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Gene Cloning And Protein Expression Of IDH1 Wild-Type And IDH1 R132H Coding Sequence In Prokaryotic E.Coli System From RGBM Cell Line

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Introduction:
The discovery of IDH1 mutations in GBMs have make a significant impact on glioma patients' diagnosis, prognosis and treatments. This study was conducted to determine the IDH1 mutation status in local established RGBM cell line, generate plasmid construct of IDH1 wild-type and IDH1 R132H coding sequence for cDNA library, and induce proteins expression.

Methods:
IDH1 mutation screening was performed on RGBM cell line via conventional PCR and DNA sequencing. IDH1 wild-type coding sequence was constructed via reverse-transcriptional PCR. Site-directed mutagenesis was performed at 395th nucleotide(G>A) on IDH1 wild-type gene to synthesis IDH1 R132H mutant and verified via DNA sequencing. E.coli system was used to express IDH1 wild-type and IDH1 R132H mutant proteins, purified via immobilized metal affinity chromatography and confirmed with Western assay.

Results:
We discovered that the RGBM cell line harbors IDH1 wild-type gene. IDH1 wild-type and IDH1 R132H mutant coding sequence were successfully synthesized and inserted into plasmid. IDH1 wild-type and IDH1 R132H mutant coding sequence were able to express proteins in prokaryotic E.coli system.

Conclusion:
In summary, we have determined that the RGBM cell line harbor IDH1 wild-type gene, constructed IDH1 wild-type and IDH1 R132H mutant cDNA library and expressed the proteins. All these will serve as an important milestone for better understanding about the role of IDH1 gene in glioma and development of more novel treatments.