Can we use rare-earth nanocrystals to target glycans for the visualization of melanoma?

“...unique optical and physical properties of rare-earth nanocrystals with a specific targeting approach can lead to new prospective technologies for melanoma treatment.”

Keywords: biomarker • glycans • lectin • melanoma • plasma membrane • rare-earth nanocrystals

Melanomas are one of the most dangerous tumors, being the cause of 55,000 deaths in 2012 [1]. The tumor which has a defined pigmentation and superficial location from the first glance looks like an easy target for a skilled surgeon. But this first impression is not true, since melanoma can very easily grow to the deep layer of the skin rich in blood vessels, penetrate them and propagate to other soft tissues like brain, lung and bowel causing deadly metastasis. And since doctors for a long time used pigmentation as the best trait of melanoma tumor we still do not know any reliable markers and tests of melanoma either on its surface (for direct detection) or released into the bloodstream (for a screening test of melanoma at early stages) and discrimination between moles and tumors.

The pigmented cells, used to define melanoma margins, are usually already well-developed melanocytes and are preceded with proliferating cells that still have not started to produce the pigment but already have spread wider than pigmentation region, besides people with light-colored hair might have difficult-to-diagnose amelanotic melanomas.

Since at early stage melanoma is very localized tumor, and no effective systemic therapy exists to cure melanoma at an advanced stage, surgical removal of thin early-stage melanoma remains the best solution for a recovery. Due to its high metastatic potential, a precise visualization of tumor contours during surgical incision or radio therapy is important to ensure and control its complete removal and save unaffected (healthy) tissue [2,3]. To achieve this goal, we must found a proper melanoma vector and practical probe for the visualization of tumor boundaries.

Molecular markers of melanoma

The tests for molecular markers of melanoma, including protein S-100 (and others like HMB-45, MART-1/Melan-A, tyrosinase), are located inside the cells and thus their identification is usually connected with post factum histological analysis of the tissue removed from the body [4]. More important, the nature of melanoma, as a malignancy arising from (nonspecific) damage of cell DNA by UV light, excludes the possibility of any specific marker to be synthesized by melanomas and exposed on the cells to be assessable for analysis. Recent analysis of 25 melanoma genomes confirmed multiple and abound DNA damage in each case [5], finding the reliable matrix-based marker (e.g., protein/RNA) for melanoma being unrealistic goal. To solve this problem very recently we paid attention to melanoma glycans [3] and found them as promising markers of melanoma cells. Glycans possess an enormous amount of information and thus can serve as specific molecular markers of specific cells and tissues [6]. Glycan synthesis is usually very complex and thus is often disrupted in tumor cells, usually providing cells with not fully differentiated glycophenotype [6]. Specific glycosylation changes attributable to melanoma tumors have been reported recently [7]. In addition, we discovered a novel glycan marker on dying cells and deciphered molecular mechanisms connected with their appearance and influence on...
immune system [8]. Since cell death also accompanies developing tumors (in zones of apoptosis and necrosis), this approach was effective in finding specific glycan markers of model mouse melanoma and its detection. Fortunately, we can relatively easy detect specific glycans on tumor cells with either antibodies or naturally occurring glycan-recognizing proteins – lectins. Since, usually the lectin affinity binding constant with glycan (∼10^{-7}M) is two orders of magnitude lower than that for monoclonal antibodies with corresponding antigen (∼10^{-9}M), lectin binding to tissue can be disrupted with the use of specific sugar inhibitors (usually non-harmful, sweet compounds acting at 100 nanomolar range) [9]. Thus, lectins can be used for induction of reversible binding to tumor tissues (needed during diagnostic stage), while antibodies will produce strong, irreversible binding, which is needed during photodynamic therapy aimed to destroy the melanoma tissue. However, specific human melanoma glycans suitable for melanoma detection are still needed to be discovered and validated in different populations, being a good challenge for glycobiologists.

“...growing the small sizes of fluoride nanocrystals is a problematic issue when high-quality nanocrystals are desired, and is a current challenge for nanotechnology.”

**Rare-earth-based nanocrystals for infrared imaging**

The other challenge in cancer detection is on the side of development of medical tools and probes for diagnostic purposes. This is especially true for detection of various cancer types for better and more efficient detection and treatment what would enable a better understanding of cancer biology, earlier and more precise diagnosis and could help to navigate surgeon during the cancer treatment.

However, to bring the nanoprobes to clinic, several criteria must be fulfilled by the probes themselves. The probes should be small (with size below 10 nm), nontoxic, their biodistribution should be controlled (most expected are biodegradable probes), they should be bright and optically stable (no photobleaching and blinking) and these probes should bring some new functionalities to became a serious competitor for label-free diagnostic approaches.

Recently used molecular probes, including organic dyes, fluorescent proteins (like GFP) or chelates containing lanthanide ions in most of the cases do not bring new functionalities but has the advantage that they can be reasonably easy removed from or assimilated by living organisms becoming biodegradable in one way or another. One of the alternatives for molecular markers are inorganic quantum dots (QDs; i.e., CdSe, CdS), which are recently commonly used in many academic works [10], especially when high optical stability is required (i.e., single cell imaging). However, most of the QDs available these days base on Cd atoms what makes them potentially toxic [11] (even chemically), they are forbidden for the commercial use in the European Union and their inorganic nature makes their biodegradability a serious issue. Except the toxicity related issues, the multimodality in QDs is difficult to be achieved what makes these probes still not so much attractive for clinical use.

As the solution for many above-mentioned problems is the use of fluoride-based nanocrystals (NCs) doped with lanthanide ions (RENCs). Several works [12] have demonstrated that fluoride (NaYF₄, and NaGdF₄, among others) NCs doped with rare-earth ions (i.e., Eu³⁺, Tb³⁺, Tm³⁺, Er³⁺) can be much better candidates for design of new diagnostic probes with no evidence of chemical toxicity and biotoxicity so far [13,14]. In addition, these NCs are characterized with no blinking, no bleaching, and can be easily tuned spectrally with the narrow emission lines (<10 nm) from UV to infrared both as downconverting as well as upconverting RENCs and long emission decay times (∼100 ns–10 ms). Moreover, it is very important that they can be designed as the multimodal probes (UPC, MRI, CT, PET and PSCT, among others). Thus, the absence of chemical toxicity and unique physical properties make RENCs very promising candidates for clinical use as diagnostic probes since all these advantages can balance their lack of biodegradability. In addition, this serious disadvantage, common for all inorganic probes, can be reduced in consequences by precise control of NC size in range between 1 and 10 nm and their biodistribution in the body combined with reversible targeting system.

The size control is very important from the toxicity and biodistribution point of view. Unfortunately, growing the small sizes of fluoride NCs is a problematic issue when high-quality NCs are desired, and is a current challenge for nanotechnology.

The most common fluoride NCs reported so far are NaYF₄:RE³⁺ (Er³⁺, Tm³⁺, Eu³⁺) [15–18]. These are mainly upconverting NCs which appear in α-cubic and, more preferential for upconversion, in hexagonal β-phase, with sizes between 10–30 nm. These kinds of NCs are characterized with low emission quantum yield (1–5%) which dramatically drops for NCs smaller than 10 nm – the size being preferential for efficient clearance from the body. By definition, the absorption cross-section for individual lanthanide ion is very low (10⁻³³ cm²) and the maximum emission efficiency of upconversion process is 50%.
Can we use rare-earth nanocrystals to target glycans for the visualization of melanoma? Editorial

upon reduction of NC size, the ions surface aggregation, crystallinity degradation (including defects formation), reduces the excitation and emission processes significantly. Thus, recently obtained Cd-based QDs with more than 90% of emission efficiency still have easily outperformed lanthanide-doped NCs.

In consequence, preferential small size of these particles is possible to achieve, but at the same time their clinical perspective is long if not impossible because of significant reduction in emission intensity when the size is reduced below 10 nm.

Keeping these limitations in mind, very recently [19] we proposed a much simpler fluoride-based probes including high concentrations of \( \text{Eu}^{3+} \) ions (up to NaEuF\(_4\) phase). With this combination we were able to obtain bright and ultra-small (the smallest 3 nm in diameter) hexagonal NCs with high contamination of \( \text{Eu}^{3+} \) ions. This high concentration of ions per NC allowed to obtain high effective absorption cross section calculated as the ion absorption cross section multiplied by the number of ions in one NC. Taking these facts into account, we can obtain the absorption cross section per NC of the same value as for the rhodamine molecule [20]. Moreover, we have shown recently [19] that except widely used red emission band of europium also its emission band at 700 nm can be used for the optical imaging as much better suited in biological optical window. This activity in near-infrared is very important since the infrared light has a longer penetration depth, do not excite the tissues, reducing auto-fluorescence, while excitation light is not harmful for the tissue and also scattering is much smaller. Moreover, other modalities for this probe like MRI and x-ray excitation allows to inject one probe only and make more complementary in diagnostic measurements simultaneously, providing new quality of data and saving time and money of the patient.

Thus in the nearest future, combination of unique optical and physical properties of rare-earth NCs with a specific targeting approach can lead to new prospective technologies for melanoma treatment.

Financial & competing interests disclosure

The authors would like to thank the National Centre for Research and Development for their financial support under the LIDER project no. 014/L-2/10 and to West-Ukrainian Biomedical Research Center. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References


