Feasibility and Quality of Biological Banking of Human Normal and Tumor Tissue Specimens as Sources of DNA for the Malmö Diet and Cancer Study


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Abstract

Human tumor and normal tissue specimens, which were collected from autopsy material 1–6 days postmortem, were compared with similar tissue specimens collected within 2 h after surgical resection and transport to the pathology department. The end point criteria used to evaluate the quality of the specimens for biological banking purposes were the extractability and yield of high molecular weight DNA and UV absorption ratios at 260:280 after collection and immediate storage of the specimens at −80°C. The data demonstrated that autopsy material was a quality source of DNA, although of not such high quality as surgical biopsy specimens <2 h after resection. The advantages of using autopsy material to supplement surgical specimen collection sent to pathology, as opposed to using specimen collection at surgery wards or formalin-fixed material, as sources of DNA are: (a) large amounts of tumor and normal tissues from a variety of organ sites can be obtained without regard to the patient’s health status; (b) a higher percentage of retrieval of incident cases of cancer in prospective designed trials is more likely to be achieved; and (c) the extractable DNA is of sufficiently high enough quality to permit direct analyses by molecular hybridization and sequence methodologies.

Introduction

Prospective studies involving cancer as an end point in the analyses often involve the enrollment of thousands of individuals. This is the case with the Malmö Diet and Cancer Study where approximately 63,000 individuals were being invited into the cohort (1) of which 30,383 (45%) participated. In an effort to increase the scientific approaches of this study, substantial effort has been made to establish a biological banking system so that biochemical and molecular markers sensitive to the etiology of cancer, but that are also influenced by dietary factor fluctuations, can be identified and studied in the future (2).

It is commonly accepted in the medical and scientific communities that rapid autolytic degradation of mammalian tissues and organs occurs after death. Therefore, the collection of tissue samples has been routinely carried out during or soon after surgery to insure the best possible preservation for research purposes. However, it is not always practical, possible, predictable, or cost-effective to arrange for the collection of biopsy/surgical samples important to prospective epidemiological studies that involve the evaluation of biochemical and molecular markers. One possible source for improving the recovery of human biological specimens is to consider the use of autopsy material. Here, we report on the evaluation of whether autopsy material can be used as a satisfactory source of DNA for biomarker studies.

There are some distinct advantages to using autopsy material as a source for collection of human specimens for future research studies. They include: (a) the opportunity to collect tumor specimens that reflect biochemical or molecular changes that have occurred during tumor progression from diagnosis to death; (b) retrieve numerous normal tissues not in the vicinity of the tumor that are ethically not available during biopsy/surgery; and (c) large amounts of tissue are available and can be preserved under appropriate conditions for immortalization experiments.

Materials and Methods

Materials. Protease K and RNase were purchased from Sigma Chemical Co. (St. Louis, MO); LE Agarose (Sea Kem) was from FMC BioProducts (Rockland, ME); and λ DNA from Boehringer Mannheim (Mannheim, Germany). All general chemicals were of analytical grade.

Source of Human Tissue Samples. All tissue samples were prepared by a pathologist from the Department of Pathology at the Malmö General Hospital. Biopsy specimens from tumor tissues (breast, rectum, and colon) and from normal tissue (colon) were transported directly from the surgical ward on ice within 2 h after surgery to the Department of Pathology, and after pathological examination and preparation of slides of the tissue specimens, the remaining portion of the samples were immediately frozen and stored in 2-ml cryotubes as 0.1–1 g pieces at −80°C. Autopsy specimens obtained from 1–6-day-old corpses were dissected free of unwanted tissues and then prepared as tumor tissue specimens (colon, breast, bronchus, etc.).
bladder, rectum, lung, and liver), as well as normal tissue specimens (colon, breast, bronchus, prostate, and lymph nodes), in a manner identical to the handling of the biopsy specimens. Some of the samples including both autopsy and biopsy specimens were also fixed in standard formalin solution (as described in Ref. 3) for 24 h before storage under the same conditions as already described for the unfixed material.

**DNA Extraction of Directly Frozen (Unfixed) Autopsy and Biopsy Specimens.** All tissue samples were extracted by standard phenol-chloroform procedure (4) with minor modifications. Small pieces of tissue 0.1–1 g were cut into smaller pieces and homogenized on ice with a sintered glass Potter in 2–2 ml of TE-buffer (10 mM Tris, 1 mM EDTA, pH = 8.0), followed by the addition of 320 μl of a 25% SDS solution to achieve a final concentration of 2%, and then the samples were incubated for 10 min at 60°C. Added were 800 μl of 5 M NaCl, which was carefully mixed before the addition of 400 μl of saturated Tris solution (pH = 8.5). The resulting solution was extracted by the addition of 5 ml of phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v), and the aqueous phase (5 ml) was transferred to a 50-ml polyallomer tube before centrifugation. The DNA was precipitated by the addition of 2 volumes of 100% ethanol, and the high molecular weight DNA was removed from the solution by centrifugation (2000 x g). The precipitated DNA was washed in 80%, 90%, and finally 100% ethanol, dried, and transferred to an Eppendorf tube. The DNA was redissolved in 800 μl of TE-buffer, and after the addition of RNase A at a final concentration of 50 μg/ml (40 μl of a 1 mg/ml stock solution), the sample was first incubated for 2 h at 37°C followed by an additional 4-h incubation with 400 μg/ml proteinase K (16 μl of a 20 mg/ml stock solution) at 37°C. Next, the ionic strength was increased to 0.5 M NaCl, and 1 ml of phenol-chloroform-isoamyl alcohol alcohol was added and mixed for 10 min before centrifugation at 14,000 x g in an Eppendorf centrifuge. The aqueous phase was precipitated with 100% ethanol, spooled, and washed in ethanol. The amount of DNA and the purity was quantified by measuring the UV absorption at 280 and 260 nm. A260/A280 = 1 was used to estimate 50 μg of DNA.

**DNA Extraction from Formalin-fixed Specimens.** Formalin-fixed samples from normal and tumor tissue were carefully minced with scissors before incubation at room temperature for 24 h in 50 ml of TEN buffer (100 mM Tris-HCl, 40 mM EDTA, 10 mM NaCl, pH = 8.0) in the presence of 400 μg/ml proteinase K (5). The samples were then pelleted at 1000 x g, and the pellet was homogenized in a sintered glass potter with 3 ml of TEN buffer before incubation for another 24 h at room temperature in a final volume of 4 ml adjusted to contain 400 μg/ml proteinase K and 1% SDS. The SDS concentration was raised to 2% before incubation was continued at 60°C for 10 min.

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*Spooled DNA was judged to be of high quality based on the fact that the bulk of the DNA sample had a molecular weight similar to λ DNA after agarose gel electrophoresis.

*Mean ± SD.

* Frequency distribution difference, Chi-square, *P* = 0.09.

* t-test, *P* < 0.005.
Next, the incubation mixture was adjusted by the addition of 800 μl of 5 M NaClO4 and 400 μl of saturated Tris solution, and then the remaining DNA extraction procedures were carried out identically as described above for the directly frozen (unfixed) samples.

**Gel Electrophoresis.** DNA (1 μg) from each sample was placed into ethidium bromide stained 0.7% agarose minigels with λ DNA as a high molecular weight standard. The samples were then migrated into the agarose by electrophoresis in 0.5 X Tris, 1 mM EDTA, 45 mM boric acid) at 70–80 V for 1–2 h (front = 5–6 cm).

**Results**

The data are presented in Table 1. As expected, when the DNA was evaluated by high molecular weight and yield, both normal and tumor tissue specimens collected at biopsy/surgery were of the highest quality (P < 0.05). However, the autopsy specimens having an average collection date of 3.1–3.2 days after death yielded surprisingly high quality DNA. Although in most cases biopsy DNA samples were significantly better, the autopsy specimens still gave on the average 79–81% high molecular weight DNA. As expected, when the DNA originating from autopsy material could be spooled, they were nonetheless of high quality because high molecular weight DNA could be collected by centrifugation, even if the DNA did not spool from the ethanol precipitating solution. On the basis of these data, it was concluded that although formalin fixation greatly reduced the quality of DNA, this type of specimen could nevertheless serve as a supplemental source to more reliable tissue specimens. Moreover, if the pathologist was motivated to directly freeze portions of specimens being prepared for formalin fixation, the recovery of quality DNA samples would be significantly enhanced.

**Discussion**

There are two choices for retrieval of human tumor tissue specimens when the objective is to establish a source of biological material for future study; namely, either after resection at the surgery department or after histological preparation and examination at the pathology department. Because it was not possible to obtain any scientific reports regarding the appropriateness of using normal or tumor tissue specimens collected after arrival at pathology departments, we have studied if samples collected at autopsy, or if biopsy material sent to pathology from surgical wards, could be used as a quality source of DNA for biological banking purposes.

The feasibility of using human tumor and normal tissue specimens as source material for quality DNA preparations when collected at a pathology department, where there is a time delay before samples can be processed and stored at ~80°C, has been demonstrated by the data presented in Tables 1 and 3. To our knowledge, this is the first study that shows that autopsy specimens sent to the pathology department and collected in the morgue after 1–6 days of storage are good sources of reasonably high quality DNA.

Our experience in this study has been that biopsy/surgery specimens produce the highest quality DNA, whereas formalin-fixed material yielded inferior quality DNA samples (Tables 1 and 3). Formalin fixation and autopsy are the primary responsibilities of pathology departments and, as such, these activities are primarily located there. Hence, there is considerable justification to supplementing normal and tumor tissue specimen collections of formalin-fixed material with the collection of
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autopsy material because it offers an increased yield and quality of DNA. Prospectively designed epidemiological studies involving biomarker development often have as a major objective to recover biological samples from as many as possible incident cases. The data in Table 1 clearly identify autopsy material as another major source of DNA easily accessible to researchers and available through normal clinical routines, which was not previously believed to be satisfactory. In addition, autopsy material provides opportunities to study tumor progression and normal organ involvement that are not options when specimens are collected only via biopsy/surgery or after formalin fixation.

In summation, it is quite important to focus our conclusions to be primarily relevant to investigators planning a biological bank for detailed molecular epidemiological studies. The approach we are recommending does not emphasize retrospective studies where formalin-fixed specimens already routinely prepared by pathology departments for other purposes, would clearly be a readily available and cost-effective source of tumor tissue. However, directly freezing unfixed autopsy or biopsy specimens arriving at the pathology department has a great advantage for prospective studies because these specimens could serve as sources for both (a) DNA and molecular genetic analyses of normal or tumor tissues when present, and (b) immortalization by xenografting to athymic mice for future in vitro or in vivo biological analysis. For example, it is well known that athymic mice accept xenografts from human normal and tumor tissues, and the morphology of these xenografts are maintained for up to 715 days (6). Although formalin-fixed tumor tissues have the retrospective advantage of being available, they cannot serve as a potential source for growing tumors either in vitro or in vivo.

References

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