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GENISTEIN INHIBITS THE CONTACT-STIMULATED MIGRATION OF PROSTATE CANCER CELLS

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Abstract: The results of several epidemiological studies have suggested that a soybean-based diet is associated with a lower risk of prostate cancer. We investigated the effect of the soy isoflavone genistein on the proliferation and contact-stimulated migration of rat prostatic carcinoma MAT-LyLu and AT-2 cell lines. Genistein almost completely inhibited the growth of both MAT-LyLu and AT-2 cells in the concentration range from 25 to 100 μM, but the addition of 1 μM genistein to the medium significantly stimulated the proliferation of both cell lines. Additionally, at concentrations above 25 μM, genistein showed a potent cytotoxic effect. However, the central finding of this study is that at physiologically relevant concentrations (1 μM and 10 μM), genistein inhibits the motility of prostate cancer cells stimulated by homo- and heterotypic contacts. These results show that at physiological concentrations, genistein exerts an inhibitory effect on the migration of prostate cancer cells and suggest that it may be one of the factors responsible for the anti-metastatic activity of plant isoflavonoids

Key words: Cell movement, Metastasis, Contact-stimulation, Prostate cancer, Genistein

INTRODUCTION

The results of several epidemiological studies have suggested that a soybean-based diet is associated with a lower risk of prostate cancer [1]. Moreover, experimental investigations have demonstrated that soybean isoflavones inhibit

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Abbreviations used: CME – coefficient of movement efficiency; DiI – 1,1'-dioctadecyl-3,3,3',3'-tetrametylindocarbo-cyanine perchlorate; FCS – fetal calf serum

chemically induced prostate cancer formation in rats [2]. Genistein is the main soy isoflavone, and it is suggested to be an anticancer component of the soy diet.

The suggested preventive and/or chemotherapeutic effect of genistein is the consequence of its multiple biological activities. Genistein displays several properties that inhibit tumour cell proliferation, including the suppression of topoisomerases, protein tyrosine kinases, oncogene product activity and growth-factor action, and the induction of apoptosis [3]. Apart from its postulated antiproliferative properties, genistein was also suggested to have anti-metastatic activity. Demographic data implies that genistein consumption is associated with a lower incidence of clinical prostate cancer metastasis [4] and the potential effect of genistein on the metastatic activity of tumour cells was confirmed in several experimental studies [5-8]. However, the molecular and cellular mechanism of genistein-mediated suppression of metastasis has yet to be completely elucidated.

Tumour cell metastasis is a multistep process which is a major cause of death amongst cancer patients. The origin and development of metastasis includes the release of cancer cells from the original site, followed by their entry into circulation, arrest in the blood vessels of the target tissue, extravasation, and subsequent growth in that tissue [9]. It is postulated that the induction of tumour cell migration is a key step in tumour metastasis, and numerous studies demonstrated a correlation between the motility of tumour cells and their metastatic potential [10, 11]. Tumour cell migration can be stimulated by several physiological factors, including chemoattractants, growth factors, cytokines, extracellular matrix components, substrate anisotropy and electric fields [12-14]. In addition, the migration of cancer cells is affected by physical contact with both normal and tumour cells, and the motility of some tumour cells can be increased due to cell-to-cell contacts [13, 15, 16]. In our previous reports [15, 17], we showed that heterotypic contacts between migrating prostatic cancer cells and normal fibroblasts, and homotypic contacts between prostatic cancer cells strongly stimulate their motile activity. The investigated cancer cells showed only limited motile activity when moving as single cells without contacts with neighbouring ones. When the cells migrated at higher cell densities, homotypic collisions between prostatic cancer cells strongly stimulated the speed of their migration. They moved above, below and around each other, to a final migration distance several times greater than in sparse cultures [15]. Moreover, we reported that both MAT-LyLu and AT-2 prostatic cancer cell lines plated onto the surface of aligned fibroblasts showed contact guidance, migrating along the long axes of fibroblasts, leading to more effective tumour cell displacement than without contact with normal or cancer cells (i.e. migration stimulated by heterotypic contacts) [17]. The contact-stimulated migration of cancer cells was also observed in the case of sarcoma XC [13, 16], melanoma B-16 [16] and Walker carcinosarcoma cells [18].

As metastatic cancer cells often migrate in physical contact with neighbouring cells, the inhibition of contact-stimulated migration of prostate cancer cells could

be a promising target for the suppression of invasion and metastasis. Therefore, the specific aim of this study was to characterize the effect of genistein on the contact-stimulated migration of rat prostate MAT-LyLu and AT-2 cells. Our results suggest that at physiologically relevant concentrations (1-10 μM), genistein inhibits the motility of prostate cancer cells, stimulated both by homo- and heterotypic contacts.

MATERIALS AND METHODS

Cell culture

Experiments were performed on two rat prostate cell lines, MAT-LyLu and AT-2 (Dunning rat model), which have markedly different metastatic abilities. The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 (Sigma, St. Louis, MO, USA), as described earlier [15], supplemented with 100 I.U./ml penicillin and 100 μg/ml streptomycin (Polfa, Tarchomin, Poland) in the presence of 1% heat-inactivated fetal calf serum (FCS; Gibco Lab., NY, USA). In the experiments on cell motility, proliferation and viability, the concentration of FCS was increased to 5% to facilitate cell attachment and movement. Human skin fibroblasts were cultured in Eagle's Minimal Essential Medium (MEM) (Sigma, St. Louis, MO, USA), supplemented with 100 I.U./ml penicillin and 100 μg/ml streptomycin in the presence of 10% fetal calf serum.

Assessment of cell viability and proliferation

The MAT-LyLu and AT-2 cells were seeded at a density of 15 x 10^3/well in 12-well plates. Twenty-four hours after the cells were subcultured, the original medium was removed and the cells were incubated in the same fresh medium (RPMI containing 5% FCS) with genistein (Sigma, St. Louis, MO, USA) in the concentration range from 1-100 μM. The cells were counted in a hemocytometer 24, 48, 72 and 96 h after genistein treatment. To evaluate cell viability, the cells were stained with ethidium bromide (Sigma, St. Louis, MO, USA) and fluorescein diacetate (Sigma, St. Louis, MO, USA), as described previously [19].

Time-lapse monitoring of movement of individual cells

The cells were examined with an inverted Olympus IMT-2 microscope. The cells growing on plastic in Corning flasks were observed using phase contrast optics, while those growing on glass (in Rose's perfusion chambers) were studied using Nomarski differential interference contrast. All the experiments were carried out at 37ºC.

MAT-LyLu and AT-2 cells were plated in culture dishes at density of 1080 cells/mm^2. Co-cultures of rat prostate cancer cells and human skin fibroblasts were initiated by plating the MAT-LyLu or AT-2 cells (80 cells/mm^2) onto a confluent monolayer of aligned fibroblasts. After 24 hours, the medium was changed and the movement of cells was recorded for 4 hours at 5-minute time intervals. After this time, genistein was added at the desired concentrations, and the cell movements were recorded for a further 4 hours. As a result, we could compare the behaviour of the same cells before and after genistein treatment.

However, as the results of the analysis of the motility of the control cells in all the separate experiments revealed similar values for the parameters describing motility, we decided to sum up all the control trajectories as one control population. Cell images were recorded with a Hitachi CCD camera, and digitized and processed as previously described [13]. In the co-culture experiments, the AT-2 and MAT-LyLu cells were stained with DiI to be distinguishable from the underlying fibroblasts [17].

The tracks of individual cells were determined from the series of changes in the cell centroid positions, as previously described [13, 16, 20], and the trajectories of cells from no less than three independent experiments were pooled (for cells migrating on fibroblasts, from no less than five experiments). "Mathematica" (Wolfram Research Inc, Champaign, IL, USA) was used to calculate the following parameters of cell movement:

(i) the total length of the cell trajectory (μm), i.e. the sum of a sequence of 'n' straight-line segments, each corresponding to the cell centroid translocation in a given time interval;

(ii) the total length of cell displacement (μm), i.e. the direct distance from the starting point to the final position of the cell;

(iii) the average speed of cell movement, i.e. the total length of the cell trajectory/time of recording (4 h);

(iv) the average speed of cell displacement, i.e. the distance from the starting point directly to the cell's final position/time of recording (4 h);

(v) the coefficient of movement efficiency (CME), corresponding to the ratio of cell displacement to cell trajectory length (the CME would equal 1 for cells moving persistently along a single straight line in a given direction and 0 for random movement);

(vi) the average directional cosine ($\sum_n \cos 2\theta/n$) - θ, defined as the directional angle between the OX axis (parallel to the long axes of the fibroblasts) and the vector AB, where A and B are the first and subsequent positions of the cell, respectively (the parameter would equal 1 for cells moving parallel to the OX axes and 0 for random movement); and

(vii) the average directional cosine ($\sum_n \cos 2\theta/n$) of the final position, where θ is defined as the directional angle between the OX axis (parallel to the long axes of fibroblasts) and the vector AB, where A and B are the first and final positions of the cell, respectively (the parameter would equal 1 for cells moving parallel to the OX axes and 0 for random movement).

Cell locomotion was also characterized using the persistent random walk analysis and the augmented diffusion constant (D*), computed from the plot of the mean square displacement against time, given by the equation:

$$<L^2> = 4D^*\{t - t^*[1 - \exp[-t/t^*)]\},$$

where D* and t* are constants, L is the length of cell displacement from the starting point to its subsequent position, and t is time.

Statistical analysis

Each parameter was calculated as the mean and standard error of the mean (SEM). The statistical significance was determined by the non-parametric Mann-Whitney test. Values of $p < 0.05$ were considered to be significant. The significance of orientation of cell locomotion against random movement was calculated using Rayleigh's distribution. The probability that the cell locomotion is random is given by: $P = e^{-(L^2 n)(10^{-4})}$;

where $L = [(\sum_n \sin 2\theta)^2 + (\sum_n \cos 2\theta)^2]^{1/2}/n$ (0.01), where n is the total number of cells [13]. The statistical significance tests for evaluating changes in D* were determined as described earlier [19].

RESULTS

Genistein inhibits proliferation and induces cytotoxicity in MAT-LyLu and AT-2 cells

Several *in vitro* studies indicate that genistein inhibits the proliferation of a wide range of cancer [21-23] and several normal cells including fibroblasts [24], smooth muscle [25] and osteoblastic cells [26]. However, the inhibitory effect of genistein is often reported to be at concentrations exceeding its physiological level. Moreover, the estimation of inhibition of cell growth by genistein is

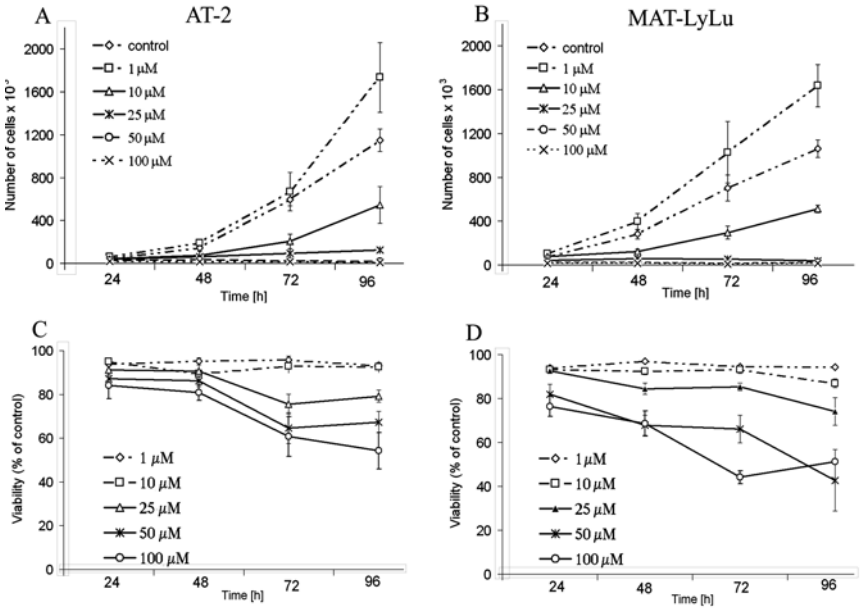

Fig. 1. The effects of genistein on the proliferation (A, B) and viability (C, D) of AT-2 (A, C) and MAT-LyLu (B, D) cells. The cells were treated with genistein at the indicated concentrations. After 24, 48, 72 and 96 h, the number of cells and their viability was determined as described in the Material and methods section. Each experiment was repeated at least three times. Every experimental point represents the mean (± SEM) of all the experiments.

frequently, at least in part, affected by the induction of cell death by a high concentration of genistein. As shown in Fig. 1, genistein almost completely inhibited the growth of both MAT-LyLu and AT-2 cells in the concentration range from 25 to 100 μM. Interestingly, the addition of 1 μM genistein to the medium significantly stimulated the proliferation of both cell lines. Additionally to the effect on cell proliferation, at concentrations above 25 μM, genistein showed a potent cytotoxic effect (Fig. 1C, D). Since the aim of our study was to characterise the effect of genistein on cell motility in physiologically relevant and non-toxic concentrations, in subsequent experiments, genistein was used at concentrations of 1 μM and 10 μM.

Genistein inhibits the migration of MAT-LyLu and AT-2 cells stimulated by homotypic contacts

In our earlier report, we demonstrated that MAT-LyLu and AT-2 cells cultured at low cell densities in the presence of 5% serum showed only limited motile activity. However, at higher densities, as a result of homotypic cell-to-cell contacts, the motility of the investigated cells significantly increased [15]. In subsequent experiments, we examined, by analysis of single cell motility, the effect of genistein on the migration of MAT-LyLu and AT-2 cells stimulated by homotypic contacts. The typical morphology of both cell lines moving at high cell density (with physical contact with neighbouring cells) is shown in Fig. 2 A and D. The trajectories of cells moving under control conditions and in the presence of 1 μM and 10 μM genistein are illustrated as circular diagrams in Fig. 2 C and F. An analysis of the individual trajectories of the two cell lines showed that both the speed of movement and the cell displacement decreased as the cell moved in the presence of genistein (Tab. 1). The results of the whole trajectory analysis were confirmed by the persistent random walk model study (Fig. 2 B, E). The value of the augmented diffusion constant D* (the parameter describing motile activity) was significantly decreased after genistein treatment (Tab. 1).

Tab. 1. Summary of quantitative data showing the effect of genistein on the migration of MAT-LyLu and AT-2 cells stimulated by homotypic contacts.

Parameters (± SEM)	AT-2			MAT-LyLu		
	Control	1 μM	10 μM	Control	1 μM	10 μM
L [μm] [1]	110.1 ± 1.9	89.2 ± 3.2 *	86.6 ± 2.8*	134.6 ± 2.3	117 ± 2.6 *	84.8 ± 3.1 *
V_l [μm/h] [2]	27.5 ± 0.5	22.3 ± 0.8 *	21.6 ± 0.7 *	33.6 ± 0.6	29.2 ± 0.6 *	21.2 ± 0.7 *
D [μm] [3]	40.0 ± 1.7	30 ± 3.1 *	30.5 ± 3.4 *	42.2 ± 1.7	37.9 ± 3.3	27.5 ± 2.9 *
V_d [μm/h] [4]	10.0 ± 0.4	7.5 ± 0.8 *	7.6 ± 0.8 *	10.5 ± 0.4	9.5 ± 0.8	6.9 ± 0.7 *
CME [5]	0.3 ± 0.01	0.31 ± 0.03	0.33 ± 0.03	0.3 ± 0.01	0.32 ± 0.03	0.32 ± 0.03
D* [μm^2/h] [6]	152.9 ± 3.4	92.4 ± 2.3	103.1 ± 1.4	156.1 ± 3.1	134.1 ± 5.9	88.0 ± 4.7

[1]L – total length of cell trajectory, [2]V_l – average speed of cell movement, [3]D – total length of cell displacement, [4]V_d – average rate of cell displacement, [5]CME – coefficient of movement efficiency, [6]D* – augmented diffusion constant.

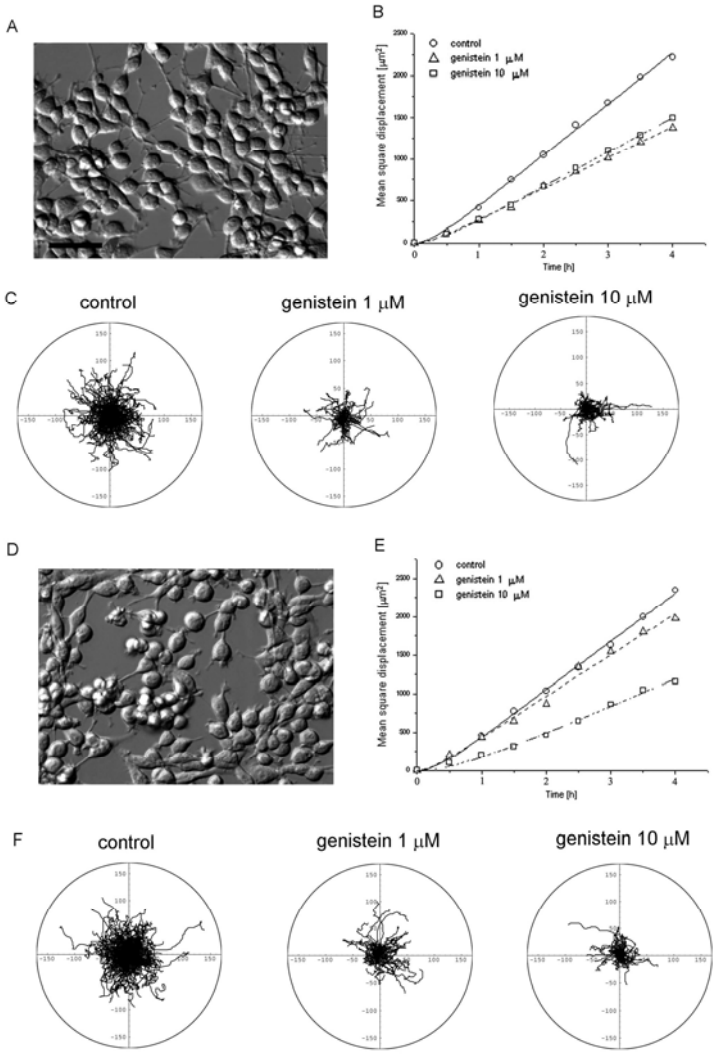

Fig. 2. The effects of genistein on the migration of MAT-LyLu and AT-2 cells stimulated by homotypic contacts. A, D – morphology of AT-2 (A) and MAT-LyLu (D) cells moving on the surface of a culture dish. The movement of cells was recorded for 4 hours at 5-minute intervals, and after this period of time, genistein was added and the cell movements were recorded for the next 4 hours. Scale bar = 50 μm. C, F – composite trajectories of the AT-2 (C) and MAT-LyLu (F) cells migrating on the isotropic surfaces of culture dishes under control conditions and after genistein treatment, displayed in circular diagrams drawn with the initial point of each trajectory placed at the origin of the plot. The panels show the trajectories of 50 (genistein) or 200 (control) individual cells. Axis scale in μm. B, E – persistent random walk analysis of AT-2 (B) and MAT-LyLu (E) cells. The mean square displacements are shown as a function of time. The experimental points are defined in the insets; the best fits were obtained according to equation given in the Materials and methods section.

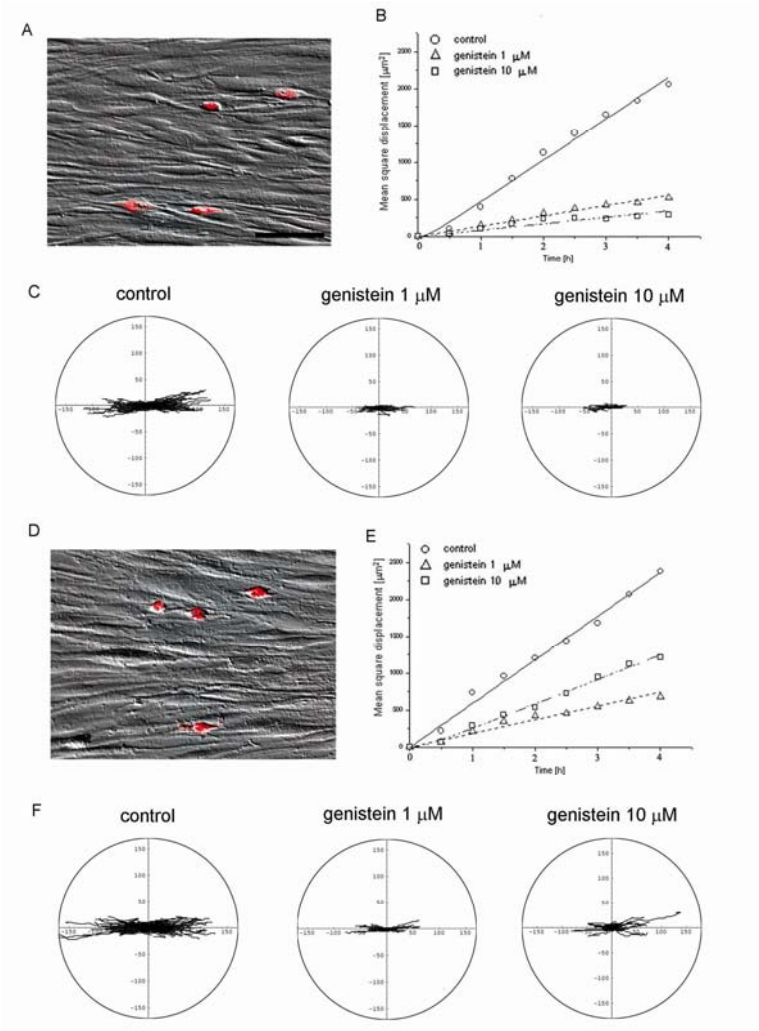

Fig. 3. The effects of genistein on the migration of MAT-LyLu and AT-2 cells stimulated by heterotypic contacts. A, D – morphology of AT-2 (A) and MAT-LyLu (D) cells moving on the surfaces of the underlying contact-inhibited human skin fibroblasts. AT-2 and MAT-LyLu cells were plated onto a confluent monolayer of aligned fibroblasts, and the cell movement was recorded for 4 hours at 5-minute intervals. After this period, genistein was added and the cell movements were recorded for the next 4 hours. The AT-2 and MAT-LyLu cells were stained with DiI to be distinguishable from the underlying fibroblasts. The cancer cells are shown in red (A, D). Scale bar = 100 μm. C, F – composite trajectories of the AT-2 (C) and MAT-LyLu (F) cells migrating on the surface of the underlying contact-inhibited human skin fibroblasts under control conditions and after genistein treatment, displayed in circular diagrams drawn with the initial point of each trajectory placed at the origin of the plot. The panels show the trajectories of 50 (genistein) or 200 (control) individual cells. Axis scale in μm. B, E – persistent random walk analysis of AT-2 (B) and MAT-LyLu (E) cells. The mean square displacements are shown for cells moving on the surfaces of the underlying contact-inhibited human skin fibroblasts as a function of time. The experimental points are defined in the insets; the best fits were obtained according to the equation given in the Materials and methods section.

Genistein inhibits the migration of MAT-LyLu and AT-2 cells stimulated by heterotypic contacts

During metastasis, tumour cells interact with various host cells, often having to migrate on the surfaces of normal cells. As we reported earlier [17], MAT-LyLu and AT-2 cells displayed significantly greater cell displacement moving on the surface of fibroblasts than on plastic substrata thanks to contact guidance and increased speed. Since the direct contact of cancer cells with normal cells may facilitate their migration during invasion, we examined the effect of genistein on the migration of prostate cancer cells on the surfaces of normal fibroblasts. For the estimation of tumour cell motility, MAT-LyLu and AT-2 cells were fluorescently stained to distinguish them from fibroblasts (Fig. 3 A, D), and computer-aided time lap analyses of cell migration were carried out. The trajectories of the Mat-LyLu and AT-2 cells were oriented along the OX axis as a result of the migration of cancer cells primarily along the long axis of the fibroblasts (Fig. 3 C, F).

Tab. 2. Summary of the quantitative data showing the effect of genistein on the migration of MAT-LyLu and AT-2 cells stimulated by heterotypic contacts.

Parameters (± SEM)	AT-2			MAT-LyLu		
	Control	1 μM	10 μM	Control	1 μM	10 μM
L [μm] [1]	68.6 ± 1.5	47.4 ± 1.9*	45.2 ± 2.1*	95.8 ± 2.1	59.4 ± 2.2 *	69.3 ± 4.1 *
V_l [μm/h] [2]	17.1 ± 0.4	11.8 ± 0.5 *	11.3 ± 0.5 *	23.9 ± 0.5	14.8 ± 0.4 *	17.3 ± 1.0 *
D [μm] [3]	35.3 ± 2.0	18.1 ± 2 *	12.9 ± 1.6 *	36.9 ± 2.3	18.9 ± 2.6 *	23.8 ± 3.7 *
V_d [μm/h] [4]	8.8 ± 0.5	4.5 ± 0.5 *	3.2 ± 0.4 *	9.2 ± 0.6	4.7 ± 0.6 *	5.9 ± 0.9 *
CME [5]	0.5 ± 0.02	0.4 ± 0.04	0.3 ± 0.03 *	0.4 ± 0.02	0.3 ± 0.03	0.31 ± 0.03
D* [μm^2/h] [6]	140.5 ± 6.7	34.7 ± 2.2	21.8 ± 3.3	147.9 ± 6.0	46.2 ± 3.3	82.7 ± 3.3
C [7]	0.83 ± 0.004	0.78 ± 0.01	0.83 ± 0.008	0.79 ± 0.004	0.83 ± 0.008	0.65 ± 0.01
C_f [8]	0.82 ± 0.002	0.72 ± 0.07	0.72 ± 0.05	0.76 ± 0.03	0.72 ± 0.07	0.5 ± 0.08 *
F [9]	1.1 x 10^{-51}	3.4 x 10^{-12}	1.6 x 10^{-6}	7.1 x 10^{-60}	3 x 10^{-12}	5.2 x 10^{-12}

[1]L – total length of cell trajectory, [2]V_l – average speed of cell movement, [3]D – total length of cell displacement, [4]V_d – average rate of cell displacement, [5]CME – coefficient of movement efficiency, [6]D* – augmented diffusion constant, [7]C – average directional cosine ($\sum_n \cos 2\theta/n$), [8]C_f – average directional cosine for final position ($\sum_n \cos 2\theta/n$), [9]F – probability of random final distribution, *significant at $p < 0.05$ (control cells vs cells with genistein).

Genistein treatment (1 μM and 10 μM) resulted in significant inhibition of the velocity of cell movement and the average cell displacement (Tab. 2). Similar results were obtained with the persistant random walk analysis (Fig. 3 B, E, Tab. 2). On the other hand, genistein treatment only had a marginal effect on the directionality of movement (c.f. directional cosine, Tab. 2). Interestingly, 1 μM genistein had a more prominent inhibitory effect on the migration of prostate cancer cells on the surfaces of fibroblasts than on the surface of a plastic culture dish (Tab. 1 and 2).

DISCUSSION

Numerous reports show that genistein inhibits the proliferation and induces the cell death of prostate cancer cells *in vitro* [3, 22, 23, 27]. The results of our studies confirm the inhibitory effect of genistein on the proliferation of prostate cancer cells. Moreover, the observation that a low concentration of genistein (1 μM) stimulates the proliferation of cancer cells is also consistent with the results of earlier studies [28]. However, the central finding of this study is that at physiologically relevant concentrations, genistein inhibits the motility of prostate cancer cells stimulated by homo- and heterotypic contacts. Apart from providing protection against cancer, epidemiological studies suggest that genistein has anti-metastatic activity [4]. The hypothesis that genistein consumption reduces the incidence of cancer metastasis is supported by experimental data. Schleicher *et al.* [5] reported on the inhibitory effect of genistein on the metastasis of a transplantable rat accessory sex gland carcinoma. Genistein treatment also reduced the experimental metastasis of melanoma [29] and intestinal adenocarcinoma [8] *in vivo*. Moreover, genistein significantly reduced the invasive properties of cancer cells *in vitro* [30]. Genistein can protect against cancer metastasis via several different mechanisms. The postulated anti-metastatic activity of genistein may be, at least in part, a function of the inhibition of tumour growth. However, much experimental data suggests that genistein directly interferes with the metastatic process. It was suggested that its anti-metastatic activity may be the result of genistein-induced down-regulation of matrix metalloproteinases [31] and its antiangiogenic potential [32, 33]. Additionally, Li and Sarkar [34] reported that genistein down-regulates invasion and angiogensesis-related genes in PC3 prostate cancer cells. It was further demonstrated that genistein increases cell adhesion, thereby antagonizing the first stages of metastasis, wherein cells detach [6].

Nevertheless, genistein may also exert its anti-metastatic activity *in vivo* by the inhibition of cancer cell motility. The migration of tumour cells is one of the key factors responsible for cancer metastasis. Therefore, the inhibition of invasive cancer migration can decrease the metastatic spread of tumour cells. Valachovicova *et al.* [7] showed that genistein inhibits the adhesion and motility of highly invasive MDA-MB-231 breast cancer cells through NF-κB- and AP-1-dependent mechanisms. In our earlier report [35], we demonstrated that flavonoid apigenin inhibits the motility and invasiveness of carcinoma cells *in vitro*. However, to the best of our knowledge, this is the first demonstration that genistein may inhibit the contact-stimulated migration of prostate cancer cells.

Unlike the majority of normal cells, invasive tumour cells are able to crawl over the dorsal surface of normal cells and do not show contact inhibition of locomotion [36]. It was reported that heterotypic contacts between cancer and normal cells and homotypic contacts between cancer cells increase the motility of some tumour cells [13, 15, 16]. During invasion *in vivo*, tumour cells very often migrate in physical contact with other cells; therefore, contact-stimulated migration seems to be important for their metastatic activity. Our results suggest

that the anti-metastatic effect of genistein may be due to the inhibition of this characteristic. Moreover, we showed that migration of prostate cancer cells on the surfaces of normal fibroblasts was significantly inhibited by the relatively low (1 μM) and physiologically relevant concentration of genistein. Plasma concentrations of genistein in heavy soy consumers are lower than usually required to reduce cancer cell proliferation [22, 23]. Adlercreutz *et al.* [37] reported that the average plasma concentration of genistein in Japanese people consuming a soy-rich diet was 0.276 μmol/L, and the maximal observed concentration was 2.4 μmol/L. Even if isoflavones are concentrated several-fold in the prostatic fluid relative to plasma concentrations [38], the physiologically relevant concentration of genistein does not exceed the micromolar level. Our results suggest that genistein can induce the inhibition of cancer cell migration at concentrations that are attained with dietary consumption, which may be responsible for its anti-metastatic effects observed *in vivo*.

Interestingly, we observed no significant difference between the high and the low metastastic cell line with regard to their inhibition by genistein. However, it should be noted that both cell lines show similar contact-stimulated motility and growth rates. It suggests that these two characteristics are similar in MAT-LyLu and AT-2 cells and as a result similarly inhibited by genistein. Moreover, we investigated only the *in vitro* effects of genistein on the motility, proliferation and viability of the two cell lines; the *in vivo* effects of genistein on metastasis formation may be more complex. The molecular mechanism of genistein action with regard to the inhibition of contact-stimulated migration is not understood, and due to the broad biological activity of genistein, it is difficult to conclude about the specific regulatory pathways involved in the inhibition of cell migration under genistein stress. However, it is known that, as an inhibitor of tyrosine kinases, genistein interferes with several signalling pathways involved in the regulation of cell motility [3]. Moreover, cell migration is modulated by matrix metalloproteinases which may be down-regulated by genistein [31]. As it was suggested that sex steroids control cell movement [39], estrogen receptor signalling, which is affected by genistein, may be involved in the regulation of cell migration. However, further experiments elucidating the mechanism of inhibition of cellular movement by genistein are required to gain some insight into the underlying signalling and regulatory pathways.

In conclusion, although several reports suggest the association between a genistein-rich diet and prostate cancer risk, the mechanisms of this phenomenon have not been fully elucidated. Our results demonstrate that at physiological concentrations, genistein exerts an inhibitory effect on the migration of prostate cancer cells, and we postulate that this may be one of the factors responsible for the anti-metastatic activity of plant isoflavonoids.

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