

Title

Comprehensive study on molecular characterization of somatic genomic variants, circulating tumor DNA and gut microbiota associated with colorectal cancer

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Introduction

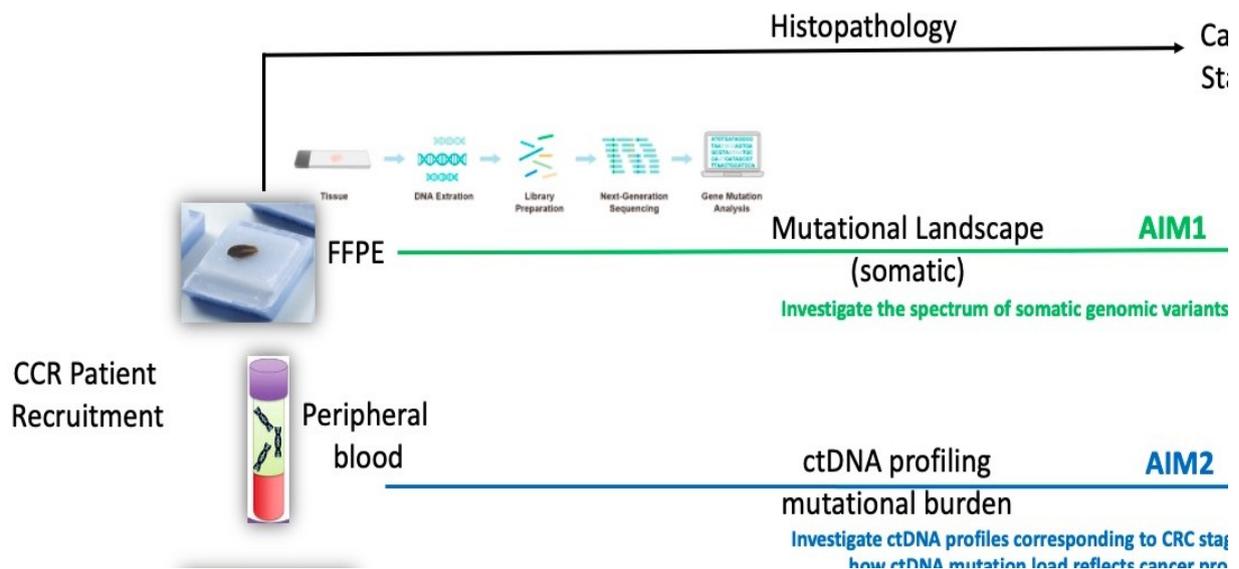
Globally, the incidence of colorectal cancer(CRC) is varied among ethnic groups suggesting strong association between genetic makeup of the population and lifestyle factors including dietary habits. Number of previous studies testify that prognosis and survival outcome of CRC are heavily dependent on the success of early detection, and optimal management using precision medicine modalities. In Sri Lanka, CRC is the third highest incident cancer accounting for 7.6% and 7.5% of all male and female cancers, respectively. Over the last decade, its incidence has doubled with a sharp increase among those aged below 50 years. Nearly 75% of CRC cases are diagnosed at an advanced stage due to late presentation, leading to poor treatment outcomes and reduced survival rates. CRC is characterized by multiple genomic variations and metabolic dysregulation that give rise to clonal evolution and heterogeneity of the disease. The genomic alterations interfere with several signaling pathways that contribute to inter/intra-tumoral heterogeneity and influence responsiveness or resistance to anti-tumour agents.

In CRC, profile of the gut microbiome has a major role in regulating the macro environment in the intestine and interfering with gene expressions through different mechanisms - genotoxin, metabolism and inflammation. Gut microbiota which is known to correlate with development and progression of CRC has shown varied abundance at different stages of CRC in diverse ethnic groups. A clear understanding of the role of particular microbiome species in modulating genetic variation is vital for informed decision making and implementation of targeted therapy. This is because heterogeneity of CRC often makes generalized management protocols less effective. Further, tumour tissue biopsy - the current gold standard in cancer diagnosis has serious limitations. Genetic analysis of tissue biopsy generally provides only a snapshot of a minimal area with genomic aberrations in the tumour and fails to capture genetic heterogeneity or temporal association of various factors in the tumour microenvironment. However, liquid biopsy-based methods, especially targeting circulating tumour DNA (ctDNA) in peripheral blood, has shown promising results in the identification of molecular markers associated with CRC. Being minimally invasive, ctDNA can be repeatedly sampled to monitor tumour progression and/or treatment response. Hence incorporation of recent techniques using ctDNA in peripheral blood for describing somatic variations would augment the results of tissue biopsy. Further, correlating the spectrum of genetic variations with gut microbiome profile can provide a comprehensive understanding of CRC for development of targeted therapy. We hypothesize that analysis of the genomic variants in tumour tissue, ctDNA profiles in peripheral blood and gut microbiota associated with CRC clinicopathological features in different stages would lead to the discovery of novel molecular markers of CRC in the Sri Lankan population.

Vision

Due to marked genetic heterogeneity, the molecular profile of CRC has been shown to differ among population groups. At present, there is little molecular marker information available on CRC in Sri Lanka. We propose to carry out a study on tumor-specific molecular markers associated with different stages of CRC through analysis of genomic variants in tumor tissue, gut microbiota in stool samples and ctDNA profiles in the peripheral blood. Patients with histopathologically confirmed CRC would be recruited into the study with ethical approval. Next Generation Sequencing (NGS)-based analysis of tumour tissue, ctDNA analysis in plasma and 16S rRNA sequencing in stool samples in patients with histopathologically confirmed primary CRC prior to commencement of treatment would be conducted. This study would facilitate the discovery of novel molecular markers with potential future applications in personalized therapy resulting in improved CRC diagnosis, clinical and survival outcome, thereby helping to minimize the morbidity and mortality of CRC in Sri Lanka.

Study Flow Chart



Aims

- Investigate the spectrum of somatic genomic variants associated with colorectal cancer in Sri Lanka through genetic analysis of tissue biopsy supplemented by circulating tumor DNA profile in peripheral blood.
- Identify the gut microbiome species associated with specific genetic variation in Sri Lankan cohort.
- Identify targetable molecular markers associated with different stages of colorectal cancer in Sri Lankan population.

Impact

This is the first large scale study to be conducted in Sri Lanka to identify novel molecular markers associated with CRC clinic-pathological features in different stages through analysis of somatic genomic variants, gut microbiota and ctDNA profiles in a cohort of Sri Lankan patients.

The results from this study would help fill the gaps in the existing molecular knowledge about specific CRC molecular markers in the local population. The research team and the local scientific community in general would immensely benefit from exposure to the novel methodologies adopted in the proposed project. Identification of targetable biomarkers would assist in implementing personalized therapy which would reduce the adverse consequences of generalized drugs which often come with huge economic burden on the low-income sections of the population. It would also lead to capacity building in cancer genomic medicine at the host institution with widespread benefits locally and internationally.

Executive Plan

National regulatory approvals for the study have already been obtained. On commencement of the project, purchase orders for consumables and equipment would be placed and the supply is expected within the first quarter. Hands-on training on tumour tissue sequencing, ctDNA analysis and microbiota analysis at the International Centre for Genetic Engineering and Biotechnology (ICGEB) Laboratory, New Delhi is also scheduled in the first quarter. Recruitment of patients and collection of samples will commence from the beginning of second quarter. Samples from 60 patients would be completed in the third quarter. DNA would be extracted from tumor tissue blocks, peripheral blood and stool samples and stored for analysis. DNA sequencing would be undertaken in the third quarter. DNA extracted from tumour tissue blocks would be used to identify somatic genomic variants using validated sequencing kits. ctDNA fractions from plasma samples would be sequenced using validated ctDNA kits. Distinct patterns of microbiota expression would be identified using metagenomics analysis by targeted sequencing of 16S rRNA in stool DNA. Bioinformatic analysis, interpretation of sequence data and identification of molecular biomarkers would be carried out in third and fourth quarters. Study results would be published and presented at national and international scientific platforms to disseminate the study outcomes.

Project Matrix				
Quarters	1	2	3	4
Regulatory approvals - already obtained				
Procurement: consumables & equipment				
Training				
Patient recruitment & sample collection				
Genomic sequencing/ microbiota analysis				
Bioinformatic analysis/ data interpretation				
Publication of results / seminars				

Partners: G2MC members (individual/country affiliation)

The study would be carried out at the Human Genetics Unit (HGU), Faculty of Medicine, University of Colombo, Sri Lanka in collaboration with the National Hospital of Sri Lanka. Two G2MC Steering Committee members who are affiliated with the HGU with special interest in cancer genomics will provide supervision and guidance for the proposed project.

Other Organizations: Non-G2MC collaborators (individuals/industry/NGOs, etc)

The collaborative links HGU has established with local clinicians, pathologists, scientists and researchers in the cancer field would support the successful implementation of the proposed project. Collaboration with ICGEB Laboratory, New Delhi would be beneficial in providing

training of project staff on the recent tools and informatics pipeline used for tumour tissue sequencing, ctDNA characterization and microbiota dysbiosis analysis.

Future funding: Plans for follow-on funding for continued support. [1 paragraph]

Follow-up funding would be sought from national funding bodies as and when the call for grant applications are made available by them.

Budget with brief

The total project cost estimated at USD 34,000.

Personnel (either as salary + benefits or hourly rate per activity) - Nil

The study would be carried out by a research student and research scientists attached to the Human Genetics Unit, Faculty of Medicine, University of Colombo who are graduates of molecular life sciences, zoology and bioinformatics. They will be actively involved in patient recruitment, sample collection, processing, laboratory testing and bioinformatics analysis under the supervision and guidance of two HGU affiliated academicians involved in cancer research who are members of the G2MC. Hence salary of personnel involved in the study would be borne by the local institution.

Materials and supplies – USD 31,000

The project proposes to use a third generation Sequencer preferably Oxford Nanopore MinION MK1C, which is a cost-effective sequencing platform and provides possibilities for single-molecule detection and rapid DNA sequencing for the purpose of validating genomic variants identified through Next-generation sequencing. It would cost approximately USD 5,000. Since the project requires higher computational capabilities for genome assembly, annotation and interpretation of sequence, provision of a sequencing control system and software for bioinformatics analysis is critical for effective implementation of the project. USD1,000 would be required for the procurement of a suitable computing platform. Proposed consumables include general lab supplies, chemicals, micropipettes, DNA extraction kits, DNA purification and quantification kits, library preparation kits, reagent kits for targeted amplification of genes of interest, barcodes / unique identifiers, AmpliSeq Cancer Hotspot Panel v2 sequencing kits and targeted 16S rRNA sequencing kits. Cost for consumables is estimated at USD25,000.

Support for local/country involvement - Nil

Travel and meeting support (if needed) – USD 2,000

Hands-on training on tissue sequencing, ctDNA analysis and microbiota analysis would be arranged by the Translational Health Group, International Centre for Genetic Engineering and Biotechnology (ICGEB) Laboratory, New Delhi. USD 2,000 would be earmarked for meeting cost of travel and fees undertaken for the training.

Miscellaneous -USD 1,000

A miscellaneous fund of USD 1,000 would be earmarked for assistance to publish the findings in international and national journals. The amount also would be utilized for arranging workshops / seminars for dissemination of study findings with other stake holders in the country. Any unforeseen expenditures would also be met from funds available under this head.