Introduction

The vascular endothelial growth factor receptors (VEGFR1, VEGFR2, and VEGFR3) have been reported to play a crucial role in promoting tumor angiogenesis, which promotes tumor proliferation, metastasis, and invasion. 

Materials and methods

In vitro cell signaling inhibition: VEGF phosphorylation induced by VEGF-A, VEGF-P, was detected in H295R-VEGFR2 cell line (established in Hutchison) with DELFIA assay. M-CSF stimulated CSF1 receptor phosphorylation in Raw 264.7 cells (ATCC) was evaluated with Western blot.

HUV EC proliferation: The post-thrombotic HUVEC cell line (ATCC, CRL-1730) was inoculated into the Matrigel (BD) and cultured in DMEM containing 20% FBS. 

HUV EC tube formation: The basement membrane matrix (BD BioSciences, 354244) were added into 96-well plates and incubated for 30 minutes at 37°C to form gel. Primary HUV ECs were subsequently incubated in a 5% CO2, 37°C incubator for 18 hours. The result was recorded by photographing under a microscope with 4X/10X magnification.

Chick embryo choriomammalian cell (CAM) assay: Matured chicken eggs were incubated at 37°C with 5% humidity for 24 hours. The following day, a small window (1 x 1 cm) was made in the shell under aseptic conditions. The abdomen was opened with 10-12 physiological saline containing various concentrations of sulfatinib were placed on the top of the growing CAM. The eggs were incubated at 37°C for 24 hours. The result was recorded by photographing under a microscope with 4X/10X magnification.

In vivo tumor inhibition: After treatment with a single oral dose of sulfatinib, inhibition on VEGFR2 expression in lung tissues of nude mice was determined with Western blot after stimulated by VEGF-A injection. 

In vivo anti-tumor efficacy studies: Different human tumor lines, BGC823, HT29, H460 and Cali-1 cells were subcutaneously inoculated to the right flank of Balb/c nude mice. 

Summary

Sulfatinib could simultaneously block tumor angiogenesis and modulate cancer immunity, which might support sulfatinib as an attractive candidate for exploration of possible combinations with checkpoint inhibitors against various cancers.