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Association of Breast Cancer and In situ expression of Interleukin-4 (IL-4) and IL-10.

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الخلاصة:

تقدير مستويات التعبير في الموضع لكل من الانترلوكين 4 و10 في مرضى سرطان الثدي.

استخدمت تقنية التهجين في الموضع لكشف وتحديد مستويات كل من الانترلوكين 4 و10 في النماذج النسيجية التي تم الحصول عليها من 60حالة مرضية و المحفوظة بالبارافين والتي تم الحصول عليها من أرشيف اثنين من مستشفيات بغداد ز قسمت النماذج إلى قسمين : 30 حالة مرضية لسرطان الثدي الخبيث و30 حالة مرضية لسرطان الثدي الحميد كمجموعة سيطرة.

كانت مستويات التعبير في الموضع لكل من الانترلوكين 4 و10 ذات زيادة ملحوظة في المجموعة الأولى مقارنة بالمجموعة الثانية ($p<0.01$) ولا توجد علاقة بين الانترلوكين 4 و10في كافة المجاميع المدروسة($p>0.05$) .

تبين الدراسة إلى إن الانترلوكين 4 و10 يزداد تعبيره في حالة سرطان الثدي الخبيث وبذلك قد يكون لهما تأثيراً في النشوء المرضي لسرطان الثدي الخبيث.

Abstract:

Estimation of the *in situ* hybridization expression of IL-4and IL-10 in patients with malignant breast cancer.

A technique utilizing *in situ* hybridization (ISH) was performed to detect and determine the *in situ* expression of IL-4 and IL-10 mRNA using paraffin embedded sections .Samples obtained from archive of department of pathology of two hospitals in Baghdad for 60 patients, who were divided into two groups: 30 with malignant breast cancer (group-1) and 30 woman with non-malignant as a control group (group-2).

The levels of the *in situ* expression of both IL-4 and IL-10 mRNA were found to be a highly significant increased in group 1 as compared with group 2 ($p<0.001$), with no significant correlation between IL-4 and IL-10 ($p>0.05$) in all studied group.

The increasing expression of IL-4 and IL-10 in malignant breast cancer tissue might be influence in the pathogenesis of malignant breast cancer.

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Introduction:

Breast cancer represents the leading cause of cancer death among women in developed countries[1] .Epidemiologically, breast cancer is the most frequently diagnosed cancer and the second leading cause of death from cancer, after lung cancer in American women[2]. All women are at risk of developing breast cancer; approximately 50% of women develop breast cancer without identifiable risk factor[3]. Among the various prognostic factors, lack of estrogen receptor (ER) has consistently been associated with poorer prognosis[4], cytokines are now emerging as factors that are potentially involved in breast carcinogenesis[5,6]. Cytokines constitute a diverse group of proteins that include haematopoietic growth factors, interferons, lymphokines and chemokines[7].

Cytokines are groups of intercellular short acting soluble mediators between cells of the immune system, as well as other non-immune cells. Multiple Cytokines appear to have a dominant role in human breast cancer formation [8]. Cytokines are produced by many cell populations, but the predominant suppliers are T-helper (Th) cells and macrophages. Th cells have two important functions: to stimulate cellular immunity and inflammation and to stimulate B cells to produce antibodies. Two functionally distinct subsets of Th cells (Th1and Th2) secrete cytokines that promote these different activities. Th1 cells produce IL-2 and IFN-γ, which activate cytotoxic lymphocytes and macrophages to stimulate cellular immunity and inflammation [9]. Th2 cells secrete IL-4 and IL-10, which stimulate antibody production by B cells. It has become evident that cancer tissues also produce cytokines [5,10].

It has been reported that interleukin (IL)-4 acts as an autocrine growth factor in pancreatic cancer cells by promoting the activation of AKT-1, signal transducers and activators of transcription (Stat) 3, and mitogen-activated protein kinase [11].

In human primary prostate, breast, and bladder cancer cells, IL-4 induces up-regulation of cFLIP and Bcl-XL, which confer resistance to death receptor and chemotherapeutic drug induced apoptosis [12].

Another cytokine, IL10 is over expressed in breast tumors[13] and exogenous administration can mediate regression of tumor growth and breast cancer metastases in mice models[14]. Mononuclear cells from breast cancer patients' exhibit increased IL10 production[15], and IL10 serum levels correlate with stage of the disease[16].

In the present study, I assessed the expression of IL-4 and IL-10 mRNA in tissue of malignant breast cancer patients and non- malignant women and their correlation with malignant breast cancer.

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Materials and Methods:

Patients: The study included 60 patients from two hospitals in Baghdad (Al-Yarmook and Baghdad Teaching Hospital). Patients' ages ranged between 28-65 years. They were separated into two groups:

Group 1: 30 women with malignant breast cancer.

Group 2: 30 women with non- malignant as a control group.

Samples: Breast tumor biopsies were selected from each patients and control included in this study .Serial sections from paraffin embedded block were taken from the archive of department of pathology of these two hospitals. Tissue sections cut into 5µm thickness, put on Fisherbrand positively charched slides.

In situ hybridization: For *in situ* hybridization technique (ISH), DNA Probe Hybridization/Detection System in situ kit (Maxim Biotech, Inc., USA) was used.

The probes were biotin-labeled DNA probes for human IL-4 (225 bp) and human IL-10 (223 bp), (Maxim Biotech, Inc., USA).

In situ hybridization (ISH) is a technique used the high specificity of complementary nucleic acid binding to detect specific DNA or RNA sequence in the cell [17].

For detection of this markers, the biotinylated DNA probe hybridize the target sequence (IL-4 and IL-10 mRNA sequence) then a streptavidin-AP (streptavidin- alkaline phosphatase) conjugate is applied followed by addition of the substrate promo–chloro–indolyl-phosphate/nitro – blutetrazolium (BCIP/NBT) which yields an intense blue- black signal appears at the specific site of the hybridized probe [18].

This directly streptavidin-AP conjugate like the biotinylated probe provides a rapid and highly sensitive detection method. Evaluation of ISH signal was done with the assistance of a histopathologist .The expression of both IL-4 and IL-10 mRNA was measured by the same scoring system, counting of the number of the positive cells in the tissue that has given a blue-black (BCIP/NBT) nuclear staining under the light microscope. The score was the average from 10 distinct high-power fields observed under ×100 magnification. The percentage of positively stained cell was calculated for each case by taking the mean of the percentages of the positively stained cell in the 10 fields. A score of 0 was given when no staining was detected, 1 if there was weak to moderate staining in less than 10% of cells, 2 if moderate to strong staining was present in 11 to 50% of cells, and 3 if strong staining in more than 50% of cells was detected [19].

Statistical analysis: Student test (t-test) and Chi-square (χ^2) were used for the quantitative data. The relationship between the indicators was measured qualitatively by using the correlation coefficient(r). The lowest level of significance was when the probability ($p<0.05$) and the highly significance was ($p<0.01$) [20].

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Results:

In the current study, the t-test analysis table (1) and (2) show the highly significant difference (p<0.01) in the mean percentages of IL-4 and IL-10 in *situ* expression respectively, in the tissue of studied group.

In table (3), Chi-square test of significant was conducted to examine the association between IL-4 and IL-10 mRNA expression in tissue in the two groups, it was found that highly significant association (p<0.001) between them in the four scoring levels.

In addition, no correlation between IL-4 and IL-10 in all studied group was observed (table-4).

The expression of IL-4 and IL-10 was heterogeneous blue-black nuclear staining in the tissue, as shown in (figure-1).

Studied groups	**No.**	**Mean± Std. Error**	**Comparison of Significant**	
			***P*-value**	**Sig.**
Group1	30	51.3±2.9	0.000	Highly Sig. (P<0.01)
Group2	30	3.8±0.5		
Total	60			

Table-1: Mean percent of the expression of IL-4 (ISH assay) among studied groups.
****= highly significant difference (p<0.01)**

Studied groups	**No.**	**Mean± Std. Error**	**Comparison of Significant**	
			***P*-value**	**Sig.**
Group1	30	43.2±2.7	0.000	Highly Sig. (P<0.01)
Group2	30	2.1±0.3		
Total	60			

Table-2: Mean percent of the expression of IL-10 (ISH assay) among studied groups.
****= highly significant difference (p<0.01)**

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variable	score[a]	Studied groups		Total (No =60)	Chi-Square *P*- value
		1(No =30) No (%)	2(No =30) No (%)		
IL-4	1	0(0%)	18(60%)	18	0.000**
	2	1(3.3%)	12(40%)	13	
	3	21(70%)	0(0%)	21	
	4	8(26.7%)	0(0%)	8	
IL-10	1	0(0%)	16(53.3%)	16	0.000**
	2	2(6.7%)	14(46.7%)	16	
	3	19(63.3%)	0(0%)	19	
	4	9(30%)	0(0%)	9	

Table-3: Comparison of the prevalence of IL-4 and IL-10 mRNA(ISH assay) in tissue of studied groups depend on the scoring level.
Score[a]: 1(<0%); 2(<10 %); 3(11-50 %),4(>50 %); **= highly significant difference (*p*<0.01)

Variable	Studied groups	Correlation Coefficient r =	*P* value
IL-4 and IL-10	Group1	0.412	>0.05
	Group2	0.389	>0.05
	Total	0.561	>0.05

Table-4: Pearson correlation (r) between IL-4 and IL-10 in studied groups. *P*>0.05 = no significant difference

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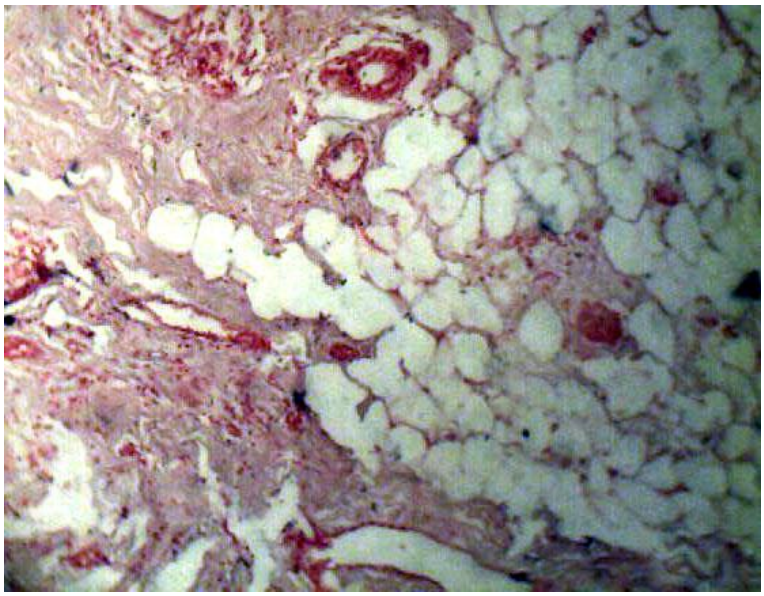
A



B

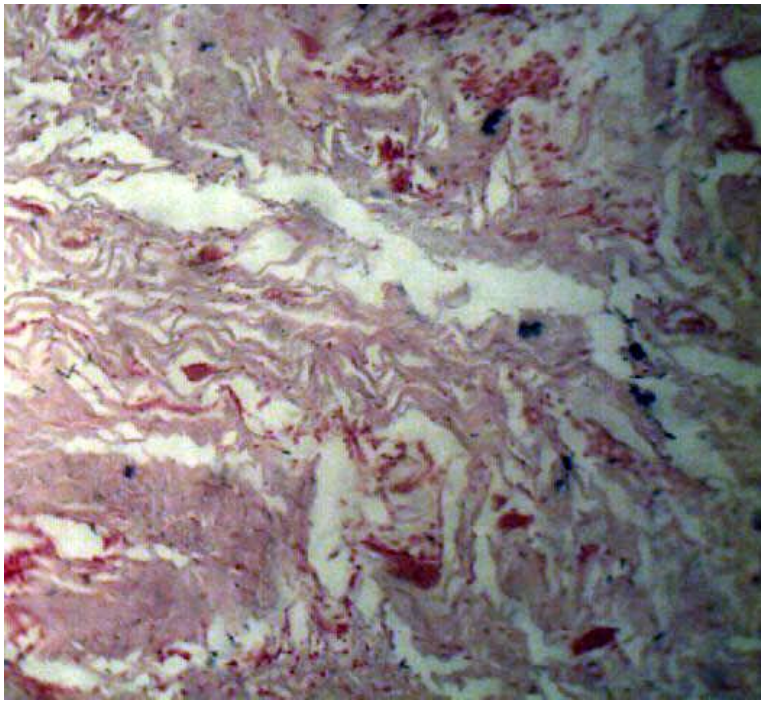


Figure-1: Detection of IL-4 and IL-10 in studied groups by in situ hybridization (ISH). Staining of IL-4 and IL-10 mRNA by BCIP/NBT (blue-black) counterstained with nuclear fast red. (A) Tissue from breast cancer patients shows positive IL-4 hybridization signals (X400). (B) Tissue from breast cancer patients shows positive IL-10 hybridization signals (X400).

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Discussion:

It is well known that the interactions of tumor cells with their microenvironment may affect tumor growth and metastasis formation. Among these, inflammatory cells and cytokines were recently suggested to play a key role in breast carcinoma [5]. Cytokines play varied roles in cancer pathogenesis with increasing evidence suggesting their involvement in tumor initiation, growth and metastasis [21].

In this study, highly significant increased expression (p<0.01) of IL-4 in patients with malignant breast cancer compared with non –malignant group. This finding is in line with previous study that demonstrated that specifically, high levels of IL-4 production have been detected among the tumor-infiltrating T lymphocytes (TILs) of several advanced solid cancers [22]. IL-4 stimulates B cell proliferation, an antiproliferative effect in breast cancer cells has been documented [23,24]. Other study found that cytokines produced by Th2 lymphocytes have been proposed to promote cell survival by influencing the expression of proteins involved in the regulation of apoptosis. Expansion of a peculiar Th2 lymphocyte subset with increased IL-4 production has been found in patients with B cell chronic lymphocytic leukemia [25]. Because IL-4 induces Bcl-2 expression in B cell chronic lymphocytic leukemia cells and inhibits spontaneous and hydrocortisone-induced apoptosis, it has been suggested that IL-4 prevents death of malignant B cells through a Bcl-2-dependent pathway [26].

Conticello and colleagues found that Th2 cytokines do not simply act through the modulation of the immune response, but they may promote cancer cell survival through the up-regulation of antiapoptotic genes [12].

Furthermore, in the present study, highly significant increased expression of IL-10 mRNA was found with malignant breast cancer (p<0.01) compared with non-malignant. This is consistent with studies that reported the increased levels of the cytokines IL-6, IL-8 and IL-10 have been observed in patients suffering from breast cancer as compared with healthy women [27,28,16].

IL-10 is thought to play a potential pathogenic or therapeutic role in a number of human conditions, such as inflammation, autoimmunity, and cancer[29]. The immunomodulatory effects of IL-10 have yielded mixed results in various tumor systems. On one hand, because many tumor types express IL-10, its role in helping tumors evade immunosurveillance has been suggested[30,31]. IL-10 inhibits the tumoricidal capacity of macrophages and the cytotoxicity and cytokine production of tumor-specific T cells and blocks the presentation of tumor antigens by antigen-presenting cells [32, 33]. On the other hand, in vivo studies in different animal models have demonstrated that IL-10 is a potent inhibitor of tumor growth and metastasis [14, 34].

Additionally, systemic administration of IL-10 has inhibited tumor metastasis and stimulated antitumor immune responses in murine models [35]. The mechanisms behind IL-10 antitumor effects might include inhibition of

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angiogenesis, stimulation of tissue inhibitor of matrix metalloproteinases (TIMPs), inhibition of MMP secretion, and inhibition of macrophage activity[30,36,37].

Moreover, IL-10 is able to affect the activities of NK cells, and NK cells were recently shown to contribute to the antiangiogenic effects of IL-12 through the killing of endothelial cells [38].

It is well known that, IL-10 is spontaneously secreted by a variety of cancer cells, including melanoma and glioblastoma, where IL-10 has been proposed to promote tumor cell survival, proliferation, and migration [39, 40].

In addition, no correlation between IL-4 and IL-10 in all studied group was observed in the current study. This result might be suggesting that increased expression of IL-4 not reflect increased expression of IL-10 but each one may indeed have an active role in pathogenesis of breast cancer. Numbers of study confirmed that, IL-4 and its receptor [41], IL-6 [42] and IL-10 [13], are important candidate genes as they play an important role in breast cancer pathogenesis[12].

Conclusions:

The increasing expression of IL-4 and IL-10 in malignant breast cancer tissue might be influence in the pathogenesis of malignant breast cancer.

References:

1 - Parkin, D.M.; Bray, F.; Ferlay, J. and Pisani, P. (2005). Global cancer statistics. CA Cancer J Clin. 55:74-108.

2 - Deshmukh, P.; Click, R.P. and Lichtor, T. (2001). Immunogene therapy with Interleukin–2 secreting fibroblasts for intracerebrally metastasizing breast cancer in mice. J Neurosurg. 94(2): 287-292.

3 - Madigan, M.; Zielger, R. and Benichon, et. al. (1995). Proportion of breast cancer cases in the United States explained by well-established risk factors. J Natl Cancer Inst .87:1681-1687.

4 - Skoog, L.; Humla, S.; Axelsson, M.; Frost, M.; Norman, A.; Nordenskjold, B. and Wallgren, A. (1987). Estrogen receptor levels and survival of breast cancer patients. A study on patients participating in randomized trials of adjuvant therapy. Acta Oncol. 26:95-100.

5 - Wilson, J. and Balkwill, F. (2002). The role of cytokines in the epithelial cancer microenvironment . Semin Cancer Biol. 12:113-120.

6 - Boon, T. and Van den, Eynde, B. (2003). Tumour immunology. Curr Opin Immunol. 15:129-130.

7 - Romagnani, P.; Lasagni, L.; Annunziato, F.; Serio, M.and Romagnani, S. (2004). CXC chemokines: the regulatory link between inflammation and angiogenesis. Trends Immunol. 25:201-209.

8 - Shea,J.J.; Frucht, D. M .and Duchett, C. S. (2001). Cytokines and Cytokine receptors In Clinical Immunology, principles and practice. (Editors) Rich,

Accepted 25 May 2009

R.R.; Fleisher, T. A.; Schwartz, B. D.; Shearer, W. T.; Kotzin, B. L. and Schroeder, H. W. (2ndedition) Mosby. Ch12 (1): 1-22

9 - Elenkov, I. J. and Chrousos, G. P. (1999). Stress hormones, Th1/Th2 patterns, Pro/Anti-inflammatory Cytokines and Susceptibility to Disease. Trends Endocrinol Metab 10:359-368.

10 - Freund, A.; Chauveau, C.; Brouillet, J.P.; Lucas, A.; Lacroix, M.; Licznar, A.; Vignon, F. and Lazennec, G.(2003). IL-8 expression and its possible relationship with estrogen-receptor-negative status of breast cancer cells. Oncogene .22:256-265.

11 - Prokopchuk, O.; Liu, Y.; Henne-Bruns, D. and Kornmann, M. (2005). Interleukin-4 enhances proliferation of human pancreatic cancer cells: evidence for autocrine and paracrine actions. Br.J.Cancer. 92:921–8.

12 - Conticello, C.; Pedini, F. and Zeuner, A. (2004). IL-4 protects tumor cells from anti-CD95 and chemotherapeutic agents via up-regulation of antiapoptotic proteins. J Immunol. 172:5467–77.

13 - Venetsanakos, E.; Beckman, I.; Bradley, J.and Skinner, J.M. (1997). High incidence of interleukin 10 mRNA but not interleukin 2 Mrna detected in human breast tumours. Br J Cancer. 75(12):1826-1830.

14 - Kundu, N.; Beaty, T.L.; Jackson, M.J.and Fulton, A.M. (1996). Antimetastatic and antitumor activities of interleukin 10 in a murine model of breast cancer. J Natl Cancer Inst. 88(8):536-541.

15 - Merendino, R.A.; Gangemi, S.; Misefari, A.; Arena, A.; Capozza, A.B; Chillemi, S.and D'Ambrosio, F.P. (1999). Interleukin-12 and interleukin-10 production by mononuclear phagocytic cells from breast cancer patients. Immunol Lett. 68(2-3):355-358.

16 - Kozlowski, L.; Zakrzewska, I.; Tokajuk, P.and Wojtukiewicz, M.Z.(2003). Concentration of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in blood serum of breast cancer patients. Rocz Akad Med Bialymst 48:82-84.

17 - Maritette, P. C.; Roeland, H. D.; Rob, P. M.; Van, Erica, B.; Clavida, M. H.;Roelof, A. P.; Jim, E.L. and Anton, K. R.(1998). Sensitive mRNA detection fluorescence in situ hybridization using horseradish peroxidase – labeled Oligodeoxy nucleotides and tyramid signal amplification. J. Histochem. Cytochem. 46: 1249-1259.

18 - Yoshiyyki, R.O.; Yoshiko, I.; and Akira, M. (2002). Aplication of plastic embedding to electron microscopic immunocytochemistry and in situ hybridization in observation of production and secretion of peptide hormones. J. Histochem. Cytochem. 98: 885-891.

19 - Nakopoulou, L.; Lazaris, A. C.; Kavantzas, N.; Alexandrou, P.; Athanassiadou, P.;Keramopoulos, A. and Davaris, P.(2000). DNA topoisomerase II-αimmunoreactivity as a marker of tumor aggressiveness in invasive breast cancer. Pathobiology. 68:137-143

Accepted 25 May 2009

20 - Sorlie, D.E. (1995). Medical biostatistics & epidemiology: Examination & board review. First ed. Norwalk, Connecticut, Appleton & Lange: 47-88.

21 - Smyth, M.J.; Cretney, E.; Kershaw, M. H. and Hayakawa, Y. (2004). Cytokines in cancer immunity and immunotherapy. Immunol Rev . 202:275-293.

22 - Shurin, M. R.; Lu, P.; Kalinski, A. M.and Stewart-Akers, M. T. (1999). Th1/Th2 balance in cancer, transplantation and pregnancy. Springer Semin. Immunopathol. 21:339.

23 - Toi, M.; Bicknell, R. and Harris, A.L . (1992).Inhibition of colon and breast carcinoma cell growth by interleukin-4. Cancer Res. 52:275–279.

24 - Blais, Y.; Gingras, S.; Haagensen, D.E.; Labrie, F. and Simard ,J. (1996). Interleukin-4 and interleukin-13 inhibit estrogen-induced breast cancer cell proliferation and stimulate GCDFP-15 expression in human breast cancer cells. Mol Cell Endocrinol. 121:11–18

25 - De Totero, D. G.; Reato, F.; Mauro, A.; Cignetti, S.; Ferrini, A.; Guarini, M.; Gobbi, C. E.and Grossi, R. (1999). IL4 production and increased CD30 expression by a unique CD8+ T-cell subset in B-cell chronic lymphocytic leukemia. Br. J. Haematol. 104:589.

26 - Dancescu, M. M.; Rubio-Trujillo, G.; Biron, D.; Bron, G.and Delespesse, M. Sarfati, (1992) Interleukin 4 protects chronic lymphocytic leukemic B cells from death by apoptosis and up-regulates Bcl-2 expression. J. Exp. Med. 176:1319.

27 - Luca, M.; Huang, S.; Gershenwald, J.E.; Singh, R.K.; Reich, R.and Bar-Eli ,M. (1997). Expression of interleukin-8 by human melanoma cells up-regulates MMP-2 activity and increases tumor growth and metastasis. Am J Pathol. 151: 1105-13.

28 - Moore, K.W.; de Waal Malefyt, R.; Coffman, R.L.and O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 19: 683-765.

29 - Howard, M. and O'Garra, A. (1992) Biological properties of interleukin 10. Immunol. Today. 13: 198-200.

30 - Smith, D. R.; Kunkel, S.L.; Burdick, M.D.; Wilke, C. A.; Orringer, M. B. ;Whyte, R. I.and Strieter, R. M. (1994). Production of interleukin-10 by human bronchogenic carcinoma. Am. J. Pathol. 145: 18-25.

31 - Young, M. R.; Wright, M. A.; Lozano, Y.; Matthews, J.P.; Benefield, J. and Prechel, M .M. (1996). Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. Int. J. Cancer. 67: 333-338,.

32 - Rohrer, J. W. and Coggin, J.H. (1995). CD81 T cell clones inhibit antitumor T cell function by secreting IL-10. J. Immunol., 155: 5719-5727.

Accepted 25 May 2009

33 - Beissert, S.; Hosoi, J.; Grabbe, S.; Asahina ,A. and Granstein, R. D. (1995) IL-10 inhibits tumor antigen presentation by epidermal antigen presenting cells. J. Immunol.154: 1280-1286.

34 - Giovarelli, M.; Musiani, P.; Modesti ,A.; Dellabona, P.; Casorati, G.; Allione, A.; Consalvo, M.; Cavallo, F.; di Pierro, F.; De Giovanni, C.; Musso, T. and Forni, G. (1995). Local release of IL-10 by transfected mouse mammary adenocarcinoma cells does not suppress but enhances antitumor reaction and elicits a strong cytotoxic lymphocyte and antibody-dependent immune memory. J. Immunol., 155: 3112-3123.

35 - Berman, R. M.; Susuki. T.; Tahara, H.; Robbins, P. D.; Narula, S. K. andLotze, M. T. (1996). Systemic administration of cellular IL-10 induces an effective, specific, and long-lived immune response against established tumors in mice. J. Immunol. 157: 231-238.

36 - Cervenak, L.; Morbidelli, L.; Donati, D.; Donnini, S.; Kambayashi, T.; Wilson, J. L.; Axelson, H.; Castanos-Velez, E.; Ljunggren, H, G.; Malefyt, R.D.; Granger, H.J.; Ziche, M. and Bejarano,M.T. (2000). Abolished angiogenicity and tumorigenicity of Burkitt lymphoma by interleukin-10. Blood. 96: 2568-2573.

37 - Di Carlo, E.; Coletti, A.; Modesti, A.; Giovarelli, M.; Forni, G.and Musiani, P. (1998). Local release of interleukin-10 by transfected mouse adenocarcinoma cells exhibit pro- and anti-inflammatory activity and results in a delayed tumor rejection. Eur. Cytokine Netw. 9: 61-68.

38 - Yao, L.; Sagadari, C.; Furuke, K.; Bloom, E. T.; Teruya-Feldstein, J.; and Tosato, G. (1999). Contribution of natural killer cells to inhibition of angiogenesis by IL-12. Blood 93: 1612-1621,

39 - Nitta, T.; Hishii, M.; Sato, K.; and Okumura, K.(1994). Selective expression of interleukin-10 gene within glioblastoma multiforme. Brain Res. 649:122–8.

40 - Sredni,B.;Weil,M. and Khomenok, G. et.al. (2004). Ammonium trichloro (dioxoethylene-*o,o'*) tellurate (AS101) sensitizes tumors to chemotherapy by inhibiting the tumor interleukin 10 autocrine loop. Cancer Res. 64:1843–52.

41 - Gooch, J. L.; Lee, A.V.; and Yee, D. (1998). Interleukin 4 inhibits growth andinduces apoptosis in human breast cancer cells. Cancer Res. 58(18):4199-4205.

42 - Salgado, R.; Junius, S.; Benoy, I.; Van, Dam, P.; Vermeulen, P.; Van Marck, E.; Huget, P.; and Dirix, L.Y. (2003). Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. Int J Cancer , 103(5):642-646.