HLA Class I Expression Changes in Different Types of Cultured Parathyroid Cells

Beyza Goncu,[1] Emrah Yucesan,[2]* Erhan Aysan,[3] Nur Ozten Kandas[4]*

Abstract

Objectives: **Tissue-specific immunogenicity can be characterized by the determination of human leukocyte antigens (HLA). Parathyroid hyperplasia tissue cells are presumed to have the ability to lose HLA class I expression profile during cultivation, whereas healthy parathyroid cells are presumed to already express HLA class I molecules at low levels. However, there are conflicting results about the expression of HLA class I antigens. In this study, our aim was to evaluate different patterns of HLA class I expression in different parathyroid tissue cells.**

Materials and Methods: **Parathyroid tissue cells were isolated enzymatically and cultured in vitro. Expression of HLA class I (HLA-A, HLA-B, HLA-C) mRNA and protein levels were studied in 7 parathyroid adenomas and 9 parathyroid hyperplasia tissue samples by reverse transcriptase-polymerase chain reaction and Western blot analyses.**

Results: **HLA-A protein expression remained stable in parathyroid adenoma and hyperplasia tissue, but HLA-A mRNA expression decreased in adenoma tissue. In parathyroid hyperplasia tissue, HLA-B protein expression remained stable, although mRNA expression levels decreased during cultivation. HLA-C mRNA expression was steady in parathyroid adenoma yet significantly decreased in hyperplasia tissue samples. HLA-C protein expression levels were below 30 pg for both types of parathyroid tissue during cultivation.**

Conclusions: **HLA class I expression levels of parathyroid hyperplasia and adenoma tissue were not found to be similar. Parathyroid hyperplasia tissue is the donor tissue for the treatment of permanent hypoparathyroidism. Therefore, expression patterns of HLA class I are directly relevant to the transplant process. In particular, the HLA region is highly polymorphic, and, as a consequence of this, heterogeneous correlations among HLA-A, HLA-B, and HLA-C expression patterns of parathyroid tissue should be evaluated in detail before transplant for future studies.**

Key words: *Human leukocyte antigen class I, Immunologic characteristics, Parathyroid adenoma, Parathyroid hyperplasia, Transplantation*

Introduction

The parathyroid gland is a member of the endocrine system and is localized in the posterior side of the thyroid gland. Healthy individuals normally have 4 parathyroid glands. The parathyroid gland secretes parathyroid hormone (PTH), which is the main regulator of calcium and phosphate homeostasis.[1,2] Parathyroid tissue is composed of chief, oxyphil, and water clear cells, and the percentage of cell types in each parathyroid gland among individuals is highly heterogeneous.[3,4]

The immunohistochemical detection of parathyroid glands seems difficult because differentiated parathyroid tissue shows a histologic pattern similar to Hurtle cell carcinoma. Thus, the diagnosis of the parathyroid glands with biopsies, as well as frozen section specimens, is particularly challenging.[4-6] The classification of the parathyroid gland is composed of 4 main subtypes: atypical parathyroid adenoma, parathyroid adenoma, parathyroid hyperplasia, and parathyroid carcinoma. Moreover, overexpression of PTH causes hyperfunctioning of the parathyroid glands. This abnormality is known as parathyroid adenoma (which often occurs in a single gland) or parathyroid hyperplasia. Parathyroid hyperplasia

From the [1]Experimental Research Center, the [2]Institute of Life Sciences and Biotechnology, the [3]Faculty of Medicine, Department of General Surgery, and the [4]Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Bezmialem Vakif University, Istanbul, Turkey

Acknowledgements: *The authors highly appreciate the efforts of Monica Ann Ozkan, MSN, RN, and CPAN in editing this article. We thank the head of Microbiology Research Laboratory Prof. M. Ziya Doymaz for equipment support. This work was funded by Bezmialem Vakif University Scientific Research Projects Unit (project number: 3.2016/7). The authors have no conflicts of interest to declare.*

**B. Goncu and E. Yucesan contributed equally to this work.*

Corresponding author: *Beyza Goncu, Adnan Menderes Bulvari, Vatan Caddesi, 34093, Fatih, Istanbul, Turkey*

Phone: *+90 212 523 22 88/1465* ***E-mail:*** *bsgoncu@gmail.com or bgoncu@bezmialem.edu.tr*

Experimental and Clinical Transplantation (2019)

DOI: 10.6002/ect.2018.0388

can be classified as primary, secondary (as a result of an extrinsic factor such as patients with chronic kidney disease), or tertiary hyperparathyroidism.[7-9] Nuclear pleomorphism, dilated follicular growth with eosinophilic colloid-like material in parathyroid adenoma,[10] diffuse abnormalities, nodules, and a noninvasive pattern in parathyroid hyperplasia are common features in histologic grading.[11] Differentiated tissues with distinct histology can vary in tumors and also in parathyroid tissue. Molecular mechanisms underlying parathyroid tissue differences still require further characterization.

Major histocompatibility complex encodes human leukocyte antigens (HLA) and enables the immune system to categorize between "self" or "non-self" molecules.[12] Major histocompatibility complex genes are the most polymorphic region of the human genome and are located on the short arm of chromosome 6 and contain more than 220 genes; a total of 10 956 HLA alleles have been recognized for HLA class I.[13,14] The HLA domain is frequently related to pathogenesis of more than 100 diseases, including celiac disease,[15] rheumatoid arthritis,[16] Behçet disease,[17] sarcoidosis,[14] and progression of cancer.[16,18,19] Moreover, one of the most conspicuous features of HLA allele-based disease association does not involve antigen recognition or pathogenesis. Therefore, disease severity cannot be explained by allelic-dose impact, and this mostly is the result of improper antigen recognition.[14,20] On the other hand, HLA molecules may enhance the immune response to a pathogen such as malaria or slow the progression of human immunodeficiency virus infection.[20]

Furthermore, HLA class I molecules play an important role in recognition of allopeptides, which are monitored closely by cytotoxic T lymphocytes and natural killer cells.[21,22] HLA class I genes encode HLA-A, HLA-B, and HLA-C, and these molecules permit T lymphocytes or natural killer cells to see the inner sides of other cells[23] for required responses such as rejection/elimination or tolerance.[24]

In solid-organ transplantation, cultured endocrine tissue cells are presumed to be passenger-leukocyte free and have low expression profiles of HLA molecules, ensuring prolonged graft survival. However, even with cultivation, endocrine tissue is still rejected, with limited survival rates after parathyroid allotransplant (PA).[25] Parathyroid allotransplant has been routinely performed in our center since 2013. Immune-monitoring criteria for PA requires ABO compatibility, negative complement, and T and B crossmatch tests for preevaluation between recipient and donor. Criteria were initially defined by Yucesan and colleagues.[26] During the immune-monitoring process after PA, recipients develop de novo donor-specific alloantibody against HLA class I (unpublished data). Therefore, with the experience gained from follow-up data, we have questioned HLA class I patterns in different parathyroid tissues.

In this study, the HLA class I molecules (HLA-A, HLA-B, and HLA-C), including both gene and protein expression changes, were analyzed and compared during cultivation in parathyroid adenoma and parathyroid hyperplasia tissues.

Materials and Methods

This study was conducted after approval was received from our Local Human Ethics Committee (approval number: 71306642-050.01.04). All of the protocols conformed to the ethical guidelines of the Helsinki Declaration, and written informed consent was obtained from all participants.

Donor recruitment

Nine patients with parathyroid hyperplasia due to chronic renal failure (5 male and 4 female patients; mean age of 41 years; range, 38-65 years) and 7 patients (3 male and 4 female patients; mean age of 33 years; range, 29-54 years) with parathyroid adenoma due to primary hyperparathyroidism who were referred from the nephrology outpatient clinic to the general surgery department for surgical approach were enrolled in our study. Patients were scheduled for parathyroidectomy. Patients who had their parathyroid tissue damaged during surgery or who had hepatitis B, hepatitis C, or human immunodeficiency virus infections were excluded from the study. Tissues were first evaluated morphologically, and then half of each of the resected gland was delivered to the pathology department for diagnosis. The remaining parts of the glands were transported to the parathyroid cell culture laboratory unit and stored at -80°C. After diagnosis with confirmed pathology, tissues were prepared for the further processes.

Cell isolation

Each tissue was cut and washed with 1× phosphate-buffered saline solution (Thermo Fisher Scientific,

Boston, MA, USA) (pH 7.4) and minced in a Petri dish on ice. The minced preparation was combined with 2 mL of 100 μg/mL bovine serum albumin (Merck Millipore, Darmstadt, Germany), 215 μM collagenase type II (Thermo Fisher Scientific), 0.32 μM DNase I (AppliChem, Gatersleben, Germany), and 1 mL of Ham's F10 supplement (Thermo Fisher Scientific). Samples were transferred to an incubator (CCL-170B-8; ESCO, Singapore) at 37°C with humidified atmosphere containing 5% CO_2, where they were incubated overnight. Each sample was filtered into a 15-mL conical tube using a sterile cell strainer (70 μm, Falcon, BD Biosciences, Franklin Lakes, NJ, USA) with 10 mL of McCoy's 5A (modified) medium (Thermo Fisher Scientific) with 1% sodium pyruvate, 1% penicillin-streptomycin, and 10% fetal bovine serum. The solution was centrifuged at 424*g* for 15 minutes to obtain a cell pellet. Cells were suspended in 1 mL of culture medium, and cell viability was assessed using a Muse cell analyzer (Merck Millipore) with a Muse Count & Viability assay kit (Merck Millipore). The process of gating in the Muse cell analyzer was adjusted for each tissue separately through the cell population profile, which maintained the gating profile through determination of viability.

Cultivation

After viability assessment, 75 × 10^6 cells were separated and marked as day 0 for each group. The remaining cells were cultivated in 25-cm^2 T flasks for 9 days, and the medium was changed every 3 days. Supernatant samples were collected and kept at -80°C for further experiments, and around 75 × 10^6 cells were separated between each medium renewal at the indicated time intervals (days 3, 6, and 9). Each separated cell group was immediately prepared for total protein and total RNA isolation processes.

Preparation of total protein extracts

A 500-μL complete RIPA lysis buffer (containing 5 μL of phenylmethylsulfonyl fluoride, 5 μL of sodium orthovanadate solution, and 5 μL of protease inhibitor cocktail) (Santa Cruz, Santa Cruz, CA, USA) was used per 50 × 10^6 cell suspension. The suspension was frozen overnight at -80°C. The extract was centrifuged for 15 minutes at 16000*g*, and then the supernatant was collected. Total protein concentrations were measured using a Coomassie (Bradford) protein assay kit (Thermo Fisher Scientific).

Western blot analyses

A 50-μg sample of total protein extract (4%-17% for HLA-A and 4%-12% for HLA-B, HLA-C, and β-actin) was separated on sodium dodecyl sulfate-polyacrylamide gels and transferred to 0.2 μm polyvinyl difluoride membranes (Bio-Rad, Hercules, CA, USA) in 1× transfer buffer at 1.8A for 30 minutes with the Trans-Blot Turbo Transfer System (Bio-Rad). The membranes were blocked overnight at +4°C with Tris-buffered saline plus 0.02% Tween 20 containing 5% nonfat milk. The primary antibody was rabbit monoclonal anti-HLA-A antibody for HLA-A (0.02 μg/mL; Abcam, Cambridge, UK), rabbit polyclonal anti-HLA-B antibody for HLA-B (0.1 μg/mL, Thermo Fisher Scientific), rabbit monoclonal anti-HLA-C antibody for HLA-C (1:1000 dilution; Abcam), and rabbit polyclonal for β-actin (1:1000 dilution; Cell Signaling, Danvers, MA, USA). Secondary antibodies were anti-rabbit immunoglobulin G and horseradish peroxidase-linked antibody (1:3000 dilution; Cell Signaling). The membranes were washed 5 times each (5 × 5 min) between antibody incubations with Tris-buffered saline plus 0.02% Tween 20. We developed the blots using the enhanced chemoluminescence detection kit (Bio-Rad).

Total RNA isolation

Extraction of total RNA from 25 × 10^6 cells was performed with a High Pure RNA isolation kit (Roche, Mannheim, Germany) according to the manufacturer's recommendations. Quantitative analysis of RNA was conducted by measuring optical density at 260 nm and 280 nm using Multiskan GO (Thermo Fisher Scientific).

Reverse transcriptase-polymerase chain reaction

The isolated RNA was used as a template for cDNA synthesis in reverse transcription reactions using a First-Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. We conducted reverse transcriptase-polymerase chain reaction (RT-PCR) analyses using SensiFast SYBR No-ROX reagents (Bioline Reagent, Ltd., London, UK) and the Bio-Rad CFX96 instrument. Primer sequences were as follows: TTGAGAGCCTACCTGGATGG (forward) and TGGTGGGTCATATGTGTCTTG (reverse) for HLA-A, CTTCAAGAGCCTCTGGCATC (forward) and AGGGGTCACAGTGGACACA (reverse) for HLA-B, and ATACCTGGAGAACGGGAAGG (forward)

and ATACCTGGAGAACGGGAAGG (reverse) for HLA-C. We conducted RT-PCR using the following protocol: 95°C for 2 minutes followed by 40 cycles of denaturing at 95°C for 5 seconds, annealing at 61°C (HLA-A) or 60°C (HLA-B) or 60.3°C (HLA-C) for 15 seconds, and extension at 72°C for 10 seconds, with a final extension step at 72°C for 2 minutes. The ratios of HLA-A, HLA-B, and HLA-C expression levels relative to β-actin in each sample were calculated.

Parathyroid hormone enzyme immunoassay

Parathyroid hormone in cell culture supernatant samples was determined with a commercially available human enzyme immunoassay kit (RayBiotech Inc., Norcross, GA, USA). This kit is a quantitative assay for detecting PTH peptide based on the competitive enzyme immunoassay principle. All samples and standards were studied in duplicate.

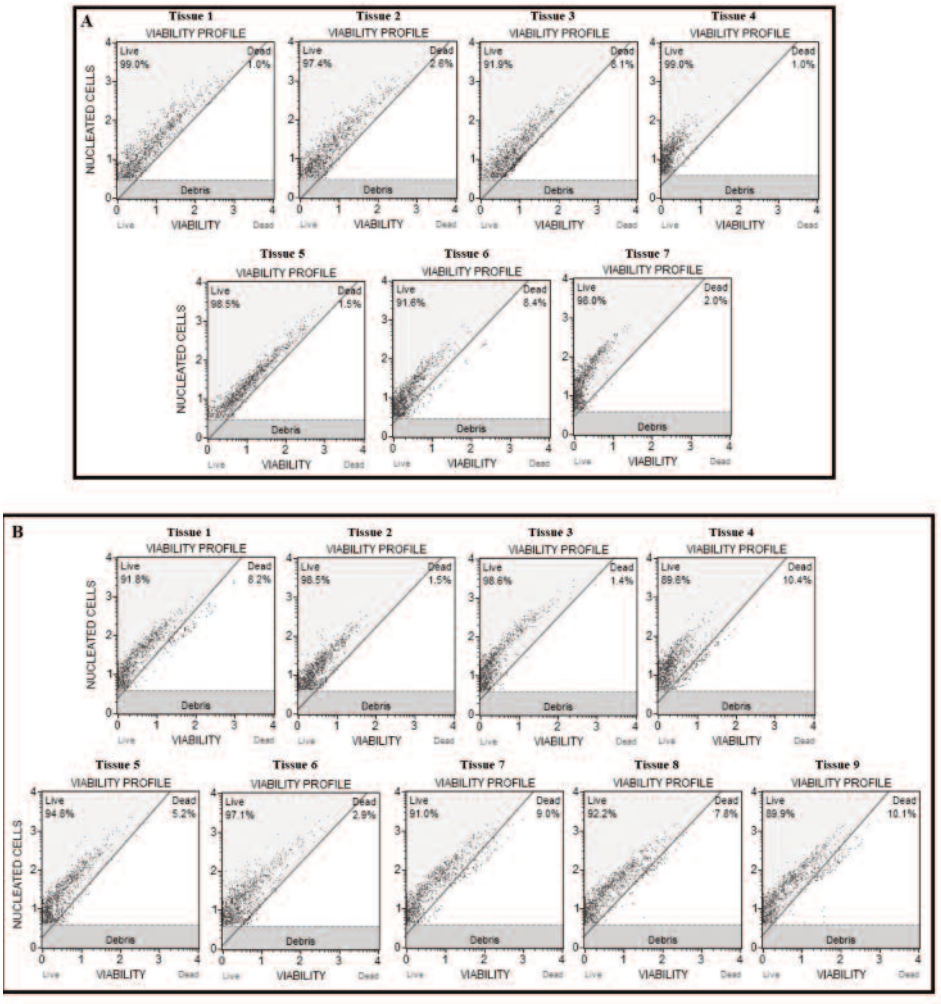
Statistical analyses

The ImageJ (National Institutes of Health) program[27] was used for analysis of Western blot images. One-way analyses of variance (GraphPad Software, Inc., La Jolla, CA, USA) were used for analyses of quantitation of Western blots and RT-PCR.

Results

Viability profiles of adenoma and hyperplasia tissues were determined after enzymatic cell isolation. As shown in Figure 1A and 1B, the mean cell viabilities were 91.5% and 98.3%, respectively. Parathyroid hormone released from each adenoma and

Figure 1. Cell Viability Profile of Nucleated Cells of Parathyroid Tissue

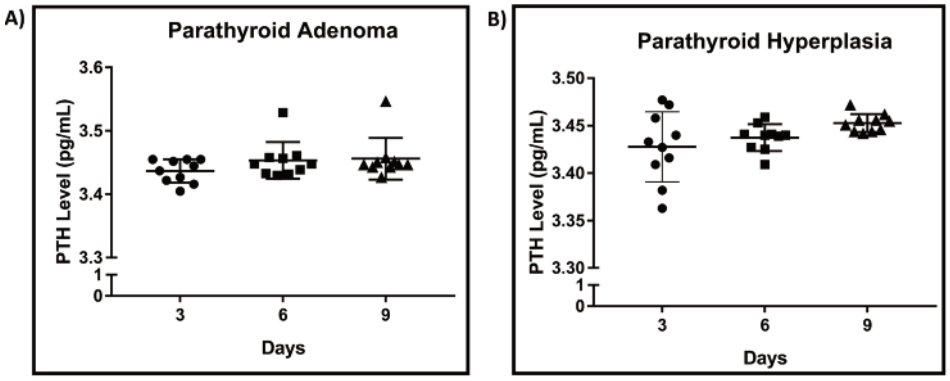


Each isolated parathyroid cell viability profile was adjusted according to the cell size index ratio. **(A)** Cell viability determined after isolation from 7 different parathyroid adenoma tissues. **(B)** Cell viability determined after isolation from 9 different parathyroid hyperplasia tissues.

hyperplasia tissue remained stable during cultivation (Figure 2; $P > .05$). In addition, protein samples extracted from isolated cells immediately after cell viability assessment for each time interval, which were indicated as day 0 (freshly isolated cells accepted as day 0), continued on day 3, day 6, and day 9 during cultivation. Protein concentration was determined by Coomassie (Bradford) protein assay, with loading of 50 μg of total proteins. Equal loading in each lane was confirmed with an anti-β-actin antibody.

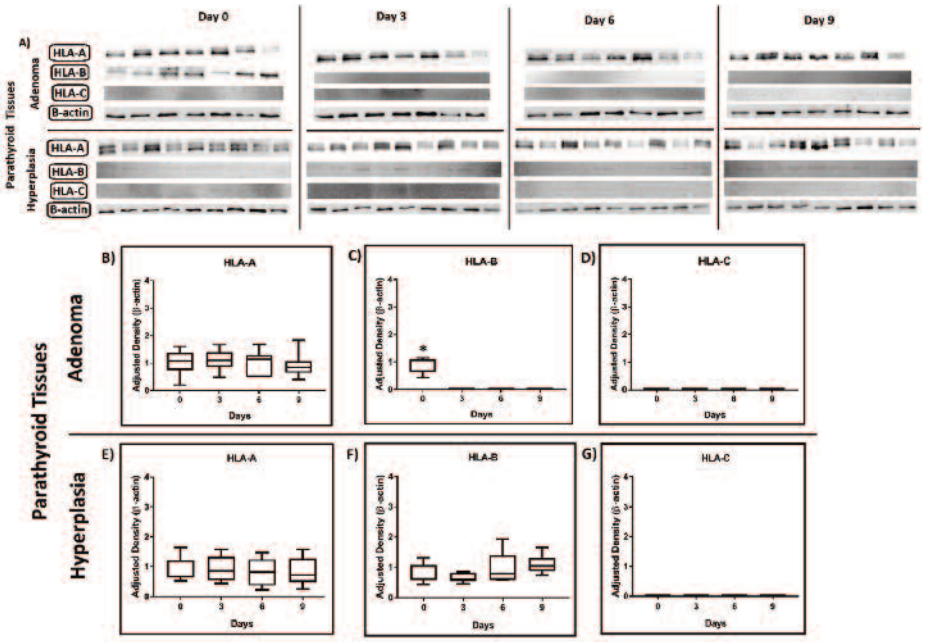
We first investigated HLA class I mRNA and protein expression levels (including HLA-A, HLA-B, and HLA-C) in parathyroid adenoma and hyperplasia tissues, with all immunoblot images compiled in Figure 3A. Each tissue's mRNA and protein expression patterns were varied during

Figure 2. Measurement of Parathyroid Hormone Levels in Parathyroid Tissue



Supernatant samples were collected on days 3, 6, and 9. Parathyroid hormone (PTH) levels were determined with competitive enzyme immunoassay principle. **(A)** PTH levels of 7 different parathyroid adenoma cells during cultivation. **(B)** PTH levels of 9 different parathyroid hyperplasia cells during cultivation.

Figure 3. Immunoblot Images and Resulting Analyses

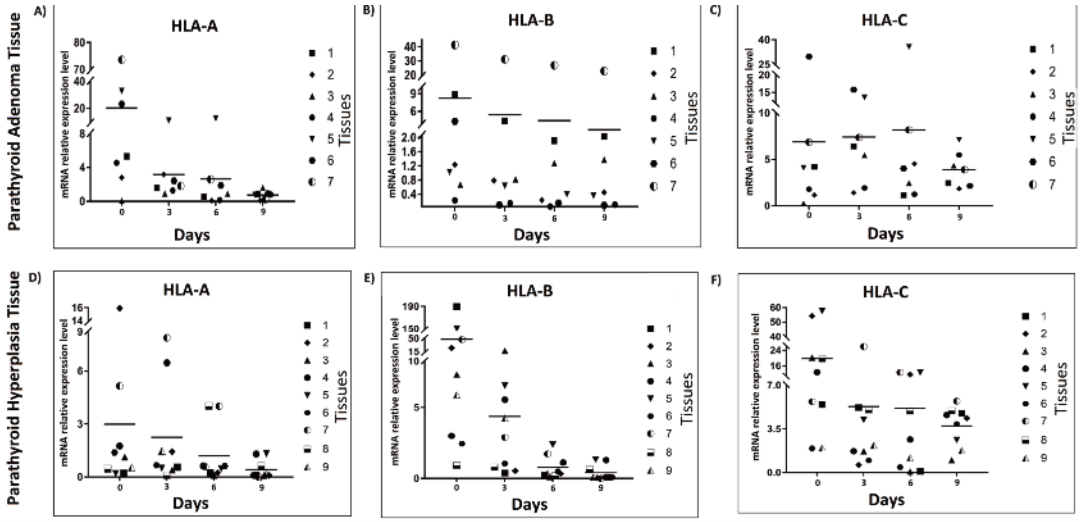


(A) Immunoblot images of HLA-A, HLA-B, HLA-C, and β-actin protein expression during cultivation in parathyroid adenoma and hyperplasia tissue. **(B)** Relative intensity (β-actin) of Western blot analysis of HLA-A in parathyroid adenoma tissue during cultivation ($P = .8616$). **(C)** Relative intensity (β-actin) of Western blot analysis of HLA-B in parathyroid adenoma tissue during cultivation ($P < .001$). **(D)** Relative intensity (β-actin) of Western blot analysis of HLA-C in parathyroid adenoma tissue during cultivation. **(E)** Relative intensity (β-actin) of Western blot analysis of HLA-A in parathyroid hyperplasia tissue during cultivation ($P = .5424$). **(F)** Relative intensity (β-actin) of Western blot analysis of HLA-B in parathyroid hyperplasia tissue during cultivation (P = .5624). **(G)** Relative intensity (β-actin) of Western blot analysis of HLA-C in parathyroid hyperplasia tissue during cultivation.

cultivation. Moreover, Western blot results of HLA-A molecules showed doublets in adenoma and hyperplasia tissues in each time interval. Depending on the size of the marker, doublets were predicted as 45 kDa and 43 kDa (upper and lower bands, respectively).

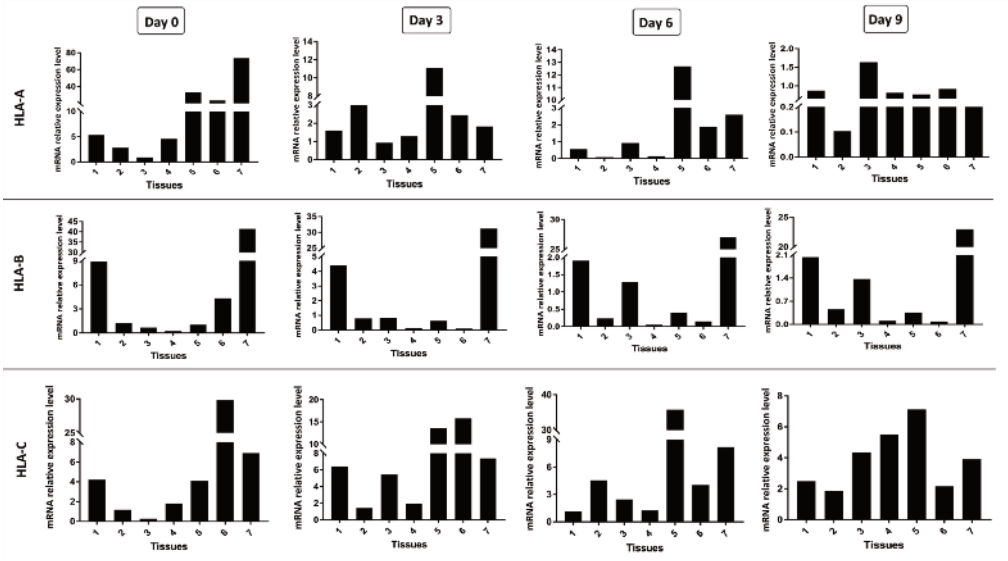
In the 7 parathyroid adenoma tissue samples, the HLA-A mRNA expression level was significantly decreased (P = .0494; Figure 4A); however, we did not observe statistical significance in protein expression of HLA-A doublets during cultivation (P = .8616; Figure 3B). On the other hand, HLA-B mRNA expression was not significant (P = .8953; Figure 4B). HLA-B protein expression levels decreased and were only detected on day 0 (P < .001; Figure 3C). Over 9 days, band intensities decreased below the detection limit. HLA-C mRNA expression levels remained stable (P = .8006; Figure 4C), and protein expression levels remained below the chemiluminescent detection limit (sensitivity was > 30 pg), with no bands detected with 50 μg of total protein (Figure 3A and 3D). The mRNA expression levels at each time interval for HLA-A, HLA-B, and HLA-C in parathyroid adenoma tissue are indicated in Figure 5.

Figure 4. mRNA Expression Levels of Parathyroid Tissue During Cultivation



(A) Parathyroid adenoma tissue HLA-A mRNA relative expression levels (P = .0494). **(B)** Parathyroid adenoma tissue HLA-B mRNA relative expression levels (P = .8953). **(C)** Parathyroid adenoma tissue HLA-C mRNA relative expression levels (P = .8006). **(D)** Parathyroid hyperplasia tissue HLA-A mRNA relative expression levels (P = .4560). **(E)** Parathyroid hyperplasia tissue HLA-B mRNA relative expression levels (P = .0199). **(F)** Parathyroid hyperplasia tissue HLA-C mRNA relative expression levels (P = .0203).

Figure 5. Parathyroid Adenoma Tissue mRNA Expression Levels in Each Tissue During Cultivation for HLA-A, HLA-B, and HLA-C



In the 9 parathyroid hyperplasia tissue samples, HLA-A mRNA (Figure 4D) and protein (Figure 3A and 3E) expression levels remained stable throughout cultivation (P = .4560 and P = .5424, respectively). In addition, HLA-B mRNA expression levels decreased (P = .0199; Figure 4E); however, protein expression levels did not change (P = .5624; Figure 3F) during cultivation. Furthermore, HLA-C mRNA expression levels also decreased (P = .0203; Figure 4F), and protein expression levels remained below the chemiluminescent detection limit (sensitivity was > 30 pg), with no bands detected with 50 μg of total protein (Figure 3A and 3G). mRNA expression levels at each time interval for parathyroid hyperplasia tissue samples are indicated in Figure 6.

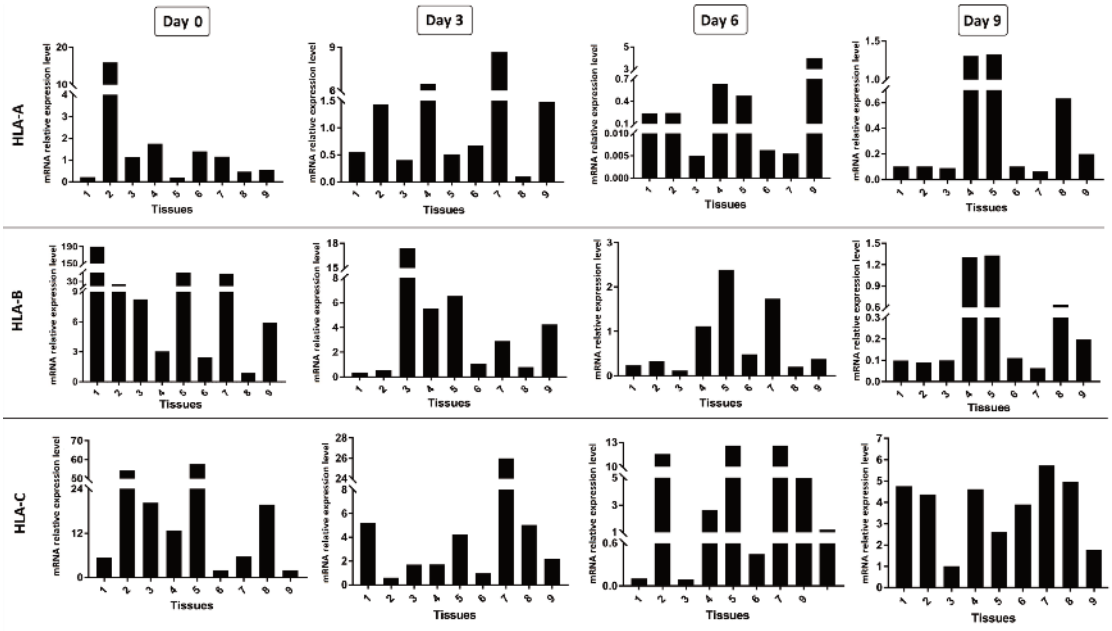
Discussion

Induction, regulation, and/or selection of immune responses is mostly controlled by HLA molecules. HLA molecules present foreign peptide fragments to enable immunosurveillance. The exact mode of action is different between HLAs. Peptide fragments from foreign molecules such as microbial fragments or from allotransplants are processed by antigen presenting cells.[28] This notion is unpredictable, particularly for the allo-peptides from the transplanted graft. However, determination of tissue-specific HLA molecules may highlight how tissue cells or tissue-derived factors could shape outcomes of the immune response. Thus far, upregulated/downregulated HLA expression has been shown to vary between different tissues and has been shown to be mainly related to disease progression,[29] with HLA and disease associations identified by population studies.

The reasons why parathyroid adenoma becomes malignant have remained unclear. Notably, this is the first study about HLA class I molecules for parathyroid adenoma tissue. In addition, no reports are available about parathyroid hyperplasia/adenoma transdifferentiation that could be affected with variable HLA class I patterns.

Permanent hypoparathyroidism is a serious clinical problem and requires lifelong medication. Nowadays, the only curative option is PA.[30] In addition, cell culture is an essential process for PA due to the potential for decreased HLA expression during cultivation. The current strategies began with the delicate work from Tołłoczko and associates.[31] Pathologically diffused parathyroid hyperplasia tissue provides the donor tissue. Although there are a few case reports about parathyroid adenoma tissue transplant, none have reported the malignancy risk.[32,33] Results from our 5-year experience encompassing 90 PA cases showed that each 1 of the 5 recipients developed donor-specific antibody against HLA class I after PA (unpublished data). Therefore, this study is largely based on the general idea that HLA class I expression

Figure 6. Parathyroid Hyperplasia Tissue mRNA Expression Levels in Each Tissue During Cultivation for HLA-A, HLA-B, and HLA-C



in parathyroid tissue could be different than already known. There are limited data regarding HLA class I molecules showing weak or no protein expression for adenoma and hyperplasia tissue, with percentages shown to be low since only HLA class I molecules had been used at that time.[34,35]

In this study, we demonstrated that HLA-A protein expression remained stable in both parathyroid adenoma and hyperplasia tissue; however, mRNA expression only decreased in adenoma tissue. In HLA-B adenoma tissue, mRNA expression levels were stable despite decreased protein levels, with hyperplasia tissue showing stable protein expression levels even though decreased mRNA expression occurred with cultivation. Moreover, in HLA-C, the mRNA expression level was stable in adenoma tissue, although it was significantly decreased in hyperplasia tissue. However, HLA-C protein expression levels for both tissues were below the limit of chemiluminescent detection, meaning that protein levels were < 30 pg. Previous studies have reported that HLA class I molecules are not transcribed with the same motion, with the cytoplasmic tail of HLA-C molecule particularly having the faster rate of endocytosis and lysosomal degradation than HLA-A and HLA-B.[36] Furthermore, our immunoblot results showed that HLA-B and HLA-C had a low expression profile, but HLA-A doublets for both types of parathyroid tissue remained stable even with cultivation. It is likely that de novo donor-specific alloantibody positivity may be associated directly with HLA-A.

The stability of HLA class I is controlled during posttranscriptional modifications by function-related stabilization centers of each molecule via ubiquitination such as MEX3C (RNA-binding E3 ubiquitin ligase, which is responsible for mRNA decay of all HLA-A molecules without affecting the expression of HLA-B and HLA-C)[22,36,37] or tapasin (which has a primary role to stabilize empty HLA class I molecules and promote binding affinity).[38] Particularly, tapasin association reconstitutes the stability of HLA class I through peptide editing.[38-40] As already known, the HLA region is highly polymorphic, and as a consequence posttranscriptional modification mechanisms vary in each type of cell. In addition, the parathyroid tissue is composed of 3 types of cells (oxyphil, chief, and water clear cells).[3] The HLA class I expression is mostly different at every stage, turning mRNA into proteins for parathyroid cells. Analyses of mRNA and protein expression levels together are important whether or not differentially expressed in different parathyroid tissues. Thus, our analysis does address this question of mRNA and protein level inconsistency. This is mainly due to the regulation control at different levels between mRNA and its encoded proteins.

A limitation of our study is the lack of healthy parathyroid tissue for evaluation. Healthy parathyroid glands are small, with an average weight of 35 to 55 mg. After deceased parathyroid donation from healthy individuals to our transplant center, we tested cell quantity for cultivation process; however, we found that 4 healthy parathyroid glands contained approximately 7.01×10^6 cells (unpublished data). Therefore, it was not possible to cultivate or to detect both mRNA and protein expression changes of HLA-A, HLA-B, and HLA-C molecules separately. In addition, Natali and colleagues showed that healthy parathyroid cells do not express HLA class I molecules.[41]

Our results are distinctive for 2 independent reasons. First, different parathyroid HLA class I mRNA and protein expression changes were evaluated for the first time in the literature. The determination of HLA class I, particularly HLA-A, stability will require examination during donor tissue evaluation pretransplant. Second, our comparison of parathyroid adenoma and hyperplasia tissue may explain the characteristics of tissue differentiation via HLAs as preliminary data. This study presents unique and valuable results and an update for immunologic characteristics of parathyroid tissue.

Conclusions

HLA class I expression patterns were different at every stage between different tissues. Further studies are needed to assess the outcomes shown with parathyroid adenoma tissue patterns and should be compared with malignant parathyroid tissue. Parathyroid hyperplasia tissue should be evaluated in detail with regard to expression patterns of HLA class I and II for allorecognition before transplant.

References

1. Lofrese JJ, Lappin SL. *Physiology, Endocrine, Parathyroid*. Treasure Island, FL: StatPearls; 2018.
2. Khan M, Sharma S. *Physiology, Parathyroid Hormone (PTH)*. Treasure Island, FL: StatPearls; 2018.

3. Ritter CS, Haughey BH, Miller B, Brown AJ. Differential gene expression by oxyphil and chief cells of human parathyroid glands. *J Clin Endocrinol Metab.* 2012;97(8):E1499-E1505.
4. Erickson LA, Mete O. Immunohistochemistry in diagnostic parathyroid pathology. *Endocr Pathol.* 2018;29(2):113-129.
5. Osawa N, Onoda N, Kawajiri H, et al. Diagnosis of parathyroid carcinoma using immunohistochemical staining against hTERT. *Int J Mol Med.* 2009;24(6):733-741.
6. Rodriguez C, Naderi S, Hans C, Badoual C. Parathyroid carcinoma: a difficult histological diagnosis. *Eur Ann Otorhinolaryngol Head Neck Dis.* 2012;129(3):157-159.
7. Lawrence DA. A histological comparison of adenomatous and hyperplastic parathyroid glands. *J Clin Pathol.* 1978;31(7):626-632.
8. Ghandur-Mnaymneh L, Kimura N. The parathyroid adenoma. A histopathologic definition with a study of 172 cases of primary hyperparathyroidism. *Am J Pathol.* 1984;115(1):70-83.
9. Arcot R, Moorthy SN, Rao S. Hyperparathyroidism revisited - Old wine in new bottles! Indian *J Endocrinol Metab.* 2011;15(3):194-197.
10. Carlson D. Parathyroid pathology: hyperparathyroidism and parathyroid tumors. *Arch Pathol Lab Med.* 2010;134(11):1639-1644.
11. Jager MD, Serttas M, Beneke J, et al. Risk-factors for nodular hyperplasia of parathyroid glands in sHPT patients. *PLoS One.* 2017;12(10):e0186093.
12. Erlich H. HLA DNA typing: past, present, and future. *Tissue Antigens.* 2012;80(1):1-11.
13. Robinson J, Guethlein LA, Cereb N, et al. Distinguishing functional polymorphism from random variation in the sequences of >10,000 HLA-A, -B and -C alleles. *PLoS Genet.* 2017;13(6):e1006862.
14. Kishore A, Petrek M. Next-generation sequencing based HLA typing: deciphering immunogenetic aspects of sarcoidosis. *Front Genet.* 2018;9:503.
15. Lazar-Molnar E, Snyder M. The role of human leukocyte antigen in celiac disease diagnostics. *Clin Lab Med.* 2018;38(4):655-668.
16. Bodis G, Toth V, Schwarting A. Role of human leukocyte antigens (HLA) in autoimmune diseases. *Rheumatol Ther*. 2018;5(1):5-20.
17. Wallace GR. HLA-B*51 the primary risk in Behcet disease. *Proc Natl Acad Sci U S A.* 2014;111(24):8706-8707.
18. Mezquita L, Charrier M, Faivre L, et al. Prognostic value of HLA-A2 status in advanced non-small cell lung cancer patients. *Lung Cancer.* 2017;112:10-15.
19. Ledys F, Klopfenstein Q, Truntzer C, et al. RAS status and neoadjuvant chemotherapy impact CD8+ cells and tumor HLA class I expression in liver metastatic colorectal cancer. *J Immunother Cancer.* 2018;6(1):123.
20. Mosaad YM. Clinical role of human leukocyte antigen in health and disease. *Scand J Immunol*. 2015;82(4):283-306.
21. Afzali B, Lombardi G, Lechler RI. Pathways of major histocompatibility complex allorecognition. *Curr Opin Organ Transplant*. 2008;13(4):438-444.
22. Cano F, Lehner PJ. A novel post-transcriptional role for ubiquitin in the differential regulation of MHC class I allotypes. *Mol Immunol*. 2013;55(2):135-138.
23. Campbell EC, Antoniou AN, Powis SJ. The multi-faceted nature of HLA class I dimer molecules. *Immunology*. 2012;136(4):380-384.
24. Yang JY, Sarwal MM. Transplant genetics and genomics. *Nat Rev Genet.* 2017;18(5):309-326.
25. Barczynski M, Golkowski F, Nawrot I. Parathyroid transplantation in thyroid surgery. Gland Surg. 2017;6(5):530-536.
26. Yucesan E, Goncu B, Basoglu H, et al. Fresh tissue parathyroid allotransplantation with short-term immunosuppression: 1-year follow-up. *Clin Transplant*. 2017;31(11).
27. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012;9(7):671-675.
28. Rock KL, Reits E, Neefjes J. Present yourself! By MHC class I and MHC class II molecules. *Trends Immunol.* 2016;37(11):724-737.
29. Lee HJ, Song IH, Park IA, et al. Differential expression of major histocompatibility complex class I in subtypes of breast cancer is associated with estrogen receptor and interferon signaling. *Oncotarget.* 2016;7(21):30119-30132.
30. Aysan E, Altug B, Ercan C, Kesgin Toka C, Idiz UO, Muslumanoglu M. Parathyroid allotransplant with a new technique: a prospective clinical trial. *Exp Clin Transplant.* 2016;14(4):431-435.
31. Tolloczko T, Wozniewicz B, Gorski A, et al. Cultured parathyroid cells allotransplantation without immunosuppression for treatment of intractable hypoparathyroidism. *Ann Transplant.* 1996;1(1):51-53.
32. Decker GA, Stark JH, Botha JR, Margolius LP, Decker G. Allotransplantation of parathyroid cells. *Lancet.* 1995;345(8942):124.
33. Duarte B, Mozes MF, John E, Aronson I, Pollak R, Jonasson O. Parathyroid allotransplantation in the treatment of complicated idiopathic primary hypoparathyroidism. *Surgery.* 1985;98(6):1072-1076.
34. Bjerneroth G, Juhlin C, Rastad J, Akerstrom G, Klareskog L. MHC class I and II antigen expression on parathyroid cells and prospects for their allogenic transplantation. *Transplantation.* 1993;56(3):717-721.
35. Timm S, Hamelmann W, Otto C, et al. Influence of donor MHC class I antigen expression on graft survival after rat parathyroid allotransplantation. *Langenbecks Arch Surg.* 2001;386(6):430-433.
36. Prevosto C, Usmani MF, McDonald S, et al. Allele-independent turnover of human leukocyte antigen (HLA) class Ia molecules. *PLoS One.* 2016;11(8):e0161011.
37. Simon A, Dosztányi Zs, Rajnavölgyi E, Simon I. Function-related regulation of the stability of MHC proteins. *Biophys J.* 2000;79(5):2305-2313.
38. Wearsch PA, Cresswell P. The quality control of MHC class I peptide loading. *Curr Opin Cell Biol.* 2008;20(6):624-631.
39. Hein Z, Uchtenhagen H, Abualrous ET, et al. Peptide-independent stabilization of MHC class I molecules breaches cellular quality control. *J Cell Sci.* 2014;127(Pt 13):2885-2897.
40. Hein Z, Borchert B, Abualrous ET, Springer S. Distinct mechanisms survey the structural integrity of HLA-B*27:05 intracellularly and at the surface. *PLoS One.* 2018;13(8):e0200811.
41. Natali PG, Bigotti A, Nicotra MR, Viora M, Manfredi D, Ferrone S. Distribution of human Class I (HLA-A,B,C) histocompatibility antigens in normal and malignant tissues of nonlymphoid origin. *Cancer Res.* 1984;44(10):4679-4687.