Open camera or QR reader and
scan code to access this article
and other resources online.

Impact of Washing Processes on RNA Quantity and Quality in Patient-Derived Colorectal Cancer Tissues

Onchira Acharayothin,[1,*] Benjarat Thiengtrong,[1,2,*] Panudeth Juengwiwattanakitti,[1,2] Panatna Anekwiang,[3] Woramin Riansuwan,[1] Vitoon Chinswangwatanakul,[1,2] and Pariyada Tanjak[1,2]

Background: Colorectal cancer (CRC) is a common and lethal cancer worldwide. Extraction of high-quality RNA from CRC samples plays a key role in scientific research and translational medicine. Specimen collection and washing methods that do not compromise RNA quality or quantity are needed to ensure high quality specimens for gene expression analysis and other RNA-based downstream applications. We investigated the effect of tissue specimen collection and different preparation processes on the quality and quantity of RNA extracted from surgical CRC tissues.

Materials and Methods: After surgical resection, tissues were harvested and prepared with various washing processes in a room adjacent to the operating room. One hundred fourteen tissues from 36 CRC patients were separately washed in either cold phosphate-buffered saline reagent ($n = 34$) or Dulbecco's modified Eagle's medium (DMEM; $n = 34$) for 2–3 minutes until the stool was removed, and unwashed specimens served as controls ($n = 34$). Six tissue specimens were washed and immersed in DMEM for up to 1 hour at 4°C. Before RNA extraction, all specimens were kept in the stabilizing reagent for 3 months at −80°C. RNA was extracted, and the concentration per milligram of tissue was measured. RNA quality was assessed using the RNA integrity number (RIN) value.

Results: Different washing processes did not result in significant differences in RNA quantity or RIN values. In the six tissues that were washed and immersed in DMEM for 1 hour, RIN values significantly decreased. The quality of the extracted RNA from most specimens was excellent with the average RIN greater than 7.

Conclusions: RNA is stable in specimens washed in different processes for short periods, but RIN values may decrease with prolonged wash times.

Keywords: biobanking, colorectal cancer, RNA integrity number, RNA concentration, stabilizing reagent

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death and the third most common type of cancer worldwide.[1] CRC is a heterogeneous disease in terms of clinical behavior and response to therapy and is associated with various genetic and epigenetic alterations.[2–4] Therefore, targeted and personalized treatments are needed to decrease CRC mortality.[5] Nucleic acids extracted from CRC patients are essential for genomic and transcrip- tomic studies. Low quality RNA can result in decayed gene expression levels and decreased library complexity in RNA sequencing processes.[6] Specimen collection and washing methods that do not compromise RNA quality or quantity are needed to ensure high quality specimens.

Most cancer tissues dissected in hospitals are fixed in formalin and stored as paraffin-embedded (FFPE) tissue blocks in the pathology department.[7] The degradation and fragmentation of RNA extracted from FFPE tissues make subsequent molecular analyses difficult.[8–10] When surgical tissues

[1]Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
[2]Siriraj Cancer Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
[3]Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
*These authors contributed equally to this work and co-first authors.

are frozen in liquid nitrogen, the quality of RNA is reduced during thawing.[11] RNA stabilizing reagents are widely used to preserve human tissue.[11,12] The stabilizing reagent contains a high concentration of quaternary ammonium sulfate and cesium sulfate at controlled pH (5.2) that protects against the denaturation of nucleic acids and proteins from RNase, DNase, and protease.[13–15] Tissues stored in the RNA stabilizing reagent are suitable for transcriptome analyses such as quantitative PCR[16] and RNA-based microarray.[17]

In addition to investigating the genome and transcriptome of CRC, surgical resection can generate primary cell lines (2D and organoids) that are essential for exploring cancer biology and drug development.[18–21] Bacterial contamination is a major cause of CRC organoid failure.[22] Therefore, dissected tissues are immediately washed and then immersed into cell culture medium with an antiseptic agent to facilitate transport and generation of primary cell lines. Afterward, tissues may be divided into small portions and stored in the RNA stabilizing reagent for further study.

We investigated the quality and quantity of RNA extracted from CRC specimens kept in the RNA stabilizing reagent at −80°C. Before storage, we washed tissues in phosphate-buffered saline (PBS) or Dulbecco's modified Eagle's medium (DMEM), and unwashed specimens served as controls. We also observed the RNA stability of specimens immersed in DMEM for 1 hour before generating organoids and stored in the RNA stabilizing reagent.

Materials and Methods

Patients and tissues

Surgical colorectal tissues from 36 patients were collected at the Faculty of Medicine Siriraj Hospital, Mahidol University. Written informed consent was given by all patients. All experiments were performed in accordance with the ethical requirements and regulations of the Siriraj Institutional Review Board (SiIRB), protocol number 785/2559 (EC3) and certificate of approval number Si 076/2017. SiIRB is in compliance with international guidelines for human research protection such as the Declaration of Helsinki. The cohort included patients ≥18 years of age with adenocarcinoma. All patients had complete pathology and treatment records. Pathology stages I, II, III, and IV were defined according to the American Joint Committee on Cancer (AJCC) staging system (7th Ed.).

Tissue preparation and collection

From the resected colorectal segment, the tissues were cut into 5–10 mm pieces and washed with cold PBS (Thermo Fisher Scientific, MA), washed with cold DMEM (Invitrogen, MA), or wiped with gauze. Both PBS and DMEM were supplemented with 1 × antibiotic–antimycotic solution (Invitrogen). After washing and wiping until the stool was removed (usually <5 minutes to avoid RNA degradation), the tissues were immediately placed in the stabilizing reagent RNA*later* (Invitrogen) and stored at −80°C until RNA extraction. Six specimens were washed and placed in DMEM, transported to the laboratory where they were immediately generated to organoid culture, and then cut and stored in the RNA stabilizing reagent (Fig. 1).

RNA extraction

Tissues were cut into 15–30 mg pieces and placed into lysis buffer with lysing matrix Z (MP Biomedicals, CA). They were homogenized using the FastPrep-24™ 5G Instrument (MP Biomedicals) at 6.0 m/s for 40 seconds ON and 3 minutes OFF cycles. During the OFF phase, the tube was moved and placed on ice. The homogenization was performed with two cycles until the lysate was clear. Then, RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA was stored at −80°C.

RNA purity and quantity

The concentration and purity of extracted RNA were determined using the NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific). RNA purity was assessed by

FIG. 1. Experimental design. The cancer tissues from 36 CRC patients were collected from heterogenous sites and washed for 2–3 minutes with PBS, DMEM, or remained unwashed. Then, they were preserved in stabilization reagent. Six tissues from 6 patients (among the 36 patients) were minced, washed in DMEM for 1 hour, and sent for organoid generation. CRC, colorectal cancer; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagle's medium.
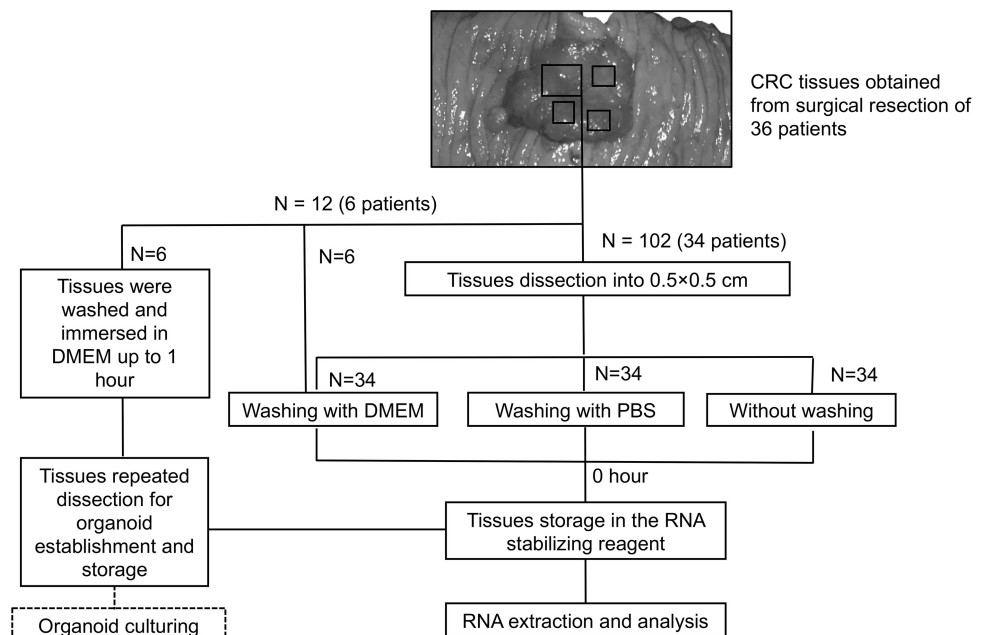
measuring absorbance at 260 nm (A260), 280 nm (A280), and 230 nm (A230). A ratio of A260/A280 of ~ 2.0 is generally accepted as "pure" and evaluated as "excellent".[23] Expected 260/230 values are in the range of 2.0–2.2.

We performed Qubit fluorometry with selective fluorescent dye stain for transcriptome sequencing, which is more specific than spectrophotometric Nanodrop.[24] Total RNA yield was analyzed using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and the Qubit RNA High Sensitivity Kit (Thermo Fisher Scientific) according to the manufacturer's standard protocol. Total RNA yield was defined as total RNA concentration divided by tissue weight (μg/mg).

RNA integrity

The RNA integrity was analyzed using an electrophoresis trace of the Agilent 2100 Bioanalyzer RNA 6000 Nano Chip (Agilent Technologies, CA). The electrophoresis and the ratio of 28s and 18s ribosomal RNA (28s:18s rRNA) bands were analyzed and reported. RNA integrity number (RIN) was calculated using the 2100 Expert Software (Agilent Technologies). RIN values range from 10 (intact RNA) to 1 (completely degraded).[25] Values greater than 7 are generally considered to be acceptable for RNA sequencing.

Statistical analysis

Statistical analysis was performed using IBM SPSS ver.25.0 (SPSS, Inc., IL). The Shapiro–Wilk test was used to determine if data were normally distributed. The clinical characteristics and the average RIN of washing condition groups were tested using the one-way analysis of variance or the Kruskal–Wallis H-test. Comparison of the three washing processes was performed using the Friedman test. The

Wilcoxon signed-rank test was used for individual comparisons. Data with statistical values of $p < 0.05$ were considered to be statistically significant.

Results

Effect of patient characteristics on RNA integrity

CRC specimens ($N = 102$) were taken from 34 CRC patients (14 males and 20 females) with a tumor size >2 cm (Fig. 1 and Table 1). The median age at diagnosis was 66.5 years (38–82). The majority of patients had moderately differentiated adenocarcinoma ($N = 31$) and were in stage III ($N = 20$). All stage IV patients had liver metastasis. Most patients had tumor location sites at the sigmoid colon ($N = 12$) or rectum ($N = 8$). In female CRC patients, the average RIN of PBS-washed tissues was significantly higher than with other processes ($p < 0.05$).

Effect of three tissue washing processes on RNA purity and yield

We compared the purity and yield of RNA isolated from tissues processed with PBS or DMEM, and unwashed specimens served as controls. A260/A280 ratios showed no statistical differences among the three processes; PBS = 1.962 ± 0.131, DMEM = 1.949 ± 0.128, and unwashed = 1.936 ± 0.250 ($p = 0.620$, Table 2 and Fig. 2A). The A260/A230 ratios were ~ 1.80 and were not significantly different (Fig. 2B), suggesting that all RNA samples were pure and suitable for use in downstream applications.

The RNA yields of the three processing conditions were not statistically significantly different (Fig. 2C and Table 2). This suggests that the wash processes did not affect the

TABLE 1. EFFECT OF CLINICOPATHOLOGICAL CHARACTERISTICS OF COLORECTAL CANCER PATIENTS ON THE AVERAGE RNA INTEGRITY NUMBER

Characteristics	N	RIN Mean ± SD PBS	Mean ± SD DMEM	Mean ± SD None[a]
Sex				
Male	14	8.03 ± 0.92	8.08 ± 1.34	8.23 ± 0.79
Female	20	8.96 ± 0.89[b]	8.75 ± 0.87	8.70 ± 0.91
Age (median age)	38–62 (66.5)			
>50	26	8.53 ± 1.03	8.41 ± 1.10	8.43 ± 0.80
≤50	8	8.72 ± 0.96	8.70 ± 1.24	8.73 ± 1.15
Location				
Colon	21	8.84 ± 0.85	8.63 ± 1.24	8.63 ± 0.82
Rectum	13	8.15 ± 1.11	8.22 ± 0.87	8.30 ± 0.96
Pathological grade				
Moderately differentiated	31	8.59 ± 1.04	8.53 ± 1.12	8.51 ± 0.88
Poorly differentiated	3	8.43 ± 0.49	7.86 ± 1.05	8.43 ± 1.01
Tumor stage				
I	6	8.30 ± 1.45	8.76 ± 0.87	8.56 ± 1.04
II	6	8.30 ± 1.08	8.68 ± 1.27	8.20 ± 0.66
IV	20	8.70 ± 0.89	8.34 ± 1.22	8.59 ± 0.95
V	2	9.05 ± 0.21	8.35 ± 0.07	8.45 ± 0.35
Size (cm)				
>5	15	8.60 ± 0.83	8.69 ± 0.97	8.42 ± 0.71
≤5	19	8.56 ± 1.14	8.31 ± 1.22	8.57 ± 1.01

[a]Unwashed.
[b]$p < 0.05$, Kruskal–Wallis H-test.
DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; SD, standard deviation.

TABLE 2. EFFECT OF DIFFERENT WASH PROCESSES ON RNA QUALITY AND QUANTITY OF COLORECTAL CANCER TISSUES

	Wash reagent	N	Mean ± SD	p[a]
A260/280	PBS	34	1.962 ± 0.131	0.620
	DMEM	34	1.949 ± 0.128	
	None[b]	34	1.936 ± 0.250	
A260/230	PBS	34	1.818 ± 0.295	0.627
	DMEM	34	1.827 ± 0.191	
	None	34	1.749 ± 0.383	
Yield (μg/mg)	PBS	34	3.429 ± 1.547	0.682
	DMEM	34	3.793 ± 1.268	
	None	34	3.527 ± 1.493	
28s:18s rRNA ratio	PBS	34	2.197 ± 0.440	0.091
	DMEM	34	2.003 ± 0.580	
	None	34	2.118 ± 0.452	
RIN[c]	PBS	34	8.579 ± 1.005	0.755
	DMEM	34	8.479 ± 1.123	
	None	34	8.509 ± 0.884	

[a]Friedman test.
[b]Unwashed.
[c]Average RNA integrity number.

purity and quantity of RNA. All RNA samples were considered to be excellent. The total RNA yield was >1 μg/mg, and the average A260/A280 and A260/A230 ratios were at least 1.8 and sufficient for molecular analysis.

RNA integrity of three tissue washing processes

Generally, RNA is considered to be high quality when the 28s:18s ribosomal RNA ratio is about 2.0 or higher.[25] We evaluated the 28s:18s rRNA ratios and the number of samples that reached a 28s:18s rRNA ratio ≥2 (Fig. 3A). DMEM-washed tissue RNA had the lowest rRNA ratio, and PBS-washed tissue had the highest ratio. Interestingly, only 16 of 34 DMEM-washed tissues had an rRNA ratio ≥2. This indicates that the large subunit of rRNA extracted from

DMEM-washed tissues was partially degraded. However, the ratios of 28s:18s among the three processes were not significantly different (PBS = 2.197 ± 0.440, DMEM = 2.003 ± 0.580, None = 2.118 ± 0.452, $p = 0.091$).

The average RIN value of RNA extracted using PBS washing was 8.579 ± 1.005; DMEM was 8.479 ± 1.123, and for the unwashed process was 8.509 ± 0.884, ($p = 0.755$) (Table 2 and Fig. 3B). More than 70% of extracted RNA ($N = 102$) from the three different processes had RIN values higher than 7, suggesting that the RNA quality was excellent. No extracted RNA had a RIN score lower than 4. All washing processes resulted in high quality RNA that was suitable for molecular applications.

Lower RIN values in DMEM condition for 1 hour

To generate organoids, we immersed the dissected specimens in DMEM, cell culturing medium, for ~1 hour, then collected and preserved them in the RNA stabilizing reagent (Fig. 1). To determine if immersion time affects RNA quality and quantity, RNA from six specimens was immersed in DMEM for ~1 hour before fixing in the RNA stabilizing reagent. The average yields of six samples at 0- and 1-hour time points were 2.795 and 3.329 μg/mg, respectively, ($p = 0.463$) (Fig. 4A). RNA concentration remained stable after 1 hour (Table 3 detailed in Supplementary Table S1), but the RIN values and the 28s:18s rRNA were significantly decreased at the 0- and 1-hour time points ($p < 0.05$) (Table 3). However, the RNA integrity of 83.3% of samples ($N = 12$) was still above seven and acceptable for RNA sequencing (Fig. 4B).

Discussion

Improvements in genomic, transcriptomic, and proteomic technologies have led to the identification of the molecular basis of various diseases, including cancers, and resulted in rapid advances in cancer research.[26] Banking of high-quality biological specimens is essential for cancer research.

FIG. 2. Comparison of the purity and yield of extracted RNA. **(A)** A260/A280 ratio from all wash conditions. **(B)** A260/A230 ratio from all wash conditions. **(C)** RNA yield from all wash conditions (μg/mg). The yield of isolated RNA was calculated by total concentration divided by tissue weight. Data are shown as mean ± SD of 34 independent samples. n.s., no statistically significant difference; None, unwashed; SD, standard deviation.
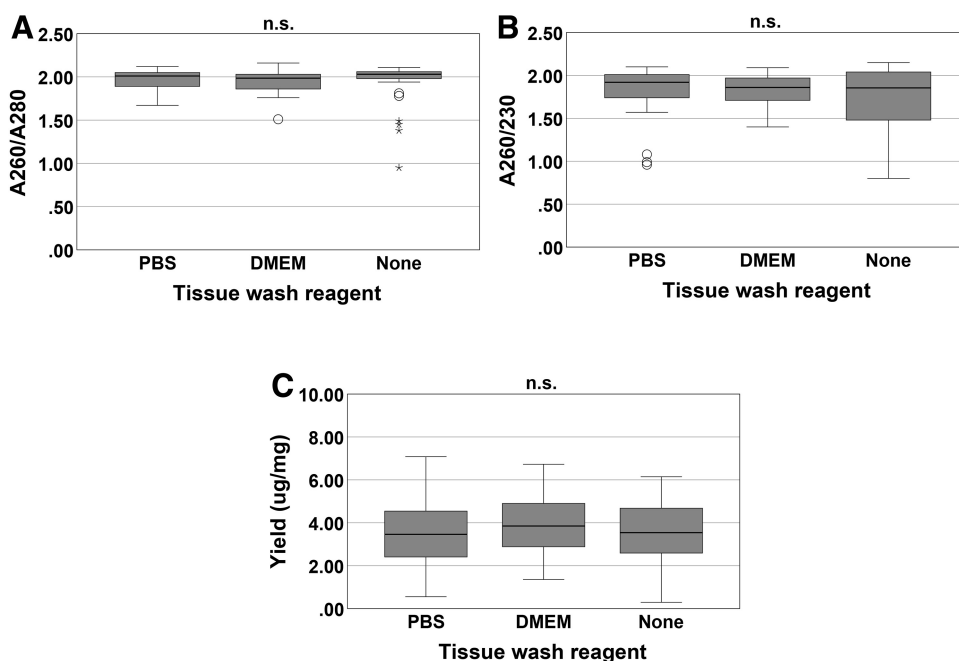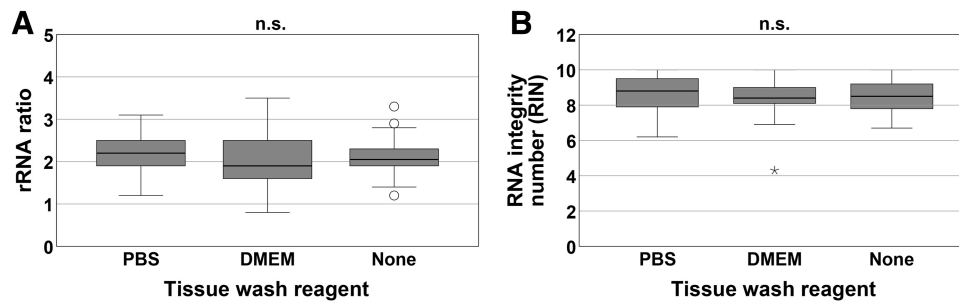
FIG. 3. RNA quality of RNA extracted from three different wash CRC tissues. **(A)** The ratio of 28s:18s rRNA (rRNA) of total extracted RNA. **(B)** The average of RIN from different wash conditions. Data are shown as mean ± SD of 34 independent samples. n.s., no statistically significant difference; RIN, RNA integrity number.

Careful collection and storage of specimens helps to prevent RNA, DNA, and protein degradation, especially RNA that is rapidly degraded by RNase.

A CRC biobank was established in 2014 at the Faculty of Medicine of Siriraj Hospital. The protocols for CRC fresh tissue collection are continually being improved to support our live CRC biobank.[27] Dissected CRC specimens are washed with saline before preservation by snap-freezing or immersion in a stabilizing reagent. Remnant specimens are cut into pieces, rinsed with DMEM, transported to the laboratory where the specimen is minced, and washed again with DMEM for organoid generation. Normally, transport to the laboratory is completed within 1 hour to prevent biomolecule degradation. Therefore, we compared the effect of different wash processes on the quality and quantity of extracted RNA from human CRC that was stored in RNA stabilizing reagent.

RNA stabilizing reagents help to maintain the integrity of RNA in stored tissues,[16,17] but optimal specimen collection and handling are the first steps to reduce RNA degradation. To maintain RNA stability, PBS may be used to remove cell debris and blood in some solid cancers.[28] In the intestinal tissues of rats and human children, washing has been reported to reduce RNA degradation, and different washing solutions, media, and PBS had no effect on RIN.[29]

In children, the RNA of proximal intestinal tissue degrades much faster than that from distal parts of the intestine.[29] This differs from our finding that RIN was slightly lower in colon tissue than from the rectum. This might be explained by the different bowel content in children and adult CRC patients. Digestive enzymes may contribute to RNA degradation in children,[29] while undigested or unabsorbed food may affect RNA degradation in CRC tissues. Therefore, washing the bowel content from cut tissues could help improve RNA stability.

Consistent with other reports, we observed that different wash processes of 5-minute duration did not affect RNA yield, purity, or integrity. For example, the Cooperative Human Tissue Network reported that the RNA quality from various epithelial cancer specimens, including CRC, was stable within 5 hours after surgery.[30] Micke et al. found that RNA remained stable in colon tissues for 6–16 hours under various conditions, including being left at room temperature and kept in normal saline.[31] Song et al. demonstrated that the quality of tissue stored for 6 years was not adversely influenced by cold ischemia times of <1 hour.[32] Guo et al. reported that the RNA of ischemia tumor tissues remained stable after 2 hours at room temperature, and degradation began at 4 hours.[28]

We found that RNA integrity was decreased after a 1-hour immersion in DMEM, but the RNA yield was unchanged. This suggests that specimens placed in DMEM for up to 1 hour will remain sufficient for biobanking. Most (83.3%) CRC tissues we assessed had a RIN score of >7. This is consistent with other studies that reported an average RIN >7 in CRC tissues.[33,34] Several studies have demonstrated that RIN values are organ dependent.[35] Fleige et al. reported higher RIN in extracted RNA from organ tissue and lower RIN in RNA extracted from connective tissue.[36] Bertilsson et al. reported that in prostate cancer, lower RIN was found from the stroma but higher RIN was found in the epithelium.[37] This suggests that our CRC tissues were mostly epithelial-rich cancers.

Our study has limitations. First, we did not have access to pathology data that could demonstrate tumor cell content. The tissues were dissected separately because heterogeneity within a single tumor was needed, but this meant that tissues subjected to different wash conditions had slightly different origins. Although we found high quality RNA in most samples, we cannot be certain that these specimens had a high
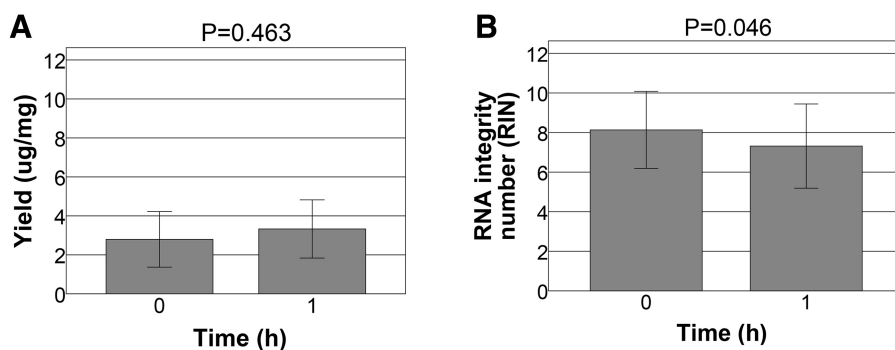
FIG. 4. Impact of tissue immersion in DMEM for 1 hour on RNA quality. **(A)** The average RNA yield at 0 and 1 hour (μg/mg). **(B)** The average RIN at 0 and 1 hour. The yield of isolated RNA was calculated by total concentration divided by tissue weight. Data of yield are shown as mean ± SD of six independent samples. *p*, the *p*-value of mean of six independent samples at 0 and 1 hour.

TABLE 3. RNA QUALITY AND QUANTITY
OF COLORECTAL CANCER TISSUES PLACED
IN DULBECCO'S MODIFIED EAGLE'S MEDIUM
0 AND 1 HOUR

	Time point (hour)	N	Mean ± SD	p[a]
A260/A280	0	6	1.935 ± 0.120	0.753
	1	6	1.893 ± 0.313	
A260/A230	0	6	1.730 ± 0.216	0.600
	1	6	1.828 ± 0.313	
Yield (μg/mg)	0	6	2.795 ± 1.426	0.463
	1	6	3.329 ± 1.492	
RIN[b]	0	6	8.133 ± 1.945	0.046
	1	6	7.316 ± 2.127	
28s:18s rRNA ratio	0	6	2.433 ± 0.987	0.042
	1	6	2.000 ± 0.975	

[a]Wilcoxon signed-rank.
[b]Average RNA integrity number.

tumor cell content. Zheng et al. reported a significant correlation between RNA integrity and the percentage of colorectal tumor cells with tumor cell content ≥75% and a RIN ≥7.[38] Zhang et al. reported that higher RIN values were observed in samples with higher tumor cell percentage or lower stroma percentage.[39] Second, we did not conduct gene expression analysis and so we cannot prove that the extracted RNA was functional. Third, our analysis was limited by small sample sizes in each wash condition group.

Consistent with our findings, previous studies have reported that RNA degradation of frozen tissues is time dependent.[28,32,33] However, another study concluded that RNA degradation is a minor problem in real-time PCR during handling of fresh human tissues before biobanking.[31] Additional analysis is needed to determine if immersing specimens in reagents for a prolonged period affects whole transcriptome analysis.

Conclusions

Different tissue washing processes did not affect the quality or quantity of RNA extracted from CRC tissues. Most samples had similar quality in terms of overall RNA yields and integrity. Our results suggest that a wash duration of 1 hour in DMEM can reduce RNA integrity. The integrity of extracted RNA is best protected by a wash period of not >5 minutes.

Acknowledgments

Authors' Contributions

O.A.: Methodology, investigation, visualization, project administration, funding acquirement. B.T.: Methodology, investigation, formal analysis, writing-original draft, writing-review, and edition. P.J.: Investigation, writing-review, and edition. P.A.: Providing resources. W.R.: Providing specimen resources. V.C.: Resources, funding acquirement, writing-review, and edition. P.T.: Conceptualization, supervision, writing-review, and edition.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

Supplementary Material

Supplementary Table S1

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
2. De Rosa M, Pace U, Rega D, et al. Genetics, diagnosis and management of colorectal cancer (Review). Oncol Rep 2015;34:1087–1096.
3. Bogaert J, Prenen H. Molecular genetics of colorectal cancer. Ann Gastroenterol 2014;27:9–14.
4. Budinska E, Popovici V, Tejpar S, et al. Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. J Pathol 2013;231:63–76.
5. Kuipers EJ, Grady WM, Lieberman D, et al. Colorectal cancer. Nat Rev Dis Primers 2015;1:15065.
6. Gallego RI, Pai AA, Tung J, et al. RNA-seq: Impact of RNA degradation on transcript quantification. BMC Biol 2014;12:42.
7. Choi Y, Kim A, Kim J, et al. Optimization of RNA extraction from formalin-fixed paraffin-embedded blocks for targeted next-generation sequencing. J Breast Cancer 2017;20:393–399.
8. Evers DL, Fowler CB, Cunningham BR, et al. The effect of formaldehyde fixation on RNA: Optimization of formaldehyde adduct removal. J Mol Diagn 2011;13:282–288.
9. Madabusi LV, Latham GJ, Andruss BF. RNA extraction for arrays. Methods Enzymol 2006;411:1–14.
10. Chen H, Luthra R, Goswami RS, et al. Analysis of preanalytic factors affecting the success of clinical next-generation sequencing of solid organ malignancies. Cancers (Basel) 2015;7:1699–1715.
11. Mutter GL, Zahrieh D, Liu C, et al. Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. BMC Genomics 2004;5:88.
12. Florell SR, Coffin CM, Holden JA, et al. Preservation of RNA for functional genomic studies: A multidisciplinary tumor bank protocol. Modern Pathol 2001;14:116–128.
13. Van Eijsden RG, Stassen C, Daenen L, et al. A universal fixation method based on quaternary ammonium salts (RNAlater) for omics-technologies: *Saccharomyces cerevisiae* as a case study. Biotechnol Lett 2013;35:891–900.
14. Drakulovski P, Locatelli S, Butel C, et al. Use of RNAlater as a preservation method for parasitic coprology studies in wild-living chimpanzees. Exp Parasitol 2013;135:257–261.

15. Bennike TB, Kastaniegaard K, Padurariu S, et al. Comparing the proteome of snap frozen, RNAlater preserved, and formalin-fixed paraffin-embedded human tissue samples. EuPA Open Proteom 2016;10:9–18.
16. Lindner M, Morresi-Hauf A, Stowasser A, et al. Quality assessment of tissue samples stored in a specialized human lung biobank. PLos One 2019;14:e0203977.
17. Hatzis C, Sun H, Yao H, et al. Effects of tissue handling on RNA integrity and microarray measurements from resected breast cancers. J Nati Cancer Inst 2011;103:1871–1883.
18. Sandberg R, Ernberg I. Assessment of tumor characteristic gene expression in cell lines using a tissue similarity index (TSI). PNAS 2005;102:2052–2057.
19. Barretina J, Caponigro G, Stransky N, et al. The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012;483:603–607.
20. Domcke S, Sinha R, Levine DA, et al. Evaluating cell lines as tumour models by comparison of genomic profiles. Nat Commun 2013;4:2126.
21. Goodspeed A, Heiser LM, Gray JW, et al. Tumor-derived cell lines as molecular models of cancer pharmacogenomics. Mol Cancer Res 2016;14:3–13.
22. Li X, Larsson P, Ljuslinder I, et al. Ex vivo organoid cultures reveal the importance of the tumor microenvironment for maintenance of colorectal cancer stem cells. Cancers (Basel) 2020;12:923.
23. Desjardins P, Conklin D. NanoDrop microvolume quantitation of nucleic acids. J Vis Exp 2010;2565.
24. Landolt L, Marti HP, Beisland C, et al. RNA extraction for RNA sequencing of archival renal tissues. Scand J Clin Lab Invest 2016;76:426–434.
25. Schroeder A, Mueller O, Stocker S, et al. The RIN: An RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 2006;7:3.
26. Horgan RP, Kenny LC. 'Omic' technologies: Genomics, transcriptomics, proteomics and metabolomics. Obstet Gynecol 2011;13:189–195.
27. Tanjak P, Rung in S, Acharayothin O, et al. 1955P - Live biobank of patient-derived organoids from Thai colorectal cancer patients enables clinical outcome prediction. Ann Oncol 2019;30:v787.
28. Guo D, Wang A, Xie T, et al. Effects of ex vivo ischemia time and delayed processing on quality of specimens in tissue biobank. Mol Med Rep 2020;22:4278–4288.
29. Heumuller-Klug S, Sticht C, Kaiser K, et al. Degradation of intestinal mRNA: A matter of treatment. World J Gastroenterol 2015;21:3499–3508.
30. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol 2002;161:1961–1971.
31. Micke P, Ohshima M, Tahmasebpoor S, et al. Biobanking of fresh frozen tissue: RNA is stable in nonfixed surgical specimens. Lab Invest 2006;86:202–211.
32. Song SY, Jun J, Park M, et al. Biobanking of fresh-frozen cancer tissue: RNA is stable independent of tissue type with less than 1 hour of cold ischemia. Biopreserv Biobank 2018;16:28–35.
33. Hong SH, Baek HA, Jang KY, et al. Effects of delay in the snap freezing of colorectal cancer tissues on the quality of DNA and RNA. J Korean Soc Coloproctol 2010;26:316–323.
34. Bao WG, Zhang X, Zhang JG, et al. Biobanking of fresh-frozen human colon tissues: Impact of tissue ex-vivo ischemia times and storage periods on RNA quality. Ann Surg Oncol 2013;20:1737–1744.
35. Walker DG, Whetzel AM, Serrano G, et al. Characterization of RNA isolated from eighteen different human tissues: Results from a rapid human autopsy program. Cell Tissue Bank 2016;17:361–375.
36. Fleige S, Walf V, Huch S, et al. Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. Biotechnol Lett 2006;28:1601–1613.
37. Bertilsson H, Angelsen A, Viset T, et al. RNA quality in fresh frozen prostate tissue from patients operated with radical prostatectomy. Scand J Clin Lab Invest 2010;70:45–53.
38. Zheng XH, Zhang SD, Zhang PF, et al. Tumor cell content and RNA Integrity of surgical tissues from different types of tumors and its correlation with ex vivo and in vivo ischemia. Ann Surg Oncol 2018;25:3764–3770.
39. Zhang H, Ha QY, Zhao ZH, et al. Biobanking of fresh-frozen gastric cancer tissues: Impact of long-term storage and clinicopathological variables on RNA quality. Biopreserv Biobank 2019;17:58–63.

Address correspondence to:
Pariyada Tanjak, PhD
Department of Surgery
Faculty of Medicine Siriraj Hospital
Mahidol University
2 Wanglang Road
Bangkok Noi
Bangkok 10700
Thailand

E-mail: pariyada.tan@mahidol.edu