Abstract

Purpose: Identification of regional node metastasis is important for accurate staging and optimal treatment of early melanoma. We hypothesize that the nodal profile of immunoregulatory cytokines can confirm the identity of the first tumor-draining regional node, i.e., the sentinel node (SN) and indicate its tumor status.

Experimental Design: RNA was extracted from freshly dissected and preserved nodal tissue of 13 tumor-negative SNs, 10 tumor-positive SNs (micrometastases <2 mm), and 11 tumor-negative non-SNs (NSN). RNA was converted into cDNA and then amplified by PCR. Expression of 96 cytokines and chemokines was assessed using cDNA microarray and compared by using hierarchical clustering.

Results: Fifty-seven genes were expressed at significantly ($P < 0.05$) different levels in SNs and NSNs (4 genes had higher expression, and 53 genes had lower expression in SNs). Expression levels of interleukin-13 (IL-13), leptin, lymphotoxin $\beta$ receptor (LT$\beta$R), and macrophage inflammatory protein 1b (MIP1b) were significantly higher ($P < 0.04$, $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively), and expression level of IL-11Ra was lower ($P < 0.03$) for tumor-positive as compared with tumor-negative SN. Receiver-operator characteristics curve analyses showed that the area under the curve (AUC) for IL-13, leptin, LT$\beta$R, MIP1b, and IL-11Ra was 0.79, 0.83, 0.75, 0.81, and 0.77, respectively. The AUC for the five genes in combination was 0.973, suggesting high concordance of gene-expression profiles with SN staging.

Conclusions: SNs have a different immunoregulatory cytokine profile than NSNs. The cytokine profile of tumor-positive SNs; increased expression of IL-13, leptin, LT$\beta$R, and MIP1b and decreased expression of IL-11Ra, may provide clues to the local tumor lymph node interaction seen in the earliest steps of melanoma metastasis.
micrometastasis could be characterized by a distinct cytokine profile.

Unlike direct markers of nodal metastasis, which can only be identified in tumor foci, a marker based on a tumor’s microenvironment cytokine profile may be identified in any section of a node that contains micrometastasis. In this study, we used a cDNA microarray of 96 immunoregulatory genes to assess the gene expression profile of SNs and non-SNs (NSNs) from patients with clinically localized melanoma.

**Materials and Methods**

**Patient population and tissue collection.** Nodal specimens were obtained intraoperatively from 49 patients who underwent wide local excision. SN biopsy, and removal of an adjacent NSN. A total of 28 patient samples were for cDNA microarray study, and an additional 21 patient samples were for quantitative real-time reverse transcription-PCR (qRT-PCR) for confirmation and verification of microarray data. The protocol was approved by the Institutional Review Board of Saint John’s Health Center/John Wayne Cancer Institute, and all specimens were obtained after patients had provided written informed consent. No patient had a history of myeloproliferative disease or primary or secondary immunodeficiency.

One to four hours before surgery, patients underwent injection of 99mTc-labeled sulfur colloid for lymphoscintigraphic identification of the nodal basin at risk for metastasis. During intraoperative lymphatic mapping, each SN was identified by observing the path of the blue dye (Lymphazurin, Tyco International) and by using a hand-held gamma counter (Intra Medical Imaging) to measure radioactive counts over the nodal basin as previously described (8, 9). A single SN (nonblue and nonradioactive) within 2 cm of the SN was also removed (5). All SNs and NSNs were evaluated for tumor by staining with H&E and by immunohistochemical staining with antibodies against S-100, HMB-45, and MART-1. All NSNs were obtained from stage II patients. None of the NSNs contained melanoma metastasis.

Data obtained from operative and pathology reports included patient's age and gender, histopathologic characteristics of the primary melanoma, and the presence of postbiopsy residual melanoma (PRM), defined as a pathologically positive margin from the initial diagnostic biopsy specimen of the primary melanoma, pathologically identifiable residual melanoma in the wide excision specimen, or a tumor-positive SN (6).

**Tissue processing and RNA extraction.** Tangential peripheral portions of freshly collected SNs and NSNs were placed immediately in RNAlater (Qiagen) for RNA preservation. RNA from each tissue specimen was extracted and purified using RNeasy kit (Qiagen) according to the manufacturer’s instructions. The initial RNA concentration and quality were assessed by optical densitometry at 260 and 280 nm. The final concentration, quality, and purity of total RNA were determined by using the RNA 6000 Nano Assay Kit on the Agilent 2100 Bioanalyzer (Agilent Technologies).

**Cell culture and RNA extraction.** Four melanoma cell lines were obtained to verify cytokine/chemokine expression profile of pure melanoma cells. Human CRL-7425 and CRL-7426 cells were obtained from the American Type Culture Collection. CRL-7425 was derived from a primary tumor, and CRL-7426 was derived from an inguinal lymph node metastasis obtained from the same patient. Human IGR-39 and IGR-37 cells were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. IGR-39 was derived from a primary amelanotic cutaneous tumor, and IGR-37 was derived from an inguinal lymph node metastasis in the same patient. CRL-7425 and CRL-7426 cell lines were grown in 90% DMEM with 4 mmol/L l-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, and 10% fetal bovine serum. IGR-39 and IGR-37 cell lines were grown in 85% DMEM with 4 mmol/L l-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, and 15% fetal bovine serum. All cell lines were grown in a humidified atmosphere of 5% CO2 until growth was 90% confluent. Cells were then washed twice with PBS, and RNA was extracted using RNeasy kit (Qiagen) according to the manufacturer’s instructions.

**Functionally focused cDNA microarray.** GEArray Q series–Human inflammatory Cytokines & Receptors Gene Array kits (SuperArray) were used per manufacturer’s instructions, with minor modifications as described by Liu et al. (10). Each Q series array evaluates the expression of 96 functionally focused and relevant genes; the array includes 10 positive controls, 3 blanks, and 3 negative controls. Each gene-spot is subspotted four times to assure uniform hybridization. This cDNA microarray platform has been cross-validated with RT-PCR by the manufacturer. Typically, 2.5 μg of extracted and purified RNA was reverse-transcribed into cDNA and then amplified into biotinylated (biotin-16-UTP. Roche) cDNA by LPR amplification protocol. The resultant biotinylated cDNA was placed on a microarray membrane that had been prehybridized with heat-denatured ultrapure salmon sperm DNA (Invitrogen) and continuously digested at 10 rpm overnight at 60°C. The next day, the hybridized cDNA was washed and bound to alkaline phosphatase–conjugated streptavidin, both supplied by the manufacturer. The expression levels of individual genes were detected by Molecular Dynamics Storm 860 imaging station (Amersham Biosciences). The photofluorescence signal intensities, which correspond to the quantity of cDNA bound to the array, were analyzed by using ScanAnalyze v.2.50 image analysis software (Lawrence Berkeley National Laboratory). Each gene-expression level was normalized to β-actin, which was chosen due to highly consistent and uniform intersample expression levels.

**Synthesis of plasmid controls to validate microarray data.** Sample RNA was processed by RT-PCR and 2% agarose gel electrophoresis; the cDNA was extracted using the QiAquick gel extraction method (Qiagen). CD3, Melan A, interleukin-13 (IL-13), leptin, lymphotoxin β receptor (LTbR), macrophage inflammatory protein 1b (MIP1b), and IL-11Ra cDNA were ligated into pCR II-TOPO cloning vector (Invitrogen); the cDNA clones were transformed into Escherichia coli DH5α cells, and cultures were expanded as described elsewhere (11). We selected CD3 to verify T-cell infiltration in lymph nodes and Melan A to avoid the possibility of our using a portion of NSN and SN samples contained the melanoma cells. IL-13, leptin, LTbR, MIP1b, and IL-11Ra genes are used to validate microarray data. Plasmids containing the target gene were purified and quantified for use in the qRT-PCR setup. To confirm the correct size of the inserted PCR product, plasmids were digested with specific restriction enzymes, and the cDNA clone PCR products were then evaluated on 2% agarose gel electrophoresis.

**Quantitative real-time PCR to measure gene expression.** Two primer and probe sequences were designed and purchased from Applied Biosystems and used in accordance with the manufacturer's protocol: Melan A, CACGAAAGTGTCTCCTCAAGAGAAAA (forward), CTCA- TAAGCGAGGTGGACATGTG (reverse), 6FAMCTGTGAACCTGTGGTTC (probe); and CD3, GCCCGGCGACCAAGG (forward), TCGGATGGCCTCATAGTCT (reverse), 6FAMAGAGGCCACCCACTGTGTC (probe). Primers for IL-13, leptin, LTbR, MIP1b, and IL-11Ra were purchased from Superarray Bioscience Corp. and used in accordance with the manufacturer’s protocol. Briefly, 2 μl of cDNA (reverse transcription mixture), 25× target primer (2 μl) and 2× RT Real-Time SYBR Green PCR Master Mix were analyzed in 50 μl volume. The thermocycler parameters were 90°C for 15 min (for initial denaturation), followed by denaturation at 95°C for 30 s; 55°C for 30 s; and 72°C for 30 s (40 cycles). The PCR amplification of the 1.010 serially diluted plasmid cDNA standard templates of each marker showed a logarithmic signal increase. The standard curve was generated using the threshold cycle (CT) of templates in known numbers of copies. The CT of each sample was plotted on the standard curve, and the mRNA copy number was calculated by the ABI Prism 7000 SDS software (Applied Biosystems).
Statistical analysis. Kruskal-Wallis test, Fisher’s exact test, and ANOVA were used to compare clinical characteristics among patients in NSN, tumor-positive SN, and tumor-negative SN groups. Wilcoxon rank-sum test was used to analyze differences in gene expression between patients and cell lines, between patients with NSNs and SNs, and between patients with tumor-positive and tumor-negative SNs. Gene expression was analyzed with CLUSTER (12) using average linkage metric and displayed using Java Tree-View.4 Gene expression was normalized by β-actin and log-transformed (base 2) using hierarchical clustering; the resulting cluster data tables were imported into the TreeView software. Univariate logistic regression analysis of predictive accuracy for individual genes and combined genes used receiver-operator characteristics (ROC) methods.

Results

A total of 34 lymph nodes were obtained for microarray study: 13 were tumor-free SNs, 11 were tumor-free NSNs, and 10 were tumor-positive SNs. Six tumor-free SNs from six patients were matched with six NSNs from the same patients. All of the tumor-positive SNs contained micrometastases (<2 mm). The clinical features of the patients, such as gender, age, primary lesion site, histology, Breslow, Clark level, ulceration, and interval between biopsy date and surgery, were similar in the three groups. A total of 5 out of 11 NSN patients, 10 out of 13 tumor-negative SN patients, and 5 out of 10 tumor-positive SN patients have pathologically identifiable residual melanoma in the wide excision specimen.

Immunosuppressive cytokine profile of SNs versus NSNs. Of the 96 human inflammatory cytokine and receptor genes evaluated, 39 were expressed at comparable levels in NSNs and SNs, 4 were up-regulated in SNs, and 53 were down-regulated in SNs (Table 1). IL-10, IL-12 receptor b2 (IL-12Rb2), CCR5, and tumor necrosis factor R2 (TNFR2) were expressed at higher levels in SNs than in NSNs. A total of 14 ILs, 12 IL receptors, 14 chemokines, 6 chemokine receptors, 4 transforming growth factor (TGF) ligands, and 3 other related genes were expressed at lower levels in SNs than in NSNs.

Inflammatory cytokine array analysis using hierarchical clustering revealed different profiles for the NSNs, tumor-negative SNs, and tumor-positive SNs (Fig. 1). Expression patterns for NSNs were a distinct group, but tumor-negative and tumor-positive SNs had overlap in their patterns. One NSN sample migrated to the left side of the heat map; this patient developed other anatomic site primary melanoma 3 months later. The first primary melanoma was on the left upper arm (draining to axilla), and the second primary melanoma developed on the ipsilateral thigh (draining to the groin). In the SN group, we found two different expression patterns from the heat map, each on alternative sides. The left-side groups had higher and the right-side groups had lower expression levels of cytokines and chemokines. When comparing these two groups, all the right-side group patients had pathologically positive margin from the initial diagnostic specimen of primary melanoma. Positive margins were found in eight out of eight (100%) from the right-side group and 7 out of 15 (47%) from the left-side group (P < 0.03). All head and neck SNs (n = 4) showed patterns migrating to the right of the heat map (maybe related all four having positive margin). Other factors such as age, gender, Breslow, Clark level, ulceration, histology, and interval between biopsy date and surgery and overall survival were not different in the two groups.

We did cDNA array analysis of two primary and two metastatic melanoma cell lines. IL-13, IL-17, IL-2, IL-20, IL-21, IL-2RA, IL-2Rb, IL-2Ry, IL-4, IL-9Ra, P10, and I-TAC were not detected in any of melanoma cell lines, which suggest that these genes are likely a product of the lymph node environment and not produced by melanoma cells. IL-12Rb2 was detected in only one primary cell line, whereas CCR5, IL-10, and TNFR2 were detected in all metastatic cell lines.

Immunosuppressive cytokine profile of tumor-positive versus tumor-negative SNs. When comparing tumor-negative to tumor-positive SNs, five genes were expressed at significantly different levels. Expression levels of IL-13, lepin, LTβR, and MIP1b were 24.4-fold (P < 0.04), 3.5-fold (P < 0.01), 2.3-fold (P < 0.05), and 2.1-fold (P < 0.01) higher, respectively, comparing tumor-positive and tumor-negative SNs (Table 2). The expression level of IL-11Ra in tumor-positive SNs was only 0.1-fold of its expression in tumor-negative SNs (P < 0.03; Table 2).

qRT-PCR is one of the most widely used techniques for the detection and quantification of gene expression, as well as the confirmation and verification of microarray data. We used the array-designed PCR primers and probe set to confirm the microarray data by qRT-PCR. To increase sample size, this study was done with an additional 21 patients (12 patients who have tumor-negative SNs and 9 patients who had tumor-positive SNs). All five genes (IL-13, lepin, LTβR, MIP1b, and IL-11Ra) showed similar trends as the microarray data. IL-13, lepin, and MIP1b expression was significantly higher in tumor-positive than tumor-negative SNs (P < 0.02, P < 0.05, P < 0.03, respectively; Fig. 2). IL-11Ra was significantly lower in tumor-positive SNs than tumor-negative SNs (P < 0.05). Expression of LTβR was not significantly (P = 0.08) different, but still showed the trends observed from the microarray data.

To assess the potential of these genes to correctly identify regional node metastasis, ROC curves were constructed based on microarray data. ROC curve analyses showed that the areas under the curve (AUC) for IL-13, lepin, LTβR, MIP1b, and IL-11Ra were 0.79, 0.83, 0.75, 0.81, and 0.77, respectively (Fig. 3A). When the five genes were combined for analysis, AUC was 0.973, suggesting high concordance between gene expression and SN staging (Fig. 3B). A permutation test confirmed that the set of five markers predicted tumor-positive SNs better than chance alone (P = 0.012).

T-cell infiltration and melanoma cell marker expression level in NSNs and SNs. To verify T-cell infiltration or to avoid the possibility of our using NSNs and SNs samples containing melanoma cells, we used the T-cell marker CD3 and the melanoma cell marker Melan A to identify T cells and melanoma cells by qRT-PCR. Although all of the tumor-positive SNs had micrometastases (<2 mm), we used melanoma cell-free areas of these lymph nodes to detect cytokines/chemokines profiles that reflect the SNs microenvironment.

CD3 expression levels were similar among the three lymph node groups: 232,844 ± 73,682 copy numbers/μg RNA for

4 This can be accessed at http://genetics.stanford.edu/~alok/TreeView.
<table>
<thead>
<tr>
<th>Genename</th>
<th>GenBank*</th>
<th>Average signal intensity (mean ± SE) NSNs</th>
<th>Average signal intensity (mean ± SE) SNs</th>
<th>Fold change †</th>
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<td>IL-10</td>
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<td>CCR1</td>
<td>NM_001295</td>
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<td>CCR4</td>
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<td>CCR9</td>
<td>NM_006641</td>
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<td>CX3CR1</td>
<td>NM_001337</td>
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<td>21.14 ± 3.19</td>
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<td>CXCR4</td>
<td>NM_003467</td>
<td>224.28 ± 17.57</td>
<td>102.66 ± 11.40</td>
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*GenBank accession number. † Mean signal ratio of SN to NSN. ‡ Wilcoxon rank-sum test.
NSNs; 177,811 ± 29,442 copy numbers/µg RNA for tumor-negative SNs, and 175,128 ± 28,832 copy numbers/µg RNA for tumor-positive SNs (SN+). Melan A was not detected in any of the three groups but was expressed in high levels in the IGR37 melanoma cell line ($P = 0.0005$), which served as a positive control (Fig. 4B). These results verify our findings and suggest that the cytokine profile reflects the lymph node microenvironment.
Discussion

Cytokines and chemokines are regulatory proteins secreted by inflammatory and other cells. Their pleiotropic regulatory effects on hematopoietic cells defend the host from exogenous/endogenous insults, restore homeostasis, and mediate immune cascade reactions. However, primary and metastatic melanoma cells may express cytokines and chemokines that regulate tumor progression, angiogenesis, and lymphangiogenesis (13, 14) and therefore promote malignancy (14, 15).

A tumor's microenvironment is the result of tumor factors and host responses. The microenvironment is significantly different from that of normal tissue and plays a critical role in tumor initiation and progression (16, 17). Melanoma cells can down-regulate antigen-presenting cells and thereby create an immunosuppressive microenvironment (18) associated with SN metastases. There is evidence that SNs immunosuppression can be reversed by immunostimulatory agents such as the granulocyte-macrophage colony-stimulating factor (6, 19). We previously reported that IL-10, IFN-γ, and the dendritic cell enzyme indoleamine 2,3-dioxygenase were key agents for SN immunosuppression. The presence of PRM was associated with significantly higher levels of IL-10 and IFN-γ in SNs than NSNs (6). In the present study, signal intensity of IL-10 was 139.65 ± 30.3 in tumor-negative SNs as compared with 180.12 ± 35.5 in tumor-positive SNs. Similarly, the signal intensity of IFN-γ was 26.29 ± 7.6 and 50.21 ± 13.8 in tumor-negative and tumor-positive SNs, respectively. Because 10 of 13 patients with tumor-negative SNs had a positive biopsy margin, i.e., PRM, SNs in these patients might already have been immunosuppressed, which would decrease the difference in cytokine levels between tumor-positive and tumor-negative SNs.

The results of our study indicate that the inflammatory cytokine profile of SNs is different from that of NSNs and predisposes the SN to immunosuppression. There were two separate subtypes of SNs identified by hierarchical clustering. PRM-positive and head and neck primary melanoma seem to have a more immunosuppressive phenotype. Certain anatomic sites such as those from severely sun-damaged site have shown a different chromosomal aberration pattern, which may explain the difference in lymph node profiles (20). We also found that the presence of metastatic foci in an SN changes that node's profile of cytokine and chemokine immunoregulatory markers. Thus, tumor-positive SNs had significantly higher gene expression of IL-13, leptin, LTbR, and MIP1b and significantly lower expression of IL-11Ra as compared with tumor-negative SNs.

Unlike direct markers of nodal metastasis, which are subject to sampling error unless the entire node is sectioned for analysis, markers of the tumor microenvironment should be found throughout the node. Thus, the presence of nodal micrometastasis can be determined by examining a single section from any part of the node. To confirm this, we examined levels of CD3, a T-cell marker, and Melan A, a melanoma cell marker. Levels of both markers were not significantly different in patients with tumor-negative SNs, tumor-positive SNs, and NSNs.

Because IL-13 was not expressed in melanoma cell lines, this cytokine is probably not a product of melanoma cell gene expression. IL-13, closely related to IL-4, is produced not only by CD4+ Th2 cells, but also by Th0 and CD8+ T cells (21, 22). It can suppress the cytotoxic functions of monocytes/macrophages

Table 2. Genes showing consistently different expression levels in SNs

<table>
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<th>Gene name</th>
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<th>Average signal intensity (mean ± SE)</th>
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<th>P value ‡</th>
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<td>Positive SNs</td>
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<td>IL-13</td>
<td>NM_002188</td>
<td>0.29 ± 0.29</td>
<td>7.19 ± 2.83</td>
<td>24.4</td>
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<tr>
<td>Leptin</td>
<td>NM_000230</td>
<td>2.47 ± 1.01</td>
<td>8.26 ± 2.06</td>
<td>3.5</td>
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<tr>
<td>LTbR</td>
<td>L04270</td>
<td>5.38 ± 1.67</td>
<td>12.18 ± 2.67</td>
<td>2.3</td>
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<td>MIP1b</td>
<td>NM_002984</td>
<td>8.95 ± 1.92</td>
<td>18.57 ± 3.10</td>
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<td>IL-11Ra</td>
<td>NM004512.1</td>
<td>2.14 ± 0.76</td>
<td>0.00 ± 0.00</td>
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</table>

*GenBank accession number.
† Mean signal ratio represents of tumor-negative SNs to tumor-positive SNs.
‡ Wilcoxon two-sample test.

Fig. 2. qRT-PCR validation of microarray results for IL-13, leptin, LTbR, MIP1b, and IL-11Ra. Expression levels of IL-13, leptin, LTbR, and MIP1b were higher, whereas expression level of IL-11Ra was lower when the SN contained micrometastasis. * P < 0.05; ** P < 0.03; *** P < 0.02. Gene expression (copy number/mg RNA) was normalized by β-actin; expression levels of IL-13, leptin, MIP1b, and IL-11Ra are 10 times smaller than indicated on the y-axis.
and the production of pro-inflammatory cytokines, and it can up-regulate the production of IL-1R (22). In this study, expression levels of IL-4 and IL-1R2 in SNs tended to be higher when these nodes contained tumor cells (data not shown). IL-1R2 acts as a decoy receptor that inhibits the activity of its ligand IL-1, a pro-inflammatory cytokine (23).

Leptin is a family member of the helical cytokines and has a physical structure similar to IL-2 (24). Most data indicate that leptin increases proinflammatory immune responses by an effect on T cells and macrophages (25). In this study, leptin expression was higher in NSNs than SNs. However, Lord et al. (26) showed that naive and memory T cells were differentially affected by leptin: the anti-CD3-driven proliferation of memory T cells was inhibited, whereas the proliferation of naive T cells was markedly enhanced. These data show that leptin can inhibit certain immune responses.

LTbR, a member of the TNF family of receptors, is expressed on the surface of most cell types, including macrophages and melanoma cells, but not on T and B lymphocytes (27). LTbR signaling is essential for embryonic development and organization of secondary lymphoid tissues and LTbR inhibitor-blocked angiogenesis (28, 29).

MIP1b proteins are expressed primarily in T cells, B cells, and monocytes after antigen or mitogen stimulation (30). MIP1b is a monocyte chemoattractant; it also has chemotactic and adhesive effects on lymphocytes, CD8+ T cells, CD4+ T cells and melanoma cells in vitro (31–33). This suggests that MIP1b can play an important role in tumor invasion.

IL-11Ra is a member of the hematopoietic cytokine receptor family, and its extracellular domain is closely related to that of IL-6Ra (24% amino acid identity) and p40 subset of IL-12 (16% amino acid identity; ref. 34). The low affinity of IL-11Ra for IL-11 is increased by interaction with gp130 (34). In our study, the tumor status of the SN did not affect expression of gp130 (data not shown). IL-11 and IL-11Ra play a role in the maturation/activation of macrophages (35).

These five genes might reflect the tumor microenvironment associated with early stages of nodal metastasis, and their expression may favor nodal invasion. ROC analysis based on array data showed that expression levels of these genes distinguished patients with tumor-negative nodes from those with nodal metastasis (AUCs = 0.79, 0.83, 0.75, 0.81, and 0.77 for IL-13, leptin, LTbR, MIP1b, and IL-11Ra, respectively). When the five genes were considered in combination, the AUC increased to 0.973, which suggests high levels of concordance between gene expression and SN staging. We also constructed the ROC curve based on qRT-PCR data. ROC curve analyses showed the AUC for IL-13, leptin, LTbR, MIP1b, and IL-11Ra was 0.73, 0.68, 0.63, 0.68, and 0.58, respectively. When the five...
genes were combined for analysis, AUC was 0.785, still suggesting high concordance between gene expression and SN staging.

Our data strongly suggest that the cytokine/chemokine microenvironment can distinguish SNs from NSNs in melanoma patients. These results have potentially important implications for the development of postoperative adjuvant immunotherapies that target specific genes expressed in the regional lymph nodes (36–38). Activation or down-regulation of genes for cytokines and chemokines and their receptors may control the immunosuppressive microenvironment that facilitates the metastasis of melanoma.

References

Molecular Characterization of Inflammatory Genes in Sentinel and Nonsentinel Nodes in Melanoma


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