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METHODS

DNA and RNA Isolated from Tissues Processed by Microwave-Accelerated Apparatus MFX-800-3 are Suitable for Subsequent PCR and Q-RT-PCR Amplification

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Over the past decade, methods of molecular biology have appeared in diagnostic pathology and are routinely applied on formalin-fixed, paraffin-embedded histological samples, processed via conventional embedding methods. Due to its reagent- and cost-effectiveness, embedding techniques that utilize microwave acceleration in one or more steps of histoprocessing are increasingly used by numerous laboratories. The demand arises that tissues processed this way should also be suitable for the requirements of molecular pathology. In this study, both conventionally embedded and MFX-800-3 machine-processed tissue samples from the same source were used for isolation of DNA and RNA and for performing PCR and real-time PCR. PCR amplification of the β-globin gene, as well as the real-time PCR amplification of the ABL mRNA was successful in all cases. Our conclusion is that samples processed by the vacuum assisted automatic microwave histoprocessor MFX-800-3 are perfectly applicable for DNA and RNA isolation and provide appropriate templates for further PCR and real-time PCR studies. (Pathology Oncology Research Vol 13, No 2, 149–152)

Key words: paraffin embedding, MFX-800-3 histoprocessor, real-time PCR, molecular pathology

Introduction

The methods of molecular pathology became routinely used in numerous histopathological laboratories over the past decade.[5] Recent advances in molecular technologies allow the utilization of formalin-fixed, paraffin-embedded histological samples for DNA and/or RNA analysis. Most of these molecular methods were optimized and validated on samples processed via the conventional embedding methods, which proved that conventional methods are in fact compatible with molecular diagnostics.[13]

At the same time an increasing number of laboratories apply embedding techniques that utilize microwave acceleration in one or more steps of histoprocessing.[3,9] The demand arises that tissues processed this way should also be suitable for the requirements of molecular pathology.

Surprisingly, only limited amount of information is available in the relevant literature regarding microwave histoprocessing and molecular pathology.[6,8,12] Numerous laboratories use the vacuum-assisted automatic microwave histoprocessor MFX-800-3; therefore detailed knowledge would be needed about the possible application of tissues processed by this apparatus for methods of molecular pathology.

In our study, both conventionally embedded and MFX-800-3 machine-processed tissue samples from the same source were used for isolation of DNA and RNA and for performing PCR and real-time PCR.

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Materials and Methods

Tissue samples

Four surgically removed tissue samples (two colon carcinomas, one gallbladder showing chronic inflammation and one normal liver tissue) were each divided into two

parts. One part was fixed with 8% neutral formalin and embedded into paraffin using a TCP 15 Tissue Processor (Medite, Burgdorf, Germany). The other part was processed by means of the MFX-800-3 apparatus, as described earlier.[10]

DNA isolation and PCR amplification of the β-globin gene

The paraffin embedded tissue sections were de-waxed using xylene and absolute ethanol washes (3x5 min). Genomic DNA isolation from tissue specimens was performed according to the standard salting-out procedure.[14] Each PCR reaction contained 100 ng of DNA. Reactions were carried out using the AmpliTaq Gold™ enzyme system (Applied Biosystems, Weiterstadt, Germany). Primer sequences for amplification of the human β-globin gene were as follows: forward (GH20): 5'-GAAGAGCCAAGGACAGGTAC-3' and reverse (PCO4): 5'-CAACTTCATCCACGTTCACC-3', generating a 267 base pair (bp) long product.

RNA isolation and real-time PCR amplification of the ABL gene

Total RNA was extracted using the High Pure RNA Paraffin kit (Roche Diagnostics, Mannheim, Germany) as recommended by the manufacturer. One μg of RNA was reverse transcribed to cDNA using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). The quantitative real-time PCR assay was performed with ABI Prism® 7300 Sequence Detection System (Applied Biosystems). For amplification of mRNA of the Abelson (ABL) gene, the TaqMan®-based technology was used.

The TaqMan® technology is an absolutely specific and highly reproducible system for detecting target genes, since signal is only detected if the target sequence is complementary to the probe used. In this case the primer-probe set was designed to flank an exon-exon boundary, which rules out the possibility of genomic DNA amplification. The sequences of the probe and the primers were as follows: TaqMan probe: 5'-FAM-CCATTTTTGGTTTGGGCTTCACACCATT-TAMRA-*3'*, forward primer: 5'-TGGAGATAACACTCTAAGCATAACTAAAGGT-3' and reverse primer: 5'- GATGTAGTTGCTTGGGACCCA-3'.[1] The estimated product size was 123 bp. All samples were run in triplicate, in a 20 μl reaction volume containing 100 ng of cDNA. The reaction plate also included a non-template control sample, which contained all reaction components except the cDNA template. Sequence Detection Software version 1.0 (Applied Biosystems) was used to analyze the data after amplification.

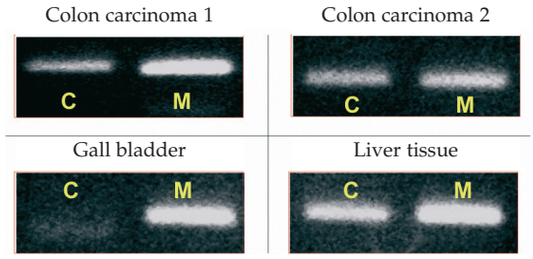

Figure 1. *The agarose gel electrophoresis image shows the results of β-globin gene amplification in different tissues using conventional and microwave-accelerated histoprocessing. The amplification was successful in all cases, only the gallbladder sample processed via the conventional method showed a slightly lighter band. (C: conventional embedding; M: microwave-accelerated embedding)*

Results

DNA isolation and PCR amplification of the β-globin gene

DNA isolation and the subsequent PCR amplification were successful from both the conventionally prepared tissue blocks as well as from blocks prepared via the microwave-accelerated technique. In both cases we were able to obtain 15-20 μg of genomic DNA with a purity of 1.6-1.7 OD_{260}/OD_{280} from 5 tissue sections of 30 μm thickness. The results of the β-globin gene amplification are depicted in *Fig. 1*. The reaction was carried out successfully in all 4 tissue samples, only the gallbladder sample processed via the conventional method showed a slightly lighter band.

RNA isolation and real-time PCR amplification of the ABL gene

Using five 30-μm-thick tissue sections we were able to obtain 2-5 μg of total RNA (OD_{260}/OD_{280} = 1.7-1.8) from tissue blocks prepared with either embedding techniques. The real-time PCR amplifications were successful in all cases *(Fig. 2)*. The average C_T values obtained for each sample are summarized in *Table 1*. The results of the ABL mRNA amplification clearly reflect that RNA isolated from the same tissue processed by two different embedding methods provides quantitatively and qualitatively similar results. The reaction kinetics was also identical, therefore, the quantitative results are directly comparable.

Discussion

Histoprocessing by use of microwave-based techniques reduces processing time compared to conventional methods, and results in approximately ten-fold decrease in the

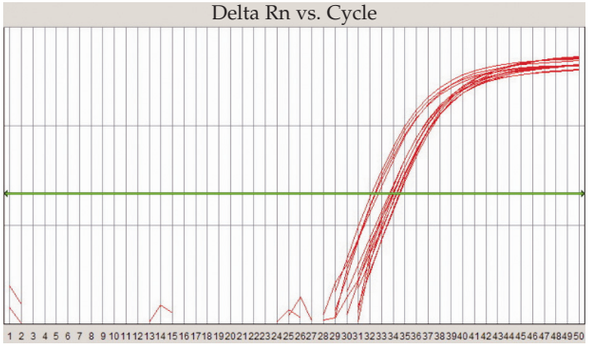

Figure 2. *The amplification plots show successful amplifications of the ABL mRNA from different tissues using conventional and microwave-accelerated histoprocessing. Similar C_T values were obtained from samples processed by either embedding methods (conventional vs. microwave-accelerated).*

cost of chemicals, and a perfect preservation of tissue and cellular structures. Our results show that isolation of DNA and RNA as well as real-time PCR is possible using tissue samples processed in a vacuum-assisted microwave apparatus, and the quality of these reactions are at least as high as that obtained by the conventional histoprocessing. The templates after isolation of DNA and RNA are sufficient for PCR and RT-PCR studies. These results confirm the data of Hsu et al, who reported high quality of DNA retrieved for Southern blot hybridization from microwave-fixed, paraffin-embedded liver tissues.[8] Man and Burgar described a novel antigen unmasking protocol for immunohistochemistry and subsequent PCR amplification, in part also utilizing microwave oven irradiation.[12] Lou et al used microwave and thermal cycler boiling methods for preparation of cell samples prior to PCR, for human papillomavirus detection.[11] Ekuni et al found RNA integrity and successful RT-PCR amplification in dento-alveolar tissues after microwave-accelerated demineralization.[6]

Table 1. **The average CT values obtained for each sample**

Sample	*Embedding*	*Average C_T*
Colon carcinoma	C	34.10
	M	33.74
Colon carcinoma 2	C	33.40
	M	33.63
Gall Bladder	C	32.21
	M	32.70
Liver tissue	C	34.52
	M	33.92

C: conventional embedding; M: microwave embedding

Prior to both embedding techniques, formalin, which is known for its nucleic acid fragmenting effects, was used as the primary fixative reagent; therefore, we did not expect any further improvement using the microwave-accelerated histoprocessing compared to the conventional embedding regarding DNA or RNA integrity. The literature describes certain alternative fixatives that cause less nucleic acid fragmentation;[2,16] we are planning to integrate these reagents into our microwave-assisted histoprocessing procedure in order to obtain more intact DNA and/or RNA samples.

The utilization of formalin-fixed, paraffin-embedded tissues for Q-PCR studies is controversial. However, some studies have successfully used this technique for gene expression analyses.[4,7,15] Since Q-PCR plays a significant role in modern pathological diagnostics, we aimed to demonstrate that RNA isolated with microwave technique is suitable for quantitative expression analysis.

Our results not only confirm previously published data, but show that the entire fixation and embedding process performed using a vacuum-assisted microwave apparatus results in samples perfectly applicable for DNA and RNA isolation in order to perform PCR and RT-PCR studies.

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