Génétique et génomique du mélanome uvéal, une tumeur rare et grave de l’adulte

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Uveal Melanoma (UM)

- A primary intraocular malignancy of adults
- Incidence ~5-7 cases per million population per year.
- ~300 new cases per year of UM are treated at Institut Curie
- Blue eye, fair skin, European ancestry
- Brachytherapy, proton-therapy and enucleation are effective for local control of the disease
- Metastasis occurs in >30% of UM (~invariably in liver), with median survival only ~12 months
- No effective therapy in metastatic setting
- No improvement in overall survival during the last 30 years
Simple and stereotyped oncogenic pathways in UM

Uveal melanocyte → **Gαq activation** → Naevus → **BESS event** → Uveal melanoma
Gαq activation in UM

Activating mutations in:
GNAQ or GNA11 (~85 %)
Van Raamsdonk et al. Nature 2009; NEJM 2010
PLCB4 (€ %)
Johansson et al. Oncotarget 2017
CYSLTR2 (€ %)
Moore et al. Nat Genet 2016

Key oncogenic activation of the YAP pathway
BESS event

- Deleterious *BAP1* mutation & Monosomy 3
  - Harbour *et al.* Science 2010
  - ~50%

- *SF3B1* mutation
  - Martin *et al.* Nat Genet 2013
  - Furney *et al.* Cancer Discov 2013
  - Harbour *et al.* Nat Genet 2013
  - ~25%

- *SRSF1* mutation
  - Robertson *et al.* Cancer Cell 2017
  - ~1%

- *EIF1AX* mutation
  - Martin *et al.* Nat Genet 2013
  - ~20%
BESS event

Gαq activated Naevus

Deleterious $BAP1$ mutation & Monosomy 3
Harbour et al. Science 2010

High risk UM

$SF3B1$ mutation
Martin et al. Nat Genet 2013
Furney et al. Cancer Discov 2013
Harbour et al. Nat Genet 2013

Moderate risk UM

$SRSF1$ mutation
Robertson et al. Cancer Cell 2017

Low risk UM

$EIF1AX$ mutation
Martin et al. Nat Genet 2013
BESS event

Gαq activated Naevus

Deleterious *BAP1* mutation & Monosomy 3
Harbour *et al.* Science 2010

Chromatin modifier

*SF3B1* mutation
Martin *et al.* Nat Genet 2013
Furney *et al.* Cancer Discov 2013
Harbour *et al.* Nat Genet 2013

Splicing factors

*SRSF2* mutation
Robertson *et al.* Cancer Cell 2017

*EIF1AX* mutation
Martin *et al.* Nat Genet 2013

Translation factor
UM has a very low mutation rate

- Mutation rate <1 per Mb
- No UV signature

## Uveal Melanoma: defining the genetic landscape

<table>
<thead>
<tr>
<th>Sample</th>
<th>Candidate SNVs</th>
<th>Gαq mutation</th>
<th>BAP1 inactivation</th>
<th>Others</th>
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<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>GNA11</td>
<td>BAP1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>GNA11</td>
<td>BAP1</td>
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</tr>
<tr>
<td>3</td>
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<td>GNAQ</td>
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</tr>
<tr>
<td>4</td>
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<td>GNA11</td>
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</tr>
<tr>
<td>5</td>
<td>23</td>
<td>GNA11</td>
<td>BAP1</td>
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</tr>
<tr>
<td>6</td>
<td>22</td>
<td>GNAQ</td>
<td></td>
<td>SF3B1</td>
</tr>
<tr>
<td>7</td>
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<td>BAP1</td>
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</tr>
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<td>GNA11</td>
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<tr>
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<td>BAP1</td>
<td></td>
<td>SF3B1</td>
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<tr>
<td>12</td>
<td>24</td>
<td>GNAQ</td>
<td></td>
<td>SF3B1</td>
</tr>
</tbody>
</table>

UM epidemiology

- Relative Risk (RR) >10 of European versus Afro-American or Asian
- Fair skin and blue/gray eyes are significantly associated with UM

Furney et al. Cancer Discov, 2013
UM epidemiology

- Relative Risk (RR) >10 of European versus Afro-American or Asian
- Fair skin and blue/gray eyes are significantly associated with UM
- Incidence of UM is stable in Europe, North America and Australia
- Absence of UV mutation signature and very low mutation burden

Furney et al. Cancer Discov, 2013
Genome-wide association study (GWAS) in UM

➢ A GWAS could identify potential genetic risk factors in a given population.
GWAS results

<table>
<thead>
<tr>
<th>SNP</th>
<th>P-value</th>
<th>OR</th>
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<tbody>
<tr>
<td>rs421284</td>
<td>7 x 10^-8</td>
<td>1.95</td>
</tr>
<tr>
<td>rs452932</td>
<td>1 x 10^-7</td>
<td>1.91</td>
</tr>
</tbody>
</table>

5p15.3
TERT/CLPTM1L
Correlation between gene expression and rs421284 genotype

eQTL analysis of 5p15.33
A GWAS in uveal melanoma identifies risk polymorphisms in the *CLPTM1L* locus

Lenha Mobuchon¹, Aude Battistella¹, Claire Bardel²,³, Ghislaine Scelo⁴, Alexia Renoud⁵, Alexandre Houy¹, Nathalie Cassoux¹, Maud Milder¹, Géraldine Cancel-Tassinô, Olivier Cussenot⁶, Olivier Delattre¹, Céline Besse⁷, Anne Boland⁷, Jean-François Deleuze⁷, David G. Cox⁵ and Marc-Henri Stern¹

Uveal melanoma, a rare malignant tumor of the eye, is predominantly observed in populations of European ancestry. A genome-wide association study of 259 uveal melanoma patients compared to 401 controls all of European ancestry revealed a candidate locus at chromosome 5p15.33 (region rs421284: OR = 1.7, CI 1.43–2.05). This locus was replicated in an independent set of 276 cases and 184 controls. In addition, risk variants from this region were positively associated with higher expression of *CLPTM1L*. In conclusion, the *CLPTM1L* region contains risk alleles for uveal melanoma susceptibility, suggesting that *CLPTM1L* could play a role in uveal melanoma oncogenesis.

npj Genomic Medicine (2017)2:5; doi:10.1038/s41525-017-0008-5
SF3B1 and splicing

- SF3B1 mutations are recurrent in UM [Furney, Cancer Discov, 2013]

➢ What are the consequences of SF3B1 mutations?
Splice factor mutations
SF3B1 and splicing

**Filters:**
- Standard deviation ≠ 0
- Median of one sample group (SF3B1 WT or mutant) ≥ 5
- At least in 15 samples with SF3B1 mutation

**Normalization (DESeq2)**
- Geometrical mean of each junction
- Median of each sample

**Differential analysis (DESeq2)**
- Comparison of junction coverage between sample with or without SF3B1 mutation
  - Adjusted p-value ≤ 10^-5
  - Log₂ fold change (log₂ fc) ≤ -1 or ≥ 1

**1,469 differential junctions**
SF3B1 WT  SF3B1 mut

Acceptors

Adjusted p-value = 1.64x10^{-70}

Log₂ fold-change = 3.85
SF3B1 mutations lead to recognition of cryptic branchpoints and alternative acceptors sites

Defining metastatic UM genetic landscape and tumor heterogeneity

23 patients
14 primary samples
79 metastatic samples

13 Trios (CPM)
An exceptional response to immune therapy in a metastatic UM
A novel mutator phenotype linked to MBD4 inactivation

Rodrigues et al. In revision
A novel mutator phenotype linked to *MBD4* inactivation

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**Institut Curie UM**

- **UVM_IC**
- No. of mutations:
  - Primary
  - Metastasis
  - Resistant
- % of CpG to TpG:
  - CpG>TpG
  - Other

**TCGA UM**

- **UVM_1**
- % of all SNVs:
  - C > A
  - C > G
  - C > T
  - T > A
  - T > C
  - T > G

**TCGA glioblastoma**

- **GBM_4**
- % of all SNVs:
  - ACG
  - CCG
  - GCG
  - TCG

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**MBD4**

*Rodrigues et al. In revision*
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The Cancer Genome Atlas
All patients and families from these series

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