Pathogenesis of selected multiple primary neoplasms

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ABSTRACT

Introduction and aim. Multiple primary tumors are defined as having more than one primary tumor in a different organ location in the same person. Therefore, it is important to know pathogenesis of multiple primary neoplasms to discover new forms of primary prevention and secondary prevention, especially connected with genetic tests which are important for the future of medicine as a part of personalized medicine. The aim of the study is to present selected aspects of the pathogenesis of multiple primary neoplasms.

Material and methods. PubMed databases and Google Scholar were searched.

Analysis of the literature. The rising risk of developing multiple primary cancers is a consequence of the progressive growth and ageing of the population and development of cancer in patients previously treated for cancer. The formation of secondary neoplasms may be multifactorial – to a large extent it is associated with genetic factors that may facilitate neoplastic transformation, for example as a result of radiation therapy, chemotherapy, inherited syndromes, environmental factors such as tobacco or alcohol, sometimes random somatic mutations.

Conclusion. Knowledge of the pathogenesis of multiple primary tumors can contribute to a better understanding of the problem, as well as help in the prevention or early diagnosis of multiple primary tumors (primary and secondary prevention).

Keywords. pathogenesis, multiple primary neoplasms, multiple primary tumors, oncogenetics, personalized medicine, tumor biology

Introduction

Multiple tumors are understood to mean two or more primary tumors in an individual that come from a primary place or tissue and are not extension, recurrence or metastatic. In cancer patients, the risk of developing new primary cancer is 20% higher than in the general population. Of those who have had cancer over the age of 60, about a third are diagnosed with more than one other neoplasm.1 Multiple primary neoplasms (MPN) are divided into three groups (Fig. 1): (1) neoplasms related to previous treatment, (2) neoplasms associated with disease syndromes, accompanied by an increased risk of cancer, (3) neoplasms in which typical factors play a role in the development of environmental or genetic predispositions. Moreover, multiple tumors can also develop by chance.2

Aim

The aim of the study is to present selected mechanisms of the pathogenesis of MPN.

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Received: 23.02.2023 / Revised: 2.05.2023 / Accepted: 9.05.2023 / Published: 30.09.2023


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Material and methods

There were searched PubMed databases and Google Scholar.

Analysis of the literature

Multiple treatment-related primary neoplasms

Secondary gliomas developing after radiation therapy

Gliomas are common secondary neoplasms associated with previous radiotherapy to the area of the brain or the cerebrospinal axis. Most often they belong to high-grade astrocytomas, which have a poor prognosis. López et al. conducted genetic profiling of 12 gliomas associated with prior radiotherapy to determine their molecular pathogenesis. The primary tumors were medulloblastoma, intracranial germinoma, leukemia, Hodgkin's lymphoma, craniopharyngioma, and pineocytoma. The interval between radiotherapy and secondary diagnosis of glioblastoma was between 4 to 41 years (mean 16 years). Ten of the secondary gliomas were high-grade infiltrating astrocytomas - they showed clearly aneuploid genomes, with significantly increased numbers of intrachromosomal breakpoints and focal amplification/homozygous deletions. The changes found were probably the result of DNA double-strand breaks caused by ionizing radiation. In secondary gliomas, a high frequency of TP53 mutations, CDK4 amplification or homozygous CDKN2A deletion, and amplification or rearrangement of receptor tyrosine kinase and Ras-Raf-MAP kinase pathway genes (PDGFRA, MET, BRAF, and RRAS2) were noted. It is worth mention that changes in the CDKN2A and CDK4 genes as well as a similar activation of the receptor tyrosine kinase and Ras-Raf-MAP pathway genes typically occur in high-grade spontaneous gliomas in both children and adults. López et al. speculated that the TP53 mutation is selected early in the development of radiation-induced gliomas, which allows the neoplasm initiating cell to survive and expand with numerous chromosomal rearrangements induced by ionizing radiation. It is worth mention that TP53 mutations have also been identified with high frequency in radiation-induced sarcomas. Of note, in secondary neoplasms, no changes were found in IDH1, IDH2, H3F3A, HIST1H3B, HIST1H3C, TERT (containing the promoter region) and PTEN, which are typical of the major subtypes of diffuse gliomas in children and adults. Unlike wild-type IDH gliomas in the adult cerebral hemispheres, high-grade radiation-associated gliomas only rarely contain an EGFR amplification or mutation, and are instead more likely to contain PDGFRA or MET amplification or mutations. Compared to both high-grade gliomas in children and wild-type IDH glioblastomas in adults, high-grade gliomas associated with radiation only rarely contain NF1 inactivation, and instead more frequently show BRAF rearrangement or high-grade focal RRAS2 amplification.

The other two types of gliomas observed in the study were anaplastic astrocytoma and glioblastoma multiform. It is worth mentioning that in both of these low-grade gliomas, there was no increase in DNA intrachromosomal copy number breakpoints, and focal amplification/homozygous deletions that are typically seen in radiation-associated high-grade gliomas. It is therefore uncertain whether these two tumors were actually caused by radiation. However, pathogenic changes identified in both tumors (KIAA1549-BRAF fusion and homozygous SMARCB1 deletion) were due to chromosome breaks rather than single nucleotide variants, could have been induced by ionizing radiation during treatment of the primary malignant neoplasm.

Secondary leukemias associated with previous chemotherapy

The definition of MPN corresponds to tumors. However, the secondary primary malignancies can also be hematological malignancies such as leukemia, lymphomas, and Hodgkin's lymphomas are the three most frequently primary cancers that develop secondary AML after treatment. The incidence of secondary AML associated with prior oncological treatment ranges from 0.8 to 6.3% after 20 years of conventional therapy, with a significant reduction in risk after 10 years. Secondary acute myeloid leukemia has a worse prognosis than its de novo counterparts, with a 5-year overall survival <30% despite advanced insight into pathogenesis and new treatment methods available. Poor prognosis in these cancers depends both on patient-related factors and those related to AML.

Genetic aberrations in hematopoietic stem cells and progenitor cells exposed to cytotoxic therapy may provide a survival advantage that leads to clonal proliferation and eventually manifests as secondary AML. The bone marrow microenvironment, including pluripotent mesenchymal cells and their descendants, endothelioma of bone marrow sinusoids, fibroblasts, reticular cells, adipocytes and catecholaminergic nerve fibers, regulates almost all functions of hematopoietic stem cells - altered functions of these cells, caused by the therapy, may also contribute to the pathogenesis of secondary AML. Cytotoxic drugs cause the release of many pro-inflammatory cytokines (TNF alpha, IL-6, TGF beta) and the generation of free radicals that damage both enzyme cells and niche autonomic nerve fibers. Their altered functions in several mice models have been shown to be sufficient for the occurrence of AML.

The use of drugs belonging to alkylating compounds and topoisomerase II inhibitors (TOPII inhibitors) is as-
associated with an increased risk of developing secondary AML. The mechanism behind this is due to the formation of double-strand breaks, which result in cell growth and apoptosis arrest. If double-strand breaks are not repaired, can generate chromosomal changes and genomic instability characteristic of these drugs.5

**Alkylating agents**

Alkylating compounds form cross-link DNA, leading to single-strand and double-strand breaks during the repair process. The result of these lesions are deletions in chromosomes 5 or 7 and haploinsufficiency of the EGR1 neoplasm suppressor gene on chromosome 5q, which are events initiating the pathogenesis of AML.6 The development of AML seems to be gradual, is often preceded by MDS and occurs after a period of about 5-7 years, caused by the deactivation of many suppressor genes.5

**TOPII inhibitors**

The mechanism of topoisomerase II inhibitors responsible for the development of secondary AML is based on their interference with DNA re-ligation and chromosome breakage.6 This results in chromosomal translocations that most often include the KMT2A genes on chromosome 11q23, RUNX1 on 21q22, and PML/RARA. Leukemia takes 1-3 years to develop and is almost never preceded by MDS. This short time is due to translocations that result in a dominant oncogene such as KMT2A fusing at 11q23, so fewer subsequent mutations are needed to transform to a leukemic phenotype.5

**Radiotherapy-related breast cancer**

Ionizing radiation directly and indirectly causes DNA damage and increases the production of reactive oxygen and nitrogen species (RONS). RONS lead to DNA damage and epigenetic changes that result in mutations and genomic instability. The proliferation of RONS increases the effects of DNA damage and mutations. This causes inflammation which additionally contributes to further carcinogenesis.7

We know that treatment neoplasms in childhood is connect with more recently occurrence of secondary primary tumors in the future. Brown et al. indicate in his study that people who have been treated at neoplasm in their childhood - have secondary primary neoplasms including breast cancers with poorer overall survivor due to poor prognosis risk factors. Although less than 3% of childhood neoplasm survivors develop second primary neoplasm within 15 years of their initial diagnosis, the cumulative incidence approaches 10% by 30 years after diagnosis in high-risk populations.8 Hodgkin lymphoma survivors had greater risk of secondary breast cancer influenced by age at treatment (higher among women exposed early to irradiation with the highest relative risk at 15 years or younger, and to be not significant among women diagnosed after the age of 40 or 50 years) and importantly time since treatment and dose (>40 Gy yielding greatest risk).9 Moskowitz et al. claim that highest risk of developing breast cancer have patients who received lower doses of radiotherapy (14 Gy) to a large area of the chest (whole lung field), which meant that a larger area of breast tissue was covered. Receive high-dose radiotherapy (30-40 Gy) to a smaller area of the chest (mantle field) have a similar or lower risk of developing breast cancer (mediastinal field), but an increased risk compared to women who have not been previously irradiated.7 Thukraa Shanmugalingam et al. suggest a IGF-1 role in increasing breast cancer risk which is the most common second primary cancer that develops in young women treated for Hodgkin's lymphoma with supradiaphragmatic irradiation. Pubertal growth of the mammary gland is mediated predominantly by the actions of IGF-1 and GH via estrogen. Among younger women more prone to developing a secondary breast cancer due to the increased levels of both IGF-1 and estrogen during puberty, and the promoting effects of IGF-1. In breast cancer, IGF-1 is mainly expressed in the stromal cells (especially fibroblasts) and also rarely in the breast epithelium. Consider also an endocrine role of IGF-1 effects on the malignant transformation of breast tissue. IGF-1 protect breast cancer cells from apoptosis and induces survival. Also suggest that IGF-1 produced by the stromal cells is increased in breast cancer. This may then promote growth of a second primary breast cancer by endocrine way.10 Radiotherapy breaks DNA, especially microsatellite alterations, which is more common in secondary than not-related with radiotherapy.11 Studies suggest increased survival of mammary epithelial cells with accumulating DNA damage and stepwise carcinogenesis due to premenopausal women with high IGF-1 levels were at risk of higher IGF1R activation in these cells.10

**MPN associated with syndromes**

Pathogenesis of MPN on the example of germ cell tumors - Wilms tumor and hepatoma in Beckwith-Wiedemann syndrome

**Nephroblastoma**

Wilms' tumor - also known as nephroblastoma, is a malignant neoplasm, the second most frequent in the pediatric population. This solid tumor occurs with a frequency of about 1 in 10,000 cases, while a bilateral Wilms tumor occurs in about 6 out of 100 children. This tumor is more common in girls than in boys, and the vast majority of cases, about 90%, are detected in children under 7 years of age.12 The family form occurs only in about 1% of patients.

The pathogenesis of this neoplasm is well described in the literature. A significant relationship has been shown between the presence of nephroma and the pres-
Hepatoblastoma is a malignant tumor of the liver that occurs most frequently in children under 2 years of age. This tumor originates from immature liver precursor cells, and in terms of histology, two main types are distinguished: epithelial and mesenchymal.

The epithelial type includes the following subtypes: fetal, pleomorphic, embryonal, macrotrabecular, small cell undifferentiated, cholangioblastic and mixed epithelial variants. The mesenchymal type, on the other hand, is divided into a subtype with and without teratoid features. Increased serum levels of alpha-fetoprotein (AFP) are found in 90% of patients.

In about 30% of patients with hepatoblastoma, coexistence of Beckwith-Wiedemann syndrome, FAP, Edwards syndrome, Li-Fraumeni syndrome, nephroblastoma and trisomy of 21 pair of chromosomes is found. However, most cases of hepatoblastoma occur sporadically.

Literature data indicate the involvement of a defect in the WNT signaling pathway in the pathomechanism. This abnormality is responsible for the accumulation of beta-catenin. Moreover, in the case of aggressive hepatoblastoma, activation of TERT and MYC has also been demonstrated.

In Beckwith-Wiedemann syndrome, a significant role of factors predisposing to the formation of hepatoblastoma, such as cell cycle regulators, MAPK kinase and (PI3K)/AKT, has been demonstrated.

Factors specific to hepatoblastoma oncogenesis – Beckwith-Wiedemann syndrome oncogenesis network – have been identified. Changes in 11p15 participate in hepatoblastoma oncogenesis by initially disrupting the balance of cell cycle regulators and chromatin organizers, including histone deacetylase 1 (HDAC1), ATP-dependent helicase X, and F-Box and WD repeat domain. In addition, oncogenic factors such as dickkopf WNT 1 and 4 signaling pathway inhibitor, WNT16, forkhead box O3 (FOXO3) and MAPK10 are differentially expressed in 11p15 altered HB both against BWS and non-syndromic background.

When comparing nephroblastoma and hepatoblastoma in terms of pathomechanism, an association consisting in the presence of a defect on chromosome 11 in both of these neoplasms can be noticed.

Pathogenesis of MPN on the example of neoplasms of epithelial origin – breast cancer and ovarian cancer in HBOC syndrome

Hereditary breast and ovarian cancer (HBOC) associated with BRCA1 and BRCA2 variants is characterized by an increased risk of breast cancer in women and men, ovarian cancer (including fallopian tube cancer and primary peritoneal cancer) and, to a lesser extent, other cancers such as prostate cancer, pancreatic cancer and melanoma, mainly in people with the pathogenic BRCA2 variant. The risk of concomitant cancer varies depending on whether HBOC is caused by the BRCA1 or BRCA2 pathogenic variant.

HBOC is a genetic cancer syndrome most commonly caused by germline mutations in the BRCA1 and BRCA2 genes. These are tumor suppressor genes located on chromosomes 17q21 and 13q12.3 respectively. These genes encode proteins involved in the repair of DNA double-strand breaks through homologous recombination, which is one of the key mechanisms for maintaining DNA integrity. To perform this function, BRCA proteins interact with many other molecules that together form a protein complex; without a functional BRCA complex, the cell relies on alternative DNA repair mechanisms, some of which are prone to errors and may further contribute to the development of genetic aberrations. Because of this phenomenon, HBOC patients with germline BRCA1 and BRCA2 mutations have an increased risk of developing a number of neoplasms, particularly those arising in the breast, as well as the ovaries and fallopian tubes.

BRCA1 and BRCA2 encode proteins, subject to expression in the cell nucleus in different tissues during S and G2 phases. BRCA genes are essential for maintaining chromosome structure. Therefore, due to their neoplasm suppressor function, they have been called “caretakers” of the genome because correct structure and function ensures genome stability. In contrast, any abnormality of the “caretakers” results in genome instability, which is an important factor in the pathomechanism of neoplasm.

The starting point for elucidating the functions of BRCA1 and BRCA2 relevant to cancer predisposition is the observation that mouse cells with deficient of the BRCA2 homologue show spontaneous aberrations in chromosome structure that accumulate during division in culture. Microscopically, abnormalities include not only...
broken chromosomes and chromatids, but also triple and quadruple-stranded structures, markers of defective mitotic recombination typical of human diseases, Bloom syndrome and Fanconi anemia, also associated with increased susceptibility to neoplasms (including breast cancer)\textsuperscript{22}.

Individuals with germline mutations in any of the BRCA1/2 genes are at higher risk of developing certain types of neoplasms compared to the general population. Most BRCA mutation-induced cancers develop in the breast or ovary. Some studies indicate a role for oxidative stress during the menstrual cycle in the development of ovarian neoplasms. In addition, hormone regulation, especially estrogen, increases the frequency of double-strand brakes, indicating tissue specificity.\textsuperscript{23}

Both BRCA genes are involved in DNA repair. Expression levels of BRCA1, BRCA2 and RAD51 increase in cells when they enter S phase, which indicate that they function during or after DNA replication. This means that BRCA1 and BRCA2 function in a common pathway that is responsible for genome integrity and maintaining chromosome stability. They form complexes that activate double-strand break repair and initiate homologous recombination. RAD51, on the other hand, is a key component of this mechanism. However, it appears that the roles played by BRCA1 and BRCA2 in this process are different.

Ubiquitination is the process by which proteins are tagged for degradation by the proteasome. BRCA1 acts with BARD1 in this ubiquitination process. Small ubiquitin-like ligases are required to localize BRCA1 in locations of DNA damage, and BRCA1 itself, post-translationally modified by sumoylation, acts with BARD1 as an E3 ligase and further ubiquitinates local proteins.

BRCA1 is part of the BRCA1-associated genome surveillance complex (BASC). This complex includes MSH2, MSH6, MLH1, ATM, BLM, the RAD50-MRE11-NBS1 complex and DNA replication factor C. All members of this complex play roles in the recognition of abnormal or damaged DNA, suggesting that BASC may serve as a sensor of DNA damage and as a regulator of the post-replication repair process.

BRCA1 also acts as a checkpoint, playing a crucial role in cell survival by preventing the propagation of DNA damage through cell cycle progression before DNA repair occurs.

BRCA1 is an integral part of the DNA damage signaling cascade, suggesting the existence of a positive feedback loop amplifying the DNA damage response. In addition, BRCA1 regulates the expression of G2M cell cycle checkpoint proteins, preventing an unplanned transition of the cell into mitosis at multiple levels of regulation. BRCA1 also plays a role in both transcription-coupled repair and global genome repair.\textsuperscript{24}

BRCA2 plays a role through its close interaction with RAD51 via BRC repeats. In addition, RAD51 also interacts with the C-terminal region of BRCA2, TR2. This part of BRCA2 play a regulatory role in recombination repair. Phosphorylation of this part of BRCA2 may have a dual function, causing inhibition or activation during homologous recombination. BRCA2 also plays a role in homologous recombination at meiosis by interacting with RAD51 and DMC1. This suggests that BRCA2 not only plays a role in carcinogenesis, but additionally contributes to fertility problems in affected carriers. In summary, both BRCA genes are involved in DNA repair and both act on a common pathway that is responsible for genome integrity and maintaining chromosome stability.\textsuperscript{21}

**BRCA1 breast cancers**

Most BRCA1 mutation-associated neoplasms are invasive ductal adenocarcinomas, accounting for 74\%\textsuperscript{21}. Morphologically, they are highly malignant carcinomas with no special type and show minimal, if any, tubular or glandular formation, markedly pleomorphic nuclei (significant variation in size and shape), vesicular chromatin, prominent nucleoli and high mitotic activity.\textsuperscript{24} However, compared to sporadic breast cancer, a much higher incidence of BRCA1-associated neoplasms are classified as medullary carcinomas, 2\% vs 13\%, respectively. The other histological types of breast cancer are found more or less equally in tumors associated with BRCA1 mutations as in sporadic breast cancer. With regard to other histopathological features, it is observed that BRCA1 mutations more often accompanied by a low degree of differentiation (G3), high mitotic activity, frequent areas of necrosis and reduced tubular formation, with a higher degree of pleomorphism. All these features indicate a more aggressive phenotype. In addition, the tumors are often well delimited and show a significant degree of lymphoplasmocyte infiltration and a high incidence of lymphatic vessel invasion.

HER-2/neu amplification is rarely found in tumors with BRCA1 mutations. One explanation may be that in the BRCA1 germline mutation background, HER-2/neu is lost during loss of heterozygosity at the BRCA1 locus because HER-2/neu is located close to BRCA1 on chromosome 17. Gene expression profile analysis has provided a tools to distinguish between different breast cancer subtypes. Based on this data, BRCA1-related breast cancers are classified as basal like type. The gene expression profile of BRCA1-associated neoplasms includes genes that have been found to have functions in proliferation, angiogenesis, cell motility, cell adhesion, transcription and DNA repair. As mentioned above, breast tumors with BRCA1 mutations show expression of basal markers such as CK 5/6, CK14, EGFR, P-cadherin and caveolin 1, vimentin and laminin, confirming the basal subtype specified by immunohistochemistry. These data additional highlight that carcinogenesis in BRCA1 ger-
mline mutation carriers very often occurs as part of the „basal” pathway of progression.

Promoter hypermethylation of tumor suppressor genes has been shown to be slightly less abundant in breast cancers associated with a germline BRCA1 mutation, although it is still markedly higher than in normal tissue.

Copy number alterations commonly found in BRCA1-associated breast cancers are gain of 3q, 7p, 8q, 10p, 12p, 16p and 17q and loss of 2q, 3p, 4p, 4q, 5q, 12q, 16p and 18q. This only partly coincides with the copy number changes found in sporadic breast cancers associated with germline BRCA2 mutations.21

BRCA2 breast cancers
Similar to BRCA1-related breast cancers, the most common histological type of BRCA2 tumors is invasive ductal carcinoma (76%). Reports have been published of a higher incidence of neoplasms belonging to the invasive (pleomorphic) lobular, tubular and situs carcinomas in BRCA2-associated breast cancers compared with sporadic breast cancer. BRCA2 tumors are more often moderately or poorly differentiated carcinomas (grades 2 and 3) due to less tubular formation, greater nuclear pleomorphism and higher mitotic rates compared to sporadic neoplasms.

In a study using gene expression analysis to distinguish BRCA2-related neoplasms, the differentiating genes were those related to transcription, signal transduction, cell proliferation, cell adhesion and extracellular matrix remodelling. In this study, relatively high expression of FGF1 and FGFR2 was observed, which was confirmed by immunohistochemistry.21 Using the gene expression profile mentioned earlier, the majority of BRCA2-associated breast cancers were classified as luminal. Looking more specifically at the molecular genetics, BRCA2-associated breast cancers show patterns of chromosome copy number gain and loss that do not occur in sporadic breast cancers. Copy number alterations more common in BRCA2-associated breast cancers are gain of 8q, 17q22-q24 and 20q13 and loss of 8p, 6q, 11q and 13q.21

BRCA1/2 ovarian cancers
BRCA1/2 germline mutations are found in approximately 15% of women with epithelial ovarian cancers, of which tubo-ovarian tumor is the most common subtype. The characteristic histopathological diagnosis of HBOC-associated fallopian tube-ovarian cancer with a BRCA mutation is high-grade serous carcinoma, and the prevalence of germline BRCA1 and BRCA2 mutations rises to approximately 25% in patients diagnosed with these neoplasms.

Morphologically, classic high-grade serous carcinoma shows expansive and infiltrative glandular and papillary growth with slit-like spaces. Cell nuclei are generally enlarged and irregular, with prominent nucleoli and active mitoses, including atypical forms. Immunohistochemically, serous carcinomas with a high degree of malignancy show p53 expression in an abnormal pattern (usually nuclear overexpression or complete absence of expression, and less often expression according to a cytoplasmic pattern), in addition to CK7, PAX8 and WT-1. P16 expression is usually diffuse, strong and block-like.24

In the context of BRCA-associated high-grade serous carcinoma, a variety of morphological features have been described. It was shown that tubo-ovarian carcinomas of BRCA1 germline mutation carriers tended to show high-grade and serous/undifferentiated histology, prominence of tumor-infiltrating lymphocytes, prominent nuclear atypia with giant/weird forms and abundant mitotic figures; these features had a negative predictive value of >94% and a positive predictive value of 21% for BRCA1 germline mutation status. Tumors with BRCA2 mutations also had pseudo-endometrioid and transitional features, but tended towards relative tumor-infiltrating lymphocytes deficiency and necrosis.24

MPN arising from environmental factors
Head and neck cancers are mainly squamous cell carcinoma and consisits oral cavity, pharynx (nasopharynx, oropharynx, hypopharynx), larynx, paranasal sinuses and nasal cavity, salivary glands (but salivary glands, sinuses, or muscles or nerves of head and neck cancers are not squamous cell carcinoma and much less common). Head and neck cancers share the same risk factors and it seems due to the anatomical proximity of each part and histopathology of these cancers.25,26

Contribution of tobacco consumption to the carcinogenesis of oral squamous cell carcinomas
Oral squamous cell carcinoma accounts for more than 90% of histopathological cases of oral cancer.27 Cianfriglia et al. described 200 cases of patients who had the first primary neoplasm as oral squamous cell carcinoma. In median follow-up of 3.2 years, the incidence rate of second primary neoplasm was 14%: 39% arose in the oral cavity, 18% in the oropharynx, 10% in the lung and 7% both in the lip and larynx. The second primary neoplasm in 96% of cases was also squamous cell carcinoma. One risk factor was the consumption of tobacco products.28 Eberl et al. indicated especially high larynx, pharynx and oral cavity for males and oesophagus, oral cavity and urinary tract for females secondary primary cancers smoking-related after treated lung cancer.29

Jiang et al. point to 4 possible pathways (Fig. 2) for tobacco smoke-induced carcinogenesis of squamous cell carcinoma: epigenetic changes of oral epithelial cells, inhibition of multiple systemic immune functions,
changes in oxidative stress and a possible “tobacco-virus collaboration.”

Fig. 2. Four possible pathways for tobacco smoke-induced carcinogenesis of squamous cell carcinoma and factors participate in pathogenesis

Epigenetic changes in epithelial cells
Several studies have shown that tobacco can cause abnormal expression of p53, GLUT-1, p16 (MTS, multiple tumor suppressor 1), DAPK (death-associated protein kinase), MGMT (O6-methylguanine-DNA methyltransferase), PI3K (the phosphatidylinositol 3-kinase) and other genes in the oral epithelium, which is associated with oral squamous cell carcinoma.

It has been observed that p53 aberrations are an initial alteration in the development of oral cancer. A high percentage of p53 protein overexpression has been found in pre-neoplastic lesions and oral neoplasms in epithelial cells among patients with heavy tobacco use.

Increased expression of GLUT-1, a glucose transporter required for glucose uptake by the cell, is observed in neoplasm cells. This indicates increased proliferative activity, energy requirements, aggressive develop and a poor response to ionizing radiation. GLUT-1 expression correlates significantly with the histological malignancy and stage (pTNM) of oral squamous cell carcinoma. Furthermore, GLUT-1 has been shown to increase the incidence of oral cancer in tobacco smokers.

Epigenetic alterations of the p16, DAPK, MGMT, PI3K, c-myc genes are frequently found in people with oral cancer.

Moreover, generally in squamous cell carcinoma of head and neck region, expression of alpha-7 nicotinic acetylcholine receptors (nAChRs) promotes proliferation and migration through phosphorylation of epidermal growth factor receptor (EGFR), protein kinase B (Akt), mammalian target of rapamycin (mTOR) and stimulation of beta-adrenergic receptors. Nicotine upregulates the expression of mesenchymal proteins (fibronectin and vimentin) and downregulates epithelial proteins (beta-catenin and E-cadherin), thereby supporting cell motility and invasion through induction of epithelial-mesenchymal transition (EMT). Nicotine can interfere with drug efficacy via cytochrome P450 (CYP)-mediated metabolism, glucuronidation and/or protein binding, which may affect the efficacy of anticancer drugs. Tobacco use also promotes a pro-inflammatory tumor microenvironment, which further supports tumor growth.

Inhibition of multiple systemic immune functions
Dysfunction of the immune system plays an important role in the escape of neoplasm cells from effector immune functions, leading to neoplasm development. The incidence of malignant neoplasms in immunocompromised patients is 100 times higher than in healthy individuals.

Tobacco-associated oral cancer is likely to be associated with multiple systemic immune abnormalities, particularly impaired CD4+ and CD3+ T lymphocytes and different regulation of IL-4 and IL-2 in subsets of CD8+ and CD4+ T lymphocytes in the peripheral blood. The findings suggest that tobacco may reduce IL-4 gene transcription.

The Fas receptor and FasL (Fas Ligand) system are associated with neoplasm immune escape. In tobacco-associated oral squamous cell carcinoma, up-regulation of FasL and down-regulation of the Fas receptor are observed.

Changes in oxidative stress
Tobacco, which is a foreign substance, has been proved to stimulate the body to produce more free radicals, which with increasing concentrations, can damage cellular components, ultimately leading to denaturation or mutation, parasitic infections, inflammatory diseases and neoplasms. The development of oral cancer has been proven to be linked to oxidative stress. Free radicals (i.e. reactive oxygen species - ROS, reactive nitrogen species - RNS and reactive oxygen metabolites such as hydrogen peroxide - H₂O₂, superoxide anions - O₂⁻, hydroxyl radicals - OH, nitric oxide - NO and malondialdehyde) can cause DNA damage. These include strand breaks, DNA-protein cross-links, base modifications, inhibition of DNA repair. In addition, lipid peroxides and reaction products with cell membrane fatty acids are formed. There is also reduced superoxide dismutase activity. In addition, one study found that smokers had a significantly higher risk of developing oral cancer than non-smokers based on erythrocyte glutathione reductase, superoxide dismutase, catalase and plasma thiol.

Possible cooperation between tobacco and the virus
Epstein-Barr virus (EBV), or human herpes virus 4 (HHV-4), resides in a latency period in healthy carriers and, under the influence of changing external factors, the virus can be periodically reactivated. EBV is often associated with various malignancies, such as Burkitt's

Several EBV proteins are expressed in oral squamous cell carcinoma tissues. This is associated with specific tumor phenotypes, indicating a correlation between EBV infection and neoplasm development. The induction of oral and nasopharyngeal squamous cell carcinoma is explained by tobacco-induced activation of EBV and HPV16. Further epidemiological and experimental studies are needed to confirm the interaction between tobacco use and EBV activation in cancer development and to reveal potential mechanisms.27

**Contribution of alcohol consumption and metabolism genes of this xenobiotic to the carcinogenesis of upper gastrointestinal cancers including cancers of the upper aerodigestive tract**

Lv et al. in an epidemiological study of 161 patients with multiple primary cancers indicated that the main carcinogens of gastrointestinal and respiratory cancers were tobacco and alcohol use.21

A link between chronic alcohol consumption and the incidence of cancer in humans has been proved.32 Smoking cessation and abstinence or moderation of alcohol consumption are key factors in the prevention of oral and pharyngeal and esophageal cancers.33

The mechanisms through which alcohol drinking confers an increased risk of first or second primary cancers are likely to be quite similar among cancers of the upper aerodigestive tract (oral cavity, pharynx, larynx, and esophagus).34 The mechanism of carcinogenesis in various alcohol-induced cancers is not fully understood, although likely events include genotoxic effect of acetaldheyde, generation of reactive oxygen species via cytochrome P450 2E1 (CYP2E1), abnormal folic acid and retinoid metabolism, increased estrogen levels and genetic polymorphisms.32

The results of the study indicate that in upper gastrointestinal cancer survivors, continued alcohol abuse were associated with a more than 2-fold increased risk of subsequent upper gastrointestinal cancers.34 Alcohol absorption begins in the mucosa of the upper gastrointestinal tract and takes place mainly in the stomach and small intestine. Acetaldehyde production occurs mainly in the liver, but acetaldehyde formation begins in the mouth and progresses along the gastrointestinal tract.35 Ethanol is oxidized in the cytosol to acetaldehyde by alcohol dehydrogenase, and acetaldehyde is then oxidized in the mitochondrion to acetate by aldehyde dehydrogenase.35 Exposure to acetaldehyde leads to molecular changes and mutagenesis, including the formation of DNA adducts, cross-linking of DNA-protein complexes, DNA strand breaks and chromosome aberrations. Locally, these changes can lead to dysplasia and further to oral and pharyngeal cancer.35 Various studies indicate that the disproportion between alcohol dehydrogenase and aldehyde dehydrogenase activity plays a key role in alcohol-induced cancers. Significantly higher activities of different isoforms of these enzymes are observed in esophageal cancer, liver cancer and cervical cancer, making it possible to attribute polymorphisms of related genes to these isoforms.32

Tamura et al. in a study conducted on rat epithelial cells showed that ethanol is not only an inducer of gastric epithelial cell necrosis, but is also an inducer of oxidative stress and causes increased lipid peroxidation.36 It is likely that the mechanism is related to the accumulation of lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal, which in turn form exocyclic DNA adducts. Reactive oxygen species can act as messengers in intracellular signaling pathways leading to the transformation of a normal cell into a cancer cell. These pathways affect cell cycle changes by activating NF-xB (nuclear factor kappa-light-chain-enhancer of activated B cells) and AP-1 (activator protein 1) (c-jun and c-fos) expression and promoting cell metastasis by regulating MAPK (mitogen-activated protein kinases). Accumulation of reactive oxygen species leads to up-regulation of vascular endothelial growth factor (VEGF) and monocyte chemotactic protein (MCP-1), which are key mediators of angiogenesis and tumor dissemination. Increased expression of the reactive oxygen species-mediated metalloproteinases MMP2 and MMP9 leads to extracellular matrix damage, increases cell motility and promotes distant metastasis.

**Metabolism gene variants**

Significant interactions between heavy drinking and the MTHFR (methyleneetahydrofolate reductase gene) TT genotype (homozygous variant with mutation) have been described for head and neck area cancer, esophageal cancer and colorectal cancer. Compared to people with the CC (homozygous normal) genotype, those with the TT or CT (heterozygous) genotype have MTHFR activities of approximately 30% and 65%, respectively.

An increased risk of esophageal squamous cell carcinoma in heavy drinkers is associated with the CYP2E1c1/c1 or CYP2E1c1/c2 genotype. Observations indicate that the c2 variant allele shows 10-fold higher transcriptional activity, elevated protein levels and increased enzymatic activity compared to the c1 allele.

The ADH1B gene shows several polymorphisms and is associated with the risk of developing various cancers. Two studies in Asian populations showed a significantly higher risk of upper gastrointestinal, oral cavity and hypopharynx cancer in moderate or heavy drinkers with the ADH1B*1/*1 genotype than in those with ADH1B*1/*2 or ADH1B*2/*2. The enzyme encoded by ADH1B*1/*1 has only 1% and 0.5% oxidative capacity against those encoded by ADH1B*1/*2 and ADH1B*2/*2, respectively.32

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The presence of the ALDH2 Lys allele has been shown to have a significantly increased direct effect on the risk of developing head and neck, esophageal and gastric cancers, even in people who have never smoked. In contrast, acetaldehyde-related carcinogenesis occurs locally in organs exposed to alcohol. It is assumed that alcohol abusers with the ALDH2 Lys allele are exposed to increased local acetaldehyde concentrations two to three times (via saliva) and five to six times (via gastric juice) higher than those without this allele. Increased acetaldehyde concentrations lead, among other things, to more DNA adducts and DNA damage.33

Bacterial flora and acetaldehyde metabolism
Acetaldehyde plays a major role in the carcinogenesis of alcohol-related upper gastrointestinal cancers, which is locally (oral mucosa and salivary glands) produced by microorganisms and also supplied with drinks and food products. Ethanol fermentation is a specific feature of microorganisms. Under hypoxia, food sugars such as glucose and fructose are converted to ethanol and acetaldehyde is formed as a by-product.

The microbial formation of acetaldehyde at mutagenic concentrations begins in the saliva and gastric juice in HCl-deficient stomachs immediately after alcohol consumption and continues as long as ethanol is present in the human body. The key role of the oral microbiome, particularly bacteria, is already well understood in this process.33

HPV-related secondary primary tonsil cancer
HPV infection is connected with head and neck squamous cell carcinomas. The molecular mechanisms of HPV-associated carcinogenesis involve the insertion of HPV genomic DNA into basal epithelial cells, leading to the expression of the viral oncoproteins E6 and E7. As a result, key cellular signaling pathways responsible for cell cycle control are altered by degradation of the tumor suppressor protein p53 via E6 and the retinoblastoma protein (pRb) via E7, leading to malignant transformation and immortalization of cells. In addition, HPV E6 protein interacts with c-myc to form the c-myc/E6 complex, which activates transcription of the human telomerase catalytic subunit (hTERT), contributing to tumor cell immortalization. Head and neck squamous cell carcinomas mainly are connected with tonsil cancer and likely same with HPV-related second primaries in this area apart tongue base and others. W. Strober et al. suggest that spreading HPV-related secondary tumour links with a single viral infection resulting in infection of cells at more than one anatomic location via intra-host spread (lymphatic, hematogenous, or salivary), infection of different tissue locations with the same virus at the same time, or on subsequent exposure or intralymphatic intraoropharyngeal metastases of tumor cells. Moreover, they claim that patient-specific (genetic and immunologic) and virus specific (such as particularly aggressive sublineages) could play a role. Multiple versus single primaries (younger patients, have small T1 tumor, no adenopathy N0) suggests different biological pathogenesis process but it needs more investigation.30,37

Diet and microbiome bacteria in second primary squamous cell carcinoma of head and neck
Certain bacteria are involved in the metabolic activation of carcinogenic chemicals such as acetaldehyde, which can promote carcinogenesis through genomic mutations. Chronic inflammation induced by persistent bacterial infection also supports several hallmark capabilities. Bacterial products, such as endotoxins, enzymes and metabolic waste, can cause DNA damage and consequently alter cell cycle control and signaling pathways, leading to further genomic instability and mutation. Certain immune cell responses to gut commensal bacteria are also associated with immunotherapy response. Red meat and processed meat also contain carcinogens (cooking at high temperatures produces carcinogenic substances such as PAHs, N-nitroso compounds (NOCs) and heterocyclic aromatic amines (HAA) that can cause genomic instability and mutations. All this factors can help in synchronous or metachronous carcinogenesis.30

Summary
Risk factors for the development of MPN include a previous treatment for neoplasm and should be considered an adverse effect of chemotherapy and radiotherapy. Therefore, an important element in the prophylaxis of secondary neoplasms is increased surveillance of people who have undergone neoplasm treatment, have a hereditary predisposition to neoplasms and have a specific lifestyle predisposing them to neoplasm (smoking, excessive alcohol consumption) or are exposed to environmental factors.38

Secondary neoplasms are considered the most serious complication of anticancer therapy and involve both chemotherapy and radiotherapy. The most common secondary neoplasm after chemotherapy is acute myeloid leukemia, which is usually preceded by myelodysplastic syndrome.39 Secondary leukemias are a consequence of the use of alkylating drugs (cisplatin, carboplatin, cyclophosphamide, ifosfamide) and topoisomerase II inhibitors (etoposide, irinotecan).30,41 Topoisomerase II inhibitors, including etoposide and teniposide, often cause rearrangements involving the mixed-lineage leukemia gene (MLL otherwise KMT2A) on chromosome 11q23. The prognosis is very poor for leukemias associated with rearrangements in the MLL gene, including secondary leukemias associated with etoposide. Typically, treatment-induced AML occurred after multiple
doses given in short intravenous infusions. The cumulative risk of complications over 4 to 5 years ranged from 0% to 18.4% in patients treated with cumulative doses ranging from 5200 mg/m² to 19200 mg/m².42

Radiotherapy can also have a significant impact on the development of secondary neoplasms. One example is radiotherapy for Hodgkin lymphoma. Although it significantly reduces the risk of regional recurrence and increases survival time as a part of treatment, it is associated with an increased risk of secondary neoplasm in the irradiated area. Less than 3% of childhood neoplasm survivors develop second primary neoplasm within 15 years of their initial diagnosis, the cumulative incidence approaches 10% by 30 years after diagnosis in high-risk populations.8

According to a 2017 study, the development of a second primary neoplasm in children, adolescents and young adults has a significantly worse prognosis than in older patients. The differences in survival rate after secondary and first neoplasm are most significant in those under 40 years of age.43 Therefore, it is important to know about the syndromes that run in the patient's family, which are associated with the occurrence of MPN and often manifest themselves at a young age, in order to implement appropriate prophylaxis.

Carcinogens such as tobacco smoke and alcohol are responsible for the development of numerous changes in tissues that are exposed to these substances. Smoking is a well-known factor responsible for the development of cancer in the respiratory tract, which is mainly related to direct exposure of the respiratory tract to the carcinogens contained in tobacco smoke. Long-term smoking can contribute to the simultaneous development of several neoplasms. The number of older people, as well as the number of people who have undergone treatment for neoplasm, is steadily increasing, which may result in an increased incidence of MPN in the future.1,38 Therefore, the timing of exposure to carcinogens and the age of the patient may be important factors in the occurrence of MPN.

Conclusion
MPN are the subject lots of research and scientific analysis. However, looking at the increasing incidence of both primary and secondary primary tumors, it is also a public health concern. Increasing knowledge of secondary neoplasm formation, analysis of epidemiological data and understanding of risk factors allows effective primary prevention using modern methods such as genetic screening to detect predisposing mutations, as well as secondary prevention with screening of those at increased risk of secondary neoplasms. The problem of the occurrence of MPN may become one of the elements of personalized medicine in the future.

Declarations
Funding
This research received no external funding.

Author contributions

Conflict of interest
The authors declare no conflict of interest.

Data availability
Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References
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Pathogenesis of selected multiple primary neoplasms


