Tumor cell adhesion to frozen lymph node sections—an 
in vitro correlate of lymphatic metastasis†

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Two variant sublines of the murine 3LL carcinoma with divergent potentials for lymphatic metastasis were used to assess the relationship between tumor cell potential for lymphatic metastasis and its ability to adhere specifically to lymphatic tissue. Using fresh cryostat sections of lymph nodes and spleens, it was found that tumor cell adhesion to the lymphatic tissue but not to control sections of the brain correlated well with their ability to metastasize lymphatically. On the other hand, there was no correlation between tumor cell attachment to isolated lymphocytes in vitro and their potential for lymphatic metastasis. When tumor cells were pretreated enzymatically or with the metabolic inhibitor tunicamycin with the aim of modulating cell surface carbohydrates, adhesion to the lymph node sections could be significantly reduced, implicating cell surface glycoproteins and in particular galactosyl groups in the binding. The results suggest that tumor cell attachment to lymph node cryostat sections could provide a useful tool in the study of host–tumor interactions in lymphatic metastasis.

Introduction

Many of the common human malignancies disseminate via the lymphatic circulation [9]. The extent of involvement of the regional lymph nodes has in fact become a parameter commonly used in the staging and prognosis of primary cancers [4]. However, despite its clinical importance, the cellular mechanisms underlying the process of lymphatic metastasis are still poorly understood. On the basis of published studies it is known that following invasion of the regional lymph nodes which drain the primary tumor, cells may (1) leave rapidly through efferent lymphatic vessels [13], (2) be retained temporarily but prevented from proliferating and eventually destroyed by local hostile mechanisms [10] or (3) be allowed to proliferate and give rise to local tumors [5]. The cellular and molecular mechanisms which control these events remain largely unexplored. This may be related, at least in part, to the scarcity of animal tumor models in which tumor dissemination is known to occur via the lymphatic drainage [5].

Two metastatic sublines of the Lewis lung carcinoma (3LL) are currently studied in our laboratory. Variant M-27 metastasizes exclusively to the lung while variant H-59 homes preferentially to the liver [6]. Using a lymph node implantation assay, we have previously shown that whereas the regional, tumor-draining nodes removed from animals bearing s.c. tumors of H-59 and implemented into new mice gave rise to new tumors in the majority (11/13) of the recipients, none of the lymph nodes (0/5) removed from M-27 bearing mice could give rise to tumors in recipient

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animals. Nodal infiltration by tumor H-59 cells was also repeatedly confirmed by histology [6]. These tumor variants therefore provide a model for the analysis of host–tumor cell interactions which regulate lymphatic metastases formation.

In this study we examined whether the heterogeneity noted in the metastatic patterns of the two sublines would be reflected in a difference in their potential to adhere to lymphatic tissue in vitro. Adhesion assays were carried out using isotope-labeled tumor cells and fresh cryostat sections of syngeneic normal lymph nodes. This adhesion assay was selected since studies by other laboratories have shown that the adhesion of both normal lymphocytes [8] and metastatic tumor cells [18] to organ frozen sections correlated well with their selective homing patterns in vivo. Studies by Weissman and associates [reviewed in 15] on the preferential homing of circulating lymphocytes using this model eventually led to the identification by monoclonal antibodies of a lymphoid homing receptor (MEL-14) which is involved in the recognition by lymphocytes of the high endothelial venules of peripheral lymph nodes.

Our studies have shown that there was a good correlation between tumor cell attachment to the lymph nodes in vitro and their potential for lymphatic metastasis in vivo.

Materials and methods

Reagents and culture media

The following were used: RPMI–FCS, RPMI 1640 (Gibco, Canada) supplemented with 10% FCS; 0.01 M HEPES buffer, 2 × 10⁻³ M glutamine and 0.001 per cent gentamicin sulphate; PBS–EDTA: Ca²⁺ and Mg²⁺-free phosphate-buffered saline with 0.02 per cent EDTA; ¹²⁵IUDR, 5-[¹²⁵I]iodo-2-deoxyuridine (5 Ci/μg; Amersham, Canada); FdUd, 5-fluorodeoxyuridine (Sigma Chemical, St. Louis, MO, U.S.A.); PLL, Poly-L-lysine (Sigma Chemical); SDS, sodium dodecyl sulfate (Sigma Chemical); test neuraminidase, from Vibrio cholera (Hoechst-Behring Diagnostics Canada, Montreal, Quebec); β-galactosidase, from E. coli (Boehringer-Mannheim of Canada, Montreal, Quebec); and tunicamycin (Boehringer-Mannheim of Canada).

Tumors and cell lines

The metastatic patterns and growth conditions of sublines H-59 and M-27 of tumor 3LL have been described in detail elsewhere [6]. Tumor H-59 metastasizes to the regional lymph nodes from various subcutaneous sites, while tumor M-27 does not give rise to lymphatic metastases although it forms multiple pulmonary metastases. The lines are routinely propagated in vivo by s.c. implantation of isolated metastatic nodules [6]. For this study, tumors were excised and enzymatically dispersed with a 0.02 per cent trypsin solution in PBS–EDTA. The cells derived were then seeded in tissue culture dishes (75 cm²) at a concentration of 5 × 10⁵ cells/ml, washed the following day to remove non-adherent cells and then cultured for 2 weeks prior to assay. This was done in order to obtain a highly viable tumor cell suspension devoid of the non-replicating, contaminating host cells. The tumor cells grew as a monolayer culture and were dislodged by a brief incubation in PBS–EDTA. Their viability as assessed by trypan blue exclusion dye always exceeded 95 per cent.