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ORIGINAL ARTICLE

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Variable expression of tumor necrosis factor α in human malignant melanoma localized by in situ hybridization for mRNA

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Abstract Tumor necrosis factor α (TNFα) is a cytokine, produced by lymphocytes and monocytes, with cytotoxic activity against some but not all tumor cell lines. Resistance to the cytolytic effects of TNFα has been reported in cell lines with autocrine TNFα production. The purpose of this study was to investigate whether human primary malignant melanoma and tumor infiltrating lymphocytes produce TNFα in vivo. Optimal conditions for in situ hybridization for TNFα mRNA in paraffin-embedded tissue were established. Analysis of 13 primary malignant melanomas and 3 metastatic lesions with different degrees of immunohistochemical TNFα positivity demonstrated that, in some tumors, both melanoma cells and leukocytes contained TNFα mRNA and protein. These findings demonstrate variable production of TNFα in primary and metastatic melanoma in vivo.The previously described resistance to TNFα cytolytic activity may, therefore, be clinically important.

Key words Malignant melanoma · TNFα · mRNA · Immunohistochemistry

Introduction

Tumor necrosis factor α (TNFα) was originally identified by its directly cytotoxic effect on some tumor cells [15, 18]. Production of TNFα has been detected in both activated monocytes [1, 8] and T cells [7], which possibly could participate in antitumor responses in vivo. In recent years, administration of cytokines, such as TNFα, has been used to increase antitumor responses in various animal models and in human studies (reviewed in [17, 21]). To investigate how TNFα affects tumor growth and rejection in vivo, transfer of TNFα genes into animal tumor cells has been carried out by several groups, with partially conflicting results. While certain TNFα-transfected, locally growing tumors, such as malignant melanoma, lymphomas or plasmacytomas, have been found to grow more slowly than parental cell lines or to be rejected, other TNFα-transfected tumors demonstrated an enhanced metastatic capacity [6, 16, 19]. Cell lines from human malignant melanoma have been reported to express TNFα mRNA and protein in vitro [4, 11, 12] and this expression has, in some clones, been associated with the presence of a mutated n-*ras* oncogene [3]. However, these lines have been selected for in vitro growth and may not be entirely representative of the biological variability of malignant melanoma in vivo. By the reverse transcriptase/polymerase chain reaction (RT-PCR) technique, TNFα mRNA was detected in a few metastatic melanoma lesions but the cellular source of the cytokine was not identified [9]. We therefore decided to look in situ for the production of this cytokine in tumor cells and infiltrating leukocytes of primary and metastatic melanoma of the skin. Since the primary tumors were small when diagnosed, optimal conditions for detection of TNFα mRNA in formalin-fixed and paraffin-embedded tissue were established.

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Materials and methods

Tissues

Formalin-fixed, paraffin-embedded tissues were obtained from the files of the Laboratory for Pathology, University Hospital, Linköping. To optimize the process of in situ hybridization for TNFα mRNA detection, a lymph node metastasis of malignant melanoma was used. The primary tumor from this metastasis and 12 additional primary malignant melanomas obtained between 1979 and 1995 were further studied. The diagnoses were established by using standard histological criteria (Table 1).

Immunohistochemical detection of TNFα protein

For TNFα staining a microwave method was used for antigen retrieval [20]. In short, 5-μm-thick tissue sections were cut, placed on positively charged glass microscopic slides (Superfrost/Plus, Gerhard Menzel

Table 1 Characterization of primary malignant melanomas. The age of the patient at the time of diagnosis is shown. The mitotic rate is the number of mitoses in ten high-power microscopic fields (magnification ×400)

No.	Age (years)	Sex	Localization	Type	Ulcerated	Breslow index (mm)	Clark level	Mitotic rate
1	63	F	Leg	SSM	No	0.5	III	<1
2	82	F	Shoulder	SSM	Yes	7.6	V	32
3	49	M	Leg	NMM	Yes	8	IV	17
4	57	F	Trunk	NMM	Yes	3.8	III	3
5	75	F	Foot	NMM	Yes	2.8	IV	5
6	69	F	Trunk	NMM	No	3.9	IV	3
7	83	M	Trunk	NMM	Yes	6	IV	21
8	40	M	Leg	NMM	Yes	3.6	III	30
9	69	F	Leg	NMM	No	1.8	IV	1
10	36	M	Trunk	NMM	Yes	3.1	IV	1
11	58	M	Arm	NMM	Yes	1.6	III	1
12	84	M	Trunk	NMM	No	4.0	IV	4
13	69	F	Arm	NMM	No	2.0	IV	0

Glasbearbeitungswerk GmbH, Braunschweig, Germany), deparaffinized by xylene and rehydrated through a graded series of ethanol concentrations to H_2O. Endogenous peroxidase was blocked by incubation of the slides with 3% H_2O_2 in H_2O for 5 min, followed by rinsing in tap water. Sections were slowly boiled for 10 + 5 min in a microwave oven in a 0.01 M citrate buffer, pH 6.0, and thereafter rinsed in phosphate-buffered saline (PBS). Sections were incubated in succession with mouse monoclonal anti-(human TNFα) antibody CY-014 (mouse IgG1, Innogenetics Laboratory, Belgium) diluted 1:200 in phosphate-buffered saline containing 1% bovine serum albumin (Sigma St. Louis, Mo. USA) (PBS/BSA) or, as a control, irrelevant mouse monoclonal IgG1 (Dakopatts) diluted 1:50 in PBS/BSA, and biotin-labelled rabbit anti-(mouse Ig) antibody (Dakopatts, Glostrup, Denmark) 1:200 in PBS with 4% human AB serum and peroxidase-conjugated streptavidin (Dakopatts) 1:500 in PBS/BSA for 30 min in a moist chamber at room temperature. Staining was developed using diaminobenzidine (Sigma) in phosphate buffer pH 7.6. Sections were counterstained using Mayers hematoxylin.

Probes

TNFα

A 0.8-kb *Eco*R1 cDNA fragment containing the entire human TNFα coding region (λ 42-4) [18] had been inserted into a Sp 64 vector containing the Sp6 promoter (Genentech Inc., San Francisco, Calif.). The template DNA was linearized at a *Pvu*II (Boehringer-Mannheim, Germany) site, generating a 185-bp segment containing bp 337–522 of TNFα. A Digoxigenin-UTP-labelled anti-sense RNA probe was prepared, by transcribing linearised template DNA using a Dig RNA Labeling kit Sp6/T7 (Boehringer-Mannheim) according to the manufacturer's instructions, and used at a concentration of 0.25 ng/ml hybridization buffer.

Positive control

Hybridization with a 21-nucleotide-long 3′-end digoxigenin-labelled oligo-(dT) probe (University of Chicago, Chicago, Ill.) was performed to ensure that mRNA was well preserved in the tissue.

Negative controls

No TNFα sense probe could be transcribed from the Sp 64 vector. Instead we used a 320-bp anti-sense RNA probe directed against human islet amyloid polypeptide (IAPP) [25] as a control probe on tissue sections of primary and metastatic melanoma to ascertain the specificity of the TNFα hybridization signals. As additional negative controls, sections were treated with RNase prior to hybridization or hybridized with buffer where the probe was excluded.

In situ hybridization for TNFα mRNA

Paraffin-sections 7 μm thick were cut, placed on PLL(Sigma)-coated slides, dried overnight at 50 °C, deparaffinized through sequential incubations in xylene (2×5 min), 100% ethanol (2×5 min), washed in 95% ethanol, 70% ethanol and finally H_20 and then rinsed in PBS. Sections were treated with 0.05% pepsin in 1 M HCl at 37 °C for 5 min, followed by post-fixation in 4% paraformaldehyde for 3 min. Sections were acetylated for 20 min in 0.1 M triethanolamine, pH 8, containing 0.25% acetic anhydride, rinsed in H_2O and pre-hybridized for 1 h at room temperature. The pre-hybridization and hybridization buffer contained 50% formamide, 5 × standard saline citrate (SSC), 10% (w/v) dextran sulfate, 5 × Denhardt's solution (100 × Denhardt's solution: 10 g Ficoll, 10 g polyvinylpyrrolidone and 10 g BSA in 500 ml H_2O), 2% sodium dodecyl sulfate (SDS) and 0.1 mg/ml sheared salmon sperm DNA. Hybridization with the digoxigenin-labelled probe was performed for 10–16 h at 50 °C, during which time the sections were covered by parafilm. After hybridization, the slides were washed four times for 5 min in 2 × SSC containing 0.1% SDS at room temperature and twice for 10 min in 0.1 SSC containing 0.1% SDS, rinsed in 2 × SSC and treated with 10 mg/ml RNase in 2 × SSC for 15 min at 37 °C. Thereafter the slides were washed in 2 × SSC, treated with 3% BSA in TRIS-buffered saline pH 7.5 for 30 min, and incubated with an alkaline-phosphatase conjugated anti-digoxigenin Fab fragment (Boehringer-Mannheim) diluted 1:500 in TRIS-buffered saline/BSA for 2 h. Slides were then washed in the same buffer for 10 min, rinsed in TRIS/saline pH 7.5 and finally washed in TRIS/saline pH 9.5 (0.1M TRIS pH 9.5, 0.1 M NaCl and 0.05 M $MgCl_2$) for 10 min. The reaction was visualized by incubating the slides for 45 min in darkness at room temperature in substrate solution: TRIS/saline pH 9.5 containing 2.5 mg/ml levamisole (Sigma, St. Louis, Mo.), 3.5 mg/ml nitroblue tetrazolium salt (Boehringer-Mannheim) and 1.7 mg/ml 5-bromo-4-chloro-3-indolyl-phosphatase (Boehringer-Mannheim). Slides were then rinsed in H_2O and mounted in Kaiser's water-soluble mounting medium containing glycerin/gelatin.

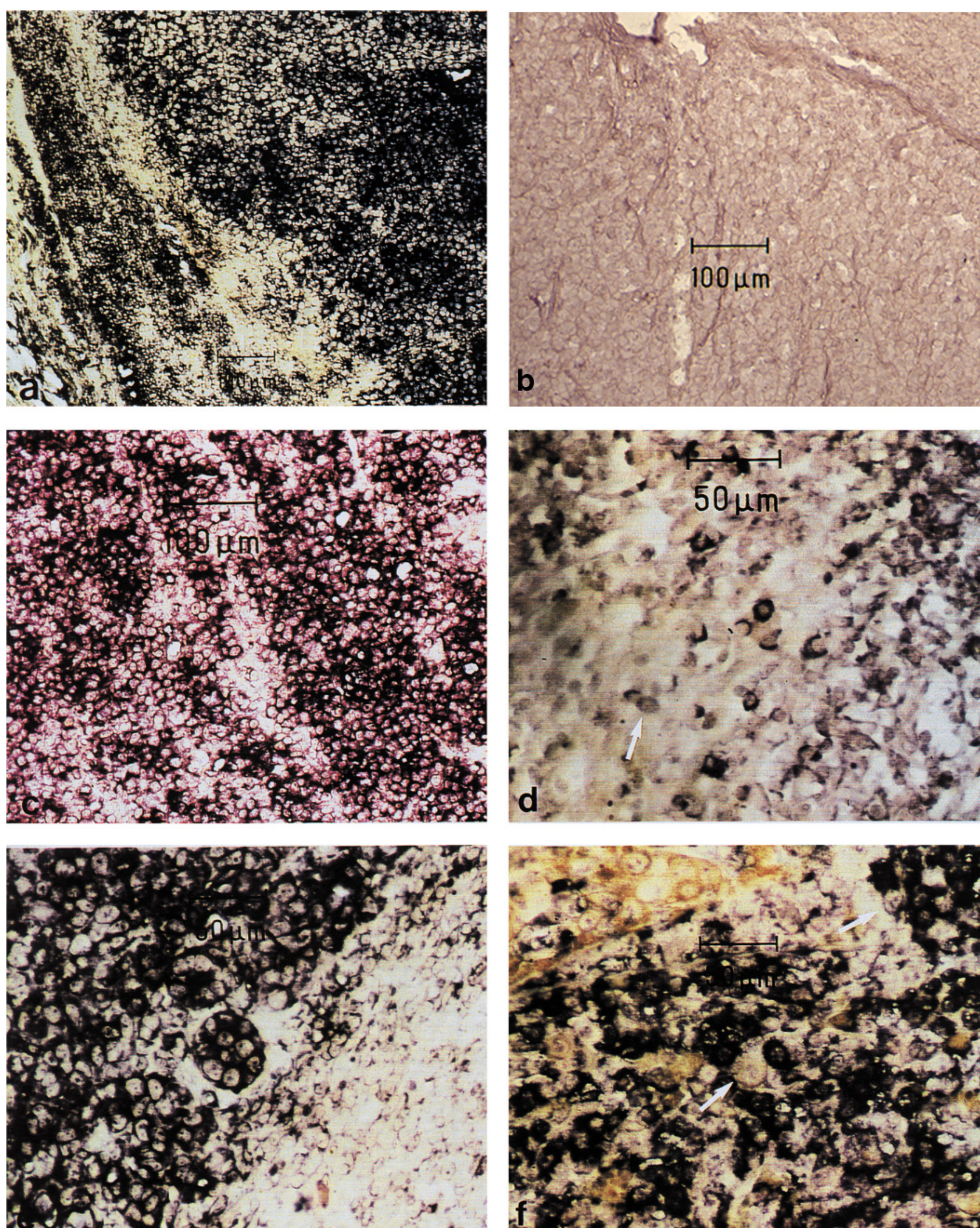
Hybridization with the oligo(dT) probe

The sections were pre-treated as above for TNFα except that the pepsin concentration was 0.25%. Pre-hybridization was performed with pre-hybridization buffer containing 10% dextran sulfate and 0.02 M sodium phosphate buffer in 1 × Denhart's solution for 1 h at room temperature. The hybridization buffer contained 3 × SSC, 10% dextran sulfate and 0.1 mg/ml sheared salmon sperm DNA in 1 × Denhart's solution. The digoxigenin-labelled probe was diluted to 4.5 ng/μl and hybridization was performed under Parafilm overnight at room temperature. After hybridization, sections were washed extensively in 2 × SSC and thereafter in 1 × SSC for 2 × 10 min, followed by 0.5 × SSC. Detection was done as for TNFα.

Results

TNFα mRNA detected in metastatic melanoma

To optimize the in situ hybridization technique on paraffin-embedded tissue a synchronous lymph node metastasis from patient no. 3 (Table 1) was used. Immunostaining for TNFα had demonstrated that this metastasis expressed TNFα protein. In order to increase the accessibility of the probes while still preserving the morphology of the tissue,



the de-paraffinized sections were partially enzyme-digested with pepsin before hybridization. In situ hybridization with a riboprobe for human TNFα showed that there were cells expressing mRNA for TNFα (Fig. 1a) both among lymphocytes (Fig. 1c, d) and among melanoma cells (Fig. 1e, f). However, among both lymphocytes and melanoma cells TNFα mRNA expression varied and negative cells could also easily be identified (Fig. 1d, f). RNase treatment completely abolished the signal in both cell types (data

Fig. 1a–f In situ hybridization of a lymph node metastasis of malignant melanoma (**a**–**e**) or a primary melanoma (**f**). Hybridization with an antisense tumor-necrosis-factor-α(TNFα)-specific RNA probe (**a**) revealed a strong histochemical reaction in lymphocytes (to the *left* in the photograph) as well as in melanoma cells (to the *right*). No specific signal was obtained after hybridization with an antisense islet-amyloid-polypeptide(IAPP)-specific probe of similar length (**b**). Higher magnification demonstrated that both lymphocytes (**c**, **d**) and melanocytes (**e**, **f**) contained TNFα mRNA, visualized as a brown staining of the cytoplasm. Note that among both lymphocytes (**d**) and melanocytes (**f**), both positive and negative cells (*white arrows*) were easily detected

Table 2 Detection of tumor necrosis factor α (*TNF*α) protein and mRNA in malignant melanoma and leukocytes. Intensity of TNFα signal by immunostaining or in situ hybridization for mRNA in malignant melanoma: *0* negative, *1* weak, *2* strong, *3* very strong. Number of TNFα+ granulocytes, lymphocytes or macrophages: – absent, (+) sparse, + moderate, ++ frequent

Patient	TNFα in melanoma		TNFα+ leukocytes	
	Protein	mRNA	Protein	mRNA
Primary melanoma				
1	1	1	+	++
2	2	2	++	++
3	1	1	+	+
4	0, (1)[a]	0, (1)	(+)	+
5	1	1	(+)	+
6	1	1	++	+
7	1	1	+	++
8	1	1	++	++
9	2	ND	(+)	ND
10	1	ND	(+)	ND
11	2	ND	++	ND
12	2	ND	++	ND
13	0	ND	+	ND
Metastatic melanoma				
3 (lymph node)	1	3	++	++
9 (cutaneous)	0–1	0	–	–
14 (lymph node)	2	3	++	++

[a] A small area of the melanoma was weakly positive in immunostaining and in in situ hybridization

not shown) and hybridization with buffer or irrelevant probes, such as that for human IAPP, was negative (Fig. 1b).

Variable expression of TNFα mRNA and protein in primary malignant melanoma

Thirteen cutaneous malignant melanomas (Table 1) with differing expression of TNFα protein, as determined by immunohistochemistry (Table 2), were selected. In all sections at least a few TNFα+ leukocytes were identified, serving as an internal positive control (Table 2). Keratinocytes were usually negative or only faintly stained for TNFα (data not shown).

In order to identify the source of the melanoma-cell-associated TNFα, it was necessary to evaluate the tumor cells for TNFα production. In 1 of the melanomas there was insufficient material for further investigations. In 4 of the tumors, the levels of total mRNA detected were very low, as assayed by in situ hybridization with the oligo(dT) probe, possibly because of degradation of mRNA in these samples. In the remaining 8 primary melanomas, TNFα mRNA was detected in melanoma cells and lymphocytes (Fig. 2, Table 2) as well as in keratinocytes and sweat gland epithelium (data not shown). The TNFα mRNA expression in the primary melanomas was variable, and in 1 of the tumors (tumor 4), the majority of cells were negative for TNFα mRNA while still strongly reacting with the oligo(dT) probe. In one melanoma (no. 3) hybridization with the oligo(dT) probe was weak and gave a clearly positive signal only in some areas, possibly because of degradation of mRNA in certain parts of the tumor. Therefore, in this melanoma only part of the tumor could be evaluated.

TNFα expression in metastatic melanoma

To investigate whether TNFα expression was also variable in metastatic lesions, two metastases, one lymph node and one subcutaneous metastasis, were selected. Immunohistochemical staining and in situ hybridization revealed different expressions of TNFα (Table 2). The lymph node metastasis was strongly TNFα+ while, in the cutaneous metastasis, both melanoma cells and the surrounding leukocytes were TNFα–. However, keratinocytes flanking the tumor nodule expressed TNFα mRNA but not protein, while keratinocytes overlying the metastasis were negative both in immunohistochemistry and in in situ hybridization. Hybridization with an oligo(dT) probe gave a strong signal, demonstrating that mRNA was still preserved in the tissue.

Discussion

Previous studies have demonstrated that human malignant melanoma cell lines produceTNFα in vitro, either spontaneously or after stimulation [4, 11]. We have now documented the expression of TNFα mRNA and protein in untreated primary and metastatic cutaneous melanoma in vivo. In general, there was a good correlation between mRNA and protein expression both in melanoma cells and in lymphocytes. However, in most samples, keratinocytes contained TNFα mRNA but no immunohistochemically detectable protein.

It has previously been demonstrated that the TNFα gene is transcriptionally active and TNFα mRNA is detectable by Northern analysis in thioglycollate-elicited mouse macrophages. This mRNA is not translated into protein, indicating that TNFα gene expression is regulated at the translational level in these cells [2]. Similar findings have been reported in vivo, both in rat [24] and in man [23], and could explain the discrepancy detected between TNFα mRNA and protein expression in keratinocytes.

One of the principal findings in this report is that TNFα production in melanoma is variable both within and between tumors. Tumors that were predominantly TNFα+ often contained a few cells that did not express TNFα mRNA. In other tumors TNFα expression was detectable in only a few surrounding leukocytes, while the melanoma cells were TNFα–. However, since the tumors analyzed in this study were not randomly selected, the frequency of TNFα-expressing primary melanomas could not be estimated. In a previous study, immunohistochemical staining of TNFα protein was demonstrated in approximately 2/3 of primary malignant melanomas of the nodular and superficial spreading subtypes [20]. Although it is difficult to judge precisely the sensitivity of the in situ hybridization and immunostaining procedures, the good correlation be-

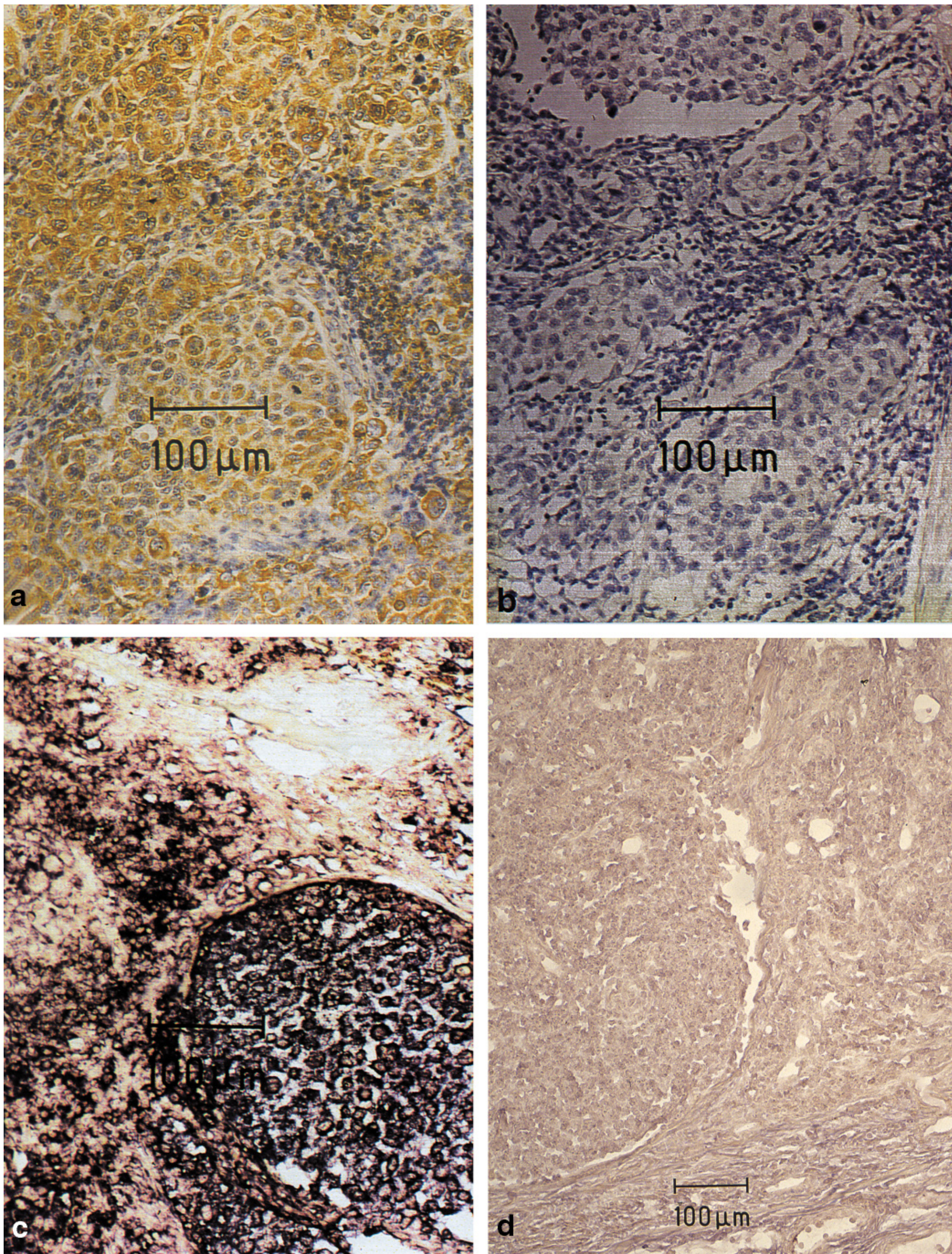


Fig. 2a–d Melanocytes and some of the small surrounding lymphocytes in primary malignant melanoma expressed TNFα protein (brown cytoplasmic staining) as assayed by immunohistochemistry using a TNFα-specific mAb (**a**) while control staining with an irrelevant subclass-matched control was negative (**b**). In situ hybridization with an antisense TNFα probe was strongly positive both in melanocytes and in some of the surrounding lymphocytes (**c**) while hybridization with an antisense IAPP probe was negative (**d**)

tween expression of mRNA and protein in the tumor cells strongly suggests that the observed variability is real.

The expression of TNFα may be important, since production of TNFα protein in cultured, transformed cells, such as malignant melanoma, has been reported to be associated with resistance to the cytolytic effects of TNFα [4, 22]. Several TNF-protective proteins, such as

manganous superoxide dismutase [26] and soluble TNF receptors, the so-called TNF-binding proteins, have been identified. It has recently been suggested that human malignant melanoma cells, in contrast to normal melanocytes, produce two types of TNF-binding proteins that both inhibit TNFα binding to TNF receptors and protect the cells from TNFα-mediated cytotoxicity [13]. Melanomas with high expression of TNFα may, therefore, be particularly resistant to immunotherapy that is directly or indirectly aimed at enhancing the TNFα concentration in the tumor.

In addition to its direct growth-regulatory effects on tumors, TNFα may facilitate tumor development, invasion and metastasis by promoting angiogenesis [10], stimulating breakdown of connective tissue [5] and inducing high expression of adhesion molecules such as VCAM-1 on endothelial cells, and its ligand VLA-4 on tumor cells [14]. In several animal tumor models, including murine melanoma, gene transfer of TNFα into the malignant cells has been reported to enhance experimental metastasis [14, 16, 19], while others have reported that TNFα transfection induced growth suppression or tumor rejection [6, 17, 19]. In this small study we were not able to investigate how TNFα production correlates to the clinical behavior of the tumor. Further prospective studies are planned to investigate this.

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References

1. Andersson U, Adolf G, Dohlsten M, Müller G, Sjögren H-O (1992) Characterization of individual tumor necrosis factor alpha and beta producing cells after polyclonal T cell activation. J Immunol Methods 123:233
2. Beutler B, Krochin N, Milsark IW, Luedke C, Cerami A (1986) Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science 232:997
3. Castelli C, Sensi M, Lupetti R, Mortarini R, Panceri P, Anichini A, Parmiani G (1994) Expression of interleukin 1α, interleukin 6, and tumor necrosis factor α genes in human melanoma clones is associated with that of a mutated N-RAS oncogene. Cancer Res 54:4785
4. Colombo MP, Maccalli C, Mattei S, Melani C, Radrizzani M, Parmiani G (1992) Expression of cytokine genes, including IL-6, in human malignant melanoma cell lines. Melanoma Res 2:181
5. Dayer JM, Beutler B, Cerami A (1985) Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and fibroblasts. J Exp Med 162:2163
6. Hock H, Dorsch M, Kundzendorf U, Qin Z, Diamantstein T, Blankenstein T (1993) Mechanisms of rejection induced by tumor cell-targeted gene transfer of interleukin 2, interleukin 4, interleukin 7, tumor necrosis factor or interferon γ. Proc Natl Acad Sci USA 90:2774
7. Kinkhabwala M, Sehejpal P, Skolnik E, Smith D, Sharma VK, Vlassara H, Cerami A, Suthanthiran M (1990) A novel addition to the T cell repertory. Cell surface expression of tumor necrosis factor/cachectin by activated normal human T cells. J Exp Med 171:941
8. Kriegler M, Perez C, De Fay K, Albert I, Lu SD (1988) A novel form of TNF/cachexin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 53:45
9. Lattime EC, Mastrangelo MJ, Bagasra O, Li W, Berd D (1995) Expression of cytokine mRNA in human melanoma tissue. Cancer Immunol Immunother 41:151
10. Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shiveley V, Nuseir N (1987) Macrophage-induced angiogenesis is mediated by tumor necrosis factor a. Nature 329:630
11. Lugassy C, Escande J-P (1991) Immunolocation of TNF-alpha/Cachectin in human melanoma cells: studies of co-cultivated malignant melanoma. J Invest Dermatol 96:238
12. Mattei S, Colombo MP, Melani C, Silvani A, Parmiani G, Herlyn M (1994) Expression of cytokine/growth factors and their receptors in human melanoma and melanocytes. Int J Cancer 56:853
13. Neuner P, Klosner G, Pourmojib M, Trautinger F, Knobler R (1994) Selective release of tumor necrosis factor binding protein II by malignant epidermal cells reveals protection from tumor necrosis factor a-mediated cytotoxicity. Cancer Res 54:6001
14. Okahara H, Yagita H, Miyake K, Okumura K (1994) Involvement of very late activation antigen 4 (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1) in tumor necrosis factor alpha enhancement of experimental metastasis. Cancer Res 54:3233
15. Old LJ (1985) Tumor necrosis factor. Science 230:630
16. Orosz P, Krüger A, Hubbe M, Rüschoff J, Von Hoegen P, Männel D (1995) Promotion of experimental liver metastasis by tumor necrosis factor. Int J Cancer 60:867
17. Pardoll D (1995) Paracrine cytokine adjuvants in cancer immunotherapy. Annu Rev Immunol 13:399
18. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynk R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV (1984) Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature 312:724
19. Qin Z, Krüger-Krasagakes, Kunzendorf U, Hock H, Diamantstein T, Blankenstein T (1993) Expression of tumor necrosis factor by different tumor cell lines results either in tumor suppression or augmented metastasis. J Exp Med 178:355
20. Sander B, Boeryd B (1996) Tumor necrosis factor α expression in human primary malignant melanoma and its relationship to tumor infiltration by $CD3^+$ cells. Int J Cancer 66:42
21. Schmidt-Wolf G, Schmidt-Wolf IGH (1995) Cytokines and clinical gene therapy. Eur J Immunol 25:137
22. Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA, Shepard HM (1985) Recombinant human tumor necrosis factor-α: effects on proliferation of normal and transformed cells in vitro. Science 230:943
23. Tovey MG, Content J, Gresser I, Gugenheim J, Blanchard B, Guymarho J, Poupart P, Gigou M, Shaw A, Fiers W (1988) Genes for IFN-β-2 (IL-6), tumor necrosis factor, and IL-1 are expressed at high levels in the organs of normal individuals. J Immunol 141:3106
24. Ulich TR, Guo K, Castillo J del (1989) Endotoxin-induced cytokine gene expression in vivo. Am J Pathol 134:11
25. Westermark GT, Christmanson L, Terenghi G, Permerth J, Betsholtz C, Larsson J, Polak JM, Westermark P (1993) Islet amyloid polypeptide: demonstration of mRNA in human pancreatic islets by in situ hybridization in islets with and without amyloid deposits. Diabetologia 36:323
26. Wong GHW, Kamb A, Elwell JH, Oberley LW, Goeddel DV (1992) MnSOD induction by TNF and its protective role. In: B. Butler (ed) Tumor necrosis factors: the molecules and their emerging role in medicine. Raven, New York, p 473