Clinicopathological characteristics and mutational landscape of APC, HOXB13, and KRAS among Rwandan patients with colorectal cancer

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論文題目

Clinicopathological characteristics and mutational landscape of *APC, HOXB13* and *KRAS* among Rwandan patients with colorectal cancer

（ルワンダの大腸癌患者における臨床病理学的特徴と *APC*、*HOXB13*、および *KRAS* 遺伝子の変異ランドスケープ）

論文の内容の要旨

[Introduction]

Cancer research in Rwanda is estimated to be less than 1% of the total African cancer research, with limited research on colorectal cancer (CRC). Rwandan patients with CRC are young. Females are more affected than males. In addition, most of the patients present with advanced disease. Given the paucity of studies on genetic alterations in Rwandan cancer patients, we investigated mutations in CRC tissues. We focused on the *adenomatous polyposis coli* (*APC*), *Kirsten rat sarcoma* (*KRAS*) and *homeobox B13* (*HOXB13*) genes. Our aim was to determine whether there were any differences between Rwandan patients and other populations.

[Patients and Methods]

This study was approved by the Institutional Review Board (IRB) of the University of Rwanda College of Medicine and Health Sciences (Approval notice No. 048/CMHS IRB/2020, No. 113/CMHS IRB/2021, and No. 176/CMHS IRB/2022), the Ethics Committee of the University Teaching Hospital of Kigali (Ref.: EC/CHUK/2/064/2020) and the Ethics Committee of the Hamamatsu University School of Medicine (EC HUSM number: 20-011). All participants signed informed consent forms prior to participation in this study.

From December 2020 to September 2022, among 148 consecutive patients who underwent colonoscopic biopsy for suspected CRC at University Teaching Hospital of Kigali, 129 (87.1%) signed an informed consent form to participate in our study. Fifty-eight of 129 (44.9%) subject had CRC confirmed by biopsy, but four cases were excluded as they unmet all requirement for analysis. We finally analysed and presented results for 54 and all were naïve to cancer therapy.

At HUSM, we used QIAamp DNA FFPE Advanced UNG Kits to extract DNA from formalin fixed paraffin embedded tissue. For *APC* gene, our sequencing targeted the mutation cluster region (MCR). We also sequenced both exons (exons 1 and 2) of the *HOXB13* gene. For *KRAS*, we sequenced exons 2 to 4 and a fragment of exon 5 extending from GRCh 38: 25209690 to 25209999. After PCR amplification of DNA, we performed Sanger sequencing in both directions. Sequence files were aligned to the

respective genomic sequences. Then, genetic variants were annotated as per the recommendations of the Human Genome Variation Society. Novel variants were analyzed using MutationTaster2021. Each detected mutation was confirmed in replicate independent experiments. Cases of insertion/deletion were subcloned using a pGEM-T Easy vector system to confirm the presence of mutation.

[Results]

For 54 patients (mean age: 60 years) with CRC, most tumors were located in the rectum (83.3%) and 92.6% were low grade. Most patients (38/54, 70.4%) had never smoked and 61.1% (33/54) had consumed alcohol. We identified 27 variants for *APC*, including three novel mutations (c.4310_4319delAAACACCTCC, c.4463_4470delinsA, and c.4506_4507delT). All three novel mutations are classified as deleterious by MutationTaster2021. We found four synonymous variants (c.330C>A, c.366C>T, c.513T>C, and c.735G>A) of *HOXB13*. For *KRAS*, we found six variants (Asp173=, Gly13Asp, Gly12Ala, Gly12Asp, Gly12Val, and Gln61His), the last four of which are pathogenic.

[Discussion]

Globally, CRC is reported to be more common in males than in females. In this study, we found that CRC was more common in females than in males (i.e., 63.0% versus 37.0%). Information about family history of cancer was self-reported. Although none of our participants had a positive family history of cancer, this may not necessarily mean the absence of familial cancer cases, especially because of limited cancer registries in the past. Therefore, data on family history of cancer should be interpreted with caution. Of 3 genes we analysed, we reported 3 novel mutations in the *APC* gene. All of these novel mutations are frameshift mutations, which are predicted to cause a premature termination codon, produce truncated proteins and be deleterious.

Twenty percent of participants reported ever having smoked and 61.1% reported ever having drunk alcohol. Both proportions are higher than 6.9% (for smokers) and 14.7% (for drinkers) reported by Wismayer et al. in the Ugandan population neighboring Rwanda. The risk of developing CRC has been associated with a high intake of red meat, processed meat, sweetened beverages, a diet low in fiber, and a low intake of dairy products. Although we did not assess the diets of our study participants, it is worth noting that Rwandans rarely consume meat, fish, fruit, and dairy products; their diets may be rich in starchy foods.

[Conclusion]

We contribute new data on genetic variation and clinicopathological information relevant to CRC patients in Rwanda. This genetic information does not yet reflect specific environmental (i.e., dietary and other) components. However, additional studies

are required to improve the quality of scientific data characterizing CRC cases in the Rwandan population to improve evidence-based management of this disease. The genetic data provided in this paper also represent a valuable resource for the study of CRC in understudied populations.