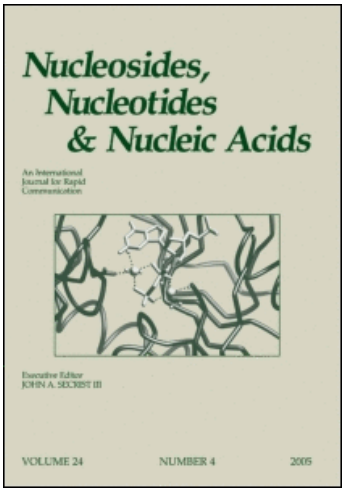

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Ecto-5'-Nucleotidase (eN, CD73) is Coexpressed with Metastasis Promoting Antigens in Human Melanoma Cells

R. Sadej[a]; J. Spychala[b]; A. C. Skladanowski[a]
[a] Department of Enzymology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland [b] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

To cite this Article Sadej, R. , Spychala, J. and Skladanowski, A. C.(2006) 'Ecto-5'-Nucleotidase (eN, CD73) is Coexpressed with Metastasis Promoting Antigens in Human Melanoma Cells', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 1119 — 1123
To link to this Article: DOI: 10.1080/15257770600894188
URL: http://dx.doi.org/10.1080/15257770600894188

Nucleosides, Nucleotides, and Nucleic Acids, 25:1119–1123, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600894188

Taylor & Francis
Taylor & Francis Group

ECTO-5′-NUCLEOTIDASE (eN, CD73) IS COEXPRESSED WITH METASTASIS PROMOTING ANTIGENS IN HUMAN MELANOMA CELLS

R. Sadej □ *Department of Enzymology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland*

J. Spychala □ *Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA*

A. C. Skladanowski □ *Department of Enzymology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland*

□ *Upregulated expression of eN has been found in the highly invasive human melanoma cell lines but neither in melanocytes nor in primary tumor cells. Membrane proteins associated with cell adhesion and metastasis: α_5-, β_1-, β_3-integrins, and CD44 were elevated gradually in accordance with increasing metastatic potential. α_v-integrin was seen mostly in aggressive melanomas. The expression of eN correlated with a number of metastasis-related markers and thus may have a function in the process. eN activity went parallel with its amount in all cells. Concanavalin A strongly inhibited the enzyme in a noncompetitive way. Clustering of eN protein in overexpressing cells by ConA-treatment increased the enzyme association with the heavy cytoskeletal complexes. A similar shift towards cytoskeletal fractions took also place with other membrane proteins coexpressed with eN. This ConA-induced association may reflect a putative interaction of eN with physiological ligand, that upon interaction, aggregates protein components of lipid rafts and triggers signaling pathway that may be intrinsically involved in cell-stroma adhesion.*

Keywords Ecto-5′-nucleotidase; Melanoma, Concanavalin A

INTRODUCTION

Ecto-5′-nucleotidase (EC 3.1.3.5) is a widely distributed enzyme anchored to the outer leaf of plasma membrane via glycosylphosphatidylinositol linkage. It produces nucleosides from their monophosphates in the extracellular space. Most abundant, adenosine, acting through specific receptors is implicated in many physiological and pathophysiological

Address correspondence to R. Sadej, Department of Enzymology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, ul. Debinki 1, 80-211 Gdansk, Poland. E-mail: rsadej@amg.gda.pl

processes including regulation of tumorigenesis. Highly active enzyme was detected in breast carcinoma, gastric cancer, pancreatic cancer, chronic myelogenous leukemia, cutaneous T-cell lymphoma, and in Walker 256 carcinoma.[1] In contrary, diminished eN activity was found in lymphocytes from patients affected by B-cell chronic lymphocytic leukemia comparing with control (CD73+) lymphocytes.[2] eN has been suggested to serve as lymphangiogenic marker in lymphatic spread of colorectal cancer cells to regional lymph nodes.[3] Changes of eN activity during melanocyte differentiation into melanoma occur but reports are contradictory.[1,4]

Studies with human lymphocytes proved that CD73 mediates adhesion of lymphocytes to endothelial layer.[5] eN is localized in lipid rafts, domains rich in sphingolipids and cholesterol, lipid signaling molecules, transmembrane receptors, and GPI-anchored proteins. Redistribution of protein molecules may control cell-cell or cell-extracellular matrix interaction, so rafts are proposed to function as platforms capable of facilitating efficient and specific signal transduction. Aim of this paper is to correlate expression of various proteins in the plasma membrane of malignant melanomas with ecto-5'-nucleotidase. We suggest that incubation of highly metastatic melanoma cell lines with ConA (eN binding lectin) results in rearrangement of membrane distribution of important tumor promoting antigens.

MATERIALS AND METHODS

Chemicals and Antibodies

All were obtained from commercial sources with exception of polyclonal rabbit antibodies against human eN which were raised and purified as described earlier.[6] Antibodies against actin, α_3-, β_1- and β_3-integrins, and secondary antibodies were from Santa Cruz (USA), antibodies against CD44 from R&D (Germany) and antibodies against α_v- and α_5-integrin from Chemicon (USA).

Cell Lines and Culture Conditions

The human melanoma cell lines WM35, WM902b, WM9, A375, Hs294T, Sk-Mel-2, and RPMI-7951 were cultured in standard conditions. Lysates of normal human melanocytes were gifts from Dr A. Aplin (Albany Medical College, Rochester, NY, USA).

Fractionation of Cell Lysates and Western Blotting

Growing cells were scraped in the presence of ice-cold PBS/protease inhibitors and lysed in PBS/1% TritonX-100. Lysates were fractionated

in Nycodenz gradient for separation of lipid rafts from other cellular compartments.[7] After centrifugation 10 fractions were analysed for distribution of different proteins by Western blotting. Equal volumes of samples (20 μl) were loaded per lane, separated by SDS-PAGE and transferred onto PVDF membrane. Proteins were probed with specific antibodies in PBS/0.2% Tween20. Secondary antibodies conjugated to HRP and BM Chemiluminescence Western Blotting Kit (Roche, Germany) were used to develop images on Kodak X-Omat AR film.

eN Assaying

eN activity was measured as previously described.[8] When necessary, ConA (20 μg/mL) was added 20 minutes before initiation of the reaction.

RESULTS AND DISCUSSION

We made a key observation that eN expression (and activity, not shown) is moderate in normal melanocytes and primary melanomas (WM35 and WM902b) and increases to a multiple fold higher in cells originated from secondary tumors (A375, RPMI7951, WM9). Further we analyzed adhesion and metastasis associated integrins and found that α_3-type is expressed ubiquitously and on a similar level in each line, α_5-, β_1-, and β_3- subunits elevated gradually in accordance with increasing metastatic potential of the tumor and expression of α_v-subunit was restricted only to highly metastatic cells. CD44, a hyaluronic acid receptor, appeared concurrent with eN expression profile. Then tumor invasion promoting antigens tended to co-express with eN in various melanoma lines (Figure 1).

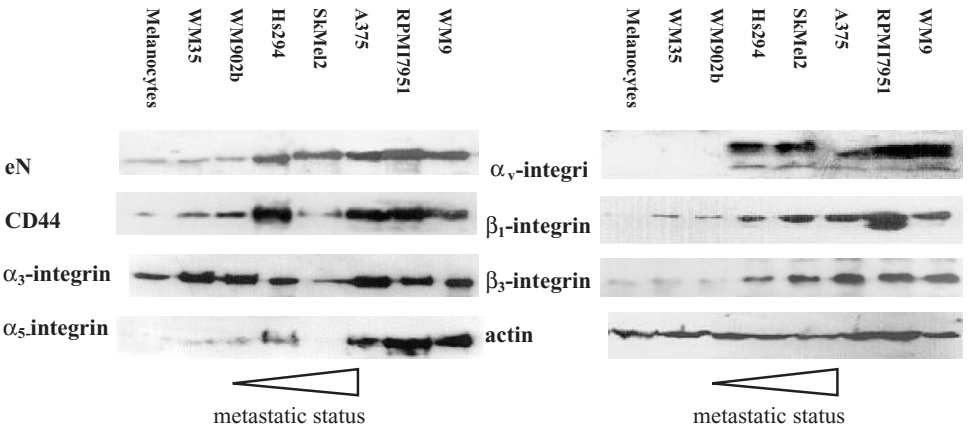

FIGURE 1 Expression of eN and other membrane proteins characteristic for high invasiveness in melanocytes and cultured melanoma cell lines.

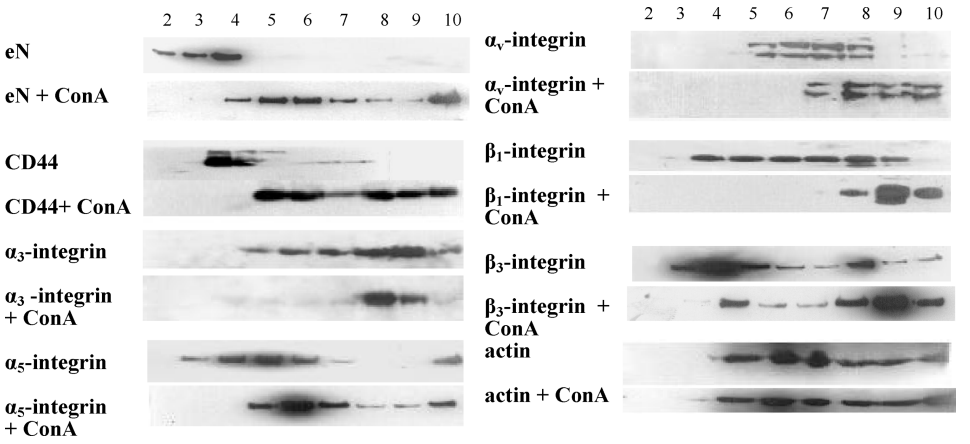

FIGURE 2 20 μg/mL ConA-stimulated relocalization of eN and other membrane proteins in WM9 melanoma cell line. TritonX-100 lysates were applied into the middle fraction of Nycodenz step gradient.

Preincubation of each cell line with Concanavalin A (20 μg/mL) resulted in ~70–80% inhibition of eN activity (not shown). Moreover in high eN expressing WM9 cell line, this interaction triggered the process of complexes formation between eN, membrane components and cytoskeletal structures. This was visible as synchronized shifts of those proteins in Nycodenz gradient (Figure 2). The same process was observed with CD44 and the analyzed integrins. We conclude that ConA-binding to eN which can mimic natural, still unknown ligand, results in clustering of neighbouring/interacting proteins what can trigger intracellular effects. The last could contribute to effective signal transduction, adhesion or migration process. It is supported by fact that the proteins which were shifted to heavier fractions together with eN are regarded as highly tumor promoting molecules.

REFERENCES

1. Spychala, J. Tumor-promoting functions of adenosine. *Pharmacology and Therapeutics* **2000**, 87, 161–173.
2. Rosi, F.; Tabucchi, A.; Carlucci, F.; Galieni, P.; Lauria, F.; Zanoni, L.; Guerranti, R.; Marinello, E.; Pagani, R. 5′-nucleotidase activity in lymphocytes from patients affected by B-cell chronic lymphocytic leukemia. *Clin. Biochem.* **1998**, 31, 269–272.
3. Parr, C.; Jiang, W.G. Quantitative analysis of lymphangiogenic markers in human colorectal cancer. *Int. J. Oncol.* **2003**, 23, 533–539.
4. Moody, D.; Bijwaard, K.E.; Gersten, D.M. Absence of murine melanoma 5'-nucleotidase activity is not attributable to a detectable inhibitor. *Pigment Cell Research* **1989**, 2, 502–513.
5. Airas, L.; Hellman, J.; Salmi, M.; Bono, P.; Puurunen, T.; Smith, D.J.; Jalkanen, S. CD73 is involved in lymphocyte binding to the endothelium: characterization of lymphocyte-vascular adhesion protein 2 identifies it as CD73. *J. Exp. Med.* **1995**, 182, 1603–1608.
6. Yegutkin, G.G.; Henttinen, T.; Samburski, S.S.; Spychala, J.; Jalkanen, S. The evidence for two opposite, ATP-generating and ATP-consuming, extracellular pathways on endothelial and lymphoid cells. *Biochem. J.* **2002**, 367, 121–128.

7. Hostager, B.S.; Catlett, I.M.; Bishop, G.A. Recruitment of CD40 and tumor necrosis factor receptor-associated factors 2 and 3 to membrane microdomains during CD40 signaling. *J. Biol. Chem.* **2000**, 275, 15392–15398.
8. Spychala, J.; Lazarowski, E.; Ostapkowicz, A.; Ayscue, L.H.; Jin, A.; Mitchell, B.S. Role of estrogen receptor in the regulation of ecto-5′-nucleotidase and adenosine in breast cancer. *Clin. Cancer Res.* **2004**, 10, 708–717.