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Correlation of hepatocyte growth factor-induced proliferation and calcium-activated potassium current in human gastric cancer cells

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Abstract

Hepatocyte growth factor (HGF) has been found to stimulate proliferation and migration of human gastric carcinoma cells. Whether the HGF-induced responses are correlated with the expressed level of HGF receptors or the changes of ionic currents is not clear. The present study investigated the effects of HGF on the proliferation and ionic currents of two human gastric adenocarcinoma cell lines, which were found to express different amounts of HGF receptor. Results showed that HGF induced a dose-dependent growth stimulation and accelerated cell cycle progression in SC-M1 cells. In patch clamp study, HGF treatment induced an outward K^+ current and increased the slope conductance at -80 mV from 110 ± 15 pS/pF to 207 ± 15 pS/pF. The HGF-induced K^+ current was abolished when tetraethylammonium chloride was added in bathing solution or a low Ca^{2+} solution was included in the recording pipette. Furthermore, HGF (10 ng/ml) induced an oscillatory Ca^{2+}-activated K^+ current with a lag period of 5 ± 3 min in SC-M1 cells. In contrast, HGF did not induce mitogenesis, cell cycle progression and changes in ionic currents in KATO-III cells, although this cell line expressed a higher level of HGF receptors than SC-M1 cells did. These findings provide evidence that the activity of Ca^{2+}-activated K^+ channel may be involved in the HGF-induced cell proliferation in human gastric cancer cells, but it did not correlate with the density of HGF receptors. © 1998 Elsevier Science B.V.

Keywords: Gastric cancer; Hepatocyte growth factor; Ca^{2+}-activated K^+ channel; Mitogenesis

1. Introduction

The expression and alteration of growth factors and their receptors play a crucial role in development and progression of gastric carcinoma [1]. Hepatocyte growth factor (HGF), which has multiple functions on cell proliferation, migration and morphogenesis [2] in different epithelial cells, has received great attention since its characterization. Binding of HGF with its receptor, a 190 kDa transmembrane heterodimer, triggers the diverse biological effects. The HGF re-

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ceptor (HGFR) has been identified to be the protein encoded by protooncogene *c-met* [3] and its overexpression has been linked to the malignant potential of gastric cancer [4]. It has also been shown that HGF stimulates both cell proliferation and migration in human gastric adenocarcinoma cell line MKN-74 [5]. Accordingly, HGF with its receptor may play an important role in the progression and metastasis of gastric cancer.

Calcium has been known as an important regulator of cell proliferation for more than a decade [6]. Extracellular Ca^{2+} is not only essential for initiating DNA synthesis and proliferation in regenerating hepatocytes [7], but also important for stimulation of cell proliferation in osteoblast [8]. It has been demonstrated that many hormones induce transient increases in the concentration of free cytosolic Ca^{2+} ($[Ca^{2+}]_i$) via either influx of extracellular Ca^{2+} into cells through voltage- or receptor-gated channels, or release of Ca^{2+} from intracellular pools via the action of inositol triphosphate (IP_3) [9]. In cultured rat hepatocytes, HGF treatment resulted in an increase in $[Ca^{2+}]_i$ through release of intracellular Ca^{2+} stores [10] or through extracellular Ca^{2+} influx [11]. This calcium response induced by HGF is considered to be mediated by the HGFR/*c-met* [12]. The HGF-induced changes may lead to alteration of membrane potential and ion channels, and the transient changes in ion membrane permeability and cell ion content are known to play an essential role in cell proliferation [10,13]. In gastrointestinal epithelial cells, however, the role of Ca^{2+} and ionic signals in HGF/*c-met* signal transduction pathway that leads to proliferation remains to be elucidated.

Previous studies have found several types of ion channels in gastrointestinal epithelial cell lines [14]. The second messenger-independent ion channels may determine the resting electrical properties, while the ion channels regulated by second messengers may play an important role in the stimulation of proliferation or differentiation coupling process in these cells [15–18]. Activation of Ca^{2+}-dependent K^+ channels has been found to be involved in secretion of gastric cancer cells [15] as well as migration in transformed MDCK cells [19]. These findings suggest that Ca^{2+}, a second messenger, -dependent ion channel activities play important roles in functional changes of transformed cells.

The present study was performed to investigate the roles of ionic currents in HGF-stimulated proliferation of two human gastric carcinoma cell lines which expressed different amount of HGFR. The KATO-III cell line, which was originated from signet ring cell carcinoma [20], has been known to show 3 to 5-fold amplification of HGFR/*c-met* gene [4]. On the contrary, the SC-M1 cells exhibited lower level of HGFR expression as examined by indirect immunofluorescent study. Interestingly, we found that the presence of HGF dose dependently stimulated cell proliferation and accelerated cell cycle progression in SC-M1 cells, while KATO-III cells showed minimal response to HGF. The proliferative responses to HGF shown in SC-M1 cells were found to correlate with the activities of an outward current that was identified as Ca^{2+}-activated K^+ current. Thus, we provide the first evidence that in human gastric cancer cells, HGF is capable of increasing the activity of Ca^{2+}-activated K^+ channels and the activation of Ca^{2+}-activated K^+ channels may be involved in the HGF-induced cell cycle progression and cell proliferation. The SC-M1 cell line may also provide a good model for the study of HGF-induced signalling pathways.

2. Materials and methods

2.1. Cell culture and mitogenic assay

Two human gastric adenocarcinoma cell lines were used. The KATO-III cell line was cultured from metastasis in pleural effusion of a Japanese patient with signet-ring cell carcinoma [20]. The SC-M1 cell line was established from a Chinese patient with gastric adenocarcinoma [21]. Cells were maintained at 37°C in 95% air/5% CO_2 in RPMI 1640 (GIBCO BRL, Grand Island, NY) containing 10% fetal bovine serum (FBS; Hyclone, Utah) and 10 μg/ml gentamycin sulfate (GIBCO BRL) throughout the study. Mitogenic responses of cells to recombinant human hepatocyte growth factor (r-HGF, R&D Systems, MN) were analyzed using 25 cm^2 culture flasks (Corning, NY). Two hundred thousand cells were placed in each flask with 0.5% FBS-RPMI 1640 fresh medium containing various concentrations (0, 1, 10 ng/ml) of HGF. The number of viable cells was

counted at 24 and 48 h after incubation using the trypan blue dye exclusion method.

2.2. Flow cytometric analysis of cell cycle

Two million exponentially growing SC-M1 and KATO-III cells were first synchronized in G0–G1 phase by the addition of 400 μM *l*-mimosine (Sigma, St. Louis, MO) for 24 h. The synchronized cells were then stimulated to begin cycling by replating into fresh medium containing 0.5% FBS. HGF (10 ng/ml) was then added and cells were harvested after 12, 24 and 48 h of treatment. The DNA content of the harvested cells was analyzed as described previously [22] with some modification. Briefly, the cells were washed twice with PBS and resuspended in PBS to a concentration of 2×10^6 cells/ml. To the sample was added 0.5 ml staining solution A (pH 7.2) containing 50 μg/ml propidium iodide (PI, Sigma), 3% polyethyleneglycol 6000 (Merck, Darmstadt, Germany), 0.1% Triton X-100 (Merck), 180 U/ml RNase (Sigma) and 4 mM sodium citrate (Merck), and incubated at 37°C for 20 min. This was followed by the addition of solution B (pH 7.2) which contained 50 μg/ml PI, 3% polyethyleneglycol 6000, 0.1% Triton X-100 and 0.4 M NaCl (Merck). The sample was then stored at 4°C for at least 1 h, filtered through 60 μm nylon mesh (Spectrum Medical Industries, CA), and then analyzed on a Becton Dickinson FACScan flow cytometer (Becton-Dickinson, Mountain View, CA) supplemented with an argon-ion laser (15 mW) with incident beam at 488 nm. Red fluorescence (PI) was collected through a 585 nm filter. Data were analyzed with Cell-Fit and Lysis-II softwares on an HP-310 computer (Hewlett-Packard, Sunnyvale, CA). Quantitative analysis was performed using the sum-of-broadened-rectangle model program provided by the manufacturer. The proliferative index was the ratio derived from dividing the percentage of cells in S and G2-M phases by the percentage of cells in G0–G1 phases.

2.3. Electrophysiological measurement

In experiments designed to investigate the long-term effect of HGF, the synchronized cells were pre-incubated with 10 ng/ml of HGF in 0.5% FBS for 48 h and transferred to a recording chamber that was mounted on the stage of an inverted microscope (Diaphot 200; Nikon, Tokyo, Japan). The immediate effect of HGF was evaluated using synchronized cells without pre-incubation. To monitor the changes in cell size, the microscope was coupled to a video camera system with magnification up to 1500×. Cells were bathed at room temperature (20–25°C) and continuously superfused at a rate of 2 ml/min with Tyrode's solution containing (in mM) NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, Hepes 5, with pH adjusted to 7.4 with NaOH. Patch pipettes were prepared by pulling out heated Kimax glass capillaries (Kimble Products, Vineland, NJ) using a two-step, vertical micropipette puller (PB-7, Narishige Scientific Instruments, Tokyo, Japan). Two types of internal pipette solutions with different Ca^{2+}-buffering capacity were used. Both solutions contained (in mM): K-aspartate 130; Na_2ATP 3; $MgCl_2$ 2; Hepes 5, with pH buffered to 7.2. Varying amounts of $CaCl_2$ and EGTA were added to the pipette solution to yield free Ca^{2+} ($[Ca^{2+}]_{free}$) of 10 nM (0.9 mM $CaCl_2$ and 10 mM EGTA) and 1 μM (0.14 mM $CaCl_2$ and 0.15 mM EGTA). $[Ca^{2+}]_{free}$ was calculated assuming a dissociation constant for EGTA and Ca^{2+} at pH 7.2 of 10^{-7} M [23]. Their DC resistance varied between 3 and 5 MΩ when the pipettes were filled with the internal solution. The pipettes were connected to the input stage of a patch-clamp amplifier (RK-400; Biologic, Claix, France). A three-dimensional micromanipulator (WR-6; Narishige Scientific Instruments) which was mounted on the fixed stage of the inverted microscope, was used to position the pipette near the cell. The perforated patch configuration [24,25] was used to record whole cell currents. In this configuration, the membrane of attached patch was permeabilized with 100 μg/ml of nystatin (Research Biochemicals International, Natick, MA) to preserve the cytoplasmic content. After filtering through a 5-pole Tchebitchoff low-pass filter (−3 dB at 3 kHz), data were monitored on a storage oscilloscope (model HM205-3; Hameg Instruments, Anaheim, CA) and simultaneously stored on a digital tape recorder (DTR1204; Biologic). The stored data were analyzed off-line with the aid of pClamp 6.03 (Axon Instruments, Foster City, CA). In experiments designed to construct the current vs. voltage (I–V) relationship,

either square command pulses with a duration of 300 msec from the holding potential to various potentials or ramp command pulses with a duration of 100 msec from −80 mV to +40 mV were used. The square or ramp voltage-step command signals were generated at a rate of 0.2 Hz by a programmable stimulator (SMP311; Biologic).

2.4. Statistical analysis

All values are reported as mean ± standard deviation. The paired or unpaired Student's *t* test and one-way analysis of variance (ANOVA) with least-significant-difference method for multiple comparison were used for statistical evaluation of differences among means. Differences between the values were considered significant when $p < 0.05$.

3. Results

3.1. HGF stimulates proliferation of SC-M1 cells

The mitogenic activity of HGF on the SC-M1 and KATO-III gastric carcinoma cells is shown in Fig. 1. After SC-M1 cells were exposed to various concentrations of HGF (0, 1 and 10 ng/ml) for 48 h, a significant ($p < 0.05$, one-way ANOVA) dose-dependent growth stimulation was observed. However, treatment with HGF for 24 h did not significantly stimulate the proliferation of SC-M1 cells ($p = 0.37$). In KATO-III cells, no significant stimulatory effect on the cell proliferation was observed at 24 h ($p = 0.07$) and 48 h ($p = 0.18$) after HGF treatment (Fig. 1).

3.2. HGF accelerates SC-M1 cell cycle progression

The mitogenic stimulatory effect of HGF on SC-M1 and KATO-III cells was further examined by flow cytometric analysis (Fig. 2). After being synchronized by 400 μM *l*-mimosine for 24 h, 85.7% of SC-M1 and 72.3% of KATO-III cells were found at G0–G1 phases, respectively (Fig. 2(A)). When these cells were released into fresh medium containing 0.5% FBS, the cells began cycling again. The HGF-treated SC-M1 cells were found to cycle more rapidly than the vehicle control, while no significant stimula-

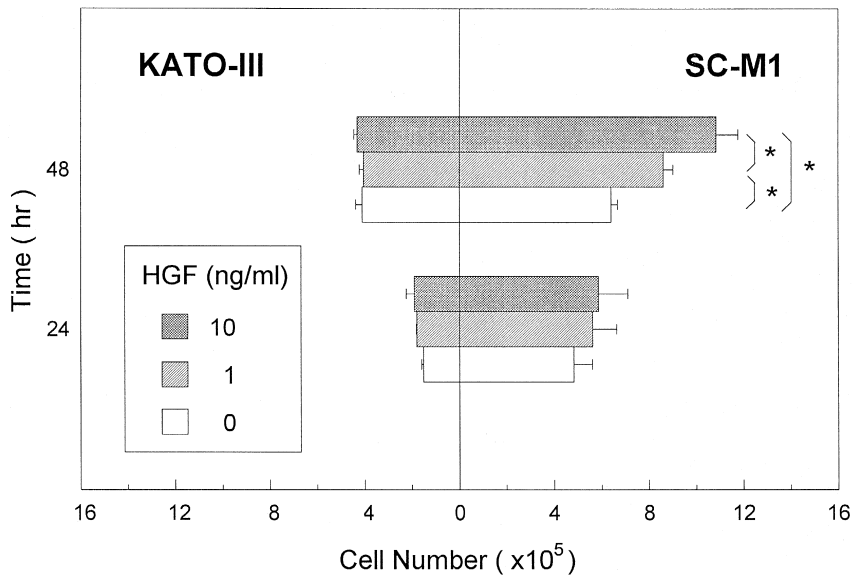

Fig. 1. Effect of HGF on the growth of SC-M1 and KATO-III cells. The number of viable cells was counted at 24 and 48 h after being incubated with 0, 1 and 10 ng/ml HGF. Data represent the mean (column) and standard deviation (error bar) from four independent experiments with triplicate samples. The asterisks indicate statistically significant difference ($P < 0.05$) using one-way ANOVA with least-significant-difference method for multiple comparison, which were found in SC-M1 cells treated with different concentrations of HGF for 48 h.

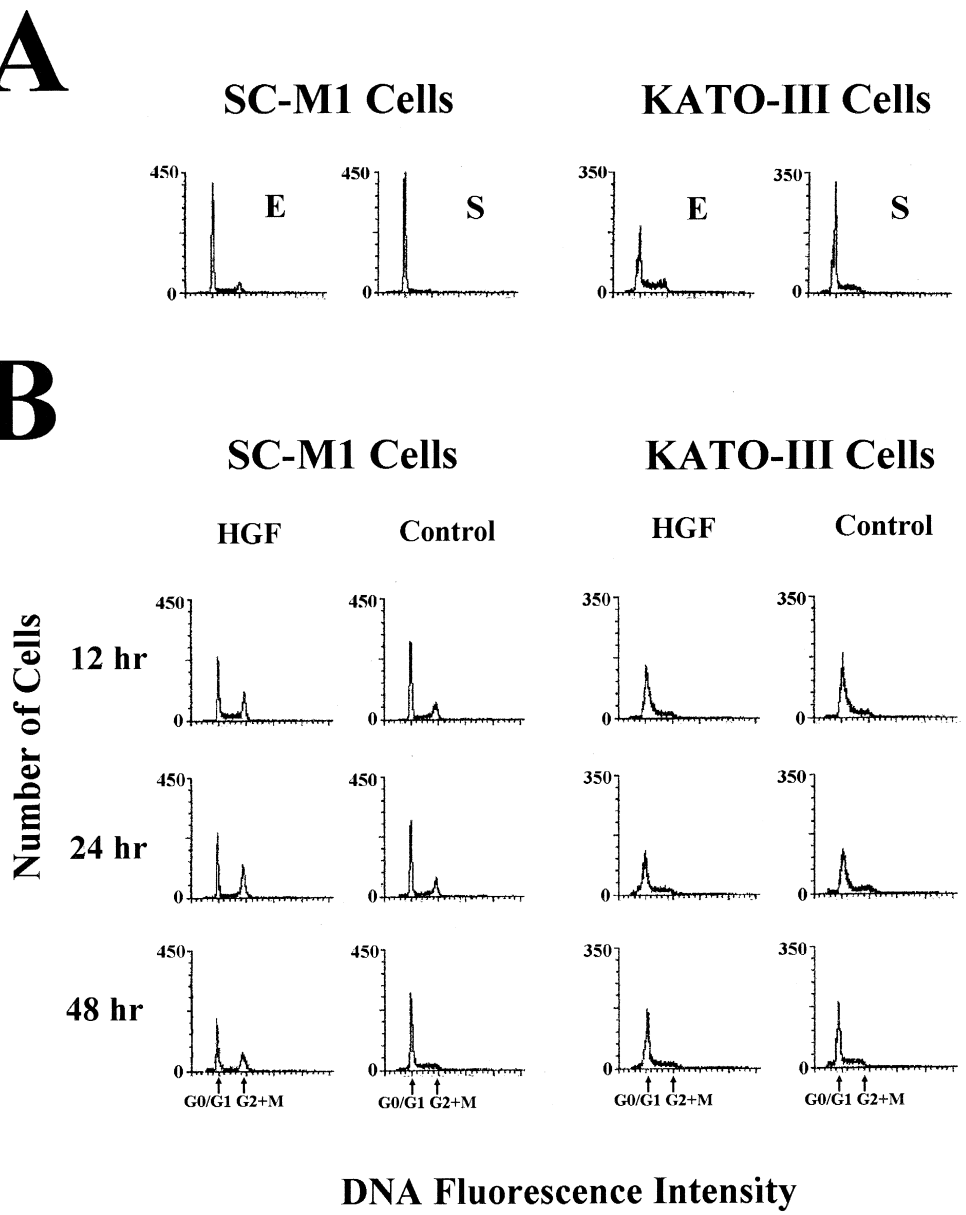

Fig. 2. Effect of HGF on cell cycle progression in SC-M1 and KATO-III cells. (A) DNA content of exponentially growing (E) and *l*-mimosine synchronized (S) SC-M1 and KATO-III cells. (B) Cell cycle progression in SC-M1 and KATO-III cells with and without the treatment of HGF. Cells were harvested at different time intervals after being released from *l*-mimosine synchronization. Ordinate and abscissa of each plot, respectively, show the number of cells tested and the DNA content detected with propidium iodide. Of note, HGF accelerated cell cycle progression in SC-M1 cells, but not in KATO-III cells.

tory effect of HGF on cell cycle progression was observed in KATO-III cells (Fig. 2(B)). In quantitative analysis derived from 4 independent experiments, the mean proliferative index of HGF-treated SC-M1 cells was significantly higher than that of the vehicle control at 48 h (0.84 ± 0.11 vs. 0.54 ± 0.06, $p < 0.05$). However, at the same time point, the proliferative indices of HGF-treated and vehicle control KATO-III cells did not significantly differ (0.72 ± 0.07 vs. 0.69 ± 0.03, $p = 0.54$).

3.3. HGF induced an oscillatory outward K^+ current in SC-M1 cells

The perforated-patch clamp method was used to investigate the effect of HGF on ionic currents in SC-M1 and KATO-III cells. The effect of HGF on the membrane current of SC-M1 cells at 0 mV is shown in Fig. 3. In experiments performed with high Ca^{2+} pipette solution ($[Ca^{2+}]_{free} = 1\,\mu M$), the addition of HGF (10 ng/ml) to the bathing solution induced a repetitive outward current in 52% (13 out of 25) of cells (Fig. 3(A)). The lag period between the addition of HGF and the onset of oscillatory outward current was variable. The mean lag time was 5 ± 3 min ($n = 12$). The mean lag time was shortened to 2 ± 3 min when the concentration of HGF was increased to 100 ng/ml ($n = 7$). In 36% of cells (9 out of 25 cells), the presence of HGF (10 ng/ml) induced a large sustained rise in outward current. The further addition of tetraethylammonium chloride (TEA, 10 mM) fully suppressed the amplitude of HGF-induced increase in this membrane current (Fig. 3(B)). Three of the 25 tested cells exhibited no changes in membrane current in response to HGF stimulation during an observation period of no less than 20 min.

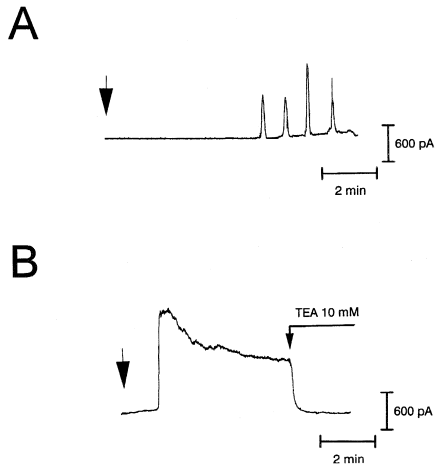

Fig. 3. Effect of HGF on outward current in SC-M1 cells. Each cell was held at 0 mV. The arrows indicate the time when of HGF (10 ng/ml) was added. The pattern and latency for the development of oscillatory outward currents were quite variable, and a representative oscillatory current is shown (panel A). The HGF-induced increase in outward current can be effectively abolished by the addition of 10 mM TEA (panel B).

When the bathing solution was replaced with Ca^{2+}-free Tyrode's solution ($n = 12$), or in cells ($n = 11$) studied with a low Ca^{2+} ($[Ca^{2+}]_{free} = 10$ nM) internal pipette solution, the HGF-induced outward currents were no longer observed in SC-M1 cells. This result suggests that the HGF-induced outward current in SC-M1 cells is mainly attributed to the excitation of Ca^{2+}-activated K^+ current. In contrast, HGF (10 ng/ml) did not produce any observable effect on the membrane current in KATO-III cells ($n = 11$).

3.4. Current–voltage (I–V) relations of K^+ currents in control and HGF-treated SC-M1 cells

As shown in Fig. 4(A), in experiments performed with a high Ca^{2+} pipette solution ($[Ca^{2+}]_{free} = 1\,\mu M$) and when the cell was held at -80 mV, a series of voltage steps elicited sustained and time-independent currents. The I–V relation in SC-M1 cells was almost linear. No voltage-gated inward or outward currents could be demonstrated in SC-M1 and KATO-III cells. When cells were treated with HGF (10 ng/ml) for 48 h, the I–V relation of SC-M1 cells was significantly altered (Fig. 4(B)). The measured potentials at which current crossed the zero-current level in control and HGF-treated cells were -19 mV and -50 mV, respectively. The slope conductance of I–V curve in HGF-treated SC-M1 cells was significantly increased to about 2-fold of the control and the reversal potential became hyperpolarized, whereas there was no significant difference in other parameters, including cell diameter, cell capacitance, membrane time constant and input resistance (Table 1). In contrast, the parameters of electric properties, including reversal potential and slope conductance between control and HGF-treated KATO-III cells did not significantly differ. In experiments performed with a low Ca^{2+} pipette solution ($[Ca^{2+}]_{free} = 10$ nM), the changes in the slope conductance and reversal potential in response to HGF stimulation in SC-M1 cells were not observed ($n = 7$). When A23187 (Sigma), a Ca^{2+} ionophore, was added to the bathing solution in a concentration of 10 μM, the slope conductance of I–V curve of SC-M1 cells and KATO-III cells was increased to 538 ± 75 pS/pF ($n = 5$) and 512 ± 63 pS/pF ($n = 4$), respectively. The reversal poten-

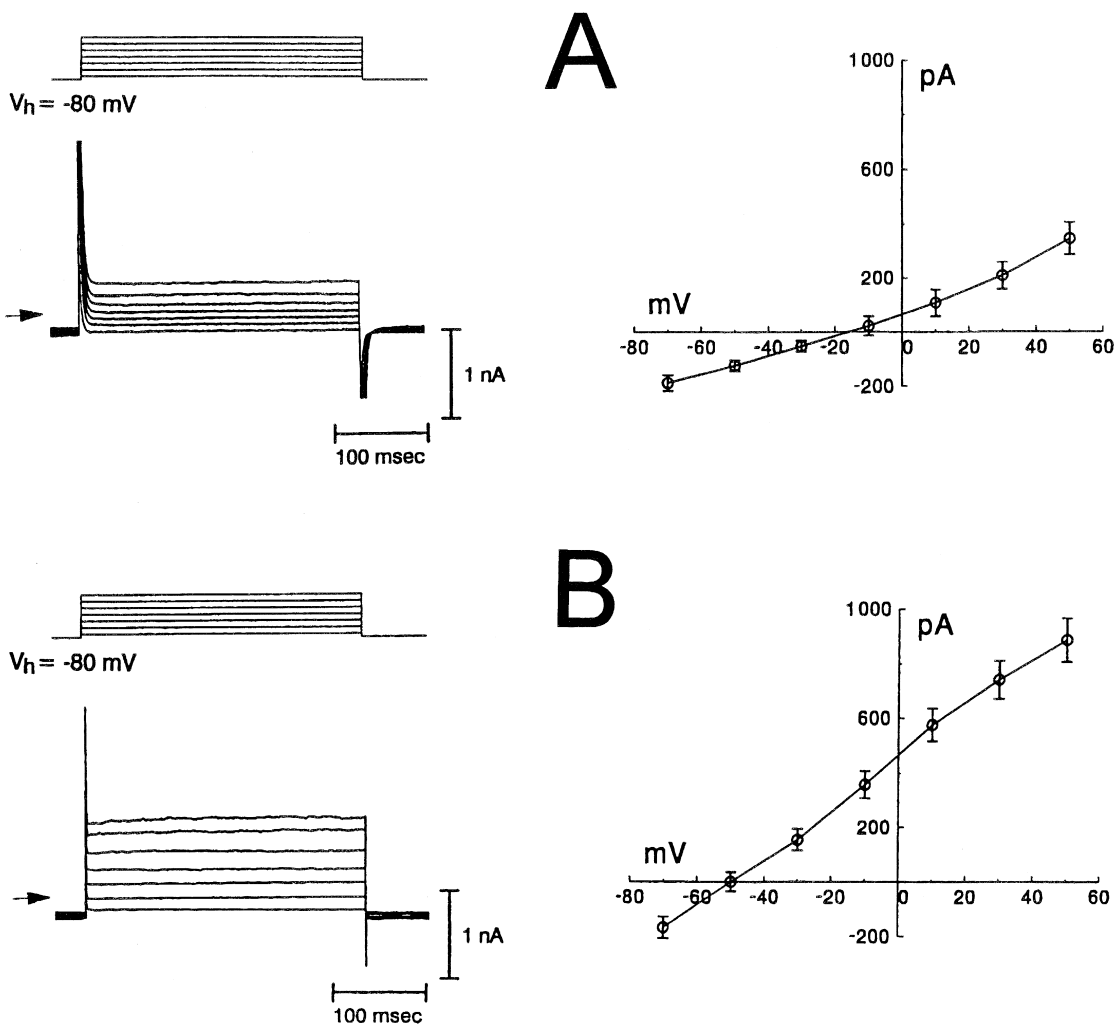

Fig. 4. Comparison of current–voltage relationships between the control (panel A) and the HGF-treated (panel B) SC-M1 cells. The superimposed voltage and current traces were shown in the left side of each panel. Each cell was held at the level of −80 mV and various voltage steps with a duration of 300 msec at 0.2 Hz were then applied. The arrows shown in left side of each panel indicate the zero current level. Each data point shown in right side of each panel is the mean ± standard deviation of four or five cells. Of note, the slope conductance in cells treated with HGF for 48 h was significantly ($p < 0.05$) greater than that in control cells and the reversal potential of HGF-treated cells was significantly ($p < 0.05$) shifted to a more negative level.

Table 1
Comparison of electric properties of SC-M1 and KATO-III cells after treatment with HGF for 48 h

	SC-M1 cells		KATO-III cells	
	Control	HGF-treated	Control	HGF-treated
Cell diameter(μm)	21 ± 4 ($n = 15$)	22 ± 5 ($n = 6$)	22 ± 6 ($n = 18$)	23 ± 7 ($n = 10$)
Reversal potential (mV)	-19 ± 5 ($n = 12$)	-50 ± 6 ($n = 5$)*	-21 ± 4 ($n = 15$)	-22 ± 5 ($n = 10$)
Cell capacitance (pF)	41 ± 5 ($n = 11$)	40 ± 6 ($n = 7$)	48 ± 5 ($n = 7$)	47 ± 6 ($n = 8$)
Membrane time constant (msec)	0.4 ± 0.1 ($n = 10$)	0.4 ± 0.1 ($n = 6$)	0.5 ± 0.2 ($n = 8$)	0.5 ± 0.1 ($n = 8$)
Input resistance (GΩ)	1.3 ± 0.1 ($n = 10$)	1.2 ± 0.1 ($n = 6$)	1.4 ± 0.1 ($n = 11$)	1.3 ± 0.2 ($n = 6$)
Slope conductance (pS/pF)	110 ± 15 ($n = 11$)	207 ± 15 ($n = 6$)*	105 ± 14($n = 11$)	114 ± 15($n = 6$)

Parentheses denote number of cells tested. *Asterisks indicate values significantly different ($p < 0.05$) from control.

tials measured in the presence of A23187 with 5.4 mM, 50 mM and 100 mM extracellular K^+ were -79 ± 6 mV ($n = 3$), -19 ± 4 mV ($n = 3$) and -7 ± 3 mV ($n = 3$) respectively in SC-M1 cells, and were -77 ± 6 mV ($n = 3$), -21 ± 5 mV ($n = 3$) and -8 ± 3 mV ($n = 3$) respectively in KATO-III cells. Thus, the measured outward current after the addition of A23187 was mainly attributed to Ca^{2+}-activated K^+ current.

3.5. Characteristics of the oscillatory outward currents in HGF-treated cells

In SC-M1 cells pretreated with HGF (10 ng/ml) for 48 h, a repetitive oscillatory outward current was observed at a frequency of 0.02 Hz when a high Ca^{2+} solution ($[Ca^{2+}]_{free} = 1\,\mu M$) was used as internal pipette solution and the cells were held at 0 mV (Fig. 5(A)). When the cell was held at -40 mV, the amplitude of HGF-induced outward current was reduced, but the frequency of oscillatory outward current was increased to about 0.1 Hz (Fig. 5(B)). Under the current-clamp condition, the oscillatory change in membrane potential can also be observed in HGF-treated cells. To further clarify the properties of this repetitive oscillatory current, the ramp voltage pulses from -80 mV to $+40$ mV with a duration of 100 msec were applied. As shown in upper panel of (Fig. 5(C)), the slope conductance of current–voltage (I–V) relation was increased by 2-fold from the bottom level to peak level of membrane current. At the level of $+40$ mV, the amplitude of membrane current was increased from 180 pA to 900 pA, whereas the amplitude of the holding current at the level of -80 mV was not significantly altered. To determine the true reversal potential of the conductance, the difference between bottom and peak levels of holding current was taken. The HGF-induced component of current was then constructed (Fig. 5(C), lower panel). The reversal potential of this net current was -74 mV, the value of which is close to the equilibrium potential of K^+ ions. In experiments performed with low Ca^{+2} pipette solution ($[Ca^{2+}]_{free} = 10$ nM) or when the cells were bathed in Ca^{2+}-free solution, the oscillatory current no longer occurred. Since the pipette solution did not contain Cl^- ion, the possibility of Ca^{2+}-activated Cl^- current was excluded. In fact, when the K-aspartate in pipette was replaced with KCl (130 mM), the results were the same. These findings strongly indicate that this oscillatory outward current was mainly due to the passage of K^+

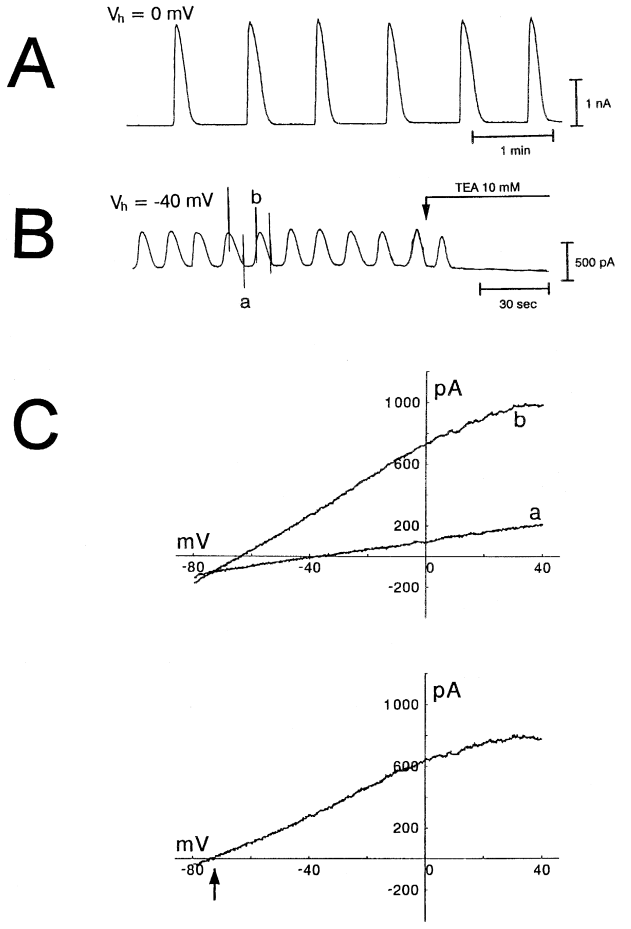

Fig. 5. Induction of oscillatory outward currents in SC-M1 cells treated with HGF for 48 h. In panel A, the cell was held at 0 mV, and a repetitive oscillatory outward current at a frequency of about 0.02 Hz was observed. In panel B, another cell was held at -40 mV, an oscillatory outward current at a frequency of about 0.1 Hz was noted and terminated by the addition of TEA (10 mM). During the bottom (labeled a) and peak (labeled b) of oscillatory current, the ramp pulses from -80 mV to $+40$ mV with a duration of 100 msec were applied. Upper part of panel C shows the original traces of current–voltage relations during the bottom and peak of oscillatory current. The net increase in outward current, i.e., trace b − trace a, was shown in lower part of panel C. The arrow indicates that the level of reversal potential for HGF-induced current is -74 mV.

ions through the membrane which is dependent on cytosolic Ca^{2+} concentration.

4. Discussion

The involvement of K^+ channel in cell proliferation has been recognized in several types of mammalian cells, such as normal human T lymphocytes [26], human breast carcinoma cells [27,28] and rat malignant T lymphocytes [29]. The types of K^+ channel involved in the mitogenesis were, however, different in various cell systems. There has a large body of evidence that voltage-dependent K^+ channel activity is a key determinant for the cell cycle progression thus effectively controlling DNA synthesis and cell proliferation [13]. In the present study, we found that the HGF-stimulated Ca^{2+}-activated K^+ channel activity in gastric cancer cells correlated well with the HGF-induced responses in cell proliferation. Based on the criteria of reversal potential, lack of voltage-gating and sensitivity to A23187, both SC-M1 and KATO-III gastric cancer cell lines were found to exhibit Ca^{2+}-activated K^+ channels. In response to HGF stimulation for 48 h, the outward K^+ current was increased, leading to an increased slope conductance and more negative reversal potential in SC-M1 cells (Fig. 4). However, this membrane hyperpolarization effect of HGF was not observed in KATO-III cells. Based on the flow cytometric analysis, the presence of HGF was also found to accelerate cell cycle progression in SC-M1 cells, while not in KATO-III cells. Together with the findings in cell proliferation assay, these results suggest that the membrane hyperpolarization resulting from the increased activity of Ca^{2+}-activated K^+ channels might be related to the HGF-induced mitogenesis of gastric cancer cells.

It is interesting that when SC-M1 cells were exposed to HGF, an oscillatory pattern of the Ca^{2+}-activated K^+ current was observed. Signal transduction by HGF has been reported to involve multiple intracellular signaling pathways including activation of *ras* [30], phosphatidylinositol 3-kinase [31] and phospholipase C − γ [12,32]. In rat hepatocytes, the activation of phospholipase C − γ generates IP_3 which in turn elicits calcium oscillation in variable frequencies [10,33]. Activation of protein kinase C has also been linked to calcium oscillation in NIH3T3 fibroblasts [34]. The detailed regulation of this oscillatory channel activity is not clear, but difference in the activation of *ras*, phosphatidylinositol 3-kinase or phospholipase C − γ may contribute to the different response to HGF between SC-M1 and KATO-III cells. The increased intracellular Ca^{2+} concentration induced by HGF may be a result of release of intracellular Ca^{2+} pool [10,35] or Ca^{2+} influx from extracellular space [11]. In the present study we have found that the oscillatory Ca^{2+}-activated K^+ current was dependent on extracellular Ca^{2+}, suggesting that HGF induces the oscillation through Ca^{2+} influx from extracellular space in SC-M1 cells. However, the oscillatory outward current was not observed in HGF-treated KATO-III cells. These results are consistent with the findings that HGF did not have any effects on the reversal potential, slope conductance as well as cell proliferation in KATO-III cells. Interestingly, overexpression of HGFR/*c-met* has been found in KATO-III cells [4] and confirmed by us with the aid of indirect immunofluorescent staining (data not shown). In contrast to the electrophysiologic and flow cytometric results, the level of HGF receptors existing in SC-M1 cells was much lower than that of KATO-III cells. It is thus suggested that the overexpressed HGF receptors in KATO-III cells were not functionally coupled to both the activation of Ca^{2+}-activated K^+ current and the induction of cell proliferation by HGF. Whether this is the result of mutation of the HGFR, defect in the HGF-induced signal transduction pathway, or both, is worthy of further investigation.

The present study clearly demonstrates that HGF stimulated Ca^{2+}-activated K^+ channel activity and mitogenesis in gastric cancer cells. This observation suggests a link, but not necessarily a causal relationship between Ca^{2+}-activated K^+ channel activity and HGF-induced mitogenesis. The activation of Ca^{2+}-activated K^+ channels may play a specific and important role in the HGF-induced signalling process, as seen in the activated Jurkat lymphocyte cell line in which the Ca^{2+}-activated K^+ channels were assumed to maintain a large negative membrane potential and act as a battery to drive Ca^{2+} influx through CRAC channels [36,37]. Alternatively, the signalling pathway induced by HGF might not directly involve in

the activation of Ca^{2+}-activated K^+ channels, although this activation might always be seen during the signalling process. However, we have demonstrated that when a low Ca^{2+} solution ($[Ca^{2+}]_{free}$ = 10 nM) was included in the recording pipette, the membrane hyperpolarization response of SC-M1 cells to HGF no longer occurred. This result thus suggests that the change in the amplitude of Ca^{2+}-activated K^+ current in HGF-treated SC-M1 cells was related to the level of $[Ca^{2+}]_i$. However, it is possible that the expression of Ca^{2+}-activated K^+ channels may have been up-regulated by HGF, or the Ca^{2+}-activated K^+ channels may have increased sensitivity to $[Ca^{2+}]_i$ by HGF stimulation. In any case, it is clear that there is a signalling mechanism which involves $[Ca^{2+}]_i$ oscillation in HGF-treated SC-M1 cells, and this oscillation may cause parallel activation of Ca^{2+}-activated K^+ channels. These findings gave support to that the alteration in the activity of Ca^{2+}-activated K^+ channels is related to functional changes of transformed cells.

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