DIFFERENCES IN ADHESION PHENOTYPE OF NORMAL T HUMAN LYMPHOCYTES AND CELLS FROM HIGH METASTATIC CELL LINE JURKAT UP TO STATIC AND DYNAMIC CONDITIONS

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ABSTRACT

Metastatic tumor spread is pathologic process with series of adhesion/de-adhesion events. In this context, adhesion to, and detachment from the components of the ECM could be important measure for the invasive potential of cancer keratynocytes cells. Human T lymphocytes and Jurkat cell line were used to study the rates of adhesion to fibronectin and other ECM proteins under static and dynamic conditions. To measure the critical share force resulting in detachment of cancer cells from the protein-coated substrata, a special flow chamber was applied. Thus, we found correlation between adhesive behavior and metastatic potential of cancer cells in respect of fibronectin in static and dynamic conditions: In static condition both cell preferably adhere to it. In dynamic conditionshuman T lymphocytes showed better adhesiveness to fibronectin to the flow rate then high metastatic Jurkat cells. Our results provide new evidences that in some distinct stage of invasion and metastasis tumor cells might perform different adhesive features.

KEY WORDS. *cell adhesion,lymphocytes, metastatic potential, electroporation*

INTRODUCTION

An important feature of malignant cells is the invasive growth. This allows them to leave the compartment to which they normally are restricted, gain access to the connective tissue and the blood vessels and to complete the initial phase of the process of metastasis [Lester et all 1992]. Metastasis is a multistep process that includes detachment of cells from the tumor mass, degradation of basement membranes, migration, adhesion and proliferation at the second site [Akiyama et al., 1995]. All these steps require series of cell adhesive interaction or loss of adhesion [Van der Flier et al., 2001]. Tumor cell adhesion to the components of the extracellular matrix (ECM) and basement membranes is mediated by specific cell surface receptors, integrins [Pozzi et al., 2003] that bind to ECM adhesive proteins such as fibronectin (FN) [Wu et al., 1995], laminin and collagens [White et al., 2004]. Fibronectin is an adhesive heterodimeric glycoprotein present in the ECM of connective tissues in disulfide cross-linked insoluble fibrils and in the blood in dimeric soluble form [Danen et al., 2001]. The addition of FN to tumor-derived cultured cells improves cell adhesion and induces ECM and cytoskeleton organization, supporting the normal cell morphology, FN has been associated with the normal cell phenotype.[Brooks et al., 1995]. The main FN-receptor, α5β1 is suppressed on the surface of cancer cells and predominantly they adhere via αVβ3-integrin receptor, known as integrin receptor for vitronectin [Stwert et al., 2004]. The integrin repertoire expressed in tumor cells differs also to that of their non-neoplastic counterparts [Forgerty et al., 1990] and the most consistent change accompanying tumor progression being the shift to expression of αvβ3 integrins [Brooks et al., 1995]. It is well documented that cancer cells alter their adhesive properties to the extracellular matrix components [Forgerty et al., 1990], and, particularly, the loss of adhesion to fibronectin is associated with neoplastic changes [Danen et al., 2001]. The present study is focused on the possible differences in the properties of cancer cells adhered to immobilized different ECM proteins, under static and dynamic conditions. For this purpose we choseT human lymphocytes and high metastatic Jurkat cell line because of the existing of high metastatic lymphoma Jurkat. Details of this investigation are reported herein.

Cell Cultures

Jurkat, Clone E6-1 cells were maintained in 10% FBS (Hyclone) in RPMI-1640 (ATCC) supplemented with 100 units/ml penicillin and 0.1 mg/ml streptomycin (Sigma-Aldrich). The cells were kept in a humidified atmosphere, at 37°C, containing 5% CO2. The cells were seeded at a cell density of 3×10^5per ml for all experimental designs. with Trypsin-EDTA during the log-phase growth according to standart protocols [Owens et al., 1987] before seeding on FN-coated surfaces (see below).

Purifiaction of fibronectin

FN was prepared from human plasma by affinity chromatography on gelatin- Sepharose 4B columns according to the method of Engvall and Ruoslahti and stored in 4M urea. Before use, the FN was transferred to 120 mM NaCl (Boeringer), 50 mM Tris, pH 7, 3 by gel filtration with Sephadex G25, and stored at 4C.

Preparation of protein coated surfaces

Proteins were dissolved in PBS phosphate buffered saline of the following composition: 150 mM NaCL, 5, 8 mM Na2HPO4, pH=7, 4. the final concentrationof the proteins were FN (20 μg/ml) was chosen to ensure surface saturation using protein adsorption data from the literature [14], BSA100μg/ml, FBG 100μg/ml, VN10 μg/ml. The adsorption procedure was performed as follow: glass coverslips (22x22 mm, Assistent, Germany) were placed in 6 well tissue culture plates (Costar) and coated with proteins for 30 min at RT. Then the plates were washed with PBS three times and 1 ml suspension of $5x10^5$ of cancer cells.Jurkat or T human lymphocytes was added left to spread for 4h in humidified CO2 incubator. This protocol was used for study the static adhesion.

Cell Adhesion Assay

Glass coverslips were placed in 6 well tissue culture plates (Costar) coated with FN as above, $5x10^4$ human T lymphocytes or cancer cells. Jurkat suspended in 1 ml DMEM medium containing 10% serum were added and leaved to attach for 4 h at 5%/CO_2/95% air humified atmosphere. Cell adhesion was quantified via counting the mean cell number in 15 randomly chosen 10x10 mm squares onthree low magnification (10x) pictures per sample. Statistical analysis was carried out using GraphPad InStat software and data are expressed as means and standard deviation (S.D.).

Measurment of cell detachment under flow conditions

In previous study [Altankov, G., and T. Groth, 1994] we have useded special flow chamber for studying the strength of lymphocyte attachment to different protein-coated substrata. Here we apply the same method to characterize the tumor cells interaction with FNand FNG. The general construction of the flow chamber has been described elsewhere [Altankov, G., and T. Groth, 1994]. Briefly,PBS from an elevated reservoir was passed through a rectangular flow channel in which a glass coversleep formed the upper wall. The flow rate through the chamber was regulated by means of precision micrometer flow valve (Nunpro type M, purchased from North London Valve and Fittings, London, UK). Under the experimental conditions, laminarity of flow was confirmed both theoretically and practically for the conduit [Owens et al., 1987].

RESULTS

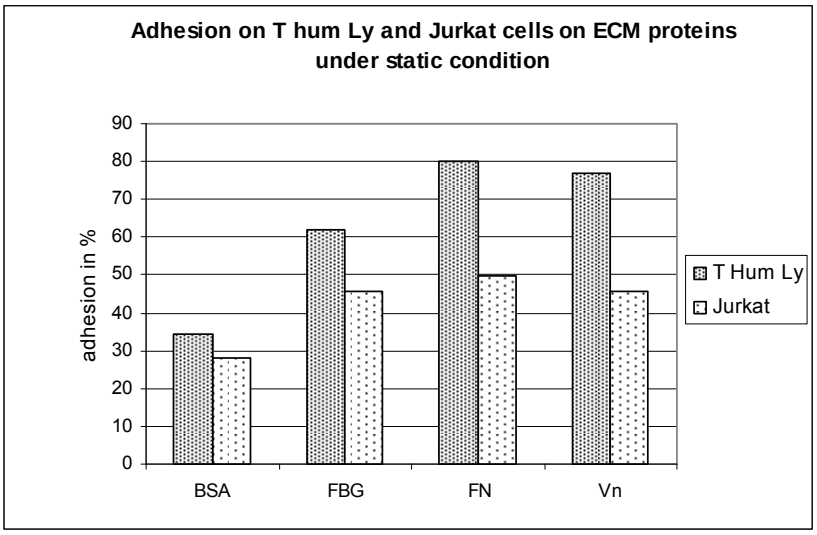

Fig. 1. *Adhesion of* of cancer *cells. Jurkat and Human T lymphocytes line on different ECM proteins under static conditions*

This chart represents the adhesive behavior of of cancer cells. Jurkat and Human T lymphocytes on different extracellular matrix proteins, including (FN), fibrinogen (FBG), vitronektin (Vn) and albumin (BSA). In comparison to the control (BSA coated glass), both cell lines show clear tendency to adhere well on these proteins, but fibronectin appears to be preferable adhesive subtsrate, also for Jurkat cells. This is was an additional reason to focus our furtherstudies on adhesive interactions of cancer cell with FN.

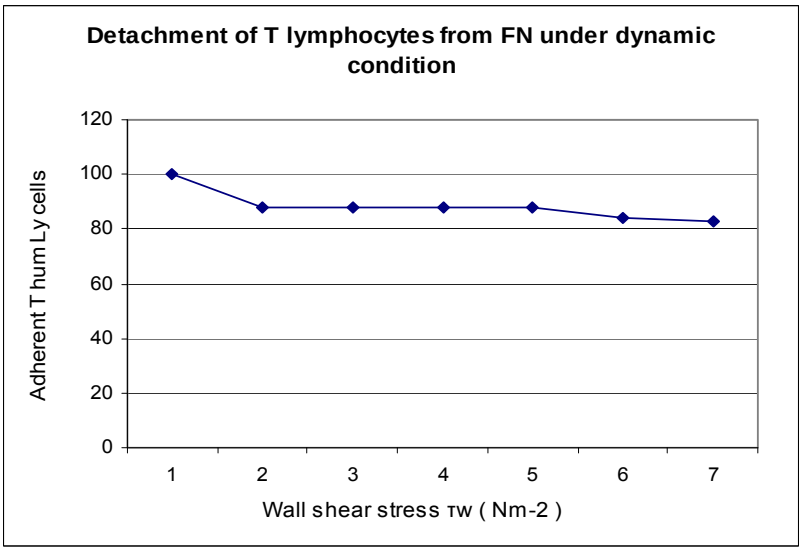

Fig. 2. *Deatchment of Human T lymphocytes from FN under dynamic flow conditions*

This chart demonstrates the detachment kinetics of Human T lymphocytes with increasing of the dynamic flow rate. The percent of adherent cells remaining on the substratum at the maximal shear force (10 N/smI) is significantly higherr, about 80% interactions with FN in the comparison to the Jurkat cells(50%).

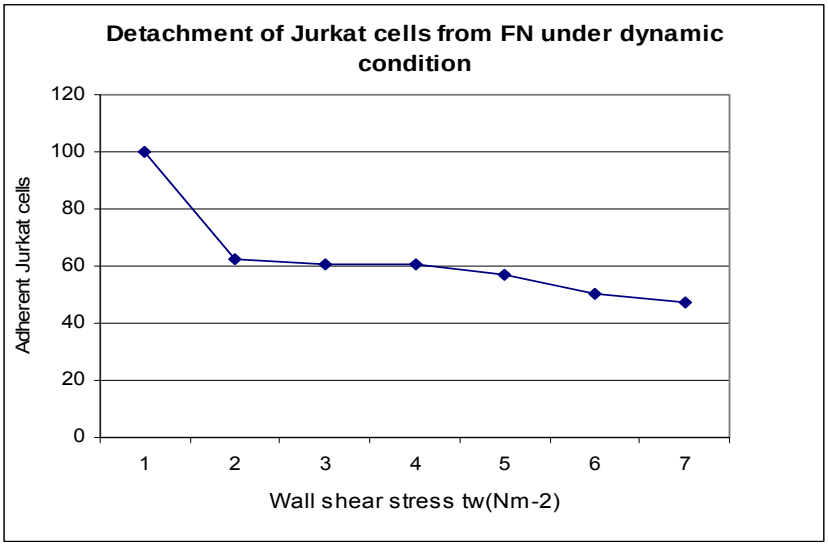

Fig.3. *Deatchment of Jurkat cells cultured on FN under dynamic flow conditions (seeMaterials&Methods).*

The Jurkat cells again demonstrated low adhesive interaction as in static adhesion assay. Approximately only half of the cells remains attached to the FN-substrate (50%) after applying the maximum shear force (10 N/sm2)). But it is high percent for cancer high metastatic cells, because as knows metastatic cells have very low adhesion affinity to ECM proteins.

DISCUSSION

The location of secondary tumors is determined, in part, by anatomical blood flow and by interactions between tumor cells and host organs [Lester et all 1992]. The adhesive molecules, such as integrin receptors and matrix proteins are involved in the tumor progression. These adhesive interactions are between the tumor cells themselves, as well as between tumor cells and the target tissue [Felding-Haberman B., 2003]. The interaction with fibronectin seems to play important role, because it reported to be strongly influenced by the process of carcinogenesis [Van der Flier et al., 2001]. It is difficult to follow these adhesive interactions *in vivo*, and in this work we developed a model cell system that is closer to in vivo conditions. The cell line used in this investigation provides another

advantage, an opportunity to compare adhesive behavior of cells with different metastatic potential in vivo. Our data demonstrated that high metastatic cells have lower affinity to FN.

This results is contradictory to the expectations that high metastasis cancer cells. Jurkat should be less associated to the matrix, and hence, easier to leave the place of primary tumor. On the other hand, metastasizing cancer cells need to be attached, hence to increase its adhesiveness to the ECM. Under dynamic flow conditions the of Human T lymphocytes show higher adhesiveness to fibronectin that might result in the subsequent instability to the flow rate.

Under dynamic flow conditions the of Human T lymphocytes and Jurkat cells are in condition near to blood stream. It is one good possibility to use electroporation with anticancer drugs for lymphoma treatment.

In conclusion, our results point to the possibility that in some distinct stage of progression tumor cells gradually change their adhesive phenotype. It likely appear in the moment of “switch” from invasion to metastasis, reflecting the series of adhesionde- adhesion events of cancer cells with surrounding ECM matrix.

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