Minireview

Uncovering the complexities of Kaposi's sarcoma through genome-wide expression analysis

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Abstract

Gene-expression profiling of endothelial cells infected with Kaposi's sarcoma-associated herpesvirus has led to a greater understanding of the histogenesis of Kaposi's sarcoma and the cellular reprogramming events that occur as a result of viral infection and that may play important roles in viral pathogenesis.

Kaposi's sarcoma (KS) is a peculiar neoplasm that has a diverse cellular makeup and an extensive neovasculature. Because of the complexity of this vascular proliferative disorder there are several questions that remain unanswered. Recently, several groups have used gene-expression microarrays in an effort to better understand the nature and pathogenesis of KS [1-5]. KS was first identified in 1872 by Moritz Kaposi, a Hungarian dermatologist who described it as an "idiopathic multiple pigmented sarcoma of the skin", which was lethal in his patients [6]. This 'classical' form of KS was further described as a rare, indolent disease predominantly found in older men of Mediterranean and Eastern European descent. In the 1950s, 'endemic' KS, a more aggressive form of KS, was identified in parts of sub-Saharan Africa. Shortly thereafter, an 'iatrogenic' form of KS was diagnosed in immunosuppressed organ-transplant patients. A fourth form of KS, 'epidemic' KS, was identified in the 1980s, initially in homosexual men with acquired immune deficiency syndrome (AIDS). The epidemiology of KS - in particular its geographical distribution as well as its prevalence in gay men - suggested that there was an infectious etiological agent for this disease. In 1994, a novel human γ-herpesvirus, Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV8), was identified through representational difference analysis whereby KS tissue was compared with normal tissue from the same individual [7]. KSHV is invariably found in the spindle-shaped cells present in all KS tumors.

Histogenesis of KS

The histopathology of KS is similar for all four clinical forms of KS and three stages have been identified in the progression of the lesion. Early on, in the 'patch' stage, thin-walled vascular spaces are visible in the upper dermis with a sparse mononuclear cell infiltrate of lymphocytes, plasma cells and macrophages. In the 'plaque' stage, these vascular spaces increase in number and spindle-cell bundles accumulate around them. In the late 'nodular' stage, the tumor is more solid and consists of large fascicles of spindle-shaped cells with fewer and more compact vascular slits. The mononuclear cell infiltrate is no longer prominent and few extravasated erythrocytes and macrophages are present between spindle cells.

Controversy has surrounded the cellular origin and nature of KS. Some studies have shown that KS spindle cells express markers characteristic of endothelial cells [8-10]. Other researchers have argued that they may be comprised of a more heterogeneous population that includes dendritic cells, macrophages, smooth muscle cells, cells from lymphatic junctions or fibroblasts [11-13]. Similarly, there has been much discussion over whether KS represents a clonal neoplasm or

a hyperproliferative reactive response. Support for the latter comes from studies which show that cultured KS cells are dependent on exogenous growth factors and do not produce tumors when transplanted into nude mice but rather induce an inflammatory and angiogenic response [14]. The lack of identifiable chromosomal abnormalities and the occasional regression of KS spontaneously or upon restoration of immune function [15] all contribute to the notion that KS is a result of an inflammatory response. On the other hand, KS lesions have been found to be monoclonal in a subset of advanced cases [16], and three cell lines with chromosomal alterations have been successfully established from KS lesions, suggesting that at least some advanced cases are neoplastic [17,18]. A current model is that KS develops from a proliferative inflammatory response that later, under certain selective pressures and/or as a result of cellular genetic alterations, gives rise to a neoplastic monoclonal lesion [19].

Recent studies have suggested that KS spindle cells are derived from lymphatic endothelial cells (LECs) rather than blood vascular endothelial cells (BECs) on the basis of their expression of vascular endothelial growth factor receptor-3 (VEGFR-3), a marker of lymphatic endothelium [8,10]. VEGFR-3, however, can also be expressed by precursor endothelial cells, so the precise histogenesis of KS lesions has not been definitively clarified. Recently, Wang *et al.* [1] have weighed in on this issue in a report in the July 2004 issue of *Nature Genetics*. In this elegant study [1], oligonucleotide microarray analysis was performed on nodular KS biopsy samples in comparison with several normal tissue types. The nodular KS lesions used in this study were composed of over 80% spindle cells, thus minimizing dermal and epidermal contaminants. The expression profile of KS lesions most closely resembled endothelial cells on a multidimensional scaling (MDS) plot. Furthermore, the KS gene-expression 'signature' was examined for the expression of 114 genes that had previously been found to differentiate LECs from BECs, and the authors found that KS spindle cells were more closely related to LECs. KS cells may not represent a pure population of LECs, however, as their expression signature contained some markers for BECs as well.

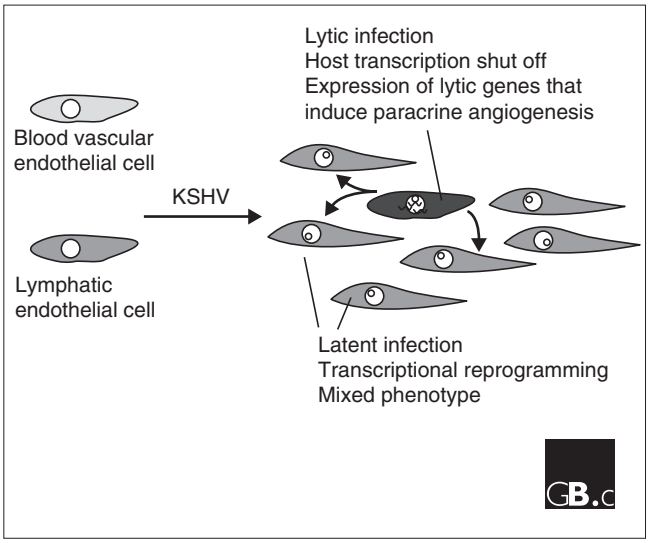

Figure 1
Infection of blood vascular endothelial cells and lymphatic endothelial cells with KSHV. The study by Wang *et al.* [1] found that the infected cells have a transcription profile that lies between those of the two starting cell types but is closer to that of lymphatic endothelium. In most cells the virus establishes a latent infection, whereas a small proportion develop a lytic infection.

To examine whether KSHV alters the transcriptional program in KS spindle cells and thereby produces an indeterminate endothelial cell phenotype, Wang *et al.* [1] infected both LECs and BECs with KSHV and compared their expression profiles to those of uninfected counterparts. They found that the genetic profiles of both KSHV-infected endothelial cell populations differed from those of the uninfected populations. Moreover, upon KSHV infection, the transcriptional program of these two populations became more similar to each other (Figure 1). These findings suggest two possibilities: that KS lesions are composed of both infected LEC and BEC populations, or, alternatively, that KSHV infects precursor endothelial cells and drives them to become more LEC-like.

Pathogenesis of KS

Other groups have used *in vitro* systems in which endothelial cells have been infected with KSHV [20-23]. These methods provide a relevant model of KS as they share some characteristics with KS lesions. Upon infection, endothelial cells change from a cobblestone appearance to the elongated, spindle-shaped morphology seen in KS lesions. Like KS spindle cells, most infected endothelial cells express latency-associated nuclear antigen (LANA), indicative of KSHV latency, whereas a smaller percentage stain positively for lytic-cycle proteins. Flore *et al.* [20] found that infection of endothelial cells resulted in upregulation of telomerase and long-term survival. Moreover, the infected cells, which represented only 1-6% of the population in this system, provided a proliferative advantage to uninfected cells in the culture, possibly through a paracrine mechanism involving the upregulation of kinase-insert domain receptor (KDR), a VEGF receptor. Other investigators have developed more robust *in vitro* infection models, using normal or immortalized endothelial cells [21-23]. Recently, microarrays have been used along with these *in vitro* systems to gain a more comprehensive picture of early cellular events and signaling pathways that are affected by KSHV infection and that may play a role in the pathogenesis of KS. This approach allows a direct comparison between endothelial cells infected with KSHV and matched uninfected cells, and study of the early events that follow infection, the latter being impossible with KS biopsies.

Gene-expression profiling by four separate groups has uncovered complex transcriptional remodeling that takes place in endothelial cells as a result of KSHV infection (Table 1) [2-5]. Using a model of lytic infection, Glaunsinger and Ganem [24] showed that cellular gene expression is dramatically inhibited by KSHV via a mechanism that involves acceleration of global mRNA turnover. To identify whether any host genes are capable of escaping this shutoff, in a separate study the same authors used microarray analysis on telomerase-immortalized microvascular endothelial (TIME) cells infected with KSHV, and subsequently lytically reactivated the virus by ectopic expression of its replication and transcriptional activator (RTA) [2]. They identified a subset of genes that were upregulated despite KSHV-mediated shutoff. A KSHV lytic gene product thought to be critical for KS development, vGPCR, has been shown to activate a number of signaling pathways, such as phosphatidylinositol 3´-kinase (PI3K), c-Jun N-amino terminal kinase (JNK) and p38, and to induce expression of multiple genes [25-27]. Interestingly, the expression of many of the host genes that are upregulated by vGPCR is blocked in KSHV-infected TIME cells. These data suggest that host gene expression is dictated by a complex interplay of viral genes that cannot be fully understood by examining the effects of a single viral gene product.

Others studies have evaluated the effect of KSHV infection in largely latently infected endothelial cells. Poole *et al.* [3] infected primary dermal microvascular endothelial cells (DMVECs) and looked at changes in their gene expression after 3 and 5 weeks. Moses *et al.* [4] used a method of infecting DMVECs that had previously been immortalized with the E6 and E7 genes of human papillomavirus (HPV); their analysis was done 4 weeks after KSHV infection. Another group was interested in evaluating changes in host gene expression during acute infection: Naranatt *et al.* [5] infected primary DMVECs and looked at host-cell transcriptional reprogramming 2 and 4 hours after KSHV infection. Together, the data from these four studies [2-5] reveal the ability of KSHV to modify the expression of genes involved in immune defense and inflammation, apoptosis, remodeling of the extracellular matrix, processing and stability of proteins, angiogenesis, and regulation of the cell cycle. Comparison of the data reveals about a dozen genes (IRF7, Mx1, IFN-induced transmembrane protein, guanylate binding protein 1, Mx R2, SSI-3, vEts transcription factor, tissue plasminogen activator, IL-8, Bcl-3, nucleoside phosphorylase, and tissue inhibitor of metalloproteinase-1) that were upregulated in both the Poole *et al.* [3] and Naranatt *et al.* [5] studies, and seven genes (RDC-1, LIM domain only 2, MADS box transciption factor, galactoside binding lectin, gap junction protein α1, interleukin 1 receptor type 1, and CC-chemokine 14) upregulated and three downregulated (stromal cell derived factor 1, urokinase plasminogen activator, and thioredoxin reductase 1) in both the Poole *et al.* [3] and the Moses *et al.* [4] studies.

The available gene-expression data on KSHV-infected endothelial cells provide interesting insights into the molecules that may play an important role in KS tumor progression, validate previously established players and offer new potential targets for treatment. Moses *et al.* [4] found c-Kit, a receptor tyrosine kinase for the mast cell growth factor SCF, to be upregulated in their assays. Upon

Table 1

Summary of the recent microarray studies of Kaposi's sarcoma (KS)

Study	Cells used	Time analyzed (post-infection)	Access to database	Major findings
Wang *et al.* [1]	Primary LECs and BECs	2 and 7 days	ArrayExpress [29] Accession number: E-MEXP-66	Expression profile of KS lesions closely resembles that of endothelial cells; infection of BECs and LECs results in an intermediate gene-expression profile
Glaunsinger and Ganem [2]	TIME cells (induced lytic replication 2 h post-KSHV infection)	6, 12 and 20 h	Gene Expression Omnibus [30] Accession number: GSE1406	Widespread shutoff of host genes during KSHV lytic replication; a subset of genes resistant to shutoff are upregulated during lytic reactivation
Naranatt *et al.* [5]	Primary DMVECs	2 and 4 h	Contact Dr Bala Chandran (University of Kansas Medical Center, Kansas City USA)	215 genes upregulated; 109 genes downregulated
Poole *et al.* [3]	Primary DMVECs	3 and 5 weeks	Pevsner lab website [31]	Around 2% of genes upregulated and 2% downregulated
Moses *et al.* [4]	DMVECs previously immortalized with HPV proteins E6 and E7	4 weeks	Früh lab website [32]	124 genes upregulated; 60 genes downregulated

LEC, lymphatic endothelial cells; BEC, blood vascular endothelial cells; TIME, telomerase-immortalized microvascular endothelial; KSHV, Kaposi's sarcoma-associated herpesvirus; DMVEC, dermal microvascular endothelial cells; HPV, human papillomavirus.

further investigation, they found that c-Kit is involved in the transformed phenotype of DMVECs infected with KSHV and that it provides the cells with a proliferative advantage in response to exogenous SCF. As a result of these studies, clinical trials are ongoing to evaluate the effect of inhibiting c-Kit with Gleevec (Imatinib) in the treatment of KS. KDR, which was previously reported to be upregulated by KSHV [20] and provides a proliferative advantage to infected endothelial cells [28], was also found to be upregulated [4]. CD36, a receptor that binds to, among other things, the angiogenesis inhibitor thrombospondin-1 (TSP-1), is highly induced as a result of KSHV infection [4]. TSP-1 is a potent inhibitor of angiogenesis and effectively inhibits a wide variety of angiogenic stimuli, including VEGF. Treatment with exogenous TSP-1 may, therefore, be an effective way of limiting the vascularization of KS lesions.

Direct comparison between the microarrays used in the various recent studies is complicated by the small overlap of genes represented on the different arrays. Also, differences in the systems of KSHV infection and in the time after infection that the arrays were performed contribute to variability between the assays. Nevertheless, each of these studies provides us with a greater understanding of the complex nature of transcriptional reprogramming that takes place at the hands of KSHV during the infection process and how changes induced by KSHV lead to angiogenic lesions characteristic of KS.

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