



Protective Biology and Engineering

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Protective biology and engineering are an integrated discipline aiming to understand the naturally occurring protective mechanisms established through an evolution in response to environmental insults and genetic defects (protective biology), and develop and use engineering strategies and technologies to optimize protective processes against cell death in injury and disease based on the naturally occurring protective mechanisms (protective engineering). There exist systems protective mechanisms in mammals, including regional mechanisms activated in a disordered organ and distant mechanisms in non-disordered organs, both acting in coordination to support cell survival and prevent cell death in the disordered organ. However, these mechanisms are not all optimized for promptness and effectiveness. Protective engineering strategies can be developed and used to correct natural deficiencies and optimize protective mechanisms. This paper addresses the fundamental concepts and potential protective engineering strategies by using two examples of diseases—heart attack and ischemic stroke, leading causes of human morbidity and mortality.
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Introduction

Protective biology and engineering are an integrated discipline aiming to identify, characterize, and understand the naturally occurring protective mechanisms responsible for preventing cell death and organ failure under environmental and genetic insults (defined as protective biology), and develop and use engineering strategies and technologies based on protective biology to control and optimize protective mechanisms for preventing cell death (defined as protective engineering). This discipline stems from experimental observations that almost all organisms tested to date possess protective mechanisms that can be activated in response to cell injury, representing an essential life-supporting system [1]. Recognized protective mechanisms include non-homologous DNA end-joining and homologous recombination for repairing double-strand DNA breaks induced by irradiation, chemical agents, and reactive oxygen species [2–4]; upregulation and/or release of cell survival-supporting factors to protect injured cells from death [5–7]; cell regeneration in response to cell injury and death [8–10]; cell and organ hypertrophy in response to elevated mechanical stress to alleviate cell work-overload and injury [11–13]; and inflammatory responses to injury to facilitate wound healing [14,15]. However, the naturally occurring protective mechanisms are not optimized for promptness and effectiveness. For instance, lethal gene mutations occur in spite of the presence of gene repairing mechanisms; the expression and/or release of protective factors often lags behind cell death and the released protective factors are unable to reach optimal levels within the period during which cell death occurs. Protective engineering strategies can be

developed and used to correct, at least partially, these natural deficiencies and minimize the level of cell injury and death.

Protective Biology—Systems Protective Mechanisms

Injury or disorders, such as heart attack and ischemic stroke, can activate simultaneously two forms of protection—regional and distant protection, both defined collectively as *systems protective mechanisms* (Fig. 1). Regional protection takes place within the disordered organ, involving the release of paracrine factors such as adenosine [16,17], opioids [18,19], and/or bradykinin [20,21] to suppress cell death processes; upregulation and secretion of cytokines [22,23] and growth factors [24–26] to support cell survival; and activation of resident stem cells to regenerate cells in the event of cell death [27–29]. Distant protection occurs in non-disordered organs in response to messengers from the disordered organ, involving upregulation and secretion of endocrine protective factors (primarily growth factors) [30–33] and mobilization of protective cells (often from organs with a high capability of regeneration such as the liver and bone marrow) to support cell protection in the disordered organ [6,7,34]. Selected cytokines from the disordered organ can serve as messengers to activate the distant protective mechanisms [6,35]. Both regional and distant protective mechanisms act in coordination and synergy to prevent cell death and support cell survival and regeneration in the disordered organ.

The naturally occurring protective mechanisms have been recognized in experimental and clinical investigations. A striking observation is that an induced mild-level injury, defined as preconditioning injury, can significantly prevent cell death in a subsequent severe injury, representing the most effective and reproducible protective strategy against cell death to date [36,37]. The underlying mechanisms are that the induced preconditioning injury can activate all needed naturally occurring protective mechanisms within several

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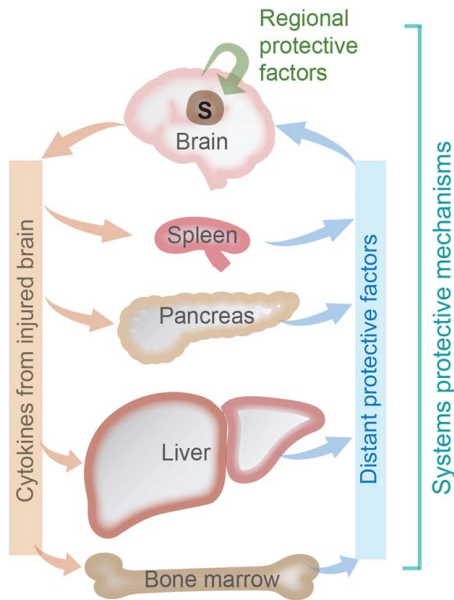


Fig. 1 Systems protective mechanisms, including regional and distant mechanisms, activated in response to ischemic stroke (S)

days after preconditioning [5,6,36,37]. The presence of the distant protective mechanisms is supported by the observations that a preconditioning injury in a distant organ, such as the hind limb, can alleviate subsequent ischemic myocardial, skeletal muscle, and brain infarction [38–41], as the preconditioning injury activates endocrine protective factors that can reach the subsequently injured organ through the circulatory system to support cell protection. These observations suggest the significance of identifying the naturally occurring systems protective mechanisms and the associated protective factors—providing a foundation for developing protective engineering strategies. Such strategies may potentially be as effective as, or possibly more effective than, the preconditioning injury procedure by engineering optimization of the types, levels, timing, and coordination of the protective factors.

Among the aforementioned protective factors, adenosine, opioids, and bradykinin act by binding to cognate G protein-coupled receptors, stimulating the cell survival-supporting and cell death-suppressing signaling pathways, such as the G protein—diacylglycerol—protein kinase C—mitogen-activated protein kinase (MAPK) pathway (supporting cell survival) and the phosphoinositide 3-kinase (PI3K)—phosphatidylinositol 3,4,5-trisphosphate—Akt serine/threonine kinase 1—Bcl2-associated agonist of cell death pathway (suppressing cell death) [5,6,37]. Selected cytokines, such as interleukin 6 and cardiotrophin 1, support cell survival via the Janus kinase—signal transducer and activator of the transcription signaling pathway [6,7,35]. Growth factors promote cell survival by activating the protein tyrosine kinase (PTK) receptor—MAPK and the PTK receptor—PI3K signaling pathways [5,6,42]. Cells mobilized from distant organs, including the bone marrow [43], spleen [44], and liver [7,34], can engraft to the disordered organ, such as the ischemic heart, releasing protective factors regionally to support cell survival and suppress cell death processes (Fig. 2). All regional and distant protective factors act together in coordination and synergy to boost cell protection. However, there are two deficiencies in the naturally occurring protective mechanisms—delayed activation and suboptimal effectiveness. These deficiencies suggest the necessity of developing protective engineering strategies.

Evolution of Protective Mechanisms

The naturally occurring protective mechanisms have been established through evolution to support the survival of organisms. The

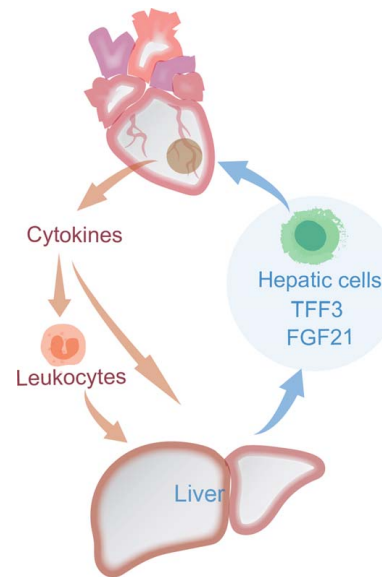


Fig. 2 Hepatic cell mobilization in response to ischemic myocardial injury. Myocardial injury causes upregulation of cytokines, which can be released into the circulatory system to activate leukocytes [34]. Activated leukocytes can migrate into the liver parenchyma to upregulate and secrete matrix metalloproteinase 2, a proteinase that can degrade collagen, causing hepatic cell mobilization into the circulatory system [34] and the ischemic myocardium [45]. The hepatic cells may release protective factors directly to the ischemic myocardium [7,34,45]. Selected cytokines may also stimulate hepatic cells to upregulate and secrete FGF21 and trefoil factor 3, which can reach the ischemic myocardium to protect cardiomyocytes from death [6,30].

protective system develops in response to constant exposure to environmental insults [1]. A potential mechanism of evolution for the protective system, as that for other biological systems [46,47], is exploitation—recruiting existing molecules and cells originally designed for other functions to serve as protective factors. The exploitation theory is supported by several observations. Adenosine is an ancient nucleoside used for energy transfer via glycolysis-based formation of ATP and the construction of the primitive RNA genome—essential processes required for the initiation and continuation of living organisms [48], probably at the very beginning of the unicellular lives about 4 billion years ago. Adenosine as a protective factor arose much later since its protective action is dependent on G protein-coupled receptors [6,7,35], which developed in *Choanoflagellates* (a Metazoan-related unicellular and colonial organism) several hundred million years ago and in multicellular organisms later [47,49,50]. Thus, adenosine was likely exploited by G protein-coupled receptors to serve as a protective ligand. Other molecular protective systems established possibly by exploitation include the glucocorticoid and mineralocorticoid receptor signaling pathways [46] and the protein tyrosine kinase signaling pathways [47], which regulate inflammation, glucose release and metabolism, water balance, cell proliferation, and cell differentiation. These processes are all related to cell protection, suggesting that the cell protective system intends to exploit molecules involved in processes that support cell protection. An example of cell types exploited to the protective system is hepatic cells [7,34]. These cells, originally established for metabolism, detoxification, blood filtration, and bile production, can be mobilized to the circulatory system and an injured organ, such as the ischemic heart, to deliver protective factors [7,34]. These observations support the exploitation theory for the establishment of the naturally occurring protective system.

An important feature of the naturally occurring protective mechanisms is labor division—various protective factors are being

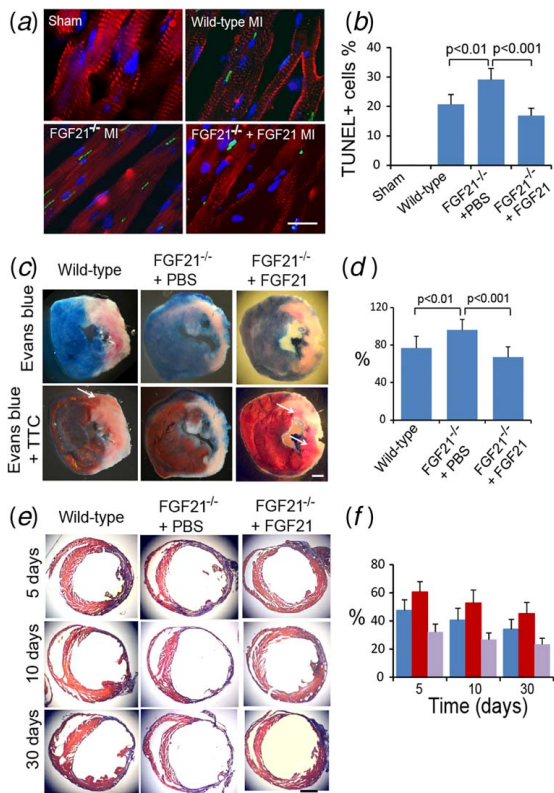


Fig. 3 Cardioprotective action of FGF21 in myocardial ischemia—reperfusion injury: (a) immunofluorescence micrographs showing cells undergoing DNA fragmentation by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay in the ischemic myocardium of wild-type mice and FGF21^{-/-} mice with PBS or recombinant FGF21 delivery at 24 h after myocardial ischemia—reperfusion injury. Red: cardiac troponin I, green: TUNEL-positive cell nuclei, and blue: cell nuclei. Scale: 10 μ m, (b) graphic representation of the fraction of TUNEL-positive cell nuclei in the ischemic myocardium calculated in reference to the total cell nuclei. Means and standard deviations are presented ($n = 8$), (c) left ventricular slices from wild-type mice and FGF21^{-/-} mice with PBS or recombinant FGF21 delivery at 24 h after myocardial ischemia—reperfusion injury, showing the influence of FGF21 on the fraction of acute myocardial infarcts (by the triphenyl tetrazolium chloride (TTC) assay) in reference to the area at risk (by the Evans blue assay). Arrows: TTC-positive or vital myocardium (red) within the area at risk (not being perfused). Scale: 1 mm, (d) graphic representation of the influence of FGF21 on the fraction of acute myocardial infarcts in reference to the area at risk. Means and standard deviations are presented ($n = 8$), (e) Azan trichrome assay-stained left ventricular sections from wild-type mice and FGF21^{-/-} mice with PBS or recombinant FGF21 delivery at 5, 10, and 30 days after myocardial ischemia—reperfusion injury. Red: intact myocardium, and blue: myocardial infarcts and fibrous tissue. Scale bar: 1 mm, (f) graphic representation of the fraction of myocardial infarcts in wild-type mice (blue) and FGF21^{-/-} mice with phosphate buffered saline (PBS, red) or recombinant FGF21 (purple) delivery at 5, 10, and 30 days after myocardial ischemia—reperfusion injury. Means and standard deviations are presented ($n = 6$). The p -value is <0.0001 for both treatment- and time-based comparisons by analysis of variance (ANOVA). Material from: Liu, S. Q., et al. Endocrine protection of ischemic myocardium by FGF21 from the liver and adipose tissue. *Sci. Rep.* 3: 2767, 2013, Springer Nature [32].

expressed and released from multiple organs to support the survival of an injured organ [6,32–35]. Cells in a single organ may not be able to provide all needed protective factors in the event of injury. This problem enforces mutual reliance between different organ systems—a systems mechanism analogous to the hormone-

based endocrine mechanisms for inter-organ communications and functional control. Such a mutual reliance has been considered a critical mechanism that drives the evolution of multicellular organisms and preventing evolutionary reversion to unicellular organisms [51], a form probably less competitive for survival in a hostile environment. Furthermore, a large number of protective factors are required for cell protection in injury and disease [5,6]. It is costly in energy consumption to construct all needed protective machineries and generate all needed protective factors in every individual cell, a circumstance against the principle of energy minimization. Thus, the establishment of multi-cell and multi-organ-based systems protective mechanisms is advantageous to the evolution of multicellular organisms.

Protective Engineering—Optimization of Protective Mechanisms

Protective engineering is to develop engineering strategies and technologies to control and optimize protective mechanisms against cell death in injury and disease. Protective engineering strategies can be established based on the naturally occurring protective mechanisms. These strategies can be categorized into molecular, cellular, and tissue-level protective engineering. *Molecular protective engineering* is to modify the types, levels, activation timing, and coordination of protective factors by controlled protein delivery, gene transfer, gene editing, RNA interference, epigenetic modifications, and/or other molecular engineering strategies, thereby optimizing cell protection processes and minimizing cell death in injury and disease. An example of molecular protective engineering is prompt delivery of the cardioprotective protein fibroblast growth factor 21 (FGF21) into the ischemic myocardium to prevent cardiomyocyte death in heart attack [32] (Fig. 3). *Cellular protective*

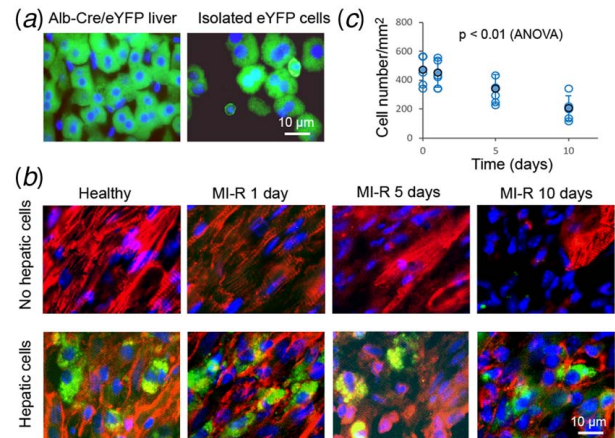


Fig. 4 Transplantation of enhanced yellow fluorescent protein (eYFP)-positive hepatic cells to the myocardium of C57BL/6J mice with and without myocardial ischemia—reperfusion injury: (a) fluorescence micrographs showing eYFP-positive hepatic cells (green) in the liver of a transgenic mouse (Alb-Cre/eYFP) expressing hepatic cell-specific eYFP and isolated eYFP-positive hepatic cells in PBS. The length bar is for both images, (b) fluorescence micrographs showing eYFP-positive hepatic cells (green) transplanted into the myocardium of a healthy mouse and into the ischemic myocardium core of mice with MI-R injury. Red: Antibody-labeled cardiac troponin I, blue: cell nuclei for both panels (a) and (b), (c) graphic representation of the density of eYFP-positive hepatic cells transplanted to the myocardium of C57BL/6J mice. Means and standard deviations are presented ($n = 6$). The solid and open circles represent the means and individual data points. Material from: Liu SQ, et al. Hepatic cell mobilization for protection against ischemic myocardial injury. *Sci. Rep.* 11: 15830, 2021. Springer Nature [45].

engineering is to introduce stem or somatic cells to an injured organ to protect cells from death. Bone marrow-derived stem cells have been utilized for transplantation into the ischemic myocardium to release cardioprotective factors, an effective approach to alleviate myocardial infarction [52,53]. Transplantation of somatic hepatic cells into the ischemic myocardium can alleviate myocardial infarction [45] (Figs. 4 and 5). *Tissue-level protective engineering* is to protect and support an injured organ by implantation of a tissue construct. A common strategy is to implant a protective factor- and/or cell-containing extracellular matrix or synthetic construct to an injured organ, such as the heart [54] and brain [55], to provide structural and/or functional support and prevent cell death. Overall, these molecular, cellular, and tissue-level engineering strategies can be designed and used for precise control of the types, levels, timing, and coordination of systems protective actions, thereby optimizing the protective processes, minimizing cell death, and facilitating recovery from injury and disease.

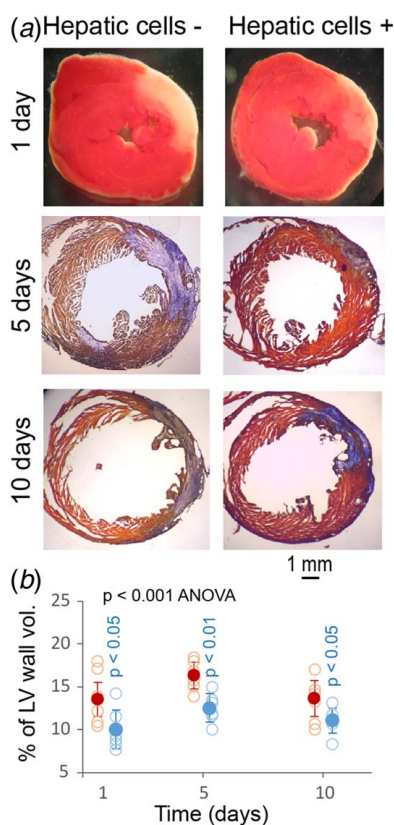


Fig. 5 Mitigation of myocardial infarction in response to hepatic cell transplantation: (a) left ventricular specimens at day 1 of myocardial ischemia—reperfusion injury were treated with TTC for demonstrating acute myocardial infarcts (pale), and left ventricular specimens at days 5 and 10 of MI-R were stained with orange-red and methylene blue (Azan trichrome assay) for demonstrating myocardial fibrosis (blue) in mice with and without hepatic cell transplantation, and **(b)** graphic representation of the influence of hepatic cell transplantation on the level of myocardial infarction. Solid red and blue circles: Means and standard deviations from individual mice without (red) and with (blue) hepatic cell transplantation, respectively. Open red and blue circles: Raw data points ($n=6$) from individual mice without and with hepatic cell transplantation, respectively. The blue-colored p -value at each time point is for comparison between mice with and without hepatic cell transplantation. Material from: Liu SQ, et. al. Hepatic cell mobilization for protection against ischemic myocardial injury. *Sci. Rep.* 11: 15830, 2021. Springer Nature [45].

Protective Versus Regenerative Engineering

Protective engineering is closely related to another engineering discipline—regenerative engineering. However, the two disciplines focus on distinct biological and engineering aspects. Protective engineering aims to optimize protective actions prior to cell death [1], whereas regenerative engineering is to induce and/or control cell regeneration after cell death [1,9]. In a broader sense, regenerative engineering is protective—protecting an injured organ from failure by regenerating cells and extracellular matrix. The ultimate goal of both types of engineering is the same—supporting the structure and function of an injured organ and facilitating recovery from injury and disease. In terms of engineering outcomes, protective engineering prevents cell death and sustains the native structure and function of an injured organ. Regenerative engineering, in contrast, induces new cell formation by activating, mobilizing, and/or transplanting stem or somatic cells to an injured organ to restore its structure and function. To be effective, the regenerated cells must survive and adapt to the native environment, integrate into the native architecture, and establish the native function of an injured organ. These demands impose significant challenges to regenerative engineering. The development of optimized protective engineering strategies can ultimately reduce the demand for regenerative engineering. However, cell death occurs inevitably despite protective engineering modulations in severe injury and disease. Thus, both protective and regenerative engineering strategies are required to maintain and restore the structure and function of an injured organ.

Concluding Remarks

Protective biology and engineering are an emerging discipline based on knowledge primarily from experimental studies. Although various protective engineering strategies have been established and tested in experimental models, few have exerted a significant clinical impact [5,56,57]. One obstacle is the lack of complete understanding of the naturally occurring systems protective mechanisms [6,7]. Current clinical treatment strategies deviate considerably from the natural mechanisms of protection [5,56,57]. Whereas a multitude of time-dependent protective molecules and cell types are required for the effective natural form of protection [6,7,30], a single “protective agent” targeting a selected molecule or pathogenic process is commonly used in clinical tests, a potential problem for the failure of most protective clinical trials [5,56,57]. This point is supported by the testimony that a preconditioning injury, which causes activation of all necessary protective factors and mechanisms, represents the most effective and reproducible treatment strategy for protection against a subsequent injury in humans and animal models [5,6,56,57]. However, a list of naturally occurring protective factors remains incomplete and their mechanisms of action have not been fully understood. To develop protective engineering strategies that maximize the capacity of cell protection, it is necessary to identify naturally occurring protective factors and understand the underlying mechanisms of action. Such information can be used to identify and correct the deficiencies of the naturally occurring protective mechanisms by engineering optimization.

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Conflict of Interest

There are no conflicts of interest. This article does not include research in which human participants were involved. Informed consent was not applicable. This article includes research with animal participants. All the applicable international, national, and

institutional guidelines for the care and use of animals were followed. Documentation provided upon request.

Data Availability Statement

The datasets generated and supporting the findings of this article are obtainable from the corresponding author upon reasonable request.

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