Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2010; 35 (4) ; 319–324.

Research Article [Araştırma Makalesi]

Yayın tarihi 15 Aralık, 2010 © TurkJBiochem.com

[Published online 15 December, 2010]

Arginase and Ornithine in Human Benign and Malignant Skin Tumors

[İnsan İyi ve Kötü Huylu Cilt Tümörlerinde Arginaz ve Ornitin]

Prof.Dr.Selma Süer Gökmen[1],
Cemal Kazezoğlu[1],
A.Cemal Aygit[2],
Reyhan Yildiz[1],
Beyhan Çakir[2],
Mevlüt Türe[3],
Şendoğan Gülen[1],

Departments of [1]Biochemistry, [2]Plastic and Reconstructive Surgery and [3]Biostatistics, Trakya University School of Medicine, Edirne, Turkey

ABSTRACT

Objectives: Arginase activity and ornithine concentration have been shown to be elevated in experimentally-induced benign tumors in mice. The aim of the study is to investigate arginase activity and ornithine concentration in human benign and malignant skin tumors and to evaluate their role for prognosis of skin tumors.

Patients and Methods: We have investigated arginase activity and ornithine concentration in supernatant of homogenates of benign tumors (nevus) of the skin from 13 patients and of malignant tumors (squamous cell or basal cell carcinomas) from 29 patients. Total arginase activity, ornithine and total protein concentration in supernatant were determined by the methods of Geyer, Chinard and Lowry, respectively.

Results: Arginase activity (p=0.006) and ornithine concentration (p=0.007) in nevus were significantly higher than in adjacent normal tissue. There was no significant difference between their levels in basal cell carcinoma and in nevus (p>0.05). There was no significant difference between ornithine concentration in squamous cell carcinoma and in nevus (p>0.05). However, arginase activity in this carcinoma was significantly higher than in nevus (p=0.018).

Conclusion: The significant difference between tissue arginase activities in squamous cell carcinoma and in nevus indicates that determination of arginase activity could be useful for prognosis of skin tumors.

Key words: Arginase, ornithine, nevus, squamous cell carcinoma, basal cell carcinoma.

Yazışma Adresi
[Correspondence Address]

Prof. Dr. Selma SÜER GÖKMEN (Ph.D.),

Department of Biochemistry, Trakya University School of Medicine, Edirne 22030, TURKEY
Tel: +90-284-2357642 / 1618
Fax: +90-284-2351564
E -mail: selmasuer@hotmail.com

ÖZET

Amaç: Farelerde deneysel olarak oluşturulmuş benign deri tümörlerinde arginaz aktivitesi ve ornitin konsantrasyonunun arttığı gösterilmiştir. Bu çalışmanın amacı, insan benign ve malign deri tümörlerinde arginaz aktivitesi ve ornitin konsantrasyonunu incelemek ve bunların deri tümörlerinin izlenmesindeki rollerini irdelemektir.

Hastalar ve Yöntemler: Arginaz aktivitesini ve ornitin konsantrasyonunu 13 hastadan elde edilen benign cilt tümörü ve 29 hastadan elde edilen malign cilt tümörü (sküamöz hücre veya bazal hücre kanseri) homojenatlarının süpernatantlarında inceledik. Süpernatantlardaki total arginaz aktivitesi, ornitin ve total protein konsantrasyonu sırasıyla Geyer, Chinard ve Lowry metotları ile ölçüldü.

Bulgular: Nevüsteki arginaz aktivitesi (p=0.006) ve ornitin konsantrasyonu (p=0.007) çevre sağlıklı dokuya göre anlamlı olarak yüksekti. Nevüsteki ve bazal hücre kanserindeki düzeyleri arasında fark yoktu (p>0.05). Nevüsün ve sküamöz hücre kanserinin ornitin konsantrasyonu arasında anlamlı fark yoktu (p>0.05). Bununla birlikte, sküamöz hücre kanserindeki arginaz aktivitesi nevüsten anlamlı olarak yüksek bulundu (p=0.018).

Sonuç: Sküamöz hücre kanserinin ve nevüsün doku arginaz aktiviteleri arasındaki anlamlı fark arginaz aktivitesi ölçümünün cilt tümörlerinin prognozunda faydalı olabileceğini göstermektedir.

Anahtar Kelimeler: Arginaz, ornitin, nevüs, sküamöz hücre kanseri, bazal hücre kanseri

Date registered: 26 January 2009; Date accepted: 10 August 2010

[Kayıt Tarihi : 26 Ocak 2009; Kabul Tarihi : 10 Ağustos 2010]

 ISSN 1303–829X (electronic) 0250–4685 (printed)

Introduction

Arginase (L-Arginine amidinohydrolase; E.C.3.5.3.1), the final enzyme in the urea cycle, is responsible for the detoxification of ammonia in mammalian liver. It hydrolyses arginine to ornithine and urea (1). It is also present in kidney, brain, intestine, mammary gland, erythrocytes, and skin (1,2). In extrahepatic mammalian tissues, arginase supplies the cell with ornithine, an important metabolite in biosynthesis of glutamic acid, proline and polyamines (1,3). Polyamines are important in regulation of cell proliferation and differentiation (3) and may play a role in neoplastic transformation of cells (4). Polyamine concentrations and ornithine decarboxylase activity (which converts ornithine to putrescine, a precursor for polyamine biosynthesis), are elevated during carcinogenesis (5). Arginase activity is markedly elevated in prostatic carcinoma (6), gastric cancer (7), colorectal cancer (8), chronic lymphocytic leukemia (9), non-small cell lung carcinoma (10) and breast cancer (11). Increased arginase activity has also been reported in cancer metastases (12,13). Other than arginase, enzymes involved in the production of urea have not been found in human epidermal tissue (14).

Increased arginase activity in mouse epidermis may provide ornithine for polyamine biosynthesis and for production of glutamate and proline and of keratinous proteins (15). Epidermal arginase activity is increased in psoriasis, an inflammatory disease of the skin characterized by epidermal hyperproliferation (16).

Most skin tumors are benign, e.g., verruca, nevi, keratosis, cysts, and skin tags, but a significant number are premalignant or malignant. A small percentage of actinic keratoses and the cellular-type lesions of blue nevi and junction nevi may undergo malignant transformation (17,18).

Arginase activity and ornithine concentration are elevated in chemically-induced benign tumors in mice (19). We have also found them to be elevated in malignant tumors of the human skin (20). We are not aware of any studies of arginase activity and ornithine concentration in benign tumors of the human skin.

We have investigated arginase activity and ornithine concentration in supernatant of homogenized benign and of malignant skin tumors.

Material and Methods

We obtained nevus and normal skin tissue from 13 patients (5 men and 8 women, aged 26 to 56 years, 36.08±11.06) and from basal cell carcinoma from 16 patients (9 men and 7 women, aged 31 to 68 years, 49.81±10.94) and squamous cell carcinoma from 13 patients (9 men and 4 women, aged 33 to 68 years, 56.23±10.90) attending the Department of Plastic and Reconstructive Surgery of our hospital. No patient had received chemotherapy or radiotherapy. The study was approved by the institutional Ethics Committee on human research.

Surgical removal of tumor with intact margins was performed. Depending on the size of the primary excision, suture, skin grafts or flaps were performed. Adjacent normal tissue was obtained only from the patients with nevus. Tissue specimens were washed twice with % 0.9 NaCl solution to remove blood immediately after removal, then placed in cold 0.9% NaCl solution and stored at -76^{0}C until analyzed. Each frozen tissue was weighed and then homogenized in 10 volume of cold 0.05 mol/L Tris/HCl buffer (pH 8.05) in a Potter-type homogenizer and then centrifuged at 11.000 g for 20 minutes at 4^{0}C.

All reagents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemie (Steinheim, Germany) and were of analytical grade. Total arginase activity and ornithine concentration in supernatant were determined twice by the procedure reported in (21), and in (22), respectively. For arginase, 50 mM arginine, 0.1 M HCl, 100 mM $Na_2CO_3/NaHCO_3$ buffer (pH 9.7), 9 mM $MnCl.2H_2O$, 0.1 μmol/mL urea standard, the color reagent and the acid reagent were used. The color reagent consisted of 0.0036 M thiosemicarbazide and 0.0617 M diacetylmonoxim. The acid reagent consisted of 1 mL of ferric chloride-phosphoric acid solution (0.12 M $FeCl_3$ in 56.7% H_3PO_4) added to 999 mL of 20% (V/V) H_2SO_4. 0.1 mL of supernatant was pre-incubated with 0.9 mL of $MnCl.2H_2O$ at 55 ^{0}C for 20 min. After pre-incubation, 0.2 mL of the upper solution was incubated with arginine and $Na_2CO_3/NaHCO_3$ buffer (pH 9.7) at 37^{0}C for 15 min in a swinging water bath. Color development was initiated by delivering the color reagent into the tube, followed by the acid reagent. The samples were placed in boiling water bath for 10 min and subsequently chilled in an ice bath for 5 min. The optical density was measured against a reagent blank at 520 nm. One unit of arginase was defined as the amount that catalyzes formation of 1 μmol of urea for 1 hour at 37^{0}C. For ornithine, 10% trichloroacetic acid, 0.3 μmol/mL ornithine standard, the acid mixture contained 6 M H_3PO_4 and glacial acetic acid and reagent solution prepared by adding 25 mg of ninhydrin to per mL of the acid mixture were used. 1 mL of supernatant was added 1 mL of trichloroacetic acid, then centrifuged at 3000 g for 15 min at 4^{0}C. To the supernatant was added glacial acetic acid and reagent solution. After mixing, the tube was capped and heated in a water bath at 100^{0}C for 60 min. After adding glacial acetic acid, the tube was cooled to room temperature. The optical density was measured at 515 nm. The protein concentration of supernatant was determined as in (23). Folin-Ciocalteu's Phenol reagent (Merck), 2% Na_2CO_3 in 0.1 N NaOH, 0.5 % $CuSO_4.5H_2O$ in 1% sodium tartrate and albumin standard were used in the assay.

Statistical analysis used MINITAB INC. Data were expressed as mean±SD. Statistical differences between nevus and their adjacent normal tissue were evaluated using Paired-Samples T test. Because of the difference in ages of patients with nevus and those with malign skin tumors, each result was corrected using the formula: patient's age X patient's arginase activity/mean group age (24). For corrected data that were non-normally distrib-

uted, Kruskal-Wallis test (25) was performed between three groups; benign skin tumor, basal cell carcinoma and squamous cell carcinoma groups. When statistical significance was determined for a multiple comparison, Dunn's post hoc test was performed to determine which samples were different. We considered p values less than 0.05 to be statistically significant.

Results

Table 1 shows the gender and age of patients and the type of benign and malignant skin tumors removed.

Arginase activity and ornithine concentration in supernatant of homogenate from nevus and from adjacent normal tissues are seen in Table 2. Arginase activity (p=0.006) and ornithine concentration (p=0.007) in nevus were significantly higher than those in adjacent normal tissues.

Arginase activity and ornithine concentration of supernatant of homogenate from malignant and benign skin tumors are seen in Table 3. There was no significant

Table 1: Characteristics of patients with nevus and malignant skin tumors.

NEVUS	
Age, years (mean±SD)	26-56 (36.08±11.06)
Women (number)	8
Men (number)	5
Types, ratio (%)	
Compound nevus	9/13 (69.23)
Blue nevus	2/13 (15.38)
Intradermal nevus	2/13 (15.38)
MALIGNANT TUMOR	
Basal cell carcinoma	
Age, years (mean±SD)	31-68 (49.81±10.94)
Women (number)	7
Men (number)	9
Squamous cell carcinoma	
Age, years (mean±SD)	33-68 (56.23±10.90)
Women (number)	4
Men (number)	9

Table 2: Arginase activity and ornithine concentration in supernatant of homogenate obtained from nevus and adjacent normal tissue.

Variable	Range	Mean±SD	Median
NEVUS			
Arginase (U/mg protein, n=13)	3.90-17.73	10.32±3.93*	11.59
Ornithine (nmol/mg protein, n=13)	4.96-67.72	31.57±19.88**	30.08
NORMAL TISSUE			
Arginase (U/mg protein, n=13)	0.77-9.55	5.11±3.04	4.66
Ornithine (nmol/mg protein, n=13)	5.66-26.85	14.98±7.33	12.96

*p=0.006, **p=0.007
(Paired sample T test)

Table 3: Arginase activity and ornithine concentration in supernatant of homogenate obtained from nevus and malignant skin tumor.

Variable	Range	Mean±SD	Median
NEVUS			
Arginase (U/mg protein, n=13)	3.90-17.73	10.32±3.93	11.59
Ornithine (nmol/mg protein, n=13)	4.96-67.72	31.57±19.88	30.08
MALIGNANT TUMOR			
Squamous cell carcinoma			
Arginase (U/mg protein, n=13)	9.37-44.36	20.55±12.47*	15.43
Ornithine (nmol/mg protein, n=13)	10.38-72.48	31.29± 19.17	23.73
Basal cell carcinoma			
Arginase (U/mg protein, n=16)	6.91-33.82	12.40±6.25	10.21
Ornithine (nmol/mg protein, n=16)	16.46-61.93	33.45±14.05	31.43

*p=0.018
(Kruskal-Wallis test and Dunn's post hoc test)

difference between results of these assays in basal cell carcinoma and in nevus (p>0.05). There was no significant difference between ornithine concentration in squamous cell carcinoma and in nevus (p>0.05). However, arginase activity in squamous cell carcinoma was significantly higher than in nevus (p=0.018) (Figure 1).

Discussion

The metabolic fates of arginase are differentially expressed according to cell type, age and developmental stage, diet and state of health and disease (26). Arginase is a necessary precursor for the synthesis of L-ornithine, polyamines, L-proline, creatine, agmatine and nitric oxide (26,27).

There are two mammalian isoforms of arginase: arginase I (cytosolic) is highly expressed in the liver and to a much lesser extent in a few other cell types, whereas arginase II (mitochondrial) has a wide tissue distribution. The function of arginase I in urea synthesis has been very well characterized whereas arginase II is involved in the formation of ornithine, a precursor of polyamines, glutamate and proline (28-31).

It has been suggested that arginase I may preferentially direct ornithine to polyamine synthesis whereas arginase II may direct it to proline and glutamate production (31). Arginase II may play an important role in the regulation of hepatic ureagenesis by furnishing ornithine for net synthesis of N-acetylglutamate, citrulline and aspartate (30). The role of arginase in the epidermis is probably different from that in tissues containing the urea cycle, being to provide ornithine (15) for biosynthesis of polyamines (3).

Arginase activity increases during hyperkeratinization and in pathologic skin diseases such as psoriasis, verruca vulgaris, ichthyosis, and in plantar epidermis (16,32). Although, total polyamines and ornithine concentrations and arginase activity has been shown to be elevated in chemically-induced benign tumors of mouse skin (19), we could find no information on arginase activity and ornithine concentration in benign tumors of the human skin. The present study found both to be increased (p=0.006 and p=0.007, respectively) in nevus compared to adjacent normal skin. These increases may have resulted from uncontrolled cell proliferation in nevus or from the increased production of ornithine and consequently of polyamine and hence of proteins and of cell proliferation.

Although a single application of tumor-promoting agent to mouse epidermis caused a rapid and transient induction of ornithine decarboxylase activity, the activity of arginase remained unaffected (15). However, epidermal arginase activity was increased in experimentally-induced hyperplasia with stimulation of DNA synthesis preceding that increase (32). This led those authors to conclude that arginase may regulate the concentrations of arginine and ornithine.

Nitric oxide (NO), formed from L-arginine by nitric oxide synthase (NOS), is a potent regulator of growth and

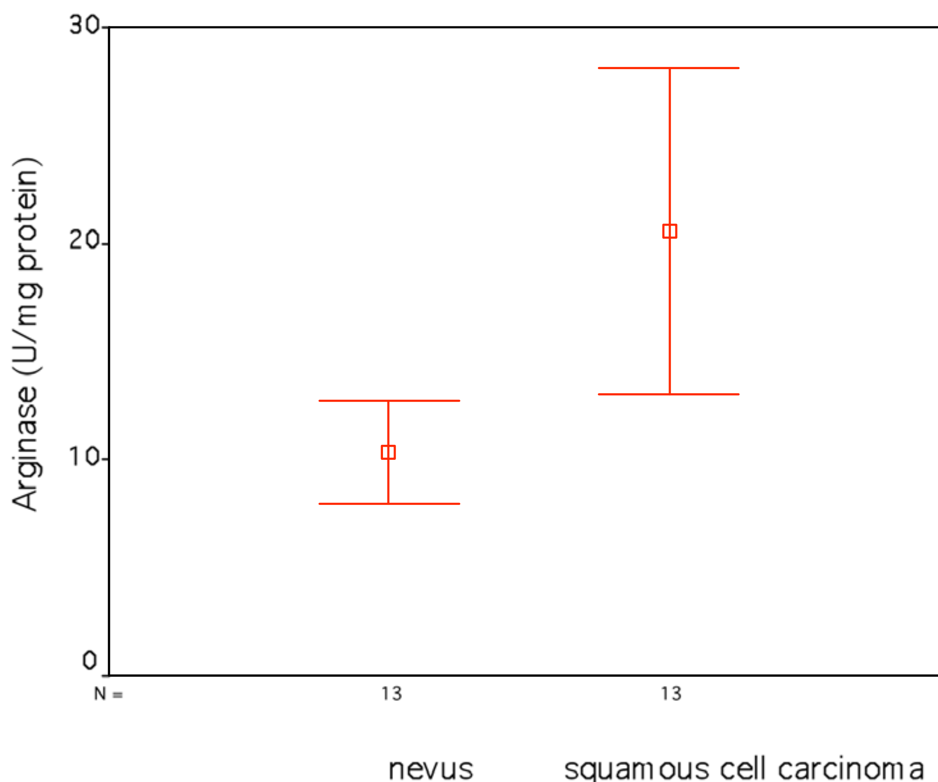

Figure 1. Arginase activity in supernatant of homogenate obtained from nevus and squamous cell carcinoma (mean±SD).

differentiation in skin-derived keratinocytes (33). NO is released from keratinocytes at high concentration and is a key inhibitor of cellular proliferation (16). Thus limitation of NO formation caused by increased activity of arginase may be responsible for an increase in cell proliferation.

Arginase activity has been reported to be elevated in cancer (6-11) and metastases (12,13). Although we have reported that arginase activity and ornithine concentration are elevated in malignant skin tumors compared to adjacent normal tissues (20), there has been no information on them in benign and malign tumors of human skin. In the present study, only arginase activity of squamous cell carcinoma was higher than that of nevus (p=0.018). This agrees with published reports (6, 34) for prostatic carcinomas compared to hypertrophic prostates. Recently, arginase I was reported to be a sensitive and specific marker of benign and malignant hepatocytes (35). The arginase II gene has demonstrated significant up-regulation and down-regulation, respectively, in malignant compared to benign prostate tissue, whereas arginase I is more often present in cancer than benign samples (36). Arginase II expression is increased in most malignant thyroid tumors, but absent in benign lesions and normal tissues (37).

We found a significant difference between arginase activity of squamous cell carcinoma and nevus but not between arginase activity or ornithine concentration of basal cell carcinoma and nevus. The arginase activity of basal cell tumors was intermediate between that for squamous cell tumors and for nevus. Basal cell carcinoma is more benign than squamous cell carcinoma, but less benign than nevus. This suggests that determination of arginase activity could be useful for prognosis of skin tumors. This determination in morphea-like basal cell carcinoma, slow-growing squamous cell carcinoma and rapid-growing squamous cell carcinoma with tendency to ulceration in early stages may help to explain the role of arginase in the prognosis of skin tumors.

References

[1] Berueter J, Colombo JP, Bachmann C. (2005) Purification and properties of arginase from human liver and erythrocytes. Biochem J 175:449-454.

[2] Yu H, Yoo PK, Aguirre CC, Tsoa RW, Kern RM, Grody WW, Cederbaum SD, Iyer RK. (2003) Widespread expression of arginase I in mouse tissues. Biochemical and physiological implications. J Histochem Cytochem 51:1151-1160.

[3] Pegg AE. (2009) Mammalian polyamine metabolism and function. IUBMB Life 61:880-894.

[4] Gerner EW, Meyskens FL Jr. (2004) Polyamines and cancer: old molecules, new understanding. Nat Rev Cancer 4:781-792.

[5] Jansen AP, Dreckschmidt NE, Verwiebe EG, Wheeler DL, Oberley TD, Verma AK. (2001) Relation of the induction of epidermal ornithine decarboxylase and hyperplasia to the different skin tumor-promotion susceptibilities of protein kinase C alpha,-delta and-epsilon transgenic mice. Int J Cancer 93:635-643.

[6] Keskineğe A, Elgün S, Yılmaz E. (2001) Possible implications of arginase and diamine oxidase in prostatic carcinoma. Cancer Detect Prev 25:76-79.

[7] Wu CW, Chi CW, Lin EC, Lui WY, P'eng FK, Wang SR. (1994) Serum arginase level in patients with gastric cancer. J Clin Gastroenterol 18:84-85.

[8] del Ara RM, Gonzalez-Polo RA, Coro A, del Amo E, Paloma L, Hernandez E, Soler G, Fuentes JM. (2002) Diagnostic performance of arginase activity in colorectal cancer. Clin Exp Med 2:53-57.

[9] Konarska L, Widzynska I, Zienkiewicz H, Sulek K. (1993) Arginase activity alterations in peripheral blood lymphocytes in the human chronic lymhocytic leukaemia. Acta Biochim Pol 40:160-163.

[10] Süer Gökmen S, Yörük Y, Çakır E, Yorulmaz F, Gülen Ş. (1999) Arginase and ornithine, as markers in human non-small cell lung carcinoma. Cancer Biochem Biophys 17:125-131.

[11] Porembska Z, Luboinski G, Chrzanowska A, Mielczarek M, Magnuska J, Baranczyk-Kuzma A (2003) Arginase in patients with breast cancer. Clin Chim Acta 328:105-111.

[12] Mielczarek M, Chrzanowska A, Scibior D, Skwarek A, Ashamiss F, Lewandowska K, Baranczyk-Kuzma A. (2006) Arginase as a useful factor for the diagnosis of colorectal cancer liver metastases. Int J Biol Markers 21:40-44.

[13] Shukla VK, Tandon A, Ratha BK, Sharma D, Singh TB, Basu S. (2009) Arginase activity in carcinoma of the gallbladder: a pilot study. Eur J Cancer Prev 18:199-202.

[14] Crounse RG, Rothberg S. (1961) Evaluation of the enzymes of the Krebs-Henseleit urea cycle in human epidermis. J Invest Dermatol 36:287-292.

[15] Verma AK, Boutwell RK. (1981) Characterization of arginase activity from mouse epidermis and its relation to ornithine decarboxylase induction by the tumor promoting agent, 12-o-tetradecanoylphorbol-13-acetate. Biochim Biophys Acta 677:184-189.

[16] Bruch-Gerharz D, Schnorr O, Suschek C, Beck KF, Pfeilschifter J, Ruzicka T, Kolb-Bachofen V. (2003) Arginase 1 over expression in psoriasis: limitation of inducible nitric oxide synthase activity as a molecular mechanism for keratinocyte hyperproliferation. Am J Pathol 162:203-211.

[17] Popkin GL. (1990) Tumors of the skin: A dermatologist's viewpoint. In: Mc Carthy JG, May JW, Littler JW, editors. Plastic Surgery, vol 5, s.3560-3613, Saunders Company, USA.

[18] Stal S, Spira M. (1997) Basal and squamous cell carcinoma of the skin. In: Aston SJ. Beasly RW, Thorne CHM, editors. Grabb and Smith's Plastic Surgery, 5th ed, s.107-120, Lippincott-Raven, New York.

[19] Koza RA, Megosh LC, Palmieri M, O'Brien TG. (1991) Constitutively elevated levels of ornithine and polyamines in mouse epidermal papillomas. Carcinogenesis 12:1619-1625.

[20] Süer Gökmen S, Aygıt AC, Ayhan MS, Yorulmaz F, Gülen Ş. (2001) Significance of arginase and ornithine in malignant tumors of the human skin. J Lab Clin Med 137:340-344.

[21] Geyer JW, Dabich D. (1971) Rapid method for determination of arginase activity in tissue homogenates. Anal Biochem 39:412-417.

[22] Chinard FP. (1952) Photometric estimation of proline and ornithine. J Biol Chem 199:91-95.

[23] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

[24] Ozdamar K. (2004) Ortak değişkenli faktöriyel denemelerin analizi. İçinde: Paket Programlar İle İstatistiksel Veri Analizi, 5.baskı, sf.390-391. Kaan Kitabevi, Eskişehir.

[25] Forthofer RN, Lee ES, Hernandez M. (2007) Biostatistics: A Guide to Design, Analysis and Discovery, 2nd Ed., p. 261-262. Elsevier Academic Press, USA.

[26] Morris SM Jr. (2007) Arginine metabolism: boundaries of our knowledge. J Nutr 137 (6 Suppl 2):1602S-1609S.

[27] Durante W, Johnson FK, Johnson RA. (2007) Arginase: a critical regulator of nitric oxide synthesis and vascular function. Clin Exp Pharmacol Physiol 34:906-911.

[28] Ash DE. (2004) Structure and function of arginases. J Nutr 134(10 Suppl):2760S-2764S.

[29] Cederbaum SD, Yu H, Grody WW, Kern RM, Yoo P, Iyer RK. (2004) Arginases I and II: do their functions overlap? Mol Genet Metab 81 Suppl 1:S38-44.

[30] Nissim I, Luhovyy B, Horyn O, Daikhin Y, Nissim I, Yudkoff M. (2005) The role of mitochondrially bound arginase in the regulation of urea synthesis: studies with [U-15N4]arginine, isolated mitochondria, and perfused rat liver. J Biol Chem 280:17715-17724.

[31] Li H, Meininger CJ, Hawker JR Jr, Haynes TE, Kepka-Lenhart D, Mistry SK, Morris SM Jr, Wu G. (2001) Regulatory role of arginase I and II in nitric oxide, polyamine, and proline syntheses in endothelial cells. Am J Physiol Endocrinol Metab 280:E75-82.

[32] Redmond AF, Rothberg S. (1978) Arginase activity and other cellular events associated with epidermal hyperplasia. J Cell Physiol 94:99-104.

[33] Krischel V, Bruch-Gerharz D, Suschek C, Kröncke KD, Ruzicka T, Kolb-Bachofen V. (1998) Biphasic effect of exogenous nitric oxide on proliferation and differentiation in skin derived keratinocytes but not fibroblasts. J Invest Dermatol 111:286-291.

[34] Harris BE, Pretlow TP, Bradley EL, Whitehurst GB, Pretlow TG. (1983) Arginase activity in prostatic tissue of patients with benign prostatic hyperplasia and prostatic carcinoma. Cancer Res 43:3008-3012.

[35] Yan BC, Gong C, Song J, Krausz T, Tretiakova M, Hyjek E, Al-Ahmadie H, Alves V, Xiao SY, Anders RA, Hart JA. (2010) Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol 34:1147-1154.

[36] Reschner A, Harlin H, Laven B, Eriksson F, Pisa P, Egevad L. (2009) Expression of immunomodulating genes in prostate cancer and benign prostatic tissue. Anal Quant Cytol Histol 31:74-82.

[37] Cerutti JM, Delcelo R, Amadei MJ, Nakabashi C, Maciel RM, Peterson B, Shoemaker J, Riggins GJ. (2004) A preoperative diagnostic test that distinguishes benign from malignant thyroid carcinoma based on gene expression. J Clin Invest 113:1234-42.