

ENVIRONMENTAL INDUCTION OF TUMOR PHENOTYPE IN A PUTATIVE KAPOSI SARCOMA PROGENITOR CELL

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ABSTRACT

Many features of AIDS-related Kaposi sarcoma (AIDS-KS), e.g., multifocal lesional presentation at sites perfused by the microvasculature, suggest that AIDS-KS is initially a hyperplasia that subsequently progresses to a neoplasia. We propose that the unique AIDS environment, which contains high levels of circulating factors such as viral cytokines, is key in initiating the KS lesion. Further, we maintain that due to their physiological function, human microvascular endothelial cells (HMECs) are both likely target cells for the AIDS-related cytokines, and are putative AIDS-KS progenitor cells. Previously, we have shown that as a component of HMEC transition between proliferative and differentiated growth, HMECs modulate their nucleotide and glutathione levels. After attaining contact inhibition, HMECs enter a state of differentiation, which is characterized by cellular entrance into a G₀, quiescent growth state, a decrease in cellular bioenergetic profiles, and spontaneous formation of microtubules. In contrast, when cultured in a "KS milieu", HMECs fail to differentiate. Instead, the "KS milieu" cultured cells assume a "growth relaxed" phenotype and demonstrate a lack of contact inhibition, loss of anchorage dependence, and retention of a "proliferative" bioenergetic profile despite culture confluence.

Our results imply both that HMECs are responsive to AIDS-related cytokines, and that the local environment is key to instigating a relaxation of cellular growth controls.

The advent of the acquired immunodeficiency syndrome (AIDS) epidemic has markedly increased the incidence and severity of Kaposi sarcoma (KS) and subsequently stimulated interest in KS pathobiology (1,2). Clinically, AIDS-KS most frequently presents as multifocal lesions at sites perfused by the microvasculature, suggesting a systemic, circulating component to this disease (3,4). Further, unlike most malignancies, which establish a primary tumor prior to metastasizing, the multifocal presentation of AIDS-KS suggests, that at least in its early stages, AIDS-KS is a hyperplastic process (4,5).

The primary sites affected by AIDS-KS, mucosa and skin, are areas richly perfused by the microcirculation (4,6). Because most blood-tissue exchange occurs at the microcirculatory interface where blood flow rate is low and the vascular surface area to blood volume ratio is high, there are ample opportunities for cellular contact and cellular adhesions (6). Due to their anatomic location and physiological function, human microvascular endothelial cells (HMECs) are prime cellular targets for the AIDS generated cytokines and human immunodeficiency virus

TABLE 1
Environmental Modulation of Human Microvascular Endothelial Cell (HMEC)
Bioenergetic Status During Postconfluence Growth

| Nucleotide | Proliferative Preconfluent, Control Medium (PRO) | Differentiated Postconfluent Control Medium (DIF) | "Growth Relaxed" Postconfluent, KS Milieu ("GR"-KSM) |
|-------------------------------------|---|--|---|
| AMP | 0.92±0.44 | 0.49±0.29 | 0.44±0.08 |
| ADP | 2.37±0.43* | 1.72±0.15*# | 2.20±0.28# |
| ATP | 60.40±3.55* | 32.60±5.01*@ | 53.79±4.23@ |
| TOTAL (ATP+ADP+AMP) | 63.69 | 34.81 | 56.43 |
| NAD ⁺ | 8.79±1.47 | 5.72±1.46# | 9.43±1.52# |
| NADH | 6.45±1.29 | 4.44±1.10 | 6.17±0.71 |
| TOTAL (NAD ⁺ +NADH) | 15.24 | 10.16 | 15.60 |
| NADP ⁺ | 0.36±0.11 | 0.28±0.11 | 0.19±0.04 |
| NADPH | 1.81±0.45* | 0.98±0.19* | 1.27±0.37 |
| TOTAL (NADP ⁺ +NADPH) | 2.17 | 1.26 | 1.46 |
| GTP | 8.72±0.51* | 5.80±1.02*@ | 8.11±0.75@ |
| UTP & CTP | 4.84±0.34* | 3.10±0.53*@ | 4.76±0.44@ |
| GSH | 66.5±5.3* | 32.5±3.8* | N.C. |

HPLC nucleotide analyses of HMECs assayed during the following growth states: proliferative, (PRO)=preconfluent in control medium, n=15; differentiated (DIF)=postconfluent in control medium, n=8; "growth relaxed" (GR-KSM)=postconfluent during culture in KS milieu (n=10). Statistical analyses were conducted with the Kruskal-Wallis one way analysis of variance, followed by the Mann Whitney U test. *=p<0.001, PRO vs DIF, @=p<0.001, DIF vs GR-KSM, #=p<0.002, DIF vs GR-KSM. Results are expressed as nmol/mg protein, ±S.D. N.C.=not completed. Note similarity between proliferative and "growth relaxed"-KSM.

(HIV) tat gene product (5,6). Therefore, we propose that a pluripotent, microvascular endothelial stem cell is a probable KS progenitor cell, and that this cell is integral to the initiation and development of the AIDS-KS lesions. We further suggest that the AIDS-KS cytokine and viral product rich milieu is an instigating factor that facilitates the

relaxation of growth control in KS, thereby promoting lesional progression from hyperplasia to neoplasia.

MATERIALS AND METHODS

Isolation and Culture of Human Microvascular Endothelial Cells and AIDS-KS Cells

HMECs were isolated from the chorio-capillaris of the human eye. Excisional biopsies of lesions, clinically suggestive of KS, were obtained from HIV+ patients. A portion of the biopsy was submitted for light microscopic examination to confirm or rule out the diagnosis of KS. Six KS cultures (obtained from a range of low to high grade lesions) have been established.

To evaluate the effects of external environment on cellular growth regulation, the HMEC and KS strains were cultured in both a "KS milieu" (KSM) and a control medium. The KSM consisted of M-199 (GIBCO), supplemented with 15mM HEPES, Na pyruvate (0.11mg/ml), L-glutamine (0.29mg/ml), Na heparin (Sigma, 90µg/ml), endothelial cell growth supplement (150µg/ml), 15% heat inactivated fetal bovine serum (HYCLONE), 5% pooled, heat inactivated male human serum, 20% conditioned media derived from MOT, an HTLV II infected cell line, and human fibronectin (hFN) that contained 5% MOT conditioned medium. The control medium consisted of the same base medium, supplements, and serum, but did not contain any viral products in either the medium or hFN.

Determination of Cellular Bioenergetic Status

Cellular levels of nucleotides and nucleosides were determined by high performance liquid chromatography (HPLC) by a modification of the method of Harsem et al (7) (Table 1).

Determination of Total Cellular Glutathione (GSH)

Cellular levels of total GSH were determined according to the method of Eyer and Podhradsky (8) (Table 1).

RESULTS AND CONCLUSIONS

We have previously demonstrated that our HMECs were capable of growth state transi-

tions between differentiation and proliferation, and therefore possessed a growth state reciprocity (9). When maintained as contact inhibited cultures, HMECs entered a differentiated growth state and showed spontaneous formation of microtubules. After subculture, these formerly differentiated HMECs readily (within 24 hours) returned to a state of proliferative growth. As a component of their growth state transitions, HMECs modulated their bioenergetic status and GSH levels. It was determined that proliferative HMECs possessed statistically significant higher levels of high energy phosphates (ATP, GTP, UTP, CTP), as well as NADPH and GSH in comparison to differentiated HMECs (personal observations). We proposed that the inherent biochemical plasticity present in HMECs was an integral cellular feature that permitted these growth state transitions.

The results of this current component of our study demonstrates that HMECs are exquisitely sensitive to environmental influences. When cultured in the KS milieu [HMECs-(KS)], HMECs fail to spontaneously differentiate. In fact, postconfluent HMECs-(KS) retain a "proliferative" bioenergetic profile which is strikingly similar, with regard to high energy phosphates and total adenine and nicotinamide adenine dinucleotides, to the bioenergetic status of proliferative HMECs cultured in control medium (Table 1, "growth relaxed" vs proliferative). In contrast, postconfluent HMECs cultured in the control medium show significant reductions in high energy phosphates and reducing equivalents (Table 1, differentiated). In addition, we observed that in a 3 dimensional Matrigel assay designed to induce differentiation/tubule formation, HMECs-(KS) maintained a proliferative phenotype, with ongoing mitosis and delayed tubule formation. Furthermore, HMECs-(KS) showed a loss of contact inhibition, with some cultures demonstrating a loss of anchorage dependence. These anchorage independent HMECs-(KS) cultures formed grossly visible, viable, cellular nodules which, when disaggregated, were capable of

sustained subculture (data not shown). Studies are ongoing to investigate the extent of the HMEC phenotypic alteration induced by the KS milieu.

The results obtained to date demonstrate that the component cells of the microvasculature are markedly affected by the AIDS-KS milieu, and support the hypothesis that the local cellular environment is integral in the development of the growth relaxation characteristic of AIDS-KS. In addition, these findings corroborate our selection of a human microvascular endothelial stem cell as a putative KS progenitor cell.

Results of experiments conducted on our KS cell strains have shown that there is an association between the KS histological grade and cellular growth characteristics in culture. KS strains isolated from higher grade, more advanced lesions, have demonstrated growth independence by prolific growth in control medium apparently via autologous production (autocrine/paracrine) of necessary growth factors. These strains show similar growth kinetics and cellular morphology in both control and KS environments. In contrast, those strains isolated from early KS lesions are distinctly dependent upon components present in the KS milieu to maintain cellular proliferation rates and prevent culture crisis and subsequent cell death.

Therefore, the concept that during its inception KS is initially a hyperplasia is supported by our findings with regard to the association between the KS histological grade and *in vitro* growth characteristics. Clarification of the causative factors that are permissive for this transition from hyperplasia to neoplasia will help target KS therapy.

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