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MOLECULAR DESIGN OF SUGAR-FREE MIGRACIN ANALOG MIGRACINAL THAT INHIBITS OVARIAN CANCER CELL MIGRATION AND INVASION

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Introduction. *Cancer metastasis consists of several steps including detachment from the primary tumor, migration, invasion, transport in the blood or lymphatic vessels, attachment at the secondary site, and growth of secondary tumor. Migration and invasion areinvolved in the mechanism of all types of cancer metastasis. We previously isolated novel cellular migration inhibitor migracin A and B from a culture filtrate of Streptomyces sp. Migracin A was shown to inhibit IGF-1-mediated cellular migration and invasion in ovarian carcinoma cells. However, it is difficult to prepare large amount of migracin A. Migracin A consists of substituted benzene and an alkylated sugar moiety. In the present research, we have designed and synthesized a simplified dialdehydederivative of migracin called migracinal having no sugar moiety.*

Material and methods. *Migracinal was purchased from Techno Chem Co., Ltd., Tokyo, Japan. Migracinal was prepared from 2,4-dihydroxybenzaldehyde (2,4-DHBA). The structure was confirmed by proton and carbon NMR spectra and ESI mass spectroscopy. The antitumor activity of the new derivative was studied by standard tests under conditions in vitro.*

Results. *Migracinal inhibited cellular migration and invasion in ovarian clear cell carcinoma ES-2 cells. It also inhibited IGF-1 expression as migracin A. Moreover, it induced anoikis rather than apoptosis in ES-2 cells.*

Conclusions. *Migracinal is easier to prepare than migracins, and it may be useful for the mechanistic study and suppression of metastasis.*

Keywords: *Migracinal, Migracin, Migration, Invasion, Anoikis, Ovarian clear cell carcinoma*

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МОЛЕКУЛЯРНЫЙ ДИЗАЙН НЕ СОДЕРЖАЩЕГО УГЛЕВОДНОГО КОМПОНЕНТА АНАЛОГА МИГРАЦИНА, ПРЕПЯТСТВУЮЩЕГО МИГРАЦИИ И ИНВАЗИИ КЛЕТОК РАКА ЯИЧНИКОВ

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Введение. *Процесс метастазирования рака состоит из нескольких этапов: отсоединение клеток от первичной опухоли, миграцию, инвазию, перемещение в крови или лимфатических сосудах, присоединение и рост вторичной опухоли. Механизмы миграции и инвазии универсальны для всех видов рака. Ранее из культуры Streptomyces SP мы выделили Migracin A и B - новые ингибиторы клеточной миграции. Было продемонстрировано как Migracin A ингибирует IGF-1-опосредованную миграцию и инвазию клеток рака яичников. Однако большое количество Migracin A, состоящего из замещенного бензола и алкилированного углеводного фрагмента, синтезировать трудоемко. В настоящем исследовании мы разработали и синтезировали упрощенное производное диальдегида Migracin, не имеющего углеводного компонента, названное Migracinal.*

Материалы и методы. *Migracin приобретался у компании «ТехноХим Со., Лтд» (Токио, Япония). Производное Migracinal получали взаимодействием Migracin с 2,4-дигидроксибензалдегидом. Структура была подтверждена спектрами ЯМР и масс-спектроскопией. Противоопухолевая активность нового производного изучалась стандартными тестами в условиях in vitro.*

Результаты. *Установлено, что Migracinal ингибирует клеточную миграцию и инвазию клеток ES-2 рака яичника и аналогично Migracin A ингибирует IGF-1 экспрессию. Кроме того, он индуцировал аноикис, а не апоптоз в клетках ES-2.*

Заключение. *Синтез Migracinal легче в сравнении с Migracin, а спектр противоопухолевой активности идентичен, что может быть использовано для подавления процессов метастазирования.*

Ключевые слова: *Migracinal, Migracin, миграции, инвазия, аноикис, рак яичника*
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INTRODUCTION

Cancer metastasis consists of several steps including detachment from the primary tumor, migration, invasion, transport in the blood or lymphatic vessels, attachment at the secondary site, and growth of secondary tumor. Migration and invasion areinvolved in the mechanism of all types of cancer metastasis. Therefore, we looked for cellular migration inhibitors of low molecular weight from microbial culture filtrates. As a result, we discovered novel compounds, migracin A and B, from the culture filtrate of Streptomyces sp [1]. Migracin A and B are closely related in structure (Fig. 1A), and show similar inhibitory activities. Migracin A (Fig. 1) and B inhibited migration of breast carcinoma MDA-MB-231, non-small cell lung carcinoma A549, and fibrosarcoma HT1080 cells without any toxicity. Migracin A and B showed similar activity.

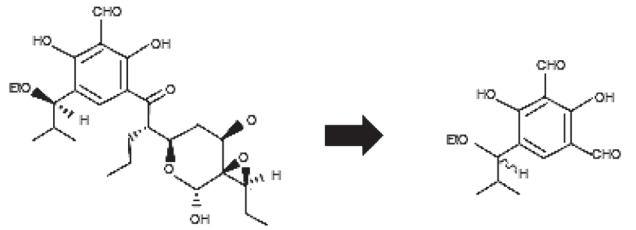

Figure 1 – Molecular design of migracinal based on the structure of migracin A.

In one hand, ovarian cancer is the most common cause of gynecologic disease-related death. Ovarian carcinomas often metastasize to neighboring organs such as lung, liver and the peritoneal cavity through direct extension, migration, invasion, and lymphatic vessel transport [2,3]. Moreover, clear cell ovarian tumors are part of the epithelial tumor group of ovarian cancers, and these tumors may have a worse prognosis than other epithelial type-ovarian tumors [4]. Therefore, effective metastasis inhibitors with low toxicity should be useful for therapy against ovarian clear cell cancer.Later we have reported that migracin A inhibits migration and invasion of ovarian clear cell carcinoma ES-2 cells via inhibition of IGF-1 expression [5]. It inhibited the cellular Akt activity that is downstream signaling of IGF-1/IGF-1 receptor. However, migracin A did not inhibit colony formation of ES-2 cells. Although migracin A may become a candidate of anti-metastasis agent, yield by the producing organism is limited. Also chemical synthesis of migracin A appears not easy, since the structure is complicated. Therefore, we looked for a simpler derivative of migracin A. In the present research, we designed a sugar-free analog of migracin A. We named the synthesized compoundmigracinal (Fig. 1), since a new aldehyde moiety was added in the structure. We have also evaluated the cellular anti-metastasis and anoikis-inducing activities of this compound.

MATERIALS AND METHODS

Migracinal. Migracinal was purchased from Techno Chem Co., Ltd., Tokyo, Japan. Migracinal was prepared from 2,4-dihydroxybenzaldehyde (2,4-DHBA). The synthetic procedure of migracinal will be reported later. It was obtained as a brown oily residue. The structure was confirmed by proton and carbon NMR spectra and ESI mass spectroscopy.

Cell culture:

Human ovarian clear cell carcinoma ES-2 cells (ATCC, Manassas, VA) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and penicillin/streptomycin at 37°C in a humidified incubator with 5% CO2.

Wound healing assay:

Cells in 24-well plates were allowed to reach confluence before the surface was uniformly scratched across the center of each well with a pipette tip. The wells were then rinsed twice with serum-free media to remove floating cells and growth media, after which the cells were cultured in serum-free media for 24 h. The initial wounded area and movement of the cells into the scratched area were recorded. Experiments were performed in triplicate in three independent experiments.

Cell invasion assay:

ES-2 cells were suspended in 500 l of serum-free medium containing migracin A or the DMSO and seeded into the upper chambers coated with BD Matrigel Basement Membrane Matrix (Corning Inc., Corning, NY). The lower chambers were filled with 750 µl of medium containing 10% FBS and incubated for 24 h at 37°C in a humidified CO2 incubator. Then, after fixation of theinvading cells, non-invading cells remaining on the upper surface were removed by wiping with a cotton swab. Invading cells attached to the underside were stained with Diff-Quick solution (Sysmex, Kobe, Japan), and counted.

Soft agar colony formation:

A soft agar colony formation assay was employed according to the manufacturer's instructions using CytoSelect 96-well Cell Transformation Assay kit (Cell Biolabs, San Diego, CA). Each well contained 50 µl of 0.6% agar in a complete medium as the bottom and feeder layer, and 75 l of 0.4% agar in a complete medium with 1 104 cells as the top layer. After 6 days colonies were lysed with lysis buffer and detected with CyQuant GR dye for the quantification of anchorage-independent growth andthe fluorescence was measured with a fluorometer using the 485/520 filter set SpectraMax M5 (Molecular Devices, Sunnyvale, CA).

Anoikis:

Cells death by anoikis were identified and quantified using the CytoSelect 24-well Anoikis Assay Kit (Cell Biolabs) according to the manufacturer's instructions. For the determination of anoikis, ovarian clear cell carcinoma ES-2 cells were cultured in serum-starved medium in normal or poly (2-hydroxyethyl methacrylate) (poly-HEMA)-coated 24-well plates. Anoikis-induced cell death was measured by ethidium homodimer (EthD-1) [6].

RESULTS

Molecular design of migracinal:

Migracin A has a similar structure of luminacin C. In case of luminacin C, a sugar-free dialdehydederivative was synthesized, and it showed the inhibitory activity on HUVEC tube formation [7]. Therefore, we designed the structure of migracinalas the luminacin analog having ethoxy moiety instead of methoxymoiety. Thus, migracinal(2,4-dihydroxy-5-(1-ethoxy-2-methylpropyl)benzene-1,2-dicarbaldehyde) contains the ethoxy moiety instead of the methoxy moiety in theluminacin C derivative [7].

Inhibition of cellular migration and invasion:

Migracinal did not decrease the viability of ES-2 cells below 10 g/ml (Fig. 2A). In one hand, it inhibited the cellular migration monitored by wound healing assay at 10 g/ml (Fig. 2B). Next, it inhibited the cellular invasion at 1-10 g/ml more effectively than migration in the Matrigel chamber assay (Fig. 2C). Previously, migracin A was found to lower the IGF-1 expression by the PCR array analysis to inhibit migration and invasion. Migracinal also inhibited the expression of IGF-1, as shown in Fig. 2D. It also inhibited the downstream signaling Akt phosphorylation.

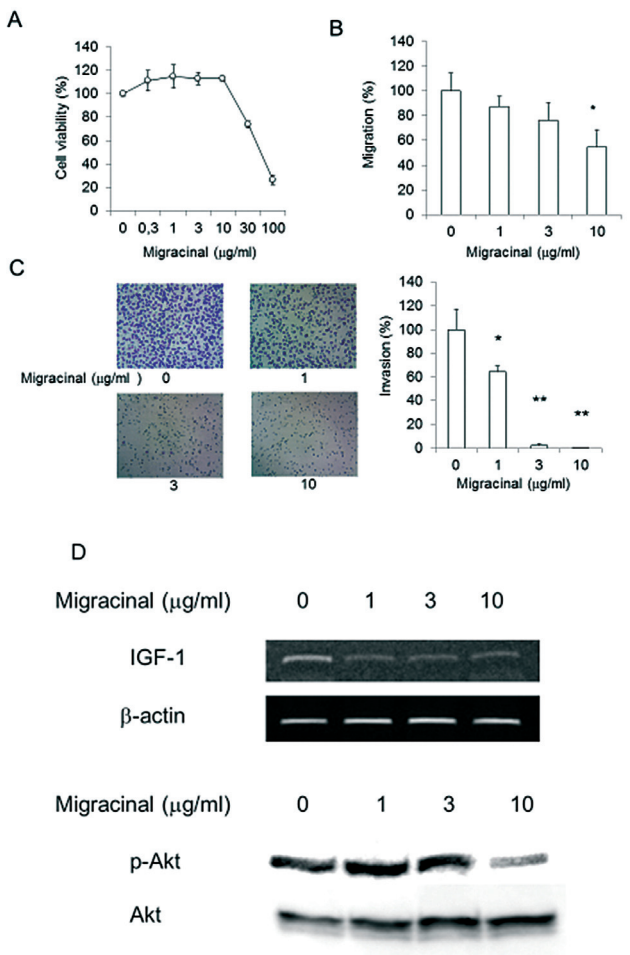

Figure 2 – Inhibition of cellular migration and invasion by migracinal in ES-2 cells. (A) Effect on viability. The cells were incubated for 24 h. (B) Inhibition of cellular migration. The cells were incubated for 24 h, and the cellular migration was measured by wound healing assay. (C) Inhibition of cellular invasion. The cells were incubated for 24 h, and the cellular invasion was measured by Matrigel chamber assay. (D) Inhibition of IGF-1 expression and Akt phosphorylation. The cells were incubated for 24 h, and the expression was measured by PCR (IGF-1) or Western blotting (phosphorylated Akt). Statistical evaluation, *P<0.05, ** P<0.01.

Inhibition of Anoikis:

Inhibition of anoikis is important to inhibit cancer metastasis. Migracinal induced anoikis at 3-10 g/ml, as shown in Fig. 3. It did not induce apoptosis at the same concentrations.

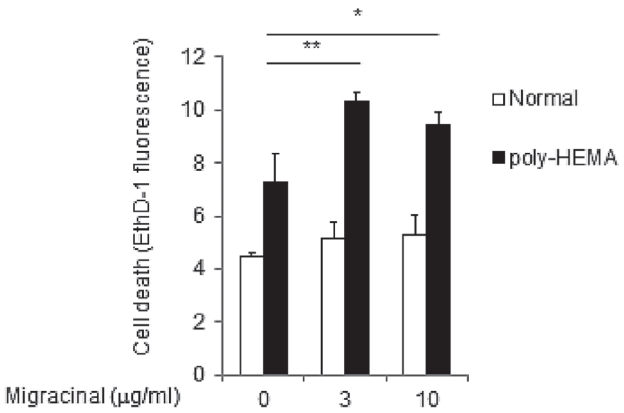

Figure 3 – Induction of anoikis. The cells were treated with migracinal for 24 h without or with poly-HEMA coating, and the cell death was measured as described in the Materials and methods. * P<0.05, ** P<0.01.

Inhibition of colony formation in soft agar:

In one hand, migracin A did not inhibit colony formation of ES-2 cells in soft agar even at 100 g/ml [5]. Interestingly, migracinal inhibited the colony formation effectively at 30-100 g/ml, as shown in Fig. 4. Then, migracinal may have cytostatic activity in addition to the anti-metastatic activity.

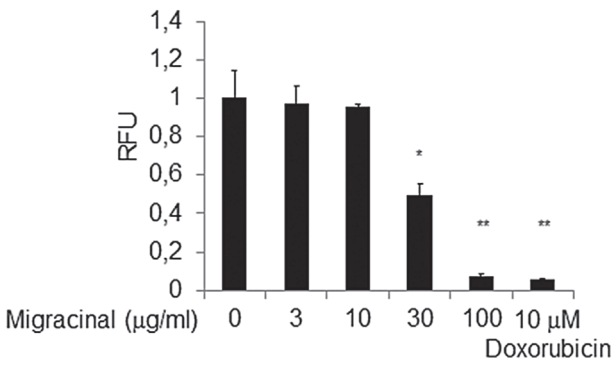

Figure 4 – Effect on growth in soft agar. The cells were incubated in soft agar for 6 days. Doxorubicin is a positive control. * P<0.01, ** P<0.001.

DISCUSSION

The structure of migracin A is closely related to that of luminacin C. Luminacin C was isolated from Streptomyces sp. as an inhibitor of capillary tube formation in human umbilical vein endothelial cells (HUVEC) [8, 9]. Since a sugar-free derivative of luminacin C did not lose the biological activity [7], we also prepared the similar structure based on migracin A.

Anoikis is the subset of apoptosis triggered by inadequate cell-matrix contacts [10]. Anoikis is essential for the regulation of tissue homeostasis in tissue remodeling, development, and especially tumor metastasis, so that the acquisition of anoikisresistance is considered to be important for achieving the successful metastasis for cancer cells. Because cancer cells should survive without binding to matrix during the metastasis. Therefore, anoikis-inducing agents may become new anti-metastasis agents. Migracinal inhibited the IGF-1 expression, as shown in Fig. 2 D. It has been reported that IGF-1 and the downstream Akt activity are essential to prevent anoikis in breast cancer cells [11].

In the present study, we employed the mixture of migracinaldiastereomers. We are planning to prepare stereo-specific migracinal for the further studies. Migracinal has simpler structure compared with migracin A, and can be prepared more easily. It may become a seed of anti-metastasis agents.

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Conflict of interest. The authors declare no conflict of interest.

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