FUSOBACTERIUM NUCLEATUM IN BIOPSIED TISSUES FROM COLORECTAL CANCER OF KAZAKHSTANI PATIENTS

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Colorectal cancer (CRC) poses a significant public health challenge, ranking third in incidence and second in mortality among all cancers in 2020. While genetic factors contribute to a portion of CRC risk, the majority is influenced by environmental factors, with the gut microbiota playing a significant role. Infections by certain pathogens like pathogenic *E. coli, Salmonella enterica,* toxigenic *Bacteroides fragilis, Fusobacterium nucleatum* (*Fn*), *Peptostreptococcus anaerobius*, and *Helicobacter pylori* have been linked to CRC risk. Among these, *F. nucleatum*, a gram-negative anaerobic bacillus, has garnered attention for its association with cancer. Recently studies show an increased presence of *Fn* in human CRC compared to healthy tissues, and higher levels of *Fn* in CRC tumors are linked to poorer survival rates. Animal and cellular models support the cancer-promoting role of *Fn*, with FadA, a protein it produces, shown to bind to E-cadherin, activating β-catenin signaling and influencing inflammatory and oncogenic responses. The overexpression of the fadA gene in colon tissue from CRC patients further supports its involvement in cancer development. However, the precise mechanisms driving the progression of cancer initiated by this bacterium are not yet fully understood. In this study, we aimed to determine the prevalence of CRC-associated bacteria *F. nucleatum* in the biopsied tissues of patients with CRC using quantitative real-time PCR (qPCR) and the fold change *Fn* abundance in colorectal cancer of Kazakhstani patients. Furthemore, we determined the association between the epidemiological characteristics of CRC and the presence of *F. nucleatum*.

In our investigation were recruited a total of 80 patients with histologically confirmed colorectal adenocarcinoma (males 38 and females 42, age range 26-86 years) undergoing surgical resections at the National Research Oncology Center, Astana, Kazakhstan between Oktober 2022 and March 2024. Demographic and clinical factors were included such as age, gender, high consumption of red meat, obesity, smoking, and alcohol intake. Prior to the study, informed consent was obtained from all patients in accordance with the guidelines set forth by the RSE Ethics Commission, «National Center for Biotechnology» under the Ministry of Health of the Republic of Kazakhstan. Biopsied tissues from each patient were obtained from colorectal carcinoma tissues, adjacent tissues, and distal normal tissues. In total, 240 number of biopsies were collected. Q-PCR was applied to detect *F. nucleatum* in CRC and normal tissues. The amount of *Fusobacterium* between two groups was assessed using the Student's t-test and Wilcoxon rank sum test.

The median abundance of *F. nucleatum* determined by 2-ΔΔCT in CRC tissues 19.4 (1.9-354.3) was significantly higher than that in normal controls 4.13 (0.98-23.57) ($P < 0.001$). *F. nucleatum* was over-represented in 67/80 (83.8%) CRC samples. The location of CRC and tumor size were significantly associated with abundance of *Fn* ($P < 0.051$, $P < 0.023$, respectively). No significant association of *F. nucleatum* with other clinico-pathological variables was observed ($P > 0.05$). To conclude, *F. nucleatum* was enriched in CRC tissues and associated with CRC development. These results imply that assessing *F. nucleatum* levels could aid in predicting clinical outcomes for colorectal cancer patients.