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Biomarkers for Melanoma Diagnosis and the Technologies Used to Identify Them

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1. Introduction

Melanoma is a malignant tumor originating from melanocytes (pigment-producing cells). Although the tumor is mainly detected in skin (cutaneous melanoma), it can also be detected in the eye (uveal melanoma), gastrointestinal (GI) tract and oral mucosa and genital tract (mucosal melanoma) (Landreville et al., 2008; Akaraviputh et al., 2010; Bakalian et al., 2008; Rigel et al., 2010; Seetharamu et al., 2010). Melanoma can be classified as belonging to one of four subtypes: superficial spreading, nodular, lentigo maligna, and acral lentiginous melanoma. These subtypes are characterized based on prognosis, incidence of metastasis and the frequency of gene mutations (e.g., *BRAF* and *NRAS*) (Saldanha et al., 2006; Jaeger et al., 2007; Markovic et al., 2007; Jönsson et al., 2010). Superficial spreading melanoma is the most common form of melanoma found in Caucasian populations, while the acral lentiginous melanoma is frequently detected in Asian and African populations (Cress and Holly, 1997; Weyers et al., 1999).

Several important risk factors that have been linked to the development of melanoma have been identified. Of these risk factors, most can be considered to be either environmental factors, such as exposure to ultraviolet (UV) radiation, especially in childhood, or other host factors such as family history and melanocytic nevi (Markovic et al., 2007; Schulman and Fisher, 2009), but other cancer risk factors such as smoking (Osterlind et al., 1988), diet (Osterlind et al., 1988; Veierod et al., 1997) or hormone therapy (Naldi et al., 2005) have not been found to be associated with an increased risk of melanoma. The risk of developing melanoma is higher in Caucasian than in Asian or African populations. This is closely related to skin pigmentation as melanin has been shown to have a protective function for UV-induced melanoma and Caucasian populations show low levels of melanogenesis (Lens and Dawes, 2004; Hu et al., 2008; Jemal et al., 2010). In general, the melanocortin-1 receptor (MC1R), which is a G-protein-coupled receptor (GPCR), stimulates melanogenesis through the activation of adenylate cyclase and protein kinase A (PKA) (Jordan and Jackson, 1998; Rouzaud et al., 2003). Its genetic variants are associated with melanoma

incidence and sun sensitivity (Box et al., 2001; Markovic et al., 2007). Moreover, the risk of developing melanoma is greater in males than in females over the age of 40, although the opposite effect is observed in patients under 40 years old (Lens and Dawes, 2004; Jemal et al., 2010).

The global incidence of melanoma has increased over the past decades (Markovic et al., 2007; Jemal et al., 2010, Rigel et al., 2010). The 5-year survival rate for melanoma is higher than for other prominent cancers such as tumors of the prostate, ovary, liver and bile duct, lung and bronchus, colon and rectum, and stomach. Yet, the early diagnosis and treatment of melanoma is crucial to increasing the survival rate (Jemal et al., 2010, Rigel et al., 2010).

An important early diagnostic methodology for melanoma is the ABCDE criteria, which is defined by describing changes to the appearance of the suspected lesion based on the following features: Asymmetry, Border (irregularity), Color (variegation), Diameter and Evolution (over time). Other diagnostic strategies also typically utilized include histological and/or molecular analysis (e.g., genes or proteins profiling) of biopsied material, dermoscopy (also known as dermatoscopy or epiluminescent microscopy) using a light-based magnification or digital (computer)-assisted device, ultrasound imaging and magnetic resonance imaging (Abbasi et al., 2004; Rigel et al., 2005; Markovic et al., 2007; Psaty and Halpern, 2009; Rigel et al., 2010).

Recently there has also been a move toward establishing biomarkers for malignant melanoma. These types of biological markers are not only beneficial for the diagnosis of melanoma, but also allow physicians to monitor the recurrence of melanoma after surgical resection, or to monitor the effect of radiation or anticancer drug therapies. To identify putative melanoma biomarkers in tissue samples or body fluids, a number of methodologies can be utilized, including two-dimensional gel electrophoresis (2-DE) and high throughput microarray technology.

2. Melanoma biomarkers

2.1 Cellular signals and tissue biomarkers (immunohistochemical biomarkers)

Signal transduction pathways are the mechanism through which cells respond to the extracellular signals (ligands) required to regulate or modulate downstream gene expression. These extracellular signals activate signal transduction pathways by either penetrating the cellular membrane or binding to specific receptors. The activated receptors are then able to change the quantity or intracellular distribution of the second messengers through the use of effector molecules. Second messengers also activate protein targets, which control downstream gene expression. In these cellular signal transduction pathways, phosphorylation of the target proteins (by protein kinases), or dephosphorylation or proteolytic cleavage (by proteases) play a key role in cell division and motility, apoptosis and carcinogenesis.

In melanoma cells, major signal transduction pathways are RAS/RAF/MEK/ERK and the PI3K/AKT (as known as protein kinase B)/mTOR pathway; however, other pathways such as PLC/DAG/PKC or Wnt/β-catenin pathway have also been identified (Figure 1). The interaction of a number of different ligands [e.g., fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), or epidermal growth factor (EGF)] with their respective receptors [e.g., growth factor receptors (GFRs) (tyrosine kinase receptors)] leads to the stimulation of the RAS target protein, which has three members (HRAS, KRAS, and NRAS).

Phosphorylation of RAF kinase by RAS activates downstream targets the MAPK extracellular signal-regulated kinase-1 and -2 (MEK1 and MEK2), which causes the phosphorylation of extracellular signal-regulated kinase-1 and -2 (ERK1 and ERK2). Activated ERK1 and 2 has been found to modulate the gene expression necessary for survival and proliferation of melanoma cells, and has been linked to the increase resistance of melanoma cells to apoptosis by inhibiting the activation of caspase 8 (Becker et al., 2006; Sekulic et al., 2008). The *BRAF* mutation is necessary for ERK-mediated survival and proliferation and participates in the reduction of proapoptotic proteins (e.g., Bcl-2 family), while *RAF* genes consist of *ARAF*, *BRAF*, and *CRAF* (also known as *Raf-1*) (Becker et al., 2006; Cartlidge et al., 2008; Sekulic et al., 2008). The *BRAF* mutation, predominantly V600E (substitution of glutamate to valine; previously known to V599E), frequently occurs in melanoma and is strongly related to exposure to UV radiation (Tsao et al., 2004; Wan et al., 2004; Gray-Schopfer et al., 2005; Becker et al., 2006; Sekulic et al., 2008).

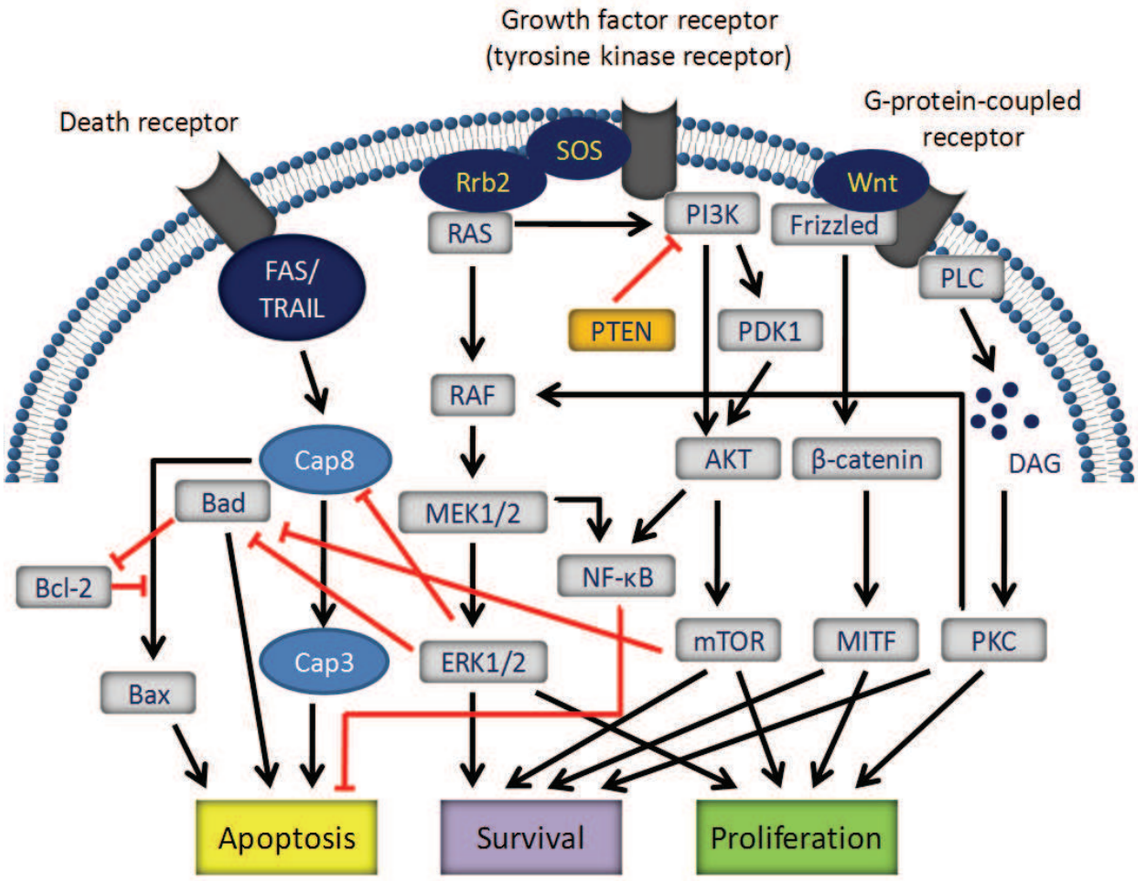

Fig. 1. Intracellular signal transduction of melanoma. RAS/RAF/MEK/ERK, PI3K/AKT/mTOR, PLC/DAG/PKC, and Wnt/β-catenin pathways are associated with survival, proliferation angiogenesis, and apoptosis of melanoma cells. Melanoma cells show an increase in the expression of survival or proliferation-associated signals and angiogenesis-associated signals, but a decrease in the expression of tumor suppressor or apoptosis-associated signals

Biomarkers	Changes of expression	
Survival or proliferation-associated molecules		
GFRs (e.g., EGFR, VEGFR, FGFR, and PIGFR) 1)	Increased	Lacal et al., Odorisio et
GPCRs (e.g., Wnt/fizzled receptor and chemokine receptors) 1)	Increased	Lee et al., 2
c-kit, one of GPCRs	Increased, but decreased in metastatic melanoma	Janku et al.
Activated PI3K	Increased	Becker et a
Activated AKT	Increased	Stahl et al.,
Activated ERK1/2	Increased	Cohen et al
Activated protein kinase C (PKC)α	Increased	Lahn and S
β-catenin 2)	Increased	Sanders et
Cytokines (e.g., IL-1, IL-6, IL-8, and IL-10)	Increased	Ciotti et al.
Heat shock proteins (HSPs) (e.g., HSP 27 or 90)	Increased	McCarthy
Microphthalmia transcription factor (MITF)	Increased, but decreased in metastatic melanoma	Garraway Fecker et a
Apoptosis-associated molecules		
Antiapoptotic Bcl-2 family (Bcl-2, Bcl-XL, and Mcl-1)	Increased 3)	Leiter et al.
Proapoptotic Bcl-2 family (multidomain proteins; Bax and Bak)	Decreased	Fecker et a
Proapoptotic Bcl-2 family (BH3-only proteins; Bad, Bid, Bim, PUMA, and NOXA)	Decreased	Eisenmann Ley et al., 2
TRAIL-R1 (DR4) and TRAIL-R2 (DR5)	Decreased	Zhang et a
Activated NF-κB	Increased	Ueda and I
Tumor suppressor-associated molecules		
p4ARF	Decreased	Krimpenfo
p16INK4A	Decreased	Krimpenfo
PTEN	Decreased	Stahl et al.,
Inhibitor of growth family member 3 (ING3)	Decreased	Wang et al.
Angiogenesis-associated molecules 4)		
Chemokine receptors (CXCR1 and CXCR2); one family of GPCRs	Increased	Scala et al., Richmond
Matrix metalloproteinases (MMPs)	Increased	Hofmann e
Urokinase plasminogen activator receptor (uPAR)	Increased	de Vries et
Metastasis-associated proteins		
Chemokine receptors (CXCR4, CCR7, and CCR10) 5)	Increased	Payne and Scala et al.,
Cell adhesion-associated molecules		
Cytoskeleton/structure proteins (e.g., vimentin)	Increased	Coupland

1) These receptors also stimulate melanoma angiogenesis. 2) The β-catenin functions as a cell adhesion-associated molecule. 3) Other studies suggested a decrease in the expression of antiapoptotic Bcl-2 in metastatic melanoma (Fecker et al., 2006; Zhuang et al., 2007). 4) The angiogenesis-associated molecules take part in melanoma metastasis. 5) The chemokine receptors also play an important role in melanoma growth.

Table 1. (continues on next page) Tissue biomarkers (immunohistochemical biomarkers) for the diagnosis of melanoma and changes in their expression levels

Biomarkers	Changes of expression	
MUC18	Increased	Lai et al., 200
Integrin αvβ3	Increased	Hieken et al.,
Integrin α6β4	Increased	Nikolopoulos
N-cadherin	Increased	Li et al., 2001
P-cadherin	Decreased	Sanders et al.
E-cadherin 6)	Decreased	Sanders et al.
		Molina-Ortiz
Antigens 7)		
Melanocyte lineage/differentiation antigens		
TRP1/gp75	Increased	Thomson et a
		Rad et al., 200
TRP2	Increased	Winder et al.,
Melan-A/MART-1	Increased	Chen et al., 1
Tyrosinase	Increased	Sonesson et a
gp100/pmel-17	Increased	Pardo et al., 2
S100 proteins (e.g., S100B)	Increased	Henze et al.,
Melanoma inhibitory activity (MIA)	Increased	Bosserhoff et
		Schmidt and
Cancer/testis antigens		
BAGE family	Increased	Simpson et al
GAGE family	Increased	Simpson et al
MAGE family	Increased	Simpson et al
NY-ESO-1	Increased	Chen et al., 1
		Barrow et al.,
Melanoma-associated antigens		
A 90-kDa glycoprotein (e.g., TA-90 and periostin)	Increased	Rote et al., 19
		Paulitschke e
Survivin (one of apoptosis inhibitors gene family)	Increased	Tas et al., 200
Other antigens		
Cytotoxic T-lymphocyte antigen-4 (CTLA-4) (a negative regulator for T cells)	Increased	O'Day et al.,
Galectin-3 (a β-galactoside-binding protein)	Increased	Prieto et al., 2
Preferentially expressed antigen of melanoma (PRAME) (a repressor of retinoic acid)	Increased	Epping and E
Multiple myeloma1 (MUM1) (melanoma associated antigen)	Increased	Natkunam et
Other tissue biomarkers		
Nodal/Cripto-1 (nodal coreceptor)	Increased	Topczewska

6) The E-cadherin also functions as a tumor suppressor-associated molecule. 7) The antigens are found mainly in metastatic melanoma.

Table 1. (continues) Tissue biomarkers (immunohistochemical biomarkers) for the diagnosis of melanoma and changes in their expression levels

Moreover, phosphorylation of AKT kinase by phosphatidyl inositol 3-kinase (PI3K) stimulates the mammalian target of rapamycin (mTOR), which leads to the survival and proliferation of melanoma cells. The AKT/mTOR pathway suppresses apoptosis by decreasing the levels of pro-apoptotic proteins (e.g., BAD and caspase-9). Among the three AKT members (AKT1, AKT2, and AKT3), it is AKT3 that is often overexpressed in melanoma (Stahl et al., 2004) and is regulated by the phosphatase and tensin homolog (PTEN), which degrades the products of PI3K (Wu et al., 2003; Becker et al., 2006; Sekulic et al., 2008).

In addition, many antigens, which have immunostimulatory or activator roles in tumorigenesis, have been identified in metastatic melanoma. Important antigens include the melanocyte lineage/differentiation antigens [e.g., tyrosinase-related protein-1 (TRP1)/gp75, TRP2, Melan-A/MART-1] (Thomson et al., 1985; Winder et al., 1994; Murer et al., 2004; Rad et al., 2004) and cancer/testis antigens (e.g., BAGE family, GAGE family, MAGE family, and NY-ESO-1) (Chen et al., 1998; Simpson et al., 2005; Barrow et al., 2006). Melanocyte lineage/differentiation antigens are associated with the production of melanin pigments and have been identified in both normal melanocytes and melanoma (Thomson et al., 1985; Houghton et al., 1988). Cancer/testis antigens are abundant in normal tissues during development, but in mature cells, their expression is restricted to the male germ cells in the testis and to various tumors (Simpson et al., 2005).

As indicated, several gene mutations such as *NRAS* (Q61K/R), *BRAF* (V600E), *PTEN*, and *CDKN2A* mutation play an important role in the occurrence of melanoma (Tsao et al., 2004; Wan et al., 2004; Gray-Schopfer et al., 2005; Becker et al., 2006; Sekulic et al., 2008). These mutations are excellent targets for the diagnosis of melanoma. Moreover, in the context of melanoma prognosis, melanoma cells show an increase in the expression of survival or proliferation-associated molecules, angiogenesis-associated molecules, and in the expression of antigens, but a decrease in the expression of tumor suppressor-associated proteins (e.g., PTEN) or proapoptotic proteins (e.g., Bax and Bak) is observed (Table 1). Thus, these molecules, which show altered expression levels in melanoma relative to normal cells, are useful tissue biomarkers (immunohistochemical biomarkers) for melanoma diagnosis. However, in spite of these discoveries, these markers are not specific to melanoma and there are few melanoma-specific tissue biomarkers, excluding melanocyte lineage/differentiation antigens and melanoma-associated antigens (e.g., TRP1/gp75 and 2, Melan-A/MART-1, and TA-90) that are overexpressed in metastatic melanoma.

2.2 Serologic biomarkers for melanoma diagnosis

Many researchers have identified putative serologic biomarkers for melanoma diagnosis (Table 2), which play a key role in the growth and survival of melanoma cells. Typically, these markers activate survival and/or proliferation-associated and angiogenesis-associated signal transduction pathways after binding with their receptors. The most important receptors of this kind are GFRs (e.g., VEGFR and FGFR) and GPCRs (e.g., MC1R, Wnt/frizzled receptor, and chemokine receptor) (Halaban, 1996; Lee et al., 2008).

The primary antigens that have been observed in the serum of melanoma patients are melanocyte lineage/differentiation antigens and melanoma-associated antigens. The existence of these antigens in serum is closely associated with melanoma progression and low survival rates. Thus, these types of markers are useful as prognostic biomarkers and have potential to act as therapeutic targets.

In the absence of vascularization, the growth of melanoma is limited to 0.2 – 0.3 cm due to the limited diffusion of oxygen and nutrients into the tumor. For additional growth, angiogenesis is essential for providing adequate blood supply to the growing lesion. Angiogenesis is regulated by proangiogenic factors, such as VEGF, FGF, tumor necrosis factor (TNF), and interleukin-8 (IL-8) and by antiangiogenic factors, such as interferons (IFNs) and angiostatin. An increase in vascular density provides a greater supply of oxygen and nutrients to cells, leading to melanoma growth (Folkman, 2007; Mahabeleshwar and Byzova, 2007). High levels of proangiogenic factors in the serum of melanoma patients can be used as an indicator of melanoma at diagnosis.

Multiple cytokines (e.g., IL-1, 4, 6, 8, 10 and 14), which are correlated with melanoma growth, angiogenesis and metastasis, have been observed in the serum of melanoma patient at both the protein and/or mRNA level. Serum levels of these cytokines are increased in metastatic melanoma patients, suggesting that they can be used as an indicator of melanoma progression (Porter et al., 2001; Varney et al., 2006; Yurkovetsky et al., 2007). Moreover, the serum concentration of the soluble IL-2 receptor is elevated in patients with metastatic melanoma and elevated serum IL-2 receptor levels are associated with lowered survival rates (Boyano et al., 1997; Ottaiano et al., 2006). Interestingly, IFNs are soluble cytokines, but possess antiangiogenic and antitumor activities. An increase in the melanoma progression-associated cytokines leads to a reduction in IFNs levels, but there is a decrease in the serum levels of melanoma progression-associated cytokines in melanoma patients following immunomodulatory therapy with IFNs (mainly IFN-α2b)(Singh and Varner, 1998; Jonasch and Haluska, 2001; Yurkovetsky et al., 2007; Dummer and Mangana, 2009; Hofmann et al., 2011). Thus, the analysis of a number of different serum cytokines may be a useful means of monitoring the efficacy of immunomodulatory therapy.

2.3 Urinary biomarkers for melanoma diagnosis

Urinary biomarkers for melanoma diagnosis have received much greater interest because of the relative ease of sample collection and handling compared with the analysis of blood or tissue samples, but this form of sample may lack the sensitivity required for a diagnostic biomarker. Of the urinary biomarkers of melanoma already identified (Table 3), 5SCD and 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI2C), are intermediate metabolites in melanin pigment formation, and have been the most extensively studied. An increase in urine levels of these markers has been associated with tumor progression and low survival rates (Kärnell et al., 1997; Bánfalvi et al., 2000; Wakamatsu et al., 2002). In healthy patients, the urinary levels of these markers are influenced by age (Meyerhöffer et al., 1998), sex (Morishima and Hanawa, 1981; Kågedal et al., 1992), skin color (Wirestrand et al., 1985) and season (Ito et al., 1987), but not by pregnancy (Carstam et al., 1985). Although both 5SCD and 6H5MI2C have been detected in the urine of melanoma patients, because of the higher levels of 5SCD, this marker is considered a more reliable urinary biomarker for melanoma than the 6H5MI2C (Kärnell et al., 1997, 2000; Wakamatsu et al., 2006). Moreover, the 90-kDa glycoprotein (TA-90)(Rote et al., 1980; Euhus et al., 1989), S100A7 (Brouard et al., 2002) and β-human chorionic gonadotropin (Carter et al., 1995) have also been identified in the urine of patients with melanoma (Table 3).

Of these urinary biomarkers, 5SCD, 6H5MI2C, and S100A7 can be considered the most melanoma-specific of the urinary biomarkers (Kärnell et al., 1997; Bánfalvi et al., 2000; Brouard et al., 2002; Wakamatsu et al., 2002).

Biomarkers	Functions
Proangiogenic factors	
VEGF 1)	VEGF receptor (VEGFR) ligand
FGF 1)	FGF receptor (FGFR) ligand
EGF 1)	EGF receptor (EGFR) ligand
Placental growth factor (PIGF) 1)	Neuropilin-1 and -2 receptor ligand
TNF 1)	GPCR ligand
IL-8 (CXCL8) 1)	GPCR (specially, chemokine receptor CXCR1 and 2) ligand
Laminin-5 2)	Laminin receptor (e.g., integrin α6β4 and α7β1) ligand
Osteopontin 2)	Integrin αvβ3 ligand
uPA 2)	uPAR ligand
Antigens 3)	
Melanocyte lineage/Differentiation antigens	
Tyrosinase	Regulator enzyme in melanin synthesis; Increased in metastatic prognosis
gp100/pmel-17	Melanin synthesis-associated melanosomal matrix glycoprotein; Increased in metastatic prognosis
S100 proteins (e.g., S100B)	Cell division and differentiation-associated acidic calcium-binding protein; Increased in metastatic prognosis
MIA	A small soluble protein; Increased in metastatic prognosis
L-dopa/L-tyrosine ratio	An index of tyrosinase functional activity; Increased in metastatic prognosis
Melanoma-associated antigens	
TA-90	Potential immunostimulator or antineoplastic activator; Increased in metastatic prognosis
Survivin (one of apoptosis inhibitors gene family)	Apoptosis inhibition; Increased in metastatic prognosis
Cytoplasmic/high-molecular-weight melanoma-associated antigen (CYT-MAA/HMW-MAA)	Unknown exactly, but may relate to melanoma progression

[1)] These proangiogenic factors also function as an important stimulator for melanoma growth.[2)] These proangiogenic factors also take part in melanoma metastasis.

Table 2. (continues on next page) Serologic biomarkers for the diagnosis of melanoma

Biomarkers	Functions
Other antigens	
Galectin-3	A β-galactoside-binding protein; Increased in metastatic prognosis
Synovial sarcoma X breakpoint-2 (SSX-2)	A family of highly homologous synovial sarcoma X (SSX) breakpoint proteins and repressive gene regulator
Gangliosides (GM2, GD2, GM3, and GD3)	Group of glycosphingolipids; Relate to interactions between melanoma cells
Cytokines and cytokine receptors 4)	
IL-1	Survival or proliferation-associated factor
IL-4	Survival or proliferation-associated factor
IL-6	Survival or proliferation-associated factor
IL-10	Survival or proliferation-associated factor
IL-12	Survival or proliferation-associated factor
Soluble IL-2 receptor	Survival or proliferation-associated factor
Other serologic biomarkers	
YKL-40	Unknown exactly, but may function as a survival or proliferation-associated factor
C reactive protein (CRP)	Unknown exactly, but may relate to tumor-associated inflammatory response
Lactate dehydrogenase (LDH)	An indicator for liver metastasis; a prognostic indicator in metastatic melanoma
Glypican-3 (GPC3)	Unknown exactly, but may function as a survival or proliferation-associated factor
PKCα	A survival or proliferation-associated protein
5SCD	A precursor of melanin; Increased in metastatic prognosis
6H5MI2C	A precursor of melanin; Increased in metastatic prognosis
Serum amyloid A (SAA)	A superfamily of acute-phase proteins and proinflammatory adipokine
Cystatin C	A potent inhibitor of cysteine proteases; Increased primary and metastatic melanoma

[3] Antigens that are found in melanoma tissues become good immunohistochemical biomarkers. [4] The cytokines also take part in melanoma metastasis.

Table 2. (continues) Serologic biomarkers for the diagnosis of melanoma

Biomarkers	References
5SCD	Yamada et al., 1992; Kärnell et al., 1997; Bánfalvi et al., 2000; Wakamatsu et al., 2002
6H5MI2C	Yamada et al., 1992; Kärnell et al., 1997
TA-90	Rote et al., 1980; Euhus et al., 1989
S100A7	Brouard et al., 2002
β-human chorionic gonadotropin	Carter et al., 1995

Table 3. Urinal biomarker for the diagnosis of melanoma

2.4 Biomarkers for early melanoma diagnosis

The early diagnosis of melanoma is closely related to an increase in survival rate. Although many prognostic biomarkers (mainly metastatic prognosis biomarkers) of melanoma have been reported, there are very few capable of allowing an early diagnosis. Glypican-3 (GPC3) is a membrane-bound heparin sulfate proteoglycan which is overexpressed in several tumors. It has been suggested that GPC3 may be a useful early stage biomarker for patients with the early stages of the disease (0 - II) (Nakatsura et al., 2004; Ikuta et al., 2005). Moreover, cyclooxygenase-2 (COX-2) (Chwirot and Kuźbicki, 2007), serum amyloid A (SAA) (Mian et al., 2005; Findeisen et al., 2009) and DNA methylation profiling (Conway et al., 2011) can be used to distinguish between early melanomas and benign nevi.

3. Screening techniques of melanoma biomarkers

Before the development of high throughput proteomics techniques, biomarker candidates were identified based on known melanoma molecular pathways and validated by traditional techniques such as western blotting, ELISA and immunohistochemical analysis. However, the recent development of proteomics has enabled novel biomarkers to be screened from across a much larger section of the proteome. The most widely used technique for this form of screening is one whereby the samples are first separated by 2-DE and then each protein is identified by mass spectrometry (MS). Recently, simple gel-free techniques such as shotgun proteomics (Liu et al., 2002) and surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS)(Petricoin et al., 2002) have been developed. These techniques employ simple separation procedures such as capillary chromatography and surface chromatography prior MS analysis.

3.1 Screening for tumor tissues and cell lines

Biomarkers discovered from tissue samples and cultured cell lines also have utility for the development of diagnostic and prognostic assays. Tissue microarray is a high throughput technique in which many tissue samples can be screened simultaneously. This technique is suitable for the validation of candidate biomarkers, which are first obtained by other proteomics techniques. Several biomarkers of melanoma such as HSP 90 (McCarthy et al., 2008), ING3 (Wang et al., 2007), and the epidermal growth factor receptor family member HER3 (Reschke et al., 2008) were validated using this technique.

For comprehensive screening of biomarkers from lysates collected from tumor tissue and cultured cells, 2-DE combining MS is used as a standard method. The representative attempts of biomarker screening of melanoma lysates are summarized in Table 4. Since

tumor tissue is heterogeneous mixture of several cell types, cultured cell lines may be preferable for the screening of biomarkers. To search for biomarkers useful for the diagnosis of metastases, primary and metastatic cell lines were compared and several candidate biomarkers were successfully discovered (Table 4) (Bernard et al., 2003; Zuidervaart et al., 2006; Al-Ghoul et al., 2008). Proteomics analysis of melanoma-associated fibroblast stromal cells revealed the aberrant expression of several proteins not detected in normal fibroblasts (Paulitschke et al., 2009). These proteins may promote tumor progression. However, the passages number of the melanoma cell lines tested was found to produce changes in the proteome, which may underscore the invasive character observed in melanoma cells line that have been passaged many times (Pardo et al., 2006).

Screening methods	Samples	Biomarkers	Remarks	References
2-DE/MALDI-TOF-MS	Primary and metastatic melanoma cell lines, and normal melanocyte lines	Hepatoma-derived growth factor (HDGF) Nucleophosmin B23	Increased in melanoma	Bernard et al., 2003
2-DE/LC-MS/MS	Uveal malignant melanoma (UM) cell lines with varying passages	MUC18 HMG-1	Increased in higher passages	Pardo et al., 2006
2-DE/MALDI-TOF/ TOF-MS	UM primary and metastatic cell lines	HSP 27 Galectin-1	Increased in metastases	Zuidervaart et al., 2006
2-DE/MS/MS	Primary and metastatic melanoma cell lines	Cyclophilin A	Increased in metastatic melanoma	Al-Ghoul et al., 2008
LC-MS/MS	Melanoma-associated fibroblasts and normal fibroblast	Periostin (a 90-kDa glycoprotein) Stanniocalcin-1 (a 56-kDa glycoprotein)	Increased; melanoma-associated antigens	Paulitschke et al., 2009
2-DE/MS/MS	UM with monosomy 3 and disomy 3	HSP 27 Vimentin	Increased in disomy UM	Coupland et al., 2010

Table 4. Biomarker screening for tumor tissues and cell lines

3.2 Screening biomarkers obtained from serum and secreted from cultured cell lines

Because serum samples are far less invasive to obtain than biopsied material, the discovery of serological biomarkers has received a great deal of attention. Well-defined biomarkers enable early detection, allow the appropriate classification of tumor types (which provides the clinician insight into the best choice of therapy), and enable the patient to be more thoroughly monitored for progression and regression. However, there are several difficulties with screening serological biomarkers: (1) the presence of abundant blood proteins, which may inhibit the detection of biomarkers, and (2) the low serum concentration of the biomarker after it is secreted from tumor tissue and diluted in the bloodstream (Simpson et al., 2008). Hence, processes by which the abundant blood proteins are removed from the sample or the target proteins are concentrated are essential to address these problems. As a result of these difficulties inherent in serum proteomics, the secretome has received much attention recently.

Table 5 summarizes a number of different reports in which comprehensive screening of biomarkers from serum and the secretome occurred. By comparing the secretome between melanoma and normal melanocyte, several potential biomarkers were successfully discovered (Pardo et al., 2007; Paulitschke et al., 2009). The traditional serological biomarkers of melanoma such as LDH, S100B and CRP lack sensitivity as early stage melanoma biomarkers. To search for early stage biomarkers, Findeisen et al. extensively analyzed the serum proteome of about 600 melanoma patients at each stage of the disease (stages I - IV) by SELDI-TOF-MS technique (Mian et al., 2005; Findeisen et al., 2009). This analysis led to the discovery of a new biomarker, SAA, which was found to be highly sensitive for detecting early stage melanoma.

Screening methods	Samples	Biomarkers	Remarks	References
2D-GE/LC-MS/MS	UM cell lines and normal melanocytes	gp100/pmel-17 Cathepsin D Mad-9 (syntenin 1)	Increased in melanoma	Pardo et al., 2007
Multiplex immunobaed assay	Serum of melanoma patients and healthy individuals	Cytokines (e.g. IL-1, IL-6, TNF-α)	Increased in patients with longer relapse-free survival (RFS) values	Yurkovetsky et al., 2007
LC-MS/MS	Malanoma cell lines and normal melanocyte	Glutathione peroxidase	Increased in malanoma	Paulitschke et al., 2009
SELDI-TOF-MS	Serum of melanoma patients with different stages	SAA	Increased in early stages	Mian et al., 2005 Findeisen et al., 2009

Table 5. Biomarker screening for serum and secretome of cell lines

4. Summary and overall conclusions

Melanoma biomarkers have the capacity not only to diagnose melanoma, but also to allow patients to be monitored for recurrence after surgical resection and to allow the effect of anticancer drug treatments to be evaluated. Advanced technologies (e.g., high throughput technologies in genomics or proteomics) have contributed much to the hunt for melanoma biomarkers in tissue samples or body fluids and have typically been MS-based or array-based technologies.

Several immunohistochemical, serologic and urinary biomarkers have been reported to be very useful diagnostic and prognostic biomarkers. However, there is a paucity of data on melanoma-specific biomarkers, with the exception of the melanocyte lineage/differentiation antigens and melanoma-associated antigens, and some urinary biomarkers such as 5SCD, 6H5MI2C and S100A7.

Moreover, several melanoma biomarkers with prognostic capabilities, mainly for the detection of metastatic disease, have been applied to clinical use. However, there is s still a great need to identify melanoma early-stage melanoma biomarkers, as early detection of the

disease is key to increasing the survival rate. Of several melanoma biomarkers identified, GPC3, COX-2, SAA and DNA methylation profiling may hold promise for the diagnosis of early melanoma.

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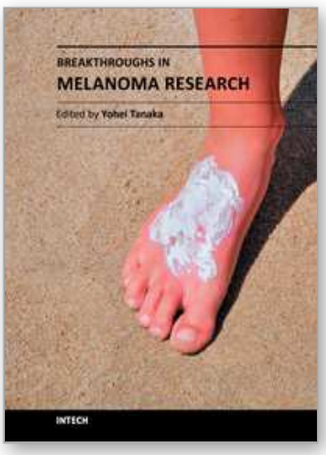

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Melanoma is considered to be one of the most aggressive forms of skin neoplasms. Despite aggressive researches towards finding treatments, no effective therapy exists to inhibit the metastatic spread of malignant melanoma. The 5-year survival rate 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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