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Original contribution

mTOR, VEGF, PDGFR, and c-kit signaling pathway activation in Kaposi sarcoma☆,☆☆,★

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Summary Kaposi sarcoma (KS) is a locally progressive, intermediate-grade vascular neoplasm with no known cure, high recurrence rates, and potential for wide dissemination. Low efficacy and high toxicity limit current therapeutic options for advanced disease. Activation of mammalian target of rapamycin (mTOR), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and c-kit signaling pathways has been implicated in KS pathogenesis and may suggest a role for targeted inhibitors. KS cases were retrospectively retrieved (N = 274), most (90%) associated with human immunodeficiency virus. Tissue microarray slides were stained with human herpes virus-8, Friend leukemia integration 1 transcription factor, CD117 (c-kit), phospho-S6 (pS6), PDGF receptor-*β*, VEGF, and phospho-mTOR. Both intensity and extent of staining were scored. Multiplying these scores for each core yielded total staining H-scores. Human herpes virus-8 was positive in 87% and Friend leukemia integration 1 transcription factor in 95.7% of cases. Most were also VEGF+ (97.6%), pS6+ (95.7%), CD117+ (92.5%), and PDGFRB+ (87.4%). Approximately half (55.6%) were phospho-mTOR+. There was no significant difference in staining among patients with low (<500 cells/mm^3) or preserved CD4 T-cell counts. Immunohistochemistry confirms upregulation of the mTOR, PDGF, VEGF, and c-kit pathways in a large cohort of KS samples. Of proteins tested, pS6, downstream of mTOR, demonstrated the highest proportion of strong positivity (67.1%). These results support the possibility of using targeted inhibitors in KS. Overexpression was independent of CD4 count, suggesting that even patients with low counts may be targeted therapy candidates.

1. Introduction

Kaposi sarcoma (KS) is an intermediate-grade angioproliferative neoplasm with no known cure caused by infection with human herpes virus-8 (HHV-8), otherwise known as *Kaposi sarcoma–associated herpes virus*, frequently arising in the context of immunosuppression. It occurs in 1 of 4 forms: classic, acquired immune deficiency syndrome (AIDS)

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associated, endemic (African), and iatrogenic (transplant associated) [1,2]. Clinically, KS most commonly involves adults, with a male predilection, and lesions evolve from early macules (patch stage) to plaques (plaque stage) and larger nodules (tumor stage). They typically involve mucocutaneous sites but may also involve visceral locations, and virtually all organs may be affected by KS [3].

Histologically, KS lesions are characterized by variable proportions of spindle-shaped tumor cells, vessels, chronic inflammatory cells, and extravasated red blood cells. The tumor cells are of host blood endothelial origin, thought to have been reprogrammed by HHV-8 to resemble lymphatic endothelial cells [2]. They generally lack significant mitotic activity, cytologic pleomorphism, and necrosis. By immunohistochemistry (IHC), the spindle cells stain positively with vascular markers CD31, CD34, and Friend leukemia virus integration 1 (FLI-1), as well as lymphatic endothelial markers such as D2-40 [2,4]. Positive nuclear signal for HHV-8 latency-associated nuclear antigen (LNA-1) is generally considered the most diagnostically useful IHC stain [2,5].

Biologically, KS is locally progressive, with high recurrence rates following surgical excision and the potential for wide dissemination in HIV-positive patients, as HIV infection promotes HHV-8 replication. KS is the most common malignancy in patients with AIDS, and the incidence of KS varies and parallels the incidence of AIDS throughout the world [2,6]. Current treatment strategies for KS frequently provide short-term responses and disease control; however, recurrence is common, and progression-free intervals are short [3]. For HIV-related KS, optimal control of HIV infection with highly active antiretroviral therapy is a standard and crucial component of therapy. Highly active antiretroviral therapy often leads to regression of KS in patients with limited disease who are treatment naive; however, patients with symptomatic, advanced KS require additional therapy [6]. Similarly, modification or decrease in immunosuppression may be necessary for treatment of iatrogenic KS. Treatment goals include symptom palliation, prevention of progression, improvement in cosmesis, decreasing edema, and ameliorating organ compromise [3,6]. Novel therapies such as thalidomide, interleukin-6 inhibitors, anti–herpes virus agents, imatinib mesylate, and matrix metalloproteinase inhibitors have been used with some success [2,3,6]. At present, therapeutic options for advanced disease include radiation, chemotherapy, and interferon-α, which are limited by low efficacy and high toxicity.

Activation of mammalian target of rapamycin (mTOR), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and c-kit signaling pathway molecules has been implicated in the pathogenesis of KS (Fig. 1) and may suggest a therapeutic role for mTOR inhibitors such as rapamycin (sirolimus) and c-kit/VEGF inhibitors such as sunitinib malate [1,3]. Despite the biologic rationale and interest in inhibiting these pathways in KS, thus far, a relatively limited number of human tumor samples have been analyzed for overexpression of mTOR, VEGF, PDGF, and c-kit signaling pathway proteins. Studying human tumor samples for overexpression of these signaling pathway proteins will be a useful approach to assess how commonly and to what level these pathway components are overexpressed. If overexpression is confirmed, these findings would lend additional support to the notion that drugs currently in use could be employed in a novel way to fill a significant deficit in the medical treatment of KS. In addition, studying a large series of tumors may inform which antibodies and targets are most reliably detected to be upregulated in KS in formalin-fixed, paraffin-embedded (FFPE) tissue, data that may be informative in assessing or monitoring response to therapy.

In this study, we characterize the pattern and level of mTOR, VEGF, PDGF, and c-kit pathway protein expression in a large cohort of human KS samples (N = 274). We correlate protein staining patterns with pathway activation and examine potential links between the various pathways activated in KS. Finally, we assess for potential correlations between histologic and IHC findings, HIV status, and CD4 T-cell counts in cases with available clinical information.

2. Materials and methods

Following approval of the Aga Khan University East Africa Research Ethics Committee and the Massachusetts General Hospital/Partners Healthcare Institutional Review Board, cases of KS obtained for diagnosis as part of routine clinical care were retrospectively retrieved from the archives of the Departments of Pathology, Aga Khan Hospital, Kisumu, Kenya, and the Massachusetts General Hospital, Boston, MA. Glass slides from these archived samples were reviewed by at least 3 study coauthors to confirm the diagnosis of KS, and 274 samples (272 from Kenya and 2 from the United States) with sufficient tissue for analysis were identified. Tumor-rich areas were marked on the glass slides to indicate the site of core retrieval for inclusion in the tissue microarray (TMA). Tissue cores (2 mm in diameter) were obtained from the corresponding FFPE tissue blocks and were transferred onto 6 "master blocks." Each master block contained 2 samples of normal skin to serve as controls for IHC. The 2 US cases, which were included in the overall analysis, also served as controls to ensure that staining characteristics between Kenyan and US cases were comparable in terms of presence or absence of background staining and pattern of positive staining (nuclear versus cytoplasmic).

Histologic sections cut from the master blocks were stained with hematoxylin and eosin, HHV-8 LNA-1 (HHV-8), and FLI-1 IHC for additional corroboration of the original diagnosis. The specific antibodies used to assess for pathway overexpression were PDGF receptor-β (PDGFRB), VEGF, c-kit (CD117), phospho-mTOR (p-mTOR), and phospho-S6 (pS6). Staining was performed using the standard avidin-biotin-peroxidase complex technique on the Dako Autostainer Plus (Dako North America, Inc, Carpinteria, CA) or Leica Bond (Leica Biosystems Inc, Buffalo Grove, IL) platforms following deparaffinization of 5-μm FFPE sections of KS TMA