J Cancer Res Clin Oncol (2000) 126:699–706

ORIGINAL PAPER

Jörg Haier · Garth L. Nicolson

Tumor cell adhesion of human colon carcinoma cells with different metastatic properties to extracellular matrix under dynamic conditions of laminar flow

Received: 23 November 1999 / Accepted: 4 May 2000

Abstract ***Purpose:*** Shear forces have an important influence on cell adhesion and other cellular functions, and malignant cell lines appear to possess different adhesive properties under static and dynamic conditions. Thus, we analyzed human colon carcinoma cell adhesion under dynamic conditions and examined the interactions of HT-29 colon carcinoma cells of different metastatic properties with various immobilized ECM components. ***Methods:*** Wall shear adhesion threshold (WSAT), dynamic adhesion rate (DAR), and adhesion stabilization rate (ASR) were compared between the cell lines using dynamic conditions in a laminar flow chamber by decreasing the flow (wall shear stress) of cell suspensions. Patterns of cell adhesion under dynamic conditions were compared to adhesive interactions in static microtiter-plate assays. ***Results:*** Poorly metastatic HT-29P cells adhered six times more than highly metastatic cells to type I collagen under laminar fluid flow, whereas only highly metastatic HT-29LMM showed adhesive interactions with fibronectin under static and dynamic conditions. High rates of cell adhesion to collagen IV were found under static, but not under dynamic, conditions. ***Conclusions:*** Although poorly and highly metastatic HT-29 cells express similar patterns of integrins, they differ in their adhesive properties to ECM components under static and dynamic conditions. Hydrodynamic shear forces appear to influence adhesive properties of HT-29 cells, and differences between dynamic and static cell adhesion were found.

J. Haier · G. L. Nicolson
The Institute for Molecular Medicine,
15162 Triton Lane,
Huntington Beach, CA 92649, USA

J. Haier (✉)
Dept. of Surgery, University Hospital B. Franklin,
Free University of Berlin,
Hindenburgdamm 30, 12200 Berlin, Germany
Tel.: +49-30-84452543; Fax: +49-30-84452740
e-mail: jhaier@ukbf.fu-berlin.de

Key words Colon carcinoma · Metastasis · Extracellular matrix · Integrins · Laminar flow · Dynamic adhesion

Introduction

The sequential model for the development of metastases involves tumor growth, neovascularization, and invasion at the primary sites, followed by penetration into lymphatics and blood vessels or through the peritoneum (Nicolson 1989). Tumor cells must detach from primary sites, and at the end of this process, circulating tumor cells must adhere to vessel walls of distant host organs, invade surrounding tissues, and survive and grow (Nicolson 1988). During these steps different interactions between tumor cells and the extracellular matrix (ECM) or surrounding cells are required at primary and secondary sites (Nicolson 1995). Various molecules on tumor cell surfaces mediate these interactions, either by direct adhesive contacts, such as integrins or other adhesion molecules, or indirectly, such as receptors for soluble peptide hormones or receptors for various growth factors that can modulate cell adhesion (Burtin et al. 1983; Lindmark et al. 1993; Takazawa 1995). Usually the same adhesion and receptor molecules are also found on normal cells where they function in the maintenance of normal tissue structure and cellular regeneration (Agrez and Bates 1994).

Integrins mediate adhesion through various ECM-binding sites, depending on the involved matrix components, and these adhesive interactions are important determinants of organ-specific metastasis (Nicolson 1991). Recently, we found that poorly and highly metastatic colorectal carcinoma cells possessed different patterns of integrin-mediated adhesion to various ECM substrates (Haier 1999a), although these cell lines expressed similar patterns of integrins (Haier 1999b). Integrins and other cell adhesion receptors on tumor cells can generate cellular regulatory signals that allow them to control cell migration and invasion into host organs (Hanks and Polte 1996; Richardson and Parson 1995).

Thus, distinct intracellular events during the adhesion of carcinoma cells to host organs may be required (Miyamoto et al. 1995a), such as those that induce various functional cellular responses, including tyrosine phosphorylation and subsequent activation of various signaling cascades (Schwarz et al. 1995). For example, integrin-mediated signal transduction is often required for adhesive properties, and the regulation and modulation of integrin-binding affinity and kinetics (Haier 1999a). The functional status of integrins, which are known for their activation or increased affinity to bind to ECM components (Ginsberg et al. 1990), appears to be regulated by complex interactions with a number of cytosolic, cytoskeletal, and membrane-bound proteins (Chen et al. 1994; Miyamoto et al. 1995b).

Shear forces that act on cells under flow conditions in the circulation can modify various cellular functions, including phosphorylation events and cytoskeletal alterations. For example, local alterations in the hemodynamic environment, such as different shear forces, can regulate endothelial cell (EC) functions (Ishida et al. 1996). Pathways that are responsible for these shear stress-mediated responses during integrin interactions between circulating cells and ECM components include: activation of ion channels and various tyrosine kinases (such as c-Src, focal adhesion kinase [FAK], and mitogen-activated protein kinases [MAPK]) and autocrine production and release of growth factors (Lehoux and Tedgui 1998; Takahashi and Berk 1996). The focal adhesion complex plays an important role in shear stress-induced signaling, and signal transduction might involve cross-talk signaling induced by integrins, or it could be directly mediated by these adhesion molecules (Ishida et al. 1997). For example, it has been shown that various signaling proteins related to integrin function, such as FAK, can alter their phosphorylation status in EC depending on applied shear forces (Ishida et al. 1996). Furthermore, it appears that the structural integrity or organization of cytoskeletal components, such as actin filaments, can be important in shear stress-induced responses (Okuyama et al. 1996; Li et al. 1997).

Static cell adhesion assays are widely used for characterization of adhesive properties of various normal and tumor cell lines. Unfortunately, these assays do not consider hydrodynamic forces, such as wall shear stress (WSS) or the parabolic form of laminar flow that occurs within the microvasculature. Using specific parallel plate laminar flow chambers for hydrodynamic adhesion assays the different phases of adhesive interactions between circulating cells and vascular surfaces (EC or ECM) can be studied separately (Menter et al. 1995). The conditions of hydrodynamic adhesion in these reports revealed that both biophysical and biochemical interactions were involved in cell adhesion. In this study, we investigated the adhesive interactions of human HT-29 colon carcinoma cells of different metastatic properties to various ECM components under dynamic conditions of laminar fluid flow and compared these results with static tumor cell adhesion.

Materials and methods

Tumor cell lines and materials

Cell adhesion properties were studied using human HT-29 colon carcinoma cells. Poorly metastatic HT-29P and highly metastatic HT-29LMM cells (Price et al. 1989) were cultured in a 1:1 mixture of Dulbecco-modified Eagle's medium/F12 medium (DME/F12, 1:1 v/v) containing 5% fetal bovine serum (FBS) without antibiotics in humidified 5% CO_2/95% air at 37 °C. Cells were harvested with Trypsin/EDTA during the log-phase of growth. After trypsinization, the cells were resuspended in serum-free adhesion medium (DME/F12, containing 1% bovine serum albumin [BSA]) for reconstitution of surface proteins, and they were then washed extensively in calcium-magnesium-free phosphate buffered saline solution (CMF-PBS).

DME/F12 was purchased from Irvine Scientific (Irvine, Calif., USA); FBS from GIBCO-BRL (Rockville, Md., USA); collagen I (C I), collagen IV (C IV) and poly-L-lysine were obtained from Sigma (St. Louis, Miss., USA), and mouse laminin (LN), human fibronectin (FN) and Matrigel were from Collaborative Biomedical Products (Bedford, Mass. USA).

Laminar flow chamber for dynamic tumor cell-ECM interactions

Using a ECM-coated glass support and a polycarbonate shear deck to form a uniform channel with a small height-to-width ratio, a parallel plate flow chamber was constructed as described (Haier et al. 1999c). Briefly, the parallel plate flow chamber was built with a specific polycarbonate shear deck (Grace Bio-Labs, Bend, Ore., USA) as the upper surface, and glass slides as the chamber bottom. The distance between the shear deck and glass slide was ensured by a medical grade silicon gasket. The geometry of the channel between the two parallel plates resulted in an internal flow chamber ($40 \times 22 \times 0.2$ mm) with a high width-to-height ratio and allowed an approximation of laminar flow. Both parts were held in place by metal clamps that allowed the use of the flow chamber on an upright microscope under low-power magnification (1:63.5). A uniform fluid flow of single cell suspensions was maintained using a standard syringe pump (Fresenius, Bad Homburg, Germany) that was connected to one end of the shear deck with silicone tubes. Fluid flow Q between 0.18–1 ml/min was used; this occurred as a laminar flow with a parabolic velocity within the chamber (Reynolds number < 2). Under these conditions the wall shear stress τ was calculated for Newtonian fluids as follows:

$$\tau = 6\,\mu Q/h^2 w = (6\,\mu/h^2 w)Q$$

[Viscosity μ for H_2O at 37 °C = 0.7 cP; τ = wall shear stress (dyn/cm2); h = channel height; w = channel width.]

Protein-coated glass slides

Glass slides (75×25 mm) were cleaned using detergent solution (0.1% sodium dodecylsulfate in CMF-PBS), rinsed with distilled H_2O, followed by 95% ethanol. ECM components C I, C IV, LN, or FN (2.5 μg/cm^2) were applied to dried glass slides in a 10 cm^2 area that approximated the area of the parallel plate flow chamber. Alternatively, Matrigel (4 μg/cm^2) as a mixture of various ECM components or poly-L-lysine (2.5 μg/cm^2) as nonspecific adhesive substrate were also used. The slides were allowed to dry completely, and rehydrated (CMF-PBS) slides were used immediately. BSA-coated glass slides (0.5 ml CMF-PBS containing 1% BSA) were used as negative controls.

Flow experiments

Tumor cells were resuspended in serum-free adhesion medium at a concentration of 2×10^5 cells/ml. The temperature of the cell suspension was kept at 37 °C. Flow experiments were performed with

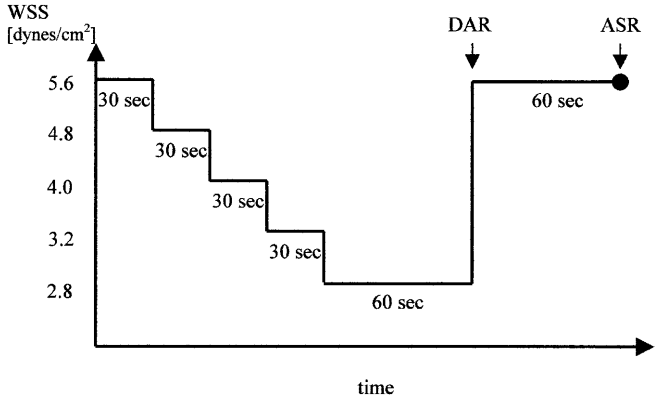

Fig. 1 Profile of wall shear stress for evaluation of dynamic adhesion. Fluid flow was initiated at a high level and decreased in 30 s increments. Adhesive properties of tumor cells were analyzed using WSS levels at which the first occurrence of different types of cell-ECM interactions occurred, and the total number of adherent cells found under low flow conditions, and relative numbers of cells with stabilized adhesion were calculated. Dynamic adhesion rate (DAR) was determined as the number of adherent cells at the end of the low flow period, adhesion stabilization rate (ASR) was calculated as the relative number of cells that were able to resist high flow rates at the end of the low flow period

variable fluid flow starting at a WSS of 5.6 dynes/cm^2. Flow rates were decreased in 30 s intervals so that the WSS equaled 4.8, 4.0, 3.2, or 2.4 dynes/cm^2. The WSS at the point of initial cell adhesion was used to measure wall shear adhesion threshold (WSAT). The dynamic adhesion rate (DAR) represented the total number of adherent cells after 60 s at a flow rate of 2.4 dynes/cm^2 which was approximately 50% of WSAT and in the range of microvascular flow conditions. Following this low flow interval, the flow rate was increased immediately to the starting level in an attempt to detach adherent cells that had not achieved adhesion stabilization during the low flow interval. The relative percentage of cells remaining adherent after 60 s in relation to DAR was calculated as the adhesion stabilization rate (ASR). Figure 1 shows the time course of WSS during the flow experiments. All experiments were repeated five times and results are shown as mean ± SD.

Static adhesion assay

Microtiter plates (96-well, Corning) were coated with C I (50 μg/ml, 150 μl/well) or 1% BSA (negative control) at 37 °C for 3 h. Effective concentrations were determined in previous experiments (data not shown). Blocking of nonspecific binding sites was performed using 1% BSA (37 °C, 30 min, 200 μl/well). During reconstitution of surface proteins in adhesion medium, cells were fluorescent-labeled with CalceinAM (20 μg/15 ml). After washing, cells were resuspended in adhesion medium at a final concentration of 0.5×10^6 cells/ml. Adhesion experiments were performed with 150 μl cell suspension/well for different times (10–120 min) as previously described. (Haier et al. 1998).

Cell spreading

Microtiter plates (24-well, Corning) were coated and blocked as described previously (Haier, 1999a, b). Cells were resuspended in serum-free adhesion medium at a final concentration of 1×10^5 cells/ml and added to the wells. After different adhesion time periods (30–90 min), the medium was carefully removed, and the cells were fixed using ice-cold 3% paraformaldehyde. Plates were kept refrigerated until evaluation. Assessment of cell spreading was performed on an inverted phase-contrast microscope (Nikon TMS) under 200× magnification.

The relative numbers of cells that showed cell spreading were evaluated in three different randomly chosen fields. A total number of 250–300 cells was investigated for each well. Specific morphological criteria for cell spreading were loss of sharp cell borders, development of pseudopodia, alteration in cell diameters, and changes of nuclear:cytoplasmic ratios. Three different categories of cell spreading were defined as follows: (a) no cell spreading; (b) intermediate status (some indications of cell spreading); and (c) clear signs of cell spreading. Intermediate status was defined mainly if the cell diameter was increased, but the nucleus was still more than 2/3 of the cell width. Loss of sharp cell borders and development of pseudopodia were used as clear signs of cell spreading. Only cells with clear signs of spreading were considered for further analysis. All experiments were repeated three times and results were shown as mean ± SD.

Statistical analysis

Statistical analysis was performed using the StatMost32 statistical program (DataMost, Los Angeles, Calif., USA). P values were calculated according to Student's t-test. Significant differences were accepted for $P < 0.05$.

Results

Dynamic adhesion to ECM components

We investigated the adhesive properties of both cell lines under dynamic conditions of laminar fluid flow. During the first series of experiments different ECM components were used to compare adhesive interactions between poorly and highly metastatic cells. Different rates of WSS resulted in distinctive levels of interactions between HT-29 cells and ECM-coated surfaces. We observed three different types of cell-ECM interactions: (1) cell rolling: this was independent from WSS at all flow rates and type of coating and was characterized by rolling of cells on the lower surface of the laminar flow chamber without stopping. Cell rolling was defined as significant reduction of speed of cell movement upon cell contact with the ECM surface compared to the average speed of cells that did not contact the surface. These cells demonstrated a rotation along the laminar flow, whereas free flowing cells did not show this; (2) cell sticking or initial adhesion: the decrease in WSS allowed cells to arrest for less than 1 s without definitive adhesion, or they demonstrated very slow movement with the flow or initiated crawling on the ECM surface. Both types of initial adhesion occurred independent from each other. Initial cell sticking was also found to immobilized BSA (nonspecific control), but at lower WSS than to ECM components; (3) definitive or stabilized cell adhesion: further reduction of WSS enabled cells to establish definitive adhesion (for >1 s). Cells achieved stabilized adhesion independent from crawling or sticking, and the WSS where first cells reached stabilized adhesion was defined as WSAT. This type of adhesion was specific for ECM components, and definitive adhesion to BSA was found for only a few cells (Fig. 2). Initial specific

cell-ECM interactions occurred below WSS of 5.6 dynes/cm^2; however, by using different ECM components there were remarkable differences in dynamic adhesion.

The highest rates of dynamic adhesion were seen for HT-29P cells if C I was used as an adhesive substrate. The number of HT-29P cells that stabilized their adhesion to C IV at lower WSS was slightly increased compared to BSA-coated surfaces, but these differences were not significant. Using LN or FN as adhesive substrates stabilized adhesion of HT-29P was not observed. Cells were able to resist increased WSS at the end of the low flow period only if they were bound to C I, and ASR was determined to be 73 ± 12% of initially adherent cells for C I compared to 35 ± 25% for BSA-coated slides. In contrast, highly metastatic HT-29LMM cells did not show stabilized adhesion to C I, C IV or LN. Using FN the initial interactions of HT-29LMM were increased, and the number of adherent cells was significant higher at high WSS compared to BSA-coated surfaces ($P < 0.05$). This observation was supported by a significantly higher WSAT for dynamic adhesion of HT-29LMM cells to FN (FN, 4.8 ± 0.7 dynes/cm^2; BSA, 3.5 ± 0.4 dynes/cm^2; $P < 0.05$). At lower WSS, however, the number of cells that were able to stabilize their adhesion to FN was only slightly increased compared to BSA and the other ECM components. We also observed that HT-29LMM cells demonstrated considerably increased sticking and crawling on C IV, but this behavior did not result in increased WSAT or stabilized adhesion (Table 1). In Fig. 2 the previously reported dynamic cell adhesion of HT-29P (Haier, 1999c) is compared to dynamic cell adhesion of highly metastatic HT-29LMM cells to various ECM components under the same conditions.

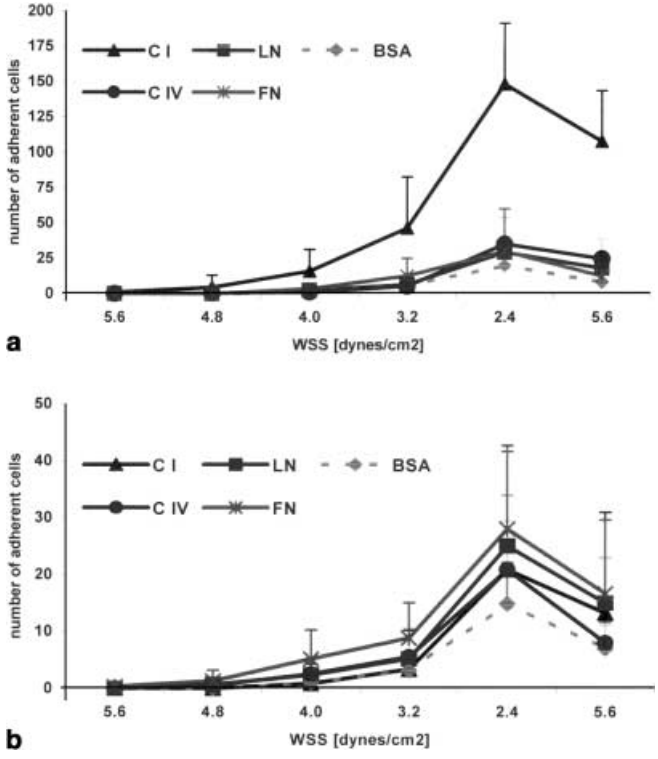

Fig. 2a, b Dynamic adhesion of HT-29 colon carcinoma cells to various immobilized ECM components. (**a**) HT-29P as previously reported (Haier, 1999c); (**b**) HT-29LMM. Numbers of adherent cells to each ECM component at the end of each time period are given from five different experiments

To further evaluate the specificity of the different types of adhesive interactions between tumor cells and ECM we used Matrigel and poly-L-lysine as adhesive substrates in dynamic adhesion experiments. Matrigel is a mixture of various ECM components, mainly C IV and LN. Both cell lines did not adhere to Matrigel-coated surfaces under dynamic conditions, and all parameters were comparable to BSA. Poly-L-lysine is a nonspecific adhesive substrate and integrins are not involved in specific cell interactions with this substrate. Both cell lines showed similar adhesive properties in these experiments. Immediate adhesion of very high numbers of cells (>300) was observed at all flow rates. Although cell rolling on the poly-L-lysine surface was seen in the same manner as seen with the other substrates, the cells never demonstrated sticking or crawling. We were also unable to remove cells from this

Table 1 Dynamic cell adhesion to various ECM components: HT-29P and HT-29LMM. Mean and SD are given from five different experiments. Adhesion to ECM components was compared to BSA-coated surfaces using Student's t-test (*$P < 0.05$; ** $P < 0.01$) for each cell line. *PL* poly-L-lysine

ECM	Rolling	Sticking	WSAT (dynes/cm^2)	DAR (number of adherent cells)	ASR (%)
HT-29P					
BSA	+	–	3.4 ± 0.7	20 ± 13	35 ± 25
C I	+	+	4.5 ± 0.7*	148 ± 43**	73 ± 12**
C IV	+	+	3.5 ± 1.2	33 ± 18	48 ± 18
LN	+	+	3.5 ± 0.4	26 ± 18	52 ± 18
FN	+	+	4.0 ± 0.0	24 ± 9	39 ± 8
PL	+	+	> 5.6	> 300	100%
HT-29LMM					
BSA	+	–	3.5 ± 0.4	15 ± 7	49 ± 18
C I	+	+	3.6 ± 0.7	21 ± 13	59 ± 14
C IV	+	+	3.9 ± 0.8	21 ± 9	40 ± 12
LN	+	+	4.0 ± 0.9	25 ± 18	50 ± 23
FN	+	+	4.8 ± 0.7*	28 ± 14	53 ± 20
PL	+	+	> 5.6	> 300	100%

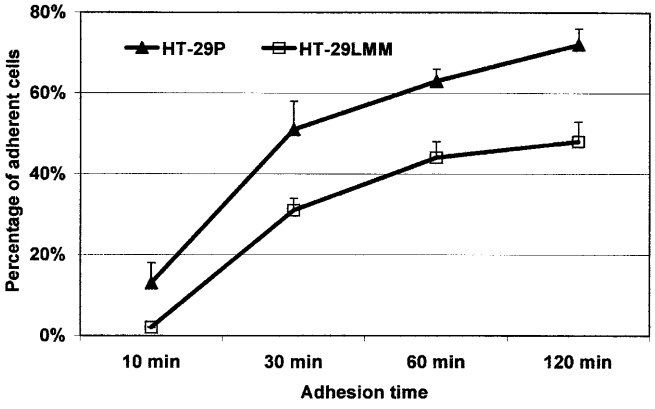

Fig. 3 Relative adhesion of poorly (HT-29P) and highly (HT-29LMM) metastatic cells to C I. Adhesion is expressed as relative fluorescence at different times compared to total fluorescence in the cell suspension; background fluorescence was calculated using adhesion to BSA-coated surface and was subtracted; background fluorescence below 1% in all experiments. Differences were significant ($P < 0.05$) at all times

substrate, even at WSS of 10 dynes/cm^2. Obviously, adhesion stabilization occurred immediately after initial cell contacts with immobilized poly-L-lysine. This resulted in WSAT > 5.6 dynes/cm2, DAR > 300 cells and ASR = 100% for both cell lines.

Adhesion to collagen

Time-dependent adhesion to C I was measured after 10–120 min, and after 60–90 min plateau values were observed. Extension of adhesion assays for more than 120 min resulted in decreased adhesion, probably because of the release of proteases and degradation of ECM. HT-29LMM cells had significantly poorer rates of adhesion to C I ($P < 0.05$) than HT-29P cells (Fig. 3).

Cell spreading

C I and C IV mediated specific HT-29P and HT-29LMM cell spreading. Similar rates of spreading cells were found for both cell lines using C IV as an adhesive substrate, whereas more HT-29LMM spread on C I compared to HT-29P cells. After 90 min about 50% of both cell lines fulfilled the criteria for cell spreading if they were plated on C IV, and 27% (HT-29P) to 49% (HT-29LMM) of cells demonstrated these signs after 90 min using C I. Consistent with our previous results that HT-29LMM cells adhered to FN, whereas HT-29P cells did not show adhesive interactions with this ECM component, HT-29LMM but not HT-29P cells spread on FN. Similar results were obtained if LN was used as an adhesive substrate, and HT-29P cells did not show significant cell spreading on this ECM component. However, 23% of HT-29LMM cells spread on LN after

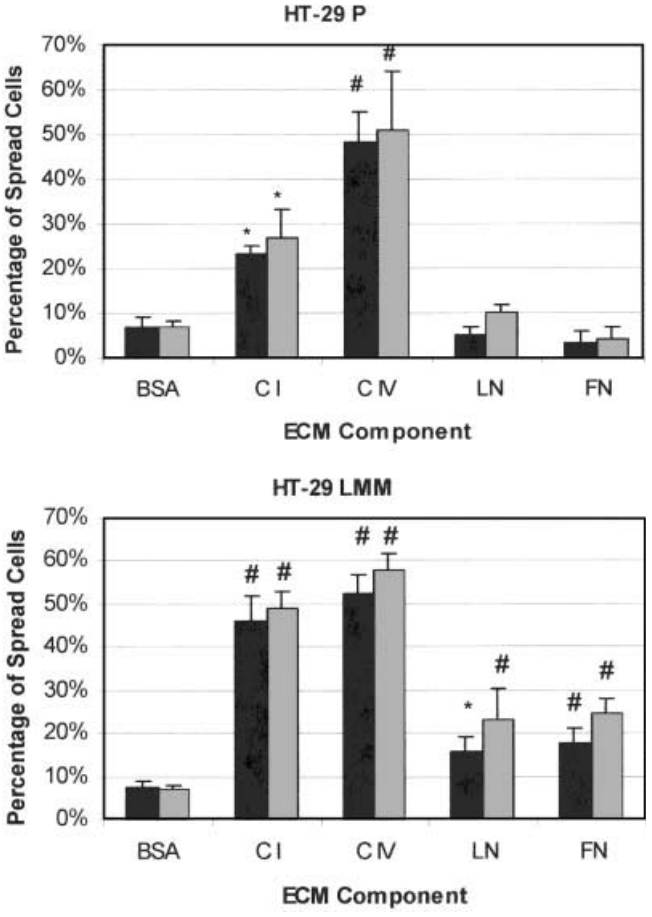

Fig. 4a, b Percentage of spread HT-29 cells on various immobilized ECM components after different adhesion periods. (**a**) HT-29P; (**b**) HT-29LMM. Results are shown as mean ± SD from three different experiments after 30 min (*full bars*) and 90 min (*hatched bars*). Statistical analysis was performed to compare spreading on ECM components with BSA using Student's *t*-test (*$P < 0.05$; ** $P < 0.01$)

90 min. Less than 8% of cells showed clear signs of cell spreading if plated onto BSA-coated surfaces (negative control) (Fig. 4).

Discussion

Fluid shear stress can modulate cellular functions and structures by stimulating mechanosensitive cell signaling (Takahashi et al. 1997). Cell adhesion mediated by integrin-matrix interactions also regulates intracellular signaling by mechanosensitive events (Takahashi et al. 1996). Two mechanisms have been proposed for regulating dynamic interactions of adhesion molecules with vascular walls under flow conditions: (a) the intrinsic kinetics of bond establishment; and (b) the susceptibility of the bond dissociation reaction (Alon et al. 1997). During blood-borne metastasis, tumor cells have to detach from the primary site by breaking adhesive interactions with cells and ECM and subsequently successfully arrest in the microvasculature of target

organs. This later step requires stable adhesive interactions with EC and ECM at the secondary site in the presence of hydrodynamic shear forces (Pauli et al. 1990). The ability of metastasizing tumor cells to form stable adhesions that can withstand shear forces is likely to be more important for blood-borne metastasis formation than adhesive properties measured under static conditions. Different types of adhesion receptors and related molecules can mediate or modulate distinct adhesive events, such as initial cell rolling along the endothelium and subsequent adhesion stabilization (McIntire 1994). For example, selectins appear to be mainly responsible for initial contacts between flowing cells and EC, whereas integrins are more likely to be involved in adhesion stabilization (Lawrence and Springer 1991).

Changes in cell adhesion may influence cell motility at the primary tumor locations and the establishment of new adhesive contacts at secondary tumor sites. Reduced tumor cell adhesion can lead to increased motility of tumor cells (Danecker et al. 1989; Aznavoorian et al. 1990), but at secondary sites new adhesions have to be established. Increased adhesive properties towards certain components may be a prerequisite for metastasizing cells, allowing access to certain secondary locations, but only certain levels of adhesion may allow those cells to leave the circulation. The formation of stabilized adhesions to the vascular wall under shear flow conditions appears to be a specific requirement for blood-borne cells that have the capacity to form secondary tumors.

Our results showed higher dynamic adhesion of poorly metastatic HT-29P cells to C I compared to highly metastatic HT-29LMM cells. This may indicate that metastatic cells that must detach from primary sites may possess less adhesive strength to interstitial collagens. On the other hand, comparable to static adhesion assays metastatic HT-29LMM cells demonstrated adhesive interactions with FN, but poorly metastatic HT-29P cells did not show these properties. In addition, we were able to differentiate between the early events of adhesive interactions, such as initial contacts to ECM, and adhesion stabilization. The advantage of HT-29P over HT-29LMM cells in C I-mediated adhesion involved all phases of initial cell adhesion and adhesion stabilization, whereas increased dynamic adhesion to FN was mainly limited to early events or initial adhesive contacts.

We have previously shown that biophysical factors seem to be responsible for the reduction of tumor cell velocity under flow if the cells become close to the ECM surface (Haier et al. 1999c). The parabolic curve of shear forces and nonspecific cell membrane-ECM interactions appear to result in the observed rotation of cells occurring as rolling on the surface, whereas free flowing cells did not demonstrate rotation. This phenomenon is different from 'rolling' of leukocytes on EC, where specific adhesive interactions lead to short term-stops of cell movement. However, these interactions do not require rotation of cells in the direction of flow, and we described the short-term binding as sticking of cells to the surface. Once tumor cells reach a critical threshold of WSS, specific biochemical interactions between tumor cell integrins and ECM components can be formed (sticking and stable adhesions).

Major differences between static and dynamic adhesion occurred if C IV was used as adhesive substrate. HT-29 cells showed very high rates of static adhesion to C IV, but the same ECM component was unable to mediate dynamic cell adhesion. The conformation of ECM components, such as the collagens, appear to be important for cell adhesion under both static conditions and dynamic fluid flow. For example, Verkleij et al. (1998) have shown that platelets adhered to various collagen-related peptides under static conditions; however, there was a total lack of adhesion under flow conditions. Thus, differing interactions of platelets with collagens seem to be mediated at different shear levels, and the low-affinity interactions were unable to withstand shear forces. They also found that the collagen triple helix was essential in the overall process of adhesion to collagen under flow, and different types of collagens receptors may be responsible for the different binding affinities. For example, although the $\alpha_2\beta_1$-integrin mediated adhesion to collagen under flow independent of collagen conformation, recognition of the triple helix resulted in strengthening of attachment and platelet activation (Verkleij et al. 1998).

Various colon carcinoma cell lines, including HT-29 cells, demonstrated 'rolling' with short-term stops on EC that was inhibitable by mAb against E-selectin (Tözeren et al. 1995). In addition, Tözeren et al. (1994) described that HT-29 cells stacked and adhered to immobilized LN, but not to C I, C IV, or FN. These dynamic interactions with LN were mediated by the $\alpha_6\beta_4$ integrin and were inhibited by mAb against these integrin subunits. In contrast, we found cell rolling, sticking, stabilized adhesion, and crawling of HT-29P cells to immobilized C I under flow conditions, and specific interactions were inhibited by β_1-integrin mAb (Haier et al. 1999c). We also found that specific and nonspecific tumor cell-ECM interactions may occur in the presence of flow comparable to the conditions in the microcirculation, and these interactions were distinct in cells lines with metastatic properties. Similar correlations were previously reported using other cell systems, such as melanoma and large cell lymphoma cells with different metastatic behaviors (Menter et al. 1995; Menter et al. 1992; Yun et al. 1997). The dynamic adhesive properties as well as cell adhesion of HT-29 cells under static conditions are apparently mediated to a large degree by β_1-integrins (Haier 1999c). The involvement of specific integrin-mediated interactions was also supported by the findings that flow adhesion to the nonspecific substrate poly-L-lysine occurred in a completely different pattern.

The mechanisms that are involved in or determine the various steps and types of cell adhesion of tumor cells to ECM appear to be distinctive (Gumbiner 1996). Our results revealed that adhesive interactions of colon carcinoma cells with ECM components occurred in several steps with different characteristics. Since we have pre-

viously shown that HT-29P and HT-29LMM cells have similar patterns of integrin expression, environmental conditions, such as WSS and ECM conformation, seem to take part in the regulation of integrin binding affinity or avidity that enables these adhesion molecules to recognize ECM components and/or stabilize their binding. The influence of shear forces that occur under fluid flow conditions was supported by the differences that we found between static and dynamic adhesion assays. Static adhesion assays and cell spreading produced similar results, but these were different from those obtained from dynamic adhesion experiments.

References

Agrez MV, Bates RC (1994) Colorectal cancer and the integrin family of cell adhesion receptors: current status and future directions. Eur J Cancer 14: 2166–2170

Alon R, Chen S, Puri KD, Finger EB, Springer TA (1997) The kinetics of L-selectin tethers and the mechanics of selectin-mediated rolling. J Cell Biol 138: 1169–1180

Aznavoorian S, Liotta L, Kupchik HZ (1990) Characteristics of invasive and noninvasive human colorectal adenocarcinoma cells. J Natl Cancer Inst 82: 1485–1492

Burtin P, Chavanel G, Foidart JM (1983) Immunofluorescence study of the antigens of the basement membrane and the peritumoral stroma in human colonic adenocarcinomas. Ann N Y Acad Sci 420: 229–236

Chen YP, O'Toole TE, Shipley T, Forsyth J, LaFlamme SE, Yamada KM, Shattil SJ, Ginsberg MH (1994) "Inside-out" signal transduction inhibited by isolated integrin cytoplasmic domains. J Biol Chem 269: 18307–18310

Danecker GW, Piazza AJ, Stelle GD, Mercurio AM (1989) Relationship between extracellular matrix interactions and degree of differentiation in human colon carcinoma cell lines. Cancer Res 49: 681–686

Ginsberg MH, Loftus JC, D'Souza S, Plow EF (1990) Ligand binding to integrins: common and ligand specific recognition mechanisms. Cell Diff Develop 32: 203–214

Gumbiner BM (1996) Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell 84: 345–357

Haier J, Nasralla M, Buhr HJ, Nicolson GL (1998) Differential integrin-associated adhesion to extracellular matrix in colon cancer cells with low metastatic potential or highly metastatic potential to the liver. Langenbecks Arch Chir [Suppl, Conf Vol] 309: 307–313

Haier J, Nasralla M, Nicolson GL (1999a) Influence of phosphotyrosine kinase inhibitors on adhesive properties of highly and poorly metastatic HT-29 colon carcinoma cells to collagen. Int J Colorect Dis 14: 119–127

Haier J, Nasralla M, Nicolson GL (1999b) Different adhesion properties of highly and poorly metastatic HT-29 colon carcinoma cells with extracellular matrix components: role of integrin expression and cytoskeletal components. Br J Cancer 80: 1867–1874

Haier J, Nasralla M, Nicolson GL (1999c) β_1-integrin mediated dynamic adhesion of colon carcinoma cells to extracellular matrix under laminar flow. Clin Exp Metastasis 17: 377–388

Hanks S, Polte T (1996) Signaling through focal adhesion kinase. Bioessays 19: 137–145

Ishida T, Peterson TE, Kovach NL, Berk BC (1996) MAP kinase activation by flow in endothelial cells. Role of beta 1 integrins and tyrosine kinases. Circ Res 79: 310–316

Ishida T, Takahashi M, Corson MA, Berk BC (1997) Fluid shear stress-mediated signal transduction: how do endothelial cells transduce mechanical force into biological responses? Ann N Y Acad Sci 811: 12–23

Lawrence MB, Springer TA (1991) Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell 65: 859–873

Lehoux S, Tedgui A (1998) Signal transduction of mechanical stresses in the vascular wall. Hypertension 32: 338–345

Li S, Kim M, Hu YL, Jalali S, Schlaepfer DD, Hunter T, Chien S, Shyy JY (1997) Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. J Biol Chem 272: 30455–30462

Lindmark G, Gerdin B, Pahlman L, Glimelius B, Gehlsen K, Rubin K (1993) Interconnection of integrins alpha 2 and alpha 3 and structure of the basal membrane in colorectal cancer: relation to survival. Eur J Surg Oncol 19: 50–60

McIntire LV (1994) Bioengineering and vascular biology. Ann Biomed Eng 22: 2–13

Menter DG, Patton JT, Updyke TV, Kerbel RS, Maamer M, McIntire LV, Nicolson GL (1992) Transglutaminase stabilizes melanoma adhesion under laminar flow. Cell Biophys 18: 123–143

Menter DG, Fitzgerald L, Patton J, McIntire LV, Nicolson GL (1995) Human melanoma integrins contribute to arrest and stabilization potential while flowing over extracellular matrix. Immunol Cell Biol 73: 575–583

Miyamoto S, Teramoto H, Coso OA, Gutkind JS, Burbelo PD, Akiyama SK, Yamada KM (1995a) Integrin function: molecular hierarchies of cytoskeletal and signaling molecules. J Cell Biol 131: 791–805

Miyamoto S, Akiyama SK, Yamada KM (1995b) Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. Science 267: 883–885

Nicolson GL (1988) Organ specificity of tumor metastasis: role of preferential adhesion, invasion, and growth of malignant cells at specific secondary sites. Cancer Metastasis Rev 7: 143–188

Nicolson GL (1989) Metastatic tumor cell interactions with endothelium, basement membrane, and tissue. Curr Op Cell Biol 1: 1009–1019

Nicolson GL (1991) Tumor and host molecules important in the organ preference of metastasis. Cancer Biol 2: 143–154

Nicolson GL (1995) Tumor cell interaction with the vascular endothelium and their role in cancer metastasis. In: Goldberg ID, Rosen EM (eds) Epithelial-mesenchymal interactions in cancer. Birkhäuser, Basel, pp 123–156

Okuyama M, Ohta Y, Kambayashi J, Monden M (1996) Fluid shear stress induces actin polymerization in human neutrophils. J Cell Biochem 63: 432–441

Pauli BU, Augustin-Voss HG, El-Sabban ME, Johnson RC, Hammer DA (1990) Organ-preference of metastasis: the role of endothelial cell adhesion molecules. Cancer Metastasis Rev 9: 175–189

Price JE, Daniels LM, Campbell DE, Giavazzi R (1989) Organ distribution of experimental metastasis of a human colorectal carcinoma injected in nude mice. Clin Exp Metastasis 7: 55–68

Richardson A, Parson JT (1995) Signal transduction through integrins: a central role for focal adhesion kinase. Bioessays 17: 229–236

Schwarz MA, Schaller MD, Ginsberg MH (1995) Integrins: emerging paradigms of signal transduction. Ann Rev Cell Dev Biol 11: 549–599

Takahashi M, Berk BC (1996) Mitogen-activated protein kinase (ERK1/2) activation by shear stress and adhesion in endothelial cells. Essential role for a herbimycin-sensitive kinase. J Clin Invest 98: 2623–2631

Takahashi M, Ishida T, Traub O, Corson MA, Berk BC (1997) Mechanotransduction in endothelial cells: temporal signaling events in response to shear stress. J Vasc Res 34: 212–219

Takazawa H (1995) Association between expression of integrin (VLA-3, VLA-5) and malignancy in human colon-cancer. Nippon Rinsho 53: 1672–1677

Tözeren A, Kleinman HK, Wu S, Mercurio AM, Byers SW (1994) Integrin $\alpha 6\beta 4$ mediates dynamic interactions with laminin. J Cell Sci 107: 3153–3163

Tözeren A, Kleinman HK, Grant DS, Morales D, Mercurio AM, Byers SW (1995) E-selectin-mediated dynamic interactions of breast- and colon-cancer cells with endothelial-cell monolayers. Int J Cancer 60: 426–431

Verkleij MW, Morton LF, Knight CG, de Groot PG, Barnes MJ, Sixma JJ (1998) Simple collagen-like peptides support platelet adhesion under static but not under flow conditions: interaction via alpha2 beta1 and von Willebrand factor with specific sequences in native collagen is a requirement to resist shear forces. Blood 91: 3808–3816

Yun Z, Smith TW, Menter DG, McIntire LV, Nicolson GL (1997) Differential adhesion of metastatic RAW117 large-cell lymphoma under static conditions: role of the $\alpha v\beta 3$ integrin. Clin Exp Metastasis 15: 3–11