Effect of rG3 Protein/PGA Ionic Conjugate on Breast Cancer Tumors

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Statement of Purpose: The use of microparticulate polyglycolic acid (PGA or A-6) as an anion-exchanger to modulate the release profile of basic antimicrobials was first reported by Shalaby in 2002.[1] Since then, such anion-exchanger, denoted A-6, was used to form solid ionic conjugates with a number of basic bioactive agents, including antibiotics, oligopeptides, proteins, and antineoplastic drugs in pharmaceutical formulations for treating periodontitis, inhibiting tumor growth, or tumor immunotherapy.[1-6] Meanwhile, the basic recombinant G3 protein (rG3) was prepared by Scott and coworkers and was shown to interrupt the propagation of breast cancer cell lines.[7] Recent findings reflecting the potential use of rG3 for suppressing the growth of breast cancer tumors and the established use of A-6 to form ionic conjugates with basic bioactive agents to modulate their release profile provided a strong incentive to pursue the study, subject of this report, on the effect of rG3 protein/A-6 ionic conjugate on breast cancer tumors.

Methods: The G3 domain of the rat Laminin-5 rG3 α3 chain was generated through polymerase chain reaction (PCR) amplification from the plasmid pHB9 containing a length of the G3 domain previously shown through nucleotide alignments with the α3 chain of Laminin-5. ArcticExpress™ Competent Cells (Stratagene, La Jolla, CA) were transformed according to manufacturer instructions and soluble rG3 expression was confirmed by SDS-PAGE. Following cell lysis and removal of insoluble protein, the soluble protein fraction was purified using a G50 Sephadex column for separation by size exclusion. The rG3 containing fractions were stored at -20 ºC overnight, and then freeze-dried to remove the ammonium acetate buffer. The fractions were then re-suspended in PBS and stored at -20 ºC for future use.

For the nude mice study, 2 million MDA-MB-231 human breast cancer cells were injected into the left mammary pad of each mouse. Twenty-four hours later mice were divided into treatment groups of 8. The treatments were A-6 conjugated with rG3 and A-6 without conjugate. Controls were mice that had no treatment. Treatments were delivered by 100 µL injection on Week 4 and 6 of the study. Tumor length and width was recorded weekly, and tumor volumes were calculated.

Results: Tumor volume increased in all treatment groups through Week 6 of the study (Figure 1), but by Week 7 the rG3 treated mice began to exhibit reduction in tumor volume from what was observed at Week 6. Tumor growth was reduced in the rG3 group from Week 6 to 7, which followed the final treatment injection.

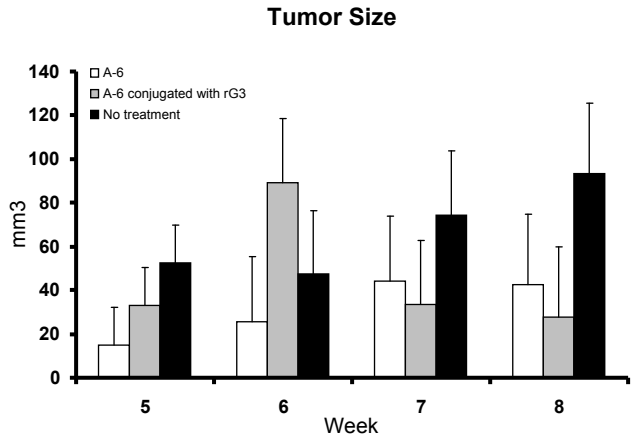

Figure 1: Size (volume) of tumors at various times post-injection of 2 x 10^6 MDA-MB-231 human breast cancer cells. [Volume = (width^2 x length)/2].

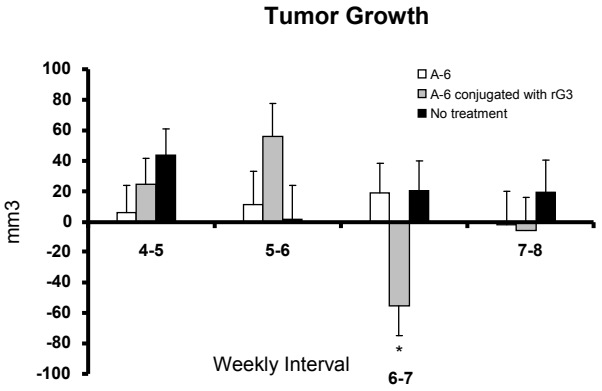

Figure 2: Growth of tumors at various times post-injection of 2 x 10^6 MDA-MB-231 human breast cancer cells. (Growth = current week volume—previous week volume, *Indicates $P \leq 0.05$).

Conclusions: Available results demonstrate the effectiveness of the rG3/A-6 ionic conjugate in suppressing the breast cancer tumor growth.

References:

[1]Shalaby,S.W., U.S. Patent No. 6,413,539 (2002).
[2]Shalaby, W.S.W. et al., *Trans. Soc. Biomater.*, 29, 142 (2006).
[3]Corbett, J.T. et al., *Trans. Soc. Biomater.*, 26, 71 (2002)
[4]Salz, U. et al., *Trans. Soc. Biomater.*, 24, 294 (2001).
[5]Shalaby, W.S.W. et al., *Trans. Soc. Biomater.*, 25, 71 (2002)
[6]Shalaby, W.S.W. et al., *Trans. Soc. Biomater.*, *J. Control Rel.*, 91, 209 (2003).
[8]Scott, T.R., Internal Report, Department of Animal & Veterinary Science, Clemson University (2008).