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"Loss of Microsatellite", a New Type of Microsatellitic Change in the Telomeric Region of Chromosomes, as a Possible Characteristic Feature of Papillary Thyroid Cancer – Preliminary Report

Loss of microsatellite, nowy typ zmian mikrosatelitarnych chromosomowych rejonów telomerowych jako charakterystyczna cecha raka brodawkowatego tarczycy – doniesienie wstępne

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Abstract

Background. Papillary thyroid cancer is the most common type of thyroid malignancy. The prognosis is favorable, but only in cases where the illness is detected and qualified for surgical treatment very early. Therefore, early and reliable preoperative diagnostic tools of the most common thyroid cancer are still demanded. In this study changes in the telomeric region of chromosomes were observed which can be detected in various kinds of thyroid tumors by PCR. A third type of microsatellitic change was identified, not described until now as a separate unit, where the subtelomeric microsatellite disappears. The name LOM (Loss of Microsatellite) is proposed for this phenomenon.
Objectives. The aim of the study was to investigate LOM as a possible characteristic feature of papillary thyroid cancer.
Material and Methods. Genetic material from fifteen patients with three of the most common types of thyroid lesions (nodular goiter, follicular thyroid adenoma and papillary thyroid cancer) was investigated. DNA isolated from tumors tissues remaining after performing all necessary routine diagnostic tests was used. As a comparative material, DNA from the blood of the patients where tumor was removed surgically was used. PCR was applied.
Results. LOM was observed in 7 (46.7%) patients with papillary thyroid carcinoma, 4 (30.76%) with follicular thyroid adenoma, and 4 (30.76%) with nodular goiter.
Conclusions. LOM occurred in papillary thyroid cancer more frequently than in benign thyroid lesions (thyroid goiters and follicular thyroid adenomas). This is a preliminary report and the authors intend to examine LOM in a larger group of patients (**Adv Clin Exp Med 2008, 17, 1, 27–31**).

Key words: microsatellite, instability, papillary thyroid cancer.

Streszczenie

Wprowadzenie. Rak brodawkowaty jest najczęściej występującym nowotworem złośliwym tarczycy. Rokowanie jest dobre w przypadkach, kiedy zmiana jest wcześnie wykryta i zakwalifikowana do leczenia operacyjnego. Dlatego nadal poszukuje się doskonalszych i czulszych metod wczesnej diagnostyki raka brodawkowatego tarczycy. W przeprowadzonym badaniu za pomocą technik reakcji PCR autorzy zaobserwowali zmiany zakresu regionów telomerowych chromosomów w materiale otrzymanym z tkanek różnych guzów tarczycy. Po dokładnej analizie

obserwowanych zmian stwierdzono, że jest to trzeci typ zmian mikrosatelitarnych, które dotychczas nie były opisywane jako osobne zjawisko. Zjawisko to autorzy nazwali LOM *(Loss of Microsatellite).*
Cel pracy. Dokładna analiza zjawiska LOM jako potencjalnej cechy charakterystycznej komórek raka brodawkowatego tarczycy.
Materiał i metody. Do badania użyto DNA pobranego od pacjentów z trzema najczęstszymi patologiami gruczołu tarczowego (wole guzowate, gruczolak pęcherzykowy tarczycy, rak brodawkowaty tarczycy). DNA izolowano z tkanki guza pobranej podczas zabiegu chirurgicznego. Materiał porównawczy stanowiło DNA uzyskane z krwi obwodowej od tych samych chorych. Do badania wykorzystano technikę PCR.
Wyniki. Obserwowano zjawisko LOM u 7 (46,7%) chorych z rakiem brodawkowatym tarczycy, u 4 (30,76%) chorych z gruczolakiem pęcherzykowym tarczycy oraz także u 4 (30,76%) z wolem guzowatym.
Wnioski. Zjawisko LOM występuje częściej u chorych z rakiem brodawkowatym tarczycy w porównaniu z pacjentami ze zmianami łagodnymi (wole guzowate, gruczolak pęcherzykowy). Jest to doniesienie wstępne, dlatego autorzy chcą zbadać zjawisko LOM na większej liczbie chorych (**Adv Clin Exp Med 2008, 17, 1, 27–31**).

Słowa kluczowe: mikrosatelity, niestabilność, rak brodawkowaty tarczycy.

Thyroid nodules are a very frequent pathology and occur in 4–7% of the general population [1]. Some data suggest that thyroid nodules are presented in 30–67% of ultrasonography examinations and almost 7% of them are malignant [1, 2]. Thyroid cancer is the most common endocrine malignant disease and its incidence is increasing [3]. Papillary thyroid cancer (PTC) is the most common type of thyroid malignancy and makes up about 50–80% of all thyroid cancers [4]. The prognosis in PTC is favorable, but only in cases where illness is detected and qualified for surgical treatment very early. Therefore, early and reliable preoperative diagnostic tools of this most common thyroid cancer are still demanded.

Microsatellitic changes occurring in cancer tissue were described until now as microsatellite instability (MSI) or loss of heterozygosity (LOH) [5]. Microsatellite instability, identified with additional "alleles" which are different from those occurring in healthy tissues of the same patient, occurs commonly (with a frequency to 2/3) in various types of familial and sporadic cancers [6, 7]. Loss of heterozygosity (LOH) is identified with a total loss or significant decrease in the amount of DNA forming one of the alleles found in the cancer cells (in relation to the healthy tissue of the examined patient).

In sporadic cancers, LOH is considered fundamental for the initiation of cancerogenesis [8, 9]. Analysis of LOH is inseparably associated with the interpretation of the obtained results. Because fragments of surgically removed cancer tissue remaining after performing routine diagnostic tests are commonly used for LOH testing, the selection of the fragment designated for analysis is crucial. Proper surgical techniques require the removal of the tumor with the proper margin of healthy tissue left surrounding the cancer. Isolation of DNA from such a large piece of material gives a mixture of cancer DNA and DNA from healthy tissue. This makes interpretation of the results difficult. Attempts to solve this problem include genetic analyzers and primers labeled with fluorochromes or by making densitometric diagrams of electrophoretic analysis and dividing the total area under particular DNA bands from healthy tissue cells by the total area under analogous bands of DNA from cancer cells. A quotient smaller than 0.6 allows one to accept the presence of LOH [10]. Results with the lowest quotients, according to the proposition of Berti et al. [11], may be called AIM (allelic imbalance) and are uninformative.

During the progress of malignancy, developing tumors gradually accumulate chromosomal aberrations as a result of partial or complete loss of chromosomes. Loss of heterozygosity caused by microdeletion in subtelomeric regions of chromosomes were also reported by other authors, for instance on 1q [7], 2q [8], 3p [9], 3q [8], 4p [10], 4q [8], 5q [8,11], 6p, 6q, 7p [8], 8p [12] and 9p [8,13], and on 11q, 12p, 14q, 15q, 17p, 17q, 20p,

Table 1. Types of thyroid tumors investigated in the study

Tabela 1. Rodzaje zbadanych guzów tarczycy

Type of tumors (Rodzaj guza)	Number of cases investigated (Liczba zbadanych zmian)	Detected LOM percentage (Odsetek wykrytych LOM)
Papillary thyroid carcinoma (Rak brodawkowaty tarczycy)	15	46.7
Follicular thyroid adenoma (Gruczolak pęcherzykowy tarczycy)	13	30.76
Nodular goiter (Wole guzowate)	13	30.76

20q, 22q, and Xq [8]. Theoretically, a double LOH (on both chromosomes) is possible, so a vanishing of the microsatellite may occur on each chromosome and at each place. This phenomenon, described in 2006 by Berti et al. [11] and named DEL (complete deletion), has been observed in 2% of lung cancer cases. Other researchers involved in the genetic studies of microsatellites have probably also noticed this phenomenon but, according to our knowledge, have never published, especially as a separate type of the change. This report builds on this phenomenon in more detail, limited to microsatellites from subtelomeric regions of different chromosomes, especially from the p arm of chromosomes 3, 8, and 17, that seem to play a particular role in pathogenesis, at least in head and neck cancers [14]. Some authors suggest [15] that position 17p13.3 is occupied by some tumor-suppressor gene.

The aim of this study was to investigate LOM as a possible characteristic feature of papillary thyroid cancer.

Table 2. Chromosomal localization of the tested microsatellite systems and primer sequences

Tabela 2. Umiejscowienie chromosomalne analizowanych układów mikrosatelitarnych oraz sekwencje starterów

System	Location (Umiejscowienie)	Primers (Startery)
1QTEL19	1q	GGA GTT AAG GTT GAA GAG CC TTC ACG TAC AAC AGT ATC TC
2PTEL12	2p	CTG TGC TTC TGC AGG TTA GA TAC CTA GGT GAG AGT TAT CC
3PTEL01	3p	AGA GTT CTC TAG AGG GAC AG TTG CCT GCA GTG CTT CTG CC
3QTEL05	3q	TCA CAG TGG CCA AGA TAT CA TCC ATG TTG CTG CAA ATG AC
3QTEL06	3q	TCA CAA GGG AAA TAA CTG TTT TAC TTC CTG TAA CCC TCC AAA AT
4PTEL04	4p	CTA GTC TTG ATT CTA TTG ACC GGT CTA AAT CAA TGA CCT AAG C
5QTEL70	5q	CTA TTT TTA TTT CAG TTG GCT GTT T AAG GAA ACG TTC CTC TAA GTT ATT A
6QTEL54	6q	CAG AAC AGA TTA AGA CTC AG GCA TTT ATC AAC TTG TGT CC
9QTEL33	9q	ATC TGT GTT GGA TTC TTG GC ACT GAA CAC ACC TGT ACA GG
10PTEL35	10p	CAG AGA CTG GCA TTC CCA A GTC TTG AAA GTC ACC AGT CC
12QTEL82	12q	GTT CCA AGG GAG AGTTTC AT TAA AAT GAT AGT TTG CAC AAT AAT GG
13QTEL56	13q	TTG CAG TGA GCT GAT ATC GC TAA CAG GAT CTG TGT AAG CG
14QTEL23	14q	GAT CAC GCC AAA TAG TAT GT TGA GAT CTG TCT TGG AAA CC
17PTEL49	17p	AGT AGG TTT CAG TTG CCT TTT C AGA GAC ACA CAC AAT GAC AAT TAG
17QTEL13	17q	CTG GCC ACT CAA ATA TAA AC CAA AAT AAA AAC TGC AAG CAA TAT A
18QTEL11	18q	CCT ATT TAA GTT TCT GTA AGG ATG GTG TAG ACC CTG TGG AA
21QTEL14	21q	CTA AGG ACA CAT GCC CAA TG ACA GAG AAG GTG GGA GAT TG
22Q	22q	TTG CAG ACA GCA GAC TAC AGG TTC AGT CTG TGG CTG TCC AG
XYQ	XYq	GGC CTG AAT TCA TTT ATT CTA ATA G GAA CAG GCA AAG ATG CCC ACT CTC

Material and Methods

Genetic material from fifteen patients with the three most common types of thyroid lesions (nodular goiter, follicular thyroid adenoma, and papillary thyroid cancer) was investigated. These were obtained from the Institute of Oncology in Gliwice and from the Department of General, Gastrointestinal, and Endocrinological Surgery of Silesian Piasts University of Medicine in Wrocław. In these studies, DNA isolated from tumors tissues remaining after performing all necessary routine diagnostic tests was used. DNA from the blood of patients where tumor was removed surgically was used as a comparative material. The types of thyroid tumors are listed in Table 1 (see previous page).

The DNA used in this study, both from tumors and from blood, was isolated using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) or Sherlock AX (DNA Gdańsk, Gdańsk, Poland). Very small amounts (ca. ¼ mm^3) of the tested material was used to minimize the danger of obtaining a mixture of DNA from cancer and healthy tissues. However, this requires from the surgeon the removal of very small pieces of tissue, definitely originating from the tumor itself.

The investigations were accomplished by the PCR method. The primer compositions are described in Table 2. The primer sequences were according to those used by Rosenberg et al. [16] and Colleaux et al. [12]. PCR was run using the Qiagen Multiplex PCR Kit and Hybaid OmniGene thermocycler. The amplification program consisted of an initial 2 min. denaturation at 94°C followed by 32 cycles of 94°C for 1 min., 60°C (or 63°C for 1QTEL19) for 45 s, and 72°C for 45 s. Upon completion of the 32 cycles the reaction was finished with 20 min. of elongation at 72°C.

Results

LOM was observed in 46.7% of the patients with papillary thyroid carcinoma, 30.7% with follicular thyroid adenoma, and 30.76% with nodular goiter. The results (LOH and LOM) are shown in Figure 1 and Table 1.

Discussion

This study suggests that a new type of microsatellitic change exists, distinct from LOH, and involving subtelomeric regions. It can have considerable significance for the early diagnosis of cancer changes. It seems important that the diagnosis of LOM may be done by inexpensive and simple agarosis minigel electrophoresis as well as using genetic analyzers (DNA sequencers).

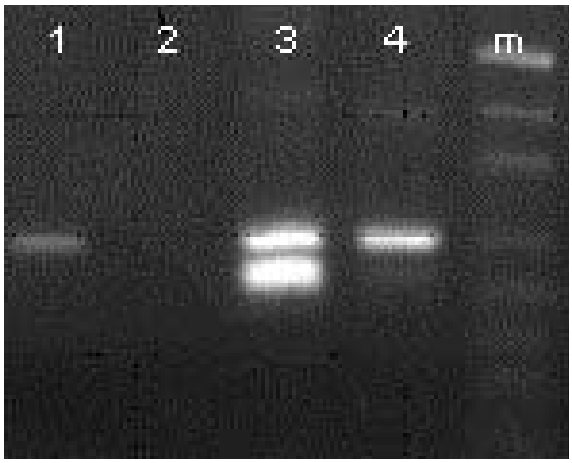

Fig. 1. Sample results: 1 – example of normal homozygote from blood, 2 – example of LOM in the same patient from tumor, 3 – example of normal heterozygote from blood, 4 – example of LOH in the same patient from tumor, m – size marker

Ryc 1. Przykładowy wynik otrzymanych obrazów: 1 – prawidłowa homozygota, badanie krwi, 2 – zjawisko LOM u tego samego pacjenta, badanie tkanki guza, 3 – prawidłowa heterozygota, badanie krwi, 4 – zjawisko LOH u tego samego pacjenta, badanie tkanki guza, m – obraz markera

The current explanation for the "immortality" of cancer cells is structural rearrangements in the chromosome, for instance by cryptic translocation [13, 17], in patients with myeloid disorders [16]. The present authors suppose that another mechanism is also possible: malignancy of a particular cancer depends significantly on its ability to reconstruct telomeres consumed during chromosomal division. Perhaps the "awakening" of fetal telomerase takes place in the cancer cell after consumption of the last telomeric unit. At this moment the normal cell would normally undergo apoptosis, but the "awakened" telomerase prevents this. The new, rebuilt telomeres do not terminate an intact chromosome, but a damaged one with shortened subtelomeric regions. These deletions could be a very early change characteristic in papillary thyroid cancers, and such a feature has been sought for early diagnosis and prediction. Further investigations should clarify if subtelomeric LOM is really a diagnostically useful early molecular change.

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