Chinese Journal of Cancer Research 8(2): 91 — 94, 1996

THE EFFECT OF RETINOIC ACID ON CELL MEMBRANE AND METASTATIC ABILITY OF MOUSE FORE-STOMACH CARCINOMA CELL

Yu Xiaodan 于晓妧 Zhao Xuemei 赵雪梅 Gao Jin 高进

Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. School of Basic Medicine, Peking Union Medical College, Beijing 100005

We studied the effect of all-trans-retinoic acid (RA) on the expression of several surface lectin receptors and cell membrane fluidity of mouse forestomach carcinoma cell line (MFC) *in vitro*. The results showed that cells treated with RA manifested decreased expression of lectin receptors, increased membrane fluidity and reduced spontaneous metastasis. These results suggest that the effect of RA on tumor cell membrane may be one of the mechanisms involved in the alternation of cell metastatic phenotype.

Key words: Retinoic acid, Cell membrane fluidity, Tumor metastasis

The cell membrane is the place that the interactions between cell to cell or cell to extracellular matrix take place. It is also involved in a variety of physiological properties such as cell growth, division, communication, movement, etc. Accompanied by the cell transformation, the function and structure of cell membrane have been changed greatly, including cell surface composition, cellular transport and the dynamics of surface receptors.[1] As the cell membrane plays an important role in cell growth regulation, the alteration of cell surface may directly affect the characteristics of tumor cells, such as the ability of invasion and metastasis. In this paper. we detected the effect of retinoic acid on tumor cell membrane during the cell differentiation and investigated the possible mechanisms.

MATERIALS AND METHODS

Tumor Cells and Culture Condition

The murine forestomach carcinoma cell line MFC cells were mantained in RPMI 1640 supplemented with 10% fetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2.

RA and Cells

All-trans-retinoic acid (obtained from Dr. Huan Rui. Institute of Drug and Medicine Science, Chinese academy of Medical Sciences.) was dissolved in 95% ethanol and add to the growth medium at the concentration of 10 μ mol/ml. Equal amounts of 95% ethanol were added to the control medium. Subdued lighting was used in all experiments. The medium was changed every 48-72 hour. The tested cells were treated by RA for 5-60 days.

Lectin Receptor Assay

Accepted February 9, 1996

* The study was supported by the grant of the national 8th five key project (85-914-03-07)

RA treated and untreated MCF cells were trypsinized to make single cell suspension, then washed twice with phosphate-buffered saline (PBS), counted, and resuspended at the concentration of 1×10^6/ml. The cells then incubated with 1 of 5 biotin labeled lectins (Vector) at 10 μg/ml/10^6 viable cells in 1 ml HEPE buffer. The lectins used were ConA, SBA, UEA, WGA and PSA. The cells were incubated on ice for 40 minutes, washed twice with washing buffer, then incubated with 5 μg/ml Avidin-FITC/PBS (Sigma) on ice for another 40 minute and washed again with PBS. Flow cytometric analysis of stained tumor cells was performed with the use of a Coulter 541 flow cytometer. Excitation was provided with an argon ion laser operating at 300 mw at 488 nm. After setting light scatter to exclude debris, we collect I-parameter integral log green fluorescence histograms for 10,000 cells. The mean fluorescence intensity was calculated for each group of cells stained with the various lectins, and the two-side student's t-test was employed to determine if the control group was significantly different from the RA-treated groups.

Cell Membrane Fluidity Assay

Using 1,6-diphenyl-1,3,5-hexatrience (DPH) as a probe, the degrees of fluorescence polarization of treated and untreated cells were compared. Collect the cells and make single-cell suspension, adjust the concentration to 1×10^7/ml and mixed with 2 volume of DPH (20 μ mol/L in PBS). Incubate the cells at 25°C water bath for 40 minutes, then washed with PBS and measured the degree of fluorescence polarization P in a HITACHI 850 apparatus. Excitation was with polarized light at 359 nm and injection was at 428 nm. The fluorescence intensities (I) of horizontal(h) or vertical (v) were measured. Fluorescence polarization P and microviscosity M were calculated as follow: P=(Ivv- GIvh)/(Ivv+GIvh) G is a correction factor, G=Ihv/Ihh. M=2P/(0.46-P) 0.46 is the limiting value of P.[2]

Tumorigenicity and Metastasis

Male 615 mice were obtained from the Institute of Experimental Animal, CAMS. age among 6-8 weeks and weight between 18-20g. The experimental groups contained 20 mice that inoculated with RA treated cells (15 or 30 days), control group contained 10 mice that inoculated with untreated cells, each mouse was injected with 1×10^6 cells. The mice died after injecting 21 days in the control group and 27 days in the experimental group. Make the histology section of the lung and lymph node of the mice to determine the spontaneous metastasis rate.

RESULTS

Effect of RA on the Cell Surface Lectin Receptors

Lectins are a kind of carbohydrate on the cell surface. We inspect the change of RA treated cells in their lectin receptors' expression. After 15 day of growth in RA-supplemented medium, the MFC cells demonstrated decreased ability to bind with ConA, SBA and UEA but not with PSA and WGA. The mean fluorescence intensities of 3 of 5 lectins were decreased statistically significant from the control cells (Table 1).

Table 1. The mean fluorescence intensity of lectins on MFC cells

	ConA	SBA	UEA	PSA	WGA
Control group	94.89± 6.05	70.17± 3.32	81.80± 2.50	122.89± 10.05	107.65± 13.12
RAtreated group	75.27± 11.21*	48.56± 8.90**	61.11± 1.15*	116.08± 20.01	99.70± 19.01

Effect of RA on Cell Membrane Fluidity

According to the hypothesis of Fluid Mosaic Model, cell membrane is a two-dimensional fluid where the motions of individual molecules (lipids and proteins) are hampered by a viscous drag which should be expressible as a 'microviscosity'. The term 'microviscosity' is used to express the order and the rotational rate of lipid-chain motion.[3] As the microviscosity is negative correlation with the membrane fluidity, we can use the reciprocal of microviscosity to represent the membrane fluidity. As

show in Table 2 the cell membrane fluidity of RA-treated cells is greatly increased than that of control. Although the change could be detectable after treating with RA for 5 days, it did not show a time-dependent increase when the cells cultured with RA for as long as 60 days.

Table 2. Effect of RA on cell membrane fluidity

	Control	Treated with RA (days)			Removal of RA(days)	
		5	15	60	5	10
P	0.157	0.12	0.122	0.123	0.165	0.159
M	1.035	0.708	0.719	0.724	0.124	1.057
F	0.965	1.412	1.39	1.381	0.89	0.946

Effect of RA on the Spontaneous Metastatic Potential of MFC Cells

By 15 or 30 days of growth in RA-supplemented medium, the tumor cells demonstrated a prolonged latent period of tumorigenesis and a decreased spontaneous metastatic ability. The metastatic rate of MFC cells treated by RA for 30 days is 50% lower than that of controls. No lymph node metastasis was observed in any mouse (Table 3).

Table 3. Effect of RA on the metastatic potential of MFC cells

Group	latent period (days)	tumorigenesis	metastatic rate (%)
control	2.9	10/10	7/10 (70)
RA group (15d)	5.2	9/10	6/9 (66)
RA group (30d)	6.2	10/10	3/10 (30)

DISCUSSION

Tumor invasion and metastasis is a complex 'cascade' process, which involved in multistep, multicomponent and multigenes.[4-6] The interaction of tumor cells- extracellular matrix and cell-cell play an important role in this process.[5] A the function and structure of cell membrane affecting many steps of metastasis, such as cell adhesion, motility and cell recognition, the changes of component of cell surface may directly influence the cells' metastatic ability. In the last decade, some evidence has been obtained that the alteration of cell surface carbohydrates may affect the metastatic process by influencing cell-cell and cell-substratum interations.[7-9] Couch, et al. found that after 15 days of growth in the medium with 13-cis-retinoic acid, malignant KLN 205 cells demonstrated greatly increased expression of several lectin receptors and a greatly decreased proclivity to metastasize, as measured by the lung colony assay.[10] Opposed with that of Couch, our result showed that the expression of three lectin receptors on MFC cells treated by RA had been greatly decreased. At the same time, the spontaneous matastatic potential is redued either. In the process of cell transformation,a well-known property of most tumor or transformed cells is that they generally agglutinate at a much lower concentration of lectins compared to their untransformed counterparts.[1] It also be confirmed that the expression of lectin receptors on cell surface are increased gradually from normal, dysplastic and then malignant tissues.[11] So the changes of cell membrane lectin receptors induced by RA may indicate some changes on tumor cell membrane composition or function. What we know is that the changes on cell surface do correlate with the matastatic potential of tumor cells. The cell membrane fluidity is a general index that denotes the cell membrane composition and state. From the data in the literature, it appears that, with a few exceptions, tumor cells from solid tissues have a lower membrane fluidity than their normal analogues, while tumors of flowing cells (e.g. leukemia) have a higher membrane fluidity than their normal analogues.[3] As the MFC cells being from solid tumor, the increased membrane fluidity may be a sign that the cells have differentiated. On the other side, the changes of cell membrane fluidity may influence the position of membrane proteins, expose the tumor antigens that had been masked before, and up-regulate

the immunogenicity. The increased immunogenicity of tumor cells can incite the antitumor immunity of the host, and indirectly diminish the metastatic potential of tumor cells.[12] So the effect of RA on tumor membrane may be one of the mechanisms that inhibit tumor growth and metstasis.

REFERENCES

1. Nicolson GL. Transmembrane Control of the Receptors on Normal and Tumor Cells. Biochem Biophys Acta 1976; 458:1.
2. Wan HS, Zhaso XM, Gao J. Isolation of Clones with Different Metastatic Potentials and Study on Their Membrane Lipid Properties. Chinese J of Laboratory Animal Science 1993; 3:102.
3. Shinitzky M. Membrane Fluidity in Malignancy. Biochem Biophys Acta 1984; 738:251.
4. Starkey JR. Cell-matrix Interactions during Tumor Invasion. Cancer Metastasis Rev 1990; 9:113.
5. Glinsky GV. Cell Adhesion and Metastasis: Is The Site Specificity of Cancer Metastasis Determined by Leukocyte-Endothelial Cell Recognition and Adhesion? Crit Rev Oncol/Hematol 1993; 14:229.
6. Yamamoto T. Molecular Basis of Cancer: Oncogenes and Tumor Suppressor Genes. Microbiol Immu 1993; 37:11.
7. Inokuchi JI, Jimbo M, Monosaki K, et al. Inhibiti of Experimental Metastasis of Murine Lewis Lu Carcinoma by An Inhibitor of Glucosylcerami Synthase And Its Possible Mechanism of Actic Cancer Res 1990; 30:6731.
8. Zhang L, Underhill CB, Chen L. Hyaluronan on t Surface of Tumor Cells Is Correlated with Metastat Behavior. Cancer Res 1995; 55: 428.
9. Edward M, Gold Ja, Mackie RM. Retinoic Aci Induced Inhibition of Metastatic Melanoma Cell Lur Colonization and Adhesion to Endothelium ar Subendothelial Extracellular Matrix. Clin Ex Metastasis 1992; 10: 61.
10. Couch MJ, Pauli BU, Weinstein RS, et a Modulation of The Metastatic Phenotype by 13-*cis* retinoic Acid. JNCI 1987; 78:971.
11. Lu LM, wang FR, Wang ZY, et al. Study of Th Pisum Sativum Agglutinin Receptors and DNA Ploid During Carcinogenesis in Esophageal Epithelium b Flow Cytometry. Chinese J Pathol 1992; 21:355.
12. Kroemer G, Alboran IM, Gonzalo JA, et al Immunoregulation by Cytokines. Crit Rev Immuno 1993; 13:163.