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Resveratrol inhibits proliferation, migration and invasion via Akt and ERK1/2 signaling pathways in renal cell carcinoma cells

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ABSTRACT

Recent studies have shown that resveratrol (RES) inhibits cancer cell growth, migration and invasion. Here, we evaluated RES in two human renal cell carcinoma (RCC) cell lines, ACHN and A498. We investigated the effects of RES on proliferation, cell morphology, colony formation, migration, and invasion. We used a proliferation assay to demonstrate that RES inhibited cell growth with IC_{50} values 132.9 ± 1.064 μM in ACHN, and 112.8 ± 1.191 μM in A498, respectively. Using inverted contrast microscopy, we showed that RES reduced cell-to-cell contact and inhibited formation of filopodia. A wound healing assay showed that RES inhibited migration of RCC cells. A Transwell assay showed that RES inhibited RCC migration and invasion. Western blot analysis showed that RES suppresses expression of N-cadherin, Vimentin, Snail, MMP-2, MMP-9, p-Akt and p-ERK1/2, but increased expression of E-cadherin and TIMP-1. In the presence of PD98059, the inhibitor of ERK1/2 pathway, we repeated all of the above experiments, showed that RES acted via the ERK1/2 pathway. Taken together, our results suggested that RES suppressed RCC cell proliferation, migration, and invasion in a concentration- and time-dependent manner. These effects likely resulted from inactivation of the Akt and ERK1/2 signaling pathways.

1. Introduction

Renal cell carcinoma (RCC) is the third most frequent urologic malignancy, after prostate and bladder cancer [1]. Surgery, radiation, and chemotherapy are the current preferred therapeutic approaches. For patients with advanced RCC who are not suitable for surgery, radiation and chemotherapy are the treatments of choice [2,3]. Nevertheless, clinical experience has shown that the sensitivity RCC to radiation and chemotherapy is extremely low [4]. In patients with RCC, approximately 30% have metastatic disease at the time of diagnosis, and another 20–30% develops metastasis following surgery [5]. Despite our understanding of primary cancer development and progression, metastasis remains the leading cause of cancer-associated death [6].

Epithelial-mesenchymal transition (EMT) is thought to represent the key process driving metastasis. It is characterized by loss of epithelial markers, increased expression of mesenchymal markers, and enhanced migratory and invasive behaviors [7,8]. E-cadherin, a marker of EMT, belongs to the classic cadherin subfamily. It is thought to maintain cell polarity and to strengthen intercellular adhesion. It also acts as a suppressor of cellular invasion. N-cadherin and vimentin, other characteristic mesenchymal markers of EMT, show increased expression in metastatic cancer [9]. The expression of E-cadherin, N-cadherin, and vimentin are induced by several transcriptional repressors, including Twist, Snail1, Snail2/Slug, E47, ZEB1/TCF8, and ZEB2/SIP1. These repressors bind to E-boxes in the E-cadherin promoter [10,11]. E-cadherin, N-cadherin, and vimentin expression are associated with ERK1/2 activation, mediating tumor cell migration and invasion [12–14].

Matrix metalloproteinases (MMPs) play important roles in tumor metastasis, by degrading extracellular matrix (ECM) components. MMPs allow cells to traverse the ECM to reach distant target sites. Expression and activation of MMPs is increased in virtually all human cancer cells [15,16]. In particular, MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) are involved in the invasive metastatic possess of tumor cells, including RCC. Extracellular signal-regulated protein kinase (ERK)1/2, one of the important members of the mitogen-activated protein kinase (MAPK) family, is an essential signaling pathway

Abbreviations: RES, resveratrol; RCC, renal cell carcinoma; EMT, epithelial-mesenchymal transition; MMP, matrix metalloproteinase; ECM, extracellular matrix; ERK, extracellular signal-regulated protein kinase; MAPK, mitogen-activated protein kinases; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; PD98059, the inhibitor of ERK1/2 pathway; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactate dehydrogenase; PMSF, phenylmethanesulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate- polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; TBST, Tris buffered saline tween

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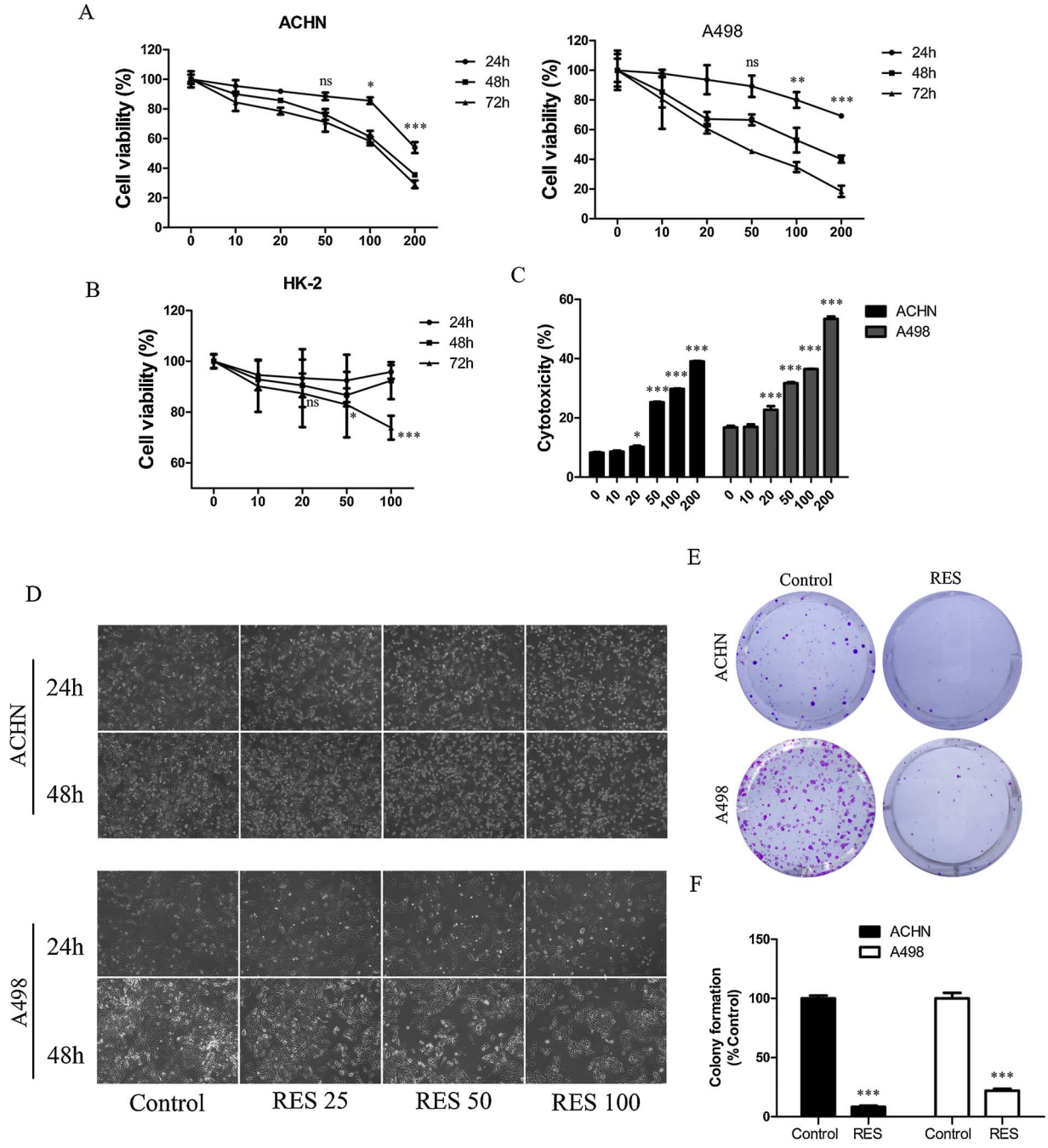

Fig. 1. The effects of RES on RCC cell proliferation, morphology and colony formation. RCC cell lines ACHN and A498 were exposed to various concentrations of RES (0, 10, 20, 50, 100 and 200 μM) for 24, 48 and 72 h. RCC (A), but not HK-2 (B), cell proliferation was suppressed in a concentration- and time- dependent manner by RES treatment, as assessed by MTT assay. (C) RES cytotoxicity was determined by an LDH assay. RES killed RCC cells in a concentration-dependent manner. ACHN and A498 cells were cultured in various concentrations (0, 25, 50, 100 μM) of RES for 24 and 48 h. (D) RES-induced morphologic alteration and number of cells decreased in RCC cells at 24 h and 48 h as visualized by microscopy (100×). (E and F) Cells were either untreated or treated with RES (100 μM) for 24 h. Colony formations were stained and counted. Cells treated with DMSO were used as a control group with cell viability set at 100%. Data are expressed as mean ± SD of three tests. *T*-test, ns (not significantly different), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the control group (DMSO-treated).

regulating cell survival, differentiation, apoptosis, proliferation, migration and invasion [17–19]. MMP expression is likely regulated by ERK1/2 in invasive carcinomas [20]. One study demonstrated that ERK1/2 phosphorylation decreased expression and activity of MMP-2 in glioblastoma cells [21].

Resveratrol (RES) is a natural polyphenolic antioxidant, found in a wide variety of fruits, including grapes, and raspberries. Numerous studies suggest that RES has chemopreventive therapeutic properties [22,23], and anticancer activities [24]. Several *in vitro* and *in vivo* studies have demonstrated growth-inhibitory effects of RES in several types of cancer, including leukemia, breast, colon, liver, lung, thyroid, and other epithelial cancers [25,26].

In the present study, we investigated the effects of RES *in vitro* on morphology, cell growth, migration, and invasion of RCC cell lines (ACHN and A498). We analyzed alterations in epithelial and mesenchymal markers. Finally, we measured the effects of RES on RCC cell growth, migration and invasion via the Akt and ERK1/2 signal pathways.