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Cell Fasting: Cellular Response and Application of Serum Starvation

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ABSTRACT

Humans suffer transient or persistent starvation due to a lack of food intake, either because of fasting, voluntary dieting, or due to the scarcity of available food. At the cellular level it is possible to possess pathological starvation during ischemia and solid tumors. Blood provides many nutrients to our cells, and researchers provide these nutrients to cells in culture in the form of enriched culture medium plus serum from animal sources. In response to starvation, animals use hormonal cues to mobilize stored resources to provide nutrients to individual cells. Besides whole-body responses to nutrient deprivation, individual cells sense and react to lack of nutrients. At the cellular level, starvation triggers different responses such as cell cycle arrest and apoptosis. Stop cycling for proliferating cells is the primary response to nutrient deprivation. Under certain conditions, the cell reacts to nutrient deprivation by engaging the mitochondrial pathway of apoptosis. Thus, serum starvation is regarded as a procedure to prepare cells for an experiment in serum-free conditions such as induction cell cycle synchronization. Several researchers have used serum starvation as a tool to study molecular mechanisms involved in different cellular process, metabolic researches and evaluation of a drug effect.

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Introduction

Food is essential for living. All organisms have therefore developed mechanisms to detect, react to and, if necessary, survive the lack of it. In multicellular organisms, responses to lack of nutrients in blood are managed by hormonal cues. However, individual cells also sense and respond to nutrient deprivation, which happens under pathological or physiological situations.

Cells require four types of "building blocks" to develop and proliferate: fatty acids to make membranes, amino acids for protein synthesis, nucleotides to build nucleic acids, and sugars to produce multiple macromolecules and ATP. Blood provides all of these nutrients to cells in mammals, and researchers provide these nutrients to cells in *in vivo* as a form of enriched culture medium plus serum from animal blood.

Since cells have to maintain the equilibrium between anabolism and catabolism, they need to sense the energetic state of the cell and react to starvation. Starvation is linked to our first contact with our external world, since there is a period of mild starvation in the newborn before breastfeeding is started.

Living organisms are continuously subjected to periods of starvation in nature. This starvation is transient or persistent due to a lack of food intake, either because of voluntary dieting, fasting, or due to the shortage of available food.

At the cellular level it is feasible to observe pathological starvation during ischemia, which is a severe form of starvation due to simultaneous nutrient and oxygen deprivation. Cells in solid tumors are also very commonly exposed to starvation, because the tumor mass grows faster than the blood vessels (1,2).

Methods

Starvation and Activation

Both the starvation and activation of cells will strongly depend on the desired experimental conditions.

The mammalian cells could be subjected to nutrient deprivation based on standard protocols such as below:

1. Warm serum-free media to 37°C.
2. Remove complete media from 96 well plate by aspiration or manual displacement.

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3. Replace media with 200 µl of pre-warmed, serum-free media per well.
4. Allow cells to incubate an additional 16 to 24 hours to ensure metabolism of complete media constituents.
5. Remove starvation media from plate wells by aspiration or manual displacement.
6. Add either serum-free media plus 1% BSA for resting cells or serum-free media plus 1% BSA with stimulation ligand such as EGF for activated cells. Use 100 µl of resting/activation media per well.
7. Allow incubation at 37°C for desired stimulation time.

There are different starvation and activation protocols. It is common, but by no means universal, to perform serum starvation in serum free basal medium with or without bovine serum albumin. Sometimes specific growth factors and hormones are added to serum-free starvation medium. Alternatively, serum starvation may be carried out in medium with low serum concentration (relative to normal growth medium), typically 0.1–0.5%, but sometimes as high as 2–5% or as low as 0.05%. The time frame for serum starvation is equally varied and can include anything from 15 to 30 minutes up to several weeks. Furthermore, while alternative designations such as serum withdrawal or deprivation are sometimes used synonymously and interchangeably with serum starvation, others make a clear distinction based on serum concentration (5).

Results

During the period of scarcity, unicellular organisms try to save energy by undergoing specialized forms of hibernation such as sporulation. Multicellular organisms generally have specialized cells that recognize nutrient deprivation –such as pancreatic and liver cells in animals-, and specialized storage tissues for instance fatty tissue. In response to starvation, animals use hormonal cues to metabolize stored energy sources to provide nutrients to individual cells. Besides whole-body responses to nutrient deprivation -such as the sensation of hunger which drives us to seek for food-, individual cells sense and react to lack of nutrients. At the cellular level, starvation of specific nutrients triggers different responses in different tissues, since some tissues like the brain prefer blood sugar as a source of energy, while other tissues such as the heart or resting muscles prefer lipids as fuel.

Cell cycle arrest

When cells are deprived of nutrients, their first response is typically to stop cycling (if they were proliferating), and try to stay alive using the least amount of energy and building blocks (1).The cyclin-dependent kinase inhibitor p21 is a multifunctional protein known to promote cell cycle arrest and survival in response to p53-dependent and p53-independent stimuli. By DNA damage, the protective effect of p21 is due to its transcriptional activation by p53.The p21 protein expression level severely increases in wild-type HCT116 cells placed under serum-nutrient starvation conditions. Starvation induced increase of p21 protein level is independent from p53 and it has been seen in p53 deficient HCT116 cell line (3).

Apoptosis

Normally, cells survive nutrient deprivation by lowering their energy and carbon requirements and by recycling structural components. However, under certain conditions, the cell reacts to nutrient deprivation by engaging the mitochondrial pathway of apoptosis (1). It has been shown that withdrawal of stimulatory growth factors induces apoptotic cell death in several cell types in *in vitro*. At least two distinct modes of cell death have been recognized in *in vivo*: necrosis and apoptosis. Apoptosis is a sort of physiologic cell death in which single cells are deleted among healthy tissue cells (4).

Besides, it is activated in animals in response to cell-damaging agents, or to developmental cues. The fact that many human cells die by apoptosis when deprived of a particular nutrient is quite interesting. Cells die by apoptosis when they are not needed anymore (for example after an immune response, or when the pathogen has been eliminated), or when they are damaged (for example after a DNA-damaging insult or viral infection). Thus, it is still unknown how and why some cells undergo the apoptotic machinery instead of arresting and waiting for "better times". Moreover, cells which undergo tumorigenic transformation are more susceptible to be eliminated than their normal counterparts (1,2).

Discussion

Serum starvation is one of the most commonly performed procedures in molecular and cell biology and there are literally many studies reporting its use.

The term serum starvation, or simply starvation, as well as serum deprivation, depletion, restriction, removal, withdrawal, and serum limitation have been used to represent various procedures that include growing cells in either reduced serum, serum-free, or serum- and protein-free medium. Serum starvation is often, at least unconditionally, regarded as a routine procedure performed to prepare cells for an experiment in serum-free conditions and therefore not an experiment by itself.

Although serum provides optimal conditions for cell growth, its poorly defined complex and above all variable composition represents an important and undesirable confounding factor while performing bioassays. Elimination of serum from culture medium provides more reproducible experimental conditions (5).

Since stopping cell cycle was primary response to starvation, serum starvation-induced synchronization, followed by serum restimulation or preceded by serum shock (e.g., 50% serum), has been extensively used in circadian rhythm research and cell cycle (5).

Serum Starvation Induced Cell Cycle Synchronization facilitates Human Somatic Cells Reprogramming. Human induced pluripotent stem cells (iPSCs) provide a precious model for regenerative medicine and research in human disease. To date, however, the reprogramming efficiency of human adult cells is still insignificant. As it has been shown that retroviruses such as the Moloney murine leukemia virus (MoMLV) require cell division to integrate into the host genome and replicate, whereas the target primary cells for reprogramming are a mixture of different cell types with several cell cycle rhythms. Using transient serum starvation induced synchronization, it has been revealed that serum starvation generates a reversible cell cycle arrest and synchronously leads to G2/M phase after release, substantially improving retroviral infection efficiency (6).

Also, many scientists have used serum starvation as a tool to study molecular cellular mechanisms involved in cellular stress response, protein degradation, autophagy, apoptosis and/or to simulate particular pathological conditions (5). For example in a study by Epstein D. et al, it was proposed that protein breakdown is accelerated upon the restriction of serum and this enhancement is withdrawn by either insulin or cycloheximide (7).

Researchers have used serum starvation in combination with hypoxia and/or lowered glucose content as an experimental model to mimic clinical conditions such as myocardial infarction and stroke or to generate a poorly vascularized, growth factor-, nutrient-, and oxygen-deficient core of tumors (5). For example in a study that was designed to investigate the role of TLR4 activation by lipopolysaccharide in protecting cardiomyocytes against apoptosis in an in vitro model of ischemia, serum starvation was used to prepare the desired condition (8).

The usefulness of serum starvation goes beyond basic molecular biology and includes metabolic research, where the introduction of serum starvation-based protocols displayed the physiological response to insulin in cultured skeletal muscle cells, while earlier studies of molecular mechanisms of insulin action had been hampered by the presence of serum (5,9).

One of the most common applications of cell culture under serum starved condition is evaluation of a drug effect on cells, but it is important to note that if a drug has an apoptotic effect, serum starvation may not be the right choice since it drives the cells to apoptosis as well (10).

To sum up, serum starvation could be used as a potent tool in molecular and cellular biology experiments.

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