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Review

Single-Cell Gel Electrophoresis (SCG)-A Review and Discussion

Yuquan LU, Tatsuya TAKESHITA and Kanehisa MORIMOTO

Department of Hygiene and Preventive Medicine, Osaka University School of Medicine, Suita

Abstract

Single-cell gel electrophoresis (SCG) is a simple, sensitive and effective technique. Being able to reflect quantitatively the genotoxicity of many hazardous agents, it is promising for application in environmental genotoxic monitoring and the study of carcinogenesis. In clinics, it can be used to evaluate the DNA repair ability and monitor DNA breaks during cancer therapy. As a biomarker, it has its own merits and limitations, being different from other biomarkers such as sister chromatid exchange (SCE) test and micronuclei (MN) assay. In many studies, it is more sensitive than SCE or MN. Combination studies with other biomarkers like SCE, MN, chromosomal aberration, bcl-2 and genetic polymorphisms have begun to demonstrate its great importance for the understanding of carcinogenesis and the genotoxicities of environmental factors.

Key words: Single-cell gel electrophoresis, Comet assay, Biomarker, DNA strand breaks, Genotoxicity

Introduction

SCG, since its development by Singh and colleagues in 1988[1], has rapidly found wide application in biomedical science. Because of its simplicity, sensitivity and practicality, it has been applied in many fields, including epidemiology[2], environmental pollution[3], industrial hygiene[4], biomonitoring[4, 5], cancer research[6-8] and so on. SCG, as a biomarker to indicate the degree of DNA damage, is nearer to the endpoint effect and may represent a different aspect from other biomarkers such as DNA adducts, SCE and the micronucleus assay. The recent years of study and application of the method merit discussion so that it can be applied more broadly and effectively in the future.

1. The Method

SCG, also known as the comet assay from the appearance of the damaged DNA after electrophoresis, is based on the observation of single cells and gives quantitative data mainly showing a DNA single-strand (including some double-strand) breaks by means of microelectrophoresis (Fig. 1). The degree of DNA damage can be well reflected through measuring the parameters such as cell tail length or tail moment (Fig. 2). As a biomarker to judge the genotoxicity of the exogenous and endogenous[9] hazardous agents in the human body, it has many advantages and some limitations of its own.

(1) Advantages

First, SCG is a sensitive biomarker. It is estimated that as few as 0.1 DNA breaks per 10^9 daltons can be detected in UV-C-irradiated HeLa cells[10]. DNA damage in newborn infants can also be detected if the mother smoked when pregnant[11]. To detect the DNA damage caused by smoking, it is more sensitive than SCE[12, 13]. In addition, even physical activities can be detected to cause an increase of the tail length in the peripheral white blood cells of healthy volunteers[14]. Second, using SCG, we can monitor the cell ability to repair DNA breaks, which will aid our understanding of the complex processes of mutagenesis and carcinogenesis[15]. Third, SCG can monitor the stability of DNA samples. In the study of cancer and toxicology, it is reported that the basal levels of DNA damage in frozen tissues are higher than in fresh tissues. Evaluation of DNA damage in tumors stored using cryopreservation may produce artificially exaggerated levels of damage. Alkaline SCG enables us to monitor the DNA damage of the frozen tissues, results in greater accuracy in examination of such tissues[16].

As a biomarker, one of its characteristics is the variability that widely exists among different individuals, different kinds of cells[17] and different cells of the same kind[18] (Fig. 1). This offers us a way to study the differences among different cell sub-populations in different conditions. An example is that SCG can show the different basal levels and induced DNA damage rates between lymphocytes and monocytes. For the basal level, the SCG is 2.9-

Reprint requests to: Kanehisa Morimoto,
Department of Hygiene and Preventive Medicine, Osaka University School of Medicine,
2-2 Yamadaoka, Suita, Osaka 565, Japan
Phone: +81-6-879-3920
FAX: +81-6-879-3929

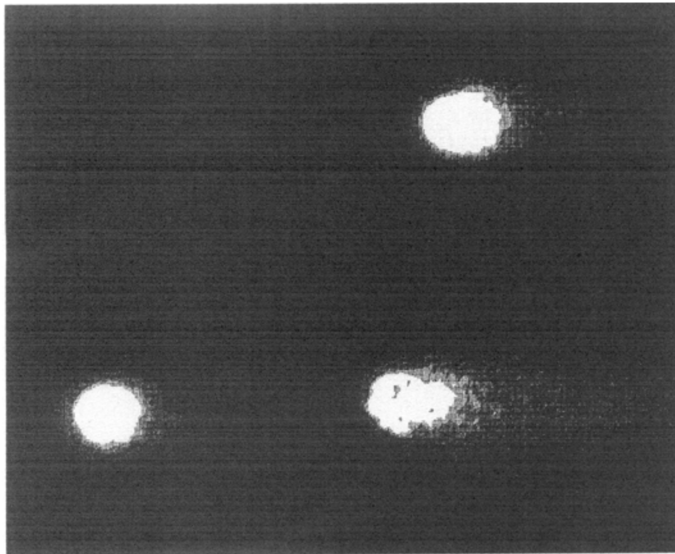

Fig. 1 The DNA Damage of Chinese Hamster Lung (CHL) cells induced by 2 × 10^{-6} M CH_3HgCl. (The degree of the DNA damage can be judged from the length of the cell tail.) Three cells derived from the same kind of CHL cells are presented on a single microgel slide. All three cells were exposed to the same dose of CH_3HgCl under the same experimental conditions. The difference of the tail length between different cells shows variability of the degree of DNA damage among individual cells.

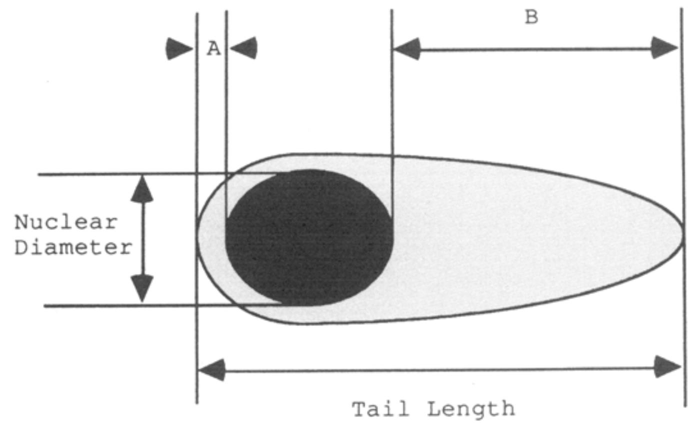

Fig. 2 The Parameters of the Single Cell Gel Electrophoresis (SCG)
A+B=DNA Migration

Ratio: The luminance of part: $\frac{\square}{\square + \blacksquare}$ × 100%

Tail Moment: The distance between the heavy center of the nucleus (■) and the damaged part (□) × Ratio

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fold higher in lymphocytes than that in monocytes. Under 10 micromole H_2O_2, it is 11.3-fold higher in lymphocytes than in monocytes [19].

Last, SCG is a simple, economical and time-saving biomarker. It has a wide range of choices of cell species including plants, earthworms and fish cells as samples in addition to mammalian cells.

(2) Limitations

SCG seems not to be sensitive to the cross-linking damage in environmental monitoring. Although we can reach a positive conclusion when seeing the cell "tail" after electrophoresis, we have to be more careful to explain the results when we fail to see the "tail." The damage caused by cross-linkers is difficult to detect with the method [20]. Sometimes, cross-linkers could also hide the toxicity of other agents [21]. In the environmental monitoring of genotoxicity, this might be a fatal limitation.

2. Application of SCG in Different Fields

(1) Study of Environmental Factors

Most studies are conducted in the laboratory. Biomarkers can detect the genetic toxicity of many environmental factors, including hazardous ones such as benzene [22], radiation [1, 5, 23], arylamines, m-phenylenediamine (mPDA), 2-aminofluorene (2-AF) [24] and salted, sun-dried, deep-fried fish or mutton [25]. On the other hand, many other factors, such as vitamin C [26], vitamin E [2, 27, 28], beta-carotene [2], toluene [29], lime and onion extracts [25] have been reported to have protective effects against the DNA breaks caused by different agents. Fruits and vegetables are also said to show a clear-cut cancer-protective effect via decreasing oxidative damage to DNA as demonstrated with SCG [2].

In the investigation and monitoring of human environmental pollution, several published papers indicate that SCG is a promising biomarker. To monitor the genotoxicity of environmental contaminants, some scientists proposed that when using endemic organisms such as bullheads and carp as sentinels, SCG should be useful because of its extreme sensitivity [3]. If the bullheads and carp are suitable to detect pollutants in water, other

Table 1 Environmental Factors Assessed by Single-Cell Gel Electrophoresis (SCG)

Agents	Subject Name	Result*	Refs.
Water samples	Raji cells (a human promyelocytic cell line)	positive and sensitive	[32]
Polluted Soil Sample	Coelomocytes of earthworms	very valuable for monitoring genotoxic compounds in the terrestrial ecosystem	[30]
Insecticide (lindane)	Cells of the gastric and nasal mucosa of the rat	positive	[33]
Herbicides, alachlor, atrazine, maleic hydrazide, paraquat and trifluralin pH solution	Human peripheral lymphocytes	Genotoxicity depends largely on the pH	[34]
Salted, sun-dried & deep-fried fish & mutton	Hepatocytes & lymphocytes of rats	positive: more hepatocyte damaged than lymphocytes	[25]

Nitric oxide	Rat islets of Langerhans & insulin-containing HIT-T15 cells	positive	[9]
X-rays	Human peripheral blood cells	positive dose-relationship	[4]
Radiation & AQ4N[1]	T50/80 murine tumors implanted in BDF mice	positive	[35]
12 mJ pulses (524 J m-2) of an XeCl excimer laser (308 nm)	Human lymphocytes	positive	[36]
Gamma-rays (0.05-0.5 Gy)	Human lymphocytes & granulocytes	dose-dependent increase	[37]
Gamma-rays	Human blood lymphocytes	rapid, sensitive and useful	[38]
Alpha particles (radon)	Chinese hamster ovary(CHO)& human-hamster hybrid (AL) cells	positive	[39]
Xe-Cl excimer laser (308 nm)	Human lymphocytes	positive	[40]
ACNU[2] & irradiation	C6 glioma cells	dose-dependent increase	[41]
Ionizing radiation	Human tumor cells (HT144 melanoma, HT29 adenocarcinoma, DU145 prostate carcinoma and U87 glioma)	positive (the radiosensitivities of the cell lines differ)	[42]
UV-B	T-lymphocytes from three normal human donors	positive	[43]
2.17-MHz ultrasound	CHO cells	dose-response increase	[44]
Peroxyacetyl nitrate (PAN)	Murine peripheral blood lymphocytes	positive	[45]
DMSO or dimethyl-nitrosamine	Cultured hepatocytes of rats	strongly positive	[46]
m-Phenylenediamine (mPDA) & 2-aminofluorene (2-AF)	Salmonella typhimurium strain YG1024 & Human lymphocytes	positive (TXIMX's protection)[3]	[24]
Benzene	Peripheral blood & liver cells of Female BDF1 mice	positive	[47]
Benzene and its metabolites	Human lymphocytes	positive	[22]
Chlorobenzene	Peripheral lymphocytes bone marrow cells (C57BL/6 female mice)	positive	[48]
Styrene	Human blood and urine	positive	[49]
Styrene-7, 8-oxide (SO)	Human peripheral blood lymphocytes	dose-dependent decrease	[50]
KMnO4	Human peripheral blood lymphocytes	positive	[51]
Sulfuric acid (trypsinization & scraping) X-rays, hydrogen Peroxide	Human fibroblasts	dose-dependent increase	[52]
Vanadium pentoxide (V2O5)	Human lymphocytes	dose-response increase	[53]
1, 2-Dibromo-3-chloropropane (DBCP)	Rat germ cells	positive	[54]
Oxidant damage	Rat tracheal epithelial and mesothelial cell DNA	dose-related increase	[55]

1. AQ4N: 1, 4-bis-([2-(dimethylamino-N- oxide) ethyl] amino) 5, 8-dihydroxyanthracene-9, 10-dione
2. ACNU: 1-(4-amino-2-methyl-5-pyrimidinyl) methyl-3-(2-chloro-ethyl)-3-nitrosourea hydrochloride, an alkylating agent
3. TX1MX: a cell-free plant activating mixture

*: Positive: $P<0.05$

endemic organisms such as earthworms[30] and plant cells[31] will be suitable to detect the genotoxic effects of pollutants in the soil and in the plant systems (Table 1).

(2) Relation of the Biomarker to Disease

Cells from some genetic diseases have been examined to determine the resistance of their DNAs to DNA breaking-agents. One report suggests that cells from ataxia-telangiectasia (AT) patients are more sensitive (detected by SCG) than those from normal individuals to a number of compounds that induce DNA damage via oxygen-derived free radical attack[56]. In addition, AT patients are at elevated risk of sustaining DNA damage in tissues undergoing inflammatory reactions as detected by SCG. By contrast, Bloom syndrome, well known for its genetic instability detected by SCE, has nearly normal baseline SCG and the ability to reseal breaks caused by irradiation with gamma rays (^{60}Co) or treatment with xanthine plus xinthine oxidase[57].

For the study of cancers, although many papers have shown that many carcinogens and precarcinogens can induce single-strand (including some double-strand) DNA breaks, there are still no data clarifying the direct relationship of this biomarker to cancers of any kind. Between the DNA strand breaks and development of cancer, too much remains unknown. Till now, the research on cancer with SCG has mainly focused on the hypoxic fraction of the cancer tissues[7, 58, 59], monitoring of the DNA damage in peripheral blood lymphocytes of cancer patients and animal tumor tissues after treatment with drugs[8, 60] or radiation[6].

(3) Apoptosis

DNA fragmentation is one of the characteristics of apoptotic cells. It is reported that the DNA fragmentation during apoptosis is caused by frequent single-strand breaks[61]. In the detection of apoptotic DNA fragmentation in the scrapie-infected sheep brain, compared to other methods, SCG seems to be simpler, rapider and more economical[62]. There is also a report that SCG is more sensitive to detect apoptotic TK6 human B lymphoblast cells than a flow cytometry method[63]. In tumor cells undergoing apoptosis induced by NK cells, the DNA damage measured with SCG in target cells is proportionate to the time of co-culture with NK cells[64].

3. Comparative Study With Other Biomarkers (Table 2)

In a population study in Italy, SCG of human lymphocytes was more sensitive than SCE as a biomarker to detect damage induced by smoking[12, 13]. For the detection of the genetic toxicity of CdSO4 in vitro, SCG also showed a positive reaction, although at a relatively high concentration, while SCE failed to show any reaction to the agent. However, for the detection of $NaAsO_2$, SCE is much more sensitive than SCG. Here, the two endpoints for the determination of genotoxic effects in vitro (human blood cultures) differed markedly[66]. From early studies, it is well known that mitomycin C (MMC) has a clear-cut effect on the formation of SCE[67], but fails to have a clear-cut effect in SCG[20].

SCG is sometimes more sensitive than the micronucleus (MN) assay. One example is that when vitamin C shows protective activities against ionizing radiation in the human body with SCG, MN could not demonstrate any difference in the same experiment[20]. While in the study of the defects in loci on chromosome 11, possibly associated with tumourigenicity, introduction of the normal chromosome 11 into defective cells can greatly decrease the micronuclei in the target cells, while having no effect in the SCG assay[69]. In a clinical study, after irradiation of healthy and cancer patients' lymphocytes, no association between the MN frequency and tail length was found. However, the author suggested that there may be some association between the repair proficiency of the DNA breaks and the mean frequency of the MN per binucleate cell in the lymphocytes of cancer patients[70].

Some scientists recently reported that statistically significant correlations were found between the DNA adducts in lymphocytes and three of its DNA strand break parameters (tail length; percentage of DNA in the tail and tail moment) in blood samples from nine workers exposed to styrene[49].

Table 2 Differences in the Sensitivities of Different Biomarkers

Agent	Sample[1]	Biomarker[2]	Refs.
Benzyl isothiocyanate	CHO	CA (++++)* SCE (+) SCG (+)	[65]
Physical activity	Human PMLs	SCE (-) SCG (++)	[14]
Smoking	Human PMLs	SCE (-) SCG (++)	[12]
NaAsO2	Human PMLs	SCE (++++) SCG (+)	[66]
CdSO4	Human PMLs	SCE (-) SCG (+)	
Radiation+Vitamin C	Human PMLs	MN (-) SCG (++)	[26]
Styrene	Murine PBLs	SCE (+) SCG (-) MN (-) CA (-)	[68]
2-AF[3]	S. typhimurium mutation (-)		[24]
2-AF + TX1MX	S. typhimurium mutation (+++)		
2-AF	Human PBLs	SCG (+)	
2-AF + TX1MX	Human PBLs	SCG (--)	

1: CHO: Chinese hamster ovary; PMLs: peripheral mononuclear leukocytes; PBLs: peripheral blood lymphocytes
2: CA: chromosomal aberration; SCE: sister chromatid exchange; SCG: single cell gel electrophoresis;
3: 2-AF: 2-aminofluorene; TX1MX: a cell-free plant activating mixture;
*: +: Positive; -: Negative

4. SCG and Genetic Polymorphisms

There are hardly any papers on combination studies of SCG and polymorphisms. The latest report is on CYP2E1[71], which is said to contribute to the formation of metabolites easily inducing DNA breaks. Addition of propylene glycol is reported to be able to reduce the tail length through inhibition of CYP2E1 activity, but the direct relation between CYP2E1 genetic polymorphisms and tail length formation has not been reported yet.

In studies with SCG, the relations of target cells and environmental agents are usually complicated. For example, TX1MX is necessary for mPDA and 2AF to induce S. Typhimurium

mutation, while preventing the DNA breaks in human lymphocytes caused by the two promutagenic arylamines [24]. Mitomycin C, a cross-linker, can produce peroxide, which makes DNA breaks while it cross-links the DNA [21]. Thus, when using SCG to judge the genotoxicity of some unknown hazardous agent, it is better to combine it with other biomarkers to gain a comprehensive understanding of the genotoxic characteristics of the hazardous agent.

5. Afterword

The technique of SCG is still under development. The combination of confocal laser scanning microscopy analysis with the SCG technique makes SCG more rapid, sensitive, and reproducible in quantitative study of single-strand breaks in the DNA of single cells [72]. A statistically significant dose-response relationship has been clearly observed [73].

The choices of samples in nature are relatively wide. From plant cells and earthworm cells to human blood cells used to monitor the genotoxicity of the environmental pollutants, it will come to be considered to be a biomarker of ecosystems, and research work in this field has just begun. In the laboratory, various cell lines, animal tissues [62, 74] and human blood cells are widely used in vitro (or in vivo for animals). In clinical studies, human lymphocytes and cancer tissues are usually preferred (Table 1).

Studies employing SCG with other biomarkers such as DNA adducts, genetic polymorphisms and some proteins like bcl-2 [75], are beginning to demonstrate the efficacy of this biomarker in many more applications in the study of genotoxicity and carcinogenesis.

In addition, the biomarker reminds us that the cells themselves may be at different risk levels for mutagenesis. Thus, epidemiological monitoring of cellular populations will be necessary to understand the developmental process of cancer.

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