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Review / Derleme

Use of Zebrafish As a Model for Understanding the Interplay Between Inflammation and Stem Cells

Enflamasyon ve kök hücre arasındaki etkileşimi anlamak için zebrabalığı modelinin kullanımı

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Inflammation is a natural response mechanism to various stimuli in animals. The initiation and progression of inflammatory cascade may follow similar routes within the phylogenetic classes. However, the regulation of the chronicity or the consequences of the inflammatory milieu on the tissue microenvironment varies among organisms. Since stem cells are integral to the maintenance and restoration of tissues, the effects of inflammation on stem cells is an important aspect in various disciplines such as immunology, cell biology or medical science. Additionally, stem cells and the disease states are closely associated, as the majority of the diseases contain stem cells that ultimately malfunction. Therefore, elaborating on the interplay between the stem cells and inflammation would be instrumental in designing regenerative therapies in humans. In this review, the modes of regulation of stem cells upon inflammatory cues and the relationship of inflammation to the disease conditions will be discussed with an outlook on regenerative medicine.

Key words: Disease; inflammation; regeneration; stem cell.

Enflamasyon hayvanlarda çeşitli uyaranlara karşı doğal bir yanıt mekanizmasıdır. Enflamatuvar kaskadın başlaması ve progresyonunda, filogenetik sınıflar içerisinde benzer yollar izlenebilir. Bununla birlikte, kronikliğin düzenlenmesi veya enflamatuvar ortamın mikro doku çevresi üzerindeki sonuçları organizmadan organizmaya değişiklik gösterir. Kök hücreler dokuların korunması ve restorasyonu için bütünleyici olduğundan, enflamasyonun kök hücreler üzerindeki etkileri immünoloji, hücre biyolojisi veya tıp bilimi gibi çeşitli disiplinler için de önemlidir. Ayrıca bu hastalıkların birçoğunda nihayetinde işlevi bozuk olan kök hücreler mevcut olduğu için, kök hücreler ve hastalık durumları birbirleriyle yakından ilişkilidir. Bu nedenle, kök hücreler ve enflamasyon arasındaki etkileşimin aydınlatılması, insanlarda rejeneratif tedavilerin geliştirilmesine yardımcı olacaktır. Bu derlemede, kök hücrelerin enflamatuvar işaretler üzerindeki düzenlenme modları ve enflamasyonun hastalık durumları ile olan ilişkisi, rejeneratif tıp perspektifinden irdelendi.

Anahtar sözcükler: Hastalık; enflamasyon; rejenerasyon; kök hücre.

Inflammation is a non-physiological response to the compromise of tissues in situations related to pathogen invasion, injury, or toxic chemicals.[1] The acute phase of inflammation is an interim response that is elicited by macrophages residing in the tissue and the dendritic cells via the secretion of pro-inflammatory cytokines, which recruit neutrophils and macrophages to the site of interest and activate the complement system.[2] Acute inflammation functions to restrain the initial threat to the tissue and is resolved by the activity of anti-inflammatory agents such as interleukin (IL)-10, transforming growth factor beta (TGF-β), and regulatory T cells. In the long-term, the adaptive immune system is stimulated, and the activity of the B, T, and natural killer (NK) cells generates a memory of the antigen.[3] Invasive injuries or tissue degeneration may lead to extended periods of inflammatory reaction, which causes chronic inflammation that is detrimental for tissue integrity and homeostasis in mammals.[4] Several diseases, such as diabetes, neurodegenerative disorders, and cancer, are products of chronic inflammation. In addition, all cell types are affected by inflammatory conditions; however, being the main repository, stem cells are vital for basic and clinical research. Therefore, focusing on stem cells

would help to understand why in general inflammation has a negative effect on tissue restoration in mammals.

STEM CELLS AND INFLAMMATION

Stem cells are regulated by their niche via different mechanisms, one of which is inflammation. In many systems, stem cell activity has been hampered by inflammation.[5-8] The question of whether there is a way to modify the inflammatory milieu in the tissues to allow the stem cells to function properly and restore lost cell types in cases of traumatic injuries, chronic degeneration, or metabolic disorders has yet to be answered.

Inflammation has been shown to regulate the proliferation and differentiation capacity of various stem cell niches. For instance, mesenchymal stem cells, the multipotent stromal cells of the bone marrow, adipose tissue, umbilical cord, and muscle, respond to inflammation. Furthermore, IL-1 from macrophages directs mesenchymal stem cells toward muscle cells,[9] and intestinal stem cells of the crypt increase their proliferation rate upon IL-6 secretion by the dendritic cells.[10] Similarly, IL-17 from the T-lymphocytes leads to the hyperproliferation of crypts.[11] In addition, satellite cells of the skeletal muscle are regulated by pro-inflammatory cytokine signaling through the CX3C chemokine receptor 1 (CX3CR1) and the monocyte chemoattractant protein-1 (MCP1) expressed either by the myofibers or the resident leukocytes.[12] Another example occurs when liver stem cells respond to tumor necrosis factor-alpha (TNF-α) that is derived from macrophages or interferon gamma (IFN-γ) via cytotoxic T-cells by enhancing their expansion capacity.[13] Moreover, CD34-positive hair follicle cells gain a reduced proliferative state upon the expression of MCP1 by macrophages,[14] Neural stem cell potential in mammals was also found to be suppressed via inflammation,[15-17] which has been verified by several studies on rodents showing that immunosuppression via genetic modifications or drug treatments can enhance the neurogenic outcome after various insults in mammalian brains.[17-20] Although these studies proposed that inflammation has a harmful effect on stem cell activity, various other studies have suggested otherwise. For example, Belmadani et al.[21] found that in a mouse hippocampus, neural stem cells proliferate and migrate to the injury site upon C-C chemokine receptor type 2 (CCR2) and MCP1 expression and that enterotoxin-mediated inflammation helps hippocampal progenitors enhance their proliferation rates.[22] These results suggest that the role of inflammation on stem cell activity is context-dependent and can be modified to increase the beneficial outcomes and suppress the negative consequences.

DISEASE CONDITIONS AND INFLAMMATION

As a non-homeostatic measure, inflammation also manifests in disease states such as metabolic disorders, neurodegeneration, and cancer by exacerbating the etiological progression. For instance, type-2 diabetes is triggered by uncontrolled inflammation and macrophage recruitment.[23,24] The cascade of cell death mechanisms in β-cells through nuclear factor kappa B (NFκB), the NLR family, pyrin domain-containing 3 (NLRP3) gene, and the Fas ligand is caused by an M1-macrophage-derived inflammatory milieu.[4,25] In addition, the rampant activity of microglia, the resident macrophages of the central nervous system, is connected to neurodegeneration[26] as the CD4+ T cells are induced by reactive oxygen species upon cytotoxicity[27] or motor neuron degeneration involving the accumulation of pro-inflammatory macrophages and T cells through TNF-α and IL-1β.[28] Similarly, in cancer progression, inflammation has a mediator effect. For example, IL-22 leads to tumor development in the intestine,[29] and IL-6 activates the signal transducer and activator of transcription 3 (STAT3) oncogene in hepatocellular carcinoma.[30] Therefore, understanding how the effects of inflammation on stem/progenitor cells could be offset for the cause of tissue regeneration is of great clinical value but is also scientifically challenging.

THE ZEBRAFISH AS A MODEL ORGANISM FOR EXPERIMENTATION REGARDING INFLAMMATION AND TISSUE REGENERATION

The zebrafish has a widespread regenerative capacity that is not manifested in mammals.[31] As an adult, zebrafish can regenerate many organs, including their appendages, heart, liver, skin, kidneys, retina, spinal cord, and brain.[32] Given that mammals and zebrafish have similar genome architecture, developmental programs, and cell types, what sets up this disparity in regenerative ability is a challenging question, but unraveling this mystery could help us understand how we could harness those molecular programs by initiating the regenerative ability to tweak the mammalian stem cells so that they could contribute to the regeneration of lost structures and cells in mammals. The central nervous system, for example, is one such tissue where the lack of regeneration of lost neurons exacerbates neurodegenerative disorders or acute traumatic insults. The zebrafish brain contains constitutive stem cell regions throughout the brain axis, whereas mammals have only two such zones in their forebrain.[33-38] Kyritsis et al.[39] found that the activity of zebrafish neural stem cells in the brain is regulated by acute inflammation

after traumatic injuries. This effect is in part mediated by the activity of the inflammatory lipid leukotriene C4 and its receptor cysteinyl leukotriene receptor 1 (Cystlr1).[39] This receptor is expressed at very low levels in zebrafish neural stem cells in the physiological state, but it is highly upregulated after the lesion. When the lesion is omitted and sterile inflammation is induced using a cerebroventricular injection[40,41] of zymosan A molecules, the stem cells initiate a similar response to that of the acute lesion as they increase their proliferation rate and form more neurons that functionally integrate into the existing circuitry. Additionally, when leukotriene C4 (LTC4) is injected into the unlesioned brain, the stem cells get activated and mimic a regeneration situation, suggesting that acute inflammation through a LTC4/Cystlr1 mechanism is sufficient for a regenerative response of the neural stem cells.[39] When the fish is immunosuppressed with dexamethasone or the leukotriene signaling mechanism is blocked using pranlukast, the regenerative response is also blocked, indicating that inflammation and leukotriene signaling are required for stem cell function. Additionally, Kizil et al.[42] discovered a special molecular program that involves the activity of the transcription factor gata3 that is activated only after injury in zebrafish tissues. Furthermore, the gata3 function is strictly required for zebrafish neural stem cells to respond to injury by reforming the lost neurons.[42] Moreover, gata3 is a downstream target of LTC4 signaling, suggesting that acute inflammation initiates a special molecular program in neural stem cells and enables them to have a regenerative capacity.[39,42,43] Several studies also identified other players in the immune system that have an effect on the activity of the neural stem cells of the fish brain, including chemokine signaling.[44-46] This indicates that the immune system and neural stem cells have an intricate relationship which might be responsible for establishing the regenerative capacity.

THE RAMIFICATIONS OF THE RELATIONSHIP BETWEEN INFLAMMATION AND STEM CELLS ON REGENERATIVE MEDICINE

Inflammation usually has a negative effect on stem cell behavior and hampers the regenerative capacity since when combined with pro-inflammatory cytokines, it has been shown to impede repair.[47-50] However, counterarguments suggest that inflammation may also have a stimulatory function on stem cell-based regeneration.[39,51-53] These findings indicate that the role of inflammation on stem cell activity is highly context-dependent based upon the type of cells involved and the timing of the inflammatory response. Therefore, organisms like zebrafish pose a great opportunity to study how the unfavorable conditions of inflammation can be circumvented with the help of special molecular programs of stem cells. They also help determine which stages of the damaged mammalian tissues are the most appropriate for conducting an intervention to repair or restore the tissue integrity and function. Furthermore, additional research that focuses on zebrafish might provide beneficial information that could be used for eliciting a regenerative response in otherwise compromised tissues. These questions along with many others are laying the foundation for stem cell research that focuses on regenerative therapies as a realistic *in vivo* treatment.

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