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Invasive Activity and Chemotactic Response to Growth Factors by Kaposi's Sarcoma Cells

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Kaposi's sarcoma (KS) is a relatively low grade neoplasm, classically occurring in the skin of elderly men. A more virulent and invasive form of Kaposi's sarcoma has been described in patients with acquired immune deficiency syndrome (AIDS). The origin and identification of the tumor cells in these lesions is controversial. Here we have studied the behavior of cells derived from KS lesions in an in vitro assay which measures the ability of cells to invade through a reconstituted basement membrane. In agreement with previous work, KS cells obtained under selective culture conditions were invasive showing activity comparable to that of malignant tumor cells. Normal fibroblasts, smooth muscle cells, and endothelial cells did not demonstrate invasive behavior under the same experimental conditions. To characterize further the nature of the KS cells we tested the chemotactic response of cells from the most invasive line to a variety of growth factors and compared their response to those of fibroblasts, smooth muscle, and endothelial cells. These studies suggest that normal cells respond to a unique repertoire of chemotactic factors. The chemotactic response of the KS cells most closely resembled that of smooth muscle cells and was quite distinct from endothelial cells. These results indicate that the KS-derived cultures contain invasive cells with a smooth muscle cell-like phenotype.

Key words: Kaposi's sarcoma, chemotaxis, invasion, growth factors

Kaposi's sarcoma (KS) presents as a progressive mesenchymal tumor involving the skin, mucosa, and digestive tract of up to 30% of patients with AIDS [1–4]. Kaposi's sarcoma differs from typical neoplasms in several characteristic ways. These

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include the frequent regression of the lesion, the lack of aneuploidy in the cells, and the occurrence of multiple lesions of apparent multicentric origin [5–12]. Histological studies show that the lesions are principally composed of endothelial cells and spindle-shaped cells, although inflammatory cells are present in early lesions [13–18]. Consequently, some authors define KS as being an invasive granulation tissue, but KS has also been described as a hemangiosarcoma or highly vascularized fibrosarcoma. Thus, whether KS is indeed a neoplasm is still controversial [5,12].

Recently we have developed a method for establishing cell cultures from KS lesions using low serum to favor the growth of transformed cells over normal cells [17]. The cells selected in this fashion demonstrate anchorage-independent growth and exhibit invasive behavior when added to cultured skeletal muscle fragments. However, cells isolated from the KS lesions in this manner did not produce tumors when injected into nude mice.

The cultured cells morphologically resemble smooth muscle cells and possess myofibrils, which stained specifically by the Masson trichrome method. They show dense bodies by electron microscopy and do not differentiate to form capillaries in vitro or possess Weibel-Palade body or factor VIII-related antigen. Thus, these cultured cells are distinct from endothelial cells and resemble smooth muscle cells [17].

The aim of the present work was to investigate further the nature of the cultured KS cell populations by evaluating their invasive potential in vitro and their chemotactic response to specific growth factors. Our results confirm the previous suggestion that KS cells from patients with AIDS are invasive and show smooth muscle-like characteristics [17].

MATERIALS AND METHODS

Biopsies and Explant Culture

Skin specimens from four AIDS patients with Kaposi's sarcoma were obtained from the Clinical Center, National Institutes of Health. Tumor tissue was separated from normal dermis and epidermis, minced, and placed in culture in flasks with Medium 199 supplemented with 20% fetal bovine serum (FBS), antibiotics, 300 μg/ml of a crude preparation of endothelial cell growth factor (Meloy Labs., Springfield, VA), and 50 μg/ml of heparin (Hynson, Wescott & Dunning, MD). After a sufficient outgrowth of cells had appeared, they were removed with trypsin and replated. After one to five passages, cells derived from each biopsy were passaged in Medium 199 containing 2.5% serum, antibiotics, and 150 μg/ml of endothelial cell growth factor (ECGF), plus 50 μg/ml heparin. The majority of the cells in the flask failed to survive under these conditions, while the surviving cells proliferated and formed a confluent sheet after about 3 weeks. These cells were subsequently cultured in media containing 20% serum, 150 μg/ml of ECGF, and 50 μg/ml of heparin.

Other Cells

Normal skin fibroblasts were derived from skin biopsies. Human endothelial cells were derived from umbilical veins [19]. Human intestinal smooth muscle cells were obtained from Dr. H. Perr, Department of Pediatrics, Medical College of Virginia. Rat aorta smooth muscle cells were a gift of Dr. White, Department of Anesthesiology, Uniformed Services University for the Health Sciences (USUHS).

Human and rat smooth muscle cells behaved in a similar way in preliminary assays and consequently both lines were used. Fibrosarcoma cells (HT 1080), adenocarcinoma cells (SW 620), melanoma cells (Malme 3M), and a rhabdomyosarcoma cell line (A 204) were purchased from Am Type Culture Collection, Rockville, MD.

Matrigel Invasion Assay

This assay was performed in Boyden chambers, as previously described [20]. Polyvinylpyrrolidone-free polycarbonate filters (12 μm pore size) were coated with varying amounts of matrigel [21] (a gift from H. K. Kleinman, NIDR, NIH). Matrigel is a urea extract of the Englebrecht-Holm-Swarm tumor, containing laminin, type IV collagen, entactin, and heparan sulphate proteoglycan, the major components of basement membranes. When dialyzed against PBS or serum-free medium, it is liquid at 4°C and polymerizes in a three-dimensional matrix at 37°C. Fibroblast conditioned medium was placed in the lower compartment of Boyden chambers to stimulate migration. Cells were harvested with trypsin, resuspended in 0.1% bovine serum albumin (BSA) in Dulbecco's Minimum Essential Medium (DMEM), and placed in the upper compartment at 1.5 to 2 $\times$ 10^5 cells per chamber. The cells were allowed to migrate for 6 h at 37°C in air plus 5% CO_2 in a humidified incubator. At the end of the incubation period, the cells on the upper surface of the filter were wiped away, and the filters were fixed in methanol and stained with hematoxylin and eosin. The mean number of migrated cells per field was determined by counting five microscopic fields. All assays were run in duplicate.

Chemotaxis

The assay was run in Boyden chambers as previously described [22]. The filters were soaked in a diluted solution of collagen IV (50 μg/ml), which coats the surface and pores and promotes the adhesion and migration of cells. In some studies fibroblast-conditioned medium was used as a source of chemoattractants [22]. In addition, the following growth factors were used as chemoattractants at concentrations found to promote cell migration: platelet derived growth factor (PDGF), 1–10 ng/ml (Collaborative Research); epidermal growth factor (EGF), 5–50 ng/ml (Collaborative Research); endothelial cell growth factor (ECGF), 10–100 ng/ml (Meloy Labs., Springfield, MD); transforming growth factor, beta (TGFβ), 1–10 ng/ml (a gift of A. Roberts, NCI, NIH); transforming growth factor, alpha (TGFα), 1–10 ng/ml (a gift of G. Todaro, Seattle, WA). These were added, along with 0.1% BSA as carrier, to the lower compartment of the Boyden chamber. After the termination of the migration period, the filters were fixed, stained, and counted in the same way as for the invasion assays.

RESULTS

Invasion

Four cell lines were established from KS tissue from four patients and were selected by their ability to grow in culture at low serum levels. The ability of the KS culture to invade a matrix of basement membrane components reconstituted onto a porous filter was measured both before and after selection [20]. In these studies, fibroblast-conditioned media containing a mixture of potent chemoattractants was placed in the chambers below the filter. KS cells were able to invade through varying

amounts of basement membrane matrix (Fig. 1-a,b). Normal human cells, including human fibroblasts, endothelial cells, and smooth muscle cells, were also studied, as well as malignant cells from various human tumors. The invasiveness of the KS cells varied among the different lines, but the range of activity was comparable to that observed with metastatic tumor cells, including those from a fibrosarcoma and a melanoma (Table I). Human intestinal and rat aorta smooth muscle cells, human umbilical vein endothelial cells, and human fibroblasts were not invasive when tested in this assay. However, they showed a significant migration through the filter in the absence of the layer of basement membrane (Fig. 1b).

To investigate the effect of the selection protocol, we compared the invasiveness of cells in the original outgrowth with those cells selected by growth in media with reduced serum content. The selected cultures were 3–7-fold more invasive than the original population of cells (Table II).

Chemotaxis

We also tested the chemotactic response of the most invasive KS cell strain (Patient C) to several growth factors as compared to primary fibroblasts, smooth muscle, and endothelial cells (Table III). These studies were performed in a manner similar to the invasion assays, but without the layer of basement membrane imposed as a barrier over the porous filter. To allow easier comparison, the data have been normalized to the response obtained using conditioned media as an attractant with each cell type studied. The absolute values varied, with the endothelial cells showing a much lower response than the other cells tested (i.e., 10 cells/field vs. 30 or 40 cells/field with the other cells). As shown in Table III, each cell type responded differently to this panel of attractants. For each growth factor a range of concentra-

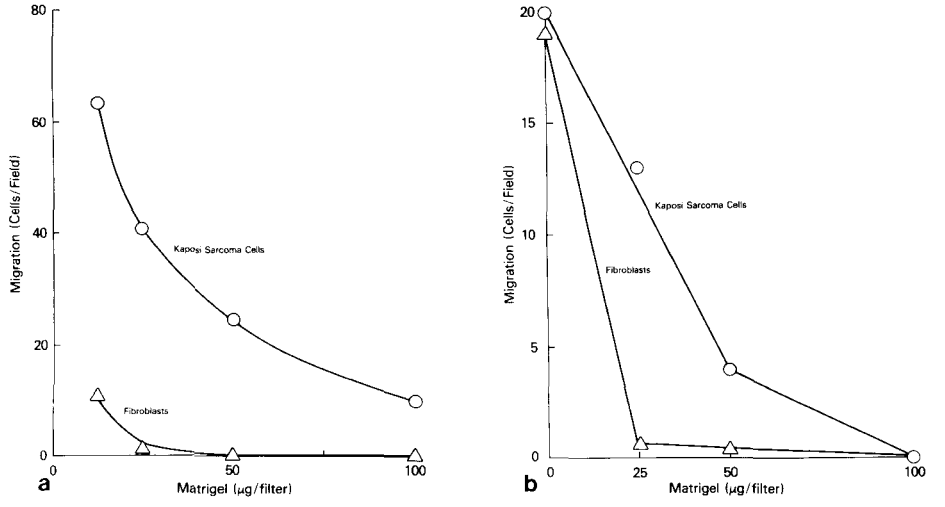

Fig. 1. Invasiveness of KS cells in **(a)** Patient C and **(b)** Patient L. Various amounts of Matrigel were coated onto porous filters. KS cells and fibroblasts were put in the upper compartment of the chemotaxis chamber and incubated for 6 h. Fibroblast-conditioned media were used as a source of chemoattractants. KS cells were more invasive than fibroblasts for all amounts of matrigel tested (**a** and **b**). In the absence of matrigel only chemotaxis is being assayed **(b)**. Data are expressed as cells invaded/field. Five random fields per filter were counted and symbols represent average value of two experiments. Standard error was less than 5 cells.

TABLE I. Invasiveness of Kaposi's Sarcoma Cells as Compared to Normal Cells and to Human Tumor Cells*

Kaposi's Sarcoma cells	Patient L	19[a] ± 3
	Patient C	32 ± 4
	Patient B	7 ± 1
	Patient M	invasive[b]
Normal cells	Primary fibroblasts	0.5 ± 0.4
	Smooth muscle cells	1 ± 0.2
	Endothelial cells	0
Invasive tumor cells	Fibrosarcoma (HT-1080)	16 ± 3
	Melanoma (Malme 3M)	28 ± 6
	Rhabdomyosarcoma (A 204)	56 ± 8
	Adenocarcinoma (SW 620)	60 ± 7

*Cells derived from biopsy specimen of Kaposi's patients (as well as control cells) were tested for their ability to invade through a reconstituted basement membrane (Matrigel) in the chemoinvasion assay as adapted for the Boyden chamber (20). Fibroblast-conditioned medium was used as chemoattractant and was placed in the bottom chamber.

[a]Data are expressed as migrated cells/field, with the mean of 5 fields from each of 2 assays reported.

[b]This study was conducted in a separate experiment and showed invasive cells, but the data cannot be directly compared, and this cell line was subsequently lost.

TABLE II. Effect of Selection in Low Serum on the Invasiveness of KS Cells*

Cells	Unselected	Selected
Control fibroblasts	0.5 ± 0.4[a]	NA[b]
Patient L	5 ± 2	17 ± 4
Patient B	1 ± 0.5	7 ± 1

*Cells derived from cultured biopsy specimens in 20% FBS were selected by growth in low serum (2.5% FBS) to enrich for tumor cells. Cultures of the original outgrowth and from the selected lines were tested for invasive activity in the chemoinvasion assay.

[a]Data are expressed as migrated cells/fields (mean of 5 fields from each of two assays).

[b]The control cells did not survive in the low serum media.

TABLE III. Chemotaxis of Kaposi's Sarcoma Cell to Growth Factors*

	Cell Type			
Attractant	Fibroblast	Endothelial	Smooth muscle	Kaposi's
BSA (1 mg/ml)	0	0	0	0
PDGF (10 ng/ml)	52 ± 9	0	179 ± 19	139 ± 18
ECGF (75 ng/ml)	8 ± 3	100 ± 19	85 ± 4	80 ± 5
EGF (50 ng/ml)	14 ± 2	40 ± 4	31 ± 1	71 ± 5
TGF α (10 ng/ml)	9 ± 4	3 ± 2	18 ± 3	36 ± 7
TGF β (10 ng/ml)	58 ± 8	0	19 ± 5	22 ± 4
CM	100	100	100	100

*Cells from the most invasive Kaposi's cell strain (Patient C) were tested for their chemotactic response to several growth factors in the Boyden chamber assay. A large range of concentrations was investigated in preliminary experiments (4–6 log). The responses to an optimal concentration in the active range are reported. Data are expressed as % of cells that migrated relative to the number of cells migrating toward fibroblast-conditioned medium (100%) and BSA (0%).

tions of 4–6 logs was tested in preliminary experiments, and the migration observed at an optimal concentration of each attractant is reported. Under these conditions, smooth muscle cells preferentially migrated to PDGF and to ECGF, and less to the other factors. As expected, endothelial cells migrated preferentially to ECGF, to a lesser extent to EGF, and not at all to PDGF or TGFβ. Fibroblasts were found to respond to PDGF, TGFβ, and only minimally to ECGF or TGFα. KS cells were highly responsive to PDGF, ECGF, and EGF, and showed a lesser response to TGFα. TGFβ exerted little attraction on the KS cells.

These data suggest that the selected KS cultures contain a mixed population of cells, with smooth muscle-like cells the most abundant. Alternatively, the KS cells may have originated from smooth muscle cells but later acquired some additional growth factor responsiveness.

DISCUSSION

Although generally thought to be a malignancy, KS exhibits certain features that are not typical of tumors; for example, KS may regress, it can present with multicentric lesions, and its cells are not always karyotypically abnormal [5], although they may show some instability [23]. We cultured cells from KS lesions obtained from AIDS patients and selected a subpopulation of cells by their ability to survive and proliferate at a lower serum concentration. The selected cells were shown to be capable of anchorage-independent growth and to be able to invade muscle tissue [17]. Such behavior is consistent with a transformed phenotype.

The appearance of the cells in culture and the presence of certain characteristic markers suggested a smooth muscle cell origin for the KS cells examined here, rather than an endothelial one [17]. These KS cells in culture stained with Masson trichrome. Dense bodies representing characteristics typical of myoblasts were observed by electron microscopy. The cells did not form capillary structures like endothelial cells, they lacked Weibel-Palade bodies, and did not express Factor VIII-related antigen or receptors for *Ulex europeaus* I-a lectin [17].

To characterize these cells further, we investigated their ability to invade through a reconstituted basement membrane barrier and the chemotactic response of the most invasive line to various growth factors. The breaching of basement membranes is an important step in the spread of malignant cells [24]. Such invasive activity is dependent on the ability of the cells to attach to the basement membrane, to produce enzymes that degrade this barrier, and to recognize chemoattractants which activate and direct their motile response [25]. Our assay uses a Boyden chamber with basement membrane proteins reconstituted onto the surface of a porous filter and chemoattractants placed in the lower chamber. The selectivity of the assay can be increased by using a thicker barrier, which requires longer periods for cells to cross. Using fibroblast-conditioned medium as a source of attractants, we assessed the invasiveness of cells cultured from Kaposi's lesions from four patients as well as normal cells and cells from metastatic cancers. The KS cells, selected by their ability to grow in media with a lower serum content, were invasive. Invasiveness varied among the cell lines, but all four cell lines showed a greater capacity for penetration of the barrier than normal cells, and in two cases their invasiveness was comparable to that observed with metastatic tumor cells used as a positive control. Normal cells, including fibroblasts, smooth muscle cells and endothelial cells, were not invasive.

Cells from two KS lesions were studied both before and after selection by growth at a lower serum concentration and showed a significant increase in invasiveness after selection. The most likely explanation for these results is that a mixed population of cells is obtained in the initial outgrowth from the lesions, and that the most invasive cells survive the selection process.

Since chemotaxis is a receptor-mediated event, considerable specificity is encountered in the response of cells to various attractants [see 26]. Recent studies indicate that certain growth factors exhibit chemotactic activity [see 27]. Thus, PDGF is both an attractant and a mitogen for fibroblasts and smooth muscle cells [28,29] and ECGF induces both the migration and proliferation of endothelial cells [30]. TGFβ has a higher chemotactic activity for fibroblasts [31] than for the other cell types studied here. The chemotactic response of the cells occurs prior to DNA synthesis and occurs even when DNA synthesis is inhibited [28,29], but it is thought to be mediated via similar initial reactions.

Here we tested several growth factors for chemotactic activity with a KS cell strain and with various normal cells. Distinctive patterns were observed with fibroblasts, endothelial cells, and smooth muscle cells. PDGF was a potent chemoattractant for fibroblasts, smooth muscle, and KS cells, but not endothelial cells. ECGF was active with endothelial cells, smooth muscle cells, and KS cells. TGFα induced a significant response for smooth muscle and KS cells, while TGFβ was very active for fibroblasts but only minimally for KS cells. Based on these observations (and particularly on their attraction toward PDGF, to which endothelial cells show no response) we suggest that the KS cells respond in this assay as smooth muscle cells. This is in agreement with previous work suggesting a smooth muscle cell-like phenotype for KS cells [17].

It should be noted, however, that the Kaposi cells showed quite a strong response to EGF, and in this regard differ from the adult smooth muscle cells studied here. While the lineage through which smooth muscle cells develop is not fully established, it is known that their phenotype can vary. Thus, the state to which the KS cells have developed may include the retention of EGF responsiveness and be related to their aberrant behavior in creating these lesions.

Current concepts suggest that the repertoire of receptors for mitogens and chemoattractants is related to a cell's function and therefore may represent a rather specific profile [27]. The studies reported in this paper support the previous suggestion [17] that the malignant cells in the Kaposi's lesions are a transformed mesenchymal cell resembling smooth muscle cells.

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