The Development of Nanoparticles for Targeted Head and Neck Cancer Detection with Molecular Imaging

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the 5th most common cancer worldwide. In the United States, there are approximately 40,000 new cases and 11,000 deaths annually from cancers generated in the oral cavity, pharynx and larynx. In the United States, head and neck squamous cell carcinoma, most common followed by tumors arising in the oral cavity, larynx and hypopharynx, the overall survival rate has not improved in the past 30 years. Patients presenting with locally advanced stage III and IV tumors remain a significant therapeutic challenge in HNSCC. About 40–60% of patients with locoregional recurrence has a 20–30% 5-year survival rate in patients with HNSCC. Therefore, early detection of local spreading and distance metastasis tumors and early management may significantly impact the treatment outcome and survival for patients with HNSCC.

Nanoparticles (NPs), such as liposomes, are particularly useful as imaging probes because of their unique chemical properties and proven safety profiles. The surface of NPs can be easily functionalized with targeting moieties, giving them a clear advantage over traditional molecular probes. Furthermore, NPs can be designed to avoid the mononuclear phagocyte system, something that is crucial for in vivo imaging. These details make NPs an interesting alternative in the detection of HNSCC; however, the application of NPs in targeted head and neck cancer with molecular imaging (MI) requires both the ability of the NP to target the site of interest and the ability of the tumor to be imaged. This unique strategy and sensitivity of these NPs in vitro using MI is the unique tumor-specific NP-based imaging probe system could be applied for non-invasive in vivo detection of HNSCC.

METHODOLOGY

Transformation: EGFR expression plasmids were transformed into Rosetta cells according to the Rosetta protocol. (Gifted from Tsoukas lab, Penn Engineering, University of Pennsylvania, Philadelphia, PA)

Protein expression: A plasmid of the expression vector was used to obtain 20 μL of LB-Amp in ten 25-ml-scale flasks and grown overnight at 37°C with 250 rpm shaking. Each 25-ml culture is then added to shaker flasks containing 250 mL LB culture media and grown to an OD 600 of 8.1 at 37°C with 250 rpm shaking. Culture is collected with 30% ammonium hydroxide (NH4OH) and cultured at 4°C for 30 min. The pellets are resuspended in a 1x PBS buffer (Sigma Madin Darby, 300mM NaCl) supplemented with 10μg/mL of lysozyme and 1x EDTA-free protease inhibitor tablet (Roche). The pellets are then resuspended with 1x PBS buffer and incubated for 30 min at RT, followed by overnight incubation at 4°C.

Purification and conjugation: Cells are then harvested at 1°C. 150 mM NaCl is added and incubated for 30 min at RT. Lysate is then centrifuged for 2 cycles of 10 min each, at 16,000 x g. Supernatant is decanted in between. Lysate is centrifuged at 16,000 x g for 30 min and the supernatant is dialyzed with 1 mL to 5x formaldehyde. The sample is then resuspended in a 1x PBS buffer (50mM NaCl, 300mM pEG) and further centrifuged at 10,000 x g for 60 min. The pellet is then resuspended in a 1x PBS buffer. The resulting fluorescent EGRF–Allobody conjugate was collected and concentrated using membrane technology.

Liposome nanoparticles formation: The HNSCC nanoparticles (HNSCCNPs) were mixed with HSPC and Rhodamine-DSEF. Once the lipids are thoroughly mixed in the organic solvent, moisture was evaporated using a nitrogen dryer to yield a lipid film. The lipid film was thoroughly dried to remove residual organic solvent by placing the vial in a vacuum pump overnight. PBS then added for hydration and kept at 25°C for 2 min sonication in water, 5°C color changes. Lipid eutonum done per protocol (in which a lipid suspension is forced through a polycarbonate filter with a defined pore size to yield particles having a similar diameter). Supernatant is then centrifuged using Amicon column resuspended with 0.2 μM HEPES buffer (pH 8.5-9.2). A 10% AEG was added to NH4-PEG and stirred at room temperature overnight. Supernatant was filtered with 100 μm Amicon columns to remove unbound AEGD while replacing HEPES with PBS. Finally, liposome nanoparticles were clicked to the fluorescent EGRF–Allobody (with) and kept overnight on thermometer at room temperature.

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RESULTS

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REFERENCES


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