Genomics of Malignant Melanoma

Margit Balázs

Department of Preventive Medicine
University of Debrecen, Debrecen

The currently used diagnostic and prognostic methods for the early detection of human cuataneous melanoma, which rely primarily on microscopic tissue morphology (depth and level of invasion, ulceration, radial versus vertical growth phase, regression, dermal mitotic activity, tumor infiltrating lymphocyte response, angiolymphatic invasion, satellites) supplemented by sentinel lymph node biopsy can define prognosis of the disease and the most optimal surgical treatment. However, in many cases, do not provide accurate, individualized assessment of risk of melanoma progression and are often not helpful in defining systemic or adjuvant therapy. Thus, we still need more accurate, personalized clinical tools for disease subclassification and staging that can offer precise information on prognosis, clinical management (therapeutic) and discoveries of novel molecular targets for targeted treatment and prevention of the disease (1).
It was clearly summarized by Linda Chin that what are the critical biological questions facing the melanoma research community (2). Those include:

1. ***What genetic and environmental factors contribute to and/or modulate risk of melanoma development in man?***
2. ***What biological or molecular features (biomarkers) in early lesions can predict high risk of subsequent metastasis?***
3. ***What genetic events underlie its propensity for metastasis and treatment resistance (phenotype)?***
4. ***Which genetic alterations responsible for development and progression of melanoma are also essential for maintenance of established disease?***
5. ***Finally, what maintenance-essential biological or molecular pathways/networks might prove amenable to preventive and/or therapeutic intervention in man?***

In order to design rational therapies, it is of critical importance to identify the genetic determinants and to define the functionally important genetic drivers that drive melanoma formation and progression (3). Advances in genetic and genomic strategies during the past decade have exponentially increased our understanding of the molecular alterations associated with the disease.

Melanoma genetics and targeted therapy, BRAF mutations as therapeutic option?

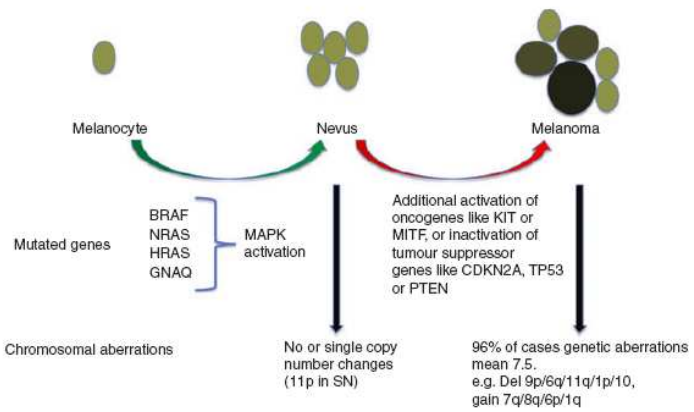


Figure 1. Schematic events in the development of melanocytic tumours. (*Histopathology 2010, 56, 121–132.)*

Using chromosome banding, fluorescence in situ hybridization, comparative genomic hybridization and other molecular approaches several studies were performed to define genetic alterations underlying melanoma development and progression. Some other studies aimed

to characterize the different and similar genetic alterations on benign and malignant melanocytic lesions. As a result these studies have implicated a large number of genes that are involved in melanoma development and progression The current knowledge about molecular cytogenetic alterations (Figure 1) was recently summarized by Blokx et al. (4). It is well demonstrated that development of a normal melanocyte into the malignant phenotype is characterized by certain histological, and so far only incompletely discovered genetic alterations. At a molecular level, the mitogen-activated protein kinase (MAPK) signalling pathway and PTEN/AKT pathway are both involved in the growth control of melanocytic cells. Activation of these pathways via somatic mutations in the RAS and RAF genes is thought to be one of the first steps in the development of common naevi. Point mutation in the BRAF gene (most frequently the V600E codon is thought to be an early event and possibly is induced by intermittent type of sun exposure (however we have to note that over 30 distinct BRAF mutations, varying in biological activity, have been found and may be predictive of clinically relevant tumor differences).

On the other hand *BRAF* mutations are rare in melanomas that are chronically exposed to the sun or on acral skin and mucosal membranes that are seldom or never exposed to the sun (5,6). In addition, several studies have shown that melanomas of the palms and soles and mucosal membranes have distinctive patterns of chromosomal aberrations as compared with those at other sites (7,8). Certain benign naevi such as blue-, congenital- and Spitz naevi do not or rarely contain *BRAF* mutations, but exhibit other mutations, e.g. in the NRAS or HRAS genes. Very recently somatic mutations of GNAQ, occurring exclusively in codon 209 in the RAS-like domain, were found in uveal melanomas and blue naevi, both lacking BRAF or NRAS mutations. Like mutations in BRAF, these RAS and GNAQ mutations can cause MAPK activation and form an alternative route for melanocytic neoplasia. In our recent study we found that 56% of primary melanomas had either BRAF or NRAS mutations, but these two mutations were not present in any of the lesions analyzed (9). All of these mutations seem to be early events in the development of melanocytic tumors, but by themselves are insufficient to cause progression towards melanoma.

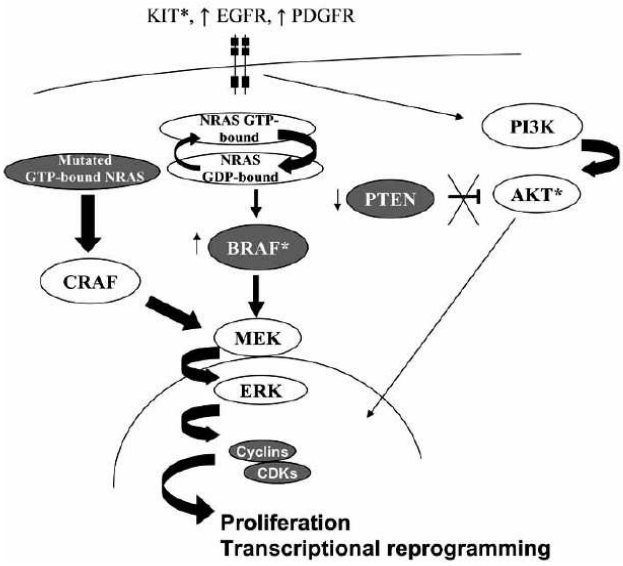


Figure 2. Receptor tyrosine kinase (RTK) pathway dysregulation in melanoma pathogenesis (*Current Genomics*, 2009, *10*, 231-239)

BRAF mutations are associated with the constitutive stimulation of *RAF/MEK* (mitogen-activated *ERK*-activating kinase)/*ERK* (extracellular signal-regulated kinase) pathway activation. Preclinical and early clinical studies predict that *RAF/MEK/ERK* pathway inhibitors will have therapeutic activity towards melanoma, but that tumor subclassification by *BRAF/NRAS* mutational status may be necessary to evaluate their efficacy (6,10). A mutation-targeted molecular therapy has shown promise against melanoma as it was published a few month ago in Science, but how it works is unclear (10). A new targeted molecular therapy (*PLX4032*) is under clinical trial. *PLX4032* is a selective *BRAF(V600E)* kinase inhibitor, it binds to and inactivates the BRAF protein. It binds to the mutant form of BRAF much better than it does to the normal, "wild-type" BRAF protein. A group from Genentech presented data at the Molecular Targets and Cancer Therapeutics conference

(Boston November 2009, USA) showing that the same RAF inhibitors that block the pathway in BRAF mutant cells activate the pathway in non-mutant cells (Figure 3). Three other research groups have recently reported similar activating activity for RAF inhibitors in normal cells. This pathway activation in normal cells could explain why side effects don't appear at high doses of the drug.

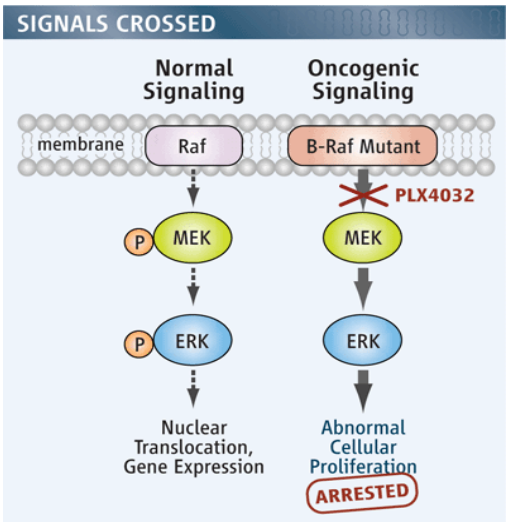


Figure 3 Plexxikon's drug interrupts a key signaling pathway in tumor cells, but not normal cells. *(*Science *2009:326:5960,1619)*

After the initiative mutations in further development from melanocytic naevus towards dysplastic naevus additional activation of oncogenes (*c-KIT, MITF*) and inactivation of tumor suppressor genes (*CDKN2A, p53, INK4A, PTEN* and/or loss of 6q, deletions on 11q) are necessary (Figure 1).

CDKN2A is one of the major gene that are involved in familial and sporadic cutaneous melanoma also. Approximately 5-10% of melanomas have familial history, and the molecular defects in most of these cases involve cell cycle regulators, particularly cyclin-dependent kinases (*CDKs*) and the *CDK* inhibitor p16 (*CDKN2A*, also named as multiple tumor suppressor gene) in their molecular pathogenesis. BRAF also cooperates with *CDKN2A* which is a component of the CyclinD1-RB pathway, and the tumor suppressor *p14CDKN2A*, which has been functionally linked to the MDM2-p53 pathway. The overall prevalence of melanoma patients who carry a *CDKN2A* mutation is between 0.2% and 2%. The penetrance of *CDKN2A* mutations is also greatly influenced by geographic location, with reported rates of 13% in Europe, 50% in the US, and 32% in Australia by 50 years of age; and 58% in Europe, 76% in the US, and 91% in Australia by age 80 (11). *CDKN2A* mutations are more frequent in patients with a strong familial history of melanoma (three or more affected family members; 35.5%) compared with patients without any history (8.2%). Correlations between the *CDKN2A* mutation status and melanoma risk factors in North American melanoma-prone families have shown the melanoma risk associated with sunburn was higher in individuals in genetically susceptible families than in non-susceptible individuals. This finding suggests that there are common mechanisms and/or interactions between the *CDKN2A* pathway and the UV-sensitivity.

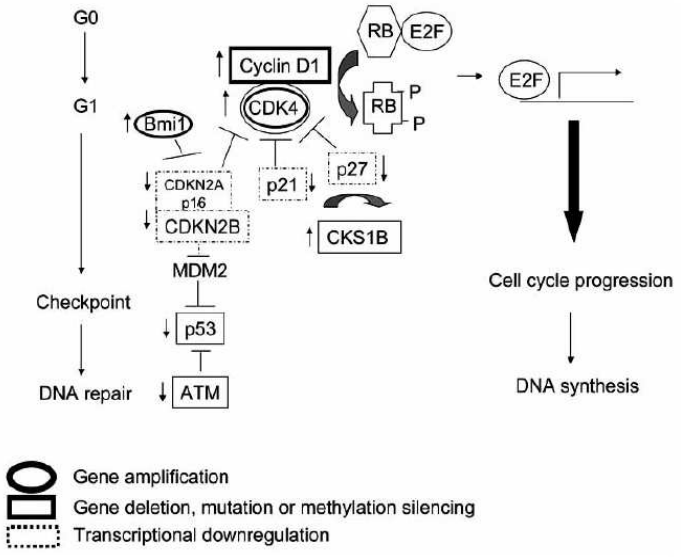


Figure 4. Cell cycle dysregulation in melanoma progression. (*Current Genomics*, 2009, *10*, 231-239)

The second melanoma susceptibility gene is the Cyclin-Dependent Kinase 4, which is located at 12q13.6, and which encodes a protein interacting with the *p16CDKN2A* gene product. *CDK4* is a rare high-penetrance melanoma predisposition gene. A recent publication

summarizes the efforts that have led to the current understanding of melanoma susceptibility as the result of complex gene-gene and gene-environment interactions (12).
Cytogenetic and molecular genetic findings suggest that 10qdeletion is a frequent and early event in malignant melanomas. ***PTEN,*** a tumorsuppressor gene is located at this region (10q23.3) which is activated through deletion and/or mutation. The *PTEN* protein has at least two biochemical functions: lipid phosphatase and protein phosphatase. The lipid phosphatase activity of *PTEN* seems to have a role in tumorigenesis by inducing a decrease in the function of the downstream *AKT* protein and is able to degrade the products of *PI3K*, suggesting that *PTEN* functions may directly antagonize the activity of *P13K*/ *AKT* pathway (Current Genomics, 2009, 10, 231-239). A widespread overview of all pathways involved in melanoma genesis are outlined in the following manuscript (13).

High throughput techniques used to analyse alterations in the melanoma genome

Recent advances in high-resolution genome-wide molecular techniques have greatly increased our ability to define molecular alterations and to understand molecular signalling networks and pathways in cancer, including melanoma.

Array CGH is a technique for the discovery of genetic alterations in melanoma

The development of high-resolution molecular biological techniques have revolutionized our ability to detect genetic alterations in the whole genome which often contain genes whose changes in copy numbers has been selected during tumor development and/or progression. These alterations can be mapped onto a representation of the normal genome by using comparative genomic hybridization (CGH). CGH was first described in 1992 as a revolutionary technique to detect and map DNA sequence copy number changes throughout the genome onto a cytogenetic map represented by normal metaphase chromosomes (14). In 1998 variation of chromosomal CGH was developed in which metaphase chromosomes are replaced by arrays of genomic bacterial artificial chromosome (BAC) clones Figure 1. (15). During the process total genomic DNA is isolated from test (tumor or other diseases) and reference (healthy individuals) samples, and labeled with green and red fluorochromes, respectively. The mixture of denatured test and reference DNA can be hybridized to a microarray of mapped clones of genomic DNA. The ratio of the fluorescence intensity for each array element provides the relative copy numbers for the genomic locus represented by the array element. The overall resolution of the array depends on the genomic distance between the clones and their lengths (16). Array CGH has already been applied on a large number of solid tumors; however, only limited articles were published for primary melanomas (17-23). Array CGH is highly effective in cataloging recurrent chromosomal

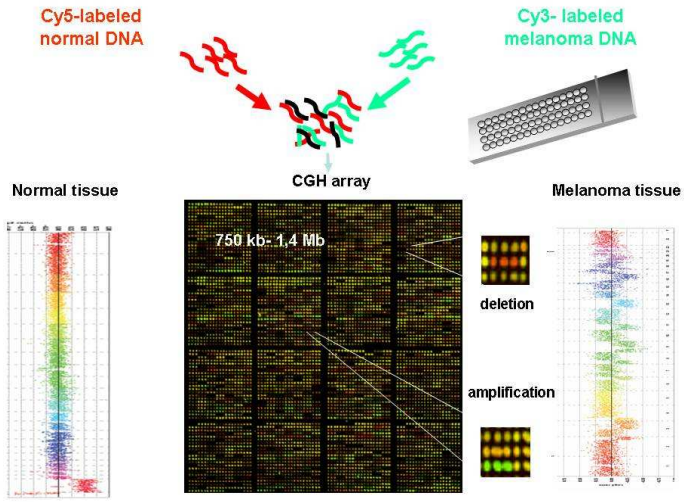


Figure 5 Scheme of array comparative genomic hybridization
(Balázs et al submitted for publication 2010

numerical aberrations associated with different tumor types in human (24-28) it can also be used to distinguish genetically and histopathologically homogeneous melanomas. The largest outcome study was published by Curtin et al. They assumed that clinical heterogeneity of the disease is explained by genetically distinct types of melanoma with different susceptibility to ultraviolet light. They compared the copy number alterations DNA and mutational status of BRAF and N-RAS in 126 melanomas from four subgroups in which the degree of exposure to ultraviolet light differs *(1. melanomas from skin with chronic sun-induced damage, 2. without chronic sun-induced damage, 3. lesions from palms, soles and subungual (acral) sites, and 4. mucosal melanomas).* The genetic alterations identified at different sites and with different levels of sun exposure indicate that there are distinct genetic pathways in the development of melanoma. The group of melanomas on skin without chronic sun-induced damage frequently had a mutation in *BRAF* together with chromosome 10 (site of *PTEN*) loss or mutations in *NRAS* alone. In contrast, melanomas in the group of tumors arising form skin with chronic sun-induced damage, mucosal- and acral melanomas did not have mutations in *BRAF* and *NRAS* but instead had increased numbers of copies of *CCND1* or *CDK4*. These findings indicate distinct genetic pathways in the development of melanoma which will affect the design of targeted therapeutic intervention in the future (29).

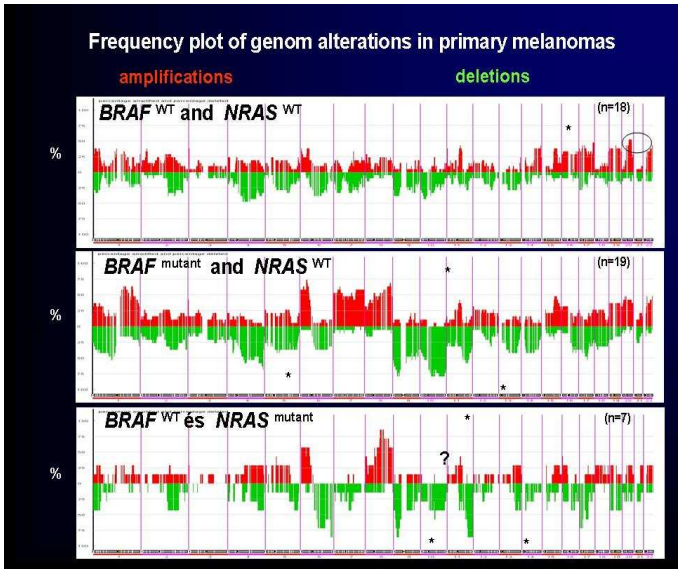


Figure 6 Genomic progression of malignant melanoma as detected by array CGH *(Balazs et al 2010. submitted)*

In our recent study we aimed to further refine clonal genetic alterations in connection with *BRAF* or *NRAS* mutation status and UV-exposure in a series of primary melanomas (n=47) array CGH (Balazs et al. submitted). The frequency patterns of copy number changes for each group across the entire genome are shown in Figure 2. Alterations exclusively associated with BRAF mutation were gains of 7p22.3-p13, 7q21.3 and 7q31.2-7q36.3, and losses of 10q21.3, 10q26.13 and 10q26.3. We found two major genetic subgroups of primary melanomas using unsupervised clustering of array CGH data for chromosome 7, clearly distinguishing tumors with BRAF mutations from tumors with NRAS mutations or without mutations Interestingly, a group of concomitant genetic alterations (e.g. 11q13, 9p21, 17q21.32, 22q13) was found frequently deleted in the left cluster of the tree (Figure 7) in contrast to the right cluster where the gain of chromosome 7 was seen. Cluster A and mainly contained patients with stage IV-V and cluster B was characteristic for Clark's level I-II-III malignant melanoma. This is clearly shown on Figure 7. (Balazs et al. submitted, 2010). Our array CGH experiments were performed on HumArray 3.2 obtained from the University of California, San

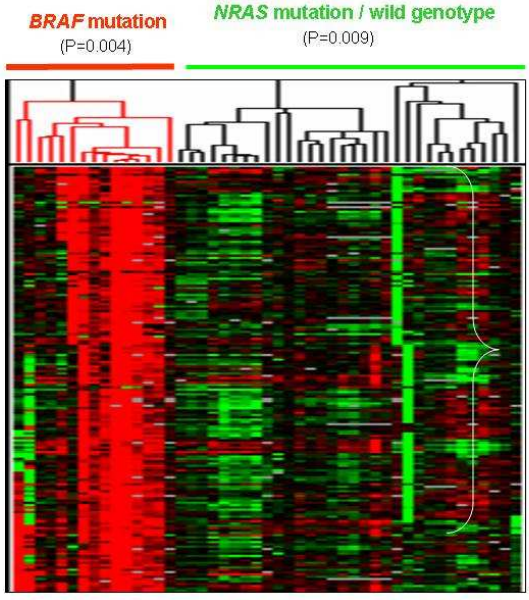


Figure 7 Cluster analysis of chromosome 7 array CGH data *(Balázset al. submitted, 2010)*

Francisco Cancer Center Array Core. This array contains 2464 BAC and P1 clones printed in triplicates covering the genome at roughly 1.4 Mb resolution. High level amplification and homozygous deletion exceeding the upper or lower thresholds (log_2 ratio $\geq$0.55 or $\leq$–0.8, respectively) included oncogenes (e.g., *CCND1*, *TAOS1*, *FGF19***,** *CTTN*, *VEGFA*, *CCND3* and *CUL1*) and a common homozygous deletion of the 17q21.32 region was observed in 26% of tumors. Frequent focused gains involving the 20q13.2 and 13q12 locus, losses involving regions of 3p, 5q and 8p chromosome arm and the 11q13.4 locus were significantly more common in the tumor group arising from chronically sun exposed sites than in the group arising from intermittently sun exposed site.
In conclusion, comparative genomic hybridization is a powerful method to detect and map DNA copy number changes in DNA extracted from archival tissue. The DNA copy number changes in melanoma and melanocytic nevi differ remarkably from each other. These differences can be exploited for diagnostic purposes as well as for discovery of genetic mechanisms in melanoma progression (16).

Gene expression profiling of melanoma

Molecular classification of cutaneous malignant melanoma by gene expression profiling was first described by Bittner et al in 2000 (30). They identified two major clusters, but no correlation was found between the cluster group and any clinical variable of their tumor sets. An other group investigated the relationship between gene expression profiles and clinical outcome of 58 primary melanomas, 254 genes were found whose expression might have predictor role the clinical outcome of melanoma patients (31). The most recent paper published by Jeffs et al. (32). The major aim of their study was to identify new prognostic markers and therapeutic targets that might aid clinical cancer diagnosis and management. Global transcript profiling identified a signature featuring decreased expression of developmental and lineage specification genes including *MITF*, *EDNRB*, *DCT*, and *TYR*, and increased expression of genes involved in interaction with the extracellular environment, such as *PLAUR*, *VCAN*, and *HIF1a*. Migration assays showed that the gene signature correlated with the invasive potential of the cell lines, and external validation by using publicly available data indicated that tumours with the invasive gene signature were less melanocytic and may be more aggressive. It is very important that the invasion signature could be detected in both primary and metastatic tumours suggesting that gene expression conferring increased invasive potential in melanoma may occur independently of tumour stage (32). In the most recent review about the impact of genomics in understanding human melanoma progression and metastasis was summarized and showed that several groups have shown distinct differences in the gene expression patterns along the spectrum of melanoma tumor progression, with many able to show a distinct set or group of over- and underexpressed genes that are validated as having a distinct and key role in this neoplastic process. Some have attempted to develop large gene classifier sets, composed of several hundreds, often thousands, of genes. For instance, the Melanoma Group of the European Organization for Research and Treatment of Cancer (EORTC) recently reported that melanoma patients with an average PCM thickness of 2.0 mm had a favourable prognosis, whereas patients in a second group with a thickness of 5.3 mm had an unfavourable prognosis (33). The most recent summary about molecular profiling of melanoma was published just a few weeks ago entitled: Melanoma transcriptome reveals novel genomic alterations not seen before using the latest high-throughput DNA sequencing technologies. This group developed a systematic approach to characterize the spectrum of cancer-associated mRNA alterations through integration of transcriptomic and structural genomic data, and applied this approach to generate new insights into melanoma biology. They identified 721 novel, nonsynonymous coding variants in melanoma, though

only a subset was subjected to validation to determine whether they are bona fide somatic mutations. Based on the results above, it was expected that most are inherited SNPs, whereas ;30% are somatic mutations. Nonetheless, the set of variants is interesting. One mutation observed in melanoma cell line 501 Mel (*CTNNB1, chr3:41241117, C!T*) is noted 135 times in the COSMIC database of somatic mutations in cancer. Using paired-end massively parallel sequencing of cDNA (RNA-seq) together with analyses of high-resolution chromosomal copy number data, they identified 11 novel melanoma gene fusions produced by underlying genomic rearrangements, as well as 12 novel readthrough transcripts. They mapped these chimeric transcripts to base-pair resolution and traced them to their genomic origins using matched chromosomal copy number information. They also used these data to discover and validate base-pair mutations that accumulated in these melanomas, revealing a surprisingly high rate of somatic mutation and lending support to the notion that point mutations constitute the major driver of melanoma progression. Taken together, these results may indicate new avenues for target discovery in melanoma, while also providing a template for large-scale transcriptome studies across many tumor types.

Although these methods are mainly used in research laboratories, they are used in the clinical field as methods of patient selection in clinical trials and as predictive tests for selection of treatment in some malignancies (eg, Oncotype DX; Genomic Health, Redwood City, CA; MammaPrint, Agendia, Amsterdam, Netherlands).

Integration of genomic data sets from different platforms, such as gene copy number and expression profiling data, represents a powerful method for identification of functionally relevant molecular aberrations. However, genomic techniques do not supplant more targeted analytical methods. The complementary use of both these approaches will be essential to identify and exploit molecular changes in cancer for improved diagnosis and treatment.

References

1. Viros A, Fridlyand J, Bauer J, Lasithiotakis K, Garbe C, Pinkel D, Bastian B. Improving melanoma classification by integrating genetic and morphologic features. PLoS Med 2008;5(6):e120.

2. Chin L, Garraway L, Fisher D. Malignant melanoma: genetics and therapeutics in the genomic era. Genes Dev 2006;20(16):2149-82.

3. Ghosh P, Chin L. Genetics and genomics of melanoma. Expert Rev Dermatol 2009;4(2):131-143.

4. Blokx W, van DM, Ruiter D. Molecular cytogenetics of cutaneous melanocytic lesions - diagnostic, prognostic and therapeutic aspects. Histopathology;56(1):121-32.

5. Maldonado J, Fridlyand J, Patel H, Jain A, Busam K, Kageshita T, Ono T, Albertson D, Pinkel D, Bastian B. Determinants of BRAF mutations in primary melanomas. J Natl Cancer Inst 2003;95(24):1878-90.

6. Thomas N. BRAF somatic mutations in malignant melanoma and melanocytic naevi. Melanoma Res 2006;16(2):97-103.

7. Bastian B, Kashani-Sabet M, Hamm H, Godfrey T, Moore D, 2nd, Brocker E, LeBoit P, Pinkel D. Gene amplifications characterize acral melanoma and permit the detection of occult tumor cells in the surrounding skin. Cancer Res 2000;60(7):1968-73.

8. van DM, Sprenger S, Rombout P, Marres H, Kaanders J, Jeuken J, Ruiter D. Distinct chromosomal aberrations in sinonasal mucosal melanoma as detected by comparative genomic hybridization. Genes Chromosomes Cancer 2003;36(2):151-8.

9. Lazar V, Ecsedi S, Szollosi A, Toth R, Vizkeleti L, Rakosy Z, Begany A, Adany R, Balazs M. Characterization of candidate gene copy number alterations in the 11q13 region along with BRAF and NRAS mutations in human melanoma. Mod Pathol 2009;22(10):1367-78.

10. Garber K. Cancer research. Melanoma drug vindicates targeted approach. Science 2009;326(5960):1619.

11. Puig S, Malvehy J, Badenas C, Ruiz A, Jimenez D, Cuellar F, Azon A, Gonzalez U, Castel T, Campoy A and others. Role of the CDKN2A locus in patients with multiple primary melanomas. J Clin Oncol 2005;23(13):3043-51.

12. Meyle K, Guldberg P. Genetic risk factors for melanoma. Hum Genet 2009;126(4):499-510.

13. Carlson J, Ross J, Slominski A. New techniques in dermatopathology that help to diagnose and prognosticate melanoma. Clin Dermatol 2009;27(1):75-102.

14. Kallioniemi A, Kallioniemi O, Sudar D, Rutovitz D, Gray J, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992;258(5083):818-21.

15. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo W, Chen C, Zhai Y and others. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 1998;20(2):207-11.

16. Bauer J, Bastian B. Distinguishing melanocytic nevi from melanoma by DNA copy number changes: comparative genomic hybridization as a research and diagnostic tool. Dermatol Ther 2006;19(1):40-9.

17. Hughes S, Damato B, Giddings I, Hiscott P, Humphreys J, Houlston R. Microarray comparative genomic hybridisation analysis of intraocular uveal melanomas identifies distinctive imbalances associated with loss of chromosome 3. Br J Cancer 2005;93(10):1191-6.

18. O'Hagan R, Brennan C, Strahs A, Zhang X, Kannan K, Donovan M, Cauwels C, Sharpless N, Wong W, Chin L. Array comparative genome hybridization for tumor classification and gene discovery in mouse models of malignant melanoma. Cancer Res 2003;63(17):5352-6.

19. Nambiar S, Mirmohammadsadegh A, Hengge U. Cutaneous melanoma: fishing with chips. Curr Mol Med 2008;8(3):235-43.

20. Harvell J, Kohler S, Zhu S, Hernandez-Boussard T, Pollack J, van dRM. High-resolution array-based comparative genomic hybridization for distinguishing paraffin-embedded Spitz nevi and melanomas. Diagn Mol Pathol 2004;13(1):22-5.

21. Treszl A, Ladanyi A, Rakosy Z, Buczko Z, Adany R, Balazs M. Molecular cytogenetic characterization of a novel cell line established from a superficial spreading melanoma. Front Biosci 2006;11:1844-53.

22. Curtin J, Busam K, Pinkel D, Bastian B. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 2006;24(26):4340-6.

23. North J, Kageshita T, Pinkel D, LeBoit P, Bastian B. Distribution and significance of occult intraepidermal tumor cells surrounding primary melanoma. J Invest Dermatol 2008;128(8):2024-30.

24. Albertson DG, Ylstra B, Segraves R, Collins C, Dairkee SH, Kowbel D, Kuo WL, Gray JW, Pinkel D. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. Nat Genet 2000;25(2):144-6.

25. Hui AB, Lo KW, Teo PM, To KF, Huang DP. Genome wide detection of oncogene amplifications in nasopharyngeal carcinoma by array based comparative genomic hybridization. Int J Oncol 2002;20(3):467-73.

26. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y and others. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 1998;20(2):207-11.

27. Takeo S, Arai H, Kusano N, Harada T, Furuya T, Kawauchi S, Oga A, Hirano T, Yoshida T, Okita K and others. Examination of oncogene amplification by genomic DNA microarray in hepatocellular carcinomas: comparison with comparative genomic hybridization analysis. Cancer Genet Cytogenet 2001;130(2):127-32.

28. Wilhelm M, Veltman JA, Olshen AB, Jain AN, Moore DH, Presti JC, Jr., Kovacs G, Waldman FM. Array-based comparative genomic hybridization for the differential diagnosis of renal cell cancer. Cancer Res 2002;62(4):957-60.

29. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Brocker EB, LeBoit PE and others. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005;353(20):2135-47.

30. Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, Radmacher M, Simon R, Yakhini Z, Ben-Dor A and others. Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature 2000;406(6795):536-40.

31. Winnepenninckx V, Lazar V, Michiels S, Dessen P, Stas M, Alonso S, Avril M, Ortiz RP, Robert T, Balacescu O and others. Gene expression profiling of primary cutaneous melanoma and clinical outcome. J Natl Cancer Inst 2006;98(7):472-82.

32. Jeffs A, Glover A, Slobbe L, Wang L, He S, Hazlett J, Awasthi A, Woolley A, Marshall E, Joseph W and others. A gene expression signature of invasive potential in metastatic melanoma cells. PLoS One 2009;4(12):e8461.

33. Ren S, Liu S, Howell P, Jr., Xi Y, Enkemann S, Ju J, Riker A. The impact of genomics in understanding human melanoma progression and metastasis. Cancer Control 2008;15(3):202-15.

Contributions:

Array CGH analysis was done by Szilvia Ecsedi, Laura Vízkleti and Viktória Lázár whereas gene expression anaylis was performed by Zsuzsa Rákosy, University of Debrecen Medical and health Science Center Department of preventive Medicine, Hungary

Growth Arrest of Melanocytic Nevus and Malignant Transformation to Melanoma

W.J. Mooi

Department of Pathology
VU University Medical Center, Amsterdam

Oncogene-induced senescence

- What is cellular senescence?
- What are the causes of cellular senescence?
- Is there a cancer-protective effect of oncogene-induced senescence?
- Does oncogene-induced senescence play a role in halting the outgrowth of pituitary tumours?

Looking at the BRAF oncogene: simple arithmetics…..

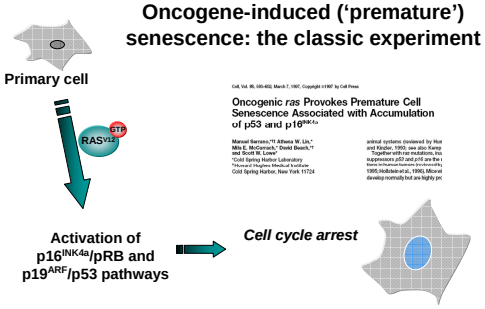
- Number of cell divisions in one lifetime: 10^{16}
- Chance of unrepaired mismatch during DNA replication, at every individual base position in DNA: 10^{-9}
- Oncogenic BRAF mutation: U→A in second position of codon 300, i.e., 1/3 of all possible mismatches
- Number of cells arising during one's lifetime harbouring a *de novo* oncogenic BRAF mutation: $10^{16} \times 10^{-9} \times 1/3 =$ 3,300.000, i.e., one cell every 12 minutes
- That cell will have increased mitogenic signaling through the MAPK pathway

Why are tumours not far more common??

Cells harboring oncogenic mutations are safeguarded by several tumor-suppressive processes

- The affected cell is irreversibly committed to terminal differentiation
- Mutated BRAF does not lead to increased MAPK pathway signaling, or that signaling does not result in increased proliferation in that particular cell type
- Elimination through intrinsic induction of apoptosis (e.g., P53-induced)
- Elimination through CTL-mediated induction of apoptosis
- Telomere attrition-associated cellular senescence leading to mitotic arrest
- Lack of induction of supportive fibrovascular stroma
- **Oncogene-induced cellular senescence leading to mitotic arrest**

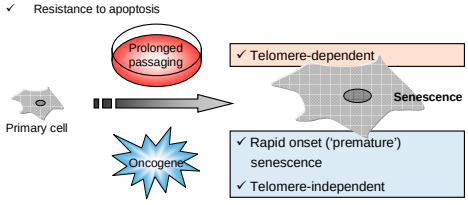
Oncogene-induced ('premature') senescence: the classic experiment



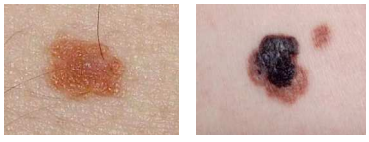
Replicative senescence *versus* Oncogene-Induced Senescence (OIS)

General characteristics of cellular senescence:

- ✓ Lack of proliferation
- ✓ Induction of tumour suppressor network including p16^{INK4A}, RB, ARF, p53
- ✓ SA-β-galactosidase (SA-β-GAL) activity; senescence-associated heterochromatin foci (SAHFs)
- ✓ Large cell size
- ✓ Resistance to apoptosis

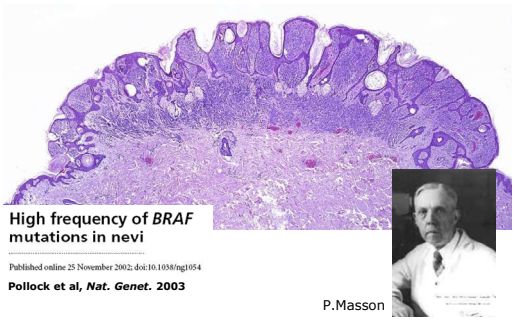


Nevus (skin 'mole') *versus* melanoma



Prototypical benign *versus* malignant neoplasm

Melanocytic nevus cells: neoplastic, but mitotically inactive



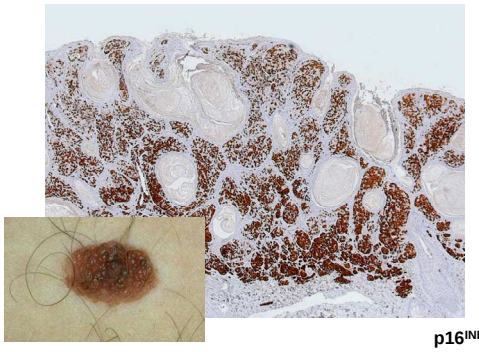
High frequency of *BRAF* mutations in nevi

Published online 25 November 2002; doi:10.1038/ng1054

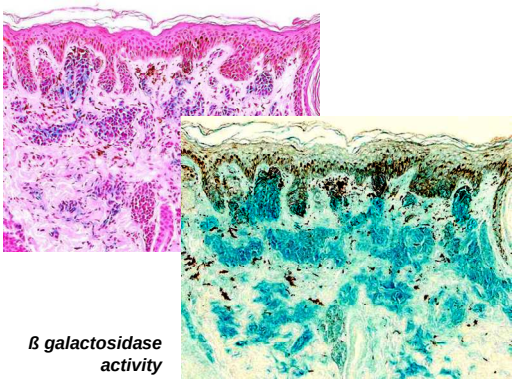
Pollock et al, *Nat. Genet.* 2003

P.Masson

Melanocytic nevus: p16^{INK4A}

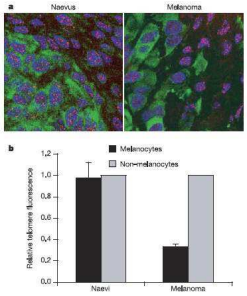


p16^{INK4A}

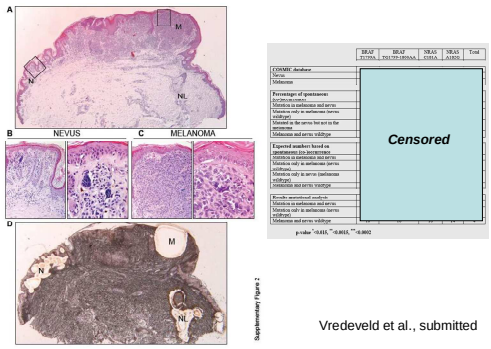


ß galactosidase activity

No apparent telomere attrition in melanocytic nevi

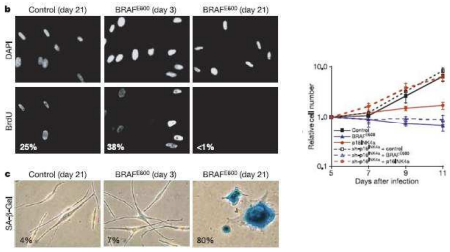


Michaloglou et al, *Nature* 2005



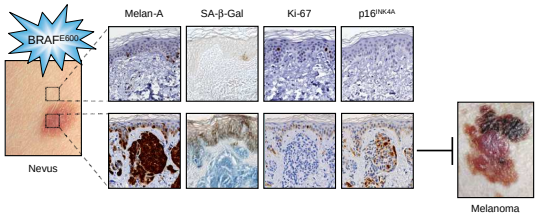
Vredeveld et al., submitted

Oncogene-induced cellular senescence (OIS) illustrated *in vitro* (BRAF^{E600}-induced OIS in cultured melanocytes)



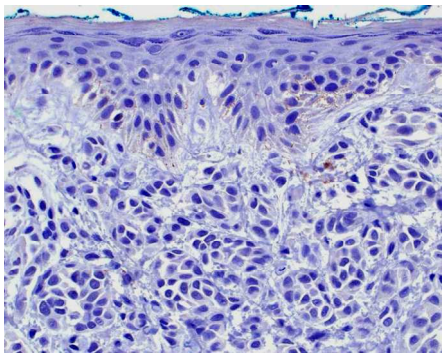
Michaloglou, Vredeveld et al., *Nature* 2005

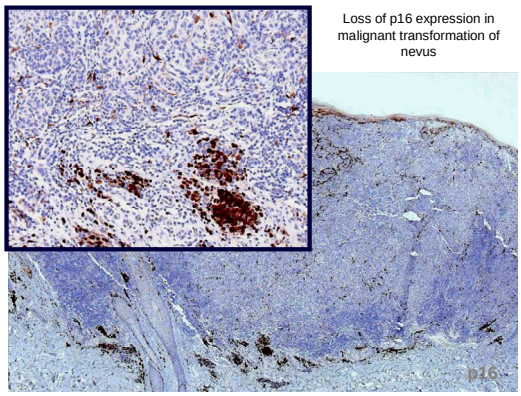
Human melanocytic nevi display OIS *in vivo*



Michaloglou *et al.*, Nature (2005)
Gray-Schopfer *et al.*, Br J Cancer (2006)

P16 immunoreactivity is absent from most melanomas





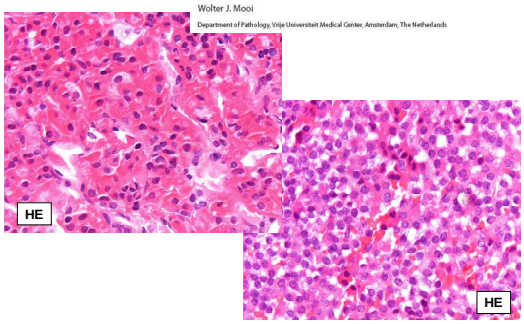
Loss of p16 expression in malignant transformation of nevus

OIS *in vivo* : an emerging theme

- **Human melanocytic nevi** (BRAF; Michaloglou *et al.*, 2005; Gray-Schopfer *et al.*, 2006)
- **Human serrated colorectal adenomas** (BRAF, KRAS; Minoo & Jass 2006)
- **Zebrafish nevi** (BRAF; Patton *et al.*, 2005)
- **Murine pancreatic & lung adenomas** (K-RAS; Collado *et al.*, 2005)
- **Murine lymphomas** (N-RAS; Braig *et al.*, 2005)
- **Murine pituitary hyperplasia** (E2F3; Lazzerini-Denchi *et al.*, 2005)
- **Murine early melanomas** (HGF; Ha *et al.*, 2007)
- **Murine lung adenomas** (BRAF; Dankort *et al.*, 2007)\
- **Pituitary adenoma????**

Oncogene-Induced Cellular Senescence: Causal Factor in the Growth Arrest of Pituitary Microadenomas?

Wolter J. Mooi
Department of Pathology, Vrije Universiteit Medical Center, Amsterdam, The Netherlands



Oncogene-Induced Cellular Senescence: Causal Factor in the Growth Arrest of Pituitary Microadenomas?

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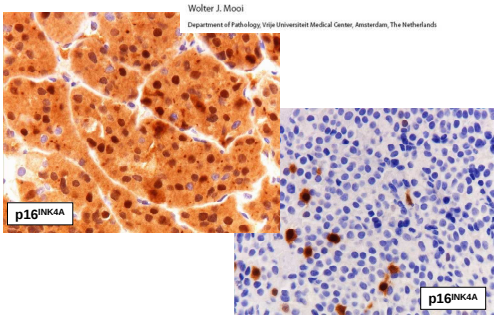
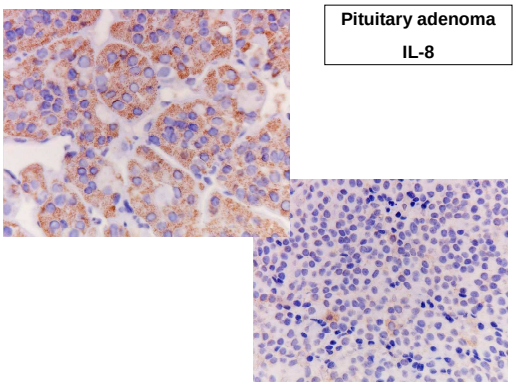
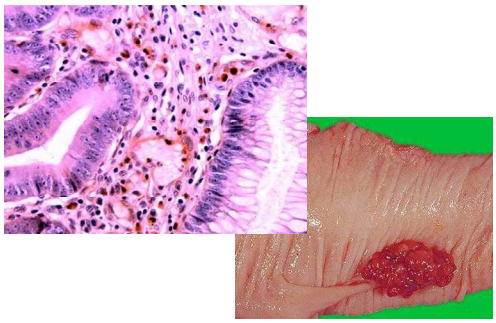


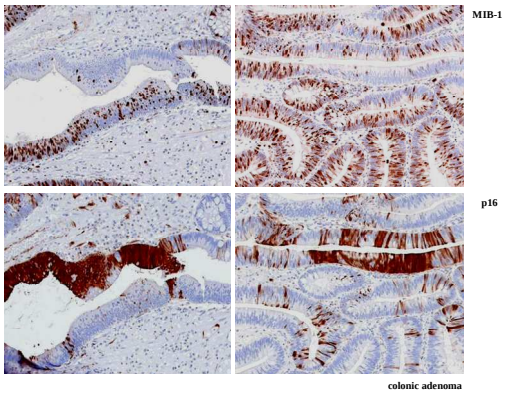


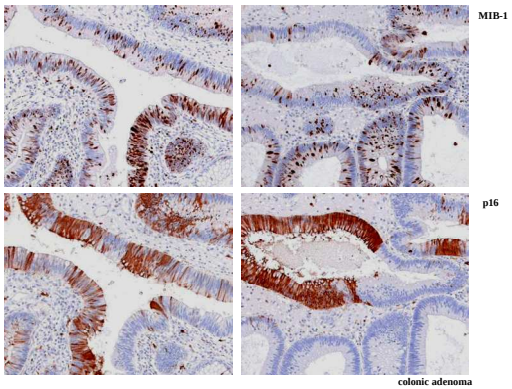
Pituitary adenoma

IL-8

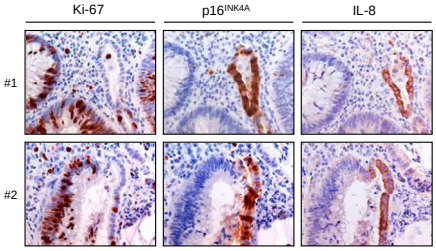
Colonic adenoma







Co-localization of IL-8 with growth-arrested, p16^{INK4A}-positive human colon adenoma cells



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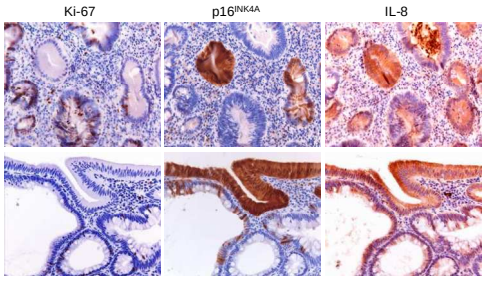
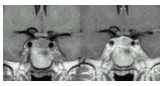
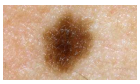




Pituitary tumours

- Extremely common: prevalence prevalence about 11-14% in autopsy series; 22% of live adults (> 3 mm, MRI data)
- Almost all are adenomas; carcinomas are extremely rare
- The vast majority of adenomas are growth-arested microadenomas (< 1 cm); problems of local mass effect are rare
- Activating point mutations in GNAS1, RAS &c



Melanocytic tumours

- Extremely common: prevalence amost 100 % in adults
- Almost all are naevi; melanomas are rare
- The vast majority of naevi are still small (< 1 cm) when they reach growth arrest
- Activating point mutations in BRAF, NRAS, HRAS

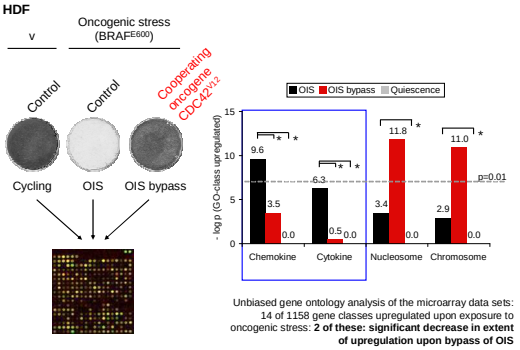
Cell

Oncogene-Induced Senescence Relayed by an Interleukin-Dependent Inflammatory Network

Thomas Kuilman,[1] Chrysilis Michaloglou,[1,4] Liesbeth C.W. Vredeveld,[1,4] Sirith Douma,[1] Remco van Doorn,[1,5] Christophe J. Desmet,[1] Lucien A. Aarden,[2,4] Wolter J. Mooi,[3,4] and Daniel S. Peeper[1,*]
[1]Division of Molecular Genetics, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands
[2]Sanquin-AMC Landsteiner Laboratory, 1066 CX Amsterdam, The Netherlands
[3]Department of Pathology, VU University Medical Center, 1081 HV Amsterdam, The Netherlands
[4]These authors contributed equally to this work
[5]Present address: Department of Dermatology, Leiden University Medical Center, 2300 RC Leiden, The Netherlands
*Correspondence: d.peeper@nki.nl
DOI 10.1016/j.cell.2008.03.039

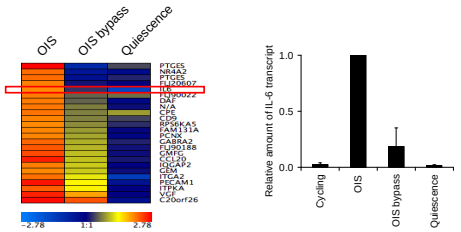
Cell 2008; **133**: 1019-31.

Gene expression profiling reveals an inflammatory response associated with OIS



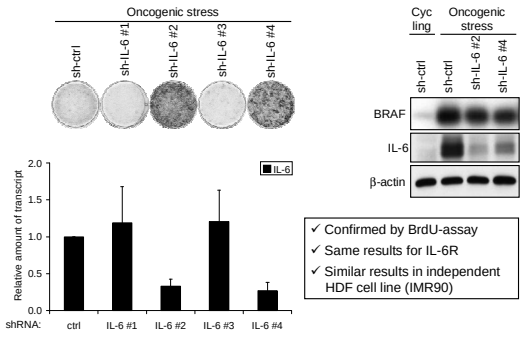
Unbiased gene ontology analysis of the microarray data sets: 14 of 1158 gene classes upregulated upon exposure to oncogenic stress: **2 of these: significant decrease in extent of upregulation upon bypass of OIS**

Interleukin-6 is specifically upregulated in OIS



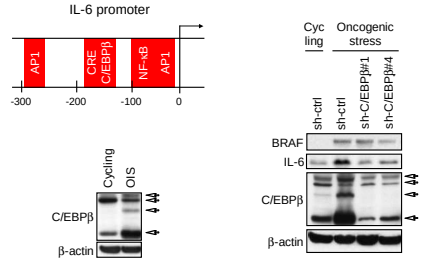
Single genes strongly associated with OIS (induced by oncogenic stress, significantly less induced during OIS bypass and quiescence: 24 genes, including IL-6

IL-6 is required for OIS

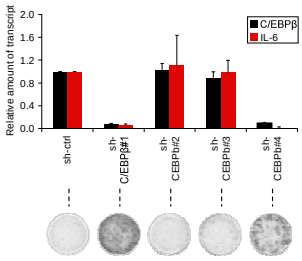


- ✓ Confirmed by BrdU-assay
- ✓ Same results for IL-6R
- ✓ Similar results in independent HDF cell line (IMR90)

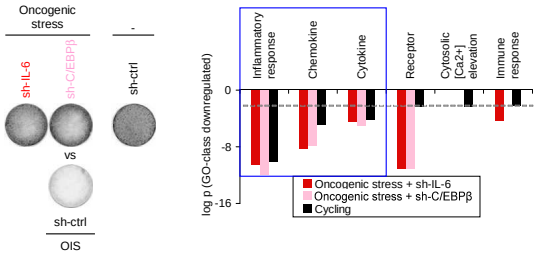
C/EBPβ is a critical regulator of IL-6



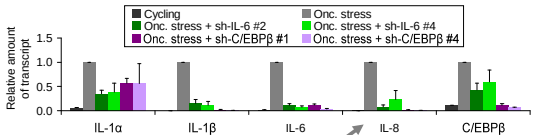
C/EBPβ mediates OIS

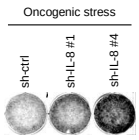


IL-6 is a central regulator of an inflammatory network mediating OIS



IL-6 and C/EBPβ amplify signaling towards the inflammatory transcriptome associated with OIS





Like IL-6, IL-8 mediates OIS bypass and is transcriptionally activated by C/EBPβ

Acknowledgements: (OIS screens and IL work)

NKI:
Thomas Kuilman
Chrysiis Michaloglou,
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Sirith Douma,
Christophe Desmet,
Remco van Doorn (LUMC),
Daniel Peeper

CLB:
Els de Groot
Lucien Aarden

VUmc:
Pim Kortman
Angelique Verlaan

Pagetoid Melanocytosis: When is it Significant?

Thomas Krausz

Department of Pathology
University of Chicago, Chicago, IL

Pagetoid melanocytosis
Definition

- A histologic pattern defined as ascent or upward scatter of single melanocytes and small groups of melanocytes, into suprabasal layers of the epidermis, including the granular layer and above
- Although it is generally considered to be a diagnostic hallmark of melanoma, it may also be seen in certain melanocytic nevi

Pagetoid melanocytosis
Histologic criteria

- Cells in the superficial layers of the epidermis must be clearly recognizable as melanocytes
- Melanocytes in the superficial epidermis must not occur as direct extension from the junctional component but must appear discontinuous
- Melanocytes must be located above a line parallel to the skin surface, placed at the level above the basal epidermal layer overlying the most superficial dermal papilla (Haupt and Stern, 1995)

Pagetoid melanocytosis
Histologic Criteria

- Melanocytes below this line may represent junctional melanocytes that falsely appear to be within the suprabasal epidermis due to off-center or tangentional sectioning of rete ridges
- If no rete ridges: suprabasal melanocytes separated by at least one layer of keratinocytes from the basal cells can be regarded as evidence of discontinuous melanocytic spread

Pagetoid cells in the epidermis
non-melanocytic

Mammary Paget's disease
Extramammary Paget's disease
Pagetoid squamous cell ca. in situ
Clear cells of Toker
Pagetoid dyskeratosis
Clear cell papulosis
Sebaceous carcinoma
Eccrine porocarcinoma
Merkel cell carcinoma
Cutaneous T cell lymphoma
Histiocytosis-X

Pagetoid cells in the epidermis
melanocytic

- Malignant melanoma, mostly superficial spreading type
- Congenital nevi in early infancy
- Spitz nevi
- Reed nevi (pigmented spindle cell nevus)
- Recurrent nevi
- Nevi following UV exposure
- Acral nevi
- Nevi on genitalia, breast and flexural sites

Frequency of pagetoid melanocytosis

- Malignant melanoma: 96 %
- Spitz nevi: 38 %
- Pigmented spindle cell nevi: 20 %
- Nevi of palms and soles: 61%
- Vulvar nevi: 80%
- Acquired ordinary nevi: none

Haupt HM, Stern JB. Am J Surg Pathol 1995; 19: 792-797

Pagetoid melanocytosis
in primary cutaneous melanoma

- Review of 340 melanomas
- Conspicuous pagetoid infiltration: 32.1%
- Occasional melanocytes in stratum spinosum: 23.5%
- No melanocytes above basal layer: 44.4%

(Fallowfield and Cook, 1992)

Worrying histologic features including pagetoid spread
in congenital nevi of infancy

- Extensive, irregular junctional component which usually extends along skin appendages
- Architectural disorder with irregularly distributed nests combined with extensive lentiginous proliferation
- Some cytologic atypia with cell enlargement and rare mitotic figures
- Pagetoid spread is usually limited but may be prominent
- Papillary dermis with large sometimes hyperchromatic and focally pigmented nevus cells forming nests or spread as solitary cells accompanied by mild inflammation and fibrosis
- Growth within arrector pili muscles, adnexa, perineurium, walls of lymphatics and blood vessels; proliferative nodules

Worrying histologic features
of congenital nevi in early infancy

- Architectural disorder with unevenly distributed irregular junctional nests combined with extensive lentiginous proliferation which usually extends along skin appendages
- Some cytologic atypia with cell enlargement and rare mitotic figures
- Pagetoid spread is usually limited but occasionally prominent
- Papillary dermis with large sometimes hyperchromatic and focally pigmented nevus cells forming nests or spread as solitary cells
- Growth within arrector pili muscles, adnexa, perineurium, walls of lymphatics and blood vessels; proliferative nodules

Giant congenital nevi of infancy

- Present at birth; large size (> 20 cm); *NRAS* mutation
- Deep dermal penetration, spread to subcutis
- More variable and worrying histologic features than most acquired nevi
- Subsequent biopsy may show less atypical features
- Malignant melanoma arising in early infancy is extremely rare, usually located in the dermis and may show divergent differentiation
- Most melanomas in congenital nevi develop after puberty
- Lifetime melanoma risk of a few percent at most

Pagetoid melanocytosis
in Spitz nevi

- Especially in superficial/junctional Spitz nevi
- Lower density upward spread than in melanoma
- Limited to the center of the lesion
- Often admixed with transepidermal elimination of melanocytic nests
- No high grade cytologic atypia
- Marked epidermal hyperplasia and hypergranulosis
- Terminal junctional epidermal nests at the lateral edge of the lesion, characteristic “spitzoid” cell type, symmetry, Kamino bodies

Pagetoid melanocytosis
in Pigmented spindle cell nevi

- Pigmented spindle cell nevus: confluent, nested proliferation of vertically oriented pigmented spindle shaped melanocytes
- Hyperplastic, hyperkeratotic epidermis
- Clefts between junctional nests and keratinocytes
- Pagetoid melanocytosis: in 20% of cases
- Usually limited to the central portion of the lesion
- Transepidermal elimination of pigmented melanocytic nests is frequent:

  large melanocytic nests reach from the dermo-epidermal junction to the cornified layer

Pagetoid melanocytosis
in Recurrent Nevi

- Recurrent nevus: single melanocytes and irregularly spaced and shaped nests at the dermo-epidermal junction
- Enlarged melanocytes, but less nuclear atypia and mitotic activity than in melanoma
- Marked hyperpigmentation
- Horizontal dermal scar
- Pagetoid ascent in 60 % of cases
- Atypical melanocytic proliferation does not extend beyond the scar on either side
- Remnants of the original nevus are present

Pagetoid melanocytosis
in Nevi following UV exposure

- UV irradiation influences proliferative and metabolic activity of melanocytes and keratinocytes
- Increased suprabasal location of melanocytes, enlargement of nuclei, enhanced HMB-45 staining
- Apoptosis in selected melanocytes and keratinocytes
- Histologic changes resembling those of melanoma in situ
- Clinical history: sunburn or phototherapy

Pagetoid melanocytosis
in Acral nevi

- Acral nevi: frequent architectural atypia, irregular pigmentation
- Absence of significant nuclear atypia
- No mitotic activity
- No inflammatory infiltrate
- Well-defined vertical columns of melanin in the cornified layer
- Pagetoid spread: limited to a few solitary melanocytes; also transepidermal elimination of melanocytic nests
- Limited to central region of epidermis

Pagetoid melanocytosis
in Acral nevi

- Palms
- Soles
- Nailbeds
- Dorsal surfaces of hands and feet
- Elbows
- Knees

Acral nevus *versus* melanoma
Histologic features favoring melanoma

- Nuclear atypia of the ascending cells
- Ascending cells retaining abundant pale cytoplasm
- Pagetoid melanocytosis beyond the lateral border of the intradermal part of the lesion
- Pagetoid spread across the entire lesion

Melanocytic Nevi of Palms and Soles:
a histologic study according to the plane of section

Signoretti S et al, Am J Surg Pathol 1999; 23:283-287

- Study of the spatial distribution of melanocytes and the effects of sectioning along different planes in relation to skin markings (dermatoglyphics): perpendicular versus parallel to ridges and furrows
- Junctional nevi of palms and soles often display a striate appearance because the pigment tends to be mostly concentrated in the furrows
- Nevi sectioned perpendicularly to dermatoglyphics: symmetry, circumscription, melanin columns
- In nevi intraepidermal scatter of melanocytes and melanin columns are concentrated in the furrows

Pagetoid melanocytosis
in Acral nevi

- Signoretti et al: 79 %
- Haupt and Stern: 61 %
- Boyd and Rapini: 38 %
- Unlike in melanoma, melanin predominates over melanocytes and both tend to be arranged in vertical columns in the horny layer
- Presence of melanin columns in melanocytic lesions of volar skin a histologic sign of benignity

Pagetoid melanocytosis
in melanocytic nevi on Genitalia

- Limited pagetoid spread: solitary units and nests of melanocytes Haupt and Stern: in 80% of cases
- Some melanocytes with enlarged nuclei, abundant pale cytoplasm
- Pagetoid spread associated with architectural atypia: focally confluent junctional nests vary in shape and size, diminished cohesion of melanocytes, increase of solitary melanocytes at dermo-epidermal junction
- Focal irregular pigmentation

Pagetoid melanocytosis
in melanocytic nevi on the Breast

- Limited pagetoid scatter of melanocytes
- Atypical architectural and cytological features
- Papillary fibroplasia

Rongioletti F et al, J Cutan Pathol 2004; 31: 137-140

Pagetoid Melanocytosis
in melanocytic nevi on Flexural sites

- Axilla
- Inguinal creases
- Umbilicus
- Pubis
- Scrotum
- Perianal area
- Irregular nested and dishesive pattern, similar to genital nevi
- Limited pagetoid spread in only rare cases

Pagetoid melanocytosis
Pathogenetic considerations

- The mechanism of the upward spread of melanocytes within the epidermis is poorly understood
- Active infiltrative process (destructive, mutidirectional)
- Passive "passenger" mechanism (vertical flow using the maturing keratinocytes as carriers)

Pagetoid melanocytosis
Pathogenetic considerations

- In the normal epidermis and in most stable nevi: the melanocytes are relatively immobile, anchored to the basement membrane and/or to each other in the melanocytic nests, hence they can resist the flow of maturing keratinocytes during epidermal turnover
- This stability is maintained by interaction between melanocytes, keratinocytes, and extracellular matrix
- Key role of of matricellular protein CCN3 for the spatial localization of melanocytes to the basement membrane through DDR1 (discoidin domain receptor)

Pagetoid melanocytosis
Pathogenetic considerations

- Under the influence of various stimuli (UV light, mechanical trauma, etc) this interactively anchored setting may temporarily change to a dynamic one through increased cell proliferation and altered expression of integrins/adhesion molecules
- Similarly, nevi in their active/growing phase (nevi in infancy, junctional/compound Spitz nevi, etc) also have a fluctuating epidermal setting, partly as a result of increased cell proliferation of both keratinocytes and melanocytes

Pagetoid melanocytosis
Key factors in pathogenesis

- Transcription factors
- Cell proliferation of both melanocytes and adjacent keratinocytes
- Adhesion molecule/integrin expression of normal skin and melanocytic tumors
- Close functional relationship between melanocytes and adjacent keratinocytes
- Keratinocyte-derived factors (endothelin-1)
- Endothelin-B receptor on melanocytes
- Matrix-metalloproteinases (MMP and TIMP)

Pagetoid melanocytosis
Conclusions

- Pagetoid melanocytosis may occur not only in melanoma but also in nevi
- However, a diffuse and dense scatter of intraepidermal melanocytes with prominent cytologic atypia and suprabasal lateral extension beyond the junctional component favor melanoma
- The correct pathologic diagnosis of melanocytic lesions must be based not only on the presence of pagetoid melanocytosis but on all the pertinent histological and clinical findings
- Key factors in the pathogenesis of pagetoid melanocytosis have been identified but the specific mechanisms are still poorly understood

Pagetoid melanocytosis
Future directions

- Existing data indicate that molecular mechanisms that govern embryonic development of the epidermis are reused during postnatal life to regulate the balance between stem cell activation and differentiation
- The molecular mechanisms that govern embryonic migration and differentiation of neural crest-derived melanoblasts in the dermis and epidermis are also probably operational in postnatal life
- In order to understand the pathogenesis of pagetoid melanocytosis the altered interaction of keratinocyte and melanocyte within the concept of Unna's Abtropfung ("drop down") versus the alternative proposal Hochsteigerung (upward climb) by Cramer has to be clarified
- Meantime some diagnostically "impossible" cases exhibiting pagetoid spread perhaps can be worked out by using FISH as an ancillary diagnostic tool to demonstrate DNA copy number alterations of melanoma

References

- Abernethy JL, Soyer HP, Kerl H et al. Epidermotropic metastatic malignant melanoma simulating melanoma in situ. Am J Surg Pathol 1994; 18:1140-1149
- Haupt HM, Stern JB. Pagetoid melanocytosis. Histologic features in benign and malignant lesions. Am J Surg Pathol 1995; 19:792-797
- Signoretti S, Annessi G, Puddu P et al. Melanocytic nevi of palms and soles: a histologic study according to the plane of section. Am J Surg Pathol 1999; 23:283-287
- Urso C. Pseudoinfiltration: an underrecognized feature in melanocytic lesions. Am J Dermatopathol 2002; 24:183-184
- Cramer SF. Pagetoid infiltration and pseudoinfiltration – another point of view. Am J Dermatopathol 2002; 24: 451
- Petronic-Rosic V, Shea CR, Krausz T. Pagetoid melanocytosis: when is it significant? Pathology 2004; 36: 435-444

References

- Khalifeh I, Taraif S, Reed JA et al. A subgroup of melanocytic nevi on the distal lower extremity (ankle) shares features of acral nevi, dysplastic nevi, and melanoma in situ. Am J Surg Pathol 2007; 31:1130-1136
- Mooi WJ, Krausz T. Pathology of Melanocytic Disorders. Hodder Arnold, 2007
- Gleason BC, Hirsch MS, Nucci MR et al. Atypical genital nevi. A clinicopathologic analysis of 56 cases. Am J Surg Pathol 2008; 32:51-57
- Cramer SF. Consumption of the epidermis – what is its place in the spectrum of aberrant melanocyte-keratinocyte interactions? Am J Dermatopathol 2008; 30:200-2002
- Viros A, Fridlyand J, Bauer J et al. Improving melanoma classification by integrating genetic and morphologic features. PloS Medicine 2008; 5:941-952

References

- Khalifeh I, Taraif S, Reed JA et al. A subgroup of melanocytic nevi on the distal lower extremity (ankle) shares features of acral nevi, dysplastic nevi, and melanoma in situ. Am J Surg Pathol 2007; 31:1130-1136
- Mooi WJ, Krausz T. Pathology of Melanocytic Disorders. Hodder Arnold, 2007
- Gleason BC, Hirsch MS, Nucci MR et al. Atypical genital nevi. A clinicopathologic analysis of 56 cases. Am J Surg Pathol 2008; 32:51-57
- Cramer SF. Consumption of the epidermis – what is its place in the spectrum of aberrant melanocyte-keratinocyte interactions?
  Am J Dermatopathol 2008; 30:200-2002
- Viros A, Fridlyand J, Bauer J et al. Improving melanoma classification by integrating genetic and morphologic features. PloS Medicine 2008; 5:941-952

Prognostic and Predictive Pathology of Skin Melanoma

József Tímár

2nd Department of Pathology
Semmelweis University, Budapest

Clinicopathology of melanoma progression

Local invasion of skin melanoma occurs in two directions, invasion of the covering epidermis and invasion of the dermal tissues. Today, ulceration of the covering epidermis of melanoma is one of the most sensitive indicators of the metastatic potential (1-3). On the other hand, thickness of the tumor in the dermis (expressed in mm, Breslow) is the most sensitive indicator of progression potential. A 4 mm thick melanoma has a 50% chance for developing visceral metastasis within 10 years, however, with further increase in thickness it does not result in an appropriately worst outcome, it seems that at 14 mm thickness the metastatic rate reaches a plateau of 70% (1-3) meaning that the size of the primary tumor *per se* is not the most sensitive prognostic marker for visceral metastic potential. Primary growth of skin melanoma is characterized by two patterns, superficial spreading (maintaining contact with the epidermis) and nodular type of growth or symmetrical growth. In case of superficial spreading type of tumors there are two types of growth, radial and vertical, the last reflecting deep dermal invasive potential. Although the nodular type of growth is associated with an increased metastatic potential, vertical growth is not a validated prognostic factor for skin melanoma clinicopathologically.

The next step in local spreading is the development of independent satellites in the subcutaneous fatty tissue or dermis. The dermis is rich in lymphatics and a primary melanoma has a potential to initiate lymphatic neoangiogenesis. Accordingly the intra- and peritumoral lymphatic microvessel density (LMDV) is found to be increased in primary tumors. (4) High LMVD is associated with the increased risk of developing regional lymph node metastases, therefore it is a negative prognostic factor. This potential is closely correlated with the expression of VEGF-D and-C in melanoma. (5)

Lymphatic invasion in the primary tumor also has a negative prognostic potential and is correlated with the onset of regional lymphatic metastases. In skin melanoma sentinel node positivity is a negative prognostic factor, but it seems to serve more as an indicator of the metastatic potential, rather than a source for systemic dissemination, since surgical removal of the sentinel metastatic lymph nodes does not affect the survival of melanoma patients. (4)

Skin melanoma cannot grow–beyond 1-2 mm without having proper vasculature. However, the dermis is rich in microcapillaries providing enough dense network of vessels for tissue expansion. Skin melanoma is characterized by an angiogenic phenotype (expression of VEGFs, bFGF, IL-8), inducing massive peritumoral neoangiogenesis (4), but studies indicated that during tissue expansion the primary tumor predominantly incorporates the peritumoral preexisting or induced microvasculature. (6) Therefore peritumoral microvessel density is not a prognostic factor in skin melanoma. On the other hand, microvessel invasion is a negative prognosticator for survival and development of visceral metastases suggesting that the incorporated peri- or intratumoral microvessels are the source for hematogenous dissemination. (1-3) During the growth of primary skin melanoma incorporation of microvessels provides continuous support for growth and hypoxia and consecutive necrosis is rare in the primary tumor. Accordingly, tissue hypoxia is most probably not the primary inducer of the expression of angiogenic cytokines, rather it is due to genetic alterations which developed during melanomagenesis.

Skin melanoma has an imminent potential to give rise to visceral metastases into almost every tissue type of the body. This is very different from other tumor types which may have a few or even only one preferred metastatic site i.e. prostate cancer (bone), colorectal cancer (liver). Since hematogenous dissemination from the various skin sites occurs at the venous site of circulation, the first filter organ in hematogenous dissemination is the lung, but melanoma does not stop spreading at this stage and continues its course into the systemic circulation. Clinical data indicate that melanoma patients with single lung metastases show longer survival rate than patients with other types of visceral metastases. (1-3) On the other hand, multiplex organ metastases determine the fate of melanoma patients, where the liver, brain, bones and any other

organ can be involved. Analysis of clinicopathology data, however, suggest that there are practically two types of skin melanoma: visceral metastatic and non-metastatic. Two sets of data indicate that increased thickness of the primary tumor does not lead to 100% metastatic efficiency and around 50% of regional lymph node positive skin melanoma patients never develop visceral metastases. (1-3)

Host factors influencing melanoma progression

Anatomy (1-3)

Melanomas on the trunk and upper extremities have a higher risk for developing the first distant metastases as compared with the head and neck and lower extremities. In the former cases the chance of the first distant metastases is 30%, while in other localizations the satellite lesions (local invasion) and in-transit local lymphatic metastases develop first. The factors determining the differences in biological behaviour of melanomas at various anatomic locations are unclear yet. UV exposure of the skin is different at various locations: the head and neck region is exposed to UV radiation to a greater extent than other locations (intermittent forms) which has recently been proved to affect the genotype of skin melanomas (CSD versus non-CSD melanomas (7). Another possible explanation for the different biological behaviour of melanomas could be the variability of lymphatics at various sites and the localization of regional lymph nodes and their potential connection to the systemic blood circulation.

Sex

Hormonal millieu may also affect progression of skin melanomas. Skin melanomas tend to develop more frequently on the lower extremities in females and in trunk region in males, and as indicated above, the progression at those sites is different. Data also indicate that there is an increased risk for the development of regional lymph node metastases in male as compared with female patients. Furthermore, epidemiological data suggest that the progression of skin melanomas is dependent on the hormonal state of woman, premenopausal status carries a lower risk as compared with postmenopausal status. (8)

Immune surveillance

It is long known to pathologists that skin melanomas are characterized by lymphoid infiltrates to various degrees and a brisk infiltrate is a good prognosis indicator. (9) On the other hand, regression of the primary melanoma due to heavy lymphoid infiltrates is a common clinicopathological feature, but data on its prognostic significance are highly controversial. Tumor infiltrating cells in melanomas contain T, B, as well as dendritic cells and macrophages. High density of T cells in skin melanomas is a weak prognostic factor (10), but in combination with a decrease in dendritic cells it becomes a much stronger prognosticator. (11) Interestingly, the density of dendritic cells in melanomas decreases parallel to increased tumor thickness unlike the T cells. Regression of the primary melanoma is accompanied by infiltration of CD4+ T cells expressing IL2R, suggestive of the process being mediated by an active antitumoral immune response.

Molecular determinants of melanoma progression

Among the oncogenes suspected to be involved in the metastatic progression of human skin melanomas, mutated or amplified ones may have significance. It may be unexpected, but from the oncogenes involved in melanomagenesis, none were proved to be involved in the later phase of progression (mutated B-RAF or N-RAS, **12**).

WNT5A was demonstrated to be overexpressed during the progression of malignant melanoma and the canonical pathway targets MITF transcription factor regulating melanocytic differenciation (13). On the other hand, in nodular melanomas a significant proportion of tumors harbor EGFR and C-MYC amplifications. Interestingly, EGFR amplification may be a supporter of the metastasic potential, (**14**) while C-MYC amplification may be an inhibitor of this potential (15). Cyclins and their inhibitors are likely involved in melanoma progression, but only a few clinical studies were able to prove this, pointing to the importance of the overexpression of cyclinE (low molecular weight variant, **ref.16**)**.** Oncosuppressors have been suspected to be involved in the progression of skin melanomas, but none were found unequivocally in the literature.

Metastasis genes

CD44 is expressed in a significant proportion of human skin melanomas but analysis of its splicing pattern suggested that instead of the v6-containing variant involved in colorectal cancer progression, the v3-containing version may play a significant prognostic role (**17**). TWIST, a master transcriptional regulator of epithelial/mesenchymal transition is overexpressed in a fraction of human primary melanomas and predicts a poor prognosis (18). Among the metastasis suppressor genes, NM23/NME1/NDP kinase may not be a significant player, since its expression is maintained in thick primary tumors toward lymphatic metastases (**19**). On the other hand, loss of expression of KISS-1 coding for metastin, ligand of the GPR54, is a frequent phenomenon in metastatic human melanomas due to either chromosomal deletions or malfuntion of its transcriptional co-activator, DRIP130. (**20**)

Alteration of the expression of cell adhesion molecules is a hallmark of metastatic progression. In human skin melanoma loss of E-cadherin and emerging expression of N-cadherin is associated with metastatic progression, which could well be the consequence of TWIST overexpression. (**21**) Although several matrix receptors have been studied in human melanoma progression, only β3 integrin overexpression was confirmed in multiple analyses. It seems that avb3 integrin overexpression is associated with malignant transformation of melanocytes in the human skin, while in a later phase of progression aberrant expression of the megakariocytic αIIb integrin chain leads to the expression of αIIbβ3 ectopic integrin. (**22**). Furthermore, aberrant/constitutive integrin signaling could also be a hallmark of melanoma progression characterized by ILK and FAK overexpressions (**23**). Recently, a novel melanoma metastasis gene was identified using a rodent melanoma model. NEDD9 is located on the 6p24-25 region of the chromosome, relatively frequently demonstrating gains in metastatic human melanoma (36%). (24) The NEDD9 protein is localized to focal contacts where it binds to FAK and is involved in invasion processes.

Progression of malignant tumors requires the expression and function of proteases, interestingly, human skin melanomas rely almost exclusively on the expression of MMP2 (25). Invasive phenotype requires upregulation of the motile phenotype, in skin melanomas C-MET overexpression could be responsible for the disregulation of the

paracrine form while overexpression of AMFR/gp78 chemokine receptor could be responsible for the emergence of an autocrine form. (**26**)

Apoptosis

Melanomas, similar to melanocytes, are characterized by constitutive resistance to apoptosis, which is determined/regulated by the expression of MITF-controlled anti-apoptotic BCL2. (27) However, BCL2 is overexpressed only in a small fraction of skin melanomas and controversial data are available on its role as prognosticator (28). Recent studies provided evidence that the expression of antiapoptotic protein Survivin is progressively increased during melanoma metastatization and its expression is a strong independent prognosticator. (29)

Stemness

Cancer stem cells emerge as significant determinants of malignant progression. Melanocyte stem cells are supposed to be characterized by a CD20+ phenotype.(**30**) Other studies demonstrated the expression of the stem cell specific ABCB5 transporter in a small fraction of melanoma cells parallel to the other stem cell marker, CD133. Furthermore, human melanoma cells are characterized by constitutive expression of several bone marrow stem cell marker genes including C-KIT, WT1, even CD34 (as part of their vasculogenic mimicry genotype), megakariocytic genes, such as integrin αIIb, platelet-12-LOX, thrombin receptor, the expression of almost all of which has been shown to be involved in melanoma progression. (**31)**

Vasculogenic mimicry

“Vasculogenic mimicry” is defined by a unique ability of aggressive melanoma cells to express an endothelial phenotype and to form vessel-like networks in 3-D culture. (**32)** Expression profiling revealed the genotype of melanoma cells of endothelial phenotype, including the expressions of VE-cadherin, EphA2, MT-MMP-1, MMP2 and laminin 5γ2, and the loss of expression of the melanoma marker MART1. Furthermore, it was later also shown that the integrin signaling regulator FAK (23) is involved in the maintenance

of this transdifferentiated phenotype together with tissue factor pathway inhibitor-2 (TFPI-2). (33)

Genomics of melanoma progression

Genes involved in tumor metastasis can now be divided into two cathegories, metastasis initiator- and metastasis maintenance ones. Gene signatures associated with thin and thick primary tumors or VGP versus RGP tumors characterize predominantly invasive potentials (corresponding to metastasis initiators) but may also contain signatures for lymphatic and vascular metastatic ability as well as signatures for lymphatic and vascular metastasis maintenace. Regional (sentinel) lymphatic metastasis of skin melanoma on the other hand carries a more restricted gene signature of lymphatic metastasis maintenance genes and vascular metastatic potentials. Lastly, visceral metastasis contains organ metastasis maintenance signatures exclusively. Skin melanoma is unique in local (skin) metastatic propensity manifested as in-transit or cutaneous metastases which contain mixture of lymphatic and hematogenous metastatic gene signatures (1,2).

When genomic analysis of melanoma tissues are performed, the origin of the tumor tissue is critical and limits those questions which can be answered. The quick overview of the microarray studies on human skin melanoma revealed that they were performed on a very heterogenous patient cohort and pathological sample collections, containing primary tumors, cutaneous metastases as well as lymphatic and in some instances visceral metastases. In case of the primary tumors thickness comparisons or VGP/RGP comparisons have also been performed. It is another problem that various histological forms of skin melanoma are not considered separately, although some of the studies revealed that their signatures are completely different from each other (SSM melanoma versus nodular melanoma versus rare non-UV forms).

<u>Prognostic gene signature: metastasis initiating genes</u>

Four such studies have been published, however on various sample types: only one study used primary tumors, some studies used cutaneous metastases and/or lymphatic metastases or even visceral metastases which contained various gene signatures (see earlier). Accordingly, these signatures although called prognostic signatures, are actually

different form each other, since they contain various mixtures of „prognostic" signatures: the study done on primary skin tumors contains a heterogenous signature of invasiveness and lymphatic/hematogenous metastatic potential, the study done on lymph node metastases contains signature of hematogenous metastatic potential exclusively, while the study done on a mixture of cutaneous mets and lymphatic metastases containes a mixture of signatures of lymphatic and hematogenous metastatic potentials. The first major microarray study on human melanoma metastasis analysed visceral metastases providing information on a visceral metastasis gene signature (**34**). It is then not surprising, that these signatures are different form each other, since they represent prognostic signatures at various stages of the progression (from the primary tumor to organ metastases) and may contain overlapping genes only accidentally (MCM3 and NFKBIZ).

Determination of invasive melanoma signature based on tumor thickness or VGP/RGP comparison have been performed in four studies, however without considering histological subtypes. Again, these invasive melanoma signatures are very heterogenous, in some studies are characterized by predominantly downregulated genes, whereas other studies contain various upregulated genes where a 9-gene overlap can be identified. These invasive melanoma signatures are completely different from the prognostic signatures identified. In one well-performed study a significant proportion of the invasiveness-signature overlapped with the prognostic signature (145/254 sets), **see selected gene list on *Table 4*).** (35)

Prognostic signatures: metastasis maintenance genes (35)

Five studies attempted to define a metastasis signature, unfortunately one study did not compare it to primary tumors and analysed immun response genes exclusively. It is another variable that in three studies cutaneous and lymphatic metastases were mixed together providing a mixed signature, only one study defined the cutaneous metastasis signature, while none were able to identify the lymphatic metastasis signature. Since studies on visceral metastases are very rare, we do not have a visceral metastasis signature of human melanoma yet, although the data obtained early on (**34**) may contain elements of that signature. It is of note, that these metastasis-signatures of human skin melanoma are predominated by downregulated genes and only by a much more limited

list of upregulated genes. Again, a minimal number of genes can be found to overlap among those signatures *(*AQP3, LGALS7 and SFN).

Meta-analysis of genomic studies on metastasis maintenance gene sets (35)

Seven published studies describing individual gene sets with discriminatory potential for melanoma metastases were included in the study. However, only six of the seven studies made the list of genes in their predictor available. The analysis was performed independently for all six published discriminatory gene sets. Hierarchical clustering was performed to assess the heterogeneity of the melanoma and primary tumor samples. All together 2475 transcripts were associated with melanoma metastasis (cutaneous or lymphatic). Of these, 350 genes were identified in more than one study. Only 19 probe sets representing 17 genes were identified in 3 studies (**see *Table 8***) and only CDC28 was identified in 4 studies.

DSC3 is a cell adhesion molecule involved in melanocyte-keratinocyte communication, suggesting the importance of this gene in the development of invasiveness and RGP/VGP transition in melanoma. (36) Early events in melanoma invasion and metastasis may involve alterations in cell proliferation (15) where genes in volved in cell cycle regulation are important: CDC6 and CDC28/CSK2 are two representative of those genes. It is of note, that two probe sets of EGFR can be found in this consensus-signature. EGFR was considered to be an important regulator of the biology of melanocytes and melanoma, however a few experimental or pathological studes were able to define its precise role in progression (37). This meta-analysis further strengthen those observations that EGFR can be a significant factor in melanoma progression. The involvement of S100 proteins in human melanoma progression is well established, where various members may have different roles. S100B is a marker of melanoma while S100A family members are more relate to progression mostly the downregulation of their expression. (38) Important factors in early phase of melanoma progression may also involve various nuclear proteins such as H2AFV, AHNAK or β-catenin-binding protein. The stromal involvement in early phase of melanoma progression is indicated by the presence of CXCL14 chemokine in the signature. The interconverted phenotype of melanoma is characterized by cytokerin expression involved in melanoma progression (39). The role for WNT5A in melanoma

progression is well documented in the literature (13), similar to the anti-apoptotic protein BCL2 (27). Finally, the integrin ligand OPN was repeatedly shown to be involved in the motility signaling of melanoma cells (40) indicating its role in progression.

Conclusion

Microarray studies on human skin melanoma produced a pletora of data without resulting in breakthrough in melanoma diagnosis or management of melanoma patients. Our crtitical analysis of these studies revealed several factors which might be responsible for such a failure. It is evident, that these studies must be based on rigorous sample collection and basic pathological considerations, where divergent histological types of melanoma cannot be analysed universally (see the example of non-small-cell lung cancers and the recent data on the genetic diversity of various melanoma subtypes. Secondly, the majority of these studies did not follow basic considerations of the metastasis biology, since they were rarely based on primary tumors but frequently on various types of regional metastases. Third, successful expression profiling studies on other tumors such as breast cancer, provided evidences that the homogeneity of the patient cohort at least by clinicopathological stage is a critical element when defining prognostic signature. On the other hand, human skin melanoma is a unique cancer type since no effective chemotherapy exist and only cytokines (IL-2 and IFNα2) can help to a certain extent, accordingly the course of the disease follows a natural path. This is the reason why the classical clinocopathological factors are still the best prognosticators for skin melanoma with a quite poor missclassification rates. Massive cooperation between clinicians, pathologist and molecular biologist is necessary to change this trend.

References:

1. Balch CM, Soong S-J, Thompson JF (2004). The natural history of melanoma and factors predicting outcome. In: Textbook of Melanoma. Eds: Thompson JF, Morton DL, Kroon BBR. Martin Dunitz Taylor & Francis Group, London, New York, pp, 181-199.
2. Cochran AJ, Bailly C, Paul E, Remotti F, Bhuta S (1997). (Eds). Characteristics that relate to prognosis. In: Melanocytic Tumors. A Guide to Diagnosis. Lippincott – Raven Publ, Philadelphia, New York, pp. 261-286.

3. Manola J, Atkins M, Ibrahim J, Kirkwood J (2002). Prognostic factors in metastatic melanoma: A pooled analysis of Eastern Cooperative Oncology Group. J Clin Oncol 7:434-446.

4. Streit M, Detmar M (2003). Angiogenesis, lymphangiogenesis, and melanoma metastasis. Oncogene 22:3172-3179.

5. Skobe M, Hamberg LM, Hawighorst T, Schirner M, Wolf GL, Alitalo K, Detmar M (2001). Concurrent induction of lymphangiogenesis, angiogenesis, and macrophage recruitment by vascular endothelial growth factor-C in melanoma. Am J Pathol 159:893-903.

6. Döme B, Paku S, Somlai B, Tímár J (2002). Vascularization of cutaneous melanoma involves vessel co-option and has clinical significance. J Pathol 197:355-362.

7. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho K-H, Aiba S, Bröcker E-B, LeBoit PE, Pinkel D, Bastian BC (2006). Distinct sets of genetic alterations in melanoma. N Engl J Med 353:2135-2147.

8. Downing A, Yu XQ, Newton-Bishop J, Forman D (2008). Trends in prognostic factors and survival from cutaneous melanoma in Yorkshire, UK and New South Wales, Australia between 1993 and 2003. Int J Cancer. 123(4):861-6.

9. Slingluff CL Jr, Chianese-Bullock KA, Bullock TN, Grosh WW, Mullins DW, Nichols L, Olson W, Petroni G, Smolkin M, Engelhard VH (2006). Immunity to melanoma antigens: from self-tolerance to immunotherapy. Adv Immunol 90:243-295.

10. Ladányi A, Somlai B, Gilde K, Fejős Z, Gaudi I, Tímár J (2004). T-cell activation marker expression on tumor-infiltrating lymphocytes as prognostic factor in cutaneous malignant melanoma. Clin Cancer Res 10:521-530.

11. Ladányi A, Kiss J, Somlai B, Gilde K, Fejős Zs, Mohos A, Gaudi I, Tímár J (2007). Density of DC-LAMP+ mature dendritic cells in combination with activated T lymphocytes infiltrating primary cutaneous melanoma is a strong independent prognostic factor. Cancer Immunol Immunother 56:1459-1469.

12. Chudnovsky Y, Khavari PA, Adams AE (2005). Melanoma genetics and the development of rational therapeutics. J Clin Invest 115:813-8245.

13. Weeraratna AT (2005). A Wnt-er wonderland--the complexity of Wnt signaling in melanoma. Cancer Metastasis Rev. 24(2):237-50.

14. Udart M, Utikal J, Krähn GM, Peter RU (2001). Chromosome 7 aneusomy. A merker for metastatic melanoma? Expression of the epidermal growth factor receptor gene and chromosome 7 aneusomy in nevi, primary malignant melanomas and metastases. Neoplasma 3:245-254.

15. Rákosy Z, Vízkeleti L, Ecsedi S, Vokó Z, Bégány A, Barok M, Krekk Z, Gallai M, Szentirmay Z, Ádány R, Balázs M (2007). EGFR gene copy number

alterations in primary cutaneous malignant melanomas are associated with poor prognosis. Int J Cancer 121:1729-1737.

16. Bales E, Mills L, Milam N, McGahren-Murray M, Bandyopadhyay D, Chen D, Reed JA, Timchenko N, van den Oord JJ, Bar-Eli M, Keyomarsi K, Medrano EE (2005). The low molecular weight cyclin E isoforms augment angiogenesis and metastasis of human melanoma cells in vivo. Cancer Res 65:692-697.

17. Döme B, Somlai B, Ladányi A, Fazekas K, Zöller M, Tímár J (2001). Expression of CD44v3 splice variant is associated with the visceral metastatic phenotype of human melanoma. Virchow Arch 439:628-635.

18. Hoek K, Rimm DL, Williams KR, Zhao H, Ariyan S, Lin A, Kluger HM, Berger AJ, Cheng E, Trombetta ES, Wu T, Niinobe M, Yoshikawa K, Hannigan GE, Halaban R (2004). Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. Cancer Res. 64(15):5270-82.

19. Winnepenninckx V, Lazar V, Michiels S, Dessen P, Stas M, Alonso SR, Avril MF, Romero PLO, Robert T, Balacescu O, Eggermont AMM, Lenoir G, Sarasin A, Tursz T, van den Oord JJ, Spatz A (2006). Gene Expression Profiling of Primary Cutaneous Melanoma and Clinical Outcome.. J Natl Cancer Inst. 98(7):472-482

20. Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR (1996). KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. J Natl Cancer Inst. 88(23):1731-7.

21. Bachmann IM, Straume O, Puntervoll HE, Kalvenes MB, Akslen LA (2005). Importance of P-cadherin, beta-catenin, and Wnt5a/frizzled for progression of melanocytic tumors and prognosis in cutaneous melanoma. Clin Cancer Res. 11(24 Pt 1):8606-14.

22. Albelda SM, Mette SA, Elder DE, Stewart R, Damjanovich L, Herlyn M, Buck CA (1990). Integrin distribution in malignant melanoma: association of the beta 3 subunit with tumor progression. Cancer Res. 50(20):6757-64.

23. Hess AR, Seftor EA, Seftor REB and Hendrix MJC (2003). Phosphoinositide 3-kinase regulates MT1-MMP and MMP-2 activity during melanoma cell vasculogenic mimicry. Cancer Res. 63:4757-62.

24. Kim M, Gans JD, Nogueira C, Wang A, Paik JH, Feng B, Brennan C, Hahn WC, Cordon-Cardo C, Wagner SN, Flotte TJ, Duncan LM, Granter SR, Chin L (2006). Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene. Cell. 125(7):1269-81.

25. Hofmann UB, Westphal JR, Van Muijen GN, Ruiter DJ (2000). Matrix etalloproteinases in human melanoma. J Invest Dermatol. 115(3):337-44.

26. Tímár J, Rásó E, Döme B, Ladányi A, Bánfalvi T, Gilde K, Raz A (2002). Expression and function of the AMF receptor by human melanoma in experimental and clinical systems. Clin Exp Metast 19:225-232.

27. McGill GG, Horstmann M, Widlund HR, Du J, Motyckova G, Nishimura EK, Lin YL, Ramaswamy S, Avery W, Ding HF, Jordan SA, Jackson IJ, Korsmeyer SJ, Golub TR, Fisher DE (2002). Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability.Cell. 109(6):707-18.

28. Mikhail M, Velazquez E, Shapiro R, Berman R, Pavlick A, Sorhaindo L, Spira J, Mir C, Panageas KS, Polsky D, Osman I (2005). PTEN expression in melanoma: relationship with patient survival, Bcl-2 expression, and proliferation.Clin Cancer Res. 11(14):5153-7.

29. Piras F, Murtas D, Minerba L, Ugalde J, Floris C, Maxia C, Colombari R, Perra MT, Sirigu P (2007). Nuclear survivin is associated with disease recurrence and poor survival in patients with cutaneous malignant melanoma. Histopathology. 50(7):835-42.

30. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn M (2005). A tumorigenic subpopulation with stem cell properties in melanomas.Cancer Res. 65(20):9328-37.

31. Tímár J, Tóvári J, Rásó E, Mészáros L, Bereczky B, Lapis K (2005). Platelet-mimicry of cancer cells: epiphenomenon with clinical significance. Oncology 69:185-201.

32. Hendrix MJC, Seftor EA, Hess AR and Seftor REB (2003). Vasculogenic mimicry and tumour-cell plasticity: Lessons from melanoma. Nature Rev Cancer 3:411-21.

33. Ruf W, Seftor EA, Prtrovan R, Weiss RM, Gruman LM, Margaryan NV, Seftor REB, Miyagi Y and Hendrix MJC (2003). Differential role of tissue factor pathway inhibitor-1 and 2 (TFPI-1 and 2) in melanoma vasculogenic mimicry. Cancer Res 63:5381-9.

34. Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, Radmacher M, Simon R, Yakhini Z, Ben-Dor A, Sampas N, Dougherty E, Wang E, Marincola F, Gooden C, Lueders J, Glatfelter A, Pollock P, Carpten J, Gillanders E, Leja D, Dietrich K, Beaudry C, Berens M, Alberts D, Sondak V, Hayward N, Trent J (2000). Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature 406, 536-40.

35. Tímár et al. CEM 2010

36. Schmitt CJ, Franke WW, Goerdt S, Falkowska-Hansen B, Rickelt S, Peitsch WK (2007). Homo- and heterotypic cell contacts in malignant melanoma cells and desmoglein-2 as a novel solitary surface glycoprotein.J Invest Dermatol. 127(9):2191-206.

37. Franco-Hernandez C, Martinez-Glez V, Arjona D, de Campos JM, Isla A, Gutierrez M, Vaquero J, Rey JA (2007). EGFR sequence variations and real-time quantitative polymerase chain reaction analysis of gene dosage in brain metastases of solid tumors.Cancer Genet Cytogenet. 173(1):63-7

38. Maelandsmo GM, Flørenes VA, Mellingsaeter T, Hovig E, Kerbel RS, Fodstad O (1997). Differential expression patterns of S100A2, S100A4 and S100A6 during progression of human malignant melanoma. Int J Cancer. 74(4):464-9.

39. Chu YW, Seftor EA, Romer LH, Hendrix MJ (1996). Experimental coexpression of vimentin and keratin intermediate filaments in human melanoma cells augments motility. Am J Pathol. 148(1):63-9.

40. Geissinger E, Weisser C, Fischer P, Schartl M, Wellbrock C (2002). Autocrine stimulation by osteopontin contributes to antiapoptotic signalling of melanocytes in dermal collagen. Cancer Res. 62(16):4820-8.

Table 1. Meta-analysis of genes associated with melanoma metastasis: result of 3-study threshold

Affymetrix ID	Genbank	UniGene	Gene Title	Symbol	Gene Ontology Biological Process
204170_s_at	NM_001827	Hs.83758	CDC28 protein kinase regulatory subunit 2	CKS2	regulation of cyclin-dependent protein kinase activity // cell cycle // spindle organization
206032_at	AI797281	Hs.41690	desmocollin 3	DSC3	cell adhesion
201984_s_at, 201983_s_at	NM_005228	Hs.488293	epidermal growth factor receptor	EGFR	ossification // electron transport // protein amino acid phosphorylation // response to stress
203968_s_at	NM_001254	Hs.405958	cell division cycle 6 homolog	CDC6	DNA replication checkpoint // regulation of cyclin-dependent protein kinase activity // DNA replication
203081_at	NM_020248	Hs.463759	catenin, beta interacting protein 1	CTNNBIP1	regulation of transcription, DNA-dependent // signal transduction
202487_s_at	NM_012412	Hs.488189	H2A histone family, member V	H2AFV	nucleosome assembly
218002_s_at	NM_004887	Hs.483444	chemokine (C-X-C motif) ligand 14	CXCL14	chemotaxis // inflammatory response // immune response //signal transduction
206033_s_at	NM_001941	Hs.41690	desmocollin 3	DSC3	cell adhesion // homophilic cell adhesion
220445_s_at	NM_004909	Hs.522810	CSAG family, member 2 /// CSAG family, member 3B	CSAG2	response to drug
205990_s_at	NM_003392	Hs.696364	wingless-type MMTV integration site family, member 5A	WNT5A	signal transduction // Wnt receptor signaling pathway, calcium modulating pathway// JNK cascade
209875_s_at	M83248	Hs.313	secreted phosphoprotein 1	SPP1	ossification// cell adhesion // cell-matrix adhesion
219529_at	NM_004669	Hs.64746	chloride intracellular channel 3	CLIC3	transport // ion transport
210198_s_at	BC002665	Hs.1787	proteolipid protein 1	PLP1	synaptic transmission // axon ensheathment
203300_x_at	NM_003916	Hs.656471	adaptor-related protein complex 1, sigma 2 subunit	AP1S2	protein complex assembly // transport // intracellular protein transport
205681_at	NM_004049	Hs.227817	BCL2-related protein A1	BCL2A1	apoptosis // anti-apoptosis
220016_at	NM_024060	Hs.502756	AHNAK nucleoprotein	AHNAK	nervous system development
204268_at	NM_005978	Hs.516484	S100 calcium binding protein A2	S100A2	endothelial cell migration
204734_at	NM_002275	Hs.654570	keratin 15	KRT15	epidermis development

Malignant Melanoma: Clinical Aspects

Zita Battyáni

Department of Dermatology,
Venereology and Oncodermatology
University of Pécs, Medical School, Pécs

Malignant melanoma

- Definition:
  - arise from melanocytes
  - the most serious oncological problem
  - incidence and mortality rise
  - affects relatively younger population
  - great tendency to early metastasis
  - the only treatment is the early recognition and the surgical excision
  - advanced tumor responds poorly

Epidemiology

- Incidence dramatically increase
  - Australia: 50/ 100 000
  - Europe: 15-20/ 100 000
  - Mexico 40 /100 000 (above 2000 m)
  - Hungary 2110 / year (2008)
- F>M
- Age affected : ~40-60 years (increased 20-30 y)
- Among blacks is very rare, mainly localized subungual, on palm, soles and mucosa
- Life time risk in USA
  - 1935 1:1500
  - 1980 1:250
  - 2000 1:70
  - 2010 1:50
- Life time risk in Australia
  - 2000 1:60

Etiology and pathogenesis

- The exact cause is unclear
- Genetical and environmental factors
- 10% show familial occurence
- Interaction within tumor cells and stroma
- Iatrogenic or acquired immunosuppression
  - Melanoma risk increased 3x

Etiology, pathogenesis

- UV irradiation- UVA-pirimidin dimers
  - Single, high dose exposition
  - Sunburns, mainly in childhood
    - >3 sunburns, melanoma RR increased 3x
- Presence of nevi
  - 25-40% of melanoma arise from nevi
  - > 50 nevi melanoma risk is 5X higher
  - Atypical or dysplastic nevi,
  - Giant congenital nevi
  - Mechanical irritation and repeated damages

Etiology, pathogenesis

- Chromosomal alterations:
  - 9p21- cell cycle regulation (CDK2A)
  - BRAF és RAS mutation
  - Raf-MAPK kinase-ERK (RAF-MEK-ERK)
  - PI3K/**PTEN**/AKT pathway (leads to apoptosis blockade)

Clinical form of malignant melanoma

- Lentigo maligna melanoma 1%
- SSM 70%
- Nodular melanoma 21%
- Acrolentiginous melanoma 5%
- Non classifiable 3%
  (mucosal, amelanotyc, desmoplastic)

Clinical forms and prognosis

- Lentigo maligna: favorable
- SSM
  - in horizontal growth phase good
  - In vertical growth phase poor
- Nodular : poor
- Acrolentiginous: poor
- Amelanotic: poor

Diagnostic possibilities

- Clinical pictures
- Digital dermoscopy - dermoscopy
- 22 MHz ultrasound investigation
- MRI

Differential diagnosis

- Nevi
- Dysplastic nevi
- Pigmented basal cell carcinoma
- Verruca seborrhea
- Pyogen granuloma
- Hemangioma

Clinical prognostic factors of melanoma

- Clinical type (LMM, SSM, ALM, NM)
- Tumor location (extremities, BANS region)
  - BANS: back, arm, neck, scalp
  - Multi-directional lymph drainage
- Age of patients (prognosis worsens with age)
- Sex (male is unfavorable)
- *Worse prognosis*
- Ulceration
- Regression
- Bleeding

Histological prognostic factors

- Tumor thickness
- Invasion level- mitotic rate <; > 1/mm^2
- Number of mitoses HPF
- Micro-ulceration (important in stage I-II-III)
- Lymphocytes infiltration (lack of infiltration)
- Satellites, in transit metastases
- Vascular invasion

NEW 7th TNM classification AJCC 2009. pT

pT	Tumor thickness	Ulceration
T1	≤ 1,0 mm	a: without ulc. (*Clark II/III*) mitosis<1/mm^2 b: with ulc. or (*Clark IV/V*) mitosis<1/mm^2
T2	1,01 – 2,0 mm	a: without ulc . b: with ulc .
T3	2,01 – 4,0 mm	a: without ulc. b: with ulc.
T4	> 4,0 mm	a: without ulc. b: with ulc.

Balch CM .J Clin Oncol. 27:6199-6206 2009

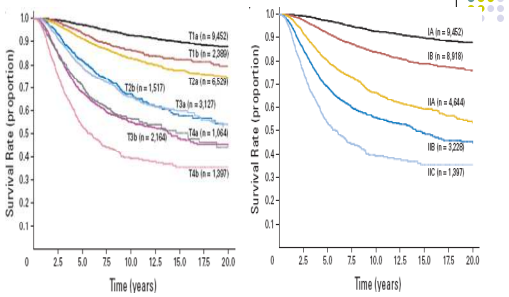
TNM classification pN

	Number of metastatic lymph node		Tumor mass lymph nodes
N1	1	lymph node	a: micromet b: macromet.
N2	2-3	lymph node	a: micromet. b: macromet c: in transit/satellita met. without lymph nod
N3	≥ 4	lymph node lymph node conglomerate or in transit/satellita metast. with lymph node metast.	

TNM classification pM

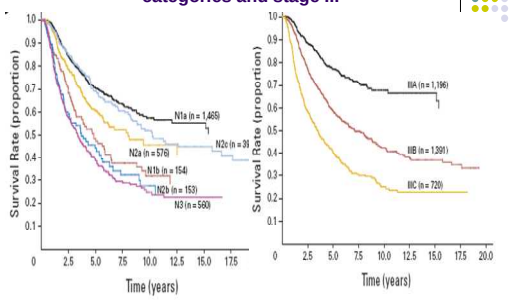
	Sites	LDH
Mo	No distant metastasis	not applicable
M1a	Distant skin, subcutaneous nodal metastasis	normal
M1b	Lung metastases	normal
M1c	All other visceral metastases Any distant metastases	normal elevated

Survival rate comparing the different T categories and stage I and II



Balch CM .J Clin Oncol. 27:6199-6206 2009.

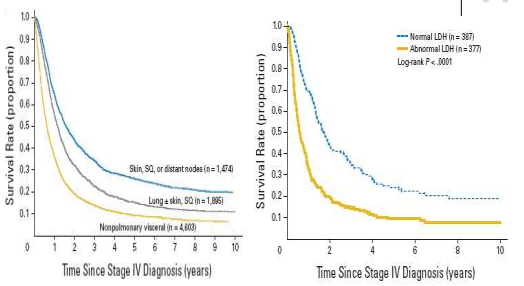
Survival rate comparing the different N categories and stage III



Balch CM .J Clin Oncol. 27:6199-6206 2009.

Survival curves with metastatic melanoma

at distant site **and serum LDH level**



Balch CM .J Clin Oncol. 27:6199-6206 2009

Anatomic stage Groupings for cutaneous Melanoma

Clinical staging				Pathologic staging			
	T	N	M		T	N	M
0	Tis	N0	M0	0	Tis	N0	M0
IA	T1a	N0	M0	IA	T1a	N0	M0
IB	T1b	N0	M0	IB	T1b	N0	M0
	T2a	N0	M0		T2a	N0	M0
II.A	T2b	N0	M0	IIA	T2b	N0	M0
	T3a	N0	M0		T3a	N0	M0
IIB	T3b	N0	M0	II.B	T3b	N0	M0
	T4a	N0	M0		T4a	N0	M0
II.C	T4b	N0	M0	II.C	T4b	N0	M0
III.	Any T	N>N0	M0	III.A	T1-4a	N1a, N2a	M0
				III. B	T1-4b	N1a, N2a	M0
					T1-4a	N1b, N2b	M0
					T1-4a	N2c	M0
				III. C	T1-4b	N1b, N2b	M0
					T1-4b	N2c	M0
					AnyT	N3	M0
IV	Any T	An N	M1	IV.	Any T	Any N	M1

New findings and definitions in the new version of staging

- In patients with localized melanoma most dominant factors
  - Tumor thickness
  - Mitotic rate(mitosis/mm^2)
  - Ulceration
- Mitotic rate replaces level of invasion as a primary criterion for T1b melanoma

The role of histology in the diagnosis of malignant melanoma

- Melanocytic vs. non melanocytic lesion
- Benign vs. malignant pigmented lesion
- In situ vs. invasive tumor
- Characteristics of primary tumor
  - Tumor thickness
  - Mitotic rate(mitoses/mm^2)
  - Lymphocytic infiltrations
  - Vascular or lymphatic invasion
- Specification of the lymph node status

Treatment of malignant melanoma Primary tumor

- Plastic surgical excision
  - Electric knife
  - To fascia of muscle
  - Safety margin depends on the tumor thickness
    - In situ melanoma 0,5 cm
    - 1-2 mm 1,0 cm
    - >2 mm 2,0 cm

INCISIONS BIOPSY PROHIBITED

Sentinel lymph node biopsy

- Indispensable
  - Together with primer tumor surgery, general anesthesia
- Indications:
  - tumor >1 mm
  - tumor < 1mm, ulceration, regression
- Regional lymph node dissection
  - by histological positive sentinel lymph node
  - palpable or detectable lymph node
- Role in the stage determination

Uncertain diagnosis of MM

- Excision with 5 mm safety margin
- Histological examination
- Further surgical treatment
  - Depends on the tumor thickness

INCISIONS BIOPSY PROHIBITED

Adjuvant treatment of melanoma

Interferon alpha 2a, 2b

- Indication
  - II. A, B,C, (pT2b, pT3,pT4)
  - III.A,B,C after tumor resection
- Low dose: 3 x 3 -10MU/ week sc. for 18 months
- High dose
  - 20MU/m^2 iv. 5x/week 1 Month (induction)
  - 10 MU/m^2 sc. 3x/ week 11 Month (maintain)

Chemotherapy

- Indication: stage IV
  5 years survival 6%
  main survival 7,5 months
- Monochemotherapy
- Polychemotherapy

Monochemotherapy

- After the (+) lymph node dissection
- Skin and pulmonal met.
- *Dacarbasin (DTIC)*
- Remission rate 10-25%

Dosage:

5 days 250mg/m^2 /day
4 weekly, 6 cycles,
or to regression of the tumor

Polychemotherapy

- Indication: other distance metastases
- BOLD regime : 35-40%
  - Bleomycin, DTIC, CCNU,Vincristin

  5 days treatment, repeated 6 weekly, in 6 cycles

Many side effects, no better clinical affectivity as DTIC

Radiations treatment

- Palliative treatment
  - Vascular invasion
  - Multiple lymph node metastasis with capsule involves
  - Cerebral metastases
  - Symptomatic treatment
- Treatment modalities
  - Whole brain irradiation
  - Stereo-taxis irradiation
  - After loading treatment

Special treatment modalities

- Isolated limb perfusion,
  - In case of isolated limb metastases
- Chemo-embolisation of liver

New treatment modalities and future

- Vaccination
- Monoclonal antibodies
  - (anti-CTLA-4) ipilimumab

New treatment modalities and future

Prevention of chemo- resistance (clinical trial)

- BRAF inhibitors
  - Sorafenib
- Anti-sens BCl2,
  - Oblimerzen
- Anti-angiogenic treatment
  - Semaximab, Bevacizumab
- mTOR inhibitors
  - CCL-779, in combination with low dose INFα

New treatment modalities and future

- Proteosoma inhibitor
  - Bortezomib
- MEK inhibitors
  - PD0325901 (Phase I.)
  - AZD6244 (Phase II.)
- Thalidomide (lenalidomide CC-4047)
  - Immunomdulatory
  - Antiangiognic
  - Antiproliferative
  - Proapoptotic properties