THE BRAF1796A TRANSVERSION IS A PREVALENT MUTATIONAL EVENT IN HUMAN THYROID MICROCARCINOMA

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The results show that BRAF1796A mutation is a prevalent event in human thyroid microcarcinomas irrespectively of the genetic background of adult patients. Mutated BRAF might be involved in the control of tumors architecture and growth pattern but its presence does not correlate with manifestations of papillary microcarcinomas (PMC) aggressiveness.

Whereas BRAF mutation comprise a common genetic alteration in conventional papillary thyroid carcinoma (PTC) and PMC, a number of differences in mutation-related associations between the two types of tumors rather supports the hypothesis that not all macroscopic PTCs may arise from papillary microcarcinoma.

Key words: papillary microcarcinoma of thyroid cancer, PCR, BRAF mutation.
Introduction

Among the human thyroid malignancies, papillary thyroid carcinoma (PTC) is the most prevalent [1]. Tumors measuring less than 1 cm in maximal dimension are referred to as microcarcinomas. Thyroid papillary microcarcinomas (PMCs) are mostly regarded as incidental findings of indolent behavior [2]. However, on rare occasions such tumors may behave aggressively and give rise to local nodal disease and distant metastases [3].

The $\text{BRAF}^{T796A}$ transversion is one of the particular targets for investigations in differentiated and anaplastic thyroid cancer [4] but less is known about PMCs.

This study was set out to determine the prevalence of $\text{BRAF}$ exon 15 mutation in PMCs and to elucidate the association between the presence of mutated $\text{BRAF}$ and PMCs’ morphology and indices of aggressiveness.

Material and methods

Thyroid tissues. The study included 17 and 31 Japanese and Russian cases of PMC, respectively.

DNA extraction. Microcarcinoma tissue was microdissected from formalin-fixed paraffin-embedded tissue sections under microscopic control. DNA was extracted using Proteinase K/phenol protocol.

$\text{BRAF}$ mutation screening by direct sequencing. Mutational analysis of $\text{BRAF}$ exon 15 was performed by PCR followed by direct sequencing. Genomic DNA was amplified in two 40-cycle rounds of identical PCR. The products were resolved in agarose gel, excised, purified and sequenced.

Statistical analysis was performed using Mann-Whitney rank sum test, Fisher’s exact test and logistic regression analysis as appropriate.

Results

In the 48 microcarcinomas, $\text{BRAF}^{T799A}$ missense mutation was found totally in 13/48 (27.1%) of cases: 4/17 (23.5%) and 9/31 (29.0%) in Japanese and Russian patients, respectively.

The patients’ gender, age at presentation, lymph node involvement, presence of distant metastases and histological variants of tumors are summarized in table 1.
Association of $BRAF^{T1799A}$ mutation with patients’ clinical profile and tumor morphology in the series of thyroid papillary microcarcinoma

<table>
<thead>
<tr>
<th></th>
<th>$BRAF^{T1799A}$</th>
<th>Wild-type $BRAF$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients, n=48</td>
<td>13</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Japanese/Russian</td>
<td>4/9</td>
<td>13/22</td>
<td>0.747$^a$</td>
</tr>
<tr>
<td>Median age, years$^b$</td>
<td>45.0</td>
<td>52.0</td>
<td>0.354</td>
</tr>
<tr>
<td>Gender, Female/Male</td>
<td>10/3</td>
<td>29/6</td>
<td>0.669</td>
</tr>
<tr>
<td>Lymph Node Metastasis, No/Yes</td>
<td>10/3</td>
<td>27/8</td>
<td>1.000</td>
</tr>
<tr>
<td>Distant Metastases, No/Yes</td>
<td>12/1</td>
<td>31/4</td>
<td>1.000</td>
</tr>
<tr>
<td>Tumor histotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>3</td>
<td>10</td>
<td>1.000</td>
</tr>
<tr>
<td>Follicular</td>
<td>1</td>
<td>15</td>
<td>0.036</td>
</tr>
<tr>
<td>Mixed papillary and follicular</td>
<td>3</td>
<td>5</td>
<td>0.664</td>
</tr>
<tr>
<td>Solid/trabecular variant</td>
<td>0</td>
<td>1</td>
<td>1.000</td>
</tr>
<tr>
<td>Mixed with solid/trabecular structures</td>
<td>6</td>
<td>4</td>
<td>0.015</td>
</tr>
</tbody>
</table>

$^a$ — Difference in the $BRAF$ mutational rate between Japanese and Russian groups.

$^b$ — Here and hereafter results are presented for the combined Japanese and Russian PMC series.

Discussion

$BRAF$ mutational rate in PMCs from Japanese and Russian patients accounted for about less than 30% of cases displaying insignificant difference between the two groups with distinct genetic background. Thus, $BRAF$ mutation is rather prevalent in PMC but in contrast to macroscopic PTCs its rate is generally lower than that in the latter entity [5, 6]. In spite of this circumstance, our results suggest determination of $BRAF$ mutation in the biological material derived even from small sized thyroid tumors may be informative as a means of preoperative diagnosis of PMC.

In the analyzed series there was a negative correlation between the $BRAF$ mutational frequency and follicular histotype of the tumors, and on the contrary, the $BRAF^{T1799A}$ rate was elevated in the PMC with mixed morphology characterized by the co-occurrence of well-differentiated and less differentiated (solid/trabecular) structures. In PTC, $BRAF$ mutation is known to associate with conventional histotype and it is nearly absent from the tumors with follicular architecture. PMCs are partly similar to PTCs in respect of negative association between $BRAF$ mutation and follicular morphology but do not prove correlation with papillary growth pattern.

Analysis of nodal disease and distant metastases association with BRAF mutation showed a lack of such in PMCs demonstrating that the presence of mutation cannot confine an aggressive phenotype of a small tumor and in this way it is not a predictor of metastatic potential.

Taken together, our data provide an additional argument indicative of certain differences in PMCs’s and PTC’s biological behavior.

Conclusion

The results show that $BRAF^{T1799A}$ mutation is a prevalent event in human thyroid microcarcinomas irrespectively of the genetic background of adult patients. Mutated $BRAF$ might be involved in the control of tumors architecture and growth pattern but its presence does not correlate with manifestations of PMC aggressiveness.

Whereas $BRAF$ mutation comprise a common genetic alteration in conventional PTC and PMC, a number of differences in mutation-related associations between the two types of tumors rather supports the hypothesis that not all macroscopic PTCs may arise from papillary microcarcinoma.

REFERENCES


Postupila 15.03.2006

УДК 574:539.1.04

СОСТОЯНИЕ РЕПРОДУКТИВНОЙ СИСТЕМЫ И ТИРЕОИДНОГО ОБМЕНА КРЫС-САМЦОВ ПОТОМСТВА I ПОКОЛЕНИЯ, ПОЛУЧЕННОГО ОТ РОДИТЕЛЕЙ, ДЛИТЕЛЬНОЕ ВРЕМЯ НАХОДИВШИХСЯ В ЗОНЕ ОТЧУЖДЕНИЯ ЧАЭС

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Анализировали морфофункциональное состояние репродуктивной системы и тиреоидного обмена у крыс-самцов первого поколения (F1), полученных от родителей, длительное время находившихся в условиях радиоактивного загрязнения, и внутриутробное развитие которых проходило в зоне отчуждения ЧАЭС. Установлено, что у опытных животных F1 в возрасте 3 и 6 мес отмечается повышение относительной массы семенников и их придатков, количества сперматогенных клеток, уровня тестостерона в сыворотке крови, активности ЛДГ и содержания ДНК (3 мес) в тестикулярной ткани. Одновременно у животных F1 выявляется гипотиреоидное состояние, что выражается в снижении уровня тиреоидных гормонов в сыворотке крови и активности дионидазы в тканях печени и почек. Полученные данные свидетельствуют об возникновении нарушений в исследуемых системах организма крыс потомства первого поколения.

Ключевые слова: катастрофа на ЧАЭС, потомство крыс I поколения, репродуктивная система самцов, ЛДГ, СДГ, нуклеиновые кислоты, тестостерон, тироксин, трийодтиронин.

ANALYSIS OF REPRODUCTIVE SYSTEM AND THYROID METABOLISM STATE OF RAT MALE PROGENY I GENERATION, OBTAINED AFTER LONG LOW-INTENSITY IRRADIATION THEIR PARENTS IN CHERNOBYL ZONE

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It rats progeny of first generation in the age of 3 and 6 month wich parents were exposed to long low irradiation in Chernobyl zone testes weight indexes, amount of spermatogenous cells of all types and their total number in a suspension of testes tissue, content of DNA and RNA, activity of LDH and SDH in the testes tissue, contents of testosterone, T3 and T4 in blood serum and activity of deiodinase in tissue liver and kidneys were studies. The changes in morphofunctional state of testes and thyroid status of progeny rats received from the parents (males and females) were found.

Key words: Chernobyl accident, rat progeny of I generation, male reproductve system, LDH, SDH, nucleic acids, testosterone, thyroxin, triiodtyronin.