Potential of epigenetic mechanisms in AMD pathology

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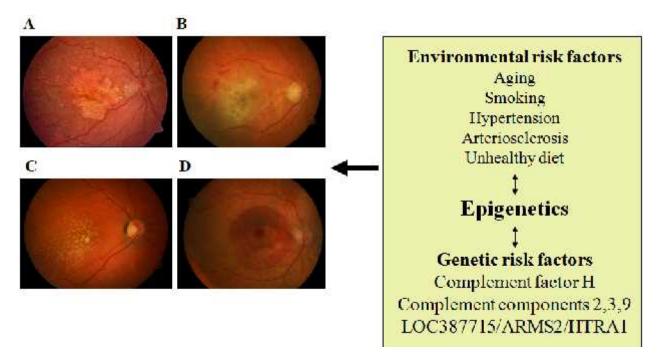
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1. ABSTRACT

Age-related macular degeneration (AMD) is an ocular disease and the main reason for sight loss in the elderly in the developed countries. The pathogenesis of the disease is complex and not fully understood, but involves several environmental and genetic risk factors**.** However, little is known about the role of epigenetics in this disease although it is recognized that epigenetic alterations often precede genetic changes in many pathological conditions and regulate aging and the developmental processes. There is experimental evidence for the involvement of DNA methylation and histone modifications in the pathogenesis of drusen formation, a central hallmark of AMD. However, the main impact of epigenetic modifications, including persistent lysine methylation of the H3 histone, is exerted during retinal embryonic development. This interplay opens an exciting possibility to manipulate the epigenetic pattern and to develop novel AMD therapies by physical, pharmacological or genetic interventions. One of the most intriguing questions is why different individuals develop different AMD phenotypes. Epigenetic regulation might open new perspectives into these changes in AMD pathology.

2. AGE-RELATED MACULAR DEGENERATION: EPIDEMIOLOGY, PATHOGENESIS AND THERAPY

AMD is the leading cause of irreversible blindness in the elderly in the developed countries and its prevalence has been estimated to increase over the coming decades (1). The disease is a medical and social problem i.e. epidemiological data in the US has indicated that about 2 million people are affected, this being forecasted to increase to 7 million (2). AMD results in an inability to read, recognize faces, drive, or move freely.

The increased AMD prevalence can be traced to its age-association and its incidence increases in parallel with the growing elderly population, especially in the Western World.

AMD has a progressive character and may develop into a dry (non-exudative) or wet (exudative) form. Both forms share certain common clinical features – the degeneration of retinal pigment epithelial layer (RPE) and the accumulation of extracellular drusen between the RPE and the inner collagenous layer of the Bruch's membrane

Figure 1. Schematic presentation of risk factors to develop different AMD phenotypes (A) geographic dry AMD, (B) fibrotic wet AMD with small haemorrhage, (C) drusen maculopathy in dry AMD, (D) wet AMD with large haemorrhage.

(Figure 1; 3). The dry form of the disease is more prevalent and it accounts for up to 90% of all cases. In this form, most patients experience a slow, gradual but progressive and inexorable decline in central vision over a period of months or years. Geographic atrophy is an advanced form of dry AMD and evokes severe central visual loss (Figure 1A). In some cases, the dry form may be converted to the wet form, in which the visual loss is sudden and occurs during a time frame of days or weeks (Figure 1B,D). Choroidal neovascularization sprouting into the retina is a diagnostic sign of wet AMD. Central visual loss is due to photoreceptor damage that results from RPE degeneration, RPE detachment, retinal swelling, accumulation of retinal exudates, choroidal neovascularization, subretinal hemorrhage, and finally fibrovascular disciform scarring (Figure 1).

The pathophysiology of AMD is not completely understood, but is strongly associated with oxidative stress and chronic inflammation. It is probably influenced by the interaction between many environmental and genetic factors. Increasing age is considered as the most significant AMD risk factor (2). However, the process of aging is associated with increasing oxidative stress resulting in oxidative damage to many biomolecules (Figure 1; 4). The next environmental/life style factor which can be involved in the AMD pathogenesis – smoking, also can be linked with oxidative stress (5). Tobacco smoke contains many chemical substances including more than 60 known carcinogens, i.e. polycyclic hydrocarbons, nitrosoamines, aromatic amines, aldehydes, volaite organic compounds, metals and others (6). Because the smoke also contains unstable free radicals and reactive oxygen species (ROS), oxidative damage may be induced in the cells of smokers

(7). A diet rich in fat can be an important risk factor for obesity and many diseases, including AMD (8, 9). However, a fat-rich diet is also associated with cholesterol, saturated fats, oxidative stress and inflammation. Thus, dietary cholesterol can increase the susceptibility of low-density lipoprotein to oxidation and potentiate the harmful effects of dietary saturated fats (10). The involvement of diet-derived oxidative stress as a pathogenic AMD factor is supported by the observation of protective effects against AMD development exerted by consuming a diet rich in antioxidants, fish and omega-3 fatty acids (11, 12). Furthermore, exposure to sunlight, especially its blue component, may be associated with the increased risk of AMD and induction of the oxidative stress (13, 14). Other environmental/life style AMD risk factors not directly associated with oxidative stress are Caucasian origin, light iris color, female sex, history of cardiovascular disease, hypertension, increased plasma fibrinogen level, diabetes, history of cerebrovascular disease (15).

Genetic and environmental factors of AMD predispose the post-mitotic RPE cells to impaired protein clearance that evoke the accumulation of lysosomal lipofuscin and extracellular drusen. Lipofuscin and drusen deposits are hallmarks of AMD and reflect the severity of the disease (15-17). Recently, autophagy has been linked to many neurodegenerative diseases and age-associated pathological conditions that are obviously involved in AMD development as well (16). Autophagy is activated during AMD-associated stress conditions, including hypoxia and oxidative stress. However, during aging, autophagy may be epigenetically regulated and this can lead to impaired autophagy and detrimental protein aggregation (17).

Familial studies, especially twin studies and segregation analyses, point to an important role of genetic factors in the development of AMD (18). AMD is considered as a complex disease in which many genes may contribute to susceptibility to this disease (19). More detailed description of a complex nature of AMD will be given in section 6. There are some difficulties encountered in studies on the role of genetic factors in AMD pathogenesis which are attributable to the basic nature of the disease and its late onset. Due to this fact, an AMD-affected individual may be exposed to many environmental factors for a relatively long time leading to the accumulation of effects and induction of threshold-sensitive changes. Moreover, the late onset of the disease, means that only one generation is available for genetic analysis, which is a serious problem in genetic research.

The importance of the genetic component in the pathogenesis of AMD originates from familial aggregation studies, twin studies, and segregation analyses (20, 21, 22, 23, 24). These studies have provided us with information on the familial nature, heritability, and mode of transmission of this disease. However these types of studies provided no information on the possible genetic locus associated with the risk of AMD. Linkage studies have indicated that almost all human chromosomes are implicated with AMD, although the most widely replicated linkage findings have focused on chromosomal regions 1q31-32 and 10q26 (18, 25).

Association studies have identified several AMD-candidate genes on the basis of linkage studies and gene functions. The complement factor H gene (*CFH*) on chromosome 1 is probably the best recognized gene linked with AMD and studies on this linkage are consistent and convincing (Figure 1; 26, 27). Many complement component variants have been associated with increased risk of AMD, establishing the complement system as the main genetic factor in AMD pathogenesis (18). The epsilon allele of the apolipoprotein E gene (*APOE*) seems to exert a protective effect against AMD development (28). Toll-like receptors 3 and 4 genes (*TLR3* and *TLR4*) are the next candidate genes reported to have risk alleles for AMD (29,30). The variability in the tissue inhibitor of the metalloproteinase 3 gene (*TIMP-3*), which codes for a product essential in the maintenance of the extracellular matrix structure, may be disturbed during AMD pathogenesis (31). We described an association between polymorphism of the vascular endothelial growth factor gene (*VEGF*) and AMD risk (32). Since oxidative stress is a recognized risk factor in AMD, the genes encoding products involved in antioxidative defense are candidates for being associated with the risk of AMD. The manganese superoxide dismutase 2 gene (*SOD2*) may be linked with the risk of AMD, although its involvement was not definitely resolved (33). We showed that the extent of oxidative damage to DNA bases in lymphocytes of AMD patients was greater than that measured in controls (34). Moreover, the cells of AMD patients showed a higher sensitivity than controls to hydrogen peroxide, a known source of ROS. Finally, the efficacy of the repair of DNA damaged by UV radiation and hydrogen peroxide was lower in AMD patients than in controls. Therefore, the lower efficiency of impaired general DNA repair may combine with enhanced sensitivity of RPE cells to blue and UV light and contribute to the pathogenesis of AMD (35). In addition, metabolism of mitochondrial DNA has been postulated to be involved in AMD pathogenesis (36). However, the Ser326Cys polymorphism of the base excision repair *hOGG1* gene, which is crucial for removing oxidative damage to the DNA bases, is not associated with AMD risk (37). Although oxidative stress is a recognized AMD pathogenesis factor, its source has not been fully identified. One should be aware that several factors may contribute to oxidative stress, for instance smoking, sunlight exposure, diet oxidants. Since ROS are the main "executors" of the stress and they may be produced in the iron-catalyzed Fenton reaction, iron ions are candidates for a source of oxidative stress and may play a role in AMD pathogenesis (38, 39). This hypothesis was suggested after observation that the eyes of AMD-affected individuals contained higher concentrations of iron than controls (40). In an attempt to clarify this hypothesis we sought an association between polymorphism of genes of iron homeostasis and AMD occurrence and progression and found a positive association of AMD with the A allele of the -576G>A polymorphism of the transferrin gene in smokers (41). However, we did not find any association between variability of the heme oxygenase-2 gene (*HMOX2*) and AMD (42).

Several actions may help slow the progression of macular degeneration but in general no definite treatment capable of reversing AMD has been identified. The maintenance of optimal blood pressure, eliminating smoking, avoiding excessive sun exposure, consuming a healthy diet have been proposed to play a role in reducing the risk of AMD (43). A number of clinical interventions are being undertaken in AMD patients, especially in patients with advanced forms of the disease, i.e. the wet form and geographic atrophy. In the wet form, after the establishment of neovascularization, several pro- and antiangiogenic factors start to play important roles. VEGFs are potent regulators of angiogenesis, which can be targeted in AMD therapy by their inhibitors: ranibizumab, bevacizumab, VEGF Trap, pegaptanib sodium and another compound currently being investigated (44). In addition, hepatocyte growth factor, platelet-derived growth factor, fibroblast growth factor-2, tumor necrosis factor alpha can be considered as targets in AMD therapy (45). Photodynamic therapy and thermal laser treatment are options reported to reduce the risk of moderate visual loss in some patients, but they may have severe negative results, including permanent and immediate vision loss (46, 47, 48). Therefore, they are used cautiously in ophthalmological clinics. Different combination therapies, polyphenolic compounds, feeder-vessel laser, antiinflammatory drugs, gene therapy and surgical interventions have also been studied in AMD curing (16, 49, 50).

3. EPIGENETIC REGULATORY MECHANISMS IN NORMAL AND PATHOLOGICAL CONDITIONS

Classically, epigenetics is understood to involve changes in gene expression which are unrelated to alterations in DNA sequences (51). A more precise

definition describes epigenetics as a term relating covalent modifications (marks) of the genome, changing the structure and activity of a gene, but not altering its sequence. The complete spectrum of such modifications is termed the epigenome (52). When epigenetic modifications of an individual allele are being considered, their spectrum is referred to as the epiallele (53). Epigenetic modifications which can be linked with a disease, epimutations, can occur for an allele with a normal DNA sequence. Links between epigenetic marking systems appear to be developmentally regulated contributing to phenotypic plasticity (54). However, some marks are stably inherited by the next generation, these are termed as constitutive germline epimutations (55).

Epigenetic mechanisms are usually considered to involve DNA methylation, histone modifications, gene-silencing by non-coding RNA, ATP-dependent chromatin remodeling. The specific epigenetic pattern produced as the result of the changes underlined by these mechanisms is passed on from one generation to the next but may be influenced by environmental factors (56). Therefore, the central dogma of molecular biology should not be limited to the primary DNA/RNA sequence, but should also include DNA methylation, histone modifications, the action of interfering RNAs and some proteins. In general, the changes introduced by epigenetic mechanisms are reversible and they change during specification of individual cell lineages and differentiation in response to various stimuli. Our knowledge on the molecular mechanisms of epigenetics has significantly increased during a past few years and one of the most important "take home messages" is the recognition that epigenetic alterations may influence the phenotype, in particular they may be associated with the induction and development of many diseases (57). This is very important because the error rate for the replication of nuclear DNA in dividing cells equals approximately 1 per 10^6 bases, representing a rare mechanism of genetic diversity, which may result in a phenotypic change. However, the error rate for replicating epigenetic marks in the genome during cell division is 1 per 10^3, creating ample opportunity for phenotypic changes (58). Today it is known that many fundamental processes, including tissue-specific gene expression, imprinting and the X chromosome inactivation, are regulated by epigenetic mechanisms (59, 60). If one wishes to understand the epigenetic aspects of a disease, it is necessary to define the mechanisms underlying the epigenetic modifications. At present, two kinds of epigenetic alterations, DNA methylation and histone modifications, seem to be strongly associated with many pathological states.

3.1. DNA methylation

In humans, DNA methylation is restricted to cytosine bases, most commonly in the context of the CpG sequences. This dinucleotide is underrepresented in the human genome due to mutagenic effect of cytosine methylation; the methylated base, 5meC, is prone to deamination producing thymine. This is the reason why the regions which are rich in CpG dinucleotides (CpG islands) are mostly depleted of 5meC. The CpG islands are frequently found upstream of a gene and the methylation
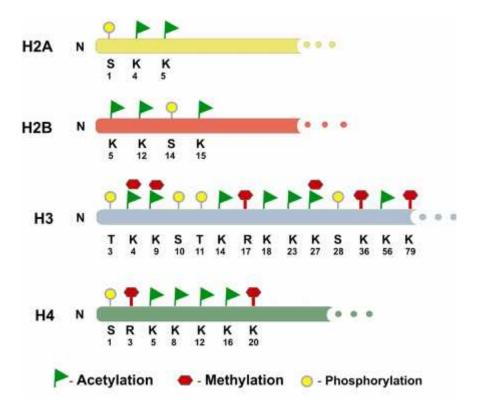
status of these regions is associated with the transcriptional potential of this gene (61). Humans have two kinds of enzymes involved in the establishment and maintenance of DNA methylation pattern: *de novo* and maintenance DNA methyltransferases (DNMTs). The former are DNMT3A and DNMT3B and they establish the methylation pattern in the embryonic development, whereas the latter, DNMT1s, copy this pattern during each DNA replication. However, in order to maintain the correct methylation profile, one needs the concerted action of both classes of enzymes (62). DNA methylation of cytosine in its 5 position at CpG islands is present in the promoters of approximately 60% of human genes (63,64). Promoter CpG islands are targets for functional differential DNA methylation and hence they are key regions of epigenetic changes related to chromatin modifications.

DNA methylation is strongly associated with the transcriptional activity of the gene, but it is difficult to establish a uniform relationship between these two quantities. Usually, methylation of the promoter is associated with the silencing of the downstream gene, but methylation of the coding sequence in the gene is often positively correlated with its transcriptional activity (65). However, these associations are not always the case and we should consider DNA methylation as a rather complex indicator of transcriptional activity of the gene, especially that it interplays with other kinds of epigenetic modifications in the regulation of gene expression.

3.2. Histone modifications

Post-translational covalent modifications of histones are considered to be a major source of epigenetic information regulating gene expression. These processes are acetylation, phosphorylation, methylation, ADP-ribosylation, sumoylation, citrullination and ubiquitination. They are imposed on the histone N-terminal tails. In general, the methylation of histones is often associated with the silencing of a gene, whereas acetylation has an opposite effect (66). However, these alterations should not be considered separately from the remaining histone modifications. This led to the concept of a histone code (Figure 2; 67). According to this proposal, it is the combination of histone modifications at given locus that determines the pattern of expression of a gene regulated by this locus. Therefore, the current collective pattern of all modifications imposed on histone tails provides information about ongoing expressional, mainly transcriptional, activity of a given locus. However, this is a matter of debate. whether the effect of histone modifications is a combination of modifications or whether it has a cumulative character (68). Whichever is the case, the histone code must be produced (written) and then its information must be read in order for it to be effective. Some of the components of the histone code, e.g. lysine acetylation and methylation as well as phosphorylation of serine and threonine have been relatively well characterized.

Histone acetylation and methylation are controlled by histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone

Figure 2. Schematic presentation of histone codes and post-translational modifications for acetylation, methylation and phosphorylation. For the sake of clarity only monomethylation is presented, but arginine can be mono- or dimethylated in an asymmetric or symmetric manner and lysines can be mono-, di- or trimethylated.

demethylases (HDMs) and methyl-binding proteins (MBDs). The enzymes involved in methylation and acetylation are recruited by DNA-binding transcription factors, whereas the members of the MBD family recruit histone-modification and chromatin-remodeling complexes to the methylation sites. Histone methylation and acetylation do not alter the structure of chromatin by themselves, instead they are recognized by non-histone proteins having the chromo- and bromodomain in their structure, respectively (69, 70). These proteins remodel the chromatin, changing the accessibility of DNA towards transcriptional machinery. In general, acetylation of the histones, executed at critical amino acids by HATs, can be associated with loosening of the chromatin and activation of transcription, whereas histone deacetylation, performed by HDACs seems to achieve an opposite effect. Generally, histone methylation may be associated with gene silencing, although this is not always the case, e.g. methylation of the H3 histone at lysine 9 (K9) may evoke an opposite effect than di-methylation of the same histone at the same position (71, 72).

Since AMD is a disease associated with aging and the eye, it is justified to take a closer look at the role of the epigenetic control of aging and eye development.

4. EPIGENETICS OF AGING

Although the precise relationship between aging, understood as progressive physical, psychological and social changes of the organism, and cellular senescence has not been established, it is clear that this relationship should be taken into account when one assesses the process of aging from a molecular point of view. The maintenance of the length of telomeres seems to be critical in senescence and it may depend on the epigenetic modifications of both DNA and histones (73, 74). These modifications include DNA and histone methylation as well as the action of long telomeric RNA (TERRA) (75). TERRA synthesis was reported to be stimulated by p53 and mixed lineage leukemia (MLL) proteins after telomere uncapping (76). The latter protein displays HMT activity and methylates the H3 histone at lysine 4. There is an accumulation of mutations with aging and they may affect the key genes involved in the regulation of the cell cycle directly modulating cellular senescence (77). These mutations may also alter the function of genes controlling the epigenetic factors, which may result in deregulation of many genes controlling senescence (78). During senescence, both maintenance and *de novo* methylating activity of DNMT, as well as the expression of the enzyme, undergo pronounced changes (79).

The source of methyl groups in epigenetic modifications is S-adenosylmethionine (SAM), where the carbon on its methyl group is the target of the catalytic action of DNMTs (80). SAM is a substrate for the production of S-adenosylhomocysteine (SAH), which is further metabolised to homocysteine and adenosine by S-

adenosylhomocysteine hydrolase (SAHH), which is the only enzyme capable of performing this reaction, preferring the reaction of methylation. SAHH has been reported to contribute to the bypassing of the proliferation arrest induced by p16^{INK4} after its downregulation (81). Since the activity of SAHH is closely related to its level, the diminished activity of the enzyme must be attributable to methylation (82). The importance of epigenetic modifications performed by SAHH is highlighted by reports suggesting that a lack of the enzyme seems to be lethal (83). In summary, the close relationship between the activity of SAHH, an enzyme playing an important role in the general methylation reaction, and senescence points to an important role of epigenetics in this process. Therefore, epigenetic modification may be significant for cellular aging, although relationship between SAHH activity and senescence requires further studies.

Senescence may be also influenced by epigenetic modification of histones. SUV39H1 is a human homolog of the *Drosophila* Su(var)3-9 protein responsible for position-effect variegation. This protein displays selective methyltransferase activity and methylates lysine-9 of the H3 histone (84). Cell with increased expression of full-length SUV39H1 are characterized by having serious disturbances in the progression of mitosis and the segregation of chromosomes. During interphase, SUV39H1 is preferentially distributed within heterochromatic foci and is located within the centromeric region during metaphase (85). Thus, SUV39H1 may take part in both epigenetic regulation of cell division and maintaining the structure of higher order chromatin and be involved in cellular senescence (86). As mentioned earlier, telomere length is a crucial factor in senescence, and there is evidence that mice lacking homologs of SUV39H1 display changes in telomere length and functions, which supports the involvement of the protein in senescence.

Apart from the HDACs, there is another considerably different, class of histone deacetylases: sirtuins (87). The involvement of the sirtuin proteins in the aging process has been well documented, especially for Sirt1, the most extensively studied member of the sirtuin family (88). It is believed that the Sirt1-mediated deacetylation of many proteins, including p53 and survivin, is involved in cellular senescence (89, 90).

In general, the human genome is gradually demethylated with age [91] that may inhibit transcription of certain genes due to hypomethylation of the gene promoters. The most convincing supports for this thesis comes from research performed on long iterspersed nuclear elements (LINE), including LINE-1 repeats, and short interspersed nuclear elements (SINE) present in the human genome, including Alu repeats (92). Repetitive element methylation was claimed to correlate with total genomic content (93). Although the genome is progressively hypomethylated with age, it seems that CpG islands may be, at least in part, excluded from this process and they may become progressively hypermethylated (94).

In summary, there is a growing body of evidence for the involvement of epigenetics in the process of cellular senescence and more generally in the process of aging. Interestingly, age-related changes in DNA-methylation patterns in the promoters of some genes have been reported to be similar to those found in the diseased tissue from younger individuals (95). This is especially manifested in age-related diseases and cancer, but with the exception of AMD, this issue is beyond the scope of this review.

5. EPIGENETICS IN EYE DEVELOPMENT AND DISEASES

The eye of *Drosophila* is probably the most widely studied visual organ and its development clearly reveals the involvement of epigenetic regulatory mechanisms (96). However, there are considerable differences in the general mechanisms of eye development in *Drosphila* and vertebrates, so we will not focus on this topic in the present review.

The vertebrate eye is produced from cells originating from the neuronal ectoderm, giving rise to the retina and epithelium of iris, whereas lens progenitor cells and corneal epithelial cells are provided by surface ectoderm. The crucial stages in the formation of ocular progenitor cells and their subsequent proliferation and differentiation are regulated by specific transcription factors (TFs). The list of TFs genes which may be important for eye development, includes over 50 genes, but surprisingly, no truly eye-specific TF has been identified (97). The functions of these genes related to the lens and retina are controlled by 7 transduction pathways: FGF/MAPK/Ras, JAK/STAT, Notch, nuclear receptors, Shh, TGF- , and Wnt as well as through the interaction between some co-expressed DNA-binding TFs (97). Extracellular signals control the expression of these TFs and their epigenetic, post-translational modifications, including acetylation, glycosylation, phosphorylation, sumoylation, and ubiquitination.

A collective epigenetic silencing of entire groups of genes at the terminal step of differentiation may account for the differentiation of precursor cells into either rod or cones (98). Although both undifferentiated and differentiated cells possess a "cell memory" of their identity and this is required for the maintenance of the differentiated state and for the pluripotency of uncommitted cells, some ocular cells, including RPE cells, can "transdifferentiate" into lens cells (99). Homeostasis of both embryonic and adult stem cells is governed by precise epigenetic regulatory mechanisms (100).

Epigenetic mechanisms have been shown to play an important role in lens development (101). Lens vehicles are formed by the proliferation and morphogenetic movements of lens progenitors. The structure formed by the vehicles is polarized with its anterior cells, but the posterior progenitors initiate terminal differentiation, forming the primary lens fibers. The expression and accumulation of crystalline and other structural proteins characterizes the differentiation of lens. The expression of

the crystalline gene is controlled mainly at the transcription stage by the interaction of general and lens-specific transcription factors and the recruitment of HATs, CBP and p30 as well as chromatin remodeling complexes SWI/SNF and ISWI.

As described in the previous section, the epigenetic pattern may be age-related and can influence several eye diseases. It has been hypothesized that environmental factors influence DNA methylation of the genes required for normal visual system, but DNA methylation may be altered during development and aging process and the accumulation of these alterations may finally leads to different eye diseases (102-106).

6. EPIGENETICS AND AMD

AMD is often referred to as a complex disease, with many susceptibility genes and environmental effects, i.e. the pathological phenotype inherited in a non-Mendelian fashion with increasing risk as one ages. The susceptibility genes do not seem to interact additively or epistatically, highlighting the truly complex character of this disease (107, 108). The importance of genetic factors in AMD pathogenesis is now well established and the main candidate genes have been identified at chromosome regions 1q31-32 and 10q26, accounting for more than half of the genetic risk associated with this disease (18, 25, 109). Apart from these two regions, many genes in the nuclear genome and some variants in the mitochondrial genome have been reported to be associated with the AMD risk (110). As mentioned, genetic factors interact with certain environmental conditions in AMD pathogenesis. However, the impact of environmental factors seems to depend on the individual. In other words, an individual may or may not have mutation(s) in gene(s) identified as risk factors for AMD. Therefore, his/her genetic susceptibility to AMD can be determined to some extent. On the other hand, an individual can be exposed to recognized AMD environmental factors. The outcome of these factors exposure would be determined by genetic (mutations) and epigenetic properties of the genes (111, 112). Therefore, the total AMD risk is exerted through an array of many genes, which can contribute to the direct susceptibility to this disease and can modulate the action of environmental factors. For instance, iron function as a catalyst of the Fenton reaction producing ROS, which may play an important role in AMD pathogenesis. There is a concept of biochip, which would assess the significance of variability of iron homeostasis genes in AMD (113). However, the function of the gene is determined also by epigenetic mechanisms, which could account for the so called *missing heritability* involved in the discordant phenotypes of monozygotic twins with AMD (110). This can be explained by epigenetic drift, spontaneous change of cellular methylation patterns in time, which occur both in dividing and non-dividing cells (114, 115).

Since AMD affects the central part of the retina, its genesis may be associated with some general developmental processes in this organ. Retinal development is a precisely regulated process in which retinal progenitor cells generate a set of glia and at least seven types of retinal neurons in an ordered sequence (116). The precise way of generating neurons and glia is ensured by histone lysine methylation at the K4, K9 and K27 residues, which is be mono-, di- or trimethylated (Figure 2; 117). Moreover, this epigenetic mechanism accounts also for the ability of neural progenitor cells to self-renew (118). Despite the well established role of epigenetic regulation in the development of the retina, little is known about the precise mechanisms of retinal histone lysine methylation performed by HMTs. Since our knowledge on the AMD pathogenesis is not complete, it is impossible to exclude the possibility that this disease is initiated during the stage of retinal development, in which histone lysine methylation may still play an important role. Therefore, one could hypothesize that aberrant histone methylation may result in abnormal retina development and create a background for the appearance of pathological conditions associated with AMD. The disruption of the function of the HMT providing 3 methyl groups to lysine-9 of the H3 histone in zebrafish morpholinos resulted in improper maturation of the retinal cells (119). Although the main impact of epigenetic modifications is expected in embryonic retina, H3K4me3 and H3K27me3 modifications are reported to persist in adult retina (120). Some of histone methylation marks are lost during development via several mechanisms. First, a mark is "diluted" during DNA replications in mitotic cells (121). Second, enzymatic demethylation removes the mark (122). Third, the decreased expression of HMTs contributes to this effect (123). In addition, an important regulatory mechanism of gene transcription is tri-methylation of lysine 27 on H3K27me3, which is catalysed and maintained by Polycomb Repressive Complex 2 (PRC2). There are distinct profiles of H3K27 that correlate with its transcriptional activity. The H3K27me3 has inhibitory effects to transcription, it marks transcription start site and has enrichment profile with a peak in the gene promoter and is associated with active transcription (124). Genes with any of these profiles are cell type dependent. Irrespective of the mechanism, disturbed epigenetic processes may result in disturbed retinal development and support the appearance of pathological conditions. This may be especially interesting in AMD cases with a family history.

As mentioned above, Sirt1 is a deacetylase involved in several physiological processes including development and aging. In an attempt to clarify the role of this protein in retinal degeneration, a retinal degeneration 10 (rd10) mouse model of retinitis pigmentosa has been devised (125). A distinct difference was observed in the immunolocalization of Sirt1 between control and rd10 retinas and the pathological pattern of immunoreactivity correlated with the beginning of retinal degeneration. The results obtained suggest an association between Sirt1 expression and retinal degeneration. The protective neural effect of Sirt1 in mouse retina and its anti-apoptotic effect are underlined by its involvement in the DNA damage response via the regulation of p53 and interaction with NBS1, a component of the MRN complex consisting of MRE11, RAD50 and NBS1 proteins, which play an

important role in the repair of DNA double strand breaks (126-129). Interestingly, we previously demonstrated a decreased efficacy of DNA repair in peripheral blood lymphocytes of AMD patients as compared with age-matched controls (130). This kind of general decrease in the DNA damage response may affect also the retina, where it could be potentiated by epigenetic modifications. It is possible, that the Sirt1-mediated effect on the response of the retina cells to DNA damage is accentuated by the remodeling of chromatin induced by this damage (125).

The clinical hallmark of AMD are drusen, consisting of round shaped and yellowish extracellular deposits of degenerative material. Clinically, hard drusen, i.e. drusen which have clear margins, are the earliest sign of AMD and represent a major risk factor for the development of AMD complications (131). Local inflammation play an important role in the formation of drusens and inflammation is also known to be associated with AMD (132). Clusterin (apolipoprotein J) is a multifunctional chaperone and one of the major proteins present in drusen (133,134). However, the precise role of clusterin in drusen formation has not yet been established nor has the regulation of its expression in the retina. However, its gene is known to contain CpG islands in its promoter. During our investigation of the role of epigenetic regulatory mechanism in AMD pathogenesis, we examined whether the induction of DNA hypomethylation with 5-aza-2'-deoxycytidine (AZA) and histone hyperacetylation with trichostatin A (TSA) could affect clusterin gene transcription and the level of its protein in retinal pigment epithelium, ARPE-19, cells (134). Treatment with either AZA or TSA induced a significant increase in the clusterin mRNA and protein levels. Furthermore, valproic acid, an HDAC inhibitor, also induced an increase in the clusterin expression and secretion in RPE cells. Thus, aging associated hypomethylation might increase clusterin secretion and this could be a triggering factor in drusen biogenesis and AMD advancing (135). Since HDAC inhibitors are known to decrease the process of angiogenesis, which can be also inhibited by clusterin, our results suggest that epigenetic regulatory mechanisms may play an important role in the pathogenesis of AMD through the inhibition of angiogenesis and inflammation. However, these results should be carefully interpreted, since histone modifications and variants contribute to diversification of a chromatin landscape shaped by dynamic processes that are driven primarily by transcription and nucleosome remodeling. It is not completely clear whether histone modifications are responsible for differences between chromatin states, or are differences in modifications mostly consequences of dynamic processes, such as transcription and nucleosome remodeling (136).

7. SUMMARY AND PERSPECTIVE

AMD is a disease for which there is no efficient cure and which affects an increasing fraction of population. Therefore, efforts should be aimed at understanding mechanisms involved in its pathogenesis of the disease, since this would help to improve its diagnosis and therapy (137). AMD is a complex disease underlined by the complex interaction between many genetic and environmental factors, and it offers many potential treatment opportunities, both with single and combined therapy. The genetic element in AMD pathogenesis is usually determined statistically, i.e. specific sequence of a fragment of the genome is correlated with AMD risk. However, the DNA sequence is generally the same in all cells of the organism, but there are striking differences between the functions performed by the cells in different organs and tissues. This is due to different patterns of gene expression in different tissues at various developmental stages. This expression depends not only on the DNA sequence, but on different modifications directly or indirectly related to the DNA molecule, collectively described as epigenetics (137,138). This new appreciation of the importance of epigenetic modifications in AMD pathogenesis has opened new perspectives for the treatment of the disease through changes in the epigenetic pattern. Today, several epigenetically-oriented anticancer drugs have been already approved by the FDA and the EMEA for cancer treatment and several other clinical applications of such drugs are being developed (139). This strengthens the need and hope that it will be possible to achieve successful targeting of epigenetic modifications in other diseases. Therefore, further studies on the involvement of epigenetic mechanisms in retinal degeneration are needed. These studies should be initially aimed at those genes which are associated with AMD risk. Combining information on genetic and epigenetic properties of an AMD susceptibility locus may help to devise a treatment focusing on this locus. This treatment could well be physical, pharmacological or genetic and it may involve the epigenetic modifications themselves or it may target some protein involved in the establishment or erasure of these modifications. Thus, Sirt1, a histone deacetylase playing an important role in the development of age-related maculopathy, could be considered as one possible promising target.

8. ACKNOWLEDGEMENTS

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Key Words: Aging, Degeneration, Epigenetics, Macula, Retina, Review

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