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# The combination of Jun N-terminal kinase inhibitor and TNP-470 blocks carcinosarcoma-induced endothelial cell tube formation in a synergistic manner

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**Abstract**

We assessed the usefulness of Jun N-terminal kinase inhibitor (JNK-I) as an anti-angiogenic agent against a human uterine carcinosarcoma cell line (FU-MMT-1). JNK-I blocked FU-MMT-1-induced human arterial endothelial cell (HAEC) tube formation in an in vitro co-culture model. Cell proliferation of FU-MMT-1 or HAEC was inhibited by JNK-I. In addition, JNK-I blocked matrix metalloproteinase production but not vascular endothelial growth factor (VEGF) secretion in HAECs. Although low concentrations of JNK-I or TNP-470, an anti-cancer agent, did not separately block FU-MMT-1-induced tube formation, such tube formation was blocked by the combination of low concentrations of JNK-I and TNP-470 because TNP-470 blocked VEGF production, suggesting that JNK-I and TNP-470 had a synergistic effect and might be effective in patients with carcinosarcoma.


*Keywords:* Jun N-terminal kinase inhibitor; TNP-470; FU-MMT-1; Matrix metalloproteinases

## 1. Introduction

Jun N-terminal kinase (JNK) is a stress-activated protein kinase that can be induced by inflammatory cytokines, bacterial endotoxin, osmotic shock and hypoxia. JNK is a serine threonine protein kinase that phosphorylates c-Jun [1], a component of the transcription factor activator protein-1 (AP-1) [2]. AP-1 regulates the transcription of numerous genes including vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs). JNK inhibitor (JNK-I) blocked MMPs and bone destruction in an animal model of arthritis [3]. JNK-I also suppressed phorbol 13-myristate 12-acetate (PMA)-inducible MMP-9 expression [4]. JNK is a member of the mitogen-activated protein kinase family that includes the extracellular regulated kinases and p38 kinases. JNK activity is critical for both the immune

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response [5] and programmed cell death [6]. The therapeutic inhibition of JNK may be clinically beneficial in diseases as diverse as arthritis, inflammatory bowel disease, chronic pulmonary disease, stroke, ischemic injury and myocardial infarction. Since angiogenesis, the process of postnatal neovascularization, is a critical component of several human diseases, including ischemic heart disease, cancer, diabetic microvascular disease, rheumatoid arthritis and psoriasis [7], it is possible that JNK-I may also be clinically beneficial in these diseases, particularly cancer.

TNP-470, a class 1 inhibitor, is a synthetic derivative of fumagillin that inhibits methionine aminopeptidase-2 [8], cell proliferation and cell migration [9], thus blocking angiogenesis both in vitro and in vivo [10]. We previously reported [11] that TNP-470 directly inhibited the growth of FU-MMT-1, an established cell line derived from a primary carcinosarcoma of the human uterus [12], but not the growth of human artery endothelial cells (HAECs), accompanied by the inhibition of VEGF production, and subsequently induced anti-angiogenesis in HAECs.

Therefore, in an in vitro co-culture model of FU-MMT-1-stimulated HAEC tube formation on a matrix gel, we showed that the JNK-I-inhibited proliferation of FU-MMT-1 cells suppressed MMPs, and the combination of low concentrations of JNK-I and TNP-470 blocked FU-MMT-1-induced tube formation in a synergistic manner. This is the first demonstration that JNK-I is an anti-angiogenic agent.

## 2. Materials and methods

### 2.1. Materials

TNP-470 was a generous gift from Takeda Chemical Industries, Ltd. (Osaka, Japan) [13]. A specific inhibitor of JNK was purchased from Calbiochem (San Diego, CA).

### 2.2. Cell culture

The human uterine carcinoma line FU-MMT-1 [12] and FU-MMT-3 [14] used has been described previously. HAECs were purchased from Clonetics (San Diego, CA). HAECs were cultured in media supplemented with 5% FBS, P/S, and EC growth supplement (Takara Co., Osaka, Japan) at 37 °C under 5% $CO_2$.

### 2.3. Angiogenesis assay on Matrigel

An angiogenesis assay on Matrigel was performed as described previously [15,16]. Briefly, matrix gels (Chemicon International, Inc., Temecula, CA) were allowed to polymerize in plates. FU-MMT-1 and FU-MMT-3 cells and HAECs were seeded and grown in medium supplemented with 0.2% FBS and without EC growth supplement for 18 h in a humidified 37 °C, 5% $CO_2$ incubator. In some experiments, cells were cultured in the presence or absence of different kinds of reagents for 18 h. After the cells were washed, tube formation was observed using a light microscope, and pictures were captured with a computer system. We performed a 'pixel analysis' of the area of tube formation according to a procedure described previously [15,16].

### 2.4. Cell proliferation assay

Cells were plated on a 96-well plate and cultured under 5% serum conditions. After 48 h, the cells were cultured for 18 h in the presence or absence of JNK-I in EBM supplemented with 0.2% FBS and without endothelial cell growth supplement at 37 °C and 5% $CO_2$. After 18 h, the cells were stained with CellTiter 96 One Solution Reagent (a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] inner salt; MTS assay) (Promega) for 4 h at 37 °C and 5% $CO_2$, and absorbance at 490 nm was recorded with 96-well plate reader.

### 2.5. Enzyme immunoassay

Concentrations of VEGF in medium were determined as described previously [11] in duplicate by specific enzyme immunoassays (R&D Systems) according to the manufacturer's instructions.