Ultra-sensitive Monitoring of Minimal Residual Disease (MRD) for Cancer Patients, using superRCA Mutation Assays with Flow Cytometry Readout

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INTRODUCTION

- Colorectal cancer is a leading cause of cancer death globally.
- Recurrence monitoring is crucial for timely intervention.
- Liquid biopsy using circulating tumor DNA in blood plasma offers a non-invasive monitoring tool.

OBJECTIVE

To demonstrate the efficacy of multiplex superRCA assays for detecting recurrence of colorectal cancer by analyzing circulating tumor DNA for hotspot mutations in blood plasma.

METHOD

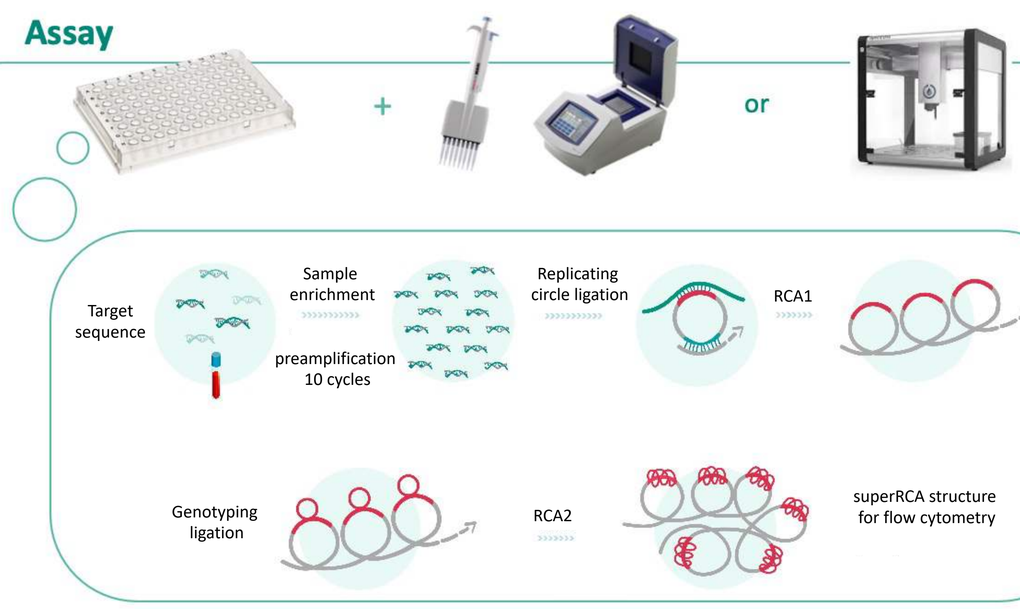
Tumor Tissue and Plasma Samples

- Samples were sourced from the Uppsala-Umeå Comprehensive Cancer Consortium (U-CAN), including 74 plasma samples from 25 colorectal cancer (CRC) patients.
- Mutations in BRAF, KRAS, NRAS, and PIK3CA were identified from medical records.
- Control genomic DNA was extracted from both normal tissue and primary tumor tissue post-surgery.

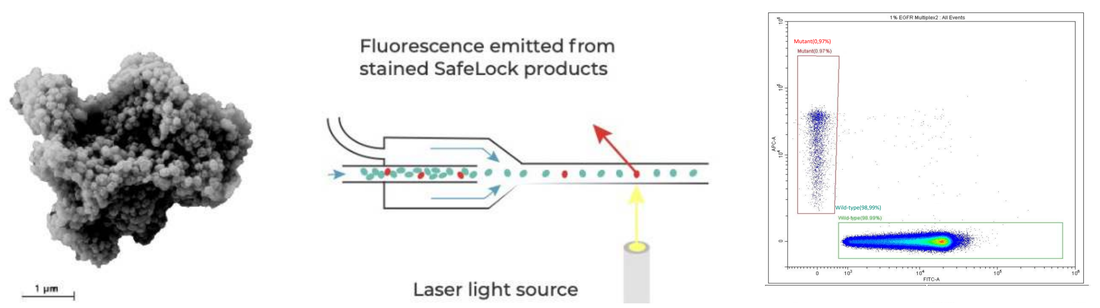
DNA Preparation

- Genomic DNA (gDNA) from tumor and normal tissue was prepared using extraction kits.
- Cell-free DNA was purified from pooled aliquots of plasma using the QIAamp Circulating Nucleic Acid Kit, and quantified.

Assay



Readout

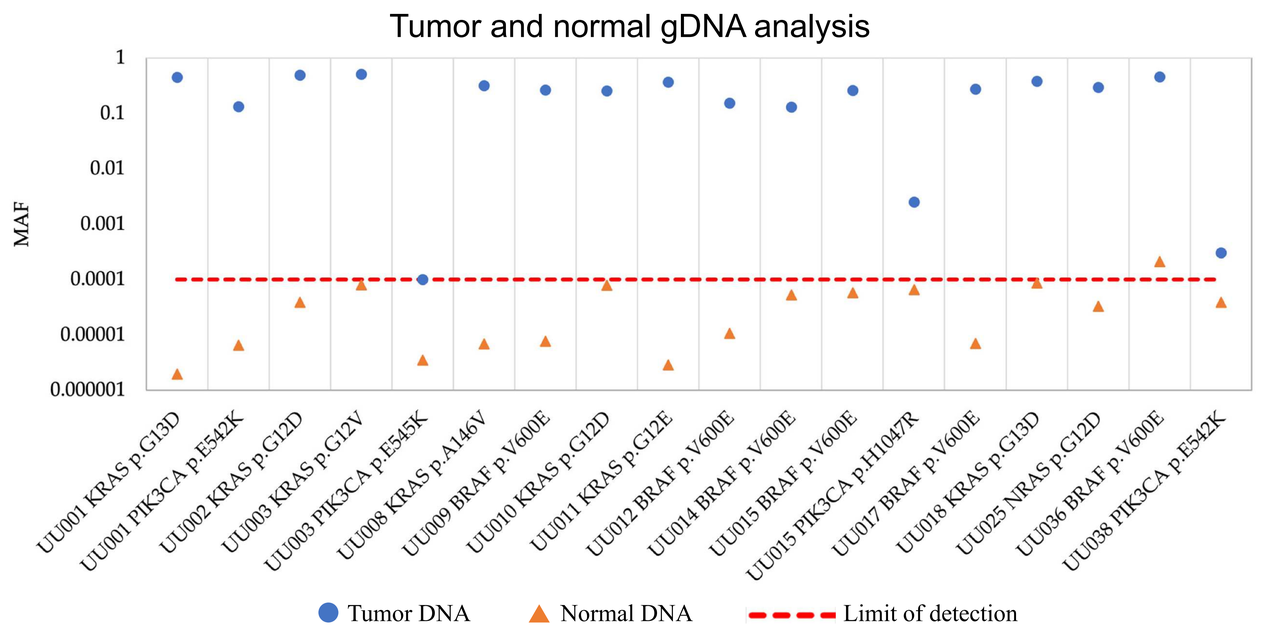


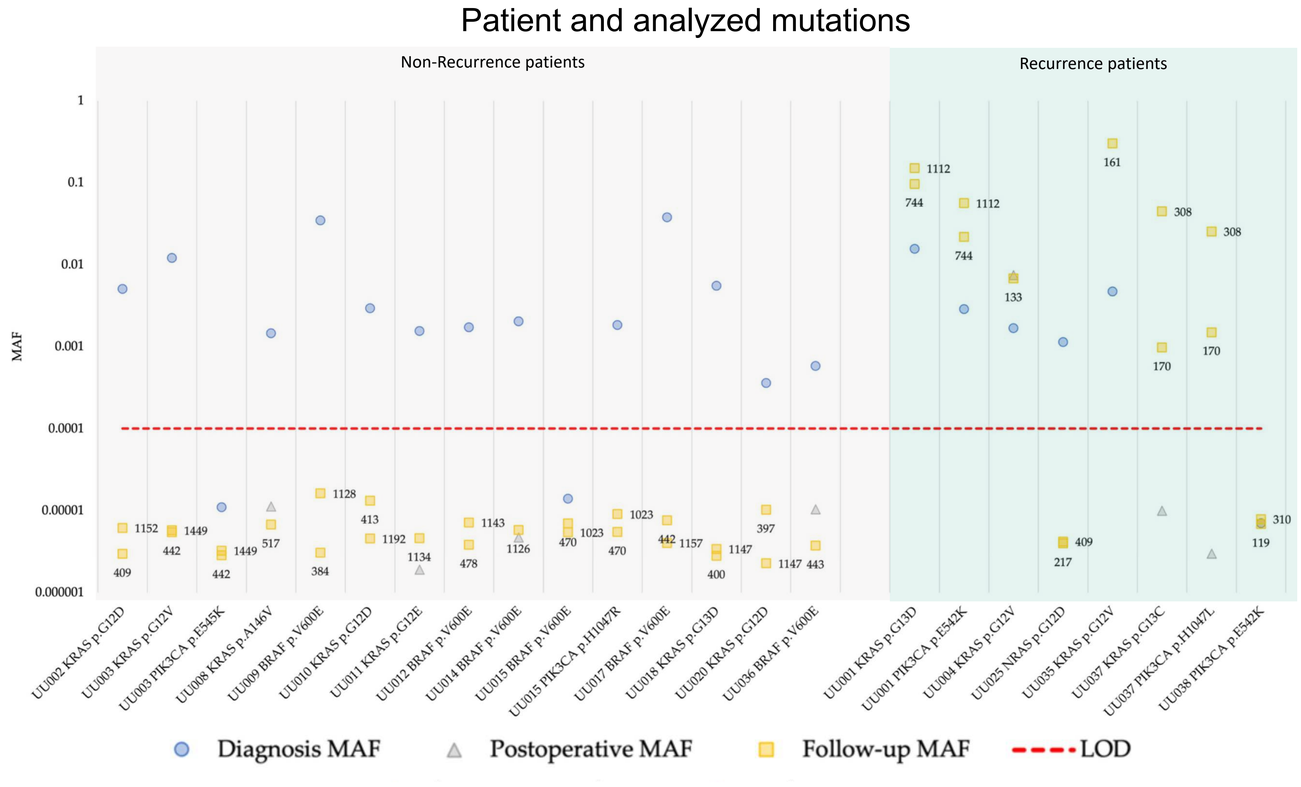
The superRCA assay starts with purified DNA that undergoes ten cycles PCR amplification covering sequences of interest. Then ligation templates are introduced to convert amplified DNA strands into DNA circles. These DNA circles are then subjected to rolling circle amplification (RCA). Next, the RCA products, each containing hundreds of copies of a sequence of interest, are genotyped using padlock probes specific for mutant or wildtype sequences. Padlock probes that have recognized their targets and formed DNA circles are then subjected to a second RCA, generating, for each starting DNA circle, large DNA structures called superRCA particles. These particles are identified and counted in a conventional flow cytometer, presenting the Mutant Allele Frequency as the ratio between wildtype and mutant particles.

RESULT

- Samples were obtained from 25 patients at diagnosis, postoperative, and follow-up, average cfDNA recovered 22,43ng, 15,91ng, and 26,62 respectively.
- Mutant allele frequencies (MAF) were analyzed in tumor and normal DNA samples, and sensitivity was demonstrated
- Hotspot mutations were detected in plasma DNA at diagnosis for 17 of 19 patients.
- Increased mutant allele frequencies correlated with clinical relapse, illustrating the suitability of superRCA for monitoring recurrence.

Patient ID	Stage	Recurrence	Hotspot Mutations	gDNA Availability		Plasma cfDNA Amount (ng)			
				Tumor	Normal	Diagnosis	Postoperative	Follow-Up	
UU001	III	Yes	*KRAS* p.G13D + *PIK3CA* p.E542K	Yes	Yes	35.7	N/A	27.0	18.0
UU002	III	No	*KRAS* p.G12D	Yes	Yes	32.7	N/A	16.2	12.3
UU003	III	No	*KRAS* p.G12V + *PIK3CA* p.Q545K	Yes	Yes	12.8	N/A	8.4	12.6
UU004	IV	Yes	*KRAS* p.G12V	No	No	15.3	8.18	9.83	N/A
UU005	III	Yes	Wild-type	Yes	Yes	24.9	17.4	41.7	N/A
UU006	III	Yes	Wild-type	No	No	20.2	N/A	24.0	43.7
UU007	III	Yes	Wild-type	Yes	Yes	15.9	N/A	12.3	Leaky Tube
UU008	II	No	*KRAS* p.A146V	Yes	Yes	16.5	9.83	18.3	N/A
UU009	III	No	*BRAF* p.V600E	Yes	Yes	19.3	N/A	10.5	17.7
UU010	III	No	*KRAS* p.G12D	Yes	Yes	29.0	N/A	13.2	30.6
UU011	III	No	*KRAS* p.G12E	Yes	Yes	29.0	16.8	10.5	N/A
UU012	III	No	*BRAF* p.V600E	Yes	Yes	38.7	N/A	11.6	21.3
UU013	II	No	Wild-type	Yes	Yes	16.5	N/A	21.3	28.2
UU014	III	No	*BRAF* p.V600E	Yes	Yes	21.3	11.5	21.6	N/A
UU015	II	No	*BRAF* p.V600E + *PIK3CA* p.H1047R	Yes	Yes	18.5	N/A	27.0	12.0
UU016	I	No	Wild-type	Yes	Yes	15.5	N/A	30.9	20.4
UU017	II	No	*BRAF* p.V600E	Yes	Yes	25.3	N/A	50.1	37.8
UU018	I	No	*KRAS* p.G13D	Yes	Yes	19.6	N/A	13.8	23.4
UU019	II	No	Wild-type	Yes	Yes	11.1	12.6	7.13	N/A
UU020	I	No	*KRAS* p.G12D	No	No	32.1	N/A	20.4	20.7
UU025	IV	Yes	*NRAS* p.G12D	Yes	Yes	35.7	N/A	32.1	26.7
UU035	III	Yes	*KRAS* p.G12V	No	No	13.5	N/A	N/A	222.9
UU036	III	No	*BRAF* p.V600E	Yes	Yes	16.5	27.0	15.9	N/A
UU037	III	Yes	*KRAS* p.G13C + *PIK3CA* p.H1047L	No	No	N/A	24.0	18.0	23.4
UU038	III	Yes	*PIK3CA* p.E542K	Yes	Yes	22.8	N/A	27.0	30.9





CONCLUSION

Multiplex superRCA is a highly sensitive and specific method for monitoring colorectal cancer recurrence. The technique offers a promising approach for early, non-invasive detection of disease recurrence, to guide timely therapeutic intervention.

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