

## Short Communication

# The single-cell gel electrophoresis assay to determine apoptosis induced by siRNA in Colo 320 cells

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**The aim of the present study was to determine if the pattern of DNA fragmentation determined by the single cell gel electrophoresis assay can be used to determine apoptosis induced by siRNA in Colo 320 cells. When the frequency of appearance of apoptotic cells following was observed over a period of time, there was a significant increase in appearance of apoptosis when using single cell gel electrophoresis assay. The present report demonstrates that the characteristic pattern of apoptotic comets detected by the comet assay corresponds to cells undergoing apoptosis.**

**Key words:** The single cell gel electrophoresis assay, Colo 320 cells, apoptosis, comets

## INTRODUCTION

The single cell gel electrophoresis or comet assay is a powerful tool for the measurement of both DNA strand breaks and oxidative base damage and has recently become available commercially for these purposes (Henderson et al., 1998; Robert et al., 1998). It is also considered that this method could be used for detecting DNA fragmentation in an apoptotic cell (Gopalakrishna and Khar, 1995; Humar et al., 1997; Olive et al., 1998). As the frequency of occurrence of apoptosis increases, the tail moment and tail length, which are parameters correlated to the degree of DNA damage measured by the comet assay both increased (Cregan et al., 1999; Dikomey et al., 1998). In practice, an image of a different type from the normal comet image is observed. Therefore, the comet assay can be used to detect a cell undergoing apoptosis (Godard et al., 1999). This study was undertaken in human in Colo 320 cells, a human colon cancer cell line which has previously been shown to undergo apoptosis when treated with siRNA directed against c-myc. The alkaline version of the single cell gel electrophoresis assay was used for this study.

## MATERIALS AND METHODS

### Cell culture

The colon cancer cell line Colo 320 was obtained from China center for type culture collection (GDC298). The cells were grown in RPMI-1640 medium (GIBCO, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Gibco BRL), 50 units/ml penicillin and 50 µg/ml streptomycin. The Colo 320 cells were maintained in a humidified 37°C incubator with 5% CO<sub>2</sub>, fed every 3 days with complete medium and subcultured when confluence was reached. The cells were routinely passaged every 1 or 2 days.

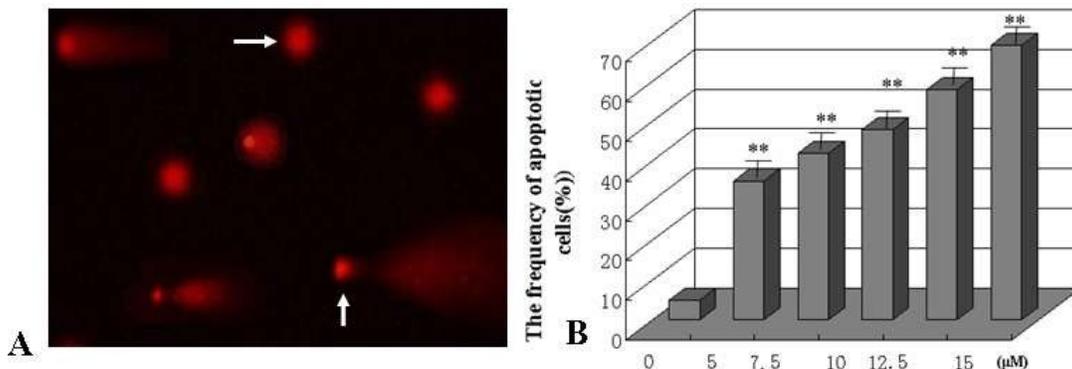
### Transfection

For transfection,  $2 \times 10^5$  cells were seeded into each well of a 6 well tissue culture plate (Costar). The next day (when the cells were 70 - 80% confluent), the culture medium was aspirated and the cell monolayer was washed with pre-warmed sterile phosphate-buffered saline (PBS). Cells were transfected with the pGensil-c-Myc-1 (Huang Hao et al., 2008) by using lipofectamine 2000 (Invitrogen) reagent (Invitrogen) in accordance with the manufacturer's protocol. Cells were continuously cultured until harvest for analysis.

### Comet assay

Transfected cells were then placed in lysis solution (containing 2.5 M NaCl, 100 mM EDTA, 10 Mm tris (hydroxymethyl) amino-methane, 1% (v/v) Triton X-100, and 10% (v/v) dimethyl sulphoxide, pH 10) for 1 h at 4°C, followed by 40 min in alkaline solution (1 mM EDTA/300 mM NaOH) at 4°C, to allow DNA unwinding to occur. Electrophoresis was performed, without changing the alkaline

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**Figure 1.** Apoptotic cells transfected was detected by comet assay measured. As described in materials and methods. After cell electrophoresis and staining with EB, Colo 320 cells were photographed by fluorescent microscopy: The upper cell represents a normal comet image. The lower cell is considered to be an image of a typical comet undergoing apoptosis that has a small head and a spread tail. This image is called an apoptotic comet (A). The frequency of apoptotic cells detected by the comet assays (B). All data were obtained from three independent experiments. Error bars represent means  $\pm$  SEM. Significantly different from the corresponding control and vector (\*\* $P < 0.01$ , vs control. ## $P < 0.01$ , vs vector).

solution, at 20 V for 25 min. The slides were neutralized (400 mM tris (hydroxymethyl) aminomethane, pH 7.4) and stained with ethidium bromide. Using a fluorescence microscope equipped with a green filter (Olympus, Tokyo), the frequency of apoptotic cells was detected by scoring apoptotic comets and non-apoptotic comets. About 100 cell were scored and at least 3 replicates were used within each experiment.

#### Statistical analysis

Differences between assays are analyzed using one-tailed or two-tailed pair t-test as appropriate (GraphPad Prism). Probability values of  $\square 0.05$  are considered to represent significant differences.

## RESULTS AND DISCUSSION

As the single cell gel electrophoresis assay has been reported to be a good method of detecting apoptosis, not only as a result of its early detection of strand breaks, but also because of the distinctive shape of the comets produced (Roser et al., 2001; Singh, 2000; O'Callaghan et al., 2001). We had hoped to see an increase in DNA strand breaks prior to cell lysis in Colo 320 cells undergoing apoptosis. Figure 1A is a photograph of a comet observed 24 h following transfection with siRNA. The photograph in Figure 1A shows a typical image of a migrated cell nucleus with DNA strand breaks and that the greater portion of the DNA has fragmented and has migrated sufficiently to make the tail. This characteristic image is called a teardrop, pear-shaped, or large fan-like tail. It is understood that this finely cut, spread, migrated DNA is indicative of an apoptotic cell. In this paper we refer to this image as an apoptotic comet. On the other hand, comet assay also reveal that Colo 320 cells transfected with siRNA have more apoptotic comet than those in the non-transfected controls. Transfection of different concentrations lead to resulted in the significant incre-

ment of the mean frequency of apoptotic comet (Figure 1B,  $P < 0.01$ ). Though it is difficult to distinguish apoptotic cells and non-apoptotic cells with morphological assay, it is easy to distinguish with the comet assay. This indicates that not only can DNA damage and repair be measured, but also early apoptosis in vivo can be observed with good specificity with the comet assay.

## Conclusion

In conclusion, not all apoptotic cells are detectable by the comet assay. However, we have shown that the apoptotic comets observed by the comet assay are apoptotic cells.

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