Review paper

Mechanisms of carcinogenesis in colorectal cancer

Mechanizmy kancerogenezy w raku odbytnicy

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Abstract

Colorectal cancer should be considered as a heterogeneous disease that leads to many different genetic changes, resulting in the existence of molecular subtypes that differ in response to the same treatment and have different prognosis. For this reason, research into new, sensitive, and specific molecular prognostic factors has been intensified. It is now clear that there are many pathways leading to tumour formation in this organ because only about 10% of intestinal tumours have mutations in three "classic" Fearon-Vogelstein genes: APC, KRAS, and P53. The study of the relationship between molecular changes and clinical and pathological features reflects the evolution of the disease. Effective care for the sick depends on appropriate pathological evaluation and the ability to perform effective research on disease mechanisms.

Streszczenie

Rak jelita grubego powinien być uważany za chorobę heterogeniczną, do której powstania prowadzi wiele różnych zmian genetycznych, skutkiem czego jest istnienie podtypów molekularnych, odmiennie odpowiadających na taką samą terapię oraz mających różne rokowania. Z tego powodu intensyfikuje się badania nad poszukiwaniem nowych, czułych i swoistych molekularnych czynników prognostycznych. Obecnie wiadomo, że istnieje wiele szlaków prowadzących do powstania nowotworu w tym narządzie. Okazało się, że tylko ok. 10% nowotworów jelita ma mutacje w trzech „klasycznych" genach modelu Fearona-Vogelsteina: APC, KRAS i P53. Badanie związku między zmianami molekularnymi a cechami kliniczno-patologicznymi odzwierciedla ewolucję choroby. Od właściwej oceny patologicznej i trafnej kwalifikacji choroby zależy efektywna opieka nad chorym oraz możliwość przeprowadzenia skutecznych badań nad mechanizmami powstawania choroby.

Introduction

The genetic model of the development of tumours in the large intestine, although simplified, has set a sort of "action plan" in their study. It allowed the designation of further stages in the planning of research into this complex disease and has led to attempts to discover molecular markers of the early stages of neoplasm development, markers for colonies released from the outer layers of tumour formation in faecal specimens: mutations KRAS, P53, or APC, and marker of microsatellite instability Bat-26. The assumption has survived as a linear model of fundamental principles guiding the process of neoplasm in the large intestine [1].

Theme extension

Fearon and Vogelstein models

Colorectal cancer (CRC) cancer was the first such well-characterised molecular model in cancer research history. E. R. Fearon and B. Vogelstein noted that CRC is an excellent model to illustrate the effect of genetic changes on the process of tumour formation [1–3]. Previous clinical and histopathological studies suggested that the majority of malignant colorectal malignancies arise from pre-existing benign tumours (adenomas). Patients can be found at various stages of development, from small adenomas to large metastatic tumours. Since both the hereditary and the environmental factors have implications for the carcinogenesis of colorectal cancer, it allowed for the examination of hereditary and somatic genetic changes leading to neoplasm. The model proposed by Fearon and Vogelstein was as follows:

– colostral tumours are primarily caused by oncogenic mutation and inactivating suppressor genes;
– to develop malignant tumour, at least 4–5 genes must mutate; fewer changes are sufficient to produce a benign tumour;

– although genetic changes often occur in some preferred order, the biological properties of the tumour correspond to the total number and type of acquired lesions, not their sequence. In some cases, mutant suppressor genes produce a phenotypic effect even when they are in the heterozygous state [1, 2, 4].

Fearon and Vogelstein's model was developed by histopathology and clinical observations, showing that most of the malignant tumours in the large intestine are formed from pre-existing adenomas that gradually increase their invasiveness. Progression of the cancer occurs continuously, however, the authors, in order to maintain the simplicity of the model, gave it a staged form. The process of acquiring subsequent genetic changes usually lasts in decades, this is confirmed by studies on the incidence of cancer depending on the age, which show that the rate of tumor development is proportional to 4–6. the power of time that has passed, which in turn suggests the need for 4–6 independent events (acquisition of appropriate changes in the genome) [1, 2].

KRAS mutations

One of the most common genetic changes in colorectal tumours is the somatic mutations in the RAS family (mainly KRAS) [5–7]; about 50% of colon adenocarcinomas and adenomas larger than 1 cm have mutations in genes belonging to this family [8]. Based on this observation, Fearon and Vogelstein concluded that activation of RAS gene mutations results in the transition from the small benign tumour stage to the larger, more invasive stage through clonal expansion of the cell in which such mutation occurs. RAS proteins are small proteins that transmit signals from the receptors on the cell surface to the inside of the cell, which results in its normal proliferation and maturation. K-ras is a 21 kD protein, located in the cytoplasmic portion of the cell membrane and having GTPase activity encoded by a gene located on the short arm of chromosome 12. Activation of K-ras occurs after its binding to guanosine-3-phosphate (GTP), whereas its inactivation occurs after the distribution of GTP to guanosine-2-phosphate (GDP). In the case of an oncogenic mutation, most often within codons 12,13 and 61, the GTP binding domain is damaged and the K-ras is constantly activated, and thus also the constant transmission of the signal to the inside of the cell. Mutations of this type occur in about 50% of sporadic colorectal cancers [5, 6]. The K-RAS gene is one of the most commonly mutated proto-oncogenes in human cancers: colorectal, pancreas, and lung. It is located on the short arm of chromosome 12 locus 12.1. Gen is constructed of six exons, the first exon (denoted 0) is a non-translational region, the so-called UTR region (untranslated region), while the remaining five exons are codenamed 1 to 4. K-RAS proteins belong to the so-called family of small G proteins. They have high ability to bind GDP and GTP, and low GTP hydrolysis activity [4]. Activation of RAS proteins requires the reversal of the dissociation of GDP from the inactive protein form and GTP binding, which activates RAS and enables its interaction with the effectors [5, 7]. Control of cyclic GDP/GTP transformations, between the active form of the protein and the inactive form, is regulated by regulatory proteins. These include:

– GEFs (guanine nucleotide exchange factors) e.g. SOS responsible for GDP disconnection and inclusion of GTP, considered as small G protein activators;
– GAPs (as-activating proteins), e.g. NF1, p120, responsible for activating the inner GTP activity of small G proteins [4].

Regulators are affected by receptors differentiated on the surface of the cell membrane, such as the EGFR epidermal growth factor receptor, PDGF plate growth factor receptor, heterotrimeric G protein linked to integrin receptors, and the cytokine receptor, e.g. interleukin-2 (IL-2). The above-mentioned receptors are receptor or non-receptor tyrosine kinases involved in the transfer of information to the cell nucleus. Stimulation of the receptors by the corresponding ligands results in stimulation of various (ligand-dependent) cytoplasmic activation pathways. On these routes, the RAS protein acts as a specific nodal point. This means that stimulation, regardless of the aforementioned receptors, always leads to the activation of RAS proteins that bind and activate numerous effectors. The RAS protein effector binds to the active form of the protein. The interaction between RAS and the effector is possible by interactions between the RAS effector domain (32-40a) and the RAS binding domain (RAS) or RA (Ras-association) domain present in most RAS effectors [7]. So far at least 10 RAS effector kits have been identified, of which three are the family of serine-threonine kinases of Raf, RAL-GEFs, and PI 3-K 3-phosphotidylinositol kinase. The RAF serine-threonine kinase family includes: c-RAF1, ARAF, and BRAF. As the RAS protein effectors have an RBD domain, they can interact with the RAS protein. For the full activation of RAF, the interaction between RAS and the region of RAF-rich cysteine protein (i.e. 139–184 aa) is necessary. To date, no part of the Ras protein has been involved in this interaction. RAF kits are involved in regulating the MAP signalling path. RAS proteins control the processes of cell proliferation and differentiation (by determining the activity of transcription factors involved in the monitoring of gene expression involved in these processes). Recent research results suggest that the RAS/RAF complex may affect both the inhibitory and the activating apoptosis, depending on the type of cells [4–6].

Another RAS effector is 3-phosphatidylinositol (phosphoinositide 3-kinases PI3K) kinase. The PI3K family is made up of three classes; mainly class I is activated by RAS proteins. In this group of proteins,

the RBD domain is the p110 subunit. The family of these enzymes catalyses the reactions that produce PIP3 acting as a II-row relay. It has the ability to bind a number of kinases, causing them to change their conformation, activating them, and altering their cellular location. In addition, PI3-K activates protein kinase A PKB/Akt. This results in the phosphorylation of BAP proapoptotic proteins, which ultimately inhibits apoptosis [5, 9].

The RAS, whose biochemical function is not well established, is a family of GEFs that serve as RAL protein activators. This signal path modulates the activity of such proteins as RHO and RAC Cdc42. Through them, RAS proteins play a role in cellular cytoskeleton modelling and regulate the activity of transcription factors [10, 11].

Mutations in the K-RAS gene are considered to be an early marker of colorectal cancer development, but their usefulness in this aspect makes sense when simultaneously identifying the correctness of other genes that are mutated during carcinogenesis [9, 12].

According to the multidimensional model of colon cancer, in the sequence of events from adenoma to cancer, mutations occur in a number of different genes. According to numerous studies on the development of colorectal cancer, K-RAS oncogene is activated at an early stage [13].

Codons most commonly affected by mutations in the K-RAS gene are 12 and 13 in exon 1. Numerous studies indicate that, depending on which one is mutated, there will be other biological consequences. Mutation in codon 12 is associated with the mucosal histopathological type of colon cancer, while mutation in codon 13 is associated with a more aggressive tumour type and greater metastatic potential. It is also likely that mutations in the K-RAS gene induce more invasive cancer cell behaviour. The mutation rate at both K-RAS and BRAF mutations increases with the depth of the infiltration. In addition, studies show that K-RAS mutations are more common in the presence of lymph node metastases and distant metastases, which also confirms the hypothesis of increased cell mobility for K-RAS mutations [4–6].

The multicentre RASCAL study, published in 1998, aimed to explain the predictive value of the presence of mutations in the K-RAS gene. It was then suggested that the mutation in K-RAS proto-oncogene was related to a more aggressive course of the disease. There is as much as a 25% increase in the risk of death in patients with mutated genes, as compared to individuals with a genotype of the unmutated type. In patients with genetic predisposition for colorectal cancer, the K-RAS oncogene mutation was detected at a similar frequency as in patients with sporadic bowel cancer. Diet, lifestyle, and environmental factors may influence the onset of K-RAS mutations at early stages of cancer development [5].

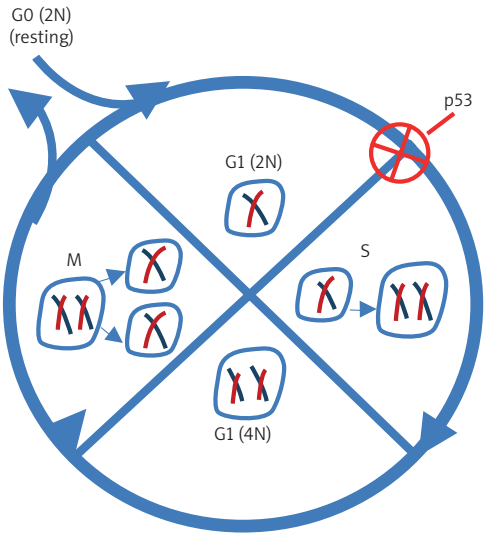

Figure 1. P53 mutations

P53 mutations

The opposite is the case with suppressor genes, where loss of function is most often associated with the loss of one of the alleles. One of the most common chromosome fragments in colorectal cancer (CRC) is the 17p region [14, 15]. The rate of loss or change in this region is up to 75% in malignant tumours, while in benign lesions it is up to about 10–30%. In the minimal deletion region, the P53 gene (the official symbol: TP53) has been identified. It is currently one of the most widely known suppressor genes, and the changes described above for colon cancer have been documented for most cancers. P53 is the most commonly damaged gene in human oncogenesis. Its protein product is called the genome guard because it is responsible for preserving and transmitting correct genetic information to the offspring. DNA damage activates the protein by P53 of the WAF1 gene encoding the P21 protein (WAF1).

This protein inhibits cyclin-dependent kinase (CDK) activity, which is required to pass from G1 cell cycle to S phase, further affects proliferating cell nuclear antigen (PCNA) and blocks DNA replication, which causes cell cycle arrest in G1 and enables DNA repair. If the DNA damage is too serious, P53 directs the cell to the path of apoptosis, i.e. controlled death by the proapoptotic action of the Bax protein and the imbalance between Bcl-2 and Bax. If P53 is inactive, aberrant DNA is transmitted to daughter cells and accumulation of genetic changes in subsequent generations [7]. P53 deficiency occurs late in Fearon and Vogelstein models and is directly related to malignant transformation in cancer, as evidenced by the fact that only 4% to 26% of adenomas and up to 75% of colorectal cancers show mutations in P53 [5] (Figure 1).

Within P53, many different point mutations have also been observed that result in amino acid substitutions, resulting in the formation of non-functional proteins. Thus, a hypothesis was made that a mutated P53 allele would provide a selective advantage, leading to tumour progression even in the presence of a second unmatched allele. The wild-type (WT) loss of wild allele is most commonly associated with the transition from adenoma to malignancy [11, 16–18].

DCC, SMAD2, and SMAD4 mutation

The second region, after 17p, most commonly showing the loss of at least one of the CRC alleles is 18q, where the loss of one of the alleles occurs in more than 70% of colorectal cancers and nearly 50% of late adenomas. Fearon and Vogelstein identified DCC gene (deleted in colorectal carcinoma) on this arm, which lies exactly in the 18q21.3 region [1]. The protein encoded by this gene has a significant similarity to the family of adhesion proteins and exhibits increased adhesion to the substrate. Initially it was thought that its loss was due to increased tumour metastatic potential, while subsequent studies did not confirm its significance, and two other genes were identified in this region: SMAD2 and SMAD4. Their products inhibit signal transduction for transforming growth factor (TGF-β), thereby affecting growth, differentiation, and cell apoptosis [4, 19].

APC mutations

The third most frequent loss of alleles in CRC is by adenomatous polyposis coli (APC). Adenomatous polyposis coli is a suppressor gene located on the long arm of chromosome 5, encoding a protein of 2843 amino acids with a mass of 312 kDa. This protein performs many functions in the cell, and interacts with β-catenin, 3β glycogen synthase kinase (GSK-3β), binding protein 1 (EB1), and Bub kinases. Based on studies of hereditary syndromes as well as sporadic cases of colorectal cancer, it is recognised that the primary events in the cancer-adenoma event sequence are disorders of regulation of APC and β-catenin complexes. In the normal cell, β-catenin forms an unstable complex with GSK-3β and APC, which results in proper degradation of this protein, whereas in the case of APC mutation, β-catenin accumulation occurs in the cell. The basic task of β-catenin is to form a complex with α-catenin and E-cadherin, whereas the extracellular domain of E-cadherin is responsible for intercellular adhesion. Disabling the functions of catenin and cadherin – as well as reducing the ability of cell adhesion – also affects their differentiation and their ability to invade. Also important for the development of cancer is the acquisition by APC of a phenotype of chromosomal instability associated with loss of heterozygosity, significant karyotype abnormalities, and abnormalities in DNA in the nucleus. This is probably due to abnormal binding of mutant APC to mitotic spindle microtubules, and EB 1 protein plays a very important role in this process. APC mutations are detected in 60% to 80% of sporadic colorectal cancers and the same number of adenomas, indicating the impact of this disorder on the early stages of CRC carcinogenesis. The normal APC protein is responsible for the antagonistic effect of the Wnt signalling pathway. Inherited mutations within APC cause familial adenomatous polyposis (FAP), which manifests itself in the formation of hundreds of adenomas in the large intestine. This gene is found in the region 5q21-q22, and the rate of loss of alleles within this arm is respectively: up to 50% in malignant tumors of the large intestine, about 30% in sporadic intestinal adenomas, while in adenomas arising in patients with familial adenomatous polyposis the loss of any of the alleles in this region is extremely rare. Familial adenomatous polyposis syndrome is inherited in an autosomal dominant way, and its essence is the presence in the large intestine and other portions of the gastrointestinal tract of hundreds to thousands of adenomatous polyps at about 20 years of age, followed by approximately 35–40 years of age with colorectal cancer, with malignant transformation occurring almost in all cases. It is estimated that 1% of colorectal cancers occur on FAP medium [4, 19].

Other chromosome aberrations

Studies of CRC mutations revealed that in addition to the aforementioned changes in arms 5q, 17p, and 18q, there were also fewer rarer aberrations, mainly deletions within lq, 4p, 6p, 6q, 8p, 9q, and 22q. This complex pattern of changes in the genome reflects two processes. First, some regions of chromosomes, or genes that are depleted or otherwise inactivated (mutations, methylation), appear to contain suppressor genes that are the "targets" of these adverse changes. Secondly, many of the delays in other regions, in a more complex way, may be partially generated in a "random" way as a result of previous changes and have no particular effect on the phenotype of the cell (although it cannot be ruled out that genes may be present in these regions. suppressive, and their loss may exacerbate changes in neoplastic phenotype [4, 9].

Knudson's theory

In the classic "double blow" theory, Knudson assumed that suppressor genes act in a recessive manner, i.e. both the maternal and allelic alleles must be inactivated to completely remove the suppressive function. So far, it has been thought that genetic syndromes that predispose to tumours arise from the hereditary inactivation of one of the alleles of the suppressor gene (unique to each syndrome). Tumours originating in patients with such genetic predisposition teams should have inactivated a second wild type allele in the region

specific to this suppressor gene cluster. In occasional tumours, the model of recessive suppressor genes presupposes that at least two genetic changes must occur to produce a noticeable phenotypic effect – and as a result of each of these events, inactivation of one allele of the suppressor gene occurs (e.g. through point mutation, mitotic recombination, or loss of part chromosome). However, such a reasoning implies a significant contradiction in the pattern of recessive character of suppressor genes in sporadic tumours, namely that it implies the effect of positive selective pressure after the first, even the smallest, genetic change, in this case – inactivation of the first allele. If the mutation of the first allele did not give rise to any selective advantage, the probability of a sufficient number of cells for the second mutation would be very small. This situation is perfectly visible on the example of mutations within P53: initial observations have already shown that the mutated mouse p53 introduced into the normal version of this gene (p53 WT) of primary rat cells together with the ras gene can give these cells tumor traits, despite the expression of wild p53 in these cells. For this reason, the assumption was made that at the cellular level mutations in P53 may function negatively dominating rather than recessively. This effect can be partially explained by the oligomerization of proteins formed from the mutant allele with normal proteins, causing inactivation of the WT allele product. These observations were hypothesised to provide tumour cells with selective growth advantage by mutation in P53, even with simultaneous presence of wild allele. The subsequent loss of WT alleles is often associated with progression from adenoma to malignant tumour and to the enhancement of the selective growth advantage provided by the P53 mutation.

Another evidence for such a model is the observation of tumors, which are an intermediate step between benign adenoma and malignant adenocarcinoma phenotype: the first of these tumors contained one mutant P53 allele and one WT allele that were expressed, giving mRNA at a more or less the same level. The P53 mutation was in all tumour cells, probably as a result of clonal cell expansion in which this mutation occurred. Fearon and Vogelstein, based on this, hypothesised that if the tumour was not removed, probably one of the cells would have lost a wild-type P53 allele, and as a result of the clonal expansion of the cell, the entire tumour would consist exclusively of this type of cell. In addition, loss of the wild allele would lead to tumour progression because intestinal tumours that have been lost within the 17p region are more aggressive than those in which there is no change in the region [4, 20].

Microsatellite instability

It is now known that there are many pathways leading to the development of rectal cancer. It transpired that only about 10% of intestinal tumours have mutations in three "classic" Fearon-Vogelstein genes: APC, KRAS, and P53 [20]. So the search for this additional, "unclassified" mechanism, which proved to be genetic instability, led to the loss of critical mechanisms to maintain DNA fidelity during cell division, and to remove the mechanisms that triggered apoptosis. The best illustration of the need for such an additional factor in the form of genetic instability is FAP. Although it is only functional abstinence of the APC gene that initiates the formation of hundreds of thousands of polyps, the vast majority of them will grow for several decades without serious injury to the organism. Looking at this situation in the context of sporadic CRC, single polyps (adenomas) appear to have a significantly higher relative frequency of transformation into malignant tumours.

The assumption of having similar-looking cancer lesions of important biological differences has originated from another genetic hereditary non-polyposis colorectal cancer (HNPCC). In this syndrome, unlike FAP, most polyps are rapidly transformed into malignant forms if they do not work before. The majority of adenomas in HNPCC patients exhibit loss of MSH2 or MLH1 protein activity (responsible for repairing misleading DNA bases) and exhibit one form of genetic instability, characterised by accumulation of numerous mutations, primarily in the repetitive DNA sequences. These sequences are most often located in non-coding microsatellite regions, hence the name of this phenomenon: microsatellite instability [21]. This mechanism was first described in colorectal cancer [4]. The syndrome is caused by the mutation of genes encoding DNA repair proteins. The mutant or repair genes are:

– MSH2 (human MutS homolog 2) on the short arm of chromosome 2,
– MLH1 (human MutL homolog 1) on the short arm of chromosome 3,
– PMS1 (human postmeiotic segregation 1) on the long arm of chromosome 2,
– PMS2 (human postmeiotic segregation 2) on the long arm of chromosome 7,
– MSH3 (human MutS homolog 3) on the long arm of chromosome 7, and
– MSH6 (human MutS homolog 6) on the short arm of chromosome 2, sometimes called G-T binding protein (GTBP) [20].

The MSH2 and MSH6 proteins bind to a heterodimer, known as hMutSα, which has the ability to find damaged DNA and connect to an abnormal fragment. Sometimes in this complex MSH2 and MSH3 combine to form hMutSβ, but this is much less frequent. Then, another heterodimer is added to hMutS, which consists of MLH1 and PMS2, termed hMutL, producing a large enzyme complex consisting of four proteins. In such a form it is capable of removing abnormal

DNA and replacing it with the correct sequence and complementing the complementary strand through the helix, nucleus, polymerase, and ligase acting in the cell nucleus. About 80% in cases of HNPCC, mutations undergo MLH1 and MSH2, most often exon 16 and 12 respectively. The result of these mutations is a phenomenon sometimes described as mutator, associated with two types of genetic disorder: microsatellite instability (MSI) and rapid accumulation of mutations of different genes [4]. Microsatellites are called single- to fourfold repetitions, which are scattered throughout the genome in an estimated number of 50 to 100,000, most commonly repetitive sequences of An and Can.

Correction of the repair gene functions leads to the blockage of the abnormal number of microsatellite repairs, and a phenotype, referred to as microsatellite instability, is very important in the disease process and diagnostics. After primary disabling of some MMR pathway genes, it is possible to detect high frequency MSI mutations in the whole genome, which is called high microsatellite instability (MSI-high – MSI-H), but if it is found in a smaller percentage of regions, it is a low instability (L-MSI). Tumours that do not show microsatellite disturbances are called microsatellite-stable. Short, repetitive sequences are also present in the coding regions of some suppressor genes, such as TGF-βRII or BAX. Thus, in the "MSI-H" phenotypes, these genes can be mutated and inactivated [20]. Most often, intestinal malignancies with the "MSI-H" phenotype are diploidal, with little loss or duplication. The difference in genetic instability at two levels: microsatellite (MSI-H), subtler, with respect to DNA sequences and chromosomal instability (CIN) for all chromosomes, or at least their arms should be emphasised. Both forms of genetic instability are mutually exclusive, so the CIN phenotypes will simultaneously belong to the MSS phenotype (microsatellite stable) [4]. At the same time, despite the existence of this opposition, it was found that the Fearon and Vogelstein model assuming the progression of all types of intestinal cancers through a sequence of similar events is hit to some extent – there were cases of patients and MSI-H cancer cell lines in which APC, KRAS and TP53 were mutated [1–3].

Epigenetic changes

In addition to the described MSI-H phenotype, there is a large – about 85% of all CRC cases – group of patients with MSS phenotype. It only accounts for about 10% of cases with the "classic" mutation kit APC, KRAS, and TP53. For the rest of the cases, a large group of DNA methylation [19, 22] can be isolated. The basic epigenetic change in man is methylation of cysteine in cysteine–guanine pairs (CpG). They are abundant in the promoter regions of about half of the genes – hence the name "CpG island methylator phenotype" (CpG) – in which hypermethylation causes loss of gene function due to blockade of transcription [23]. Like a mutation, hypermethylation is an irreversible process. It has been shown that some of the colon cancers are produced on the basis of this epigenetic disorder, with the genes associated with both Fearon and Vogelstein adenomatous mutation pathways and genes associated with microsatellite instability [23].

Hypermethylation was detected in the MLH1, P16, P14, and APC gene promoter regions. In most patients with sporadic colorectal carcinoma characterised by a microsatellite instability phenotype, MLH1 promotes hypermethylation of the MLH1 promoter region and suppresses its function [23]. This can be as much as 85% of these tumours. Tumours that have been found to be hypermethylated are more likely to have late-stage females, are proximal to the spleen bundle, are less histopathologically different, rarely have P53 and K-mutations in them, and the prognosis is worse. In these tumours, BRAF gene mutation (affects cell division, differentiation, and secretion) and chromosome-level stability are also very common [24]. Interestingly, tumors with the MSI-H phenotype but simultaneously being "CIMP-high" showed very similar clinical and pathological features: occurrence more often in women, later presentation age, lower degree of differentiation (higher grading), mucus form, round and vesicular nucleus cell with a distinct nucleus.

On the other hand, differences between the two groups have been identified that underline the need for patients to be considered for satellite instability: MSI-H/CIMP-H tumours are more likely to be diagnosed late than advanced, cancer cells lose connectivity during growth, lymphocytes infiltrate the tumour, but they respond well to adjuvant therapy with 5-fluorouracil (5-FU). The exact mechanisms responsible for the CIMP-H phenotype have not been fully identified, but some common features of patients with this phenotype have been recognised, suggesting a genetic background for CIMP. In addition to the frequent occurrence of BRAF mutations in these patients, colorectal cancer is more common in these patients.

In addition, strong methylation of DNA was observed in normal mucosal patients with hyperplastic polyps. Some patients with hyperplastic polyps develop several cancers, each with phenotypes of MMS, MSI-Low, or MSI-High. In view of this, it is possible that the syndrome of hyperplastic polyps is inherited as an autosomal recessive disease associated with numerous polyps and malignant tumours. Patients with one allele of the mutant gene may develop several polyps and thus may have a higher risk of developing CIMP-H. The early stages of CIMP-H tumours appear to be the same regardless of their MSI status – genetic modifiers may then affect the probability of methylation and gene inactivation, such as MLH1 or MGMT (enzyme responsible for repairing DNA after expo-

sure to alkylating carcinogens), which consequently will decide whether the tumour will pass on to the MSS, MSI-L, or MSI-H phenotype. CIMP-H or BRAF mutations may have the same genetic background and systemic factors, which is a good example of their prevalence in women. In addition, some environmental factors may affect the pathogenesis of this type of cancer. The increase in the risk of smoking-related CRC can largely be explained by having a BRAF mutation and/or a CIMP-H phenotype. Smoking is also associated with hyperplastic polyps, which may prove that the increase in risk is due to the earliest stages of disease development [19].

The distribution of colorectal cancers according to Jass

Due to the characteristics described above, i.e. the type of instability (or lack of it) and the presence or absence of methylation of DNA, colon cancer according to Jassa can generally be divided into five groups:

1. "Sporadic MSI-H" – MSI-H, CIMP-H, MLH1 methylation, BRAF mutation, stable at chromosomal level, developed from serrated polyps.
2. MSS or MSI-L, CIMP-H, partial methylation of MLH1, BRAF mutation, stable at chromosomal level, developed from serrated polyps.
3. MSS or MSI-L, CIMP-L, MGMT methylation, KRAS mutation, chromosomally unstable, developed from adenomas or serous polyps.
4. Negative IMP, CIN, mainly MSS, originated from adenomas (sporadic and associated with hereditary syndromes: FAP and MUTYH).
5. "Family MSI-H" – associated with Lynch syndrome, CIMP negative, no mutation in BRAF, chromosomal stable, MSI-H, formed from adenomas.

In addition to the aforementioned molecular features, the Jassa group is characterised by a variety of morphological and clinical features, including the type of precursor change, the degree of differentiation, and the ability to so-called "Budding" of the tumour. Modifications to the Jassa classification were introduced by Ogino and Goel. They proposed updating Jass's research according to the results of a new study and slightly differentiated between the groups that were also separated by MSI and CIMP status. The theoretical number of possible groups on this basis is nine (3 types of MSI × 3 CIMP types). However, Jassa argued that the analysis of the phenotype and clinical and pathological properties justified the existence of at most six distinct groups [25].

Conclusions

It is now clear that there are many pathways leading to tumour formation in this organ, because only about 10% of intestinal tumours have mutations in three "classic" Fearon-Vogelstein genes: APC, KRAS, and P53 [1–3]. Therefore, the following versions of general models of cancer were made: one of the most frequently cited works, in which the current state of knowledge on this subject is summarised, is the the work of Hanahan and Weinberg. The authors identified six categories of functional changes in the metabolism or physiology of cells, which must be acquired by them to give a fully malignant phenotype. These are:

- self-sufficiency in relation to growth signals (e.g. RAS oncogene activation);
- loss of susceptibility to growth inhibitory signals (e.g. loss of activity by the RB suppressor gene);
- ability to avoid apoptosis (e.g. carbohydrate production of growth factor IGF);
- unlimited growth potential (e.g. telomerase activation);
- ability for angiogenesis (e.g. VEGF production);
- ability to invade tissues and metastases (e.g. inactivation of E-cadherin).

The authors underline the variability and diversity of pathways leading to malignant potential [4, 26–28].

Conflict of interest

The author declares no conflict of interest.

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