Signal-Adjusted ctDNA Methylation Profiling Captures Epigenetic Dynamics of Treatment Resistance in Metastatic Colorectal Cancer

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Background

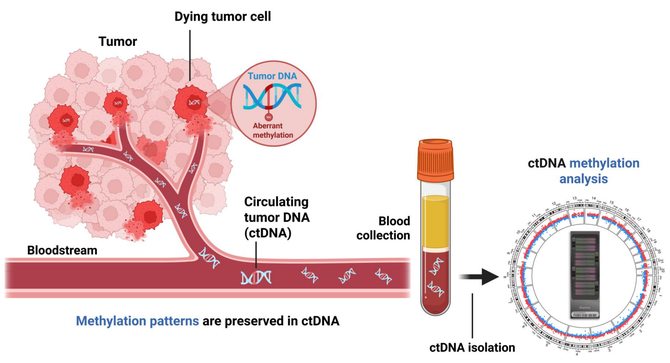
A clinical problem

Nearly all patients with **metastatic colorectal cancer (mCRC)** eventually develop **resistance to systemic therapies**, critically limiting long-term survival. This process is driven by tumor plasticity, which enables malignant cells to adapt under therapeutic pressure.

A biological solution

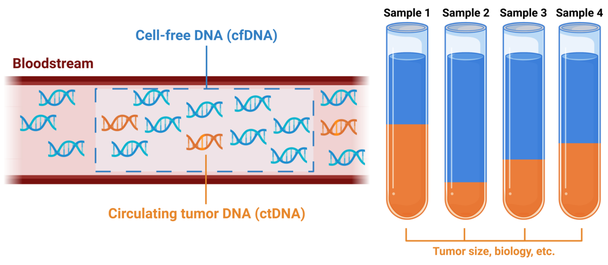
Emerging evidence highlights **dynamic epigenetic reprogramming**—particularly alterations in DNA methylation—as a key mechanism underlying these adaptive responses, yet its contribution in the clinical setting remains poorly understood.

Liquid biopsy has transformed cancer monitoring by enabling **non-invasive, longitudinal sampling** of cell-free DNA (cfDNA). Beyond genetic alterations, cfDNA captures the methylation patterns of the tumor and its analysis would provide a unique opportunity to dynamically assess the methylomic landscape of mCRC and its changes with the course of the disease.



A technical challenge

A fundamental challenge, however, is that only a variable fraction of cfDNA originates from tumor cells (ctDNA), while the remainder derives from normal tissues. The exact proportion of ctDNA within cfDNA varies across samples (e.g., baseline vs. progression), can obscure tumor-specific methylation signals, confound biomarker discovery and complicate interpretation across serial samples.

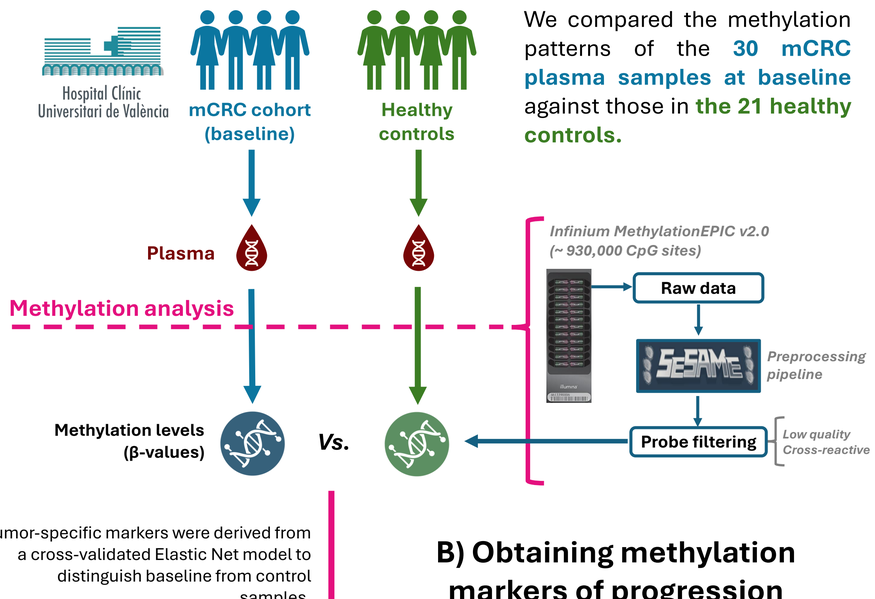


Understanding and accounting for these ctDNA fraction dynamics is therefore essential to reliably capture the epigenetic trajectories of mCRC and to develop clinically meaningful biomarkers of resistance.

Methods

We recruited a cohort of 30 mCRC patients and 21 healthy controls. For the patients, plasma samples were taken at both baseline (diagnosis) and progression to 1st line of therapy. Methylation in plasma samples was assessed with Illumina Infinium MethylationEPIC v2.0 BeadChips following a custom plasma-adapted workflow.

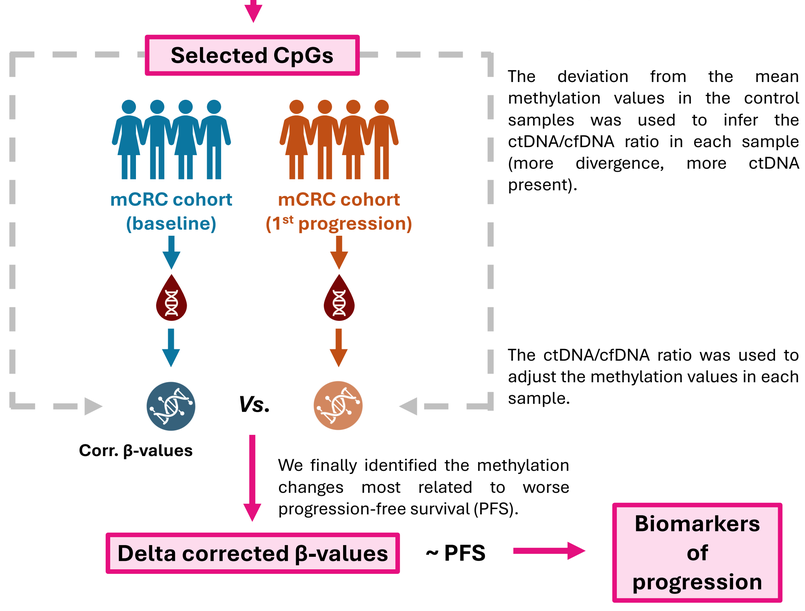
A) Obtaining a mCRC-specific methylation signature



We compared the methylation patterns of the **30 mCRC plasma samples at baseline** against those in **the 21 healthy controls.**

Tumor-specific markers were derived from a cross-validated Elastic Net model to distinguish baseline from control samples.

B) Obtaining methylation markers of progression



The deviation from the mean methylation values in the control samples was used to infer the ctDNA/cfDNA ratio in each sample (more divergence, more ctDNA present).

The ctDNA/cfDNA ratio was used to adjust the methylation values in each sample.

We finally identified the methylation changes most related to worse progression-free survival (PFS).

Delta corrected β-values ~ PFS → **Biomarkers of progression**

Results

Methylation patterns specific to colon and liver tissue (the main site of metastasis in mCRC patients) can be found within the plasma samples of the patients via methylation-based deconvolution, less so in the healthy controls (**Figure 1A** and **Figure 1B**). The higher signal would indicate cell death processes in these organs, as expected.

This signal is insufficient to completely distinguish and separate the ctDNA-specific signal in the mCRC plasma samples but suggests the plausibility of identifying tumor biomarkers with this methodology.

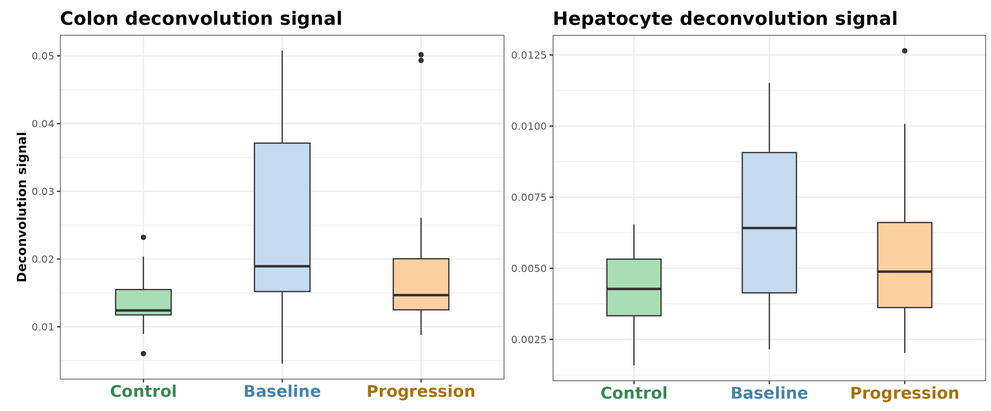


Figure 1. Tissue signal deconvoluted from total plasma cfDNA for A) colon tissue and B) liver tissue.

To identify an mCRC-specific methylation signal we put in place a penalized regression model that allowed us to identify a signature of 40 CpGs (hereinafter **mCRC-40**) capable of predicting whether a plasma sample comes from mCRC or not, clearly separating them (**Figure 2**). These CpGs mapped to potentially regulatory sites associated to genes relevant to mCRC, such as *KDM2B*, *TMEFF2*, and *CHL1*.

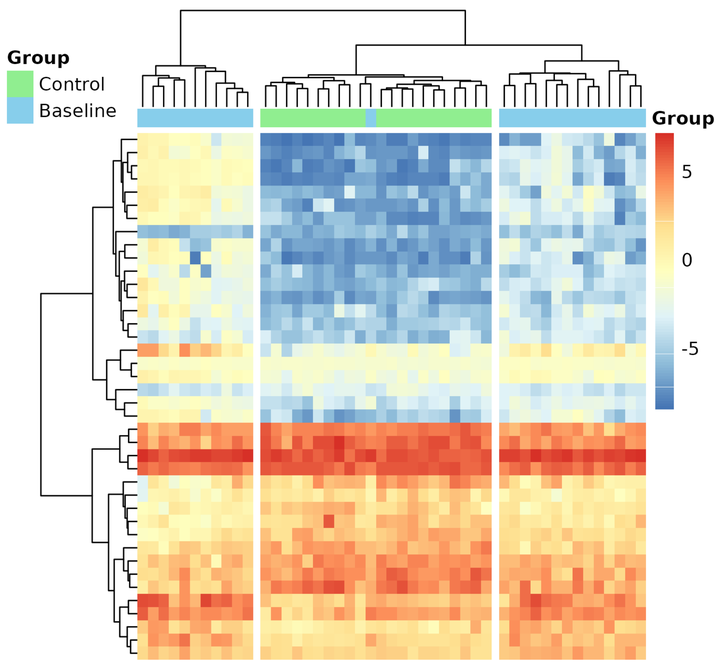


Figure 2. Heatmap of methylation values for the probes in the mCRC-40 signature, separating control from baseline mCRC samples.

For each sample, the mean of the β-values in the 40 CpGs was computed to obtain the mCRC-40. This score remained stable in controls (median = 0.43; IQR: 0.42–0.44) but was significantly higher in both baseline (median = 0.49; IQR: 0.46–0.56) and progression samples (median = 0.45; IQR: 0.43–0.49) compared with controls ($p < 0.001$), indicating this is a hypermethylation-driven signature (**Figure 3**).

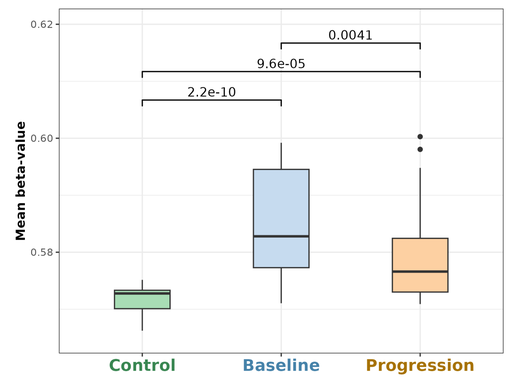


Figure 3. Distribution of the mCRC-40 across groups.

The deviation from the normal values of mCRC-40 correlated well with the signal obtained from colon + liver via deconvolution (**Figure 4**), further suggesting the signature is meaningful.

After adjusting the methylation values in all the samples by their deviation from the normal mCRC-40, the patterns seen in baseline and progression samples differed more from those in the healthy controls, when initially all three groups had them convoluted (**Figure 5**).

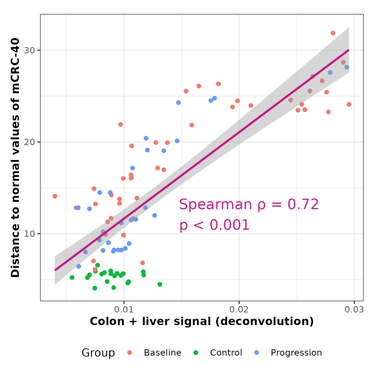


Figure 4. Correlation between the distance to a normal mCRC-40 and colon + liver tissue signal.

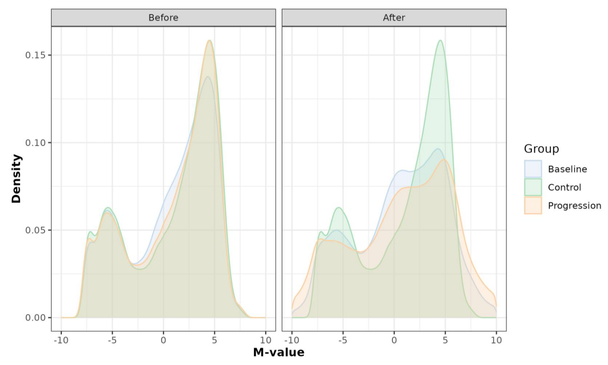


Figure 5. Distribution of the methylation values in each group of the study before and after adjusting for mCRC-40.

After normalizing for mCRC-40, 50 CpGs showed progression-associated changes linked to proliferation and therapy resistance (*CYP46A1, TM9SF1, MPO, DNAJB1*), and cell stemness (*SMURF1, ALDH1A3*) (main genes in **Figure 6**). A score based on these CpGs stratified patients into high- and low-risk groups (HR = 10.85, 95% CI: 3.68–32.05; $p < 0.001$) (**Figure 7**).

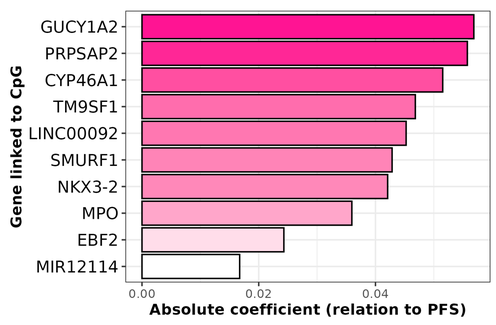


Figure 6. Coefficients (contributions) to the model of the top 10 genes whose methylation changes in the associated CpGs led to lower PFS.

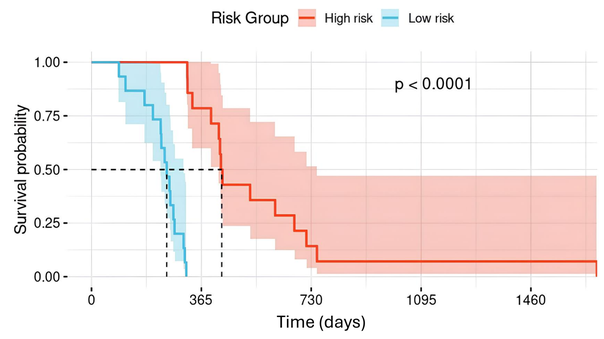


Figure 7. Comparison of the PFS between the high-risk and low-risk groups defined by the 50-CpG signature.

Conclusions

Plasma ctDNA methylation profiling distinguishes mCRC patients from controls and reveals a hypermethylation-driven signature (40 CpGs).

A set of 50 progression-associated CpGs most relate to patient progression-free survival, highlighting genes linked to proliferation, therapy resistance, and stemness.

A CpG-derived score stratifies patients into prognostic groups, underscoring the clinical potential of methylation biomarkers.

Future validation of this approximation in an external cohort will shed light on its clinical relevance.

DOI: The presenting author (D.G. Camblor) reports no conflicts of interest.

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