Sclerostin plays a vital role in tumor progression and metastasis in triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer with limited treatment options. It is essential to continue advancing well-defined molecular targets in TNBC. Sclerostin (*SOST*) is mostly concerned as an osteocyte secreted protein and a drug target for osteoporosis. Recently, sclerostin was reported to be expressed in a majority of clinical TNBC tissues but not in normal breast tissue. Upregulated *SOST* expression was reported to be associated with worse prognosis in breast cancer patients. This study aimed to investigate the role of sclerostin in TNBC.

Methods: Cell proliferative ability was evaluated by colony formation assay. Cell migratory and invasive abilities were evaluated by transwell assay. The tumor growth and metastasis were evaluated in 4T1 orthotopic and metastatic mouse models. The cellular uptake of antibody and aptamer was evaluated by fluorescence microscopy.

Results: It was found that sclerostin knockout demonstrated inhibitory effects on proliferation and migration/invasion in both human (MDA-MB-231) and murine (4T1) TNBC cell lines *in vitro*. Surprisingly, no tumor growth and metastasis were found in 4T1-*sost* ^{KO} inoculated orthotopic and metastatic mouse models compared to their wildtype counterparts. It suggested the vital role of sclerostin in TNBC.

Anti-sclerostin antibody (Scl-Ab) could not be internalized by TNBC cells and demonstrated no effects on proliferation and migration/invasion *in vitro.* On the other hand, no difference in proliferation and migration/invasion was also observed between TNBC cells with and without supplementing sclerostin *in vitro*. Genetically, there was no difference in tumor growth between 4T1 inoculated WT mice and *SOST* $^{-/-}$ mice. Pharmacologically, no difference was also observed in tumor growth between Scl-Ab treatment and IgG control in 4T1 inoculated mice. It suggested that the role of extracellular and systemic sclerostin could be excluded in TNBC.

Aptamers are single-strand nucleic acids which bind to specific targets. It was found that sclerostin-specific aptamer (Scl-Apt) could be internalized by TNBC cells and demonstrated inhibitory effects on proliferation and migration *in vitro*. Consistently, Scl-Apt demonstrated inhibitory effects on tumor growth in 4T1 inoculated mice. It suggested that the role of intracellular sclerostin could not be excluded in TNBC.

Conclusions: Sclerostin plays a vital role in TNBC progression and metastasis. The role of intracellular sclerostin could not be excluded.

Legal entity responsible for the study: The authors.

Funding: This work was supported by Theme-based Research Scheme from the Research Grants Council of the

Hong Kong Special Administrative Region, China (Project No. T12-201/20-R, A.L.), HKSAR Government's Innovation and Technology Commission (UIM/298 and UIM/328). Hong Kong General Research Fund from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. 12102120, Y.Y.; Project No. 12102322, Y.Y.), Area of Excellence from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. AoE/M-401/20, Z.G.).

Disclosure: All authors have declared no conflicts of interest.