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Identification of genes involved in the regulation of 14-deoxy-11,12-didehydroandrographolide-induced toxicity in T-47D mammary cells

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ABSTRACT

14-Deoxy-11,12-didehydroandrographolide is one of the principle compounds of the medicinal plant, *Andrographis paniculata* Nees. This study explored the mechanisms of 14-deoxy-11,12-didehydroandrographolide-induced toxicity and non-apoptotic cell death in T-47D breast carcinoma cells. Gene expression analysis revealed that 14-deoxy-11,12-didehydroandrographolide exerted its cytotoxic effects by regulating genes that inhibit the cell cycle or promote cell cycle arrest. This compound regulated genes that are known to reduce/inhibit cell proliferation, induce growth arrest and suppress cell growth. The growth suppression activities of this compound were demonstrated by a downregulation of several genes normally found to be over-expressed in cancers. Microscopic analysis revealed positive monodansylcadaverine (MDC) staining at 8 h, indicating possible autophagosomes. TEM analysis revealed that the treated cells were highly vacuolated, thereby suggesting that 14-deoxy-11,12-didehydroandrographolide may cause autophagic morphology in these cells. This morphology may be correlated with the concurrent expression of genes known to affect lysosomal activity, ion transport, protein degradation and vesicle transport. Interestingly, some apoptotic-like bodies were found, and these bodies contained multiple large vacuoles, suggesting that this compound is capable of eliciting a combination of apoptotic and autophagic-like morphological characteristics.

1. Introduction

14-Deoxy-11,12-didehydroandrographolide is isolated from a medicinal plant known as *Andrographis paniculata* Nees (Acanthaceae) or "Hempedu Bumi" in Malaysia. This herb has an extreme bitter taste. Hence, it is also known as "King of Bitters" and is traditionally used to treat various ailments in Asia. It has been used to treat the following conditions: common cold, fever, cough, mouth ulcers, upper respiratory tract infections, sore throat, parasite infestations in the gastrointestinal tract and urinary infections (Pole, 2006; Tang and Eisenbrand, 1992). It is a predominant constituent of at least 26 Ayurvedic formulations used to treat liver disorders (Varma et al., 2009). Interestingly, it is also one of the herbs mentioned in Ayurvedic literature and is indicated for the treatment of neoplasms (Balachandran and Govindarajan, 2005).

An array of scientific evaluations performed on this plant concurs with its traditional usage. Andrographolide and related diterpenoids isolated from this plant have shown some degree of anti-pyretic, anti-malarial and anti-inflammatory activities (Barret, 2007; Jain et al., 2000). Andrographolide, the principle compound of this plant, has been reported to protect against alcohol and carbontetrachloride-induced hepatotoxicity (Choudhury et al., 1987; Choudhury and Poddar, 1984). Several bioactive constituents in this plant have also demonstrated anti-tumor properties, such as potent cell differentiation-inducing activity on myeloid leukemia cells (Matsuda et al., 1994), potent stimulation of the immune response (Puri et al., 1993), activation of the cytotoxic T lymphocyte responses and attenuation of *in vivo* tumor growth (Sheeja and Kuttan, 2007). These diterpenoids trigger cell cycle arrests in HepG2 hepatoma cells (Li et al., 2007a), Jurkat cells (Geethangili et al., 2008) and human leukemic HL-60 cells (Cheung et al., 2005).

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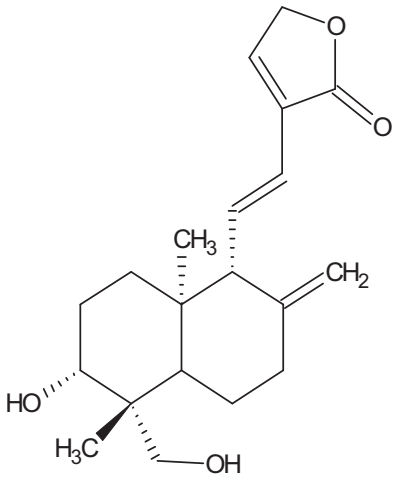
As one of the principle compound of *A. paniculata* Nees,14-deoxy-11,12-didehydroandrographolide is more cytotoxic against T-47D breast carcinoma cells compared to other diterpenoids such as andrographolide, andrographiside, neoandrographolide, deoxy-andrographiside and 14-deoxy-12-methoxyandrographolide (Tan et al., 2005). Interestingly, this diterpenoid also triggers a non-apoptotic type of cell death in T-47D breast cells in which the cell death is not preceded by DNA fragmentation or necrotic features. Although non-apoptotic cell deaths may suggest either autophagic cell death or programmed necrosis, the exact molecular mechanisms and characteristics of the non-apoptotic cell death remain to be determined.

This study further explored the mechanisms of the 14-deoxy-11,12-didehydroandrographolide-induced toxicity in breast carcinoma cells as previously described (Tan et al., 2005). The application of microarrays is considered to be a highly practical approach for revealing biological pathways and networks associated with toxicity induced by these herbal components, thereby elucidating potential mechanisms (Guo et al., 2010). Global gene expression was examined to characterize and identify transcriptional changes associated with the toxicity and cell death in a time series manner. The morphological characteristics of the breast cells treated with the compound were investigated.



14-deoxy-11,12-didehydroandrographolide

Fig. 1. The chemical structure of 14-deoxy-11,12-didehydroandrographolide. Source: Matsuda et al. (1994).

2. Materials and methods

2.1. Chemicals and reagents

The T-47D (human breast carcinoma) cell line was purchased from the American Type Culture Collection (ATCC, USA). RPMI 1640 and cell culture additives were obtained from either Hyclone (USA) or Sigma–Aldrich (USA). The RNeasy Mini Kit for total RNA isolation was obtained from Qiagen (USA). One-Cycle Target Labeling and Control Kit and Genechip Human Genome U133 Plus 2.0 arrays were obtained from Affymetrix (USA). Monodansylcadaverine (MDC) was obtained from Sigma–Aldrich (USA). 14-Deoxy-11,12-didehydroandrographolide was obtained as previously described (Tan et al., 2005). The chemical structure of 14-deoxy-11,12-didehydroandrographolide is shown in Fig. 1.

Table 1
Oligonucleotide primers and optimized annealing temperature used for quantitative RT-PCR analysis.

No	Gene symbol	Genbank accession no.	Nucleotide sequence (position)		Amplification size	Annealing temperature (^{0}C in 30 s)
			Forward primers	Reverse primers		
1	DDIT3/CHOP	NM_004083	5′-TACTGACTACCCTCTCACTA-3′	5′-TGTCTACTCCAAGCCTTC-3′	77	54.1
2	GADD45A	NM_001924	5′-CGGTGATGGCATCTGAAT-3′	5′-GGAGTAACTGCTTGAGTAACT-3′	86	54.1
3	RIT1	NM_006912	5′-CTTCTATTCACACTATGCTTCCT-3′	5′-TTGGCACTGATCTCATTAGC-3′	77	54.1
4	JMY	BF447037	5′-CACAAAGCTAGTAAATGGT-3′	5′-AAGGAGACTGACATGATT-3′	82	51.1
5	SNX16	NM_022133	5′-GCTTCTTCCATTGAGTATTC-3′	5′-CCAGTATAGTAGGTGTAGATG-3′	100	55.7
6	HERPUD1	AF217990	5′-CGAGATTGGTTGGATTGGA-3′	5-GGAGGAGTAGAAGTAGAGGAT-3′	75	55.7
7	RORC	NM_005060	5′-GAGGCTGGAGATAATTGT-3′	5′-ATGTCTGAGATGCTCTTG-3′	76	51.1
8	IFRD1	AA747426	5′-TTTGTTGATGCCAGTGAC-3′	5′-ACAAGAGTTCTGGGTACA-3′	109	55.7
9	SFPQ	NM_005066	5′-CATCTGGAGGCTAAGTATTG-3′	5′-CAGCGTGCTTCCTATATG-3′	112	54.1
10	FUS/TLS	NM_004960	5′-AGAGTTACAGTGGTTATAGC-3′	5′-GAGTTGACTGAGTTCCAT-3′	108	54.1
11	TSTA3	NM_003313	5′-ACGACCTACCCGATAGAT-3′	5′-TCTTGGCATACGAGTACC-3′	79	55.7
12	MYBL2	NM_002466	5′-CTCTGGCTCTTGACATTGT-3′	5′-TCGGCAAGGATAGAGACTT-3′	75	54.1
13	PSPC1	AI872384	5′-CTTCCAGATAACAGGTAA-3′	5′-GCGTCGTAGATATTAAAC-3′	93	51.1
14	HMGCS2	NM_005518	5′-TCCCGTCTAAAGGTGTTC-3′	5′-GGCTACTATGTCGATTCAA-3′	134	54.1
15	WT1	NM_024426	5′-TCTGACTCTCCACTCCTC-3′	5′-GGTATCTTGTCTTGGAAGTTG-3′	76	55.7
16	RHOD	NM_014578	5′-GAGCGGTACATGGTCAAC-3′	5′-TCATAGTCATCTTGCCCTG-3′	83	55.7
17	OPRS1	NM_147157	5′-TTACAGACAGGGACATAC-3′	5′-CACAGGTCTCAGTATCTATA-3′	129	55.7
18	CXCL12	NM_000609	5′-TTAGAGATTACCTCCTGAG-3′	5′-GTCCAATGAGATCCAATG-3′	117	54.1
19	IMMPIL	AA977197	5′-CAATCACAATGTCACCTCTT-3′	5′-GTGTTCTGGACCATCAATG-3′	107	55.7
20	SFXN2	AL530504	5′-TCACAGTTAGTGGACTTATCTT-3′	5′-ACCTATTCTTGGAATCTTCTCT-3′	92	55.7
21	OLFM1	NM_006334	5′-AGGCAGTTTAAGGGCTAA-3′	5′-TGGCTCTCTTTCATGGTTA-3′	91	54.1
22	MSTO1	NM_018116	5′-TGTCTTCATGCAGAGGAG-3′	5′-TTGGTCTGATGGCACATA-3′	117	55.7
23	CLPTM1	BC004865	5′-AGCACGAGCACTTTACAG-3′	5′-TCCAGTCGCCATACACAA-3′	78	54.1
24	ALDOA	NM_000034	5′-CACACGTCAACGATTCTA-3′	5′-CAGACTCCTCCTATAACA-3′	79	54.1
25	ß-ACTIN	NM_001101.3	5′-ATCACCATTGGCAATGAG-3′	5′-GATGGAGTTGAAGGTAGTT-3′	105	51.1–55.7