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Expression of 2-nd Receptor of Tumor Necrosis Factor Cytokine (TNFR-II) in Canine Mammary Neoplasms

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Abstract. Actual study proposes an actual and controversial theme in oncology, such as expression and distribution of TNF cytokine 2-nd receptor in canine mammary cancer, and its involvement in cancer progression and prognosis. TNFR-II was evaluated by LSAB immunohistochemistry technique using anti-TNFR-II marker. Tumor malignancy was appreciated using histology grading (I to III), mitotic index, nuclear grade, tumor size, and histology type. In the study were elected benign and malign canine mammary tumors. Distribution and localization of TNFR-II was restricted in both inflammatory cells from the sustaining stroma of studied tumors (macrophage, lymphocyte), and in cancerous epithelial or myoepithelial cells. Obtained dates demonstrate a direct relation between histologic grade and TNFR-II expression; TNFR-II had a lower expression in benign and differentiated grade I canine mammary tumors (cancerous epithelial and myoepithelial cells), and a higher expression in poorly differentiated ones (grade II cancers).

Keywords: TNFR-II, TNF, bitch, cytokine, necrosis

INTRODUCTION

Mammary cancer is one of the main causes of dying in bitches having about three folds higher incidence than breast cancer in women. We approach this type of cancer because of its high incidence, representing the main cause of death in female dogs.

The goal of the study is to look after the distribution of the 2-nd receptor of TNFα cytokine (TNFR-II) and interrelation (if there is one) of TNFR-II with canine mammary cancer malignancy markers (histology grading, mitotic index, tumor size, and histology type). The TNF (tumor necrosis factor) is a cytokine involved in immunity and inflammation, and often presented in tumors. These active cytokines acts using tow receptors such as: TNFR-I and TNFR-II. There are many studies involving distribution and the role of the TNFR-I. In cancer TNFR-I mediate cytotoxic effect of TNF on tumoral cells inducing cell death or apoptosis. Regarding TNFR-II, biologic role and its distribution in tumoral cells is still unclear. Despite of the name seems that TNF (tumor necrosis factor) cytokine could have carcinogenic effect respectively to initiate and favor cancer onset by involvement in chronic inflammation, which generate increased oxidative stress known as carcinogenic agent. On the other hand, TNF clearly possesses antitumor effects not only in preclinical models but also in the clinical setting by inducing intratumor necrosis (Waterston Ashita *et al*., 2004).

MATERIALS AND METHODS

Sample harvesting, fixation, paraffin embedding, paraffined slices preparation and staining. Mammary tumor formations had been provided by corps reached to Pathology

department from the University of Agricultural Science and Veterinary Medicine, Faculty of Veterinary Medicine Cluj-Napoca, Romania. There were utilized 7 malign and 2 benign mammary tumors provided by different bitch breeds, such as: German Sheppard, Cocker Spaniel, Stray dogs, Mioritic Sheppard, Boxer and Irish Setter. For histopathology exam the samples had been fixed in 10% buffered formalin and processed by paraffin technique, and stained by hematoxilin-eozin and trichrome Masson technique. The mammary tumors are from 9 bitches, with the age of 2-13 years. To make an appropriate histology grading of malignant tumors based on WHO classification, were evaluated nuclear degree, tubule formation and mitotic index. Canine mammary tumors were framed in three histological grades such as: grade I (less aggressive), grade II and grade III (high aggressivity).

Immunohistochemistry. TNFR-II and tumor proliferation were evaluated by LSAB immunohistochemistry technique using anti-TNFR-II marker (polyclonal antibodies – Abcam U.S.A. – clone ab15563-500, Rabbit polyclonal antihuman to TNF Receptor II). Histological slides had about 5 μm thicknesses and were fixed on silanized slides (Dako) during 24 hours in 37°C, followed by deparaffination in xylen. Antigen retriever had been made using a pressurized cooker in citrate solution, pH=6.0 (Dako); endogenous peroxidase was inactivated by peroxidase blocking reagent (Dako - Peroxidase blocking reagent 3%) during 5 minutes at the room temperature. Primary monoclonal antibodies (anti-TNFR-II) were maintained overnight, during 18 hours at 4°C, using a dilution of 1:50 in antibody diluent (Dako). The visualization of immunological reaction was performed using Universal LSAB+Kit/HRP, Rb/Mo/Goat (DAB+) system (Dako); the counterstaining was performed by Mayer hematoxylin. To evaluate the antibody specificity was used negative control (replacing the primary antibody with antibody diluent).

Microscopic examination and quantification. The microscopic images were obtained by Olympus BX51 microscope, connected to a photo digital camera (Olympus DP-25). TNFR-II quantification was realized by following cells with immunomarked membrane, where the receptor is. Positive cells had a brown membrane of variable intensities. Counting of TNFR-II positive cells had been made in about three high powered microscopic fields (400 folds magnification) for every studied tumor, being evaluated about 900-1300 cells/tumor. There were monitored and elected microscopic fields with less connective sustentacular tissue. In every monitored microscopic field were counted only epithelial and myoepithelial cells, and weren't counted positive stromal cells or inflammatory cells from tumoral stroma. We used a semiautomatic computerized analysis technique (Olympus Soft imaging solutions Cell B).

RESULTS AND DISCUSSIONS

In the Table 1 are presented the results obtained from every canine mammary tumor taken in study, and these results will be debated later.

Tumor necrosis factor (TNF) is a proinflammatory cytokine that have an important role in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis or Crohn diseases in humans. TNF is found as a 26kd membrane bound molecule which, when cleaved by the TNF converting enzyme (TACE), forms soluble TNF consisting of the 76 aminoterminal residues with a molecular weight of 17kd (Waterston Ashita *et al.*, 2004). Under native conditions bound and soluble TNF exist as a monomer, dimer and trimer in equilibrium, with the trimer being the biologically active form. TNF belongs to the TNF superfamily, which includes Lymphotoxin a and b, Fas ligand, CD40 ligand, and two apoptosis inducing ligands, TRAIL/Apo-2 ligand (Waterston Ashita *et al.*, 2004) and LIGHT,

which is also involved in T cell activation (Mauri *et al.*, 1998). These proteins are all ligands for the TNF receptor superfamily.

Tab 1.

Summary of the results obtained by evaluation of studied canine mammary tumors.

Case nr.	Tubular structure (grade)	NP (grade)	MI	Histopathologic diagnose	HG	TNFR-II (%)			
						Field 1	Field 2	Field 3	**Average**
1	-	-	-	simple adenoma	-	7,45	5,61	6,93	6,66
2	1	1	14	simple cystic papillary carcinoma	I	12,6	5,9	9,35	9,28
3	1	2	19	simple tubular carcinoma	I	5,55	4,88	2,39	4,24
4	2	2	12	complex tubule-papillary carcinoma	II	8,39	5,70	11,26	8,45
5	1	1	10	complex tubule-papillary carcinoma	I	7,98	4,40	5,93	6,10
6	2	2	14	complex tubule-papillary carcinoma	II	10,55	7,61	5,53	7,89
7	-	-	-	simple adenoma	-	7,87	7,86	5,83	7,18
8	1	3	16	simple tubule-papillary carcinoma	II	9,37	7,87	6,27	7,83
9	2	3	19	cystic papillary cystic carcinoma in benign mixed tumor	II	10,54	11,67	7,27	9,82

NP: nuclear polymorphism.
MI: mitotic index – number of mitoses/10 microscopic fields magnified of 400 folds.
HG: histologic grade.
TNFR-II (%): percentage value obtained by quantification of tumoral cells/3 microscopic fields magnified of 400 folds.

TNF binds with high affinity to two cell surface receptors, a 55kd protein (p55TNF-R or TNFR-I) and a 75kd protein (p75TNF-R or TNFR-II), both are expressed by most cell lines and primary tissues. However, the level of receptor expression varies with cell type. The p55TNF-R expression is dominant on most cells, except for haemopoetic cells, and is relatively constant, while the p75TNF-R expression fluctuates (Waterston Ashita *et al*., 2004). It is thought that p55TNF-R is the major signal transducer of soluble TNF responses, due to the abundance and binding avidity of this receptor; while p75TNF-R is preferentially activated by membrane bound TNF (Grell *et al*., 1995; Smith *et al*., 1994).

The major sources of TNF are macrophages and to a lesser extent T lymphocytes, proliferating B cells, natural killer (NK) cells, mast cells and stimulated neutrophils (English *et al*., 1991; Gemlo *et al.*, 1988; Gordon *et al*., 1990). Non-immune cells such as keratinocytes, smooth muscle cells, astrocytes and microglial cells have all been shown to produce TNF upon lipopolysaccharides stimulation *in vitro* (Kock *et al*., 1990; Waterston Ashita *et al*., 2004). TNF is a pleiotropic cytokine, which acts on a large variety of cells with wide ranging effects on individual cells. TNF promotes the pro-inflammatory cascade, by inducing the release of pro-inflammatory cytokines such as the chemokine IL-8 (Nickoloff *et*

al., 1991), IL-6 (Waterston Ashita *et al*., 2004), and adhesion molecules such as VCAM important in metastasis (Osborn *et al*., 1990).

TNF in large quantities may lead to tumor necrosis, antitumor effects being a consequence of vascular induced tumor necrosis, or the consequence of apoptosis initiation by activating caspases 8 and 10, or finally the consequence of direct cytotoxic effects induced by free radicals and lysosomal enzymes (Enari *et al*., 1998; Waterston Ashita *et al*., 2004).

The studies that investigated the effects of these cytokines are diverse and controversial. Also, it had been proved antitumor effect of TNF in combination with some other cytokines or chimiotherapeutic agents, aspects observed in oncologic clinic in some sarcomas and melanomas (Waterston Ashita *et al*., 2004). On the other hand, despite of the name it seems that TNF can act as pro-carcinogenic agent favoring tumor onset and subsequent cancerous progression, tumor associated cahexy being the consequence of proteolysis and lipids metabolization (Aggarwal *et al*., 2003; Balkwill *et al*., 2006; Mocellin *et al*., 2008). Concluding, it seems that TNF can act not only as anti-cancerous agent but as pro-carcinogenic agent initiating and favoring tumor progression especially in aged animals. Furthermore, in experimental models of cancer induction realized in rats TNF favor not only the metastasis but intratumor angiogenesis as well. These strong pro-cancerous effects could be the consequence of deregulation of TNF production such as overproduction of this cytokine (Balkwill *et al*., 1992).

The mechanism and signalling events associated with carcinogenesis are still being elucidated. TNF, along with other proinflammatory cytokines, induces nitric oxide synthetase in a cholangiocarcinoma cell line. This enzyme produces nitric oxide, which can increase DNA damage by inhibiting sensitive DNA repair enzymes, and thereby contributes to an increase in genetic mutations (Jaiswal *et al*., 2000). Other studies have shown that the presence of iNOS in gynecological tumours correlates with dedifferentiation (Waterston Ashita *et al*., 2004). Therefore, the production of nitric oxide through TNF induction of iNOS may not only lead to tumour cell apoptosis, as described previously, but may also promote carcinogenesis. The signalling pathways induced by TNF have also been examined in rat mammary cells. TNF stimulated growth and morphogenesis of normal rat mammary epithelial cells as well as transformed mammary epithelial tumours. TNF may induce carcinogenesis by up-regulating of some proteins (NF-kB) that cause cell proliferation and morphogenesis (Waterston Ashita *et al*., 2004).

Further, the TNF seems to have an important role as well in metastasis. TNF is a potent proinflammatory cytokine that can be utilized by tumours to induce some downstream molecules involved in the metastatic process. Recombinant TNF injected into mice inoculated with a methylcholanthrene-induced fibrosarcoma increased the number of lung metastases (Orosz *et al*., 1993).

Regarding the TNF involvement in tumor neovascularisation and angiogenesis, it was shown that some chemokines such as IL-8 and Groα as well as other growth factors e.g. FGF, PDGF and thymidine phosphorylase are important in neovascularisation (Nagy *et al*., 1995; Waterston Ashita *et al*., 2004). They attract endothelial cells and cause the migration of capillaries into the tumours. TNF has been found to increase the expression of IL-8 and Groα in a number of different cell types (Strieter *et al*., 1995). In histological samples of malignant breast cancer, increased TNF staining correlated with increased thymidine phosphorylase an important enzyme in angiogenesis (Waterston Ashita *et al*., 2004).

Having these paradoxes about TNF activities in different tumor types, this study will show some aspects regarding TNFR-II in canine spontaneous mammary cancer. Obtained

dates will be compared with some classic parameters for this type of tumor, such as histologic type, and histologic grade (grades I to III).

Our dates was realized on mammary tumors provided by bitches of different ages (2-13 years old) and breeds, respectively German Sheppard, Cocker Spaniel, stray dogs (every one about 22,20% from examined cases), Mioritic Sheppard, Boxer and Irish Setter (every one about 11,10% from examined cases) (Table 1). In the study were elected benign and malign canine mammary tumors, such as: two simple adenomas (22,2% from all tumors), three complex tubulopapillary carcinomas (33,3% from all tumors), two simple tubule-papillary carcinomas (22,2% from all tumors), one simple cystic carcinoma (11,1% from all tumors), and one carcinoma in benign mixed tumor (11,1% from all tumors). Malign tumors were framed in histological grades for a better understanding of their malignancies, such as: three grade I mammary tumors (cases 2, 3, 5) and four grade II mammary tumors (cases 4, 6, 8, 9).

Concerning localization and distribution of TNF cytokine 2-nd receptor, particularly of TNFR-II, it was encountered in both inflammatory cells from the sustaining stroma of studied tumors (macrophage, lymphocyte – Fig. 2 and 4), and in cancerous epithelial or myoepithelial cells (Fig. 1). There was an increased incidence of TNFR-II especially in cancerous cells placed circa intra-tumoral necrotized areas indicating, probably, it's involvement in induction of tumoral apoptosis and necrosis. The tumors with tubular cancerous structures had numerous intraductal TNFR-II positive desquamated cells indicating apoptosis (Fig 3). In fact it is known from bibliographic dates mentioned earlier that TNF cytokine can induce intratumor necrosis, this ability being utilized by some therapeutic antitumor protocols (Waterston Ashita *et al*., 2004). On the other hand, the presence of TNFR-II in inflammatory cells from tumoral sustaining stroma could indicate TNF potential in cancer developments, progression and metastasis, aspects highlighted as well in the literature (Mocellin *et al*., 2008; Waterston Ashita *et al*., 2004). Deregulated TNF expression within the tumor microenvironment appears to favor malignant cell tissue invasion, migration and ultimately metastasis formation. TNF clearly possesses antitumor effects not only in preclinical models but also in the clinical setting. These conflicting findings and apparently paradoxical TNF activities, such as anticancer as well as cancer-promoting TNF effects, are realties observed in clinical and animal models being quite dificult to explain the coexistence of these (Aggarwal *et al*., 2003; Balkwill *et al*., 2006; Waterston Ashita *et al*., 2004).

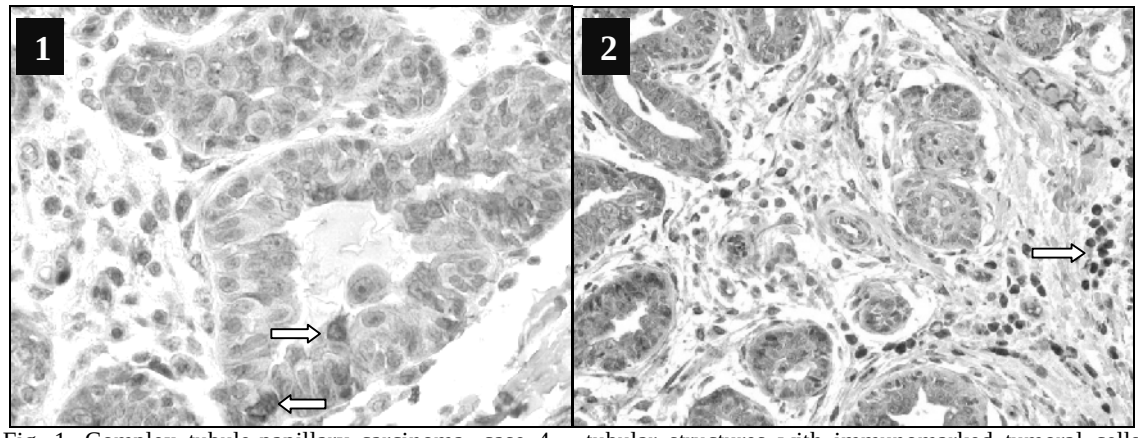

Fig. 1. Complex tubule-papillary carcinoma, case 4 – tubular structures with immunomarked tumoral cells (arrows); IHC reaction anti-TNFR-II, 400x.

Fig. 2. Complex tubule-papillary carcinoma, case 4 – expression of TNF 2-nd receptor in inflammatory cells from sustaining stroma (macrophages); IHC reaction anti-TNFR-II, 100x.

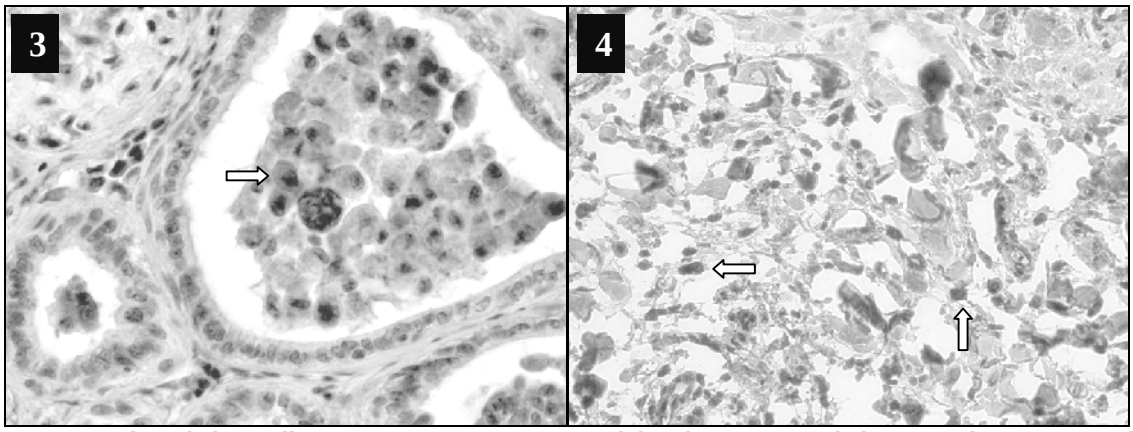

Fig. 3. Complex tubule-papillary carcinoma, case 6 – tumoral ductal structures with dequamated (apoptotic) and immunomarked cancerous cells (arrow); IHC reaction anti-TNFR-II, 400x.
Fig. 4. Simple tubule-papillary carcinoma, case 8 – TNFR-II immunopositive macrophages in tumoral capsule; IHC reaction anti-TNFR-II, 200x.

Distribution of TNFR-II in cancerous mammary cells and correlation between its incidence with histologic grade can be observed in figure 5. There is obvious a direct relationship between the two parameters. Also, benign tumors and differentiated mammary carcinomas have the fewest TNFR-II immunomarked tumoral cells (values ranging from 4,24% to 9,28%) comparing with poorly differentiated canine mammary carcinomas (grade II) where TNFR-II incidence had greater values (values ranging from 7,83% to 9,82%). The only difference is represented by the case 2, which despite being a differentiated one have high percentage of cells with TNFR-II (TNFR-II positive cells - 9,28%).

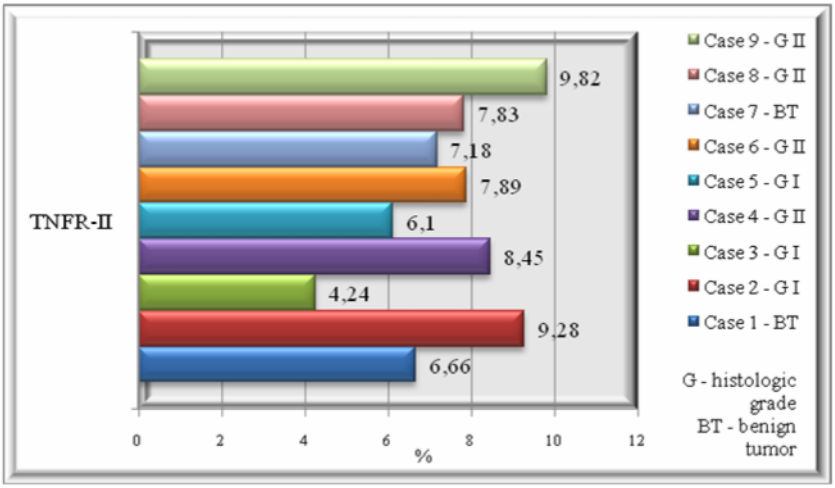

Figure 5. Correlation between histologic grade and average percentage value of TNFR-II.

Obtained dates regarding TNFR-II deliver some additional important data regarding intratumor distribution in different types of benign and malign canine mammary tumors. The bibliographic dates is poor in information about intratumor distribution and role of the 2-nd receptor of TNF-α cytokine (TNFR-II or p75 TNFR). There is a high amount of information about the first receptor of TNF-α (TNFR-I or p55 TNFR). We will present some bibliographic data concerning distribution, role and significance of the TNFR-I in different tumor types.

TNF has been detected in a number of different tumour types such as ovarian and breast tissue as well as haematological malignancies (Miles *et al*., 1994; Naylor *et al*., 1993; Warzocha *et al*., 2000). Both mRNA expression and TNF protein has been found in human epithelial ovarian tumour cells as well as within the infiltrating macrophages. The p55 TNFR has also been detected within ovarian tumour cells and infiltrating macrophages but not stromal macrophages whilst the p75 TNFR has only been found within the infiltrating macrophages (Naylor *et al*., 1993). Compared to ovarian human tumors, actual study proved that p75 TNFR (TNFR-II) was expresses within mammary tumoral cells (cancerous epithelial and myoepithelial cells) and in infiltrating stromal inflammatory cells. There was observed a direct relation between malignancy and expression of TNFR-II. Also, histologic grade and especially proliferating marker Ki-67 values increased in the same time with expression of TNFR-II. According to this, overexpression of TNFR-II can be correlated with a poor prognosis and high malignancy degree in the case of canine mammary cancer. A similar picture of increased production of TNF correlating with worse prognosis has been identified in patients with prostrate cancer. In these patients, raised serum TNF levels were associated with a reduction in body mass index and other factors associated with cachexia as well as a significantly increased mortality (Nakashima *et al*., 1998; Mizokami *et al*., 2000).

CONCLUSIONS

Distribution and localization of TNFR-II was restricted in both inflammatory cells from the sustaining stroma of studied tumors (macrophages, lymphocytes), and in cancerous epithelial or myoepithelial cells.

There was an increased incidence of TNFR-II especially in cancerous cells placed circa intratumoral necrotized areas and in desquamated intraductal tumoral cells indicating TNFR-II involvement in induction of tumoral apoptosis and necrosis.

Obtained dates demonstrate a direct relation between histologic grade and TNFR-II expression in cancerous epithelial and myoepithelial cells; TNFR-II had a lower expression in benign and differentiated grade I canine mammary tumors, and a higher expression in poorly differentiated ones (grade II cancers).

The study demonstrate, comparing with bibliographic dates, that TNFR-II can be met not only in the inflammatory cells of the canine mammary carcinoma but as well in cancerous mammary tumors (epithelial and myoepithelial cancerous cells).

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REFERENCES

1. Aggarwal, B.B., (2003). Signalling pathways of the TNF superfamily: a double-edged sword. Nat. Rev. Immunol. 3: 745-756.

2. Baba A.I., C. Cătoi, (2007). Comparative Oncology, Romanian Academy Ed, 87-407.

3. Balkwill F., (2006). TNF-alpha in promotion and progression of cáncer, Cancer Metastasis Rev. Sep;25(3):409-16.

4. Balkwill FR, (1992). Tumour necrosis factor and cáncer, Prog Growth Factor Res., 4(2):121-37.

5. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S, (1998). A caspase-activated DNase that degrades DNA during apoptosis and its inhibitor ICAD [published erratum appears in Nature 1998, May 28;393(6683):396]. Nature 391, 43-50.

6. English BK Weaver WM, and Wilson CB, (1991). Differential regulation of lymphotoxin and tumor necrosis factor genes in human T lymphocytes. J Biol Chem 266, 7108-13.

7. Gemlo BT, Palladino MAJ, Jaffe HS, Espevik TP, and Rayner AA, (1988). Circulating cytokines in patients with metastatic cancer trated with recombinant interleukin 2, and lymphokine-activated killer cells. Cancer Res 48, 5864-67.

8. Gordon JR, Gallis SJ, (1990). Mast cells as A, source of both preformed and immunologically inducible TNF-a/cachectin. Nature 346, 274-76.

9. Grell M Douni E Wajant H Lohden M Clauss M Maxeiner B Georgopoulos S Lesslauer W Kollias G Pfizenmaier K. (1995). The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80, kDa tumor necrosis factor receptor. Cell 83 793-802.

10. Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ, (2000). Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by A, nitric oxidedependent mechanism. Cancer Res 60, 184-90.

11. Kock A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, Ansel JC, Luger TA, (1990). Human keratinocytes are A, source of tumor necrosis factor a evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J Exp Med 172, 1609-14.

12. Mauri DN, Ebner R, Montgomery RI, Kochel KD, Cheung TC, Yu GL, Ruben S, Murphy M, Eisenberg RJ, Cohen GH, Spear PG, Ware CF, (**1998**). LIGHT A, new member of the TNF superfamily and lymphotoxin a are ligands for herpesvirus entry mediator. Immunity 8, 21-30.

13. Miles DW, Happerfield LC, Naylor MS, Bobrow LG, Rubens RD, Balkwill FR, (1994). Expression of tumour necrosis factor (TNFa) and its receptors in benign and malignant breast tissue. Int J Cancer 56, 777-82.

14. Mizokami A, Gotoh A, Yamada H, Keller ET, Matsumoto T, (2000). Tumor necrosis factor-a represses androgen sensitivity in the LNCaP prostate cancer cell line. J Urol 164, 800-5.

15. Mocellin S, Nitti D., (2008). TNF and cancer: the two sides of the coin, Front Biosci. 1;13:2774-83.

16. Nagy JA, Masse EM, Herzberg KT, Meyers MS, Yeo KT, Yeo TK, Sioussat TM, Dvorak HF, (1995). Pathogenesis of ascites tumor growth: vascular permeability factor vascular hyperpermeability and ascites fluid accumulation. Cancer Res 55, 360-8.

17. Nakashima J, Tachibana M, Ueno M, Miyajima A, Baba S, Murai M, (1998). Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer. Clin Cancer Res 4, 1743-8.

18. Naylor MS, Stamp GW, Foulkes WD, Eccles D, Balkwill FR, (1993). Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. J Clin Invest 91, 2194-206.

19. Nickoloff BJ, Karabin GD, Barker JN, Griffiths CE, Sarma V, Mitra RS, Elder JT, Kunkel SL, Dixit VM, (1991). Cellular localization of interleukin-8, and its inducer tumor necrosis factor-a in psoriasis. Am J Pathol 138, 129-40.

20. Orosz P, Echtenacher B, Werner F, Ruschoff J, Weber D, Mannel DN, (1993). Enhancement of experimental metastasis by tumor necrosis factor. J. Exp. Med 177, 1391-1398.

21. Osborn L, (1990). Leukocyte adhesion to endothelium in inflammation. Cell 62, 3-6.

22. Smith CA, Farrah T, and Goodwin RG, (1994). The TNF receptor superfamily of cellular and viral proteins: activation costimulation and death. [26, refs]. Cell 76, 959-62.

23. Strieter RM, Polverini PJ, Kunkel SL, Arenberg DA, Burdick MD, Kasper J, Dzuiba J, Van Damme J, Walz A, Marriott D, (1995). The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 270, 27348-57.

24. Warzocha K, Salles G, Bienvenu J, Bastion Y, Dumontet C, Renard N, Neidhardt-Berard EM, Coiffier B, (1997). Tumor necrosis factor ligand-receptor system can predict treatment outcome in lymphoma patients. J Clin Oncol 15, 499-508.

25. Waterston Ashita, Bower M. (2004). TNF and cancer: good or bad?, Cancer Therapy Vol 2, 131-148.