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Melanoma-Induced Endothelial Cell Growth Involves Phospholipase A$_2$ and COX$_2$ Upregulation

Mario Alberghina, Carla Motta, Gabriella Lupo,
Carmelina D. Anfuso and Renato Bernardini
Department of Clinical and Molecular Biomedicine, University of Catania, Catania,
Italy

1. Introduction

Angiogenesis, the process of formation of new vessels, is fundamental in many biological processes including development, reproduction and wound repair. In tumor biology, key biochemical questions remain to be answered, in particular the molecular and cellular mechanisms responsible for establishing melanoma new vessels. The search of role for already identified soluble factors in experimental cell models encompassing brain endothelial cells, murine melanoma cell lines and pericytes could expand our molecular understanding of cerebral melanoma proliferation. The purpose of this chapter is to summarize existing knowledge of biochemical mechanisms of melanoma adhesion and angiogenesis, as well as new experimental data on the enzymatic and signaling cross-talk between tumor and microvascular cells.

2. Mechanisms of melanoma adhesion and transendothelial migration

During hematogeneous metastasis the blood borne malignant cells must successfully arrest in the microcirculation, invade the endothelium and escape from the vascular system. The process of extravasation (formation of dynamic, F-actin-rich pseudopodia that penetrate capillary endothelial walls) occurs when malignant cells, adherent to vascular endothelial cells (ECs), cause retraction of these cells and exposure of the underlying basal lamina. Models to studying the interaction between malignant cells and normal endothelium have been developed in vitro, using monolayers of vascular ECs, monolayers of vascular ECs on smooth muscle cell multilayers, or multicell biological membranes such as chorioallantoic or amniotic membranes (Nicholson, 1982).

Initial interactions of highly metastatic tumor cells with ECs cause morphological changes in ECs and involve mechanical contact and transient adhesion, mediated by endothelial selectins and their ligands on the neoplastic cells. This contact initiates a sequence of activation pathways that involves cytokines, growth factors, bioactive lipids, and reactive oxygen species produced by either the cancer cells or the endothelium. These molecules elicit expression of integrin adhesion molecules in cancer cells and ECs, matrix metalloproteinases, and chemotactic factors that promote, by gradient, the attachment of

tumor cells to the vessel wall and/or transvascular penetration. Induction of endothelial free radicals can be cytotoxic to cancer cells. Afterwards, the adhesion of tumor cells to endothelial basal lamina, where they rapidly spread by solubilizing the protein structure, appears to be mediated by specific cell molecules such as fibronectin, laminin, collagen and glycosaminoglycans. Considerable information on the regulation of the adhesive and migratory properties of melanoma cells on the endothelial floor is available (Cangara et al., 2010; Klemke et al., 2007).

2.1 Integrins

Several modes of integrin-mediated interaction between melanoma cells and ECs have been documented, e.g. between LFA-1 (αLβ2), VLA-4 (α4β1), and α4β7 on melanoma cells and their major receptors, ICAM-1, VCAM-1, and MAdCAM-1, respectively, on ECs. Adhesion of integrin/CD44 to hyaluronan is also important for the extravasation of melanoma cells into tissues. Therefore, not only the combination of chemokine receptors, but also adhesion molecules expressed on melanoma cells can determine their selective migration toward a particular site. Integrins are a superfamily of transmembrane heterodymeric (α and β subunits) surface receptors involved in cell-matrix and cell-cell adhesion. So far, at least, 18 α subunits and 8 β subunits have been isolated and characterized. Metastatic melanoma cells do express multiple adhesion receptors which can be either extremely specific for a single ligand or capable of binding multiple ligands. It is likely that the tumor cell's repertoire of adhesion receptors may influence not only its adhesive properties, but its metastatic characteristics as well. α6β1 integrin is expressed on the highly metastatic cell line B16/129 melanoma (Ruiz et al., 1993). It was suggested that α6 integrins play a dual role in the metastatic process, mediating the adhesion of tumor cells to the luminal surface of the endothelium and the adhesion to laminin in the subendothelial extracellular matrix (ECM) during extravasation. Malignant phenotype of melanoma cells expresses a melanoma-specific integrin (α7β1) that binds laminin and is not detectable in normal melanocytes (Kramer et al., 1991).

The expression of several cell adhesion molecules, notably vitronectin binding receptor αvβ3, has been associated with the metastatic potential of tumor cells. Confocal microscopy revealed the presence of the integrin αvβ3 on melanoma membrane protrusions and pseudopods penetrating the endothelial junction. αvβ3 was also enriched in heterotypic contacts between ECs and melanoma cells (Voura et al., 2001) (Fig. 1). However, many human melanoma cells do not express β3 integrins. Human melanoma cells with different metastatic potency, which do not express β2 or β3 integrins, express the VCAM-1 receptor α4β1. VCAM-1 is up-regulated on activated ECs and promotes transendothelial migration (Klemke et al., 2007). On the other hand, α6β1 and α6β4 integrins are present at a high level on the luminal and basolateral side of vascular endothelium. Human melanoma cell lines that express high constitutive levels of the metastasis-associated marker ICAM-1 were found to secrete IL-1 in vitro. The IL-1 present in melanoma-conditioned medium induced the expression of VCAM-1, endothelial-leukocyte adhesion molecule 1, and ICAM-1 on ECs in culture, and increased the rate at which melanoma cells and ECs adhered to each other (Burrows et al., 1991).

2.2 Signal transduction

Tumour cell transition from fluid to initial adhesive conditions to the endothelium involves an early polarization event and major rearrangements of the submembrane cytoskeleton

that remain poorly understood. PKC is involved in the metastatic characteristics of melanoma cells. Among PKC isoforms, the α isoform is postulated to increase motility of melanoma cells (Oka & Kikkawa, 2005). Reduced PKCβ and increased PKCζ and PKCι expression, at both protein and mRNA levels in melanoma cell lines, was found (Voris et al., 2010). As a molecular mechanism for enhancement of invasion, PKC was proposed to mediate the signal of 12-(S)-hydroxyeicosatetraenoic acid (HETE), a 12-lipoxygenase metabolite of arachidonic acid, that upregulates expression of integrin molecules. In metastatic melanoma cells, misregulated expression of PKCα and PKCδ, and elevated Src activity are required for efficient αvβ3-mediated invasion.

Activation of signal transduction through the PKC/MEK/ERK during melanoma cell line B16BL6 cell invasion and metastasis has been reported (Tsubaki et al., 2007). In this context, results of Sandoval et al. (2001) suggest a critical role for Ca^{2+} signaling and activation of PKCα in mediating the disruption of VE-cadherin junctions, and thereby in the mechanism of increased endothelial permeability (Fig. 1).

In integrin-ECM interactions, downstream signaling events include Ras, phosphatidylinositol-3 kinase (PI3K), MAP kinases, FAK, Src, Akt, Abl and Rac, Rho and cdc42 small GTPases. Signaling pathway via α5β1 and αvβ3 integrins may be mediated by cross-talk with receptor tyrosine kinases associated with growth factor ligands (Soung et al., 2010). Overexpression of the small GTPase, RhoC, in various human cancers has been correlated with high metastatic ability and poor prognosis. Rho-kinase (ROCK) is an important effector of Rho GTPases. The oncogenic serine/threonine kinase Akt is a downstream effector of PI3K. Akt activation contributes to the neoplastic phenotype by promoting cell cycle progression, increasing antiapoptotic functions, and enhancing tumor cell invasion. Using a human melanoma cell line WM35, results suggest that RhoC promotes invasion in part via activation of the PI3K/Akt pathway, in a manner independent of ROCK signaling (Ruth et al., 2006). In addition, PKCα and Src enhance αvβ3-mediated invasion in part by increasing the GTPase activity of Rac relative to RhoA. PKCα influences focal adhesion formation, while PKCδ controls stress fibers (Putman et al., 2009). PKC interacts with Rho GTPases in the regulation of the actin cytoskeleton. The PKC-α isozyme binds the Rho GTPase cdc42, and both are coordinated with the Rac-PI3K signaling pathway in melanoma cell invasion and migration on extracellular matrix proteins (Byers et al., 2010).

Transendothelial migration (TEM) is blocked by pharmacological inhibitors of Src family protein-tyrosine kinases (PP2 and herbimycin A), PI3K (wortmannin), and phosphatidylinositol-specific phospholipase C (U73122). These data indicate that there are signaling pathways for TEM independent of chemokine attraction, but through adhesion molecules including CD44 (Katakai et al., 2002). Integrins regulate cell adhesion process partially through control of extracellular signal-regulated kinases 1 and 2 (ERK1/2). In mutant B-Raf-expressing melanoma cells (SKMEL-24 and SKMEL-28), the ERK1/2 pathway was constitutively active, and ECM adhesion-dependent regulation of ERK1/2 activity was by-passed. Furthermore, in melanoma cells, ERK1/2 translocates to the nucleus and regulated transcription events in an adhesion-independent manner (Conner et al., 2003). On the other hand, PI3 kinase/Akt pathway is downstream of the major vascular endothelial growth factor (VEGF) receptors in endothelial cells, and it is activated in EC migration process in response to tumor efferent stimulation (Anfuso et al., 2009; Brader & Eccles, 2004) (Fig. 1).

Contact of melanoma cells to HUVEC triggered rapid endothelial [Ca^{2+}]$_i$ response through PLC-IP3 pathway. In addition, alternation of endothelial adherens junctions following

contact of melanoma cells was evidenced by changes in immunological staining patterns of vascular endothelial (VE)-cadherin. Inhibition of PI3K resulted in a reduction of melanoma cell transmigration (Peng et al., 2005).
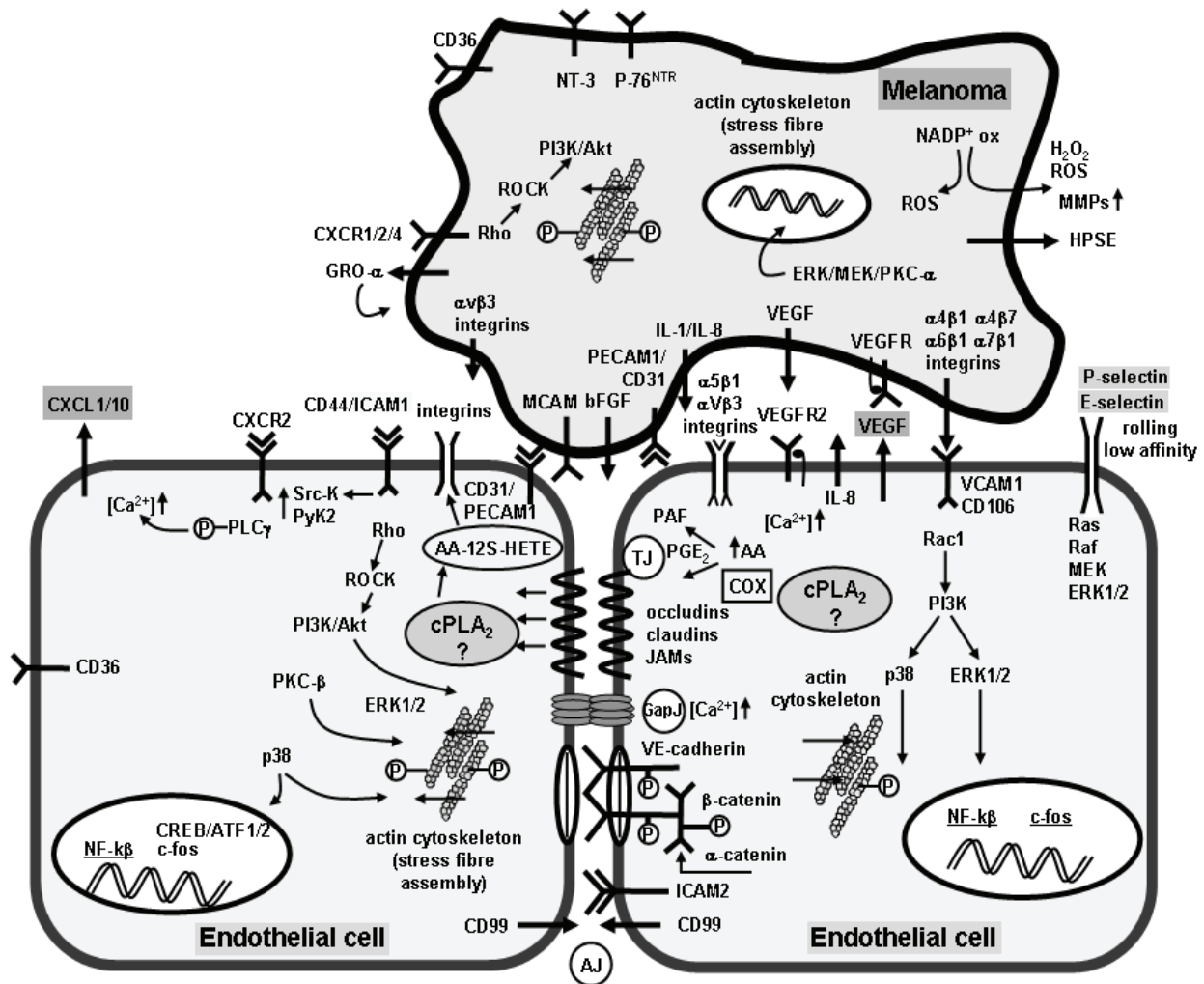

Fig. 1. Cell surface interactions and cellular responses between melanoma cells and endothelial cells (ECs), and ECs–ECs in blood vessels. During dissemination of malignant cells, the attachment of cancer cells to the ECs on microvasculature is considered to be an essential step. A spectrum of growth factors/chemokines is able to induce intracellular signaling responses in both ECs and melanoma cells which reciprocally secrete similar autocrine and paracrine growth factors and chemokines. Signaling responses include functional cross-talk between growth factor/growth factor receptors and integrins, and actin/myosin cytoskeleton reorganization. ECs exploit cytoskeletal elements to ensure the integrity of the cell monolayer in quiescent endothelium, and to enable the disintegration of the former barrier in response to various agonists. Functional association between VEGFR2 and integrin αvβ3 is of reciprocal nature since each receptor is able to promote activation of its counterpart. Potential tumor cell response includes enhanced cell proliferation, proteolytic activity, migration and invasion. Potential EC response contributes with tumor neovascularization and loosing of permeability properties of the microvascular barrier at the tight and gap junctions, and adherens junctions (cadherins)

2.3 Cadherins and selectins

E-cadherin is a protein with extracellular, transmembrane and cytoplasmic domains. The surface glycoprotein of E-cadherin acts to connect neighboring cells whereas the cytoplasmic tail is noncovalently linked to the actin cytoskeleton via catenins. E-cadherin not only acts as molecular glue, but also mediates intracellular signaling through β-catenin. Growth factor signaling pathways are important in regulating the cadherin-catenin complex through phosphorylation and dephosphorylation of β-catenin. Melanoma cells induce vascular VE-cadherin junction disassembly through heterotypic contact with human umbilical vein ECs (HUVEC) in co-culture. Melanoma-induced VE-cadherin disassembly and upregulation of p38 MAP kinase in ECs are regulated by both soluble factors from melanomas, particularly interleukin IL-8, IL-6, and IL-1β, VEGF, sVEGFR-1, bFGF, and through vascular cell adhesion molecule-1 (Khanna et al., 2010; Ruffini et al., 2011).

During cell-cell adhesion process, attachment of melanoma cells on the endothelium induces a twofold increase in transmembrane N-cadherin expression in melanoma cells and the redistribution of N-cadherin to the heterotypic contacts. Whereas N-cadherin and β-catenin colocalize in the contact regions between melanoma cells and HMVEC during the initial stages of attachment, β-catenin disappears from the heterotypic contacts during transmigration of melanoma cells. Immunolocalization and immunoprecipitation studies indicate that N-cadherin becomes tyrosine-phosphorylated, resulting in the dissociation of β-catenin from these contact regions. Concomitantly, an increase in the nuclear level of β-catenin occurs in melanoma cells, together with a sixfold increase in β-catenin-dependent transcription (Qi et al., 2005).

E-selectin and P-selectin, expressed on the EC surface, are considered to play also an important role in hematogenous metastasis, and are a marker for proliferating endothelium (Ludwig et al., 2004). E-selectin and P-selectin are barely expressed in unstimulated endothelial cells. Whereas E-selectin is not expressed in ECs in vivo, unless cells are stimulated by an inflammatory cytokine such as IL1β or tumor necrosis factor α, E-selectin is expressed in response to cytokines secreted by tumor cells in cancer patients (Kannagi et al., 1997). The adhesion of cancer cells to E-selectin expressed by ECs regulates the barrier function of these cells. An increase in the activity of endothelial ERK and p38 mitogen-activated protein kinases, with concomitant enhancement of TEM and migration of cancer cells has been observed (Tremblay et al., 2006).

On the other hand, activation of ERK by E-selectin modulates the opening of interendothelial spaces by initiating the activation of Src kinase activities and the dissociation of the VE-cadherin/β-catenin complex.

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Melanoma cellular adhesion molecule (MCAM), also known as MUC18, is a membrane glycoprotein that functions as a Ca^{2+}-independent adhesion molecule, and mediates homotypic and heterotypic adhesion between melanoma cells and ECs, respectively (McGary et al., 2002). Up-regulation of receptors and signaling molecules not found on melanocytes, but important for melanoma-melanoma and melanoma-EC interactions such

as MCAM, N-cadherin and zonula occludens protein-1 (ZO-1), is operative in this context (Haass et al., 2005). In addition, transcription factors CREB/ATF-1 and ATF-2 are up-regulated in these cells. The expression of genes involved in angiogenesis, invasion and apoptosis such as bFGF, IL-8, EGF-R, PAR-1 correlates with higher metastatic potential of human melanoma cells (Melnikova et al., 2009) (Fig. 1).

2.4 Chemokines

Migration of melanoma cells into tissues occurs via a complex and sequential interaction between melanoma and ECs. If melanoma cells and the endothelium express pairs of tethering molecules such as L-, P-, and E-selectins and their sialylated carbohydrate ligands, melanoma cells flowing through the blood stream become tethered to a vessel wall and can roll along the endothelium. Subsequently, integrin activation and cell arrest in the endothelium are induced by chemokines, soluble or matrix-bound chemotactic factors produced by the surrounding tissue or ECs, and presented on the luminal surface of the vessel wall. EC-secreted chemoattractants can induce, in fact, melanoma cell chemotaxis.

A large number of chemokines have been discovered in recent decades. CXC chemokines are heparin-binding proteins that display unique disparate roles in the regulation of angiogenesis. Members (CXCL1-8) that contain the ERL motif bind to CXC chemokine receptor 2 (CXCR2) on endothelium and are angiogenic (Vandercappellen et al., 2008). For example, a serial mechanism for the maintenance of angiogenic microenvironment encompasses VEGF activation on ECs which can lead to upregulation of antiapoptotic molecule, Bcl-2, that in turn promotes the expression of EC-derived CXCL8; the upregulated expression of CXCL8 functions in an autocrine and paracrine manner to maintain the angiogenic EC phenotype. The growth-related oncogene (GRO) subgroup of chemokines (CXCL1/GROα, CXCL2/GROβ, CXCL3/GROγ) was originally identified from culture supernatant of melanoma cell lines (Richmond & Thomas, 1988), and acts as autocrine growth factor for the melanoma and other tumors (Fig. 1).

Some chemokines such as IL-8, RANTES (CCL5), MIP-1β (CCL4), and IP-10 (CXCL10) selectively attract melanoma cells. Chemotactic response to IL-8 is mediated by CXC-chemokine receptor CXCR1 (Ramjeesingh et al., 2003). CXCL1,2,3 are important mediators of tumorigenesis related to melanoma. CXCR1 or CXCR2 are stably overexpressed in human melanoma cell lines, and CXCR1- or CXCR2-induced modulation of the melanoma cell proliferation and migration was observed to be mediated through stimulated ERK1/2 phosphorylation (Singh et al., 2009) and PKA, PI3K/Akt, PKC, Ras/Raf/MEK/JNK/p38/ERK2 enhanced activities (Strieter et al., 2006). On the other hand, tumor ECs secrete high levels of CXCL9 in all, and CXCL10 in most melanoma metastases (Amatschek et al., 2011). In this context, tumor-derived chemokines further determine influx of ECs into the tumor. In HUVEC, GRO-α markedly increases metalloproteinase MMP-1 and -2, VEGF, angiopoietin-2, CD31, and receptor KDR and CXCr2 (Caunt et al., 2006).

Chemokines deliver downstream signals via heterotrimeric G-protein-coupled receptors, and not only increase the affinity of integrins for their ligands, but also stimulate cell motility. CXCL12/CXCR4 is the most commonly expressed chemokine/chemokine receptor pair in human cancers, in which it regulates cell adhesion, extravasation, metastatic colonization, angiogenesis, and proliferation. All of these processes require activation of signaling pathways that include G proteins, PI3K, JAK kinases, Rho GTPases, and focal adhesion-associated proteins.

In a human melanoma cell line, PI3Kγ regulates tumor cell adhesion in response to CXCL12 stimulation, through mechanisms different from those involved in cell invasion. Data indicate that, following CXCR4 activation after CXCL12 binding, the invasion and adhesion processes are regulated differently by distinct downstream events in these signaling cascades (Monterrubio et al., 2009).

3. Extracellular matrix degradative enzymes (heparanase, metalloproteinases) and oxidative stress

Heparan sulfate proteoglycans (HSPGs) are ubiquitous molecules present as cell surface components anchored in the plasma membrane, as ingredients of the insoluble ECM or as soluble molecules present in ECM and serum (Lander & Selleck, 2000). Cell surface HSPGs play a role in the cell signaling integration and influence biological processes by interacting with a large number of physiologically macromolecules (e.g., growth factors, chemokines) that regulate cell behavior in normal and pathological processes (Iozzo & San Antonio, 2001).

It has become increasingly clear that heparan sulfates (HSs) and HSPGs play important roles in regulating disease processes including tumor progression and invasion (Sasisekharan et al., 2002). Metastatic melanomas show aberrant modulation of several key HS biosynthetic enzymes such as 3-O-sulfotransferase and 6-O-sulfotransferase, and also catabolic enzymes such as HSulf-1, HSulf-2 and heparanase (HPSE), the mammalian endoglucuronidase whose promotion activity of aggressive tumor behavior has been widely implicated in cancer metastasis (Arvatz et al., 2011). In ECs, heparanase colocalizes with lysosomes predominately around the nucleus, and angiogenic factors cause its dispersion towards the plasma membrane for subsequent secretion. Extracellular heparanase, secreted by melanoma and ECs, is able to cleaving heparan sulfate side chains of HSPGs including syndecans, thus contributing to degradation of ECM and basement membrane underlying epithelial and ECs, and cell invasion. The enzyme produces HS fragments which are biologically active, for example, able to bind growth and angiogenic factors. On the other hand, heparanase contributes to melanoma metastasis and angiogenesis by generating bioactive HS from the cell-surface (Raman & Kuberan, 2010; Roy & Marchetti, 2009). Inhibition of HPSE-1 expression inhibits tumor cell invasion by metastatic melanoma cells (Roy et al., 2005).

HS sequence and length are regulators of fibroblast growth factor-2 (FGF-2) activity, an important mediator of melanoma angiogenesis and progression (Herlyn, 2005). HPSE is able to both enhance and inhibit FGF-2 binding and activity in an HPSE concentration-dependent manner. Cell exposure to HPSE or to HPSE-degraded HS modulates FGF-2-induced angiogenesis in melanoma (Reiland et al., 2006). In addition, heparin and HS have profound effects on VEGF$_{165}$ function (Robinson & Stringer, 2001). Two heparin-binding sites of the VEGF$_{165}$ dimer interact simultaneously with highly sulfated S-domain regions of the HS chain linked through a stretch of transition sequence (Robinson et al., 2006).

Treatment with exogenous heparanase downregulated brain metastatic melanoma (BMM) cell invasion. Extracellular HPSE modulates BMM cell signaling by involving syndecans (SDCs)1/4 carboxy terminal-associated proteins and downstream targets. Small GTPase guanine nucleotide exchange factor-H1 (GEF-H1) is a new component of a SDC signaling complex that is differentially expressed in BMM cells compared to corresponding nonmetastatic counterparts. Knockdown of GEF-H1, SDC1, or SDC4 decreased BMM cell invasiveness and GEF-H1 modulated small GTPase activity of Rac1 and RhoA in conjunction with heparanase treatment (Ridgway et al., 2010).

Notably, although heparanase inhibitors attenuated tumor progression and metastasis, other studies revealed that heparanase also functions in an enzymatic activity-independent manner. Thus, inactive heparanase was noted to improve adhesion and migration of primary ECs and to promote phosphorylation of signaling molecules such as Akt and Src, facilitating gene transcription (i.e. VEGF) and phosphorylation of selected Src substrates (i.e. VEGF receptors). The concept of enzymatic activity-independent function of HPSE had gained substantial support by the recent identification of the HPSE C-terminus domain as the molecular determinant behind its signaling capacity (Barash et al., 2010).

Among enzymatic-independent functions are the induction of Akt/PKB phosphorylation noted in endothelial- and tumor-derived cells, stimulation of PI3K- and p38-dependent EC migration, and upregulation of VEGF, all responses contributing to its potent pro-angiogenic activity. Protein domains of heparanase required for signaling are not identified to date, nor are identified heparanase binding proteins/receptors capable of transmitting heparanase signals. The possible function of mannose 6-phosphate receptor (MPR) and low-density lipoprotein-receptor related protein (LRP), recently implicated in cellular uptake of heparanase, as heparanase receptors mediating Akt phosphorylation, has been examined. HPSE addition to MPR- and LRP-deficient fibroblasts elicited Akt activation indistinguishable from control fibroblasts. In contrast, disruption of lipid rafts abrogated Akt/PKB phosphorylation following HPSE addition. These results suggest that lipid raft-resident receptor mediates heparanase signaling (Ben-Zaken et al., 2007).

Cancer metastasis is accompanied by orchestrated proteolytic activity executed by array of proteases such as matrix metalloproteases (MMP). The repertoire of the cellular molecules expressed on the cell surface (E-cadherin silencing, N-cadherin and VCAM-1, ICAM-1 overexpression, melanotransferrin and integrin $\alpha v\beta 3$ upregulation, CD9 tetraspanin redistribution) may promote invasion and metastasis through an interaction with increased MMP-2 (Fig. 1). This MMP plays a role in assisting melanoma cells to degrade type IV collagen in the basement membrane, an early step in metastasis cascade that promotes tumor cell disassociation and invasion. One of the mechanism by which integrins modulate tumor progression is the transduction of signals regulating expression of matrix-specific MMP (Munshi & Stack, 2006). $\alpha 5\beta 1$ integrin, a fibronectin-binding specific integrin, interacts with MMP-2 collagenase on the surface of SKMEL-147 human melanoma cells, suggesting that it controls cell invasion via regulation of MMP-2 collagenase expression. This control can occur either through signaling pathways involving PI3K, Akt, ERK protein kinases and the c-Jun, or via direct recruitment of MMP-2 to the cell surface (Morozevich et al., 2009).

While the importance of specific degradative proteases such as MMPs is well documented, there is some evidence that reactive oxygen species (ROS) are also involved in tumor-cell-endothelial interactions. Recent observations have demonstrated that neoplastic cells, in particular melanomas, produce increased levels of ROS and pyrrolic end products of lipid oxidation, and thus themselves have the capability to damage ECs (Sander et al., 2003; West et al., 2010; Wittgen & van Kempen, 2007).

4. Pericytes

In tumor vascular bed, proliferating ECs and pericytes are detected, but angiogenesis is present with characteristic and significant differences among the malignant tumor types. Particularly significant are the varying degrees of pericyte recruitment indicating differences

in the functional status of the tumor vasculature. In addition, they show multiple abnormalities, i.e. loose association with ECs, extended cytoplasmic processes deep into the tumor tissue and altered expression of marker proteins (NG2, 3G5), vessel leakiness (Morikawa et al., 2002). The regulatory role of pericytes in angiogenesis is very poorly appreciated at molecular level, despite the fact that tumor metastasis invasion and intratumoral vessel growth need the perturbation of pericyte-EC cell-cell interactions and promotion of pericyte invasion during neovascularization by ECM degradation. Paracrine PDGF production by B16 mouse melanoma stimulates pericyte recruitment to tumor vessels, suggesting that pericyte abundance influences tumor cell apoptosis and tumor growth (Furuhashi et al., 2004).

5. Factors released by melanoma cells (VEGF, bFGF, PDGFα/β-TGF, IL-1, IL-8)

To promote angiogenesis, tumors secrete a variety of growth factors such as VEGF, bFGF, platelet-derived growth factor (PDGF) that induce EC activation in nearby vessels. VEGF is a major inducer of angiogenesis in tumors. In human melanoma cell lines, VEGF and bFGF expression, heterogeneous in levels, was observed (Danielsen & Rofstad, 1998). Melanoma and colorectal carcinoma cells express functional EGF/TGF- α receptors, and produce TGF-α, indicating that this growth factor is synthesized for autocrine stimulation. By screening a panel of 8 primary and 21 metastatic melanoma cell lines for constitutive secretion of cytokines, it was found that melanomas expressed bioactivity for transforming growth factor (TGF- β) (8/25 lines) and IFN (7/12), but not IL-2. Immunoassays detected interleukin IL-1α (4/25), IL-1β (12/25), IL-6 (13/29), IL-8 (29/29), TGF-β2 (5/12) and GM-CSF (11/29), but not IL-3, IL-4, TNF-α, or IFN-γ. IL-8 was produced by all lines tested. The data demonstrated that cultured melanoma cells produce a variety of cytokines which are potentially capable of influencing tumor growth in vivo (Bennicelli & Guerry, 1993). In tissue culture of choroidal melanomas and two established skin melanoma cell lines, high secretion of IL-6 was detectable in choroidal melanoma cultures, but not in the cell lines. IL-8 secretion was found in all melanoma cultures (Fig. 1). However, IL-10 was only secreted by one skin melanoma cell line and in choroidal melanoma cell cultures. Secretion of bFGF by choroidal melanomas was higher than by other cell lines. No differences were seen in the amount of TGF-β1 produced by melanoma cells (Enzmann et al., 1998). Secretion of interleukin-1 receptor antagonist (sIL-1ra), IL-6, IL-8, IL-10, TGF-α, TGF-β, VEGF, PDGF, bFGF, angiopoietin-1 and angiopoietin-2 was found in seven human uveal melanoma cell lines (Ijland et al., 1999).

In a study of a large number of uveal tumors, it was observed that while most tumor cells expressed bFGF at the protein level (89%), relatively few (22%) expressed VEGF. All 20 tumors tested by RT-PCR contained mRNA for both bFGF and VEGF. Co-culture experiments using an ATP based bioassay showed that uveal melanomas could support the growth of a rat brain endothelial cell line and HUVEC (Boyd et al., 2001). VEGF receptors VEGFR1, VEGFR2 and neurophilin-1 are expressed in A375 melanoma cells. Overproduction of VEGF$_{165}$ concomitantly expressed with its receptors favors cell growth and survival of melanoma cells through MAPK and PI3K signaling pathways (Graells et al., 2004).

PDGF receptor signaling participates in the stimulation of tumor angiogenesis. PDGF-BB is produced by ECs, and PDGFR-β is expressed by mural cells, including pericytes. PDGF-BB is produced by most types of solid tumors, and PDGF receptor signaling participates in various processes, including autocrine stimulation of tumor cell growth and recruitment of

tumor stroma fibroblasts. Furthermore, PDGF-BB-producing tumors are characterized by increased pericyte abundance and accelerated tumor growth (Suzuki et al., 2007).

By using an antibody-based proteomics strategy, galectin-1, a protein member of the group of lectins that bind to β-galactosides, was identified as protein with enhanced expression in cells from the melanocytic lineage and cancer tissues, and as a proangiogenic factor (Bolander et al., 2008). Indeed, in invasive B16 melanoma supernanants, proteomic analysis allowed the identification of 18 differential proteins, among which the proteins with a higher concentration, lactate dehydrogenase B, M2 pyruvate kinase, cathepsin D, and galectin-1 (Rondepierre et al., 2010). Uptake of galectin-1 by cultured endothelial cells specifically promotes H-Ras signaling to the Raf/MAP kinase/Mek/ERK cascade and stimulates EC proliferation and migration (Thijssen et al., 2010).

6. Cell co-culture models

The cross-talk between melanoma and ECs has been assessed in in vitro co-culture models in which signal transduction mechanisms can be closely studied. ECs are able to communicate with tumor cells through gap junctions, allowing cytosolic exchange of peptides and other small molecules (Saito-Katsuragi et al., 2007). Melanoma cells seeded on confluent lymphatic endothelial cells (LECs) were able to adhere by pseudopodia, and to induce endothelial junction dissolution and retraction. The passage of the tumor cells through the opened gap and the migration under LECs suggest that tumor cells could metastasize through the lymphatic vessel by destroying intercellular junctions (Ding Z. et al., 2005).

An interesting work demonstrated that B16 melanoma cells, together with Lewis lung carcinoma cells, produce the lipoxygenase metabolite 12(S)-HETE during in vitro interaction with CD3 microvascular ECs, which in turn induce EC retraction (Honn et al., 1994). These results suggest that 12(S)-HETE synthesis during tumor cell–endothelial cell interactions may represent a key contributory factor in cancer metastasis.

The supporting role of soluble pro-angiogenic cytokines such as bFGF and VEGF in the endothelial growth and their expression by co-cultured melanomas have been well described. Rat brain and human EC lines show significant enhancement of cell growth by 48–120 h of co-culture with melanoma cells without cell–cell contact (Boyd et al., 2002). In a co-culture system based on transwell indirect model, bone-marrow-derived mesenchymal stem cells (BMSCs) acquired endothelial phenotype and expressed VEGFR-1, VEGFR-2 and factor VIII after co-culture with B16 cells (Sun et al., 2008).

Treatments with angiogenesis inhibitors markedly suppressed in vitro tube formation by human endothelial cells and HUVEC, whereas tubular network formation by human melanoma MUM-2B and C8161 cells was relatively unaffected. The differential response of the two cell types was probably due to higher mRNA and protein endothelial expression for α5 integrin and heparin sulfate proteoglycan 2 than melanoma cells (van der Schaft et al., 2004). On the other hand, analyses of malignant melanomas in co-culture with microvascular ECs revealed a strong expression of bone morphogenic proteins and their capability to induce tube formation and migratory efficiency (Rothhammer et al., 2007).

A critical role in the regulation of melanoma-endothelial intercellular communication is interpreted by neuropilin-2 (NRP2), a co-receptor for VEGF and the semaphorin (SEMA) families. Overexpression of NRP2 in primary human ECs promoted cell survival induced by VEGF-A and VEGF-C. In contrast, SEMA3F, another ligand for NRP2, was able to inhibit

human EC survival and migration induced by VEGF-A and VEGF-C (Favier et al., 2006). In melanoma cells, NRP2 is upregulated in a two-dimensional co-culture systems of melanoma and ECs, suggesting that NRP2 assists melanoma cells in EC recruitment towards the development of a functional new vasculature, thus enhancing melanoma survival and providing potential routes for metastasis (Stine et al., 2011).

Studies using two-dimensional melanoma-EC cultures have greatly advanced our knowledge of angiogenesis, and will continue to be used extensively. Furthermore, results generated in two-dimensional cultures can be furthered by the use of three-dimensional model systems that mimic the natural tumor environment and may result in the development of treatments with better therapeutic outcomes. Collagen-implanted melanoma spheroid consists of cell aggregates grown in a collagen matrix. Melanoma spheres mimic an in vivo tumor with cells having differing access to oxygen and nutrients depending on position in the spheroid. Similar to a tumor in vivo, the proliferating cells are found on the outside of the spheroid, while the innermost cells have downregulated ERK and cyclin-dependent kinase activity (Haass et al., 2008).

In a 3-D co-culture system, human ECs and GFP-expressing melanoma cells formed distinct tumor clusters with integrated endothelial networks when seeded on tumor-derived extracellular matrix. In contrast, an entirely different phenotype was exhibited when the tumor matrix was replaced with collagen, suggesting that the extracellular matrix impinges on cellular function, possibly by an Akt-mediated mechanism (Sengupta et al., 2004). Furthermore, in melanoma cell lines co-cultured with vascular ECs in three dimensional spheroids, VEGF-induced angiogenesis was inhibited after treatment with resveratrol. This effect was associated with increased melanoma cell expression of p53 and matrix protein TSP1, as well as decreased hypoxia-driven expression of hypoxia inducible factor-1α, and inhibition of VEGF production (Trapp et al., 2010).

7. Phospholipase A$_2$ activation

Cytosolic phospholipase A$_2$ (cPLA$_2$), which regulates the provision of arachidonic acid (AA), cyclooxygenase-2 (COX-2), and prostaglandin E$_2$ release, is highly upregulated in angiogenic ECs during tumor progression (Wendum et al., 2005), promoting integrin αvβ3-mediated EC adhesion, spreading, and angiogenesis through prostaglandin cAMP-PKA dependent activation of the small GTPase Rac (Bogatcheva et al., 2005). Phospholipases A$_2$ are a diverse group of enzymes that catalyze the hydrolysis of the sn-2 substituent from glycerophospholipid substrates to yield a free fatty acid and 2-lysophospholipid acceptors (Balsinde et al., 2002). They are the rate limiting step for the AA production from membrane phospholipids, which in turn is the major precursor to prostaglandins, leukotrienes, and hydroxyeicosatetraenoic acids (via the cyclooxygenase, lipoxygenase, and epoxygenase pathways, respectively) that increase cell proliferation in response to various agonists in different cell types (Anfuso et al., 2007; Balsinde et al., 2002; Chakraborti, 2003; Lupo et al., 2002).

Among the PLA$_2$s is an 85 kDa cPLA$_2$ that requires Ca^{2+} for catalysis, and a calcium independent PLA$_2$, iPLA$_2$β, for which several potential functions have been proposed, including a housekeeping role in phospholipid remodeling and a signaling role in cell growth, apoptosis, secretion, inflammation, and oxidant-induced cell injury (Chakraborti, 2003). A number of observations that iPLA$_2$ has a functional role in cellular signaling in addition to its roles in AA release and phospholipid remodeling have been reported (Akiba

& Sato, 2004). iPLA$_2$ mediates the phosphorylation of transcription factors through a PKA-dependent pathway (Martinson et al., 2003).

We recently demonstrated that human melanoma cells highly express PLA$_2$, COX-1/COX-2, and produce higher levels of prostaglandins than normal melanocytes; this response correlated with higher proliferation rate and was blocked by specific inhibitors of PLA$_2$ and cyclooxygenase (Scuderi et al., 2008). It was also demonstrated that in vitro ECs/ melanoma co-culture or EC cultures enriched with melanoma conditioned medium (CM) recapitulate the signals that arise from the relationship between these cell types in an intact vascularized tumor (Anfuso et al., 2009). In that study, it was performed cell culture experiments in which CMs prepared from two human melanoma lines, SKMEL-28 and OCM-1, were incubated with quiescent GP8.3 rat brain immortalized ECs, in order to study the CM effect on EC proliferation and migration and on the expression of phospholipases, and activation of the ERK1/2 and PI3K/AKT pathways. Melanoma CMs significant increased endothelial AA release versus control unstimulated cells. This result indirectly demonstrated that melanoma CM-stimulated AA release in ECs was mediated by activation of phospholipase A$_2$ enzymes. The use of the specific iPLA$_2$ inhibitor, bromoenol lactone, allowed to discriminate between the c- and iPLA$_2$ activity contribution. A major contribution of cPLA$_2$ enzyme activity in mediating AA release of melanoma CM-stimulated ECs was found.

Additional findings suggested that the mitogen-activated protein kinase ERK1/2 and PI3K are involved in the signaling pathways that mainly activate cPLA$_2$. Furthermore, it was observed, by confocal microscopy, activation of cPLA$_2$ in perinuclear and membrane regions of ECs grown in CM-stimulated cultures, suggesting that cPLA$_2$ and its downstream products, after long-lasting stimulation of ECs by melanoma CMs, play significant roles in nuclear functions such as regulation of gene expression and cell proliferation.

In order to clarify the roles of cPLA$_2$ and iPLA$_2$ in ECs stimulated by melanoma in in vitro models (Fig. 2), recently we were able to demonstrate that the non-contact presence of melanoma cells in EC co-cultures, in the absence (double co-cultures) or presence (triple co-cultures) of microvascular pericytes, increased cPLA$_2$ and iPLA$_2$ protein expression (Fig. 3). In cultured ECs, 48 hour exposure to melanoma cells significantly increased cPLA$_2$ expression, with a 2.7-fold ($p<0.01$) and 1.6-fold ($p<0.01$) increase in iPLA$_2$ total protein, respectively (Fig. 3 A,B). Furthermore, melanoma-stimulated GP8.3 ECs showed an increase in the constitutive phosphorylated form of cPLA$_2$ by 8.3-fold ($p<0.01$) compared to unstimulated control cells. Thus, the increase of cPLA$_2$ synthesis and phosphorylation (p-cPLA$_2$/cPLA$_2$ ratio from 0.20 to 1.66 for melanoma-stimulated ECs) may support an increase in cPLA$_2$ activity.

Since endothelial PKCα might be involved in stimulated endothelial cPLA$_2$ activity and p42/p44 MAPK phosphorylation by melanoma cells via the Raf-MEK pathway, we further evaluated the PKCα and ERK1/2 protein expression and activation. Tumor cells had no significant effect on total PKCα expression compared with unstimulated control ECs (Fig. 3C); however, the presence of melanoma did induce a 2.1-fold ($p<0.01$) increase in phosphorylated PKCα form expression in ECs, with basal phosphorylation levels around 70% in unstimulated ECs. In addition, as shown in Fig. 3D, the presence of melanoma cells in EC co-cultures did not induce changes or increases in endothelial total p42/p44 MAPK (ERK1/2, double band) protein expression. On the contrary, melanoma-stimulated GP8.3 cells showed an increase in phosphorylated form of ERK1/2 by 3.1-fold (p42, $p<0.01$), compared to control unstimulated ECs grown alone. These data indicate that in ECs the ERK kinase phosphorylation and PLA$_2$ activation are coincident. These results suggest that
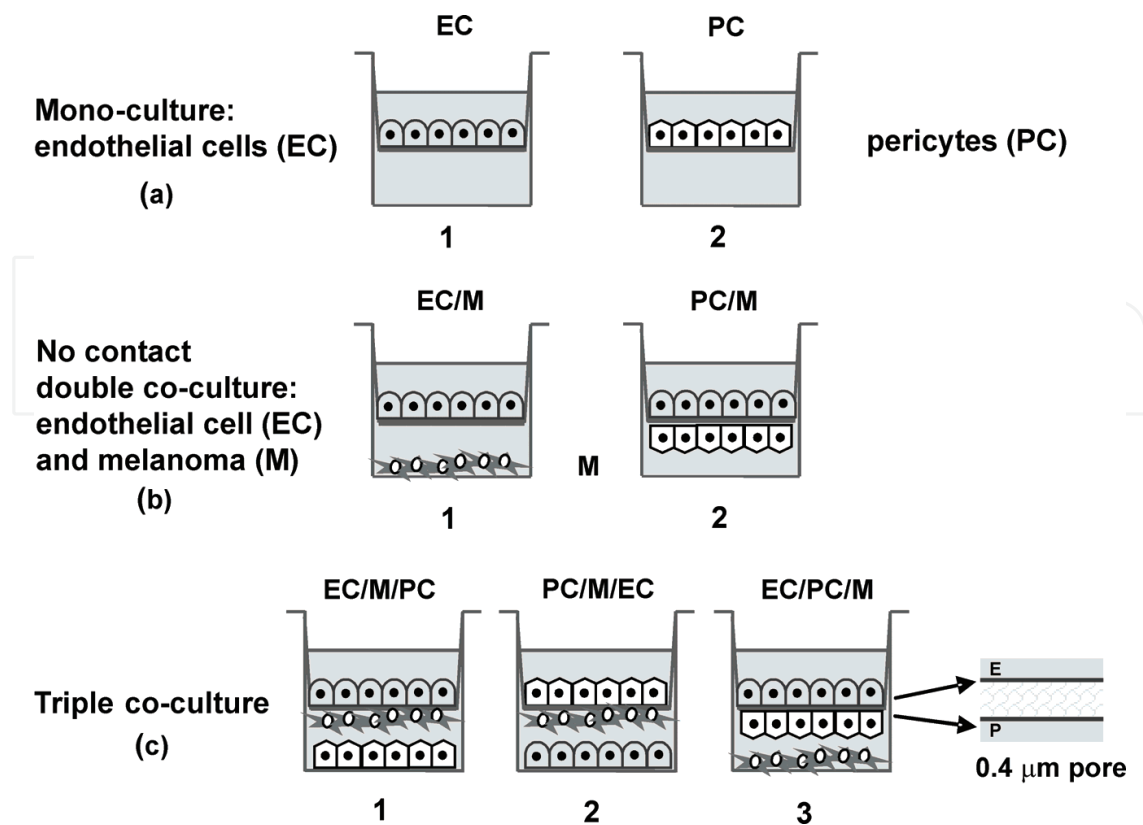
Fig. 2. Scheme of different Transwell systems for co-cultures of endothelial cells (ECs) and melanoma derived cells in monolayers. Immortalized rat brain endothelial cells (GP8.3) were fed with F10 HAM's medium supplemented with 10% FBS, 80 µg/ml heparin, 2 mM glutamine and antibiotics. Cultures of pericytes isolated from microvessels were prepared from bovine retinas as described previously (Lupo et al., 2001). The isolated cells were then cultured in DMEM supplemented with 2 mM glutamine, 10% FBS and antibiotics. COLO38 is a malignant melanoma cell line which expresses the MPG antigen and was grown in RPMI medium containing 2 mM glutamine, 10% FBS and antibiotics. Culture plates and inserts were coated on the upper and the bottom side with collagen. (a) EC or pericyte monolayers (1 and 2). (b) Melanoma cells are placed on the bottom of the wells, while ECs are present in the semipermeable filter insert (1). Pericytes are seeded on the lower side of the membrane insert, while ECs are present in the apical side of the filter insert (2). (c) Melanoma cells are seeded on the lower side of the membrane filter (1 and 2), and then ECs (1) or pericytes (2) are seeded in the upper compartment. In the third system, pericytes are seeded on the lower side of the filter, and then ECs are seeded in the upper compartment (3). In all systems, cells on both sides are exposed to conditioned medium from the cell type growing on the bottom of the wells. In panels (b)-2 and (c)-3, pericytes were first plated on the outside of the membrane filter (80,000 cells). 4 hour after, the inserts were placed into six-well plates with the culture medium. ECs were plated on the top surface of the same inserts (80,000 cells) on which pericytes have been plated two days before. Three days after, the inserts were placed into wells where COLO 38 melanoma cells have been already cultured for at least 2 days. As controls, ECs were also cultured with either COLO38 cells or pericytes alone (double co-culture) and without any other cell type. All control- and three cell type-cultures [panel (a)-1, panel (b)-1,2, panel (c)-3] were fed for 24 h in a serum-free DMEM-F10-HAM's (1:1) plus glutamine medium
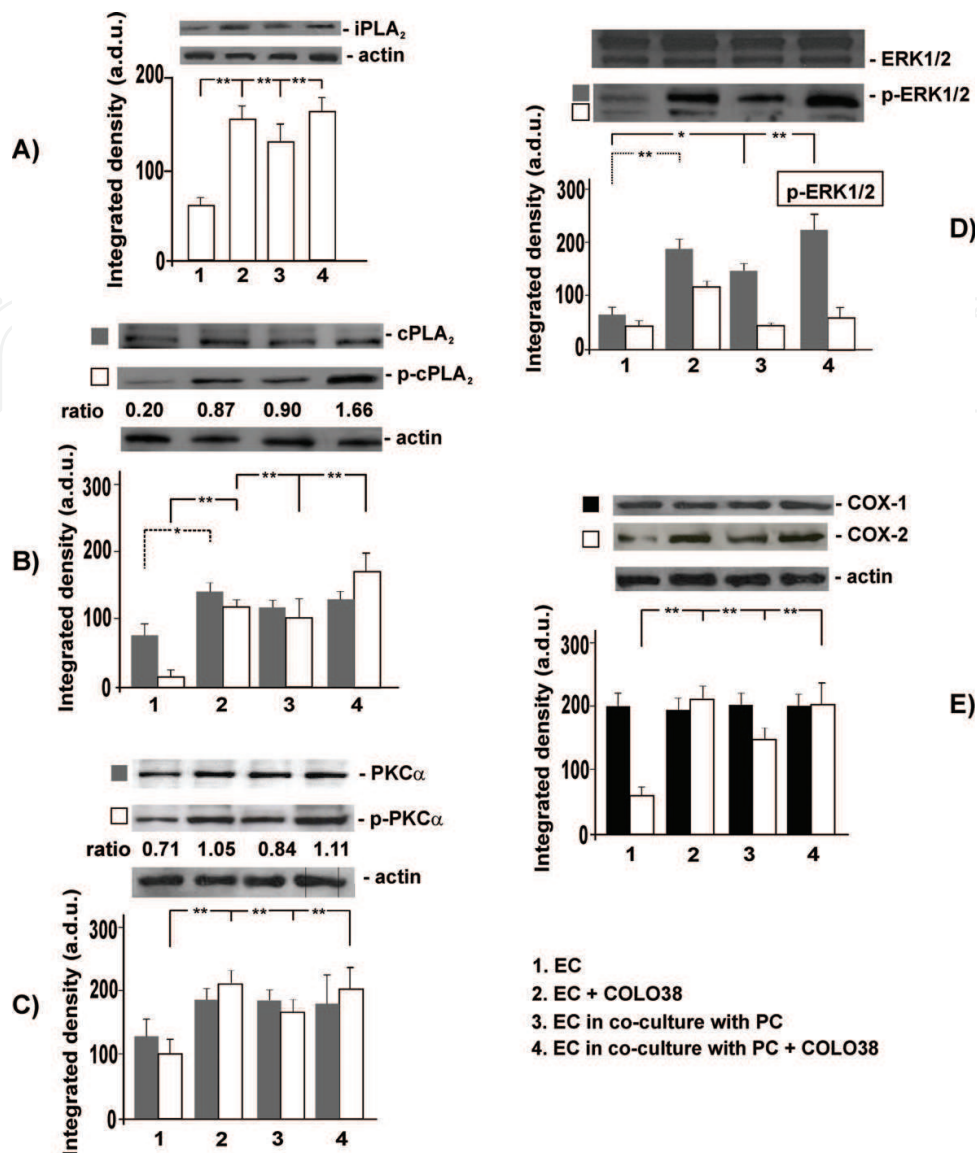
Fig. 3. Western blot analyses of cPLA$_2$, calcium-independent intracellular phospholipase A$_2$ (iPLA$_2$), total extracellular signal-regulated kinases (ERK1/2), PKCα, COX-1/-2 and phosphorylated forms of ERK1/2, cPLA$_2$ and PKCα protein expression in GP8.39 EC/bovine retinal pericyte (PC) co-cultures in the presence of COLO38 melanoma cells (triple co-culture). In the in vitro model of control blood brain barrier or tumor-conditioned blood brain barrier (see Fig. 2, panel b-2, c-3), 48 hours after incubation ECs were scraped from the insert with a rubber policeman, and lysates were resolved by SDS/PAGE (Lupo et al., 2005). Expressed proteins were independently revealed with cPLA$_2$, p-cPLA$_2$, PKCα, COX-2 or p-ERK monoclonal antibodies, and iPLA$_2$, p-PKCα, COX-1 or ERK1/2 polyclonal antibodies. The ratios of the band intensities, phospho-cPLA$_2$/total cPLA$_2$ and phospho-PKCα/total PKCα, are indicated. The values (bar graphs) expressed as arbitrary densitometric unit (a.d.u.), were obtained by the reading of blots using the Image J program, and are means ± S.E.M. from three independent experiments. Representative gels are shown; statistically significant differences by pairwise Student's t-test are indicated by asterisks (*p<0.05, **p<0.01), comparing EC/PC cultures in presence of COLO38 with EC monoculture or EC/PC co-cultures

ERK1/2 pathway is involved in enhanced AA liberation and GP8.3 EC proliferation (sequential activation of ERK1/2 and cPLA$_2$) induced by melanoma cell presence in the culture medium.

Because COX-1 and COX-2 govern the rate limiting step in the conversion of AA to its downstream prostanoid effectors, we also evaluated COX-1 and COX-2 expression in ECs in response to the presence of COLO38 cells in co-cultures. Melanoma-stimulated ECs, for 48 h, significantly expressed COX-2 total protein at levels higher (3.3-fold; p<0.01) than control unstimulated EC cultures (no additions) (Fig. 3E). Melanoma cells did not induce any change in COX-1 protein expression. These results indicate substantial contribution of COX-2 activity, chiefly stimulated by growth factors such as VEGF, to endothelial prostanoid synthesis, cell proliferation and motility in melanoma-conditioned ECs.

8. COX-2 and 12-lipoxygenase expression

COX-1 and COX-2 mediate the rate limiting step in arachidonic acid metabolism. Both in vitro and in vivo studies indicate that either COX-2 or COX-1 overexpression upregulates angiogenic factors in neoplastic cells and promotes tumor angiogenesis (Tsuji et al., 2001).

COX-2 promotes integrin αvβ3-mediated EC adhesion, spreading, migration and angiogenesis through the prostaglandin-cAMP-PKA dependent activation of the small GTPase Rac (Ruegg et al., 2004).

There are several lines of evidence indicating that increased expression of COX-2 plays a functional role in the development and progression of malignant epithelial cancer (Kuźbicki et al., 2006). COX-2 is expressed in the vasculature of surgically resected human tumors. In tumor endothelial cells (TECs) from human melanoma, compared to normal endothelial cells (NECs), COX-2 mRNA was upregulated. Cell migration and proliferation were suppressed by COX-2 inhibitor NS398 in TECs but not in NECs. The number of CD133^{+}/VEGF-2^{+} cells in the circulation was significantly suppressed by COX-2 inhibition. In addition, the number of progenitor marker-positive cells decreased in the tumor blood vessels after COX-2 treatment, which suggests that NS398 specifically targets both TECs and vascular progenitor cells without affecting NECs (Muraki et al., 2011).

COX-2 has also recently been reported as a marker of malignant melanoma (MM) in a study on 40 archival cases of MM and 35 cases of benign melanocytic lesions. The MM group and the benign melanocytic nevi group showed a highly statistically significant difference in the intensity of COX-2 expression. Staining intensity in the dermal component of MM cases also showed a tendency to increase with increasing tumor depth. By contrast, the intensity of the dermal component in the melanocytic nevi group decreased with increasing depth as the nevus cells matured from type A to type C cells (Minami et al., 2011). Furthermore, Kaplan-Meier curves illustrated a significant correlation between staining intensity and disease-specific survival (Becker, 2009).

In melanoma cell lines, A375 and Hs294, overexpression of COX-2 and its metabolite prostaglandin E$_2$ (PGE$_2$) receptors promotes cells migration. Treatment of A375 and Hs294 cells with berberine, an isoquinoline alkaloid, resulted in concentration-dependent inhibition of migration of these cells, which was associated with a reduction in the levels of COX-2 and PGE$_2$ receptors (EP$_2$ and EP$_4$). Treatment of the cells with 12-O-tetradecanoylphorbol-13-acetate (TPA), an inducer of COX-2 or PGE$_2$, enhanced cell migration, whereas berberine inhibited TPA- or PGE$_2$ promoted cell migration (Singh et al., 2011).

Specimens from dysplastic nevi, melanoma at different stages and melanoma metastasis lymphnodes overexpressed COX-2 compared to normal melanocytes. COX-2 was consistently observed in keratinocytes, dermal fibroblasts and inflammatory cells in regions adjacent to benign nevi and primary cutaneous melanomas (Goulet et al., 2003).

Omega-3 and omega-6 PUFA regulated COX-2 mRNA expression and PGE_2 production in 70W, a human melanoma cell line that metastasizes to the brain in nude mice. Additionally, PGE_2 increased in vitro Matrigel invasion, whereas exposure to PGE_3 significantly decreased invasion (Denkins et al., 2005).

12-Lipoxygenase (12-LOX), through its metabolite 12-HETE, has been demonstrated to play a pivotal role in experimental melanoma invasion and metastasis. Differences in 12-LOX protein expression during the progression of melanoma from human skin melanocytes to benign and dysplastic nevi have been found. Melanomas had higher levels of expression compared with dysplastic nevi, suggesting that 12-LOX may be an important marker of cancer progression (Winer et al., 2002).

9. Arachidonic acid metabolites: prostaglandins, 12(S)-HETE, leukotrienes

Lipid mediators derived from the arachidonic acid/5-LOX metabolism have a fundamental stimulatory role in melanoma progression associated with inflammation, whereas ω3 PUFA–derived metabolites have opposite effects (Bachi et al., 2009). Inhibitors of $cPLA_2$, 5-LOX and COX-2 reduced pulmonary metastasis formation by B16F10 melanoma cells in a dose-dependent manner. Importantly, all these inhibitors reduced PAF-induced angiogenesis in an in vivo mouse model employing Matrigel of basement membrane injected subcutaneously, and also reduced expression of MMP-2 and MMP-9 in the lungs (Jeong et al., 2010). Studies of adhesion of a highly metastatic melanoma cell line (WM-1617) demostrated that it contains at least two types of close intercellular adhesions: classic focal adhesions, and more extensive, irregularly shaped adhesions along lamellipodial edges. These adhesions are highly dynamic and highly sensitive to PKCε activation, for example, by the 12(S)-HETE. Eicosanoid-induced cell detachment seems to be triggered by myristoylated alanine-rich C-kinase substrate (MARCKS) phosphorylation (Estrada-Bernal et al., 2009). 12(S)-HETE stimulation of human epithelial carcinoma cell (A431) migration involved ERK1/2, PKC, PI3K and Src kinase. Focal adhesion kinase, a key organizer of focal cell adhesions, was tyrosine phosphorylated in response of 12(S)-HETE treatment, phosphorylation which required Src, but not PKC, PI3K or ERK1/2 enhanced activity (Szekeres et al., 2002).

Prostaglandins (PG) and arachidonic acid metabolites affect melanocyte dendricity, melanization, and proliferation (Kashiwagi et al., 2002; Parsad et al., 2002). PGD2, leukotriene (LT) B4, LTC4, LTD4, LTE4, thromboxane B2, and 12-(S)HETE increased dendricity and tyrosinase in human epidermal melanocytes whereas PGE_1, PGF_{2a}, and 6-ketoPGF$_{1a}$ had no stimulatory effects (Tomita et al., 1992). In B16F10-induced melanoma metastasis, PGD2 reduced the protective effect of α-galactosylceramide, an effect depending on IFN-γ production by invariant NK T cells (Torres et al., 2008). Nimesulide, a selective inhibitor of COX-2 that causes the breakdown of proinflammatory 2-series prostaglandins, adversely affected the growth of B16F10 melanoma cells through the induction of differentiation, indicating that the antineoplastic activity observed for nimesulide may be ascribed to intracellular changes in PG level (Tabolacci et al., 2010).

10. Future research

The events that follow tumor cell adhesion to the endothelium and lead to either the estrablishment of secondary tumor colonies or angiogenesis are poorly understood. The availability of cell cultures of melanoma cell lines, endothelial cells, pericytes and astrocytes, and combinations in co-cultures of three or four cell types in an in vitro multicellular systems may greatly contribute to the elucidation of the interplay mechanisms during melanoma cell adhesion and migration through endothelial cells and surrounding pericytes in blood vessels. Although these triple or quadruple systems are still lacking, it is very likely that the use of them may allow in future to discover new and unexpected alterations of the gene and protein expression patterns during proinvasive, prometastatic melanoma migration as well as endothelial recruitment mediated by intracellular signaling. These findings may contribute to the development of new antivascular therapeutic agents that target both angiogenesis and tumor cell vasculogenic mimicry.

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Melanoma is considered to be one of the most aggressive forms of skin neoplasms. Despite aggressive researches towards finding treatments, no effective therapy exists to inhibit the metastatic spread of malignant melanoma. The 5-year survival rate of metastatic melanoma is still significantly low, and there has been an earnest need to develop more effective therapies with greater anti-melanoma activity. Through the accomplishment of over 100 distinguished and respected researchers from 19 different countries, this book covers a wide range of aspects from various standpoints and issues related to melanoma. These include the biology of melanoma, pigmentations, pathways, receptors and diagnosis, and the latest treatments and therapies to make potential new therapies. Not only will this be beneficial for readers, but it will also contribute to scientists making further breakthroughs in melanoma research.

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