Gene signature and outcome of patients with melanoma

Alain Spatz, MD
Gustave-Roussy Institute, Villejuif, France
EORTC Melanoma group Chair
Four questions

1. Does the multistep model apply to melanoma?
2. What have we learnt from genomics studies?
3. Is there a unifying concept behind molecular pathogenesis and immunomodulation of melanoma progression?
4. Where do we stand with genotypic/phenotypic correlations in melanoma?
Multistep model of melanoma progression

After Miller & Mihm, NEJM, 355, 2006
BRAF mutations in melanoma

- BRAF (and NRAS) mutations arise early during melanoma pathogenesis.
- A single aa substitution (V600E) accounts for a vast majority of mutations: activ. BRAF kinase.
- Mutations are preserved throughout tumor progression.
- Oncogene if additional genetic lesions.
- Role for BRAF\textsuperscript{E600} in senescence: even if p53 is KO.

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Cancers with \textit{BRAF} mutations

% carrying a \textit{BRAF} mutation

\begin{itemize}
  \item Melanoma
  \item Colorectal
  \item Glioma
  \item Lung
  \item Sarcoma
  \item Breast
  \item Ovarian
  \item Others
\end{itemize}

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BRAF pathway

Michaloglou et al., Oncogene, 2007
BRAF$^{E600}$ as a therapeutical target
Is $BRAF^{E600}$ a therapeutical target?

- Sorafenib is a multi-kinase inhibitor that targets ser/thr and RTKs in both the tumor cells & vasculature.
- More specific anti-BRAF molecules in development.
- What is the good anti-BRAF combination?
  - Problem of BRAF isoforms (alternative splicing): i.e., B2-RAF and B3-RAF have antagonist actions on MEK.
  - Property issues.

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Multistep model of melanoma progression

<table>
<thead>
<tr>
<th>Stage</th>
<th>Benign Nevus</th>
<th>Dysplastic Nevus</th>
<th>Radial-Growth Phase</th>
<th>Vertical-Growth Phase</th>
<th>Metastatic Melanoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Limited growth</td>
<td>Premalignant</td>
<td>Decreased differentiation</td>
<td>Crosses basement membrane</td>
<td>Metastasis to lung, liver, or brain</td>
</tr>
<tr>
<td></td>
<td>Lesions may regress</td>
<td>Random atypia</td>
<td>Unlimited hyperplasia</td>
<td>Grows in soft agar</td>
<td>Grows at distant sites</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clonal proliferation</td>
<td>Forms tumor</td>
<td></td>
</tr>
</tbody>
</table>

**Biologic Events**
- **BRAF mutation**
- **CDKN2A loss**
- **PTEN loss**
- **Increased CD1**
- **E-cadherin loss**
- **N-cadherin expression**
- **αVβ3 integrin expression**
- **MMP-2 expression**
- **Survivin**
- **Reduced TRPM1**
- **Absent TRPM1**

After Miller & Mihm, NEJM, 355, 2006
Other pathways

After Miller & Mihm, NEJM, 355, 2006
Microphthalmia-associated transcription factor

- Master regulator of melanocyte development, function and survival.
- Activates the pigmentation machinery in the presence of P53.
- Amplified oncogene in a fraction of melanoma (5-20%).
- Role in melanoma progression *in vivo* ill-known; no clear relation with prognosis nor response.
- Regulatory interactions RAF-MEK-ERK / Beta-cat.-MITF

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Objectives of high throughput technology in melanoma

- To study if there is a transcriptional signature associated with prognosis of cutaneous primary melanomas.
- To characterize key molecular events during tumor progression (transition nevus-primary melanoma & primary melanoma-metastasis).
- To identify new therapeutical targets.
Supervised analysis: distant metastasis free survival at 4yrs

- 254 out of the 11,043 filtered genes have a differential expression ($p=10^{-4}$) between patients with DMFS $<$ or $> 4$yrs.
- Signature with 60 top genes highly discriminant.
- Expression clusters associated with phenotypic variables.

Winnepenninckx et al., J Natl Cancer Inst 2006
Replication-controling pathways

- Replication licensing and origins firing are key in melanoma progression
- hPTTG/securin overexpression is associated with VGP/RGP:
  - blocks sister chromatid separation: aneuploidy
  - induction of angiogenesis (through βFGF and VEGF)
  - downregulation of p53 transcription

Winnepenninckx *et al.*, J Natl Cancer Inst, 2006
Winnepenninckx *et al.*, Mod Pathol, 2006
Replication origins firing pathway

- Global overexpression of the ROF-related genes: 4/9
  
  *Mini-Chromosome Maintenance* genes (2, 3, 4, 6)
  
  and *geminin*

- *MCM-4* and -6 overexpression are prognostic for
  
  DMFS and OS independently from age, sex,
  
  ulceration, thickness (Cox model)

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Dysregulation of the replication origins firing pathway: MCM-4

Patients at risk

<=average  68  56  41  27  18  
>average  108  84  61  45  35

P=0.0016
Replication origins firing pathway

After Gonzalez et al., Nat Rev Cancer, 2005
BRCA1-IRIS controls GEM-CDC6 interaction and MCM2

After ElShamy & Livingstone, Nat Cell Biol, 2004
Differential expressions for the DNA repair pathway

Repair All
3.92E-17
DNA-repair pathway

◆ SBIME analysis of 231 genes involved in DNA repair: expression for 47 genes differs between groups

◆ Almost all genes have higher expression in the group with poor prognosis

◆ **TOP2A** is the most overexpressed gene in tumors with short survival: relieves DNA torsional stress, resistance to alkylating agents.

Kauffmann *et al.*, Oncogene, In Press
Telomeric repeat binding factor 2 gene

- TRF2 expression strongly decreases with tumor Breslow and metastatic potential
- Overexpression of TRF2 gene leads to hypersensitivity to UV or crosslinking agents and chromosome instability (Bradshaw et al., Nat Genet, 2005:37)
- What is the mechanism for TRF2 gene underexpression?

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Epigenetic changes in melanoma

- Micro-RNAs extracted from the same specimens than RNAs and DNAs
- Three miRNAs are associated with thickness, and differentiate mets. from corresponding primary tumor (underexpressed during evolution):
  - Hsa-miR-200c (TF8, E-Cadherin): fdr $p=0.02$
  - Hsa-miR-203 (ovarian cancers, expression regulated by DNA methylation: Lorio et al., Cancer Res 2007): fdr $p=0.002$ for thickness, 0.005 for DMFS
  - hsa-miR-200a: fdr $p=0.05$
- Some of them are located in hot spots for aCGH modifications
- Are miRNAs related gene-silencing involved in melanoma pathogenesis:
  - genetic effects
  - but also immunomodulation (Cancer-Testis Antigens, i.e., MAGE)
Genetic aberrations of miRNAs in human cancer

Zhang et al., PNAS, 2006
Overexpression of has-miR-200c decreases TF8 and increases E-Cadherin expression

- miR-200c seems to be strongly involved in EMT
- Loss of 200c is likely to play a significant role in invasive phenotype
- Is miR-200c downregulation linked with DNA changes?
- How to restablish miR-200c expression?

Hurteau et al., Cancer Res, 67, 2007
B-Raf signature

- Genes expression associated with BRAF status (FDR<0.01, P=0.95): CD63 (FDR P=0.0001!), HSP70, MAGE-D2, Melanoma Inhibitory Activity, SERPINE2.

- CD63: tetraspanins family, colocalizes with integrins

- HSP70B’: BRAF-dependent response to HSP70 inducers?

- MAGE-D2: interacts physically with $p53^{wt}$ (p53-dissector)

- Enhanced motility and vesiculization: modulation of adaptative UV response?

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Impact of molecular pathology on the taxonomy of melanocytic tumors.
The classification of malignant melanoma and its histologic reporting.

Vincent J. McGovern, Martin C. Mihm Jr., Christiane Bailly, et al.

Cancer 1973, 32:1446
Melanomas in sun-exposed skin

- Separation based on the lateral epidermal component is often difficult: some LMMs have prominent nesting, or epidermal spreading at later stage.
- No prognostic difference between sub-types in sun-exposed skin.

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Melanomas in sun-exposed skin: morphological overlapping
Melanomas in sun-exposed skin: *p53* pathway (*xeroderma pigmentosum* model)
Role of $p53$ in the suntan response

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Cui et al., Cell, 2007
Melanomas in sun-exposed skin

- Solar elastolysis is strongly and independently associated with better prognosis
- Melanomas in sun-exposed skin have $p53$ gene mutations (UV-induced).
- Mutations in $KIT$ (26%) and MAP kinase-related genes

No justification to maintain LMMs in a separate category!

Berwick et al., JNCI, 2005
Cutin et al., NEJM 2005 & J Clin Oncol 2006
At least three diseases

- **Solar melanomas**: better prognosis, UV-induced $p53$ mutations, $KIT$ mutations, XP melanomas as a model (involvement of XP genes?)

- **Acral melanomas (glabrous skin)**: gains in chromosomes 5p and 11q (oncogenes?), less frequency of $BRAF$ mutations

- **Other melanomas**: Growth phase pattern overcomes histogenetic classification. Dysregulation of replication origins firing.
PIGMENTATION

Images of cytoplasmic pigmentation:

0

1

2

3

4
Conclusion

- A primary melanoma that is going to metastasize is a machinery to replicate DNA: maximum efficiency and minimal mistakes are necessary to overcome dysfunctional replication or telomeres maintenance.

- The increased expression of repair proteins may partly explain melanoma resistance toward radiotherapy and chemotherapy.

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Conclusion (cont’d)

- Mitotic activity is the phenotypic reflect of dysregulation in the replication regulation: ROF, chromatid separation
- Stabilization of Geminin/CDT1/CDC6 is key to regulation replication
- Impact of BRAF mutations on ser kin –related genes (CD63?)
- Role of hsa-miR-200c downregulation during melanoma progression
- Melanoma is a heterogeneous group of diseases: phenotypic variables to discriminate between categories

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