Review

Cellular origin of Kaposi's sarcoma and Kaposi's sarcoma-associated herpesvirus-induced cell reprogramming

Laila Cancian, Amy Hansen*, and Chris Boshoff

UCL Cancer Institute, 72 Huntley Street, University College London, London WC1E 6BT, UK

Kaposi's sarcoma (KS) is the most common malignancy in untreated HIV patients. KS is characterised by abnormal neoangiogenesis, inflammation, and proliferation of tumour cells [KS spindle cells (SCs)]. Kaposi's sarcoma-associated herpesvirus (KSHV) is the aetiological agent of KS. KS SCs are the predominant KSHV-infected cells in KS lesions. In this review, we report advances in understanding of the cellular origin of the KS SC, a contentious topic in KSHV research. KS SCs are now known to be of endothelial cell (EC) origin, phenotypically most similar to lymphatic ECs (LECs), but poorly differentiated. We focus on recent insights into KSHV's ability to exploit *the* normal differentiation pathway and intrinsic plasticity *of ECs*, through manipulation of EC-specific transcriptional regulators [i.e., *prospero homeobox 1* (PROX1) and MAF] and discuss how this may contribute to viral persistence and KS sarcomagenesis.

Kaposi's sarcoma: background

In 1872, the Hungarian dermatologist Moritz Kaposi reported five cases of 'idiopathic multiple pigmented sarcomas of the skin' [1] and in 1895 the term Kaposi's sarcoma (KS) was coined. In 1981, cases of disseminated KS, as well as *Pneumocystis carinii* pneumonia, were reported from New York City and Los Angeles, heralding the HIV/AIDS pandemic [2,3].

KS is classified into four distinct clinico-epidemiological forms. Classic KS is typically an indolent tumour occurring in elderly men of Mediterranean, Eastern European, or Jewish descent [4]. A more aggressive form of KS, named endemic KS, preceded the HIV/AIDS pandemic and often affects children in Equatorial and East Africa [5]. The most aggressive form of KS occurs in immunosuppressed individuals, including HIV-infected people (AIDS-KS or epidemic KS) and transplant patients (iatrogenic or post-transplant KS) [6,7].

In 1994, the aetiological agent of KS was identified using a PCR-based technique to isolate unique DNA sequences from AIDS-KS lesions [8]. The DNA represented a novel gammaherpesvirus, termed Kaposi's sarcoma-associated herpesvirus (KSHV), also called human herpesvirus 8 (HHV-8). KSHV is classified as a gamma-2-herpesvirus. Its structure and genomic organisation are related to the prototypical human oncogenic virus, Epstein–Barr virus (EBV), a gamma-1-herpesvirus. Seroepidemiological studies confirmed the causal link between KSHV infection and KS development [9–12]. KS is unusual in healthy KSHV-infected individuals, supporting the notion that additional cofactors (e.g., immunosuppression) are required for KS to arise [13]. Although the incidence of AIDS-KS has rapidly declined in Western populations due to the availability of antiretroviral therapy, KS remains a significant burden in developing countries [14]. In addition to KS, KSHV is also linked to the pathogenesis of certain B cell lymphoproliferations that occur mainly in immunosuppressed individuals, including primary effusion lymphoma (PEL) [15] and multicentric Castleman's disease (MCD) [16].

The origin of the main tumour cell of KS remains contentious. Here, we summarise the current evidence supporting an EC origin for KS. We report advances in our understanding of the molecular mechanisms adopted by KSHV to reprogram EC fate and discuss how this may contribute to KS pathogenesis. We describe how other oncogenic viruses have usurped cell differentiation mechanisms to ensure viral persistence and, finally, we discuss the possible role of either mature blood/lymphatic ECs or endothelial progenitor cells as precursors of KS tumour cells.

KS histogenesis: a lymphatic derivation for KS SCs?

KS is a multifocal, oligoclonal disease presenting as red/purplish lesions on the skin or oral mucosa. Lesions can progress to involve lymph nodes and visceral organs [17–19]. Tumours comprise immature blood vessels, an abundant inflammatory infiltrate, and proliferating SCs (also known as KS cells), so called due to their elongated morphology [9,13,20] (Box 1). SCs are the predominant KSHV-infected cells in KS lesions [21–23]. In early lesions, 10% of SCs are KSHV positive, but this increases to >90% in

Corresponding author: Boshoff, C. (c.boshoff@ucl.ac.uk).
* *Current address:* Harvard Medical School, Department of Genetics, 77 Avenue Louis Pasteur, NRB 260I, Boston, MA 02115, USA.
Keywords: Kaposi's sarcoma; KSHV/HHV8; endothelial cells; cell differentiation; cell plasticity.oncogenic viruses.

0962-8924/$ – see front matter
 http://dx.doi.org/10.1016/j.tcb.2013.04.001

Box 1. Composition and pathogenesis of KS lesions: reactive inflammation or true neoplasm?

If left untreated, KS lesions progress through three stages: patch, plaque, and nodular. However, lesions of all three stages can occur simultaneously in an individual patient. Early patch-stage KS presents as flat, red-brown lesions in the dermis characterised by a significant inflammatory infiltrate and abundant blood vessels. Lesion development is associated with neoangiogenesis and the appearance of spindle-shaped ECs, which are the KS tumour cells [9,19]. These SCs proliferate with disease progression and neovascularisation continues along with extravasation of red blood cells and haemosiderin, contributing to the red colour of the lesions [25]. Nodular KS lesions are dominated by SCs, although immune cells are also present and are interspersed by abnormal slit-like vasculature spaces [25].

Aberrant angiogenesis, proliferation of KSHV-infected SCs and inflammation are the hallmarks of KS lesions. Early-stage KS is a reactive, polyclonal inflammatory process that often arises at sites of previous inflammation (the Koebner phenomenon) [80]. The inflammatory infiltrate can appear before SC formation, highlighting the importance of inflammatory cells in KS pathogenesis [9]. The immune infiltrate largely comprises CD8+ T cells and monocyte-macrophages. Dendritic cells, CD4+ T cells, and B cells are also present, although less abundant [9]. KS lesions are typified by high levels of proinflammatory cytokines, (interferon gamma [IFNγ], tumour necrosis factor alpha (TNF-α), IL-1, IL-6, and granulocyte-macrophage colony-stimulating factor [GM-CSF]), chemokines (monocyte chemoattractant protein 1 [MCP-1] and IL-8), and proangiogenic growth factors (VEGF family, platelet-derived growth factor [PDGF], beta fibroblast growth factor [βFGF], transforming growth factor beta [TGFβ], cyclooxygenase 2, angiogenin, and angiopoietin family members [30,34,81–85]) secreted by immune cells and/or KSHV-infected SCs [9] (see Figure 3 in main text). This cytokine profile may contribute to KS pathogenesis because it influences SC activation/proliferation, induces further recruitment of T cells and monocytes, and induces angiogenesis. The virus contributes directly to the proinflammatory/angiogenic environment by encoding its own array of cytokines such as vIL6 and the viral chemokines (vCCL1–3) [37]. Additionally, HIV infection might promote directly KS SC proliferation, by augmenting cytokine and growth factor production from infected lymphocytes and macrophages (see Figure 3 in main text) [9].

Early KS lesions are polyclonal, whereas late-stage lesions, within a given patient, can be oligo/monoclonal [17,18,86]. KS probably evolves as a polyclonal proliferation of KSHV-infected SCs, accompanied by a prominent inflammatory/angiogenic reaction, that subsequently – in the presence of selective pressures, genetic alterations, or immunosuppression – progresses to a true monoclonal malignancy [9]. The proliferative potential of KSHV-infected SCs is mainly driven by viral latent genes (including vcyclin, vFLIP, Kaposins, LANA, and KSHV miRNAs) that manipulate cell proliferation, differentiation, and survival (see Figure 3 in main text). Deregulated expression of cellular oncogenes/tumour suppressors (i.e., p53) has been observed in only a few late-stage KS tumours [20]. Whether these rare somatic events act as driver mutations remains to be established.

advanced nodular lesions [21–23], indicating that these infected cells have a proliferative advantage.

The origin of the SC lineage remains contentious. Initial immunohistochemistry studies showed that KS SCs are poorly differentiated cells of endothelial origin, as evidenced by the expression of the panendothelial cell markers CD31, CD34, and Factor VIII and reactivity with the endothelial cell-specific mAb PAL-E [9,19,20,24]. However, a fraction of SCs also express markers of smooth muscle cells, fibroblasts, monocytes/macrophages (CD68, PAM-1), and dendritic cells (Factor XIII), suggesting that the SC population is heterogeneous or shows differentiation plasticity [19,20,25]. Nevertheless, the most widely accepted conclusion from these studies is that SCs derive from the EC lineage. Specifically, immunohistochemistry supports a lymphatic EC origin for KS SCs. SCs lack expression of blood vessel-specific markers, but do express markers of LECs, including lymphatic vessel endothelial receptor 1 (LYVE-1), D2-40, podoplanin, and vascular endothelial growth factor receptor 3 (VEGFR3) [19,20]. Anatomical evidence further supports a lymphatic association: the unique distribution of KS along the lengths of lymphatic vessels; the predilection of KS for lymph nodes; and the lack of lesions in tissues devoid of lymphatics (i.e., central nervous system and cornea) [26]. Furthermore, within tumours, KSHV colocalises with the lymphatic-specific growth factor receptor VEGFR3, contrasting with the paucity of KSHV staining in normal vascular endothelium [27]. KS tumours express the lymphangiogenic ligand VEGFC, which signals mainly through VEGFR3, and addition of VEGFC to KS tumour cells stimulates their *in vitro* proliferation [28,29]. Moreover, serum levels of the other VEGFR3 ligand, VEGFD, correlate with disease burden and are significantly reduced on resolution of AIDS-KS during antiretroviral therapy, corroborating the hypothesis that KS SCs are of LEC origin and are dependent on lymphangiogenic growth factors [30].

KSHV-induced EC fate reprogramming

Molecular studies added a twist to the view that KS SCs originate from the lymphatic cell lineage. A gene expression microarray profiling analysis comparing nodular KS (>80% SCs) with the mRNA expression profiles of LECs and blood ECs (BECs) indicated that the transcriptional profile of KS is closest to that of LECs [30]. However, some BEC-specific markers were also found in the expression profile of KS SCs, suggesting that these cells do not faithfully represent either cell lineage [30]. KSHV infection of LECs *in vitro* induces transcriptional reprogramming, driving LECs away from their mature differentiated state towards the opposing BEC lineage [30]. The resulting transcriptional profile of KSHV-LECs is more similar to BECs than uninfected cells, whereby levels of genes typically associated with the blood endothelium are increased and lymphatic markers downregulated [30]. This transcriptional reprogramming, or dedifferentiation, is also observed on KSHV infection of BECs, whereby BEC-specific genes (i.e., *CXCR4* and *neuropilin-1*) are downregulated and a reciprocal increase in LEC markers including *PROX1*, a master regulator of lymphatic development (Boxes 2 and 3), *VEGFR3*, *PDPN* (encoding podoplanin), and *LYVE-1* is observed [31,32]. Mechanistically, this reprogramming is likely to be due to virus-induced changes in cell fate-specifying transcription factors (TFs); recent studies unveiled key roles played by PROX1 and MAF.

PROX1

The upregulation of *PROX1* on KSHV infection of BECs [31,32] suggests that the transcriptional activity downstream of this master regulator of lymphatic identity switches on LEC-specific genes in the blood vasculature [32]. Silencing *PROX1* during infection of BECs partially inhibits KSHV-mediated upregulation of multiple key