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Formalin-fixed paraffin-embedded sample conditions for deep next generation sequencing

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ABSTRACT

Introduction: Precision medicine is only possible in oncology practice if targetable genes in fragmented DNA, such as DNA from formalin-fixed paraffin-embedded (FFPE) samples, can be sequenced using next generation sequencing (NGS). The aim of this study was to examine the quality and quantity of DNA from FFPE cancerous tissue samples from surgically resected and biopsy specimens.

Methods: DNA was extracted from unstained FFPE tissue sections prepared from surgically resected specimens of breast, colorectal and gastric cancer, and biopsy specimens of breast cancer. A total quantity of DNA ≥60 ng from a sample was considered adequate for NGS. The DNA quality was assessed by Q-ratios, with a Q-ratio >0.1 considered sufficient for NGS.

Results: The Q-ratio for DNA from FFPE tissue processed with neutral-buffered formalin was significantly better than that processed with unbuffered formalin. All Q-ratios for DNA

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from breast, colorectal and gastric cancer samples indicated DNA levels sufficient for NGS. DNA extracted from gastric cancer FFPE samples prepared within the last 7 years is suitable for NGS analysis, whereas those older than 7 years may not be suitable. Our data suggested that adequate amounts of DNA can be extracted from FFPE samples, not only of surgically resected tissue but also of biopsy specimens.

Conclusions: The type of formalin used for fixation and the time since FFPE sample preparation affect DNA quality. Sufficient amounts of DNA can be extracted from FFPE samples of both surgically resected and biopsy tissue, thus expanding the potential diagnostic uses of NGS in a clinical setting.

Introduction

Over the past 10 years, the Human Genome Project, followed by The Cancer Genome Atlas project, have revealed that cancer is a disease of the genome,[1-8] and that genomic analysis may be used to identify mutations in tumor tissue that indicate appropriate treatments tailored for an individual patient.[9,10] In 2015, President Obama announced the Precision Medicine Initiative, in which individual variability and heterogeneity are considered in the prevention and treatment of cancer.[11,12] Precision medicine utilizing next generation sequencing (NGS) is expected to bring a paradigm shift from a pathological microscopic-based approach to a genetic signature-based diagnosis.[13] The essence of precision medicine is to minimize the toxicity of cancer treatment while maximizing benefit by subgrouping patients according to genomic alterations that can be treated with specific molecular-targeted therapy.[14] Given its concept, precision medicine is expected to provide cost-effectiveness by improving treatment efficacy while avoiding ineffective treatments.[15]

Although there are great expectations for the treatment of cancer patients with precision medicine, it is only possible in oncology practice if commonly available clinical samples can be analyzed by deep sequencing with NGS.[16] It is only possible to apply NGS analysis for precision medicine if tumor DNA within stored samples is maintained in conditions that are appropriate for meaningful NGS analysis, and if only minimal artifacts are produced. In reality, however, the vast majority of clinical samples are stored as formalin-fixed, paraffin-embedded (FFPE) tissue in which DNA necessary for NGS is often fragmented.[17] Recently, it has been reported that the quality of FFPE samples varies depending on how surgical specimens have been prepared and preserved.[18] Therefore, it is extremely important that the next generation of surgeons who will be harvesting samples for NGS know how to appropriately handle samples for FFPE processing. Furthermore, it is important to clarify how much DNA can be harvested from a minimally sized sample, because often only a small amount of tissue is available in clinical settings such as from a biopsy or after neoadjuvant therapy.

The aim of this study was to examine the quality and quantity of FFPE cancer tissue samples obtained from surgically resected or biopsy specimens for deep sequencing with NGS. In this study, we validated the quality and quantity of DNA extracted from FFPE samples of breast, gastric, and colorectal cancer.

Materials and methods

Patients

FFPE tissue blocks were processed using surgically resected specimens obtained from patients with breast cancer (BC; $n = 35$), colorectal cancer (CRC; $n = 134$), or gastric cancer (GC) ($n = 138$), after operations at Niigata University Medical and Dental Hospital or Niigata Cancer Center Hospital from 2009 through to 2016. The choice of the specific organ tissues was made based on the prevalence in Japan. Indeed, top three most prevalent cancers in Japan are gastric, colon, and lung cancer in male, and breast, colon, and gastric cancer in female. FFPE tissue blocks were also prepared using biopsy specimens obtained from patients with BC ($n = 20$) at the same institutions from 2015 through to 2016. This study protocol was approved by the Institutional Review Board of Niigata University Medical and Dental Hospital. Informed consent was obtained from all the patients.

FFPE processing

Surgically resected and biopsy specimens were fixed in neutral-buffered formalin (NBF) or unbuffered formalin (only for the experiments shown in Fig. 2), and FFPE sample blocks were prepared by fixation, dehydration, clearing, and embedding steps, according to the standard protocol summarized in Figure 1. Briefly, tissue samples were fixed with NBF containing PhosSTOP (Roche) to preserve phosphorylated proteins. The fixation time was kept strictly to within 24 hours to avoid over-fixation which can result in extensive cross-linking. Five dehydration steps were performed in gradually increasing concentrations of ethanol, followed by three steps of clearing with xylene and four steps of embedding in paraffin wax.

DNA extraction

Serial sections were prepared from each FFPE block: six unstained tissue sections (20 μm) for DNA extraction and one