Differential Expression of Chemokine Receptors on Uveal Melanoma Cells and Their Metastases

Haochuan Li, Hassan Alizadeh, and Jerry Y. Niederkorn

PURPOSE. To determine the expression of the chemokine receptors CXCR4 and CCR7 on human uveal melanoma cells and their metastases and the effect of liver-borne factors on the chemotactic responses of uveal melanoma cells.

METHODS. Four human uveal melanoma cell lines and three cell lines of uveal melanoma metastases were examined by RT-PCR and flow cytometry for their constitutive expression of CXCR4 and CCR7. The effect of the liver and liver-borne factors on the expression of CXCR4 and CCR7 was determined after intracamer, intrasplenic, and subcutaneous transplantation of uveal melanoma cells in nude mice. Chemotactic responses of melanoma cells to liver-borne factors were determined by in vitro chemotaxis assays using protein extracts of hepatocytes and striated muscle tissue.

RESULTS. All the primary uveal melanoma cell lines expressed CXCR4 and CCR7 message and protein, whereas the metastases cell lines expressed little or no chemokine receptor. Extracts of human liver cells stimulated chemotaxis of uveal melanoma cells, which could be inhibited by anti–CXCR4 antibody. Liver-borne factors also induced the downregulation of CXCR4 and CCR7 on uveal melanoma cells. Uveal melanoma cells maintained their high expression of CXCR4 and CCR7 after intracameral transplantation. However, CXCR4 and CCR7 expression was sharply reduced in liver metastases arising from intraocular melanomas.

CONCLUSIONS. CXCR4 and CCR7 provide directional migration of uveal melanoma cells toward the liver, the most common site for the formation of uveal melanoma metastases. However, soluble factors elaborated by hepatocytes induce the downregulation of CXCR4 and CCR7 on metastatic uveal melanoma cells. (Invest Ophthalmol Vis Sci. 2008;49:636–643) DOI:10.1167/iovs.07-1035

Uveal melanoma is the most common intraocular malignant neoplasm in adults and occurs at a frequency of 6 to 7 cases per 1 million adults.1 Although cutaneous and uveal melanomas arise from neural crest progenitors, they differ significantly in their epidemiologic,2 cytogenetic,3 metastatic,4–6 and immunologic7 characteristics. One of the remarkable differences between cutaneous and uveal melanomas is their metastatic behavior. Although skin melanomas metastasize to almost any organ, uveal melanoma displays a propensity to spread to the liver. Indeed, liver metastases are present in up to 95% of the patients who die of uveal melanoma.7–10

Metastasis is a sequential process that culminates in the nonrandom dissemination of tumor cells to secondary organs. The predictable and selective pattern of metastases in different malignancies led Paget to propose the “seed and soil” hypothesis, which, as the name implies, proposes that certain organs provide a more fertile milieu than other organs for the establishment and growth of metastases.11 An equally appealing theory holds that different organs elaborate chemoattractants that promote the arrest and extravasation of blood-borne tumor cells, thus accounting for the organ specificity of metastasis. Recent reports from several laboratories have demonstrated that a wide variety of tumors express chemokine receptors, which correspond with the expression of their respective ligands in organs that have the highest frequency for developing metastases.

Chemokines are a superfamily of small polypeptides that are signaling molecules that function in a wide range of cell-trafficking events. They are classified into four subgroups—C, CC, CXC, and CX3C—based on the positioning of their cysteine residues. Activation of chemokine receptors leads to growth, adhesion, and directional migration of tumor and inflammatory cells.12 Under normal conditions, leukocytes express chemokine receptors, which direct migration of T cells to lymph nodes and to sites of inflammation. Many tumors also express chemokine receptors, and a growing body of evidence indicates that chemokine receptor/chemokine interactions have profound effects on the organ specificity of metastases.13–16 The propensity of certain tumors to consistently metastasize to specific organs is influenced by chemokine receptor/ligand interactions. Colon cancer, like uveal melanoma, has a propensity to metastasize to the liver. In vitro studies have demonstrated that cellular extracts of the liver parenchyma have high concentrations of CXCL12, the chemokine ligand for CXCR4, which is expressed on colon cancer cells.17,18 Moreover, CXCR4 expression is significantly elevated in liver metastases in patients with colorectal cancer, and a high expression of CXCR4 on primary colorectal tumor specimens is correlated with poor prognosis.17 By contrast, lymph nodes express CCL21, the ligand for CCR7, which is expressed on tumors that metastasize to lymph nodes. CCR7-expressing B16 murine skin melanoma cells metastasize 700-fold more efficiently to lymph nodes than do B16 melanoma cells not expressing this receptor.19

CXCR4 has been detected on histologic specimens of almost 60% of the 44 patients examined with primary human uveal melanoma.20 Intensity of CXCR4 staining correlated with an epithelioid morphology, which is closely associated with poor prognosis.21

The present study explored the expression and possible function of chemokine receptors on human uveal melanoma cells and their metastases. CXCR4 was selected because it is associated with the metastasis of colon cancer, which, like uveal melanoma, metastasizes to the liver. CCR7 was also examined because of its importance in the metastasis of skin melanomas, which, like uveal melanomas, arise from neural crest progenitors.22
METHODS

Mice

Beige nude mice were purchased from Charles River Laboratories (Wilmington, MA) and Taconic Farms (Germantown, NY). All mice were housed and cared for in accordance with the NIH Guidelines on Laboratory Animal Welfare and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Human Uveal Melanoma Cell Lines

Four primary uveal melanoma cell lines—designated OCM3, OCM8, MEL270, and MEL290—and three cell lines—designated OMM1, OMM1.5, and OMM2.3 and isolated from metastatic lesions in uveal melanoma patients—were used. OCM3 and OCM8 primary uveal melanoma cell lines were kindly provided by June Kan-Mitchell (University of California, San Diego, CA).25 MEL270, MEL290, OMM1, and OMM2.3 cell lines were kindly provided by Bruce Ksander (Schepens Eye Research Institute, Boston, MA). OMM1 cells were isolated from a skin metastasis arising in a uveal melanoma patient and were a gift from Gregorius Luyten (University Hospital Rotterdam, Rotterdam, The Netherlands).25 Both the primary cell line and the metastasis cell line were maintained in complete RPMI 1640 (JRH Biosciences, Lenexa, KS).26

Generation of Liver Metastasis Cell Lines

Liver metastases of OCM8 melanomas were generated in nude mice. Briefly, OCM8 cells (1 × 10⁶ cells in 100 µL) were transplanted under the spleen capsule in nude mice. Mice underwent necropsy 35 to 42 days later, and macroscopic metastatic foci were aseptically isolated from the livers and cultured in complete RPMI 1640. The metastatic cell culture was designated OCM8.LM.

Flow Cytometric Analysis

Expression of human CXCR4 and CCR7 was assessed by flow cytometry, as previously described.27 Single melanoma cell suspensions were prepared and washed in fluorescence-activated cell sorter (FACS) buffer consisting of phosphate-buffered saline (PBS; pH 7.2) containing 1% bovine serum albumin and 0.02% sodium azide. Cells (1 × 10⁶) were incubated with monoclonal anti–CXCR4, anti–CCR7, or normal mouse IgG (1 µg/mL; Sigma-Aldrich, St. Louis, MO) for 30 minutes on ice, washed three times, and incubated with FITC-labeled goat anti–mouse IgG (Accurate Chemical and Scientific Co., Westbury, NY) for 20 minutes at 4°C. Cells were then washed three additional times in PBS, fixed in 1% paraformaldehyde, and assessed for fluorescence in a flow cytometer (FACScan; BD Biosciences, San Diego, CA).

RT-PCR Detection of Chemokine Transcripts

For RT-PCR analysis of CXCR4 and CCR7 gene expression, mRNA of human uveal melanoma cells was isolated (OligoFast Direct mRNA Mini Kit; Qiagen, Valencia, CA). Samples were treated with DNase I (0.2 U/µL; Ambion, Austin, TX) to remove possible DNA contamination. RT-PCR was carried out using one-step quantitative RT-PCR (SuperScript One-Step RT-PCR with Platinum Taq; Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Protein concentration was determined with the use of a protein assay (BCA Protein Assay Kit; Pierce, Rockford, IL) according to the manufacturer’s instructions. For real-time PCR, cDNA samples were preincubated with various concentrations of specific primers (anti–human CXCR4 or CCR7) to determine the number of cells in 10 random fields using direct light microscopy. Results were expressed as the mean number of cells per high-power field (HPF). All assays were performed in quadruplicate.

Protein Extracts of Liver Parenchymal Cells and Smooth Muscle Cells

Proteins were extracted from human liver and smooth muscle tissues (National Disease Research, Interchange, PA) using an extraction kit (ReadyPrep Protein Extraction Kit; Bio-Rad Laboratories, Hercules, CA) according to the manufacturer’s instructions. Protein concentration was determined with the use of a protein assay (BCA Protein Assay Kit; Pierce, Rockford, IL) according to the manufacturer’s instructions. For neutralization studies, cells were preincubated with various concentrations of anti–human CXCR4 or CCR7 monoclonal antibody (4+7+17.111, IgG1; or anti–human CCR7 monoclonal antibody (goat IgG; both R&D Systems).

Melanoma Cell Migration and Invasion Assays

Melanoma cell migration in response to protein extracts of liver and smooth muscle cells was performed using 24-well transwell chambers (Costar, Corning Inc., Corning, NY). Melanoma cells (1 × 10⁴) were plated in the top chambers, and protein extracts (40 µg/mL) of human liver or human smooth muscle cells were added to the bottom chambers. Upper and lower chambers were separated by 8-µm pore membranes. In some experiments, anti–CXCR4, anti–CCR7, or an isotype control antibody was added to the lower chamber (20 µg/mL). Plates were incubated for 24 hours at 37°C in 5% CO₂. The top chambers were removed, and the number of melanoma cells that had migrated to the bottom chambers was determined by counting the number of cells in 10 random fields using direct light microscopy. Results were expressed as the mean number of cells per high-power field (HPF). All assays were performed in quadruplicate.

Transwell chambers were used for invasion assays to evaluate the capacity of melanoma cells to penetrate a synthetic basement membrane. The top chamber of the transwell 8-µm pore membrane was coated with 60-µL basement membrane preparation (Matrigel; Collaborative Biomedical Products, Bedford, MA) for 30 minutes at 37°C. Excess basement membrane preparation (Matrigel; Collaborative Biomedical Products) was removed, and the membranes were allowed to dry at room temperature. Melanoma cells (2 × 10⁵) were added to the top chamber, and protein extracts (40 µg/mL) of human liver or smooth muscle were added to the lower chambers. Anti–chemokine and isotype control antibodies were added to the bottom chambers, as described, and the number of melanoma cells that penetrated the membrane was determined as in the chemotaxis assays.

Immunohistochemistry

Tumor-containing eyes, ears, and subcutaneous tumors were removed from euthanatized mice, fixed in formalin, embedded in paraffin, and cut into 5-µm sections. Sections were deparaffinized, and endogenous peroxidase was quenched by incubating the slides in 3% hydrogen peroxide (Sigma Chemical, St. Louis, MO) for 12 hours at room temperature. Slides were washed twice in PBS, incubated in blocking serum (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA), washed once in PBS, and incubated for 30 minutes at 37°C in anti–CXCR4 (R&D Systems), anti–CCR7 (R&D Systems), anti–HMB45/50 (Laboratory Vision Corporation, Fremont, CA), or isotype control (0.25 µg/mL) antibodies. Slides were washed in PBS and incubated in biotinylated anti–rabbit IgG (Vectastain Elite ABC Kit; Vector Laboratories), and then they were washed in PBS/0.1% Tween-20 and incubated in Vectastain ABC reagent for 30 minutes at 37°C. Slides were washed as before and developed in peroxidase substrate (DAB Peroxidase Substrate Kit; Vector Laboratories) for 2 to 10 minutes at room temperature. Slides were washed and counterstained in methyl green.

Tumor Transplantation and Derivation of In Vivo–Derived Melanoma Cell Lines

OCM8 uveal melanoma cells were transplanted transsclerally, immediately posterior to the ciliary body of mice, as described previously.28
This technique produces intraocular melanoma that invades the retina and choroid and metastasizes to the liver. Briefly, mice were deeply anesthetized with 0.66 mg/kg ketamine hydrochloride (Vetalar; Parke Davis & Co., Detroit, MI) administered intramuscularly. With the use of a Hamilton (Reno, NV) syringe fitted with a 35-gauge glass needle, a tunnel was prepared from the cornea at the limbus, along the sclera and ciliary body to the choroid, under a dissection microscope. Tumor cells (10$^5$ cells/2.5$^3$/H9262) were injected into the choroid and subretinal space. Eyes were examined two or three times per week, and tumor growth was examined under the dissecting microscope. Mice were humanely killed 35 to 42 days later, and the tumor-bearing eyes were enucleated. Ocular tumors were immediately microdissected from the enucleated eyes and placed in the culture, as described. OCM8 uveal melanoma cells were also transplanted into the spleen capsules (1 × 10$^7$ cells/100 µL) or subcutaneously (1 × 10$^5$ cells/100 µL) into other nude mice. Mice were humanely killed 35 to 42 days later, and the liver metastases and subcutaneous tumors were isolated and cultured in vitro, as described. The three melanoma cultures (ocular, liver, and subcutaneous) were examined by flow cytometry for surface expression of CXCR4 and CCR7.

**Statistical Analysis**

The Student t-test was used to determine significance in differences between experimental groups and controls.

**RESULTS**

Expression of Chemokine Transcripts and Protein in Human Uveal Melanoma Cells and Cells from Uveal Melanoma Metastases

All four cell lines derived from primary human uveal melanomas expressed CXCR4 and CCR7 transcripts (Fig. 1). By contrast, faint expression of the CCR4 transcript was found in only one of the three metastasis cell lines (OMM2.5), and CCR7 was expressed only weakly in OMM1 cells. Flow cytometric analysis of six of the cell lines confirmed that CXCR4 and CCR7 proteins were expressed on all three primary uveal melanoma cell lines but were absent or only weakly expressed on the metastases cell lines (Fig. 2).

![Figure 1. CCR7 and CXCR4 mRNA expression in human uveal melanoma cell lines and cell lines derived from uveal melanoma metastases. Lane 1: 100-base pair (bp) DNA ladder. Lane 2: MEL 270. Lane 3: MEL 290. Lane 4: OCM8. Lane 5: OCM3. Lane 6: OMM1. Lane 7: OMM2.5. Lane 8: OMM2.3. CXCR4, 367 bp; CCR7, 338 bp.](image)

![Figure 2. Surface expression of CXCR4 and CCR7 in human uveal melanoma cell lines and cell lines derived from uveal melanoma metastases detected by flow cytometry. (A) CXCR4 expression. (B) CCR7 expression. Filled profile: isotype control. Open profile: anti-CXCR4. Each of these flow cytometry analyses has been performed three to five times, with similar results each time.](image)
Effect of Extracts from Liver Parenchymal Cells on Uveal Melanoma Chemotaxis and Invasion of Basement Membranes

Previous studies have demonstrated that extracts from liver parenchyma contain relatively high concentrations of CXCL12, the ligand for CXCR4. In addition, liver metastases of colorectal cancer express higher amounts of CCR4 than do primary colorectal cancers. Therefore, primary uveal melanoma cells and cells from uveal melanoma metastases were examined for their chemotactic responses to protein extracts from human liver parenchymal cells. Protein extracts from human striated muscle tissue served as a control for the chemotaxis assays. As anticipated, all four of the primary uveal melanoma cell lines responded to extracts from liver parenchymal cells but did not significantly respond to extracts from smooth muscle tissue (Fig. 3). Moreover, chemotaxis of uveal melanoma cells toward liver extracts was significantly inhibited by anti-CXCR4 antibody (Fig. 3). By contrast, none of the three metastasis cell lines displayed significant chemotactic responses to liver or muscle extracts (data not shown). Invasion assays were performed to confirm chemotactic responses to liver extracts and to determine whether the uveal melanoma cells and metastases were able to penetrate a synthetic basement membrane (Matrigel; Collaborative Biomedical Products). As in the previous experiment, all three primary uveal melanoma cell lines demonstrated a propensity to respond to liver extracts but not to muscle extracts (Fig. 4). As before, melanoma cells displayed insignificant responses to muscle extracts.

Effect of Liver Extracts on Uveal Melanoma Expression of CXCR4 and CCR7

The striking difference between the expression of CXCR4 in primary uveal melanoma cells and in cells from metastases raised the possibility that perhaps factors in the liver influenced CXCR4 gene expression. Accordingly, the four primary uveal melanoma cell lines were cultured for 4 days in the presence of liver or muscle extracts and then were assessed for the expression of CXCR4 and CCR7 transcripts. As previously shown, MEL270, MEL290, OCM8, and OCM3 expressed CXCR4 and CCR7 transcripts (Fig. 5). However, expression of...
both chemokine receptor transcripts was sharply reduced in all uveal melanoma cell lines cultured in liver extract. CXCR4 expression by MEL270, OCM8 and OCM3 cultured with muscle extract remained unchanged whereas expression by MEL290 was reduced. CCR7 expression by MEL270, MEL290 and OCM8 cultured with muscle extract was reduced while expression by OCM3 remained unchanged. Flow cytometric analysis confirmed that both primary uveal melanoma cell lines cultured in liver extracts displayed significantly reduced levels of CXCR4 protein and CCR7 protein compared with cells cultured in muscle extracts (Fig. 6). CXCR4 and CCR7 protein expression on the three melanoma metastasis cell lines was unaffected by in vitro culture in liver or muscle extracts (data not shown).

**Downregulation of Chemokine Receptor Expression after Metastasis**

Additional experiments were performed to confirm that metastasis to the liver alters the expression of chemokine receptors on uveal melanoma cells. We used the nude mouse model of intraocular melanoma to recapitulate the downregulation of CXCR4 expression that we think occurs when uveal melanoma cells metastasize from the eyes to the liver in humans. Accordingly, OCM8 cells were injected into the posterior compartment in the eyes of nude mice. Melanoma-containing eyes were enucleated 28 days later and were processed for immunohistochemistry. Mice were humanely killed 32 days after enucleation, and the livers were processed for immunohistochemistry. Tumor-containing eyes and livers were stained with HMB45/50, CXCR4, CCR7, or an isotype control. The HMB45/50 antibody has been reported to stain more than 95% of human uveal melanomas and was used to confirm the presence of melanomas in the posterior portion of the eye and the presence of discrete metastatic melanoma foci in the liver (Fig. 7). Although most cells in the primary OCM8 ocular melanomas stained with both CXCR4 and CCR7, the cells that ultimately formed liver metastases did not express either chemokine receptor, suggesting that the local environment in the liver downregulated chemokine receptor expression.

Additional experiments were performed to determine whether the putative modulation of chemokine receptor expression was unique to metastases arising from the eye or whether uveal melanoma cells metastasizing from other routes would also undergo similar downregulation of chemokine receptor expression. Accordingly, OCM8 melanoma cells were transplanted under the spleen capsule of nude mice. The spleen was chosen for tumor transplantation because tumors injected under the spleen capsule have ready access to the venous portal circulation and, thus, have a greater likelihood of forming liver metastases. Mice were humanely killed 35 to 42 days after tumor implantation under the spleen capsule, and the liver metastases were isolated and cultured in vitro. The in vitro cell cultures were expanded and examined by flow cytometry for the expression of CXCR4 and CCR7. Results clearly demonstrated that OCM8 liver metastases, created by intrasplenic injection, had significantly reduced expression of CXCR4 and CCR7 compared with parental cells (Fig. 8). Addi-
tional experiments considered the hypothesis that the micro-
vironment of the liver was responsible for the downregu-
lation of CXCR4 and CCR7 expression, whereas the
microenvironment at other sites would not promote the down-
regulation of either chemokine receptor. Accordingly, OCM8
cells were transplanted intracamerally or subcutaneously into
nude mice. Tumor-containing eyes and subcutaneous tumors
were extirpated 30 days later, cultured in vitro, and examined
by flow cytometry for CXCR4 and CCR7 expression. As previ-
ously shown, OCM8 melanomas sustained their high expres-
sion of CXCR4 and CCR7 after intracameral transplantation.
Similarly, subcutaneous OCM8 melanomas also retained their
expression of both chemokine receptors (Fig. 8). Thus, the
microenvironment of the liver and soluble factors produced by
hepatocytes led to steep reduction in the expression of CXCR4
and CCR7 message and protein.

DISCUSSION

The nonrandom nature of metastasis was recognized by Paget
more than 100 years ago and remains an intriguing riddle that
may hold the key for major breakthroughs in the treatment of
many malignancies.11 Recent findings have demonstrated that
the expression of chemokine receptors on tumor cells corre-
lates with organ-specific metastasis and, as a result, have rekin-
dled interest in Paget’s “seed and soil” hypothesis.15,16 Tumor
cells and leukocytes express various chemokine receptors and
respond to chemokine gradients, which may explain the non-
random nature of tumor metastasis.18,50 Although 19 human
chemokine receptors have been identified, CXCR4 and CCR7
are the ones most frequently linked to tumor metastasis.16
CCR7 is expressed on cutaneous melanoma18 and, as shown
here, on uveal melanoma. However, the role of CCR7 in me-
tastasis appears to be limited to tumors that disseminate to
lymph nodes, where its ligand, CCL21, is expressed.16 By
contrast, CXCR4 is expressed on at least 23 different types of
human cancers of epithelial, mesenchymal, neuroectodermal,
and hematopoietic origin and is associated with metastasis to
the lung, liver, bone marrow, and lymph node, where its
ligand, CXCL12, is highly expressed.14–16 Our findings indicate
that CXCR4 is also expressed on human uveal melanoma but is
downregulated or absent in uveal melanoma metastases. The
only known ligand for CXCR4 is CXCL12, which is strongly
expressed in the liver. Interactions between CXCR4 and
CXCL12 stimulate tumor cell migration and invasiveness
through artificial extracellular matrices, such as basement
membrane preparation (Matrigel; Collaborative Biomedical
Products).18 CXCL12 also stimulates the proliferation and pro-

**FIGURE 7.** In situ expression of che-
mokine receptors on OCM8 human
uveal melanoma cells transplanted
into nude mice. OCM8 uveal melano-
ma cells were transplanted into
the eyes of nude mice on day 0. Eyes
were enucleated between days 28
and 35 and were processed for im-
munohistochemical staining of che-
mokine receptors. Mice underwent
necropsy 30 days after enucleation,
and the livers were examined by im-
munohistochemistry for expression
of CXCR4 and CCR7. HMB45/50 an-
tibody was used as a generic stain for
identifying human melanoma cells in
the eyes (UM) and livers (arrows).

**FIGURE 8.** Effect of microenvironment on CXCR4 and CCR7 expression on uveal melanoma cells. OCM8 uveal melanoma cells were transplanted
into eyes, spleen capsules, or subcutaneous sites in nude mice. Mice were humanely killed 35 to 42 days later, and ocular tumors, liver metastases,
and subcutaneous tumors were isolated, cultured in vitro, and examined by flow cytometry for surface expression of CXCR4 and CCR7. OCM8 eye,
OCM8LM, liver metastases; OCM8SC, subcutaneous tumors. **P < 0.01
motes the survival of CXCR4+ tumor cells. A growing body of evidence suggests that CXCR4-expressing metastatic cells migrate to organs containing high levels of CXCL12, but tumor cells form progressive metastatic tumors only if high levels of CXCL12 are maintained.

Progressively growing primary tumors contain large areas of hypoxia, leading to the production of hypoxia-induced factor-1α (HIF-1α). A recently published study reported that HIF-1α is expressed at higher levels in primary breast cancers than in breast cancer metastases. This study also found that breast cancer cells downregulated their expression of CXCR4 after metastasizing to the lymph node, which expresses large amounts of CXCL12 (the only known ligand for CXCR4) or when incubated with CXCL12 in vitro. This is similar to the results reported here comparing primary uveal melanomas with their liver metastases.

Chemokine receptors and their ligands not only shed light on the biology of metastasis, they may be important therapeutic targets. Systemic administration of antibodies to CXCR4 or its ligand, CXCL12, inhibited the spread of breast cancer and lung cancer cells in mice. Systemic administration of the CXCR4 antagonist AMD3100 inhibited the growth of human glioblastoma and medulloblastoma xenografts and increased tumor cell apoptosis in SCID mice. AMD3100 also inhibited the spontaneous proliferation and CXCL12-induced proliferation of cell lines isolated from human cutaneous melanoma metastases.

In the present study, the absence of detectable CXCR4 message or protein on cell lines derived from human uveal melanoma metastases and liver metastases generated in nude mice was in sharp contrast to recent findings that detected significant CXCR4 protein expression on cell lines isolated from human cutaneous melanoma metastases. Our results were, however, consistent with a recent study by Scala et al., who reported the expression of CXCR4 on approximately 60% of the primary human uveal melanomas examined. This dichotomy in chemokine receptor expression in cutaneous and uveal melanoma metastases represents yet one more example of how these two neural crest–derived pigmented tumors differ in parameters that can have profound effects on their malignant behavior.

CXCR4 expression on primary uveal melanoma cells is consistent with the presence of its ligand, CCL12, which is expressed in high levels in the liver. Moreover, our finding that anti–CXCR4 antibody inhibits the chemotactic responses of uveal melanoma cells to liver extracts and their invasion of basement membrane preparation (Matrigel; Collaborative Biomedical Products; data not shown) further supports the role of CXCR4 in the formation of liver metastases. In addition to facilitating chemotactic responses to chemokines elaborated in the liver, CXCR4/CXCL12 interactions lead to increased activation of matrix metalloproteinases (MMPs), which are known to facilitate tumor cell invasiveness and the development of metastases. It has been shown that uveal melanoma cells express CXCR4 and secrete MMPs. These results are similar to the observed association between the expression of CXCR4 and the development of liver metastases in colorectal cancer. However, unlike liver metastases in colorectal cancer in which CXCR4 expression increases, liver metastases in uveal melanomas have reduced expression of both CXCR4 and CCR7. In vivo and in vitro findings strongly suggest that the downregulation of CXCR4 on uveal melanoma metastases is the result of factors elaborated in the liver. Exposure to liver extracts resulted in a sharp reduction in CXCR4 gene transcription and a commensurate reduction in CXCR4 protein expression. Results from experiments in nude mice revealed that CXCR4 protein expression remained high in ocular and subcutaneous tumors but was sharply reduced in liver metastases that arose from intraocular tumors or that were produced by intrasplenic injection of uveal melanoma cells. By contrast, uveal melanoma cells transplanted into the posterior compartment of the eye or subcutaneously maintained CXCR4 and CCR7 expression. The dampening of chemokine receptor expression in liver metastases suggests that modulation of CXCR4 occurs after the tumor cells arrive in the liver and have invaded the parenchyma, two processes known to be facilitated by the chemotactic function of CXCR4 and the elaborate MMPs initiated by CXCR4/CXCL12 interactions.

This conclusion is supported by in vitro experiments that demonstrated brief culturing in protein extracts of human hepatocytes resulted in a steep reduction in CXCR4 gene transcripts and a commensurate diminution in CXCR4 protein expression. It remains a mystery why the putative liver-borne factors do not exert a similar effect on liver metastases in patients with colorectal cancer.

The expression of CXCR4 on uveal melanoma cells and high levels of its ligand, CXCL12, in the liver offer an attractive explanation for the selective colonization of the liver. However, additional receptor/ligand interactions might also contribute to the formation of liver metastases in uveal melanoma-bearing hosts. Among these is c-Met, which is the receptor for hepatocyte growth factor-scatter factor (HGF/SF). C-Met is expressed on murine melanoma cells that preferentially form liver metastases. Although c-Met does not influence the homing of blood-borne murine melanoma cells to the liver, engagement of c-Met with its ligand, HGF/SF, promotes tumor cell growth and invasiveness. Indeed, compelling evidence indicates that interaction between c-Met and its ligand on hepatocytes produces paracrine growth effects on murine melanoma cells. Human uveal melanoma cell lines also express c-Met, with the highest levels occurring on those cell lines with invasive phenotypes and the greatest chemotactic responses to HGF/SF. Thus, at least two surface molecules expressed on human uveal melanoma cells can promote the development of liver metastases. One molecule, CXCR4, facilitates the accumulation of uveal melanoma cells in the liver, and the second molecule, c-Met, promotes invasion and stimulates tumor growth through a paracrine effect produced by hepatocytes. Both molecules are potential therapeutic targets for the prevention and management of liver metastases arising from uveal melanoma, for which there is currently no effective treatment.

References

9. COMS. Assessment of metastatic disease status at death in 455 patients with large choroidal melanoma in the Collaborative Ocular Melanoma Study (COMS): COMS report no. 15. Arch Ophthal- 