research article

Functional polymorphisms in antioxidant genes in Hurthle cell thyroid neoplasm - an association of *GPX1* polymorphism and recurrent Hurthle cell thyroid carcinoma

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Radiol Oncol 2016; 50(3): 289-296.

Received 4 January 2016
Accepted 2 May 2016

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Disclosure: The authors declare no conflict of interest.

Background. Hurthle cells of the thyroid gland are very rich in mitochondria and oxidative enzymes. As a high level oxidative metabolism may lead to higher level of oxidative stress and can be associated with an increased risk for cancer, we investigated whether common functional polymorphisms in antioxidant genes (*SOD2*, *CAT*, *GPX*, *GSTP1*, *GSTM1* and *GSTT1*) are associated with the development or clinical course of Hurthle cell thyroid carcinoma (HCTC).

Methods. A retrospective study was performed in 139 patients treated by thyroid surgery for a Hurthle cell neoplasm. HCTC, Hurthle cell thyroid adenoma (HCTA) or Hurthle cell thyroid nodule (HCTN) were diagnosed by pathomorphology. DNA was extracted from cores of histologically confirmed normal tissue obtained from formalin-fixed paraffin-embedded specimens and genotyped for investigated polymorphisms. Logistic regression was used to compare genotype distributions between patient groups.

Results. HCTC, HCTA and HCTN were diagnosed in 53, 47 and 21 patients, respectively. Metastatic disease and recurrence of HCTC were diagnosed in 20 and 16 HCTC patients, respectively. Genotypes and allele frequencies of investigated polymorphisms did not deviate from Hardy-Weinberg equilibrium in patients with HCTC, HCTA and HCTN. Under the dominant genetic model we observed no differences in the genotype frequency distribution of the investigated polymorphisms when the HCTA and HCTN group was compared to the HCTC group for diagnosis of HCTC or for the presence of metastatic disease. However, *GPX1* polymorphism was associated with the occurrence of recurrent disease ($p = 0.040$).

Conclusions. *GPX1* polymorphism may influence the risk for recurrent disease in HCTC.

Key words: Hurthle cell thyroid carcinoma; Hurthle cell neoplasm; thyroid; oxidative stress; antioxidant genes

Introduction

Hurthle cell thyroid carcinoma (HCTC) is a rare type of differentiated thyroid cancer (DTC). Traditionally, HCTC was regarded as a subtype of follicular thyroid cancer, while new evidence indicates that HCTCs may have a distinct molecular profile compared to other DTCs.[1]

Clinically, and compared to other DTCs, HCTCs are considered more aggressive, with worse prognosis, requiring more stringent follow-up. HCTCs are also more likely to metastasize to neck soft tissue and distant sites, are more iodine resistant and have higher tumour-related mortality.[1-4] A definitive way to differentiate a HCTC from a benign Hurthle cell thyroid adenoma (HCTA) is based on vascular and/or transcapsular invasion.[5-9] For HCTA, a lobectomy is a sufficient surgical procedure. However, if a HCTC is diagnosed on histologic sections after a lobectomy, then a complete

thyroidectomy is performed as a second surgical procedure. Therefore, when a follicular neoplasm is detected with a cytological analysis of material obtained by fine-needle aspiration biopsy, the use of predictive clinical[4,10] or genetic markers[11] has been proposed, before deciding on the extent of the thyroid surgical procedure.

A Hurthle (oncocytic) cell has abundant granular eosinophilic cytoplasm, which has such an appearance because of the accumulation of a large number of mitochondria. A full-blown Hurthle cell has 4000 to 5000 mitochondria, while a human cell rich in mitochondria (oocyte) has about 1500 mitochondria only.[7,12] Enzyme histochemistry studies have shown that Hurthle cells contain high concentrations of oxidant enzymes.[13]

The respiratory redox chain in the mitochondria is considered the major source of reactive oxygen species (ROS) and other free radicals in the cell.[14] ROS and other free radicals can oxidize target cellular proteins, membrane lipids, nucleic acids and damage their cellular structure and function. Effective protective mechanisms, comprising antioxidative molecules and compartmentalization of potentially toxic molecules, have been developed to maintain a balance between generation and detoxification of reactive oxygen species (ROS) under physiological conditions. In case of excessive ROS oxidative stress occurs.[15,16] To prevent this, complex defence mechanisms including many enzymes, proteins and antioxidants are involved. Antioxidant enzymes such as manganese superoxide dismutase (Mn-SOD), glutathione peroxidase (GPX) and catalase (CAT) directly eliminate ROS, while glutathione-S-transferases (GSTs) detoxify cytotoxic secondary metabolites. Numerous functional polymorphisms in the genes coding for antioxidant enzymes have been described that may also modify their ROS detoxification capacity.[17]

Oxidative stress and ROS have been associated with several cancers and also many complex diseases like cardiovascular disease, diabetes mellitus and neurodegenerative disorders.[15,18] Several studies also found a connection between oxidative stress and thyroid diseases including neoplasia and thyroid cancer.[16,19-25] However all these studies have been done on papillary thyroid carcinoma and/or follicular thyroid carcinoma. As Hurthle cells are very rich in mitochondria and oxidative enzymes, it is possible that antioxidant enzymes may have an important role in defence against oxidative stress. To our knowledge, there are no data in the literature about oxidative stress and HCTC or HCTA. Furthermore, there are no data about the association between HCTC/HCTA and polymorphisms of genes coding for antioxidant enzymes.

Patients and methods

Patients

A retrospective study included Slovenian patients treated by thyroid surgery for a Hurthle cell neoplasm at the Institute of Oncology Ljubljana. The medical records of all the patients were reviewed and a total of 167 patients with cytological features for a Hurthle cell neoplasm were selected for molecular analysis. As 28 patients had no sufficient formalin-fixed and paraffin-embedded (FFPE) material for DNA extraction, they were excluded from the study. Eventually, 139 patients were included.

All the patients had a Hurthle cell neoplasm diagnosed by fine-needle aspiration cytology and the majority of fine-needle aspiration biopsies were ultrasound guided.[4,10] The cytological criteria for Hurthle cell neoplasms were hypercellularity, with a predominance of Hurthle cells (at least 75%), few or no lymphocytes, and scant or no colloid.[26] Cytological slides were examined by cytopathologist, experienced in thyroid pathomorphology.

Final diagnosis of HCTC/HCTA/other was obtained by definitive histology of thyroid tissue obtained by surgical procedure. The histological features for HCTC were based on vascular invasion and/or transcapsular invasion.[26] Histology slides were examined by a pathologist, experienced in thyroid pathomorphology.

All patients with HCTC diagnosis were regularly monitored for possible recurrent or metastatic disease. The median follow-up time was 105 (1–337) months.[4]

The study was reviewed and approved by the Slovenian Ethics Committee for Research and Medicine (No: KME 32/12/11) and was carried out according to the Declaration of Helsinki. The study was also approved by the Institute of Oncology Ljubljana Protocol Review Board.

Methods

Hematoxylin and eosin (H&E) stained slides from FFPE samples were examined by a pathologist, experienced in thyroid pathomorphology, to confirm the diagnosis and to select areas representative of normal tissue. Two to three cores (1 mm in diameter) of histologically confirmed normal tissue were obtained from each specimen for DNA extraction using a QiaAmp Mini kit (Qiagen, Hilden,

Germany) according to the manufacturer's instructions.

Genotyping of SNPs in *SOD2* rs4880 (c.47C>T; p.Val16Ala), *CAT* rs1001179 (c.-262C>T; c.-262G>A), *GPX1* rs1050450 (c.599C>T; p.Pro200Leu), *GSTP1* rs1695 (c.341C>T; p.Ile105Val) and *GSTP1* rs1138272 (c.313A>G; p.Ala114Val) was carried out using a fluorescence-based competitive allele-specific (KASPar) assay (Kbiosciences, Herts, UK) according to the manufacturer's instructions. Amplifications were performed in a PCR system 9700 AB (Applied Biosystems, California, USA) as recommended by the manufacturer (Kbiosciences). Fluorescence was measured on a 7500 Real Time PCR System AB and allele discrimination data analyzed with 7500 System SDS Software (both Applied Biosystems).

GSTM1 and *GSTT1* gene deletions were detected using a multiplex PCR simultaneously amplifying *GSTM1*, *GSTT1* and *BGLO* genes as described previously.[27] With this approach, we could identify homozygous *GSTM1* or *GSTT1* gene deletion, but we were not able to distinguish between carriers of one or two copies of each gene. Genotyping was repeated in 20% of samples to check for genotyping accuracy.

Statistical analysis

Median and interquartile ranges were used to describe central tendency and variability of continuous variables, while frequencies were used to describe the distribution of categorical variables. A standard chi-square test was used to assess the deviation from Hardy-Weinberg equilibrium (HWE). Logistic regression was used to compare genotype distributions between patient groups and to calculate odds rations (ORs) and 95% confidence intervals (CIs). All statistical analyses were carried out using IBM SPPS Statistics version 19.0 (IBM Corporation, Armonk, NY, USA). A dominant genetic model was used in all statistical analyses and the level of statistical significance was set at 0.05. Haplotype analysis was performed using Thesias software[28] as previously described.[29]

Results

In total 139 patients with cytological features for Hurthle cell neoplasm were included in the study. The female to male sex ratio was 3.8:1. Median (range) age was 54 (42–66) years. Median diameter of the tumour was 28 (20–45) mm. The final diagnosis was established by definitive histology of the thyroid tissue obtained by a surgical procedure. Patients were diagnosed as follows: 53 (38.1%) had HCTC, 47 (33.8%) HCTA, 21 (15.1%) Hurthle cell thyroid nodule (HCTN), 11 (7.9%) multi nodular goiter, 4 (2.9%) follicular thyroid adenoma, while 2 (1.4%) patients had lymphocyitic thyroiditis. In 46 (33%) patients, concomitant disease was recorded: 31 (22.3%) had Hashimoto thyroiditis, 12 (8.6%) diabetes mellitus, 7 (5.0%) Graves' disease, and 4 (2.9%) patients had other malignant disease not present in the thyroid tissue.

Only patients with a final diagnosis of HCTC, HCTA or HCTN were selected for molecular analysis. The group of patients with HCTA or HCTN was compared to the group of patients with HCTC. Altogether 20 of 53 (37.7%) patients with HCTC had metastatic disease. Recurrent disease was observed in 16 (30.0%) patients with HCTC. The clinical and demographic characteristics of those patients are summarized in Table 1.

The patients from the HCTC group had a different gender (F/M) ratio ($p = 0.043$), were older ($p = 0.004$) and had a larger tumour diameter ($p < 0.001$) in comparison to the patients from the HCTA or HCTN group (Table 2). In the HCTC group, independent risk factors for both metastatic disease and recurrent disease were the patient's age and tumour diameter as shown by logistic regression analysis (Table 2).

Genotype frequencies of the investigated polymorphisms in patients with HCTC, HCTA and

TABLE 1. Clinical and demographic characteristics of patients with Hurthle cell neoplasms

	HCTA + HCTN	HCTC
Number [N] (%)	68 (56.2)	53 (43.8)
Median age [years] (range)	49.5 (38.5–57.8)	62 (45.5–70.5)
Gender F/M [N] (%)	58/10 (85.3/14.7)	37/16 (69.8/30.2)
Median tumor diameter [mm] (range)	26.0 (16.0–34.8)	40.0 (25.5–65.0)
Metastasis (%)	/	20 (37.7)
Recurrence (%)	/	16 (30.2)
Concomitant disease N (%)	16 (23.5)	20 (37.7)
Hashimoto thyroiditis	11 (16.2)	12 (22.6)
Diabetes mellitus	1 (1.5)	7 (13.2)
Graves' disease	2 (2.9)	3 (5.7)
Non-thyroid Malignancy	2 (2.9)	2 (3.8)

F= female; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; M = male

TABLE 2. Association of clinical and demographic characteristics with Hurthle cell thyroid neoplasms, metastatic disease and recurrent disease

	HCTA+HCTN versus HCTC		Metastatic disease		Recurrent disease	
	OR (95% CI)	p[a]	OR (95% CI)	p[a]	OR (95% CI)	p[a]
Gender	2.51 (1.03–6.12)	0.043	2.08 (0.63–6.90)	0.230	2.42 (0.70–8.37)	0.163
Age	1.04 (1.01–1.06)	0.004	1.07 (1.02–1.12)	0.005	1.05 (1.01–1.10)	0.026
Tumor diameter	1.05 (1.02–1.07)	< 0.001	1.09 (1.04–1.14)	< 0.001	1.04 (1.01–1.07)	0.005
Concomitant disease	1.97 (0.90–4.34)	0.092	0.83 (0.26–2.63)	0.749	0.83 (0.26–2.63)	0.523

CI = confidence interval; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio; [a] = p less than 0.05 was considered statistically significant

HCTN are shown in Table 3. The observed genotype frequencies did not deviate from Hardy-Weinberg equilibrium in the whole cohort of patients (p > 0.050, Table 3).

The association of *SOD2*, *CAT*, *GPX1* and *GST* polymorphisms with diagnosis of Hurthle cell neoplasm and with the presence of metastatic or recurrent disease are presented in Table 4. These associations were also adjusted for clinical parameters. Since gender, age and tumour diameter were correlated in a multivariable model, only tumour diameter was used for adjustment.

Under the dominant genetic model, no significant differences in the genotype frequency distribution of the investigated polymorphisms were observed when the HCTA and HCTN group was compared to the HCTC group (all p > 0.050). These polymorphisms were also not associated with metastatic disease (all p > 0.050). However, *GPX1* polymorphism was associated with the presence of recurrent disease (p = 0.040). The association of *GPX1* polymorphism and recurrent disease was even greater when adjusted for tumour diameter (p = 0.036).

TABLE 3. Genotype frequencies in patients with Hurthle cell neoplasms

Gene	Polymorphism	Genotype	All patients (%)	P_{HWE}	HCTA+HCTN (%)	HCTC (%)
SOD2	rs4880; c.47C>T; p.Val16Ala	CC	26 (21.7)	0.903	12 (17.9)	14 (26.4)
		CT	59 (49.2)		34 (50.7)	25 (47.2)
		TT	35 (29.2)		21 (31.3)	14 (26.4)
CAT	rs1001179; c.-262C>T; c.-262G>A	CC	70 (58.3)	0.907	35 (52.2)	35 (66.0)
		CT	43 (35.8)		30 (44.8)	13 (24.5)
		TT	7 (5.8)		2 (3)	5 (9.4)
GPX1	rs1050450; c.599C>T; p.Pro200Leu	CC	63 (52.1)	0.424	35 (51.5)	28 (52.8)
		CT	51 (42.1)		32 (47.1)	19 (35.8)
		TT	7 (5.8)		1 (1.5)	6 (11.3)
GSTP1	rs1695; c.341C>T; p.Ile105Val	CC	54 (44.6)	0.653	28 (41.2)	26 (49.1)
		CT	52 (43.0)		32 (47.1)	20 (37.7)
		TT	15 (12.4)		8 (11.8)	7 (13.2)
GSTP1	rs1138272; c.313A>G; p.Ala114Val	AA	103 (85.1)	0.159	58 (85.3)	45 (84.9)
		AG	16 (13.2)		8 (11.8)	8 (15.1)
		GG	2 (1.7)		2 (2.9)	0 (0)
GSTM1	Gene deletion	Wild type	55 (50.9)	/[a]	33 (50.8)	22 (51.2)
		Gene deletion	53 (49.1)		32 (49.2)	21 (48.8)
GSTT1	Gene deletion	Wild type	93 (86.1)	/[a]	54 (83.1)	39 (90.7)
		Gene deletion	15 (13.9)		11 (16.9)	4 (9.3)

HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; HWE = Hardy-Weinberg equilibrium
[a] HWE could not be evaluated for GSTM1 and GSTT1 as we were not able to distinguish between carriers of one or two copies of each gene.

TABLE 4. Association of *SOD2*, *CAT*, GPX1 and *GST* polymorphisms with diagnosis of Hurthle cell neoplasm, presence of metastatic disease and occurrence of recurrent disease

Gene	Genotype	Diagnosis (HCTA+HCTN vs. HCTC)				Metastatic disease				Recurrent disease			
		OR (95% CI)	p[a]	OR-adj[b] (95% CI)	p-adj[b]	OR (95% CI)	p[a]	OR-adj[b] (95% CI)	p-adj[b]	OR (95% CI)	p[a]	OR-adj[b] (95% CI)	p-adj[b]
SOD2 rs4880	CC CT+TT	0.61 (0.25–1.46)	0.264	0.65 (0.25–1.67)	0.373	1.12 (0.32–4.00)	0.856	0.72 (0.12–4.09)	0.706	1.11 (0.29–4.26)	0.878	0.82 (0.18–3.62)	0.788
CAT rs1001179	CC CT+TT	0.56 (0.27–1.18)	0.129	0.81 (0.36–1.81)	0.600	0.34 (0.09–1.24)	0.102	0.57 (0.11–2.91)	0.499	1.25 (0.37–4.25)	0.721	2.95 (0.66–13.1)	0.155
GPX1 rs1050450	CC CT+TT	0.95 (0.46–1.94)	0.882	1.02 (0.46–2.24)	0.962	0.63 (0.20–1.93)	0.417	0.72 (0.15–3.52)	0.682	0.25 (0.07–0.94)	0.040	0.19 (0.04–0.89)	0.036
GSTP1 rs1695	CC CT+TT	0.73 (0.35–1.50)	0.388	0.82 (0.37–1.82)	0.628	1.30 (0.43–3.96)	0.646	2.40 (0.47–12.13)	0.291	0.46 (0.14–1.52)	0.202	0.49 (0.13–1.89)	0.300
GSTP1 rs1138272	AA AG+GG	1.03 (0.38–2.83)	0.952	1.15 (0.39–3.45)	0.800	0.99 (0.21–4.67)	0.988	1.24 (0.17–9.19)	0.836	0.29 (0.03–2.54)	0.261	0.24 (0.02–2.64)	0.244
GSTM1	Wild type Gene deletion	0.98 (0.46–2.13)	0.968	0.91 (0.39–2.12)	0.819	1.59 (0.47–5.39)	0.456	1.40 (0.23–8.57)	0.716	1.32 (0.38–4.64)	0.666	1.24 (0.28–5.41)	0.774
GSTT1	Wild type Gene deletion	0.50 (0.15–1.70)	0.269	0.44 (0.11–1.82)	0.257	1.44 (0.18–11.29)	0.730	0.83 (0.02–39.34)	0.923	2.00 (0.25–15.85)	0.512	1.42 (0.1–20.98)	0.798

CI = confidence interval; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio; [a] = p less than 0.05 was considered statistically significant; [b] = adjusted for tumor diameter

TABLE 5. Association of *GSTP1* haplotypes and diagnosis of Hurthle cell neoplasm, presence of metastatic disease and occurrence of recurrent disease

Haplotype	Estimated frequency	Diagnosis (HCTA+HCTN vs. HCTC)		Metastatic disease		Recurrent disease	
		OR (95% CI)	p[a]	OR (95% CI)	p[a]	OR (95% CI)	p[a]
AC	0.68	Reference		Reference		Reference	
GC	0.25	0.88 (0.49–1.60)	0.686	1.04 (0.38–2.86)	0.935	0.45 (0.13–1.64)	0.230
GT	0.07	0.83 (0.33–2.13)	0.704	0.99 (0.21–4.72)	0.988	028 (0.03–2.89)	0.288

CI = confidence interval.; HCTC = Hurthle cell thyroid carcinoma; HCTA = Hurthle cell thyroid adenoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio
[a] - p less than 0.05 was considered statistically significant

Haplotype analysis was performed to assess the combined effect of SNPs within the *GSTP1* gene. As shown in Table 5, no associations were observed between *GSTP1* haplotypes and diagnosis of HCTA/HCTN versus HCTC, the presence of metastatic disease or the occurrence of recurrent disease.

Discussion

In the present study, we investigated whether common functional polymorphisms in antioxidant genes could be used as molecular markers for the development of HCTC or its clinical course in patients with Hurthle cell neoplasms.

In patients with cytological features for Hurthle cell neoplasm, different final diagnoses are made by definitive histology of thyroid tissue obtained by a surgical procedure. In our study group, 87% of patients with cytological features for Hurthle cell thyroid neoplasm had HCTC, HCTA or HCTN and were eligible for our study. Patient groups with benign HCTA and HCTN were combined and compared to a group with HCTC. The malignancy rate in our HCTC group was 44% and within the incidence rate of malignancy reported in the literature, where it ranged from 13%[30] up to 70%.[31] A significant difference in age was observed between the HCTA+HCTN group and the HCTC group, with patients in the HCTC group being nearly 12 years older and having a significantly larger median size of initial tumour (26 versus 40 mm). These findings are consistent with previous reports.[10,32,33] We also found a small gender difference, with a significantly larger F/M ratio in the HCTA+HCTN group as compared to the HCTC group. The two groups did not differ regarding the presence of concomitant disease. Metastases were diagnosed in 38% of patients with HCTC. Furthermore, 30% of patients developed a recurrent disease. These two groups of patients had a significantly larger initial

tumour diameter (69 versus 30 mm and 62 versus 30 mm, respectively) or were significantly older (67 versus 53 years and 65 versus 54 years, respectively) at initial diagnosis than the HCTC patients that did not have metastatic or recurrent disease. However, it has to be noted that our HCTC group with metastatic or recurrent disease was relatively small compared to non-metastatic or non-recurrent HCTC group.

To establish whether common functional polymorphisms in genes coding for antioxidant genes could be used as molecular markers for the development of HCTC or its clinical course, we investigated associations between *SOD2*, *CAT*, *GSTP1*, *GSTM1*, *GSTT1* and *GPX1* genotypes and the clinical characteristics of patients with definite diagnosis of HCTC, HCTA or HCTN.

CAT -262C>T genotype frequencies observed in our patient group were in accordance with those previously published for a healthy population.[34-36] In our study *CAT* -262C>T polymorphism was not associated with HCTC, or metastatic or recurrent disease. To our knowledge *CAT* -262C>T has not been studied in HCTC, but higher CAT activity has been associated with papillary thyroid carcinoma and follicular carcinoma.[24,37] It has been demonstrated that *CAT* -262C>T polymorphism influences the binding of transcriptional factors and is associated with a decrease in enzyme expression[35,38,39], but also with higher CAT activity.[34,40]

Also *SOD2* Val16Ala genotype frequencies in our patients with Hurthle cell neoplasms were similar to frequencies previously reported in Caucasian patients.[41-44] In our study *SOD2* Val16Ala polymorphism was not associated with the occurrence of HCTC, or with metastatic or recurrent disease. To our knowledge this polymorphism has not been studied in HCTC yet. *SOD2* Val16Ala polymorphism leads to less efficient transport of SOD2 into mitochondrial matrix in vitro[45], but association studies of SOD2 in thyroid cancers gave inconclusive results. Two groups showed an increased SOD2/SOD level or activity in follicular and papillary thyroid cancer, while one group found no change of SOD activity in papillary thyroid cancer.[37,46,47] On the other hand a reduced level of SOD2 was found in poorly differentiated thyroid cancers.[48]

Frequencies of *GSTP1*, *GSTM1* and *GSTT1* polymorphisms in our patients were similar to previously reported studies.[49] However, in the HCTC group we noticed a lower percentage of *GSTT1* gene deletion, compared to the HCTN/HCTA group. *GSTP1* genotypes and haplotypes as well as *GSTT1* and *GSTM1* deletions were not associated with the occurrence of HCTC and neither with metastatic nor recurrent disease. Our findings are in agreement with a previous study that also found no association between *GSTM1* and *GSTT1* polymorphisms and HCTC.[50] Both *GSTP1* Ile105Val and *GSTP1* Ala114Val decrease enzymatic activity[51,52], while *GSTM1* and *GSTT1* deletion polymorphisms result in the complete loss of enzymatic activity in homozygous carriers.[53] Some previous studies have shown possible associations of *GSTP1*, *GSTM1* or *GSTT1* polymorphisms, or a combination of *GSTT1* and *GSTM1* null allele with papillary and/or follicular thyroid cancer[54-58], while others found no association between these polymorphisms and primary or secondary thyroid cancers.[59-62]

Frequencies of *GPX1* Pro198Leu genotypes in our study group were also similar to the ones previously reported.[63] We did not find any association of *GPX1* Pro198Leu polymorphism with the occurrence of HCTC or with metastatic disease, even though several groups have found decreased activity or decreased expression of GPX1 in thyroid carcinomas[24,47,64,65], while one group reported increased levels of GPX1 in papillary thyroid carcinoma.[37] Several groups also reported that Leu variant could lead to lower GPX1 activity in patients with lung cancer, breast cancer, prostate cancer, bladder cancer and some other cancers.[66-68] We observed an interesting association between *GPX1* Pro198Leu polymorphism and lower probability for recurrent disease. Our findings are consistent with a previous report on the association of *GPX1* 198Leu variant with lower risk of recurrence in cancer patients.[69] A possible explanation may be that some HCTC therapies (radioiodine ablation and radiotherapy) are large ROS generators with antineoplastic effects and may also influence the patient's prognosis after these treatments. As GPX1 198Leu variant is associated with reduced removal of ROS and their secondary products produced by some HCTC therapies, patients with variant allele may have a better prognosis and longer recurrence-free survival time.

To sum up, in our study we did not find any association between common functional polymorphism antioxidant genes (*SOD2*, *CAT*, *GPX1*, *GSTP1*, *GSTM1*, and *GSTT1*) and the development of HCTC. A possible explanation could be that these polymorphisms may influence an initial and shared phase of HCTC and HCTA/HCTN development. Common functional polymorphisms in *SOD2*, *CAT*, *GSTP1*, *GSTM1* or *GSTT1* were also

not associated with metastatic or recurrent disease development, while *GPX1* Pro198Leu polymorphism may modulate the risk of HCTC recurrence. However, the group of patients with recurrent disease was relatively small, so it is possible that the results may result from sampling error. Ideally, our findings relating both to statistically significant associations and not significant associations, should be confirmed in an independent sample cohort. Because of the rarity of these tumours, it was impossible to perform a validation study in a single institution. Further research in a larger group is needed before we can conclude that *GPX1* Pro198Leu polymorphism could be used as an additional molecular marker in clinical practice to support decisions about follow-up procedures in patients with HCTC.

Conclusions

In conclusion, *GPX1* Pro198Leu polymorphism may influence the risk for recurrent disease in HCTC, however, these results must be validated in an independent sample cohort.

Acknowledgments

This work was financially supported by the Slovenian Research Agency (ARRS Grant No. P3-0289 and P1-0170).

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Radiol Oncol 2016; 50(3): 289-296.
doi:10.1515/raon-2016-0031

Funkcionalni polimorfizmi antioksidativnih genov pri neoplazmi Huerthlejevih celic ščitnice - povezava med polimorfizmom gena *GPX1* in ponovitvijo raka Huerthlejevih celic ščitnice

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Izhodišča. Za Huerthlejeve celice ščitnice je značilno veliko število mitohondrijev in oksidativnih encimov. Ker povečan oksidativni metabolizem lahko vodi v povečan oksidativni stres oziroma ga lahko povezujemo z večjo verjetnostjo razvoja raka, smo v naši raziskavi preverjali, ali obstaja povezava med funkcionalnimi polimorfizmi antioksidativnih genov (*SOD2*, *CAT*, *GPX*, *GSTP1*, *GSTM1* in *GSTT1*) in nastankom ali kliničnim potekom raka Huerthlejevih celic ščitnice (HCTC).

Bolniki in metode. Retrospektivno raziskavo smo izvedli pri 139 bolnikih, pri katerih smo zaradi suma na neoplazmo Huerthlejevih celic ščitnice opravili operacijo ščitnice. Diagnozo HCTC, adenoma Huerthlejevih celic ščitnice (HCTA) ali gomolja Huerthlejevih celic ščitnice (HCTN) smo postavili s histopatomorfološko analizo. DNA smo izolirali iz stebričkov histološko potrjenega zdravega dela ščitnice, pridobljenega iz arhiviranih parafinskih blokov tumorjev, fiksiranih v formalinu. S postopki genotipizacije smo določali prisotnost polimorfizmov v antioksidativnih genih. Z logistično regresijo pa smo primerjali porazdelitve posameznih genotipov med različnimi skupinami bolnikov.

Rezultati. HCTC smo ugotovili pri 53, HCTA pri 47 in HCTN pri 21 bolnikih. Pri 20 bolnikih s HCTC smo ugotovili prisotnost zasevkov, pri 16 pa ponovitev bolezni. Pri skupinah bolnikov s HCTC, HCTA in HCTN frekvence genotipov in alelov preučevanih polimorfizmov niso odstopale od Hardy-Weinbergovega ravnotežja. Dominantni genetski model ni pokazal povezave med porazdelitvijo frekvenc genotipov preučevanih polimorfizmov in prisotnostjo HCTC v primerjavi s HCTA in HCTN, prav tako ni bilo povezave s prisotnostjo zasevkov pri HCTC. Ugotovili pa smo povezavo med polimorfizmom *GPX1* in ponovitvijo HCTC (p = 0,040).

Zaključki. Polimorfizem *GPX1* lahko vpliva na možnost ponovitve HCTC.

Radiol Oncol 2016; 50(3): 297-307.
doi:10.1515/raon-2015-0047

Vpliv polimorfizmov v segregacijskih genih BUB1B in TTK na dovzetnost za razvoj želodčnega raka

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Izhodišča. Maligna preobrazba normalnih želodčnih celic je zapleten večstopenjski proces, ki vodi v nastanek heterogenih tumorjev. Na razvoj želodčnega raka vplivajo poleg dejavnikov okolja tudi genetsko ozadje in genetske spremembe. Polimorfizmi enega baznega para (*angl. Single nucleotide polymorphisms*, SNP) v mitotskih segregacijskih genih bi lahko bili odgovorni za počasno kopičenje genetskih sprememb, ki vodijo v genomsko nestabilnost.

Bolniki in metode. V raziskavi primerov s kontrolami smo opredelili vpliv polimorfizmov rs151658 v mitotski kinazi *TTK* in rs1031963 ter rs1801376 v kinazi *BUB1B* na razvoj želodčnega raka. Z metodo imunskega odtisa smo določili količino TTK v rakavih tkivih bolnikov.

Rezultati. Odkrili smo, da genotipa C/G in G/G polimorfizma rs151658 značilno vplivata na dovzetnost za razvoj difuzne oziroma intestinalne oblike želodčnega raka (p = 0,049). Genotip A/A polimorfizma rs1801376 je bil značilno povezan z višjim tveganjem za razvoj želodčnega raka pri bolnicah (0,007), medtem ko se je pri moških z želodčnim rakom pogosteje pojavljal le pri preiskovancih, pri katerih so tumorske celice preraščale v subserozo (0,009). Pri nosilcih genotipa T/T polimorfizma rs1031963 so se pogosteje razvili dobro diferencirani tumorji (0,035). V dominantnem modelu sta bila genotipa TT+CT polimorfizma rs1031963 (razmerje obetov [OR] = 2,929, 95 % interval zaupanja [CI]: 1,281–6,700; p = 0,017) in genotipa GG+AG polimorfizma rs1801376 (OR = 0,364, 95 % CI: 0,192–0,691; p = 0,003) značilno povezana z višjim tveganjem za razvoj bolezni.

Zaključki. Rezultati raziskave kažejo, da polimorfizmi v mitotskih kinazah *TTK* in *BUB1B* v naši skupini preiskovancev statistično značilno prispevajo k povišanemu tveganju za razvoj želodčnega raka in mogoče vplivajo na potek razvoja tumorjev. Za opredelitev njihove klinične uporabnosti so potrebne nadaljnje raziskave v večjih skupinah bolnikov z želodčnim rakom različnih ras.