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Circulating Melanoma Cells

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1. Introduction

Cutaneous Melanoma is an aggressive cancer which accounts for 80% of skin cancer deaths. Australia has the highest incidence world-wide and rates are increasing annually (10,000 new cases and 1,700 deaths per year). Notably it is the most common cancer in 15-39 year olds and the leading cancer related cause of death in young males (WA Cancer Registry 2007). In the USA, incidence is rising faster than other cancers, with 60,000 new cases and 8,000 deaths in 2007 [1].

Mortality rates remain high due to metastasis of the tumour. Once melanoma has metastasised, survival is commonly 6 to 9 months, with 5-year survival rate less than 40% [2-4]. Several prognostic factors have been identified, based upon pathological evaluation of the primary tumour [5] and lymph node metastases [6]. However, metastatic disease particularly micrometastasis, is difficult to detect and occurs in over 30% of patients, including 8-30% of patients with *in situ* melanoma [7-9] and 15% of patients whose lymph nodes are negative at the time of surgical intervention [10,11]. In addition, metastasis can occur up to 35 years after diagnosis [12]. Effective therapies providing long term survival are very limited (<20%), often due to drug resistance associated with prevailing undetected mutations or acquisition of new mutations. Surprisingly few studies [13-15] have monitored circulating cells as a means of determining disease progression, and/or treatment efficacy or for design of personalised treatment strategies.

A great deal of information has been compiled in an attempt to identify prognostic factors that correlate with clinical outcomes. Current clinical staging is performed using measures of the pathological features of the primary tumour, lymph node and distant metastases [6], as well as LDH levels and now also, genetic changes in the primary tumour [16-19]. The inability to accurately predict melanoma progression, may be related to the fact that the majority of studies use primary and less often, metastatic tumour tissue to stratify patients and delineate prognostic markers. Very few studies analyse the circulating tumour cell (CTC) phenotype that gives rise to the metastases. There is therefore an unmet need for detailed analyses of CTCs to provide early identification of metastatic risk and prognosis and evaluation of adjuvant therapies.

2. Circulating tumour cells

Millions of cells are shed from a tumour every day (approx 4×10^6 cells/g primary tumour) [20], and these cells invade lymphatic and/or venous circulatory systems. Once they

have entered the blood stream they become a circulating melanoma cell population. The fact that metastasis can occur many years after surgery indicates that disseminated tumour cells may remain in the blood stream for decades, evading the immune system and apparently remaining quiescent. Interestingly, few establish clinically diagnosed metastasis [21,22]. Those that do, particularly after many years, must acquire some activating change [12].

Since most cancer patients die as a result of metastatic spread rather than from their primary tumours [22,23], metastatic inefficiency of the primary tumour is likely overcome by the large number of tumour cells that enter the systemic circulation daily, estimated to be up to ~4 × 10^6 tumour cells released per gram of primary tumour [20,24]. Several studies have shown that the number of CTCs in patient peripheral blood increases with increasing stages of melanoma [25,26-29, 30]. Yet the variety and phenotype of these CTCs, their ability to remain in circulation for many years thus evading the immune system, and their activation causing metastasis, remain largely unexplored, particularly for melanoma.

3. Melanoma metastasis

When cancer cells detach from the primary tumour and enter into blood vessels (or the lymphatic system), they can do so actively or passively [7]. Passive cell intravasation, where cells are simply dislodged from the primary tumour, occurs as a result of increased hemodynamic flow [22,31,32]. By contrast, active migration occurs in cells which have separated from their neighbours and actively migrate. In addition, many circulating cells are apoptotic or necrotic and are unlikely to survive immune cell destruction [33-37].

The question remains then, is there a phenotypic and/or genotypic difference between cells that are able to survive in the circulation and metastasise from those that cannot? Fundamentally, gene expression signatures that prompt a melanoma cell to proliferate *in situ* must be different from those that permit a cell to actively migrate, and survive as a circulating cell, and then establish a secondary tumour. Several studies have documented the differential gene expression associated with malignant progression of melanoma. Pathways associated with initiation of melanoma metastasis include the transition from radial to vertical growth phase, epithelial to mesenchymal transitions, alterations in cell adhesion properties and suppression of apoptosis [38,39]. Included are several key steps; loss of adhesion, dermal invasion, migration from the primary site, intravasation followed by survival in the blood, migration into target tissues, and increased proliferation in the new tissue microenvironment followed by orchestration of angiogenesis at the new site [40].

Normal melanocyte cells in the epidermis are tethered tightly to other melanocyte cells and surrounding keratinocytes by cell surface molecules [41]. Once they become proliferative and malignant, melanoma cells lose many of the cell surface proteins that secure the tight epithelial cell–cell adhesive interactions [33]. One of the key cell surface proteins, CDH1 (Cadherin 1, E-cadherin), is bound via its cytoplasmic tail to α-catenin and β-catenin, and thus to the actin cytoskeleton to maintain close cell junctions [42]. Once melanoma cells become invasive, they no longer express CDH1, but express rather CDH5 (V-cadherin) or CDH2 (N-cadherin), proteins synonymous with the start of an epithelial to- mesenchymal transition (EMT) [43]. The EMT process, commonly utilised by migrating cells during embryonic development, involves switching of polarised epithelial cells to contractile, motile mesenchymal progenitor cells, and is triggered by secretion of growth factors (EGF (epithelial growth factor), FGF (fibroblast growth factor)) and chemotactic/pro-migratory factors SF/HGF (hepatocyte growth factor) and chemokines from stromal fibroblasts and

macrophages. This secretion induces intracellular transduction pathways (Wnt, Notch) which in turn activate transcription factors (Twist and SNAI1 and 2) [44,45] bringing about the invasion of melanoma cells from the epidermal, dermal border to invasion of the dermis and entry of the cells into the circulation and metastasis [27,46]. The invasive process is also the result of activated signalling cascades such as the NEDD9-DOCK3-Rac (Neural precursor cell expressed developmentally down-regulated protein 9- Dorsocross3 - Rac) pathway. The movement of these cells through the extracellular matrix and their migration towards blood vessels is assisted by integrin and matrix metalloproteinases [27,47-49].

The next step in the active migration process is the attraction of tumour cells to lymph and blood vessels, a process mediated by ligand-receptor interactions between tumour cells and the stroma or endothelial cells. Tumour cells secrete CSF1 (colony stimulating factor 1) and growth factors such as EGF which activate the formation and proliferation of tumour-associated macrophages in the stroma. These cells in turn secrete chemokines including SDF-1 (stromal-cell-derived factor 1), SCL/CCL21 (chemokine C-C motif ligand 21) and I309/CCL1 (chemokine C-C motif ligand 1), which assist with chemotaxis of tumour cells expressing the appropriate receptors, CXCR4, CCR7 and CCR8, into blood vessels [27,50,51].

Whether cells actively move toward and into nearby blood vessels or whether the process is passive and coincidental may be of some significance. Expression of specific genes that assist entry into the circulation, either passively or actively, may determine cell survival and metastatic ability. That is cells expressing genes associated with EMT, or cell migration, may be more prone to tumourigenesis and metastasis.

Model systems that quantify circulating cells leaving the tumour, show, in fact, that $3–4\times10^6$ malignant cells/day are shed per gram of tumour suggesting that millions of cells may be shed from a tumour every day [24,52,53]. Characterisation of these circulating tumour cells in patients with metastatic prostate and breast cancer indicates that they are predominantly apoptotic [37] or necrotic and unlikely to survive [34,35,36]. Furthermore, circulating cells are often sheared and destroyed as they leave the tumour and enter the circulation. Moreover, immune cells in the circulation destroy the bulk of circulating cells and prevent all but the most active from producing metastases [33,54].

Some cells do however survive for long periods of time in the vasculature [55,33] where they are usually found in clumps or clusters known as circulating tumour microemboli, surrounded by a "cloak" of platelets and leukocytes which assists tumour cell survival for some time by evasion of the immune system [56-58]. Moreover, melanoma cell evasion of the immune system and thus survival in the blood stream is also due to the intracellular localisation of the ligand which typically activates NKD2D receptors on natural killer (NK) cells [59]. Thus evasion from attack by natural killer (NK) cells affords melanoma cells a powerful means of protection from NK cell mediated cytotoxicity. Metastatic melanoma cells also develop resistance to inhibitory cytokines through the modification of oncostatin M receptors [60,61]. With such an arsenal of survival mechanisms, melanoma cells may indeed survive in the circulation for long periods of time.

4. Detection of CTCs

Various methods have been used to quantify and characterise CTCs, including indirect methods, namely qRT-PCR [25,62-65], and direct analyses such as immunomagnetic bead capture, or fibre-optic array scanning technology [28,29,66,67,68]. From these results it is obvious that; a) CTCs are present at relatively low concentrations; one tumour cell per 10^6 to 10^7

normal blood cells or on average, 1 cell per ml of blood [69,70,71]; b) the number of cells appears to be related to stage [2,25,72]; c) melanoma cell markers differ with respect to stage [73]; and c) CTC gene expression differs from that of the primary tumour [28,29].

Quantitative RT-PCR has typically detected expression of melanocytic genes such as tyrosinase (TYR) [74, 75] since normal melanocytes are not thought to circulate in peripheral blood and therefore detection of transcripts from melanocytic genes should correlate to identification of CTCs [72,76]. The sensitivity and specificity of PCR for circulating melanoma cells is increased by analysis of multiple markers [76], and these commonly include melan-A (MLANA), beta-1,4-N-acetyl-galactosaminyl transferase 1 (B4GALNT1), silver homolog (SILV), melanoma cell adhesion molecule (MCAM), melanoma associated antigen p97 (MFI2), melanoma antigen family A3 (MAGEA3) and microphthalmia-associated transcription factor 4 (MITF4) [63,64,77,78]. Several studies have shown that levels of gene expression associated with melanoma CTCs in patient blood correlate to AJCC stage, survival and disease recurrence [2,25,72].

Alternately, CTCs can be positively selected from whole blood using immunomagnetic beads [66]. With this system, CTC numbers are shown to positively correlate with cell stage and be an independent prognostic indicator of progression-free and overall survival for breast cancer [79], melanoma [28] and many other cancers [71].

5. Differential gene expression profiles of circulating

The question remains then, how do we differentiate those cells that are able to survive in the circulation from those that cannot? Moreover, can we differentiate actively metastatic melanoma cells from those that are quiescent or apoptotic and is there a need to do so? It is thought that any cancer cell can acquire the ability to disseminate at any time, even early and prior to overt tumour formation [11,80]. For heterogeneous tumours such as melanoma, an unstable, genetically-variant, invasive cell may metastasise at any time [27]. Whether this is possible for all melanoma cells remains to be confirmed, but recent experiments suggest that all melanoma cells can initiate a new tumour when xenotransplanted into immunocompromised mice [81].

By contrast a plethora of information gathered over many decades indicates that melanoma cells express stage related markers that are associated with a more or less invasive tumour [73]. In Fig 1, for example, we have shows differential stage related expression of the melanoma cell adhesion molecule, MCAM. At early disease stages, MCAM is found in <50% cells of 4/10 primary tumours, as opposed to metastatic tissue, where all cells are MCAM+ (5/5, 93% cells).[82]. MCSP, on the other hand, is found on >80% of melanoma cells at all stages I-IV [14,83-85] so its expression is not stage related. CTCs are also likely to have differential gene expression signatures related to stage and these may differ relative to those of the primary tumour as previously demonstrated [28,29]. It is apparent that merely identifying the presence of CTCs using for example MCSP, does not necessarily provide evidence of disease progression. It may be necessary to analyse CTC phenotype or genotype to obtain more prognostic information.

In recent years a number of researchers have shown the existence of a sub-set of tumour initiating or melanoma stem cells within the primary tumour [86-90]. These cells are believed to be responsible for relapse and metastasis by virtue of their ability to survive treatment and initiate new tumour formation. Rare cancer stem cells would therefore be capable of effectively managing the metastatic process [91] and would act as stem cells for metastasis

formation at a new site [69]. Melanoma stem cell markers include JARID1B (jumonji, AT rich interactive domain 1B) [92], ABCB5 (ATP-Binding Cassette Subfamily B (MDR/TAP) Member 5), ABCG2 (ATP-binding cassette sub-family G member 2), MDR1 (Multi-Drug Resistance 1) [87,93-99] and more recently CD271 [100].

6. Markers of metastasis – Can they be identified in CTCs?

A plethora of studies have focused on identification of markers with sufficient specificity to accurately predict melanoma progression. Although many of these were identified using primary tissue or melanoma cell lines, they have been used for the multitude of CTC studies conducted thus far [72,101-103]. For qRT-PCR analysis of CTCs include SILV, MLANA, TYR, MAGEA3 and MAGE-A10 [25,62-64,72,104], or more recently, ABCB5 [86,87 105]. From high throughput analyses of melanoma gene expression, several key progression pathways have been identified [38,39,103,106,107] but remain to be tested as informative for CTC analysis. Key amongst these pathways are: tyrosine kinase receptors (TKRs) (e.g. VEGFR, ERBB2, TGF-betaR), the Ras / Raf / MEK / ERK pathway, the PI3K / Akt / PTEN / mTOR pathway, cell cycle regulation pathways (Rb / p53 / p16INKA / p14ARF / HDM2), epigenetic gene expression regulation and DNA repair pathways (DNA methylation, histone acetylation, RNA interference), apoptotic pathways (e.g. death receptors: FAS, TRAILR, TNFR; mitochondrial pathway: Bcl2 family), common apoptosis effectors, protein chaperoning, degradation (HSP, proteasome) [25,108,109] and epithelial to mesenchymal transition (reviewed in [110]). A thorough screening of CTCs from metastatic melanoma patients for these activated pathways needs to be performed so as to establish their involvement in CTC survival, proliferation and intra- and extravasation. By detecting the presence and/or levels of genes associated with these activated / metastatic pathways, CTC analyses might be significantly enhanced.

Several reports also suggest that, in melanoma cells, altered regulation of melanocyte developmental pathways, are key to the acquisition of metastatic potential [111-116]. Indeed, melanoma metastasis reflects to some extent the migratory capacity of melanoblast developmental precursors, the neural crest cells. Moreover, genes that are critical for melanocyte development have been recognised as important factors of melanoma growth, for example MITF, DCT and SOX10 all function to maintain the stem or progenitor cell population of melanoblasts during migration from the neural crest and during melanoblast survival in the hair follicle niche [72,110,117-119] and may be equally important in melanoma cell maintenance and migration. It is important then to identify the expression of these developmental genes in CTCs and assess their association with metastasis.

7. Mutations in circulating melanoma cells

There is increasing evidence that melanoma metastasis is activated by mutations in multiple pathways including MAPK-ERK, PI3/AKT, PTEN and retinoblastoma pathways that regulate cyclin-dependent kinases (CDKs) (Table 1) [120,121]. MITF, a key transcription factor, is amplified in melanoma, and also regulated by c-KIT via MAPK/ERK and PI3/AKT pathways [122]. Differing mutations in multiple pathways have also now been identified for different melanoma subtypes [120,123,124]. It remains for stage and subtype related mutations to be profiled in CTCs.

There are currently eight defined subtypes of melanoma, based on mutations in key melanoma genes, but not all melanoma cases fit into these subtypes [125]. The majority of

melanomas will have a mutation in either *BRAF* (57%) or NRAS (17%) but rarely have both [124]. Further details about known mutations are described below.

	Pathway(s) activated by mutations
1.	MAPK
2.	c-KIT
3.	GNAQ/GNA11
4.	NRAS
5.	MITF
6.	AKT/PI3K
7.	CDK
8.	P53/BCL
9.	Undetermined

Table 1. Melanoma subtypes – adapted from Vidwans et al. [125]

Many new treatments being developed for melanoma target these specific molecular pathways which are associated with tumour progression. Unfortunately the effectiveness of these potential treatments has so far been limited by drug resistance as a result of newly acquired mutations [126] or the inadequate analysis of existing additional mutations. Since the presence or absence of certain mutations can drastically effect the success of targeted treatments it would be of benefit to develop a detailed profile of mutations that exist in an individual patient to maximise efficacy of treatment. One possibility is to use CTCs for stage and subtype related mutation analysis.

8. Conclusion

From our own (Fig. 1) and many other reported studies [33-36,54], it is clear that positivity *per se* is not necessarily a prognostic indicator i.e. it is possible that not all circulating cells establish successful tissue metastases. Thus a more comprehensive set of experiments and additional markers are required to better understand the diagnostic and prognostic significance of circulating melanoma cells. These issues are best addressed by isolation, characterisation and quantification of circulating melanoma cells. Additionally, newly identified prognostic markers need to be measured in CTCs and assessed relative to patient outcome to delineate the metastatic potential of circulating melanoma cells and their usefulness as a prognostic indicator [72,127].

We hypothesise that the ability of circulating melanoma cells to become activated, proliferative and migratory from a quiescent cell depends on several key genes. An alternate hypothesis is that malignant cells disseminate from the primary tumour early in tumourigenesis and remain in a clinically latent state until either the cells themselves or the host environment is receptive to the development of metastases. Quintana and colleagues [81] and more recently Roesch et al., [92] show that single human melanoma cells with no specifically identifiable gene signature can re-establish melanoma tumours when xenotransplanted into severely immunocompromised mice. It is of paramount importance therefore that we identify pathways associated with metastasis of circulating cells, ie those pathways that confer metastatic properties on quiescent melanoma stem cells capable of evading human anti-tumour immune responses. Furthermore, it is necessary to identify whether CTC numbers, gene expression profiles, or a combination of both, are key factors in

patient outcome. It also remains to be seen whether tumour related DNA mutations and the resultant activated proteins provide more accurate measures of melanoma progression. A combination of marker types is likely to be more accurate than measures that determine the presence and quantity of CTCs alone. Future studies in this field will need to be performed to address the multitude of issues alluded to in this chapter.

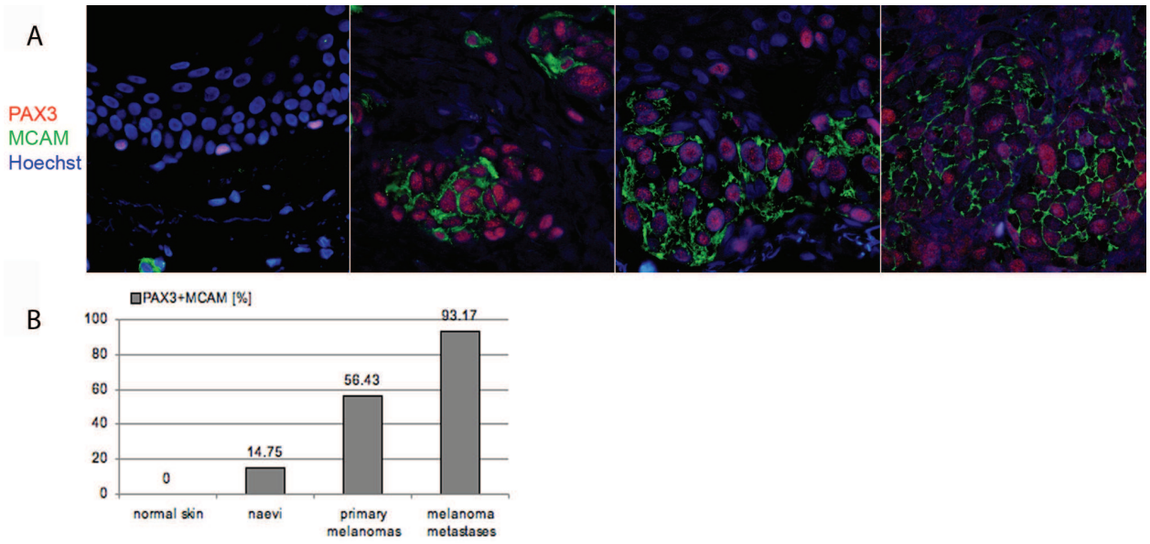


Fig. 1. A) Double immunofluorescent staining showing PAX3 (mouse monoclonal antibody, DSHB) and MCAM co-expression in normal skin (epidermal melanocytes), naevus, primary melanoma and melanoma metastasis respectively. Lines in (A) demarcate the epidermal-dermal border (EDB) or epidermal surface (ES). B) Graph showing the overall number of PAX3, MCAM double-labelled cells in normal skin, naevi, primary melanomas and melanoma metastases. Each column represents a percentage of PAX3-positive, MCAM-positive, averaged across all samples. Note: MCAM positive cells were all PAX3 positive (revised from [73])

9. References

[1] Lee KB, Weinstock MA, Risica PM. Components of a successful intervention for monthly skin self-examination for early detection of melanoma: the "Check It Out" trial. *J Am Acad Dermatol.* Jun 2008;58(6):1006-1012.

[2] Hoon DS, Bostick P, Kuo C, et al. Molecular markers in blood as surrogate prognostic indicators of melanoma recurrence. *Cancer Res.* Apr 15 2000;60(8):2253-2257.

[3] Postovit LM, Seftor EA, Seftor RE, Hendrix MJ. Targeting Nodal in malignant melanoma cells. *Expert Opin Ther Targets.* Apr 2007;11(4):497-505.

[4] Shivers SC, Wang X, Li W, et al. Molecular staging of malignant melanoma: correlation with clinical outcome. *JAMA.* Oct 28 1998;280(16):1410-1415.

[5] Breslow A. Tumor thickness, level of invasion and node dissection in stage I cutaneous melanoma. *Ann Surg.* Nov 1975;182(5):572-575.

[6] Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol.* Aug 15 2001;19(16):3635-3648.

[7] Michaelson JS, Cheongsiatmoy JA, Dewey F, et al. Spread of human cancer cells occurs with probabilities indicative of a nongenetic mechanism. *Br J Cancer.* Nov 28 2005;93(11):1244-1249.

[8] Koyanagi K, Kuo C, Nakagawa T, et al. Multimarker quantitative real-time PCR detection of circulating melanoma cells in peripheral blood: relation to disease stage in melanoma patients. *Clin Chem.* Jun 2005;51(6):981-988.

[9] Zalaudek I, Horn M, Richtig E, Hodl S, Kerl H, Smolle J. Local recurrence in melanoma in situ: influence of sex, age, site of involvement and therapeutic modalities. *Br J Dermatol.* Apr 2003;148(4):703-708.

[10] Jack A, Boyes C, Aydin N, Alam K, Wallack M. The treatment of melanoma with an emphasis on immunotherapeutic strategies. *Surg Oncol.* Jul 2006;15(1):13-24.

[11] Weight RM, Viator JA, Dale PS, Caldwell CW, Lisle AE. Photoacoustic detection of metastatic melanoma cells in the human circulatory system. *Opt Lett.* Oct 15 2006;31(20):2998-3000.

[12] Singh AD, Rennie IG, Kivela T, Seregard S, Grossniklaus H. The Zimmerman-McLean-Foster hypothesis: 25 years later. *Br J Ophthalmol.* Jul 2004;88(7):962-967.

[13] Pinzani P, Mazzini C, Salvianti F, et al. Tyrosinase mRNA levels in the blood of uveal melanoma patients: correlation with the number of circulating tumor cells and tumor progression. *Melanoma Res.* Aug 2010;20(4):303-310.

[14] Kitago M, Koyanagi K, Nakamura T, et al. mRNA expression and BRAF mutation in circulating melanoma cells isolated from peripheral blood with high molecular weight melanoma-associated antigen-specific monoclonal antibody beads. *Clin Chem.* Apr 2009;55(4):757-764.

[15] Board RE, Ellison G, Orr MC, et al. Detection of BRAF mutations in the tumour and serum of patients enrolled in the AZD6244 (ARRY-142886) advanced melanoma phase II study. *Br J Cancer.* Nov 17 2009;101(10):1724-1730.

[16] Eton O, Legha SS, Moon TE, et al. Prognostic factors for survival of patients treated systemically for disseminated melanoma. *J Clin Oncol.* Mar 1998;16(3):1103-1111.

[17] Francken AB, Accortt NA, Shaw HM, et al. Prognosis and determinants of outcome following locoregional or distant recurrence in patients with cutaneous melanoma. *Ann Surg Oncol.* May 2008;15(5):1476-1484.

[18] Leiter U, Meier F, Schittek B, Garbe C. The natural course of cutaneous melanoma. *J Surg Oncol.* Jul 1 2004;86(4):172-178.

[19] Thompson JF, Scolyer RA, Kefford RF. Cutaneous melanoma in the era of molecular profiling. *Lancet.* Aug 1 2009;374(9687):362-365.

[20] Fidler IJ, Yano S, Zhang RD, Fujimaki T, Bucana CD. The seed and soil hypothesis: vascularisation and brain metastases. *Lancet Oncol.* Jan 2002;3(1):53-57.

[21] Chen Y, Clegg NJ, Scher HI. Anti-androgens and androgen-depleting therapies in prostate cancer: new agents for an established target. *Lancet Oncol.* Oct 2009;10(10):981-991.

[22] Bockhorn M, Jain RK, Munn LL. Active versus passive mechanisms in metastasis: do cancer cells crawl into vessels, or are they pushed? *Lancet Oncol.* May 2007;8(5):444-448.

[23] Chen LL, Blumm N, Christakis NA, Barabasi AL, Deisboeck TS. Cancer metastasis networks and the prediction of progression patterns. *Br J Cancer.* Sep 1 2009;101(5):749-758.

[24] Butler TP, Gullino PM. Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. *Cancer Res.* Mar 1975;35(3):512-516.
[25] Mocellin S, Hoon D, Ambrosi A, Nitti D, Rossi CR. The prognostic value of circulating tumor cells in patients with melanoma: a systematic review and meta-analysis. *Clin Cancer Res.* Aug 1 2006;12(15):4605-4613.
[26] Ikuta Y, Nakatsura T, Kageshita T, et al. Highly sensitive detection of melanoma at an early stage based on the increased serum secreted protein acidic and rich in cysteine and glypican-3 levels. *Clin Cancer Res.* Nov 15 2005;11(22):8079-8088.
[27] Chiang AC, Massague J. Molecular basis of metastasis. *N Engl J Med.* Dec 25 2008;359(26):2814-2823.
[28] Ulmer A, Schmidt-Kittler O, Fischer J, et al. Immunomagnetic enrichment, genomic characterization, and prognostic impact of circulating melanoma cells. *Clin Cancer Res.* Jan 15 2004;10(2):531-537.
[29] Ulmer A, Beutel J, Susskind D, et al. Visualization of circulating melanoma cells in peripheral blood of patients with primary uveal melanoma. *Clin Cancer Res.* Jul 15 2008;14(14):4469-4474.
[30] Husemann Y, Geigl JB, Schubert F, et al. Systemic spread is an early step in breast cancer. *Cancer Cell.* Jan 2008;13(1):58-68.
[31] Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med.* Dec 25 1986;315(26):1650-1659.
[32] Liang S, Slattery MJ, Wagner D, Simon SI, Dong C. Hydrodynamic shear rate regulates melanoma-leukocyte aggregation, melanoma adhesion to the endothelium, and subsequent extravasation. *Ann Biomed Eng.* Apr 2008;36(4):661-671.
[33] Paterlini-Brechot P, Benali NL. Circulating tumor cells (CTC) detection: clinical impact and future directions. *Cancer Lett.* Aug 18 2007;253(2):180-204.
[34] Swartz MA, Kristensen CA, Melder RJ, et al. Cells shed from tumours show reduced clonogenicity, resistance to apoptosis, and in vivo tumorigenicity. *Br J Cancer.* Nov 1999;81(5):756-759.
[35] Larson CJ, Moreno JG, Pienta KJ, et al. Apoptosis of circulating tumor cells in prostate cancer patients. *Cytometry A.* Nov 2004;62(1):46-53.
[36] Mehes G, Witt A, Kubista E, Ambros PF. Circulating breast cancer cells are frequently apoptotic. *Am J Pathol.* Jul 2001;159(1):17-20.
[37] Glinsky GV. Apoptosis in metastatic cancer cells. *Crit Rev Oncol Hematol.* Apr 1997;25(3):175-186.
[38] Hoek KS, Schlegel NC, Brafford P, et al. Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. *Pigment Cell Res.* Aug 2006;19(4):290-302.
[39] Mandruzzato S, Callegaro A, Turcatel G, et al. A gene expression signature associated with survival in metastatic melanoma. *J Transl Med.* 2006;4:50.
[40] Mazzocca A, Carloni V. The metastatic process: methodological advances and pharmacological challenges. *Curr Med Chem.* 2009;16(14):1704-1717.
[41] Van Den Bossche K, Naeyaert JM, Lambert J. The quest for the mechanism of melanin transfer. *Traffic.* Jul 2006;7(7):769-778.
[42] Vogelmann R, Nguyen-Tat MD, Giehl K, Adler G, Wedlich D, Menke A. TGFbeta-induced downregulation of E-cadherin-based cell-cell adhesion depends on PI3-kinase and PTEN. *J Cell Sci.* Oct 15 2005;118(Pt 20):4901-4912.

[43] Moustakas A, Heldin CH. Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. *Cancer Sci.* Oct 2007;98(10):1512-1520.

[44] Peinado H, Portillo F, Cano A. Transcriptional regulation of cadherins during development and carcinogenesis. *Int J Dev Biol.* 2004;48(5-6):365-375.

[45] Cheng GZ, Chan J, Wang Q, Zhang W, Sun CD, Wang LH. Twist transcriptionally up-regulates AKT2 in breast cancer cells leading to increased migration, invasion, and resistance to paclitaxel. *Cancer Res.* Mar 1 2007;67(5):1979-1987.

[46] Hsu MY, Meier FE, Nesbit M, et al. E-cadherin expression in melanoma cells restores keratinocyte-mediated growth control and down-regulates expression of invasion-related adhesion receptors. *Am J Pathol.* May 2000;156(5):1515-1525.

[47] Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer.* Mar 2002;2(3):161-174.

[48] Bartolome RA, Galvez BG, Longo N, et al. Stromal cell-derived factor-1alpha promotes melanoma cell invasion across basement membranes involving stimulation of membrane-type 1 matrix metalloproteinase and Rho GTPase activities. *Cancer Res.* Apr 1 2004;64(7):2534-2543.

[49] Sanz-Moreno V, Gadea G, Ahn J, et al. Rac activation and inactivation control plasticity of tumor cell movement. *Cell.* Oct 31 2008;135(3):510-523.

[50] Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res.* Jan 15 2006;66(2):605-612.

[51] Pittet MJ. Behavior of immune players in the tumor microenvironment. *Curr Opin Oncol.* Jan 2009;21(1):53-59.

[52] Liotta LA, Kleinerman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. *Cancer Res.* May 1974;34(5):997-1004.

[53] Bockhorn M, Roberge S, Sousa C, Jain RK, Munn LL. Differential gene expression in metastasizing cells shed from kidney tumors. *Cancer Res.* Apr 1 2004;64(7):2469-2473.

[54] Holmgren L, O'Reilly MS, Folkman J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nat Med.* Feb 1995;1(2):149-153.

[55] Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer.* Apr 2009;9(4):274-284.

[56] Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A, Muschel RJ. Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. *Nat Med.* Jan 2000;6(1):100-102.

[57] Borsig L, Wong R, Hynes RO, Varki NM, Varki A. Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis. *Proc Natl Acad Sci U S A.* Feb 19 2002;99(4):2193-2198.

[58] Laubli H, Stevenson JL, Varki A, Varki NM, Borsig L. L-selectin facilitation of metastasis involves temporal induction of Fut7-dependent ligands at sites of tumor cell arrest. *Cancer Res.* Feb 1 2006;66(3):1536-1542.

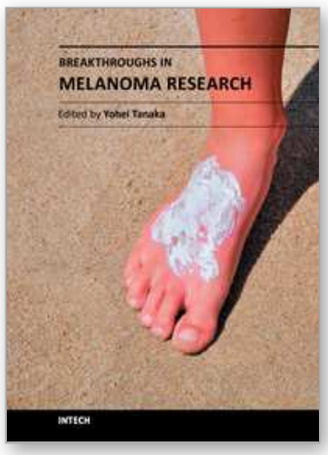
[59] Fuertes MB, Girart MV, Molinero LL, et al. Intracellular retention of the NKG2D ligand MHC class I chain-related gene A in human melanomas confers immune privilege and prevents NK cell-mediated cytotoxicity. *J Immunol.* Apr 1 2008;180(7):4606-4614.

[60] Lacreusette A, Nguyen JM, Pandolfino MC, et al. Loss of oncostatin M receptor beta in metastatic melanoma cells. *Oncogene.* Feb 8 2007;26(6):881-892.
[61] Zbytek B, Carlson JA, Granese J, Ross J, Mihm MC, Slominski A. Current concepts of metastasis in melanoma. *Expert Rev Dermatol.* Oct 2008;3(5):569-585.
[62] Koyanagi K, O'Day SJ, Gonzalez R, et al. Serial monitoring of circulating melanoma cells during neoadjuvant biochemotherapy for stage III melanoma: outcome prediction in a multicenter trial. *J Clin Oncol.* Nov 1 2005;23(31):8057-8064.
[63] Koyanagi K, Mori T, O'Day SJ, Martinez SR, Wang HJ, Hoon DS. Association of circulating tumor cells with serum tumor-related methylated DNA in peripheral blood of melanoma patients. *Cancer Res.* Jun 15 2006;66(12):6111-6117.
[64] Koyanagi K, O'Day SJ, Gonzalez R, et al. Microphthalmia transcription factor as a molecular marker for circulating tumor cell detection in blood of melanoma patients. *Clin Cancer Res.* Feb 15 2006;12(4):1137-1143.
[65] Voit CA, Schafer-Hesterberg G, Kron M, et al. Impact of molecular staging methods in primary melanoma: reverse-transcriptase polymerase chain reaction (RT-PCR) of ultrasound-guided aspirate of the sentinel node does not improve diagnostic accuracy, but RT-PCR of peripheral blood does predict survival. *J Clin Oncol.* Dec 10 2008;26(35):5742-5747.
[66] Krivacic RT, Ladanyi A, Curry DN, et al. A rare-cell detector for cancer. *Proc Natl Acad Sci U S A.* Jul 20 2004;101(29):10501-10504.
[67] Cools-Lartigue JJ, McCauley CS, Marshall JC, et al. Immunomagnetic isolation and in vitro expansion of human uveal melanoma cell lines. *Mol Vis.* 2008;14:50-55.
[68] Weight RM, Dale PS, Viator JA. Detection of circulating melanoma cells in human blood using photoacoustic flowmetry. *Conf Proc IEEE Eng Med Biol Soc.* 2009;2009:106-109.
[69] Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer.* May 2008;8(5):329-340.
[70] Pantel K, Otte M. Occult micrometastasis: enrichment, identification and characterization of single disseminated tumour cells. *Semin Cancer Biol.* Oct 2001;11(5):327-337.
[71] Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res.* Oct 15 2004;10(20):6897-6904.
[72] Medic S, Pearce RL, Heenan PJ, Ziman M. Molecular markers of circulating melanoma cells. *Pigment Cell Res.* Apr 2007;20(2):80-91.
[73] Medic S, Ziman M. PAX3 expression in normal skin melanocytes and melanocytic lesions (naevi and melanomas). *PLoS One.* 2010;5(4):e9977.
[74] Palmieri G, Ascierto PA, Perrone F, et al. Prognostic value of circulating melanoma cells detected by reverse transcriptase-polymerase chain reaction. *J Clin Oncol.* Mar 1 2003;21(5):767-773.
[75] Gradilone A, Cigna E, Agliano AM, Frati L. Tyrosinase Expression as a Molecular Marker for Investigating the Presence of Circulating Tumor Cells in Melanoma Patients. *Curr Cancer Drug Targets.* Apr 12 2010.
[76] Keilholz U, Goldin-Lang P, Bechrakis NE, et al. Quantitative detection of circulating tumor cells in cutaneous and ocular melanoma and quality assessment by real-time reverse transcriptase-polymerase chain reaction. *Clin Cancer Res.* Mar 1 2004;10(5):1605-1612.

[77] Reynolds SR, Albrecht J, Shapiro RL, et al. Changes in the presence of multiple markers of circulating melanoma cells correlate with clinical outcome in patients with melanoma. *Clin Cancer Res.* Apr 2003;9(4):1497-1502.
[78] Samija I, Lukac J, Maric-Brozic J, et al. Prognostic value of microphthalmia-associated transcription factor and tyrosinase as markers for circulating tumor cells detection in patients with melanoma. *Melanoma Res.* Mar 30 2010.
[79] Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med.* Aug 19 2004;351(8):781-791.
[80] Bernards R, Weinberg RA. A progression puzzle. *Nature.* Aug 22 2002;418(6900):823.
[81] Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature.* Dec 4 2008;456(7222):593-598.
[82] Rapanotti MC, Bianchi L, Ricozzi I, et al. Melanoma-associated markers expression in blood: MUC-18 is associated with advanced stages in melanoma patients. *Br J Dermatol.* Feb 2009;160(2):338-344.
[83] Pluschke G, Vanek M, Evans A, et al. Molecular cloning of a human melanoma-associated chondroitin sulfate proteoglycan. *Proc Natl Acad Sci U S A.* Sep 3 1996;93(18):9710-9715.
[84] Yang J, Price MA, Li GY, et al. Melanoma proteoglycan modifies gene expression to stimulate tumor cell motility, growth, and epithelial-to-mesenchymal transition. *Cancer Res.* Oct 1 2009;69(19):7538-7547.
[85] Erfurt C, Muller E, Emmerling S, et al. Melanoma-associated chondroitin sulphate proteoglycan as a new target antigen for CD4+ T cells in melanoma patients. *Int J Cancer.* May 15 2009;124(10):2341-2346.
[86] Schatton T, Frank MH. Cancer stem cells and human malignant melanoma. *Pigment Cell Melanoma Res.* Feb 2008;21(1):39-55.
[87] Schatton T, Murphy GF, Frank NY, et al. Identification of cells initiating human melanomas. *Nature.* Jan 17 2008;451(7176):345-349.
[88] Grichnik JM, Burch JA, Schulteis RD, et al. Melanoma, a tumor based on a mutant stem cell? *J Invest Dermatol.* Jan 2006;126(1):142-153.
[89] Monzani E, Facchetti F, Galmozzi E, et al. Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. *Eur J Cancer.* Mar 2007;43(5):935-946.
[90] Zabierowski SE, Herlyn M. Melanoma stem cells: the dark seed of melanoma. *J Clin Oncol.* Jun 10 2008;26(17):2890-2894.
[91] Wicha MS. Cancer stem cells and metastasis: lethal seeds. *Clin Cancer Res.* Oct 1 2006;12(19):5606-5607.
[92] Roesch A, Fukunaga-Kalabis M, Schmidt EC, et al. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell.* May 14 2010;141(4):583-594.
[93] Herlyn M. Driving in the melanoma landscape. *Exp Dermatol.* Jun 2009;18(6):506-508.
[94] Passegue E, Rafii S, Herlyn M. Cancer stem cells are everywhere. *Nat Med.* Jan 2009;15(1):23.
[95] Smalley KS, Herlyn M. Integrating tumor-initiating cells into the paradigm for melanoma targeted therapy. *Int J Cancer.* Mar 15 2009;124(6):1245-1250.
[96] Vultur A, Herlyn M. Cracking the system: melanoma complexity demands new therapeutic approaches. *Pigment Cell Melanoma Res.* Feb 2009;22(1):4-5.

[97] Keshet GI, Goldstein I, Itzhaki O, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun.* Apr 18 2008;368(4):930-936.
[98] Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res.* Oct 15 2005;65(20):9328-9337.
[99] La Porta C. Cancer stem cells: lessons from melanoma. *Stem Cell Rev.* Mar 2009;5(1):61-65.
[100] Boiko AD, Razorenova OV, van de Rijn M, et al. Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. *Nature.* Jul 1 2010;466(7302):133-137.
[101] Bosserhoff AK. Novel biomarkers in malignant melanoma. *Clin Chim Acta.* May 2006;367(1-2):28-35.
[102] Bennett DC. How to make a melanoma: what do we know of the primary clonal events? *Pigment Cell Melanoma Res.* Feb 2008;21(1):27-38.
[103] Gogas H, Eggermont AM, Hauschild A, et al. Biomarkers in melanoma. *Ann Oncol.* Aug 2009;20 Suppl 6:vi8-13.
[104] Xi L, Nicastri DG, El-Hefnawy T, Hughes SJ, Luketich JD, Godfrey TE. Optimal markers for real-time quantitative reverse transcription PCR detection of circulating tumor cells from melanoma, breast, colon, esophageal, head and neck, and lung cancers. *Clin Chem.* Jul 2007;53(7):1206-1215.
[105] Ma J, Lin JY, Alloo A, et al. Isolation of tumorigenic circulating melanoma cells. *Biochem Biophys Res Commun.* Nov 26 2010;402(4):711-717.
[106] Talantov D, Mazumder A, Yu JX, et al. Novel genes associated with malignant melanoma but not benign melanocytic lesions. *Clin Cancer Res.* Oct 15 2005;11(20):7234-7242.
[107] Alonso SR, Tracey L, Ortiz P, et al. A high-throughput study in melanoma identifies epithelial-mesenchymal transition as a major determinant of metastasis. *Cancer Res.* Apr 1 2007;67(7):3450-3460.
[108] Winnepenninckx V, Lazar V, Michiels S, et al. Gene expression profiling of primary cutaneous melanoma and clinical outcome. *J Natl Cancer Inst.* Apr 5 2006;98(7):472-482.
[109] Kauffmann A, Rosselli F, Lazar V, et al. High expression of DNA repair pathways is associated with metastasis in melanoma patients. *Oncogene.* Jan 24 2008;27(5):565-573.
[110] Medic S, Ziman M. PAX3 across the spectrum: from melanoblast to melanoma. *Crit Rev Biochem Mol Biol.* Apr 28 2009:1-13.
[111] Gupta PB, Kuperwasser C, Brunet JP, et al. The melanocyte differentiation program predisposes to metastasis after neoplastic transformation. *Nat Genet.* Oct 2005;37(10):1047-1054.
[112] Gupta PB, Mani S, Yang J, Hartwell K, Weinberg RA. The evolving portrait of cancer metastasis. *Cold Spring Harb Symp Quant Biol.* 2005;70:291-297.
[113] White RM, Zon LI. Melanocytes in development, regeneration, and cancer. *Cell Stem Cell.* Sep 11 2008;3(3):242-252.
[114] Topczewska JM, Postovit LM, Margaryan NV, et al. Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat Med.* Aug 2006;12(8):925-932.
[115] McArdle L, Rafferty MM, Satyamoorthy K, et al. Microarray analysis of phosphatase gene expression in human melanoma. *Br J Dermatol.* May 2005;152(5):925-930.

[116] Carreira S, Goodall J, Denat L, et al. Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. *Genes Dev.* Dec 15 2006;20(24):3426-3439.
[117] Kubic JD, Young KP, Plummer RS, Ludvik AE, Lang D. Pigmentation PAX-ways: the role of Pax3 in melanogenesis, melanocyte stem cell maintenance, and disease. *Pigment Cell Melanoma Res.* Dec 2008;21(6):627-645.
[118] Plummer RS, Shea CR, Nelson M, et al. PAX3 expression in primary melanomas and nevi. *Mod Pathol.* May 2008;21(5):525-530.
[119] Lang D, Lu MM, Huang L, et al. Pax3 functions at a nodal point in melanocyte stem cell differentiation. *Nature.* Feb 24 2005;433(7028):884-887.
[120] Whiteman DC, Zhou XP, Cummings MC, Pavey S, Hayward NK, Eng C. Nuclear PTEN expression and clinicopathologic features in a population-based series of primary cutaneous melanoma. *Int J Cancer.* May 1 2002;99(1):63-67.
[121] Duncan LM. The classification of cutaneous melanoma. *Hematol Oncol Clin North Am.* Jun 2009;23(3):501-513, ix.
[122] Levy C, Khaled M, Fisher DE. MITF: master regulator of melanocyte development and melanoma oncogene. *Trends Mol Med.* Sep 2006;12(9):406-414.
[123] Platz A, Egyhazi S, Ringborg U, Hansson J. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. *Mol Oncol.* Apr 2008;1(4):395-405.
[124] Goel VK, Lazar AJ, Warneke CL, Redston MS, Haluska FG. Examination of mutations in BRAF, NRAS, and PTEN in primary cutaneous melanoma. *J Invest Dermatol.* Jan 2006;126(1):154-160.
[125] Vidwans SJ, Flaherty KT, Fisher DE, Tenenbaum JM, Travers MD, Shrager J. The Melanoma Disease Model: Subtypes. 2010; http://mmdm.cancercommons.org/smw/index.php/The_Melanoma_Disease_Model:_Subtypes. Accessed 7 February 2011.
[126] Ireland A, Millward M, Pearce R, Lee M, Ziman M. Genetic factors in metastatic progression of cutaneous melanoma: the future role of circulating melanoma cells in prognosis and management. *Clin Exp Metastasis.* Feb 11 2011.
[127] Fusi A, Collette S, Busse A, et al. Circulating melanoma cells and distant metastasis-free survival in stage III melanoma patients with or without adjuvant interferon treatment (EORTC 18991 side study). *Eur J Cancer.* Dec 2009;45(18):3189-3197.



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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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