行政院國家科學委員會專題研究計畫果報告書

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★　計畫名稱：石蠟塊切片的基因型鑑定　★

★　(The genomic identification of paraffin section)　★

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計畫類別：個別型計畫

計畫編號：NSC-88-2314-B002-187

執行期間：民國 87 年 8 月 1 日至民國 88 年 7 月 31 日

個別型計畫：　計畫主持人：吳木榮

共同主持人：

處理方式：□可立即對外提供參考
□一年後可對外提供參考
□兩年後可對外提供參考

執行單位：台大醫學院　法醫學科

中華民國 八十八 年 七 月 三十一 日

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執行機構： 台大醫學院

執行單位： 法醫學科

關鍵詞：基因型鑑定、石蠟塊切片、法醫病理學

目的：研究由石蠟塊組織中萃取 DNA **以進行基因型鑑定的方法。**

材料：從 1998 **年** 1 **月到** 1998 **年** 12 **月底，由臺大醫院病理部的手術切除肝臟的標本中，選擇適合研究的肝臟標本** 20 **例，作為研究的對象。每個案例均收集病人的血液、新鮮肝臟組織及石蠟塊組織，進行** DNA **基因型的鑑定及比較研究。**

方法：

實驗組：將 20 **個案例的肝臟組織，經過** 10%**中性福馬林固定後，包埋於石蠟塊中，切成** 6-8 μm **的切片作病理學觀察，然後在由石蠟塊中稱抽取組織 的** DNA **後，依照** Amplitype ® PM PCR Amplification and Typing Kit (Perkin Elmer; Roche Molecular Systems, Inc. Branchburg, NJ) **的操作步驟進行** PCR **的** amplification**，其所用來作** PCR amplification **的** genetic locus **分別為：**

HLA DQA1 PCR product size 242/239 bp

Low density lipoprotein receptor (LDLR；PCR product size 214 bp)

Glycophorin A (GYPA； PCR product size 190 bp)

Hemoglobulin G Gammaglobulin (HBGG；PCR product size 172 bp)

D7S8 (PCR product size 151 bp)

Group Specific Component (GC；PCR product size 138 bp)

經過一系列 PCR amplification **後，在** polymarker probe strips **上顯示其結果，經判讀之後加以分析。**

控制組：將 20 **個案例的血液及新鮮標本抽取** DNA **後，利用** Amplitype ® PM PCR Amplification and Typing Kit (Perkin Elmer; Roche Molecular Systems, Inc. Branchburg, NJ) **同**

樣進行其 DNA 的 typing, 將其結果作為控制組, 與實驗組 --- 石蠟塊組織的基因標本比較，分析其差異。

結果：

在 20 例研究案例中，有 15 例為男性，5 例為女性，年齡由 31 至 88 歲不等，其平均年齡為 57.1 歲。其中有 16 例為肝細胞癌，9 例有肝硬化 (佔 56.25%)，7 例為慢性肝炎，無肝硬化。另外，有 2 例為結腸轉移至肝臟，肝細胞無肝炎；1 例為良性腫瘤，肝細胞無肝炎；最後 1 例為肝移植後肝硬化，肝細胞有肝炎。

以上 20 例的 PM DNA 基因型分別為：

LDLR： AA --- 1 例；AB --- 9 例；BB --- 10 例；

GYPA： AA --- 7 例；AB --- 8 例；BB --- 5 例；

HBGG： AA --- 0 例；AB --- 8 例；BB --- 12 例；

D7S8： AA ---10 例；AB --- 9 例；BB --- 1 例；

GC：AA --- 0 例；AB --- 9 例；AC --- 4 例；AC --- 2 例；

BB --- 2 例；BC ---3 例；CC --- 2 例。

以上實驗結果發現，利用石蠟塊切片組織來進行 DNA 基因鑑定是一種可行的方法。藉由本研究的方法, 人們可以進行各種相關的研究, 也可應用於法醫學的鑑定工作和人身鑑別，實在是非常有價值的一種方法。

目　錄

中 文 摘 要

在 20 **例研究案例中，藉由案例中血液的** POLYMARKER DNA **基因型，與同一人石蠟塊切片的** POLYMARKER DNA **基因型的交叉校對後，所有** 20 **例研究案例均能順利的由石蠟塊切片中獲知其** POLYMARKER DNA **基因型，其結果與血液的結果吻合。**

以上實驗結果顯示，利用石蠟塊切片組織來進行 DNA **基因鑑定是一種可行而有價值的的方法。藉由本研究的方法，我們可以進一步地推動各種相關的研究，也可應用於法醫學的鑑定和人身鑑別工作，實在是非常有值得利用的一種方法。**

關鍵詞：石蠟塊切片、POLYMARKER DNA **基因型，法醫病理學**

ABSTRACT

From 1998 to 1999, 20 cases of resected liver tissue in the Department of Patholology of National Taiwan University Hospital were studied for their tissue genomic type in the paraffinized sections. All these 20 cases were successful to extract their tissue DNA and typing by the Amplitype® PM PCR Amplification and Typing Kit.

The Amplitype® PM PCR Amplification and Typing Kit is a very powerful and useful tool to identify the individual genetic pattern in forensic fields. By using the application of Amplitype® PM PCR Amplification and Typing Kit, we demonstrate a clinical, practical way to resolve the problems of genomic typing in the paraffinized tissue in the forensic pathology. Also, it is a quickly, easy, specific and scientific way to perform in the molecular laboratories within 3 days.

In conclusion, it is possible to resolve the clinical and medicolegal problems of genomic typing in paraffin-embedded biopsies by using the Amplitype® PM PCR Amplification and Typing Kit now.

Key words: Genomic typing, Polymarker, Paraffinized section, Forensic pathology

INTRODUCTION

One of the most important developments in the field of human identify testing is the use DNA typing to analyze biological evidence.[1,2] In particular, the powerful GeneAmp Polymerase Chain Reaction (PCR)[3] is used to analyze samples which can't be typed by other methods, such as samples containing minute amounts of human DNA and very old and/or degraded DNA.[4-8] The Amplitype® PM PCR Amplification Typing Kit is a commercially available human identify testing product based on the reverse dot blot typing technology[9] for forensic casework analysis.[10-11]

Currently, most identified human materials for genomic typing are blood, muscles and bones. However, the majority of human tissue studied in the field of forensic pathology are fixed and paraffinized sections. If there are not other tissue available, paraffinized tissue is the only way to identify victim's genomic type. In addition, mixed up tissue specimens during the processing for microscopic examination do occur accidentally. If there is any question, the differentiation of which is which is very critical and important. The histological findings are skillful and useful to distinguish the mixed up, however, it is impossible or doubtful to completely separate these mixed up tissue specimens.

In order to evaluate the possibility of genomic typing in paraffinized tissue, we design this study to explore the way to perform by application of Amplitype® PM PCR Amplification Typing Kit in our forensic pathological laboratories and set up a quick, scientific method for future further application in these kinds of situation.

MATERIALS AND METHODS

Histology:

Twenty cases of patients with resected liver specimens were collected and studied from 1998-1999. The specimens includes the snapped blood present in the resected liver, the fresh frozen liver tissue and formalin-fixed paraffin blocks. The paraffinized sections were stained with routine hematoxylin-eosin staining. Then, they are observed under the microscope and recorded.

DNA Isolation:

The snapped blood DNA was extracted by Genomix Kit. DNA from paraffin-embedded tissue was isolated by 4-hour incubation of six 10 μm sections, without deparaffinization, in 100 μl 10 mM Tri.HCl, pH 8.3, 0.5% Tween 20, and 1 mM EDTA 10% proteinase-K (wt/vol) at 56°C,[12] followed by a 10-minutes incubation at 100°C to inactivate the proteinase-K. The tubes were centrifuged directly while the samples were hot to attain a proper separation of the liquid and solid phases. The liquid phase was carefully decanted through the solid paraffin residue.

Amplitype® PM PCR Amplification Typing Protocols

The extracted DNA was amplified by using DNA thermal cycler 480 with total reaction volume of reagents supplied in the typing kit in μl/tube, up to 100 μl plus 2 drops mineral oil. The amplification program was set on 32 cycle. Each step-cycle includes 60 sec 94°C, 30 sec 60°C, 30 sec 72°C with time delay of 7 minutes 72°C. The Amplitype® PM reactions were then verified by 3% agarose gel electrophoresis in minigel electrophoresis apparatus. The amplified DNA was then proceeding to the DNA hybridization including 1) hybridization of amplified DNA to DNA probe strips; 2) binding of HRP-SA Enzyme Conjugate to hybridized PCR products; and 3) stringent wash to remove non-specifically bound PCR products. Color development was performed by using TBM and hydrogen peroxide.

Interpretation of Results

The pattern of blue dots from each wet DNA probe stripe was recorded and take photographs. The results were analyzed.

Amplitype® Genetic Marker Characteristics

	LDLR	GYPA	HBGG	D7S8	GC
Chromosomal location	19p13.1-13.3	4q28-31	11p15.5	7q22-31.1	4q11-13

Power of discrimination for Amplitype® Genetic Marker Systems

U.S. Caucasian	African American	U.S. Hispanic	Japanese
0.9954	0.9948	0.9961	0.9938

Evaluation of genomic type in paraffinized sections and compare with it of snapped blood

After genomic typing of paraffinized section, we compare with the previous genomic studies of snapped blood for its accuracy.

RESULTS

Clinical information and Histology：

The 20 cases are 15 males and 5 females, ranged from 31 to 88 years old (mean: 57.05 years). There are 16 cases of hepatocellular carcinoma, 9 (56.25%) cases with cirrhotic liver among these 16 cases. The others include 2 metastatic adencarcinoma of colorectal cancer, an inflammatory pseudotumor and a posttranplanted liver cirrhosis.

Amplitype® PM PCR Amplification Typing Results

The Amplitype® PM PCR Amplification Typing results are list as follow:

	LDLR	GYPA	HGBB	D7S8	GC
	A B	A B	A B C	A B	A B C
Case 1	AA	BB	AB	AA	AC
Case 2	AB	AA	AB	AA	AB
Case 3	AB	BB	BB	AA	BC
Case 4	AB	AB	AB	AA	AB
Case 5	AB	BB	BB	AA	AB
Case 6	AB	AB	BB	AB	CC
Case 7	AB	AB	BB	AA	BC
Case 8	AB	AA	AB	AB	AB
Case 9	AB	BB	BB	AB	CC
Case 10	AB	AA	AB	AA	AC
Case 11	BB	BB	BB	AA	AB
Case 12	BB	AA	AB	AA	AB
Case 13	BB	AA	AB	AA	BB
Case 14	BB	AB	BB	AB	AB
Case 15	BB	AB	BB	AB	BB
Case 16	BB	AB	BB	AA	AC
Case 17	BB	AA	BB	AA	BC
Case 18	BB	AB	AB	AA	BC
Case 19	BB	AA	BB	AB	AB
Case 20	BB	BB	BB	BB	AC

Comparison of Genomic Typing Between Blood and Paraffin Sections

Each pairs of polymarker DNA typing in paraffinized section and blood are the same. They can duplicated each other, i.e., they can be reconstructed and confirmed by the individual genetic typing of blood. The total genomic typing rate of paraffinized section is 100%.

DISCUSSION

As a forensic colleague says, “DNA is in everywhere”. Today, DNA typing become a vital identification card in our society. You can’t destroy it, but the forensic scientist may not find it. Hence, our study demonstrates that genomic typing in the paraffinized sections is very difficult but still possible. By using our method, we can do the genomic typing of each paraffinized sections and are corresponded to the genomic type of snapped blood. These results are very encouraged to the forensic pathologists because they are dealing with paraffinized sections every day. From now on, they can distinguish which section belongs to whom. Furthermore, they can perform personal identification from the sections.

Although mismanagement of surgical specimens for diagnostic purposes should be prevented insofar as possible by good laboratory practices, mixing up labels or double use of numbers can occur. Problems proceeding from this can be very serious, ranging from being obligated to take new biopsies to the impossibility of making a diagnosis, for example, in total resections. To identify mix-up specimens correctly, a method is required that is fast, sensitive, and specific. Sex-chromosome-specific analysis on sections, such as Barr body counting, is unreliable because of sections contain mainly partly cut nuclei. In situ hybridization with X-chromosome-specific probe is limited to cases of different sexes. Chromosome-specific polymorphic microsatellite markers circumvent this limitation, and these highly polymorphic markers can also be used on specimens from patients of the same sex. Moreover, this PCR-based PM genetic typing can be performed on very small amounts of paraffin-embedded tissue. Shibata et al.[13] had used allele-specific oligomer probe to specifically detect polymorphism in the HLA DQα region and LDLR gene after PCR amplification. Because fewer alleles can be detected, this method has a lower specificity than Polymarker genetic typing.

In summary, our study reveals that genomic typing of paraffinized sections is available, valuable and practical in forensic pathology. We, forensic pathologists, can give the paraffin block a new life.

計畫成果自評

本研究是以實用性為主導的研究計畫，目的在於利用無生命的石蠟塊切片，以現代分子生物學的方法, 將其轉換為法醫病理學上有用的鑑定物質, 不但可以用於疾病的診斷與治療，而且可以進行人身鑑別的依據，實有無比的學術價值！

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