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ATF4 regulates CCL2 expression to promote endometrial cancer growth by controlling macrophage infiltration

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ABSTRACT

Activating transcription factor 4 (ATF4), an endoplasmic reticulum stress-inducible transcription factor, plays important roles in cancer progression and resistance to therapy. However, no report is available about its roles in endometrial cancer (EC). In this study, we found that ATF4 is commonly expressed in EC cell lines. Loss-of-function studies in two EC cell lines showed that ATF4 knockdown suppresses tumor growth of EC in vivo without influencing cell proliferation in vitro. And xenograft tumors derived from ATF4-knockdown cells had reduced M2 macrophage infiltration. In clinical specimens, ATF4-high expressing tumors indeed contained more macrophage infiltration compared to those with lower ATF4 expression. Moreover, we identified that ATF4-mediated chemokine CCL2 expression ultimately results in macrophage infiltration and tumor growth of EC. Taken together, our findings suggest that ATF4 contributes to tumor growth of EC by promoting CCL2 and subsequent recruitment of macrophage, and that ATF4/CCL2 axis might be a potential therapeutic target for EC.

1. Introduction

Endometrial cancer (EC) is one of the most common gynecological malignancies, and affects approximately 150,000 women each year estimated in Europe and the United States combined [1]. Although the prognosis of EC is favorable, the disease in a small proportion of EC patients is progressed due to metastasis, recurrence and resistance to chemotherapy [2,3]. Therefore, unraveling the crucial steps associated with tumor initiation and progression may represent an important achievement in the identification of new therapeutic targets for EC treatment.

The tumor microenvironment is characterized by transient fluctuations in oxygen, nutrients and pH [4,5]. Hypoxic microenvironment contributes to endoplasmic reticulum (ER) stress, which further results in the activation of the unfolded protein response (UPR) [5–8]. The UPR consists of three different parallel signaling branches that are triggered by different sensory ER transmembrane protein including protein kinase R (PKR)-like ER kinase (PERK), inositolrequiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6). Specifically, the PERK pathway is best known route of UPR [6]. PERK activation leads to phosphorylation of the eukaryotic translation initiator factor 2α (eIF2α), which subsequent inhibits global protein synthesis and thus preventing accumulation of misfolded proteins in the ER [9,10]. Several mRNAs can escape the translational inhibition by eIF2α and are part of the UPR such as the activating transcription factor 4 (ATF4) [11–13].

In cancers, ATF4 expression is elevated in tumor tissues compared with matched normal tissues [14,15]. Notably, ATF4 expression can be induced by the stresses including oxygen, glucose or amino acid deficiency in the tumor microenvironment [16,17]. As an adaptation to microenvironmental stress, ATF4 promotes expression of genes involved in redox balance, angiogenesis and autophagy [18]. Meanwhile, increased expression of ATF4 is associated with resistance to multiple current chemotherapeutic drugs, such as DNA damaging agents, non-steroidal anti-inflammatory drugs and proteasome inhibitors [17].

In most human cancers, macrophages compose the prominent component of stromal cells in the tumor microenvironment. Once infiltrated into tumor tissues, macrophages are acclimated to tumor associated macrophages (TAMs), which plays key roles in tumor growth and progression [19,20]. In this study, we for the first time identified the immunomodulatory function of ATF4 in EC. Genetic silencing of ATF4 in EC cells failed to influence cell proliferation but significantly suppressed tumor growth in vivo. Subsequently, we revealed that ATF4 promoted CCL2 production and recruited macrophages to infiltrate into tumor tissues, and finally facilitated tumor growth of EC.

2. Materials and methods

2.1. Cell culture and EC specimens

The human EC cells (AN3CA, ECC-1, KLE and Ishikawa) were all

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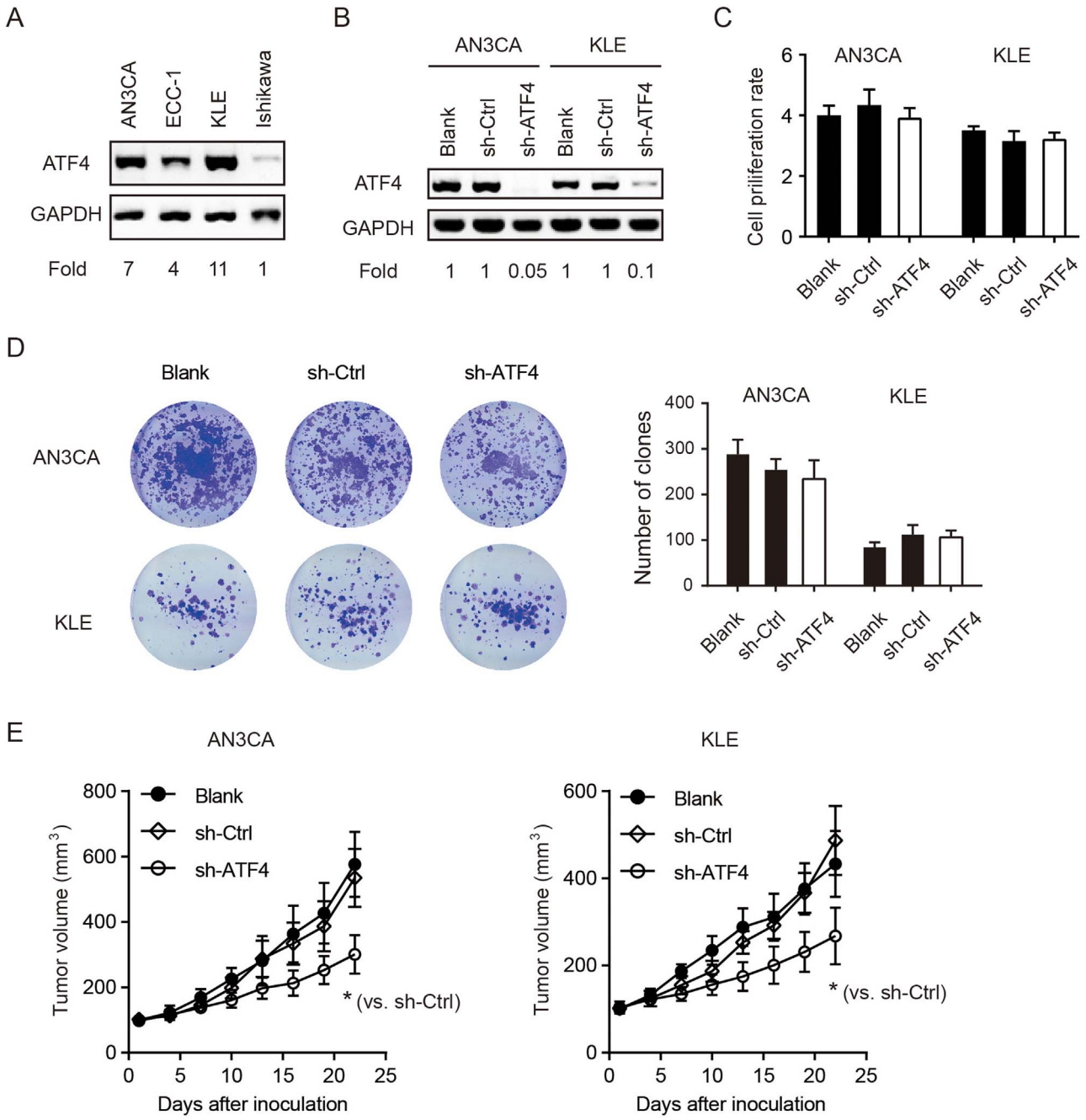

Fig. 1. Effects of ATF4 knockdown on growth of endometrial cancer (EC) in vitro and in vivo. (A) The protein expression of ATF4 in 4 EC cell lines was analyzed by western blotting. (B) The knockdown efficacy of ATF4 in AN3CA and KLE cells was determined by western blotting. (C) The effect of ATF4 knockdown on the proliferation rate of AN3CA and KLE cells. (D) The effect of ATF4 knockdown on the clone formation abilities of AN3CA and KLE cells. (E) Tumor growth curve was built based on tumor xenograft volume in the blank, sh-Ctrl or sh-ATF4 tumors from AN3CA and KLE cells. $^{*}P < 0.05$.

obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 0.5% penicillin and streptomycin. Cells were grown at 37 °C and 5% CO_2 in a humidified atmosphere. The human EC tissue microarray containing 97 cases of EC tissues was purchased from Superbiotek Inc (Shanghai, China).

2.2. Plasmid construction and transfection

The pSIH1-H1-Puro lentiviral short hairpin RNA vector (System Biosciences, Palo Alto, CA) was used to generate ATF4 stable knockdown EC cells. Three packaging vectors (pCMV-VSVG, pRRE, and pRSVREV) were purchased from Biosettia Company (San Diego, CA, USA). The constructed vectors were transfected into 293 T cells to produce viruses. Then cells seeded into 6-well plates at a density of 3×10^5 per well were incubated with the mixture of 1 ml complete medium and 1 ml lentivirus supernatant. Two days later, supernatants were replaced with complete medium, followed by selection with 2 μg/ml puromycin. For genetic silencing of CCL2, two small interfering RNAs (siRNAs) targeting CCL2 were synthesized by GenePharma (Shanghai, China). The Transfection Reagent Lipofectamine 2000 (Invitrogen, USA) was applied to transfect siRNAs into cells according to the manufacture's protocol.