Biomaterial-mediated cancer-specific DNA delivery to liver cell cultures using synthetic poly(beta-amino esters)

Stephany Y. Tzeng,[1] Luke J. Higgins,[2] Martin G. Pomper,[2] Jordan J. Green[1]
[1]Dept. of Biomedical Engineering, [2]Dept. of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD

Statement of Purpose: Liver cancer is a leading cause of cancer death. Most patients are treated by injection of emboli, providing a convenient avenue for local treatment by novel therapies such as gene therapy. Hepatoma cells (MCA-RH7777) and hepatocytes (BRL-3A) were transfected with eGFP or luciferase DNA using synthetic poly(β-amino esters) (PBAEs). PBAEs caused higher transfection in hepatoma cells and lower toxicity in hepatocytes than optimized formulations of commercial reagents. Top PBAEs also transfected a greater percentage of hepatoma cells than hepatocytes, as well as causing several hundred-fold higher expression intensity with eGFP and luciferase DNA, respectively. When transfected in co-culture with eGFP DNA, very high cancer selectivity was observed.

Methods: An array of PBAEs was synthesized as previously reported[1] and mixed with eGFP DNA at pH 5 to form nanoparticles. Particles were used to transfect MCA-RH7777s in a high-throughput screen. Transfection was measured by flow cytometry at 48 hr (% cells positive, intensity). Viability was measured by cell count at 24 hr. Top polymers were purified and used for further transfections on MCA-RH7777s and BRL-3As with eGFP and luciferase DNA. For co-culture studies, MCA-RH7777s were labeled with H2B-Cherry using the Piggybac transposon system and sorted by FACS after 4 wk. Labeled MCA-RH7777s and unlabeled BRL-3As were seeded together and transfected with eGFP DNA. Flow cytometry was used to determine transfection efficacy in each of the two cell types.

Results: The top polymer (457e, 1.1:1) transfected 98±0.4% of MCA-RH7777s hepatoma cells and 68±4% of BRL-3As hepatocytes when cultured separately. The highest transfection efficacy seen in BRL-3As was 77±1% (537e, 1.05:1, 75 w/w), while 10 of the 21 PBAE formulations tested transfected >90% of the MCA-RH7777s. Polymers causing >10% toxicity to BRL-3As were excluded from further study. While lipid-based commercial reagents were effective for MCA-RH7777 transfection, they were also cytotoxic to BRL-3As. At the dose required for >90% transfection of MCA-RH7777s, <20% BRL-3As remained alive. Most striking was the difference in expression intensity. The geometric mean fluorescence of MCA-RH7777s as measured by flow cytometry was significantly higher than the fluorescence of BRL-3As in many of the PBAE formulations tested. In top conditions, at least 10-fold higher eGFP expression was seen in hepatoma cells, with some conditions showing up to 330±96-fold higher expression. Luminescence from cells transfected with luciferase DNA showed this even more strongly (at least 200-fold higher luminescence in MCA-RH7777s compared with BRL-3As). Polymer 537e, 1.05:1, had the highest expression and the most cell-type specificity (up to 470±25-fold).

In co-cultures, in addition to the H2B-Cherry dye, labeled MCA-RH7777s could be distinguished from BRL-3As by the multicellular structures formed. Even though both cell types formed relatively even monolayers when grown separately, the hepatoma cells formed dense clusters when in co-culture with hepatocytes, while hepatocytes remained in monolayer. Fluorescence microscopy showed that green eGFP signal overlapped almost exclusively with the clusters of red H2B-Cherry signal (Figure 1), indicating that transfection occurred largely in hepatoma cells. Very few BRL-3As were transfected compared to MCA-RH7777s, and the expression intensity in those was very low. Flow cytometry quantitatively confirmed these results and showed that specificity was even higher in the co-culture system, likely because the hepatoma cells so readily took up nanoparticles and were transfected, leaving fewer available particles per hepatocyte.

Conclusions: PBAEs are highly effective for DNA delivery to hepatoma cells, with nearly 100% transfection of liver cancer cells with low cytotoxicity and low rates of transfection in hepatocytes. The co-culture experiments more closely mimic the *in vivo* situation and show the highest efficacy and specificity. PBAEs are a promising potential tool for targeted therapy of liver cancer.

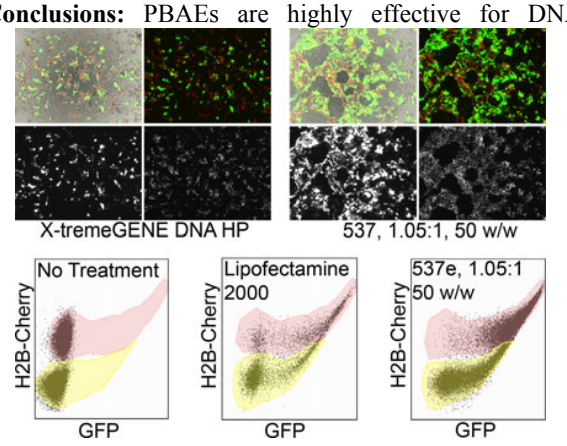

Figure 1. Hepatoma (red) and hepatocyte (no label) co-cultures transfected with GFP (green). In each set, top left: merge; top right: GFP+H2B-cherry; bottom left: GFP; bottom right: H2B-cherry. Flow cytometry and imaging showed toxicity by commercial reagents and high hepatoma transfection by PBAEs.

Acknowledgements: This work was supported in part by the NIH (R21CA152473). SYT thanks the National Science Foundation for fellowship support. We thank Karl Wahlin for assistance with Piggybac transposon transfections.

References: 1. Tzeng SY, Guerrero-Cazares H, Martinez EE, Sunshine JC, Quinones-Hinojosa A, Green JJ. *Biomaterials* 32 (2011) 5402-10.