Original Paper

Inhibition of Colonic Tumor Growth by the Selective SGK Inhibitor EMD638683

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Key Words

Colon carcinoma • Serum and glucocorticoid inducible kinase • Cell volume • Apoptosis • Mitochondrial potential • Caspase 3 • Radiation

Abstract

Background: The serum and glucocorticoid inducible kinase SGK1, which was originally cloned from mammary tumor cells, is highly expressed in some but not all tumors. SGK1 confers survival to several tumor cells. Along those lines, the number of colonic tumors following chemical carcinogenesis was decreased in SGK1 knockout mice. Recently, a highly selective SGK inhibitor (EMD638683) has been developed. The present study explored whether EMD638683 affects survival of colon carcinoma cells *in vitro* and impacts on development of colonic tumors *in vivo*. **Methods:** Colon carcinoma (Caco-2) cells were exposed to EMD638683 with or without exposure to radiation (3 Gray) and cell volume was estimated from forward scatter, phosphatidylserine exposure from annexin V binding, mitochondrial potential from JC-9 fluorescence, caspase 3 activity from CaspGlow Fluorescein staining, DNA degradation from propidium iodide staining as well as late apoptosis from annexin-V FITC and propidium iodide double staining. *In vivo* tumor growth was determined in wild type mice subjected to chemical carcinogenesis (intraperitoneal injection of 20 mg/kg 1,2-dimethylhydrazine followed by three cycles of 30 g/L synthetic dextran sulfate sodium in drinking water for 7 days). **Results:** EMD638683 treatment significantly augmented the radiation-induced decrease of forward scatter, increase of phosphatidylserine exposure, decrease of mitochondrial potential, increase of caspase 3 activity, increase of DNA fragmentation and increase of late apoptosis. The *in vivo* development of tumors following chemical carcinogenesis was significantly blunted by treatment with EMD638683. **Conclusions:** EMD638683 promotes radiation-induced suicidal death of colon tumor cells *in vitro* and decreases the number of colonic tumors following chemical carcinogenesis *in vivo*.

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Introduction

SGK1 was originally cloned as a gene transcriptionally up-regulated by serum and glucocorticoids in rat mammary tumor cells [1]. Human SGK1 was discovered as a cell volume-regulated gene, up-regulated by cell shrinkage [2]. SGK1 transcription is stimulated by several additional hormones and mediators including mineralocorticoids, gonadotropins, calcitriol, transforming growth factor β (TGF- β), interleukin 4, fibroblast growth factor and platelet-derived growth factor [3]. SGK1 is expressed in all tissues tested thus far [2]. SGK1 is activated by growth factors, an effect involving phosphatidylinositol (PI) 3-kinase and phosphoinositide-dependent kinase 1 (PDK1) signaling [3].

SGK1 is highly expressed in several tumor cells [4], such as colon cancer [4], myeloma [5], medulloblastoma [6], prostate cancer [7], ovarian tumors [8] and non-small cell lung cancer [9]. On the other hand, down-regulation of SGK1 in some tumors has been reported, including adenomatous polyposis coli (APC) [3, 4, 10]. SGK1 expression is apparently not required for the survival of those tumor cells. Nevertheless, genetic knockout of SGK1 significantly decreased the number of spontaneous tumors in APC-deficient mice [11] and of colonic tumors following chemical carcinogenesis [12].

Several observations point to a role of SGK1 in cell survival [13-15]. SGK1 has been shown to inhibit apoptosis of Madin-Darby canine kidney (MDCK) [14] and breast cancer [15] cells and to confer interleukin (IL)-6-dependent survival of cholangiocarcinoma cells [16]. Transformation of intestinal epithelial cells by oncogenic β -catenin was followed by increased SGK1 expression [17, 18].

The selective SGK1 inhibitor GSK650394 proved to be effective *in vitro* against prostate cancer cells [19] and LY294002 against breast cancer [20]. The IC₅₀ of GSK 650394 is 0.6 μ M [19]. More recently, the highly selective SGK inhibitor EMD638683 has been developed, which is effective *in vivo* [21]. In HeLa cells, EMD638683 was tested with regard to the SGK1-dependent phosphorylation of NDRG1 (N-Myc downstream-regulated gene 1) [21]. The IC₅₀ value of EMD638683 for this (*in vitro*) activity is 3 μ M. While EMD638683 may inhibit the other two SGK isoforms with similar potency [21], in a biochemical assay (measuring inhibition of enzyme activity), more than one order of magnitude higher concentrations are required to inhibit mitogen- and stress-activated protein kinase 1 MSK1 and protein kinase A [21]. More than two orders of magnitude higher concentrations were required for any of the other kinases tested [21]. EMD638683 is readily soluble in water and is the first SGK inhibitor shown to be effective *in vivo* [21]. The substance has previously been shown to reverse the hypertension following hyper-insulinemia and salt excess [21]. The present study explored whether EMD638683 could foster suicidal death of cultured colon carcinoma cells *in vitro* and counteract colonic tumor growth *in vivo*. To this end, chemical carcinogenesis has been induced by 1,2-dimethylhydrazine/dextran sulfate sodium treatment in mice without or with EMD638683 treatment.

Materials and Methods

Experiments in CaCo-2 cells

Colon carcinoma (CaCo-2) cells were grown in complete DMEM medium containing 10% fetal calf serum, 1% sodium pyruvate, 1% penicillin-streptomycin and 1% non-essential amino acids (all from PAA, Austria) under standard culture conditions (37°C, 5% CO₂). 10⁵ cells were seeded in 6 well plates and cultured with fresh culture medium for 24 h, after which EMD638683 (50 μ M) was applied for 24 hours. For comparison, the cells were treated with the solvent (0.2 μ l DMSO) and one solvent control was analysed with each set of experiments. The cells were subsequently exposed to 3.18 min radiation (3 Gray). After further incubation for 72 h in the presence or absence of EMD638683 (50 μ M) the cells were analyzed utilizing flow cytometry (BD FACS Calibur, BD Biosciences, USA).

For FACS analysis the cultured cells were detached from the 6-well plate by treatment with 1x trypsin-EDTA (PAA, Austria) for 15 minutes. The cells were washed with DMEM media with 10% FBS

by centrifugation at 1600 RPM for 3 minutes. 10^5 cells were suspended in 200 μ l PBS. Cell volume was estimated from forward scatter in FACS analysis.

Depolarization of the outer membrane of mitochondria was measured by incubating 10^5 cells in 10ng/ml concentration of JC9 (Invitrogen, USA) in the dark for 10 minutes at 37°C. The cells were washed once in PBS at 1600 RPM for 3 minutes and measured immediately by flow cytometry. JC-9 is a cationic dye which accumulates selectively in the mitochondria and undergoes a potential-dependent shift of fluorescence emission from 525 nm (FL1) to 590 nm (FL2). Therefore, cells with hyperpolarized mitochondria are in the upper right quadrant of an FL1 vs FL2 dot plot whereas cells with depolarized mitochondria are in the lower quadrants (Product Information for JC-9, Invitrogen, USA).

Active caspase-3 was measured by CaspGlow Fluorescein Active Caspase-3 Staining kit from BioVision (CA, USA) adhering to the manufacturer's instruction. Briefly, 10^5 cells were suspended in 100 μ l complete DMEM, stained for 1 hour with 0.1 μ l active caspase-3 FITC antibody under cell culture conditions. The cells were then washed once in 200 μ l wash buffer supplied in the kit and resuspended in 200 μ l wash buffer for immediate FACS analysis.

To determine apoptosis, 10^5 cells/100 μ l in complete DMEM were incubated in 70% ethanol (molecular grade, Sigma) on ice for 30 minutes, centrifuged at 1600 RPM for 3 minutes at 4°C, added to 200 μ l of hypotonic buffer (0.1% sodium citrate, 0.1% triton X-100, 2mM CaCl₂, 20U/ml RNase A in deionized water) together with 24 μ l/ml Annexin V FITC (Mabtag, Germany) and 50 μ g/ml propidium iodide (Mabtag, Germany) and incubated on ice in the dark for 60 minutes. The cells were washed once at 1600 RPM for 3 minutes, resuspended in PBS-1% BSA and measured immediately yielding a FL-1 vs FL-2 dot plot in FACS.

Experiments in mice

Experiments were carried out on wild type mice as previously described [12]. The animals were housed under controlled environmental conditions (22–24°C, 50–70% humidity and a 12 h light/dark cycle). Throughout the study mice had free access to tap water and pelleted food. The animals received either placebo food or food containing EMD638683 (4460 ppm in the chow, which resulted in a dose of approx. 600 mg/kg/day). All animal experiments were conducted according to the guidelines of the American Physiological Society and the German law for the care and welfare of animals and were approved by local authorities (Regierungspräsidium Tübingen). Colonic tumors were generated as described previously [22]. At the age of 8 weeks the animals were treated intra-peritoneally with 20 mg/kg 1,2-dimethylhydrazine (DMH; Sigma-Aldrich Corp. St.Louis MO, USA, preparation in 0.9% saline) and subsequently by three cycles of alternating administration of drinking water containing 30 g/L synthetic dextran sulfate sodium (DSS; molecular mass 5000 g/mol; Wako Pure Chemical Industries, Ltd. Japan) for 7 days followed by drinking water lacking DSS for subsequent 14 days. The treatment leads to appearance of tumors specifically in colonic tissue with predominant localization in the distal part of the colon [22, 23]. All mice were anesthetized with ether and sacrificed at the age of 20 weeks. After death, the entire colorectum from the colocolic junction to the anal verge was examined. Fresh specimens were placed in liquid nitrogen and subsequently stored at -80 °C for further analysis. The colon length was measured, as inflammation and tumor growth results in shortening of the colon [22, 24]. Then, the colon was opened longitudinally, washed with PBS, and divided into three portions (proximal, middle and distal). After macroscopic inspection the colon was fixed in a formaldehyde buffer solution (pH 7.4).

Statistical analysis

Data are provided as means $\pm$ SD, *n* represents the number of independent experiments. Data were tested for significance using ANOVA (one-way ANOVA) and paired student's *t*-test. Differences were considered statistically significant, if *p*-values were < 0.05.

Results

In a first series of experiments the effect of the selective SGK inhibitor EMD638683 was tested *in vitro*. To this end, human colon carcinoma (CaCo-2) cells were exposed for 24 hours to 50 μ M EMD638683 prior to radiation or, for comparison, to 0.2 μ l solvent DMSO. The cells were subsequently exposed for 3.18 minutes to radiation (3 Gray). As illustrated in Fig. 1,

Fig. 1. Effect of radiation and/or EMD638683 on CaCo-2 cell forward scatter. A. Original histograms of forward scatter of colon carcinoma (Caco-2) cells without (left panels) and with (right panels) a 3.18 minute exposure to radiation (3 Gray) 72 h prior to measurements without and with a 24 h exposure to 50 μ M EMD638683. B. Arithmetic means $\pm$ SD ($n = 9$) of the average forward scatter of Caco-2 cells following a 24 h exposure in the absence (control) or presence of 50 μ M EMD638683 (EMD) or 0.2 μ l solvent Dimethyl sulfoxide (DMSO) without (white bars) and with (black bars) an additional 3.18 min exposure to radiation (3 Gray). Forward scatter measured in FACS is indicative of cell volume. * ($p < 0.05$) and *** ($p < 0.001$) indicates significant difference to respective value of untreated control and ## ($p < 0.05$) indicates significant difference to respective value without radiation (t-test) and \$\$ ($p < 0.05$) indicates significant difference to DMSO.

EMD638683 treatment alone did not significantly modify the forward scatter of CaCo-2 cells, but significantly augmented the cell shrinkage following radiation.

As shown in Fig. 2, EMD638683 treatment alone tended to enhance the percentage of CaCo-2 cells with depolarized mitochondria. Similarly, radiation alone tended to enhance the percentage of CaCo-2 cells with depolarized mitochondria. The percentage of CaCo-2 cells with depolarized mitochondria was significantly increased by radiation of EMD638683-treated CaCo-2 cells. The same observations were made on caspase activity (Fig. 3). EMD638683 treatment alone tended to enhance caspase activity. Similarly, radiation alone tended to enhance caspase activity. The caspase activity was significantly increased by radiation of EMD638683-treated CaCo-2 cells.

To identify cells undergoing apoptosis, cell membrane scrambling with exposure of phosphatidylserine (PS) on the membrane surface was elucidated by determination of annexin-V binding and the degradation of nuclear DNA by propidium iodide staining (Fig. 4A). As illustrated in Fig. 4B, treatment with EMD638683 tended to enhance the percentage of annexin V binding CaCo-2 cells. Radiation significantly increased the percentage of annexin V binding CaCo-2 cells both, in the presence and absence of EMD638683. The percentage annexin V binding radiated CaCo-2 cells was, however, significantly higher in the presence than the absence of EMD638683. Thus, EMD638683 treatment significantly augmented the annexin V binding following radiation.

The same observations were made on late apoptosis. In both, control and EMD638683-treated CaCo-2 cells, radiation significantly increased the percentage of CaCo-2 cells undergoing late apoptosis (Fig. 4C). EMD638683 treatment alone tended to enhance the percentage of apoptotic CaCo-2 cells. Following radiation the percentage of apoptotic

Fig. 2. Effect of radiation and/or EMD638683 on mitochondrial potential in CaCo-2 cells. A. Original dot plot of mitochondrial potential of colon carcinoma (Caco-2) cells without (left panel) and with (right panel) a 3.18 minutes exposure to radiation (3 Gray) 72 h prior to measurements without (-) and with (+) a 24 h exposure to 50 μ M EMD638683. B. Arithmetic means $\pm$ SD ($n = 11$) of Caco-2 cells with decreased mitochondrial potential following a 24 h exposure in the absence (control) or presence of 50 μ M EMD638683 (EMD) or 0.2 μ l solvent Dimethyl sulfoxide (DMSO) without (white bars) and with (black bars) a 3.18 minutes exposure to radiation (3 Gray). * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) indicates significant difference to respective value of untreated control; ## ($p < 0.01$) indicates significant difference to respective value without radiation and \$\$ ($p < 0.01$) indicates significant difference to DMSO (t-test).

Fig. 3. Effect of radiation and/or EMD638683 on caspase-3 activity in CaCo-2 cells. A. Original dot plots of caspase-3 activity in colon carcinoma (Caco-2) cells without (left panel) and with (right panel) a 3.18 minutes exposure to radiation (3 Gray) 72 h prior to measurements without (-) and with (+) a 24 h exposure to 50 μ M EMD638683. B. Arithmetic means $\pm$ SD ($n = 8$) of caspase-3 activity in Caco-2 cells following a 24 h exposure in the absence (control) or presence of 50 μ M EMD638683 (EMD) or 0.2 μ l solvent Dimethyl sulfoxide (DMSO) without (white bars) and with (black bars) an additional 3.18 minutes exposure to radiation (3 Gray). * ($p < 0.05$), ** ($p < 0.01$) indicates significant difference to respective value of untreated control and # ($p < 0.05$) indicates significant difference to respective value without radiation and \$ ($p < 0.05$) represents significant difference from DMSO (t-test).

Fig. 4. Effect of radiation and/or EMD638683 on apoptosis of CaCo-2 cells. A. Original dot plots of apoptotic cells in colon carcinoma (Caco-2) cells without (left panel) and with (right panel) a 3.18 minutes exposure to radiation (3 Gray) 72 h prior to measurements without (-) and with (+) a 24 h exposure to 50 µM EMD638683. The cells without externalized phosphatidylserine on the cell membrane and without degraded DNA appear on the lower left quadrant of the dot plot. B. Arithmetic means $\pm$ SD ($n = 9$) of the percentage Caco-2 cells binding annexin-V following a 24 h exposure in the absence (control) or presence of 50 µM EMD638683 (EMD) or 0.2 µl solvent Dimethyl sulfoxide (DMSO) without (white bars) and with (black bars) an additional 3.18 minutes exposure to radiation (3 Gray). Annexin V-FITC binds to externalized phosphatidylserine (PS). * ($p < 0.05$), ** ($p < 0.01$) indicates significant difference to respective value of untreated control and # ($p < 0.05$), ## ($p < 0.01$) indicates significant difference from respective value without radiation and \$ ($p < 0.05$) indicates significant difference from DMSO (t-test). C. Arithmetic means $\pm$ SD ($n = 9$) of the percentage Caco-2 cells undergoing late apoptosis following a 24 h exposure in the absence (control) or presence of 50 µM EMD638683 (EMD) or 0.2 µl solvent Dimethyl sulfoxide (DMSO) without (white bars) and with (black bars) an additional 3.18 minutes exposure to radiation (3 Gray). Cells with degraded DNA allow propidium iodide (PI) to enter the cell. ** ($p < 0.05$) and *** ($p < 0.001$) indicates significant difference to respective value of untreated control and # ($p < 0.05$) and ## ($p < 0.01$) indicates significant difference to respective value without radiation and \$\$ represents significant difference from DMSO (t-test).

EMD638683-treated CaCo-2 cells was significantly higher than the percentage of apoptotic control cells. Thus, EMD638683 treatment significantly augmented the apoptosis following radiation.

In a second series of experiments the influence of EMD638683 on tumor growth was tested *in vivo*. To this end, wild type mice received either placebo food or food containing EMD638683 (4460 ppm in the chow, which resulted in a dose of approx. 600 mg/kg/day). The animals were exposed to chemical carcinogenesis by intra-peritoneal injection of the carcinogenic drug 1,2-dimethylhydrazine in 0.9% saline followed by 3 cycles of dextran sulfate sodium in the drinking water. As listed in Table 1, body weight was not significantly different between

Table 1. Effect of chemical carcinogenesis on body and organ weight, as well as colon length. Body and organ weight as well as colon length of mice prior to (Start) and 12 weeks after treatment with the carcinogenic drug 1,2-dimethylhydrazine followed by dextran sulfate sodium (End) without (-EMD638683) and with (+EMD638683) treatment with EMD638683. Values are given with standard error of the mean. * indicates statistically significant difference between the respective value prior to and 12 weeks after the beginning of the 1,2-dimethylhydrazine + dextran sulfate sodium treatment; # indicates statistically significant difference between -EMD638683 and +EMD638683

	-EMD638683		+EMD638683	
	Start (n=11)	End (n=8)	Start (n=12)	End (n=11)
Body weight [g]	19.58 ± 1.21	23.73 ± 0.53*	18.52 ± 1.04	23.99 ± 0.40*
Colon length [cm]		9.38 ± 0.16		10.50 ± 0.22#
Colon weight [g]		0.96 ± 0.04		0.80 ± 0.03#
Small intestine weight [g]		1.31 ± 0.11		1.16 ± 0.04
Liver weight [g]		1.21 ± 0.06		1.29 ± 0.06
Spleen weight [g]		0.15 ± 0.01		0.15 ± 0.01
Stomach weight [g]		0.18 ± 0.01		0.15 ± 0.01#
Lung weight [g]		0.23 ± 0.03		0.20 ± 0.01
Heart weight [g]		0.14 ± 0.01		0.14 ± 0.01
Kidney weight [g]		0.36 ± 0.02		0.36 ± 0.02

Fig. 5. Effect of chemical carcinogenesis on tumor incidence in absence and presence of SGK1 inhibitor EMD638683. Arithmetic means ± SD (n = 8-11) of the number of colonic tumors in wild type mice 12 weeks after treatment with the carcinogenic drug 1,2-dimethylhydrazine followed by dextran sulfate sodium treatment without (white bar) and with (black bar) EMD638683 (600 mg/kg/day) treatment. # indicates significant difference from untreated mice (t-test).

mice treated with EMD638683 and mice treated with placebo. Three animals did not survive in the group receiving placebo, and one animal did not survive in the EMD638683-treated group.

The colon was significantly longer and the colon weight significantly lower in EMD638683-treated mice than in placebo-treated mice (Table 1), a finding pointing to an influence of EMD638683 on tumor growth following chemical carcinogenesis. In addition, the stomach weight was significantly lower in the EMD treated group. Most importantly, the number of developing tumors following carcinogenic treatment was significantly blunted by EMD638683 treatment (Fig. 5).

Discussion

The present study reveals that the highly selective SGK inhibitor EMD638683 fosters the development of CaCo-2 cell apoptosis and sensitizes CaCo-2 cells to the apoptotic effect of radiation. Moreover, this is the first demonstration that pharmacological inhibition of SGK decreases tumor growth and/or survival *in vivo*.

We show that 50 μ M EMD638683 triggers and/or enhances the effect of radiation on decrease in the cell volume (Fig. 1), depolarization of mitochondria (Fig. 2), activation of caspase-3 (Fig. 3) and induction of late apoptosis (Fig. 4). We further demonstrate that EMD638683 treatment decreases the development of tumors following chemical carcinogenesis *in vivo* (Fig. 5).

The effect of EMD638683 on CaCo-2 cell apoptosis reached statistical significance following radiation. According to an earlier study [25], SGK1 transcription is stimulated by radiation of radiation-resistant tumor cells but not in radiation-sensitive tumor cells. Thus, the survival of tumor cells following radiation is a function of their ability to up-regulate SGK1 expression. SGK1 expression is further up-regulated by ultraviolet (UV) radiation [16]. SGK1 protects tumor cells not only against radiation but as well against chemotherapy. SGK1 silencing increases the toxicity of chemotherapeutic drugs [16]. Conversely, over-expression of SGK1 by glucocorticoids inhibits chemotherapy-induced apoptosis of breast cancer cells [26].

SGK1 modifies tumor growth via several mechanisms. SGK1 phosphorylates and thus inhibits the transcription factor FKRHL1 (FOXO3) [14, 17, 27-29], which in turn counteracts cell survival and fosters cell growth [30-32]. FOXO3a triggers apoptosis at least in part by stimulating the expression of the Bcl2-interacting mediator BIM (Bcl-2-interacting mediator of cell death) [33], a protein stimulating apoptosis and inhibiting cell proliferation [34, 35]. SGK1 further interacts with apoptosis by inhibiting FAS and FASL expression and thus FAS-dependent activation of caspase 8 [36]. SGK1 contributes to the phosphorylation and inhibition of glycogen synthase kinase (GSK) 3 [37], which in turn phosphorylates the oncogenic transcription factor β -catenin [38]. Additional mechanisms presumably contributing to SGK1-dependent tumor cell survival include p53 [39-41], N-myc downstream regulated 1 gene (NDRG1) [42, 43] and NF- κ B [15].

SGK1-dependent NF- κ B [15, 44] stimulates the Ca^{2+} release activated Ca^{2+} channel (I_{CRAC}) Orai1 [45, 46], a channel decisive in the regulation of cell proliferation [47, 48]. SGK1 further up-regulates several K^{+} channels, such as the voltage sensitive K^{+} channel Kv1.3 [3, 4, 49, 50]. The K^{+} channels maintain the cell membrane potential, which is in turn required for the function of I_{CRAC} [3, 4].

SGK1 may further affect cell survival by stimulating cellular nutrient uptake. SGK1 up-regulates a wide variety of nutrient transporters [3] and may thus contribute to the excessive nutrient uptake by tumor cells. In view of the excessive glucose demand of tumor cells, SGK1-dependent stimulation of cellular glucose uptake may be particularly important for tumor cell survival [3]. SGK1 further up-regulates the activity of the $\text{Na}^{+}/\text{H}^{+}$ exchanger [51]. The subsequent cytosolic alkalization supports the excessive glycolytic flux in tumor cells [52].

SGK1 is up-regulated by ischemia [53, 54] and may participate in the stimulation of tumor vascularization [55].

The inhibition of tumor growth by EMD638683 is in full agreement with the former observations that lack of SGK1 decreases the number of spontaneous tumors in APC-deficient mice [11] and of colonic tumors following chemical carcinogenesis [12]. The present demonstration confirms that SGK1 is indeed an attractive pharmacological target in the treatment of tumors *in vivo* and that EMD638683 or a related substance could be effective *in vivo*. Notably, in the *in vivo* experiments, EMD638683 was applied without additional radiation, i.e. the substance was effective even without additional challenge of tumor cell survival.

Those observations are in seeming contrast to the reports of decreased SGK1 expression in prostate cancer [56], ovarian tumors [57], hepatocellular carcinoma [58] and adenomatous polyposis coli (APC) [3, 4, 10]. It has been speculated [12] that tumor cells with enhanced activation of the PI3K/ PKB/Akt cascade [59-61] are not dependent on SGK1 for survival. Loss-of-function mutations of the phosphatase and tensin homolog PTEN [62-64], for instance, result in excessive activation of PKB [60]. In view of the partially overlapping specificity of PKB/Akt and SGK1 [3], SGK1 may be dispensable in tumor cells with excessive PKB/Akt activity.

In conclusion, pharmacological inhibition of SGK1 with EMD638683 counteracts CaCo-2 cell survival *in vitro* and colonic tumor growth *in vivo*. Thus, SGK1 may be an attractive pharmacological target in the treatment of malignancy.

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