Polymalic Acid

Maximal safe resection of glioma remains the single most effective treatment. Tools to guide the resection while avoiding removal of normal brain tissues can aid neurosurgeons in achieving optimal results. One strategy to achieve this goal is to rely upon intraoperative fluorescence staining of tumor cells in vivo, that can be visualized by the surgeon during resection. Towards this goal we have designed a biodegradable fluorescent mini nano imaging agent (NIA) with high specificity for U87MG glioma cells and previously unmet high light emission. The NIA is the conjugate of polymalic acid (PMLA) with chlorotoxin for tumor targeting, indocyanine green (ICG) for NIR fluorescence and the tri-leucine peptide as fluorescence enhancer. PMLA as a multivalent platform carries several molecules of ICG and the other ligands. The NIA recognizes multiple sites on glioma cell surface, demonstrated by the effects of single and combined competitors. Systemic IV injection into xenogeneic mouse model carrying human U87MG glioblastoma indicated vivid tumor cell binding and internalization of NIA resulting in intensive and long-lasting tumor fluorescence. The NIA is shown to greatly improve tumor removal supporting its utility in clinical applications [1)].

Ding et al., developed tritryptophan containing copolymer (P/WWW) of polymalic acid (PMLA) that permeates membranes by a mechanism different from previously described PMLA copolymers of trileucine (P/LLL) and leucine ethyl ester (P/LOEt) that use the “barrel stave” and “carpet” mechanism, respectively. The novel mechanism leads to solubilization of membranes by forming copolymer “belts” around planar membrane “packages.” The formation of such packages is supported by results obtained from studies including size-exclusion chromatography, confocal microscopy, and fluorescence energy transfer. According to this “belt” mechanism, it is hypothesized that P/WWW first attaches to the membrane surface. Subsequently the hydrophobic tryptophan side chains translocate into the periphery and insert into the lipid bilayer thereby cutting the membrane into packages. The reaction is driven by the high affinity between the tryptophan residues and lipid side chains resulting in a stable configuration. The formation of the membrane packages requires physical agitation suggesting that the success of the translocation depends on the fluidity of the membrane. It is emphasized that the “belt” mechanism could specifically function in the recognition of abnormal cells with high membrane fluidity and in response to hyperthermia [2)]:

To inhibit GBMs more effectively, polymalic acid-based blood-brain barrier crossing nanobioconjugates were synthesized that are delivered to the cytoplasm of cancer cells and specifically inhibit the master regulator serine/threonine protein kinase CK2 and the wild-type/mutated epidermal growth factor receptor (EGFR/EGFRvIII), which are overexpressed in gliomas according to The Cancer Genome Atlas (TCGA) GBM database. Two xenogeneic mouse models bearing intracranial human GBMs from cell lines LN229 and U87MG that expressed both CK2 and EGFR at different levels were used. Simultaneous knockdown of CK2α and EGFR/EGFRvIII suppressed their downstream prosurvival signaling. Treatment also markedly reduced the expression of programmed death-ligand 1 (PD-L1), a negative regulator of cytotoxic lymphocytes. Downregulation of CK2 and EGFR also caused deactivation of heat shock protein 90 (Hsp90) co-chaperone Cdc37, which may suppress the activity of key cellular kinases. Inhibition of either target was associated with downregulation of the other target as well, which may underlie the increased efficacy of the dual nanobioconjugate that is directed against both CK2 and EGFR. Importantly, the single nanodrugs, and especially the dual nanodrug, markedly suppressed the expression of the cancer stem cell markers c-Myc, CD133, and

nestin, which could contribute to the efficacy of the treatments. In both tumor models, the nanobioconjugates significantly increased (up to 2-fold) animal survival compared with the PBS-treated control group. The versatile nanobioconjugates developed in this study, with the abilities of anti-cancer drug delivery across biobarriers and the inhibition of key tumor regulators, offer a promising nanotherapeutic approach to treat GBMs, and to potentially prevent drug resistance and retard the recurrence of brain tumors [3)].

Tumor-specific targeting using achievements of nanotechnology is a mainstay of increasing efficacy of anti-tumor drugs. To improve drug targeting we covalently conjugated for the first time two different monoclonal antibodies, an anti-mouse transferrin receptor antibody and a mouse autoimmune anti-nucleosome antibody 2C5, onto the drug delivery nanoplatform, poly(beta-L-malic acid). The active anti-tumor drug components attached to the same carrier molecule were antisense oligonucleotides to vascular protein laminin-8. The resulting drug, a new Polycefin variant, was administered intravenously into glioma-bearing xenogeneic animals. The drug delivery system was targeted across mouse endothelial system by the anti-mouse transferring receptor antibody and to the tumor cell surface by the anti-nucleosome antibody 2C5. The targeting efficacies of the Polycefin variants bearing either two antibodies or each single antibody were compared in vitro and in vivo. ELISA confirmed the co-existence of two antibodies on the same nanoplatform molecule and their functional activities. Fluorescence imaging analysis after 24 h of intravenous injection demonstrated significantly higher tumor accumulation of Polycefin variants with the tandem configuration of antibodies than with single antibodies. The results suggest improved efficacy for tandem configuration of antibodies than for single configurations carried by a drug delivery vehicle [4)].

A new prototype of polymer-derived drug delivery system, the nanoconjugate Polycefin, was tested for its ability to accumulate in tumors based on enhanced permeability and retention (EPR) effect and receptor mediated endocytosis. Polycefin was synthesized for targeted delivery of Morpholino antisense oligonucleotides into certain tumors. It consists of units that are covalently conjugated with poly(beta-l-malic acid) (M(w) 50,000, M(w)/M(n) 1.3) highly purified from cultures of myxomycete Physarum polycephalum. The units are active in endosomal uptake, disruption of endosomal membranes, oligonucleotide release in the cytoplasm, and protection against enzymatic degradation in the vascular system. The polymer is biodegradable, non-immunogenic and non-toxic. Polycefin was also coupled with AlexaFluor 680 C2-maleimide dye for in vivo detection. Nude mice received subcutaneous injections of MDA-MB 468 human breast cancer cells into the left posterior mid-dorsum or intracranial injections of human glioma cell line U87MG. Polycefin at concentration of 2.5mg/kg was injected via the tail vein. In vivo fluorescence tumor imaging was performed at different time points, 0-180 min up to 24h after the drug injection. The custom-made macro-illumination imaging MISTI system was used to examine the in vivo drug accumulation in animals bearing human breast and brain tumors. In breast tumors the fluorescence signal in large blood vessels and in the tumor increased rapidly until 60 min and remained in the tumor at a level 6 times higher than in non-tumor tissue (180 min) ($p<0.003$). In brain tumors drug accumulated selectively in 24h without any detectable signal in non-tumor areas. The results of live imaging were corroborated histologically by fluorescence microscopic examination of various organs. In addition to tumors, only kidney and liver showed some fluorescent signal [5)].

Unclassified

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