Induction of Monocyte Chemoattractant Protein-1 and Interleukin-10 by TGFβ1 in Melanoma Enhances Tumor Infiltration and Immunosuppression

Nancy Díaz-Valdés1, María Basagoiti1, Javier Dotor2, Fernando Aranda1, Iñaki Monreal1, José Ignacio Riezú-Boj1, Francisco Borrás-Cuesta1, Pablo Sarobe1, and Esperanza Feijoo2

Abstract

Melanoma progression is associated with the expression of different growth factors, cytokines, and chemokines. Because TGFβ1 is a pleiotropic cytokine involved not only in physiologic processes but also in cancer development, we analyzed in A375 human melanoma cells, the effect of TGFβ1 on monocyte chemoattractant protein-1 (MCP-1) and interleukin-10 (IL-10) expression, two known factors responsible for melanoma progression. TGFβ1 increased the expression of MCP-1 and IL-10 in A375 cells, an effect mediated by the cross-talk between Smad, PI3K (phosphoinositide 3-kinase)/AKT, and BRAF-MAPK (mitogen activated protein kinase) signaling pathways. Supernatants from TGFβ1-treated A375 cells enhanced MCP-1–dependent migration of monocytes, which, in turn, expressed high levels of TGFβ1, bFGF, and VEGF mRNA. Moreover, these supernatants also inhibited functional properties of dendritic cells through IL-10–dependent mechanisms. When using in vitro, the TGFβ1-blocking peptide P144, TGFβ1-dependent Smad3 phosphorylation, and expression of MCP-1 and IL-10 were inhibited. In vivo, treatment of A375 tumor–bearing athymic mice with P144 significantly reduced tumor growth, associated with a lower macrophage infiltrate and decreased intratumor MCP-1 and VEGF levels, as well as angiogenesis. Finally, in C57BL/6 mice with B16-OVA melanoma tumors, when administered with immunotherapy, P144 decreased tumor growth and angiogenesis. These results show new effects of TGFβ1 on melanoma cells, which promote tumor progression and immunosuppression, strongly reinforcing the relevance of this cytokine as a molecular target in melanoma.

Cancer Res; 71(3); 812–21. ©2011 AACR.

Introduction

TGFβ1 is a pleiotropic cytokine that plays a relevant role in the progression of cancer due to its effect on cell proliferation, angiogenesis, epithelial-to-mesenchymal transition, and metastasis (1). Also, it has been described that TGFβ1 induces the expression of integrins and alters the composition of extracellular matrix by activating the production of matrix metalloproteinases, which favor the invasion of tumor cells. Moreover, TGFβ1 enhances angiogenesis by activating the expression of proangiogenic factors such as interleukin (IL)-8 and VEGF (2). This molecule also exerts immunoregulatory functions by inhibiting proliferation, activation, and differentiation of lymphocytes (3) as well as the function of natural killer (NK) cells (4) and dendritic cells (DC; ref. 5), which leads to the suppression of the antitumor immune response.

TGFβ1 has been described as an important factor in the development of melanoma. Although it has antiproliferative effects on premalignant cells, as that occurs in normal melanocytes, advanced melanoma cells become resistant to this suppressive effect and they can use TGFβ1 to enhance their invasive and metastatic phenotype (6). Tumor cells evade the suppressive effect of TGFβ1 through receptor- or Smad-inactivating mutations (7, 8) or by downstream signaling alterations. These modifications avoid the suppressive pathway and induce prometastatic processes (9). Although no genetic mutations of TGFβ1 classical signaling molecules have been identified in melanoma, a cross-talk between the Smad signaling pathway and other pathways, including Ras/MEK [MAP/ extracellular signal regulated kinase (ERK) kinase]//ERK, and Akt, which are constitutively activated in a large percentage of cutaneous melanomas, has been described (2, 10).

The majority of malignant melanoma cells overexpress different growth factors and cytokines, which enhance growth rate and invasiveness by their autocrine and paracrine effects.
and stimulated with TGF-β and IL-10. In some experiments, TGF-β was supplemented with 10% FBS and antibiotics. After preincubation at 37°C for 30 minutes before the addition of TGF-β, Expression of phosphorylated forms of Smad3, AKT, and ERK1/2 induced by TGF-β (2 ng/mL) was assessed by Western blotting in A375 lysates from cells cultured in 6-well plates (3 × 10⁵ per well) in serum-free DMEM for 1 hour. In some experiments, TGF-β was preincubated at 37°C for 1 hour with P144 or control peptide (50–200 μg/mL).

Generation of melanoma conditioned medium from stimulated A375 cells
Melanoma conditioned media from A375 cells treated with or without TGF-β (TGF-β-MCM and MCM, respectively) used in functional in vitro experiments with monocytes and monocyte-derived DCs were produced by culturing A375 cells in 6-well plates (2.5 × 10⁵ per well) in DMEM with or without TGF-β (2 ng/mL) for 24 hours. Then, cells were washed twice with medium to remove TGF-β and maintained in culture for an additional 48 hours.

Western blot analysis
One hour after cell stimulation with TGF-β as described earlier, cells were washed with PBS, lysed with RIPA (radio-immunoprecipitation assay) buffer in the presence of protease and phosphatase inhibitors (Roche Diagnostic GmbH), and precipitated proteins (30 μg) were resolved on 10% SDS-polyacrylamide gels. After transference onto nitrocellulose membranes (Bio-Rad), nonspecific binding was blocked with 10% nonfat milk in 10 mmol/L Tris-buffered saline containing 0.1% Tween 20. Phosphorylated Smad3, AKT, and ERK1/2 were detected using rabbit polyclonal antibodies anti-phospho Smad3 (1:1,000; Chemicon), AKT (1:1,000; Cell Signaling), and MAP kinase 1/2 (ERK1/2; 1:1,000; Cell Signaling). Actin was also analyzed for control evaluation with rabbit anti-actin (1:2,000; Sigma-Aldrich) antibodies. Finally, an ECL anti-rabbit IgG linked to horseradish peroxidase (Amersham Biosciences) plus the Lumi-Light Western Blotting Substrate (Roche Diagnostic GmbH) was used to develop the proteins bands in films scanned with the Curix-60 (AGFA).

Monocyte migration assay
Monocytes were obtained from peripheral blood mononuclear cells by positive selection with CD14-coated beads (Miltenyi). In all cases, peripheral blood mononuclear cells were obtained from healthy donors after informed consent, following a protocol in accordance with the Declaration of Helsinki. Then, monocytes (5 × 10⁴) were plated in 100 μL of complete medium (RPMI 1640 supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 mg/mL streptomycin) in the top chamber of 5-μm pore membrane Transwells (Costar), with 5 μg/mL rat anti-human MCP-1 antibody (R&D Systems) or rat IgG control isotype (BD-Pharmingen) when required. The bottom chamber was filled with 500 μL of DMEM, MCM, or TGF-β1-MCM. In some cases, melanoma conditioned media were incubated with...
anti-human MCP-1 antibody or rat IgG for 1 hour at 37°C prior to the addition of media to Transwell plates. After 4 hours, the number of monocytes in the lower chamber was counted by flow cytometry as described (18).

**Culture of DCs in TGFβ1-MCM**
Monocyte-derived DCs obtained as described (18) were cultured in 96-well plates (10⁵ in 200 μL per well) in RPMI with MEM, MCM, or TGFβ1-MCM (proportion 4:1) for 6 hours. Then, DCs were stimulated with lipopolysaccharide (LPS; 1 μg/mL, Sigma-Aldrich) and after 24 hours, production of TNFα and IL-12 was measured in culture supernatants by ELISA. The role of IL-10 was assessed by preincubating MCM with rat anti-human IL-10 monoclonal antibody (Bioscience) or control isotype (BD-Pharmingen; 1 μg/mL) before their addition to DC cultures.

**Mixed leukocyte reaction**
Mixed lymphoblast reactions (MLR) were carried out by stimulating in 96-well plates nonadherent (CD14−) cells (2 × 10⁵ per well) obtained from healthy donors with 10⁴ allogeneic DCs for 5 days. DCs were previously matured with LPS (1 μg/mL) in the presence of A375 MCM (4:1) or TGFβ1-MCM (4:1) for 24 hours. Cells were pulsed with 0.5 μCi per well of [³H]thymidine for the last 18 hours and proliferation was measured. To measure the production of IFNγ by stimulated lymphocytes, 100 μL of culture supernatants was harvested on day 3.

**ELISA**
Human or murine TGFβ1, MCP-1, IL-10, VEGF, TNFα, IL-12, and IFNγ from tumor homogenates, serum, or supernatants of DC, NK, or T-cell cultures were measured by ELISA kits (BD Biosciences) following the manufacturer instructions.

**RT-PCR**
Monocytes and DC (1 × 10⁷/200 μL per well) were cultured for 24 hours in 96-well plates in RPMI with MEM, MCM, or TGFβ1-MCM (proportion 1:1). Total RNA extraction from cultures and real-time PCR were carried out as described (19) using specific primers for each gene (Table 1). Results were normalized according to β-actin. The amount of each transcript was expressed by the formula: 2^−ΔΔCt [ΔΔCt = Ct (β-actin) – Ct(gene)].

### Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primer</th>
<th>Antisense primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβ1</td>
<td>TGCTAATGTTGAAACCCAC</td>
<td>ATCCGCCAGGAATTTGCTG</td>
</tr>
<tr>
<td>bFGF</td>
<td>ACCGTACCCTGGACTGAG</td>
<td>CTTGTTATTTCTCGAGG</td>
</tr>
<tr>
<td>VEGF</td>
<td>CTGCTGCTTGGGTGACATTG</td>
<td>CACCGCTCCGGCTGTCA</td>
</tr>
<tr>
<td>COX-2</td>
<td>CAAAACCTGGGAAAGCTTCT</td>
<td>CCTCGCTTATGATGTCTCTG</td>
</tr>
<tr>
<td>PDL-1</td>
<td>ACAAGCACGTGACCATGCAAG</td>
<td>AGAGTGTCTGGGATGAC</td>
</tr>
<tr>
<td>IDO</td>
<td>TGGCACACGCCTATGGAAC</td>
<td>ATGCATTCCAGAACTAGAC</td>
</tr>
</tbody>
</table>
days later, plugs were dissected and hemoglobin content was measured as described (21). In some experiments, mice received 35 μg of anti-MCP-1 neutralizing antibodies (R&D Systems) or isotype control (BioXcell) 1, 4, and 6 days after tumor inoculation.

**IFNγ production by murine T cells**

IFNγ production by T cells was measured by ELISA after a 2-day stimulation in 96-well plates of splenocytes (4 × 10⁵) with the CD8 epitope OVA(257–264; 1 μg/mL).

**Statistical analysis**

Data were analyzed using the nonparametric Mann–Whitney test with the GraphPad software program. Survival curves of animals treated with different protocols were plotted according to the Kaplan–Meier method and were compared using the log-rank test. Statistical significance was considered when \( P < 0.05 \).

### Results

**TGFβ1 increases MCP-1 and IL-10 expression in A375 cells by the cross-talk between Smad, AKT, and MAPK/ERK signaling pathways**

To study the effect of TGFβ1 on MCP-1 and IL-10, two known factors associated with melanoma progression, A375 melanoma cells were treated with this cytokine. Although this cell line does not produce TGFβ1 in vitro (data not shown), we found that it can be detected both in A375 tumor homogenates (181 ± 77 pg/mg of protein) and in the serum of A375 tumor-bearing athymic mice (13.5 ± 2.3 ng/mL vs. 7.3 ng/mL in control mice). Although MCP-1 was constitutively expressed by A375 cells, the addition of TGFβ1 significantly increased its expression after 48 hours of culture (Fig. 1A). No detectable expression was found in untreated cultures in the presence of IL-10, whereas a clear induction was observed at 24 and 48 hours in the presence of TGFβ1 (Fig. 1B).

![Figure 1. Effect of TGFβ1 on the expression of MCP-1 and IL-10 in A375 melanoma cells.](image-url)

A and B, A375 cells (3 × 10⁴ per well) were stimulated with TGFβ1 (2 ng/mL) during 24 and 48 hours. Supernatants (n = 6 per group) were then collected to analyze MCP-1 and IL-10 concentrations by ELISA. Results are expressed as the mean ± SEM. **, \( P < 0.01 \).

C, A375 melanoma cells were stimulated with TGFβ1 (1 ng/mL) for 1 hour and phosphorylation of Smad3, AKT, and ERK1/2 was determined by Western blotting. Actin expression was analyzed as the control. D, effect of inhibiting Akt (LY294002), ERK (U0126), and Smad3 (SIS3) on the production of MCP-1 and IL-10 in A375 cells stimulated with TGFβ1 (1 ng/mL). DMSO was used as a control. Results are expressed as the mean ± SEM. *, \( P < 0.05 \); **, \( P < 0.01 \). One representative experiment of at least 3 independent experiments is shown.
To characterize signaling pathways involved in the induction of these factors, Western blot experiments were carried out to measure phosphorylation of Smad3 as well as AKT and ERK1/2 kinases. It was found that in A375 cells, TGFβ1-induced phosphorylation of the canonical pathway molecule Smad3 but no increase in phosphorylated AKT and ERK kinases over basal constitutive levels was observed (Fig. 1C). However, by using specific inhibitors of Smad3 (SIS3), PI3K/AKT (LY294002), and MAPK/ERK (U0126) during stimulation experiments, it was found that MCP-1 production was dependent on all 3 molecules whereas IL-10 production depended on Smad3 and MAPK/ERK-related pathways (Fig. 1D).

**TGFβ1 enhances MCP-1–mediated monocyte migration**

Because TGFβ1 increased the production of MCP-1 in A375 cells, we tested whether MCM from A375 cell cultures could enhance monocyte migration. *In vitro* Transwell experiments showed that TGFβ1-MCM from A375 cells had a higher chemoattractant activity on monocytes than that observed with MCM from untreated A375 cells or complete medium (DMEM), an MCP-1–dependent effect (Fig. 2A). To evaluate the relevance of an increase in intratumor monocyte infiltration mediated by TGFβ1, we measured the expression of VEGF and bFGF in monocytes, two essential factors involved in the progression of melanoma. Interestingly, MCM and TGFβ1-MCM induced a similar increase in mRNA expression of both proangiogenic factors (Fig. 2B and C). Moreover, culture supernatants from untreated or TGFβ1-stimulated A375 cells increased the expression of TGFβ1 mRNA in monocytes (Fig. 2D). Thus, although TGFβ1 does not directly enhance the ability of monocytes to produce angiogenic factors, it may enhance the proangiogenic environment by increasing the number of infiltrating monocytes, a similar phenomenon as that described for smooth muscle cells (22, 23).

**Inhibition of DC functions by TGFβ1-induced IL-10**

Although TGFβ1-induced IL-10 may have an autocrine stimulatory effect on melanoma cells by increasing their proliferation (24), it may also behave as a suppressor factor,
mainly for antigen-presenting cells. We thus measured the effect that the addition of TGF\(\beta\)1 to tumor cells had on the activation of DCs, a cell population that links innate and adaptive immunity and activates antitumor immunity. An inhibition on LPS-induced TNF\(\alpha\) and IL-12 production was observed after pretreatment of DCs with MCM, which was stronger when using TGF\(\beta\)1-MCM, as compared with culture medium (Fig. 3A). The inhibitory effect of TGF\(\beta\)1-MCM was restored by anti-IL-10 blocking antibodies, confirming the important role of IL-10 as a suppressive factor in TGF\(\beta\)1-MCM.

Similarly, TGF\(\beta\)1-MCM induced upregulation of immunosuppressive molecules such as enzymes COX-2 and IDO (indoleamine-2,3-dioxygenase) and the membrane molecule PD-L1 (Fig. 3B). In this case, upregulation of COX2 and PD-L1 was dependent on IL-10 whereas IDO expression depended on other TGF\(\beta\)1-induced unknown factors.

We finally studied the ability of these DCs to activate T lymphocytes. Although DCs treated with MCM, with or without TGF\(\beta\)1, had a lower capacity to induce allogeneic T-cell proliferation, a higher inhibition in IFN\(\gamma\) production was observed when using DCs treated with TGF\(\beta\)1-MCM.
(Fig. 3C), in agreement with the lowest levels of IL-12 produced by these DCs.

**In vitro and in vivo antitumor effects of the TGF-β1 inhibitor peptide P144**

Because TGFβ1 plays an important role in the induction of factors relevant for melanoma progression, we analyzed the *in vitro* and *in vivo* effects of the TGFβ1 inhibitor peptide P144 (17). *In vitro* analysis of the classical TGFβ1 signaling pathway showed that phosphorylation of Smad3 in A375 cells was decreased after treatment with P144 (Fig. 4A). Accordingly, TGFβ1-induced MCP-1 and IL-10 production in A375 cell cultures was specifically inhibited by this peptide (Fig. 4B).

Because of the inhibitory effects that P144 had on A375 cells *in vitro*, we tested this peptide in *in vivo* tumor models. We first implanted A375 cells subcutaneously in athymic mice, and when tumor diameter reached 5 mm, animals were given P144 injections daily for 3 weeks. TGFβ1 is detected both in the serum of these animals and in tumor homogenates, suggesting that this cytokine could be relevant in this *in vivo* model and susceptible to be considered a target. A significant delay in tumor growth and enhanced survival was observed in those mice treated with P144 (Fig. 5A). They also had a lower number of tumor-infiltrating macrophages, associated with lower MCP-1 levels (Fig. 5B). A similar decrease in MCP-1 levels was also observed in the serum (Supplementary Fig. S1A). Moreover, treatment with P144 reduced intratumor VEGF levels (Supplementary Fig. S1B) and inhibited tumor angiogenesis, measured as a hemoglobin content (Fig. 5C) or CD31 staining (Supplementary Fig. S1C). Finally, *in vivo* inhibition of MCP-1 with neutralizing antibodies decreased tumor angiogenesis, showing the role of this chemokine in this tumor model (Fig. 5D).
The immunosuppressive effect of TGFβ1 was studied in immunocompetent C57BL/6 mice bearing B16-OVA melanoma tumors, in which the production of TGFβ1 (25) and IL-10 (26) has been reported. Although treatment of tumor-bearing mice with OVA antigen plus adjuvants poly(I:C) and anti-CD40 delayed tumor growth, combination of this treatment with P144 promoted a higher tumor growth inhibition (Fig. 6A). This decreased tumor growth was associated with lower intratumor IL-10 (Fig. 6B). The analysis of innate immunity in the spleen of tumor-bearing mice showed that P144 enhanced the proportion of activated CD86+ DCs induced by immunotherapy, as well as the activity, measured as IFNγ production, of NK cells (Supplementary Fig. S2). Moreover, splenic T cells from P144-treated mice produced higher IFNγ levels after stimulation with the CD8+ epitope OVA(257–264) (Fig. 6C). This enhanced activation of innate and adaptive immunity resulted in higher intratumor levels of IFNγ (Fig. 6D).

Discussion

In the present study, we showed that TGFβ1 enhances the production of MCP-1 and IL-10 by A375 melanoma cells, two factors associated with the progression of this malignancy (27). It has been described that MCP-1 increases tumor-associated monocyte/macrophages, leading to a higher degree of angiogenesis (28). Moreover, IL-10 has been detected as elevated levels in sera from patients with melanoma and is associated with a shorter survival rate in advanced melanoma patients (16). However, to our knowledge, the present work is the first to report the induction of MCP-1 and IL-10 by TGFβ1 in melanoma cells. Preliminary experiments, carried out using primary tumor lines obtained from patients with metastatic melanoma, have also shown an increase in MCP-1 induction by TGFβ1 (E. Feijoo, unpublished results), which confirms the clinical relevance of this finding.

Cross-talk between different signaling pathways has been described to explain the effect of TGFβ1 on different factors related to cancer progression. Besides the TGFβ1-Smad pathway, several groups have also observed the TGFβ1-mediated activation of Smad-independent pathways (29–31). We have determined that TGFβ1-dependent Smad3 phosphorylation is responsible for the MCP-1 increase and IL-10 induction in A375 cells. Because IL-10 is induced by TGFβ1 and it has been previously described that MAP/ERK signaling pathway was implicated in the expression of IL-10 mRNA in A375 cells (32), we hypothesized that this signaling pathway could interact with the Smad3 signaling pathway induced by TGFβ1. We have shown that the MAP/ERK1/2 pathway, which is constitutively activated in this melanoma cell line due to the BRAFV600E mutation, a mutation detected in 66% of melanomas (33), is not activated by TGFβ1. However, using specific inhibitors, we found that both Smad3 and MAP/ERK1/2 pathways were essential for the expression of IL-10. We have also found that A375 cells show constitutive expression of IL-8, a factor dependent on the MAPK/ERK1/2 pathway but independent on TGFβ1 (data not shown). Because PI3K/AKT signaling supports the survival of melanoma cells (34) and has been implicated in the progression of human melanoma (35), we tested the role of this pathway in the production of MCP-1 and IL-10. Even though TGFβ1 has no effect on the phosphorylation of AKT, by using a specific inhibitor of PI3K, we observed that MCP-1 production by A375 cells is triggered by this signaling pathway as well as by the Smad3 and MAPK/ERK signaling pathways. These results suggest the existence of a downstream cross-talk between Smad3, PI3K/AKT, and MAP/ERK1/2 signaling pathways in the expression of MCP-1 and IL-10 in A375 melanoma cells.

Besides the direct effects of TGFβ1 on tumor cells, our studies show new indirect mechanisms induced by TGFβ1 that facilitate melanoma progression. Indeed, TGFβ1-dependent upregulation of MCP-1 resulted in a higher attraction of monocytes, which have been conditioned by tumor cells to...
produce proangiogenic factors VEGF and bFGF, suggesting that TGFβ1 may promote mechanisms that not only favor monocyte infiltration but also condition these cells toward a phenotype that facilitates melanoma progression. There are a number of publications indicating the relevant role of macrophage polarization from M1 to M2 in tumor progression (36, 37). According to this, our results support that monocytes could enhance tumor growth directly by the production of growth factors such as bFGF (38) and VEGF (39), and, indirectly, by their proangiogenic effect (13). This possibility seems to be in agreement with a previous work showing that MCP-1 blockade prevented angiogenesis and tumor growth in human malignant melanoma (40). Moreover, the induction of TGFβ1 mRNA in monocytes cultured with A375 melanoma conditioned medium might suggest that tumor-infiltrating monocytes could be a source of this cytokine in patients with melanoma. As it has been described in this work, this TGFβ production may constitute a positive loop that further enhances tumor progression.

Consistent with the immunosuppressive effect of IL-10, we have shown that TGFβ1-MCM induced an IL-10-dependent functional impairment of dendritic cells, characterized by a lower production of proinflammatory factors TNFα and IL-12 and the upregulation of immunoregulatory molecules PDL-1 and COX-2. Moreover, we have also observed that TGFβ1-MCM induced other suppressive factors such as IDO in DCs, which may cause their impairment, although in this case, it was independent of the production of IL-10. As a result, a decreased ability to activate IFNγ-producing T lymphocytes was observed in DCs cultured with TGFβ1-MCM. Thus, these results show that in melanoma, TGFβ1 not only acts on DCs through the already described direct immunosuppressive mechanism (5) but also through indirect pathways mediated by IL-10 and other unknown factors which will enhance the final negative effect on this important cell population.

The multifaceted effect of TGFβ1 on tumor progression suggests that inhibition of this cytokine should be considered when planning therapeutic strategies in melanoma patients. Indeed, we have reported that inhibition of this cytokine in combination with immunotherapy had some beneficial effects in a murine melanoma model (41). Because we found several mechanisms induced by TGFβ1 on A375 cells favoring tumor progression, we tested the effect of the TGFβ1 inhibitor peptide P144. In vitro assays showed that P144 inhibited TGFβ1-induced Smad3 phosphorylation and MCP-1 and IL-10 expression in A375 cells cultures. More important, in vivo administration of P144 in two melanoma models significantly delayed tumor growth. In athymic mice bearing A375 tumors, P144 treatment decreased macrophage infiltrate, intratumor MCP-1, and VEGF production as well as tumor angiogenesis, reinforcing the role of TGFβ1 and MCP-1 on this tumor growth mechanism. Moreover, in immunocompetent mice, P144 downregulated intratumor IL-10 expression, associated with stronger innate and adaptive immunity. These data show new roles of TGFβ1 in the progression of melanoma, such as those responsible for the induction of MCP-1 and IL-10. They suggest that its inhibition in combination with other therapies could be indicated in patients with metastatic melanoma in accordance with recently published data showing that inhibition of TGFβ1 enhances the efficacy of immunotherapy (42–44).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Edurne Elizalde, Uxue Latasa, Lucia Martinez, Virginia Belsue, Verónica Fernández, and Laura Guembe for their excellent technical assistance with RT-PCR, Western blotting, and immunohistologic analysis, and Matías Avila and Juan José Lasarte for their interesting discussion.

Grant Support

This work was supported by ‘UTE project CIMA’ to N. Díaz-Valdés, M. Basagoiti, I. Monreal, J.I. Riezu-Boj, F. Borras-Cuesta, and P. Sarobe.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 23, 2010; revised November 4, 2010; accepted November 18, 2010; published OnlineFirst December 15, 2010.

References

Induction of MCP-1 and IL-10 by TGFβ1 in Melanoma


Induction of Monocyte Chemoattractant Protein-1 and Interleukin-10 by TGF β1 in Melanoma Enhances Tumor Infiltration and Immunosuppression

Nancy Díaz-Valdés, María Basagoiti, Javier Dotor, et al.