The cGAS/STING pathway: a sensor of senescence-associated DNA damage and trigger of inflammation in early age-related macular degeneration

Yan Wu1,2,∗
Qingquan Wei1,∗
Jing Yu1,3

1Department of Ophthalmology, Shanghai Tenth People’s Hospital Affiliated with Tongji University, Shanghai, People’s Republic of China; 2Department of Ophthalmology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, People’s Republic of China; 3Department of Ophthalmology, Ninghai First Hospital, Zhejiang, People’s Republic of China

∗These authors contributed equally to this work

Abstract: Age-related macular degeneration (AMD) is the leading cause of irreversible blindness among the elderly. Considering the relatively limited effect of therapy on early AMD, it is important to focus on the pathogenesis of AMD, especially early AMD. Ageing is one of the strongest risk factors for AMD, and analysis of the impact of ageing on AMD development is valuable. Among all the ageing hallmarks, increased DNA damage accumulation is regarded as the beginning of cellular senescence and is related to abnormal expression of inflammatory cytokines, which is called the senescence-associated secretory phenotype (SASP). The exact pathway for DNA damage that triggers senescence-associated hallmarks is poorly understood. Recently, mounting evidence has shown that the cGAS/STING pathway is an important DNA sensor related to proinflammatory factor secretion and is associated with another hallmark of ageing, SASP. Thus, we hypothesized that the cGAS/STING pathway is a vital signalling pathway for early AMD development and that inhibition of STING might be a potential therapeutic strategy for AMD cases.

Keywords: age-related macular degeneration, cGAS/STING pathway, DNA damage, inflammation

Background
Age-related macular degeneration (AMD) is the leading cause of irreversible blindness among the elderly in developed countries.1 As a chronic, degenerative disorder in the macular region of the retina, AMD leads to progressive central vision loss from the early stage (medium-sized drusen and retinal pigment epithelium abnormality) to the late stage (neovascular AMD and geographic atrophy).2 The number of patients with AMD is expected to be approximately 200 million globally by 2020. There are a large number of elderly patients with visual impairment caused by AMD, which is likely to increase with time. The levels of vision described amount to considerable visual compromise and constitute a major public health burden, resulting in increased social isolation, depression, restriction of daily activities, risk of falling and hip fracture.3 And this condition will become a major public health issue with substantial socio-economic losses.

Anti-vascular endothelial growth factor (VEGF) agents (such as ranibizumab, aflibercept, or bevacizumab) are the main treatments for neovascular AMD and provide significant therapeutic effects.4 Ranibizumab 0.5 mg (Lucentis®; Novartis
And smoking cessation is unequivocally important in the prevention of AMD, especially early AMD. AMD is caused by multiple factors (genes, ageing, smoking, family history, dietary habits, oxidative stress, and hypertension). What’s more, several genetic and molecular studies have shown the participation of inflammatory molecules, immune cells, and complement proteins in the development and progression of the disease. In addition, different genes (IL-6, IL-8, CFH, CFI, C2, C3, and ARMS2) that play an important role in the inflammatory pathway have been related with AMD risk. Also, the sample cohort has been subjected to a large genotyping analysis of 20 genetic variants which are known to be associated with AMD among European and Asiatic populations. This study revealed that 8 genetic variants (IL-8, CFH, TIMP3, SLC16A8, RAD51B, ARMS2, VEGFA and COL8A1) were significantly related with AMD susceptibility. Among all the associated risk factors, ageing and smoking are by far the strongest risk factors. The results from different observations showed that smoking is significantly associated with the incidence of AMD. And smoking cessation is unequivocally cost-effective in terms of its impact on AMD development and progression. Both in vivo and in vitro experimental models based on smoke exposure and tobacco extract are available. Thus, focusing on the impact of ageing on the development of AMD is valuable. More information about the ageing-associated or driven...
molecular progress of AMD would contribute to the successful prevention or treatment of AMD, and this knowledge might help in other human ageing diseases.

Ageing, which is a time-related, degenerative process beginning in adulthood, occurs in most organisms and eventually ends life. Ageing is associated with a series of diseases, including cardiovascular disease, neurodegeneration, carcinoma, and osteoarthritis. In a previous review, the hallmarks of ageing included genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Among all the ageing hallmarks, increased DNA damage accumulation was regarded as a beginning of cellular senescence and was related to abnormal expression of inflammatory cytokines, which is called the senescence-associated secretory phenotype (SASP). DNA sensors can sense senescence-associated DNA damage and trigger inflammation. cGMP-AMP (cGAMP) synthase (cGAS) and the adaptor stimulator of interferon genes (STING) were shown to be involved in the regulation of senescence. In addition, a recent study by Nagaraj Kerur et al indicated that cGAS responded to mitochondrial damage-induced inflammasome activation and thus played an important role in the regulation of geographic atrophy. However, the exact role of the cGAS/STING pathway in the development of early AMD as a senescence-associated DNA damage sensor remains unclear.

Presentation of the hypothesis

Ageing, which is one of the strongest risk factors of AMD, influences both the anatomy and function of the retina. Retinal pigmented epithelium (RPE) cells, which are a monolayer of cells that provide trophic support to photoreceptors, are regarded as one of the earliest influenced cells in the retina. RPE dysregulation is associated with various kinds of retinal diseases, including AMD, diabetic retinopathy and proliferative vitreoretinopathy. The abnormal expression of growth factors and cytokines secreted by RPE cells is involved in the dysfunction of RPE cells. As reported in previous studies, these growth factors include vascular endothelial cell growth factor (VEGF), pigment epithelium-derived factor (PEDF), and transforming growth factor beta 2 (TGF-β2). The cytokines include interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-17A (IL-17A) and interleukin-1β (IL-1β). SASP is regarded as a key hallmark of ageing, and abnormal expression of various factors, such as IL-1α, IL-1β, IL-6, IL-8, and matrix metalloproteinases (MMP1 and MMP3), is involved. There was a wide-ranging overlap between RPE dysfunction-associated factors and SASP-associated factors. Thus, the dysfunction of RPE was, at least partly, caused by cell senescence, and the abnormal expression of growth factors and cytokines were signs of SASP.

Throughout an organism’s lifespan, DNA is exposed to both exogenous and endogenous harmful factors, such as chemicals, radiation and different kinds of metabolic products, leading to different types of DNA damage and genomic instability. Many different factors, such as tobacco smoking, which is a strong environmental risk factor, has been reported to be associated to increased DNA damage in retina. DNA damage is regarded as both an important hallmark and a key trigger of senescence. A persistent DNA damage response (DDR) in ageing cells leads to senescent DNA damage foci (SDF) and telomere dysfunction-induced foci (TIF). DNA damage includes nuclear DNA and mitochondrial DNA (mtDNA) damage. There is a modulated balance between DNA damage and DNA repair in normal cells, and the absence of this balance leads to the accumulation of damaged DNA. mtDNA is a closed-loop DNA molecule independent of nuclear DNA in cells. Mitochondrial DNA, which exists without the protection of histone and DNA binding proteins, is vulnerable to oxygen free radical damage. In addition, mtDNA is not easily repaired due to the lack of a repair system. Therefore, mtDNA is more easily affected by influencing factors and accumulates more harmful mutations compared with nuclear DNA. A published review has focused on the role of DNA damage in the effect of cellular senescence in AMD. It concluded that oxidative stress can induce DDR and cell senescence, promoting AMD incidence. Thus, how the DNA damage signal is detected by the cell could answer questions about the progression of ageing or senescence-associated signs.

Increasing evidence has demonstrated the important role of the cGAS/STING pathway as a cytoplasmic DNA sensor, and its classical function is to promote the production of type I interferons (IFNs) and immune factors, which is important in antiviral and antineoplastic processes. Advanced studies have also shown that this pathway is involved in the incidence and progression of autoimmune diseases, carcinoma and ageing. When cellular senescence was considered, the regulatory effect of the cGAS/STING pathway also demonstrated a significant effect. Activation of the cGAS/STING pathway leads to two independent...
downstream pathways: type I IFNs through interferon regulatory factor 3 (IRF3) and proinflammatory responses through NFkB16. Both the IRF3- and NF-kB-dependent pathways lead to the production of inflammatory growth factors and cytokines, leading to SASP.

Based on the above absorbing viewpoints and studies, we hypothesized that the cGAS/STING pathway is the sensor of senescence-associated DNA damage and trigger of inflammation in early AMD (Figure 1). cGAS/STING functioned as an important bridge to connect ageing-related DNA damage and AMD incidence, and thus, the inhibition of this pathway would help in drug development in the future.

**Testing the hypothesis**

1. To demonstrate the feasibility and stability of this hypothesis, we will first detect the association between RPE cellular senescence and the incidence of ageing and early AMD. Young rats (2 months), ageing rats (24 months) and early AMD rat models (24 months + smoke exposure) will be obtained. Smoke expose will be conducted using a smoking machine for 2 months and following experiments will conducted as a smoke expose group. The average concentration of total suspended particulates was 130 mg/m³ and was monitored twice daily. The retinal structure will be conducted using retina sections and retinal function would be detected with multifocal visual electrophysiology examining system. Next generation sequence will be conducted to detect the general RNA expression in the three groups. The expression of the cell senescence biomarkers SA-β-Gal activity and p16Ink4a will be detected by immunohistochemistry of retinal tissues and Western blot analyses of extracted RPE cells. This aim will be provided evidence for the relationship between RPE cell ageing and the incidence of AMD.

2. Second, the DNA damage was linked to the AMD-like phenotype both in-vivo and in-vitro. Both the extracted primary cultured RPE cells from young, old and AMD rat models and in vitro cellular models (normal or tobacco extracted treatment group) will be obtained for detection. Cytoplasmic dsDNA will be detected by staining with a primary antibody against dsDNA. Mitochondrial DNA PCR will be obtained

**Figure 1** Molecular mechanisms of the cGAS/STING pathway as a senescence-associated DNA damage sensor and inflammation trigger. DNA is exposed to both exogenous and endogenous harmful factors, such as chemicals, radiation and different kinds of metabolic products. DNA damage includes nuclear DNA and mitochondrial DNA (mtDNA) damage. The cGAS/STING pathway is a cytoplasmic DNA sensor, and activation of the cGAS/STING pathway leads to two independent downstream pathways: type I IFNs through IRF3 and proinflammatory responses through NFkB16. Both the IRF3- and NF-kB-dependent pathways lead to the production of inflammatory growth factors and cytokines, leading to SASP.

**Abbreviations:** SASP, senescence-associated secretory phenotype; RPE, retinal pigmented epithelium; ER, endoplasmic reticulum; ROS, reactive oxygen species.
for the detection of cytoplasmic mtDNA. Both cytoplasmic dsDNA and damaged mtDNA will be upregulated in ageing retina and early AMD models.

3. Third, cGAS/STING and its downstream IRF3- and NF-κB-dependent pathway-related key proteins will be detected in an in vitro model. Tobacco extract treatment will be used in the in vitro model construction, and the dose-response effect of tobacco extract will also be detected. For the SASP-associated factors, the cellular and secreted growth factors, including IL-1α, IL-1β, IL-6, IL-8, and matrix metalloproteinases (MMP1 and MMP3), will be detected by Western blots and ELISAs.

4. In the end, we assessed inhibition of the cGAS/STING pathway as a treatment of early AMD. As reported in a recent study, covalent small molecules can inhibit STING,\(^6,^5\) the specific STING inhibitors will be used both in vivo and in vitro to test their potential therapeutic effects. If an inhibitor could reverse retinal ageing signs and release SASP markers, these molecules will be a potential drug for early AMD.

**Conclusion**

Ageing is one of the strongest risk factors for AMD incidence, but the exact role of ageing remains unclear. DNA damage, including nuclear and mtDNA damage, is important in the progression of senescence. The extract pathway for DNA damage in the trigger of senescence-associated hallmarks is poorly understood. Recently, mounting evidence has shown that the cGAS/STING pathway is an important DNA sensor related to proinflammatory factor secretion and is associated with another hallmark of ageing, SASP. Thus, we hypothesized that the cGAS/STING pathway is a vital signalling pathway for early AMD development and that inhibition of STING might be a potential therapeutic strategy for AMD cases.

**Acknowledgments**

This work was supported in whole or in part by the Project supported by the National Science Foundation for Young Scientists of China (Grant No. 81700804) and the Foundation for Young Medical Talents of Jiangsu Province, 2016 (Grant No. QNRC2016211).

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


