Reviewers report

Title: A small molecular agent YL529 inhibits VEGF-D-induced lymphangiogenesis and metastasis in preclinical tumor models in addition to its known antitumor activities

Version: 6 Date: 20 December 2014

Reviewer: Yasuyoshi Miyata

Reviewers report:

The authors investigated the anti-tumoral effect of a small molecular agent, YL529, in VEGF-D-induced lymphangiogenesis and lung cancer cells with VEGF-D over-expression. They used in vitro lymph node metastasis models and in vivo subcutaneous tumor models in mice. In addition, they paid attention to various molecules to understand its anti-tumoral mechanism. They found that YL529 can inhibit vascular endothelial growth factor (VEGF)-D-induced pro-lymphangiogenic activities including survival, proliferation, and tube-formation of lymphatic endothelial cells. Furthermore, YL-529 inhibited lymphangiogenesis and metastasis in vivo studies. They also found that YL529 can down-regulated p-VEGFR-3, p-JNK, and Bax while up-regulated Bcl-2. Finally, they concluded that YL529 might be an effective drug for inhibition of tumor progression and regulation of lymphangiogenesis and metastasis.

I appreciated that this manuscript interesting information to understand detailed pathological roles of YL529 in processes of metastasis including lymphangiogenesis. However, I have several questions and I would like to know your opinion.

(Major)

1. I agree with the opinion that VEGF-D is one of important regulators of lymphangiogenesis in various pathological conditions including cancers. However, in general, VEGF-C is most well-known as lymphangiogenesis-related molecule. So, I recommend adding and/or emphasis of the information on pathological significance and prognostic roles of VEGF-D in several cancers into “Introduction” section.

2. In migration assay, you showed that YL529 inhibited the migration of cells compared with that of untreated cells (Figure 2A). However, unfortunately, I cannot confirm your opinion in Figure 2A. Please show clearer and enlarged Figure of cell scratch assay.

3. In Figure 4D, Kaplan-Meier survival curves were shown. In this study, number of mice in each group was just 10. I have a senesce of discomfort with that such small data is showed by Kaplan-Meier survival curve. So, I recommend showing the relationship between YL529 and survival by the other method. I would like to know your opinion.
4. Please add the new photo with higher magnification (X400~600) or change the Figure 6 because I am afraid that your original photo is too small to understand your results.

5. In Figure 6A, I felt that pathological findings of cancer tissues was different between vehicle tissue and treated one. Please explain your opinion.

(Minor)
1. In Figure 7, I think that “metastasis” should be show on out of double lines (cell membrane).

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'