Gold Nanoparticles as a Tool for Detecting Tumor Margins of Glioblastoma Multiforme

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Introduction

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults. Complete resection is nearly impossible. Overexpression of epithelial growth factor receptor (EGFR) has been associated with level of malignancy and with possible prognosis. In this study we use EGFR overexpression to mark tumor margins and infiltration to adjacent tissue, using anti-EGFR antibodies conjugated to gold nano-particles (GNPs). Immuno-staining is used in conjunction with molecular analysis of EGFR over-expression.

Methods

Fourteen GBM samples were collected according to Institutional and National ethic committee's approval. DNA was extracted from fresh tumor samples and analyzed for EGFR overexpression using real time PCR. The gene expression status (polysomy or amplification of EGFR) was analyzed by calculating the ratio of expression between the following genes: EGFR, GPER and RNaseP. Immuno-staining with anti EGFR-GNP conjugated antibodies was carried out in parallel. Stained tumor cells were detected using hyperspectral imaging. These samples were compared with routine hematoxylin eosin (H&E) staining.
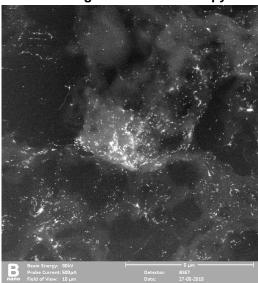
Results

Ten of the 14 (71%) GBM samples had EGFR overexpression. Amplification was detected in 50%, polysomy in 50% and one sample showed both amplification and polysomy. Four samples showed normal EGFR expression (29%). Tumor borders determined by anti EGFR-GNP conjugated staining extended into tissue previously considered "healthy" by pathological analysis.
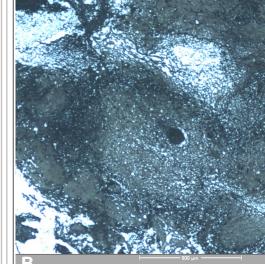
Patient Data and EGFR Overexpression Status

Pt. no.	Sex	Age (yr)	Date of diagnosis	Date of last f-u or death	Length of survival (mos)	EGFR over-expression	Mechanism
1	F	71	04.2014	2.2015	10	Yes	Amplification
2	F	59	03.2013	Alive	>30	Yes	Amplification
3	F	70	5.2014	Alive	>18	No	--
4	M	35	2015	N/A	N/A	No	--
5	M	86	07.2014	08.2015	11	Yes	Polysomy
6	F	76	07.2014	04.2015	9	No	--
7	M	84	07.2014	10.2014	3	Yes	Amplification
8	M	60	07.2014	04.2015	9	Yes	Amplification
9	M	62	08.2014	Alive	<15	No	--
10	M	47	10.2014	03.2015	5	Yes	Polysomy
11	N/A	70	10.2014	Alive	N/A	Yes	Amplification
12	N/A	71	10.2014	Alive	N/A	Yes	Amplification & polysomy
13	M	64	09.2014	Alive	<2	Yes	Polysomy
14	F	49	09.2014	N/A	N/A	Yes	Polysomy

Scanning Electron Microscopy

Gold nanoparticles coated with anti-EGFR preferentially infiltrating into brain tissue effected by GBM overexpressing EGFR.

Dynamic light scattering spectroscopy

Infiltration of GNPs into GBM

Conclusions

The high level of expression of EGFR in GBM in the majority (71%) of the samples is in line with recent publications, and enables a new method to identify tumor borders, using anti EGFR antibodies conjugated to GNPs. We found a higher infiltration rate of tumor cells to the surrounding neural tissue. Staining with GNPs depends on the functional status of the tumor and may correlate with level of malignancy and prognosis. In the future this technology may enable in-vivo detection of the real tumor margins to facilitate complete resection of GBM.

Selected References

1. Ankri, R., Duadi, H., Motiei, M., Fixler, D., 2012a. In vivo tumor detection using diffusion reflection measurements of targeted gold nanorods - a quantitative study. J. Biophotonics 5, 263-273.
2. Ankri R, Peretz V, Motiei M, Popovtzer R, Fixler D, 2012b. A new method for cancer detection based on diffusion reflection measurements of targeted gold nanorods. Int. J. Nanomedicine 7, 449-455.
3. Bienkowski, M., Piaskowski, S., Stoczynska-Fidelus, E., Szybka, M., Banaszczyk, M., Witusik-Perkowska, M., Jesien-Lewandowicz, E., Jaskólski, D.J., Radomiak-Zaluska, A., Jesionek-Kupnicka, D., Sikorska, B., Papierz, W., Rieske, P., Liberski, P.P., 2013. Screening for EGFR amplifications with a novel method and their significance for the outcome of glioblastoma patients. PLoS One 8, e65444.