Molecular Pathways Mediating Liver Metastasis in Patients with Uveal Melanoma

Silvin Bakalian, Jean-Claude Marshall, Patrick Logan, Dana Faingold, Shawn Maloney
Sebastian Di Cesare, Claudia Martins, Bruno F. Fernandes, and Miguel N. Burnier, Jr.

Abstract   Uveal melanoma arises from melanocytes located in the uveal tract of the eye and is the most common primary intraocular tumor in adults. Metastatic liver disease is the overwhelming cause of death in uveal melanoma patients, with almost 50% of patients developing liver metastases up to 15 years after diagnosis. Most of these patients do not present with any evidence of overt metastasis at the time of initial diagnosis although it is assumed that they have undetectable micrometastases. Currently, there are no therapeutic modalities to prevent or efficiently treat the metastatic disease in uveal melanoma patients. Recent discoveries have shed light on the molecular pathways that may contribute to the progression of liver metastasis. The aim of this review is to describe new insights into the genetic and molecular pathways that may play a role in the development of liver metastases in uveal melanoma patients.

Background

Uveal melanoma is the most common primary intraocular malignant tumor in adults, with an incidence of roughly seven cases per million (1). Tumors arise in the uveal tract, which comprises the iris, ciliary body, and the choroid. In contrast with skin melanoma, epidemiologic studies have failed to show an association between exposure to sunlight and an increased incidence of uveal melanoma (2–5). Despite this lack of correlation, recent studies have implicated blue light exposure as a possible risk factor for uveal melanoma, but further studies are necessary to draw definitive conclusions (6–8).

Histopathologically, uveal melanoma tumors are composed of either spindle or epithelioid cells. Callender's classification, modified at the Armed Forces Institute of Pathology, is one of the most reliable prognosticators. Tumors composed exclusively of spindle cells carry a better prognosis than those that contain epithelioid cells in any proportion (9, 10).

Despite advances in the diagnosis and treatment of the primary tumor, the 5-year mortality rate of uveal melanoma patients has not significantly changed since 1973 (11). The survival rates at 5, 10, and 15 years are 65%, 52%, and 46%, respectively (12, 13). The principal target organ for metastasis is the liver, which is involved in 71.4% to 87% of patients with metastatic disease (14–16). The liver is the exclusive site of systemic metastasis in ~40% of the patients and is often the first metastatic site in patients (17). Unfortunately, when liver metastases are diagnosed, treatment options are limited and life expectancy is poor. Reports have differed on the median survival time, ranging between 2.2 and 12.5 months, probably reflecting technological advances that detect metastasis earlier (16, 18).

Cells that escape the primary tumor do so by hematogenous dissemination, due to the lack of lymphatics in the eye itself (19). The predominance of liver metastasis cannot be solely explained by circulation because the lungs are the first set of capillary beds that these cells would encounter. Therefore, uveal melanoma offers a unique setting in which to study the hematogenous dissemination of cells and the subsequent homing of these cells, or their preferential survival, in the liver of patients. Understanding of the mechanisms underlying this phenomenon could have vast implications for the large number of patients who are at a high risk for the development of liver metastasis from other cancers, such as colorectal or breast carcinomas (20, 21). Substantial questions remain regarding the specific liver metastasis in uveal melanoma patients. Is it a reflection of homing of cells to the liver or simply preferential growth and survival of these uveal melanoma cells in that microenvironment? As in many things, it may be a mixture of the two.

Vasculogenic Mimicry

The discovery by Maniotis and colleagues (22) that uveal melanoma cells had the ability to line, or even create, vascular-like channels ideally illustrates the utility of this disease as a model system. The lack of lymphatics in the eye made uveal melanoma an ideal system to study the ability of tumor cells to create vascular-like channels and three-dimensional matrices (23). The idea of vasculogenic mimicry has changed the way we view angiogenesis and has important implications for therapeutic targeting of angiogenesis in a wide variety of cancers.

Gene Expression Profiling

There have been several recent advances in studying the metastatic process of uveal melanoma cells. Genetic assays such as microarray analysis of tumor samples have yielded new directions of study and confirmed previous theories about the malignant transformation of uveal melanocytes. These studies were able to classify patients into two groups: class 1, tumors associated with a low risk of metastatic death, and class 2, tumors associated with a high risk of metastatic death (24). Down-regulation of melanoma-specific genes including the helix-loop-helix inhibitor *ID2* was discovered in class 2 tumors along with a corresponding increase in E-cadherin expression (25). A colocalization of E-cadherin and β-catenin to the plasma membrane in high-risk patients was observed, possibly implicating a Wnt signaling pathway.

In addition to these patient studies, animal models have also been used to describe the metastatic process. Recently, a transcriptional study of tumor cells isolated from an intraocular tumor, circulating malignant cells, and subsequent metastasis in a xenograft albino rabbit model of uveal melanoma was published (26). This study identified several factors that were up-regulated from intraocular tumor to circulating malignant cells, and from circulating malignant cells to metastasis (26). Some of these highly up-regulated genes include *insulin receptor substrate-2*, *fibronectin 1*, and *cytokeratin 18* (26). Conversely, a decrease in vimentin and melanoma-specific markers, such as MelanA and CD63, was seen.

These studies, combined with others using a variety of techniques such as immunohistochemical studies of tissue sections, orthotopic animal models, and *in vitro* assays, have provided hints as to the possible pathways implicated in liver metastasis from uveal melanoma.

Hepatocyte Growth Factor/Scatter Factor and c-Met

One of the molecules that has long been suspected of playing a role in specific growth of cells in the liver is hepatocyte growth factor (HGF), also known as scatter factor, and its corresponding receptor, c-Met (Fig. 1). Expression of c-Met by uveal melanoma cells was shown to be correlated with their expression of epithelial specific cytokeratin, previously described as the interconverted phenotype (27, 28).

Studies have shown that increased levels of c-Met expression in the primary tumors of patients significantly increased the risk of those patients to develop subsequent liver metastasis (29). Uveal melanoma cells have also been shown to become highly motile and invasive when HGF was used as a chemoattractant (30, 31). The exposure of uveal melanoma cells to tumor associated macrophages, the presence of which is an indicator of worse prognosis in uveal melanoma, was also shown to increase the expression of HGF by tumor cells (32).

On activation of c-Met by HGF, c-Met is autophosphorylated on two tyrosine residues. This initiates the formation of a docking site that can recruit intracellular adapter proteins, such
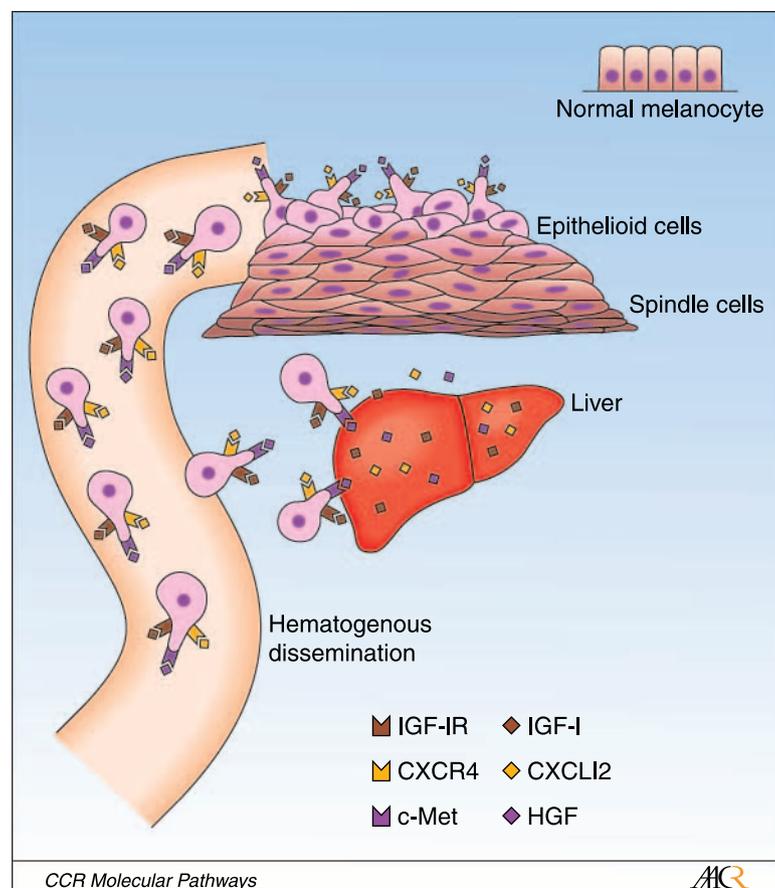

Fig. 1. Compared with normal melanocytes, uveal melanoma cells have increased expression of c-Met, IGF-IR, and CXCR4. The liver is the only organ that highly expresses the corresponding ligands of these receptors (HGF, IGF, and CXCL12), indicating that these pathways may be highly involved in the liver-specific metastases in uveal melanoma.
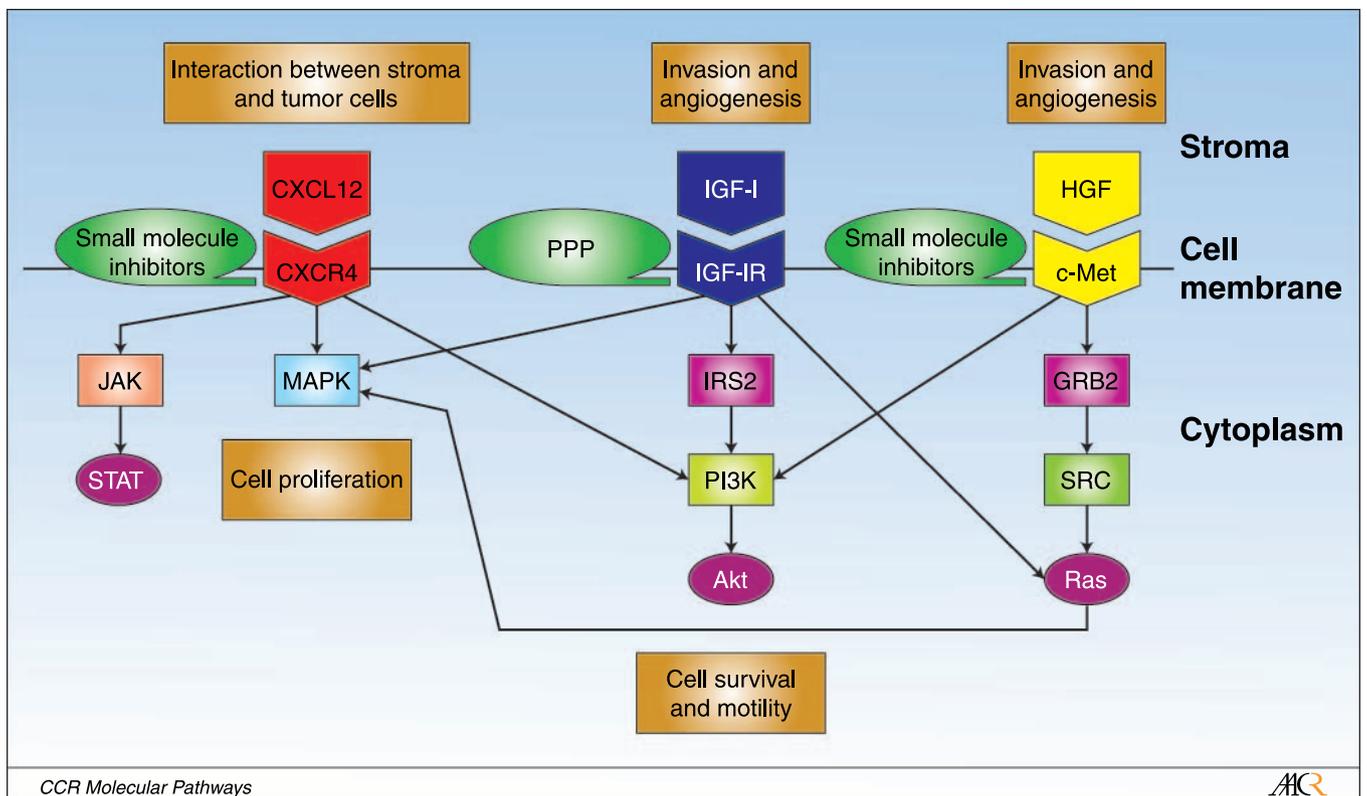
Fig. 2. Several pathways may be involved in the formation of liver metastasis in uveal melanoma patients with potential therapeutic targets of each pathway.

as growth factor receptor bound protein 2, phosphatidylinositol 3-kinase, Shc, and Src (33). This leads to multiple downstream signaling pathways, including the Ras protein kinase pathway. These pathways lead to the up-regulation of multiple genes and can increase cellular proliferation, cell cycle progression, protection from apoptosis, increased cellular motility, and invasive ability (ref. 33; Fig. 2).

The expression of HGF alone, however, does not explain the predominance of liver-specific metastasis. A study by Economou and co-workers (34) described the interrelation of insulin-like growth factor I receptor (IGF-IR) and c-Met from uveal melanoma samples. Interestingly, although the coexpression of these two molecules was highly predictive of liver metastasis by univariate analysis, only IGF-IR expression had a significant prognostic value when analyzed by multivariate analysis (ref. 34; Fig. 1).

IGF-I and IGF-IR

Molecules of more recent interest in uveal melanoma are IGF-I and its receptor as expression of these have been shown to correlate with worse prognosis (35). Similar to HGF, IGF-I is mainly produced by the liver and may help explain the preferential homing or growth of cells to the liver in uveal melanoma patients. IGF-I binds to IGF-IR, a heterotetrameric plasma membrane glycoprotein, which is composed of two α and two β subunits (36). When stimulated by ligand binding, the intrinsic tyrosine kinase activity of the β subunit is activated and phosphorylation of several intracellular proteins takes place (37).

At least nine substrates of the insulin/IGF-I receptors have been described. It is believed that the phosphorylation of these diverse substrates may have different cellular effects depending on the substrate that is activated (38). The insulin receptor substrate family members bear structural and functional similarities; however, insulin receptor substrate-1 is believed to mediate the mitogenic effects of the IGF-I receptor, whereas insulin receptor substrate-2 is believed to play a role in metabolic and proliferative signals triggered by the insulin receptors (38). It is also interesting to note that insulin receptor substrate-2 is the major effector of insulin signaling in the liver. Among the downstream pathways that can be stimulated by insulin receptor substrate-2 is the phosphorylation of Akt via phosphatidylinositol 3-kinase. Phosphorylated Akt has also been shown to be a prognostic indicator of increased metastasis in patients with uveal melanoma. It is possible that this increased mortality rate may reflect the activation of Akt through the IGF-I pathway. Other downstream pathways that have been shown to be activated are Ras and mitogen-activated protein kinase (ref. 38; Fig. 2).

Activation of IGF-IR has been shown to play a role in cellular proliferation, protection from apoptosis, migration, integrin-mediated adhesion to the extracellular matrix, and invasion of basement membranes (39). These are all essential steps in the formation of metastasis. Targeting of this pathway by using a specific inhibitor of IGF-IR tyrosine phosphorylation called cyclolignan picropodophyllin was shown to cause tumor regression in a xenograft mouse model (40). Targeting this receptor pathway may be a good choice for preventing or at least decreasing the chance of metastasis in patients (Fig. 2).

Table 1. Summary of recent therapeutic targets and agents that have been used *in vitro* and in clinical trials for various types of cancers including uveal melanoma

Strategies	Potential targets	Implicated cancers	Therapeutic agents	Phase of development/ clinical trials
Inhibit induction of invasion and angiogenesis	c-Met	Bladder (58), breast (58), cervical (58), colorectal (58), lung (58), liver (58), adult T-cell leukemia (58), multiple myeloma (58), glioblastomas/ astrocytomas (58), melanoma (58)	PHA665752— several (59, 60)	*In vitro*
	IGF-IR	Breast (61), prostate (62), uveal melanoma (63)	Nordihydroguaiaretic acid—breast cancer (61)	*In vitro*
			Gene therapy (IGF-IR dominant negative)—gastric cancer (64)	*In vivo* (mouse)
			PPP—uveal melanoma (40)	*In vitro/in vivo* (mouse)
Inhibit interactions between disseminated tumor cells and stoma at metastatic site	Chemokines (CXCR4/CXCL12), integrins	Breast (42), uveal melanoma (50)	AMD3100 and other small-molecule inhibitors (65)	Phase II
Inhibit signaling pathways implicated in cell survival and motility	PI3K	Breast (66), colon (67), uveal melanoma (68)	LY294002—ovarian cancer (69)	*In vitro/in vivo* (mouse)
			Wortmannin— breast cancer (70)	*In vivo* (murine)
	Akt	Prostate (71), uveal melanoma (46)	API-2—melanoma (72)	*In vitro*
			Celecoxib analogues (73)	*In vitro*

Abbreviations: PI3K, phosphatidylinositol 3-kinase; PPP, picropodophyllin.

CXCR4 and CXCL12

An emerging field of study has been the family of chemokines. Of these, the CXCL12 or stromal derived factor-1 may be the most interesting. The major receptor for CXCL12 is believed to be CXCR4, a cell surface G-protein–coupled seven-span transmembrane receptor (41). This receptor has been widely reported in many different cancer types, including breast, prostate, and colon cancer (42–44). Currently, CXCL12 is the only ligand that has been described for CXCR4, making it unique among chemokines and chemokine receptors that typically have several potential ligands and receptors.

Activation of CXCR4 by CXCL12 has been shown to lead to a variety of intracellular signal transduction pathways and regulation of cellular survival, proliferation, migration, and adhesion (45). Among the multiple pathways that are activated is phosphatidylinositol 3-kinase, which subsequently phosphorylates Akt. As mentioned previously, activated Akt is associated with worse prognosis in uveal melanoma and plays a role in proliferation of cells as well as migration (46). Other pathways of interest that have been shown to be activated by CXCL12/CXCR4 are mitogen-activated protein (MAP) kinase and Janus-activated kinase (JAK)/signal transducer and

activator of transcription (STAT). Activation of the MAP kinase pathway is common in uveal melanoma, although a report by Zuidervaart and colleagues (47) showed that, in contrast to cutaneous melanoma, this pathway is seldom activated by BRAF or Ras. Future experiments will have to be carried out to establish if this often-seen activation of MAP kinase in uveal melanoma is caused by CXCL12 signaling or if a second, currently undefined, ligand is responsible (Fig. 2).

Recently, it has been shown that cancer cells are capable of exploiting and hijacking this system to facilitate their movement and extravasation out of the primary site and into systemic circulation (48). It has also been hypothesized and widely believed that the CXCR4/CXCL12 mono-axis may play a critical role in guiding circulating malignant cells (CXCR4-positive cells) to organ-specific locations that actively secrete CXCL12, such as bone, brain, lungs, and, most importantly, the liver (ref. 49; Fig. 1). An animal model of breast cancer in which the CXCR4/CXCL12 axis was inhibited showed repression of the metastatic process (48). Recent work in uveal melanoma has shown that, although cell lines typically do not express CXCL12, tumors can express CXCR4, and this expression correlates with markers of poor prognosis (30, 50). In addition, it has been shown that TN14003 inhibits

migration of uveal melanoma cell lines when chemoattracted by CXCL12 (51). More work will have to be done to categorize the exact pathways of activation in uveal melanoma and establish for which part of liver-specific metastasis CXCL12 is responsible.

Clinical-Translational Advances

There are currently several treatments for primary uveal melanomas that are located in the posterior part of the eye. Historically, uveal melanoma has been treated by enucleation of the affected globe, which in addition to loss of vision requires the implantation of prosthesis. This has been supplanted by the use of globe-sparing treatments such as plaque radiotherapy, in which a shield containing a radioactive isotope such as ruthenium is implanted opposite the base of the tumor on the sclera of the affected eye for several days. No significant change in patient mortality has been shown for either treatment option (52). The majority of patients are treated by plaque radiotherapy, although a percentage of patients still undergo enucleation because of failure of the primary treatment or the size of tumor on diagnosis.

Although treatment of the primary tumor is well established and usually results in local control, treating metastatic disease is a much more daunting task. At diagnosis, few patients present with clinically detectable metastatic lesions regardless of the clinical prognostic factors such as tumor height (18). As mentioned previously, the outlook for patients becomes increasingly poor as follow-up time increases, with an almost 50% mortality rate due to metastatic disease after 15 years. Minor improvements have been seen in some patients after surgical resection of the liver metastasis, when possible, followed by intrahepatic arterial chemotherapy, with all of the associated morbidity. Clinical trials assessing potential therapeutic agents, such as dacarbazine, treosulfan in combination with gemcitabine, thalidomide with IFN α2b, temozolomide, and 9-nitrocamtothecin, have also been similarly disappointing with only minor improvements in some patients (53–57).

We have described here several pathways (Fig. 2) that we believe play a role in the majority of uveal melanoma patients who go on to develop liver metastasis as the first detectable metastasis and the major cause of mortality among uveal melanoma patients (Fig. 1). These pathways represent opportunities to halt the progression of this disease and to use uveal melanoma as a model system for hematogenous dissemination and liver metastasis formation in other cancers. It seems unlikely, from the evidence given, that targeting only one of these molecular pathways would be sufficient to improve patient mortality. It is far more likely that it will take a combined approach, targeting at least two of these pathways that may induce liver-specific metastasis, to lead to an improvement in patient mortality rates. Several inhibitors of these specific pathways are in development, with a handful already in phase II clinical trials for other diseases. Table 1 shows the status of several of these molecules that are specific for the pathways that have been discussed above, including inhibitors targeting c-Met, IGF-IR, phosphatidylinositol 3-kinase, Akt, and CXCL12/CXCR4. It would be of great importance to test these molecular targets in the context of uveal melanoma and study the effects that they would have not only on liver metastasis but also on hematogenous dissemination of tumor cells.

To date, the majority of clinical trials have been run with metastatic uveal melanoma patients with diagnosed liver metastasis. These trials have been disappointing, showing little or no effect of drug treatments on reducing the metastatic burden. We believe that an adjuvant trial, beginning at diagnosis, would yield better results for patients. These trials would, by the nature of the disease, have to run for up to 10 years to establish efficacy. Biomarkers of disease progression and response could also be used, such as melanoma inhibitory activity, S100, or the detection of circulating malignant cells. It is perhaps naïve to believe that compounds will be able to cure established metastatic nodules of the liver. Unlike other cancer types, however, there is a large window in which to operate for uveal melanoma.

References

1. Egan KM, Seddon JM, Glynn RJ, Gragoudas ES, Albert DM. Epidemiologic aspects of uveal melanoma. Surv Ophthalmol 1988;32:239–51.
2. Ivry GB, Ogle CA, Shim EK. Role of sun exposure in melanoma. Dermatol Surg 2006;32:481–92.
3. Shah CP, Weis E, Lajous M, Shields JA, Shields CL. Intermittent and chronic ultraviolet light exposure and uveal melanoma: a meta-analysis. Ophthalmology 2005;112:1599–607.
4. Holly EA, Aston DA, Char DH, Kristiansen JJ, Ahn DK. Uveal melanoma in relation to ultraviolet light exposure and host factors. Cancer Res 1990;50: 5773–7.
5. Seddon JM, Albert DM, Lavin PT, Robinson N. A prognostic factor study of disease-free interval and survival following enucleation for uveal melanoma. Arch Ophthalmol 1983;101:1894–9.
6. Manning WS, Jr., Greenlee PG, Norton JN. Ocular melanoma in a Long Evans rat. Contemp Top Lab Anim Sci 2004;43:44–6.
7. Fernandes BF, Marshall JC, Burnier MN, Jr. Blue light exposure and uveal melanoma. Ophthalmology 2006; 113:1062 e1; author reply.
8. Marshall JC, Gordon KD, McCauley CS, de Souza Filho JP, Burnier MN. The effect of blue light

exposure and use of intraocular lenses on human uveal melanoma cell lines. Melanoma Res 2006;16: 537–41.
9. Callender GR. Malignant melanotic tumors of the eye: a study of histologic types of 111 cases. Trans Am Acad Ophthalmol Otolaryngol 1931;36:131–42.
10. McLean IW, Foster WD, Zimmerman LE, Gamel JW. Modifications of Callender's classification of uveal melanoma at the Armed Forces Institute of Pathology. Am J Ophthalmol 1983;96:502–9.
11. Singh AD, Topham A. Survival rates with uveal melanoma in the United States: 1973-1997. Ophthalmology 2003;110:962–5.
12. Jensen OA. Malignant melanomas of the human uvea: 25-year follow-up of cases in Denmark, 1943-1952. Acta Ophthalmol (Copenh) 1982;60:161–82.
13. Raivio I. Uveal melanoma in Finland. An epidemiological, clinical, histological and prognostic study. Acta Ophthalmol Suppl 1977;133:1–64.
14. Kath R, Hayungs J, Bornfeld N, Sauerwein W, Höffken K, Seeber S. Prognosis and treatment of disseminated uveal melanoma. Cancer 1993;72: 2219–23.
15. Lorigan JG, Wallace S, Mavligit GM. The prevalence and location of metastases from ocular melanoma:

imaging study in 110 patients. AJR Am J Roentgenol 1991;157:1279–81.
16. Rajpal S, Moore R, Karakousis CP. Survival in metastatic ocular melanoma. Cancer 1983;52:334–6.
17. Bedikian AY. Metastatic uveal melanoma therapy: current options. Int Ophthalmol Clin 2006;46: 151–66.
18. Rietschel P, Panageas KS, Hanlon C, Patel A, Abramson DH, Chapman PB. Variates of survival in metastatic uveal melanoma. J Clin Oncol 2005;23: 8076–80.
19. Woll E, Bedikian A, Legha SS. Uveal melanoma: natural history and treatment options for metastatic disease. Melanoma Res 1999;9:575–81.
20. Nicolini A, Giardino R, Carpi A, et al. Metastatic breast cancer: an updating. Biomed Pharmacother 2006;60:548–56.
21. Widel MS, Widel M. [Mechanisms of metastasis and molecular markers of malignant tumor progression. I. Colorectal cancer]. Postepy Hig Med Dosw (Online) 2006;60:453–70.
22. Maniotis AJ, Folberg R, Hess A, et al. Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry. Am J Pathol 1999; 155:739–52.

23. Hendrix MJ, Seftor EA, Hess AR, Seftor RE. Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. Nat Rev Cancer 2003;3:411 – 21.

24. Onken MD, Worley LA, Ehlers JP, Harbour JW. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. Cancer Res 2004;64:7205 – 9.

25. Onken MD, Ehlers JP, Worley LA, Makita J, Yokota Y, Harbour JW. Functional gene expression analysis uncovers phenotypic switch in aggressive uveal melanomas. Cancer Res 2006;66:4602 – 9.

26. Marshall JC, Nantel A, Blanco P, Ash J, Cruess SR, Burnier MN, Jr. Transcriptional profiling of human uveal melanoma from cell lines to intraocular tumors to metastasis. Clin Exp Metastasis 2007;24:353 – 62.

27. Hendrix MJ, Seftor EA, Seftor RE, et al. Regulation of uveal melanoma interconverted phenotype by hepatocyte growth factor/scatter factor (HGF/SF). Am J Pathol 1998;152:855 – 63.

28. Hendrix MJ, Seftor EA, Seftor RE, et al. Biologic determinants of uveal melanoma metastatic phenotype: role of intermediate filaments as predictive markers. Lab Invest 1998;78:153 – 63.

29. Mallikarjuna K, Pushparaj V, Biswas J, Krishnakumar S. Expression of epidermal growth factor receptor, ezrin, hepatocyte growth factor, and c-Met in uveal melanoma: an immunohistochemical study. Curr Eye Res 2007;32:281 – 90.

30. Di Cesare S, Marshall JC, Logan P, et al. Expression and migratory analysis of 5 human uveal melanoma cell lines for CXCL12, CXCL8, CXCL1, and HGF. J Carcinog 2007;6:2.

31. Woodward JK, Elshaw SR, Murray AK, et al. Stimulation and inhibition of uveal melanoma invasion by HGF, GRO, IL-1α and TGF-β. Invest Ophthalmol Vis Sci 2002;43:3144 – 52.

32. Cools-Lartigue J, Marshall JC, Caissie AL, Saraiva VS, Burnier MN, Jr. Secretion of hepatocyte growth factor and vascular endothelial growth factor during uveal melanoma-monocyte *in vitro* interactions. Melanoma Res 2005;15:141 – 5.

33. Peruzzi B, Bottaro DP. Targeting the c-Met signaling pathway in cancer. Clin Cancer Res 2006;12:3657 – 60.

34. Economou MA, All-Ericsson C, Bykov V, et al. Receptors for the liver synthesized growth factors IGF-I and HGF/SF in uveal melanoma: intercorrelation and prognostic implications. Invest Ophthalmol Vis Sci 2005;46:4372 – 5.

35. All-Ericsson C, Girnita L, Seregard S, Bartolazzi A, Jager MJ, Larsson O. Insulin-like growth factor-1 receptor in uveal melanoma: a predictor for metastatic disease and a potential therapeutic target. Invest Ophthalmol Vis Sci 2002;43:1 – 8.

36. Pollak MN. Insulin-like growth factors and neoplasia. Novartis Found Symp 2004;262:84 – 98; discussion-107, 265 – 8.

37. Adhami VM, Afaq F, Mukhtar H. Insulin-like growth factor-I axis as a pathway for cancer chemoprevention. Clin Cancer Res 2006;12:5611 – 4.

38. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414:799 – 806.

39. Riedemann J, Macaulay VM. IGF1R signalling and its inhibition. Endocr Relat Cancer 2006;13 Suppl 1: S33 – 43.

40. Girnita A, All-Ericsson C, Economou MA, et al. The insulin-like growth factor-I receptor inhibitor picropodophyllin causes tumor regression and attenuates mechanisms involved in invasion of uveal melanoma cells. Clin Cancer Res 2006;12:1383 – 91.

41. Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood 2006;107:1761 – 7.

42. Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50 – 6.

43. Wang J, Wang J, Sun Y, et al. Diverse signaling pathways through the SDF-1/CXCR4 chemokine axis in prostate cancer cell lines leads to altered patterns of cytokine secretion and angiogenesis. Cell Signal 2005;17:1578 – 92.

44. Zeelenberg IS, Ruuls-Van Stalle L, Roos E. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. Cancer Res 2003; 63:3833 – 9.

45. Yasumoto K, Koizumi K, Kawashima A, et al. Role of the CXCL12/CXCR4 axis in peritoneal carcinomatosis of gastric cancer. Cancer Res 2006;66:2181 – 7.

46. Saraiva VS, Caissie AL, Segal L, Edelstein C, Burnier MN, Jr. Immunohistochemical expression of phospho-Akt in uveal melanoma. Melanoma Res 2005;15: 245 – 50.

47. Zuidervaart W, van der Velden PA, Hurks MH, et al. Gene expression profiling identifies tumour markers potentially playing a role in uveal melanoma development. Br J Cancer 2003;89:1914 – 9.

48. Liang Z, Wu T, Lou H, et al. Inhibition of breast cancer metastasis by selective synthetic polypeptide against CXCR4. Cancer Res 2004;64:4302 – 8.

49. Dhawan P, Richmond A. Role of CXCL1 in tumorigenesis of melanoma. J Leukoc Biol 2002;72:9 – 18.

50. Scala S, Ierano C, Ottaiano A, et al. CXC chemokine receptor 4 is expressed in uveal malignant melanoma and correlates with the epithelioid-mixed cell type. Cancer Immunol Immunother 2007;56:1589 – 95.

51. Di Cesare S, Marshall JC, Fernandes BF, et al. *In vitro* characterization and inhibition of the CXCR4/CXCL12 chemokine axis in human uveal melanoma cell lines. Cancer Cell Int 2007;7:16.

52. Diener-West M, Earle JD, Fine SL, et al. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma, III: initial mortality findings. COMS Report No. 18. Arch Ophthalmol 2001;119:969 – 82.

53. O'Neill PA, Butt M, Eswar CV, Gillis P, Marshall E. A prospective single arm phase II study of dacarbazine and treosulfan as first-line therapy in metastatic uveal melanoma. Melanoma Res 2006;16:245 – 8.

54. Schmittel A, Schuster R, Bechrakis NE, et al. A two-cohort phase II clinical trial of gemcitabine plus treosulfan in patients with metastatic uveal melanoma. Melanoma Res 2005;15:447 – 51.

55. Solti M, Berd D, Mastrangelo MJ, Sato T. A pilot study of low-dose thalidomide and interferon α-2b in patients with metastatic melanoma who failed prior treatment. Melanoma Res 2007;17:225 – 31.

56. Bedikian AY, Papadopoulos N, Plager C, Eton O, Ring S. Phase II evaluation of temozolomide in metastatic choroidal melanoma. Melanoma Res 2003;13: 303 – 6.

57. Ellerhorst JA, Bedikian AY, Smith TM, Papadopoulos NE, Plager C, Eton O. Phase II trial of 9-nitrocamptothecin (RFS 2000) for patients with metastatic cutaneous or uveal melanoma. Anticancer Drugs 2002;13: 169 – 72.

58. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev 2003;4:915 – 25.

59. Ma PC, Schaefer E, Christensen JG, Salgia R. A selective small molecule c-MET Inhibitor, PHA665752, cooperates with rapamycin. Clin Cancer Res 2005;11: 2312 – 9.

60. Smolen GA, Sordella R, Muir B, et al. Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. Proc Natl Acad Sci U S A 2006;103: 2316 – 21.

61. Youngren JF, Gable K, Penaranda C, et al. Nordihydroguaiaretic acid (NDGA) inhibits the IGF-1 and c-erbB2/HER2/neu receptors and suppresses growth in breast cancer cells. Breast Cancer Res Treat 2005; 94:37 – 46.

62. Hellawell GO, Turner GD, Davies DR, Poulsom R, Brewster SF, Macaulay VM. Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. Cancer Res 2002;62:2942 – 50.

63. Mallikarjuna K, Pushparaj V, Biswas J, Krishnakumar S. Expression of insulin-like growth factor receptor (IGF-1R), c-Fos, and c-Jun in uveal melanoma: an immunohistochemical study. Curr Eye Res 2006;31: 875 – 83.

64. Min Y, Adachi Y, Yamamoto H, et al. Insulin-like growth factor I receptor blockade enhances chemotherapy and radiation responses and inhibits tumour growth in human gastric cancer xenografts. Gut 2005;54:591 – 600.

65. Cashen AF, Nervi B, DiPersio J. AMD3100: CXCR4 antagonist and rapid stem cell-mobilizing agent. Future Oncol (London, England) 2007;3:19 – 27.

66. Wu G, Xing M, Mambo E, et al. Somatic mutation and gain of copy number of PIK3CA in human breast cancer. Breast Cancer Res 2005;7:R609 – 16.

67. Velho S, Oliveira C, Ferreira A, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. Eur J Cancer 2005;41:1649 – 54.

68. Hess AR, Seftor EA, Seftor RE, Hendrix MJ. Phosphoinositide 3-kinase regulates membrane type 1-matrix metalloproteinase (MMP) and MMP-2 activity during melanoma cell vasculogenic mimicry. Cancer Res 2003;63:4757 – 62.

69. Hu L, Hofmann J, Lu Y, Mills GB, Jaffe RB. Inhibition of phosphatidylinositol 3′-kinase increases efficacy of paclitaxel in *in vitro* and *in vivo* ovarian cancer models. Cancer Res 2002;62:1087 – 92.

70. Lemke LE, Paine-Murrieta GD, Taylor CW, Powis G. Wortmannin inhibits the growth of mammary tumors despite the existence of a novel wortmannin-insensitive phosphatidylinositol-3-kinase. Cancer Chemother Pharmacol 1999;44:491 – 7.

71. Shukla S, Maclennan GT, Hartman DJ, Fu P, Resnick MI, Gupta S. Activation of PI3K-Akt signaling pathway promotes prostate cancer cell invasion. Int J Cancer 2007;121:1424 – 32.

72. Yang L, Dan HC, Sun M, et al. Akt/protein kinase B signaling inhibitor-2, a selective small molecule inhibitor of Akt signaling with antitumor activity in cancer cells overexpressing Akt. Cancer Res 2004;64: 4394 – 9.

73. Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. J Biol Chem 2000;275:11397 – 403.