ORIGINAL ARTICLE

A Novel Fixative Improves Opportunities of Nucleic Acids and Proteomic Analysis in Human Archive's Tissues

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Abstract: All tissues from biopsy or surgery origin are fixed and paraffin embedded as a routine procedure in the hospital departments of pathology. The traditional method of tissue preservation is the fixation in formalin, followed by paraffin embedding. In this way tissue's integrity is ensured also for future analyses, because there is no further chemical degradation of nucleic acids and proteins in tissues embedded in paraffin. After few sections for the histopathological examination the tissues are stored for decades in the hospital archives. Even if formalin fixation compromises the quality and integrity of nucleic acids, it has already been demonstrated that it is possible to recover and analyze DNA and RNA from these archive's tissues, even of autopsy origin. Protein analysis is on the contrary completely blocked, due to the fact that formalin fixation creates covalent links between proteins and the only way to study protein expression is immunohistochemistry. In this study we present our results concerning the use of a new formalin free fixative, the FineFIX. After extraction of nucleic acids, PCR and RT-PCR analyses were performed in DNA and RNA respectively. For DNA analysis it was possible to obtain amplicons of 2400 bps, while in formalin-fixed samples the maximum length achieved was less than 400 bps. RT-PCR analysis show that it was possible to study RNA fragments of 600 bs from FineFIX fixed tissues, against a maximum length of about 150 bs achieved by formalin-fixed tissues. These tissues were analyzed also by Western Blot analysis, showing that the proteins obtained from FineFIX treated samples are amenable and comparable in quality with those obtained from fresh frozen tissues. Protein extracts from FineFix© treated tissues were also compared with fresh tissues'ones by two dimensional electrophoresis, demonstrating that the protein pattern were well comparable for number and distribution of the spots.

Key Words: paraffin embedded tissues, ethanol based fixation, functional genomics, proteomics, twodimensional electrophoresis

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Tissue fixation is a crucial procedure for the molecular preservation of human specimens stored in pathology department archives. As a routine, every tissue from biopsy or surgical origin is fixed and paraffin wax–embedded. These tissues constitute a huge amount of human material, amenable for research in molecular medicine. It is possible to extract and analyze nucleic acids from paraffin-embedded tissues.[1–3] Biopsies and surgical specimens, as well as postmortem tissue samples, represent an important resource, especially for studies of molecular epidemiology, rare diseases, neuropathology, and case studies with very long follow-up periods and with known therapy outcomes. By using archive tissues, it is also possible to study the natural history of diseases at a molecular level because samples from patients are collected at different stages of the disease.[4]

A major advantage in using paraffin-embedded material is that it is easier to collect tissues from already existing archives in comparison to frozen fresh tissues that need a specific collection, with dedicated spaces and specific equipment.

The common fixation procedure includes the use of formalin or a few other fixatives, such as Bouin solution. The formalin fixation process leads to the formation of a net of covalent cross-links between side chains of proteins by methylenic bridge formation. Therefore, the only way to analyze proteins in formalin-fixed and paraffin-embedded samples is immunohistochemistry, a technique that provides the intracellular localization of a protein,[5] but offers only a poor quantitative analysis of protein expression. A more sensitive and precise quantitative analysis of proteins requires them to be extracted from cell lines or fresh tissues.

Ethanol-based fixatives do not create covalent bonds between proteins; they eliminate the specific hydration water molecules which surround proteins that collaborate to maintain their conformation and function. As a consequence, the proteins coagulate and all the enzymatic functions are stopped all of a sudden. Therefore, proteins should be amenable to conventional proteomic techniques. Few studies have been performed on molecular analysis of tissues fixed with formalin-free reagents to date.[6–9] The problems in the use of ethanol fixation or similar are usually connected with the possibility of properly fixing only very small tissue fragments and with the poor quality of the morphological analysis, such as coartation of cells and migration of cell

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organelles inside the cell. We present the results obtained in the molecular analysis of samples treated with a new ethanol-based fixative (FineFIX, Milestone SrL, Bergamo, Italy) that overcomes the over-reported problems and allows improved molecular analysis.

MATERIAL AND METHODS

Chemicals and reagents

4–7 IPG buffer, cover fluid and 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS), Taq DNA polymerase, and ECL Western Blotting Detection Reagent were purchased from Amersham Biosciences (Uppsala, Sweden); AMV retrotranscriptase from Promega (Madison, Wisconsin, USA); 4 to 7 IPG strips, Tween 20, methanol, acetic acid, glycine, glycerol, DTT, urea, SDS, bromophenol blue, iodoacetamide, ethanol, sodium thiosulphate, and silver nitrate were purchased from Sigma-Aldrich (Steinheim, Germany). Tris base was purchased from Invitrogen (Carlsbad, CA, USA).

Samples

Five colon cancer samples were obtained from the Pathology Department of the University of Turin. Each biopsy was split into 2 parts: one half was formalin fixed and paraffin embedded as the routine procedure of the clinical laboratories, and the other half was fixed with FineFIX (Milestone SrL, Bergamo, Italy) and paraffin embedded. FineFIX is a mixture of ethanol (65% to 75% w/v), distilled water, glycerol, polyvinyl alcohol, and monomeric carbohydrates (European patent N. EP 1 455174B1 – December 15th 2004), which maintains good morphological results. Fresh tissue specimens (received directly from the surgical theater) were immersed in FineFIX fixative (28 ml concentrated FineFIX reagent added upon use to 72 ml of absolute ethanol) for 30 minutes at room temperature, then transferred, always in FineFIX fixative, into the microwave processing apparatus (RHS-2, Milestone SrL, Bergamo, Italy). The procedure was set to maintain the temperature constant at 50°C for 30 minutes. Therefore, tissues were routinely embedded in paraffin wax and stored at room temperature. Samples used for this study were stored for an average time of 6 months.

Human hepatoma cell line (HEPG2) and fresh frozen colon cancer tissues were used as controls.

Immunohistochemistry

Two μm tissue sections were cut using a conventional microtome. Sections were then stained for keratin in immuno-peroxidase (ABC procedure) with KL1 mouse monoclonal antibody (Immunotech, Marseille, France). No antigen retrieval was performed.

DNA extraction and amplification

DNA was extracted from 10 μm sections of paraffin wax–embedded tissues, both from FineFIX and formalin-fixed tissues. Fifteen sections were cut with standard microtome for every paraffin wax block. The DNA extraction was performed as previously reported.[10] For fresh tissues controls, tissues frozen after surgical resection were reduced to powder in a mortar; tissue powder was split in 2 different tubes, 1 for DNA extraction and 1 for RNA. The DNA extraction was performed as previously described.[11]

Every sample was amplified using specific primers for human TTR gene (transthyretin gene GeneBank M11844). PCR condition and amplicon lengths are reported in Table 1.

PCR was performed in a 50 μl final volume using standard conditions. Every reaction included 250 ng of DNA, 15 pmoles of each primer, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris/HCl (pH 9 at room temperature), 200 mM of each dNTP, and 1 U of Taq DNA polymerase. The following amplification programme was used for every PCR analysis: denaturation at 95°C for 3 minutes; 5 cycles of 95°C for 1 minute, annealing temperature for 1 minute, and 72°C for 1 minute; then 45 cycles of 95°C for 30 seconds, annealing temperature for 30 seconds, and 72°C for 30 seconds; 1 elongation step at 72°C for 8 minutes.

Ten microliters of the amplification products were separated in 8% polyacrylamide gels. Gels were stained with ethidium bromide (5 mg/ml) and recorded using a VersaDoc imaging system (Bio-Rad Laboratories, Hercules, California, USA).

TABLE 1. Primers Used for DNA Amplification

Gene	Primer Sequence 5′–3′	Length of Products	Ta*	Cycles
TTR	Forward: CAG CAG GTT TGC AGT CAG AT Reverse: GGT ACC CTT GCC CTA GTA AT	291 bp	60	45
TTR	Forward: TGG TGG AAA TGG ATC TGT CTG Reverse: TGG AAG GGA CAA TAA GGG AAT	339 bp	60	45
TTR	Forward: the same primer used for TTR 291 bases Reverse: TGA TCC ATT ATC AGG AAC G	1000 bp	55	60
TTR	Forward: the same primer used for TTR 291 bases Reverse: AAG CCA CTC TTG CACATG AA	1939 bp	60	45
TTR	Forward: the same primer used for TTR 291 bases Reverse: TAT GCC CCT CCT CCT TTC TT	2395 bp	58	60

*Temperature of annealing.

TABLE 2. Primers Used for RNA Amplification

Gene	Primer Sequence 5′–3′	Length of Products	Ta*	Cycles
Beta actin	Forward: ATC ACT GCC CTG GCA CCC A Reverse: CCG ATC CAC ACG GAG TAC TTG	77 bp	60	30
Beta actin	Forward: CTG GAC TTC GAG CAA GAG AT Reverse: GAA GGT AGT TTC GTG CAT CG	170 bp	58	40
Beta actin	Forward: GAG AAG CTG TCC TAC GTC G Reverse: the same primer used for beta actin 170 bases	200 bp	59	45
Beta actin	Forward: GTT GCT ATC CAG GCT GTG CT Reverse: the same primer used for beta actin 170 bases	438 bp	57	50
Beta actin	Forward: GGC ATC CTC ACC CTG AAG TA Reverse: the same primer used for beta actin 170 bases	651 bp	58	45

*Temperature of annealing

AQ10 RNA extraction and RT-PCR for RNA analysis

RNA was extracted as previously reported.[2,3] DNase I digestion was performed to eliminate genomic DNA from the RNA solution as previously reported.[1] For fresh tissues controls, the tissue powder was processed as described by Chomczynski and Sacchi.[12]

Reverse transcription was performed as previously described,[13] using 500 ng of total RNA, AMV reverse transcriptase, and sequence specific antisense primer as reported in Table 2.

For β-actin (GeneBank M10277), 5 systems were analyzed. The lengths of the RT-PCR products were 77, 170, 200, 438, and 651 bases. Ten microliters of PCR products were separated on 8% polyacrylamide gels and stained with ethidium bromide (5 mg/ml). Gels were recorded using a VersaDoc imaging system (Bio-Rad Laboratories).

Protein sample preparation

Paraffin-embedded tissues

Thirty sections (10 mm thick) were cut and put into an Eppendorf tube. One milliliter of xylene was then added for each sample, and the tubes vortexed extensively and centrifuged at 16,000*g* for 10 minutes at room temperature. The supernatant was eliminated, and this first step was repeated. The pellet obtained was washed with 1 ml of 100% ethanol, vortexed, and centrifuged at 16,000*g* for 10 minutes. After the elimination of the supernatant, this step of washing was performed with 1 ml of 80% ethanol. The pellet was then resuspended in a solution containing 500 µl of water- saturated phenol, 10 µl of 10% SDS and 20 µl of β-mercaptoethanol, and sonicated extensively. Three hundred microliters of 40% sucrose were then added to the homogenate, which was centrifuged for 3 minutes at 3000*g*. The phenol upper phase was transferred into a new tube containing 1.5 ml of methanol and 2 µl of 1% Ficoll as carrier. The sample was then centrifuged at 3000*g* for 3 minutes. The protein pellet was then washed with methanol, and air dried. Pellets were then resuspended in 300 µl of a solution containing 8 M Urea, 2% CHAPS, and a mixture of protease inhibitors.

Liquid nitrogen frozen tissues

The frozen tissues were placed into 800 µl of a solution containing 8 M Urea, 2% CHAPS, and a protease inhibitor cocktail. The tissue was homogenized using an Ultra-Turrax T25 homogenizer (Janke and Kunkle), 5 × 20 seconds, high setting. The sample was then sonicated and centrifuged at 20,000*g* for 15 minutes. The supernatant was finally recovered and stored at −80°C.

HepG2 lysate

Approximately 10^6 cells were lysed in 500 µl of the same solution used for the fresh tissues. The sample was then sonicated and centrifuged at 20,000*g* for 15 minutes. The supernatant was finally recovered and stored at −80°C.

Western blot

About 70 µg of whole protein extract was loaded onto a 10% polyacrylamide separating gel. The gel was run until the bromophenol dye reached the end of the gel; then the proteins were transferred to a nitrocellulose membrane. The membrane was blocked for 1 hour at room temperature in 5% nonfat milk powder in PBS AQ11 added with 0.2% Tween 20 (PBST), and then incubated AQ12 for 1 hour at room temperature with a mouse anti-α-tubulin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and diluted 1:10,000 in 5% nonfat milk powder in PBST. After incubation with the primary antibody, the membrane was washed 5 times for 5 minutes with PBST, and then incubated for 1 hour with the secondary antimouse HRP-conjugated antibody AQ13 (Dako, Glostrup, Denmark), and diluted 1:15,000 in 5% nonfat milk powder in PBST. The membrane was washed 5 times for 5 minutes with PBST, incubated for 1 minute with the ECL detection reagent, and finally developed on a Kodak Biomat XAR film. The same AQ14 protocol was followed with monoclonal anti-tissue transglutaminase antibody (purified from a secerning

hybridoma, kindly provided by Daniele Sblattero), diluted 1:3000 in 5% nonfat milk powder in PBST.

Two-dimensional electrophoresis

Isoelectric focusing (IEF) was performed using 18 cm ProteoGel IPG Strips, pH 4 to 7. Passive rehydration was applied resuspending 500 µg of proteins in a solution containing 8 M urea, 2% CHAPS, 40 mM DTT, 0.6% carrier ampholyte, and traces of bromophenol blue. Each IPG strip was rehydrated for at least 12 hours.

The IEF was carried on according to the following protocol: 500 V for 500 Vh, 1000 V for 1000 Vh, followed by 8000 V to achieve a total amount of 56 kVh at 20°C (Protean IEF Cell, Bio-Rad Laboratories). When the focusing process was completed, the strips were equilibrated for 20 minutes in a solution containing 6 M Urea, 30% Glycerol, 2% SDS, 50 mM Tris-Cl pH 8.8, 1% DTT, traces of bromophenol blue, rinsed with double distilled water, and then transferred for 20 minutes more in the same solution containing 2.5% iodoacetamide instead of the DTT.

Second dimension SDS-PAGE was performed on 12% separating gel, at 15 mA/gel for 15 minutes, and then at 30 mA/gel until the bromophenol dye front reached the bottom of the gel.

Gels were silver stained by the procedure described by Mortz et al[14]

Gel images were acquired at a VersDoc system, digitalized and analyzed using the PDQuest software (Bio-Rad Laboratories).

RESULTS

Immunohistochemistry

Immunohistochemistry analysis was performed in parallel on routinely formalin-fixed and FineFIX-fixed tissues. Two micrometer sections were cut from the paraffin blocks, and then conventionally stained for Keratin with a KL-1 antibody. The higher intensity of the staining obtained after FineFIX microwave-assisted fixation (Fig. 1), is index of a better maintenance of the antigenic properties of the tissue.

Nucleic acids analysis

Nucleic acids were extracted from 10 µm sections of paraffin wax–embedded blocks. Fifteen sections were cut from every sample. On average, 510 ng of DNA from every section of FineFIX and 300 ng of DNA from the formalin-fixed section were obtained. For RNA, on average, 390 ng were recruited from the FineFIX section and 180 ng from the formalin section. Every PCR and RT-PCR analysis was repeated twice to confirm the reproducibility of the results.

DNA samples were amplified with standard PCR amplification. DNA was successfully amplified from all samples, and treated with both formalin and FineFIX, for amplicons in a range between 291 bp and 339 bp. For amplifications longer than 339 bp (TTR 1000 bp, TTR 1900 bp, and TTR 2400 bp), no amplification was obtained in any formalin-fixed tissues, although all the tissues fixed with the new fixative were positively amplified (Fig. 2 and Table 3).

The PCR product was also purified and sequenced to confirm the presence of the correct amplification product.

For RNA analysis, all amplicons that amplified successfully were no longer than 170 bs in formalin-fixed tissues; for longer fragments, no amplification products were detected. FineFIX-fixed samples showed completely different results. Every tissue presented a positive amplification in the range from 200 to 450 bases. By increasing the length of the amplicon to 600 bs, 3 out 5 samples resulted still positive (Fig. 3 and Table 4).

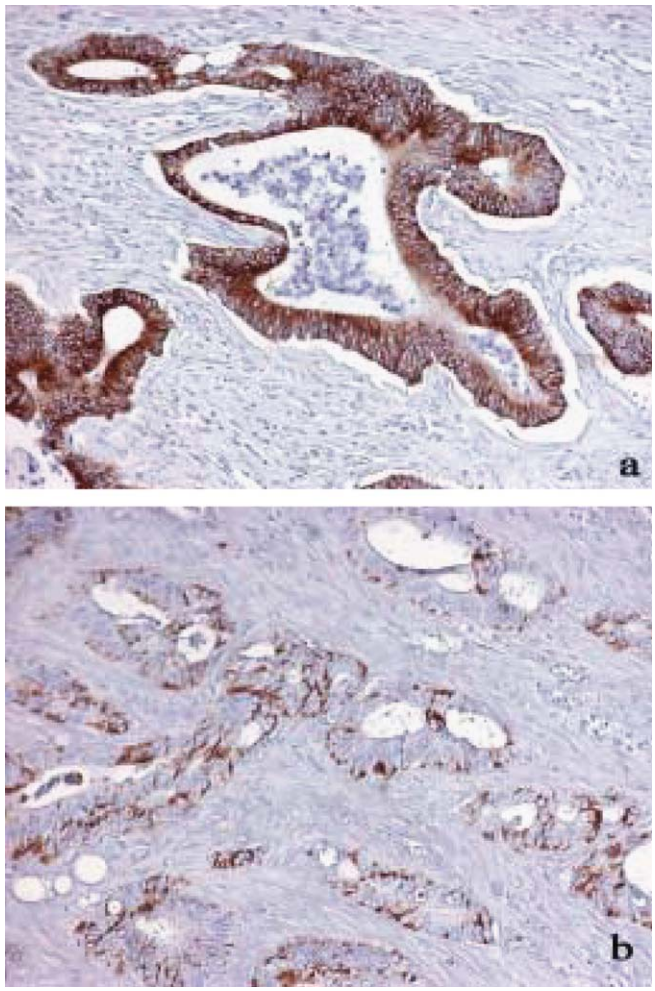


FIGURE 1. Colon carcinoma. Near-by tissue specimens were alternatively fixed in FineFIX fixative followed by MW treatment or (a) routinely fixed in buffered formalin and (b) then embedded in paraffin. Sections were stained for keratin in immuno-peroxidase (ABC procedure) with KL1 mouse monoclonal antibody (Immunotech, Marseille, France). No antigen retrieval was performed. The staining is much stronger in MW treated tissue, proving the superior preservation of antigenic properties.

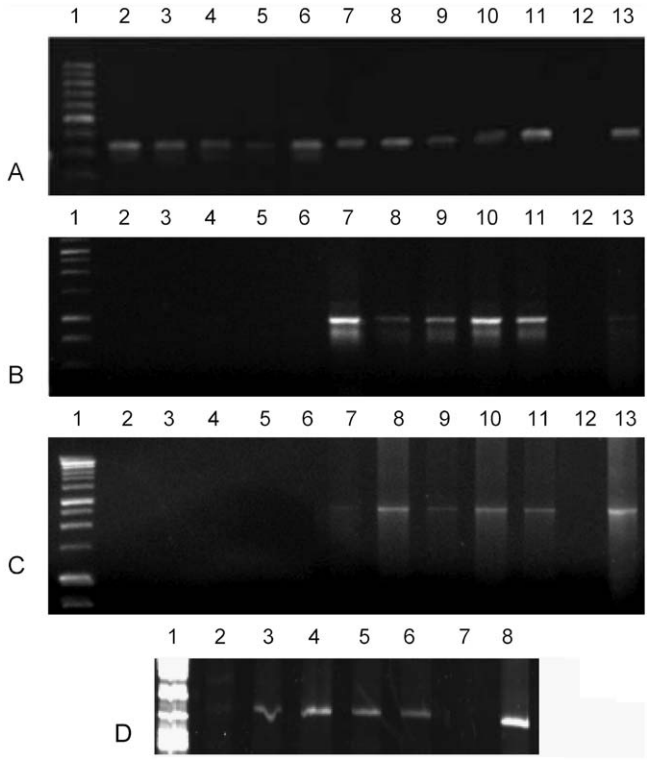


FIGURE 2. Polymerase chain reaction for DNA from formalin and FineFIX tissues.(A) TTR DNA (339 bp). Lane 1, molecular size marker; lanes 2 to 6, DNA from formalin-fixed tissues; lanes 7 to 11, DNA from FineFIX-fixed tissues; lane 12, negative control; lane 13, DNA from HepG2 cells, positive control. (B) TTR DNA (1000 bp). Lane 1, molecular size marker; lanes 2 to 6, DNA from formalin-fixed tissues; lanes 7 to 11, DNA from FineFIX-fixed tissues; lane 12, negative control; lane 13, DNA from HepG2 cells, positive control. (C) TTR DNA (2400 bp). Lane 1, molecular size marker; lanes 2 to 6, DNA from formalin-fixed tissues; lanes 7 to11, DNA from FineFIX-fixed tissues; lane 12, negative control; lane 13, DNA from HepG2 cells, positive control. (D) TTR DNA (2400 bp). Lane 1, molecular size marker; lanes 2 to 6, DNA from frozen colonic mucosa; lane 7, negative control; lane 8, DNA from HepG2 cells, positive control.

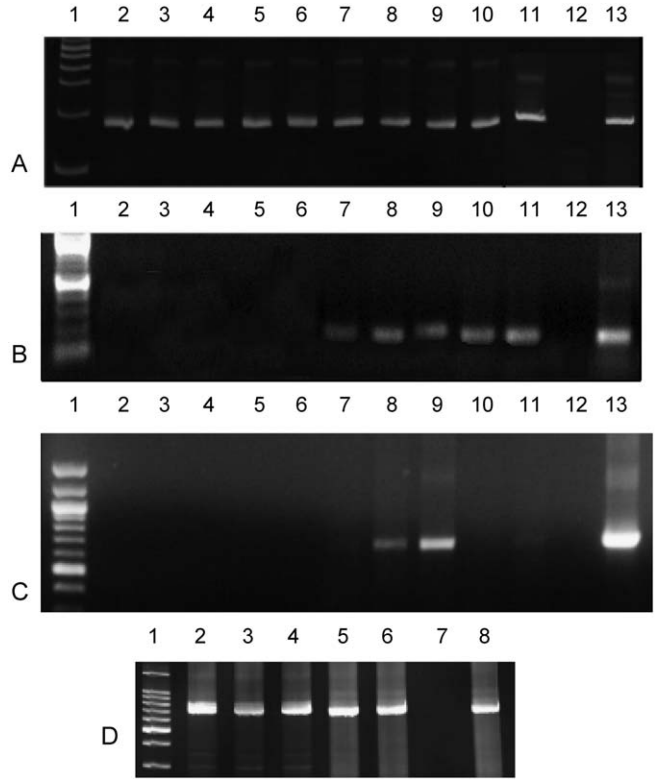


FIGURE 3. RT-PCR analysis in formalin and FineFIX tissues. (A) mRNA analysis fot the β actin gene (170 bp). Lane 1, molecular size marker; lanes 2 to 6, RNA obtained from formalin-fixed tissues; lanes 7 to 11, RNA obtained from FineFIX-fixed tissues; lane 12, negative control; lane 13, mRNA from HepG2 cells, positive control (B) mRNA analysis for the β actin gene (200 bp). Lane 1, molecular size marker; lanes 2 to 6, RNA obtained from formalin-fixed tissues; lanes 7 to 11, RNA obtained from FineFIX-fixed tissues; lane 12, negative control; lane 13, mRNA from HepG2 cells, positive control. (C) mRNA analysis for the β actin gene (600 bp). Lane 1, molecular size marker; lanes 2 to 6, RNA obtained from formalin-fixed tissues; lanes 7 to 11, RNA obtained from FineFIX-fixed tissues; lane 12, negative control; lane 13, RNA from HepG2 cells, positive control. (D) mRNA analysis for the β actin gene (753 bp). Lane 1, Molecular size marker; lanes 2 to 6, RNA obtained from frozen colonic mucosa; lane 7, negative control; lane 8, RNA from HepG2 cells, positive control.

Protein analysis

Western blot

Western Blot analysis was used to determine the quality of the protein extract obtained. A single well-defined band was obtained after SDS-PAGE electrophoresis and Western Blot from both the cell culture lysate and the FineFIX treated tissues, although in fresh frozen tissue protein extraction, a second lower band, caused by degradation products, was sometimes present. No proteins were obtained from the formalin-fixed tissues treated with the same extraction procedure (Fig.4(a)).

A second Western Blot analysis was performed against a higher molecular weight protein, identified as human tissue transglutaminase (tTG). Again, no proteins were detected from the formalin-fixed samples, although the extracts from fresh tissues and from specimens treated with the new fixative showed the same good quality (Fig.4(b)).

TABLE 3. DNA Amplification Comparison Between Formalin and FineFIX, Number of Positive Results After PCR Amplification

Length (Bases)	Formalin Fixed	New Fixative
291	5/5	5/5
339	5/5	5/5
1000	0/5	5/5
1900	0/5	5/5
2400	0/5	5/5

TABLE 4. RNA Amplification Comparison Between Formalin and FineFIX, Number of Positive Results After RT-PCR Amplification

Length (Bases)	Formalin Fixed	New Fixative
77	5/5	5/5
170	5/5	5/5
200	0/5	5/5
450	0/5	5/5
600	0/5	3/5

Two-dimensional electrophoresis

Two-dimensional electrophoresis was performed to test the compatibility of proteins extracted from FineFIX treated tissues with conventional proteomic analysis, compared with the same process using a fresh tissue sample. The gels obtained from the analyzed samples showed a good distribution of protein spots in the given pH and molecular weight range in both samples. Figure 4 shows gels analyzed with PDQuest. Approximately 1000 protein spots were identified and found to be present in both gels, with a difference in the computational analysis confined to 14 protein spots out of 1000 included in the analysis (1.4%) (Fig. 5).

DISCUSSION

Nucleic acid degradation depends on tissue and environmental origin, enzymes, and temperature, in addition to chemical factors related to fixative solutions. Until now, the most widely used fixative in histopathology laboratories has been buffered formalin, but other fixatives have also been employed as a routine or for special purposes. Different fixatives exhibit varying levels of nucleic acids degradation.[1,15] Even if formalin and, more extensively, Bouin's solution fixation compromises the quality and integrity of nucleic acids, it has already been reported that it is possible to extract and analyze DNA[1,10,11,16] and RNA,[1–3,17] even from tissues of autopsy origin.[16,17]

The proposal to use new types of alcoholic fixatives, specially developed for rapid fixation procedures, gives us the opportunity to improve the quality of molecular analysis, without decrements of the quality of the histopathology analysis (Fig. 1). As reported in Figure 2, upon routine formalin fixation, the DNA fragment of maximum length is around 350 bases, although the FineFIX treatment allows the amplification of over 2400 bases of DNA. This gives the opportunity to perform efficient DNA molecular analysis from archive tissues, which is comparable to fresh tissue extraction, with the advantage of a precise tissue morphology analysis and an accurate microdissection.

In our experience, formalin-fixed paraffin-embedded tissues result in RNA fragments ranging between 100 and 200 bases. In FineFIX treated specimens, the RNA sequence length reaches 600 bases (Fig. 3). Quantitative analysis of specific RNAs can be performed in formalin-fixed specimens by relative RT-PCR analysis.[18–20] However, an absolute quantification analysis is not available

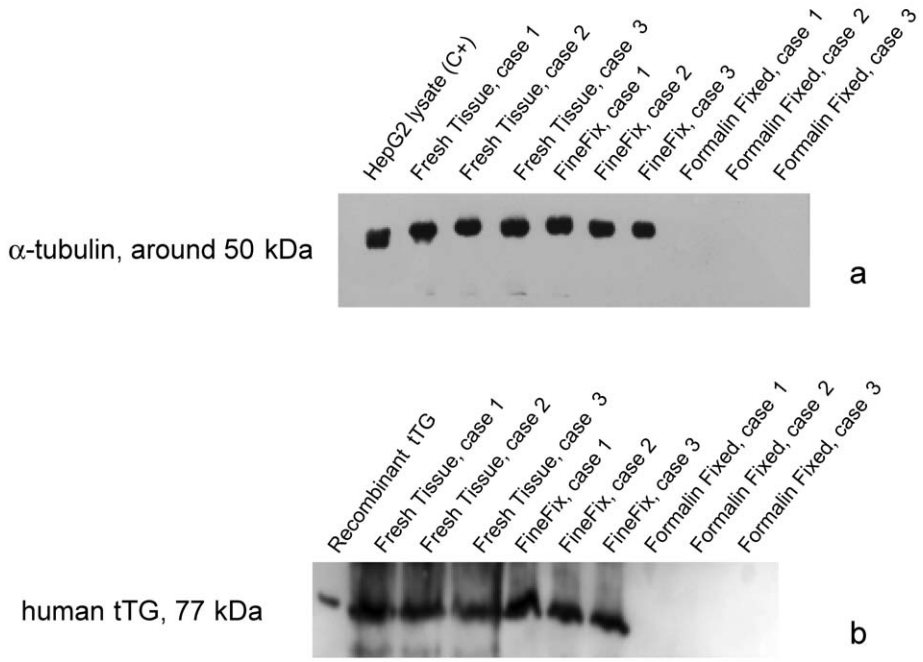
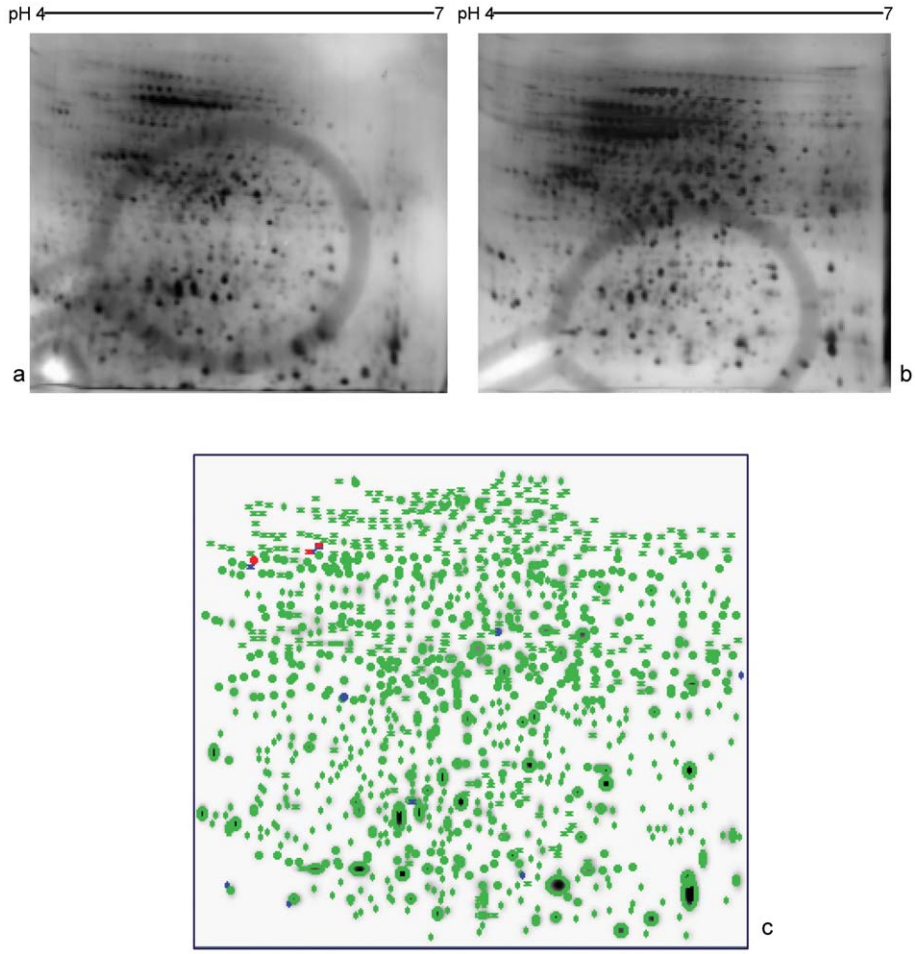


FIGURE 4. Western Blot analysis performed using monoclonal antibodies against α-tubulin (a) and human tissue transglutaminase (b).



AQ21 **FIGURE 5.** Representative two-dimensional electrophoretic maps from FineFIX-fixed paraffin-embedded colon cancer tissue (A) and fresh colon cancer tissue (B) performed using 4 to 7 pH gradient IPG strips for the first dimension and a 12% acrylamide gel for the second dimension, and silver staining. C shows the result of the PDQuest analysis performed on the 1000 more intense protein spots, where spots present in both gels are indicated in green; spots present only in gels obtained from FineFix treated samples are indicated in red, and spots present only in gels obtained from fresh tissue samples are indicated in blue.

in these tissues because of the extensive random degradation. FineFIX solution fixed material show a decreased amount of degradation also at the RNA level with the extraction of long sequences, in comparison with formalin fixation. The RNA and DNA quality obtained from these tissues is comparable to that from fresh tissues.

The formalin fixation process does not allow the extraction of tissue-derived proteins, owing to structural changes induced in these molecules. The use of formalin leads to chemical reactions in tissues, such as the formation of methylenic bridges between the side chains of the proteins.[15,21] Therefore, only in situ protein examination is amenable to immunohistochemisty, which provides information about protein expression in a specific cellular population and intracellular localization AQ18 of the analyzed protein,[5] but with a very poor quantitative information.[22,23] A technique was developed[24] to allow mass spectrometric protein expression profiling directly on tissue samples. Though this technique is limited by the need for fresh tissue, its development is a confirmation of the interest and of the necessity for modern pathology to analyze protein expression levels. Therefore, new fixatives designed for the preservation of proteins can represent an interesting innovation in pathology research, owing to the possibility of combining accurate microdissection and protein expression profiles. The results presented in this study show that the FineFIX treated tissues are suitable for protein preservation, allowing protein extraction and conventional proteomic analysis. In fact, the fixation procedure with ethanol-based reagents induces substitution of hydrating water molecules around the proteins with the fixative molecules; this causes structural modifications of the proteins with denaturation, precipitation, and consequent inactivation of every enzyme, including proteases. The latter, are in

fact, the main cause of protein degradation. This mechanism of protein preservation is confirmed by our results. Both the Western Blot (Fig. 4) and the 2D-PAGE analysis (Fig. 5), in fact, show that the quality of the proteins obtained from FineFIX treated samples is comparable to those obtained from frozen tissues.

One of the main problems in proteomic analysis using fresh tissues is the difficulty in obtaining a homogeneous population of cells, because normal and pathological structures are often strictly mixed. Therefore, the data obtained from differential proteomic analysis can be influenced by the composition of the sample analyzed. In FineFIX-fixed paraffin-embedded tissues, it is easy to obtain a well-defined recognition of the morphological structure of the specimen with a mechanical or a more sophisticated laser capture microdissection. The possibility of extracting and analyzing proteins from the same tissue used for the histopathology morphological characterization offers the opportunity, after microdissection, to study a selected specimen with a well-defined cell population.

Protein expression analysis and mRNA quantitative analysis do not give the same type of information. The 2 types of analyses give complementary data. In fact, the functional regulation of the protein expression cannot be predicted unequivocally by the mRNA expression level.[25,26] mRNA levels, in fact, can reflect reactive modifications of cells and can undergo also to specific regulatory mechanisms, which determine the amount of translated protein. Therefore, this new fixative offers the opportunity to gain a more complete characterization of a particular physiopathological condition, analyzing both mRNA and protein expression levels, with the advantage also of storing the samples at room temperature.

In conclusion, the use of this fixative allows more extensive molecular analysis of biological samples. DNA, RNA, and proteins are preserved, with a quality comparable to fresh tissues. Moreover, the use of fixed and paraffin-embedded tissues gives access to morphological analyses such as traditional histology and immunohistochemistry, with a better specimen definition. Pathologic alterations in nucleic acids and proteins can therefore be studied in well-chosen microdissected samples, integrating histopathology analysis with a complete molecular profile of the same specimen, and improving the characterization of diseases in molecular medicine research.

In addition, this reagent does not modify the routine processing of the surgical samples; this fact implies that any new useful molecular biomarker can be applied in a short time to clinical practice.

Samples can be directly treated with the fixative and paraffin embedded without freezing or other particular types of storage. This characteristic also points out a potential application in the development of new tissue biobanks, where the costs can be reduced because tissues can be stored at room temperature, with minimal space needs.

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