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Magnolol suppresses hypoxia-induced angiogenesis *via* inhibition of HIF-1α/VEGF signaling pathway in human bladder cancer cells

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ABSTRACT

The hypoxic environment in tumors is an important factor causing tumor angiogenesis by activating the key transcription factor, hypoxia-inducible factors-1α (HIF-1α). Magnolol isolated from *Magnolia officinalis* has been reported to exhibit an anticancer activity *via* elevation of apoptosis. However, whether magnolol inhibits tumor angiogenesis remains unknown. In the present study, we demonstrated that magnolol significantly inhibited angiogenesis *in vitro* and *in vivo* evidenced by the attenuation of hypoxia and vascular endothelial growth factor (VEGF)-induced tube formation of human umbilical vascular endothelial cells, vasculature generation in chicken chorioallantoic membrane and Matrigel plug. In hypoxic human bladder cancer cells (T24), treatment with magnolol inhibited hypoxia-stimulated H_2O_2 formation, HIF-1α induction including mRNA, protein expression, and transcriptional activity as well as VEGF secretion. Additionally, the enhanced degradation of HIF-1α protein *via* enhancing prolyl hydroxylase activity and the decreased newly-synthesized HIF-1α protein in hypoxic T24 cells may involve the reduction of HIF-1α protein accumulation by magnolol. Interestingly, magnolol also acts as a VEGFR2 antagonist, and subsequently attenuates the down-stream AKT/mTOR/p70S6K/4E-BP-1 kinase activation both in hypoxic T24 cells and tumor tissues. As expected, administration of magnolol greatly attenuated tumor growth, angiogenesis and the protein expression of HIF-1α, VEGF, CD31, a marker of endothelial cells, and carbonic anhydrase IX, an endogenous marker for hypoxia, in the T24 xenograft mouse model. Collectively, these findings strongly indicate that the anti-agngiogenic activity of magnolol is, at least in part, mediated by suppressing HIF-1α/VEGF-dependent pathways, and suggest that magnolol may be a potential drug for human bladder cancer therapy.

1. Introduction

Angiogenesis, the formation of new blood vessels, is essential for tumor progression by supplying sufficient oxygen and nutrients required for tumor growth and metastasis [1]. Rapid growth of tumor can quickly outstrip its vasculature, resulting in area of inadequate oxygen perfusion (hypoxia). The intratumoral hypoxia (0.05–5% O_2) often detected in advanced solid tumors is a leading cause of angiogenesis that is closely associated with tumor progression, resistance to radiation and chemotherapy as well as poor prognosis [2,3]. Although several transcription factors are implicated in the cellular responses to hypoxia, hypoxia-inducible factors-1 (HIF-1) has been regarded as the most important transcriptional factor promoting tumor angiogenesis by up-regulating pro-angiogenic genes such as vascular endothelial growth factor (VEGF) [4–6]. HIF-1 is a heterodimeric factor consisting of an inducible oxygen-sensitive alpha subunit (HIF-1α) and a constitutive oxygen-insensitive beta subunit (HIF-1β/ARNT). It is known that the expression of HIF-1β is not affected by changes of oxygen pressure. In contrast, the protein level of HIF-1α is tightly regulated by cellular oxygen concentration [7,8]. Under normoxic condition, the proline residues in oxygen dependent degradation domain (ODDD) of HIF-1α are hydroxylated by prolyl hydroxylase (PHD). Subsequently, the hydroxylated HIF-1α is recognized by the Von Hippel-lindau (VHL) tumor suppressor protein, leading to ubiquitination and degradation by ubiquitin–proteasome system, and thereby abolishing HIF-1α protein accumulation. However, under hypoxia, the prolyl hydroxylation of HIF-1α is impaired, which reduces VHL-ubiquitination and thereby enhancing HIF-1α protein stability [9]. Several studies have demonstrated that HIF-1α activation is critical in the tumorgenesis of bladder cancer, and there was a close correlation

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of HIF-1α level with bladder tumor grade, VEGF expression, microvessel density and proliferation index [10,11]. Blocking HIF-1α activation markedly suppressed the angiogenesis and progression in several human tumors [12,13]. Collectively, HIF-1α may be a promising target for cancer treatment.

Magnolol, a phenolic compound isolated from Chinese herbal plant *Magnolia officinalis* (Fig. 1A), has been reported to exhibit a variety of pharmacological activities, including anti-oxidant, anti-inflammatory, and anti-atherogenic activities [14–16]. Recent researches showed that magnolol also exerts an anticancer activity in human colon, liver cancer and ovarian cancer cells *via* induction of apoptosis or down-regulation of HER2 expression [17–19]. However, whether magnolol has an ability to inhibit the tumor angiogenesis in bladder cancer remains unknown. In the present study, we demonstrated for the first time that magnolol significantly inhibited hypoxia-stimulated HIF-1α expression, transcriptional activity, VEGF synthesis, and VEGF binding to VEGFR2 in human bladder cancer cell line (T24) as well as angiogenesis *in vitro* and *in vivo*. In addition, the underlying mechanisms involved may include the

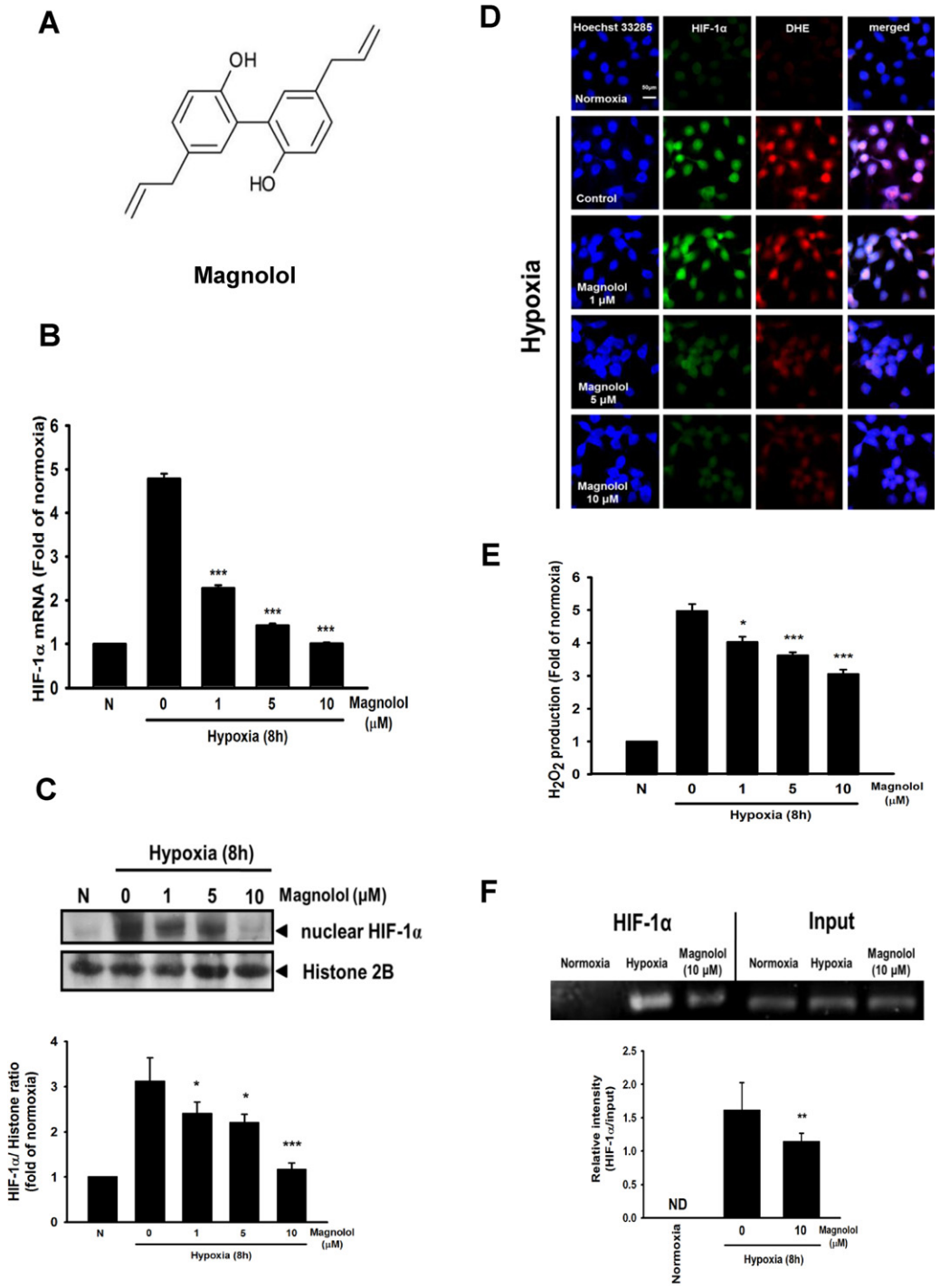

Fig. 1. Magnolol inhibited hypoxia-induced HIF-1α induction and transcriptional activity in T24 cells. (A) The chemical structure of magnolol. T24 cells were treated with solvent or different concentrations of magnolol for 8 h under normoxic or hypoxic condition. Then, the HIF-1α mRNA (B), nuclear HIF-1α protein level (C), HIF-1α nuclear translocation and superoxide production (D), H_2O_2 formation (E), and HIF-1α transcriptional activity (F) were determined as described in Section 2. Data were expressed as mean ± SEM ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* hypoxia-treated alone group. N: normoxia.