THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PH.D)

SIGNIFICANCE OF IMMUNOLOGIC, CYTO- AND MOLECULAR GENETIC INVESTIGATIONS IN THE PATHOGENETICS AND DEVELOPMENT OF THYROID TUMORS

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INTRODUCTION

The malignant degeneration of normal tissues is a multifactorial process in which both genetic host and environmental elements contribute to produce clinical cancer; the relative contributions of host and environmental vary considerably both among tumor types and among individual patients.

Carcinogenesis is a complex genetic and epigenetic process that involves changes not only in characteristics of the tumor cell but also in it's interaction with the cellular environment. The response of the host, in turn, determines the survival, continued growth, and spread of tumor cells. The immune response in this scenario is being appreciated increasingly.

The cellular and humoral immune responses triggered by tumor cell-specific neoantigens are evaded by growing tumors through a number of strategies. These include the physical exclusion of immune cells, reduced immunogenicity due to the expression of major histocompatibility complex (MHC), costimulatory molecules and disruption of natural killer and the natural killer T cell. Some tumors elaborate cytokines or growth factors that interfere with antigen presenting cells or block the production of proinflammatory molecules. The progressing tumor is immunoedited as a consequence of this interaction with the host by the selection of poorly immunogenic and/or immuno-resistant malignant cells.

Although thyroid carcinoma is an unusual malignancy, it is receiving increasing attention, because it currently is the malignancy with fastest rate of increase among women. Our understanding of the molecular basis of the thyroid carcinoma is advancing rapidly, as are our strategies in treating it. A better understanding of the response of the immune system to thyroid carcinoma may enhance future treatment options, particularly in patients with poorly differentiated or anaplastic tumors.

In wiew of their pivotal role in the immune response several investigators have studied the association of thyroid carcinoma with HLA alleles.

HLA antigens, predominantly the Class II HLA DR molecules, have been associated with well-differentiated thyroid carcinoma. Conversely, no associations were found in some earlier studies. Most studies have not found skewed associations with histology or tumor behavior.

HLA antigens have been implicated as genetic factors influencing development or various types of malignant tumors. The mechanism by which the major histocompatibility comlex (MHC) genes are related to cancer pathogenesis are obscure, but the interaction between environment and MHC genes to enhance tumor genesis is well-documented in some cases.

There are still important unanswered questions „Which are the environmental factors influencing development of thyroid carcinoma?" or „Which are the HLA genes involved and are there genes outside the MHC may be involved in inherited tumor susceptibility.

Medullary thyroid carcinoma

Medullary thyroid carcinoma (MTC), representing 5–10% of all thyroid malignancies, derives from the parafollicular C-cells of the thyroid. High level calcitonin secretion is observed which serves as a reliable marker for neoplastic tissue diagnosis. Prognosis mainly depends on the stage of tumor progression at the time of diagnosis, with a mean 10-year survival of approx. 75%. MTC occurs in sporadic (approx. 75%) and hereditary forms with autosomal dominant trait (approx. 25%). Familial MTC occurs as part of three different clinical phenotypes: multiple endocrine neoplasia (MEN) type 2A and type 2B and familial MTC-only syndrome. Medullary thyroid carcinoma is characterized by dominant activating mutations in the RET proto-oncogene. Currently therapy is restricted to surgical removal of all neoplastic tissue lacking alternative forms of treatment such as chemotherapy or radiotherapy.

Cytogenetics of benign tumors and carcinoma of the thyroid

Knowledge of the cytogenetics of solid tumors has lagged behind the rapid advances made in studies of hematological malignancies. Only 1% of all reported cases have dealt with primary epithelial tumors.

Only 11 cases of thyroid carcinoma, most of them medullary and anaplastic carcinoma have previously been cytogenetically analyzed.

Teyssier et al. examined thyroid adenoma and carcinoma tissues. No chromosome anomalies were found in the former, but the thyroid carcinoma cells proved to be hypodiploid (35 to 43 chromosomes) with consistent structural rearrangement involving chromosomes 1 and 2.

To our knowledge, there was no previous report of banding analyses of papillary thyroid carcinomas. Only Mark et al. mentioned unpublished data about the cytogenetics of one primary and one metastic tumor. In these two direct preparations only normal karyotypes were observed.

ras oncogene mutation in thyroid neoplasia.

ras is a key component of several signaling pathways in vertebrates, invertebrates and yeast where it plays critical roles in development, proliferation, differentiation and survival. In the twenty years since the first identification of mutated ras genes in human tumors, intensive effort has been devoted to understanding how ras promotes neoplastic transformation. The incidence of ras mutations in thyroid tumors and their frequency in specific histologic types varies widely in different series. Mutations in all three cellular ras genes (H-, K- and N-ras) have been identified in benign and malignant thyroid tumors. Unlikely that the ras oncogen mutation is the single cause of the tumor development in pathogenesis of thyroid neoplasia.

THE OBJECTIVES OF OUR WORK WAS THE FOLLOWING:

1.) HLA antigen typing was performed in differentiated and medullary type of thyroid carcinoma to be found an allel which is/are characteristic for the histology type, onset of the disease, spreading of the malignant cells and their localisation, or related to the response of the immunsystem.

2.) the IgG heavy chain (Gm) allotypes. was investigated from the same points of wiew

3.) To succeed in our object we analysed our data by using of proper mathematical analysis.

4.) HLA typing, basal/stimulated calcitonin test and family study was performed to determine associations among MTC patients and in their relatives.

5.) We wished to determine whether the contrast in biological behavior and oncogene activation pattern might be associated with a difference in the cytogenetic picture.

6.) Are there any differencies in the cytogenetic picture of benign and malignant thyroid neoplasia ?

7.) Could indicate the cytogenetic picture's changes of the progression of thyroid diseases ?

8.) The mutation frequency of Ha, N, K *ras* oncogens was studied in well differentiated malignant and benign follicular neoplasia.

9.) Does the *ras* oncogene activation play a critical role in development, proliferation differentiation and survival of thyroid neoplasia or not ?

10.) Are there any consequencies of different dietary iodine intake /in *Eastern Hungary and New-Foundland/Canada*/ related to *ras* mutations.

MATERIALS AND METHODS

Fifty-two patients with thyroid epithelial cell cancer were studied for evidence of association with human leukocyte antigens (HLA). Fifty-two consecutive patients with thyroid carcinoma attending to the first Department of Surgery, University of Debrecen, Hungary were included in the first study of ours. All 52 patients were typed for 58 HLA-A, B, C, and DR antigens. The distribution of the patient's HLA phenotypes were compared with those of 380 healthy controls typed for HLA-A, B, C antigens and 160 typed for HLA-A, B, C, DR antigens

HLA antigens were typed by standard microcytoxicity method (Terasaki,McLeland). Relative risk were calculated by standard method (Woolf)

Gm typing of the sera was performed by haemagglutination inhibition test on microfolliculation slides (van Loghem). Using our reagents 13 phenotypes were observed in this material. The distribution of the patient's HLA phenotypes were compared with those of 160 healthy controls typed for H LA-A, B, C DR antigens. HLA phenotype information was also available for 81 out of 168 controls typed for Gm.

In the following investigation, we used DNA typing for HLA-DR allels. All 75 patients who were included in this study were from the same area, from eastern part of the country. Genomic DNA was amplified and alllels were identified by polymerase chain reaction. The frequency distribution of HLA-DR antigens were compared with frequency in 170 healthy blood donors who had no personal or familial history of thyroid disease from the same region.

Statistical analysis

Consideration of fundamental statistical principles is necessary in the design and interpretation of HLA and disease studies. These principles will influence the size of the study, the choice of a population-based and selection of controls.

If individuals with different HLA antigens have varying risk of disease, then a difference should be seen in the distribution of HLA types among patients and

healthy controls. We used the chi-square probe (Woolf) and also we calculated the RR (relative risk) by Haldane.

Discriminant function analysis was performed using the SPSS software package. The following variables were evaluated as predictors: patient age, tumor histology, HLA status, lymphocytic infiltration, lymph node and distant metastases and tumor size. Positive and negative predictive values were calculated by standard using formulae.

HLA typing, basal and stimulated calcitonin examinations and family studies in medullary carcinoma of thyroid

30 patients with medullary cancer of the thyroid gland operated on between 1950 and 1990 at the I-st University Department of Surgery at Debrecen.were studied HLA typing and serum calcitonin examinations were performed on 12 patients. In 5 of 12 patients family accumulation was found. HLA-A, B, C, and DR antigens were determined in the patients and their families in addition to basic and stimulated calcitonin. Family studies were done not only in FMTC but in sporadic medullary cancer

Cytogenetics of four thyroid neoplasia

Cytogenetic analyses were attempted in papillary carcinomas and adenomas of thyroid from 6 patients. Only primary lesions were processed. In only 4 of the 6 patients were analyzable metaphases obtained. For the cytogenetic analysis, primary tumor tissue was carefully cleaned of fibrous tissue and fat, minced with scissors, and washed twice in Hanks' balanced salt solution (HESS). When the number of cells of the culture appeared to be suitable (after 7 to 60 days). Colcemid was added and the cells were incubated. At harvest, the cells were centrifuged and resuspended for 20 minutes. After fixation the cells were centrifuged and resuspended in fresh fixative. Slides were stained by the ASG-trypsin banding method and a fluorescent banding method of Caspersson et al. The nomenclature follows that of ISCN.

Molecular genetics in differentiated carcinoma and in benign adenomas of the thyroid

Paraffin-embedded tissue blocks from a variety of thyroid tumors were randomly selected from the archives of Pathology Department Health Sciences Centre, St. John's Newfoundland, Canada, a high dietary iodine intake area

(dietary iodide, 190-550 microgr/day) and from those of the I st Department of Surgery, Debrecen, Hungary, a low dietary iodide intake area (dietary iodide, 46-70 microgr/day).

Synthetic oligonucleotides and polymerase chain reaction

The oligomers were purchased from Clouted' Company (CA). Five- µm sections were cut from paraffin blocks and processed for polymerase chain reaction as described by Shibata et al. with some modifications. The primers were 20 bases long, each pair enclosing an amplified region of 100 base pairs. Samples were denatured and then cooled rapidly. Forty cycles of amplification were used. Al of the amplified products was run on 2% agarose gel to ascertain that specific amplification had occurred.

Southern Blot and Oligonucleotide Probe Hybridization

After an initial quantitation on the gel each amplified product were run on agarose gel and transferred to nylon membrane. Replicate filters were prepared and DNA was fixed by UV illumination. Hybridization was performed. The filters were then subjected to autoradiography over-night.

RESULTS AND DISCUSSION

In our first publication (Juhász et al 1986) the differentiated thyroid tumors and their significant association to HLA DR1 was established. HLA-DR1 was detected in 53.8% of the patients compared to 19.4% of the healthy controls. These proportions yield a relative risk of 4.85 (χ^2 = 21.26, p < 0.0001). The result was highly significant. When we related the biologic behavior of thyroid tumor to HLA phenotypes, some important differences emerged. Thus, 10 of 12 (83.3%) patients with metastatic disease were DR 1-positive. This proportion was significantly different from that of the 18 of 40 (45%) patients without metastases (RR = 6.1, = 4.67, p < 0.05).

χ^2

The distribution of Gm haplotypes among the control and epithelial cancer group showed no significant deviation. In order to examine possible interaction of Gm phenotypes and HLA in modifying susceptibility to thyroid cancer, we classified patients and controls based on HLA-DR 1 status and homozygozity for Gm fb. Using the proportion of DR1- fb - as baseline, we calculated relative risk for thyroid cancer of 37.5, 6.0, and 2.6 for DR1+ fb+, DR1+ fb -, DR1- fb - groups respectively. Thus Gm and HLA interact to enhance greatly the risk of thyroid cancer.

In a recent study (Juhász et al 2005), we revisited the association of MHC Class II antigens with well differentiated thyroid carcinoma in eastern Hungary using DNA technology and found an association with HLA-DR11. We previously documented an association with DR1 (Juhász et al 1986, 1989), which was detected serologically, in the same population irrespective of thyroid tumor histotypes. That this is unlikely to be a technical problem is proven by the consistency in HLA antigen designation in bone marrow transplantation donors when we changed from serologic typing to DNA typing for MHC II antigens.

HLA-DR11 was the only antigen that showed significant deviation from the background population. The finding that deviation in the prevalence of other antigens was not skewed by the increase in HLA-DR11 was sustained when the relative risk (RR) was recalculated after excluding tumors that were positive for HLA-DR11.

We found no apparent influence of the DR11 phenotype on tumor size, degree of lymphocytic infiltration, metastases, or lymphnode involvement. We examined the influence of various parameters on the progression from microtumor to a clinically relevant carcinoma. Only two predictors in discriminant function analysis were related significantly to tumor size: Distant metastases had a positive relation (minimum $F = 6.647$; function coefficient = 0.841; $p = 0.0119$), and the degree of lymphocytic infiltration had a negative relation (minimum $F = - 0.565$; $p = 0.0109$).

The latter finding suggests that the emergence of clinical tumors is associated with impairment of the immune response, as manifested by lymphocytic infiltration. Other variables, such as HLA-DR phenotypes, histology, age, and gender, did not demonstrate discriminant value.

This study raises two basic and immediate questions: What is the relevance of the association of HLA alleles with thyroid carcinoma; and why have so many alleles been reported in different studies, including our own from eastern Hungary? HLA Class II molecules play central roles in the immune response as restricting elements for peptides presented by antigen-presenting cells to T cells and because their polymorphism and linkage disequilibrium of alleles in linked loci (haplotypes) may determine the quantitative response to downstream products, particularly cytokines.

It has been reported recently that the rearranged in transformation (RET)/PTC oncogene induced the expression of MHC Class II through a signal transducer and activator of transcription 1-mediated mechanism. It is likely that the associated elaboration of cytokines by thyroid tumor cells promotes lymphocytic infiltration. This was demonstrated previously with the overexpression of MET reported in both PTC and FTC, explaining why MHC II expression and lymphocytic infiltration is not limited to PTC. It is noteworthy that wild type p53 may have a stabilizing effect on MHC II expression, which is impaired in mutants. The induction of MHC II through some carcinoma genes explains why previous studies have found that MHC II expression was regulated differently in thyroid carcinoma cells compared with normal thyroctyes. To allow for an effective immune response, the infiltrating mononuclear cells serve to provide costimulatory molecules that are not expressed otherwise by thyroid carcinoma cells. However, these infiltrating mononuclear cells may be a double-edged sword, in that proinflammatory mediators can enhance tumor growth in a paracrine fashion.

There is evidence for humoral and cellular immune responses to thyroid carcinoma-specific neoantigens (RET/PTC) that may be successful in eliminating cells bearing mutant oncogenes in the process of immunomodulation. Immunomodulation emphasizes the reshaping of surviving tumors under the selective pressure of the immune system, resulting in a tumor that differs radically from the original malignancy.

The difference in MHC Class II antigens may be related to patient stratification or to the impact of changing environmental risk factors for thyroid carcinoma targeting different susceptible populations. Sridama et al in an example of heterogeneity noted that DR7-positive patients with radiation-associated thyroid carcinoma had a much

shorter latency period from exposure than DR7-negative patients. In the current study, it is instructive that 7 patients who had PTC with a vigorous and apparently successful immune response to the tumor burden all were positive for DR1, the antigen that was incriminated in our earlier study.

HLA typing and basal serum calcitonin examinations were performed on 12 patients of 30 patients with medullary thyroid cancer operated on between 1950 and 1990 at the 1st University Department of Surgery at Debrecen In 5 of latter patients family accumulation has been confirmed . HLA-A, B. C, and DR antigens were determined in the patients and their families in addition to basic and stimulated calcitonin. On the basis of our results a relationship was found between the HLA-DR7 and the calcitonin level seemingly might have shown a susceptibility to the development of medullary cancer of the thyroid. The relative risk was not possible to estimate because of the small number of the MTC patient but the tendency (from 12 MTC patient 9 was DR7 positive) is rather a remarkable findings. A family study was performed to supporting the assumption that the DR7 association to the medullary cancer is significant. 14 family member was typed for HLA-DR antigens and the basal/stimulated calcitonin level was determined. The basal/stimulated calcitonin level in 7 family member was higher then normal but only two of them showed a significant peak which allow us to diagnose FMTC. After informed consent both underwent total thyroidectomy On the first postoperative day the calcitonin level decreased to normal. Histological finding in both showed C cell hyperplasia which is a precancerous and it should be diagnosed/treated as a relevant medullary carcinoma. Molecular genetics was not available yet at the time of these investigations. MEN 2a, 2b syndromes were not to be verified in any of the patients. Accordingly these, patients with familial carcinoma were ranked into the non-MEN type FMTC showing family accumulation. We concluded that family studies advisable in case of sporadic medullary cancer as well.

On the basis of our results we can established that the modal chromosome number in each of our four specimens was in the diploid range. In one of the papillary carcinomas pseudodiploidy was observed; in the second hypodiploidy with 44 to 45 chromosomes, and in the third cells with 45 to 46 chromosomes were observed. The tumor cells from the follicular adenoma were diploid and appeared to have a normal

karyotype. Other investigators also observed a tendency for hypodiploidy in medullary carcinomas, whereas cells from anaplastic carcinoma after 27 to 412 days in culture proved to be hypotetraploid-tetraploid-hypertetraploid and hexaploid. or near-hexaploid. Consistent losses of chromosomes were observed in only one of the three papillary carcinomas.

To our knowledge, there were no previous reports of banding analyses of papillary thyroid carcinomas. Only Mark et al mentioned unpublished data about the cytogenetics of one primary and one metastic tumor. In these two direct preparations only normal karyotypes were observed.

Consistent losses of chromosomes were observed in only one of the three papillary carcinomas. Most cells of the tumor from patient 1 showed loss of one or both chromosome 10 and less frequently showed loss of Y. Of the markers, the deletion of the long arm of chromosome 11. which was detected in two of the three specimens of papillary carcinoma and suspected in the third specimen, and the rearrangement of chromosome 1 leading to a partial duplication of the long arm in the tumor cells of patient 1 are of particular interest.

The deletion of the long arm of chromosome 11 is the single common aberration of the three specimens of papillary carcinomas. Although the breakpoints involved in the three cases were not exactly the same, their location and the fact that the llq deletion could be detected in most cells suggest that loss of the terminal part of the long arm of chromosome 11 may be specific for papillary carcinoma of the thyroid.

The most frequent aberration in tumor cells of patient 1 was a rearrangement of chromosome 1 (mar 1) resulting in a partial duplication of 1q:dup(1)(g23→qter). The suspected breakpoints were 1p35,1q23. A similar structural aberration of chromosome 1 was found by Wurster-Hill et al in thyroid medullary carcinoma. That the chromosome 1 aberration could be detected in most cells indicates that it may play an important role in the tumorigenesis. That this aberration appeared only in one of the three cases with a papillary carcinoma and was associated with several other markers suggests, however, that case 1 may represent a later stage of the disease. This hypothesis and the frequent and nonspecific occurrence of chromosome 1 rearrangements in human tumors suggest that chromosome 1 anomalies may be important during tumor progression.

In some of the cells of each patient with papillary carcinoma, the signs of gene amplification, double minut's were present.

The current data raise the possibility that 11q21—23 deletions are specific to papillary thyroid carcinoma. That the deletions were detected at different culture times in two patients makes it unlikely that these abnormalities are in vitro artefacts. Because we have not examined follicular carcinoma tissue, this conclusion must be tempered. The patterns of familial accumulation and oncogene activation are consistent with this assertion, however.

Comparing ras activation data in an iodide-sufficient and iodide-insufficient territory is still a unique publication in the literature and cited rather often. The histology of specimens from Debrecen was reviewed in St. John's before PCR amplification to exclude differences in diagnostic criteria. Since the activating mutations so far identified in naturally occurring tumors are at the codons 12, 13, and 61 (8), selective amplification of both regions was performed. Amplified DNA was analyzed with a set of oligomers each designed to be complementary to a different point mutation within these co-dons. This allows for the detection of all possible single base pair substitutions. Mutated ras oncogenes were identified in 1 of 13 thyroid adenomas (85%) and 3 of 6 follicular carcinomas (50%) obtained from Debrecen. Most of the adenomas (80%) were macrofollicular and the rest were mixtures of micro- and macro-follicular adenoma. No mutations in any of the three ras oncogenes were found in 12 papillary carcinomas. The most common mutation site was codon 61 with Gln → Arg. Mg substitution accounting for 93% of the mutations found at that position. No mutations were detected in codons 12 or 13. One specimen displayed two ras mutations in codon 61 (Gin→ Lys in N-ras and Gin→ Arg in Ha-ras). Its histology was typical of a microfollicular adenoma.

The frequency of ras oncogene mutations was much lower in the specimens from St. John's than in the Eastern Hungarian material. Only 2 mutants in 12 specimens (16%) were identified in adenomas with the same histological pattern as the Hungarian material. This incidence is significantly lower than that in the Hungarian material ($chi2 = 5.695$, $p < 0.02$). In only 1 of 10 (10%) follicular carcinomas was a ras mutant detected. The difference between the incidence of ras mutations in Newfoundland and Eastern Hungary was not significant ($chi2 = 1.423$, $p< 0.02$),

probably related to the small number of Hungarian specimens. Codon 61 with Gin→ Arg substitution was still the most common target for mutation. As in the iodide-deficient area, no mutations were found in 10 papillary carcinoma specimens examined.

The ras protooncogene family includes three genes that have been designated Ha-ras-1, K-ras-2, and N-ras. These genes appear to be functionally involved in regulating cellular growth and cellular growth and differentiation. Mutations at codons 12, 13, or 61 of one of the three ras genes convert them into active oncogenes. Mutation at codon 61 is the most proficient in changing the conformation of ras proteins which is required to catalyze GTP hydrolysis (GTPase-inhibiting mutation). The mutant proteins can bind to the GTPase-activating protein which mediates the signal transducing effect of ras protein on the cell.

Dietary iodine intake in Eastern Hungary is suboptimal, whereas in North America it is more than sufficient. Our results show that ray genes are more frequently activated in follicular thyroid tumors in Eastern Hungary than in Newfoundland, and that they are activated at similar frequency in the early stages (adenoma) as in the late stages (follicular carcinoma) of tumorigenesis. In view of small sample numbers we combined our Hungarian data with those reported from Cardiff, United Kingdom (2) and compared them with the Newfoundland material. The ras oncogene mutation rate (11 of 21) was significantly greater than that from Newfoundland (1 of 10) (chi2 = 14.03; p < 0,0001). This exercise is justified by the borderline (mean, 90 pg/day) urinary iodide excretion in Wales, reflecting iodide intake below that recommended (150—300 pg/ (day), as well as the similarities in ras mutation rates. Iodide deficiency is known to produce thyroid hyperplasia, nodule formation, and ultimately malignancy in experimental animals. Some studies have also shown increased numbers of thyroid carcinomas in endemic goiter regions. The excess of differentiated thyroid tumor is attributable to those with follicular histology (22, 24) in which ras mutations were found in this study. Our data suggested that in dietary iodine-deficient area ras oncogene activation may play a more important role in tumor initiation and/or maintenance. Additional factors are, however, necessary to initiate carcinogenesis.

THE THESIS IS BASED ON THE FOLLOWING PUBLICATION

1. F. Juhász, Gy. Balázs, Valéria Stenszky, L. Kozma and N.R. Farid. The relation of susceptibility to and biologic behavior of thyroid epithelial cell cancer to HLA. Cancer. 1986, 58: 52-54. IF: 2, 33
2. F. Juhász, Gy. Balázs, L. Kozma, E. Kraszits, Valéria Stenszky and N.R. Farid. Interaction of IgG Heavy-chain allotypes /Gm/ and HLA in conferring susceptibility to thyroid carcinoma. Clinical Endocrinology. 1986, 25: 17-21. IF: 2, 388
3. F. Juhász, P. Boros, Gy. Szegedi, Gy. Balázs, P. Surányi, E. Kraszits, Valéria Stenszky and N.R. Farid. Immungenetics and immunologic studies of differentiated thyroid cancer. Cancer. 1989, 63: 1318-1326. IF: 2, 313
4. Éva Oláh, F. Juhász, F. Boján, Valéria Stenszky, N.R. Farid. Cytogenetic analyses of three papillary carcinomas and a follicular adenoma of the thyroid. Cancer Genet Cytogenet. 1990, 44: 119-129. IF: 2, 392
5. Oláh É., Balogh E., Boján F., Pásti G., Losonczi L., Juhász F., Stenszky V., Farid N.R. Kromoszómavizsgálat pajzsmirigy papilláris karcinómában és follikuláris adenomában. Orvosi Hetilap. 1991, 132 (6): 281-336.
6. Shi Y.F., Zou M.J., Schmidt H., Juhasz F., Stenszky V., Robb D., Farid N.R. High rates of ras codon 61 mutation in thyroid tumors in an iodide-deficient area. Cancer Res. 1991 May 15, 51 (10): P 2690-3. IF: 4, 383
7. Juhász F., Stenszky V., Lukács G., Győry F., Lenkey Á. Családvizsgálatok medulláris pajzsmirigyrákokban. Magyar Sebészet. 1993, 46: 361-368.
8. F. Juhász, L. Kozma, V. Stenszky, F. Győry, G. Lukács, N.R. Farid. Well differentiated thyroid carcinoma is associated with human leucocyte antigen D-related 11 in Eastern Hungarians. Cancer. 2005 Oct 15, 104 (8): 1603-8. IF: 4, 434

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FURTHER PUBLICATION RELATED TO THE SUBJECT

1. E. Bodolay, Gy. Szegedi, P. Surányi, F. Juhász, V. Stenszky, Cs. Balázs, N. R. Farid. Expression of HLA-DR antigens by thyroid cells: the effect of Graves IgG. Immunology Letters. 1987. 15. 77-81 IF: 1, 241
2. Fábián E., Balázs Gy., Lukács G., Csáky G., Juhász F. Pajzsmirigydaganatok preoperatív igazolása finomtű-aspirationval. Magyar Sebészet. 1987, 40: 121-126
3. V. Stenszky, C. Balázs, E. Kraszits, F. Juhász, L. Kozma, Gy. Balázs and N. R. Farid. Association of goitrous autoimmune thyroiditis with HLA-DR 3 in Eastern Hungary. Journal of Immunogenetics. 1987, 14: 143-148 IF: 1, 250
4. Balázs Gy., Lukács G., Fábián E., Csáky G., Juhász F. A pajzsmirigy hideg göbök differenciáldiagnosztikája. Orvosképzés. 1987, 62: 471-477
5. Edith Bodolay, Péter Surányi, Ferenc Juhász, Valeria Stenszky, Csaba Balázs, N. R.Farid. Methimazol blocks Graves IgG but not interferon-y-HLA-DR expression by thyroid cells. Immunology Letters. 1988, 18: 167-172
   IF: 1, 137
6. Surányi P., Szegedi Gy., Damjanovich S., Juhász F., Stenszky V., Farid N.R. B_Lymphocyte subsets in Hashimoto Thyroiditis. Immunology Letters. 1989, 22(2): 147-1450. IF: 1, 241
7. Lukács G., Balázs Gy., Juhász F. A radikalitás szempontjai a papilláris pajzsmirigyrákok regionális nyirokcsomó-metasztázisainak kezelésében. Magyar Sebészet. 1991, 44: 113-117
8. Lukács G., Balázs Gy., Mikó T., Juhász F., Pálffy A. Klinikum és morfológia a follikuláris szerkezetű pajzsmirigytumorok szelektív műtéti kezelésében. Magyar Sebészet. 1991, 44: 165-171
9. Lukács G., Balázs Gy., Molnár F., Juhász F., Győry F. Pajzsmirigykarcinóma és benignus pajzsmirigybetegség együttes előfordulása. Magyar Sebészet. 1991, 44: 281-287.
10. Lukács G., Balázs Gy., Juhász F. A radikalitás szempontjai a papilláris típusú pajzsmirigyrákok regionalis nyirokcsomó-metasztázisainak kezelésében Magyar Sebészet. 1991, 44: 113-117

11. Lukács G., Balázs Gy., Uray É., Juhász F. Differenciált szerkezetű pajzsmirigyrákok tüdő-és csontáttéteinek kezelése. Orvosi Hetilap. 1991, 132: 2687-2690
12. Gy. Balázs, E. Fábián, G. Lukács, F. Juhász. Rapid cytological diagnosis during thyroid surgery. European Journal of Surgical Oncology. 1992, 18: 1-6
IF: 0,450
13. Lukács G., Balázs Gy., Juhász F., Thomázy V., Győry F. A medulláris pajzsmirigyrák kezelése és prognózisa. Magyar Sebészet. 1992, 45: 313-320
14. Lukács G., Miltényi L., Uray É., Győry F., Juhász F., Molnár P., Balázs Gy. Interdiszciplináris együttműködés a magas malignitású pajzsmirigy tumorok kezelésében. Magyar Sebészet. 1993, 46: 1-6
15. Lukács G., Győry F., Juhász F. Differenciált szerkezetű pajzsmirigyrákok recidiváinak prognosztikai jelentősége. Magyar Sebészet. 1993, 46: 351-360.
16. Juhász F., Balázs Gy., Lukács G., Lenkey Á., Győry F. Thyreoglobulin meghatározások értéke differenciált pajzsmirigycarcinomában szenvedő betegek utókezelésében. Orvosi Hetilap. 1994, 135: 849-853
17. A.F. Ahmad, V. Stenszky, F. Juhász, Gy. Balázs, N. R. Farid. No Mutations in the Translated Region of Exon 1 in the TSH Receptor in Graves Thyroid Glands. Thyroid 1994, 4: N. 2. IF: 1,695
18. Gy. Balázs.,G. Lukács,.F. Juhász., F. Győry,. Éva Oláh, Erzsébet Balogh. Special features of childhood and juvenile thyroid carcinomas Surgery Today Jpn J Surg. 1996, 26: 536-540 IF: 0,171
19. Győry F., Lukács G., Nagy V. E, Juhász F., Mezősi E., Szakáll Sz., Máth J.,Balázs Gy. Differenciált pajzsmirigy carcinoma: prognosztikai faktorok vizsgálata. Orvosi Hetilap. 2001, 54: 69-74
20. Győry F., Lukács G., Juhász F., Mezősi E., Szakáll Sz., T. Végh, Máth J., Balázs Gy. Surgically treated Hashimoto Thyroiditis. Acta Chir Hungarica. 1999, 38 3-4: pp. 243-247
21. Győry F, Balázs G, Nagy EV, Juhász F, Mezősi E, Szakáll S, Máth J, Lukács G. Differentiated thyroid cancer and outcome in iodine deficiency. Eur J Surg Oncol. 2004 Apr, 30 (3): 325-31 IF: 1.882

22. Puskas LG, Juhasz F, Zarva A, Hackler L, Farid J, Farid N. Gene profiling identifies genes specific for well-differentiated epithelial thyroid tumors. Cell Mol Biol 2005 Sep 5, 51-2: 177-186 IF: 0. 873

Impact factor: 9,930

BOOK REFERENCIES

1. T. Mikó, G. Lukács, Gy. Balázs, F. Juhász. Reliability of frozen section diagnosis of the thyroid. Verh Dtsch Krebs Ges. 1983, 4: 629. Gustav Fischer Verlag Stuttgart New York
2. Balázs György, Juhász Ferenc. Pajzsmirigygyulladások. 1989, 57-59,. Medicina Szerk. : Balázs György A pajzsmirigy és a mellékpajzsmirigy sebészete
3. G. Lukács, G. Balázs, P.Molnár, F. Juhász, F. Győry. Gleichzeitiges Auftreten von Schilddrüsenkarzinomen und benigner Schilddrüsenerkrankung. In: W. Pimpl, Derzeitiger Stand in Diagnose und Therapie der Struma Maligna Springer Verlag Berlin 1993, 27-37
4. F. Juhász, Zs. Kincses, F. Győry, Zs. Kanyári, B. Megyeri and Gy. Balázs. Surgical and medical treatment of abdominal carcinoid tumors 8 th World Congress of the International Gastro-Surgical Club Strasbourg (France), 1998, April 15-18, International Proceedings Division Eds: H. Bismuth, J. P.Galmiche, M. Huguier, D. Jaeck (Monduzzi Editore)
5. L.G. Puskas, F. Juhasz, A. Zarva, L. Hackler, Jr. and N. R. Farid. Distinction Between Graves' Disease and Hashimoto's Thyroiditis by Gene Profiling The Journal of Endocrine Genetics 2005, Volume 4: No. 1,

ABSTRACT, SUPPLEMENTUM, COMMENT

1. Juhász F., Stenszky V., Bartha I., Balázs Gy.: A complex clinical and genetic analysis of patients with medullary thyroid cancer. Acta Endocrinologica. 1983,. 102:. Suppl. 252 IF: 2, 241
2. F. Juhász, Valéria Stenszky, Gy. Balázs, L. Kozma and N. R. Farid. Epithelial thyroid cell carcinoma is associated with DR 1. International Meeting on Immunogenetics of endocrine Disorders. St.John,s , New Foundland. Canada. 1985. augusztus 24-27. Abstracts, 23.
3. V. Stenszky, F. Juhász, Gy. Balázs, B. Larsen, N. R. Farid. HLA and IgG heavy chain markers /GM/ in differentiated thyroid carcinoma. XIII. Congress of the European Academy of Allergology and Clinical Immunology. Abstracts. 1986.
4. F. Juhász, V. Stenszky, Gy. Balázs, B. Larsen, N. R. Farid. HLA and IgG heavy chain markers (Gm) in DTC. 14-th International Cancer Congress. Budapest, 1986. augusztus 21-27. Abstract of Lectures. Vol. 3. 4527.
5. V. Stenszky, C. Balázs, E. Kraszits, F. Juhász, L. Kozma, Gy. Balázs and N. R. Farid. Association of goitrous autoimmune thyroiditis with HLA-DR 3 in Eastern Hungary.Journal of Immunogenetics. 1987, 14: 143-148
6. Balázs Gy., Lukács G., Fábián E., Csáky G., Juhász F. A pajzsmirigy hideg göbök differenciáldiagnosztikája. Orvosképzés. 1987, 62. 471-477.
7. Sápy P., Péter M., Balogh E., Juhász F. Schwere chronische Entzündung im Pankreaskopf - negatives ERCP. Acta Chir Austriaca. 1988, 3: 153-154
8. Gy. Balázs, G. Lukács, G. Csáky, F. Juhász and E. Fábián. Reeingriffe bei papillären Schilddrüsenkarzinom. Acta Chir Austriaca. 1988, Sonderheft/Jahrgang. 20.
9. F.Juhász und L.Varga. Anwendungsmöglichkeiten des Ebrimycin-gels in der septischen Abteilung der allgemeinen Chirurgie? Acta Chir Austriaca. 1988, Heft 3.
10. Gy. Balázs, G. Lukács, F. Juhász, Éva Uray. Komplexe Behandlung des papillären Schilddrüsenkarzinom ohne Radiojodtherapie. Acta Chir Austriaca. 1988, Heft 3.

11. G. Lukács, F. Juhász, Valeria Stenszky, Gy. Balázs, B. Larsen, Farid N.R. HLA and IgG heavy chain markers (Gm) in differentiated thyroid carcinoma. Medicine, Biologie, Environment. 1990, 18: 35-37.
12. F. Juhász, I. Erdei, Zs. Kanyári. Metal allergy as a possible cause for the early reoperation after laparoscopic cholecystectomy. 4-th International Congress of the European Association for Endoscopic Surgery Trondheim Norway 1996. junius 23-26. Abstract.
13. Juhász F., Farid N. R. Immune response to papillary thyroid carcinoma. (Letter: Comment) J Clin Endocrinol Metab. 1996 Nov, 81(11): 4175-6. IF: 5.778
14. G. L. Lukács, Gy. Balázs, F. Győry, F. Juhász, Sz. Szakáll. Chirurgische Strategie bei Strumen im Kindesalter. Acta Chir.Austriaca. Suppl. Nor. 141. 8. 1998.
15. Juhász F.,Kanyári Zs.,Győry F.,Lukács G. Laparoszkópos adrenalectomia. Learning curve. Magyar Sebészet 2000 október, 46: III. évf. Supplementum.
16. G. L. Lukács, F. Györy, F. Juhász, Sz. Szakáll. Pediatric thyroid cancer European Journal of Cancer 2001 October, Vol 37: (Suppl.6) page 108. Abstracts IF: 3.302

Impact factor: 11,321