

Extra Views

Altered Epigenetic Patterning Leading to Replicative Senescence and Reduced Longevity

A Role of a Novel SNF2 Factor, PASG

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ABSTRACT

Understanding the biological mechanisms underlying aging and cancer predisposition remains a fundamentally important goal in biomedicine. The generation of a PASG hypomorphic mutant mouse model shows that PASG, an SNF2 family member, is essential for properly maintaining normal DNA methylation and gene expression patterns. Disruption of PASG leads to decreased incorporation of BrdU, accumulation of senescence-associated tumor suppressor genes, and increased senescence-associated β -galactosidase as well as age-related phenotypes. These observations demonstrate that PASG plays a critical role in maintenance of tissue homeostasis, normal growth and longevity.

DNA methylation is an epigenetic modification that contributes to the regulations of gene expression and consequently cellular physiology.¹⁻³ In mammalian cells, DNA methylation involves the addition of a methyl group to deoxycytosine (dC) at the five position to form deoxymethylcytosine (d^mC). This reaction is catalyzed by the methyltransferases. There are three major methyltransferases in mammalian cells. DNMT3a and DNMT3b are primarily de novo methyltransferases, which transfer methyl groups to cytosines at CpG dinucleotides on both DNA strands. DNMT1 has a preference for hemimethylated double-strand DNA after replication, which restores methylated cytosines at CpG sites on the newly duplicated strand. Therefore, DNMT1 is considered to be primarily important in maintenance of DNA methylation. In vivo mutation studies have demonstrated that these DNA methyltransferase genes are necessary for embryonic development and postnatal life.⁴⁻⁶

The covalent modification of DNA by interaction of DNA and methyltransferase represents a complex process in mammalian cells. Nucleosomal DNA interacts with core histone modifying and ATP-dependent chromatin remodeling proteins and complexes. Different chromatin structures, resulting from histone modifications and nucleosome positioning mediated by ATP-dependent chromatin proteins significantly influence DNA methylation patterns. Histones are subject to posttranslational modifications including acetylation, methylation, phosphorylation, ubiquitination, and ADP-ribosylation, that change the accessibility of regulatory factors to DNA resulting in altered gene expression and cellular physiology.^{7,8} Chromatin remodeling complexes, which contain proteins with ATPase activity, are able to disrupt histone-DNA interactions, allowing for physical movement, or sliding of nucleosomes along the DNA using the energy from ATP hydrolysis. These chromatin changes increase the accessibility of DNA to various proteins that regulate gene transcription, DNA replication and repair.⁹⁻¹¹ Chromatin remodeling complexes can be divided into three main classes based on the identity of their catalytic ATPase subunit: SWI2/SNF2, ISWI and Mi-2 families.¹² SNF2 family members are characterized by the presence of seven conserved structural motifs. DDM1, ATRX and PASG (*Ish*) belong to the SNF2 superfamily and have been demonstrated to be involved in the modulation of DNA methylation.

DDM1 (decrease in DNA methylation), is not a methyltransferase, but has been shown to facilitate DNA methylation in the flowering plant *Arabidopsis thaliana*. Inactivating mutations of DDM1 result in the loss of 70% of the total methyl cytosine of the genome, primarily in repetitive sequences, followed by single copy genes. DDM1 mutant plants display defects in flowering time, floral and leaf morphology as well as fertility.¹³ DDM1 is also required to maintain histone H3 methylation patterns. In DDM1 mutant plants, methylation of histone H3 lysine 9 is largely replaced by methylation of lysine 4.¹⁴ ATRX is the gene mutated in a human disorder known as X-linked, α -thalassemia, mental retardation (ATR-X). Mutations of ATRX give rise to changes in

the methylation pattern of selective genes, including hypomethylation of ribosomal repeated sequences and hypermethylation of Y chromosomal-specific repeats.¹⁵

PASG (Proliferation Associated SNF-2-like Gene, *lsh*, *hells*), another member of SWI2/SNF2 subfamily of helicase, is closely related in sequence and function to DDM1.¹⁶⁻¹⁸ The open reading frame of human and murine PASG is 2,514 (838 amino acids) and 2,466 (822 amino acids) nucleotides, respectively.^{16,17} *lsh* (PASG), initially designated as being a lymphoid specific helicase,¹⁹ was subsequently shown to be ubiquitously expressed in a wide variety of embryonic tissues and tumor cell lines.¹⁷ PASG has been also shown to be linked to cell proliferation and replicative senescence.^{17,18} The genetic locus on chromosome 10q23-24 in human and chromosome 19C3-D1 in mouse, has been termed SMARCA6.^{16,20}

The absence of normal PASG expression during development results in global genomic hypomethylation^{18,21} and reexpression of subsets of genes, including senescence-associated genes.¹⁸ PASG mutant mice display a loss of 33 to 43% of total genomic methylcytosine transcriptionally.^{18,21} The normally hypermethylated genes such intracisternal A-particle (IAP) and minor satellite centromeric repetitive sequences are demethylated and repressed in PASG mutant mice and fibroblasts.^{18,22,23} PASG mutant mice also display a significant increase in expression of senescence-related genes such p16^{INK4a}, p19^{ARF}, p53 and p21.¹⁸ However, the increased expression of tumor suppressor genes such as p16^{INK4a} is not related to promoter demethylation, but instead is associated with downregulation of *bmi-1* gene, a negative regulator of p16^{INK4a}¹⁸ and, possibly, chromatin changes. *Bmi-1* is a member of another group of epigenetic regulatory proteins, the Polycomb-trithorax (Pc-G/trx) protein complexes.^{24,25}

Hypomorphic PASG mutant mice display growth retardation and premature aging phenotypes.¹⁸ Growth retardation is detectable during mid-gestation and continues into postnatal life. PASG mutant mice have a low birth weight and show failure to thrive; they also display an early onset of phenotypes associated with aging, including graying and loss of hair, reduced skin fat deposition, osteoporosis, kyphosis, cachexia, ataxia, and premature death. Fibroblasts derived from PASG mutant embryos have a replicative senescence phenotype.^{18,26} Although decreased cell proliferation has been demonstrated in both PASG mutant mice and fibroblasts, cell cycle checkpoints appear to be intact. The reduced proliferation capacity is due to replicative senescence, not due to defects in cell cycle progression.¹⁸

Senescence is a state in which a cell no longer has the ability to proliferate and is accompanied by specific changes in cell morphology and gene expression. To maintain tissue homeostasis during the lifetime of an animal, stem cells have developed strict regulatory mechanisms to self-renew, differentiate, and prevent premature senescence. Disruption of PASG leads to decreased incorporation of BrdU, accumulation of senescence-associated tumor suppressor genes, and increased senescence-associated β -galactosidase as well as the age-related phenotypes, demonstrating that PASG plays a critical role in maintenance of tissue homeostasis.¹⁸

Imperfect maintenance of genome integrity may be an important cause of senescence or premature aging.²⁷ DNA methylation governs several distinct processes including genomic stability, retroviral repeated element suppression and gene promoter regulation. During DNA replication, cells replicate both DNA as well as the DNA methylation pattern. Errors in replication of DNA methylation patterns, such as observed in mutant PASG mice, may destabilize the genome and activate cellular self defense mechanisms that prevent

cells from entering S-phase. Altered gene expression, reduced cell proliferation and abnormal embryonic development are also consequences. However, other mechanisms may also contribute to the observed senescence phenotypes in PASG mutant mice. For example, *bmi-1*, a transcriptional regulator, may provide an alternative mechanism to DNA methylation in regulating the expression of downstream genes playing essential roles in establishing and/or maintaining a replicative senescence phenotype.²⁵ Furthermore, altered chromatin modification, structure and function may also play an important role in regulating cell proliferation and senescence.^{28,29}

There are still many questions remaining to be answered. How does PASG interact with methyltransferases or their complexes to maintain proper methylation patterns? How does PASG regulate senescence-associated gene expression? Will downregulation of PASG with siRNA or small molecule inhibitors affect tumor cell growth? We hope that further studies using PASG mutant mice will help us elucidate the mechanisms of aging and cancer predisposition as well as epigenetic regulation during embryonic development, postnatal life and neoplastic transformation.

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