Cytotoxic Effect of Epidermal Growth Factor-Gold Nanoparticle Conjugates

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Abstract:

Cancer cells stemming from an overexpression of the epidermal growth factor receptor (EGFR) surface protein expression often develop resistance to conventional EGFR inhibitors. Epidermal Growth Factor-Gold Nanoparticle (EGF-GNP) conjugates offer a potential alternative, demonstrating significant cytotoxicity against drug-resistant cells at different diameters (60 nm, 80 nm, and 200 nm) when compared to media. While the exact mechanism remains unclear, optimizing the nanoparticle's effectiveness could provide patients with a valuable additional cancer-fighting option.

Summary of Research:

Background and Motivation. Epidermal growth factor receptor (EGFR) is a vital cell surface protein involved in signaling pathways within human cells, impacting functions like cell proliferation, inflammation, and extracellular matrix regulation [1]. Dysregulation of EGFR signaling contributes to excessive cell growth, cancer progression through processes like angiogenesis and metastasis, and resistance to apoptosis [2]. EGFR inhibitors are developed to block its activation and hinder tumor cell proliferation, but they come with notable side effects, including folliculitis, xerosis, pruritus, and alopecia [3]. Resistance often develops, especially after epithelial-mesenchymal transition or EGFR mutations occur, posing challenges in treating EGFR-driven cancers. To address this, researchers have explored conjugating Epidermal Growth Factor (EGF) to gold nanoparticles (GNP) to reduce cancer cell viability, with previous studies showing cytotoxicity at 15 and 50 nm [5,6]. This study aims to investigate how the size of Epidermal Growth Factor-Gold Nanoparticle (EGF-GNP) conjugates affects their cytotoxicity against cancer cells.

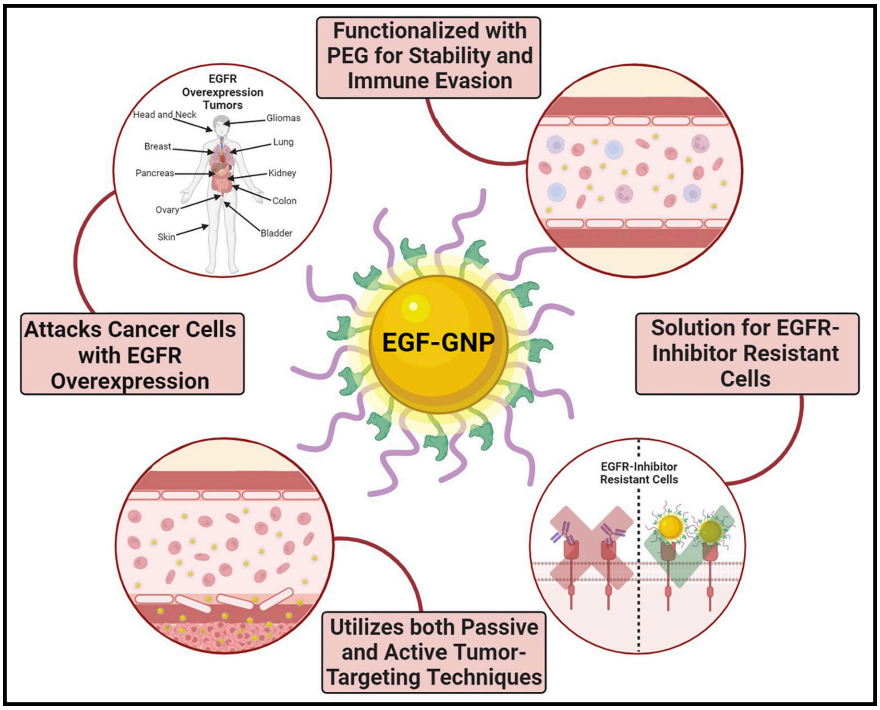

Figure 1: A graphic highlighting the advantages of EGF-GNP.

Materials and Methods. Epidermal growth factor-nanoparticle conjugates were prepared as per established methods [7]. Growth analysis between functionalization was done to confirm successful conjugation. Initially, GNPs were functionalized with PEG-DSU and HS-PEG-COOH, introducing PEG for stability and immune evasion, thereby forming PEG-GNP, as shown in Figure 2 [8]. Subsequently, EDC/NHS was used to activate HS-PEG-COOH's carboxyl group, enabling EGF binding and creating EGF-GNP, as shown in Figure 2. DLS measurements at each functionalization step showed a significant size increase, validating successful conjugation of EGF. Following functionalization, both EGF-GNP and PEG-GNP of various hydrodynamic diameters

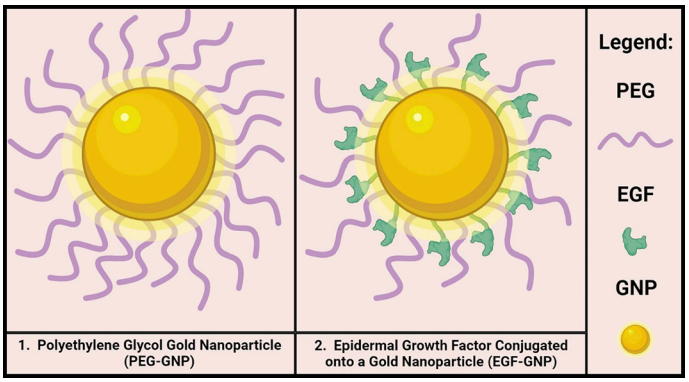

Figure 2: A graphic showcasing the functionalization of PEG and conjugation of EGF onto the GNP.

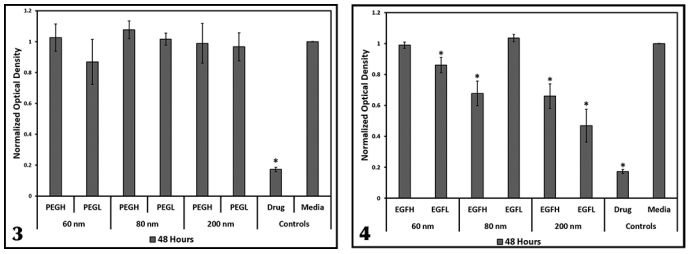

Figure 3, left: Normalized optical density of all PEG-GNP samples after 48 hours. The asterisk above drug represents a significant difference against the media. Figure 4, right: Normalized optical density of all EGF-GNP samples after 48 hours. The asterisk above certain conditions represents significant difference against the media.

(60 nm, 80 nm, and 200 nm) were introduced to cancer cell cultures at high and low concentrations, with the former having ten times the GNPs. After 48 hours of incubation, Cell Counting Kit-8 was used to quantify live cancer cells, and a microplate reader measured optical density differences for each condition. Due to the GNP's ability to absorb and scatter light efficiently, controls were set in place to subtract the effect of the GNP on the optical density.

Results and Discussion. To explore the cytotoxicity related to EGF-GNPs' size, we compared them to PEG-GNPs of the same diameter and media. The optical density in each sample was normalized against the media to emphasize the impact of each condition. Figure 3 indicates that none of the listed conditions significantly differed from the media, which aligns with expectations since PEG's presence shouldn't induce cytotoxicity. However, some EGF-GNP conditions exhibited statistically significant decreases in cell viability, as shown in Figure 4. Incubation with 60-EGFL, 80-EGFH, 200-EGFH, and 200-EGFL resulted in notable reductions in optical density and, consequently, cell viability. Even though 80-EGFL's optical density appears similar to the media, it's likely that this is an outlier and replicating this experiment would reveal a reduction in measured optical density.

These findings align with current research regarding the impact of EGF on cell viability and support the claim that EGF is solely responsible for the cytotoxic effects.

Conclusions and Future Steps:

The novelty of this project arises from the significant enhancement in cytotoxic efficiency while increasing GNP diameter. Although the exact cytotoxic mechanism remains unknown, it is believed that the increase in surface area from GNPs with larger diameters allows for greater PEG and EGF functionalization, increasing the potential for EGF-GNP binding to EGFR and subsequent cytotoxic effects. Future research can focus on enhancing EGF-GNP by modifying PEG molecular weight, adjusting EGF or PEG surface densities, exploring more diameters, or varying incubation times. Ultimately, gaining insights into the underlying cytotoxic mechanisms and using this knowledge to optimize this technology would offer a safer alternative for treating cancers driven by EGFR overexpression, benefiting patients.

References:

[1] Rayego-Mateos, S., et al. Role of Epidermal Growth Factor Receptor (EGFR) and Its Ligands in Kidney Inflammation and Damage. Mediators Inflamm 2018, 8739473 (2018).

[2] Sasaki, T., et al. The Role of Epidermal Growth Factor Receptor in Cancer Metastasis and Microenvironment. BioMed Research International 2013, e546318 (2013).

[3] Hirsh, V. Managing treatment-related adverse events associated with EGFR tyrosine kinase inhibitors in advanced non-small-cell lung cancer. Curr Oncol 18, 126-138 (2011).

[4] Weng, C.-H. et al. Epithelial-mesenchymal transition (EMT) beyond EGFR mutations per se is a common mechanism for acquired resistance to EGFR TKI. Oncogene 38, 455-468 (2019).

[5] Zhang A, Nakanishi J. Improved anti-cancer effect of epidermal growth factor-gold nanoparticle conjugates by protein orientation through site-specific mutagenesis. Sci Technol Adv Mater. 2021 Sep 6;22(1):616-626. doi: 10.1080/14686996.2021.1944783. PMID: 34512175; PMCID: PMC8425683.

[6] Yamamoto S, Nakanishi J. Epidermal Growth Factor-gold Nanoparticle Conjugates-induced Cellular Responses: Effect of Interfacial Parameters between Cell and Nanoparticle. Anal Sci. 2021 May 10;37(5):741-745. doi: 10.2116/analsci.20SCP16. Epub 2020 Dec 25. PMID: 33390415.

[7] Wu L, Xu F, Reinhard BM. Nanoconjugation prolongs endosomal signaling of the epidermal growth factor receptor and enhances apoptosis. Nanoscale. 2016 Jul 14;8(28):13755-68. doi: 10.1039/c6nr02974d. PMID: 27378391; PMCID: PMC5081566.

[8] Li, M., et al. Brush Conformation of Polyethylene Glycol Determines the Stealth Effect of Nanocarriers in the Low Protein Adsorption Regime. Nano Lett. 2021 Feb 24;21(4):1591-1598. doi: 10.1021/acs.nanolett.0c03756. Epub 2021 Feb 9. PMID: 33560851; PMCID: PMC8023711.