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Quercetin Induces Recombinational Mutations in Cultured Cells as Detected by DNA Fingerprinting

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Quercetin, a flavonoid, is found in many fruits and vegetables. This drug was previously shown to affect the metastatic potential of mouse tumor cells. Mutagenicity of quercetin was examined by means of DNA fingerprint analysis using the Pc-1 probe that efficiently detects mutations due to recombination. Treatment of BMT-11 and FM3A tumor cells with 55 μM quercetin resulted in gain and loss of bands in the fingerprints in both cell lines. The frequencies of the clones having undergone mutation were 3/11 and 6/26, respectively. This suggests that quercetin is mutagenic and induces recombination. This result seems to provide a molecular basis for the phenotypic variations of BMT-11 tumor cells induced by quercetin.

Key words: Quercetin — Recombinational mutation — Tumor progression — DNA fingerprinting

Quercetin, a member of the flavonoid family found in plants, is widely distributed in many edible fruits and vegetables.[1] Mutagenic and carcinogenic activities of quercetin have been extensively studied,[2–12] and it has been concluded that the chemical exhibits mutagenic ability but fails to show carcinogenic activity.[12] However, flavonoids are still of considerable interest, because they influence the tumorigenic and metastatic capacities of tumor cells and inhibit growth of human malignant cells.[13–17] When BMT-11 mouse fibrosarcoma cells with a low metastatic potential were treated with quercetin, non-tumorigenic regressed clones and more malignant progressed clones developed frequently, and the latter metastasized to lungs at a high rate.[17] This suggests that quercetin affects the malignancy of tumor cells.

Recently we have isolated a minisatellite sequence, Pc-1, which detects mutations efficiently when used as a probe in DNA fingerprinting.[18] The Pc-1 sequence consists of tandem repeats of the GGGCA sequence and its homologous sequences are present throughout the mouse genome. Mutation at these sequences reflects DNA rearrangements due to recombination between the tandem repeats. Although the effects of quercetin on phenotypes of cells, chromosomes and enzymes have been studied,[4, 19–21] its effect on DNA was not investigated. Hence, using DNA fingerprinting, we tested whether or not quercetin can induce mutation.

BMT-11 tumor cells derived from a C57BL/6 mouse were freshly cloned and a clone was exposed to 55 μM quercetin for two days, which was reported to affect the metastatic character of the cells.[17] The cells were then cultured in fresh medium without quercetin for five days and subjected to cloning. DNA was extracted, digested with *Hae* III, and analyzed by DNA fingerprinting. Three out of the eleven clones examined exhibited a shifted band, loss of a band and an extra band, respectively (indicated by arrowheads in Fig. 1A). These changes are ascribed to unequal homologous recombination between Pc-1-related DNA fragments. Ten untreated clones did not reveal any change on the DNA fingerprint (Fig. 1B). A band indicated by an arrow in Fig. 1B was not found in Fig. 1A, and may have been caused by different washing conditions. These results suggest that quercetin induces recombinational mutations at a significant frequency, which may be responsible for the change in the malignant character of BMT-11 tumor cells.

In order to confirm the above results, we used another cell line, FM3A cells, and tested for the effect of quercetin. FM3A cells originated from a mammary tumor induced in a C3H/He mouse. The freshly cloned cells were grown at a density of 2×10^5/ml and exposed to 55 μM quercetin for two days. The cells were then cultured in fresh medium without quercetin for four days and cloned in soft agarose gel. Twenty-six clones were isolated and analyzed by DNA fingerprinting and six of them were found to have mutations. Figure 2 shows the results for the six that underwent mutations. The quercetin treatment produced both disappearance and addition of bands in the fingerprint (marked by arrowheads). No mutation was detected in twenty control clones from untreated cultures (data not shown). These results are

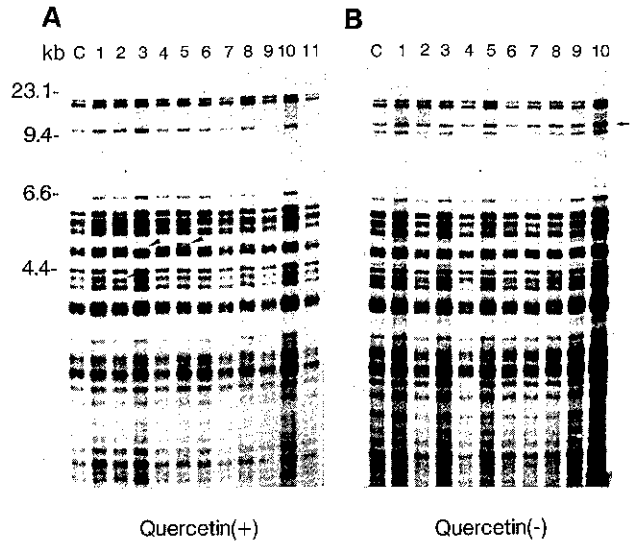

Fig. 1. Quercetin-induced mutations detected by DNA fingerprinting. A. BMT-11 clones from quercetin-treated cultures. B: untreated control clones. C denotes the original clone. Arrowheads indicate mutated bands. The band marked by an arrow was not detected in some cases in Fig. 1A and this was considered to be due to the difference in washing conditions. BMT-11 clone 9-5 cells were plated at 1×10^6 cells/10 cm dish. One day after plating, the medium was replaced with fresh medium (MEM) containing 55 μM quercetin (obtained from Nacalai Tesque, Inc., Kyoto), and the cells were allowed to grow for two days. The medium was then replaced with fresh medium without quercetin and the cells were incubated for five days thereafter. The cells were seeded into 96-well flat-bottomed microtiter plates at an average concentration of 0.4 cell/well. Eleven clones were isolated, and DNA was extracted from cells by a standard method using proteinase K and phenol:chloroform extraction, then digested with *Hinf* I under the conditions recommended by the supplier (Takara, Inc.). The resultant DNA fragments were electrophoresed through 1.3% agarose gel and transferred to a nylon membrane filter.[31] The filter was hybridized to a ^{32}P-labeled Pc-1 minisatellite probe in 4×SSC, 1% SDS at 65°C for 18 h and washed in 1×SSC, 0.1% SDS at 65°C. Pc-1 was isolated from a BALB/c mouse genomic library as described previously.[18] Pc-1 clone contains a 2.0 kb insert consisting of a tandem repeat sequence, GGGCA. Then 25 ng of insert DNA was labeled by the oligoprimer method and used as a probe.[32]

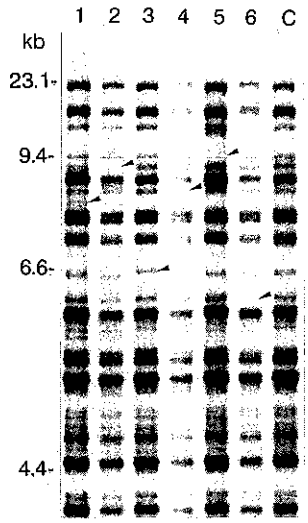

Fig. 2. Mutations induced by quercetin in FM3A cells. FM3A cells were suspended at a density of 2×10^5 cells/ml in ES medium with 5% calf serum containing 55 μM quercetin and were allowed to grow for two days. Then, the cells were cultured in fresh medium without quercetin for four days and plated on 0.5% agarose gels containing ES medium with 10% fetal bovine serum. DNA was isolated from twenty-six colonies and subjected to DNA fingerprint analysis as described in the legend to Fig. 1. The results for six clones having undergone mutations and an original clone are shown here. C denotes the original clone. Arrowheads indicate mutated bands.

consistent with those for BMT-11 cells and indicate that quercetin induces recombination of Pc-1-related sequences.

The treatment, however, reduced the survival of the cells to 82% as assayed by colony formation (Table IA). It is therefore possible that the mutations may have been induced secondarily in cells with impaired viability. To exclude this possibility, the cells receiving UV irradiation were isolated and analyzed by DNA fingerprinting. Table

Table I. The Survival Fractions of FM3A Cells after Treatments with Quercetin (A) and Ultraviolet Light (B)

	Plating no.	Colony no.	PE	SF
A) quercetin				
55 μM	200	127	63.5	79.4
55 μM	400	236	59.0	73.8
0 μM	200	160	80.0	/
B) irradiation				
10 s	1000	80	8.0	10.0
10 s	10000	700	7.0	8.8
12 s	1000	4	0.4	0.5
12 s	10000	50	0.5	0.6
0 s	400	320	80	/

Cloned FM3A cells were treated with quercetin as described in the legend to Fig. 2. The cells were also irradiated with UV at doses giving cell survival of 1%–30%. The indicated numbers of the cells were plated on agarose gels and the resulting colonies were counted after one week. The plating efficiency was obtained from the number of colonies corrected for the number of viable cells plated. The survival fraction was normalized with respect to the plating efficiency of the control population. PE (%): plating efficiency, SF (%): survival fraction.

IB shows the plating efficiency and the survival fraction. Forty-four clones were isolated after 12 s UV irradiation that decreased the viability to 0.6%. Despite such low survival, only one of them revealed an extra band (data not shown). These results imply that the mutations are not due to the secondary effect of cell killing but are due to specific action of quercetin on DNA.

The DNA fingerprint analysis presented here has revealed that quercetin has a mutagenic activity and induces recombination of minisatellite sequences. The sensitivity of this method using Pc-1 is higher than that of the method using Vr DNA probe that we developed before.[22] Therefore, this assay can be applied to drug-treated cells at moderate levels of viability. This is an important feature for an assay used to monitor mutations.

Mutations of the type detected here are implicated in genetic diseases involving the red- and green-cone opsins,[23] the low-density-lipoprotein receptor[24] and the α, β, γ-globin complex.[25,26] This type of mutation is also found in human cancer cells.[27] Hence, it may also be involved in impairment of genes such as tumor suppressor genes. In addition, telomeres and centromeres, which consist of tandem repeat sequences, may also be susceptible to recombinational mutation, as the Pc-1 minisatellite is. Telomeres and centromeres are required for keeping chromosomes intact during cell cycles. Mutations at these sequences could cause loss of the chromosomal integrity and abnormal partition of chromosomes,[28-30] which are often seen in tumor cells. Therefore, these mutations can confer malignant properties upon tumor cells and may provide a molecular basis for the effect of quercetin on the tumorigenic and metastatic properties of BMT-11 tumor cells.[17]

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