

**Appendix 1 Based on bioinformatics and molecular docking, the role of triphenyl phosphate in promoting the progression of glioma by influencing GRM3 and IDH2 was explored**

**Research Plan**

1. **Verification of the expression of GRM3 and IDH2 in glioma tissues**  
Verify that there may be a relationship between GRM3 and IDH2 in the occurrence of glioma.
  - 1) Samples: glioma tissues and adjacent cancer tissues from patients with glioma
  - 2) Grouping: ① Glioma tissue ② para-carcinoma tissue
  - 3) detecting cancer tissue adjacent to content: the expression of GRM3 and IDH2 in tissues was detected by IHC staining (n≥6)
2. **The effects of triphenyl 2-phosphate (TPHP) and its metabolite diphenyl phosphate (DPHP) on the expression of GRM3 and IDH2 in glioma cells**  
Verify that low concentrations of triphenyl phosphate and its metabolites may promote the development of glioma through GRM3 and IDH2
  - 2.1 Screening of the concentration of triphenyl phosphate (TPHP) and its metabolite diphenyl phosphate (DPHP) for promoting the proliferation of glioma cells
    - 1) Cells: U251, T98G
    - 2) Grouping:
      - ① Blank control group
      - ② TPHP (0.5, 1, 5, 10, 20, 40 μM)
      - ③ DPHP (0.5, 1, 5, 10, 20, 40 μM)
    - 3) Detection content: the cell viability was detected by CCK-8 at 24, 48 and 72 h, and the concentration range of promoting proliferation was screened.
  - 2.2 The effect of TPHP on the expression of GRM3 and IDH2 in glioma cells
    - 1) Cells: U251, T98G (can be replaced with cells requested by the customer or existing ones of the company)
    - 2) Grouping:
      - ① blank control group
      - ② Low-dose TPHP group
      - ③ TPHP medium-dose group
      - ④ High-dose TPHP group
    - 3) Detection content: the effect of WB on the expression of GRM3 and IDH2 in cells.
  - 2.3 The effect of DPHP on the expression of GRM3 and IDH2 in glioma cells
    - 1) Cells: U251, T98G (can be replaced with cells requested by the customer or existing ones of the company)
    - 2) Grouping:
      - ① blank control group
      - ② Low-dose DPHP group
      - ③ DPHP medium-dose group
      - ④ High-dose DPHP group
    - 3) Detection content: The effect of WB on the expression of GRM3 and IDH2 in cells.
  - 2.4 TPHP and its metabolite DPHP affect the malignant biological behavior of glioma cells by influencing the expression of GRM3 and IDH2
    - 2.4.1 Screening and validation of GRM3 and IDH2 knockdown gene Sequences (with three guarantees for one)
      - 1) Cell: U251
      - 2) Group 1:
        - ① si-NC group
        - ② si-GRM3#1 group
        - ③ si-GRM3#2 group
        - ④ si-GRM3#3 group
      - 3) Group 2:
        - ① si-NC group
        - ② si-IDH2#1 group
        - ③ si-IDH2#2 group
        - ④ si-IDH2#3 group
      - 4) Detection content: PCR was used to detect the mRNA levels of GRM3 (Group 1) or IDH2 (group 2) within cells, and the best knockdown sequences were screened.
    - 2.4.2 TPHP and its metabolite DPHP affect the malignant biological behavior of glioma cells by influencing the expression of GRM3 and IDH2
      - 1) Cell: U251
      - 2) Group 1:
        - ① si-NC group
        - ② si-GRM3 group
        - ③ si-NC + TPHP group
        - ④ si-GRM3 + TPHP group
        - ⑤ si-NC + DPHP group
        - ⑥ si-GRM3 + DPHP group
      - 3) Group 2:
        - ① si-NC group
        - ② si-IDH2 group
        - ③ si-NC + TPHP group
        - ④ si-IDH2 + TPHP group
        - ⑤ si-NC + DPHP group
        - ⑥ si-IDH2 + DPHP group
      - 4) Test content:
        - a. CCK-8 was used to detect cell proliferation activity;
        - b. The scratch test was used to detect the cell migration ability;
        - c. Transwell detection of cell invasion ability;
        - d. WB was used to detect the expression changes of GRM3 (Group 1) or IDH2 (Group 2) in cells.