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The activity of 5'deiodinase type I is remarkably increased in breast cancer

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Introduction. The aim of the study was to compare the activity of type 1 5'iodothyronine deiodinase (5'D1) in breast cancer tissue and in non-cancerous breast tissue taken from the opposite side to the location of the tumour.
Material. The material consisted of 36 samples of breast cancer tissues (grades G1 to G3) and non-cancerous control tissues, which were collected during radical mastectomy or local tumour resection.
Methods. Tissues were homogenized and protein concentration was estimated acc. to the Bradford method. 5'D1 activity was measured by quantification of the radioactive iodide released from 3'5'[^{125}I]-rT3.
Results. The activity of 5'D1 in non-cancerous breast tissues was found to be very low or non-measurable. On the contrary, in cancer tissues from the same breasts – especially in G1 and G2 tumours, the enzyme activity was significantly increased. Also in small tumours (first and second grade clinical stage) 5'D1 activity was high.
Conclusions. Our data may suggest that increased activity of 5-deiodinase type I can lead to elevated de novo production of triiodothyronine in breast cancer tissues and that the thyroid hormone plays a part especially in early stage of carcinogenesis.

Aktywność 5'dejodynazy typu pierwszego jest znacząco podwyższona w raku sutka

Wstęp. Celem pracy było porównanie aktywności enzymatycznej 5' jodotyroninowej dejodynazy typu pierwszego (5'D1) w tkance raków piersi i w niezmienionej nowotworowo tkance gruczołu piersiowego, wziętej z przeciwnego bieguna w stosunku do lokalizacji guza.
Materiał. Materiał stanowiło 36 próbek tkanek guzów sutka (stopnie złośliwości G1 do G3) oraz ta sama ilość tkanek gruczołu piersiowego, niezmienionych nowotworowo, zebranych podczas radykalnej mastektomii lub lokalnej tumorektomii.
Metody. Uzyskane tkanki zostały poddane homogenizacji w celu izolacji białka enzymatycznego. Stężenie białka oznaczono metodą Bradforda. Aktywność 5'D1 została zmierzona za pomocą zliczania jodu radioaktywnego, uwolnionego z 3'5'[^{125}I]-rT3, który służył do przeprowadzenia reakcji enzymatycznej.
Wyniki. Aktywność 5'D1 w nienowotworowych tkankach piersi była bardzo niska lub niewykrywalna. W przeciwieństwie do tego, tkanki raków pochodzące z tych samych gruczołów piersiowych wykazywały podwyższoną aktywność 5'D1, w szczególności w guzach G1 i G2. Również w małych guzach, w pierwszym i drugim stopniu klasyfikacji klinicznej, obserwowano znaczący wzrost aktywności 5'D1.
Wnioski. Nasze dane mogą sugerować, że podwyższona aktywność 5-dejodynazy typu I może prowadzić do podwyższonej produkcji trijodotyroniny w tkance guzów piersi i że hormony tarczycy mogą mogą pełnić jakąś rolę, szczególnie we wczesnej fazie karcinogenezy.

Key words: iodothyronine deiodinase, *hdio 1* gene, breast cancer
Słowa kluczowe: jodotyroninowa dejodynaza, gen *hdio*, rak sutka

Introduction

A possible relationship between the thyroid gland and its hormones and human breast cancer has been suggested for many years, although published data is still confusing.

In the past it has been reported [1-3] that Japanese women suffering from Hashimoto's thyroiditis have a five times higher risk of developing breast cancer than women without thyroid disorders. The authors of this report suggested [4] that mild hypothyroidism present in

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Hashimoto's patients might predispose to breast cancer. However, in another retrospective study Goldman et al. found that patients with Hashimoto's thyroiditis have had the lowest percent of deaths due to breast cancer. The possible link between development of breast cancer and hypothyroidism was also neglected.

In a prospective, 18-year long study of 10.000 women residing on Guernsey, all those who developed breast cancer had a negative history of thyroid pathologies and presented no abnormalities of the serum level of triiodothyronine (T3), thyroxine (T4) and pituitary TSH in the decade before their malignancy was diagnosed [4]).

Most recently, due to the molecular findings the possible relationship between breast carcinogenesis and thyroid hormones was again brought to attention [Puzianowska et al. 2001 – 5]. Firstly, high expression of Na/I symporter (the membrane iodide transporter present in thyroid cell membranes) was discovered both in healthy and cancerous breast tissues [6-8]. The possibility that the expression of thyroid hormone and function of its nuclear receptors [TRs] in cancer tissues can be disturbed was supported by studies on liver cancer [Lin KH et al. – 9], renal clear cell cancer [Puzianowska et al. – 10, Kamiya et al. – 11], thyroid cancers [Puzianowska et al. – 12] and other tumours. The possibilities that triiodothyronine receptor function is changed in breast cancer was also cofirmed by Ali et al. [13] and Futreal et al. [14], who discovered the loss of heterozygosity on chromosome 3 and on chromosome 17 i.e. in regions of chomosomes where genes for TRβ and TRα are present.

In addition it is well known that biologically active thyroid hormone – T3, is a product of local thyroxine monodeiodination – the reaction which is catalyzed by 5'-deiodinase type I, the enzyme present in almost all human cells [15, 16]. Enzymatic activity and its gene expression have been examined in some cancers and a significant decrease of gene expression and enzyme activity was found in renal clear cell cancer and thyroid cancer [Pachucki et al. 17 Ambrodziak et al. 17]. As there exists no data on local triiodothyronine formation in human breast tissues we decided to investigate the 5'-deiodinase type I activity both in human non-cancerous breast tissue and in breast cancer of different grades of differentiation.

Material

Tissues were obtained from patients with breast cancer, who underwent surgery as the first step of oncological treatment. Samples from post-chemotherapy patients were not included. The patients had no history of thyroid disease in the past, therefore we could be certain that breast cancer cell metabolism was not disturbed by cytostatic drugs or thyroid disorders.

During radical mastectomy or tumour resection 36 tissue samples were obtained. All patients had samples taken from the tumour, as well as from the side opposite to the tumour – i.e. macroscopically normal tissue. These non-cancerous tissues were treated as controls. Immediately after surgery the samples were frozen in liquid nitrogen and kept at -70° C until needed.

Morphologically different types of cancers were present including intraductal, lobular, tubulo-lobular, tubular, medular, mucinous and apocrine carcinoma.

According to the histopathological classification three differentiation grades were represented in the study group (G1 – 14 samples, G2 – 13 samples and G3 – 9 samples). According to TNM classification there were 13 stage 1, 21 stage 2 and 2 stage 3 tumours.

At hospital admission each patient had blood sample taken in order to measure the levels of thyroid hormones. After the clot was formed, the blood sample was centrifuged. Obtained plasma was collected and stored at -70° C until needed. All patients were clinically euthyroid and remained in good health prior to the operation. There was no postoperative mortality. The study was approved by the Ethical Committee of the Medical University of Warsaw (KB/210/2001).

Methods

Sample preparation

Tissue samples were defrozen and then homogenized in ice bath. Homogenization was performed in ice-old homogenisation buffer (0.02 M TRIS-HCl, 1mmEDTA, 10mMDTT-dithiothreitol and 0.25 M glucose, pH 7.0). Protein concentration was estimated by Bradford's method, using bovine albumin as a standard.

Deiodinase assay

5'D1 activity was measured by quantification of the radioactive iodide released from 3'5'[^{125}I]-rT3. Before each assay, water diluted 3'5'[^{125}I]-rT3 was purified by *Sephadex LH 20* mini-column chromatography by successive washing with 20 ml of H_2O followed by elution with 2 ml of 75% ethanol in 0.5 ml fractions. From the fraction with the highest activity (30 000 CPM to 40 000 CPM per 60 μl), 60 μl were added to each reaction tube. This amount of 75% alcohol did not affect the reaction. Incubations were done at 37° C in 0.1 M phosphate buffer, pH=7.0 (0.1 M KPO4 and 1 mM EDTA) with 1 μM unlabeled rT3 and 20 mM DTT. Incubation time was 45 minutes. Reactions were stopped by placing the samples in the ice bath. Iodothyronines were then precipitated by adding 2 volumes of horse serum and 1 volume of 50% TCA. Samples were vortexed for 1.5 minutes and centrifuged (15 000G, 10 minutes). An aliquot of the supernatant containing [^{125}I] (not iodothyronines) was counted in a gamma counter (Wizard-1470, Wallac). Each assay was performed twice. At least one internal control was measured in all assays (human liver tissue with high activity level).

fT3,fT4, TSH assays

To measure plasma thyroid hormone concentration we used fT3, fT4, TSH electrochemiluminescence immunoassays "ECLIA" (*Roche*). All sera were measured in one series and in identical conditions.

Statistical analysis

Statistical analysis was performed with *STATISTICA* software. All data is reported as median and minimal and maximal value are given. Groups were compared with the Mann-Whitney non-parametrical test for independent trials.

Results

5'D1 activity in normal, non-cancerous breast tissue was low in 32 samples – 89% and below detectable values in 4 samples – 11%. Non-cancerous tissues with measurable levels of enzyme activity were treated as controls. Contrary to the non-cancerous tissues we found high 5'D1 activity in 80.% tumours, which according to histopathological grading included: 79% of G1 tumours, 100% of G2 tumours and 56% of G3 tumours. 5'D1 activity in group G1 was 22 times higher than in controls. The difference was statistically significant (p=0.003). 5'D1 activity in group G2 was 3 times higher than in controls, and the result was also statistically significant (p=0.0003). We also observed slightly increased activities of the enzymes in group G3 but it was not statistically significant (Figure 1). The median value for G1 was 12.75 fmol/mg*min compared with 0.57 fmol/mg*min in non-cancerous tissues. The median value for G2 was 2.36 fmol/mg*min compared with 0.72 fmol/mg*min in non-cancerous tissues. The median value for G3 was 0.7 fmol/mg*min compared with 0.59 fmol/mg*min in non-cancerous tissues.

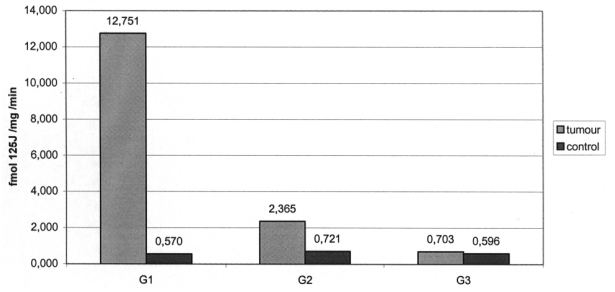

Figure 1. 5'D1 activity according to histopathological grading

We found that in 85% of small tumours (up to 2 cm in diameter) median 5'D1 activity was 10 times higher than in control tissues. The difference is statistically significant (p=0.004). In 76% of medium-sized tumours (2 cm to 5cm in diameter) 5'D1 activity was 4 times higher than in control tissues. The difference is statistically significant (p=0.002) (Figure 2). There were only 2 large tumours (over 5 cm in diameter) and they were discarded from further analysis. The median value for small tumours was 5.53 fmol/mg*min compared with 0.57 fmol/mg*min in control tissues. The median value for medium-sized tumours was 2.99 fmol/mg*min compared with 0.71 fmol/mg*min in control tissues.

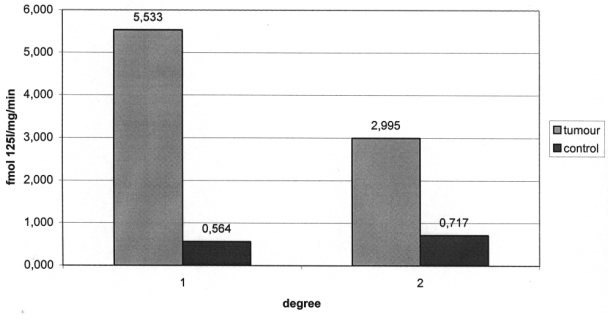

Figure 2. 5'D1 activity according to clinical classification

To check if these findings implicate changes in blood thyroid hormones levels we performed fT3, fT4, TSH immunoassays. FT3 levels were normal in all patients (Figure 3). Only one patient had a little lower FT4 level (Figure 4). In 4 cases TSH was elevated and in two decreased in comparison with normal range (Figure 5).

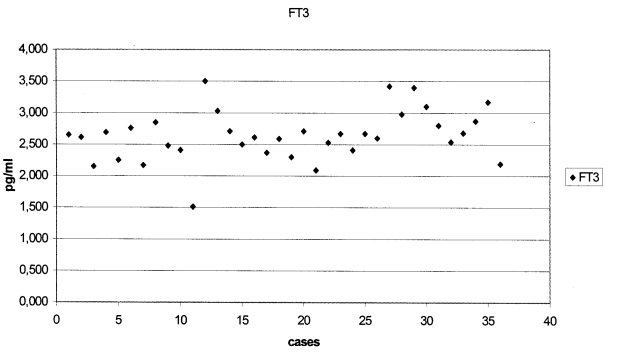

Figure 3. FT3 levels (reference values 1,8 – 4,62)

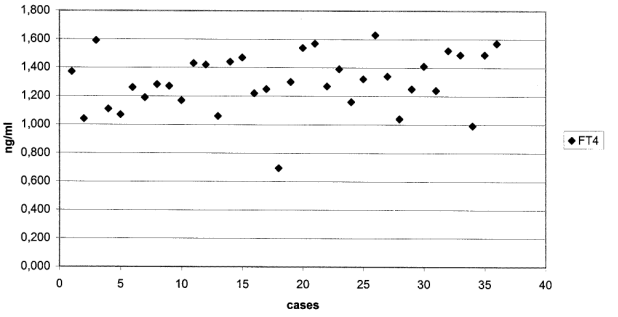

Figure 4. FT4 levels (reference values 0,93 – 1,7)

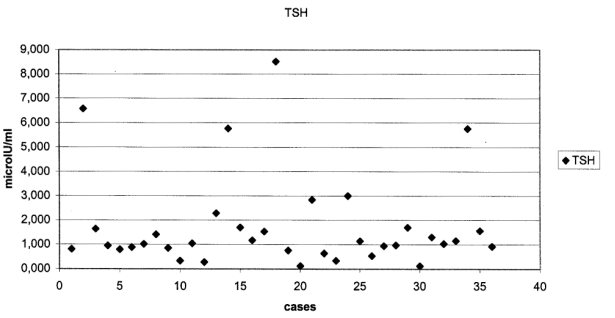

Figure 5. TSH levels (reference values 0,27-4,2)

Discussion and conclusions

5'D1 is present in many different organs and the highest 5'D1 activity was found in the thyroid, kidneys and liver [15-18]. There is no data available on 5'D1 activity in human healthy breast tissue. There is only one report proving the presence of 5'D1 in rat breast tissue [19]. The results of our study identifying the activity of 5'D1 in

human breast tissue are the first published in literature. We have found that in non-cancerous tissue the activity of the enzyme is very low and in some cases below the limit of detection. Values remained low even though different protein concentration, incubation time, reagents concentrations were used. Decreased 5'D1 activity was described in thyroid cancer, renal clear cell carcinoma [17, 20] and primary liver cancer [unpublished own data] but in healthy tissues of these organs 5'D1 activity remains relatively high [20]. On the contrary, we report increased 5'D1 activity in breast cancer. It is of interest that significantly elevated values have been found in highly differentiated tumours of G1 and G2.

The 5'D1 expression is strongly stimulated by thyroid hormone and the promotor of human 5'D1 gene has two thyroid hormone responsive elements – TRE [21, 22]. Overexpression of *hdio* gene may be a simple explanation of increased 5'D1 activity, but it needs further studies on mRNA level. We may suspect high promotor activity via TREs or some mutations which may activate the promotor of *hdio* gene. Moreover, mutated TR (thyroid receptors) in breast cancer may activate TREs. Many other factors such as cytokines, glucocorticoids, retinoids, cAMP are known to regulate, directly or indirectly, 5'D1 activity in various rat organs [16, 18, 19, 23] and probably in humans. They possibly play role in stimulating or inhibiting human deiodinase. We do not have an immediate explanation for our findings show a higher activity of the enzyme than those reported in other tissues. High 5'D1 activity in most breast cancers may be one of the signals that intracellular metabolism is changed by neoplasmatic process.

In many neoplasmatic conditions changed values of fT3, fT4 and TSH are described and referred to as the "sick euthyroid syndrome" [24]. As abnormal thyroid hormones levels were also reported in patients with breast cancer but published data were controversial [3, 24, 25] we decided to measure thyroid hormones levels in the entire group of patients from whom samples had been obtained.

All our patients remained hormonally euthyroid. We conclude that monodeiodination of tertaiodothyronine by increased 5'D1 activity in breast cancer tissue is not sufficient to influence systemic fT3, fT4 and TSH levels.

In conclusion, the present study shows high enzymatic activity of 5'D1 in human breast cancer tissue but further research on mRNA level is necessary to assess the expression of the *hdio* gene.

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References

1. Nass SJ, Davidson NE. The biology of breast cancer. *Hematol Oncol Clin North Am* 1999 13: 311-32.
2. Henderson BE, Feigelson HS. Hormonal carcinogenesis. *Carcinogenesis* March 2000, 21: 427-33.
3. Strain JJ, Bokje E, van't Veer P et al. Thyroid hormones and selenium status in breast cancer. *Nutrition and Cancer* 1997; 27: 48-52.
4. Mustacchi P, Greenspan FS. Thyroid Disease and Breast Cancer. In: *The Thyroid*. Sidney H Ingbar, Lewis E Braveman (eds.). Philadelphia: J.B. Lippincott Company, 1986: 1453-57.
5. Puzianowska-Kuźnicka M, Madej A, Krystyniak A et al. Triiodothyronine and its nuclear receptors in tumorogenesis. *Postępy Biologii Komórki* 2002; 28:183-96
6. Spitzweg C, Joba W, Eisenmenger W et al. Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complementary deoxyribonucleic acids from salivary gland, mammary gland and gastric mucosa. *Journal of Clin Endocrin and Met* 1998; 83: 1746-51.
7. Tazebay UH, Wapnir IL, Levy O et al The mammary gland iodide transporter is expressed during lactaction and in breast cancer. *Nature Medicine* 2000; 6: 871-8.
8. Cho JY, Leveillee R, Kao R et al. Hormonal regulation of radioiodide uptake activity and Na/I symporter expression in mammary glands. *The Journal of Clin Endocr and Metab* 2000; 85: 2936-43.
9. Lin KH, Lin YW, Parkinson C et al. Stimulation and proliferation by 3,5,3'-triiodothyronine in poorly differentiated human hepatocarcinoma cells overexpressing thyroid hormone receptor. *Cancer Lett* 1994; 85: 189-94.
10. Puzianowska-Kuźnicka M, Nauman A, Madej A et al. Expression of thyroid hormone receptors is disturbed in human renal clear cell carcinoma. *Cancer Lett* 2000; 155: 145-52.
11. Kamiya Y, Puzianowka-Kuźnicka M, McPhie P et al. Expression of mutant thyroid hormone nuclear receptors are associated with human renal clear cell carcinoma *Carcinogenesis* 2002; 23: 25-33.
12. Puzianowska-Kużnicka M, Krystynia A, Madej A et al. Functionally impaired TR mutants arę present in thyroid papillary cancer *J. Clin Endocrinol. Metab* 2002; 87: 1120-8.
13. Ali IU, Lidereau R, Całłahan R. Presence of two members of c-erb A receptor gene family in smallest region of somatic homozygosity on chromosome 3p21-p25 in human breast cancer. *J Natl Cancer Inst* 1989; 81:1815-20.
14. Futreal PA, Soderquist P, Marks JR et al. Detection of frequent allelic loss on proximal chromosome 17q in sporadic breast carcinoma using microsatellite length polymorphism *Cancer Res* 1992; 52: 2624-7.
15. Macke-Nauman A. Monodejodynacja tyroksyny do trijodotyroniny – specyficzność tkankowa. Regulacyjny wpływ katecholamin. – Rozprawa habilitacyjna. *Endokrynologia Polska* 1990; 41 suppl 1.
16. Kohrle J. Thyroid hormone deidinases – a selenoenzyme family acting as a gatekeeper to thyroid hormone action. *Acta Med Austriaca* 1996; 23: 17-30.
17. Pachucki J, Ambroziak M, Tański Z et al. Type I 5'-iodothyromne deiodinase activity and its mRNA are remarkably reduced in human renal clear cell carcinoma *J Endocrinol Investigation* 2001; 24: 253-61.
18. Sabatino L, Iervasi G, Ferrazzi P et al. A study of iodothyronine 5'-monodeiodinase activities in normal and pathological tissues in man and their comparison with activities in rat tissues. *Life Sci* 2000; 68: 191-202.
19. Slebodzinski AB, Brzezinska-Slebodzinska E, Styczynska E et al. Presence of thyroxine deiodinases in mammary gland: possible modulation of the enzyme-deiodinating activity by somatotropin. *Domest Anim Endocrinol* 1999; 17: 161-9.
20. Ambrodziak M, Nauman A. Ekspresja i funkcja ludzkiej jodotyroninowej 5'dejodynazy typu I (5'DI). Regulacja ekspresji genu *hdio 1. Endokrynologia Polska* 1998; 49: 241-51.
21. Jacobs TC, Schmutzler C, Meissner J et al. The promotor of the human type I 5'-deiodinase gene: Mapping of the transcription star site and identification of a DR+4 thyroid-hormone-response- element. *Eur J Biochem* 1997; 247: 288-97.
22. Zhang CY, Kim S, Harney JW, Larsen PR. Further characterisation of thyroid hormone response elements in the human type I iodothyronine deiodinase gene. *Endocrinology* 1998; 139: 1156-1163
23. St. Germain DL, Galton VA. The deiodinase family selenoproteins. *Thyroid* 1997; 7: 655-68.
24. Camacho PM, Dwarkanathan AA. Sick euthyroid syndrome. *Postgraduate Medicine* 1999; 105: 215-219.

25. Berger MM, Lemarchand-Beraud T, Cavadini C et al. R. Relations between the selenium status and the low T3 syndrome after major trauma. *Intensive Care Med* 1996; 22: 575-81.

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