Review

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Reprogramming antitumor immunity

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Regenerative medicine holds great promise in replacing tissues and organs lost to degenerative disease and injury. Application of the principles of cellular reprogramming for the treatment of cancer, however, is not well established. Here, we present an overview of cellular reprogramming techniques used in regenerative medicine, and within this context, envision how the scope of regenerative medicine may be expanded to treat metastatic cancer by revitalizing an exhausted and senescent immune system.

Regenerative medicine as a therapy for cancer

The ability of tumor cells to evade immune destruction is an emerging hallmark of cancer [1]. The theory of immune surveillance posits that an ever vigilant immune system eliminates nascent cancer cells [2]. Tumor-specific T cells can become exhausted and senescent with chronic antigen challenge (Box 1), however, allowing malignant cells to persist and develop into invasive and widespread cancer. Immune-based approaches such as adoptive cellular immunotherapy (ACT) help to overcome T cell exhaustion and senescence by surgically isolating T cells from the tumor microenvironment and expanding them *ex vivo* prior to adoptive transfer into autologous patients [3]. ACT is emerging as a potentially curative therapy for patients with advanced cancer, but one of the main limitations to improving the efficacy of ACT is to ensure that T cells maintain the capacity for self-renewal and are able to continually produce progeny capable of eradicating tumor after adoptive transfer into patients [4].

Herein, we envision how reprogramming techniques developed in stem cell biology may be used to treat metastatic cancer by revitalizing an exhausted and senescent immune system. Applying techniques of cellular reprogramming may endow features of stemness to adoptively-transferred T cells—namely enhanced self-renewal and multipotency to produce a continual supply of cytolytic effector progeny—thereby improving the ability of antitumor T cells to sustain a prolonged attack on advanced cancer. This article has a three-prong focus: first, we offer an overview of cell-based reprogramming techniques to provide a conceptual framework and vocabulary that can be used to understand approaches in regenerative medicine. A discussion of ACT and mechanisms underlying exhaustion and senescence of the immune system follows. Finally, we sharpen our focus to explore regenerative medicine techniques that may revitalize an exhausted immune response and have the potential to enhance the antitumor efficacy of cell-based immunotherapy.

Language of plasticity

Although the field of regenerative medicine has deep historical roots, there are often conflicting definitions regarding terms of cellular reprogramming (Table 1). What is at stake, however, is clear: the plasticity of a cell. Plasticity is the ability of a cell to convert from one cell type into another and, in the context of regenerative medicine, ultimately reconstitute tissues. This definition of plasticity rests on at least two assumptions. First, that there are discrete cell types (or discrete cell lineages), and second, that a differentiated cell can alter its phenotype, whether within a lineage or between lineages [5].

In 1957 Conrad Waddington conceptualized the process of cellular differentiation as a ball (representing a cell) placed at the top of a hill [6]. Using the Waddington model, the plasticity of a cell can be conceptualized with reference to its lineage. Totipotent stem cells reside at the top peak with the ability to differentiate into any cell type or extraembryonic tissue [7]. As a cell begins to travel from its undifferentiated state, a series of extracellular cues and gene expression programs determines the path of the cell until it arrives at a differentiated valley, representing a distinct cellular lineage (Figure 1). The prevailing paradigm is that somatic cells become increasingly, and irreversibly, committed to their somatic fate and lose potency as they travel down the hill. That is, a mature skin cell, at least in the physiological setting, cannot give rise to a heart cell, and vice versa [8].

There are several experimental techniques in regenerative medicine that can induce plasticity and alter the fate of cells that would otherwise be subject to physiological dictates. These cellular reprogramming techniques can be grouped into two broad approaches: reprogramming to pluripotency and lineage reprogramming [7]. Reprogramming to pluripotency includes cell fusion, somatic cell nuclear transfer, induction of pluripotency by ectopic gene expression, and stimulus-triggered acquisition of

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Box 1. Exhaustion and senescence of T cells

A hallmark of adaptive cellular immunity is the ability of T cells to undergo a robust clonal response with secondary antigen challenge [83]. Repeated and chronic antigenic stimulation in the tumor microenvironment seems to attenuate this response as T cells become increasingly exhausted and senescent [35]. Senescence defines a loss of replicative capacity that is associated with DNA damage and telomere erosion [84,85]. Exhaustion refers to compromised functional capability of T cells [86]. Traditionally considered to be passive phenomena that weaken an immune response, there is now increasing evidence that both exhaustion and senescence are distinct processes controlled by active molecular pathways [87].

Exhaustion was first described in mice with chronic infection of lymphocytic choriomeningitis virus (LCMV) and later validated in models of human T lymphotropic virus (HTLV)1, HIV, hepatitis B virus (HBV), simian immunodeficiency virus (SIV), and hepatitis C virus (HCV) [87]. Exhaustion of T cells in mice and humans with high tumor burden have also been observed [36].

Exhausted $CD8^+$ T cells in mice and humans are characterized by attenuated expression of receptors for IL-15 and IL-7, CCR7, and L-selectin (also known as CD62L), consistent with T_{EFF} cell phenotype [36]. Interestingly, exhaustion occurs in distinct stages of functional impairment: IL-2 production is initially lost, followed by tumor necrosis factor (TNF) expression, and finally IFN-γ in the most severe state of exhaustion [88].

Cellular senescence was first recognized when Hayflick observed a limitation to the replicative capacity of fibroblasts that was later found to be due to shortening of telomeres and triggering of the DNA damage response (DDR) [89]. Senescent T cells are characterized by a shortening of telomeres, decreased expression of telomerase, and increased expression of killer cell lectin-like receptor subfamily G, number 1 (KLRG1) [36]. Reversal of senescence in fibroblasts by antagonizing the cell cycle arrest protein checkpoint kinase 2 homolog (CHK2) and key mediators such as p21, p53, and p38 [90] suggest it is possible to reverse or delay senescence in T cells. For an excellent review on T cell exhaustion in the tumor microenvironment, see [91].

pluripotency—the common denominator being a reversion to a pluripotent state [9]. Lineage reprogramming encompasses approaches of dedifferentiation, transdifferentiation, and transdetermination, and refers to conversion of a cell from one type to another in the same lineage or different lineages without reversion to pluripotency [10] (Figure 1).

The process of dedifferentiation occurs when a terminally differentiated cell reverts to a less-differentiated precursor within its own lineage [10]. The Waddington 'ball', so to speak, rolls back up the hill, but not all the way to the top (to pluripotency). Ectopic expression of Lin-28 homolog B (Lin28), for example, has been shown to reprogram adult hematopoietic stem/progenitor cells (HSPCs) into fetal-like hematopoietic stem cells that have enhanced capacity for multilineage reconstitution [11]. Another example of dedifferentiation within the lymphoid lineage was observed when conditional deletion of paired box gene (Pax)5 in mice enabled mature B cells from peripheral lymphoid organs to dedifferentiate *in vivo* to early uncommitted progenitors in the bone marrow and ultimately rescue T lymphopoiesis in the thymus of T cell-deficient mice. The B cell-derived T cells showed evidence of immunoglobin gene rearrangement and maintained the capacity to form germinal centers in immunized mice [12].

Transdetermination is similar to dedifferentiation, but the proverbial Waddington ball does not roll back to the same valley from whence it came. The ball rolls down a different valley. In other words, it dedifferentiates to an earlier progenitor (without a pluripotent intermediate) and then switches lineages to differentiate to a cell of a distinct lineage. An impressive example of transdetermination was demonstrated when human dermal fibroblasts were converted to multilineage blood progenitors by ectopic expression of octamer-binding transcription factor (OCT)4 in addition to specific cytokine treatment [13]. The fibroblast-derived cells expressed the panleukocyte marker CD45 and gave rise to erythroid, megakaryocytic, monocytic, and granulocytic lineages that maintained the capacity for *in vivo* engraftment. Notably, the adult

Table 1. Language of plasticity.

Stem cell	Cell with enhanced properties of self-renewal and potency
Lineage	Cells of same developmental origin with common phenotype and function.
Differentiation	The process by which a cell loses its potency and capacity for self-renewal and ultimately becomes a mature and discrete cell type within a discrete lineage.
Reprogramming to pluripotency	Reprogramming of a cell to a pluripotent state. Techniques include somatic cell transfer, cell–cell fusion, and direct reprogramming; the common denominator being a reversion to a pluripotent cell.
Lineage reprogramming	Conversion of a cell from one type to another in the same lineage or different lineages without reversion to pluripotency. Techniques include dedifferentiation, transdifferentiation, and transdetermination.
Dedifferentiation	The process by which a cell reverts to a less specialized progenitor state within a discrete lineage.
Transdifferentiation	Switch from one cell lineage to another without moving through a dedifferentiated or pluripotent intermediate.
Nuclear transfer	Transplantation of a nucleus from a somatic cell to an enucleated oocyte where the somatic cell nucleus is reprogrammed in the environment of the oocyte.
Plasticity	Ability of a cell to convert from one discrete cell type or lineage into another.
Totipotency	Ability of cell to produce all differentiated cells in an organism (including extraembryonic tissue)
Pluripotency	Capacity to give rise to any of the three germ layers: endoderm, mesoderm, and ectoderm.
Multipotency	Capacity to give rise to cells of multiple lineages or cell subsets.
Senescence	A growth-arrest program that limits the lifespan of mammalian cells and prevents unlimited cell proliferation.
Cell fusion	Occurs when two distinct cell types combine to form a single entity. The only form of nuclear reprogramming observed in nature.
Exhaustion	T cell exhaustion is a state of T cell dysfunction that arises during chronic infection and cancer. It is defined by poor effector function, sustained expression of inhibitory receptors, and a transcriptional program distinct from that of functional T_{EFF} or T_{CM} cells.
Transdetermination	Dedifferentiation of cell to less committed progenitor state which switches lineages to redifferentiate to a cell type in a new lineage.