Characteristics and Prospect of Skin Aging Cell Model

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Abstract. Aging is a necessary stage of the body, accompanied by the decline and abnormal state of skin, nerves, organs and viscera. Skin aging is one of the most remarkable organs in the aging process of the body, its research has become an unfailing hot spot. With the deepening of research and the exploration of aging mechanism, the research on cell level becomes more and more important. In order to facilitate the study of cell level mechanism and model selection, this paper summarizes the replication and characteristics of skin aging cell model, so as to simplify model selection and promote new skin cell model replication methods.

Introduction

Aging is a normal life phenomenon, accompanied by degenerative changes in body tissues and organs, which is related to the gradual loss of the integrity of genome, proteome and metabolome. As one of the most significant changes in the aging process of the body, skin is mainly manifested by skin relaxation, folding, and pigmentation [1]. Based on the pursuit of aesthetics and the improvement of material living standards, delays aging has become the common pursuit of different groups at different ages. The existing models used for skin aging mainly include animal models and cell models, both of which can be used for drug research to delay skin aging. Because animal skin aging research is relatively convenient, and simple operation, relatively low technical content; Cell culture has high environmental requirements, complicated operation and relatively high technical content. Therefore, at the present stage, the replication and research of animal skin aging model is in a common state, while the cell aging model are relatively few. The study of cellular level molecular mechanism is an essential step to reveal the micro state and fundamental step of aging, and is also the most critical step to verify the effectiveness of drugs. Based on the characteristics of cell selection and model, this paper summarizes and prospects the cell model of skin aging, in order to promote the research on the level of skin aging cells.

Source and Selection of Model Cells

So-called skin aging, the source of cells should be skin tissue. There are two types of cell sources: animal and human skin. Cells used in experiments can be purchased, or directly from animals or humans. At present, the cells used in the study of skin aging mainly include fibroblasts and keratinocytes derived from human skin tissue. Skin fibroblasts from animals.

Human Skin Tissue Aging Cell Model

Human Keratinocytes Aging Model

Modeling by irradiation of long wave UVA (320~400 nm) [2-4]: Taking logarithmic growth of HaCaT cells, the irradiation dose of UVA was 20mJ/cm²~30m J/cm², and the vertical irradiation distance between the light source and cells was adjusted to 15 cm, and the irradiation time was 44min~6h.

Modeling by irradiation of medium wave UVB (290~320 nm) [5~6]: Logarithmic growth of HaCaT cells was taken and the cover of the culture dish was removed and placed under the ultraviolet lamp. The cell was about 6~20 cm away from the lamp source, and the total irradiation dose was 0.5 mJ/cm²~60 mJ/cm², and the irradiation time was 1.5min~30min.
Modeling by irradiation of short wave UVC (200~290 nm): When the cells were fused to 80%~90%, they were uniformly inoculated into 6-well or 96-well plates for UVC irradiation. Generally, the radiation distance is 70 cm, the radiation dose is 20 J/m², and the radiation dose is 30 min [7].

The Characteristics of Human Keratinocytes Aging Caused by Different Methods

All of the above three modeling methods can stimulate cells to produce ROS and superoxides, which can lead to oxidative stress reaction of cells and damage cells, leading to their death. Under the common character of oxidative stress injury, the three modeling methods can be used in the research of drug intervention in oxidative stress. However, it also has different characteristics and principles, so it is suitable for different research designs. Long-wave ultraviolet radiation can activate membrane receptors and promote the change of proliferative and apoptosis-related pathways, which is suitable for the study of drug intervention in specific pathways [8]. Medium-wave ultraviolet radiation mainly stimulates keratinocytes to secrete excessive reactive oxygen, activate oxygen free radicals, cause oxidative damage of cells, and promote the occurrence and development of skin photoaging [9], which is suitable for the study of the effect of antioxidant direction. In the case of short-wave ultraviolet radiation, DNA can directly absorb UVC energy and then damage cells [10], which is suitable for the study on genes and proteins. Therefore, the smooth progress of the experiment and the consistency between the actual experimental results and the expected experimental results are influenced by the modeling method, which should be selected according to the experimental scheme and the characteristics of the tested drug.

Human Skin Fibroblast Aging Model

Modeling by irradiation of long wave UVA (320~400 nm): At the UVA dose of 0~20J/cm², the cell viability was inversely proportional to the irradiation dose. But when the irradiation dose is greater than 30J/cm², the cells dry out. Therefore, when the cells were fused by 70%~80%, a UVA dose of 10J/cm²~20J/cm² was irradiated for about 2 weeks to achieve a satisfactory effect [11].

Modeling by irradiation of medium wave UVA (290~320 nm)[12]: Cells inoculated in 96-well plates (about 50%~60% of cells growing in a cell adherent layer) or other culture containers (80%~90%) were placed under UVB light with a distance of 30cm, and UVB irradiation dose was adjusted to be 30~300m J/cm² with an irradiation time of 1~3d.

Modeling by irradiation medium and long wave UV: Select the light source composed of 2 UVA and 1 UVB lamp, adjust the radiation distance to 15cm, and set the radiation dose of UVA and UVB to be 20J/cm² and 40J/cm², respectively. Irradiation of human skin fibroblasts growing to 80%~90%. Exposure time depends on the specific situation [13].

Hydrogen Peroxide Induced Moulding

Excessive oxidative stress can lead to DNA oxidative damage, tissue damage, and is a major cause of aging and many diseases. H2O2 was used as an oxidant to induce human skin fibroblasts (HDF) damage, and HDF cell oxidative damage model can be well established by 4h culture of DMEM medium containing 200μmol/LH₂O₂ for the study of photoaging [14].

The Aging Characteristics of Human Skin Fibroblasts Caused by Different Methods.

Long-term oxidative stress conditions can lead to normal human skin fibroblasts aging, morphological changes and abnormal gene expression. UVB has a strong penetrating ability, which can cause apoptosis of dermal fibroblasts, pigmentation, skin photosensitivity, photoaging and skin cancer [15]. Oxidative stress and lipid peroxidation caused by excessive production of ROS (including OH, O₂-, H₂O₂ and ¹O₂, etc.) from UVA and UVB are important causes of skin photoaging. Therefore, the control of ROS level is the first step to protect against UVA and UVB light damage. After H₂O₂ enters cells, oxygen free radicals are generated in the cells through various metabolic pathways, resulting in accumulation of free radicals in the cells, which leads to cell oxidative damage and photoaging. All the above four modeling methods are the pharmacodynamics research on the direction of oxidative stress injury at the level of the free radical theory of aging.
Model of Aging in Animal Skin Fibroblasts

Glycosylated end products (AGEs) induce moulding: The mice skin fibroblasts L929 were stimulated by MEM culture medium containing 0.100 g/L AGEs, and the aging model was prepared by continuous culture for 48 h. According to the literature reports, when the selection of the mold making concentration for AGEs is carried out, its concentration can be adjusted to 0.050 g/L~0.15g/L, and the time can be around 2d [16].

D-galactose induced moulding: The mouse back skin was cut (fat free) and fibroblast cells were isolated for culture, and the cells were cultured to the state of subfusion. Join with D-galactose 40 g/L DMEM culture based on 37 ℃ and 5% CO₂ incubator for 48 h [17].

The aging characteristics of animal skin fibroblasts induced by different methods: Glycosylation end products can be through the induction of apoptosis, oxidative stress affects the vitality of skin fibroblasts and state, but also with protein, free amino crosslinking and change of protein structure, disrupt the normal skin fibroblasts of structure, disrupt the normal physiological function of cell/tissue, causing cell aging and even death [18]. D-galactose is a kind of reduced monosaccharide, which can metabolize glucose under normal conditions, but produces ROS and advanced glycosylated end products (AGEs) in excess, thus promoting animal aging [19]. Long-term injection of D-gal can cause systemic metabolic disorder and decline of various organs, which can effectively simulate natural aging on physiological, biochemical and other indicators. As a commonly used experimental model of aging in aging research, it is suitable for pharmacodynamics research and confirmation of different aging theories.

Matters Needing Attention

The source of keratinocytes and fibroblasts involves the age of the provider (day or month age of the animal), health status, preservation conditions, and number of generations, which can lead to differences in molding conditions. In addition, different wavelengths, different irradiation intensity and irradiation distance, as well as different glycation end products, d-galactose and hydrogen peroxide concentrations also affect irradiation time inconsistencies. Even though the above modeling methods have some limitations, they can narrow down the scope of selecting molding conditions so as to determine the best molding conditions in the shortest time.

Selection of Experimental Cells

Keratinocytes are the main target of UVB radiation and are involved in the pathophysiological process of the skin. Studies have shown that the aging of HaCaT cells involves the changes of toll-like receptor 9 (TLR9), Nrf2/Bach1 pathway, extracellular regulated protein kinase (ERK) signaling pathway, NF-κB signaling pathway, and related p38 mitogen-activated protein kinase (p38MAPK) signaling pathway, but not limited to general changes of cell oxidative factors and inflammatory factors. The most important cell components in the dermis of skin fibroblasts are most susceptible to UVA, and the changes in its number, morphology and secretory synthesis function can reflect the aging of cells. As research on the ageing mechanism of HaCaT cells, existing research on the mechanisms of aging research has involved ROS-PTEN and PI3K, Smad3-collagen, HDFs size/mechanical force - Tb RII, mi RNAs, NF-κB - Collal-Itype I collagen and so on the many kinds of mechanism research.

At present, the pharmacodynamics research models for the aging of skin are mostly limited to human keratinocytes and fibroblasts, and animal cells are rarely used. The reasons were analyzed as follows: first, the toxicity and efficacy of drugs were studied by using animals. On the premise that the drugs were proved to be effective by experiments in vivo, the cross-repeatability of the experiments was avoided and the mechanism of drug in vitro action was not suitable for animal cells. The purpose of drug research is to achieve clinical wide application, that is, the ultimate object of drug research is human. Therefore, on the premise that the pharmacodynamic screening in the animal body has been carried out, it is of better significance to carry out the culture of human
cell tissue to verify the pharmacodynamic conclusion obtained in the animal experiment and to study the response of human tissue to drugs and the mechanism of action of drugs at the cell level.

Summary

Aging involves a variety of collective metabolism, secretion, and synthesis of changes, skin aging is the most direct and most intuitive body aging of the external performance. So far, the research on anti-aging medicine has never stopped. The research on anti-aging medicine gradually develops from the crude ingredients of decoction or alcohol extraction into the total component or monomer component of a certain kind of compound. With the deepening of research, the mechanisms of aging gradually show diversity, including the overall level of the body, the level of organs, and even the level of cells and molecules. Other studies have shown that skin aging is accompanied by the changes and stability of DNA repair, extracellular matrix, lipid synthesis, ubiquitin-induced protein decomposition and cell metabolism, cell cycle and apoptosis, and other important biological processes.

Studies in laboratories (in animals) of various drugs have shown good anti-aging properties, however, very few can actually be developed for human use. In other words, under the premise of large expenditure and animal experiment, there is no corresponding achievement transformation. The main cause of the above problems is that most of the researches are stagnated in the laboratory research stage of animals. However, the study of pharmacodynamic effect at the cellular level (especially the human tissue cells) can reflect the metabolism of drugs in human cells and the response of human cells to drugs to some extent. Compared with animal studies, the study at the cellular level is closer to the actual application.

There are individual differences, whether animal or human. The modeling methods of cell aging used in various literatures have certain differences in radiation intensity, radiation time, the severity of mold formation and the density of cell growth. Therefore, on the premise that the modeling method is basically fixed, it is necessary to screen the radiation intensity, time, the severity of the modeling and the cell survival rate in a small range according to the literature method before the formal experiment, so as to determine the best modeling conditions for cells. It takes sufficient time and some patience to carry out the selection of modeling methods and conditions within a large range, in which uncertainties may affect the experiment. Under the time pressure, a certain range of cell modeling methods and selection can help researchers to make better choices in the shortest time. Therefore, it is necessary to summarize the replication and characteristics of cellular aging model, so that the model can be traced and followed by rules. Furthermore, researchers should be encouraged to make use of cells to study and discuss the mechanism of deep aging, so as to avoid research at the animal research stage.

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