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The effect of a possible glioblastoma treatment on somatic cells

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Introduction

Glioblastoma is a deadly intracranial cancer with less than 10% of patients surviving over 3 years

Hypothesis

Glioblastoma multiforme (GBM) has specific cellular receptors, Fn-14. Superparamagnetic iron oxide nanoparticles (SPIONs) can be coated and bioconjugated with a polymer (polyethylene glycol) that attaches to the TWEAK protein through streptavidin, and TWEAK will bind to the Fn-14 receptors. The SPION can then be taken into the cell via endocytosis and exposure to a magnetic field induced by a solenoid will cause the SPION to rotate and give off heat, disrupting the cell and causing apoptosis, ultimately killing the cancerous cells.

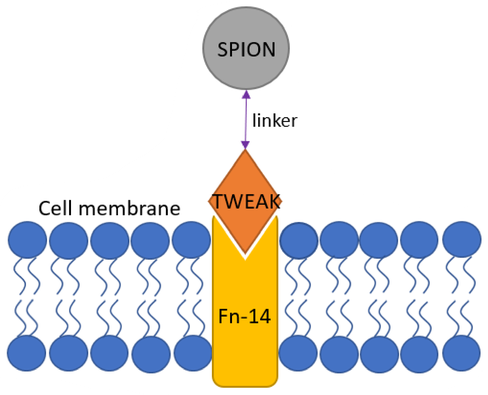

Figure 1. A depiction of the binding of the SPION-TWEAK to the Fn-14 GBM receptor

Previous Experimentation

- Superparamagnetic iron oxide nanoparticles (SPIONs) synthesis
  - Iron oxide is non-toxic in the body in small quantities and the body can naturally excrete it
  - Size is directly related to synthesis time
    - Experiments showed 20 nm has the largest temperature gradient and greatest magnetic properties

- Nanoparticle – Biopolymer Conjugation
  - Structure
    - Confirmed by fluorescence quenching and infrared spectra

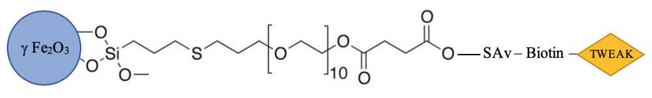

Figure 2. The chemical structure of the nanoparticle-polymer complex.

- Cellular Effect
  - Transmission Electron Microscopy (TEM)

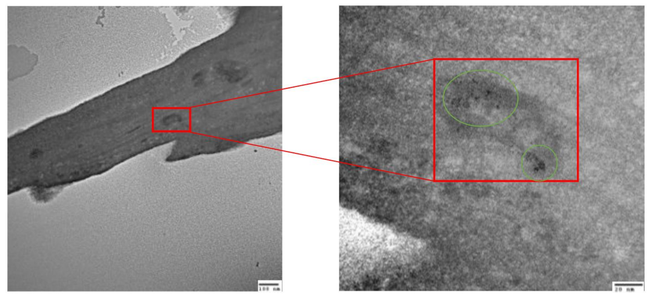

Figure 3. Evidence of SPION accumulation in the mitochondria of GBM.

- Immunofluorescence Assay (IFA)
  - IFA post-SPION-TWEAK incubation and magnetic field exposure demonstrated that GBM undergoes apoptosis

Experimental

Experiments were performed to further ensure that the SPION-TWEAK complex did not induce apoptosis in non-cancerous cells

- Incubation of SPIONs with human embryonic vein endothelial cells (HUVEC) and exposure to magnetic field
  - Followed by immunofluorescence assay (IFA) to visualize presence or lack of cell death

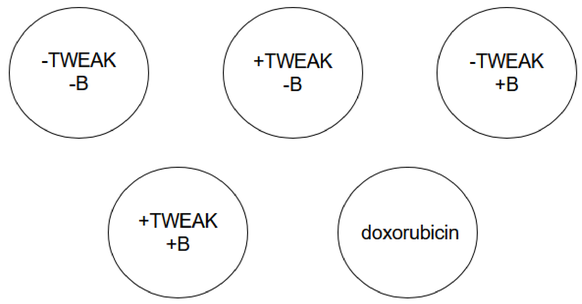

Figure 4. Apoptotic studies via IFA. TWEAK represents the nanoparticle-polymer complex, B represents the magnetic field (- = no field present; + = field present), and doxorubicin acts as the positive control as it induces apoptosis in cells.

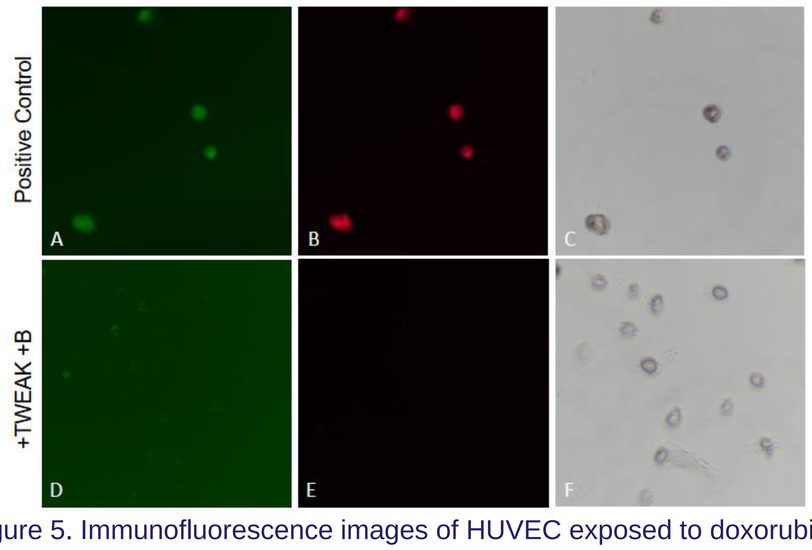

Figure 5. Immunofluorescence images of HUVEC exposed to doxorubicin under green light (A), red light (B), and white light (C), and HUVEC exposed to TWEAK and a magnetic field under green light (D), red light (E) and white light (F).

Discussion

HUVEC exposure to the SPION-TWEAK complex under magnetic conditions

- Doxorubicin, a chemiapoptotic agent, acted as a positive control as it induces apoptosis in cells. The HUVEC are shown under the green light and white light. Exposure to the red light demonstrates if a cell is undergoing apoptosis. As seen in figure 5B, the HUVEC were undergoing apoptosis, thus, red fluorescence was observed.
- The HUVEC that were exposed to the SPION-TWEAK showed no indication of apoptosis. The cells are visible under green and white light (figure 5D and 5F), but are not visible under the red light; therefore, apoptosis did not occur.
- These results indicate that the SPION-TWEAK complex, which has successfully induced apoptosis in GBM, has no effect on HUVEC. This supports the hypothesis that the complex can only interact with and kill GBM cells, as the complex is specific to the Fn-14 receptor on GBM.
- Previous studies have shown that the physicochemical properties and integrity of nanoparticles undergo drastic changes *in vitro* and *in vivo,* and these changes are attributed to cellular lysosomal degradation.

Future Research

It would be optimal to carry out more experiments with HUVEC under improved conditions, such as solenoid exposure rather than simple magnet exposure. It is also important to test the complex on cell lines other than HUVEC to ensure that it does not affect other cell lines. Transmission electron microscopy studies should also be conducted to determine whether the biopolymer from the SPION-TWEAK complex is affected by the magnetic field presence, or if the biopolymer is shed by lysosomal degradation.

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References

1) Tovar, M.; Parkhurst, A.; Matuczinski, E.; Ballenger, S.; Giancarlo, L. Synthesis of a superparamagnetic iron oxide based nano-complex for targeted cell death of glioblastoma cells. *Nanotechnology*, 2019, *30,* 465101