Analyse génomique intégrée des tumeurs

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CARPEM
CANCER RESEARCH FOR PERSONALIZED MEDICINE

PLAN

1. Les technologies de la génomique à haut débit et leur application à l'étude des cancers

2. Analyse génomique intégrée des paragangliomes – lien entre méthylation de l'ADN et métabolisme cellulaire

PLAN

1. Les technologies de la génomique à haut débit et leur application à l'étude des cancers

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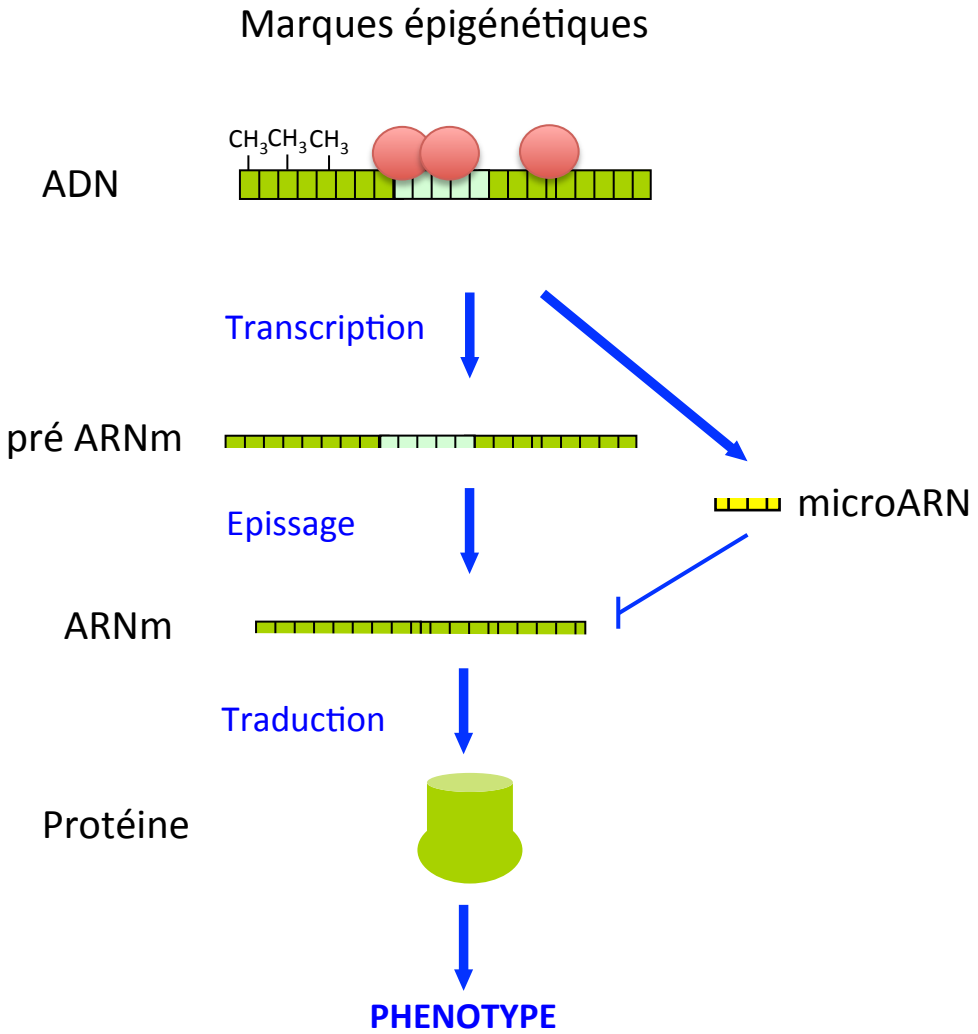
Analyses génomiques des tumeurs : que mesure-t-on, et comment ?

CELLULE NORMALE



ALTÉRATIONS

- Méthylation différentielle
- Remodelage de la chromatine

- Mutations
- Aberrations chromosomiques

- Dérégulation de l'expression des ARNm et microARN

- Epissage alternatif

Impact sur l'expression et la fonction des protéines

PHÉNOTYPE TUMORAL

Puces vs. séquençage

Puces à ADN

Sample (e.g. frozen tumor)

↓

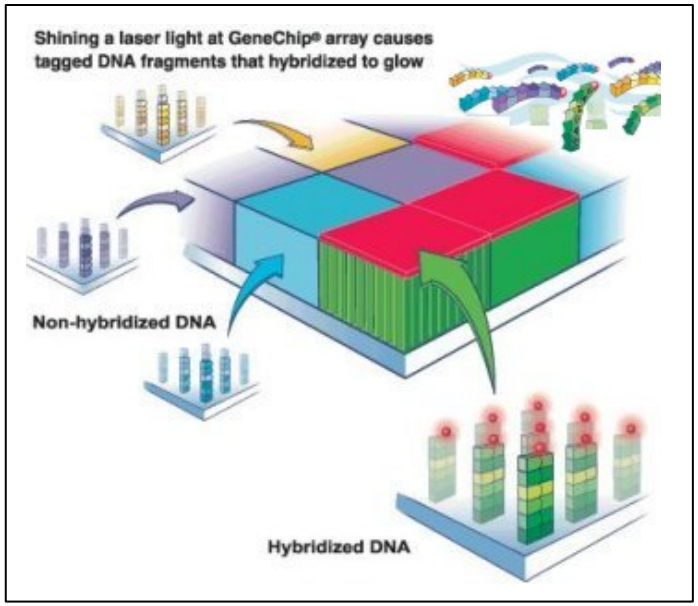
Extract molecules to quantify (DNA, mRNA, small RNAs…)

↓

Potential chemical treatment (e.g. bisulfite)

↓

Hybridization



↓

Analysis (signal quantification, normalization…)

Séquençage à haut débit

Sample (e.g. frozen tumor)

↓

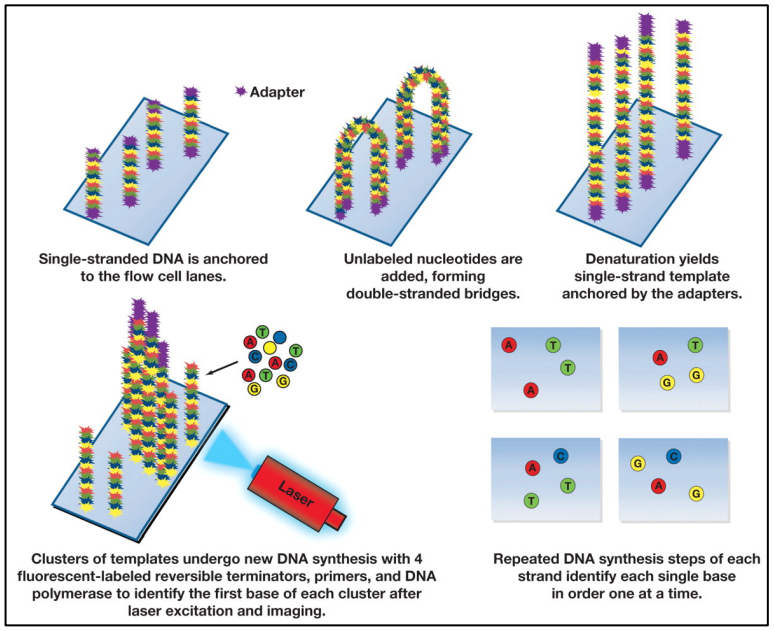
Extract molecules to sequence (DNA, mRNA, small RNAs…)

↓

Purify particular regions (capture kit, immunoprecipitation…)

↓

Sequencing



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Analysis (alignement, quantification, variant discovery…)

Analyse des mutations

- **Goal:** Identify genes with recurrent mutations in cancer, which may correspond to driver genes.

- **Data:** Whole exome (~50 Mb) or whole genome (>3 Gb) sequences.

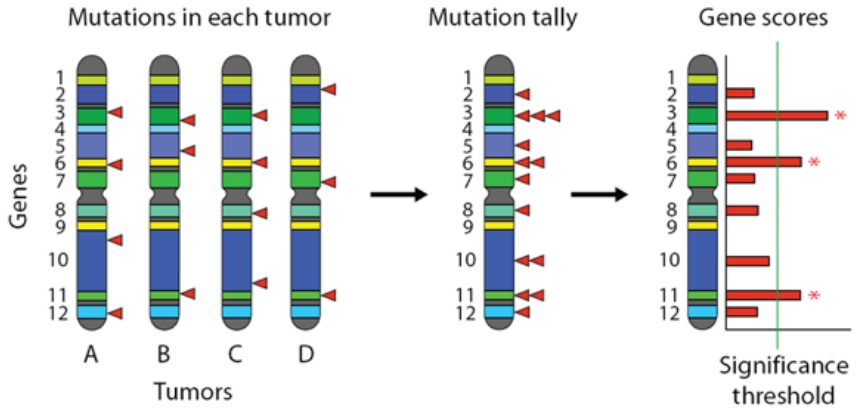
- **Analysis:**

  1) Identify somatic variants
     - Align reads to the human genome
     - Detect single nucleotide variant(s) and small indels
     - Remove low quality variants (read depth, Qphred…)
     - Compare normal and tumor sequences to detect somatic variants
     - Annotate the variants (referenced, predicted functional impact…)

  2) Interpret the variants
     - Examine the mutational spectrum
     - Find significantly mutated genes or pathways
     - Integrate with clinical annotations and other omics data

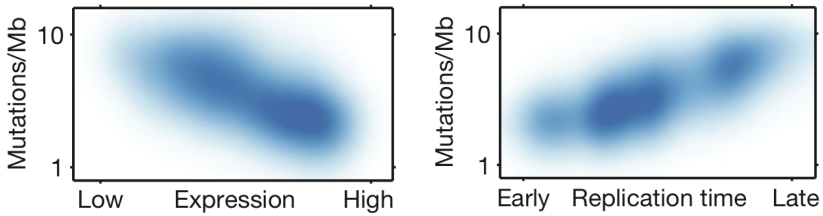
MÉTHODE : Identification des gènes significativement mutés

- We want to find genes that harbor **more mutations than expected by chance**, reflecting **selection** of driver genes.



- The number of mutations expected by chance depends on the **size of the gene** and on the **background mutation rate** (BMR).

- The BMR depends on the **sample**, the **mutation category**, and the **genomic context** (expression level, replication time, chromatin context).

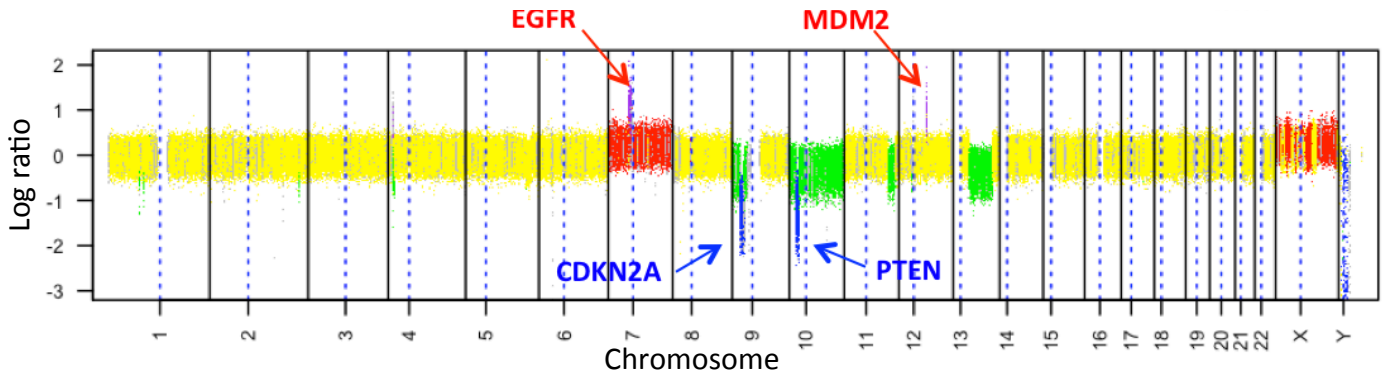


Lawrence *et al.*, Nature 2013

- The **MutSigCV algorithm** evaluates the mutation rate $\mu_{g,c,p}$ for each gene g, category c and patient p. A p-value is then computed for each gene, taking into account these mutation rates.

Analyse des aberrations chromosomiques

- **Goal:** Identify recurrent copy-number changes (gains, losses), which may point out tumor suppressors and oncogenes.



- **Data:** SNP array, exome or whole-genome sequences.

- **Analysis:**

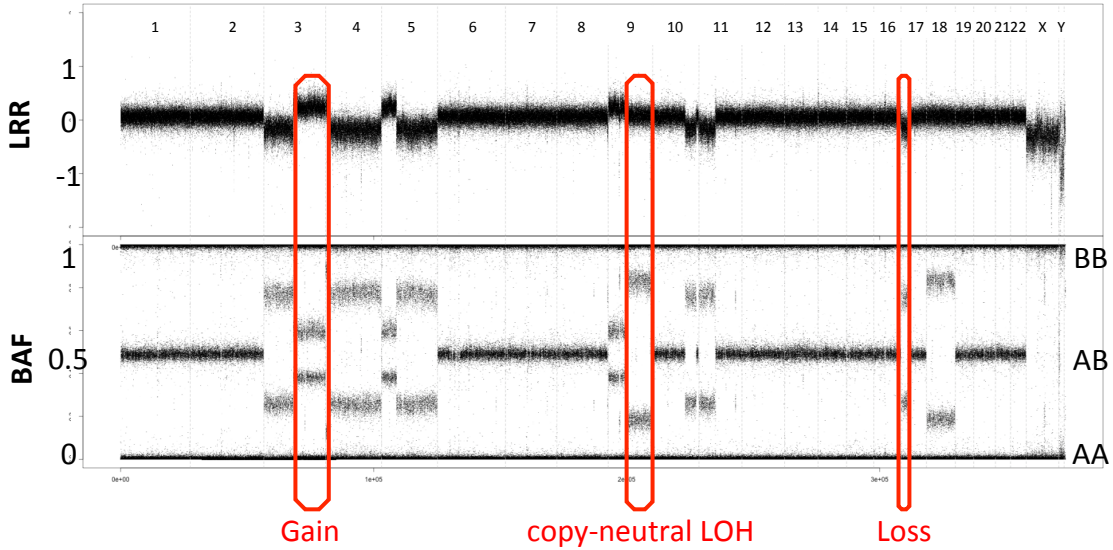
1) Identify copy-number alterations (CNAs) in each tumor

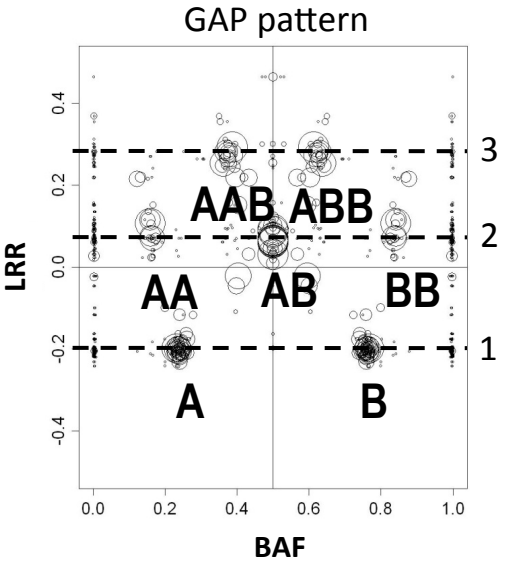
2) Integrate CNAs across the cohort to find recurrent alterations and candidate driver genes

MÉTHODE : Utilisation des SNPs pour détecter les aberrations chromosomiques

- SNP profiles are analyzed using both the log R ratio (LRR) and B allele frequency (BAF) profiles:

$$LRR = \log\left(\frac{(A+B)_{tum}}{(A+B)_{ref}}\right) \qquad BAF = \frac{B}{A+B}$$

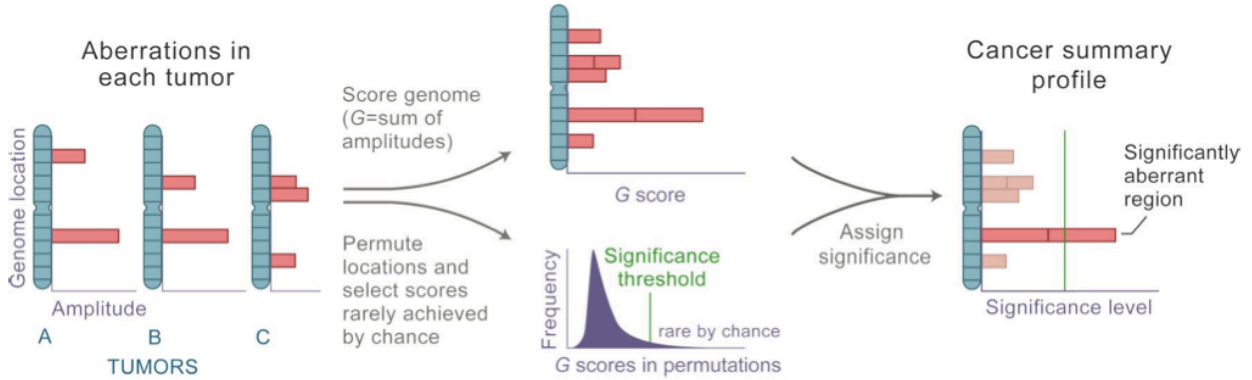




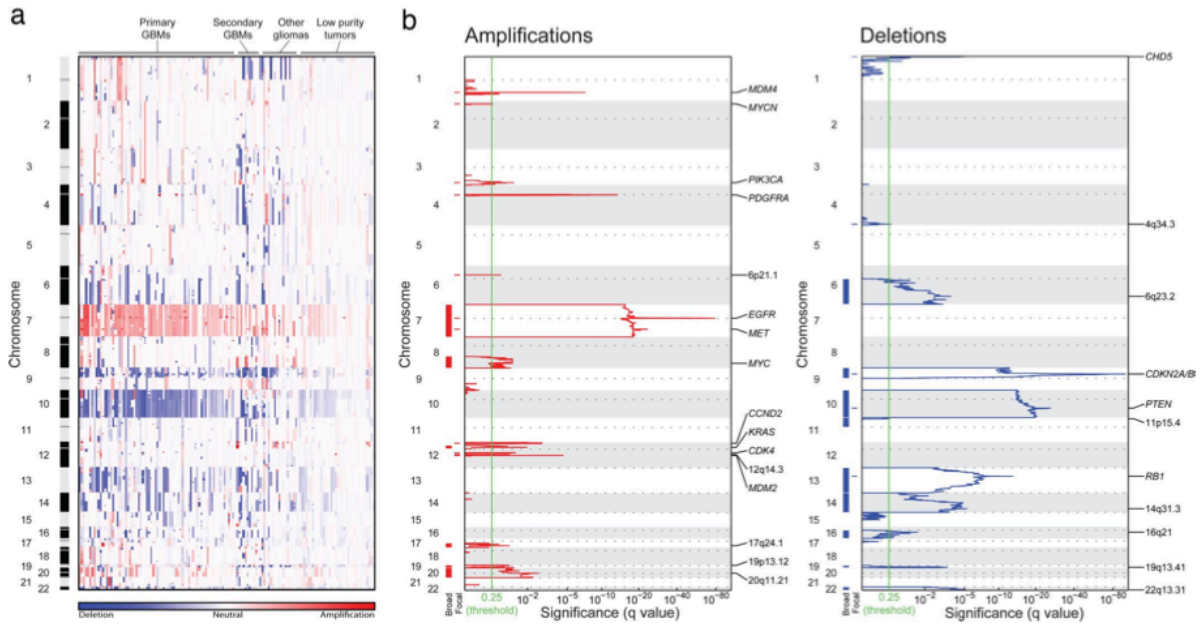
-> The **GAP method** estimates the **absolute copy-number** of each segment, the **ploidy** of the sample, and the level of **contamination** by normal cells (Popova *et al.*, Genome Biol 2009).

MÉTHODE : Détection des aberrations chromosomiques récurrentes

- We want to find chromosome gains and losses that are **significantly r**ecurrent across the dataset.
- The **GISTIC algorithm** uses both aberration frequencies and amplitudes to derive a G-score, which significance is determined as compared to a null distribution:



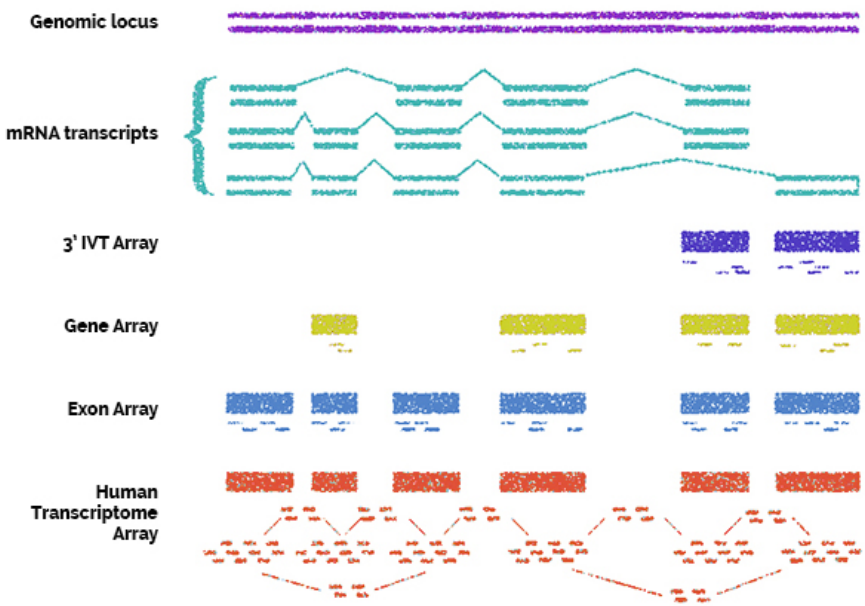
- GISTIC analysis of glioblastomas:



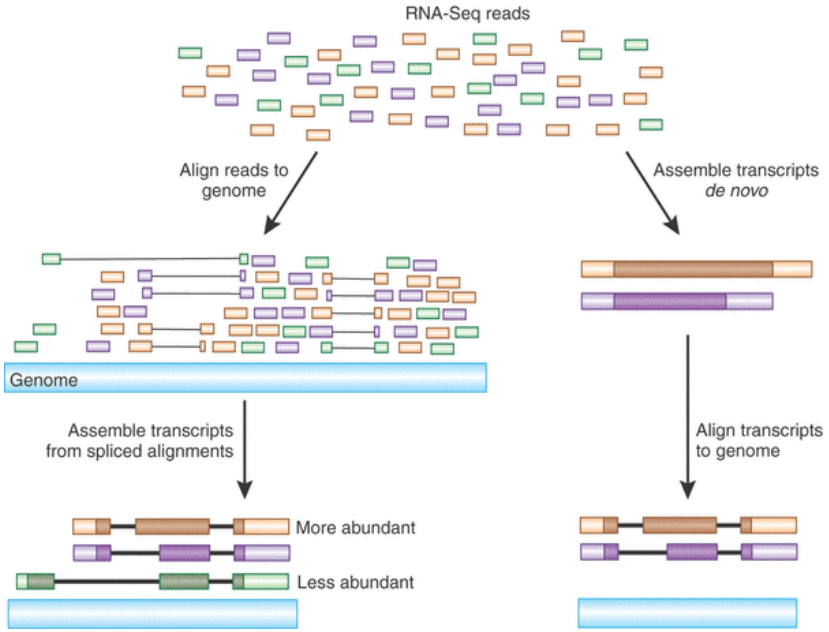
Analyse du transcriptome

- **Goal:** Detect gene expression changes (mRNA or miRNA) and identify molecular subgroups.
- **Data:** mRNA or miRNA expression arrays, RNA-seq.

Microarrays



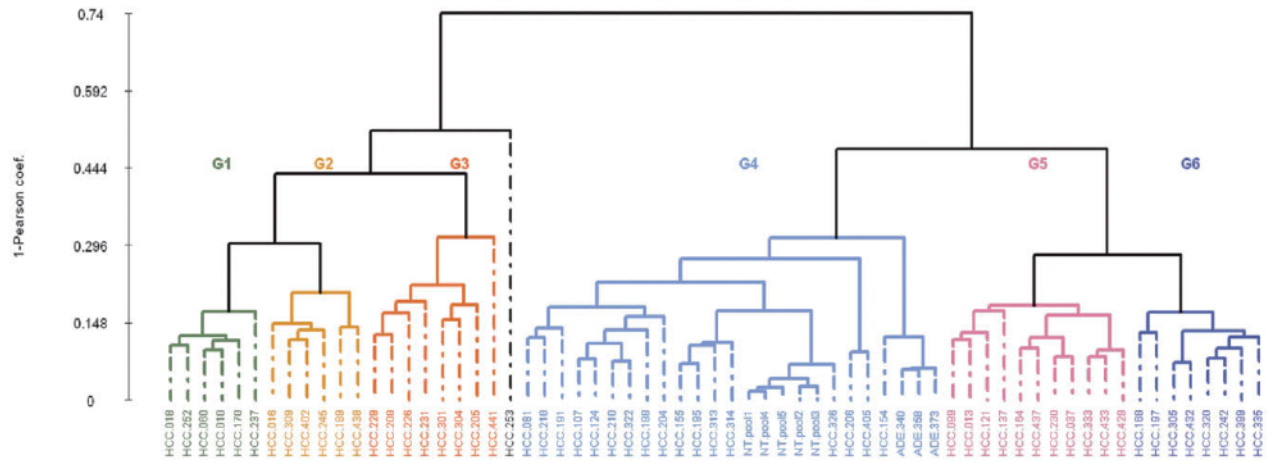
RNA-seq



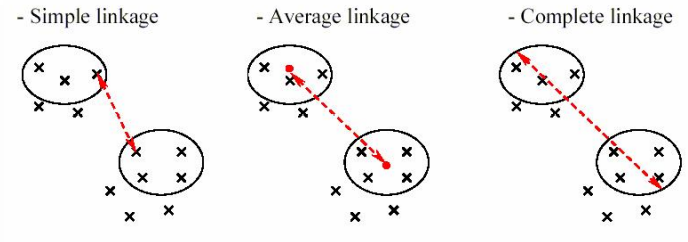
- **Analysis:**
  - Identify molecular subgroups (unsupervised)
  - Identify deregulated genes and pathways (supervised)
  - Define molecular predictors of diagnosis and prognosis

MÉTHODE : Clustering hiérarchique

- **Hierarchical clustering** is a classical approach to identify molecular subgroups of tumors.



- This bottom-up agglomerative algorithm requires a **distance metric** (Euclidean, Pearson correlation…) and a **linkage criteria** (simple, average, complete…).



- <u>BUT</u> -> How stable are the clusters?
  -> What partition should be selected?

- **Consensus clustering** is an approach to investigate the stability of clusters and determine the best partition.

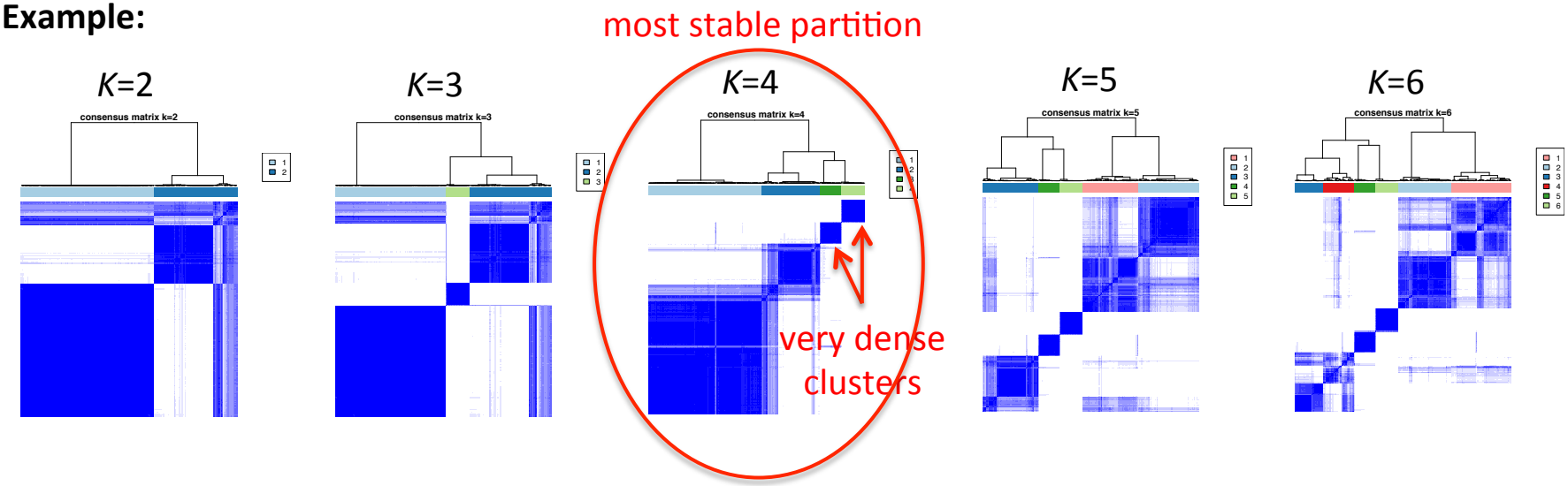
- **Method:**

  1) For each number *K* of clusters, repeat 1,000 times:
     - randomly select x % of probes and y % of samples
     - compute a partition in *K* clusters

  2) Calculate the co-classification value (0-1) for each pair of samples

  3) Clusterize the samples using the co-classification values.

- **Example:**



- Consensus clustering can also be applied to **other omics data** (e.g. DNA methylation profiles).

MÉTHODE : Analyse d'enrichissement – voies cellulaires

Goal: Extract the biological meaning of a set of genes deregulated in a cancer/subgroup.

Input

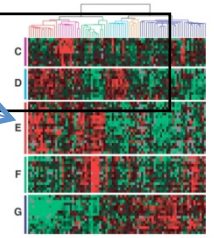
« Pathway »
corresponding to a set of genes involved in a common function / signaling cascade, etc …

Ex. Cell cycle pathway ={CCND1, CDK4, RB1, TP53, etc…}

OR

Ordered signature
Ex. : Tumor vs Normal T-statistics ordered genes

List
Ex. : Significantly up-regulated genes



Two main approaches

Are the genes from this pathway uniformely distributed in the ordered signature ?

T-statistics order

=> summarized score (ex: GSEA)

OR

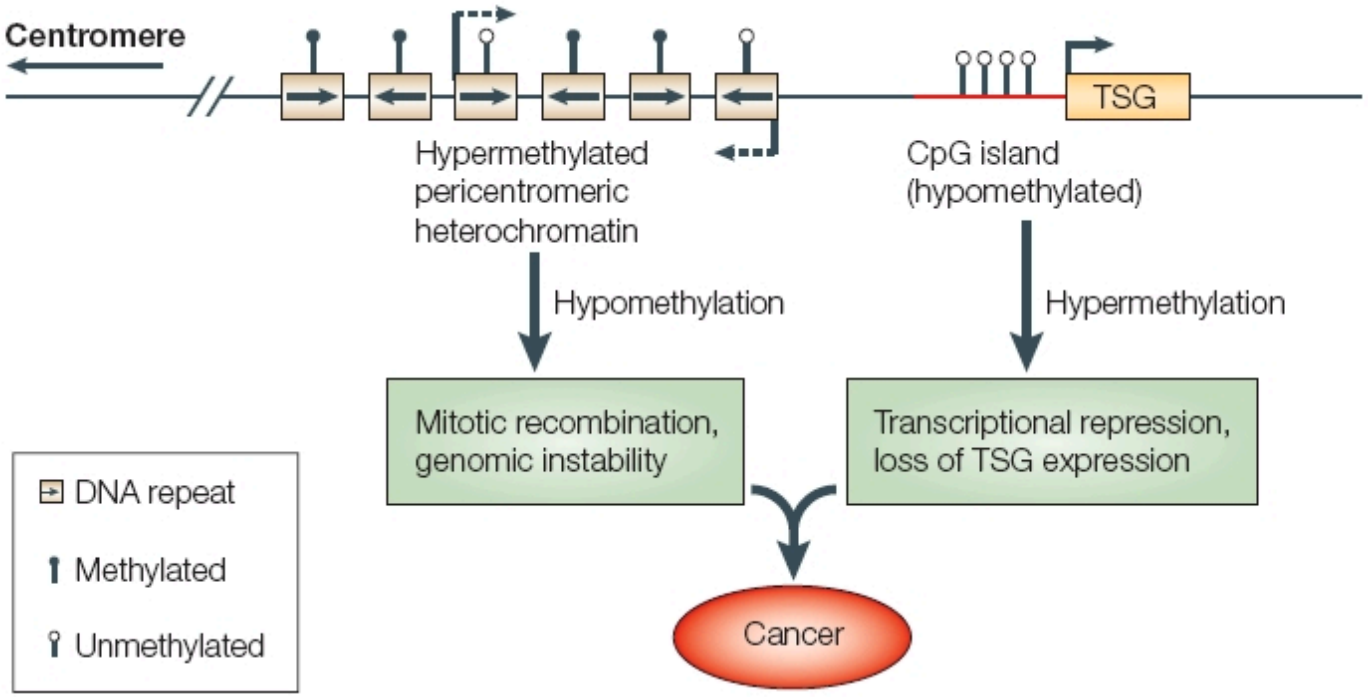
Are the genes from this pathway « enriched » in the list (*vs* outside) ?

	# in pathway	# out of pathway
# in list	a	b
# out of list	c	d

=> Hypergeometric test

ALTÉRATIONS DE LA MÉTHYLATION DE L'ADN DANS LES CANCERS

- General features:



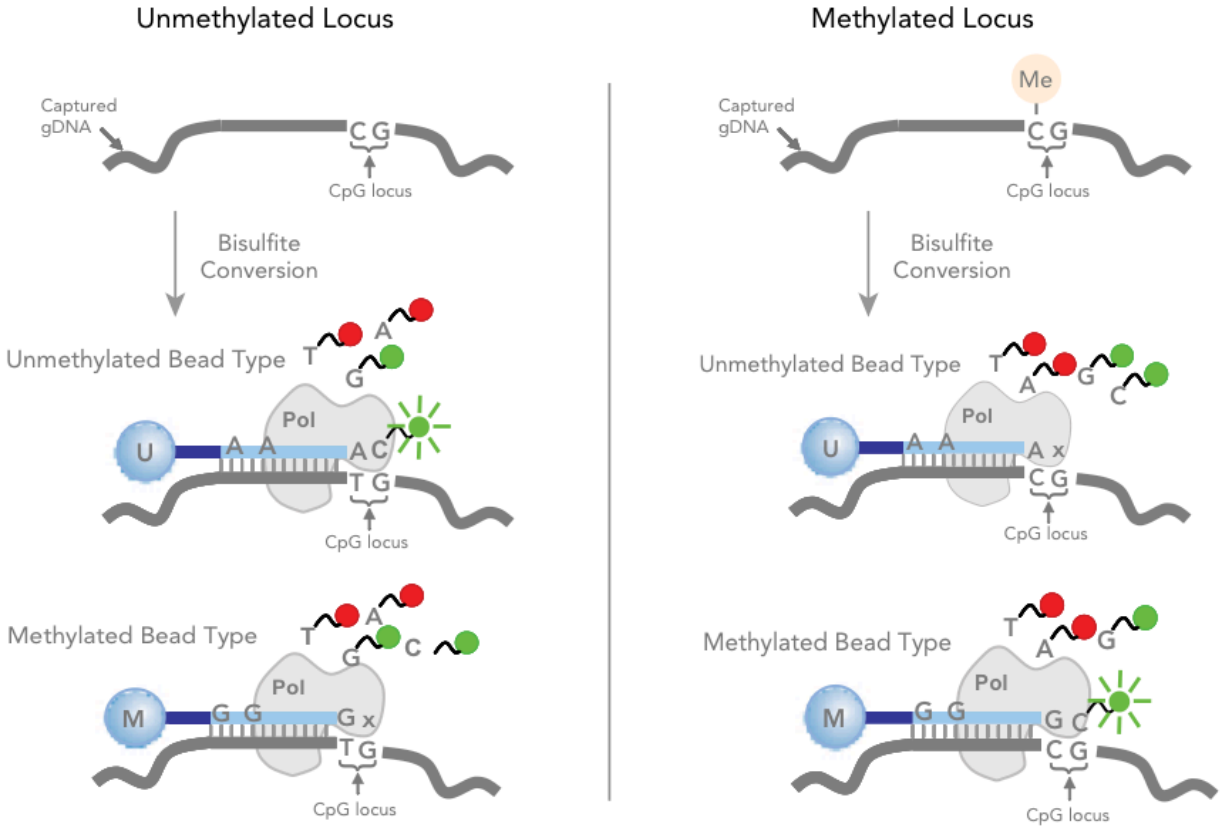
Phillips *et al.*, Nat Education 2008

- **CpG island methylator phenotypes (CIMP)** have been described in colorectal cancer (Toyota *et al.*, PNAS 1999), glioma (Noushmehr *et al.*, Cancer Cell 2010) and other cancers, characterized by the concerted hypermethylation of a large number of genes.

- The glioma CIMP phenotype is associated with isocitrate dehydrogenase (IDH) mutations.

Puces "méthylation"

- The most widely used DNA methylation arrays are Illumina bead arrays (450,000 CpG sites):

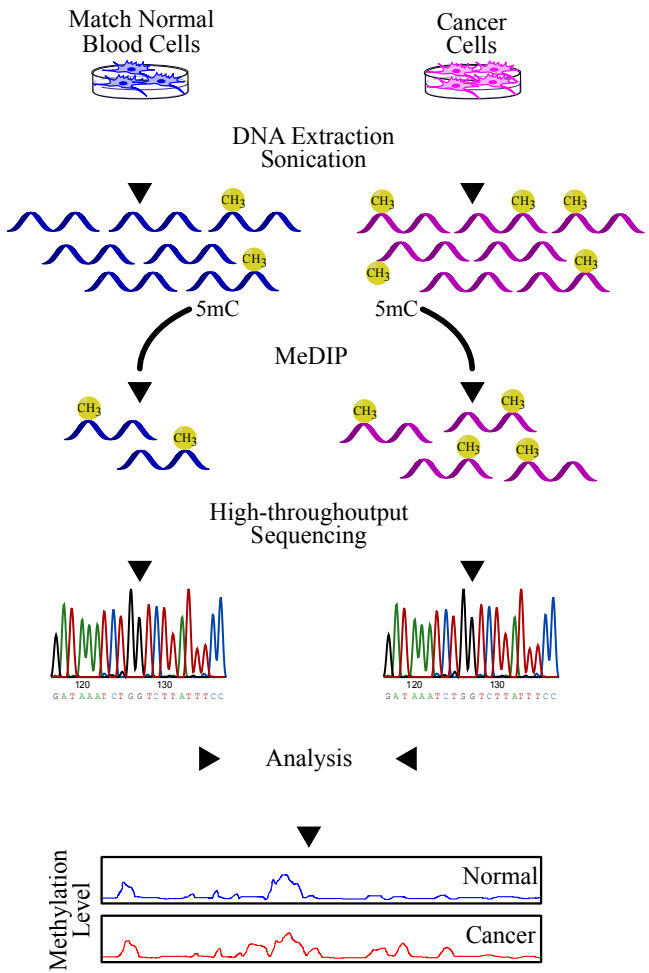


- Methylation at each CpG site is quantified as a beta value: $\beta = \frac{M}{M+U}$

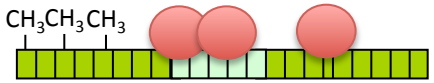
Analyse de méthylation par séquençage

Two main approaches:

- **Bisulfite sequencing** = bisulfite conversion followed by NGS (whole genome or RRBS)
- **MeDIP-seq** = immunoprecipitation-based approach.

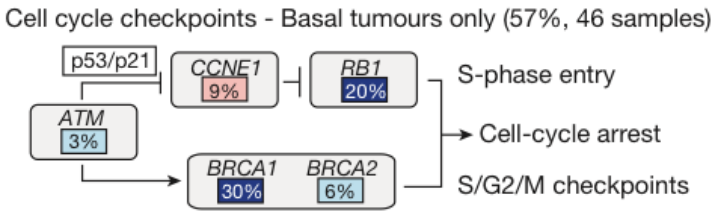


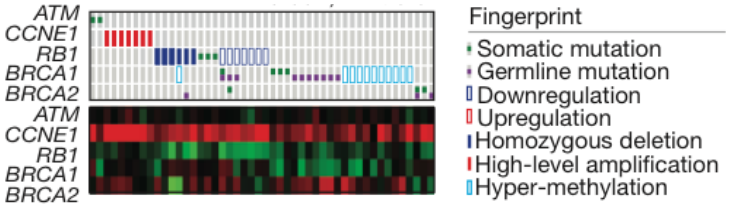
Bilan : Les technologies "omics" et leurs applications

NORMAL CELL	ALTERATIONS	ARRAYS	NGS
Epigenetic regulation	• DNA methylation changes • Chromatin remodeling	• methylation	• MeDIP-seq, BS • ChIP-seq
 DNA	• Mutations • Chromosome aberrations	• SNP	• WES, WGS • WES, WGS
Transcription ↓			
 mRNA  miRNA	• Gene expression changes	• mRNA • miRNA	• RNA-seq • RNA-seq
	• Alternative splicing	• exon	• RNA-seq
Translation ↓	↓		
 Protein	Impact on protein expression and function		
↓	↓		
PHENOTYPE	**TUMOR PHENOTYPE**		

Intégration multi-omics

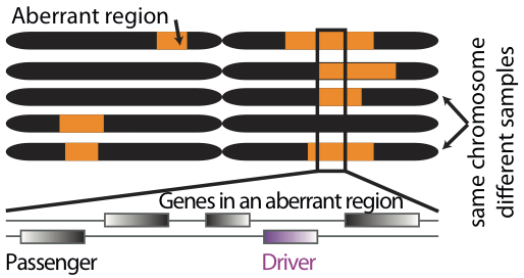
- Integrating several omics data is important to **refine oncogenic mechanisms** and **tumor subgroups**.
- **Example 1:** Several types of alteration can alter the same gene/pathway.

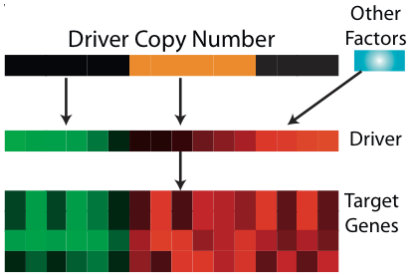




TCGA, Nature 2012

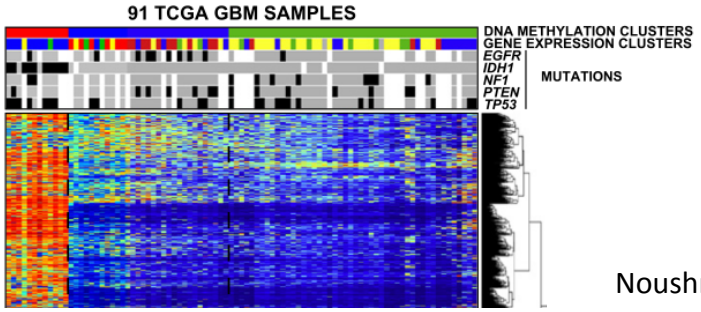
- **Example 2:** CONEXIC combines copy-number and expression data to identify driver genes.





Akavia *et al.*, Cell 2010

- **Example 3:** Association between a mutation and a DNA methylation subgroup.



Noushmehr *et al.*, Cancer Cell 2010

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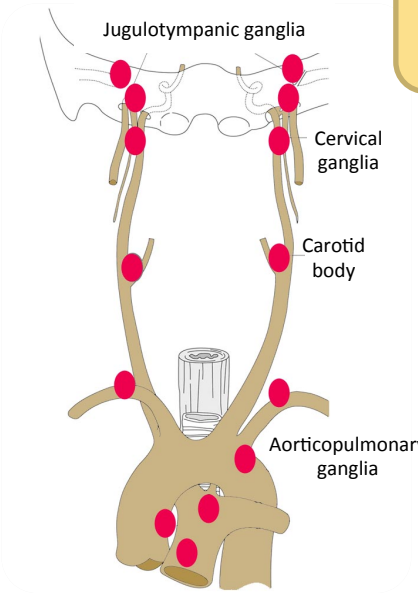
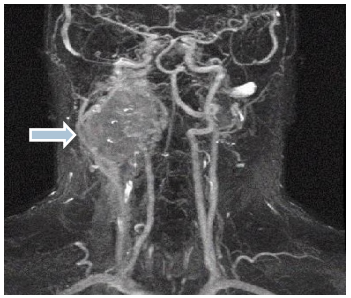
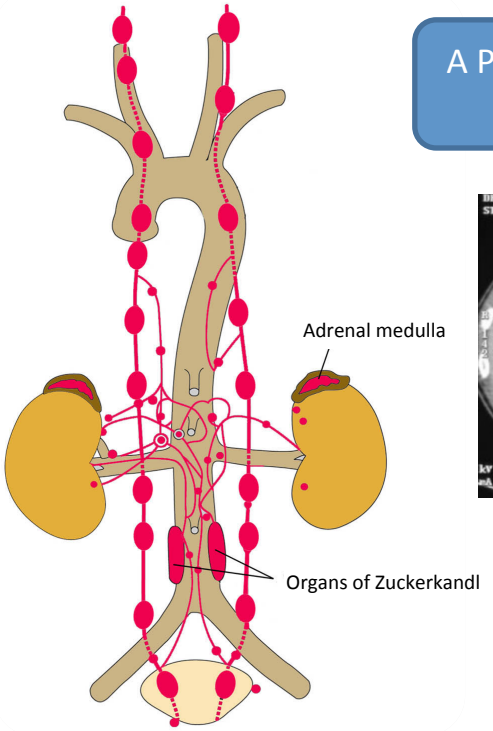
Phéochromocytomes et paragangliomes

- Pheochromocytomas (PCC) and paragangliomas (PGL) are rare tumors (2-8 cases per million) arising from neural crest-derived tissues.

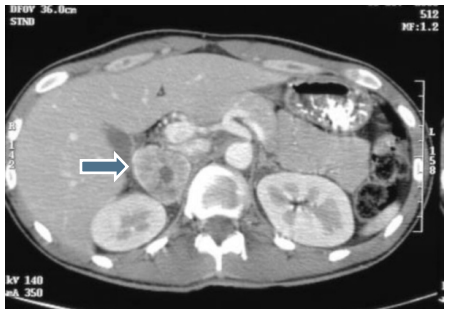


PGL arise from parasympathetic or sympathetic ganglia





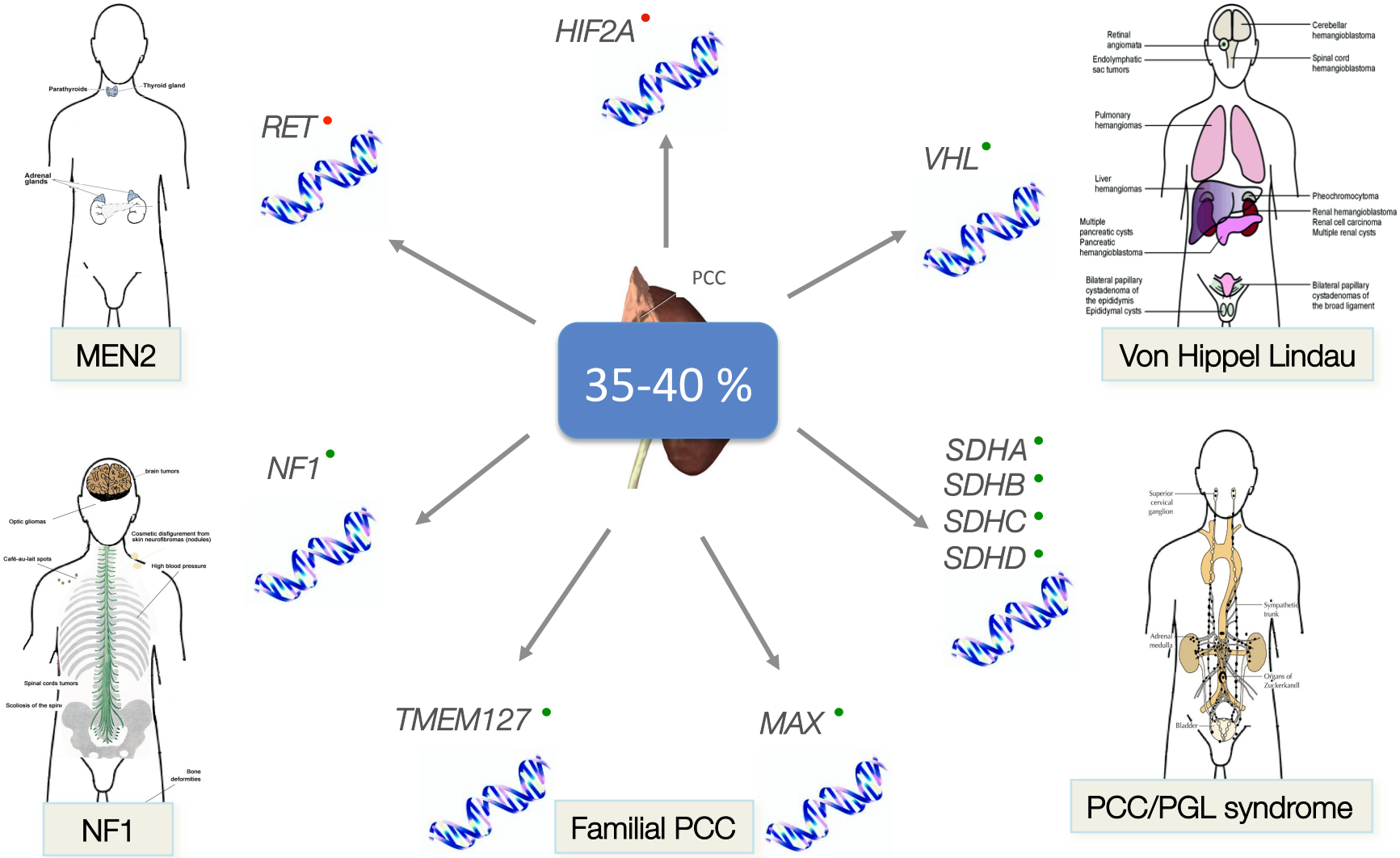
A PCC is a PGL that develops in the adrenal medulla



- PGL/PCC are neuroendocrine tumors often secreting catecholamines, resulting in hypertension, headache, sweating, palpitations…

Prédisposition génétique des paragangliomes

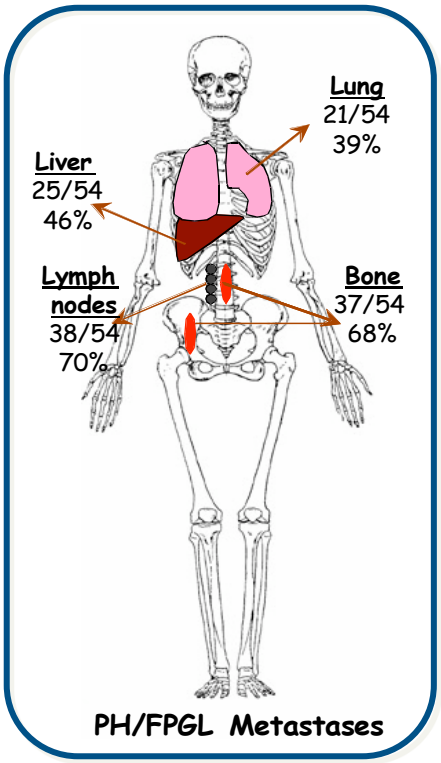
- PGL/PCC are often associated with hereditary syndromes:



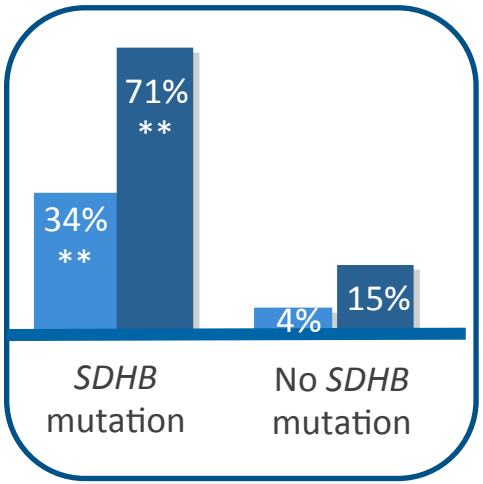
Les tumeurs mutées SDH sont particulièrement agressives ?

- PGL/PCC are generally benign but 10-15% of all cases develop metastases.
- SDHB mutations confer a much higher risk of metastasis and a shorter survival than patients with malignant tumors but no SDHB mutation.

Malignancy: 10-15% of cases



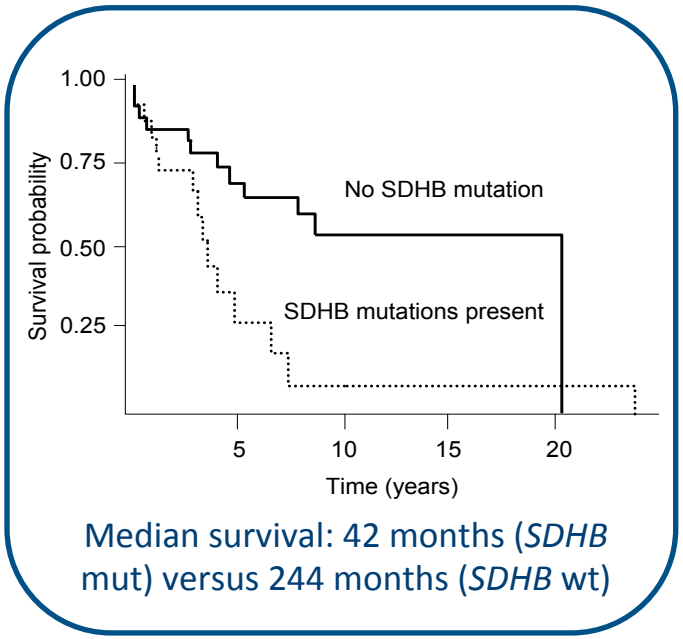
Metastatic progression



Neumann *et al.*, JAMA 2004

Amar *et al.*, J Clin Oncol 2005

Survival in malignant PGL/PCC

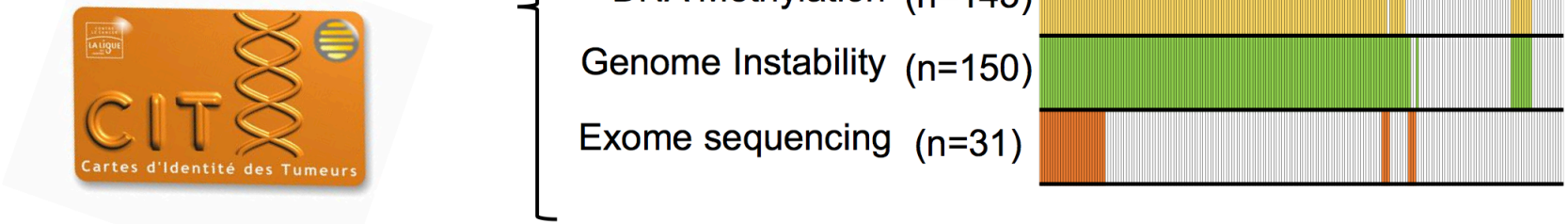


Median survival: 42 months (*SDHB* mut) versus 244 months (*SDHB* wt)

Gimenez-Roqueplo et al., Cancer Res 2003
Amar et al., J Clin Oncol 2005
Amar et al., J Clin Endocrinol Metab 2007

Analyse génomique intégrée des paragangliomes (cohorte COMETE)

COMETE Network
HEGP/Cochin - 1993/2008
190 patients – 202 tumor samples

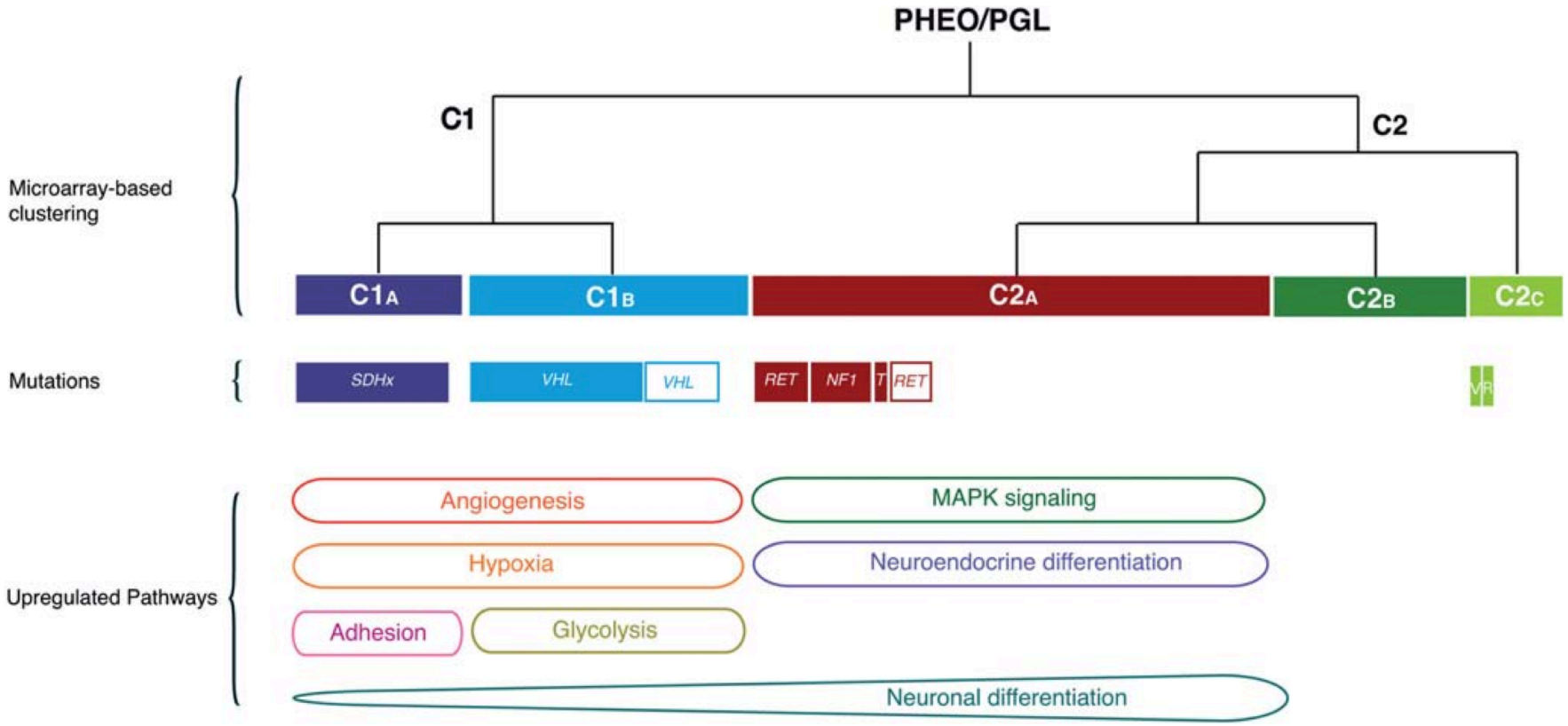


Main goals of the project:

- Identify new driver genes and oncogenic mechanisms
- Define the spectrum of alterations underlying tumorigenesis
- Explain the agressiveness of *SDHB*-mutated case

Les groupes transcriptomiques de paragangliomes sont très associés aux gènes de prédisposition

- Transcriptome profiling of the CIT/COMETE cohort:

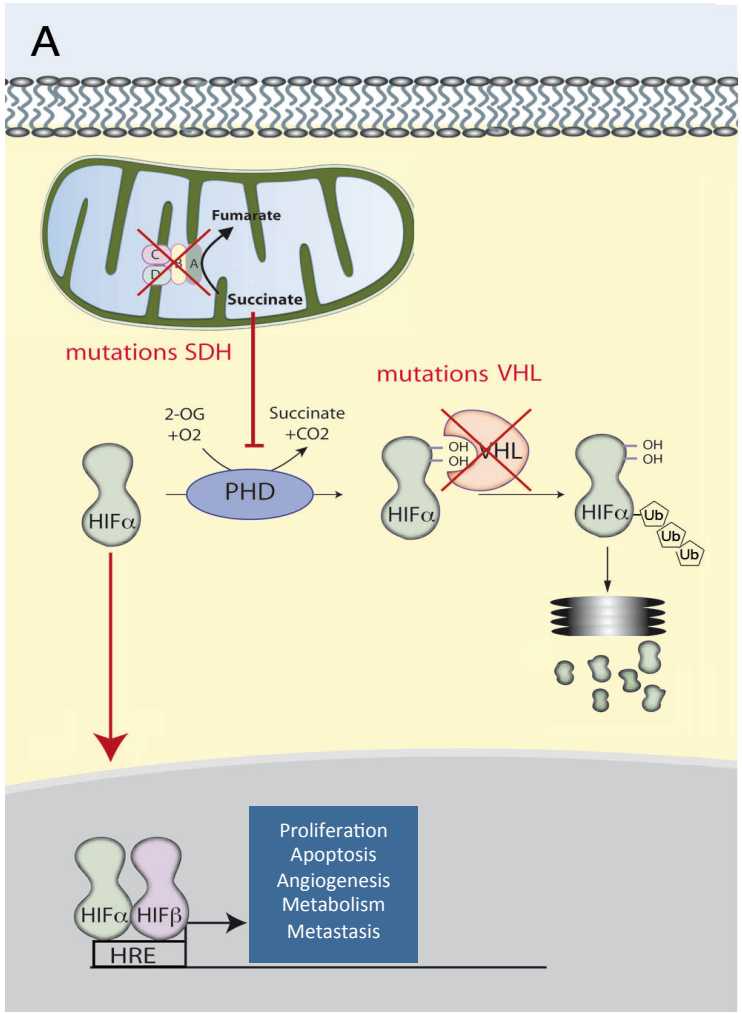


Burnichon *et al.*, HMG 2011

-> Tumors of the C1 cluster (*SDHx*, *VHL*) display an activation of hypoxia, whereas tumors of the C2A cluster (*RET*, *NF1*) display an activation of the MAP kinase pathway.

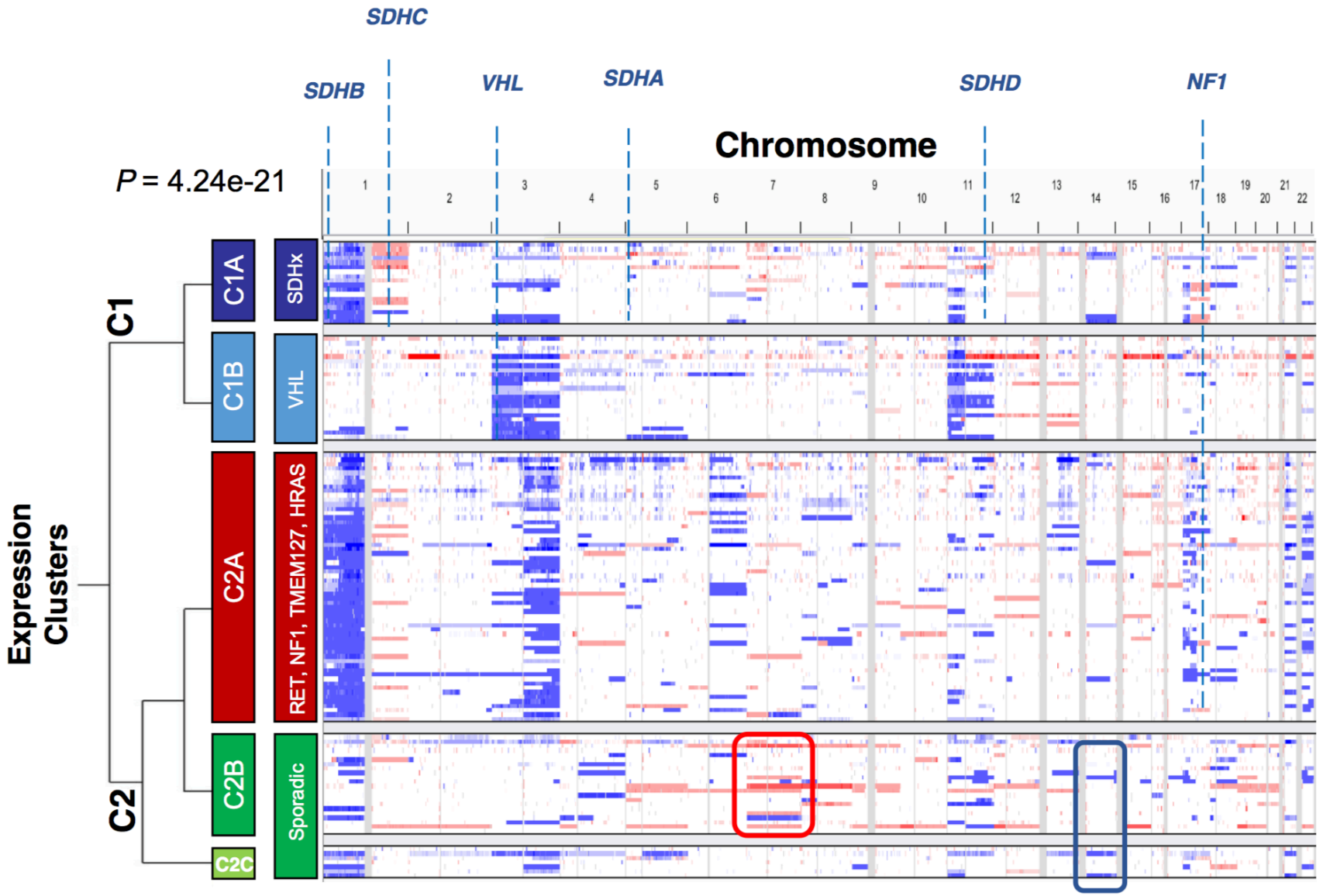
Deux voies cellulaires principales impliquées dans les paragangliomes

Cluster C1: hypoxia



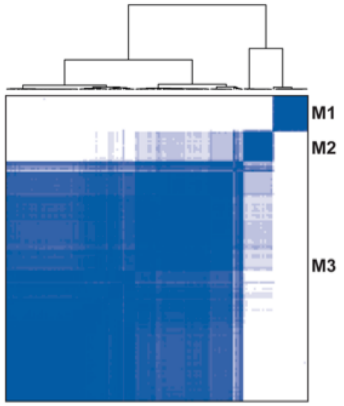
Adapted from Favier and Gimenez-Roqueplo, Médecine Sciences, 2012

Les aberrations chromosomiques sont très associées aux groupes moléculaires

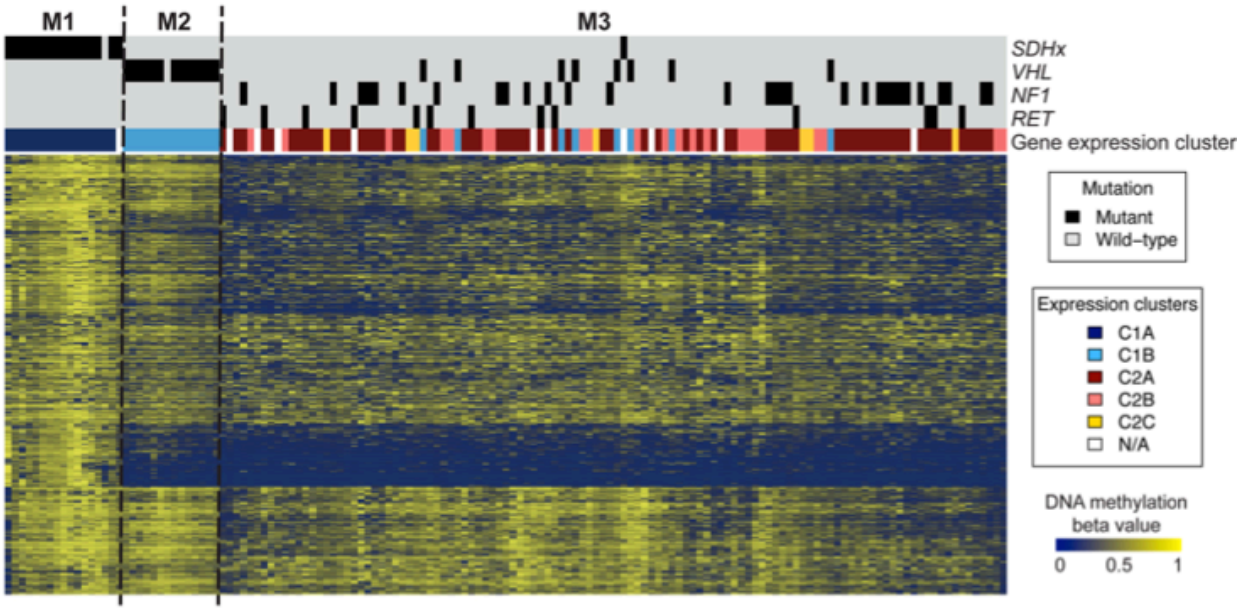


Classification du méthylome des paragangliomes

- Consensus clustering reveals 3 homogeneous tumor clusters:



- DNA methylation subgroups are highly associated with molecular subtypes:

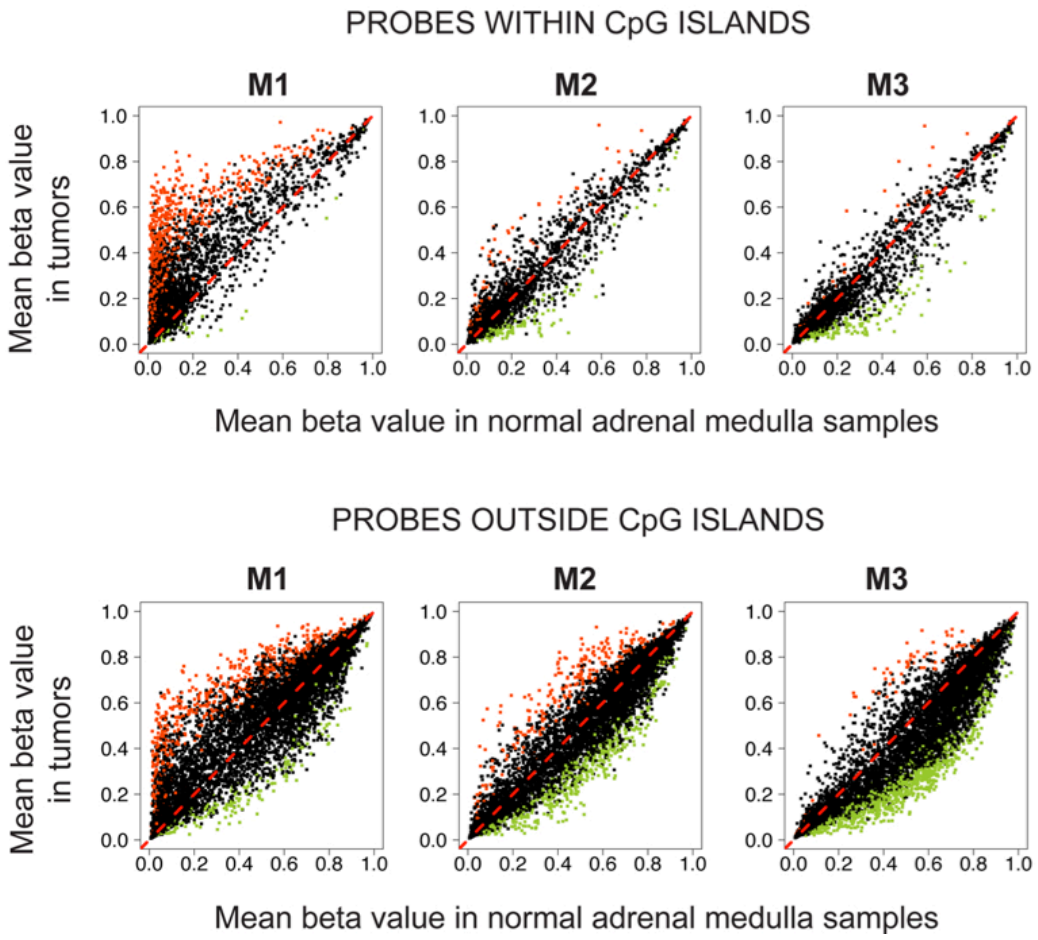


Letouzé*, Martinelli* *et al.*
Cancer Cell 2013

-> Clusters M1 and M2 respectively correspond to SDH and VHL-related tumors. Other tumors are comprised in cluster M3.

Les tumeurs mutées *SDHx* présentent un phénotype hyperméthylateur

- DNA methylation changes in each tumor subgroup:

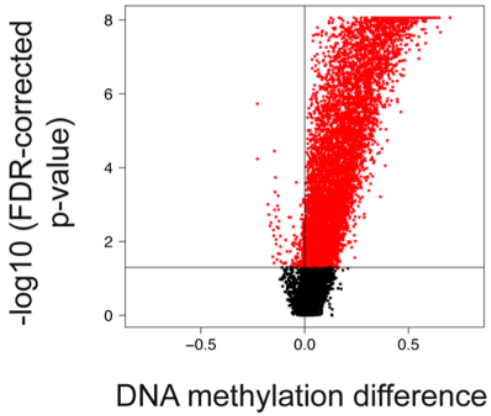


-> M1 tumors display widespread hypermethylation both within and outside CpG islands.

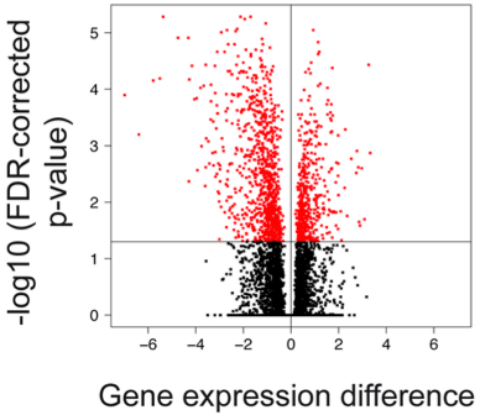
Les tumeurs *SDHx* présentent un phénotype hyperméthylateur associé à une inhibition transcriptionnelle

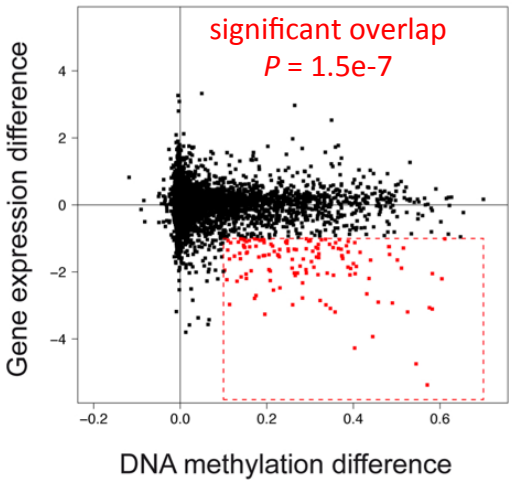
- DNA methylation and gene expression changes between M1 (*SDHx*) and non-M1 tumors:

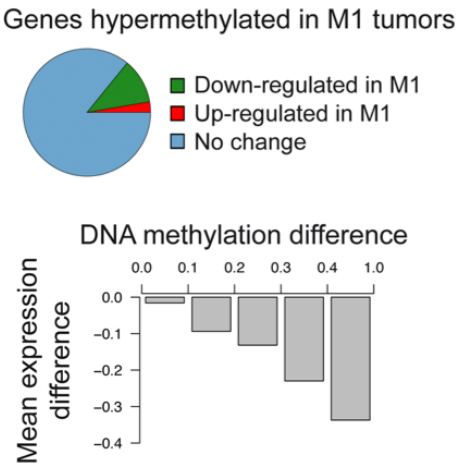
4,663 genes hypermethylated in M1



623 genes down-regulated in M1







-> 11.5% of hypermethylated genes (beta-value difference > 0.1) are down-regulated in M1 tumors.

Gènes inhibés par hyperméthylation dans les tumeurs *SDHx*

- 191 genes are significantly hypermethylated and down-regulated in *SDHx* tumors:

	DNA METHYLATION			GENE EXPRESSION		
Gene symbol	Illumina CpG ID	Beta value difference (M1-non M1)	Methylation FDR-adjusted p-value	Affymetrix probeset ID	Log2 fold change (M1/non-M1)	Expression FDR-adjusted p-value
NPY	cg00355281	0.3	0.000298922	206001_at	-6.39	0.000631472
CRYBA2	cg02805994	0.35	0.000298922	220136_s_at	-5.37	5.22E-06
KRT19	cg02893823	0.45	0.000594842	201650_at	-4.74	1.23E-05
EFS	cg18454133	0.4	0.004512674	204400_at	-4.27	6.73E-05
SYT13	cg21074260	0.25	0.001551809	226086_at	-3.96	0.00014417
PNMT	cg06596543	0.25	0.000298922	206793_at	-3.93	0.002706443
DNAJA4	cg05392364	0.37	0.000408954	225061_at	-3.8	9.46E-05
GNG8	cg21072025	0.28	0.007184473	234284_at	-3.61	0.005169372
RET	cg06559368	0.39	0.00208515	205879_x_at	-3.58	3.69E-05
JAKMIP1	cg05382097	0.18	0.000298922	238600_at	-3.42	0.002065611
TMEM45B	cg27294678	0.04	0.157509306	230323_s_at	-3.37	0.000343782
GDF10	cg04110601	0.32	0.016436798	206159_at	-3.26	0.000373051
RPP25	cg16064478	0.42	0.000298922	219143_s_at	-3.19	3.85E-05
RBP1	cg13099330	0.51	0.000298922	203423_at	-3.11	0.003154997
SLC6A2	cg04874129	0.25	0.007184473	239394_at	-3.09	0.000268757
C2orf40	cg06499647	0.1	0.01098253	223623_at	-3.07	0.000268764
MAPK13	cg06100227	0.34	0.002718498	210059_s_at	-3.07	0.006474365
RAB27B	cg09052983	0.1	0.00208515	228708_at	-3.01	0.009740778
CALY	cg10780164	0.23	0.000408954	219896_at	-3.01	0.045421315
SHC3	cg27044506	0.08	0.016436798	229824_at	-2.98	0.002231716
SPOCK2	cg17958658	0.25	0.004512674	202524_s_at	-2.98	0.002604168

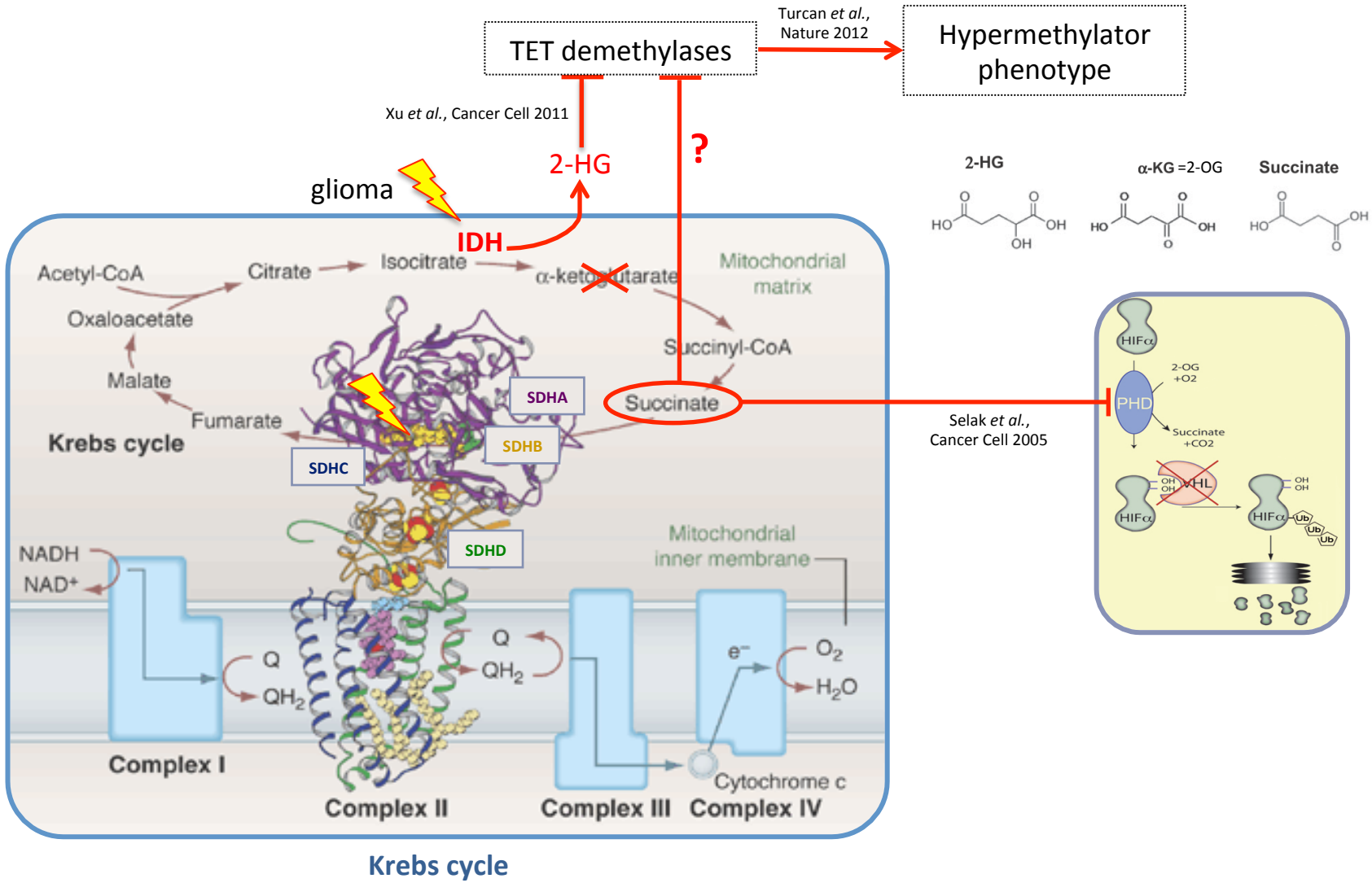
Catecholamine metabolic process

Epithelial-mesenchymal transition

Known tumor suppressors

-> Epigenetic silencing of genes implicated in chromaffin cell differentiation and EMT explains the phenotypic characteristics of these tumors.

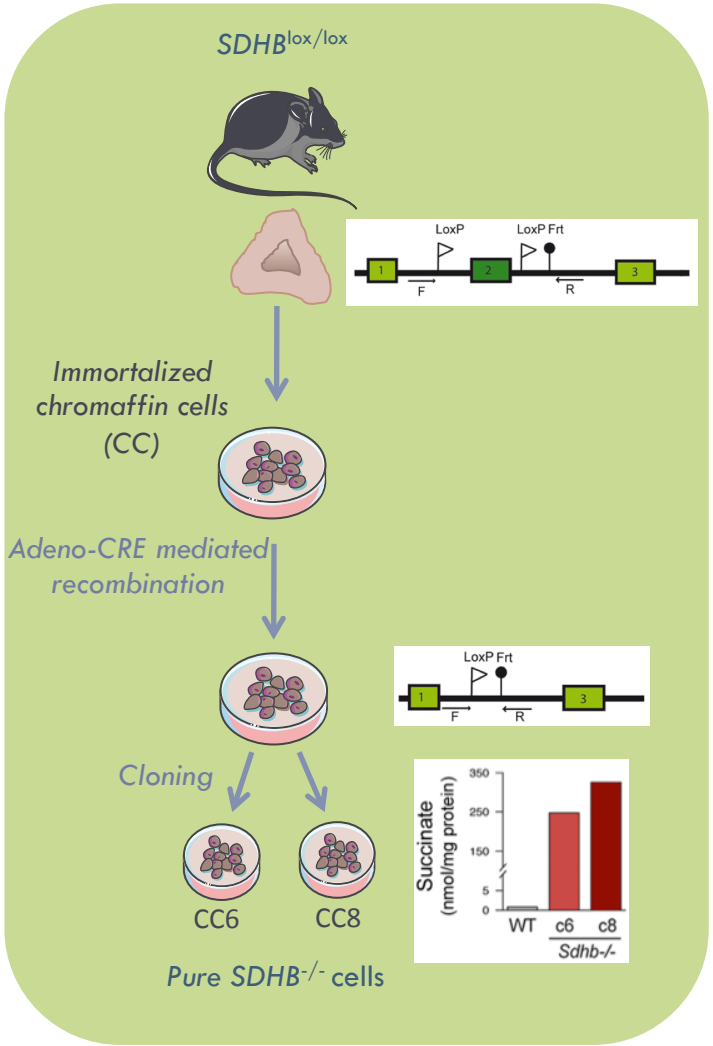
Quel mécanisme relie les mutations *SDH* et la méthylation de l'ADN ?



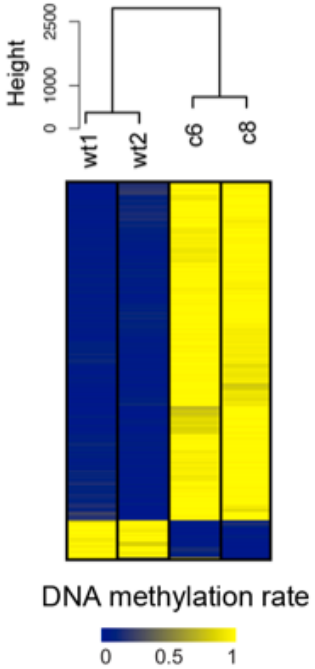
Krebs cycle

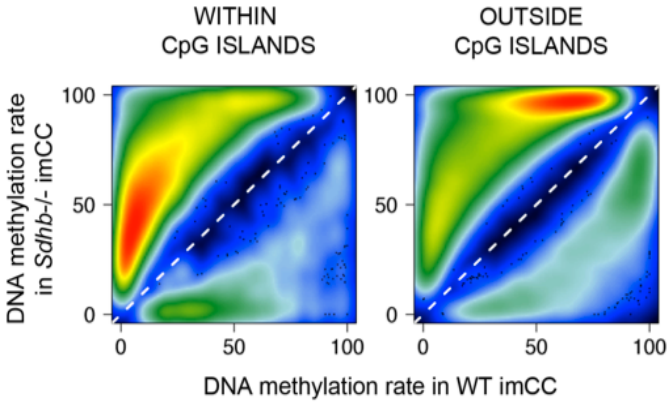
-> Does succinate also inhibit KDM and TET enzymes in SDH-related PGL/PCC?

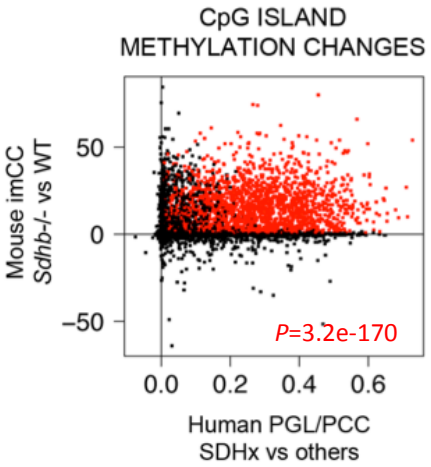
Création et analyse de cellules murines *Sdhb-/-*



- Reduced representation bisulfite sequencing (RRBS) analysis of mouse chromaffin cells:



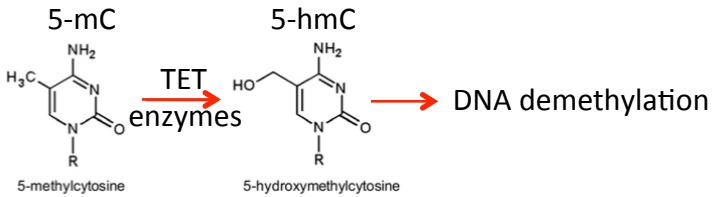


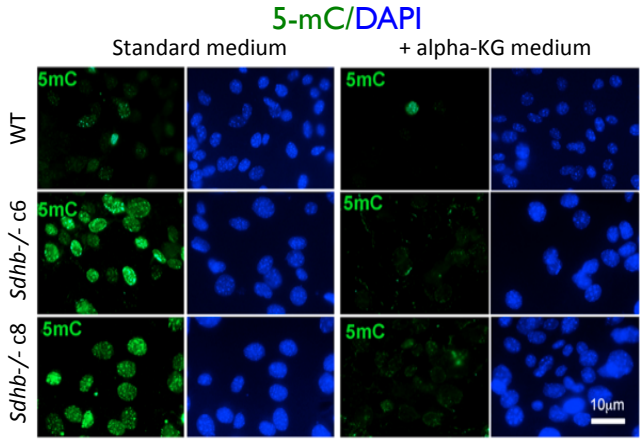


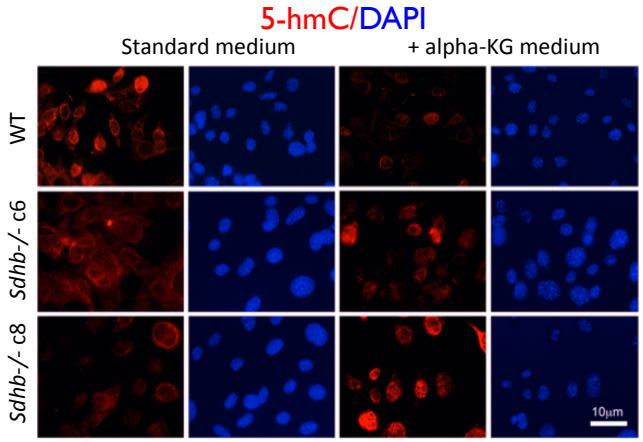
-> *Sdhb-/-* mouse chromaffin cells recapitulate the hypermethylator phenotype seen in human tumors, with 1,014 hypermethylated genes in common.

Ratio 5mC/5-hmC dans les cellules *Sdhb-/-*

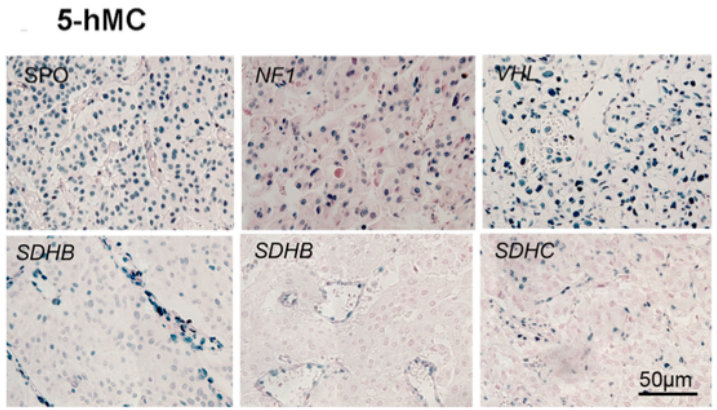
- 5-mC/5-hmC ratio in *Sdhb-/-* cells:





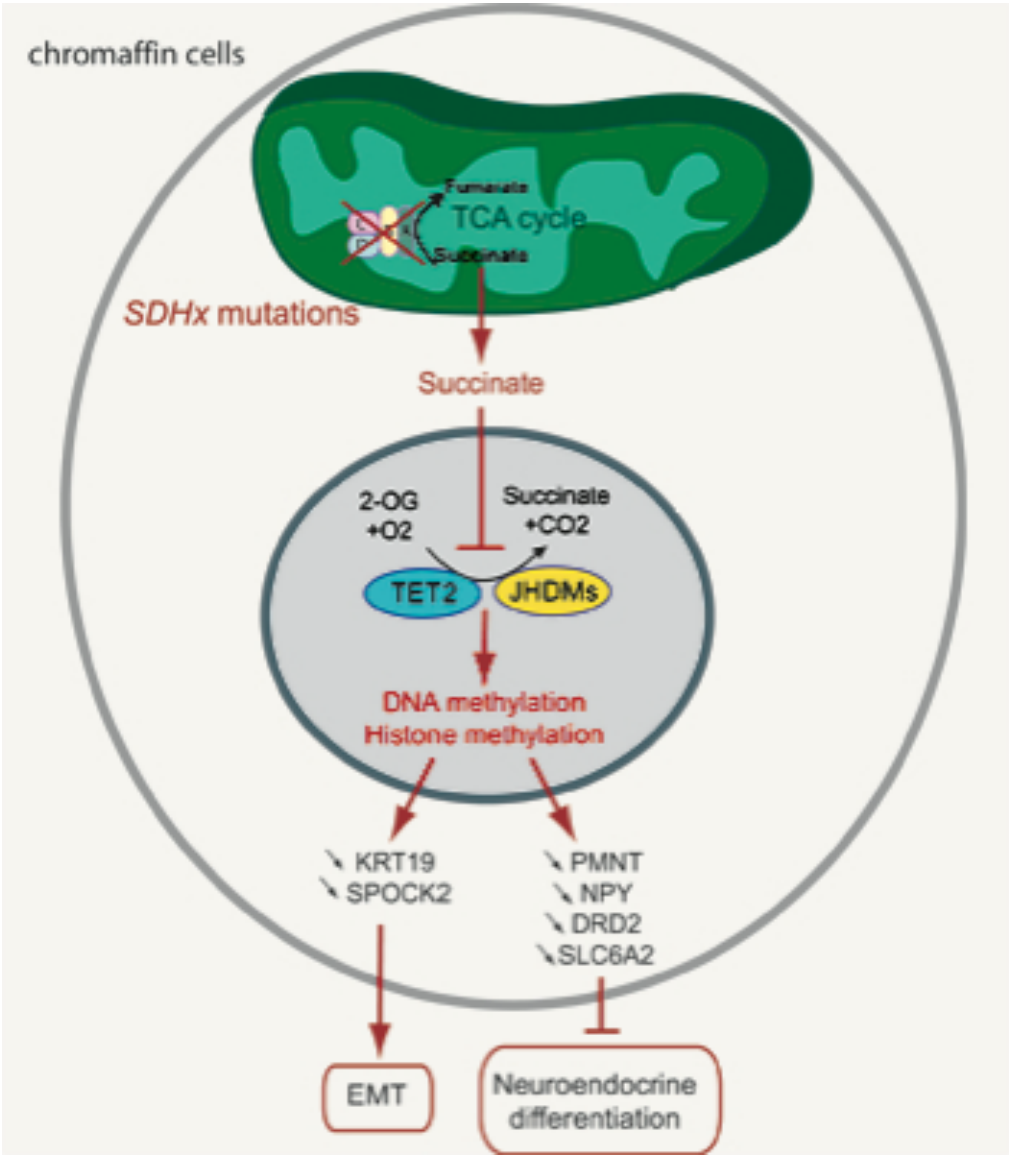


- 5-hmC staining of human PGL/PCC:



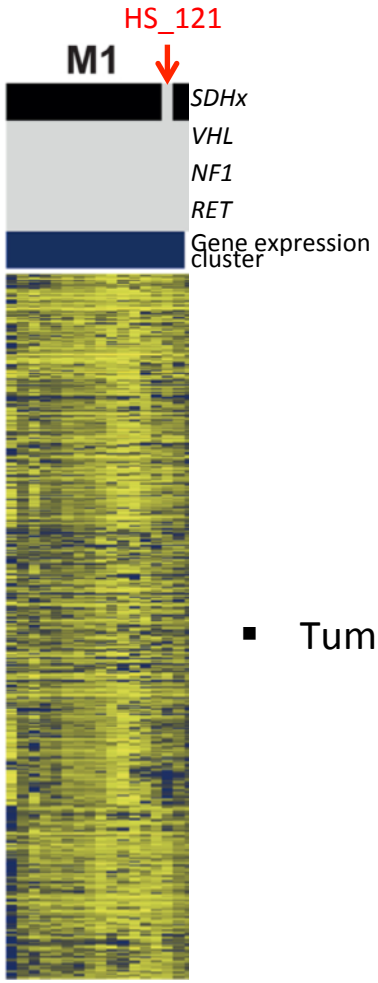
-> Inhibition of TET demethylases establishes the hypermethylator phenotype in SDH-deficient PGL/PCC.

Le mécanisme oncogénique des mutations *SDHx*

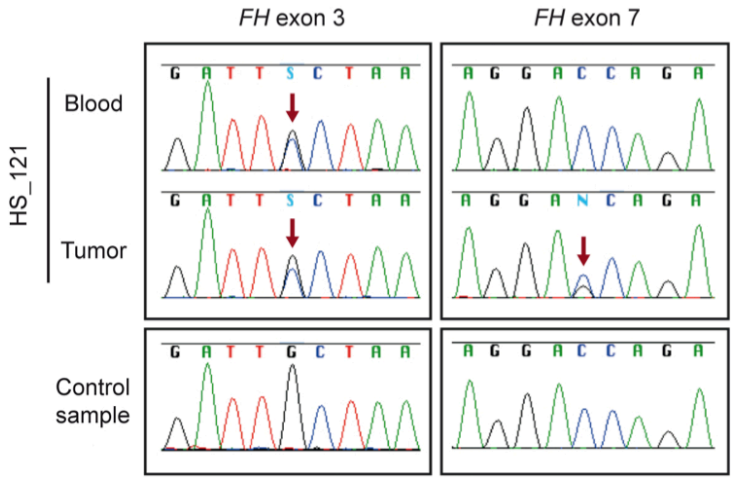


Letouzé*, Martinelli* *et al.* Cancer Cell 2013

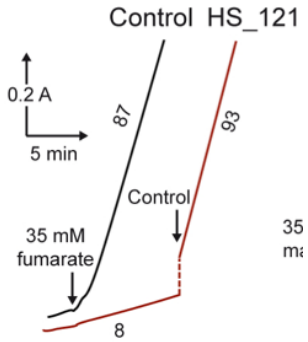
Le profil hyperméthylateur révèle un nouveau gène de prédisposition aux PGL/PCC

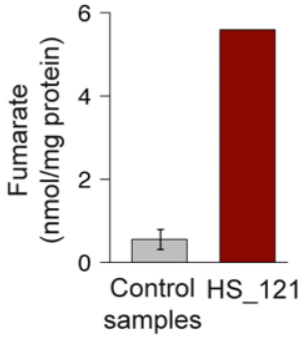


- Exome sequencing identifies two *FH* mutations in HS_121:



- Tumor HS_121 shows reduced fumarase activity and increased fumarate concentration:



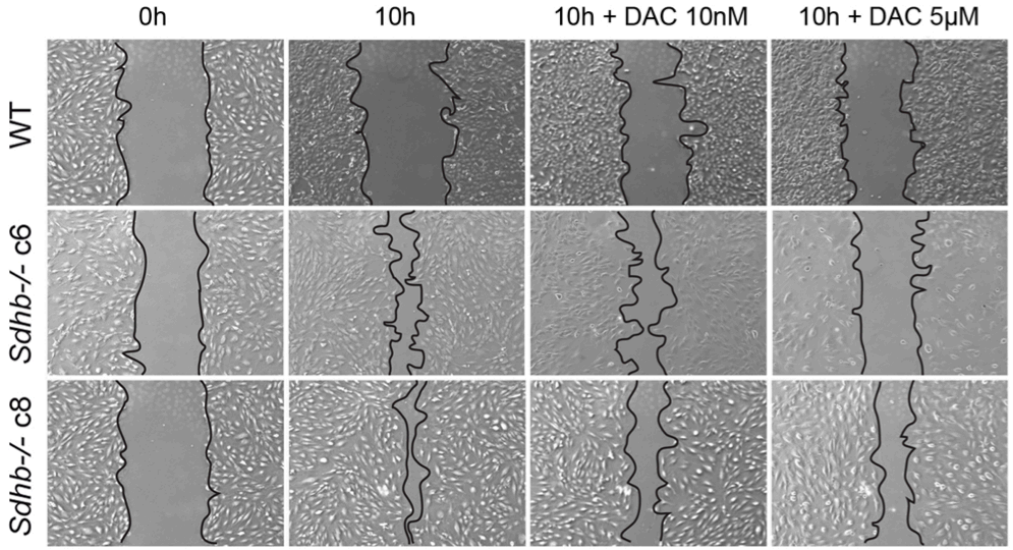


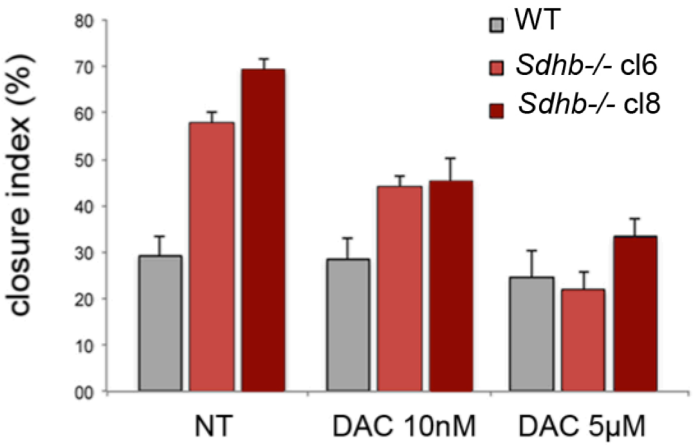
-> *FH* mutations cause PGL/PCC by establishing a hypermethylator phenotype.

-> This new predisposing gene accounts for 0.8% of patients (Castro-Vega *et al.*, Hum Mol Genet 2014).

Une nouvelle option thérapeutique pour les tumeurs *SDHx*

CELL MIGRATION ASSAY



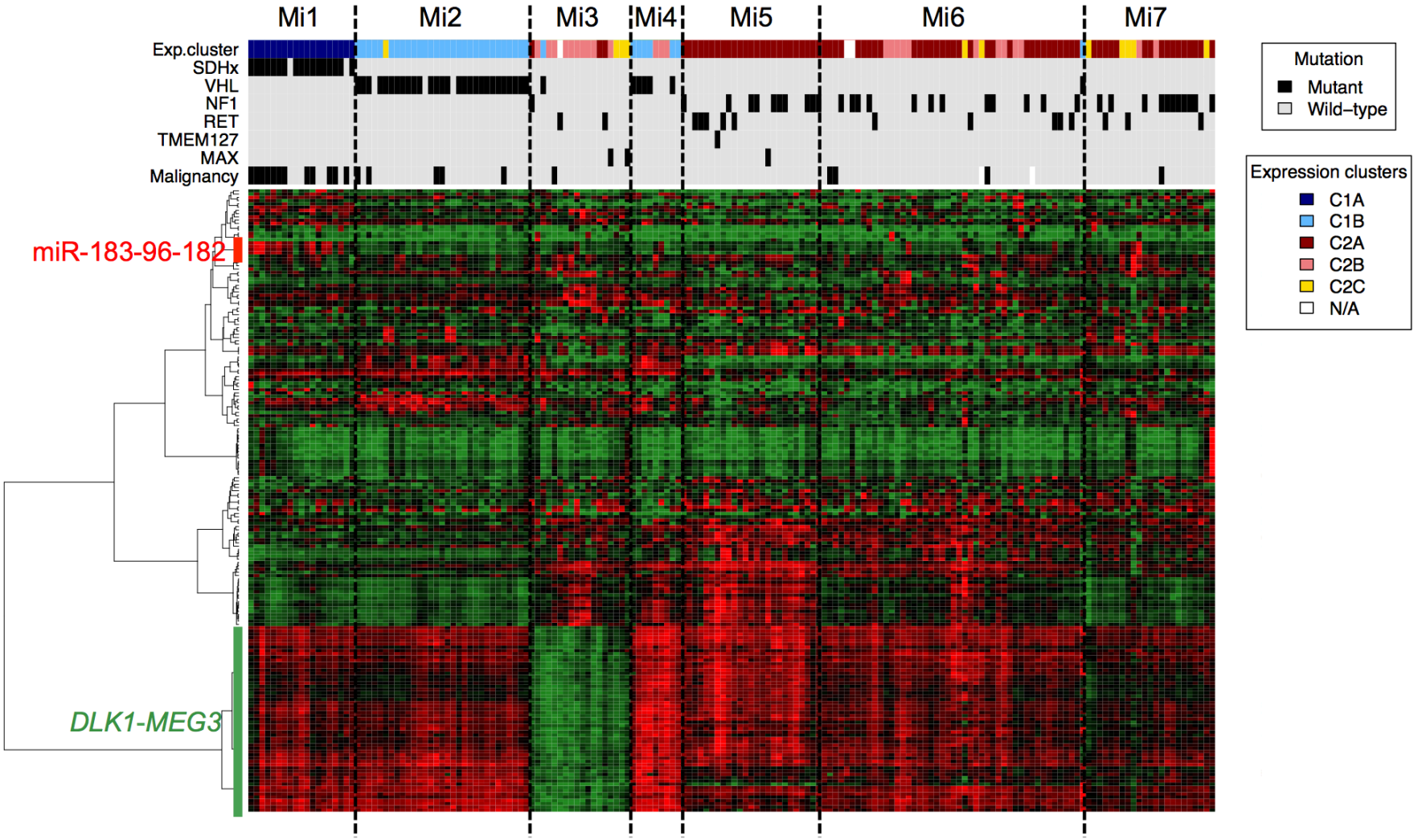


-> Like SDH-related tumors, *Sdhb-/-* cells do not proliferate fast but display a migratory phenotype.

-> Reversal of this phenotype by decitabin (DAC) suggests the use of demethylating agents to treat these aggressive tumors.

miRNA profiling of PGL/PCC

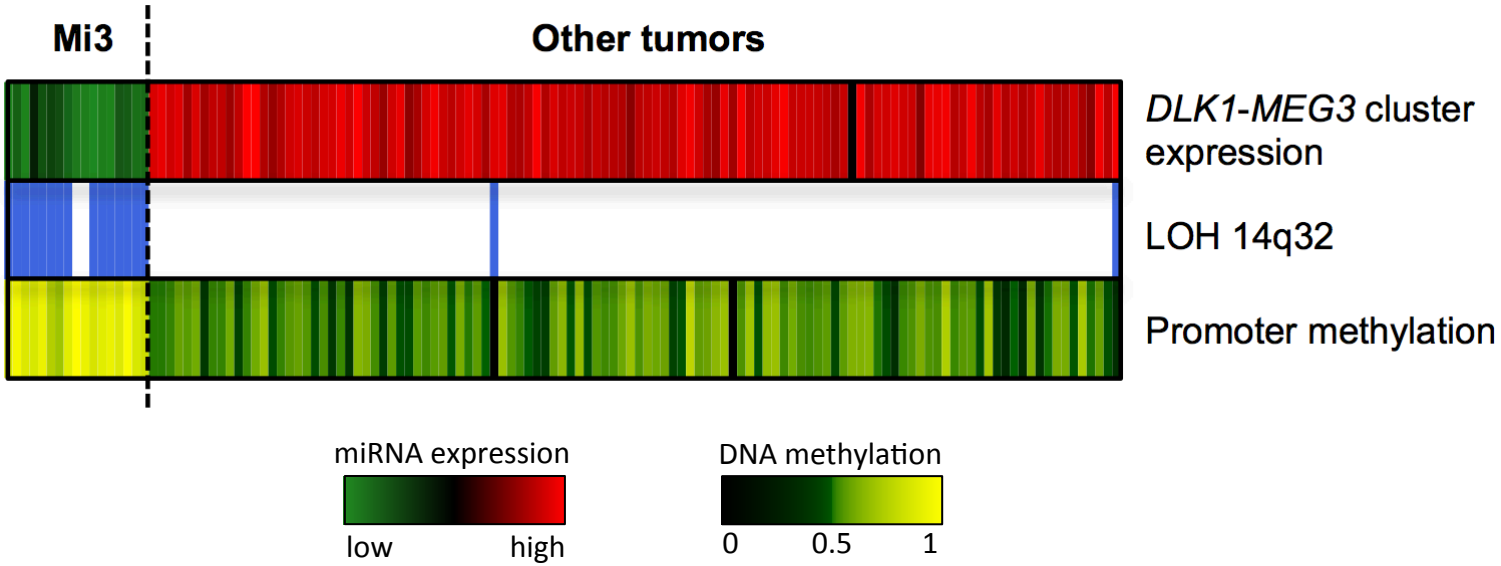
- miRNA profiling reveals 7 sable clusters with different miRNA expression patterns:



Mécanisme expliquant l'extinction du cluster *DLK1-MEG3* dans les tumeurs du groupe Mi3

- The *DLK1-MEG3* cluster is **imprinted**: the maternal allele is expressed, whereas the paternal allele is methylated.

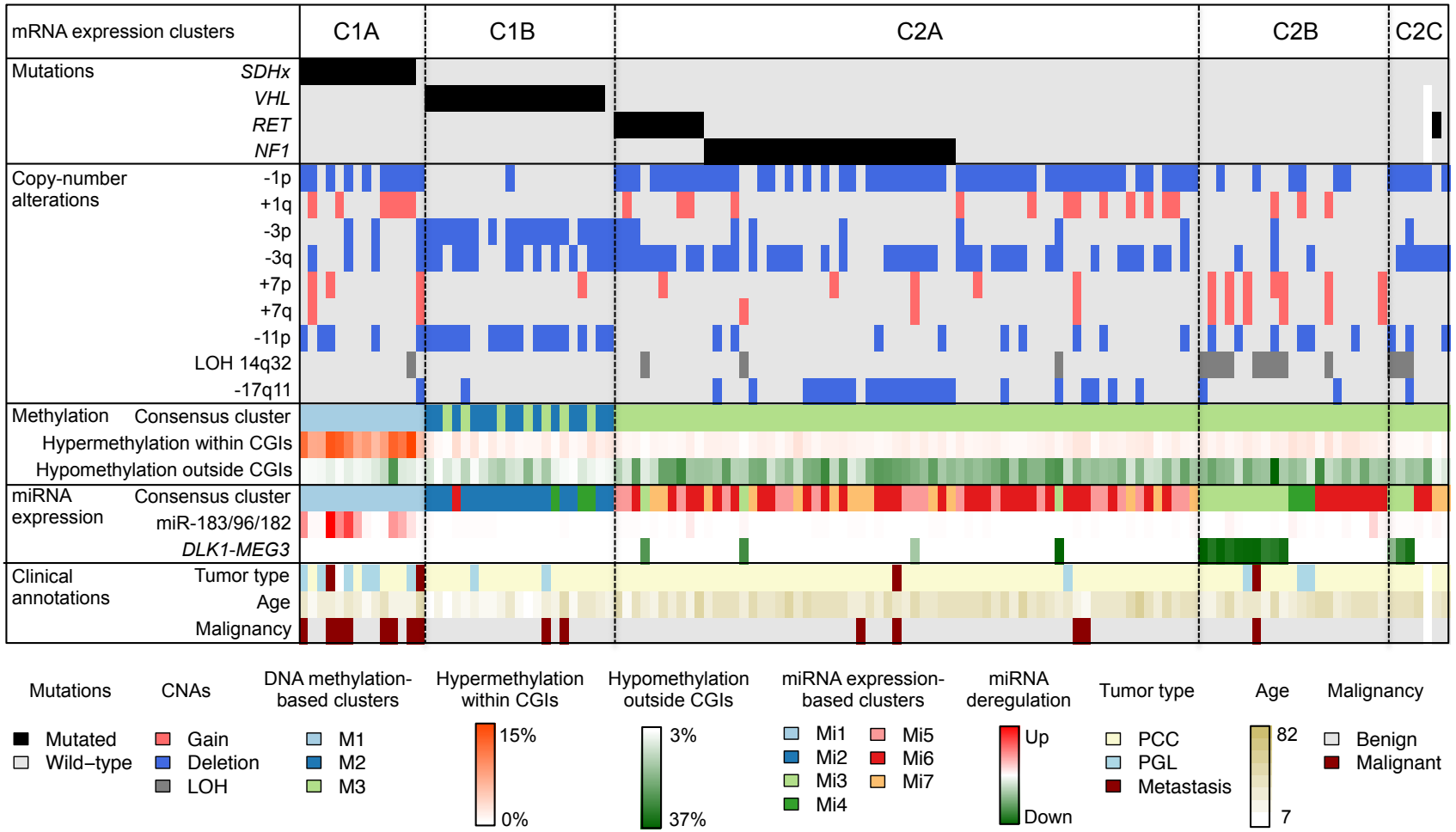
- Tumors of the Mi3 subgroup display an **LOH of chromosome arm 14q** associated with a **high methylation** of the *DLK1-MEG3* promoter:



-> In Mi1 tumors, loss of the maternal unmethylated allele leads to the inhibition of the *DLK1-MEG3* cluster.

Conclusion – Analyse génomique intégrée des paragangliomes

- Our analysis defines **5 molecular subgroups** of PGL/PCC, strongly associated with mutations in known driver genes, and displaying completely different molecular features:



Castro-Vega*, Letouzé*, Burnichon* *et al.* Nat Commun 2015

Conclusion générale

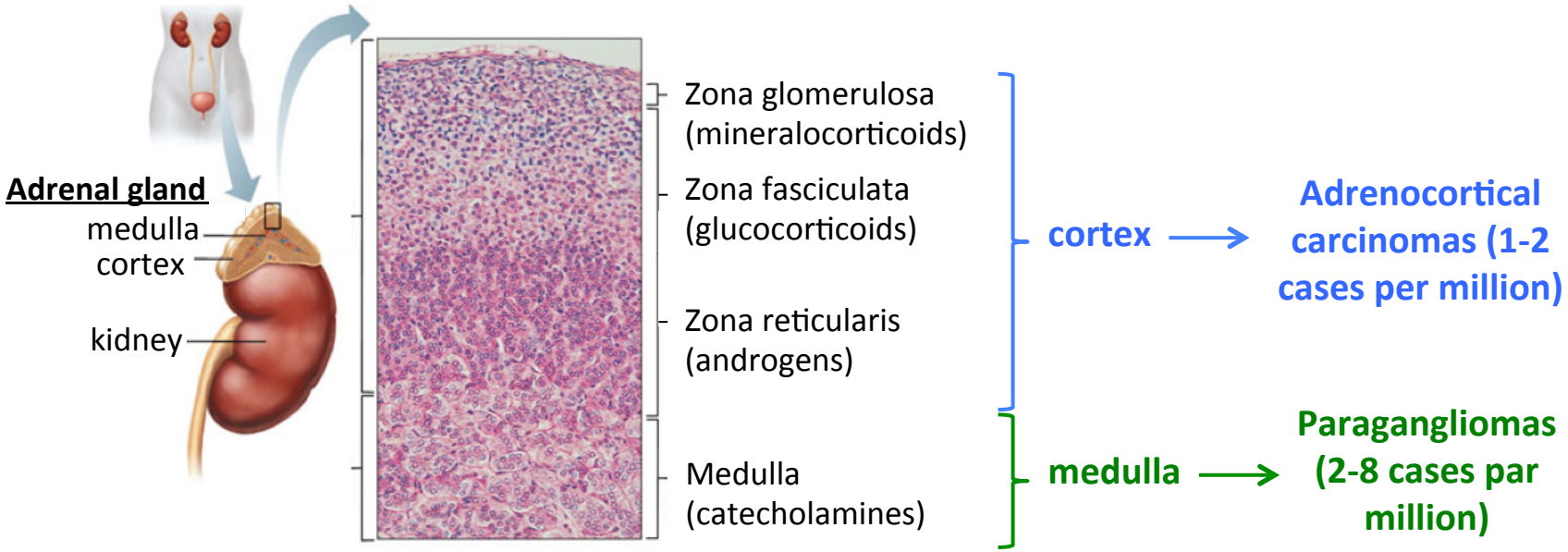
ANALYSE GÉNOMIQUE INTÉGRÉE

- Les données génomiques permettent d'étudier différents niveaux de dérégulations (épi)génétiques dans les cancers.
- Analyser de façon intégrée les différents omics est essentiel pour mieux comprendre la biologie des tumeurs.
- Les analyses multi-omics permettent de définir des sous-groupes homogènes de tumeurs et d'identifier les mécanismes oncogéniques impliqués.

APPLICATION AUX TUMEURS DE LA GLANDE SURRÉNALE

- Les données multi-omics révèlent des groupes de tumeurs très distincts d'un point de vue moléculaire et clinique.
- Dans les paragangliomes, l'inhibition des enzymes TET par le succinate relie méthylation de l'ADN et métabolisme.
- L'identification du phénotype hyperméthylateur ouvre de nouvelles perspectives thérapeutiques pour les paragnalgiomes mutés *SDHx*.

Analyse génomique intégrée des cortico-surrénalomes



Introduction: Tumeurs du cortex surrénalien

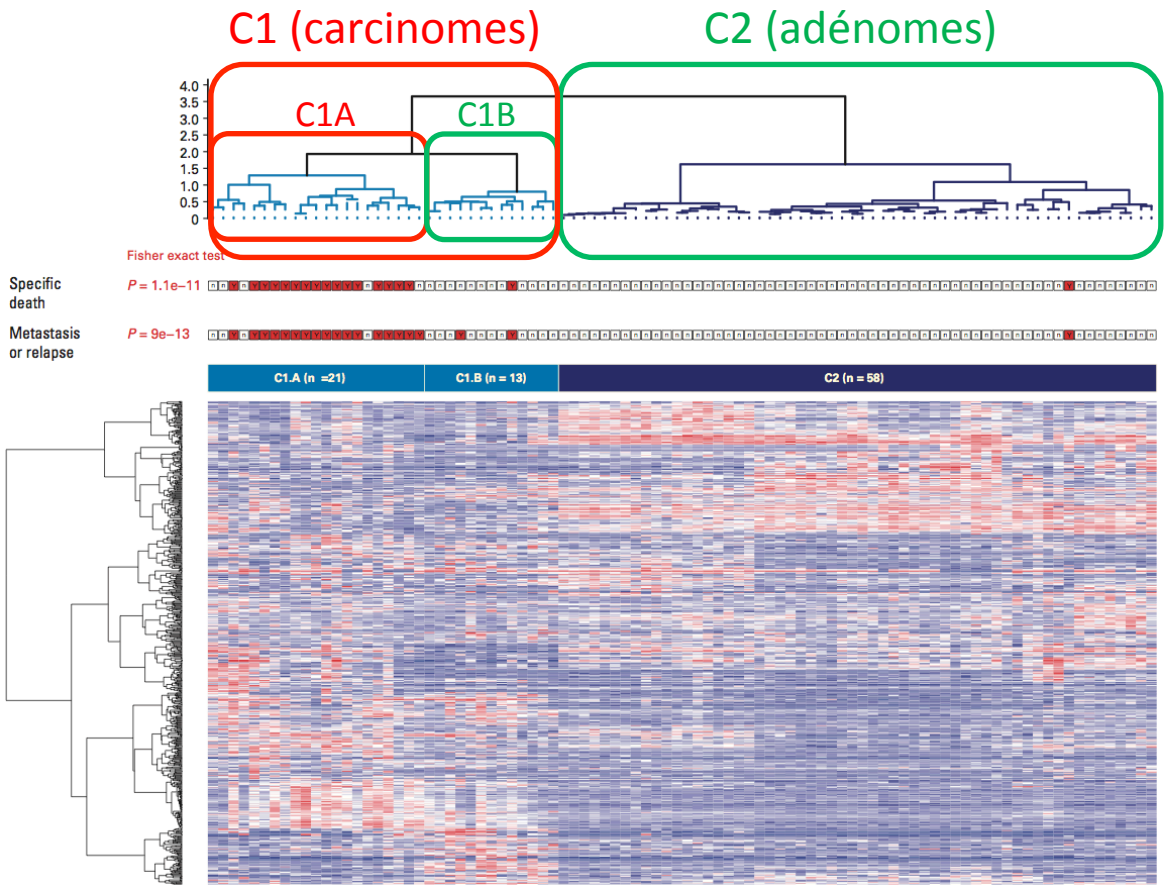
Two types of adrenocortical tumors:

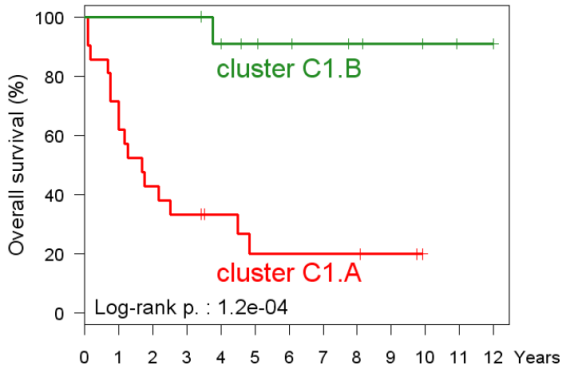
Adrenocortical tumors

Carcinomas (malignant)	Adenomas (benign)
Incidence ~2 cases/million adults/year **Age** 2 peaks: 3.5 ans et 30-50 ans **Sex ratio** 1.5 F / 1 M **5-year survival** < 30%	**Prevalence** ~3 % after 50 years Most often discovered incidentally (autopsy)

Classification des tumeurs basée sur le transcriptome

Transcriptome analysis of 92 adrenocortical tumors:





de Reyniès*, Assié* *et al.*, JCO 2009

-> L'expression des gènes permet de séparer les adénomes des carcinomes, et révèle 2 groupes de carcinomes de pronostic totalement différent (**C1A** and **C1B**).

-> Un prédicteur basé sur seulement 3 gènes permet de distinguer les tumeurs C1A, C1B et C2.

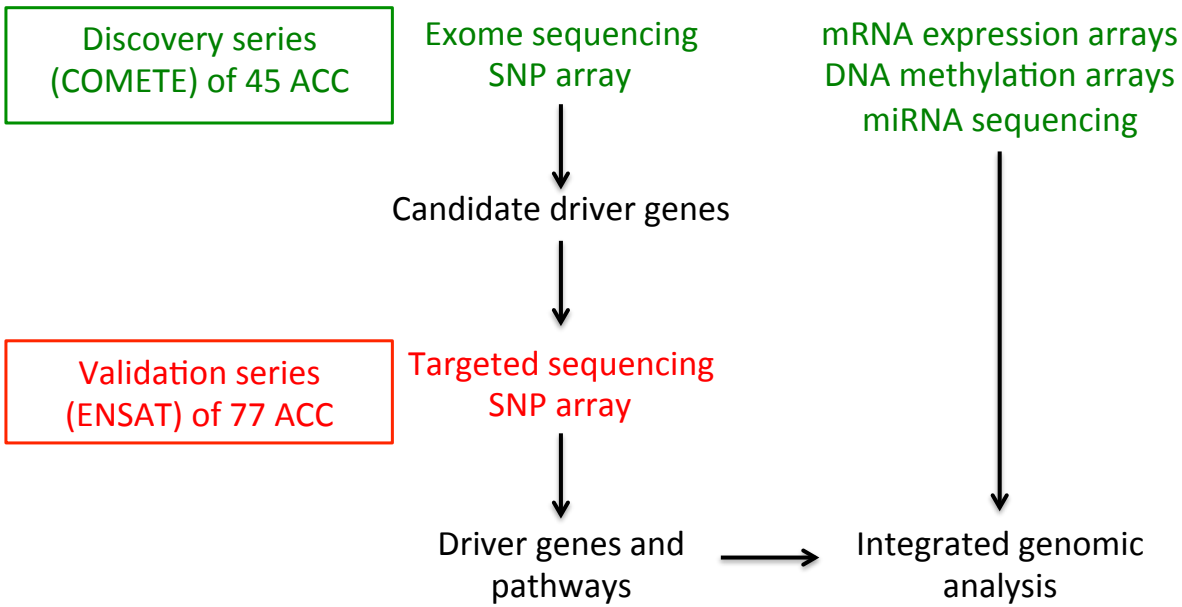
Other known molecular features

- Recurrent activating mutations of ***CTNNB1*** and inactivating mutations of ***TP53***.
- A CpG island methylator phenotype (**CIMP**) has been described, associated with a poor prognosis..
- Several studies identified deregulated **miRNAs** associated with tumor type and prognosis.

Goals of the present study:

-> PART 1: Comprehensive identification of **driver genes and pathways** by a combined analysis of mutations (exome sequencing) and focal copy number changes (SNP array).

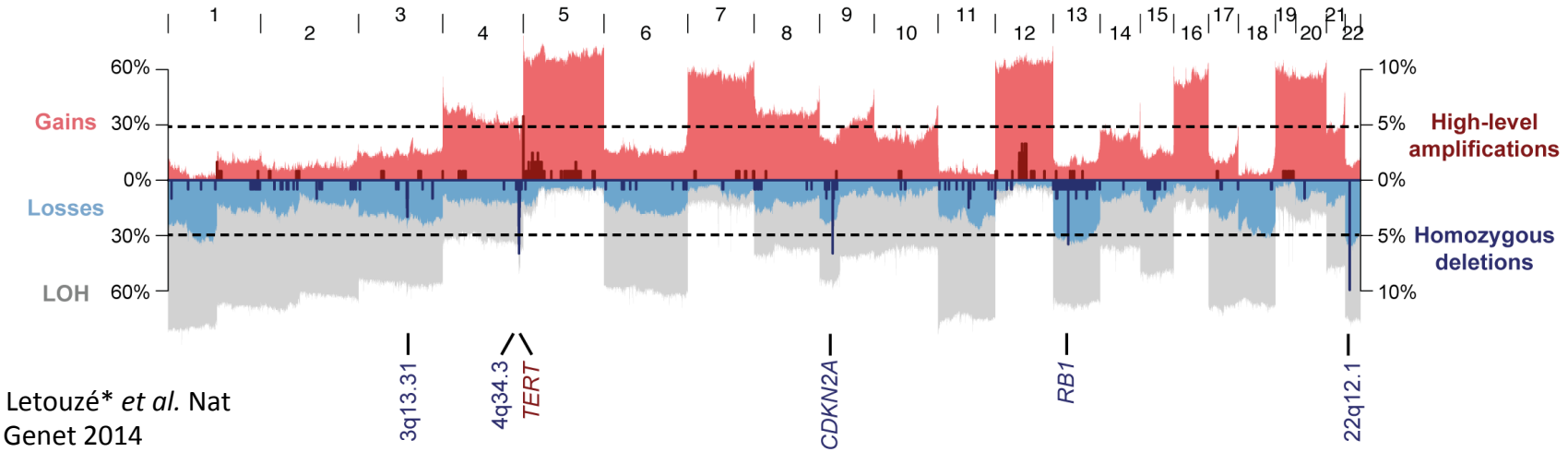
-> PART 2: Integrated multi-omics analysis of a single ACC cohort.



PARTIE 1 : Identification des gènes et pathways drivers

Analyse des aberrations chromosomiques

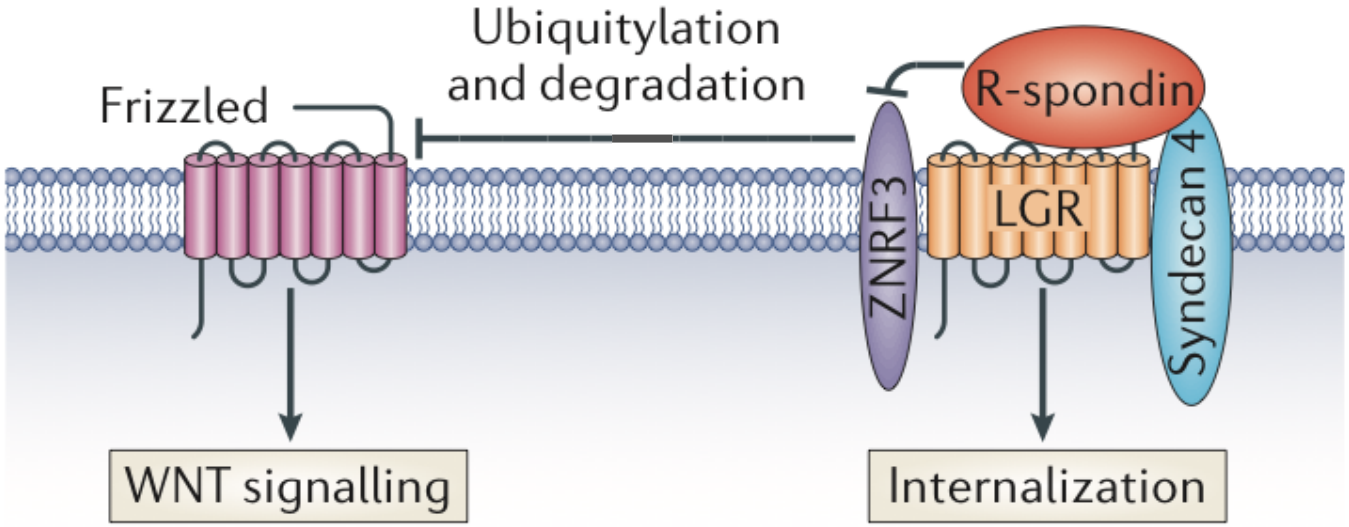
- ACC are extremely altered (mean=85% of the genome) and frequently polyploid (43%).



Assié*, Letouzé* *et al.* Nat Genet 2014

ZNRF3 est un régulateur négatif de la voie Wnt/ß-caténine

- *ZNRF3* negatively regulates the Wnt pathway by promoting the turnover of Frizzled receptors (Hao *et al.*, Nature 2012):

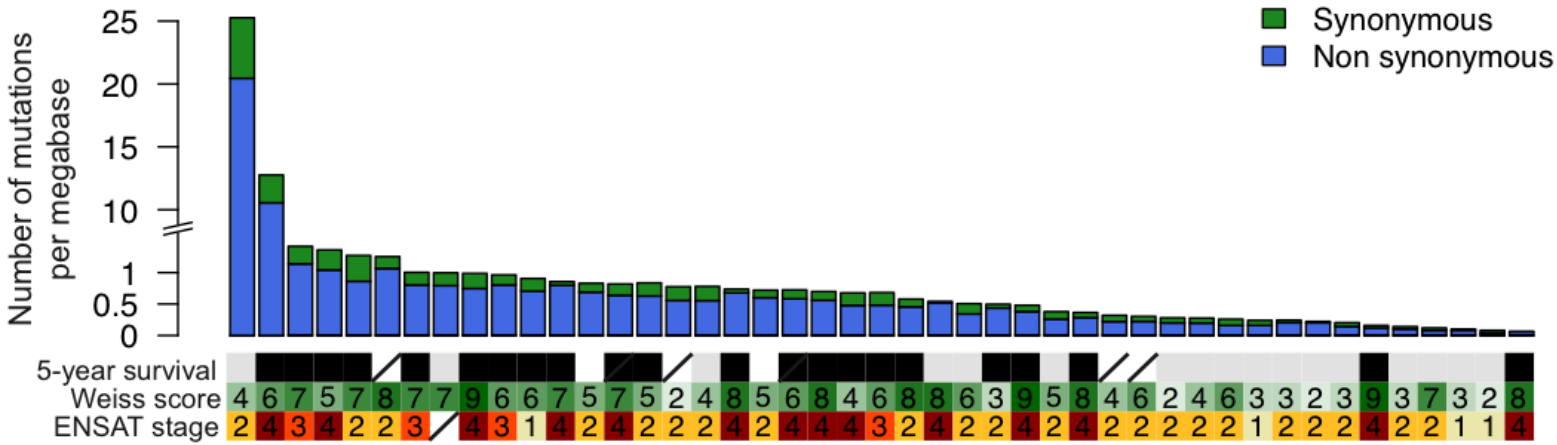


-> *ZNRF3* inactivation is another means to activate the Wnt/ß-catenin pathway in ACC, in addition to *CTNNB1* mutations.

Analyse des mutations somatiques (séquençage exome)

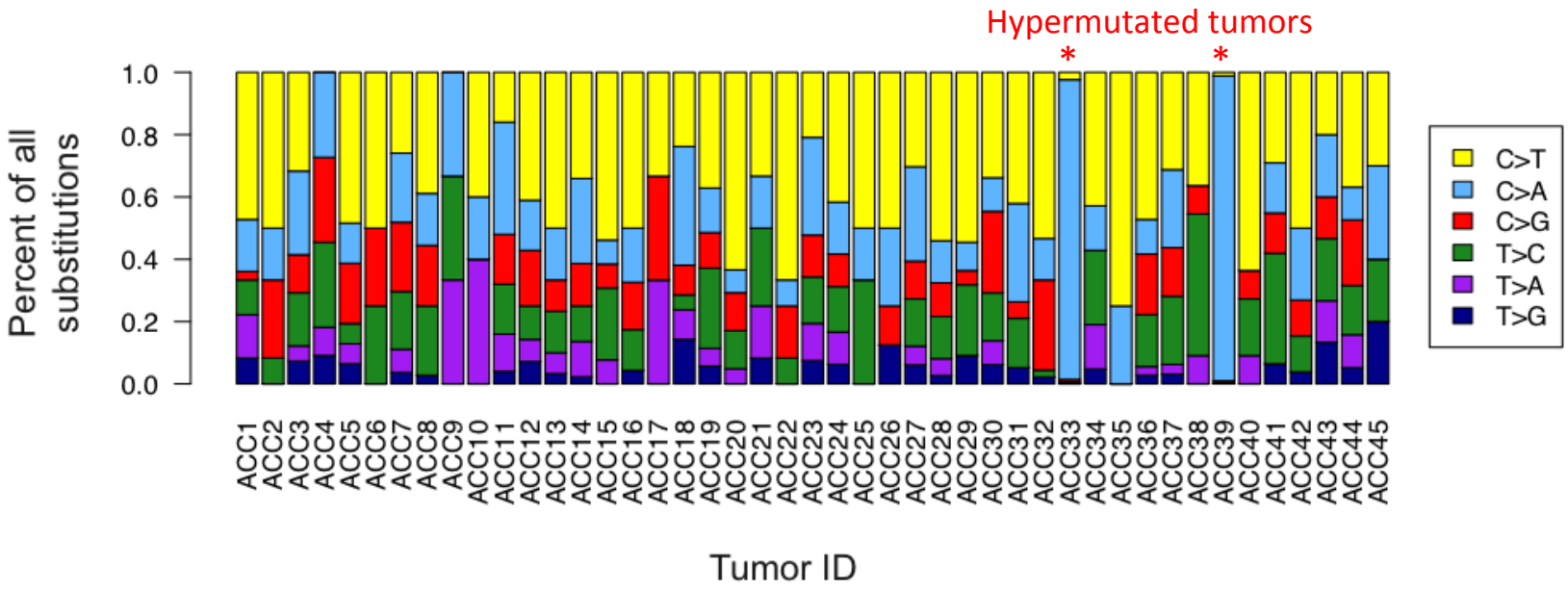
- We sequenced 70 Mb of exonic sequences (20,975 genes) to a mean depth of 90x.

- We found a median of **30 mutations per tumor** in coding sequences. The mutation rate, relatively low (0.6 mutations/Mb), is significantly associated with tumor stage and prognosis:



Spectre mutationnel des ACC

- Frequency of each type of substitution in 45 ACC:

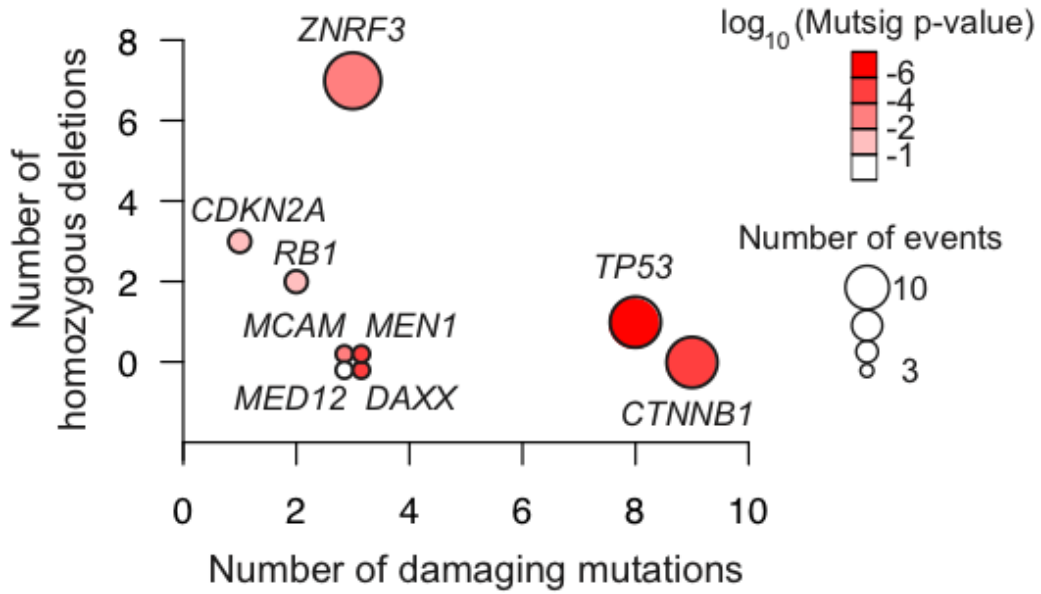


-> As in most cancers, C>T transitions are the most frequent point mutations. Hypermutated tumors have a high rate of C>A transversions, presumably due to the exposure to a mutagen that remains to be identified.

Sélection des gènes drivers candidats

- To identify candidate driver genes, we integrated **damaging mutations** (nonsense, indel, or missense mutations with functional consequences predicted by Polyphen2 software) and **homozygous deletions**.

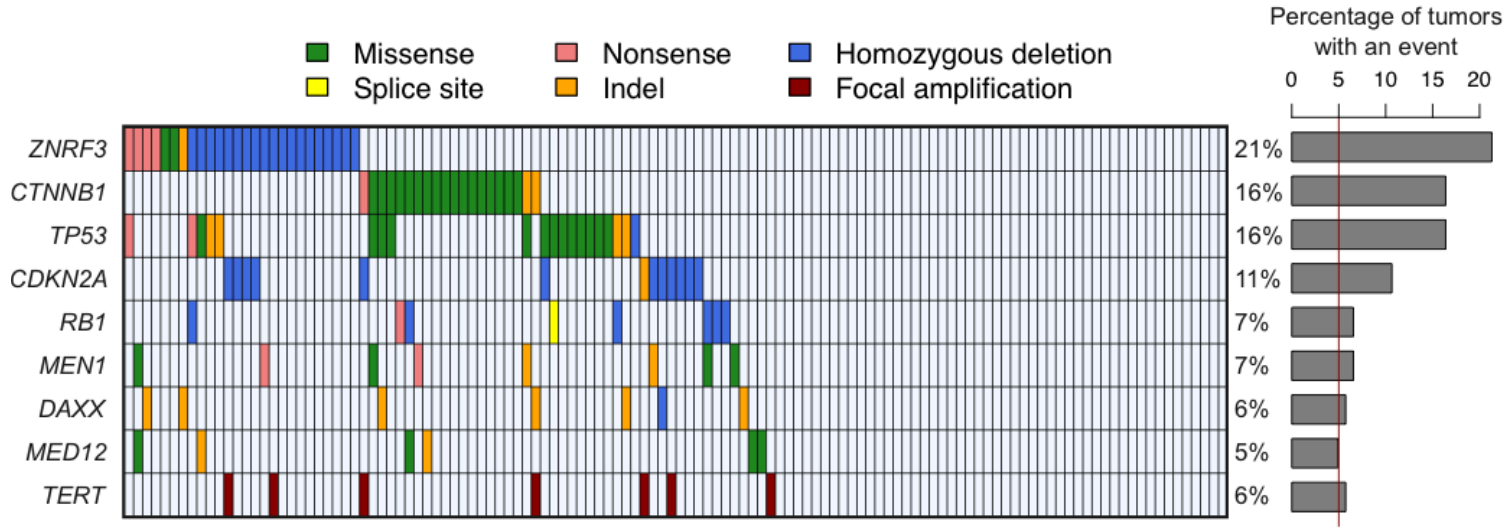
- We selected genes altered in ≥3 tumors of the discovery set (n=45):



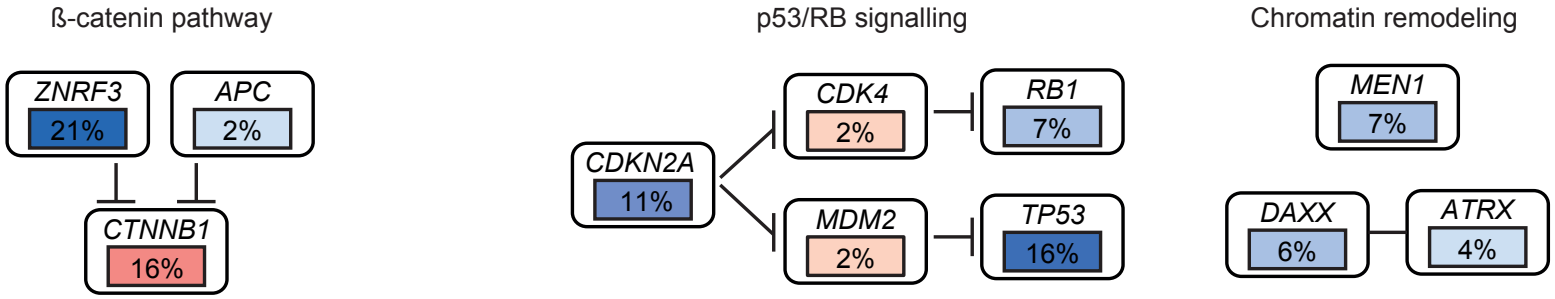
-> These genes were analyzed by targed sequencing in the validation series of 77 ACC.

Gènes et voies cellulaires mutées dans la série complète de 122 ACCs

- In the entire cohort, 9 genes are altered by mutation, homozygous deletion or high-level amplification in ≥5% of ACC :



- These driver genes belong to three main pathways:



47 ACC (39%) with at least one alteration

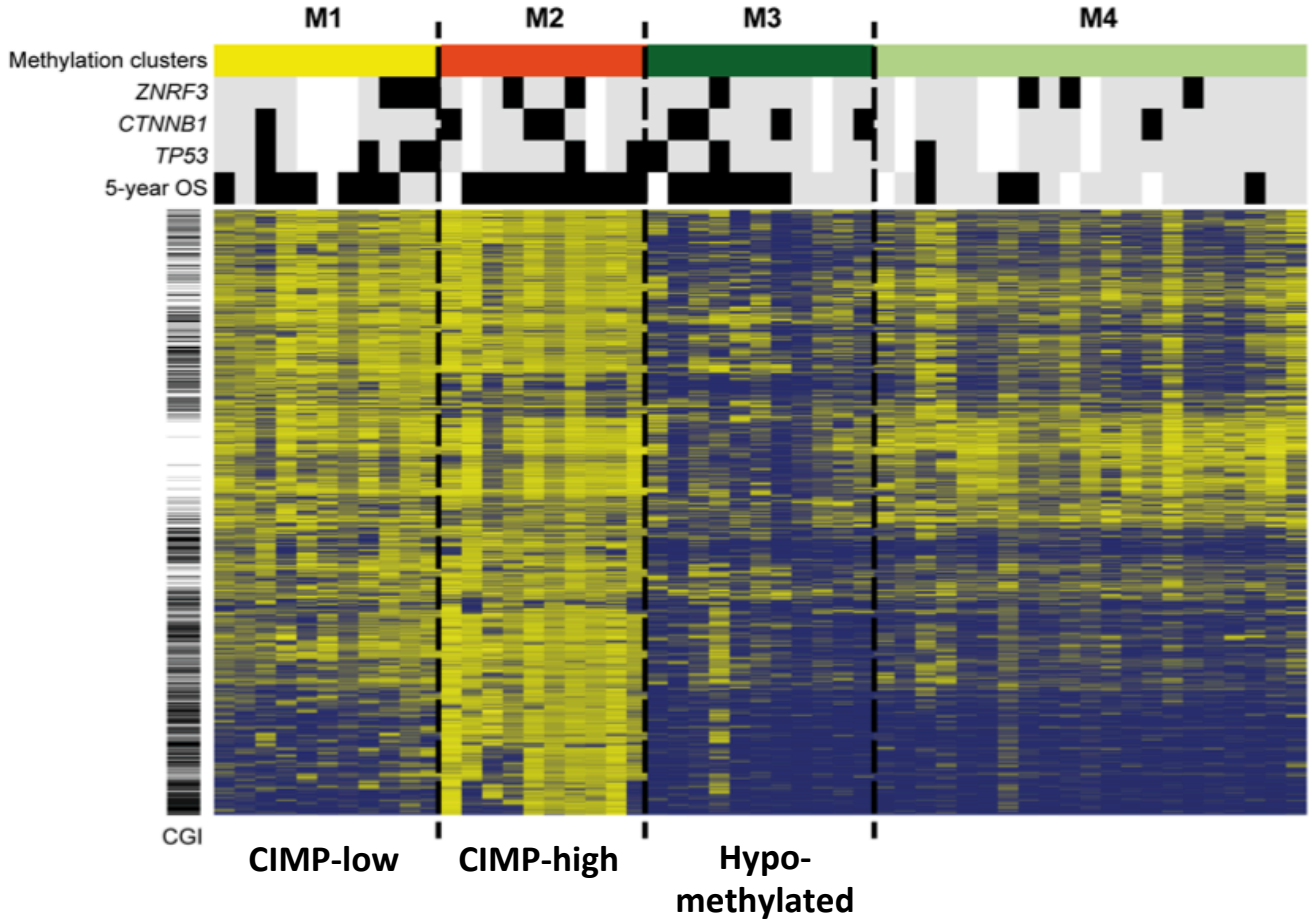
40 ACC (33%) with at least one alteration

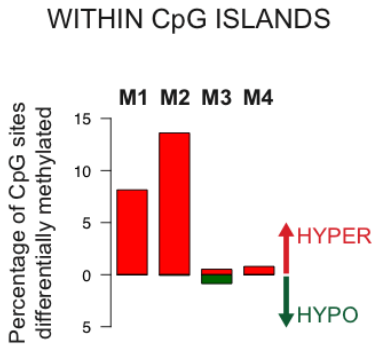
+13 genes mutated in a single tumor

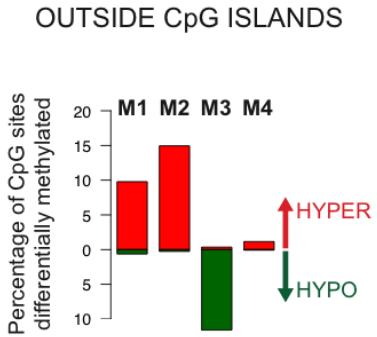
PARTIE 2 : Analyse multi-omics intégrée des ACC

Analyse de la méthylation de l'ADN

- Tumors of the discovery set were analyzed with Illlumina Meth27 arrays (27,000 probes).
- We identified 4 homogeneous DNA methylation-based ACC subgroups using the RPMM method (Houseman *et al.*, BMC Bioinformatics 2008).



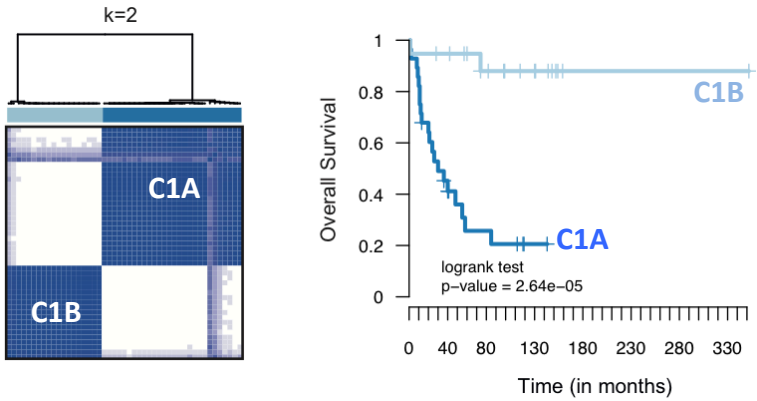




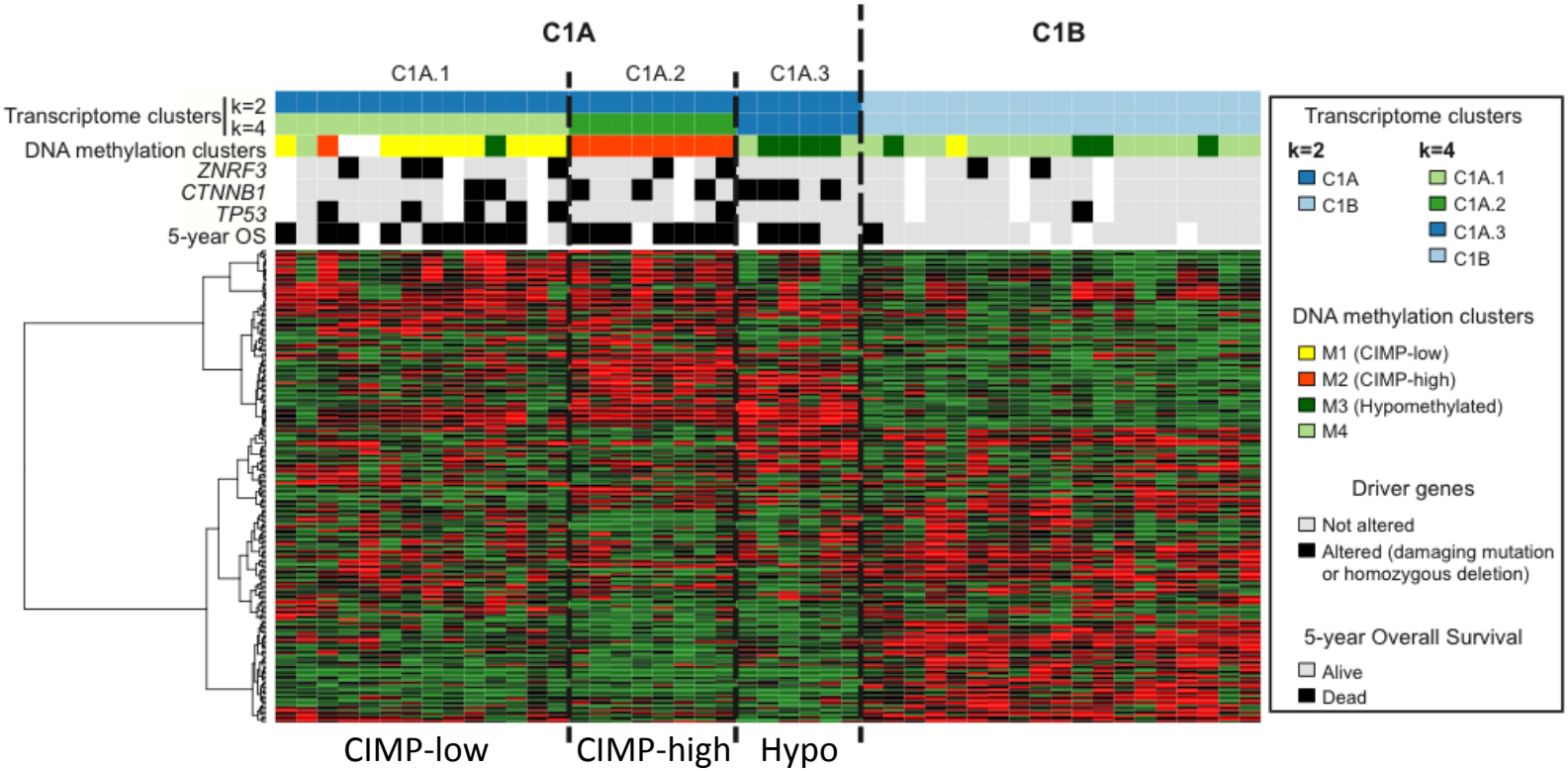
-> We identified the two previously described CIMP clusters, and a new ACC subgroup strongly hypomethylated outside CpG islands.

Analyse de l'expression des gènes (ARNm)

- A consensus clustering confirms the existence of 2 main clusters (C1A and C1B):

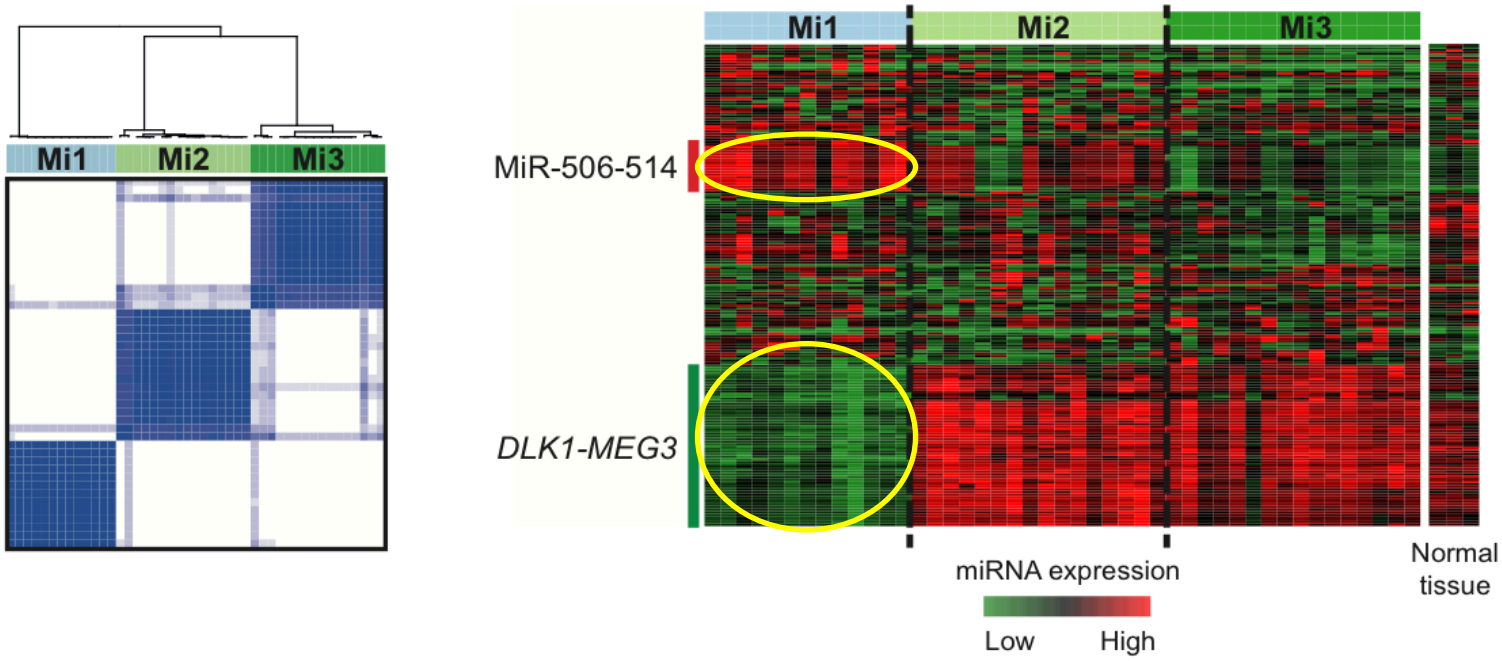


- The C1A group can be divided in 3 subgroups, strongly associated with DNA methylation clusters:



Analyse de l'expression des microARN

- A **consensus clustering** identifies 3 homogeneous ACC subgroups based on miRNA expression:

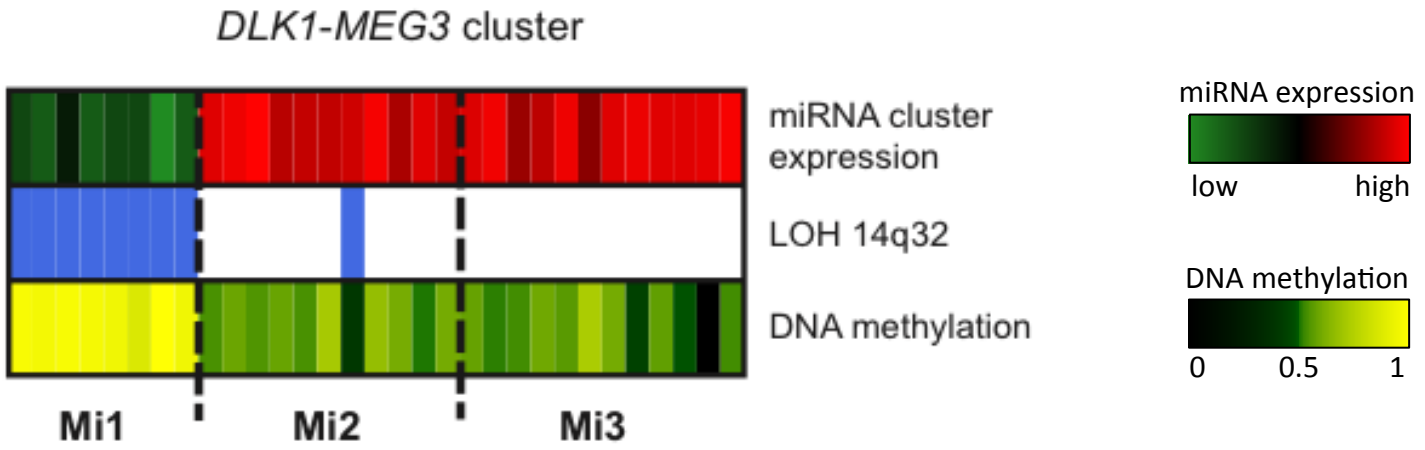


-> Mi1 tumors display the strongest miRNA expression changes with the deregulation of 2 miRNA clusters.

Mécanisme expliquant l'extinction du cluster *DLK1-MEG3*

- The *DLK1-MEG3* cluster is **imprinted**: the maternal allele is expressed, whereas the paternal allele is methylated.

- All tumors of the Mi1 tumors have an **LOH of chromosome arm 14q** vs only 1/23 non-Mi1 tumors (*P*=1.1e-6), and display a **hypermethylation** of the *DLK1-MEG3* promoter:



-> In Mi1 tumors, loss of the maternal unmethylated allele leads to the inhibition of the *DLK1-MEG3* cluster.

Conclusions

- Integrated exome/SNP analysis reveals **9 major driver genes** (*ZNRF3*, *CTNNB1, TP53, CDKN2A*, *RB1*, *MEN1*, *DAXX*, *MED12* and *TERT*), involved in **3 major pathways** (ß-catenin, p53/Rb, and chormatin remodeling).

- Our **DNA methylation** analysis confirms the existence of CIMP-high and CIMP-low phenotypes, and reveals a new hypomethylated subgroup.

- **mRNA expression** analysis confirms the existence of 2 main clusters (C1A and C1B). The C1A cluster can be subdivided in 3 subgroups strongly associated with CIMP-high, CIMP-low and hypomethylated tumors.

- **miRNA expression** reveals a strong deregulation of miR-506-514 (oncogenic) and *DLK1-MEG3* (tumor suppressive) in a subgroup of ACC.

- ***DLK1-MEG3*** silencing results from the loss of the maternal allele by LOH of chromosome arm 14q.

Intégration multi-omics

- Integrative genomic analysis reveals the existence of **two distinct molecular and clinical entities** of ACC, driven by specific oncogenic alterations:
  - **C1A** tumors of bad prognosis, heavily mutated, often CIMP
  - **C1B** tumors of good prognosis, with a deregulation of miR-506-514 and *DLK1-MEG3* clusters.