Hypothesis

More Precise Mapping of Gliobalstoma Based on a Nanoprobe-Decorated Drug Molecule

Seyedeh Sara Esnaashari[1], Samira Raminfard[2], Zeinab Gharaylou[2], Simzar Hosseinzadeh[1]*

[1]Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Neuroscience and addiction studies, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

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Corresponding author:
Simzar Hosseinzadeh
Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
s_hosseinzadeh@razi.tums.ac.ir

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Abstract

Glioblastoma is considered as the most aggressive type of gliomas. Its invasive character involves adjacent tissues and hinders the treatment procedure. Although surgical resection followed by radiotherapy and chemotherapy have been the standard therapeutic protocol, the incompetency of detection methods to delineate the exact tumor margins results in recurrence of the tumor. NKCC1 (Sodium-Potassium-Chloride Cotransporter) is a transmembrane channel, which overexpress in pathological conditions like glioma and helps the tumor cells to change their shape for easier migration. Such a channel can play the role of a specific marker for infiltrating tumor cells and using a paired moiety against this transporter may possibly improve the precision of detection methods including Magnetic Resonance Imaging (MRI) contrast agents like SPNs (Superparamagnetic nanoparticles). Bumetanide, under the trade name of Bumex, is a diuretic drug that can block NKCC1. It has been demonstrated that in in-vivo context, bumetanide have the potency to decrease the migration of glioma cells. We have hypothesized that bumetanide can pair with NKCC1 and accumulate around the glioma cells. Hence, it seems that MRI contrast agents loaded with bumex on their surface can be proposed for more accurate tumor margins detection whilst providing additional therapeutic effects. The proposed theranostic nanostructure may further be improved and tested both in-vitro and in-vivo to prove its applicability.

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Introduction

With ongoing progress in nanoscience, scientists are looking for new ways for specific treatments of diseases through nanotools. In recent years, targeted drug delivery methods have added a new approach to the professional therapy field. Besides targeted therapy, these novel methods are also used for targeted diagnosis. As an example, magnetic nanoparticle utilized in MRI is one of the diagnostic tools, having the potential to be decorated with targeting moieties for specific tissues.

Since cancer diseases are invasive and results in high death rate, an early and precise diagnosis of the tumors position can considerably help the treatment process.

Glioma

Gliomas are considered as primary brain tumors originated from glial cells. They have many characteristics in common with glial cells (1) including their migration style, which is similar to glial cells migration during the embryonic period(2).

Based on the severity, the World Health Organization (3) classified gliomas into 4 grades, I, II, III, or IV according to their histological features. The grade IV, named glioblastoma multiforme (GBM)(4), is one of the most aggressive malignant tumors with very high invasive power(5).

Surgical resection followed by radiotherapy and chemotherapy known to be the most promising therapeutic methods for GBM. Conducting a surgery or designing a treatment strategy for radiotherapy requires tumor margins to be determined precisely by imaging via CT scan and/or MRI(6). However, the microscopic extend of the glioma tumors cannot be clarified even by injecting contrast agents in MRI(6).

Although in radiotherapy planning protocols, an approximately 2-3 centimeter further the tumor margins (depicted in MRI or CT images) is considered in therapeutic field, the recurrence of the tumor is indicative of failed therapeutic protocols. These findings demonstrate that GBM cells infiltrate through normal tissues of the brain resulting in tumor recurrence (7).

In fact, the main problem is the infiltrating cells which migrate but not proliferate; therefore, the destructive treatments for dividing cells (especially radiotherapy and chemotherapy) cannot efficiently affect infiltrating cells (7).

Based on the above, the exact detection of tumor extent is not accessible by convenient methods or even the developed ones like MRI. Hence, there is still the need for a method to enable the detection of tumor margins and even the infiltrating cell to adjacent tissues with a high precision.

NKCC1 as a marker of GBM

Cells should undergo a series of structural changes during migration including cell volume alteration. By altering genes expression, cytoskeletal components and ions homeostasis are affected; consequently cellular shape transforms from normal to elongated form (8).

NKCC1 (9) is a cotransporter, which imports Na, K and Cl^- ions into cell and is expressed in neurons and glial cells (10). This transmembrane channel has different roles in cell such as facilitating cell figure transformation and migration (11). NKCC1 expression decreases after birth, but it has shown an upward trend during various pathologic conditions like epilepsy (12), migraine (13), cerebral ischemia (14) and brain tumors(15). It has also seen that the expression of this channel in glial cells, lets the cells to change their volume. Actually this channel causes influx of ions especially Cl and water uptake inside the cells resulting in cell volume expansion which is necessary for migration (16).

Additionally, NKCC1 expression increases in glioma cells especially in GBM and anaplastic astrocytoma ones. Molecular studies have also reported that the expression of NKCC1 at the extending site of the migrated cells is more than other sides (17).

Bumex as a moiety for targeting glioblastoma

Bumex is a FDA approved diuretic, which specifically suppresses both NKCC isoforms (18).

This drug is used for hypertension, while due to its suppressive effect on the NKCC1, its indication in other pathologic states is under investigation (19). Previous studies have shown that using bumex in case of brain tumors can prevent ion importation to the cells by blocking NKCC1 and inhibiting the cell migration(9).

The effect of NKCC1 expression on shape transformation of glial cells (from normal to elongated form) which helps cell migration and also the inhibitory role of bumex on NKCC1 are outlined in Figure 1.

Improvement of MRI quality using SPNs contrast agents applied in MRI are the chelating agents of paramagnetic materials. Two types of contrast agents are available: T1 and T2 agents. Paramagnetic metals such as gadolinium (Gd^{3+}) are T1 agents, which produce bright positive signals by altering the longitudinal relaxation times of water protons.

The efficiency of the magnetic nanoparticles as contrast agent largely depends on their physicochemical properties such as size and surface chemistry. However, there are some limitations in using these particles for instance causing negative contrast (inducing dark field in image) and requiring millions of these nanoparticles for developing the desired contrast with high magnetization(20). Superparamagnetism (SPM) is a state that ferromagnetic or ferrimagnetic particles present in small size ranging from a few nanometers to a couple of tenth of nanometers.

Superparamagnet nanoparticles are single-domain and the total magnetic moment of corresponding nanoparticles is approximately indicative of uniaxial anisotropy (21). The obtained strong anisotropy is one of the most important reasons for the application of these nanoparticles in clinic and research area that cancels space averaging of resulted MRI signals. The adequate nanoparticle trafficking is useful for tracking cells by distinction between region of interest and environment in MRI.

The approved mechanism of SPN in enhancement of MRI is summarized in Figure 2.

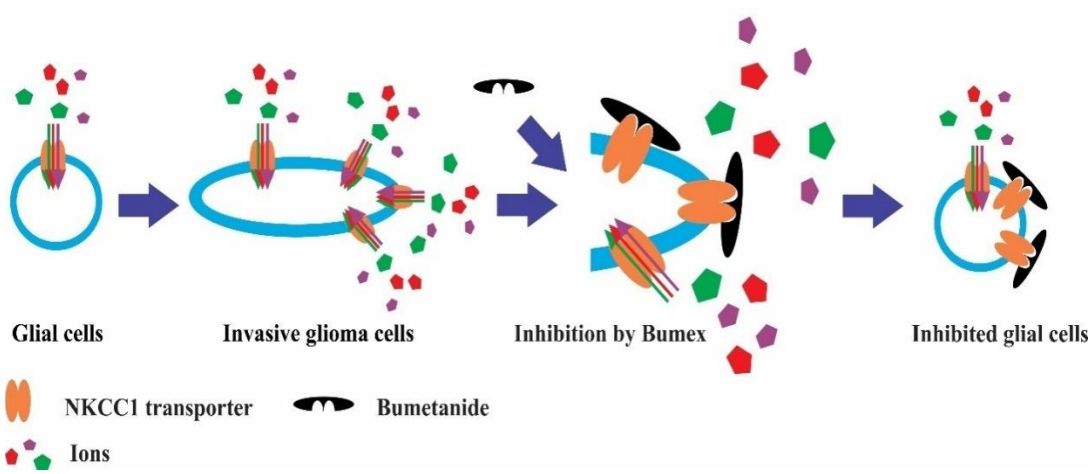

Figure 1: The high ratio of NKCC1 expression in glial cells which can be blocked using Bumex.

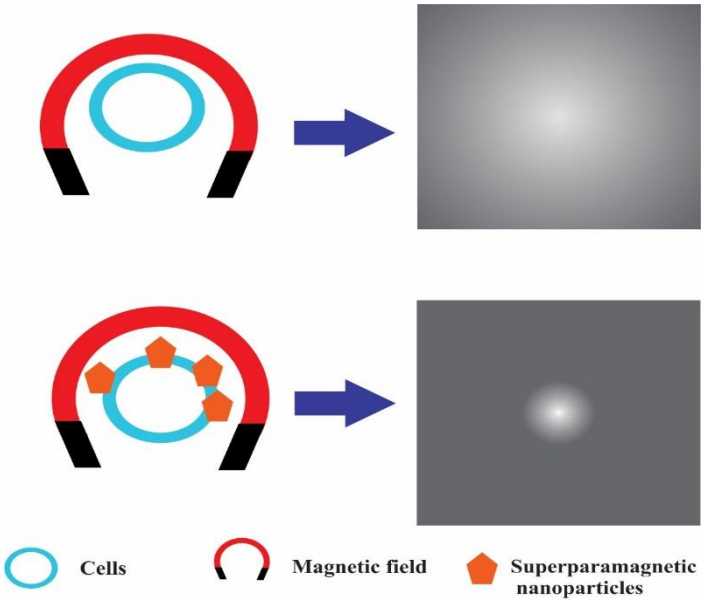

Figure 2. Untargeted and targeted SPNs used in MRI and the resulted images show that tumor margins are clearer in targeted imaging.

An engineered carrier for escaping the immune system

Nanostructures are approximately sized 10-100 nm, estimated to be more appropriate for in vivo application. Nanoparticles < 10 nm are quickly filtered through glomeruli whereas the ones larger than 100 nm are easily recognized by reticuloendothelial system and collected from circulation (22). Other determinative factors in nanoparticles fate during its journey through body is its composition, surface properties and morphology.

In the case of superparamagnetic iron oxide nanoparticle, a wide range of carriers has been studied (23) including hydrophilic polymers (24) to inorganic stealth (25). A proper coating for parenteral administered nanoparicles should assure stability of the nanoparticle both inside and outside of the body. Among the examined carriers, a mesoporous silica shell with a magnetic core of

SPN can serve our nanostructure requirement by providing both a biocompatible and biodegradable reservoir for SPN(26). The surface modification of mesoporous silica shell by polyethylene glycol (PEG) provides a large surface for binding bumex molecules. Furthermore, PEG would confine the nonspecific uptake by macrophages and interaction with plasma proteins, which results in more prolonged circulation time and more exposure to tumor cells.

Hypothesis

In pathologic conditions like tumors, some molecular and cellular alterations which occur in tumor bed could be used as a marker for diagnosing via advanced molecular imaging. The goal of new diagnostic approach in tumor field is finding the best procedure to improve early detection of tumor and it would not be achieved, unless through the identification of tumor specific markers.

Total removal of the tumor by therapeutic approaches either by the use of drug or through surgery has remained challenging. Meanwhile, biomarker-specific imaging probe offers a most prognostic way for delineation of invasive tumor margin. Glioblastoma has been classified among progressive tumors and detection of exact tumor margin has been failed through common imaging modalities such as MRI. It has been reported that in pathologic conditions like glioblastoma, NKCC1is overexpressed and enforces the migration of tumor cells. Since the corresponding cotransporter is expressed in glioblastoma more than glial cells, it can be considered as a marker. Hence, if a proper moiety is used against this transporter, the tumor cells will be detected specifically. In fact, due to the specific interaction between bumex and NKCC1 and the aggregation of bumex around these cells, they can be tracked even more precisely.

As already mentioned, bumex is well known as a specific NKCC1 antagonist that binds to chloride site of the transporter and reduces influx of Cl^-.

In general, taking advantage of an anticancer as a biomarker beside its therapeutic intervention can be regarded as a new promising strategy to govern the drug distribution.

Ultra small super paramagnetic particles which have been listed among the new class of contrast agent for MRI, can be combined with a medication for healing aim and tracking tumor extend simultaneously.

Here, we speculate that the combination of SPN and bumex can label the invasive GBM cells through binding to NKCC1. This emerging area would improve drug-delivery besides monitoring tumor upon the treatment course.

Such an insight may be used to adjust the dose based on personal conditions possibly resulting in no or minimal adverse effect of medications. In addition, the accurate tumor margins inform the location of invasive cells, which mostly relate with the high risk of residual cell population after the gross resection of the tumor tissue.

As mentioned above, tracking materials with no magnetic properties in MRI is impossible. Therefore, our hypothesis is formed based on the possibility of conjugating bumex with a particle with superparamagnetic property, which can be detectable via MRI giving rise to better quality images. We may hope that through the specificity value of this drug for GBM, tumor extension is clarified with higher precision. This would then be helpful for more accurate diagnosis in GBM patients while the standard of care drug continues to render its therapeutic value.

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