REVIEW

Роль механизмов «метаболической памяти» в развитии и прогрессировании сосудистых осложнений сахарного диабета

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Изучение сахарного диабета (СД), его осложнений и смежных патологий непрерывно ведется в течение многих лет. Однако несмотря на большую работу и выдающиеся достижения в изучении механизмов развития СД, а также успехи в разработке новых лекарственных препаратов для контроля гликемии, проблемы, связанные с поздними осложнениями СД, нарастают. Значение гликемического контроля на ранних стадиях СД для развития осложнений проявляется только через достаточно длительный период наблюдения. Такой отсроченный эффект первичного хорошего или неудовлетворительного метаболического контроля, во многом формирующий клиническую судьбу пациента, определяют термином «метаболическая память». Развивающиеся под воздействием гипергликемии нарушения сохраняются длительное время после нормализации показателей углеводного обмена, а эффект предшествующей гипергликемии растягивается на следующие 20 и даже 30 лет. Предметом изысканий в настоящее время является изучение возможных механизмов развития метаболической памяти, в том числе окислительного стресса, конечных продуктов гликирования и эпигенетические механизмы. Их исследование позволит определить потенциальные маркеры раннего развития и прогрессирования сосудистых осложнений, а в перспективе, и новых терапевтических возможностей. Однако более важным является определение вероятной «точки невозврата», которая подразумевает под собой грань, переступая которую, остановить прогрессирование сосудистых осложнений СД не представляется возможным. Результаты многочисленных экспериментальных исследований демонстрируют предпосылки для использования компонентов «метаболической памяти» в качестве потенциальных маркеров прогрессирования осложнений СД, а также в качестве потенциальных терапевтических стратегий таргетного воздействия.

***Ключевые слова**: сахарный диабет; метаболическая память; окислительный стресс; эпигенетика; конечные продукты гликирования*

The role of «metabolic memory» mechanisms in the development and progression of vascular complications of diabetes mellitus

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The study of diabetes mellitus (DM), its complications and related pathologies has been continuously performed for many years; however, despite the substantial work and outstanding achievements in studying the mechanisms of DM development and the success of new medicinal products for controlling glycaemia, the problems associated with the late complications of DM continue to increase. The importance of glycaemic control in the early stages of DM for the development of complications is seen only after a sufficiently long period of observation. Such a delayed effect of primary good or unsatisfactory metabolic control, which shapes the patient's clinical fate to a greater extent, is termed 'metabolic memory'. The disorders developed under the influence of hyperglycaemia persist for long periods after the normalisation of carbohydrate metabolism; moreover, the effect of previous hyperglycaemia extends over the next 20 and even 30 years. Current research is focused on the possible mechanisms of metabolic memory development, including oxidative stress, advanced glycation end products and epigenetic mechanisms. This research will provide insight into potential markers for the early development and progression of vascular complications and new therapeutic possibilities for the future. However, determining the probable 'point of no return' is more important, which implies that a point exists; after this point is crossed, the progression of vascular complications associated with DM cannot be prevented or reversed. The results of numerous experimental studies demonstrate that the prerequisite components of metabolic memory can be used as potential markers of the progression of DM complications, and may be potential therapeutic targets.

***Key words:** diabetes mellitus; metabolic memory; oxidative stress; epigenetics; advanced glycation end products*

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According to the International Diabetes Federation, diabetes mellitus (DM) affects 415 million people (1 in every 11), a number that will ultimately increase to 642 million (almost 10% of the total population) by 2040 [1]. Despite the progress in understanding the developmental mechanisms of DM and the remarkable results in the development of new medications for glycaemic control, problems associated with diabetes are still increasing. Social and economic encumbrances determined by the development of micro- and macrovascular complications are one such example. The existence and progression of specific microvascular complications (retinopathy, nephropathy and neuropathy) are associated with the quality of glycaemic control, whereas those of macrovascular complications are associated with a comprehensive programme to reduce cardiovascular risk, which includes quitting smoking, glycaemic and blood pressure control, lipid lowering and antiplatelet therapy. This is important because cardiovascular diseases remain the leading cause of death in patients with diabetes. Compared with those without diabetes, the relative risk for cardiovascular events and mortality in adults with DM is one to three and two to five in men and women, respectively [2].

The impact of glycaemic control on the development of complications during the early stages of DM is manifested only after a sufficiently long follow-up period. Such a delayed effect of careful primary metabolic control, which determines to a great extent the patient's clinical outcome, is defined by the term 'metabolic memory' or 'heritage effect'. This concept is applicable to all microvascular complications for which the benefits of stable metabolic control become apparent after at least 10 years. The DCCT/EDIC study has demonstrated the greater advantageous effects of better glycaemic control for retinopathy, nephropathy and autonomic neuropathy in patients with type 1 diabetes (DM1) [3]. Compared with the traditional one, the primary prevention cohort had a 76% reduction in the risk of retinopathy development after intensive therapy for more than 6 years, while the second cohort (secondary prevention) had a 54% decrease in the indicator, with a corresponding 47% decrease in the risk of proliferative and pre-proliferative retinopathy. The intensive therapy group had a 39% and 55% decrease in the development of microalbuminuria and proteinuria, respectively, compared with the traditional therapy group. Similar results were obtained for other complications. Advantages were also observed in the EDIC observational study despite the absence of differences in glycated haemoglobin [3, 4].

Further observation (EDIC 30) for up to 30 years showed that despite the alignment of glycated haemoglobin in both groups, the protective effect of previous good glycaemic control against the risk of microvascular complications persisted. The concept of 'metabolic memory' turned out to be applicable to macrovascular complications, which was estimated by measuring intima-media thickness and coronary calcium. Moreover, the intensive control group showed a significant 58% reduction in the risk of cardiovascular events (fatal and nonfatal heart attacks and strokes) after 18 years of follow-up [3, 4].

Thus, the positive 'metabolic memory' mechanism provided protection against the development of vascular complications almost two decades after the completion of the DCCT study. In other words, the risk of complications in patients with DM1 over a given period depends on their glycaemic control during the preceding 10–20 years.

Further development of this concept was observed in the ADVANCE and ACCORD studies. The former revealed a significant reduction in the risk for terminal renal failure development in patients with type 2 diabetes (DM2) who received intensive antihyperglycaemic gliclazide therapy during the study, without evidence for an increase or decrease in the risk for cardiovascular events or death [5, 6].

The ACCORD study, which included patients with prolonged DM2 and high cardiovascular risk, demonstrated that intensive glycaemic control for 3.7 years had a neutral effect on major cardiovascular endpoints. Meanwhile, an increase in overall and cardiovascular mortality led to the early termination of randomised glycaemic control. However, improvements in retinopathy and other microvascular endpoints had already become apparent in the intensive therapy group. Notably, total mortality did not significantly increase after 9 years of follow-up, whereas cardiovascular mortality with less progress remained significant [6].

The researchers concluded that intensive glycaemic control did not show cardiovascular benefits for those with long-term DM2 and high cardiovascular event risk. However, these conclusions cannot be generally accepted, given that the antihyperglycaemic intervention in the ACCORD study was extremely intensive [6].

Thus, long-term studies support the 'heritage effect' concept of early intensive glycaemic control for the prevention of micro- and macrovascular complications, in case these were absent during the early stages of DM monitoring. However, the latter statement was somewhat deterred by the results of the EMPA-REG OUTCOME study, which unexpectedly showed an improvement in cardiovascular benefit for patients with long-term DM2 and high cardiovascular event risk who received empagliflozin, an SGLT-2 inhibitor [7]. The study demonstrated a 14% decrease in the risk for combined endpoints (cardiovascular death, nonfatal infarction and nonfatal stroke) and a 35% decrease in hospitalisations due to heart failure. Certainly, it has been suggested that glycaemic control with empagliflozin alone cannot fully account for such results. It is important to consider the effects of weight loss and haemodynamics, which is determined by a decrease in extracellular volume and blood pressure.

Thus, the effect of early intensive DM treatment, which is aimed at achieving target glycaemic parameters, significantly exceeds that of late compensation for carbohydrate metabolism after a long period of unsatisfactory glycaemic parameters. Current extensive research has focussed on possible mechanisms for the

development of 'metabolic memory', which would identify both potential markers for the progression of vascular complications and new therapeutic possibilities, and the definition of a possible 'point of no return' wherein the progression of vascular DM complications would be impossible to stop.

OXIDATIVE STRESS

Hyperglycaemia affects the tissues through five main mechanisms: increased glucose flow through the polyol pathway, increased intracellular formation of the end products of enhanced glycation, increased receptor expression to the final products of enhanced glycation and their activating ligands, activation of protein kinase C and increased hexosamine pathway activity. Some studies have shown that all aforementioned mechanisms activate oxidative stress. Normally, free oxygen radicals (FORs), which can act as redox signal transmitters and disrupt normal cell signalling, are continuously formed during the metabolism of cells. Free radicals include superoxide (O2-), HOCl, NO and ONOO. Superoxide has been suggested to initiate this process, given that it can be converted into more reactive forms of FOR after its formation in mitochondria. Under pathological conditions, such as exposure to persistent hyperglycaemia, the balance between the production and detoxification of FOR is disturbed, leading to an imbalance in the system. Superoxide, a molecule that interacts with proteins, lipids and nucleic acids in a span of just min, leads to the formation of molecules that have a half-life much longer than that for superoxide. A modification in these molecules can lead to prolonged cellular dysfunction, which partly explains the phenomenon of metabolic memory [8]. Excessive production of free radicals, stimulated by hyperglycaemia, has been shown to remain under experimental conditions even after normalisation of glycaemia, accompanied by the induction of protein kinase C, NAD(P)H oxidase, collagen, fibronectin and 3-nitrotyrosine [9].

Mitochondria have their own DNA, which, unlike the nuclear DNA, is stored in the form of an open chromatin. The open structure of the DNA increases its sensitivity to the damaging effects of FOR [10]. Hyperglycaemia disrupts the formation of ATP in the mitochondria through the electron transport chain, which transports electrons to oxygen-like molecules (O2), leading to the formation of superoxide (O2-) and other FOR. FOR can interact with mitochondrial proteins, including those within the electron transport chain, and disrupt their function. Hydrogen peroxide and peroxynitrite (ONOO-) can pass through membranes and damage molecules in other areas of the cell [11].

Zheng et al.'s experiment demonstrated that in both the cell culture and animal model, the hyperglycaemia-induced increase in the levels of Bax protein and nuclear factor κB persisted even after normalisation of glycaemia. Moreover, hyperglycaemia was found to induce the activation of poly(ADP-ribose)-polymerase, which can

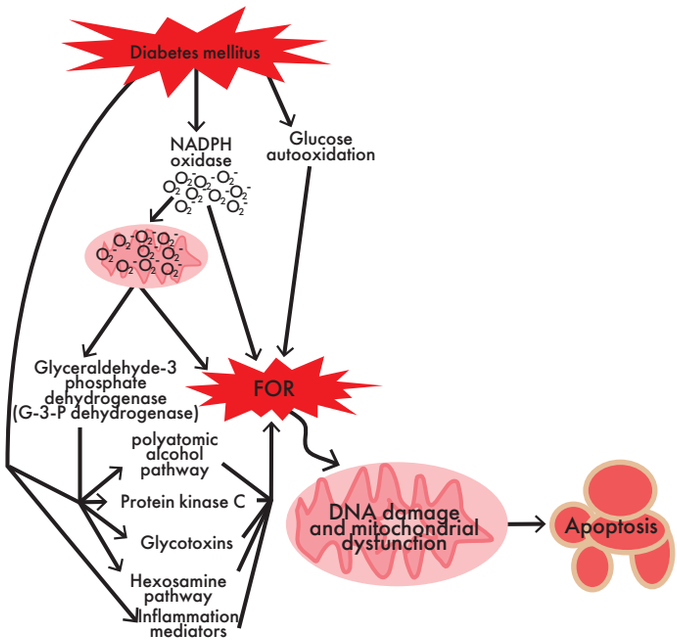

Fig. 1. Role of oxidative stress in the development of mitochondrial dysfunction in DM (adapted from [13]).

in turn inhibit sirtuin 1 (SIRT1). This causes a potential feedback loop that enhances the production of FOR in the mitochondria, which in turn exacerbates the effect of oxidative stress [12].

Summarising the information, one can suggest a positive feedback: free radicals formed during hyperglycaemic conditions disrupt mitochondrial mechanisms, thereby strengthening their own formation (Figure 1) [13].

After nitrogen radicals, including ONOO- and nitrogen dioxide, react with tyrosine residues, a nitro group is added to these residues, forming 3-nitrotyrosine, the final product of the reaction between ONOO- and free tyrosine or proteins containing tyrosine. Elevated 3-nitrotyrosine levels serve as a marker of oxidative stress in DM complications. The formation of 3-nitrotyrosine in enzymes, including Ca2+-ATP-ase of the sarcoplasmic reticulum, manganese superoxide dismutase, protacycline synthase, tyrosine hydroxylase and aldolase A, leads to the suppression of their normal function. Moreover, 3-nitrotirozin disturbs mitochondrial function in the myocardium [14], while protein and lipoprotein nitration can play a direct pathophysiological role in the development of atherosclerosis. For example, nitrated low-density lipoproteins (LDLs) absorbed by macrophages lead to the accelerated formation of foam cells [8].

Thus, Kowluru et al. (2007) showed that after 6 months of hyperglycaemia followed by 6 months of good glycaemic control, rats with Streptozotocin-induced DM had no significant reduction in retinal 3-nitrotirozine levels compared with those having poor glycaemic control throughout the study. Similar results were obtained for caspase-3-induced hyperglycaemia and nuclear factor κB [15].

Furthermore, the experiment demonstrated that the normalisation of carbohydrate metabolism indices in rats with induced DM after a certain period of decompensation did not lead to a significant improvement (markers of oxidative stress remained elevated in both urine and kidney cortical substance) [16].

Thus, oxidative stress, which disrupts fundamental cellular functions, is one of the essential pathophysiological processes involved in the formation of DM complications. Such complications persist for a long period even after the normalisation of carbohydrate metabolism indices, reflecting the phenomenon of metabolic memory in the development of vascular DM complications.

FINAL GLYCATION PRODUCTS

Glycotoxins (AGEs) are formed during the Maillard process, a non-enzymatic reaction between carbohydrates and the amino group of proteins, lipids and nucleic acids. During hyperglycaemia and/or oxidative stress, this process begins with the conversion of reversible Schiff basic adducts into more stable, covalently bound products of the Amadori reorganisation. After a while, the Amadori products are converted into fluorescent secondary macroproteins called glycotoxins. These glycotoxins then slowly decompose and remain in the vessels for a long period, even after euglycaemia has been achieved [17].

The accumulation and prolonged existence of AGEs formed during hyperglycaemic conditions can be one of the very important factors involved in metabolic memory. AGEs directly induce the crosslinking of long-lived proteins, such as collagen, which aggravates the stiffness of the vascular wall, thus contributing to the progression of micro- and macrovascular complications of DM [18].

AGEs have their own receptor (RAGE), which is located on the cell surface in a free state and belongs to the superfamily of immunoglobulins. RAGEs are direct signal transmitters for AGE. RAGE and AGE binding has been repeatedly shown to promote the progression of oxidative stress [19], as well as an increase in the level of the main pro-inflammatory and prosclerotic cytokines. Moreover, AGE formation can cause irreversible modification in mitochondrial proteins, which contributes to mitochondrial dysfunction through excessive free radical formation [18].

Given that continuous RAGE activation is one of the mechanisms of 'metabolic memory', the development of metabolic disorders can be attributed to persistent inflammation in combination with oxidative stress, in which the AGE–RAGE axis plays one of the fundamental roles. Moreover, soluble RAGE (sRAGE) and endogenously secreted RAGE can serve as biomarkers and therapeutic targets for the prevention of vascular DM complications, particularly chronic kidney disease [20].

The blockade of RAGE through the administration of genetically engineered sRAGE successfully prevented the development of micro- and macrovascular diabetes complications [21].

AGE-modified proteins not only increase the density of the vascular wall and myocardium but also lead to various organ system dysfunctions, including those involved in the pathogenesis of isolated systolic hypertension and diastolic heart failure. AGEs inhibit the expression of endothelial nitric oxide synthase in endothelial cells while stimulating the production of ONOO- [22, 23].

During LDL glycation, their clearance is decreased, and the normal metabolic pathway is disrupted. This leads to an increase in the LDL lifespan and the formation of foam cells [24]. Accordingly, AGEs inhibit the release of cholesterol from the macrophages into apolipoprotein (apo) AI and high-density lipoproteins. This confirms the role of AGEs and their receptors in cholesterol transport dysfunction and acceleration of foamy cell formation inside atherosclerotic plaques [25].

The interaction between glycotoxins and their receptors suppresses Akt protein kinase and cyclooxygenase-2, which consequently accelerates apoptosis and inhibits the migration and formation of a tube of endothelial progenitor cells [26]. Blocking this interaction leads to the propagation and migration of progenitor cells through glycation of the Arg-Gly-Asp fibronectin sequence, which causes a disorder in the vascular reducing ability [27].

Moreover, although one of the glycation products, glycated haemoglobin A1c, was revealed to have been partially enzymatically deglycosylated, this was not observed in other AGEs. The formation of mitochondrial AGEs could perhaps be an irreversible phenomenon and may partly be responsible for the prolonged nature of 'metabolic memory' through the formation of excessive amounts of reactive oxygen species. This can in turn lead to catastrophic damage to mitochondrial DNA and the inhibition of respiratory chain function, which increase the damaging effects of oxidative stress on the cells, triggering a glucose-independent cascade of reactions that contribute to the progression of diabetes complications within the framework of 'metabolic memory' [28].

EPIGENETIC MECHANISMS

Epigenetic mechanisms include post-translational histone modifications, changes in the access to chromatin due to DNA methylation and control of gene expression by non-coding microRNAs. Together, the above processes allow cells to quickly react to and 'remember' environmental changes when stimuli influence termination. In particular, epigenetic changes in DNA/histone complexes are important modulators of inflammatory and oxidative genes, thus leading to continuous oxidative stress and endothelial dysfunction. Consequently, possible epigenetic changes that develop under hyperglycaemic conditions are of great interest particularly in the study of the 'metabolic memory' phenomenon in DM (Fig. 2).

HISTONE MODIFICATIONS

The nucleosome, which is the structural unit of chromatin, consists of 147 DNA nucleotide pairs around

histone octamers (two copies of H2A, H2B, H3 and H4). More than 100 post-translational histone modifications are methylated and/or acetylated for lysine residues. Acetylation neutralises the positive charge of lysine residues, which strongly affects chromatin structure, and is associated with transcriptional activation of the genes. Initial studies on epigenetic modifications and changes in gene transcription in DM focussed on acetylation and methylation.

A single histone modification has no significant effect on the regulation of gene transcription, which depends on the sum of all histone modifications in this locus. To understand the epigenetic control of altered persistent gene expression and 'metabolic memory' in DM, a complete chromatin profile at each locus must be identified. This was shown in a study by Zhong and Kowluru [30], in which the authors tested histone modifications on retinal manganese superoxide dismutase and Sod2 (type 2 superoxide dismutase gene) and found a decrease in its expression in response to hyperglycaemia. The study was performed on mice with Streptozotocin- or galactosis-induced DM. The first group of mice displayed poor glycaemic control for the first 4 months, followed by 4 months of good glycaemic control. The second group had 2 months of hyperglycaemia and 2 months of euglycaemia (formation of metabolic memory). An increase in H3K9 acetylation and p65 transcription factor recruitment in its promoter/enhancer was found. Increased trimethylation of H4K20 me3 (a repressive histone modification) was also detected at the Sod2 locus, which may presumably overlap with the activating modifications. The third histone deacetylase class is SIRT1, a multifunctional protein that deacetylates not only histone tails but also many nonhistone substrates, including transcription factors and co-regulators [31]. SIRT1 can regulate many cellular processes, including inflammatory response and FOR levels. This led Zheng et al. [32] to the assumption that SIRT1 can play a significant role in metabolic memory. The authors investigated this hypothesis by incubating bovine retinal endothelial capillaries under normal glucose levels for 3 weeks, acute hyperglycaemia for 3 weeks or hyperglycaemia for 1 week and normoglycaemia for 2 weeks (modelling the 'metabolic memory'). They also examined the retinas from normal mice, those with Streptozotocin-induced DM without insulin therapy for 6 weeks (hyperglycaemia group) and those with DM without insulin therapy for 2 weeks who then received insulin for the next 4 weeks (metabolic memory group). SIRT1 expression and activity decreased in response to hyperglycaemia, which was probably irreversible with 'metabolic memory'. Moreover, p65 experiments revealed that nuclear factor κB and apoptosis factor Bax (the gene that triggers cell apoptosis) were increased due to hyperglycaemia-induced FOR and remained elevated even after normoglycaemia was achieved. The study showed SIRT1's ability to deacetylate and stimulate AMP-activated protein kinase, which in turn activates manganese superoxide dismutase and uncoupling protein 2. Together, they catalyse the reduction in FOR. Elevation of SIRT1 expression or activation with metformin decreased FOR, the nuclear factor and Bax expression. Vahtola et al. [33] showed that during hyperglycaemia, mice with diabetes had elevated SIRT1 levels in their cardiomyocytes. All of these suggest tissue-specific epigenetic control and the importance of determining the epigenetic profile in all tissues to determine the possibility of a therapeutic effect.

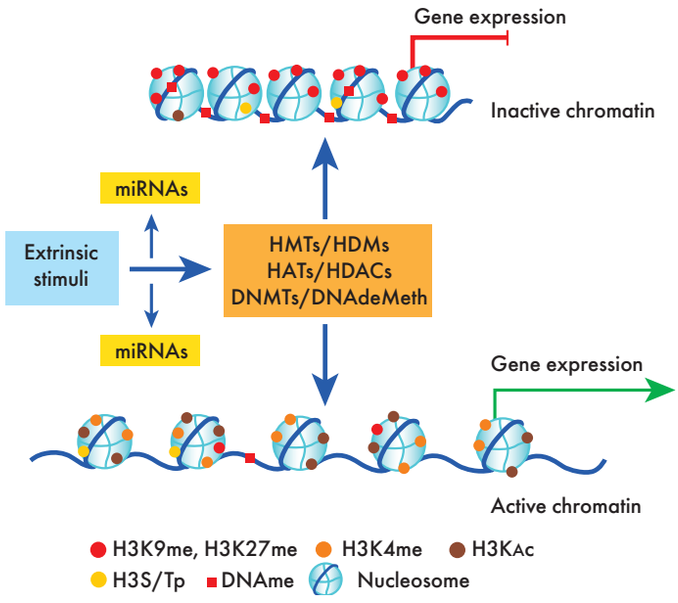

Fig. 2. Epigenetic mechanisms of gene expression regulation (adapted from [29]).

Manipulations in epigenetic mechanisms were investigated in the context of diabetic nephropathy. Epigenetic modifications are believed to precede and promote increased albuminuria (the first clinical sign of kidney damage), podocyte loss, glomerular hypertrophy and growth of the mesangial matrix. The epidermal growth factor and its receptor are involved in hyperglycaemia-induced mesangial expansion [34–36]. Together, these factors led Gilbert et al. [37] to conduct a study on histone deacetylase inhibitors, such as Vorinostat, as a possible means of inhibiting kidney hypertrophy during hyperglycaemia. During the cultivation of proximal tubule cells with Vorinostat, the epidermal growth factor receptor and its mRNA decreased, while cell proliferation was suppressed. Moreover, daily use of Vorinostat for 4 weeks in rats with Streptozotocin-induced DM significantly reduced renal and glomerular hypertrophy.

MICRORNA

MicroRNA is a family of short (19–24 nucleotides in length), non-coding single-stranded RNA molecules that regulate gene expression by attaching to specific sites on the 3′-untranslated region (3′-UTR) of the targeted matrix RNA (mRNA), leading to repression of translation or degradation of the mRNA [38]. Each microRNA can regulate up to 200 mRNA individually or in combination with other microRNAs. These molecules can control the expression of more than 60% of the genes encoding proteins [39].

Some studies have confirmed that microRNAs contribute to diabetic nephropathy. Long et al. identified 10 microRNAs whose activity is selectively increased in the renal glomeruli of mice with the db/db genotype, renal vascular endothelial cells and podocytes during hyperglycaemia [40]. Convincing evidence showed that one of these microRNAs, miR-29c, specifically affects Sproutyhomolog 1 (Spry1) and inhibits its synthesis in response to hyperglycaemia. This is critically important for diabetic nephropathy, because SPRY1 suppresses Rho-kinases whose activity is associated with diabetic nephropathy through the induction of podocyte apoptosis and the synthesis of mesangial fibronectin. This study used chemically modified technologies for knocking out microRNA29c expression, which correlated with improved albuminuria in db/db mice. Dey et al. observed that hyperglycaemia induces microRNA-21 expression, which in turn inhibits the expression of PTEN (an oncogenesis suppressor gene), triggering mesangial hypertrophy and excess fibronectin [41].

It was shown that 3′-UTR transforming growth factor-β2 (TGF-β2) includes a target region for the microRNA-141/200a family. Moreover, this study showed that increased ectopic expression of miRNA-141 or miRNA-200a decreased TGF-β2 expression, possibly lowering the synthesis of extracellular matrix genes in vitro. As additional evidence of the involvement of the microRNA141/200a family in diabetic nephropathy, the authors showed that the levels of these microRNAs were reduced in the renal cortex of apoE mice with diabetes. Together, these data confirm that microRNA-200a plays a central role in extracellular matrix accumulation in diabetic nephropathy [42].

Natarajan et al. conducted a study on increased mRNA levels in the context of diabetic nephropathy and provided evidence for the possible modulation of microRNA expression therein [43]. Their previous work had shown that microRNA-192 can be the main regulator in diabetic nephropathy as it precedes the cascade of events leading to glomerular hypertrophy. This microRNA suppresses E-box repressors, such as Zeb1 and Zeb2, in mesangial cells and glomeruli of mice with hyperglycaemia [44]. Given that some E-boxes are found in regions preceding the extracellular matrix protein promoter (collagen I 2a and collagen IV a1), TGF-β, a connective tissue growth factor, fibronectin and other microRNAs (microRNA-216a/217 and microRNA-200 family) that modulate microRNA-192 levels can have pleiotropic effects. Putta et al. [43] tested the effectiveness of using microRNA-192 inhibitors modified with locked nucleic acids (LNAs) on murine models of diabetic nephropathy. The use of LNA-modified anti-microRNA-192 (for 17 weeks from the time of DM manifestation) significantly decreased microRNA-192 levels in the kidneys of mice with DM and those in the control group. Moreover, a concomitant increase in Zeb1/2 was achieved, which reduced the expression of gene collagen, TGF-β and fibronectin. From the diabetic nephropathy standpoint, the treatment of DM with LNA-modified anti-microRNA-192 reduces proteinuria. This study revealed that pharmacological intervention aimed at this epigenetic mechanism can be used for the treatment of diabetic nephropathy in the future.

Feng et al., after culturing human umbilical vein endothelial cells, revealed an increase in fibronectin expression with a simultaneous decrease in microRNA146a expression [45]. The 3′-UTR mRNA sites of fibronectin include target sites for microRNA146a. This was shown in transfection studies that involved microRNA146a binding and its control over fibronectin expression. A decrease in microRNA146a expression occurred in mice with Streptozotocin-induced DM in vivo. Moreover, the authors suggested that glucose-induced inhibition was mediated via histone deacetylase p300 and that the fibronectin/p300/microRNA146a triad in both the kidney and the heart should be considered. This study showed microRNA's control over fibronectin expression, as well as a functional link between the control of microRNA gene expression and histone modification, which had not been previously described.

DNA METHYLATION

DNA methylation occurs in CpG dinucleotides. Among vertebrates, genomic DNA methylation is determined throughout the genome, except for short, unmethylated regions called CpG islands located predominantly in promoters [45, 46]. Historically, the central function of DNA methylation has been the stabilisation of DNA in repressed regions and, consequently, the inhibition of promoter activity. However, recent studies on DNA methylation have shown that methylation on 'bodies' of active genes is much higher than that on inactive ones [47, 48]. This is most likely necessary to suppress unwanted transcription, regulate RNA splicing, modulate elongation and regulate the activity of a tissue-specific alternative promoter [49].

Variations of 'normal' DNA methylation can correlate with various aspects of DM, including a tendency to develop diabetes [50, 51], insulin resistance [52], development of complications [53] and the possibility of early diagnosis [54]. The overall profile of genomic DNA methylation among patients with DM2 includes 276 CpG loci. These loci are significantly hypomethylated and can play a role in disturbing the regulation and pathogenesis of the disease [55].

A causal relationship between hyperglycaemia and changes in DNA methylation was demonstrated, wherein DNA hypomethylation was induced in the liver of rats with DM1 2 weeks after the manifestation of hyperglycaemia [56]. However, the DM2 model (Zucker diabetic fatty rats) also demonstrated DNA hypermethylation in the liver at week 12 [57]. Pirola et al. [58] studied primary aortic endothelial cells under hyperglycaemia in vitro and performed a complete analysis of both histone acetylation and DNA methylation. This study showed significant changes in DNA methylation and that induced hypermethylation is localised in regions within 5 kb of the

transcriptional starting sites. They also showed extensive changes in H3K9/K14 acetylation. It was interesting that the localisation of hyperacetylation correlated with DNA hypomethylation and gene induction in response to hyperglycaemia. However, none of these studies considered the effects of prolonged hyperglycaemia or the metabolic memory phenomenon.

Changes in DNA methylation induced by hyperglycaemia, which are persistent in metabolic memory, were studied in the zebra danio family of carps with Streptozotocin-induced DM1 [59]. Given their ability to regenerate affected pancreatic cells, these fish are unique for studying metabolic memory after pancreatic cell regeneration and restoration of euglycaemia. Another advantage of this model is that mitotically transmitted components (epigenetic modifications) can be considered without the need for considering other hyperglycaemic factors (AGE, FOR, etc.). This study observed a dysfunction in caudal fin regeneration after the restoration of euglycaemia. Significant demethylation was demonstrated, which was maintained after achieving euglycaemia, that is, modulation of the metabolic memory phenomenon. Moreover, DNA hypomethylation was evenly distributed among promoters and intra- and intergenic sites. Examining these data in the context of a global change in gene expression revealed a correlation with a group of genes. The most interesting fact is that at least three members of the epigenetic code replication mechanism complex, which is responsible for doubling the epigenetic code during replication [60], had been modified during their expression. Thus, a mechanism that allows for the transfer of epigenetic changes in DNA methylation, the modification of histones in hyperglycaemia and the maintenance of these changes in cell division had been found, which indicates the involvement of epigenetic factors in the phenomenon of metabolic memory.

A number of experimental studies have identified multiple changes in epigenetic gene regulation mechanisms that can allow for the formation of the metabolic memory phenomenon. Thus far, the treatment regimens existing for most diabetic complications have been ineffective. Therefore, the study of epigenetic mechanisms is warranted, because it enables obtaining new information on the developmental mechanisms of vascular DM complications and the likely identification of new targets for drug treatment (e.g. epigenetic drugs and microRNA modulators) given that epigenetic changes are potentially reversible in nature.

CONCLUSION

The currently accumulated data on the mechanisms of 'metabolic' memory are still ambiguous, which may be due to the differences in their specific effects depending on the type of the models used. However, there are prerequisites for using the components of 'metabolic memory' as potential biomarkers for the progression of DM complications and as potential therapeutic strategies for targeting exposure. Further decoding of the mechanisms to elucidate the action of individual components and their combination is required to improve the prognosis of patients with DM.

ADDITIONAL INFORMATION

FINANCING OF THE WORK

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CONFLICT OF INTEREST

The authors declare no obvious and potential conflicts of interest related to the publication of this article.

PARTICIPATION OF AUTHORS

Chernikov AA analysed the literature and wrote the text; Severina AS analysed the literature, wrote the text and performed editorial revisions; Shamkhalova MSh wrote the text and performed editorial revisions; and Shestakova MV performed editorial revisions.

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