COMMUNICATIONS TO THE EDITOR

Apoptolidin, a New Apoptosis Inducer in Transformed Cells from *Nocardiopsis* sp.

Sir:

Several oncogenes including *myc*, *E2F* and the adenovirus E1A have been demonstrated to sensitize cells to apoptosis[1~3]. Thus, specific apoptosis inducers in cells expressing such oncogenes may be useful as anticancer agents for treating certain types of tumors. In the course of our screening for apoptosis inducers using E1A-transformed cells[4], an actinomycete identified as *Nocardiopsis* sp. was found to produce a new active compound designated apoptolidin (Fig. 1). In this report, we describe the production, isolation, physico-chemical properties and biological activity of apoptolidin.

The producing organism was cultivated in 500 ml-Erlenmeyer flasks containing 100 ml of a medium consisting of glycerol 2.0%, molasses 1.0%, casein 0.5%, Polypepton 0.1% and $CaCO_3$ 0.4% (pH 7.2) on a rotary shaker at 27°C for 5 days.

The culture broth (2 liters) was centrifuged and the supernatant was extracted with EtOAc. The mycelial acetone extract was concentrated to a small volume and then extracted with EtOAc. The combined extract was subjected to silica gel column chromatography with $CHCl_3$ - MeOH (10 : 1). The active eluate was chromatographed on a Sephadex LH-20 column with MeOH and further purified by HPLC using a YMC-Pack D-ODS-7 column with 70% MeOH. The active fraction was evaporated to dryness to give a colorless powder of apoptolidin (218 mg).

The physico-chemical properties of apoptolidin are summarized in Table 1. The molecular formula of apoptolidin was determined as $C_{58}H_{96}O_{21}$ by high-resolution FAB-MS. The IR absorption peaks at 3450 and 1665 cm^{-1} indicated the presence of hydroxyl and conjugated carbonyl groups, respectively. ^{13}C and ^{1}H NMR data summary are presented in Table 2. Details of the structure determination will be reported in due course.

During apoptosis, loss of membrane integrity is typically preceded by chromatin condensation and internucleosomal cleavage of genomic DNA[5]. Significant numbers of E1A-transformed rat glia cells (RG-E1A-7)[4] treated with 1 μg/ml of apoptolidin for 24 hours contained condensed chromatin and fragmented nuclei as visualized by staining with Hoechst Dye 33258 (Fig. 2). The extract of these cells contained a large amount of fragmented DNA (Fig. 3). These data suggest that cell death induced by apoptolidin resulted from apoptosis. The cytotoxic activity of apoptolidin against normal and transformed cells is summarized in Table 3. Apoptolidin induced apoptotic cell death in cells transformed with the adenovirus type 12 oncogenes including E1A but not in normal cells or 3Y1 rat fibroblasts transformed with other oncogenes[6~8]. The adenovirus type 12 E1B gene encodes two major proteins of 19 kDa and 54 kDa, both of which independently can suppress apoptosis induced

Fig. 1. Structure of apoptolidin.

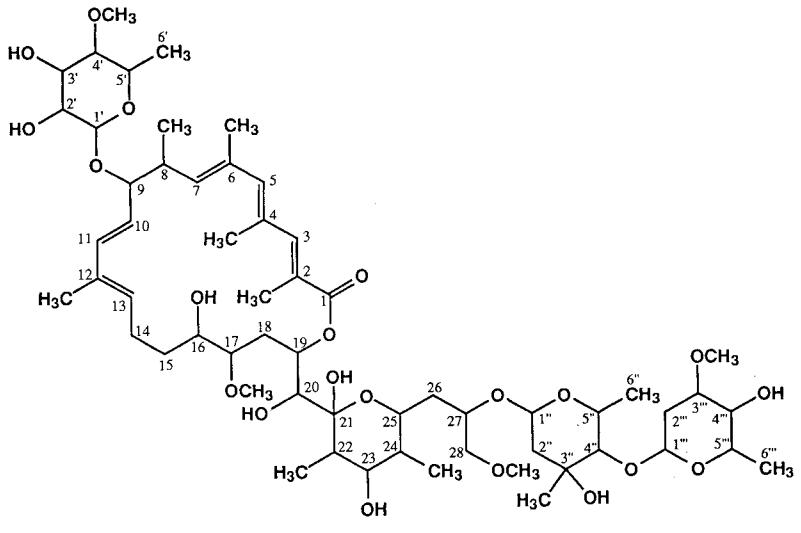


Table 1. Physico-chemical properties of apoptolidin.

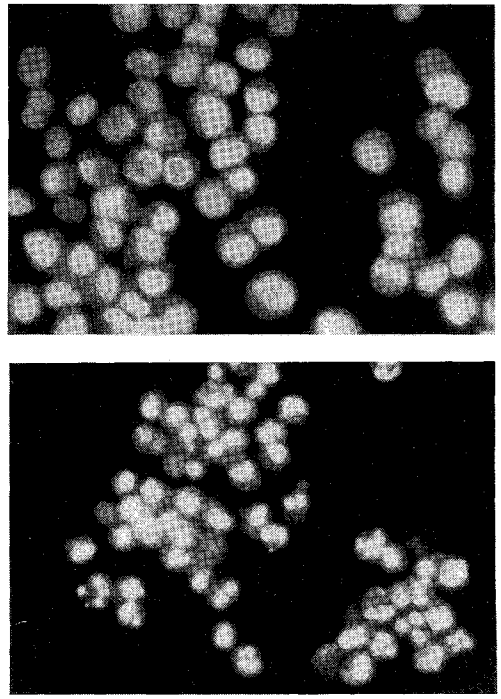
Appearance	Colorless powder
MP	128~130°C
$[\alpha]_D^{21}$	−5.2° (*c* 1.0, MeOH)
Molecular formula	$C_{58}H_{96}O_{21}$
HRFAB-MS	
Found:	1151.6357 $(M+Na)^+$
Calcd.:	1151.6342
UV λ_{max}^{MeOH} nm (ε)	234 (28,300), 320 (22,000)
IR ν_{max} (KBr) cm^{-1}	3450, 1665

Table 2. ^{13}C and ^{1}H data for apoptolidin in CD_3OD.

No.	δ_C	δ_H	No.	δ_C	δ_H
1	172.7		6-Me	16.6	1.97
2	123.7		8-Me	18.4	1.17
3	149.2	7.41	12-Me	12.2	1.71
4	133.1		22-Me	12.4	1.06
5	147.0	6.23	24-Me	5.3	0.92
6	133.4		17-OMe	61.4	3.40
7	142.9	5.27	28-OMe	59.5	3.30
8	38.9	2.79			
9	84.2	3.87	1′	96.0	4.85
10	126.4	5.26	2′	73.6	3.44
11	141.2	6.21	3′	74.9	3.76
12	134.8		4′	87.4	2.76
13	133.3	5.71	5′	68.2	3.78
14	24.7	2.50	6′	18.4	1.29
		2.09	4′-OMe	61.1	3.61
15	36.4	1.52			
		1.44	1″	99.5	4.97
16	74.6	3.47	2″	45.5	1.96
17	83.8	2.75			1.84
18	38.4	2.20	3″	73.0	
		1.78	4″	85.8	3.37
19	72.4	5.32	5″	67.4	3.70
20	75.4	3.57	6″	19.0	1.25
21	101.3		3″-Me	22.9	1.36
22	36.4	2.08			
23	73.8	3.76	1‴	101.9	4.86
24	40.6	1.76	2‴	37.2	2.47
25	69.4	3.99			1.32
26	37.2	1.62	3‴	82.0	3.21
		1.49	4‴	77.1	3.01
27	76.8	3.48	5‴	73.2	3.24
28	76.8	3.36	6‴	18.4	1.31
2-Me	14.2	2.14	3‴-OMe	57.4	3.46
4-Me	18.0	2.21			

Chemical shifts are given in ppm using TMS as internal standard.

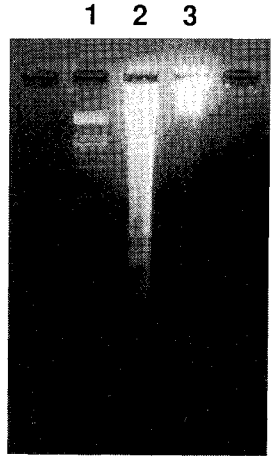
Fig. 2. Fluorescence micrographs of E1A-transformed rat glia cells stained with Hoechst Dye 33258.



Cells were cultured for 24 hours with (top) or without (bottom) 1 μg/ml of apoptolidin.

Fig. 3. Ethidium bromide-stained DNA extracted from E1A-transformed rat glia cells.

1: Marker, 2: apoptolidin (1 μg/ml, 24 hours), 3: control.



by E1A[3,9]. However, expression of these gene products was insufficient to suppress apoptosis induced by apoptolidin (Table 3). Further studies on the biological activities of apoptolidin are in progress.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research and a Grant-in-Aid for Scientific Research on Priority Areas, The Ministry of Education, Science, Sports and Culture, Japan, and Research for Future, Japan Society for the Promotion of Science.

Table 3. Cytotoxicity of apoptolidin against normal and transformed cells.

Cell line	Oncogene	IC_{50} (ng/ml)
Glia		>100,000
RG-E1A-7	E1A	11
RG-E1A19K-2	E1A, E1B19K	10
RG-E1A54K-9	E1A, E1B54K	13
RG-E1-4	E1A, E1B19K, E1B54K	10
3Y1		>100,000
Ad12-3Y1	E1A, E1B19K, E1B54K	17
HR-3Y1	H-*ras*	>100,000
SR-3Y1	v-*src*	>100,000
SV-3Y1	SV40 large T antigen	>100,000

Cells were cultured for 3 days with various concentrations of apoptolidin, and the cell growth was measured with formazan formation after 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) treatment.

JIN WOO KIM
HISASI ADACHI
KAZUO SHIN-YA
YOICHI HAYAKAWA*
HARUO SETO

Institute of Molecular and Cellular Biosciences,
The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

(Received April 24, 1997)

References

1) EVAN, G. I.; A. H. WYLLIE, C. S. GILBERT, T. D. LITTLEWOOD, H. LAND, M. BROOKS, C. M. WATERS, L. Z. PENN & D. C. HANCOCK: Induction of apoptosis in fibroblast by c-myc protein. Cell 69: 119~128, 1992
2) QIN, X.-Q.; D. M. LIVINGSTON, W. G. KAELIN, Jr. & P. D. ADAMS: Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. Proc. Natl. Acad. Sci. USA 91: 10918~10922, 1994
3) DEBBAS, M. & E. WHITE: Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B. Genes Dev. 7: 546~554, 1993
4) HAYAKAWA, Y.; K. SOHDA, K. FURIHATA, T. KUZUYAMA, K. SHIN-YA & H. SETO: Studies on new antitumor antibiotics, leptofuranins A, B, C and D. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 49: 974~979, 1996
5) WYLLIE, A. H.: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284: 555~556, 1980
6) KIMURA, G.; A. ITAGAKI & J. SUMMERS: Rat cell line 3Y1 and its virogenic polyoma- and SV40-transformed derivatives. Int. J. Cancer 15: 694~706, 1975
7) ZAITSU, H.; H. TANAKA, T. MITSUDOMI, A. MATSUZAKI, M. OHTSU & G. KIMURA: Differences in proliferation properties among sublines of rat 3Y1 fibroblasts transformed by various agents *in vitro*. Biomed. Res. 9: 181~197, 1988
8) SHIMURA, H.; T. MITSUDOMI, A. MATSUZAKI, M. KABEMURA, A. OKUDA & G. KIMURA: Transformation by v-H-*ras* does not restore proliferation of a set of temperature-sensitive cell cycle mutants of rat 3Y1 fibroblasts. Cell Struct. Funct. 15: 211~219, 1990
9) YEW, P. R. & A. J. BERK: Inhibition of p53 transactivation required for transformation by adenovirus early 1B protein. Nature 357: 82~85, 1992