Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Science Letters:

Brain metastases of melanoma—mechanisms of attack on their defence system by engineered stem cells in the microenvironment#

DIMITROV Borislav D.†[1], ATANASSOVA Penka A.[2], RACHKOVA Mariana I.[3]

([1]*BORDANI, Alzano Lombardo, Bergamo 24022, Italy*)

([2]*Department of Neurology, Medical University, Plovdiv 4000, Bulgaria*)

([3]*ASSON-93 Research Co., Chicago, IL 60056, USA*)

†E-mail: bdd11@yahoo.com

Received Aug. 2, 2007; revision accepted Aug. 10, 2007

Abstract: This report gives a better emphasis on the role of targeted effectors (e.g. a combination of 5-FC with CD-NSPCs as compared to the application of NSPCs alone) and how such delivery of pro-drug activating enzymes and other tumor-killing substances may overcome melanocytic defence system, interact with and promote the host defence and immune response modulations not only in melanoma but, potentially, in other highly-metastatic cancers.

Key words: Melanoma, Brain metastases, Neural stem/progenitor cells (NSPCs), Cytosine deaminase
doi:10.1631/jzus.2007.B0609 **Document code:** A **CLC number:** R74

We would like to bring attention to a timely and intriguing paper published recently by Aboody *et al*.(2006). This report presented original and interesting proof-of-concept study on how an engineered system of neural stem/progenitor cells (NSPCs)-expressing cytosine deaminase (CD) may be used to target brain metastases in melanoma model.

Notably, the brain metastatic process is the major cause of morbidity and mortality in patients with melanoma and, unfortunately, the molecular mechanisms that lead to brain metastases remain poorly understood. We are convinced that using stem-cell chemotactic "conveyor" systems is a very promising approach. It is quite interesting to note the (relative) percentage decreases of 71% and 69% in the tumor burden as obtained by CD-NSPCs and 5-FC treatment, respectively, as compared to both control groups, although they may seem, to a certain extent, surprisingly high [see p.123, Fig.4E in Aboody *et al*.(2006)]. To enhance the importance and reliability of these results, in such designs where the distributions of the data may deviate from normality, one idea is to apply a log-transformation of the original data (Altman and Bland, 1995) or to use various other alternatives such as paired comparisons, non-parametric tests, or more complex analysis-of-variance models for other possible interactions that may have caused the higher variability to be taken into account (e.g., weight on day 19 after injection as a random individual-subject effect). In this way, this report will be able to give a

An earlier development of these novel theoretical conjectures may be traced back to one of authors' original peer-reviewed works that was accepted for publication just within 9 days from receipt by the *Medical Hypotheses* (Dimitrov, 1993) as well as to the second one in the *European Journal of Dermatology* (Dimitrov, 1997) with the critical comments by Prof. Patrick A. Riley from UCLMS (England). The most recent description of Melanocytic Defence System (MDS) research and development was presented in part at the 10th PASPCR Annual Meeting in Minneapolis (USA) by Dimitrov and Rachkova (2001) (http://dx.doi.org/10.1034/j.1600-0749.2001.140313.x). Detailed references for each of the above assumptions are presented within the authors' poster at this meeting; both are available upon written request to the authors at pp_atanassova@yahoo.com or bdd11@yahoo.com. The biochemically-based 4-step tyrosinase model of "host-matched interaction" for melanoma metastases by Rachkova and Dimitrov (1999) represented one of the most important works on which the 2003 US Patent No. 7030129 is based (Miller *et al*., 2003; available from the authors as well as upon free registration at http://www.freepatentsonline.com/7030129.html).

better emphasis on the role of the combination of 5-FC with CD-NSPCs as compared to the application of NSPCs alone and how such delivery of pro-drug activating enzymes and other tumor-killing substances may interact with and promote the host defence and immune response modulations.

In this line of thought, we suggested earlier (Dimitrov and Rachkova, 2001) and presented here in a tabular form the novel framework structure for a Melanocytic Defence System (MDS) that may operate not only at the process of transformation of melanocytes to melanoma cells (Table 1), but also at the steps of transformation of melanoma cells to metastatic cells with a potential for higher malignancy and dissemination throughout the body, including the brain. Our theoretical concept includes two main flows of substances and energy (Table 1), which, if applied in the light of a stem-cell chemotactic "conveyor" approach, may not only foster the above-mentioned promotion of defence cellular and body mechanisms, but also further clarify the role of such non-specific signal (energy?) transduction pathways as that of Stat3 protein activation as recently reported by Xie *et al*.(2006). It is interesting to mention that this is an almost parallel reporting of Stat3 activity as a potential target for a therapy in (human) melanoma brain metastases, which may transcriptionally regulate such a promoter as vascular endothelial growth factor (VEGF). VEGF is produced by the melanoma cells and represents, at the same time, notably, a powerful chemotactic substance for NSPCs (Aboody *et al*., 2006). These developments in the melanoma-to-brain metastasis theory, and possible therapeutic applications might be very conveniently considered within our previously described 4-step tyrosinase model of "host-matched interaction" (Rachkova and Dimitrov, 1999). Such embeddings may be seen as a very good example of tumor-"host-stromal" relationships on the grounds of the renown Paget's "seed and soil" hypothesis of breast cancer (i.e., melanoma appears not only on the skin but also on covered tissue areas and/or in immunocompromized patients). Conceivably, the pigment cells are from neuro-ectodermal origin and the melanocytes as well as all phenol-oxidase (PO) positive cells are (potential) melanin formers. Thus, it is very probable that NSPCs "match" very closely with the melanoma metastases (as "hosts") in the brain by a biochemical similarity (PO+) and encoded (embedded) similar early-stage patterns of development and levels of (de)differentiation in both cell types. Moreover, the chemotaxis of NSPCs to metastatic melanoma cells in the brain with conversion of 5-FC to 5-FU by CD could be provoked not only by biochemical signaling axes alone, but it may be also triggered through their own thermodynamic "self-similarity" nature as within the system "(melanocyte)-(melanoma cell)-(metastatic cell)" as a local "hot-tissue area" according to our 7-phase models of energy storage (Dimitrov, 1993; 1997). An analogy could be made to the described targeted tumor-stromal interactions that may reciprocally modulate gene expression patterns during metastasis as well. This recent example refers to a xenogeneic breast cancer model (Montel *et al*., 2006); to note, the breast cancer is one of the tumors that preferentially spreads and forms metastases in the brain as a host (as a "selected" organ). In this line of thought, we should note that we had postulated earlier also that, the melanocytes divide and bifurcate normally under normal conditions and, by exporting energy (entropy?) and substances, the cells are progressing slowly within the developmental choice of optimization and synchronization to the surrounding microenvironment. However, in critical conditions, the melanocytes (and, possibly, melanoma metastatic cells in the brain) may neither utilize the highest possible work value of their own energy nor bifurcate normally (Dimitrov, 1997). In such situations, by possibly increasing also in size (dimension), the transforming or already transformed malignant cells may die by apoptosis, aggregate, or start switching-on their own "defence" system to work outwards (Table 1) with (over)expressing various chemotactic substances (e.g., secretory glycoproteins, arachidonic acid, etc.; see Dimitrov and Rachkova, 2001) in the attempt to maintain their own internal thermodynamic balance.

Notably, such an "overloaded" unstable status of the metastatic melanoma cells might be the best moment to be targeted by NSPCs and pro-drug agents' attacks. The paper (Aboody *et al*., 2006) may actually be seen as a further theoretical background on a proof-of-principle approach for a tumor (probably, metastatic) size reduction that might have interfered with survival (p.123, left column, line 5) as based on the above-mentioned therapeutic effect (i.e.,

the decrease of the tumor burden in the experimental group as shown on Fig.4E). The work by Aboody *et al*.(2006) may be considered as an additional emphasis on further possible implications of their own target-based engineered NSPCs system, and in particular, whether their system can also apply to other silent but highly metastatic and deadly tumors (e.g., lung cancer, breast cancer, etc.) in the brain, and/or to the utilization of such other potential therapeutic agents as spin-labeled free stable nitroxyl radical derivatives that may accumulate in melanoma (Raikov *et al*., 2003), as well.

Table 1 Directions of energy flux within melanocytes and mechanisms of Melanocytic Defence System (MDS) in the view of attacking (melanoma) metastasis cells by engineered neuronal stem/progenitor cells*

	Mechanisms of MDS
Directions	
Outwards	(To the borders of the system and cell microenvironment)
	⇦ by dissipation as heat
	⇦ by secretion and production of 'abundant reactive molecules'
	⇦ by intercellular secretion, transport and production of glycoproteins onto premelanosomes/cell surface
	⇦ by transfer of melanosomes (to keratinocytes)
Inwards	(To the centre of the system and cell nucleus)
	⇨ by transfer of heat towards/within cell nucleus and DNA
	⇨ by transport of 'abundant molecules' towards cell nucleus and accumulation around DNA
	⇨ by transport of free radical species (OH·, RO·) towards cell nucleus and DNA
	⇨ by melanin action as a source of free radical and their accumulation within cell nucleus and around DNA
	⇨ by melanin transport and its action as a free radical towards/within cell nucleus and around DNA
	⇨ by direct accumulation of (UVR) energy within cell nucleus and around DNA, etc.
Levels	
1	Membrane fluidity changes
2	Release of arachidonic acid
3	Transport of secretory glycoproteins
4	Maximisation of tyrosinase activity
5	Activation of glycosylation of tyrosinase
6	Synthesis of tyrosinase
7	Formation of melanin
8	Formation of indoles by (photo)lysis
9	Melanin trapping of free radicals/energy absorption

*Note: From Dimitrov and Rachkova (2001) with modifications. Detailed references for each of above assumptions are presented in the paper by Dimitrov and Rachkova (2001)

References

Aboody, K.S., Najbauer, J., Ole Schmidt, N., Yang, W., Wu, J.K., Zhuge, Y., Przylecki, W., Carroll, R., Black, P.M., Perides, G., 2006. Targeting of melanoma brain metastases using engineered neural stem/progenitor cells. *Neuro-Oncology*, **8**(2):119-126. [doi:10.1215/15228517-2005-012]

Altman, D.G., Bland, J.M., 1995. The normal distribution. *British Medical Journal*, **310**:298.

Dimitrov, B.D., 1993. The storage of energy as a cause of malignant transformation: a 7-phase model of carcinogenesis. *Medical Hypotheses*, **41**(5):425-433. [doi:10.1016/0306-9877(93)90119-B]

Dimitrov, B.D., 1997. UVR and energy storage within melanocytes: a 7-phase model of melanoma genesis and metastasis. *European Journal of Dermatology*, **7**: 461-463.

Dimitrov, B.D., Rachkova, M.I., 2001. Defence Mechanisms of Melanocytes against Radiation as Based on the Rules of Non-Equilibrium Thermodynamics. *In*: Tenth Annual Meeting of the Pan-American Society for Pigment Cell Research. 2001 June, Minneapolis, MN, USA, p.20.

Miller, R.L., Gaspari, A.A., Gillis, J.A., 2003. Method of Reducing and Treating UVB-Induced Immunosuppression. US Patent No. 7030129.

Montel, V., Mose, E.S., Tarin, D., 2006. Tumor-stromal interactions reciprocally modulate gene expression patterns during carcinogenesis and metastasis. *International Journal of Cancer*, **119**(2):251-263. [doi:10.1002/ijc.21757]

Rachkova, M.I., Dimitrov, B.D., 1999. Free radical production, immunosuppression and the role of tyrosinase in melanoma genesis and metastasis. *Periodicum Biologorum*, **101**:187-192.

Raikov, Z.D., Atanasov, A.T., Raikova, E.T., 2003. Nitroxyl radicals and malignant pigment melanoma. *Medical Hypotheses*, **60**(3):387-388. [doi:10.1016/S0306-9877(02)00407-3]

Xie, T.X., Huang, F.J., Aldape, K.D., Kang, S.H., Liu, M., Gershenwald, J.E., Xie, K., Sawaya, R., Huang, S., 2006. Activation of Stat3 in human melanoma promotes brain metastasis. *Cancer Research*, **66**(6):3188-3196. [doi:10.1158/0008-5472.CAN-05-2674]