S9-3

# Anti-Angiogenic Cancer Gene Therapy with Apolipoprotein (a) Kringle Genes

Kyuhyun Lee, Sung-Tae Yun, and Eui-Cheol Jo*

*Biomolecular Engineering Division, MOGAM Biotechnology Research Institute*

**Background**

Apolipoprotein (a) (apo(a)) is a glycoprotein composed of multiple kringle domains and a protease-like domain homologous to plasminogen. Earlier studies suggested a possible role for apo(a) in bridging atherogenesis and angiogenesis *in vivo*, by showing an anti-angiogenic activity of apo(a). We previously demonstrated the anti-angiogenic activities of apo (a) kringles LK68 and LK8 (LKs) *in vitro* and *in vivo*, and further elucidated a potential anti-metastatic, anti-tumor activity of these molecules.1-5 As anti-angiogenic cancer therapy requires chronic administration of relatively high concentrations of proteins, gene therapy approach constitute an ideal solution for therapeutic purpose.

**Methods**

We generated a series of recombinant AAV carrying genes encoding LKs (rAAV-LKs) according to the standard vector construction procedures. *In vitro* anti-angiogenic activity of LKs was examined by the HUVEC proliferation assay and the wound migration assay. We investigated anti-metastatic property of rAAV-LKs in two experimental animal models of tumor metastasis. We then explored the use of rAAV-LK as a potential treatment for established subcutaneous tumor xenograft models derived from human HCC and colon cancer cells.

**Results**

The LK-containing supernatant inhibited proliferation of HUVECs, which supported the unique activity of LKs as anti-angiogenic molecules *in vitro*. Intramuscular administration of rAAV-LKs substantially reduced the number of tumor nodules in both the pulmonary and the hepatic metastasis models in the immuno-deficient mice, and also gave substantial suppression of tumor growth in mice bearing subcutaneously transplanted human HCC and colon tumor cells, respectively. The median survival of rAAV-LK treated mice bearing HCC tumors was substantially higher than from control groups. The tumors relapsed in the survivor mice. H&E-stained sections from various organs of the survivors denoted

that these mice were rescued from tumor.

**Conclusions**

A single administration of rAAV-LK gave rise to persistent expression of LKs, inhibited tumor angiogenesis, triggered tumor apoptosis, and substantially suppressed tumor mestastasis and growth in several experimental animal models. These results collectively suggested that rAAV-LKs hold potential for a gene therapeutic agent that may bring up long-term anti-angiogenic anti-tumor activity in clinical settings.

**Keywords**: AAV, cancer gene therapy, apoptosis, LK, anti-angiogenic, metastasis, hepatocellular carcinoma, colon cancer

## References

1. Kim JS, et al. J Biol Chem 2003;278:29000–29008.
2. Yu HK, et al. Cancer Res 2004;64:7092-7098.
3. Yu HK, et al. J Gene Med 2004;7:39-49.
4. Kim JS, et al. Biochem Biophys Res Commun 2004;313:534–540.
5. Ahn JH, et al. J Biol Chem 2004;279:21808–21814.