Published in final edited form as:
Dokl Biochem Biophys. 2012 ; 446: 235–237. doi:10.1134/S1607672912050146.

STUDY OF EFFICIENCY OF THE MODULAR NANOTRANSPORTER FOR TARGETED DELIVERY OF PHOTOSENSITIZERS TO MELANOMA CELL NUCLEI *IN VIVO*

T. A. Slastnikova[a,b], **A. A. Rosenkranz**[a,b], **T. N. Lupanova**[a,b], **P. V. Gulak**[†], **N. V. Gnuchev**[a], and **A. S. Sobolev**[a,b]

[a]Institute of Gene Biology, Russian Academy of Sciences, ul. Vavilova 34/5, Moscow, 119334 Russia

[b]Biological Faculty, Moscow State University, Moscow, 119992 Russia

Photodynamic therapy (PDT) is a promising treatment method, which was shown to be effective for various types of cancer [1]. This method is based on selective accumulation of photosensitizers in cancer cells, with subsequent irradiation of the tumor at one of the absorption peaks of photosensitizers [2]. The active cytotoxic principle of photosensitizers are reactive oxygen species (ROS), primarily singlet oxygen and hydroxyl radical, whose radius of action is limited to 20 nm [2]. The most sensitive cellular compartment to the damaging effect of ROS, and consequently, to the photocytotoxic effect of photosensitizers is the cell nucleus [3, 4]. However, it was shown that free photosensitizers do not accumulate in the nucleus in detectable amount [2]. Thus in order to reach the maximum effect of PDT with the minimum dose of a photosensitizer (and, thus, to minimize the side effects of therapy), it seems reasonable to ensure not only selective accumulation of photosensitizers in tumor cells but also their targeted delivery into the cell nucleus. Applying this strategy there were developed modular nanotransporters (MNTs) utilizing natural intracellular transport and macromolecule sorting machinery for selective targeted delivery of locally acting drugs to the nucleus of melanoma cells. MNTs are recombinant polypeptides whose functional modules ensure (1) selective recognition of the target cell with its subsequent endocytosis, due to the ligand module, α-melanocyte stimulating hormone (αMSH), a ligand for internalized melanocortin receptors-1 overexpressed on the surface of melanoma cells [4]; (2) release from the endosome into the cytoplasm, due to the translocation domain of the diphtheria toxin (DTox); (3) transport into the nucleus, due to the optimized nuclear localization sequence (NLS) of the SV40 large T antigen; and (4) effective attachment of the delivered drug, ensured by the carrier module, hemoglobin like protein (HMP) of *E. coli*. Earlier, it was shown that the photosensitizers conjugated to MNTs were two orders of magnitude more efficient than the free photosensitizers on the melanoma cells *in vitro* [4].

It is worth mentioning that PDT of pigmented melanoma is challenging, due to the presence of melanin, which determines its pigmentation and absorbs light, thereby hampering light penetration into deeper layers of tissue [5]. However, the pronounced effect of MNTs on melanoma cells *in vitro* [4, 6] allowed us to assume that the small amounts of light penetrating the tumor may be sufficient for the manifestation of the effect of a photosensitizer delivered by MNT into the nucleus, the most sensitive compartment. In this study, photosensitizer bacteriochlorin p, synthesized under the supervision of Professor A.F. Mironov, Lomonosov State Academy of Fine Chemical Technology, Moscow, was used. It

[†]Deceased

was chosen due to the long wavelength absorption maximum (761 nm) shifted to the region where the melanin absorbtion is weaker and the tissue is more transparent [7]. Taking into account the fact that melanomas are highly heterogeneous in the content of melanin (from amelanotic to completely black), in this study we used two models of melanoma with different levels of pigmentation — weakly pigmented Cloudman S91 melanoma (clone M3) and highly pigmented melanoma B16-F1. The modular nanotransporter DTox–HMP–NLS–αMSH was synthesized and purified as described previously [4]. To study the distribution of the MNT in the tissues of tumor bearing mice, MNT was radioactively labeled using the iodination agent N-succinimidyl 3-[^{125}I]iodobenzoate [8], which yields a labeled product resistant to deiodination *in vivo* [8].

For PDT experiments, MNT was conjugated to the photosensitizer bacteriochlorin p [4]. Since the delivered photosensitizer exerts its photocytotoxic effect only within the irradiated area, the distribution of the transporter between the tumor and its immediate environment is the most important for PDT. The selectivity of accumulation of the MNT in the B16-F1 tumor transplanted to C57Black/6J mice (Tables 1, 2) significantly increased with MNT doses starting from 9.7 mg/kg body weight and reached the optimal tumor-to-muscle ratio of specific radioactivities (13.4 ± 1.7) at a dose of 38.6 mg/kg as early as 3 h after intravenous injection. Despite the tendency to an increased accumulation of the label in the skin compared to the muscle, presumably due to the presence of melanocortin receptors on melanocytes, the optimal ratios of tumor-to-skin specific°radioactivities (9.8 ± 1.8) at a dose of 9.7 mg/kg were 3–8 times greater than the published ratios for the free photosensitizers for the same B16 melanoma model [9–11].

As the time elapsed after the injection increased to 15 h, the tumor-to-surrounding tissue specific radioactivities ratio of the specific radioactivities tended to increase, although the absolute content of the label both in the tumor and in other tissues significantly decreased over time, dropping down by more than 90% over the period from 3 to 15 h. Taking this into account, for the therapy of C57Black/6J mice with transplanted melanoma B16-F1, we used several different schemes of irradiation. In the first scheme, mice were irradiated three times (3, 6, and 9 h after the injection of the photosensitizer conjugated to MNT) with increasing light dose from the first to the third exposure, which ensured a high efficacy of therapy with the use of the photosensitizer conjugated to MNT (Table 3). Another scheme, consisting of a single light exposure 3 h after intravenous injection of the photosensitizer conjugated to MNT, showed a similar effect of PDT with a considerable simplification of the treatment setting (Table 3). Even a more pronounced inhibition of tumor growth and increased survival rates were obtained using the less pigmented Cloudman S91 melanoma (Table 3).

To study the intracellular distribution of MNT at the time of irradiation, we performed immunofluorescent staining of tumor sections. Subsequent analysis of the sections under an LSM 510 Meta NLO confocal laser scanning microscope (Carl Zeiss, Germany) revealed the accumulation of the MNT in the nuclei of tumor cells (Table 4).

Thus, in this study we demonstrated highly selective accumulation of MNT in melanoma as compared to the surrounding healthy tissue and its significant concentration in the nuclei of tumor cells *in vivo*, which ensured 93% melanoma growth inhibition and a significant increase in the lifespan of animals that were treated with the photosensitizer conjugated to the MNT as compared to the animals treated with the free photosensitizer.

Acknowledgments

We are grateful to N.A. Shevkun (Biological Faculty, Moscow State University) for assistance in conducting the experiment. Experiments were performed using the equipment of IGB RAS facilities supported by the Ministry of Science and Education of the Russian Federation (grant N 16.552.11.7067). This work was supported by the

Russian Foundation for Basic Research (project no. 10-04-01037a), US National Institute of Health (NIH) (grant no. P50 NS20023), and the state contract no. 16.512.12.2004.

References

1. Davids LM, Kleemann B. Cancer Treat Rev. 2011; 37(6):465–475. [PubMed: 21168280]
2. Sobolev AS, Rozenkrants AA, Gilyazova DG. Biophysics. 2004; 49(2):351–379.
3. Liang H, Shin DS, Lee YE, et al. Lasers Med Sci. 2000; 15:109–122.
4. Rosenkranz AA, Lunin VG, Gulak PV, et al. FASEB J. 2003; 17(9):1121–1123. [PubMed: 12692081]
5. Lim DS, Ko SH, Lee WY. J Photochem Photobiol. 2004; 74(1):1–6.
6. Rozenkrants AA, Lunin VG, Sergienko OV, et al. Russ J Genet. 2003; 39(2):259–268.
7. Michailov N, Peeva M, Angelov I, et al. J Photochem Photobiol. 1997; 37(1/2):154–157.
8. Vaidyanathan G, Zalutsky MR. Nat Prot. 2006; 1(2):707–713.
9. Fabris C, Vicente MG, Hao E, et al. J Photochem Photobiol. 2007; 89(2/3):131–138.
10. Jori G, Soncin M, Friso E, et al. Appl Radiat Isot. 2009; 67(7/8, suppl):S321–S324. [PubMed: 19376726]
11. Woodburn KW, Fan Q, Kessel D, et al. J Investigat Dermatol. 1998; 110(5):746–751.

Table 1

Melanoma B16-F1/healthy surrounding tissue radioactivity ratio as a function of time elapsed after intravenous injection of labeled MNT at a dose of 0.5 mg/kg (the mean value and the standard data of the mean, mean ± SE, n = 3)

Time after injection of labeled MNT, h	Radioactivity ratios:	
	tumor/muscle	tumor/skin
1 h 10 min	2.2±0.6	2.1±0.8
3 h	4.7±0.2	1.4±0.4
15 h	6.9±1.7	3.3±0.5

Table 2

Melanoma B16-F1/healthy surrounding tissue radioactivity ratio 3 h after intravenous injection of different amounts of labeled MNT (mean ± SE, n = 3)

Amount of injected labeled MNT, mg/kg	Radioactivity ratios:	
	tumor/muscle	tumor/skin
0.5	4.7±0.2	1.4±0.4
9.7	8.2±2.0	9.8±1.8
38.6	13.4±1.7	4.9±2.3

Dokl Biochem Biophys. Author manuscript; available in PMC 2013 May 07.

Table 3

Effectiveness of photodynamic therapy (PDT) with the use of the photosensitizer bacteriochlorin p conjugated to MNT (MNT–BChl)

Mouse melanoma	Number of PDT sessions (number of exposures per session)	Tumor growth inhibition, % relative to:		Lifespan since tumor inoculation, days (mean ± SE)		
		control	Bchl	MNT-Bchl	Bchl	control
B16-F1	4 (3)	80	85	29±3*	18±3	18±3
	5 (1)	82	89	34±4*	18±1	20±3
Cloudman S91 (clone M3)	5 (1)	98	93	52±17**	35±10	21±2

Note: BChl—bacteriochlorin.

Differences from the respective group “BChl” were significant at

* $p < 0.002$ and

** $p < 0.05$ (according to the Mantel–Hansel test).

Table 4

Percentage of fields (mean ± SE) with a signal specific for MNT in the nuclei and cytoplasm of Cloudman S91 melanoma cells (n = 89) on a tumor section

		% of areas with the signal intensity above the threshold
Tumor	Nucleus	84±0.10
	Cytoplasm	98±0.02