Dasatinib-induced SRC inhibition blocks non-angiogenic vascularization and potentiates antibody-drug conjugates in high-grade breast cancer

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Abstract #927

Background Non-angiogenic vascularization (NAV) is a process in which cancer cells obtain blood supply without using neoangiogenesis. NAV is evident in high-grade breast cancer including ERBB2-positive breast cancer and triple-negative breast cancer. The aim of this study was to identify a small-molecule compound that suppress NAV including vascular mimicry (VM) and vessel co-option (VCO).

Methods Trastuzumab-resistant ERBB2-positive JIMT-1 and triple-negative MDA-MB-231 cell lines were used. Tube formation assay was employed to evaluate the ability of VM in vitro. For VCO, the ability of cancer cells to adhere on tubes derived from human umbilical vein endothelial cells was evaluated.

Results Screening of a small-molecule compound panel comprising 259 drugs using in vitro tube formation assay identified three FDA-approved drugs including dasatinib, bosutinib, and ponatinib, all of which are SRC kinase inhibitors, as potent inhibitors for tube formation. Dasatinib exhibited the most potent inhibition of tube formation (IC50 = 1.4 nM and 7.3 nM for JIMT-1 and MDA-MB-231, respectively) and vessel adherence. Western blotting of SRC kinase pathway and RNAi for SRC kinase confirmed that SRC kinase inhibition resulted in such effects. In mouse xenograft models, dasatinib significantly suppressed tumor growth. TUNEL staining of tumors revealed that dasatinib did not increase apoptotic tumor cells, indicating that dasatinib had minimal cytotoxic effect on breast cancer cells. However, in tumors of the dasatinib group, mouse tumor vessels were significantly decreased compared with control tumors. A NAV marker SerpinE2-positive tumor cells were also significantly decreased in the dasatinib group. Moreover, dasatinib significantly decreased CD31-positive mature vessels, while it did not affect immature CD105-positive vessels. Dasatinib increased the efficacy of trastuzumab-emtansine (T-DM1), an antibody-drug conjugate, on the growth of JIMT-1 tumors. While T-DM1 alone did not affect tumor vessels, T-DM1 in combination with dasatinib significantly suppressed the CD31-positive vessels.

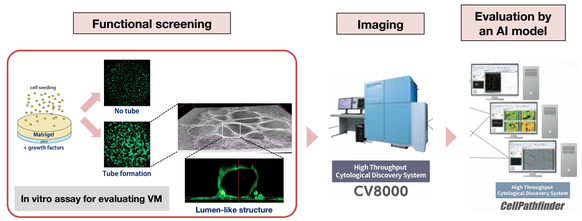
Conclusion Our results indicate that dasatinib suppresses VCO by inhibiting SRC kinase and eventually suppresses tumor growth. Combining dasatinib with ADC may enhance the efficacy of the ADC, which warrants further study.

Introduction

- Tumor growth requires a sustained blood supply.
- To that end, tumors can exploit not only sprouting angiogenesis, but also **non-angiogenic vascularization (NAV)**, mainly,
  - ✓ **vessel co-option (VCO)**, where tumor cells hijack and grow along pre-existing host vessels; and
  - ✓ **vascular mimicry (VM)**, where tumor cells form perfused, vessel-like channels without endothelial cells.
- NAV can circumvent antiangiogenic therapies (e.g., anti-VEGF), contributing to primary or adaptive resistance.
- NAV leads to tumor progression, metastasis, and relapse.
- Strategies that can directly disrupt NAV or resensitize tumors to antiangiogenic therapies remain limited.
- In this study, we aimed to identify compounds that inhibit NAV.

Methods

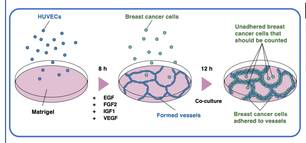
1. Establishing functional screening method for NAV inhibitors



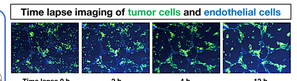
Tube formation assay

The extent of tube formation in test wells was scored from 0 to 9 through AI–based image analysis (Yokogawa Electric, Tokyo, Japan).

2. Establishing quantitative *in vitro* assay for VCO



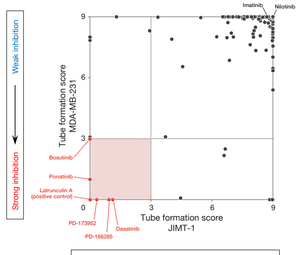
In vitro VCO assay



The assay would permit quantitative evaluation of tumor cell–endothelial cell interaction.

Results

1. Identification of tube-formation inhibitors



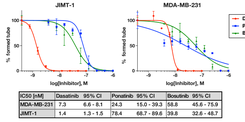
Using MDA-MB-231 and JIMT-1 cells, 259 small-molecule compounds were screened.

"Hits" were defined as tube-formation scores of 3 or less in both cell lines (light red area).

Five compounds met this criterion and were designated as tube-formation inhibitors.

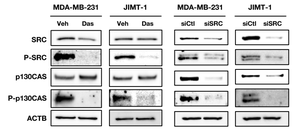
2. Determination of dasatinib's mechanism of action

IC50 of three oral SRC inhibitors

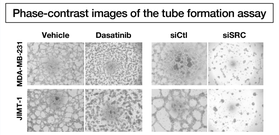


IC50 [nM]	Dasatinib	95% CI	Ponatinib	95% CI	Bosutinib	95% CI
MDA-MB-231	7.3	6.6 - 8.1	24.3	15.0 - 39.3	58.8	45.6 - 75.9
JIMT-1	1.4	1.3 - 1.5	78.4	68.7 - 89.6	39.8	32.6 - 48.7

WB for SRC and its target p130CAS



Phase-contrast images of the tube formation assay

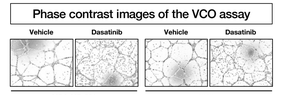


Dasatinib was found to be the most potent among three prescription SRC inhibitors.

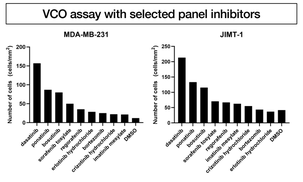
SRC knockdown and western blotting analysis revealed that the inhibitory effect of dasatinib depended on SRC inhibition.

3. Effect of dasatinib on tumor cell–endothelial cell adhesion

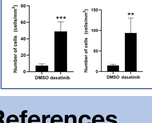
Phase contrast images of the VCO assay



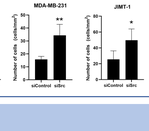
VCO assay with selected panel inhibitors



VCO assay with dasatinib

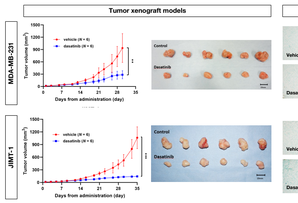


VCO assay following SRC knockdown



Dasatinib strongly inhibited tumor cell–endothelial cell adhesion, a crucial process in VCO.

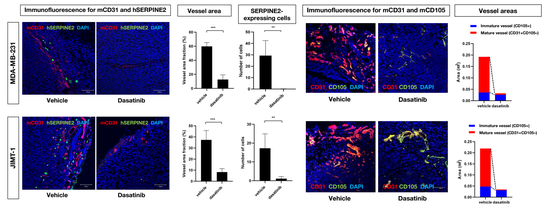
4. Effect of dasatinib on tumor growth in mouse xenograft models



Tumor cells were inoculated into the mammary fat pads of SCID mice, and dasatinib was administered orally twice daily.

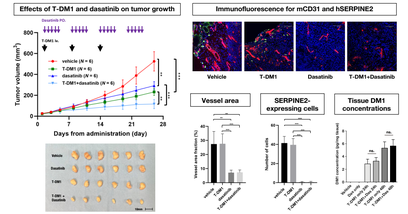
Dasatinib suppressed tumor growth without significantly inducing apoptosis.

5. Effect of dasatinib on tumor vasculature



Dasatinib significantly reduced the number of mature tumor vessels as well as tumor cells positive for the NAV marker, SERPINE2.

6. Effect of dasatinib + T-DM1 on trastuzumab-resistant tumors



Dasatinib sensitized trastuzumab-resistant tumors to T-DM1 through reduced tumor vasculature.

Despite the reduction in tumor vessels with dasatinib administration, the tissue payload concentration of DM1 was not diminished.

Conclusions

- SRC kinase constitutes an effective therapeutic target for inhibiting NAV in breast carcinomas.
- Inhibition of SRC kinase might also enhance the efficacy of antibody–drug conjugates in tumors resistant to the carrier antibodies.

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