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ADAM and ADAMTS family proteins and their role in the colorectal cancer etiopathogenesis

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The ADAM and ADAMTS families, also called adamalysins belong to an important group of extracellular matrix proteins. The ADAMs family belong to both the transmembrane and secreted proteins, while ADAMTS family only contains secreted forms. Adamalysins play an important role in the cell phenotype regulation via their activities in signaling pathways, cell adhesion and migration. The human proteome contains 21 ADAM, and 19 ADAMTS proteins, which are involved in extracellular matrix remodeling, shedding of various substrates such as: adhesion ligands, growth factors, their receptors and diverse cytokines. Recent studies provide evidence that adamalysins play a crucial role in colorectal cancer (CRC) etiopathogenesis. It seems possible that adamalysins might be used as CRC prediction markers or potential pharmaceutical targets. [BMB Reports 2013; 46(3): 139-150]

INTRODUCTION

Cancers, after the disorders of the cardiovascular system, are the second leading cause of mortality and therefore are also called one of the twenty-first century epidemics. Permanent growth in the number of cases prompts the scientists to deepen their knowledge of the etiology, diagnosis and cancer treatment.

The mechanism of tumor formation is sophisticated and closely linked to the accumulation of genetic changes, whose etiology is not completely known. The tumor development is mainly related to the abnormal regulation of tumor cell processes such as cell cycle control, apoptosis, angiogenesis and remodeling of extracellular matrix (ECM) and finally the metastasis, which is associated with these processes. Until now all research performed to clarify the basic mechanisms, causes and course of carcinogenesis, give as many answers as new questions (1). Two leading theories are currently applied to explain the basics of the cancerogenesis: clonal theory and stem cells theory. Despite the differences between these theories, according to both the trigger for the process of carcinogenesis is largely associated with the accumulation of the genetic changes such as aberrations, point mutations and/or epigenetic changes (changing CpG island methylation status and acetylation of histone proteins) (2).

The diversity of current molecular biology research tools allows us study not only the role of individual genes involved in carcinogenesis, but also whole families of genes. Usage of a broad-spectrum of methods and holistic approach in the analysis of data highlighted the significant role of zinc-dependent proteases (metzincins) (3), including matrix metalloproteinases (MMPs - matrix metalloproteinases) in the pathogenesis of cancer (4).

Metzincins consist of a large heterogeneous superfamily of proteolytic proteins present in the extracellular matrix. These proteins can be classified taking into account various criteria such as mechanism of carried catalytic reaction, substrate preferences, resultant products and structural homology. The structural criterion has been applied for the purposes of this article (5, 6). The major structural homology which was found in all proteins of this superfamily is highly conservative motif **HEXXHXXGXXH** present within the active site of these proteins (3, 7). Majority of differences between zinc-dependent proteases is associated with the occurrence of additional domains within the C-terminus of these proteins. Those C-terminus variants are linked to MMP's location and their function performed within the cells and tissues. Described more than half century ago, MMPs were the first known zinc-dependent proteases family (8). They were followed by ADAM (a-disintergin and metalloproteinase), and ADAMTS (a-disintergin and metalloproteinase with thrombospondin motifs) (9) families; other closely related protease families are also serralysins and astacins (3, 10). Recent studies indicate the important roles of the ADAM and ADAMTS metalloproteinases family in the tumor formation and development (11).

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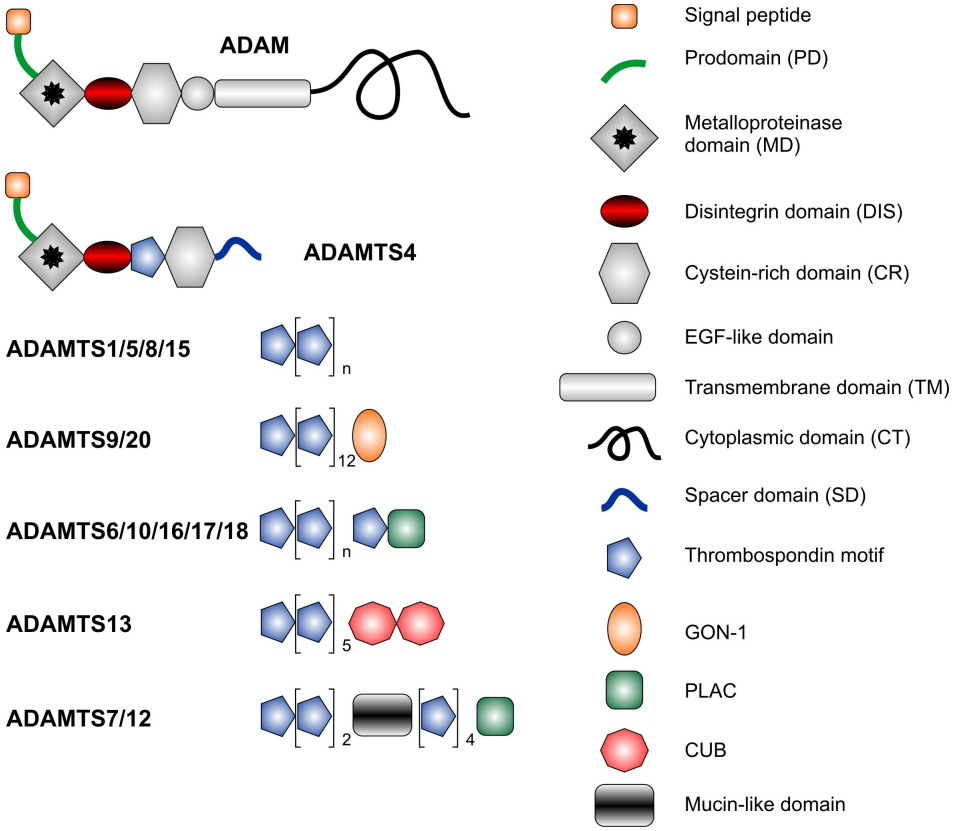

Fig. 1. Schematic structure of ADAM and ADAMTS proteins families.

STRUCTURE OF PROTEINS BELONGING TO THE ADAM AND ADAMTS FAMILIES

ADAM and ADAMTS, which together are known as adamalysins, are the matrix zinc-dependent metalloproteinases (12), which also include the metalloproteinases present in snake venom (SVMP). The first protein of this group have been identified in mammalian gonads (5, 13). Occurrence of the adamalysins have been described in many organisms, including *Homo sapiens*, yeast (*S. cerevisie*) and nematodes (*C. elegans*) (14). Until now in the human proteome, 21 ADAM (15), and 19 ADAMTS (16) proteinases have been identified. Most of the ADAM metalloproteinases are transmembrane proteins while ADAMTS are secretion ones (see Fig. 1). They are mainly localized in the extracellular matrix (ECM), with which they can be linked by spacer domain (SD) or thrombospondin motif (TS). All adamalysins (ADAM and ADAMTS) are initially synthesized as precursors, and most of them can be activated by furin (13).

Both ADAM and ADAMTS proteins show a multidomain structure (see Fig. 1). Despite many similarities found in the structure of these proteins, they individually possess a lot of variable motives and structures which ensure their function and tissue specificity. In the structure of proteins from both families a specific N-terminal sequences can be distinguished. These sequences are responsible for the transport of protein precursors to the trans-Golgi network, where they are converted into active form and then targeted to secretory pathways (17). Another common motif is predomain (PD), which primarily plays a role as an inhibitor of metalloprteinase domain (MP) proteolytic activity. Highly conserved cysteine in the PD sequence preferentially binds to the zinc atom within the active center of the MP. Created in this way protein conformation blocks its proteolytic activity (18). To activate the catalytic center it is necessary to change the coordination bonds with the zinc atom in the active site what using the cysteine-switch mechanism (18) or after prodomain removal (17, 19). Some proteases from ADAM(TS) families have a short, highly conservative furin-recognition motif **Rx(R/K)R** located between MP and PD domains. This motif is identified and cleaved by protein convertases (PCs), among others by furin, which initiate enzyme maturation (15). However, not all metalloproteinases require furin/protease cleavage for activation; ADAM8 and ADAM28 are activated by autocatalytic mechanism (20). It should be noted that the occurrence of cysteine-switch mecha-

nism has been proven only for the ADAM family. Other role of the PD domain is that it probably also participates in the correct folding of adamalysin (21).

Metalloproteinase domain is another common motif in structure of proteins belonging to ADAM(TS) families. In its active site MP domain contains, as mentioned earlier, highly conservative motif, **HEXXHXXGXXH**, which is responsible for proteolytic activity. This motif contains three histidines surrounded by methionines in so-called methionine loop. Such zinc binding motif is characteristic for zinc-dependent metalloproteinases of the reprolysin family. In addition to zinc atom MP domain active site, possess, also coordinatively bound water molecule – crucial for hydrolysis of proteins (3, 7).

Disintegrin domain (DIS) is about 90aa long protein fragment which is also characteristic for the adamalysin's structure. Disintegrin itself is a protein that inhibits platelet aggregation. Interaction between disintegrin and integrin (22) prevents binding plates with their natural ligands such as fibrinogen. Adamalysin's DIS structure is homologous to the similar domain in the SVMP, where it determines the high toxicity and is probably the cause of hemorrhage (23). DIS domain, in both ADAM and ADAMTS, plays a crucial role in substrate recognition and it confers protease activity. It has been proven that some ADAM family proteins use the DIS domain to specifically connect with integrins such as: $\alpha 2\beta 1$, $\alpha 4\beta 1$, $\alpha 4\beta 7$, $\alpha 5\beta 1$, $\alpha 6\beta 1$, $\alpha 6\beta 4$, $\alpha 9\beta 1$, $\alpha 2\beta 1$, $\alpha V\beta 3$, $\alpha V\beta 5$. This may point to the important role of those integrins in cell adhesion and cell-cell interactions (24).

Cysteine-rich domain (CR) is the least common motif that could be found in the structure of ADAM and ADAMTS proteins. The function of this domain is not fully known. It was observed that the CR domain of ADAM13 regulates the proteolytic activity; it might have a similar functions in all adamalysins. In ADAM8 the CR domain together with PD is involved in autocatalytic activation of the metalloproteinase (20). Furthermore, it was proved that ADAM's PD could bind to normal and tumor cells (25). It is likely that this binding is mediated by fibronectin (26).

In the ADAM's proteins structure, next to the CR a EGF-like domain can be distinguished. This is a rather conservative domain, containing ten cysteines in its sequence. Until now the role of this domain remains unclear. Recent studies indicate that EGF-like domain might mediate the multimerisation of ADAM17 in cell membrane, which in turn may play a critical role in protease activation. It is worth to emphasize however, that this phenomenon has been proven for ADAM17 only (27).

As it was mentioned above most of ADAM proteinases are cell membrane integral proteins and therefore they possess the transmembrane domain (TM), located behind EGF-like domain. The last, C-terminal fragment of ADAM proteins, located in the intracellular space is called a cytoplasmic tail (CT). This domain is playing a key role in intracellular signaling. Analytical structure of this protein fragment in different members of ADAM family shows that this it is presumably the most variable protein part, both in size and in aminoacid sequence. Despite this, it was found that PXXP motifs present within the CT sequence allow to bind proteins containing SH3 domain (28). CT domain includes also several potential phosphorylation sites (29). Cytoplasmic tail shows also regulatory properties, among others it can regulate the rate of MAPK cascade activity (30). All together, EGF-like, TM and CT domain, form a carboxylic region of the ADAM metalloproteinase family. ADAMTSs C-terminal region contains the TS, CR, and SD domains.

In contrast to the previously described ADAM's structures, the thrombospondin type 1-like repeat (TS) next to DIS domain occurs in ADAMTS structure. TS shows a high homology to the region I of thrombospondin 1 and 2 (31). This region allows ADAMTS to bind to the extracellular matrix (32) and it can probably be also involved in apoptosis and angiogenesis, which can be concluded from its homology to thrombospondin 1 and 2 (33). Different members of the ADAMTS family possess variable number of thrombospondin type 1-like motif repeats in their structures. This number varies between one, in ADAMTS4, and fifteen in ADAMTS20 and ADAMTS9 (15).

Another structural diversity difference between the ADAM and ADAMTS proteins is spacer region, which is found only in ADAMTS. This region is located after CR domain and it is responsible for the interaction of enzyme with substrate (34).

Within the ADAMTS' C-terminus region four additional domains were described; three of them (GON-1, CUB, PLAC, Mucin-like) are located after the last repetition of the TS domain, only the mucin-like domain occurs between repetitions of the TS domain. These domains are unique and occur only in some proteases of ADAMTS family. GON-1 domain, found in ADAMTS9 and ADAMTS20, shows high homology to a GON-1 from *C. elegans* metalloproteinase, where it is necessary for distal tip cells migration during the gonads formation. Within its sequence, GON-1 contains ten conservative cysteines. The function of this domain in the human ADAMTS9 and ADAMTS20 has not yet been clarified (31, 35).

The PLAC (protease and lacunin) domain occurs in the sequences of several proteins from the ADAMTS family (6, 7, 10, 12, 16, 17, 18 and 19) (36). This domain was originally described in lacunin, which is hightly expressed in the ECM during *Manduca sexta* embryogenesis (37). In its sequence, PLAC domain has six cysteines which resemble an arrangement known from some PCs (38). ADAMTS13 is the only protein in this family with two CUB (Complement-Uegf-BMP-1) domains located in its C-terminus (39). This domain has also been found in proteins such as spermathesine and TSG-6 (tumor necrosis factor-stimulated gene-6) where it is probably involved in proteine-proteine interactions (40). The last of the additional domains present in ADAMTS is mucin-like domain. This domain is found in ADAMTS7 and ADAMTS12 and is located between the third and fourth repetition of the TS. Mucin-like domain is a region rich in serine, proline, and threonine and undergoes o-glycosylation. It is suggested to be involved in

metalloproteinase-substrate interaction (38).

ADAM AND ADAMTS: BIOLOGICAL FUNCTIONS AND THEIR PERTURBATIONS

Ever since the primary studies carried on adamalysins the expression of these proteins in various tissues has been demonstrated. For some of them, like ADAMTS1, expression was traceable in almost all tissues (36, 41). On the other hand some of ADAM(TS) are tissue-specific, such as ADAMTS17 whose expression was observed exclusively in the ovary (42). Some disorders in collagen-rich tissues are connected with adamalysins' dysfunction. For example, mutations in ADAMTS10 have been characterized as a Weill-Marchesani syndrome background, whereas ADAMTS2 mutations are recognized as a background of Ehlers-Danlos syndrome type VII C (12).

Among the two metalloproteinase families described in this article, the ADAM's function has been first discovered and characterized; these proteins are also likely to be evolutionarily older (15). The first ADAM-related reports have been associated with the studies of such sperm proteins, as fertillin, associated with the processes of spermatogenesis and sperm-egg fusion. Studies performed on mice showed that the Adam1 (fertillin-α) and Adam2 (fertillin-β) proteins are involved in spermatogenesis and presumably might play a crucial in this process (43). This finding, however, could not be directly transferred to humans because *ADAM1* appeared to be a pseudogene. Adam2 and Adam1 present on the surface of mice spermatozoids are cleaved between MP and DIS domains (43). Retained DIS domain binds the α6β1-integrin on the surface of the egg and thus allows sperm-egg fusion. It is not known whether Adam1 and Adam2 are the only adamalysins involved in this process, since a similar feature is also postulated for ADAM3. Studies of this case are difficult because silencing of *Adam2* and *Adam3* also induces down-regulation of other adamalysins including Adam5-7, which also are involved in spermatogenesis, and result in dysfunction of sperm (44, 45). It is worth to note that humans' *ADAM3*, similarly to *ADAM1*, proved to be a pseudognes (46), which again leads to the conclusion that often results of certain studies cannot be generalized to other species. Nineteen of the so far described ADAM's family proteins (1-7, 14, 16, 18-21, 24-26, 30, 32, 34) are involved in spermatogenesis and in fertilization (12, 47); whereas ADAM8, -9, -10, -12, -15 and -17 may be involved in embryo implantation in the uterus (48). The above mentioned experimental data clearly demonstrates the important role played by ADAM proteins in fertilization and in embryonic development. In addition, the expression of different members of the ADAM family has been demonstrated in many tissues or organs, where they play certain roles in proper functioning. These organs include: heart (ADAM9, 17, 19) (49), kidney (ADAM19), lung (ADAM33), teeth (ADAM28) and pancreas (ADAM9, 10, 17) (14). Research conducted on mice showed that *Adam19* mutations are lethal, which proves the key role of this protein in organs' formation. Homozygous *Adam19*-/- mouse embryos died due to abnormalities of the heart and others cardiovascular system disorders.

The functions of some of the ADAM and ADAMTS proteins have not yet been carefully examined – below we describe the examples whose function is at least partially identified and the pathological function or expression changes were found in colorectal cancer (CRC) cell lines or tissues.

ADAM9 (MDC9, meltrin γ)

The first reports on ADAM9 protein (50) initiated many studies on its function, location and development in normal and pathological tissues. Like most adamlysins, ADAM9 is synthesized as a precursor and is directed to posttranslational processing in *trans*-Golgi. During this process metalloproteinase's precursor is cleaved, by furin or other PCs, between PD and MP domains (51). In the same study it was shown that ADAM9 is catalytically active and *in vitro* it cleaves the insulin β-chain. On the other study the α-secretase activity of ADAM9 was postulated because its shortened form ADAM9s (lacking TD and cytoplasmic domain) expressed in COS cells cleaved proamyloid protein APP (52). In several *in vivo* studies the following substrates for ADAM9 were found: FGFR2iiib, TNF (53) and fibronectin (54). Within a CT domain, ADAM9 has a potential SH3 binding site but, so far, its function has not been demonstrated *in vivo* (15, 51). *Adam9* also showed high expression in mouse embryo; those data suggests its role in embryogenesis and embryo implantation (48). ADAM9 is the only protein of this family, which has been shown to interact with integrin α2β1 and αVβ5 (24).

ADAM10 (MADM, kuz)

In early studies ADAM10, by analogy to ADAM2, was supposed to be associated with spermatogenesis (55). Now these assumptions generally seem not to be valid although, along with other proteins including the aforementioned ADAM9, ADAM10 is also involved in embryo implantation (48). The structure of this protein is not typical because it does not contain EGF-like domain, while other elements typical for ADAM family are preserved. Like most proteins of the ADAM family, ADAM10 manifests its proteolytic activity. Its substrate range is broad and contains the six EGFR ligands, TNF, epireguline, HB-EGF and EGF (56). Similarly to ADAM9, ADAM10 cleaves APP; this evidence suggests that these proteins might influence the formation of amyloid inclusions and Alzheimer's disease development (57). ADAM10 together with ADAM17 are involved in cleavage of transmembrane protein Klotho (KL), which is involved in aging process. Additionally, cleavage of KL by ADAM10 is stimulated by insulin (58).

The last on the list but probably the most important function of ADAM10 is its involvement in Regulated Intramembrane Proteolysis (RIP), which is part of the Notch/Delta signaling pathway. There exists some evidence that ADAM10 plays crucial role in cleavage of Notch membrane receptor (59).

Adam10 silencing is lethal in the early stage of mouse embryogenesis and produces high neurological damage in aborted embryos (60). So far no protein-protein interactions between ADAM10 and integrins, were demonstrated (14).

ADAM12 (meltrin-α)

ADAM12 is a protein which can be found in a human organism in the two following forms: a long form (ADAM12-L) and short (ADAM12-S). Presence of those two versions is a result of an alternative splicing. ADAM12-L is a transmembrane protein, while ADAM12-S is the secretion one (61). Both splicing versions have gelatinase activity and their substrates include mainly ECM proteins such as collagen type IV and fibronectin (62). Early studies on *ADAM12* gene have shown its expression in muscles and emphasized its role in muscle tissue formation via its influence on mioblast fusion (63). However, no change was found within the muscle tissue of a mouse after silencing the *Adam12* expression, moreover, 30% of mice offspring with *Adam12-/-* double knockout has died off within the first week of birth (64). Further research performed on mice suffering from muscular dystrophy showed that an overexpression of *Adam12* decreases the disease's symptoms in the early age of the mouse (65). Such results might suggest that ADAM12 has no influence on embryogenesis. Like other proteins from this family ADAM12 interacts with integrins: an interaction between this protein and integrins like α9β1 i α5β1was found (24).

ADAM17 (TACE, TNF-convertase)

ADAM17 is the second ADAM family member which, next to ADAM10, does not contain a EGF-like domain in its structure. The proteolytic activity was also shown for this protein and one of its first discovered substrates were pro-TNF i TNF-α (66). As in ADAM10, the substrate specificity of ADAM17 was revealed for six EGF-like growing factors (56). Because of the similarity in the substrate specificity of ADAM17 to ADAM10, it may be assumed that ADAM17 can also take part in Notch signaling pathway as it was exhibited via *in vitro*. Yet, there was no change observed within this pathway in a mouse with *Adam17-/-* double gene knockout (67). ADAM17 shows also some characteristics of α-secretase which is to cut APP, thus suggesting its relation with the occurrence of Alzheimer's disease (57). The main activity of ADAM17 in *in vitro* studies is the TNFα conversion. It is proved that, proper function of ADAM17 is crucial for the transformation of inactive pro-TNFα into its active form and it is also crucial for the proper function of EGFR (68). The proteolytic activity of ADAM17 is regulated by the mechanism of oxidative stress (69).

ADAM23

The structure of this protein is typical for the (ADAMs' family) but it does not show proteolytic activity and, within MP domain of this protein, a high-conservative zinc-binding motif was not detected. The first reports of ADAM23 were connected with its high expression in the brain tissue (70). It can take part in a proper nervous system development via interaction with αVβ3 integrin (71). Research made on mice with *Adam23-/-* gene double knockout revealed increased embryonic mortality and many cases of ataxia in individuals born alive (72).

ADAM29

ADAM29 does not show any proteolytic activity and, like ADAM23, it does not contain a high-conservative zinc-binding motif (73). Gene *ADAM29* is located in chromosome 4 and its greatest expression is detected in testis (74). According to the conducted research, a human ADAM29 is found in three splicing forms containing 820 aa, 786 aa and 767 aa. Structural differences between particular isoforms are located within the CT domain (75). Results of RT-PCR experiments performed using the material taken from patients with chronic lymphocytic leukemia (CLL) show a higher *ADAM29* expression in these patients. Some authors suggest, that the level of this gene expression may have a significant prognostic value for patients with CLL (76). Recent studies found an increased number of mutations within *ADAM29* gene among patients with melanoma. In addition, it was proved that mutated ADAM29 protein possess lower collagen adhesion, which may influence increased frequency and speed of metastasis formation (77).

ADAMTS1 (METH-1)

ADAMTS1 is one of the most important and best described protein in the whole ADAMTS family; the studies on its structure and function started in 1997 (78). It is confirmed that its spacer-domain has a strong affinity with ECM and the TS domain shows high affinity with heparin (32). It is also found that ADAMTS1 has some proteolytic activity and shows a certain substrate specificity among others with aggrecan, although the places of cleavages made by ADAMTS1 vary from other aggrecans (79). At the beginning of research, the inhibition of angiogenesis was specified as a basic function of ADAMTS1 (41). It was proved that this protein silences the proliferation signal caused by VEGF and FGF2 via the inhibition of VEGFR2 phosphorylation (80). This is not the only pathway of action of this protein on neovascularisation because due to its proteolytic activity ADAMTS1 can dissociate from thrombospondin type I and II a short polypetide with antiangiogenic activity a (81). These data may suggest that ADAMTS1 has a strong influence on angiogenesis, and as a result, on the speed of cancer development. It was also proved that ADAMTS1 possesses a metastasis promotion and tumor progression activity (82). Possible cause of such phenomenon could be shedding off amphiregulin and HB-EGF (83).

ADAMTS9

In human organism, the ADAMTS9 protein occurs in two secretion forms differing in number of TS motifs. The so-called long form of ADAMTS9 has got 14 repetitions of TS motif while the so-called short form has got only three such repeti-

tions in its structure (35). Additionally, there is an untypical *gon-1*-like motif C-terminus of ADAMTS9. The first studies on ADAMTS9 metalloproteinase were published at the beginning of this century and resulted in finding a high gene homology to *gon-1* metalloproteinase in *C. elegans*. It is stated that, like *gon-1*, *ADAMTS9* could take part in gametogenesis (84), however, this result was not confirmed in studies on mammals. The ADAMTS9 protein shows proteolytic activity and is also classified as aggrecanase and versicanase (35). In the case of mice, *Adamts9* gene exhibits high expression during embryogenesis. Mouse embryos with double knockout of *Adamts9-/-* gene died off before gastrulating (85). This fact confirms the essential role of this gene in embryogenesis. As a contrast, mice with a single silenced *Adamts9* allele showed a spontaneous neovascularisation, thus confirming the antiangiogenic activity of ADAMTS9. As it is assumed in the latest research, *ADAMTS9* gene might be a novel gene supressor; its silencing or deletion can be found in almost 50% of esophagus cancers (86).

ADAMTS12

ADAMTS12 is one of two, next to ADAMTS7, glycoproteins in ADAMTS family with an unique, in this family, mucine-like domain in its structure (38). ADAMTS12 also reveals proteolytic activity and is one of the main metalloproteins which play a role in COMP (cartilage oligomeric matrix protein) degradation, what can explain the role of this protein in arthritis development (87). Other substrates of this metalloproteinase are, among others, VEGF and HGF; their shedding results in Ras-MAPK pathway blocking. *ADAMTS12* role in Ras-MAPK pathway regulation and some evidence of epigenetic silencing of the gene in cancer cells suggests that *ADAMTS12* may be a novel gene suppressor (88). This thesis seems to be proved by the fact that cancer cells injected into a mouse with silenced *Adamts12* gene proliferate much faster and produce more developed neovascular system (89).

ADAMTS15

ADAMTS15 is the least examined protein from the whole ADAMTS family. This metalloproteinase is a quite short secretion protein with only two repetitions of TS motif of C-terminus. High homology of its gene to *ADAMTS1* was also detected (42). It was also showed that this protein has aggrecanese activity however, this fact was not sufficiently proved (36, 90). Research on prostate cancer cell lines revealed lower *ADAMTS15* expression in this cancer (90) and regulation of *ADAMTS15* expression under influence of androgens. Clinical studies on patients with breast cancer exhibited that the lower *ADAMTS15* expression correlates with a higher probability of cancer development and increased mortality (91). The results of this research might emphasize a crucial role of ADAMTS15 in development of cancers.

ADAMTS18

The function of ADAMTS18 protein is so far unspecified. First research on ADAMTS18 showed its substantial structural similarity to ADAMTS16 (42). It is known that in its structure, ADAMTS18 possess a motif allowing glycoaminoglycane binding; yet the *in vitro* based research proved that such binding is inefficient. *In vitro* ADAMTS18 manifests its proteolytic activity as an aggrecanase but this enzymatic activity is rather low and manifests itself at ADAMTS18 high concentrations (92). Studies on cancer cell lines indicated that *ADAMTS18* gene is frequently epigenetically silenced , what was confirmed by research on cancer tissue obtained from patients (93). It may be claimed, according to this research, that *ADAMTS18* might be a novel gene suppressor. Recent studies prove that ADAMTS18 plays a role in maintenance of homeostasis via activation of platelet aggregation (94).

THE ROLE OF ADAMALYSINES IN COLORECTAL CANCER (CRC)

As it was proved by the research, proteins from ADAM and ADAMTS families play a significant role in such processes as intercellular signaling, angiogenesis, formation of proper tissue structure, embryogenesis and cell migration; what might indicate that any perturbation of function of these proteins might be essential in cancerogenesis (12).

There is about one million of new cases of colorectal cancer worldwide every year. Globally, it is the fourth in men and third in women most frequent cancer. The biggest amount of CRC is registered in such areas as Northern America, Australia and Western Europe (95). Statistical analysis shows that the number of CRC cases is constantly rising (96). Several long-time studies conducted on etiopathogenesis of this cancer show that it may be influenced by lifestyle, environment, socioeconomic status and genetic background in which the CRC occurs (95, 97).

A number of information about the role of adamalysines in etiopathogenesis of colorectal cancer were published. In the most of the cell lines studies are presented. In these papers the influence of the ADAM9s secretion form on the increasing invasive phenotype of Clone A cell lines was emphasized. The DIS domain of ADAM9 may directly bind to $\alpha 6\beta 4$ and $\alpha 2\beta 1$ integrins and proteolytic activity of this adamalysin allows to cleave laminines. The *in vitro* studies on CRC cell lines reveled the influence of ADAM9s overexpression on increased invasiveness of cancer cells (98). The investigation on CRC HT29 cell line pointed out the influence of overexpression of ADAM9 transmembrane forms on the HB-EGF-induced increased proliferation and decreased cell adhesion via E-cadherin degradation. It was found that ADAM9 strengthens the cell proliferation signal and decreases the contact suppression. Additionally, metastasis may be caused by higher E-cadherin degradation (99). Tissue Chip Assay studies show some correlation between the amount of ADAM10 and the cancer stage.

The results of these studies revealed also an increase in number of ADAM10 on the CRC cell surface in the late stages of the cancer (100). It was also found that one of the factors that modulate tetraspanine network within CRC tumors is ADAM10 (101). The investigation on cancer cell lines (293T, SW480 and HCT116) indicated that there is a significant influence of *ADAM10* overexpression on metastasis strengthening; moreover, this effect was amplified by simultaneous L1-CAM expression (neural cell adhesion receptor) (102). The most recent histopathological examination conducted on tumors collected from whole gastrointestinal tract, including CRC, showed the *ADAM10* overexpression. What more, the protein level correlated with the frequency of metastasis to lymph nodes and other organs. However, these studies do not show any correlation between *ADAM10* expression level and the 5-year survival rate (103). In the case of role of ADAM12 in CRC, a higher expression of this protein was detected on the outer surface of cancer cells. However, the research was conducted on cell lines, thus it is not reliable to show the full spectrum of this protein activity *in vivo*. It is also suggested that the activity of CR domain in ADAM12 may influence cancer cell adhesion to ECM and promote metastasis (25). The investigation on expression changes of CRC liver metastasis revealed increased level of *ADAM17* mRNA in most metastasis tumors in comparison with primary tumors which do not form such metastasis. It confirms a significant influence of ADAM17 on liver metastasis formation frequency (104). According to experiments conducted *in vitro* ADAM17 could become a potential pharmaceutical target in CRC treatment. Carcinoma cell lines grown in presence of 5-fluorouracil, a chemotherapeutic used in CRC treatment, show growth factors overexpression. These factors are the main reason of chemotherapy resistance of the cancer cells, as it as shown both *in vitro* and *in vivo* studies. It was also found that silencing of *ADAM17* by siRNA causes an enhancement of chemotherapy response (105, 106). In other study it was shown that the alternation in methylation status results in silencing of *ADAM23* gene. This phenomenon was found in both cell lines and cancer tissues obtained from patients. Re-expression of *ADAM23* was detected after cell line passage with the 5-aza-20-deoxycytidin dimethylating agent treatment. What is more, some of the cells expressing *ADAM23* have lost their cancer phenotype (107). Downregulation of DNA-methyltransferase-1 (*DNMT1*) and, as a result, *ADAM23* reactivation may be obtained by regaining a proper expression of miR342 (miRNA silenced in some CRC cell lines). Decreased proliferation and cancer cells' vitality was also observed in this experiment (108). According to research conducted on a large group of CRC tissues *ADAM29* gene undergoes frequent mutations. Unfortunately in this paper the mutation types were not described and additionally their influence on *ADAM29* protein and its expression, was not explained (109). On the other hand aCGH based studies point out that ADAM29 is frequently amplified in CRC tumors. Also in this paper the role of this phenomenon in the cancer development is not suggested (110).

Data obtained from a material collected from patients with CRC and with adenoma exhibited that *ADAMTS1* gene promoter was hypermethylated in both cancer types in comparison to a normal tissue of the same patient. It is well known that promoter hypermethylation, in most cases, causes the gene silencing (111, 112). The recent studies with the use of methylate bead-chip array-based technology confirmed increased methylation in case of *ADAMTS5* gene promoter. In addition the expression micro-chip method revealed (4) that the amount of the gene's mRNA is decreased in a tumor tissue. Micro-chip arrays allow to gain a large number of information at one time, what often is essential to indicate pathways of further research. It has to be stated however, that this results obtained using this technology need to be confirmed by other, more accurate methods. Investigation on a bigger group of CRC conducted using the novel HRM (high-resolution melting) method revealed increased *ADAMTS9* gene promoter methylation (113). It was also found that *ADAMTS12* gene promoter is significantly hypermethylated in comparison to of a normal tissue (88). The amount of proteins on the tumor cell surface increases according to the growth of distance from the tumor center (114). Taking into account the both above cited papers it can be concluded that ADAMTS12 may be a sufficient marker for an intraoperative examination in order to determinate the operation margin; it can also be treated as a prognostic marker. *In vitro* and *in vivo* research on tissues obtained from patients showed hypermethylation of *ADAMTS15* gene promoter and a correlation between this phenomenon and ADAMTS15 protein depreciation of the expression. Histopathological and immunohistochemical studies have shown that the level of ADAMTS15 protein expression is inversely proportional to the histological grade of tumor differentiation (115). Such results may suggest that the gene might be a novel suppressor. Moreover, *ADAMTS18* gene is also epigenetically silenced within CRC what has been emphasized by the HRM method; it is yet not proved via immunohistochemical studies (94). Furthermore, it has been observed that both *ADAMTS15* and *ADAMTS18* undergo frequent mutations in CRC cells but it is still unknown what type of mutations are they or how their function influences further expression or function of newly formed proteins (109).

As it was introduced in this review, adamalysines play essential role in CRC etiopathogenesis and metastasis. The quoted results seem to suggest that the ADAM(TS) may be potential tumor markers and predictors. This potential may be widely used in monitoring of treatment progression and specification of operation margins while CRC tumor resection. Further studies on adamalysines seem to be essential to explain the molecular mechanisms of this cancer which is seen in the role of adamalysines in cancer formation especially in the CRC etiopathogenesis. The large amount of various articles on ADAM(TS) proteins point out their essential role in physiological processes and in pathology. Unfortunately there is not

much research which would explain the correlations between this clinical data and adamalysines' function disorders. The fact that the ADAM(TS) are not separated proteins but they work as a part of various complex processes needs further studies as their full activity and regulations are still unknown.

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