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ORIGINAL ARTICLE

Method for preservation of DNA stability of liquid-based cytology specimens from a lung adenocarcinoma cell line

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Abstract
Liquid-based cytology (LBC) specimens of lung adenocarcinoma have the potential to be widely used for genetic analysis. However, formaldehyde contained in some LBC preservation solutions can cause DNA fragmentation during specimen storage, rendering the samples unsuitable for molecular analysis. To investigate a novel preservation technique for improved DNA stability, which was evaluated by mutation analysis of epidermal growth factor receptor (*EGFR*) gene in human lung adenocarcinoma cell lines. Cells were fixed in CytoRich Red preservation solution. After 30 min of fixation, cells were either stored using the conventional method (suspended in preservation solution) or washed in phosphate-buffered saline and stored as a cell pellet (newly proposed method). The effect of storage was evaluated after 5, 7, and 9 days of storage at ambient temperature. The cell pellet group was also tested after 14 and 28 days. Specifically, we evaluated the DNA stability, DNA yield, and sample suitability for polymerase chain reaction (PCR), and *EGFR* mutation detection. The DNA yields and degree of stability from the cell pellet group were higher than those from the suspension group at every time point examined. PCR amplification from the cell pellet group was successful up to day 28. Mutation detection using the Cycleave PCR method indicated that the Ct values of the cell pellet group were significantly lower than those of the suspension group. Storing LBC specimens as a cell pellet post-fixation can maintain the DNA quality for a longer period than the conventional method, making it a promising strategy for molecular analysis.

Keywords Cell pellet · DNA stability · Epidermal growth factor receptor · Liquid-based cytology · Lung cancer

Introduction

Lung cancer is the most common cause of death in Japan and worldwide [1]. Since the 2000s, the effectiveness of molecular targeted therapy has improved for those who harbour certain active cancer-driving mutations that can be specifically targeted in comparison with the usual anticancer drug treatment [2–4]. Typically, non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (*EGFR)* active mutation is highly responsive to *EGFR* tyrosine kinase inhibitors (TKIs). Thus, mutation testing for the selection of patients who can receive molecular targeted therapy has already been standardized for therapeutic strategies in recent years [5, 6].

Liquid-based cytology (LBC) method for clinical diagnosis began to be widely used in the early 2000s. The technical advantages over the conventional Papanicolaou smear method include uniform distribution of cells, elimination of drying of samples, and reduced loss of specimens [7–12]. Studies have reported promising results for the utilization of LBC for genetic analysis of lung cancer specimens [13–17]. Thus, in the near future, LBC specimens of lung adenocarcinoma for genetic analysis may become widely used. Some commercial preservative solutions for LBC include small amounts of formaldehyde, which is known to improve the imaging of specimens for diagnosis by preservation of cellular morphology. However, formaldehyde is also well known to cause

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degradation of DNA in formalin-fixed paraffin-embedded (FFPE) specimens [18–21]. The small amount of formaldehyde in LBC preservation solution may also cause some DNA degradation. Therefore, numerous studies have examined whether specimens fixed in preservative solution containing formaldehyde are suitable for genomic analyses. These studies have investigated various factors, such as required cell number [22], storage duration [23–26], preparation techniques [27], and DNA quality for next-generation sequencing [26]. However, till date, there has been no clear evidence indicating whether LBC specimens can be utilized for genetic analysis under certain conditions.

Therefore, in the current study, we evaluated whether storing LBC specimens in a state of cell pellet could improve the DNA stability compared with the conventional method, which stores LBC specimens as a suspension in the preservation solution. Particularly, we examined the quality of DNA and mutation analysis of *EGFR* using human lung adenocarcinoma cell lines.

Materials and methods

Ethics statement

The current study was preformed according to the Declaration of Helsinki and was approved by the ethics committee of Kitasato University Medical Ethics Organization (Approval No. KME B14-155).

Cell lines

The human lung adenocarcinoma cell line H1975 was purchased from the American Type Culture Collection (ATCC; Manassas, Virginia). H1975 cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640; Merck, Darmstadt, Germany) supplemented with 10% foetal calf serum (Biosera, Nuaille, France), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific, Waltham, Massachusetts), and incubated at 37 °C in 5% CO_2/95% air. H1975 is known to contain the *EGFR* double mutation: T790M in exon 20 and L858R in exon 21.

Sample fixation and storage

The cultured cells were collected using TrypLE Express (Thermo Fisher Scientific) and washed with phosphate-buffered saline (PBS) (Thermo Fisher Scientific). The collected cells were immersed in CytoRich Red preservative (CytoRich Red; Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) at 1×10^6 cells/mL. CytoRich Red consisted mainly of 23.3% isopropanol, 10% methanol, 6.7% ethylene glycol, and 0.4% formaldehyde, with a pH of 7.5. After fixation time of 30 min, the fixed cells were split into two groups: group of suspension in CytoRich Red (S-CR) and group of cell pellet (CP). The cells in each group were stored for 5, 7, and 9 days. In the case of the CP group, cells were stored for an additional 14 and 28 days. In each case, 1 mL of the fixed cells (1×10^6) was dispensed into a 1.5-mL tube; ten specimens each were prepared for the 5, 7, and 9 days of storage, and 8 specimens for the 14 and 28 days of storage. The cells in the S-CR group were stored as cells immersed in preservation solution at ambient temperature (conventional method, recommended by manufacturer). The cells of CP group were centrifuged at 2000 ×*g* for 5 min at 23 °C, followed by removal of CytoRich Red by aspiration. Subsequently, the cells were washed once with PBS and spun down. After removal of the supernatant, the cell pellets were stored at ambient temperature.

Genomic DNA extraction

Genomic DNA from cells was purified using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Extraction protocol "for tissues" was used based on our previous study [28]. In the case of the S-CR group, cells were spun down, washed once with PBS, and then resuspended in 80 μL PBS, before continuing with the extraction procedure. In the case of the CP group, the cell pellet was resuspended in 80 μL PBS, and without washing was subjected to the extraction procedure. Extracted DNA was quantified using the NanoDrop ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific). The DNA quality was analyzed using a 4200 TapeStation system (Agilent Technologies; Santa Clara, CA), which measures DNA integrity number (DIN) and quantifies double-stranded (ds) DNA by electrophoretic separation. The gel electrophoresis was performed using Genomic DNA ScreenTape (Agilent Technologies). The DIN is represented from 1 to 10, with a higher number representing highly intact DNA and smaller number representing highly degraded DNA.

DNA fragmentation analysis and PCR amplification

To analyze DNA fragmentation, genomic DNA samples (500 ng/lane, quantified by NanoDrop) were separated by gel electrophoresis using a 0.8% agarose gel in 0.5 × tris-borate-EDTA buffer. Next, the L858R mutation region was amplified using genomic DNA (100 ng, quantified by NanoDrop). The primer sequences for exon 21 of the *EGFR* gene are as follows: forward primer, 5′-AGGG CATGAACTACTTGGA-3′; reverse primer 5′-AAAT GCTGGCTGACCTAAAG-3′ (amplicon size: 190 bp). The DNA was amplified using AmpliTaq Gold 360 PCR Master Mix (Thermo Fisher Scientific) in a reaction volume of 20 μL and the cycling conditions were as follows: initial denaturation at 95 °C for 10 min followed by 35 cycles of 95 °C for

30 s, 60 °C for 30 s, and 72 °C for 30 s, and then a final extension at 72 °C for 7 min using a TaKaRa PCR Thermal Cycler Dice® Touch (Takara Bio, Shiga, Japan). Then, 3 μL of the PCR product was loaded in each lane of a 1.5% agarose gel in 0.5 × tris-borate-EDTA buffer and separated by gel electrophoresis. The agarose gels were stained with ethidium bromide solution, and the amplified DNA bands were semi-quantified.

EGFR mutation analysis

To determine whether genomic DNA extracted from cells of both groups can be utilized for detecting *EGFR* mutations, we analyzed the mutation in exon 20 (T790M) and exon 21 (L858R) by Cycleave PCR using a C1000 Touch thermal cycler (Bio-Rad Laboratories, Inc., Hercules, California). The primer and probe sequences for exon 20 are as follows: forward primer, 5′-TGCGAAGCCACACTGAC-3′; reverse primer, 5′-TCTGCACACACCAGTTGA-3′; and probe, 5′-CTGCATGATG (FAM)-3′. The primer and probe sequences for exon 21 are as follows: forward primer, 5′-GCAG CATGTCAAGATCAC-3′; reverse primer, 5′-TGAC CTAAAGCCACCTC-3′, and probe 5′-GCCCGCC (FAM)-3′. The genomic DNA samples (5 ng, quantified by NanoDrop) were amplified using Cycleave PCR Reaction Mix (Takara Bio) and the following cycling conditions: initial denaturation at 95 °C for 20 s, followed by 50 cycles of 95 °C for 5 s, 60 °C for 30 s. DNA damage and fragmentation were evaluated by comparing the Ct (threshold cycle) values for each preservation time.

Statistical analysis

All data are expressed as the mean ± standard error of the mean. Statistical analysis was performed using the JMPPro 14.2 software (SAS Institute Inc., Cary, North Carolina). The Wilcoxon comparison test was used to compare the groups. P value < 0.05 was considered to be statistically significant.

Results

Genomic DNA yield and quality

The yield of nucleic acids and dsDNA extracted from cells in the CP group was significantly higher than that from the S-CR group for storage durations of 5, 7, and 9 days. Further, the yields tended to decrease with increasing storage duration. The tendency of the A260 value and amount of dsDNA were almost the same. However, in the S-CR group, the value of A260 increased approximately twofold at day 9 compared with that at days 5 and 7, while the yields at days 5 and 7 were similar for both groups (Fig. 1a). Meanwhile, the dsDNA yield increased only slightly at day 9 compared with that at days 5 and 7 (Fig. 1b). The average DNA purity (ratio of the absorbance at 260 and 280 nm) on the days 5, 7, and 9 of the S-CR group and days 5, 7, 9, 14, and 28 of the CP group were 2.22, 2.05, 2.10, 2.06, 2.02, 2.04, 2.02, and 2.00, respectively.

The DNA extracted from cells in the CP group showed significantly higher DIN than that from the S-CR group at storage durations of 5, 7, and 9 days. In the CP group, the DINs were almost equivalent at all time points. In contrast, the average DIN on day 9 in the S-CR group was increased compared with that at days 5 and 7 (Fig. 1c). In the CP group, the yield of nucleic acids and dsDNA was decreased after day 9, and the decrease in the A260 value and dsDNA from day 9 to day 28 was approximately 70% and 30%, respectively. However, the DNA qualities were maintained high from day 5 to day 28, evident from the similar DIN values.

Genomic DNA visualized by agarose gel electrophoresis is shown in Fig. 2 a. In the S-CR group, the DNA fragmentation pattern was markedly observed at all preservation durations and almost no residue of intact high molecular weight genomic DNA band was seen. In the CP group, undegraded genomic DNA band was observed in all storage duration samples. The smear bands observed below 2 kbp on days 5, 7 and 9 for both groups were decreased in the CP group on days 14 and 28 (Fig. 2a). The dsDNA visualized by electrophoresis using TapeStation is shown Fig. 2 b. The tendencies were same with agarose gel electrophoresis. In the CP group, the bands of intact dsDNA at high molecular weight were observed markedly at all storage durations. In the S-CR group, the high molecular weight bands disappeared at all storage durations (Supplementary Fig. 1).

PCR and mutation analyses of the *EGFR* gene

High levels of the amplified PCR products (exon 21 pf *EGFR* gene) were detected in the S-CR group for days 5 and 7, but the band for day 9 was weaker than that for day 5 or 7. In contrast, in the CP group, high levels of amplified PCR products were detected for all samples (day 5 through 28) (Supplementary Fig. 2).

To assess efficiency of mutation detection for each preservation time, we compared the Ct values of quantitative PCR by Cycleave PCR method. In the S-CR group, detection rates of the *EGFR* exon 20 (T790M) mutation were 100% (10 of 10 specimens) on day 5, 80% (8 of 10 specimens) on day 7, and 20% (2 of 10 specimens) on day 9, while those of *EGFR* exon 21 (L858R) mutation were 90% (9 of 10 specimens) on day 5, 50% (5 of 10 specimens) on day 7, and 20% (2 of 10 specimens) on day 9. In the CP group, detections of both exon 20 and 21 mutations were possible in all specimens (all storage durations). Ct value of the samples from the CP group was significantly lower than that of samples from the S-CR group

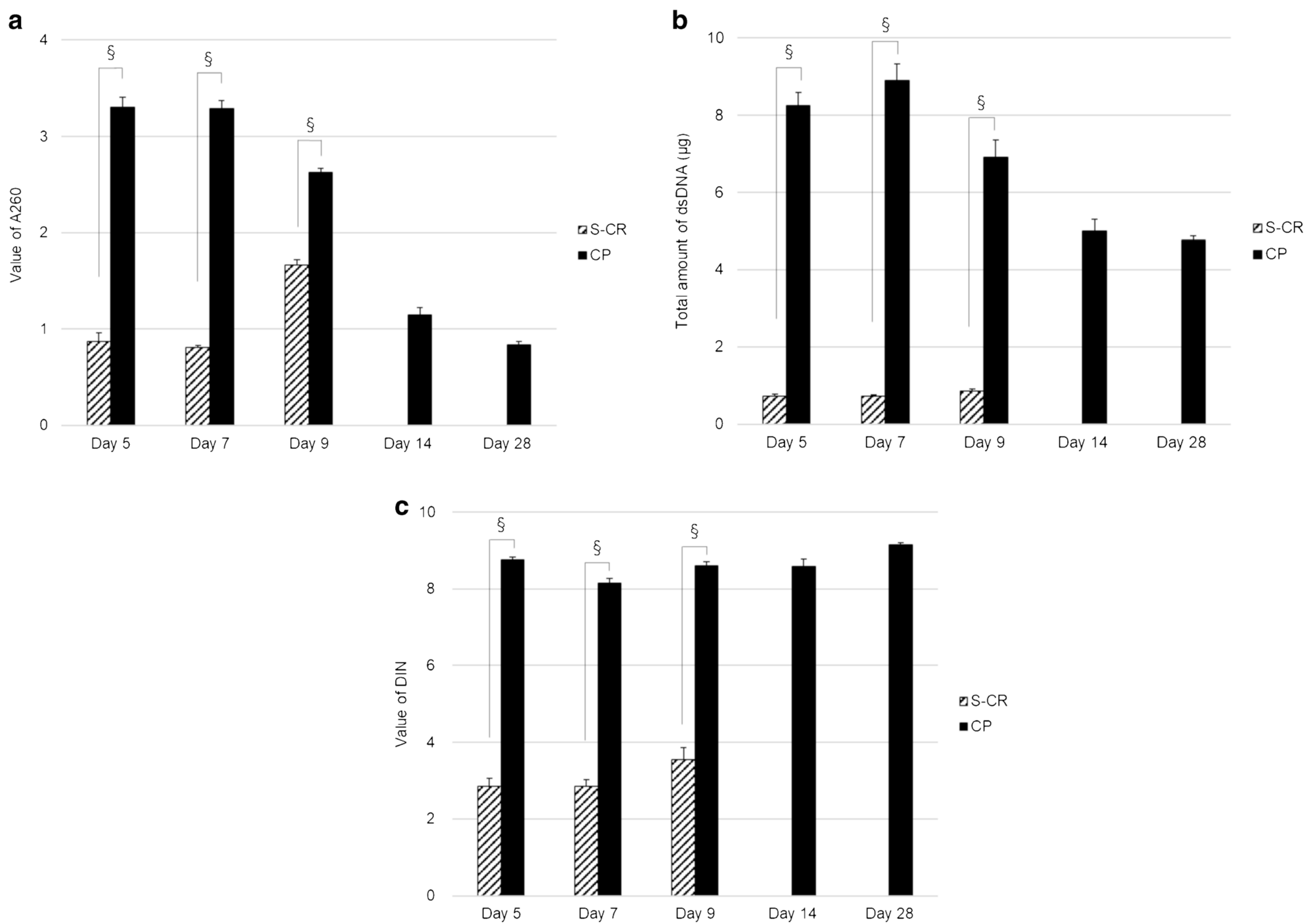

Fig. 1 **a** Absorbance values measured at 260 nm (A260) of DNA, **b** total amount of dsDNA, and **c** value of DIN from 1×10^6 cells after fixation at indicated time points. In the S-CR group, the specimens were stored by immersing in the preservation solution (CytoRich Red) for 5, 7, and 9 days. In the CP group, the specimens were washed with PBS after 30 min of fixation and then stored as cell pellets for 5, 7, 9, 14, and 28 days. ($n = 10$ for samples stored for 5, 7, and 9 days, $n = 8$ for samples stored for 14 and 28 days; n is lower owing to insufficient numbers of cells). In the SC-R group, the DINs were undetectable in 20% (2 of 10 specimens) on day 5 and 10% (1 of 10 specimens) samples on day 9. $^{\S}p < 0.001$

for days 5 to 9 for both exon 20 (T790M) and 21 (L858R) mutations (Table 1). The difference in CT values of S-CR and CP was approximately 9 to 10, indicating that the DNA amounts in the CP group were ~ 500 to 1000 times more than those in the S-CR group.

Discussion

In this study, we showed that storing LBC specimens of lung cancer cell in a cell pellet state improved the stability of the DNA in the sample. Important steps include the washing of fixed cells with PBS and removing the preservation solution immediately to avoid over fixation. Although CytoRich Red contains 0.4% formaldehyde, the novel method yielded higher amounts of DNA as well as better stability of the extracted DNA than storing specimens in the state of suspension. In the S-CR group, the A260 values were increased approximately twofold at day 9 compared with that at days 5 and 7, whereas the values were decreased with the preservation time in the CP group. This result can be explained by various factors. Firstly, UV absorbance can increase by hyperchromic effect caused by denaturation of DNA due to formaldehyde. The increase in the A260 value on day 9 was larger than that at days 5 and 7 in the case of dsDNA. Therefore, the increased UV absorbance on day 9 could be caused by DNA or RNA denaturation and secondly, reduction of dsDNA by unknown causes, effect of collection rate, and loss of cells or DNA during extraction. Therefore, the amount of DNA on day 9 could be the same as that on day 5 or 7. A similar tendency was observed in our previous study using the same cell line H1975 [28]. Our results indicated that DNA extracted from the pellet did not degrade and retained high quality during storage at ambient temperature.

Moreover, in the S-CR group, the DIN value on the day 9 was higher than that on days 5 and 7. Some specimens showed DIN value around 4 on day 9; this might be due to fluorescence caused by denaturation of higher order structures of

DNA or RNA. PCR efficiency on day 9 was lower than that on days 5 and 7, whereas the DIN on day 9 was almost the same as that on days 5 and 7. Thus, these results indicated that PCR efficiencies on day 9 were inhibited by excessive denaturation of DNA or RNA. Therefore, when analyzing stored specimens, both A260 value and DIN should be determined. The smear bands below 2 kbp were decreased on days 14 and 28 in the CP group on agarose gel electrophoresis. The

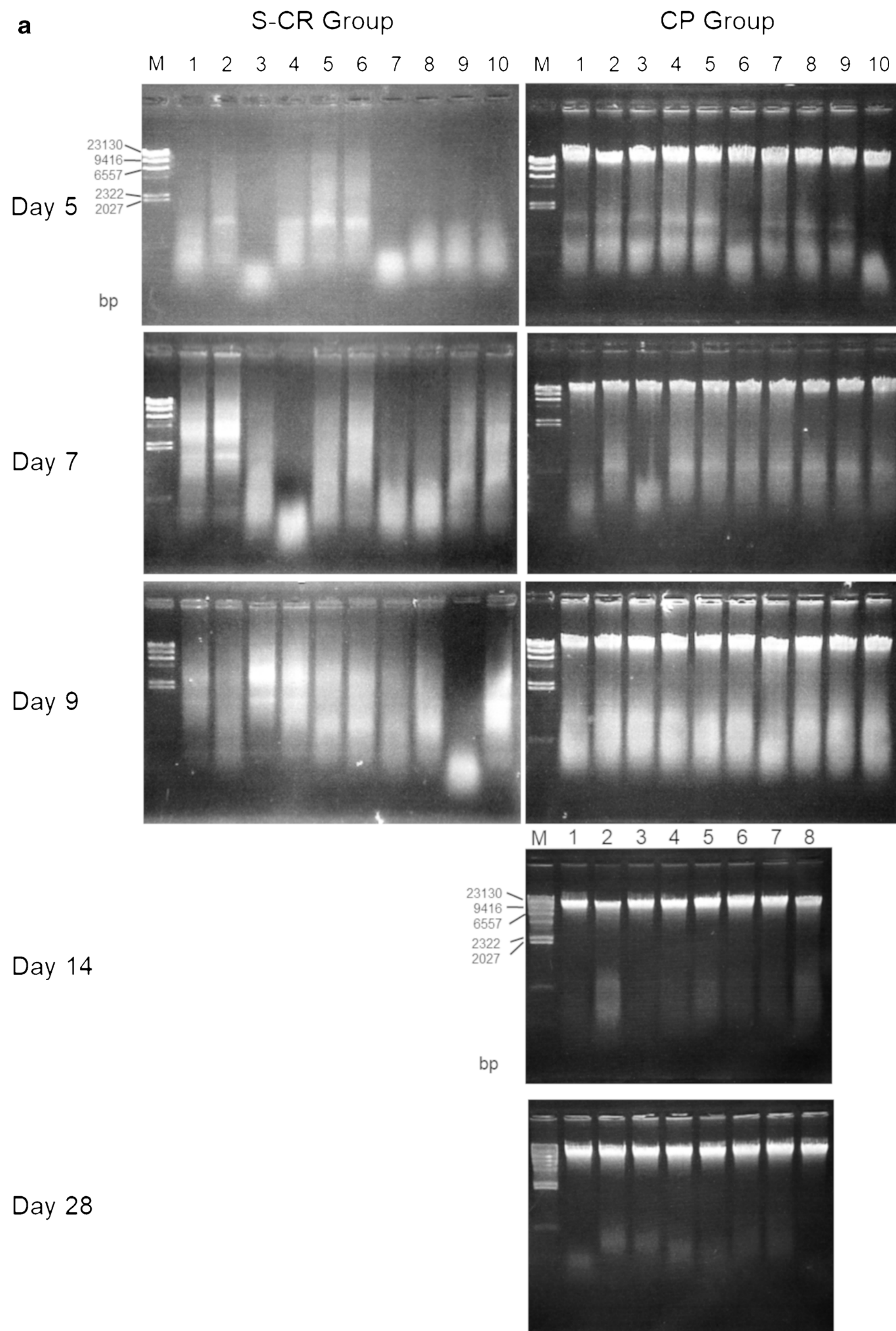

Fig. 2 Images of DNA fragmentation for each preservation time. Numbers 1 to 10 indicate the sample number ($n = 10$ for samples stored for 5, 7, and 9 days, $n = 8$ for samples stored for 14 and 28 days). **a** DNA (500 ng) was electrophoresed using 0.8% agarose gels. Lane M shows the λ DNA *Hin*dIII digest marker. **b** DNA (1 μL) was electrophoresed using Genomic DNA ScreenTape. Lane M shows the marker included in the Genomic DNA Reagents (Agilent Technologies)

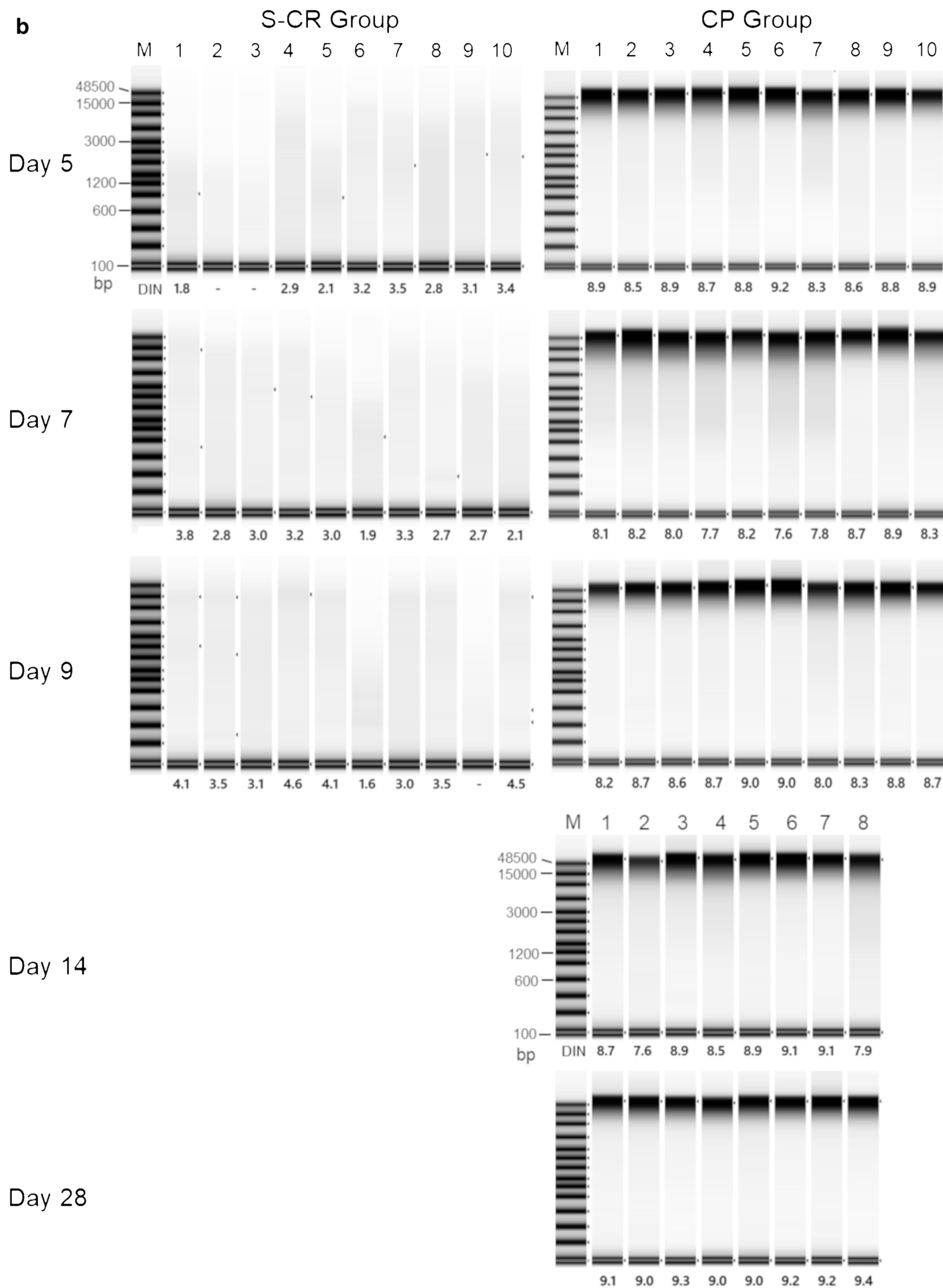

Fig. 2 (continued)

decrease indicated a lack of RNA, because the degraded RNA could not have mingled with the collected DNA. Moreover, the decreasing rate of A260 values from day 9 to days 14 and 28 in the CP group was higher than that of dsDNA yields, further suggesting that the decreasing A260 values reflected a lack of RNA.

Table 1 Ct values obtained with Cycleave PCR method for epidermal growth factor receptor (*EGFR*) mutations for each preservation duration. (a) Analysis of *EGFR* exon 20 (T790M) and (b) exon 21 (L858R)

a

T790M

	Day 5		Day 7		Day 9		Day 14		Day 28	
Sample number	S-CR	CP	S-CR	CP	S-CR	CP	S-CR	CP	S-CR	CP
1	38.39	29.34	N.A.	29.50	N.A.	30.14	N.D.	28.43	N.D.	28.56
2	38.20	29.59	39.67	29.14	N.A.	29.81	N.D.	29.50	N.D.	28.43
3	39.00	29.71	38.20	29.34	41.67	29.43	N.D.	27.35	N.D.	27.96
4	37.34	29.52	38.66	29.43	N.A.	29.25	N.D.	28.21	N.D.	28.19
5	35.96	29.57	36.59	29.18	N.A.	29.11	N.D.	28.45	N.D.	28.26
6	39.51	29.43	40.74	29.64	N.A.	29.32	N.D.	28.17	N.D.	28.20
7	38.76	29.16	40.13	29.35	N.A.	28.92	N.D.	28.30	N.D.	28.15
8	38.71	29.98	N.A.	29.65	43.68	29.82	N.D.	29.15	N.D.	27.84
9	39.35	29.24	38.04	29.02	N.A.	29.62	N.D.	N.D.	N.D.	N.D.
10	37.99	29.89	38.82	29.05	N.A.	29.41	N.D.	N.D.	N.D.	N.D.
Mean ± SEM	38.32 ± 1.05	29.54 ± 0.26	38.85 ± 1.32	29.33 ± 0.23	42.68 ± 1.42	29.48 ± 0.37	N.D.	28.44 ± 0.65	N.D.	28.20 ± 0.23

b

L858R

Sample number	Day 5		Day 7		Day 9		Day 14		Day 28	
	S-CR	CP	S-CR	CP	S-CR	CP	S-CR	CP	S-CR	CP
1	37.25	28.91	N.A.	28.91	N.A.	29.37	N.D.	28.16	N.D.	28.65
2	37.50	28.70	37.88	28.70	37.88	29.14	N.D.	29.04	N.D.	28.25
3	37.50	28.56	N.A.	28.56	N.A.	28.77	N.D.	27.17	N.D.	27.75
4	38.02	28.50	N.A.	28.50	N.A.	28.39	N.D.	27.83	N.D.	27.72
5	36.12	28.42	40.44	28.42	40.44	28.59	N.D.	27.79	N.D.	27.91
6	42.02	28.81	N.A.	28.81	N.A.	28.59	N.D.	27.60	N.D.	27.93
7	N.A.	28.72	N.A.	28.72	N.A.	28.36	N.D.	27.70	N.D.	27.90
8	37.02	28.71	N.A.	28.71	N.A.	29.18	N.D.	28.47	N.D.	28.00
9	35.89	28.34	N.A.	28.34	N.A.	29.09	N.D.	N.D.	N.D.	N.D.
10	37.79	28.33	N.A.	28.33	N.A.	28.88	N.D.	N.D.	N.D.	N.D.
Mean ± SEM	37.68 ± 1.78	28.60 ± 0.20	39.16 ± 1.81	28.60 ± 0.20	39.16 ± 1.81	28.84 ± 0.35	N.D.	27.97 ± 0.58	N.D.	28.02 ± 0.31

CP group wherein specimen is stored as a cell pellet, *N.A* not available, *N.D* not determined, *S-CR* group wherein specimen is stored suspended in CytoRich Red. Data are presented as mean ± standard error of mean (SEM). ($n = 10$ for days 5, 7, and 9, and $n = 8$ for days 14 and 28)

LBC specimens fixed in CytoRich Red are recommended to be stored at ambient temperature for approximately 30 days and a maximum of 6 months for morphological stabilization by the manufacturer. In our previous study, we investigated the effect of storage time from 30 min to 9 days after fixation in CytoRich using two extraction protocols. The results indicated that genomic DNA extracted from human adenocarcinoma cell lines fixed in CytoRich Red can be utilized for *EGFR* mutation detection, although yields of DNA tended to decrease with the storage time and the DNA showed fragmentation after 1 h in the fixation solution. In addition, we found that the DNA extraction protocol for tissue (QIAamp DNA Mini Kit) is suitable for cells fixed in CytoRich Red [28].

Generally, biopsy specimens are stored at below 4 °C for stability of DNA. However, previous studies reported that there was no difference in the suitability of LBC specimens for human papillomavirus DNA test between ambient and refrigerated temperature when fixed in SurePath preservation solution [29, 30]. We also attempted to maintain the quality of LBC specimens immersed in CytoRich Red preservation solution by storing the samples in the refrigerator or freezer using 3 cell lines: H1975, A549, and PC-9 (Supplementary Fig. 3). Specifically, we compared fragmentation by agarose gel electrophoresis and *EGFR* PCR product of the DNA extracted from suspension and pellet specimens after 3 days of storage at ambient temperatures, 4, − 20, and − 80 °C. DNA fragmentation was observed only in the suspension specimen stored at ambient temperature (Supplementary Fig. 3a). *EGFR* PCR product was successfully detected in all specimens at each storage condition, although suspension specimen stored at ambient temperature showed a weak signal (Supplementary Fig. 3b).

Recently, Kawahara et al. reported that DNA extracted from LBC specimens of cell lines preserved for 5 or 40 days

at room temperature can be used to detect *EGFR* mutations by PNA–LNA PCR clamp and TaqMan assays, but details were not included [24]. Akahane et al. reported that DNA extracted from specimens fixed in LBC preservation solution and stored at 4 °C could be successfully subjected to next-generation sequencing of either lines stored for 90 days and residual LBC specimens for 1 year [26]. We demonstrated only a molecular monogenic test such as *EGFR* mutation in this study. Multiple or comprehensive gene analysis by NGS would be succeed by both CP- and S-CR-treated specimens. However, CP-treated specimens show extremely higher value of DIN than S-CR-treated specimens (Fig. 1c), suggesting that CP-treated genomic DNA might be suitable for such whole exome analysis. Clinical liquid cytology specimens treated by CP method such as pleural effusion or ascites would be useful for comprehensive gene analysis in the near future. As a result of our preliminary experiment using the 3 cell lines including H1975, the qualities of DNA extracted from cells stored at ambient temperature compared favourably with those of DNA extracted from cells stored at other temperatures in the case of the CP groups (Supplementary Fig. 3a). In addition, it is more practical to store clinical specimens at ambient temperature. Therefore, our method of storing residual LBC specimens in microtubes at ambient temperature could be beneficial, easy, and economical for use in clinical laboratories.

The results of the current study indicated that pellet specimens could be stored for longer than suspension specimens at ambient temperature for obtaining sufficient quality for molecular analysis. Therefore, we considered that the condition of DNA extracted from pellet is similar to that from specimens scratched from the LBC slide, and both specimens should be stored separately from the preservation solution after fixation for further analyses. Kim et al. reported that DNA extracted from LBC slide of thyroid fine needle aspiration biopsy stored for 6 months could be successfully used for PCR amplification, whereas storage for 9 months failed to provide DNA of sufficient quality [31]. We found that the DNA quality of LBC specimens is maintained for longer when the preservative solution is removed (thereby removing formaldehyde) than when it is stored immersed in the solution. Thus, the present study suggested that the improvement of DNA stability was achieved by avoiding over fixation. Over fixation using formalin is already well known to cause deterioration of DNA and protein quality. Generally, each specimen type has to be observed after a recommended fixation time for efficient molecular testing [18, 19, 32, 33]. However, the new storage method could allow longer storage durations of specimens for molecular testing.

There are some limitations and considerations in this study. First, our study was limited to using only CytoRich Red preservation solution, and to the detection of only *EGFR* mutations using only one lung adenocarcinoma cell line with high allelic fraction of the mutation, with sufficient cell numbers. Therefore, future studies should test other preservation solutions and various kinds of mutations using residual clinical LBC specimens including some impurities such as blood or other components to confirm the effective utilization of LBC specimens. Moreover, we will perform mutation analyses using low allele cells mixed with known number of mutant types, such as H1975, and wild type artificially in the future. Second, the following consideration should be made: LBC slides cannot be duplicated for morphological diagnosis, which was the original purpose; therefore, if one needs to also carry out morphological examination, then the specimens can be left suspended in the preservation solution at ambient temperature for 3 to 4 days, and DNA extracted from it can be used for molecular testing, according to our previous study [28].

In conclusion, LBC specimens fixed in preservation solution containing a low concentration of formaldehyde can be stably preserved for good-quality DNA for 4 weeks as a cell pellet after PBS washing. Thus, our demonstrated method is promising for obtaining good-quality DNA from residual LBC specimens for genomic analysis. Thus, using this method, LBC specimens of lung cancer could be used not only for morphological diagnosis, but also for molecular testing after a long period of preservation.

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Authors' contributions Yukiko Matsuo: Conception and design of this study, acquisition, analysis, and interpretation of data, writing—original draft, and writing—review and editing. Kazuya Yamashita: Conception and design of this study, acquisition, analysis, and interpretation of data, and writing—review and editing. Tsutomu Yoshida: Conception and design of this study, interpretation of data, and writing—review and editing. Yukitoshi Satoh: Conception and design of this study, interpretation of data, project administration, and writing—review and editing. All authors approved the final version of the manuscript to be published and are accountable for all aspects of the work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The current study was preformed according to the Declaration of Helsinki and was approved by the ethics committee of Kitasato University Medical Ethics Organization (Approval No. KME B14-155).

Consent to participate Not applicable.

Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

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