Bexell et al.[33] found no evidence of MSC homing to intracranial gliomas following intravenous injections and suggested that MSC should be administered by intratumoral implantation to achieve a therapeutic effect. In a work by a different group, MSC genetically modified for the expression of TNFSF10/TRAIL (MSC^{TRAIL}) inhibited colon carcinoma tumor growth after subcutaneous coinjection, but systemic application of MSC^{TRAIL} had no effect, which appeared to be due to lung entrapment and low rate of tumor grafting.[34]

Intratumoral/peritumoral MSC have been validated for the delivery of CX3CL1, IFN-β, IL-2, IL-7, IL-12, IL-18, TNF α and TRAIL to subcutaneous or intracranial tumors. Using a different experimental approach, Studeny et al.[35] reported that intravenous-injected, IFN-β expressing MSC ($MSC^{IFN\beta}$) significantly extended animal survival in mice with human melanoma lung metastasis. These results were supported by Ren et al.,[36] who demonstrated the therapeutic effect of systemically administered murine $MSC^{IFN\beta}$ in a mouse model for prostate cancer metastasis.

A therapeutic effect was also reported by Chen et al.,[37] who injected different tumor cells into the footpad of syngenic mice and after intravenous administration of $MSC^{IL\text{-}12}$ observed inhibition of tumor growth and spontaneous metastasis. MSC could be detected into the tumors five weeks after administration but interestingly they were absent from normal tissues such as lung and liver. As an alternative route of systemic delivery, $MSC^{IL\text{-}12}$ were administered intraperitoneally before intravenous inoculation of melanoma cell.[33] Treatment led to a considerable decrease in the number of lung metastasis, but unfortunately no data about potential MSC homing or increased IL-12 serum levels were reported. Recently, it was shown that adipose-derived MSC expressing TRAIL, intratumoral- or intravenous-administered in mice bearing subcutaneous tumors, caused a reduction in tumor burden.[38] Presence of MSC^{TRAIL} in tumors after intravenous administration was demonstrated, but no data on potential localization of MSC in normal tissues were provided. In a different setting, MSC^{LIGHT} exhibited a notable prophylactic and therapeutic effect when administered subcutaneous in the flank contralateral to tumors. However, migration of MSC from the left flank to the right one where tumor cells were inoculated is not addressed in this work.[39]

Considering that it is difficult to estimate the percentage of MSC effectively homing to tumors in these models (probably very low), and given the antitumoral effect observed in most of them, these results could be attributed, at least in part, to therapeutic protein production in locations other than tumors. Therefore, perhaps MSC as cell factories for soluble proteins can be "outsourced" from the tumor while preserving their therapeutic effect.

If MSC tumor homing is not a requirement, perhaps we could implant them in a determined location, within a controlled environment providing clues that could enhance their engraftment and survival. Moreover, some MSC properties (e.g., immunosupresion, metastasis and angiogenesis promotion) would strongly recommend avoiding the direct contact between MSC and tumor cells.[40,41]

Confined Administration of Ex Vivo Gene-Modified MSC for Cancer Immunotherapy

In fact, for strategies where MSC are used as cell factories for therapeutic antibodies, ex vivo gene-modified producer cells can be confined within a controlled environment providing clues that could enhance their engraftment and survival. The scaffold would keep cells at the implantation site, with the therapeutic protein acting at distance (immunotherapeutic organoid), and could be retrieved once the therapeutic effect is fulfilled (**Fig. 2**). A seminal work by Eliopoulos et al. reported that transduced MSC secreting EPO (MSC^{EPO}), when delivered freely in the subcutaneous or intraperitoneal spaces led to a temporary hematocrit increase.[42] In contrast, subcutaneous implantation of MSC^{EPO} embedded in Matrigel led to a sustained pharmacological effect. This systemic effect of locally produced proteins (IL-2, IL-12, PEX, sIGF-IR and TRAIL) has also been reported in the context

of cancer therapy (**Table 1**). For example, Wang et al.[43] reported that Matrigel-embedded sIGF-IR-secreting MSC subcutaneous implanted, provided sustained delivery of this decoy receptor in vivo. The protein achieved therapeutically effective concentrations, resulting in marked reductions in the ability of three different highly metastatic tumor cell types to colonize the liver.

Matrigel is a reconstituted extracellular matrix (ECM) preparation extracted from a murine sarcoma cell line, not suitable for MSC immobilization in a clinical setting. For this reason we used a chemically defined and non-immunogenic synthetic ECM (sECM) for in vivo MSC engraftment in a bsAb-based cancer immunotherapy approach. MSC were transduced ex vivo with a lentiviral vector expressing a recombinant anti-CEA x anti-CD3 diabody, embedded in a sECM crosslinkable in situ (Extracel-X) and inoculated in the ventral subcutaneous space of nude mice. The antibody was released into the bloodstream at detectable levels for at least 7 weeks. MSC-secreted diabody activated tumor-specific T cells and reduced the growth of CEA-expressing human colon carcinoma cells.[40]

In previous works, two immunotherapeutic MSC-based organoids had been reported for the expression of IL-2 and IL-12.[74,75] Matrix-embedded IL-2-producing MSC inoculated in the vicinity of B16 melanoma led to inhibition of tumor growth.[74] Similarly, gene-modified MSC to express IL-12, embedded in a matrix, and implanted peritumorally in a model of breast cancer caused a significant decrease of tumor growth.[75] Although $MSC^{IL\text{-}12}$ scaffolds determined increased IL-12 plasma levels, the observed therapeutic benefit was not due to a systemic effect, since $MSC^{IL\text{-}12}$ implanted contralaterally did not inhibit significantly tumor growth. In contrast, MSC-secreted diabody demonstrated the systemic effect of a locally produced protein.[40]

In summary, the necessary components for generating a long-lasting immunotherapeutic organoid (**Fig. 2**) are: (1) gene/s encoding a soluble protein/s with immune-modulating activity, (2) an efficient and safe transfer vector, (3) a suitable an easily transduced long-lived cell and (4) a non-immunogenic synthetic matrix scaffold that guarantees the survival of the cell vehicle at the point of implantation.

Future Prospect

AAV have demonstrated excellent safety and tolerability in human trials and two phase 1 trials currently ongoing involve the use of AAV for the expression of anti-HIV antibodies from muscle tissues.[79] However, the possibility of rare adverse events is still a concern. On the other hand, cell-based approaches face challenges such as loss of transgene expression over time due to limited life-span of producer cells or host immune responses against them. MSC are emerging as the best cell choice for the generation of long-lasting cell factories (**Fig. 2**). Perhaps the safest and most practical approach might be the use of scaffolds that keep genetically modified autologous MSC at the implantation site (immunotherapeutic organoids). However, in terms of cost-effectiveness, the only potential approach to apply these cell factories to the clinical setting would imply the use of "off-the-shelf" stocks of gene-modified MSC ready to be used in a series of patients. In principle, MSC would be perfect candidates for this strategy due to low immunogenicity and immunomodulatory properties, but some reports point out that allogenic MSC are not so "invisible" to immunocompetent hosts.[43,44] Therefore, the use of encapsulation systems (**Fig. 2**) to shield producer MSC from the host immune system (immunotherapeutic capsules) would be highly desirable in order to obtain long-term systemic protein delivery. In a recent work, Goren et al.[77] demonstrated the feasibility of this approach for the in vivo production of PEX, an inhibitor of angiogenesis, by encapsulated MSC.[77] The technical and biological advances may lead to the realization of the full potential of cell encapsulation.[80]

In summary, the transfer of genes encoding antibodies, both in vivo and ex vivo, is a promising strategy that can be applied to treat clinical conditions in which continuous production of antibodies is required. Administration of ex vivo gene-modified cells embedded in appropriate scaffolds, such as hydrogels, can help to improve their therapeutic potential.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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