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Versatile cell ablation tools and their applications to study loss of cell functions

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Abstract

Targeted cell ablation is a powerful approach for studying the role of specific cell populations in a variety of organotypic functions, including cell differentiation, and organ generation and regeneration. Emerging tools for permanently or conditionally ablating targeted cell populations and transiently inhibiting neuronal activities exhibit a diversity of application and utility. Each tool has distinct features, and none can be universally applied to study different cell types in various tissue compartments. Although these tools have been developed for over 30 years, they require additional improvement. Currently, there is no consensus on how to select the tools to answer the specific scientific questions of interest. Selecting the appropriate cell ablation technique to study the function of a targeted cell population is less straightforward than selecting the method to study a gene's functions. In this review, we discuss the features of the various tools for targeted cell ablation and provide recommendations for optimal application of specific approaches.

Keywords

Animal models; Optogenetics; Chemogenetics; Clodronate-liposome; Thymidine kinase (TK); Bacterial nitroreductase (NTR). Caspase; Diphtheria toxin (DT); human CD59; intermedilysin (ILY); cell interaction

Introduction

Targeted cell ablation is a powerful approach for studying cell type specific functions in tissues, cell differentiation, and tissue regeneration ***in*** *vivo*[1–10] Investigation of these fundamental processes has benefited greatly from the development of a wide array of technologies and reagents. Molecular engineering tools to effect targeted gene knockout and gene over-expression methods have been developed over 30 years for studying the function of individual and multiple genes *in vivo*. Importantly, CRISPR/Cas9 technology has rapidly augmented existing tools and significantly accelerated our understanding of gene functions across multiple species. For loss of function (LOF) studies of individual cell populations and cell-to-cell interactions, we need methods for the *in vivo* elimination of targeted cell populations or inhibition of their activities. As compared to techniques for studying *in vivo* gene functionality, the tools for studying cell functionalities are disparate and technically complex. Table 1 shows the distinct features of the methods that have been developed over the past 30 years. Due to the complexity of the methods used for targeted cell ablation, and the accessibility and intrinsic properties of the targeted cell populations, none of these methods can be universally applied for studying all cell types in the context of tissue compartments *in vivo*. Importantly, the method best suited for a particular biological question, for example, investigating the role of specific cell type in disease pathogenesis, in regeneration, or in development, may be very different. To facilitate the selection of a cell ablation tool best suited for studying the scientific question at hand, we will review the features of the various tools for targeted cell ablation and neuronal activity inhibition and make recommendations on how best to apply these tools.

Versatile cell ablation tools

1. Laser ablation

Laser ablation is one of the earliest approaches used for killing cells of interest. The intense light produced by a laser is absorbed within a short time period and converted into heat, which damages cellular proteins and other macromolecules and leads to cell death within seconds[11]. Laser pulses can damage the biological material on different scales. The power, repetition rate, and duration of the pulses will determine the extent of the damage[12]. The main advantage of laser-mediated cell ablation over the chemical and genetic cell ablation methods described below is flexibility; a laser can eliminate every kind of cell in any physiological state[13]. Laser ablation allows for a precise control over the time of ablation and the ability to ablate cells at any stage of development, which are less feasible with chemical or genetic cell ablation[12, 14] The technique has been employed extensively in developmental model systems like *C. elegans* and zebrafish[151617] However, it has its limitations; it is time consuming, labor-intensive, and requires expensive equipment[12]. Because it needs to be combined with microscopic techniques, only targeted cell groups that can be visualized by microscopy are amenable to ablation [18] Development of vital fluorescent imaging systems in the past two decades has increased its efficiency and versatility[18, 19] The most significant and obvious limitation of laser ablation is unavoidable damage to adjacent cells due to cytoplasmic boiling and gas bubbles generated by the high energy laser power[12]. Second, ablating multiple cells in an individual animal is a tedious, time-consuming and labor-intensive task. Third, ablation of multiple cells can be inefficient since there are significant differences of laser light absorbance levels among cell types[12], and ablation of cells in deep locations requires higher levels of laser power than superficially-located cells [12] For these reasons, laser ablation has been seldom applied to studying cell function in adult animals, but is still utilized for addressing fundamental questions in early development and in *in vitro* organ culture [20, 21].

2. Optogenetic ablation

Optogenetic or photo-inducible cell ablation has been developed recently by combining genetic and laser ablation methods (Fig. 1) [11]. This technique often uses genetically encoded photosensitizers, which produce reactive oxygen species (ROS) upon light excitation (Fig. 1A and 1B) [11,22] Photosensitizers, which include a red fluorescent KillerRed (Fig. 1A) [2324] and a green fluorescent mini singlet oxygen generator (miniSOG) (Fig. 1B)[25], transmit energy from the absorbed green or blue lights to activate molecules in the acute cell necrosis[11]. Precise photo-inducible ablation of cells such as neurons *in vivo* can also be achieved through cell-specific expression of a light-activated caspase-3, engineered by exploiting its spring-loaded activation mechanism through insertion of the light-sensitive protein (LOV2) domain that expands upon blue light exposure (Fig. 1C)[26]. Optogenetic cell ablation methods are effective at single-cell resolution, with precise temporal control [11], and have minimal off-target/non-specific cell death since they utilize a lower intensity of light than the laser ablation method. These optogenetic methods allow for selective ablation of cells in a temporally and spatially precise manner, facilitating the study of cell function in different tissues and developmental stages in various model systems, including vertebrates. However, the ability to photo-ablate cells is also limited by the accessibility and

transparency of tissues for focused illumination of a region of interest. Optogenetics can be used for cell ablation by combining genetics and light stimulation, enabling the execution of well-defined events within genetically defined populations of cells, with precise temporal and spatial resolution.

3. Optogenetic and chemogenetic approaches for transient inhibition or activation of the neuronal activity

Optogenetic approaches can be used for manipulating neuronal excitability transiently, which is an efficient way to probe causal relationships between specific neuronal cells and behavior. Chemogenetic tools have also been developed for this purpose. Both techniques have been widely used in the central nervous system (CNS) and peripheral sensory ganglia to manipulate neuronal activity in a cell-type-specific fashion both *in vitro* and *in vivo,* to determine functions of specific neuronal populations[27–32]. Although these tools do not ablate neurons, they can rapidly silence them. Therefore, we will briefly discuss these two methods.

Optogenetically-mediated neuronal inhibition or activation—A major contributing factor to the development of optogenetics was the discovery of microbial opsins, which act as membrane-bound light-driven ion pumps. Microbial opsin expression in mammalian CNS neurons can effectively regulate membrane potentials and action potential firing in response to light illumination, enabling light-mediated control of neuronal excitability. In neurons, light activation of archaerhodopsin or halorhodopsin promptly hyperpolarizes the cell membrane and eliminates action potentials, leading to neuronal inhibition. Blue light activation of channelrhodopsin in CNS neurons, on the other hand, depolarizes membrane and dramatically increases action potentials[29, 33, 34], leading to neuronal activation.

Chemogenetically mediated neuronal inhibition or activation—Chemogenetics combines genetics and pharmacology to manipulate the cell's behavior. It can be used to control neuronal function using small molecules that activate engineered receptors that can be genetically targeted to specific cell types. These engineered receptors include nucleic acid hybrids, a variety of metabolic enzymes, ion channels and G-protein coupled receptors (GPCRs)[28, 35]

Designer receptors exclusively activated by designer drugs (DREADDS) is a chemogenetic technique that has been widely used to manipulate genetically defined subsets of neurons through engineered GPCRs that can activate or silence neuronal firing[28]. DREADD technology has emerged as a powerful tool to study function of genetically defined cell populations *in vitro* and *in vivo*. The excitatory G-protein (Gq)-DREADD (hM3Dq) is a mutated muscarinic acetylcholine receptor that initiates Gq signaling cascades upon activation. Activation of the inhibitory G-protein (Gi)-DREADD (hM4Di) initiates Gi-protein signaling cascades, leading to inhibition of adenylyl cyclase and voltage-gated Ca^{2+} channels (VGCCs), and activation of Gi-protein regulated K^+ channels (GIRKs). Both hM3Dq and hM4Di receptors are exclusively controlled by the biologically inert compound CNO and are unresponsive to endogenous acetylcholine[27], and devoid of constitutive activity[36].

Although chemogenetic and optogenetic techniques can both be used to selectively manipulate cellular functions in a genetically defined cell population, there are major differences that can dictate the method of choice for particular experiments. First, optogenetics, which directly affects the region illuminated by light (i.e., a restricted area), chemogenetics uses genetically engineered receptors and exogenous molecules specific for those receptors, affecting the activity of cells in a large area. Therefore, chemogenetics is advantageous for studying functions of neurons that are diffusely scattered[27, 28] Second, while optogenetics allows rapid and reversible control of neuronal activity within milliseconds, CNO activation of hM3Dq and hM4Di receptors affects neuronal activity over a long period of time (around 1 to 10 hours)[36, 37] Third, chemogenetics provides considerably more accessibility and flexibility than optogenetic, in which the animal typically has a tethered implant, which may restrict measurement of certain behaviors. Fourth, optogenetic studies require specialized light equipment, including waveform generators, optic fibers, and other devices[38]. While chemogenetic studies are easier to implement and require no light usage. Furthermore, chemogenetic ligands can be administered via several routes (injection, food, water, or pump). Therefore, both chemogenetics and optogenetics have inherent advantages and disadvantages. The experimental question and the available resources decide which technology should be used or if both techniques can be used within the same experimental design.

4. Chemical-mediated ablation models

Some cell populations are especially vulnerable to specific chemical agents, which therefore can be used as toxins to target and eliminate those cells. We will briefly discuss some of these tools in the context of some of the diseases and/or cell types in which they have been applied.

Chemical-mediated Parkinson's disease model—Parkinson's disease results primarily from the gradual loss of dopaminergic neurons (DN) in the substantia nigra[39], but the causes of the initiation and progression of the dopaminergic neurodegeneration are unknown. A better understanding of the underlying molecular mechanisms requires use of animal models in which dopaminergic neurons are specifically destroyed *in vivo* [40] Neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat, 6-hydroxydopamine (6-OHDA), and rotenone eliminate dopaminergic neurons by creating toxic metabolites[41, 42] and remain the most popular tools for producing selective neuronal death in both *in vitro* and *in vivo* systems[42].

Clodronate-liposome (CL)-mediated monocyte ablation—Liposome-mediated intracellular delivery of clodronate to phagocytes is a powerful tool to deplete monocytes/macrophages *in vivo.* Clodronate-liposomes (CLs) are artificially prepared lipid vesicles, which consist of concentric phospholipid bilayers encapsulating clodronate, a non-toxic bisphosphonate[43]. After injection, CLs are selectively taken up by monocytes/macrophages via endocytosis; digested and released as free clodronate within the cells. Accumulation of clodronate above a threshold concentration induces apoptosis[44]. Therefore CLs administered by different routes can efficiently eliminate monocytes and macrophages in such organs as liver, spleen, lung, and peritoneum [45] Intravenous injection of CLs can deplete peripheral

monocytes by 24 hours post-treatment, but regeneration begins by 2 days; multiple injections are therefore needed to maintain effective ablation[46]. Since neutrophils and lymphocytes will not ingest CLs, the ablation is specific for monocytes[44, 45] CLs greatly facilitate loss-of-function studies of monocytes/macrophages, but their toxic effects are a cause for concern. One study reported a 33.3% mortality rate after multiple injections of CLs in mice[48]. Also, because CL cannot penetrate vascular barriers, intravenous delivery of CLs does not adequately deplete macrophages residing in tissue compartments [49, 50] Local injection has been employed to reach these cells, but procedures like intranasal injection require special skills, which hinder their widespread use. Moreover, because the working dose of CL varies among different administration routes, careful titrations are needed. Lastly, CL-induced cell apoptosis usually requires days to achieve efficient ablation, resulting in cell injury and regeneration occurring at the same time[45, 51]. Therefore, it is difficult to use this strategy to investigate the dynamic response to acute cellular loss.

PHZ and BE-mediated hemolytic anemia—Phenylhydrazine (PHZ) has been used in rats and 2-butoxyethanol (BE) in mice to induce hemolysis[52–54]. PHZ destroys red blood cells by increasing production of ROS, which induces oxidative stress and the multiple cellular reactions that cause hemolytic anemia[54]. BE's metabolites, including butoxyacetic acid (BAA), can also induce hemolysis[55]. Erythrocyte ablation by either method requires repeated injections [54] However, PHZ can also act as a mitogen, stimulating lymphocytes and monocytes following multiple injections[56, 57] PHZ-treated mouse erythrocytes can be used to model cell deformation, but their aggregation is inhibited, whereas aggregation is markedly increased in patients with thalassemia[54, 58] The response to BE in rats is delayed, variable, and age-dependent; younger rats exhibit resistance to hemolysis [55]. Therefore, there is a need for a more precisely targeted agent which can specifically ablate erythrocytes in a rapid manner that more closely models red blood cell diseases[54]. As noted previously, the specific mechanisms of action of chemical-mediated cell ablation agents limit their applicability to specific cell populations.

5. Targeted recombination between inverted *loxP* sites (TRIP)

Cre-mediated recombination of molecularly engineered *loxP* sites in animals has been widely used to generate a variety of targeted genomic rearrangements. Cre-mediated recombination with inverted *loxP* sites, which causes chromosomal loss and triggers apoptosis, is known as the targeted recombination between inverted *loxP* sites (TRIP) method of cell ablation[59]. The targeted cell population is defined by both transgenic Cre-expression and the knock-in of inverted *loxP* sites in an animal's genome. TRIP-mediated cell ablation is useful to investigate the function of a large variety of proliferating cell populations in a whole organism[59]. Although this technique is promising, it has not yet been used widely. Limitations include: 1) its ability to only target proliferating cell populations, and 2) the potential for lethality if Cre is expressed constitutively or leaky in early stages of development.

6. Caspase-mediated cell ablation

Apoptosis is a morphologically distinct form of programmed cell death. It occurs in a wide range of mammalian cell types and plays important roles in both physiological and

pathological conditions[60]. Since apoptosis is an intrinsic process involving controlled dismantling of intracellular components while avoiding inflammation and damage to surrounding cells, manipulating it by targeting its central components, caspases, can provide an ideal approach to induce "suicide" of target cells with few bystander effects[61]. Caspases that are initially synthesized as inactive monomeric zymogens (pro-caspases) induce cell apoptosis after the pro-caspases are activated through dimerization and often oligomerization[62]. Taking advantage of cell killing by pro-caspase dimerization, several groups constructed pro-caspases fused to FK506-binding protein (FKBP), a chemically induced dimerization (CID)-binding site. Addition of FK506 (or its homodimer, FK1012), a nontoxic and lipid-permeable dimerizer, then aggregates and activates the pro-caspases [61, 63] Several groups have successfully used this method to deplete hepatocytes[64], adipocytes[65], pancreatic β-cells[66], retinal ganglion cells[67], and mixed populations of specific cell types[68]. However, this tool can be affected by antiapoptotic factors *in vivo*. For example, adiponectin decreases caspase-8-mediated death of cardiac myocytes and pancreatic β-cells, whereas genetic ablation of adiponectin enhances caspase-8-induced apoptosis *in vivo*[69]. FK506 is also a potent immunosuppressive agent with significant nephrotoxic properties[70]. Finally, FKBPs are involved in regulating a wide range of biological and pathological processes in mammals without the binding of FK506[71]. These intrinsic features have the potential to confound experimental results.

7. Antibody (Ab)-mediated cell ablation

Antibody (Ab)-mediated cell ablation based on targeting specific antigens expressed on immune cells has been used to deplete cells, including neutrophils, B cells, and T cells, in mice. The most commonly used antibody for neutrophil ablation is anti-Ly6G mAb, clone 1A8. In contrast to clone RB6–8C5, which cross-reacts with Ly6C, a receptor also present on other myeloid cells and lymphocytes[72, 73], clone 1A8 specifically recognizes and depletes Ly6G+ neutrophils, but not other cells[74]. Anti-ly6G-mediated depletion of neutrophils *in vivo* depends on the function of macrophages but not the activation of complement [75]. Therapeutic B cell depletion by targeting CD20 has significantly improved treatment outcomes of non-Hodgkin's lymphoma in humans[76]. Anti-mouse CD20 mAb is also widely used to eliminate B cells in mice[77]; a single dose depletes >95% of mature B cells in circulation and tissue by monocyte-mediated cellular cytotoxicity[77, 78] Like B cells, CD4+ and CD8+ T cells can be eliminated *in vivo* by injection of CD4 or CD8 mAb, respectively, but the mechanisms responsible remain unknown[79, 80] The available data indicates that, although antibody-mediated ablation is efficient, only cells expressing specific surface markers can be targeted. This strategy therefore cannot be applied to most myeloid cells, including dendritic cells and monocytes, because they lack appropriate antigens, and it is also limited by the availability of specific antibodies. Furthermore, because different antibodies kill cells by different mechanisms, it can be problematic to compare the functions of two distinct cell populations based on LOF studies with two different antibodies.

8. Herpes simplex virus thymidine kinase (HSV-TK)-mediated cell ablation

Application of the HSV-TK method to study the function of targeted cell populations evolved from anti-cancer therapy using the suicide gene HSV-TK[81]. HSV-TK phosphorylates nucleoside analogs into nucleotides. It phosphorylates the human nucleoside

analog, ganciclovir (GCV), to form deoxythymidine triphosphate[82], which is incorporated into the genome and causes cell death by inhibiting DNA synthesis only in proliferative cells (Fig. 2) [83].HSV-TK cell ablation is achieved by oral administration of GCV to an animal, usually mice, engineered to express HSV-1-TK in a specific cell population (Fig. 2) [83, 84] This strategy will eliminate only cells that actively synthesize DNA and can therefore be used to ablate dividing, but not quiescent, cells. Also, this strategy has following limitations: 1) the toxic side effects of GCV on bone marrow cells; and 2) the off target effect on adjacent cells through transporting the metabolite triphosphate to nearby cells[85] (Fig. 2).

9. Bacterial nitroreductase (NTR)/pro-drug-mediated cell ablation

Bacterial nitroreductase (NTR)-induced cell ablation kills both actively dividing cells and nondividing cells. Bacterial NTR can activate the pro-drug CB1954 (5-aziridin-1 -yl-2–4-dinitrobezamide) into two DNA-damaging, cytotoxic metabolites, 2-hydroxylamine and 4-hydroxylamine[86] that can kill dividing and non-dividing cells[87]. Various NTRs are found in nature. The most commonly used NTR is obtained from *E. coli*[87] NTR/prodrug CB1954-mediated cell ablation uses a transgenic strategy to express NTR in a specific cell population with administration of the pro-drug Metronidazole and CB1954[88] This method has been used to ablate post-mitotic cells such as neurons, astrocytes and adipocytes[89- 91]. Its main limitations come from the effects of the toxic metabolites[88]. 2-hydroxylamine can cause unavoidable bystander effects by entering adjacent non-targeted cell populations through gap junctions [92, 93]

10. Diphtheria toxin-mediated cell ablation

Diphtheria toxin (DT) is a 535 amino acid polyprotein that is cleaved into to two fragments, A and B [1]. The A fragment is the amino terminal 190 amino acids containing the catalytically active toxin domain, whereas the B fragment (345 amino acids) contains the domain interacting with the cell surface receptor for DT. DT-mediated cell ablation model is generated by either transgenic expression of the DT-receptor (DTR) in the targeted cell population coupled with injection of DT to the animal (DT/DTR-mediated cell ablation) or by expressing active A fragment within cell populations [2, 94], in the absence of the B fragment (*DTA* expressing cell ablation) (Fig. 3). Mice are not normally sensitive to DT since the Epithelial Growth Factor Receptor in mice does not bind DT as it does in other species [1]. In susceptible hosts, B fragment binding to the receptor causes the internalization and cleavage of A fragment (Fig. 3A)[1]. The A fragment is internalized and binds to protein synthesis machinery, inhibiting protein synthesis leading to cell death via apoptotic pathway[1]. Because the B fragment is inactive in rodents, researchers have primarily used the selective expression of DT-A chain to accomplish cell ablation in mice(Fig. 3A). Although the *DT/DTR* cell ablation model has been used to study cellular functionalities *in vivo* for more than 30 years[1, 2], it has limitations. It has a narrow pharmacological window, which prohibits dose dependent studies. This narrow range is primarily due to the extreme toxicity of DT, where even a single molecule may kill a cell[95]. Several groups have recently reported that DT administration of only 2- to 3-fold higher doses than the effective dose results in significant off-target effects, including local lung and renal toxicity and significant weight loss, resulting in morbidity and mortality independent of the DTR[9697] The narrow pharmacological window and off-target effects of the DT-mediated cell-ablation model often

make it difficult to distinguish target dependent effects from off-target effects upon DT delivery in DTR transgenic mice. This method cannot be used in intermediate- to large-sized animals such as rabbits, sheep, or pigs, because in these animals, endogenous DTR binds to DT, causing apoptosis independently of transgene expression[1, 2, 98] These facts underscore an unmet need to develop a new model that specifically ablates cells *in vivo* with higher efficiency and fewer off-target effects.

Transgenic expression of the diphtheria toxin fragment A (DT-A) subunit driven by a cell specific promoter is another useful cell ablation method (Fig. 3B)[294] Since DT-A directly inhibits protein synthesis, DT-A within a cell will lead to cell death via apoptotic pathway, and no further activation steps are needed(Fig. 3B). This makes DT-A an attractive tool for the specific ablation of cells. The DT-A gene used for eliminating cells is engineered so that the protein product will be retained within the cytoplasm. In the absence of DT-B, any DT-A released from dying cells does not affect neighboring cells. By this method, groups have successfully killed pancreatic cells (Fig. 3B)[99], growth hormone-expressing cells[100] and brown adipose tissue[101]. However, this method easily causes lethality when the promoter driving DT-A is active in multiple cell types or in cell types that are essential for embryo viability[102]. In addition, because of the extreme toxicity of DT-A, lethality or side effects may occur if the so-called tissue- or cell-specific promoter has any ectopic DT-A gene expression outside the intended lineage[103]. In order to improve this method, researchers have tried to generate attenuated DT-A. Attenuated DT-A requires a substantially higher level of expression than its nonattenuated form to produce a lethal event [104] Generation of the conditional expression of DT-A mice employing Cre recombinase and Cre/ER system can tightly control the expression of DT-A in target cells at specific life stage (Fig. 3B)[103, 105–107] By genetic modulation, DT-A mediated cell ablation has been successfully applied in depleting T cells[108], retinal pigment epithelium[107] and myogenic cell populations[109].

Except for the laser-mediated technique, all of the methods so far discussed produce cell ablation that occurs relatively slowly, from several hours up to a day[110]. This is a disadvantage when studying relatively rapid cellular events occurring over a time course of seconds to minutes during development or normal physiological function. We next describe a novel method for mediating rapid cell ablation.

11. Intermedilysin (ILY)/human CD59 (hCD59)-mediated rapid cell ablation:

We recently created a new method of conditional and rapid cell ablation[98]. This model is based on findings that intermedilysin (ILY)[111], exclusively lyses human cells but not cells from any other species through binding to the human complement regulatory protein CD59 (hCD59). ILY is a cholesterol-dependent cytolysin that is secreted by *Streptococcus intermedius*. ILY specifically lyse human cells by binding to hCD59 and forming a pore (Fig. 4). The C-terminus of ILY domain 4 (D4) encounters the membrane first[112, 113] The binding of D4 triggers a structural rearrangement in which ILY monomers oligomerize and form a pre-pore complex that causes lysis of the cells[112]. ILY oligomerizes with as many as 50 other ILY molecules to form large diameter pores[112, 113] through which ILY rapidly lyses the cells by necrosis (Fig. 4) [112] Interestingly, ILY does not lyse CD59 positive (CD59+)

cells from 9 other animal species (cat, chicken, cow, dog, horse, rabbit, sheep, rat, and mouse) that were tested because it does not bind to the CD59 protein of these species[111, 112] Additionally, we have documented that human serum contains ILY-specific neutralizing antibodies not found in any other animal species[98]. Another exciting characteristic of ILY is its large pharmacological window[98]. No cell lysis was observed even when the ILY concentration was 5,000-fold greater than the dose that is required to lyse 50% of human erythrocytes[98].

We have taken advantage of ILY's unique binding to hCD59 as well as the absence of the neutralizing Ab against ILY in any other animal species and developed an ILY- mediated hCD59-expressing cell ablation model in mice [98] (Fig. 4). Recently, we also confirmed its effectiveness by generating hCD59 transgenic rats that specifically express hCD59 only on the surface of their red blood cells under the control of the hemoglobin promoter[54, 98] CD59 is a glycosylphosphatidylinositol-linked (GPI-linked) membrane protein that inhibits formation of the membrane attack complex (MAC) in the complement system by binding to complement (C) proteins C8 and C9, preventing C9 incorporation and polymerization[114]. Over the past fifteen years, our group has focused on generating *CD59* knockout[115–118] and *hCD59* transgenic mice to investigate the role of CD59 in the pathogenesis of human diseases[119–122]. Importantly, over-expression of CD59 does not cause any abnormalities *in vitro* or *in vivo*[98, 116, 122–127]

To increase the usefulness of ILY/hCD59 cell ablation, we have explored the potentially broad application of the ILY-mediated cell-ablation model by generating Cre- inducible hCD59 transgenic mice (Fig. 4) [128] Specifically, we generated a line of Cre- inducible floxed STOP-hCD59 transgenic mice, where specific hCD59 expression occurs following Cre-mediated recombination (ihCD59). By crossing ihCD59 transgenic mice with transgenic mice that express Cre in a cell-specific manner or after delivery of an adenovirus expressing Cre, we obtained several lines that specifically expressed ihCD59 in a spatially regulated manner on the surface of immune, epithelial, or neuronal cells[128]. ILY injection resulted in conditionally specific ablation of various types of cells without detectable off-target effects, including on evidence of lysis to cells in adjacent tissues [128] Moreover, we tested this ablation technique in diverse disease models and found it valuable for the study of cellular functions, regeneration, and neuronal and tissue injury[128].

Together, in the section, we have reviewed versatile tools for ablating the targeted cell populations including the neuronal cells and discussed their features (Table 1). In the next, we will discuss how to apply these tools for the studies.

Application of cell ablation tools

Selection of the appropriate cell ablation tool for studying function of a targeted cell population is less straightforward than selection of a tool for studying gene function. As discussed above, each method can be applied to study diverse cells in different tissue compartments with varying efficacies, mechanisms, and off-target effects. The following elements are crucial for selecting the best strategy: (1) The specific scientific question. Does the scientific question relate to the development, regeneration, and/or pathogenesis of human

diseases? (2) The location of the cells of interest. Are they in the CNS or peripheral cells? Are they circulating cells or cells found in solid organs? (3) Whether the targeted cells are proliferative and to what degree. (4) The properties of the candidate technique. Does the technique ablate the targeted cells conditionally or continually? What is the duration of ablation? Are there any off-target effects? Is a Cre line necessary and how specific is its expression for the cells of interest? Based on these elements, we will make some general recommendations in the following sections for selecting techniques to study targeted cell populations under physiological and pathological conditions, in microenvironment for tumorigenesis, during regeneration and development, as well as to generate the disease models in multiple species including not only rodents but also large animal species.

1. Physiological and pathological conditions

Dissecting normal cell function and normal cellular interactions is fundamental to understanding multi-cellular life. Thorough characterization of a targeted cell population's function under physiological conditions also facilitates understanding of its pathogenic role and of how to cause it to regenerate after ablation. The LOF approach is essential for achieving these goals.

For cells in the CNS—CNS consists of a network of neurons and a large number of additional types of cells, including oligodendrocytes, microglia, astrocytes, and ependymal cells. The blood brain barrier (BBB) is a highly selective, semi-permeable membrane that is a significant determent for selecting the most suitable ablation method for the CNS. Formed by brain endothelial cells and astrocytes, it allows selective passage of water, some gases, and lipid-soluble molecules by passive diffusion, in addition to selective transport of molecules, such as glucose and amino acids, that are crucial for neuronal function[129]. The selectivity of the BBB is maintained by tight junctions between capillary cells that do not exist in normal circulation[129]. This barrier also includes a thick basement membrane and astrocytic endfeet[130] Biological toxins such as ILY cannot easily pass the BBB to access neurons and other type of cells that may be targeted in the CNS[128]. Ablation of CNS cells may require systemic injections of higher doses of biological toxins, including DT, than ablation of peripheral cells. However, since DT has a narrow pharmaceutical window [96,97], a higher dose may cause off-target effects that can confound interpretation of results. Local injections of these toxins to the CNS may provide an alternative strategy to overcome this problem. Other strategies using administration of small molecules, including ganciclovir (GCV) for HSV-1-TK and NTR/pro-drug-mediated cell ablation, are well-established techniques for ablating post-mitotic cells such as neurons and astrocytes[89–91, 131132] Additional considerations include the minimal capacity of neurons to regenerate their axons and their unique feature of propagating electrical signals (action potentials) along their axons. For these reasons, chemogenetic or optogenetic techniques are the best choice for transient inhibition of neuronal activity, as we discussed above. Optogenetics-mediated cell ablation can be used to further study the function of neuronal cells and circuitry. HSV-1-TK, NTR/pro-drug and caspase-mediated cell ablation can also be used for dissecting the function of proliferative cells such as glial cells. Finally, toxin-based approaches can serve as an alternative to confirm the phenotypes of targeted CNS neurons and glial cells obtained by these techniques.

For the cell populations in the circulation—Numerous tools are available to ablate targeted cell populations in the circulation or peripheral organs. Circulatory cells include immune cells, erythrocytes and endothelial cells. Immune cells are more easily accessed by all of the tools listed in Table 1 than the cells in the peripheral organs. Therefore, almost all techniques listed in Table 1, except the laser-mediated cell ablation model can be considered to ablate immune cells. In contrast, most of these techniques, except the ILY-hCD59 method, are not suitable to ablate erythrocytes which do not actively synthesize DNA and protein (Fig. 4) [9812254]

ILY-mediated cell ablation is one current strategy to destroy endothelial cells[98]. A continuous monolayer of vascular endothelial cells (VECs) protects blood vessels from uncontrolled paracellular permeability[133, 134] and vital organs from invasion by micropathogens such as HIV1 virus[135, 136] This endothelium participates in diverse homeostatic and cellular functions essential to its own and systemic integrity. For example, the endothelium controls leukocyte adhesion, platelet reactivity, capillary permeability, the regulation of vascular smooth muscle cells, and blood clotting [98] In addition, VECs actively and reactively participate in hemostasis and immune and inflammatory reactions. They are involved in the manifestations of atherogenesis, autoimmune diseases, and infectious processes. They produce and react to various cytokines and adhesion molecules and mount anti- and pro-inflammatory responses as the key immunoreactive cells[137].

Available evidence indicates that hematopoietic stem cells (HSCs) are the source of both blood cells and VECs[138]. Studies of embryonic stem cells *in vitro* demonstrate that a bipotential progenitor cell, or hemangioblast, differentiates into endothelium and cells of the hematopoietic lineages[139, 140] Thus these investigations show that VECs are an intrinsic component of myeloid lineage differentiation and underscore the close functional relationship between the hematopoietic and vascular systems[141].

As stated previously, we have been crossing *ihCD59* with transgenic mice that express Cre in a cell-specific manner or by delivering an adenovirus expressing Cre to obtain lines of mice in which *ihCD59* is specifically expressed in a spatially regulated manner on the surface of immune cells, epithelial cells, or neural cells (**Step 1 in** Fig. 4) [128]. However, because VECs differentiate from common myeloid and granulocyte/macrophage progenitors, it is a challenge to use the same protocol to generate a VEC ablation model. Crossing Tek-Cre mice with *ihCD59* mice to generate compound mice *(ihCD59*$^{+/-}$*/Tek-Cre* $^{+/-}$, hemizygous for each transgene), in which Cre is under the control of Tek promoter. In the compound mice, the Cre-mediated expression results in *hCD59* expression in endothelial cells. However, because Cre expression would occur prior to differentiation of VECs and immune cells from the progenitor cells common to VECs and blood cells, the transgene would be introduced into both hematopoietic and endothelial cells in transgenic ihCD59+/−/Tek-Cre+/− mice. To preclude this problem, we have included additional steps to obtain an ILY-hCD59 VEC-ablation model: the ihCD59+/−/Tek-Cre+/− mice are irradiated and then receive a bone marrow transplantation (BMT) from wild type mice[142]. Thorough investigations have confirmed that this strategy provides the first specific VEC ablation model.

For cells in peripheral organs—The selection of an appropriate method for ablating cells in peripheral organs is more complicated than for ablating circulating cells. The blood vascular barrier is also the key determinant for selecting a strategy for ablating cells in peripheral organs. These barriers control the trafficking of plasma contents, including water, vitamins, ions, and proteins[143]. Structural differences in the extent to which they are continuous, fenestrated, or sinusoidal cause the properties of blood vessels in different tissues to vary [143] In general, the permeability of the blood vessels in various tissues ranges from high to low in the following order: sinusoidal in liver and spleen > fenestrated open pore without diaphragm in kidney > fenestrated with diaphragm in skin, gut, and lymph nodes > continuous in thymus, skin, adipose tissue and lung[143]. We have demonstrated that ILY cell ablation is effective for targeted cells in liver and spleen [128], kidney (unpublished data), and adipocytes[144], but not in other tissues such as thymus[128].

Because specific characteristics of liver sinusoidal endothelial cells, such as the presence of fenestrae and absence of diaphragm and basement membrane, make them the most permeable endothelial cells in mammals, drugs and toxins (e.g., ILY and DT) easily reach hepatocytes, biliary epithelial cells, and hepatic stellate cells. Indeed, injection of ILY rapidly and efficiently injures hepatocytes and biliary epithelial cells in several strains of Cre +ihCD59 mice [128] This strategy therefore provides an excellent model in which to study hepatocyte and biliary epithelial cell regeneration after acute injury. Lack of a specific Cre for hepatic stellate cells prevented us from specifically deleting them in ihCD59 mice. It has been documented that expressing Cre under control of the Lrat gene promoter (Lrat-Cre) can be used to delete the floxed gene in hepatic stellate cells [145], but also deletes the floxed gene in the lungs and many additional tissues (Feng et al. unpublished data). We have generated LratCre+ihCD59 mice via crossing Lrat-Cre with ihCD59 mice. Treatment with ILY rapidly and efficiently killed hepatic stellate cells isolated from the LratCre+ihCD59 mice, but injection of ILY rapidly killed LratCre+ihCD59 mice, probably because of respiratory failure (Feng et al., unpublished data). It will be necessary to cross ihCD59 mice with a more specific Cre-expressing transgenic strain in order to generate hepatic stellate cell-specific hCD59 transgenic mice. We also recently applied ILY-mediated cell ablation to generate an acute, selective adipocyte death model and demonstrated that rapid adipocyte death preferentially induces liver injury and inflammation by activating CCR2+ macrophages and lipolysis[144] This new finding and the newly generated adipocyte damage model will stimulate further studies of adipocyte death and lipolysis in patients with nonalcoholic steatohepatitis (NASH) [144]

ILY-mediated cell ablation can be used to kill cells in the liver, spleen [128], and kidney (Liu et al, unpublished data) and adipocytes[144], but not in thymus and certain other tissues [128] These compartmental issues are related to the distribution of toxins such as DT and ILY within these tissues. For example, in skin and thymus, in which impermeable vasculature precludes use of DT and ILY, methods such as HSV-1-TK, NTR/pro-drug and caspase-mediated cell ablation can be considered, because they do not require the blood vessels to be permeable to a toxin (as shown in Table 1).

Importantly, the timing of cell ablation depends on the specific research goals. For example, to examine the role of immune cells in the pathogenesis of mouse models of human

diseases, these cells should be ablated before the disease is established, whereas determining their therapeutic value is likely to require ablation after the diseases are established.

2. Microenvironment for tumorigenesis

Cell ablation tools are also powerful for studying the role of the microenvironment in tumorigenesis. Tumorigenesis, also called oncogenesis is the process that transforms normal cells into malignant cancer cells. This intricate process is associated with cellular, genetic, and epigenetic changes and abnormal cell division[146]. The role of the microenvironment in tumor survival, progression, metastasis, immune evasion, and therapy resistance is only recently beginning to be better appreciated. Tumors are complex cellular structures that incorporate cancer cells, host cells of various lineages, and stroma. Host cells in tumors include endothelial cells that form blood and lymphatic vessels, tumor-associated macrophages (TAMs), fibroblasts, circulating blood cells, extravasated white blood cells, and platelets. The microenvironment is essential for tumor establishment, growth, infiltration of neighboring tissues, immune evasion, and metastasis. Without blood vessels the maximum volume that can be attained by a tumor is ~1mm^3; recruitment of new blood vessels through angiogenesis and/or vasculogenesis is therefore essential for tumor growth. It is now evident that these new blood vessels are formed by activation and proliferation of vessel wall stem/progenitor cells (VWSPC) or mature endothelial cells, both derived from local blood vessels, rather than by cells originating in bone marrow (references).

Understanding the contribution of various cellular components to tumor establishment, growth, invasion, and metastasis will require development of tools that can specifically ablate each of the cell types *in vivo* or in an *ex vivo* organ culture model. ILY-mediated cell ablation can be applied to both situations, particularly to an *ex vivo* organ culture model of new blood vessel formation. In the *ex vivo* aortic ring organ culture model, for example, abdominal aorta from mice is cut into ~1mm thick circular pieces and embedded into collagen in endothelial cell growth media with or without serum growth factors such vascular endothelial growth factors (VEGF). The aortic ring contains all layers of a large blood vessel, including endothelium, intima, smooth muscle, and adventitia, which acts as a reservoir of diverse types of cells, including VWSPC cells and tissue resident macrophages. Collagen-embedded aortic rings can sprout cells and form capillary-like structures when stimulated with growth factors and/or in the presence of appropriately sized tumor organoids. Formation of new capillaries begins with cell sprouting from the aortic rings within the first 24–48 hours. These cells then organize into capillary-like structures that can branch into a capillary network. It is likely that not only VWSPC and/or endothelial cells but also cells in the intima and particularly in the adventia play important roles in capillary formation. Deployment of rapid, inducible, and cell-type specific cell ablation can facilitate understanding of the role that each of these cell types plays in the formation of new blood vessels. The speed of ablation is important because the first stage of new capillary formation, activation of VWSPC or endothelial cells, occurs within 24–48 hours of culture. Techniques that require prolonged incubation of the inducer will therefore not be useful. Diphtheria toxin-based cell ablation, for example, cannot be used because it requires nearly three days, when capillary formation in aortic ring organ culture model is already well established

3. Regeneration and Development

Cell ablation has been used extensively to investigate processes related to development and regeneration in several model systems from worm to mice (Table 1). Before the advent of genetic cell ablation methods, laser ablation was used to examine the role of the anchor cell in vulval induction and uterine development in *C. elegans* [147, 148149], and of floor plate cells and motor neurons in neural patterning in the zebrafish embryo [150151152] Moreover, optogenetic cell ablation is the preferred method for transecting axons in axon regeneration studies in worms and zebrafish (Table 1 and Fig. 1) [153154155156157] Genetically-mediated cell ablation has been used extensively in zebrafish in several contexts: 1) to ablate putative stem cells and study their roles in the spinal cord and fins [158159]; 2) to ablate endothelial cells and study their role in regenerating cardiac tissue [88, 160]; and 3) to ablate motor neurons and evaluate their contribution to specific behaviors [15] In *Drosophila*, genetically-mediated cell ablation has been employed to study neuronal circuits [1126] The various chemical-genetic ablation methods used in these studies require several hours to be fully effective and therefore are not suited for investigating dynamic events like cell motility and neuronal activity. Due to its rapid action, ILY-hCD59 mediated cell death represents a powerful alternative approach to generating fundamental insights into dynamic cellular processes and functions (Fig. 4).

4. Generation of models of human disease

Cell ablation tools are not normally used to create mouse models of human diseases because only a few are caused by deficiency of a single cell lineage. Examples include type I diabetes, which results from loss of pancreatic β-cells; Parkinson's disease, from loss of dopaminergic neurons; hemolytic anemia, from loss of mature erythrocytes; ribosomopathies such Diamond-Blackfan anemia, from loss of myelogenic progenitors. Streptozotocin (STZ)-induced type I diabetes[161] and chemically induced Parkinson's disease [41, 42] have been extensively used to study the pathogenesis of these diseases. STZ specifically targets and damages pancreatic p-cells by interaction with the GLUT2 receptor[162], which is abundant on p-cell plasma membranes, but also expressed in liver and kidney to a lesser extent[162]. Caspase-mediated cell ablation has generated an animal model of inducible β-cell destruction, which is potentially useful in many areas of diabetes research (Wang ZV et al., 2008). As discussed earlier, injection of MPTP, paraquat, 6-OHDA or rotenone to mammalian CNS eliminates dopaminergic neurons and provides animal models of Parkinson's disease[41, 42]

Generation of a model of intravascular hemolysis in mouse is important for better understanding the pathogenesis of the sequelae of intravascular hemolysis in human hematological diseases. These sequelae include such important clinical manifestations as abdominal pain, dysphasia, erectile and endothelial dysfunction, pulmonary arterial hypertension (PAH), renal failure, platelet activation/thrombosis [163], and even unexpected sudden death[164–167]. This accounts for 12–26% of all deaths in sickle cell disease (SCD)[168–172] and occurs frequently in other acute and chronic hemolytic diseases, such as thalassemia, hemolytic uremic syndrome (HUS), paroxysmal nocturnal hemoglobinuria (PNH), ABO mismatch transfusion reaction, malaria, and autoimmune hemolytic anemia[173–177]. Pulmonary arterial hypertension (PAH) is suspected to be a strong

determinant of mortality in hemolytic disorders[178–180], although high mortality with only mild-to-moderate increases in systolic pulmonary artery pressure remains an unresolved paradox[181]. Acute hemolytic disorders such as ABO mismatch transfusion reaction and HUS associated with escherichia coli O157:H7 infection are less commonly seen in clinic than chronic hemolytic disorders, but also are associated with sudden death[174–177]. The underlying mechanism by which hemolysis contributes to the development of PAH in SCD and other hemolytic disorders still remains unclear[182, 183] Additionally, the risk factors for a fatal attack are poorly defined due to the lack of a rapid, conditional hemolysis model[52, 53] To address these questions, we developed an unique hemolysis model, in which sudden death and varying degrees of hemolysis can be conditionally induced in the *ThCD59*^{RBC} by administration of ILY to mice[98] and rats [54] We used this approach to demonstrate the critical roles of NO insufficiency and platelet activation in the pathogenesis of PAH and sudden death in the acute massive hemolytic model [122] These results also show that ILY-mediated rapid erythrocyte damage model is very useful for dissecting the pathogenesis of hemolysis-associated complications.

5. Tools used in large animal species

Most of the cell ablation techniques, except DT/DT-A, can be applied to study the function of targeted cell populations in large animal species such as monkey, pig, and rabbit. The DT/DT-A method cannot be used in animals other than mice and rats because other species express an endogenous DT receptor that makes their cells sensitive to DT-mediated cell apoptosis. The ILY-induced technique, however, can be used to generate cell ablation models in a large animal species [98] Broad use of this strategy in species besides mice and rats is achievable due to the following specific features[98]: (1) ILY does not damage cells in animals other than humans[111] and (2) Absence of ILY-neutralizing antibodies (Abs) in species other than humans allows it to be used in transgenic animals such as mice, rats, and pigs[98]. Importantly, hCD59 overexpression caused no obvious abnormalities in *hCD59* transgenic mice [98, 122]

Conclusions and future directions

Emerging tools for permanently or conditionally ablating targeted cell populations and transiently inhibiting neuronal activities have varied and distinct features. None of the present cell ablation models can be universally applied to study different cell types in various tissue compartments *in vivo*. All of them require further development and improvement to meet the urgent need for better understanding of the mechanisms underlying normal cell and tissue regeneration and development and the pathogenesis of human diseases. Each strategy has advantages and disadvantages. Selection of the best technique for the particular question of interest should be based on the specific question, the anatomic location and proliferative status of the targeted cell population, whether ablation is to be conditional or permanent, rapid or slow, the pharmacological window and off-target effects of the various methods, the mechanism by which the method causes cell death, and whether there is leaky expression in non-targeted cell populations. Some of these cell ablation methods will be more suitable for studying certain cell populations or specific questions than others.

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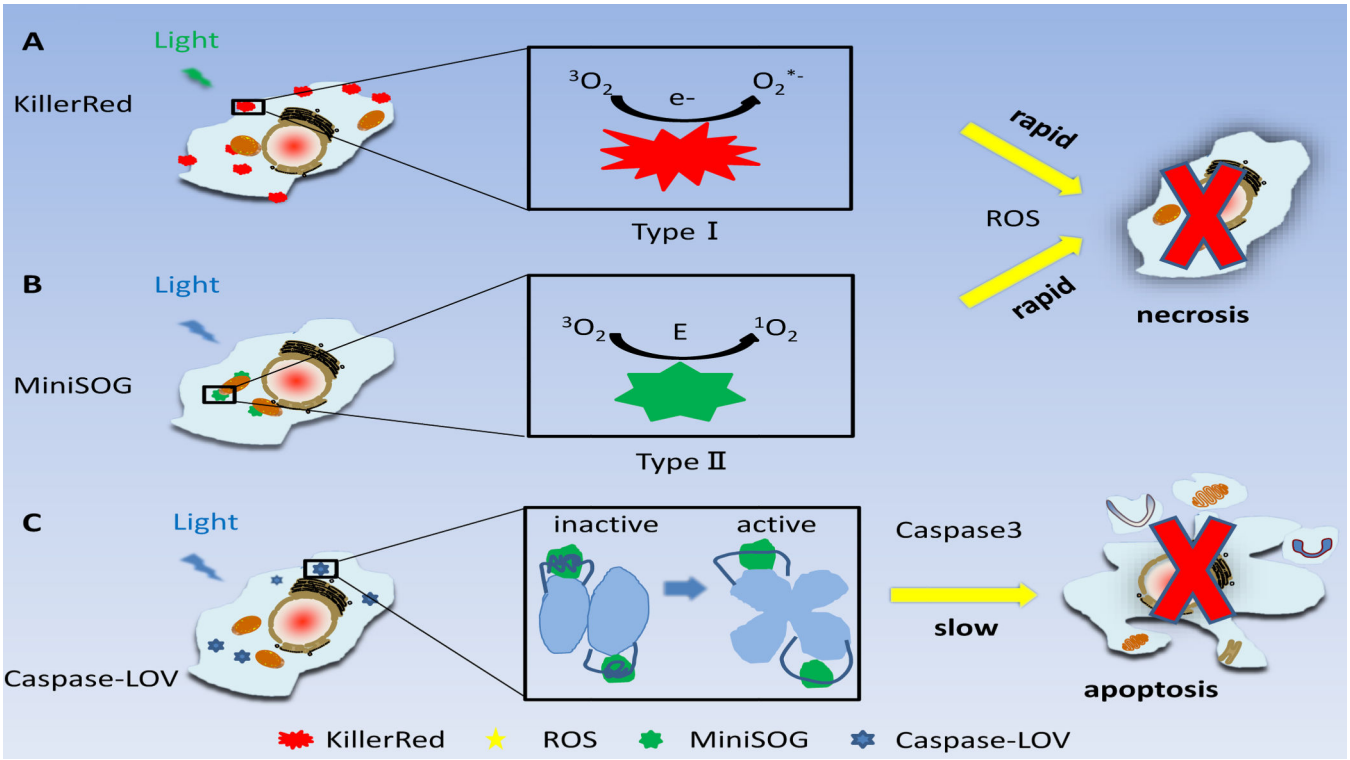

Figure 1: Optogenetic cell ablation.

A. Illumination with green light causes the rapid necrosis or death of cells expressing KillerRed on plasma membrane or mitochondria via the production of reactive oxygen species (ROS) by Type I photoreaction. **B.** Illumination with blue light causes the rapid necrosis or death of cells expressing mini singlet oxygen generator (miniSOG) on mitochondria via the production of ROS by Type II photoreaction. **C.** Illumination with blue light causes the apoptosis of the cells expressing a light-activated human caspase-3 (Caspase-LOV). Upon illumination, the rational insertion of the light- sensitive LOV2 domain expands the spring to activate pro-caspase 3 to active caspase 3, thereby leading to caspase-induced cell death.

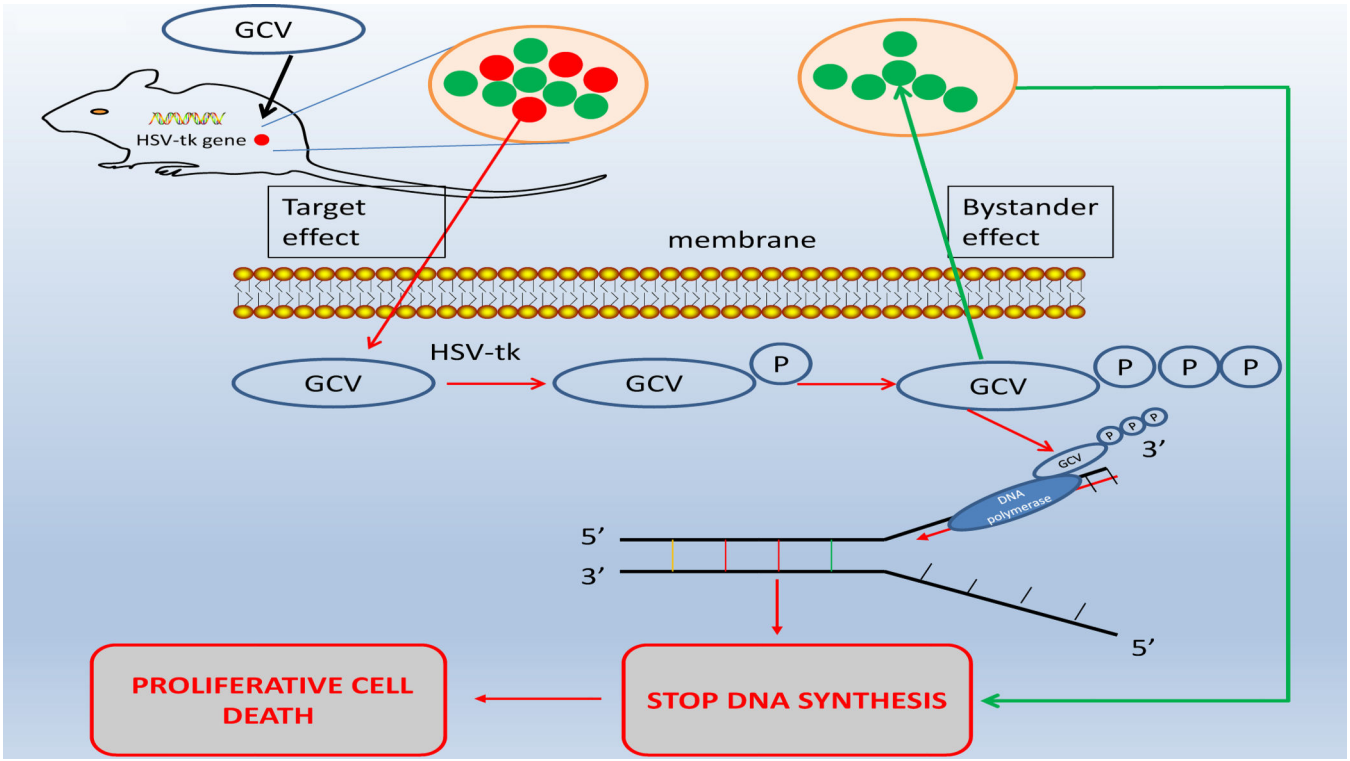

Figure 2: HSV-TK-mediated cell ablation:

GCV administration to *HSK-TK* transgenic mice induces death of target cells (red cycle) expressing HSK-TK proteins (target effect) as well as death of the adjacent cells (bystander effect). In the cell (red cycle) expressing HSK-TK, HSK-TK phosphorylates the human nucleoside analog, ganciclovir (GCV), to form deoxythymidine triphosphate, which is incorporated into the genome and causes proliferative cell death by inhibiting DNA synthesis. The deoxythymidine triphosphate causes the death on adjacent cells (green cycle) through transporting the metabolite triphosphate to nearby cells.

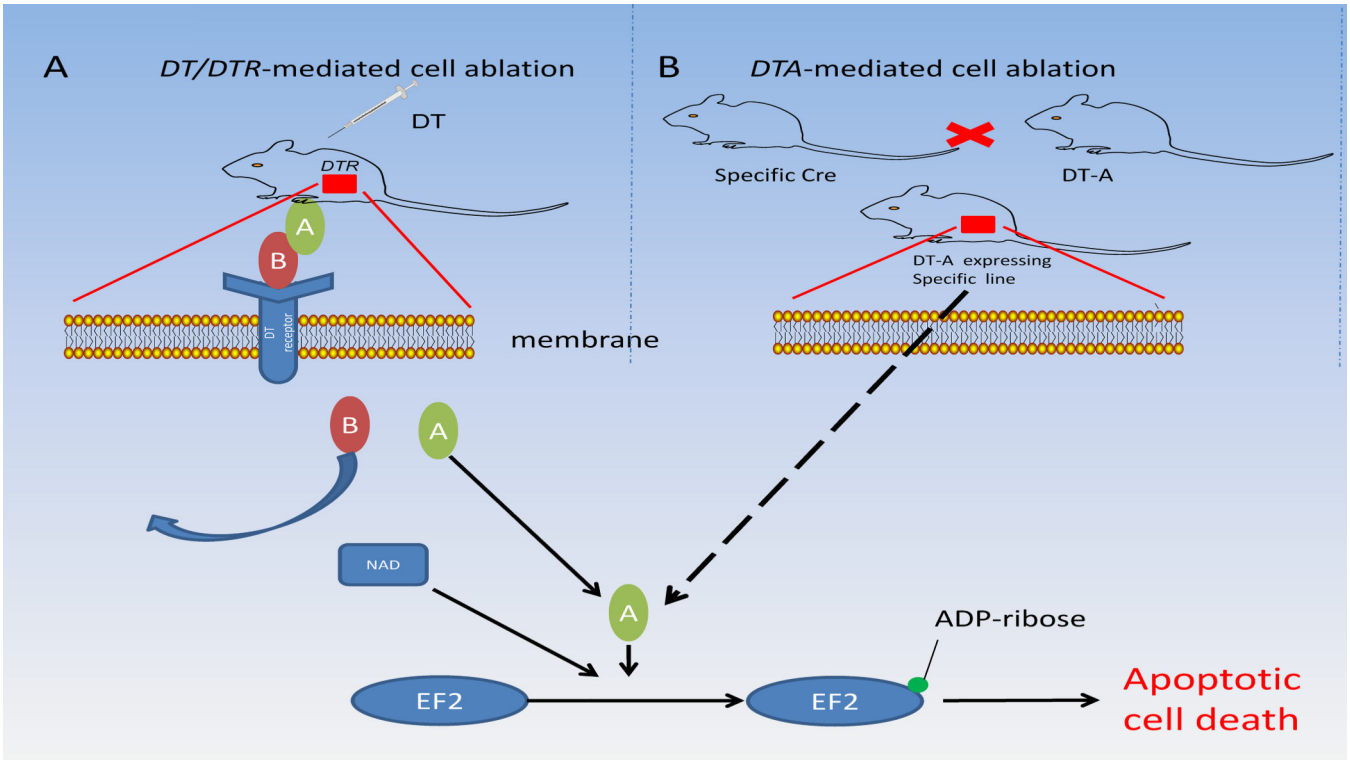

Figure 3: Diphtheria toxin-mediated cell ablation.

A. DT/DTR-mediated cell ablation. DT: Diphtheria toxin, A: fragment A, and B: fragment B. **B.** Transgenic expression of the diphtheria toxin fragment A (DT-A) mediates cell ablation.

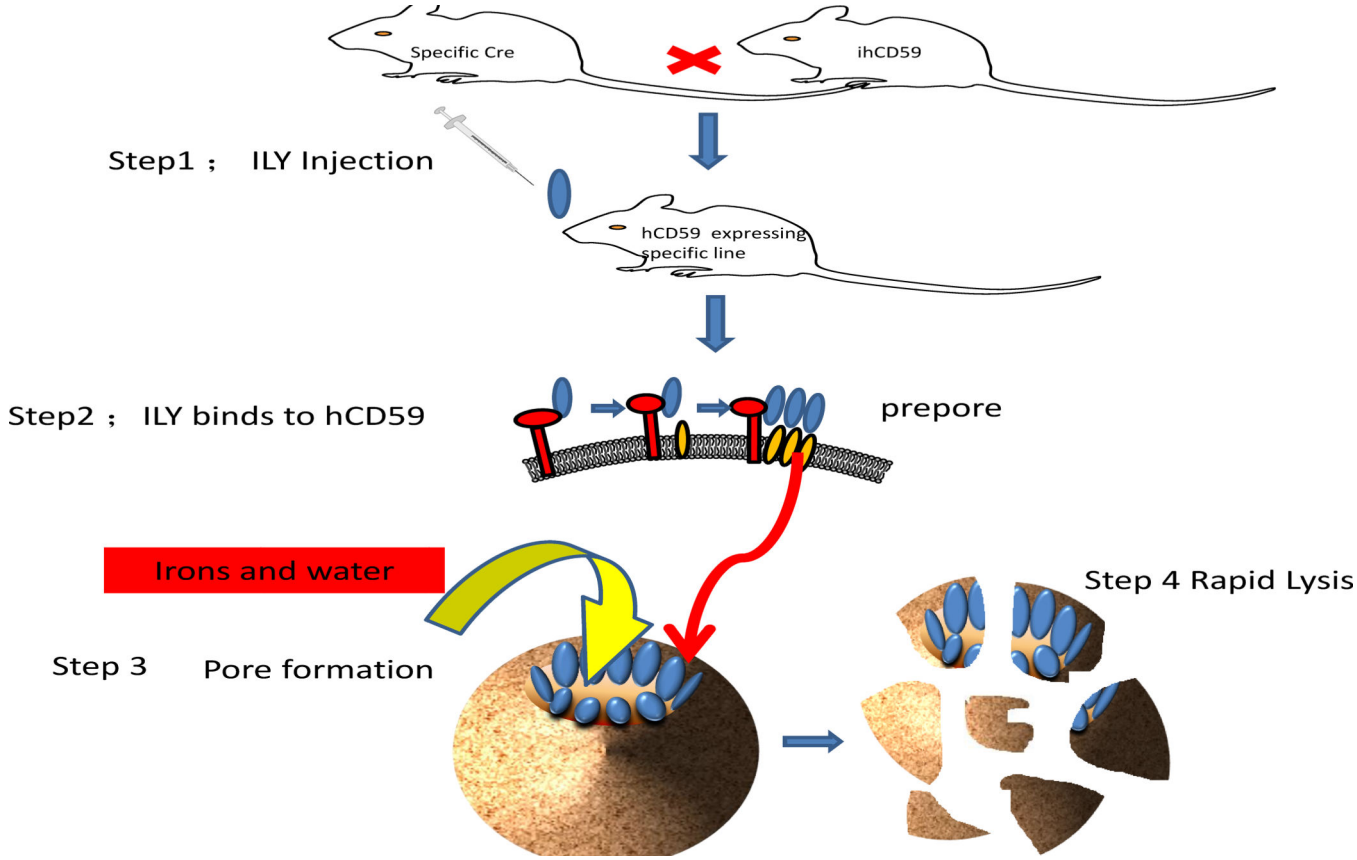

Figure. 4: Schematic of steps for ILY-mediated rapid cell ablation:

Step 1: ILY is administered to the transgenic mice expressing hCD59 on specific cell populations (hCD59-expressing specific line). These animals are generated by crossing Cre-inducible floxed STOP-hCD59 transgenic mice (ihCD59) with specific Cre mice. **Step 2**: ILY binds to cells expressing hCD59 on their surface, leading to initiation of ILY interaction and prepore formation. **Step 3**: Polymerization of ILY (n > 50) results in the pore formation in the hCD59 expressing cells. **Step 4**. Influx of iron and water into the cell induces rapid cell lysis.

Table 1:

Tools for cell ablation and inhibition:

CL: Clodronate-liposomes, PHZ: Phenylhydrazine; BE: 2-butoxyethanol; TRIP: Targeted recombination between inverted loxP sites; Ab: antibody; HSV-TK: Herpes simplex virus thymidine kinase; NTR: Bacterial nitroreductase; ILY: Intermedilysin; hCD59; human CD59; miniSOG: mini singlet oxygen generator; DREADDS: Designer receptors exclusively activated by designer drugs; DN: Dopaminergic neurons; ROS: Reactive oxygen species; and DT-A: Diphtheria toxin fragment A.

Tools	Mechanism	Features		Applications for
		Strength	Weakness	
Laser ablation	Laser power-mediated cell structure damage	Flexible	Inefficient in ablation of multiple cells in animals and unavoidable damage to adjacent cells	Early development and in in vitro organ culture
Optogenetic ablations				
1) Photo-activated killerRed or miniSOG	Acute cell necrosis via the production of ROS	High resolution, rapid effect, and less off-target effect	Limited accessibility and transparency of tissues	Early development and CNS
2) Photo-activated caspase-3	Cell apoptosis	High resolution, slow effect, and less off-target effect	Limited accessibility and transparency of tissues	Early development and CNS
Optogenetic Neuronal inhibition	Photo-activated opsins	High resoluation, rapid and reversible effect and less off-target effect	Changed natural property of cell membrane by opsin and limited studying area (light covering area)	Neuronal activities and circuitry studies
Chemogenetic neuronal inhibition	DREADDS	Accessible, flexible, and wider studying area coverage	Slow effect and control compound-mediated non specific effect	Neuronal activities and circuitry studies
Chemical-mediated Parkinson's disease model	Neurotoxin-generated toxic metabolites in DN	Easy	Only DN neuron	Parkinson's disease studies
CL-mediated monocyte ablation	CL-induced apoptosis	Easy and specific to monocytes and macrophages	CL-mediated non specific effect	Monocytes and macrophages
PHZ and BE-mediated hemolytic anemia models	PHZ or BE-mediated ROS production	Easy and specific to red blood cells	PHZ or BE-mediated non specific effect on monocytes and lymphocytes	Establishing hemolytic animal model
TRIP technology	Cre-mediated chromosomal loss leading to apoptosis	Functional study of a large variety of proliferating cell populations in vivo	Only target for proliferating cell populations and potential lethality	Development and regeneration
Caspase-mediated cell ablation	Caspase-activated apoptosis	Specific to targeted cells	Influenced by antiapoptotic factors such as adiponectin and immune suppressive effect	Physiological and pathological conditions
Ab-mediated ablation	Interaction of antibody with antigen	Easy	Limited by the availability of specific antibodies and Ab specific cell killing mechanism	Studying the function of immune cells
HSV-TK-mediated ablation	HSV-TK-mediated interference in DNA replication	Ablating proliferative cells	Non applicable for quiescent cells and off target effect on adjcent cells	Physiological and pathological conditions
(NTR)/pro-drug-mediated ablation	NTR activated cytotoxic metabolites	Ablating dividing and non dividing cells	Unavoidable bystander effects in adjacent non-targeted cell populations	Development, physiological and pathological conditions
Diphtheria toxin-mediated ablation	DT-A-mediated protein synthesis inhibition	Very potent, and able to ablating dividing and non dividing cells	The narrow pharmacological window, off-target effects and potential lethality	Physiological and pathological conditions

Tools	Mechanism	Features		Applications for
		Strength	Weakness	
ILY/hCD59-mediated rapid ablation	ILY-mediated rapid cell necrosis	Rapidity, larger pharmacological window, ability to ablating dividing and non dividing cells in multiple species	Compartmental issues	Early development, physiological and pathological conditions, in vitro organ culture system, generation of hemolytic model