Synthesis of Layer-by-Layer Nanoparticles for Targeting of Epithelial Cancers

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Background
- Gonadoblastoma are a typically benign cancer that affects the gonads (ovaries or testicles)
- Commonly develops in people with mixed gonadal dysgenesis
- More common in phenotypic females (80%) than males
- Around 10% become malignant and metastatic
- Immunotherapeutic techniques can be applied for treatment of gonadoblastoma cancers
- Tumor microenvironments can be targeted using nanoparticles with altered surface chemistries
- Charged polymers can be added to NPs using a layer-by-layer technique to increase tumor targeting and decrease intrinsic medicine toxicity
- Affinity for cancer cell surface of interest for delivery of cytokines are other immunostimulatory molecules

Experimental Procedure
- Synthesis of liposome nanoparticles (LNP) with alternating charged polymer layers added using a layer-by-layer (LbL) method
- Tetramethyl rhodamine (TMR) added as a fluorescent tag to the NP liposome core
- All LNPs made with a positive poly-L-arginine (PLR) inner layer
- LNPs sized using extrusion with a 150 nm, 100 nm, and finally 50 nm filter paper after addition of each layer
- Five outer layers tested: unlayered (UL), hyaluronic acid (HA), poly(acrylic) acid (PAA), poly-L-aspartic acid (PLD), and poly-L-glutamic acid (PLE).

DLS & Plate Reader Assay
- DLS data collected after each layer addition to measure particle size and charge
- HA displayed above average size and PDI. Charge and size between PAA, PLD, and PLE are similar
- ELOG-1 cells analyzed at three dilutions using a plate reader fluorescence assay

Flow Cytometry Results
- ELOG-1 flow cytometry (FC) performed to test intensity and confirm plate reader assay data
- Only the 100 μL LbL-NP dosage used
- FC performed with DAPI to test for living cells
- FC data analyzed using gates for singlets, forward vs. side scatter, DAPI, and positive and negative TMR fluorescence readings

Conclusions
- Successful synthesis of five TMR-NPs for use in targeting of gonadoblastoma ELOG-1 cancer cell line
- Increased affinity to ELOG-1 cells with PLD and PLE outer-layered LbL-NPs
- Similar percent positive signal normalized to unlabeled signal from FC data
- Similar MFI between all five TMR-NPs, but highest in PLD- and PLE-TMR-NPs

Path Forward
- Synthesis of NPs with drug loadings such as IL-2, IL-12, or IL-15
- Testing of affinity of the TMR-NPs to other cells involved in immune response
- Testing of these NPs on different gonadoblastoma cancer cell lines
- In vitro testing of LbL-NPs using mice models with ELOG-1 cancer cells

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