http://www.cjmb.org

Open Access Original Article

Crescent Journal of Medical and Biological Sciences
Vol. 5, No. 1, January 2018, 25–28
eISSN 2148-9696

Comparison of Methods of RNA Extraction From Breast and Gastric Cancer Tissues

Seyed Amin Norollahi[1], Parviz Kokhaee[2,3], Ali Rashidy-Pour[4,5], Vida Hojati[1], Seyedeh Elham Norollahi[4], Laleh Vahedi Larijani[6], Ali Akbar Samadani[4,3*]

Abstract

Objective: Optimal quality and quantity of extracted RNA is the first step in molecular biology analysis and investigation. In this way, several methods have been proposed in order to obtain the best quality of RNA in different cases. On the other hand, RNA extraction from cells and tissues is different.

Materials and Methods: In this study, the effects of 4 common RNA extraction kits including Trizol, AccuZol, Ribozol and TriPure and also the effect of RNAlater and liquid nitrogen were compared and studied on 50 breast cancer and 50 gastric cancer tissues. Remarkably, the quality of the extracted RNA was investigated using real-time PCR TaqMan assay on *HER2* gene.

Results: The results showed better relative quality of extracted RNA with Trizol kit compared to other kits in this study.

Conclusion: Conspicuously, fewer amount of tissues between 10 to 30 mg lead to gain a much better quality of RNA. Meanwhile, the expression of *HER2* gene indicates a suitable performance of extracted RNA qualitatively and quantitatively. Notably, *GAPDH* gene was used as internal control in all samples.

Keywords: RNA extraction, Histopathological, Gastric cancer, Breast cancer.

Introduction

The first step in gene expression and molecular research study is having a good purity and quality of extracted RNA (1,2). Relatively, RNA has an unstable and sensitive structure in comparison with DNA. RNase enzyme can degrade RNA easily. In this account, compatible RNA extraction techniques with high quality in many applications have been considered with much attention recently (3,4). The RNA extraction from various tissues and cells is different and is associated with their certain situations. Therefore, optimization of the extraction of RNA from animal and human tissues (especially cancerous tissues) is specifically challenging and important in some cases (5,6). As it was mentioned, the expression level of RNA is a good indicator of physiological status of cells and tissues and the accuracy of gene expression measurement is greatly affected by the quantity and quality of extracted RNA (7,8). Relatively, success in each analysis (e.g. real-time PCR, microarray etc) is based on the purity and integrity of extracted RNA and appropriate quantity (9,10). In recent years, the quality and quantity of RNA analysis are important parts of studies which are assigned to different methods of extraction. For example, using homogenizer or liquid nitrogen in order to grind the samples can create different quality and quantity of RNA (11,12). The source of RNA (human, plants, tissues, cells etc) for extraction affects the quantity and quality of final product.

Correspondingly, using Trizol commercial solution for extracting RNA obtain lower quality of RNA compared with column-based techniques (13,14). Since the extraction of RNA from human tissue specimens, which are extremely valuable, is the starting point in studies such as the study of gene expression. The aim of this study is to investigate the optimization of RNA extraction methods in gastric and breast cancer tissues and also the evaluation of extracted RNA, employing *HER2* gene by TaqMan assay.

Materials and Methods

In order to protect the tissues from RNase activities, all the surgical tools were placed in a diethylpyrocarbonate (DEPC) solution for at least 1 hour.

For removing the toxic properties of DEPC solution, all

Received 6 June 2016, **Accepted** 2 September 2017, **Available online** 17 September 2017

[1]Department of Bioloy, Damghan Branch, Islamic Azad University, Damghan, Iran. [2]Faculty of Medicine, Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran. [3]Faculty of Medicine, Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran. [4]Faculty of Medicine, Research Center of Physiology, Semnan University of Medical Sciences, Semnan, Iran. [5]Faculty of Medicine, Department of Physiology, Semnan University of Medical Sciences, Semnan, Iran. [6]Department of Pathology, BoAli Sina Hospital, Mazandaran University of Medical Sciences, Sari, Iran.
***Corresponding Author:** Ali Akbar Samadani, Tel: +989113534399, Email: a_a_hormoz@yahoo.com

the tools and devices were kept in the oven for 3 hours. After obtaining informed written consent from the patients, tissues were obtained directly by gastrectomy and mastectomy in surgery room. Tissues were cut into the dimensions of a few millimeters apart and then some of them were put into special pipes called cryotubes and then they were quickly moved into the liquid nitrogen tank. A number of other tissues were placed in RNAlater solution and then all of them were transported to the laboratory. Finally, all the samples were deep frozen.

All the tissues in the laboratory were ground to a homogenous powder using a mortar and pestle under liquid nitrogen, according to the manufacturer's protocol and once again it was done by homogenizer set. All the tissues were divided into 4 groups in terms of value as follows: 10-30, 30-50, 50-70 and 70-90 mg.

Quality and Quantity of Extracted RNA

In order to verify the quality of extracted RNA, electrophoresis was performed on 2% agarose gel in order to observe the 5, 18 and 28S bands. Then, for quantity verification, all the RNAs were measured by a NanoDrop spectrophotometer and their OD was evaluated.

In addition, other variables such as the effects of coldness, temperature and speed of operation were evaluated.

cDNA and Real-Time PCR

In order to ensure the quality and quantity of extracted RNA, 10 suitable samples with high quality and quantity were selected for cDNA synthesis and real-time PCR reaction in order to observe the fluctuations of *HER2* gene expression in both gastric and breast cancer tissues.

In this way, cDNA QuantiTect Reverse Transcription kit (QIAGEN, USA) was employed and 2 ng of RNA was used and then the PCR reaction was done by real-time PCR (ABI 7300, USA), TaqMan assay, with specific primers. The specific primers were designed by Oligo version 7.56 as follows:

The Sequence of *HER2* Gene

- Forward> 5' GAATGGAACTGCATGATCTCC -3'
- Reverse> 5' ACAGGTGGTGTCACAGGATC -3'
- Probe>FAM-CTCTCTTTGGTGCCGCTGCTCAC-TAMRA

Statistical Analysis

Statistical analysis of obtained data and results was analyzed by SPSS statistics software version 16.0 (SPSS Inc, Chicago, USA).

Analysis of variance (ANOVA) test was used and *P*-value of less than 0.05 was considered significant.

Results

Quantitative Analysis of Extracted RNA

The results of OD which were obtained by a spectrophotometer, Nanodrop set, for cancerous breast tissues (Table 1) indicated a different data in comparison with the data obtained for gastric cancer tissues (Table 2).

Qualitative Analysis of Extracted RNA

The results of using liquid nitrogen (Figure 1), in order to make powder the gastric and cancerous breast tissues, in comparison with using homogenizer set instead of liquid nitrogen and also the maintenance quality of tissues with RNAlater solution and liquid nitrogen (Figure 2), electrophoresis of tissues on 2% agarose gel (Figure 3), quality of RNA in both tissues according to different amount (Figure 4), the relative quality of RNA samples with Trizol kit indicates a significant difference (Figure 5) According to Figure 4, it is concluded that less amount of tissue can lead to a better quality of RNA due to the lack of RNase enzyme.

The Results of the cDNA and Real Time PCR

In order to ensure the quality and quantity of extracted RNA, 10 samples were selected for cDNA. In this way, cDNA kit Quantitect Reverse Transcription was employed (QIAGEN ,USA). Relatively, 2 ng of extracted RNA of each sample was used for cDNA synthesis and real time PCR (ABI, 73000, USA) with TaqMan assay was employed for each reaction with specific primers for *HER2* gene.

Discussion

Considering that the first step in gene expression is having a suitable and great quality of extracted RNA, numerous

Table 1. The Quantitative Results of Extracted RNA in Breast Cancer Tissues

The Minimum Concentration, ng/µL	The Maximum Concentration, ng/µL	260/280	Mean	SD
458.2	2445.6	2.1	1296.73	845.11

Table 2. The Quantitative Results of Extracted RNA in Gastric Cancer Tissues

The Minimum Concentration, ng/µL	The Maximum Concentration, ng/µL	260/280	Mean	SD
465.8	2223.5	2.1	1143.2	728.2

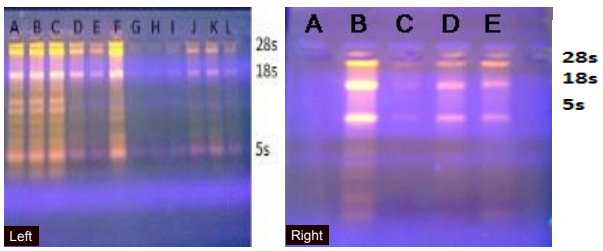

Figure 1. The samples of RNA extraction from breast tissues (Left) and gastric cancerous tissues (Right) were powdered with liquid nitrogen and Homogenizer set which were electrophoresed on 2% agarose gel. ABCDE samples were powdered with liquid nitrogen and FGHIJ samples were powdered with homogenizer set. Sample D in the left figure indicates the size marker.

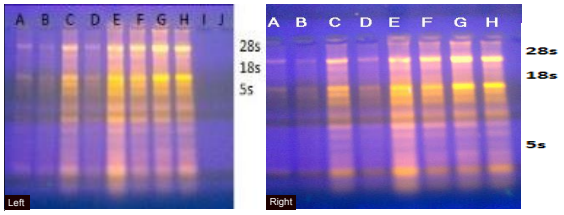

Figure 2. The samples of RNA extraction from breast tissues (Left) and cancerous gastric tissues (Right) which were kept in RNAlater and liquid nitrogen. ABCDE samples were kept in RNAlater and EFGHIJ samples were kept in liquid nitrogen. Sample D in the left figure indicates the size marker.

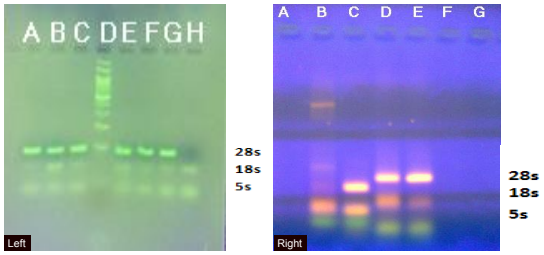

Figure 3. RNA extraction from breast tissues (Left) and gastric tissues (Right). All bands including 5s, 18s and 28s are determined by electrophoresis on 2% agarose gel.

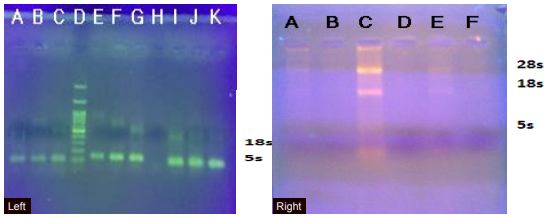

Figure 4. RNA Extraction with different amounts. A) 10-30 mg breast tissue, (B) 30-50 mg breast tissue, (C) 50-70 mg breast tissue, (D) 70-90 mg breast tissue (Left). (A) 70-90 mg gastric tissue, (B) 50-70mg gastric tissue, (C) 10-30 mg gastric tissue (Right).

methods have been studied in this category recently. However, the way of RNA extraction from the blood cells is far different from tissue cells and RNase enzyme is also really important in destruction of RNA (14,15). As it has been found in researches, the purity of RNAs is different and it can be concluded from different types of the tissues including adipose, soft and cartilaginous tissues and also the existence of RNase enzyme. Conspicuously, RNA extraction from tissue is much more difficult than its extraction from blood cells. It should also be mentioned that extraction from cancerous tissues is different and is really very complicated and unstable (16). Correspondingly, it is found in this project that RNA extraction from breast cancer tissues is a little easier compared with that from gastric cancer tissues. It could be because of the softness of this types of tissue (17). Moreover, the tissues which have percentages of fat and adipose tissue will be much easier to extract RNA from (18). RNA extraction kits are

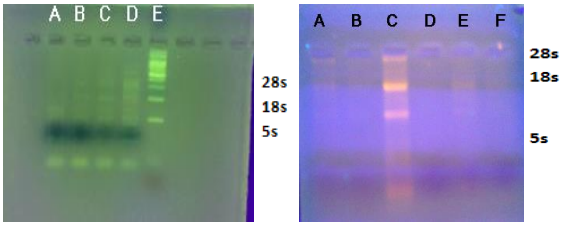

Figure 5. The sample of extracted RNA with 10-30 mg amount of breast tissues (Left) and gastric tissue (Right) by Trizol kit. E in the left figure is the size marker.

really attractive in research and therapeutic environments specially in gene expression and study of microRNAs (19) In this research, AccuZol, TriPure, Ribozol and Trizol kits were investigated and checked and it could be noted that Trizol had a better relative percentage in comparison to the other extraction kits. The significant point is that, the purity of the extracted RNA for making cDNA have a great importance; it means the higher the quality and quantity of extracted RNAs without RNAse enzyme, the better the quality of molecular investigations like real-time PCR. Remarkably, extracted RNA with better purities and by making cDNA and real-time PCR action on *HER2* gene from both breast and gastric tumors indicated compatible results.

Conclusion

It was found that gastric cancer tissues have more difficulties in comparison with breast cancer tissues because of having hard tissues. Trizol extraction kits also result in a better quality of final product. It is necessary to mention that a variety of other kits that are used in research such as kits that we have chosen, are based on phenol chloroform, however according to their qualities and also the manufacturer's instructions which slightly differ, some of them have somehow a better quality. Less amount of tissue between 10 to 30 mg have a better quality because of possessing fewer RNase enzymes.

Conflict of Interests

The authors declare that the preference of one kit over other ones does not imply the bias of authors and it is only the result of authors' experiment. In other words, the experiments of other researchers on other cell lines using different kits may provide different results.

Ethical Issues

The Vice Chancellery of Research & Technology of Azad university of Damghan approved the study.

Financial Support

This project was the post graduation thesis of Mr Seyed Amin Norollahi and was supported financialy by him.

Acknowledgements

This data was collected from Mr Seyed Amin Norollahi's

post graduation thesis. The authors are grateful to those people who were engaged in this study.

References

1. Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present. J Biomed Biotechnol. 2009;2009:574398. doi:10.1155/2009/574398
2. Peirson SN, Butler JN. RNA extraction from mammalian tissues. Methods Mol Biol. 2007;362:315-327. doi:10.1007/978-1-59745-257-1_22
3. Samadani AA, Nikbakhsh N, Fattahi S, et al. RNA Extraction from Animal and Human's Cancerous Tissues: Does Tissue Matter? Int J Mol Cell Med. 2015;4(1):54-59.
4. Griffin M, Abu-El-Haija M, Abu-El-Haija M, Rokhlina T, Uc A. Simplified and versatile method for isolation of high-quality RNA from pancreas. Biotechniques. 2012;52(5):332-334. doi:10.2144/0000113862
5. Skidmore AF, Beebee TJ. Characterization and use of the potent ribonuclease inhibitor aurintricarboxylic acid for the isolation of RNA from animal tissues. Biochem J. 1989;263(1):73-80.
6. Mukhopadhyay T, Roth JA. Isolation of total RNA from tissues or cell lines. Visualization in gel. Methods Mol Biol. 1998;86:55-59. doi:10.1385/0-89603-494-1:55
7. Fleige S, Pfaffl MW. RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med. 2006;27(2-3):126-139. doi:10.1016/j.mam.2005.12.003
8. Gill SS, Aubin RA, Bura CA, Curran IH, Matula TI. Ensuring recovery of intact RNA from rat pancreas. Mol Biotechnol. 1996;6(3):359-362. doi:10.1007/bf02761714
9. Kiba T, Kintaka Y, Nakada E, Suzuki Y, Inoue S, Ishigaki Y. High-quality RNA extraction from rat pancreas for microarray analysis. Pancreas. 2007;35(1):98-100. doi:10.1097/01.mpa.0000278690.93930.b6
10. Sparmann G, Jaschke A, Loehr M, Liebe S, Emmrich J. Tissue homogenization as a key step in extracting RNA from human and rat pancreatic tissue. Biotechniques. 1997;22(3):408-410, 412.
11. Mullin AE, Soukatcheva G, Verchere CB, Chantler JK. Application of in situ ductal perfusion to facilitate isolation of high-quality RNA from mouse pancreas. Biotechniques. 2006;40(5):617-621.
12. Hernandez GE, Mondala TS, Head SR. Assessing a novel room-temperature RNA storage medium for compatibility in microarray gene expression analysis. Biotechniques. 2009;47(2):667-668, 670. doi:10.2144/000113209
13. Li D, Ren W, Wang X, et al. A modified method using TRIzol reagent and liquid nitrogen produces high-quality RNA from rat pancreas. Appl Biochem Biotechnol. 2009;158(2):253-261. doi:10.1007/s12010-008-8391-0
14. Samadani AA, Nikbakhsh N, Pilehchian M, Fattahi S, Akhavan-Niaki H. Epigenetic changes of CDX2 in gastric adenocarcinoma. J Cell Commun Signal. 2016;10(4):267-272. doi:10.1007/s12079-016-0327-9
15. Jackson DP, Lewis FA, Taylor GR, Boylston AW, Quirke P. Tissue extraction of DNA and RNA and analysis by the polymerase chain reaction. J Clin Pathol. 1990;43(6):499-504.
16. Botling J, Micke P. Fresh frozen tissue: RNA extraction and quality control. Methods Mol Biol. 2011;675:405-413. doi:10.1007/978-1-59745-423-0_25
17. Micke P, Ohshima M, Tahmasebpoor S, et al. Biobanking of fresh frozen tissue: RNA is stable in nonfixed surgical specimens. Lab Invest. 2006;86(2):202-211. doi:10.1038/labinvest.3700372
18. 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(5):316-323. doi:10.3393/jksc.2010.26.5.316
19. Norollahi SE, Alipour M, Nikbakhsh N, et al. The Role of MicroRNAs in Cancer Progression. J Clin Exp Oncol. 2016;5(2). doi:10.4172/2324-9110.1000155