PAX8-directed nanotherapeutics for high-grade serous ovarian cancer

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OBJECTIVE

High-grade serous ovarian cancer (HGSOC) is the most common epithelial ovarian cancer subtype. Targeted therapy for HGSOC is challenging because the disease is characterized by copy number variation rather than recurrent somatic mutations. HGSOC displays well-conserved epigenetic features such as expression of transcription factor PAX8. We propose employing epigenetic therapies that capitalize on interactions between PAX8 and specific DNA-binding sites to develop potential therapeutic approaches to target HGSOC. Our hypothesis is to use a biomimetic approach wherein PAX8 is induced to transcribe an ectopically transfected CRISPR-base editor (BE)-guide RNA (gRNA) plasmid containing a PAX8 binding site in its promoter. The gRNA will impart a dominant-negative allele of Polo Like Kinase 1 (PLK1) or Ataxia telangiectasia and Rad3-related (ATR) which has been shown to be synergistically lethal with deficient tp53 in cancer cells. In the presence of PAX8, the plasmid will be transcribed, while in the absence of PAX8, the plasmid is degraded, with minimal toxicity observed in healthy cells.

METHODS

Polyplexes were synthesized by combining the plasmid of interest with a biocompatible polyamine polymer, PPLG-g-azidopropylamine (PPLG). PAX8-expressing ovarian cancer cell lines including COV362, Kuramochi, OVCAR8 and OVSAHO or PAX8-negative control cell lines including JEG3, MCF-7, HeLa and HEK293T were transfected with polyplexes bearing the plasmid of interest. Polyplex diameter, polydispersity indices and surface zeta potentials were measured on a Malvern Zetasizer. Polyplexes were imaged for uptake and functionality using fluorescence microscopy and Amnis ImageStream. Polyplex toxicity was assessed *in vitro* using cell viability assays. Preliminary *in vivo* experiments to demonstrate luciferase plasmid-bearing polyplexes have been performed in NOD-SCID mice. To monitor *in vivo* transfection efficiencies, we will deliver fluorescently labeled plasmids or luciferase-encoding plasmids to test bio-distribution and pharmacokinetics.

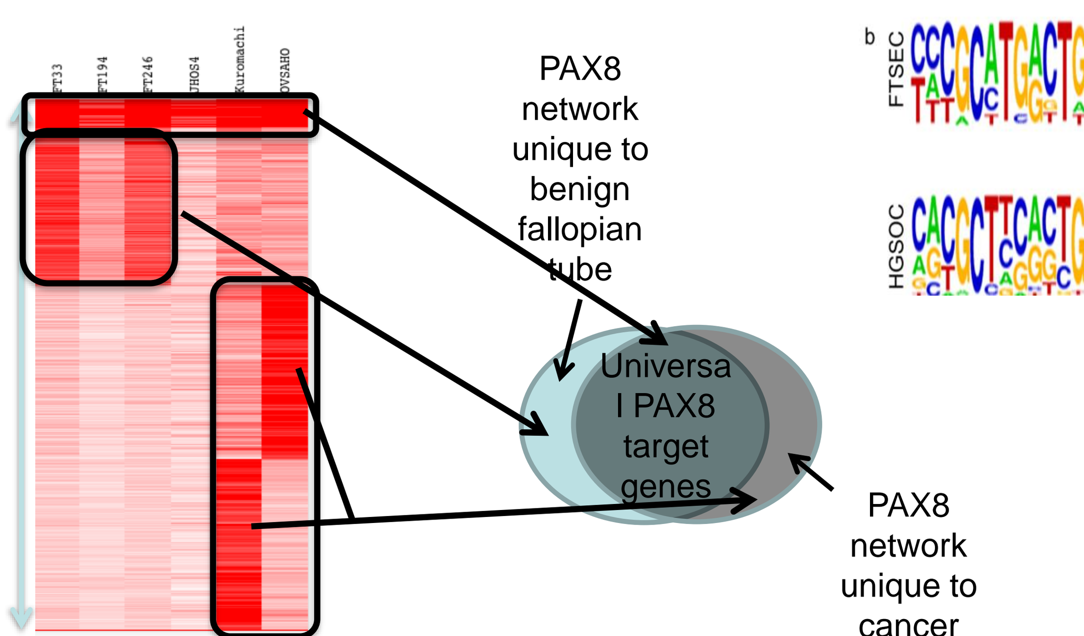


Figure 1. Heatmap of genome wide PAX8 binding sites determined by ChIP-seq. 3 types of PAX8 targets were identified – targets common between benign and malignant states, targets unique to benign FT and those unique to HGSOC. This suggests that the differential access of PAX8 to chromatin is a fundamental part of ovarian cancer pathogenesis.

RESULTS

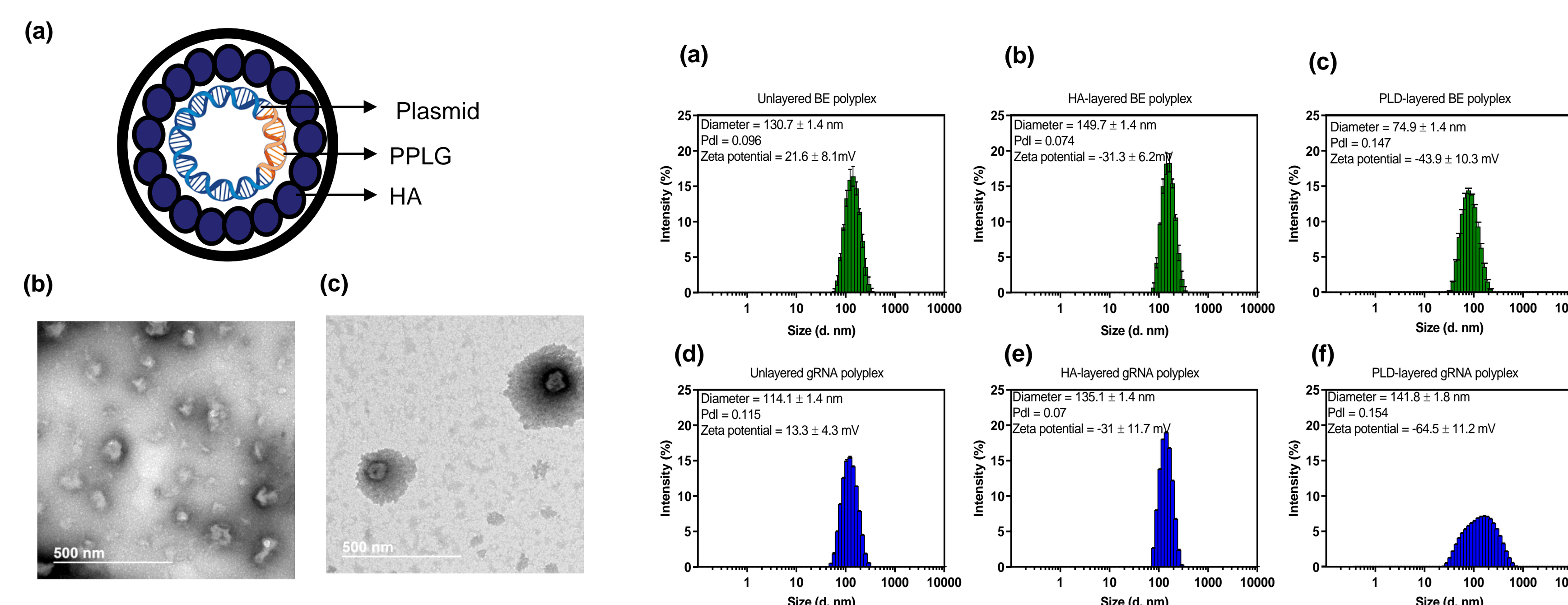


Figure 2. (a) PAX8-activated plasmid that encodes for Cas9, with or without the gRNA in the same plasmid complexed with PPLG can be layered with hyaluronic acid (HA) to form polyplexes. Representative CryoEM images of **(b)** unlayered polyplex and **(c)** layered polyplex.

Figure 3. Comparison of average diameter, zeta potential (surface charge) and polydispersity index (PdI) of polyplexes before and after layering with HA. **(a)** Unlayered base editor (BE) polyplex; **(b)** HA-Layered BE polyplex; **(c)** PLD Layered BE polyplex; **(d)** Unlayered -/- PLK1 gRNA polyplex; **(e)** HA-Layered gRNA polyplex; **(f)** PLD-Layered gRNA polyplex

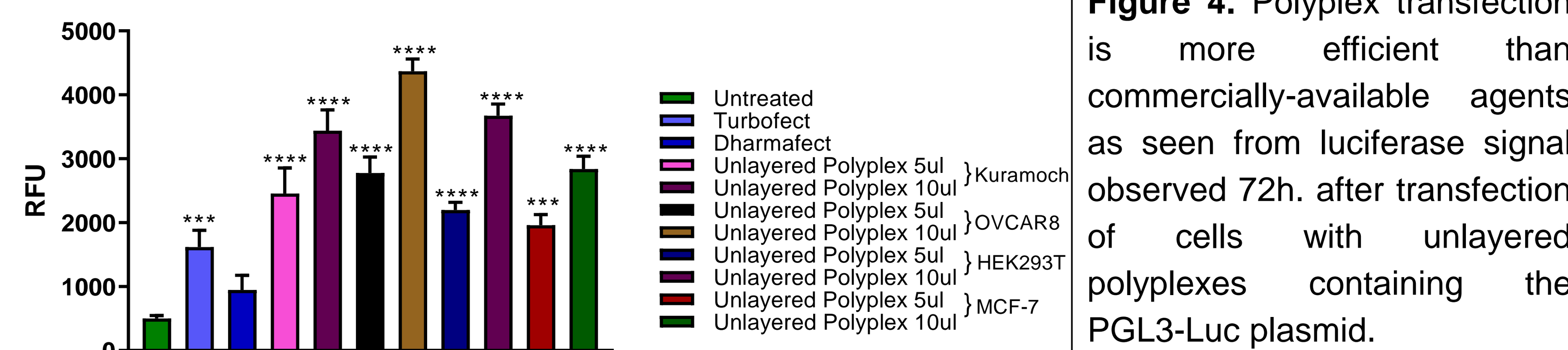


Figure 4. Polyplex transfection is more efficient than commercially-available agents as seen from luciferase signal observed 72h. after transfection of cells with unlayered polyplexes containing the PGL3-Luc plasmid.

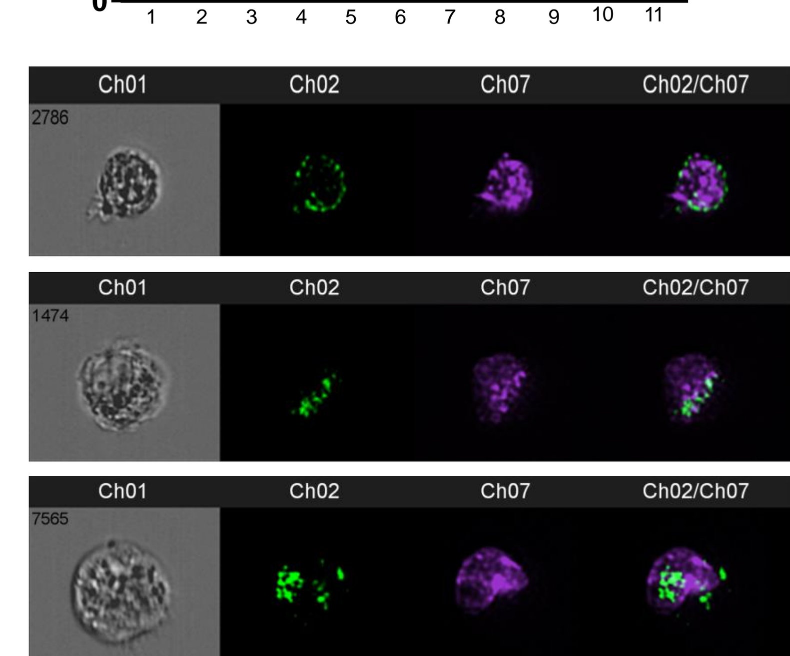


Figure 5. Representative cell panels showing accumulation of polyplexes in OVCAR8 cells 72h. after transfection with unlayered BE (containing fusion GFP as seen in Ch02) polyplexes synthesized using Cy-7 labeled polymer (Ch12) as measured using an ImageStream imaging flow cytometer. Panel 1: BF; Ch 02: GFP; Ch07: DAPI;

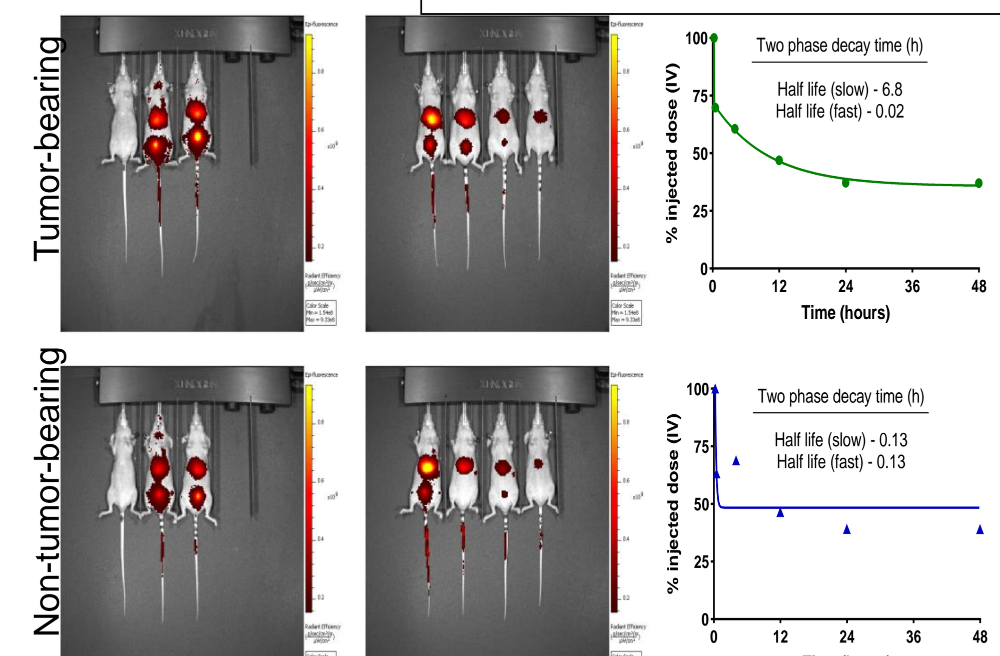
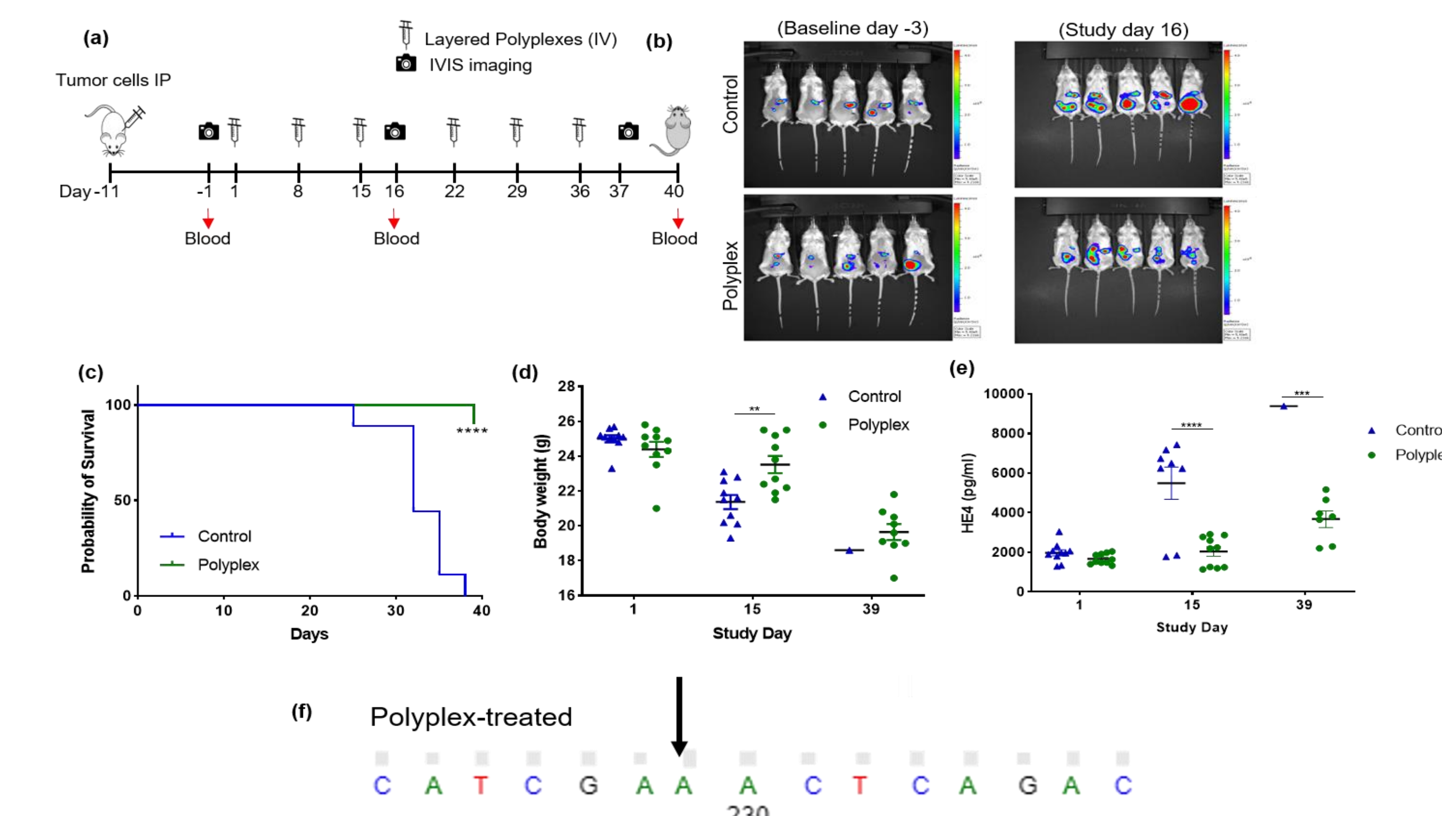


Figure 6. Polyplex biodistribution *in vivo* and calculation of half-lives. Cy7-labeled layered polyplex particles were dosed intravenously at specific time points (15 minutes, 30 minutes, 4 hours, 12 hours, 24 hours, 48 hours) into nude mice that were OVCAR8-tumor bearing or non-tumor bearing. Time-dependent systemic clearance and accumulation in the liver, kidney, and spleen area or the bladder were observed.

RESULTS



(f) Polyplex-treated

C A T C G A A A C T C A G A C

230

Figure 7. *In vivo* delivery and efficacy of polyplexes. NOD-SCID mice were inoculated with HGSOC cells. After 6 weekly IV injection of polyplexes, the mice receiving polyplex particle treatment lived significantly longer and had visible reduction in tumor burdens (b-c) as observed during necropsies compared to the no treatment tumor bearing mice (d). This was further substantiated by polyplex-treated mice losing body weights at a reduced pace (d) and increased HE4 (tumor marker) levels in control mice compared to treatment and observed gene edit (f).

ONGOING WORK & CONCLUSIONS

Ongoing work: Ongoing work includes polyplex injection *in vivo* to determine therapeutic efficiency, repetitive polyplex injections *in vitro* and *in vivo,* quantifying percentage of total sequencing reads with target base pair conversions after treatment, determination of plasmid loading efficiency, and testing ATR gRNAs against other targets for efficacy in ovarian cancer targeting.

Conclusions: Well-defined lineage transcription factors have the potential to serve as novel targets for gene editing therapies in ovarian cancer and other malignancies. Combining nanotechnology and gene editing can improve specificity toward ovarian cancer cells as well as incorporate precision therapy aimed at the underlying genetic architecture of this disease.

REFERENCES

Elias, K. M. *et al.* (2016) 'Epigenetic remodeling regulates transcriptional changes between ovarian cancer and benign precursors', *JCI Insight*, 1(13), pp. 33–43. doi: 10.1172/jci.insight.87988.

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Patent Pending

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