**Purpose**

- The Renin-Angiotensin System (RAS) has two opposing axes:
  - Classical RAS axis composed of ACE/Ang II/AT1R, promoting proliferation, migration, angiogenesis, vasocostriction and inflammation.
  - Protective axis composed of ACE2/Ang-1-7/MasR, promoting anti-proliferation, anti-angiogenesis, vasodilation and anti-inflammation effects.
- Imbalance between these two axes underlies angiogenesis, tumor growth and metastasis, etc.1,2
- Ang II have been associated with ovarian3, renal11, colorectal8, breast-cancer and other cancers.4
- Ang-1-7 is an antitumor agent, acts as a negative regulator of Ang II activity.6,8
- Therapeutic potential of Ang-1-7 is severally hampered by its short half-life and low bioavailability due to rapid degradation by peptidases.
- We developed a novel bone-targeting Ang-1-7 Conjugate (Ang ConJ), with 10-fold longer half-life which upon administration loads on bone and releases the active peptide in a sustained manner.
- The purpose of current work was to study in vitro effects of Ang ConJ on cell proliferation and gene expression of RAS components in different cancer cell lines. Due to promising results of in vitro study, this compound was tested in an animal model of syngeneic sarcoma as well.

**Objectives**

- To study the effects of Ang ConJ on cell proliferation and gene and protein expression of the RAS components in different cancer cell lines.
- To study the effect of Ang ConJ on progression of syngeneic sarcoma cancer using a mice model.

**Methods**

**In-vitro Study**

- Cell viability test was done on various cancer cell lines such as, FLYURI, MOJO, HSYSL, and SYHO to study anti-proliferative effects of Ang ConJ using the MTT assay across multiple concentrations and time points and compared with native Ang-1-7 as positive control and Ang II as negative control.
- The RAS components and apoptosis markers gene and protein expressions were studied using qPCR and Protein quantification using Western blotting (WB), respectively.

**In-vivo Study**

- Anti-cancer efficacy of Ang ConJ was tested on syngeneic sarcoma mice models. Tumor-bearing animals were divided into 3 groups and treated subcutaneously with vehicle (n=3), 400 µg/kg Ang-1-7 (n=4) and equivalent dose of Ang ConJ (n=4), twice daily for 3 weeks.
- Body weight and tumor size were measured every other day.
- qPCR and WB assay was performed on tumor tissues to quantify the mRNA and protein level, respectively.
- Statistical Analysis: Data was analyzed using an unpaired Student t-test or One-way ANOVA with Dunnet post hoc test, p < 0.05.

**Results**

- Effect of Ang ConJ and Ang-1-7 on cell viability of cancer cells
  - Fig. 1 Panel A presents the results of cell viability study and indicates that treatment with Ang ConJ or Ang-1-7 resulted in a concentration-dependent inhibition of cell proliferation compared with Ang II, specifically in HSYSL cell line. This effect was more pronounced in Ang ConJ treated cells.
  - Effect of Ang ConJ and Ang-1-7 on the RAS components gene expression in HSYSL cell line
    - Fig. 1 Panel B presents gene expression of the components of the RAS.
      - The expression levels of protective arm (ACE2 and MasR) genes were expressed more than 8-fold higher than control after Ang ConJ treatment, while they were down regulated 2- and 3-fold due to treatment with Ang-1-7 and Ang II, respectively.
      - The components of classical arm (ACE and AT1R) genes expressing were increased by 3- and 1.5-fold after Ang ConJ treatment, respectively, while ACE by 500- and 4-fold and AT1R 1- or 5-fold were under expressed after treatment with Ang-1-7 or Ang II, respectively.
    - Effect of Ang ConJ and Ang-1-7 treatment on tumor size change in syngeneic sarcoma bearing mice
    - Fig. 2 presents the weight reduction in control mice bearing tumor treated with vehicle. Ang ConJ and Ang-1-7 did not change the percent body weight. On the other hand, tumor size increased in control group and decreased in both Ang 1-7 and Ang ConJ treated groups.
    - Effect of Ang ConJ and Ang-1-7 on the RAS gene expression in tumor tissue from syngeneic sarcoma-bearing mice
    - Fig. 3 presents the significant increase in ACE2 gene expression after Ang 1-7 and Ang ConJ treatments. Ang ConJ increased MasR expression but it was not significantly different from other groups. There was a trend on AT1R gene expression reduction due to treatment as well.
    - Effect of Ang ConJ and Ang-1-7 on the RAS and apoptosis protein expression in tumor tissue from syngeneic sarcoma-bearing mice
    - Fig. 4 presents the significant effect of Ang ConJ and Ang-1-7 on increasing ACE2 and Caspase-3 and decreasing of AT1R protein expression.

**Conclusion**

- This study provides preliminary evidence that:
  - Ang ConJ, effectively reduce proliferation of some cancer cell lines. Considering the lack of target (bone) for binding of bone-seeking moiety of Ang ConJ, the enhanced observed efficacy compared with native Ang 1-7 can be attributed to its improved stability and sustained release of native peptide.
  - Ang-1-7 presents its beneficial effects such as anti-proliferative activity through binding to the MasR. In this study the Ang ConJ ability in increasing the gene expression of ACE2 enzyme, which is responsible for conversion of Ang II to Ang-1-7, and also increasing the MasR expression, can explain the observed anti-proliferative effects.
  - Ang ConJ has better stability and efficacy than native peptide on suppressing the cancer progression in vivo which is presented by significantly reducing the tumor size. This effect could be attributed to switching the balance between two arms of the RAS in favor of protective arm which results in anti-proliferation, anti-angiogenesis, vasodilation and anti-inflammation effects.
  - This switch in balance was done by increasing ACE2 and decreasing AT1R gene and protein expression due to Ang ConJ treatment. Additionally overexpression of Caspase-3 protein as a marker of apoptosis is in concert with reduction of tumor size.
  - The observed milder effects of Ang 1-7 in comparison with Ang ConJ could be attributed to difference in half-life and stability of these compounds. The improved stability through conjugation of Ang-1-7 resulted in a profound anti-proliferation and anticancer actions of Ang ConJ.

**References**


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**Figures**

- Fig. 1: Effects of different concentrations of Ang-1-7 and Ang ConJ on cell proliferation.
  - Panel A: Effect of Ang ConJ and Ang-1-7 on the in vitro proliferation levels of the RAS components in a representative cancer cell line HSYSL.

- Fig. 2: Effect of Ang ConJ and Ang-1-7 on cell viability of cancer cells

- Fig. 3: Effect of Ang ConJ and Ang-1-7 on cell viability of cancer cells

- Fig. 4: Effect of vehicle, Ang ConJ, and Ang-1-7 on the in vitro and apoptosis gene expressions in HSYSL human sarcoma cells. Data are presented as mean ± standard error. *p < 0.05 compared with control.

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**In-vitro and in-vivo study of novel bone-targeting angiotensin (1-7) conjugate effects on cell survival, mRNA, and protein expression of different components of renin-angiotensin system**

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