Hemostatic disorders of the menopausal period: the role of microRNA

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Abstract

Adverse changes in hemostasis of menopausal women, observed e.g. in atherosclerotic or neoplastic cases, are of multicausal origin. It is believed that in the development and regulation of these processes, an important role is played by microRNA particles, which presence is ascertained in endothelial cells, atherosclerotic plaques and systemic circulation. Discovered for the first time over 20 years ago, up to now over two and a half thousand types of microRNA have been identified in the human body. MicroRNAs are single stranded RNA molecules of 20-24 nucleotides, encoded by the cell’s genome and then transcribed by polymerase II. They regulate the expression of a large gene pool, approximately 30% of all genes, in the human body. MicroRNA molecules, like other bioactive molecules – RNA, protein – both play important roles in tumor invasion, metastasis, inflammation, coagulation, and regeneration. What is important, they can be detected not only in tissues (e.g. tumor tissues), but also in circulation (blood serum), where they are released. Accurate understanding of the role played by certain types of microRNA (e.g. miR-126, miR-17-92, miR-33, miR-613, miR-27a/b, miR-143, miR-335, miR-370, miR-122, miR-19b, miR-520, or miR-220) in hemostatic processes may allow in the future for their use not only as specific biomarkers of cardiovascular diseases but also as the target for innovative gene therapies.

Key words: microRNA, menopause, hemostasis, atherosclerosis, endothelium.

With age in women, but slightly later than in men, adverse effects are observed in hemostasis, which directly translates into an increased risk of diseases of the circulatory system. This risk primarily involves the increased incidence of thromboembolic events, both arterial and venous, being in many cases a result of the accumulation during menopause (understood broadly as pre-, peri- and postmenopause) of a number of cardiovascular risk factors. The list of known factors is particularly long in the case of the arterial system. Even the age of menopausal women itself is a risk factor for both arterial and venous diseases. Thus, it is not surprising that menopause is the period in which women often begin to develop diseases that are derived from adverse changes in hemostasis, including coronary heart disease, high blood pressure, or venous thromboembolism (VTE) [1-3].

Let us recall that among traditional risk factors for arterial thrombosis in women the following stand out: age > 50 years, hypertension, abdominal obesity, hypercholesterolemia, impaired glucose tolerance and hyperinsulinemia, smoking, lack of physical activity. Whereas newer risk factors include: left ventricular hypertrophy, hypertriglyceridemia, elevated Lp(a), hyperhomocysteinemia, elevated serum concentrations of C-reactive protein (CRP), hyperfibrinogenemia. There are also the so-called hemostatic risk factors which, in addition to elevated levels of fibrinogen, include also increased activity of factors VII, VIII and von Willebrand factor (VWF), reduced fibrinolytic activity (↑ plasminogen activator inhibitor-1 [PAI-1], tissue plasminogen activator [t-PA] and t-PA/PAI-1 complexes), increased platelet activation and endothelial cell dysfunction (e.g. ↑ vascular cell adhesion molecule 1 [VCAM-1] or E-selectin) [4-7].

It is also emphasized that certain genetic polymorphisms of selected coagulation factors (e.g. factor V gene and the G20210A prothrombin gene), although not being independent risk factors for thrombosis, they increase this risk very much (e.g. the risk of myocardial infarction in smokers of tobacco, or in persons with metabolic risk factors) [8].

In recent times, more and more attention has been paid to the potential role of another genetic factor in the regulation of hemostasis, namely microRNA (miRNA). MicroRNAs are single stranded RNA molecules of 20-24 nucleotides, encoded by the cell’s genome and then transcribed (just as mRNA) by polymerase II. The miRNA biosynthetic pathway is as follows: within the nucleus, from the genomic DNA (transcription with the use of RNA polymerase II), the longer thread of primary miRNA (pri-miRNA) is first formed, which subsequently under the influence of Drosha (complex of RNase III and Pasha protein, or DGCR8) is converted into pre-mature miRNA (pre-miRNA). Pre-miRNA is transferred from the
nucleus to cytoplasm by the protein XPOS (exportin-5), which is then converted to a mature miRNA, involving ribonuclease III – Dicer. MicroRNAs thus formed are fitted into the RISC complex (RNA-induced silencing complex), which post-transcriptionally inhibits the expression of target mRNA by binding to its 3’ untranslated region (3’UTR) [9]. Binding of miRNA to the 3’UTR of specific RNA (complementary to its sequence) inhibits translation or promotes degradation of mRNA [10].

For the first time miRNA was discovered in 1993 in Caenorhabditis elegans (a small – about 1 mm in length – non-parasitic nematode, living in soils of the temperate climate) [11]. They were discovered then in humans, and for the next 20 years, over two and a half thousand various types of miRNA have been identified in the human body [12].

It has been shown that miRNAs are important regulators of many, both physiological and pathophysiological, processes in the human body. They are involved, inter alia, in the negative regulation of gene expression during development. They specifically block, in the mechanism described previously, translation of the mRNA, give them their specificity, and are also considered to be mediators of the interference mechanism of mRNA translation (RNA interference – RNAi). Their importance is based on the regulation of expression of a large gene pool (approximately 30% of all genes) in the human body. What is important, they can be detected not only in tissues (e.g. tumor tissues), but also in the circulation, blood serum, where they are released. MicroRNA molecules, like other bioactive molecules – RNA, protein – both play important roles in tumor invasion, metastasis, inflammation, coagulation, and regeneration (using stem cells) [13].

In the context of menopause and the associated hemostatic disorders, first it is worth following the studies concerning the involvement of miRNAs in atherosclerotic processes and hemostatic disorders associated with neoplastic disease.

**Atherosclerotic disease**

Cardiovascular diseases (CVDs), being a derivative of the atherosclerotic process, are still not widely perceived as the greatest threat to the health and life of women. However, it is them, and not cancer (e.g. breast cancer), as is commonly believed, that is the major cause of mortality. According to the American Heart Association, CVDs arising from atherosclerosis are responsible for over 30% of all deaths in the U.S. [14].

Atherosclerosis is a slow process, which gives the first signs after many years of duration. A morphological sign of atherosclerosis is endothelial damage. A special role in the development of atherosclerotic lesions is attributed to inflammatory processes and disorders of lipoprotein metabolism. The fact that elevated blood cholesterol is a risk factor for heart attack, has been known since 1961 from reports of the Framingham Heart Study. In the development of atherogenic processes several steps are involved. They include: 1) endothelial cell dysfunction; 2) penetration of LDL into the subendothelial space and their modification; 3) migration of monocytes into the subendothelial layer, transforming them into foam cells and disintegration of the cells; 4) thrombogenesis and platelet release of growth factors and chemotactic substances; 5) middle layer → inner layer migration of myocytes in the vessel (with their subsequent growth); and 6) formation of intercellular connective tissue [15].

The view on the pathogenesis of atherosclerosis has evolved from assigning the leading role of lipids to recognize vascular inflammation as the main factor in the emergence and development of atherogenic changes. Any inflammatory response, also in the vessel wall, occurs in several stages, including:

- margination, which is dependent only on the physical blood properties;
- rolling – a process involving some selectins (L-selectin, P-selectin, E-selectin);
- activation – where the main role is played by cytokines, in particular a large group known as chemokines, including platelet factor 4 (PF4), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1);
- adhesion, dependent mainly on integrins and
- diapedesis – a process of leukocyte transition through the endothelial barrier and then through tissues into location of the antigen [16].

Modified in this way, endothelium begins to produce VCAM-1, by which monocytes and lymphocytes T are attached to the endothelial surface. Leukocyte migration across the endothelium is accomplished by monocyte chemoattractant protein-1 (MCP-1). Monocytes in the intima capture modified lipoprotein particles, transform into foam cells, then begin to produce cytokines and matrix metalloproteases (MMPs). Matrix metalloproteases are assigned a large role in plaque rupture, parietal thrombosis and the occurrence of acute cardiovascular events [17].

It is postulated that miRNA also participates in atherosclerotic processes. This applies in particular to endothelial cells (ECs) involved in most aspects of vascular biology ranging from inflammation, vasodilation to angiogenesis [18]. Endothelial cells dysfunction may result, among others, in the occurrence of hypertension, thrombosis and atherosclerosis [19]. Results of the last few years clearly show that, in the regulation of almost all EC functions, the key role is played by miRNAs. These include, among others, miR-126 and miR-17-92 [20].

MicroR-126 was one of the first miRNA detected in endothelium. It affects the control of vascular inflammation by inhibiting adhesion molecules VCAM-1 [21].
It plays also a central role in the regulation of angio-
genmic signaling and vascular integrity, directly inhib-
iding negative regulators of the VEGF signaling pathway,
such as Sprouty-related protein 1 (SPRED) or phospha-
tinostol-3 kinase regulatory subunit 2 (PIK3R2/p85β)
[22]. It was also shown that miR-126, generated by
ECs, is secreted into microparticles and apoptotic bod-
ies, transferring a paracrine signal to target cells in the
vessel. Apoptotic bodies created and released from ECs
during atherosclerotic processes, stimulate in the above
mechanism the expression of CXCL12 – a gene located
on chromosome 10, responsible for the production of
stromal-derived factor-1 (SDF-1) protein, a cytokine
which is highly chemotactic to lymphocytes, taking part
among others in angiogenesis [23]. Administration of
apoptotic bodies, or miR-126 results in inhibiting the
atherosclerotic process (by promoting the incorpora-
tion of precursor cells, Sca-1+, to a vessel wall) [24].

Other known miRNAs affecting activation of ECs or par-
ticipating in vascular inflammatory processes include
miR-181b, miR-17-3, and miR-31 (expression of the last
two is increased by TNF-α, which, by influencing ICAM-1
and E-selectin, will lead to inhibition of the inflamma-
tory response) [25].

An important role in atherogenesis is also played by
a polycistronic cluster of several miRNA named miR-17-
92, consisting of seven mature miRNA, namely miR-17-
3p, miR-17-5p, miR-18a, miR19a, miR19b, miR-20a and
miR-92a. This applies in particular to miR-92a, which
regulates endothelial nitric oxide synthase (eNOS), an
enzyme producing NO in ECs, so that vascular tone and
angiogenesis is regulated. A molecular mechanism of
regulating the expression of ENOS by miR-92a is com-
plex. It has been shown that atheroprotective, laminar
blood flow reduces the levels of miR-92a, which in turn
inhibits Krüppel-like factor 2 (KLF2), a transcription fac-
tor regulating the expression of ENOS [26]. Krüppel-like
factor 2 in turn increases intraepithelial expression of
miR-143/145 which are secreted into microbubbles,
and reaching vascular smooth muscle cells (VSCMs) as
an anti-atherogenic factor, inhibiting the expression of
the E twenty-six (ETS) oncogene family – ELK1, Krüpp-
el-like factor 4 (KLF4), calcium/calmodulin-dependent
protein kinase II delta (CAMK2d), slingshot homolog 2
(SSH2), phosphatase actin regulator 4 (PHACTR4) and
cofilin 1 (CFL1) [27].

Studies conducted in animal models indicate that
miRNAs also have an impact on lipid metabolism. Mi-
croRNA-33 (miR-33) is the first to demonstrate a re-
lationship with high-density lipoprotein (HDL) metabo-
lism, bile excretion, as well as with the so-called reverse
cholesterol transport (RCT):

- miRNA-33 embedded within intron-2 of sterol-regu-
latory-element-binding protein-2 (SREBP-2), a tran-
scription factor functioning as the main intracellular
regulator of cholesterol homeostasis, which promotes
gene expression for cholesterol biosynthesis and up-
take, including e.g.: 1) HMG-CoA reductase (HMGR)
– an enzyme limiting cholesterol biosynthesis, 2) LDL
receptor, or 3) proprotein convertase subtilisin/kexin
type 9 (PCSK9) – an enzyme which, when attached
to the LDL receptor, destroys it as well the LDL mol-
cule bound to it. The clinical significance of the
SREBP-2 path is by reducing serum LDL levels. It
has been demonstrated that under physiological
conditions the expression of miR-33 is regulated by
intracellular cholesterol levels, and stimulated by
statin treatment. Preliminary research also shows
that miR-33 will suppress the expression of cho-
lesterol transporter – ATP-binding cassette trans-
porter 1 (ABCA-1); 3’UTR sequence of ABCA-1, which
binds to miR-33, was then identified. ABCA-1
plays an essential role during the early stages of the
biogenesis of HDL, mobilizing intracellular cholesterol
into circulating ApoA-1. In mice with overexpressed
miR-33, reduced hepatic levels of ABCA-1 were ob-
erved. More importantly, the suppression of he-
patic miR-33 (using antisense nucleotides) leads to
increased, hepatic levels of ABCA-1 (both miRNA and
protein) as well as to elevated blood levels of HDL.

These results suggest that persistent suppression of
hepatic miR-33 can be effective in the treatment of
patients with hypercholesterolemia in order to raise
levels of anti-atherogenic HDL and thereby to reduce
the risk of CVD [28, 29];

- miR-33 also controls the expression of sterol trans-
porters secreted into bile, including ATP8B1 and
ABCB11. Severe functional mutations of the above
factors result in familial progressive intrahepa-
tic cholestasis – progressive familial intrahepa-
tic cholestasis (PFIC) type 1 and 2. Whereas lighter mu-
tations lead to mild, recurrent intrahepatic choles-
tasis – called benign recurrent intrahepatic cholestasis
(BRIC), also type 1 and 2. In animal models, manipu-
lations of hepatic miR-33 resulted in changes in the
levels of bile secretion as well as changes in the total
bile collection from the gallbladder. It was also shown
that the use of statins in mice or Pagen diet (rich in
fat, cholesterol and sodium chelates) resulted in the
occurrence of the cholestatic phenotype. Important-
ly, this hepatic phenotype can be maintained using
pre-treatment in the form of anti-miR-33 oligonu-
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expression of pro-inflammatory agents, or angiogenic, but also affect coagulation, by changing e.g. the levels of tissue factor (TF) or PAI-1. This process is facilitated by the simultaneous loss of function of tumor suppressor genes (P53, PTEN) and changes in miRNA – for example, miR-19b or miR-520. Tumor cells themselves may start producing certain clotting factors – such as factor VII, or affect procoagulant properties of microparticles (MPs). Oncogenes may also deregulate functions of protease-activated receptors (PAR-1/2), which will affect the transmission of signals in the coagulation system. In addition, these processes involve microenvironment factors of the tumor (hypoxia), its response to stress factors (applied therapy), and mechanisms such as epithelial-to-mesenchymal transitions (EMT) and tumor initiating cell (TIC). The altered coagulation system will affect early (initiation of the process, angiogenesis), middle (growth, invasion) and late (metastasis, recurrence) stages of cancer’s development [36].

Previously mentioned TF plays an important role not only in the clotting process, but also shows a number of other, both physiological and pathophysiological, functions in vascular wall hemostasis, angiogenesis, and tumorigenesis. Research in recent years has shown that miRNA, which plays a key role in post-transcriptional control of protein production by regulating the expression of one third of all human genes, also modulates the expression of biological functions of TF, both in physiological and pathophysiological processes, in different types of cancers [37].

It is postulated that the key component of the coagulation system – thrombin – is an enzyme that can be considered as a factor involved in carcinogenesis, promoting angiogenesis, stimulating platelet adhesion to endothelium as well as inducing tumor’s growth and metastases. It is known that thrombin receptors (of the aforementioned PAR group) are present on the surface of tumor cell lines. Thrombin itself has a direct effect on cancer cells by activating the cell cycle through down-regulation of p27 (Kip1) and induction of Skp2, cyclin D and A. MicroR-220, which inhibits p27 (Kip1) is up-regulated by thrombin. The inhibition of thrombin can lead to the arrest of tumor growth and may explain the inhibition of tumor growth and metastasis development with the use of anticoagulants (as observed in animal models), preferably affecting the survival rate in cancer patients treated with oral anticoagulants in several clinical studies [38].

Neoplasms

Not since today it has been known that the neoplastic process affects hemostasis, including coagulation and fibrinolysis. Oncogenes (e.g. HER-2, RAS, EGFR, MET) presented by tumor cells not only alter the expression of pro-inflammatory agents, or angiogenic, but also affect coagulation, by changing e.g. the levels of tissue factor (TF) or PAI-1. This process is facilitated by the simultaneous loss of function of tumor suppressor genes (P53, PTEN) and changes in miRNA – for example, miR-19b or miR-520. Tumor cells themselves may start producing certain clotting factors – such as factor VII, or affect procoagulant properties of microparticles (MPs). Oncogenes may also deregulate functions of protease-activated receptors (PAR-1/2), which will affect the transmission of signals in the coagulation system. In addition, these processes involve microenvironment factors of the tumor (hypoxia), its response to stress factors (applied therapy), and mechanisms such as epithelial-to-mesenchymal transitions (EMT) and tumor initiating cell (TIC). The altered coagulation system will affect early (initiation of the process, angiogenesis), middle (growth, invasion) and late (metastasis, recurrence) stages of cancer’s development [36].

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