Genomic Instability and Epigenomic Dysregulation as Hallmarks of mCRC Therapy Resistance

Findings from Broad ctDNA Molecular Profiling (METACC study)

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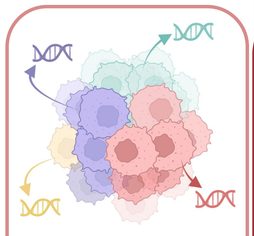
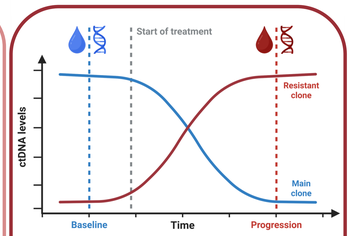
1. Background

Metastatic colorectal cancer (mCRC) —particularly **microsatellite-stable (MSS)** mCRC— remains a major clinical challenge, with high incidence, limited therapeutic options, and poor long-term survival. Resistance to standard treatments is common and driven by complex, evolving tumor biology.

Circulating tumor DNA (ctDNA) has emerged as a reliable, minimally invasive tool for detecting key resistance mutations (e.g., *RAS*, *BRAF*), showing high concordance with tissue-based testing. But ctDNA has greater potential to explore therapy resistance mechanisms (both genomic and epigenomic).



ctDNA better captures intratumoral heterogeneity

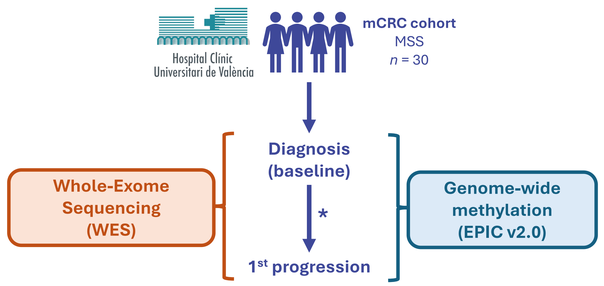


ctDNA allows longitudinal sampling along tumor progression

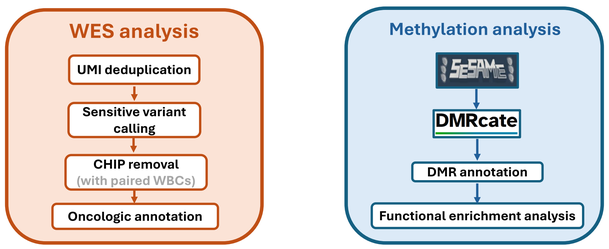
The METACC study aims to leverage ctDNA to understand genomic and epigenomic therapy resistance in mCRC

2. Methodology

We analyzed a retrospective cohort of **30 MSS mCRC patients**, with plasma samples collected at diagnosis and first progression. We compared the **genomic** and **methylomic** landscape between both conditions.



*** 1st line of treatment:** Chemotherapy ± Anti-VEGF or Anti-EGFR



3.1. Results: Whole-Exome Sequencing

***ARID1A* mutations** were the most widespread acquired event (Figure 1), which shortened time to progression (Figure 2). Variant allele frequency (VAF) gains in *ARID1A* correlated with increases in the tumor mutational burden (TMB) (Figure 3), in line with the gene's role in maintaining genomic integrity. **ARID1A loss has implications for immunotherapy.**

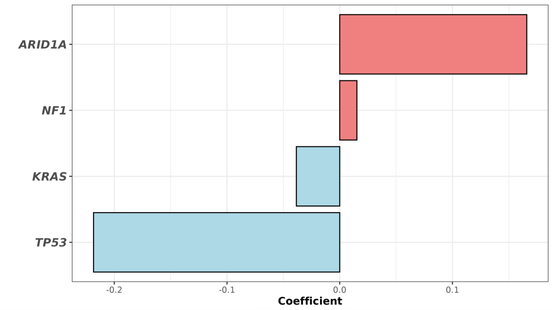


Figure 1. Top gene coefficients from lasso-regularized logistic regression. **Positive:** VAF related to progression. **Negative:** VAF related to baseline.

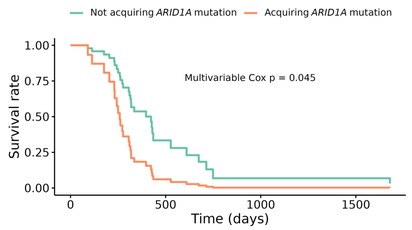


Figure 2. Multivariable-adjusted Kaplan-Meier curves for patients that acquire and do not acquire *ARID1A* mutations.

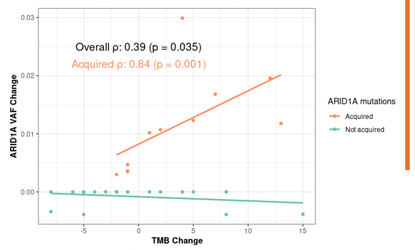


Figure 3. *ARID1A* VAF changes correlate with TMB changes (progression vs. baseline)

Patients also gained mutations in genes related to **therapy resistance** and **epigenomic regulation** (Figure 4).

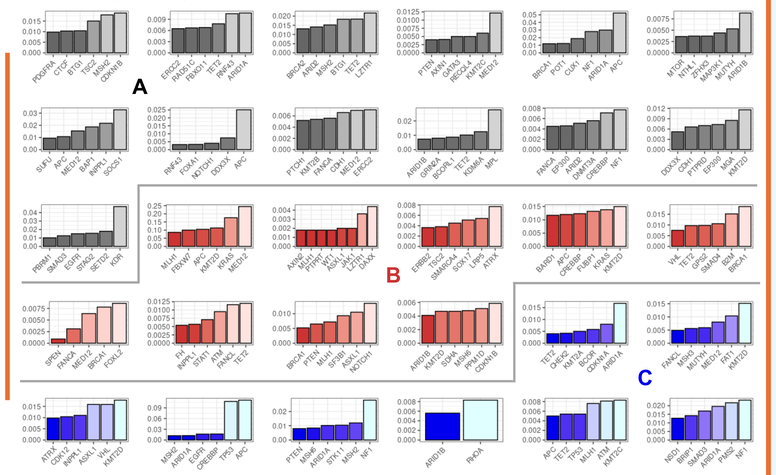


Figure 4. Top VAF increases associated with progression in each patient. **A)** Patients receiving chemotherapy; **B)** Chemotherapy + Anti-VEGF; **C)** Chemotherapy + Anti-EGFR

3.2. Results: Methylation assessment (EPIC v2.0)

Differential methylation analysis identified 16,026 differentially methylated regions (DMRs) (**198 with |Δβ| > 0.2**) (Figure 5), linked to different genomic regions (Figure 6, Figure 7), including the **hypermethylation of an *ARID1A* enhancer**.

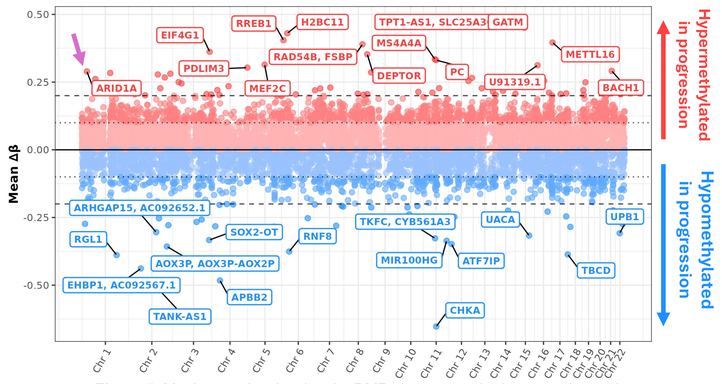


Figure 5. Manhattan plot showing the DMRs between paired progression and baseline samples. Highlighted are regions with |Δβ| > 0.2.

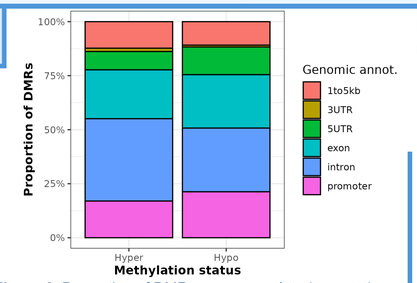


Figure 6. Proportion of DMRs per gene-related annotation.

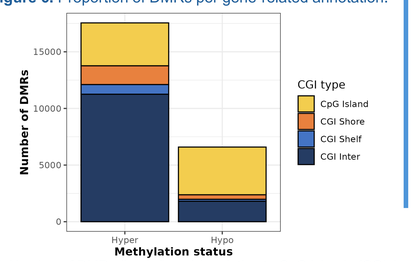


Figure 7. Number of DMRs (hyper/hypo) in relation to CpG islands (CGIs).

Enrichment analysis linked DMRs to: **1)** tumor metabolism and microenvironment, **2)** therapy resistance, and **3)** cell longevity (Figure 8).

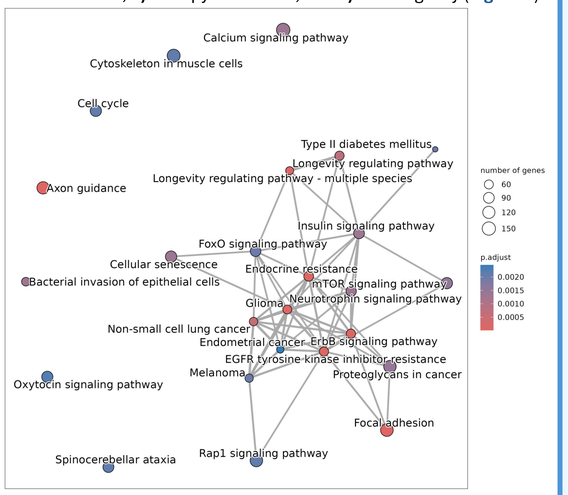


Figure 8. KEGG pathways resulting from DMR-associated gene set analysis.

4. Conclusions

ctDNA shows great promise as a tool to uncover therapy resistance mechanisms in mCRC

WES

- ***ARID1A* mutations** are globally related to **progression**.
- At the individual level, progression occurs with mutations in genes related to **therapy resistance** and **epigenetic regulation**.

Methylation

- There is **great variation** in methylation markers that is acquired with progression and may **dynamically favor resistance to therapy**.
- ***ARID1A*** may also be silenced via a **hypermethylation-dependent mechanism**.

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