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MSI and LOH in the development and prognosis of follicular cell-derived thyroid tumours

MSI i LOH w rozwoju i prognozowaniu przebiegu nowotworów wywodzących się z komórki pęcherzykowej tarczycy

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Abstract

Microsatellite instability (MSI) and loss of heterozygosity (LOH) represent molecular disorders acquired by the cell during neoplastic transformation. Both are associated with genetic instability. Functional silencing of tumour suppressor genes may be the consequence of genomic instability, particularly of the globally occurring LOH phenomenon. Numerous studies have confirmed the role of MSI/LOH at both the early and the late stages of thyroid tumourigenesis. This paper reviews the available study results on MSI/LOH significance and prevalence in thyroid neoplasms. Additionally, it summarises the knowledge regarding the practical usage of the study findings on MSI/LOH in aspects of cancer risk assessment as well as the development of prognostic markers for thyroid neoplasms.
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Key words: *microsatellite instability, loss of heterozygosity, thyroid neoplasms, prognostic marker*

Streszczenie

Niestabilność mikrosatelitarna (MSI) i utrata heterozygotyczności (LOH) to zaburzenia molekularne, które w znacznym stopniu wiążą się z nabywaniem przez komórkę nowotworową niestabilności genetycznej. Funkcjonalną konsekwencją niestabilności w genomie — a w szczególności globalnie występującego LOH — może być utrata funkcji genów supresorowych. Przeprowadzone liczne badania procesu nowotworzenia w gruczole tarczowym potwierdziły rolę MSI/LOH zarówno we wczesnych, jak i poźnych stadiach karcynogenezy. W pracy przedstawiono wyniki badań dotyczących znaczenia i częstości występowania MSI/LOH w procesie nowotworzenia w gruczole tarczowym. Artykuł jest także podsumowaniem stanu wiedzy na temat praktycznego wykorzystania rezultatów badań dotyczących MSI/LOH w ocenie ryzyka wystąpienia nowotworu i w opracowaniu markerów prognostycznych przebiegu choroby nowotworowej tarczycy. **(Endokrynol Pol 2012; 63 (2): 126–136)**

Słowa kluczowe: *niestabilność mikrosatelitarna, utrata heterozygotyczności, nowotwory tarczycy, marker prognostyczny*

Introduction

Thyroid cancer accounts for the majority of endocrine malignancy in the world, and its incidence is still increasing [1]. Follicular cell-derived thyroid carcinoma includes several morphological types: (1) well-differentiated thyroid carcinoma (WDTC), (2) poorly differentiated thyroid carcinoma (PDTC), and (3) undifferentiated (anaplastic) thyroid carcinoma (UTC; ATC) [2–4]. These types are phenotypically distinct, revealing different potential degrees of malignancy, from relatively indolent (as PTC, papillary thyroid carcinoma, belonging to WDTC) to highly aggressive tumours (UTC) with a poor prognosis. PDTCs displays intermediate biological and clinical features, and show a high tendency towards recurrence of symptoms, metastasis and progressive dedifferentiation of follicular cells [3, 5–7]. ATC is characterised by dynamic growth, local infiltration and multiple distinct metastases, as well as high lethality [8, 9].

In the WDTC group, about 90% of newly diagnosed cases represent PTC, and about 10% represent follicular thyroid carcinoma (FTC) [10]. FTC is the more aggressive tumour, with a poorer prognosis than PTC, displaying more rapid growth, local infiltration and higher rate of distinct metastases [11].

According to classic carcinogenesis theory, the development of a tumour derived from well-differentiated cells is associated with dedifferentiation, uncontrolled proliferation and loss of apoptosis ability. There are clinical, epidemiologic, and genetic evidences supporting the hypothesis of gradual progression and dedifferentiation of thyroid cancer derived from follicular thyroid cells [12, 13].

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Although understanding of the possible molecular mechanisms underlying this process is still insufficient, there have been several studies assessing the molecular factors that may play an essential role in the dedifferentiation of WDTC [13–15].

It is believed that one of these factors is genetic instability. This term refers to an accumulation of chromosomal aberrations and/or genetic mutations in cells undergoing neoplastic transformation [16–18].

Microsatellite sequences and MSI/LOH

Microsatellite sequences consist of long, tandem repeats of between one and six nucleotides. The number of nucleotide repeats varies, ranging, on average, from five to 100 segments, with a total length of repetitive DNA of 100–600 bp [19]. In the human genome, it has been demonstrated that the cytosine/adenine (CA)n sequence is a repetitive unit of the highest prevalence, being spread throughout thousands of locations [19–20]. Microsatellite sequences are found in all chromosomes, with an average density of about 14.000 bp/Mbp [21].

The changes in lengths of microsatellite sequences, for which the mutation rate is higher than in other regions of the genome, are the basis of genetic instability [18]. Microsatellite instability (MSI), defined as the occurrence of an allele of a new, and thus never observed, length, is one of the mechanisms associated with genetic instability [16]. The major contributor to microsatellite mutagenesis is DNA polymerase slippage during replication. It results in errors within microsatellites with a frequency 10- to 100-fold higher than the frequency of frameshifts in coding sequences [18, 20]. This process leads either to DNA transcript elongation or its shortening, depending whether wrongly paired base pairs are localised on a template strand or a daughter strand. Additionally, the loss of function of the DNA mismatch repair (MMR) genes, mainly of *MLH1*, *MSH2*, *MSH6*, the mutations in which are confirmed in a high percentage of neoplasms, is also the cause of microsatellite instability, manifested by constant and irreversible molecular changes [22]. The molecular mechanism of MSI is shown in Figure 1.

Loss of heterozygosity (LOH) is a phenomenon contrary to MSI. In this genetic disorder, one of the gene alleles is lost in a neoplastic cell [16]. The mechanism of LOH is chromosome-specific, and it

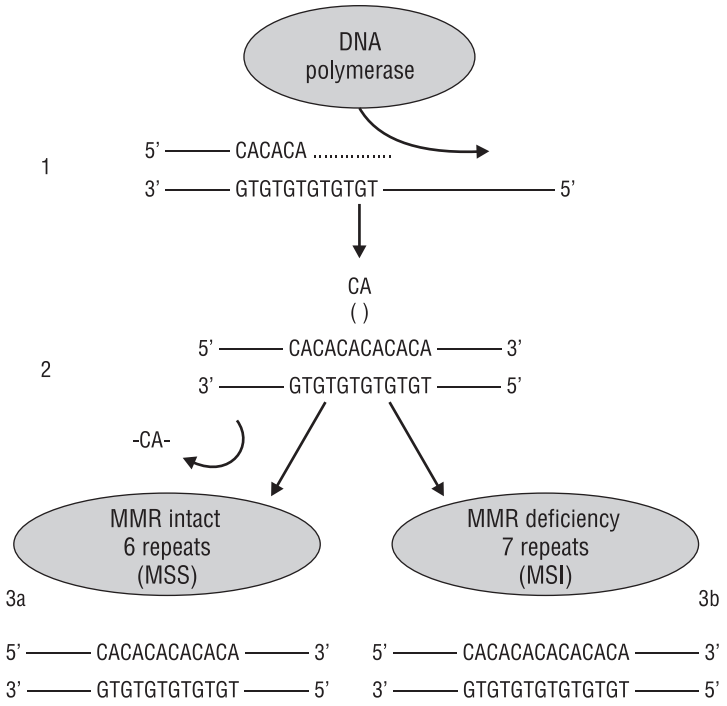

Figure 1. *Mechanism of microsatellite instability (MSI) formation. Modified on [20]. (**1**) DNA replication. (**2**) CA repeat, wrongly incorporated into the chain of replicated DNA. (**3a**) Maintained microsatellite stability (MSS) by an effective MMR system. (**3b**) MMR system defect: lack of elimination of wrongly incorporated into DNA nucleotides and resulting MSI*

Rycina 1. *Mechanizm powstawania niestabilności mikrosatelitarnej (MSI). Zmodyfikowano na podstawie [20]. (**1**) Replikacja DNA. (**2**) Błędnie wbudowane powtórzenie CA w łańcuch replikowanego DNA. (**3a**) Zachowanie stabilności sekwencji mikrosatelitarnych (MSS) poprzez działanie sprawnego systemu MMR. (**3b**) Defekt MMR; brak usuwania błędnie wstawionych nukleotydów w czasie replikacji DNA, powstanie MSI*

may concern the entire chromosome. However, loss of genetic material is more often associated with deletion of a fragment of chromosome, leading to LOH regarded as a generalised form of allelic imbalance. Loss of function of suppressor as well as mutator genes may be a functional consequence of the globally occurring LOH [17]. According to the "two hit" hypothesis postulated by Knudson [23], loss of function of both tumour suppressor gene (TSG) copies is required for an uncontrolled proliferation of modified cells and, eventally, for neoplastic transformation. In the classical mechanism of TSG inactivation, one allele is inactivated either by promoter hypermethylation or point mutation (e.g. substitution) or intragenic microdeletion, while the second allele is lost via LOH [17, 23]. Figure 2 shows genetic changes leading to silencing of suppressor gene functions in neoplastic transformation.

In MSI/LOH analysis, highly polymorphic microsatellite *loci*, called microsatellite markers, are used. These studies compare the changes in the length of microsatellite alleles between DNA isolated from neoplastic tissue and DNA from macroscopically unchanged tissue (control) of the same patient. The MSI/LOH phenomenon can be identified only when it simultaneously occurs in many tissues.

Then, it may serve as an indicator of the clonal expansion of cells, typical for a given neoplasm [20]. MSI is the basis of the so-called replication error phenotype (RER), and the neoplasms with confirmed microsatellite instability are classified as RER+ [16]. In turn, studies on LOH, using cytogenetics and molecular biology techniques, can identify the minimally deleted regions (MDR) in chromosomes. In neoplastic cells, loss of genetic material in MDR indicates the presence of suppressor or mutator genes [24, 25]. A practical use of the studies on MSI/LOH is associated with the possibility of neoplasm risk evaluation and with the development of informative diagnostic and predictive markers of the neoplastic disease course.

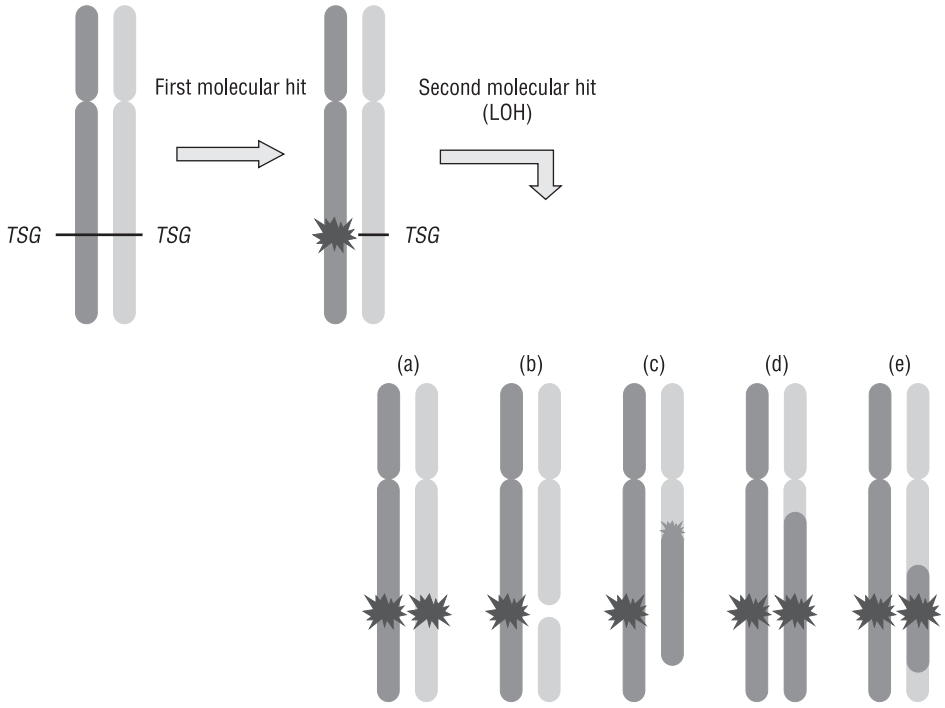

Figure 2. *TSG inactivating genetic changes, following Knudson's "two-hit" hypothesis. Modified on [17]. The first molecular event is the rare mutation in gene sequence (microdeletion or substitution), which — transferred in the germinal line — can become a cause of hereditary cancer. The second molecular event, LOH, is a mechanism frequently observed in somatic cells, leading to either hemi- or homozygosity of the chromosomal region via: (**a**) nondisjunction with reduplication of mutated TSG containing chromosome, (**b**) subchromosomal deletions, (**c**) imbalanced translocations, (**d**, **e**), miotic recombinations*

Rycina 2. *Genetyczne zmiany inaktywujące TSGs według teorii „dwóch zdarzeń" Knudsona. Zmodyfikowano na podstawie [17]. Pierwszym zdarzeniem molekularnym jest rzadka mutacja w sekwencji DNA genu (mikrodelecja lub substytucja), która może być przekazywana w linii germinalnej i może stać się przyczyną dziedzicznej formy raka. Drugie zdarzenie molekularne — LOH — jest częstym mechanizmem w komórkach somatycznych, prowadzącym do hemi- lub homozygotyczności regionu chromosomu na drodze: (**a**) nondysjunkcji z reduplikacją chromosomu zawierającego zmutowany TSG, (**b**) subchromosomalnych delecji, (**c**) niezrównoważonych translokacji, (**d**, **e**), miotycznych rekombinacji*

MSI in thyroid carcinogenesis

There have been few reports concerning the participation of microsatellite instability in the neoplastic transformation of thyroid follicular cells. The first study — with the use of three microsatellite markers (D2S123, D2S119, D2S147) localised on chromosome 2 and including the *MSH2 locus* — did not confirm MSI participation in the process of neoplasia in the thyroid gland [26].

However, later studies provided evidence for the presence of microsatellite instability both in benign and malignant lesions of the gland. They also proved a different incidence (0–25%) of MSI between the histopathological types of thyroid neoplasm [27–31]. Out of the various types of neoplastic thyroid lesions (PDTC, FTC, PTC, FA), the lowest percentage of samples with MSI within at least one *locus* was observed in poorly differentiated thyroid carcinomas [27]. Thus, microsatellite instability does not seem to play any key role in PDTC progression. MSI seems to play a rather limited role in the development of papillary thyroid carcinoma, for which, the percentage of samples with microsatellite instability is 7–17% [27–29, 31–33]. Moreover, the studies employing the Bethesda panel did not prove any significant role of MSI in the aetiopathogenesis of PTC, while emphasising the lack of relationship between MSI and mutations of repair genes, *MLH1* and *MSH2* [34].

A slightly higher incidence of microsatellite instability in at least one *locus* is observed in follicular thyroid carcinoma (8–25%) [27, 31]. There are indeed reports which indicate a close relationship between MSI in a given microsatellite *locus* (D2S123) on chromosome 2 (2p16.3) and FTC phenotype [35, 36]. These reports confirmed MSI in 100% of the studied FTC samples within the D2S123 *locus*.

Additionally, microsatellite instability in the D2S123 *locus*, demonstrated also in follicular adenomas (FAs), may support the view on MSI role in neoplastic transformation of the thyroid gland [35, 36].

It is worth noting that MSI has been observed not only in DNA from either FA or FTC, but also in preneoplastic lesions (i.e. in nodular goitre — NG or hyperplastic goitre) [27, 30]. It must also be emphasised that MSI occurs more often in non-neoplastic tissue than in either PTC or FTC [37]. These results suggest that microsatellite instability is a molecular mechanism associated with an early stage of carcinogenesis in the thyroid gland. Figure 3 presents the incidence of microsatellite instability, observed in at least in one *locus*, in selected lesions of the thyroid gland, according to the results of different studies.

It is also significant that, of all the studied thyroid neoplastic lesions characterised by MSI, 7–50% of tumours demonstrate microsatellite instability in more than 30% of used microsatellite *loci*, which qualifies these cases to the RER+ phenotype [27, 28, 36]. However, because of the varying numbers of employed microsatellite *loci*, the different techniques used for MSI analysis, and varying group sizes, the results of these studies are somewhat controversial and difficult to compare. Additionally, a precise technique of selection of neoplastic cells plays a significant role in MSI analysis. Such a method, employed to acquire and differentiate neoplastic cells from the surrounding neoplastically unchanged cells, is laser capture microdissection (LCM).

LCM has been used to prove a high incidence of malignant thyroid tumours (PTC — 64.2% and FTC — 62.5%) characterised by microsatellite instability — high (MSI-H) phenotype [36]. Following the criteria of MSI classification in neoplasms, MSI-H tumours are classified if two out of five studied markers, or

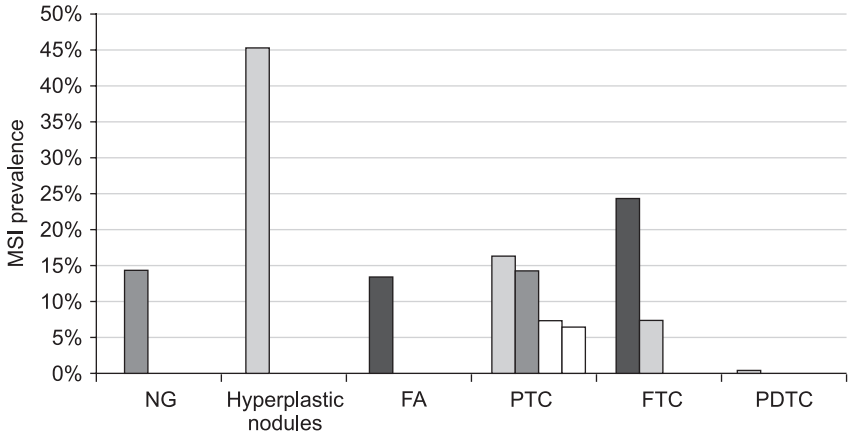

Figure 3. *Prevalence of MSI (in at least one locus) in neoplastic and non-neoplastic thyroid tissue, according to literature data [27–31]*

Rycina 3. *Częstość występowania MSI (w co najmniej jednym locus) w tkance nowotworowej i nienowotworowej gruczołu tarczowego, na podstawie danych z piśmiennictwa [27–31]*

more than 30–40% (in cases where a higher number of *loci* is evaluated) demonstrate instability [16, 20, 22]. Moreover, using the LCM method, it was found that 90% of FAs revealed microsatellite stability [36]. This suggests that the MSI status of cells can be a marker differentiating neoplastic lesions in the thyroid gland. Other reports, based on autoradiographic evaluation of electrophoretically separated PCR products, have confirmed an elevated incidence of MSI-H in follicular neoplasms (FA, FTC; 33%) as compared with PTC (3%) or Hurthle cell lesions (0%) [28]. These observations do not concur with the results obtained by Soares et al. [27], who observed MSI-H in 8% of studied PTCs, while not finding it in any FTC.

To sum up, due to the rather small number of studies concerning microsatellite instability analysis in thyroid neoplasms, as well as differing methods and analysed *loci* and, finally, to the controversial results of the studies, MSI can neither be regarded as a marker differentiating benign lesions from malignant ones, nor as a marker differentiating particular histopathological types of neoplasms.

MSI prognostic values in thyroid cancers

Attempts have been made to answer the question as to whether MSI can be a useful prognostic marker of thyroid cancer. On the one hand, no statistically significant relationship has been observed between the status of MSI and the patient's age at the time of disease diagnosis [36]; on the other hand, a correlation has been observed between the incidence of MSI and the advanced age of patients, when MSI analysis involved the *loci* of genes particularly important for thyroid functions [35].

Statistically significant differences in MSI incidence have been observed in the thyreotropin receptor (*TSHR*) gene *locus* in thyroid carcinoma in patients aged above 70 and below 70 (50% and 14.7%, respectively). It has been found that elderly patients, characterised by a higher incidence of MSI, have a better prognosis, indicating the usefulness of MSI as a prognostic tool [35].

The occurrence of MSI in the *TSHR locus* also demonstrates its significance in the process of thyroid cancer metastasis. MSI in the *TSHR locus* was significantly more often observed in the group of patients with lymph nodes metastases more distant from the primary tumour (N3–4), compared to either a group of patients without metastases (N0) or those with N1–2 metastases [35].

While analysing the significance of MSI as a prognostic factor for primary tumour size, it has been confirmed that thyroid tumours of size > 4 cm are more often characterised by stable microsatellites (MSS) [36]. The authors of this study suggested two different types of malignant thyroid neoplasms. The first included tumours in size 4 cm, which — following the histopathological identification — were probably qualified for immediate surgical removal; their status might have been that of MSI-H. The second type, i.e. tumours of size > 4 cm, previously submitted to observation and non-invasive prophylactic treatment, developed probably from benign precursor change (adenomas), and might have represented the status of MSS [36]. Therefore, it may well be assumed that, regarding the tumour progression from benign neoplastic to malignant form, microsatellite instability is neither a key nor a sufficiently causative molecular event. However, it should be kept in mind that other genetic factors (e.g. mutations in *RET, RAS, BRAF, MET* or *TP53*), also participate in the mutational pathway of thyroid neoplasms [38–42]. Additionally, beside mutations in the *RET* or *TP53* genes, the presence of MSI in the *loci* of both genes is very important for their inactivation. MSI in the RET *locus* has been observed in young (< 30) patients with PTC, who had in the past been exposed to radiation (i.e. the Chernobyl explosion) [43]. In turn, the presence of MSI in the *TP53 locus* [35] was associated with gene inactivation — regarded as the main genetic event in the dedifferentiation process of thyroid carcinoma cells towards ATC [44]. It is important that a high prevalence of MSI (36.5–45.9%) was also observed in the *locus* of triiodothyronine nuclear receptor (*THR 1*), exon 9 of which contains repetitive sequences of (CA)18.

However, no correlation has been observed between the presence of MSI in the *locus* of the gene with clinical features of the patients or with histopathological type of thyroid cancer [35, 36].

Survival data confirms a better prognosis for patients with thyroid neoplasms characterised by MSI than from thyroid neoplasms characterised by MSS phenotype [35]. This observation suggests the usefulness of MSI status as a prognostic factor.

LOH in thyroid carcinogenesis

The results of studies concerning the role of LOH in the process of neoplastic transformation of thyroid cells have demonstrated that inactivation of various genes by deletions in their *loci* is already present in preneoplastic lesions, being associated with the process of carcinogenesis initiation in the thyroid gland. However, the role of that phenomenon varies, depending on chromosomal region and the significance of the LOH-affected *loci* in the physiology of the thyroid gland.

LOH analysis in the *TPO locus* (2p24-25) — the key important gene in thyroid hormone synthesis — has revealed its presence in thyroid nodules. The lack of simultaneous somatic mutations in the gene suggested the possibility of deletions within other genes

of that region, participating in the process of neoplastic transformation [45]. Additionally, LOH has been demonstrated in colloid nodules of the thyroid gland and confirmed within the 10q22.2 *locus* [46]. Deletions of the 10q22-24 region have often been observed in thyroid follicular adenomas [46–49]. Moreover, studies performed in benign neoplasms, concerning different histopathological types, have demonstrated the highest percentage (75%) of LOH in 10q22-23 in atypical adenomas vs. benign lesions originating from the Hurthle cells (Hurthle cell adenoma — HA) (11%), or typical follicular adenomas (13%) [48]. These results suggest not only the possibility of using LOH as a marker differentiating histopathological subtypes of follicular adenoma; they also confirm the fact that FA progression from typical to atypical form may be associated with inactivation of TSGs in the 10q22-23 region [48]. It is known that this *locus* contains the *PTEN* gene, the dysfunction of which leads to uncontrolled cell proliferation [50]. So, it can be assumed that LOH in this *locus* may be associated with the progression of an atypical adenoma into a malignant neoplasm. However, the results of the studies are far from unequivocal. Some reports describe more frequent LOH in the 10q22-24 region in FAs than in FTCs [46, 49], while others have reported the opposite: a much higher percentage of LOH in this region in FTCs (100%) *vs.* FAs (13%) [51]. Probably, a slow progression from the stage of benign into malignant lesion — beside *PTEN* inactivation — requires also dysfunction of other genes [49, 51].

Loss of heterozygosity in other chromosomal regions, such as: 11p15.5, 6q24-q26 and 1p31.2 (83%, 50% and 60%; respectively) has also been more frequently found in atypical FAs *vs.* typical FAs.

Based on the above-mentioned studies, it may be concluded that certain potential suppressor genes, playing a key role in neoplastic transformation in the thyroid gland, are localised in those regions. Regarding the 11p15 region, the following imprinted genes are postulated: *CDKN1C*, *KCNQ1*, *IGF2*, *IGF2-AS*, *H19* and *INS* [52]. Understanding the mechanism of *KCNQ1* inactivation seems to be particularly important, mainly for its participation in thyroid hormone synthesis control. It is known that the *KCNQ1* gene product plays a significant role, together with the sodium-iodide symporter (NIS), in iodide transport in the cellular membranes of thyrocytes [53, 54]. Moreover, it has already been proven that kcnq1 protein deficit disturbs cell proliferation in the thyroid gland [55].

Despite the fact that the development of thyroid follicular adenoma may have strong associations with LOH, both within the long arm of chromosome 10 (10q) and the short arm of chromosome 11 (11p), this does not exclude the involvement of other deletion regions (e.g. 1p13, 3p14-25, 7q21.1-q31.2, 11p, 11q23 or 17p13).

The prevalence of LOH in selected chromosomal regions in cases of thyroid follicular adenoma is shown in Figure 4.

An interesting hypothesis as to the molecular sequence of genetic events in carcinogenesis in the thyroid gland was proposed by Sarquis et al. [52]. They suggested the role of the 15q11-q13 region, as a hot LOH site, associated with inactivation of *IPW*, *UBE3A*, *ATP10* genes and leading to a neoplastic transformation of normal thyroid cells towards adenoma. In turn, the imprinted genes: *ARHI* (1p31.2), *CDKN1C* (11p15.5), *IPW* (15q11-12), *SNRPN* (15q11.2), *SNURF* and *NDN* (15q13.1), in case of which a decreased expression was observed in FTC as compared with FA, take part in

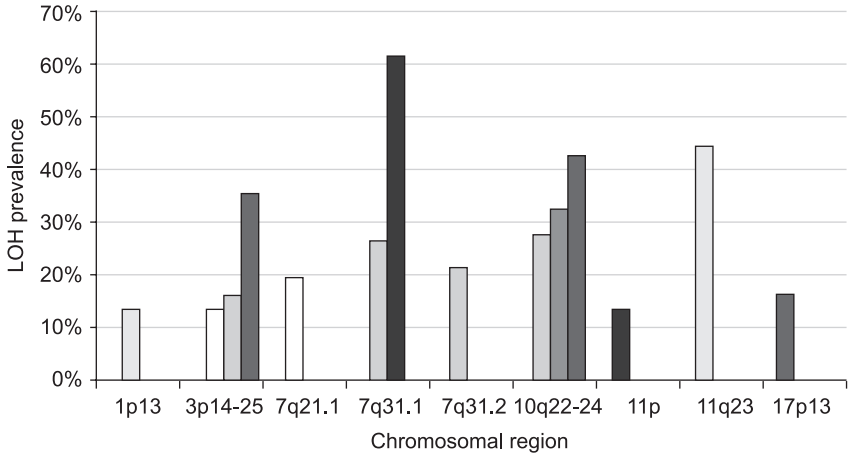

Figure 4. *Prevalence of LOH in various chromosomal regions in FA, according to literature data [31, 46, 47, 49, 56–58]*

Rycina 4. *Częstość występowania LOH w różnych regionach chromosomów w FA, na podstawie danych z piśmiennictwa [31, 46, 47, 49, 56–58]*

differentiation of typical adenomas into an atypical form, thus confirming their role in the early stage of neoplastic transformation towards malignant lesion (FTC) [52].

Loss of heterozygosity in the 7q21.1-q31.2 region is also regarded as an early and probably initiating event in the development of follicular adenoma [46, 56–60]. The progressive character of the process is particularly evident for LOH in the 7q31.2 *locus*, which is assigned the role in FA transformation towards follicular thyroid carcinoma [46, 56, 57]. The high incidence of the loss of heterozygosity in FTC (50–55%) and lower in FA (22–27%) — confirmed for D7S480 and D7S490 markers, which flank the region — suggest that the 7q31.2 *locus* could be the MDR, specific for FTC [56], similarly to the 7q21.2 (D7S492) region [60]. Moreover, the higher percentage of malignant tumours with LOH observed in those *loci*, vs. benign lesions may confirm the hypothesis that the development of FA and FTC is associated with a clonal event, occurring in a single precursor cell [56, 60]. Most probably, LOH in the 7q region also predisposes FTC cells to undergo dedifferentiation towards anaplastic thyroid carcinoma (ATC) [46, 59–60].

It should be emphasised that in thyroid tumours (ATC, FTC and PTC), within which LOH was studied in 7q, as well as the expression levels of HGF (hepatocyte growth factor) and of its receptor, HGF-R (*c-MET*) (7q21, 7q31; respectively), gene overexpression with a simultaneous lack of LOH was demonstrated in the PTC group only. Opposing results were obtained in FTC and ATC cases, where loss of expression and LOH presence were observed in the studied *loci* [59]. The results of that study suggest a certain usefulness of the microsatellite markers, localised in the 7q region, in differentiating FTCs vs. PTCs. They also reflect a genetic distinction of cells in these two types of thyroid neoplasm. Moreover, based on the above presented results, Trovato et al. [59] proposed a hypothesis regarding the localisation of other suppressors participating in the mutational pathway (FA → FTC → ATC), to be close to *HGF* and *HGF-R* genes. While it is true that a subsequent work by Trovato et al. [60] did not reveal any new genes within the 7q region which could play a significant role in neoplastic transformation of thyroid follicular cells, it did provide some important data, proving that LOH in the 7q21 *locus* is the key event in the FA → FTC → ATC mutational pathway. The observed mean fractional allelic loss (FAL) values were similar in goitres and hyperplastic lesions, while significantly increasing with neoplastic transformation, i.e. higher FAL values were calculated for FTC and ATC than for FA. The allelic loss at 7q21 – more frequent in FTC as compared with PTC — was found to be related to iodine-deficient geographical areas. Additionally, Trovato et al. observed that the mean size of thyroid gland with LOH was increased by up to 10 cm. Therefore, it was claimed that LOH in thyroid glands predisposed to intense growth stimuli [60]. Other authors have suggested that iodine deficiency influences MSI/LOH, and may serve as an indicator of defective MMR functions in thyroid tumourigenesis [61].

Beside the above-mentioned chromosome 7q — undergoing a selective loss in FTC — deletions in other chromosomal *loci* have also been described as a causative factor of follicular thyroid carcinoma development. These concern the following regions: 1p34.2, 2p, 2q, 11p13-15, 17p13 and 22q, in which, the mapped TSGs, e.g. *L-MYC1* (1p34.2), *WT1* (11p13), *TP53* (17p13.1), *NF2* (22q12.2) or *SNF5/INI1* (22q11.2) may be significant for FTC development [47, 51, 58, 62, 63]. In FTC, a high percentage of samples with LOH was observed, as much as 30-86%, depending on the studied chromosomal region (Figure 5).

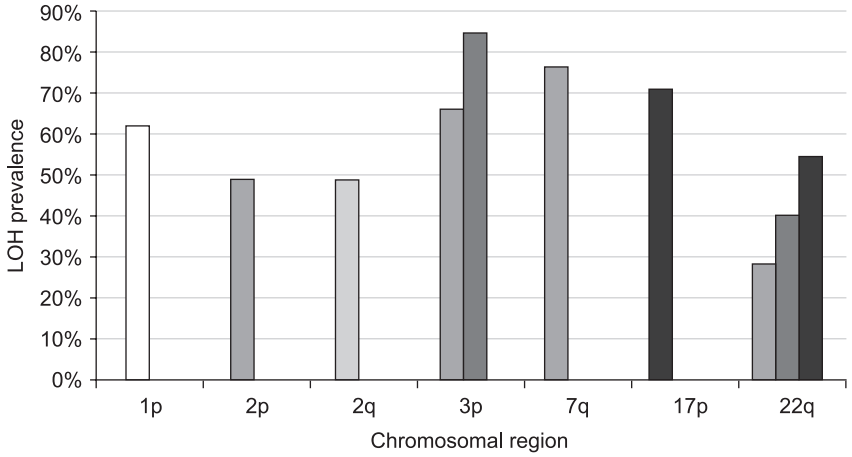

Figure 5. *Prevalence of LOH in FTC, in various chromosomal regions, according to literature data [31, 47, 56, 62–64]*

Rycina 5. *Częstość występowania LOH w różnych regionach chromosomów w FTC, na podstawie danych z piśmiennictwa [31, 47, 56, 62–64]*

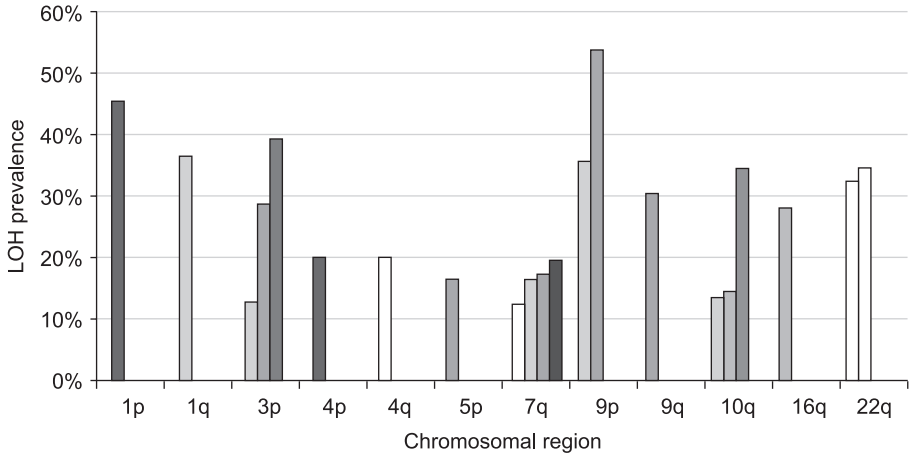

Figure 6. *Prevalence of LOH in PTC in various chromosomal regions, according to literature data [31–33, 46, 47, 57, 64, 67–69]*

Rycina 6. *Częstość występowania LOH w różnych regionach chromosomów w PTC, na podstawie danych z piśmiennictwa [31–33, 46, 47, 57, 64, 67–69]*

It also seems that a specific feature for FTC is LOH within the short arm of chromosome 3 (3p), where a few deletion regions are considered: 3p13-cen, 3p14.2~p14.1, 3p21.1, 3p24-25 and 3p26.3 [31, 47, 64]. The obtained results are, however, controversial, as in the study conducted by Rodrigues-Serpa et al. [31], 3p25.3~pter and 3p21.2-p12 regions were specific for FTC, while a relatively high prevalence of LOH in 3p25.3-p24.2 was confirmed in PTC development. In turn, other authors have pointed to 3p25~pter as a region of considerable loss of heterozygosity in PTC development [47]. The observations of LOH in the 3p region in FTC and FA become fairly interesting from the perspective of the possible identification of potential suppressor genes within this chromosomal region. Especially interesting given that their deletions may turn out to be helpful in improving the differential diagnostics of thyroid follicular neoplasms. LOH in the *VHL locus* (3p25.3), more frequently found in widely invasive FTC and angioinvasive HTC, turned out to be a molecular marker specific for these malignant neoplasms [65]. This fact was very significant regarding the function of *VHL* as a suppressor gene. It was proven that a loss of function of the gene was associated with disturbed control of cell survival, the progression of cell cycle, angiogenesis, cell migration and invasion [66].

An analysis of the loss of heterozygosity was also undertaken in cases of papillary thyroid carcinoma. In that type of neoplasm, the presence of LOH was estimated to be 13–54%, depending on the studied chromosomal region (Figure 6).

The relatively high prevalence of LOH in 9p in PTC has prompted a search for potential genes localised in that particular chromosomal region. It is postulated that LOH in *CDKN2A* and *CDKN2B* genes (9p21) could be the main mechanism, inactivating these genes, recognised as neoplastic suppressors and — probably — playing an important role in PTC development [33, 68, 69]. It is not excluded that inactivation of other genes, such as: *CMM* (1p36.2), *L-MYC1* (1p34.2), *PTCH1* (9q22.1), *TP53* (17p13) and *NF2* (22q12.2), localised in the *loci* with confirmed LOH, take part in PTC development and progression [68, 70]. Recently, the participation of LOH in *hOGG1* (major repair gene for free radical-induced oxidative DNA damages) *locus* in PTC development has been suggested. A high incidence of LOH in *hOGG1* has been found in PTCs and also in Hashimoto's thyroiditis (HTs), but not in benign goitres. This supports the hypothesis that thyroid follicular epithelium accumulates aberrant genetic changes in long-standing HT, which might represent a precursor lesion of PTC [71].

Studies to date have emphasised the considerable differences in LOH prevalence between PTC and FTC [31, 47, 58, 59, 64]. These observations not only suggest a different molecular pathway of development of these main two thyroid carcinoma types, but they also indicate their different biological features.

LOH prognostic value in thyroid cancers

There have been few reports regarding the prognostic significance of LOH in the thyroid gland. However, some researchers have pointed to progressive LOH incidence along with neoplastic transformation. The highest LOH incidence was observed for the more malignant carcinomas, such as HCC or FTC [60, 72]. For example, in the region of 17p13.1, loss of heterozygosity was more often associated with a higher stage of FTC development, as well as with its higher growth rate. Moreover, in that chromosomal area, a narrow mini-

mally deleted region, characteristic for the Hurthle cell derived carcinoma (regarded as more aggressive than FTC) was identified [58]. It should also be emphasised that in anaplastic thyroid carcinoma, a high percentage of LOH is found in various chromosomal regions [46, 60, 73–75]. Results of these reports suggest that LOH may be regarded as a late event in thyroid tumourigenesis, associated with the loss of tumour differentiation and increased degree of aggressiveness.

Some reports have suggested a hypothesis that LOH in a specific chromosomal region occurs selectively for a given histopathological type of thyroid carcinoma. Allelic deletions in 22q have been found to be more frequent in highly invasive FTCs with poor prognosis [76]. The author of that study did not find LOH in this region in case of PTCs, thus suggesting the usefulness of LOH analysis in 22q as a prognostic marker specific only for FTC. This hypothesis has, however, proved controversial, as other researchers have indicated LOH in this chromosomal region in various histopathological types of thyroid carcinoma, i.e. PTC, FTC and ATC, suggesting a participation of deletions in the 22q region as a key genetic factor in their development [69, 74]. Additionally, LOH within that region was associated with the mortality of patients in whom mainly PTC was diagnosed [69]. On the other hand, it has been accepted that LOH in the 3p25-26 *locus* (*VHL*) can be a good prognostic marker for patients with FTC and HTC, due to its association with tumour metastases, neoplastic disease recurrence and the mortality of patients [65].

Many reports analysing LOH as a prognostic factor of neoplastic disease, have pointed to the fact of simultaneous loss of heterozygosity in many chromosomal *loci*.

This observation seems fairly plausible, taking into account that carcinogenesis is a multistep process and its biologic potential is unlikely to be unravelled with the use of a single genetic marker. In particular, it is emphasised that the coexistence of genetic material loss within the following chromosomes: 1q, 4p, 7p, 9p, 9q and 16q is a more frequent feature of tumours in patients who have died of PTC [69]. Subsequent studies, which considered the consequences of genetic material loss on the biological course of multifocal PTC, prove a more frequent role of LOH in the following regions: 1p, 7q21, 17p13, 10q23 and 22q13 in spreading neoplastic foci within the gland [68]. A lower value of the FAL index (35%) was also demonstrated in DNA from patients with neoplastic disease (FTC) in their history, in whom neither thyroglobulin levels nor radioiodine examination indicated any recurrence of the disease. A considerably higher FAL index was observed not only in patients with documented distant metastases (78%), but also in those who died from FTC (67%) [77]. These observations may point to LOH coincidence in various chromosomal regions, leading to the development of aggressive forms of thyroid carcinoma and reflecting the clinical progression of neoplasm.

So far, only a few publications have reported a coincidence of LOH and/or MSI with a patient's age as a prognostic factor. On the one hand, there is a tendency towards more frequent allelic losses in tumours from older patients (> 45 years) with PTC [46], which is particularly important, as the risk of disease recurrence demonstrates a linear growth after the age of 45–50 [78]. It is also postulated that an increased incidence of LOH may contribute to clinical aggressiveness of carcinoma in these patients [46]. However, there are also publications which have either reported a tendency towards more frequent occurrence of LOH/MSI in tumours from patients aged below 45 [30] or indicated a lack of any association between LOH/MSI and age [32, 33].

A prognosis in the course of PTC may also vary, depending on its histopathological variants. LOH analysis in multiple regions (1p34-36, 3p24-26, 3p12-24, 7p31, 9p21, 10q23, 17p13, 17q21, 18q21, 21q22 and 22q13), performed within PTC, demonstrated that LOH coincidence in the *loci* of 3p24, 9p21, 17q21 and 21q22 might reflect a worse prognosis of disease course, particularly in the case of the diffuse sclerosis variant of PTC vs. the classical or follicular variant of PTC [68]. The observation of increasing LOH incidence (expressed as FAL index values), reflecting a worse prognosis, has also been confirmed in the oncocytic or tall-cell variant of PTC [70]. Very few publications have reported a correlation between the coexistence of *BRAF* mutation and LOH in 1p36, 18q21 and 22q13 and tumour size as a prognostic factor: they were significantly higher in PTC > 1 cm than in PTC ≤ 1 cm. The authors concluded that these genetic lesions might play some role in the faster growth of PTC [68]. Similarly, the frequency of allelic loss was statistically associated with tumour size > 4 cm, showing an average FAL of 30% and tumours < 4 cm with an average FAL of 11% [70].

In turn, the simultaneous loss of heterozygosity in 10q23, 17p13, 17q21 and 22q13 suggests an association of LOH in these *loci* with PTC metastases into lymph nodes [68]. Such an observation may be a hint to extend studies to search for markers differentiating the histopathological variants of PTC, or for prognostic markers of disease course.

Summary

Loss of heterozygosity seems to be a more frequent phenomenon than MSI, inducing genetic instability in thyroid neoplasms in many chromosomal regions. MSI may be causatively associated with the initiation of molecular changes only, which may later

lead to neoplasia, whereas the incidence of LOH overtly increases with tumour progression. The incidence of LOH and the numbers of *loci*, in which the loss of heterozygosity occurs, can coexist with mutations (*BRAF, RET*). This phenomenon increases with the degree of neoplastic progression, which indicates a successive accumulation of molecular disorders in cells and a coincidence of LOH/mutations in thyroid tumourigenesis. In the light of the actual studies, taking into account the controversial aspects of the available data, MSI and/or LOH cannot be regarded as informative prognostic markers.

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