

Technical report

Amplification of FISH signals using intermittent microwave irradiation for analysis of chromosomal instability in gastric cancer

Y Kitayama, H Igarashi, H Sugimura

Abstract

Gastrointestinal tract tumours are notorious for their difficulty in relation to conventional cytogenetic analysis. In particular, necrosis, the presence of stromal inflammatory and other cells, and poor attachment of tumour cells have led to problems with the quality and reliability of cytogenetic preparations, even with the recently developed fluorescence in situ hybridisation (FISH) technique. Furthermore, background autofluorescence masks the weak hybridisation signals in the nuclei. To overcome this problem, brief microwave treatment was applied for the identification of centromeres by in situ hybridisation in gastric cancer cells. Using this technique, a panel of 17 centromeric specific α -satellite probes was used to detect chromosomal instability in these cells. Lymphocyte controls and cancer cells subjected to irradiation achieved the hybridisation threshold in 30 minutes, providing a significant difference when compared with the non-irradiated samples (mean (SD) frequency of diploid cells 97% (2.1%) v 76% (4.6%), respectively). Therefore, this protocol of intermittent microwave treatment is recommended as a simple, rapid, and highly reproducible technique for application to various types of probe. It also gives well defined hybridisation signals and reduces background "noise".

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Keywords: fluorescence in situ hybridisation; microwave irradiation; gastric cancer

Numerical chromosomal abnormalities are a well known characteristic of human cancer, but only a few studies have examined chromosomal abnormalities in gastric cancer by means of fluorescence in situ hybridisation (FISH).^{1,2} Although FISH is a powerful tool for the evaluation of chromosomal abnormality, the standard FISH technique, originally developed by Pinkel *et al*,^{3,4} includes overnight (12 hours) hybridisation, even with a directly fluorochrome labelled probe. Microwave oven heating of tissue sections has been used recently for

"antigen retrieval" from formalin fixed, paraffin wax embedded tissues. Immunohistochemical techniques on such tissues often have improved staining with various antibodies.⁵⁻⁷ In our study, we applied intermittent microwave irradiation to the FISH technique. This new procedure takes only one hour and gave consistent and distinct signals, without fluctuation in intensities, as encountered with conventional methods.

Materials and methods

TISSUE SAMPLES

All samples were obtained from surgically resected specimens at the Hamamatsu University Hospital and its affiliated hospitals. Touch preparations of fresh gastric cancerous tissue and control lymphocytes from normal lymph nodes were used for FISH analysis.

DNA PROBES

A panel of 17 centromeric α -satellite DNA probes (D1Z5, D2Z, D3Z1, D4Z1, D6Z1, D7Z1, D8Z2, D10Z1, D11Z1, D12Z3, D15Z, D16Z2, D17Z1, D18Z1, D20Z1, DXZ1, and DYZ3) derived from chromosomes 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 15, 16, 17, 18, 20, X, and Y, respectively, was purchased from Oncor Inc (Gaithersburg, Maryland, USA) and all the probes were labelled with digoxigenin-11-dUTP by nick translation.⁸

FISH WITH MICROWAVE AMPLIFICATION

The samples were fixed in methanol/acetic acid (3/1) and then DNA was denatured at 75°C for two minutes. After treating with ethanol, a 10 μ l mixed solution with probe (7 μ l Hybrisole IV, 0.5 μ l carrier DNA, 1 μ l probe, and 1.5 μ l double distilled water) was placed on to a slide glass with a cover slip over it. The samples were then put into a microwave processor (MI-77; Azumaya, Tokyo, Japan). The device was set to give irradiation at intervals of four seconds on and seven seconds off, at a frequency of 2.45 GHz at 300 W output power, with the temperature sensor set at 38°C. Seven hybridisation times were tested. Thus, each probe was hybridised with microwave irradiation for five minutes, 15 minutes, 30 minutes, one hour, three, six, and 12 hours, and these samples were compared with those without irradiation.

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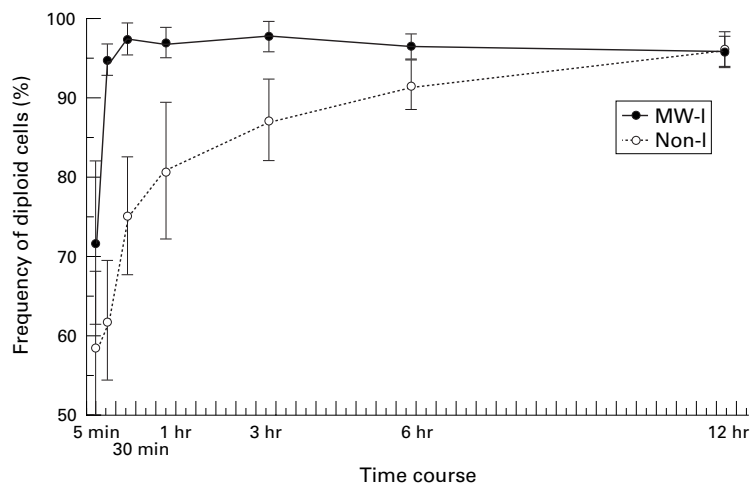


Figure 1 Mean (SD) percentage of two spot signal of lymphocyte controls in 17 probes with microwave irradiated and non-irradiated samples. Significant difference obtained at 30 minutes ($p < 0.001$). MW-I, microwave irradiated; non-I, non-irradiated.

Fluorescent staining by preincubation and a fluorescein isothiocyanate (FITC)-digoxigenin labelled probe was applied to each sample after hybridisation. Propidium iodide (1 $\mu\text{l/ml}$; Sigma, St Louis, Missouri, USA) was used for nuclear staining. The samples were observed promptly after staining.

EVALUATION

The numbers of nuclei capable of being analysed by each probe were calculated from 200 cell nuclei and also simultaneously compared for signal intensity with an Olympus photomicroscope equipped with epifluorescence (BH-2; Olympus, Tokyo, Japan). After fluorescent staining, the frequency of diploid lymphocytes seen in microwave irradiated samples was compared with the number seen in non-irradiated samples, according to the time course.

Results and discussion

Lymphocyte controls subjected to irradiation attained the hybridisation threshold in 30 minutes, providing a significant difference in relation to the non-irradiated samples (mean (SD) frequency of diploid cells, 97% (2.1%) *v* 76% (4.6%), respectively; $p < 0.001$). All probes showed a distinct two spot signal, with no variations in staining intensity for an average of 96% of the nuclei. After six hours of microwave intermittent irradiation, the signals appeared to decrease (fig 1). A significant difference was obtained during the time course in probing eight gastric cancer tissues, monitored after microwave irradiation for up to 12 hours (fig 2). The α -satellite probes for chromosomes

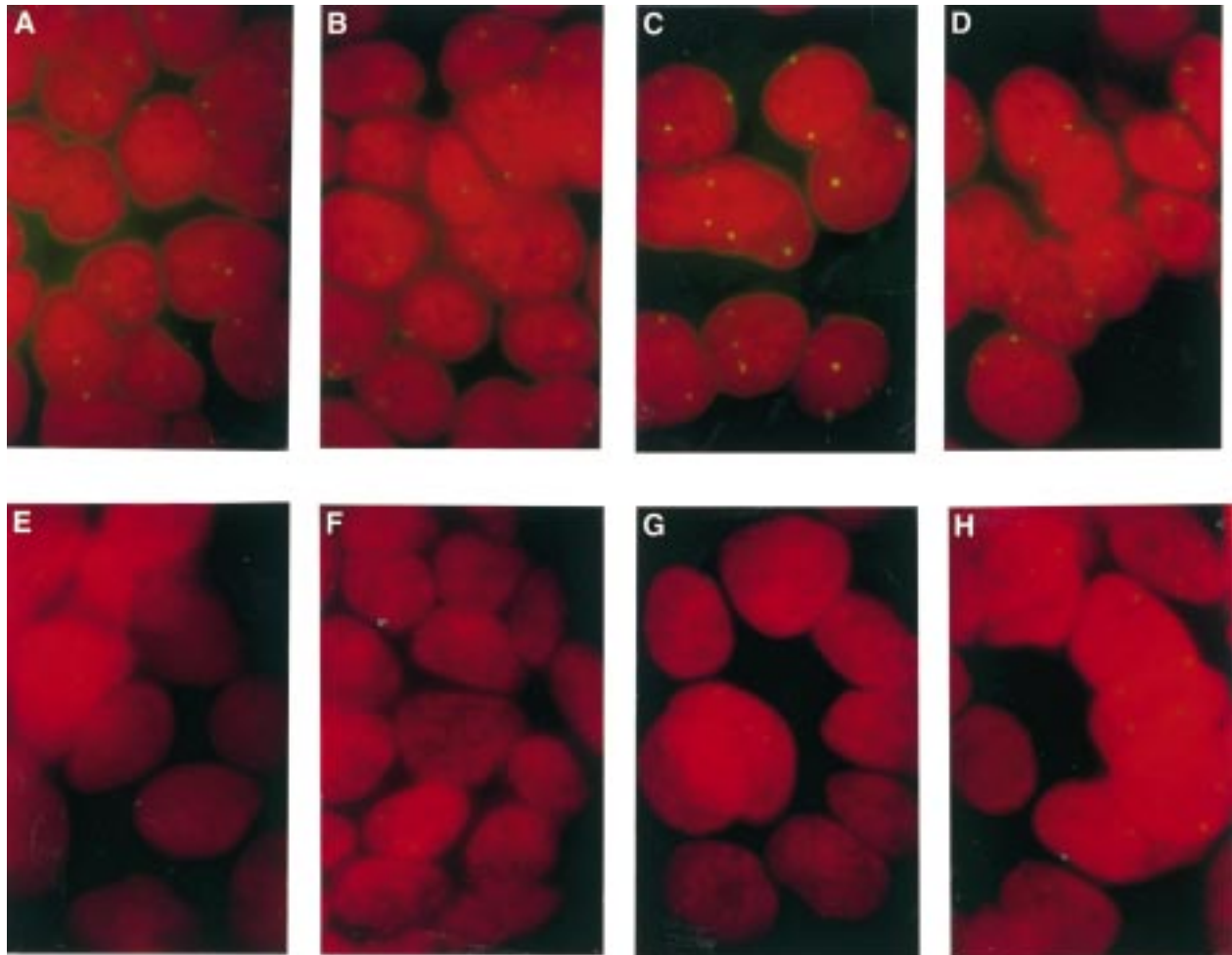


Figure 2 Chromosome 2 centromere in a case of gastric cancer with microwave irradiated and non-irradiated samples. Comparison of time course and signal intensity of cancer cells in fluorescence in situ hybridisation analysis with microwave irradiation (A, B, C, and D) and without irradiation (E, F, G, and H). Time course: A and E, 30 minutes; B and F, one hour; C and G, three hours; D and H, 12 hours.

1, 2, 16, 20, and Y usually had poor staining signals, and in many cases the identification of these chromosome was difficult using conventional procedures because of weak signals. However, with our method there was a distinct signal, compatible with those obtained with other chromosome probes. Importantly, microwave irradiation was performed at intervals to keep as uniform a temperature as possible, and also to prevent the slides from drying. Results showed that hybridisation, even for the shortest time (15 minutes), yielded satisfactory results for analysis; furthermore, the intermittent microwave irradiation protocol allows the entire process to be completed within one hour. This sequence also decreases background "noise" to a minimum. Although the mechanistic basis for improved sensitivity is not clearly understood, it might be the result of enhancement of intermolecular movement and the improvement of nick translation and binding.^{9 10} The signals with microwave irradiation diminished slightly after six hours. The fluorescence seen with in situ hybridisation occurs at specific points and "loose", non-specific binding might be disrupted by excessive microwave vibration. The application of our approach to more difficult ISH targets is a challenge. We are currently evaluating the application of this method to paraffin wax embedded and frozen sections. We are also studying penetrability of probes. We have tested this procedure only in gastric epithelium and normal lymph node lymphocytes, and it would be interesting to see whether the effect is in any way tissue dependent. We believe that this

rapid FISH technique using microwave antigen retrieval will facilitate the speedy and accurate analysis of chromosomal abnormalities in human cancer.

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