wjpmr, 2018,4(9), 120-124

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Review Article

ISSN 2455-3301

WJPMR

HYPOXIA INDUCIBLE FACTOR AND OSMOREGULATORY MOLECULES AFFECT CELL BLEBBING; IMPLICATIONS FOR SENESCENCE AND AGEING PROCESS

Manoj G. Tyagi*

Department of Biotechnology & Paramedical Technology Vellore Institute of Technology, Vellore, TN & National Institute of Medical Sciences Jaipur, Rajasthan, India.

***Corresponding Author: Manoj G. Tyagi**
Department of Biotechnology & Paramedical Technology Vellore Institute of Technology, Vellore, TN & National Institute of Medical Sciences Jaipur, Rajasthan, India.

Article Received on 28/06/2018 **Article Revised on 18/07/2018** **Article Accepted on 08/08/2018**

ABSTRACT

Cellular senescence, which features irreversible growth arrest in response to various stresses, is a fundamental mechanism that mediates age-related dysfunctions and chronic diseases.An important mechanism of cellular osmoregulation occurs through the cellular uptake/production of organic osmolyteslike sorbitol, betaine, and myo-inositol. Betaine and myo-inositol are transported bythe proteins BGT-1 and SMIT, respectively. Sorbitol on the other, is synthesized inside the cell by the enzyme aldose reductase. These three proteins are regulated atthe transcriptional level by the transcription factor, NFAT5/TonEBP. Other osmoregulatory molecules are the natriuretic peptides and aquaporin water channel modulators. On the other hand hypoxia inducible factor is an inducible molecular oxygen sensor during hypoxia and plays an important role in apoptosis. This review outlines the interaction between NFAT 5 and HIF in cell blebbing and eventual implication for the ageing process.

KEYWORDS: NFAT 5, Cell blebbing, hypoxia, protein, ageing, blebbing.

INTRODUCTION

Senescence is a process by which the cells cease to divide and there are several suggested mechanisms for cellular senescence which may ultimately lead to the ageing process.[1-3] Gene expression of these of the osmoregulatory genes are increased by cell shrinkage in a hypertonic environment. Upregulation of gene transcription is carried out by multiple enhancers known as the osmotic-reponse element (ORE) or tonicity-response enhancer (TonE) that are located in the regulatory domains of these genes.[4-5] The original transcription factor for ORE/TonE was identified as TonE-binding protein (TonEBP)/ORE-binding protein (OREBP), whichshows sequence homology to a family of transcription factors called the Nuclear Factorof Activated T cells (NFAT), and therefore it was named NFAT5.[6-7] NFAT5 has been discovered to be ubiquitouslyexpressed throughout the body and has been shown to be sensitive to hypertonicity inall cell types.[8-9] NFAT 5 and natriuretic/ aquaporin water channel modulators may affect the cell morphology and in conjunction with hypoxia inducible factors like the HIF1 and HIF2 may play an important role in the long term changes in the cell and its sustainenace and replacement, this may Intricately affect the ageing process. This review and hypothetical article suggests that osmolar changes and hypoxic alterations in metabolism mediate morphological changes in the cell and mediate the long term process of ageing in living organisms.

Genetic theory of Ageing: Experimental evidence supporting theories of genetically programmed cell ageing were provided in the researchers of Pereira-Smith and Smith, and Sugawara et al.[10-11] By fusing different immortal human cell lines Pereira- Smith and Smith suggest that the loss or inactivation of one of the many genes allows cells to avoid ageing process (12). If this hypothesis is confirmed it would allow the mapping of genes involved in cell ageing (13).Preliminary mapping by Sugawara et al. of the gene of ageing on the chromosome 1.was presented with the use of three independent experimental methods using human cells, among others, and immortal hamster cells. But whether these are the genes which regulate the osmolar in the cell and milieu interior and the hypoxia in cells which ultimately affects the wear and tear and induce ageing needs to be thoroughly probed. One of the techniques which can repair the DNA of the mutated gene is the Base Excision Repair Mechanism (BER) is instrumental in repairing damaged DNA by removing mutated/damaged bases and can be a mechanism for prospective anti-ageing techniques as well.[14]

NFAT 5 and hypoxia inducible factor and cell morphology: The transcription factor NFAT 5 is critical

to maintain cellular osmolality and is known to be expressed ubiquitously in several regions of the body.It appears from the recent studies that NFAT5 is linked with hypoxia also and may contribute in the survival of the cells.Upregulation of gene transcription is carried out by multiple enhancers known as the osmotic-reponse element (ORE) or tonicity-response enhancer (TonE) that are located in the regulatory domains of these genes (Fig.1). The original transcription factor for ORE/TonE was identified as TonE-binding protein (TonEBP)/ORE-binding protein (OREBP), which shows sequence homology to a family of transcription factors called the Nuclear Factor of Activated T cells (NFAT), and therefore it was named NFAT5 The cells are under hypertonic stress, and in order to avoid significant shriveling and shrinking that leads to cell death, cells elicit a genetic program of osmoregulation. Normally, high extracellular osmolality causes water flux from cells resulting in cell shrinkage, an early event of apoptotic cell death.[15] However, cells possess several adaptive mechanisms that allow them to survive in osmotic stress by restoration of the osmotic balance. Cellular survival under high-salt conditions is initially maintained by activation of ion transport systems and thereafter by intracellular accumulation of small organic osmolytes and increased abundance of heat shock proteins and AQPs.[16,17] The classical cellular response to high extracellular osmolality involves the transcription of target genes by the nuclear factor of activated T cell 5 (NFAT5), also known as tonicity-responsive enhancer binding protein (TonEBP/OREBP).This response gradually replaced electrolytes like Na+, Cl-, and K+ by small, uncharged organic osmolytes like sorbitol, betaine, and myo-inositol . These organic osmolytes play an integral role in osmoregulation due to their ability to accumulate without disturbing cellular structure and function. In fact, when kidney-derived Madin-Darby canine kidney (MDCK) cells were cultured in hypertonic solution, the concentration of betaine in the cell increased to 1,000 times the medium concentration.[18] Therefore, organic osmolytes accumulate as a response to cell shrinkage, and they are released following swelling,[19] In a study conducted by Senavirathna it was clearly shown that NFAT 5 mRNA levels increased at day 6 after an increase in cell hypoxia.

Hypoxia inducible factor and ageing: Ageing cellular physiology and gene expression in response to changing oxygen availability is essential for survival. The hypoxia inducible factor (HIF) is highly conserved transcriptional regulator of genes involved in the process.[20-21] HIF exists as a heterodimer of the regulated HIF alpha subunit and the constitutively expressed HIF beta subunit.[22] Under normoxic conditions, HIF-apha is ubiquitinated by a cullin E3 ligase complex containing the von Hippel Lindau protein (VHL) substrate recognition subunit and targeted for proteasomal degradation. This ubiquitination reaction is inhibited by hypoxia, leading to stabilization of HIF-a and induction of the hypoxic response.[23] The mechanism of ubiquitin mediated regulation of HIF-alpha stability is conserved from nematodes to humans.Ageing is characterized by a general decrease in O_2 supply to tissues and a reduction in tissue pO_2. A diminished vascularization in ageing alters the diffusion of O_2 at the capillary tissue level, and at an advanced stage, this can lead to tissue hypoxia. Molecular O_2 sensors mediate the response to O_2 deprivation through the regulation of protein expression, enzyme activities,andmetabolic regulating factors.[24] HIF1α is perhaps the best-studied responsive mechanism. A critical rolein vascularization and angiogenesis is played by VEGF, a HIF1α target. This crucial proangiogenic factor is alsoregulated at the mRNA level by a non-HIF pathway, DEAD-box RNA helicase.[25] Deficient O_2 supplytogether with a reduced ability to induce HIF1α expression may contribute to the aetiology of ageing. On the other hand, low O_2 levels lead to neovascularization that can contribute to pathologicalevents such as tumour growth and macular oedema. Furthermore, intermittent or continuous cellular hypoxia represents a source for oxygen radical production that is responsible for the inexorable decline of tissue morphology and physiology with age. In ageing, neovascularization appears to be attenuated whichmight be linked to impaired HIF1α induction. Other compensatory mechanisms might also take place inconditions of O_2 limitation.[26]

Cell blebbing and ageing: Cellular blebbing, first described in 1919 as hyaline blisters or bubbles, was subsequently characterized as smooth circular extensions (2–15 μm in diameter) of the plasma membrane that expand from the cytoplasm and retract to the initial site of origin.[27] These protrusions have received more attention in recent years due to their occurrence in widely differing cell types and their association with various physiological and pathological conditions.[28-29] Blebs have been seen in numerous cell types including fibroblasts, endothelial and mesenchymal cells, cancer cells, immune cells, germ cells, amoeba,[30-32] parasites and bacteria.[33] It was originally assumed that blebbing was related solely to pathological conditions in response to nonspecific cellular insults such as lipid peroxidation, anoxia. The bleb life cycle can be subdivided into three phases:(a) nucleation, (b) expansion i.e cytosol flowing from the cell body into the bleb and (c) retraction driven by myosin. They are formed when the plasma membrane separates from the underlying actin cortex and ispushed outwards by fluid pressure exerted by contraction of the cell cortex. However, other models have suggestedthat their formation and extension occur as a resultof an osmotic flux followed by actin polymerization.Fedier and Keller showed that decreasing free water content inside Walker carcinoma cells by 39% inhibited bleb formationand locomotion. Various stimuli and molecules involved in the formation of apoptotic and nonapoptotic blebs.[34] (Fig.2). Cellular blebbing can be induced in response to various mechanical and chemical stimuli and leads to the activation of many downstream signaling molecules depending on the stimuli and cell type.[35-36]

These protrusions have been shown to play an important role in various physiological and pathophysiological conditions, such as immune-related conditionsand cancer pathogenesis. Apoptotic cells exhibit uniform blebbing that eventually results in cell lysis, where as nonapoptotic blebs are reversible and involved in motile functions necessary for directional cellular migration.[37] In the case of endocrine-resistant cancer cells, a high external pH can induce either condition depending upon the time of exposure The molecules that have been implicated in blebformation. hydrostatic pressure, increased viscoelastic resistance topassive and swelling deformation and decreased space betweencytoplasmic components, without a significant increasein viscosity of the aqueous phase or any change inthe amount of F-actin. Pseudopod-like blebs were alsoseen in U937 monocytes stimulated by permeabilization of the cellular membrane with a nanosecond-pulsed electricfield; this was inhibited by partial isosmotic replacement of extracellular sodium chloride for a larger solute such as sucrose, suggesting that colloid-osmotic water uptake is the driving force for bleb formation.

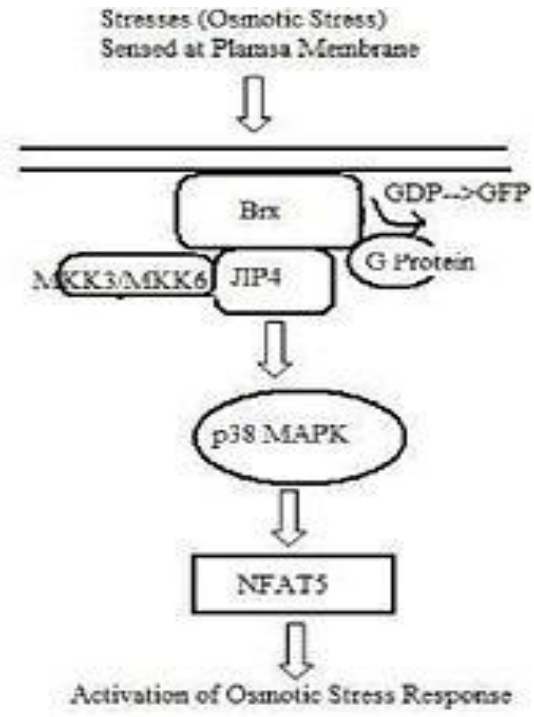

Fig. 1:

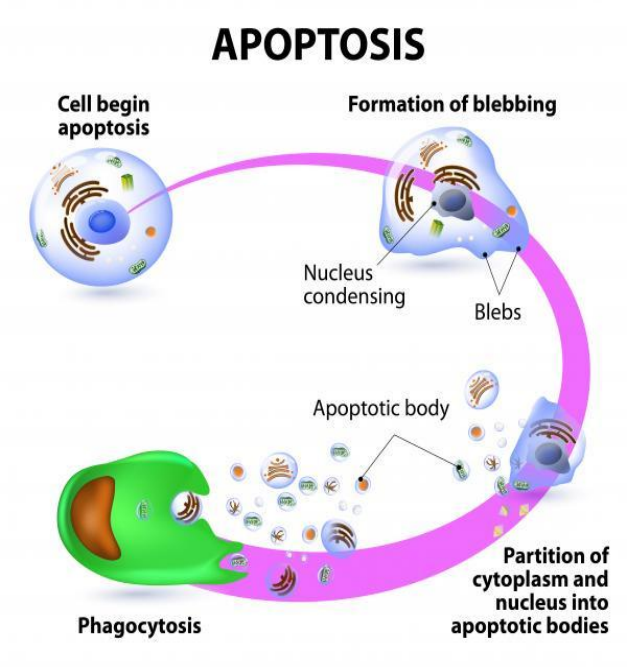

Fig. 2:

CONCLUSION

It is assumed that the ageing of cells also occurs in vivo and that the life expectancy andreplicative lifetime of cells are under common genetic control. The cellular process of aging is supposedly controlled by many genes. Important in the regulation of genes in this process are mutated and deactivation immortal cancer cells that have avoided aging. Morphologically, senescent cells become flat, vacuolated, andincrease in size.This process can be restored in these cells by the introduction of normal chromosomes, enabling the mapping and cloning of genes. The basic functions of these genes are helpful in explaining of regulation, cell cycle control and regulation of telomerase. All these factors are important inducers of genetic pathways of aging.In the current study the authors have stressed the importance of osmolality and hypoxia in particular the transcription factor NFAT5 and HIF1 and HIF2 and also the effects on cell morphology. Cellular blebbing over a period of time induces sensescent changes and may lead to wear and tear and play an important role in the ageing process in a living organism including higher mammals and humans. The exact molecular mechanism need to be explored but these are possibly key mechanisms regulating the ageing process i.e hypoxia and osmolality modulations leading to long term deleterious effects on cell survival and division.

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