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EDITORIAL

Therapeutic approaches to cellular stress response

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Usually, human life begins with a cry and respiratory distress and then extends to a diverse set of extrinsic and intrinsic stressors, of which infection, DNA replication stress, and oxidative stress are the most common. These stresses converge to aging and age-related diseases and ultimately toward death, which is the final and profound challenge for human beings. Although immunology has achieved its glory in responding to infectious microorganisms, the roles of the immune system in combating various cellular stresses, as well as in generating cellular stress with each heartbeat, are not negligible and need to be further highlighted. Here, we report a summary of more than a decade of our research on serum starvation, replicative cell senescence, and heat shock-mediated cellular stress responses using some advanced laboratory methods and a wide range of preclinical models, suggesting that the threat of cellular stress can be exploited as an opportunity to treat some age-related diseases, such as cancer and atherosclerosis. In this brief, the clinical applications of fibroblasts and immune cells' responses to three stressors: serum starvation, replicative cell senescence, and heat shock are discussed.

Background

In 2012, in response to a reviewer's comment in the Journal of Biotechnology and Applied Biochemistry about our finding regarding the positive effects of co-culture of collagenase-induced-injured human fibroblasts and mice pancreatic islet cells on the injured cells' survival (1), we accidentally discovered that during a 16 h of serum starvation stress, the human neonatal skin fibroblasts secreted a protein cocktail consisting of more than 300 protein components. These proteins had relative molecular weights of 10–120 kDa and pI in the range of 4–6 based on two-dimensional electrophoresis (2DE) analysis (2). We call it SFBC, which is abbreviated for Starved Fibroblasts Biomolecule Complex. SFBC protected fibroblast survival under stressful conditions of serum starvation and increased the level of fibroblast proliferation after serum refeeding. This phenomenon was abrogated by heating SFBC. To find a molecular basis to explain these observations, we hypothesized that those fibroblasts that had been adapted in a growth factor-enriched medium supplied with 10% fetal bovine serum (FBS) were suddenly exposed to growth factor deprivation stress. To alleviate this metabolic stress, cells activated the endoplasmic reticulum stress response program, ensuring the production of only the essential growth factors required for survival and adaptation under challenging conditions. Interestingly, this hypothesis was confirmed by atomic absorption-based analysis in which SFBC induced a sustained intracellular calcium influx for one hour in fibroblasts, to the same extent as the growth factor control, FBS (2). We conducted this analysis based on previous knowledge, in which growth factors exert their diverse biological functions by binding to and activating a well-defined group of cell surface receptors with intrinsic protein kinase activity, the so-called receptor tyrosine kinases (RTKs). Some RTKs can be coupled to calcium channels, which in turn mediate a sustained influx of calcium upon ligand binding. Calcium ions mediate almost all functions of cellular physiology.

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Furthermore, LC-ESI tandem mass spectrometry-based analysis of SFBC offered additional evidence in support of this hypothesis. This analysis revealed the presence of some growth factors, such as insulin-like growth factor binding protein (IGFBP-4, -5, -6, and -7), in SFBC (3). Although the molecular mechanisms leading to the production of such a set of proteins have not yet been elucidated, we know that, in contrast to 16 hours of starvation, 6 hours of serum starvation stress not only failed to induce enhanced proliferation but also significantly reduced fibroblast viability after serum refeeding. Moreover, the culture supernatant of such fibroblasts, unlike the supernatant prepared from 16-hour serum-free fibroblast culture SFBC, reduced fibroblast viability (2, 4). Therefore, within a period of 6-16 hours of culturing fibroblasts under serum starvation, these vital proteins should be produced. Investigating the molecular mechanisms underlying this difference is one of our laboratory research interests (Figure 1).

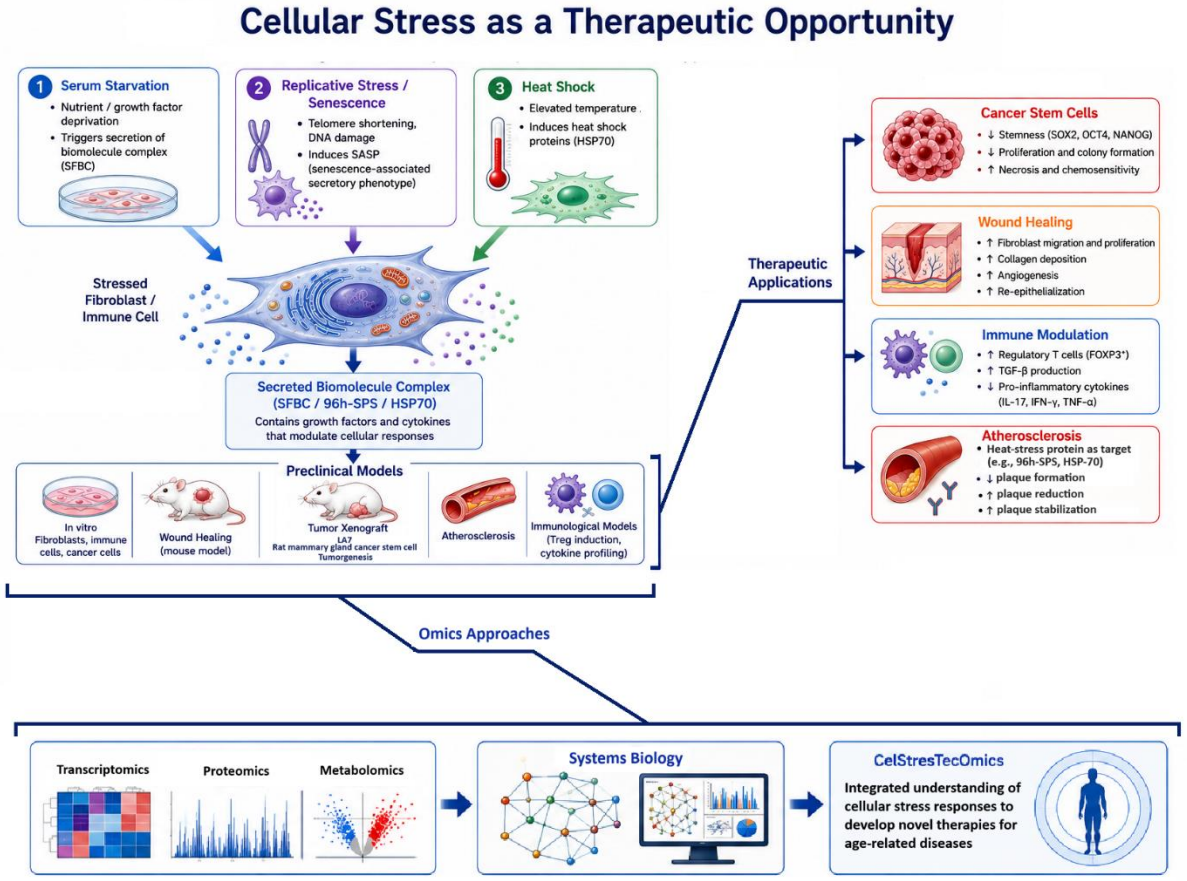

Figure 1. Cellular Stress as a Therapeutic Opportunity. Schematic representation of the transition from cellular stress signals to adaptive cellular responses and potential therapeutic applications. SFBC, starved fibroblast biomolecule complex; 96h-SPS, 96-hour starved peripheral blood mononuclear cell (PBMC) culture supernatant. Image generated with ChatGPT (OpenAI), 2026.

The potential of SFBC in clinical applications

Wound healing

Given the lessons in knowledge translation that I learned from my esteemed professors, Professor Huber Kolb and Professor Nanette Catherine Schloot, while completing my doctoral thesis at the German Diabetes Research Institute in Düsseldorf, we decided to translate this knowledge into medical applications. Thus, to investigate the clinical application of SFBC, a tissue injury wound model was used, first in vitro through a scratch test with human skin fibroblasts, and then in vivo using a mouse wound healing model. We chose the wound model based on our reasoning that

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due to impaired blood circulation in wounds, various cells, including fibroblasts, may experience some degree of nutrient deficiency. Interestingly, we found that SFBC induced fibroblast migration and promoted angiogenesis, leading to improved wound healing without scarring (2, 3, 5).

Cancer

Given that cancer is often described as an incurable wound, and based on our previous observation of the proangiogenic effects of SFBC on wound healing, we were inspired to take the next step in investigating the effects of SFBC on tumorigenesis, utilizing both in vitro and in vivo models of LA7 rat mammary cancer stem cells. Noteworthily, in the in vitro model, SFBC reduced the expression of stemness-related genes such as *Oct4*, *Sox2,* and *C-myc* but not *Nanog*, indicating the potential of this protein complex in cancer differentiation therapy. It also showed cytotoxic effects on LA7 in an MTT assay (6). Furthermore, downregulation of stemness genes, reduction of tumor size, and induction of necrosis were observed in LA7 cancer stem cell-derived tumors treated with SFBC in our in vivo study (7). Finally, we extended our study to human cancers. SFBC had significant cytotoxicity on breast cancer cell lines, such as MCF-7 and MDA-MB-231, but not on the normal breast cells, MCF10A. It also showed cytotoxic effects on pancreatic cancer cell lines, brain and colon, but not prostate cancer cells (manuscript in preparation).

Starved immune cells derived biomolecules and clinical applications

We also investigated the effects of serum starvation stress on non-fibroblast cells, namely human peripheral blood mononuclear cells (PBMCS). Overall, PBMCs were more resistant to serum starvation-induced cell death and produced 294 distinct proteins after 96 hours of cell culture under serum starvation-induced metabolic stress, of which 54 proteins were shared with SFBC, with albumin at the top (3, 8). We called this condition medium 96 h–SPS, which stands for 96 h Starved PBMCs-culture Supernatant.

Anti-tumorigenesis effects of 96 h–SPS

The anti-cancer effects of 96 h–SPS on rat LA7 mammary gland cancer stem cell models were also evaluated in vitro and in vivo. The 96 h-SPS solution reduced LA7 cell viability, proliferation, migration, and metabolic gene expression, such as *Gch1* and *Spr, in vitro*, whereas the stemness gene *Oct4* was up-regulated. The intra-tumor lactate was significantly decreased in the 96 h-SPS-treated group. In this group, *Gch1* and *Spr* were significantly down-regulated in tumors, whereas *Sox2* and *Oct4* expression were unchanged. significantly. The number of vessels and mitosis ($Ki67^+$ cells) in the 96 h–SPS–treated group was significantly reduced. The increased rate of necrosis in this group was statistically significant (9).

Immunomodulation and bone marrow transplantation

Furthermore, in another study, starved PBMCs harbored high levels of CD4+CD25+CD127-FOXP3+ regulatory T cells and increased production of the cytokine transforming growth factor beta (TGF-β) in our cell culture analysis (10). These findings suggest an immunomodulatory property of serum starvation stressors. Consistent with this result, the splenocytes' allogenicity of mice under serum starvation stress was significantly reduced (11). Moreover, in a mouse model of bone marrow transplantation, we demonstrated a reduced level of graft-versus-host disease in mice that received allogeneic bone marrow cell transplantation (MSc thesis). Also, the effect of serum starvation on immunogenic cell death (ICD) was recently evaluated in our laboratory using a LA7 cancer stem cell model. LA7 cells were directly subjected to serum starvation stress or treated with SFBC; in both cases, the ICD marker calreticulin was increased at the cell surface in our flow cytometric analysis (unpublished data). This finding suggests that serum starvation could be considered a tool in vaccination against tumors. In fact, one of our collaborative laboratories independently reached the same conclusion with 96 h-SPS in their in vitro and in vivo studies with mouse colon cancer cells (ready to be published).

Replication stress and heat shock response in clinical applications

On fibroblasts, in addition to cellular stress induced by serum starvation, we explored the therapeutic potential of two other cellular stressors, namely replication stress and heat shock response. These stressors play significant roles in aging and age-related diseases, including cancer and atherosclerosis. Replication stress is now considered a driver for aging

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and cellular senescence (12), and it is well known that the Senescence-Associated Secretory Pattern (SASP) plays some important roles in cancer development and progression. Therefore, the genotherapy approach, which involves the direct removal of senescent cells from the body or indirectly the inhibition of SASP, represents a promising strategy to combat age-related diseases.

Anticancer effects of replicative senescent fibroblasts

In our laboratory, fibroblasts isolated from the foreskin were replicated in cell culture for 14 passages until most of the cells were no longer able to divide and showed senescent morphology. The culture supernatant of such cells was harvested and designated P14, short for passage 14 (6). We previously observed that serum-starved fibroblasts, as well as fibroblasts in high cell culture passages, secrete high levels of exosomes (MSc thesis-unpublished data). We investigated the anti-cancer activity of P14 stem cells in vitro using the rat LA7 cancer stem cell model. After incubation with LA7 for 24 h, it showed strong cytotoxicity and downregulated all the indicated stem cell-related genes, *including Oct4*, *Nanog*, *C-myc*, and *Sox2* (6).

Heat shock stress response and atherosclerosis

Considering that heat shock proteins (HSPs) may be produced by skin fibroblasts in response to abundant and potentially harmful environmental stresses, including UV radiation, we used fibroblasts isolated from the foreskin to produce HSPs with a relative molecular weight of 70 kDa. Interestingly, we showed that patients with coronary artery disease had lower levels of antibodies against heat-stressed fibroblast-derived proteins than healthy subjects (13). This suggests a protective role for such antibodies in atherosclerosis, and it is possible that immunizing people at risk of this disease with those proteins may have beneficial effects for them.

Effects of human ovarian follicular fluid on cancer stem cells

On the other hand, we have studied the tumorigenesis effects of human Ovarian Follicular Fluid (OFF) using the LA7 breast cancer stem cell model. We know that OFF naturally regulates the egg cell cycle and differentiation in women from embryonic times to around age 54. Therefore, OFF can be considered as a solution consisting of a set of biomolecules produced by cells under normal physiological conditions. Compared to other biological fluids prepared from stressed cells, such as SFBC and P14, it showed the lowest cytotoxicity. Even after 24 hours of cell incubation with its diluted solution (from 1/2 to 1/16 in cell culture medium), it can improve the viability of cancer stem cells. The effect of OFF on stem cell-related genes was heterogeneous, with Nanog gene expression significantly reduced after 24 h of cell treatment and then this reduction was lost, and Oct4 was upregulated after 7 days of cell treatment with OFF (6). Together, we expect OFF to play a role in tumorigenesis. We need an in vivo study to support this conclusion. We believe that understanding the underlying molecular mechanism in OFF-mediated tumorigenesis may help us better understand the pathogenesis of cancer-related diseases in women, especially breast and ovarian cancer. Furthermore, we repeatedly observed that OFF-treated LA7 cells displayed endothelial-like cell morphology, suggesting its potential in cancer differentiation therapy, especially in combination with SFBC. Further study is required to explore this application for OFF.

Modes of action

We have already evaluated the potential modes of action of SFBC mainly and 96h-SPS partially in inhibiting tumorigenesis using the LA7 cancer stem cell model for tumor induction. This evaluation, based on our repeatable and reproducible data, showed that SFBC has cytotoxic effects mainly on cancer cells and is completely safe for normal cells. This finding then raised the question: What makes a normal cell a cancer cell? Cancer dependency on aerobic glycolysis (Warburg effect) and the window of oxidative stress are certainly two of the main differences between normal and cancer cells. Furthermore, inhibition of cell death, ability to induce migration and metastasize, angiogenesis, recruitment of mesenchymal stem cells, and turning the immune system from a foe into a friend are other features that distinguish a cancer cell from a normal cell. According to the cancer stem cell hypothesis, these properties originate from cancer stem cells, which, despite the low prevalence in tumor bulk, render the cancer resistant to chemo and radiotherapy and consequently lead to cancer-related disease relapse. Thus, we have focused on the effects of SFBC

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and 96h-SPS on these properties in different studies. Collectively, the data obtained from these studies showed that metabolic changes, oxidative stress fluctuations, stemness/differentiation alternation, reduction of angiogenesis, inhibition of migration, immune modulation, and mesenchymal stem cell infiltration were the most effective modes involved in SFBC and 96h-SPS-mediated tumor development in the rat breast cancer model.

Furthermore, our proteome analysis, bioinformatic prediction, and experimental evidence revealed an immune response down-regulation function in serum-starved human fibroblasts. Under serum starvation-induced metabolic stress, both PBMCs and fibroblasts produced molecules like heat shock proteins and amyloid-beta, which can play pathogenic roles in auto-inflammatory diseases such as rheumatoid arthritis, type 1 diabetes mellitus, systemic lupus erythematosus, aging, and cancer. However, starved fibroblasts showed immunosuppressive activity both in silico analysis through human non-classical leukocyte antigen-G and –E expression and in an in vitro model of allogeneic transplantation through classical class I human leukocyte antigens, suggesting their potential to modify such adverse reactions by downregulating the immune system (3).

We need omics-based technology and the use of systems biology-based algorithms to draw a comprehensive picture of the upstream cellular signaling that led to the production of SFBC, 96h-SPS, and therapeutic proteins in replicative senescence, or to downstream signaling for the aforementioned anticancer effects. We can call this program CelStresTecOmics, an acronym for cell stress technology and omics, specifically transcriptomics, proteomics, and metabolomics. The lipid components of these therapeutic biological fluids and their therapeutic effects need to be analyzed in the future.

Conclusions

In summary, our fourteen years of experience in the field of cellular stress response have led us to conclude that while immune cells and fibroblasts share some commonalities, they typically display distinct programs in response to different cellular stressors. If we can find the optimal levels of a cellular stressor appropriate for each cell type, we can exploit the cellular stress response in immune regulation and in the treatment of age-related diseases such as cancer, atherosclerosis, and wounds, possibly diabetic wounds, or some other diseases, using the senotherapy approach. We are currently interested in providing more details in this area by mimicking the microenvironment of solid tumors using a simple multi-cell culture system. Bioinformatics and systems biology-based approaches may be useful in this area (Figure 1).

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